Organically-Bound Sulphur in the Geosphere: A Molecular Approach

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JACOBUS SMEDE SINNINGHE DAMSTÉ
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aan Wenda

Zoals bij vrijwel ieder proefschrift is ook de totstandkoming van de voor u liggende dissertatie alleen mogelijk geweest dankzij bijdragen van vele mensen. Op deze plaats wil ik hen dan ook hartelijk bedanken voor hun grote of kleine, wetenschappelijke of technische inbreng, hun morele steun en de samenwerking, die ik als zeer prettig ervaren heb.

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Contents

9. Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils

10. Quenching of labile functionalised lipids with inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis

11. Identification of homologous series of alkylated thiophenes, thiolanes and benzothiophenes present in pyrolysates of sulphur-rich kerogens

12. Organic sulphur in macromolecular sedimentary organic matter.I. Structure and origin of sulphur-containigg moieties in kerogen, asphaltenes and coal as revealed by flash pyrolysis

Abbreviations

ATI	Alkyl Thiophene Index
BAI	Branched Alkane Index
CC	Column Chromatography
DABT	2,4-Di-*n*-AlkylBenzo[*b*]Thiophene(s)
DATL	2,5-Di-*n*-AlkylThioLane(s)
DATN	2,6-Di-*n*-AlkylThiaNe(s)
DATP	2,5-Di-*n*-AlkylThioPhene(s)
DMDS	DiMethylDiSulphide
DMF	DiMethylFormamide
DSDP	Deep Sea Drilling Project
FID	Flame Ionization Detector
FPD	Flame Photometric Detector
GC	Gas Chromatography
HBIT	Highly Branched Isoprenoid Thiophene(s)
HMWA	High Molecular Weight Aromatic
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
I	retention Index
IR	Infra Red spectroscopy
JED	Jurf Ed Darawish oil shale
LMWA	Low Molecular Weight Aromatic
MS	Mass Spectrometry
MTTC	2-Methyl-2-(4,8,12-TrimethylTridecyl)Chroman(s)
NMR	Nuclear Magnetic Resonance
OSC	Organic Sulphur Compound(s)
OSPP	Organic Sulphur Pyrolysis Product(s)
Py	Pyrolysis
RI	Retention Index
RPO	Rozel Point Oil
THF	TetraHydroFuran
TIC	Total Ion Current
TMEDA	TetraMethylEneDiAmine
TLC	Thin Layer Chromatography
UV	Ultra Violet

Summary

In this thesis the results of molecular organic geochemical investigations into the structure and origin of organic sulphur compounds (OSC) and sulphur-containing moieties in macromolecular organic matter (*i.e.* kerogen, coal and asphaltenes) occurring in the geosphere are described. In the first part of the thesis (chapters 2 to 10) a considerable number of novel OSC is identified in immature bitumens and immature crude oils by gas chromatography with sulphur-selective flame photometric detection, gas chromatography-mass spectrometry, Raney-Ni desulphurisation, NMR spectroscopy and, in several cases, synthesis of authentic standards. In the second part (chapters 11 and 12) experiments are described which focus on the nature of organic sulphur in sedimentary macromolecular organic matter as revealed by flash pyrolysis-gas chromatography with sulphur-selective flame photometric detection and flash pyrolysis-gas chromatography-mass spectrometry.

In chapter 2 it is demonstrated that a C_{20} isoprenoid thiophene (3-methyl-2-(3,7,11-trimethyldodecyl)thiophene) occurs widespread in both recent and ancient deep-sea sediments as a limited number of the possible stereoisomers. Its presence is explained by incorporation of sulphur into chlorophyll-derived phytol or archaebacterial phytenes or their diagenetical products. A similar mechanism of formation is proposed in chapter 3 for series of *n*-alkyl and isoprenoid thiophenes and thiolanes occurring in a sample from a marl layer from a Messinian evaporitic basin (Northern Apennines, Italy). C_{19}-C_{28} isoprenoid thiophenes were major compounds. The most abundant isoprenoid thiophene (2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene) occurring in this and other samples was unambigiously identified by synthesis (chapter 4).

The OSC present in the Rozel Point Oil, an immature oil from a shallow reservoir in Utah (U.S.A.), are described in detail in chapters 5 and 6. Chapter 5 deals with the the origin and fate of C_{20} and C_{15} isoprenoid thiophenes, thiolanes, benzothiophenes, bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes identified in the Rozel Point Oil and other oils and bitumens. Their origin is explained by sulphur incorporation into unsaturated phytanols and farnesol and subsequent cyclisation and aromatisation reactions. OSC containing two sulphur atoms are thought to originate from polyunsaturated phytenols, compounds especially abundant in

hypersaline depositional environments due to the presence of archaebacteria and green and purple sulphur bacteria.

Chapter 6 gives a detailed description of the almost 1000 individual OSC occurring in the "aromatic" fraction of the Rozel Point Oil. They include, besides the OSC described in chapter 5, 2,5-di-*n*-alkylthiolanes, 2,6-di-*n*-alkylthianes, 2,4-di-*n*-alkylbenzothiophenes and thiophene and thiolane steroids. Because the carbon skeletons of the OSC identified have the same structures as well known geologically-occurring hydrocarbons (*n*-alkanes, isoprenoid hydrocarbons, steranes) these compounds are thought to be products of early diagenetic sulphur incorporation into specific functionalised precursors.

The widespread occurrence of the OSC described above in immature bitumens and immature crude oils is demonstrated in chapter 7. The OSC present in samples from different geographical locations and of different ages (Miocene to Permian) are described; the relative amounts and distributions of the various classes of OSC vary considerably as a result of different organic input and different degrees of maturation. Therefore, OSC may be useful as molecular indicators of organic input, depositional environment and maturity and in oil-oil and oil-source rock correlation studies.

The hydrocarbons produced by desulphurisation of the OSC present in these samples exhibited distribution patterns different from those of the hydrocarbons originally present (chapter 8). Therefore reaction of elemental sulphur with these hydrocarbons at elevated temperatures as proposed in the literature must be considered as an unlikely origin for these OSC. Desulphurisation of high molecular weight fractions of bitumens and oils also produced hydrocarbons, suggesting that these substances are formed by similar sulphur incorporation reactions as OSC, but in an intermolecular fashion.

Eight C_{20} and two C_{25} highly branched isoprenoid thiophenes were identified in various samples (chapter 9). Only a limited number of all theoretically possible isomers were present, indicating that these compounds are probably formed by selective sulphur incorporation reactions into the widespread occurring highly branched isoprenoid alkenes. Similar observations were made for a number of other OSC groups (C_{28} and C_{37} di-*n*-alkylthiophenes, -thiolanes and -thianes and C_{19} branched thiophenes) occurring in the Jurf ed Darawish oil shale bitumen (Jordan) (chapter 10). From these observations a general model for the incorporation of sulphur into organic matter is proposed.

Chapters 11 and 12 describe a study of organic sulphur present in sedimentary macromolecular organic matter by flash pyrolysis. In chapter 11 the major organic sulphur pyrolysis products (OSPP) of sulphur-rich kerogens (*i.e.* C_4-C_9 alkylated thiophenes) are identified. The pyrolysates also contain homologous series of 2-alkylthiophenes, 2-alkyl-5-methylthiophenes, 2-alkyl-5-ethylthiophenes, 2-alkylthiolanes, 2-alkyl-5-methylthiolanes, 2-alkylthianes, 2-alkylbenzothiophenes, 2-alkyl-4-methylbenzothiophenes and 2-alkyl-4-methylbenzothiophenes through a total carbon number of 22.

The studies of distributions of OSPP generated by flash pyrolysis of macromolecular sedimentary organic matter (kerogen, coal, asphaltene) are comprised in chapter 12. Although the amounts of OSPP in the pyrolysates relative to the other products were highly variable the distribution patterns of the alkylated thiophenes were dominated by a limited number of all theoretically possible isomers. The alkyl substitution patterns of the dominant isomers bear a strong similarity to those of the OSC present in immature bitumens and immature oils (chapter 2-10). Therefore, it is suggested that these OSPP are formed by pyrolysis of related thiophenic and benzothiophenic moieties present in the macromolecular sedimentary substances. Specific examples include those with linear alkyl, *iso* and *anteiso* alkyl, isoprenoid alkyl and steroidal side-chain carbon skeletons. The presence of higher molecular weight alkylthiophenes and alkylbenzothiophenes with these same carbon skeletons in pyrolysates of sulphur-rich kerogens provided further evidence for the presence of these sulphur-containing moieties. It is likely that these moieties have been formed by abiogenic sulphur incorporation into sedimentary organic matter during early diagenesis.

Samenvatting

In dit proefschrift worden de resultaten beschreven van een organisch geochemisch onderzoek op moleculair niveau naar de structuur en oorsprong van organische zwavelverbindingen ("organic sulphur compounds", OSC) en zwavel bevattende stuctuurelementen in macromoleculair organisch materiaal (kerogeen, steenkool en asfaltenen) voorkomend in de geosfeer. In het eerste gedeelte van het proefschrift (hoofdstuk 2 tot en met 10) wordt de identificatie van een aanzienlijk aantal OSC met tot dan toe onbekende structuren in onrijpe sediment extracten en onrijpe oliën besproken. De gebruikte analytische methoden zijn gaschromatografie met zwavel-selectieve vlamfotometrische detectie, gaschromatografie-massaspectrometrie en NMR spectroscopie. Daarnaast heeft ontzwaveling met behulp van Raney-Ni en in een aantal gevallen synthese van standaarden plaatsgevonden. In het tweede gedeelte van dit proefschrift (hoofdstuk 11 en 12) worden experimenten beschreven die gericht zijn op het onderzoek naar de aard van organische zwavel in fossiel macromoleculair organisch materiaal zoals die met behulp van analytische pyrolyse-gaschromatografie met zwavel-selectieve vlamfotometrische detectie en analytische pyrolyse-gaschromatografie-massaspectrometrie aan het licht kan worden gebracht.

In hoofdstuk 2 wordt aangetoond dat een beperkt aantal van alle mogelijke stereoisomeren van een C_{20} isoprenoide thiofeen (3-methyl-2-(3,7,11-trimethyldodecyl)thiofeen) wijd verspreid voorkomt in zowel recente als oude diepzee sedimenten. Het voorkomen van deze thiofenen kan verklaard worden door de inbouw van zwavel in van chlorofyl afkomstig fytol of in archaebacteriele fytenen of in de diagenetische producten van deze verbindingen. Een vergelijkbaar vormingsmechanisme wordt in hoofdstuk 3 voorgesteld om het voorkomen van reeksen *n*-alkyl en isoprenoide thiofenen en -thiolanen in een monster van een mergellaag uit een Messinien evaporitisch bekken (Noord Apennijnen, Italie) te verklaren. C_{19}-C_{28} isoprenoide thiofenen zijn hoofdcomponenten in het extract van dit monster. De isoprenoide thiofeen die in dit en andere monsters als belangrijkste thiofeen voorkomt wordt in hoofdstuk 4 ondubbelzinnig geidentificeerd als 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiofeen door synthese van de standaard.

De OSC zoals deze voorkomen in de Rozel Point Oil, een onrijpe olie uit een ondiep reservoir in Utah (U.S.A.), worden beschreven in hoofdstukken 5 en 6. In hoofdstuk 5 wordt de oorsprong en het lot van C_{20} en C_{15} isoprenoide thiofenen, thiolanen, benzothiofenen, bithiofenen, (thienyl)alkylthiofenen en thienylthiolanen, geidentificeerd in Rozel Point Oil en andere oliën en sediment extracten, besproken. Deze verbindingen zijn waarschijnlijk gevormd door zwavel inbouw in onverzadigde fytenolen en farnesol gevolgd door ring sluiting en aromatisering. OSC die twee zwavel atomen bevatten hebben waarschijnlijk poly-onverzadigde fytenolen als precursors, die in relatief hoge concentratie voorkomen in hypersaliene afzettingsmilieus door de aanwezigheid van archaebacteriën en fotosynthetische zwavelbacteriën in dit specifieke milieu.

In hoofdstuk 6 wordt een beschrijving gegeven van de ongeveer duizend verschillende OSC die in de "aromatische" fractie van de Rozel Point Oil voorkomen. Naast de in hoofdstuk 5 beschreven verbindingen komen in deze onrijpe olie 2,5-di-*n*-alkylthiolanen, 2,6-di-*n*-dialkylthianen, 2,4-di-*n*-alkylbenzothiofenen en thiofeen en thiolaan steroiden voor. Omdat de koolstofskeletten van deze OSC dezelfde zijn als die van de algemeen bekende fossiele koolwaterstoffen (*n*-alkanen, isoprenoide alkanen, steranen) wordt aangenomen dat deze componenten gevormd zijn door inbouw van zwavel in bepaalde gefunctionaliseerde precursors tijdens de vroege diagenese.

Het algemeen voorkomen van de hierboven beschreven OSC in onrijpe sediment extracten en onrijpe oliën wordt in hoofdstuk 7 beschreven. De relatieve hoeveelheden en distributies van de verschillende klassen van OSC in monsters van verschillende geografische locaties en van verschillende ouderdom (Mioceen tot Perm) blijken aanzienlijk te verschillen als gevolg van verschillen in organische "input" en maturatie. Hierdoor kunnen deze OSC gebruikt worden als moleculaire indicatoren voor het vaststellen van de organische "input", van het afzettingsmilieu en van de maturatie en bij correlaties tusssen oliën onderling en tussen oliën enoliemoedergesteenten.

De koolwaterstoffen gevormd door het ontzwavelen van de OSC in de in hoofdstuk 7 genoemde monsters vertonen andere distributie patronen dan die van de als zodanig voorkomende koolwaterstoffen in dezelfde monsters (hoofdstuk 8). Een reactie van elementaire zwavel met verzadigde koolwaterstoffen bij verhoogde temperatuur, zoals in de literatuur is voorgesteld, moet daarom beschouwd worden als een onwaarschijnlijke vormingswijze voor deze OSC. Door ontzwaveling van fracties van oliën en sediment extracten met een hoger molecuul gewicht worden ook koolwaterstoffen gevormd. Dit suggereert dat deze hoger moleculaire verbindingen gevormd worden door inbouw van zwavel in gefunctionaliseerde lipiden waarbij deze lipiden als het ware "gecrosslinked" worden.

De identificatie van C_{20} en C_{25} vertakte isoprenoide thiofenen in een aantal monsters wordt beschreven in hoofdstuk 9. Het feit dat er slechts een beperkt aantal van alle theoretisch mogelijke isomeren wordt gevonden, wijst er op dat deze verbindingen gevormd zijn door inbouw van zwavel op specifieke plaatsen in wijd verspreid in sedimenten voorkomende vertakte isoprenoide alkenen. Vergelijkbare resultaten worden in hoofdstuk 9 beschreven voor een aantal andere klassen van OSC (C_{28} en C_{37} di-*n*-alkylthiofenen,

-thiolanes en -thianen en C_{19} vertakte thiofenen), die voorkomen in het extract van de Jurf ed Darawish olieschalie (Jordanië). In dit hoofdstuk wordt ook een algemeen model voor de inbouw van zwavel in organisch materiaal gepresenteerd.

In de hoofdstukken 11 en 12 wordt een onderzoek naar organisch gebonden zwavel zoals die voorkomt in fossiel macromoleculair organisch materiaal beschreven. Dit onderzoek is uitgevoerd met behulp van analytische pyrolyse van zwavelrijk kerogeen, gevolgd door identificatie van de gevormde zwavel bevattende pyrolyse produkten ("organic sulphur pyrolysis products", OSPP) (hoofdstuk 11). De belangrijkste OSPP zijn C_4-C_9 gealkyleerde thiofenen; daarnaast bevatten de pyrolysaten ook homologe reeksen 2-alkylthiofenen, 2-alkyl-5-methylthiofenen, 2-alkyl-5-ethylthiofenen, 2-alkylthiolanen, 2-alkyl-5-methylthiolanen, 2-alkylthianen, 2-alkylbenzothiofenen, 2-alkyl-4-methylbenzothiofenen en 2-methyl-4-alkylthiofenen oplopend tot componenten met totaal 22 koolstofatomen.

Het onderzoek naar de distributies van OSPP gevormd door pyrolyse van fossiel macromoleculair organisch materiaal (kerogeen, steenkool, asfaltenen) is beschreven in hoofdstuk 12. Hoewel de relatieve hoeveelheid van OSPP ten opzichte van de andere pyrolyse produkten nogal varieert, blijken de distributie patronen van de gealkyleerde thiofenen in alle gevallen gedomineerd te worden door een beperkt aantal van alle theoretisch mogelijke isomeren. De substitutiepatronen van de dominant aanwezige isomeren vertonen een sterke overeenkomst met die van de OSC zoals die in onrijpe sediment extracten en onrijpe oliën aanwezig zijn (hoofdstukken 2 tot en met 10). Daarom lijkt het aannemelijk dat deze OSPP gevormd zijn door pyrolytische afbraak van bepaalde zwavel bevattende eenheden in deze fossiele macromoleculaire verbindingen, die een vergelijkbare structuur vertonen als de "vrij aanwezige" OSC. Structuurelementen in deze fossiele macromoleculaire verbindingen met een lineaire alkyl, een *iso* en *anteiso* alkyl, een isoprenoide alkyl koolstofskelet en dat van de zijketen van steroiden, zijn hiervan voorbeelden. Het bestaan van deze structuurelementen wordt nog verder ondersteund door de identificatie van hoger moleculaire alkylthiofenen en alkylbenzothiofenen met identieke koolstofskeletten. Het moet waarschijnlijk worden geacht dat deze structuurelementen ook door abiogene inbouw van zwavel in organisch materiaal tijdens de vroege diagenese gevormd worden.

1. Introduction

1.1 THE ABUNDANCE AND SIGNIFICANCE OF ORGANICALLY-BOUND SULPHUR IN THE GEOSPHERE

Sulphur is often the most abundant hetero element in crude oils and bitumens; the total sulphur content (including dissolved elemental sulphur and hydrogen sulphide) varies from less than 0.05 to 14% by weight, although few produced crude oils contain more than 4% sulphur (Orr, 1978; Tissot and Welte, 1984). The distribution of the sulphur content of crude oils (based on 9347 samples) is bimodal with a minimum at 1% separating:

(i) low sulphur crudes with a sulphur content less than 1% (maximum at *ca.* 0.2%),

(ii) sulphur-rich crude oils with a sulphur content of more than 1% (maximum at *ca.* 2.1%) (Tissot and Welte, 1984, p. 398).

On a production plus reserves basis the group of sulphur-rich crude oils is probably as important as, or even more than, the group of low sulphur crude oils (Tissot and Welte, 1984, p. 398).

Most of the sulphur present in crude oils and bitumens is organically-bound sulphur, *i.e.* bound to carbon, because hydrogen sulphide and elemental sulphur dissolved in crude oil usually represent a very minor portion of the total sulphur (Orr, 1978). Organically-bound sulphur in crude oils is mainly present in medium (as single compounds) and heavy fractions (as part of complex macromolecules; *i.e.* asphaltenes and resins). The division between individual sulphur-containing compounds and sulphur-containing macromolecules is of course arbitrary; in this study we will adhere to a separation at a molecular weight of 800 daltons.

The identification of organic sulphur compounds (OSC) started in the nineteenth century with the identification of ten alkyl sulphides in Ohio crude oil (Mabery, 1891) and thiophene in Gromy crude oil (Charitschkoff, 1899). Ever since, the number and structural complexity of the OSC identified (Fig. 1.1) has increased significantly as comprehensively reviewed by Galpern (1971, 1976, 1984), Orr (1978), Thompson (1981) and Aksenov and Kamyanov (1981). A considerable step forward was the research performed by the U.S. Bureau of Mines in the fifties and sixties (A.P.I. Research Project 48). An investment of more than 1000 man-years led to the identification of 176 individual OSC in four crude oils (Thompson, 1981).

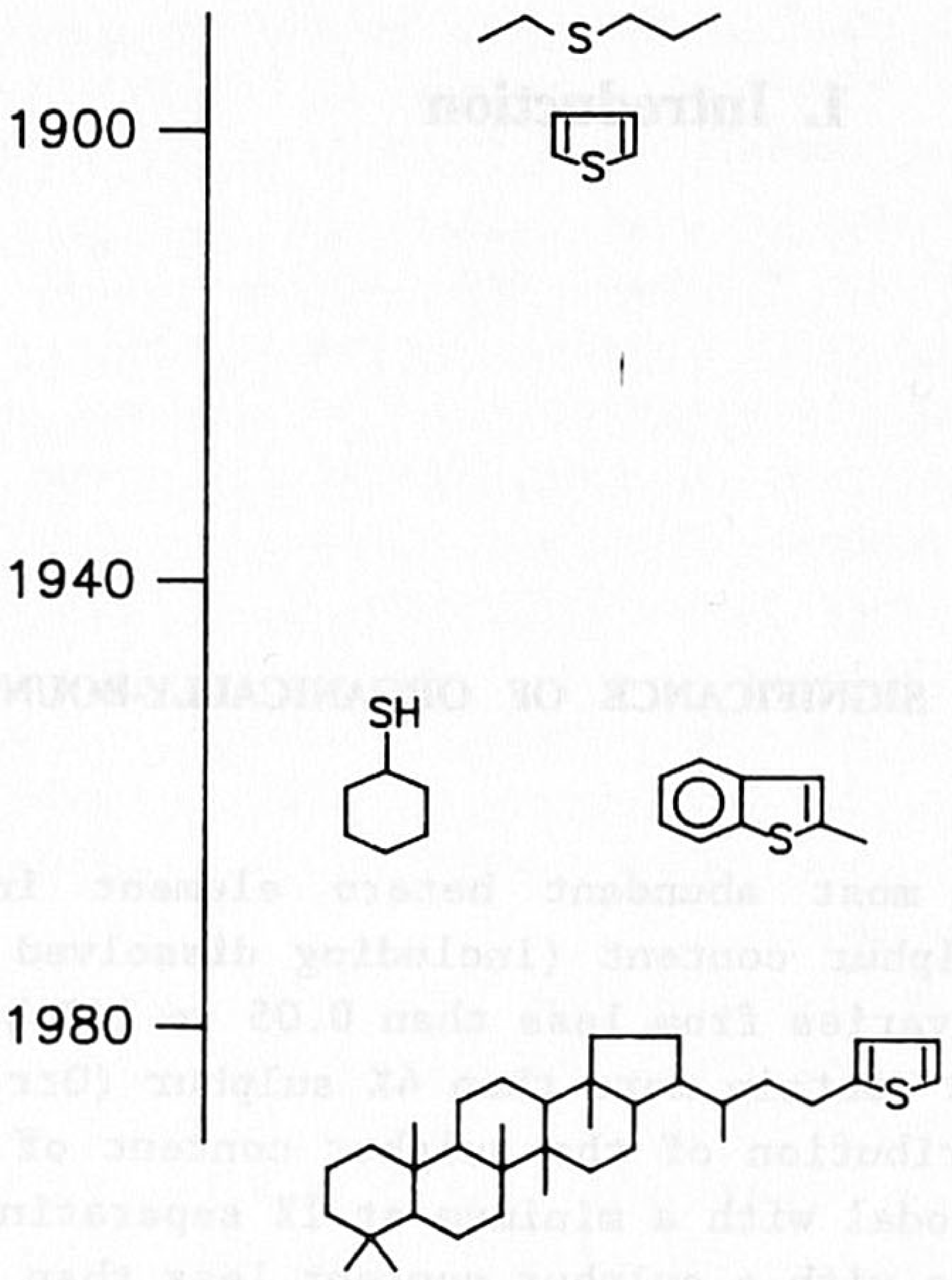

Fig 1.1. OSC identified in crude oils and sediments through the years.

The beginning of a new era in OSC research was the identification of more complex OSC related to certain biochemical and/or geochemical precursors (*e.g.* last structure in Fig. 1.1, Valisolalao *et al.*, 1984), made possible by major advances in analytical chemistry (*e.g.* high resolution capillary gas chromatography in combination with mass spectrometry, high performance liquid chromatography).

Much less is known about the nature of organically-bound sulphur present in macromolecules (*i.e.* kerogen, coal and asphaltenes) mainly due to their more complex structure and the thereby more complicated characterisation of these substances. Although oxygen is generally more abundant than sulphur in kerogen and coal, sulphur may reach 15% on a weight basis in certain kerogens (Orr, 1984).

The significance of organically-bound sulphur in fossil fuels and related materials is threefold (Orr, 1978):

(i) OSC are deleterious to the refining of petroleum and related products because they cause corrosion and poison catalysts.

(ii) Sulphur dioxide is produced during combustion of sulphur-containing fuels, which causes global environmental problems, *e.g.* acidification of forests and lakes.

(iii) Its geochemical significance, *i.e.* for understanding processes which determine its abundance, distribution and chemical form in fossil organic matter.

1.2 ORIGIN OF ORGANICALLY-BOUND SULPHUR IN THE GEOSPHERE: THE BIOLOGICAL SULPHUR CYCLE

Microorganisms and plants are able to convert sulphate into OSC (Fig. 1.2) which fullfill essential roles in living cells. Proteins and enzymes, which contain the sulphur-containing amino acids cysteine and methionine, the vitamins thiamine and biotin, and coenzyme A are examples of ubiquitous sulphur-containing primary cell constituents. The sulphur-containing amino acids represent the main form of sulphur in the living cell (Schlegel, 1986). However, biochemical OSC cannot account to a great extent for the

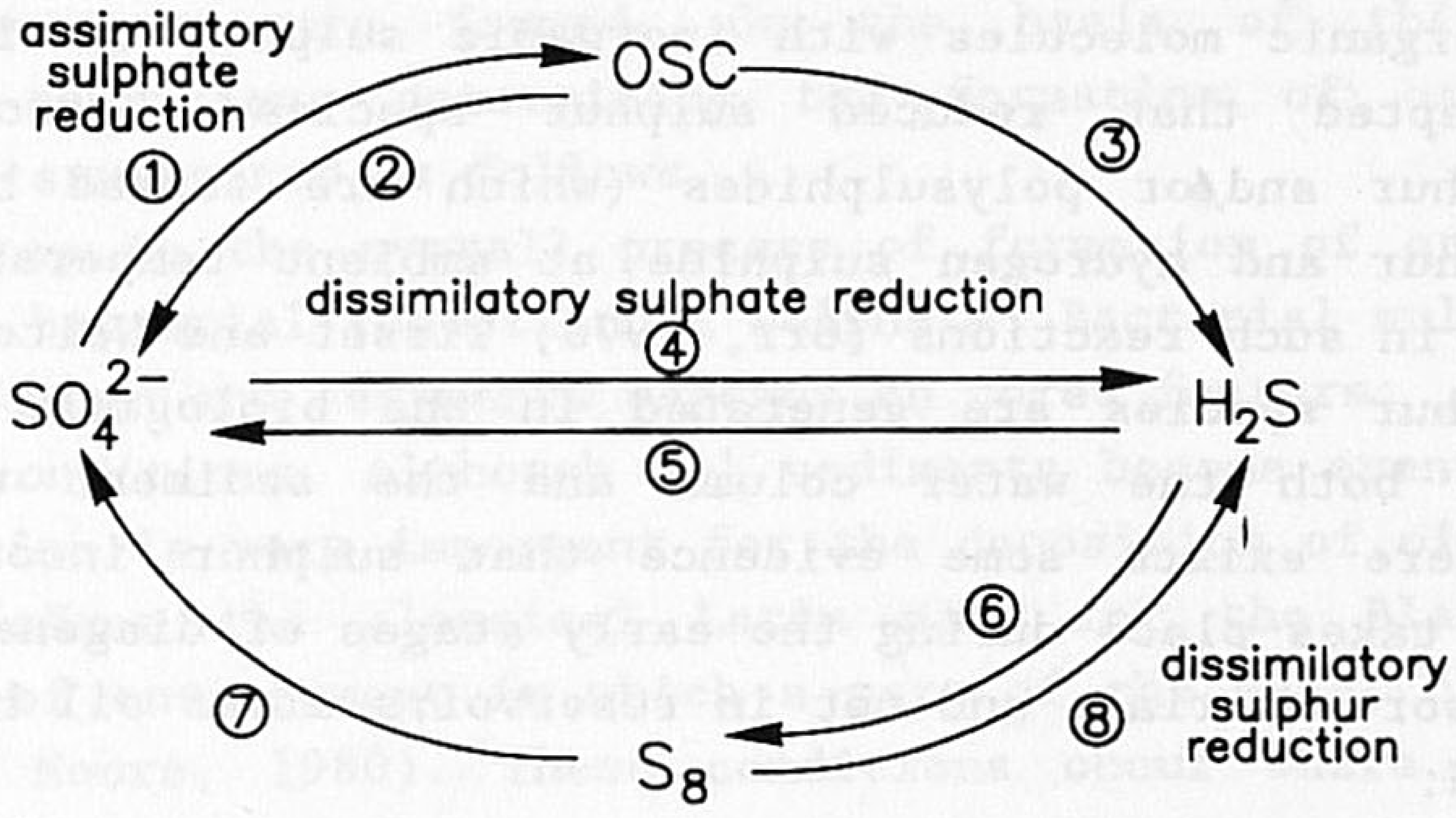

Fig 1.2. The biological sulphur cycle (Schlegel, 1986; Jørgensen, 1982; Trüper, 1979; Trüper and Fischer, 1982)

(1) Assimilatory sulphate reduction converts sulphate to the reduction level of sulphide and fixes it in organic form mainly in the proteins of living organisms. It is carried out by plants and bacteria.

(2) Biochemical oxidation by animals and microorganisms.

(3) Anaerobic degradation of organic matter yields hydrogen sulphide from OSC.

(4) Dissimilatory sulphate reduction converts sulphate to hydrogen sulphide and is carried out by the group of stricly anaerobic sulphate reducing bacteria (*Desulfovibrio*, *Desulfotomaculum*, *Desulfobacter*).

(5-7) Hydrogen sulphide can be oxidised by three groups of bacteria. The colourless sulphur bacteria oxidise hydrogen sulphide to sulphate (*Thiobacillus*, *Sulfolobus*) or elemental sulphur (*Beggiatoa*, *Thioploca*, *Thiothrix*) aerobically. The anaerobic photosynthetic green (*Chlorobium*) and purple (*Chromatium*, *Ectothiorhodospira*, *Thiocapsa*, *Thiocystis*) sulphur bacteria also oxidise hydrogen sulphide.

(8) *Desulfuromonas* can reduce elemental sulphur to hydrogen sulphide by anaerobic dissimilatory sulphate reduction. The colourless sulphur bacterium *Beggiatoa* may also reduce under anaerobic conditions accumulated elemental sulphur to hydrogen sulphide. The same holds for green and purple sulphur bacteria under dark anoxic conditions.

organically-bound sulphur in fossil organic matter for two major reasons:

(i) The sulphur content of fossil organic matter is often too high to attribute its sole source to survival of biochemical OSC. Since sulphur is the third element in petroleum but the sixth element in biomass (it constitutes about 1% of the dry matter of the cell) that would require a selective preservation of these OSC in the geosphere, whereas biochemical OSC probably have a lower fossilisation potential than for example lipids, aliphatic biopolymers and lignin.

(ii) The structures of OSC found in petroleums, bitumens and coals are very different from those biosynthesized.

Therefore it is evident that a major part of the organically-bound sulphur in sediments with a moderate or high organic sulphur content is produced by reactions of organic molecules with inorganic sulphur species. It is now generally accepted that reduced sulphur species, hydrogen sulphide, elemental sulphur and/or polysulphides (which are formed by reaction of elemental sulphur and hydrogen sulphide at ambient temperature), are the sulphur donors in such reactions (Orr, 1978; Tissot and Welte, 1984). These inorganic sulphur species are generated in the biological sulphur cycle (Fig. 1.2) in both the water column and the sediment under suitable conditions. There exists some evidence that sulphur incorporation into organic matter takes place during the early stages of diagenesis, thus into the oil precursor material, and not in reservoirs after oil has been formed and accumulated:

(i) In their classical study Gransch and Posthuma (1974) came to the conclusion that most of the sulphur in crude oils originates from organically-bound sulphur in kerogen.

(ii) Various authors have shown a sulphur enrichment of macromolecular sedimentary organic matter (humic substances, proto-kerogen, coal) at the early stages of diagenesis or in simulation experiments performed at low temperatures (Nissenbaum and Kaplan, 1972; Aizenshtat *et al.*, 1981; Francois, 1987; Casagrande *et al.*, 1979; Casagrande and Ng, 1979).

(iii) The recent identification of a thiophene-ring containing C_{35} hopanoid in immature sediments, a compound not reported to occur in organisms, suggests abiogenic sulphur incorporation into organic matter during early stages of diagenesis (Valisolalao *et al.*, 1984)[1].

Sulphur incorporation after oil has been formed is probably of minor importance (Gransch and Posthuma, 1974; Orr, 1978). Biodegradation of petroleum may result in an increase of the sulphur content by a factor of 2 or 3, but this increase is largely due to removal of non-OSC rather than to an introduction of additional sulphur (Orr, 1978).

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[1] In the course of the research described in this thesis a number of papers appeared which describe the identification of these type of OSC. These papers are referenced in the appropriate chapters of this thesis.

1.3 ENVIRONMENTAL CONDITIONS DETERMINING THE FORMATION OF ORGANICALLY-BOUND SULPHUR IN THE GEOSPHERE

Gransch and Posthuma (1974) developed a concept for the occurrence of high- and low-sulphur crude oils in which environmental conditions of the palaeo-environment of the source rock of the oils are of prime importance (Fig. 1.3). Hydrogen sulphide produced by sulphate-reducing bacteria may react with organic matter to yield sulphur-rich kerogen. Reactive iron minerals, however, may outcompete organic matter for the reduced sulphur and lead to the formation of low-sulphur kerogen in marine siliciclastic environments. In lacustrine (freshwater) environments small amounts of sulphate are present for the production of hydrogen sulphide and therefore low-sulphur kerogens are formed. On the basis of this concept the environmental conditions determining the formation of organically-bound sulphur can be summarized as follows.

The first step in the overall process of formation of organically-bound sulphur is the bacterial reduction of sulphate. Bacterial sulphate reduction in the water column and sediments depends on three factors:

(i) *anoxic conditions*. Although all sediments become eventually anoxic a special situation is very important for the deposition of oil source rocks; the euxinic (after the classical Latin name for the Black Sea; Pontus Euxinus) type of environment in which a part of the water column is anoxic (Demaison and Moore, 1980). These conditions occur where the demand for

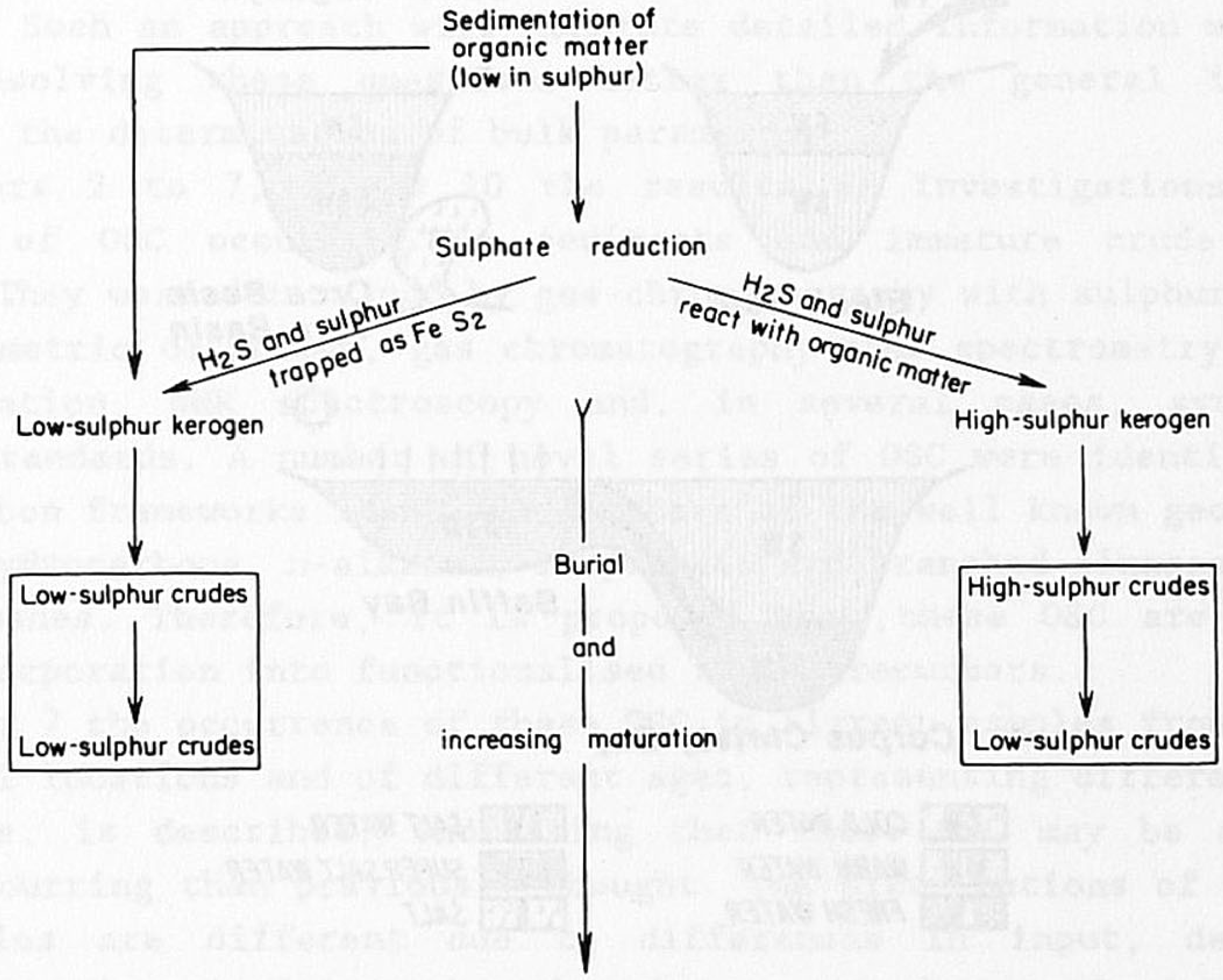

Fig 1.3. Proposed explanation for the occurrence of high- and low-sulphur crude oils in nature (after Gransch and Posthuma, 1974).

oxygen in the water column exceeds the supply. Oxygen is used by heterotrophic bacteria for the mineralisation of organic matter. Oxygen demand relates to surface biological productivity, whilst oxygen supply largely depends on water circulation. Water circulation may be restricted by temperature and/or density stratification (Fig. 1.4; Huc, 1988) and topographic features such as sills or seafloor depressions.

(ii) *presence of sulphate*. In marine sediments this is not the rate-determining factor for sulphate reduction. In lacustrine (fresh water) sediments, however, sulphate is rapidly and totally consumed *via* sulphate reduction and low amounts of hydrogen sulphide are produced.

(iii) *amount and reactivity of organic matter*. In a marine setting sulphate reduction is determined by the amount and especially the reactivity of organic matter present in the water column or in the sediment. Sulphate reducing bacteria use organic matter as a carbon source in the dissimilatory sulphate reduction.

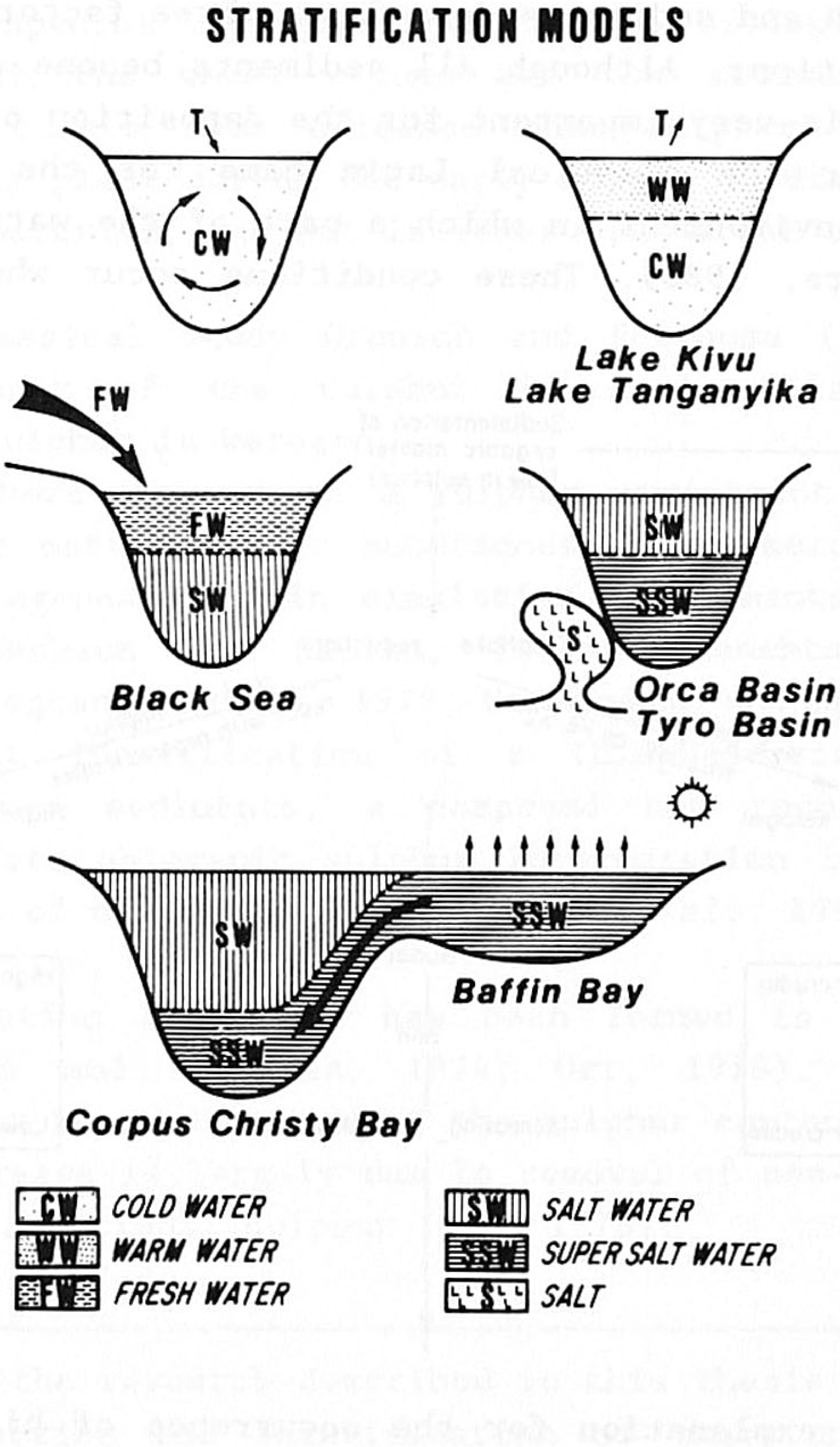

Fig 1.4. Water stratification models (after Huc, 1988).

The second environmental factor determining the formation of organically-bound sulphur is the presence of reactive iron minerals (available iron). Hydrogen sulphide reacts with detrital iron minerals to form iron sulphides (*e.g.* pyrite) during the early stages of diagenesis and, thus, these can act as a sink for hydrogen sulphide (Berner, 1984, 1985). Not all detrital iron will be converted to iron sulphides. Only the more reactive phases, especially fine-grained hydrous iron oxides, are sulphidised. If available iron is present, more than 90% of the sulphur in sediments is in the form of iron sulphides (Berner, 1985). However, in localities far removed from sources of terrigenous clays or silt hydrogen sulphide may react with organic matter.

In summary, formation of organically-bound sulphur may take place in anaerobic sediments or water columns, which receive high amounts of fresh organic matter and where the input of reactive detrital iron minerals is exceeded by the amount of produced hydrogen sulphide.

1.4 SCOPE AND FRAMEWORK OF THE THESIS

The general objective of the research described in this thesis is to provide more detailed evidence for the formation of organically-bound sulphur in the geosphere during the early stages of diagenesis and the subsequent fate of the produced sulphur-containing substances. To reach this goal a molecular approach aiming at structural elucidation of OSC and sulphur-containing moieties in sedimentary high-molecular-weight substances is applied. Such an approach will generate detailed information which might help in resolving these questions better than the general information obtained by the determination of bulk parameters.

In chapters 2 to 7, 9 and 10 the results of investigations into the structures of OSC occurring in sediments and immature crude oils are presented. They were determined by gas-chromatography with sulphur-selective flame photometric detection, gas chromatography-mass spectrometry, Raney Ni desulphurisation, NMR spectroscopy and, in several cases, synthesis of authentic standards. A number of novel series of OSC were identified. They possess carbon frameworks identical to those of the well known geologically-occurring hydrocarbons, *n*-alkanes, isoprenoid and branched alkanes, steranes and triterpanes. Therefore, it is proposed that these OSC are formed by sulphur incorporation into functionalised lipid precursors.

In chapter 7 the occurrence of these OSC in sixteen samples from different geographical locations and of different ages, representing different palaeoenvironments, is described, indicating that these OSC may be more widespreadly occurring than previously thought. The distributions of the OSC in these samples are different due to differences in input, depositional environment and maturity and, therefore, may be used as molecular indicators.

In chapter 8 the origin of these OSC is discussed in detail. An origin for these OSC from a reaction of hydrocarbons with elemental sulphur is unlikely since the distributions of the OSC and hydrocarbons are often different. Since these OSC have never been reported to occur in biota, formation by sulphur incorporation is thought to be the most likely origin. Investigation of sulphur-rich macromolecular substances indicated that these reactions also occur on a intermolecular basis.

In chapters 9 and 10 these conclusions regarding the origin of OSC are corroborated. In a number of sedimentary OSC classes the isomer distributions are dominated by a limited number of the theoretically possible isomers indicating that sulphur incorporation has taken place at specific sites of the precursors determined by the position of their functionality.

The identification of the sulphur-containing flash pyrolysis products (mainly C_4-C_9 alkylated thiophenes) of sulphur-rich kerogens is described in chapter 11. Their occurrence, abundance and geochemical significance in flash pyrolysates of sedimentary macromolecular organic matter (*i.e.* kerogen, coal and asphaltenes) is discussed in chapter 12. The major sulphur-containing pyrolysis products are probably formed by pyrolysis of thiophene moieties present in these substances, which possess linear alkyl, *iso* and *anteiso* alkyl, isoprenoid alkyl and steroidal side-chain carbon skeletons. It is likely that these moieties have been formed by abiogenic sulphur incorporation reactions into sedimentary organic matter during early diagenesis.

2. Isoprenoid thiophenes: novel products of sediment diagenesis?*

S.C. Brassell (a), C.A. Lewis (a), J.W. de Leeuw (b),
F. de Lange (b) and J.S. Sinninghe Damsté (b)

(a) Organic Geochemistry Unit, University of Bristol, School of Chemistry,
Cantock's Close, Bristol BS8 1TS, UK

(b) Organic Geochemistry Unit, Delft University of Technology, Department of
Chemistry and Chemical Engineering, de Vries van Heystplantsoen 2,
2628 RZ Delft, The Netherlands

Sulphur is a significant component of the organic matter in recent and ancient sediments and in petroleums (Tissot and Welte, 1984; Orr, 1977), yet the precise nature of its association and incorporation is poorly understood. Various sulphur-containing compounds have been recognized in petroleums (Orr, 1977; Speers and Whitehead, 1969; Ho *et al.*, 1974), but little is known about their origins and mode of generation during sediment burial, and for only a few organo-sulphur compounds with more than 15 carbon atoms have the structures been determined (Valisolalao *et al.*, 1984; Payzant *et al.*, 1985). Here we identify one of the alkylthiophenes which occur widely in both recent and ancient deep-sea sediments (Rullkötter *et al.*, 1981, 1982, 1984a and b; van Graas, 1982; Klok, 1984; ten Haven *et al.*, 1985) as 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene, occurring as a limited number of the possible stereoisomers. This compound is presumed to originate from the incorporation of sulphur into chlorophyll-derived phytol, or archaebacterial phytenes or their diagenetic products. Its recognition suggests a novel diagenetic pathway for acyclic isoprenoids involving the introduction of sulphur into specific lipid moieties. Similar, but intermolecular, sulphur incorporation might give rise to sulphur-linked macromolecular materials and thereby contribute significantly to the formation of kerogens.

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* *Nature* 320, 160-162 (1986)

Significant developments have recently occurred in the understanding of the biological origins of sedimentary acyclic isoprenoids and their subsequent diagenetic fate. It is now evident that such compounds, which were among the first clearly related to natural products to be recognized in sediments and petroleums (Dean and Whitehead, 1961; Bendoraitis *et al.*, 1962), are not wholly derived from the phytyl side-chain of chlorophyll, but may also originate from the free and ether-bound lipids of archaebacteria (Brassell *et al.*, 1981; Chappe *et al.*, 1980; Risatti *et al.*, 1984) and from the tocopherols (Goossens *et al.*, 1984) of photosynthetic organisms. Here we further expand the range of such compounds with the characterisation of acyclic isoprenoid thiophenes in sediments.

Table 2.1. Occurrence of alkylthiophenes in oceanic and other sediments

	DSDP		Compounds			
Location	Leg-Site	Age	I	II	Others[1]	Ref.[2]
Cariaco Trench	15-147	Quaternary	✓	✓	-	1
Japan Trench	56-436	Pliocene	-	-	F	1
	57-440	Pleist.-Mio.	✓	✓	E,F	1
San Miguel Gap	63-467	Plio.-Mio.	✓	✓	F	2
Gulf of California	64-474	Pleistocene	-	-	F	3
	64-478	Pleistocene	-	-	G,H	3
	64-479	Pleistocene	✓	✓	G,H	1
	64-481	Pleistocene	-	-	Unspecified[3]	3
Middle America Trench	67-496	Quaternary	✓	✓	-	1
Walvis Ridge	75-532	Quat.-Plio.	✓	✓	F	1,4
Angola Basin	75-530	Miocene	✓	✓	F	4
Mazagan Escarpment	79-545	Cenomanian	✓	-	-	5
	79-547	Eocene	✓	✓	-	5
Livello Bonarelli	NA	Cen./Tur.	✓	✓	-	6
Namibian Shelf	NA	Quaternary	✓	✓	-	7
Sarsina	NA	Miocene	✓	✓	B,C + others	8

- Not observed or reported; NA, not applicable. Pleist., Pleistocene; Mio., Miocene, Quat., Quaternary; Plio., Pliocene; Cen., Cenomanian; Tur., Turonian.

[1] The identities of compounds A to F are given in the legend of Fig. 2.1; G and H are isomeric C_{25} alkylthiophenes ($M^{+\cdot} = 378$, prominent ions with mass/charge ratios $m/z = 265$ and 125 (Rullkötter *et al.*, 1982)) of unknown structure.

[2] (1) Brassell (unpublished data), (2) Rullkötter *et al.* (1981), (3) Rullkötter *et al.* (1982), (4) Rullkötter *et al.* (1984b), (5) Rullkötter *et al.* (1984a), (6) van Graas (1982), (7) Klok (1984), (8) ten Haven *et al.* (1985).

[3] Range of mono-, di- and trialkylated thiophenes.

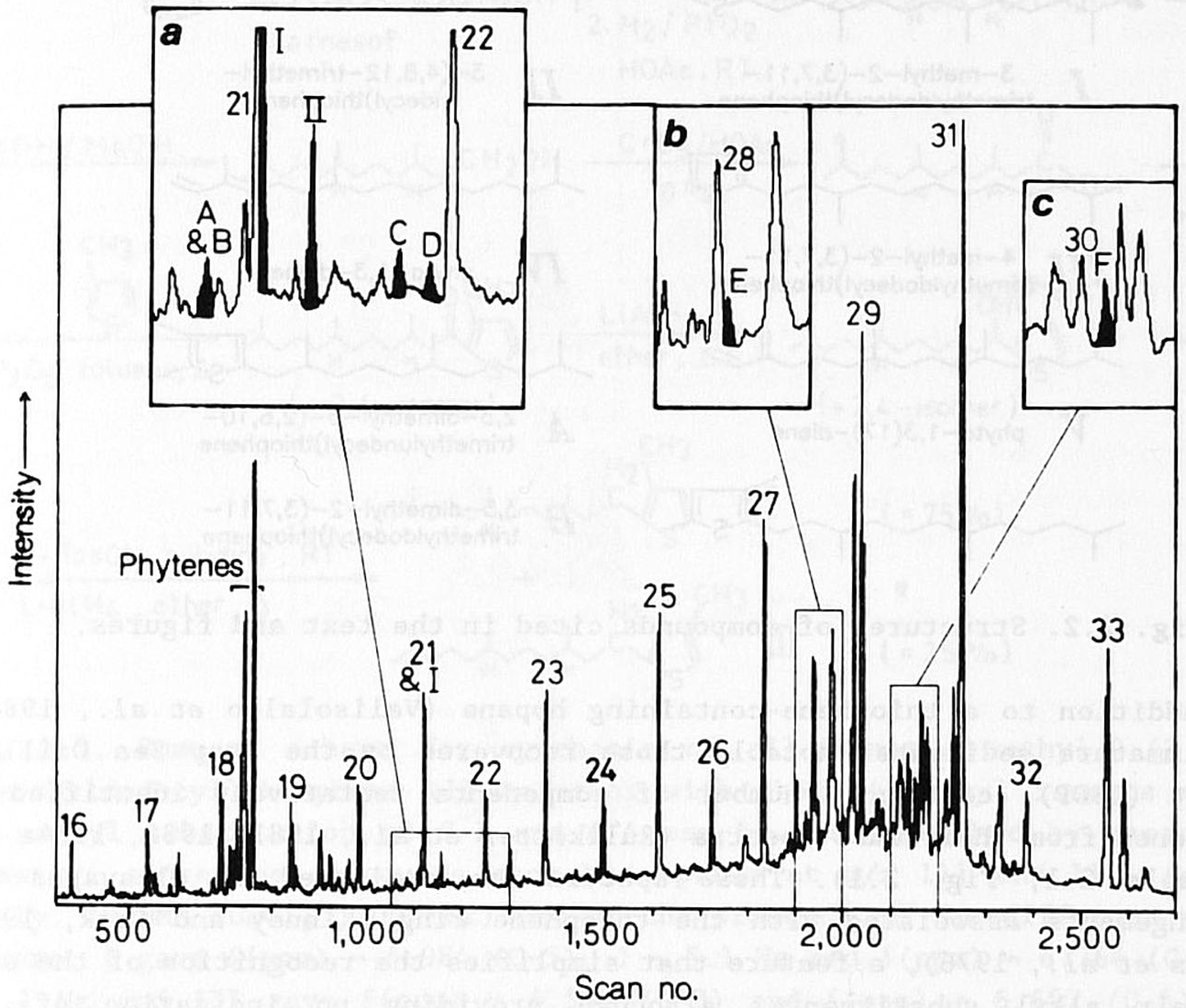

Fig. 2.1. Reconstituted ion chromatogram from GC-MS analysis of the extractable aliphatic hydrocarbon fraction of a diatomaceous ooze from Walvis Ridge (DSDP 75-532-42-3, 173 m sub-bottom depth). Insets a, b and c are expansions of the scan regions 1,050-1,300, 1,900-2,000 and 2,100-2,200, respectively. This sample contains a number of alkylthiophenes distinguished by the prominence of m/z = 97, 98, 111 or 125 in their mass spectra. Compounds I and II are discussed in the text; their mass spectra for molecular ion fragment mass 308 are dominated by m/z of 111 and 98, respectively, and are published in Rullkötter *et al.* (1984b). A, D, E and F appear to be C_{18}, C_{19}, C_{25} and C_{27} *n*-alkyl substituted thiophenes. The mass spectrum of F (M = 406) is given in Rullkötter *et al.* (1982). The minor constituents B and C are probably 2,3-dimethyl-5-(2,6,10-trimethylundecyl)-thiophene (ten Haven *et al.*, 1985) and 3,5-dimethyl-2-(3,7,11-trimethyldodecyl)thiophene, respectively (Fig. 2.2). Peaks corresponding to *n*-alkanes are designated by their carbon numbers; among other significant components note the abundance of three phytene isomers between scans 700 and 800. The GC-MS conditions were similar to those previously reported (Brassell *et al.*, 1980b).

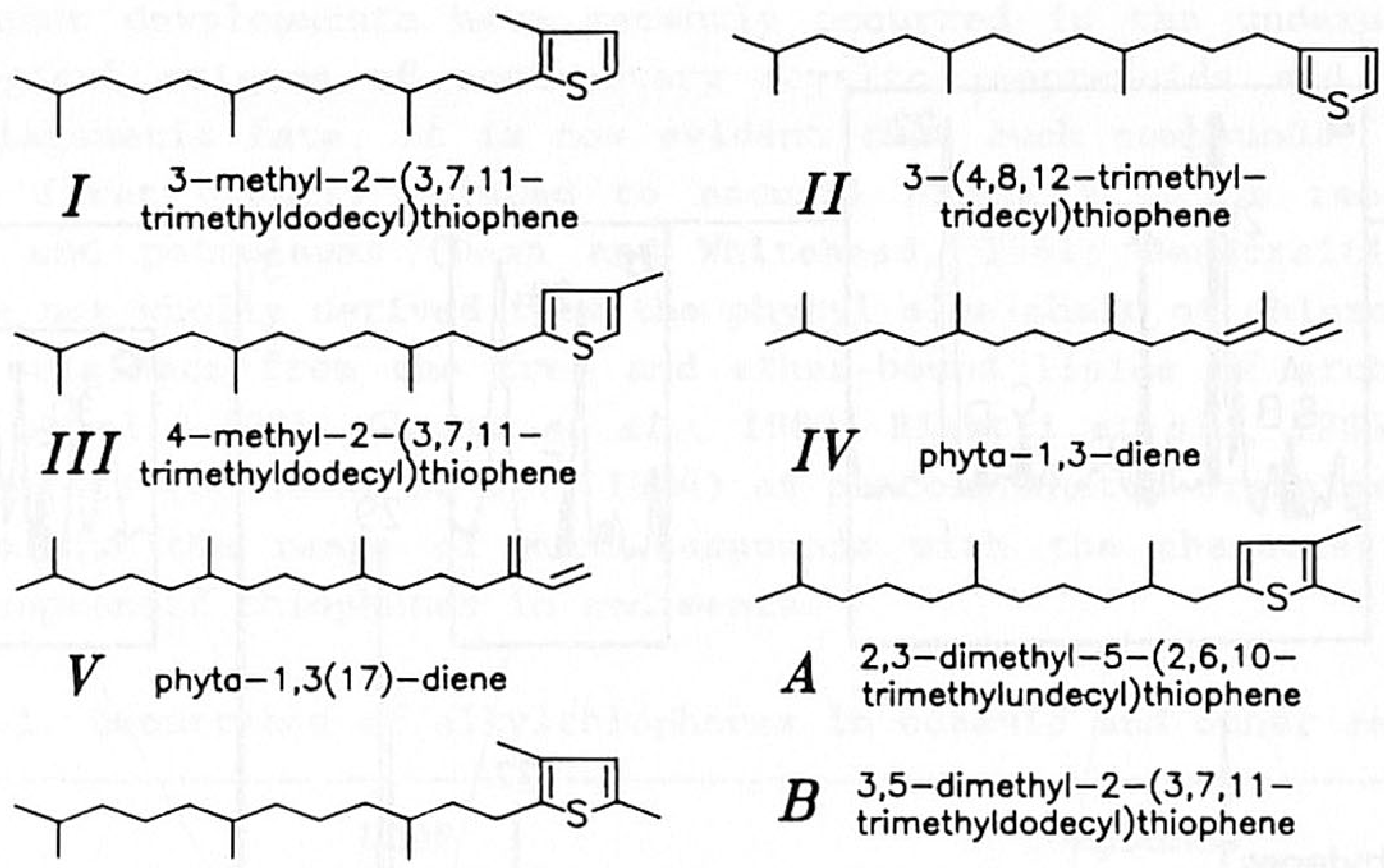

Fig. 2.2. Structures of compounds cited in the text and figures.

In addition to a thiophene-containing hopane (Valisolalao *et al.*, 1984), many immature sediments, notably those recovered by the Deep Sea Drilling Project (DSDP), contain a number of components tentatively identified as thiophenes from their mass spectra (Rullkötter *et al.*, 1981, 1982, 1984a and b) (Table 2.1, Fig. 2.1). These spectra are dominated by cleavages and rearrangements associated with the thiophene ring (Kinney and Cook, 1952; Pomonis *et al.*, 1976), a feature that simplifies the recognition of the size of their alkyl substituents, although providing no indication of the structure of these alkyl groups (whether straight-chain or branched). The widespread occurrence and relative abundance of the C_{20} alkyl thiophenes I and II suggested to us that these compounds might be related to acyclic isoprenoids. Certainly their occurrence in several sediments (for example, in the Japan Trench, Walvis Ridge and Cariaco Trench) with high concentrations of phytenes (Brassell *et al.*, 1983) and phytol (Brassell *et al.*, 1980a) provided circumstantial evidence for this relationship.

If I (Table 2.1, Fig. 2.1) is an acyclic isoprenoid thiophene, then its mass spectrum (Rullkötter *et al.*, 1984b) might correspond to that expected for 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene (Fig. 2.2). Synthesis of this compound (together with its 4-methyl isomer, III; Fig. 2.3) followed by gas chromatograph (GC) and gas chromatograph-mass spectrometer (GC-MS) coinjection (OV-1 methyl silicone fluid columns) confirmed this assignment. Such a structure might arise from the incorporation of sulphur into a phytadiene of phytol, a process also observed in heating experiments with H_2S and carbohydrates (Mango, 1983). The recognition of alkylthiophenes in shallow, immature sediments (Table 2.1) suggests, however, that elevated temperatures are not required for their formation. Rather, their occurrence in oceanic sediments is more closely analogous to that of various low-molecular-weight organo-sulphur compounds, including dimethylsulphide and

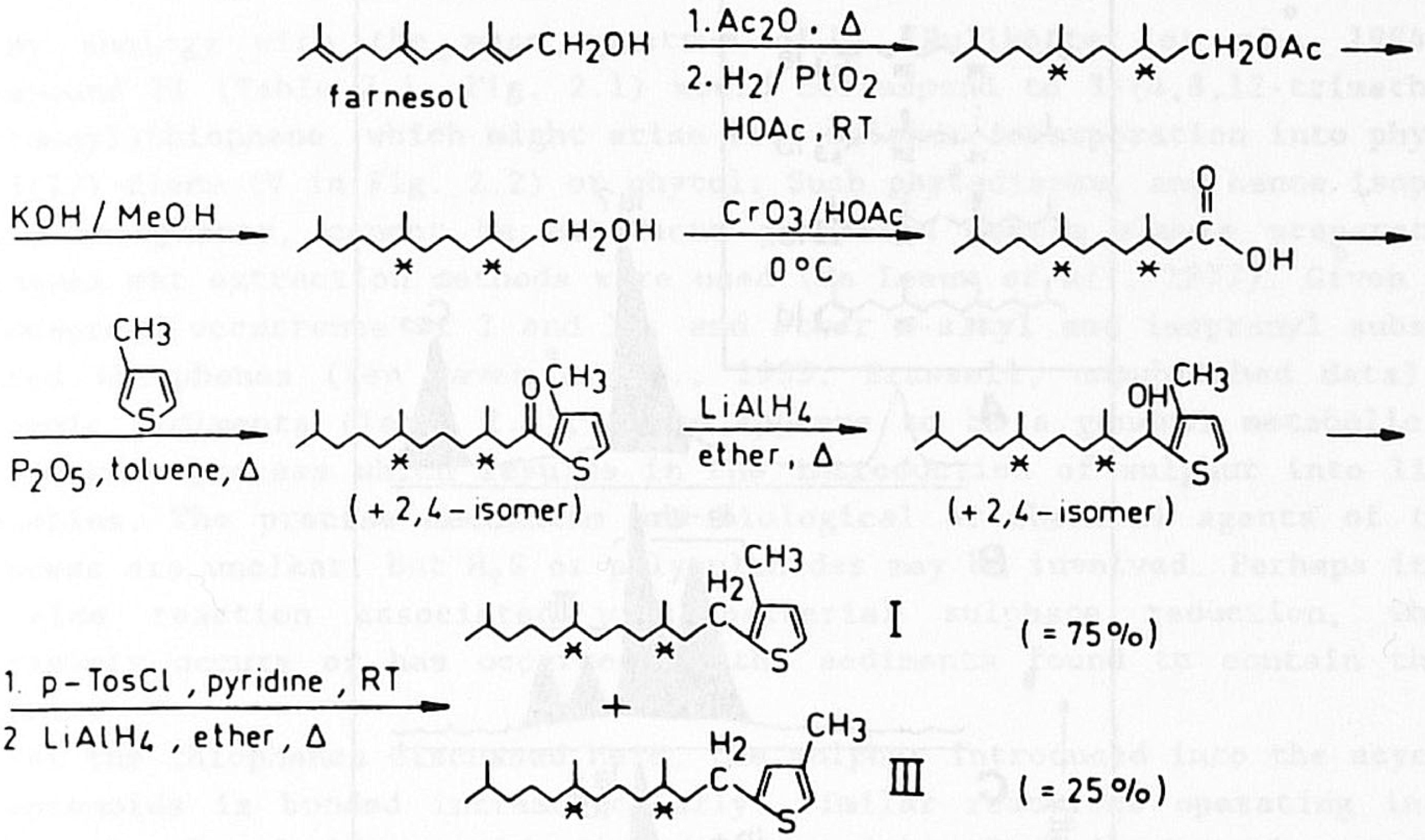

Fig. 2.3. Summary scheme for synthesis of all-isomer 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene (I) together with their 4-methyl isomers (III). The methyl substitution at C-3 and C-4 can be distinguished by mass spectrometry, since the compounds show base peaks at *m/z* 111 and 112, respectively. ^{1}H-NMR ($CDCl_3$, 200 MHz) proved the presence of the different methyl isomers: I gave δ(ppm) = 6.984 (C-5), J = 5.1 Hz and δ(ppm) = 6.766 (C-4), J = 5.1 Hz and III gave δ(ppm) = 6.589 (C-3) and δ(ppm) = 6.657 (C-5) with very small splitting, possibly caused by coupling with the C-4 methyl of the thiophene ring.

volatile thiophenes (Whelan *et al.*, 1980), which are deemed to be direct metabolic products. A biosynthetic origin of higher molecular weight thiophenes, such as I, cannot be fully excluded.

A useful method for assessing the origins and extent of thermal maturation of acyclic isoprenoids is the evaluation of the steric configuration at their chiral centres. Comparative GC on a diethylene glycol succinate/polyethylene glycol succinate (DEGS/PEGS)-coated column (McKenzie *et al.*, 1982) showed that the naturally occurring compound in a calcareous clay from the Cariaco Trench was composed of a maximum of two of the four possible stereoisomers (Ia-d, Fig. 2.4) found in the all-isomer synthetic product. It also seems probable from this analysis that the naturally occurring I in the Cariaco Trench sediment is composed, at least in part, of the isomer (3R,7R; Ia in Fig. 2.4) expected to derive from isoprenoid biosynthesis, although proof of this requires stereospecific synthesis in the laboratory. The structure and limited stereochemistry of I is consistent with sulphur incorporation into phyta-1,3-diene (IV in Fig. 2.2) or phytol.

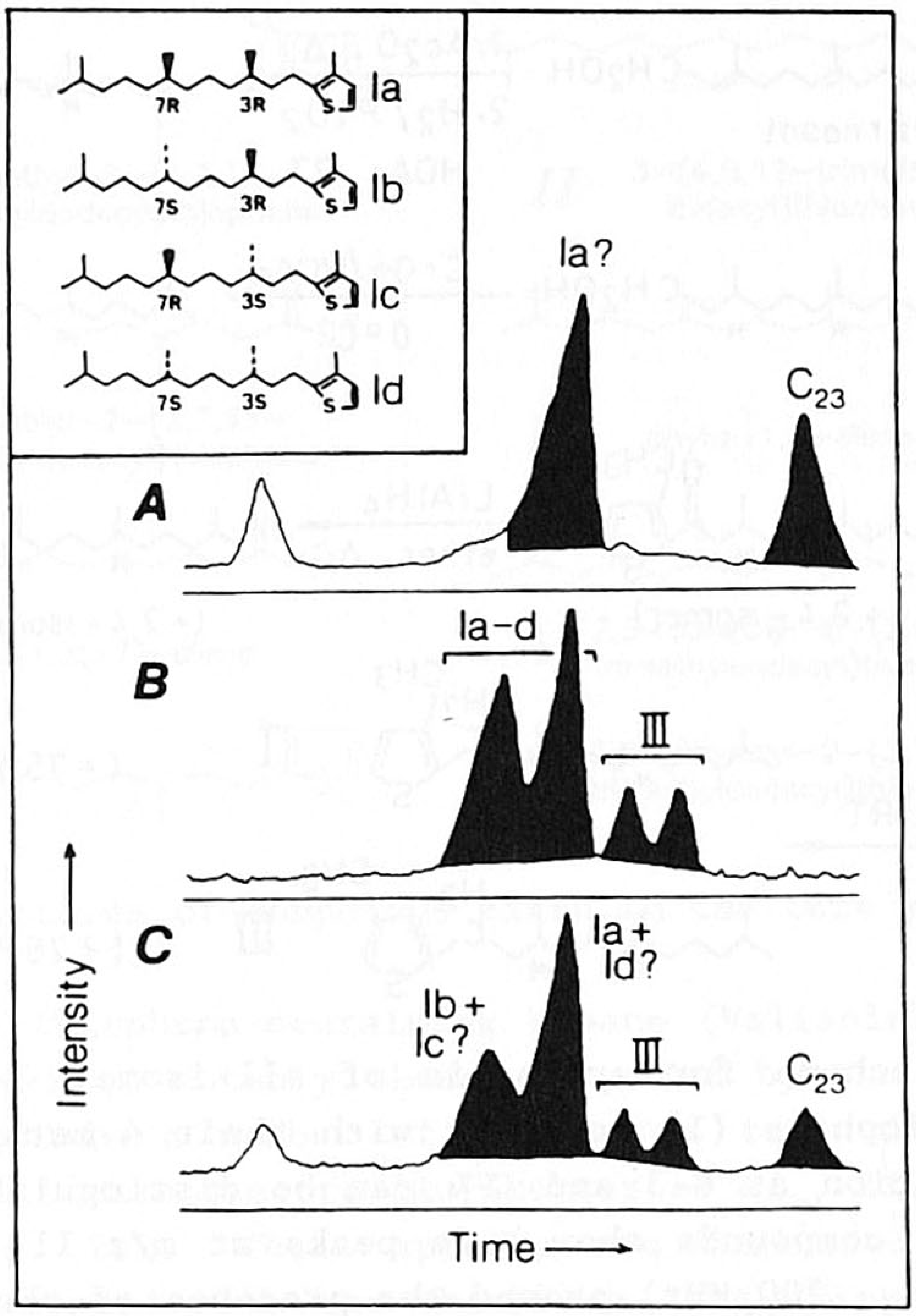

Fig. 2.4. Partial GC traces using a DEGS/PEGS column of the *n*-tricosane (*n*-C_{23}) region of: (A) aliphatic hydrocarbon fraction of a Pleistocene calcareous clay from the Cariaco Trench (DSDP 15-147C-3-3, 138 m sub-bottom depth), (B) products (I and III) of the synthesis shown in Fig. 2.3, (C) coinjection of A and B. The naturally occurring thiophene enhances the latter peak attributed to I. This evidence can be compared with that from similar GC analyses of acyclic isoprenoids where the isomer with the stereochemistry corresponding to both that of the phytyl moiety in chlorophyll-*a* (Burrell *et al.*, 1966) and archaebacterial phytanyl ether moieties (Anderson *et al.*, 1977) occurs in the peak with the greater elution time (for example 7R, 11R in dihydrophytol and phytanic acid (Anderson *et al.*, 1977; Brooks and Maxwell, 1974; Van Vleet and Quinn, 1979; Prahl *et al.*, 1984), and 6R, 10S in phytane (Patience *et al.*, 1980).

Methods: 34 m DEGS/PEGS (3:1) glass capillary column fitted in Carlo Erba FTV2150 chromatograph programmed from 20-109°C at 4°C/min and held isothermally at 109°C. Data are acquired and processed using a VG Minichrom data system. Assignment of *n*-C_{23} was made by comparison with the elution position of a reference standard. Note that the elution times of the thiophenes relative to *n*-alkanes are markedly greater with this column than with that (OV-1) employed in the GC-MS analysis (Fig. 2.1).

By analogy with the mass spectrum of I (Rullkötter *et al.*, 1984b), compound II (Table 2.1, Fig. 2.1) would correspond to 3-(4,8,12-trimethyltridecyl)thiophene, which might arise from sulphur incorporation into phyta-1,3(17)-diene (V in Fig. 2.2) or phytol. Such phytadienes, and hence isoprenoid thiophenes, cannot be artefacts generated during sample preparation because wet extraction methods were used (de Leeuw *et al.*, 1977). Given the widespread occurrence of I and II, and other *n*-alkyl and isopranyl substituted thiophenes (ten Haven *et al.*, 1985; Brassell, unpublished data) in oceanic sediments (Table 2.1), there appears to be a general metabolic or diagenetic process which results in the introduction of sulphur into lipid moieties. The precise mechanism and biological or chemical agents of this process are unclear, but H_2S or polysulphides may be involved. Perhaps it is a side reaction associated with bacterial sulphate reduction, which certainly occurs or has occurred in the sediments found to contain thiophenes.

For the thiophenes discussed here, the sulphur introduced into the acyclic isoprenoids is bonded intramolecularly. Similar reactions operating in an intermolecular fashion would give rise to sulphur-linked polymeric material within kerogens. Such bonding may survive in petroleums and occur in both their "aromatic" and asphaltene fractions; especially the latter since it is rich in sulphur. Alternatively, thermal breaking of carbon-sulphur bonds may be a significant contributory process in the generation of petroleum from kerogen. The clear indication that sulphur incorporation can affect organic compounds during early diagenesis represents a major advance in the understanding of interactions at the molecular level between the sulphur and carbon cycles within sediments. Also, the occurrence of organo-sulphur compounds indicates that organic matter, like iron (Berner, 1984), can act as a sink for sedimentary sulphur. Thus, the identification of acyclic isoprenoid thiophenes in sediments demonstrates a new, significant process in the diagenetic alteration of lipids, and presumably other compound classes.

3. Organic geochemical studies of a Messinian evaporitic basin, northern Apennines (Italy). II. Isoprenoid and n-alkyl thiophenes and thiolanes *

J.S. Sinninghe Damsté, H.L. ten Haven, J.W. de Leeuw and P.A. Schenck

Delft University of Technology, Department of Chemistry and Chemical
Engineering, Organic Geochemistry Unit, De Vries van Heystplantsoen 2,
2628 RZ Delft, The Netherlands

3.1 ABSTRACT

Series of *n*-alkyl and isoprenoid thiophenes and thiolanes, most of which have not been previously reported, have been identified in an extract from a Messinian (Upper Miocene) marl layer deposited under hypersaline, euxinic conditions. The identifications were based on mass spectra and chromatographic data of synthesized reference compounds and on comparison of mass spectra, relative retention times and response on the FPD. Their specific structures and their distribution patterns show similarities with those of the alkanes. Inorganic sulphur is therefore considered to be incorporated into specific lipid moieties from (archae)bacterial and/or algal input during diagenesis. A biosynthetic origin of these compounds is also possible, however. The organic sulphur compounds encountered are thought to be indicators of a hypersaline depositional environment.

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* in *Advances in Organic Geochemistry 1985* (D. Leythaeuser and J. Rullkötter, eds.), *Org. Geochem.* 10, 791-805 (1986)

3.2 INTRODUCTION

The origin of organic sulphur in petroleums and sedimentary organic matter is still not fully understood. The high concentrations of organic sulphur found in some petroleums and kerogens contrast with the relatively low concentrations in organisms. This discrepancy has led to the assumption that inorganic sulphur (*i.e.* sulphur in H_2S, elemental sulphur, polysulphides (S_x^{2-}), iron bound sulphur, sulphates) is incorporated into organic matter in the geosphere. The literature, as reviewed by Orr (1978) and Tissot and Welte (1984), reflects a diversity of opinions concerning the mechanism of the incorporation of sulphur. Different views concerning the diagenetical stage at which sulphur is incorporated and the environmental conditions favouring such an incorporation are reported.

The occurrence of high sulphur crude oils has been explained by a reaction of products of sulphate reducing bacteria such as H_2S and elemental sulphur, with organic matter during early diagenesis (Gransch and Posthuma, 1974). Most of the organic sulphur compounds (OSC) in crude oils are thought to originate from this organically-bound sulphur present in the kerogen of source rocks. Gransch and Posthuma (1974) illustrate this with a number of examples in which a strong correlation was observed between the sulphur content of oils and that of the kerogens in the corresponding source rocks. They therefore postulated that the sulphur content of a crude oil is determined primarily by the environment of deposition of the potential oil source rock. Potential oil source rocks deposited in fresh water (where sulphate reduction is not important) or deposited in an environment where conditions prevail which are favourable for pyrite formation (*e.g.* presence of abundant reactive iron minerals; see Berner, 1984) usually have low-sulphur kerogens and release low-sulphur crude oils.

Tissot and Welte (1984) noted the consistently higher sulphur content of crude oils produced from carbonate-evaporite source rocks (*e.g.* oils from the Middle East). They attributed this to massive sulphur incorporation into organic matter. Closed environments of carbonate-evaporite sedimentation become depleted in oxygen because of aerobic microbial activity so that anaerobic conditions are rapidly established. Large quantities of H_2S are produced from sulphate and, because iron is less abundant, sulphur combines with organic matter during diagenesis.

This view of early incorporation of sulphur into organic matter is supported by studies of sulphur isotope ratios ($^{34}S/^{32}S$) (Thode *et al.*, 1958; Thode and Monster, 1965, 1970). These studies gave evidence for a parallellism between the $\delta^{34}S$ values of sulphur in crude oils and the fluctuations of $\delta^{34}S$ of sulphate in seawater over geological periods. However, sulphur in crude oils is commonly $15^0/_{00}$ isotopically lighter than in seawater of the same age, indicating isotope fractionation during microbial reduction of sulphate at the time of deposition. Aizenshtat *et al.* (1983) also supported the idea of early incorporation of sulphur by reaction of products of sulphate reducing bacteria with organic matter at the very early stages of diagenesis. In Solar Lake, a marine hypersaline, stratified, heliothermally heated water body, the study of various sulphur species revealed enrichment of the organically bound sulphur in the sedimentary column from 1.4% at the surface to 8.2% at 80-87 cm depth.

The contrary view that a large part of the sulphur in crude oils is due to *in situ* sulphurisation in relatively shallow petroleum reservoirs resulting from microbial sulphate reduction in the reservoir, has become more and more rejected (Orr, 1978). The sulphur content of a crude oil is not only determined by the environment of deposition but is influenced by a number of other parameters and mechanisms, such as its stage of maturation or evolution (Gransch and Posthuma, 1974), water washing, biodegradation (Orr, 1978) and sulphurisation and desulphurisation processes in high temperature reservoirs (Orr, 1974). However, these additional processes are probably of minor importance (Tissot and Welte, 1984).

The analysis of OSC and kerogens from sediments deposited under euxinic conditions may provide clues to the understanding of the incorporation of sulphur into organic matter. Although almost one hundred years have passed since the first identification of OSC in petroleums was reported (Mabery and Smith, 1891), until now a relatively small number of specific OSC have been identified which help us to understand the mechanism of sulphur enrichment during early diagenesis. Payzant *et al*. (1983, 1985) identified homologous series of bicyclic and tetracyclic terpenoid sulphoxides and sulphides in Athabasca bitumen. These series were obviously related to the ubiquitous tricyclic terpanes and other cyclic terpanes in most petroleums and appear to point to some hitherto unrecognized microbial activities responsible for their formation. Valisolalao *et al*. (1984) identified a C_{35} pentacyclic triterpenoid of the hopane series containing a thiophene ring, 30-(2-methylene thienyl)-17β(H),21β(H)-hopane, in immature sediments, suggesting incorporation of bacterially formed sulphur into organic matter at an early stage of diagenesis. Brassell *et al*. (1986c) also suggested incorporation of sulphur into specific lipid moieties during early diagenesis. The isoprenoid thiophenes identified by these authors could have arisen from sulphur incorporation into chlorophyll-derived phytol or archaebacterial phytenes or their diagenetic products, although the possibility of biosynthesis of these compounds could not be completely ruled out.

We report the identification of a number of OSC in a bituminous marl layer from a sedimentary basin of Messinian age (Upper Miocene) located in the Northern Apennines (Italy). Our ultimate aim is to study sulphur incorporation into organic matter in the geosphere at a molecular level. To this end, the OSC in the sediment were characterised, and an attempt was made to understand the origin of the OSC found and the mechanism of their formation. This sediment was chosen for this investigation because it is geologically well documented. The palaeoreconstruction (ten Haven *et al*., 1985) points to hypersaline, euxinic conditions during deposition of this marl layer, conditions which favour sulphur incorporation reactions. In an earlier study, in which the hydrocarbon fraction of this sediment was described, some preliminary results on these OSC were reported (ten Haven *et al.*, 1985). It is noteworthy that a seep oil from a sulphur mine, located in the same sedimentary basin, contains an extremely high organic sulphur content of 10.5% (Colombo and Sironi, 1961).

3.3 EXPERIMENTAL

Sampling and geological setting. The sediment investigated consists of varieties of gypsum deposits interbedded with bituminous marl layers, which smell strongly of H_2S. A 10 cm thick marl layer was sampled and analysed. It consists mainly of carbonates (*ca.* 50%) and clay minerals (*ca.* 22%) of which montmorillonite predominates. Sampling and geological setting are described in detail by ten Haven *et al.* (1985).

Extraction. The sample was ground in a rotary disc mill and the powdered sample (205 g) was Soxhlet extracted with toluene/methanol (1/3, v/v) for 46 h. The residue was subsequently extracted with 75 ml MeOH, twice with 75 ml MeOH/CH_2Cl_2 (1/1, v/v) and six times with 50 ml CH_2Cl_2 using ultrasonication and centrifugation. All supernatants were combined in a separatory funnel. After addition of 50 ml distilled water the CH_2Cl_2-layer was withdrawn, combined with the Soxhlet extract, dried over anhydrous Na_2SO_4, evaporated to dryness in a rotary evaporator and weighed (1.52 g; 0.71%).

Column chromatography. An aliquot of this extract (172 mg) was fractionated by column chromatography (see Fig. 3.1 for the analytical flow diagram). The column (50 cm x 10 mm) was wet packed in pentane with equal volume of alumina overlying silica (both activated for 1 h at 150°C) and prewashed with 50 ml of pentane. The extract was taken up in CH_2Cl_2 and adsorbed on approximately 0.5 g alumina by evaporating the solvent under a gentle stream of nitrogen. This alumina was put on top of the column. This procedure was applied to avoid solution problems (when the extract is injected on the column as a pentane solution) or distortion of the chromatographic system (when CH_2Cl_2 is used as a solvent). The extract was thus separated into "saturated" (4.6 mg), "aromatic" (23.5 mg) and "polar compound" (130.7 mg) fractions using 60 ml pentane, 75 ml toluene and 75 ml toluene/methanol (1:1) respectively. A column of activated copper was used to remove elemental sulphur from the "saturated" and "aromatic" fractions. The saturated straight-chain hydrocarbons were removed by urea adduction.

An aliquot of the "aromatic" fraction was further chromatographed on activated alumina (1 h at 150°C). The column (25 cm x 10 mm) was dry packed and prewashed with 20 ml hexane. An aliquot (9.1 mg) of this "aromatic" fraction was added to the column as described above. The "aromatic" fraction was further fractionated using 75 ml hexane, 30 ml hexane/toluene (9:1, v/v) and 30 ml toluene as eluents. The first fraction (35 ml) consisted of only small amounts of phytane (the most abundant compound of the saturated

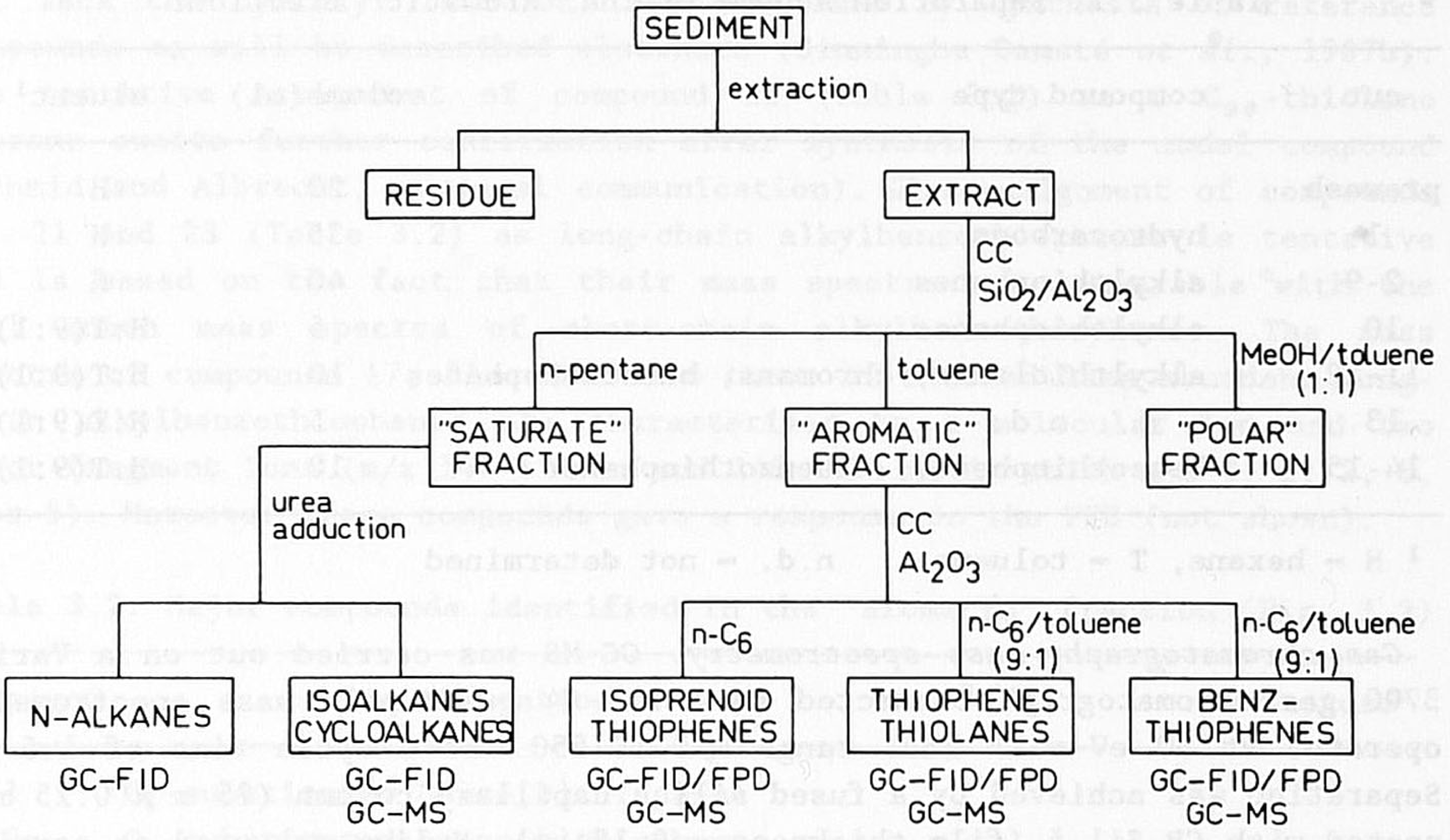

Fig. 3.1. Analytical flow diagram.

hydrocarbon fraction) and some other compounds such as extended hopenes and probably C-ring aromatic steroid hydrocarbons. The subsequent fractions (5 ml) were all analysed by GC and recombined into five distinctive fractions (see Table 3.1).

Gas chromatography. Gas chromatography was carried out on two instruments, one of them equipped with a flame photometric detector (FPD) giving a selective response for OSC. The Carlo Erba 4160 instrument was equipped with a flame ionization detector (FID) and an on-column injection system (Grob, 1978; Grob and Grob, 1978), provided with a special cooling system. A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.13 μm) was used with helium as carrier gas. Samples were injected at 80^{0}C (hexane) or 100^{0}C (ethylacetate). The oven temperature was programmed from 130^{0}C to 330^{0}C at 4^{0}C/min as soon as the solvent eluted. The Varian 3700 instrument was equipped with a FPD and a flame ionization detector. The fused silica capillary column (30 m x 0.26 mm) coated with DB-5 (film thickness = 0.1 μm) was split just before the FPD and the FID with a splitter device (8:1 respectively) (Scientific Glass Engineering) as described by Cox and Earp (1982). Helium was used as carrier gas. Both detectors were operated with helium make up gas at 15 ml/min. Samples in CH_2Cl_2 were injected at 70^{0}C with a splitless injector. After 2 min the temperature was programmed to 130^{0}C at a rate of 10^{0}C/min. Then the column temperature was programmed from 130 to 300^{0}C at 4^{0}C/min.

Table 3.1. Separation scheme of the "aromatic" fraction.

cut	compound type	volume(ml)	eluent[1]
prewash	-	20	H
1	hydrocarbons	35	H
2-9	alkylthiophenes	40	H
10	alkylthiophenes	5	H:T(9:1)
11-12	alkylthiolanes, chromans, benzothiophenes	10	H:T(9:1)
13	n.d.	5	H:T(9:1)
14-15	benzothiophenes, dibenzothiophenes	10	H:T(9:1)

[1] H = hexane, T = toluene   n.d. = not determined

Gas chromatography-mass spectrometry. GC-MS was carried out on a Varian 3700 gas chromatograph connected to a MAT-44 quadrupole mass spectrometer operated at 80 eV with mass range *m/z* 50-550 and a cycle time of 1.5 s. Separation was achieved by a fused silica capillary column (25 m x 0.25 mm) coated with CP Sil-5 (film thickness = 0.12 μm). Helium was used as carrier gas.

Synthesis of reference compounds. 2-Methyl-5-tridecylthiophene was synthesized as follows (modified from Brassell *et al*., 1986c): 2-methylthiophene was coupled with tridecanoic acid in toluene with P_2O_5 as dehydrating agent. Reduction ($LiAlH_4$) of the resulting ketone afforded 2-(1-hydroxytridecyl)-5-methylthiophene. This alcohol was reacted with *p*-toluene sulfonylchloride under reflux to 2-methyl-5-tridecen-1-ylthiophene; subsequent hydrogenation with H_2 using Pd/C (5%) as catalyst yielded the desired thiophene.

2-Dodecyl-5-ethylthiophene was synthesized following the reaction steps described above starting with dodecanoic acid and 2-ethylthiophene.

As byproducts of both hydrogenations the corresponding thiolanes, 2-methyl-5-tridecylthiolane and 2-dodecyl-5-ethylthiolane, were also obtained.

3.4 RESULTS

The gas chromatograms of the "aromatic" fraction and the important subfractions are shown in Fig. 3.2. The peak numbers correspond to those listed in Table 3.2. Identifications are based on comparison of mass spectra with reference spectra reported in the literature and those obtained for synthesized model compounds. Use was also made of relative retention times and response on the FPD (Flame Photometric Detector). An example is given in Fig. 3.3.

The assignment of compounds 14 (VIII), 16 and 18 (IX) (Table 3.2) as isoprenoid chromans (these compounds have the same skeleton as tocopherols but lack the hydroxyl function) was confirmed by synthesis of reference compounds as will be described elsewhere (Sinninghe Damsté *et al.*, 1987b). The tentative assignment of compound 22 (Table 3.2) as a C_{29}-thiolane sterane awaits further confirmation after synthesis of the model compound (Schmid and Albrecht, personal communication). The assignment of compounds 17, 21 and 23 (Table 3.2) as long-chain alkylbenzothiophenes is tentative and is based on the fact that their mass spectra are comparable with the well known mass spectra of short-chain alkylbenzothiophenes. The mass spectra of compounds 17, 21 and 23 and those of related less abundant long-chain alkylbenzothiophenes are characterised by a molecular ion and two major fragment ions (m/z 147 + 14.n and 148 + 14.n, in which n = 0, 1, 2, 3, 4 or 5). Moreover these compounds gave a response on the FPD (not shown).

Table 3.2. Major compounds identified in the "aromatic" fraction (Fig. 3.2)

compound	identification	structure[1]
1	C_3-substituted benzo[*b*]thiophene	-
2	C_4-substituted benzo[*b*]thiophene	-
3	C_4-substituted benzo[*b*]thiophene	-
4	C_5-substituted benzo[*b*]thiophene	-
5	C_5-substituted benzo[*b*]thiophene	-
6	phytane	-
7	C_6-substituted benzo[*b*]thiophene	-
8	2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene	I
9	3,5-dimethyl-2-(3,7,11-trimethyldodecyl)thiophene	III
10	5-ethyl-3-methyl-2-(3,7,11-trimethyldodecyl)thiophene	IV
11	4-methyl-2-(2,6,10,14-tetramethylpentadecyl)thiophene	VI
12	2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)thiophene	VII
13	isomer of 12	-
14	2,8-dimethyl-2-(4,8,12-trimethyltridecyl)chroman	VIII[2]
15	isomer of 12	-
16	2,8-dimethyl-2-(4,8,12-trimethyltridecyl)chroman	VIII[2]
17	C_{17}-substituted benzo[*b*]thiophene (*m/z* 372, 176, 175)	-
18	2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman	IX
19	18-nor-17β-methyl-24-ethylcholesta-8,11,13-triene	X
20	18-nor-4,17β,22-trimethylcholesta-8,11,13-triene	XI
21	C_{18}-substituted benzo[*b*]thiophene (*m/z* 386, 189, 190)	-
22	C_{29} thiolane sterane	-
23	C_{20}-substituted benzo[*b*]thiophene (*m/z* 414, 175, 176)	-
24	unknown	-

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[1] most of the structures are tentative

[2] both compounds have identical mass spectra and are thought to be stereoisomers (2α-methyl and 2β-methyl)

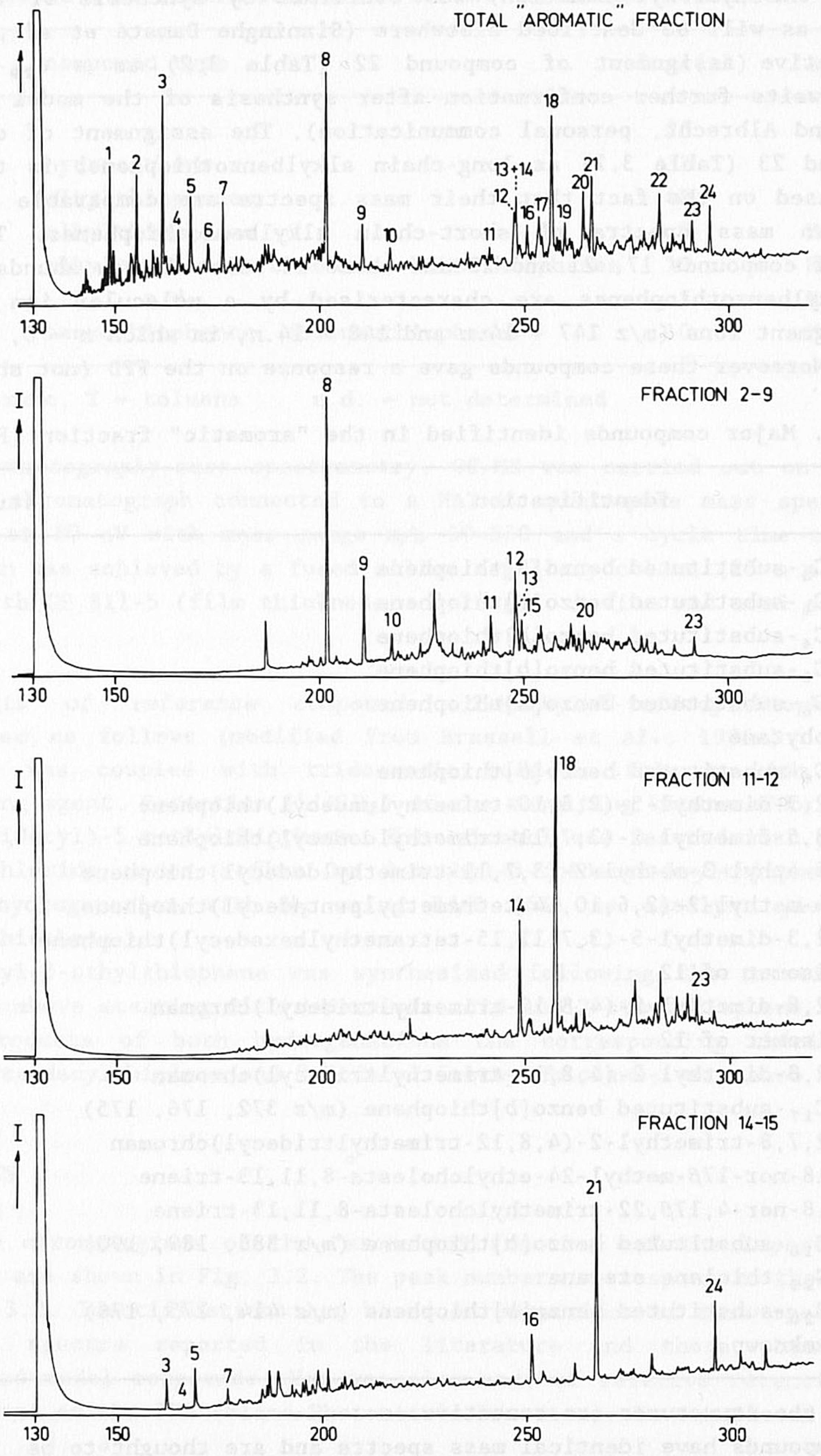


Fig. 3.2. Gas chromatograms of the "aromatic" fraction and the obtained subfractions. Identifications of numbered compounds are given in Table 3.2.

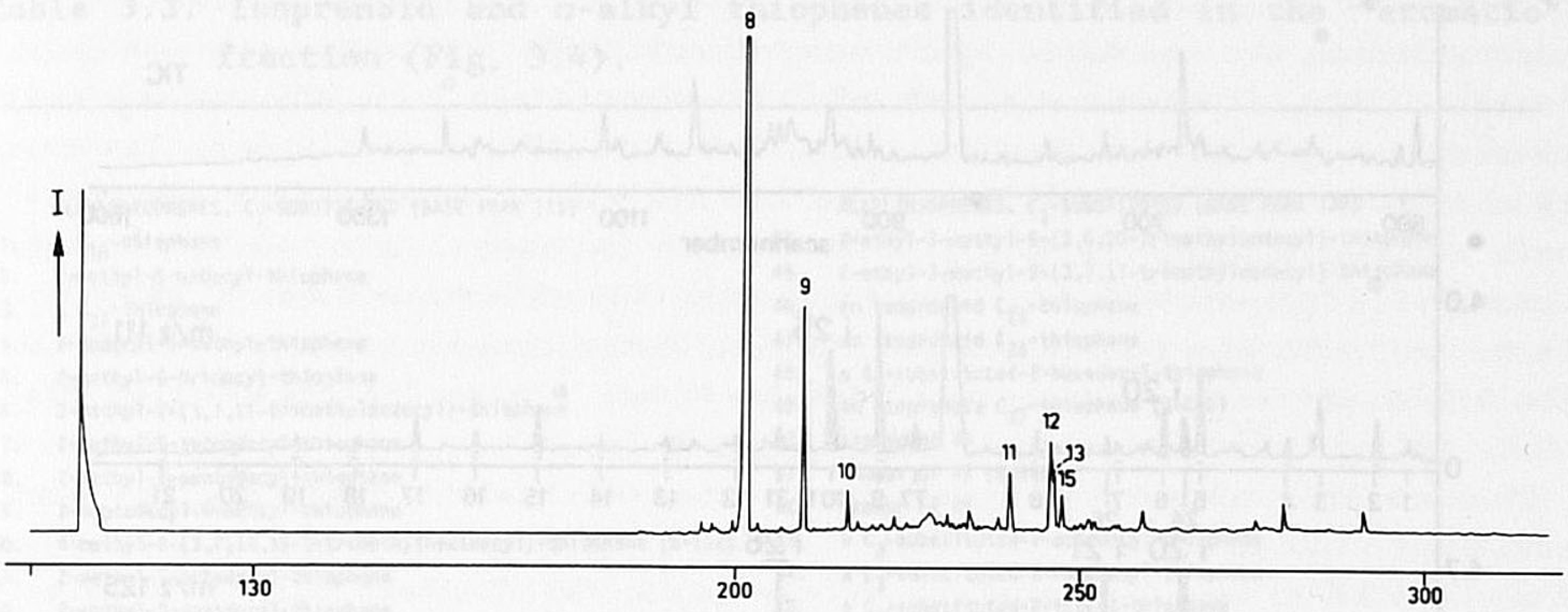


Fig. 3.3. FPD chromatogram of subfraction 2-9 of the "aromatic" fraction. Identifications of the numbered OSC are given in Table 3.2.

Separation procedure

It is evident from Fig. 3.2 that the column chromatographic procedure succeeds in fractionating the "aromatics" in distinctive subfractions. This is a useful tool to reduce the complexity of the mass spectral data. Moreover it gives valuable information on the chromatographic behaviour of the various compounds. For example, the isoprenoid thiophenes (fraction 2-9) elute very quickly which explains why these thiophenes are sometimes present in the saturated hydrocarbon fraction of extracts as reported by ten Haven *et al*. (1985), Brassell *et al*. (1986c) and Palmer and Zumberge (1981). The latter authors reported the presence of a compound (x) in the saturated hydrocarbon fraction of samples from Sicily, which is in fact an isoprenoid thiophene, presumably compound I (Zumberge, personal communication). Partial quantities of some thiophenes occur in the saturated hydrocarbon fraction if only slightly more than the required volume of pentane is used to elute this fraction. This can lead to misinterpretation of mass spectra of compounds from this fraction because the spectra of alkylcyclohexanes and alkylthiophenes resemble each other especially when there is a considerable background of alkyl fragment ions. A minor disadvantage of the fractionation of the "aromatics" is the partition of some compound classes over several fractions (*e.g.* *n*-alkylthiophenes). Data on distribution patterns of distinct compound classes were therefore mainly generated from the total "aromatic" fraction.

Thiophenes

The distribution patterns and mass spectrometric behaviour of the isoprenoid and *n*-alkylthiophenes in the "aromatic" fraction are shown in Fig. 3.4. The peak numbers in this figure correspond with those listed in Table 3.3. The most abundant thiophene (compound 24) was identified as 2,3-

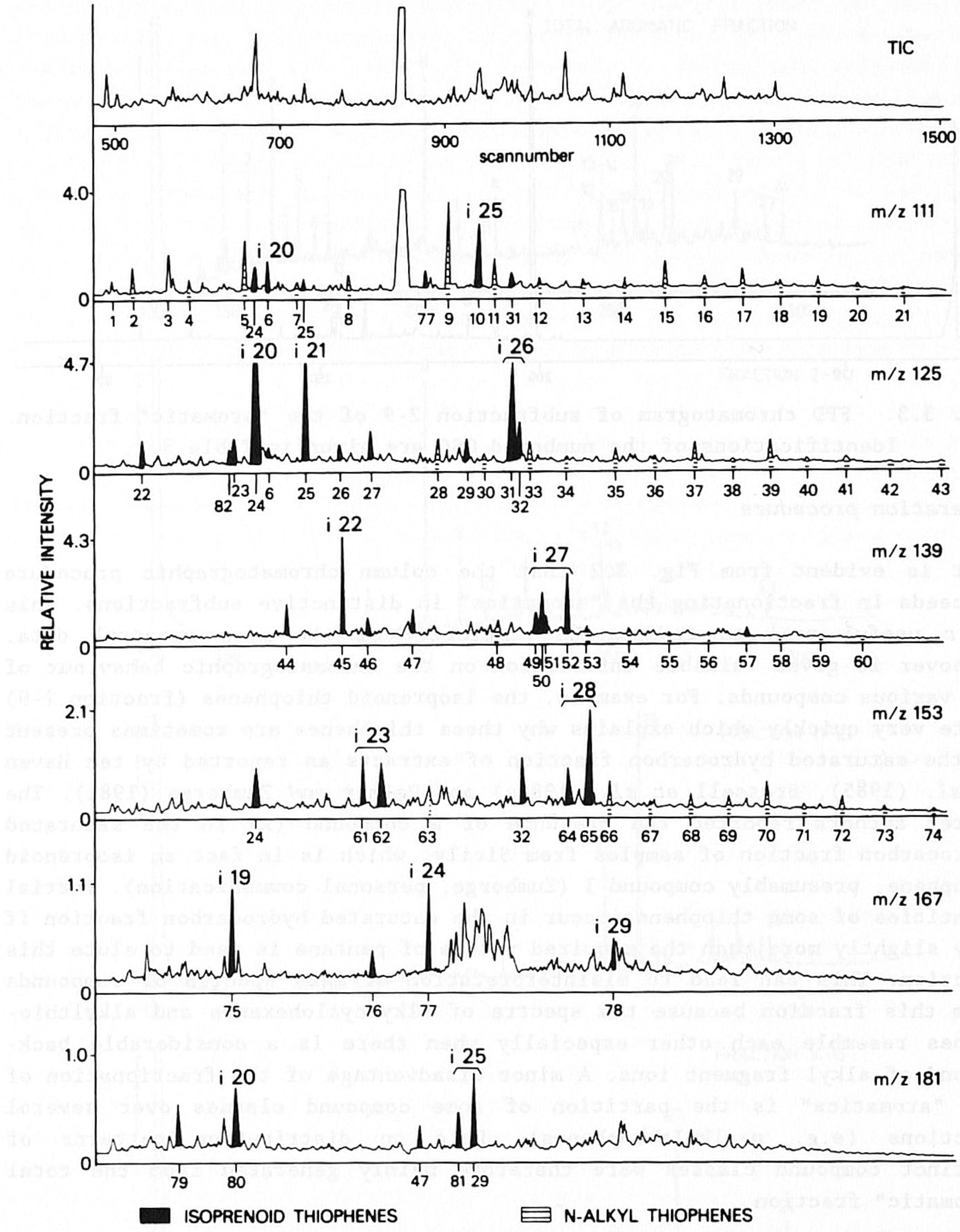


Fig. 3.4. Mass chromatograms of *m/z* 111, 125, 139, 153, 167 and 181 and the TIC (upper trace) of the "aromatic" fraction. Identifications of the numbered compounds are given in Table 3.3. The large peak at scan number 850 in the TIC and in the *m/z* 111 trace is assigned to dioctyladipate, a plastizer, and is due to contamination during the process of removal of the elemental sulphur.

Table 3.3. Isoprenoid and *n*-alkyl thiophenes identified in the "aromatic" fraction (Fig. 3.4).

ALKYLTHIOPHENES, C_1-SUBSTITUTED (BASE PEAK 111)

1. a C_{16}-thiophene
2. 2-methyl-5-undecyl-thiophene
3. a C_{17}-thiophene
4. 2-dodecyl-5-methyl-thiophene
5. 2-methyl-5-tridecyl-thiophene
6. 3-methyl-2-(3,7,11-trimethyldodecyl)-thiophene
7. 2-methyl-5-tetradecyl-thiophene
8. 2-methyl-5-pentadecyl-thiophene
9. 2-heptadecyl-5-methyl-thiophene
10. 4-methyl-2-(3,7,11,15-tetramethylhexadecyl)-thiophene (B=112)
11. 2-methyl-5-octadecyl-thiophene
12. 2-methyl-5-nonadecyl-thiophene
13. 2-icosyl-5-methyl-thiophene
14. 2-henicosyl-5-methyl-thiophene
15. 2-docosyl-5-methyl-thiophene
16. 2-methyl-5-tricosyl-thiophene
17. 2-methyl-5-tetracosyl-thiophene
18. 2-methyl-5-pentacosyl-thiophene
19. 2-hexacosyl-5-methyl-thiophene
20. 2-heptacosyl-5-methyl-thiophene
21. 2-methyl-5-octacosyl-thiophene

ALKYLTHIOPHENES, C_2-SUBSTITUTED (BASE PEAK 125)

22. an isoprenoid C_{18}-thiophene
23. an isoprenoid C_{20}-thiophene and 2-dodecyl-5-ethyl-thiophene
24. 2,3-dimethyl-5-(2,6,10-trimethylundecyl)-thiophene
25. 3,5-dimethyl-2-(3,7,11-trimethyldodecyl)-thiophene
26. an isoprenoid C_{22}-thiophene
27. an isoprenoid C_{23}-thiophene
28. 2-ethyl-5-hexadecyl-thiophene
29. 2,3-dimethyl-5-(2,6,10,14-tetramethylpentadecyl)-thiophene
30. 2-ethyl-5-heptadecyl-thiophene
31. 2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)-thiophene (B=126) (two isomers; 12 and 13 in Figs. 2 and 3)
32. isomer of 31 (B=126)
33. 2-ethyl-5-octadecyl-thiophene
34. 2-ethyl-5-nonadecyl-thiophene
35. 2-ethyl-5-icosyl-thiophene
36. 2-ethyl-5-henicosyl-thiophene
37. 2-docosyl-5-ethyl-thiophene
38. 2-ethyl-5-tricosyl-thiophene
39. 2-ethyl-5-tetracosyl-thiophene
40. 2-ethyl-5-pentacosyl-thiophene
41. 2-ethyl-5-hexacosyl-thiophene
42. 2-ethyl-5-heptacosyl-thiophene
43. 2-ethyl-5-octacosyl-thiophene

ALKYLTHIOPHENES, C_3-SUBSTITUTED (BASE PEAK 139)

44. 2-ethyl-3-methyl-5-(2,6,10-trimethylundecyl)-thiophene
45. 5-ethyl-3-methyl-2-(3,7,11-trimethyldodecyl)-thiophene
46. an isoprenoid C_{23}-thiophene
47. an isoprenoid C_{24}-thiophene
48. a C_3-substituted-2-hexadecyl-thiophene
49. an isoprenoid C_{27}-thiophene (B=140)
50. isomer of 49
51. isomer of 49 (B=140)
52. isomer of 49
53. a C_3-substituted-2-octadecyl-thiophene
54. a C_3-substituted-2-nonadecyl-thiophene
55. a C_3-substituted-2-icosyl-thiophene
56. a C_3-substituted-2-henicosyl-thiophene
57. a C_3-substituted-2-docosyl-thiophene
58. a C_3-substituted-2-tricosyl-thiophene
59. a C_3-substituted-2-tetracosyl-thiophene
60. a C_3-substituted-2-pentacosyl-thiophene

ALKYLTHIOPHENES, C_4-SUBSTITUTED (BASE PEAK 153)

61. an isoprenoid C_{23}-thiophene
62. isomer of 61
63. 5-butyl-2-tetradecyl-thiophene
64. an isoprenoid C_{28}-thiophene (B=154)
65. isomer of 64 (B=154)
66. 2-butyl-5-octadecyl-thiophene
67. 2-butyl-5-nonadecyl-thiophene
68. 2-butyl-5-icosyl-thiophene
69. 2-butyl-5-henicosyl-thiophene
70. 2-butyl-5-docosyl-thiophene
71. 2-butyl-5-tricosyl-thiophene
72. 2-butyl-5-tetracosyl-thiophene
73. 2-butyl-5-pentacosyl-thiophene
74. 2-butyl-5-hexadecyl-thiophene

ALKYLTHIOPHENES, C_5-SUBSTITUTED (BASE PEAK 167)

75. an isoprenoid C_{19}(?)-thiophene
76. 2-butyl-3-methyl-5-(2,6,19-trimethylundecyl)-thiophene
77. 5-(2-methylpropyl)-3-methyl-2-(3,7,11-trimethyldodecyl)-thiophene
78. an isoprenoid C_{29}-thiophene

ALKYLTHIOPHENES, C_6-SUBSTITUTED (BASE PEAK 181)

79. 5-(2,6-dimethylheptyl)-2-(3-methylbutyl)-3-methyl-thiophene
80. 2-(3,7-dimethyloctyl)-5-(2-methylbutyl)-3-methyl-thiophene
81. an isoprenoid C_{25}-thiophene

ALKYLTHIOPHENES, C_7-SUBSTITUTED (BASE PEAK 195)

82. 5-(2,6-dimethyl-1-(3-methyl-butyl)-heptyl)-2,3-dimethyl-thiophene and/or 5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3-methyl-thiophene

dimethyl-5-(2,6,10-trimethylundecyl)thiophene (I). This compound was earlier identified in the saturated hydrocarbon fraction of this sediment (ten Haven *et al.*, 1985). The mass spectrum of this compound (Fig. 3.5C) shows a base peak at *m/z* 125 and a molecular ion at *m/z* 308. Such a simple mass spectrum is characteristic for both isoprenoid and *n*-alkylthiophenes. Mass spectra of both compound classes (see Fig. 3.5A-C) show in general a molecular ion and one major fragmentation ion associated with the thiophene moiety. Therefore it is not possible to distinguish between these compound classes solely on the basis of their mass spectra. However, they are readily recognized from their relative retention times, as explained later.

The presence of several homologous series of *n*-alkylthiophenes has been confirmed by synthesis of two *n*-alkylthiophenes: 2-methyl-5-tridecylthiophene (XII) and 2-dodecyl-5-ethylthiophene (XIII). Both compounds reveal a mass spectrum (see Fig. 3.5B) indistinguishable from those of compounds 5 and 23 respectively (Fig. 3.4). Coinjection on a SE-52 fused silica capillary (25 m x 0.32 mm) confirmed the presence of these thiophenes in the sediment. From these assignments and the approximately linear relation between retention time and the carbon number of a homologous series combined with mass spectral data the 2-alkyl-5-methylthiophene and 2-alkyl-5-ethylthiophene homologous series were identified.

In an analogous manner it might be expected that the "139" and "153"-*n*-alkylthiophene series, *i.e.* those having m/z 139 and 153 base peaks (see Fig. 3.6), are the 2-alkyl-5-propylthiophene series and 2-alkyl-5-butylthiophene series, respectively. However, only the last one is deemed likely to be the 2-alkyl-5-butylthiophene series since the compounds of this series elute at the expected retention time, relative to the 2-alkyl-5-methylthiophene series (Sinninghe Damsté *et al.*, 1988c).

If the *n*-alkylthiophene homologous series is taken arbitrarily as a reference, the isoprenoid thiophenes are easily recognized as those alkylthiophenes which elute much earlier than might be expected from their molecular weight. This was confirmed in the case of 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene (II, C_{20}), by coinjection of the sub-fraction 2-9 on a SE-52 fused silica capillary column (25 m x 0.32 mm) with a synthetic standard (Brassell *et al.*, 1986c). This C_{20} compound (6, Table 3.3) elutes just after the C_{18} *n*-alkylthiophene, 2-methyl-5-tridecylthiophene (XII) which is similar to the retention behaviour of isoprenoid aliphatic hydrocarbons relative to *n*-alkanes. We therefore propose to use here a purposely defined "retention index": the Alkyl Thiophene Index (ATI):

$$ATI(x) = 100.z + 100.\{t(x) - t(z)\}/\{t(z+1) - t(z)\}$$

where t(x) is the retention time of the compound for which ATI is to be determined, t(z) and t(z+1) are the retention times of the *n*-alkylthiophenes which bracket the compound of interest, and z is the number of carbon atoms in the *n*-alkylthiophene that elutes just prior to the compound of interest. The ATI can be calculated with respect to several *n*-alkylthiophene series.

From these series, one has to be chosen which gives a basepeak 14 daltons lower than the isoprenoid thiophene because there is always one methyl group of an isoprenoid unit associated with the thiophene moiety (*e.g.* compare structures I and XII). In Table 3.4 the ATI of the most abundant isoprenoid thiophenes are compared with the retention indices of the isoprenoid aliphatic hydrocarbons present in the marl layer. With this method the isoprenoid thiophenes are easily distinguished from the *n*-alkylthiophenes but their structures are only partially elucidated. The reported structures of the isoprenoid thiophenes (Tables 3.2 and 3.3) are tentative except for structure II and are based on mass spectral interpretations and on the agreement of the ATI of an isoprenoid thiophene with the retention index of the corresponding isoprenoid hydrocarbon (Table 3.4). In the mass spectra of 4-methyl-2-(3,7,11,15-tetramethylhexadecyl)thiophene (VI) and 2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)thiophene (VII) (Fig. 3.5E), a rearrangement fragment (*m/z* 112 and *m/z* 126 respectively) was observed as base peak. The mass spectrum of 4-methyl-2-(3,7,11-trimethyldodecyl)thiophene (XIV), a byproduct in the synthesis of II (Brassell *et al.*, 1986c), also shows a rearrangement ion (*m/z* 112) as base peak. It is therefore suggested that the isoprenoid carbon skeletons of these compounds are comparable with that of thiophene XIV and thus characterised by a single tail-tail linkage. The other structures proposed for the isoprenoid thiophenes have regular isoprenoid carbon skeletons except for structure XVIIa.

Table 3.4. Retention data of the isoprenoid hydrocarbons and their thiophene analogues.

hydrocarbon compound	I[1]	thiophene analogue ATI[2]	I[3]
phytane	1816	1820(I), 1825(II)[4]	2093
2,6,10,14-tetramethylheptadecane	1895	1914(III)	2187
2,6,10,14-tetramethyloctadecane	1987	2007(IV)[5]	2265
2,6,10,14,18- and/or 2,6,10,14,19-pentamethyleicosane	2242	2242(V), 2246(VI)[4]	2532, 2551
2,6,10,14,18- and/or 2,6,10,14,19-pentamethyleicosane	2342	2339, 2357(VII)[6]	2628, 2651

[1] retention index measured on CP Sil-5, 130°C to 300°C at 4°C/min

[2] Alkyl Thiophene Index (ATI) measured on CP Sil-5, 130°C to 300°C at 4°C/min, with the homologous series of 2-alkyl-5-methylthiophenes as calibration standards unless mentioned otherwise

[3] retention index measured on SE-52, 150°C to 300°C at 4°C/min

[4] with the homologous series of 2-alkylthiophenes as calibration standards

[5] with the homologous series of 2-alkyl–5-ethylthiophenes as calibration standards

[6] there are two isomers (compounds 12 and 15 in Table 3.2)

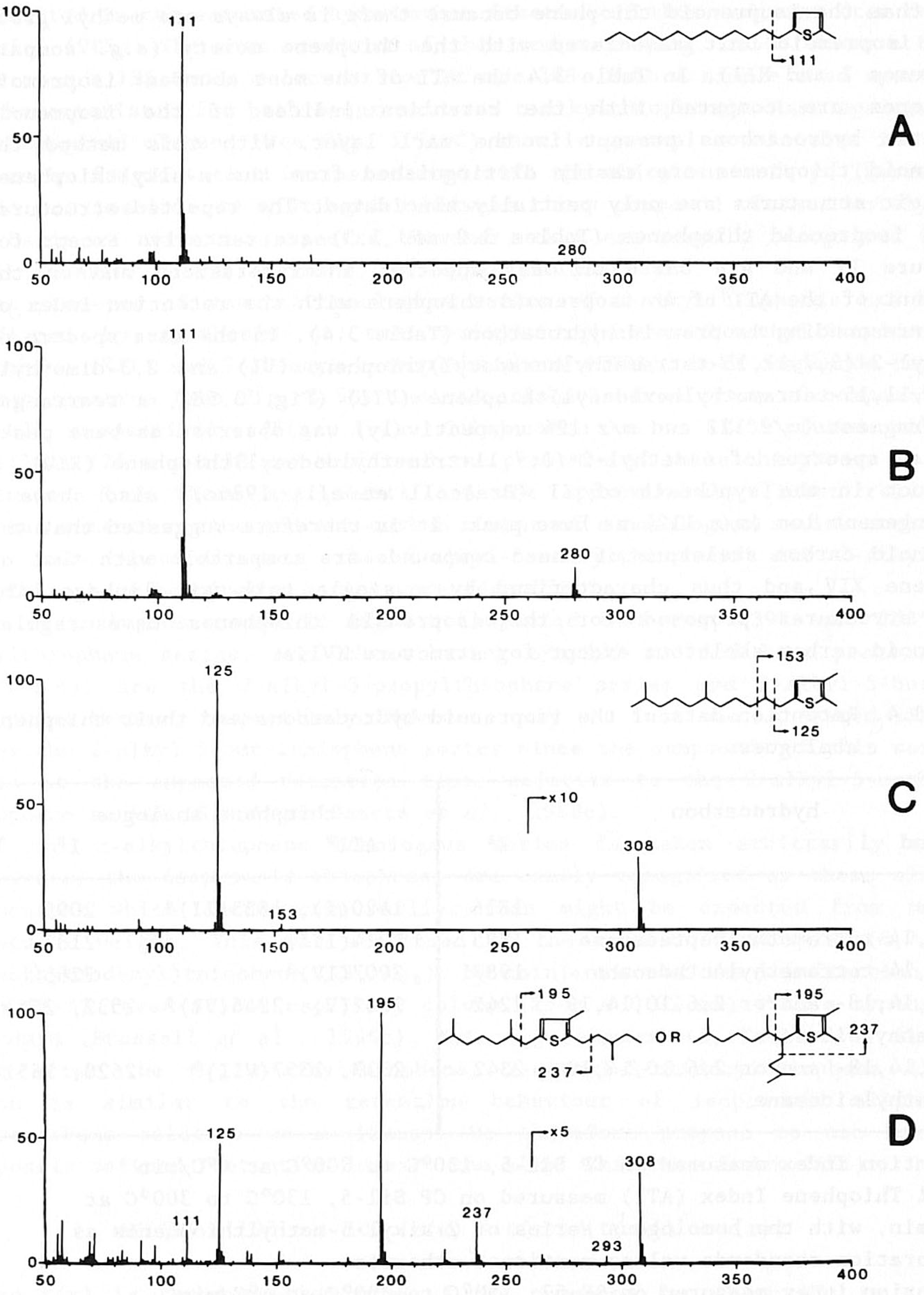
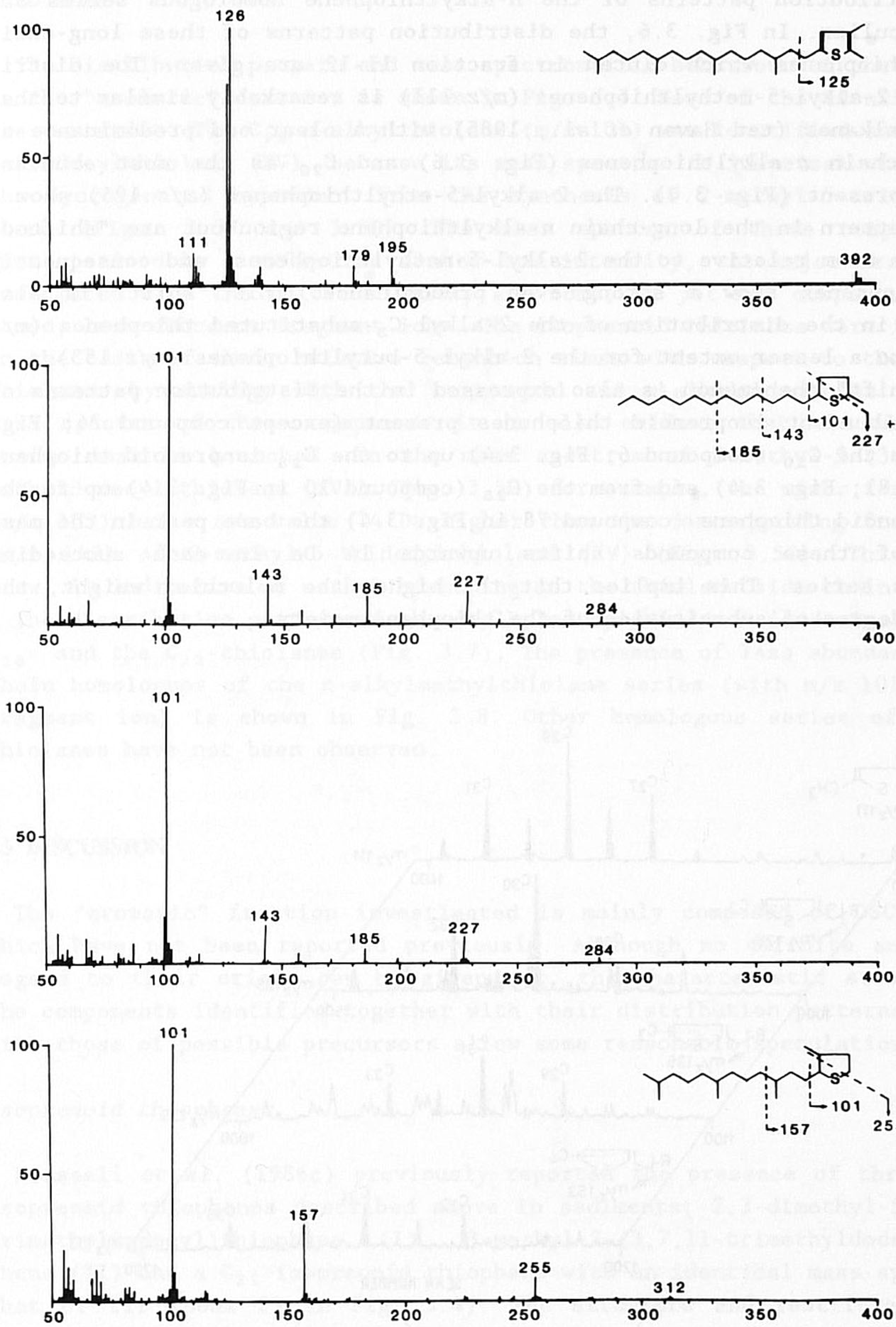


Fig. 3.5. Mass spectra, corrected for background, of (A): 2-methyl-5-tridecylthiophene (XII), (B): synthetic 2-methyl-5-tridecylthiophene, (C): 2,3-dimethyl-5-(2,6,10-trimethylundecyl)-thiophene (I), (D): 5-(2,6-dimethyl-1-(3-methylbutyl)-heptyl)-2,3-dimethylthiophene (XVIIa) and/or 5-(2,6-di-



methylheptyl)-2-(3-methylpentyl)-3-methylthiophene (XVIIb), (E): 2,3-dimethyl-5-(3,7,11,15-tetramethyl-hexadecyl)thiophene (VII), (F): 2-methyl-5-undecylthiolane (XV), (G): synthetic 2-methyl-5-undecylthiolane, (H): 3-methyl-5-(3,7,11-trimethyldodecyl)thiolane (XVI).

The distribution patterns of the *n*-alkylthiophene homologous series are rather peculiar. In Fig. 3.6, the distribution patterns of these long-chain *n*-alkyl thiophenes which eluted in fraction 11-12 are given. The distribution of 2-alkyl-5-methylthiophenes (*m/z* 111) is remarkably similar to that of the *n*-alkanes (ten Haven *et al.*, 1985) with a clear odd predominance of the long-chain *n*-alkylthiophenes (Fig. 3.6) and C_{22} as the most abundant compound present (Fig. 3.4). The 2-alkyl-5-ethylthiophenes (*m/z* 125) show a similar pattern in the long-chain *n*-alkylthiophene region but are "shifted" one carbon atom relative to the 2-alkyl-5-methylthiophenes, and consequently these thiophenes show a strong even predominance. This "shift" is also expressed in the distribution of the 2-alkyl-C_3-substituted thiophenes (*m/z* 139) and to a lesser extent for the 2-alkyl-5-butylthiophenes (*m/z* 153).

This "shift" phenomenon is also expressed in the distribution patterns of the most abundant isoprenoid thiophenes present (except compound 24; Fig. 3.4). From the C_{20} (compound 6; Fig. 3.4) up to the C_{25} isoprenoid thiophene (compound 81; Fig. 3.4) and from the C_{25} (compound 10 in Fig. 3.4) up to the C_{29} isoprenoid thiophene (compound 78 in Fig. 3.4) the base peak in the mass spectra of these compounds shifts upwards 14 Da in each succeeding homologous series. This implies that the higher the molecular weight, the more the degree of substitution of the thiophene moiety.

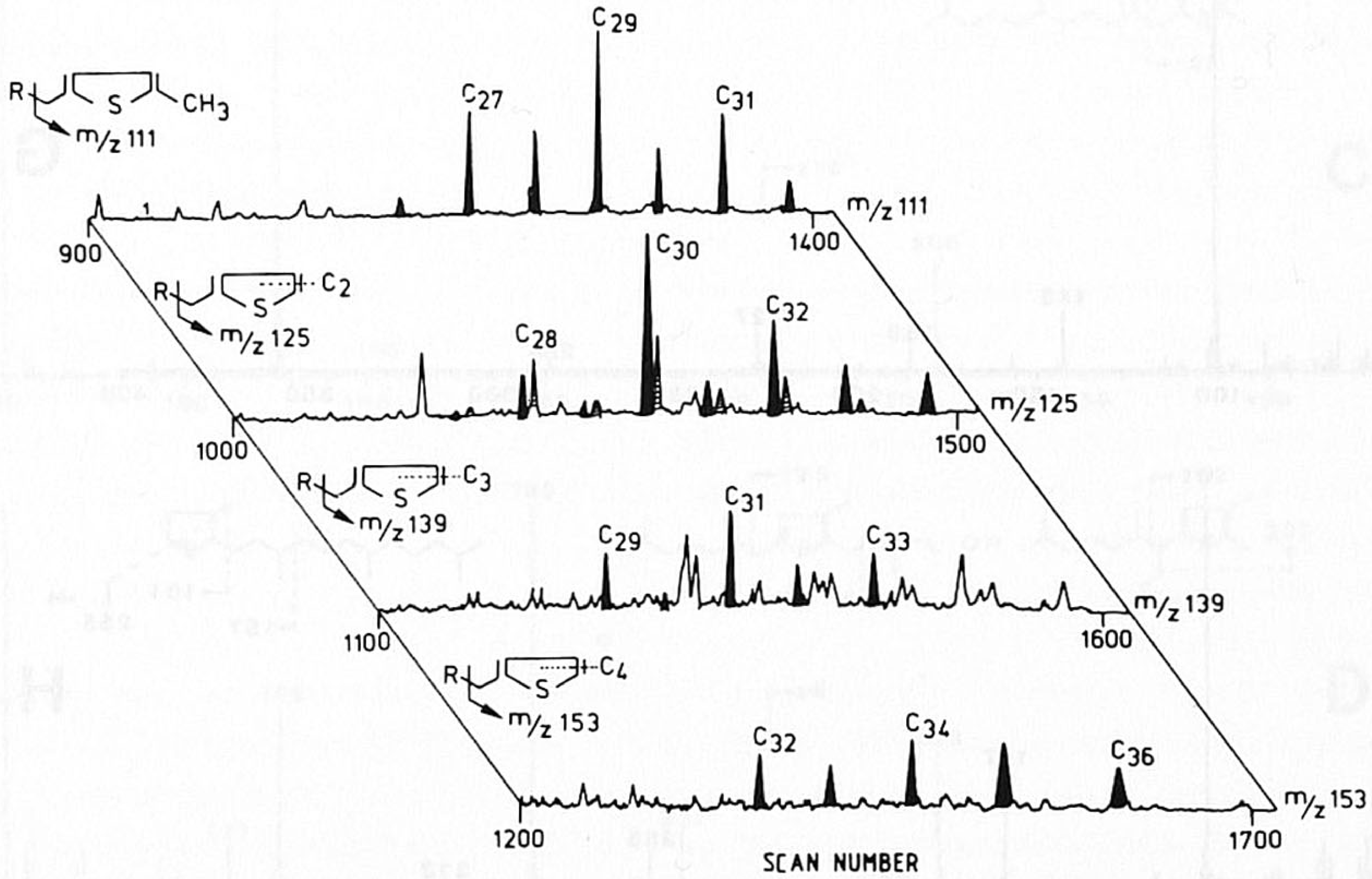


Fig. 3.6. Mass chromatograms of *m/z* 111, 125, 139 and 153 in the region where the long-chain *n*-alkylthiophenes elute in fraction 11-12. The peak indications represent the total number of carbon atoms of the *n*-alkylthiophenes.

Thiolanes

The distribution pattern and mass spectrometric behaviour of the thiolanes in the "aromatic" fraction is shown in Fig. 3.7. Most of the identifications are tentative. The C_{18} *n*-alkylthiolane (*m/z* 101) was identified as 2-methyl-5-tridecylthiolane (XV), because its mass spectrum is identical with that of the hydrogenated byproduct from the synthesis of 2-methyl-5-tridecylthiophene (Figs. 3.5F and 3.5G). The mass spectra of these compounds are characterised by a molecular ion of low intensity, one major fragment ion associated with the thiolane moiety and some, as yet not completely understood, less abundant fragment ions. The isoprenoid thiolanes were identified on the basis of their relative retention times with respect to the *n*-alkylthiolanes by analogy with the isoprenoid and *n*-alkylthiophenes. From the mass spectra of these compounds it is also evident that the alkyl side-chains must be branched. In the mass spectrum of 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (XVI) (Fig. 3.5H) for example, the minor fragment ion (*m/z* 157) has a *m/z* value 14 Da higher than the corresponding fragment ion (m/z 143) of 2-methyl-5-tridecylthiolane (XV) (Fig. 3.5G). This, in our view, is indicative of methyl branching in the thiolane side-chain.

The distribution pattern of the *n*-alkylmethylthiolanes is dominated by the C_{18}- and the C_{22}-thiolanes (Fig. 3.7). The presence of less abundant longer-chain homologues of the *n*-alkylmethylthiolane series (with m/z 101 as major fragment ion) is shown in Fig. 3.8. Other homologous series of *n*-alkylthiolanes have not been observed.

3.5 DISCUSSION

The "aromatic" fraction investigated is mainly composed of OSC, most of which have not been reported previously. Although no definite answer with regard to their origin can be given yet, the characteristic structures of the components identified together with their distribution patterns compared with those of possible precursors allow some reasonable speculations.

Isoprenoid thiophenes

Brassell *et al*. (1986c) previously reported the presence of three of the isoprenoid thiophenes described above in sediments; 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene (I), 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene (II) and a C_{21} isoprenoid thiophene with an identical mass spectrum to that of III (peak 25 in Fig. 3.4). The structure and restricted stereochemistry of II was consistent with its presumed origin as an early stage diagenetic product, arising from sulphur incorporation into chlorophyll-derived phytol or archaebacterial phytenes and/or their diagenetic products. However, biosynthesis of these thiophene compounds or functionalized precursors was not completely precluded. In this paper a large number of new

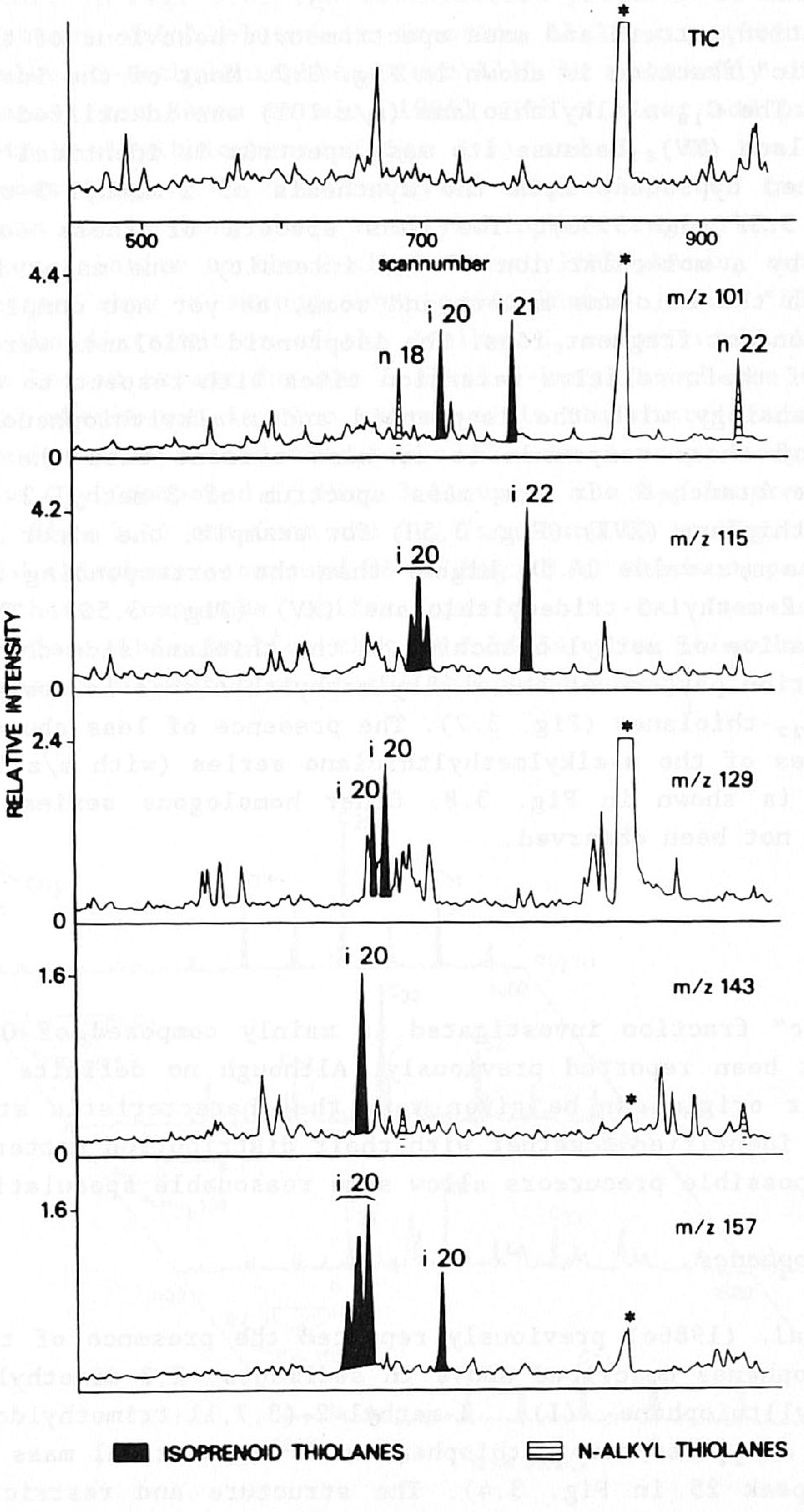


Fig. 3.7. Mass chromatograms of *m/z* 101, 115, 129, 143 and 153 and part of the TIC (upper trace) of the "aromatic" fraction. The large peak marked with an asterisk is due to dioctyladipate (see Fig. 3.4)

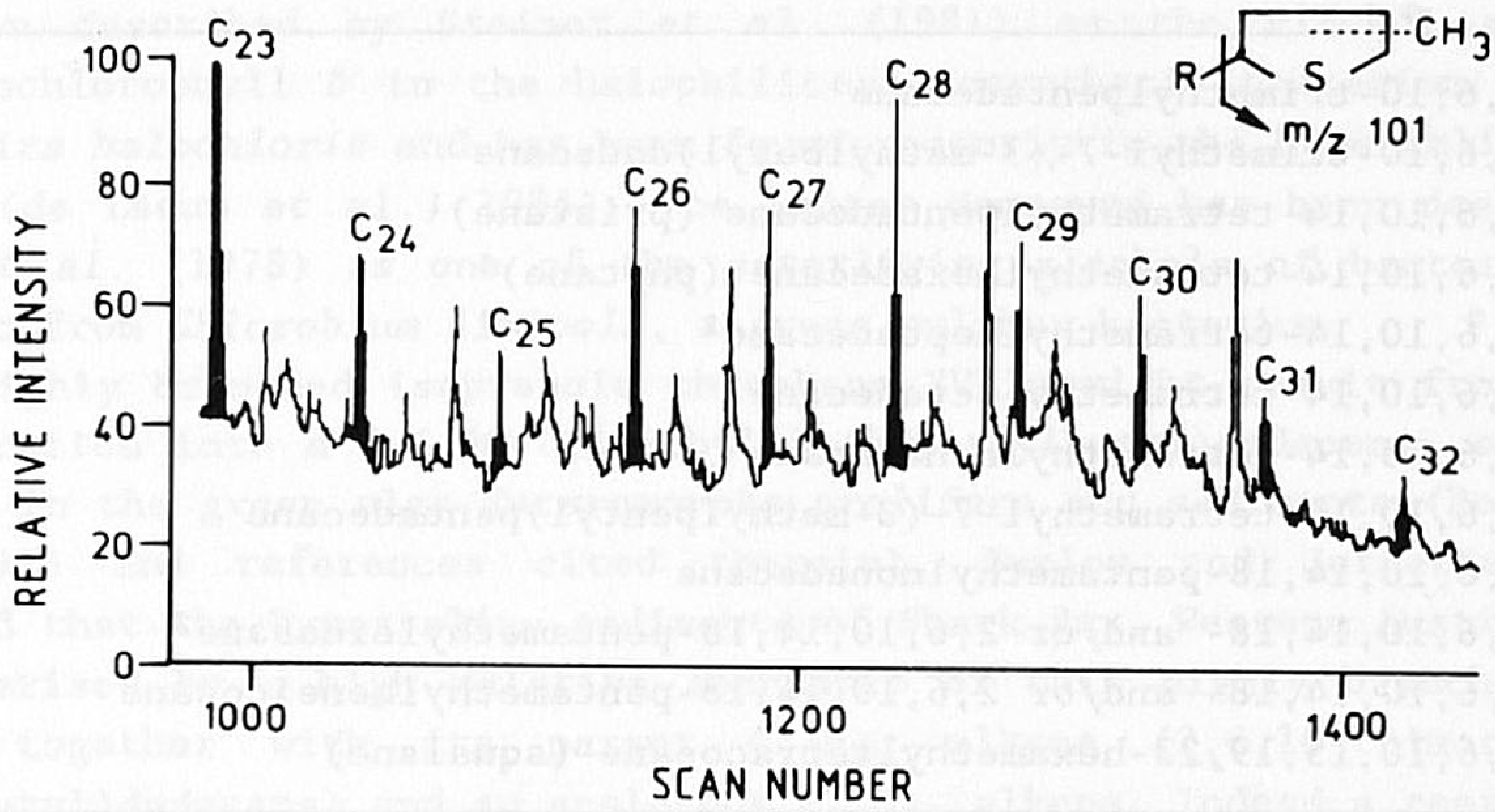


Fig. 3.8. Mass chromatogram of *m/z* 101 in the region where the long-chain alkylmethylthiolanes elute. The peak indications represent the total number of carbon atoms present in the thiolanes.

isoprenoid thiophenes ranging from C_{19} to C_{29}, with C_{20}, C_{21}, C_{22}, C_{25} and C_{26} components predominating, are reported. We agree with Brassell *et al.* (1986c) that the thiophenes found are either of biosynthetic origin or are formed as the result of a reaction of H_2S, polysulphides and/or elemental sulphur with biogenic alkenes (*i.e.* phytenes, squalenes, *i*-C_{25}-alkenes) or other functionalized isoprenoids (such as unsaturated alcohols). Such biogenic alkenes are often reported in sediments (*e.g.* Barrick *et al.*, 1980; Boon *et al.*, 1982; de Leeuw *et al.*, 1985; Requejo and Quinn, 1983; Risatti *et al.*, 1984) and archaebacteria (*e.g.* Holzer *et al.*, 1979; Risatti *et al.*, 1984). However, there are as yet no reports of analogous sulphur compounds in these bacteria.

If the isoprenoid thiophenes were formed by sulphur incorporation into functionalized and/or unsaturated isoprenoid compounds (precursors) one would expect that the distribution of the products (isoprenoid thiophenes) reflects that of the precursors. However, these precursors are not present anymore in this six million year old sediment but the carbon skeletons of the preserved isoprenoid alkanes may reflect those of the originally present functionalized and/or unsaturated isoprenoids. Therefore, the distribution of isoprenoid alkanes in this sediment was reexamined to compare it with those of the isoprenoid thiophenes. A number of minor compounds were identified (Table 3.5), in addition to the isoprenoid alkanes reported previously (ten Haven *et al.*, 1985).

It is noteworthy that both phytane and the isoprenoid C_{20} thiophene (I) are the most abundant compounds in their respective compound classes. The C_{25} isoprenoid hydrocarbon is also relatively abundant (ten Haven *et al.*, 1985). The exact structure of the C_{25} isoprenoid is not yet known, since 2,6,10,14,18- and 2,6,10,14,19-pentamethyleicosane exhibit similar mass

Table 3.5. Identified isoprenoid hydrocarbons.

2,6,10-trimethylpentadecane[1]
2,6,10-trimethyl-7-(3-methylbutyl)dodecane
2,6,10,14-tetramethylpentadecane (pristane)[1]
2,6,10,14-tetramethylhexadecane (phytane)[1]
2,6,10,14-tetramethylheptadecane[1]
2,6,10,14-tetramethyloctadecane
2,6,10,14-tetramethylnonadecane[1]
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane
2,6,10,14,18-pentamethylnonadecane
2,6,10,14,18- and/or 2,6,10,14,18-pentamethyleicosane[2]
2,6,10,14,18- and/or 2,6,10,14,18-pentamethylheneicosane
2,6,10,15,19,23-hexamethyltetracosane (squalane)

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[1] previously identified by ten Haven *et al.* (1985)
[2] previously identified as the first isomer by ten Haven *et al.* (1985)

spectra (Risatti *et al.*, 1984) and coelute on the column used. The fully separated C_{25} thiophene analogues (V and VI; 29 and 10 in Fig. 3.4) are thought to be present in the sediment. The C_{26} isoprenoid thiophene (V) is also relatively abundant but the C_{26} isoprenoid hydrocarbon is not.

Squalane is present in the "saturated" fraction, which is attributed to bacterial sources. Such bacteria usually also contain squalenes. However, no C_{30} isoprenoid thiophenes are observed. One could speculate that squalenes have undergone sulphur incorporation, and that this results in the long-chain alkylbenzothiophenes (peaks 17, 21 and 23 in Fig. 3.2), with the loss of some methyl groups during aromatisation.

Whether the regular C_{21}, C_{22}, C_{23} and C_{24} isoprenoid alkanes originate from thermal breakdown of longer isoprenoid structures (*e.g.* diphytanylglycerolethers, polyprenols) or originate from archaebacteria is unknown. However, no so called (Albaiges *et al.*, 1985) quasi-isoprenoids, compounds supposed to occur simultaneously with the regular isoprenoids after catagenetic degradation of archaebacterial lipids, were present and so the first origin is less likely. Assuming a direct origin from archaebacteria for these compounds, the corresponding isoprenoid alkenes could be present originally in the sediment since the degree of unsaturation of isoprenoid hydrocarbons synthesized by archaebacteria depends on their growth conditions (Tornabene *et al.*, 1979). Sulphur incorporation into these isoprenoid alkenes may give rise to the isoprenoid thiophenes in this sediment. The C_{21}, C_{24}, C_{26} and C_{28} isoprenoid thiophenes for example may be linked to the input of organic material from *Sulfolobus*, a photosynthetic sulphur-containing bacterium, which contains the C_{21}, C_{24}, C_{26} and C_{28} isoprenoid alkanes (Holzer *et al.*, 1979).

The "mid-chain" C_{20} isoprenoid thiophenes (XVIIb and XVIII) may result from sulphur incorporation into mid-chain unsaturated phytadienols (or their diagenetic products) such as $\Delta^{2,6}$- and $\Delta^{2,10}$-phytadienol. The first compound has been described by Steiner *et al.* (1981) as the alcohol moiety of bacteriochlorophyll *b* in the halophilic photosynthetic bacterium *Ectothiorhodospira halochloris* and has been found recently in the hypersaline Gavish Sabkha (de Leeuw *et al.*, 1985). The latter compound has been described by Caple *et al.* (1978) as one of the esterifying alcohols of bacteriochlorophylls *c* from *Chlorobium limicola*, a green sulphur bacterium.

The highly branched isoprenoid thiophene XVIIa might result from sulphur incorporation into a 2,6,10-trimethyl-7-(3-methylbutyl)dodecene, a compound present in the green alga *Enteromorpha prolifera* and sediments (Rowland *et al.*, 1985 and references cited therein). Dunlop and Jefferies (1985) reported that the hypersaline sediments of Shark Bay, Western Australia, are characterised by a high relative abundance of this highly branched $C_{20}H_{40}$ alkene together with its parant $C_{20}H_{42}$ alkane (2,6,10-trimethyl-7-(3-methylbutyl)dodecane) and an analogous $C_{25}H_{50}$ alkene. Indeed a reexamination of the hydrocarbon fraction revealed the presence of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane and 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (Table 3.5). The latter compound may result from reduction of a related C_{25} alkene, present in hypersaline sediments (Dunlop and Jefferies, 1985) and/or alkadiene, present in *Enteromorpha prolifera* and sediments (Rowland *et al.*, 1985).

The reported isoprenoid thiophenes may be products of sulphur incorporation into specific lipid moieties from (archae-)bacterial and/or algal input during diagenesis. On the other hand, the partial similarity observed between the distribution patterns of the isoprenoid alkanes and the isoprenoid thiophenes can also be the reflection of a direct biosynthetic relationship. Confirmation of the postulated structures by synthesis and sulphur incorporation experiments with model compounds (work in progress) may help to better understand the origin of the isoprenoid thiophenes.

n-*Alkylthiophenes*

The distribution of the 2-alkyl-5-methylthiophenes (Fig. 3.6, *m/z* 111) mimics that of the *n*-alkanes in this sediment as reported by ten Haven *et al.* (1985) (high *n*-C_{22}, odd predominance of the long-chain alkanes). This observation seems to support the mechanism of thermal reaction of free sulphur with alkanes (Schmid (*et al.*, 1987). However, the even-over-odd predominance of the 2-alkyl-5-ethylthiophenes and 2-alkyl-5-butylthiophenes and the odd over even predominance of the C_3-substituted 2-alkylthiophenes (the "shift"-phenomenon, Fig. 3.6) cannot be explained straightforwardly by such a mechanism. At present, we cannot explain the distribution patterns observed but the occurrence of compounds possessing long-chain *n*-alkylthiophene moieties in living organisms is one possibility. Sulphur incorporation of H_2S, CH_3SH, C_2H_5SH etc., compounds known to be produced by various microorganisms (Kadota and Ishida, 1972; Wakeham *et al.*, 1984), into certain substrates is another possible explanation. This type of reaction may also explain the observed shift phenomenon of the isoprenoid thiophenes. In this case the substrates involved may then be the biogenic alkenes (phyt(adi)ene, C_{25} isoprenoid alkenes). However, the formation of thiophenes from such reactions is not so easy to imagine.

Thiolanes

The thiolanes may reflect intermediate structures in the formation of the corresponding thiophenes, but their distribution patterns do not correspond very closely to those of the corresponding thiophenes. The distribution pattern of the alkylmethylthiolanes is comparable to that of the 2-alkyl-5-methylthiophenes in a way that the C_{18}, C_{22} and C_{23} compounds (Fig. 3.7 and 3.8) are the most abundant alkylmethylthiolanes and -thiophenes, respectively. In the C_{24}-C_{30} range, however, the 2-alkyl-5-methylthiolanes show a slight even predominance (Fig. 3.8), whereas the 2-alkyl-5-methylthiophenes show an odd-over-even predominance (Fig. 3.6). Isoprenoid thiolanes were only encountered in the C_{20}-C_{22} range in contrast to the isoprenoid thiophenes.

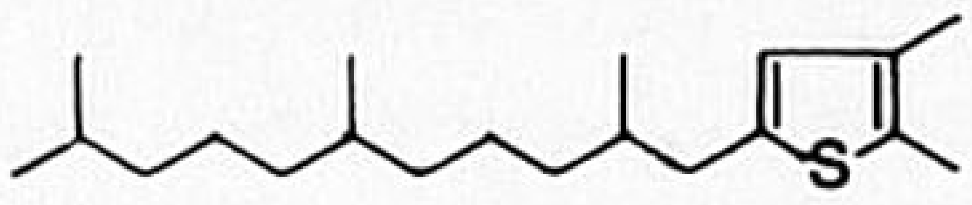
The C_{22} *n*-alkane is relatively abundant in the saturated hydrocarbon fraction (ten Haven *et al.*, 1985). The predominance of this *n*-alkane has been reported before (Schenck, 1969; Powell and McKirdy, 1973) in sediments, and the relative abundance of this compound among the *n*-alkanes was recently proposed as an indication for hypersaline environments (ten Haven *et al.*, 1985). The abundant presence of the C_{22} *n*-alkylmethylthiophenes and -thiolanes could result from sulphur incorporation into an unknown precursor of the C_{22} *n*-alkane or may reflect a biosynthetic relationship.

3.6 CONCLUSIONS

A number of *n*-alkyl and isoprenoid thiophenes and thiolanes have been identified in an extract from a Messinian (Upper Miocene) marl layer deposited under hypersaline, euxinic conditions. Their specific structures and their distribution patterns show a partial similarity with those of the corresponding alkanes. These OSC might therefore result from sulphur incorporation into specific (archae)bacterial and/or algal functionalized alkanes (*e.g.* alkenes, unsaturated alcohols) during early diagenesis. This mechanism may be extended to comparable intermolecular processes, thus leading to high-molecular-weight structures (kerogen, asphaltenes). The partial similarity observed between the alkanes and the OSC distribution patterns can, however, also be the reflection of a direct biosynthetic relationship. A reaction of elemental sulphur with the saturated alkanes during late diagenesis as proposed by Schmid *et al.* (1987) does not seem to be consistent with our results.

The OSC encountered in this sediment and in other sediments and seep oils (under investigation) are thought to reflect a hypersaline depositional environment. This observation is in agreement with the conclusion of Tissot and Welte (1984) that crude oils produced from carbonate/evaporite source rocks exhibit a consistently higher sulphur content.

APPENDIX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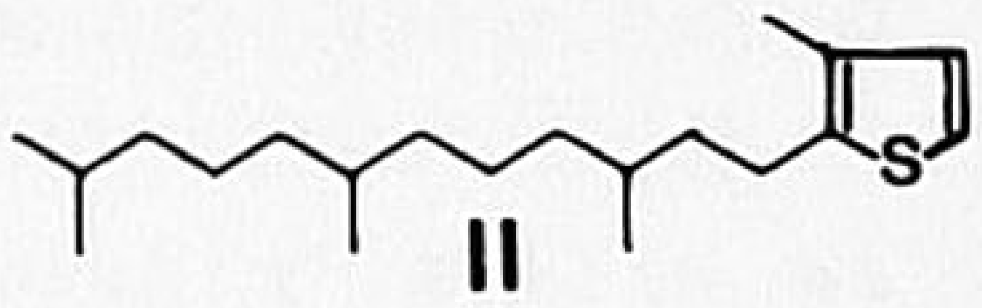
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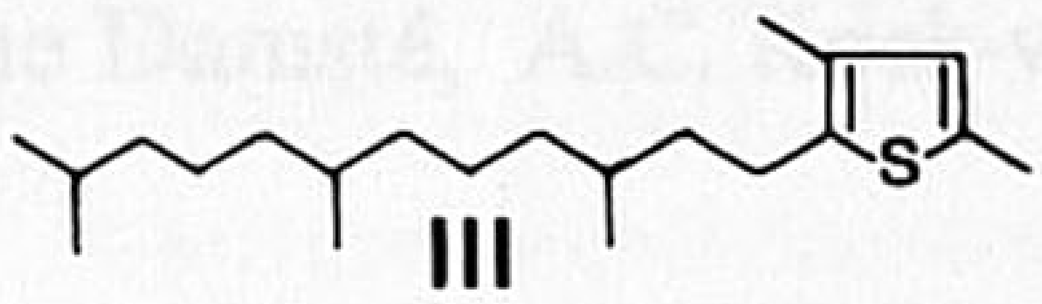
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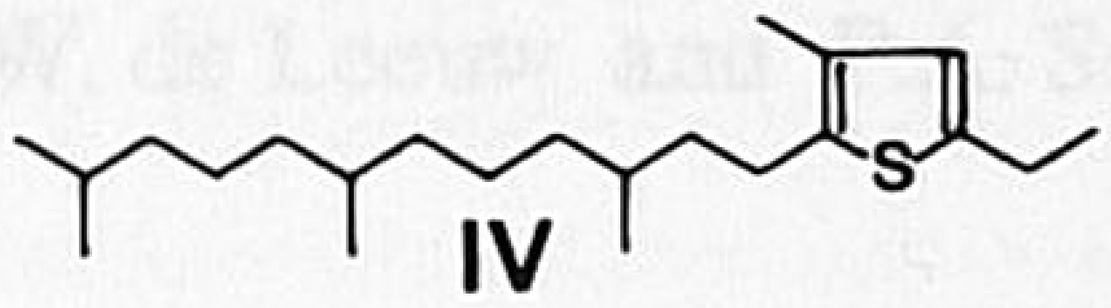
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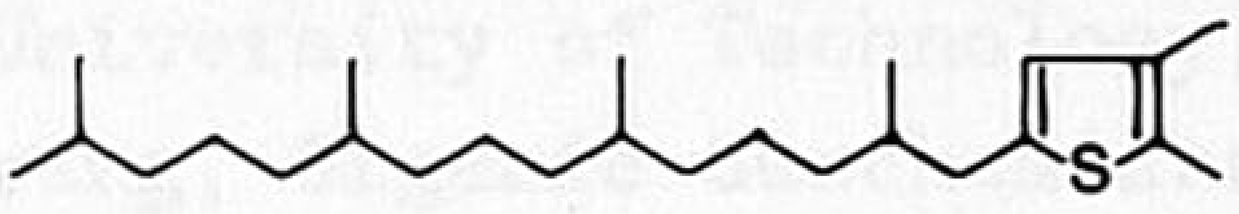
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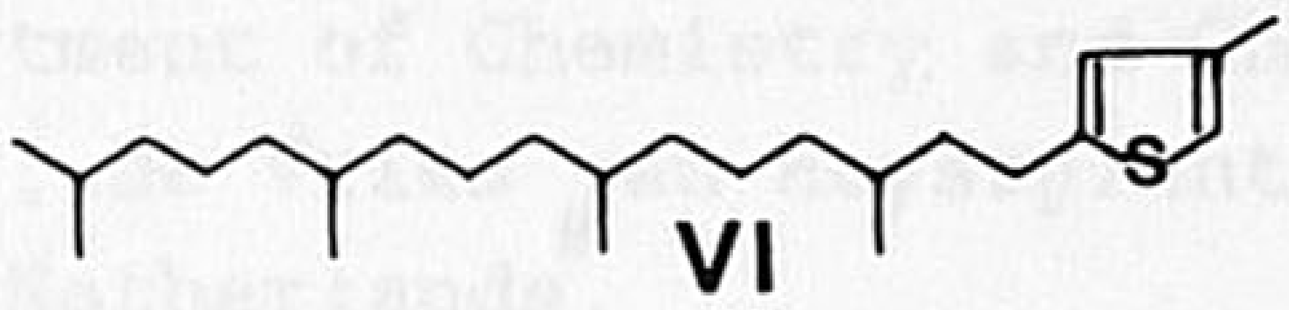
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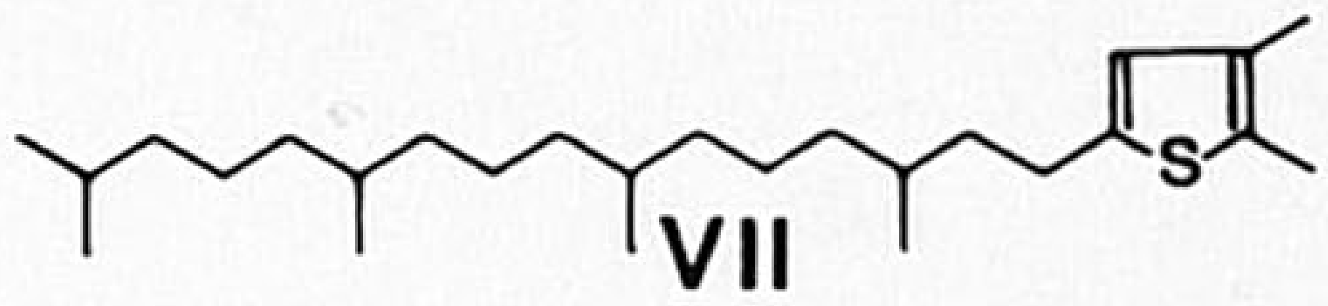
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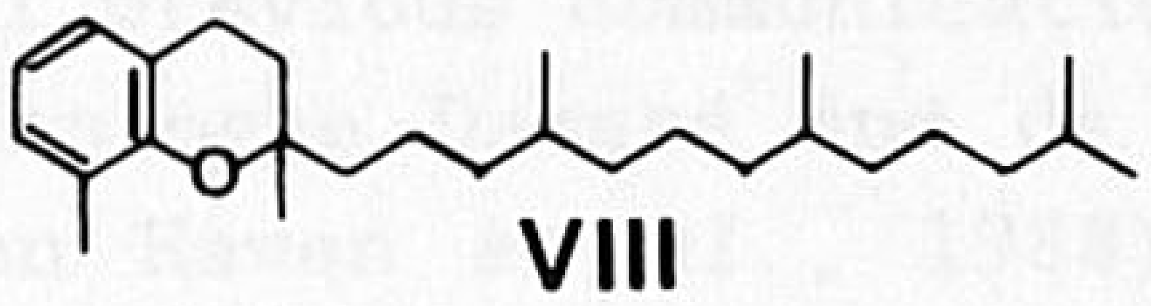
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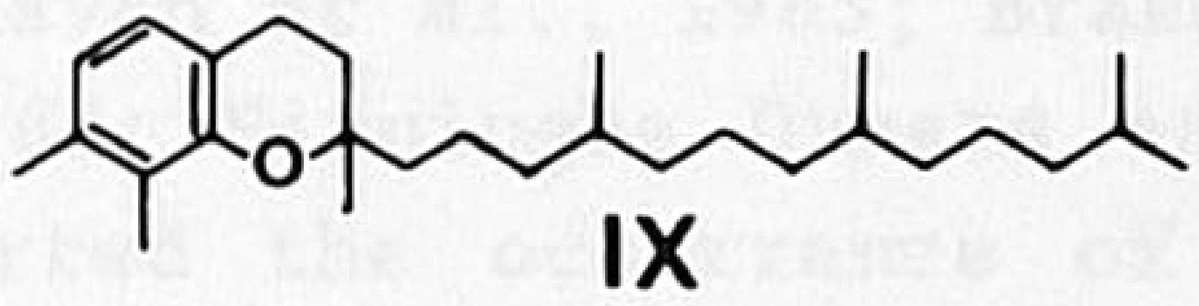
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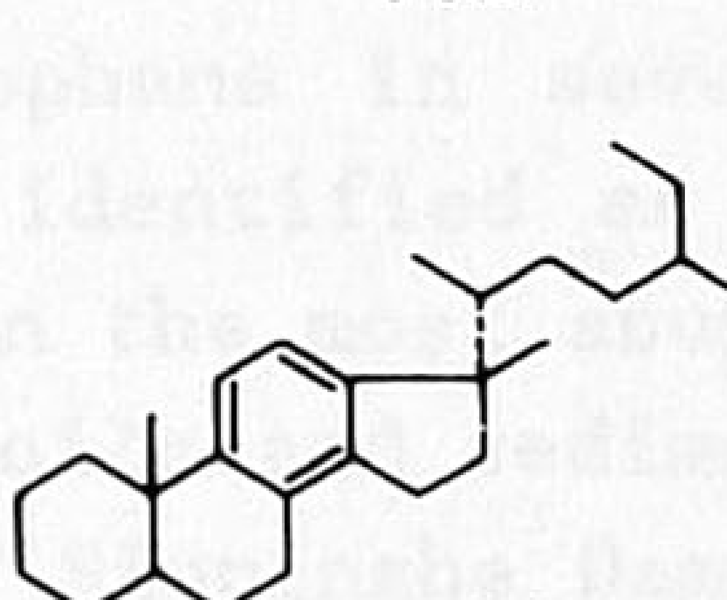
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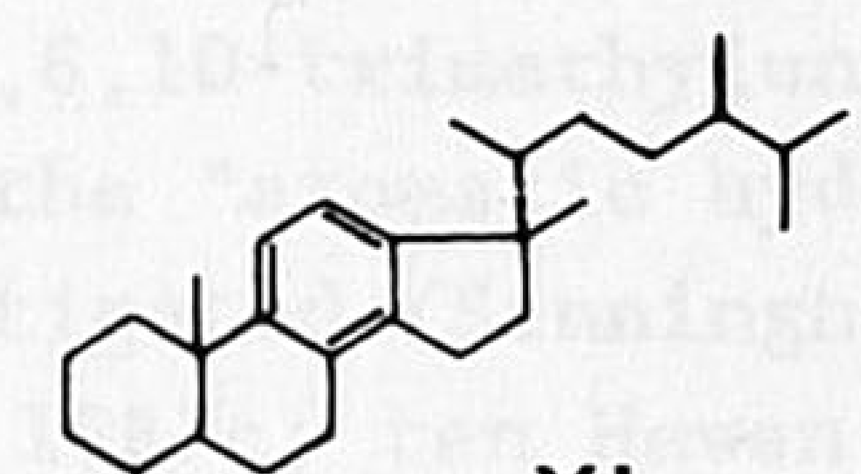
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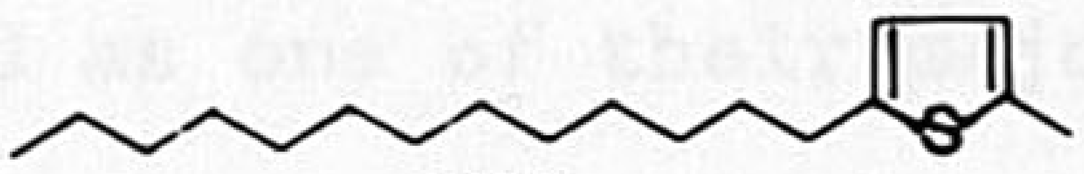
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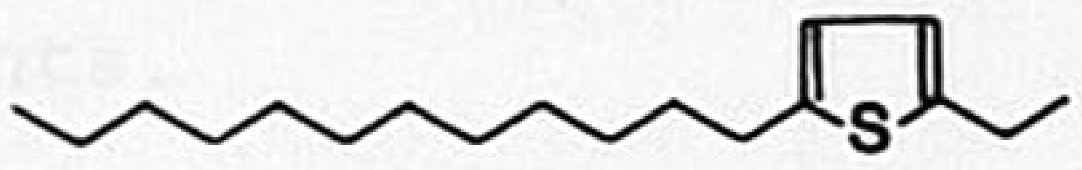
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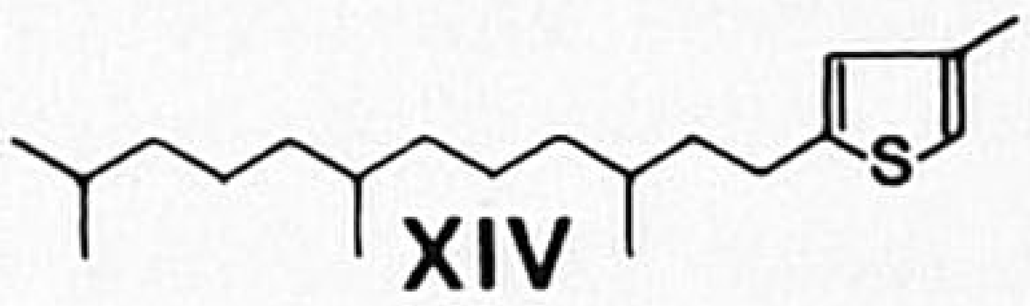
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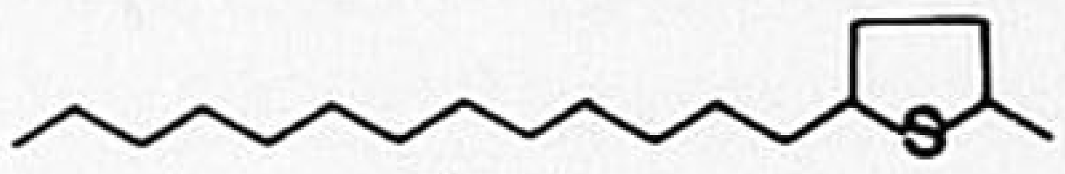
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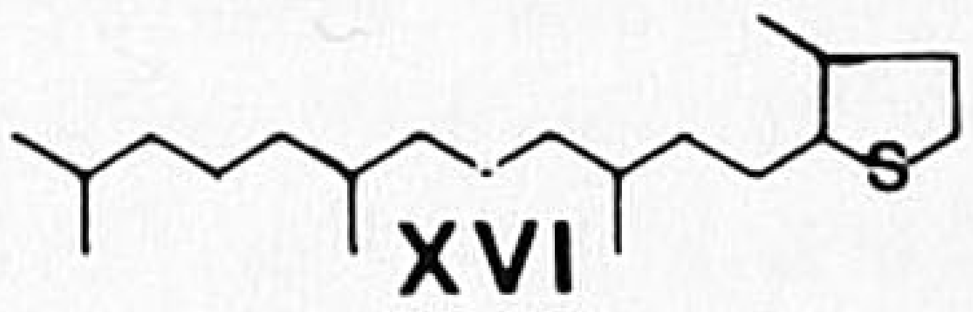
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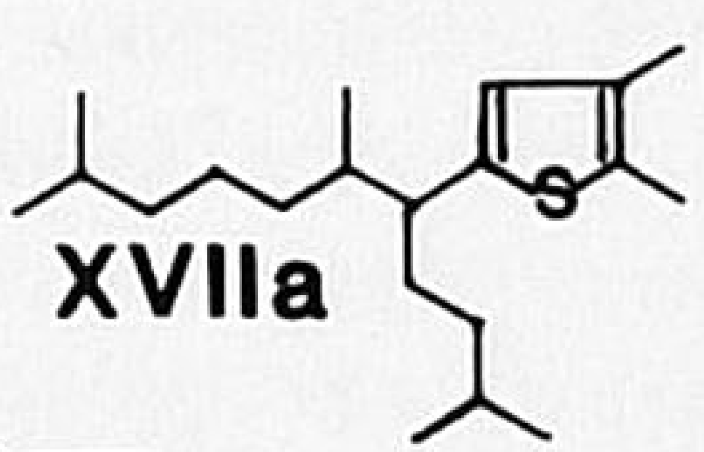
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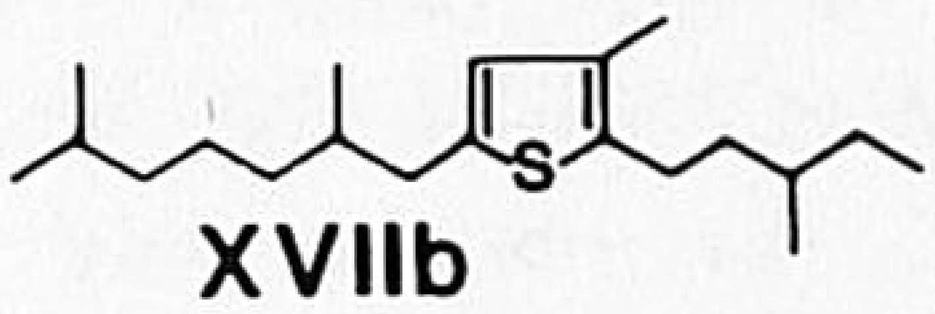
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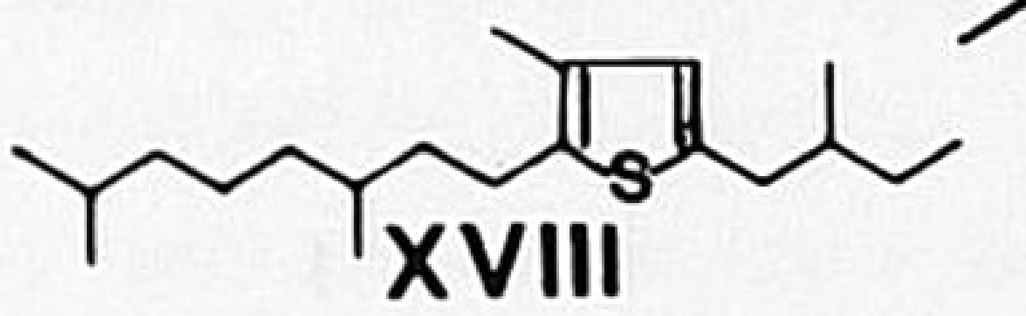
XVIII

4. The identification of 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene a novel sulphur-containing biological marker *

J.S. Sinninghe Damsté, A.C. Kock-van Dalen, J.W. de Leeuw and P.A. Schenck

Delft University of Technology, Department of Chemistry and Chemical
Engineering, Organic Geochemistry Unit, De Vries van Heystplantsoen 2,
2628 RZ Delft The Netherlands.

In several previous communications (ten Haven *et al.*, 1985; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987c; ten Haven *et al.*, 1988) we reported the occurrence of a C_{20} isoprenoid thiophene in several sediment extracts and oils. This compound, tentatively identified as 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene (I), is often the most abundant compound of the "aromatic hydrocarbon" fraction of the oils and sediment extracts investigated (Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987c; ten Haven *et al.*, 1988). Since the "aromatic hydrocarbon" fraction of these samples represented *ca.* 50% (by weight) of the total oil or sediment extract, this compound has to be considered as one of their major constituents.

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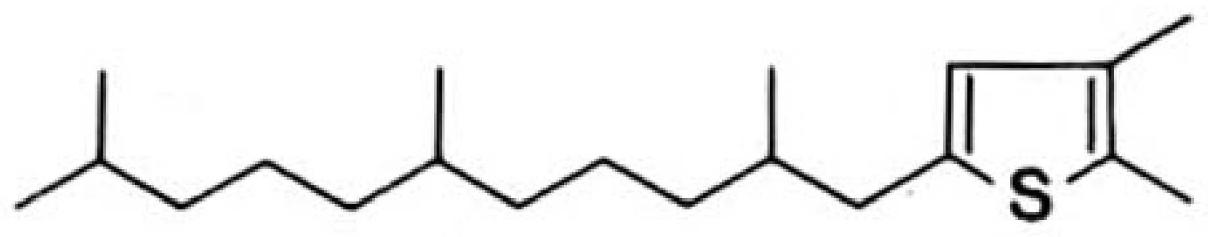
* *Tetrahedron Lett.* 28, 957-960 (1987)

The tentative identification of I was based on mass spectral and relative retention time data (Sinninghe Damsté *et al.*, 1986) in comparison with those of a previously synthesized (Brassell *et al.*, 1986c) isoprenoid thiophene (an alkylthiophene with an isoprenoidal carbon skeleton), 3-methyl-2-(3,7,11-trimethyldodecyl)thiophene (II). This identification was further supported by desulphurisation of a particular subfraction, obtained by several column chromatographic (Al_2O_3) separation steps, of the "aromatic hydrocarbon" fraction of the Rozel Point oil (a crude oil from the North Western part of Utah, USA). This fraction contained I for *ca.* 35% together with several so called "midchain" isoprenoid C_{20} thiophenes (40%). Desulphurisation of this fraction using Raney nickel afforded a mixture of hydrocarbons in which phytane (III) was the major compound (65%). This experiment proved that the carbon skeleton of I is indeed isoprenoidal. GC-HRMS (Sinninghe Damsté *et al.*, 1987c) of I showed that the elemental formula is $C_{20}H_{36}S$.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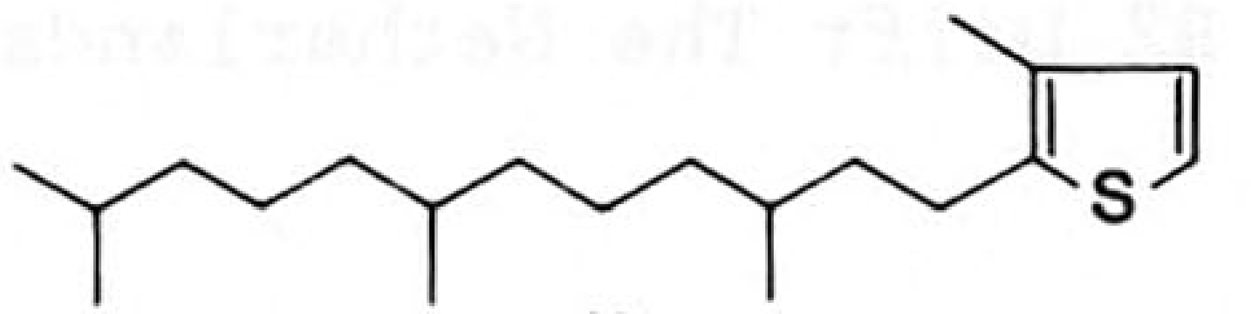
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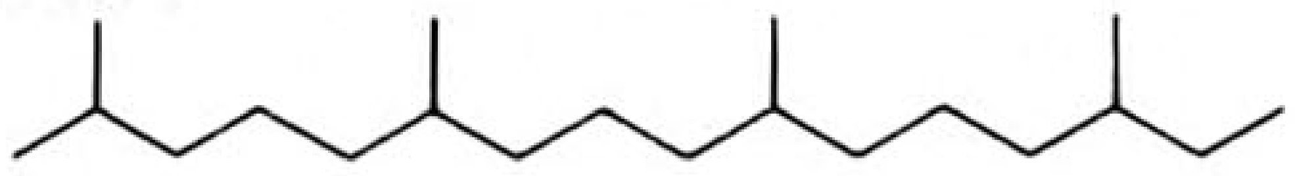
III

Confirmation of this tentative assignment came from comparison of I with a synthetic standard. The synthesis scheme is shown in Fig. 4.1. Farnesol (3,7,11-trimethyl-2,6,10-dodecatrienol, Aldrich) was catalytically hydrogenated after protection of the hydroxyl group by acetylation. Hydrolysis afforded hexahydrofarnesol (IV), which was coupled with stearyl chloride. The resulting ester was pyrolysed at 400°C yielding 3,7,11-trimethyltetradec-1-ene (V) (Smith and Rouault, 1949). V was subsequently oxidized to the isoprenoid C_{14} acid, 2,6,10-trimethylundecanoic acid (VI) (Lee and Chang, 1978). VI was coupled with 2,3-dimethylthiophene (VII), obtained *via* a modified Wolf-Kishner reduction of 3-methyl-2-thiophenecarboxaldehyde (Aldrich) (King and Nord, 1949). The resulting ketone, 2-(6,10-dimethyl)-undecyl-5'-(2',3'-dimethylthienyl)ketone (VIII) was also reduced by a modified Wolf-Kishner reaction to I. The spectral characteristics of I and several intermediate products (Appendix) are in accordance with their structures. The mass spectrum of the synthesized product was indistinguishable from that reported for the geological thiophene (Sinninghe Damsté *et al.*, 1986). The standard compound coeluted with the geological thiophene on two different capillary columns (CP Sil-5, 25 m x 0.32 mm, film thickness = 0.12 μm; CP Sil-88, 25 m x 0.32 mm, film thickness = 0.20 μm).

The structure of this C_{20} isoprenoid thiophene has thus unambiguously been established. The carbon skeleton of I, as was already shown by desulphurisation, is the same as that of phytane (III). Phytane appears widely in the geosphere. A number of precursors has been suggested for phytane including the phytyl side chain of chlorophylls and archaebacterial membrane lipids (Tissot and Welte, 1984). The structural relationship between I and phytane supports the hypothesis (Brassell *et al.*, 1986c; Sinninghe Damsté and de

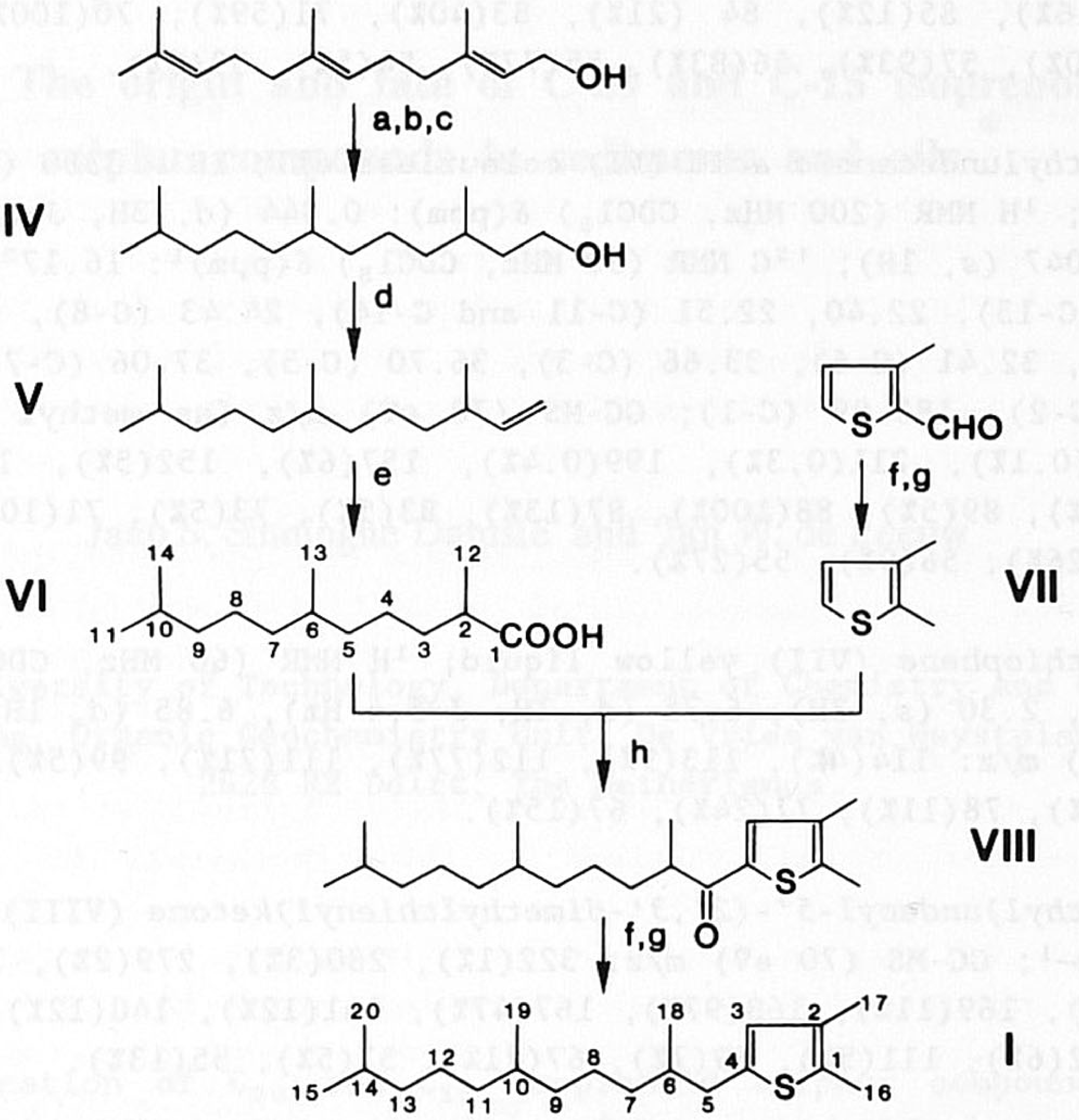

Fig. 4.1 The synthesis scheme for I: (a) Ac_2O, N,N-dimethyl-4-aminopyridine, (b) H_2, PtO_2, (c) OH^-, ΔT, (d) n-$C_{17}H_{35}COCl$, ΔT(400°C), (e) $KMnO_4$, HOAc, Adogen 464, (f) H_2N-NH_2, ΔT, (g) OH^-, ΔT, (h) P_2O_5, ΔT. The numbering of the structures VI and I has been used in the assignments of the ^{13}C NMR spectra of these compounds (Appendix).

Leeuw, 1987; Sinninghe Damsté *et al.*, 1986) that the isoprenoid thiophenes have been formed *via* incorporation of inorganic sulphur into the precursors of the isoprenoid alkanes found in sediments and oils. For example incorporation of sulphur into phytol, phytenes and/or phytadienes may lead to the formation of I. The presence of phytane as most abundant compound of the aliphatic fraction of the samples studied strongly supports this theory, since I is the most abundant compound of the "aromatic" fraction.

The reaction of inorganic sulphur in the form of *e.g.* H_2S, HS^- and/or polysulphides requires an euxinic environment of deposition. Therefore I has to be considered as a marker for such an environment.

APPENDIX

3,7,11-trimethyldodec-1-ene (V) yellow liquid; IR ν 915, 1000, 1420, 3030 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ(ppm): 4.7-5.15 (*m*, 2H), 5.4-6.0 (*m*, 1H); GC-MS (70 eV) *m/z*: 210(<1%), 140(5%), 126(10%), 125(13%), 112(6%), 111(16%), 98(8%), 97(16%), 85(12%), 84 (21%), 83(40%), 71(59%), 70(100%), 69(79%), 68(9%), 67(10%), 57(93%), 56(83%), 55(77%), 54(5%), 53(6%).

2,6,10-trimethylundecanoic acid (VI) colourless oil; IR ν 3360 (broad), 1710 (sharp) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 0.844 (*d*, 3H, J=6.2 Hz), 2.55 (*m*, 1H), 10.047 (*s*, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ(ppm)[1]: 16.17[2], 16.70[2] (C-12), 19.39 (C-13), 22.40, 22.51 (C-11 and C-14), 24.43 (C-8), 24.57 (C-4), 27.77 (C-10), 32.41 (C-6), 33.66 (C-3), 36.70 (C-5), 37.06 (C-7), 39.12 (C-9), 39.34 (C-2), 183.09 (C-1); GC-MS (70 eV) *m/z* (as methyl ester): 242 (0.4%), 227(0.1%), 211(0.3%), 199(0.4%), 157(6%), 152(5%), 129(3%), 101 (35%), 97(13%), 89(5%), 88(100%), 87(13%), 83(5%), 73(5%), 71(10%), 69(19%), 59(10%), 57(26%), 56(9%), 55(27%).

2,3-dimethylthiophene (VII) yellow liquid; ^{1}H NMR (60 MHz, $CDCl_3$) δ(ppm): 2.04 (*s*, 3H), 2.30 (*s*, 3H), 6.75 (*d*, 1H, J=5.4 Hz), 6.85 (*d*, 1H, J=5.4 Hz); GC-MS (70 eV) *m/z*: 114(4%), 113(9%), 112(77%), 111(71%), 99(5%), 98(6%), 97 (100%), 85(6%), 78(11%), 77(24%), 67(15%).

2-(6,10-dimethyl)undecyl-5'-(2',3'-dimethylthienyl)ketone (VIII) orange oil; IR ν 1665 cm^{-1}; GC-MS (70 eV) *m/z*: 322(1%), 280(3%), 279(2%), 181(9%), 180 (4%), 170(5%), 169(11%), 168(97%), 167(47%), 141(12%), 140(12%), 139(100%), 138(11%), 112(6%), 111(5%), 69(7%), 67(21%), 57(5%), 55(13%).

2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene (I) colourless liquid; ^{1}H NMR (200 MHz, $CDCl_3$) δ(ppm): 0.839, 0.843 (two *d*, 3H, J=6.4 Hz), 0.865 (*d*, 6H, J=7.0 Hz), 0.894 (*d*, 3H, J=6.4 Hz), 0.9-1.7 (*m*, ≈15H), 2.063 (*s*, 3H), 2.276 (*s*, 3H), 2.437 (*dd*, 1H, J=7.2 and 14.0 Hz), 2.650 (*dd*, 1H, J=7.2 and 14.0 Hz), 6.414 (*s*, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ(ppm)[1]: 12.77 (C-17), 13.36 (C-16), 19.27[2], 19.32[2], 19.49[2], 19.58[2] (C-18, C-19), 22.45, 22.54 (C-15, C-18), 24.29 (C-8), 24.62 (C-12), 27.80 (C-14), 32.59 (C-10), 35.07 (C-6), 36.57[2], 36.62[2], 37.04[2], 37.08[2], 37.17, 37.29[2], 37.37[2] (C-5, C-7, C-9, C-11), 39.17 (C-13), 127.71 (C-3), 129.75 (C-1), 132.01 (C-2), 139.12 (C-4); GC-MS (70 eV) *m/z*: 310(0.4%), 309(0.4%), 308(3%), 153(3%), 127(5%), 126(17%), 125 (100%), 124(6%), 111(4%), 91(6%), 55(5%).

[1] assignments are tentative and substantiated from comparison of ^{13}C NMR spectra of several isoprenoid hydrocarbons (Yon, 1981) and by using the additivity rules (Lindemans and Adams, 1971).

[2] there are two pairs of enantiomers because of the presence of two chiral centres in the molecule. These pairs of enantioisomers are recognized by ^{13}C NMR: they give rise to two peaks in the spectrum for certain carbon atoms. The ratio of these two peaks is for these carbon atoms ca. 1:1, which shows that the pairs of enantioisomers are present in a ratio of 1:1, as expected from the applied synthesis scheme.

5. The origin and fate of C-20 and C-15 isoprenoid sulphur compounds in sediments and oils *

Jaap S. Sinninghe Damsté and Jan W. de Leeuw

Delft University of Technology, Department of Chemistry and Chemical
Engineering, Organic Geochemistry Unit, De Vries van Heystplantsoen 2,
2628 RZ Delft, The Netherlands.

5.1 ABSTRACT

The identification of C_{20} and C_{15} isoprenoid sulphur compounds in Rozel Point Oil is described. The occurrence of isoprenoid thiophenes, thiolanes, benzothiophenes, bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes in this oil and in other oils and sediments is reported. These compounds are thought to originate from sulphur incorporation into unsaturated phytanols and farnesol at the early stages of diagenesis. Compounds, that contain two sulphur atoms, are thought to originate from polyunsaturated phytanols, compounds especially abundant in hypersaline environments due to the presence of archaebacteria and green and purple sulphur bacteria. A diagenetical scheme, indicating relationships between precursors and products is presented.

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5.2 INTRODUCTION

The occurrence of organic sulphur compounds (OSC) in oils and extracts of ancient and recent sediments is well known. Neither the origin nor the diagenetic fate of OSC is, however, understood. This lack of knowledge is partly due to the fact that the structures of OSC presently known are not very characteristic; the carbon skeletons do not seem to carry information about appropriate biosynthetic precursors. Only recently some OSC which seem to be related to such biosynthetic precursors were reported by several investigators (Payzant *et al.*, 1983; 1985; Valisolalao *et al.*, 1984; Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986).

We have encountered isoprenoid and straight chain alkylthiophenes, -thiolanes, -thianes, and -benzothiophenes with chain lenghts of C_{12}-C_{28} in several sediment extracts and oils (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986; de Leeuw, 1986; Sinninghe Damsté *et al.*, 1987c; ten Haven *et al.*, 1988). Especially the C_{20} isoprenoid thiophenes occurring in several sediment extracts and oils give clues to their biosynthetic origin. In this paper we concentrate on data obtained for these isoprenoid OSC.

Over the years Berner (1984) has pointed out very elegantly that bacterial sulphate reduction is the mechanism for formation of anorganic sulphur compounds in sediments. In normal marine sediments the rate of sulphate reduction is determined by the reactivity of the organic matter substrate. Hence, the presence of high quality organic matter causes a high production of H_2S and its fixation products (FeS, FeS_2). From the organic chemical point of view one can expect the interaction of H_2S or other sulphur reagents like polysulphides with suitable functional groups present in the organic compounds present. Especially when no "suitable" Fe-ions are available (*e.g.* in hypersaline evaporitic environments), one can expect relatively high abundancies of OSC since no competition for H_2S between the organic compounds and Fe-ions exists. Therefore we have investigated sediment extracts and crude oils wich were known to originate from anaerobic environments of sedimentation and from evaporitic environments of sedimentation. Especially an oil (Rozel Point Oil), the organic matter of which is thought to originate from an evaporitic playa lake environment (Meissner *et al.*, 1984; ten Haven *et al.*, 1988), was investigated because of its high abundance of OSC.

5.3 EXPERIMENTAL

Rozel Point Oil was obtained from the Geological Survey of Utah (USA). This oil came from a site in the North-western part of Utah.

Details on the fractionation of the oil and sub-fractionation of the "aromatic" fraction of the oil have been described elsewhere (Sinninghe Damsté *et al.*, 1987c). The "aromatic" fraction was also fractionated into a so called low molecular weight aromatic and a high molecular weight aromatic fraction by column chromatography (Al_2O_3, 25 cm x 10 mm) using hexane/toluene (9:1) and toluene as eluents, respectively. The low molecular weight aromatic fraction contains most of the GC amenable compounds of the "aromatic" fraction (Sinninghe Damsté *et al.*, 1987c). Gas chromatography of the obtained fractions was carried out on a Varian 3700 gas chromatograph equipped with a flame ionization (FID) and a flame photometric detector (FPD). A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was split at the end of the column with a splitter device. The conditions used were described previously (Sinninghe Damsté *et al.*, 1986).

GC-MS analysis was carried out on a Varian 3700 gas chromatograph connected to a MAT-44 quadrupole mass spectrometer as described previously (Sinninghe Damsté *et al.*, 1986).

Exact mass measurements were performed on a Varian MAT 311 double focussing mass spectrometer by peak matching using selected masses from perfluorkerosine as the reference at a resolution of 5000.

5.4 RESULTS

The Rozel Point Oil was separated by conventional techniques into so called aliphatic, aromatic and hetero fractions. Because of the very complex nature of the "aromatic" fraction a further fractionation was performed by column chromatography over alumina using several eluents sequentially. The resulting fractions (in case of the Rozel Point Oil called RPO-1, -2, etc.) were investigated by capillary gas chromatography with dual FID and FPD detection and by capillary gas chromatography-mass spectrometry. Since we will concentrate in this paper on the most abundant C_{20} isoprenoid components present, Fig. 5.1 shows the appropriate parts of the total ion current (TIC) traces of the low molecular weight aromatic fraction and the fractions RPO-1, -2, -5 and -6. The symbols used in these traces indicate compounds with structures shown in Schemes 5.2 and 5.3 and in the Appendix.

Fraction RPO-1 almost entirely consisted of C_{20} isoprenoid thiophenes and thiolanes. The major isoprenoid thiophenes were identified based on their relative GC retention times and on comparison of their mass spectra with mass spectra of synthesized isoprenoid thiophenes (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1987a). A typical mass spectrum of 2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiophene (D) is shown in Fig. 5.2A. This mass spectrum is characterised by two fragments (*m/z* 167 and 265) originating from β-cleavages of both side chains. The base peak *m/z* 125 is thought to be a secondary fragment ion originating from both primary fragments (*m/z* 167 and 265) by a rearrangement in which the other alkyl side-chain is removed as an alkene. This mass spectrum is in agreement with those of a series of 2-*n*-alkyl-3-methyl-5-(2-methylalkyl)thiophenes (Pomonis *et al.*, 1976), in which the base peak also appeared at *m/z* 125.

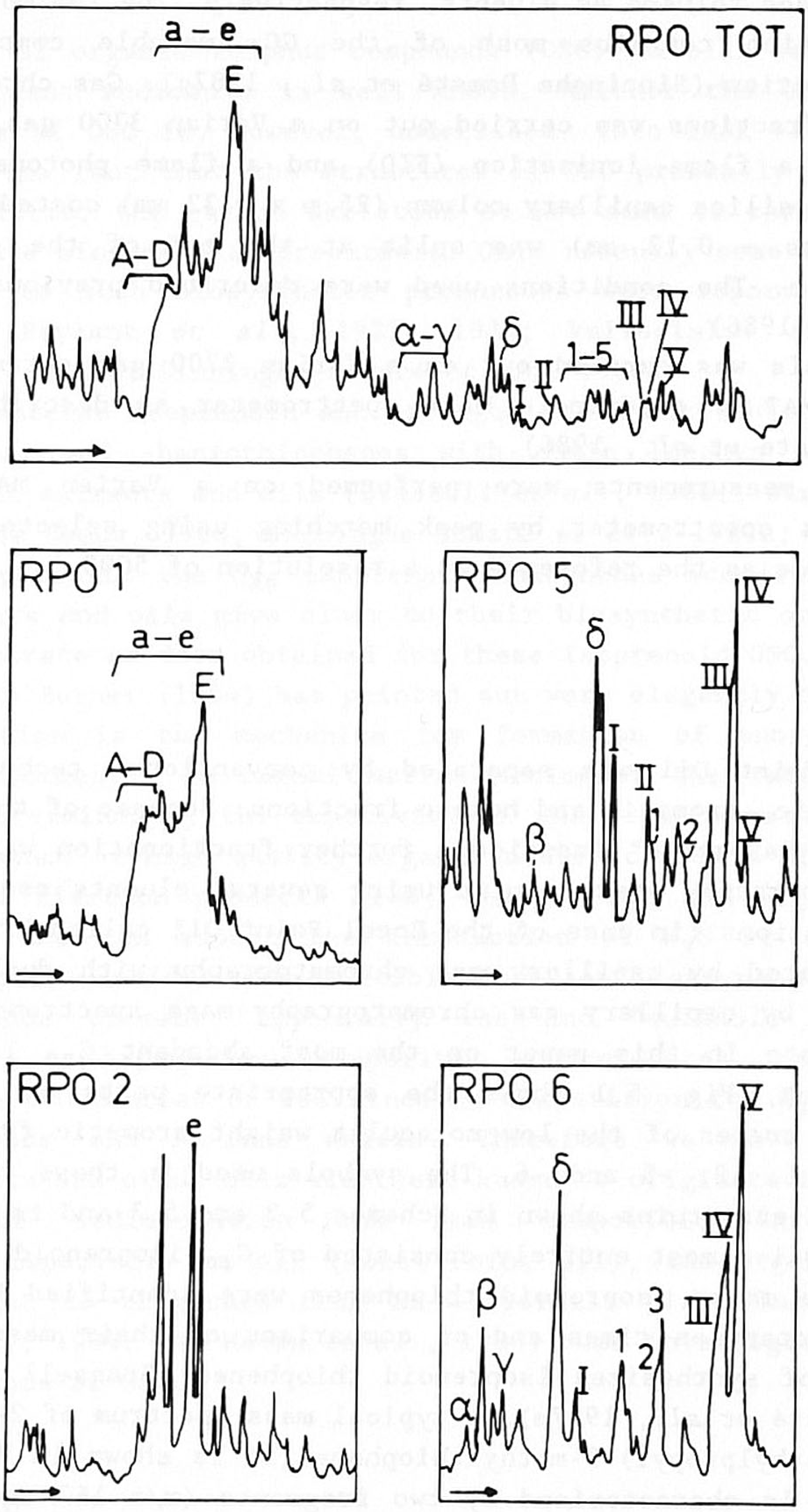


Fig. 5.1. Partial total ion current traces of appropriate fractions of the "aromatic" fraction and of the low molecular weight aromatic fraction of the Rozel Point Oil showing the distributions of the C_{20} isoprenoid sulphur compounds. Symbols refer to compounds assigned in Table 5.1.

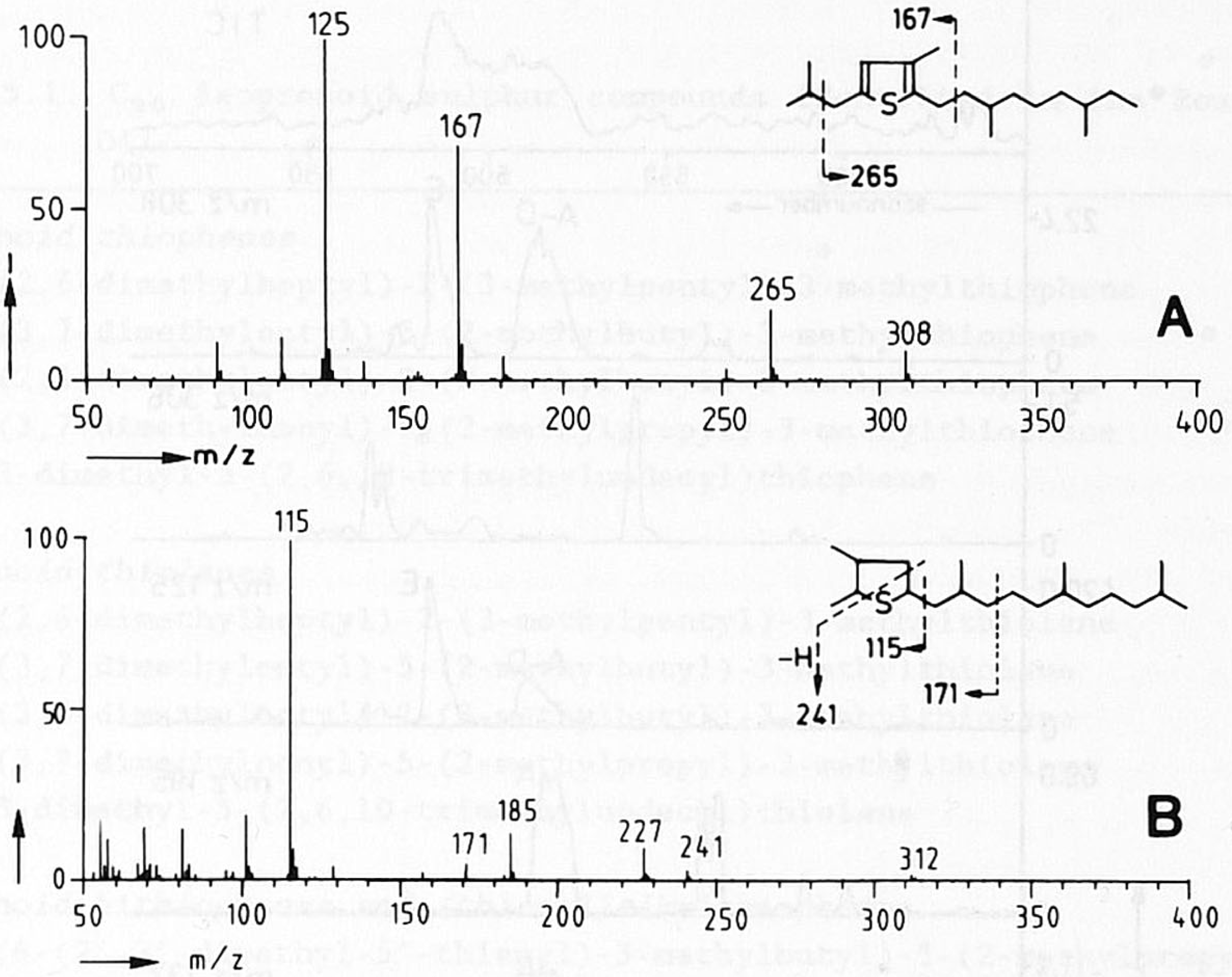


Fig. 5.2. Mass spectra of (A): 2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiophene (D), (B): a mixture of 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiolane (e) and an unknown compound (with major fragment ions at *m/z* 185 and 227 in its mass spectrum).

Appropriate mass chromatography using the *m/z* values of ions generated by characteristic β-cleavages clearly shows the presence of the major isoprenoid thiophenes (Fig. 5.3). They are listed in Table 5.1 and the structures tentatively identified are presented in Schemes 5.2 and 5.3.

The isoprenoid thiolanes were present in RPO-1 through RPO-4. The identifications of these thiolanes are based on comparison of mass spectra with those of synthesized standards (Sinninghe Damsté *et al.*, 1986; 1987c) and relative retention times with respect to the 2,5-di-*n*-alkylthiolane series. It is noteworthy that the retention behaviour on the alumina column is dependent on the position of the thiolane ring in the molecule; isoprenoid thiolanes with the thiolane ring at the end of the chain elute later than those possessing the thiolane ring in the centre of the molecule. A typical mass spectrum of 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiolane (e), is shown in Fig. 2B. The mass spectrum of this compound, exhibiting fragment ions at *m/z* 115, 171, 237 and 308, is completely analogous with that of 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane as reported by Sinninghe Damsté *et al.* (1986).

In fractions RPO-5 and -6 four new series of isoprenoid sulphur compounds were encountered; bithiophenes (III-V), (thienyl)alkylthiophenes (I-II),

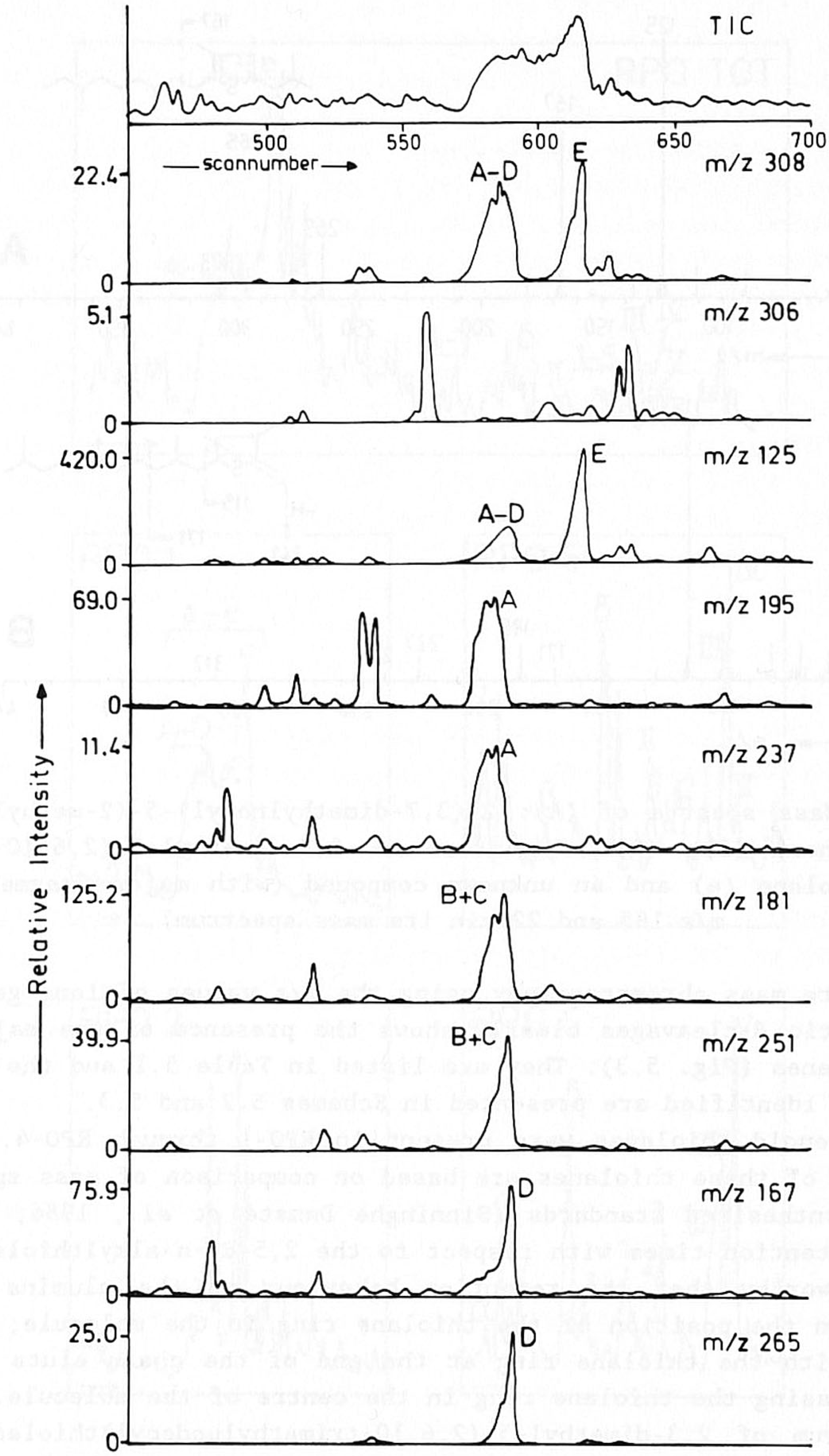


Fig. 5.3. Partial mass chromatograms of *m/z* 308, 306, 125, 195, 237, 181, 251, 167 and 265 and the partial total ion current trace (TIC) of fraction 1 of the "aromatic" fraction of the Rozel Point Oil (RPO 1). Symbols refer to compounds assigned in Table 5.1. The mass chromatogram of m/z *306* reveals the presence of several C_{20} isoprenoid alkenylthiophenes.

Table 5.1. C_{20} isoprenoid sulphur compounds identified in the Rozel Point Oil.

isoprenoid thiophenes	
A	5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3-methylthiophene
B	2-(3,7-dimethyloctyl)-5-(2-methylbutyl)-3-methylthiophene
C	5-(2,6-dimethyloctyl)-2-(3-methylbutyl)-3-methylthiophene
D	2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiophene
E	2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene
isoprenoid thiolanes	
a	5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3-methylthiolane
b	2-(3,7-dimethyloctyl)-5-(2-methylbutyl)-3-methylthiolane
c	5-(2,6-dimethyloctyl)-2-(3-methylbutyl)-3-methylthiolane
d	2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiolane
e	2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiolane
isoprenoid bithiophenes and (thienyl)alkylthiophenes	
I	2-(4-(2′,3′-dimethyl-5′-thienyl)-3-methylbutyl)-5-(2-methylpropyl)-3-methylthiophene
II	5-(3-(2′,3′-dimethyl-5′-thienyl)-2-methylpropyl)-2-(3-methylbutyl)-3-methylthiophene
III	5′-(3-methylpentyl)-5-(2-methylpropyl)-3,4′-dimethyl-2,2′-bithiophene
IV	5-(2-methylbutyl)-5′-(3-methylbutyl)-3,4′-dimethyl-2,2′-bithiophene
V	5-(2,6-dimethylheptyl)-3,4′,5′-trimethyl-2,2′-bithiophene
isoprenoid thienylthiolanes	
1	5-(5′-(2-methylbutyl)-3′-methyl-2′-thienyl)-2-(3-methylbutyl)-3-methylthiolane
2	2-(2′-(3-methylbutyl)-3′-methyl-5′-thienyl)-5-(2-methylbutyl)-3-methylthiolane
3	2-(2′-(3-methylpentyl)-3′-methyl-5′-thienyl)-5-(2-methylpropyl)-3-methylthiolane
4	5-(2,6-dimethylheptyl)-2-(2′,3′-dimethyl-5′-thienyl)-3-methylthiolane
5	isomer of 4
isoprenoid benzothiophenes	
α	4-(2,6-dimethylheptyl)-2,3,6-trimethylbenzo[*b*]thiophene
β	4-(2-methylbutyl)-2-(3-methylbutyl)-3,6-dimethylbenzo[*b*]thiophene
γ	2-(3-methylpentyl)-4-(2-methylpropyl)-3,6-dimethylbenzo[*b*]thiophene
δ	2-(3,7-dimethyloctyl)-3,6-dimethylbenzo[*b*]thiophene

benzothiophenes (α–δ) and thienylthiolanes (1-5). The assignment of the structures of these compounds is based on mass spectral data, GC-retention times and on the differences in response of flame ionization and flame photometric detection. Fig. 5.4, for example, shows the partial GC-FID and GC-FPD traces of fraction RPO-6. It is clearly seen that some compounds (*i.e.* 1, 2, III, IV and V) have a much higher response with FPD detection than is expected from the FID-trace if only one sulphur atom is present. Such an enhanced response on the FPD therefore indicates the presence of more than one sulphur atom. From a comparison of the ratio β/1 and γ/2 in the FID and FPD chromatogram, bearing in mind the quadratic response of the FPD, it was estimated that the compounds 1 and 2 contain two sulphur atoms per molecule. The relative intensity of the M+2 isotope peak in the mass spectra of the bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes (see Fig 5.5A-C) of about 10% of the intensity of the molecular ion, indicating the presence of two sulphur atoms, also supports these iden-

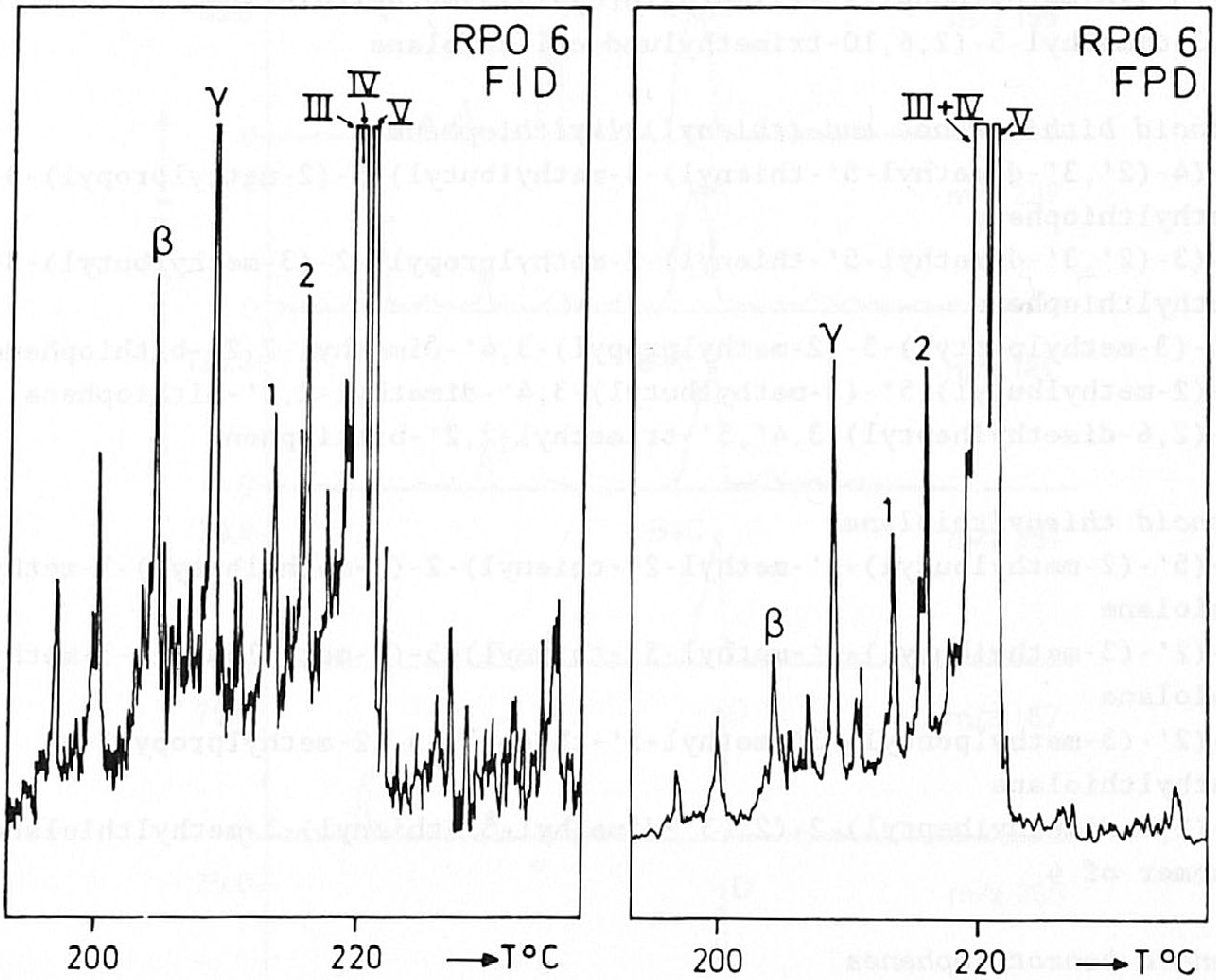
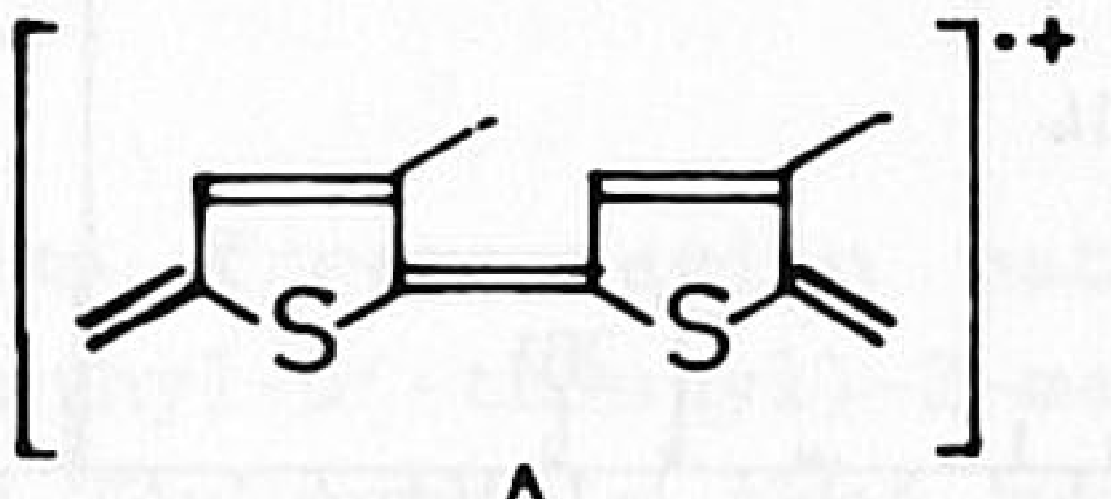


Fig. 5.4. Partial GC-FID (left) and GC-FPD (right) traces of fraction 6 of the "aromatic" fraction of the Rozel Point Oil (RPO-6). Symbols refer to compounds assigned in Table 5.1. Note the differences in the β/1 and γ/2 ratio between the two traces. These differences in response on the FID and FPD strongly support the identification of the bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes.

tifications. Exact mass measurements of the molecular ion (*m/z* 334) of the bithiophenes and a fragment ion of the bithiophenes III and IV (*m/z* 220) with high resolution mass spectrometry (exact mass: found 334.173, calculated for $C_{20}H_{30}S_2$ 334.179; exact mass: found 220.037, calculated for $C_{12}H_{12}S_2$ 220.038) established the presence of two sulphur atoms per molecule in these bithiophenes.

Typical mass spectra representing these four new series of isoprenoid sulphur compounds are shown in Figs. 5.5A-D.

Fig. 5A shows the mass spectrum of a bithiophene, 5'-(3-methylpentyl)-5-(2-methylpropyl)-3,4'-dimethyl-2,2'-bithiophene (III). This mass spectrum is characterised by a molecular ion (*m/z* 334), two primary fragments (*m/z* 291 and 263) originating from *β*-cleavages of both side chains and a fragment ion at *m/z* 220. This last fragment ion cannot be formed directly from the molecular ion because that should imply loss of the saturated C_8H_{18} since the exact mass measurements of *m/z* 220 showed that this fragment ion still contains two sulphur atoms. Therefore it is assumed that this highly aromatic ion ($C_{12}H_{12}S_2$; note also the double charged ion at *m/z* 110 in the mass spectrum) originates from the two primary fragments (*m/z* 291 and 263) due to loss of the other alkyl side chain as an alkyl radical. Although this is an unusual fragmentation, its formation is explained by the stability of the structure of the fully conjugated ion A. A similar process is observed in the mass spectral fragmentation pattern of 1,4-di-*t*-butylbenzene (van de Graaf, 1979) where a loss of two CH_3-radicals leads to the fully conjugated ion B.


A

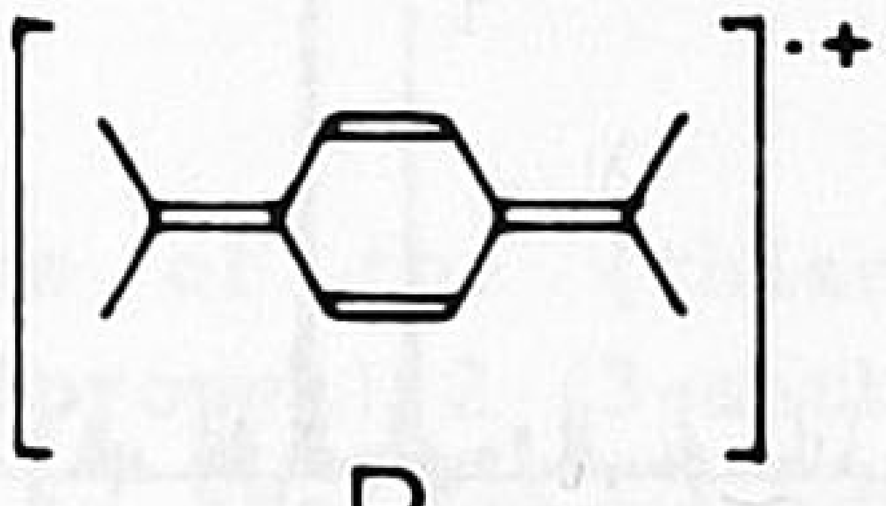

B

Fig. 5.5B shows the mass spectrum of a (thienyl)alkylthiophene, 5-(3-(2',3'-dimethyl-5'-thienyl)-2-methylpropyl)-2-(3-methylpropyl)-3-methylthiophene (II). The mass spectral fragmentation pattern of this compound is outlined in Scheme 1.

Fig. 5.5C shows the mass spectrum of a thienylthiolane. Apart from the molecular ion at *m/z* 338, two fragment ions at *m/z* 267 and 281, which originate from cleavages of the alkyl side chains at the thiophene and the thiolane moieties in the molecule, give clues to the identity of these compounds. The spectrum, however, is not assigned to a particular compound because three of the five thienylthiolanes present (1,2 and 3) explain the fragment ions at m/z 267 and 281. Four rearrangement ions (*m/z* 144, 156, 200 and 214) are also observed in this mass spectrum.

Fig. 5.5D shows the mass spectrum of a typical isoprenoid benzothiophene, 2-(3,7-dimethyloctyl)-3,6-dimethylbenzo[*b*]thiophene (*δ*). *β*-Cleavage leads to the base peak in the mass spectrum at *m/z* 175. The alkyl side chain of this compound is thought to be branched because of its relative GC retention time

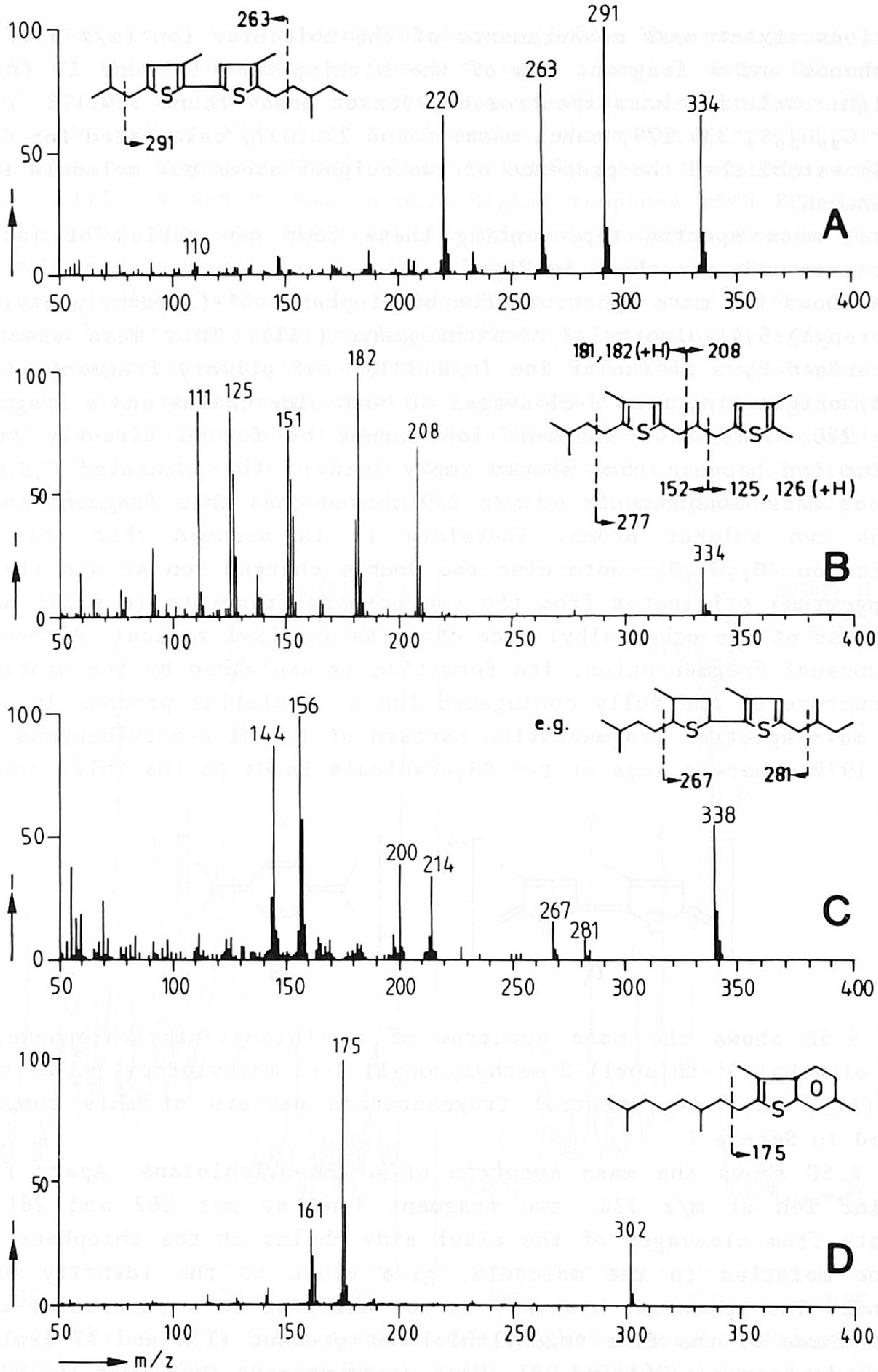
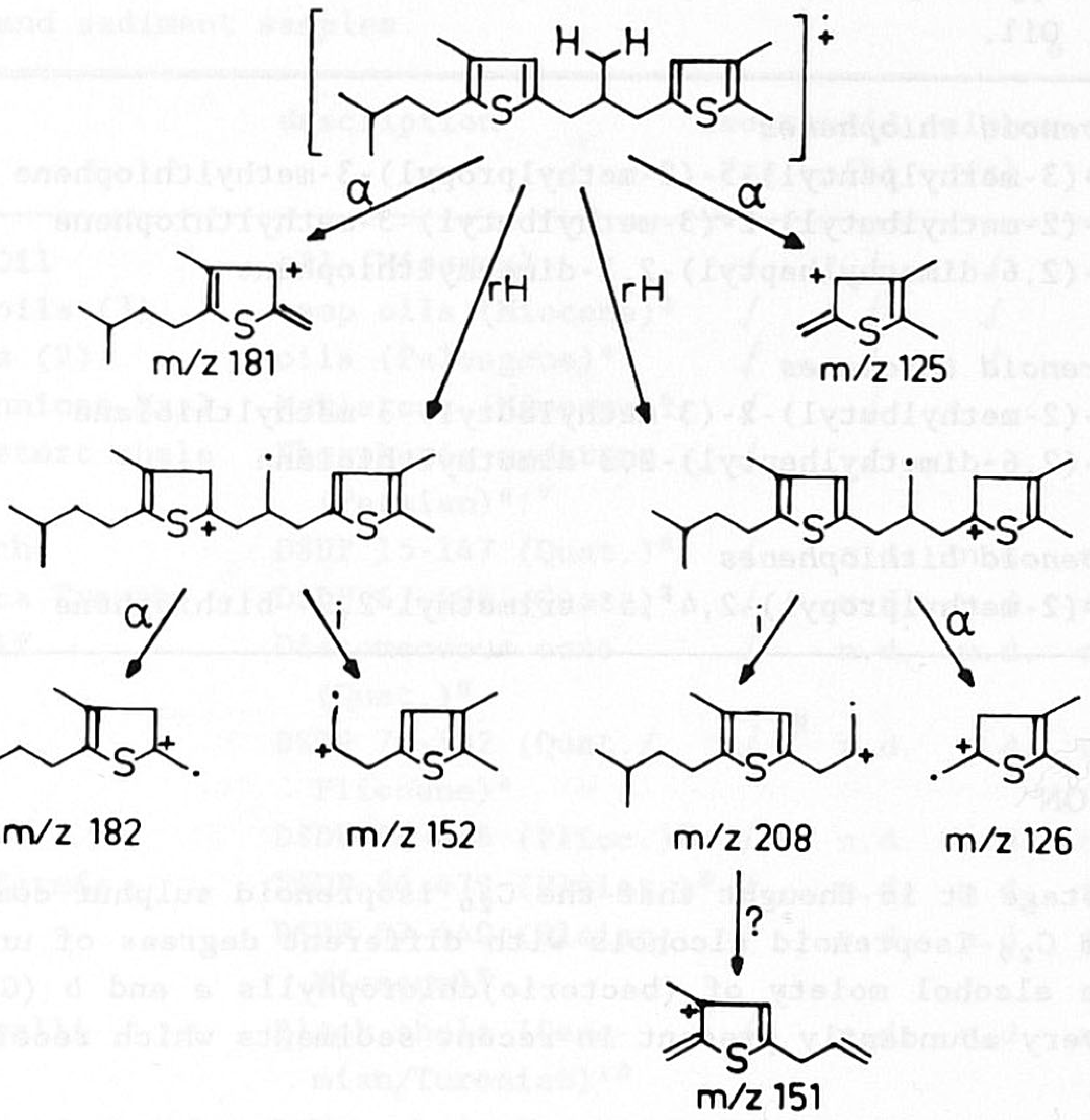


Fig. 5.5. Mass spectra corrected for background of (A): 5'-(3-methylpentyl)-5-(2-methylpropyl)-3,4'-dimethyl-2,2'-bithiophene (III), (B): 5-(3-(2',3'-dimethyl-5'-thienyl)-2-methylpropyl)-2-(3-methylbutyl)-3-methylthiophene (II), (C): a thienyl thiolane (3) and (D): 2-(3,7-dimethyloctyl)-3,6-dimethylbenzo[*b*]thiophene (*6*).



Scheme 5.1. Some fragmentation pathways of the (thienyl)alkylthiophene, 5-(3-(2′,3′-dimethyl-5′-thienyl)-2-methylpropyl)-2-(3-methylbutyl)-3-methylthiophene (II). The symbols that refer to the fragmentation reactions are adopted from McLafferty (1973).

with respect to the 4-*n*-alkyl-2-ethylbenzo[*b*]thiophene series (Sinninghe Damsté *et al.*, 1987c).

Several C_{15} isoprenoid sulphur compounds were also encountered in the Rozel Point Oil. They are listed in Table 5.2.

Although the results reported here focus on the C_{20} and C_{15} isoprenoid sulphur compounds occurring in Rozel Point Oil, most of the identified compounds were also present in other oils from China and from Sicily (Italy) and in extracts from recent and ancient sediments. In Table 5.3 an overview of the samples investigated and the presence of C_{20} isoprenoid sulphur compounds in these samples is given. The first four samples or their source rocks (in case of oils) originate from hypersaline environments.

Table 5.2. C_{15} isoprenoid sulphur compounds identified in the Rozel Point Oil.

isoprenoid thiophenes	
F	2-(3-methylpentyl)-5-(2-methylpropyl)-3-methylthiophene
G	5-(2-methylbutyl)-2-(3-methylbutyl)-3-methylthiophene
H	5-(2,6-dimethylheptyl)-2,3-dimethylthiophene
isoprenoid thiolanes	
g	5-(2-methylbutyl)-2-(3-methylbutyl)-3-methylthiolane
h	5-(2,6-dimethylheptyl)-2,3-dimethylthiolane
isoprenoid bithiophenes	
VI	5-(2-methylpropyl)-2,4',5'-trimethyl-2,2'-bithiophene

5.5 DISCUSSION

At this stage it is thought that the C_{20} isoprenoid sulphur compounds originate from C_{20} isoprenoid alcohols with different degrees of unsaturation. Phytol, the alcohol moiety of (bacterio)chlorophylls *a* and *b* (Gloe *et al.*, 1975), is very abundantly present in recent sediments which receive an input from algae.

Especiallly photosynthetic green and purple sulphur bacteria, omnipresent and sometimes abundantly present in evaporitic environments, are known to biosynthesize di- and polyunsaturated isoprenoid alcohols such as geranylgeraniol, $\Delta^{2',10}$- and $\Delta^{2',6}$-phytadienol as alcohol moieties of bacteriochlorophylls (Katz *et al.*, 1972; Gloe and Pfennig, 1974; Caple *et al.*, 1978; Steiner *et al.*, 1981). Geranylgeraniol has also been reported to occur as such in archaebacteria (Kuswaha and Kates, 1978). These C_{20} isoprenoid alcohols have been encountered in relatively high concentrations in very recent hypersaline environments like Solar Lake (Boon and de Leeuw, 1987), the Gavish Sabkha (de Leeuw *et al.*, 1985) and Roquetas de Mar (Goossens, unpublished results). Therefore an origin from these precursors is suggested. The reaction of these precursor molecules or their diagenetical products such as unsaturated phytenes might result in the formation of a number of isoprenoid thiophenes (A-E) as indicated in Scheme 5.2. These kind of sulphur incorporation reactions are postulated to occur in recent sediments to explain the presence of several isoprenoid thiophenes (Brassell *et al.*, 1986) and of a thiophene containing hopane (Valisolalao *et al.*, 1984). Whether or not the corresponding isoprenoid thiolanes (a-e) are intermediate structures in the thiophene formation remains to be seen (Scheme 5.3). A further incorporation of sulphur triggered by the presence of additional double bonds might result in the formation of bithiophenes (III-V) and (thienyl)alkylthiophenes (I and II). Supporting evidence for this dia-

Table 5.3. Occurrence of C_{20} isoprenoid sulphur compounds in several oils and sediment samples.

sample	description[1]	isoprenoid sulphur compounds[2]				
		(a)	(b)	(c)	(d)	(e)
Rozel Point Oil	oil (Miocene)	✓	✓	✓	✓	✓
Sicily Seep oils (3)	seep oils (Miocene)[3]	✓	✓	✓	✓	✓
Jianghan oils (2)	oils (Paleogene)[4]	✓	✓	✓	✓	✓
Northern Apennines Marl	Marlstone (Miocene)[5]	✓	✓	-	-	-
Phosphoria Retort shale	Phosphatic mudstone (Permian)[6,7]	✓	✓	-	-	✓
Cariaco Trench	DSDP 15-147 (Quat.)[8]	✓	n.d.	n.d.	n.d.	n.d.
Middle America Trench	DSDP 67-496 (Quat.)[8]	✓	n.d.	n.d.	n.d.	n.d.
Namibian Shelf	Diatomeceous ooze (Quat.)[9]	✓	n.d.	n.d.	n.d.	n.d.
Walvis Ridge	DSDP 75-532 (Quat./Pliocene)[8]	✓	n.d.	n.d.	n.d.	n.d.
Japan Trench	DSDP 56-436 (Plioc.)[8]	✓	n.d.	n.d.	n.d.	n.d.
Gulf of California	DSDP 64-479 (Pleist.)[8]	✓	n.d.	n.d.	n.d.	n.d.
Japan Trench	DSDP 57-440 (Pleist. Miocene)[8]	✓	n.d.	n.d.	n.d.	n.d.
Livello Bonarelli	Black shale (Cenomian/Turonian)[10]	✓	n.d.	n.d.	n.d.	n.d.

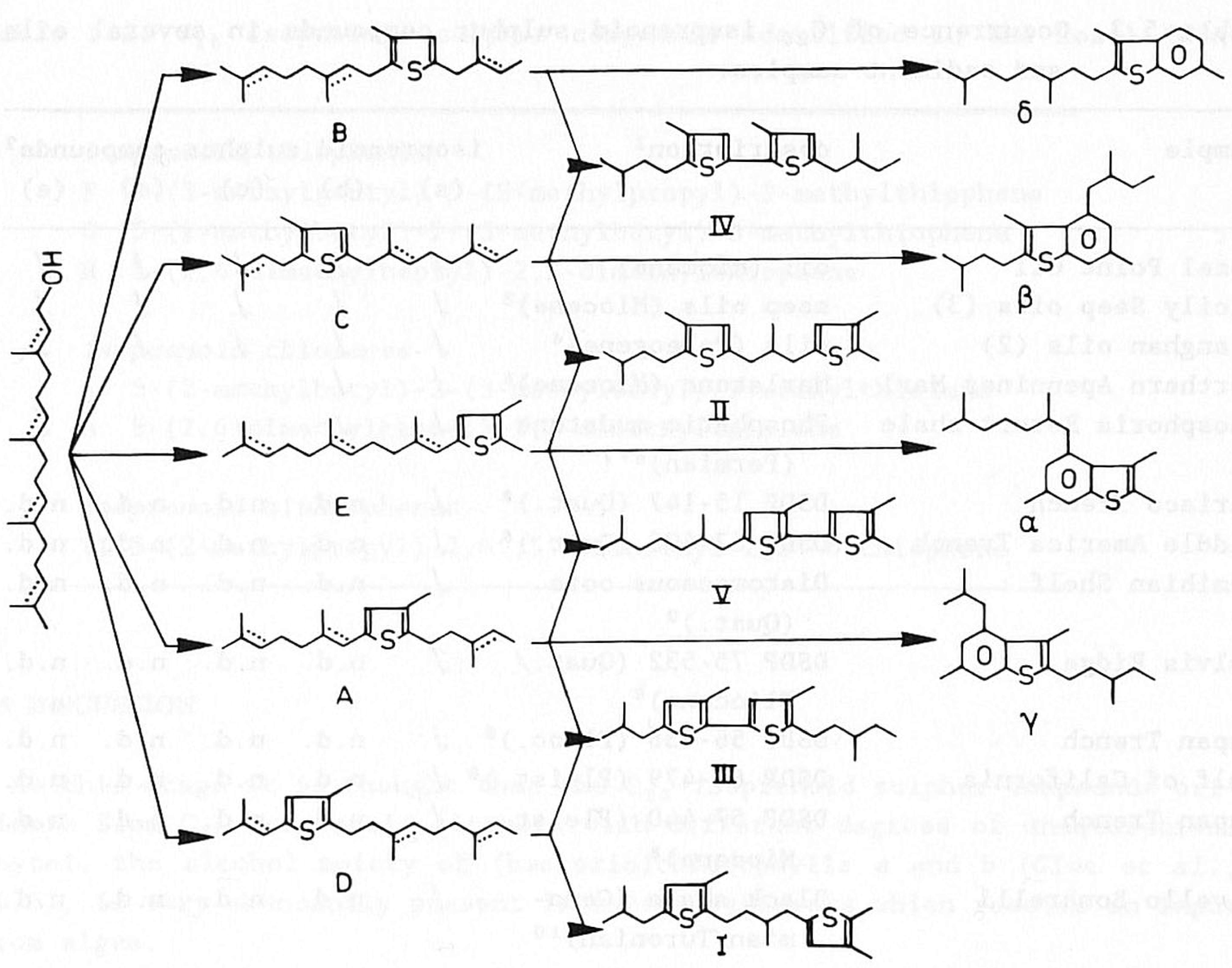
[1] Quat. = Quaternary, Plioc. = Pliocene, Pleist. = Pleistocene, DSDP = Deep Sea Drilling Project (Leg, Site)

[2] (a) thiophenes, (b) thiolanes, (c) bithiophenes, (d) thienylthiolanes, (e) benzothiophenes

[3] Palmer and Zumberge (1981), [4] Fu Jiamo *et al.* (1986), [5] ten Haven *et al.* (1985), [6] Winters *et al.* (1982), [7] Lewan (pers. commun.), [8] Brassell *et al.* (1986c), [9] Klok (1984), [10] van Graas (1982)

genetical pathway is the presence of several alkenylthiophenes in RPO-1 as revealed by the m/z 306 mass chromatogram in Fig. 5.3. Here too, it remains to be seen whether the corresponding thienylthiolanes are intermediates. Another possibility for subsequent reactions of the unsaturated isoprenoid thiophenes is the formation of the corresponding C_{20} isoprenoid benzothiophenes (α-δ) (Scheme 5.2).

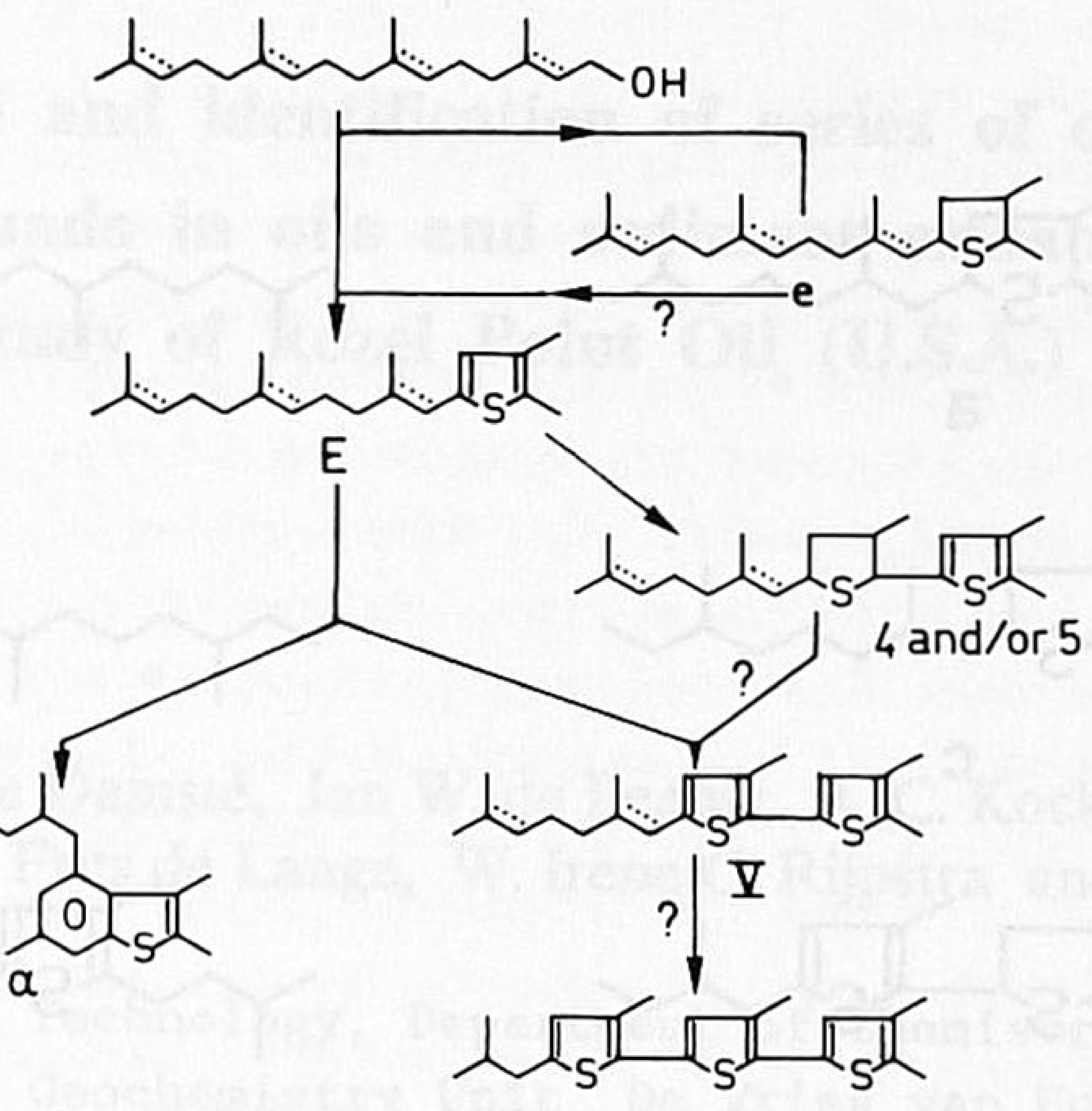
Further circumstantial evidence for this diagenetical scheme is obtained from data concerning C_{15} isoprenoid sulphur compounds. Farnesol (2,6,10-trimethyldodecatrienol) has been reported as the alcohol moiety of bacteriochlorophylls *c*, *d* and *e* (Gloe *et al.*, 1975) and those of green photosynthetic bacteria (Rapoport and Hamlow, 1961; Katz *et al.*, 1972; Caple *et al.*, 1978) and has been identified, although in much lesser abundance as the C_{20}



Scheme 5.2. Diagenetical scheme showing the possible origin and presumed pathways of several organic sulphur compounds encountered in several oils and sediment extracts starting from unsaturated phytanols. The symbols refer to the compounds assigned in Table 5.1. Dotted lines in the structures indicate the possible presence of double bonds. Symbols A-E refer to the structures with saturated alkyl side chain(s).

isoprenoid alcohols, in the microbial mat of the Gavish Sabkha (de Leeuw *et al.*, 1985). In the playa lake environment in which the source rock of the Rozel Point Oil is thought to be deposited (Meissner *et al.*, 1984; ten Haven, 1987), an input of farnesol seems highly probable. Therefore, analogous to the diagenetical pathways of the C_{20} isoprenoid alcohols, C_{15} isoprenoid sulphur compounds were expected from sulphur incorporation into farne-sol. Indeed, although much less abundant, some C_{15} isoprenoid sulphur compounds were identified (Table 5.2) in Rozel Point Oil. In addition, an C_{15} isoprenoid alkenylthiophene was encountered.

It can be speculated that an additional thiophene ring is formed upon further sulphur incorporation into bithiophene V (Scheme 5.3). The benzothiophenes and bi- and trithiophenes may upon further cyclisation give rise to the highly aromatic, one to three sulphur atoms containing compounds reported to occur in several crude oils (Coleman *et al.*, 1973; Dooley *et*



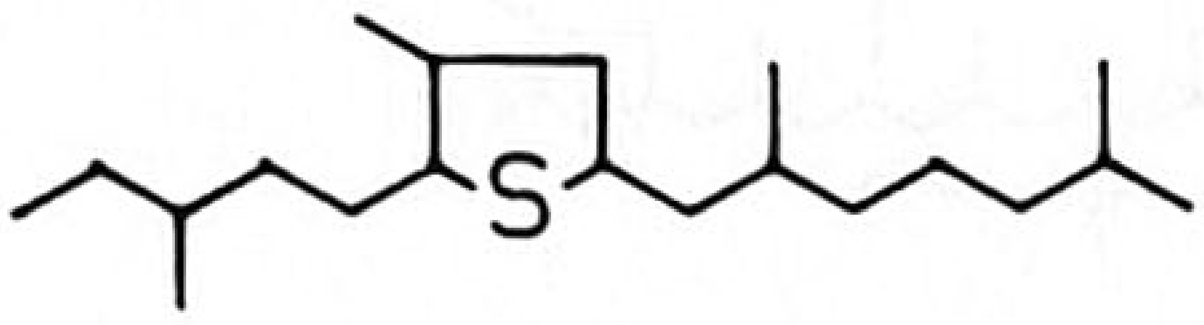
Scheme 5.3. Part of the diagenetical scheme proposed for OSC, showing the presumed role of thiolanes and thienylthiolanes as intermediates. The symbols refer to the compounds assigned in Table 5.1. Symbols refer to the structures with a saturated alkyl side chain.

al., 1974).

To summarize we can conclude that C_{20} isoprenoid sulphur compounds of types described here do occur in both recent and ancient sediments and in crude oils. Hence, in sediments anaerobically laid down or of evaporitic nature these compounds are naturally formed. These types of depositional environments show their signature in the composition of the OSC. In hypersaline environments relatively high concentrations of polyunsaturated phytanols show up, which lead to the formation of "midchain" thiophenes (A-D), -thiolanes (a-d) and two sulphur atoms containing OSC (1-5, I-V). In anaerobically deposited sediments (not hypersaline) no polyunsaturated phytanols occur and only the formation of OSC that originate from sulphur incorporation into phytol (E, e) is expected.

Erosion of more ancient sediments in the hinterland or human caused oil spills are other pathways by which the C_{20} and C_{15} isoprenoid sulphur compounds compounds can contribute to recent sediments.

APPENDIX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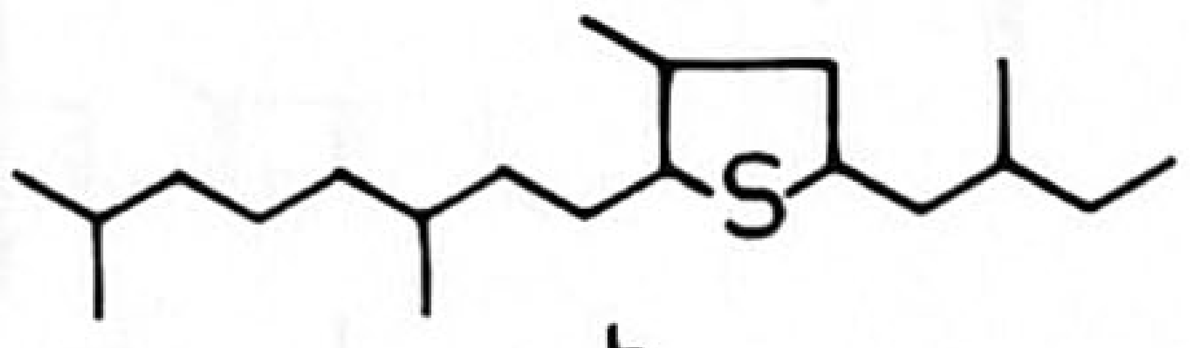
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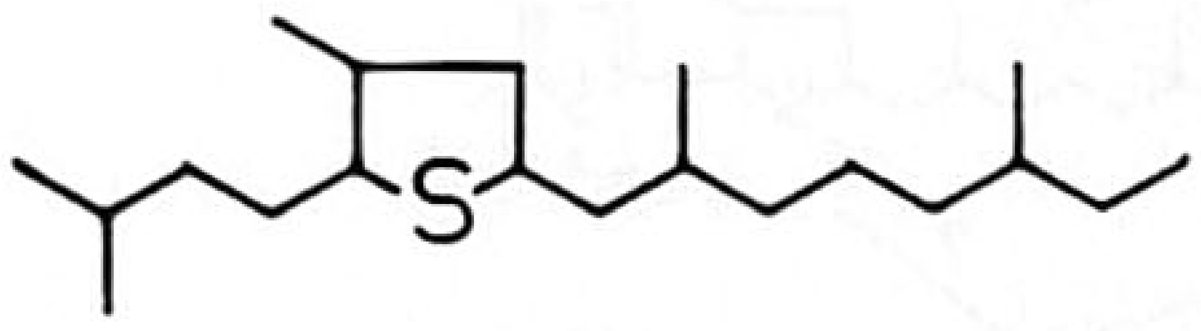
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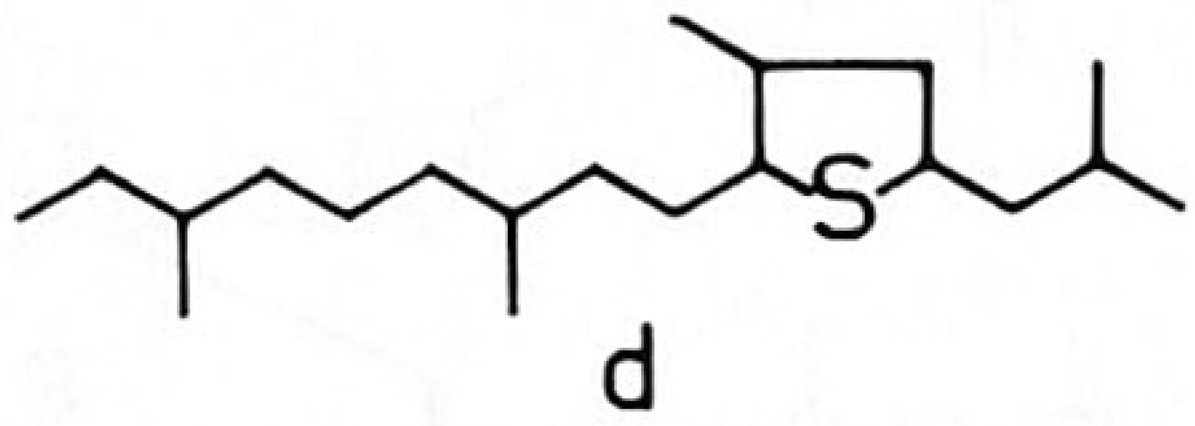
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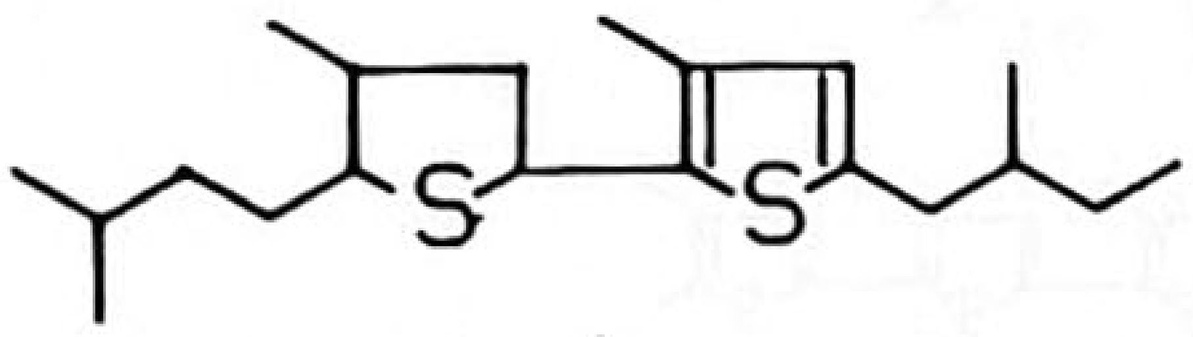
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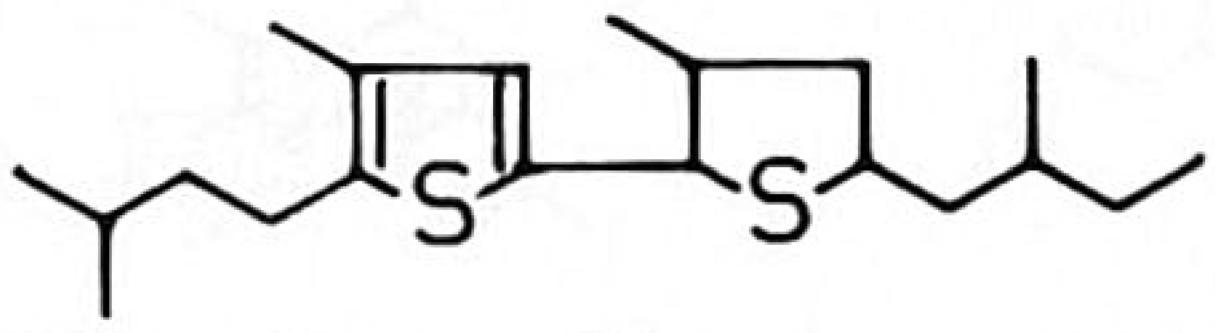
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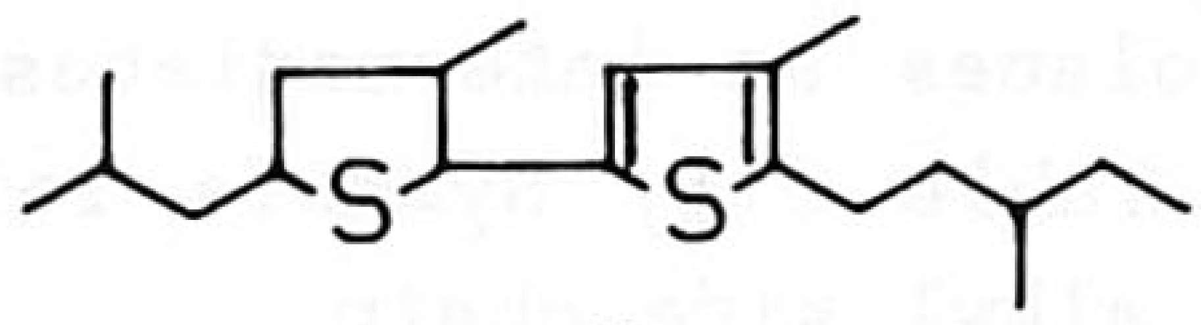
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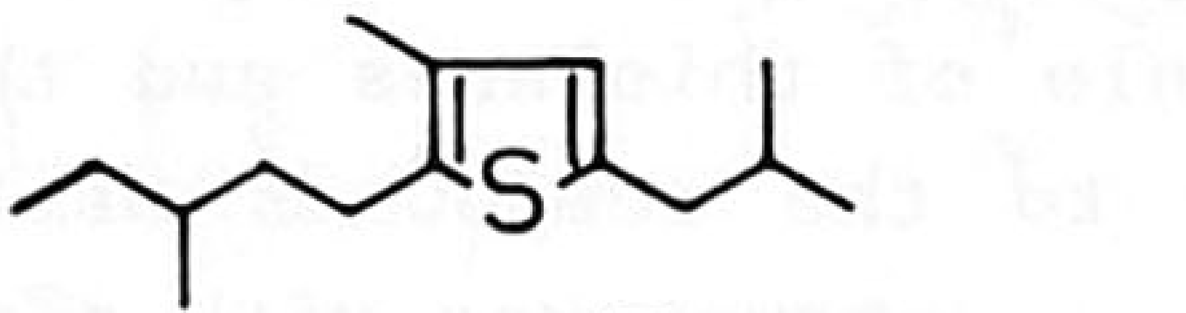
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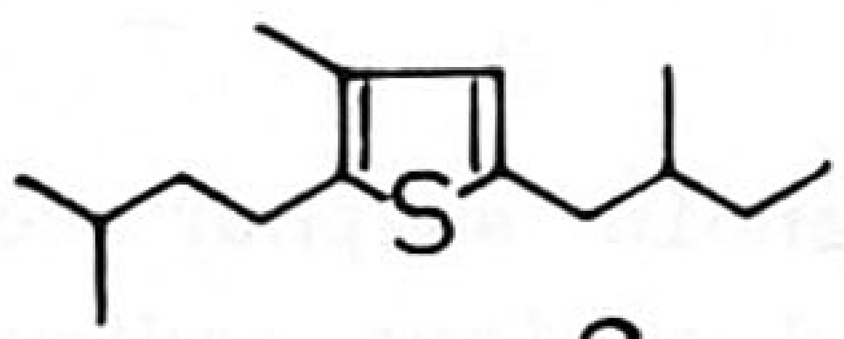
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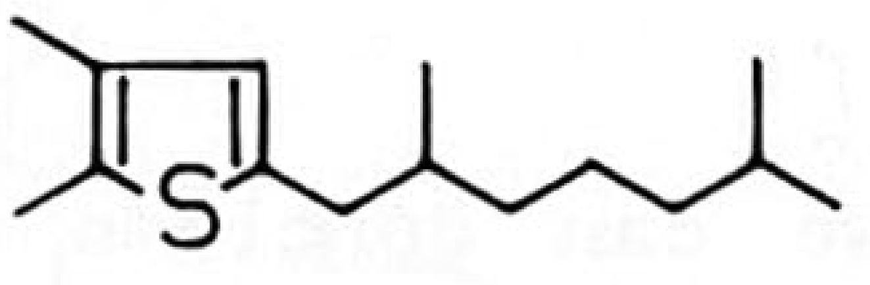
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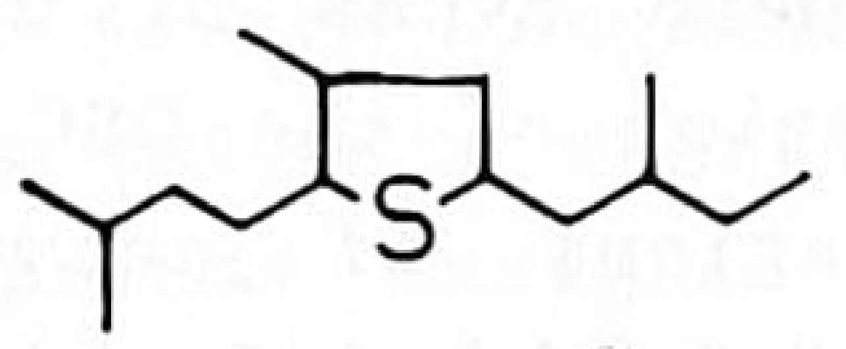
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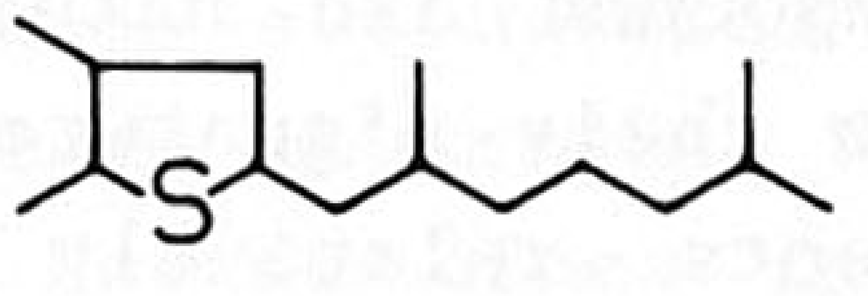
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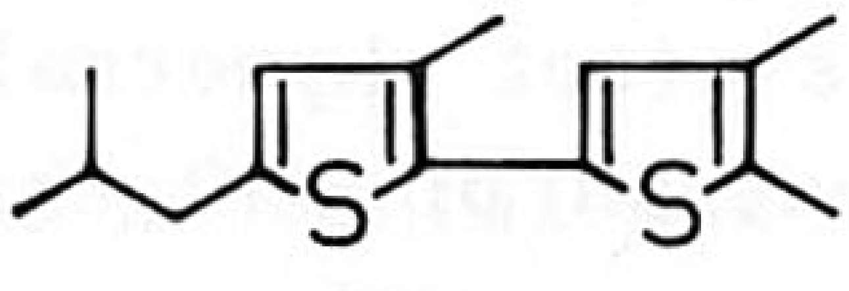
VI

6. The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts I. A study of Rozel Point Oil (U.S.A.)*

Jaap S. Sinninghe Damsté, Jan W. de Leeuw, A. C. Kock-van Dalen,
Martin A. de Zeeuw, Frits de Lange, W. Irene C. Rijpstra and P.A. Schenck

Delft University of Technology, Department of Chemistry and Chemical
Engineering, Organic Geochemistry Unit, De Vries van Heystplantsoen 2,
2628 RZ Delft, The Netherlands.

6.1 ABSTRACT

A number of novel series of organic sulphur compounds (OSC) have been identified in several oils and sediment extracts including Rozel Point Oil (Box Elder County, Utah, USA; Miocene). This oil contains these series in abundance, and the OSC were therefore studied in detail. The "aromatic" fraction was fractionated using column chromatography, and the fractions obtained were analysed by GC with simultaneous FID and FPD detection and by GC-MS. Series of isoprenoid thiophenes, isoprenoid thiolanes, isoprenoid bithiophenes, isoprenoid thienylthiolanes, isoprenoid benzothiophenes, thiophene and thiolane steranes, 2,5-di-*n*-alkylthiolanes, 2,6-di-*n*-alkylthianes and 2,4-di-*n*-alkylbenzo[*b*]thiophenes have been identified. The identifications were based on chromatographic and mass spectral data, response on the FPD, carbon skeleton determination by desulphurisation and in some cases on mass spectral data from, and coinjections with, synthetic compounds. Together, almost 1000 OSC have been identified in the "aromatic" fraction of this oil. Because the carbon skeletons of the OSC identified have the same structures as the well known hydrocarbons from geological materials (isoprenoid hydrocarbons, steranes, *n*-alkanes) these compounds are thought to be products of sulphur incorporation reactions into specific precursors.

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6.2 INTRODUCTION

In several oils and sediments organic sulphur compounds (OSC) are present in abundance. This abundance contrasts with the relatively low concentration of organically-bound sulphur in the biomass, the precursor of these geological materials. Recently we have reported that this enrichment in OSC correlates with a hypersaline environment of deposition of the sediment or source rock (Sinninghe Damsté *et al.*, 1986; ten Haven *et al.*, 1988). This correlation should therefore also hold to a large extent for the source rock of Rozel Point Oil (RPO), an oil from the north-western part of Utah, USA. Meissner *et al.* (1984) reported that the organic matter of the source rock of RPO was originally deposited in a playa lake environment. The structures and distribution patterns of the hydrocarbons present in the oil, as reported by ten Haven *et al.* (1988), are thought to be characteristic for a hypersaline environment of deposition, *e.g.* even-over-odd carbon number predominance of the *n*-alkanes, low pristane/phytane ratio (<0.1) and the presence of a series of extended hopanes and hopenes maximizing at C_{35} (ten Haven *et al.*, 1985, 1988).

The extremely high amount of organic sulphur in RPO (14 %) has been reported by Thompson (1981). This enabled us to study in detail the structures of the OSC. Since the same series have been encountered in several other oils and sediment extracts, it was thought that the identification of the individual structures might help us to understand their origin. Once the origin is understood their application as markers for several environmental settings and diagenesis can be evaluated. A preliminary report on several isoprenoid OSC in RPO has been published elsewhere (Sinninghe Damsté and de Leeuw, 1987). It should be noted that similar studies on OSC present in another RPO sample and in a French oil have been performed simultaneously by the Organic Geochemistry Unit in Strasbourg, France (Schmid, 1986; Schmid *et al.*, 1987).

6.3 EXPERIMENTAL

Sample. The Rozel Point Oil field is located in Box Elder County, Utah (USA). Various oil occurrences are known in this area, including seeps and reservoirs at shallow depths, which produce heavy oils with an unusually high sulphur content up to 14 percent by weight (Utah Geological and Mineralogical Survey, pers. commun.).

The sample described here (West Rozel #2) is from an Amoco wildcat well (T8N, R8W, section 15) in the great Salt Lake. This oil is a well sample from fractured Tertiary basalt at a depth interval between 810 and 825 m. Bulk properties include an API gravity of 4.8, a sulphur content of 7.5 weight percent and a pour point of 24°C (M.D. Lewan, pers. commun.).

Fractionation. The oil was fractionated by column chromatography as described previously for a sediment extract (Sinninghe Damsté *et al*., 1986). Briefly, the oil (214.8 mg) was separated into "saturated" (3.0 mg), "aromatic" (68.4 mg) and "polar" compounds (94.0 mg) using pentane, toluene and toluene/methanol (1:1) as eluents. The "aromatic" fraction (≈ 50 mg) was further separated on an alumina column (25 cm x 1 cm) using hexane (90 ml), hexane/toluene (9:1) (25 ml) and toluene (25 ml) respectively, as eluents. The first six sub-fractions, named RPO 1-6 (Table 6.1), were analysed by gas chromatography (GC) using flame ionization (FID) and flame photometric detection (FPD) and by gas chromatography-mass spectrometry (GC-MS). At a later stage the fractionation of the "aromatic" fraction was scaled up to obtain larger quantities of the various sub-fractions, enabling NMR analysis and desulphurisation experiments. Thus, 3.4 g of the "aromatic" fraction from 7.2 g RPO was fractionated on a column with alumina (60 cm x 3.5 cm) using hexane (1.80 l), hexane/toluene (9:1) (0.72 l) and toluene (0.72 l) as eluents respectively. A large number of sub-fractions was obtained in order to relate more accurately the desulphurisation products with the starting OSC. Where the composition of such a fraction was very similar to that of one of the fractions RPO 1-6, it is referred to as RPO 1'-6'.

The "aromatic" fraction (68.4 mg) was also separated into a so-called low molecular weight "aromatic" fraction (LMWA) (19.0 mg) and a high molecular weight "aromatic" fraction (HMWA) (43.3 mg) on a column (25 cm x 1 cm) of activated (1 h at 150°C) alumina using 50 ml hexane/toluene (9:1) and 50 ml toluene, respectively. The HMWA fraction contained hardly any GC amenable compounds and is thought to consist of asphaltenes.

The isolation procedures were repeated several times for this oil, related samples and synthetic compounds. The recovery and reproducibility were good and no artefact formation was apparent.

Table 6.1. Separation scheme of the "aromatic" fraction.

cut	major compound type(s)	volume(ml)	eluent[1]
prewash	-	20	H
1	isoprenoid thiophenes, isoprenoid thiolanes, thiophene steranes	35	H
2	2,6-di-*n*-alkylthianes	20	H
3	2,6-di-*n*-alkylthianes, 2,5-di-*n*-alkylthiolanes	20	H
4	2,5-di-*n*-alkylthiolanes, thiolane steranes	5	H:T(9:1)
5	2,4-di-*n*-alkylbenzo[*b*]thiophenes, isoprenoid bithiophenes	5	H:T(9:1)
6	isoprenoid bithiophenes, isoprenoid benzo[*b*]thiophenes	5	H:T(9:1)

[1] H = hexane, T = toluene

Synthesis. *cis*-2-Methyl-5-tridecylthiolane and *cis*-2-dodecyl-5-ethylthiolane were obtained previously as side products during the catalytic hydrogenation of the corresponding thiophenes (Sinninghe Damsté *et al.*, 1986). To obtain the *trans* isomers 2-(5-methylthienyl)dodecyl ketone and 2-(5-ethylthienyl) undecyl ketone were subjected to an ionic hydrogenation (Parnes *et al.*, 1977). The alkylthienyl ketones (20 mg) were mixed with 50 μl triethylsilane, and 100 μl of a 3 % solution of BF_3.etherate in trifluoroacetic acid was slowly added at -10°C. The reaction time was 1 h.

2,5-Diheptylthiolane was synthesized as follows: Thiophene was coupled with heptanoic acid in refluxing toluene with P_2O_5 as dehydrating agent. The resulting ketone was reduced to 2-heptylthiophene via a simplified Wolf-Kishner reduction (King and Nord, 1949). 2-Heptylthiophene was subsequently coupled with heptanoic acid in refluxing toluene with P_2O_5 as dehydrating agent. Wolf-Kishner reduction of the resulting ketone afforded 2,5-diheptylthiophene, which was hydrogenated to 2,5-diheptylthiolane using both ionic hydrogenation as well as hydrogenation in ethyl acetate with Pd/C (10%) as catalyst.

3-Methyl-2-(3,7,11-trimethyldodecyl)thiolane was synthesized from the corresponding thiophene, synthesized previously (Brassell *et al.*, 1986c), using both Pd/C (10 %) as well as triethylsilane under the conditions described above.

Raney Ni desulphurisation. Typically, 20 mg of a sub-fraction was dissolved in 3.5 ml abs. ethanol together with 0.5 ml of a suspension (0.4 mg/ml) of Raney Ni (W-6; Bilica and Adkinds, 1955) and heated under reflux for 1.5 h. The desulphurisation products (yield *ca.* 70 %) were isolated by centrifugation and subsequent extraction with diethylether (2.5 ml, x4). The combined extracts were washed (x3) against NaCl-saturated, double-distilled H_2O , dried with $MgSO_4$ and evaporated to dryness using a rotating evaporator at 20°C. The extract was taken up into a small volume of In some cases the desulphurisation products were further separated by preparative thin layer chromatography on silica plates using *n*-hexane as developer. The desulphurisation products were analysed by GC-FID, GC-MS and in some cases with ^{1}H-NMR.

^{1}*H-NMR*. ^{1}H-NMR spectra were measured at 200 MHz with a Nicolet NT 200 WB spectrometer using the FT-technique. $CDCl_3$ and CD_3COCD_3 were used as solvents.

Gas chromatography. GC was carried out on a Varian 3700 gas chromatograph using a DB-5 (30 m x 0.26 mm, film thickness = 0.1 μm) capillary column. The flow was splitted in a ratio 1:4 to the flame ionization and flame photometric detector, respectively. Helium was used as carrier gas. Samples were injected splitless at 70°C. After two minutes the oven temperature was programmed to 130°C at 10°C/min. From 130°C it was programmed at 4°C/min to 300°C.

GC of the desulphurised fractions was carried out on a Carlo Erba 4160 instrument equipped with a flame ionization detector and an on-column injector. A fused silica capillary column (25 m x 0.34 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was used with helium as carrier gas. Samples were injected at 100°C in ethyl acetate, and the oven temperature was programmed to 320°C at 4°C/min.

Gas chromatography-mass spectrometry. GC-MS was carried out on a Varian-MAT 44 instrument. Details of the conditions used have been described previously (Sinninghe Damsté *et al.*, 1986). GC-HRMS was performed on a HP-5890 A gas chromatograph coupled with a VG-70S mass spectrometer at a resolution of 5000. Mass spectra were recorded at 70 eV with a mass range from *m/z* 50 up to *m/z* 500 with a cycle time of 1 s.

6.4 RESULTS

The FPD and FID gas chromatograms of the LMWA fraction of the RPO are shown in Fig. 6.1. This fraction contained most of the GC-amenable compounds of the "aromatic" fraction and all compounds discussed hereafter. The letters correspond to the classes of compounds in Table 6.2. The numbers indicate the total number of carbon atoms. It is evident that the "aromatic" fraction is very complex and contains virtually only OSC. Therefore, a further separation step was performed. Fig. 6.2 shows the FPD chromatograms of the sub-fractions RPO 1-6 (Table 6.1). The peak indications also correspond with those given in Table 6.2. In Table 6.2 a summary of the range of occurrence of the various classes of compounds and the chain length distribution of the individual classes within a certain fraction is also given.

Isoprenoid thiophenes

A series of isoprenoid thiophenes (alkylthiophenes with an isoprenoidal carbon skeleton) have been identified on the basis of mass spectral data and relative retention times of the compounds and their desulphurised products, and by comparison of mass spectra and retention times with those of synthetic compounds (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1987a). The isoprenoid thiophenes showed up in fraction RPO 1 (Fig. 6.2). Their distribution is exemplified by Fig. 6.3, which shows a mass chromatogram of *m/z* 125, a frequently observed ion in the mass spectra of isoprenoid thiophenes. The major members are the C_{20} thiophenes I-V (see Appendix for structures and names) as described elsewhere (Sinninghe Damsté and de Leeuw, 1987). The GC-HRMS data supported these identifications (Table 6.3). Additional evidence for the assignments was obtained by Raney Ni desulphurisation of particular fractions of the RPO which contained these C_{20} isoprenoid thiophenes as primary constituents: a mixture of hydrocarbons

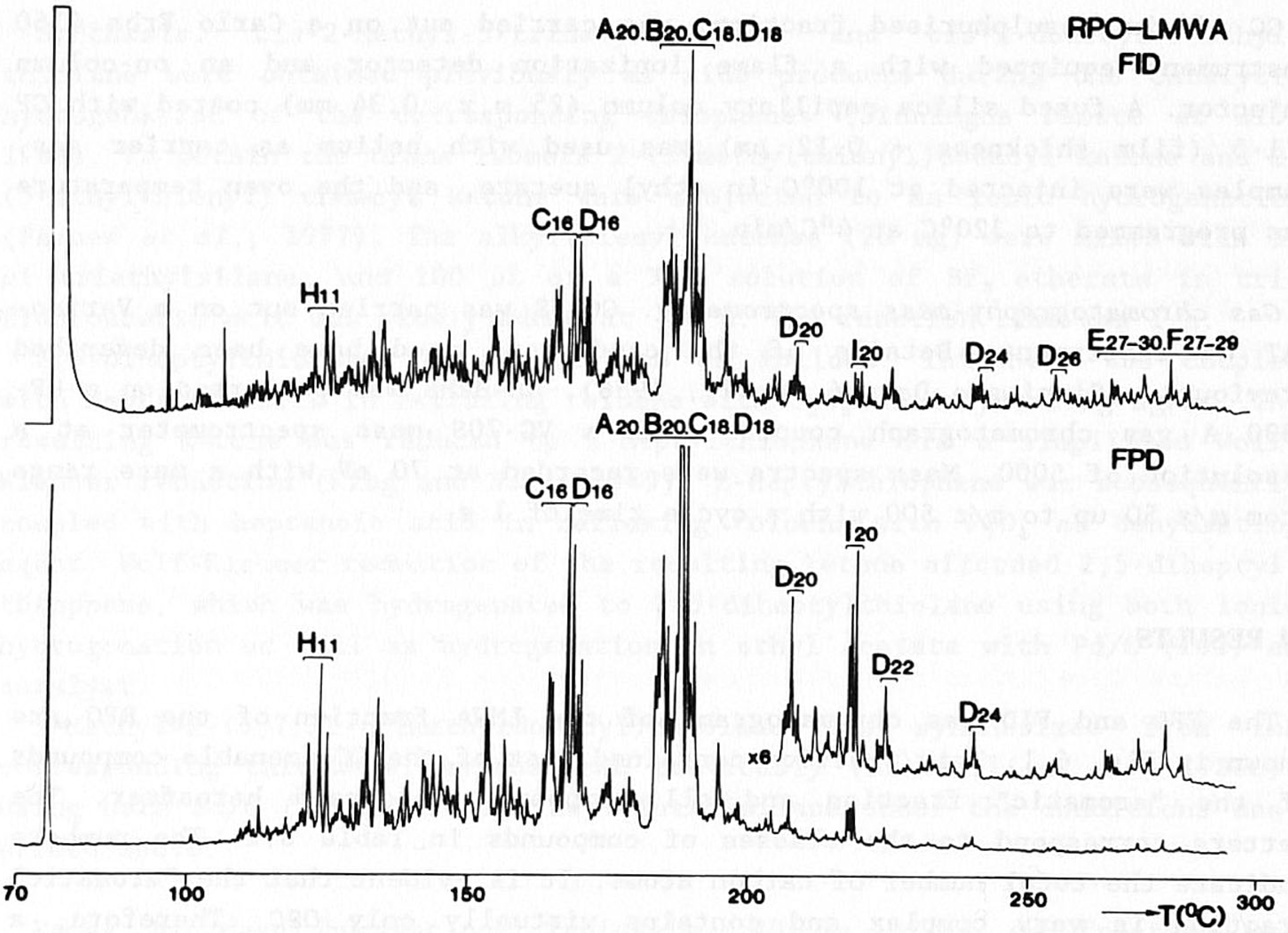


Fig. 6.1. FID (upper trace) and FPD (lower trace) chromatogram of the low molecular weight "aromatic" fraction of RPO. The letters correspond to classes listed in Table 6.2. The numbers indicate the total number of carbon atoms of the particular compound class.

dominated by phytane resulted. The occurrence of I in RPO was recently confirmed by coinjection with a synthetic standard (Sinninghe Damsté *et al.*, 1987a).

Three relatively abundant C_{15} thiophenes (VI-VIII) were identified on the basis of mass spectral data, relative retention times and the formation of farnesane upon desulphurisation.

The C_{30} and C_{35} thiophenes were also relatively abundant. The mass spectra of four C_{30} thiophenes (IX-XII) were characterised by a molecular ion at *m/z* 448 and a presumed secondary fragment at *m/z* 125. In addition, characteristic fragment ions resulting from β-cleavages of the alkyl substituents, *m/z* 167 and 405 (IX), *m/z* 181 and 391 (X), *m/z* 237 and 335 (XI)(Fig. 6.4A) and *m/z* 251, 252 and 321 (XII) were present. Desulphurisation of the mixture yielded only one C_{30} hydrocarbon, squalane.

The rearrangement ion *m/z* 252 in the mass spectrum of XII further supports this skeleton structure. This ion can be explained by a McLafferty rearrangement. The intensity enhancement compared with other isoprenoid thiophenes is thought to be due to abstraction of a tertiary hydrogen from the isoprenoid side chain in the six-membered ring transition state. Such an intensity

Table 6.2. Distribution of OSC over several fractions and within fractions.

Compound class	RPO LMWA	RPO 1	RPO 2	RPO 3	RPO 4	RPO 5	RPO 6
A isoprenoid thiophenes	15-40(20)	15-40(20)	20-21(20)	-	-	-	-
B isoprenoid thiolanes	20	15,20,30(20)	20	20	20	-	-
C 2,6-di-n-alkylthianes	11-28(18)	11-18(16)	13-22(18)	15-26(18)	15-26(18)	-	-
D 2,5-di-n-alkylthiolanes	11-28(18)	-	12-16(14)	14-21(18)	14-27(18)	16-31(24)	22-27(24)
E thiophene steranes	27-30(29)	27-30(29)	27-29(29)	-	-	-	-
F thiolane steranes	27-29(29)	27-29(29)	-	27-29(28)	27-28(28)	27-29	27-29
G isoprenoid benzo[b]thiophenes	20	-	-	-	-	20	20
H 2,4-di-n-alkylbenzo[b]-thiophenes	11-24(12)	-	-	-	-	11-29(12)	11-26(14)
I isoprenoid bithiophenes	15,18-23(20)	-	-	-	-	20	15,18-23(20)
J isoprenoid (thienyl)alkyl-thiophenes	20	-	-	-	-	20	20
K isoprenoid thienylthiolanes	20	-	-	-	-	20	20

Numbers indicate total number of carbon atoms, numbers in parentheses indicate maxima in carbon number distributions.

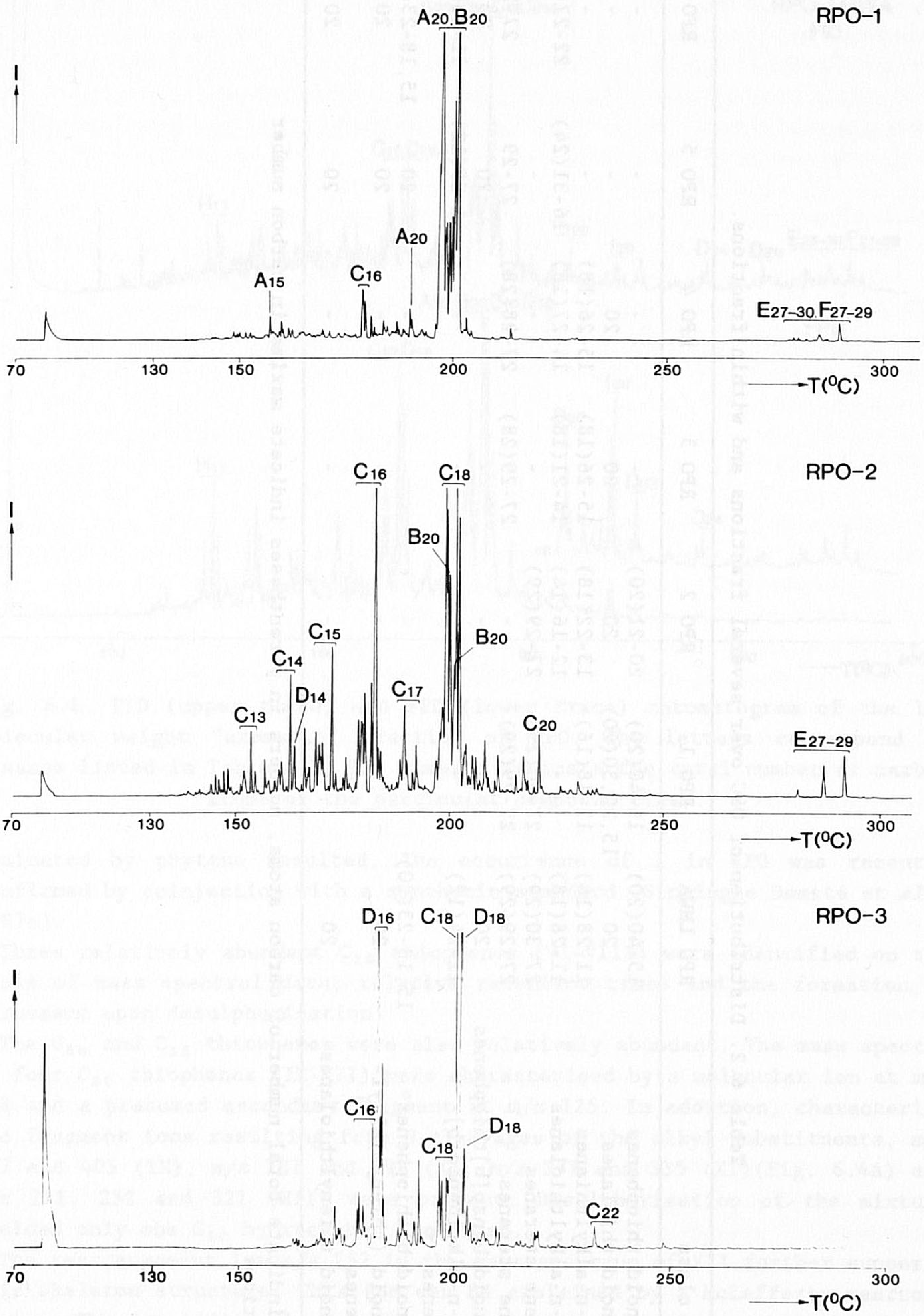


Fig. 6.2. FPD chromatograms of sub-fractions of the "aromatic" fraction of RPO. The peak designations refer to compounds as indicated in Fig. 6.1.

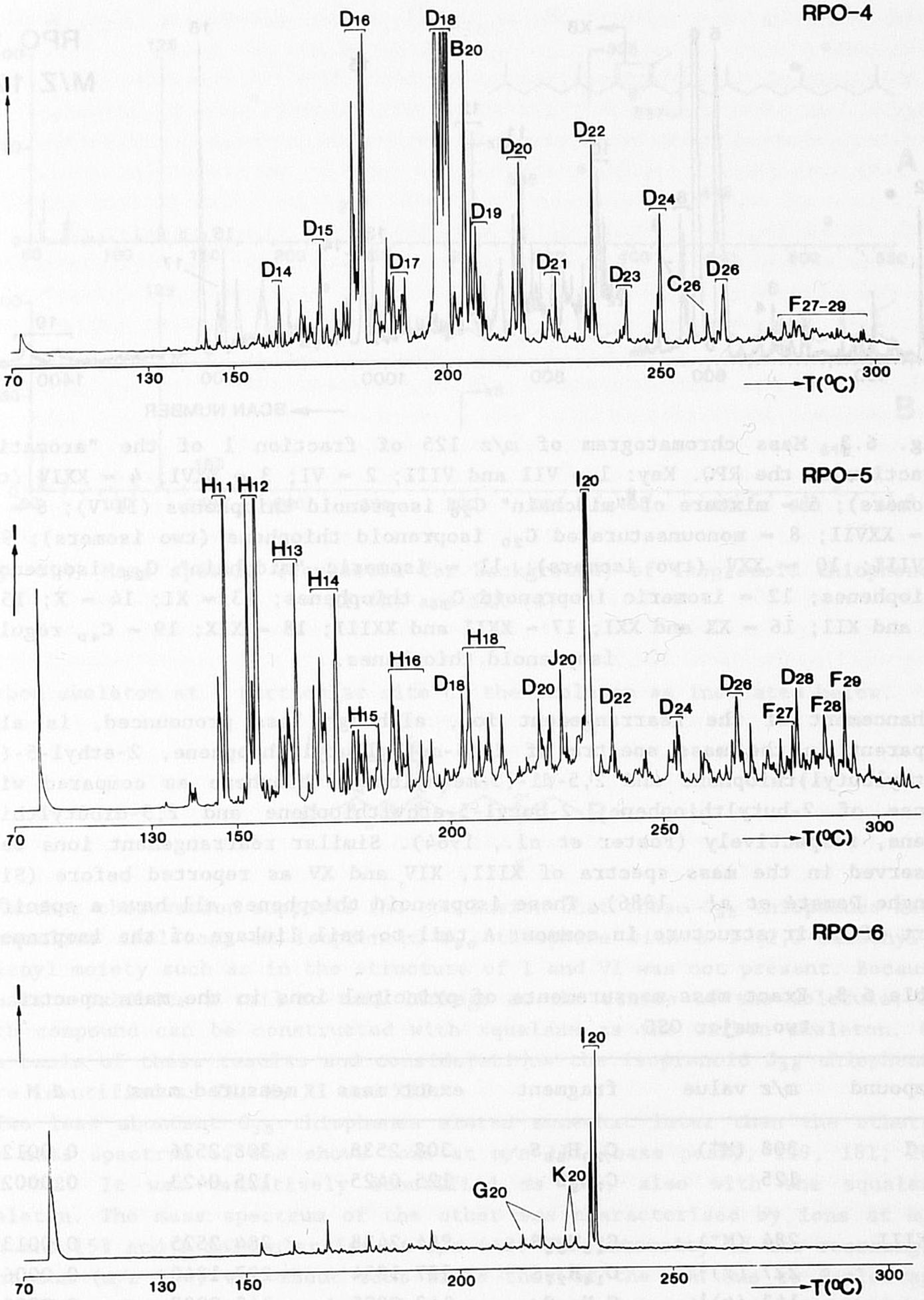


Fig. 6.2. (Continued)

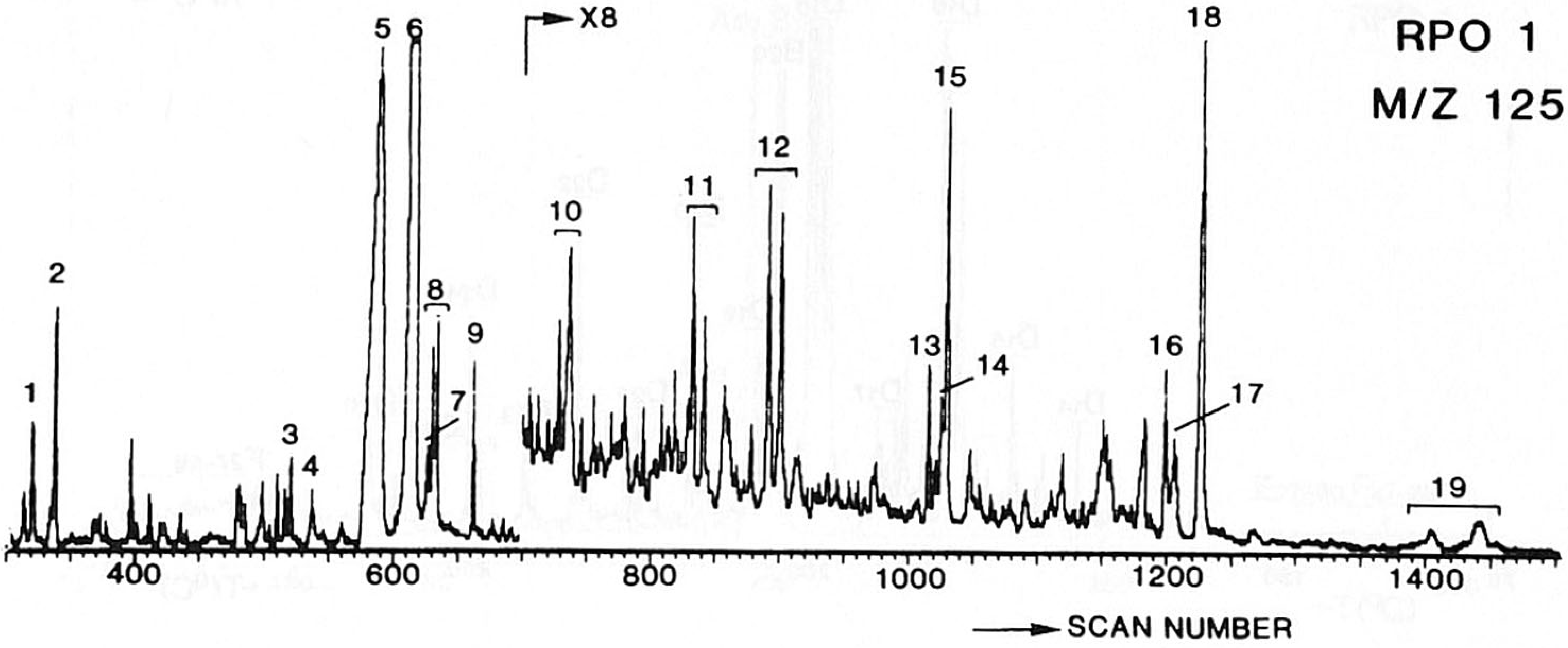


Fig. 6.3. Mass chromatogram of *m/z* 125 of fraction 1 of the "aromatic" fraction of the RPO. Key: 1 = VII and VIII; 2 = VI; 3 = XXVI; 4 = XXIV (two isomers); 5 = mixture of "midchain" C_{20} isoprenoid thiophenes (II-V); 6 = I; 7 = XXVII; 8 = monounsaturated C_{20} isoprenoid thiophene (two isomers); 9 = XXVIII; 10 = XXV (two isomers); 11 = isomeric "midchain" C_{25} isoprenoid thiophenes; 12 = isomeric isoprenoid C_{26} thiophenes; 13 = XI; 14 = X; 15 = IX and XII; 16 = XX and XXI; 17 = XXII and XXIII; 18 = XIX; 19 = C_{40} regular isoprenoid thiophenes.

enhancement of the rearrangement ion, although less pronounced, is also apparent in the mass spectra of 2-(3-methylbutyl)thiophene, 2-ethyl-5-(3-methylbutyl)thiophene and 2,5-di-(3-methylbutyl)thiophene as compared with those of 2-butylthiophene, 2-butyl-5-ethylthiophene and 2,5-dibutylthiophene, respectively (Foster *et al.*, 1964). Similar rearrangement ions were observed in the mass spectra of XIII, XIV and XV as reported before (Sinninghe Damsté *et al.*, 1986). These isoprenoid thiophenes all have a specific part of their structure in common: A tail-to-tail linkage of the isoprenoid

Table 6.3. Exact mass measurements of principal ions in the mass spectra of two major OSC.

compound	*m/z* value	fragment	exact mass	measured mass	Δ M
I	308 ($M^{+\cdot}$)	$C_{20}H_{36}S$	308.2538	308.2526	0.0012
	125	C_7H_9S	125.0425	125.0423	0.0002
XXXIII	284 ($M^{+\cdot}$)	$C_{18}H_{36}S$	284.2438	284.2525	0.0013
	227 (r)[1]	$C_{14}H_{27}S$	227.1834	227.1840	-0.0006
	143 (t)[1]	$C_8H_{15}S$	143.0895	143.0887	0.0008
	101 (q)[1]	C_5H_9S	101.0425	101.0419	0.0006

[1] letters refer to fragmentation pathways outlined in Fig. 6.5

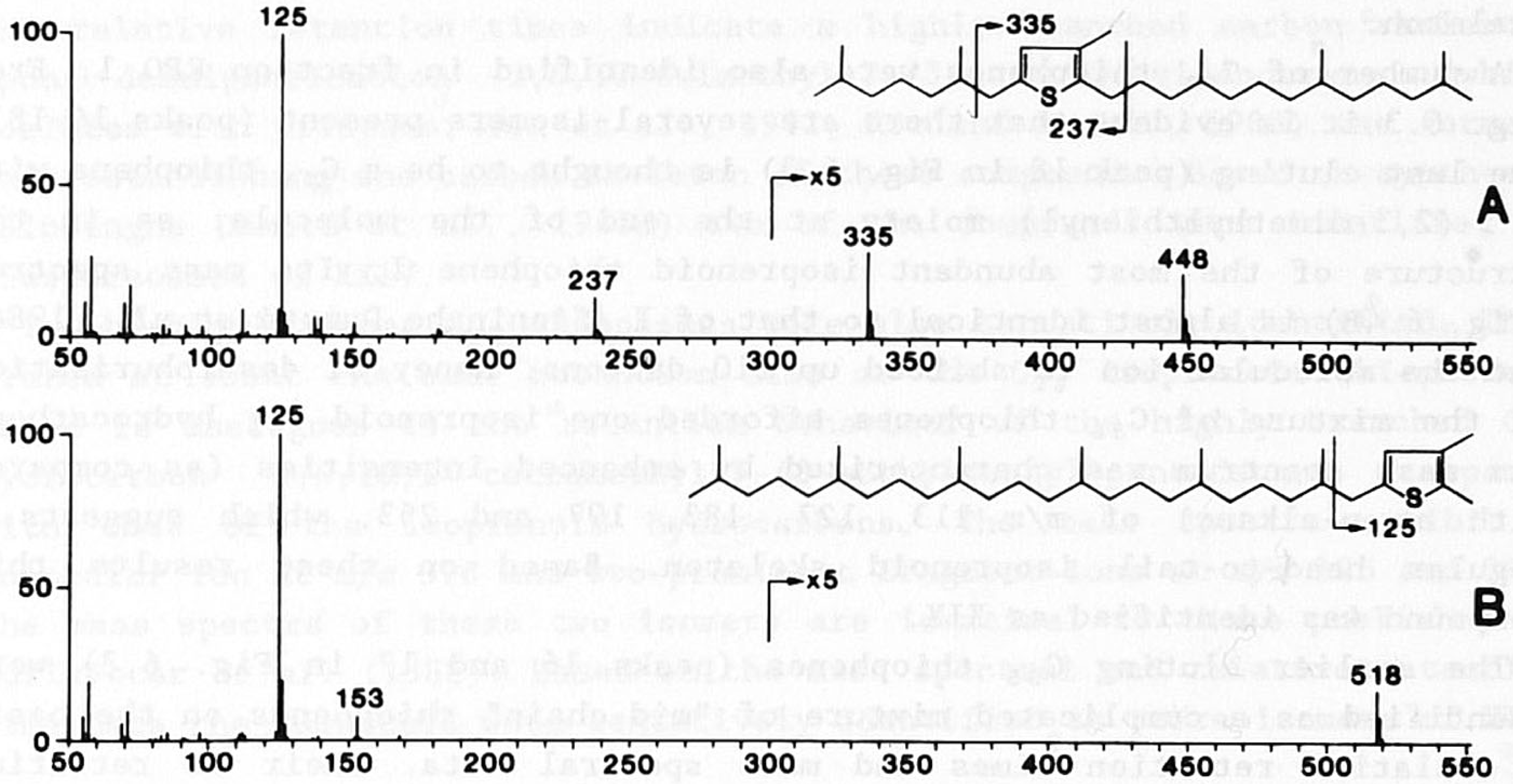
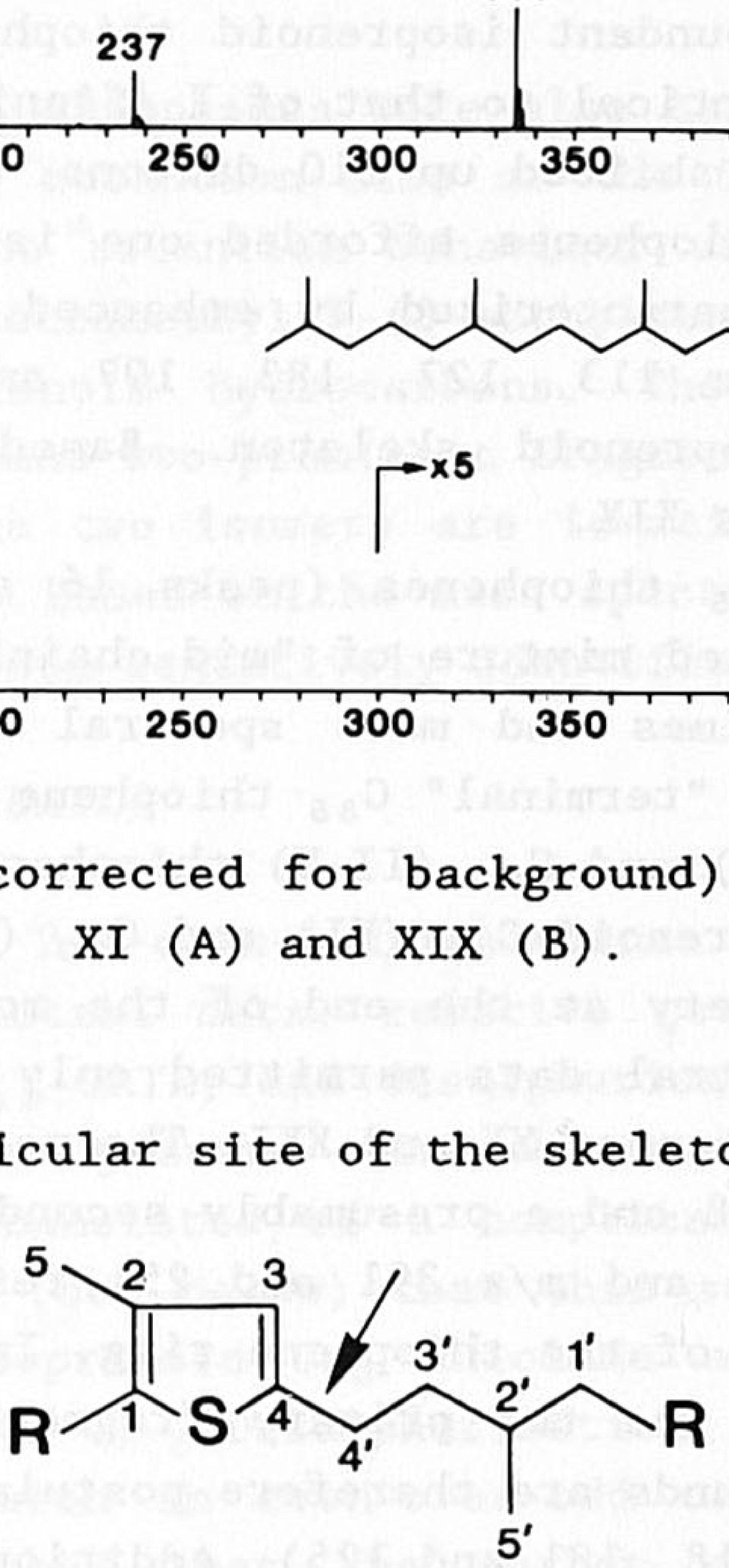


Fig. 6.4. Mass spectra (corrected for background) of isoprenoid thiophenes XI (A) and XIX (B).

carbon skeleton at a particular site of the skeleton as indicated below.



Another observation supports the conclusion that these C_{30} thiophenes have a squalane skeleton: an isoprenoid C_{30} thiophene with a 5-(2,3-dimethyl)-thienyl moiety such as in the structure of I and VI was not present. Because squalane exhibits a tail-to-tail linkage at the centre of the molecule, no such compound can be constructed with squalane as the carbon skeleton. On the basis of these results and considerations the isoprenoid C_{30} thiophenes were identified as IX, X, XI and XII.

Two less abundant C_{30} thiophenes eluted somewhat later than the others. The mass spectrum of one shows ions at *m/z* 111 (base peak), 139, 181, 209 and 448. It was tentatively identified as XVI, also with the squalane skeleton. The mass spectrum of the other was characterised by ions at *m/z* 154 and 153 and a molecular ion at *m/z* 448. The intensity of the rearrangement ion (*m/z* 154) was about four times that of the ion due to β-cleavage (*m/z* 153). This phenomenon has also been observed in the mass spectrum of XVII (Rullkötter *et al.*, 1984b; Brassell *et al.*, 1986c) and is thought to be indicative of alkyl substitution of the thiophene ring at C-3 (or C-4) in combination with no substitution at C-2 (or C-5, respectively). Therefore, this compound was identified as XVIII, also possessing the squalane skeleton.

A number of C_{35} thiophenes were also identified in fraction RPO 1. From Fig. 6.3 it is evident that there are several isomers present (peaks 16-18). The last eluting (peak 18 in Fig. 6.3) is thought to be a C_{35} thiophene with a 5-(2,3-dimethylthienyl) moiety at the end of the molecule, as in the structure of the most abundant isoprenoid thiophene I. Its mass spectrum (Fig. 6.4B) is almost identical to that of I (Sinninghe Damsté *et al.*, 1986) but the molecular ion is shifted up 210 daltons. Raney Ni desulphurisation of the mixture of C_{35} thiophenes afforded one isoprenoid C_{35} hydrocarbon. Its mass spectrum was characterized by enhanced intensities (as compared with an *n*-alkane) of *m/z* 113, 127, 183, 197 and 253, which suggests a regular head-to-tail isoprenoid skeleton. Based on these results this compound was identified as XIX.

The earlier eluting C_{35} thiophenes (peaks 16 and 17 in Fig. 6.3) were identified as a complicated mixture of "mid-chain" thiophenes on the basis of relative retention times and mass spectral data. Their GC retention behaviour relative to the "terminal" C_{35} thiophene is similar to that of the "mid-chain" C_{15} (VII-VIII) and C_{20} (II-V) thiophenes (peaks 1 and 5 in Fig. 6.3) relative to the isoprenoid C_{15} (VI) and C_{20} (I) thiophenes with the 5-(2,3-dimethylthienyl) moiety at the end of the molecule (peaks 2 and 6 in Fig. 6.3). The mass spectral data permitted only a firm identification of two "mid-chain" C_{35} thiophenes: XX and XXI. The mass spectra showed, besides a molecular ion at *m/z* 518 and a presumably secondary ion at *m/z* 125, fragments at *m/z* 377 and 265 and *m/z* 391 and 251 respectively, which are indicative for the position of the thiophene ring. In the spectra of the other two isomers only one of the two primary fragment ions was observed. The structures of these compounds are therefore postulated as XXII (*m/z* 518, 195 and 125) and XXIII (*m/z* 518, 181 and 125). Additional unknown C_{35} thiophenes were also present in low abundance.

At the end of the *m/z* 125 chromatogram of RPO 1 (Fig. 6.3) two broad peaks are present. These are ascribed to C_{40} isoprenoid thiophenes. The identifications are, however, tentative because no molecular ions were observed in the mass spectra or those of their desulphurisation product, thought to be the regular C_{40} isoprenoid hydrocarbon (enhanced intensities of *m/z* 113, 127, 183 and 197).

Isoprenoid thiophenes with C_{18}-C_{19} and C_{21}-C_{28} carbon numbers were identified as well (some of them are shown in Fig. 6.3 (peaks 4, 9 and 12)). A number of these have been encountered in the "aromatic" fraction of an extract of a marl layer from the Northern Apennines (Italy; Sinninghe Damsté *et al.*, 1986). Desulphurisation confirmed the proposed structures. The distribution of these thiophenes in RPO is similar to that in Northern Apennines Marl.

Apart from the C_{20} isoprenoid thiophenes with regular isoprenoid carbon skeletons (I-V) a number of C_{20} thiophenes were encountered which elute together with the regular C_{19} isoprenoid thiophenes. The mass spectra are characterised by m/z values 308, 195, 125 and several other fragment ions. The relative retention times indicate a highly branched carbon skeleton. Upon desulphurisation 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (which coelutes with pristane; Yon *et al.*, 1982; Rowland *et al.*, 1985) was formed, thus establishing the carbon skeleton of these compounds. Based on synthesis (Sinninghe Damsté *et al.*, 1988d) two of the compounds were identified as diastereomers of XXIV.

Two highly branched C_{25} thiophenes were also tentatively identified. They eluted at about the same retention time as the C_{23} isoprenoid thiophenes, which is analogous to the retention behaviour of the highly branched C_{25} hydrocarbon 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane compared with that of the isoprenoid hydrocarbons. The mass spectra exhibit a molecular ion at *m/z* 378 and two prominent fragment ions at *m/z* 125 and 265. The mass spectra of these two isomers are identical to those published by Rullkötter *et al.* (1982). Based on the mass spectral and relative retention time data the compounds were tentatively identified as stereoisomers of XXV.

*2,5-Di-*n*-alkylthiolanes (DATL)*

A series of C_{11}-C_{28} 2,5-di-*n*-alkylthiolanes (DATL) (XXIX, XXX) was identified from mass spectral data, relative retention times, coinjection with several synthetic C_{18} DATL, and desulphurisation experiments. Evidence for the 2,5-substitution pattern was obtained by desulphurisation of fraction RPO 4′, which consisted of a complicated mixture of DATL. Mass spectral analysis showed (see below) that this sub-fraction contained only DATL, except for one isoprenoid C_{20} thiolane and a mixture of thiolane steranes (Fig. 6.2; RPO 4). Desulphurisation gave a mixture strongly dominated by *n*-alkanes with an even-over-odd carbon number predominance. This experiment established that the carbon skeletons of the DATL in fraction RPO 4′ are mainly linear. It should be emphasized that in this fraction only very minor amounts of dialkylthianes were present, further supporting the 2,5-substitution pattern of the DATL present. The results of the desulphurisation are in complete agreement with the work of Schmid (1986) and Schmid *et al.* (1987) who desulphurised an aliphatic sulphide fraction of the Maruejols crude oil (Ales basin, France; Oligocene) and also obtained a mixture of predominantly *n*-alkanes.

Mass spectra of several low molecular weight (< 10 carbon atoms) DATL have been reported by Khvostenko and Sultanov (1964). Zimina *et al.* (1960) reported the mass spectra of a series of 2-*n*-alkylthiolanes up to 2-hexylthiolane and Zaikin *et al.* (1978) reported the mass spectra of a series of 3-*n*-alkylthiolanes up to 3-hexylthiolane. Mass spectra of several C_{18}-C_{23} DATL (Schmid, 1986; Schmid *et al.*, 1987) and 2-methyl-5-tridecylthiolane (Sinninghe Damsté *et al.*, 1986) were reported recently. The features of these spectra are in agreement with those of the long-chain DATL reported here. They are characterised by the fragmentations exemplified in Fig. 6.5; α-cleavage of the alkyl side chains results in ions p and q. The relative intensity of these ions in the mass spectrum of a DATL depends on the ratio of the length of the side chains. The spectrum of XXXI (Fig. 6.5A) exhibits a M-29 fragment (p) of low intensity. This ion becomes more important as the length of the shorter alkyl side chain increases (Fig. 6.5). Secondary fragmentations then become more important. In the spectrum of, *e.g.* XXXII (Fig. 6.5C) *m/z* 87, which probably results from loss of both alkyl side

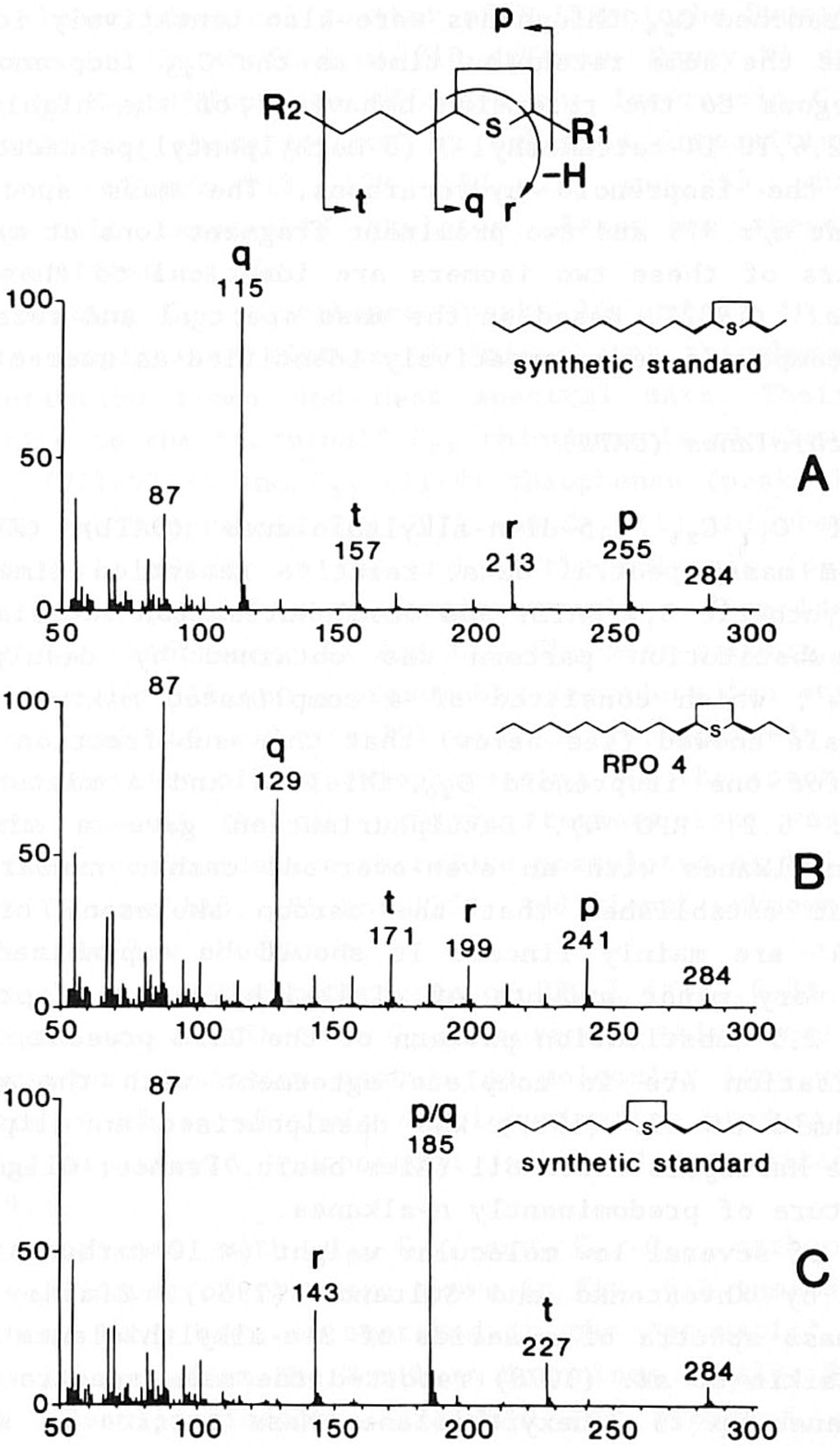
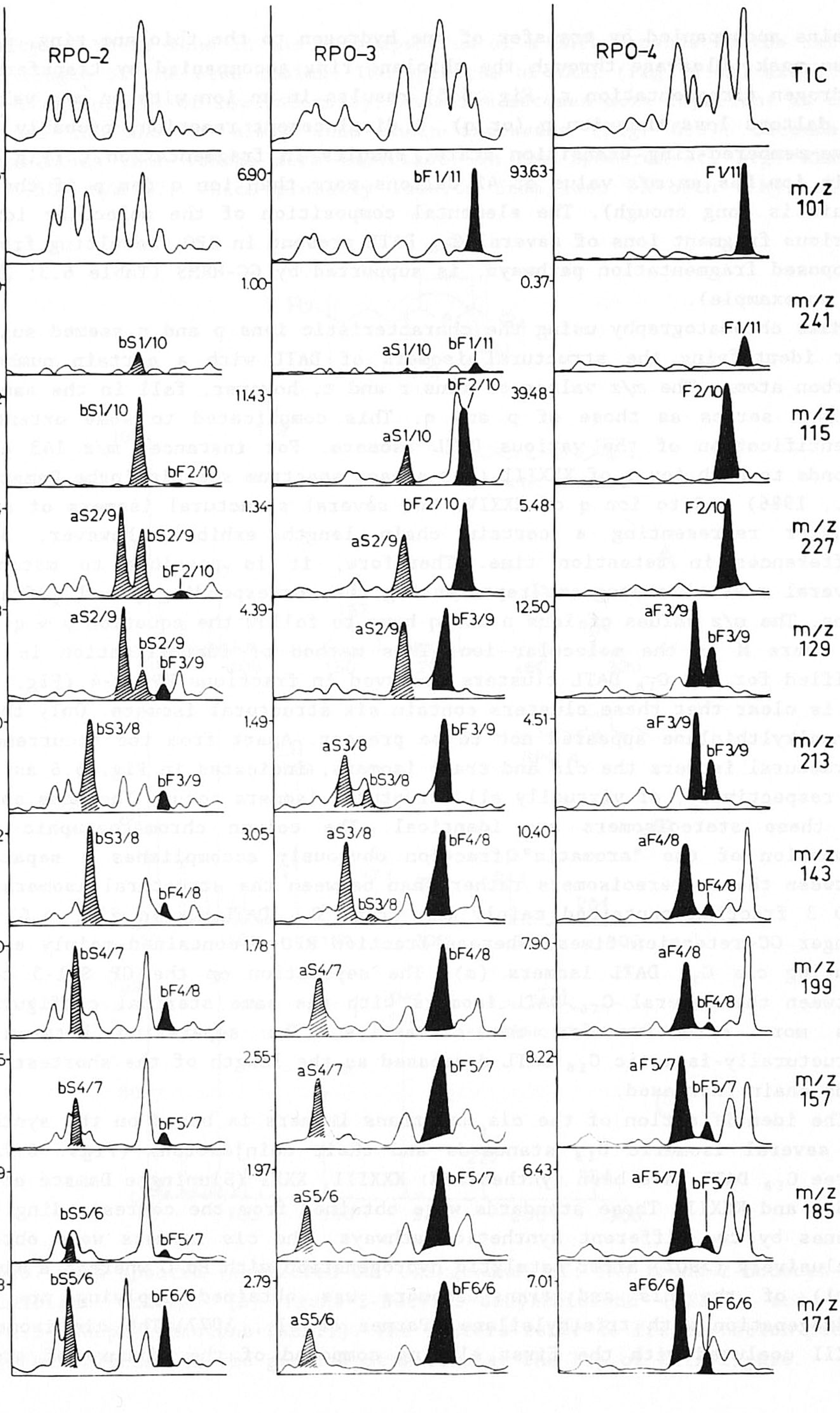


Fig. 6.5. Mass spectra (corrected for background) of: (A) *trans*-2-dodecyl-5-ethylthiolane (XXXI), (B) *trans*-2-butyl-5-decylthiolane (XXXIV) and (C) *trans*-2,5-diheptylthiolane (XXXII). The letters refer to fragmentation pathways exemplified in the general structure at the top of this figure.

chains accompanied by transfer of one hydrogen to the thiolane ring, is the base peak. Cleavage through the thiolane ring accompanied by transfer of a hydrogen (fragmentation r, Fig. 6.5) results in an ion with an *m/z* value of 42 daltons less than ion p (or q). A displacement reaction, probably *via* a five-membered-ring transition state, results in fragmentation t (Fig. 6.5) This ion has an *m/z* value of 42 daltons more than ion q (or p if the side chain is long enough). The elemental composition of the molecular ion and various fragment ions of several C_{18} DATL present in RPO, resulting from the proposed fragmentation pathways, is supported by GC-HRMS (Table 6.3; XXXIII as an example).

Mass chromatography using the characteristic ions p and q seemed suitable for identifying the structural isomers of DATL with a certain number of carbon atoms. The *m/z* values of ions r and t, however, fall in the same 101 + 14.*n* series as those of p and q. This complicated to some extent the identification of the various DATL isomers. For instance, *m/z* 143 corresponds to both ion t of XXXIII (for a mass spectrum see Sinninghe Damsté *et al.*, 1986) and to ion q of XXXIV. The several structural isomers of a DATL cluster representing a certain chain length exhibit, however, slight differences in retention time. Therefore, it is possible to match the several mass chromatograms representing the corresponding p and q fragment ions. The *m/z* values of ions p and q have to follow the equation $p + q - M = 86$ where M is the molecular ion. This method of identification is exemplified for the C_{16} DATL clusters observed in fractions RPO 2-4 (Fig. 6.6). It is clear that these clusters contain six structural isomers. Only the C_{16} monoalkylthiolane appeared not to be present. Apart from the occurrence of structural isomers the *cis* and *trans* isomers, indicated in Fig. 6.6 as a and b, respectively, of virtually all structural isomers occur. The mass spectra of these stereoisomers are identical. The column chromatographic fractionation of the "aromatic" fraction obviously accomplishes a separation between these stereoisomers rather than between the structural isomers. The RPO 3 fraction contained mainly the *trans* C_{16} DATL (b in Fig. 6.6) with longer GC retention times, whereas fraction RPO 4 contained mainly earlier eluting *cis* C_{16} DATL isomers (a). The separation on the CP Sil-5 column between the several C_{16} DATL isomers with the same sterical configuration was more effective for the *cis*-series. The separation between the structurally-isomeric C_{16} DATL decreased as the length of the shortest alkyl side chain increased.

The identification of the *cis* and *trans* isomers is based on the synthesis of several isomeric C_{18} standards and their coinjections (Figs. 6.7A-D). Three C_{18} DATL have been synthesized: XXXIII, XXXI (Sinninghe Damsté *et al.*, 1986) and XXXII. These standards were obtained from the corresponding thiophenes by two different synthetic pathways. The *cis* isomers were obtained exclusively (>90%) after catalytic hydrogenation with Pd/C whereas a mixture (1:1) of the *cis* and *trans* isomers was obtained applying an ionic hydrogenation with triethylsilane (Parnes *et al.*, 1977).The *cis* isomer of XXXII coeluted with the first eluting compound of the mixture of stereo-


RPO-2
RPO-3
RPO-4
TIC
4.95
6.90
bF1/11
93.63
F1/11
m/z
101
1.00
1.00
0.37
m/z
241
bS1/10
aS1/10
bF1/11
F1/11
49.52
bS1/10
bF2/10
11.43
bF2/10
aS1/10
39.48
F2/10
m/z
115
1.84
aS2/9
bS2/9
bF2/10
1.34
bF2/10
aS2/9
5.48
F2/10
m/z
227
10.38
aS2/9
bS2/9
bF3/9
4.39
aS2/9
bF3/9
12.50
aF3/9
bF3/9
m/z
129
1.60
bS3/8
bF3/9
1.49
bF3/9
aS3/8
bS3/8
4.51
aF3/9
bF3/9
m/z
213
4.82
bS3/8
bF4/8
3.05
aS3/8
bF4/8
bS3/8
10.40
aF4/8
bF4/8
m/z
143
1.90
bS4/7
bF4/8
1.78
bF4/8
aS4/7
7.90
aF4/8
bF4/8
m/z
199
6.25
bS4/7
bF5/7
2.55
aS4/7
bF5/7
8.22
aF5/7
bF5/7
m/z
157
6.29
bS5/6
bF5/7
1.97
bF5/7
aS5/6
6.43
aF5/7
bF5/7
m/z
185
2.98
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bF6/6
m/z
171

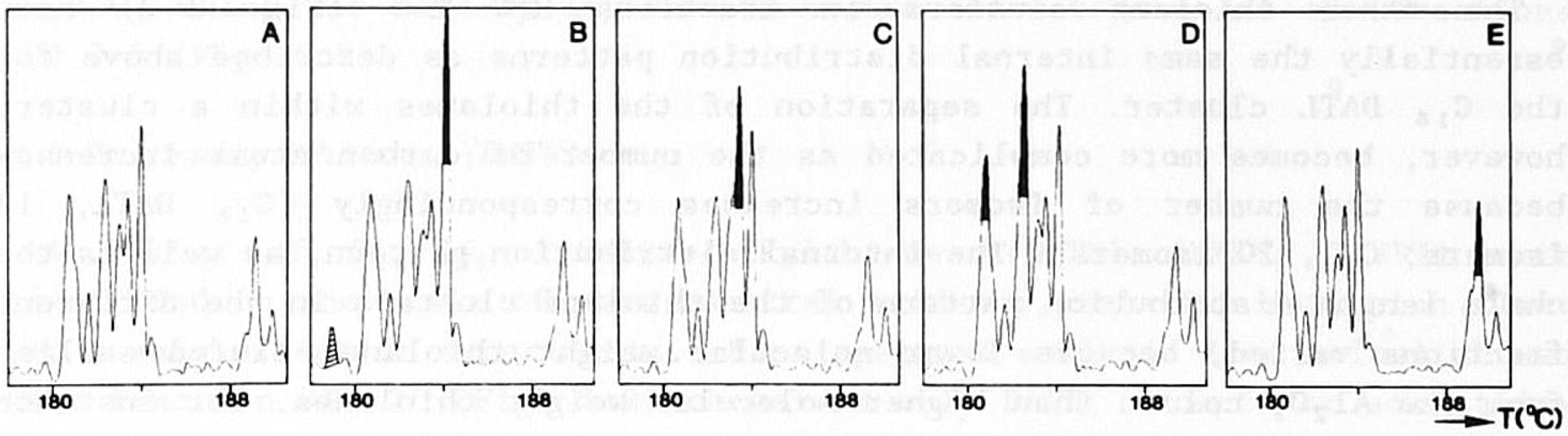

Fig. 6.7. Partial GC-traces of fraction 4 of the "aromatic" fraction of RPO (A) and its coinjection with (B) *cis*- and *trans*-2-methyl-5-tridecylthiolane (XXXIII), (C) *cis*- and *trans*-2-dodecyl-5-ethylthiolane (XXXI), (D) *cis*- and *trans*-2,5-diheptylthiolane (XXXII) and (E) *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (XXXVII). Increments of peak areas upon coinjection are coloured black.

isomers obtained by ionic reduction of 2,5-diheptylthiophene. Both the *cis* and the *trans* isomers of the synthetic XXXIII and XXXI coeluted with each other and with the corresponding structural isomers in fractions RPO 3 and 4. The mass spectra of the *cis* and *trans* isomers of XXXI, XXXII, and XXXIII were identical.

Proof of the fact that ionic hydrogenation indeed yielded a pair of coeluting *cis* and *trans* isomers was obtained from the formation of sulphoxides in the synthesis of the DATL XXXI, XXXII and XXXIII. In all three syntheses, using ionic hydrogenation as the last reaction step, a pair of two GC-separable isomeric, probably *cis* and *trans*, sulphoxides with the same carbon skeletons as the corresponding thiolanes were formed as side products. These pairs of isomeric sulphoxides exhibited identical mass spectra. The ratio of the two sulphoxides formed in the ionic reduction of 2,5-diheptylthiophene was the same as that of *cis* and *trans* 2,5-diheptylthiolane. Hydrogenation with Pd/C of 2,5-diheptylthiophene led to the formation of only one sulphoxide (and one thiolane). These two facts indicated that the *cis* and *trans* sulphoxides were probably formed via the *cis* and *trans* DATL. Because of the formation of two isomeric sulphoxides in the synthesis of thiolanes XXXI and XXXII using ionic hydrogenation, it seems highly probable that *cis* and *trans* DATL were formed but coeluted on the column used.

Fig. 6.6. Mass chromatograms of diagnostic *m/z* values for DATL (black peaks) and DATN (shaded peaks) of that particular area of the TIC of fractions 2, 3 and 4 of the "aromatic" fraction of the RPO, in which the C_{16} DATL and DATN elute. The symbol indications refer to particular isomers (see XXIX, XXX, XXXV and XXXVI, key: F = five membered ring, S = six membered ring, x and y = number of carbon atoms in alkyl side chains, a = *cis*, b = *trans*).

The other thiolane clusters in fractions RPO 2-5 (Fig. 6.2) have essentially the same internal distribution patterns as described above for the C_{16} DATL cluster. The separation of the thiolanes within a cluster, however, becomes more complicated as the number of carbon atoms increases because the number of isomers increases correspondingly (C_{16} DATL, 12 isomers; C_{24}, 20 isomers). The internal distribution pattern, as well as the chain length distribution pattern of the thiolane clusters in the different fractions varied, because lower molecular weight thiolanes eluted earlier from the Al_2O_3 column than higher molecular weight thiolanes. For instance the C_{14}-C_{18} thiolane clusters in RPO 4 consist for the greater part of the earlier GC eluting *cis*-thiolanes whereas the C_{19}-C_{27} thiolane clusters are composed for the greater part of the later GC eluting *trans*-thiolanes.
Fig. 6.8A shows the carbon-number distribution pattern of the C_{14}-C_{26} DATL, which was obtained by integrating the appropriate peaks in the mass chromatograms of m/z 87 + 14.*n* of the RPO LMWA fraction, assuming that no considerable differences occur in the response factors of the homologous series. This pattern shows the even carbon number predominance of the DATL

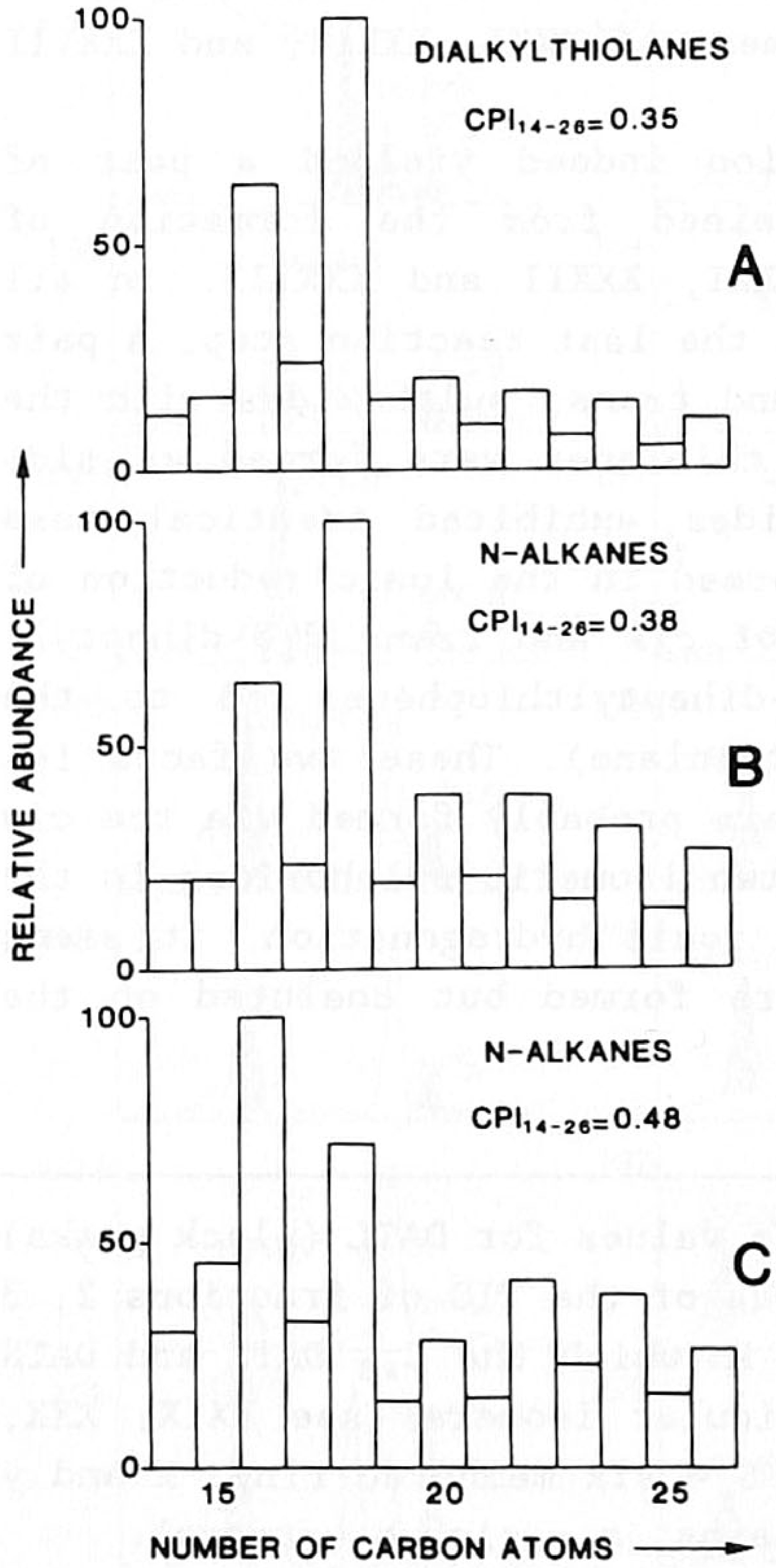

Fig. 6.8. Carbon number distribution patterns (C_{14}-C_{26}) of: (A) DATL obtained by integrating the appropriate peaks in the mass chromatograms of *m/z* 87 + 14.*n* (*n* depends on the carbon number) of the LMWA fraction of the RPO, (B) *n*-alkanes after desulphurisation of the "aromatic" fraction of the RPO. This pattern represents the distribution pattern of DATL plus DATN, (C) *n*-alkanes in the adduct of the aliphatic fraction of RPO.

in RPO, which was already apparent from the chromatograms of the fractions RPO 3-5 (Fig. 6.2).

2,6-Di-n-alkylthianes (DATN)

A series of C_{11}-C_{28} 2,6-di-*n*-alkylthianes (DATN) (XXXV, XXXVI) were identified on the basis of mass spectral evidence, relative retention times and desulphurisation experiments. Mass spectral analysis (see below) showed that fraction RPO 2′ (Fig. 6.2) contained only DATN, except for some thiophene steranes. Desulphurisation gave a mixture of hydrocarbons dominated by *n*-alkanes with even-over-odd carbon number predominance. This experiment established that the DATN are substituted exclusively at position 2 and 6 of the thiane ring. It should be emphasized that in this fraction only very minor amounts of DATL were present. The isomer distribution patterns were studied in detail by GC-MS.

No literature data are available on mass spectra of synthetic DATN. Brodskii *et al.* (1969) reported on some features of the mass spectra of 2-ethyl-, 2-hexyl- and 2-undecylthiane, which were characterized by an intense *m/z* 101 ion (23.8-33.3% of the total ion current). Zaikin *et al.* (1978) described the mass spectra of several low molecular weight 2-, 3- and 4-alkylthianes.

The mass spectra of the DATN are characterised by the same kind of fragments formed by the same pathways as described for the DATL. The α-cleavage of the alkyl side chains results in ions p and q (see Fig. 6.9). The intensity of these ions depends on the ratio of the length of the alkyl side chains. Loss of both side chains accompanied by hydrogen transfer to the thiane ring affords the secondary fragment *m/z* 101. Cleavage through the thiane ring accompanied by hydrogen transfer (fragmentation r) results in an ion with an *m/z* value 56 daltons less than that of fragment p. The formation of ion t, with an *m/z* value 42 daltons more than that of ion q, is probably fully analogous to the formation of this ion in the mass spectra of DATL. From comparison of Figs. 6.5 and 6.9 it can be concluded that the mass spectra of DATL and DATN are similar at first sight. There are, however, three major differences:

1) The equation $p + q - 86 = M$ for DATL has to be $p + q - 100 = M$ for DATN.
2) The difference between the *m/z* values of p and r is 42 daltons in the mass spectra of DATL but 56 daltons in the mass spectra of DATN.
3) In the mass spectra of DATN the secondary fragment appears at *m/z* 101 instead of *m/z* 87 in the spectra of DATL.

Mass chromatography of the *m/z* values of ions p and q is suitable for unravelling the DATN isomers, by analogy with the strategy followed for the DATL. This approach is, however, somewhat complicated by the fact that these *m/z* values are also indicative for fragment ions of DATL and for fragment ions r and t of DATN. DATN can be discriminated from DATL by their mass spectral data as indicated above and on the basis of relative retention times. DATN elute earlier than the corresponding DATL (Fig. 6.6). To discriminate the several structural isomers of a DATN cluster matching mass chromatograms of *m/z* values which fulfil the equation p + q - 100 = M have to be traced. The structural DATN isomers with the same number of carbon atoms exhibit slightly greater differences in retention time than the corresponding DATL (cf. Fig. 6.6; C_{16} cluster as an example). Apart from the

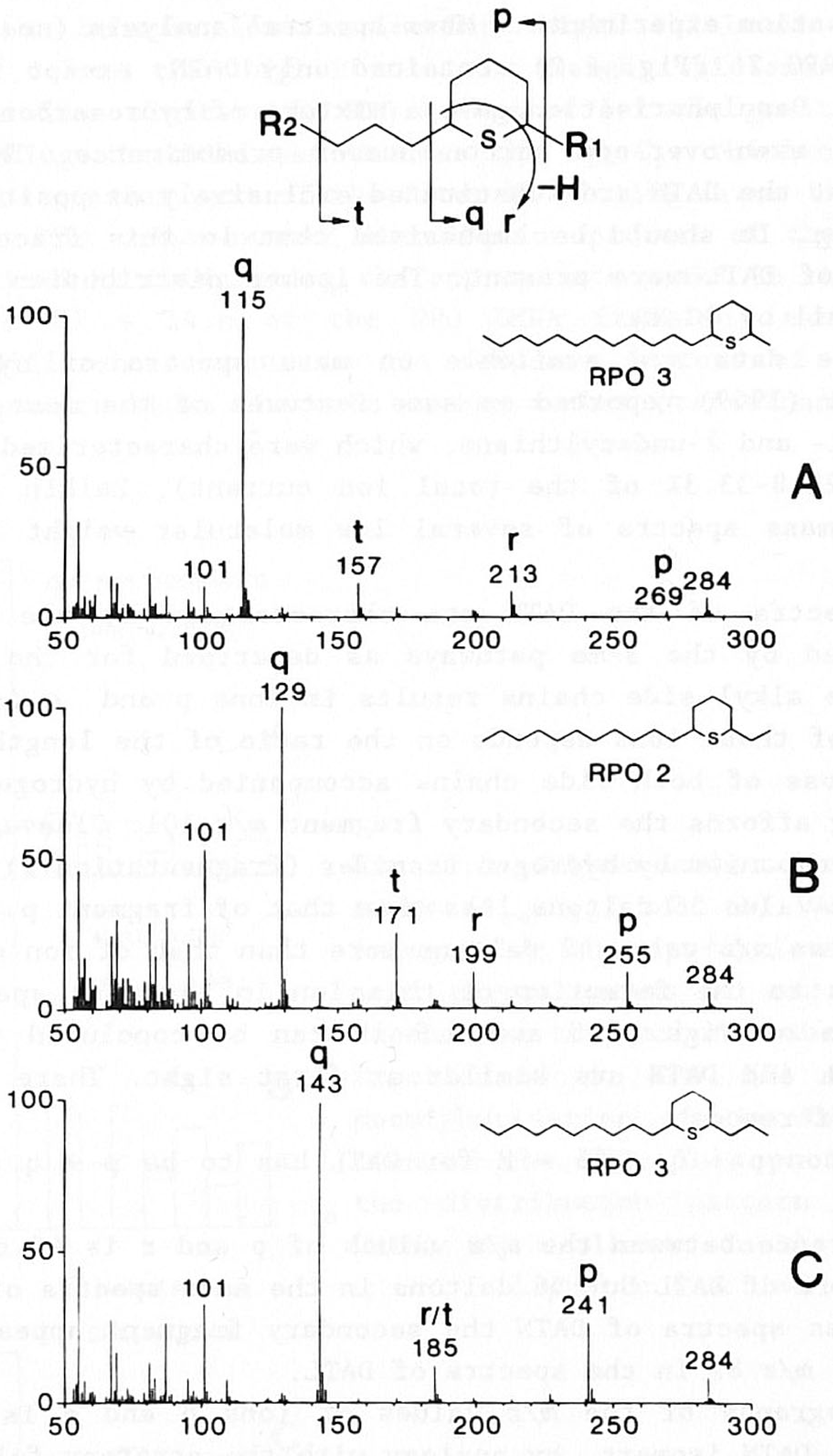

Fig. 6.9. Mass spectra (corrected for background) of: (A) 2-dodecyl-6-methylthiane (B) 6-ethyl-2-undecylthiane and (C) 2-decyl-6-propylthiane. The letters refer to fragmentation pathways exemplified in the general structure shown.

occurrence of five structural isomers (the monoalkylthiane was absent) both stereoisomers, indicated in Fig. 6.6 by a and b, of all structural isomers occur. The stereoisomers exhibit identical mass spectra. The column chromatographic separation procedure applied led to an almost complete separation of the first eluting (GC) a-series of C_{16} DATN (presumably *cis* by anology with the DATL) present in fraction RPO 2 and the later eluting (GC) b-series of C_{16} DATN (presumably *trans*) present in fraction RPO 3. The separation between the structural C_{16} DATN isomers on the CP Sil-5 capillary column was somewhat better for the a-series.

The other DATN clusters in fractions RPO 2-4 have similar distribution patterns as observed for the C_{16} DATN cluster. The distribution pattern within a given DATN cluster in a particular fraction depends on the number of carbon atoms as described for the DATL.

The carbon number distribution of the DATN could not be obtained as accurately as described for the DATL because of the presence of presumed *iso* and *anteiso* DATL which eluted at about the same retention times as the DATN. From Fig. 6.2 (RPO 2 and 3) it is obvious, however, that the DATN also show an even carbon number preference. Fig. 6.8B shows the carbon number distribution of the C_{14}-C_{26} *n*-alkanes, observed after desulphurisation of the "aromatic" fraction of RPO. This pattern reflects those of DATL and DATN added to each other. Because the concentrations of DATL and DATN are of the same order of magnitude (Fig. 6.1) and this distribution pattern resembles that of the DATL (Fig. 6.8A) it may be concluded that the distribution pattern of DATN is similar to that of the DATL.

Isoprenoid thiolanes

A complicated mixture of presumably C_{20} isoprenoid thiolanes occurs in RPO. These compounds elute at about the same retention times as the C_{18} DATL and DATN as shown in Fig. 6.10. This figure shows the high resolution mass chromatograms of *m/z* 284.2538 ($C_{18}H_{36}S$) and *m/z* 312.2851 ($C_{20}H_{40}S$) of the LMWA fraction of RPO. This retention behaviour is indicative of the isoprenoid nature of these compounds. This assignment was supported by desulphurisation of a sub-fraction of RPO 1, a fraction which did not contain any isoprenoid thiophenes; phytane was the most abundant compound.

The identification of these thiolanes was complicated by the complex composition of this compound class. Upon fractionation of the "aromatic" fraction of RPO the isoprenoid C_{20} thiolanes showed up in fractions RPO 1-4. However, the larger part eluted in fraction RPO 1 (Fig. 6.2). This complex C_{20}-cluster of isoprenoid thiolanes could not be separated by GC into individual compounds, thus preventing the acquisition of pure mass spectra. The presence of both *cis* and *trans* isomers is a possible explanation for the higher degree of complexity when compared with that observed for the isoprenoid thiophenes. Also, the possible presence of isoprenoid C_{20} thianes in this cluster cannot be ruled out. Nevertheless, a limited number of isoprenoid thiolanes could be identified. Some have been described before

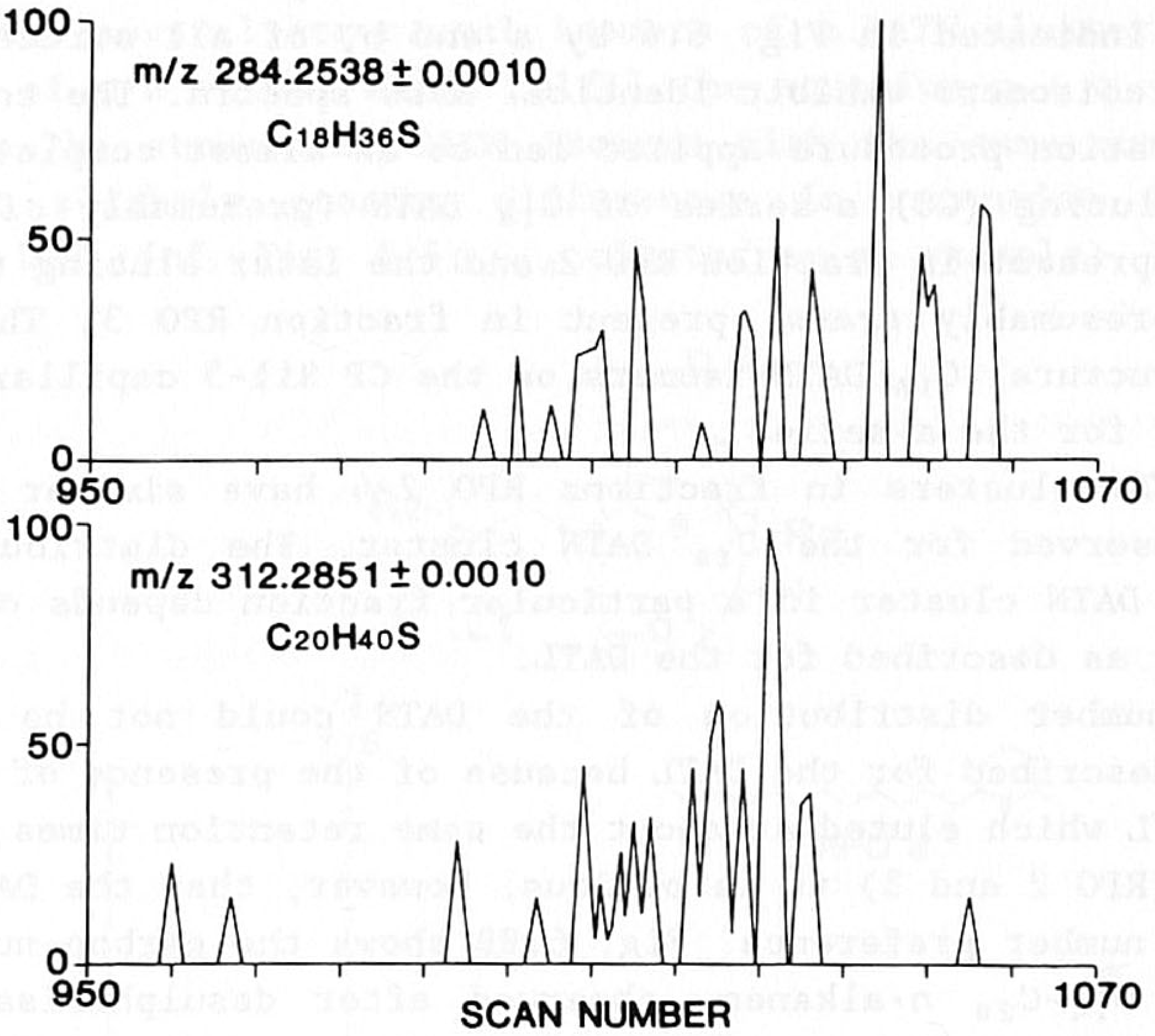

Fig. 6.10. High resolution mass chromatogram of *m/z* 284.2538±0.0010 ($C_{18}H_{36}S$) and *m/z* 312.2851±0.0010 ($C_{20}H_{40}S$) of the LMWA fraction of the RPO, showing the coelution of the isoprenoid C_{20} thiolanes with the C_{18} DATL and DATN.

(Sinninghe Damsté and de Leeuw, 1987).

An isoprenoid thiolane present in RPO 4 could be identified as XXXVII. This compound has been reported previously to occur in a sediment extract from a marl layer in the Northern Apennines (Sinninghe Damsté *et al.*, 1986). Coinjection of fraction RPO 4 with a synthetic standard proved this assignment (Figs. 6.7A and E). The standard was obtained by ionic hydrogenation of XXVII, synthesized previously (Brassell *et al.*, 1986c). This hydrogenation yielded a mixture of isoprenoid thiolanes of which presumably *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane was the primary constituent; *cis*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane was obtained by hydrogenation of XXVII with Pd/C. The mass spectral fragmentation pattern (Fig. 6.11) of this C_{20}-thiolane supports the fragmentation pattern proposed for the DATL (Fig. 6.5).

Apart from the occurrence of regular isoprenoid C_{20} thiolanes, several other isoprenoid thiolanes also occurred in fraction RPO 1: Isoprenoid C_{15} thiolanes (*e.g.* XXXVIII), a highly branched isoprenoid C_{20} thiolane (XXXIX) and isoprenoid C_{30} thiolanes possessing the squalane skeleton (*e.g.* XL). Their assignments were based on mass spectral data and carbon skeleton determinations after Raney Ni desulphurisation.

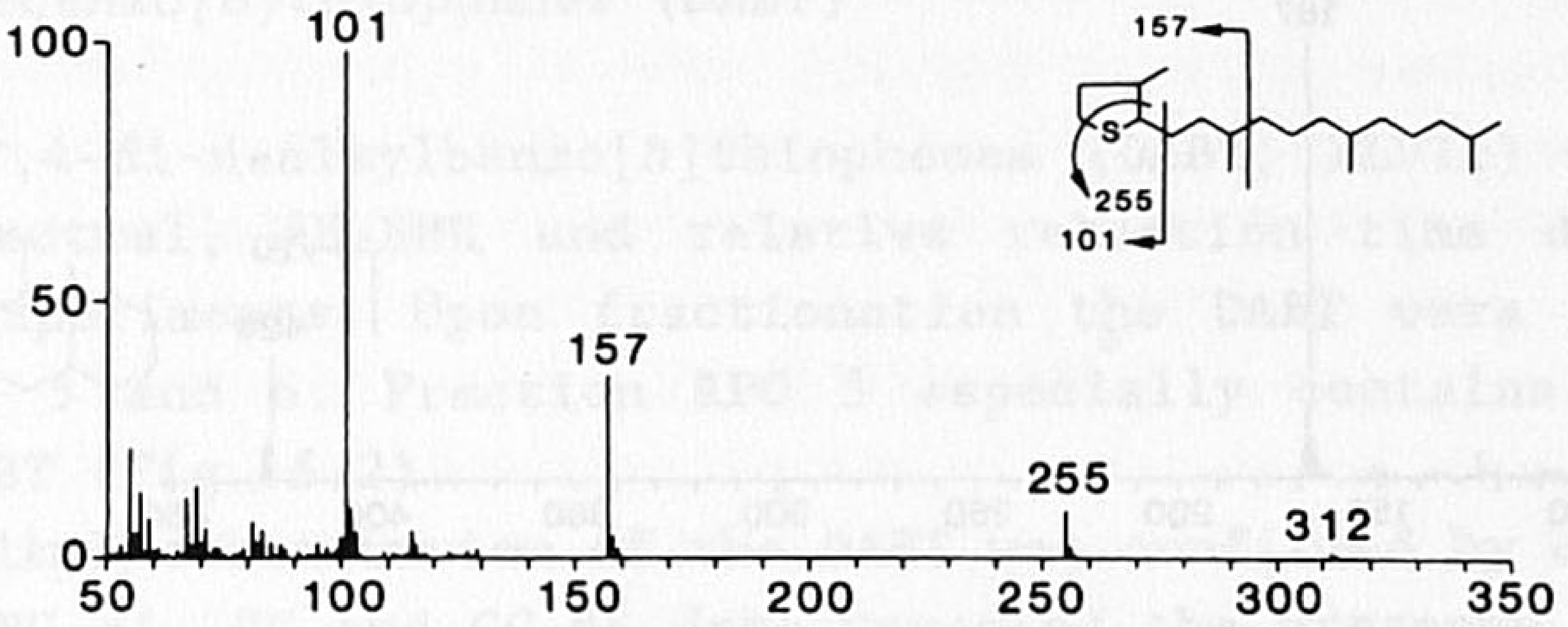

Fig. 6.11. Mass spectrum (corrected for background) of *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (XXXVII).

Thiophene steranes.

The thiophene steranes were concentrated in fractions RPO 1 and RPO 2. Their composition in RPO 2 was relatively simple. The three major members of fraction RPO 2 (Fig. 6.2) are characterised by a sterane skeleton condensed to a thiophene moiety (XLI) as determined conclusively by synthesis by Schmid (1986) in another RPO sample. Another tentatively identified C_{29} thiophene sterane (XLII) partly coelutes with the C_{29} thiophene sterane (XLIc). This compound exhibits fragment ions at *m/z* 426, 355 (instead of 341), 177 and 191 (instead of 163 and 177) in its mass spectrum.

In RPO 1 the four "normal" thiophene steranes are also present but are accompanied by several other series of thiophene steranes. The first group probably consists of stereoisomers of the thiophene steranes already discussed. They exhibit almost identical mass spectra but eluted about "one homologue" earlier.

Another group representing 4-methyl thiophene steranes (XLIII) was also encountered in this fraction. Their structures are characterised by a condensed thiophene moiety and a methyl group at C-4 of the sterane skeleton. The mass spectra show a molecular ion, a base peak at *m/z* 355 and two smaller fragment ions at *m/z* 163 and 177. These last two fragment ions are important because their presence suggested that the methyl group was not located at positions 14 or 22 of the original sterane skeleton since the ions *m/z* 177 and 191 were not enhanced in the mass spectra.

Two other groups of thiophene steranes were also tentatively identified. Both are characterised by the presence of a thiophene moiety in the side chain. The mass spectra of XLIV were characterised by the molecular ion and by one major fragment ion either at *m/z* 153 or 167 (Fig. 6.12A) originating from β-cleavage. Two stereoisomers of every structural isomer were encountered. Circumstantial evidence for the assignment of XLIV b and c was obtained by Raney Ni desulphurisation. These four isomeric C_{29} thiophene steranes were concentrated in one particular fraction, which did not contain other thiophene steranes. Desulphurisation of this fraction afforded a mixture of C_{29} steranes dominated by the $5\alpha(H),14\beta(H),17\beta(H)$-isomers.

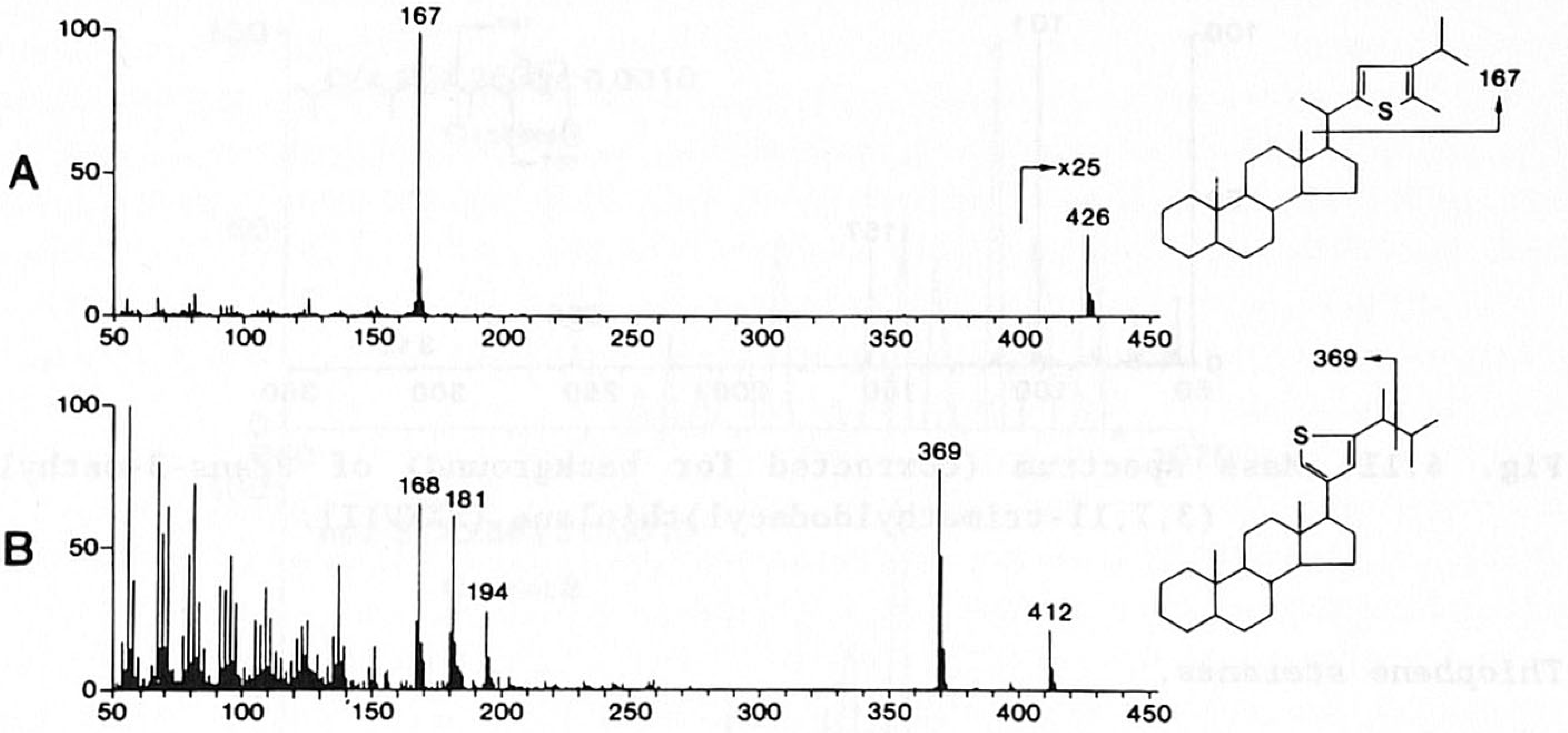

Fig. 6.12. Two selected mass spectra of tentatively identified steranes (XLIVb and XLVa) with a thiophene moiety in the side-chain.

The mass spectra of the other group of thiophene steranes (XLV) exhibited more complicated spectra. The spectra (Fig. 6.12B) were characterised by a resulting from loss of part of the alkyl side chain of the thiophene moiety, and several rearrangement ions (*m/z* 168, 181 and 194 and *m/z* 182, 195 and 208 for thiophene steranes XLVa and XLVb, respectively) thought to result from cleavage through the D-ring of the sterane skeleton.

All these thiophene steranes (except XLII) and several others were also tentatively identified by Schmid (1986) in another RPO, a sample in which the sulphur-containing steranes are much more prominent than in the RPO described here. Moreover, Schmid (1986) isolated two stereoisomers of XLIVb and studied their structures with ^{1}H-NMR. The NMR spectra are in agreement with the structure proposed here.

Thiolane steranes

In contrast with the thiophene steranes the thiolane steranes were not concentrated in particular fractions (Table 6.2). They consisted of a complicated mixture, probably because of the presence of additional stereoisomers as compared with the thiophene steranes. The mass spectra (see Schmid, 1986) of an abundant thiolane steranes series (XLVI) are characterised by an ion at *m/z* 331 resulting from α-cleavage of the alkyl side chain and a base peak at *m/z* 115, 129 and 143 for the C_{27}, C_{28} and C_{29} members, respectively. The formation of these latter ions is probably analogous to fragmentation r (Fig. 6.5), *i.e.* fragmentation through the thiolane ring. An additional ion at *m/z* 99 (C_5H_7S) was observed. Several other series, described by Schmid (1986), were present.

*2,4-Di-*n*-alkylbenzo[*b*]thiophenes (DABT)*

Series of 2,4-di-*n*-alkylbenzo[*b*]thiophenes (DABT; XLVII) were identified from mass spectral, ^{1}H-NMR and relative retention time data and desulphurisation experiments. Upon fractionation the DABT were concentrated in fractions RPO 5 and 6. Fraction RPO 5 especially contains a considerable portion of DABT (Fig. 6.2).

The 2,4-dialkyl substitution of the DABT was confirmed by desulphurisation of fraction RPO 5′. GC and GC-MS data revealed the presence of all isomeric 1,2-di-*n*-alkylbenzenes (DAB) ranging from C_{11} up to C_{26}. The mass spectra of the DAB are characterised by one or two ions, resulting from β-cleavage(s) of the alkyl side chain(s). These ions were used to identify the several isomers in a cluster of DAB. The retention behaviour of the C_{11} to C_{16} DAB was in accordance with literature data (Sojak *et al.*, 1977; Fig. 6.13). The aromatic signals in the ^{1}H-NMR spectra (in CD_3COCD_3) of the desulphurised fraction were consistent with the 1,2-substitution pattern. The aromatic signal consisted of a very complicated but symmetric signal ranging from δ = 7.05 up to δ = 7.17, which is indicative for *ortho*-substituted benzenes.

The aromatic signals in the ^{1}H-NMR spectra of the original fraction (fraction RPO 5′) were in agreement with the 2,4-substitution pattern of the DABT. These signals were characterised by two broad peaks (δ = 7.07 and 7.10, J ≈ 6.5 Hz), thought to reflect a double doublet of the proton at C-5, a triplet (δ = 7.17, J = 7.4 Hz), assigned to the proton at C-6, a singlet (δ = 7.25) assigned to the proton at C-3 and two broad peaks (δ = 7.58 and 7.62, J ≈ 7.6 Hz), thought to reflect a double doublet of the proton at C-7.

Fig. 6.14 shows the mass spectra of three isomeric 2,4-DABT. They are characterised by a molecular ion, one or two ions resulting from β-cleavage of the alkyl side chain(s) (fragments p and q in Fig. 6.14), and sometimes

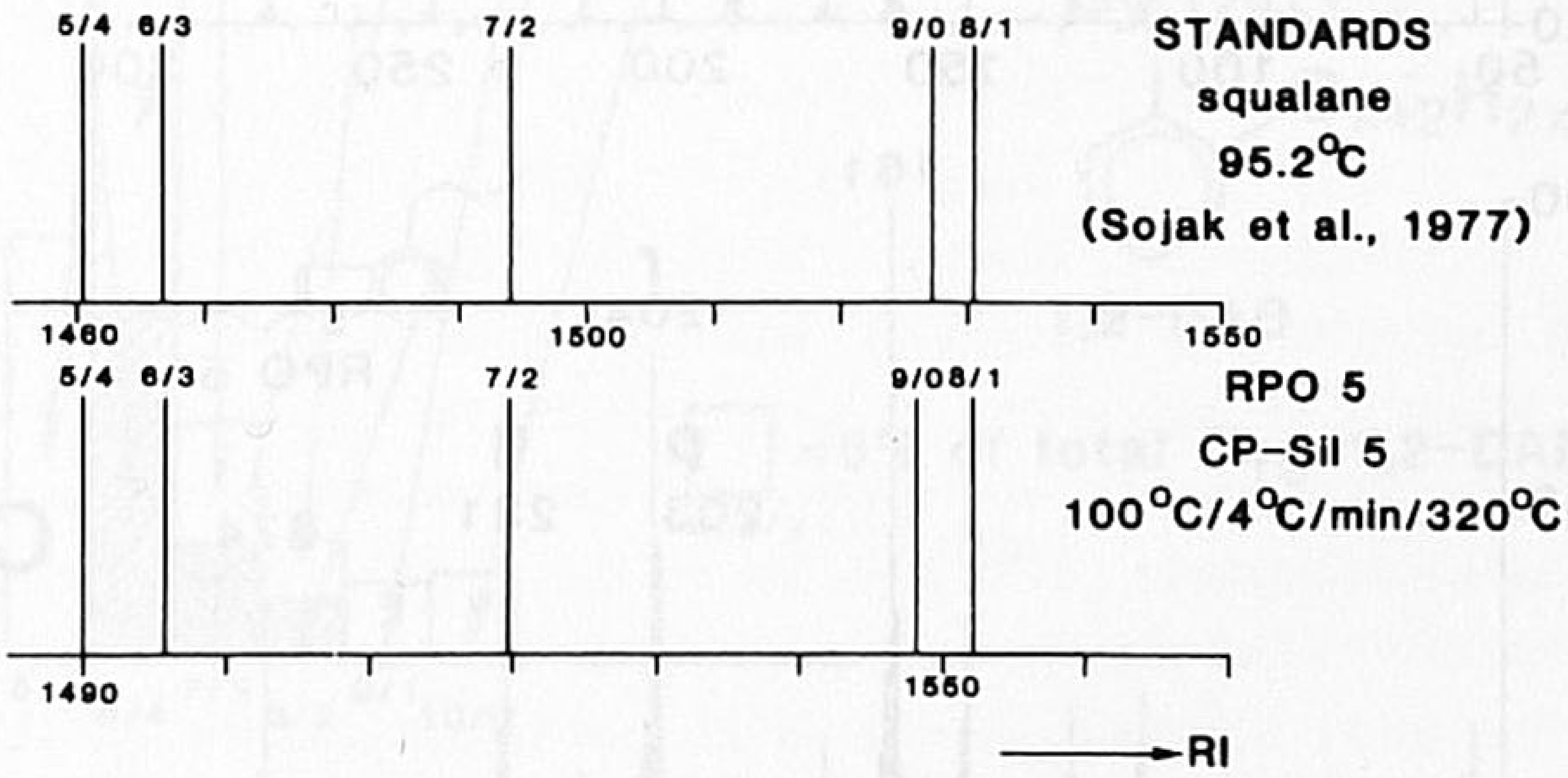

Fig. 6.13. Comparison of retention indices of the C_{15} 1,2-DAB obtained by desulphurisation of the C_{15} 2,4-DABT of fraction 5′ of the "aromatic" fraction of the RPO and those of standards as reported by Sojak *et al.* (1977). Numbers refer to the number of carbon atoms of alkyl side chain(s) of positional isomers of the C_{15} DAB at positions 1 and 2, respectively.

an ion resulting from a McLafferty rearrangement (fragment r, Fig. 6.14). Two secondary fragment ions at *m/z* 160 and 161 caused by loss of the alkyl side chains as alkyl radical and an alkene and two alkyl radical ions, respectively, were also frequently observed. The rearrangement ion (fragment r)

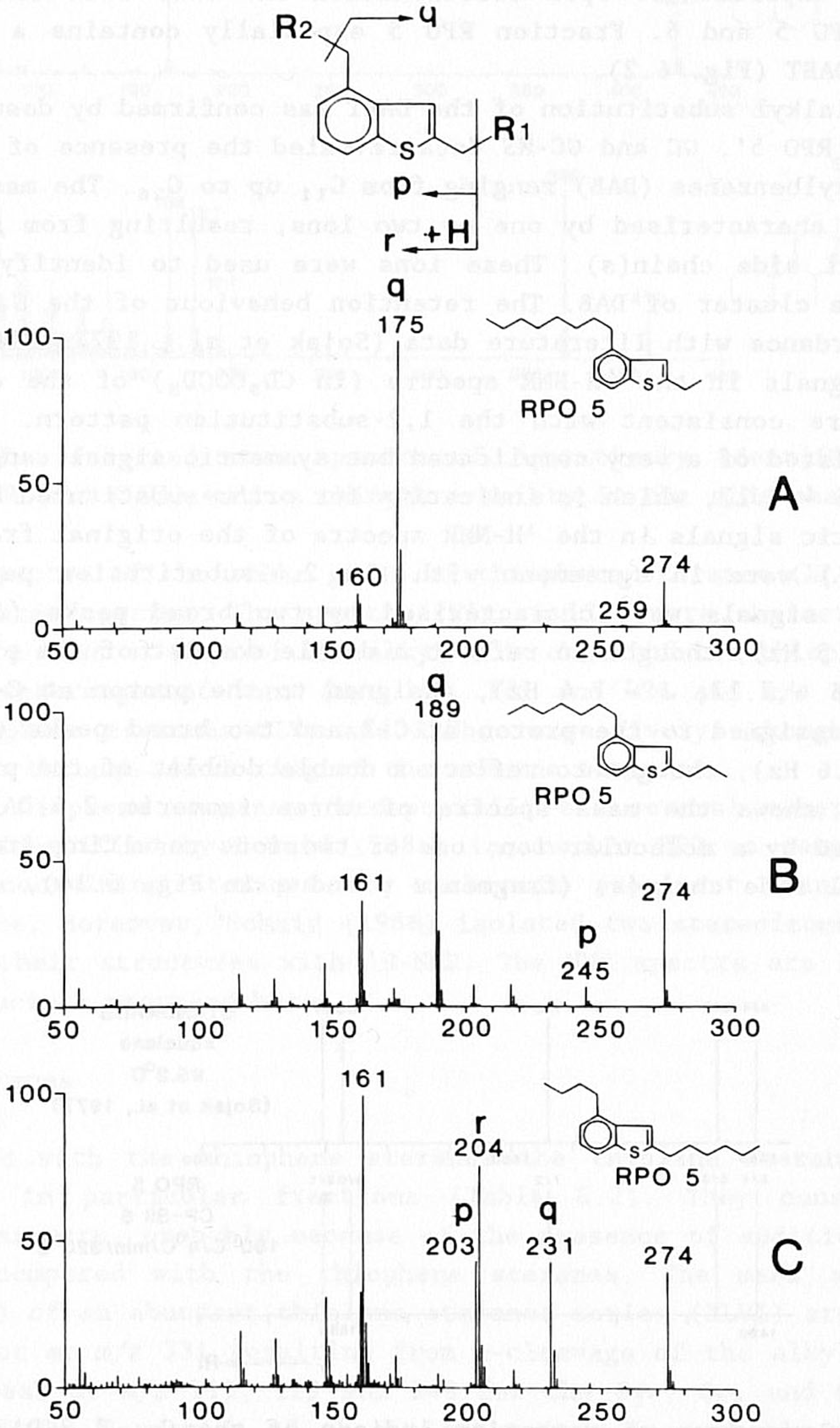

Fig. 6.14. Mass spectra (corrected for background) of: (A) 2-ethyl-4-octylbenzo[*b*]thiophene, (B) 4-heptyl-2-propylbenzo[*b*]thiophene and (C) 4-butyl-2-hexylbenzo[*b*]thiophene (XLIX). The letters refer to fragmentation pathways exemplified in the general structure shown.

could be due to a McLafferty rearrangement of both alkyl side chains. However, a rearrangement of one of these two was particularly favoured because, at the most, one such ion was observed in the spectra of DABT. To establish which alkyl side chain was involved in this rearrangement, the relative concentrations of both the C_{16} 2,4-DABT as well as their desulphurisation products, the C_{16} 1,2-DAB were compared (Fig. 6.15). Because a particular 2,4-DABT upon desulphurisation gives rise to a particular 1,2-DAB their relative concentrations (% of total 1,2-DAB or 2,4 DABT) have to be the same and therefore the 2,4-DABT could all be assigned. These assignments, in combination with the mass spectral data, indicated that the alkyl side chain at C-2 in the 2,4-DABT was involved in the rearrangement (Fig. 6.14).

All structural isomers of 2,4-DABT with a given number of carbon atoms were encountered. Fig. 6.16 shows the presence of the C_{18} 2,4-DABT using the appropriate mass chromatography. The McLafferty rearrangement of the alkyl side chain at C-2 of the benzothiophene ring was indispensible in distinguishing the two isomers with the same alkyl substituents but at the

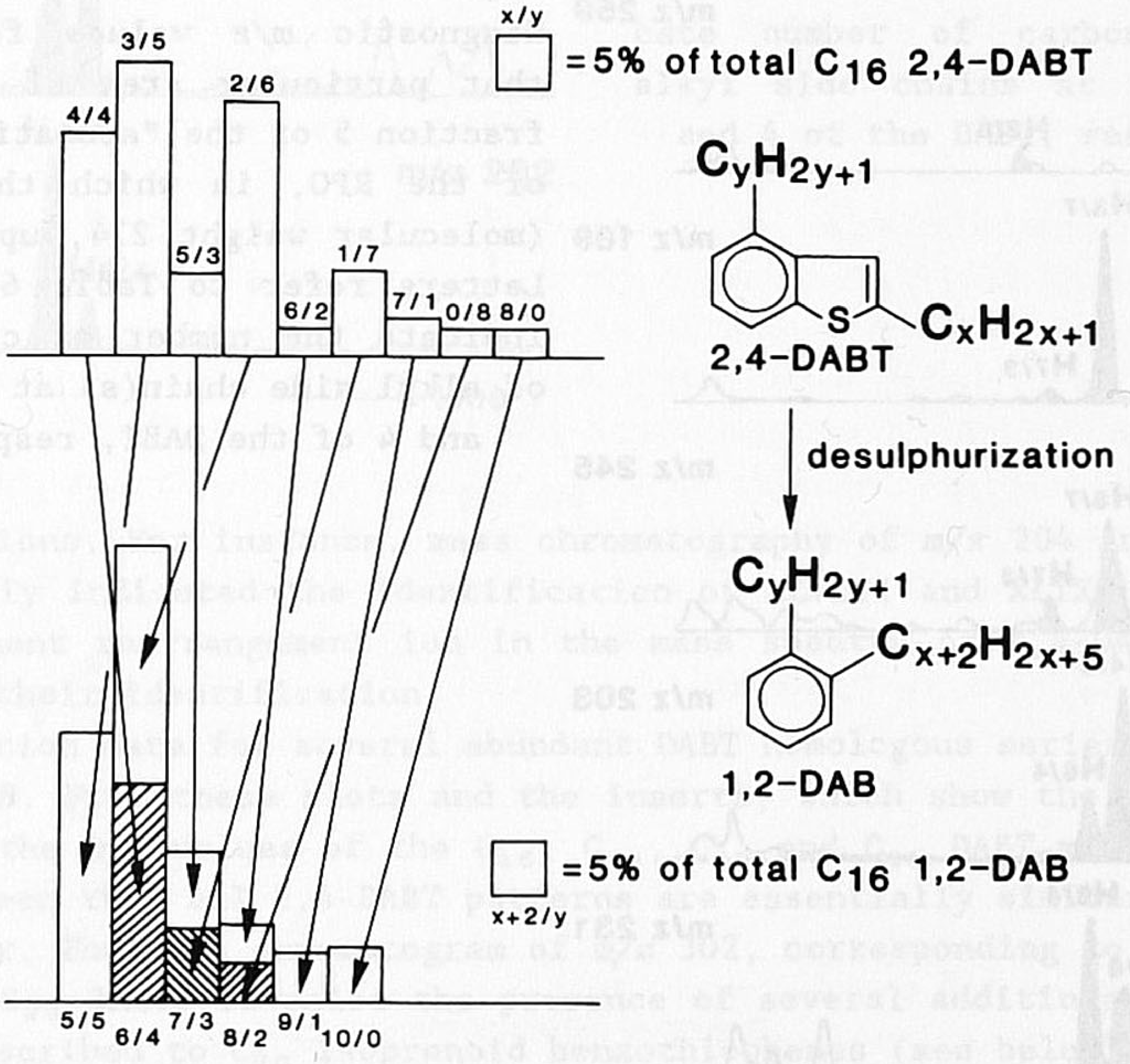

Fig. 6.15. Histograms showing distributions of C_{16} 2,4-DABT (upper) from fraction RPO 5′ and C_{16} 1,2-DAB (lower) from Raney Ni desulphurisation. The numbers refer to number of carbon atoms of alkyl side chain(s) of the positional isomers of the C_{16} DABT at position 2 and 4, respectively, and of the C_{16} DAB at position 1 and 2, respectively.

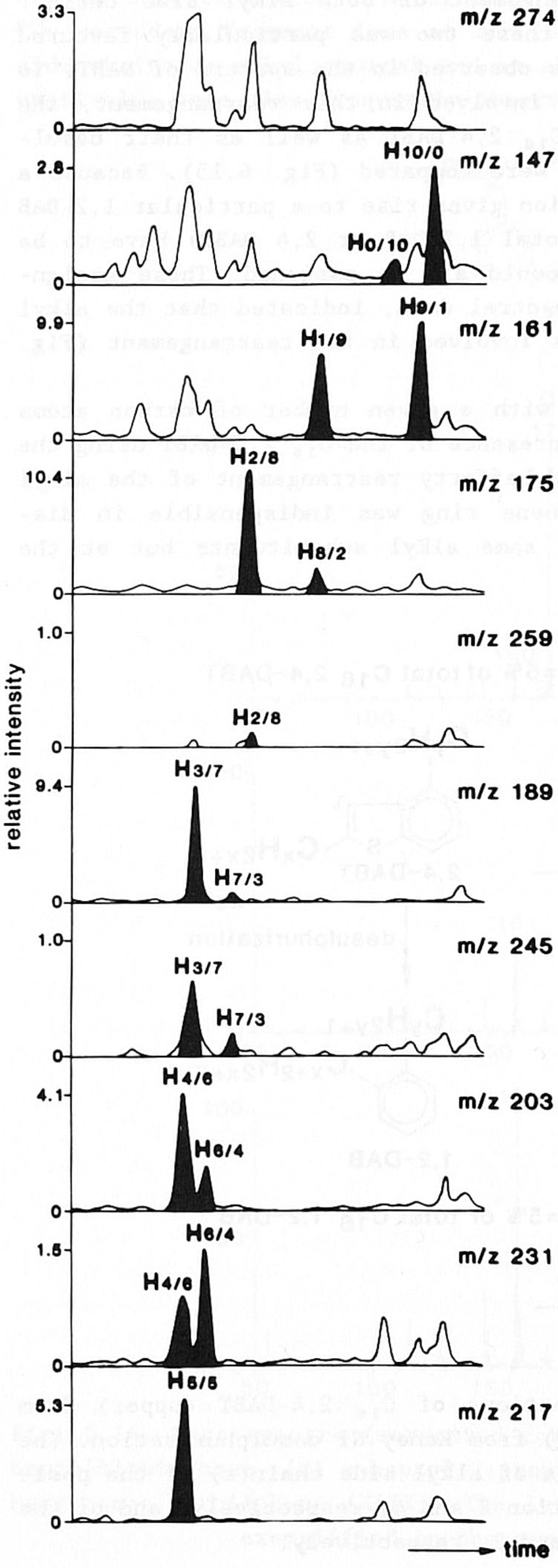

Fig. 6.16. Mass chromatograms of diagnostic *m/z* values for DABT of that particular area of the TIC of fraction 5 of the "aromatic" fraction of the RPO, in which the C_{18} DABT (molecular weight 274, upper) elute. Letters refer to Table 6.2. Numbers indicate the number of carbon atoms of alkyl side chain(s) at positions 2 and 4 of the DABT, respectively.
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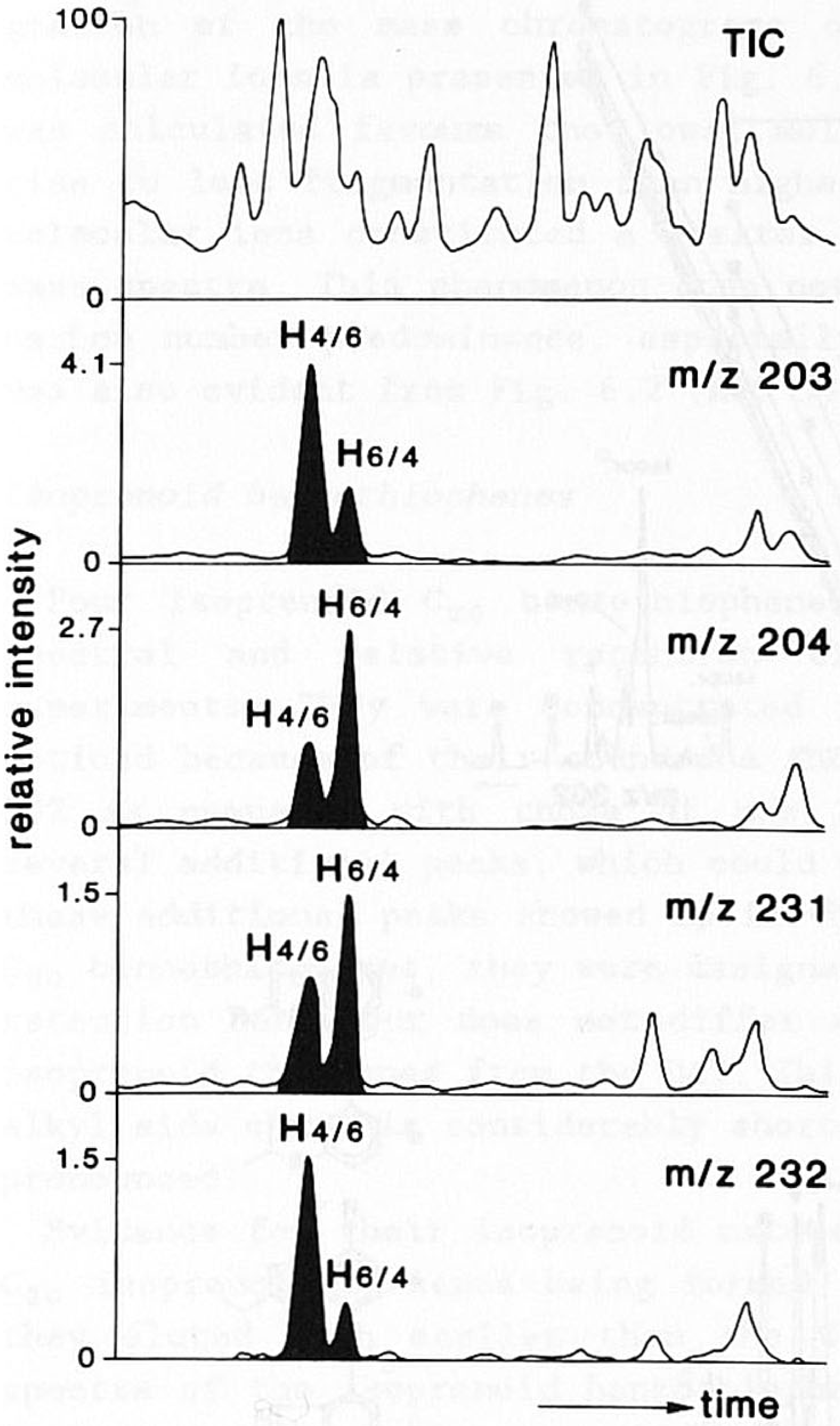


Fig. 6.17. Mass chromatograms of *m/z* 203, 204, 231 and 232 of that particular area of the TIC of fraction 5 of the "aromatic" fraction of the RPO, in which the C_{18} DABT elute. They illustrate the use of the diagnostic value of the rearrangment ion in the mass spectra of the DABT for distinguishing two isomers with the same alkyl substituents but at different positions on the molecule. Letters refer to Table 6.2. Numbers indicate number of carbon atoms of alkyl side chains at positions 2 and 4 of the DABT, respectively.

other positions. For instance, mass chromatography of *m/z* 204 and 232 (Fig. 6.17) readily indicated the identification of XLVIII and XLIX. The absence of a prominent rearrangement ion in the mass spectra of 2,4-DABT was also helpful in their identification.

The retention data for several abundant DABT homologous series are plotted in Fig. 6.18. From these plots and the inserts, which show the mass chromatograms of the *m/z* values of the C_{16}, C_{18}, C_{20} and C_{22} DABT molecular ions, it can be seen that all 2,4-DABT patterns are essentially similar to the C_{18} DABT cluster. The mass chromatogram of *m/z* 302, corresponding to the molecular ion of C_{20} DABT, revealed the presence of several additional compounds. They were ascribed to C_{20} isoprenoid benzothiophenes (see below).

In parallel with our identifications of DABT these compounds were also identified by Perakis (1986) in a very immature sediment from Marocco and in another RPO sample by synthesis of all possible C_{18}-DABT including 2- and 4-decylbenzo[*b*]thiophene. The mass spectra and relative retention times of the synthetic compounds are in complete agreement with our results.

The carbon number distribution pattern of the DABT as obtained by inte-

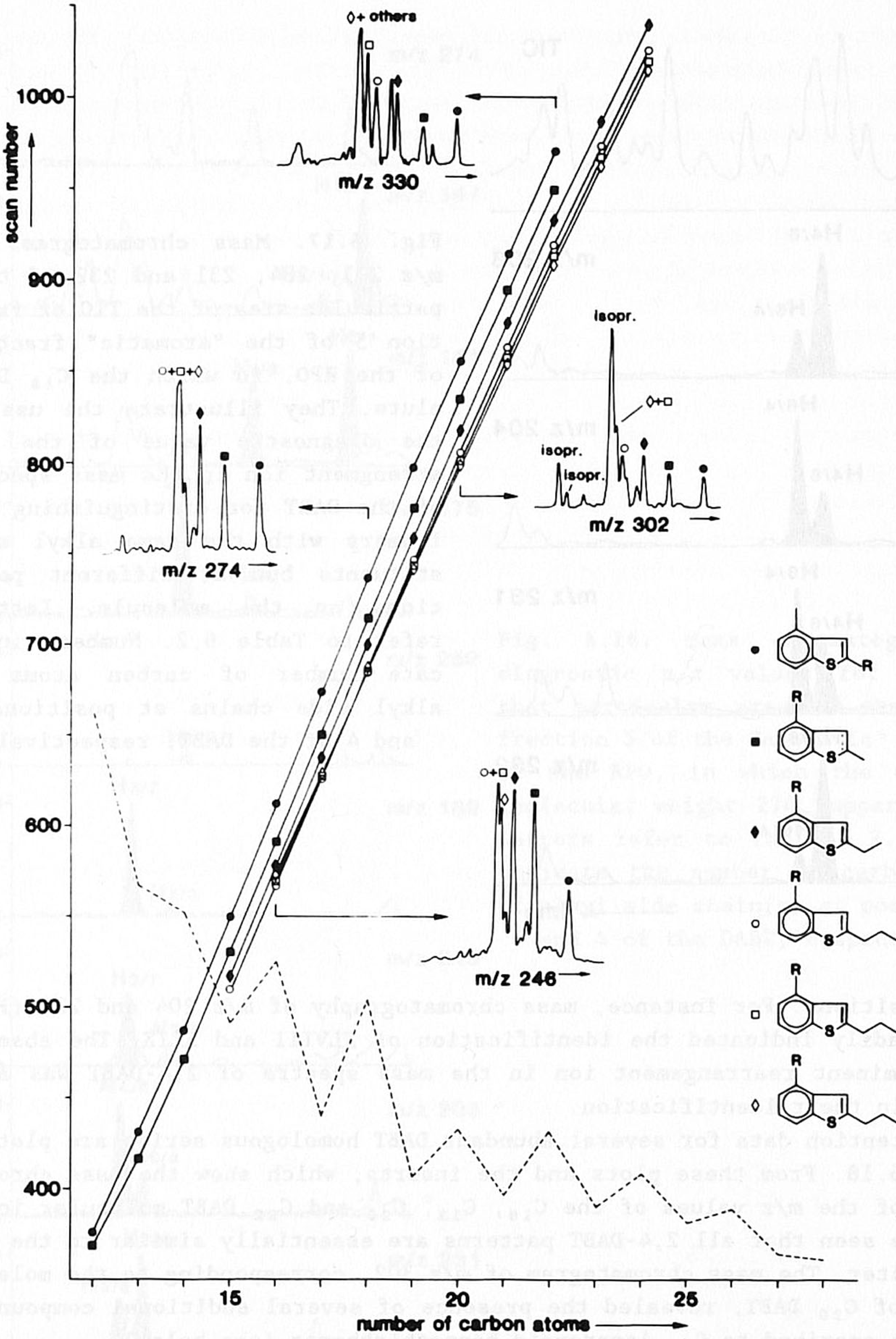


Fig. 6.18. A plot of scan numbers of a number of homologous series of 2,4-DABT in the TIC of RPO 5 as a function of number of carbon atoms. Insets show mass chromatograms of the molecular ions of the C_{16}, C_{18}, C_{20} and C_{22} DABT. Symbols refer to homologous series as indicated. Isopr. refers to the presence of isoprenoid benzothiophenes. The dashed line shows the carbon number distribution pattern of the DABT, normalized to the C_{12} DABT.

gration of the mass chromatograms of the m/z values of the respective molecular ions is presented in Fig. 6.18 (dotted line). The way in which it was calculated favours the lower molecular weight DABT because they give rise to less fragmentation than higher molecular weight DABT and hence the molecular ions constituted a greater part of the total ion yield of their mass spectra. This phenomenon does not affect extensively the even-over-odd carbon number predominance, especially that of the C_{16} and C_{18} DABT. This was also evident from Fig. 6.2 (RPO 5)

Isoprenoid benzothiophenes

Four isoprenoid C_{20} benzothiophenes (L-LIII) were identified from mass spectral and relative retention time data and from desulphurisation experiments. They were concentrated in fractions RPO 5 and 6. They were noticed because of their abundance and because the mass chromatogram of *m/z* 302 as compared with those of *m/z* 246, 274 and 330 (Fig. 6.18) showed several additional peaks, which could not be ascribed to DABT isomers. Since these additional peaks showed up in the mass chromatogram indicative of the C_{20} benzothiophenes, they were assigned as isoprenoid benzothiophenes. Their retention behaviour does not differ as much from the DABT as that of the isoprenoid thiolanes from the DAT. This is probably due to the fact that the alkyl side chain is considerably shorter and the effect of branching is less pronounced.

Evidence for their isoprenoid nature was obtained after desulphurisation, C_{20} isoprenoid benzenes being formed. They were identified as such because they eluted much earlier than the C_{20} 1,2-DAB (at about C_{18}). The mass spectra of the isoprenoid benzothiophenes were characterised by a molecular ion at *m/z* 302 and ions at *m/z* 175 (L; Sinninghe Damsté and de Leeuw, 1987), 189 (LI), 245 and 189 (LII) and 259, 231 and 189 (LIII), respectively. The mass spectral data of the desulphurised isoprenoid benzothiophenes were in accordance with these assignments. The four C_{20} isoprenoid benzenes were assigned as LIV (characteristic *m/z* values 119 and 274; derived from L), LV (characteristic *m/z* values 133, 161, 245 and 274; derived from LI), LVI (characteristic *m/z* values 133, 189 and 274; derived from LII) and LVII (characteristic *m/z* values 133, 175 and 274; derived from LIII).

Isoprenoid bithiophenes

Three C_{20} isoprenoid bithiophenes have been assigned from mass spectral data, relative response on the FPD and desulphurisation experiments. They were abundant in RPO 6 (Fig. 6.2).

The presence of two sulphur atoms per molecule was concluded from the much higher FPD response relative to compounds containing one sulphur atom, and from exact mass measurements (Sinninghe Damsté and de Leeuw, 1987). Raney Ni desulphurisation of these fractions, which consisted for a considerable part of bithiophenes, resulted in a mixture of compounds among which phytane was

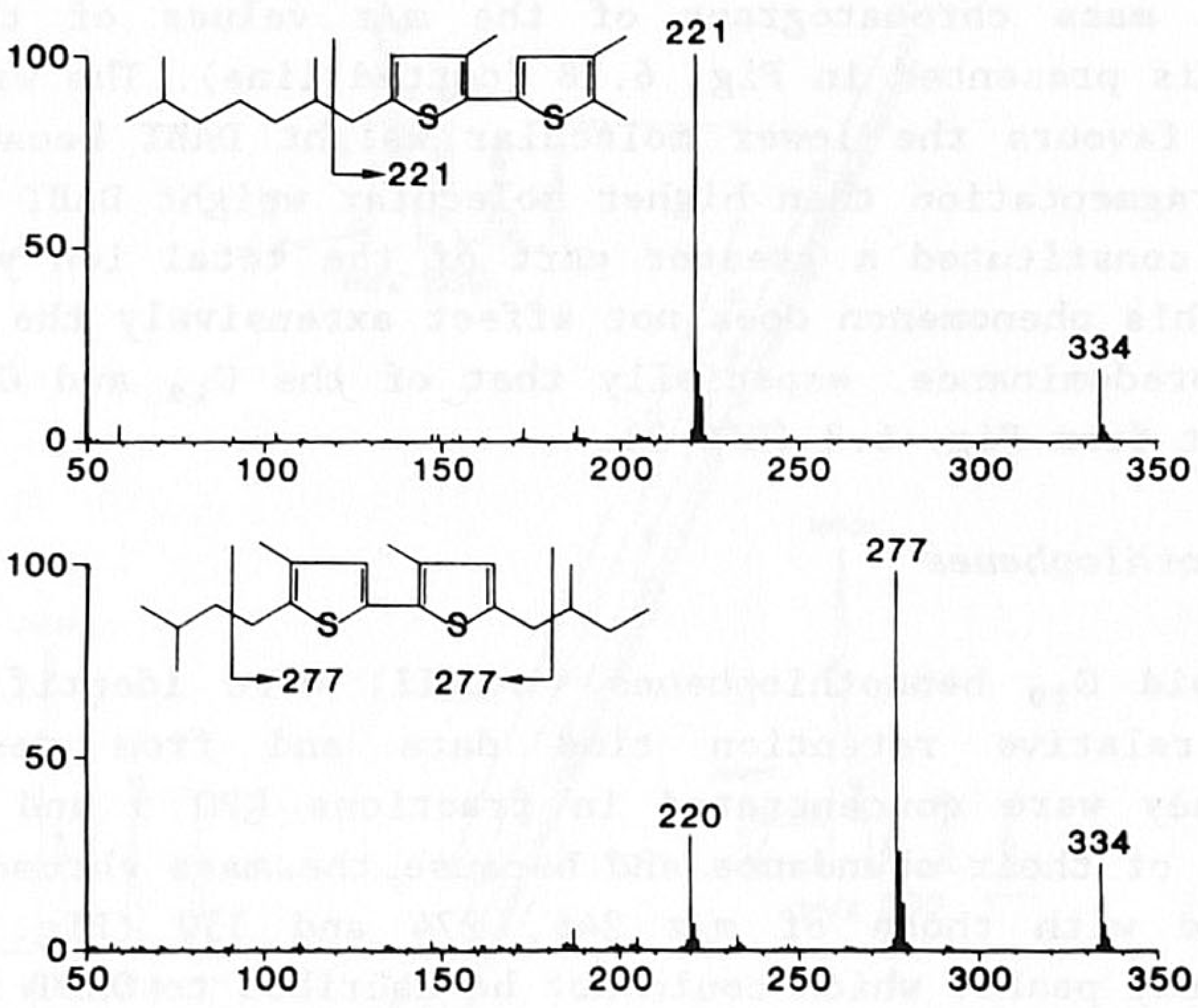


Fig. 6.19. Mass spectra (corrected for background) of isoprenoid bithiophenes LX (upper) and LIX (lower).

a dominant constituent. This established that these bithiophenes possess a phytane carbon skeleton.

Mass spectral data (Fig. 6.19) together with the above presented results led to their conclusive identification (Sinninghe Damsté and de Leeuw, 1987). They were identified as LVIII, LIX and LX. Their presence was also noted by Schmid (1986) in another RPO sample.

A C_{15} isoprenoid bithiophene (LXI) was also encountered. Its mass spectrum was characterised by a molecucular ion at m/z 264 and a fragment ion at *m/z* 211. In addition several other C_{18}, C_{19}, C_{21} and C_{22} isoprenoid bithiophenes, although much less abundant, were encountered.

Other sulphur containing compounds

Two (thienyl)alkylthiophenes (LXII and LXIII) and a number of thienylthiolanes (LXIV-LXVII) were identified as described elsewhere (Sinninghe Damsté and de Leeuw, 1987).

A number of sulphur-containing hopanoids were also encountered but were not studied in detail. Schmid (1986) has described the identification and occurrence of these compounds in another RPO sample.

6.5 DISCUSSION

The abundance of organic sulphur compounds (OSC) in Rozel Point Oil (RPO) is a reflection of the high sulphur content (7.5%). Although RPO is somewhat extreme in this respect, the OSC were studied because these compounds are present in a number of other oils and sediment extracts from different locations studied by us (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986; Sinninghe Damsté and de Leeuw, 1987; ten Haven *et al.*, 1988), an observation which will be discussed in detail in Part II of this series.

The high amount of organic sulphur made it possible for us to identify about a thousand individual OSC, most of them not previously known. These OSC differ essentially from most of the OSC previously identified in oils (*e.g.*, Thompson, 1981; Aksenov and Kamyanov, 1981; Hughes, 1984) because they have higher molecular weights and their structures seem to be related to certain bio- and/or geochemical precursors, thereby providing clues to their origin. The identification of these kinds of OSC started only recently (Payzant *et al.*, 1983; 1985; 1986; Valisolalao *et al.*, 1984; Cyr *et al.*, 1986; Schmid, 1986; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986; 1987a).

A prerequisite for the formation of these kinds of OSC is probably the presence of some reactive form of sulphur (*e.g.* H_2S, HS^-, polysulphides) in the original environment of deposition, which requires an euxinic environmental setting. Bacterial sulphate reduction leads to the production of H_2S which readily reacts with Fe ions to form inorganic sulphur compounds (Berner, 1984). However, in environments with a low Fe content (such as carbonate environments) Fe ions are readily depleted and the remaining H_2S produced is available for sulphur incorporation reactions, generating organic-sulphur-rich kerogens and/or extractable OSC (Gransch and Posthuma, 1974). This mechanism explains the fact that crude oils from carbonate-evaporite source rocks exhibit a consistently higher sulphur content (Tissot and Welte, 1984; Jones, 1984) and our observation that the high abundance of OSC in our samples (Sinninghe Damsté *et al.*, 1986; ten Haven *et al.*, 1988) seems to correlate with their hypersaline environment of deposition.

The OSC in RPO can be divided into three groups, *i.e.* those with an isoprenoidal carbon skeleton (thiophenes, thiolanes, benzothiophenes, bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes), those with a steroidal carbon skeleton (thiophene and thiolane steranes) and those possessing a linear carbon skeleton (2,5-di-*n*-alkylthiolanes, 2,6-di-*n*-alkylthianes and 2,4-di-*n*-alkylbenzo[*b*]thiophenes).

Isoprenoid OSC

The group of OSC with an isoprenoid carbon skeleton is dominant in RPO. Isoprenoid thiophene I is the most abundant OSC in the LMWA fraction of RPO. The carbon number distribution patterns of the various classes of OSC comprising this group are dominated by the C_{20} members (Table 6.2). This fact, together with the structural resemblance of all these C_{20} isoprenoid compounds (they all possess a phytane carbon skeleton) prompted us to propose a common precursor for these compounds (Sinninghe Damsté and de Leeuw, 1987). Sulphur incorporation into mono- and polyunsaturated C_{20} isoprenoid alcohols or their diagenetic products is proposed to lead to the formation of thiophenes I-V. The presence of additional double bonds in the alkyl side chain of these thiophenes may result in incorporation of another sulphur atom, leading to the formation of bithiophenes (LVIII-LX) and (thienyl)alkylthiophenes (LXII-LXIII), or in a ring closure reaction followed by aromatisation, leading to the formation of isoprenoid benzothiophenes (L-LIII). The isoprenoid thiolanes (*e.g.* XXXVII) and thienylthiolanes (LXIV-LXVIII) may be intermediate compounds. The precursors proposed for these reactions, unsaturated C_{20} isoprenoid alcohols, are widely distributed in the geosphere. The ubiquitous esterifying alcohol of (bacterio)chlorophylls *a* and *b*, phytol, would, upon sulphur incorporation, give rise to "terminal" C_{20} isoprenoid thiophenes I, XXVII and XVII. These compounds have been observed in a number of sediment extracts, including those from "normal" oceanic sediments (Brassell *et al.*, 1986c). The availability of polyunsaturated C_{20} isoprenoid alcohols, precursors proposed for the "mid-chain" thiophenes II-V, the "mid-chain" thiolanes, the bithiophenes, the (thienyl)-alkylthiophenes, the thienylthiolanes and the benzothiophenes, is, however, restricted to environments where photosynthetic sulphur bacteria and archaebacteria contribute significantly to the biomass. Hypersaline environments, such as sabkhas, are good examples in this respect.

These sulphur incorporation reactions occur at an early stage of diagenesis (Brassell *et al.*, 1986c); C_{20} isoprenoid thiophenes are already observed in a gypsum crust containing layers of living photosynthetic sulphur bacteria overlying a sulphate reducing zone (Goossens, unpublished results).

The other isoprenoid OSC are thought to be formed by the same mechanism, starting with other substrates. So, the C_{15} isoprenoid thiophenes (VI-VIII), thiolanes (*e.g.*, XXXVIII) and bithiophene (LXI) are thought to be formed from sulphur incorporation into farnesol (or its diagenetic products), the C_{30} isoprenoid thiophenes (IX-XII, XVI, XVIII) and thiolanes (*e.g.*, XL) from sulphur incorporation into squalenes and/or hydrosqualenes, and the C_{20} and C_{25} highly branched thiophenes (XXIV and XXV) and C_{20} thiolane (XXXIX) from sulphur incorporation into the C_{20} and C_{25} highly branched alkenes, widely distributed in the geosphere (Rowland *et al.*, 1985; Robson and Rowland, 1986). The regular C_{35} (XIX-XXII) and C_{40} isoprenoid thiophenes may result from sulphur incorporation into C_{35} and C_{40} polyprenols or their diagenetic products, bacterial compounds proposed to be precursors of the corresponding regular isoprenoid hydrocarbons occurring in certain petroleums (Albaiges, 1980: Albaiges *et al.*, 1985). These hydrocarbons were relatively abundant in Amposta-marino crude oil (Spain; sulphur content 4.3%; Moldowan *et al.*, 1986); Its source rock is thought to have been deposited in a hypersaline carbonate environment (Albaiges, 1980). The other isoprenoid thiophenes

(C_{18}-C_{19}, C_{21}-C_{28}) and bithiophenes (C_{18}-C_{19}, C_{21}-C_{23}) are less obviously related to particular precursors but may find an origin in unsaturated isoprenoid alkenes biosynthesized by archaebacteria (Sinninghe Damsté *et al.*, 1986).

Further evidence for the existence of this sulphur incorporation mechanism is the distribution of the saturated isoprenoid hydrocarbons (*e.g.* farnesane, phytane, squalane, 2,6,10-trimethyl-7-(3-methylbutyl)dodecane, regular C_{35} and C_{40} isoprenoid hydrocarbons), which are thought to have been formed from the same precursors as those proposed for the isoprenoid OSC. The carbon number distribution pattern of these isoprenoid hydrocarbons is remarkably similar with those of the isoprenoid thiophenes; both are for instance dominated by the C_{20} member(s).

Steroidal OSC

The OSC with a steroidal carbon skeleton also occur in RPO. The thiophene steranes XLIa-c were conclusively identified by synthesis by Schmid (1986). Apart from these thiophene steranes several other groups (XLIII-XLV) were tentatively identified. The composition of the thiolane steranes occurring in RPO is very complex. Some of these could be tentatively identified, because they are saturated counterparts of the thiophene steranes (XLI). More work on these types of thiolane steranes has been reported recently (Schmid, 1986).

The origin of these compounds is as yet unclear. Cyr *et al.* (1986) suggested a biosynthetic origin for a series of hopanoid OSC. However, an early diagenetic sulphur incorporation into *e.g.* hop-17(21)-enes and Δ^{22}-sterols and/or sterenes also may explain the formation of the hopanoid and steroid OSC, respectively. Valisolalao *et al.* (1984) identified a C_{35} pentacyclic triterpenoid of the hopane series containing a thiophene ring in its side chain in immature sediments and suggested an origin from incorporation of bacterially-formed sulphur into a suitable precursor, *e.g.* bacteriohopanetetrol, supporting this view. A formation of thiophene steranes due to a reaction of elemental sulphur at elevated temperatures with steranes is less likely (Schmid, 1986).

Linear OSC

The series of OSC with a linear carbon skeleton are all characterised by a continuous carbon number distribution from C_{11} to C_{28}, with an even-over-odd carbon number predominance. The starting and end points of these distributions, however, are both determined by the experimental conditions used. Desulphurisation experiments established that higher molecular weight members of these series are probably present.

It was recognized earlier, especially by several Russian investigators, that monocyclic sulfides are predominant types of OSC in certain oils (Gal'pern, 1971, 1976; Polyakova *et al.*, 1978; Aksenov and Kamyanov, 1981).

Some of the structures of the lower molecular weight monocyclic sulfides, called thiophanes, were identified already at the beginning of this century (Mabery and Quale, 1900, 1906). These identifications were subsequently confirmed and those of other low molecular weight monocyclic sulphides (C_4-C_8) reported (Gal'Pern, 1971; Thompson, 1981). Aksenov and Kamyanov (1981) described the C_{10}-C_{20} monocyclic sulphides in the 300-325°C cut of a West Siberian petroleum as primarily 2-*n*-alkyl or 2,5-di-*n*-alkyl monocyclic sulphides because of the formation of *n*-alkanes on desulphurisation. Based on literature data the thiolanes were estimated to be 60-70% of the monocyclic sulfides in the middle cuts distilled from various petroleums, and the thianes 30-40%. The presence of higher molecular weight di-*n*-alkylthiolanes and -thianes, although with a then unknown substitution pattern, was described by Connan *et al.* (1983) and de Leeuw (1986) in several oils.

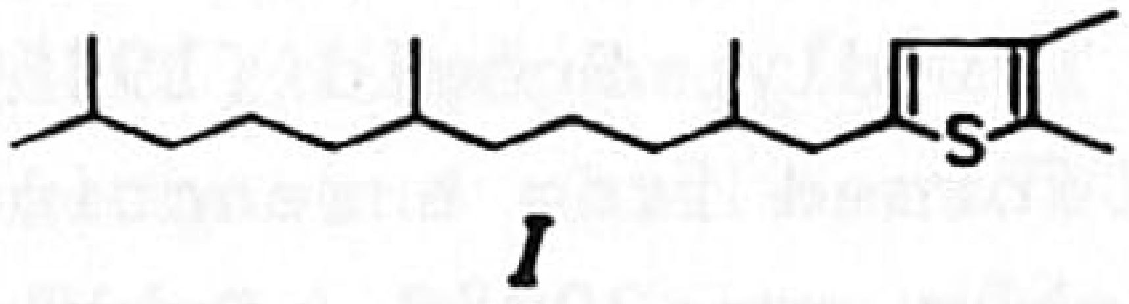
The 2,5-di-*n*-alkylthiolanes and 2,6-di-*n*-alkylthianes in RPO are characterised by the presence of all the structural and stereoisomers. The mono *n*-alkylthiolanes and -thianes were not present. Similar results, also based on synthesis of reference compounds, were reported by Schmid (1986) and Schmid *et al.* (1987) on the aliphatic sulphides of the Maruejols crude oil and another sample of RPO. The carbon number distributions of these compounds resemble that of the *n*-alkanes (Fig. 8); all distributions are dominated by the C_{16} and C_{18} members and exhibit an even-over-odd carbon number predominance. This observation is comparable with that made for the isoprenoid thiophenes and isoprenoid hydrocarbons whose distributions also resemble each other, suggesting a similar process of formation. Appropriate precursors for the DATL and DATN in such a process would be *n*-alkenes or polyunsaturated *n*-alkenes, compounds which are not so widely distributed as unsaturated isoprenoid alkanes. However, unsaturated fatty acids or alcohols (after dehydration) may also play a role as precursors of DATL and DATN. After sulphur incorporation into an unsaturated fatty acid, the acid group of the resulting intermediate product would be reduced to explain the even carbon number predominance of the DATL and DATN. Although this reaction is not a likely one (Nishimura and Baker, 1986), it has been postulated (Welte and Ebhardt, 1968; Kvenvolden, 1970; Albaiges and Torradas, 1974; Seifert, 1975; Sheng *et al.*, 1980) to explain the even-over-odd carbon number predominance of the *n*-alkanes sometimes observed. RPO is also characterised by a strong even carbon number predominance of the *n*-alkanes (ten Haven *et al.*, 1988). Recently, Verne-Mismer *et al.* (1986) also proposed a sedimentary reduction of carboxylic groups in porphyrins.

The inference that DATL and DATN are formed during early diagenesis, cannot be made definitely at the moment for several reasons. First, although the formation of isoprenoid thiophenes at the early stages of diagenesis has been shown convincingly (Brassell *et al.*, 1986c), the formation of DATL and DATN has not been observed. Futhermore, the presence of all structural isomers suggests that the double bond in the precursors was located at every possible position. Another possibility may be that, during thermal stress, all structural isomers of DATL and DATN were formed from one particular compound due to shifting of the carbon-sulphur bond. Thirdly, Schmid (1986) has demonstrated that 2,5-di-*n*-alkylthiophenes can be formed from a reaction between *n*-alkanes and *n*-alkenes with elemental sulphur at 200°C. Schmid (1986) stated that the reaction conditions were too vigorous to get thiolanes. However, at lower reaction temperatures no reaction was observed. Therefore, there is some doubt in the geochemical significance of these model experiments. The origin of the DATL and DATN remains, therefore, far from being understood.

The series of 2,4-di-*n*-alkylbenzo[*b*]thiophenes is also ranked under the OSC with a linear carbon skeleton since the substitution pattern suggests a relationship with the 2,5-di-*n*-alkylthiophenes. These compounds are present in only relatively small amounts in RPO but are much more important in an extract from a marl layer in the Northern Apennines (Italy) (Sinninghe Damsté *et al.*, 1986). The 2,4-DABT can be formed from these structurally related 2,5-di-*n*-alkylthiophenes *via* ring closure followed by aromatisation. Whether this mechanism operates in the geosphere cannot be answered at this moment. Because DABT were formed when alkanes are heated at 250°C in the presence of elemental sulphur (Schmid, 1986), Perakis (1986) suggested that the DABT might be formed from such a reaction. The carbon number distribution pattern of the 2,4-DABT (Fig. 18) is characterised by an even carbon number predominance just as that of the *n*-alkanes, DATN and DATL. In addition to the lower molecular weight DABT, the C_{16} and C_{18} members are present in abundance.

It is tempting to assume that upon diagenesis DATL and DATN are intermediates in the formation of dialkylthiophenes and that the dialkylthiophenes in their turn are intermediates in the formation of DABT. The similarities between the distribution patterns of DATL, DATN and DABT are in favour of such a hypothesis. Laboratory experiments in which the origin and formation of OSC and their presumed diagenetic pathways are simulated are presently carried out at this laboratory. Part II of this series will deal with the widespread occurrence of these OSC in the geosphere.

APPENDIX



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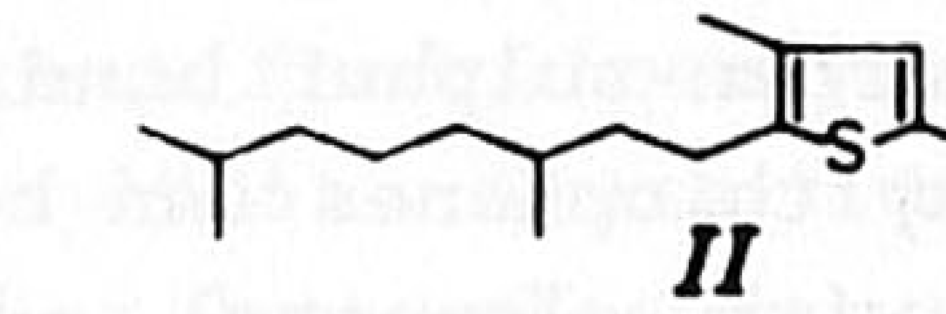

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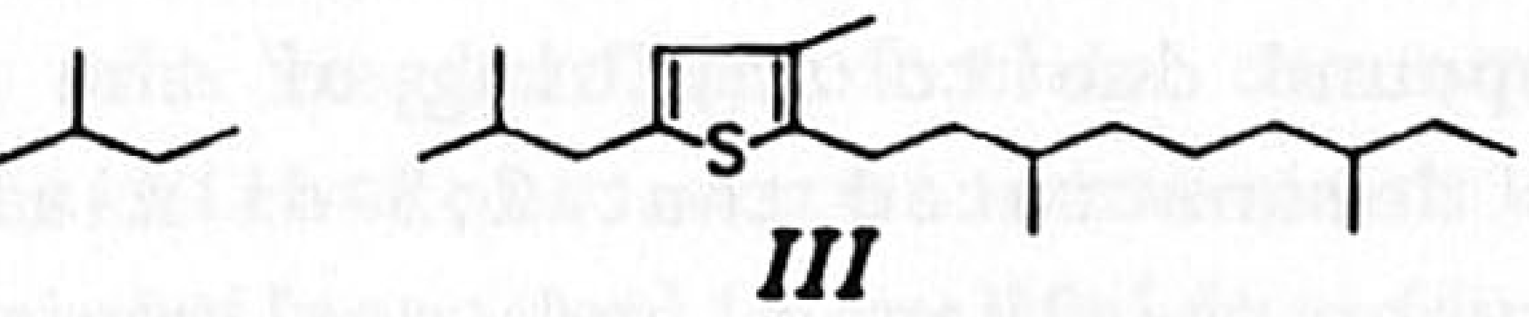

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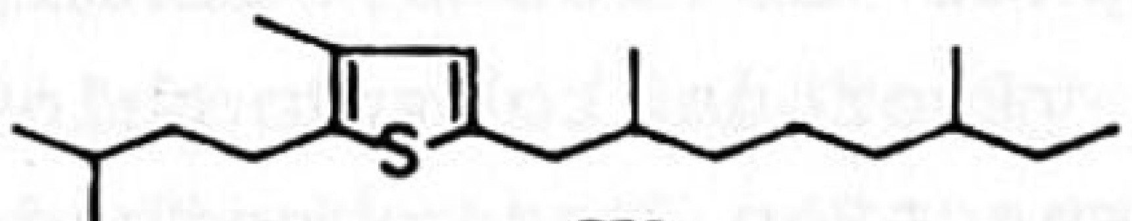

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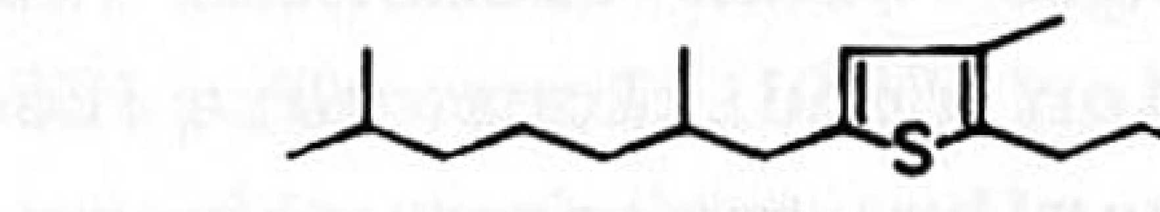

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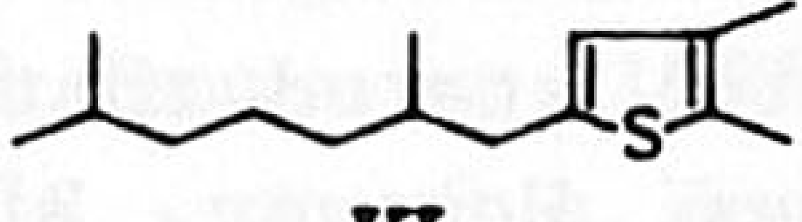

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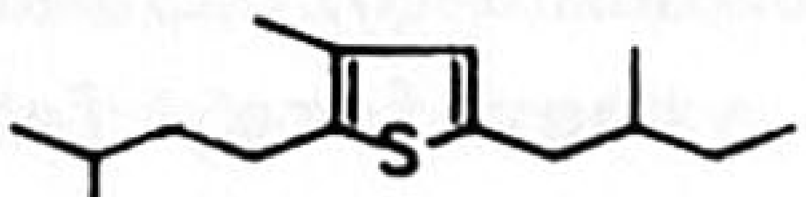

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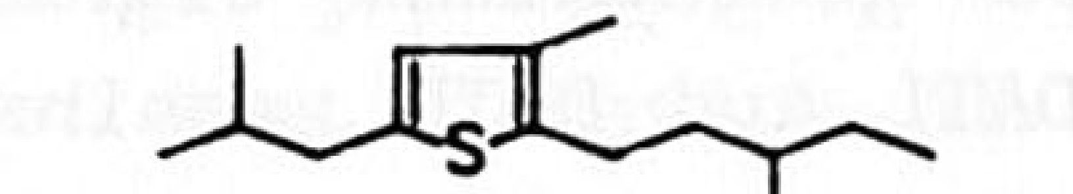

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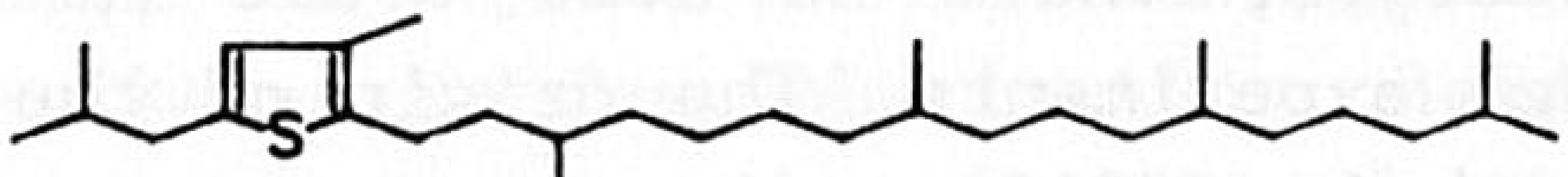

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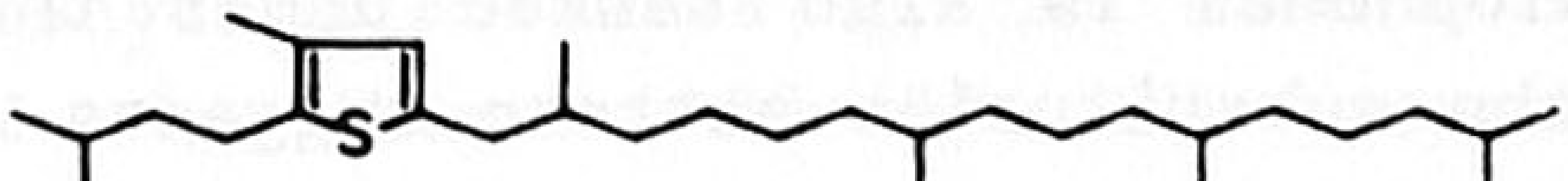

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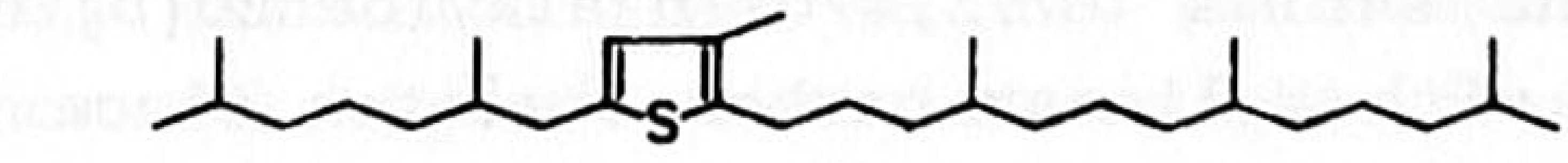

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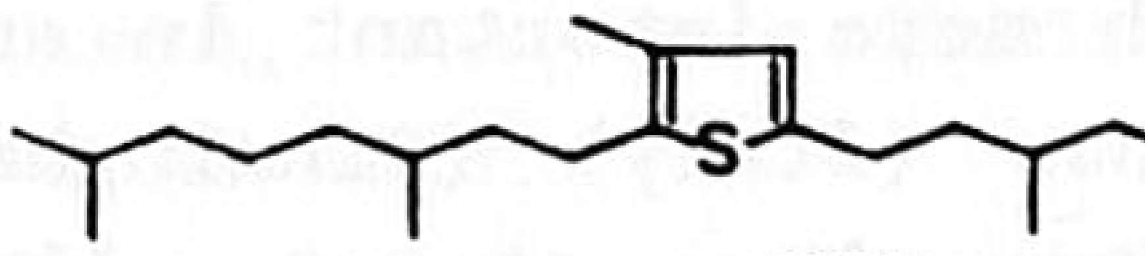

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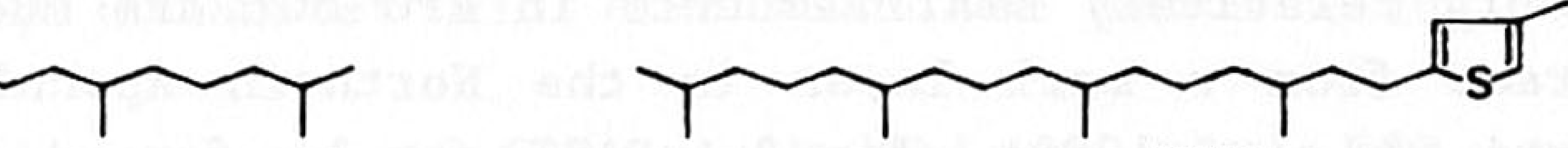

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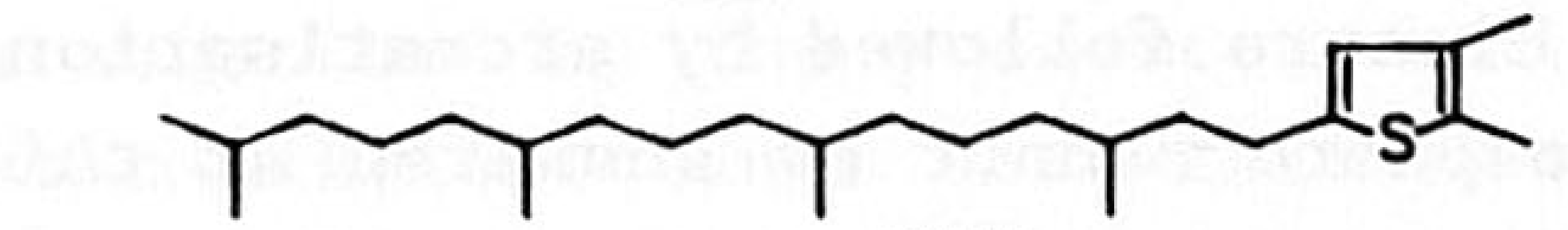

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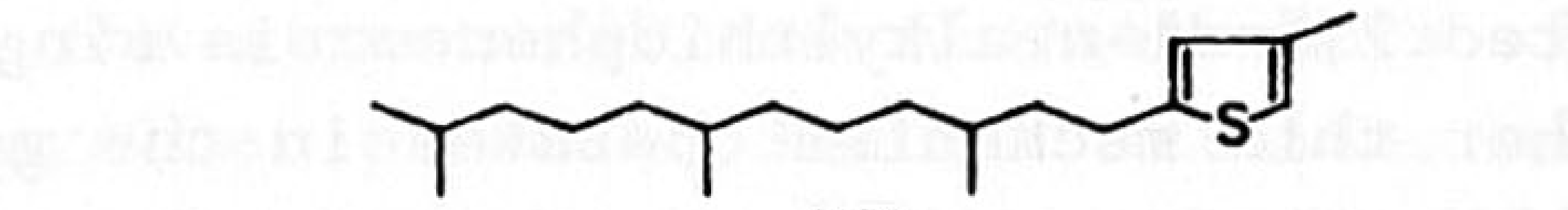

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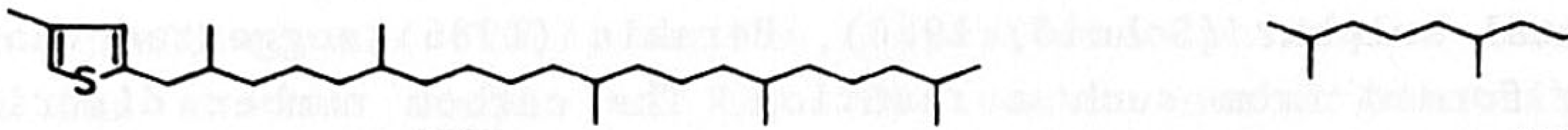

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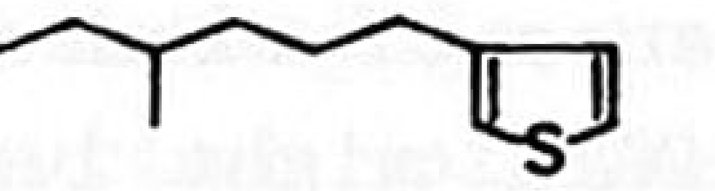

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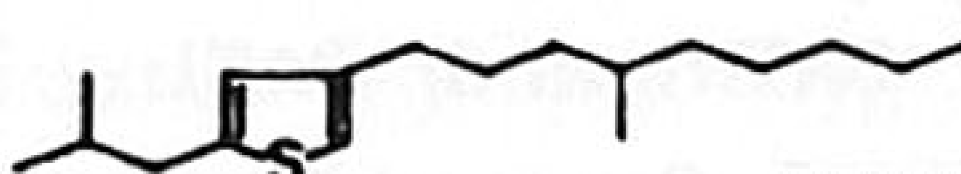

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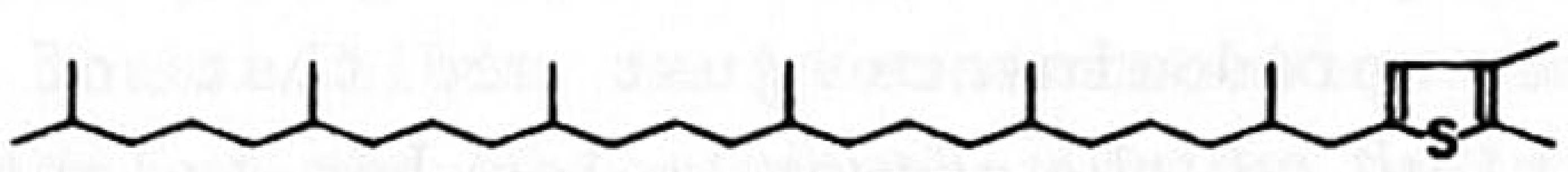

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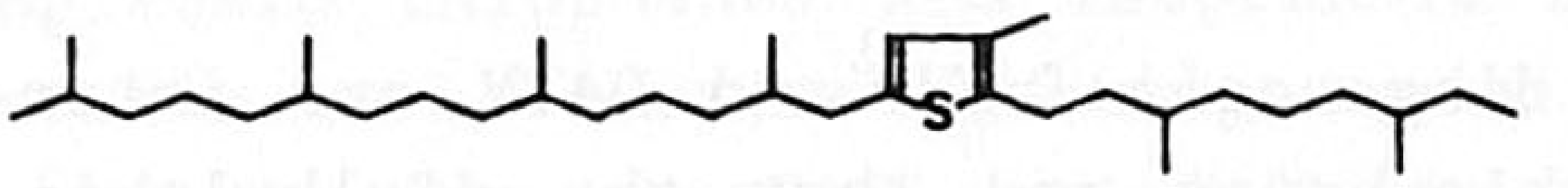

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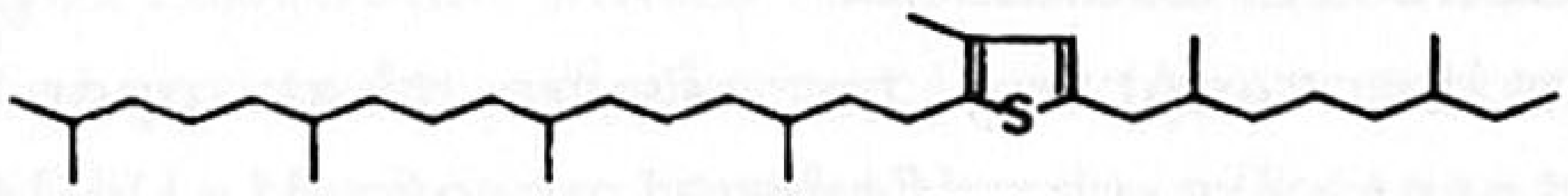

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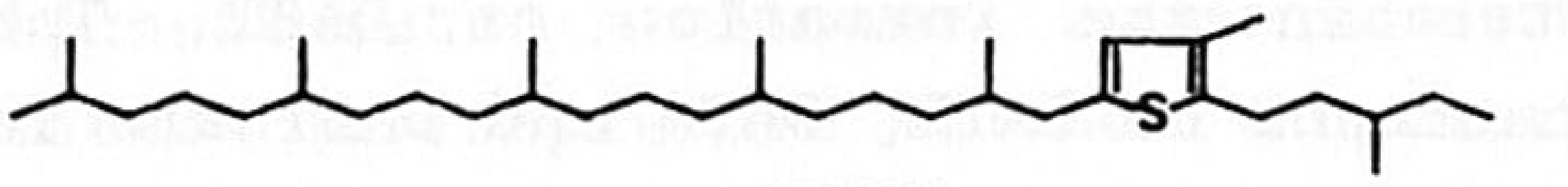

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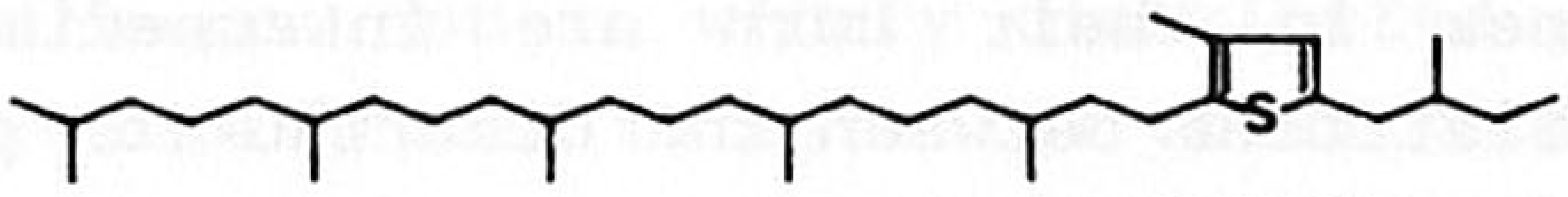

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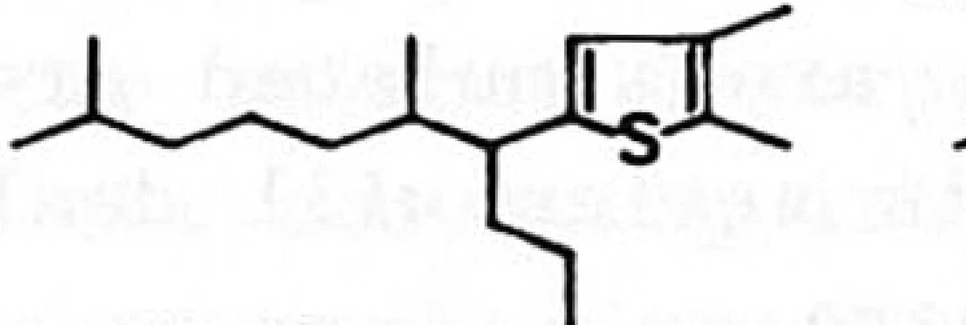

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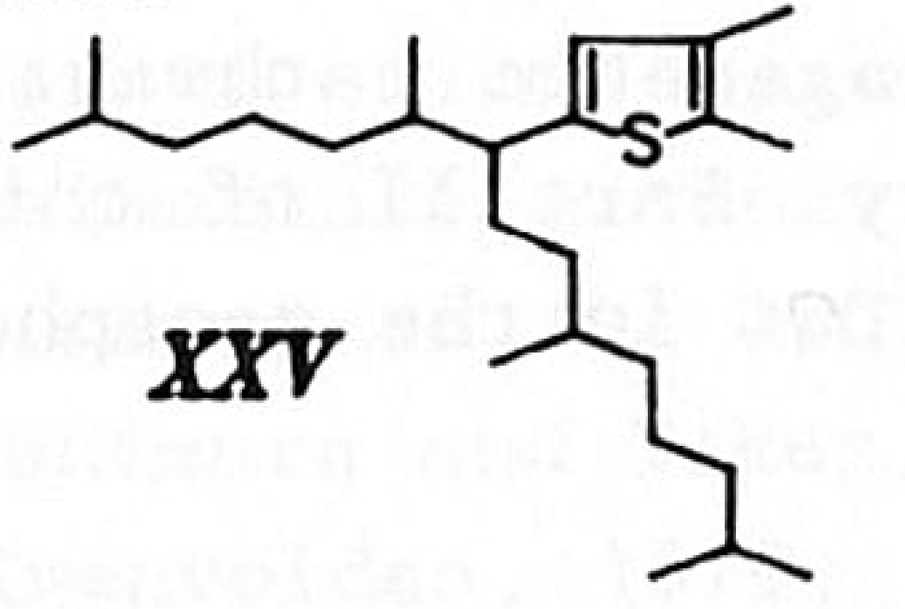

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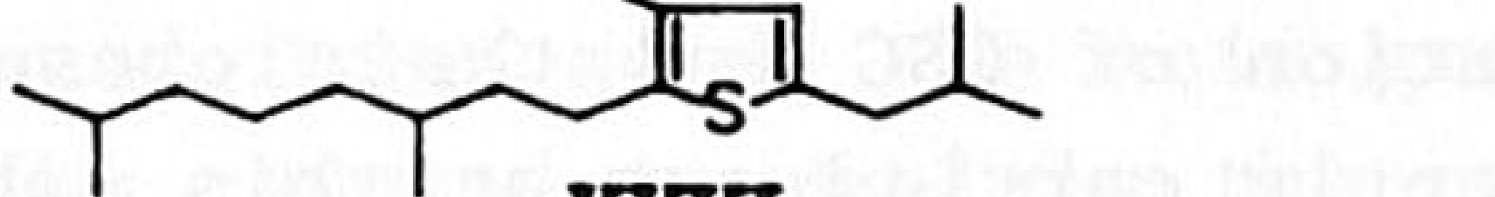

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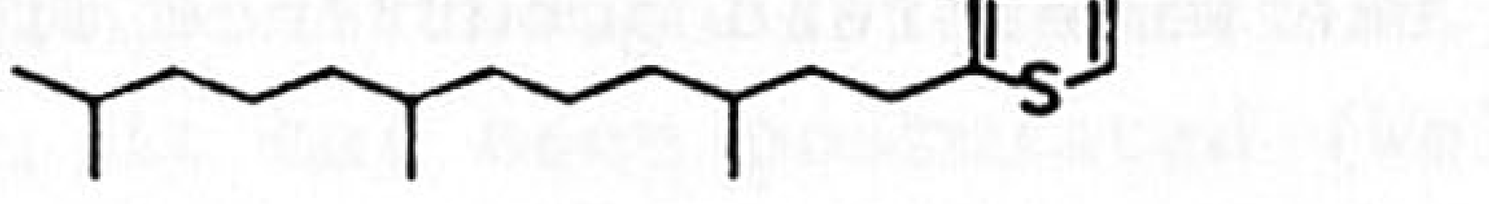

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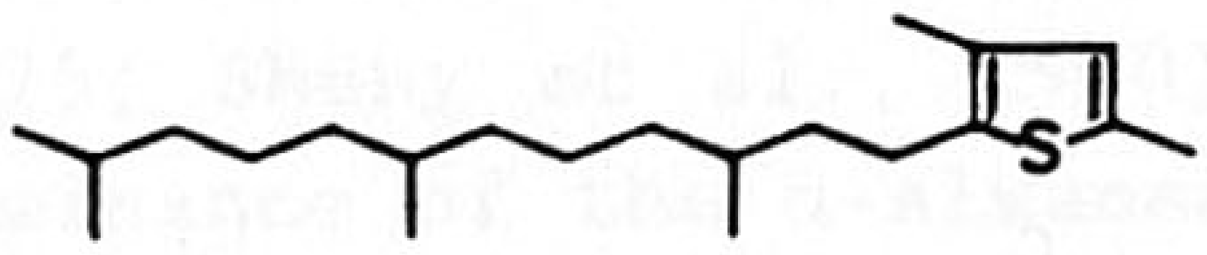

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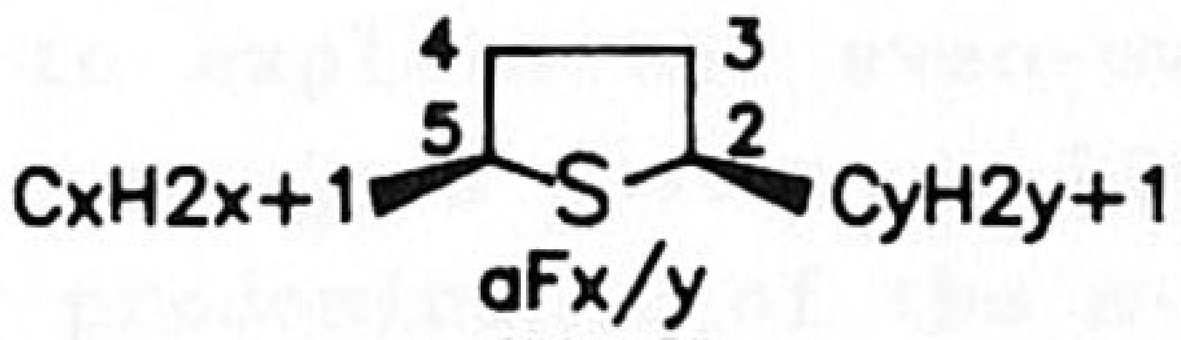

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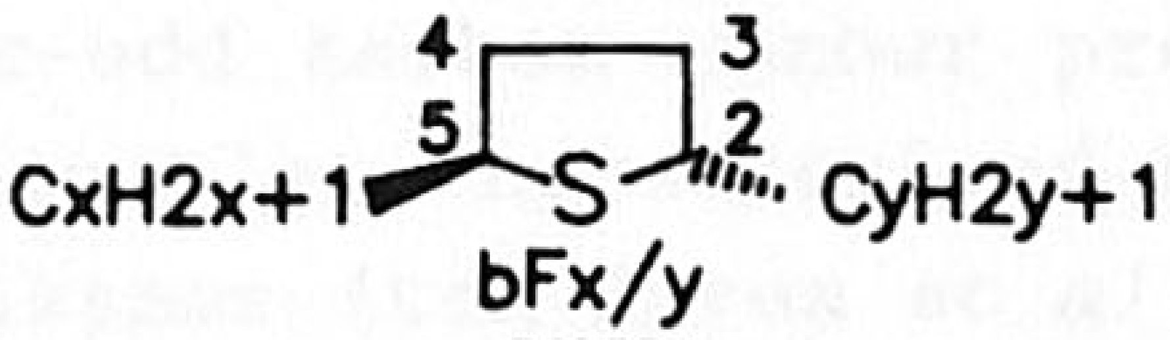

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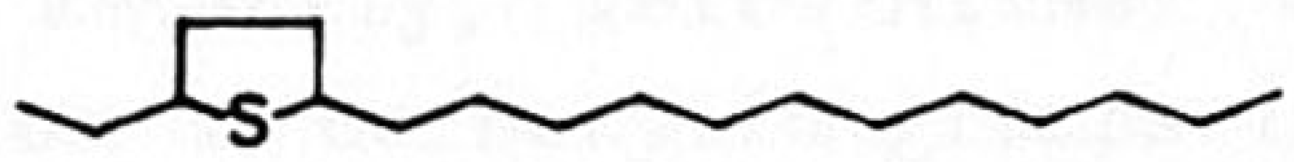

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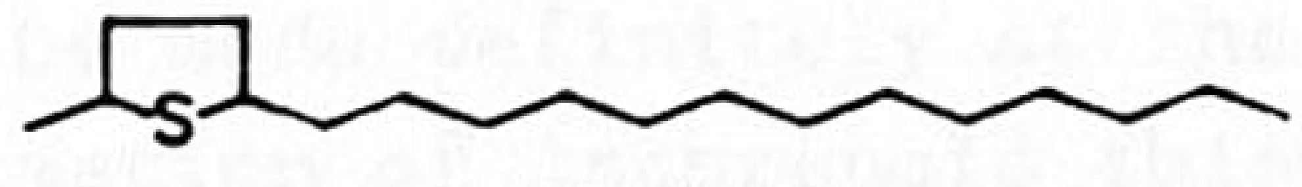

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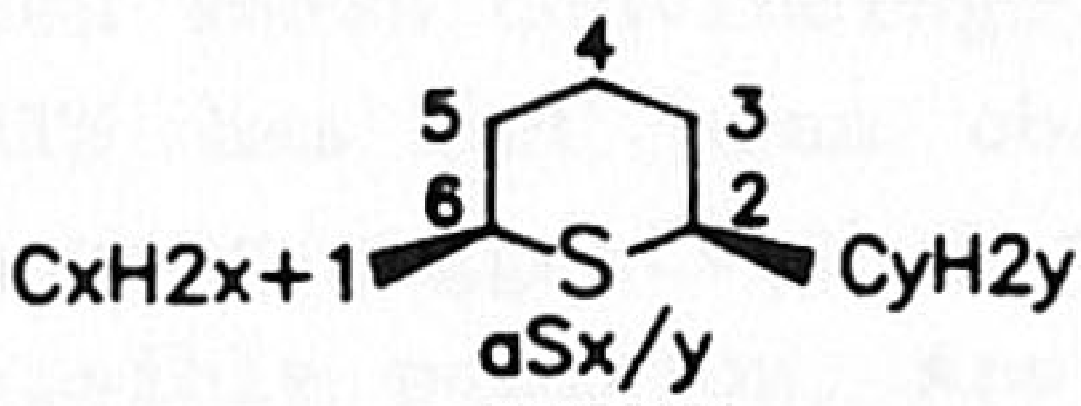

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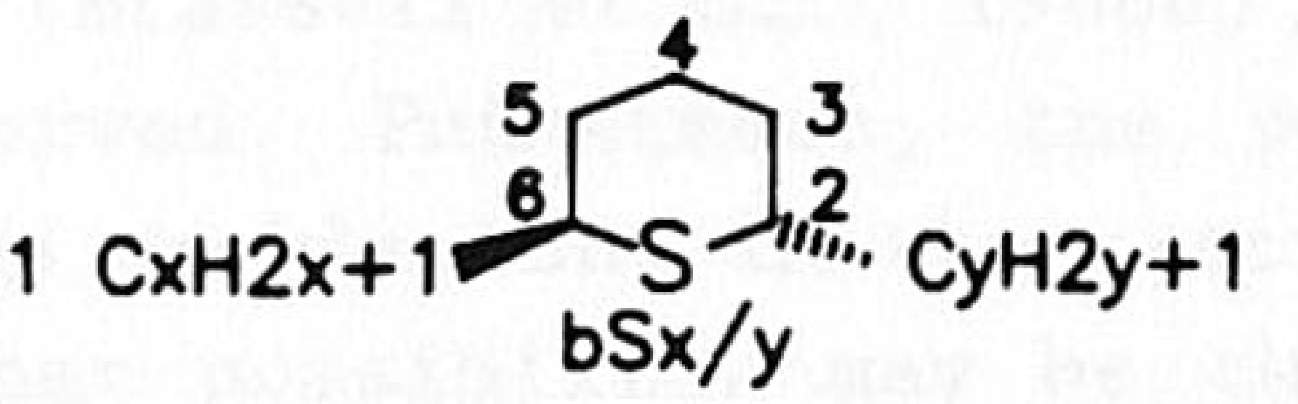

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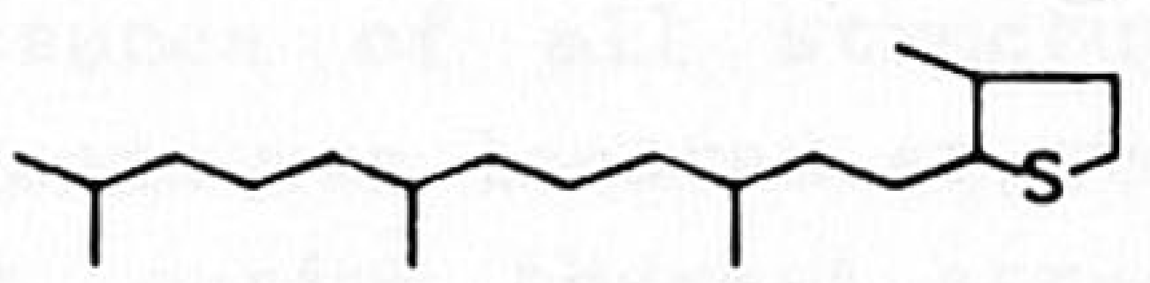

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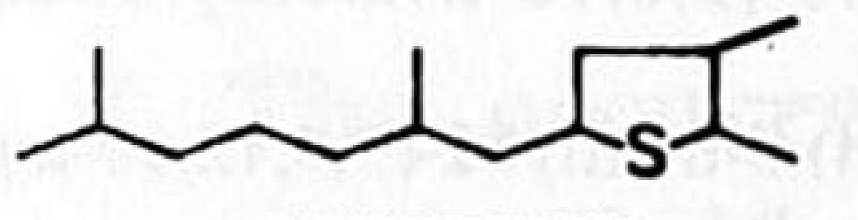
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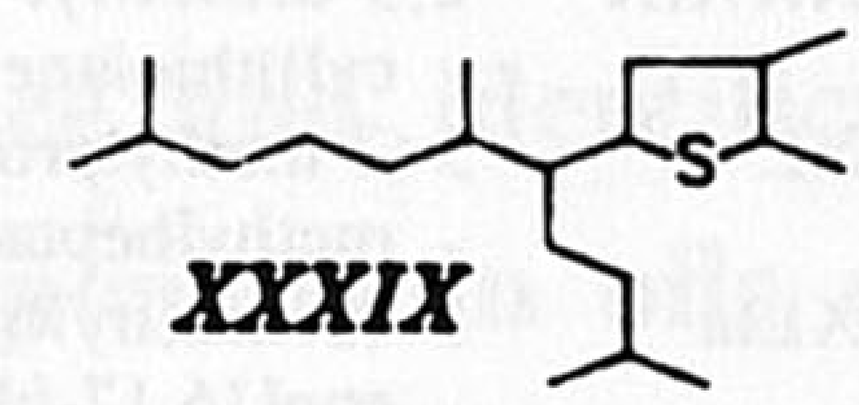
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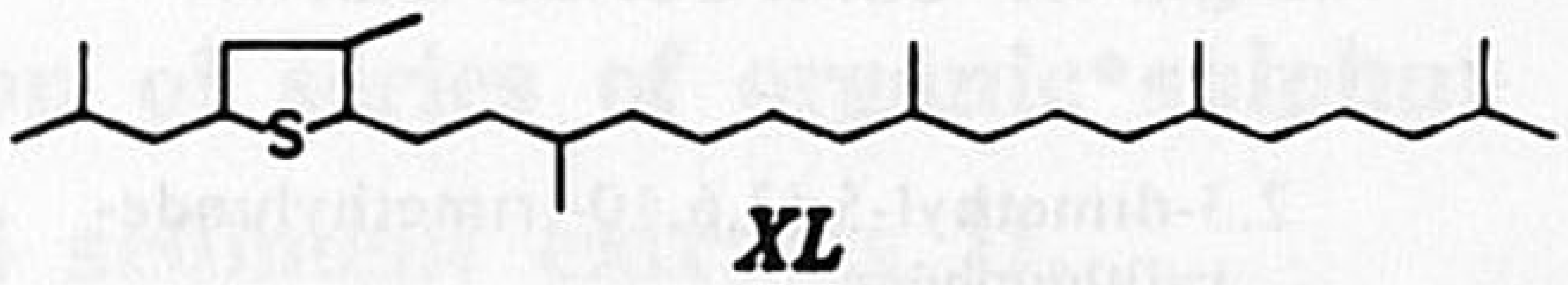
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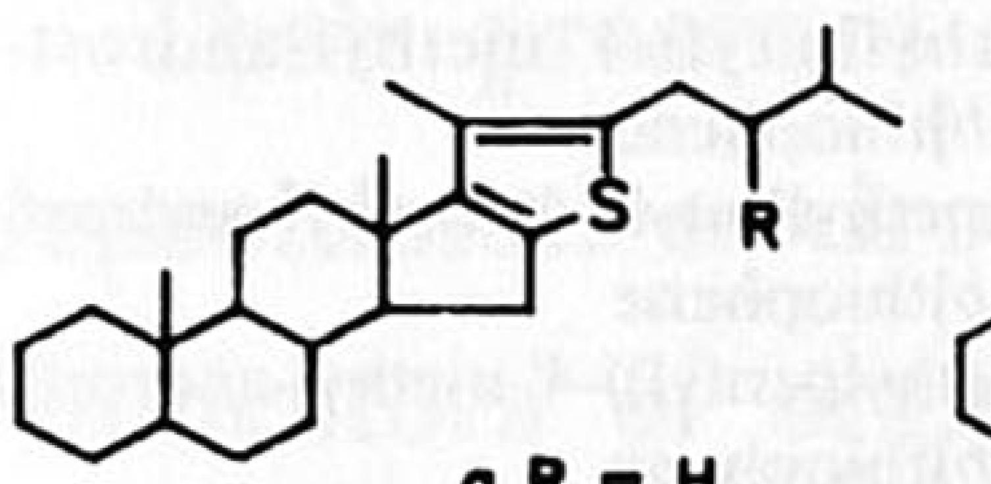
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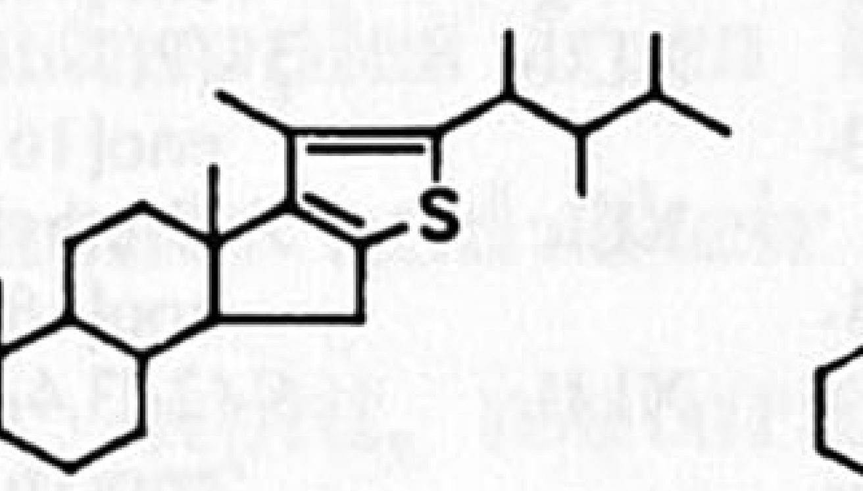
b R = CH3
c R = C2H5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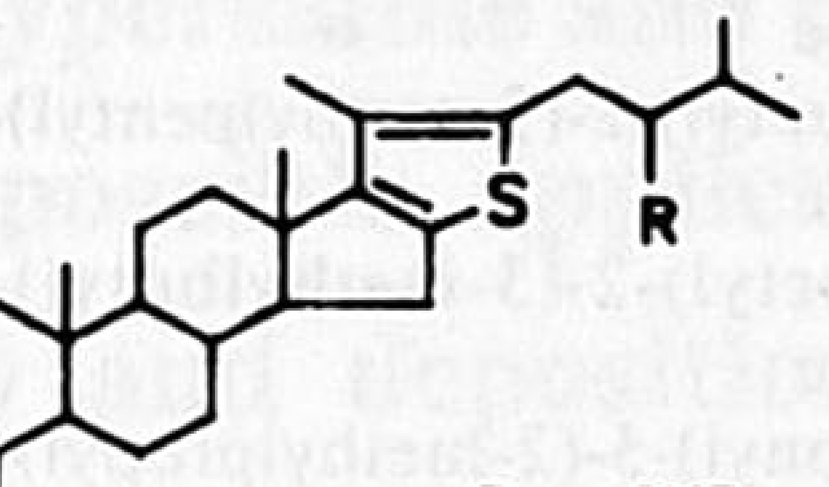
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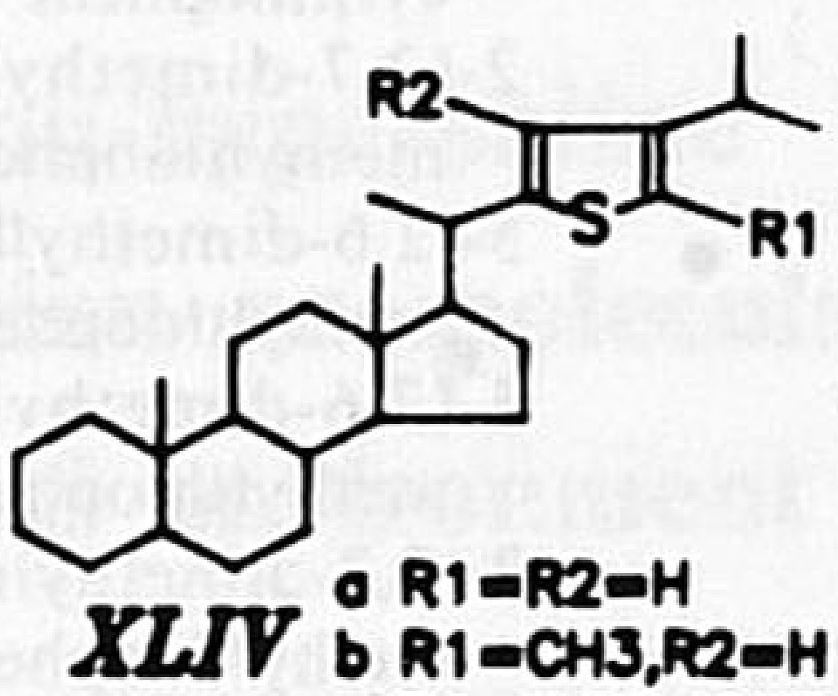
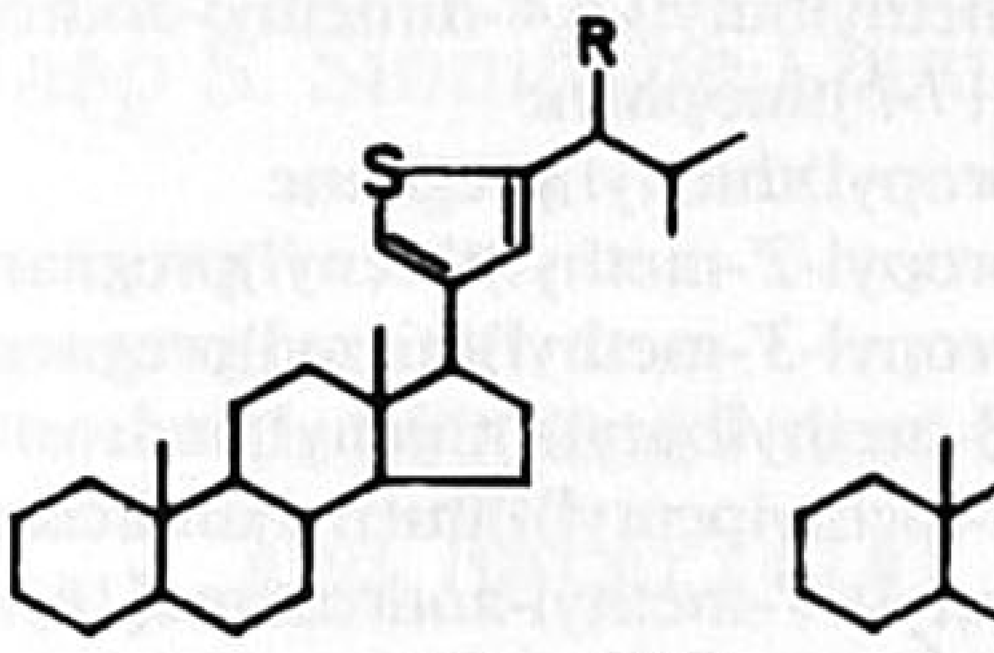

XLIV a R1=R2=H
b R1=CH3,R2=H
c R1=H,R2=CH3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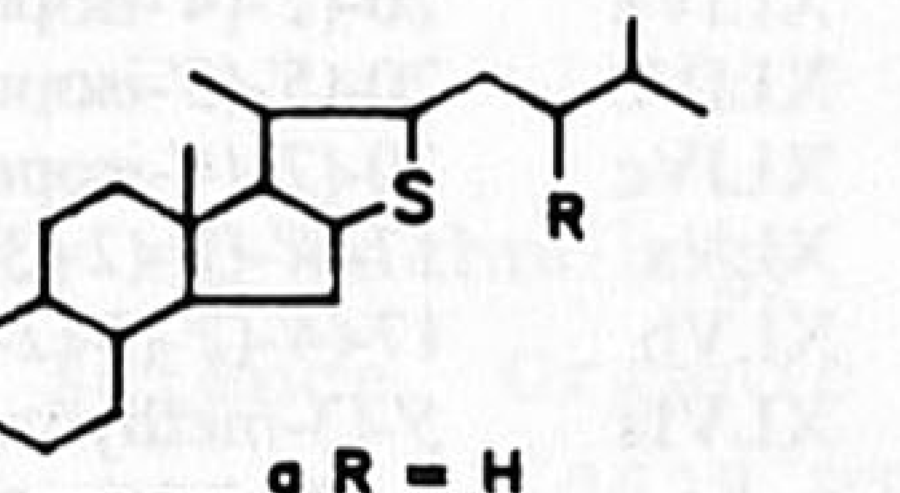
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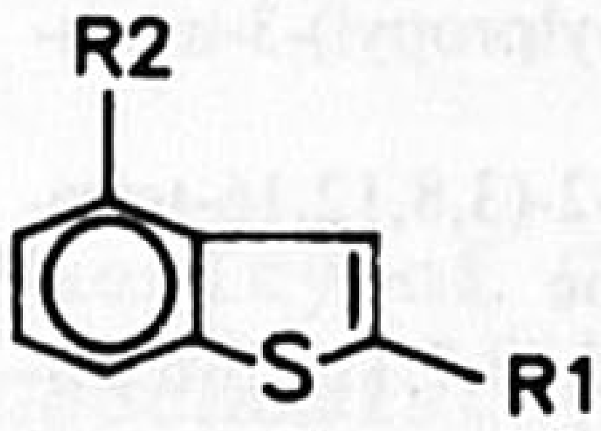
b R = CH3
c R = C2H5


R1 and R2 = alkyl

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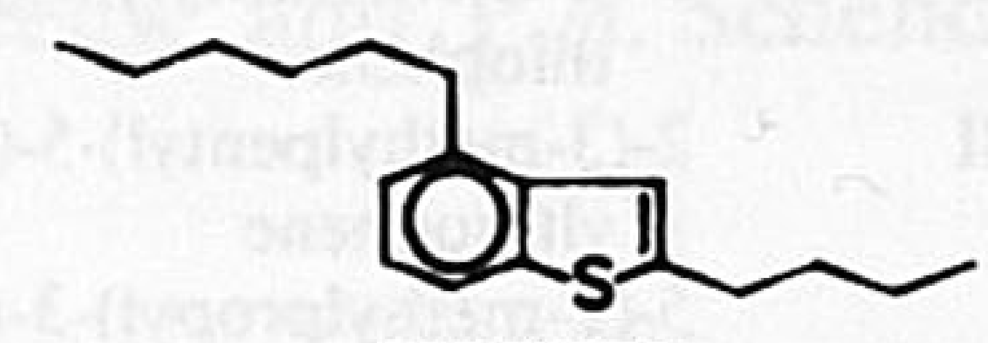
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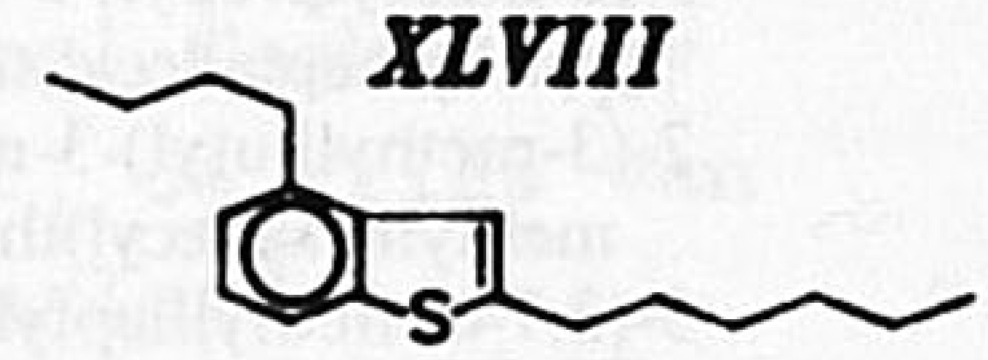
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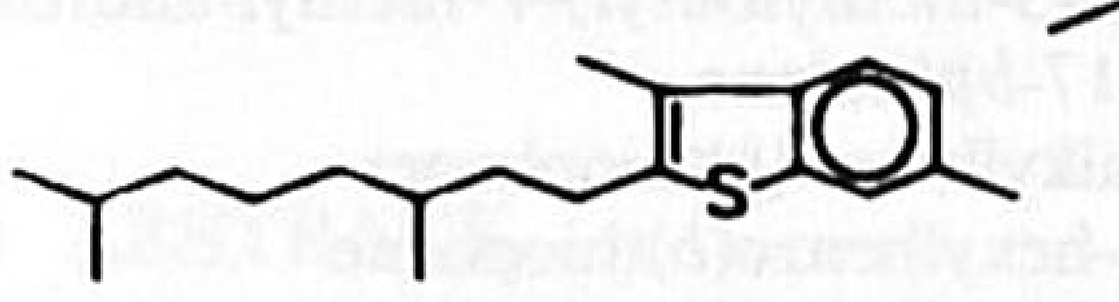
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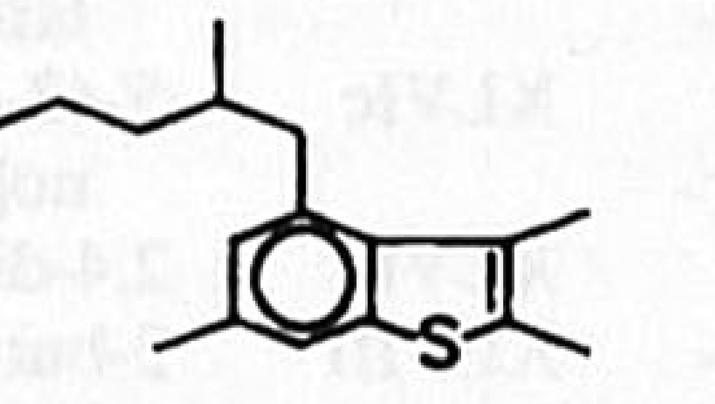
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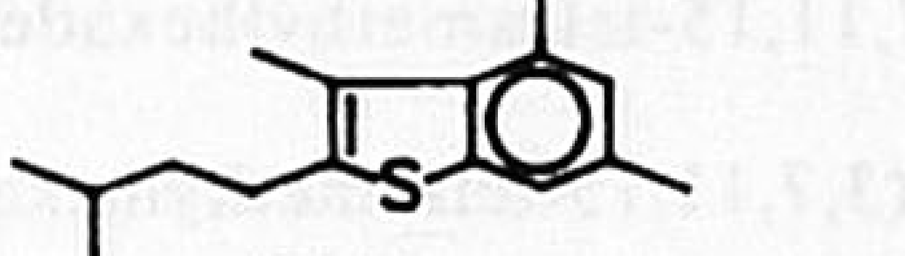
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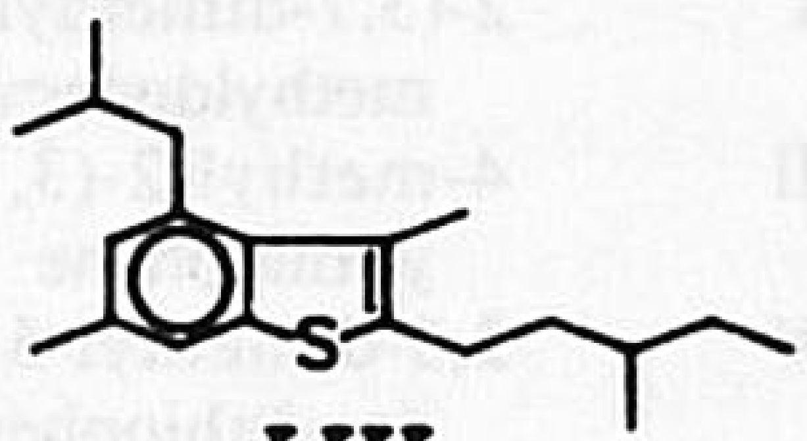
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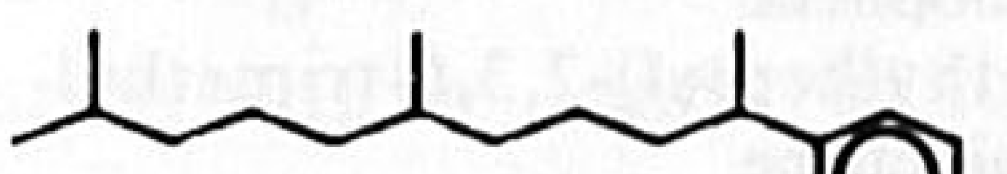
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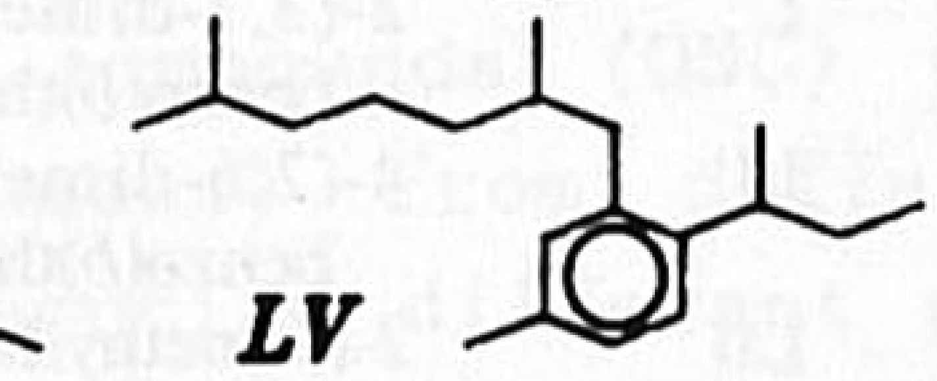
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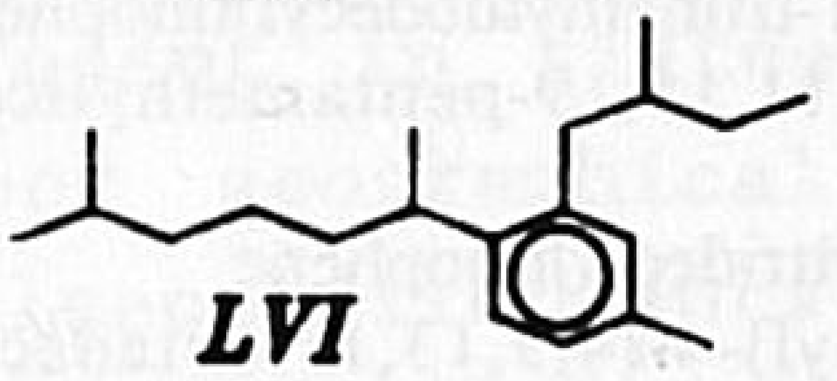
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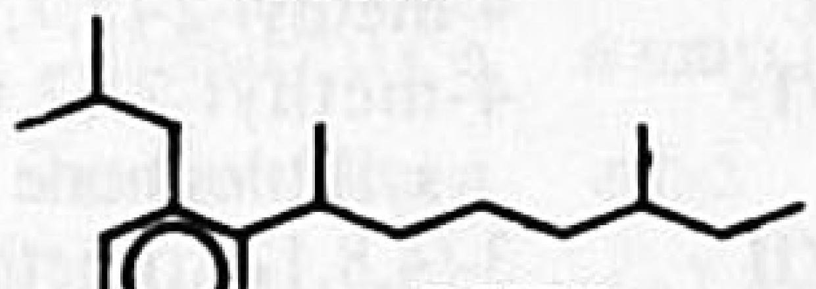
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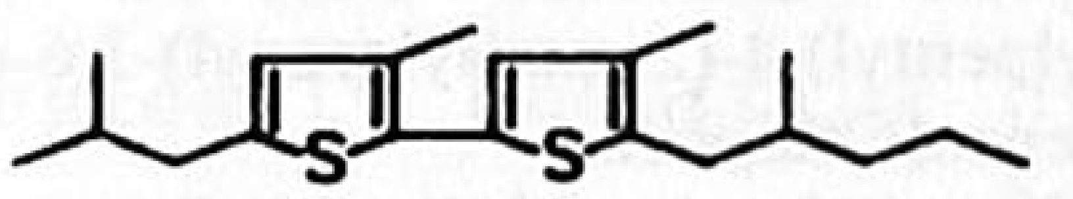
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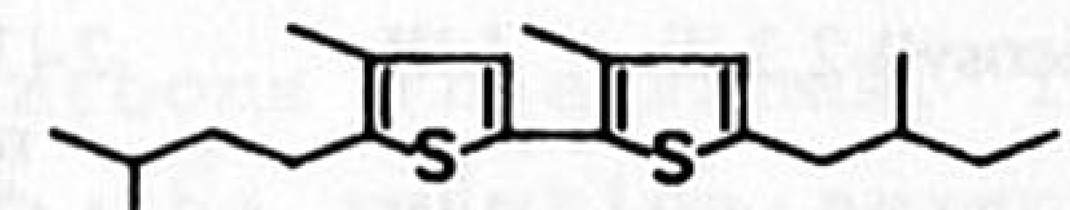
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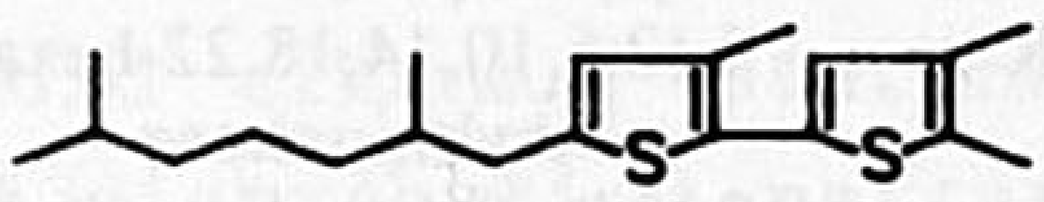
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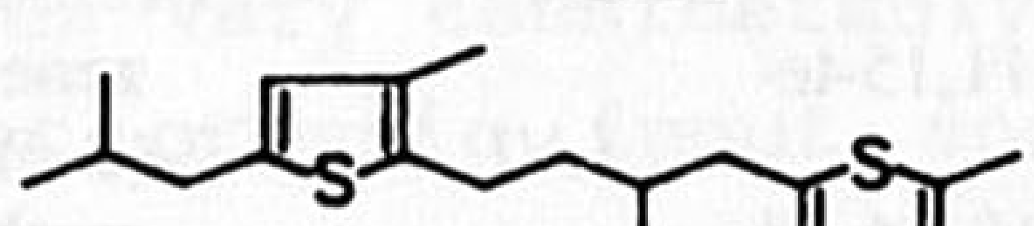
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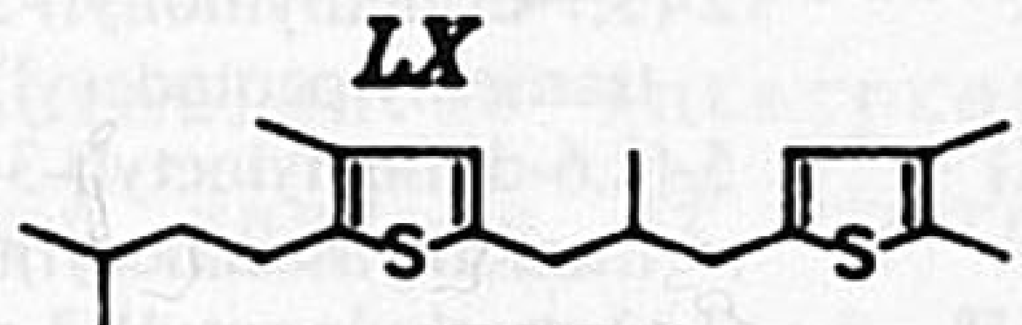
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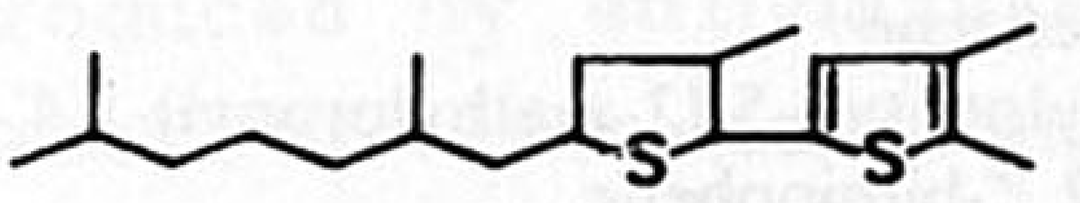
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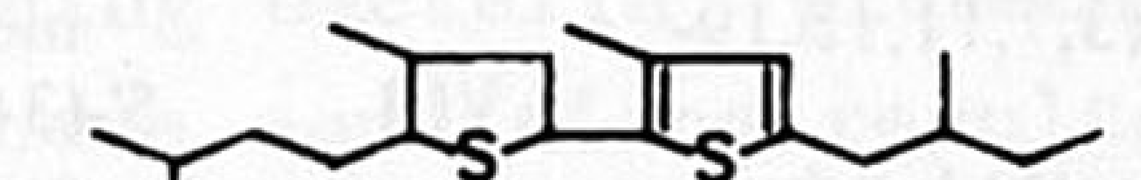
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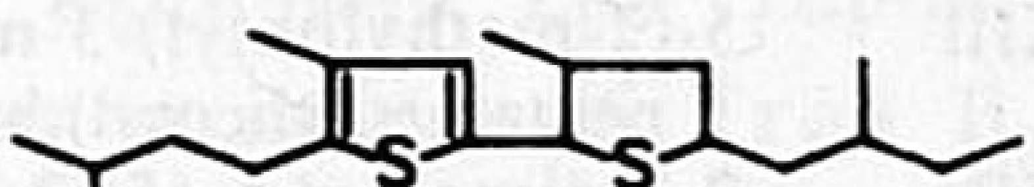
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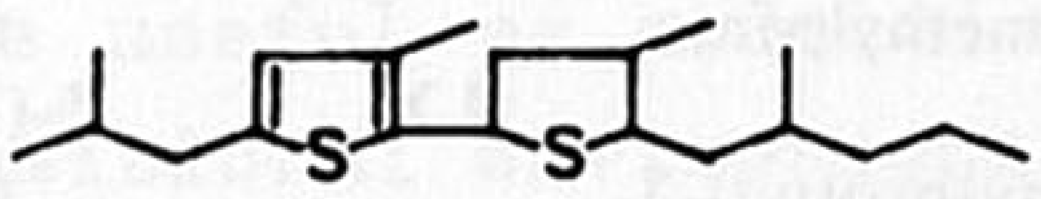
LXVIII

NAMES OF COMPOUNDS DRAWN

I	2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene
II	2-(3,7-dimethyloctyl)-5-(2-methylbutyl)-3-methylthiophene
III	5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3-methylthiophene
IV	5-(2,6-dimethyloctyl)-2-(3-methylbutyl)-3-methylthiophene
V	2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiophene
VI	5-(2,6-dimethylheptyl)-2,3-dimethylthiophene
VII	5-(2-methylbutyl)-2-(3-methylbutyl)-3-methylthiophene
VIII	2-(3-methylpentyl)-5-(2-methylpropyl)-3-methylthiophene
IX	5-(2-methylpropyl)-3-methyl-2-(3,8,12,16-tetramethylheptadecyl)thiophene
X	2-(3-methylbutyl)-3-methyl-5-(2,7,11,15-tetramethylhexadecyl)thiophene
XI	5-(2,7-dimethylheptyl)-3-methyl-5-(4,8,12-trimethyltridecyl)thiophene
XII	2-(3,7-dimethyloctyl)-3-methyl-5-(3,7,11-trimethyldodecyl)thiophene
XIII	4-methyl-2-(3,7,11,15-tetramethylhexadecyl)thiophene
XIV	2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)thiophene
XV	4-methyl-2-(3,7,11-trimethyldodecyl)thiophene
XVI	4-methyl-2-(2,6,11,15,19-pentamethylicosyl)thiophene
XVII	3-(4,8,12-trimethyltridecyl)thiophene
XVIII	2-(2-methylpropyl)-4-(4,9,13,17-octadecyl)thiophene
XIX	5-(2,6,10,14,18,22-hexamethyltricosyl)-2,3-dimethylthiophene
XX	2-(3,7-dimethylnonyl)-3-methyl-5-(2,6,10,14-tetramethylpentadecyl)thiophene
XXI	5-(2,6-dimethyloctyl)-3-methyl-2-(3,7,11,15-tetramethylhexadecyl)thiophene
XXII	2-(3-methylpentyl)-3-methyl-5-(2,6,10,14,18-pentamethylnonadecyl)thiophene
XXIII	5-(2-methylbutyl)-3-methyl-2-(3,7,11,15,19-pentamethylicosyl)thiophene
XXIV	2,3-dimethyl-5-(5-(2,6,10-trimethylundecyl))thiophene
XXV	2,3-dimethyl-5-(7-(2,6,10,14-tetramethylpentadecyl))thiophene
XXVI	2-(3,7-dimethyloctyl)-5-(2-methylpropyl)-3-methylthiophene
XXVII	3-methyl-2-(3,7,11-trimethyldodecyl)thiophene
XXVIII	3,5-dimethyl-2-(3,7,11-trimethyldodecyl)thiophene
XXIX	*cis*-2,5-di-*n*-alkylthiolanes
XXX	*trans*-2,5-di-*n*-alkylthiolanes
XXXI	2-dodecyl-5-ethylthiolane
XXXII	2,5-diheptylthiolane
XXXIII	2-methyl-5-tridecylthiolane
XXXIV	2-butyl-5-decylthiolane
XXXV	*cis*-2,6-di-*n*-alkylthianes
XXXVI	*trans*-2,6-di-*n*-alkylthianes
XXXVII	3-methyl-2-(3,7,11-trimethyldodecyl)thiolane
XXXVIII	5-(2,6-dimethylheptyl)-2,3-dimethylthiolane
XXXIX	2,3-dimethyl-5-(5-(2,6,10-trimethylundecyl))thiolane
XL	5-(2-methylpropyl)-3-methyl-2-(3,8,12,16-tetramethylheptadecyl)thiolane
XLIa	5′-(3-methylbutyl)-4′-methyl-androst-16-eno[16,17-*b*]thiophene
XLIb	5′-(2,3-dimethylbutyl)-4′-methyl-androst-16-eno[16,17-*b*]thiophene
XLIc	5′-(2-ethyl-3-methylbutyl)-4′-methyl-androst-16-eno[16,17-*b*]thiophene
XLII	5′-(2-(3,4-dimethylpentyl))-4′-methyl-androst-16-eno[16,17-*b*]thiophene
XLIIIa	5′-(2,3-dimethylbutyl)-4,4′-dimethyl-androst-16-eno[16,17-*b*]thiophene
XLIIIb	5′-(2-ethyl-3-methylbutyl)-4,4′-dimethyl-androst-16-eno[16,17-*b*]thiophene
XLIVa	20-(2′-(4′-isopropyl)thienyl)pregnane
XLIVb	20-(5′-(3′-isopropyl-2′-methyl)thienyl)pregnane
XLIVc	20-(2′-(4′-isopropyl-3′-methyl)thienyl)pregnane
XLVa	17-(4′-(2′-(2-(3-methylbutyl)))thienyl)androstane
XLVb	17-(4′-(2′-(3-(2-methylpentyl)))thienyl)androstane
XLVIa	5′-(3-methylbutyl)-4′-methyl-androstano[16,17-*b*]thiolane
XLVIb	5′-(2,3-dimethylbutyl)-4′-methyl-androstano[16,17-*b*]thiolane
XLVIc	5′-(2-ethyl-3-methylbutyl)-4′-methyl-androstano[16,17-*b*]thiolane
XLVII	2,4-di-*n*-alkylbenzo(*b*)thiophenes
XLVIII	2-butyl-4-hexylbenzo(*b*)thiophene
XLIX	4-butyl-2-hexylbenzo(*b*)thiophene
L	2-(3,7-dimethyloctyl)-3,6-dimethylbenzo(*b*)thiophene
LI	4-(2,6-dimethylheptyl)-2,3,6-trimethylbenzo(*b*)thiophene
LII	2-(3-methylbutyl)-4-(2-methylbutyl)-3,6-dimethylbenzo(*b*)thiophene
LIII	2-(3′-methylpentyl)-4-(2-methylpropyl)-3,6-dimethylbenzo(*b*)thiphene
LIV	1-(2-(6,10-dimethylundecyl))-4-methylbenzene
LV	1-(2-butyl)-2-(2,6-dimethylheptyl)-4-methylbenzene
LVI	2-(2-methylbutyl)-1-(2-(6-methylheptyl))-4-methylbenzene
LVII	1-(2-(6-methyloctyl))-2-(2-methylpropyl)-4-methylbenzene
LVIII	5′-(3-methylpentyl)-5-(2-methylpropyl)-3,4′-dimethyl-2,2′-bithiophene
LIX	5-(2-methylbutyl)-5′-(3-methylbutyl)-3,4′-dimethyl-2,2′-bithiophene
LX	5-(2,6-dimethylheptyl)-3,4′,5′-trimethyl-2,2′-bithiophene
LXI	5-(2-methylpropyl)-2,4′,5′-trimethyl-2,2′-bithiophene
LXII	2-(4-(2′,3′-dimethyl-5′-thienyl)-3-methylbutyl)-5-(2-methylpropyl)-3-methylthiophene
LXIII	5-(3-(2′,3′-dimethyl-5′-thienyl)-2-methylpropyl)-2-(3-methylbutyl)-3-methylthiophene
LXIV	5-(2,6-dimethylheptyl)-2-(2′,3′-dimethyl-5′-thienyl)-3-methylthiolane
LXV	5-(5′-(2-methylbutyl)-3′-methyl-2′-thienyl)-2-(3-methylbutyl)-3-methylthiolane
LXVI	2-(2′-(3-methylbutyl)-3′-methyl-5′-thienyl)-5-(2-methylbutyl)-3-methylthiolane
LXVII	2-(2′-(3-methylpentyl)-3′-methyl-5′-thienyl)-5-(2-methylpropyl)-3-methylthiolane

7. The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. II.

Their presence in samples from hypersaline and non-hypersaline depositional environments and their possible application as molecular indicators of organic input, maturity and depositional environment*

Jaap S. Sinninghe Damsté, W. Irene C. Rijpstra, Jan W. de Leeuw and P.A. Schenck

Delft University of Technology, Faculty of Chemical Technology
and Materials' Science, Organic Geochemistry Unit, de Vries van
Heystplantsoen 2, 2628 RZ Delft, The Netherlands.

7.1 ABSTRACT

The organic sulphur compounds (OSC) present in sixteen immature samples (both oils and bitumens) from different geographical locations and of different ages representing different palaeoenvironments have been analysed by GC-MS. In all samples OSC with structures related to well-known geologically occurring hydrocarbons (*n*-alkanes, isoprenoid alkanes, steranes, triterpanes) occur although the relative amounts and distribution patterns of the various OSC classes vary considerably. This variation is interpreted as a result of different organic input and different degrees of thermal maturation. The palaeoenvironments of the samples were anoxic and H_2S, produced by sulphate-reducing bacteria, probably has exceeded the input of reactive iron minerals. These conditions resulted in a surplus of free H_2S, which reacted with organic matter, leading to the formation of OSC. The distributions of OSC may be useful as molecular indicators for the assessment of organic input, depositional environment and maturity and for oil-oil and oil-source rock correlation studies. The distributions of the C_{20} isoprenoid thiophenes in combination with those of the methylated 2-methyl-2-(4,8,12-trimethyltridecyl)chromans can be used to discriminate non-hypersaline from hypersaline palaeoenvironments.

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* *Geochim. Cosmochim. Acta* (submitted)

7.2 INTRODUCTION

In the previous paper of this series (Sinninghe Damsté *et al.*, 1987c) it has been demonstrated that Rozel Point Oil, a sulphur-rich oil from Utah (U.S.A.) contains a number of novel series of organic sulphur compounds (OSC), together accounting for almost 1000 individual compounds. Series of 2,6-di-*n*-alkylthianes, 2,5-di-*n*-alkylthiolanes, 2,4-di-*n*-alkylbenzo[*b*]thiophenes, isoprenoid thiophenes, thiolanes, benzothiophenes and bithiophenes, and thiolane and thiophene steroids were identified. They represent the major compounds present in the "aromatic" fraction of the oil. These OSC differ essentially from most of the OSC previously identified in oils (*e.g.* Thompson, 1981; Aksenov and Kamyanov, 1981; Hughes, 1984, Arpino *et al.*, 1987) because their stuctures are related to certain bio- and geochemical precursors.

Some of the series of OSC identified in the Rozel Point Oil and several other series of OSC related to bio- and geochemical precursors have also been reported in other samples. Payzant *et al.* (1983, 1985, 1986) identified a number of terpenoid sulphides and related sulphoxides in a variety of petroleums from the Western Hemisphere. Valisolalao *et al.* (1984) reported the presence of a thiophene-ring containing hopanoid in black shales. Brassell *et al.* (1986c) identified a number of isoprenoid thiophenes in DSDP samples. Sinninghe Damsté *et al.* (1986) reported the presence of 2,5-di-*n*-alkylthiolanes, 2,5-di-*n*-alkylthiophenes, isoprenoid thiolanes and thiophenes in a marl layer from the Northern Apennines (Italy). Perakis (1986) identified a series of 2,4-di-*n*-alkylbenzo[*b*]thiophenes in an immature shale from Marocco and in the Rozel Point Seep Oil (Utah, U.S.A.). Schmid (1986) and Schmid *et al.* (1987) noted the occurrence of a series of 2,5-di-*n*-alkylthiolanes and 2,6-di-*n*-alkylthianes in the Maruejols crude oil (Ales Basin, France) and the Rozel Point Seep Oil. Schmid (1986) also identified isoprenoid thiophenes, bithiophenes and a trithiophene, various types of thiophene and thiolane steroids, hopanoid sulphides and steroid bisulphides in the Rozel Point Seep Oil. Cyr *et al.* (1986) reported the presence of a series of hopanoid sulphides ranging from C_{30} to C_{35} in heavy oils of Northern Alberta (Canada). Sinninghe Damsté and de Leeuw (1987) reported a number of C_{20} and C_{15} isoprenoid OSC occurring in sediment extracts and oils from various locations and ages. An isoprenoid C_{20} thiophene was identified as one of the most abundant OSC occurring in several oils by Sinninghe Damsté *et al.* (1987a). Sheng *et al.* (1987) reported the presence of series of alkylthiolanes and -thiophenes and isoprenoid thiolanes, thiophenes and benzothiophenes in oils from the Jianghan Basin (China).

In this paper the OSC present in a more extensive set of samples from different geographical locations and of different ages are described, indicating their widespread occurrence. The potential application of OSC as molecular indicators is also discussed.

7.3 EXPERIMENTAL

Samples. The Northern Apennines Marl is from Miocene strata in the Perticara Basin (Italy) which consists of gypsum deposits interbedded with bituminous marl layers. A 10 cm thick marl layer was sampled which is thought to have been deposited in a hypersaline environment of deposition. A detailed geochemical description of the composition of the hydrocarbon and "aromatic" fraction of the bitumen of this sample is given in ten Haven *et al*. (1985) and Sinninghe Damsté *et al*. (1986). The organic matter of this sample consists for *ca*. 60% of bitumen and is therefore thought to be an oil-stained sediment.

Four samples of the Miocene Monterey Formation (California, U.S.A.) were taken from immature outcrop sections at different locations along the coast (Shell Beach (SB), Gaviota Beach (W4), El Capitan Beach (M5), Naples (T6)). The Monterey Formation is a siliceous-phosphorite-carbonate sequence which received a considerable input of phytoplankton (*e.g.* diatoms) due to the high productivity caused by climatic cooling and intensified upwelling in the late Miocene (Isaacs *et al*., 1983).

The three samples from the Cretaceous Jurf ed Darawish Oil shale are taken from a core and are composite samples from 45-46, 100-101 and 156-157 m, respectively. Some organic and inorganic gechemical data of this oil shale have been described by Wehner and Hufnagel (1987). Its depositional environment is thought to be similar to that of the Senonian (Upper Cretaceous) bitumenous rocks in Israel. Tannenbaum and Aizenshtat (1985) reported that this sedimentary sequence was deposited in an environment of high organic productivity under reducing conditions in small basins. The source of the organic matter is thought to be mainly marine (algae) with some terrestrial contribution, reworked by bacteria.

Phosphoria Retort shale is a phosphatic mudstone and dolomitic marlstone sequence of Permian age from the North-western part of Montana (U.S.A.) which was deposited in a large embayment with water depths of probably less than 40 meters (McKelvey *et al*., 1959).

The Rozel Point Oil is from the North-western part of Utah (U.S.A.). Its source rock is thought to have been deposited in a playa lake environment (Meissner *et al*., 1984). Bulk properties of the oil and a detailed description of both the hydrocarbon and "aromatic" fraction of the oil have been described elsewhere (Sinninghe Damsté *et al*., 1987c, ten Haven *et al*., 1988).

The three related Sicily Seep Oils-E1, -E2 and -E5 seep out of marl layers of Upper Miocene evaporites deposited in an environment largely comparable with that described for the Northern Apennines marl (ten Haven *et al*., 1985, 1988). Their geological setting and a description of the hydrocarbon fractions are given by Palmer and Zumberge (1981) and ten Haven *et al*. (1988).

The two Chinese oils (B1 and W1349) from shallow depths (*ca*. 600 and 1200 m, respectively) in the Jianghan Basin are immature and are thought to originate from hypersaline lacustrine source rocks. For the location of the well sites and a description of the hydrocarbon fractions of these oils see Brassell *et al.* (1988). Some information on the OSC present in these samples can be found in Sheng *et al.* (1987).

The Amposta oil is from the Taragona Basin (Spanish Mediterranean offshore). The source rock of this oil is probably deposited during marginal hypersaline (carbonate-evaporite) episodes (Albaiges *et al.*, 1986). Data on biological markers present in this oil have been described by Albaiges and Torradas (1974) and Albaiges *et al.* (1986).

Extraction. Sediment samples were powdered in a rotary disc mill and soxhlet extracted with toluene/methanol (1:3, v/v) for approximately 50 h. The extracts were concentrated with a rotary evaporator at 30°C and weighed.

Fractionation. Ca. 200 mg of the oil or sediment extract was fractionated (without prior removing of asphaltenes) into saturated hydrocarbon, "aromatic" and hetero compound fractions by a column (50 cm x 10 mm) packed with an equal volume of alumina overlying silica (both activated for 1 h at 150°C) by elution with pentane (65 ml), toluene (75 ml) and toluene/methanol (1/1, v/v; 75 ml) as eluents. The "aromatic" fractions were further separated on a column of activated (1 h at 150°C) alumina into low-molecular weight aromatic (LMWA) and high-molecular weight aromatic fractions using 50 ml hexane/tolene (9/1, v/v) and 50 ml toluene, respectively. The LMWA fraction contained most of the GC-amenable compounds of the "aromatic" fraction including the OSC described previously (Sinninghe Damsté *et al.*, 1987c), chromans (Sinninghe Damsté *et al.*, 1987b), both *n*-alkylbenzenes and isoprenoid alkylbenzenes (Sinninghe Damsté *et al.*, 1988b) and A-, B- and C-ring monoaromatic steroids.

Gas chromatography. Gas chromatography of the saturated hydrocarbon fractions was performed on a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was used with helium as carrier gas. Detection was accomplished by both a flame ionization detector and a sulphur-selective flame photometric detector (FPD), using a stream splitter at the end of the capillary column. The FPD was used to check the absence of OSC in the saturated hydrocarbon fraction. Samples were injected at 75°C. The oven was programmed from 75 to 130°C at 20°C/min and then at 4°C/min to 320°C.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) was carried out on a Hewlett-Packard 5840 gas chromatograph connected to a VG-70S mass spectrometer operated at 70 eV with a mass range *m/z* 40-800 and a cycle time of 1.8 s. The gas chromatograph was equipped with a fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) and was operated with helium as carrier gas. LMWA fractions (1.0 μl) were on-column injected at 50 °C in ethylacetate (*ca.* 6

mg/ml) and the temperature was programmed at 10°C/min to 100°C and then at 4°C/min to 315°C at which it was held for 20 min.

7.3 RESULTS

Sixteen samples (both oils and bitumens) from different geographical locations and of different ages representing different palaeoenvironments have been analysed for OSC and other constituents. Some general and molecular data of these samples are given in Table 7.1. The composition of the hydrocarbon fractions of a number of these samples has been described elsewhere as indicated above. The hydrocarbon fractions of these samples were reanalysed by GC and GC-MS to obtain a number of molecular parameters such as the pristane/phytane ratio and the carbon preference index. These values were determined to seek correlations with OSC distributions. However, the main objective was to study the occurrence and distributions of the OSC present in these samples. The analytical approach previously used to identify the OSC in the Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c) was applied. The "aromatic" fraction obtained by standard column chromatography was separated into two fractions; the so-called Low Molecular Weight Aromatic (LMWA) fraction, which contains all the OSC previously identified, was analysed by GC-MS. Because these LMWA fractions sometimes contain about 1000 individual OSC, as shown previously for the Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c), the compounds present in these fractions were identified by mass chromatography of a number of characteristic ions (Table 7.2) rather than by inspection of individual mass spectra. These mass chromatograms were compared with those of samples previously studied in detail by separation of OSC in various sub-fractions and subsequent characterisation by gas chromatography-mass spectrometry, Raney-Ni desulphurisation and in some cases NMR spectroscopy (Sinninghe Damsté *et al.*, 1986, 1987c, 1988e). In a number of cases these identifications were confirmed by comparison of mass spectral and relative retention time data of geologically occurring OSC and other compounds with those of authentic standards (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987a, b and c, 1988b, d and e). Furthermore, the LMWA fractions of a number of samples were desulphurised using Raney-Ni providing information about the carbon skeletons of the OSC present (Sinninghe Damsté *et al.*, 1988f). Thus, all major components were identified. An overview of the occurrence, the carbon number distributions and, to some extent, the relative abundance of the various classes of compounds is given in Table 7.3. The data in Table 7.3 show that OSC are often the major compounds present in the LMWA fractions, whilst sometimes non-OSC are most abundant (*e.g.* Monterey shale bitumens). For the major classes of OSC a number of examples, in the form of characteristic mass chromatograms, are given, with the aim of showing

(i) the widespread occurrence of the OSC previously described (Sinninghe Damsté *et al.*, 1987c),

Table 7.1. Geochemical data of the samples studied.

Sample	Age[1]	Lithology	$\%R_0$[2]	TOC[3] %	EOM[4] %
Sediments					
Northern Apennines marl	Mioc.	bitumenous marl	n.d.	n.d	0.7
Monterey shale-SB	Mioc.	cristobalitic claystone	n.d.	21	2.3
Monterey shale-W4	Mioc.	muddy opal-CT stone	n.d.	6	1.2
Monterey shale-M5	Mioc.	mudstone	0.40	24	2.3
Monterey shale-T6	Mioc.	phosphatic muddy limest.	0.46	13	1.0
Jurf ed Darawish shale-45	Cret.	bitumenous limestone	n.d.	5	0.6
Jurf ed Darawish shale-100	Cret.	bitumenous limestone	n.d.	8	0.5
Jurf ed Darawish shale-156	Cret.	bitumenous calc. marl	0.30	17	1.6
Phosphoria Retort shale	Perm.	phosphatic mudstone	0.28	16	2.0
Oils					
Rozel Point Oil	Mioc.	n.a.[18]	n.a.	n.a	n.a.
Sicily Seep Oil-E1	Mioc.	n.a.	n.a.	n.a.	n.a.
Sicily Seep Oil-E2	Mioc.	n.a.	n.a.	n.a.	n.a.
Sicily Seep Oil-E5	Mioc.	n.a.	n.a.	n.a.	n.a.
Jianghan-B1 oil	Plio.	n.a.	n.a.	n.a.	n.a.
Jianghan-W1349 oil	Plio.	n.a.	n.a.	n.a.	n.a.
Amposta oil	Mioc.	n.a.	n.a	n.a.	n.a.

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[1] Mioc. - Miocene, Cret. - Cretaceous, Perm. - Permian, Plioc. - Pliocene

[2] $\% R_0$ - vitrinite reflectance

[3] TOC - Total Organic Carbon

[4] EOM - Extractable Organic Matter

[5] S - total sulphur content of bitumen or oil, this includes elemental S

[6] Sa - weight precentage of sat. hydrocarbon fraction of bitumen or oil

[7] Ar - weight percentage of "aromatic" fraction of bitumen or oil

[8] He - weight precentage of hetero compound fraction of bitumen or oil

[9] asph. - asphaltene content of bitumen or oil

(ii) differences in distributions of OSC for different samples.

2,5-Di-n-alkylthiolanes (I) and 2,6-di-n-alkylthianes (II). 2,5-Di-*n*-alkylthiolanes and 2,6-di-*n*-alkylthianes occur in most of the samples studied (Table 7.3); the distribution patterns of the various series differ, however, to a great extent. Figs. 7.1 and 7.2 show mass chromatograms of *m/z* 101 and 115 respectively. *M/z* 101 is a characteristic ion of 2-alkyl-5-methylthiolanes, 2-alkylthianes and "mid-chain" 2,6-dialkylthianes. The term "mid-chain" will be used for OSC possessing two alkyl side chains, both containing more than 3 carbon atoms.

Table 7.1. (Continued)

S[5] %	Sa[6] %	Ar[7] %	He[8] %	asph.[9] %	LMWA[10] %	CPI[11]	R_{22}[12]	Pr/Ph[13]	Ph/C_{18}[14]	%triMe[15]
10.2	3	14	76	30	6	1.38	1.89	0.06	70.3	0.04
n.d.	1	13	27	n.d.	7	n.d.	~ 1	0.60	2.0	-
n.d.	24[16]	16[16]	60[16]	77[17]	n.d.	>1.5	1.18	0.41	22.4	0.83
7.9	3[16]	15[16]	82[16]	83[17]	n.d.	>1.5	1.19	0.49	21.7	0.88
4.4	3[16]	9[16]	88[16]	82[17]	n.d.	>1.5	1.17	0.41	5.1	0.92
9.9	2	7	26	59	3	1.15	1.38	0.62	0.9	0.66
12.9	2	8	36	51	4	1.04	1.09	0.62	0.8	0.65
13.3	1	24	40	23	10	1.28	0.93	0.37	1.7	0.63
10.0	2	35	48	n.d.	17	1.32	0.98	0.64	4.0	0.53
7.5	2	43	28	n.d.	12	0.53	1.91	0.03	4.3	0.36
20.0	1	26	31	n.d.	3	0.65	3.15	0.06	18.0	0.22
20.4	3	37	n.d.	n.d.	8	0.72	2.97	0.05	17.5	0.25
19.7	2	33	29	n.d.	9	0.66	3.24	0.05	19.7	0.20
12.9	3	46	31	n.d.	18	0.52	1.65	0.05	4.6	0.21
10.4	4	45	38	n.d.	22	0.71	1.61	0.10	7.4	0.30
6.7	16	43	16	n.d.	13	0.90	1.00	0.37	0.6	0.25

[10] LMWA = weight percent of LMWA fraction of bitumen or oil

[11] CPI = Carbon Preference Index (measured from C_{15} to C_{30})

[12] $R_{22} = 2 * n\text{-}C_{22}/(n\text{-}C_{21} + n\text{-}C_{23})$

[13] Pr/Ph = pristane/phytane ratio

[14] Ph/n-C_{18} = phytane/octadecane ratio

[15] %tri Me MTTC= XXXId/total amount of methylated MTTC

[16] on the basis of total recovered material

[17] Palmer (unpublished results)

[18] n.a. = not aplicable

For the Jianghan-B1 oil the m/z 101 mass chromatogram is dominated by a homologous series of 2-alkyl-5-methylthiolanes, which shows an even-over-odd carbon number predominance with maxima at C_{18} and C_{26}. Sicily Seep Oil-E2 also shows the presence of this series although another series, *i.e.* 2-alkylthianes, is also observed. The series of 2-alkylthianes, so-called terminal thianes, was not reported previously in the Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c) but is present as well in this oil as a minor series. It is noteworthy that the Sicily Seep Oils also contain relatively high amounts of terminal thiolanes, *i.e.* 2-alkylthiolanes, which are absent in Rozel Point Oil.

Table 7.2. Characteristic ions used to identify the various compound classes present in the LMWA fractions.

compound class	characteristic ions[1]	ref.[2]
2,5-di-*n*-alkylthiolanes	*m/z* 87+14.*n*	a,b
2,6-di-*n*-alkylthianes	*m/z* 101+14.*n*	c
2,5-di-*n*-alkylthiophenes	*m/z* 97+14.*n*	a
2,4-di-*n*-alkylbenzo[*b*]thiophenes	*m/z* 147+14.*n*	c,d
isoprenoid thiolanes	*m/z* 101+14.*n*	a,c,e
isoprenoid thiophenes	*m/z* 97+14.*n*	a,c,e,f
isoprenoid benzothiophenes	*m/z* 175+14.*n*	e
isoprenoid bithiophenes	*m/z* 220,221	e
highly branched isoprenoid thiophenes	*m/z* 125,195,265	g
branched thiophenes	*m/z* 97+14.*n*	h
thiolane steroids	*m/z* 331,129,143	i
thiophene steroids	*m/z* 341,153,167,154, 168,182,287,301	c,i
thiolane hopanoids	*m/z* 191,369,168+14.*n*	i,j
thiophene hopanoids	*m/z* 191,97,111,287	k
branched octadecyl substituted benzothiophenes	*m/z* 175,189,203	l
1,2-di-*n*-alkylbenzenes	*m/z* 91+14.*n*	
isoprenoid benzenes	*m/z* 92,106,120,134	m
A- and B-ring monoaromatic steroids	*m/z* 211	n
C-ring aromatic steroids	*m/z* 253,267	o
methylated MTTC	*m/z* 121,135,149,161,175, 189	p

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[1] in combination with molecular ions

[2] (a) Sinninghe Damste *et al*. (1986), (b) Schmid *et al*. (1987), (c) Sinninghe Damste *et al*. (1987c), (d) Perakis (1986), (e) Sinninghe Damste and de Leeuw (1987), (f) Brassell *et al*. (1986c), (g) Sinninghe Damste *et al*. (1988d), (h) Sinninghe Damste *et al*. (1988f), (i) Schmid (1986), (j) Cyr *et al*. (1986), (k) Valisolalao *et al*. (1984), (l) Sinninghe Damste (unpublished results), (m) Sinninghe Damste *et al*. (1988b), (n) Hussler *et al*. (1981), Hussler and Albrecht (1983), (o) Riolo and Albrecht (1985), Riolo *et al*. (1985), Moldowan and Fago (1986), (p) Sinninghe Damste *et al*. (1987b)

In the *m/z* 101 mass chromatogram (Fig. 7.1C) of the Jurf ed Darawish Oil shale-156 bitumen a third series of OSC, the *trans* mid-chain 2,6-dialkylthianes, occurs. The three homologous series show different carbon number distributions, although all three series exhibit (sub)maxima at C_{28} and show a strong even carbon number predominance in the C_{22}-C_{30} range. Remarkably, the *cis* mid-chain 2,6-dialkylthianes (not shown in Fig. 7.1C) are much lower

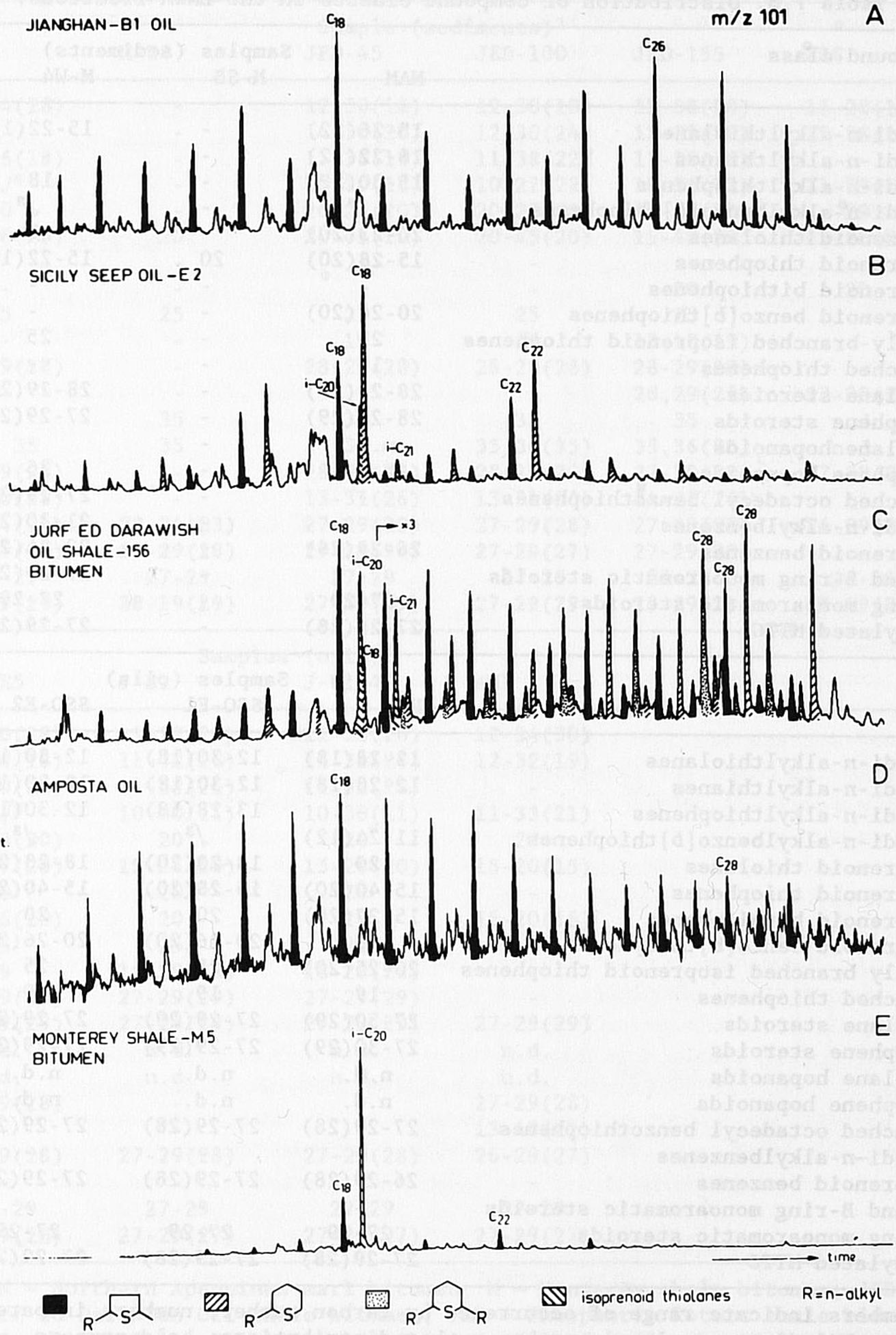

Fig. 7.1. Mass chromatograms of *m/z* 101 for the LMWA fractions of the samples indicated. The indicated mid-chain 2,6-di-*n*-alkylthianes represent the *trans* isomers.

Table 7.3. Distribution of compound classes in the LMWA fractions.

Compound class	Samples (sediments)		
	NAM	M-SB	M-W4
2,5-di-*n*-alkylthiolanes	15-26(22)	-	15-22(18)
2,6-di-*n*-alkylthianes	16-22(22)	-	-
2,5-di-*n*-alkylthiophenes	15-30(22)	-	18
2,4-di-*n*-alkylbenzo[*b*]thiophenes	√[3]	-	√[3]
isoprenoid thiolanes	20-23(20)	-	20
isoprenoid thiophenes	**15-28(20)**	20	15-22(15)
isoprenoid bithiophenes	-	-	-
isoprenoid benzo[*b*]thiophenes	20-26(20)	-	-
highly branched isoprenoid thiophenes	25	-	25
branched thiophenes	19	-	-
thiolane steroids	28-29(29)	-	28-29(28)
thiophene steroids	28-29(29)	-	27-29(28)
thiolane hopanoids	n.d.[4]	-	-
thiophene hopanoids	n.d.	-	35
branched octadecyl benzothiophenes	-	-	27-29(28)
1,2-di-*n*-alkylbenzenes	-	-	21-30(28)
isoprenoid benzenes	26-29(26)	-	22-24(23)
A- and B-ring monoaromatic steroids	-	-	**27-29(28)**
C-ring monoaromatic steroids	**27-29**	-	**27-29**
methylated MTTC	**27-29(28)**	-	**27-29(29)**
	Samples (oils)		
	RPO	SSO-E1	SSO-E2
2,5-di-*n*-alkylthiolanes	**12-28(18)**	12-30(18)	12-30(18)
2,6-di-*n*-alkylthianes	12-28(18)	**12-30(18)**	12-30(18)
2,5-di-*n*-alkylthiophenes	-	13-28(18)	12-30(18)
2,4-di-*n*-alkylbenzo[*b*]thiophenes	11-24(12)	√[3]	√[3]
isoprenoid thiolanes	**20**	**18-23(20)**	**18-23(20)**
isoprenoid thiophenes	**15-40(20)**	**15-28(20)**	**15-40(20)**
isoprenoid bithiophenes	15-22(20)	20	20
isoprenoid benzo[b]thiophenes	20	20-26(20)	20-26(20)
highly branched isoprenoid thiophenes	20-25(20)	25	25
branched thiophenes	19	19	19
thiolane steroids	27-30(29)	27-29(29)	27-29(29)
thiophene steroids	27-30(29)	27-29(29)	27-29(29)
thiolane hopanoids	n.d.	n.d.	n.d.
thiophene hopanoids	n.d.	n.d.	n.d.
branched octadecyl benzothiophenes	27-29(28)	27-29(28)	27-29(28)
1,2-di-*n*-alkylbenzenes	-	-	-
isoprenoid benzenes	26-29(28)	27-29(28)	27-29(28)
A- and B-ring monoaromatic steroids	-	-	-
C-ring monoaromatic steroids	27-29	27-29	27-29
methylated MTTC	27-29(28)	27-29(28)	27-29(28)

[1] numbers indicate range of occurrence by carbon number; numbers in parentheses indicate maxima in carbon number distributions; bold numbers indicate that a member of this compound class is one of the three most abundant components of the LMWA fraction

[3] low molecular weight (C_{10}-C_{16}) 2,4-di-*n*-alkylbenzo[*b*]thiophenes are probably present

Table 7.3. (Continued)

		Sample (sediments)[2]			
M-M5	M-T6	JED-45	JED-100	JED-155	PRS
15-24(18)	-	12-30(18)	12-30(18)	**12-38(28)**	15-28(18)
-	-	14-30(20)	12-30(24)	**12-33(28)**	15-28(18)
16-26(18)	-	11-38(18)	11-38(22)	11-38(18)	-
√[3]	-	10-20(12)	10-22(22)	10-26(12)	11-25(18)
20	-	20-21(20)	20-22(20)	20-23(20)	20
15-26(20)	*20*	20-45(20)	20-45(20)	15-45(20)	**15-20(20)**
-	-	-	-	-	-
-	-	-	-	20	20
25	25	25	25	25	-
-	-	19	19	17,19(19)	-
28-29(28)	-	28-29(28)	28-29(28)	28-29(28)	-
-	-	-	-	28,29(28)	27-29(28)
-	35	35	35	35	n.d.
35	35	35	**35,36(35)**	**35,36(35)**	n.d.
27-29(28)	-	28-29(29)	28-29(29)	27-29(29)	27-29(28)
21-30(28)	-	13-31(26)	13-31(26)	14-31(26)	-
22-24(23)	22-24(23)	27-29(28)	27-29(28)	27-29(28)	26-29(28)
27-29(28)	**27-29(28)**	**27-29(27)**	**27-29(27)**	27-29(28)	-
27-29	27-29	27-29	27-29	27-29	**27-29**
28-29(29)	**28-29(29)**	27-29(29)	27-29(29)	27-29(29)	**27-29(29)**

		Samples (oils)[2]	
SSO-E5	J-B1	J-W1349	AMP
12-30(18)	11-32(26)	11-32(26)	**12-34(30)**
12-30(18)	11-32(26)	11-32(26)	12-32(19)
13-30(18)	11-32(26)	11-32(26)	-
√[3]	10-30(11)	10-30(11)	**11-33(21)**
18-23(20)	**20**	**20**	20
15-27(20)	**15-26(20)**	**15-26(20)**	15-20(15)
20	20	20	-
20-26(20)	20	20	15-20(15)
25	25	25	-
19	18-28(28)	18-28(28)	-
27-29(29)	27-29(29)	27-29(29)	-
27-29(29)	27-29(29)	27-29(29)	27-29(29)
n.d.	n.d.	n.d.	n.d.
n.d.	n.d.	n.d.	n.d.
27-29(28)	-	-	27-29(28)
-	-	-	**13-40(21)**
27-29(28)	27-29(28)	27-29(28)	26-29(27)
-	-	-	-
27-29	27-29	27-29	**27-29**
27-29(28)	27-29(27)	27-29(27)	27-29(27)

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[2] NAM = Northern Apennines marl bitumen; M = Monterey shale bitumen; JED = Jurf ed Darawish Oil shale bitumen; PRS = Phosphoria Retort shale bitumen; RPO = Rozel Point Oil; SSO = Sicily Seep Oil; J = Jianghan Oil; AMP = Amposta oil

[4] n.d. = not determined

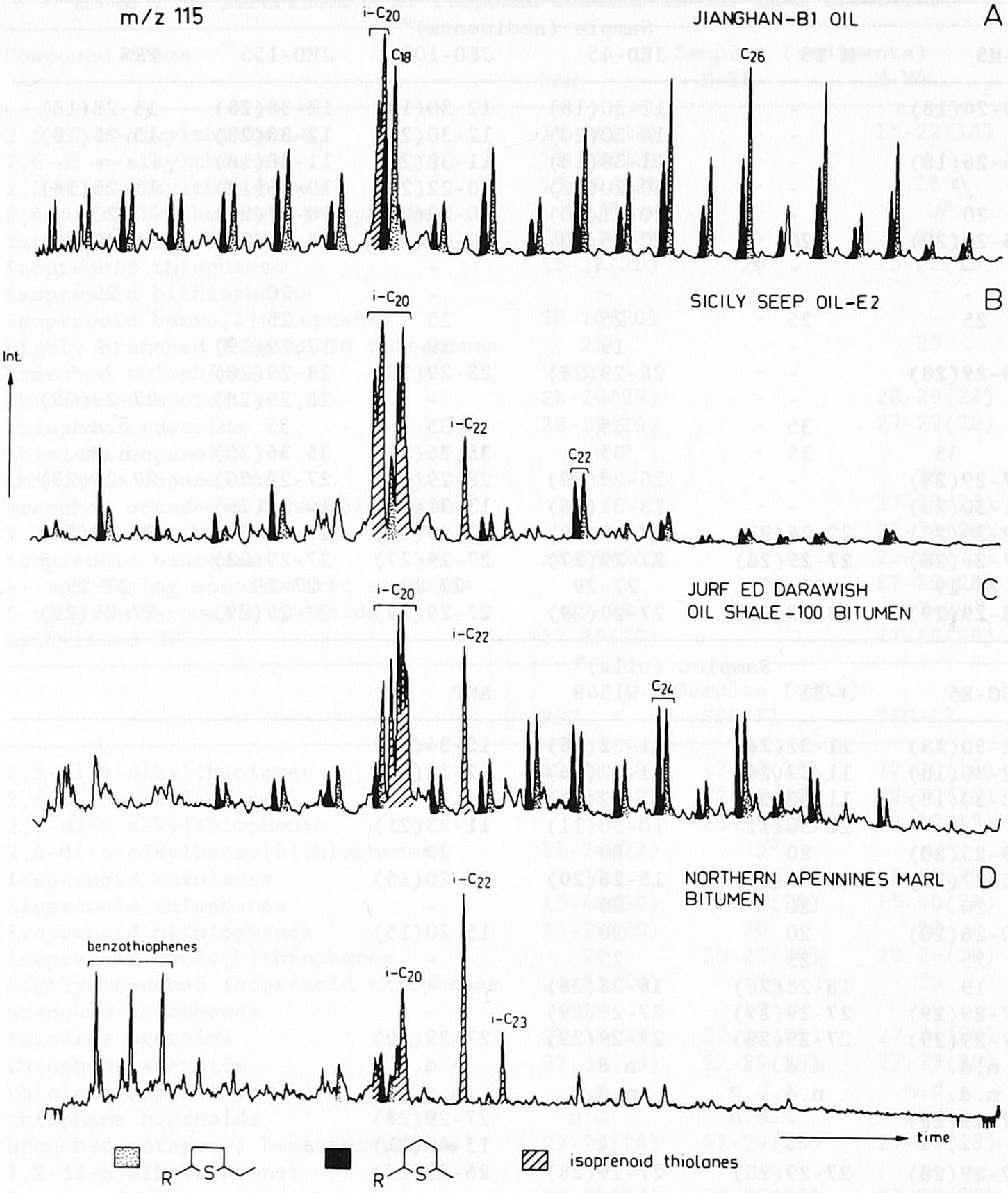


Fig. 7.2. Mass chromatograms of *m/z* 115 for the LMWA fractions of the samples indicated.

in abundance than the *trans* mid-chain 2,6-dialkylthianes.

Fig. 7.1D shows the *m/z* 101 mass chromatogram of the Amposta oil. The homologous series of 2-alkyl-5-methylthiolanes dominates; no strong even-over-odd carbon number predominance is observed as is the case for the previous examples. In the C_{24}-C_{30} range a slight even predominance is observed.

Fig. 7.2 shows mass chromatograms of *m/z* 115, a characteristic ion of, amongst other OSC, 2-alkyl-5-ethylthiolanes and 2-alkyl-6-methylthianes. The presence of these two homologous series is shown for the Jianghan-B1 oil, the Sicily Seep Oil-E2 and the Jurf ed Darawish Oil shale-100 bitumen in Figs. 7.2A-C. In the Jianghan-B1 oil and the Sicily Seep Oil-E2 these two series exhibit a strong even-over-odd carbon number predominance with maxima at C_{18} and C_{26} and C_{18} and C_{22}, respectively, whilst these two series in the Jurf ed Darawish Oil shale-100 bitumen only show an even carbon number predominance in the C_{22}-C_{30} range.

*2,5-Di-*n*-alkylthiophenes* (III). Homologous series of 2-alkyl-5-methylthiophenes, 2-alkyl-5-ethylthiophenes and 2-alkyl-5-butylthiophenes have been previously reported to occur in the Northern Apennines marl bitumen (Sinninghe Damsté *et al.*, 1986). Some of these series are also present in a number of other samples (Table 7.3). In addition to the three series, previously reported in the Northern Apennines Marl, homologous series of 2-alkyl-5-propylthiophenes, 2-alkyl-5-pentylthiophenes, 2-alkyl-5-hexylthiophenes, 2-alkyl-5-heptylthiophenes and 2-alkyl-5-octylthiophenes were identified in the Jianghan-B1 and -W1349 oils and in the Jurf ed Darawish Oil shale-156 bitumen. A series of 2-alkylthiophenes was present in the Jurf ed Darawish Oil shale bitumens.

Figs. 7.3 and 7.4 show mass chromatograms of *m/z* 111 and 125 for various samples. They provide examples of the occurrence of 2,5-dialkylthiophenes in the samples studied. *M/z* 111 is a characteristic ion of 2-alkyl-5-methylthiophenes and mid-chain 2,5-dialkylthiophenes (*e.g.* Sinninghe Damsté *et al.*, 1988f). Both series are present in the Jianghan-B1 oil and the Jurf ed Darawish Oil shale-156 bitumen (Figs. 7.3A-B). The peaks, representing the mid-chain 2,5-dialkylthiophenes, are somewhat broader than those of the 2-alkyl-5-methylthiophenes, because they comprise several structural isomers which have slightly different retention times. Both the 2-alkyl-5-methylthiophenes and the mid-chain 2,5-dialkylthiophenes in the Jianghan-B1 oil show an even-over-odd carbon number predominance with maxima at C_{18} and C_{26} and C_{26}, respectively. The 2-alkyl-5-methylthiophenes of the Jurf ed Darawish Oil shale-156 bitumen are dominated by the C_{18} member and also show a slight even-over-odd carbon number predominance in the C_{22}-C_{32} range, whilst the mid-chain dialkylthiophenes are only relatively abundant in the C_{26}-C_{37} range with a maxima at C_{28}.

The other two *m/z* 111 mass chromatograms shown (Figs. 7.3C-D; Northern Apennines marl and Monterey shale-M5 bitumens) only reveal the presence of 2-alkyl-5-methylthiophenes.

Mass chromatograms of *m/z* 125 (Fig. 7.4) reveal the presence of homologous series of 2-alkyl-5-ethylthiophenes as relatively minor compounds in a number of samples.

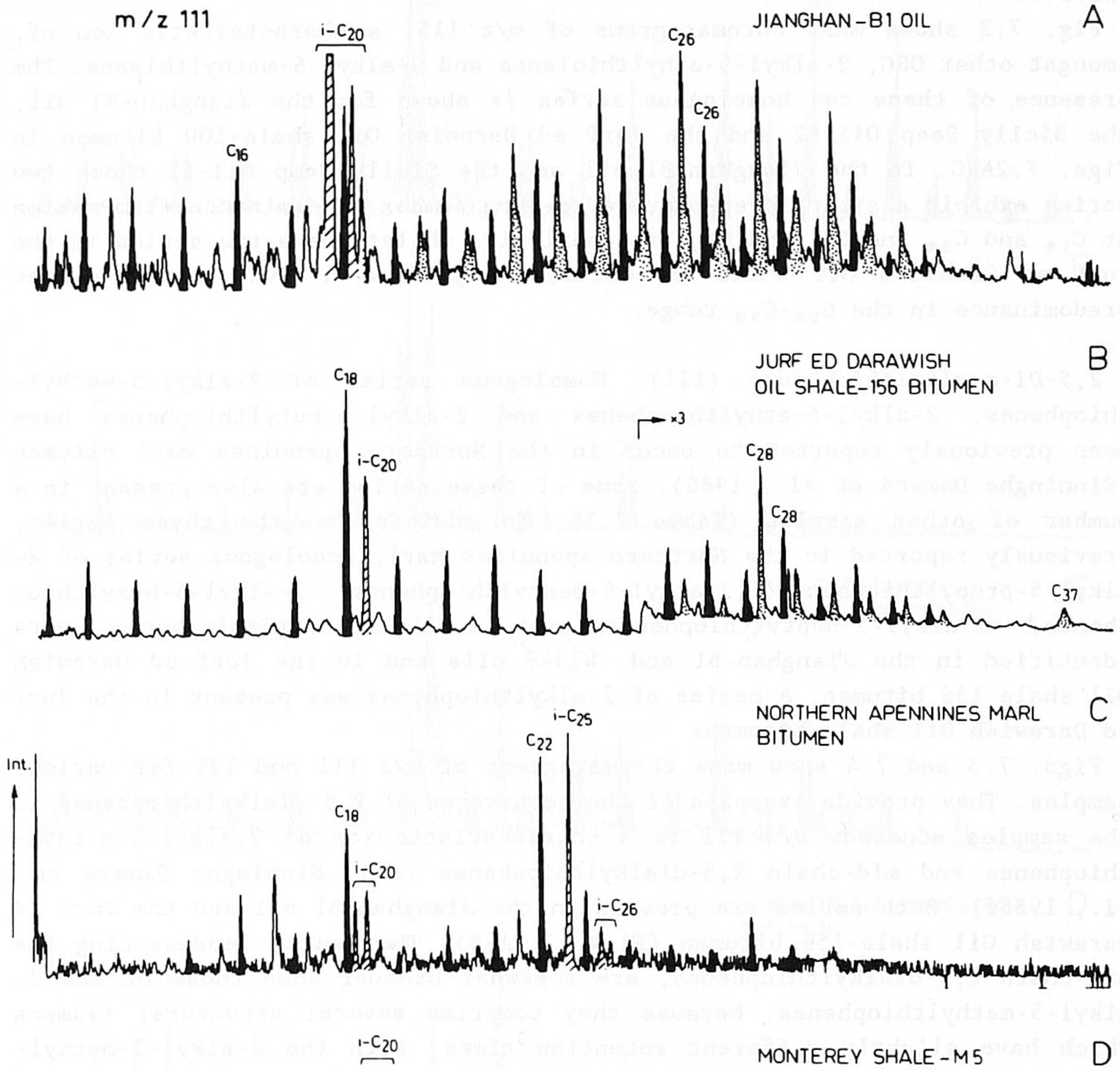


Fig. 7.3. Mass chromatograms of *m/z* 111 for the LMWA fractions of the samples indicated.

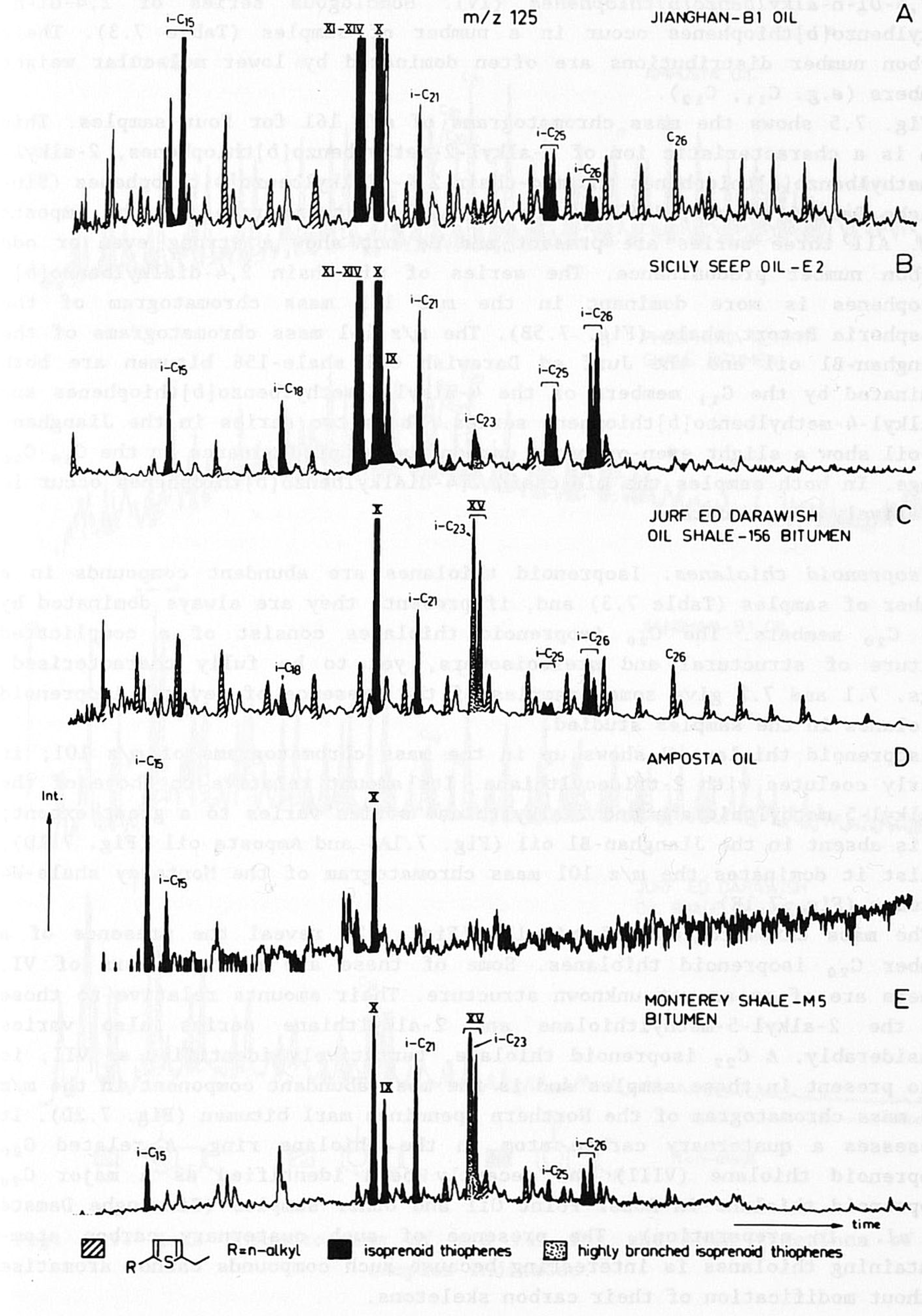


Fig. 7.4. Mass chromatograms of *m/z* 125 for the LMWA fractions of the samples indicated.

2,4-Di-n-alkylbenzo[b]thiophenes (IV). Homologous series of 2,4-di-*n*-alkylbenzo[*b*]thiophenes occur in a number of samples (Table 7.3). Their carbon number distributions are often dominated by lower molecular weight members (*e.g.* C_{11}, C_{12}).

Fig. 7.5 shows the mass chromatograms of *m/z* 161 for four samples. This ion is a characteristic ion of 4-alkyl-2-methylbenzo[*b*]thiophenes, 2-alkyl-4-methylbenzo[*b*]thiophenes and mid-chain 2,4-dialkylbenzo[*b*]thiophenes (Sinninghe Damsté *et al.*, 1987c). Fig. 7.5A shows this trace for the Amposta oil. All three series are present and do not show a strong even or odd carbon number predominance. The series of mid-chain 2,4-dialkylbenzo[*b*]-thiophenes is more dominant in the *m/z* 161 mass chromatogram of the Phosphoria Retort shale (Fig. 7.5B). The *m/z* 161 mass chromatograms of the Jianghan-B1 oil and the Jurf ed Darawish Oil shale-156 bitumen are both dominated by the C_{11} members of the 4-alkyl-2-methylbenzo[*b*]thiophenes and 2-alkyl-4-methylbenzo[*b*]thiophene series. These two series in the Jianghan-B1 oil show a slight even-over-odd carbon number predominance in the C_{16}-C_{30} range. In both samples the mid-chain 2,4-dialkylbenzo[*b*]thiophenes occur in relatively low amounts.

Isoprenoid thiolanes. Isoprenoid thiolanes are abundant compounds in a number of samples (Table 7.3) and, if present, they are always dominated by the C_{20} members. The C_{20} isoprenoid thiolanes consist of a complicated mixture of structural and stereoisomers, yet to be fully characterised. Figs. 7.1 and 7.2 give some examples of the presence of several isoprenoid thiolanes in the samples studied.

Isoprenoid thiolane V shows up in the mass chromatograms of *m/z* 101; it nearly coelutes with 2-tridecylthiane. Its amount relative to those of the 2-alkyl-5-methylthiolane and 2-alkylthiane series varies to a great extent; it is absent in the Jianghan-B1 oil (Fig. 7.1A) and Amposta oil (Fig. 7.1D), whilst it dominates the *m/z* 101 mass chromatogram of the Monterey shale-W4 bitumen (Fig. 7.1E).

The mass chromatograms of *m/z* 115 (Fig. 7.2) reveal the presence of a number C_{20} isoprenoid thiolanes. Some of these are stereiosomers of VI, others are of an as yet unknown structure. Their amounts relative to those of the 2-alkyl-5-methylthiolane and 2-alkylthiane series also varies considerably. A C_{22} isoprenoid thiolane, tentatively identified as VII, is also present in these samples and is the most abundant component in the *m/z* 115 mass chromatogram of the Northern Apennines marl bitumen (Fig. 7.2D). It possesses a quaternary carbon atom in the thiolane ring. A related C_{20} isoprenoid thiolane (VIII) has recently been identified as a major C_{20} isoprenoid thiolane in Rozel Point Oil and other samples (Sinninghe Damsté *et al.*, in preparation). The presence of such quaternary carbon atom-containing thiolanes is interesting because such compounds cannot aromatise without modification of their carbon skeletons.

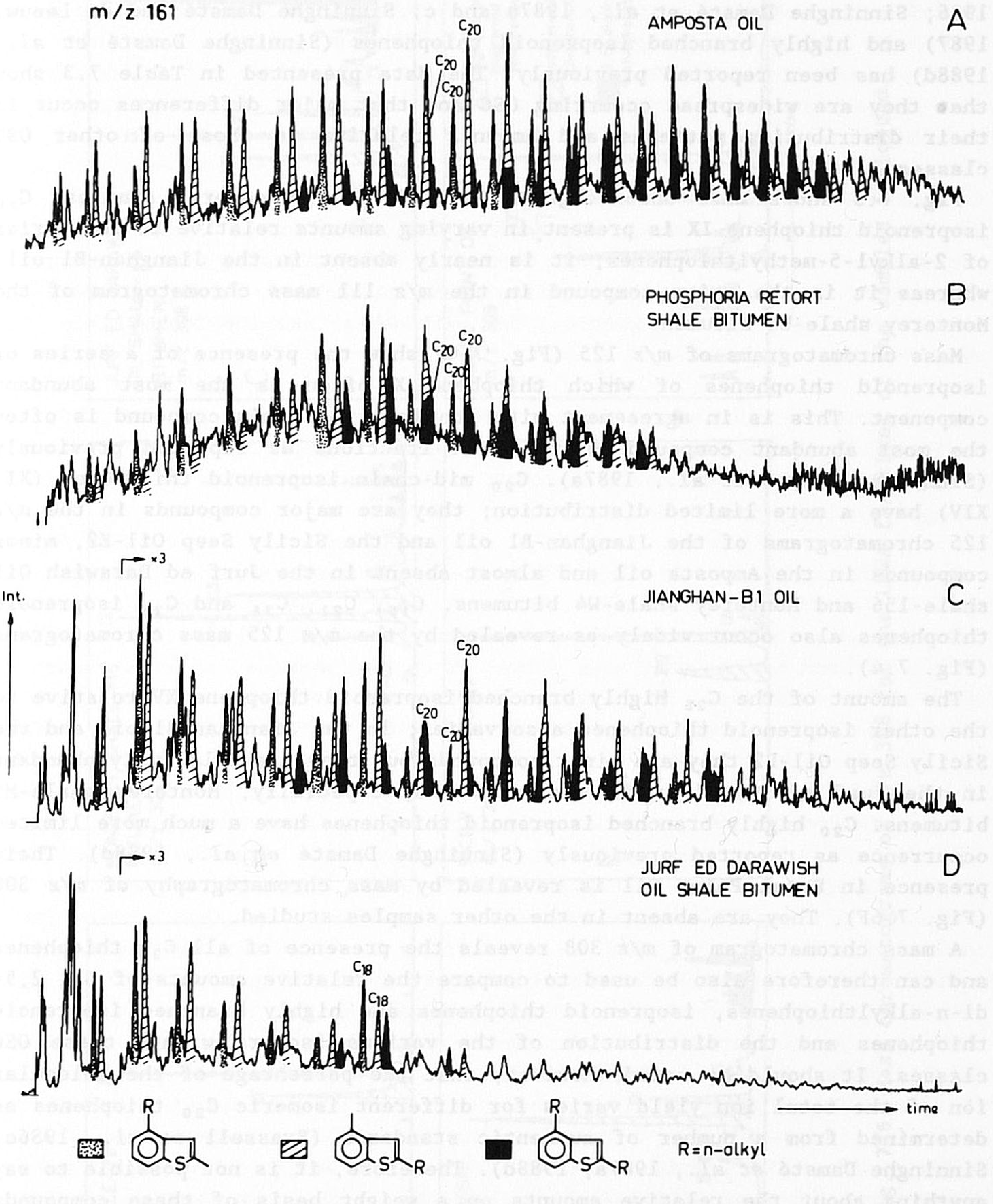


Fig. 7.5. Mass chromatograms of *m/z* 161 for the LMWA fractions of the samples indicated.

Isoprenoid and highly branched isoprenoid thiophenes. The occurrence of isoprenoid thiophenes (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986; Sinninghe Damsté *et al.*, 1987a and c; Sinninghe Damsté and de Leeuw, 1987) and highly branched isoprenoid thiophenes (Sinninghe Damsté *et al.*, 1988d) has been reported previously. The data presented in Table 7.3 show that they are widespread occurring OSC and that major differences occur in their distribution patterns and amounts relative to those of other OSC classes.

Fig. 7.3 shows mass chromatograms of *m/z* 111 for several samples. C_{20} isoprenoid thiophene IX is present in varying amounts relative to the series of 2-alkyl-5-methylthiophenes; it is nearly absent in the Jianghan-B1 oil, whereas it is the major compound in the *m/z* 111 mass chromatogram of the Monterey shale-W4 bitumen.

Mass chromatograms of *m/z* 125 (Fig. 7.4) show the presence of a series of isoprenoid thiophenes of which thiophene X often is the most abundant component. This is in agreeement with the fact that this compound is often the most abundant compound of the LMWA fractions as reported previously (Sinninghe Damsté *et al.*, 1987a). C_{20} mid-chain isoprenoid thiophenes (XI-XIV) have a more limited distribution; they are major compounds in the *m/z* 125 chromatograms of the Jianghan-B1 oil and the Sicily Seep Oil-E2, minor compounds in the Amposta oil and almost absent in the Jurf ed Darawish Oil shale-156 and Monterey shale-W4 bitumens. C_{15}, C_{21}, C_{25} and C_{26} isoprenoid thiophenes also occur widely as revealed by the *m/z* 125 mass chromatograms (Fig. 7.4).

The amount of the C_{25} highly branched isoprenoid thiophene XV relative to the other isoprenoid thiophenes also varies; in the Jianghan-B1 oil and the Sicily Seep Oil-E2 they are minor compounds but they are relatively abundant in the Jurf ed Darawish Oil shale-156 and, especially, Monterey shale-M5 bitumens. C_{20} highly branched isoprenoid thiophenes have a much more limited occurrence as reported previously (Sinninghe Damsté *et al.*, 1988d). Their presence in Rozel Point Oil is revealed by mass chromatography of *m/z* 308 (Fig. 7.6F). They are absent in the other samples studied.

A mass chromatogram of *m/z* 308 reveals the presence of all C_{20} thiophenes and can therefore also be used to compare the relative amounts of C_{20} 2,5-di-*n*-alkylthiophenes, isoprenoid thiophenes and highly branched isoprenoid thiophenes and the distribution of the various isomers within these OSC classes. It should be noted, however, that the percentage of the molecular ion of the total ion yield varies for different isomeric C_{20} thiophenes as determined from a number of authentic standards (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1987a, 1988d). Therefore, it is not possible to say anything about the relative amounts on a weight basis of these compounds from these mass chromatograms. Fig. 7.6 shows that in most of the samples studied the C_{20} thiophenes are dominated by the isoprenoid thiophenes; only in the Jurf ed Darawish Oil shale-45 bitumen the amounts of the linear dialkylthiophenes are of the same magnitude as those of the isoprenoid thiophenes. The distributions of the C_{20} isoprenoid thiophenes show major

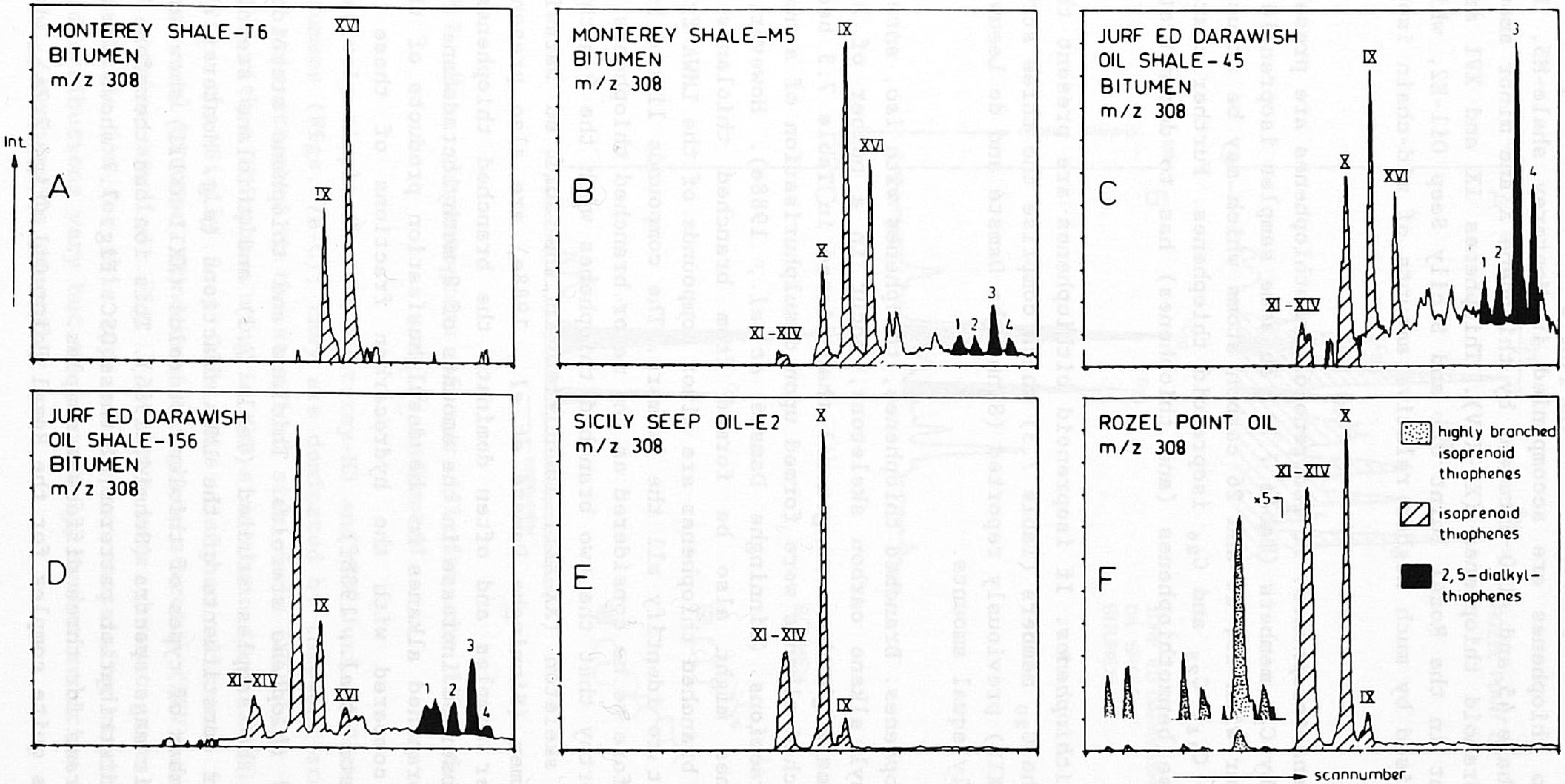


Fig. 7.6. Mass chromatograms of m/z 308 for the LMWA fractions of the samples indicated.

differences. The Monterey shale-T6 bitumen only contains thiophenes IX and XVI. These two thiophenes are accompanied in Monterey shale-M5, Jurf ed Darawish Oil shale-45 and -100 bitumens by thiophene X and minor amounts of mid-chain isoprenoid thiophenes (XI-XIV). Thiophenes IX and XVI are very minor or absent in the Rozel Point Oil and Sicily Seep Oil-E2, which are also characterised by much higher relative amounts of mid-chain isoprenoid thiophenes.

Isoprenoid benzothiophenes. If isoprenoid benzothiophenes are present they are dominated by C_{20} members (Table 7.3). In some samples isoprenoid benzothiophenes occur with 15, 25 and 26 carbon atoms which may be structurally related to the C_{15}, C_{25} and C_{26} isoprenoid thiophenes. Further characterisation of these benzothiophenes (and thiophenes) has to demonstrate this relationship.

Isoprenoid bithiophenes. If isoprenoid bithiophenes are present they are dominated by the C_{20} members (Table 7.3) which comprise the three structural isomers (XVII-XIX) previously reported (Sinninghe Damsté and de Leeuw, 1987) in approximately equal amounts.

Branched thiophenes. Branched thiophenes, thiophenes with *iso*, *anteiso* and mid-chain methyl alkane carbon skeletons, occur in a number of samples. Their occurrence might be more general than listed in Table 7.3 because a series of branched alkanes were formed upon desulphurisation of a number of these LMWA fractions (Sinninghe Damsté *et al*., 1988e). However, these branched alkanes might also be formed from branched thiolanes and/or thianes. Since branched thiophenes are minor compounds of the LMWA fractions it is difficult to identify all the isomers. The compounds listed in Table 7.3 have therefore to be considered as the major branched thiophenes.

It is noteworthy that the two branched thiophenes with the 9-methyloctadecane carbon skeleton (XX-XXI) identified in the Jurf ed Darawish Oil shale-156 bitumen (Sinninghe Damsté *et al*., 1988e) are also present in a number of other samples and often dominate the branched thiophenes. This explains the observed increase in the amounts of 9-methyloctadecane relative to the other branched alkanes in the desulphurisation products of the LMWA fractions as compared with the hydrocarbon fractions of these samples (Sinninghe Damsté *et al*., 1988f).

Thiolane and thiophene steroids. Thiolane and thiophene steroids occur widespread in the samples studied (Table 7.3) and thiolane steroids are sometimes major constituents of the LMWA fractions (*e.g*. Monterey shale-M5 bitumen). A number of types of thiolane steroids (XXII-XXIII) show an ion at *m/z* 331 in their mass spectra (Schmid, 1986). This ion can therefore be used to reveal the distribution patterns of these OSC. Fig. 7.7 shows the *m/z* 331 mass chromatograms for three different samples.

This trace is quite complex for the Rozel Point Oil (Fig. 7.7A), whilst it

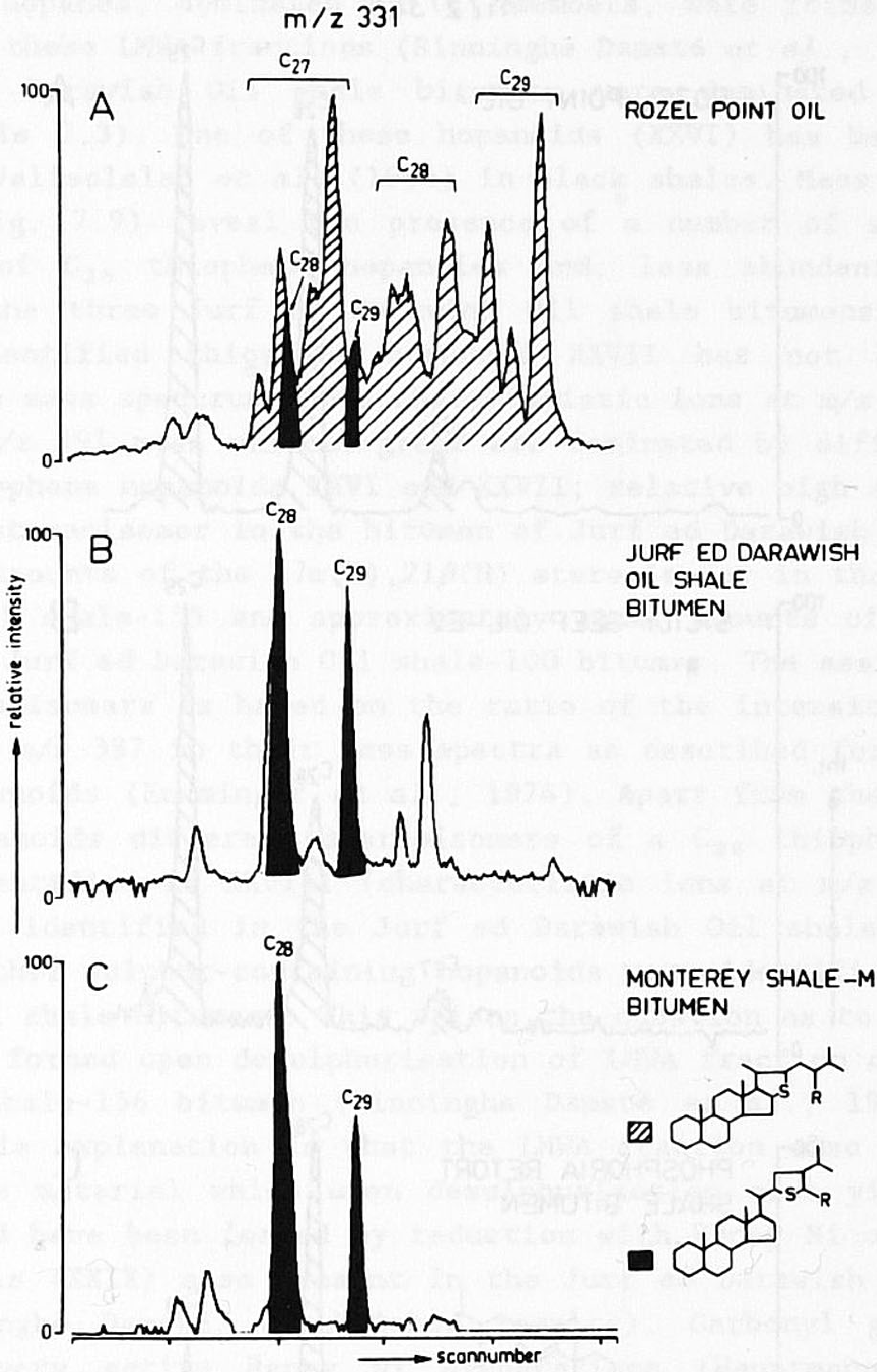


Fig. 7.7. Mass chromatograms of *m/z* 331 for the LMWA fractions of the samples indicated.

is relatively simple for the Monterey-M5 and the Jurf ed Darawish Oil shale-100 bitumens (Figs. 7B-C); they are dominated by thiolane steroids with the sulphur atom attached to the steroidal side-chain (XXIII).

Steroids with a thiophene ring in the side-chain (*e.g.* XXIV) occur in Rozel Point Oil, the Sicily Seep Oils, the Jianghan oils and in the Northern Apennines marl bitumen. Condensed thiophene steroids (XXV) occur also widely in the samples studied. Fig. 7.8 shows mass chromatograms of *m/z* 341, a characteristic ion for this type of sulphur-containing steroids. The carbon number distributions vary but the amount C_{27} member relative to those of the C_{28} and C_{29} members is generally low.

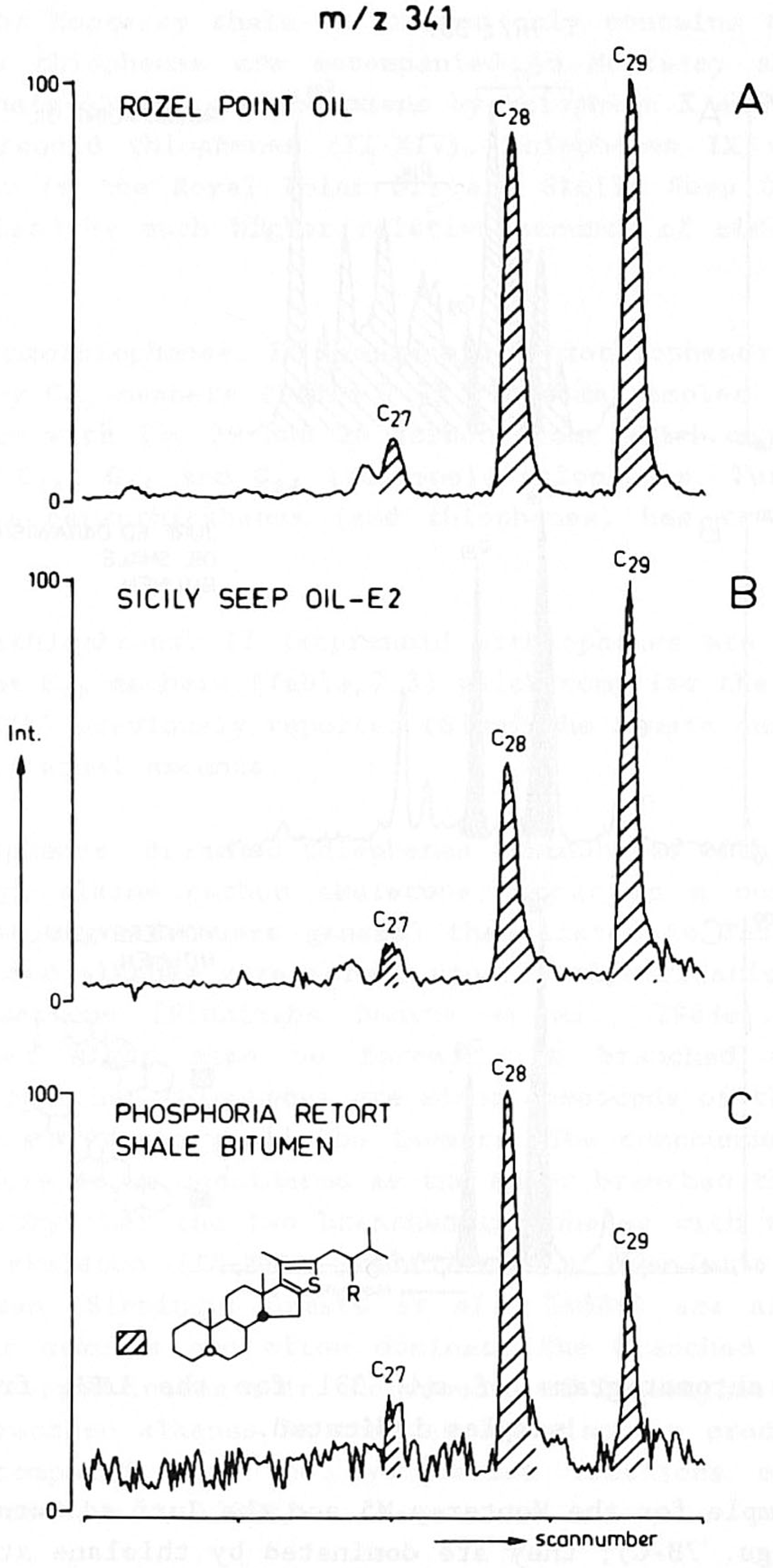


Fig. 7.8. Mass chromatograms of *m/z* 341 for the LMWA fractions of the samples indicated.

Thiolane and thiophene hopanoids. The presence of thiolane and thiophene hopanoids could only be proved in a number of samples due to problems with the GC-MS interface. In other cases, however, these types of OSC seem to be present since hopanes, dominated by C_{35} members, were formed upon desulphurisation of these LMWA fractions (Sinninghe Damsté *et al.*, 1988f).

The Jurf ed Darawish Oil shale bitumens were dominated by thiophene hopanoids (Table 7.3). One of these hopanoids (XXVI) has been previously identified by Valisolalao *et al.* (1984) in black shales. Mass chromatograms of *m/z* 191 (Fig. 7.9) reveal the presence of a number of structural and stereoisomers of C_{35} thiophene hopanoids and, less abundant, C_{35} hopane sulphides in the three Jurf ed Darawish Oil shale bitumens studied. The tentatively identified thiophene hopanoid XXVII has not been reported previously. Its mass spectrum shows characteristic ions at *m/z* 111, 191, 387 and 508. The *m/z* 191 mass chromatograms are dominated by different stereoisomers of thiophene hopanoids XXVI and XXVII; relative high amounts of the 17β(H),21β(H) stereoisomer in the bitumen of Jurf ed Darawish Oil shale-45, relative high amounts of the 17α(H),21β(H) stereoisomer in that of the Jurf ed Darawish Oil shale-155 and approximately equal amounts of both stereoisomers in the Jurf ed Darawish Oil shale-100 bitumen. The assignment of the different stereoisomers is based on the ratio of the intensity of the ions at *m/z* 191 and *m/z* 387 in their mass spectra as described for non sulphur-containing hopanoids (Ensminger *et al.*, 1974). Apart from the C_{35} sulphur-containing hopanoids different stereoisomers of a C_{36} thiophene hopanoid, tentatively identified as XXVIII (characteristic ions at *m/z* 97, 205, 287 and 522), were identified in the Jurf ed Darawish Oil shale-100 and -156 bitumens. No other sulphur-containing hopanoids were identified in the Jurf ed Darawish Oil shale bitumens. This raises the question as to where are the other hopanes, formed upon desulphurisation of LMWA fraction of the Jurf ed Darawish Oil shale-156 bitumen (Sinninghe Damsté *et al.*, 1988f), derived from. A possible explanation is that the LMWA fraction also contains some non GC-amenable material which upon desulphurisation also yields hopanes. They also could have been formed by reduction with Raney Ni of a series of hopanoid ketones (XXIX) also present in the Jurf ed Darawish Oil shale-156 bitumen (Sinninghe Damsté, unpublished results). Carbonyl groups may be reduced with very active Raney Ni preparations (Hauptmann and Walter, 1961).

The thiophene hopanoids present in several Monterey bitumens almost exclusively exist of the 17β(H),21β(H)-stereoisomer of thiophene hopanoid XXVI.

Branched octadecyl-substituted benzothiophenes. A series of benzothiophenes, not yet previously described, was present in a number of samples (Table 7.3). The structures of these compounds are tentatively identified as XXXa-d, compounds which have the same carbon skeletons as methylated 2-methyl-2-(4,8,12-trimethyltridecyl)chromans (MTTC, XXXIa-d; Sinninghe Damsté *et al.*, 1987b) and as specific isoprenoid alkylbenzenes (XXXIIa-d; Sinninghe Damsté *et al.*, 1988b). Their identification awaits further confirmation by comparison of retention time and mass spectral data with those of synthetic standards, a study presently being carried out in this laboratory. They were

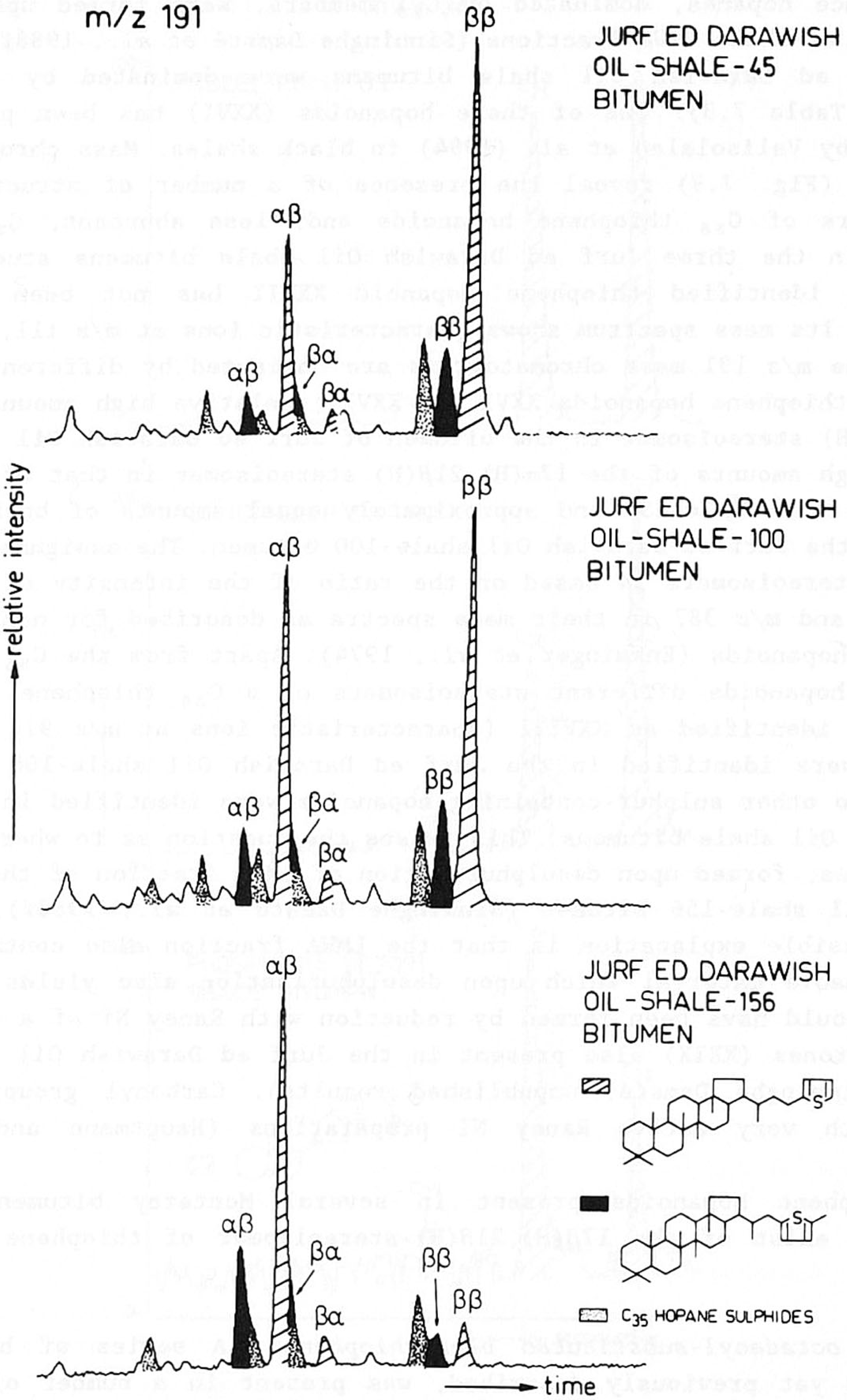


Fig. 7.9. Mass chromatograms of *m/z* 191 for the LMWA fractions of the samples indicated.

identified in the LMWA fractions by mass chromatography of *m/z* 175, 189 and 203. The carbon number distributions of this series are often dominated by the C_{28} member(s) and are not always similar to those of the structurally related MTTC and isoprenoid benzenes (Table 7.3).

Non-OSC. 1,2-Di-*n*-alkylbenzenes (XXXIII) were abundantly present in the Amposta oil and represent minor compounds in the Jurf ed Darawish Oil shale and Monterey shale bitumens. In the latter samples only series of mono alkylbenzenes were identified, whilst in the Amposta oil this series was accompanied by series of 1-alkyl-2-methylbenzenes, as reported previously by Albaiges *et al*. (1986), 1-alkyl-2-ethylbenzenes, 1-alkyl-2-propylbenzenes, 1-alkyl-2-butylbenzenes and 1-alkyl-2-pentylbenzenes. Isoprenoid alkylbenzenes (XXXII) occur in a number of samples as reported previously. It is noteworthy that C_{26}-C_{29} isoprenoid alkylbenzenes are absent in the Monterey bitumens but that C_{22}—C_{24} isoprenoid alkylbenzenes (XXXIVa-c) occur. These alkylbenzenes are structurally related to the C_{26}-C_{29} isoprenoid alkylbenzenes but instead of an C_{20} isoprenoid side-chain they possess an C_{15} isoprenoid side-chain.

C-ring monoaromatic steroids occur ubiquitously in the samples studied (Table 7.3), whilst A- and B-ring monoaromatic steroids were present in a more restricted sample suite. The carbon number distributions of the C-ring monoaromatic steroids is not given in Table 7.3 because members of this series with different carbon numbers coelute and the molecular ions in their mass spectra are very weak.

Methylated MTTC (XXXI) are ubiquitous compounds in the samples studied but their relative amounts vary; they are major compounds in most of the bitumens but minor compounds in the oils (Table 7.3). Their carbon number distributions vary; they are dominated by the C_{29} member in the bitumens (except the Northern Apennines marl bitumen), whilst those of the oils and the Northern Apennines marl bitumen show maxima at either C_{27} or C_{28}.

7.5 DISCUSSION

Geochemical characteristics of the samples

Maturity. Most of the samples are characterised by very low amounts of saturated hydrocarbons and a high asphaltene content. Although these characteristics are generally thought to be indicative of biodegraded mature oils, there are some distinct signs of immaturity: *e.g.* strong even-over-odd or odd-over-even carbon number predominance of the *n*-alkanes, high phytane/octadecane ratio, presence of relatively high amounts of sterenes and hopenes, relatively low amounts of 5α(H),14β(H),17β(H) steranes. Only the Amposta oil and Phosphoria Retort shale show about equal amounts of 20R and 20S 5α(H),14α(H),17α(H) steranes and 20R and 20S 5α(H),14β(H),17β(H) steranes. Only the complete isomerisation at C-20 of the 5α(H),14α(H),17α(H) steranes is an indication of the relatively more mature stage of the organic matter since early diagenetical reactions of Δ^7 sterols (present in relatively high amounts in recent hypersaline environments) may "rapidly" lead to the formation of 20R and 20S 5α(H),14β(H),17β(H) steranes (ten Haven *et al.*, 1986). The Amposta oil also contains higher amounts of saturated hydrocarbons, a carbon number preference index near unity and a relatively low phytane/octadecane ratio and, therefore, has to be considered more mature than the other oils. The same holds to a lesser extent for the Phosphoria Retort shale bitumen.

Input. The hydrocarbon fractions of all the samples studied (Table 7.1) do not show abundantly present biomarkers derived from higher plants such as *n*-alkanes with a marked odd-over-even carbon number predominance in the C_{25}-C_{35} range, diterpenoids and oleananes. This testifies to the low amount of terrestrial input relative to the autochthonous input of phytoplankton and bacteria. The Northern Apennines marl, the Monterey shale, Jurf ed Darawish shale and the source rock of the Sicily Seep Oils-El received some higher plant input as concluded from the slight to moderate odd-over-even carbon number predominance of the *n*-alkanes in the C_{25}–C_{35} range. However, the *n*-alkane distribution patterns of most of these immature samples maximize at C_{16}–C_{22}, indicating major marine or lacustrine sources for the organic matter.

Other biomarkers present in the samples studied are the steranes and triterpanes, dominated by hopanes. In the saturated hydrocarbon fractions of the Rozel Point Oil, Sicily Seep Oils, Jianghan oils and Northern Apennines marl bitumen the steranes dominate over the hopanoids, whereas this was the opposite in the saturated hydrocarbon fractions of Jurf ed Darawish Oil shale, Phosphoria Retort shale and Monterey bitumens. This may be interpreted as a higher input of algae (steroids) relative to the input of bacteria (hopanoids) in the first group of samples. The Monterey shale-SB was quite extreme in this respect since the saturated hydrocarbon fraction of its bitumen consisted for *ca.* 80% of 17α(H),18α(H),21β(H)-28,30-bisnorhopane.

Depositional environment. Studies on the hydrocarbon biological markers indicate that the source rocks of the Rozel Point Oil, Sicily Seep Oils and the Jianghan oils and the Northern Apennines marl were probably deposited under hypersaline conditions (ten Haven *et al.*, 1985, 1987, 1988; Brassell *et al.*, 1988). The hydrocarbon fractions of these samples are characterised by a very low pristane/phytane ratio (<0.1), an even-over-odd carbon number predominance of the *n*-alkanes (except the Northern Apennines marl bitumen) and the presence of a series of extended hopanes and hopenes maximizing at C_{35}. The methylated MTTC distributions, recently proposed to be of use in assessing palaeosalinity (Sinninghe Damsté *et al.*, 1987b), also point to a hypersaline depositional environment. The methylated MTTC distribution of the Amposta oil maximises at the C_{27} member and therefore also points to a hypersaline depositional environment of the source rock of this oil. A series of extended hopanes maximising at C_{35} is also present (Sinninghe Damsté *et al.*, 1988e) but the pristane/phytane ratio is somewhat higher and the carbon preference index much lower than for the other oil samples (Table 7.1). However, the *n*-alkanes in the C_{21}-C_{33} range show a slight even predominance (Albaiges and Torradas, 1974). These differences in biomarker characteristics might not be explained by a different depositional environment but by differences in maturity (see above). The hydrocarbon imprint of the depositional environment is, in the case of the Amposta oil, probably diluted by hydrocarbons generated from thermal degradation of kerogen and asphaltenes. This also explains the higher amount of saturated hydrocarbons in the oil. The pristane/phytane ratio might be affected by thermal generation of pristane by breakdown of macromolecularly bound tocopherols (Goossens *et al.*, 1984).

The source rocks of the Sicily Seep Oils, the Northern Apennines marl and possibly the source rock of the Amposta oil were deposited in the Late Miocene in the Mediterranean Basin. At that time the Mediterranean was a deep, desiccated basin that contained a series of isolated saline lakes in which evaporitic carbonates and evaporites were deposited under shallow but fluctuating water depths (Warren, 1986). This indicates that the conditions under which deposition of organic matter took place might be different due to differences in salinity.

The hydrocarbon biological markers from the Jurf ed Darawish Oil shale bitumens do not show all the characteristics of a hypersaline depositional environment. Although series of extended hopenes maximising at C_{35} are present, the pristane/phytane ratios are higher than those of the samples described above (Table 7.1) and no even-over-odd predominance of *n*-alkanes is observed. The methylated MTTC distributions maximise at the C_{29} member, also indicating a non-hypersaline environment of deposition. The samples consist of 40-80% $CaCO_3$ (Wehner and Hufnagel, 1987) probably deposited by oversaturation of the water column. These conditions represent, however, the first stage of hypersalinity in the water column. Therefore, the palaeoenvironment of the Jurf ed Darawish has to be considered as intermediate between a hypersaline palaeoenvironment and a "normal" marine palaeoenvironment. The lower pristane/phytane ratio of the deepest sample of the Jurf ed Darawish oil shale suggest that it was deposited under more saline conditions as the other two samples (ten Haven *et al.*, 1987). This is consistent with data presented for the related Ghareb Formation (Jordan Valley) of the same age. Spiro and Aizenshtat (1977) presented evidence that the lowermost beds of this formation were deposited under hypersaline conditions. This indicates that the change in the distribution of the methylated MTTC (Sinninghe Damsté *et al.*, 1987b) occurs at a salinity level higher than in normal sea-water.

The Monterey Formation and Phosphoria Retort shale are samples representing a non-hypersaline environment of deposition. Although they sometimes show series of extended hopanes and/or hopenes maximising at C_{35}, the pristane/phytane ratios are higher than 0.1, no even-over-odd predominance of the *n*-alkanes is observed and the methylated MTTC distributions maximise at the C_{29} member.

These changes in biological marker compositions with increasing salinity are visualised in Fig. 7.10. Fig. 7.10A shows a plot of the pristane/phytane ratio *versus* the trimethyl MTTC/total methylated MTTC ratio. Two clusters of samples are observed. One cluster represents samples from non-hypersaline to slightly hypersaline depositional environments characterised by a high trimethyl MTTC/total methylated MTTC ratio (> 0.5) in combination with a relatively high pristane/phytane ratio (> 0.3). The other cluster represents samples from hypersaline depositional environments which are characterised by a low trimethyl MTTC/total methylated MTTC ratio (< 0.4) in combination with a very low pristane/phytane ratio (< 0.1). The Amposta oil does not fit into this cluster because its pristane/phytane ratio is too high probably because of its more mature nature.

Occurrence of OSC

OSC of the types previously identified in the Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c) occur in all the samples studied (Table 7.3). These OSC are proposed to be formed by sulphur incorporation reactions during early diagenesis (Valisolalao *et al.*, 1984; Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987a and c, 1988d, e and f; Sinninghe Damsté and de Leeuw, 1987). The widespread occurrence of these types of OSC in immature bitumens and immature oils indicates that sulphur incorporation into lipid material during early diagenesis is a significant and widely occurring mechanism for the sulphur-enrichment of organic matter.

A significant finding is that sulphur incorporation into organic matter does not only takes place under hypersaline conditions of deposition but also takes place in marine, highly anoxic sediments such as those of the Monterey Formation. Of course, hypersaline conditions of deposition are in favour of such reactions because hypersalinity often induces a density stratification of the water column and thereby establishes anoxic conditions in the lower part of the water-column and at the sediment-water interface. Iron is generally less abundant in these type of environments whilst the productivity of organic matter is high and so high levels of H_2S produced by sulphate-reducing bacteria may react with the lipids biosynthesised by phytoplankton, phytobenthos and bacteria, the major producers of organic matter in hypersaline environments (Warren, 1986). However, in sediments depositing under anoxic conditions due to other reasons than hypersalinity (*e.g.* anoxia caused by upwelling; Demaison and Moore, 1980) these sulphur incorporation reactions may also take place as shown by the presence of OSC in samples from the Monterey Formation. In these cases the amounts of H_2S produced by sulphate-reducing bacteria probably exceed the amount of available iron (due to clastic input) since it is thought that H_2S quanti-

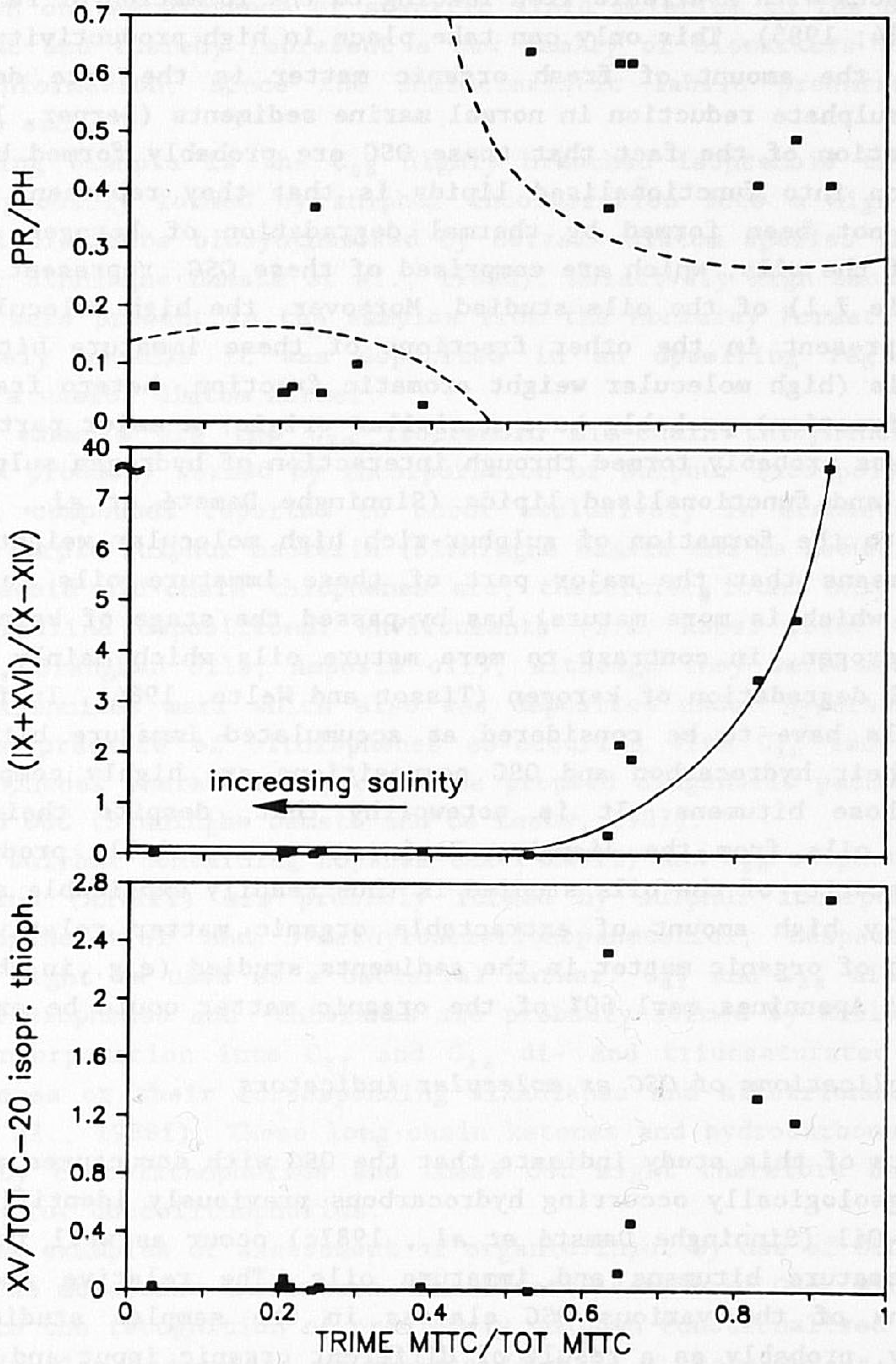


Fig. 7.10. Plots of the pristane/phytane ratio, the sum of isoprenoid thiophenes IX and XVI/the sum of isoprenoid thiophenes X-XIV ratio and the C_{25} highly branched isoprenoid thiophenes/total C_{20} isoprenoid thiophenes ratio *versus* the trimethyl MTTC/total MTTC ratio. The pristane/phytane and trimethyl MTTC/total MTTC ratios are on a weight basis. The other two ratios are not on a weight basis since they were determined by integration of mass chromatograms of characteristic ions and it is not exactly known how much these ions contribute to the total ion yield of all the compounds involved.

tatively reacts with available iron leading to the formation of FeS and FeS_2 (Berner, 1984; 1985). This only can take place in high productivity environments since the amount of fresh organic matter is the rate determining factor for sulphate reduction in normal marine sediments (Berner, 1984).

An implication of the fact that these OSC are probably formed by sulphur incorporation into functionalised lipids is that they represent compounds which have not been formed by thermal degradation of kerogen. The LMWA fractions of the oils, which are comprised of these OSC, represent 3-22% (by weight; Table 7.1) of the oils studied. Moreover, the high molecular weight substances present in the other fractions of these immature bitumens and immature oils (high molecular weight aromatic fraction, hetero fraction and asphaltene fraction) probably have a similar origin; a major part of these substances was probably formed through interaction of hydrogen sulphide with unsaturated and functionalised lipids (Sinninghe Damsté *et al.*, 1988a and e) leading to the formation of sulphur-rich high molecular weight substances. This means that the major part of these immature oils (except the Amposta oil which is more mature) has by-passed the stage of being part of insoluble kerogen, in contrast to more mature oils which mainly originate from thermal degradation of kerogen (Tissot and Welte, 1984). In fact these immature oils have to be considered as accumulated immature bitumens and therefore their hydrocarbon and OSC compositions are highly comparable to those of those bitumens. It is noteworthy that, despite their unusual origin, the oils from the Jianghan Basin are commercially produced. The unusual immaturity of the oils studied is thus readily explicable as well as the unusually high amount of extractable organic matter relative to the total amount of organic matter in the sediments studied (*e.g.* in the case of the Northern Apennines marl 60% of the organic matter could be extracted).

Possible applications of OSC as molecular indicators

The results of this study indicate that the OSC with structures related to well-known geologically occurring hydrocarbons previously identified in the Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c) occur as well in a number of other immature bitumens and immature oils. The relative amounts and distributions of the various OSC classes in the samples studied varies considerably, probably as a result of different organic input and different degrees of thermal maturation. A discussion of their possible application as molecular indicators is therefore worthwhile.

Assessment of organic input. Although the exact mechanisms of the formation of OSC are as yet unknown it is very likely that these compounds originate from a "quenching" type of reaction of functionalised lipids (*e.g.* unsaturated fatty acids, alcohols, alkenes) with H_2S during early diagenesis. Because the resulting OSC are much more stable against microbial attack and under geological conditions than the original lipids, the structures of the OSC occurring in bitumens and immature oils may provide information on the presence of specific lipid moieties in the depositional environment and thereby represent a new family of biomarkers with complementary information, since the characteristic labile precursors do not survive as such.

A striking example is the C_{25} highly branched isoprenoid thiophene XV, which is probably formed by sulphur incorporation into a highly branched isoprenoid alkadiene biosynthesised by certain diatom species (Nichols *et al.*, 1988; Sinninghe Damsté *et al.*, 1988d). Relatively high amounts of this thiophene were present in the samples from the Monterey Formation which is not unlikely because it was deposited in an upwelling region. It may represent a useful diatom marker.

Another example are the C_{20} isoprenoid mid-chain thiophenes (XI-XIV), which were probably formed by incorporation of sulphur into polyunsaturated phytenols, compounds reported to occur exclusively in archaebacteria and green and purple sulphur bacteria (Sinninghe Damsté and de Leeuw, 1987). The C_{20} isoprenoid mid-chain thiophenes are, therefore, found only in samples from hypersaline depositional environments (*i.e.* Rozel Point Oil, Sicily Seep Oils, Jianghan oils, Amposta oil), although they were absent in the Northern Apennines marl which also was deposited under hypersaline conditions. The presence of bithiophenes co-occuring with C_{20} isoprenoid mid-chain thiophenes therefore supports the propsed diagenetic pathways for C_{20} isoprenoid OSC (Sinninghe Damsté and de Leeuw, 1987).

The C_{35} sulphur-containing hopanes (XXVI-XXVII) and C_{36} sulphur-containing methylhopane (XXVIII) are probably formed by sulphur incorporation into bacteriohopanetetrol and 3-methylbacteriohopanetetrol, respectively, and therefore might be used as a bacterial marker. C_{37} and C_{38} mid-chain 2,5-di-*n*-alkylthiophenes and -thiolanes are probably formed by early diagenetic sulphur incorporation into C_{37} and C_{38} di- and triunsaturated methyl and ethyl ketones or their corresponding alkadienes and alkatrienes (Sinninghe Damsté *et al.*, 1988f). These long-chain ketones and hydrocarbons are lipids produced by coccolithophorids and these OSC might therefore be of use as biomarkers for coccolithophorids.

These few examples of assessment of organic input by use of OSC show their potential as molecular indicators. The identification of OSC, alternatively, may lead to the recognition of previously unknown functionalised lipids. For example the C_{26} isoprenoid thiophenes (Fig. 7.4) have an as yet unknown precursor.

Assessment of depositional environment. In general the presence of the OSC described in this paper are an indication of an anoxic environment of deposition where the production of H_2S exceeded the input of reactive iron minerals as discussed above. However, more detailed information seems to be available from distribution patterns regarding the assessment of palaeoenvironment.

An example are the distribution patterns of the C_{20} isoprenoid thiophenes which seem to be dependent on the salinity of the depositional environment

(Fig. 7.6). The C_{20} isoprenoid thiophenes in normal marine (not hypersaline) samples are dominated by thiophenes IX and XVI. For instance the C_{20} isoprenoid thiophene distribution of the Monterey-T6 bitumen is dominated by these two thiophenes. They have also been reported to be the major thiophenes in oceanic sediments (Brassell *et al.*, 1986c; Rullkötter *et al.*, 1988). C_{20} isoprenoid thiophene X becomes gradually more abundant in the the Monterey shale-M5, the Jurf ed Darawish Oil shale-45 and -156 bitumens, respectively (Fig. 7.6B-D). In the Rozel Point Oil and Sicily Seep Oil-E2 (but also in the other Sicily Seep Oils, the Jianghan oils and the Amposta oil; not shown in Fig. 7.6) thiophene XVI is completely absent whilst thiophene X dominates over thiophene IX (Figs. 7.6E-F). The C_{20} isoprenoid thiophene distributions of the Rozel Point Oil and Sicily Seep Oil-E2 also show relatively high amounts of isoprenoid mid-chain thiophenes (XI-XIV; Figs. 7.6E-F), which are minor compounds or absent in the other four examples (Figs. 7.6A-D). These changes in the C_{20} isoprenoid thiophene distributions seem to co-occur with changes in the salinity of the depositional environment of the samples as derived from geological and other biomarker data (see above). A plot of the (IX+XVI)/(X+XI+XII+XIII+XIV) ratio *versus* the trimethyl MTTC/total MTTC ratio (Fig. 7.10B) shows this phenomenon. In this plot the samples from the Jurf ed Darawish Oil shale (which originate from a slightly hypersaline paleoenvironment) lie indeed between those from non-hypersaline and from hypersaline depositional environments. A plot of the C_{25} highly branched isoprenoid thiophenes/total C_{20} isoprenoid thiophenes ratio *versus* the trimethyl MTTC/total MTTC ratio (Fig. 7.10C) shows a similar trend but there seems to be more variation in the C_{25} highly branched isoprenoid thiophenes/total C_{20} isoprenoid thiophenes ratio in samples from non-hypersaline to slightly hypersaline depositional environments. This is probably due to differences in input (relative contribution of diatoms to the total organic matter). Although these changes in distribution of C_{20} isoprenoid thiophenes are not yet completely understood and may also depend on maturity, they might be useful, in combination with methylated MTTC distributions, in assessing palaeosalinity, especially since C_{20} isoprenoid thiophenes are among the most widespread occurring classes of OSC (Table 7.3).

Assessment of maturity. The types of OSC described here have so far only been found in sediments and immature oils. "Normal" mature oils, however, contain sulphur compounds with thiophene rings as part of more complex ring systems (primarily benzothiophenes, dibenzothiophenes and benzonaphtothiophenes with much shorter alkyl side-chains; *e.g.* Hughes, 1984; Arpino *et al.*, 1987). These differences in OSC distributions were explained by:

(1) dilution by newly generated sulphur compounds from kerogen maturation

(2) cyclisation and aromatisation of long-chain OSC (Sinninghe Damsté *et al.*, 1988a).

Supporting evidence for both explanations is obtained from this study. The distribution patterns of the 2-alkyl-5-methylthiolanes in the Sicily Seep

Oil-E2 and Jianghan-B1 oil (Figs. 7.1A and B) are characterised by a strong even-over-odd predominance, whilst this distribution in the more mature Amposta oil (Fig. 7.1D) shows no strong predominance. The depositional environment of the source rocks of these oils is probably comparable. Therefore, it is not unlikely that the 2-alkyl-5-methylthiolanes formed during early diagenesis, exhibiting a strong even-over-odd carbon number predominance (as can be slightly seen in the C_{25}-C_{31} range; Fig. 7.1D), were diluted by thermally generated 2-alkyl-5-methylthiolanes with no carbon number predominance. Series of 2-alkyl-5-methylthiolanes were generated by flash-pyrolysis of sulphur-rich kerogens (Sinninghe Damsté *et al.*, 1988c). Hydrous-pyrolysis, a technique to simulate oil formation, also produced this series (Eglinton *et al.*, 1988f) indicating that such a generation can take place.

The possible formation of 2,4-di-*n*-alkylbenzo[*b*]thiophenes (IV) from 2,5-di-*n*-alkylthiophenes (III) (Perakis, 1986; Sinninghe Damsté *et al.*, 1987c) and isoprenoid benzothiophenes from isoprenoid thiophenes (Sinninghe Damsté and de Leeuw, 1987) by cyclisation and subsequent aromatisation reactions have been described previously. The fact that the two somewhat more mature samples (Amposta oil and Phosphoria Retort shale) contain relatively more 2,4-dialkylbenzo[*b*]thiophenes and virtually no 2,5-dialkylthiophenes (Table 7.3) strongly support this theory. The transition of 2,5-dialkylthiolanes (I) and possibly 2,6-dialkylthianes (II) into 2,5-dialkylthiophenes (III) by aromatisation is in some cases quite clear because of similar distribution patterns but in other cases less obvious since some samples contain 2,5-dialkylthiolanes and 2,4-dialkylbenzo[*b*]thiophenes but no 2,5-dialkylthiophenes (Table 7.3).

It seems that when these processes will be better understood the compounds involved may act as maturity indicators (*e.g.* benzothiophene/thiophene ratios) spanning a large range.

Another possible maturity indicator might be the isomerisation of sulphur-containing steranes and hopanes. The distributions of the thiophene hopanoids in the Jurf ed Darawish Oil shale core (Fig. 7.9) suggest such an application; the probably less stable 17β(H),21β(H) stereoisomers of XXVI and XXVII (assumed to be formed by sulphur incorporation into a precursor with natural 17β(H),21β(H) stereochemistry) are the major stereoisomers in the shallowest Jurf ed Darawish Oil shale sample, whilst the probably more stable 17α(H),21β(H) stereoismers are the most abundant stereoisomers in the deepest sample. This suggest that these changes in distribution are due to different, although small, degrees of maturity as proposed by Valisolalao *et al.* (1984). This observed trend co-occurs with a similar trend in the stereochemistry of the C_{29}-C_{31} hopanes; in the shallowest sample 17β(H), 21β(H)-hopanes dominate, whilst they are relatively minor in the deepest sample. Wehner and Hufnagel (1987) ascribed this trend, however, to a change in the depositional environment rather than to the influence of maturation. Extended hopanes with the 22R 17α(H),21β(H)-stereochemistry have, indeed, been found in lichens and fungi (Corbett and Heng, 1971) and in a sample of predominantly *Sphaghnum* peat (Taylor *et al.*, 1980). The difference in depth of the shallowest and deepest sample is only 111 m, which means that the differences in temperature history are small and probably cannot acount for the almost complete isomerisation although isomerisation of 17β(H),21β(H)-hopanes to 17α(H),21β(H)-hopanes occurs at relatively low levels of maturity (Mackenzie *et al.*, 1988). At present it is thus unknown whether the observed differences in both hopane and thiophene hopanoid distributions are related to differences in input or maturation. More research, in which the other hopanoids (*e.g.* extended hop-17(21)-enes and hopanoid ketones (XXIX)) have to be taken into account as well, might resolve this question.

Oil-oil and oil-source rock correlation studies. As outlined above differences in input and maturity have a serious impact on the OSC distributions. Therefore, these distribution can be used in fingerprinting oils and bitumens. For example, the distributions of the various classes of OSC in the related Sicily Seep oils (E1, E2 and E5) were indeed very similar (Table 7.3). A similar observation was made for the two related Jianghan oils (B1, W1349).

7.5 CONCLUSIONS

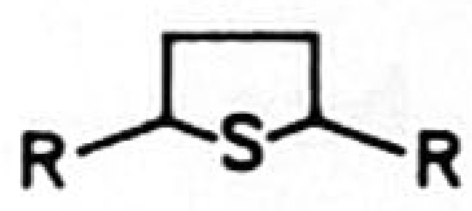
(1) Organic sulphur compounds (OSC), with carbon skeletons identical to well-known geologically occurring hydrocarbons (*n*-alkanes, isoprenoid alkanes, steranes, triterpanes), occur widely in immature bitumens and immature crude oils.

(2) The palaeoenvironments of the sediments and source rocks of the oils in which these OSC occur were anoxic and H_2S, produced by sulphate-reducing bacteria, has probably exceeded the input of available iron. These conditions caused a surplus of free H_2S, which reacted with organic matter to form OSC.

(3) The relative amounts and distributions of the OSC vary considerably as a result of different organic input and different degrees of maturation.

(4) Therefore, OSC may be useful as molecular indicators for the assessment of organic input, depositional environment and maturity and in oil-oil and oil-source rock correlation studies.

APPENDIX



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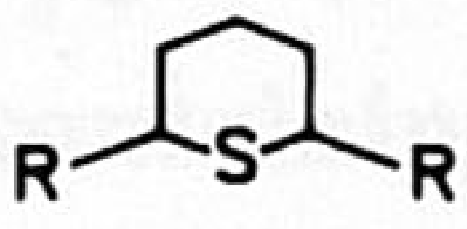

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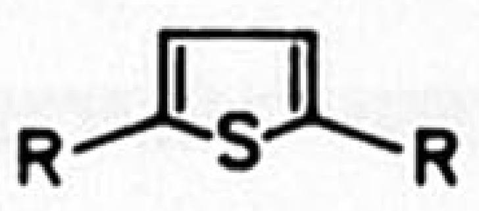

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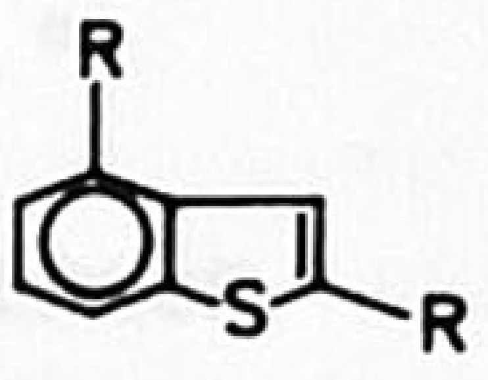

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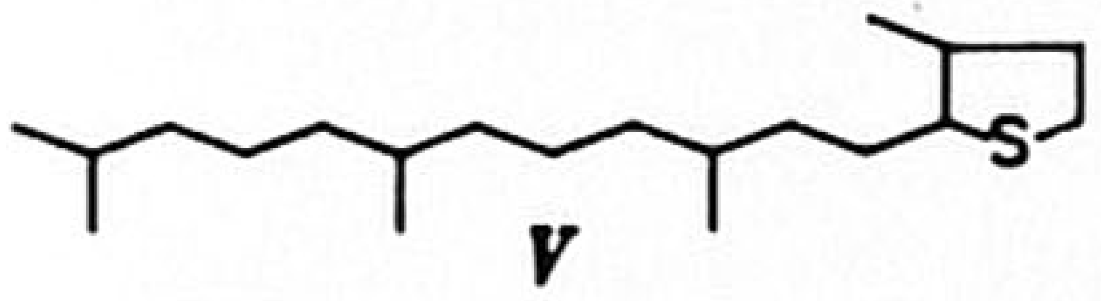

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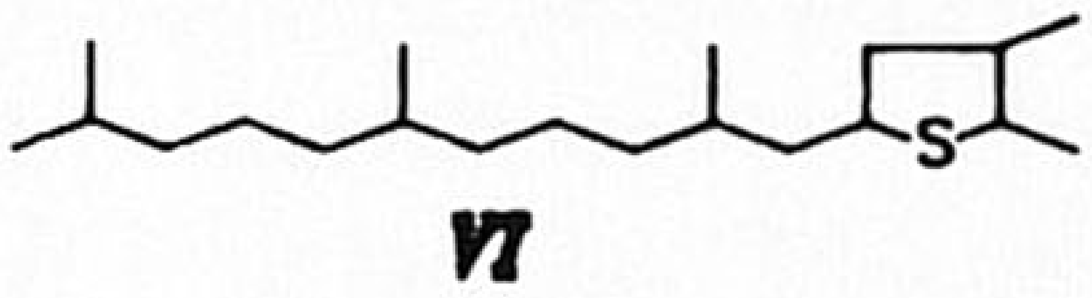

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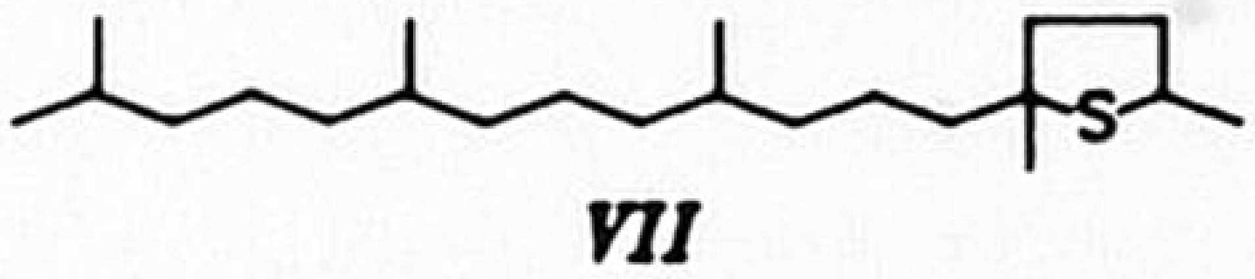

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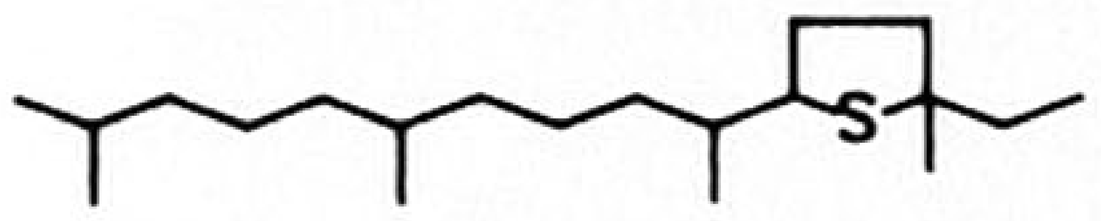

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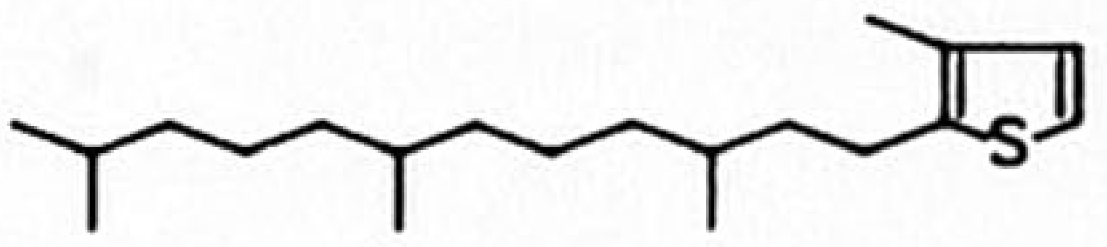

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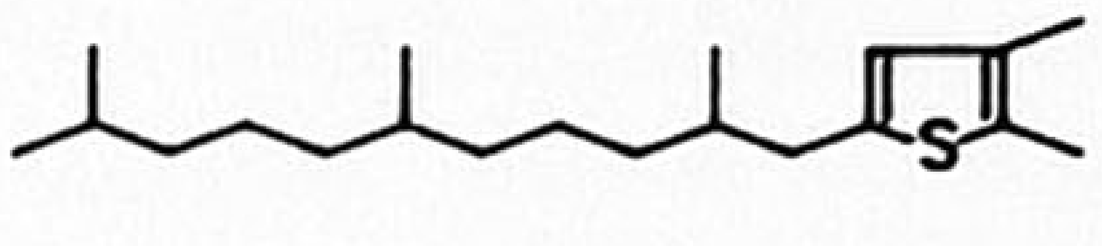

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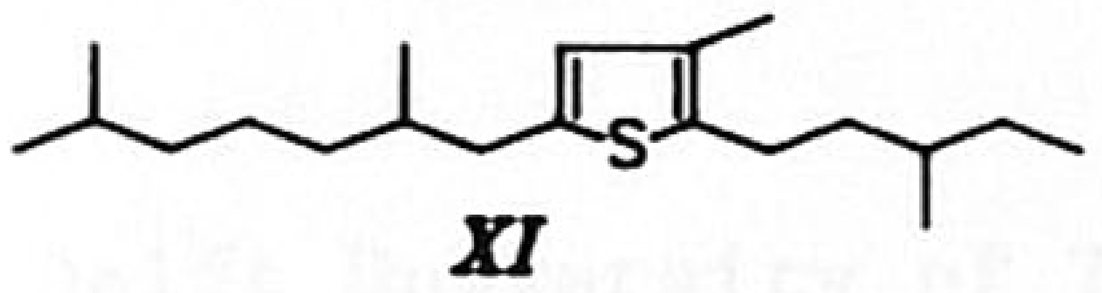

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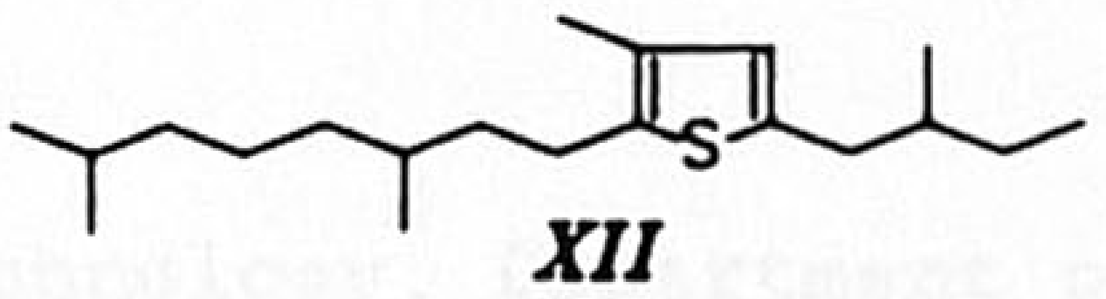

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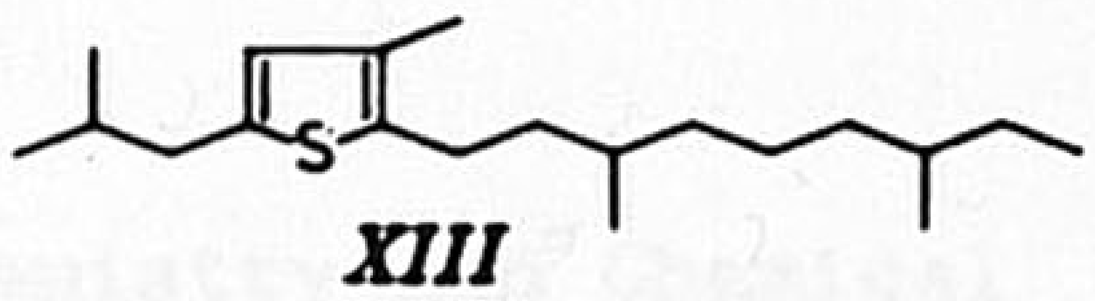

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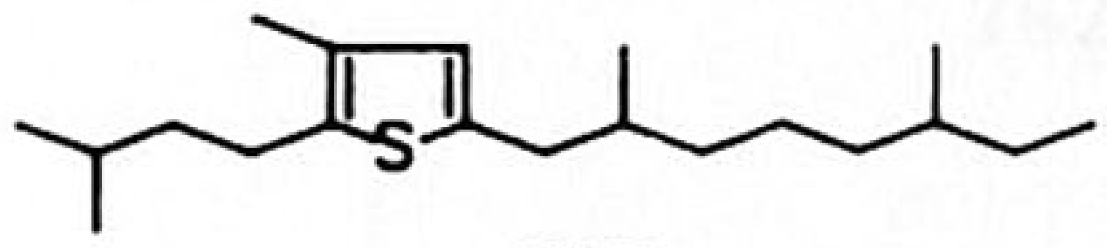

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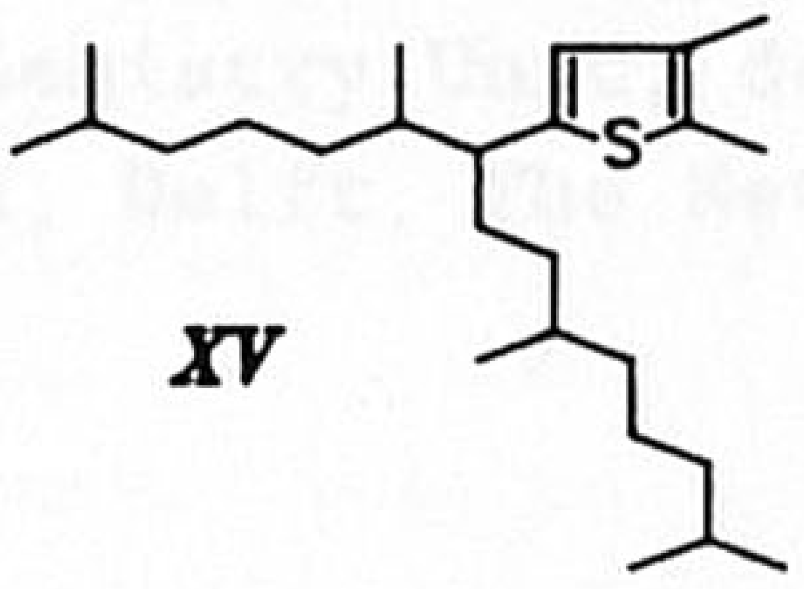

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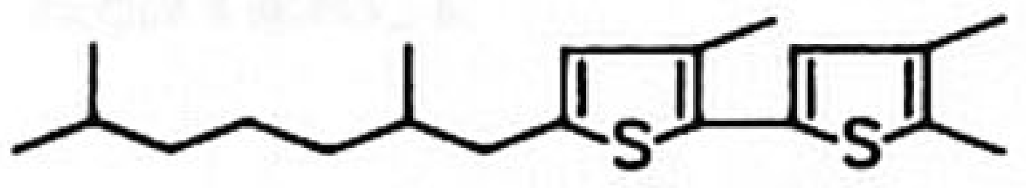

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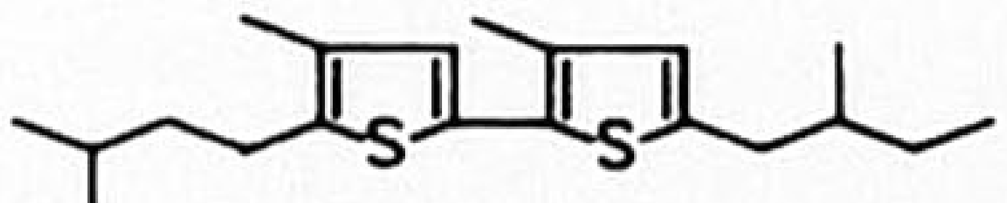

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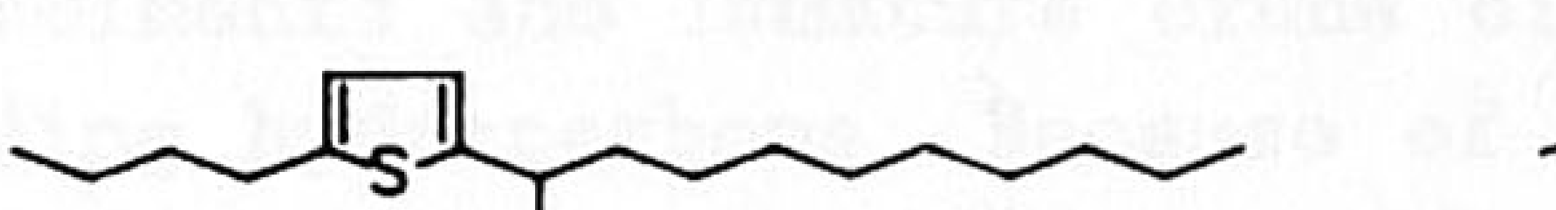

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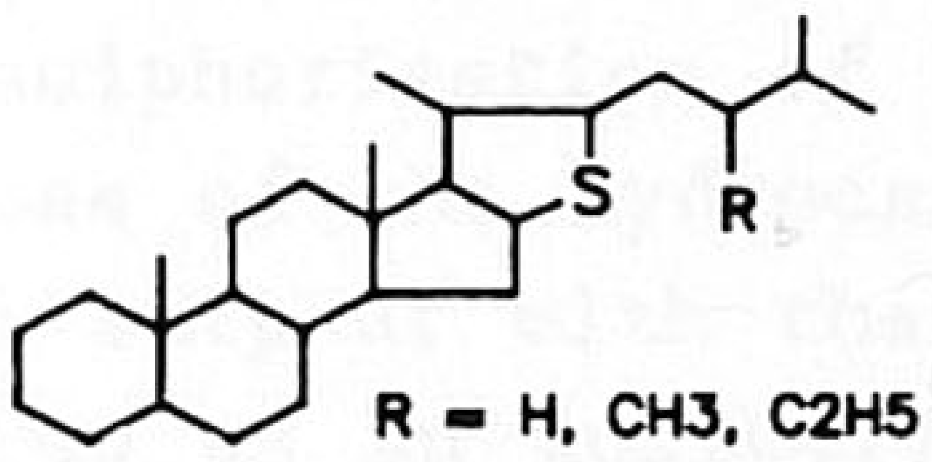

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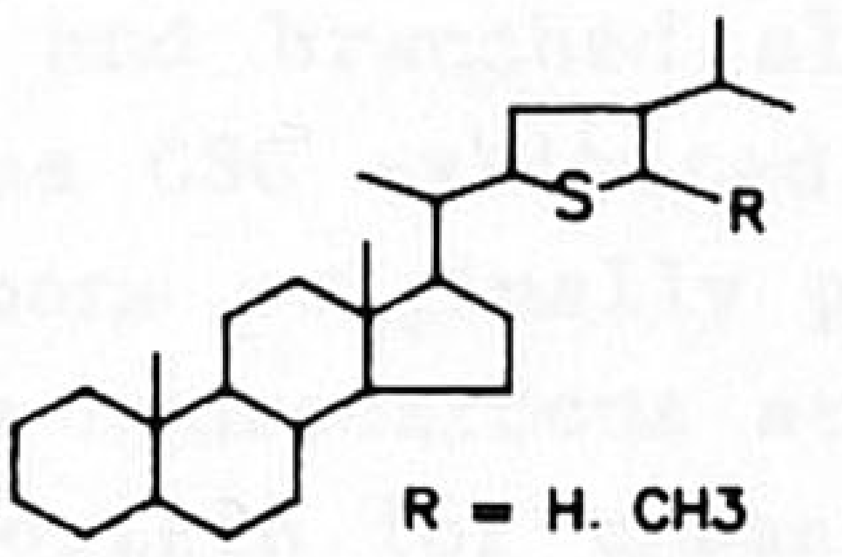

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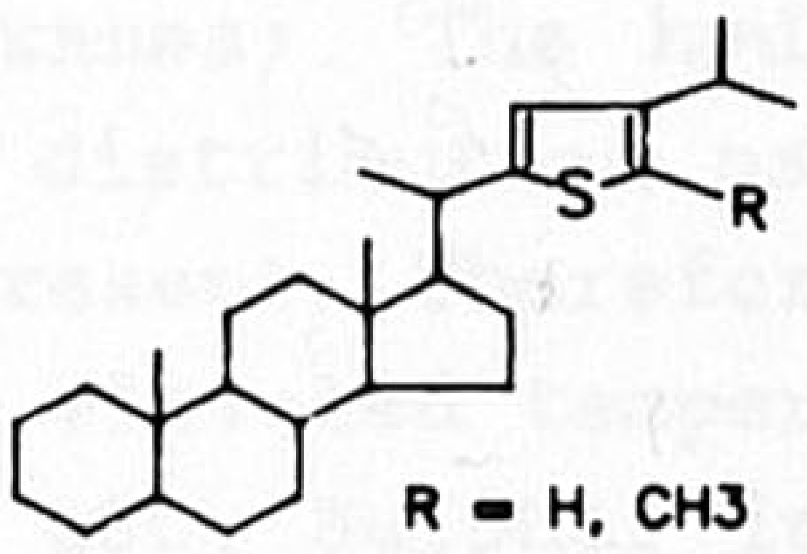

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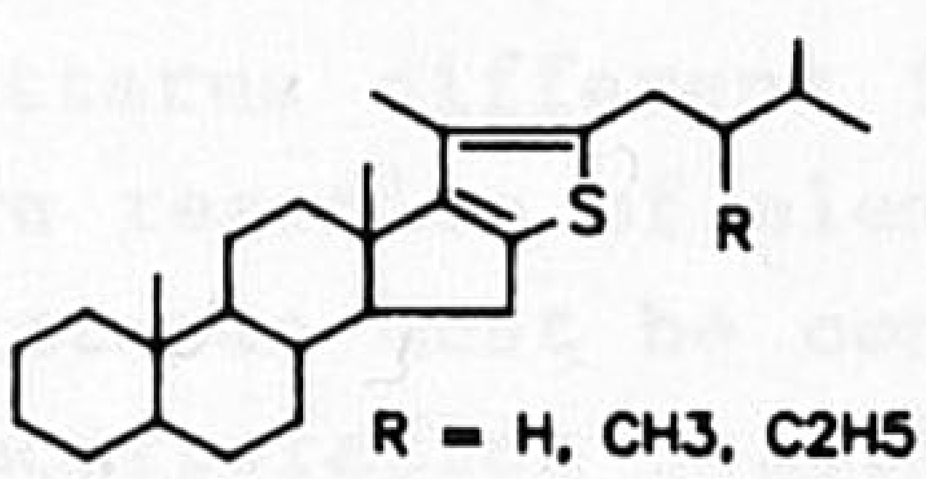

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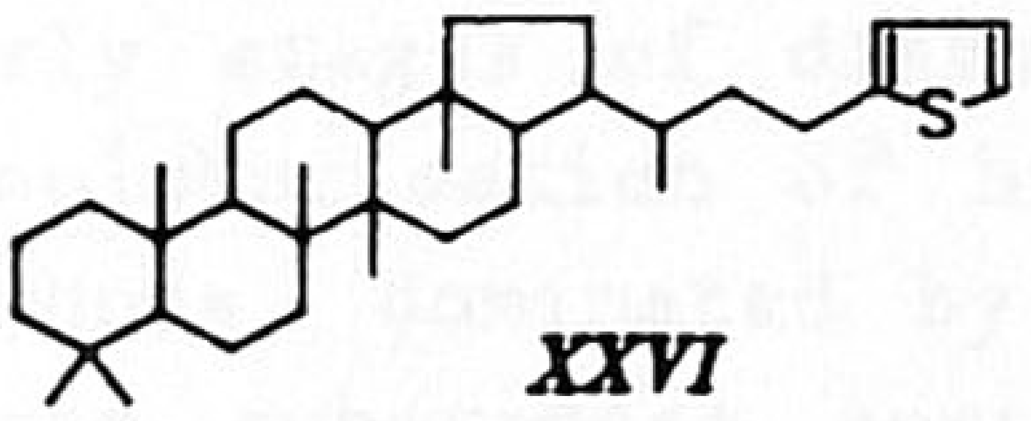

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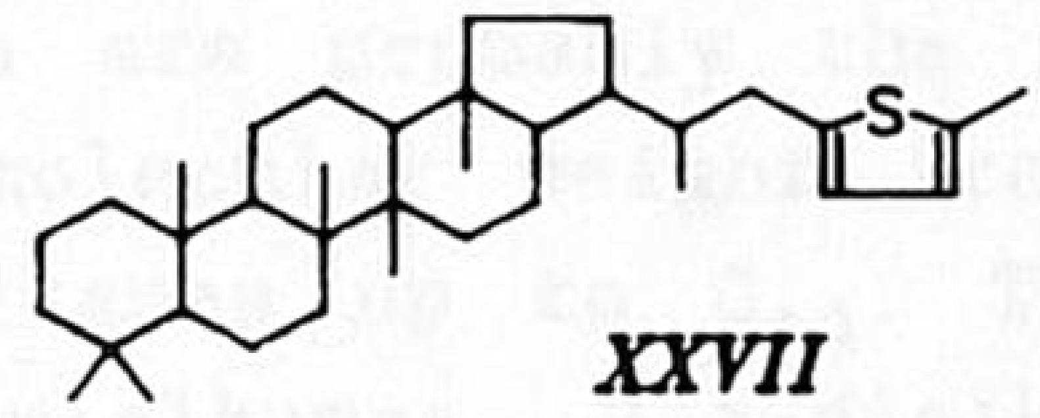

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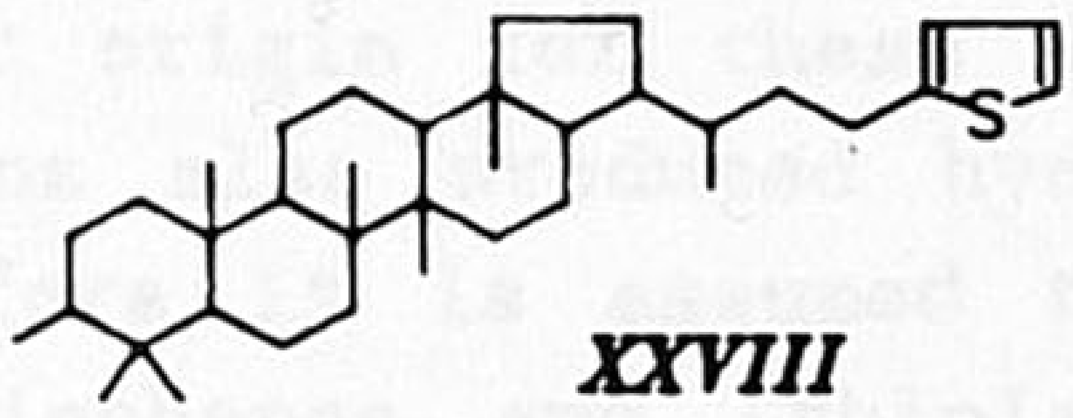

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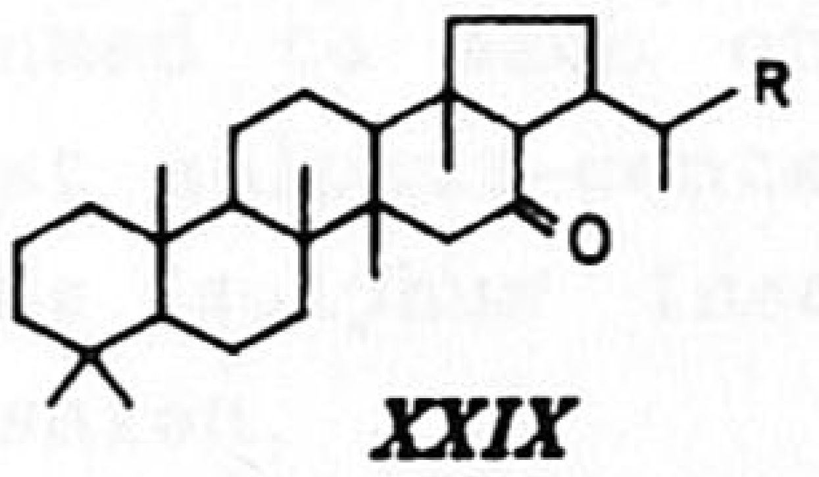

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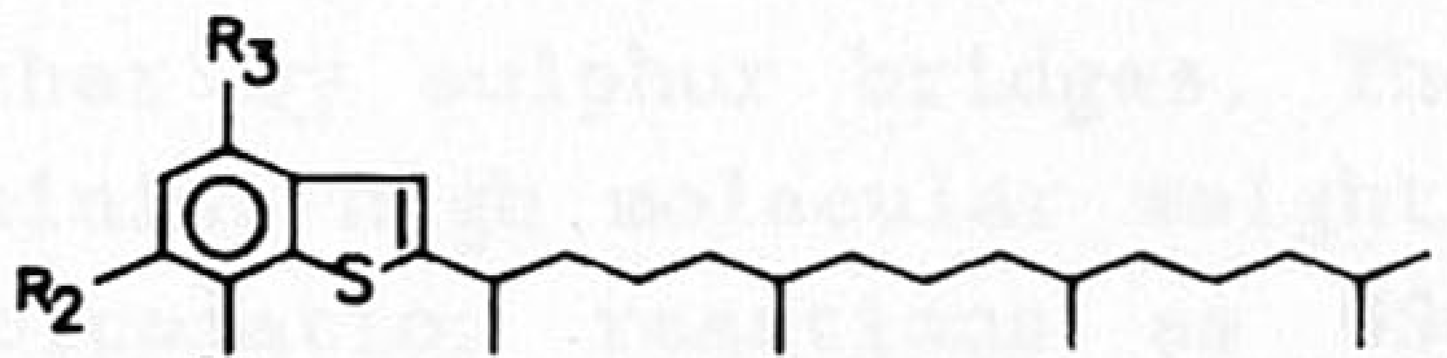

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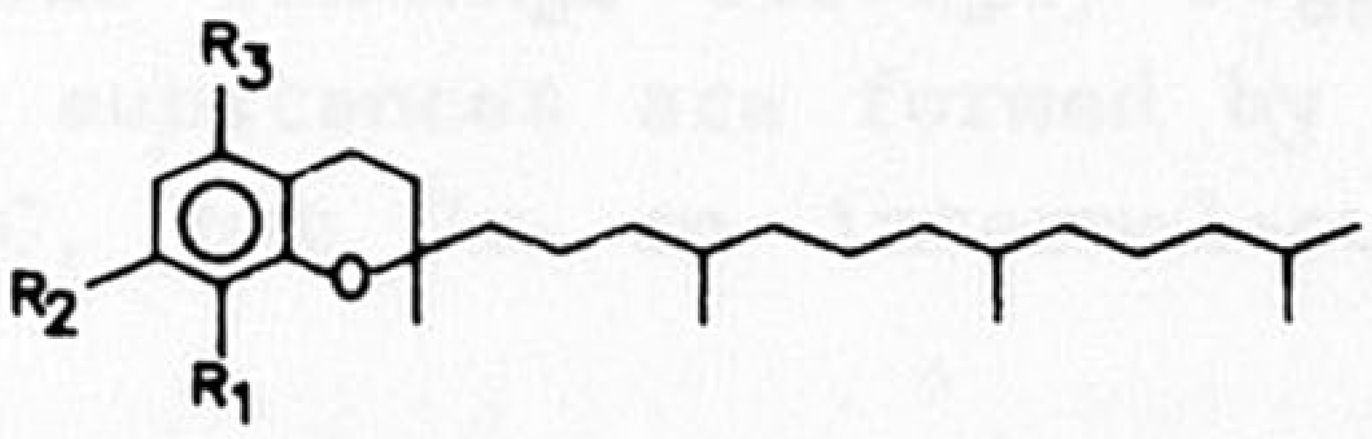
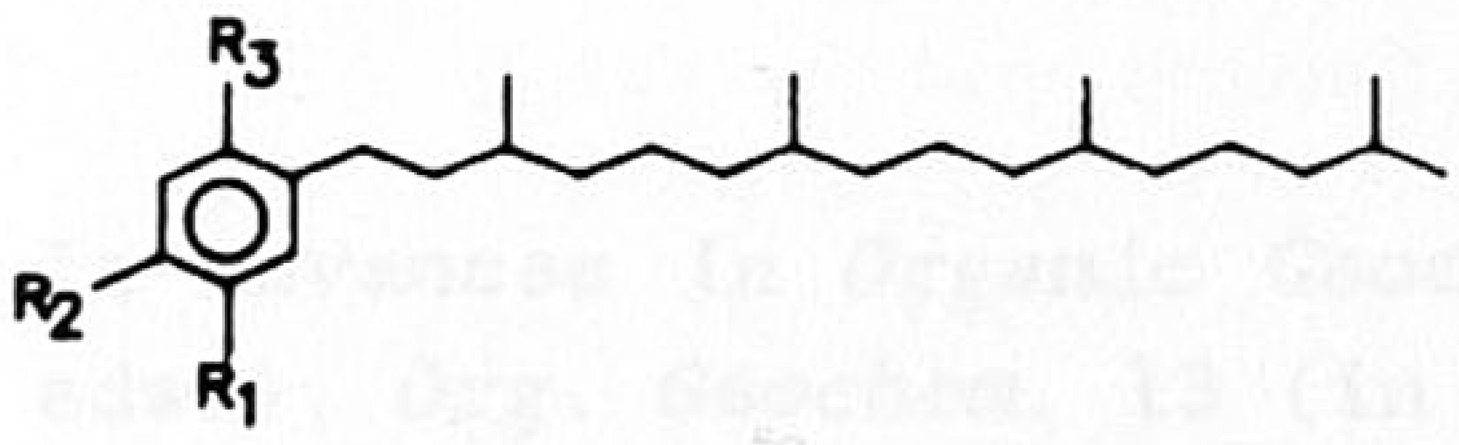
XXXb $R_1=CH_3, R_2=H, R_3=CH_3$
XXXc $R_1=CH_3, R_2=CH_3, R_3=H$
XXXd $R_1=CH_3, R_2=CH_3, R_3=CH_3$



XXXIa–d for R_1–R_3 see XXX



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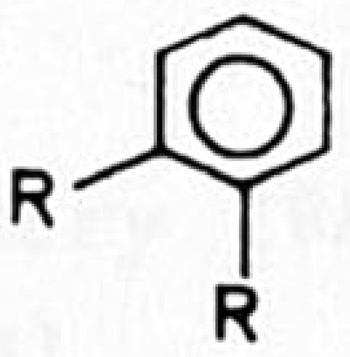

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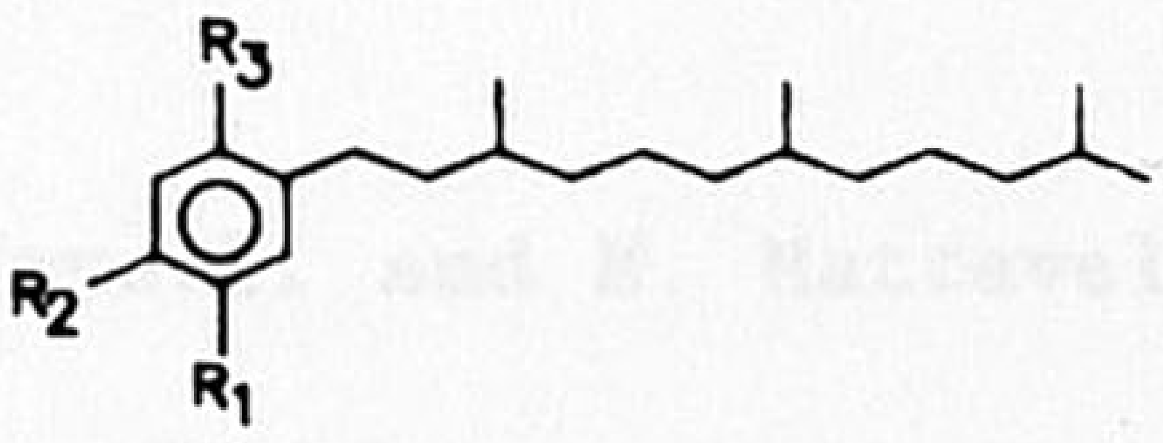

XXXIVa–d for R_1–R_3 see XXX

8. Origin of organic sulphur compounds and sulphur-containing high molecular weight substances in sediments and immature crude oils.*

Jaap S. Sinninghe Damsté, W. Irene C. Rijpstra, Jan W. de Leeuw and P.A. Schenck.

Delft University of Technology, Department of Chemistry and Chemical
Engineering, Organic Geochemistry Unit, de Vries van Heystplantsoen 2,
2628 RZ, Delft, The Netherlands.

8.1 ABSTRACT

The distribution patterns of Organic Sulphur Compounds (OSC), occurring in certain sediments and immature crude oils, were compared with those of the corresponding hydrocarbons. Because of the complexity of the OSC mixtures, they were desulphurised to hydrocarbons (*n*-alkanes, isoprenoid alkanes, steranes, triterpanes and branched alkanes). The hydrocarbons produced by desulphurisation of the OSC exhibited distribution patterns different from those of the hydrocarbons originally present. Therefore reaction of elemental sulphur with these hydrocarbons at elevated temperatures must be considered as an unlikely origin for these OSC. Sulphur incorporation reactions on an intramolecular basis with suitable functionalized precursors at the early stages of diagenesis are probably the major origin for these OSC. Desulphurisation of high molecular weight fractions also produced hydrocarbons, dominated by *n*-alkanes up to C_{40}. Therefore it is assumed that these substances contain *n*-alkanes, 2,5-dialkylthiophenes and -thiolanes linked to each other by sulphur bridges. These findings strongly suggest that sulphur-containing high molecular weight substances are formed by the same sulphur incorporation reactions as OSC, but in an intermolecular fashion.

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* In *Advances in Organic Geochemistry 1987* (M. Novelli and M. Mattavelli, eds.), *Org. Geochem.* 13 (in press)

8.2 INTRODUCTION

A recent advance in organic geochemistry is the identification of Organic Sulphur Compounds (OSC) in oils and sediment extracts which differ markedly from the previously known OSC (Aksenov and Kamyanov, 1981; Thompson, 1981; Payzant *et al.*, 1983, 1985, 1986; Valisolalao *et al.*, 1984; Brassell *et al.*, 1986c; Cyr *et al.*, 1986; Schmid, 1986; Perakis, 1986; Schmid *et al.*, 1987; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987a and c, 1988g). These OSC are characterised by their structural resemblance with well known, geologically occurring hydrocarbons and their biochemical precursors (*e.g.* bacteriohopanetetrol, sterols; Fig. 8.1). Their identification has led to a number of hypotheses regarding their origin and the interaction of the sulphur- and carbon-cycle in the geosphere in general.

At the moment there are three major lines of thoughts concerning the origin of OSC, none of which has been proved at present:

1) *Biosynthesis*. Cyr *et al.* (1986) suggested a biosynthetic origin for the bicyclic, tetracyclic and hopane sulphides present in petroleum (Payzant *et al.*, 1983, 1985, 1986; Cyr *et al.*, 1986). In components belonging to these series the authors noted that the sulphur atom is attached to the second carbon atom of the alkyl side chain of the hydrocarbon analogues (*e.g.* IX ,Fig. 8.1). This common structural feature was thought to reflect the site specificity of the biosynthetic pathway whereby sulphur is incorporated into the hydrocarbon framework. Payzant *et al.* (1986) suggested that the bicyclic and tricyclic terpenoid sulphides were accessory pigments in photosynthesis,

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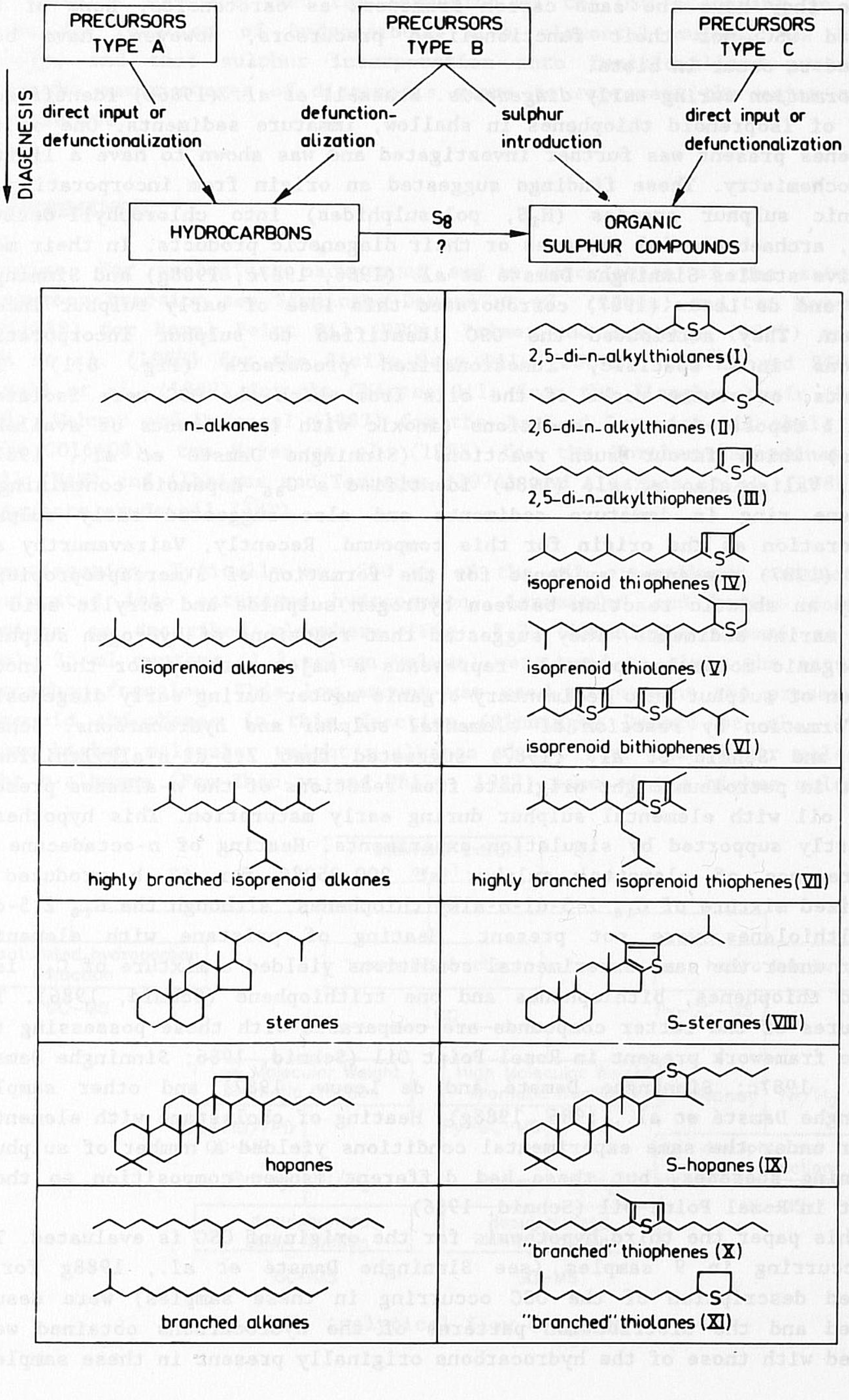
Fig. 8.1. Hypothetical diagenetic scheme showing the possible origins of OSC in sediments and immature crude oils. Precursors of type A yield only hydrocarbons upon diagenesis. Examples are terrestrial *n*-alkanes. Precursors of type B can yield both hydrocarbons and OSC. An example is phytol (or phytenes) which might give rise to both phytane and C_{20} isoprenoid thiophenes, thiolanes and bithiophenes. Precursors of type C are sulphur-containing biosynthetic compounds which survive as such or are diagenetically transformed. An example could be Caldariellaquinone, a major lipoquinone of certain archaebacteria (De Rosa *et al.*, 1977; Collins and Langworthy, 1983), which may yield alkylated benzothiophenes upon diagenesis. A number of typical examples are given, which show a hydrocarbon class and the corresponding OSC class(es). The carbon frameworks of the OSC are identical to their hydrocarbon analogues. The structures drawn are typical examples of compounds belonging to these compound classes. For a detailed description of the identification of these OSC classes the reader is referred to Brassell *et al.* (1986c; IV), Cyr *et al.* (1986; IX), Schmid (1986; I, II, IV, VI, VIII, IX), Schmid *et al.* (1987; I), Sinninghe Damsté and de Leeuw (1987; IV-VI), Sinninghe Damsté *et al.* (1987a; IV); Sinninghe Damsté *et al.* (1987c, I-VIII); Sinninghe Damsté *et al.* (1987d, I-III); Sinninghe Damsté *et al.* (1987e; I-XI) and Valisolalao *et al.* (1984; IX).


PRECURSORS TYPE A
PRECURSORS TYPE B
PRECURSORS TYPE C
DIAGENESIS
direct input or defunctionalization
defunctionalization
sulphur introduction
direct input or defunctionalization
HYDROCARBONS
S8 ?
ORGANIC SULPHUR COMPOUNDS
n-alkanes
2,5-di-n-alkylthiolanes (I)
2,6-di-n-alkylthianes (II)
2,5-di-n-alkylthiophenes (III)
isoprenoid thiophenes (IV)
isoprenoid alkanes
isoprenoid thiolanes (V)
isoprenoid bithiophenes (VI)
highly branched isoprenoid alkanes
highly branched isoprenoid thiophenes (VII)
steranes
S-steranes (VIII)
hopanes
S-hopanes (IX)
"branched" thiophenes (X)
branched alkanes
"branched" thiolanes (XI)
S

because they have the same carbon framework as carotenoids. None of the reported OSC nor their functionalized precursors, however, have been reported to occur in biota.

2) *Formation during early diagenesis*. Brassell *et al.* (1986c) identified a number of isoprenoid thiophenes in shallow, immature sediments. One of the thiophenes present was further investigated and was shown to have a limited strereochemistry. These findings suggested an origin from incorporation of inorganic sulphur species (H_2S, polysulphides) into chlorophyll-derived phytol, archaebacterial phytenes or their diagenetic products. In their more extensive studies Sinninghe Damsté *et al.* (1986, 1987c, 1988g) and Sinninghe Damsté and de Leeuw (1987) corroborated this idea of early sulphur incorporation. They attributed the OSC identified to sulphur incorporation reactions into specific, functionalized precursors (Fig. 8.1). The sediments, or source rocks of the oils from which the OSC were isolated, were all deposited under conditions (anoxic with low amounts of available Fe-ions) which favour such reactions (Sinninghe Damsté *et al.*, 1987c, 1988g). Valisolalao *et al.* (1984) identified a C_{35} hopanoid containing a thiophene ring in immature sediments and also suggested early sulphur incorporation as the origin for this compound. Recently, Vairavamurthy and Mopper (1987) presented evidence for the formation of 3-mercaptopropionic acid by an abiotic reaction between hydrogen sulphide and acrylic acid in recent marine sediments. They suggested that reactions of hydrogen sulphide with organic molecules probably represents a major pathway for the incorporation of sulphur into sedimentary organic matter during early diagenesis.

3) *Formation by reaction of elemental sulphur and hydrocarbons*. Schmid (1986) and Schmid *et al.* (1987) suggested that 2,5-di-*n*-alkylthiolanes present in petroleum might originate from reactions of the *n*-alkanes present in the oil with elemental sulphur during early maturation. This hypothesis was partly supported by simulation experiments. Heating of *n*-octadecane in the presence of elemental sulphur at 200-250°C for 62 h produced a randomized mixture of C_{18} 2,5-di-*n*-alkylthiophenes, although the C_{18} 2,5-di-*n*-alkylthiolanes were not present. Heating of pristane with elemental sulphur under the same experimental conditions yielded a mixture of C_{19} isoprenoid thiophenes, bithiophenes and one trithiophene (Schmid, 1986). The structures of the latter compounds are comparable with those possessing the phytane framework present in Rozel Point Oil (Schmid, 1986; Sinninghe Damsté *et al.*, 1987c; Sinninghe Damsté and de Leeuw, 1987) and other samples (Sinninghe Damsté *et al.*, 1986, 1988g). Heating of cholestane with elemental sulphur under the same experimental conditions yielded a number of sulphur-containing steranes, but these had different isomer composition to those present in Rozel Point Oil (Schmid, 1986).

In this paper the third hypothesis for the origin of OSC is evaluated. The OSC occurring in 9 samples (see Sinninghe Damsté *et al.*, 1988g for a detailed description of the OSC occurring in these samples) were desulphurised and the distribution patterns of the hydrocarbons obtained were compared with those of the hydrocarbons originally present in these samples.

These results indicate that the formation of the OSC occurring in these samples by reaction of hydrocarbons with elemental sulphur is highly unlikely, and that sulphur incorporation into functionalized precursors during the early stages of diagenesis seems to represent the major origin for these OSC.

8.3 EXPERIMENTAL

Samples. For geological background and a description of the saturated hydrocarbon fraction see Sinninghe Damsté *et al*. (1986c) and ten Haven *et al*. (1988) for Rozel Point Oil (RPO), Palmer and Zumberge (1981) and ten Haven *et al*. (1988) for the Sicily Seep Oils (SSO-E1, SSO-E2 and SSO-E5), Brassell *et al*. (1988) for the Chinese Oils from the Jianghan basin (B1 and W1349), Wehner and Hufnagel (1987) for the Jurf ed Darawish oil shale (JED; sample CO16408), ten Haven *et al*. (1985) for the Northern Apennines Marl sample (NAM) and Albaiges and Torradas (1974) and Albaiges *et al*. (1986) for the Amposta crude oil (AMP).

Fractionation. Typically *ca*. 200 mg of the oil or sediment extract was fractionated into saturated hydrocarbon, "aromatic" and hetero compound fractions as described elsewhere (Fig. 8.2) (Sinninghe Damsté *et al*., 1987c). 35 ml pentane (1.2 column volume) were used to elute the saturated hydrocarbon fraction. This low amount was used to prevent the presence of isoprenoid thiophenes in this fraction (Sinninghe Damsté *et al*., 1986). Because higher molecular weight *n*-alkanes elute later than lower molecular weight *n*-alkanes (Fan Zhao-an and Philp, 1987) some of the higher molecular

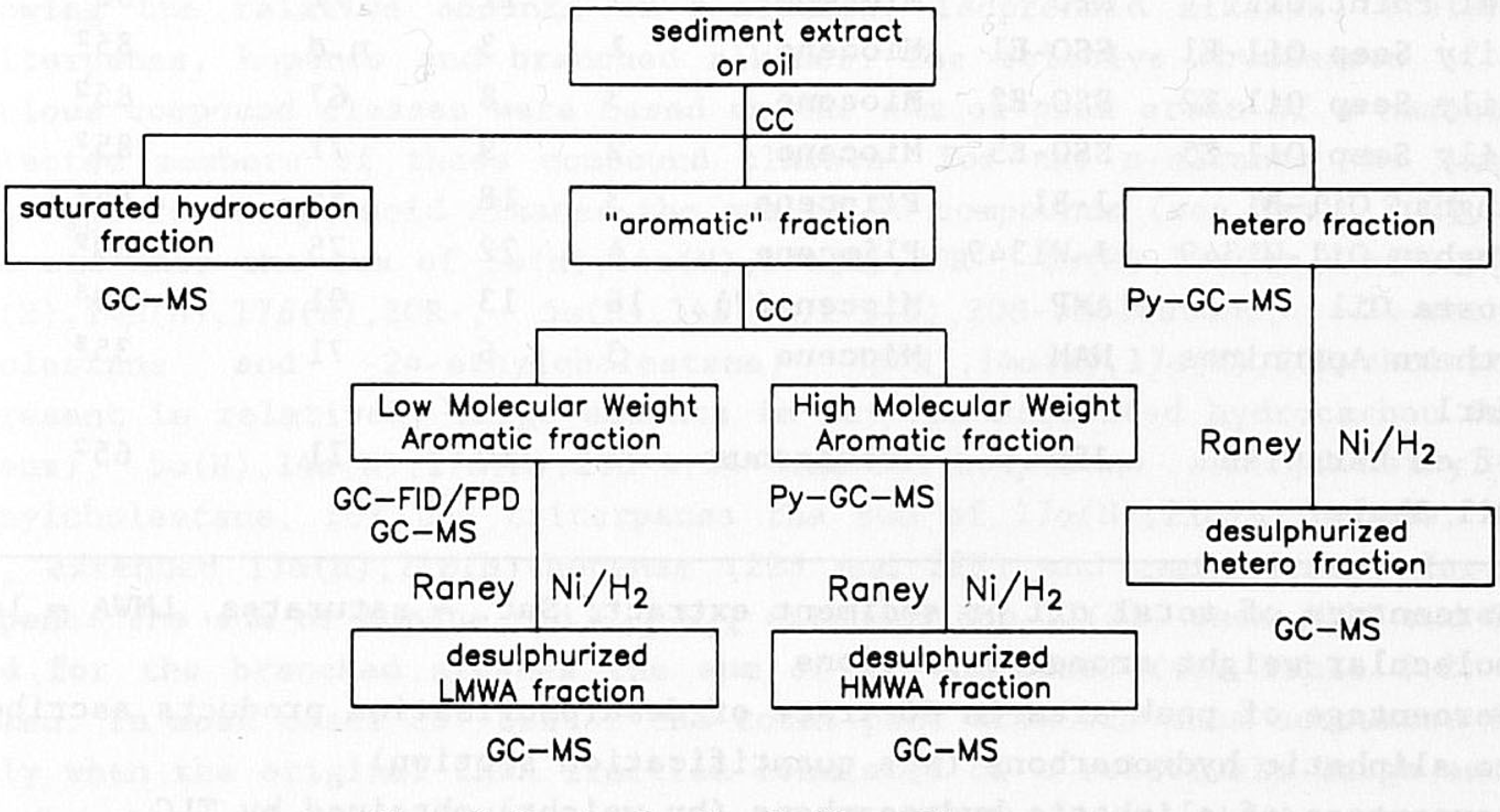


Fig. 8.2. Analytical flow diagram.

weight *n*-alkanes eluted in the "aromatic" fraction. These were always *n*-alkanes with carbon numbers higher than 30 and in most cases higher than 34. This, probably, depends strongly on the activity of the adsorbents used.

"Aromatic" fractions were sub-fractionated into a Low Molecular Weight Aromatic (LMWA) and a High Molecular Weight Aromatic (HMWA) fraction as described elsewhere (Sinninghe Damsté *et al.*, 1987c). The LMWA fractions contained the OSC and, in some cases, a number of other types of compounds (*n*-alkylbenzenes, isoprenoid alkylbenzenes, isoprenoid chromans (see Sinninghe Damsté *et al.*, 1987b; 1988b)), whereas the HMWA fractions contained hardly any GC amenable compounds. The yields of the saturated and LMWA fractions (by weight) are presented in Table 8.1.

Desulphurisation. Aliquots of the LMWA fractions were desulphurised using Raney Ni. Typically 5-10 mg were reacted with a 0.5 ml suspension (0.4 mg/ml) of Raney Ni (W-6; Bilica and Adkinds, 1955) in 2 ml refluxing abs. ethanol for 1.5 h under a nitrogen blanket. The desulphurisation products were isolated by centrifugation and subsequent extraction with CH_2Cl_2 (3 ml, x3). The combined extracts were washed with 30 ml NaCl-saturated H_2O, which was extracted thereafter with 2 ml CH_2Cl_2 (x2). The combined CH_2Cl_2-extracts were evaporated to dryness using a rotary evaporator at 20°C. The desulphurisation products were chromatographed over a silica column (4 x 0.5 cm)

Table 8.1. Sample description and some geochemical data.

Sample	Abbreviation	Age	Sat.[1]	LMWA[1]	Desulphurisation prod. yield	aliph. hydr.
			%	%	%	%
Rozel Point Oil	RPO	Miocene	1	12	71	80[2]
Sicily Seep Oil-E1	SSO-E1	Miocene	1	3	n.d.	85[2]
Sicily Seep Oil-E2	SSO-E2	Miocene	3	8	67	85[2]
Sicily Seep Oil-E5	SSO-E5	Miocene	2	9	71	85[2]
Jianghan Oil-B1	J-B1	Pliocene	3	18	85	85[2]
Jianghan Oil-W1349	J-W1349	Pliocene	4	22	75	80[2]
Amposta Oil	AMP	Miocene(?)	16	13	91	20[3]
Northern Apennines Marl	NAM	Miocene	3	6	71	35[3]
Jurf ed Darawish Oil Shale	JED	Cretaceous	1	10	71	65[2]

[1] percentage of total oil or sediment extract; Sat. = saturates, LMWA = low molecular weight aromatic fracions

[2] percentage of peak area in GC trace of desulphurisation products ascribed to aliphatic hydrocarbons (see quantification section)

[3] percentage of aliphatic hydrocarbons (by weight) obtained by TLC separation of the desulphurisation products

with some $MgSO_4$ on top of it using hexane/EtOAc (3:1) as an eluent. Sometimes the desulphurisation mixture contained small amounts of unsaturated hydrocarbons due to incomplete hydrogenation. In those cases a further hydrogenation using PtO_2 at room temperature for 4 h was performed. The overall yields of desulphurisation products are presented in Table 8.1.

Sometimes, the desulphurised LMWA fraction was separated into a saturated fraction and an "aromatic" fraction by thin layer chromatography on silica plates (0.25 mm) using hexane as developer (see Table 8.1 for yields). This was necessary if the original LMWA fraction contained other compounds (*e.g.* isoprenoid chromans, alkylbenzenes) besides the OSC, which obscured the aliphatic hydrocarbons formed upon desulphurisation.

Gas chromatography. GC was carried out on a Carlo Erba 4160 instrument, equipped with an on-column injector. A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was used with helium as carrier gas. Samples (0.6 μl, 2 mg/ml in hexane) were injected at 75°C. The oven temperature was programmed from 130 to 320°C at 4°C/min as soon as the solvent eluted.

Gas chromatography-mass spectrometry. GC-MS was carried out on a HP 5840 gas chromatograph connected with a VG-70S mass spectrometer operated at 70 eV with a mass range *m/z* 40-800 and a cycle time of 1.8 s. Separation was achieved by the same capillary column as desribed above with helium as carrier gas.

Relative quantification. Gas chromatograms of the original saturated fractions and those obtained by desulphurisation of the LMWA fractions were integrated. For every sample a bar graph was generated from these data showing the relative amounts of *n*-alkanes, isoprenoid alkanes, steranes, triterpanes, hopenes and branched alkanes. The relative abundances of the various compound classes were based on the sum of peak areas of a number of selected members of these compound classes. For the *n*-alkanes the sum of C_{15}-C_{30}, for isoprenoid alkanes the sum of 20 compounds (see Table 8.2), for the steranes the sum of 5α(H),14α(H),17α(H),20R-, 5α(H),14α(H),17α(H),20S-, 5α(H),14β(H),17β(H),20R-, 5α(H),14β(H),17β(H),20S-cholestane, -24-methylcholestane and -24-ethylcholestane, 5β(H),14α(H),17α(H),20R-cholestane (present in relatively large amounts in several saturated hydrocarbon fractions), 5α(H),14α(H),17α(H),20S-4,24-dimethylcholestane and -4-methyl-24-ethylcholestane, for the triterpanes the sum of 17α(H),21β(H)-hopane, C_{31}-C_{35} extended 17α(H),21β(H)-hopanes (22S and 22R) and gammacerane, for the hopenes the sum of hopene and C_{31}-C_{35} extended hop-17(21)-enes (22S and 22R) and for the branched alkanes the sum of 12 compounds (see Table 8.2) was taken. In most cases *ca*. 80% of the total peak area was thus accounted for. Only when the original LMWA fraction consisted of a relatively large amount of 2,4-di-*n*-alkylbenzo[*b*]thiophenes (which yield 1,2-dialkylbenzenes upon desulphurisation) and/or non-OSC was a smaller fraction accounted for (see

Table 8.2. Isoprenoid and branched alkanes identified in the saturated hydrocarbon and the desulphurised LMWA fractions.

I[1]	2,6,10-trimethylpentadecane
II	2,6,10,14-tetramethylpentadecane
III	2,6,10-trimethyl-7-(3-methylbutyl)dodecane
IV	2,6,10,14-tetramethylhexadecane
V	2,6,10,14-tetramethylheptadecane
VI	2,6,10,14-tetramethyloctadecane
VII	*i*-C23
VIII	2,6,10,14-tetramethylnonadecane
IX	2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane
X	2,6,10,14-tetramethylheneicosane
XI	2,6,10,14,19-pentamethyleicosane (I[2] = 2224)
XII	2,6,10,14,18-pentamethyleicosane (I=2237)
XIII	*i*-C25 (I=2270)
XIV	*i*-C26 (I=2311)
XV	2,6,10,14,19-pentamethylheneicosane (I=2321)
XVI	2,6,10,14,18-pentamethylheneicosane (I=2338)
XVII	*i*-C26 (I=2367)
XVIII	2,6,10,15,19,23-hexamethyltetracosane
XIX	*i*-C35
XX	*i*-C40
A	2-methyltricosane
B	3-methyltricosane
C	2-methyltetracosane
D	3-methyltetracosane
E	2-methylpentacosane
F	3-methylpentacosane
G	2-methylhexacosane
H	3-methylhexacosane
I	2-methylheptacosane
J	9-methyloctacosane
K	2-methyloctacosane
L	3-methyloctacosane

[1] Symbols refer to Figs. 8.3 and 8.4.

[2] I = retention index as measured on CP Sil-5 (130°C to 300°C at 4°C/min)

Table 8.1). In cases of coelution of compounds, mass chromatography was used to calculate (on the basis of spectra of standards) the relative contribution of each coeluting compound to the total peak area. Distribution patterns of and within the various classes of compounds were expressed as a percentage of their sum.

8.4 RESULTS AND DISCUSSION

To decipher the origin of Organic Sulphur Compounds (OSC) present in certain sediment extracts and immature oils comparison of the distribution patterns of these OSC and their hydrocarbon analogues (Fig. 8.1) seems a promising tool. If OSC are formed by reaction of elemental sulphur and hydrocarbons, as proposed for 2,5-di-*n*-alkylthiolanes by Schmid (1986) and Schmid *et al.* (1987), the distribution patterns of the reactants (hydrocarbons) would have to be very similar to those of their products (OSC). However, if OSC are formed during early diagenesis by sulphur incorporation into functionalized precursors as proposed by Valisolalao *et al.* (1984), Brassell *et al.* (1986c), Sinninghe Damsté *et al.*, (1986, 1987c, 1988g), Sinninghe Damsté and de Leeuw (1987), such a similarity is not an implicit condition. Firstly, there may be precursors (types A and C; Fig. 8.1) which only yield hydrocarbons or OSC upon diagenesis. Secondly, the reactivity of a certain member of a group of precursors towards sulphur incorporation might differ from the other members which might lead to an enrichment or a depletion of OSC with that particular carbon framework. Furthermore, the distribution patterns of the hydrocarbons and OSC derived from the same precursor(s) may also be dissimilar because of different diagenetic pathways (Fig. 8.1).

In this study 9 samples were considered in this respect. The complex composition of the OSC present in these samples (Sinninghe Damsté *et al.*, 1986, 1987c, 1988g) complicated a comparison of the distribution patterns of the OSC with their respective hydrocarbon analogues. Therefore it was decided to convert the OSC mixtures into hydrocarbons by Raney Ni desulphurisation and to compare the distribution patterns of the hydrocarbons formed with those of the hydrocarbons originally present. As a consequence of this approach different types of OSC with the same carbon framework (*e.g.* I, II and III; Fig. 8.1) and all structural isomers within a group of OSC are converted into one type of hydrocarbon (Fig. 8.1).

Figs. 8.3 and 8.4 show the relative contributions of the *n*-alkanes, isoprenoid alkanes, steranes, triterpanes, hopenes and branched alkanes to the hydrocarbons originally present and those formed by desulphurisation of the LMWA fraction. Also shown are the internal distribution patterns (except for hopenes) for each hydrocarbon class. One sample was omitted because its composition was very similar to another sample (SSO-E5, SSO-E2). Henceforth the differences observed will be discussed for the successive hydrocarbon classes.

n-*Alkanes*

The CPI values of the *n*-alkanes formed by desulphurisation of the LMWA fraction are consistently lower than those of the *n*-alkanes originally present (Fig. 8.5). All *n*-alkanes formed by desulphurisation show a strong even predominance (CPI < 0.6) except those of the Amposta crude oil. In case of the Jurf ed Darawish shale sample the difference between the two *n*-alkane distribution patterns is especially noteworthy; in the original hydrocarbons the *n*-alkanes in the C_{25}-C_{30} area are characterised by an odd carbon-number predominance whereas the *n*-alkanes formed by desulphurisation show a strong even carbon-number predominance (Fig. 8.6; upper part). Not only are the CPI values different but a shift in the distribution maxima is often observed. These results strongly suggest that OSC with a linear carbon framework are not formed by reaction of elemental sulphur with *n*-alkanes and thereby contradict the hypothesis of Schmid (1986) and Schmid *et al.* (1987).

The natural precursors of the *n*-alkanes and OSC with linear carbon skeletons might also become part of higher molecular weight substances by reaction with some inorganic sulphur species during early diagenesis (inter-

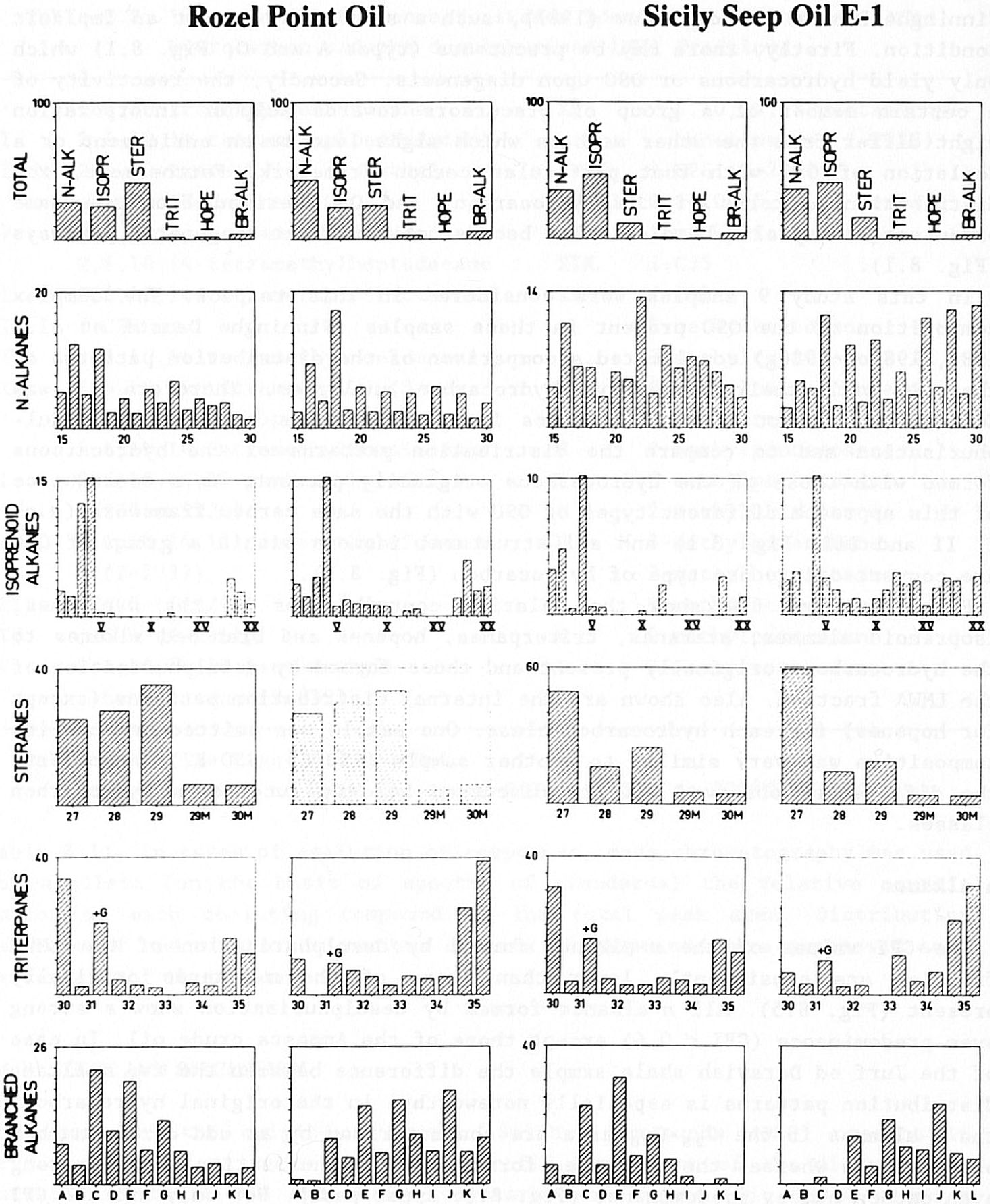
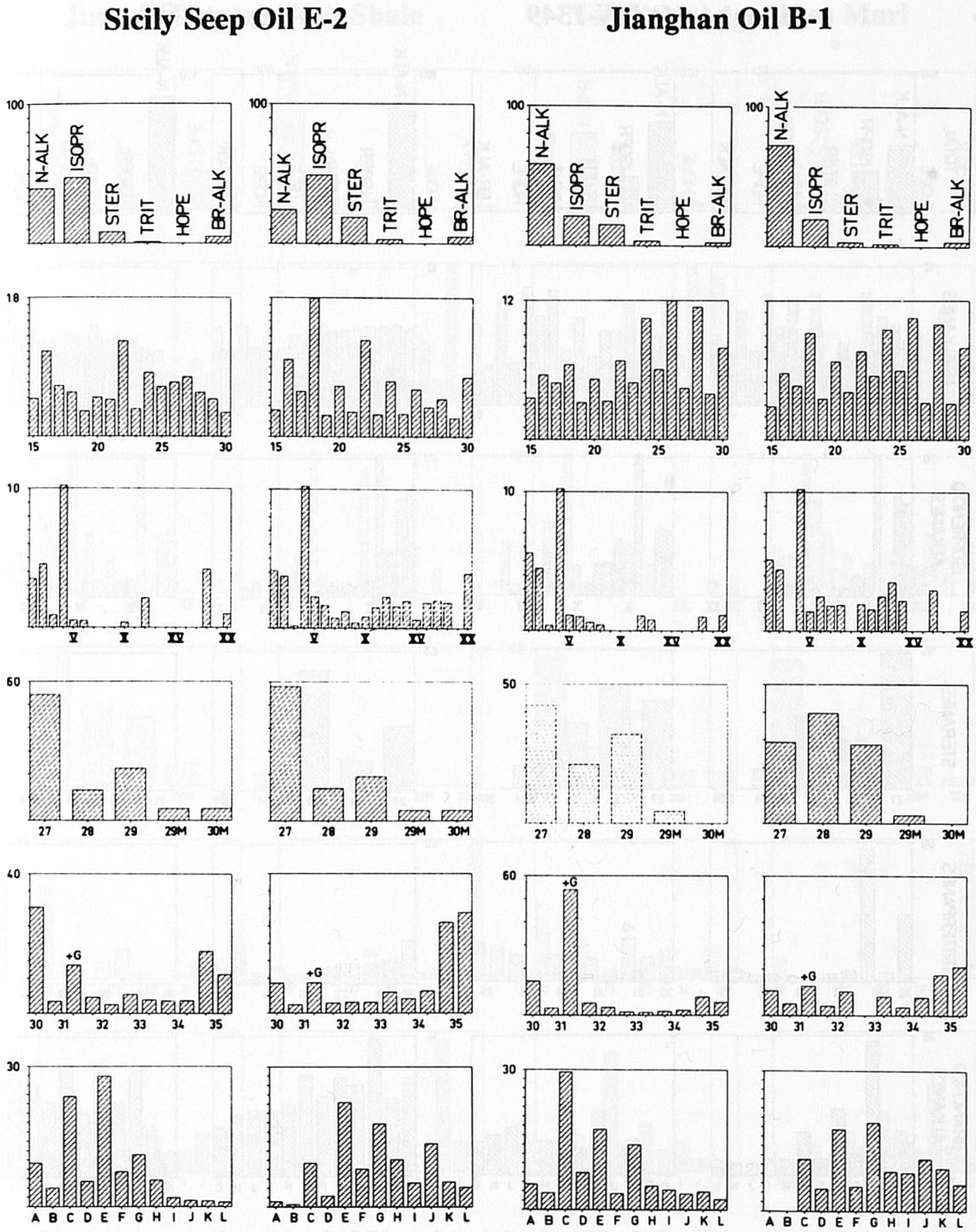


Fig. 8.3. Bar graphs showing the relative amounts of *n*-alkanes, isoprenoid alkanes, steranes, triterpanes and branched alkanes (upper bar graphs) and their internal distribution patterns (except for hopenes) of the original hydrocarbons (left bar graphs) and those formed after desulphurisation of the LMWA fractions (right bar graphs) of the Rozel Point Oil, Sicily Seep



Sicily Seep Oil E-2

Jianghan Oil B-1

Oil-E1 and -E2 and Jianghan-B1 oil. Roman numbers and Arabic letters refer to Table 8.2. Arabic numbers refer to the number of carbon atoms. G denotes gammacerane and M denotes 4-methylsterane. The Arabic numbers in the triterpane bar graphs refer to two compounds (except 30); the first one is the extended 22R-17α(H),21β(H)-hopane and the latter the 22S isomer.

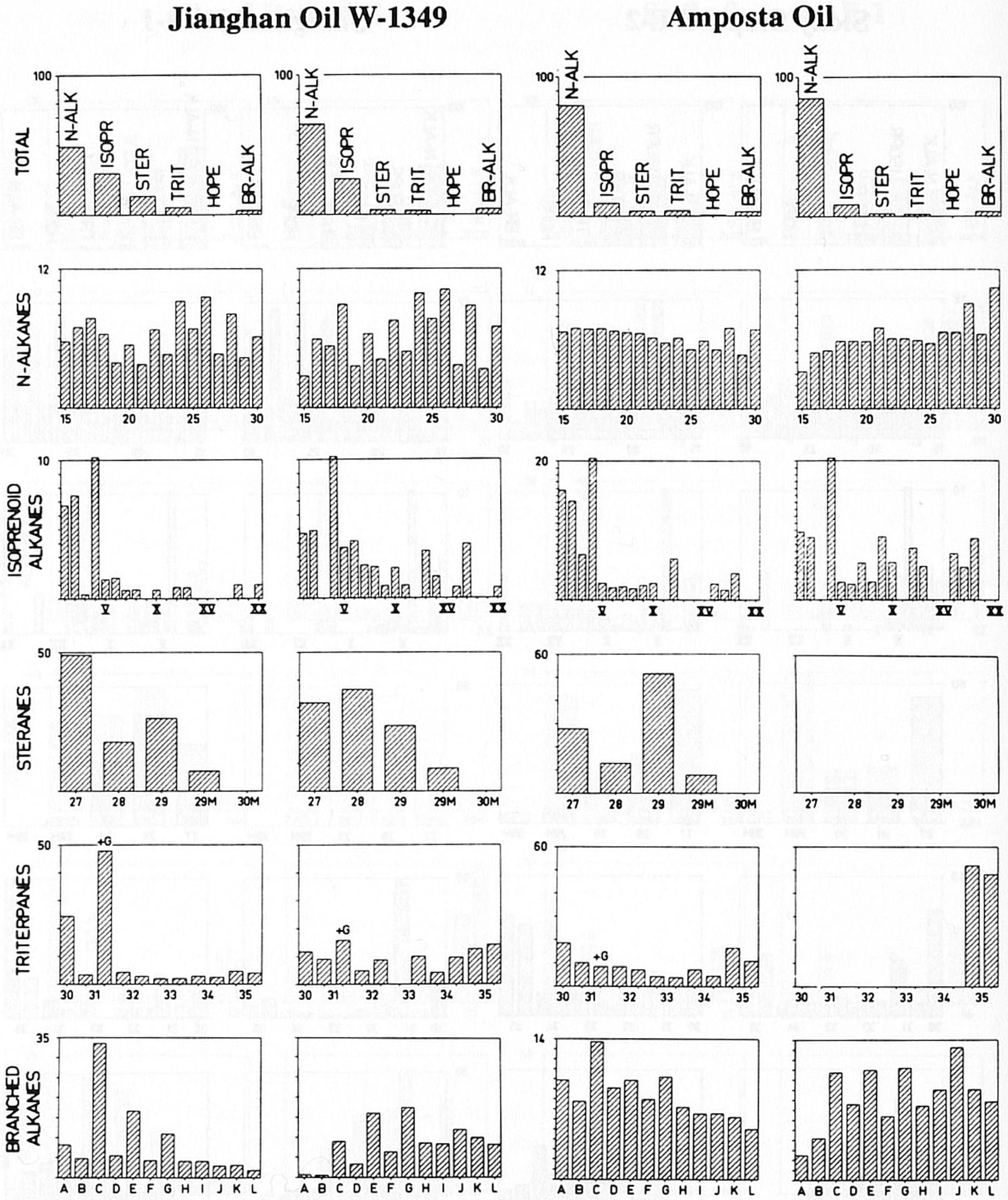


Fig. 8.4. As for Fig. 8.3 for the Jianghan-W1349 oil, the Amposta oil, the Jurf ed Darawish oil shale and the Northern Apennines marl.

molecular crosslinking; *e.g.* Aizenshtat *et al.*, 1983). An alternative origin for these higher molecular weight substances is reaction of elemental sulphur with *n*-alkanes at elevated temperatures. This possibility may be postulated as a reason for the lack in similarity between the distribution

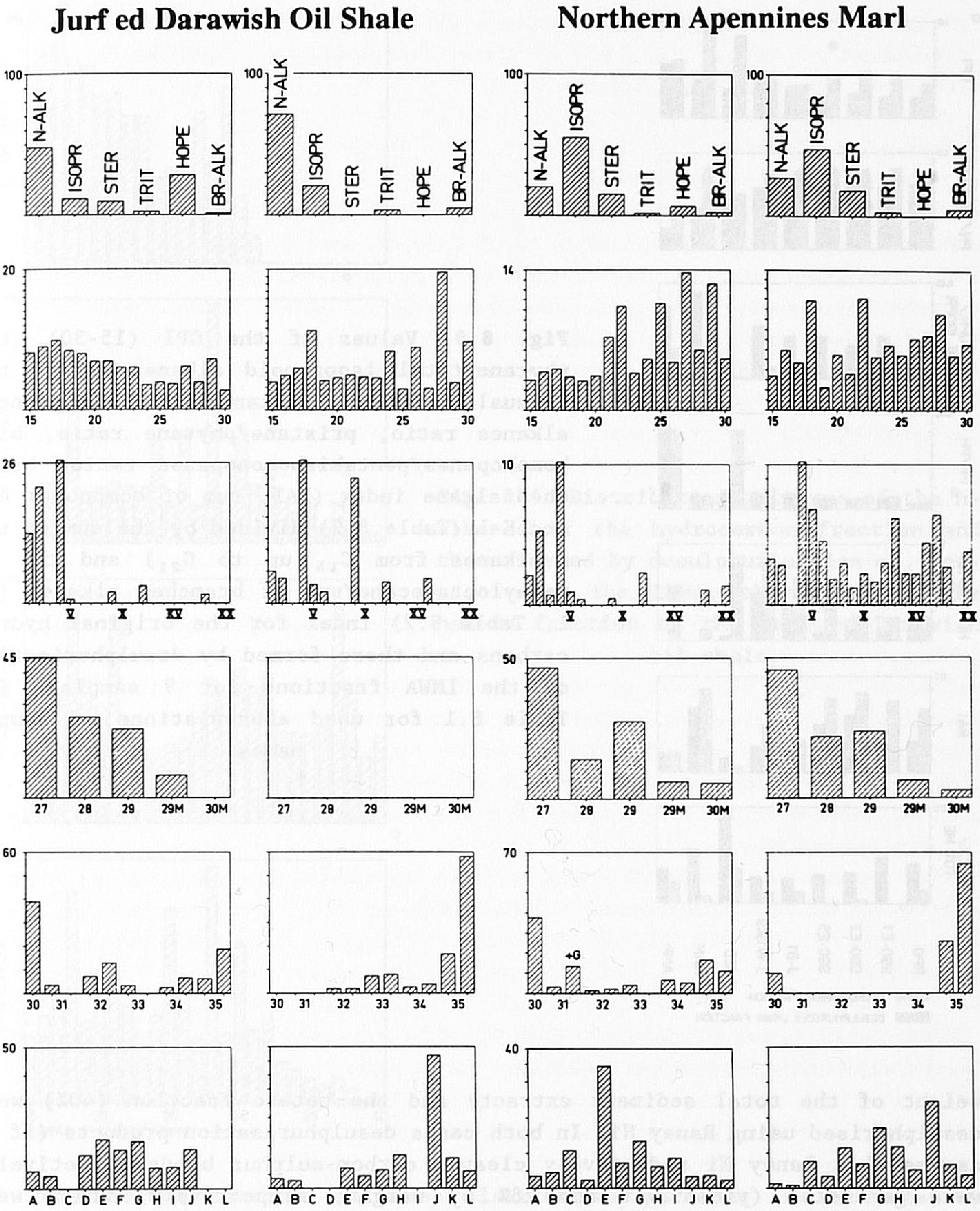


Fig. 8.4. (Continued)

patterns of the *n*-alkanes originally present and those generated by desulphurisation of the LMWA fractions. However, it is difficult to imagine that odd *n*-alkanes react more easily to form higher molecular weight substances than those with an even carbon number. Nevertheless this possibility was checked for the Jurf ed Darawish oil shale sample. The HMWA fraction (9% by

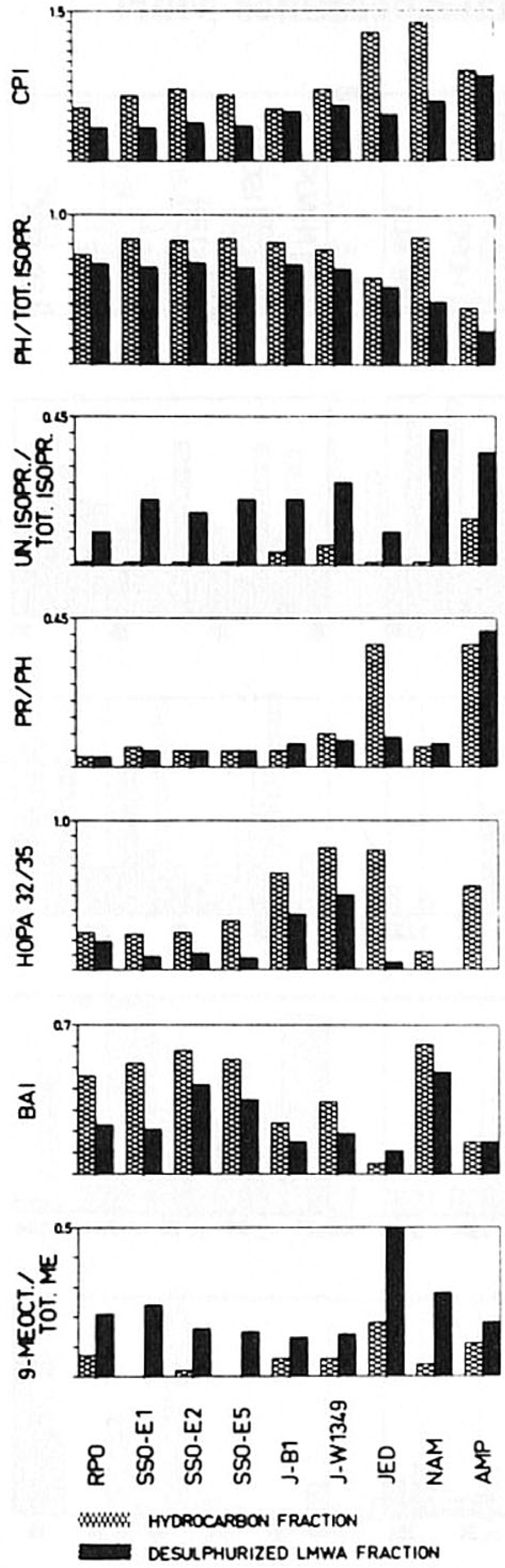


Fig. 8.5. Values of the CPI (15-30), the phytane/total isoprenoid alkanes ratio, the unusual isoprenoid alkanes/total isoprenoid alkanes ratio, pristane/phytane ratio, bishomohopanes/pentakishomohopanes ratio, branched alkane index (BAI; sum of compounds A-I and K-L (Table 8.2) divided by the sum of the *n*-alkanes from C_{16} up to C_{21}) and the 9-methyloctadecane/sum of branched alkanes (E-L, Table 8.2) index for the original hydrocarbons and those formed by desulphurisation of the LMWA fractions for 9 samples. See Table 8.1 for used abbreviations of sample names.

weight of the total sediment extract) and the hetero fraction (40%) were desulphurised using Raney Ni. In both cases desulphurisation products (if we assume that Raney Ni reductively cleaves carbon-sulphur bonds selectively) were generated (yield 43% and 28% by weight, respectively) which were dominated by *n*-alkanes. Fig. 8.6 shows the distribution patterns of the *n*-alkanes originally present and those formed by desulphurisation of the LMWA, HMWA and hetero fractions of the Jurf ed Darawish oil shale. The distribution patterns of the *n*-alkanes formed by desulphurisation of the HMWA and hetero fractions are completely different from those of the original *n*-alkanes. Therefore an origin for those structures present in the HMWA and hetero fractions, which yield *n*-alkanes upon desulphurisation, by reaction with elemental sulphur of *n*-alkanes is highly unlikely.

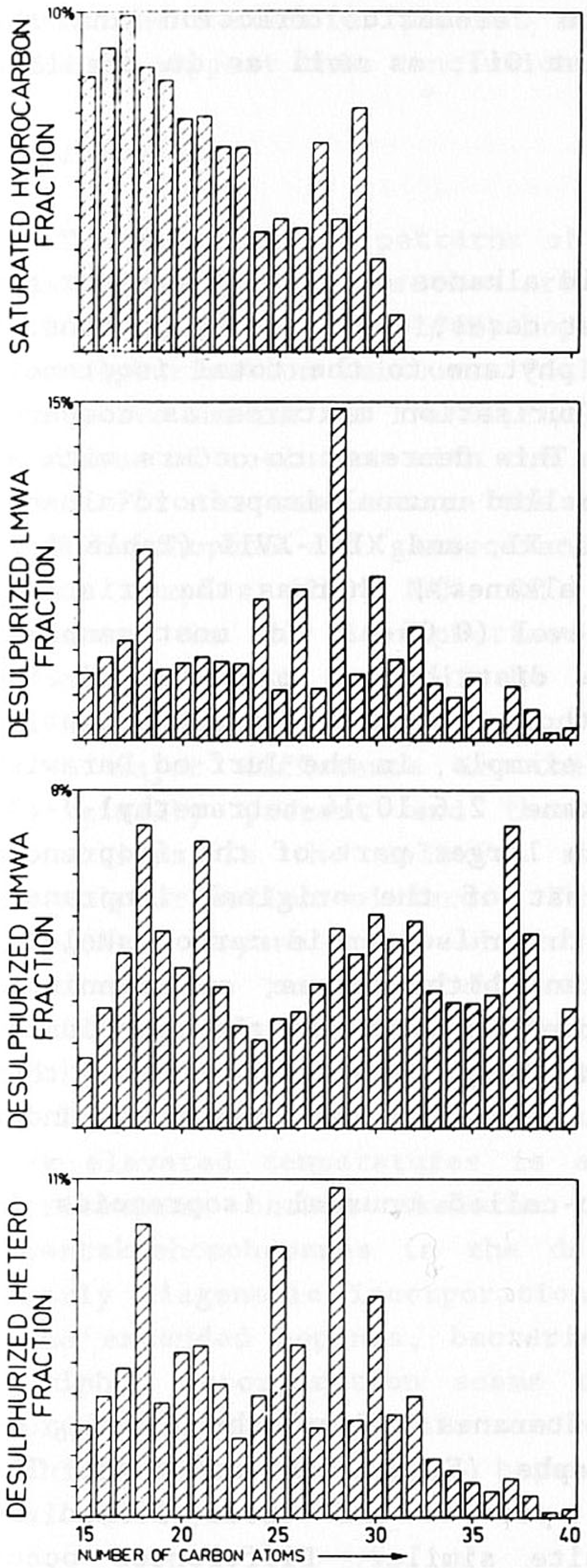


Fig. 8.6. Distribution patterns of the *n*-alkanes of the hydrocarbon fraction and those formed by desulphurisation of, respectively, the LMWA, the HMWA and the hetero fraction of the Jurf ed Darawish oil shale.

A remarkable phenomenon in these distribution patterns is the relative abundance of *n*-heptatricosane and *n*-octatricosane, which may find an origin *via* early diagenetic sulphur incorporation into long-chain alkenones (*i.e.* C_{37}-C_{38} di- and triunsaturated methyl and ethyl ketones) or their corresponding alkadienes and alkatrienes present in sediments (Boon *et al.*, 1978; de Leeuw *et al.*, 1980; Volkman *et al.*, 1980b; Marlowe *et al.*, 1984; Farrimond *et al.*, 1986; Brassell *et al.*, 1986a and b) and in the coccolithophorid *Emilania huxleyi* (Volkman *et al.*, 1980a and b). This relative abundance of *n*-heptatricosane and *n*-octatricosane was also observed in several desulphurised sub-fractions of the "aromatic" fraction and the desulphurised hetero fraction of Rozel Point Oil, as well as in the LMWA fractions of some other samples.

Isoprenoid alkanes

The distribution patterns of the isoprenoid alkanes originally present and those formed by desulphurisation are, in most cases, dominated by phytane. A consistent decrease in the contribution of phytane to the total isoprenoid alkanes is however observed in the desulphurisation mixtures as compared with the saturated hydrocarbons (Fig. 8.5). This decrease co-occurs with an increase in the relative amounts of the so-called unusual isoprenoid alkanes (defined as the sum of compounds V-VIII, X, XI, and XIII-XVII (Table 8.2) divided by the total amount of isoprenoid alkanes), whereas the pristane/phytane ratio remains constant at its low level (0.05-0.1 for most samples; Fig. 8.5). The dissimilarity between the distribution patterns of the isoprenoid alkanes originally present and those formed by desulphurisation (Figs. 8.3 and 8.4) is again evident. As an example, in the Jurf ed Darawish oil shale the highly branched isoprenoid alkane 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane, constituted a much larger part of the isoprenoid alkanes formed by desulphurisation than that of the original isoprenoid alkanes. These results indicate that OSC with an isoprenoid carbon skeleton (such as isoprenoid thiophenes, thiolanes and bithiophenes; see Sinninghe Damsté *et al.*, 1987c) are unlikely to be formed in nature by the reaction of isoprenoid alkanes with elemental sulphur, although it should be noted that Schmid (1986) has shown that this reaction occurs in the laboratory under suitable conditions.

The structural identification of the so-called unusual isoprenoids is presently being studied in this laboratory.

Steranes

The relative abundances of the C_{27}-C_{29} steranes and of the C_{29}-C_{30} 4-methylsteranes are represented by bar graphs (Figs. 8.3 and 8.4). The distributions of the steranes originally present and those formed by desulphurisation are for most samples quite similar. Differences occur however in the relative amounts of stereoisomers; an increase in the relative amounts of the C_{28} and C_{29} $5\alpha(H),14\beta(H),17\beta(H)$ steranes in comparison with the total amounts of C_{28} and C_{29} steranes was observed. This might be due to the abundant presence in the LMWA fractions of the C_{28} and C_{29} condensed thiophene steroids (*e.g.* VIII, Fig. 8.1) (Sinninghe Damsté *et al.*, 1987c), which yield primarily $5\alpha(H),14\beta(H),17\beta(H)$ steranes upon desulphurisation (Schmid, 1986). Schmid (1986) showed that thiophene steroids are formed when cholestane is heated with elemental sulphur. The thiophene steroids thus generated showed, however, a very different structural isomer composition to those present in Rozel Point Oil. Schmid therefore concluded that the S-steroids present in oils are probably not generated in this way and we support this conclusion.

Triterpanes

The distribution patterns of the triterpanes originally present and those formed by desulphurisation are all characterised by the presence of a series of extended 17α(H),21β(H)-hopanes (Figs. 8.3 and 8.4), although in some desulphurisation mixtures only the C_{35} members were encountered. The relative amount of 17α(H),21β(H)-22R-trishomohopane is sometimes not shown because of coelution with the much more abundant 22S-tetrakishomohop-17(21)-ene. For the same reason the sum of the relative amounts of 17α(H),21β(H)-22R-homohopane and gammacerane is shown. In the original hydrocarbons of some samples (JED, NAM, RPO) a series of extended hop-17(21)-enes were present in the desulphurised mixtures which show distribution patterns comparable with those of the 17α(H),21β(H)-hopanes present in the saturated hydrocarbon fractions.

A major difference in the distribution patterns of the triterpanes originally present and those formed by desulphurisation of the LMWA fractions is the relative abundance of the pentakishomohopanes in the desulphurisation mixtures. This is shown in Fig. 8.5 by the C_{32}(R+S)/ C_{35}(R+S) hopane ratio which drops consistently for all desulphurisation mixtures as compared with the saturated hydrocarbon fractions. Another difference is the 22R/22S ratio of the pentakishomohopanes which is higher for desulphurisation mixtures than for the original hydrocarbons.

These results indicate that reaction of elemental sulphur with triterpanes at elevated temperatures is an unlikely origin for the OSC possessing a triterpane carbon skeleton. Moreover, the relative abundance of the pentakishomohopanes in the desulphurisation mixtures seems to reflect an early diagenetic incorporation of sulphur into the presumed precursors of the extended hopanes, bacteriohopanetetrol (Ourisson *et al.*, 1979, 1982); sulphur incorporation seems to fix the number of carbon atoms of this presumed precursor. This conclusion is supported by the identification of a thiophene containing C_{35} hopanoid in immature sediments by Valisolalao *et al.* (1984).

Branched alkanes

The distribution patterns of the branched alkanes originally present and those formed by desulphurisation of the LMWA fractions are presented in Figs. 8.3 and 8.4. An important difference is the drop in the relative abundance of the branched alkanes as compared with the *n*-alkanes in the desulphurisation mixtures. This is represented in Fig. 8.5 by the branched alkane index (BAI), defined as the sum of compounds A-I and K-L (Table 8.2) divided by the sum of the *n*-alkanes from C_{15} up to C_{21}. If OSC with a branched carbon skeleton (see Fig. 8.1) are formed by reaction of branched alkanes with elemental sulphur, such a drop is difficult to explain; why should *n*-alkanes react more easily with elemental sulphur than structurally closely related *iso* and *anteiso* alkanes?

This decrease of the BAI co-occurs with a considerable increase in the relative amount of 9-methyloctadecane in the desulphurisation mixtures. This is the reason why this compound was not taken into account in the BAI. This increase probably reflects the existence of an unknown precursor with the same carbon framework as 9-methyloctadecane, which readily reacts with some sulphur species during early diagenesis, leading to the formation of an OSC with a carbon framework identical to that of 9-methyloctadecane (*e.g.* X, Fig. 8.1).

These results indicate that reaction of branched alkanes with elemental sulphur at elevated temperatures is an unlikely origin for OSC with branched carbon skeletons.

Sulphur-containing high molecular weight substances

The formation of *n*-alkanes upon desulphurisation of HMWA and hetero fractions seems to indicate the presence of sulphur-containing high molecular weight substances. These substances contain building blocks of lower molecular weight material which are released upon desulphurisation and are therefore thought to be bonded *via* one or more sulphur bridges. In themselves these building blocks must have a linear carbon framework because the desulphurisation mixtures are dominated by *n*-alkanes. However, they do not have to be *n*-alkanes but may also consist of 2,5-di-*n*-alkylthiophenes and/or -thiolanes. This was supported by the fact that flash pyrolysis GC-MS of these fractions yielded amongst other compounds series of *n*-alkanes, *n*-alkenes, saturated and monounsaturated 2-*n*-alkylthiophenes and -thiolanes, 2,5-di-*n*-alkylthiophenes and -thiolanes (Sinninghe Damsté, unpublished results). These series were also encountered in pyrolysates of sulphur-rich kerogens (Sinninghe Damsté *et al.*, 1988a and c). Besides *n*-alkanes other compounds were generated as well by desulphurisation of these sulphur-containing high molecular weight substances (*e.g.* phytane, 22R-pentakishomohopane and steranes) which suggests that these structures were also bound *via* sulphur linkages. Schmid (1986) obtained the same results with a high molecular weight fraction of the Rozel Point Oil ("l'anneau rouge"; 32% by weight of the total oil), which consisted for the major part of *n*-alkanes and steranes linked to each other by sulphur bridges. Schmid presented evidence that the steranes were bonded *via* sulphur bridges on position 3 of the sterane skeletons because of the formation of 3-mercaptosteranes upon heating (200°C, 48 h) of this fraction.

These findings indicate the existence in nature of a so-called "intermolecular sulphur cross-linking mechanism" as suggested earlier (Brassell *et al.*, 1986c). Besides reactions of lipids with inorganic sulphur species leading to the formation of low molecular weight OSC (MW < 800) in which the sulphur atom is bonded intramolecularly, these reactions also occur on a

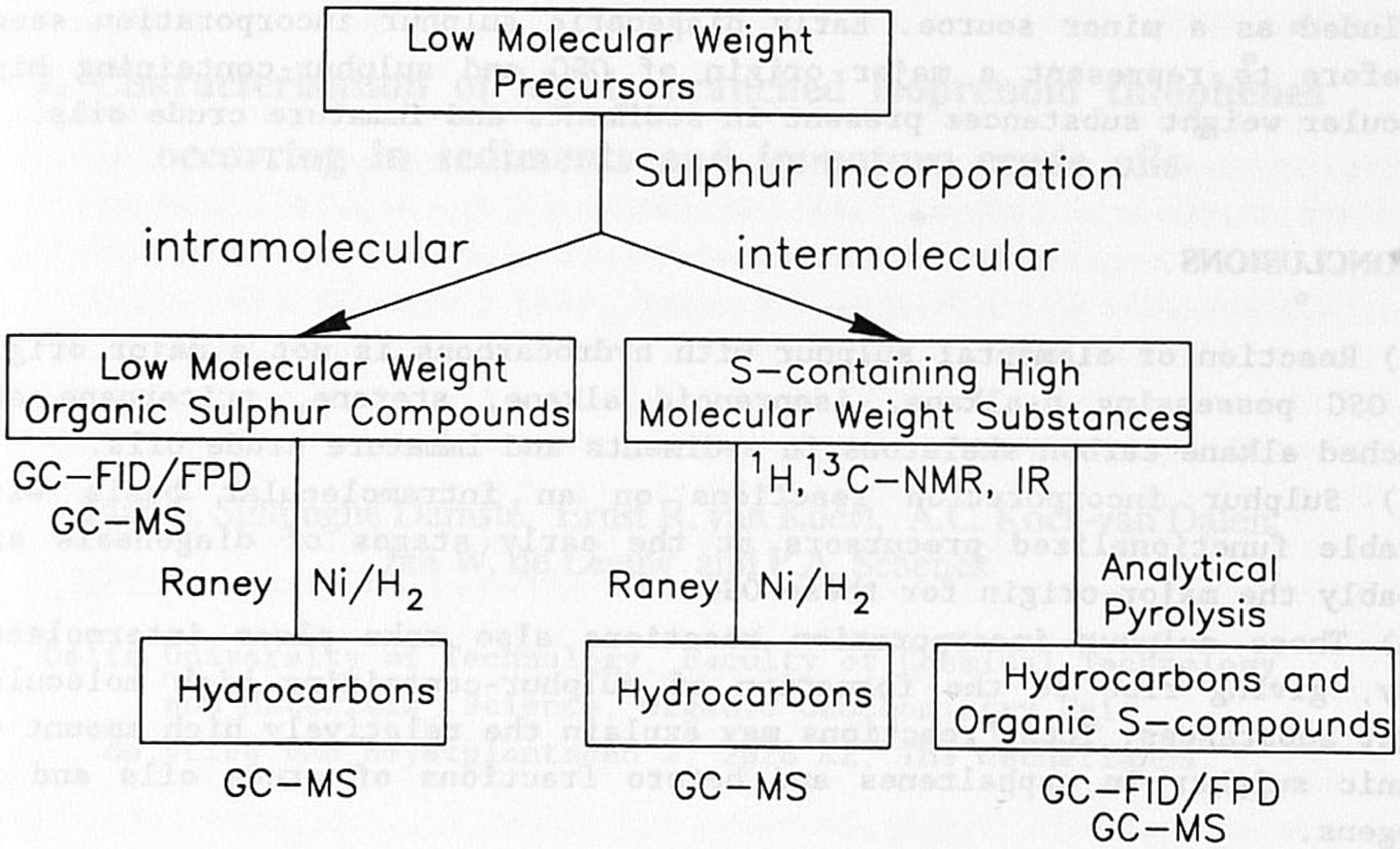


Fig. 8.7. Scheme indicating the two ways in which inorganic sulphur species might react with low molecular weight precursors to yield geologically occurring organically-bound sulphur and the methods by which this is studied.

intermolecular basis, giving rise to sulphur-containing high molecular weight substances (Fig. 8.7). That these reactions may occur during early stages of diagenesis was demonstrated by Ciereszko and Youngblood (1971). A "sediment" produced by the anaerobic decomposition of *Pseudoplexaura porosa* cortex in slowly running seawater was shown to contain a mixture of C_{16} and C_{18} *n*-alkyl disulphides, which were not present in the fresh organisms. Aizenshtat *et al.* (1981) showed that organically bonded sulphur was enriched down the sedimentary column of Solar Lake from 1.4% at 0-5 cm to 8.2% at 80-87 cm depth. This was attributed to secondary sulphur enrichment of organic matter at the early stages of diagenesis. The finding of Schmid (1986) that the steranes in the high molecular weight fraction of the Rozel Point Oil are bonded *via* sulphur bridges on position 3 of the sterane skeleton also points, in our opinion, to sulphur cross-linking of functionalized steranes (*e.g.* sterols) during early diagenesis. The even predominance of the *n*-alkanes formed after desulphurisation (*e.g.* Fig. 8.6) also points to reactions occurring during the early stages of diagenesis.

A consequence of the existence of a probably random intermolecular sulphur cross-linking mechanism, an obviously abiotic process, is that this mechanism on an intramolecular basis is a much more likely origin for the OSC found in sediments and immature crude oils than biosynthesis, although biosynthesis of OSC or sulphur-containing precursors of OSC cannot be precluded as a minor source. Early diagenetic sulphur incorporation seems therefore to represent a major origin of OSC and sulphur-containing high molecular weight substances present in sediments and immature crude oils.

8.5 CONCLUSIONS

(1) Reaction of elemental sulphur with hydrocarbons is not a major origin for OSC possessing *n*-alkane, isoprenoid alkane, sterane, triterpane and branched alkane carbon skeletons in sediments and immature crude oils.

(2) Sulphur incorporation reactions on an intramolecular basis with suitable functionalized precursors at the early stages of diagenesis are probably the major origin for these OSC.

(3) These sulphur incorporation reactions also take place intermolecularly, giving rise to the formation of sulphur-containing high molecular weight substances. These reactions may explain the relatively high amount of organic sulphur in asphaltenes and hetero fractions of crude oils and in kerogens.

9. Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils *

Jaap S. Sinninghe Damsté, Ernst R. van Koert, A.C. Kock-van Dalen,
Jan W. de Leeuw and P.A. Schenck

Delft University of Technology, Faculty of Chemical Technology
and Materials' Science, Organic Geochemistry Unit,
de Vries van Heystplantsoen 2, 2628 RZ, The Netherlands.

9.1 ABSTRACT

A number of C_{20} and C_{25} highly branched isoprenoid thiophenes (HBIT) (*i.e.* thiophenes with 2,6,10-trimethyl-7-(3'-methylbutyl)dodecane and 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane carbon skeletons) have been identified in sediments and oils from different geographical locations. Eight C_{20} and two C_{25} HBIT structural isomers were assigned on the basis of mass spectral characterisation, desulphurisation of isolated or mixtures of C_{20} and C_{25} HBIT and in some cases by comparison of mass spectral and relative retention time data with those of authentic standards. Only a limited number of all theoretically possible isomers were present, indicating that these HBIT are probably formed by selective sulphur incorporation reactions into the widely occurring highly branched isoprenoid alkenes.

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* *Org. Geochem.* (submitted)

9.2 INTRODUCTION

Over the last years considerable interest has arisen in the biological and geological occurrence of a novel group of hydrocarbons, the so-called highly branched isoprenoid hydrocarbons. So far C_{20} (I), C_{25} (II) and C_{30} (III) members of this group have been reported in recent lacustrine, marine and hypersaline sediments (Robson and Rowland, 1986 and references cited therein). I and II have been unambigiously identified by synthesis (Yon *et al.*, 1982; Robson and Rowland, 1986) whereas monounsaturated (C_{20}, C_{25}) and polyunsaturated (C_{25}) alkenes with the same carbon skeletons were identified on the basis of their hydrogenation products (Rowland *et al.*, 1985; Robson and Rowland, 1986 and references cited therein). These compounds are sometimes the major hydrocarbons present in sediments (Robson and Rowland, 1986). The origin of these compounds has not yet been unambiguously determined. Rowland *et al.* (1985) reported the biological occurrence of I (and a related monoene) and a diene with carbon skeleton II in field samples of the green alga *Enteromorpha prolifera*. Recently, however, Nichols *et al.* (1988) demonstrated that the highly branched C_{25} diene is a major hydrocarbon in sea-ice diatom communities and that the reported biological occurrence of this diene in *Enteromorpha prolifera* may have been due to the presence of epiphytic microalgae in the field sample analysed.

In previous studies (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987a and c, 1988f; Sinninghe Damsté and de Leeuw, 1987) we have shown that in euxinic environments of deposition inorganic sulphur species may react with specific functionalised precursors, such as the highly branched isoprenoid alkenes, leading to the formation of organic sulphur compounds (OSC). Consequently, these OSC present in sediment extracts and immature crude oils have similar carbon skeletons as the well known geologically occurring hydrocarbons (*e.g.* *n*-alkanes, branched and isoprenoid hydrocarbons, steranes, hopanes). Among the OSC identified in oils and sediment extracts two thiophenes (IV and V) with the highly branched isoprenoid carbon skeletons I and II, so-called highly branched isoprenoid thiophenes (HBIT), were present (Sinninghe Damsté *et al.*, 1987c). In this paper we extend the identification of this type of OSC.

9.3 EXPERIMENTAL

Samples. The Rozel Point Oil is a highly viscous, sulphur-rich seep oil from Utah, U.S.A.. The OSC of this oil have been described in detail by Schmid (1986). Its composition differs from that of the West Rozel Point Oil (Sinninghe Damsté *et al.*, 1987c), which is an oil from a shallow reservoir in the same area. The Jurf ed Darawish Oil shale is a Cretaceous deposit (thickness *ca.* 100 m) of immature bituminous calcareous marl stones and is located 130 km south of Amman (Jordan).

Details of the other samples investigated have been described elsewhere

(Sinninghe Damsté *et al.*, 1988g or references cited therein).

Gas chromatography. Gas chromatography (GC) was performed on a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was used with helium or hydrogen as carrier gas. Samples were injected at 75°C. The oven was programmed from 75 to 100°C (or 130°C) at 20°C/min and then at 4°C/min to 320°C. Detection of the separated compounds was performed by both a flame ionization detector (FID) and a sulphur-selctive flame photometric detector (FPD), using a stream splitter at the end of the capillary column.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) was performed on a HP 5840 gas chromatograph interfaced to a VG-70S mass spectrometer operated at 70 eV with a mass range *m/z* 40-800 and a cycle time of 1.8 s. Separation was achieved by the same capillary column as described above with helium as carrier gas.

Isolation of C_{20} highly branched isoprenoid thiophenes (Fig. 9.1). Rozel Point Oil (6.0 g) was fractionated by column chromatography using two columns (60 cm x 3.5 cm) packed with equal volumes of alumina overlying silica (both activated for 2 h at 150°C). Four fractions were eluted from each column using 320 ml (1.2 bed volume) pentane (hydrocarbon fraction),

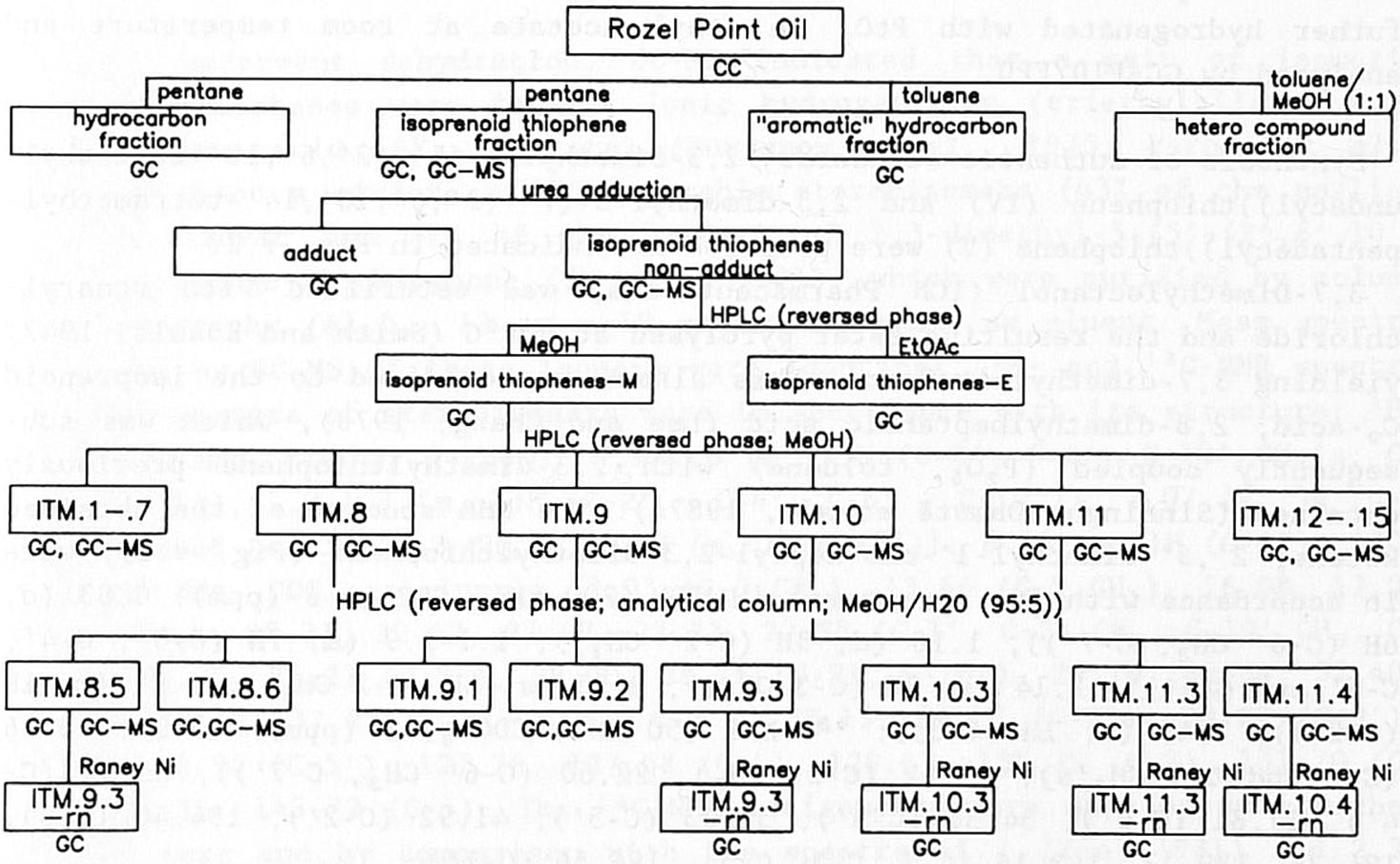

Fig. 9.1. Analytical flow diagram.

450 ml (1.7 bed volume) pentane (isoprenoid thiophene fraction), 800 ml toluene ("aromatic hydrocarbon" fraction) and 900 ml methanol/toluene (1:1) ("hetero compound" fraction). The corresponding fractions were combined and weighed. Saturated straight-chain hydrocarbons were removed from the isoprenoid thiophene fraction (52.0 mg; 0.9 % of the total oil) by urea adduction. Subsequently, the non-adduct (32.1 mg) was further separated into 2 fractions by high performance liquid chromatography (HPLC) (Water Associates equipped with a refractory detector) on a semi-preparative octadecylsilane bonded-phase column (25 cm x 16 mm; polygoSil60-C_{18}; 10 µm) using methanol and ethyl acetate, respectively, as eluents (2.0 ml/min). The sample loading was approximately 25 mg in 60 µl ethyl acetate. The methanol fraction was subsequently separated into 16 fractions using the same conditions. Four of these sub-fractions were further separated by HPLC (Water Associates equipped with an UV (254 nm) detector) on an analytical octadecylsilane bonded phase column (10 cm x 8 mm; polygoSil60-C_{18}; 5 µm) using methanol/water (95:5) as an eluent (0.6 ml/min, 1400 psi).

Raney Ni desulphurisation. Isolated HBIT were desulphurised using Raney Ni. Typically approximately 100 µg of an isolated fraction was reacted with 0.50 µl suspension (0.4 mg/ml) of Raney Ni (W-6; Bilica and Adkinds, 1955) in 400 µl refluxing abs. ethanol for 3 hours under a nitrogen blanket. The desulphurisation products were isolated by centrifugation and subsequent extraction with CH_2Cl_2 (x3). The combined extracts were washed with NaCl-saturated H_2O (3x 3 ml), dried with some anhydrous $MgSO_4$, concentrated, futher hydrogenated with PtO_2 in ethyl acetate at room temperature and analysed by GC-FID/FPD.

Synthesis of authentic standards. 2,3-Dimethyl-5-(5'-(2',6',10'-trimethylundecyl))thiophene (IV) and 2,3-dimethyl-5-(7'-(2',6',10',14'-tetramethylpentadecyl))thiophene (V) were prepared as indicated in Fig. 9.2.

3,7-Dimethyloctanol (ICN Pharmaceuticals) was esterified with stearylchloride and the resulting ester pyrolysed at 400°C (Smith and Roault, 1947) yielding 3,7-dimethyloct-1-ene. This alkene was oxidized to the isoprenoid C_9-acid, 2,8-dimethylheptanoic acid (Lee and Chang, 1978), which was subsequently coupled (P_2O_5, toluene) with 2,3-dimethylthiophene previously obtained (Sinninghe Damsté *et al.*, 1987a). The NMR spectra of the obtained ketone, 2',6'-dimethyl-1'-oxo-heptyl-2,3-dimethylthiophene (Fig. 9.2), were in accordance with its structure: ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 0.83 (*d*, 6H (C-6' CH_3, C-7')), 1.18 (*d*, 3H (C-2' CH_3)), 1.1-1.9 (*m*, 7H (C-3', C-4', C-5' and C-6')), 2.14 (*s*, 3H (C-3 CH_3)), 2.35 (*s*, 3H (C-2 CH_3)), 3.22 (*m*, 1H (C-2')), 7.45 (*s*, 1H (C-3)); ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 13.57, 13.86 (C-2 and C-3 CH_3's), 17.92 (C-2' CH_3), 22.60 (C-6' CH_3, C-7')), 25.31 (C-4'), 27.81 (C-6'), 34.36 (C-3'), 39.03 (C-5'), 41.92 (C-2'), 134.40 (C-4), 134.78, 139.11, 143.16 (C-2, C-3, C-5), 196.56 (C-1').

The ketone was alkylated with 3-methylbutyl magnesium bromide in tetrahydrofuran. During work-up of the reaction mixture the resultant tertiary

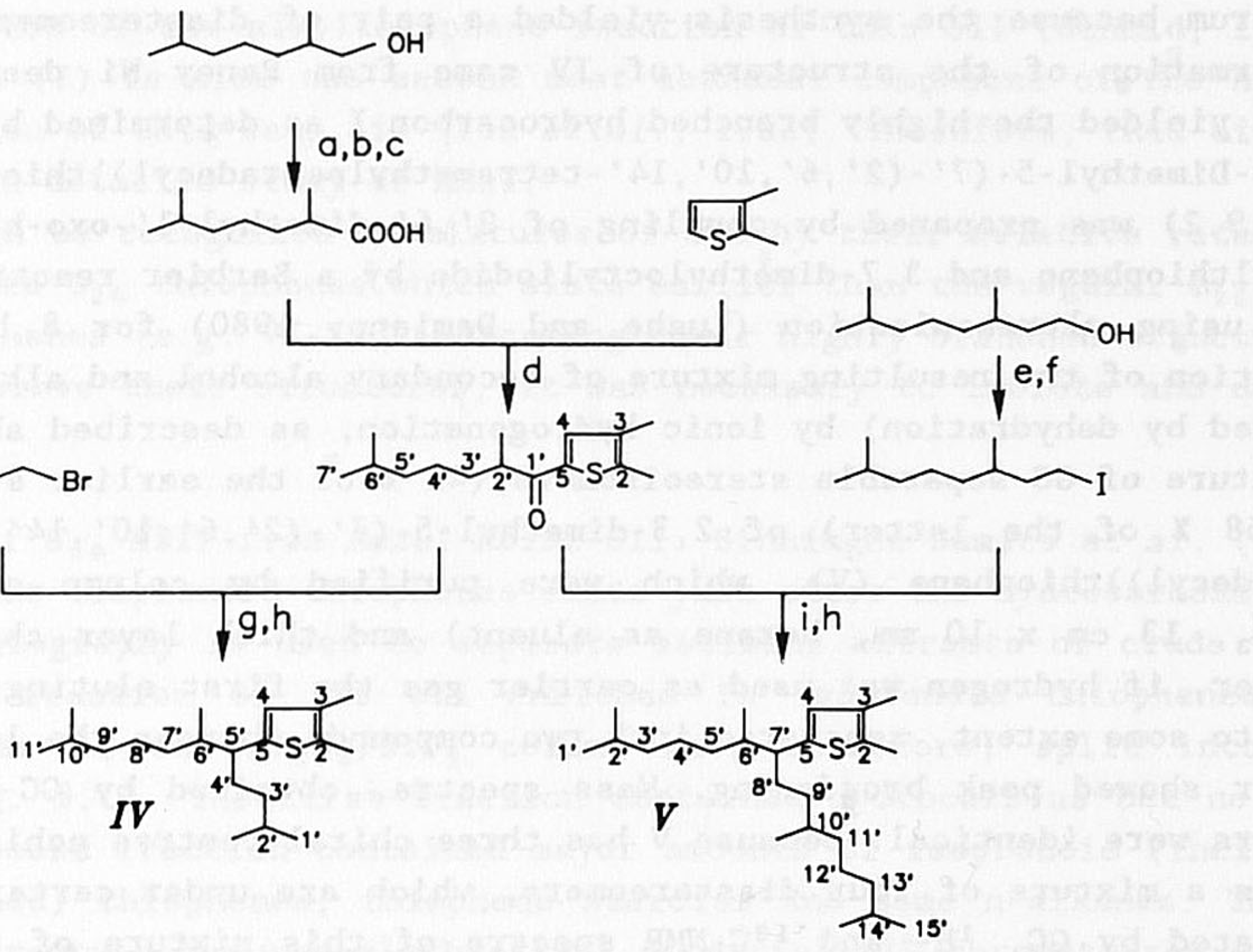

Fig. 9.2. Synthesis scheme for IV and V, a) n-$C_{17}H_{35}COCl$, b) ΔT (400°C), c)$KMnO_4$, HOAc, Adogen 464, d) P_2O_5, ΔT, e) TsCl, pyridine, 0°C, f) NaI, acetone, ΔT, g) Mg, THF, ΔT h)triethylsilane, CF_3COOH, BF_3, 0°C, i) Li, THF, 25°C.

alcohol underwent dehydration; GC-MS indicated that a pair of isomeric alkenyl thiophenes were formed. Ionic hydrogenation (triethylsilane, trifluoroacetic acid, BF_3 etherate) (Kursanov *et al.*, 1975; Parnes *et al.*, 1977) yielded a mixture of GC separable stereoisomers (43% of the earlier eluting isomer and 57% of the latter) of 2,3-dimethyl-5-(5'-(2',6',10'-trimethylundecyl))thiophene (IV; Fig. 9.2)), which were purified by column chromatography (Al_2O_3, 13 cm x 10 mm) with hexane as eluent. Mass spectra obtained by GC-MS of these isomers were identical. ^1H- and ^{13}C-NMR spectra of this mixture of stereoisomers were in accordance with its structure: ^1H-NMR (200 MHz, $CDCl_3$) δ (ppm): 0.8-1.0 (*m*, 15H (C-2', C-6', C-10' CH_3's, C-1', C-11')), 1.0-1.7 (*m*, 13H (C-2' - C-4', C-6' - C-10')), 2.07 (*s*, 3H (C-3 CH_3)), 2.28 (*s*, 3H (C-2 CH_3)), 2.60 (*m*, 1H (C-5')), 6.38 (*s*, 1H (C-4)); ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 13.03 (C-3 CH_3), 13.66 (C-2 CH_3), 16.08, 17.09 (C-6' CH_3), 22.31, 22.42, 22.62, 22.73, 22.85 (C-1', C-2' CH_3, C-10' CH_3, C-11'), 25.05, 25.21 (C-8'), 28.00, 28.16 (C-2', C-10'), 29.70, 30.64, 32.40, 34.07, 35.12, 37.22 (C-3', C-4', C-7'), 38.13, 39.17 (C-6'), 39.27 (C-9'), 46.20, 46.94 (C-5'), 127.26, 127.58 (C-4), 129.43, 129.32 (C-2), 131.70 (C-3), 142.35, 143.72 (C-5). The ^{13}C-NMR assignments were made by an attached proton test and by comparison with the spectra of I (Yon, 1981) and 2,3-dimethyl-5-(2',6',10'-trimethylundecyl)thiophene (Sinninghe Damsté *et al.*, 1987a). A number of carbon atoms gave rise to two signals in the NMR spectrum because the synthesis yielded a pair of diastereomers. A further confirmation of the structure of IV came from Raney Ni desulphurisation which yielded the highly branched hydrocarbon I as determined by GC-MS.

2,3-Dimethyl-5-(7'-(2',6',10',14'-tetramethylpentadecyl))thiophene (V; Fig. 9.2) was prepared by coupling of 2',6'-dimethyl-1'-oxo-heptyl-2,3-dimethylthiophene and 3,7-dimethyloctyliodide by a Barbier reaction in THF at 30°C using ultrasonication (Luche and Damiano, 1980) for 8 h. Subsequent reduction of the resulting mixture of secondary alcohol and alkenylthiophene (formed by dehydration) by ionic hydrogenation, as described above, yielded a mixture of GC separable stereoisomers (42 % of the earlier eluting isomer and 58 % of the latter) of 2,3-dimethyl-5-(7'-(2',6',10',14'-tetramethylpentadecyl))thiophene (V), which were purified by column chromatography (Al_2O_3, 13 cm x 10 mm, hexane as eluent) and thick layer chromatography. However, if hydrogen was used as carrier gas the first eluting stereoisomer was, to some extent, separated into two compounds whereas the latter eluting isomer showed peak broadening. Mass spectra, obtained by GC-MS, of these isomers were identical. Because V has three chiral centres achiral synthesis yields a mixture of four diastereomers, which are under certain conditions separated by GC. ^{1}H- and ^{13}C-NMR spectra of this mixture of stereoisomers were in accordance with their structures: ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 0.8-1.0 (*m*, 18H (C-2', C-6', C-10', C-14' CH_3's, C-1', C-11')), 1.0-1.7 (*m*, 20H (C-2'-C-6', C-8'-C-14')), 2.06 (*s*, 3H (C-3 CH_3)), 2.28 (*s*, 3H (C-2 CH_3)), 2.60 (*m*, 1H (C-7')), 6.38, 6.39 (*s*, 1H (C-4)); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 12.99 (C-3 CH_3), 13.62 (C-2 CH_3), 16.07, 16.22, 17.11, 17.14 (C-6' CH_3), 19.59, 19.64, 19.85, 19.91 (C-10' CH_3), 22.59, 22.62, 22.64, 22.71, 22.73 (C-2' and C-14' CH_3's, C-1', C-5'), 24.66, 24.68, 24.86, 25.04, 25.06, 25.22, 25.24 (C-4', C-12'), 28.01, 28.02 (C-2', C-14'), 30.20, 30.32, 31.95, 32.09, 35.13, 35.18, 35.22, 35.35 (C-8', C-9'), 32.79, 32.95, 32.99 (C-10'), 34.08, 34.18 (C-5'), 36.78, 36.88, 37.35, 37.43 (C-11'), 38.04, 38.35, 39.07, 39.23 (C-6'), 39.24, 39.33, 39.35, 39.36 (C-3', C-13'), 46.33, 46.34, 47.01, 47.18 (C-7'), 127.31, 127.34, 127.64, 127.68 (C-3), 129.39, 129.49 (C-2), 131.69, 131.71 (C-4), 142.49, 142.51, 143.80, 143.84 (C-5). The ^{13}C-NMR spectrum indicates that a mixture of four diastereomers was obtained indeed. A further confirmation of the structure came from Raney Ni desulphurisation of V which yielded the highly branched hydrocarbon II.

9.4 RESULTS AND DISCUSSION

Identification of C_{20} HBIT

A C_{20} highly branched isoprenoid thiophene (HBIT) (IV) has previously been identified in the West Rozel Point Oil, an immature oil with a high sulphur content (Sinninghe Damsté *et al.*, 1987c). An oil seep from the same area contains relatively high amounts of C_{20} HBIT, since the highly branched isoprenoid C_{20} hydrocarbon (I) was formed in relatively high amounts upon desulphurisation of the alkylthiophene fraction of this oil (Schmid, 1986). This compound (I) is also the second most abundant component of the hydrocarbon fraction of this seep oil (Yon *et al.*, 1982). Therefore, this oil was selected for a detailed study of HBIT.

C_{20} HBIT can be recognized in mixtures of OSC by their relative retention times; they are C_{20} thiophenes which elute earlier than the regular C_{20} isoprenoid thiophenes (*e.g.* VI-X), indicating their highly branched structures. However, to prove their structures, it was necessary to isolate and desulphurise them.

Isolation of C_{20} HBIT from Rozel Point Oil. Sinninghe Damsté *et al.* (1986) have noted that isoprenoid thiophenes elute just after the hydrocarbons when column chromatography is used to separate sediment extracts or crude oils. To obtain a fraction of the oil enriched in isoprenoid thiophenes the pentane eluate of the Al_2O_3/SiO_2 column was, therefore, split into two portions (Fig. 9.1). The first fraction contained hydrocarbons but no OSC, whilst the second fraction contained major amounts of isoprenoid (including highly branched) thiophenes, thiophene steroids and some *n*-alkanes. The *n*-alkanes were removed by urea clathration. A partial FID chromatogram of the non-adduct of the isoprenoid thiophene fraction is presented in Fig. 9.3. Peak numbers refer to compounds listed in Table 9.1. GC-MS of this fraction indicated that a considerable number of C_{20} thiophenes are present which elute earlier than the C_{20} regular isoprenoid mid-chain thiophenes (*i.e.* VII-X) and therefore have to be considered as HBIT. High performance liquid chromatography (HPLC) of the non-adduct on a reversed phase column using methanol and ethyl acetate as eluents separated the isoprenoid thiophenes from the thiophene steroids.

To further characterise these isomers the fractions containing the isoprenoid thiophenes were further separated by HPLC into sixteen fractions (Fig. 9.4), which were analysed by GC-FID/FPD and GC-MS. Four fractions contained HBIT. These fractions were subsequently separated by HPLC on an analytical reversed phase column using methanol/water (95/5) as an eluent. This separation achieved, in several cases, a complete isolation of the sedimentary HBIT (see for example Fig. 9.4). The obtained fractions were used to prove the carbon skeletons of the HBIT and to obtain pure mass spectra of these compounds.

Raney Ni desulphurisation/hydrogenation of C_{20} HBIT. Several isolated fractions containing one or more C_{20} HBIT (see Table 9.1) were submitted to Raney Ni desulphurisation and subsequent hydrogenation yielding in all but one case pure (>90%) 2,6,10-trimethyl-7-(3′-methylbutyl)dodecane (I) as determined by co-chromatography with a synthetic standard. In the latter case the fraction contained in addition a C_{20} isoprenoid thiophene which yielded phytane upon desulphurisation (*e.g.* Fig. 9.3). Thus, the isolated thiophenes were shown to be HBIT.

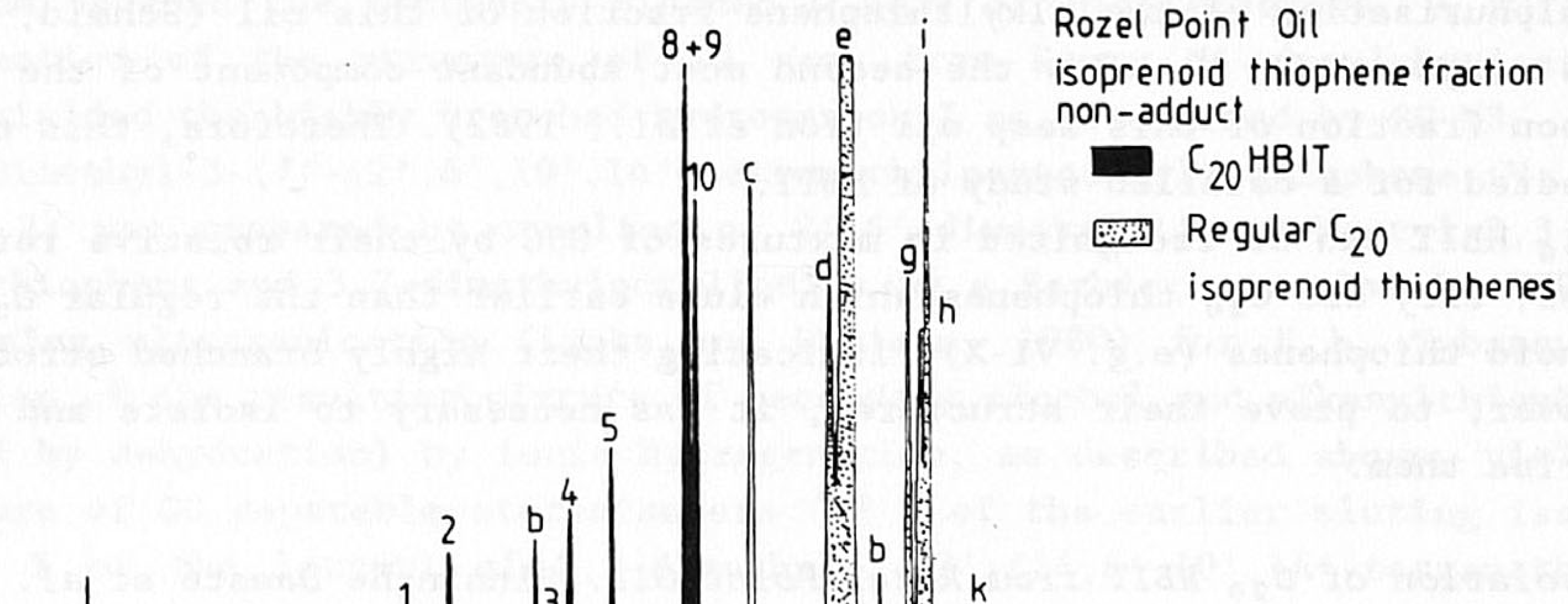

Fig. 9.3. Partial FID chromatogram of the non-adduct of the isoprenoid thiophene fraction of the Rozel Point Oil. Numbers refer to C_{20} HBIT identified in Table 9.1. Letters indicate non-HBIT. Key: a = monounsaturated C_{18} isoprenoid thiophene, b = monounsaturated C_{19} isoprenoid thiophene, c = C_{20} monounsaturated isoprenoid thiophene, d = VII, e = VIII-X, f = C_{20} monounsaturated isoprenoid thiophene, g = XXIV and XXV, h = XXVI, i = VI and XXVII (minor amounts), j and k = C_{20} monounsaturated isoprenoid thiophenes.

Mass spectral characterisation of C_{20} HBIT. The obtained mass spectra of the C_{20} HBIT are presented in Fig. 9.5. The structures of the C_{20} HBIT were assigned by considering the theoretical fragmentation pathways (as determined from data obtained previously on other thiophenes; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987c; Rullkötter *et al.*, 1988) of all possible structural isomers (13; IV, XI-XXII) and selecting the most likely isomer.

The mass spectrum of XVIII (Fig. 9.5A) shows β-cleavage fragments at *m/z* 293, 279, 265 and 223 all consistent with its proposed structure. The abundance of the ion at *m/z* 265 relative to those at *m/z* 293, 279 and 223 is most likely due to the formation of a secondary alkyl radical instead of a primary alkyl radical. The ion at *m/z* 181 is probably a secondary ion formed by loss of C_3H_6 (part of the C_4 alkyl substituent at C-2) from the ion at *m/z* 223 and/or by loss of C_6H_{12} (part of the C_8 alkyl substituent at C-3) from the ion at *m/z* 265. The formation of the ion at *m/z* 69 is at present less understood, although it probably has something to do with fragmentation of the C_8 alkyl side-chain at C-3. Similar fragmentation pathways are

Table 9.1. HBIT identified and their Kovats retention indices.

Num.[1]	name	characterisation[2]	isolated in	I[3]
1	5-(2′-butyl)-3-(2′-(6′-methylheptyl))-2-(2′-methylpropyl)thiophene (XVIII)	MS,RND	ITM.8.5	1826
2	2-(2′-methylbutyl)-3-(2′-(6′-methylheptyl))-5-(2′-propyl)thiophene (XVI)	MS,RND	ITM.8.5	1845
3	4-(5′-(2′,8′-dimethyldecyl))-2-(2′-methylpropyl)thiophene (XII)	MS,RND	ITM.8.6 and .9.3	1902
4	2,3-dimethyl-5-(5′-(2′,6′,10′-trimethylundecyl))thiophene (IV)	MS,RND,S	ITM.10.3	1905
5	2,3-dimethyl-5-(5′-(2′,6′,10′-trimethylundecyl))thiophene (IV)	MS,RND,S	ITM.11.3	1925
6	4-methyl-2-(7′-(2′,6′,10′-trimethyldodecyl))thiophene (XXII)	MS,RND	ITM.9.3	1932
7	4-methyl-2-(7′-(2′,6′,10′-trimethyldodecyl))thiophene (XXII)	MS,RND	ITM.10.3	1944
8	3-methyl-5-(2′-methylbutyl)-2,4-di-(3′-methylbutyl)thiophene (XIII)	MS,RND	ITM.11.4	1960
9	2-(2′-methylbutyl)-3-(3′-methylbutyl)-4-(4′-methylpentyl)thiophene (XX)	MS,RND	ITM.10.3	1962
10	3-methyl-2-(3′-methylbutyl)-4-(3′-methylpentyl)-5-(2′-methylpropyl)thiopene (XIV)	MS,RND	ITM.11.4	1965
11	2-(2′,6′-dimethyl-3′-(3′′-methylbutyl)-octyl)-4-methylthiophene (XI)	MS,RND	ITM.9.3 and .10.3	1967
12	2-(2′,6′-dimethyl-6-(3′′-methylbutyl)-octyl)-4-methylthiophene (XI)	MS,RND	ITM.9.3 and .10.3	1976
13	2-(2′-methylbutyl)-3,5-di(2′-(6′-methylheptyl))thiophene (XXIII)	MS,RND	-	2203
14	2,3-dimethyl-5-(7′-(2′,6′,10′,14′-tetramethylpentadecyl))thiophene (V)	MS,RND,S	-	2304, 2307
15	2,3-dimethyl-5-(7′-(2′,6′,10′,14′-tetramethylpentadecyl))thiophene (V)	MS,RND,S	-	2322

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[1] numbers refer to Figs. 9.3, 9.4 and 9.6

[2] MS = characterisation by mass spectrometry, RND = characterisation by Raney–Ni desulphurisation, S = characterisation by synthesis of authentic standard

[3] Kovats retention index determined by coinjection with a homologous series of *n*-alkanes. Temperature program: 130^0C to 320^0C at 4^0C/min. Carrier gas: hydrogen.

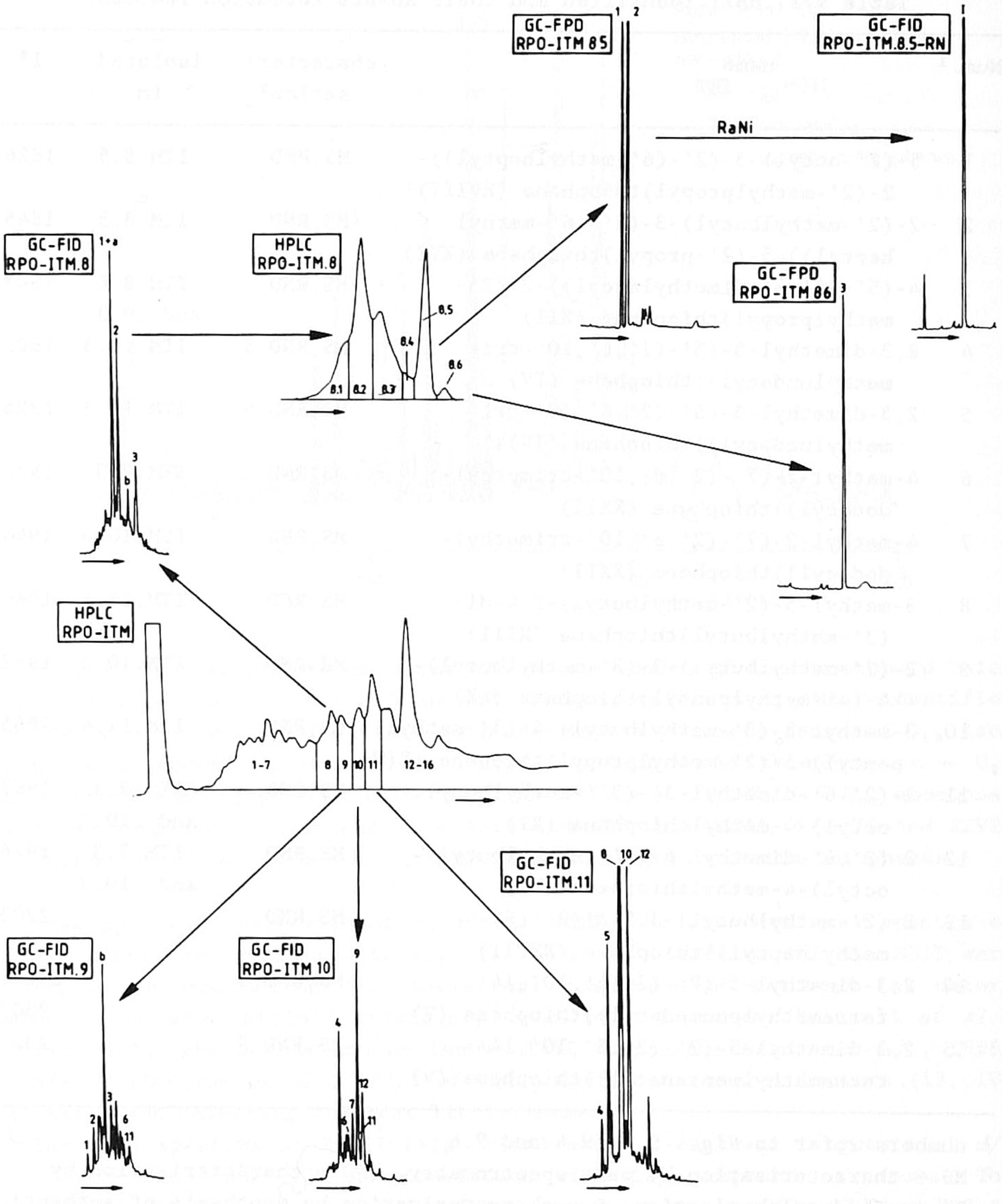


Fig. 9.4. Reversed-phase HPLC isolation of C_{20} HBIT from the methanol eluate of the non-adduct of the isoprenoid thiophene fraction of the Rozel Point Oil (see Fig. 9.1). An example is given for the further isolation of C_{20} HBIT from ITM.8 using an analytical HPLC column and $MeOH/H_2O$ (95/5) as an eluent. Numbers refer to Table 9.1. Letters indicate non-HBIT. Key: as for Fig. 9.3.

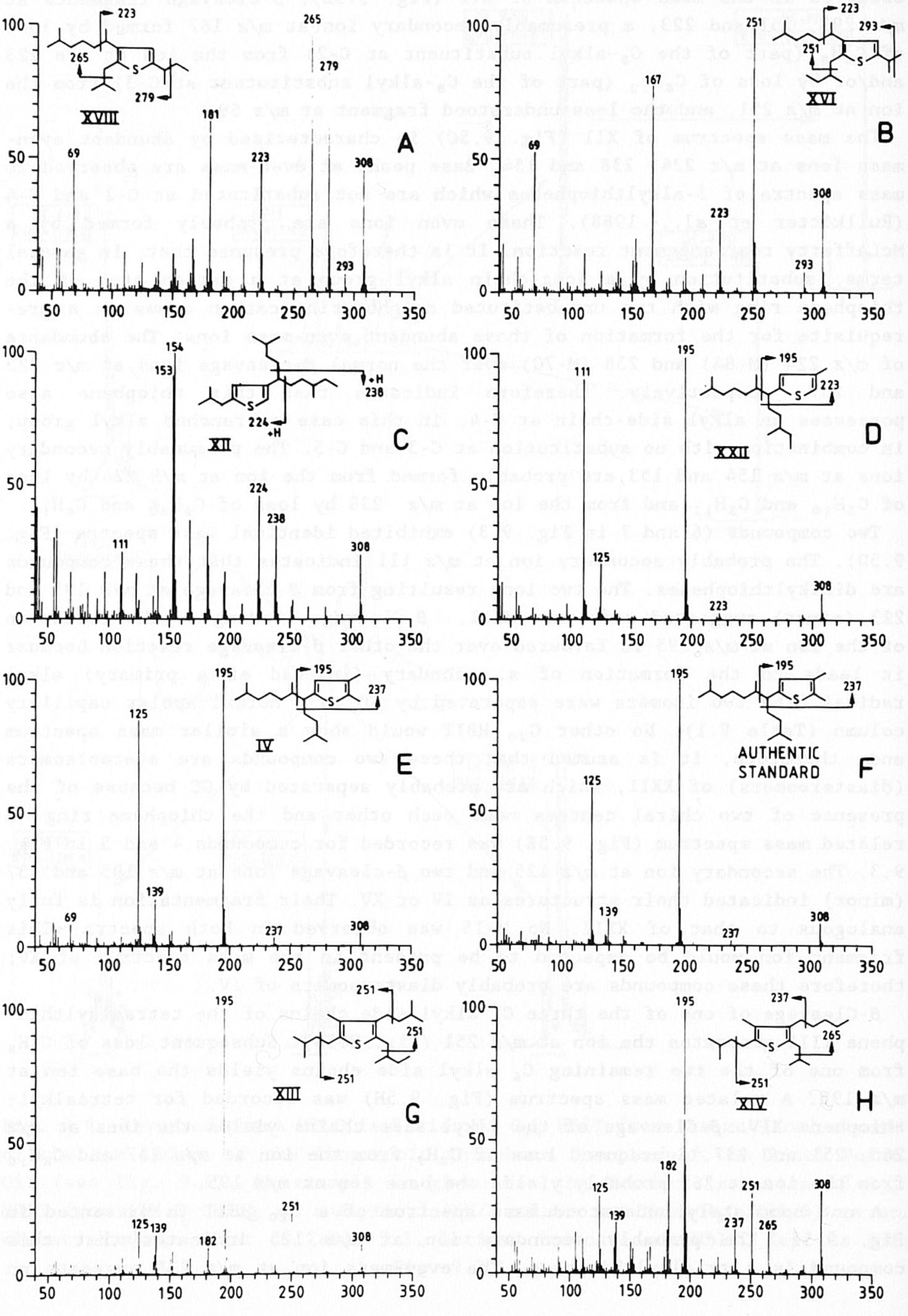
observed in the mass spectrum of XVI (Fig. 9.5B); β-cleavage fragments at *m/z* 293, 251 and 223, a presumably secondary ion at *m/z* 167 formed by loss of C_4H_8 (part of the C_5–alkyl substituent at C-2) from the ion at *m/z* 223 and/or by loss of C_6H_{12} (part of the C_8-alkyl substitutent at C-3) from the ion at *m/z* 251, and the less understood fragment at *m/z* 69.

The mass spectrum of XII (Fig. 9.5C) is characterised by abundant even-mass ions at *m/z* 224, 238 and 154. Base peaks at even-mass are observed in mass spectra of 3-alkylthiophenes which are not substituted at C-2 and C-4 (Rullkötter *et al.*, 1988). These even ions are probably formed by a McLafferty rearrangement reaction. It is therefore presumed that, in general terms, substitution by a long-chain alkyl group at a carbon atom of the thiophene ring with two unsubstituted neighbouring carbon atoms is a prerequisite for the formation of these abundant even-mass ions. The abundance of *m/z* 224 (M-84) and 238 (M-70) over the normal β-cleavage ions at *m/z* 223 and 237, respectively, therefore indicates that this thiophene also possesses an alkyl side-chain at C-4, in this case a branched alkyl group, in combination with no substitution at C-3 and C-5. The presumably secondary ions at *m/z* 154 and 153 are probably formed from the ion at *m/z* 224 by loss of C_5H_{10} and C_5H_{11} and from the ion at *m/z* 238 by loss of C_6H_{12} and C_6H_{13}.

Two compounds (6 and 7 in Fig. 9.3) exhibited identical mass spectra (Fig. 9.5D). The probably secondary ion at *m/z* 111 indicates that these compounds are dialkylthiophenes. The two ions resulting from β-cleavage at *m/z* 195 and 223 (minor) suggested structure XXII. β-Cleavage leading to the formation of the ion at *m/z* 195 is favoured over the other β-cleavage reaction because it leads to the formation of a secondary (instead of a primary) alkyl radical. The two isomers were separated by GC on a normal apolar capillary column (Table 9.1). No other C_{20} HBIT would show a similar mass spectrum and, therefore, it is asumed that these two compounds are stereoisomers (diastereomers) of XXII, which are probably separated by GC because of the presence of two chiral centres near each other and the thiophene ring. A related mass spectrum (Fig. 9.5E) was recorded for compounds 4 and 5 in Fig. 9.3. The secondary ion at *m/z* 125 and two β-cleavage ions at *m/z* 195 and 237 (minor) indicated their structures as IV or XV. Their fragmentation is fully analogous to that of XXII. No M-15 was observed in both spectra. This fragment ion would be expected to be present in the mass spectrum of XV; therefore these compounds are probably diastereomers of IV.

β-Cleavage of one of the three C_5 alkyl side chains of the tetraalkylthiophene XIII generates the ion at *m/z* 251 (Fig. 9.5G). Subsequent loss of C_4H_8 from one of the two remaining C_5 alkyl side chains yields the base ion at *m/z* 195. A related mass spectrum (Fig. 9.5H) was recorded for tetraalkylthiophene XIV. β-Cleavage of the alkyl side chains yields the ions at *m/z* 265, 251 and 237. Subsequent loss of C_3H_7 from the ion at *m/z* 237 and C_5H_{10} from the ion at 265 probably yields the base ion at *m/z* 195.

A not completely understood mass spectrum of a C_{20} HBIT is presented in Fig. 9.5I. The probably secondary ion at *m/z* 125 indicates that this compound is a trialkylthiophene. The even-mass ion at *m/z* 238 suggests an


A
XVIII
223
265
279
181
69
223
265
279
308
293
B
XVI
223
251
293
251
167
69
223
308
293
C
XII
154
153
238
224
+H
+H
111
224
238
308
D
XXII
195
223
195
111
125
308
223
E
IV
195
237
195
125
139
69
237
308
F
AUTHENTIC
STANDARD
195
237
195
125
139
237
308
G
XIII
251
251
251
195
125
139
182
251
308
H
XIV
237
265
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195
182
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265
308
100
50
0
50
100
150
200
250
300
350

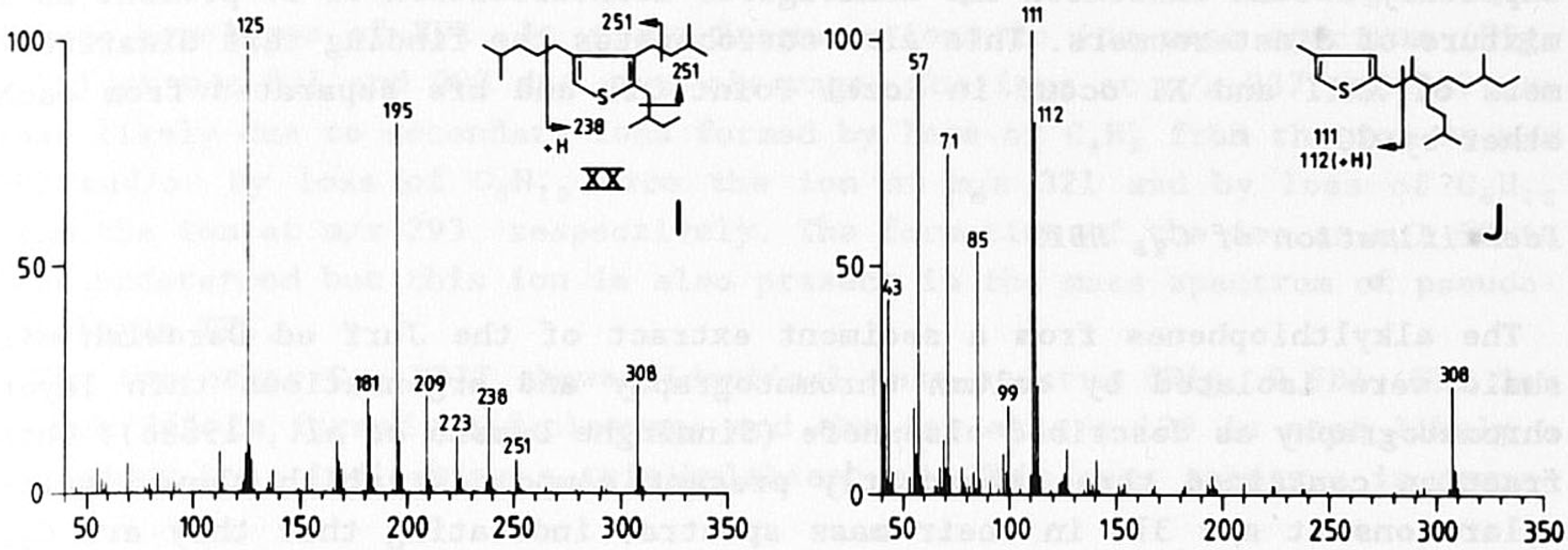


Fig. 9.5. Mass spectra (corrected for background) of C_{20} HBIT present in Rozel Point Oil (see also left page).

alkyl side-chain at C-4 in combination with no substitution at C-5. From all the possible C_{20} HBIT (IV, XI-XXII) only two isomers (XX and XXI) possess these structural features. The ion at *m/z* 195 is probably formed by loss of both alkyl side-chains at C-2 and C-3 (*e.g.* compare with Figs. 9.5G and 9.5H). XX and XXI are both likely structures for this compound. However, in the mass spectrum of XXI a relative abundant β-cleavage ion at *m/z* 265 (formation of a secondary alkyl radical) would be expected but is not observed. Therefore, this spectrum is assigned to trialkylthiophene XX.

The mass spectrum (Fig. 9.5J) of compound 11 in Fig. 9.3 shows a fragment ion at *m/z* 111 (due to β-cleavage) and an ion at *m/z* 112 (resulting from hydrogen rearrangement) of about the same intensity. This phenomenon is observed in mass spectra of γ-branched 2-alkylthiophenes, not substituted at C-3 (Brassell *et al.*, 1986c; Sinninghe Damsté, 1986, 1987c, Rullkötter *et al.*, 1988). In this specific case the McLafferty rearrangement ion is enhanced relative to non-γ-branched alkylthiophenes. The ion at *m/z* 111 indicates a dialkylthiophene. On this basis XI was thought to be the most likely structure. The ions at *m/z* 57 + 14.*n* are generated from fragmentation of the long alkyl side-chain. Another compound (12 in Fig. 9.3) was isolated and had an identical mass spectrum. By anology with compounds 6/7 and 4/5 compounds 11 and 12 are most likely diastereomers.

Co-chromatography with an authentic standard. One C_{20} HBIT (IV) has been synthesised as indicated in Fig. 9.2 to prove its geological occurrence. Two isomers were obtained which showed mass spectra (Fig. 9.5F) identical to those of compounds 4 and 5 in Fig. 9.3. The ^{13}C-NMR spectra of this synthetic mixture indicated that it was a mixture of diastereomers (*i.e.* in several cases two signals were observed for one carbon atom). No attempts were undertaken to assign the two diastereomers separable by GC. Co-chromatography with this synthetic mixture of IV and various fractions of the Rozel Point Oil obtained by the isolation procedure on an apolar phase capillary column indicated the unambigious identification of IV present as a mixture of diastereomers. This also corroborates the finding that diastereomers of XXII and XI occur in Rozel Point Oil and are separated from each other by GC.

Identification of C_{25} HBIT

The alkylthiophenes from a sediment extract of the Jurf ed Darawish oil shale were isolated by column chromatography and argentatious thin layer chromatography as described elsewhere (Sinninghe Damsté *et al.*, 1988e). This fraction contained three abundantly present compounds, which showed molecular ions at *m/z* 378 in their mass spectra, indicating that they are C_{25} alkylthiophenes. They eluted much earlier than the C_{25} regular isoprenoid thiophenes and the C_{25} 2,5-di-*n*-alkylthiophenes (Fig. 9.6). This retention behaviour indicated their highly branched structure. Raney Ni desulphurisation of this fraction yielded relatively high amounts of the C_{25} highly branched isoprenoid hydrocarbon II.

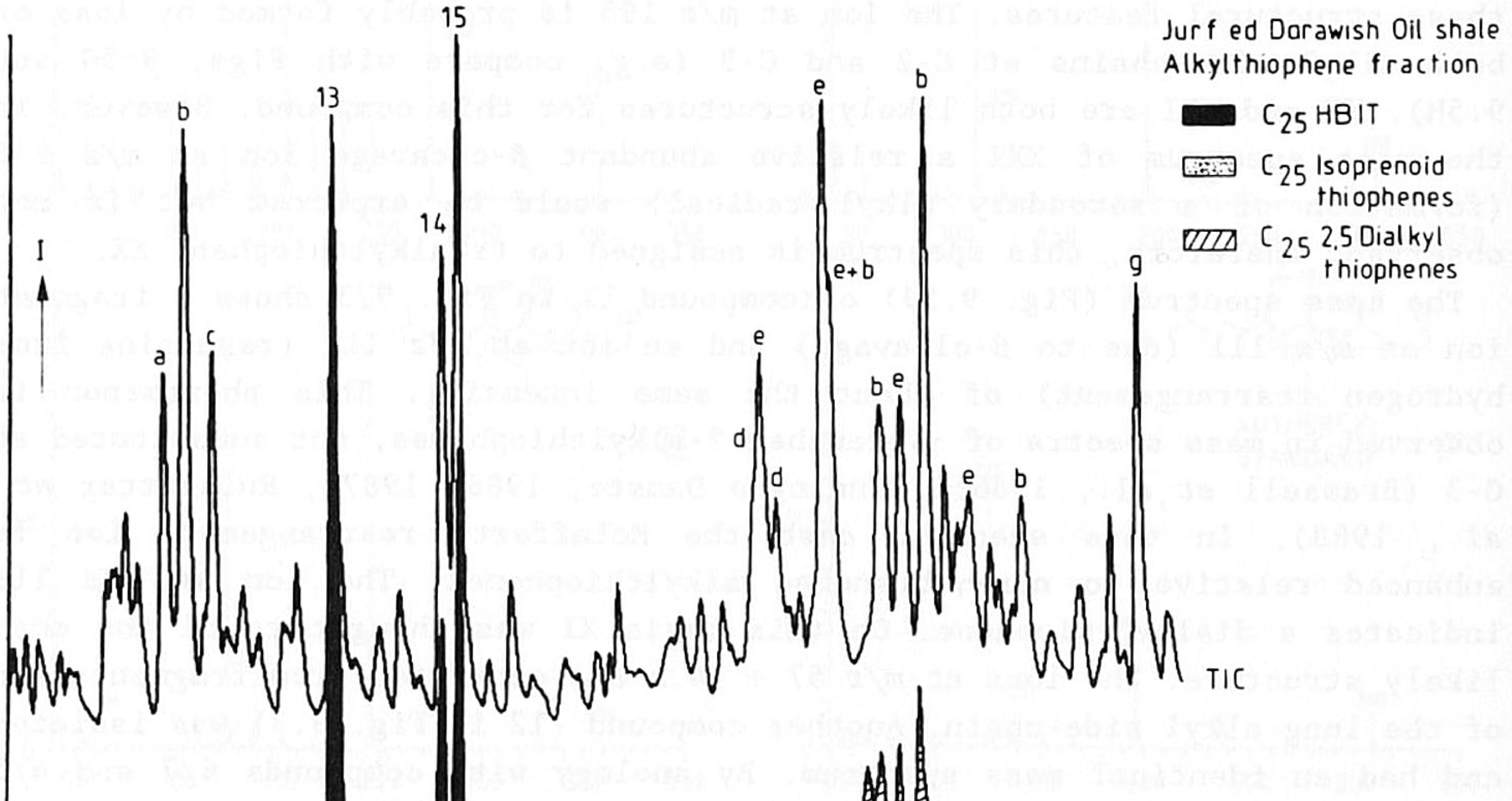


Fig. 9.6. Partial total ion current (TIC) and mass chromatogram of *m/z* 378 of the alkylthiophene fraction of the extract of the Jurf ed Darawish Oil shale. Numbers refer to C_{25} HBIT identified in Table 9.1. Letters indicate non-HBIT. Key: a - 2-methyl-5-tridecylthiophene b - VI, c - XXVIII, d - C_{26} isoprenoid thiophenes, e - C-ring monoaromatic steroids, f - C-ring monoaromatic 4-methylsteroids, g - C_{28} "mid-chain" 2,5-di-*n*-alkylthiophenes.

The first eluting isomer (compound 13 in Fig. 9.6; XXIII) is probably a pseudo-homologue of XVI. It shows fragment ions in its mass spectrum (Fig. 9.7A) at *m/z* 321 and 293 due to *β*-cleavage. The ions at *m/z* 237 and 209 are most likely due to secondary ions formed by loss of C_4H_8 from the ion at *m/z* 293 and/or by loss of C_6H_{12} from the ion at *m/z* 321 and by loss of C_6H_{12} from the ion at *m/z* 293, respectively. The formation of the ion at *m/z* 69 is less understood but this ion is also present in the mass spectrum of pseudo-homologue XVI.

The two other C_{25} HBIT showed identical mass spectra (Fig. 9.7B). The ion at *m/z* 265 is formed by *β*-cleavage and the ion at *m/z* 125 is most likely a secondary ion, indicating a trialkylthiophene. This mass spectrum is compa-

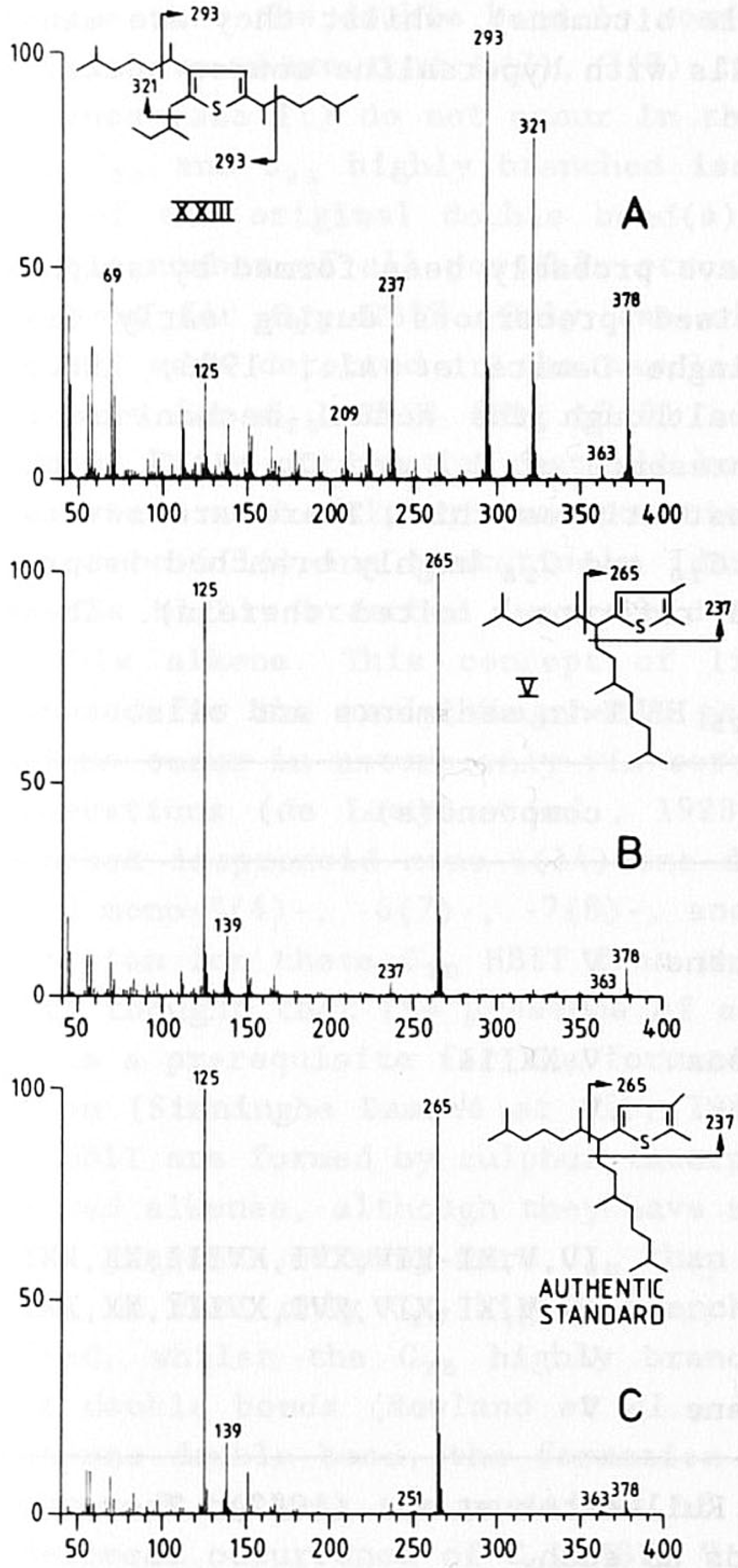


Fig. 9.7. Mass spectra (corrected for background) of C_{25} HBIT present in the extract of the Jurf ed Darawish Oil shale.

rable to that of the C_{20} HBIT IV (Fig. 9.5E) and compounds 14 and 15 (Fig. 9.7) were thus identified as a pair of diastereoisomers of V. This identification was confirmed by coinjection of a synthetic mixture of diastereoisomers of V (Fig. 9.2) with the alkylthiophene fraction of the Jurf ed Darawish oil shale and by comparison of mass spectra (Fig. 9.7C).

Occurrence of C_{20} and C_{25} HBIT

A number of these HBIT are also present in a number of sediment extracts and oils from different geographical locations which range from Pleistocene to Cretaceous (Table 9.2). A detailed description of these samples has been presented elsewhere (Sinninghe Damsté *et al.*, 1988g). The results in Table 9.2 indicate that C_{25} HBIT occur more widely than C_{20} HBIT. Furthermore, these C_{25} HBIT are sometimes major OSC in normal marine sediments (Jurf ed Darawish Oil shale bitumen, Monterey shale bitumens), whilst they are minor compounds in hypersaline sediments and oils with hypersaline source rocks.

Origin of HBIT

Previously we have reported that OSC have probably been formed by sulphur incorporation reactions into functionalised precursors during early diagenesis (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987c; Sinninghe Damsté and de Leeuw, 1987), although the actual mechanisms of their formation are still unknown. The presence of C_{20} and C_{25} HBIT in the geosphere, as reported here, further substantiates this. There are several reports which describe the occurrence of C_{20} and C_{25} highly branched isoprenoid alkenes (Rowland *et al.*, 1985 and references cited therein). These

Table 9.2. Occurrence of C_{20} and C_{25} HBIT in sediments and oils.

sample	age	compound(s)
sediments		
Gulf of California[1]	Pleistocene	V
Monterey Shale (3)	Miocene	V
Jurf ed Darawish Oil shale (3)	Cretaceous	V,XXIII
Northern Apennines Marl	Miocene	V
oils		
Rozel Point Oil seep	Miocene	IV,V,XI-XIV,XVI,XVIII,XX,XXII
West Rozel Point Oil	Miocene	IV,V,XI-XIV,XVI,XVIII,XX,XXII
Sicily Seep Oils (3)	Miocene	V
Jianghan Oils (2)	Palaeogene	V

[1] derived from mass spectral evidence of Rullkötter *et al.* (1982). These authors did not identify these compounds as such.

alkenes occur widely in recent lacustrine, marine and hypersaline sediments and may be regarded as likely precursors of the C_{20} and C_{25} HBIT described here. Addition of hydrogen sulphide (or polysulphides) to double bonds in these biogenic alkenes might initiate the formation of HBIT during early diagenesis. Such an abiogenic sulphur addition reaction to the activated double bond of acrylic acid has been shown to occur in recent sediments (Vairaivamurthy and Mopper, 1987). However, sulphur addition to non-activated double bonds will be more difficult. Nevertheless, the presence of only a limited number of all possible HBIT structural isomers provides evidence that these reactions do occur.

C_{20} and C_{25} highly branched isoprenoid alkenes with double bonds in specific positions do occur in various sediments, although the actual positions are not excactly known at present. Dunlop and Jefferies (1985) have shown by ozonolysis of one sedimentary C_{20} highly branched isoprenoid monoene that the double bond is sometimes 6(14) (see I). Robson and Rowland (1986) have shown that 6(7), 7(8) and 7(1') C_{25} highly branched isoprenoid monoenes (see II) do not occur in their sample suite. Sulphur incorporation into C_{20} and C_{25} highly branched isoprenoid alkenes would fixate the position of the original double bond(s) and would lead to the formation of a limited number of all possible structural isomers, which is most strikingly observed for C_{25} HBIT. Only two of the seventeen theoretically possible isomers were detected in the samples studied. More structural isomers were detected for C_{20} HBIT (Fig. 9.8) but a number of isomers were completely absent. It is noteworthy that all but one C_{20} HBIT present have a structure consistent with sulphur incorporation into the C_{20} highly branched isoprenoid mono-6(14)-ene tentatively identified by Dunlop and Jefferies (1985) and the highly branched isoprenoid alkenes formed by limited isomerisation of this alkene. This concept of limited isomerisation has recently been proposed for the early diagenetic pathway of steroids; double bond isomerisations occur in nature only *via* tertiary carbocations and not *via* secondary carbocations (de Leeuw *et al.*, 1988). Limited isomerisation of the highly branched isoprenoid mono-6(14)-ene during early diagenesis would thus only yield mono-5(6)-, -6(7)-, -7(8)-, and -7(1')-enes. However, the mechanism of formation for these C_{20} HBIT from these alkenes is, as yet, unclear because it is thought that the presence of at least two double bonds in the precursor is a prerequisite for the formation of OSC by abiogenic sulphur incorporation (Sinninghe Damsté *et al.*, 1988e). Another possibility is that these C_{20} HBIT are formed by sulphur incorporation into C_{20} polyunsaturated highly branced alkenes, although they have not yet been reported in sediments.

The presence of many more C_{20} than C_{25} HBIT isomers may be due to the fact that, so far, only C_{20} highly branched isoprenoid monoenes have been identified, whilst the C_{25} highly branched alkenes identified possess one to four double bonds (Rowland *et al.*, 1985). In case of the presence of more than one double bond, the formation of structural isomers of HBIT might be suppressed because the positions of sulphur attack are more determined. The widespread occurrence of C_{25} HBIT V in combination with the absence of any

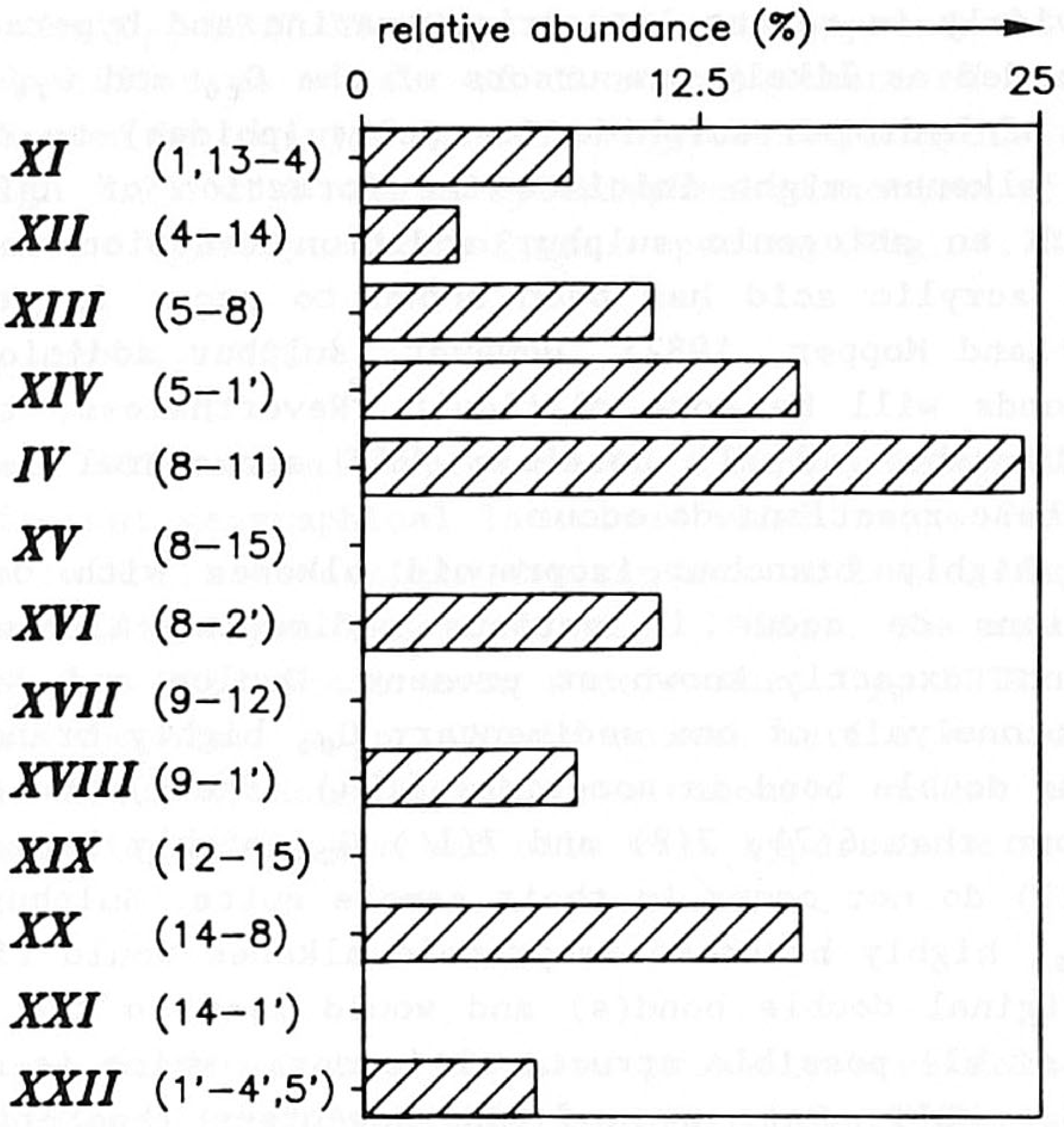
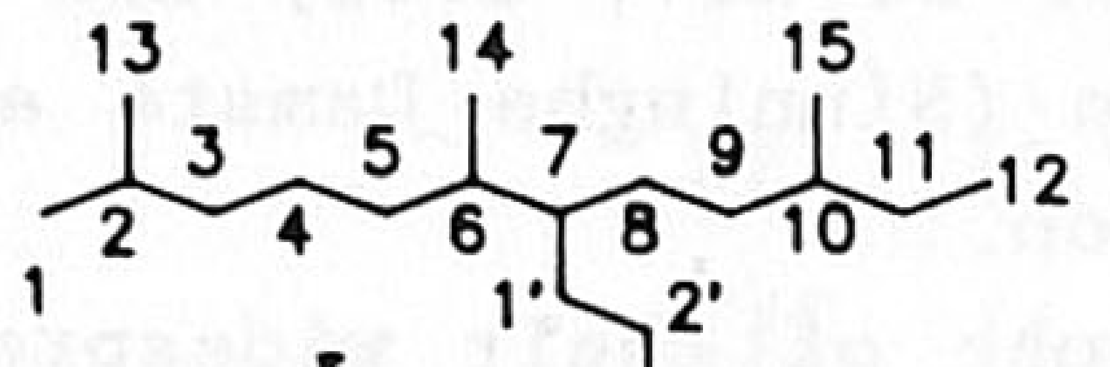


Fig. 9.8. Bar graph representing the relative abundance of all the possible structural isomers of C_{20} HBIT in Rozel Point Oil. The relative abundance of these compounds was obtained by integration of the FID chromatograms of the ITM-fraction and several sub-fractions of this fraction.

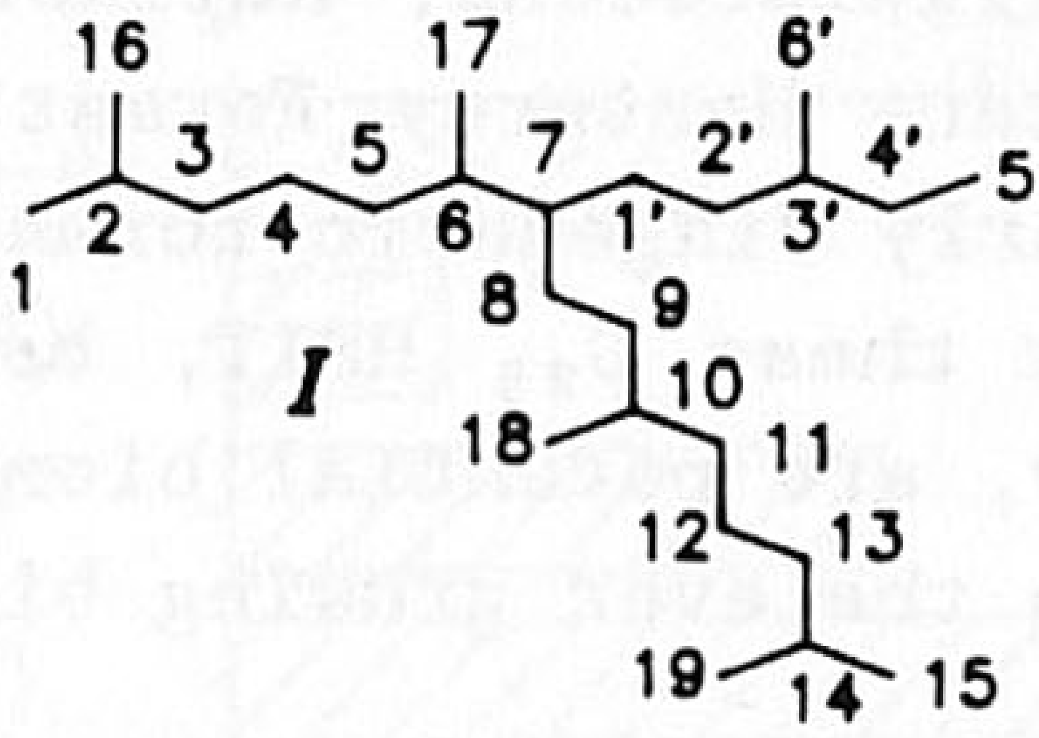
other C_{25} HBIT isomer in these samples (except for one sample from the Jurf ed Darawish Oil shale) might thus be explained by sulphur incorporation into a C_{25} highly branched isoprenoid diene with double bond positions at the C_6 part (C-1' - C-6'; see II) of the carbon skeleton. A C_{25} highly branched isoprenoid diene with a mass spectrum consistent with such double bond positions has frequently been reported to occur in recent sediments, sedimenting material and, recently, sea-ice diatoms (Requejo and Quinn, 1983; Requejo *et al.*, 1984; Albaiges *et al.*, 1984; Rowland *et al.*, 1985; Venkatesan and Kaplan, 1987; Venkatesan, 1988; Nichols *et al.*, 1988). Characteristic fragment ions of these dienes (so far two isomers with an identical mass spectrum have been reported) are *m/z* 348 (molecular ion), *m/z* 320 (M-C_2H_4)), *m/z* 266 (M-C_6H_{10}), *m/z* 235 (M-C_8H_{17}) and *m/z* 207 (M-$C_{10}H_{21}$) (*e.g.* Rowland *et al.* (1985). Interestingly enough, the C_{25} HBIT V is most abundant in anoxic marine, which likely have received a considerable input of diatoms, but not in hypersaline sediments. HBIT V might thus reflect a precursor biosynthesised by diatoms, which under anoxic conditions (with low amounts of available iron) is converted into HBIT V, a compound quite stable over geological time spans because it is also found in *e.g.* Cretaceous sediments (Table 9.2). The presence of HBIT V in immature sediment samples from the Gulf of California (Pleistocene, Rullkötter *et al.*, 1982) and in immature outcrops of the Miocene Monterey Formation (Sinninghe Damsté *et al.*, 1988g) indicates their early diagenetic formation.

It might be speculated that these C_{25} HBIT, because of their widespread occurrence and their stability, are potential biomarkers for diatoms and are thus a significant addition to the ever growing biomarker collection.

APPENDIX



I



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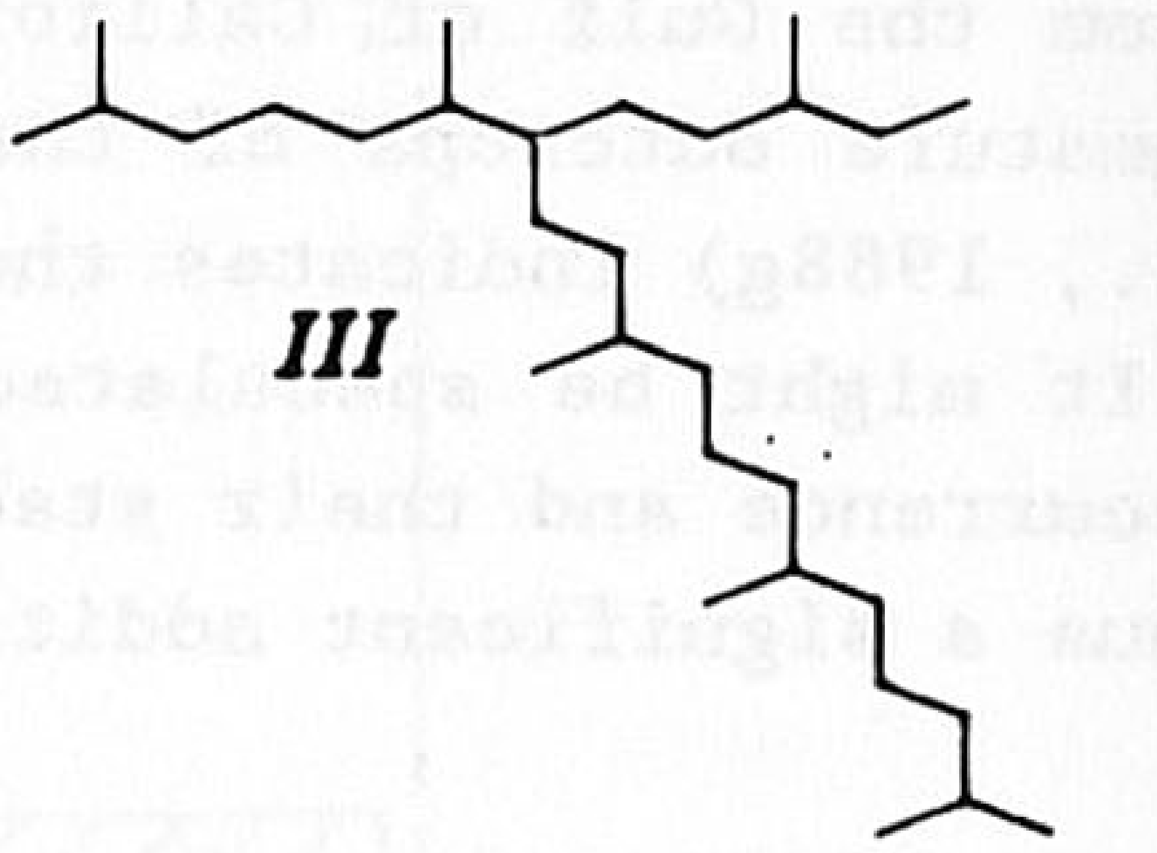
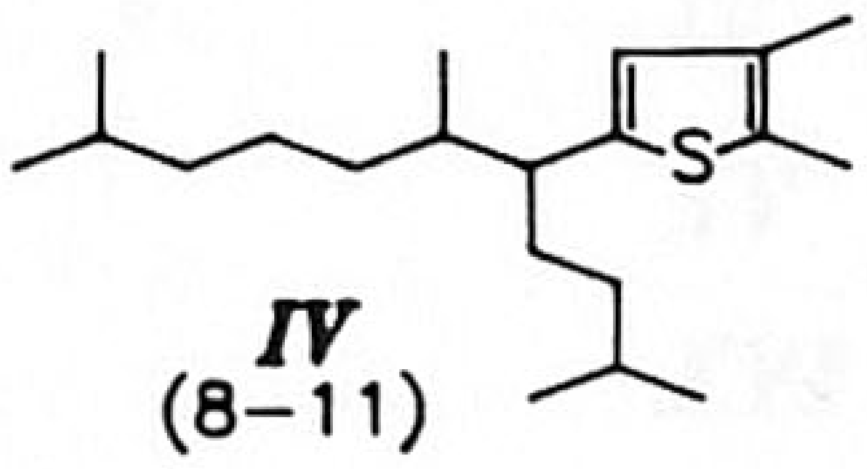
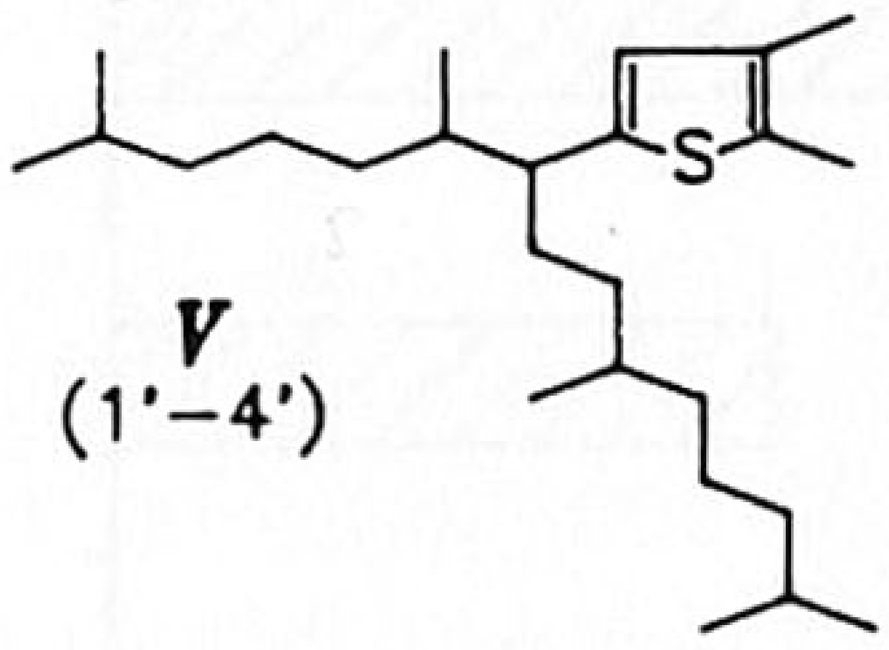


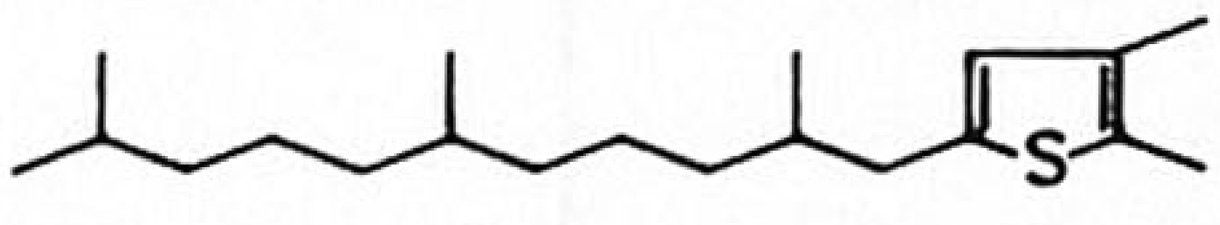
III



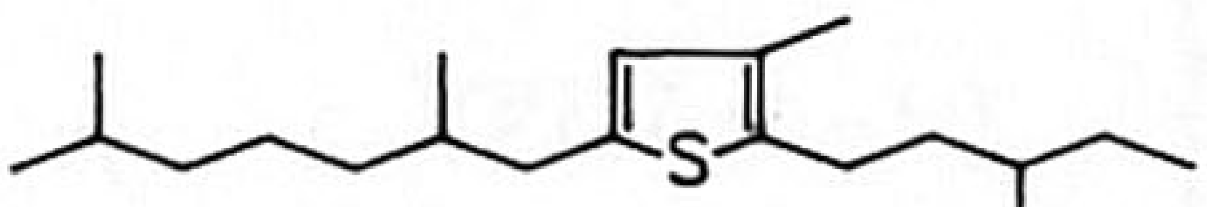
IV
(8–11)



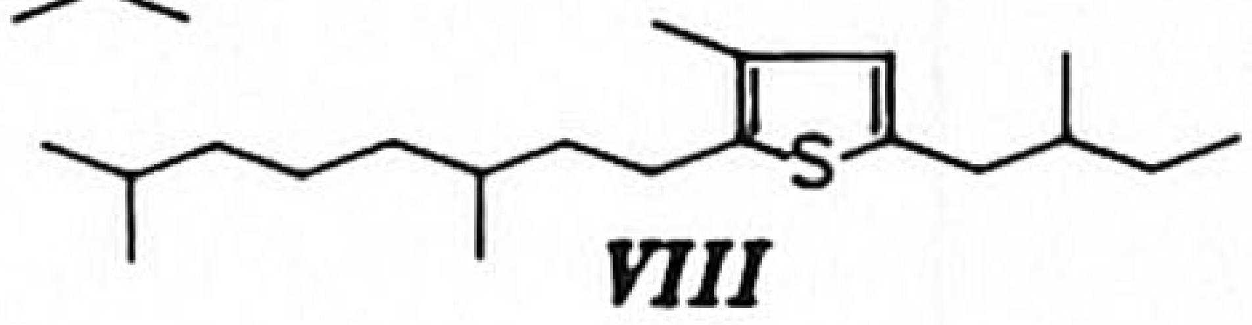
V
(1'–4')



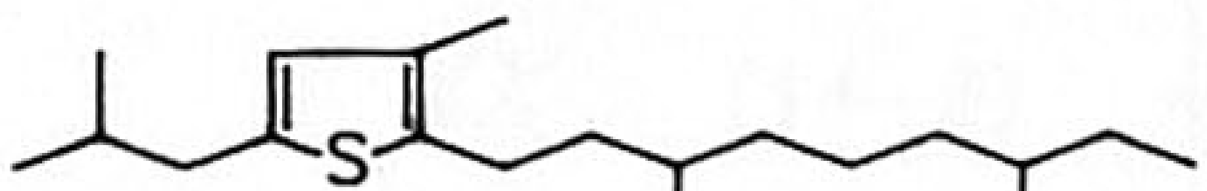
VI



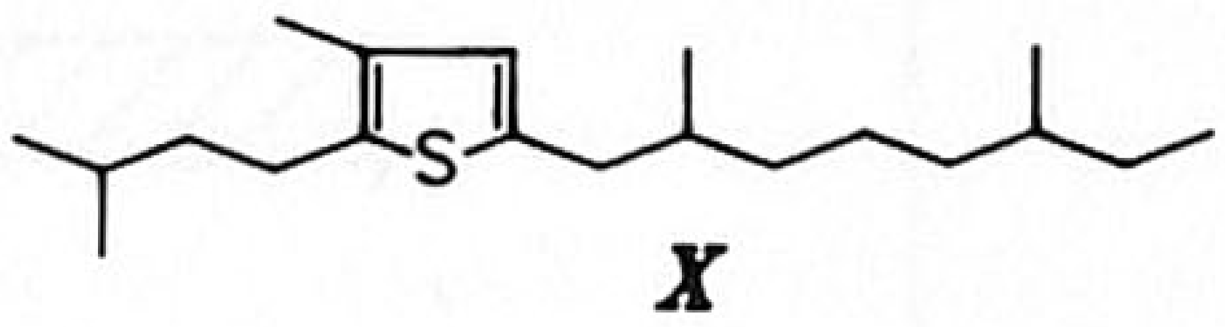
VII



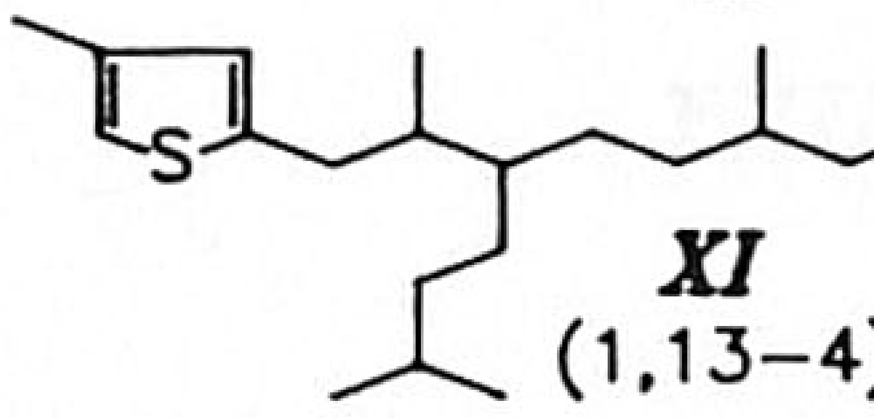
VIII



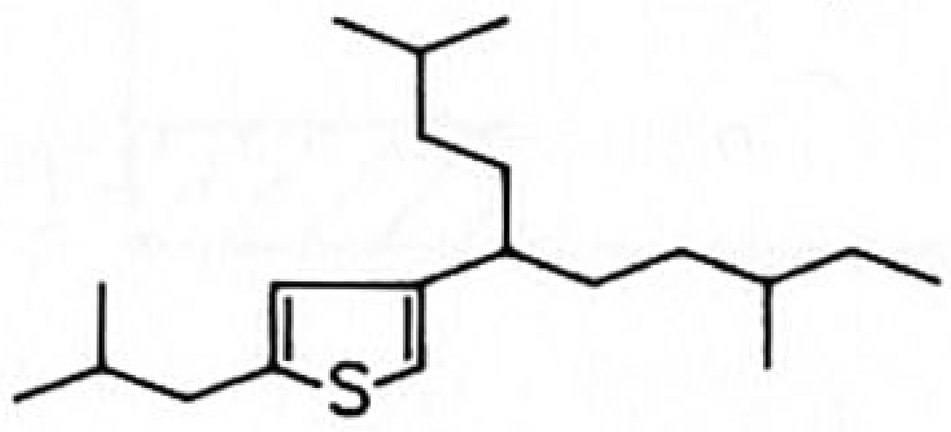
IX



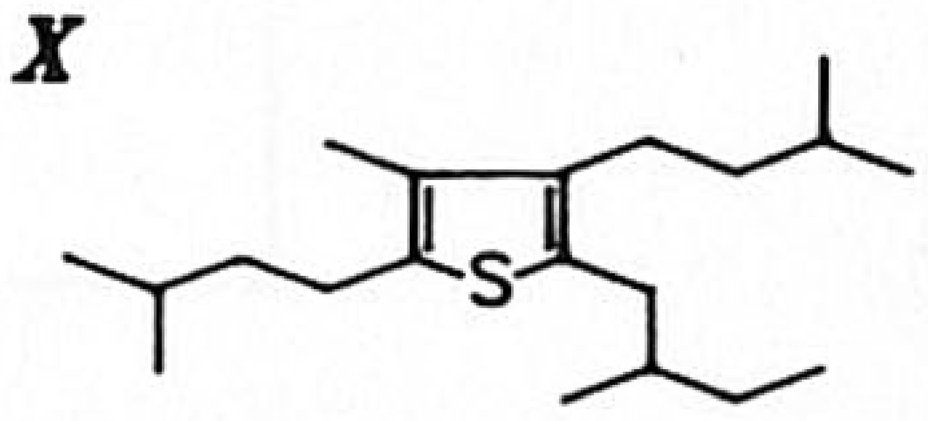
X



XI
(1,13–4)



XII (4–14)



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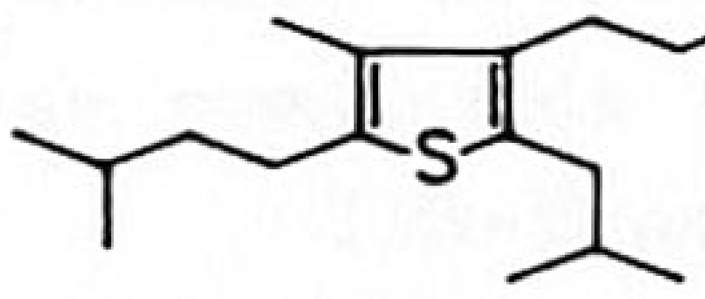
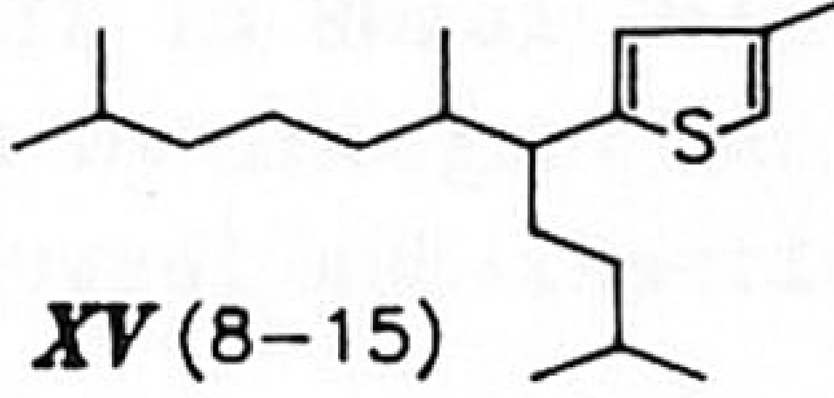
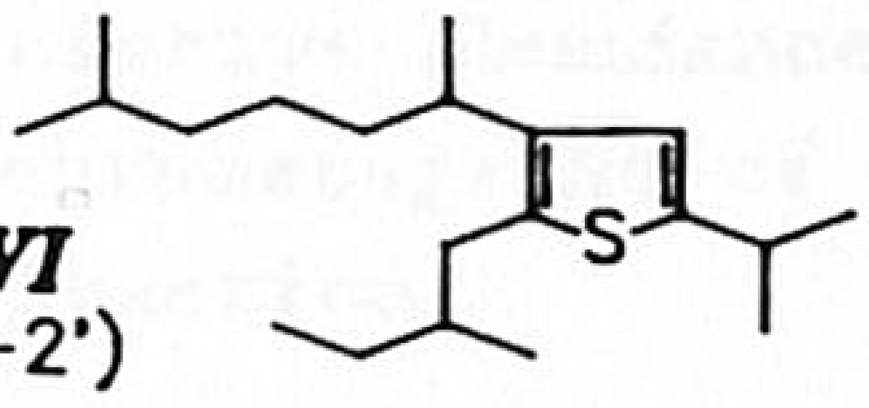


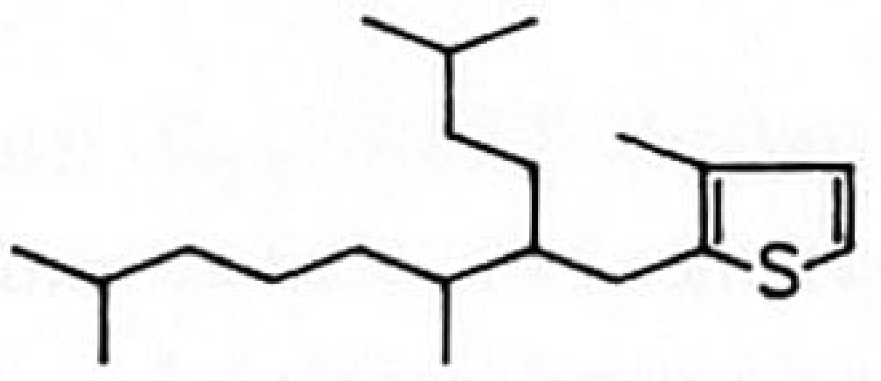
XIV (5–1')



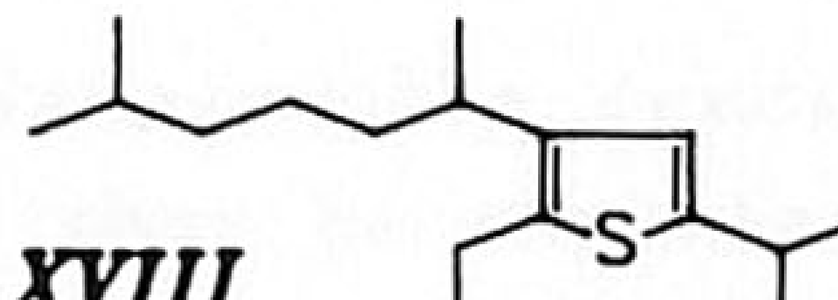
XV (8–15)



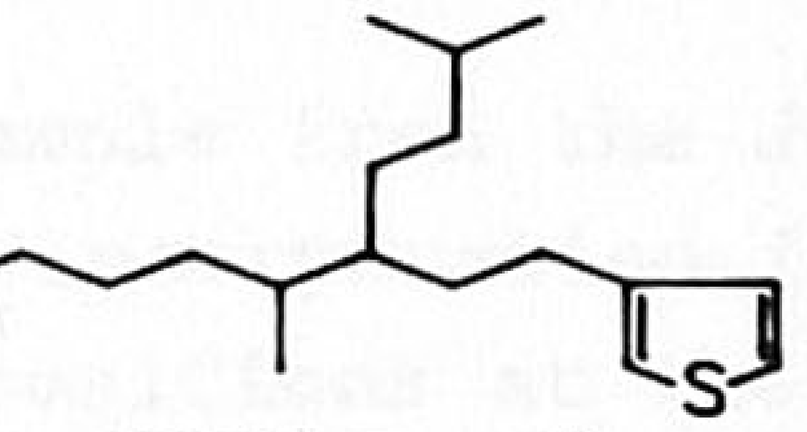
XVI
(8–2')



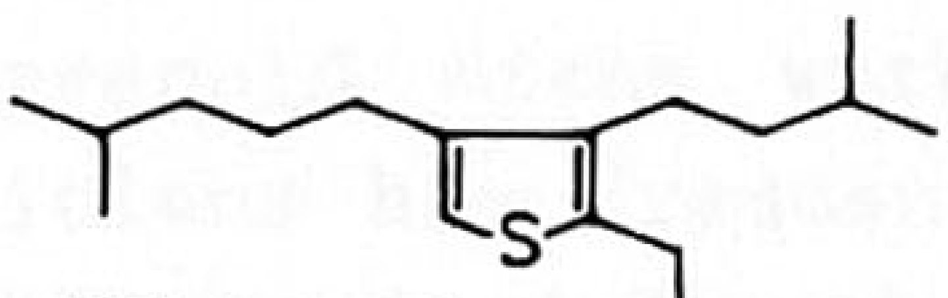
XVII (9–12)



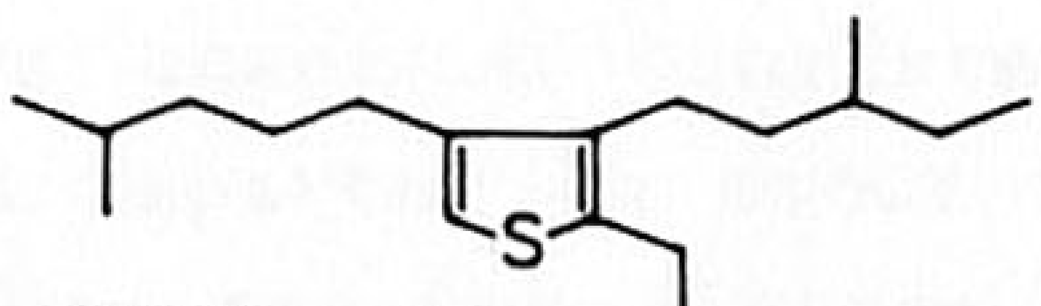
XVIII
(9–1')



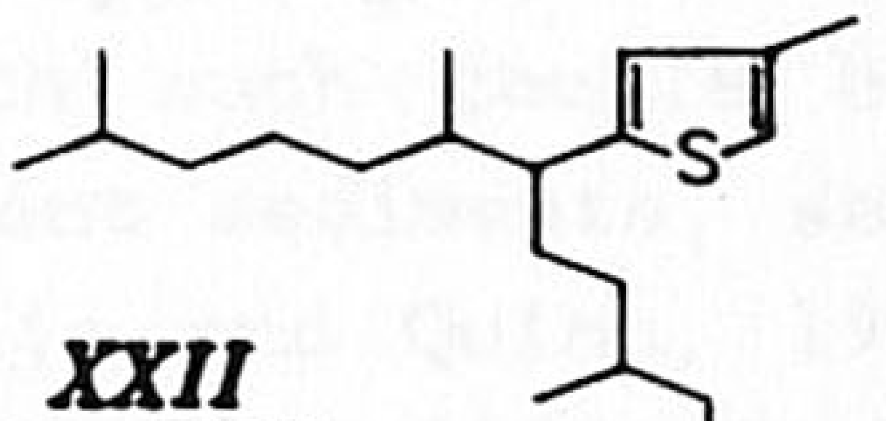
XIX (12–15)



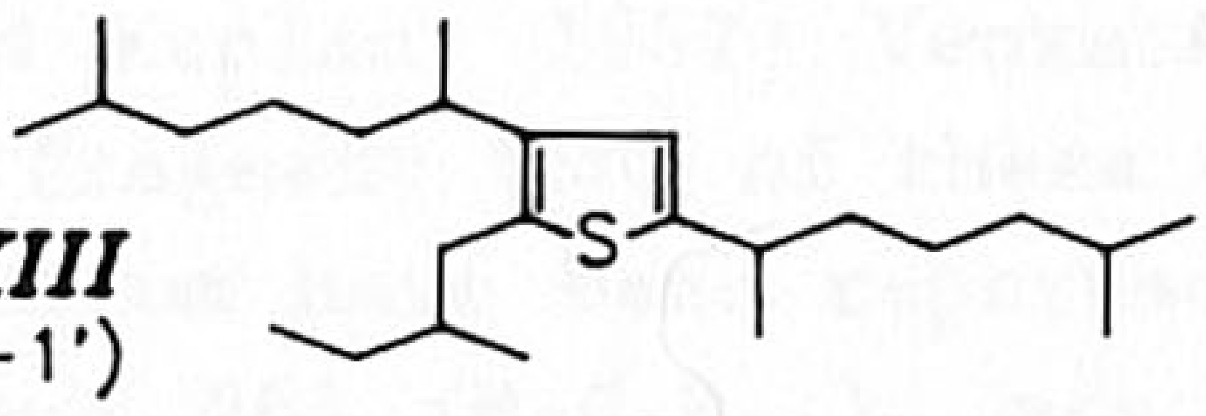
XX (14–8)



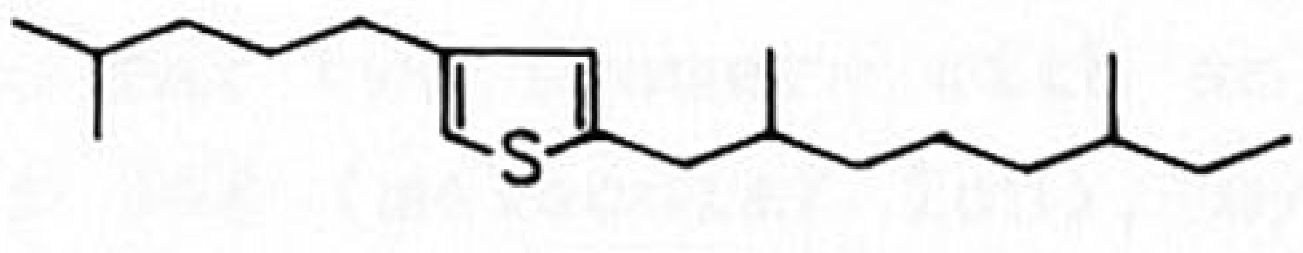
XXI (14–1')



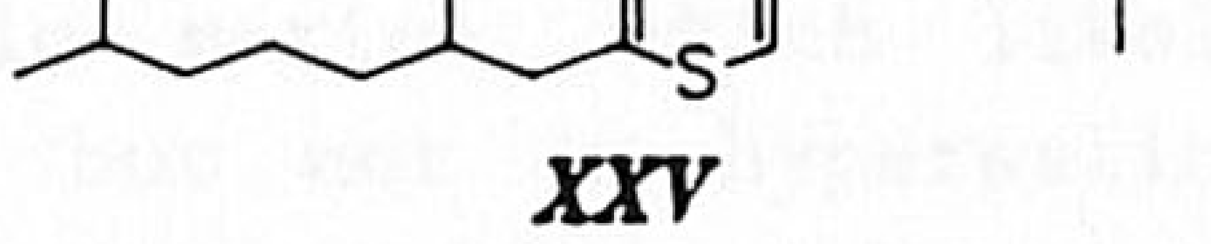
XXII
(1'–4',5')



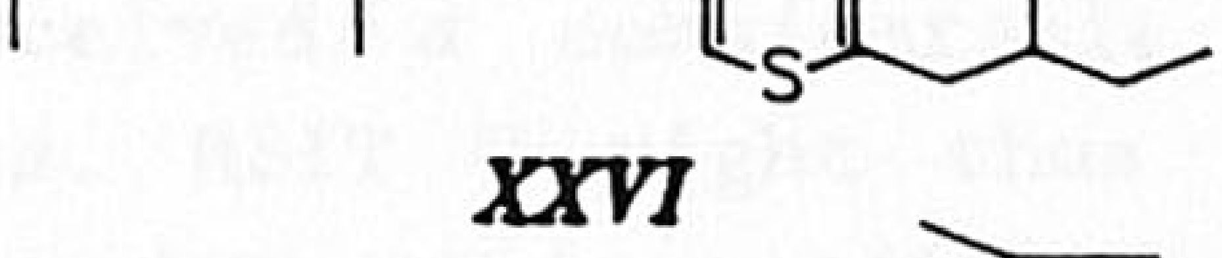
XXIII
(9–1')



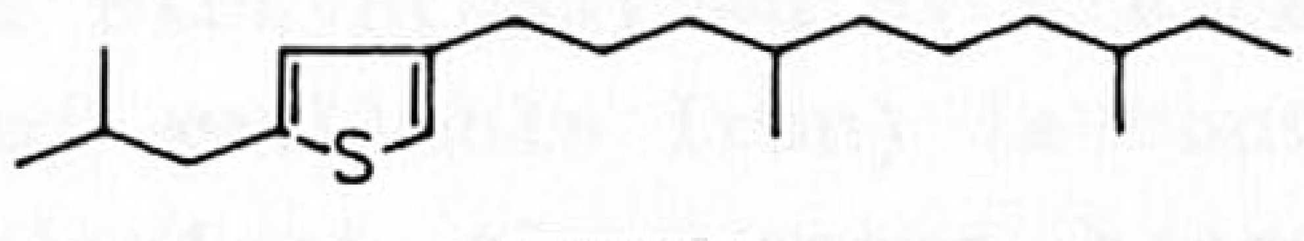
XXIV



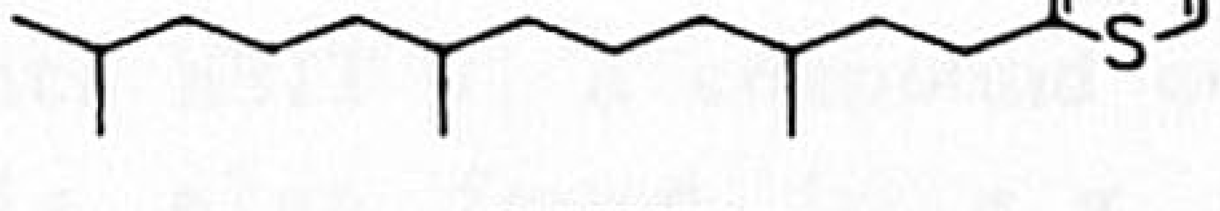
XXV



XXVI



XXVII



XXVIII

10. Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis *

Jaap S. Sinninghe Damsté, W. Irene C. Rijpstra, A.C. Kock-van Dalen,
Jan W. de Leeuw and P.A. Schenck

Delft University of Technology, Faculty of Chemical Technology
and Materials' Science, Organic Geochemistry Unit,
de Vries van Heystplantsoen 2, 2628 RZ, The Netherlands.

10.1 ABSTRACT

The bitumen of the Jurf ed Darawish Oil Shale has been analysed for organic sulphur compounds (OSC). A number of unprecedented OSC are reported: several C_{28}, C_{37} and C_{38} 2,5-di-*n*-alkylthiolanes and -thiophenes and 2,6-di-*n*-alkylthianes, and C_{19} branched thiophenes possessing the 9-methyloctadecane carbon skeleton. A number of these compounds were unambiguously identified by synthesis of authentic standards. All the OSC compound classes mentioned exhibit structural isomer distributions dominated by a limited number of all theoretically possible isomers. This provides direct evidence for the formation of these OSC by abiogenic sulphur incorporation into functionalised lipids at the early stages of diagenesis. Precursors for the OSC identified are suggested.

From these observations and from data on the occurrence of other OSC and of sulphur in high molecular weight substances a general model for the incorporation of sulphur into organic matter is proposed. Sulphur incorporation into precursors with double bonds (or other reactive functionalities) will lead to formation of OSC and sulphur-rich high molecular weight substances. Only precursors with two double bonds in favourable position for intramolecular addition of intermediate thiols can yield OSC. Double bond isomerisations by a sequence of H_2S addition and elimination reactions may play a role in this respect.

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* *Geochim. Cosmochim. Acta* (submitted)

10.2 INTRODUCTION

Recently, homologous series of 2,5-di-*n*-alkylthiolanes (I) have been unambigiously identified in immature oils and bitumens by synthesis of several members of these series (Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1986, 1987c). Detailed study of these compounds in the Maruejols (Alès Basin, France) and Rozel Point (Utah, U.S.A.) crude oils (Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1987c) showed that these compounds occur as complicated mixtures of all structural and stereoisomers. The mono 2-*n*-alkylthiolanes (I; m=0) were not present in the Maruejols crude oil (Schmid *et al.*, 1987) but they were minor compounds in the Rozel Point oil and major thiolanes in the Sicily Seep oils (Sinninghe Damsté *et al.*, 1988g). Both 2,5-di-*n*-alkylthiolanes as well as 2-*n*-alkylthiolanes have a linear carbon framework and thus yield *n*-alkanes upon desulphurisation.

The similarity between the *n*-alkanes of the immature Maruejols crude oil and those obtained by Raney nickel desulphurisation of its sulphides (which were comprised of mainly 2,5-di-*n*-alkylthiolanes) together with the "random" isomer composition of the 2,5-di-*n*-alkylthiolanes led Schmid *et al.* (1987) to suppose an origin for these compounds from a reaction between the *n*-alkanes and elemental sulphur during early maturation in the source rock. Sinninghe Damsté *et al.* (1988f) showed that the distribution patterns of the original *n*-alkanes present in immature crude oils and immature bitumens and those formed upon desulphurisation of their Organic Sulphur Compounds (OSC) are often different. This contradicts the hypothesis of Schmid *et al.* (1987) and an origin for these OSC by early diagenetic sulphur incorporation into functionalised lipids was proposed. However, the "random" isomer distribution of the 2,5-di-*n*-alkylthiolanes in the Maruejols and Rozel Point crude oils (Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1987c) is still puzzling in this respect.

In this paper the OSC present in the bitumen of the Jurf ed Darawish oil shale (Jordan) are described. The 2,5-di-*n*-alkylthiolanes present do not show a "random" isomer distribution and thereby provide more direct evidence for an origin from early diagenetic sulphur incorporation into functionalised precursors. Similar observations were made for other OSC classes and are also described in this paper.

10.3 EXPERIMENTAL

Sample. The Jurf ed Darawish Oil shale is a Cretaceous deposit (thickness *ca*. 100 m) of immature bitumenous calcareous marl stones which is located 130 km south of Amman (Wehner and Hufnagel, 1987). The sample investigated in this paper is a composite sample from 156-157 m taken from a core and is representative of the Lower Member of this oil shale. The content of organic matter is high (17.3 % determined on the solvent extracted rock sample). $CaCO_3$ (*ca*. 40%, Wehner and Hufnagel, 1987) and clay are the major inorganic constituents. Some information on the hydrocarbon biological markers (Wehner and Hufnagel, 1987) and OSC (Sinninghe Damsté *et al.*, 1988g) have been reported elsewhere. The kerogen present in this sample is marine derived (Type II) with a high sulphur content (Sinninghe Damsté *et al.*, 1988a).

Extraction. The sample was powdered in a rotary disc mill and soxhlett extracted with toluene/methanol (1:3, v/v) for 46 h. The bitumen was obtained by removing the solvent with a rotary evaporator at 30°C and weighed (1.45 g; 2.3%).

Fractionation. *Ca*. 800 mg of the bitumen of the sediment sample was fractionated (without prior removing of asphaltenes; asphaltene content was determined separately: 23%) into three fractions with a column (34 cm x 25 mm; column volume 110 ml) packed with alumina (activated for 2 h at 150°C) by elution with hexane/toluene (9:1, v/v; 500 ml; the "apolar fraction"), toluene (500 ml) and toluene/methanol (1/1, v/v; 350 ml) as eluents. An aliqout (*ca*. 10 mg) of the apolar fraction (70 mg; 9% of the total bitumen) was further separated by argentatious thin layer chromatography using hexane as developper. The $AgNO_3$-impregnated silica plate (20 x 20 cm; thickness 0.25 mm) was prepared by dipping in a solution of 1% $AgNO_3$ in $MeOH/H_2O$ (4:1, v/v) for 45 s and subsequent activation at 120°C for 1 h. Seven bands (Table 10.1) were detected by inspection under UV light after spraying with Rhodamine 6G. These bands were scrapped of the TLC plate and ultrasonically extracted with ethyl acetate (x3). These fractions were analysed by GC and GC-MS.

A so-called low-molecular weight aromatic (LMWA) fraction was also obtained from the bitumen as described elsewhere (Sinninghe Damsté *et al.*, 1987c). The LMWA fraction contained most of the GC-amenable compounds of the "aromatic" fraction including the OSC described previously (Sinninghe Damsté

Table 10.1. General composition of the sub-fractions obtained by argentatious TLC of the apolar fraction of Jurf ed Darawish Oil shale bitumen.

Fraction	R_f	major compound classes
1	0.84-0.91	saturated hydrocarbons
2	0.68-0.84	sterenes, hopenes, hopanones
3	0.50-0.68	alkylbenzenes, alkylated thiophenes, C-ring monoaromatic steroids
4	0.43-0.50	alkylated benzothiophenes, thiophene hopanoids, A- and B-ring monoaromatic steroids
5	0.22-0.43	-
6	0.08-0.22	methylated 2-methyl-2-(4,8,12-trimethyltridecyl)-chromans
7	0.00-0.08	alkylated thiolanes and thianes

et al., 1987c).

Gas chromatography. Gas chromatography (GC) was performed on a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) was used with helium as carrier gas. Detection was accomplished by both a flame ionization detector and a sulphur-selective flame photometric detector (FPD), using a stream splitter at the end of the capillary column. Samples were injected at 75°C. The oven was programmed from 75 to 130°C at 20°C/min and then at 4°C/min to 320°C.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) was carried out on a Hewlett-Packard 5840 gas chromatograph connected with a VG-70S mass spectrometer operated at 70 eV with a mass range *m/z* 40-800 and a cycle time of 1.8 s. The gas chromatograph was equipped with a fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.12 μm) and was operated with helium as carrier gas. LMWA fractions (1.0 μl) were on-column injected at 50 °C in ethyl acetate (*ca.* 6 mg/ml) and the temperature was programmed at 10°C/min to 100°C and then at 4°C/min to 315°C at which it was held for 20 min.

General procedures for the synthesis of authentic standards. 2-Hexadecyl-5-octylthiophene and 2-nonyl-5-pentadecylthiophene were prepared by a series of coupling and reduction reactions. 2-Octylthiophene, obtained by reaction of thiophene and octylbromide, was coupled with hexadecanoic acid and the obtained ketone was reduced to yield 2-hexadecyl-5-octylthiophene. 2-Nonyl-5-pentadecylthiophene was obtained by coupling of 2-nonylthiophene, prepared by coupling of thiophene with nonanoic acid and subsequent reduction, and pentadecanoic acid followed by reduction of the obtained ketone. The corresponding *cis* and *trans* thiolanes of 2-hexadecyl-5-octylthiophene and 2-nonyl-5-pentadecylthiophene were obtained by ionic hydrogenation (Parnes *et al.*, 1977). The mass spectra were in accordance with their structure and with literature data of related compounds (Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1986, 1987c).

The C_{19} "branched" alkylthiophenes were prepared as follows. Reaction of 3-methylthiophene and heptanoic acid afforded a mixture of 2-(1'-oxoheptyl)-3-methylthiophene and 2-(1'-oxoheptyl)-4-methylthiophene. This mixture was reduced and the alkylthiophenes obtained were coupled with heptanoic acid. The obtained mixture of ketones was subsequently reduced to yield 2,5-diheptyl-3-methylthiophene. The mass spectrum and ^{1}H-NMR spectrum (400 MHz, $CDCl_3$, δ(ppm): 0.88 (*t*, 6H (C-7', C-7'')), 1.2-1.4 (*m*, 16H (C-3' - C-6', C-3'' - C-6'')), 1.54-1.66 (*m*, 4H (C-2', C-2'')), 2.06 (*s*, 3H (C-2 CH_3)), 2.63 (*t*, 2H (C-1' or C-1'')), 2.68 (*t*, 2H (C-1'' or C-1')), 6.42 (*s*, 1H (C-3))) were in accordance with its structure. 2-Butyl-5-(2'-undecyl)thiophene was prepared by coupling of 2-butylthiophene, synthesised by a reaction of thiophene and butylbromide, with decanoic acid. The ketone thus obtained was reacted with methyllithium which yielded after dehydration of the resulting tertiary alcohol a mixture of isomeric 2-butyl-5-(2'-undecenyl)thiophenes. Ionic hydrogenation of this mixture aforded 2-butyl-5-(2'-undecyl)thiophene. The mass spectrum and ^{1}H-NMR spectrum (400 MHz, $CDCl_3$, δ(ppm): 0.87 (*t*, 3H (C-11'')), 0.93 (*t*, 3H (C-4')), 1.20-1.32 (*m*, 16H (C-3', C-4'' - C-10'')), 1.27 (*d*, 3H (C-1'')), 1.39 (*m*, 2H (C-3'')), 1.63 (*m*, 2H (C-2')), 2.75 (*t*, 2H (C-1')), 2.90 (*m*, 1H (C-2'')), 6.55 (*s*, 2H (C-2, C-3))) were in accordance with its structure.

Coupling of thiophene with alkylbromides. To a stirred solution of thiophene (*ca.* 4 g) in dry THF at -15°C was slowly added an equimolar amount of butyllithium. After 2 h an equimolar amount of TMEDA was slowly added. After completion a twofold excess (on a molar basis) of the alkylbromide in THF was added over *ca.* 30 min. After 1 h the the reaction mixture was refluxed for 2 h. After cooling the reaction mixture was transferred to a separatory funnel and 25 ml H_2O was added. The resulting mixture was extracted with diethylether (x2) and the combined layers were washed with 10 ml 2M HCl (x3) and 10 ml sat. NaCl solution (x2), dried with $MgSO_4$ and the solvent was evaporated with a rotatory evaporator. The obtained yellow-brown oil was purified by distillation (2-butylthiophene) or by column chromatography (2-octylthiophene)

Coupling of thiophenes with acids. The coupling reactions were performed by slowly adding 1.4 equivalent of oxalyl chloride at 20°C to a stirred solution of equimolar amounts of the thiophene and the acid in CH_2Cl_2 with a catalytic amount of DMF. After 30 min the solution was cooled to -10°C and 0.6 equivalent of $SnCl_4$ was added over *ca.* 10 min. After 2.5 h stirring at 0°C 10 ml H_2O was added, the CH_2Cl_2-layer was washed with sat. $NaHCO_3$ solution and almost evaporated to dryness. *Ca.* 10 ml diethyl ether was added and the solution was stirred with 10 ml 4M KOH for 15 min. The diethyl ether-layer was subsequently washed with H_2O (x2) and sat. NaCl solution (x2), dried with $MgSO_4$ and evaporated to dryness.

Reduction of 2-(alkyl-1'-oxo)thiophenes. The ketone was dissolved in dry ether and 1.8 molar equivalent of $AlCl_3$ in dry ether was slowly added at -10°C. After 30 min the formed complex was reduced with 1.2 molar equivalent $LiAlH_4$. After addition of a few ml 2 M HCl and stirring for 30 min the water-layer was removed and the ether-layer washed with H_2O, 5% NaCl in H_2O and a sat. NaCl in H_2O, dried with $MgSO_4$ and evaporated to dryness.

Ionic hydrogenation of dialkylthiophenes. The 2,5-di-*n*-alkylthiophenes were hydrogenated as reported elsewhere (Sinninghe Damsté *et al.*, 1987c).

10.4 RESULTS

The Organic Sulphur Compounds (OSC) present in the Jurf ed Darawish Oil shale were studied by GC-MS of the Low Molecular Weight Aromatic (LMWA) fraction of the bitumen and of sub-fractions obtained by argentatious thin layer chromatography of an apolar fraction of the bitumen. The LMWA fraction is a sub-fraction of the "aromatic" fraction of the bitumen and contains all the OSC discussed hereafter. Argentatious thin layer chromatography was used to separate certain OSC classes from each other (*e.g.* thiolanes from thiophenes). The general composition of these sub-fractions is given in Table 10.1. Coinjections with authentic standards were also performed with these sub-fractions. A general description of the compounds present in the LMWA fraction of this sample has been given elsewhere (Sinninghe Damsté *et al.*, 1988g). The overall composition of the OSC present in the LMWA fraction of the Jurf ed Darawish Oil shale is illustrated with a chromatogram of the desulphurised LMWA fraction (Fig. 10.1; Sinninghe Damsté *et al.*, 1988f). It shows that the carbon frameworks of the OSC are predominantly linear and isoprenoid, although some branched and triterpenoid hydrocarbons were formed too. Interesting phenomena are the strong even-over-odd carbon number predominance of the *n*-alkanes and the relative abundance of octacosane, phytane, 9-methyloctadecane, 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane and related alkenes, which contrasts with the distribution of the original hydrocarbons. In the following a number of examples of OSC classes which show "non-random" isomer distributions will be discussed.

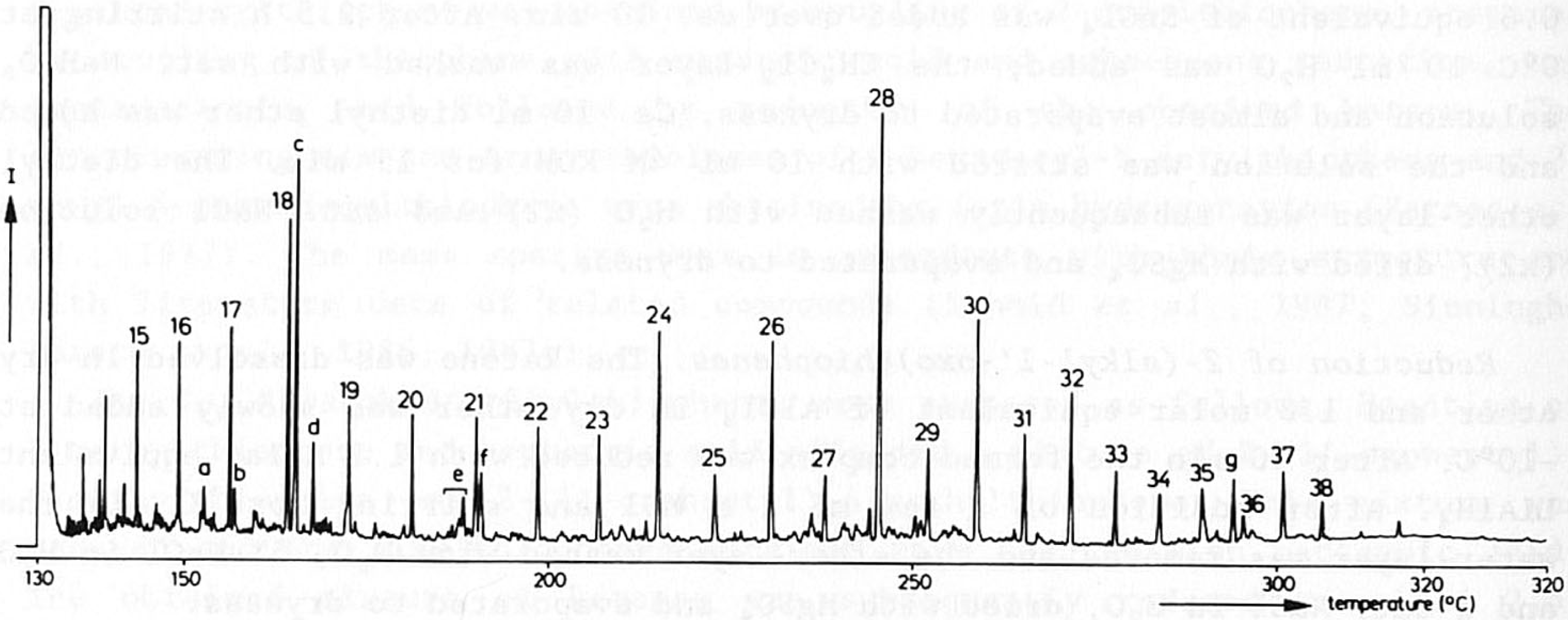


Fig. 10.1. Gas chromatogram of the desulphurised LMWA fraction of the JED bitumen. Arabic numbers indicate number of carbon atoms of *n*-alkanes. Key: a=norpristane, b=pristane, c=phytane, d=9-methyloctadecane, e=monounsaturated C_{25} highly branched isoprenoids, f=2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane, g=17α(H),21β(H)-pentakishomohopane.

C_{28} OSC with linear carbon frameworks

As described above these compounds are relatively abundant compounds in the LMWA fraction. The isomer composition of C_{28} 2,5-di-*n*-alkylthiophenes (DATP; II), 2,5-di-*n*-alkylthiolanes (DATL) and 2,6-di-*n*-alkylthianes (DATN; III) is however quite different from those with other total carbon numbers. Fig. 10.2 shows the the partial mass chromatograms of the molecular ions of the C_{24}, C_{26}, C_{28} and C_{30} DATP of sub-fraction 3 of the Jurf ed Darawish Oil shale bitumen. The isomer clusters are built up as follows. All mid-chain DATP (II, m>n, n>4) coelute, whilst the 2-alkyl-5-butylthiophenes and 2-alkyl-5-propylthiophenes are just separated from the mid-chain DATP in the C_{24} cluster but coelute with these DATP in the C_{30} cluster. The 2-alkyl-5-ethylthiophenes, 2-alkyl-5-methylthiophenes and 2-alkylthiophenes are separated from each other and the other DATP. Fig. 10.2 shows that the relative abundance of the various isomers present in these clusters differ to a great extent. The C_{28} cluster is highly dominated by the mid-chain DATP. An averaged mass spectrum of this peak (Fig. 10.3A) shows, however, that although the C_{28} mid-chain thiophenes comprise eight theoretically possible isomers (II, n=4+x; 0<x<10) two isomers (II, m=8 and n=16, m=9 and n=15) dominate. Evidence for this observation was obtained by coinjection (CP Sil-5) of authentic standards and comparison of mass spectral data. The mass spectra of the synthetic DATP (Figs. 10.3B-3C) show abundant fragment ions generated by β-cleavage and a secondary ion at *m/z* 111 caused by loss of both alkyl side-chains. This explains the abundance of the ions at *m/z* 111 and 420 in the mass spectrum of the C_{28} mid-chain thiophenes in the Jurf ed Darawish Oil shale bitumen; these ions are generated by all mid-chain

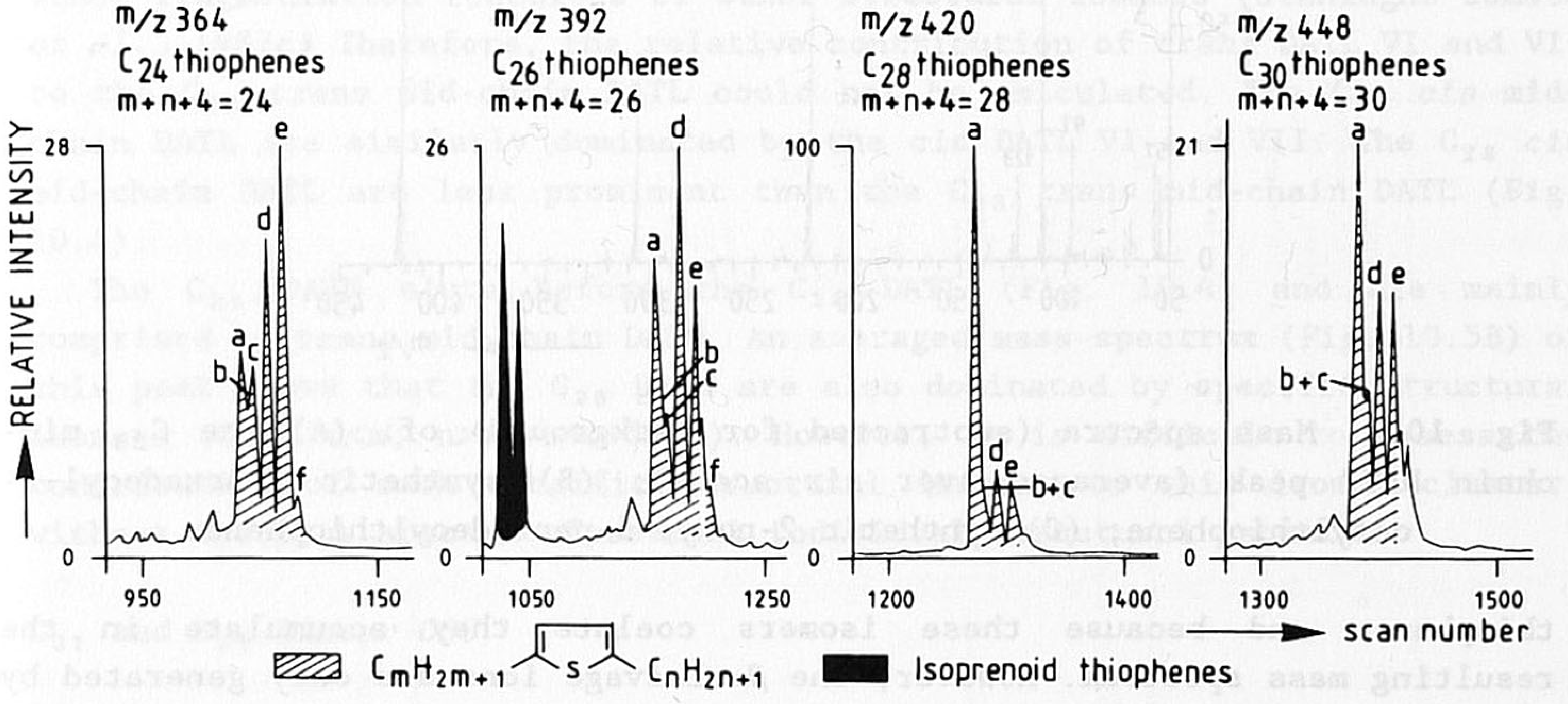


Fig. 10.2. Partial mass chromatograms of *m/z* 364, 392, 420 and 448 of sub-fraction 3 of the apolar fraction of the JED bitumen. Key: a=mid-chain thiophenes (m>n, n>4); b: n=4; c: n=3; d: n=2; e: n=1; f: n=0.

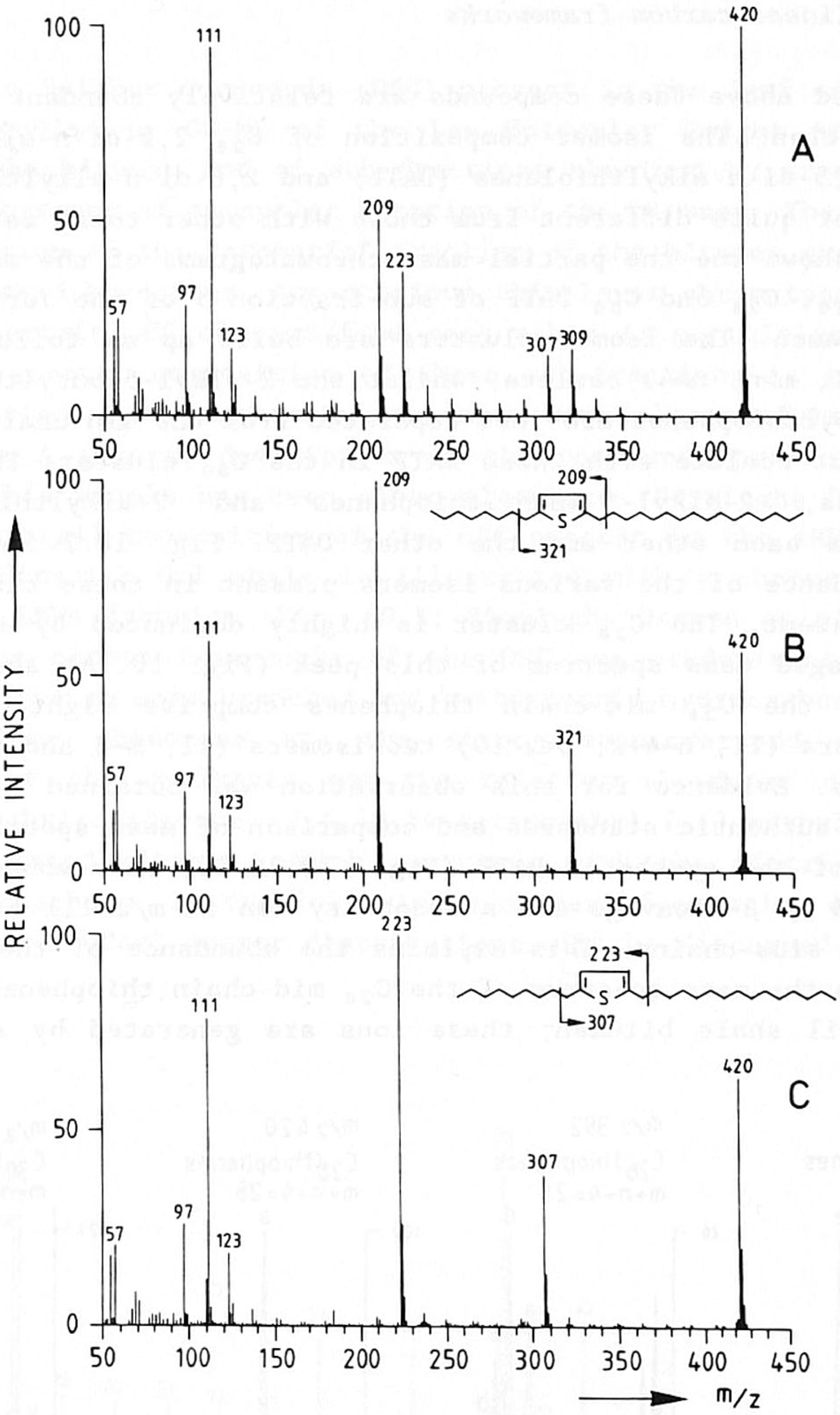


Fig. 10.3. Mass spectra (subtracted for background) of: (A) the C_{28} mid-chain DATP peak (averaged over six scans); (B) synthetic 2-hexadecyl-5-octylthiophene; (C) synthetic 2-nonyl-5-pentadecylthiophene.

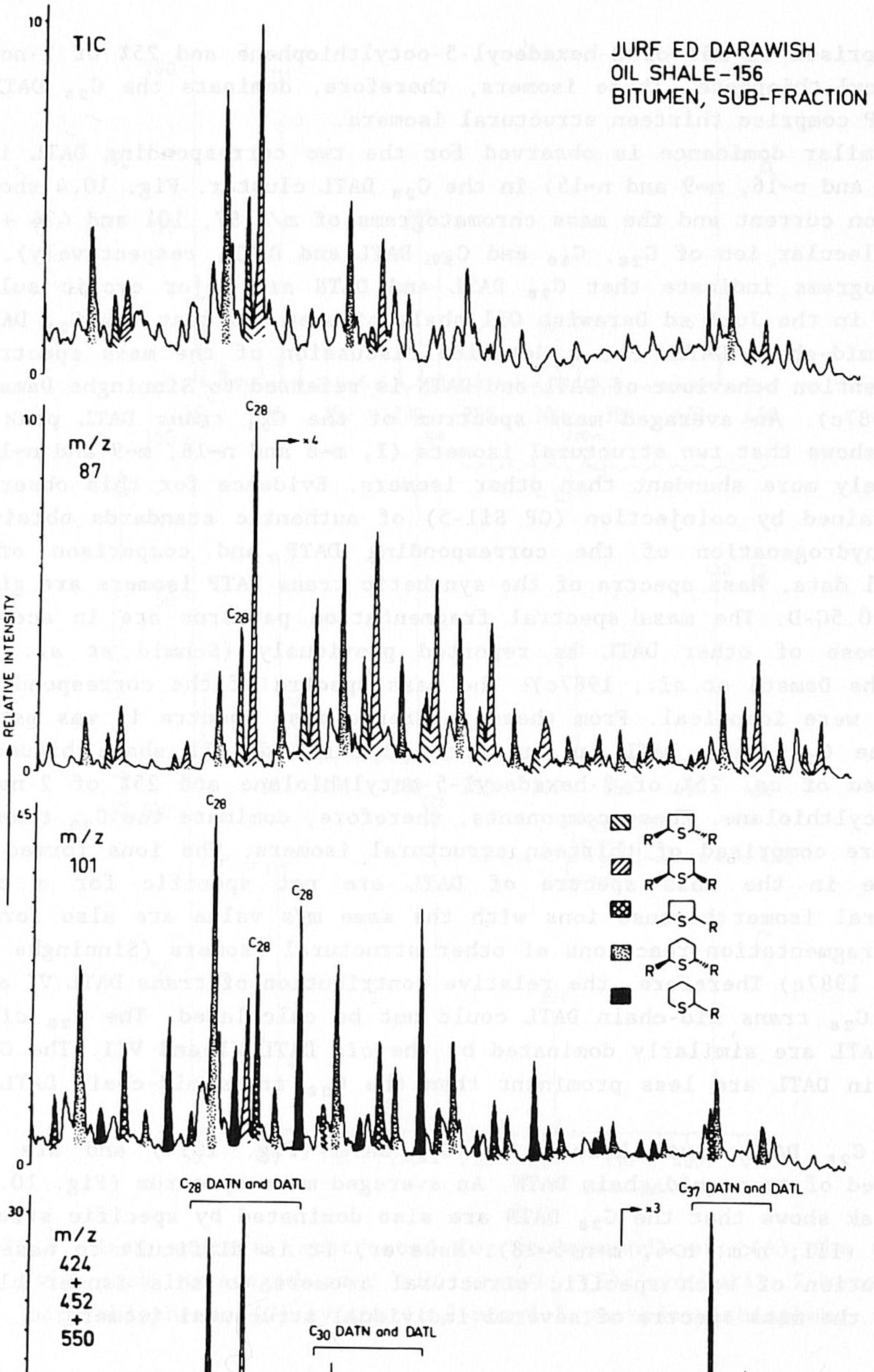
thiophenes and because these isomers coelute they accumulate in the resulting mass spectrum. However, the β-cleavage ions are only generated by one structural isomer and are therefore not very prominent in the resulting mass spectrum. From the intensity of the ions at *m/z* 111, 209, 223, 307, 321 and 420 and the mass spectra of the synthetic standards it was calculated that the C_{28} mid-chain thiophenes of the Jurf ed Darawish Oil shale bitumen are comprised of 25% of 2-hexadecyl-5-octylthiophene and 25% of 2-nonyl-5-pentadecyl-thiophene. These isomers, therefore, dominate the C_{28} DATP. The C_{28} DATP comprise thirteen structural isomers.

A similar dominance is observed for the two corresponding DATL isomers (I, m=8 and n=16, m=9 and n=15) in the C_{28} DATL cluster. Fig. 10.4 shows the total ion current and the mass chromatograms of *m/z* 87, 101 and 424 + 452 + 550 (molecular ion of C_{28}, C_{30} and C_{37} DATL and DATN, respectively). These chromatograms indicate that C_{28} DATL and DATN are major cyclic sulphides present in the Jurf ed Darawish Oil shale bitumen and that the C_{28} DATL are mainly mid-chain DATL. For a detailed discussion of the mass spectrometry and retention behaviour of DATL and DATN is referred to Sinninghe Damsté *et al.* (1987c). An averaged mass spectrum of the C_{28} *trans* DATL peak (Fig. 10.5A) shows that two structural isomers (I, m=8 and n=16, m=9 and n=15) are relatively more abundant than other isomers. Evidence for this observation was obtained by coinjection (CP Sil-5) of authentic standards obtained by ionic hydrogenation of the corresponding DATP and comparison of mass spectral data. Mass spectra of the synthetic *trans* DATP isomers are given in Figs. 10.5C-D. The mass spectral fragmentation patterns are in accordance with those of other DATL as reported previously (Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1987c). The mass spectra of the corresponding *cis* isomers were identical. From these reference mass spectra it was estimated that the C_{28} *trans* DATL in the Jurf ed Darawish Oil shale bitumen are comprised of *ca.* 25% of 2-hexadecyl-5-octylthiolane and 25% of 2-nonyl-5-pentadecylthiolane. These components, therefore, dominate the C_{28} *trans* DATL which are comprised of thirteen structural isomers. The ions formed by α-cleavage in the mass spectra of DATL are not specific for a certain structural isomer because ions with the same *m/z* value are also formed by other fragmentation reactions of other structural isomers (Sinninghe Damsté *et al.*, 1987c) Therefore, the relative contribution of *trans* DATL VI and VII to the C_{28} *trans* mid-chain DATL could not be calculated. The C_{28} *cis* mid-chain DATL are similarly dominated by the *cis* DATL VI and VII. The C_{28} *cis* mid-chain DATL are less prominent than the C_{28} *trans* mid-chain DATL (Fig. 10.4).

The C_{28} DATN elute before the C_{28} DATL (Fig. 10.4) and are mainly comprised of *trans* mid-chain DATN. An averaged mass spectrum (Fig. 10.5B) of this peak shows that the C_{28} DATN are also dominated by specific structural isomers (III; n<m; n>4, m+n+5=28). However, it is difficult to assess the contribution of each specific structural isomers to this isomer cluster, without the mass spectra of several individual structural isomers.

C_{37} and C_{38} linear OSC

Upon desulphurisation of the LMWA fraction heptatricosane and octatricosane were formed in relatively large amounts compared with the other *n*-alkanes in the C_{35}-C_{40} range (Fig. 10.1). The dominance of heptatricosane is in contrast with the observed even over odd carbon number predominance of


TIC
JURF ED DARAWISH
OIL SHALE-156
BITUMEN, SUB-FRACTION
10
0
m/z
87
C28
x4
C28
RELATIVE INTENSITY
45
m/z
101
C28
C28
C28
R S R
R S R
S R
R S R
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C28 DATN and DATL
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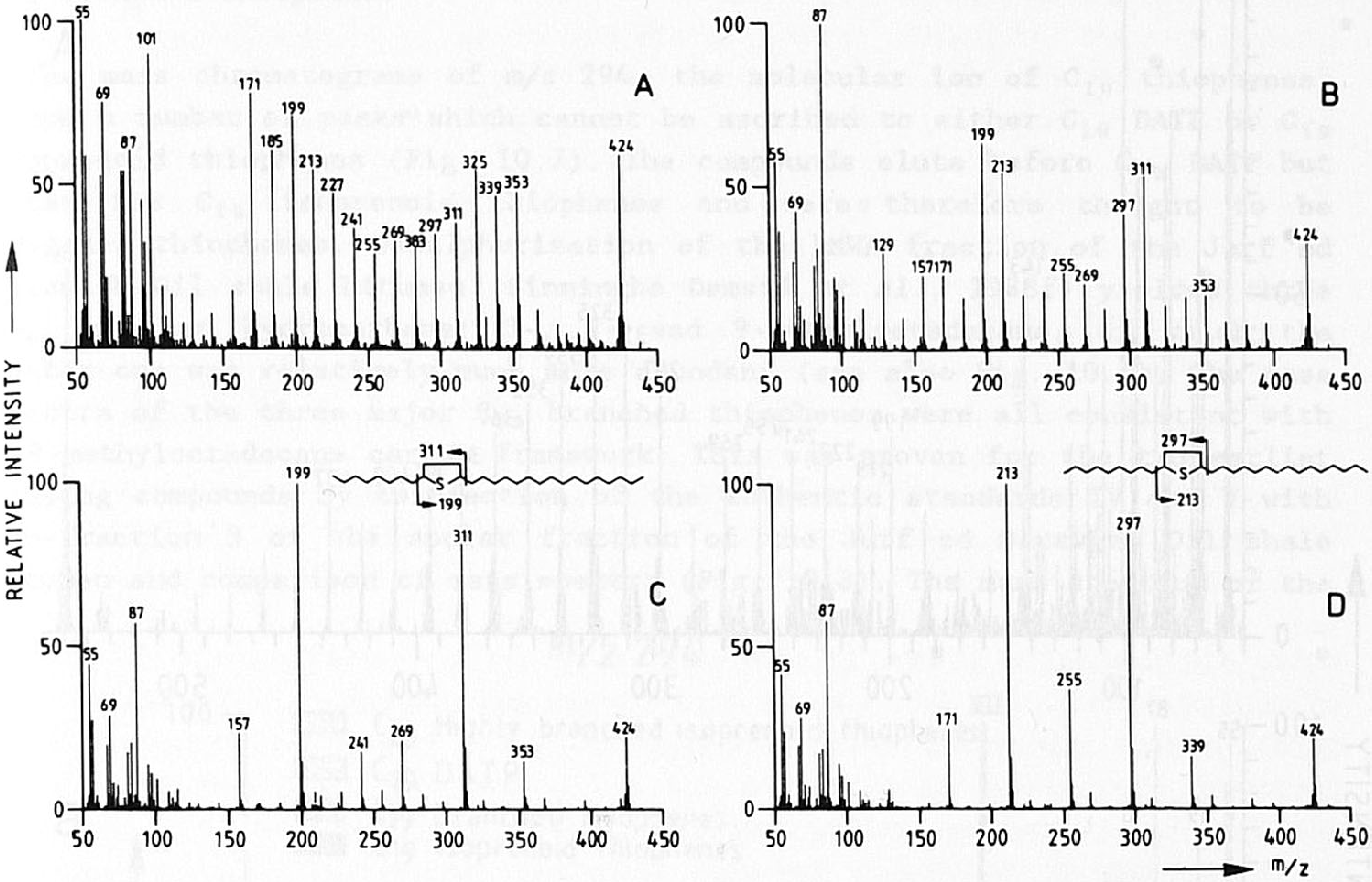


Fig. 10.5. Mass spectra (subtracted for background) of: (A) the C_{28} mid-chain DATN peak (averaged over five scans); (B) the C_{28} mid-chain DATL peak (averaged over five scans); (C) synthetic 2-hexadecyl-5-octylthiolane; (C) synthetic 2-nonyl-5-pentadecylthiolane.

the *n*-alkanes formed upon desulphurisation of the OSC.

Analysis of the OSC in the Jurf ed Darawish Oil shale bitumen indicated that especially C_{37} and C_{38} cyclic sulphides were relatively abundant (Fig. 10.4). The C_{37} cyclic sulphides are dominated by *trans* mid-chain DATN and, less abundant, *trans* mid-chain DATL. The relative concentrations of the *cis* mid-chain DATN and DATL are lower than of the corresponding *trans* compounds (Fig. 10.4). The averaged mass spectra of the *trans* mid-chain DATN and DATL peaks (Figs. 10.6A-B) indicate that they consist mainly of isomers with alkyl side chains with more than seven carbon atoms (I, $n<m$, $n>8$, $m+n+4=37$; III, $n<m$, $n>6$, ($m+n+5=37$)). This contrasts with other DATN and DATL isomer clusters because these also contain other mid-chain cyclic sulphides.

The C_{37} and C_{38} DATP were similarly dominated by mid-chain DATP with alkyl

Fig. 10.4. Partial total ion current (TIC) and partial mass chromatograms of *m/z* 87, 101, and 424+452+550 of sub-fraction 7 of the apolar fraction of the JED bitumen.

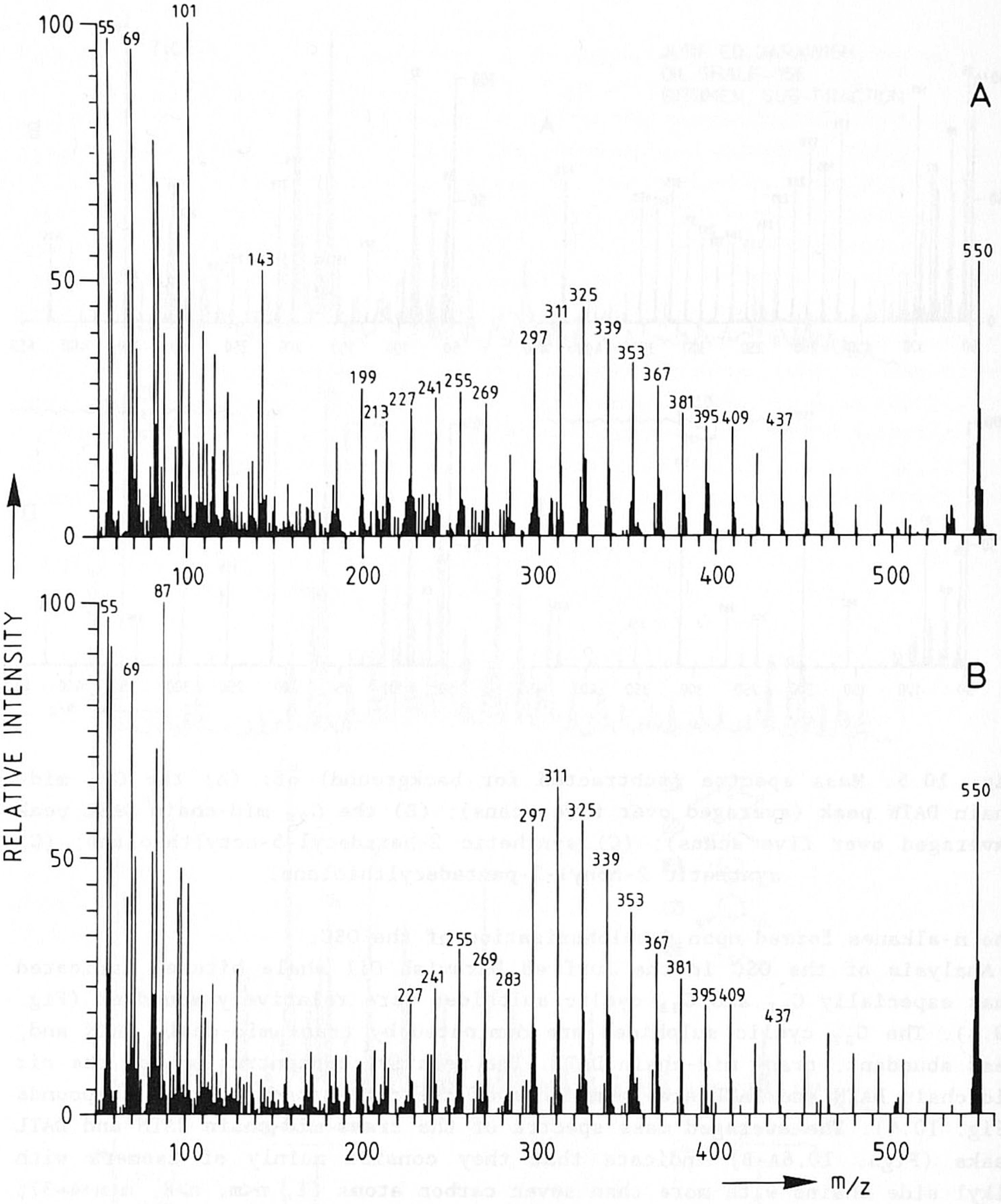


Fig. 10.6. Mass spectra (subtracted for background) of: (A) the C_{37} mid-chain DATN peak (averaged over five scans); (B) the C_{37} mid-chain DATL peak (averaged over five scans).

side-chains with more than six carbon atoms (II, n>6, n<m) but the amount of C_{37} and C_{38} DATP relative to the total DATP is less than the amount of the corresponding thiolanes and thianes to the total DATL and DATN.

C_{19} branched thiophenes

The mass chromatograms of *m/z* 294, the molecular ion of C_{19} thiophenes, shows a number of peaks which cannot be ascribed to either C_{19} DATP or C_{19} isoprenoid thiophenes (Fig. 10.7). The compounds elute before C_{19} DATP but after the C_{19} isoprenoid thiophenes and were therefore thought to be branched thiophenes. Desulphurisation of the LMWA fraction of the Jurf ed Darawish Oil shale bitumen (Sinninghe Damsté *et al.*, 1988f) yielded three C_{19} branched hydrocarbons; 3-, 2- and 9-methyloctadecane, of which the latter one was relatively much more abundant (see also Fig. 10.1). The mass spectra of the three major C_{19} branched thiophenes were all consistent with a 9-methyloctadecane carbon framework. This was proven for the two earlier eluting compounds by coinjection of the authentic standards IV and V with sub-fraction 3 of the apolar fraction of the Jurf ed Darawish Oil shale bitumen and comparison of mass spectra (Fig. 10.8). The mass spectrum of the

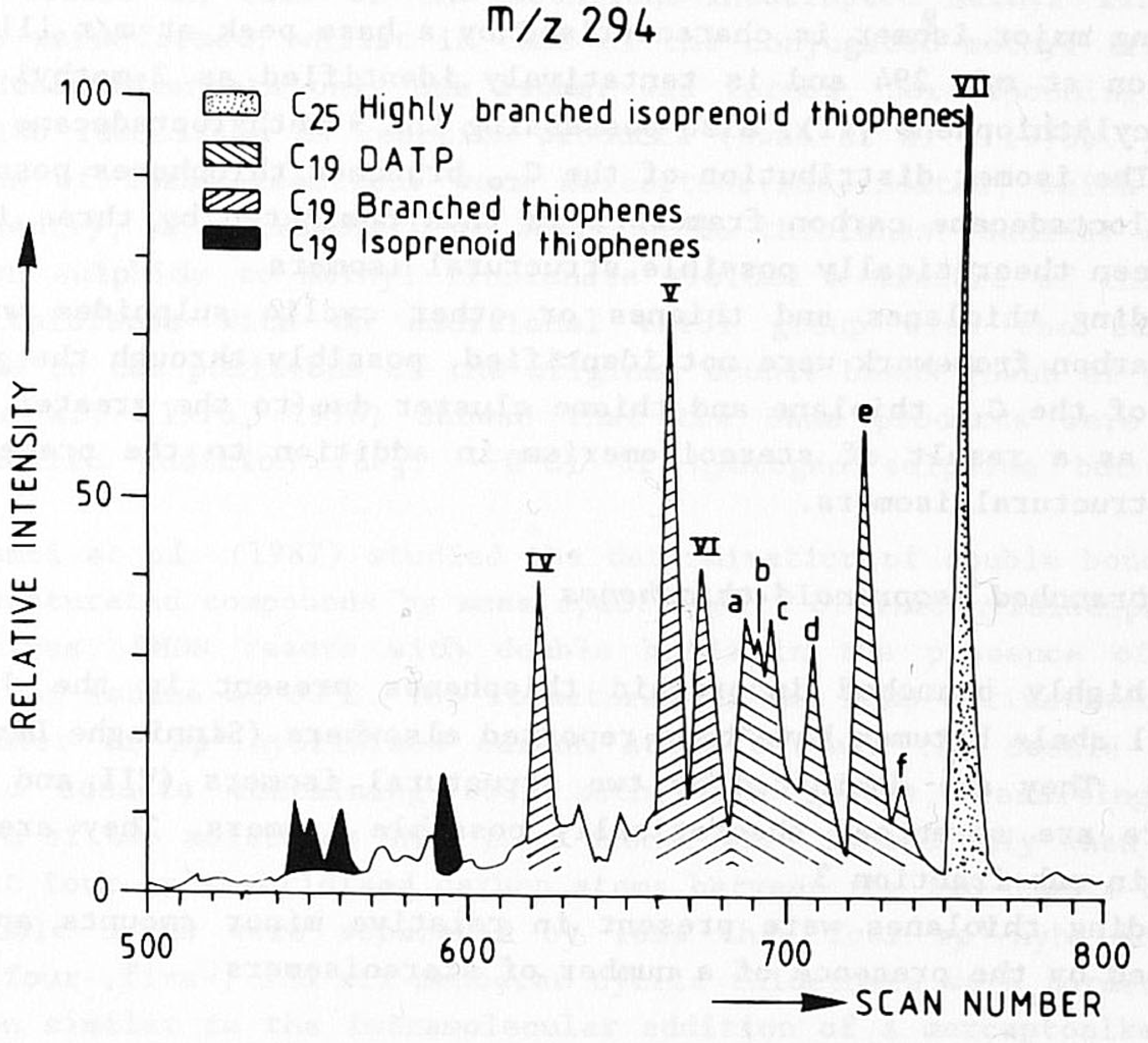


Fig. 10.7. Partial mass chromatograms of *m/z* 294 of sub-fraction 3 of the apolar fraction of the JED bitumen. Key: IV, V and VI are C_{19} branched thiophenes; a = mid-chain DATP; b = 2-butylthiophene, c = 2-dodecyl-5-propylthiophene, d = 2-ethyl-5-tridecylthiophene, e = 2-methyl-5-tetradecylthiophene, f = 2-pentadecylthiophene. C_{25} highly branched isoprenoid thiophene VII gives a signal in this mass chromatogram since *m/z* 293 is the base peak in its mass spectrum.

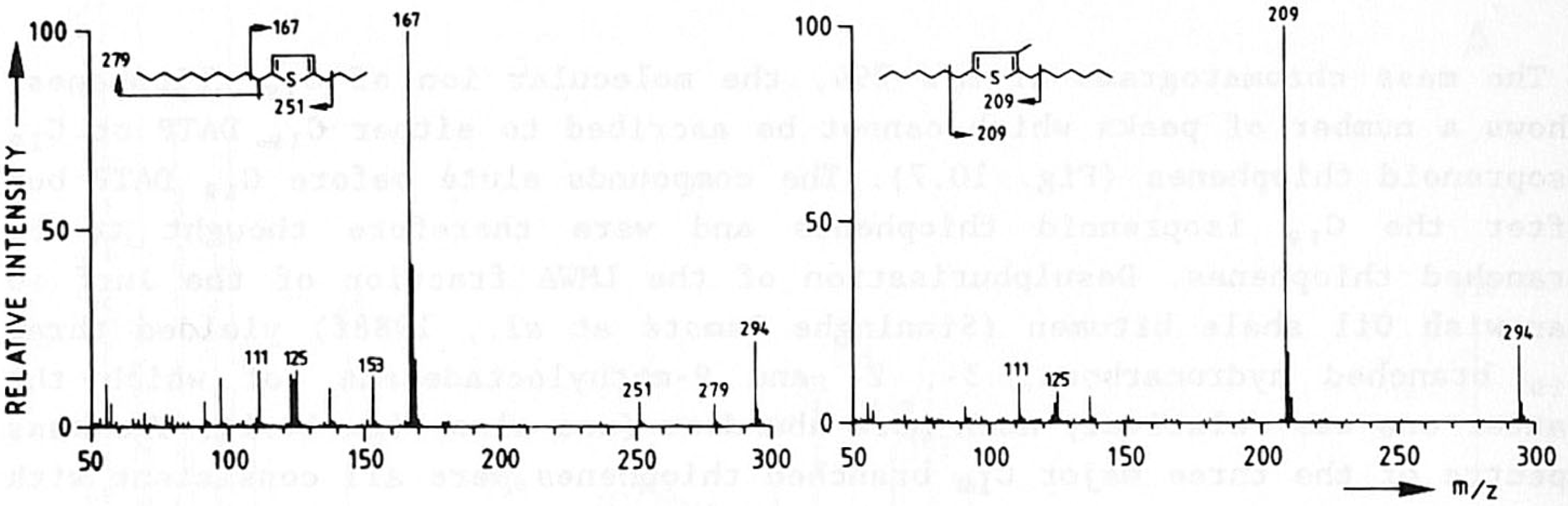


Fig. 10.8. Mass spectra (subtracted for background) of authentic C_{19} branched thiophenes possessing the 9-methyloctadecane carbon skeleton: (A) 2,5-diheptyl-3-methylthiophene; (B) 2-butyl-5-(2'-undecyl)thiophene.

third eluting major isomer is characterised by a base peak at *m/z* 111 and a molecular ion at *m/z* 294 and is tentatively identified as 2-methyl-5-(4'-methyltridecyl)thiophene (VI), also possessing the 9-methyloctadecane carbon framework. The isomer distribution of the C_{19} branched thiophenes possessing the 9-methyloctadecane carbon framework is thus dominated by three isomers of the fifteen theoretically possible structural isomers.

Corresponding thiolanes and thianes or other cyclic sulphides with an identical carbon framework were not identified, possibly through the greater complexity of the C_{19} thiolane and thiane cluster due to the greater number of isomers as a result of stereoisomerism in addition to the presence of different structural isomers.

C_{25} *highly branched isoprenoid thiophenes*

The C_{25} highly branched isoprenoid thiophenes present in the Jurf ed Darawish Oil shale bitumen have been reported elsewhere (Sinninghe Damsté *et al.*, 1988d). They are dominated by two structural isomers (VII and VIII), whilst there are seventeen theoretically possible isomers. They are major thiophenes in sub-fraction 3.

Corresponding thiolanes were present in relative minor amounts and were characterised by the presence of a number of stereoisomers.

10.5 DISCUSSION

In contrast with other samples (*e.g.* Rozel Point and Maruejols crude oils, Sinninghe Damsté *et al.*, 1987c; Schmid *et al.*, 1987) the DATN and DATL present in the Jurf ed Darawish Oil shale bitumen are not completely "randomised", *i.e.* all theoretically possible isomers are not present in approximately equal amounts. Similar observations were made for the DATP,

C_{19} branched thiophenes and C_{25} highly branched isoprenoid thiophenes present in the Jurf ed Darawish Oil shale bitumen. This provides more direct evidence for the formation of these OSC by early diagenetic sulphur incorporation into functionalised precursors as proposed previously (Valisolalao *et al.*, 1984; Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987c, 1988f; Sinninghe Damsté and de Leeuw, 1987). The dominance of certain structural isomers cannot be explained by a reaction of elemental sulphur with hydrocarbons. Sulphur incorporation at specific sites of the precursors triggered by the positions of their functionalities is a more likely origin for these OSC.

Recently, Vairavamurthy and Mopper (1987) have shown that addition of hydrogen sulphide to double bonds occurs in recent sediments. Swab *et al.* (1973, 1978) have shown that photochemical addition of hydrogen sulphide to methyl linoleate and methyl *trans*, *trans*-9,11-octadecadienoate yields thiolanes with the sulphur atom attached to the positions of the original double bonds. In case of the methylene-interrupted methyl linoleate two isomers were formed, whilst in case of the conjugated methyl *trans*, *trans*-9,11-octadecadienoate only one isomer was formed. Corresponding thiophenes were also identified as reaction products (Swab *et al.*, 1978). The initial products of these reactions were mercaptooctadecenoates, which underwent, subsequently, an intramolecular addition to thiolanes. Radical addition of hydrogen sulphide to methyl linolenate yielded a mixture of thiolanes and dialkylthiolanes with an additional thiol group with the sulphur atom attached to the positions of the original double bonds (Swab *et al.*, 1976). Swab *et al.* (1975, 1976) showed that the same products were formed by nucleophilic addition (BF_3, -70°C) of hydrogen sulphide but in higher yields.

Vincenti *et al.* (1987) studied the determination of double bond positions in diunsaturated compounds by mass spectrometry of dimethyldisulphide (DMDS) derivatives. DMDS reacts with double bonds in the presence of catalytic amounts of iodine at 60°C. The structures of the DMDS derivatives depend on the number of sp^3-hybridised carbon atoms between the double bonds. The expected adducts containing four methylthio groups, resulting from the addition of two molecules DMDS to a diene, were formed only when there were at least four sp^3-hybridised carbon atoms between the two double bonds. When the double bonds were separated by less than four sp^3-hybridised carbon atoms, four-,five-, and six-membered cyclic thioethers were formed through a reaction similar to the intramolecular addition of a mercaptoalkenoic acids described above.

Although, the reaction conditions, used in the above described experiments, are not comparable with those in sediments, these studies show that similar reactions are not unlikely to occur in nature and may trap labile functionalised lipids, thus generating geologically more stable compounds. In the following section the various compound classes, exhibiting non-random isomer distributions, will be discussed regarding their possible precursors and way of formation.

C_{28} linear OSC

The dominance of 2-hexadecyl-5-octylthiolane and 2-nonyl-5-pentadecylthiolane and the corresponding DATP suggest that a major precursor for C_{28} DATL and DATP was octacosa-9,12-diene or lipids with the same carbon skeleton (including the two double bonds) but also possessing a functional group (acids, alcohols, aldehydes). The double bond position in these precursors is favourable for the formation of the two C_{28} DATL isomers (Fig. 10.9).

Octacosa-9,12-diene has not been reported in either organisms or sediments but since long-chain polyunsaturated *n*-alkenes have been reported in algae (*e.g.* C21:6; Blumer *et al.*, 1970; Lee and Loeblich, 1971) and sediments (*e.g.* C29:2, C34:3, C34:4, C34:5; Volkman *et al.*, 1986) it seems not unreasonable to propose such a precursor. $\Delta^{9,12}$ octacosadienoic acid is also a possible precursor since the positions of the double bonds are identical to those in the widespread occurring C_{18} diunsaturated acid, linoleic acid. However, the C_{28} acid has, to our knowledge, also not been reported to occur in nature. Monounsaturated C_{28} fatty acids have been reported in the freshwater alga *Chlorella kessleri* (Δ^{10}; Rezanka and Podojil, 1986), in *Mycobacterium phlei* (Δ^{9}, Δ^{17}, Δ^{19}; Cervilla and Puzzo, 1983), in *Mycobacterium tuberculosis* (Δ^{9}; Takayama *et al.*, 1978), in freshwater sponges (Dembitskii, 1981a and b) and in a recent sediment from the Gavish Sabkha (de Leeuw *et al.*, 1985), whilst C_{28} diunsaturated fatty acids have been found in both freshwater (Dembitskii, 1981b) and marine sponges (Morales and Lichtfield, 1976). C_{28} triunsaturated fatty acids are sometimes major fatty acids in marine sponges ($\Delta^{5,9,19}$; Lichtfield and Mercantinio, 1978; Dembitskii and Nebylitsyn, 1980; $\Delta^{5,9,21}$ and $\Delta^{5,9,23}$; Walkup *et al.*, 1981) but the double bonds are not in the right positions to serve as precursors for the identified C_{28} DATP and DATL in the JED bitumen.

A consequence of assuming precursors containing a functional group is that this functionality has to be reduced to a methyl or methylene group. Such

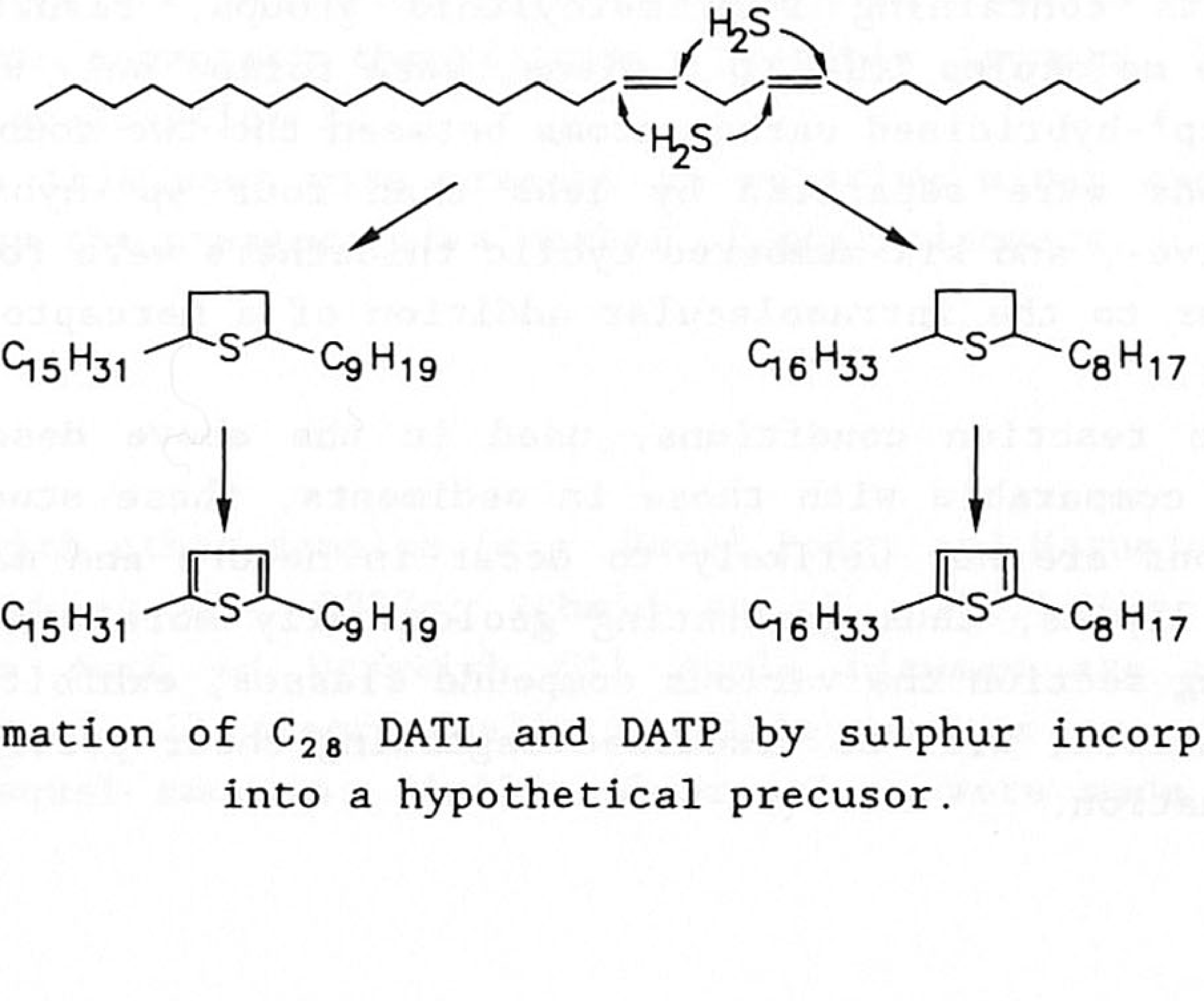


Fig. 10.9. Formation of C_{28} DATL and DATP by sulphur incorporation of H_2S into a hypothetical precusor.

reductions have been proposed to occur in nature (Sinninghe Damsté *et al.*, 1987c and references cited therein) and may explain the strong even carbon number distributions of DATL, DATN and DATP if fatty acids or alcohols are assumed as precursors.

The C_{28} DATN present seem to have other precursors than those of the dominating C_{28} DATP and DATL isomers because they are less dominated by specific structural isomers and the C_{28} DATN expected to be formed from the proprosed precursors of the C_{28} DATL and DATP, 2-octyl-6-pentadecylthiane, is not dominant at all.

C_{37} and C_{38} linear OSC

The C_{37} and C_{38} linear OSC are likely to be derived from sulphur incorporation into C_{37} and C_{38} di- and triunsaturated methyl and ethyl ketones or their corresponding alkadienes and alkatrienes present in sediments (Boon *et al.*, 1978; de Leeuw *et al.*, 1980; Volkman *et al.*, 1980b; Marlowe *et al.*, 1984; Farrimond *et al.*, 1986; Brassell *et al.*, 1986a and b) and in the coccolithophorid *Emilania huxleyi* (Volkman *et al.*, 1980a and b). In the first place this explains the carbon number distributions of the DATN, DATL and DATP in the C_{35}-C_{40} range. Secondly, the isomer composition of the C_{37} DATN, DATL and DATP is also consistent with such precursors.

The unsaturated C_{37} methylketones have double bond positions as indicated in Fig. 10.10. Addition of hydrogen sulphide to the double bonds would result in the formation of thiols. Intramolecular addition to form thianes or thiolanes is difficult because the other double bond positions are not in favourable positions. Therefore, these thiols are more likely to react intermolecularly generating sulphur-containing high molecular weight

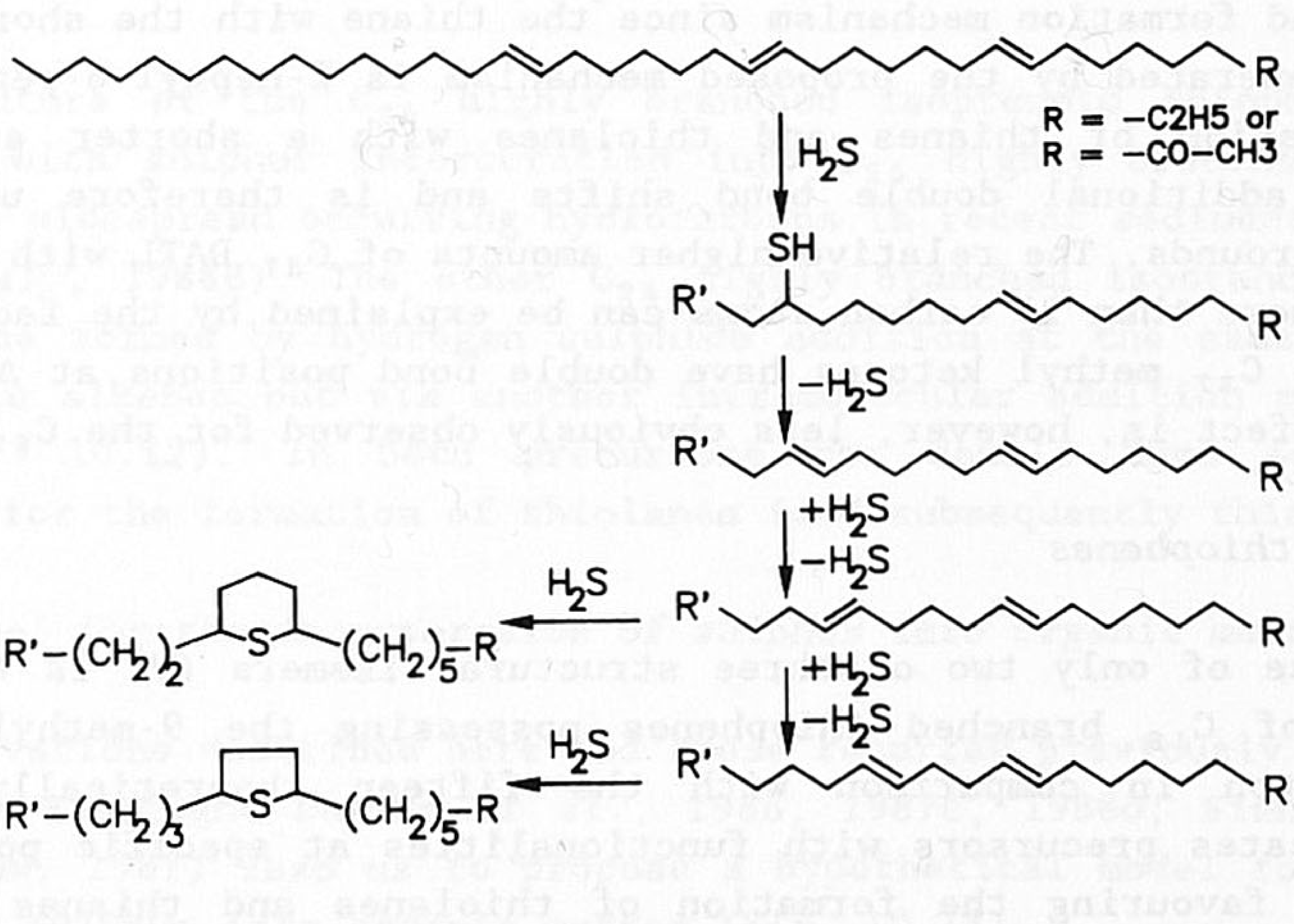


Fig. 10.10. Examples of the formation of two C_{37} DATL and DATN isomers by sulphur incorporation into a triunsaturated C_{37} methylketone or hydrocarbon.

substances. Indeed, higher molecular weight fractions of the Jurf ed Darawish Oil shale bitumen yielded upon desulphurisation significantly higher amounts of heptatricosane and octatricosane relative to the other *n*-alkanes generated (Sinninghe Damsté *et al.*, 1988f). Nevertheless, intramolecular additions of the C_{37} and C_{38} thiols also seem to occur, possibly after isomerisations of the double bonds.

However, recently it has been shown by de Leeuw *et al.* (1988) that double bond isomerisations during early diagenesis occur only *via* tertiary carbocations and not *via* secondary carbocations. This concept of limited isomerisation has been proposed for the early diagenetic pathways of steroids but also seems to be applicable to C_{37} and C_{38} unsaturated ketones. Farrimond *et al.* (1986) reported long-chain alkenones (C_{37}-C_{42}) with double bonds in the original positions in two Cretaceous black shales. In depositional environments in which free H_2S is present, however, an alternative mechanism for the isomerisation of double bonds may be operative. After addition of H_2S to a double bond, H_2S may subsequently be released *via* an elimination reaction. This can cause a shift of the double bond position (isomerisation). By this mechanism the double bonds of the unsaturated ketones may come into a favourable position for intramolecular addition (Fig. 10.10) leading to the formation of thianes and thiolanes. The fact that the C_{37} and C_{38} cyclic sulphides are dominated by DATN seems to be consistent with this explanation; they can be formed by intramolecular addition of a thiol moiety separated from a double bond by three sp^3-hybridised carbon atoms (in stead of two in case of the formation of thiolanes). An elimination reaction of thianes and thiolanes as proposed for the intermediate thiols is also possible but is less likely because HS^- is a better leaving group than RS^-.

The dominance of the C_{37} mid-chain DATN and DATP with alkyl side-chains with at least seven carbon atoms is also consistent with the proposed precursors and formation mechanism since the thiane with the shortest alkyl side-chain generated by the proposed mechanism is 2-heptyl-6-pentacosanylthiane. Formation of thianes and thiolanes with a shorter alkyl would require two additional double bond shifts and is therefore unlikely on statistical grounds. The relative higher amounts of C_{37} DATL with alkyl side chains with more than 14 carbon atoms can be explained by the fact that the diunsaturated C_{37} methyl ketones have double bond positions at Δ^{15} and Δ^{22} only. This effect is, however, less obviously observed for the C_{37} DATN.

C_{19} branched thiophenes

The presence of only two or three structural isomers (VI is tentatively identified) of C_{19} branched thiophenes possessing the 9-methyloctadecane carbon skeleton in comparison with the fifteen theoretically possible isomers indicates precursors with functionalities at specific positions of the molecule favouring the formation of thiolanes and thianes (and subsequently thiophenes)(Fig. 10.11). However, such compounds have not been found in nature, although some related compounds have been reported. A

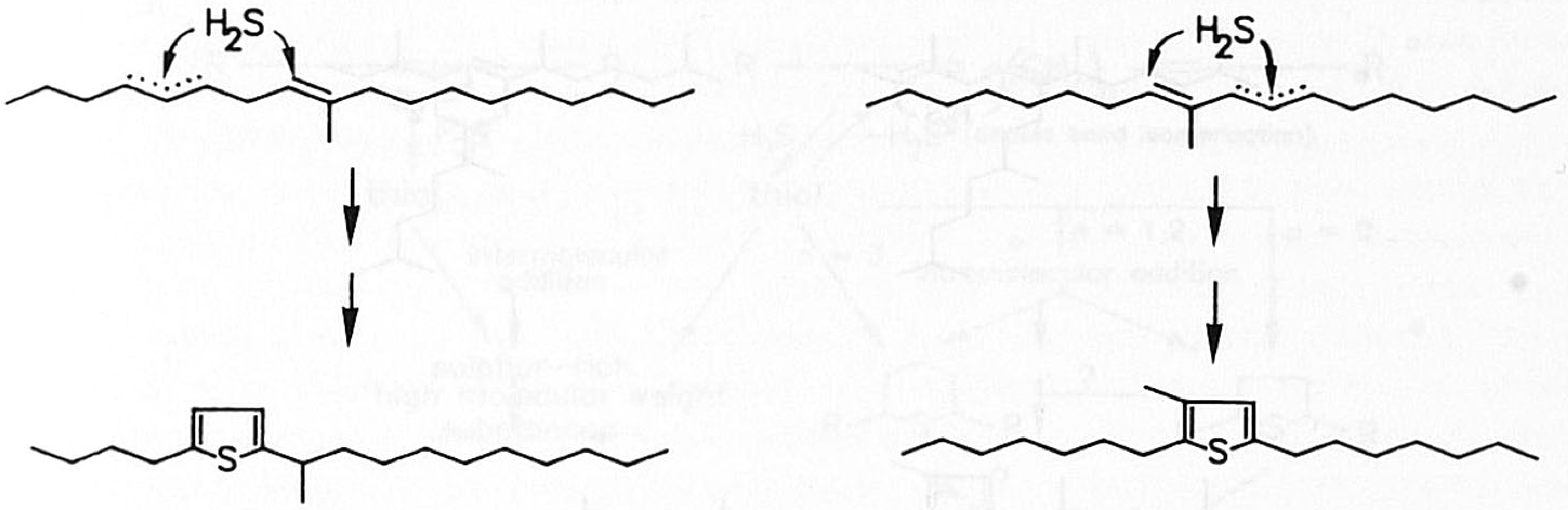


Fig. 10.11. Formation of C_{19} branched thiophenes, possessing the 9-methyloctadecane carbon skeleton, by sulphur incorporation into hypothetical precursors.

diunsaturated 9-methyloctadecanoic acid has been identified as a minor constituent of cod liver oil (Duru, 1983). Kerger *et al.* (1986) reported the presence of 10-methyloctadec-6-enoic acid (which also possesses the 9-methyloctadecane carbon skeleton) in polar lipids of the bacterium *Thiobacillus*. The unknown precursor of the C_{19} branched thiophenes seems to react more readily with inorganic sulphur species than other branched OSC precursors since the amounts of 9-methyloctadecane generated upon desulphurisation of OSC-containing fractions relative to other generated hydrocarbons is much higher than in the original hydrocarbon fractions (Sinninghe Damsté *et al.*, 1988f).

C_{25} highly branched isoprenoid thiophenes

The structure of the C_{25} highly branched isoprenoid thiophene VIII is consistent with sulphur incorporation into C_{25} highly branched isoprenoid alkadienes, widespread occurring hydrocarbons in recent sediments (Sinninghe Damsté *et al.*, 1988d). The other C_{25} highly branched isoprenoid thiophene (VII) can be formed by hydrogen sulphide addition at the same position of the biogenic alkenes but *via* another intramolecular addition of the formed thiol (Fig. 10.12). In both precursors the double bond positions are favourable for the formation of thiolanes (and subsequently thiophenes).

General model for the incorporation of sulphur into organic matter

The observations described here and those reported previously (Brassell *et al.*, 1986c; Sinninghe Damsté *et al.*, 1986, 1987c, 1988d; Sinninghe Damsté and de Leeuw, 1987) lead us to propose a hypothetical model for the incorporation of sulphur into organic matter (Fig. 10.13).

Sedimentary molecules with one double bond (or only one other reactive functionality) are not likely to serve as a precursor for OSC, although they

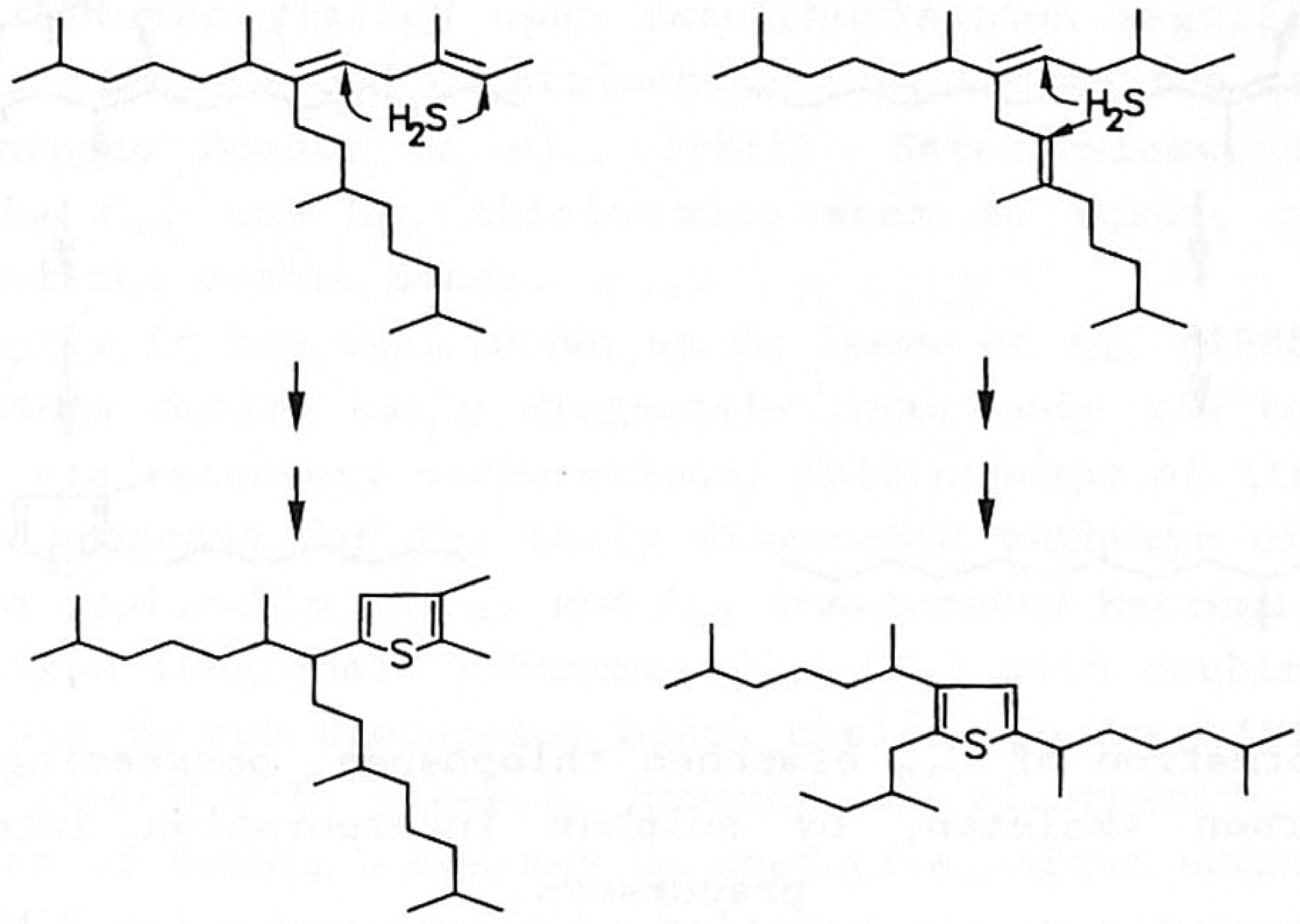


Fig. 10.12. Formation of C_{25} highly branched isoprenoid thiophenes by sulphur incorporation into highly branched isoprenoid alkadienes, ubiquitous compounds in recent sediment (Sinninghe Damsté *et al.*, 1988b and references cited therein).

may become part of sulphur-rich high molecular weight substances (*e.g.* resins, asphaltenes, kerogen) *via* H_2S addition and subsequent intermolecular addition of the resulting thiol. A prerequisite for formation of OSC by sulphur incorporation into a precursor during the early stages of diagenesis seems to be the presence of at least two double bonds (or other reactive functionalities) in the precursor molecule. If the double bonds of the precursor are in favourable position for the formation of thiolanes (Fig. 10.13; n<3) or thianes (0<n<4) sulphur incorporation leads to formation of a restricted number of isomers (*e.g.* C_{28} DATL and DATP, C_{19} branched thiophenes, C_{25} highly branched isoprenoid thiophenes). If the double bonds of the precursor are not in a favourable position (n>3) for thiolane or thiane formation a competition exists between reactions by which the precursor becomes part of sulphur-rich high molecular weight substances and a sequence of H_2S addition/elimination reactions, ultimately leading to a precursor molecule in which the double bonds are in a favourable position (n<4) for thiane and thiolane formation (*e.g.* C_{37} and C_{38} DATN and DATL). The resulting OSC mixture from these reactions is characterised by a higher amount of structural isomers. Precursors with more than two double bonds may undergo either of the two addition reactions (in the case of two intramolecular reactions leading to the formation of *e.g.* bithiophenes (Sinninghe Damsté and de Leeuw, 1987)) or both addition reactions leading to the formation of sulphur-rich higher molecular weight substances with units also containing intramolecularly incorporated sulphur (*e.g.* DATL, DATN and DATP connected with each other by sulphur bridges; Sinninghe Damsté *et al.*, 1988f).

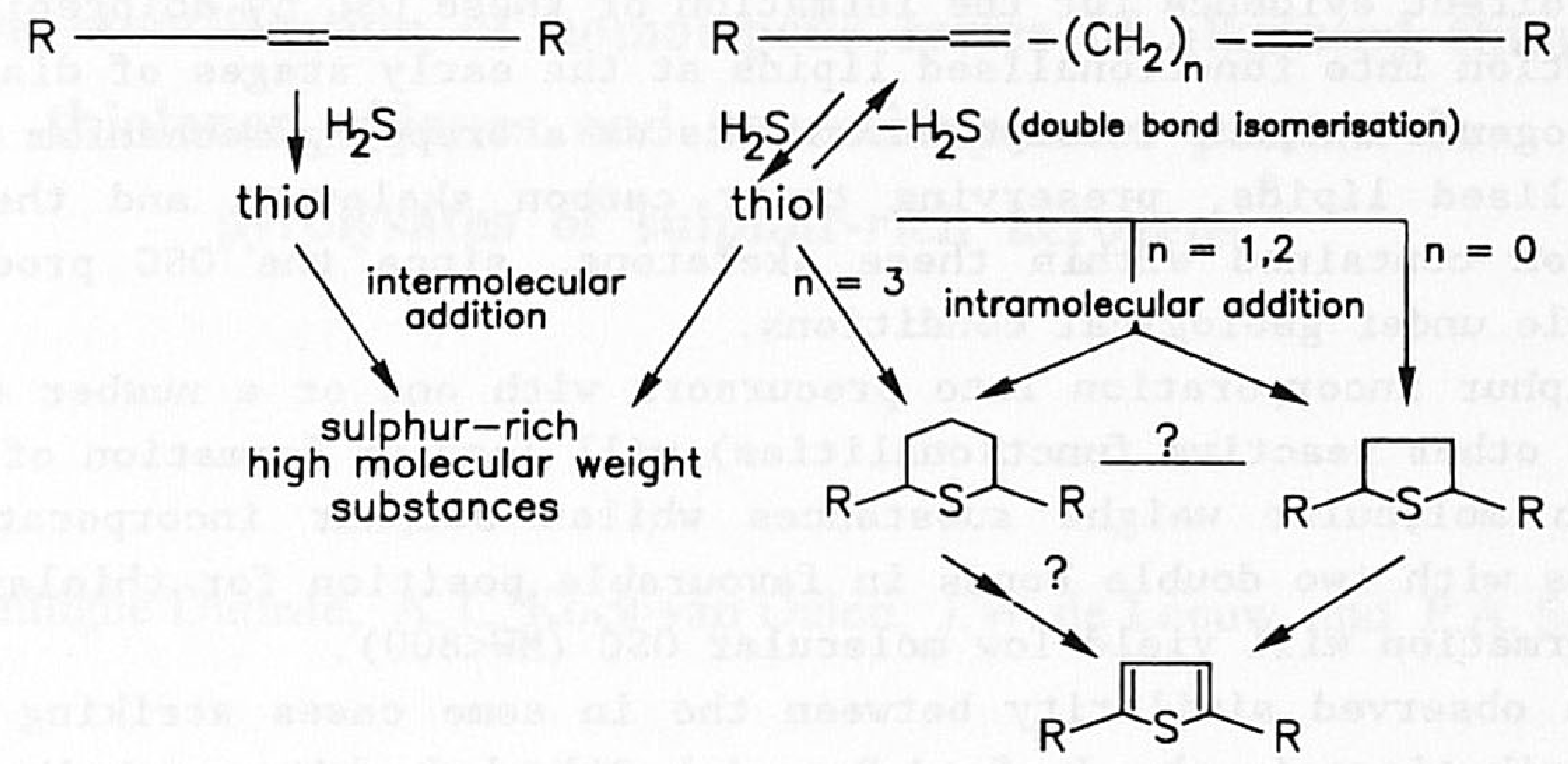


Fig. 10.13. General scheme for the sulphur incorporation into mono- and diunsaturated precursor.

For the major part of the OSC identified in sediments and oils (*e.g.* Sinninghe Damsté *et al.*, 1988g) but not described in this paper naturally occurring precursors exist which are in agreement with this proposed model. For example, C_{20} isoprenoid thiolanes and thiophenes find likely precursors in phytadienes, whilst thiolane and thiophene hopanoids are likely formed by sulphur incorporation into bacteriohopanetetrol or its diagenetic products. An exception in this respect are the thiolane and thiophene steroids since precursors with two reaction sites located in the steroidal side-chain or one located at the steroidal side-chain and the other one in the D-ring are not widespread occurring in nature, although isomerisation products of $\Delta^{7,22}$ diunsaturated steroids may account for the last mentioned precursor. The C_{20} highly branched thiophenes (Sinninghe Damsté *et al.*, 1988d) may also be exceptions in this respect because, so far, polyunsaturated C_{20} highly branched alkenes have not been reported.

Increasing thermal stress may account for the observed randomisation of DATL and DATN due to shifting of the carbon-sulphur bond. This also may lead to an increase in the relative amounts of DATL and a decrease of the relative amounts of DATN since the five-membered thiolane ring is thermodynamically more stable than the six-membered thiane ring. Indeed, samples which are characterised by randomised DATL and DATN mixtures (*e.g.* Rozel Point and Maruejols crude oils; Sinninghe Damsté *et al.*, 1987c; Schmid *et al.*, 1987) also contain higher amount of DATL relative to DATN, whilst the JED bitumen described here contains high amounts of DATN relative to DATL.

10.6 CONCLUSIONS

(1) The OSC present in the Jurf ed Darawish Oil shale bitumen are characterised by a number of compound classes the distributions of which are

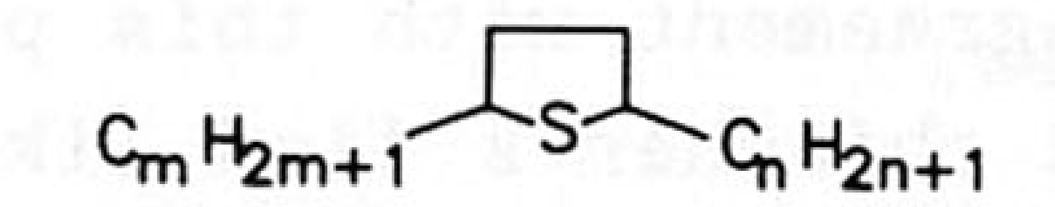
dominated by a limited number of all theoretically possible isomers. This provides direct evidence for the formation of these OSC by abiogenic sulphur incorporation into functionalised lipids at the early stages of diagenesis.

(2) Abiogenic sulphur incorporation acts as a trapping mechanism of labile functionalised lipids, preserving their carbon skeletons and thereby the information contained within these skeletons, since the OSC produced are more stable under geological conditions.

(3) Sulphur incorporation into precursors with one or a number of double bonds (or other reactive functionalities) will lead to formation of sulphur-rich high molecular weight substances whilst sulphur incorporation into precursors with two double bonds in favourable position for thiolane and/or thiane formation will yield low molecular OSC (MW<800).

(4) The observed similarity between the in some cases striking DATL and DATP distributions in the Jurf ed Darawish Oil shale bitumen indicates that DATP are under certain conditions more easily formed from DATL (and possibly DATN) than DATL are isomerised.

APPENDIX



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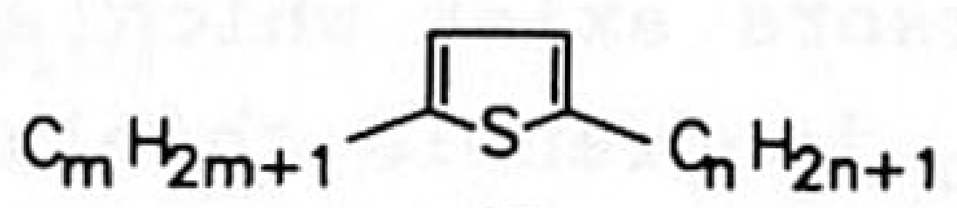

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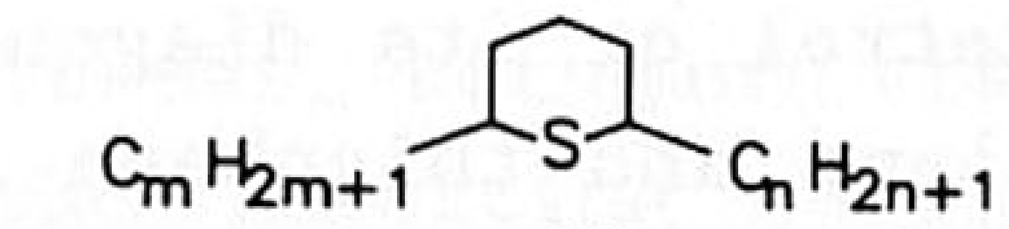

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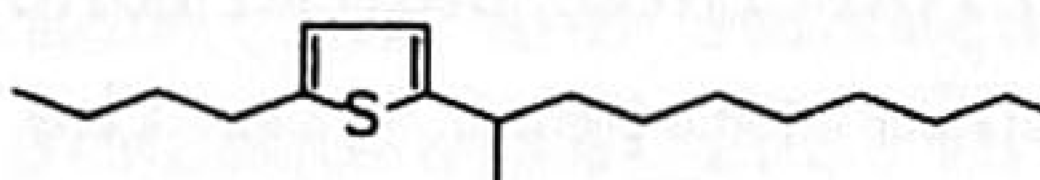

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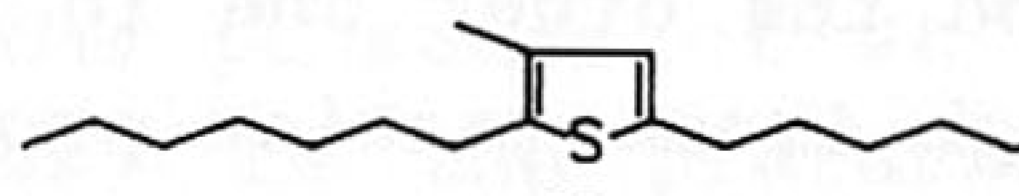

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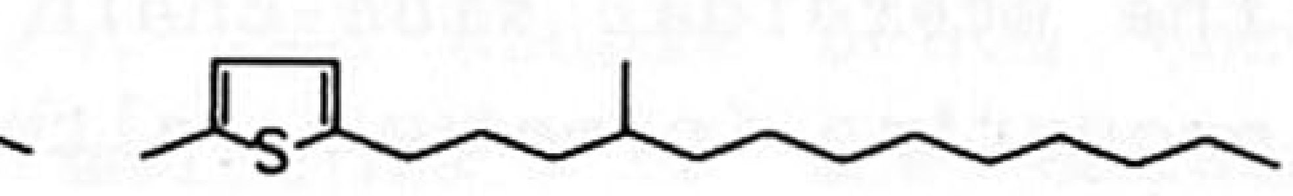

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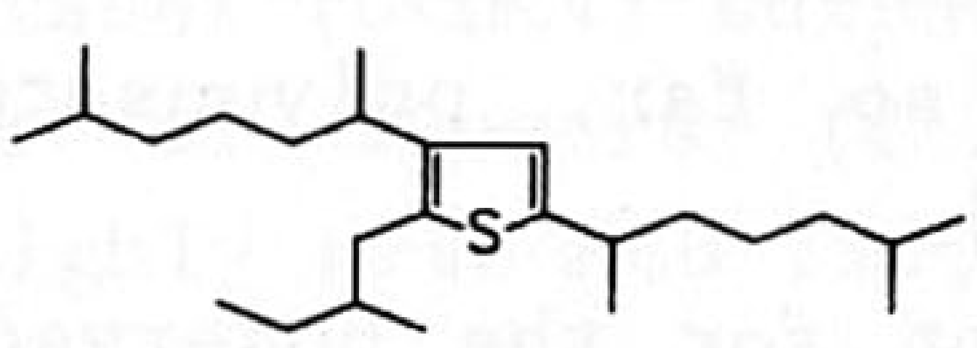

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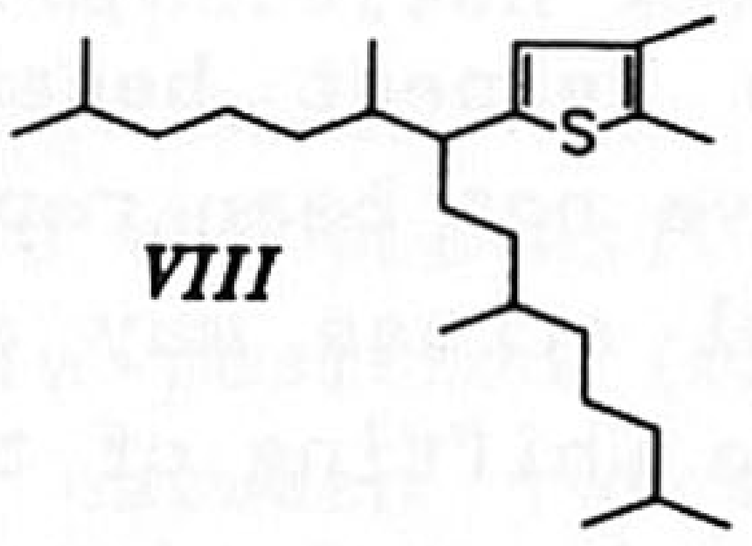

VIII

11. The identification of homologous series of alkylated thiophenes, thiolanes, thianes and benzothiophenes present in pyrolysates of sulphur-rich kerogens*

J.S. Sinninghe Damsté, A. C. Kock-van Dalen, J.W. de Leeuw and P.A. Schenck

Delft University of Technology, Department of Chemistry and Chemical Engineering, Organic Geochemistry Unit, de Vries van Heystplantsoen 2, 2628 RZ Delft, The Netherlands.

11.1 ABSTRACT

The ability of flash pyrolysis-gas chromatography (Py-GC) with dual flame ionization (FID) and flame photometric detection (FPD) and flash pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) to afford detailed information about the Organic Sulphur Pyrolysis Products (OSPP) of kerogens is exemplified through analysis of five different sulphur-rich, type II kerogens. The pyrolysates of these kerogens are extremely complex mixtures. Py-GC-FPD yields a chromatogram which shows the distribution of only OSPP in the pyrolysates. However, a considerable loss of sensitivity of flame photometric detection was apparent for higher molecular weight compounds. The use of the mass spectrometer as a detector, using characteristic *m/z* values of organic sulphur compounds for appropriate mass chromatograms, was therefore preferable. Identification of the OSPP was accomplished by sulphur-selective flame photometric detection and comparison of their mass spectral and chromatographic data with those of standard reference compounds. Chromatographic retention time data for 48 standard sulphur compounds (alkylated thiophenes, thiolanes and benzo[*b*]thiophenes) were determined. Homologous series of 2-alkylthiophenes, 2-alkyl-5-methylthiophenes, 2-alkyl-5-ethylthiophenes, 2-alkylthiolanes, 2-alkylthianes, 2-alkyl-5-methylthiolanes, 2-alkyl-4-methylbenzo[*b*]thiophenes, 4-alkyl-2-methylbenzo[*b*]thiophenes and 2-alkylbenzo[*b*]thiophenes, through a total carbon number of 22, have been identified through linear Kováts' plots and comparison of mass spectral and chromatographic data of the C_{18} members of these series with those of standard compounds.

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* *J. Chromatogr.* 435, 435-452 (1988)

11.1 INTRODUCTION

Sulphur compounds are present in almost all fossil fuels, which causes problems of all sorts. They are deleterious to the refining of petroleum and of coal liquefaction products, because they poison catalysts and cause corrosion. Furthermore, noxious sulphur dioxide is produced during combustion of sulphur containing fuels. As such they are toxic and some of them are suspected mutagens and/or carcinogens. Better knowledge of the forms in which sulphur occurs in fossil fuels might aid the development of methods for its removal.

A major part of the organic sulphur present in these materials occurs as thiophenic compounds, which makes them an important class of sulphur compounds to study. In petroleums the thiophene ring is mostly present as part of ring systems (primarily benzo- and dibenzothiophenes) (Martin and Grant, 1965; Hughes, 1984; Arpino *et al.*, 1987) but alkylated thiophenes also occur (Orr, 1978) and are the most abundant thiophenic compounds present in shale oils (Joyce and Uden, 1983). Alkylated thiophenes are also major compounds in several sediment extracts and immature crude oils (Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986; 1987a and c). Lee and co-workers (Wiley *et al.*, 1981; Kong *et al.*, 1984; Nishioka *et al.*, 1985, 1986a and b) and Burchill *et al.* (1982) have identified a number of polycyclic aromatic sulphur heterocycles in oils, synfuels, coal liquids and shale oils.

Surprisingly, no detailed chromatographic data have been published concerning alkylated thiophenes, although a number of these compounds have been synthesized for a number of reasons, *e.g.* mass spectrometric studies (Kinney and Cook, 1952; Foster *et al.*, 1964, 1965; Cook and Dinneen, 1965) and flavour evaluation (Galetto and Hoffman, 1976). We are especially interested in a firm identification of these alkylated thiophenes, because they are sometimes major products obtained by flash pyrolysis of kerogens, the insoluble macromolecular organic constituent of sedimentary rocks and precursor material for petroleum.

Flash pyrolysis, in combination with gas chromatography (Py-GC) and/or mass spectrometry (Py-GC-MS, Py-MS), has been used in organic geochemistry to characterise bio- and geopolymers such as lignins (Saiz-Jimenez and de Leeuw, 1984a, 1986a) humic substances (Saiz-Jimenez and de Leeuw, 1984b, 1986b), coals (van Graas *et al.*, 1980; Nip *et al.*, 1985, 1987), asphaltenes (Behar and Pelet, 1985; Philp and Gilbert, 1985) and kerogens (van Graas *et al.*, 1980; Philp and Gilbert, 1985; Solli and Leplat, 1986; Larter *et al.*, 1978; van de Meent *et al.*, 1980; van Graas *et al.*, 1981; Solli *et al.*, 1984; Larter, 1984) by identification of their pyrolysis products and evaluation of their relative amounts. Py-GC of the different kerogen types provides, for instance, distinct fingerprints which are useful in source rock evaluation (Larter, 1984). We have used Py-GC and Py-GC-MS to study the structure of sulphur-rich kerogens, so called Type II-S kerogens, which are inferred to be the source of most major high-sulphur crude oil accumulations

(Orr, 1986). This may provide insight into the way sulphur becomes incorporated into organic matter in the geosphere and how organic sulphur compounds in crude oils have been formed. In this paper we will focus on the analytical aspects of this work, the geochemical significance of these findings will be described elsewhere (Sinninghe Damsté *et al.*, 1988a).

11.3 EXPERIMENTAL

Samples. Five samples were studied: Phosphoria Retort Shale (Montana, USA; Permian), three outcrop samples from the Monterey formation (California, USA; Miocene) and the oil shale of Jurf ed Darawish (Jordan; Cretaceous). The samples were ground in a rotary disc mill and the powdered samples were exhaustively (40 h) Soxhlet extracted with a toluene/methanol (1:3) azeotrope.

Standards. Thiophene, 2-methylthiophene, 3-methylthiophene, 2,5-dimethylthiophene and 2-ethylthiophene were comercially available.

5-Lithio derivatives of 2-methylthiophene, 3-methylthiophene, 2,3-dimethylthiophene, 2,4-dimethylthiophene and 2-ethylthiophene were obtained by reaction of these thiophenes with *n*-butyllithium. Subsequent alkylation of these lithio derivatives with ethylbromide, 1-propylbromide and 1-butylbromide (Ramathan and Levine, 1962) yielded the 5-ethyl, 5-propyl and 5-butyl derivatives of the starting thiophenes. 2-Propylthiophene, 2-butylthiophene and 2-pentylthiophene were prepared by alkylation of 2-lithiothiophene with 1-propylbromide, 1-butylbromide and 1-pentylbromide. 2,4-Dimethylthiophene was obtained by methylation with dimethylsulphate of 2-lithio-4-methylthiophene. 2-Lithio-4-methylthiophene was obtained by reaction of *n*-butyllithium with 3-methylthiophene. 3,4-Dimethylthiophene, 2,3,4-trimethylthiophene, 2,3,5-trimethylthiophene and 2,3,4,5-tetrametylthiophene were prepared by metallation with *n*-butyllithium of respectively 3,4-dibromothiophene, 2,3,4-tribromothiophene, 2,3,5-tribromothiophene and 2,3,4,5-tetrabromothiophene, followed by subsequent methylation with di-methylsulphate (Janda *et al.*, 1972). 2,3,5-Tribromothiophene and 2,3,4,5-tetrabromothiophene were prepared by bromination of thiophene (Janda *et al.*, 1972). 3,4-Dibromothiophene and 2,3,4-tribromothiophene were prepared by a Grignard reaction of 2,3,4,5-tetrabromothiophene with Mg and 1,2-dibromoethane (Janda *et al.*, 1972).

Thiolane, 2-methylthiolane, 2-ethylthiolane, and the *cis* and *trans* isomers of 2,5-dimethylthiolane, 2-ethyl-5-methylthiolane, 2-methyl-5-propylthiolane and 2-butyl-5-methylthiolane were prepared by hydrogenation with triethylsilane of the corresponding thiophenes (Kursanov *et al.*, 1975; Parnes *et al.*, 1977).

2-Methyl-5-tridecylthiophene, 2-dodecyl-5-ethylthiophene and 2-methyl-5-tridecylthiolane were prepared previously (Sinninghe Damsté *et al.*, 1986, 1987c). 2-Tetradecylthiophene was prepared by coupling of thiophene with tetradecanoic acid with P_2O_5 as a dehydrating agent. The obtained ketone was reduced *via* a modified Wolf-Kishner reaction (King and Nord, 1949). 2-Tetradecylthiolane was prepared by hydrogenation with triethylsilane of 2-tetradecylthiophene (Kursanov *et al.*, 1975; Parnes *et al.*, 1977).

4-Nonylbenzo[*b*]thiophene, 2-decylbenzo[*b*]thiophene, 4-nonyl-2-methylbenzo-[*b*]thiophene and 2-nonyl-4-methylbenzo[*b*]thiophene were made available for this study by Dr. P. Albrecht (University of Strasbourg, France). The preparation of these compounds has been described by Perakis (1986).

Curie point pyrolysis-gas chromatography. Curie point-Py-GC analysis of extracted samples was carried out using a pyrolysis reactor, previously described by van de Meent *et al.* (1982), which was directly mounted on the injector block of a Varian 3700 gas chromatograph. The temperature of the injector block was 250°C. Samples were applied to ferromagnetic wires by pressing the dry samples on the wire (Venema and Veurink, 1985). The wires were heated within 0.1 s to their Curie temperature (in this case 610°C) and were kept at this temperature for 10 s. Separation of the pyrolysis products was performed on a fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness 0.45 μm) using helium as carrier gas. The temperature was programmed from 0°C (5 min), by using a cryogenic unit, to 300°C (20 min) at a rate of 3°C/min. The gas chromatograph was equipped with a flame ionization detector (FID) and a flame photometric detector (FPD). The flow from the capillary column was split at the end with a splitter device (FPD:FID=4:1) as described by Cox and Earp (1982).

Curie point pyrolysis-gas chromatography-mass spectrometry. Curie point Py-GC-MS analysis was performed on a Varian 3700 gas chromatograph connected to a Varian MAT 44 S quadrupole mass spectrometer by an open atmospheric split device. Electron impact mass spectra were obtained at 80 eV under the following conditions: cycle time 2 s: mass range, *m/z* 20-450 up to scan 250 and *m/z* 50-450 after scan 250; *m/z* 28, 32, 40 and 44 were omitted from the reconstructed total ion currents. The same pyrolysis reactor, capillary column and temperature conditions were used as described for the Py-GC experiments.

Retention index measurements. A function was obtained by fitting a polynome of the third order through the datapoints (retention index, scan number) of the homologous series of *n*-alkanes present in the pyrolysates of the kerogens for each run. Subsequently, the retention indices of the organic sulphur compounds of the pyrolysates were calculated by the scan number of peaks in the reconstructed total ion current or mass chromatograms corresponding to these compounds and this regression function.

The retention indices of the standards were measured using the same methodology but by coinjection of these compounds with a standard solution of *n*-alkanes on the same capillary column and using the same temperature program as described for the Py-GC and Py-GC-MS experiments.

11.4 RESULTS AND DISCUSSION

Fig. 11.1 is a typical example of an FID and FPD chromatogram obtained by flash pyrolysis of a sulphur-rich kerogen. The FPD chromatogram shows the distribution of the organic sulphur pyrolysis products (OSPP) generated by pyrolysis. Such a chromatogram of OSPP may be very useful to characterise this and other types of kerogens. A disadvantage is, however, that in the range of molecular weights of OSPP (*e.g.* thiophenes from 84 (C_4H_4S) up to 336 ($C_{22}H_{40}S$) daltons; see below) the sensitivity of the FPD for these compounds decreases considerably as a result of the decreasing atomic sulphur fraction with increasing molecular weight (Fig. 11.2). This loss of sensitivity is even more severe as a result of the approximately quadratic response of the FPD. The mass spectrometer does not have this disadvantage and is, therefore, more suitable as a detector for this type of analysis by using appropriate mass chromatography to resolve the distributions of the OSPP.

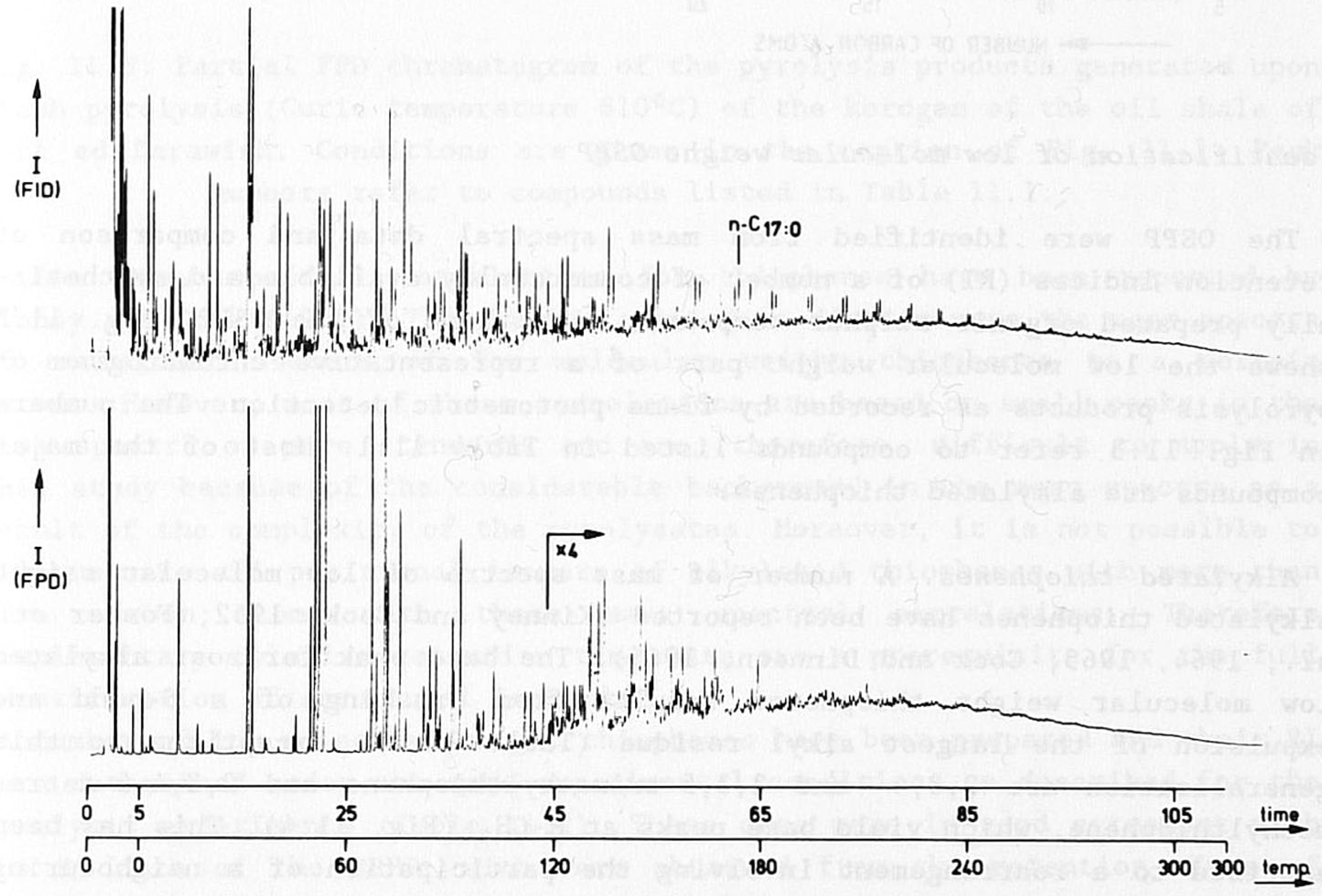

Fig. 11.1. FID and FPD chromatograms of the pyrolysis products generated upon flash pyrolysis (Curie temperature 610°C) of the kerogen of the Phosphoria Retort Shale; 25m x 0.32 mm i.d. fused silica capillary column coated with CP Sil-5 (film thickness = 0.45 μm) temperature programmed 0-300°C at 3°C/min. *n*-C-17:0 indicates heptadecane.

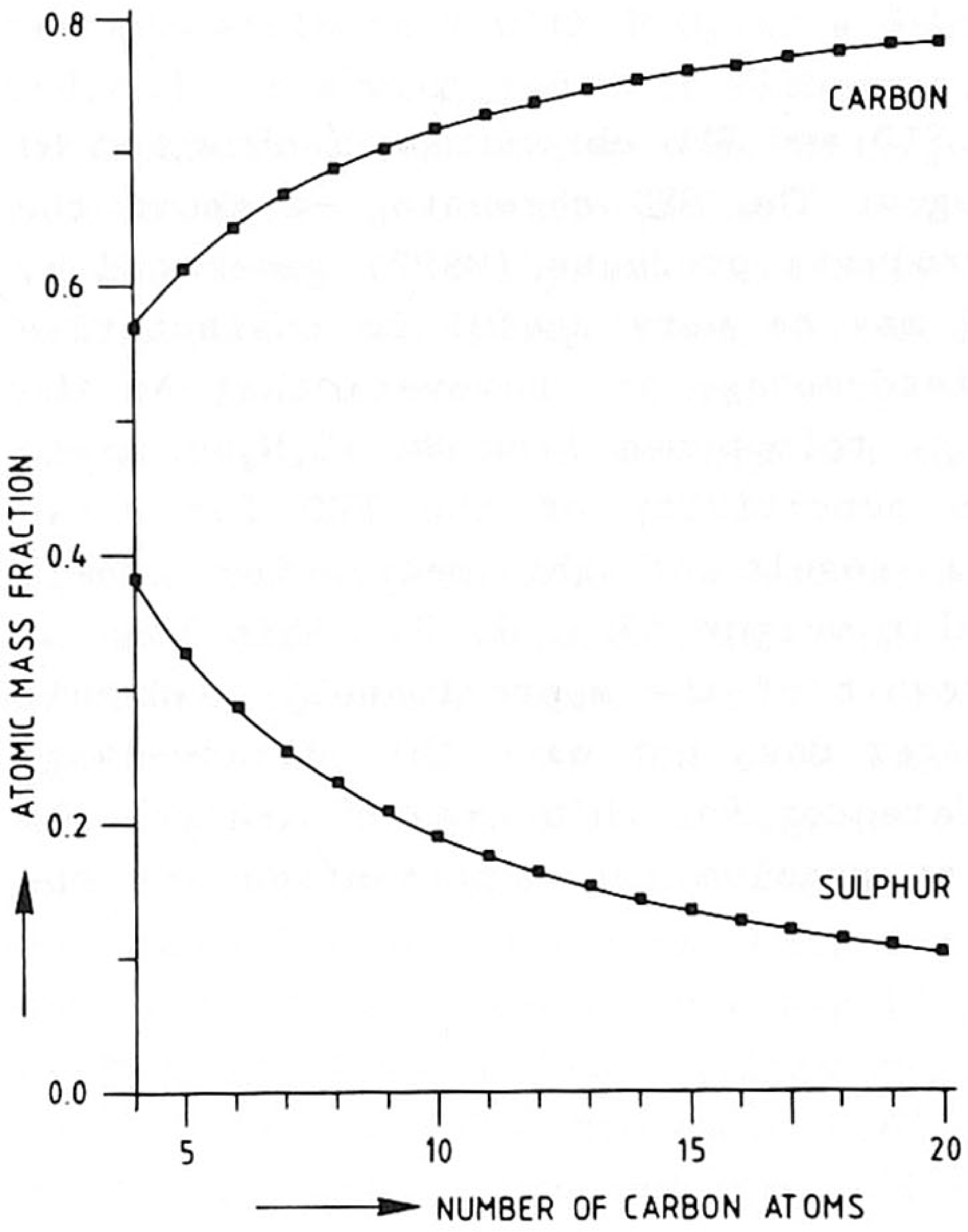

Fig. 11.2. Atomic sulphur and atomic carbon fraction (by weight) of alkylated thiophenes as a function of the molecular weight. Because the the FPD exhibits an approximately quadratic response to the mass flow (ngS/s) (Farwell and Barinaga, 1986), these curves show the decreasing sensitivity of the FPD as compared with that of the FID for detection of these thiophenes with increasing molecular weight.

Identification of low molecular weight OSPP

The OSPP were identified from mass spectral data and comparison of retention indices (RI) of a number of commercially available and synthetically prepared organic sulphur compounds with those of the OSPP. Fig. 11.3 shows the low molecular weight part of a representative chromatogram of pyrolysis products as recorded by flame photometric detection. The numbers in Fig. 11.3 refer to compounds listed in Table 11.1. Most of the major compounds are alkylated thiophenes.

Alkylated thiophenes. A number of mass spectra of low molecular weight alkylated thiophenes have been reported (Kinney and Cook, 1952; Foster *et al.*, 1964, 1965; Cook and Dinneen, 1965). The base peak for most alkylated low molecular weight thiophenes results from breaking of a β-bond and expulsion of the largest alkyl residue (Table 11.1). Exceptions to this generalisation are 2,3,4- and 2,3,5-trimethylthiophene and 2,3,4,5-tetramethylthiophene, which yield base peaks at M-CH_3 (Fig. 11.4). This has been ascribed to a rearrangement involving the participation of a neighbouring methyl group (Budzikiewicz *et al.*, 1964). All low molecular weight alkylated thiophenes show a molecular ion in their mass spectra. Therefore, mass chromatography of the *m/z* values of the molecular ions reveals the clusters of isomers of alkylated thiophenes in the pyrolysates (Fig. 11.5). Mass spectra of positional isomers often resemble each other (*e.g.* 2,3-, 2,4-, 2,5- and 3,4-dimethylthiophene; Table 11.1) which allows no full identifi-

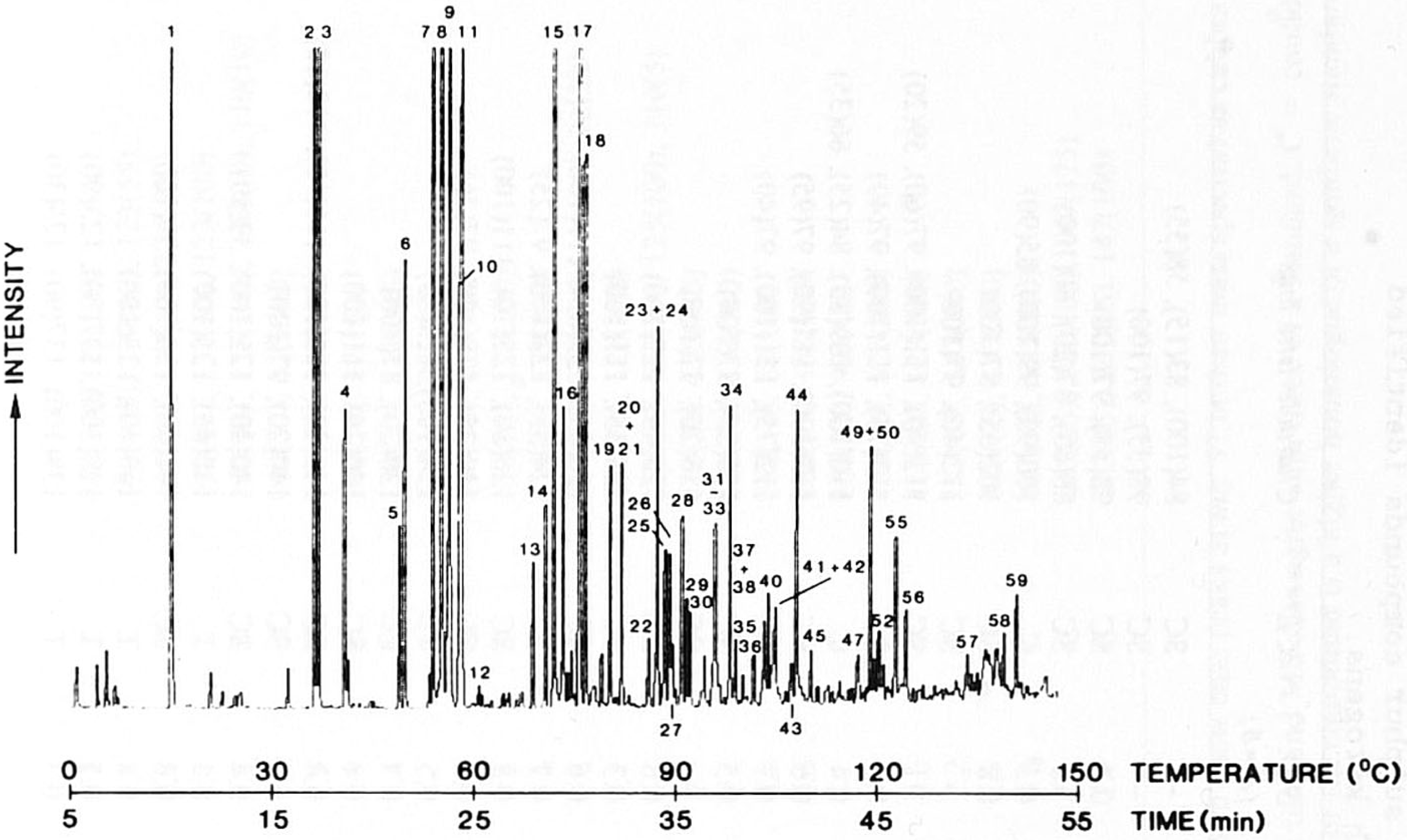

Fig. 11.3. Partial FPD chromatogram of the pyrolysis products generated upon flash pyrolysis (Curie temperature 610°C) of the kerogen of the oil shale of Jurf ed Darawish. Conditions are given in the caption of Fig. 11.1. Peak numbers refer to compounds listed in Table 11.1.

cation. Mass spectral correlations for thiophenes have been reported by Kinney and Cook (1952). They can be applied to discriminate the mass spectra of positional isomers of low molecular weight thiophenes to a certain extent. However, most of these correlations are based on small peaks in the mass spectra of pure standards and are, therefore, difficult to apply in this study because of the considerable background in the mass spectra as a result of the complexity of the pyrolysates. Moreover, it is not possible to discriminate all positional isomers of alkylated thiophenes with more than five carbon atoms with these mass spectral correlations. Therefore complementary relative retention time data are a prerequisite for the full identification of structural isomers.

A number of low molecular weight thiophenes have been prepared and their RI were determined under the same experimental conditions as described for the pyrolysis experiments (Table 11.2). These data were in good agreement with the RI data of the OSPP, wich were obtained from the retention times of these compounds and those of the homologous series of *n*-alkanes also generated by pyrolysis (Fig. 11.5). The standard deviation of the RI data of the OSPP (Table 11.1) was determined by measuring the RI of these compounds in six different Py-GC-MS runs. A considerable reduction of the standard deviation could be obtained by using the homologous series of 2-*n*-alkylthiophenes instead of *n*-alkanes as calibration standards, as proposed previously

Table 11.1. Major low-molecular weight organic sulphur compounds identified in pyrolysates of sulphur-rich kerogens.

*No.**	*Compound*	*Mol. wt.*	*I*	*S.D. (n=6)*	*ATI$_{97}$***	*S.D. (n=6)*	*CL****	*Characteristic ions*
1	Thiophene	84	643.7	1.1	–	–	SC	84(100), 83(15), 58(55)
2	2-Methylthiophene	98	750.5	2.0	500.0	–	SC	98(55), 97(100)
3	3-Methylthiophene	98	756.2	2.5	505.6	0.7	SC	98(55), 97(100)
4	Thiolane	88	774.9	2.4	525.8	1.2	SC	88(60), 87(20), 60(100)
5	Methyldihydrothiophene	100	815.6	1.0§	571.9	0.3§	C	100(90), 99(100), 85(90)
6	2-Methylthiolane	102	821.8	2.0	575.9	0.8	SC	102(55), 87(100)
7	2-Ethylthiophene	112	845.0	1.7	600.0	–	SC	112(40), 97(100)
8	2,5-Dimethylthiophene	112	852.4	1.8	608.2	1.1	SC	112(90), 111(100), 97(60), 59(20)
9	2,4-Dimethylthiophene	112	858.7	1.8	614.9	0.7	SC	112(75), 111(100), 97(40)
10	2-Vinylthiophene	110	866.9	1.6	623.0	0.4	C	110(100), 109(85), 84(25), 66(35)
11	2,3-Dimethylthiophene	112	868.2	2.4	625.2	0.6	SC	112(100), 111(90), 97(95)
12	3,4-Dimethylthiophene	112	881.5	2.0	638.9	0.3	SC	112(75), 111(100), 97(40)
13	2-Ethylthiolane	116	926.5	2.0	685.3	0.5	SC	116(25), 87(100)
14	2-Propylthiophene	126	937.0	1.6	700.0	–	SC	126(20), 97(100)
15	2-Ethyl-5-methylthiophene	126	945.3	1.5	704.5	0.6	SC	126(40), 111(100)
16	2-Ethyl-4-methylthiophene	126	952.7	1.6	712.1	0.3	SC	126(35), 111(100)
17	2,3,5-Trimethylthiophene	126	966.9	1.6	726.6	0.6	SC	126(85), 125(85), 111(100), 59(20)
18	2-Methyl-5-vinylthiophene	124	971.4	1.7	731.2	0.2	T	124(85), 123(100), 97(25)
19	2,3,4-Trimethylthiophene	126	993.4	2.1	753.4	0.8	SC	126(80), 125(70), 111(100)
20	Isopropylmethylthiophene	140	1003.5	1.8	763.7	0.6	C	140(25), 125(100), 97(25)
21	$C_{3:1}$-Thiophene	124	1003.7	1.6	763.9	0.5	T	124(100), 123(50)
22	2-Propylthiolane	130	1027.5	2.0	787.8	0.7	C	130(20), 87(100)
23	2-Methyl-5-propylthiophene	140	1034.8	1.6	795.1	0.4	SC	140(25), 111(100)
24	2,5-Diethylthiophene	140	1034.8	1.6	795.1	0.4	SC	140(40), 125(100), 111(10), 110(10)
25	2-Butylthiophene	140	1041.1	1.7	800.0	–	SC	140(20), 97(100)
26	2-Ethyl-3,5-dimethylthiophene	140	1045.3	1.6	805.6	0.4	SC	140(40), 125(100), 59(10)
27	Ethyldimethylthiophene	140	1047.1	1.5	807.5	0.5	T	140(45), 125(100)
28	5-Ethyl-2,3-dimethylthiophene	140	1056.4	1.5	816.7	0.6	SC	140(40), 125(100), 59(10)
29	Ethyldimethylthiophene	140	1060.6	1.9	820.9	0.8	T	140(40), 125(100)
30	$C_{4:1}$-Thiophene	138	1063.8	1.7	824.1	0.5	T	138(100), 137(60), 123(90)
31	$C_{4:1}$-Thiophene	138	1085.8	1.5	845.8	0.3	T	138(100), 137(90), 123(30)

32	Ethyldimethylthiophene	140	1087.0	1.6	847.0	0.5	T	140(25), 125(100)
33	$C_{4:1}$-Thiophene	138	1089.1	1.9	849.1	0.7	T	138(100), 137(80), 123(55)
34	2,3,4,5-Tetramethylthiophene	140	1101.4	1.8	861.3	0.8	SC	140(70), 139(60), 125(100)
35	$C_{4:1}$-Thiophene	138	1106.2	1.8	866.0	0.7	T	138(95), 137(60), 123(100)
36	2-Ethyl-5-propylthiophene	154	1122.5	1.3	882.0	0.5	SC	154(30), 125(100), 139(10), 110(10)
37	3,5-Dimethyl-2-propylthiophene	154	1131.3	1.6	890.7	0.5	SC	154(30), 125(100)
38	C_5-Thiophene	154	1132.9	1.3	892.6	0.5	T	154(50), 139(100)
39	2-Butylthiolane	144	1133.2	2.0	892.3	0.9	C	144(10), 87(100)
40	2-Butyl-5-methylthiophene	154	1136.5	1.3	895.7	0.4	SC	154(20), 111(100)
41	2-Pentylthiophene	154	1142.5	1.4	900.0	–	SC	154(15), 97(100)
42	2,3-Dimethyl-5-propylthiophene	154	1144.0	1.4	903.1	0.4	SC	154(30), 125(100)
43	2-Propyl-5-vinylthiophene	152	1153.7	1.5	912.6	0.5	T	152(20), 123(100)
44	C_5-Thiophene	154	1163.4	1.4	922.1	0.6	T	154(40), 139(100)
45	C_5-Thiophene	154	1177.7	1.7	935.9	0.8	T	154(35), 139(100)
46	C_6-Thiophene	168	1192.0	1.4	949.8	0.7	T	168(20), 125(100)
47	2-Butyl-5-ethylthiophene	168	1223.6	1.2	980.3	0.6	SC	168(25), 153(10), 125(100), 110(5)
48	2-Butyl-3,5-dimethylthiophene	168	1228.7	1.2	986.5	0.5	SC	168(20), 125(100)
49	2-Methyl-5-pentylthiophene	168	1236.6	1.2	993.0	0.4	C	168(20), 111(100)
50	2-Pentylthiolane	158	1237.8	2.1	994.2	0.8	C	158(10), 87(100)
51	C_5-Thiophene	168	1240.8	1.7	997.0	0.5	T	168(35), 153(100)
52	2-Hexylthiophene	168	1245.1	1.5	1000.0	–	C	168(20), 97(100)
53	5-Butyl-2,3-dimethylthiophene	168	1245.1	1.5	1000.0	–	SC	168(20), 125(100)
54	2-Butyl-5-vinylthiophene	166	1256.4	1.7	1012.0	0.5	T	166(20), 123(100)
55	Methylbenzo[*b*]thiophene	148	1264.8	3.6	1020.1	2.2	C	148(65), 147(100)
56	Methylbenzo[*b*]thiophene	148	1276.9	3.9	1031.7	2.5	C	148(65), 147(100)
57	2-Hexyl-5-methylthiophene	182	1336.8	1.3	1089.1	0.3	C	182(15), 111(100)
58	Dimethylbenzo[*b*]thiophene	162	1381.5	4.3	1131.6	2.6	C	162(85), 161(100), 147(125)
59	Dimethylbenzo[*b*]thiophene	162	1395.0	4.7	1144.5	2.9	C	162(95), 161(65), 147(100)

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* Numbers refer to Figs. 3 and 5.

** ATI_{97} = Alkyl thiophene index with the homologous series of 2-alkylthiophenes (which show base peaks at *m/z* 97 in their mass spectra) as calibration standards

*** CL = confidence level of structural assignment; SC = standard confirmed, retention time and MS data agree with those of a standard; C = confident, mass spectrum is identical with a reference mass spectrum from the literature or MS database or the compound is a higher homologue of a synthetic standard as determined by a linear Kováts plot and a similar mass spectral fragmentation; T = tentative.

§ Two determinations.

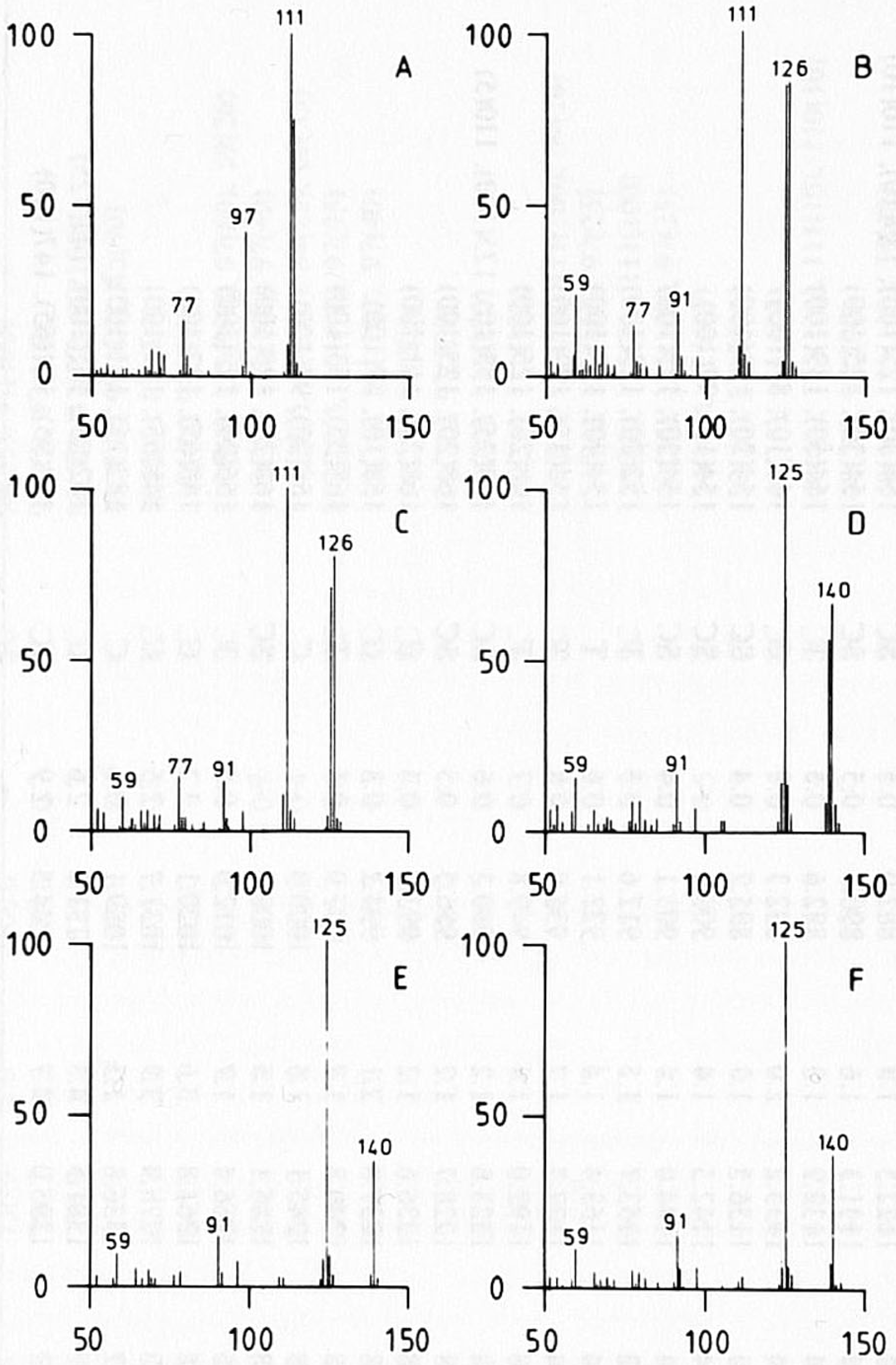


Fig. 11.4. Mass spectra (corrected for background) of several synthetic thiophenes: (A) 3,4-dimethylthiophene, (B) 2,3,5-trimethylthiophene, (C) 2,3,4-trimethylthiophene, (D) 2,3,4,5-tetramethylthiophene, (E) 2-ethyl-3,5-dimethylthiophene, (F) 5-ethyl-2,3-dimethylthiophene.

(Sinninghe Damsté *et al.*, 1986). This, so called, ATI (Alkyl Thiophene Index) was purposely defined to distinguish isoprenoid thiophenes (alkylated thiophenes with an isoprenoid carbon skeleton) from 2,5-di-*n*-alkylthiophenes. These results show that this index can also be of value as an alternative retention index system in the case of sulphur compounds.

Alkylated thiolanes and thianes. Alkylated thiolanes and thianes were present in considerably less relative quantities than the alkylated thiophenes. They were identified from their mass spectral and relative retention time data (Table 11.3) and, in case of the alkylated thiolanes from

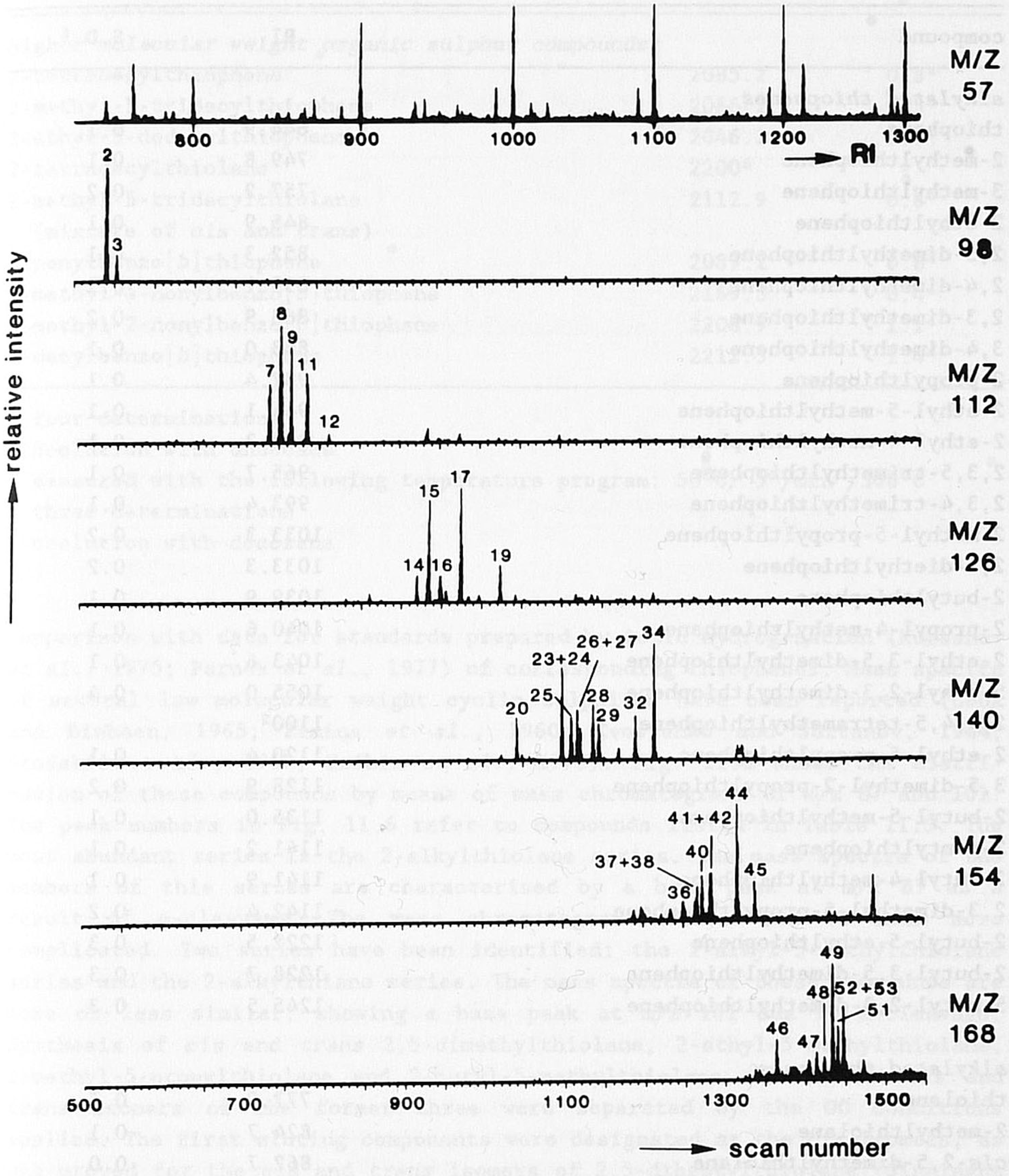


Fig. 11.5. Partial mass chromatograms showing the C_8-C_{13} *n*-alkanes (*m/z* 57) and the C_1-C_6 alkyl derivatives of thiophene (*m/z* 96, 112, 126, 140, 154 and 168) present in the pyrolysate (Curie temperature 610°C) of the kerogen of the oil shale of Jurf ed Darawish. Peak numbers refer to compounds listed in Table 11.1. Conditions are given in the caption of Fig. 11.1.

Table 11.2. Retention indices of standards.

compound	RI	S.D.[1]
alkylated thiophenes		
thiophene	644.9	0.1
2-methylthiophene	749.8	0.1
3-methylthiophene	757.2	0.2
2-ethylthiophene	845.9	0.1
2,5-dimethylthiophene	852.3	0.1
2,4-dimethylthiophene	859.1	0.1
2,3-dimethylthiophene	868.9	0.2
3,4-dimethylthiophene	883.0	0.1
2-propylthiophene	937.4	0.1
2-ethyl-5-methylthiophene	944.1	0.1
2-ethyl-4-methylthiophene	952.3	0.1
2,3,5-trimethylthiophene	965.7	0.1
2,3,4-trimethylthiophene	993.4	0.1
2-methyl-5-propylthiophene	1033.3	0.2
2,5-diethylthiophene	1033.3	0.2
2-butylthiophene	1039.9	0.1
2-propyl-4-methylthiophene	1040.6	0.1
2-ethyl-3,5-dimethylthiophene	1043.6	0.1
5-ethyl-2,3-dimethylthiophene	1055.0	0.4
2,3,4,5-tetramethylthiophene	1100[2]	-
2-ethyl-5-propylthiophene	1120.4	0.1
3,5-dimethyl-2-propylthiophene	1128.9	0.2
2-butyl-5-methylthiophene	1135.0	0.1
2-pentylthiophene	1141.2	0.1
2-butyl-4-methylthiophene	1141.9	0.1
2,3-dimethyl-5-propylthiophene	1142.4	0.2
2-butyl-5-ethylthiophene	1222.5	0.3
2-butyl-3,5-dimethylthiophene	1228.7	0.3
5-butyl-2,3-dimethylthiophene	1245.5	0.3
alkylated thiolanes		
thiolane	777.9	0.7
2-methylthiolane	824.7	0.1
cis-2,5-dimethylthiolane	862.7	0.0
trans-2,5-dimethylthiolane	866.2	0.1
2-ethylthiolane	928.3	0.1
cis-2-ethyl-5-methylthiolane	962.5	0.1
trans-2-ethyl-5-methylthiolane	966.4	0.2
cis-2-methyl-5-propylthiolane	1059.7	0.2
trans-2-methyl-5-propylthiolane	1062.3	0.2
2-butyl-5-methylthiolane	1163.7	0.1

Table 11.2. (Continued)

higher molecular weight organic sulphur compounds[3]		
2-tetradecylthiophene	2085.2	0.8[4]
2-methyl-5-tridecylthiophene	2066.2	0.5[4]
2-ethyl-5-dodecylthiophene	2046.1	0.9[4]
2-tetradecylthiolane	2200[5]	-
2-methyl-5-tridecylthiolane (mixture of *cis* and *trans*)	2112.9	0.8[4]
4-nonylbenzo[*b*]thiophene	2089.1	0.8[4]
2-methyl-4-nonylbenzo[*b*]thiophene	2169.5	0.4[4]
4-methyl-2-nonylbenzo[*b*]thiophene	2206.9	1.1[4]
2-decylbenzo[*b*]thiophene	2212.3	1.4[4]

[1] four determinations
[2] coelution with undecane
[3] measured with the following temperature program: 50°C/ 3°/min /300°C
[4] three determinations
[5] coelution with docosane

comparison with data for standards prepared by ionic hydrogenation (Kursanov *et al.*, 1975; Parnes *et al.*, 1977) of corresponding thiophenes. Mass spectra of several low molecular weight cyclic sulphides have been reported (Cook and Dinneen, 1965; Zimina *et al.*, 1960; Kvostenko and Sultanov, 1964; Brodskii *et al.*, 1969; Zaikin *et al.*, 1978). Fig. 11.6 shows the distribution of these compounds by means of mass chromatograms of *m/z* 87 and 101. The peak numbers in Fig. 11.6 refer to compounds listed in Table 11.3. The most abundant series is the 2-alkylthiolane series. The mass spectra of the members of this series are characterised by a base peak at *m/z* 87 as a result of α-cleavage. The mass chromatogram of *m/z* 101 is much more complicated. Two series have been identified: the 2-alkyl-5-methylthiolane series and the 2-alkylthiane series. The mass spectra of these compounds are more or less similar, showing a base peak at *m/z* 101 due to α-cleavage. Synthesis of *cis* and *trans* 2,5-dimethylthiolane, 2-ethyl-5-methylthiolane, 2-methyl-5-propylthiolane and 2-butyl-5-methylthiolane showed that *cis* and *trans* isomers of the former three were separated by the GC conditions applied. The first eluting components were designated as the *cis* isomers, as was proved for the *cis* and *trans* isomers of 2,5-diheptylthiolane (Sinninghe Damsté *et al.*, 1987). RI data of the synthetic 2-alkyl-5-methylthiolanes was used to identify these compounds in the pyrolysates.

The identification of the 2-alkylthiane series was not based on synthesis of appropriate standards. However, the retention behaviour of these compounds indicated their identity. From data presented here it is known that 2-alkylthiolanes elute much later than the corresponding *cis* and *trans* 2-alkyl-5-methylthiolane isomers (which coelute if the total number of

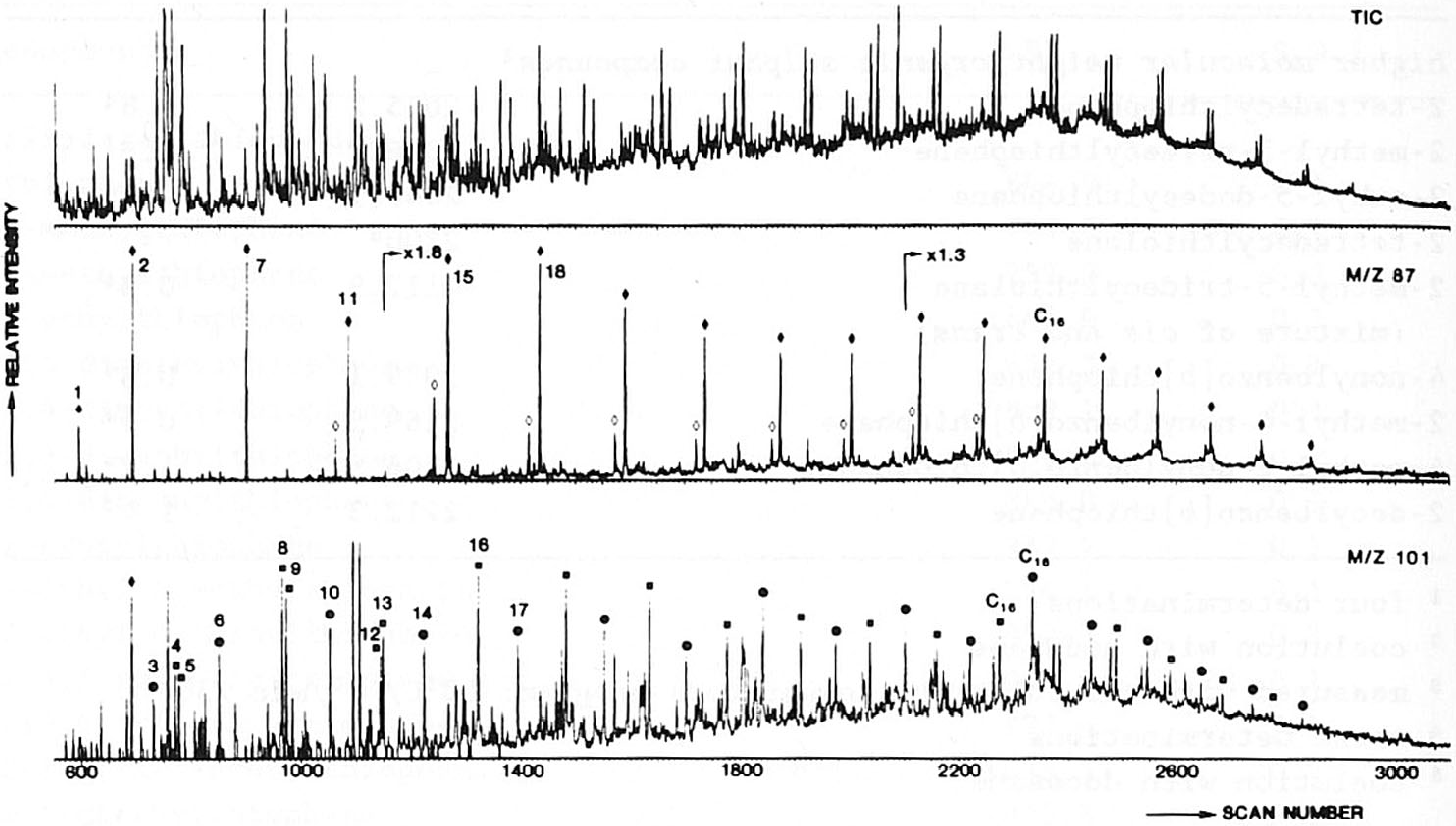


Fig. 11.6. Mass chromatograms of *m/z* 87 and 101, showing the homologous series of 2-alkylthiolanes (black diamonds), 2-alkylthianes (black circles) and 2-alkyl-5-methylthiolanes (black squares), and part of the TIC (upper trace) of the pyrolysate (Curie temperature 610°C) of the kerogen of the oil shale of Jurf ed Darawish. Peak numbers refer to compounds listed in Table 11.3. C_{16} indicates the member of the homologous series with 16 carbon atoms. The other homologous series present in the mass chromatogram of *m/z* 87 (white diamonds) is that of 2-(alkenyl)thiolanes, probably 2-(ω-alkenyl)-thiolanes. Conditions are given in the caption of Fig. 11.1.

carbon atoms is greater than seven). Earlier work (Sinninghe Damsté *et al.*, 1987c) showed that the other (both *cis* and *trans*) 2,5-di-*n*-alkylthiolane isomers elute just before the *cis* and *trans* 2-alkyl-5-methylthiolanes. It was also shown that 2,5-di-*n*-alkylthianes elute just before the corresponding isomeric thiolanes (Sinninghe Damsté *et al.*, 1987c). Because the thiane series encountered here elute before the 2-alkylthiolane series but considerably later than the 2-alkyl-5-methylthiolane series, it is deemed likely to be the 2-alkylthiane series.

Other compounds. Apart from the alkylated thiophenes, thiolanes and thianes several other low molecular OSPP were identified based on their mass spectral data: unsaturated alkylated thiophenes (compounds 10, 18, 21, 30, 31, 33, 35, 43 and 54 in Table 1), alkylated dihydrothiophenes (compound 5) and alkylated benzo[*b*]thiophenes (compounds 55, 56, 58 and 59).

Table 11.3. Low molecular weight cyclic sulphides identified in pyrolysates of sulphur-rich kerogens.

no.[1]	compound	MW[2]	RI	S.D.[3]	CL[4]
1	thiolane	88	774.9	2.4	SC
2	2-methylthiolane	102	821.8	2.0	SC
3	thiane	102	841.8	2.4	C
4	*cis*-2,5-dimethylthiolane	116	861.7	2.1	SC
5	*trans*-2,5-dimethylthiolane	116	864.7	2.1	SC
6	2-methylthiane	116	902.2	2.6	C
7	2-ethylthiolane	116	926.5	2.0	SC
8	*cis*-2-ethyl-5-methylthiolane	130	962.5	1.8	SC
9	*trans*-2-ethyl-5-methylthiolane	130	966.5	1.9	SC
10	2-ethylthiane	130	1010.6	2.5	C
11	2-propylthiolane	130	1027.5	2.0	C
12	*cis*-2-methyl-5-propylthiolane	144	1061.4	1.7	SC
13	*trans*-2-methyl-5-propylthiolane	144	1064.2	1.8	SC
14	2-propylthiane	144	1108.4	2.3	C
15	2-butylthiolane	144	1133.2	2.0	C
16	2-butyl-5-methylthiolane	158	1165.9	1.5	SC
17	2-butylthiane	158	1212.1	2.6	C
18	2-pentylthiolane	158	1237.8	2.1	C

[1] compound numbers refer to Fig. 11.6
[2] MW = Molecular Weight
[3] five determinations (in different samples)
[4] CL = Confidence Level of structural assignment, see footnote to Table 11.1

Identification of higher molecular weight OSPP

In the higher molecular weight part of the pyrograms mass chromatography of *m/z* 97, 111, 125, 139, 87, 101, 147, 148, 161, and 162 revealed the presence of several homologous series of OSPP. The mass chromatograms of *m/z* 87 and 101, *m/z* 97 and 111, and *m/z* 147, 161 and 162 of typical Py-GC-MS runs are shown in Figs. 11.6-11.8. The compounds were identified from their mass spectral and relative retention time data. The mass spectra of the higher molecular weight OSPP were characterised by the indicated base peak (Fig. 11.9) and a molecular ion. When the RI of these homologous series were plotted as a function of their carbon number straight lines were obtained (Figs. 11.10 and 11.11). The constant methylene increment of RI observed for these series strongly suggests that all compounds are indeed members of the indicated homologous series. In case of 2-alkylthiophene (I), 2-alkyl-5-methylthiophene (II), 2-alkyl-5-ethylthiophene (III), 2-alkylthiolane (V), 2-alkyl-5-methylthiolane (VI) (both *cis* and *trans*), 2-alkyl-4-methyl-

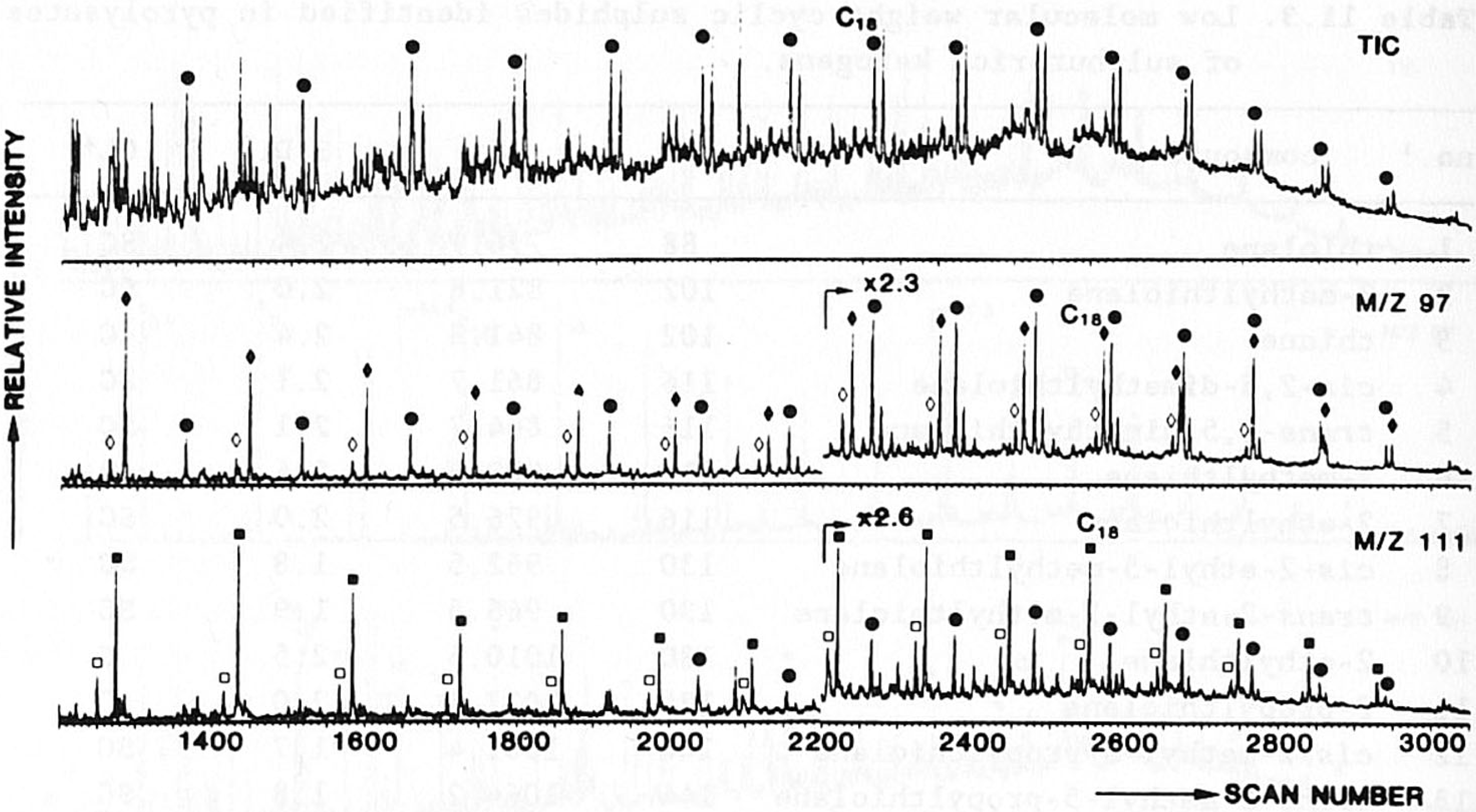


Fig. 11.7. Mass chromatograms of *m/z* 97 and 111, showing the homologous series of 2-alkylthiophenes (black diamonds), 2-alkyl-5-methylthiophenes (black squares) and *n*-alk-1-enes (black circles), and part of the TIC (upper trace) of the pyrolysate (Curie temperature 610°C) of the kerogen of the oil shale of Jurf ed Darawish. C_{18} indicates the member of the homologous series with 18 carbon atoms. The other homologous series present in the mass chromatograms are those of 2-(alkenyl)thiophenes, probably 2-(ω-alkenyl)-thiophenes (white diamonds) and 2-(alkenyl)-5-methylthiophenes, probably 2-(ω-alkenyl)-5-methylthiophenes (white squares). Conditions are given in the caption of Fig. 11.1.

benzo[*b*]thiophene (VII), 4-alkyl-2-methylbenzo[*b*]thiophene (VIII) and 2-alkylbenzo[*b*]thiophene (IX) series these identifications were based on comparison of the mass spectral and RI (Table 11.4) data with those of synthetic C_{18} standards of these homologous series (Table 11.2).

11.5 CONCLUSIONS

Py-GC-FID/FPD and Py-GC-MS analysis of five different sulphur rich, type II-S kerogens revealed the presence of large numbers of organic sulphur pyrolysis products (OSPP). Detection of these compounds by mass spectrometry is preferable over sulphur-selective flame photometric detection because of the considerable loss of sensitivity of the latter for higher molecular weight components. Mass spectral and relative retention time data of a number of synthetic standards were of vital importance for identifying most

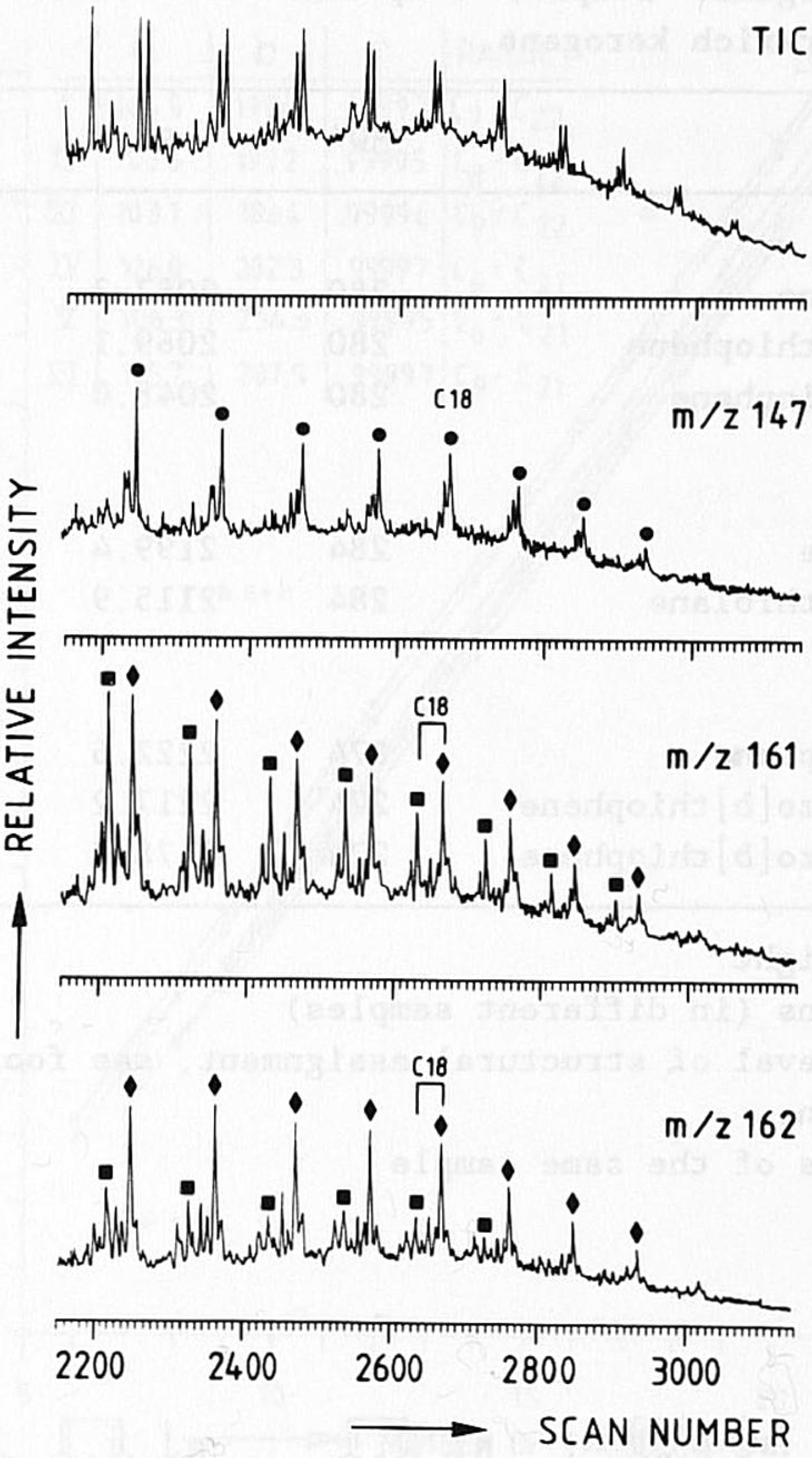


Fig. 11.8. Mass chromatograms of *m/z* 147, 161 and 162, showing the homologous series of 2-alkylbenzo[*b*]thiophenes (black circles), 2-alkyl-4-methylbenzo[*b*]thiophenes (black squares) and 4-alkyl-2-methylbenzo[*b*]thiophenes (black diamonds), and part of the TIC (upper trace) of the pyrolysate (Curie temperature 610°C) of the kerogen of the Phosphoria Retort Shale. C_{18} indicates the member of the homologous series with 18 carbon atoms. Conditions are given in the caption of Fig. 11.1.

Table 11.4. C_{18} organic sulphur compounds identified in pyrolysates of sulphur-rich kerogens.

compound	MW[1]	RI	S.D.[2]	CL[3]
thiophenes				
2-tetradecylthiophene	280	2087.3	2.9	SC
2-methyl-5-tridecylthiophene	280	2069.1	3.0	SC
2-dodecyl-5-ethylthiophene	280	2048.0	3.1	SC
thiolanes				
2-tetradecylthiolane	284	2199.4	3.3	SC
2-methyl-5-tridecylthiolane	284	2115.9	2.9[4]	SC
benzothiophenes				
2-decylbenzo[*b*]thiophene	274	2222.6	1.1[5]	SC
4-methyl-2-nonylbenzo[*b*]thiophene	274	2217.2	0.7[5]	SC
2-methyl-4-nonylbenzo[*b*]thiophene	274	2178.6	1.0[5]	SC

[1] MW = Molecular Weight

[2] five determinations (in different samples)

[3] CL = Confidence Level of structural assignment, see footnote to Table 11.1

[4] four determinations

[5] two determinations of the same sample

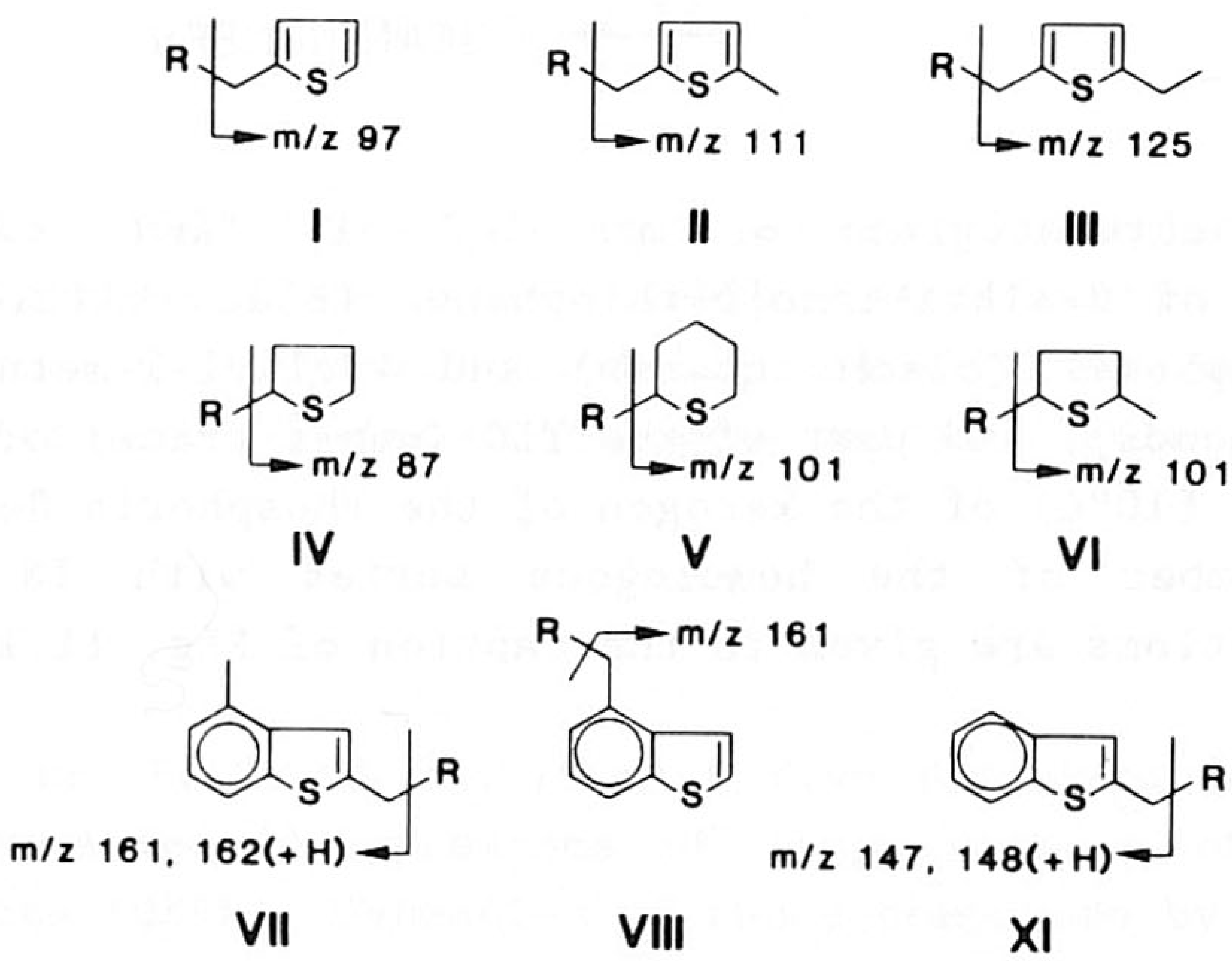
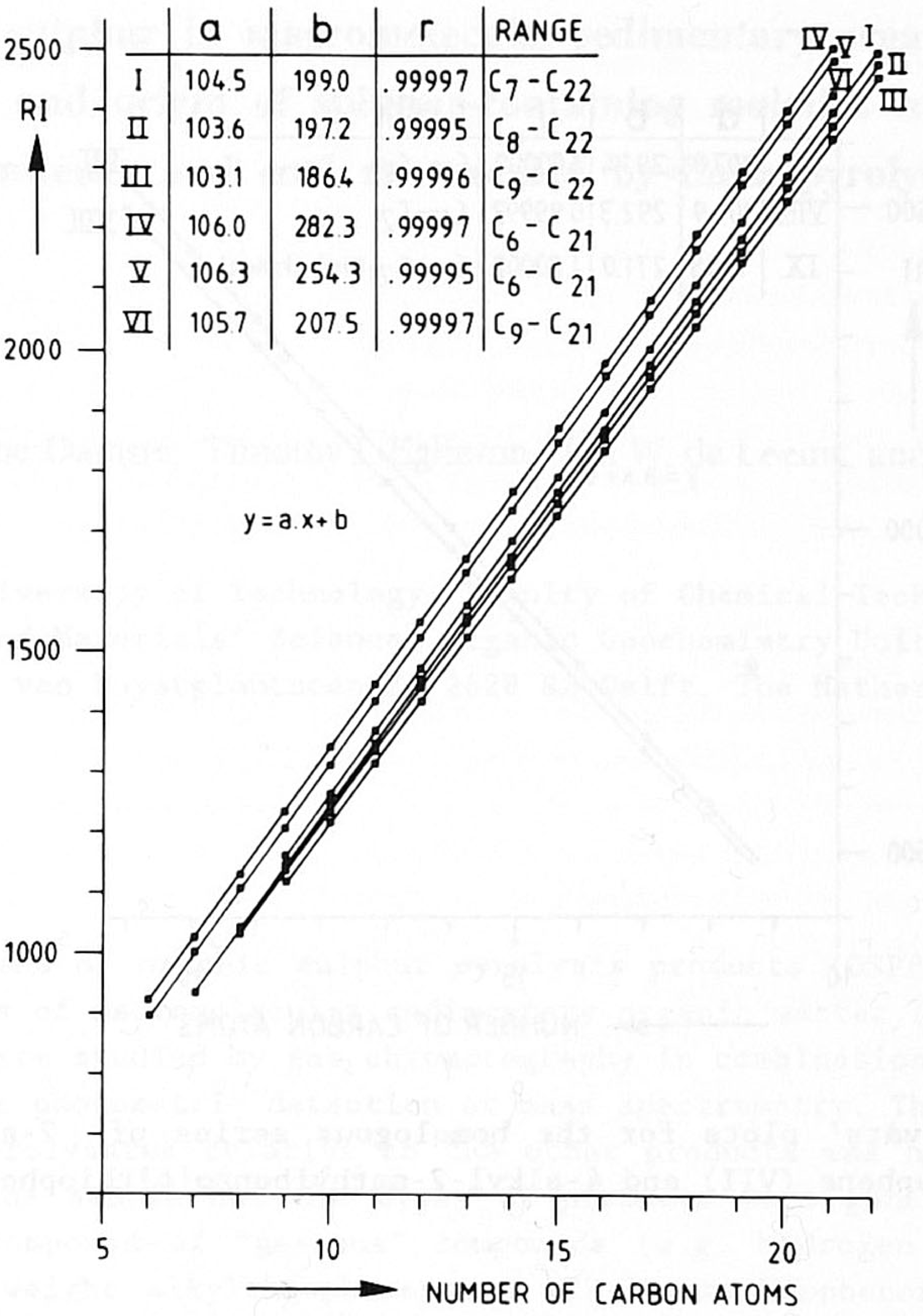


Fig. 11.9. Mass spectral fragmentation of nine homologous series of OSPP encountered in pyrolysates of sulphur-rich kerogens.



	a	b	r	RANGE
I	104.5	199.0	.99997	C_7-C_{22}
II	103.6	197.2	.99995	C_8-C_{22}
III	103.1	186.4	.99996	C_9-C_{22}
IV	106.0	282.3	.99997	C_6-C_{21}
V	106.3	254.3	.99995	C_6-C_{21}
VI	105.7	207.5	.99997	C_9-C_{21}

Fig. 11.10. Kováts' plots for the homologous series of 2-alkylthiophenes (I), 2-alkyl-5-methylthiophenes (II), 2-alkyl-5-ethylthiophenes (III), 2-alkylthiolanes (V), and 2-alkyl-5-methylthiolanes (VI).

of the low molecular weight OSPP. In the higher molecular weight range homologous series of 2-alkylthiophenes, 2-alkyl-5-methylthiophenes, 2-alkyl-5-ethylthiophenes, 2-alkylthiolanes, 2-alkylthianes, 2-alkyl-5-methylthiolanes, 2-alkyl-4-methylbenzo[*b*]thiophenes, 4-alkyl-2-methylbenzo[*b*]thiophenes and 2-alkylbenzo[*b*]thiophenes were identified by the straight lines obtained in their Kováts' plots and by comparison of mass spectral and relative retention time data of the C_{18} members of these series with those of synthetic standards (except for the 2-alkylthiane series).

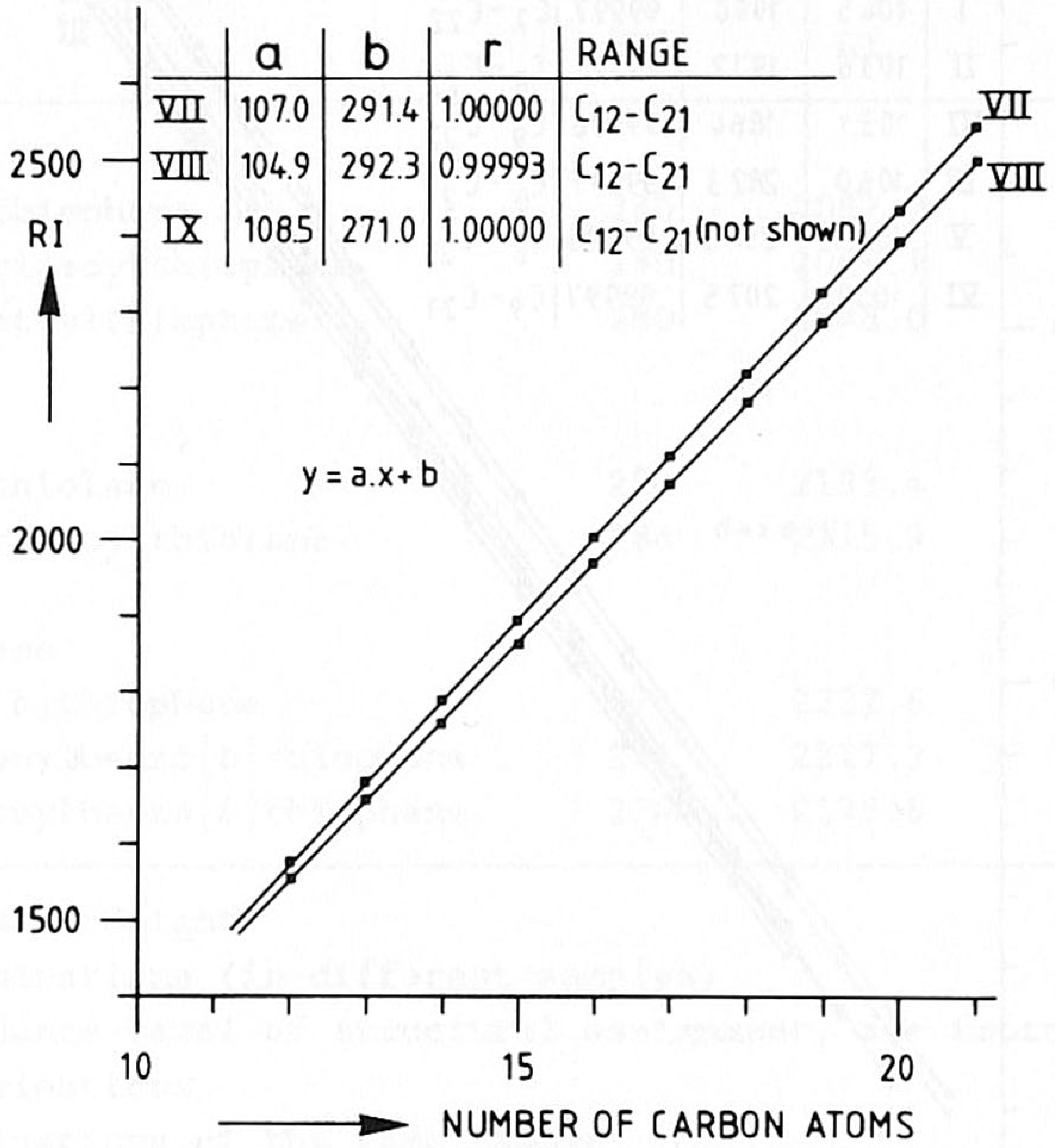


Fig. 11.11. Kováts' plots for the homologous series of 2-alkyl-4-methylbenzo[*b*]thiophene (VII) and 4-alkyl-2-methylbenzo[*b*]thiophene (VIII).

12. Organic sulphur in macromolecular sedimentary organic matter. I. Structure and origin of sulphur-containing moieties in kerogen, asphaltenes and coal as revealed by flash pyrolysis.*

Jaap S. Sinninghe Damsté, Timothy I. Eglinton, Jan W. de Leeuw and P.A. Schenck

Delft University of Technology, Faculty of Chemical Technology
and Materials' Science, Organic Geochemistry Unit,
de Vries van Heystplantsoen 2, 2628 RZ Delft, The Netherlands.

12.1 ABSTRACT

The distributions of organic sulphur pyrolysis products (OSPP) generated by flash pyrolysis of macromolecular sedimentary organic matter (kerogen, coal, asphaltenes) were studied by gas chromatography in combination with sulphur-selective flame photometric detection or mass spectrometry. The abundance of OSPP in the pyrolysates relative to the other products was highly variable depending on the sample but the types of products were generally similar, being mainly composed of "gaseous" compounds (*e.g.* hydrogen sulphide) and low molecular weight alkylthiophenes and alkylbenzothiophenes. The distribution patterns of the alkylated thiophenes were dominated by a limited number of all theoretically possible isomers. The alkyl substitution patterns of the dominant isomers bear a strong similarity to those of the organic sulphur compounds present in bitumens and immature oils. Therefore, it is suggested that these OSPP are formed by pyrolysis of related thiophenic and benzothiophenic moieties present in the macromolecular sedimentary substances. Specific examples include those with linear alkyl, *iso* and *anteiso* alkyl, isoprenoid alkyl and steroidal side-chain carbon skeletons. The presence of higher molecular weight alkylthiophenes and alkylbenzothiophenes with these same carbon skeletons in pyrolysates of sulphur-rich kerogens provided further evidence for the presence of these sulphur-containing moieties. It is likely that these moieties have been formed by abiogenic sulphur incorporation into sedimentary organic matter during early diagenesis.

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* *Geochim. Cosmochim. Acta* (submitted)

12.2 INTRODUCTION

The bulk of the organic matter in the earth's crust is in the form of kerogen. Kerogen, defined as that fraction of sedimentary organic matter which is insoluble in common organic solvents, is believed to be comprised of complex macromolecules derived from organic remains (Durand, 1980). Coal, the world's largest fossil fuel resource, may be regarded as a special type of kerogen, derived mainly from terrestrial organic matter (Durand, 1980; Larter, 1984). Petroleum, which is formed by the thermal degradation of kerogen, may also contain large amounts of complex macromolecular materials ("asphaltenes"). Recent evidence suggests that asphaltenes may be considered as small "soluble" fragments of kerogen having comparable structures (*e.g.* Behar and Pelet, 1985; Bandurski, 1982; Tissot and Welte, 1984; Philp and Gilbert, 1985).

Each of these materials is comprised mainly of carbon, hydrogen, oxygen, nitrogen and sulphur. Kerogens are usually classified only on the basis of their carbon, hydrogen and oxygen contents and three (or four) types are usually distinguished (Tissot and Welte, 1984): hydrogen-rich "Type I" kerogens are commonly derived from algal and/or bacterial organic matter deposited in lacustrine environments, "Type II" kerogens from sapropelic marine organic matter and oxygen-rich "Type III" kerogens from mainly higher plant debris.

The organic sulphur content of kerogen varies with its stage of evolution and also with type, as determined by depositional environment and precursor organisms (Durand and Monin, 1980; Tissot and Welte, 1984). In general, immature Type II kerogen in marine sediments contains the highest amount of organic sulphur, whilst the organic sulphur contents of Type I and Type III kerogens are normally quite low. Recently, Orr (1986) proposed a subclassification for Type II kerogens on the basis of their atomic organic sulphur to carbon ratios. Type II kerogens with an atomic S/C ratio greater than 0.04 were designated as "Type II-S" kerogens, because they are "sufficiently different from the classical Type II kerogens such as those of the Paris Basin". However, the organic sulphur content of kerogens is not determined routinely mainly because of the intimate association of pyrite which hinders analysis (Durand and Monin, 1980). For this reason, sulphur-rich kerogens (8-14 wt% organic sulphur) may be more common than generally recognized (Orr, 1986). The relatively high amounts of organic sulphur in kerogen from marine sediments has been explained by reaction of the products of sulphate-reducing bacteria (such as hydrogen sulphide) and elemental sulphur (formed by oxidation of hydrogen sulphide) with organic matter involved in the formation of kerogen (*e.g.* Nissenbaum and Kaplan, 1972; Gransch and Posthuma, 1974; Dinur *et al.*, 1980; Aizenshtat *et al.*, 1983; Tissot and Welte, 1984; Francois, 1987). It should be emphasised that the amount of hydrogen sulphide produced must exceed the amount of iron available in the sediment since it is thought that iron competes more successfully than organic matter as a sink for sulphur (Berner, 1985).

Therefore sulphur-rich kerogens are more likely to be formed in non-clastic environments (*e.g.* carbonates, siliceous oozes, evaporites). The organic sulphur content of coals also vary depending on their depositional environment and stage of evolution, however, it is generally higher in marine-influenced coals.

Organic sulphur in kerogen may play a particular role in oil generation since there is evidence to suggest that high-sulphur kerogens begin to generate oil at a lower rank levels than classical Type II kerogens (*e.g.* Tannenbaum and Aizenshtat, 1985; Lewan, 1985; Jones, 1984). The presence of abundant, relatively weak sulphur-sulphur and sulphur-carbon bonds is believed to lower the maximum of the activition energy distribution for the thermal degradation of the kerogen. Kerogens with a high organic sulphur content are believed to yield high sulphur oils (Gransch and Posthuma, 1974).

Despite the obvious importance of organic sulphur determining the composition and fate of macromolecular sedimentary organic matter, very little is known about the distribution of sulphur-containing functional groups in these materials. Organic sulphur in kerogen is thought to be present in sulphide and disulphide moieties and as atomic constituents of heterocycles (Tissot and Welte, 1984). Speight and Moschopedis (1981) suggest that organic sulphur in asphaltenes is present only in aromatic forms such as thiophene derivatives, while others consider sulphide sulphur to be present as well (Ignasiak *et al.*, 1977). Recently, a detailed study of the sulphur species in a representative coal, rich in organically-bound sulphur, using complimentary analytical methods revealed the presence of labile sulphur moieties such as aliphatic thiols and sulphides as well as thermally more stable forms including thiophene derivatives (Boudou *et al.*, 1987). These contradictions may be resolved by further studies.

Several approaches aimed at elucidating partial sulphur-containing structures present in kerogens and other macromolecular sedimentary organic matter can be envisaged. However, specific chemical degradation reactions (*e.g.* selective S-S or S-C cleavages) have, so-far, not been successfully applied to such intractible substances as kerogen, whilst spectroscopic techniques (*e.g.* nuclear magnetic resonance, infra-red spectroscopy) provide information about functional groups rather than information about the arrangements of atoms. Recently, Barakat and Yen (1988) found that the acid oxidation of a sulphur-rich kerogen did not provide any information about its sulphur-containing moieties. Flash-pyrolysis, in combination with gas chromatography (GC) and/or mass spectrometry (MS), has been shown previously to be a useful technique for the characterisation of complex macromolecules including kerogens (*e.g.* Larter and Douglas, 1978, van de Meent *et al.*, 1980), coals (*e.g.* Nip, 1987) and asphaltenes (*e.g.* Behar and Pelet, 1985). However, with regard to organic sulphur, pyrolysis has been less extensively employed. Examples include bulk pyrolysis studies where the total amount of pyrolysable organic sulphur in sedimentary rocks (Madec and Espitalie, 1985) and the sulphur isotope composition of the various forms of organic sulphur in macromolecular substances (Krouse *et al.*, 1987) have been determined. Boudou *et al.* (1987) studied the sulphur-containing flash pyrolysis products of an organic sulphur-rich coal at different Curie-temperatures. Eglinton *et al.* (1988b) studied variations in the distributions of sulphur-containing products from flash pyrolysis of artificially matured kerogens from the Kimmeridge Clay. The organic sulphur pyrolysis products were not identified in detail in any of these pyrolysis studies. Recently, however, the major sulphur-containing products in the flash pyrolysates of sulphur-rich kerogens have been identified (Sinninghe Damsté *et al.*, 1988c).

In this paper we report on the GC-amenable organic sulphur compounds present in flash pyrolysates of several types of kerogens, coals and asphaltenes. The structures of these organic sulphur pyrolysis products (OSPP) indicate the presence of specific structural moieties within these macromolecules. These moieties are probably formed by abiogenic sulphur incorporation into sedimentary organic matter during early diagenesis and therefore provide direct evidence on a molecular level for the generally accepted origin of sulphur in kerogen.

12.1 EXPERIMENTAL

Samples. Rock samples (Table 12.1) were powdered in a rotary disc mill, Soxhlet or ultrasonically extracted and finally dried in a vacuum stove (40°C). Some samples were treated with HCl to remove carbonate and others were more thoroughly demineralized using slightly modified standard procedures (Eglinton *et al.*, 1988a). Coal samples were pyrolysed without any pretreatment. Asphaltene fractions were prepared from crude oils and bitumens by precipitation with *n*-heptane.

Flash pyrolysis-gas chromatography. Flash pyrolysis-gas chromatography (Py-GC) was carried out as previously described (Sinninghe Damsté *et al.*, 1988c). Briefly, the samples were pyrolysed at 610°C (or 358°C) for 10 sec. using a Curie point-pyrolysis device. The pyrolysis products were separated on a fused-silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness 0.45 μm) using a temperature programme from 0°C (5 min) to 300°C (20 min) at a rate of 3°C/min. The separated pyrolysis products were detected simultaneously by both a flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD), using a stream splitter at the end of the capillary column.

The OSPP were not simply the result of secondary reactions of products generated upon pyrolysis with elemental sulphur or pyrite present in the mineral matrix as was demonstrated by pyrolysing synthetic mixtures of sulphur-lean sedimentary organic matter with elemental sulphur or pyrite (Coomans, unpublished results). Flash pyrolysis of a set of samples (whole rock, extracted rock, de-mineralised rock) showed that the kerogen isolation procedure had no significant influence on the distribution of OSPP.

Replicate analyses indicated good reproducibility for the chromatographic data.

A quantitative assessment of the relative amounts of OSPP was made by integration of peak areas in the chromatograms followed by calculation of the square roots (to account for the approximately quadratic response of the FPD detector; Farwell and Barinaga, 1986). To obtain data comparable to those acquired by using a FID, a correction for the atomic sulphur fraction in each molecule was also made.

Flash pyrolysis-gas chromatography-mass spectrometry. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was carried out using a similar Curie point-pyrolysis device and a Varian 3700 gas chromatograph connected to a Varian MAT 44 S quadrupole mass spectrometer as described previously (Sinninghe Damsté *et al.*, 1988c).

10.3 RESULTS AND DISCUSSION

For the study of organic sulphur moieties in macromolecular sedimentary organic matter, isolated kerogens, unextracted and extracted rocks, asphaltenes from oils and bitumens, and coals were subjected to flash pyrolysis (Py). The pyrolysis products were separated by capillary gas chromatography and detected by both a flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD). Sixty samples (Table 12.1) were studied in this way. All of the samples studied produced sufficient OSPP to be monitored by the FPD. The amounts of OSPP varied considerably relative to the other pyrolysis products (*e.g.* alkanes, alkenes, alkylated benzenes) (Eglinton *et al.*, 1988d), but generally correlated with the amount of sulphur present in the starting material. For example, OSPP were barely detectable in the FID chromatograms of the lacustrine Green River, Autun and Messel shales whereas alkylated thiophenes were major components in the pyrolysates of so-called Type II-S kerogens (Monterey, Kimmeridge, Phosphoria, Serpiano and Jurf ed Darawish shales) and sulphur-rich asphaltenes.

Five samples were studied in detail and selected geochemical data for these are presented in Table 12.2. They were chosen as typical examples of four types of kerogen and of the asphaltenes isolated from a high-sulphur oil. In addition, one sample was chosen to study the relationships between the OSPP from a kerogen and of the asphaltenes isolated from the corresponding bitumen and another sample (Phosphoria Retort shale) was chosen for detailed study by GC-MS. Since maturation is known to have a major influence on the organic sulphur content of a kerogen, it is likely that the OSPP distributions may partly reflect maturity, therefore only relatively immature samples were studied in detail.

The kerogen of the Ordovician Guttenberg Oil Rock is immature Type I and most probably algal-derived (Fowler and Douglas, 1984). The Lower Toarcian sediments of the Paris Basin are classical examples of marine shales

Table 12.1. Kerogens, coals and asphaltenes studied which show characteristic OSPP upon flash pyrolysis.

Name	Location	Age	Type
Kerogens			
Autun	Surmoulin, France	Permian	I
Green River	Colorada, U.S.A.	Eocene	I
Guttenberg Oil Rock	Wisconsin, U.S.A.	Ordovician	I
Heather	North Sea	Jurassic	II/III
Jurf ed Darawish (3)	Jordan	Cretaceous	II-S
Jet Rock	U.K.	Lias	II
Kimmeridge (4)	Dorset, U.K.; North Sea	Jurassic	II
Marl Slate	North Sea	Permian	II
Messel	F.R.G.	Eocene	II
Monterey (6)	California, U.S.A.	Miocene	II-S
New Albany	Indiana, U.S.A.	Devonian	II
Paris Basin (10)	France	Toarcian	II
Phosphoria Retort	Montana, U.S.A.	Permian	II-S
Posidonia	Marienburg, F.R.G.	Toarcian	II
Serpiano	Switzerland	Triassic	II-S
Tasmanite	Tasmania, Australia	Permian	I
Westfield	Lothians, U.K.	Carboniferous	I
Woodford	Oklahoma, U.S.A.	Devonian	II
coals			
Bark coal	China	Eocene	III
Mahakam (7)	Indonesia	Tertiary	III
PSOC 667	?	?	III
Raza lignite	Yugoslavia	?	III
SBN 136FR45	France	?	III
SBN 133US42	Pittsburg, U.S.A.	?	III
SBN 511US43	Illinois, U.S.A.	?	III
asphaltenes			
from oils:			
L.A. Basin	California, U.S.A.	Miocene	n.a.
Rozel Point seep oil	Utah, U.S.A.	Miocene	
from bitumens:			
Athabasca	Canada	Cretaceous	?
Jurf ed Darawish (3)	Jordan	Cretaceous	II-S[1]
Kimmeridge	Dorset, U.K.; North Sea	U. Jurassic	II-S[1]
Monterey	California, U.S.A.	Miocene	II-S[1]
New Albany	Indiana, U.S.A.	Devonian	II[1]
Northern Apennines Marl	Italy	Miocene	II-S[1]

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[1] type of organic matter of source rock

Table 11.2. Geochemical data of samples studied in detail.

Sample	Age	$\%R_0$[1]	TOC[2]	Elemental Analysis H/C	O/C	N/C	S(tot)/C	S(org)/C
Guttenberg Oil rock	Ordovician	n.a.[3]	8.0	1.48	0.10	0.013	n.d.[4]	n.d.
Paris Basin, Dontrien	Toarcian	0.45	n.d.	1.26	0.09	0.033	n.d.	0.015
Monterey shale, El Capitan Beach	Miocene	0.40[5]	23.7	1.45	n.d.	0.049	0.058[7]	n.d.
Jurf ed Darawish Oil shale-156	Cretaceous	0.30[5]	17.3[6]	1.60	n.d.	0.024	0.084[7]	n.d.
Phosphoria Retort shale	Permian	0.28[5]	16.1[6]	1.43	n.d.	0.026	0.068[8]	n.d.
Gardanne coal	-	0.48	60.2	0.81	0.13	0.020	0.032	0.024
L.A. Basin asphaltene	Miocene	n.a.	80.3	1.21	n.d.	0.038	n.a.	0.025
Jurf ed Darawish Oil shale-156 asphaltene	Cretaceous	n.a.	56.8	1.44	n.d.	0.025	0.072	n.d.

[1] $\%R_0$ = vitrinite reflectance
[2] TOC = Total Organic Carbon
[3] n.a. = not applicable
[4] n.d. = not determined
[5] vitrinite reflectance measurements may be suppressed due to bitumen impregnation
[6] determined on solvent-extracted rock samples
[7] microscopic examination indicated low pyrite content
[8] microscopic examination indicated low-moderate pyrite content

containing Type II kerogen (Durand and Monin, 1980; Tissot and Welte, 1984). The sample chosen for detailed study (Dontrien) is marginally mature (Ensminger *et al.*, 1978). The Miocene Monterey Formation is considered to contain Type II-S kerogen (Orr, 1986). The immature sample included in the present study is from an outcrop section at El Capitan Beach, California. The Permian Phosphoria Retort shale (Montana, U.S.A.; Lewan, 1985) and the Cretaceous Jurf ed Darawish oil shale (Jordan; Wehner and Hufnagel, 1987) also contain immature Type II-S kerogen. The L.A. Basin crude oil (Playa del Rey Fd., California) is believed to have originated from Miocene sulphur-rich source rocks (S. Larter, pers. commun.). The coal sample originates from the Houilleres de Province mine, France) and is of high volatile bitumenous rank.

Distributions of OSPP

FPD pyrolysis-gas chromatograms of five samples are shown in Fig. 12.1. Peak assignments are listed in Table 12.3. Most of the compounds were identified by comparison of mass spectral and relative retention time data with those of authentic standards (Sinninghe Damsté *et al.*, 1988c).

The major OSPP for all samples studied were highly volatile organic sulphur compounds (H_2S, COS, SO_2), which collectively make up the "gas" peak, alkylated thiophenes and, to a lesser extent, alkylated benzothiophenes. Minor OSPP included alkylated thiolanes and thianes, alkylated dibenzothiophenes, and unsaturated alkylated thiophenes. Despite the major variations in relative abundance, the same alkylated thiophenes dominated the pyrolysates of the samples studied in detail. In the C_3-alkylated thiophenes, for example, only eight of the possible twelve theoretical isomers were found. Of those eight, two were highly dominant; namely 2-ethyl-5-methylthiophene (compound 18) and 2,3,5-trimethylthiophene (compound 20) together accounting for between 47% and 67% of the total amount of the twelve possible isomers (Fig. 12.2). Other pyrograms were similarly dominated by these two structural isomers. It was noted that each of the alkylthiophene clusters was dominated by only a limited number of isomers. Furthermore, it was apparent that a relationship existed between alkylated thiophenes with identical substitution patterns. For example, the OSPP of the Guttenberg kerogen were characterised by relatively large amounts of 2-alkylthiophenes (compounds 3, 8, 16, 30, 47, 58, 63 and 66 in Fig. 12.1A and 12.2A). In contrast, the thiophene distribution produced from the kerogen of the Paris Basin was dominated by 2-alkyl-5-methylthiophenes (compounds 9, 17, 28, 46, 56, 62, 65 in Fig. 12.1B and 12.2B) and lesser amounts of 2-alkylthiophenes (except for 2-methylthiophene). These homologous series extend to C_{22} as reported previously (Sinninghe Damsté *et al.*, 1988c). These similarities within one sample and variations between samples of different origin suggest that OSPP reflect specific structural moieties in the kerogen structure.

Alkylated thiophenes are the most abundant thiophenic compounds in shale oils (Joyce and Uden, 1983) and are also the major organic sulphur compounds reported in several sediment extracts and immature crude oils (Schmid, 1986; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987a and c, 1988g). It is, therefore, not unlikely that a major fraction of organic sulphur in the immature macromolecular materials studied is present in thiophene moieties.

Possible mechanisms of formation of OSPP

In an attempt to explain the distributions of OSPP described above, a discussion covering the mechanisms of their formation is necessary. Our results and those of Boudou *et al.* (1987) indicate that flash pyrolysis of

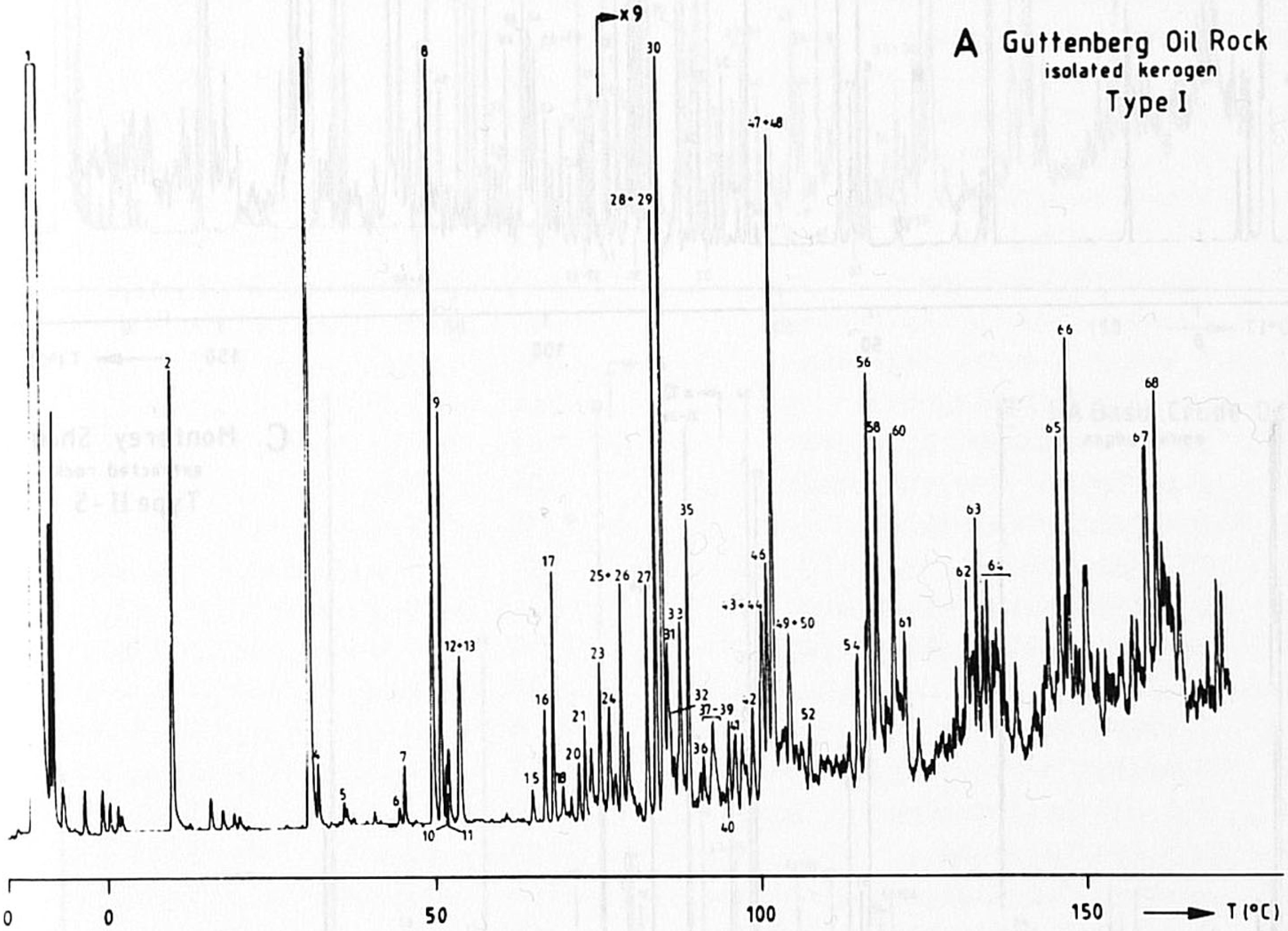


Fig. 12.1. Partial FPD chromatograms of the pyrolysis products generated by flash pyrolysis (Curie temperature 610°C) of (A) an isolated kerogen of the Guttenberg Oil Rock, (B) a de-carbonated and extracted sample from the Paris Basin shale (Dontrien), (C) an extracted sample from the Monterey shale (outcrop from El Capitan Beach), (D) a coal from the Gardanne layer, (E) an isolated asphaltene fraction from a L.A. Basin oil. Numbers refer to Table 12.3. The first parts (0°C to 75°C) of the chromatograms are normalised to the second most abundant alkylthiophene present; the second parts are normalised to the most abundant component present.

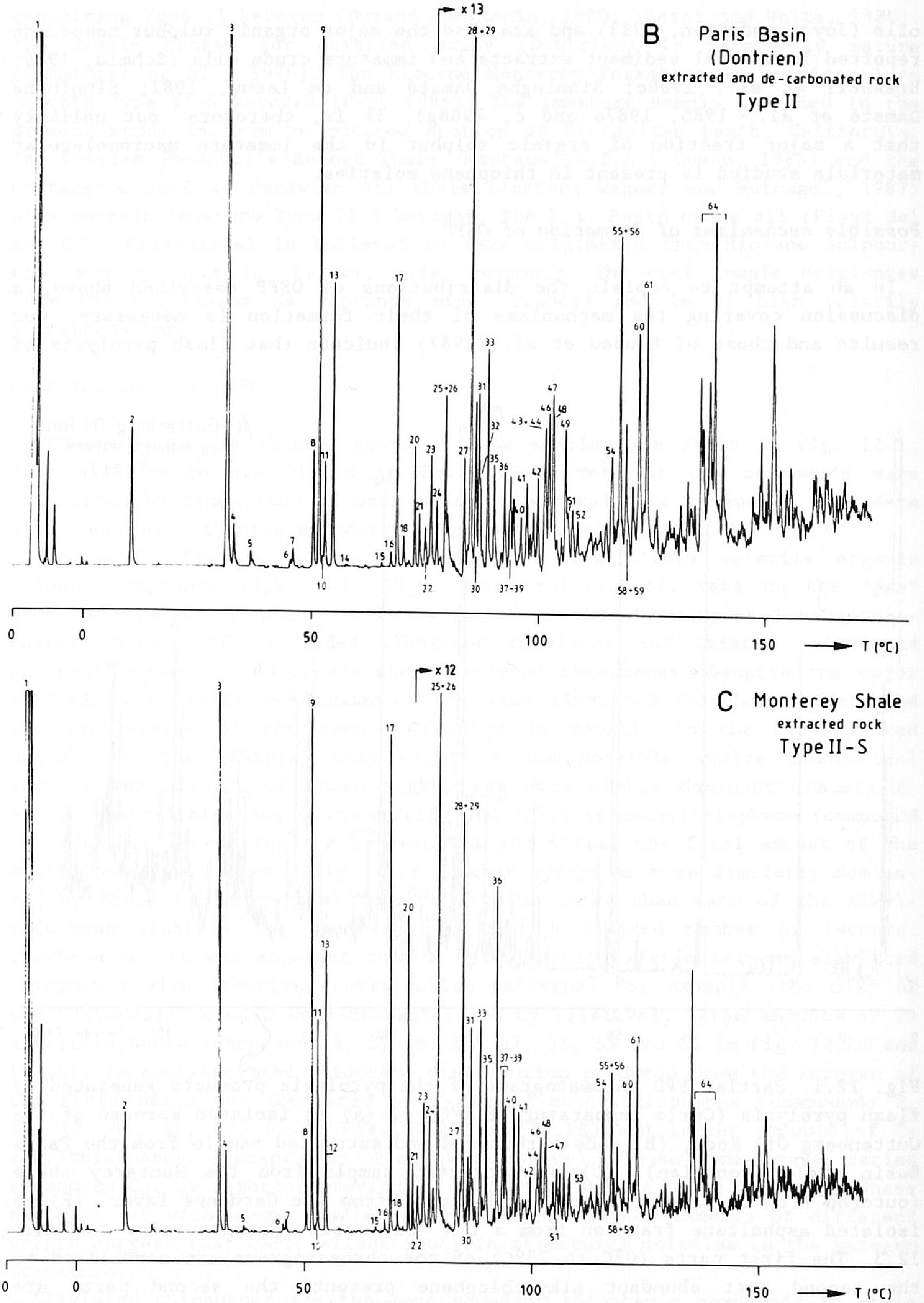


Fig. 12.1. (Continued)

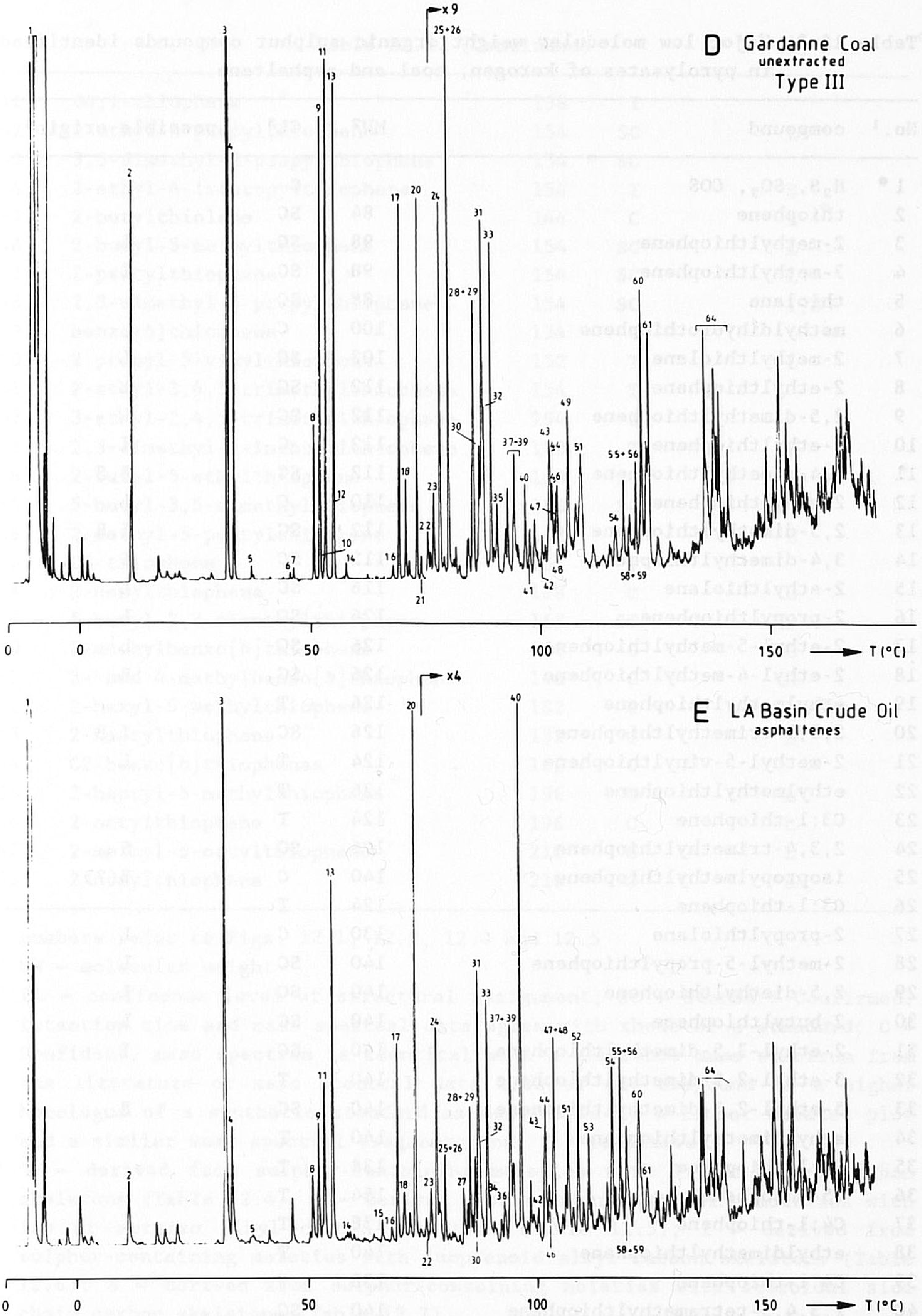


Fig. 12.1. (Continued)

Table 12.3. Major low molecular weight organic sulphur compounds identified in pyrolysates of kerogen, coal and asphaltene.

No.[1]	compound	MW[2]	CL[3]	possible origin[4]
1	H_2S, SO_2, COS	-	C	
2	thiophene	84	SC	
3	2-methylthiophene	98	SC	L
4	3-methylthiophene	98	SC	I
5	thiolane	88	SC	
6	methyldihydrothiophene	100	C	
7	2-methylthiolane	102	SC	L
8	2-ethylthiophene	112	SC	L
9	2,5-dimethylthiophene	112	SC	L
10	3-ethylthiophene	112	C	I
11	2,4-dimethylthiophene	112	SC	B,S
12	2-vinylthiophene	110	C	L
13	2,3-dimethylthiophene	112	SC	I,B
14	3,4-dimethylthiophene	112	SC	S
15	2-ethylthiolane	116	SC	L
16	2-propylthiophene	126	SC	L
17	2-ethyl-5-methylthiophene	126	SC	L
18	2-ethyl-4-methylthiophene	126	SC	B
19	ethylmethylthiophene	126	T	
20	2,3,5-trimethylthiophene	126	SC	I,B
21	2-methyl-5-vinylthiophene	124	T	L
22	ethylmethylthiophene	126	T	
23	C3:1-thiophene	124	T	
24	2,3,4-trimethylthiophene	126	SC	S
25	isopropylmethylthiophene	140	C	B(?)
26	C3:1-thiophene	124	T	
27	2-propylthiolane	130	C	L
28	2-methyl-5-propylthiophene	140	SC	L
29	2,5-diethylthiophene	140	SC	L
30	2-butylthiophene	140	SC	L
31	2-ethyl-3,5-dimethylthiophene	140	SC	I
32	3-ethyl-2,5-dimethylthiophene	140	T	
33	5-ethyl-2,3-dimethylthiophene	140	SC	B
34	ethyldimethylthiophene	140	T	
35	C4:1-thiophene	138	T	
36	C5-thiophene	154	T	
37	C4:1-thiophene	138	T	
38	ethyldimethylthiophene	140	T	
39	C4:1-thiophene	138	T	
40	2,3,4,5-tetramethylthiophene	140	SC	

Table 12.3. (Continued)

41	C4:1-thiophene	138	T	
42	2-ethyl-5-propylthiophene	154	SC	L
43	3,5-dimethyl-2-propylthiophene	154	SC	
44	2-ethyl-4-isopropylthiophene	154	T	S
45	2-butylthiolane	144	C	L
46	2-butyl-5-methylthiophene	154	SC	L
47	2-pentylthiophene	154	SC	L
48	2,3-dimethyl-5-propylthiophene	154	SC	I,B
49	benzo[*b*]thiophene	134	C	
50	2-propyl-5-vinylthiophene	152	T	L
51	2-ethyl-3,4,5-trimethylthiophene	154	T	
52	3-ethyl-2,4,5-trimethylthiophene	154	T	
53	2,3-dimethyl-5-isobutylthiophene	168	T	I
54	2-butyl-5-ethylthiophene	168	SC	L
55	5-butyl-3,5-dimethylthiophene	168	SC	
56	2-methyl-5-pentylthiophene	168	C	L
57	C6-thiophene	168	T	
58	2-hexylthiophene	168	C	L
59	5-butyl-2,3-dimethylthiophene	168	SC	B
60	2-methylbenzo[*b*]thiophene	148	C	L
61	3- and 4-methylbenzo[*b*]thiophene	148	C	L
62	2-hexyl-5-methylthiophene	182	C	L
63	2-heptylthiophene	182	C	L
64	C2-benzo[*b*]thiophenes	162	C	
65	2-heptyl-5-methylthiophene	196	C	L
66	2-octylthiophene	196	C	L
67	2-methyl-5-octylthiophene	210	C	L
68	2-nonylthiophene	210	C	L

[1] numbers refer to Figs. 12.1, 12.2, 12.4 and 12.5

[2] MW = molecular weight

[3] CL = confidence level of structural assignment; SC = Standard Confirmed, retention time and mass spectral data agree with those of a standard; C = Confident, mass spectrum is identical with a reference mass spectrum from the literature or mass spectral data base or the compound is a higher homologue of a synthetic standard as determined by a linear Kovats' plot and a similar mass spectral fragmentation; T = Tentative

[4] L = derived from sulphur-containing moieties with linear alkyl carbon skeletons (Table 12.4); B = derived from sulphur-containing moieties with *iso* or *anteiso* alkyl carbon skeletons (Table 12.5); I = derived from sulphur-containing moieties with isoprenoid alkyl carbon skeletons (Table 12.6); S = derived from sulphur-containing moieties with steroidal side chain carbon skeletons (Table 12.7).

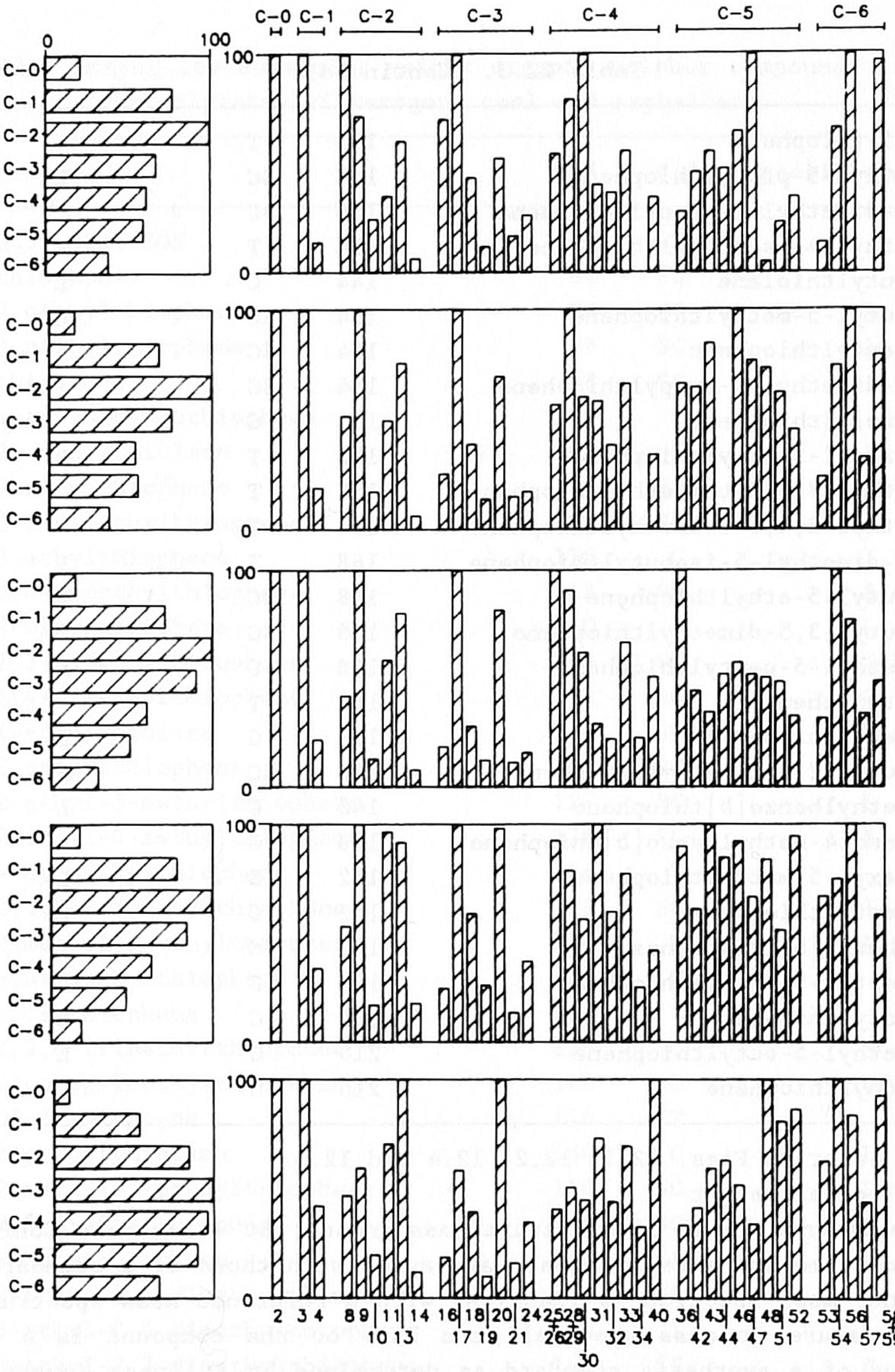


Fig. 12.2. Bar graphs showing the carbon number distributions of the alkylthiophenes generated by flash pyrolysis (left bar graphs) and their internal distribution (right bar graphs) of (A) the isolated kerogen of the Guttenberg Oil Rock, (B) a de-carbonated and extracted sample (Dontrien) of the Paris Basin shale, (C) an extracted rock sample of the Monterey shale, (D) the Gardanne coal and (E) the asphaltene fraction of the L.A. Basin crude oil. The internal distributions of the alkylated thiophenes are normalized to the most abundant component. Numbers refer to Table 12.3.

kerogens (including coals), and asphaltenes, performed with ferromagnetic wires having a Curie temperature of 358°C generates significant amounts of hydrogen sulphide and some other highly volatile sulphur compounds. Only traces of other OSPP such as alkylated thiophenes and *n*-alkanes and *n*-alk-1-enes were encountered in the pyrolysates. It is concluded that under these mild pyrolysis conditions only S-S bonds (*ca.* 275 kJ/mol) and C-S bonds (*ca.* 275 kJ/mol) are cleaved whilst C-C bonds (*ca.* 350 kJ/mol) remain intact. This assumption is substantiated by literature data on model substances and asphaltenes. Degradation of thiols, sulphides and disulphides occurs at significantly lower temperatures than the thermal degradation of thiophenes and benzothiophenes (Giraud and Bestougeff, 1967; Drushel, 1969; Braekman-Danheux, 1985). Furthermore, hydrogen sulphide is a major sulphur-containing product on thermal degradation of thiols and aliphatic sulphides (cyclic and acyclic).

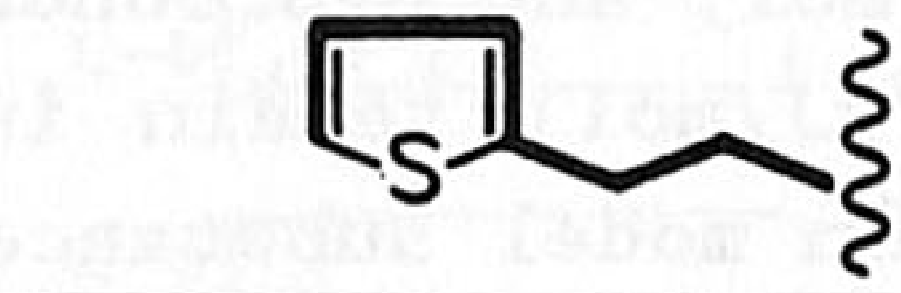
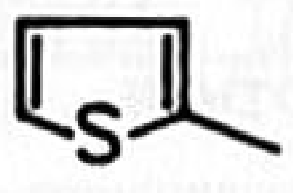
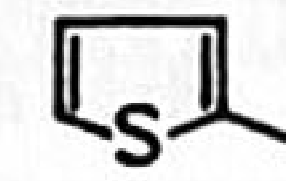
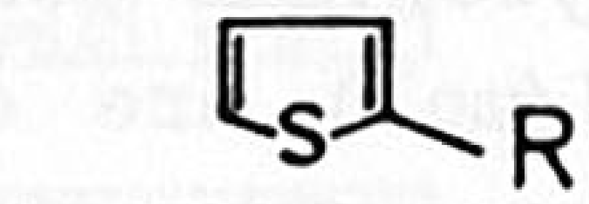
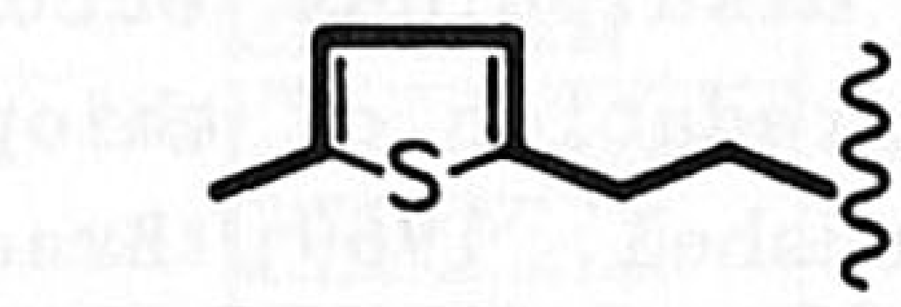
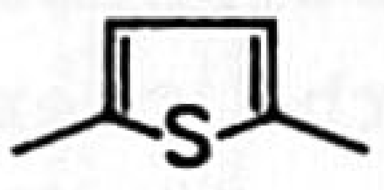
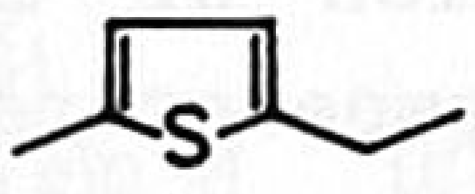
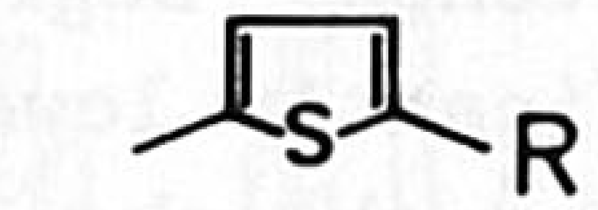
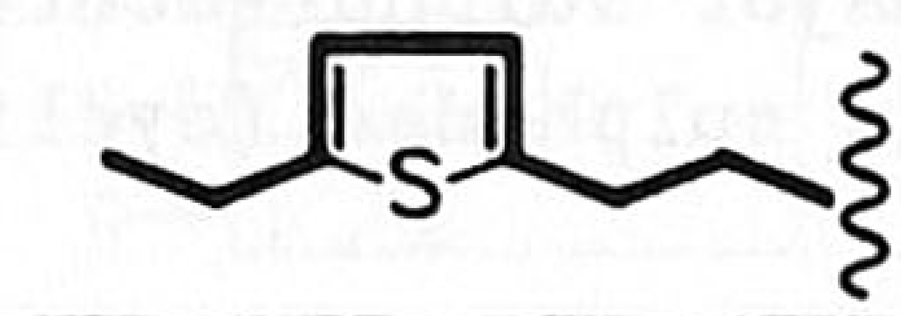
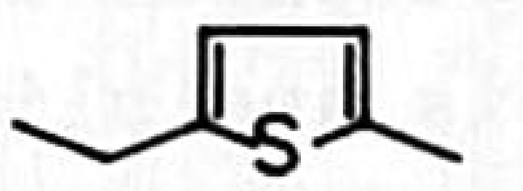
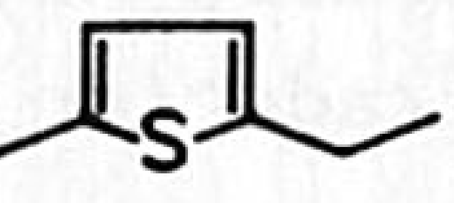
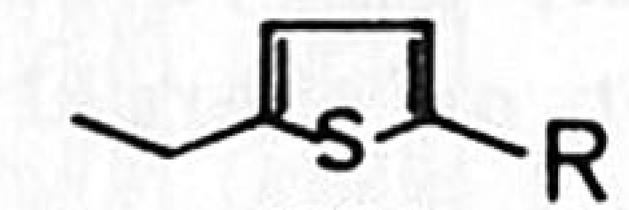
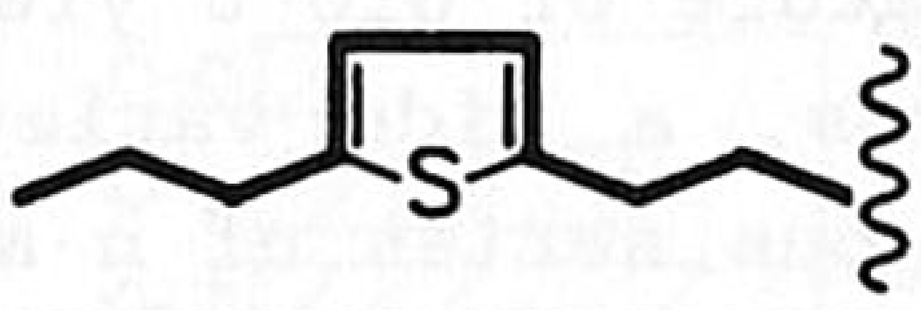
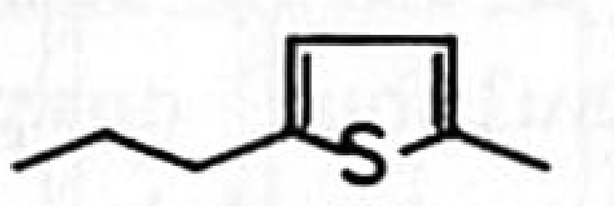
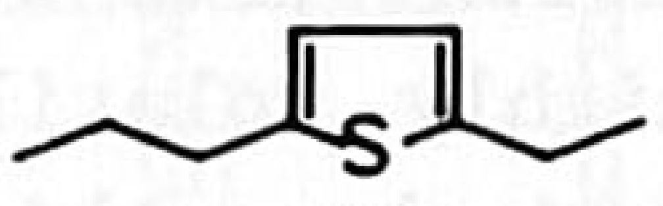
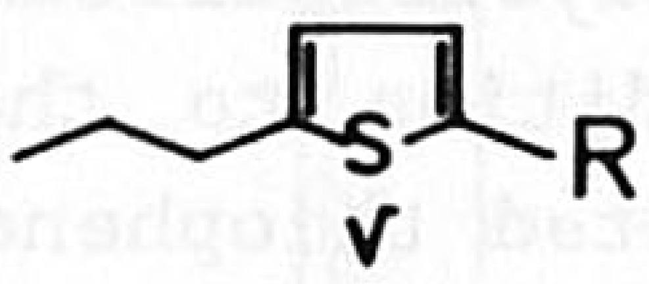
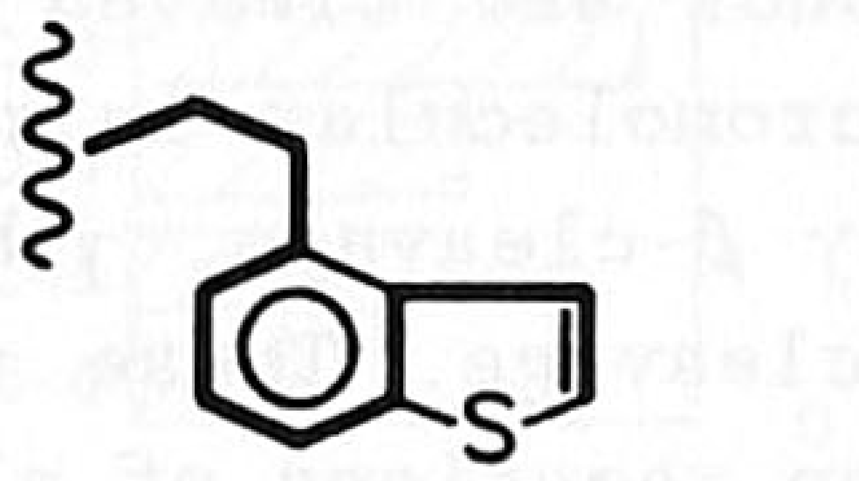
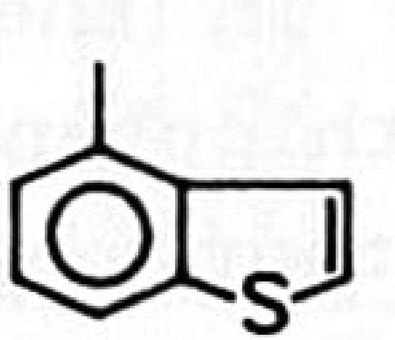
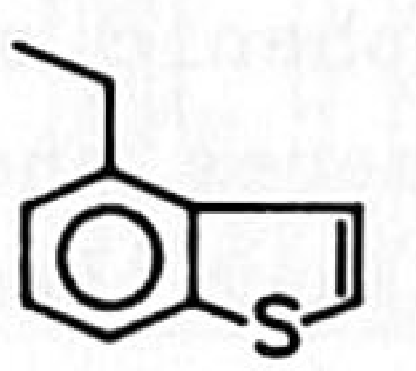
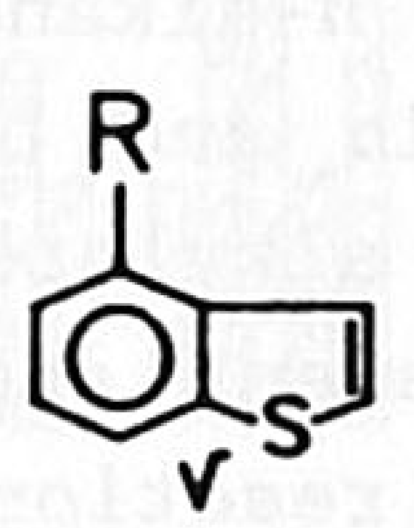
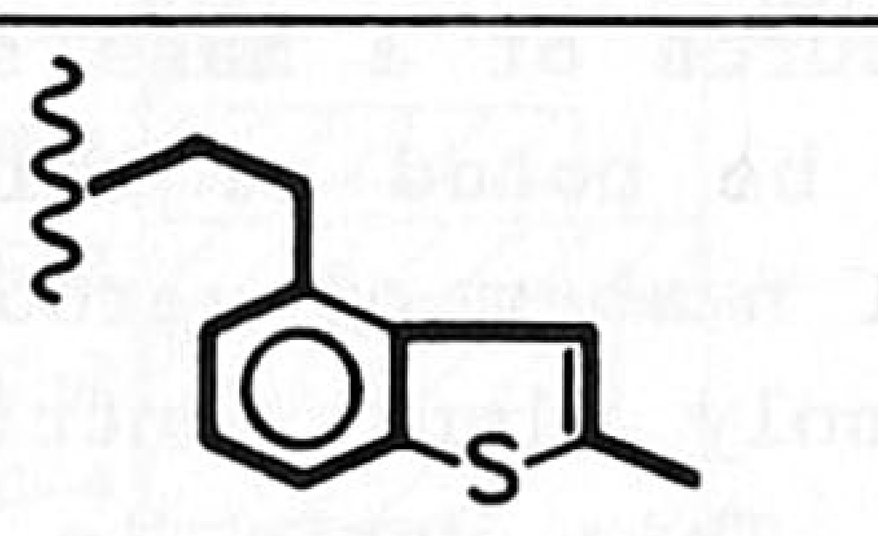
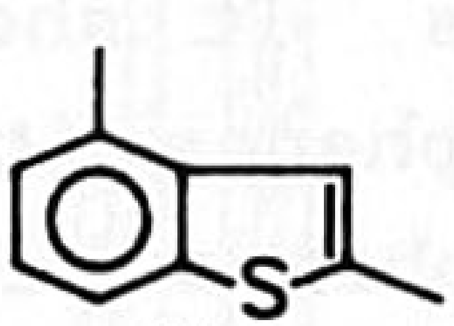
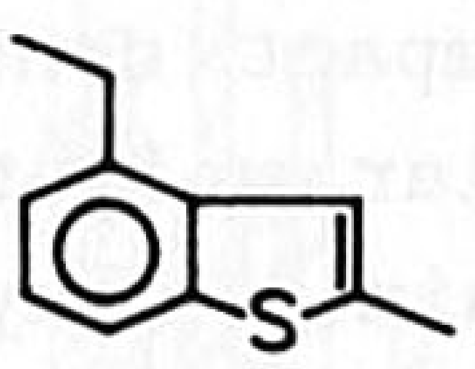
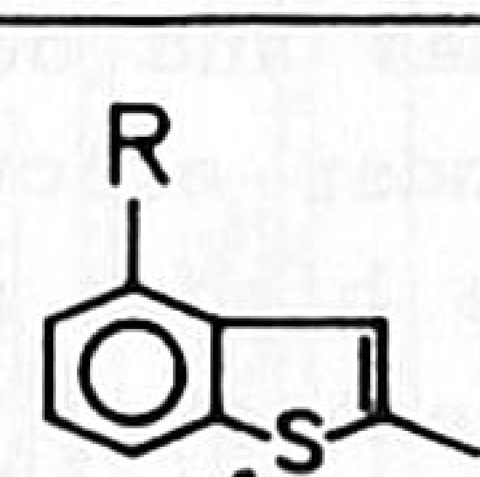
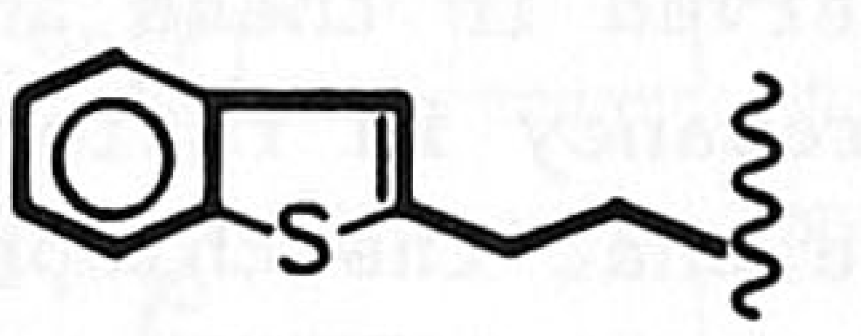
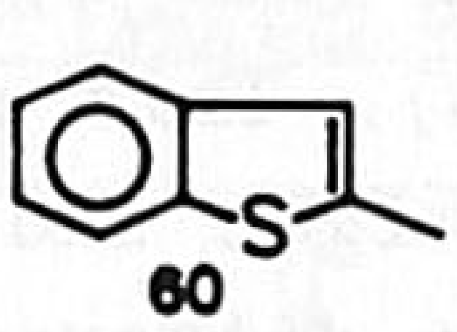
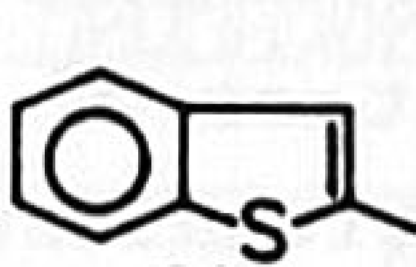
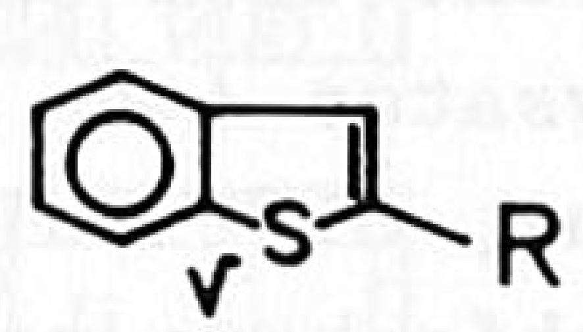
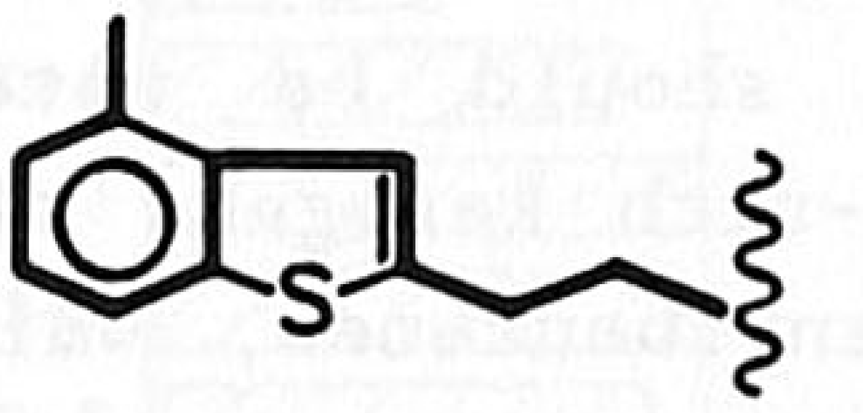
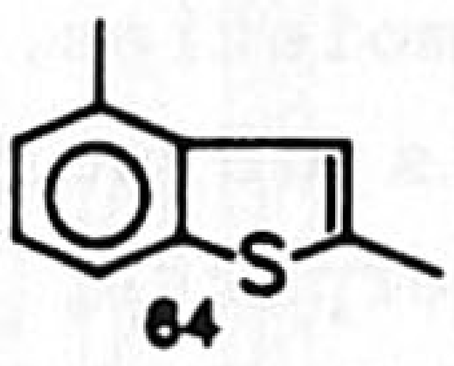
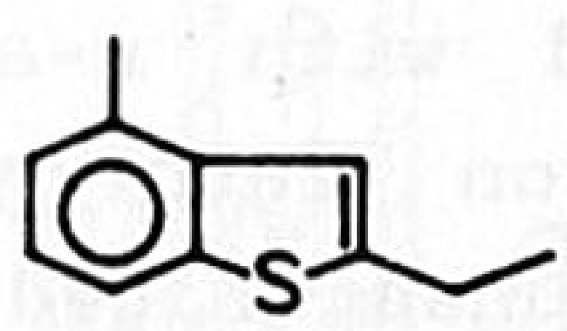
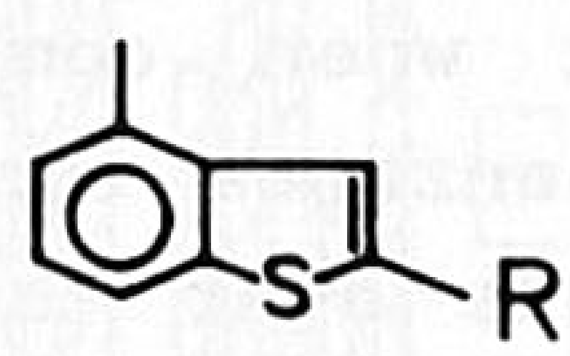
Pyrolysis performed with wires with a Curie temperature of 610°C yielded, in addition to the highly volatile sulphur compounds, a wide variety of alkylated thiophenes and benzothiophenes and homologous series of *n*-alk-1-enes and *n*-alkanes. Hence, much more stable C-C bonds are cleaved and the thiophenic and benzothiophenic moieties in the macromolecular matrix will generate alkylated thiophenes and benzothiophenes by β-cleavage, γ-hydrogen rearrangement and, to a much lesser extent, by γ-cleavage. These proposed types of reactions are analogous to the fragmentation reactions of alkylated thiophenes and benzothiophenes occurring in the source of a mass spectrometer under electron impact conditions. It should be noted at this stage that the higher molecular weight thiophenes (total number of carbon atoms > 10) generated are mainly alkylthiophenes with only minor contributions (*ca.* 20%) of the corresponding alkenylthiophenes. This ratio is clearly different from the alkene/alkane ratios (*ca.* 1) observed in these and other pyrolysates (van de Meent *et al.*, 1980). This discrepancy in ratios is, at present, difficult to rationalise; it might indicate that the thiophene and benzothiophene moieties are linked in a different way to the macromolecular matrix when compared with *n*-alkyl moieties. It should be noted that alkylbenzenes formed on flash pyrolysis of sulphur-rich kerogens similarly dominate over their unsaturated counterparts, alkenylbenzenes, whilst the alkenylbenzenes/*n*-alkylbenzene ratio of Type I kerogens is *ca.* 1 (Larter *et al.*, 1978).

It appears, therefore, that whilst flash pyrolysis provides little information on the structure of thiol, sulphide and disulphide moieties in kerogen, the presence of thiophene and benzothiophene moieties are revealed by the generation of alkylated thiophenes and benzothiophenes.

Structure of sulphur-containing moieties in kerogens.

On the basis of the mechanisms of their formation the thiophenes and benzothiophenes produced on pyrolysis may be correlated with presumed structural moieties in the kerogens (Tables 12.4-7). Four separate groups of

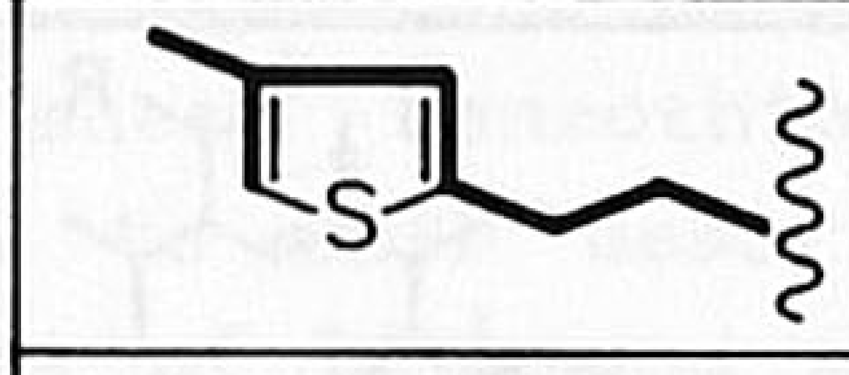
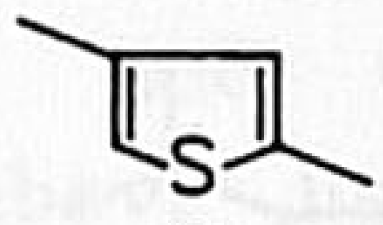
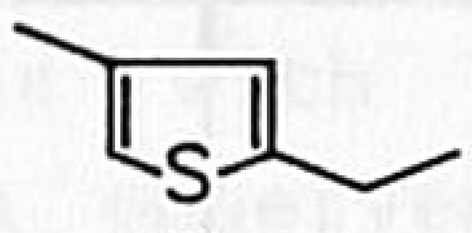
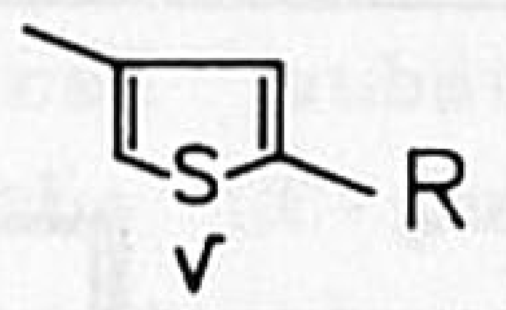
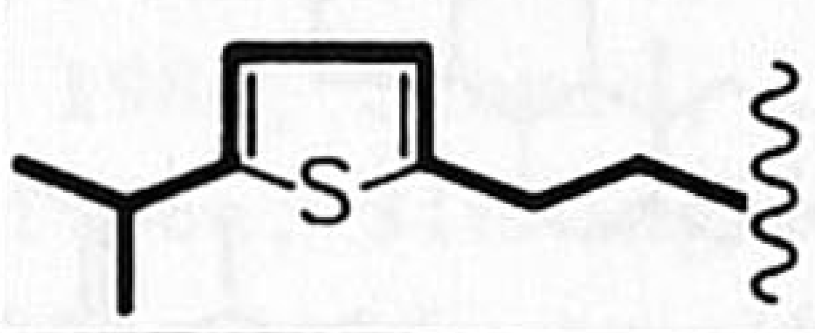
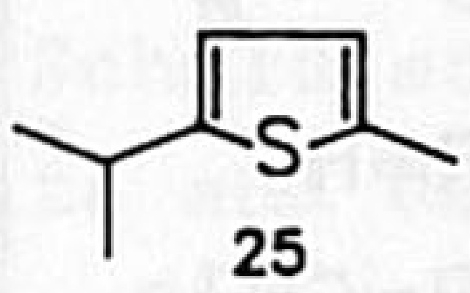
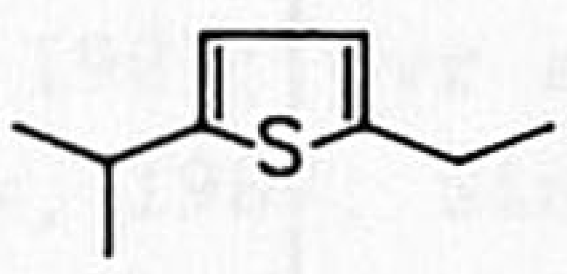
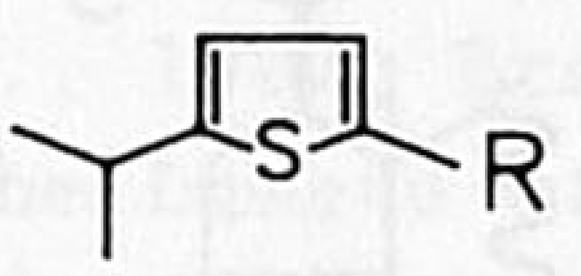
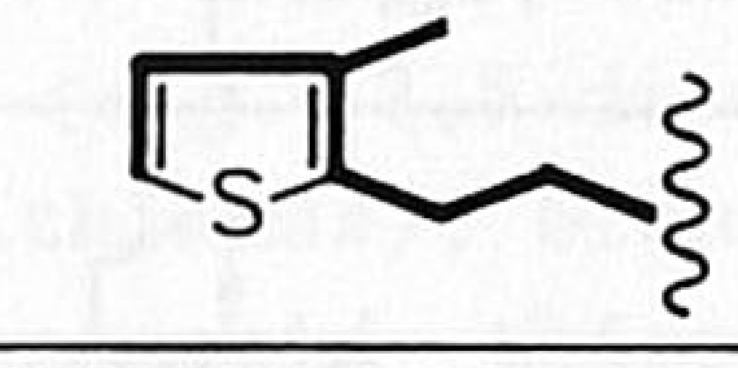
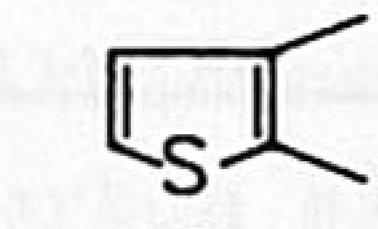
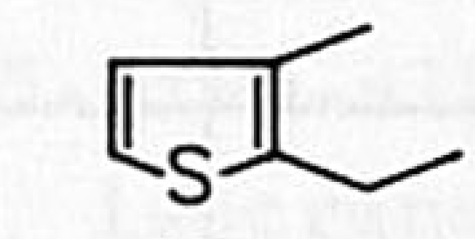
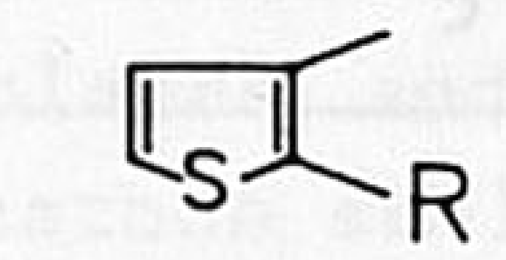
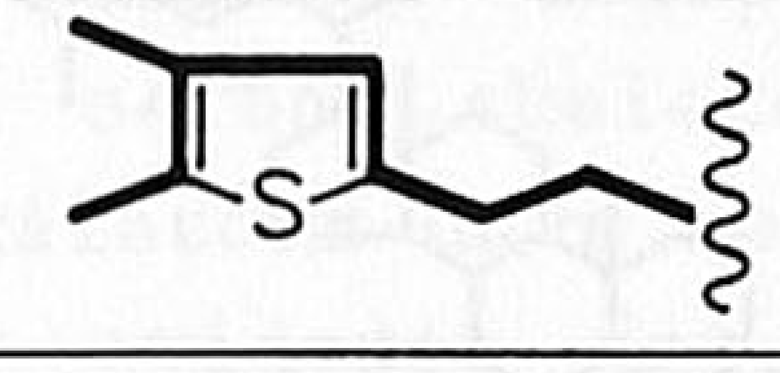
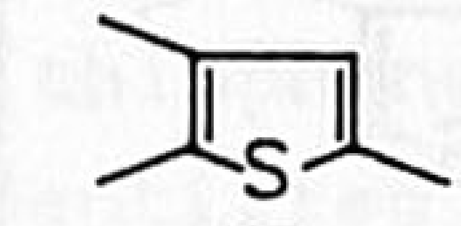
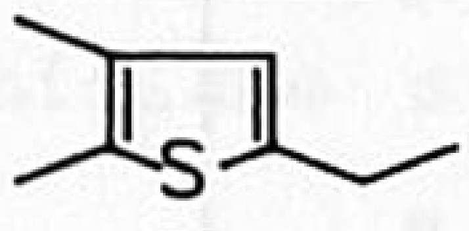
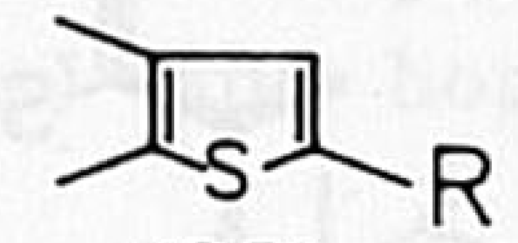
Table 12.4. Postulated products of flash pyrolysis of sulphur-containing moieties with linear carbon skeletons.

presumed moiety in kerogen[a]	postulated products of flash pyrolysis[b]		
	beta-cleavage[c]	gamma-cleavage	other cleavages
	 3	 8	 16,30,47,58,63,66
	 9	 17	 28,46,56,62,65
	 17	 29	 42,54
	 28	 42	 √
	 61	 64	 √
	 64		 √
	 60	 64	 √
	 64		

[a] carbon skeletons indicated by bold lines
[b] numbers refer to Table 3; √ = present
[c] gamma-hydrogen rearrangement yields the same products

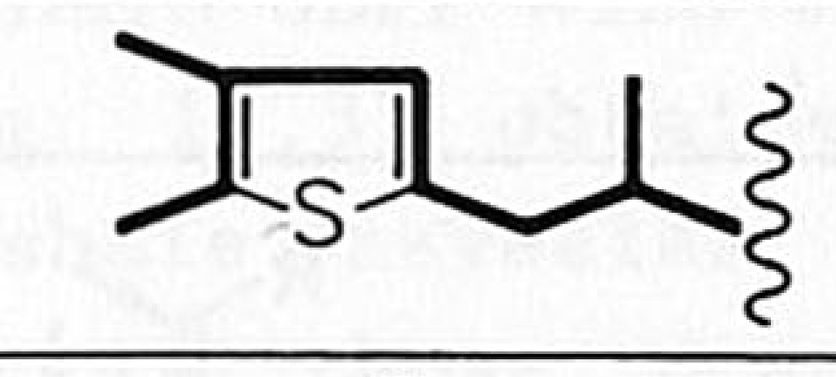
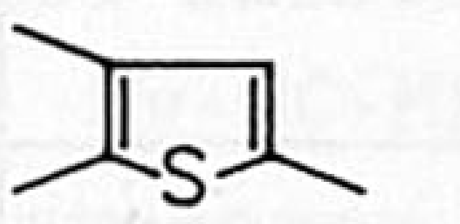
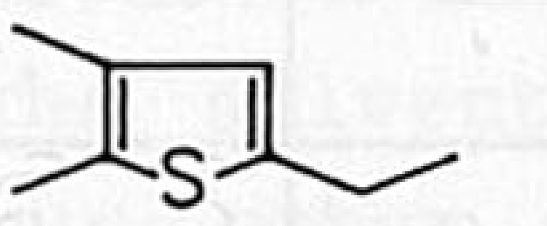
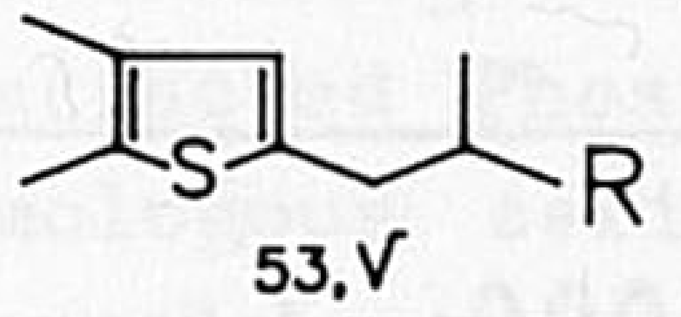
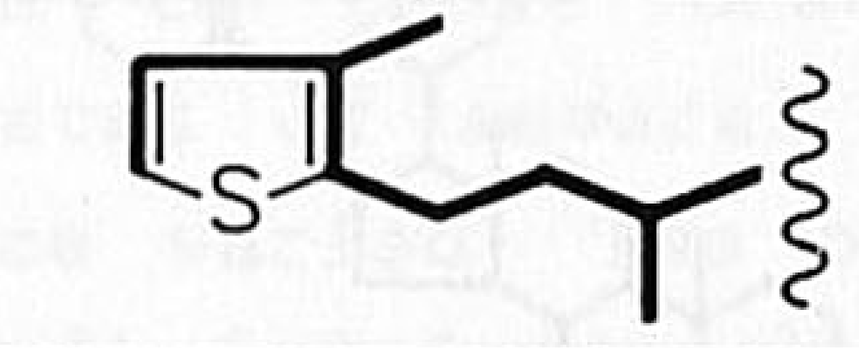
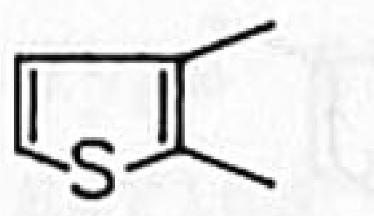
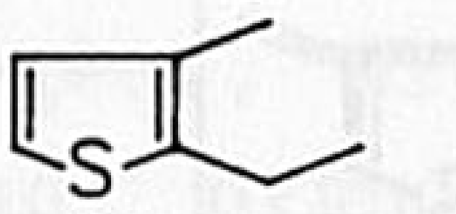
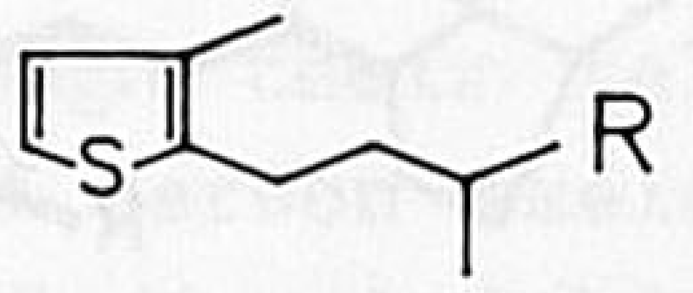
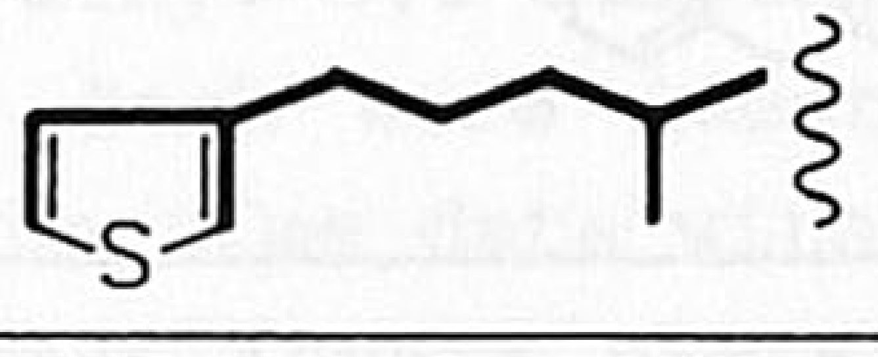
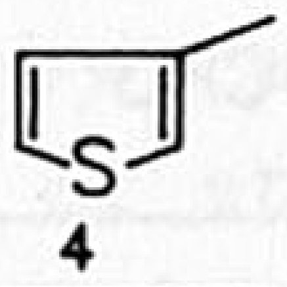
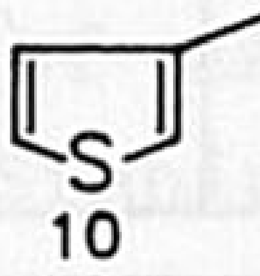
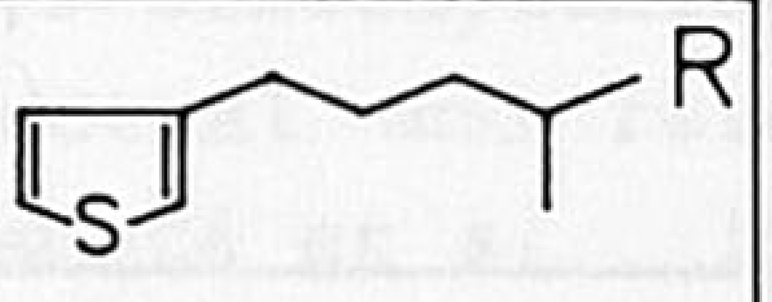
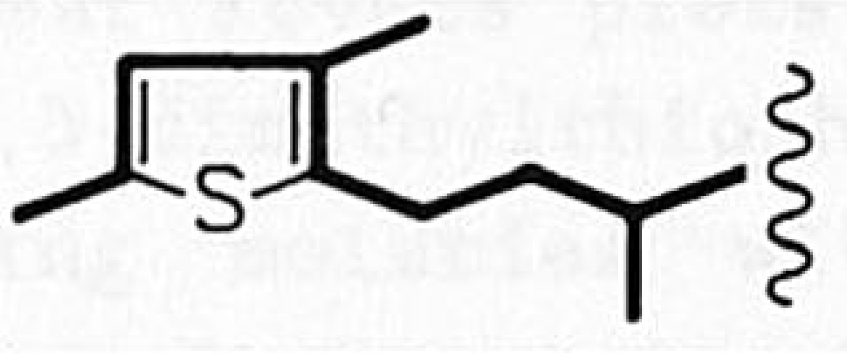
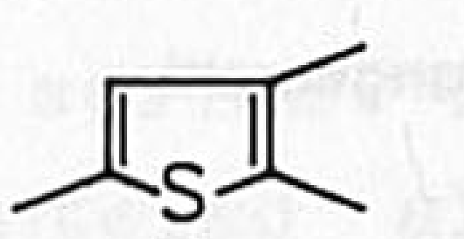
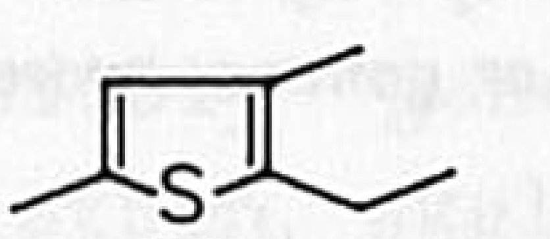
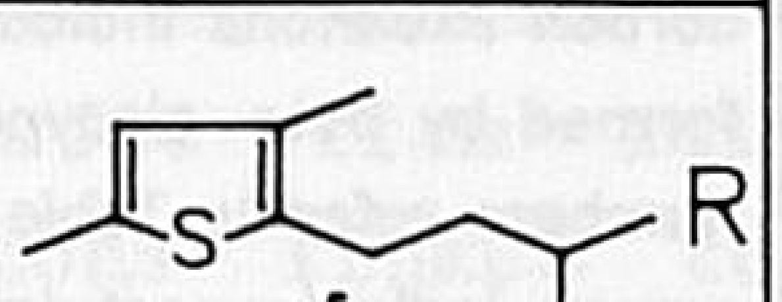
carbon skeletons can be discriminated based on the substitution patterns of the thiophenes and benzothiophenes: linear alkyl carbon skeletons (Table 12.4); *iso* and *anteiso* alkyl carbon skeletons (Table 12.5); isoprenoid alkyl carbon skeletons (Table 12.6) and steroidal side-chain carbon skeletons (Table 12.7). Almost 90% of the identified OSPP, including all major alkylated thiophenes, can thus be ascribed to a structural moiety (Table 12.3). The presence of the limited number of well-defined types of sulphur-

Table 12.5. Postulated products of flash pyrolysis of sulphur-containing moieties with branched carbon skeletons.

	presumed moiety in kerogen[a]	postulated products of flash pyrolysis[b]		
		beta-cleavage[c]	gamma-cleavage	other cleavages
iso		 11	 18	 √
		 25		
anteiso		 13	 19	
		 20	 33	 48,59

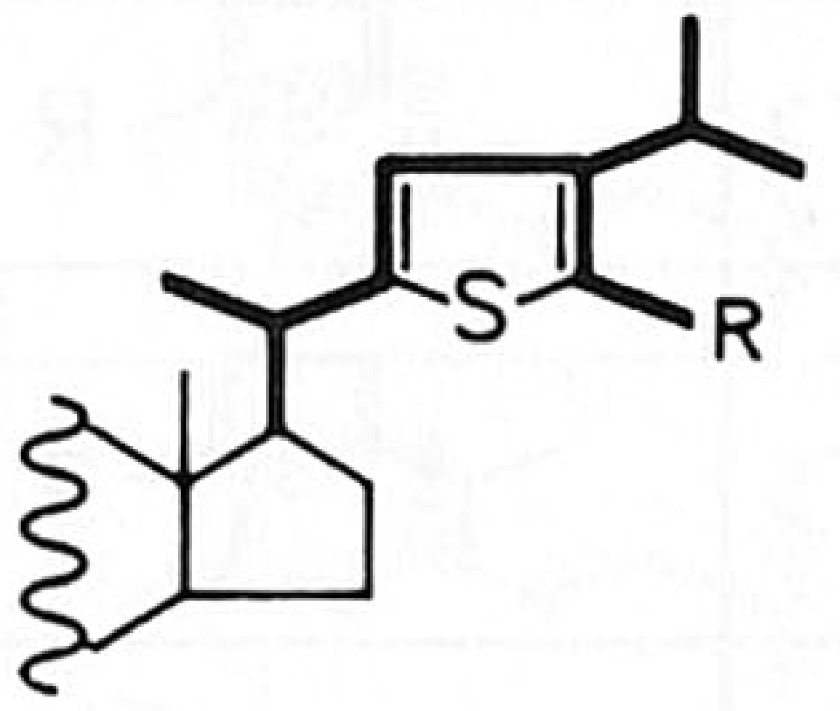
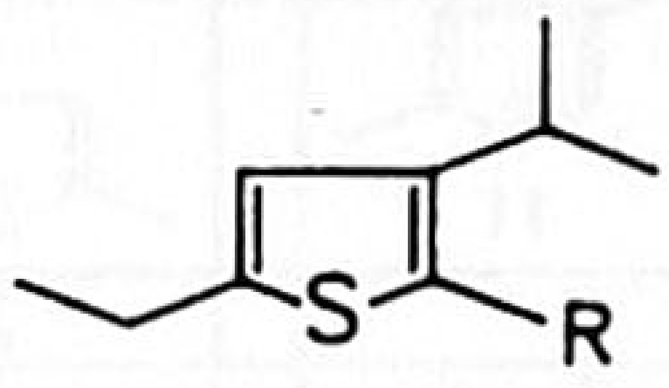
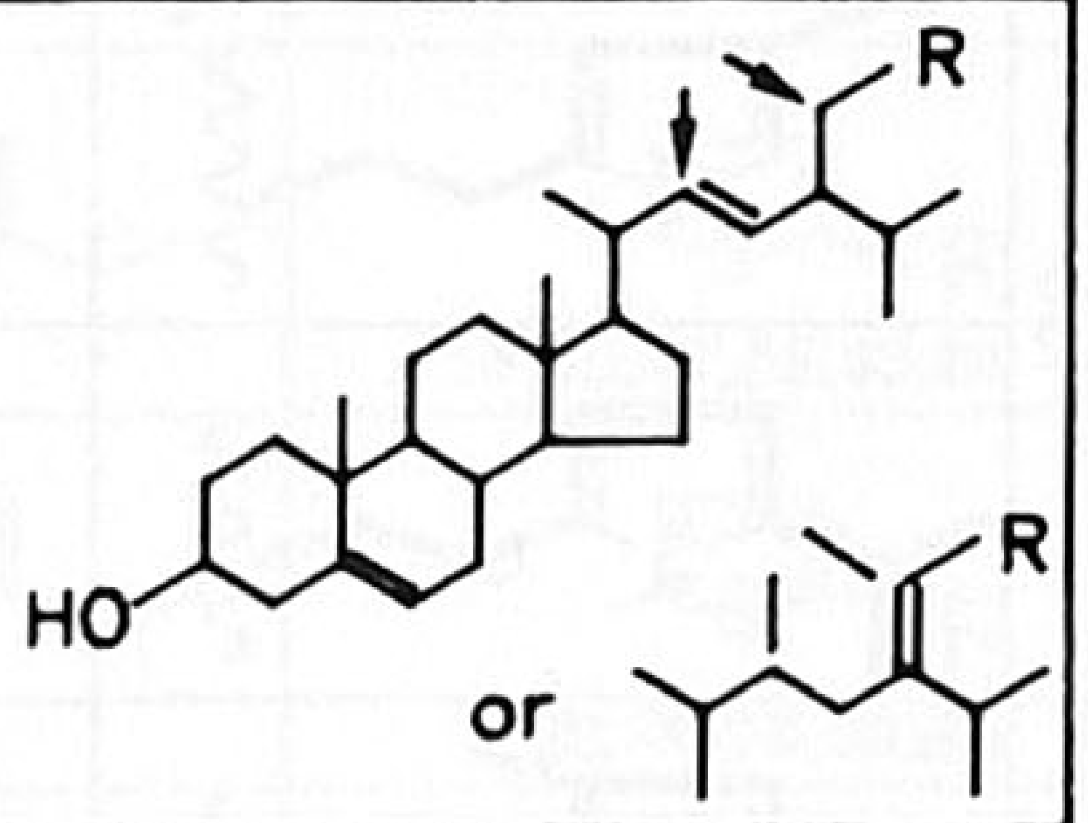
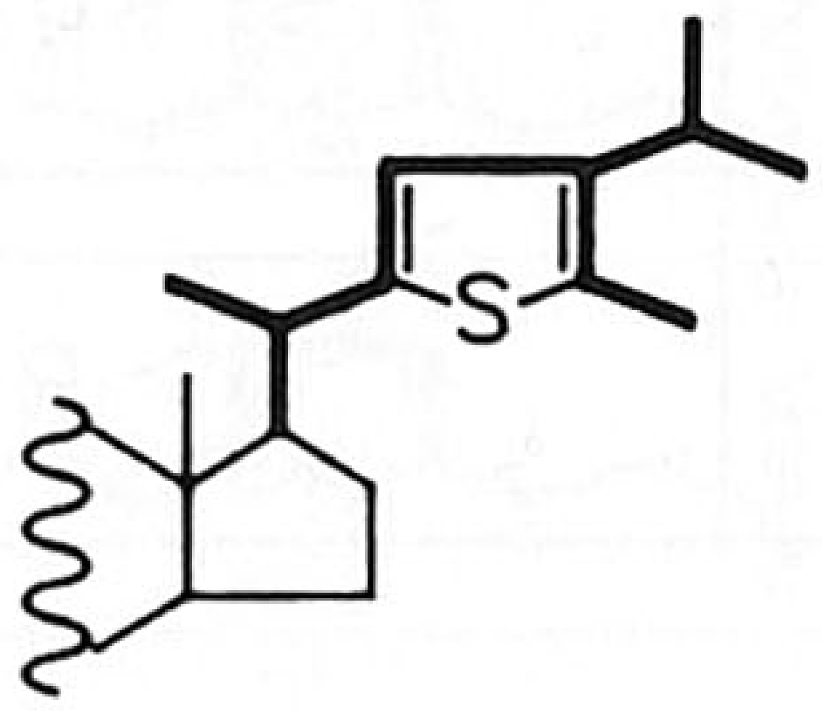
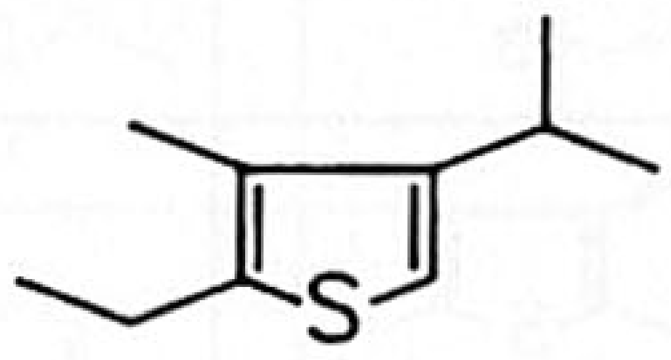
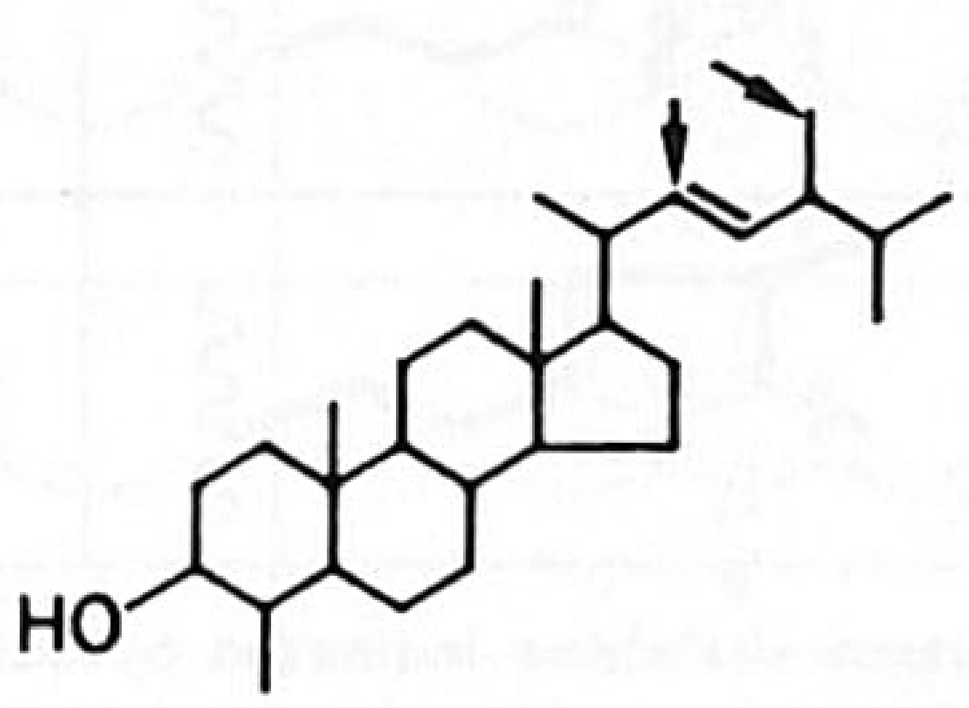
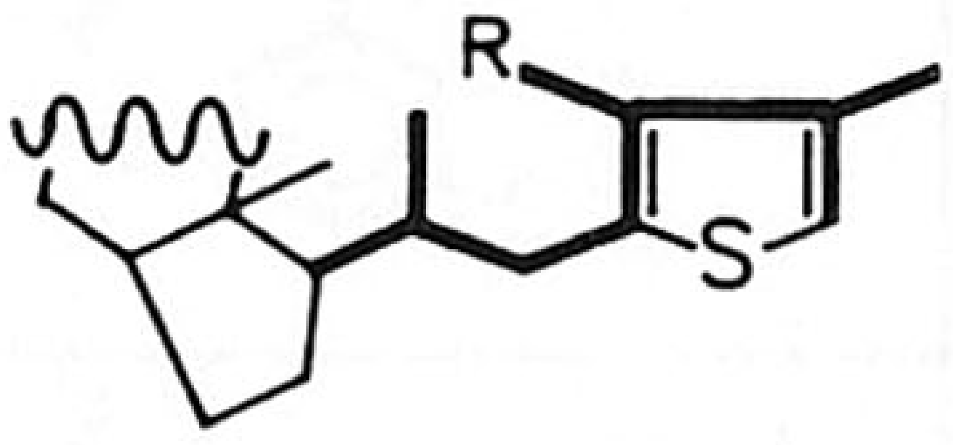
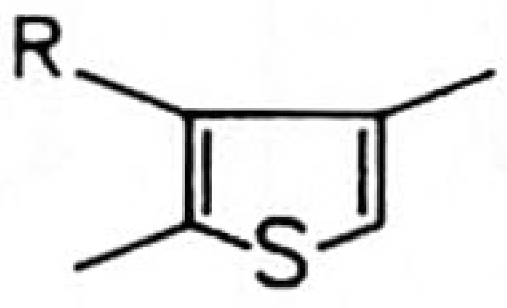
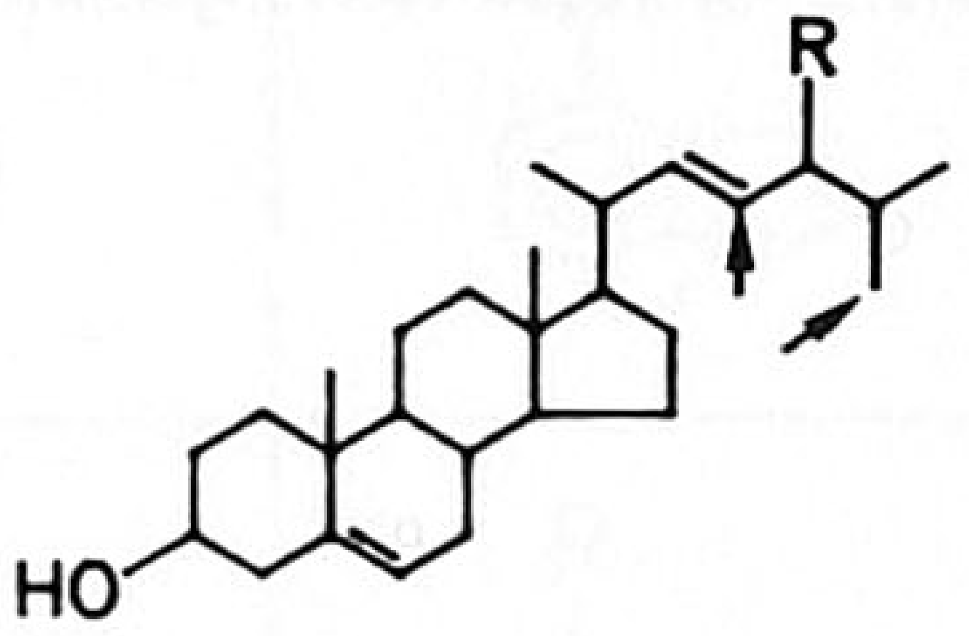
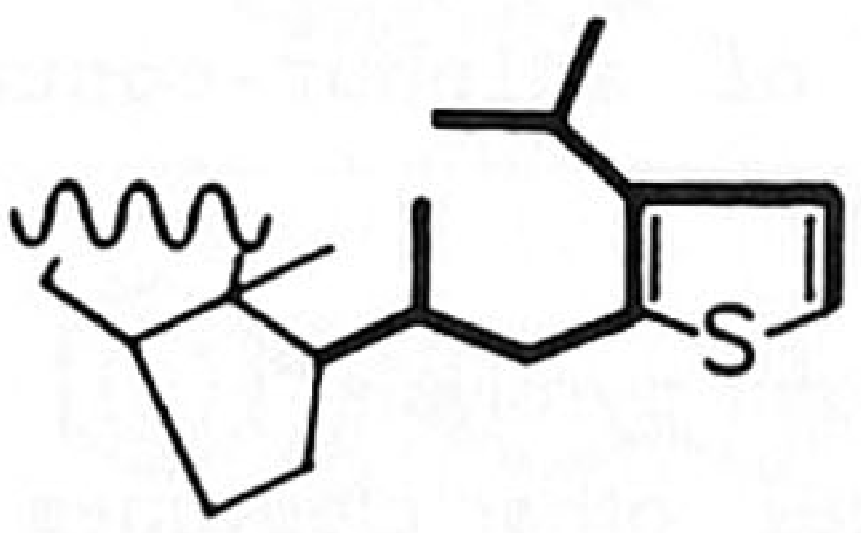
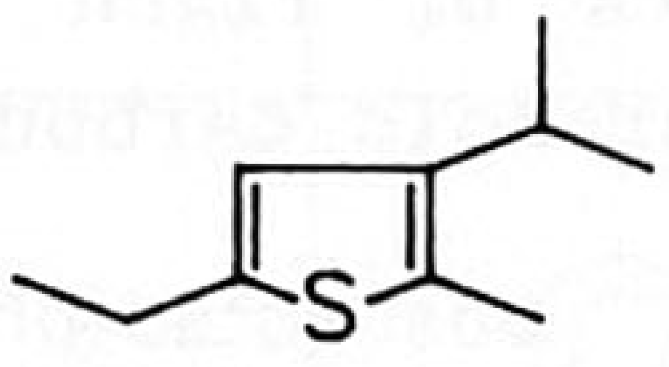
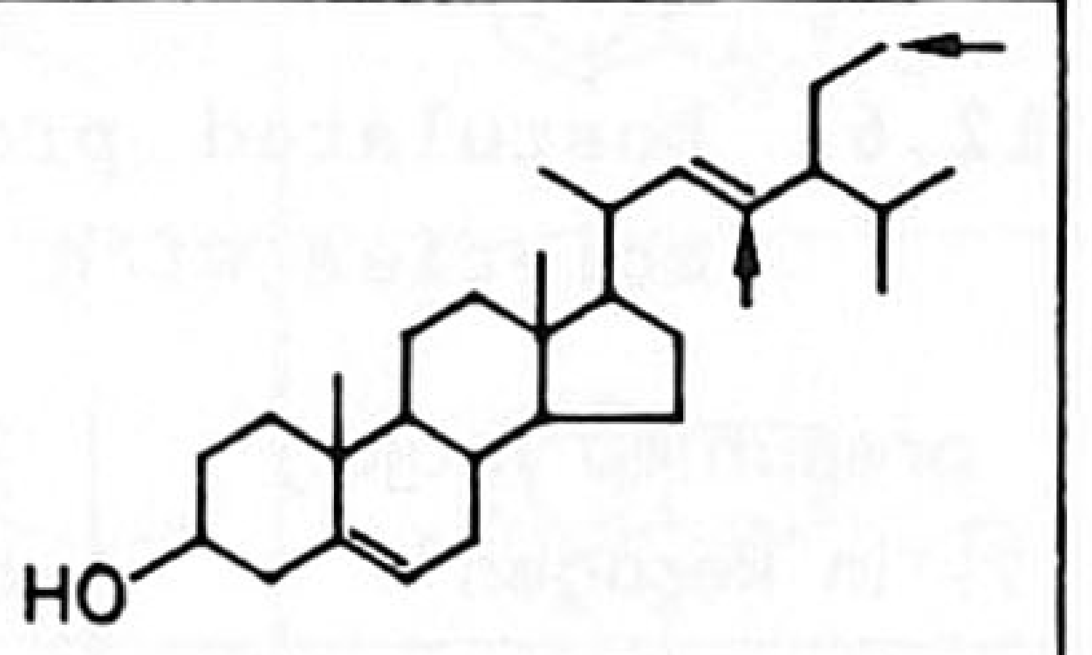
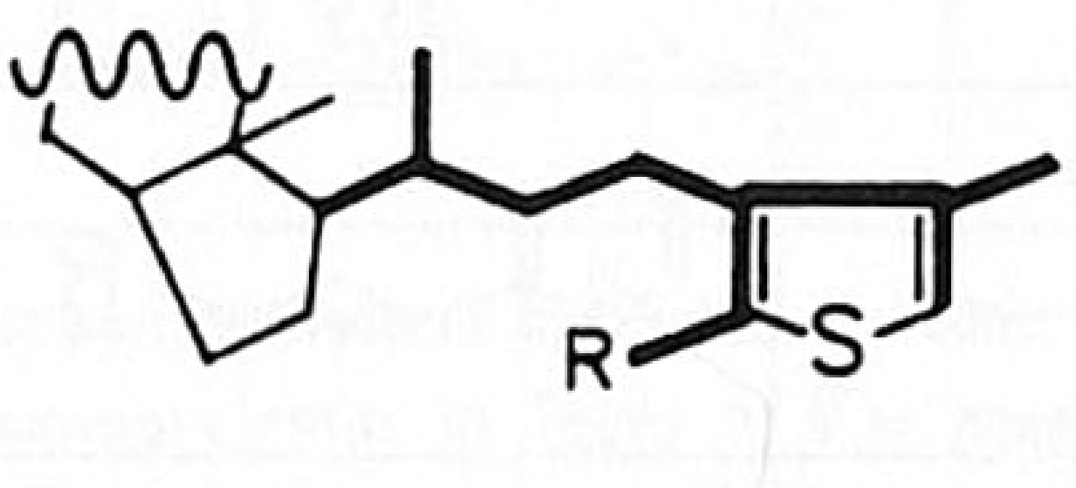
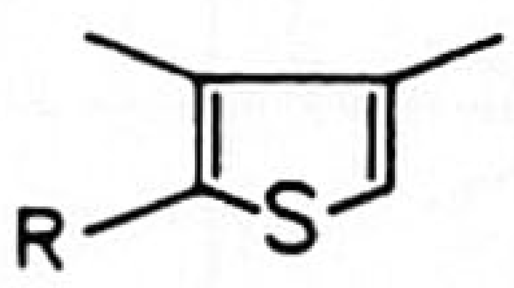
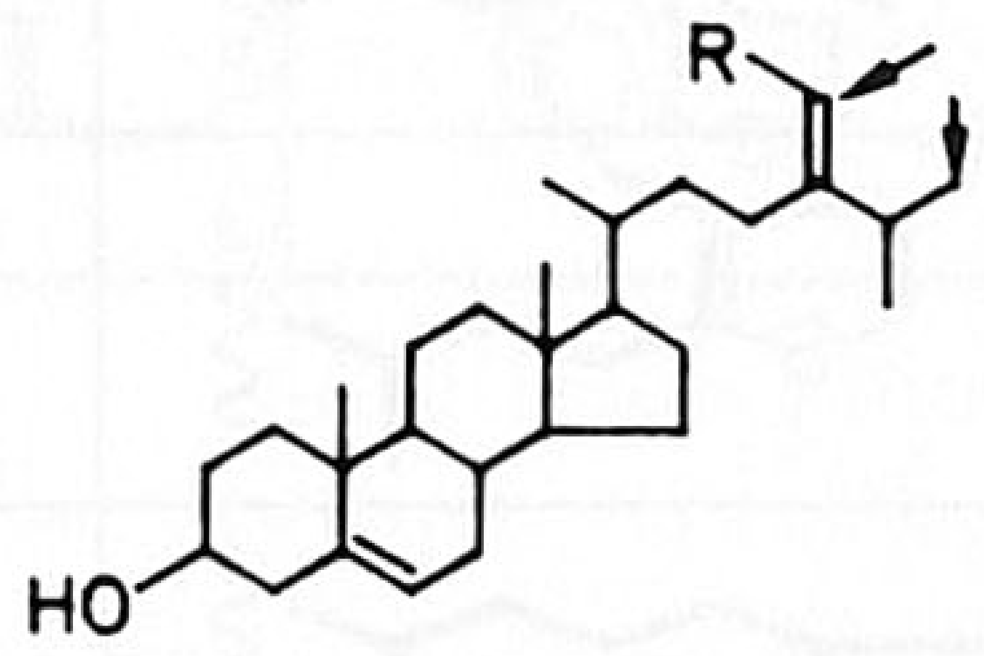
[a] carbon skeletons indicated by bold lines
[b] numbers refer to Table 3; √ = present
[c] gamma-hydrogen rearrangement yields the same products

Table 12.6. Postulated products of flash pyrolysis of sulphur-containing moieties with isoprenoid carbon skeletons.

presumed moiety in kerogen[a]	postulated products of flash pyrolysis[b]		
	beta-cleavage[c]	gamma-cleavage	other cleavages
	 20	 48	 53,√
	 13	 19	
	 4	 10	
	 20	 31	 √

[a] carbon skeletons indicated by bold lines
[b] numbers refer to Table 3; √ = present
[c] gamma-hydrogen rearrangement yields the same products

Table 12.7. Postulated products of flash pyrolysis of sulphur-containing moieties with steroid side-chain carbon skeletons.

presumed moiety in kerogen[a]	postulated products of flash pyrolysis[b,c]	possible precursor[d]
	 44 R=H R=CH3	
		
	 11 R=H 24 R=CH3 R=C2H5	
	 25	
	 14 R=H 24 R=CH3	

[a] carbon skeletons indicated by bold lines
[b] formed by beta–cleavage or gamma–hydrogen rearrangement
[c] numbers refer to Table 3
[d] arrows indicate postulated sites of sulphur attack

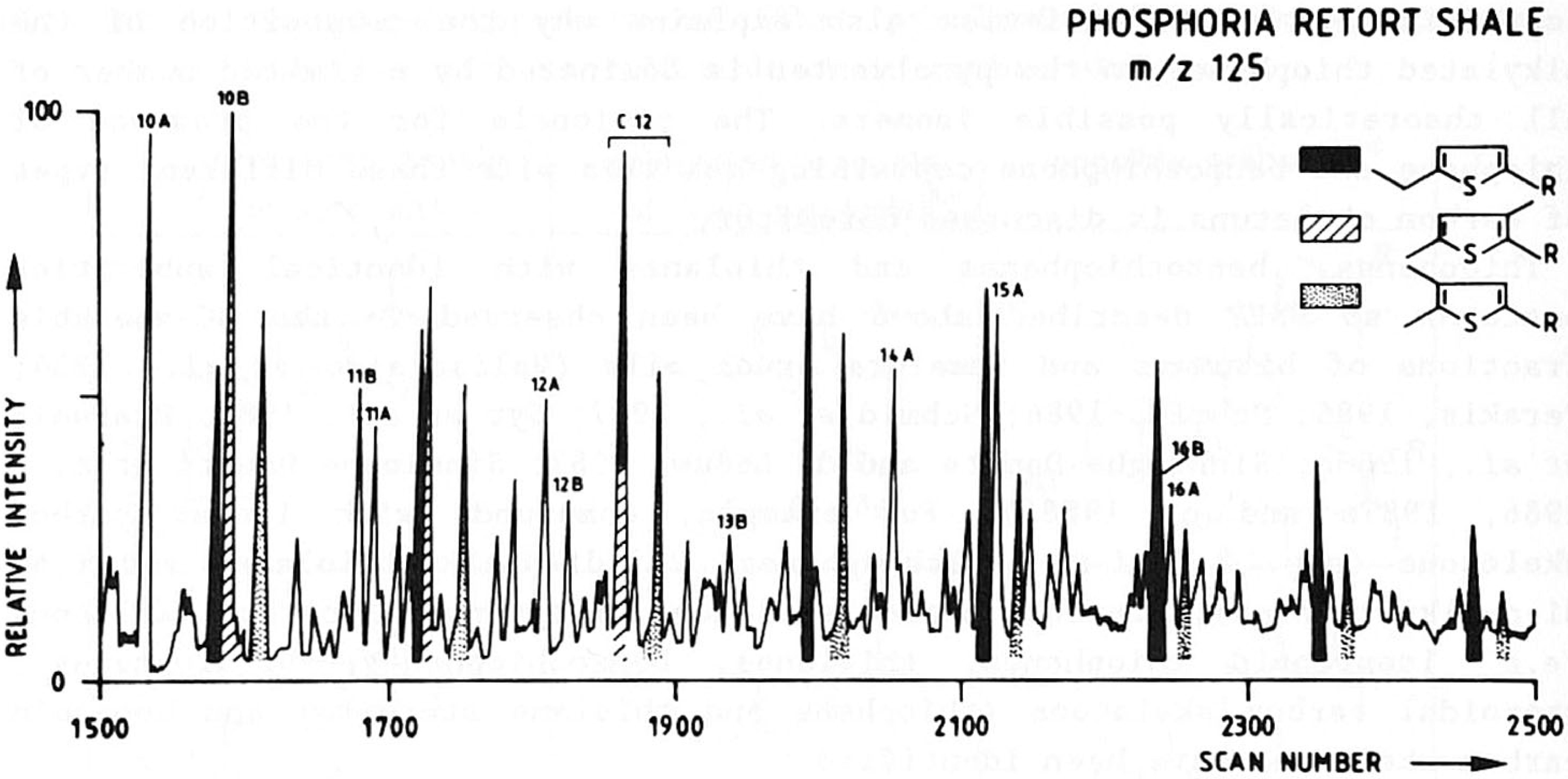
containing structural moieties also explains why the composition of the alkylated thiophenes in the pyrolysates is dominated by a limited number of all theoretically possible isomers. The rationale for the presence of thiophene and benzothiophene containing moieties with these different types of carbon skeletons is discussed hereafter.

Thiophenes, benzothiophenes and thiolanes with identical substition patterns to OSPP described above have been observed in the GC-amenable fractions of bitumens and immature crude oils (Valisolalao *et al.*, 1984; Perakis, 1986; Schmid, 1986; Schmid *et al.*, 1987; Cyr *et al.*, 1987; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987a and c, 1988f). For example, compounds with linear carbon skeletons (*e.g.* 2,5-di-*n*-alkylthiophenes, 2,5-di-*n*-alkylthiolanes and 2,6-di-*n*-alkylthianes), branched carbon skeletons, isoprenoidal carbon skeletons (*e.g.* isoprenoid thiophenes, thiolanes, benzothiophenes, bithiophenes), steroidal carbon skeletons (thiophene and thiolane steroids) and hopanoid carbon skeletons have been identified.

Further evidence for the presence of the limited number of types of thiophene and benzothiophene structures in kerogen and related materials is provided by the identification of suites of long-chain alkylthiophenes and alkylbenzothiophenes (extending up to C_{22}) in the flash pyrolysates of sulphur-rich kerogens.

Long-chain OSPP with linear carbon skeletons. It has been shown previously that flash pyrolysates of sulphur-rich kerogens contained homologous series of 2-alkylthiophenes, 2-alkyl-5-methylthiophenes, 2-alkyl-5-ethylthiophenes (see also Fig. 12.3) and probably 2-(ω-alkenyl)thiophenes and 2-(ω-alkenyl)-5-methylthiophenes (Sinninghe Damsté *et al.*, 1988c). These series indicate alkylated thiophene moieties, with linear alkyl carbon skeletons, linked to the macromolecular matrix (Table 12.5).

Long-chain OSPP with branched carbon skeletons. A mass chromatogram of *m/z* 125 (Fig. 12.3) obtained by Py-GC-MS of the solvent-extracted Phosphoria Retort shale revealed the presence of four other homologous series in addition to those described above. These series were also present in pyrolysates of several other Type II-S kerogens, although their relative abundance varied. Two of the series contained branched carbon skeletons: the 2-alkyl-3,5-dimethylthiophene and the 5-alkyl-2,3-dimethylthiophene series. Both were identified by comparison of mass spectral and relative retention time data with those of standards (Sinninghe Damsté *et al.*, 1988c) and linear Kováts plots as for the 2-alkyl-5-ethylthiophene series. The 5-alkyl-2,3-dimethylthiophene series is possibly generated from sulphur-containing moieties with *anteiso* alkyl carbon skeletons linked to the macromolecular matrix (see Table 12.5), whilst the 2-alkyl-3,5-dimethylthiophene series is derived from an unknown precursor. A homologous series of 2-alkyl-4-methylthiophenes was also observed in some sulphur-rich kerogens and probably represents the presence of sulphur-containing moieties



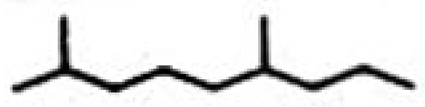
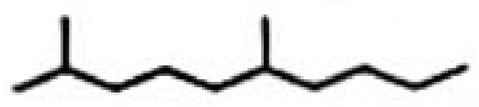
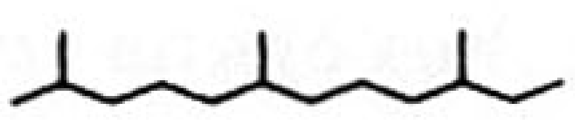
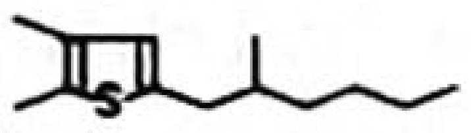
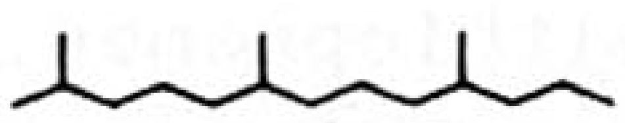
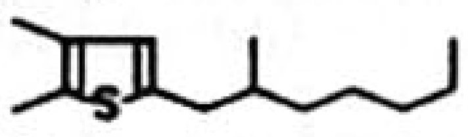
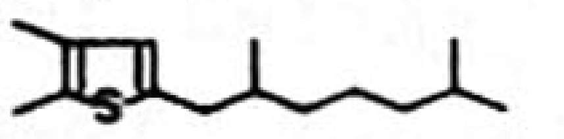
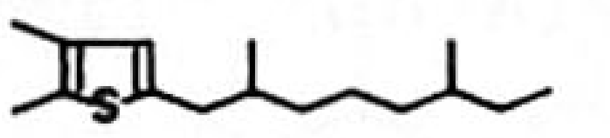
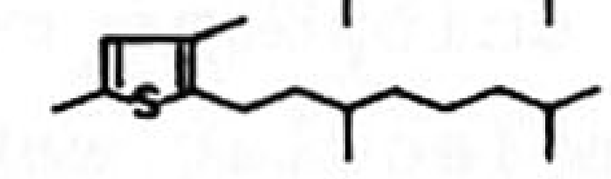
A-series of isoprenoid alkylthiophenes				isoprenoid alkanes			B-series of isoprenoid alkylthiophenes			
peak no.	compound	I	ΔI		I	ΔI	peak no.	compound	I	ΔI
					1026					
–		1145			–	190				
			45							
10a		1194			1216		10b		1231	
			105			49				62
11a		1299			1274		11b		1293	
			86			102				104
12a		1385			1376		12b		1397	
						85				88
absent		–	192		1461		13b		1485	
14a		1577			–	188	absent		–	194
			60							
15a		1637			1649		15b		1679	
			107			60				60
16a		1744			1709		16b		1739	
						105				
					1814					

Fig. 12.3. Partial mass chromatogram for *m/z* 125 obtained by Py-GC-MS (Curie temperature 610°C) of the extracted rock of the Phosphoria Retort shale showing the presence of the homologous series of 2-alkyl-5-ethylthiophenes (black peaks), 2-alkyl-3,5-dimethylthiophenes (shaded peaks) and 5-alkyl-2,3-dimethylthiophenes (dotted peaks) and two series (A and B) of isoprenoid thiophenes. The isoprenoid thiophenes are indicated with a number representing the total number of carbon atoms. Their structures are indicated in the table below the mass chromatogram. They were identified by the characteristic differences in retention indices (I) of the members of these series. Similar differences are observed in the series of isoprenoid alkanes.

with *iso* alkyl carbon skeletons linked to the macromolecular matrix.

Long-chain OSPP with isoprenoid carbon skeletons. The two other pseudo-homologous series of alkylated thiophenes detected did not show linear Kováts plots but exhibited retention behaviour typical of isoprenoidal compounds (Fig. 12.3). The 5-alkyl-2,3-dimethylthiophene series (with an isoprenoidal alkyl side chain; Fig. 12.3) is probably generated from sulphur-containing moieties with isoprenoid alkyl carbon skeletons linked to the macromolecular matrix (see Table 12.6). The C_{20} isoprenoid thiophene, 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene, which is present as a major constituent of certain petroleums and bitumens (Sinninghe Damsté *et al.*, 1987a), possesses the same thiophenic group as the series identified here. These pyrolysis products therefore possibly reflect the presence of moieties with the same structure as this C_{20} isoprenoid thiophene linked to the macromolecular matrix.

The other series of isoprenoid alkylthiophenes detected has been assigned as a 2-alkyl-3,5-dimethylthiophene series (Fig. 12.3). Their generation may therefore also indicate the presence of sulphur-containing moieties with isoprenoid alkyl carbon skeletons linked to the macromolecular matrix (see Table 12.6). Possible precursors include moieties with the same structure as 3,5-dimethyl-2-(3,7,11-trimethyldodecyl)thiophene, a major C_{21} isoprenoid thiophene in certain petroleums and bitumens (Sinninghe Damsté *et al.*, 1988g), linked to the macromolecular matrix. An alternative explanation for the formation of some members of the isoprenoid alkylthiophene series is the thermal breakdown of isoprenoid C_{20} "mid-chain" thiophenes (also major thiophenes in certain petroleums and bitumens; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1987c) linked to the macromolecular matrix.

Benzothiophenic OSPP. Apart from the alkylated thiophenes, alkylated benzothiophenes were also formed in varying amounts. The C_1-benzothiophene cluster was dominated for all samples by 2-methylbenzo[*b*]thiophene and 3- and/or 4-methylbenzo[*b*]thiophene (which co-elute on apolar stationary phases). Higher alkylated benzothiophene homologues were not fully assigned due to the lack of available authentic standards. 2- and 4-Methylbenzo[*b*]-thiophene are listed as flash pyrolysis products of sulphur-containing structural moieties with linear carbon skeletons (Table 12.4) since these moieties can be formed in macromolecular sedimentary organic matter from thiophene units with linear carbon skeletons *via* ring closure reactions followed by aromatisation. Organic sulphur compounds with a similar carbon skeleton (*i.e.* 2,4-di-*n*-alkylbenzo[*b*]thiophenes) have been identified in bitumens and immature crude oils (Perakis, 1986; Sinninghe Damsté *et al.*, 1987c, 1988g). The identification of three homologous series of long-chain alkylbenzothiophenes (2-alkylbenzo[*b*]thiophenes, 4-alkyl-2-methylbenzo[*b*]-thiophenes and 2-alkyl-4-methylbenzo[*b*]thiophenes) in the pyrolysates of sulphur-rich kerogens (Sinninghe Damsté *et al.*, 1988c) also suggests that these structural moieties are present in macromolecular sedimentary organic matter.

Cyclic sulphideic OSPP. Cyclic sulphide moieties may generate predominantly hydrogen sulphide upon flash pyrolysis without release of long-chain compounds and, as a consequence, minor amounts of alkylated cyclic sulphides were detected in the pyrolysates. These consisted mainly of 2-alkylthiolanes, but 2-alkyl-5-methylthiolanes and 2-alkylthianes were also identified (Sinninghe Damsté *et al.*, 1988c). This, therefore, provides more direct evidence to suggest that, in addition to thiophene moieties, cyclic sulphide moieties with linear carbon skeletons are present in macromolecular sedimentary organic matter. This is not surprising in view of the presence of major amounts of cyclic sulphides (*i.e.* 2,5-dialkylthiolanes, 2,6-dialkylthianes, isoprenoid thiolanes, thiolane steranes) in some bitumens and immature crude oils (Schmid, 1986; Schmid *et al.*, 1987; Sinninghe Damsté *et al.*, 1986, 1987c, 1988g; Sinninghe Damsté and de Leeuw, 1987). The presence of these sulphur-containing moieties is also supported by the production of homologous series of long-chain 2-alkylthiolanes, 2-(ω-alkenyl)thiolanes, 2-alkyl-5-methylthiolanes and 2-alkylthianes upon flash pyrolysis of high-sulphur kerogens (Sinninghe Damsté *et al.*, 1988c).

In summary, the above results indicate that, at least, a part of the organic sulphur in macromolecular sedimentary organic matter is present in moieties with structures comparable to organic sulphur compounds present in sulphur-rich bitumens and immature crude oils. Some of these sulphur-containing moieties (*i.e.* thiophene and benzothiophene units) generate, upon flash pyrolysis, specific OSPP thus explaining why only a limited number of all theoretical isomers of alkylated thiophenes and, to some extent, alkylated benzothiophenes occur in the pyrolysates. Differences in the relative amounts of these products are presumably a function of the relative abundance of the various sulphur-containing moieties present. This, in turn, will be determined by source input (*i.e.* type of organic matter) and depositional environment (*i.e.* availability of hydrogen sulphide or other reactive forms of sulphur). The distribution of these OSPP may, therefore, be of use in the characterisation and fingerprinting of macromolecular sedimentary organic substances. Results from one such exercise will be described in detail elsewhere (Eglinton *et al.*, 1988c). However, an example of this application is illustrated in Figs. 12.4 and 12.5. These show FPD chromatograms and the alkylated thiophene distributions, respectively, from flash pyrolysis of the Jurf ed Darawish Oil shale kerogen and an asphaltene fraction isolated from the corresponding bitumen. The striking similarity between the OSPP distributions supports the concept of asphaltenes as being structurally related to kerogen (*e.g.* Behar and Pelet, 1985; Bandurski, 1982; Tissot and Welte, 1984; Philp and Gilbert, 1985).

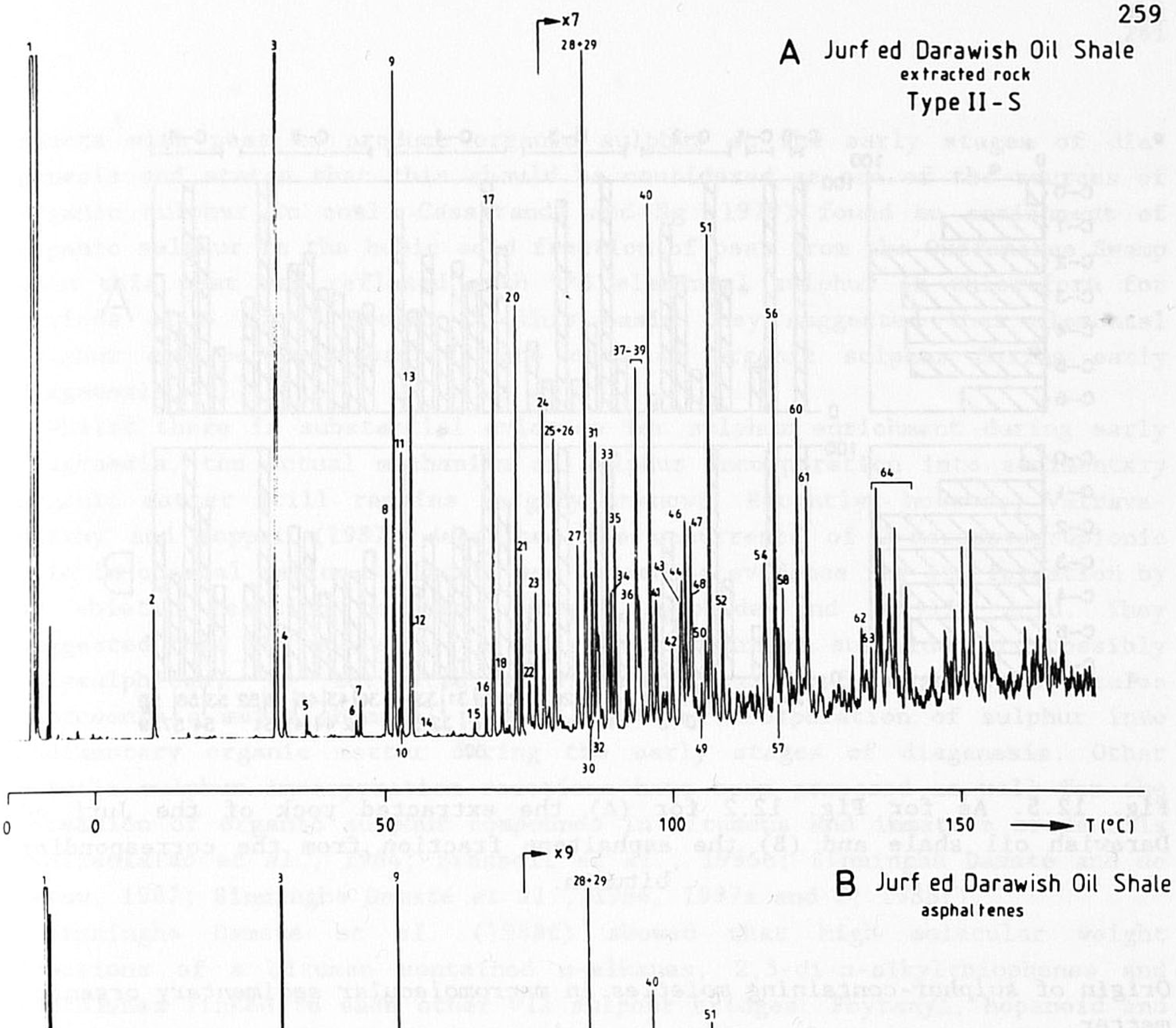


Fig. 12.4. As for Fig. 12.1 for (A) the extracted rock of the Jurf ed Darawish oil shale and (B) the asphaltene fraction from the corresponding bitumen.

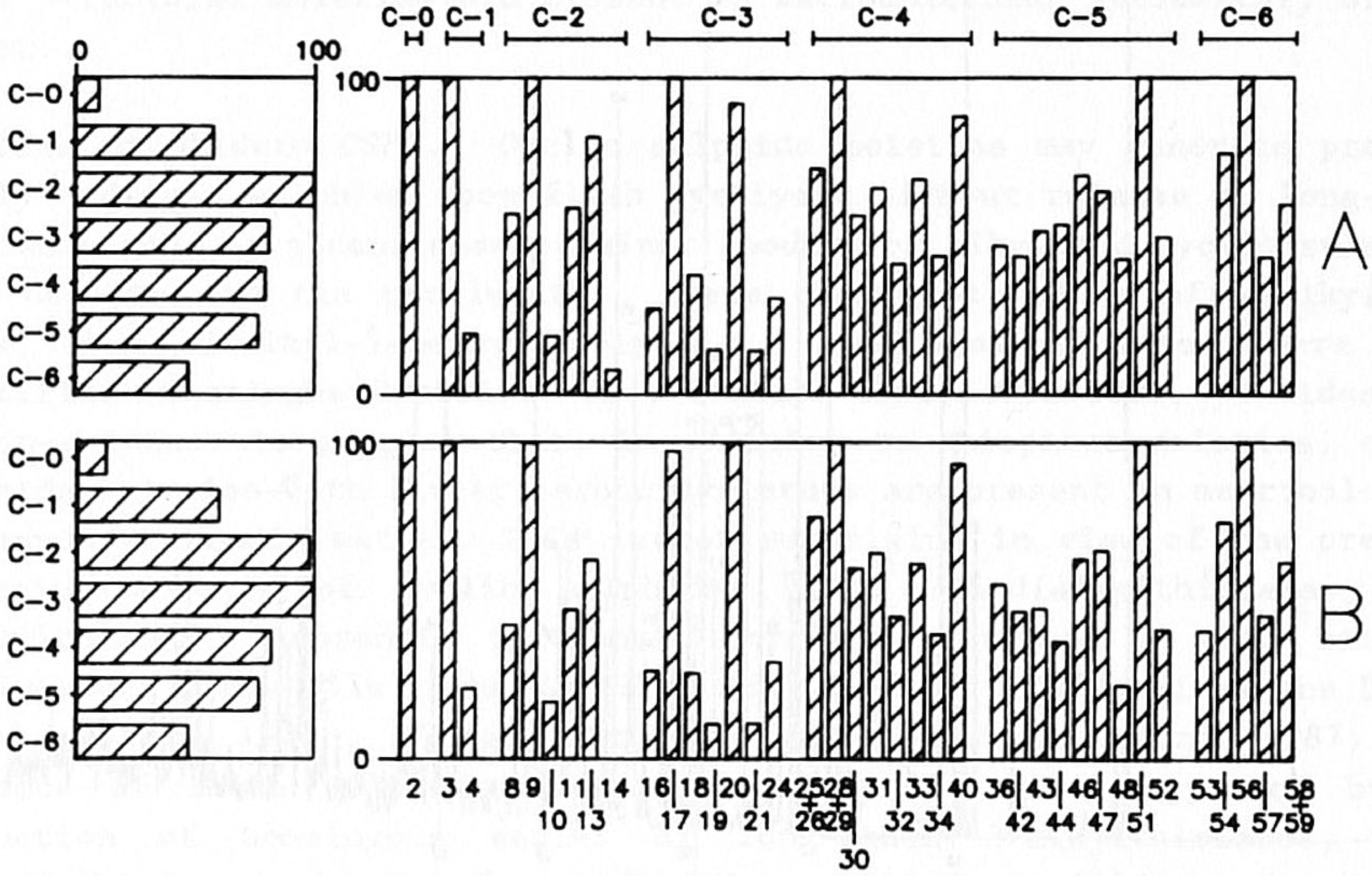


Fig. 12.5. As for Fig. 12.2 for (A) the extracted rock of the Jurf ed Darawish oil shale and (B) the asphaltene fraction from the corresponding bitumen.

Origin of sulphur-containing moieties in macromolecular sedimentary organic matter

Numerous investigators have reported the early diagenetic sulphur enrichment of macromolecular sedimentary organic matter. Nissenbaum and Kaplan (1972) provided chemical and isotopic evidence to show that the sulphur in marine humic acids is largely introduced *in situ* during early diagenesis rather than through the selective preservation of parent sulphur-containing organic material. They suggested that "the introduction of sulphur from external sources (possibly through reaction of hydrogen sulphide or elemental sulphur with organic compounds) may explain the high sulphur content of marine humic acids". Francois (1987) studied the variation in the sulphur content of humic acids extracted from a 70 cm core of a near-shore sediment. The atomic S/C ratio increased continuously with depth to values which strongly suggest sulphur addition to the humic matrix during early diagenesis by reactions between organic matter and hydrogen sulphide or its oxidation products. Aizenshtat *et al.* (1981) showed that the organically bound sulphur in "proto-kerogen" (material precursing kerogen) increased with depth in the sedimentary column of Solar Lake. This was attributed to secondary sulphur enrichment of organic matter at the early stages of diagenesis. Casagrande *et al.* (1979) showed that hydrogen sulphide reacts with peat to produce organic sulphur at the early stages of diagenesis and stated that this should be considered as one of the sources of organic sulphur in coal. Casagrande and Ng (1979) found an enrichment of organic sulphur in the humic acid fraction of peat from the Okefenokee Swamp when this peat was refluxed with ^{35}S elemental sulphur in chloroform for periods of 6 to 48 hours. On this basis they suggested that elemental sulphur can be incorporated into coal as organic sulphur during early diagenesis.

Whilst there is substantial evidence for sulphur enrichment during early diagenesis, the actual mechanism of sulphur incorporation into sedimentary organic matter still remains largely unknown. Recently, however, Vairavamurthy and Mopper (1987) described the occurrence of 3-mercaptopropionic acid in coastal marine sediments and presented evidence for its formation by an abiotic reaction between hydrogen sulphide and acrylic acid. They suggested that the nucleophilic addition of hydrogen sulphide, and possibly polysulphides, to activated unsaturated bonds of organic molecules represents a major chemical pathway for the incorporation of sulphur into sedimentary organic matter during the early stages of diagenesis. Other abiotic sulphur incorporation reactions have been proposed as well for the formation of organic sulphur compounds in bitumens and immature crude oils (Valisolalao *et al.*, 1984; Brassell *et al.*, 1986c; Sinninghe Damsté and de Leeuw, 1987; Sinninghe Damsté *et al.*, 1986, 1987a and c; 1988f).

Sinninghe Damsté *et al.* (1988f) showed that high molecular weight fractions of a bitumen contained *n*-alkanes, 2,5-di-*n*-alkylthiophenes and -thiolanes linked to each other *via* sulphur bridges. Phytanyl, hopanoid and steroid moieties were also present. These findings indicated the existence of a so-called "intermolecular sulphur cross-linking mechanism" as previously suggested by Brassell *et al.* (1986c). Evidence has been presented that this probably occurs during early diagenesis (Sinninghe Damsté *et al.*, 1988f).

The sulphur-containing moieties which we have postulated to be present in macromolecular sedimentary organic matter probably have a similar origin. Their carbon skeletons indicate that they are most likely formed by sulphur incorporation into functionalised lipids (*e.g.* normal, *iso* and *anteiso* fatty acids, steroids, isoprenoid alcohols and/or alkenes) which were, or have become, part of a macromolecular structure. Moreover, in specific cases such as in the formation of high-sulphur kerogens, sulphur incorporation reactions may act as a major pathway for the formation of macromolecular sedimentary organic matter by cross-linking of functionalised lipids *via* sulphide and disulphide bonds. Ciereszko and Youngblood (1971) have shown that such reactions can occur at the early stages of diagenesis. A "sediment" produced by the anaerobic decomposition of *Pseudoplexaura porosa* cortex in slowly running seawater was shown to contain a mixture of C_{16} and C_{18} *n*-alkyl disulphides, which were not present in the living organisms. Tissot and Welte (1984) suggested that reaction of unsaturated fatty acids with sulphur may explain the more aromatic character of Type II marine kerogens since it induces cyclisation and subsequent aromatisation. Our findings confirm this hypothesis and provide direct evidence on a molecular level for the abiogenic sulphur enrichment of macromolecular sedimentary organic matter.

An important point to note is the fact that, whilst the relative abundance of OSPP varies considerably, the same types of sulphur-containing moieties are present in a wide variety of kerogen types derived from different input material. This indicates that early diagenetic sulphur incorporation into organic matter is a ubiquitous process and is not limited to specific types of environments, although the presence of hydrogen sulphide (or possibly polysulphides) is a prerequisite.

Implications for the role of sulphur in the evolution of sedimentary organic matter

In the light of the above discussion, some further comments can be made on the role of organically-bound sulphur in the evolution of sedimentary organic matter.

The similarity between the OSPP of kerogens and asphaltenes was discussed earlier. A distinct relationship between asphaltene content and sulphur concentration has also been reported previously (Orr, 1986) with sulphur-rich bitumens and crude oils, in general, containing the highest amounts of asphaltenes. Furthermore, high-sulphur kerogens (so-called "Type II-S", Orr, 1986) reportedly generate petroleums at a relatively low level of thermal exposure (Tannenbaum and Aizenshtat, 1985; Lewan, 1985; Jones, 1984). These "immature" petroleums are also high in asphaltenes. These and other observations have led several authors to propose that asphaltenes represent intermediates between kerogen and hydrocarbons (*e.g.* Pelet *et al.*, 1986; Orr, 1986). It seems likely that the abundance of asphaltenes in immature petroleums generated from sulphur-rich sediments may be due to break down of the kerogen macromolecule at relatively weak sites (*e.g.* sulphur bridges such as sulphide and disulphide linkages). In this way smaller, but intact, fragments of kerogen (*i.e.* asphaltenes) are produced. The abundance of these labile bridges within the kerogen will determine the extent to which degradation proceeds by this mechanism. Sulphur-lean kerogens may not show significant degradation until the thermal energy required for the cleavage of stronger bonds (*e.g.* carbon-carbon bonds) is available. Under these circumstances petroleum generation may largely by-pass the asphaltene stage.

High asphaltene contents in bitumens associated with high-sulphur kerogens are, however, not restricted to sediments which have experienced catagenesis. We have noted that very immature, sulphur-rich sediments contain bitumens which are very high in asphaltenic materials. For example, the bitumens extracted from Jurf ed Darawish and Monterey shales contained more than 50% asphaltenes. This asphaltenic material is probably formed directly, by interaction of hydrogen sulphide with unsaturated and functionalised lipids and perhaps carbohydrates (Moers *et al.*, 1988), rather than through degradation of the kerogen. In this context, sulphur may play an important structural role in such materials. Moreover, when hydrogen sulphide (or other suitable donors of sulphur) is abundant, the functionalised biological precursors may be "mopped-up" becoming an integral part of the macromolecules *in statu nascendi*. This would give rise to low concentrations of free lipids. When sulphur donors are less widely available then the "mopping-up" process would be less important and unsaturated lipids would be more susceptible to reduction and therefore serve as a source for free hydrocarbons.

It appears therefore that sulphur plays a particular role in determining both the molecular weight distribution of sedimentary organic matter and its subsequent fate. Kerogens and asphaltenes may be regarded as part of the same continuum, at least as far as the sulphur-rich parts are concerned, and as such produce closely related OSPP distributions.

A second point arising from the present study concerns the differences between the types of organic sulphur compounds identified in pyrolysates, immature bitumens and oils and in mature oils. Recently it has been demonstrated that some of the major organic sulphur compounds in sulphur-rich sediment extracts and immature crude oils are alkylthiophenes and alkylthiolanes (Schmid, 1986; Schmid *et al*., 1987; Brassell *et al*., 1986c; Sinninghe Damste and de Leeuw, 1987; Sinninghe Damste *et al*., 1986; 1987a and c; 1988g). Mature oils, however, only contain sulphur compounds with thiophene rings as part of more complex ring systems (primarily benzothiophenes, dibenzothiophenes and benzonaphthothiophenes; *e.g*. Hughes, 1984; Arpino *et al*., 1987). Three possible explanations for these different organic sulphur compound distributions are considered here:

(1) thermal degradation of long-chain organic sulphur compounds to volatile compounds;

(2) dilution by newly generated sulphur compounds from kerogen maturation;

(3) cyclisation and aromatisation of long-chain organic sulphur compounds.

Evidence for (1) has been provided by Schmid (1986) who heated the unusually sulphur-rich (8 %) West Rozel Point oil at 300°C for up to 24 days and noted a shift in the organic sulphur compound distribution to lower carbon numbers. These results, however, are by no means conclusive. This particular oil contains abundant high molecular weight compounds (resins and asphaltenes; *ca*. 60%) which are also potential contributors to volatile sulphur compounds, particularly taking into account the fact that the temperature employed in these experiments is known to be sufficiently high for cracking resins and asphaltenes.

The ability of kerogens and asphaltenes to generate low molecular weight ($<C_{15}$) sulphur compounds under conditions similar to those used by Schmid (1986) has been demonstrated by Eglinton *et al*. (1988a). In this particular study, kerogens and asphaltenes were subjected to "hydrous pyrolysis", a technique which has been reported to produce "oil-like" pyrolysates (Lewan *et al*., 1979). Furthermore, the types of products from these artificial maturation experiments were similar to those observed in flash pyrolysates

(Eglinton *et al.*, 1988f). This suggests that the organic sulphur compounds produced by pyrolysis are also likely to be formed under natural conditions and, therefore, that kerogens and related macromolecules may be considered as sources of organic sulphur compounds with a relatively low molecular weight (*i.e.* explanation (2)). Although the presence of volatile sulphur compounds in association with petroleums has only been reported infrequently, some supportive evidence does exist. Thompson (1981) identified a series of low molecular weight thiophenes and thiols in a Texan crude oil, whilst Le Tran (1972) detected substantial amounts of hydrogen sulphide associated with organic matter in carbonates. Moreover, this author also suggested that the hydrogen sulphide was probably derived from the organic matter by cleavage of C-S bonds. The paucity of literature on the occurrence of low molecular weight organic sulphur compounds in fossil fuels may also partly be explained by their volatility. These compounds are easily lost during sampling and work-up. In addition, they may be transferred into the gaseous phase for the greater part with the consequence that they are less effectively trapped within petroleum reservoirs.

The final alternative (3) is supported by the identification of 2-alkyl- and 2,4-di-*n*-alkylbenzo[*b*]thiophenes in crude oils (Perakis, 1986; Sinninghe Damste *et al.*, 1987c). The disproportionately large amounts of these isomers relative to others can be explained by the cyclisation and aromatisation of 2-*n*-alkyl and 2,5-di-*n*-alkylthiophenes in which the alkyl group(s) contain at least four carbon atoms in a linear chain. Similar cyclisation and aromatisation reactions have been proposed to explain the presence of isoprenoid benzothiophenes in some bitumens and oils (Sinninghe Damste and de Leeuw, 1987) and might also occur within the kerogen structure. The high relative amounts of 2- (and possibly 4-) methylbenzo[*b*]thiophenes in the flash pyrolysates (this study) and the identification of homologous series of 2-alkyl and 2,4-dialkylbenzo[*b*]thiophenes in sulphur-rich kerogen pyrolysates (Sinninghe Damste *et al.*, 1988c) may testify to the latter phenomenon. An increased relative abundance of alkylbenzothiophenes over alkylthiophenes in flash pyrolysates of artificially matured kerogens was noted by Eglinton *et al.* (1988b). Although the absolute amounts of OSPP in the residue undoubtedly decrease as a result of this artificial maturation, the increased dominance of alkylbenzothiophenes relative to alkylthiophenes is also probably due partly to such ring closure reactions. The extent to which aromatisation reactions prevail over those which result in generation of low molecular weight sulphur compounds may be dependent upon a number of factors including thermal history. Therefore results obtained from high temperature simulation experiments must be treated with caution. Future work may resolve which of the two remaining alternatives (*i.e.* 2 and 3) are actually operative.

The preferential generation of limited numbers of isomers in the OSPP has a further implication regarding the distribution of organic sulphur compounds in petroleums and related fossil fuels. The same dominance has been observed in some immature sediment extracts (Sinninghe Damste *et al.*, 1988e) which suggests that sulphur incorporation is not a random process. Consequently, the isomer distribution of organic sulphur compounds in petroleums may, initially, be influenced by the original sites of addition in precursor molecules, although kinetic control may be the dominant factor at higher levels of maturity. There are several reports in which such isomer preferences in petroleums are mentioned. The occurrence of 2,4-di-*n*-alkylbenzo[*b*]thiophenes has already been discussed. An isomer distribution of C_6-thiophenes similar to those recorded in the OSPP was observed in a crude oil from Wilmington, California (Thompson, 1981). Gallegos (1985) also noted that the distribution of the alkylated thiophenes present in a number of oils were dominated by a limited number of all possible isomers. These data and findings are therefore at variance with the views of Arpino *et al.* (1987) who consider that organic sulphur compounds in sediments are formed by random alkylation and degradation.

12.5 CONCLUSIONS

(1) The organic sulphur pyrolysis products (OSPP) from a wide variety of macromolecular sedimentary organic materials (kerogens, asphaltenes, coals) differ markedly in absolute and relative abundance. However, all samples produced sufficient amounts of these compounds to be detected by the FPD. The analysis of OSPP from sedimentary organic materials is, therefore, not restricted to sulphur-rich samples.

(2) The types of products are generally similar, being mainly comprised of "gaseous" compounds (*e.g.* hydrogen sulphide) and low molecular weight alkylthiophenes. The latter products are dominated by a limited number of isomers and, most significantly, their alkyl substitution patterns bear strong similarity to those observed in immature sediment extracts and crude oils. This suggests, firstly, that the OSPP are related to specific moieties within the kerogen and, secondly, that sulphur incorporation into these macromolecular materials is not a random process, but occurs at specific sites in the precusor lipid moieties. This leads us to suggest that:

(3) Analysis of organic sulphur compounds produced by flash pyrolysis of kerogens and related materials may yield information on the relative amounts of the various sulphur-containing structural moieties within these macromolecules. This, in turn, may shed light both upon the contributions from the different source organisms and the role of sulphur in the formation of macromolecular organic substances. It should be emphasised, however, that postulated precursors are not absolutely categorical. Rather, they are merely intended to provide a framework for further study.

(4) The types of OSPP observed provides further proof for the early diagenetic enrichment of organic matter with sulphur, and the mechanisms by which sulphur incorporation might take place.

(5) Examination of the OSPP from kerogens and asphaltenes indicates a genetic relationship between these macromolecules suggesting that the sulphur-rich parts share a common origin and fate.

(6) In specific cases (*i.e.* high-sulphur environments) sulphur incorporation into organic matter may act as a mechanism for the formation of high molecular weight substances including kerogens and asphaltenes.

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Curriculum Vitae

Jaap S. Sinninghe Damsté werd op 1 januari 1959 te Baarn geboren. Na het behalen van het diploma Atheneum-B aan het Baarnsch Lyceum begon hij in 1977 zijn studie Elektrotechniek aan de Technische Universiteit Delft. Na één jaar besloot hij over te stappen naar de afdeling der Scheikundige Technologie waar hij in juni 1982 met goed gevolg het kandidaatsexamen aflegde. De doctoraalfase van zijn studie bestond uit een bijvak Organische Geochemie en een hoodvak analytische scheikunde (richting: intrumentele analyse). Zijn afstudeeronderzoek (Reactie van chloor met humuszuur) verrichtte hij bij prof. dr. L. de Galan onder leiding van dr. ir. E.W.B. de Leer. Na het behalen van het doctoraalexamen (met lof) in juni 1984 trad hij op 1 juli 1984 in dienst van de Technische Universiteit Delft voor het verrichten van een promotieonderzoek naar de vorming van organisch-gebonden zwavel in de geosfeer, waarvan dit proefschrift de verslaggeving omvat.

Stellingen

1

Het is, gezien de enorme consequenties van de aanwezigheid van zwavel in fossiele brandstoffen voor het milieu, verwonderlijk dat de in dit proefschrift beschreven resultaten anno 1988 nog orgineel zijn.

2

De door hun naamgeving gesuggereerde samenstelling van de "aromatische koolwaterstoffen"- en "hetero-component" fracties van oliën en sediment extracten is niet altijd in overeenstemming met de werkelijke chemische samenstelling.

3

De identificatie van een C_{25} éénmaal onverzadigde monocyclische hoog vertakte isoprenoide koolwaterstof in recente antarctische sedimenten door Venkatesan en Kaplan (1987) moet op grond van een door deze auteurs uitgevoerd hydrogeneringsexperiment als onjuist beschouwd worden.

Venkatesan M.I. and Kaplan I.R., Mar. Chem. 21, 347-375 (1987).

4

Hoewel de Amposta olie inderdaad isoprenoide alkylbenzenen bevat, zijn de door Albaigés *et al.* (1986) als isoprenoide alkylbenzenen geidentificeerde verbindingen in deze olie zeer waarschijnlijk 1,2-di-*n*-alkylbenzenen.

Albaigés J., Algaba J., Clavell E. and Grimalt J., In Advances in Organic Geochemistry 1985 (D. Leythaeuser and J. Rullkötter, eds.), Org. Geochem. 10, 441-450 (1986).

5

De door Arpino *et al.* (1987) voorgestelde "random" vorming van zwavelbevatttende polycyclische aromatische koolwaterstoffen in aardoliën stoelt op onvoldoende bewijs.

Arpino P.J., Ignatiadis I. and de Rijcke G., J. Chromatogr. 390, 329-348 *(1987)*

6

Het meer aromatische karakter van Type II en type III kerogeen t.o.v. dat van Type I kerogeen zal eerder tot uitdrukking komen in de relatieve hoeveelheid aromatische pyrolyseproducten t.o.v. alifatische producten dan in de verhouding van thiofenen t.o.v. benzothiofenen gevormd bij pyrolyse.

Philip R.P. and Bakel A., Energy Fuels 2, 59-64 (1988)

7

Het hanteren van een indicatieve richtwaarde voor de totaal concentratie aan polycyclische aromatische koolwaterstoffen (PAK) voor de beoordeling van verontreinigingsniveaus in de bodem dient op grond van de grote verschillen in fysische en chemische eigenschappen van de verschillende leden van deze groep van stoffen afgewezen te worden.

Handboek bodemsaneringstechnieken (1988)

8

Het publiceren van een wetenschappelijke "ontdekking" op basis van andermans werk is de uiterste consequentie van de "Publish or Perish" gedachte.

Sheng Guoying, Fu Jiamo, Jiang Jigang, Liang Digang, Brassell S.C. and Eglinton G., Scientia Sinica 30, 1338-1344 (1987).
Sinninghe Damste J.S., Kock-van Dalen A.C., de Leeuw J.W., Schenck P.A., Sheng Guoying and Brassell S.C., Geochim. Cosmochim. Acta 52, 2393-2400 *(1987).*

9

Bij het reconstrueren van afzettingsmilieus op basis van biomarkers dient voor milieus waar sulfaatreductie heeft plaatsgevonden de nodige voorzichtigheid betracht te worden.

10

Helaas bestaat er in Nederland nog steeds wettelijke grond voor de uitdrukking "vogelvrij verklaard zijn".

Jachtwet (1954)

11

Het plan om automobilisten ook overdag licht te laten voeren kan aangemerkt worden als een poging ons aftakelende leefmilieu tenminste nog het aanzien te geven van een uitgebreid bezochte begrafenis.

Delft, 24 November 1988 J.S. Sinninghe Damsté

Stellingen behorend bij het proefschrift *Organically-bound sulphur in the geosphere: A molecular approach.*