



Thermal stability of thiophene biomarkers as studied by hydrous pyrolysis*

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Abstract—An immature ($R_o = 0.25\%$) sulphur-rich calcareous shale from the Gessoso-solfifera Formation (Messinian) in the Vena del Gesso Basin (northern Italy) was artificially matured by hydrous pyrolysis at constant temperatures ranging from 160 to 330°C for 72 h to study the applicability of alkylthiophenes as molecular indicators during progressive diagenesis and early catagenesis. Artificial maturation at temperatures up to 330°C generated large amounts of alkylthiophenes from the thermal degradation of kerogen. The importance of the kerogen in the production of alkylthiophenes is supported by the observation that the isomer distributions of alkylthiophenes at high temperatures coincide with the isomer distributions of alkylthiophenes in the Curie-point pyrolysate of the isolated kerogen of the original sample. The generation profiles of the thiophenes with a phytane carbon skeleton and the 3,4-di-*n*-alkylthiophenes suggest that continuous degradation of alkylthiophenes is also taking place, so that the overall effect is alkylthiophene concentrations with intermediate values. The structural isomer distribution of linear alkylthiophenes does not change significantly when these compounds are generated at different temperatures. This implies that important information regarding the original positions of functionalities in the precursor molecules is retained and, thus, that alkylthiophenes are useful biomarkers at relatively high levels of thermal maturity (up to $R_o \approx 0.95$ –1.1%).

Key words—alkylthiophenes, organic sulphur compounds, biomarkers, artificial maturation, hydrous pyrolysis, diagenesis

INTRODUCTION

Several alkylthiophenes bearing an unambiguous link with biochemical precursors have been identified over the last decade (e.g. Valisolalao *et al.*, 1984; Brassell *et al.*, 1986a; Sinninghe Damsté *et al.*, 1987). In these compounds, which are formed by the incorporation of reduced inorganic sulphur species into functionalized lipids during very early diagenesis (Sinninghe Damsté *et al.*, 1989a; Kohnen *et al.*, 1990a), important information such as the carbon skeleton and the original positions of functional groups has been preserved. This has enabled the use of these organic sulphur compounds as molecular indicators of organic input into, and physical conditions of, the depositional environment (Sinninghe Damsté *et al.*, 1989b, 1990; Kohnen *et al.*, 1990b). These studies, however, have focused mainly on immature sedimentary rocks ($R_o \leq 0.3\%$). It is presently not known if the use of alkylthiophenes as molecular indicators remains valid in more mature sedimentary rocks and crude oils. However, since alkylthiophenes are much more stable under geological conditions than their biochemical precursors, it has been assumed that the information may be retained even in ancient sedimentary rocks (Sinninghe Damsté *et al.*, 1989a).

There are several aspects to the thermal stability of alkylthiophenes in the geosphere. First, isomerization of alkylthiophenes, although not very likely, may lead to different structural isomer distributions, which results in loss of important information regarding the original positions of functional groups in the biochemical precursors. Second, thermal degradation of alkylthiophenes may take place by cleavage of side chains (Sinninghe Damsté and de Leeuw, 1990). Third, alteration of alkylthiophenes may occur *via* cyclization and aromatization reactions, thereby lowering the amount of alkylthiophenes (Sinninghe Damsté *et al.*, 1989b). In all three cases, the application of alkylthiophenes as molecular indicators has lost its validity, since information about either the original position of functional groups or the carbon skeleton has been lost. Our objective is to study these three aspects of thermal stability, thereby evaluating the use of alkylthiophenes as molecular indicators over a larger maturity interval.

In order to assess the thermal stability of alkylthiophenes, artificial maturation of an immature sulphur-rich sedimentary rock from the Gessoso-solfifera Formation (Messinian) in the Vena del Gesso Basin (northern Italy) was carried out by hydrous pyrolysis over a broad temperature interval (160–330°C). The levels of thermal maturity represented by

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these experiments were estimated on the basis of vitrinite reflectance values from experimental work by Lewan (1993a) and a kinetic model by Burnham and Sweeney (1989) to be 0.65–0.85% R_o at 300°C, and 0.95–1.1% R_o at 330°C. Hydrous pyrolysis is a technique developed to simulate catagenetic processes such as oil generation and expulsion, using typical experimental temperatures between 300 and 365°C (Lewan *et al.*, 1979; Lewan, 1993b). In a few cases it has been used to establish the effect of diagenesis on relative or absolute changes in concentrations of hydrocarbon biomarkers, using temperatures as low as 200°C (Lewan *et al.*, 1986; Eglinton and Douglas, 1988; Peters *et al.*, 1990). In this work, the main interest was in the applicability of alkylthiophenes as molecular indicators during progressive diagenesis and early catagenesis. Therefore, the temperatures used were relatively low, in order to link the results to previous studies on immature sedimentary rocks (e.g. Kohnen *et al.*, 1990b).

EXPERIMENTAL

Sample

For an extensive description of the geology of the Gessoso-solfifera Formation (Messinian) in the Vena del Gesso Basin (northern Italy), see Vai and Ricci Lucchi (1977) and Sinninghe Damsté *et al.* (1995). This evaporitic basin comprises a sedimentary sequence, consisting of bituminous marl layers topped by gypsum, that is repeated 14 times. Three samples covering the complete (1.2 m thick) marl layer of evaporitic cycle IV (i.e. IV-1A, IV-1B and IV-1C) were collected from a fresh outcrop in the open-pit gypsum mine at Riolo Terme during a field trip in January 1992. A representative sample of the marl layer of cycle IV, assembled by mixing IV-1A, IV-1B and IV-1C in equal amounts, was used in this study. Rock-Eval pyrolysis yielded a total organic carbon of 1.98 wt%, a T_{max} of 404°C, and a hydrogen index of 238 mg HC/g TOC. Elemental analyses of the isolated kerogen indicated it was an immature Type II-S kerogen with an atomic H:C ratio of 1.44, an atomic O:C ratio of 0.17, and an atomic S_{org} :C ratio of 0.08.

Hydrous pyrolysis

A detailed description of the experimental procedures of a typical hydrous pyrolysis experiment has been given by Lewan (1993b). In short, a 1 l. Hastelloy-C276 reactor is filled with 90–100 g of rock chips and 475 g of distilled water. The remaining volume is purged and filled with helium at a pressure of 2.4 bar. Artificial maturation was accomplished by heating aliquots isothermally for 72 h at 160, 180, 200, 220, 239, 260, 280, 300 and 330°C, respectively. The temperatures were continuously monitored during the experiments at 30 s intervals. Standard deviations were between ± 0.2 and $\pm 0.3^\circ\text{C}$ for all experiments except for the 160°C experiment

which had a standard deviation of $\pm 0.6^\circ\text{C}$. Experiments at 300 and 330°C generated an expelled oil that was recovered from the water surface with a pipette. The reactor walls and the rock chips were rinsed with benzene to recover any sorbed oil films, which occurred in experiments at 260, 280, 300 and 330°C. 95% or more of the originally loaded rock chips was recovered from all the experiments. The residual rock chips were dried in a vacuum oven ($T \leq 50^\circ\text{C}$).

Isolation procedure

The analytical procedure has been described previously by Kohnen *et al.* (1991). In short, the residual rock chips were freeze-dried and ultrasonically extracted with dichloromethane/methanol (7.5:1 v/v). If a sorbed oil or expelled oil was present, it was combined with the extract. This combined extract was separated into a maltene and an asphaltene fraction by repeated precipitation in *n*-heptane. An aliquot of the maltene fraction (ca. 250 mg), to which an alkylthiophene standard was added for quantitative analysis (Kohnen *et al.*, 1990b), was fractionated by column chromatography with alumina into an apolar and a polar fraction by elution with *n*-hexane/dichloromethane (9:1 v/v) and dichloromethane/methanol (1:1 v/v), respectively. Further separation of the apolar fraction by argentatious thin-layer chromatography yielded the alkylthiophene fraction ($R_f = 0.4$ –0.9). The extracted rock residues of the original sample and the sample artificially matured at 300°C were demineralized using standard procedures (Lewan *et al.*, 1986) to yield the isolated kerogens for elemental analyses and Curie-point pyrolysis.

Gas chromatography

Gas chromatography (GC) was performed using a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The column effluent was monitored by a flame ionization detector (FID). The samples were injected at 70°C and the oven was subsequently programmed to 130°C at 20°C/min and then at 4°C/min to 320°C, which was held for 20 min.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) was carried out on a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range m/z 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.2 μm). Helium was used as carrier gas. The samples were injected at 60°C and the oven was subsequently programmed to 130°C at 20°C/min and then at 4°C/min to 310°C, which was held for 20 min.

Curie-point pyrolysis gas chromatography-mass spectrometry

Py-GC-MS analyses were carried out on a Hewlett-Packard 5890 gas chromatograph equipped with a FOM-3LX unit for pyrolysis and interfaced to a VG Autospec Ultima mass spectrometer. The operating conditions for the MS were identical to those described above for GC-MS. The samples were applied to a ferromagnetic wire with a Curie temperature of 610°C. The gas chromatograph, equipped with a cryogenic unit, was programmed from 0°C (5 min) to 320°C (20 min) at a rate of 3°C/min. Separation was achieved using a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.4 µm). Helium was used as carrier gas.

Quantitation

Alkylthiophenes were quantified by integration of mass chromatograms of their main fragment ions (m/z 97 + 98, 111 + 112, 125 + 126) and the main fragment ions (m/z 127 + 128) of the alkylthiophene standard, 2,3-dimethyl-5-(1',1'-d₂-hexadecyl)thiophene. Since mass spectrometric detection of compounds gives a molar response, a factor containing the molecular weights of the alkylthiophene quantified and the alkylthiophene standard was introduced to obtain absolute amounts (µg/g TOC of original rock). Because the alkylthiophenes and the alkylthiophene standard are assumed to have a similar ionization potential, and because the main fragment ions of the alkylthiophenes and the alkylthiophene standard make up a similar portion of the total ion yield (ca. 75%), the calculated amounts of alkylthiophenes are considered to be absolute. However, because the main fragment ions of mid-chain 2,5-di-*n*-alkylthiophenes (m/z 111 + 112) represent only ca. 25% of the total ion yield (Sinninghe Damsté *et al.*, 1989a), the calculated amounts of mid-chain 2,5-di-*n*-alkylthiophenes were multiplied by three.

Quantification of duplicate experiments at 280 and 300°C indicated that the standard deviation is typically 5–25%. The results for the short-chain alkylthiophenes differed more, probably due to differences in amounts of compounds that volatilized during work-up. The results for the C₂₈–C₃₂ and C₃₇–C₃₈ 2,5-di-*n*-alkylthiophenes also differed more (standard deviation up to 50%). Reported results for the experiments at 280 and 300°C are averages of the duplicate runs.

RESULTS AND DISCUSSION

An immature ($R_o = 0.25\%$) sulphur-rich sedimentary rock from the Gessoso-solfifera Formation (Messinian) in the Vena del Gesso Basin (northern Italy) was artificially matured by hydrous pyrolysis at 160, 180, 200, 220, 239, 260, 280, 300 and 330°C for 72 h. The artificially matured samples and the original sample, which acted as a reference, were analysed for

alkylthiophenes. The analysis was carried out using an internal standard (2,3-dimethyl-5-(1',1'-d₂-hexadecyl)thiophene) to enable quantification of alkylthiophenes.

Alkylthiophenes can be readily analysed by mass chromatography of their main fragment ions, resulting from cleavage β to the thiophene ring. Figure 1 shows six summed mass chromatograms (m/z 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140) of the alkylthiophene fractions of the original sample (a) and the samples artificially matured at 180, 220, 260, 300 and 330°C (b–f). The maturity-related changes in abundance and distributions of various classes of alkylthiophenes will be discussed in detail. These classes are (i) linear thiophenes, (ii) thiophenes with a phytane carbon skeleton, (iii) C₃₇–C₃₈ 2,5-di-*n*-alkylthiophenes, (iv) C₂₈–C₃₂ 2,5-di-*n*-alkylthiophenes and (v) C₃₆–C₄₄ 3,4-di-*n*-alkylthiophenes.

Linear thiophenes

This class of alkylthiophenes can be subdivided into four groups based on their chromatographic separation. These groups can be discriminated by their main fragment ions: 2-*n*-alkylthiophenes (m/z 97 + 98), 2-*n*-alkyl-5-methylthiophenes (m/z 111 + 112), 2-*n*-alkyl-5-ethylthiophenes (m/z 125 + 126) and mid-chain 2,5-di-*n*-alkylthiophenes (m/z 111 + 112). The alkylthiophenes with a particular carbon skeleton will appear as a cluster in the gas chromatogram, in which the coeluting mid-chain 2,5-di-*n*-alkylthiophenes elute first, then the 2-*n*-alkyl-5-ethylthiophene, followed by the 2-*n*-alkyl-5-methylthiophene and finally the 2-*n*-alkylthiophene (Sinninghe Damsté *et al.*, 1989a). In Fig. 1(a,d) these clusters of linear thiophenes are indicated by numbers that represent the total number of carbon atoms.

In the original sample linear thiophenes are present only in minor amounts (mostly <5 µg/g TOC). They are dominated by 2-tridecylthiophene, 2-tetradecylthiophene and 2-methyl-5-tridecylthiophene [Fig. 1(a)]. Artificial maturation at temperatures up to 239°C does not result in significant changes in the amounts of linear thiophenes. This can be seen in Table 1 and Fig. 2, which shows the summed amounts of C₁₄, C₁₇, C₂₀ and C₂₆ linear thiophenes as a function of hydrous pyrolysis temperature. Above 239°C large amounts of linear thiophenes are produced (Fig. 2). These linear thiophenes are probably derived from the thermal degradation of the kerogen. Curie-point pyrolysis of the isolated kerogen of the original sample yielded linear thiophenes up to C₂₆ (Fig. 3). Figure 1(c–f) shows that the increase in the amounts of short-chain linear thiophenes is larger than the increase in the amounts of long-chain linear thiophenes. This can already be anticipated when the Curie-point pyrolysate of the isolated kerogen of the original sample is studied, because short-chain linear thiophenes are more abundant than long-chain linear thiophenes (Fig. 3). At high temperatures, linear

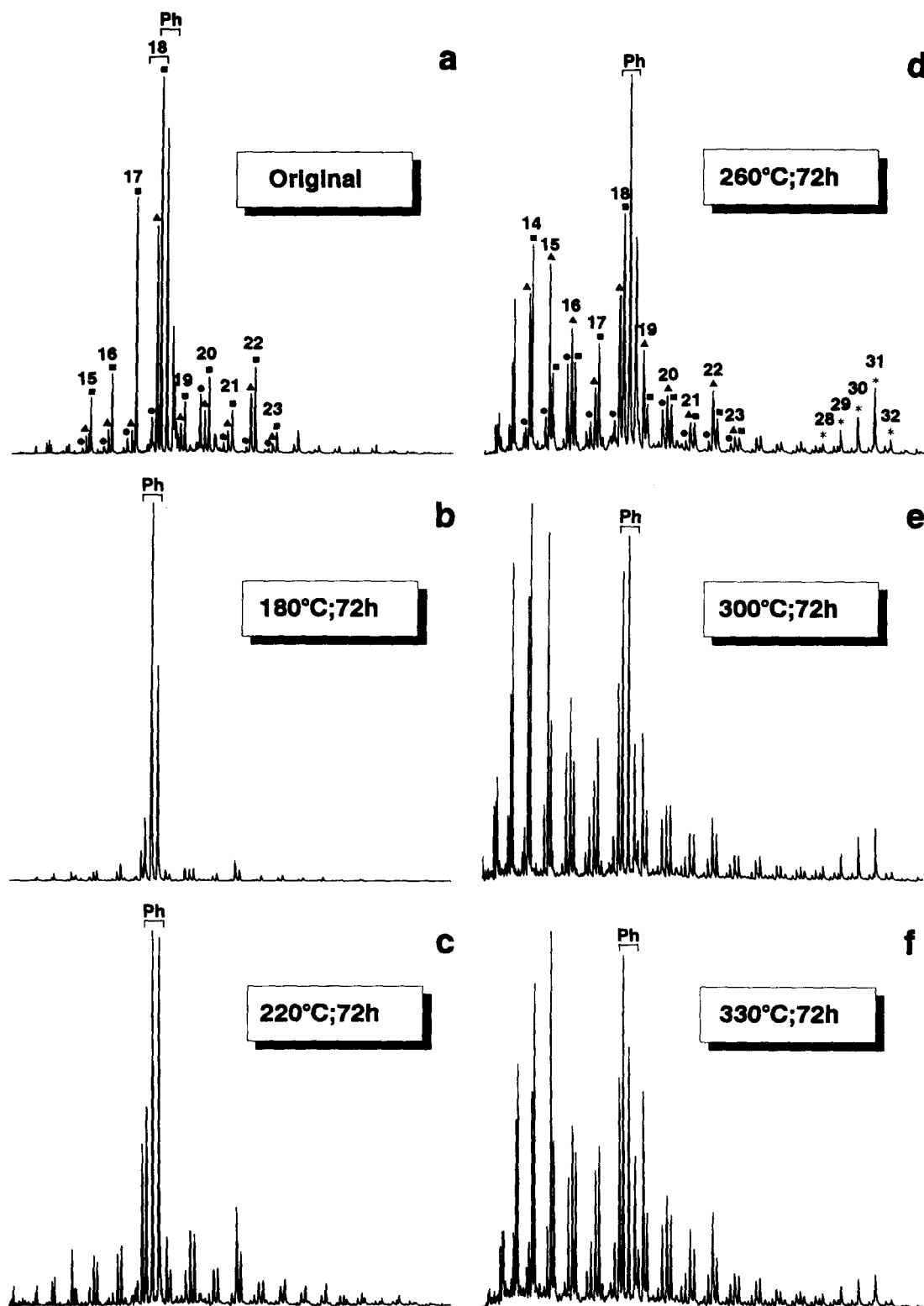


Fig. 1. Summed mass chromatograms (m/z 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140) of the alkylthiophene fractions of (a) the original unheated sample, and the samples subjected to hydrous pyrolysis for 72 h at (b) 180°C, (c) 220°C, (d) 260°C, (e) 300°C and (f) 330°C. Key: Ph = thiophenes with a phytane carbon skeleton, (●) 2-*n*-alkyl-5-ethylthiophenes, (▲) 2-*n*-alkyl-5-methylthiophenes, (■) 2-*n*-alkylthiophenes and (*) 2,5-di-*n*-alkylthiophenes. Clusters of linear alkylthiophenes are indicated by numbers that represent the total number of carbon atoms.

Table 1. Absolute amounts ($\mu\text{g/g}$ TOC of original rock) of some linear thiophenes, thiophenes with a phytane carbon skeleton (see for structures Fig. 3) and C_{28} – C_{32} mid-chain thiophenes as a function of hydrous pyrolysis temperature

Compound	Original	160°C	180°C	200°C	220°C	239°C	260°C	280°C	300°C	330°C
C_{14} linear thiophenes	0.3	1.1	2.1	1.4	2.5	6.8	1.0×10^2	1.0×10^2	2.4×10^2	2.6×10^2
C_{17} linear thiophenes	9.2	4.0	7.2	6.0	10	12	91	1.0×10^2	2.0×10^2	2.2×10^2
C_{20} linear thiophenes	6.6	8.3	16	16	20	24	86	1.0×10^2	1.8×10^2	2.4×10^2
C_{26} linear thiophenes	0.8	0.9	2.3	4.2	4.7	5.5	18	21	35	40
Phytane thiophene I	0.5	4.4	8.9	5.8	10	8.6	49	49	65	60
Phytane thiophene II	11	61	1.4×10^2	53	37	19	2.0×10^2	2.2×10^2	3.1×10^2	1.8×10^2
Phytane thiophene III	4.2	26	86	49	41	45	1.2×10^2	1.0×10^2	1.3×10^2	1.1×10^2
Total phytane thiophenes	16	91	2.4×10^2	1.1×10^2	88	72	3.6×10^2	3.7×10^2	5.0×10^2	3.6×10^2
C_{28} mid-chain thiophenes	0	0	0	1.3	2.0	2.9	12	17	53	39
C_{29} mid-chain thiophenes	0	0	0	2.6	401	10	43	33	89	83
C_{30} mid-chain thiophenes	0	0	0	2.8	3.5	12	79	51	1.3×10^2	1.2×10^2
C_{31} mid-chain thiophenes	0	0	0	4.7	5.9	24	1.4×10^2	81	1.6×10^2	1.3×10^2
C_{32} mid-chain thiophenes	0	0	0	1.8	1.2	3.3	24	18	49	27

thiophenes are present in abundances 20–1000 times higher than in the original sample.

In Table 2 the isomer distributions of the C_{17} and C_{20} linear thiophenes are given as a function of hydrous pyrolysis temperature. The isomer distribution of the C_{17} linear thiophenes in the original sample is dominated by 2-tridecylthiophene. After thermal treatment at 160°C the relative abundance of 2-dodecyl-5-methylthiophene has increased significantly, which is mainly due to the decreased amount of 2-tridecylthiophene. This results in an isomer distribution that is dominated by 2-tridecylthiophene and 2-methyl-5-dodecylthiophene, whereas 2-ethyl-5-undecylthiophene and the C_{17} mid-chain 2,5-di-*n*-alkylthiophenes are significantly less abundant (Table 2). Further thermal stress causes a slight decrease in the relative abundance of 2-tridecylthiophene and 2-methyl-5-dodecylthiophene, and a slight increase in the relative abundance of 2-ethyl-5-undecylthiophene and the C_{17} mid-chain 2,5-di-*n*-alkylthiophenes. It is interesting to note that the isomer distribution of the C_{17} linear thiophenes at 330°C is already reflected in the Curie-point pyrolysate of the isolated kerogen of the original sample (Table 2). This indicates that the originally present linear thiophenes are diluted by the quantitatively more important thiophenes generated from the bulk of the organic matter (i.e. kerogen). There are no indications for the

occurrence of isomerization reactions during hydrous pyrolysis. The compositions of the alkylthiophenes thus reflect the original sites of sulphurization. The isomer distribution of the C_{20} linear thiophenes shows more variation with temperature (Table 2), but the distribution at 330°C does not differ much from the distribution in the Curie-point pyrolysate of the isolated kerogen of the original sample. The high abundance of the linear alkylthiophenes at high maturation temperatures suggests that linear alkylthiophenes are still useful as biomarkers at relatively high levels of thermal maturity ($R_o \approx 0.95$ – 1.1%), but their original composition may be altered by dilution with thiophenes generated from the kerogen.

Thiophenes with a phytane carbon skeleton

In the alkylthiophene fraction of the original sample three thiophenes with a phytane carbon skeleton (I–III; see Fig. 3) are major compounds [Fig. 1(a)]. These isoprenoid thiophenes are widespread in sedimentary rocks and are thought to originate from the incorporation of reduced inorganic sulphur species into chlorophyll-derived phytol or its diagenetic products phytadienes and phytanal (Brassell *et al.*, 1986a; Sinnighe Damsté *et al.*, 1987; Rullkötter *et al.*, 1988; de Graaf *et al.*, 1992; Schouten *et al.*, 1993, 1994; Krein and Aizenshtat, 1994). Artificial maturation at temperatures as low as 160 and 180°C, respectively, yields a 6- and 15-fold increase in the total amount of thiophenes with a phytane carbon skeleton. These data are listed in Table 1 and shown in Fig. 4, in which the generation profiles of the three structural isomers are depicted together with the generation profile of the total amount of thiophenes with a phytane carbon skeleton.

The formation of these isoprenoid thiophenes at relatively low temperatures could result from the thermal decomposition of polysulphide-bound phytanyl units in high-molecular-weight (HMW) fractions of the rock (such as kerogen, asphaltene and the polar fraction). Schouten *et al.* (1994) found that heating (250°C; 5 min) of oligomeric polysulphide-bound phytane aggregates, formed by the reaction of phytadienes with reduced inorganic polysulphides, yielded C_{20} isoprenoid thiophenes in relatively high

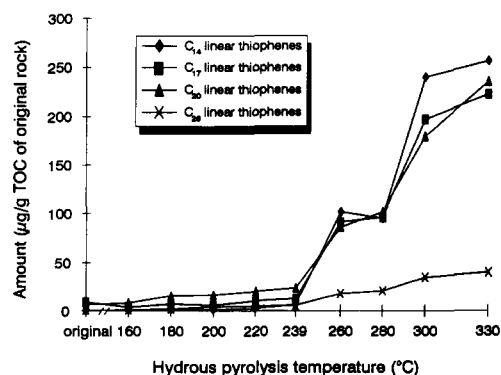


Fig. 2. Absolute amounts ($\mu\text{g/g}$ TOC of original rock) of C_{14} , C_{17} , C_{20} and C_{26} linear alkylthiophenes as a function of hydrous pyrolysis temperature.

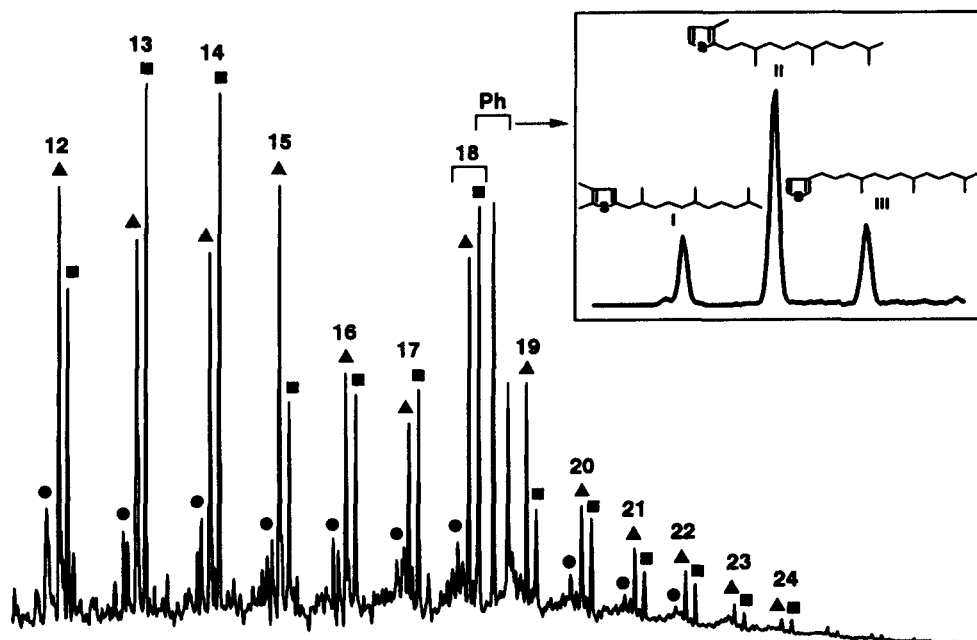


Fig. 3. Summed mass chromatogram (m/z 97 + 98 + 111 + 112 + 125 + 126) of the flash pyrolysate of the isolated kerogen of the original sample. The insert shows a partial mass chromatogram m/z 308 revealing the distribution of the thiophenes with a phytane carbon skeleton. Key: Ph = thiophenes with a phytane carbon skeleton, (●) 2-*n*-alkyl-5-ethylthiophenes, (▲) 2-*n*-alkyl-5-methylthiophenes and (■) 2-*n*-alkylthiophenes. Clusters of linear thiophenes are indicated by numbers that represent the total number of carbon atoms.

abundance. However, the dominant isomer was I, and in our experiments at 160 and 180°C this isomer is still of relatively minor importance. Krein and Aizenshtat (1994) heated (300°C; N₂ flow) oligomeric polysulphide-bound phytanyl units, formed by the reaction of phytanal with reduced inorganic polysulphides, and found only thiophenes II and III in a 3:1 ratio. These experiments suggest that the formation of HMW fractions consisting of polysulphide-bound phytanyl units during the earliest stages of diagenesis of the Vena del Gesso sediment involved reaction of reduced inorganic polysulphides with phytanal rather than phytadienes. It is noteworthy that after treatment of

the polar fraction of the original sample with MeLi/MeI, a reagent that selectively cleaves S-S bonds and subsequently methylates the produced thiols (Kohnen *et al.*, 1991), methylthioethers of phytane were among the most abundant compounds present (Koopmans *et al.*, 1995). This suggests that substantial amounts of polysulphide-bound phytane skeletons are present in the polar fraction.

Artificial maturation at temperatures between 180 and 239°C results in a decrease of the total amount of thiophenes with a phytane carbon skeleton (Fig. 4). Further heating at temperatures higher than 239°C again yields increasing amounts of thiophenes with a

Table 2. Relative amounts (%) of structural isomers of C₁₇, C₂₀ and C₃₁ linear thiophenes as a function of hydrous pyrolysis temperature. Relative amounts of these isomers in the Curie-point pyrolysate of the isolated kerogen of the original sample are listed in the column denoted KER

Compound	Original	160°C	180°C	200°C	220°C	239°C	260°C	280°C	300°C	330°C	KER
2-tridecylthiophene	83	52	56	44	38	37	41	40	37	33	41
2-dodecyl-5-methylthiophene	7	30	32	36	38	37	30	32	31	35	39
2-ethyl-5-undecylthiophene	6	8	7	9	10	14	14	13	16	17	12
C ₁₇ mid-chain thiophenes	4	10	6	12	14	12	16	15	17	15	9
2-hexadecylthiophene	34	31	32	33	30	20	24	28	29	22	33
2-methyl-5-pentadecylthiophene	25	33	30	44	45	46	38	42	33	42	42
2-ethyl-5-tetradecylthiophene	35	32	34	17	19	28	29	21	26	27	15
C ₂₀ mid-chain thiophenes	6	5	4	6	7	6	10	10	13	9	10
2-nonyl-5-octadecylthiophene	-	-	-	4	5	4	4	5	6	2	ND
2-decyl-5-heptadecylthiophene	-	-	-	5	5	6	7	10	9	7	ND
2-hexadecyl-5-undecylthiophene	-	-	-	10	12	12	12	14	13	10	ND
2-dodecyl-5-pentadecylthiophene	-	-	-	19	21	17	16	18	17	17	ND
2-tetradecyl-5-tridecylthiophene	-	-	-	62	57	61	61	54	54	64	ND

ND = not detected.

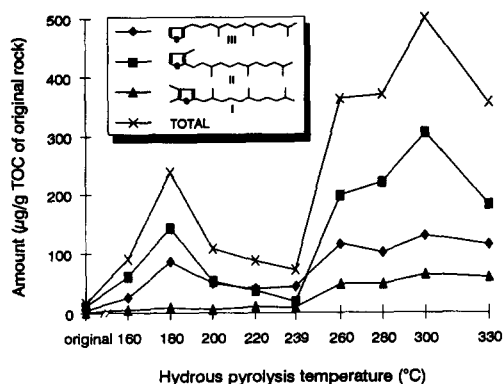


Fig. 4. Absolute amounts ($\mu\text{g/g}$ TOC of original rock) of three thiophenes with a phytane carbon skeleton and the total amount of thiophenes with a phytane carbon skeleton as a function of hydrous pyrolysis temperature.

phytane carbon skeleton, which at 330°C are about 20 times more abundant than in the original sample. These generation profiles suggest that there are two main stages of formation of thiophenes with a phytane carbon skeleton from HMW fractions with different activation energies (low E_A : original–180°C; high E_A : 239–300°C), and that continuous degradation of thiophenes with a phytane carbon skeleton is taking place with notable decreases in absolute abundance between 180 and 200°C. As was stated in the previous paragraph, the formation of thiophenes with a phytane skeleton at low temperatures (first stage of formation) could result from the thermal breakdown of polysulphide-bound phytanyl units in HMW fractions. The second stage of formation of thiophenes with a phytane skeleton could then result from the thermal breakdown of monosulphide-bound phytanyl units in HMW fractions. This coincides with the fact that S–S bonds are weaker than C–S bonds. The similarity of the generation profiles of the linear thiophenes (Fig. 2) and the thiophenes with a phytane carbon skeleton (Fig. 4) at $T > 220^\circ\text{C}$ suggests that they are formed by similar processes. The idea of continuous degradation of thiophenes is supported by two observations. First, 3,4-di-*n*-alkylthiophenes, which have been found in thermally immature sedimentary rocks but whose mid-chain dimethyl carbon skeletons have not been detected upon desulphurization of HMW fractions (Sinninghe Damsté *et al.*, 1990; Kohnen *et al.*, 1990c), were present in the original sample but could not be detected after thermal treatment at 220°C (*vide infra*). Second, off-line pyrolysis (400°C; 1 h; N_2 flow) of the isolated kerogen of the original sample yielded phytane skeletons in high amounts (ca. 13 mg/g TOC). At

300°C only 5% of the phytane skeletons present in the original kerogen had been converted to free thiophenes with a phytane carbon skeleton, and 10% to free phytane. However, Curie-point pyrolysis of the isolated kerogen of the sample that was artificially matured at 300°C yielded no detectable phytane skeletons (Koopmans *et al.*, 1995). This indicates that a considerable amount of phytane skeletons is missing, possibly due to the degradation of thiophenes with a phytane carbon skeleton. It is likely that the products of the degradation of the thiophenes with a phytane carbon skeleton are volatilized during work-up.

The isomer distribution of the thiophenes with a phytane carbon skeleton changes dramatically upon heating. This can be visualized by the isoprenoid thiophene ratio [$\text{ITR} = (\text{II} + \text{III})/(\text{I} + \text{mid-chain thiophenes with a phytane carbon skeleton})$], a parameter that is thought to reflect the (hyper)salinity of the depositional environment (Sinninghe Damsté *et al.*, 1989b) (Table 3). The decline of the ITR from 32 in the original sample to 4.9 at 330°C is probably due to the dilution of the thiophenes with a phytane carbon skeleton already present in the alkylthiophene fraction of the original sample by thiophenes with a phytane carbon skeleton that are liberated from HMW fractions because of increasing thermal stress. This is supported by the composition of the Curie-point pyrolysate of the isolated kerogen of the original sample, comprising the same three thiophenes with a phytane carbon skeleton with a distribution that corresponds to an ITR of 4.2 (Table 3; Fig. 3). Although the ITR changes considerably with temperature, it does not drop below the critical value of 0.5 which represents the upper limit for hypersaline palaeoenvironments (Sinninghe Damsté *et al.*, 1989b). A similar drop in the ITR with increasing thermal stress has been observed in an evaporite sample using confined pyrolysis (Benalioul-haj *et al.*, 1993).

C_{37} – C_{38} 2,5-di-*n*-alkylthiophenes

Linear C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes are only observed when the rock is matured at 260°C and higher temperatures. This explains why in a previous study of sedimentary rocks from the Vena del Gesso Basin these thiophenes were not discussed (Kohnen *et al.*, 1992). The C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes are present in relatively minor amounts with a maximum of 27 $\mu\text{g/g}$ TOC at 300°C, and their generation profiles are co-variant as judged from mass chromatograms (m/z 546 + 560). The C_{38} 2,5-di-*n*-alkylthiophenes occur in slightly higher amounts than the C_{37} 2,5-di-*n*-alkylthiophenes. At 330°C these compounds are not detectable, probably due to the

Table 3. Isoprenoid thiophene ratio (ITR) as a function of hydrous pyrolysis temperature. The ITR of the Curie-point pyrolysate of the isolated kerogen of the original sample is listed in the column denoted KER

	Original	160°C	180°C	200°C	220°C	239°C	260°C	280°C	300°C	330°C	KER
ITR	32	20	26	18	7.8	7.3	6.4	6.6	6.8	4.9	4.2

degradation of thiophenes as discussed in the previous section. The C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes are thought to be derived from C_{37} and C_{38} di- and tri-unsaturated methyl and ethyl ketones present in Prymnesiophyceae (Volkman *et al.*, 1980). Sulphur incorporation into these functionalized lipids has been reported to yield cyclic monosulphides, 2,5-di-*n*-alkylthiophenes (Sinninghe Damsté *et al.*, 1989a, 1990; Kohnen *et al.*, 1990b) and sulphur-bound *n*- C_{37} and *n*- C_{38} carbon skeletons in HMW fractions (Sinninghe Damsté *et al.*, 1988; Schouten *et al.*, 1993).

The formation of C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes only at relatively high temperatures (260°C) can be explained as follows. Because of the large distance between the various double bonds and the carbonyl group, which is also available for sulphurization (Schouten *et al.*, 1993), the incorporation of reduced inorganic sulphur species into C_{37} and C_{38} di- and tri-unsaturated methyl and ethyl ketones occurs predominantly in an intermolecular fashion and is likely to result in multiple sulphur linkages. This leads to the formation of a cross-linked sulphur-rich macromolecular network present in the asphaltene fraction or the kerogen. Artificial maturation at 200°C results in the cleavage of some of these (poly)sulphide bridges, forming sulphur-rich macromolecular aggregates which end up in the polar fraction. This is confirmed by the relatively large amounts of C_{37} and C_{38} *n*-alkanes that were released after desulphurization of the polar fraction of the sample matured at 200°C, which were an order of magnitude higher than the amounts released after desulphurization of the polar fraction of the original sample (Koopmans *et al.*, 1995). At temperatures higher than 220°C, the remaining (poly)sulphide bridges in these macromolecular aggregates will disintegrate, leading to the formation of free C_{37} and C_{38} *n*-alkanes (Koopmans *et al.*, 1995) and the C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes presently encountered in the alkylthiophene fractions.

The isomer distribution of the C_{38} mid-chain 2,5-di-*n*-alkylthiophenes is not random as judged from an averaged mass spectrum covering the whole peak reflecting the C_{38} mid-chain 2,5-di-*n*-alkylthiophenes [Fig. 5(a)]. 2-hexadecyl-5-octadecylthiophene (22%) and 2-nonadecyl-5-pentadecylthiophene (22%) make up a large portion of the total C_{38} mid-chain 2,5-di-*n*-alkylthiophenes, whereas the other isomers are less abundant ($\leq 12\%$). This specific isomer distribution of the C_{38} thiophenes produced after thermal treatment at 260°C is retained when the rock is heated at 280 and 300°C. A similar isomer distribution is observed for the C_{37} mid-chain 2,5-di-*n*-alkylthiophenes, but due to coelution with a more abundant C_{35} hopanoid thiophene it was difficult to quantify. An explanation for this specific isomer distribution can be given on the basis of the number of double bonds present in the precursor lipids. Prymnesiophyceae biosynthesising C_{37} and C_{38} di- and tri-unsaturated methyl and ethyl ketones

(Volkman *et al.*, 1980) adjust the relative amounts of these di- and tri-unsaturated compounds to the temperature of the surrounding water in order to maintain optimal membrane fluidity (Brassell *et al.*, 1986b; Prah and Wakeham, 1987). This results in an inverse relationship between the overall degree of unsaturation and the surface water temperature (Brassell *et al.*, 1986b; Prah and Wakeham, 1987). The Vena del Gesso sediments were deposited in an evaporitic environment (Vai and Ricci Lucchi, 1977). Therefore, it can be argued that the surface water temperatures were relatively high when the Prymnesiophyceae bloomed. As a result, these algae biosynthesized mostly di-unsaturated methyl and ethyl ketones. Sulphur incorporation into the C_{38} di-unsaturated methyl and ethyl ketone during the earliest stages of diagenesis can lead to multiple sulphur cross-linking with other functionalized lipids to form a macromolecular structure such as kerogen. Sulphur can be attached to carbon atoms 2, 16, 17, 23 and 24 (methyl ketone) or 3, 16, 17, 23 and 24 (ethyl ketone) [Fig. 5(b)] (Schouten *et al.*, 1993, 1994), assuming that the positions of the double bonds in the methyl ketone are the same as those in the ethyl ketone (de Leeuw *et al.*, 1980).

Artificial maturation at temperatures higher than 220°C leads to degradation of the (poly)sulphide linkages in the macromolecular aggregates in the polar fraction, which may yield C_{38} 2,5-di-*n*-alkylthiophenes as stable products. The dominating isomers, 2-hexadecyl-5-octadecylthiophene and 2-nonadecyl-5-pentadecylthiophene, can only be formed by ring closure directed towards the original position of the second double bond [Fig. 5(b)]. Theoretically, taking into account all eight possible products of such a ring closure, this should yield 2-hexadecyl-5-octadecylthiophene and 2-nonadecyl-5-pentadecylthiophene in a 1:2 ratio [Fig. 5(b)]. However, these isomers occur in a 1:1 ratio (both 22% of total C_{38} mid-chain 2,5-di-*n*-alkylthiophenes). The thiophenes that could be formed from ring closure directed towards the terminal carbon atom are represented only by minor peaks in the mass spectrum [Fig. 5(a,b)]. This suggests that the second double bond (or a derivative thereof) plays an important role in ring closure and, therefore, in the production of C_{38} 2,5-di-*n*-alkylthiophenes. It is noteworthy that 2-tetratriacontylthiophene, 2-methyl-5-tritriacontylthiophene and 2-ethyl-5-dotriacontylthiophene, which are present in amounts 20 times lower than the sum of the mid-chain thiophenes, have a relative abundance of 1:4:4. If this ratio is corrected for background (the average relative abundance of 2-*n*-alkylthiophenes, 2-*n*-alkyl-5-methylthiophenes and 2-*n*-alkyl-5-ethylthiophenes is ca. 2:2:1), the relative enhancement of 2-methyl-5-tritriacontylthiophene and 2-ethyl-5-dotriacontylthiophene becomes apparent. This is consistent with sulphur incorporation into the keto group at carbon number 2 (methyl ketone) or 3 (ethyl ketone) (Schouten *et al.*, 1993) and subsequent ring closure [Fig. 5(b)].

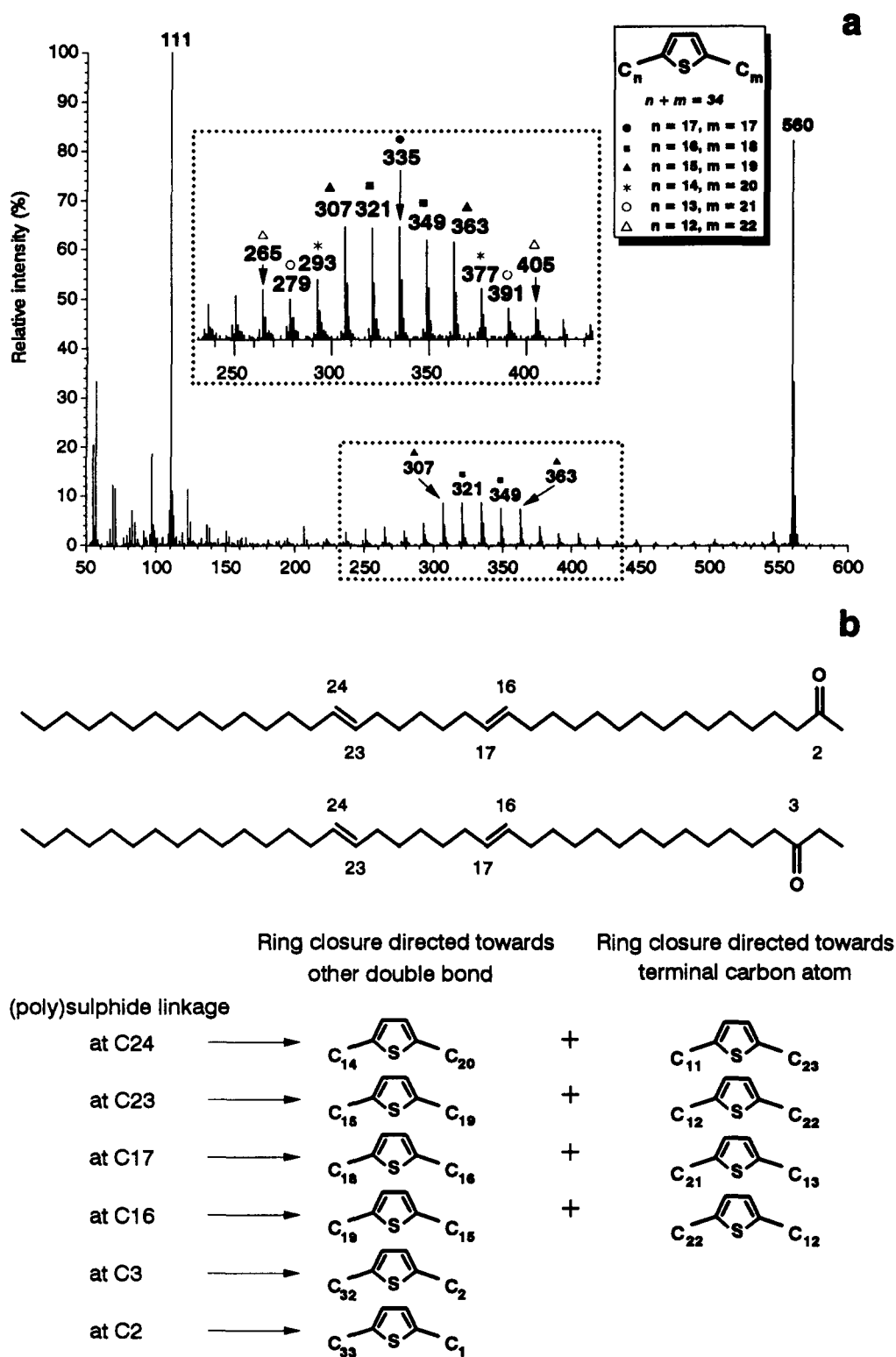


Fig. 5. (a) Averaged mass spectrum (subtracted for background; averaged over 13 scans) of C_{38} 2,5-di-*n*-alkylthiophenes from the sample artificially matured at 260°C. (b) Structures of C_{38} di-unsaturated methyl and ethyl ketones, and possible products of ring closure after cleavage of intermolecular (poly)sulphide linkages.

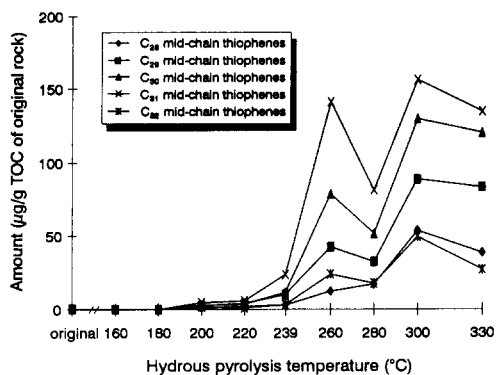


Fig. 6. Absolute amounts ($\mu\text{g/g}$ TOC of original rock) of C_{28} – C_{32} 2,5-di-*n*-alkylthiophenes as a function of hydrous pyrolysis temperature.

The C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes can thus be considered as stabilization products of multiply (poly)sulphide linked *n*- C_{37} and *n*- C_{38} carbon skeletons derived from unsaturated long-chain ketones. It should be noted that these alkylthiophenes are also present in some relatively immature rocks (Sinninghe Damsté *et al.*, 1989a, 1990; Kohnen *et al.*, 1990b), suggesting that hydrous pyrolysis simulates natural diagenesis. The explanation for the formation of these thiophenes is basically different from that proposed earlier (Sinninghe Damsté *et al.*, 1989a), and it does not require the unlikely process of double bond isomerization at the earliest stages of diagenesis as suggested by Sinninghe Damsté *et al.* (1989a). The fact that product–precursor relationships could be made at temperatures up to 300°C (including the confirmation of the original positions of the double bonds of the

unsaturated long-chain ketones) underlines the use of alkylthiophenes as molecular indicators during progressive diagenesis and early catagenesis. It should be noted that the C_{37} and C_{38} *n*-alkanes do not reveal information about the presence of original positions of functionalities, and that Curie-point pyrolysis of the isolated kerogen of the original sample did not reveal the C_{37} and C_{38} 2,5-di-*n*-alkylthiophenes.

*C*₂₈–*C*₃₂ 2,5-di-*n*-alkylthiophenes

At 200°C, 2,5-di-*n*-alkylthiophenes in the C_{28} – C_{32} range are formed. At higher temperatures, large amounts of these compounds are produced with the C_{31} members being the most abundant (Table 1; Figs 1 and 6). These compounds are not encountered in the alkylthiophene fraction of the original sample, and, therefore, it is not surprising that in a previous study of a similar marl sample from the Vena del Gesso Basin the C_{28} – C_{32} 2,5-di-*n*-alkylthiophenes were not reported (Kohnen *et al.*, 1992). At 330°C the C_{28} – C_{32} 2,5-di-*n*-alkylthiophenes are still present in considerable quantities.

In Fig. 7 an averaged mass spectrum covering the whole peak of the C_{31} mid-chain 2,5-di-*n*-alkylthiophenes is given from the alkylthiophene fraction of the sample matured at 300°C. It is apparent that the distribution of the structural isomers is not random. There is a definite predominance of 2-tetradecyl-5-tridecylthiophene over the other structural isomers. Averaged mass spectra of the other mid-chain 2,5-di-*n*-alkylthiophenes in the C_{28} – C_{32} range also show a definite predominance of the 2-*n*-alkyl-5-tetradecylthiophenes over the other structural isomers. This suggests that the precursors of these compounds

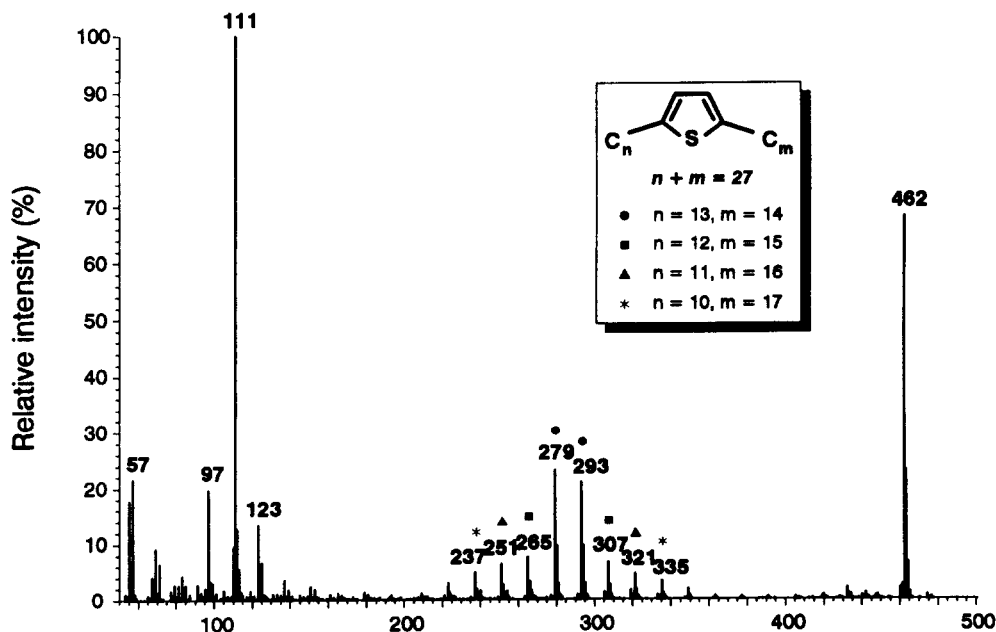


Fig. 7. Averaged mass spectrum (subtracted for background; averaged over 13 scans) of C_{31} 2,5-di-*n*-alkylthiophenes from the sample artificially matured at 300°C.

had a functional group at C-15. Possible candidates are the C₃₀–C₃₂ 1,15-diols and the C₃₀–C₃₂ 15-keto-1-ols reported earlier (e.g. de Leeuw *et al.*, 1981; ten Haven *et al.*, 1992), although it is known that hydroxyl groups do not react with reduced inorganic sulphur species (Schouten *et al.*, 1993). Reduced inorganic sulphur species could react with the keto group to form a (poly)sulphide linkage with another functionalized lipid (Schouten *et al.*, 1993). Another possibility is the dehydration of the hydroxyl group at C-15 in the C₃₀–C₃₂ 1,15-diols, which would yield a double bond either at C-14 or C-15. This allows for the incorporation of sulphur at C-14, C-15 or C-16 in a 1:2:1 ratio. Despite these possibilities to form intermolecular (poly)sulphide linkages, it is difficult to imagine how a thiophene could form from such a structure upon increasing thermal stress because no additional functionalities are available nearby. Moreover, the specific isomer distribution of the C₂₈–C₃₂ mid-chain 2,5-di-*n*-alkylthiophenes, with the 2-*n*-alkyl-5-tetradecylthiophenes as the dominant isomers, shows that the formation of the thiophene ring is directed preferentially to one side of the carbon chain, thus suggesting that an additional functionality is present between C-16 and the terminal carbon atom. Other possible precursors are the C₃₀–C₃₂ di-unsaturated alcohols and the C₃₂ mono-unsaturated 1,15-diol found in algae of the class Eustigmatophyceae (Volkman *et al.*, 1992). The position of the double bond in the C₃₂ mono-unsaturated 1,15-diol was not determined, although it was proposed that the double bond is located between C-16 and C-32 based on mass spectral data (Volkman *et al.*, 1992). This would coincide with the fact that the formation of the thiophene ring is directed towards that side of the carbon chain. However, the specific carbon number distribution of the C₂₈–C₃₂ mid-chain 2,5-di-*n*-alkylthiophenes, with the C₃₁ members as the most abundant components, is not reflected in any of the above precursors, which generally have a strong even-over-odd carbon number predominance (Volkman *et al.*, 1992). On the other hand, 2-ethyl-5-pentacosylthiophene, 2-methyl-5-hexacosylthiophene and 2-heptacosylthiophene have a relative abundance of 1:7:2, which after correction for background (see previous section) results in a definite enhancement of 2-methyl-5-hexacosylthiophene over the other two isomers. This suggests that an intermolecular (poly)sulphide linkage was present at C-2, which upon thermal stress formed a thiophene ring. This would be consistent with the presence of a terminal hydroxyl group, which upon dehydration would yield a double bond at C-1 that could react with reduced inorganic sulphur species to form a (poly)sulphide linkage preferentially at C-2 (de Graaf *et al.*, 1992; Schouten *et al.*, 1994).

In Table 2 the isomer distribution of the C₃₁ mid-chain 2,5-di-*n*-alkylthiophenes is given as a function of hydrous pyrolysis temperature for the range 200°C (when these compounds are first formed)

to 330°C. The specific isomer distribution of the C₃₁ mid-chain 2,5-di-*n*-alkylthiophenes observed at 200°C is retained at 330°C. Again, this shows that alkylthiophenes maintain their biomarker signatures at relatively high levels of thermal maturity, enabling the link between products and precursors which would not be possible using the corresponding *n*-alkanes, or compounds in the Curie-point pyrolysate of the isolated kerogen of the original sample. The specific isomer distribution also shows that the generation of organic sulphur compounds by the (random) reaction of elemental sulphur with alkanes, as proposed by Schmid *et al.* (1987), during hydrous pyrolysis should be considered highly unlikely. It is important to note that, although the reproducibility of the absolute amounts of C₂₈–C₃₂ 2,5-di-*n*-alkylthiophenes is low as determined from duplicate experiments at 280 and 300°C (see Experimental), the reproducibility of the specific isomer distribution is high. Thus, the aspect of the C₂₈–C₃₂ 2,5-di-*n*-alkylthiophenes that makes them suitable as molecular indicators is still recognisable in the duplicate experiments.

C₃₆–C₄₄ 3,4-di-*n*-alkylthiophenes

The presence of long mid-chain 3,4-di-*n*-alkylthiophenes has been reported before in immature sedimentary rocks from a.o. the Jurf ed Darawish oil shale in Jordan and the Vena del Gesso Basin (Sinninghe Damsté *et al.*, 1990; Kohnen *et al.*, 1990c). These reports speculated on the formation of these molecules from specific mid-chain dimethylalkadienes by reaction with reduced inorganic sulphur species. The fact that these carbon skeletons were not reported before was attributed to the instability of the precursor molecule, and it was concluded that the incorporation of sulphur had preserved the carbon skeleton (Sinninghe Damsté *et al.*, 1990).

In the original unheated rock mid-chain 3,4-di-*n*-alkylthiophenes were found in the C₃₆–C₄₄ range. Although the amounts are low (≤ 1 µg/g TOC), a definite even-over-odd carbon number preference is observed. Artificial maturation of the rock results in a gradual decrease of the amounts of C₃₆–C₄₄ mid-chain 3,4-di-*n*-alkylthiophenes, and at 220°C these compounds are not detectable. Again, this suggests that the degradation of thiophenes is a process that already takes place at low levels of thermal maturity. It is interesting to note that the C₃₆–C₄₄ mid-chain 3,4-di-*n*-alkylthiophenes are the only alkylthiophenes that are *not* generated at increasing temperatures. This observation is consistent with the fact that the corresponding mid-chain dimethylalkanes have never been reported upon desulphurization of sulphur-rich macromolecular aggregates. This suggests either that their precursors, the mid-chain dimethylalkadienes (Kohnen *et al.*, 1990c), are not incorporated into a macromolecular network such as kerogen from which they can be liberated upon increasing thermal stress, or that the C₃₆–C₄₄ mid-chain 3,4-di-*n*-alkylthiophenes are more

labile under geological conditions than their 2,5-di-*n*-alkyl counterparts. The latter is, however, hard to believe from a chemical point of view. It is more likely that the spacing of the original functionalities was appropriate for direct formation of a thiophene moiety.

CONCLUSIONS

After artificial maturation by hydrous pyrolysis at temperatures up to 330°C for 72 h, linear alkylthiophenes and thiophenes with a phytane carbon skeleton are present in much higher concentrations than in the original sample due to their production from HMW fractions, presumably kerogen. The importance of the kerogen in the production of alkylthiophenes is supported by the observation that the isomer distributions of alkylthiophenes at high temperatures coincide with the isomer distributions of alkylthiophenes in the Curie-point pyrolysate of the isolated kerogen of the original sample.

The structural isomer distribution of linear alkylthiophenes does not change significantly when these compounds are generated from HMW fractions at different temperatures. This implies that information regarding the original positions of functional groups in the biochemical precursors is retained at relatively high levels of thermal maturity, indicating that linear alkylthiophenes are useful biomarkers throughout diagenesis and early catagenesis. This is exemplified by the specific isomer distributions of the C₃₁ and C₃₈ 2,5-di-*n*-alkylthiophenes, which enabled the identification of possible precursor lipids, *i.e.* C₃₀–C₃₂ (unsaturated) 1,15-diols or 15-keto-1-ols in case of the C₃₁ 2,5-di-*n*-alkylthiophenes and C₃₈ di-unsaturated methyl and ethyl ketones in case of the C₃₈ 2,5-di-*n*-alkylthiophenes. These long-chain alkylthiophenes are not present in the original (unheated) sample, indicating that hydrous pyrolysis is capable of revealing sequestered information about the depositional environment. The decreasing amounts of mid-chain 3,4-di-*n*-alkylthiophenes suggest that alkylthiophenes are subject to continuous degradation, as can also be concluded from the generation profiles of the thiophenes with a phytane carbon skeleton. This means that the concentrations given here are the collective product of opposing processes involved in the production and degradation of alkylthiophenes. No direct evidence was found for side-chain cleavage, possibly due to loss of volatile compounds during work-up. The absence of (di)benzothiophenes in all samples suggests that cyclization and aromatization of alkylthiophenes is not an important "degradation" pathway.

Generation of organic sulphur compounds by the random reaction of elemental sulphur and alkanes during hydrous pyrolysis is unlikely in view of the highly specific isomer distribution of the C₃₁ and C₃₈ mid-chain 2,5-di-*n*-alkylthiophenes that does not change significantly upon increasing thermal stress.

Hydrous pyrolysis is a useful simulation method to study the thermal stability of alkylthiophenes in the geosphere during diagenesis and early catagenesis. This requires experimental temperatures, however, that are much lower than the temperatures used to simulate oil generation and expulsion processes.

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