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The combined application of organic sulphur and isotope geochemistry to assess multiple sources of palaeobiochemicals with identical carbon skeletons*

MATH E. L. KOHNEN,[1]† STEFAN SCHOUTEN,[1] JAAP S. SINNINGHE DAMSTÉ,[1]
JAN W. DE LEEUW,[1] DAWN MERRIT[2] and J. M. HAYES[2]

[1]Organic Geochemistry Unit, Faculty of Chemical Technology and Materials Science, Delft University of Technology, De Vries van Heystplantsoen 2, 2628 RZ Delft, The Netherlands and [2]Biogeochemical Laboratories, Department of Chemistry and of Geology, Geology Building, Indiana University, Bloomington, IN 47405, U.S.A.

Abstract—Five immature sediments from a Messinian evaporitic basin, representing one evaporitic cycle, were studied using molecular organic sulphur and isotope geochemistry. It is shown that a specific carbon skeleton which is present in different "modes of occurrence" ("free" hydrocarbon, alkylthiophene, alkylthiolane, alkyldithiane, alkylthiane, and sulphur-bound in macromolecules) may have different biosynthetic precursors which are possibly derived from different biota. It is demonstrated that the mode of occurrence and the carbon isotopic composition of a sedimentary lipid can be used to "reconstruct" its biochemical precursor. This novel approach of recognition of the suite of palaeobiochemicals present during the time of deposition allows for identification of the biological sources with an unprecedented specificity.

Key words—organic sulphur compounds, thiophenes, thiolanes, 1,2-dithianes, biomarkers, carbon-13, palaeoenvironmental reconstruction, palaeobiochemicals, evaporites

INTRODUCTION

One of the most challenging tasks in organic geochemistry is the detailed reconstruction of palaeoenvironments by establishment of unambiguous relationships between geolipids and precursor biolipids biosynthesised by the suite of organisms present at the time of deposition of the sediment. In principle, the high structural specificity of biochemicals warrants a direct link to their biological sources, although the recognition of such relationships is hampered to some extent by the limited knowledge of lipid compositions of organisms and by the fact that many organisms which contributed to the organic matter in ancient sediments are extinct. The specificity of biochemicals is due to three features; (i) the carbon skeleton, (ii) the nature and position of functional group(s), (iii) the stable carbon isotopic composition. The size and nature of the carbon skeleton, including its stereochemical features, in combination with the type(s) and position(s) of functional group(s) are dictated by its enzyme-controlled biosynthesis. Within each group of organisms there are common features in the biochemistry as well as some major differences, although sometimes a single species may contain an unusual lipid distribution. In general, a group of taxonomically-related species may contain an unusual lipid distribution. In general, a group of taxonomically-related organisms has distinctive and diagnostic lipid compositions (Volkman, 1988). The stable carbon isotopic compositions of biochemicals are determined by the isotopic composition of the carbon source assimiliated by the organism and the biosynthetic pathways by which they are synthesised (Hayes *et al.*, 1990).

Establishing the relationship between a geolipid and its corresponding biolipid is, however, difficult in many cases since microbially mediated and chemical transformation processes, occurring in both the sediment and the water column, can induce changes in biolipids, i.e. loss of functional groups and modification of the basic carbon skeleton. On the other hand, isotopic compositions of geolipids are likely to be close to those of their precursor biochemicals since isotopic fractionations during these processes are considered to be small since the chemical reactions occur at specific sites within the biolipids. Isotopic abundances at those sites may shift as reactions occur, but other portions of the molecule will be unaffected and their isotopic constancy will buffer the effects of isotopic shifts at the reaction sites (Hayes *et al.*, 1990).

Apart from defunctionalisation or mineralisation reactions, functionalised biolipids can also undergo reactions with reduced inorganic sulphur species

*Delft Organic Geochemistry Unit Contribution 262.
†Present address: Koninklijke/Shell Exploratie en Produktie Laboratorium, Volmerlaan 6, 2288 GD Rijswijk, The Netherlands.

(H_2S and HS_x^-), leading to formation of organic sulphur compounds (OSC) and sulphur-bound lipid moieties during very early stages of diagenesis (for a review see Sinninghe Damsté and de Leeuw, 1990). These sulphurised biolipids are more resistant towards microbial attack than their original counterparts, resulting in a better preservation of the lipid substrate (i.e. basic carbon skeleton). Furthermore, it has also been demonstrated that these sulphurised lipids provide clues about the number and positions of functional groups in the precursor biolipids (Kohnen *et al.*, 1990; Sinninghe Damsté *et al.*, 1990a). The position of C–S linkages in the carbon skeletons of sulphurised lipids are related to the positions of the original functionalities (Kohnen *et al.*, 1991a). Hence, by studying OSC and sulphur-bound lipids in macromolecules in addition to the "classical" hydrocarbon biomarkers, a more complete reconstruction of the suite of palaeobiochemicals present in the environment of deposition is obtained.

Here we report on some differences in the palaeoenvironments of five immature sediments from the Messinian Vena del Gesso basin (Italy) based on hydrocarbon, and on organic sulphur and isotope geochemistry used in a combined fashion. It is demonstrated that a particular carbon skeleton can have different biosynthetic precursors which are, in a number of cases, derived from different biological sources. These findings indicate that the commonly used approach in "classical" hydrocarbon biomarker geochemistry, in which precursor–product relationships are established on the basis of carbon skeletons, may lead to erroneous conclusions with respect to the biological community and hence the conditions in the palaeoenvironment.

EXPERIMENTAL

Samples and geological setting

The samples are from the Vena del Gesso basin in the Northern Apennines (Italy) and were taken from fresh outcrops. The geological setting of this Messinian (Upper Miocene) basin is described in detail by Vai and Ricci Luchi (1977). In brief, this evaporitic basin is filled with thick (35 m) beds of coarse crystalline gypsum associated with thinner carbonate and shaly (euxinic) intercalations. Vai and Ricci Luchi (1977) found a sedimentary sequence comprising six facies which is repeated 14 times. The idealised evaporitic depositional cycle starts with the non-evaporitic bituminous marls and ends with the deposition of gypsum due to evaporitic precipitation. The sample set consists of three subsamples of the bituminous marl [thickness 120 cm; VDG-7A1 (Marl-1), VDG-7A2 (Marl-2) and VDG-7A3 (Marl-3)], a sample from a stromatolitic limestone (thickness 15 cm; VDG-7B), and a sample from the massive gypsum (thickness *ca* 10 m; VDG-7M) from the same evaporitic cycle. The immature character of these sediments is illustrated by the low reflectance (average $R_o = 0.25\%$) of trace amounts of indigenous vitrinite particles in a marl sample. TOC values of the samples analysed are reported in Table 1.

Extraction and fractionation

The freeze-dried samples were powdered in a rotary disc mill and Soxhlet extracted with methanol/CH_2Cl_2 (1:7.5, v/v) for 40 h in a nitrogen atmosphere. The extract was obtained by removing the solvent with a rotary evaporator at 30°C. Subsequently, the extract was taken up in a small amount of CH_2Cl_2 and distilled water was added to remove salts. The organic matter was re-extracted from this mixture by extracting it four times with CH_2Cl_2. The pooled CH_2Cl_2 layers were dried with anhydrous Na_2SO_4 and evaporated to dryness (for yields, see Table 1). Separation of the asphaltenes from the maltenes was achieved by dissolving the extract in a minimum volume of CH_2Cl_2 and subsequently adding a 40-fold excess of *n*-heptane (Fig. 1). After >8 h the asphaltenes flocculated out completely and the precipitate (asphaltenes) and supernatant (maltenes) were collected separately. In order to purify the maltene fraction this precipitation step was repeated once more. To an aliquot (*ca* 200 mg) of the ultimate maltene fraction weighed amounts of four synthetic standards [6,6-D_2-2-methyleicosane, 2,3-dimethyl-5-(1,1-D_2-hexadecyl)thiophene, 2,3-dimethyl-5-(1,1-D_2-hexadecyl)thiolane and 2-methyl-2-(4,8,12-trimethyldecyl)-chroman: Kohnen *et al.*, 1990] were added. Subsequently the maltenes were separated into an apolar and a polar fraction on a column (25 cm × 2 cm; $V_0 = 35$ ml) packed with alumina

Table 1. Geochemical data of the samples studied

Samples*	TOC† (wt%)	EOM‡ (wt%)	Maltene§ (wt%)	Asphal.¶ (wt%)	Apolar‖ (wt%)	Polar** (wt%)
VDG-7A1	1.7	1.3×10^{-1}	8.3×10^{1}	8.4	6.4	5.9×10^{1}
VDG-7A2	1.3	1.2×10^{-1}	7.4×10^{1}	2.3×10^{1}	7.2	6.3×10^{1}
VDG-7A3	1.2	1.1×10^{-1}	7.7×10^{1}	1.8×10^{1}	7.3	6.3×10^{1}
VDG-7B	0.2	8.1×10^{-3}	6.2×10^{1}	1.5×10^{1}	6.4	4.2×10^{1}
VDG-7M	ND††	2.7×10^{-3}	7.3×10^{1}	2.2×10^{1}	9.3	5.6×10^{1}

Key: *VDG-7A1 = Marl-1 of Vena del Gesso evaporitic cycle 7, VDG-7A2 = Marl-2, VDG-7A3 = Marl-3, VDG-7B = stromatolitic limestone, VDG-7M = gypsum; †TOC = Total Organic Matter; ‡EOM = Extractable Organic Matter as percent of whole rock; §weight percent of maltene fraction of EOM; ¶weight percent of asphaltene fraction of EOM; ‖weight percent of apolar fraction of maltene fraction; **weight percent of polar fraction of maltene fraction; ††ND = not determined.

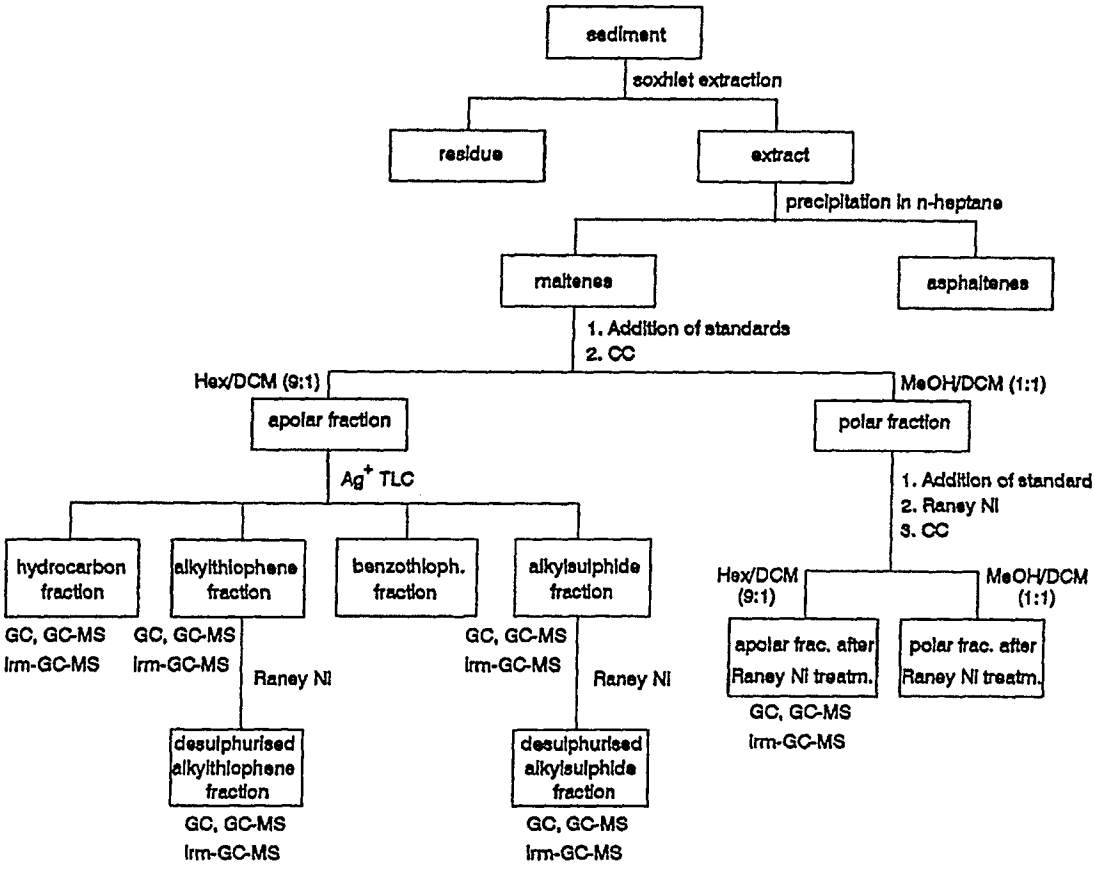

Fig. 1. Analytical flow diagram.

(activated for 2.5 h at 150°C) by elution with 150 ml hexane/CH_2Cl_2 (9:1, v/v) and 150 ml methanol/CH_2Cl_2 (1:1, v/v), respectively. An aliquot of the apolar fraction (*ca* 10 mg) was further separated by argentation thin layer chromatography (Ag^+-TLC; Kohnen *et al.*, 1990). Four bands, R_f 0.8–1.0 (hydrocarbon fraction), R_f 0.6–0.8 (alkylthiophene fraction), R_f 0.1–0.6 and R_f 0.0–0.1 (alkylsulphide fraction) were scraped off the TLC plate, ultrasonically extracted three times with ethyl acetate, and subsequently analysed quantitatively by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) using the internal standards. The fractions isolated from Marl-2 were isotopically analysed by isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC-MS).

In the case of the stromatolitic limestone and the gypsum, the yields of the apolar fractions were too low (Table 1) to perform an Ag^+-TLC separation because of the bad recovery (*ca* 60 wt%) from the $AgNO_3$ impregnated TLC plate. Therefore, a conventional TLC separation (silica plates, 20 × 20 cm, thickness 0.25 mm) using hexane as developer was performed, which yielded three fractions [R_f 0.8–1.0 (hydrocarbon fraction), R_f 0.6–0.8 (alkylthiophene fraction), R_f 0.0–0.6 (alkylsulphide fraction)]. Subsequently, these fractions were analysed by GC and GC-MS.

Raney Ni desulphurisation/hydrogenation

All five polar fractions and the alkylthiophene and the alkylsulphide of Marl-2 were desulphurised with Raney Ni and subsequently hydrogenated as described elsewhere (Kohnen *et al.*, 1992a). Before desulphurisation a known amount of the synthetic thiophene standard [2,3-dimethyl-5-(1,1-D_2-hexadecyl) thiophene] was added to an aliquot of the polar fraction (*ca* 10 mg). The resultant hydrocarbons isolated from the desulphurised polar fractions using column chromatography over Al_2O_3 were analysed by GC and GC-MS. The desulphurised fractions of Marl-2 were also analysed isotopically using irm-GC-MS.

Gas chromatography

GC was performed using a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The column effluent was monitored by both a flame ionisation detector (FID) and a sulphur-selective flame photometric

detector (FPD), using a stream-splitter at the end of the column (split ratio FID:FPD = 1:2). The samples (dissolved in ethyl acetate) were injected at 70°C and subsequently the oven was programmed to 130°C at 10°C/min and then at 4°C/min to 320°C at which it was held for 40 min.

Gas chromatography-mass spectrometry

GC-MS was carried out on a Hewlett-Packard 5480 gas chromatograph interfaced to a VG-70S mass spectrometer operated at 70 eV with a mass range m/z 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm). Helium was used as carrier gas. The samples were injected at 50°C and subsequently the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 300°C at which it was held for 10 min.

Isotope-ratio-monitoring gas chromatography-mass spectrometry

The irm-GCMS system (DELTA-S) has been described previously (Matthews and Hayes, 1978; Hayes *et al.*, 1990; Freeman *et al.*, 1990). A fused silica capillary column (50 m × 0.32 mm) coated with cross-linked methyl silicone gum (Ultra-1, film thickness 0.52 μm) was used with helium as carrier gas. The samples (in ethyl acetate) isolated from the extract of Marl-2 were injected at 70°C and subsequently the oven was programmed to 130°C at 10°C/min and then at 3.0°C/min to 320°C, at which it was held for 60 min. The isotopic values were obtained by integrating the mass 44, 45 and 46 ion currents from the carbon dioxide produced by the continuous combustion of the chromatographic effluent. The isotopic compositions are referred to the PDB ^{13}C standard by comparison with co-injected isotopic standards.

Quantitation

The concentrations of selected compounds (mg/kg bitumen) were obtained by integration of their peak areas and that of the internal standards in the FID-traces. For the compounds in the "free" hydrocarbon fractions and in the desulphurised polar fractions the internal standard was the deuterated C_{22} anteisoalkane, for the alkylthiophenes the deuterated C_{22} alkylthiophene and for the alkylsulphides the deuterated C_{22} alkylthiolane was used. On the basis of the quantitative analysis of other sediment extracts performed previously (Kohnen *et al.*, 1990) the analytical error is estimated to be 15%.

RESULTS AND DISCUSSION

The extracts of the five samples, representing one evaporitic cycle, were fractionated into a hydrocarbon-, an alkylthiophene-, an alkylsulphide and a so-called polar fraction using column chromatography and subsequent argentation thin layer chromatography after removal of the asphaltenes (Fig. 1). The polar fractions, containing macromolecularly sulphur-bound lipids, as described in detail elsewhere for another marl layer from the Vena del Gesso basin (Kohnen *et al.*, 1991a), were desulphurised using Raney Ni and the released hydrocarbons were isolated from the desulphurised mixture using column

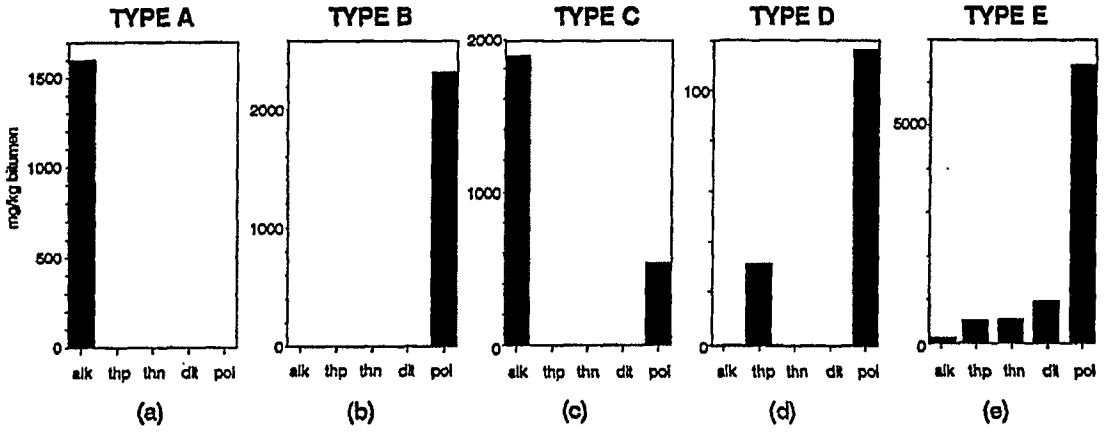

Fig. 2. The different carbon skeleton distribution types: (a) Type A, carbon skeletons which are exclusively present as "free" hydrocarbons, as an example the lycopane skeleton distribution pattern of Marl-1 is shown, (b) Type B, carbon skeletons which are exclusively present as sulphur-bound moieties in the macromolecules, as an example the dinosterane skeleton data for Marl-3 are shown, (c) Type C, carbon skeletons which are present as "free" hydrocarbon and as a sulphur-bound moiety in macromolecules, as an example the hentriacontane carbon skeleton distribution pattern of Marl-2 is shown, (d) Type D, carbon skeletons which are present as alkylthiophene and as a sulphur-bound moiety, in macromolecules as an example is the C_{25} HBI skeleton distribution pattern of Marl-2, (e) Type E, carbon skeletons which are present in at least three modes of occurrence, as an example the phytane carbon skeleton distribution pattern of Marl-2 is shown. Key: alk = alkane(ene), thp = alkylthiophene, thn = alkylthiolane and alkylthiane, dit = alkyldithiane, pol = sulphur-bound to macromolecules present in the polar fraction.

chromatography (Fig. 1). The resulting fractions were analysed quantitatively by GC using internal standards. Individual "free" hydrocarbons, alkylthiophenes, alkylthiolanes, alkylthianes, alkyldithianes and sulphur-bound lipids in macromolecules have thus been quantified. In addition, the different fractions and the corresponding Raney Ni desulphurised fractions isolated from Marl-2 (VDG-7A2) were also analysed using irm-GC-MS.

Different modes of occurrence of particular carbon skeletons

This quantitative approach yielded surprising results. For example, *ca* 70% of the phytane carbon skeleton is present as a sulphur-bound moiety in macromolecules and only *ca* 1% as the free hydrocarbon in the bitumen [Fig. 2(e)]. Furthermore, other carbon skeletons [e.g. lycopane; Fig. 2(a)] occur exclusively as "free" hydrocarbons whereas others occur exclusively as sulphur-bound moieties in macromolecules [e.g. 4α,23,24-trimethyl-5α(H)-cholestane (dinosterane); Fig. 2(b)]. These major differences in modes of occurrences of lipids have a significant impact on palaeoenvironmental reconstructions based on biological marker distributions; if the naturally sulphurised carbon skeletons go unnoticed and only the non-sulphurised compounds are considered, a heavily biased view of the palaeoenvironment is obtained. In a recent communication we demonstrated for two immature sediments from the Vena del Gesso basin and the Peruvian Upwelling Area that a palaeoenvironmental reconstruction based solely on "free" hydrocarbons is both dissimilar and incomplete relative to that which also takes into account the naturally sulphurised hydrocarbons (Kohnen *et al.*, 1991b). However, it is emphasised that palaeoenvironmental reconstruction studies based solely on the analyses of "free" hydrocarbons are at present not as frequent as in the past as far as immature sedimentary settings are concerned. This is due to the growing awareness that many lipids undergo a series of diagenetic reactions before

Table 2. Distribution types of the carbon skeletons occurring in Marl-2 and the $\delta^{13}C$ values of these particular skeletons in the different modes of occurrence

Carbon skeletons	Type*	Hydrocarbon† fraction	Alkylthiophene‡ fraction	Alkylsulphide‡§ fraction	Polar¶ fraction
n-C_{15}	E	−30.5 ± 0.5	—	−27.5 ± 1.2	−27.1 ± 1.0
n-C_{16}	E	−28.1 ± 0.9	—	−20.6 ± 0.3	−22.1 ± 1.0
n-C_{17}	E	−29.3 ± 0.6	−24.4 ± 5.1	−25.0 ± 0.2	−25.2 ± 0.4
n-C_{18}	E	−28.5 ± 0.3	−17.5 ± 3.2‖	−26.4 ± 0.3	−26.4 ± 0.4
n-C_{19}	E	−30.5 ± 0.1	−22.7 ± 0.9‖	−26.1 ± 0.5	−26.0 ± 0.9
n-C_{20}	E	−29.0 ± 1.4	−18.6 ± 5.2‖	−25.9 ± 0.3	−26.2 ± 0.5
n-C_{21}	E	−28.7 ± 0.5	−24.9 ± 1.1‖	−26.9 ± 0.6	−27.6 ± 0.4
n-C_{22}	E	−29.2 ± 0.9	−25.4 ± 0.2	−25.4 ± 0.2	−25.9 ± 0.7
n-C_{23}	E	−29.3 ± 0.8	−27.7 ± 1.1	−27.4 ± 0.2	−27.0 ± 0.6
n-C_{24}	E	−29.4 ± 0.3	−27.7 ± 0.3	−26.1 ± 0.3†††	−26.4 ± 0.5
n-C_{25}	E	−29.3 ± 0.7	−29.1 ± 0.8	−26.8 ± 0.5†††	−27.0 ± 1.4
n-C_{26}	C	−28.8 ± 0.3	−28.4 ± −.−	−27.2 ± 0.3†††	−27.1 ± 0.8
n-C_{27}	C	−28.9 ± 0.4	−27.7 ± −.−	−27.7 ± 0.6†††	−28.7 ± 0.6
n-C_{28}	C	−29.5 ± 0.2	—	−28.2 ± 1.5†††	−27.6 ± 1.2
n-C_{29}	C	−29.5 ± 0.7	—	−26.1 ± 0.7†††	−26.8 ± 0.7
n-C_{30}	C	−29.6 ± 0.8	—	−27.8 ± 0.7†††	−27.1 ± 1.5
n-C_{31}	C	−28.9 ± 0.3	—	−17.6 ± 0.3†††	−17.7 ± 1.1
n-C_{32}	C	−30.1 ± 0.6	—	−27.5 ± 1.5‡‡‡	−27.0 ± 4.9
n-C_{33}	C	−29.3 ± 0.7	—	−26.2 ± 2.6†††	−26.6 ± 0.4
Pristane	C	−31.2 ± 2.5	—	—	−27.5 ± 1.1
Phytane	E	−32.8 ± 0.7	−30.0 ± 0.6 (VIII) −30.2 ± 0.4 (VI)	−29.8 ± 2.3 (VII)†† −30.0 ± 0.3 (VII)†† −30.2 ± 0.8 (VII)††	−30.5 ± 0.4
PME	A	−25.8 ± 1.6	—	—	—
Squalane	E	−31.6 ± 0.8	−36.4 ± 1.3 (III)	−41.3 ± 0.7 (II)‡‡ −40.4 ± 2.8 (II)§§	−33.2 ± 0.1
Lycopane	A	−25.3 ± 1.0	—	—	—
C_{20} HBI	A	−17.7 ± 0.6	—	—	—
C_{25} HBI	D	—	−27.3 ± 0.9	−23.4 ± 0.8†††	−23.9 ± 0.6
Hop-17(21)ene	A	−26.6 ± 0.3	—	—	—
Cholestane¶¶	C	—	—	−26.8 ± 0.4†††	−26.3 ± 0.3
24-ethylcholestane‖‖	C	—	—	−26.6 ± 1.2†††	−26.7 ± −.−
Dinosterane	C	—	—	−26.6 ± 0.6†††	−26.7 ± 1.2
C_{35} hopane***	E	—	−26.3 ± 1.0	−25.3 ± 0.7†††	−22.7 ± 1.4
Di-aromatic carotenoid	B	—	—	−11.5 ± 1.0†††	−10.7 ± 0.6

Key: *Five different types of carbon skeleton distributions are distinghuished: i.e. Type A–E (Fig. 2); †the carbon isotope data are presented in $\delta^{13}C$ values, ‡$\delta = 10^3 [(R_x - R_s)/R_s]$ in ‰, where $R = {}^{13}C/{}^{12}C$, x designates sample, s designates PDB standard and $R_s = 0.0112372$. Entries are mean and 95% confidence interval for two to six replicate measurements; ‡for the majority of carbon skeletons the isotope-analysis are performed on desulphurised compounds, the ones which are analysed as OSC are annotated with a roman number; §the n-alkanes are derived from both low-molecular-weight alkylsulphides and macromolecularly S-bound moieties (Kohnen *et al.*, 1991d); ¶the compounds are analysed in the desulphurised polar fraction; ‖different structural isomers of linear alkylthiophenes are encountered which are distinct in their isotopic composition (Fig. 8); ††the isotopic composition of phytane in the desulphurised alkylsulphide fraction is −30.4 ± 0.3‰; ‡‡cis isomer; §§trans isomer; ¶¶5α(H),14α(H),17α(H)-cholestane, the 5β(H) has a similar $\delta^{13}C$value; ‖‖5α(H),14α(H),17α(H)-24-ethylcholestane; ***22R-17β(H),21β(H)-pentakishomohopane; †††compounds are exclusively derived from macromolecularly sulphur-bound moieties.

they are converted to hydrocarbons. Therefore, an increasing number of organic geochemical studies deal with functionalised lipids such as alcohols, acids and ketones, in addition to the conventional hydrocarbon biomarkers. Nevertheless, it is expected that these so-called "total lipid approach" studies suffer from the same bias since these oxygenated lipids also may become naturally sulphurised in sulphur-rich sediments (ten Haven *et al.*, 1986, 1989; Kohnen *et al.*, 1991b).

On the basis of the data obtained for the five samples analysed, five types of carbon skeleton distributions are distinguished [Types A-E; Fig. 2(a–e), Table 2]. Carbon skeletons which exhibit a distribution Type A are present exclusively as "free" hydrocarbons [e.g. (2,6,10-trimethyl-7-(3-methylbutyl)-dodecane, a C_{20} highly branched isoprenoid alkane (HBI); 2,6,10,15,19-pentamethyleicosane (PME) and lycopane]. Type B carbon skeletons are exclusively encountered as sulphur-bound moieties in macromolecules [e.g. dinosterane, carotenoid-derived diaromatic compounds]. Carbon skeletons of types C [e.g. pristane, hentriacontane] and D [e.g. 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane a C_{25} HBI] are both present as macromolecularly sulphur-bound moieties and as "free" hydrocarbons or alkylthiophenes, respectively. Carbon skeletons which have at least three different modes of occurrence belong to Type E (e.g. phytane, squalane, pentakishomohopane). Considering the five samples analysed, it is evident that the relative abundances of a carbon skeleton in the different modes of occurrence vary significantly throughout the sedimentary sequence. This is exemplified by the distribution of the n-$C_{31}H_{64}$ carbon skeleton as shown in Fig. 3. Furthermore, a particular carbon skeleton is not consistently related to a certain distribution type. For example, the octadecane carbon skeleton shows a Type E distribution in the marls and the stromatolitic limestone and a Type C distribution in the gypsum (Fig. 4). A similar phenomenon is noticed for the squalane carbon skeleton (Fig. 5).

The occurrence of these different distribution types can be rationalised in a straightforward manner. Type A carbon skeletons, encountered only as "free" hydrocarbons, obviously did not react with inorganic sulphur species, indicating that they were biosynthesised without a reactive functional group [e.g. PME, lycopane, hop-17(21)-ene; Table 2]. The carbon skeletons present as free hydrocarbons are therefore considered as unaltered biochemicals synthesised as such by organisms present in the ancient water column or sediment.

The carbon skeletons which are present exclusively as sulphur-bound moieties in the macromolecular fractions (Type B) must have precursors which

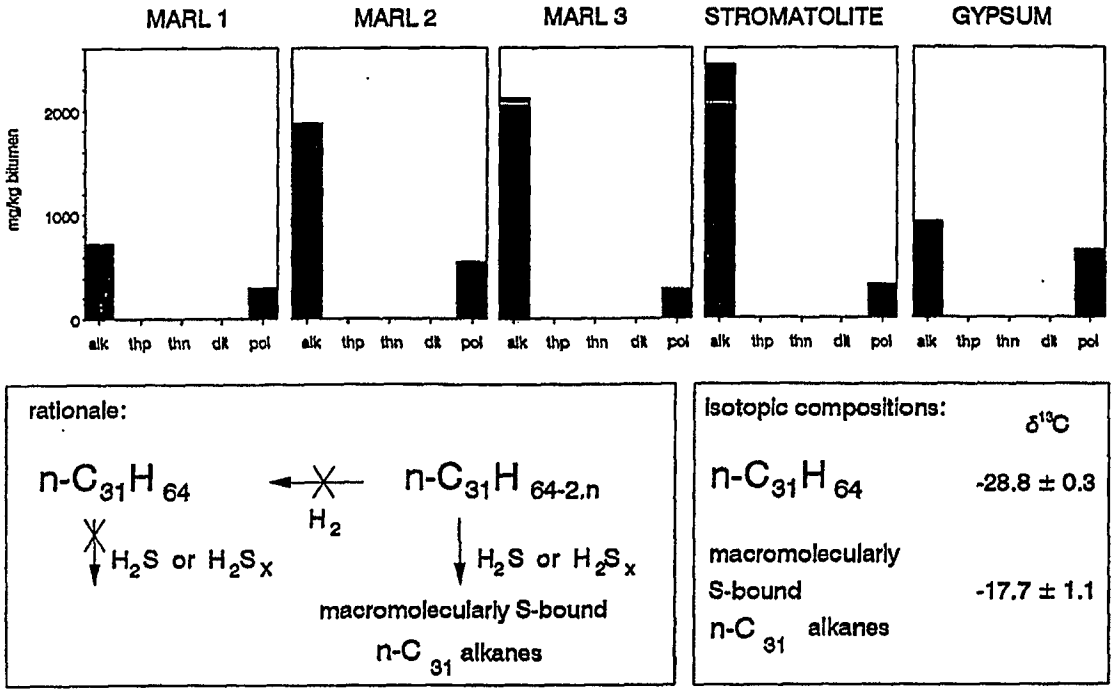

Fig. 3. Absolute abundances of the hentriacontane carbon skeleton in its different modes of occurrence in five samples from an evaporitic cycle in the Messianian Vena del Gesso basin. The ratios of the abundances of the free over the sulphur-bound hentriacontane carbon skeleton vary between 1.5 (Gypsum) and 7.8 (Stromatolite) throughout the sequence. A schematic rationale for the observed distribution of the hentriacontane carbon skeleton and the carbon isotopic compositions of the carbon skeletons in their different modes of occurrence in Marl-2 is depicted. See caption of Fig. 1 for key.

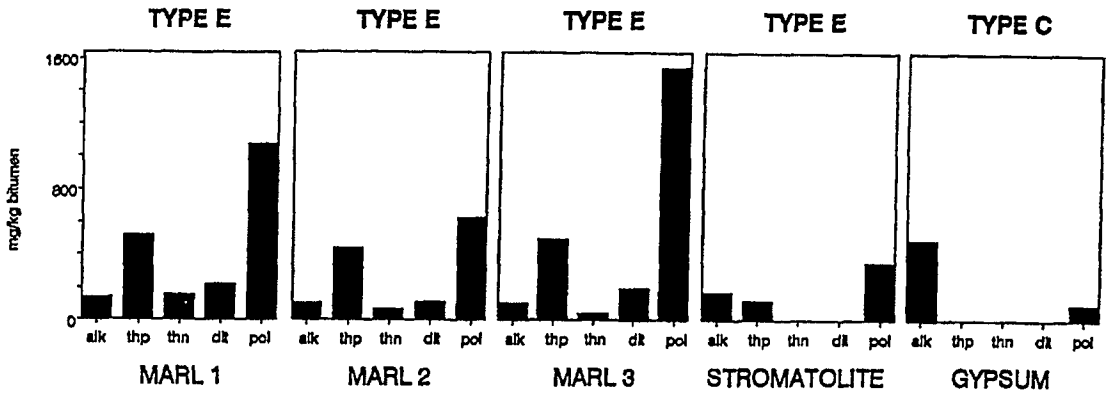

Fig. 4. Absolute abundances of the octadecane carbon skeleton in its different modes of occurrence in the five samples from the Vena del Gesso basin. It is noteworthy that in the marl and in the stromatolitic limestone the octadecane skeleton shows a Type E distribution whereas in the gypsum it shows a Type C distribution. See caption of Fig. 1 for key.

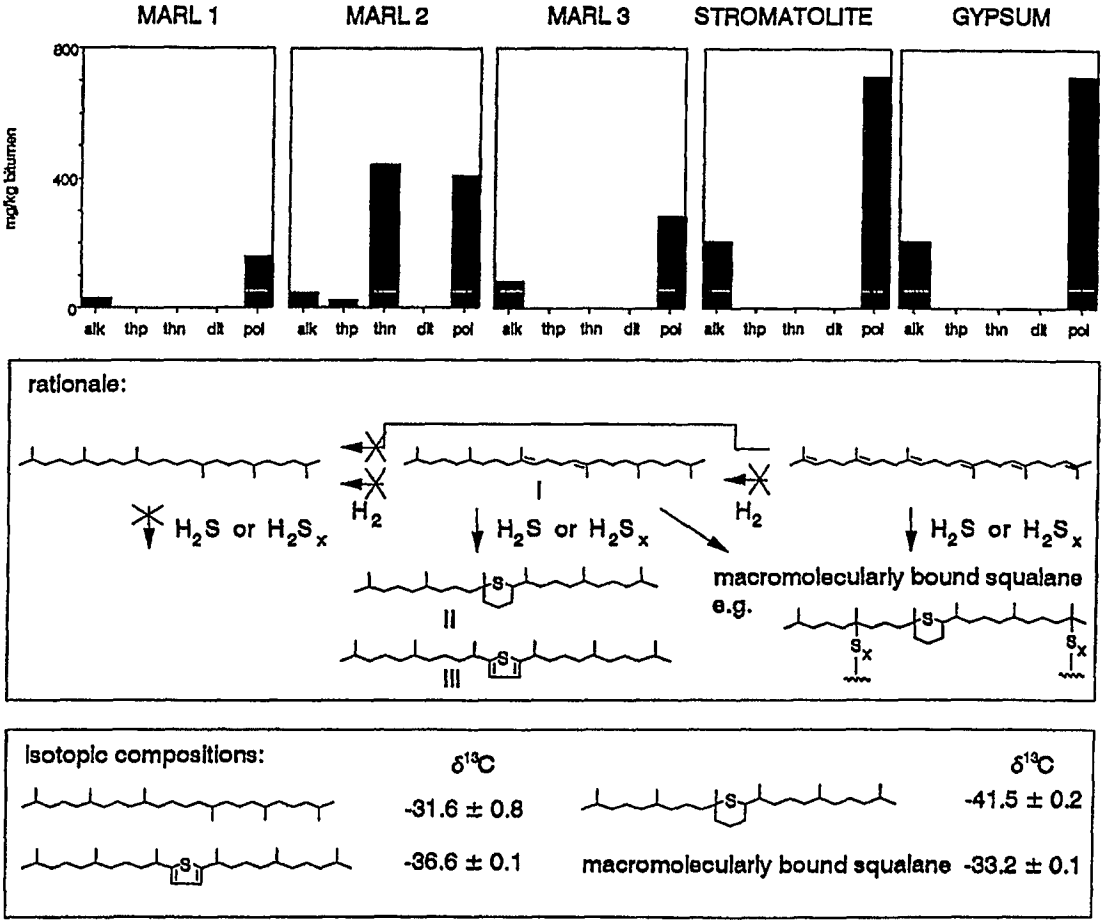

Fig. 5. Absolute abundances of the squalane carbon skeleton in its different modes of occurrence in the five samples from the Vena del Gesso basin. A schematic rationale for the observed distribution of the squalane carbon skeleton and the carbon isotopic compositions of the carbon skeletons in the different modes of occurrence in Marl-2 is depicted. The depicted macromolecularly sulphur-bound squalane carbon skeleton is an hypothetical example of the products resulting from H_2S or HS_x^- incorporation into squalene. See caption of Fig. 1 for key.

reacted with sulphur species in an intermolecular fashion. That is, the initially formed OSC (e.g. an alkylthiol) reacted with a second functionalised lipid or moiety resulting in the formation of macromolecular substances (Sinninghe Damsté *et al.*, 1989, 1990b; Kohnen *et al.*, 1991a, 1992a). This contrasts with intramolecular incorporation of sulphur by which the reactive intermediate (e.g. a thiol group) attacks another functionality (e.g. double bond) in the same carbon skeleton leading to the formation of a cyclic OSC. Therefore, the precursors of type B carbon skeletons probably possessed one or several functional groups (e.g. double bonds) which are separated by at least four sp^3-hydridised carbon atoms preventing intramolecular sulphur incorporation (Sinninghe Damsté *et al.*, 1989). For example, the sulphur-bound dinosterane carbon skeleton representing a Type B carbon skeleton originates from sulphur incorporation into dinosterol or its diagenetic product dinosterene, precursor substrates in which the functional groups at C-3 and C-22 are separated by a large number of non-functionalised carbon atoms. In addition, carbon skeletons which have precursor (bio)lipids possessing multiple double bonds (e.g. di-aromatic carotenoids) will also preferentially accumulate as sulphur-bound moieties in macromolecules; the presence of numerous reaction-sites makes the formation of at least one intermolecualr S-linkage likely, despite the fact that the double bonds are separated by less than four sp^3-hybridised carbon atoms favouring intramolecular incorporation of sulphur. Another example is provided by the macromolecularly sulphur-bound C_{20} isoprenoid thiophenes encountered in a Sicily Seep Oil (Kohnen *et al.*, 1992a). These moieties, with both intra- and intermolecular sulphur-linkages, are thought to be the result of sulphur incorporation into geranyl geraniol-derived polyenes which possess up to five double bonds (Kohnen *et al.*, 1992a). The exclusive occurrence of these polyene-related carbon skeletons as sulphur-bound moieties in macromolecules strongly suggests that hydrogenation of the precursor substrates (e.g. dinosterene) leading to the formation of "free" saturated hydrocarbons proceeded much less rapidly than the natural sulphurisation process in the Vena del Gesso sediments. This further supports the hypothesis that Type A carbon skeletons are biosynthesised as such and are not derived via hydrogenation of unsaturated alkene precursors.

Type C carbon skeletons are present as both "free" hydrocarbons and sulphur-bound moieties in macromolecules. The first characteristic requires a non-functionalised precursor as in Type A. The second indicates (a) precursor(s) with one or more functionalities that mainly reacted intermolecularly with sulphur species, similar to Type B carbon skeletons. Thus, the hentriacontane found as "free" hydrocarbon has a biosynthetic source different from that of the macromolecularly sulphur-bound hentriacontane (Fig. 3). Further evidence for this dual origin of biolipids possessing the same carbon skeleton is offered by the observed variations in relative abundances of the two modes of occurrence of the hentriacontane carbon skeleton throughout the sedimentary sequence (Fig. 3) as discussed above.

Type D carbon skeletons occur as alkylthiophene and as sulphur-bound moieties in macromolecules (e.g. Fig. 6). It is theoretically possible that these moieties have the same precursor(s) that incorporated reduced sulphur species both in an intra- and intermolecular fashion. It must be realised, however, that sulphur incorporation into different precursors with the same carbon skeleton may also lead to a Type D carbon skeleton distribution. For example, a diene with one sp^3-hybridised carbon atom between the double bonds will be a suitable precursor for the alkylthiophene, and precursor(s) with one isolated functional group or numerous reactive sites, as proposed for Type B skeletons, are likely to accumulate in the macromolecular fractions. In Fig. 6 the rationale for the distribution of the C_{25} HBI carbon skeleton (Type D) in its different modes of occurrence is depicted. Proposing different precursors with a C_{25} HBI alkane carbon skeleton with different patterns of functionalisation is in accordance with the reported occurrence of C_{25} HBI alkenes with varying degrees of unsaturation (i.e. 1–4 double bonds) in sediments or organisms (for a review see Rowland and Robson, 1990). The number and the position(s) of the double bonds in the C_{25} HBI components will control their ultimate mode of occurrence: alkylthiophene vs macromolecularly sulphur-bound.

Similarly, carbon skeletons showing a type E distribution may also have several different precursors. For example, the squalane carbon skeleton is encountered in Marl-2 as "free" hydrocarbon, as cyclic OSCs and as sulphur-bound moieites in macromolecules (Fig. 5). The rationalisation of this distribution involves at least three different precursors: squalane which has neither formed by hydrogenation of unsaturated precursors nor has reacted with inorganic sulphur species; octahydrosqualane (I) which incorporated sulphur intramolecularly; and the prominent biosynthetic product squalene with multiple double bonds and which therefore accumulated mainly as macromolecularly sulphur-bound moieties. Octahydrosqualane (I), the putative precursor of the OSC III and IV (Fig. 5), has been tentatively identified previously in particulate organic matter in the water column of the Cariaco trench (Wakeham, 1990). Incorporation of sulphur into this diene will mainly yield the alkylthiane(II) because sulphur addition to a double bond probably involves an intermediate carbocation which consequently leads to the formation of an initial C–S bond at the most substituted position (i.e. C-9; Kohnen *et al.*, 1991a, 1992a). Subsequently, the addition reaction will be completed by attack of the reactive intermediate (e.g. a thiol group) on the other double bond, forming the stable six-membered ring system. The formation of

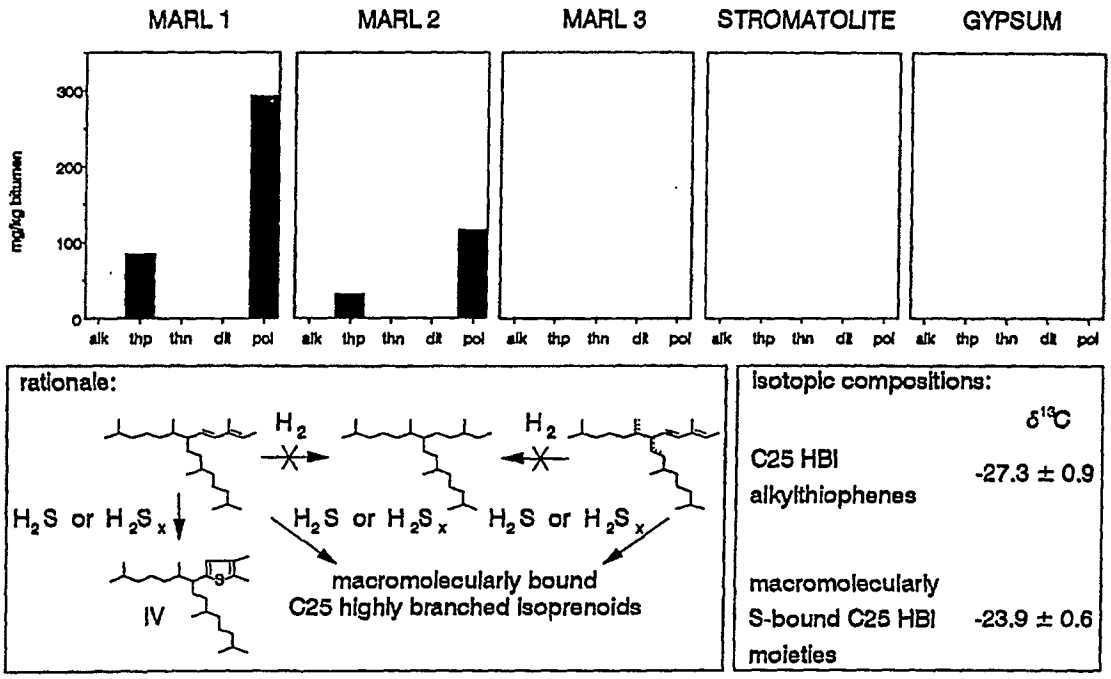

Fig. 6. Absolute abundances of the C_{25} HBI carbon skeleton in its different modes of occurrence in the five samples from the Vena del Gesso basin. A schematic rationale for the observed distribution of the C_{25} HBI carbon skeleton and the carbon isotopic compositions of the carbon skeletons in the different modes of occurrence in Marl-2 is depicted. See caption of Fig. 1 for key.

the alkylthiophene (III), on the other hand, must involve an intermediate secondary carbocation which is thermodynamically less favoured in comparison to a tertiary carbocation—the intermediate in the formation of the alkylthiane. This is reflected in the pronounced dominance of the alkylthiane over the alkylthiophene in Marl-2 (Fig. 5). These novel OSC, II and III, are tentatively identified on the basis of relative retention times, Raney Ni desulphurisation and mass spectral data. The mass spectra of alkylthiophene III and alkylthiane II are shown in Fig. 7.

The phytane carbon skeleton also exhibits a Type E distribution (Fig. 8). The sulphur-bound phytane carbon skeletons, which were shown to have the S-linkage mainly at the C-3 position (Kohnen *et al.*, 1991a, 1992a), and the OSC V–VIII can be ascribed to a reaction of chlorophyll-derived phytadienes with reduced sulphur species (Brassell *et al.*, 1986; Sinninghe Damsté *et al.*, 1986, 1987; Rullkötter *et al.*, 1988; Kohnen *et al.*, 1991a, c, 1992a). Therefore, this distribution of the phytane carbon skeleton can be explained by invoking two different precursors (i) phytane and (ii) phytol-derived phytadienes (Fig. 8).

From the above mentioned observations it can be rationalised that the number and position(s) of the functional groups of the (bio)lipids govern the ultimate number and position(s) of the sulphur-linkages, and consequently, the mode of occurrence of a specific carbon skeleton. It should be emphasised again that "free" hydrocarbons in immature S_{org}-rich sediments are lipids that did not react with sulphur during early diagenesis probably due to the fact that they were biosynthesised as such (i.e. without a reactive functional group). Moreover, if a carbon skeleton is present in different modes of occurrence it may have different biosynthetic precursors which are possibly derived from different biological sources.

Variations in carbon isotopic compositions of a carbon skeleton present in different modes of occurrence

Separate origins for particular carbon skeletons with various modes of occurrence are firmly supported by carbon isotopic analyses of the carbon skeletons in Marl-2. For example, the "free" hentriacontane and the sulphur-bound hentriacontane in macromolecular organic matter differ 11.1‰ in their carbon isotopic compositions (Fig. 3). It can be ruled out that this major isotopic difference is caused by post-depositional isotopic fractionation; isotope effects associated with the sulphur incorporation reaction, for example, would be capable of fractionating carbon isotopes only at the positions involved in the formation of C–S bonds. Isotopic shifts at these positions are, however, diluted by the unchanged isotopic compositions at the far more numerous carbon positions not participating in the reaction with reactive sulphur species, and the fractionation

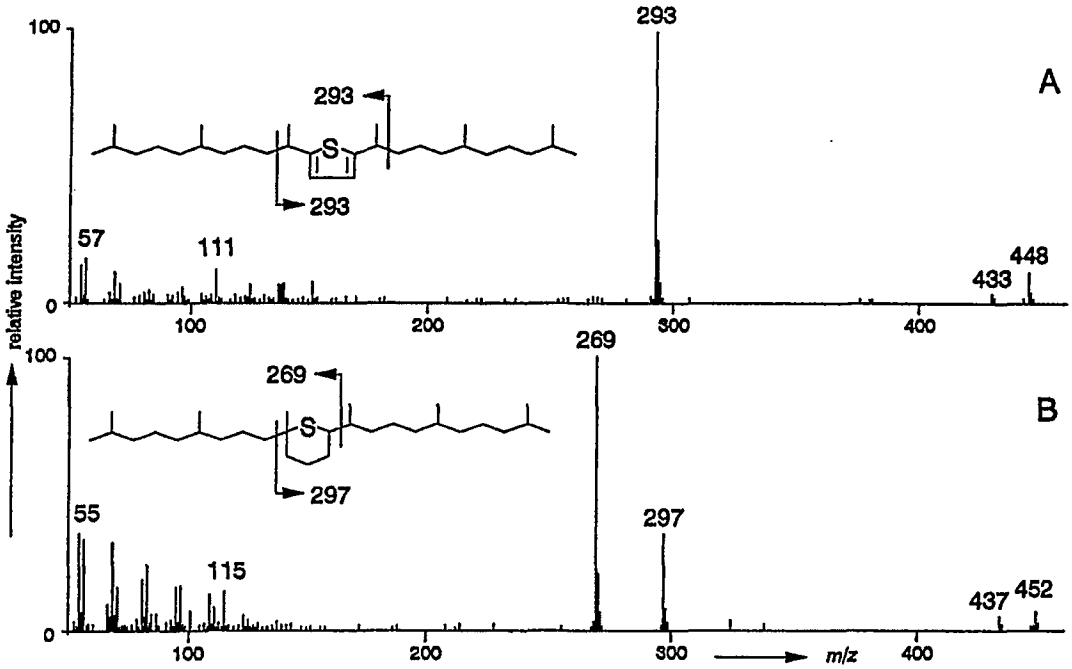

Fig. 7. Mass spectra (not corrected for background) of (A) 2,5-di-(2-(6,10-dimethylundecyl)thiophene, (B) 2-(4,8-dimethylnonyl)-6-(2-(6,10-dimethylundecyl)-2-methylthiane tentatively identified in the extract of Marl-2. Another stereomer of the alkylthiane is present and shows a similar mass spectrum. It is of note that the α-cleavage reaction leading to the formation of the ion at *m/z* 269 (mass spectrum B) is favoured over the other α-cleavage reaction (*m/z* 297) because this cleavage leads to the formation of a secondary alkyl radical instead of a primary alkyl radical as in the latter case.

required to explain an overall molecular-isotopic difference of 11.1‰ would be implausibly large. Accordingly, separate biosynthetic origins must be invoked for the identical carbon skeletons found as free hydrocarbon on the one hand and as macromolecularly sulphur-bound moiety on the other hand.

Significant differences in carbon isotope composition are also observed for the different modes of occurrence of the other *n*-alkane homologues, although not as pronounced as in the case of hentriacontane. The isotopic compositions of the *n*-alkanes present in the hydrocarbon fraction and those *n*-alkanes isolated after Raney Ni desulphurisation of the alkylthiophene, alkylsulphide and polar fractions are depicted in Fig. 9. It is worthy of note that the alkylsulphide fraction contains, in addition to linear alkylthiolanes and alkyldithianes, macromolecular substances which also comprise sulphur-bound *n*-alkanes (Kohnen *et al.*, 1991a). Hence, the *n*-alkanes in the desulphurised alkylsulphide fraction are derived from two different modes of occurrence, alkylsulphide and macromolecularly sulphur-bound. However, the carbon number of the linear alkylsulphide series ranges from C_{15} to C_{25} and all the higher *n*-alkanes in the desulphurised fraction are, thus, exclusively derived from the sulphur-bound moieties in the macromolecules. The free *n*-alkanes are isotopically distinct from the sulphurised ones and, especially at higher carbon numbers, are rather constant in isotopic composition suggesting, a common biological source distinct from the precursors of the sulphurised *n*-alkanes.

Over the whole range of homologues the $\delta^{13}C$ values of *n*-alkanes in both the desulphurised alkylsulphide and polar fractions are similar, suggesting (a) common biological source(s). This, however, does not exclude the possibility that the precursors of the alkylsulphides and those of the sulphur-bound *n*-alkanes in the macromolecules differed in position(s) and number of functional groups. The isotopic compositions of the *n*-alkanes (C_{18}–C_{21}) in the desulphurised alkylthiophene fraction differ significantly from those of the corresponding homologues in the other modes of occurrence. This is rather surprising since it has been suggested by several investigators that alkylthiophenes are diagenetic products of alkylsulphides as a result of aromatisation reactions (for a review see Sinninghe Damsté and de Leeuw, 1990). The isotopic results presented here seem to be in conflict with this hypothesis.

Analyses of the untreated alkylthiophene fraction revealed that the several structural isomers of linear alkylthiophenes with a specific carbon number are strikingly different in carbon isotopic composition (Fig. 10). For example, the two alkylthiophenes with an octadecane carbon skeleton differ in their $\delta^{13}C$

values 13.2‰. This irrefutably demonstrates that at least two precursors with an octadecane carbon skeleton, apparently biosynthesised by different organisms, were present in the palaeoenvironment. This dual source is plausible because the distinction between the structural isomers is the location of the sulphur atom in the carbon skeleton, indicating that the corresponding precursor (bio)lipids must be chemically distinct in that they had functional groups (e.g. double bonds) at different positions in their carbon skeletons. It is noteworthy that the relatively heavy series of alkylthiophenes shows a so-called "shift phenomenon". This series starts with the C_{18} monoalkylated thiophene, the first homologue is the C_{19} 2-methyl-5-tetradecylthiophene, the second is the C_{20} 2-ethyl-5-tetradecylthiophene and so on. The other series of structural isomers have $\delta^{13}C$ values ranging between -24.7 and -27.9‰, similar to those of the corresponding carbon skeletons in the desulphurised alkylsulphide fraction. This indicates we are dealing with two series of precursor lipids from distinct biological sources. It is speculated that one series of precursors yielded upon sulphur incorporation alkylsulphides which were subsequently transformed into alkylthiophenes, i.e. the -24.7‰ to -27.7‰ series. The other series, characterised by the relatively heavy $\delta^{13}C$ values, ranges from C_{18} to C_{21} and the location of the functional groups (e.g. double bonds) remained the same in the carbon skeletons [Fig. 9(B)]. The absence of corresponding alkylsulphides with these heavy carbon isotopic compositions indicates that sulphur incorporation yielded alkylthiophenes instantly and thus omitted the "alkylsulphide stage". The rather large divergence in the isotopic compositions of the isotopically heavy series of alkylthiophenes may be attributed to varying degrees of dilution with structurally identical but isotopically lighter alkylthiophenes. The C_{20} 2-ethyl-5-tetradecylthiophene is isotopically seen an end member of the isotopically heavy series. This is consistent with the fact that the isotopically lighter series does not include 2-ethyl-5-alkylthiophenes and thus does not contribute to this isomer; hence its isotopic value is considered to represent that of the series of precursor biolipids [A, Fig. 9(B)] of this isotopically heavy series of alkylthiophenes. Accordingly, it is estimated that the major C_{18} isomer, with an isotopic composition deviating only slightly from that of the C_{20} isomer, is the result of sulphur incorporation into a C_{18} diene *ca* 80% derived from the isotopically heavy series and *ca* 20% derived from the isotopically lighter series of precursor biolipids. The greater deviation of the isotopic compositions of the C_{19} and the C_{21} isomers

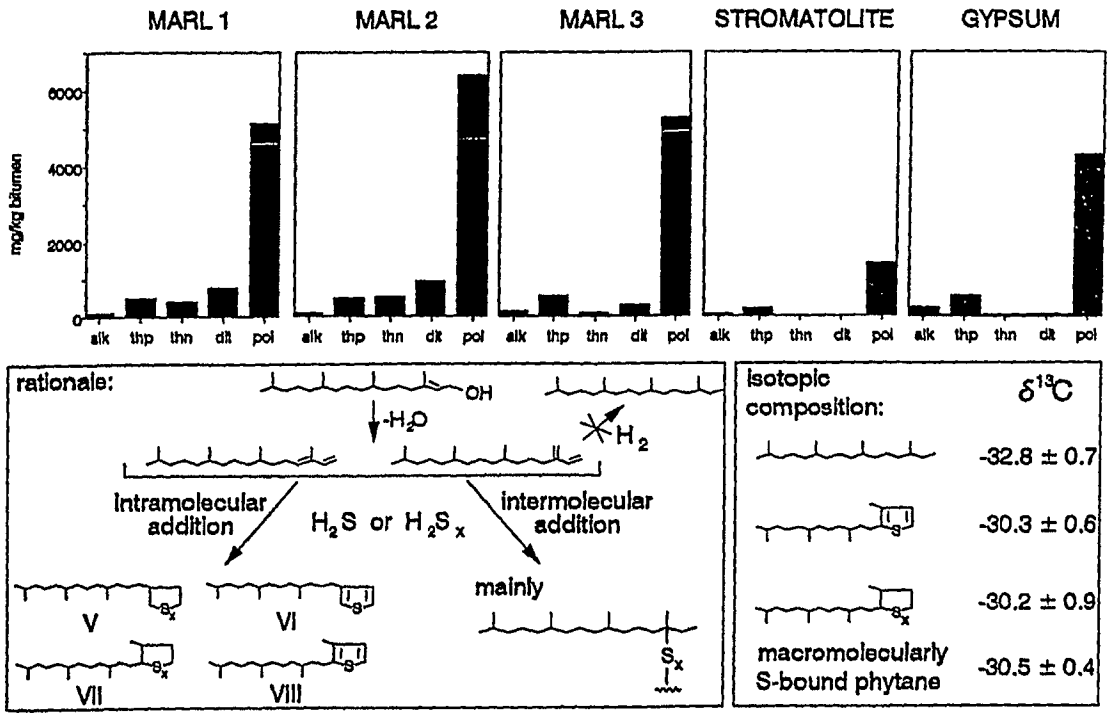

Fig. 8. Absolute abundances of the phytane carbon skeleton in its different modes of occurrence in the five samples from the Vena del Gesso basin. A schematic rationale for the observed distribution of the phytane carbon skeleton and the carbon isotopic compositions of the different modes of occurrence in Marl-2 is depicted. In addition to phytol, archaebacterial phytenyl glyceryl ethers (Volkman *et al.*, 1989) with the double bond at the same position as that in phytol may also be a source for the phytadienes. See caption of Fig. 1 for key.

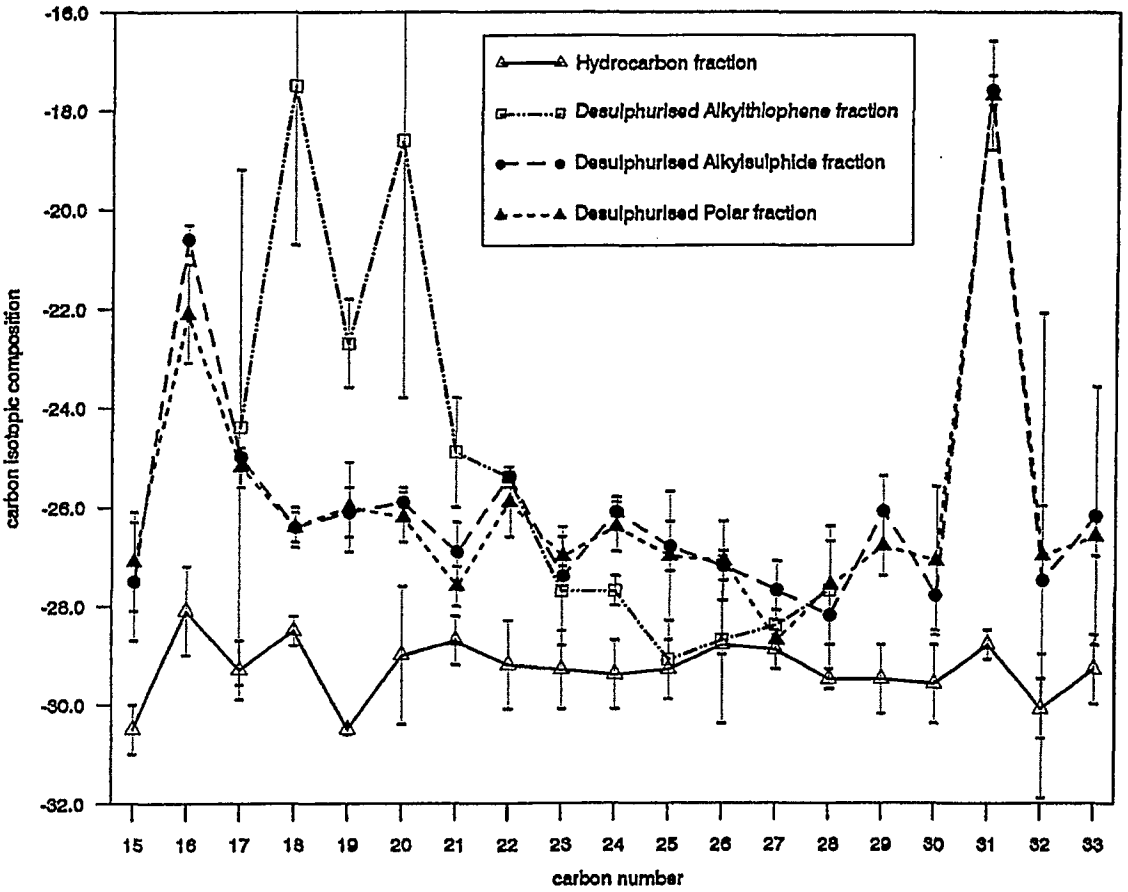

Fig. 9. $\delta^{13}C$ values and the corresponding 95% confidence intervals for the *n*-alkanes in the free hydrocarbon fraction (triangles), the desulphurised alkylthiophene fraction (squares), the desulphurised alkylsulphide fraction (solid circles), and the desulphurised polar fraction (i.e. macromolecularly sulphur-bound *n*-alkanes; solid triangles).

of this series relative to the C_{20} isomer is the result of a greater dilution with the series of isotopically lighter alkylthiophenes.

Alternatively it can be rationalised that the C_{18} and C_{20} thiophenes, with δ-values of -12.9 and -9.5‰, respectively, and the C_{19} and C_{21} thiophenes with δ-values of -17.9‰ and -18.9‰ represent two independent series of biolipids [B and C, Fig. 9(B)]. From a biosynthetic point of view this alternative may hold, since chain elongations commonly proceed with C_2 increments.

The C_{25} HBI carbon skeleton exhibits a Type D distribution, suggesting that at least two different precursors with a C_{25} HBI carbon skeleton were present in the palaeoenvironment. Isotopic analyses of both modes of occurrence, alkylthiophene and macromolecularly sulphur-bound, confirmed this hypothesis (Fig. 6, Table 2). The alkylthiophene (IV) and the sulphur-bound C_{25} HBI in the macromolecules are isotopically distinct, indicating that these different precursors originate from different biota.

At least three different precursors are proposed to explain the distribution of the squalane carbon skeleton (Fig. 5). Isotopic analysis confirm this hypothesis, though different precursors for the alkylthiophene and the alkylthiane must be postulated as well. This contradicts the proposed reaction pathways outlined in Fig. 5. Accordingly, we speculate that a hexahydrosqualene with the double bonds situated in the middle of the carbon skeleton (not shown in Fig. 5) would lead preferentially to the thiophene.

The distribution pattern of the phytane carbon skeleton (Type E) implies two different precursors, phytane and phytol-derived phytadienes (Fig. 8). Isotopic analysis support this hypothesis since all the sulphur-bound phytanes are isotopically identical and the "free" hydrocarbon phytane is isotopically distinct and thus has a different biological source (Fig. 8). It should be noted, however, that identical isotopic compositions do not necessarily imply a common biological source. It remains possible that different biological sources contributed to the

phytadiene pool but, since these phytol-derived phytadienes were chemically identical, they all reacted similarly and are represented in constant proportions in the different modes of occurrence.

In many cases, isotopic analyses of desulphurised alkylthiophene and alkylthiolane fractions yield average values for each carbon skeleton, since many different OSC with the same carbon skeleton are present. These are likely to derive from different biota and to have different ^{13}C contents; an eloquent example is provided by the striking differences in isotopic compositions of the different alkylthiophene isomers possessing the same carbon skeletons (Fig. 10). In many cases, however, the complexity of

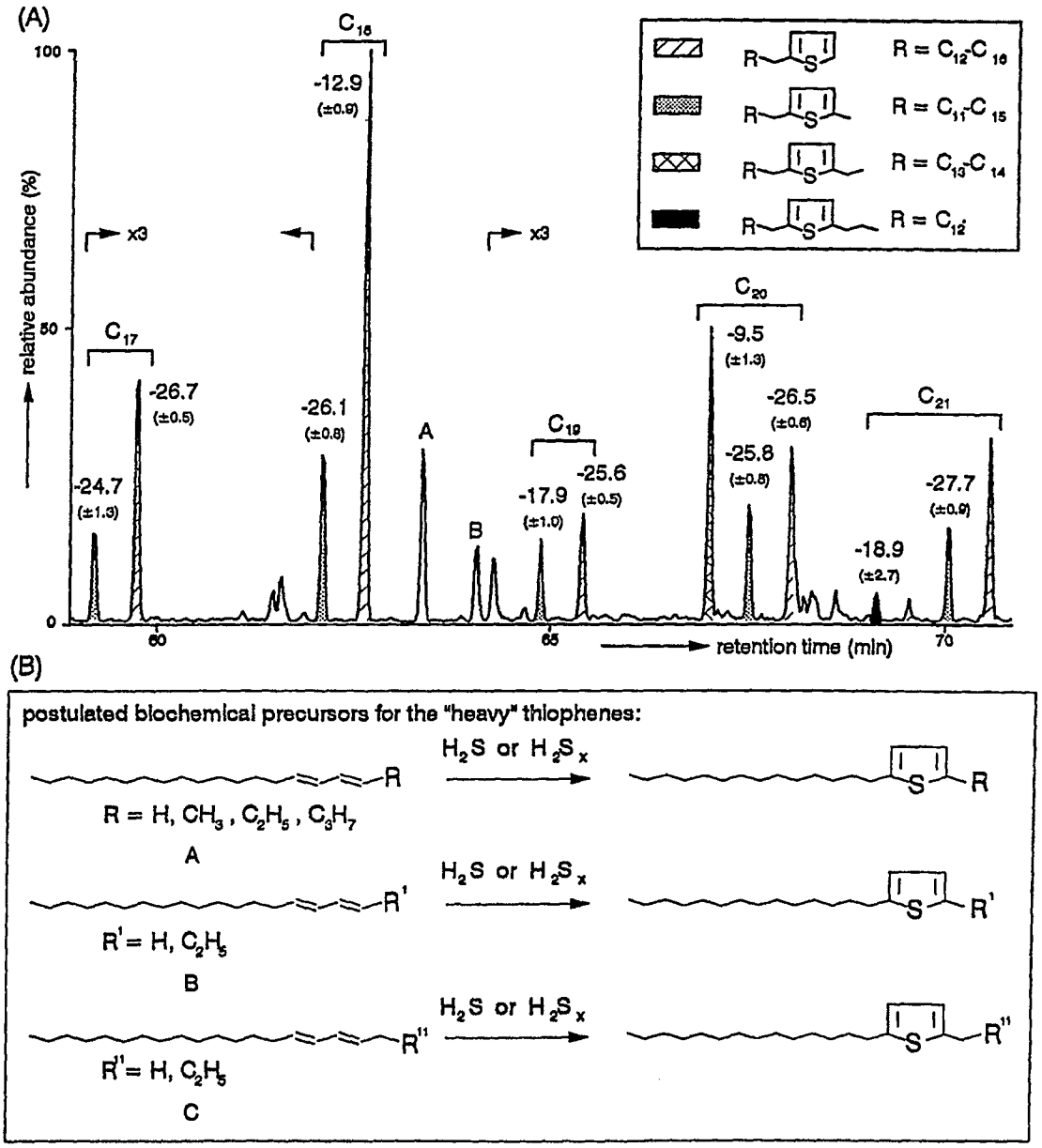

Fig. 10. (A) Partial mass chromatogram for m/z 252 + 266 + 280 + 296 + 308 + 322 of the alkylthiophene fraction isolated from the extract of Marl-2 exhibiting the distribution of the alkylthiophenes which possess n-alkyl carbon skeletons (C_{17}–C_{21}). The mean carbon isotopic compositions of the major isomers are depicted and the corresponding 95% confidence intervals from three to six replicate analyses are presented between brackets. A reliable isotopic composition of the major structural isomer in the cluster of alkylthiophenes possessing an n-C21 carbon skeleton could not be determined because of coelution problems. The peaks denoted with A and B correspond to alkylthiophenes VIII and VI possessing a phytane carbon skeleton. (B) Postulated precursor alkenes for the series of isotopically heavy alkylthiophenes.

the alkylthiophene and the alkylsulphide fractions hampers the assessment of reliable isotopic compositions for its constituents because of poor chromatographic resolution. It is expected that the carbon isotopic results obtained for the sulphur-bound lipids should also be considered as averaged values since it can be envisaged that carbon skeletons are coming from several suites of precursors. Furthermore, it was demonstrated in an earlier communication that, for example, in the polar fraction from another marl layer from the Vena del Gesso basin, the sulphur-bound *n*-alkanes in the macromolecules with a particular carbon number have sulphur-linkages at different positions of the carbon skeletons, indicating the presence of different precursors possibly biosynthesised by different biota (Kohnen *et al.*, 1991a).

The isotopic results presented here clearly confirm the hypothesis that a specific carbon skeleton present in different modes of occurrence can have different biosynthetic precursors which are possibly derived from different biota. These findings have important implications for palaeoenvironmental assessment studies using biomarkers.

Reconstruction of palaeoenvironments through the combined application of organic sulphur and isotope geochemistry

The mode of occurrence of a carbon skeleton provides information regarding the location and nature of the original functional group(s) in the precursor biolipids. In conjunction with carbon isotopic analysis, the three types of information contained in a biochemical [i.e. the carbon skeleton, functional group(s) location(s), stable carbon isotopic composition] can now be retrieved from sedimentary organic compounds. Hence, this combined approach leads to recognition of palaeobiochemicals present in the depositional environment and allows a more detailed reconstruction of the palaeobiological community. A few illustrations of this combined approach will be given here, since a more elaborate palaeoenvironmental reconstruction of the Vena del Gesso sediments by the combined application of organic sulphur and isotope geochemistry will be published elsewhere (Kohnen *et al.*, 1992b).

The mode of occurrence and structure of the dinosterane carbon skeleton (Table 2) strongly suggests that its precursor (bio)lipid is dinosterol or its early diagenetic product dinosterene (Volkman, 1988; Summons *et al.*, 1987). This specific steroid is biosynthesised exclusively by dinoflagellates (Boon *et al.*, 1979) which are photoautotrophic algae. Therefore, its $\delta^{13}C$ value (Table 2) reflects the isotopic composition of the primary biomass. Variations in the isotope ratios of the dinosterane skeleton or of other primary products may be used to trace variations in CO_2 levels (Popp *et al.*, 1989; Jasper and Hayes, 1990). The variations in concentration of the macromolecularly sulphur-bound dinosterane in the depth sequence reflect variations in abundance of the biolipid dinosterol and thus in the abundance of dinoflagellates in the palaeoenvironment [Fig. 11(a)].

The mode of occurrence of the C_{40} di-aromatic carotenoid carbon skeleton (Table 2) reveals that its precursor lipids are the poly-unsaturated di-aromatic carotenoids which are known to be biosynthesised by *Chlorobiaceae*, photosynthetic green sulphur bacteria (Liaaen-Jensen, 1978). However, aromatic

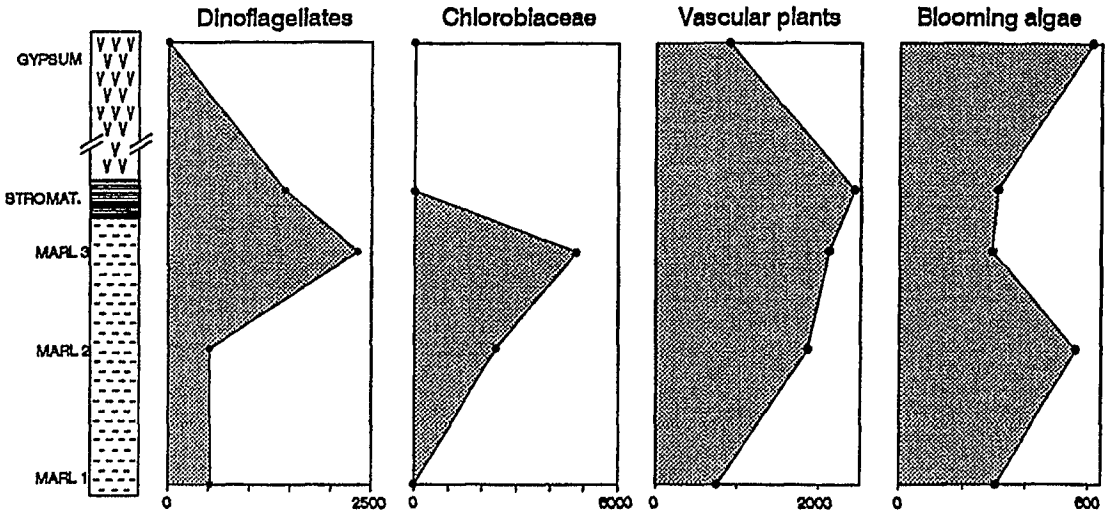

Fig. 11. Depth profiles of: (a) the macromolecularly sulphur-bound dinosterane carbon skeleton, (b) the macromolecularly sulphur-bound di-aromatic carotenoid carbon skeleton, (c) the "free" hentriacontane carbon skeleton and (d) the macromolecularly sulphur-bound hentriacontane carbon skeleton in an evaporitic cycle of the Vena del Gesso basin. Locations of Marl-1, Marl-2, Marl-3, Stromatolitic Limestone and Gypsum samples are indicated on the schematic lithological column.

carotenoids also occur abundantly in certain species of sponges (Liaaen-Jensen *et al.*, 1982), though it was acknowledged that this may be due to bacterial symbionts (Imhoff and Trüper, 1976). The $\delta^{13}C$ -value of these carotenoid-derived compounds (-10.7 ± 0.6‰) is consistent with an origin from green sulphur bacteria in which the anaerobic phototropic CO_2 fixation takes place via the reverse-TCA cycle and results in biomass with an anomalously heavy carbon isotopic composition (Quandt *et al.*, 1977; Sirevag *et al.*, 1977; Summons and Powell, 1986,. 1987). These chemical and isotopic data irrefutably mark the presence of green sulphur bacteria in the palaeoenvironment. In turn, palaeoenvironmental conditions can be deduced from the unique habitat of these bacteria; these anoxygenic photosynthetic organisms thrive in that part of the photic zone in which stable anoxic conditions (i.e. hydrogen sulphide present) are established, a situation often encountered in a stratified water column. The depth profile depicted in Fig. 11(b) reflects the variations in abundance of green sulphur bacteria and the corresponding palaeoenvironmental conditions.

Both these examples are rather straightforward since the carbon skeletons have a unique biochemistry and precursor–product relationships are therefore clearly established on the basis of the structural features of their carbon skeletons. In the case of the n-C_{31} carbon skeleton, the establishment of a product–precursor relationship is not as easy since this carbon skeleton occurs widely in the biosphere. The combined approach resolves at least two different precursors originating from different biota. The "free" hentriacontane, like the other long-chain n-alkanes (C_{25}–C_{30}), is apparently biosynthesised as such. In addition, the free long-chain n-alkanes (C_{25}–C_{30}) exhibit an odd carbon number predominance which finds its numerical expression in the "carbon preference index" [CPI, (Bray and Evans, 1961), e.g. CPI = 6.3 in Marl-2]. This high CPI is characteristic for suites of n-alkanes biosynthesised by vascular plants (Eglinton and Hamilton, 1963). The carbon isotopic composition of the "free" long-chain n-alkanes ranges between -28.8‰ and -29.6‰ and is consistent with a terrigenous source (Popp *et al.*, 1989). On the basis of its mode of occurrence and its isotopic composition, the free hentriacontane can be related to higher plants and the variations in the depth profile are therefore considered to be the sedimentary expression of the variations in the contribution of terrigenous organic matter in the Vena del Gesso basin [Fig. 11(c)]. The sulphur-bound n-C_{31} carbon skeleton in the macromolecules, on the other hand, may reflect mono- or poly-unsaturated n-C_{31} alkene(s) precursor biolipids. Long-chain n-alkenes are abundant in the coccolithophorid *Emiliania huxleyi* (Volkman *et al.*, 1980; Wakeham *et al.*, 1992) and in the (freshwater) green alga *Botryococcus braunii* (Metzger *et al.*, 1985). However, there are apparently considerable variations in the distribution of these alkenes in different strains (or due to different growth conditions) of *E. huxleyi*. For example, a recently analysed strain was dominated by 31:2, 33:3 and 34:4 alkenes and another was dominated by 31:2 and 37:3 alkenes (Volkman *et al.*, 1980; Wakeham *et al.*, 1992). In view of this, an algal source of the mono- or polyunsaturated C_{31} n-alkene(s) is feasible. However, the isotopic composition of this biolipid (-17.6‰) is rather unusual for algal lipids especially since other algae, such as the photoautotrophic dinoflagellates, biosynthesised lipids with $\delta^{13}C$ values of *ca* -26‰ (Table 2). A possible explanation for this enigma could be that the source alga(e) were periodically blooming and causing a significant drop in the concentration of CO_2 in the water. Consequently, the biosynthesised algal biomass became enriched in ^{13}C. This hypothesis is intriguing if one realises that the specific source of these biolipids is still unknown but that on the basis of their isotopic signatures information concerning the habitat of their biological sources may be revealed. The concentration profile of the macromolecularly sulphur-bound n-C_{31} carbon skeleton is thought to be representative for the abundance of yet unknown algae which were periodically blooming.

It is emphasised that in this paper the functional groups of postulated novel palaeobiochemicals are represented by double bonds, although it is possible that other functional groups may have reacted with inorganic sulphur species during the earliest stages of diagenesis. For example, Kohnen *et al.* (1992a) provided some evidence that the macromolecularly sulphur-bound steroids in a wide range of samples are, at least in part, the result of reactions of an S_N2 reaction of inorganic sulphur species with esterified sterols or stanols.

It is also noteworthy that compound-specific isotope analyses may help to establish genetic relationships between different lipids. For example, the C_{20} HBI shows an isotopic composition which is similar to that of the sulphur-bound n-C_{31} carbon skeleton in the macromolecules (Table 2). This similarity in $\delta^{13}C$ values, which are relatively unique in the sediment analysed, justifies the proposal of a common source for their precursors. An algal source for the C_{20} HBI biolipid is consistent with the fact that a C_{20} HBI alkane has been identified in field samples dominated by the green alga *Enteromorpha prolifera* (Rowland *et al.*, 1985).

In sediments where natural sulphurisation of the organic matter did not occur during early diagenesis, biolipids with the same carbon skeleton but different original functionalities are not resolved from each other since they are not "archived" in different modes of occurrence. Consequently, retrieval of the second type of information present in the precursor biochemicals (i.e. functional group information) is not possible. The widespread occurrence of OSCs

(ten Haven *et al.*, 1990) and the fact that reduced inorganic sulphur species are present in any anoxic, organic-matter-containing Recent marine sediment (Berner, 1985) suggests that natural sulphurisation of functionalised (bio)lipids is a ubiquitous process so that the above discussed "palaeobiochemical" approach is potentially a versatile method for providing valuable data on the origins of organic matter in immature marine sediments.

CONCLUSIONS

Compelling evidence is presented that a carbon skeleton which is present in different modes of occurrence may have different biosynthetic precursors with the same carbon skeleton derived from different biota. Moreover, it is demonstrated that the combined application of organic sulphur and isotope geochemistry in the analyses of fossil organic matter enables the reconstruction of suites of palaeobiochemicals present in the depositional environment and thus allows for identification of the biological sources with improved specificity.

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