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# Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy): III. Stratigraphic changes in the molecular structure of kerogen in a single marl bed as revealed by flash pyrolysis*

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**Abstract**—Kerogens of nine samples from a single marl bed of the Gessoso-solfifera Formation in the Vena del Gesso basin (Messinian, Italy) were qualitatively and quantitatively studied by analytical pyrolysis. Relationships between the nature of the pyrolysis products and the source organisms were determined. The high abundance of (i) algal-derived components (*n*-alkanes and *n*-alk-1-enes) and (ii) sulphur-containing and sulphur-bound products signifying a high degree of early diagenetic sulphurization were observed. Presence of photic zone anoxia during the deposition of these sediments was discussed via the presence of 1,2,3,4-tetramethylbenzene in the pyrolysates. Unusual distributions were found for three series of pyrolysis products (i.e. $C_{13}$ and $C_{14}$ *n*-alkylated thiophenes and thiolanes and $C_{12}$ and $C_{13}$ *n*-1,3-alkadienes were by far the major products of these series) in the pyrolysates of two samples corresponding to the middle of the cycle (samples 3 and 5) which also have the highest $\delta^{13}C_{TOC}$ values and the highest pyrolysis yields. Therefore, the contribution of organisms biosynthesizing resistant algal biopolymer was considered as much more important during the deposition of the sediments associated with samples 3 and 5. We finally proposed partial structural elements from which the specific low molecular weight compounds of samples 3 and 5 can be derived upon pyrolysis.

*Key words*—kerogen, flash pyrolysis, quantitative pyrolysis, algaenan, marine microalgae, sulphurization, evaporites

## INTRODUCTION

Free and sulphur-bound lipids, and free pigments, present in the extracts of ten samples from marl bed IV of the Vena del Gesso basin have been examined by Kenig *et al.* (1995) and Keely *et al.* (1995), respectively. Differences in component concentrations implying changes in the major source organisms and in degree of sulphurization of organic matter and changes in depositional setting were observed. However, the extracts represent only *ca* 5% of the organic matter (OM). Hence, qualitative and quantitative investigations of components which can be released from the kerogens are necessary in order to extend the geochemical informations which can be obtained about the source organisms, depositional conditions and degree of sulphurization. The products released from the kerogens by $Li/EtNH_2$ treatment have been examined and were found to contain in particular compounds of algal origin (Schaeffer *et al.*, 1995). Flash pyrolysis is considered one of the most powerful analytical tools for exploring the chemical structure of kerogens (e.g. van de Meent *et al.*, 1980; Larter, 1984; Nip *et al.*, 1988). Hence, we have used this approach, employing a method that permits quantification of the GC-amenable products obtained by Curie point pyrolysis–gas chromatography.

## EXPERIMENTAL

### *Samples*

Cycle IV of the Messinian evaporitic sequence from the Vena del Gesso sediments (see Vai and Ricci Lucchi, 1977 and Sinninghe Damsté *et al.*, 1995 for geological background) consists of a 1.3 m thick marl layer deposited under slow sedimentation rates and overlaid by a stromatolitic bed and a thick gypsum bed. The marl layer was split in 10 samples, each representing a thickness of *ca* 13 cm and are numbered 1 to 10 from base to top. Together they cover the entire marl bed of cycle IV. Sample 9 was not analysed in the present study.

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Kerogen isolation by treatment with HCl and HF/HCl is described in Schaeffer *et al.* (1995). Determination of the total organic carbon content (TOC) of the kerogen concentrates was performed by flash combustion using a Carlo Erba NA-1500 analyser (Verardo *et al.*, 1990).

*Pyrolysis methods*

The kerogen samples were crushed as finely as possible using a pestle and a mortar. A standard, 2,3-dimethyl-5-(1′,1′-$d_2$-hexadecyl)thiophene, was added and each sample was ultrasonicated in dichloromethane under a nitrogen flow. About 20 $\mu$g of standard was generally used for *ca* 20 mg of kerogen. The kerogen samples were pressed onto a flattened ferromagnetic wire and heated by inductive heating (Curie temperature 610°C) for 10 s using a Curie point high frequency generator (Fischer 9425). The gas chromatograph (Hewlett Packard HP-5890) was equipped with a cryogenic unit and programmed from 0°C (5 min) to 320°C (10 min) at a rate of 3°C/min. Separation of the products was achieved by a 25 m fused silica capillary column coated with CP-Sil 5 (0.32 mm i.d.; film thickness 0.45 $\mu$m). Helium was used as carrier gas and the temperature of the flame ionization detector (FID) was 320°C. Identification of the pyrolysis products was performed by Curie point pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) using the same pyrolysis and GC-conditions as described above. The column was coupled to the electron impact ion source of a VG Autospec Ultima mass spectrometer (mass range $m/z$ 40–800 at a resolution of 1000); cycle time 1.8 s; ionization energy 70 eV). The concentrations of the pyrolysis products which showed no evidence of coelution were determined by integration of the peak areas in the FID traces. Integration of peak areas in specific mass chromatograms of selected compounds classes, in combination with the quantified FID data, were used for quantitation. It was assumed that the MS response factors do not vary significantly within each class.

To test the validity of the quantitative pyrolysis method, 10 analyses of sample 4 were carried out at two different standard concentrations (0.92 and 2.49 mg/g kerogen). A selection of 16 peaks scanning the almost entire FID trace were quantified. The relative standard deviations of the 10 measurements for the 16 peaks varied from 17 to 31%, the latter corresponding to the less intense peak, thus causing larger integration errors.

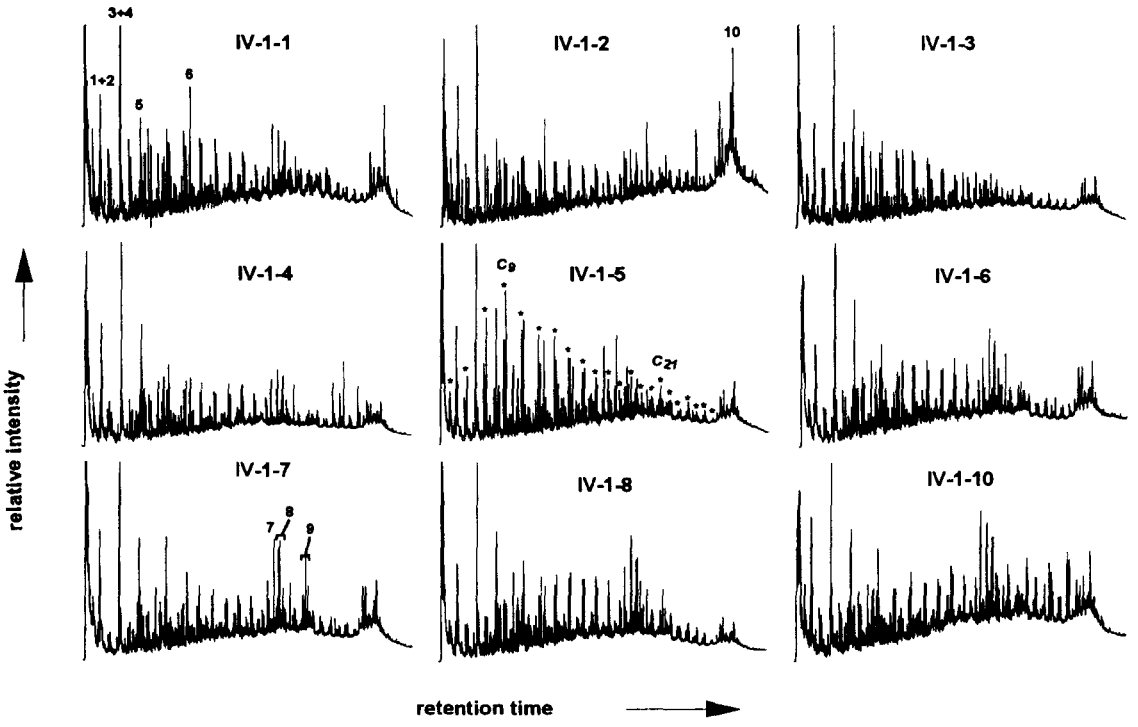


Fig. 1. FID trace of the flash pyrolysates of kerogens 1–8 and 10. Stars indicate (only shown for sample 5) the doublets of *n*-alkanes/*n*-alk-1-enes, the italic numbers indicating the carbon numbers. Peak bold numbers identify the indicated pyrolysis products as (1) benzene, (2) thiophene, (3) toluene, (4) 2-methylthiophene, (5) *m*-xylene + *p*-xylene, (6) 1,2,3,4-tetramethylbenzene, (7) prist-1-ene, (8) phytenes, (9) thiophenes with a phytanyl skeleton (10) $C_{29:1}$ sterene. Note that these chromatograms reflect analyses without internal standard.

## RESULTS AND DISCUSSION

### *Identification and significance of pyrolysis products*

The gas chromatograms of the flash pyrolysates of the nine isolated kerogens revealed contributions from several series of compounds (Fig. 1). Series of *n*-alkanes, *n*-alk-1-enes, alkylated benzenes, alkylated thiophenes, steranes, sterenes, $C_{20}$ isoprenoid thiophenes, phytenes, alkan-2-ones and fatty acids dominate the pyrolysates of all the samples. Although all the samples released the same series of products, the relative abundances vary significantly. For instance, sterenes are very abundant in the pyrolysate of sample 2, whereas they are present in relatively low abundance in the other samples.

Series of *n*-alkanes and *n*-alk-1-enes in pyrolysates typically reflect the presence of long polymethylenic chains, as shown by studies on the mechanism of pyrolysis of polyolefins (e.g. Lattimer, 1995). It is now well known that certain microalgae, especially Chlorophyceae, can produce non-hydrolysable and highly aliphatic macromolecules, termed "algaenan", which comprise the outer cell walls (Largeau *et al.*, 1984; Goth *et al.*, 1988; Derenne *et al.*, 1992; de Leeuw and Largeau, 1994). The ability of these macromolecules to resist the bacterial and chemical degradation that takes place during early diagenesis has also been demonstrated. Their potential for preservation is therefore responsible for their presence in many sediments. Furthermore, Eglinton (1994)

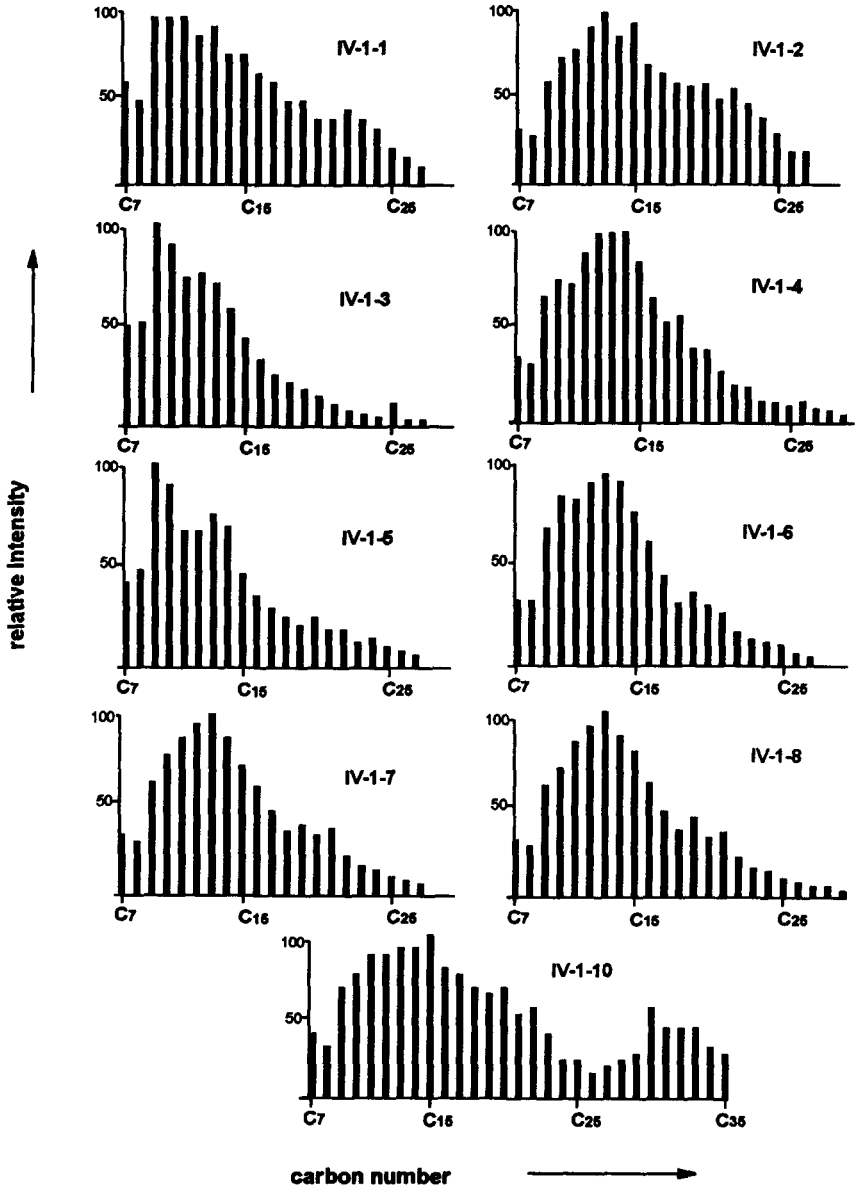


Fig. 2. Distribution of *n*-alkanes, determined from mass chromatograms (*m*/*z* 57) of the pyrolysates of kerogens 1–8 and 10.

showed for several different kerogens that the $\delta^{13}C$ values of *n*-alkanes released by pyrolysis were almost identical for a given kerogen with respect to the carbon number. In most cases the values were close to the $\delta^{13}C_{TOC}$ values, demonstrating the importance of the aliphatic macromolecules in kerogens. As shown in Fig. 2, the distributions of the *n*-alkanes in the pyrolysates are very similar for samples 4 and 6–8, with a maximum at $C_{13}$, and for samples 3 and 5, with a maximum at $C_9$, respectively. The distribution for sample 10 is different, showing a distribution up to $C_{35}$. Such long-chain *n*-alkanes are usually found only in the pyrolysates of lacustrine kerogens and of some algaenans derived from freshwater algae (e.g. Largeau *et al.*, 1986). It should be noted that desulphurization of sample 10 (and 9) by $Li/EtNH_2$ treatment released HMW *n*-alkanes up to $C_{38}$ maximizing at $C_{30}$ indicating that the products originate from sulphur-bound lipids which have an unknown source (Schaeffer *et al.*, 1995). In support of this, the desulphurized kerogen (treated with $CrCl_2$ and $Li/EtNH_2$) of sample 10 does not release such long-chain *n*-alkanes, upon flash pyrolysis (Gelin *et al.*, in preparation). Desulphurization of the other kerogens also released large amounts of *n*-alkanes (Schaeffer *et al.*, 1995). Desulphurization experiments thus show that a significant part of the linear hydrocarbons in pyrolysates can result from the cleavage of sulphur-containing bonds. These findings are supported by quantitative pyrolyses of the desulphurized kerogens 1–10, which revealed a decrease in the quantity of thermally released *n*-alk-1-enes and *n*-alkanes with respect to the non-treated kerogens (Gelin *et al.*, 1995a). Therefore, the abundance of the alkene/alkane series in the pyrolysates of all the samples is probably due to the presence of selectively preserved algaenans, although a fraction of these hydrocarbons might derive from diagenetically incorporated lipids.

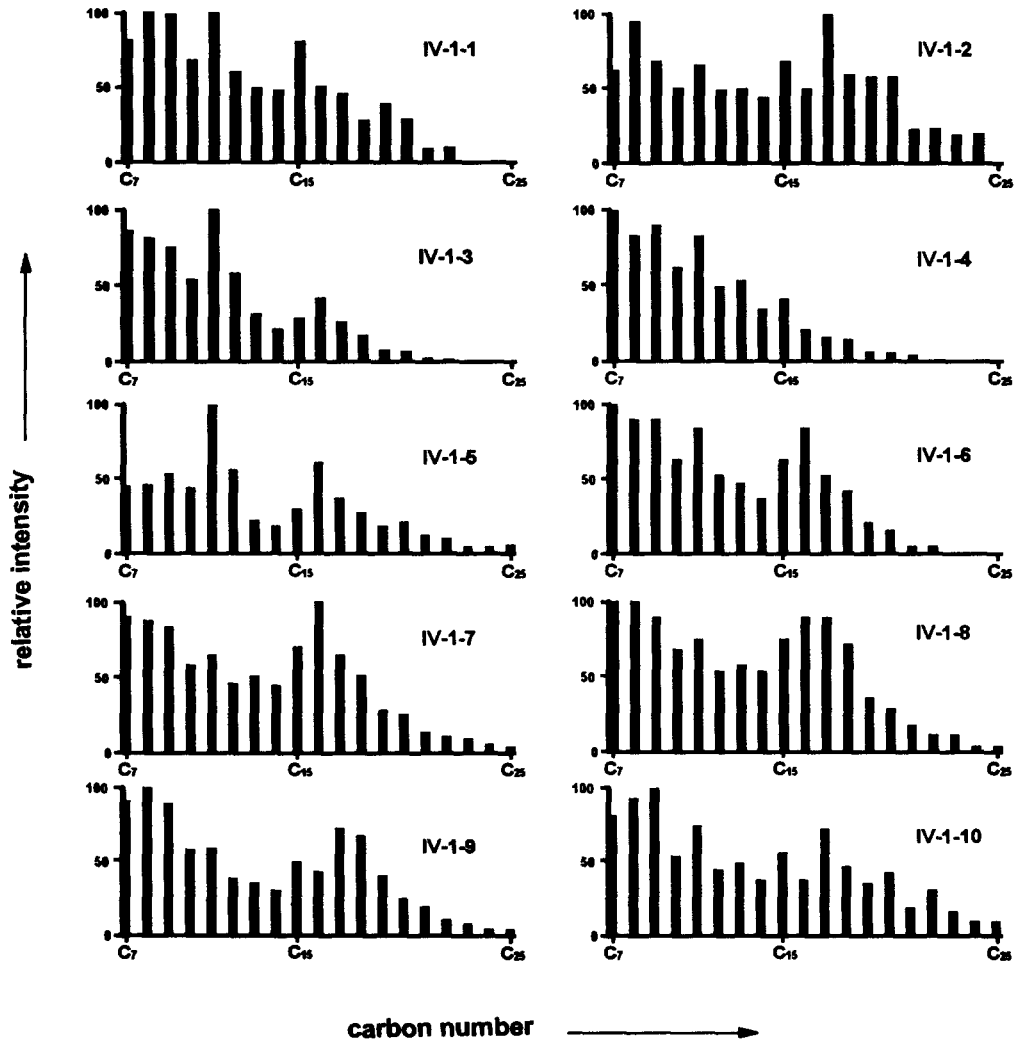


Fig. 3. Distribution of *n*-alkan-2-ones, determined from mass chromatograms (*m*/*z* 58) of the pyrolysates of kerogens 1–8 and 10.

Alkan-2-ones are commonly found in pyrolysates of algaenans and of kerogens known to be composed primarily of algaenans (e.g. Largeau *et al.*, 1986). Pyrolytic studies have shown that ketones can be formed by homolytic cleavage of ether bonds (Gelin *et al.*, 1993, 1994), which participate in the macromolecular network of many algaenans (Gatellier *et al.*, 1993; Derenne *et al.*, 1989; Gelin *et al.*, 1995b). Figure 3 shows the distributions of the alkan-2-ones in the pyrolysates, which slightly differ, although the distributions for samples 3 and 5 are almost identical.

Aromatic components are abundant products and show a dominance of the short-chain alkylated benzenes. The summed mass chromatograms of $m/z$ 78 + 91 + 92 + 105 + 106 + 119 + 120 + 133 + 134, revealing the distribution of benzene and $C_1$–$C_4$ alkylated benzenes, differ mainly with respect to the relative abundance of 1,2,3,4-tetramethylbenzene (TMB). This is shown in Fig. 4 for three representative samples (2, 5 and 10). The presence of TMB in pyrolysates is attributed to pyrolysis of macromolecularly bound aromatic carotenoids (Hartgers *et al.*, 1991; Douglas *et al.*, 1991; Requejo *et al.*, 1992). Hartgers *et al.* (1994a, b) demonstrated that TMB in the kerogen pyrolysate of the Devonian Duvernay Formation is derived from macromolecularly-bound isorenieratene and an as yet undiscovered diaromatic carotenoid with a 2,3,6- and 3,4,5-methyl substitution pattern (Hartgers *et al.*, 1993). The occurrence of this latter carotenoid seems, however, to be restricted to the Mesozoic and Paleozoic and so may not have contributed to the Vena del Gesso sediments. The absence of its carbon skeleton in the extracts after desulphurization of the polar and kerogen fractions is in line with this idea (Kenig *et al.*, 1995; Schaeffer *et al.*, 1995). Since isorenieratene is a specific carotenoid of photosynthetic green sulphur bacteria (Liaaen-Jensen, 1978), the presence of TMB in the kerogen pyrolysates suggested the presence of green sulphur bacteria in the palaeoenvironment. Further evidence comes from the presence of isorenieratane in the products from desulphurization of the polar and

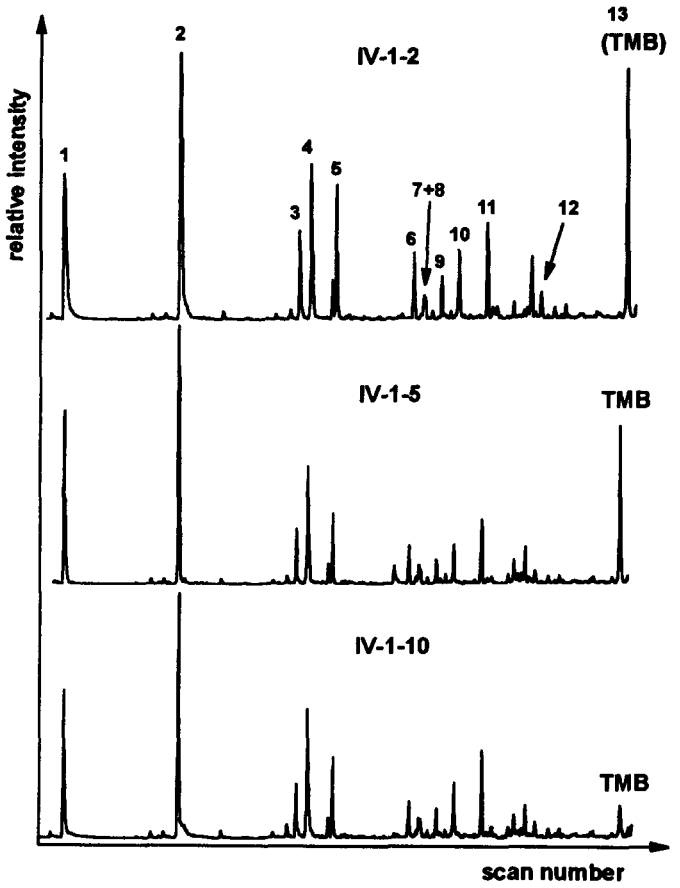


Fig. 4. Partial summed mass chromatograms of $m/z$ 78 + 91 + 92 + 105 + 106 + 119 + 120 + 133 + 134 revealing the distributions of benzene and $C_1$–$C_4$ alkylated benzenes (identified according to Hartgers *et al.*, 1991) in the flash pyrolysates of the indicated kerogens. Key: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = *m*-xylene + *p*-xylene, 5 = *o*-xylene, 6 = propylbenzene, 7 = 1-ethyl-3-methylbenzene, 8 = 1-ethyl-4-methylbenzene, 9 = 1-ethyl-2-methylbenzene, 10 = 1,2,3-trimethylbenzene, 11 = 1,2,4-trimethylbenzene, 12 = butylbenzene, 13 = 1,2,3,4-tetramethylbenzene (TMB).

asphaltene fractions of the extracts (Kenig *et al.*, 1995) and the kerogens (Schaeffer *et al.*, 1995), as well as its $^{13}C$ content. In addition, the intact carotenoid precursor occurs in the extracts (Keely *et al.*, 1995). Since the occurrence of green sulphur bacteria requires the presence of both light and hydrogen sulphide, they indicate photic zone anoxia (cf. Sinninghe Damsté *et al.*, 1993).

Sulphur-containing components are abundant in all the pyrolysates indicating a high abundance of organic sulphur compounds in the kerogens (cf. Eglinton *et al.*, 1990). Series of thiophenes and thiolanes are dominated by compounds with linear carbon skeletons (i.e. 2-alkylthiophenes and 2-alkyl-5-methylthiophenes). It has been shown that incorporation of inorganic sulphur species into aliphatic macromolecules forms thiophenes, thiolanes and benzothiophenes with linear carbon skeletons (Douglas *et al.*, 1991). Thiophenes with a phytanyl carbon skeleton are also abundant and result mainly from the pyrolysis of (poly)sulphur-bound isoprenoid $C_{20}$ compounds derived from phytol (e.g. Sinninghe Damsté *et al.*, 1987; Schouten *et al.*, 1993). Phytenes, the predominant pyrolysis products in the carbon number range $C_{17}$–$C_{25}$ are also likely to derive from such moieties. Pyrolysis of ester-bound phytol produces preferentially phytadienes (van de Meent *et al.*, 1980), which are in low abundance in the pyrolysates, suggesting that most of the phytanyl moieties are probably sulphur-linked to the matrix.

A high abundance of steranes in the desulphurized polar and asphaltene (Kenig *et al.*, 1995) and kerogen (Schaeffer *et al.*, 1995) fractions has been reported, indicating a significant extent of sulphurization of the precursor steroids. It is therefore not surprising to find these compounds in the pyrolysates. The main products were assigned as $C_{27}$ and $C_{29}$ steranes and sterenes. Furthermore, as reported by Kohnen *et al.* (1993), the flash pyrolysate of the polar fraction of another Vena del Gesso marl, the $C_{27}$ sterenes comprise a mixture of cholest-2-enes, cholest-3-enes, cholest-4-ene and cholest-5-ene, which indicates the presence of sulphur-linkages at C-2, C-3, C-4 and C-5 positions of the steranes. The high relative abundance of these pyrolysis products is probably in part the consequence of significant sulphurization of organic matter during early diagenesis. Ester-bound steroid hydrocarbons are also present in the kerogen as revealed by the Li/$EtNH_2$ treatment, which yielded high abundance of sterols (Schaeffer *et al.*, 1995). Therefore, the presence of steroid hydrocarbons in

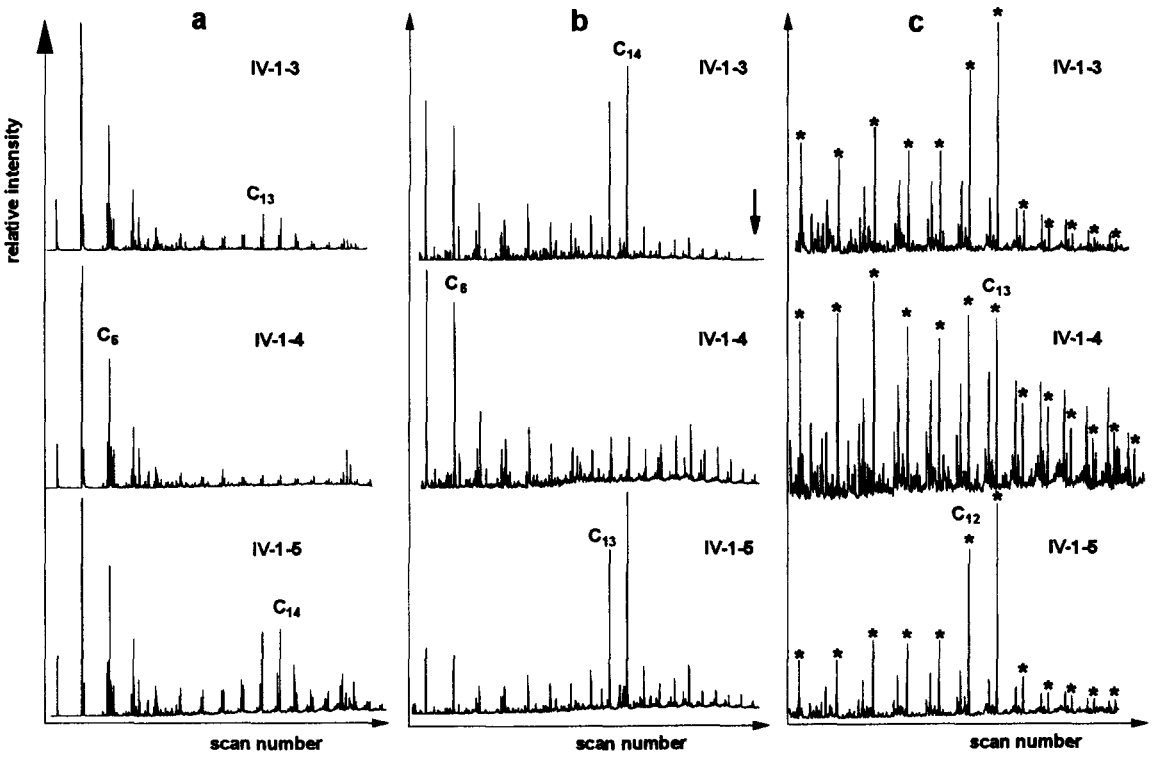


Fig. 5. (a) Partial summed mass chromatograms of $m/z$ 84 + 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140 revealing the distributions of thiophene and alkylated thiophenes in the flash pyrolysates of the indicated kerogens. (b) Partial, accurate mass chromatograms of $m/z$ 87.02 revealing the distributions of the $C_5$–$C_{23}$ thiolanes in the flash pyrolysates of the indicated kerogens. (c) Accurate mass chromatograms of $m/z$ 54.03 revealing the distributions of the $n$-1,3-alkadienes (indicated by the stars) in the flash pyrolysates of the indicated kerogens.

the pyrolysates probably reflects both sulphur- and ester-bound moieties.

Three series of compounds differ significantly from the rest in the pyrolysates of kerogens 3 and 5. In these cases, series of alkylthiophenes, alkylthiolanes and 1,3-alkadienes have distributions with two dominant compounds, the $C_{13}$ and $C_{14}$ *n*-alkylthiolanes and -thiophenes and the $C_{12}$ and $C_{13}$ 1,3-alkadienes [Figs 5(a–c)]. These Figs display only the mass chromatograms of samples 3 and 5 together with sample 4 which generated similar mass chromatograms as the six other kerogen pyrolysates. It is also noteworthy that for the three series this phenomenon is more pronounced in sample 5 than sample 3, revealing that these pyrolysis products are certainly correlated with each other.

*Quantitative pyrolysis*

The concentrations of the pyrolysis products were normalized to total organic carbon (TOC) content of the kerogens. The TOC values range from 10.1% for sample 4 to 27.7% for sample 8. The relatively low content in organic carbon is probably due to the high abundance of inorganic sulphur species occurring as pyrite and gypsum. Indeed, removal of pyrite using $CrCl_2$ from the HF/HCl-treated sediments increased significantly the TOC contents (Schaeffer *et al.*, 1995).

Figure 6(a) shows the concentrations of all the selected pyrolysis products, which comprise *n*-alkanes, *n*-alk-1-enes, benzene and $C_7$–$C_{10}$ alkylated benzenes, thiophene and $C_5$–$C_9$ alkylated thiophenes, $C_{27}$ hopene, $C_{27} + C_{29}$ steranes and sterenes, prist-1-ene, naphthalene and $C_{11}$–$C_{12}$ alkylated napthalenes, benzothiophene and $C_9$–$C_{10}$ benzothiophenes, phytenes, thiophenes with a phytanyl carbon skeleton, *n*-alkanoic acids and *n*-alkan-2-ones. It is remarkable that flash pyrolysis of the kerogens of samples 3 and 5 gave a much higher yield (more than 300 mg/g TOC for the selected products only) than the pyrolysates of the kerogens isolated from the other sediments of this marl (from 32 mg/g TOC for samples 4 and 10 to 167 mg/g TOC for sample 1).

As expected, the series of *n*-alkanes, *n*-alk-1-enes, and the alkylated benzenes and thiophenes, which represent the bulk of the pyrolysates, follow a trend similar to the total yield [cf. Figs 6(b) and 6(c) with 6(a)]. It is also not surprising that the depth profile of the *n*-alkanones [Fig. 6(d)] also follows that of the *n*-alkanes and *n*-alkenes, since these three series are believed to derive from pyrolysis of algal aliphatic macromolecules (Largeau *et al.*, 1986). The depth profile of prist-1-ene, which originates from the pyrolysis of bound tocopherol units (Goossens *et al.*, 1984), is also similar to the previous ones [Fig. 6(e)]. It is surprising, however, that the TMB depth profile [Fig. 6(f)] also follows the same trend, since the source of this compound is green sulphur bacteria and not algae. Indeed only three classes of pyrolysis products do not follow the *n*-alkane trend; these are thiophenes with a phytanyl skeleton, phytenes and sterenes [Figs 6(g,h)]. These three classes are all generated, at least in part, from S-bound moieties in the kerogens. Phytenes and sterenes are probably formed from a direct elimination of a (poly)sulphide linkage. However, sterenes may to some extent also derive from a direct elimination of ester-bound sterols (Schaeffer *et al.*, 1995). Thiophenes with a phytanyl skeleton are probably formed by pyrolysis of polysulphide-bound phytanyl moieties (Koopmans *et al.*, 1995). In contrast, *n*-alkanes and *n*-alk-1-enes derive from the pyrolytic cleavage of long polymethylenic chain as a result of a radical-chain mechanism pathway (see Poutsma, 1990 for a review).

The difference between mechanisms for the formation of phytenes, sterenes and isoprenoid thiophenes on the one hand and the rest of the pyrolysis products on the other hand could explain the different yields. Indeed, the free radical pathway which dominates in the thermal dissociation of C–C bonds leads to the formation of very unstable radicals which will "catalyse" the pyrolysis of the rest of the organic matrix by their need to stabilize by hydrogen transfer. This phenomenon could explain why the yield of pyrolysis products from different sources (e.g. alkanes vs TMB) and structures (e.g. benzenes vs thiophenes) can follow a similar trend. The depth profile of the phytenes, isoprenoid thiophenes and sterenes, which are probably formed by elimination independent from the free radical pathway is therefore different from the main trend.

The comparison of Figs 6(f) and 3 clearly demonstrates the necessity to quantify the kerogen pyrolysates. The absolute concentration of TMB in the pyrolysate of sample 5 is much higher than that in sample 2 whilst the relative intensity of TMB is higher in the mass chromatogram of sample 2 (Fig. 3). However, a stronger contribution of TMB to the pyrolysates does not necessarily indicate a higher abundance of aromatic carotenoid-derived moieties since the concentration of TMB depends on the degree of sulphurization of organic matter and on the degree of catalysis during pyrolysis (see above). The relatively high contribution of TMB in most of the pyrolysates can only be interpreted as an indication for photic zone anoxia but does not tell about the importance of the latter. However, the absence of TMB in the pyrolysate of the kerogen from the top layer of this marl (sample 10) confirms the absence of photic zone anoxia during deposition of that sediment as also revealed by studies of the extracts (Kenig *et al.*, 1995; Keely *et al.*, 1995) and the kerogens (Schaeffer *et al.*, 1995).

*Origin and structure of the main component of kerogen from samples 3 and 5*

As reported by Kenig *et al.* (1995), $\delta^{13}C_{TOC}$ values for samples 3 and 5 are more enriched than those of other samples, These excursions in $\delta$-values are followed by $C_{16}$, $C_{31}$ and $C_{32}$ *n*-alkanes released by desulphurization of the polar fractions of the extracts

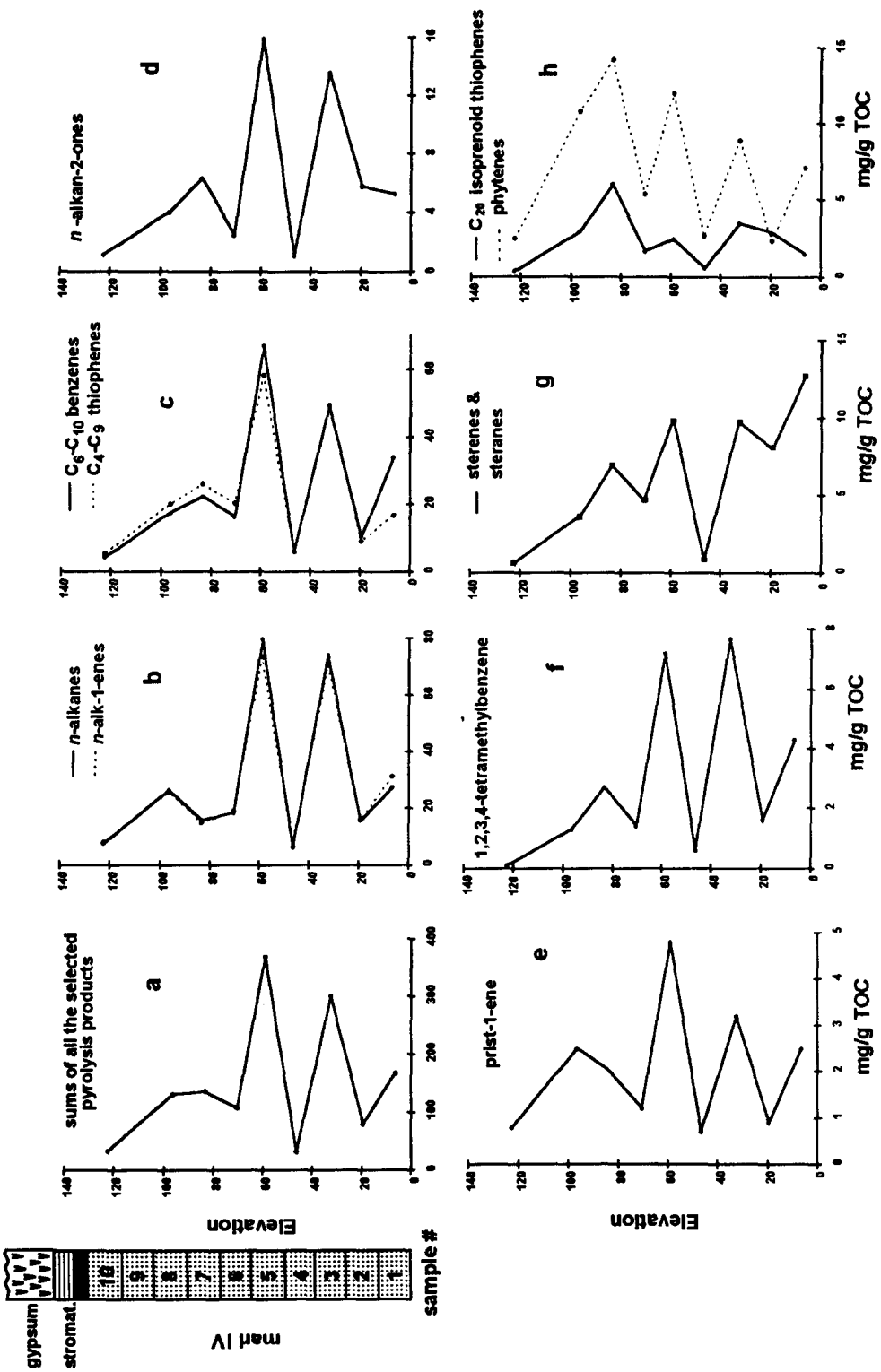


Fig. 6. Depth profiles of the concentration of products in the flash pyrolysates. (a) Sum of all selected (main) pyrolysis products, (b) *n*-alkanes and *n*-alk-1-enes, (c) $C_0$–$C_4$ alkylated benzenes and $C_0$–$C_5$ alkylated thiophenes, (d) *n*-alkan-2-ones, (e) pris-1-ene, (f) 1,2,3,4-tetramethylbenzene, (g) $C_{27} + C_{29}$ steranes and sterenes, (h) phytenes and thiophenes with a phytanyl skeleton. A lithological column is indicated for reference.

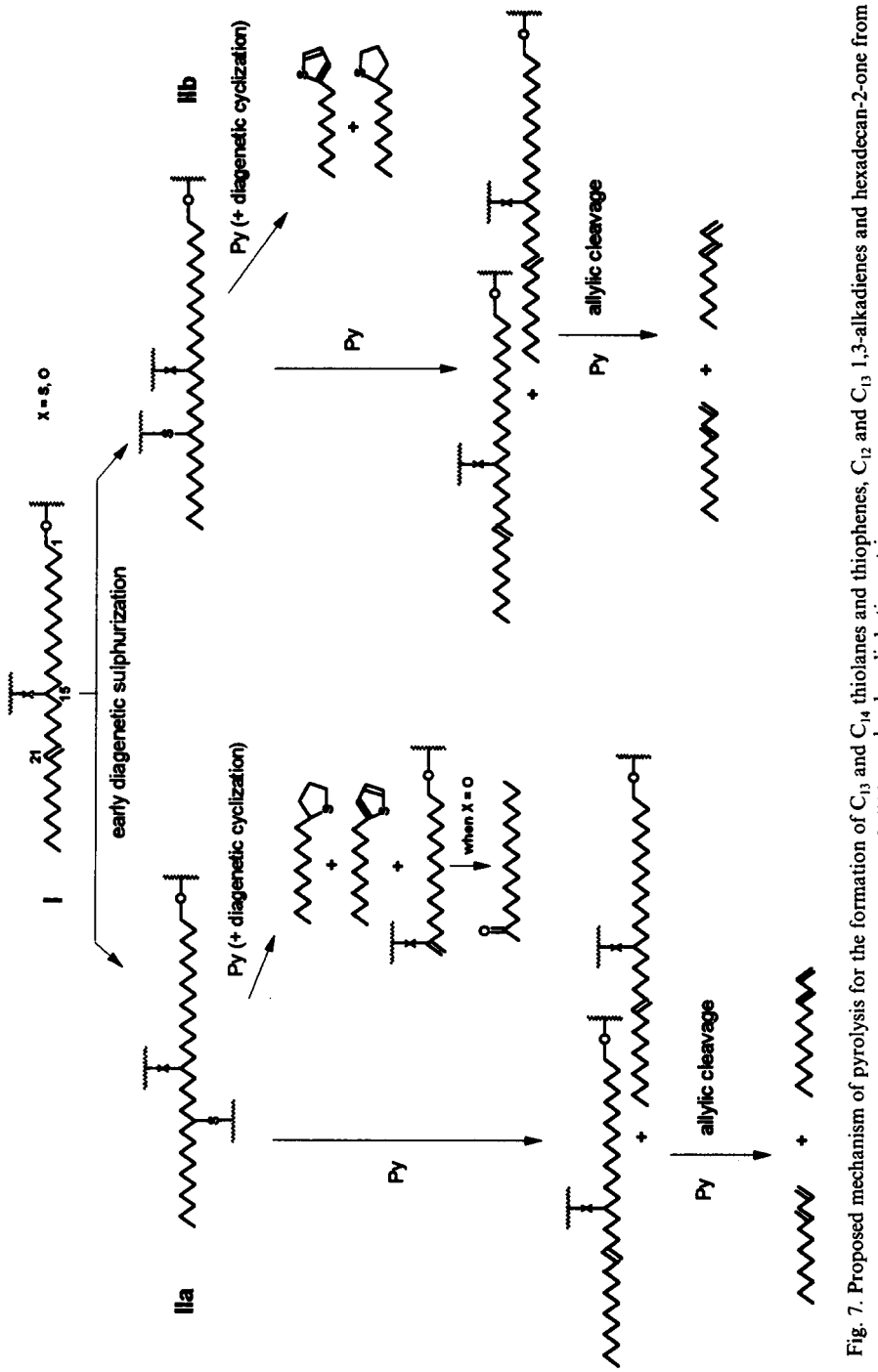


Fig. 7. Proposed mechanism of pyrolysis for the formation of $C_{13}$ and $C_{14}$ thiolanes and thiophenes, $C_{12}$ and $C_{13}$ 1,3-alkadienes and hexadecan-2-one from a macromolecular aliphatic matrix.

(Kenig *et al.*, 1995), indicating that organisms which are the precursor for these S-bound alkanes are probably also major contributors of kerogen in sample 3 and 5. Koopmans *et al.* (1995), in artificial maturation experiments with the same marl, showed the presence of long-chain thiophenes with the sulphur atom predominantly attached at position 15 of the linear carbon skeleton and attributed this to sulphurization of $C_{30}$–$C_{32}$ 1,15-alkyl diols and alkyl-1-5-one-1-ols or their diagenetic derivatives. It is well documented that saturated and mono-unsaturated $C_{30}$–$C_{32}$ 1,15-alkyl diols are present as bound lipids in marine microalgae of the class Eustigmatophyceae (Volkman *et al.*, 1992; Gelin *et al.*, 1995b). These diols and $C_{28}$–$C_{32}$ alkan-15-one-1-ols were found in many recent marine sediments (e.g. Leeuw *et al.*, 1981; Morris and Brassell, 1988). Furthermore, it was recently demonstrated that the marine eustigmatophyte *Nannochloropsis salina* biosynthesizes highly aliphatic algaenans probably composed of ether-linked $C_{30}$–$C_{32}$ units (Gelin *et al.*, 1995b). Although fossil eustigmatophytes are still unknown, Hibberd (1979) suggested that this group of algae might resemble a common ancestor of other important groups of algae, i.e. Xanthophyta and Chrysophyta. These findings suggest that a significant part of the kerogens of samples 3 and 5 derives from a similar origin which may be algaenan derived from Eustigmatophyceae microalgae or is formed by sulphurization of diols or keto-ols from Eustigmatophyceae.

The results obtained by flash pyrolysis of the kerogens strongly support this hypothesis. The kerogens from samples 3 and 5 are completely different from the kerogen of the other samples because of (i) their much higher pyrolysis yields and (ii) the characteristic distribution of the *n*-alkanes, alkan-2-ones, alkylated thiophenes, alkylated thiolanes and 1,3-alkadienes (Figs 2, 4 and 5(a–c), respectively). Both observations can be linked to the presence of algaenan derived from eustigmatophytes or sulphurized diols or keto-ols from these algae. This conclusion can be corroborated by proposing a schematic mechanism that shows the formation of 1,3-dodecadiene, 1-3-tridecadiene, 2-nonylthiolane, 2-decylthiolane, 2-nonylthiophene and 2-decylthiophene and hexadecan-2-one, as significant products in their respective compound classes, from the pyrolysis of geomacromolecules derived from unsaturated $C_{30}$ alkyl-1,15-diol or alkyl-15one-1-ol precursors (Fig. 7). Structure **I**, a hypothetical macromolecular entity based on the above considerations, could be diagenetically altered at its point of unsaturation, forming structure **IIa** with a S-bond at C-20 structure **IIb** with a S-bond at C-21. During early diagenesis and upon pyrolysis, structures **IIa** and **IIb** could cyclize and aromatize with a C–C cleavage between positions 16 and 17 for **IIa** and positions 17 and 18 for **IIb**. As shown in Fig. 7, these transformations could lead to the formation of 2-decylthiolane, 2-decylthiophene and also hexadecan-2-one on the one hand and of 2-nonylthiolane and 2-nonylthiophene on the other hand. Elimination of the S-bound moieties of **IIa** and **IIb** could create unsaturations at three different positions which could lead, after allylic cleavages and hydrogen transfer, to the formation of $C_{11}$, $C_{12}$ and $C_{13}$ *n*-1,3-alkadienes. However, the absence of abundant $C_{11}$ 1,3-alkadienes in the pyrolysates of samples 3 and 5 is not explained with this mechanism.

## CONCLUSIONS

(1) Quantitative analytical pyrolysis allowed for the determination of a large part of the insoluble organic matter (up to *ca* 400 mg/g TOC). This method revealed that kerogens of samples 3 and 5 produced a much higher yield upon pyrolysis than the other samples. This also holds for the major pyrolysis products (*n*-alkanes, *n*-alk-1-enes, alkylbenzenes and alkylthiophenes).

(2) The depth profile of three series of pyrolysis products (phytenes, sterenes and thiophenes with a phytanyl skeleton) do not follow the general trend described above. A different pyrolysis mechanism may be responsible for this difference since the formation of these three series of pyrolysis products is probably due to elimination of (poly)sulphide or ester bonds whilst the major pyrolysis products involve C–C cleavage via a free radical pathway.

(3) Flash pyrolysis revealed that the major contributors to these kerogens were biomacromolecules from microalgae. This contribution is much higher for two samples in the bottom middle of the marl bed (samples 3 and 5) as revealed by the $\delta^{13}C_{roc}$ values, the yields of pyrolysis and the very specific distribution patterns for 2-alkylthiophenes and -thiolanes, methylketones and 1,3-alkadienes. These specific distributions are thought to be related to the presence of an algaenan with 1,15-diols as major building blocks derived from algae of the class Eustigmatophyceae or/and incorporation of 1,15-diols or keto-ols in the kerogen through natural sulphurization.

(4) 1,2,3,4-Tetramethylbenzene, which is the pyrolysis product revealing the contribution of photosynthetic sulphur bacteria to the OM, is abundantly present in pyrolysates of samples 1–8. This confirms the occurrence of photic zone anoxia during the almost entire marl deposition excepting of as shown by investigations on desulphurized extracts (Kenig *et al.*, 1995; Keely *et al.*, 1995) and kerogens (Schaeffer *et al.*, 1995).

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## REFERENCES

Derenne S., Largeau C., Casadevall E. and Berkaloff C. (1989) Occurrence of a resistant biopolymer in the L race of *Botryococcus braunii*. *Phytochemistry* **28,** 1137–1142.

Derenne S., Largeau C., Berkaloff C., Rousseau B., Wilhelm C. and Hatcher P. G. (1992) Non-hydrolysable macromolecular constituents from outer walls of *Chlorella fusca* and *Nanochlorum eucaryotum*. *Phytochemistry* **31,** 1923–1929.

Douglas A. G., Sinninghe Damsté J. S., Fowler M. G., Eglinton T. I. and de Leeuw J. W. (1991) Unique distributions of hydrocarbons and sulphur compounds released by flash pyrolysis from the fossilised alga *Gloeocapsomorpha prisca*, a major constituent in one of four Ordovician kerogens. *Geochim. Cosmochim. Acta* **55,** 1–17.

Eglinton T. I. (1994) Carbon isotopic evidence for the origin of macromolecular aliphatic structures in kerogen *Org. Geochem.* **21,** 721–735.

Eglinton T. I., Sinninghe Damsté J. S., Kohnen M. E. L. and de Leeuw J. W. (1990) Rapid estimation of the organic sulphur content of kerogens, coals and asphaltenes by pyrolysis-gas chromatography. *Fuel* **69,** 1394–1404.

Gatellier J.-P. L. A., de Leeuw J. W., Sinninghe Damsté J. S., Derenne S., Largeau C. and Metzger P. (1993) Very early diagenesis of a resistant cell wall biomacromolecule of the green alga *Botryococcus braunii* (Race A) as revealed by spectroscopic and pyrolysis investigations of a Coorongite. *Geochim. Cosmochim. Acta* **57,** 2053–2068.

Gelin F., Gatellier J.-P. L. A., Sinninghe Damsté J. S., Derenne S., Largeau C., Metzger P. and de Leeuw J. W. (1993) Mechanisms of flash pyrolysis of ether lipids isolated from the green microalga *Botryococcus braunii*. *J. Anal. Appl. Pyrol.* **27,** 155–168.

Gelin F., Sinninghe Damsté J. S., Derenne S., Largeau C., Metzger P. and de Leeuw J. W. (1994) Scope and limitations of flash pyrolysis–gas chromatography/mass spectrometry as revealed by the thermal behaviour of high-molecular-weight lipids derived from the green microalga *Botryococcus braunii*. *J. Anal. Appl. Pyrol.* **28,** 183–204.

Gelin F., Sinninghe Damsté J. S., Harrison W. N., Maxwell J. R. and de Leeuw J. W. (1995a) Variations in origin and composition of kerogen constituents as revealed by analytical pyrolysis of immature kerogens before and after desulphurization. In *Proc. 17th Int. Meeting on organic geochemistry*. In press.

Gelin F., Boogers I., Noordeloos A. A. M., Sinninghe Damsté J. S., Hatcher P. G. and de Leeuw J. W. (1995b) Novel resistant microalgal polyethers: a major sink of organic carbon in the marine environment. *Nature* Submitted.

Goossens H., de Leeuw J. W., Schenck P. A. and Brassell S. C. (1984) Tocopherols as likely precursors of pristane in ancient sediments and crude oils. *Nature* **312,** 440–442.

Goth K., de Leeuw J. W., Püttmann W. and Tegelaar E. W. (1988) Origin of Messel Oil Shale Kerogen. *Nature* **336,** 759–761.

Hartgers W. A., Sinninghe Damsté J. S. and de Leeuw J. W. (1991) Mechanisms of hydrocarbon formation during flash pyrolysis of kerogen. *Prep. Am. Chem. Soc.—Div. Fuel Chem.* **36,** 790–795.

Hartgers W. A., Sinninghe Damsté J. S., Koopmans M. P. and de Leeuw J. W. (1993) Sedimentary evidence for a diaromatic carotenoid with an unprecedented aromatic substitution pattern. *J. Chem. Soc., Chem. Commun.* **23,** 1715–1716.

Hartgers W. A., Sinninghe Damsté J. S., Requejo A. G., Allan J., Hayes J. M., Ling Y., Xie T.-M., Primack J. and de Leeuw J. W. (1994a) A molecular and carbon isotopic study towards the origin and diagenetic fate of diaromatic carotenoids. In *Advances in Organic Geochemistry* 1993 (Edited by Telnoes N., van Graas G. and Øygard K.). *Org. Geochem.* **22,** 703–725. Elsevier Science, Oxford.

Hartgers W. A., Sinninghe Damsté J. S., Requejo A. G., Allan J., Hayes J. M. and de Leeuw J. W. (1994b) Evidence for only minor contributions from bacteria to sedimentary organic carbon. *Nature* **369,** 224–227.

Hibberd D. J. (1979) The structure and phylogenetic significance of the flagellar transition region in the chlorophyll c-containing algae. *BioSystems* **11,** 243–261.

Keely B. J., Blake S. R. and Maxwell J. R. (1995) Distributions of pigments in the organic matter of marls from the Vena del Gesso evaporitic sequence. (Vena del Gesso, N. Italy). *Org. Geochem.* **23,** 527–539.

Kenig F., Sinninghe Damsté J. S., Frewin N., Hayes J. M. and de Leeuw J. W. (1995) Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy). II. Stratigraphic variations in abundances and $^{13}C$ contents of free and sulphur-bound skeletons in a single marl bed. *Org. Geochem.* **23,** 485–526.

Kohnen M. E. L., Sinninghe Damsté J. S., Bass M., Kock-van Dalen A. C. and de Leeuw J. W. (1993) Sulphur-bound steroid and phytane carbon skeletons in geomacromolecules: Implications for mechanism of incorporation of sulphur into organic matter. *Geochim. Cosmochim. Acta* **57,** 2515–2528.

Koopmans M. P., Sinninghe Damsté J. S., Lewan M. D. and de Leeuw J. W. (1995) Thermal stability of thiophene biomarkers as studied by hydrous pyrolysis. *Org. Geochem.* **23,** 583–596.

Largeau C., Casadevall E., Kadouri A. and Metzger P. (1984) Formation of Botryococcus-derived kerogens. Comparative study of immature torbanites and of the extent alga *Botryococcus braunii*. In *Advances in Organic Geochemistry* 1983 (Edited by Schenck P. A., de Leeuw J. W. and Lijmbach G. M. M.). *Org. Geochem.* **6,** 327–332.

Largeau C., Derenne S., Casadevall E., Kadouri A. and Sellier N. (1986) Pyrolysis of immature torbanite and of the resistant biopolymer (PRB A) isolated from extant alga *Botryococcus braunii*. Mechanisms for the formation and structure of torbanite. In *Advances in Organic Geochemistry* 1985 (Edited by Leythaeuser D. and Rullkötter J.). *Org. Geochem.* **10,** 1023–1032.

Larter S. R. (1984) Application of analytical pyrolysis techniques to kerogen characterisation and fossil fuel exploration/exploitation. In *Analytical Pyrolysis* (Edited by Voorhees K. J.), pp. 212–275. Butterworth, London.

Lattimer R. P. (1995) Pyrolysis field ionisation mass spectrometry (Py–FI–MS) of polyolefins. *J. Anal. Appl. Pyrol.* **31,** 203–226.

de Leeuw J. W., Rijpstra W. I. C. and Schenck P. A. (1981) The occurrence and identifications of $C_{30}$, $C_{31}$ and $C_{32}$

alkan-1,15-diols and alkan-15-one-1-ols in Unit I and Unit II Black Sea sediments. *Geochim. Cosmochim. Acta* **45,** 2281–2285.

de Leeuw J. W. and Largeau C. (1994) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In *Organic Geochemistry, Principles and Applications* (Edited by M. H. Engel and S. A. Macko), pp. 23–72. Plenum Press, New York.

Liaaen-Jensen S. (1978) Chemistry of Carotenoid Pigments. In *Photosynthetic Bacteria* (Edited by Clayton R. K. and Sistrom W. R.), pp. 233–248. Plenum Press, New York.

van de Meent D., Brown S. C., Philip R. P. and Simoneit B. R. T. (1980) Pyrolysis–high resolution gas chromatography and pyrolysis gas chromatography–mass spectrometry of kerogens and kerogen precursors. *Geochim. Cosmochim. Acta* **44,** 999–1013.

Morris R. J. and Brassell S. C. (1988) Long-chain alkanediols: biological markers for cyanobacterial contributions to sediments. *Lipids* **23,** 256–258.

Nip M., de Leeuw J. W. and Schenck P. A. (1988) The characterisation of eight maceral concentrates by means of Curie-point pyrolysis gas chromatography and Curie-point pyrolysis gas chromatography–mass spectrometry. *Geochim. Cosmochim. Acta* **52,** 637–648.

Poutsma M. L. (1990) Free-radical thermolysis and hydrogenolysis of model hydrocarbons relevant to processing of coal. *Energy and Fuel* **4,** 113–131.

Requejo A. G., Allan J., Creaney S., Gray N. R. and Cole K. S. (1992) Aryl isoprenoids and diaromatic carotenoids in paleozoic source rocks and oils from the western Canada and Williston basins. *Org. Geochem.* **19,** 245–264.

Schaeffer P., Harrison W. H., Keely B. J. and Maxwell J. R. (1995) Product distributions from chemical degradations of kerogens from a marl from a Miocene evaporitic sequence (Vena del Gesso, N. Italy) *Org. Geochem.* **23,** 541–554.

Schouten S., van Driel G. B., Sinninghe Damsté J. S. and de Leeuw J. W. (1993) Natural sulphurization of ketones and aldehydes: a key reaction in the formation of organic sulphur compounds. *Geochim. Cosmochim. Acta* **57,** 5111–5116.

Sinninghe Damsté J. S., kock-van-Dalen A. C., de Leeuw J. W. and Schenck P. A. (1987) The identification of 2,3-dimethyl-5-(2,6,19-trimethylundecyl)thiophene, a novel sulphur-containing biological marker. *Tetrahedron lett.* **28,** 957–960.

Sinninghe Damsté J. S., Wakeham S. G., Kohnen M. E. L., Hayes J. M. and de Leeuw J. W. (1993) A 6000-year sedimentary molecular record of chemocline excursions in the Black Sea. *Nature* **362,** 827–829.

Sinninghe Damsté J. S., Frewin N., Kenig F., Miggins D. and de Leeuw J. W. (1995) Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy). I. General cyclic variations in extractable organic matter of eleven marl beds. *Org. Geochem.* **23,** 471–483.

Vai G. B. and Ricci Lucchi F. (1977) Algal crust, autochtonous and clastic gypsum in a cannibalistic evaporite basin: a case history from the Messinian of Northern Apennines. *Sedimentology* **24,** 211–244.

Verardo D. J., Froelich P. N. and McIntyre A. (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyser. *Deep-Sea Res.* **37,** 157–165.

Volkman J. K, Barrett S. M., Dunstan G. A. and Jeffrey S. W. (1992) $C_{30}$–$C_{32}$ alkyl diols and unsaturated alcohols in microalgae of the class Eustigmatophyceae. *Org. Geochem.* **18,** 131–138.