

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

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Abstract—The bitumen of the Jurf ed Darawish Oil Shale has been analysed for organic sulphur compounds (OSC). A number of OSC are reported for the first time: several C₂₈, C₃₇ and C₃₈ 2,5-dialkylthiolanes and -thiophenes and 2,6-di-*n*-alkylthianes, and C₁₉ branched thiophenes possessing the 9-methyloctadecane carbon skeleton. A number of these compounds were 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 low molecular weight OSC. Double bond isomerisations by a sequence of H₂S addition and elimination reactions may play a role in this respect.

INTRODUCTION

RECENTLY, homologous series of 2,5-di-*n*-alkylthiolanes (I) have been identified in immature oils and bitumens by comparison of mass spectral and relative retention time data with those of synthesised members of these series (SCHMID *et al.*, 1987; SINNINGHE DAMSTÉ *et al.*, 1986, 1987). 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.*, 1987) 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.*, 1989c). 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 cyclic sulphides (which were mainly comprised of 2,5-di-*n*-alkylthiolanes) together with the "random" isomer composition of the 2,5-di-*n*-alkylthiolanes led SCHMID *et al.* (1987) to postulate 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.* (1988) 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.*, 1987) 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 functionalised¹ precursors. Similar observations were made for other OSC classes and are also described in this paper.

EXPERIMENTAL

Sample. The Jurf ed Darawish Oil Shale is a Cretaceous deposit (thickness *ca.* 100 m) of bituminous calcareous marlstones and 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. CaCO₃ (*ca.* 40%, WEHNER and HUFNAGEL, 1987) and clay are the major inorganic constituents. The sample contains a high amount (17.3% determined on the solvent extracted rock sample) of immature organic matter (%R₀ = *ca.* 0.30). Some information on the hydrocarbon biological markers (WEHNER and HUFNAGEL, 1987) and OSC (SINNINGHE DAMSTÉ *et al.*, 1989c) have been reported elsewhere. The kerogen present in this sample is marine derived (Type II) with a high sulphur content (S/C = 0.084; SINNINGHE DAMSTÉ *et al.*, 1989a).

Extraction. The sample was powdered in a rotary disc mill and extracted in a soxhlet apparatus 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 was fractionated (without prior removal of asphaltenes; asphaltene content was determined separately: 23%) into three fractions with a column (34 cm × 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 aliquot (*ca.* 10 mg) of the apolar fraction (70 mg; 9% of total bitumen) was further separated by argentatious thin layer chromatography using hexane as developer. The AgNO₃-

¹ In this paper we define functionalised molecules as those molecules containing a functional group including double bonds (*cf.* STREITWIESER and HEATHCOCK, 1976).

impregnated silica plate (20 × 20 cm; thickness 0.25 mm) was prepared by dipping it in a solution of 1% AgNO₃ in MeOH/H₂O (4:1, v/v) for 45 s and subsequent activation at 120°C for 1 h. Seven bands (Table 1) were detected by inspection under UV light after spraying with Rhodamine 6G. These bands were scrapped off the TLC plate and ultrasonically extracted with ethyl acetate (×3). These fractions were analysed by GC and GC-MS. Coinjections with authentic standards were also performed with these sub-fractions.

A so-called low-molecular weight aromatic (LMWA) fraction was also obtained from the bitumen as described elsewhere (SINNINGHE DAMSTÉ *et al.*, 1989c). The LMWA fraction contained most of the GC-amenable compounds of the "aromatic" fraction including the OSC described previously (SINNINGHE DAMSTÉ *et al.*, 1987).

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 × 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, a cycle time of 1.8 s and a resolution of 1000. The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil 5 (film thickness = 0.12 μm) and was operated with helium as carrier gas. Fractions (1.0 μl) in ethyl acetate (*ca.* 6 mg/ml) were injected on-column at 50°C 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 ketone obtained 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 resulting 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, 1987).

The C₁₉ "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 resulting mixture of ketones was subsequently reduced to yield 2,5-diheptyl-3-methylthiophene. The mass spectrum and ¹H-NMR spectrum (400 MHz, CDCl₃, 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 CH₃)), 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 methyl lithium which after dehydration of the resulting tertiary alcohol yielded a mixture of isomeric 2-butyl-5-(2'-undecenyl)thiophenes. Ionic hydrogenation of this mixture afforded 2-butyl-5-(2'-undecyl)thiophene. The mass spectrum and ¹H-NMR spectrum (400 MHz, CDCl₃, 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 an equimolar amount of butyllithium was slowly added. After 2 h an equimolar amount of tetramethylenediamine was slowly added. After completion a two-

fold excess (on a molar basis) of the alkylbromide in THF was added over *ca.* 30 min. After 1 h the reaction mixture was refluxed for 2 h. After cooling the reaction mixture was transferred to a separatory funnel and 25 ml H₂O were added. The resulting mixture was extracted with diethylether (×2) and the combined layers were washed with 10 ml 2M HCl (×3) and 10 ml sat. NaCl solution (×2), dried with MgSO₄ and the solvent was evaporated with a rotary evaporator. The yellow-brown oil obtained 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 CH₂Cl₂ with a catalytic amount of DMF. After 30 min the solution was cooled to –10°C and 0.6 equivalent of SnCl₄ was added over *ca.* 10 min. After stirring for 2.5 h at 0°C 10 ml H₂O was added, the CH₂Cl₂-layer was washed with sat. NaHCO₃ solution and almost evaporated to dryness. *Ca.* 10 ml diethyl ether were added and the solution was stirred with 10 ml 4M KOH for 15 min. The diethyl ether layer was subsequently washed with H₂O (×2) and sat. NaCl solution (×2), dried with MgSO₄ and evaporated to dryness.

Reduction of 2-(alkyl-1'-oxo)thiophenes. The ketone was dissolved in dry ether and 1.8 molar equivalent of AlCl₃ in dry ether was slowly added at –10°C. After 30 min the complex was reduced with 1.2 molar equivalent LiAlH₄. 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 H₂O, 5% NaCl in H₂O and a sat. NaCl solution, dried with MgSO₄ and evaporated to dryness.

Ionic hydrogenation of 2,5-dialkylthiophenes. The 2,5-dialkylthiophenes were hydrogenated as reported elsewhere (SINNINGHE DAMSTÉ *et al.*, 1987).

RESULTS

The organic sulphur compounds (OSC) present in the Jurf ed Darawish Oil Shale were studied by GC-MS analysis 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 general composition of these sub-fractions is given in Table 1. A general description of the compounds present in the LMWA fraction of this sample has been given elsewhere (SINNINGHE DAMSTÉ *et al.*, 1989c). The overall composition of the OSC present in the LMWA fraction of the Jurf ed Darawish Oil Shale is illustrated by a chromatogram of the desulphurised LMWA fraction (Fig. 1; SINNINGHE DAMSTÉ *et al.*, 1988). It shows that the carbon frameworks of the OSC are predominantly linear and isoprenoid, although some

Table 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

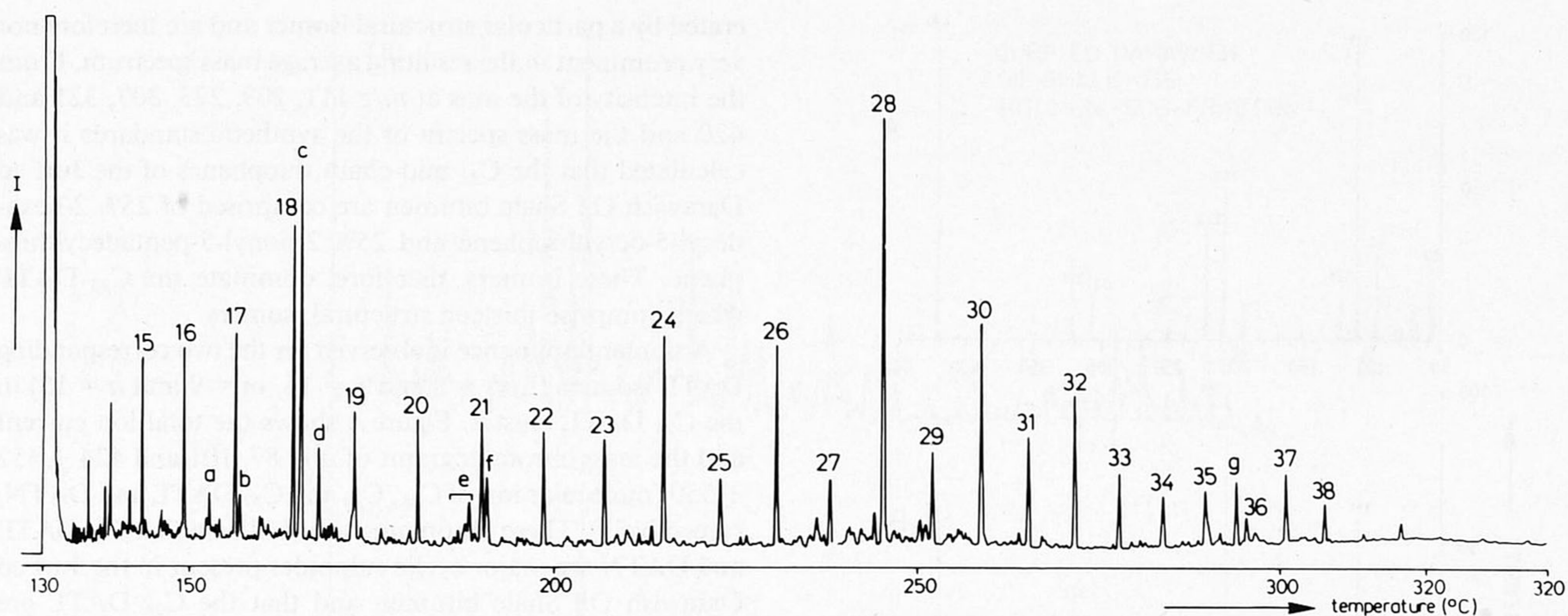


FIG. 1. Gas chromatogram of the desulphurised LMWA fraction of the JED bitumen. Arabic numbers indicate number of carbon atoms of *n*-alkanes. Key: *a* = norpristane, *b* = pristane, *c* = phytane, *d* = 9-methyloctadecane, *e* = monounsaturated C_{25} highly branched isoprenoids, *f* = 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane, *g* = 17 α (H),21 β (H)-pentakishomohopane.

branched and triterpenoid hydrocarbons also occur. 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. This contrasts with the distribution of the hydrocarbons, which are characterised by a slight odd-over-even carbon number predominance of the *n*-alkanes, a pristane/phytane ratio of 0.37, relatively high amounts (*ca.* 30% of the saturated hydrocarbon fraction) of extended hopenes maximising at C_{35} and a much lower relative abundance of octacosane, 9-methyloctadecane and 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (SINNINGHE DAMSTÉ *et al.*, 1988). In the following a number of examples of OSC classes

which show "non-random" isomer distributions will be discussed.

C_{28} OSC with linear carbon frameworks

As described above these compounds are relatively abundant in the LMWA fraction. The isomer composition of C_{28} 2,5-di-*n*-alkylthiophenes (DATP; II), 2,5-di-*n*-alkylthiolanes (DATL; I) and 2,6-di-*n*-alkylthianes (DATN; III) is, however, quite different from those with other total carbon numbers.

Figure 2 shows the partial mass chromatograms of the *m/z* values of the molecular ions of the C_{24} , C_{26} , C_{28} and C_{30} DATP for sub-fraction 3 of the Jurf ed Darawish Oil Shale bitumen. The isomer clusters are built up as follows. All mid-chain DATP (II, $m > n$, $n > 4$; *a* in Fig. 2) coelute, whilst

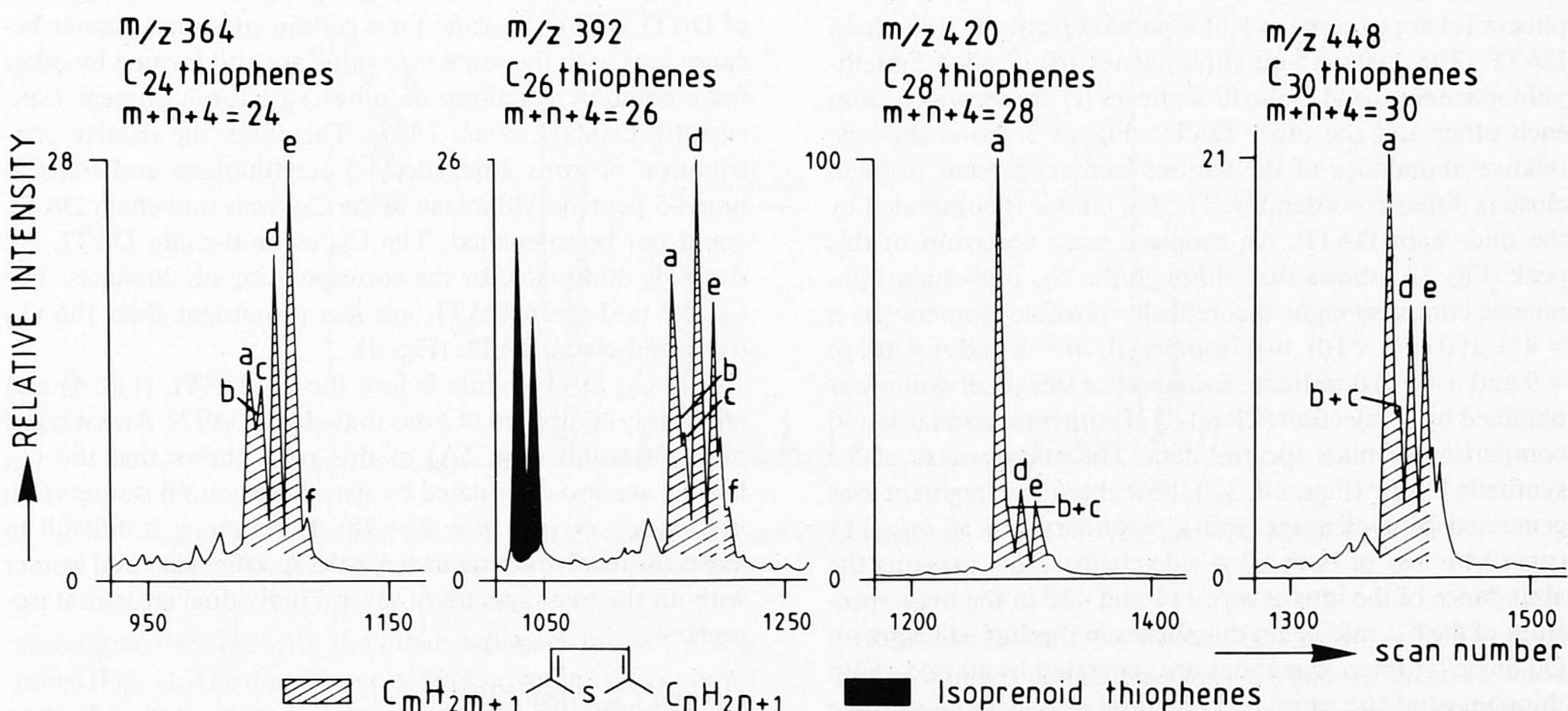


FIG. 2. Partial mass chromatograms of *m/z* 364, 392, 420 and 448 of sub-fraction 3 of the apolar fraction of the JED bitumen. Key: *a*: mid-chain thiophenes ($m > n$, $n > 4$); *b*: $n = 4$; *c*: $n = 3$; *d*: $n = 2$; *e*: $n = 1$; *f*: $n = 0$.

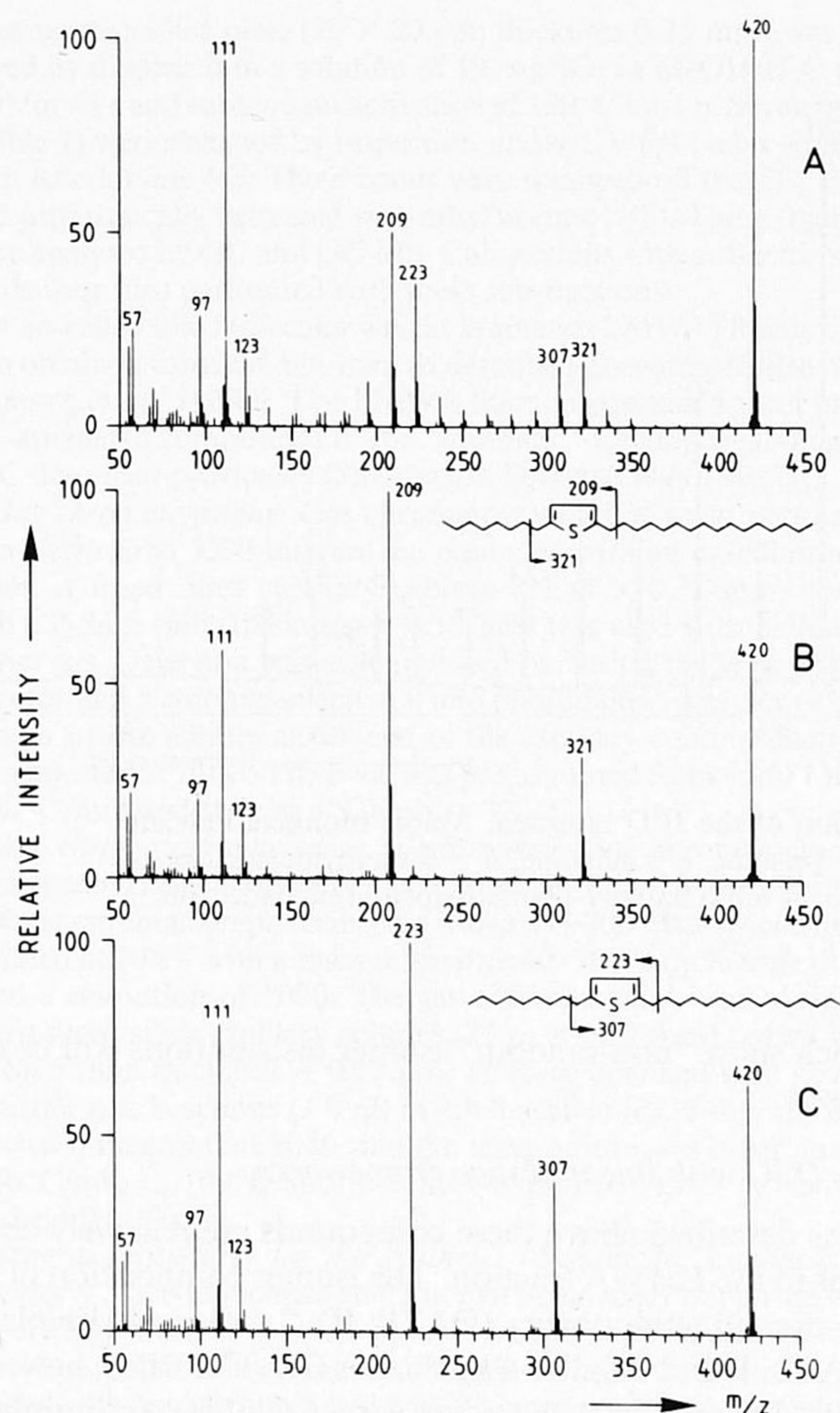


FIG. 3. Mass spectra (subtracted for background) of: (A) the C_{28} mid-chain DATP peak (averaged over six scans); (B) synthetic 2-hexadecyl-5-octylthiophene; (C) synthetic 2-nonyl-5-pentadecylthiophene.

the 2-alkyl-5-butyl-thiophenes (b) and 2-alkyl-5-propylthiophenes (c) are to some extent separated from the mid-chain DATP. The 2-alkyl-5-ethylthiophenes (d), 2-alkyl-5-methylthiophenes (e) and 2-alkylthiophenes (f) are separated from each other and the other DATP. Figure 2 shows that the relative abundance of the various isomers present in these clusters differs considerably. The C_{28} cluster is dominated by the mid-chain DATP. An averaged mass spectrum of this peak (Fig. 3A) shows 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. 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 thiophenes of the same carbon number and because these isomers coelute they accumulate in the resulting mass spectrum. On the other hand, the β -cleavage ions are only gen-

erated by a particular structural isomer and are therefore not very prominent in the resulting average 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% 2-hexadecyl-5-octylthiophene and 25% 2-nonyl-5-pentadecylthiophene. These isomers, therefore, dominate the C_{28} DATP which 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. Figure 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. A detailed discussion of the mass spectrometry and retention behaviour of DATL and DATN is given by SINNINGHE DAMSTÉ *et al.* (1987). An averaged mass spectrum of the C_{28} *trans* DATL peak (Fig. 5B) 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* DATL isomers are given in Figs. 5C and 5D. The mass spectral fragmentation patterns are in accordance with those of other DATL as reported previously (SCHMID *et al.*, 1987; SINNINGHE DAMSTÉ *et al.*, 1986, 1987). 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% 2-hexadecyl-5-octylthiolane and 25% 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.*, 1987). Therefore, the relative contribution of *trans* 2-hexadecyl-5-octylthiolane and *trans* 2-nonyl-5-pentadecylthiolane to the C_{28} *trans* mid-chain DATL could not be calculated. The C_{28} *cis* mid-chain DATL are similarly dominated by the corresponding *cis* thiolanes. The C_{28} *cis* mid-chain DATL are less prominent than the C_{28} *trans* mid-chain DATL (Fig. 4).

The C_{28} DATN elute before the C_{28} DATL (Fig. 4) and are mainly comprised of *trans* mid-chain DATN. An averaged mass spectrum (Fig. 5A) 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 relative abundance of each specific structural isomer without the mass spectra of several individual structural isomers.

C_{37} and C_{38} linear OSC

Upon desulphurisation of the LMWA fraction heptacosane and octacosane were formed in relatively large

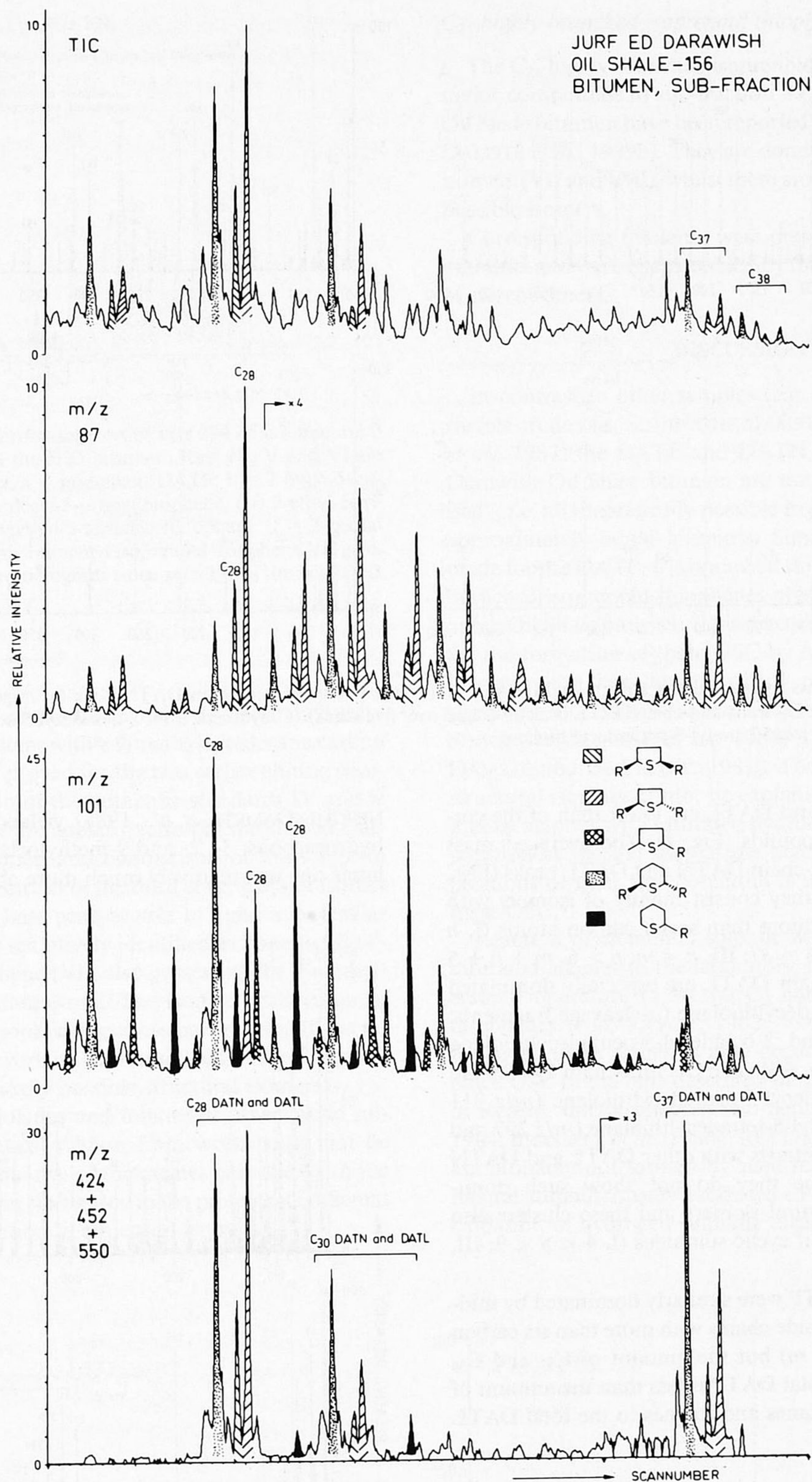


FIG. 4. Partial total ion current (TIC) and partial mass chromatograms of m/z 87, 101, and 424 + 452 + 550 of sub-fraction 7 of the apolar fraction of the JED bitumen.

amounts compared with the other n -alkanes in the C_{35} - C_{40} range (Fig. 1). The dominance of heptatricosane is in contrast with the observed even over odd carbon-number predominance of 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. 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

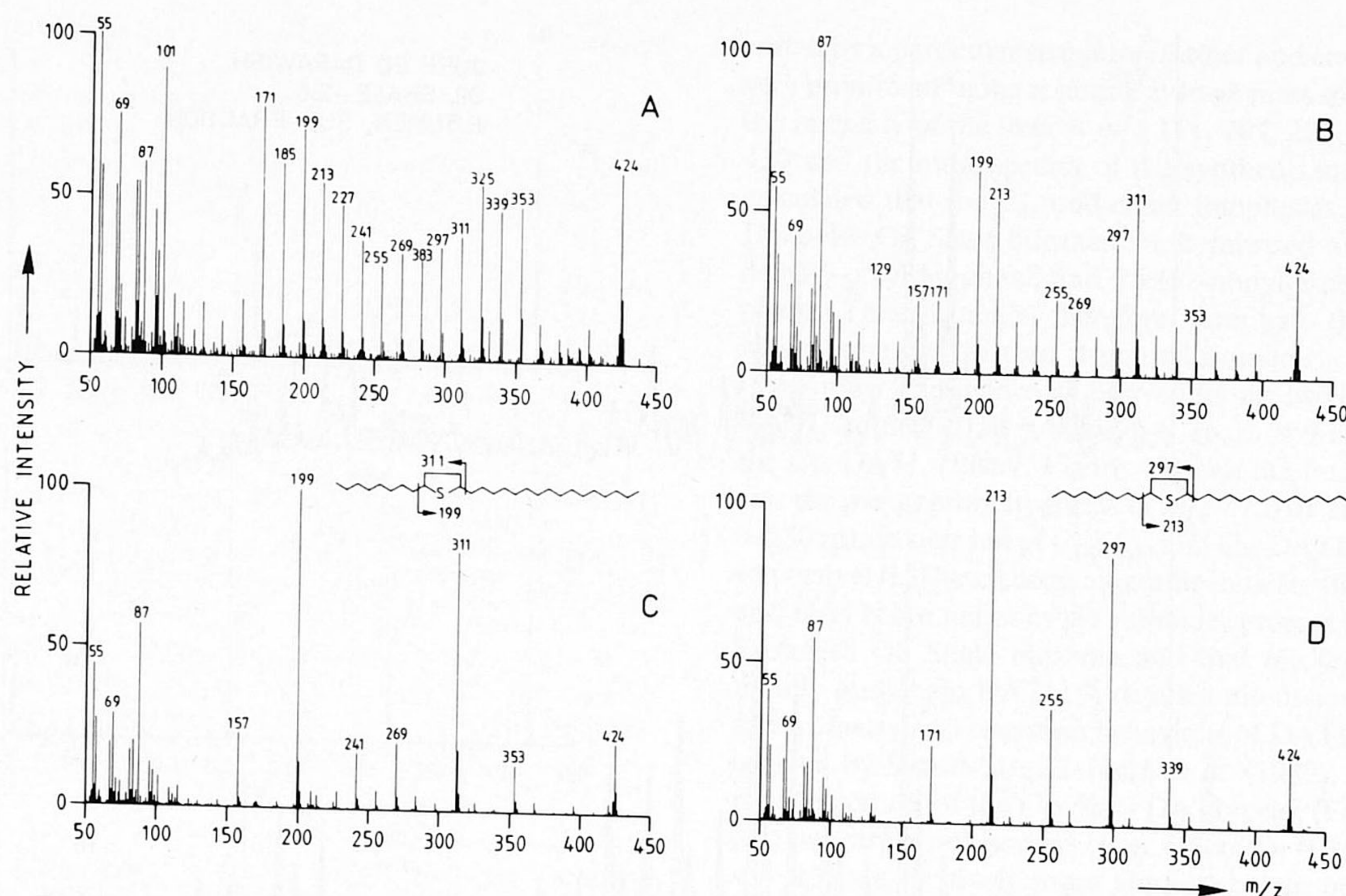


FIG. 5. Mass spectra (subtracted for background) of: (A) the C₂₈ *trans* mid-chain DATN peak (averaged over five scans); (B) the C₂₈ *trans* mid-chain DATL peak (averaged over five scans); (C) synthetic *trans* 2-hexadecyl-5-octylthiolane; (D) synthetic *trans* 2-nonyl-5-pentadecylthiolane.

cis mid-chain DATN and DATL are lower than of the corresponding *trans* compounds (Fig. 4). The averaged mass spectra of the *trans* mid-chain DATN and DATL peaks (Figs. 6A, 6B) indicate that they consist mainly of isomers with alkyl side chains with more than seven carbon atoms (I, $n < m$, $n > 7$, $m + n + 4 = 37$; III, $n < m$, $n > 6$, $m + n + 5 = 37$). The C₃₇ mid-chain DATL are especially dominated by 2-heptadecyl-5-hexadecylthiolane (α -cleavage fragments: m/z 311 and 325) and 2-octadecyl-5-pentadecylthiolane (m/z 297 and 339) (Fig. 4B). The C₃₇ mid-chain DATN are dominated by 2-heptadecyl-5-pentadecylthiolane (m/z 311 and 339) and 2-octadecyl-5-tetradecylthiolane (m/z 297 and 353) (Fig. 4A). This contrasts with other DATL and DATN isomer clusters because they do not show such dominance of specific structural isomers and these clusters also contain other mid-chain cyclic sulphides (I, $4 < n < 9$; III, $4 < n < 7$).

The C₃₇ and C₃₈ DATP were similarly dominated by mid-chain DATP with alkyl side-chains with more than six carbon atoms (II, $n > 6$, $n < m$) but the amount of C₃₇ and C₃₈ DATP relative to the total DATP is less than the amount of the corresponding thiolanes and thianes to the total DATL and DATN.

C₁₉ branched thiophenes

The mass chromatogram of m/z 294, the molecular ion of C₁₉ thiophenes, shows a number of peaks which cannot be ascribed to either C₁₉ DATP or C₁₉ isoprenoid thiophenes (Fig. 7). The compounds elute before C₁₉ DATP but after the C₁₉ isoprenoid thiophenes and were therefore thought to be branched thiophenes. Desulphurisation of the LMWA fraction of the Jurf ed Darawish Oil Shale bitumen (SIN-

NINGHE DAMSTÉ *et al.*, 1988) yielded three C₁₉ branched hydrocarbons; 3-, 2- and 9-methyloctadecane, of which the latter one was relatively much more abundant (see also Fig.

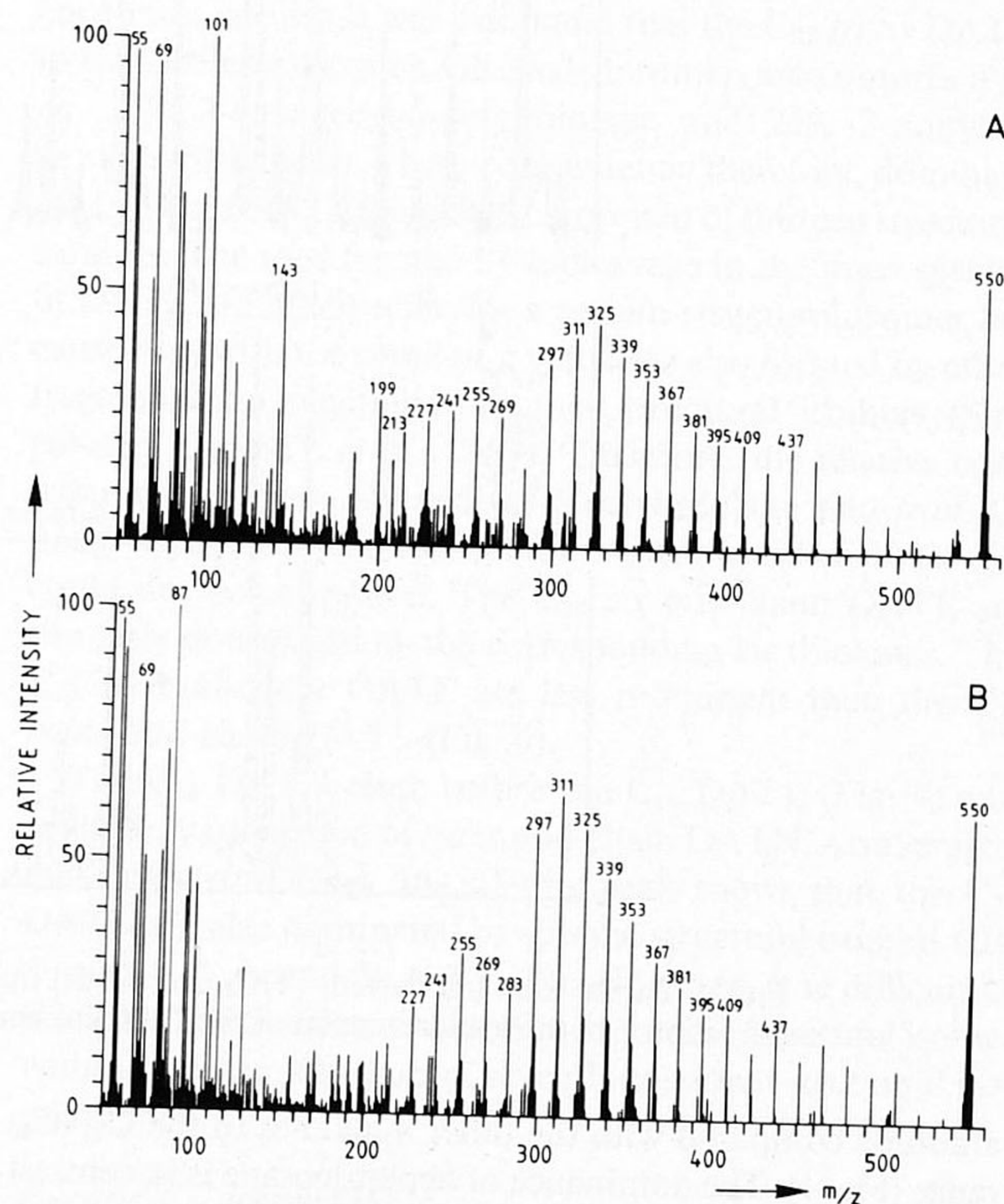


FIG. 6. Mass spectra (subtracted for background) of: (A) the C₃₇ *trans* mid-chain DATN peak (averaged over five scans); (B) the C₃₇ *trans* mid-chain DATL peak (averaged over five scans).

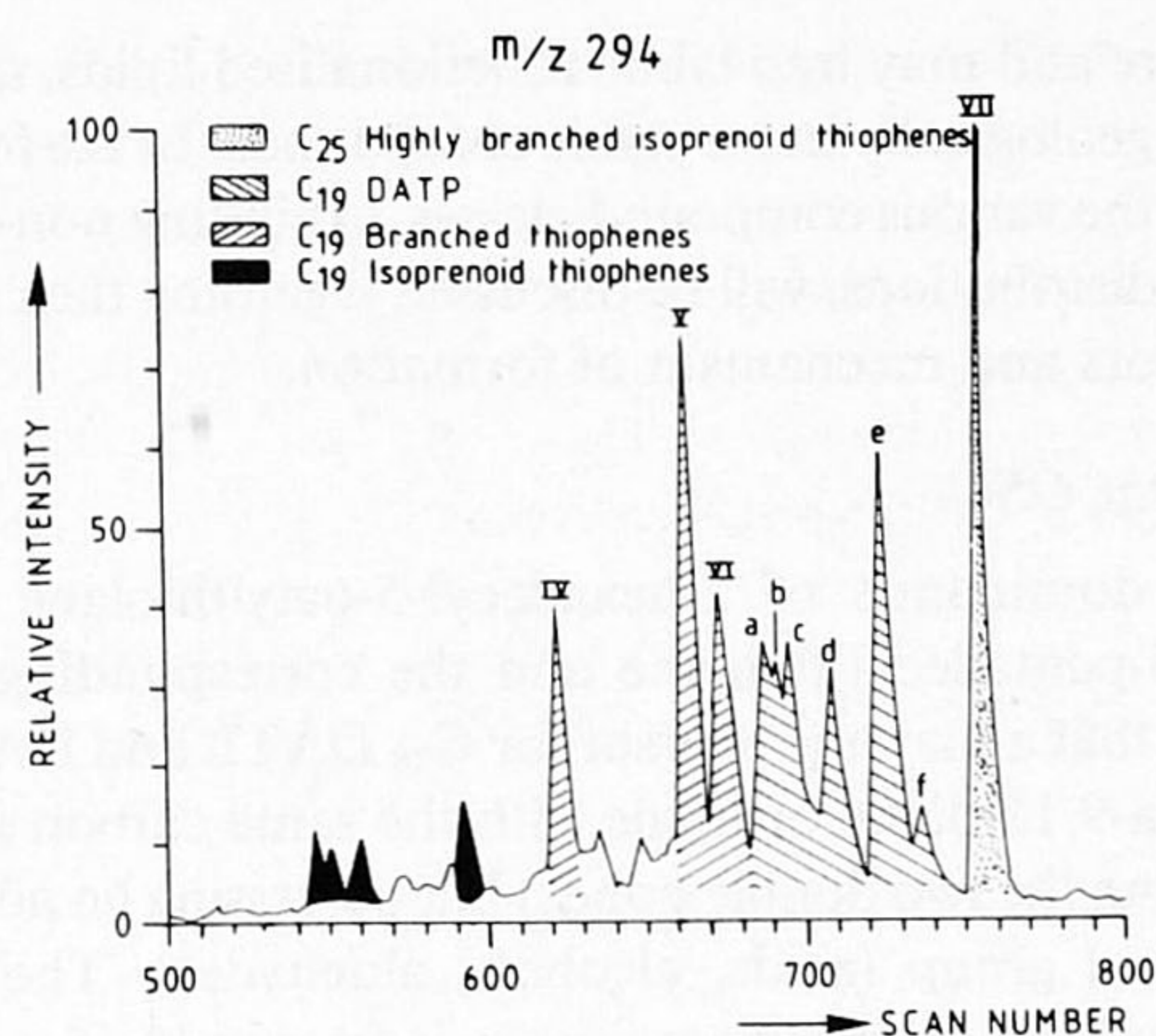


FIG. 7. Partial mass chromatograms of m/z 294 of sub-fraction 3 of the apolar fraction of the JED bitumen. Key: IV, V and VI are C_{19} branched thiophenes; a = mid-chain DATP; b = 2-butyl-5-undecylthiophene, c = 2-dodecyl-5-propylthiophene, d = 2-ethyl-5-tridecylthiophene, e = 2-methyl-5-tetradecylthiophene, 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.

1). The mass spectra of the three major C_{19} branched thiophenes were all consistent with a 9-methyloctadecane carbon framework. This was proved 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. 8). The mass spectrum of the 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 could not be identified as a consequence of the greater complexity of the C_{19} thiolane and thiane cluster due to the presence of different stereoisomers in addition to structural isomers.

C_{25} highly branched isoprenoid thiophenes

The C_{25} highly branched isoprenoid thiophenes present as major compounds in sub-fraction 3 of the Jurf ed Darawish Oil Shale bitumen have been reported elsewhere (SINNINGHE DAMSTÉ *et al.*, 1989b). They are dominated by two structural isomers (VII and VIII), whilst there are seventeen theoretically possible isomers.

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

DISCUSSION

In contrast to other samples (*e.g.*, Rozel Point and Maruejols crude oils, SINNINGHE DAMSTÉ *et al.*, 1987; SCHMID *et al.*, 1987) the DATL and DATN 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; BRASELL *et al.*, 1986c; SINNINGHE DAMSTÉ *et al.*, 1986, 1987, 1988; SINNINGHE DAMSTÉ and DE LEEUW, 1987). The dominance of certain structural isomers cannot be explained by a reaction of elemental sulphur with saturated hydrocarbons. Sulphur incorporation at specific sites of the precursors triggered by the positions of their functionalities is a more likely origin for these OSC.

Reaction of elemental sulphur with mono- and polyunsaturated alkenes in the laboratory starts at relatively moderate temperature (120–160°C) and yields sulphides and thiophenes (*e.g.*, PRYOR, 1962; VORONKOV *et al.*, 1987). However, these reactions only occur in non-aqueous media. Since OSC of the type described in this paper are also found in Recent, unconsolidated sediments (VALISOLALAO *et al.*, 1984; BRASELL *et al.*, 1986c; RULLKÖTTER *et al.*, 1988) OSC are probably not formed by such reactions. Moreover, elemental sulphur is only produced in sediments by chemical oxidation of hydrogen sulphide (unlikely in anaerobic sedi-

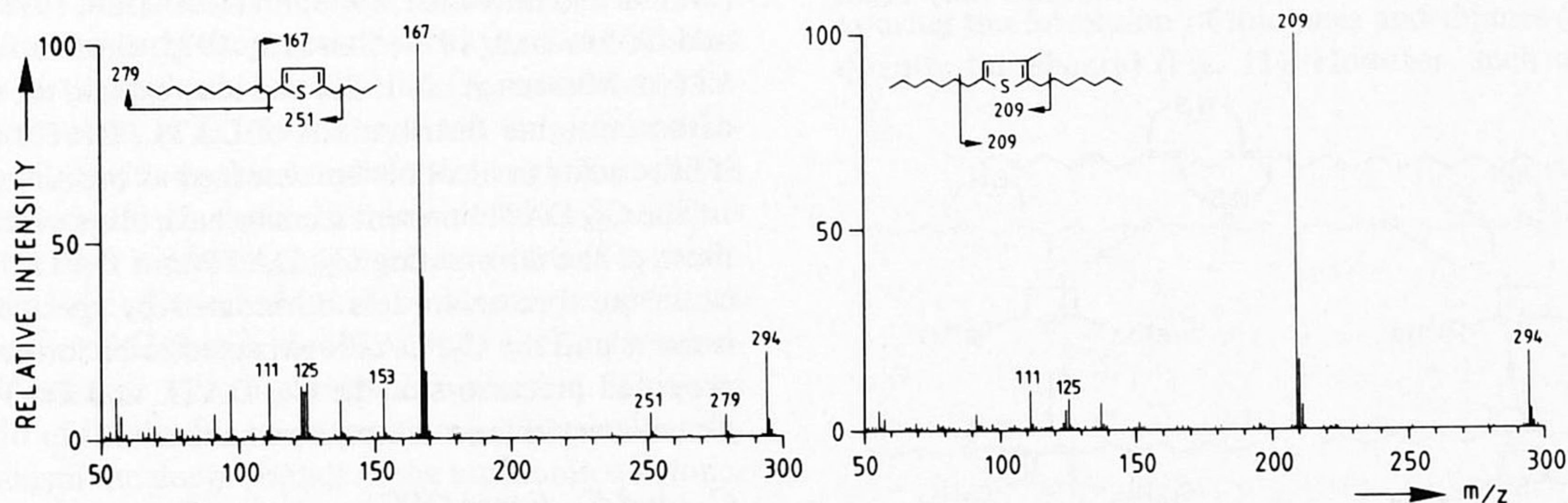


FIG. 8. Mass spectra (subtracted for background) of authentic C_{19} branched thiophenes possessing the 9-methyloctadecane carbon skeleton: (A) 2-butyl-5-(2'-undecyl)thiophene; (B) 2,5-diheptyl-3-methylthiophene.

ments) or by biochemical oxidation of hydrogen sulphide by certain sulphur bacteria. Therefore, we feel that hydrogen sulphide, produced by omnipresent sulphate-reducing bacteria, is a more likely sulphur species to react with organic matter. Recently, VAIRAVAMURTHY and MOPPER (1987) have shown that addition of hydrogen sulphide to activated double bonds occurs in Recent sediments. They stated that this process has to be considered a major chemical pathway for the introduction of sulphur into sedimentary organic matter during the early stages of diagenesis.

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 the 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 subsequently underwent an intramolecular addition to form the thiolanes. Radical addition of hydrogen sulphide to methyl linolenate yielded a mixture of thiolanes and dialkylthiolanes with an additional thiol group; both sulphur atoms were 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 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 may occur

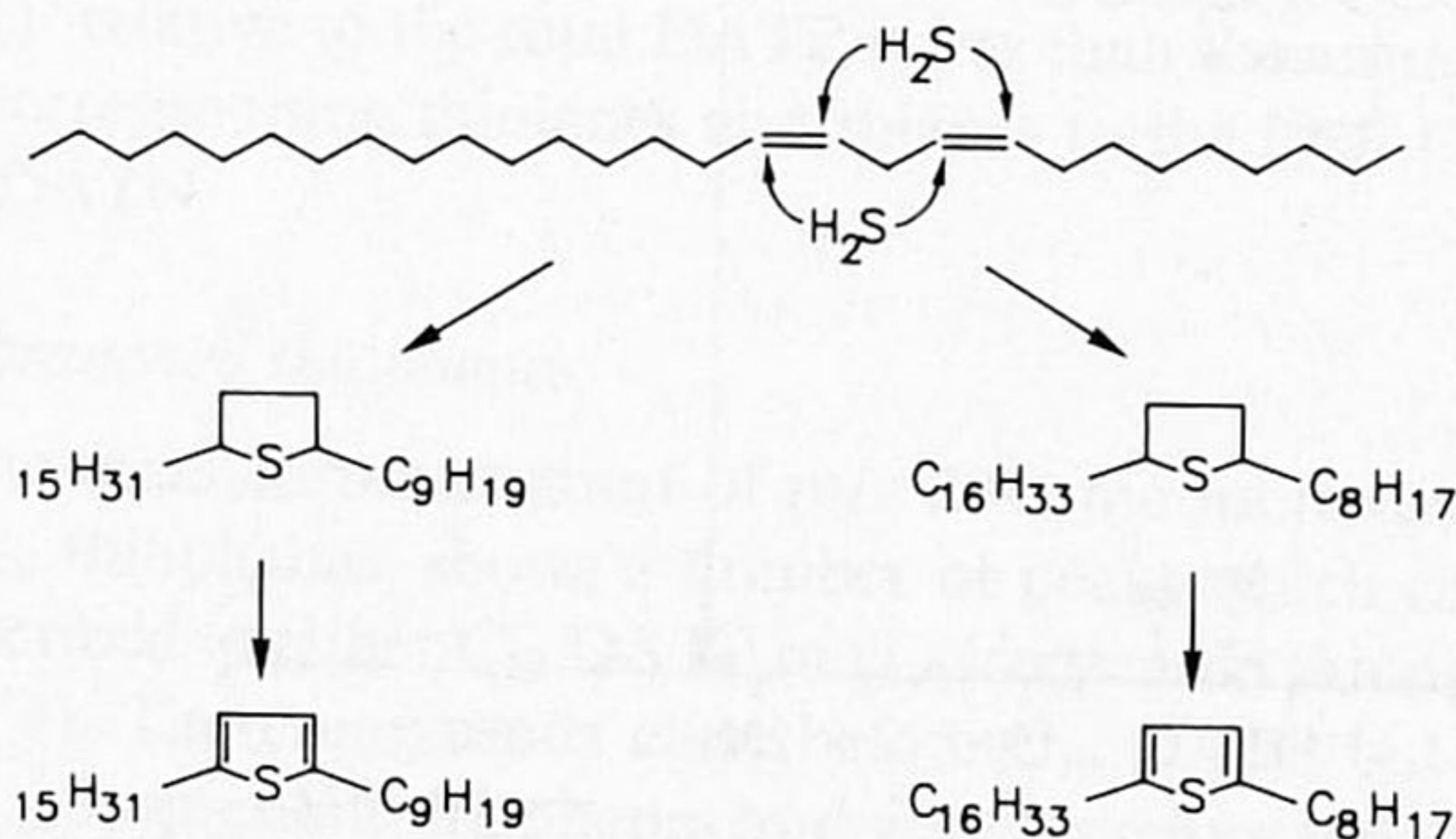


FIG. 9. Formation of C_{28} DATL and DATP by sulphur incorporation of H_2S into a hypothetical precursor.

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 mechanism 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 octacos-9,12-diene or lipids with the same carbon skeleton (including the two double bonds) but possessing an additional 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. 9).

Octacos-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.*, $\text{C}_{21:6}$; BLUMER *et al.*, 1970; LEE and LOEBLICH, 1971) and sediments (*e.g.*, $\text{C}_{29:2}$, $\text{C}_{34:3}$, $\text{C}_{34:4}$, $\text{C}_{34:5}$; 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 occurrence of $\Delta^{9,12}$ octacosadienoic acid in nature has, to our knowledge, also not been described. 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 PUZO, 1983), in *Mycobacterium tuberculosis* (Δ^9 ; TAKAYAMA *et al.*, 1978), in freshwater sponges (DEMBITSKII, 1981a,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 Jurf ed Darawish Oil Shale 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 reductions have been proposed to occur in sediments under strong reducing conditions (WELTE and EBHARDT, 1968; KVENVOLDEN, 1970; ALBAIGÉS and TORRADAS, 1974; SEIFERT, 1975; SHENG *et al.*, 1980; VERNE-MISMER *et al.*, 1986) 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 the former are less dominated by specific structural isomers and the C_{28} DATN expected to be formed from the proposed 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

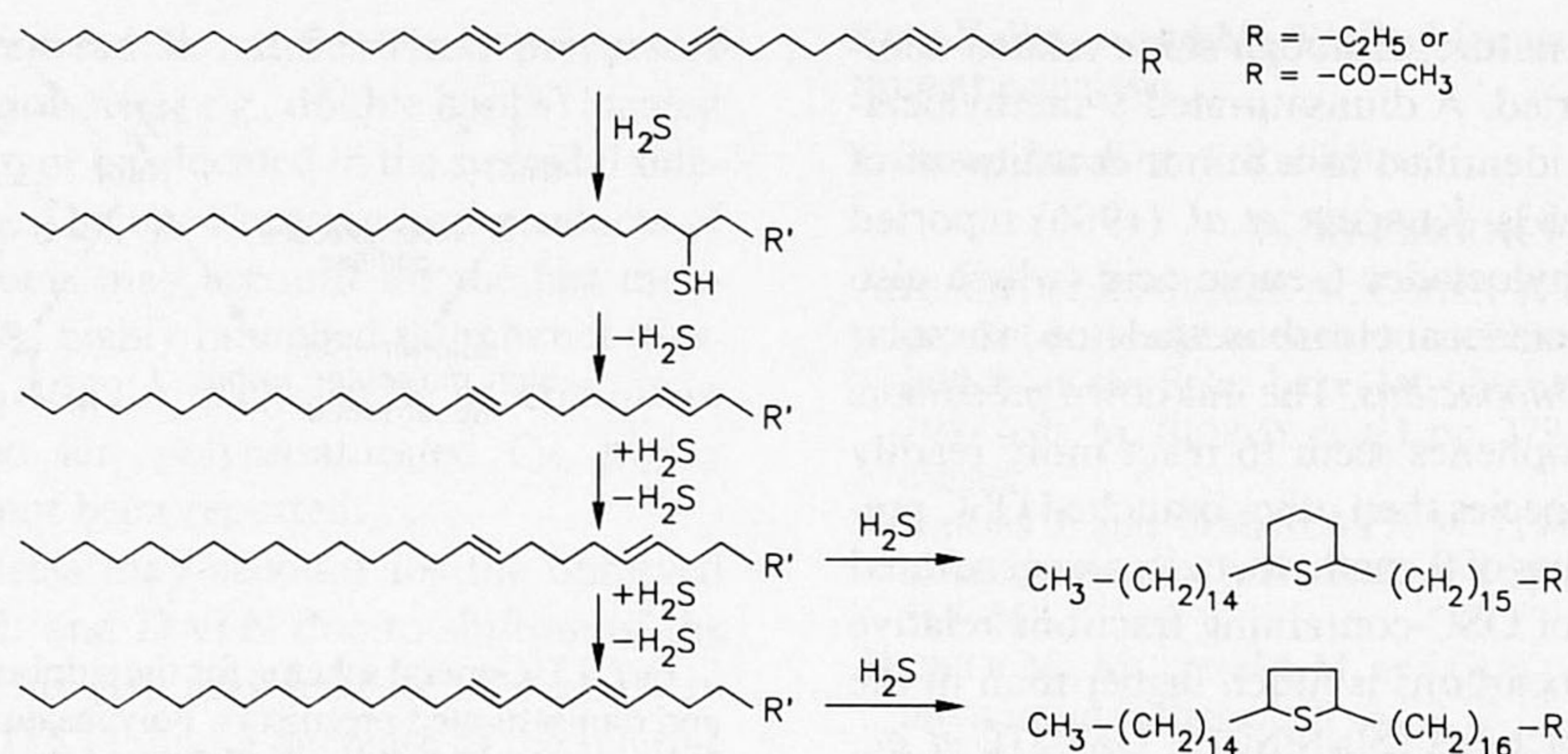


FIG. 10. Examples of the formation of two major C₃₇ DATL and DATN isomers by sulphur incorporation into a triunsaturated C₃₇ methylketone or hydrocarbon.

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,b) and in the coccolithophorid *Emiliana huxleyi* (VOLKMAN *et al.*, 1980a,b). In the first place this explains the carbon number distributions of the DATN, DATL and DAP in the C₃₅–C₄₀ range. Secondly, the isomer composition of the C₃₇ DATN, DATL and DAP is also consistent with such precursors.

The unsaturated C₃₇ methylketones have double bond positions as indicated in Fig. 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 bonds are not in favourable positions. Therefore, these thiols are more likely to react intermolecularly generating sulphur-containing high molecular weight substances. Indeed, higher molecular weight fractions of the Jurf ed Darawish Oil Shale bitumen upon desulphurisation yielded significantly higher amounts of heptatricosane and octatricosane relative to other *n*-alkanes than desulphurisation of the LMWA fraction (SINNINGHE DAMSTÉ *et al.*, 1988). Nevertheless, intramolecular additions of the C₃₇ and C₃₈ thiols also seem to occur, possibly after isomerisations of the double bonds.

However, recently it has been shown by DE LEEUW *et al.* (1989) 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 C₃₇ and C₃₈ unsaturated ketones. FARRIMOND *et al.* (1986) reported long-chain alkenones (C₃₇–C₄₂) with double bonds in the original positions in two Cretaceous black shales. In depositional environments in which free H₂S is present, however, an alternative mechanism for the isomerisation of double bonds may be operative. After addition of H₂S to a double bond, H₂S 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) leading to the formation of thianes and thiolanes. The fact that the C₃₇ and C₃₈ cyclic sulphides are dom-

inated 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 sp³-hybridised carbon atoms (instead 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 C₃₇ mid-chain DATN and DAP 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-octyl-6-tetracosanylthiane. Formation of thianes and thiolanes with a shorter alkyl would require two additional double bond shifts and is therefore unlikely on statistical grounds (although small amounts of 2-heptyl-5-pentacosanylthiane were observed). The relative higher amounts of C₃₇ DATL with alkyl side chains with more than 14 carbon atoms can be explained by the fact that the diunsaturated C₃₇ methyl ketones have double bond positions at Δ¹⁵ and Δ²² only. This effect is, however, less obvious for the C₃₇ DATN.

C₁₉ branched thiophenes

The presence of only two or three structural isomers (VI is tentatively identified) of C₁₉ 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. 11). However, such compounds

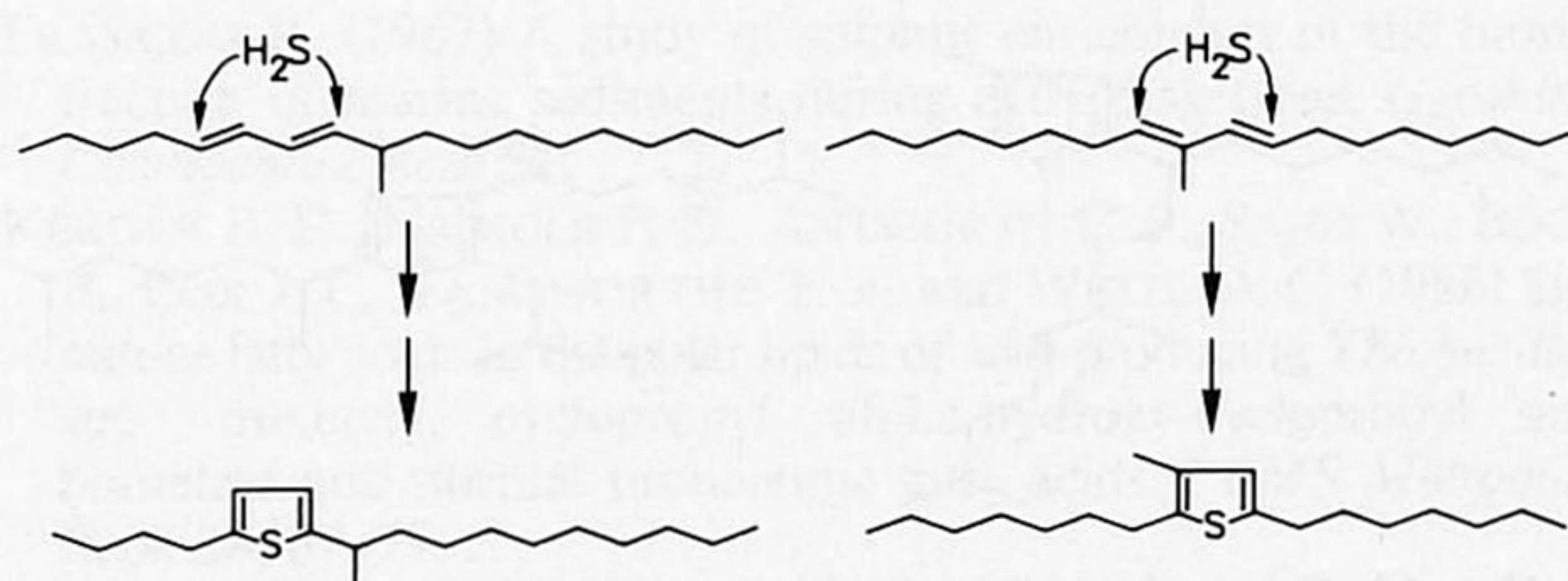


FIG. 11. Formation of C₁₉ branched thiophenes, possessing the 9-methyloctadecane carbon skeleton, by sulphur incorporation into hypothetical precursors.

have not been found in nature, although some related compounds have been reported. A 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 precursors of the C_{19} branched thiophenes seem 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.*, 1988).

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 (ROWLAND *et al.*, 1985; ROBSON and ROWLAND, 1986). The other C_{25} highly branched isoprenoid thiophene (VII) may be formed by sulphur incorporation into another structural isomer of the highly branched isoprenoid alkadienes (Fig. 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 (BRASELL *et al.*, 1986c; SINNINGHE DAMSTÉ *et al.*, 1986, 1987, 1989b; SINNINGHE DAMSTÉ and DE LEEUW, 1987) lead us to propose a hypothetical model for the incorporation of sulphur into organic matter (Fig. 13).

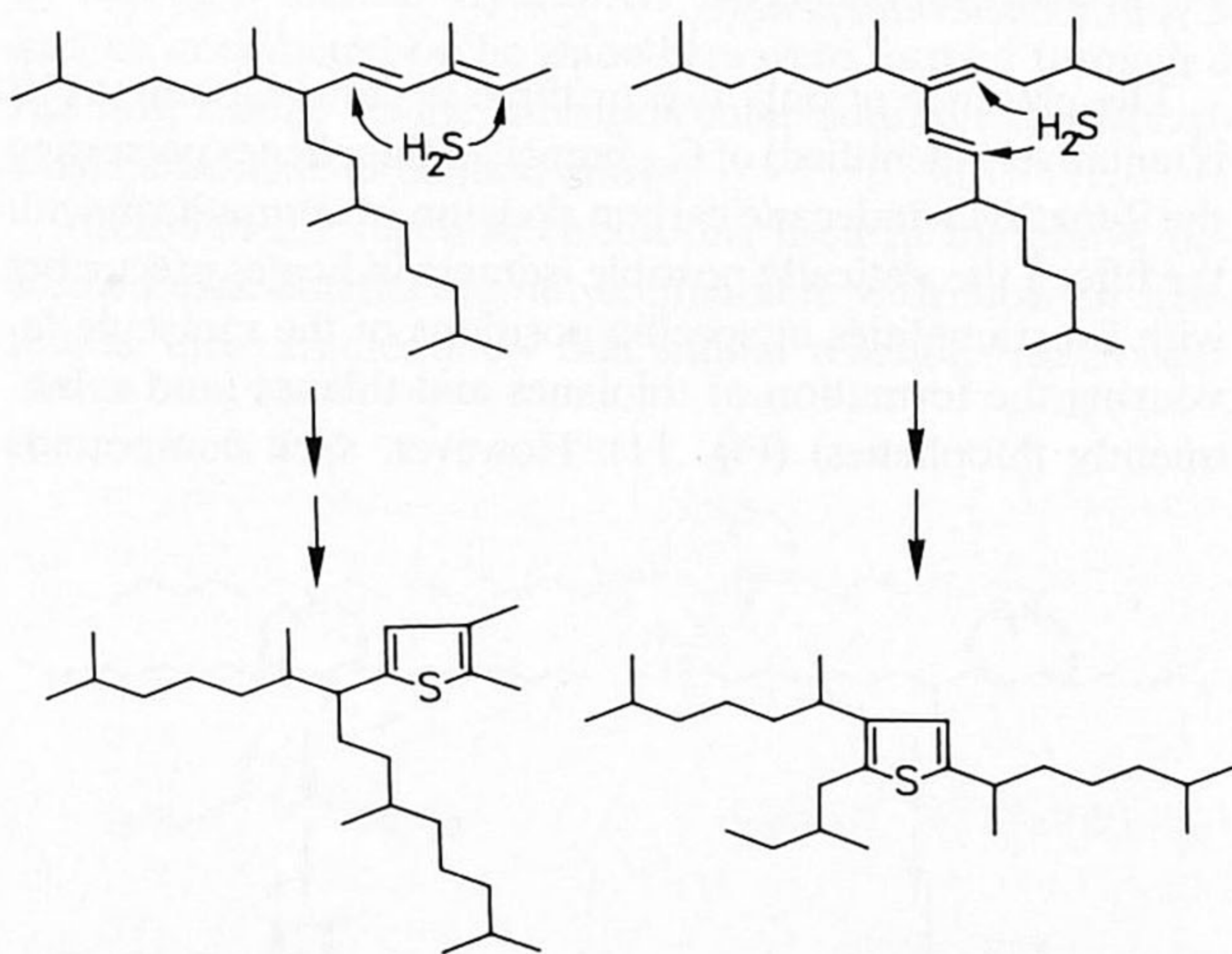


FIG. 12. Formation of C_{25} highly branched isoprenoid thiophenes by sulphur incorporation into highly branched isoprenoid alkadienes, ubiquitous compounds in Recent sediment (ROWLAND *et al.*, 1985; ROBSON and ROWLAND, 1986).

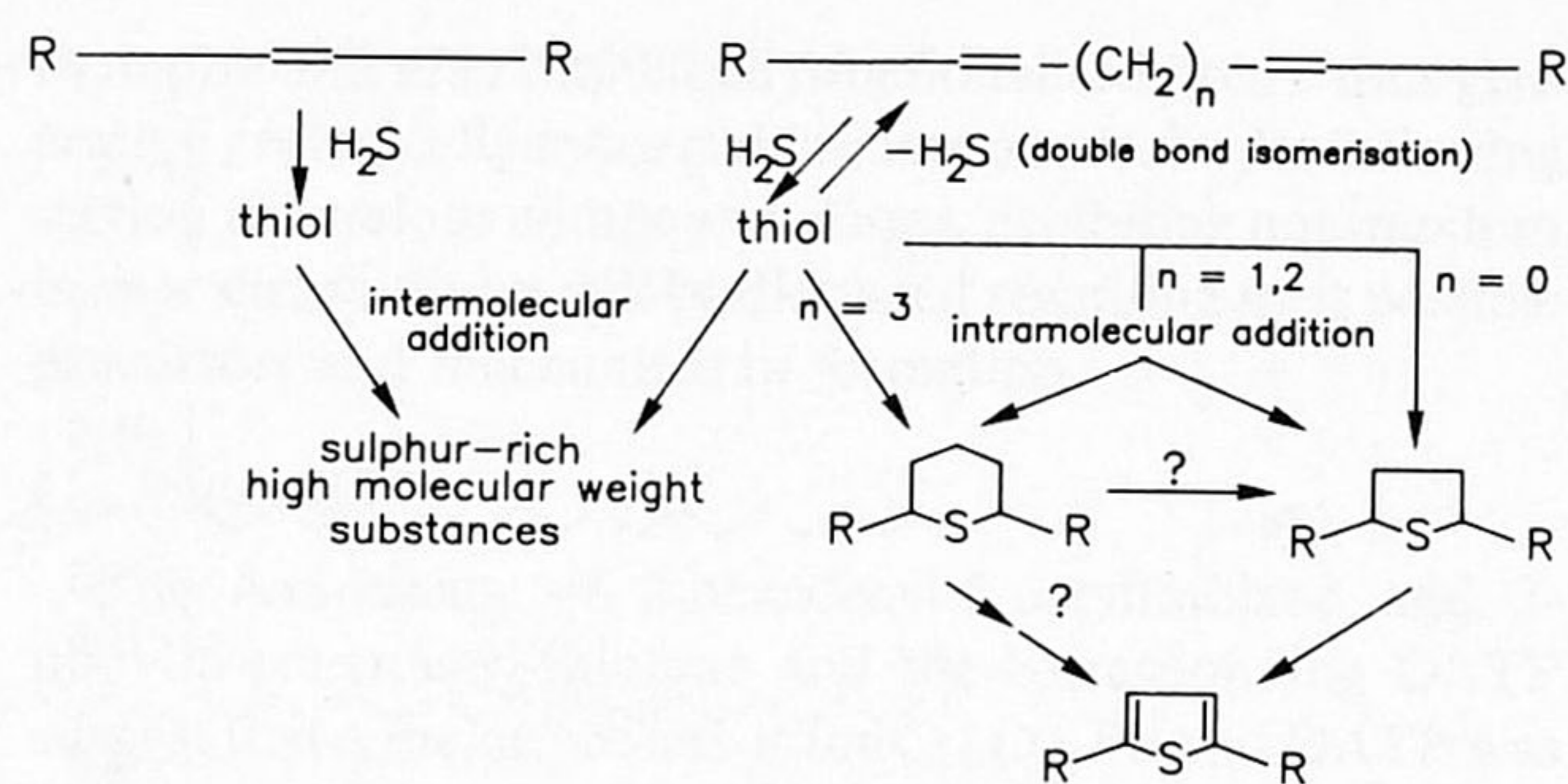


FIG. 13. General scheme for the sulphur incorporation into mono- and diunsaturated precursors. Polyunsaturated precursors (not shown in scheme) can undergo both intramolecular and intermolecular sulphur incorporation leading mainly to the formation of sulphur-rich high molecular weight substances.

Sedimentary molecules with one double bond (or only one other reactive functionality) are not likely to serve as a precursor for OSC, although they may become part of sulphur-rich high molecular weight substances (*e.g.*, resins, asphaltens, 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. 13; $n < 3$) or thianes ($0 < n < 4$) sulphur incorporation may lead 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 one 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 to each other by sulphur bridges; SINNINGHE DAMSTÉ *et al.*, 1988).

For the major part of the OSC identified in sediments and oils (*e.g.*, SINNINGHE DAMSTÉ *et al.*, 1989c) but not described in this paper, naturally occurring precursors exist which are in agreement with this proposed model. For example, the precursors of C_{20} isoprenoid thiolanes and thiophenes are probably phytadienes, whilst sulphur-containing hopanoids are likely formed by sulphur incorporation into bacteriohopanetetrol or its diagenetic products. Sulphur-containing steroids are an exception in this respect since their assumed

precursors are not widespread in nature. These precursors have to possess two reaction sites (*e.g.*, double bonds) located in the steroidal side-chain or one located in the steroidal side-chain and another in the D-ring. 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.*, 1989b) 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.*, 1987; SCHMID *et al.*, 1987) also contain higher amount of DATL relative to DATN, whilst the Jurf ed Darawish Oil Shale bitumen described here contains high amounts of DATN relative to DATL.

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 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. This conclusion is consistent with bulk studies (NISENBAUM and KAPLAN, 1972; CASAGRANDE *et al.*, 1979; CASAGRANDE and NG, 1979; AIZENSHTAT *et al.*, 1983; FRANÇOIS, 1987) which report the early diagenetic sulphur enrichment of sedimentary organic matter.

(2) Abiogenic sulphur incorporation acts as a trapping mechanism of labile functionalised lipids, preserving their carbon skeletons and information about the original sites of functionalities. The OSC produced are more stable under geological conditions than their precursors and can therefore reveal this information even in ancient sediments.

(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, in addition, low molecular OSC (MW < 800).

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

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APPENDIX

