Advances in Organic Geochemistry 1985
Org. Geochem. Vol. 10, pp. 791–805, 1986
Printed in Great Britain

0146-6380/86 $3.00 + 0.00
Pergamon Journals Ltd

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

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(*Received* 16 *September* 1985; *accepted* 21 *January* 1986)

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.

Key words: organic sulphur compounds, isoprenoid thiophenes, *n*-alkylthiophenes, isoprenoid thiolanes, *n*-alkylthiolanes, evaporitic environment, isoprenoid hydrocarbons, mass spectrometry, GC–FPD

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, elementary 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‰ 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

*For Part I see: ten Haven *et al.* (1985).

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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* sulphurization 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 sulphurization and desulphurization 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 sulfoxides and sulfides 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 diagnesis. Brassell *et al.* (1986) 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 characterized, 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).

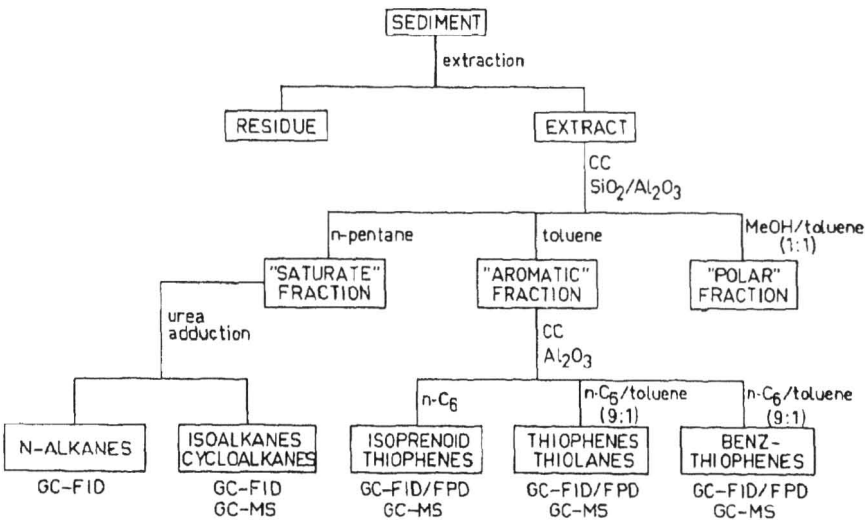


Fig. 1. Analytical flow diagram.

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 (*c.* 50%) and clay minerals (*c.* 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. 1 for the analytical flow diagram). The column (i.d. 10 mm, length 50 cm) 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, i.d. = 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 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 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 and an on-column injection system (Grob, 1978; Grob and Grob, 1978), provided with a special cooling system. A fused silica capillary column (l = 25 m, i.d. = 0.32 mm) coated with CP-Sil 5 (film thickness = 0.13 μm) was used with helium as carrier gas (p_i = 0.5 atm). Samples were injected at 80°C (hexane) or 100°C (ethylacetate). The oven temperature was programmed from 130°C to 330°C at 4°C/min as soon as the solvent eluted. The Varian 3700 instrument was equipped with a FPD and a flame ionization detector (FID). The fused silica capillary column (l = 30 m, i.d. = 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°C with a splitless injector. After 2 min the temperature was programmed to 130°C at a rate of 10°C/min. Then the column temperature was programmed from 130 to 300°C at 4°C/min.

Table 1. Separation scheme of the "aromatic" fraction

cut	compound type	volume(ml)	eluent[a]
prewash	-	20	H
1	hydrocarbons	35	H
2-9	alkylthiophenes	40	H
10	alkylthiophenes	5	H:T(9:1)
11-12	alkylthiophenes, 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)

[a] 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 (l = 25 m, i.d. = 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.*, 1986): 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.

RESULTS

The gas chromatograms of the "aromatic" fraction and the important sub-fractions are shown in Fig. 2. The peak numbers correspond to those listed in Table 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.

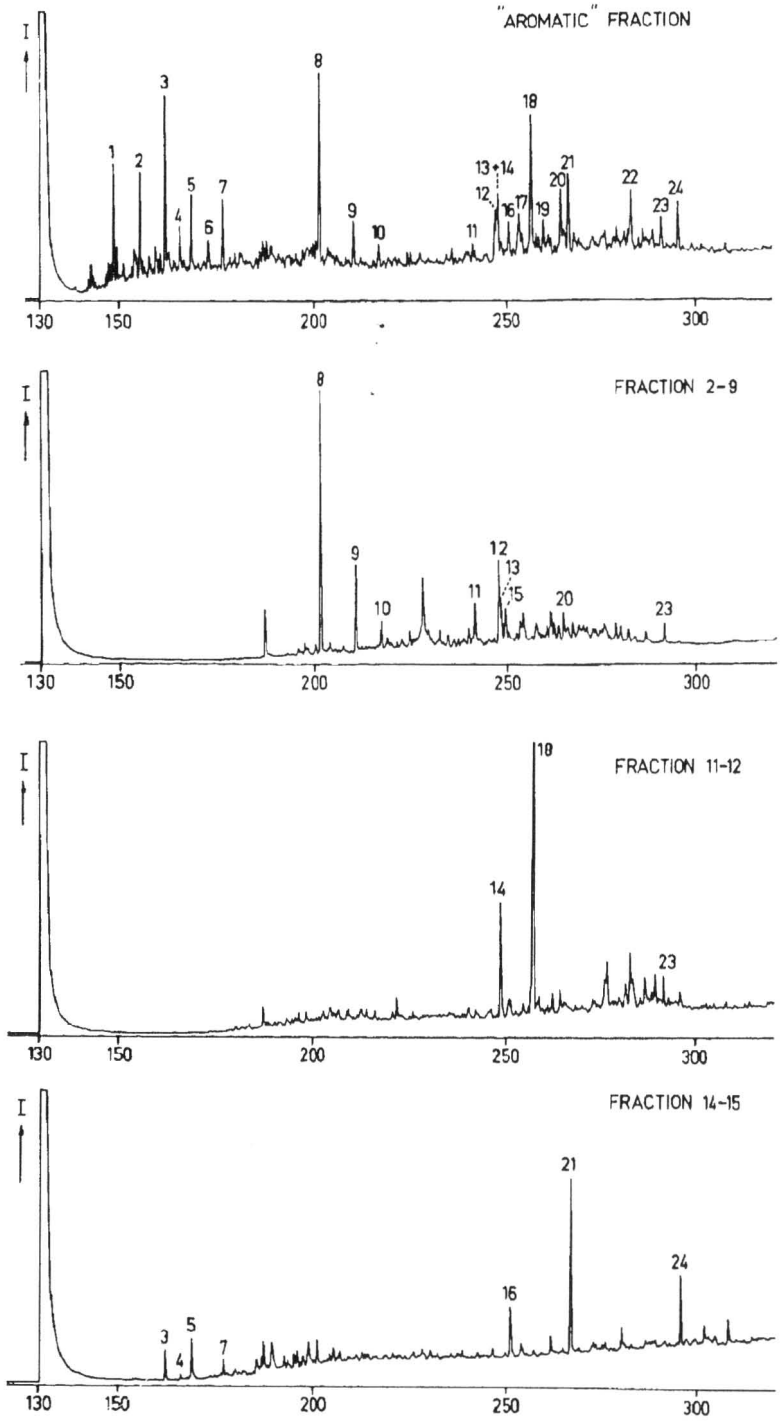


Fig. 2. Gas chromatograms (FID detection) of the "aromatic" fraction and the obtained sub-fractions. Identification of numbered compounds are given in Table 2.

The assignment of compounds 14 (VIII), 16 and 18 (IX) (Table 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.*, 1986). The tentative assignment of compound 22 (Table 2) as a C_{29} thiolanesterane awaits further confirmation after synthesis of the model compound (Schmid and Albrecht, personal communication). The assignment of compounds 17, 21 and 23 (Table 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 al-

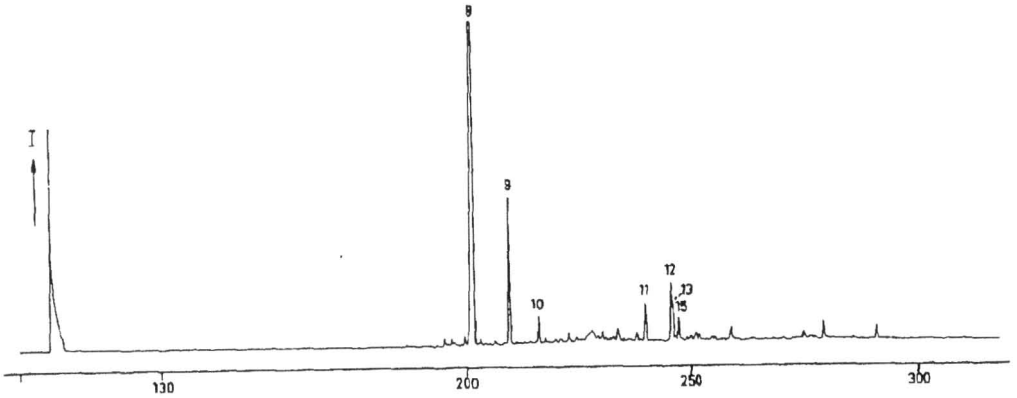


Fig. 3. FPD chromatogram of sub-fraction 2-9 of the "aromatic" fraction. Identifications of the numbered OSC are given in Table 2.

kylbenzothiophenes. The mass spectra of compounds 17, 21 and 23 and those of related less abundant long-chain alkylbenzothiophenes are characterized by a molecular ion and two major fragment ions (m/z $147 + 14n$ and $148 + 14n$, in which $n = 0, 1, 2, 3, 4,$ or 5). Moreover these compounds gave a response the FPD (not shown).

Separation procedure

It is evident from Fig. 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.* (1986) and Palmer and Zumberge (1981). The latter authors reported the presence of a compound (x) in the

Table 2. Major compounds identified in the "aromatic" fraction (Fig. 2)

compound	identification	structure[a]
1.	C_3-substituted benzothiophene	-
2.	C_4-substituted benzothiophene	-
3.	C_4-substituted benzothiophene	-
4.	C_5-substituted benzothiophene	-
5.	C_5-substituted benzothiophene	-
6.	phytane	-
7.	C_6-substituted benzothiophene	-
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[b]
15.	isomer of 12	-
16.	2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-chroman	VIII[b]
17.	C_{17}-substituted benzothiophene (m/z 372,176,175)	-
18.	2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-chroman	IX
19.	18-*nor*-17β-methyl-24-ethyl-cholesta-8,11,13-triene	X
20.	18-*nor*-4,17β,22-trimethyl-cholesta-8,11,13,triene	XI
21.	C_{18}-substituted benzothiophene (m/z 386,189,190)	-
22.	C_{29}-thiolanesterane	-
23.	C_{20}-substituted benzothiophene (m/z 414,175,176)	-
24.	unknown	-

[a] most of the structures are tentative (see text)

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

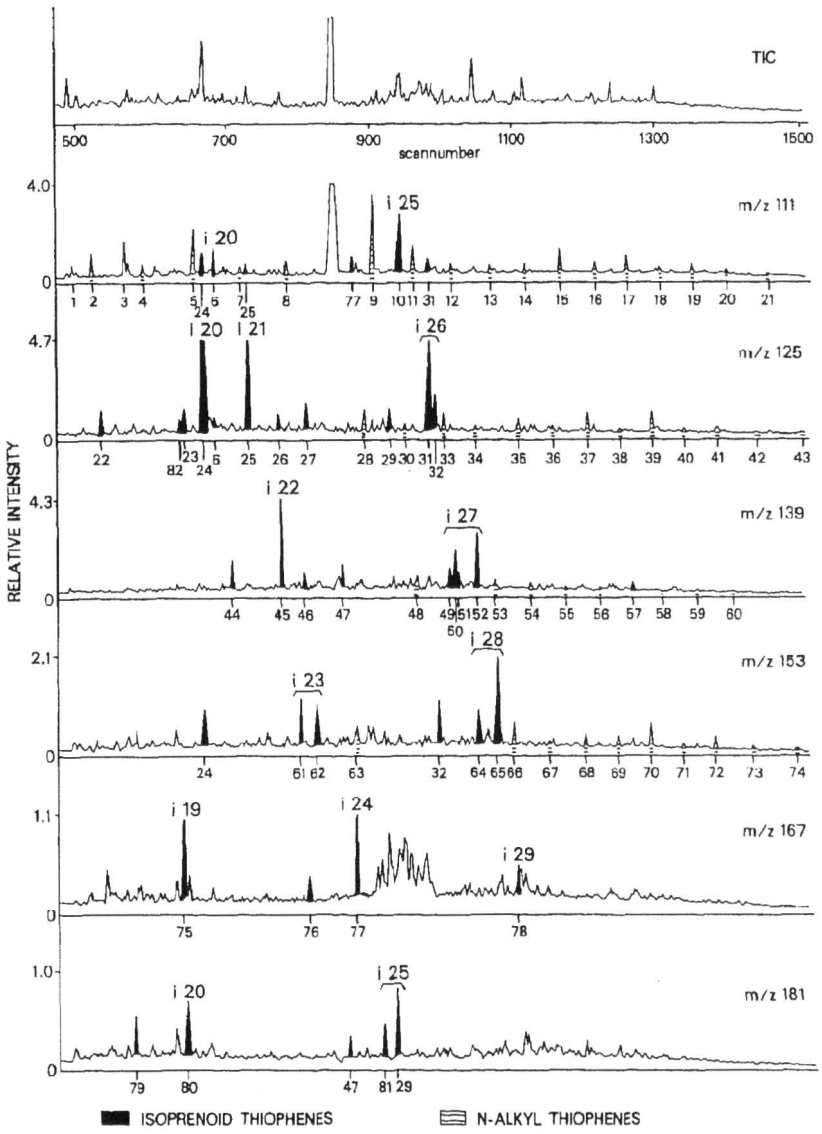


Fig. 4. Mass chromatograms of m/z 111, 125, 139, 153, 167 and 181 and the TIC (upper trace) of the "aromatic" fraction. Identifications of the numbered compounds are given in Table 3. The large peak at scan number 850 in the TIC and in the m/z 111 trace is assigned to dioctyladipate, a plastizer, and is due to contamination during the process of removal of the elemental sulphur.

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. 4. The peak numbers in this figure correspond with those listed in Table 3. The most abundant thiophene (compound 24) was identified as 2,3-dimethyl-5-(2,6,10-trimethylundecyl)-thiophene (I). This compound was previously identified in the saturated hydrocarbon fraction of this sediment (ten Haven *et al.*, 1985). The mass spectrum of this compound (Fig. 5C) shows a base peak at m/z 125 and a molecular ion at m/z 308.

TABLE 3: ISOPRENOID AND N-ALKYL THIOPHENES IDENTIFIED IN THE "AROMATIC" FRACTION (FIG. 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

Such a simple mass spectrum is characteristic for both isoprenoid and *n*-alkylthiophenes. Mass spectra of both compound classes (see Fig. 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.

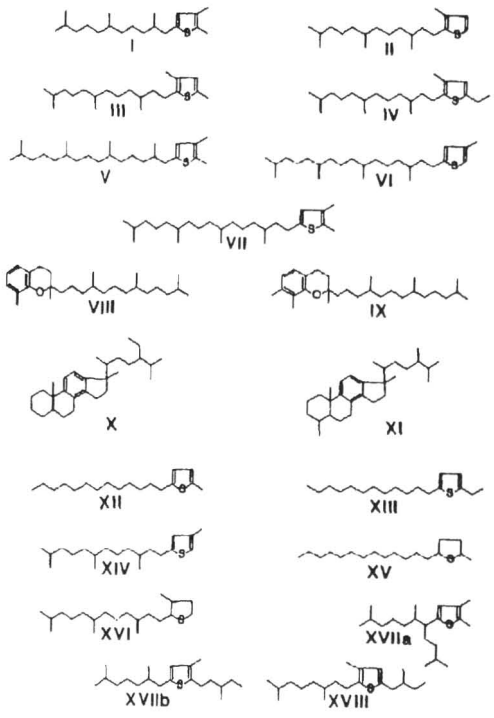
The presence of several homologous series of *n*-alkylthiophenes has been confirmed by synthesis of two *n*-alkylthiophenes: 2-methyl-5-tridecylthiophene (XII) and 2-dodecyl-5-ethylthiophene (XIII). Both compounds reveal a mass spectrum (see Fig. 5B) indistinguishable from those of compounds 5 and 23 respectively (Fig. 4). Coinjection on a SE 52 fused silica capillary (25 m × 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. 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.*, unpublished results).

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 sub-fraction 2–9 on a SE-52 fused silica capillary column (25 m × 0.32 mm) with a synthetic standard (Brassell *et al.*, 1986). This C_{20} compound (6, Table 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):

$$\mathrm{ATI(x)} = 100.z + 100\,\frac{t_r(\mathrm{x}) - t_r(z)}{t_r(z+1) - t_r(z)}$$

where t_r(x) is the retention time of the compound for which ATI is to be determined, $t_r(z)$ and $t_r(z+1)$ 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 base peak 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 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 (Table 2 and 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 4).



Scheme 1.

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. 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.*, 1986), also shows a rearrangement fragment (*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 characterized by a single tail-tail linkage. The other structures proposed for the isoprenoid thiophenes have regular isoprenoid carbon skeletons except for structure XVIIa.

The distribution patterns of the *n*-alkylthiophene homologous series are rather peculiar. In Fig. 6, the

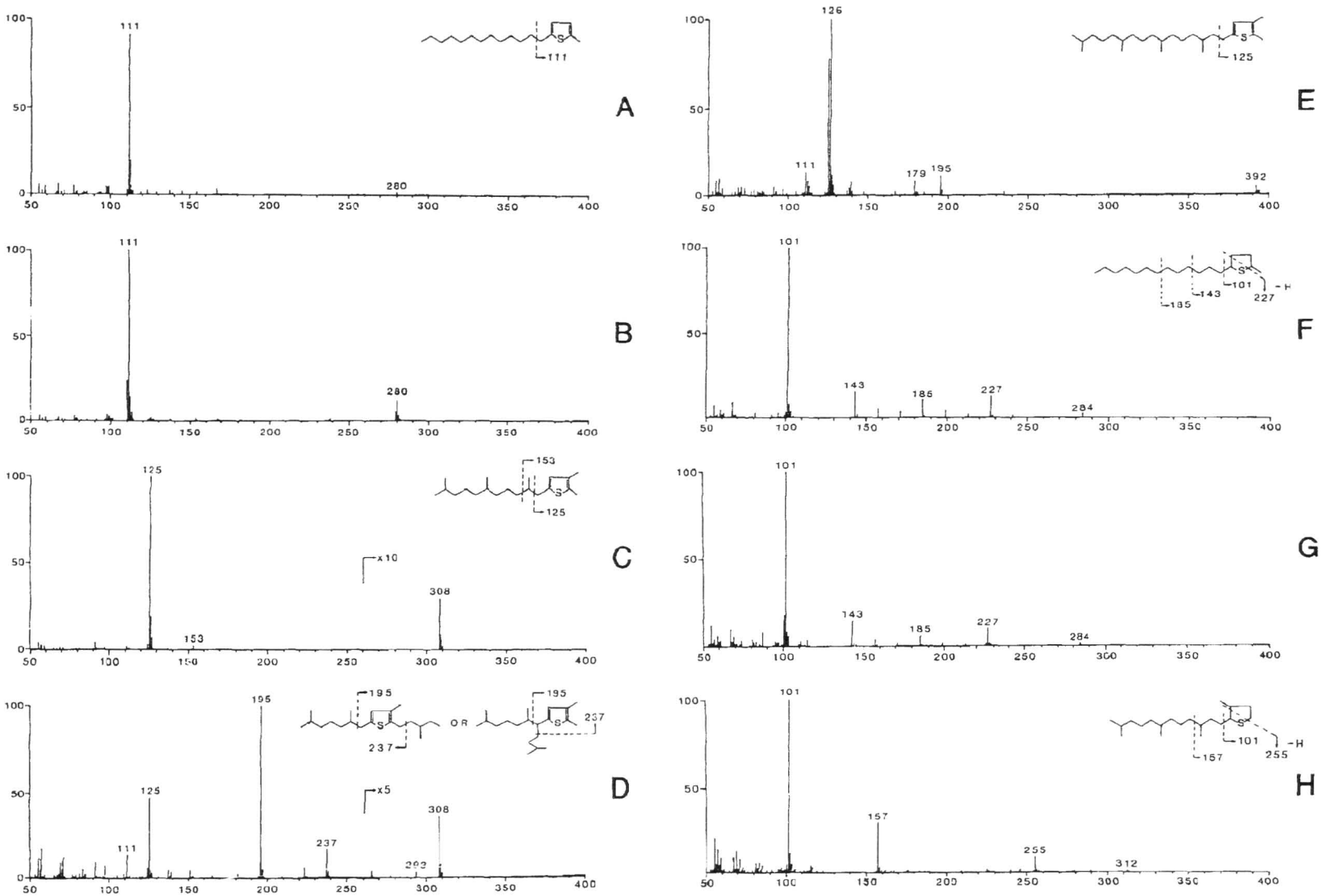


Fig. 5. Mass spectra, corrected for background, of (A): 2-methyl-5-tridecylthiophene (XII), (B): synthesized 2-methyl-5-tridecylthiophene, (C): 2,3-dimethyl-5-(2,6,10-trimethylundecyl)-thiophene (I), (D): 2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)-thiophene (VII), (E): 5-(2,6-dimethyl-1-(3-methylbutyl)-heptyl)-2,3-dimethylthiophene (XVIIa), and/or 5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3- methylthiophene (XVIIb), (F): 2-methyl-5-undecylthiolane (XV), (G): synthesized 2-methyl-5-undecylthiolane, (H): 3-methyl-5-(3,7,11-trimethyldodecyl)-thiolane (XVI).

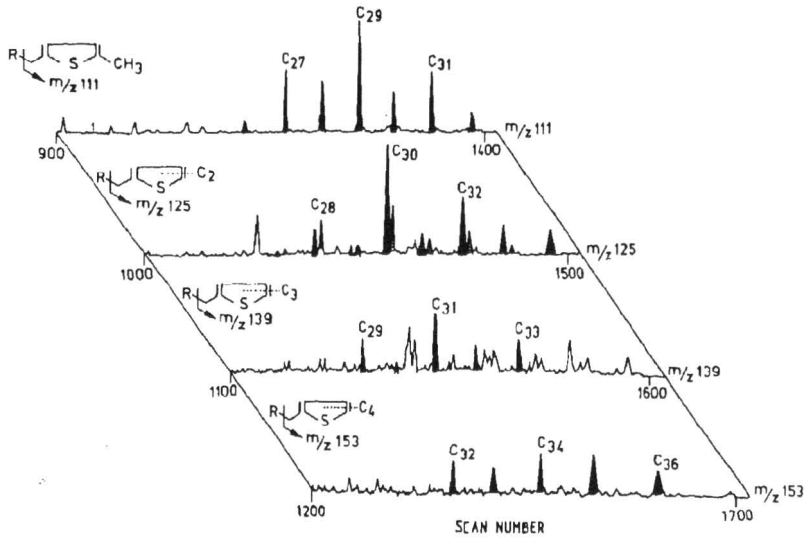


Fig. 6. Mass chromatograms of *m/z* 111, 125, 139 and 153 in the region where the long-chain *n*-alkylthiophenes elute in fraction 11-12. The peak indications represent the total number of carbon atoms of the *n*-alkylthiophenes.

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. 6) and C_{22} as the most abundant compound present (Fig. 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. 4). From the C_{20} (compound 6; Fig. 4) up to the C_{25} isoprenoid thiophene (compound 81; Fig. 4) and from the C_{25} (compound 10 in Fig. 4) up to the C_{29}

Table 4. Retention data of the isoprenoid hydrocarbons and their thiophene analogues

hydrocarbon		thiophene analogue	
compound	I[a]	ATI[b]	I[c]
phytane	1816	1820(I)	2093
		1825(II)[d]	2116
2,6,10,14-tetramethylheptadecane	1895	1914(III)	2187
2,6,10,14-tetramethyloctadecane	1987	2007(IV)[e]	2265
2,6,10,14,18- and/or 2,6,10,14,19-pentamethyleicosane	2242	2242(V)	2532
		2246(VI)[d]	2551
2,6,10,14,18- and/or 2,6,10.14,19-pentamethylheneicosane	2342	2339,2357(VII)[f]	2628,2651

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

[b] 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

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

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

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

[f] there are two isomers (compounds 12 and 15 in Table 2)

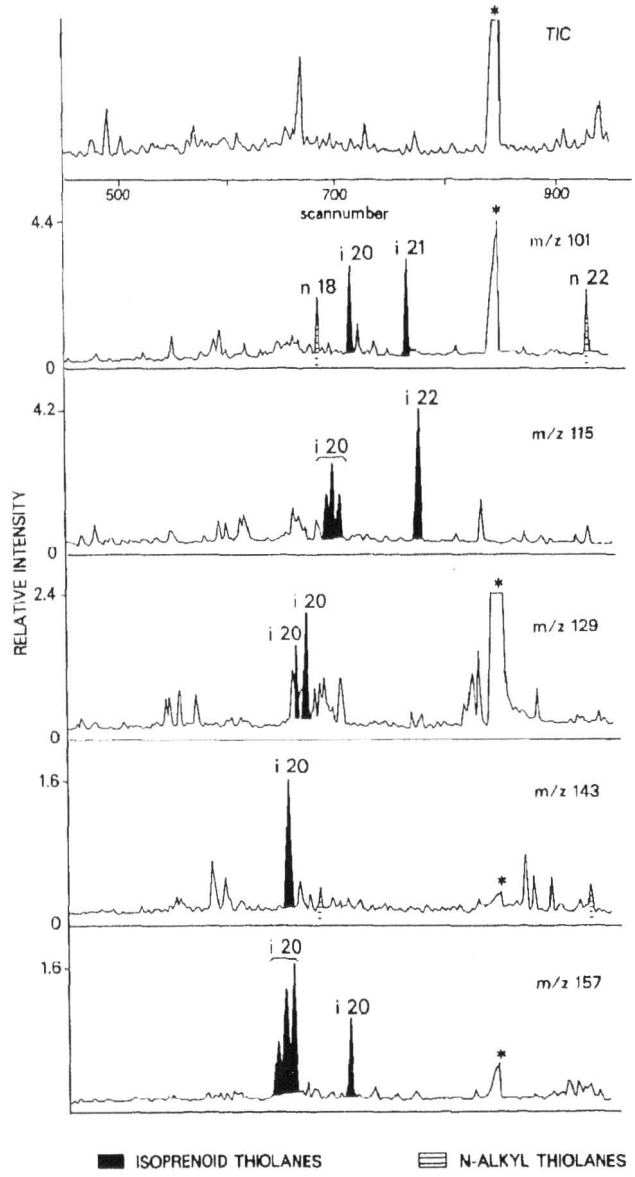


Fig. 7. Mass chromatograms of *m/z* 101, 115, 129, 143 and 153 and part of the TIC (upper trace) of the "aromatic" fraction. The large peak marked with an asterisk is due to dioctyladipate (see Fig. 4).

isoprenoid thiophene (compound 78 in Fig. 4) the base peak in the mass spectra of these compounds shifts upwards by 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.

Thiolanes

The distribution pattern and mass spectrometric behaviour of the thiolanes in the "aromatic" fraction is shown in Fig. 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. 5F and 5G). The mass spectra of these compounds are characterized 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 the 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. 5H) for example, the minor fragment ion (*m/z* 157) has a *m/z* value 14 daltons higher than the corresponding fragment ion (*m/z*

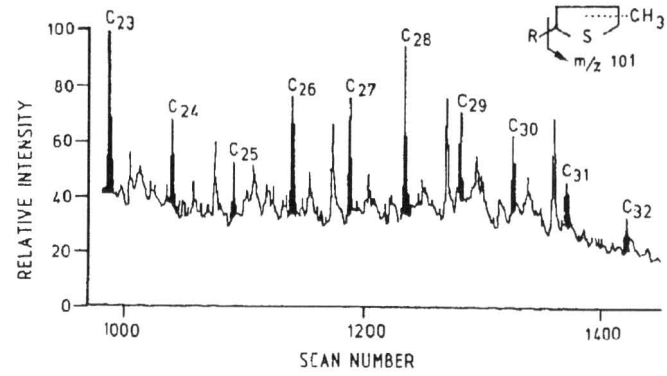


Fig. 8. Mass chromatogram of *m/z* 101 in the region where the long-chain alkylmethylthiolanes elute. The peak indications represent the total number of carbon atoms present in the thiolanes.

143) of 2-methyl-5-tridecylthiolane (XV) (Fig. 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. 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. 8. Other homologous series of *n*-alkylthiolanes have not been observed.

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.* (1986) 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. 4). The structure and restricted stereochemistry of II was consistent with its presumed origin as an early stage diagenetical 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 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.* (1986) that the thiophenes found are either of biosynthetic origin or are formed as the result of a reaction of H_2S, polysulphides (S_x^{2-}) 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). But 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 5), in addition to the

Table 5. Identified isoprenoid hydrocarbons

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

[a] previously identified by ten Haven et al. (1985)
[b] previously identified as the first isomer by ten Haven et al. (1985)

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 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. 4) are thought to be present in the sediment. The C_{26} isoprenoid thiophene (V) is also relatively abundant but the isoprenoid C_{26} 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. 2), with the loss of some methyl groups during aromatization.

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 categenetic 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 XBIII) may result from sulphur incorporation into mid-chain unsaturated phytadienols (or their diagenetic products) such as $\Delta^{2,10}$- and $\Delta^{2,6}$-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 characterized by a high relative abundance of this highly branched $C_{20}H_{40}$ alkene together with its parent $C_{20}H_{42}$ 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 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. 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 (Ocampo *et al.*, 1986). 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-alkylthiophenes (the "shift"-phenomenon, Fig. 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. 7 and 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. 8), whereas the 2-alkyl-5-methylthiophenes show an odd-over-even predominance (Fig. 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.

CONCLUSION

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 Ocampo *et al.* (1986) 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.

Acknowledgements—The Netherlands Foundation for Earth Science Research (AWON) with the financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO) is thanked for support (Grant 18.23.09) to part of this work. Dr S. C. Brassell is kindly acknowledged for his contribution to the identification of the isoprenoid chromans and Dr H. C. Cox for helpful discussions. We gratefully made use of the valuable comments by Dr J. K. Volkman. We are grateful to Mrs J. K. C. Hessels for assistance with the GC analyses, Mrs A. H. Knol-Kalkman for performing the mass spectrometric analyses and Mrs J. V. Fornerod and Mrs M. Baas for the typing.

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