Pergamon

Geochimica et Cosmochimica Acta, Vol. 61, No. 10, pp. 2065–2082, 1997

0016-7037/97 $17.00 + .00

PII S0016-7037(97)00062-8

A molecular stable carbon isotope study of organic matter in immature Miocene Monterey sediments, Pismo basin

STEFAN SCHOUTEN,[1] MARTIN SCHOELL,[2] W. IRENE C. RIJPSTRA,[1] JAAP S. SINNINGHE DAMSTÉ,[1] and JAN W. DE LEEUW[1]

[1]Department of Marine Biogeochemistry and Toxicology, Netherlands Institute for Sea Research (NIOZ), 1790 AB, Den Burg, Texel, The Netherlands

[2]Chevron Petroleum Technology Company, 1300 Beach Boulevard, La Habra, California 90631, USA

(*Received August* 8, 1996; *accepted in revised form January* 29, 1997)

Abstract—The 300 m section of the Miocene Monterey Formation outcropping at Shell Beach (Pismo basin; ca. 15–11 Ma) is composed of calcareous phosphatic (15.1–14.5 Ma) and siliceous facies (14.5–11.0 Ma). An objective of this paper is to document lateral paleoenvironmental changes in the Miocene Monterey Formation by comparing the Shell Beach (SB) profile with the Naples Beach (NB) section in the Santa Barbara-Ventura basin (Schouten et al., 1997) which is ~80 km to the south. Eight samples (one sample representing, on average, a time period of ca. 2000 y) from this section were analyzed for variations of extractable biomarkers and their carbon isotopic signatures as indicators for paleoenvironmental change during the Miocene. Saturated hydrocarbons present include 28,30-dinorhopane, phytane, *n*-alkanes (C_{17}–C_{31}), lycopane, and 17β,21β(H)-homohopane. The biomarkers released after desulfurization of the polar fractions predominantly consist of phytane, 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane, C_{17}–C_{31} *n*-alkanes, regular 5α- and 5β-steranes, dinosteranes, and (22R)-17β,21β(H)-pentakishomohopane. Steranes have similar carbon isotopic compositions (−25 to −27‰) throughout the section and are isotopically similar at both sites, indicating laterally similar and vertically stable environmental conditions for algae living in the upper part of the photic zone. Free and S-bound *n*-alkanes at SB mainly originate from marine organisms and not from terrestrial sources as in the NB section. S-bound pentakishomohopane is ca. 1–4‰ depleted compared to the steranes and is thought to be derived from the deeper water dwelling cyanobacteria. These findings are consistent with stable carbon isotopic data obtained for these compounds from Middle Miocene Monterey sediments at Naples Beach and indicates similar environmental conditions in the depositional environments of the Santa Barbara-Ventura and the Pismo basin. S-bound highly branched isoprenoids have, at both sites, different isotopic compositions indicating the presence of different diatom species, special growth conditions, or different bloom periods in the Pismo basin. The carbon isotopic composition of 28,30-dinorhopane shifts to more depleted values up section, suggesting that the dinorhopane-synthesizing organism or organisms live on CO_2, which is isotopically influenced by methane production and oxidation. The C_{31} hopane is enriched by 1–4‰ in ^{13}C compared to other hopanes and steranes. Specific bacteria, possibly heterotrophs, may have been the organisms producing this compound.

1. INTRODUCTION

The analysis of organic compounds and their stable carbon isotopic signatures has become increasingly powerful in the reconstruction of biogeochemical processes and determination of the sources of extractable sedimentary lipids (e.g., Collister et al., 1992; Freeman et al., 1990, 1994; Kohnen et al., 1992a,b; Kenig et al., 1995; Schoell et al., 1994a; Schouten et al., 1997b). Although our knowledge of processes that control carbon isotopes at the molecular level in extant organisms is still limited, stable carbon isotopic compositions of molecular fossils can provide important clues for the reconstruction of past depositional environments. As more data on extant organisms (e.g., Summons et al., 1994; Laws et al., 1995) and modern depositional settings (e.g., Freeman et al., 1994) will become available, it may be possible to reconstruct past paleoenvironments more correctly and in ever more detail.

Our studies of the distribution patterns and carbon isotopic compositions of free and S-bound hydrocarbons of the Monterey Formation outcropping at Naples Beach (Santa Barbara basin; Schoell et al., 1994a; Schouten et al., 1997a,b) revealed regional and general features of the paleoenvironment. For example, the observed difference in carbon isotopic composition of free and S-bound cholestane (**I**) and pentakishomohopane (**II**) parallels the Miocene cooling of deep photic zone waters (Schoell et al., 1994a). Carbon isotopic data confirmed a terrestrial origin for the free long-chain *n*-alkanes and enabled the assessment of the relative amount of terrestrial input into the sediments (Schouten et al., 1997b).

To further substantiate these combined applications of stable carbon isotope compositions and distribution patterns of organic compounds, it is also necessary to examine lateral variations in sedimentary formations (cf. Schoell et al., 1994b). For this purpose, a stratigraphic equivalent to the Monterey formation at Naples Beach was sampled at Shell Beach (Pismo basin, ca. 80 km from Naples Beach; Fig. 1). The comparison will help differentiate compounds recording local changes from those monotoring global changes during

Fig. 1. Map showing the geographical locations of the sample sites at Naples Beach and Shell Beach, Southern California (USA).

the Miocene. In analogy to our study of the NB section, the samples were analyzed for free and S-bound biomarkers, and their isotopic compositions were determined. In addition, the Pismo basin and Santa Barbara-Ventura basin samples were analysed using chemical degradation (Hoefs et al., 1997) for ether-bound carbon skeletons. The isotopic composition of the released carbon skeletons were analyzed as well. We will specifically discuss compounds for which recent work provided a much better understanding of the controlling factors of carbon isotopic compositions of organisms (e.g., Summons et al., 1994; Goericke et al., 1994; Laws et al., 1995).

2. EXPERIMENTAL

2.1. Extraction and Fractionation

Eight samples from the Pismo basin outcropping at Shell Beach were extracted, and the extracts were fractionated as described in Schouten et al. (1997a). Briefly, the asphaltenes were removed from the bitumens and the residual maltene fractions were then separated into apolar and polar fractions. The polar fractions were desulfurized using nickel boride (see section 2.2). The apolar fractions were further separated by argentation thin layer chromatography into an A1-fraction (saturated hydrocarbons) and an A2-fraction (unsaturated hydrocarbons and thiophenes). The fractions were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The A1-fractions were separated in adduct and nonadduct subfractions using molecular sieves 5 Å (O'Connor et al., 1962). These subfractions and selected A2-fractions were analyzed by isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC-MS; Merrit and Hayes, 1994).

2.2. Nickel Boride Desulfurization

In a typical desulfurization experiment, 20 μg of the thiophene standard [2,3-dimethyl-5-(1,1-d_2-hexadecyl)thiophene, **III**] was added to ~20 mg polar fraction. The polar fractions were then desulfurized with nickel boride as described previously (Schouten et al., 1993). The hydrocarbons released were isolated by column chromatography with Al_2O_3 by elution with hexane/dichloromethane (9:1 v/v) and quantitatively analyzed by GC and GC-MS. The residual polar fraction was eluted with dichloromethane/methanol (1:1 v/v). A selected set of hydrocarbon fractions obtained after desulfurization of the polar fractions (SB-1, SB-2, SB-3, and SB-6) were separated in adduct and nonadduct subfractions using molecular sieves 5 Å (O'Connor et al., 1962). Samples SB-13 and SB-18 were separated in adduct and nonadduct subfractions using Silicalite (PQ Zeolites; West et al., 1990). These subfractions were analyzed by irm-GC-MS.

2.3. Hydrogen Iodide Treatment

A selected set (SB-1, SB-13, and SB-18) of residual polar fractions left after desulfurization, isolated by column chromatography with Al_2O_3, was treated with hydrogen iodide to release ether-bound lipids (Hoefs et al., 1997). Briefly, the residual polar fraction was, after addition of the thiophene standard, refluxed with 56 wt% HI-solution (in H_2O) for 1 h. The released alkyliodides were isolated by column chromatography with Al_2O_3 (eluent hexane/dichloromethane 9:1 v/v) and reduced to alkanes by refluxing with $LiAlH_4$ in dioxane for 1.5 h. The obtained fractions were quantitatively analyzed by GC and GC-MS and analyzed by irm-GC-MS.

2.4. Gas Chromatography (GC)

GC was performed using a Carlo Erba 5300 or a Hewlett Packard 5890 instrument, both 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 He as carrier gas. Both a flame ionization detector (FID) and a S-selective flame photometric detector (FPD) were applied simultaneously by using a stream-splitter at the end of the column (split ratio FID:FPD = 1:2). The samples were dissolved in ethyl acetate and injected at 75°C. Subsequently, the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C where the temperature was maintained for 10 min.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was performed using a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of 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) or with an HP Ultra 1 column (50 m × 0.32 mm) coated with crosslinked methyl silicone (film thickness = 0.5 μm). The carrier gas was He. The samples were on column injected at 50°C and, in case of a CP Sil-5 column, the oven was subsequently 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. In the case of the Ultra 1 column, the oven was programmed to 130°C at 10°C/min and then at 3°C/min to 320°C/min, at which it was held for 30 min.

2.6. Carbon Isotope Analysis

The isotope ratio monotoring GC-MS equipment (irm-GC-MS; Finnigan Delta S) used for samples SB-1 to SB-6 has been described previously (Hayes et al., 1990; Schoell et al., 1992; Merrit and Hayes, 1994). The gas chromatographic column was a 50 m Hewlett Packard Ultra 1 column with 0.32 mm internal diameter and a 0.5 μm stationary phase (crosslinked methyl silicone). The carrier gas was He at a flow rate of 1.5 mL/min. The samples (dissolved in cyclohexane) were on-column injected at 50°C and, subsequently,

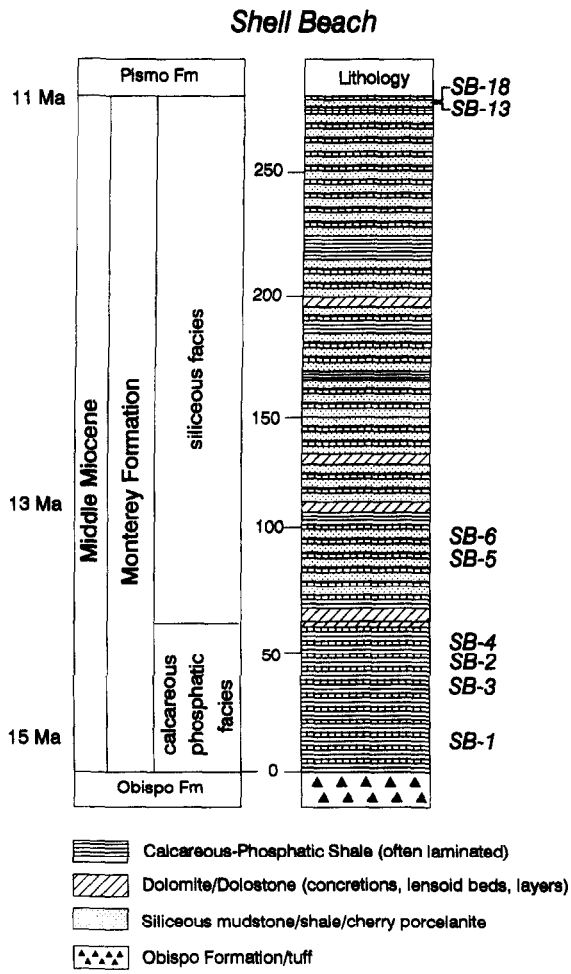


Fig. 2. Stratigraphic profile of the Monterey Formation outcropping at Shell Beach (after Omarzai, 1992).

the oven was programmed to 130°C at 10°C/min and then at 3°C/min to 320°C, at which it was held for 40 min.

Samples SB-13 and SB-18 were analyzed using a Delta C irm-GC-MS. The gas chromatographic column was a 25 m CP Sil 5 column with 0.32 mm internal diameter and 0.12 μm stationary phase. The carrier gas was He and the samples (dissolved in hexane) were on-column injected at 70°C and, subsequently, the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C, at which is was held for 15 min.

All values reported were determined by at least two analyses, and the results were averaged to obtain a mean value and to calculate the standard deviation. The stable carbon isotope compositions are reported in the delta notation against the PDB ^{13}C standard. An internal standard (6,6-d_2-2-methylheneicosane **IV**, Schouten et al., 1997a; Kohnen et al., 1990) was used for monitoring the reproducibility during the project; the pooled standard deviation and isotopic values are −24.5 ± 0.6‰ (SB-1 to SB-6, Delta S) and −25.4 ± 0.4‰ (SB-13 and SB-18, Delta C). The difference in isotope value between internal standards in samples SB-1 to SB-6 and SB-13 to SB-18 is caused by the use of two different batches of synthetic internal standards.

2.7. Quantitation

The concentrations of selected compounds (mg/kg bitumen) in free hydrocarbon fractions and desulfurized polar fractions were obtained by integrating their peak areas and that of the internal standard (6,6-d_2-2-methylheneicosane, **IV**) in the FID-chromatograms. The concentrations of compounds in hydrocarbon fractions released by HI-treatment of the residual polar fractions were obtained by integrating their peak areas and that of the internal standard (2,3-dimethyl-5-(1,1-d_2-hexadecyl)thiophene).

Table 1. General data of Monterey sediment samples outcropping at Shell Beach.

Sample	Facies	Height[1] (m)	Age (Ma)	TOC[2]
SB-1	Phosphatic	18	15.0	3.4
SB-2	Phosphatic	45	14.6	8.6
SB-3	Phosphatic	39	14.7	3.2
SB-4	Phosphatic	57	14.5	3.8
SB-5	Siliceous	84	13.2	3.9
SB-6	Siliceous	96	13.1	5.2
SB-13	Siliceous	275	11.3	14.8
SB-18	Siliceous	285	11.1	3.7

[1] from base of the Monterey Formation.
[2] TOC: Total organic carbon content in weight percent.

3. RESULTS

Eight samples were collected from the Monterey Formation of the Pismo basin outcropping at Shell Beach (Fig. 2; after Omarzai, 1992) ranging in age from 15 to 11 Ma. The age of the eight samples was estimated by projecting the measured distance of a sample from the base of the formation (i.e., the boundary between the older Obispo Formation and the Monterey Formation) into an existing palaeomagnetic age curve (Omarzai, 1992). Since the thickness of the sampled layers were ~20 cm on average and the sedimentation rate was on average ~94 m/Ma (Omarzai, 1992), it can be estimated that our samples represent time periods of ~2000 y. Four samples were taken from the phosphatic facies and four samples from the siliceous facies. Total Organic Carbon (TOC) contents of the samples vary between 3 and 14% (Table 1). The soluble organic matter is characterized by high asphaltene contents (40–60% of total bitumen; Table 2). The amount of apolar substances is accordingly low (~3% of total bitumen).

Table 2. Extraction data of Monterey sediment samples at Shell Beach.

Sample	EOM[1] (%)	Asph.[2] (%)	Malt.[2] (%)	Polar[2] (%)	Apolar[2] (%)
SB-1	3.8	50	44	25	2.6
SB-2	4.2	50	41	26	2.5
SB-3	5.9	57	43	22	2.6
SB-4	2.4	41	59	29	3.0
SB-5	2.8	45	53	27	3.0
SB-6	5.4	58	42	29	3.3
SB-13	7.1	59	33	19	4.4
SB-18	5.9	58	34	15	2.8

[1] EOM = Extractable organic matter as a percentage of TOC.
[2] As a percentage of total extract.

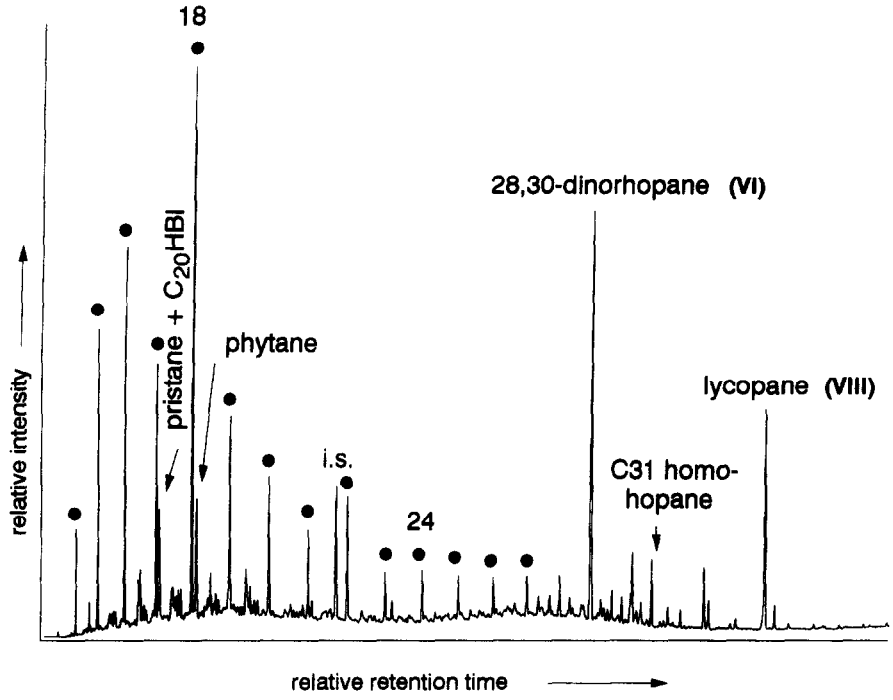


Fig. 3. Gas chromatogram of saturated hydrocarbon fractions of sample SB-1. Filled circles indicate *n*-alkanes. i.s. = internal standard. Numbers in parentheses correspond to structures shown in appendix.

3.1. Saturated Hydrocarbon Fractions

Compounds present in the hydrocarbon fractions (e.g., Fig. 3) include *n*-alkanes, pristane, phytane, a C_{20} highly branched isoprenoid [2,6,10-trimethyl-7-(3-methylbutyl)-dodecane, **V**; C_{20} HBI], 17β,21β(H)-28,30-dinorhopane (**VI**), 17β,21β(H)-homohopane (**VII**), and lycopane (**VIII**). Steranes and 17β,21β(H)-25,28,30-trinorhopane (**IX**) are present in trace amounts. The *n*-alkanes are dominated by low-molecular-weight *n*-alkanes (C_{15}-C_{22}). The higher-molecular-weight *n*-alkanes (C_{23}–C_{31}) have a slight odd-over-even carbon number predominance (Table 3).

Concentrations of dinorhopane vary widely with a maximum (~3300 mg/kg bitumen) at the top of the formation (SB-18; Fig. 4). Lycopane concentrations vary between 50 and 120 mg/kg bitumen. 17β,21β(H)-Homohopane appears in low concentrations in the lower part of the phosphatic facies and in higher concentrations in the silicious facies.

Table 3. Carbon-preference-indices as determined by GC-analyses.

Sample	CPI_{24-33} free	CPI_{24-33} S-bound
SB-1	0.9	0.7
SB-2	1.5	0.6
SB-3	1.4	0.6
SB-4	1.5	0.6
SB-5	1.7	0.5
SB-6	2.2	0.6
SB-13	1.3	1.1
SB-18	1.0	2.6

The pristane and phytane concentrations covary somewhat and are the highest in sample SB-13.

3.2. Desulfurized Polar Fractions

Hydrocarbons released by desulfurization of polar fractions (e.g., Fig. 5) are *n*-alkanes, phytane, a highly branched isoprenoid [2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane; C_{25} HBI, **X**], steranes, and some triterpenoids. The *n*-alkanes range from C_{17} to C_{30} and show a slight even-over-odd carbon number predominance for *n*-alkanes possessing more than 20 carbon atoms except for samples SB-13 and SB-18, where there is a relatively high abundance of the *n*-C_{25} and *n*-C_{27} resulting in relatively high CPI values (Table 3). The steranes range in carbon number from C_{26} to C_{30} and are composed of the 22R 5α- and 5β-steranes. The distribution of steranes in the desulfurized polar fraction of sample SB-1 is revealed by mass chromatograms of *m/z* 217 (4-desmethylsteranes; upper box, Fig. 6 and *m/z* 231 (4-methylsteranes, lower box, Fig. 6). 27-Nor-24-methyl-5α-cholestane (**XI**), previously identified by Schouten et al. (1994), elutes just after the 5α-cholestane. Just before the 24-ethyl-5α-cholestane (**Ic**) elutes 4-desmethyldinosterane (**XIIa**), which has tentatively been identified in Messinian sediments from Italy (Kenig et al., 1995; Schaeffer et al., 1995). The mass chromatogram of m/z 231 reveals the presence of 4-methylsteranes ranging in carbon number from C_{28} to C_{30}. Furthermore, two isomers of dinosterane, which were tentatively identified as 4α-20R,22R,23S-dinosterane and 4α-20R,22R,23R-dinosterane (J. M. Moldowan, pers. commun.), are present. Two unsaturated steranes are present in

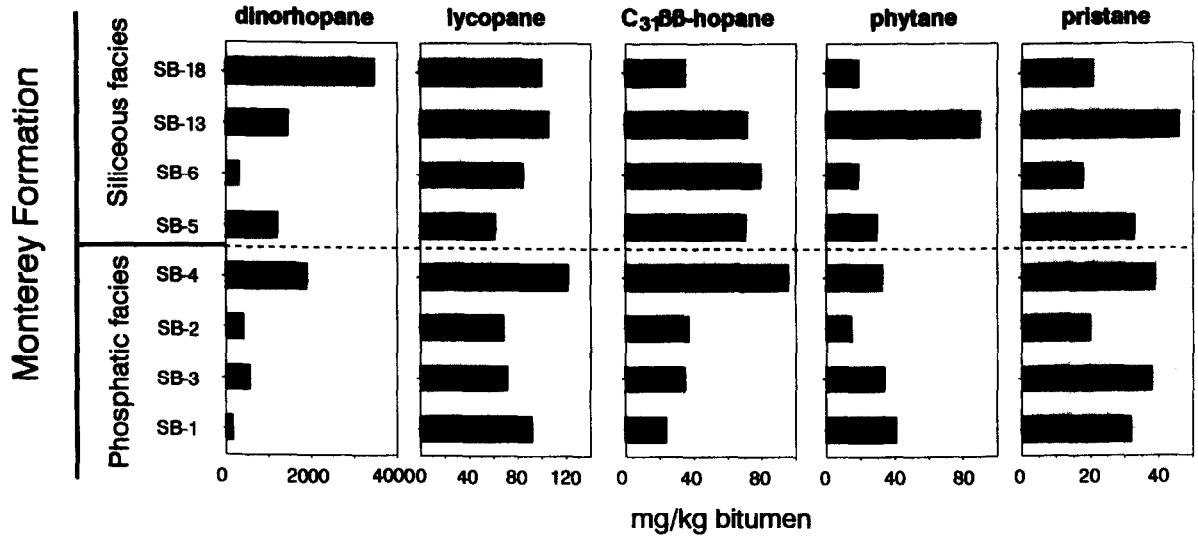


Fig. 4. Concentrations of selected free saturated hydrocarbons in sediments of the Monterey Formation from the Pismo basin (Shell Beach).

relatively high abundance: 4-desmethyldinoster-22-ene and dinoster-22-ene. Their presence confirms previous reports which indicated that dinosterenes are difficult to hydrogenate using PtO_2/H_2, probably because the double bond is sterically hindered (Kohnen et al., 1991). The triterpenoids in the desulfurized polar fraction are dominated by the 17β,21β(H)-pentakishomohopane (**II**; e.g., Fig. 5). Gammacerane is also present, though in relatively small amounts.

Recent evidence has shown that treatment of polar fractions of Recent sediments with either nickel boride (Hartgers et al., 1995) or Raney nickel (Prahl et al., 1996) can result in the formation of phytane from phytol. In our case, however, we consider the phytane released here by the nickel boride treatment is predominantly S-bound due to the facts that the Monterey samples are consolidated sediments and that low-molecular-weight S compounds with phytane carbon skeletons are ubiquitous in our samples. However, we can not exclude the possibility that phytol contributed to a minor extent.

S-bound phytane is most abundant in sample SB-18 and has a different concentration profile than free phytane (Fig. 7). S-bound C_{35} hopane maximizes in concentration in sam-

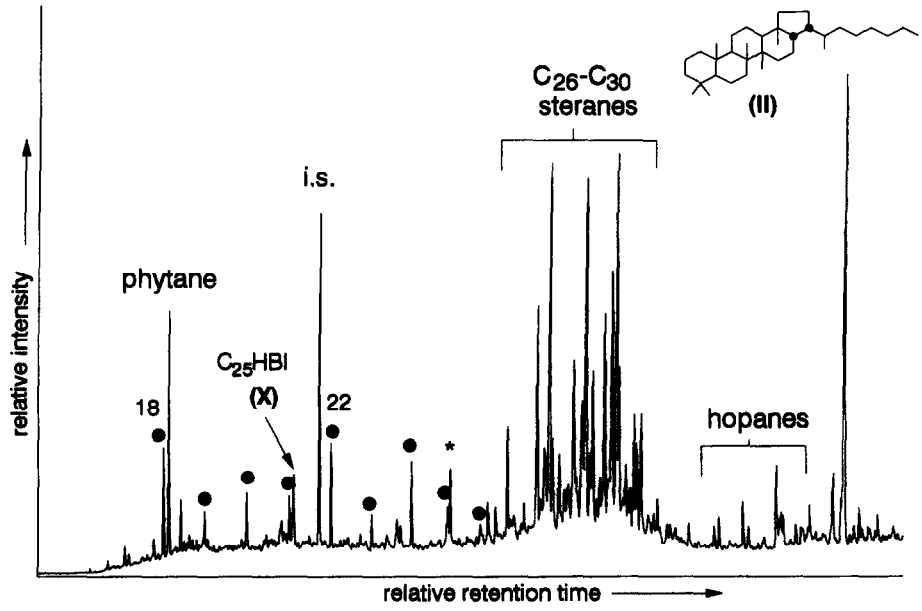


Fig. 5. Gas chromatogram of desulfurised polar fraction of sample SB-1. Filled circles indicate *n*-alkanes. Numbers indicate total number of carbon atoms. Numbers in parentheses correspond to structures shown in appendix. i.s. = internal standard. * = contamination.

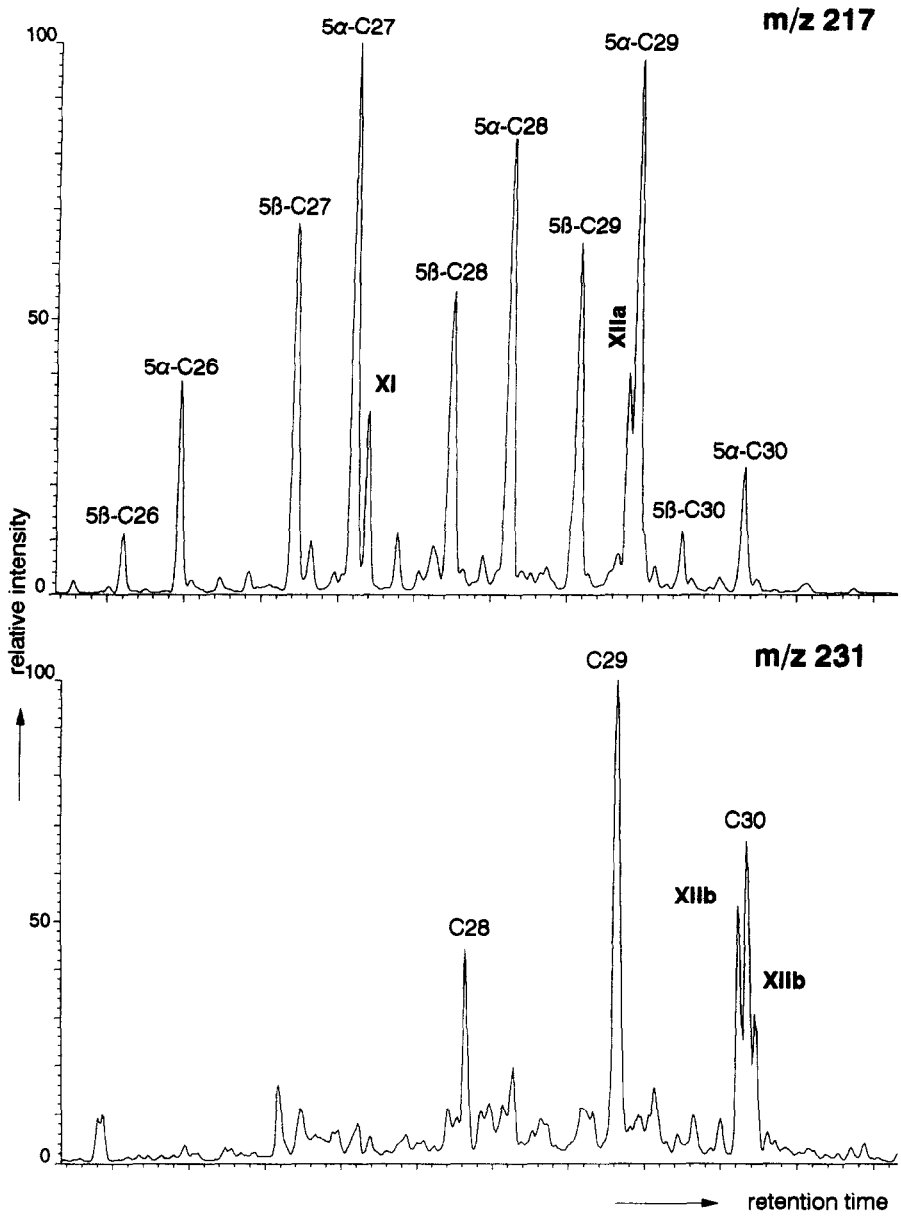


Fig. 6. Partial mass chromatograms of m/z 217 for 4-desmethylsteranes (top) and m/z 231 for ring-A methylated steranes (bottom) of the desulfurized polar fraction of sample SB-1.

ple SB-2 while the concentration of S-bound 5α-cholestane (but also the other steranes) maximizes in SB-13. The S-bound C_{25} HBI depth profile shows that the compound is present in very low concentrations in the extracts of samples SB-2 and SB-6. The concentration profile of dinosterene and 4-desmethyldinosterene are similar to each other but different when compared to the profiles of the other steranes.

3.3. HI-treated Residual Polar Fractions

The residual polar fractions after desulfurization of the polar fractions of SB-1 to SB-3, SB-13, and SB-16 were treated with HI/$LiAlH_4$, which converts alcohols and ether-bound carbon skeletons to alkanes (Hoefs et al., 1997). The hydrocarbons released by HI/$LiAlH_4$-treatment of the residual polar fractions in the case of Shell Beach usually consist of n-alkanes with an even-over-odd carbon number predominance, phytane, and a complex mixture of steranes and sterenes (e.g., Fig. 8). In some samples 2,6,10,14,18-pentamethylicosane (**XIII**) is found in addition to sometimes very high amounts of acyclic and cyclic biphytanes (**XIV**–**XVII**). The mass spectra of the acyclic, monocyclic, and bicyclic biphytanes are similar to those described by de Rosa and Gambacorta (1988). The mass spectrum of the tricyclic biphytane seems, however, different to that described by de Rosa and Gambacorta (1988) for **XVIII** and is comparable to that described by Meunier-Christmann (1988). Based on its mass spectrum, Hoefs et al. (1997) proposed a different

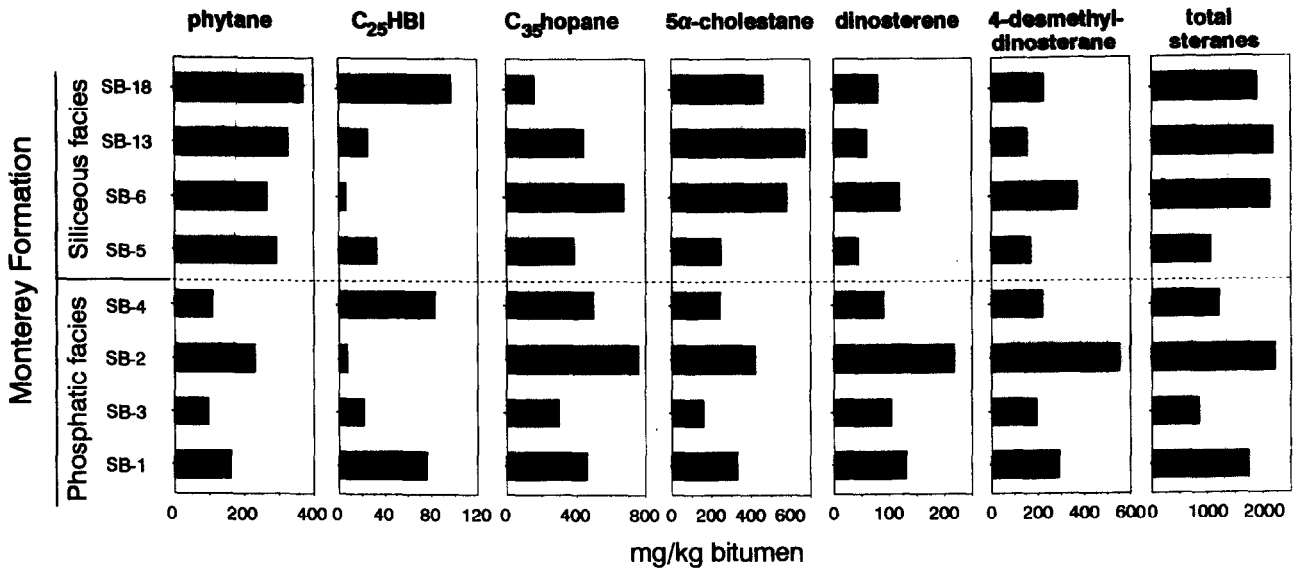


Fig. 7. Concentrations of selected S-bound carbon skeletons in Monterey samples from Pismo basin (Shell Beach).

position for one of the cyclopentane rings resulting in structure **XVII.**

3.4. Carbon Isotopic Compositions

The adduct and nonadduct fractions of free hydrocarbon fractions, selected thiophene fractions, and the adduct and nonadduct fractions of the hydrocarbon fractions released after desulfurization of the polar fractions (except for SB-4 and SB-5) and HI-treatment of the residual polar fractions of SB-1 and SB-18 were analyzed by irm-GC-MS (Tables 4–6). Several compounds, although present in relatively high amounts, were not baseline separated and their carbon isotopic compositions are, therefore, not reported. The two dinosterane isomers and 4-methyl-24-ethylcholestane are severely coeluting. However, since it is very likely that both these compounds are predominantly derived from dinoflagellates (Summons et al., 1987), the carbon isotopic composition of the whole mixture was determined. The values obtained were very similar to those of the carbon isotopic

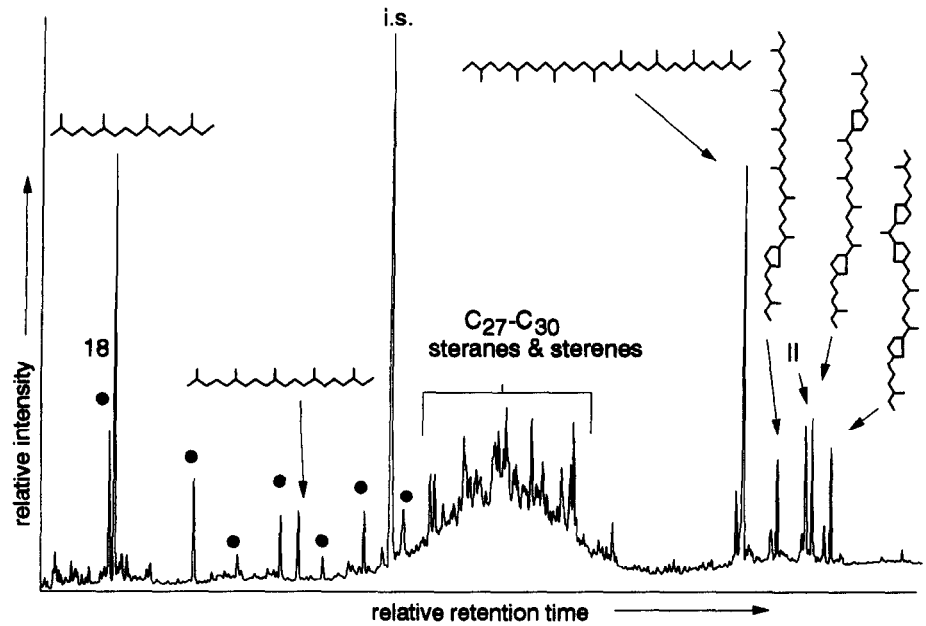


Fig. 8. Gas chromatogram of the hydrocarbon fraction obtained by HI/$LiAlH_4$-treatment of the residual polar fraction of sample SB-1. Dots indicate *n*-alkanes. Numbers indicate total number of carbon atoms. i.s. = internal standard.

Table 4. Stable carbon isotope date of free isoprenoids in Shell Beach samples.

	δ^{13}C-value			
Sample	Phytane	Dinorhopane	C_{31} hopane	Lycopane
SB-1	−28.5 ± 1.2	−26.3 ± 0.1	−24.9 ± 1.0	−25.4 ± 0.3
SB-2	−29.0 ± 0.2	−25.8 ± 0.1	−23.0 ± 0.1	−25.8 ± 0.3
SB-3	−27.9 ± 0.1	−25.8 ± 0.1	−23.0 ± 0.2	−24.7 ± 0.1
SB-4	−30.5 ± 0.3	−24.5 ± 0.1	−23.2 ± 0.6	−24.7 ± 0.3
SB-5	−27.8 ± 1.6	−25.4 ± 0.1	−22.2 ± 0.3	−23.1 ± 0.2
SB-6	−28.8 ± 0.9	−24.7 ± 0.4	−22.6 ± 0.4	−23.5 ± 0.4
SB-13	n.d.	−27.6 ± 0.1	−22.5 ± 1.2	−23.6 ± 0.8
SB-18	−30.0 ± 0.2	−28.8 ± 0.1	−22.6 ± 0.4	−24.8 ± 0.4

n.d. = not determined.

compositions calculated for 4-desmethyldinosterene and dinosterene. Therefore, these values were averaged and a carbon isotopic composition was derived which was representative of compounds which are probably derived from dinoflagellates.

4. DISCUSSION

Dinoster-22-ene, dinosterane (**XIIb**), and, to a lesser extent, 4-methyl-24-ethylcholestane are considered biomarkers for dinoflagellates (Summons et al., 1987) although the precursor of dinosterene and dinosterane, dinosterol, has also been detected in low amounts in one diatom species (Volkman et al., 1993). The carbon isotopic compositions of these products released by nickel boride treatment in the samples studied are rather constant between −25 and −26‰ (Table 5; Fig. 9a). The isotopic composition of S-bound 5α-cholestane is similar, indicating that it is also derived mainly from primary producers. It is unlikely that the sulfurization process isotopically changed the original pool of steranes since both free and S-bound steranes have rather similar isotopic compositions in Monterey sediments (Schouten et al., 1997b). Thus, we infer that variations of cholestane and dinosterane represent the average isotope variations of primary algal autotrophs (stippled pattern in Fig. 9). The 5α-24-norcholestane has an isotopic composition slightly depleted in ^{13}C compared to those of other steranes. Suzuki et al. (1993) reported an unusually high abundance of 24-nor-5α-cholestane (**XI**) in siliceous sediments from the Miocene Onnagawa formation, which is considered to be an analogue of the Monterey Formation. The authors attributed this to the abundant presence of diatoms in the palaeodepositional environment. Other authors (e.g., Goad and Withers, 1982), however, have reported this compound in marine dinoflagellates. Our data is consistent with an origin from diatoms and/or dinoflagellates.

The carbon isotopic composition of organic carbon of primary producers depends on many environmental factors (e.g., CO_2-concentration, temperature; Hayes et al., 1993) and species specific factors (e.g., cell size, growth rate; Francois et al., 1993; Goericke et al., 1994; Laws et al., 1995). It is thus striking that the ~2000 y-average carbon isotopic composition of cholestane is rather constant through time. This apparent constant carbon isotopic composition may be due to two reasons:

1) The compound is biosynthesized by numerous algae. Any species-specific factors like cell size and growth rates will be averaged and thus, if no large changes occur in the species distribution of the algal community, these factors remain constant through time.

2) The environmental factors did not change significantly at the sample resolution studied. Conditions in the upper part of the photic zone (CO_2-concentration, temperature, etc.) were thus rather constant in the depositional environment of the Pismo basin. This does not imply that conditions were stable all of the time: they may have varied significantly during short periods (certainly at a seasonal level). However, averaged out over periods of 2000 y, these signals may not be apparent in our sample set.

The δ^{13}C-values of compounds derived from dinoflagellates are also rather constant, indicating that, on average, environmental conditions for dinoflagellates were also constant through time, which in turn suggests that they occupied the same part of the water column.

The carbon isotopic composition of the S-bound C_{25} HBI (**X**) has been determined in three samples (Fig. 9b; Table 4). This compound is derived from sulfur incorporation into C_{25} HBI alkenes possessing several double bonds (Sinninghe Damsté et al., 1989; Kohnen et al., 1991). These precursors are thought to be biosynthesized by diatoms (Kohnen et al., 1992a; Summons et al., 1993; Volkman et al., 1994; Schouten et al., 1997b), primarily also since they have only been found in axenic cultures of diatoms (Volkman et al., 1994). The S-bound C_{25} HBIs have isotopic compositions slightly depleted compared to the average algal signal (i.e., cholestane). These C_{25} HBI carbon skeleton occur also in other sediments (e.g., Black Sea, Freeman et al., 1994; Vena del Gesso, Kohnen et al., 1992; Naples Beach, Schouten et al., 1997b; Shark Bay, Summons et al., 1993) but are usually enriched in ^{13}C compared to other algal-derived hydrocarbons, suggested to be due to blooming-effects, different physiology, or assimilation of bicarbonate (Kohnen et al., 1992a; Summons et al., 1993; Freeman et al., 1994; Schouten et al., 1997b). Since this is not the case for the C_{25} HBI in the Pismo basin sediments, this suggests at first glance that the C_{25} HBI-synthesizing diatoms present in the depositional

Table 5. Stable carbon isotope data of S-bound isoprenoids in Shell Beach samples.

	δ ^{13}C-value					
Sample	Phytane	C_{25} HBI	C_{26} 5α-sterane	C_{27} 5α-sterane	"Dinostr."[+]	C_{25} hopane
SB-1	−26.3 ± 0.3	−27.6	−27.2 ± 0.4	−26.2 ± 0.5	−26.1 ± 0.5	−27.7 ± 0.2
SB-2	−25.8 ± 0.1	n.d.	−27.3 ± 0.4	−26.2 ± 0.5	−25.7 ± 0.7	−26.9 ± 0.1
SB-3	−25.2 ± 0.2	−26.2 ± 0.1	−26.1 ± 0.4	−26.4 ± 0.2	−25.0 ± 0.6	−26.8 ± 0.2
SB-6[#]	−24.8	n.d.	n.d.	−25.4	−24.7	−27.9
SB-13	−24.2 ± 0.2	n.d.	n.d.	−25.2 ± 0.6	n.d.	−29.3 ± 0.8
SB-18	−24.1 ± 0.2	−28.3 ± 0.4	n.d.	−25.0 ± 0.2	n.d.	−28.4 ± 0.1

[+] Averaged isotopic composition of 4-desmethyldinosterene,dinosterene and the coeluting mixture of dinosteranes and 4-methyl-24-ethyl-cholestane.

[#] Available material was sufficient for 1 analysis only.

n.d. = not determined

environment were nonblooming species or had a different physiology. Determination of the isotopic composition of the two C_{25} HBI thiophenes present in sample SB-18 (Fig. 10) sheds, however, a different light on this matter. Their isotopic compositions differ by ~11‰ (−33.1‰ vs. −22.6‰) despite the fact that their functionalized precursors probably only differ in the position of the double bonds. Similar phenomena have, for example, been observed for Δ^5- and Δ^7-sterols (~10‰) in particulate organic matter (B. Popp, pers. commun.) and for C_{30} HBI's possessing four and five double bonds in surface sediments of the Indian Ocean (~14‰; M. J. L. Hoefs et al., unpubl. results). These two HBI thiophenes may be considered isotopic endmembers of the C_{25} HBI carbon skeletons present in SB samples, thus implying that the carbon isotopic composition of the C_{25} HBI carbon skeleton released by desulfurization of the polar fraction is a mixture of the two isotopic endmembers. The ^{13}C-enrichment of the C_{25} HBI isomer relative to the cholestane ^{13}C-concentration is consistent with previous observations of ^{13}C enrichment of C_{25} HBI carbon skeletons (Kohnen et al., 1992a; Summons et al., 1993; Freeman et al., 1994; Schouten et al., 1997b). Diatom biomass in modern settings is also characteristically enriched in ^{13}C (Fry and Wainwright, 1991; Raven et al., 1994). The C_{25} HBI isomer, substantially depleted in ^{13}C compared to cholestane (~7‰) and the other C_{25} HBI endmember (by ~10‰), poses a different problem since it can be assumed that the compound is also derived from diatoms (though likely to be quite different species) living in similar habitats. This substantial isotopic difference may be due to a combined effect induced by biological factors (Goericke et al., 1994; Laws et al., 1995) and environmental factors (Hayes, 1993):

1) Biological factors: Two of the main controlling factors of the carbon isotopic compositions of diatoms (and other algae) are growth rate (Takahashi et al., 1991; Laws et al., 1995) and cell size (Goericke et al., 1994). We infer that the C_{25} HBI endmember depleted in ^{13}C is biosynthesized by diatoms having, on average, lower growth rates and/or smaller cell sizes. Conversely, the C_{25} HBI endmember with enriched δ^{13}C-values may be sourced by diatoms which, on average, had higher growth rates (or bloomed regularly) and/or larger cell sizes. This also implies that the precursor molecules of the two thiophenes are biosynthesized by only a limited number of diatom species which had different growth rates and/or cell sizes than that of the average algal community. This is consistent with the findings of Volkman et al. (1994) and Hird and Rowland (1995) that probably only a limited number of diatom species biosynthesize HBIs.

2) Environmental factors: Although the sterane data suggest that the environmental factors averaged out over 2000 y-periods were constant, it still may be possible that conditions changed during short periods of time (certainly at a seasonal level). If the compounds were produced during different periods of time, for instance, due to upwelling events, then the isotopic compositions of the different C_{25} HBIs reflect the different environmental conditions during which these compounds were biosynthesized.

It is difficult, if not impossible, to say which factor controlled the isotopic compositions of the C_{25} HBIs. Considering the magnitude of the difference, a combined effect seems to be the most likely.

Lycopane (**VIII**) has an isotopic composition 1−3‰ enriched in ^{13}C compared to the average algal signal (Fig. 9c). Kohnen et al. (1992a,b) and Wakeham et al. (1993) recently suggested that this compound is biosynthesized by photoautotrophs in the open ocean. If this is the case in the deposi-

Table 6. Stable carbon isotope data of ether-bound isoprenoids in two Shell Beach samples.

	δ ^{13}C-value				
Sample	PME	C40:0	C40:1	C40:2	C40:3
SB-1	−28.6 ± 0.8	−21.1 ± 0.2	−20.1 ± 0.6	−20.3 ± 0.2	−18.7 ± 0.2
SB-18	−29.0 ± 0.8	−22.0 ± 0.2	−22.1 ± 0.6	−22.1 ± 0.7	−21.8 ± 0.3

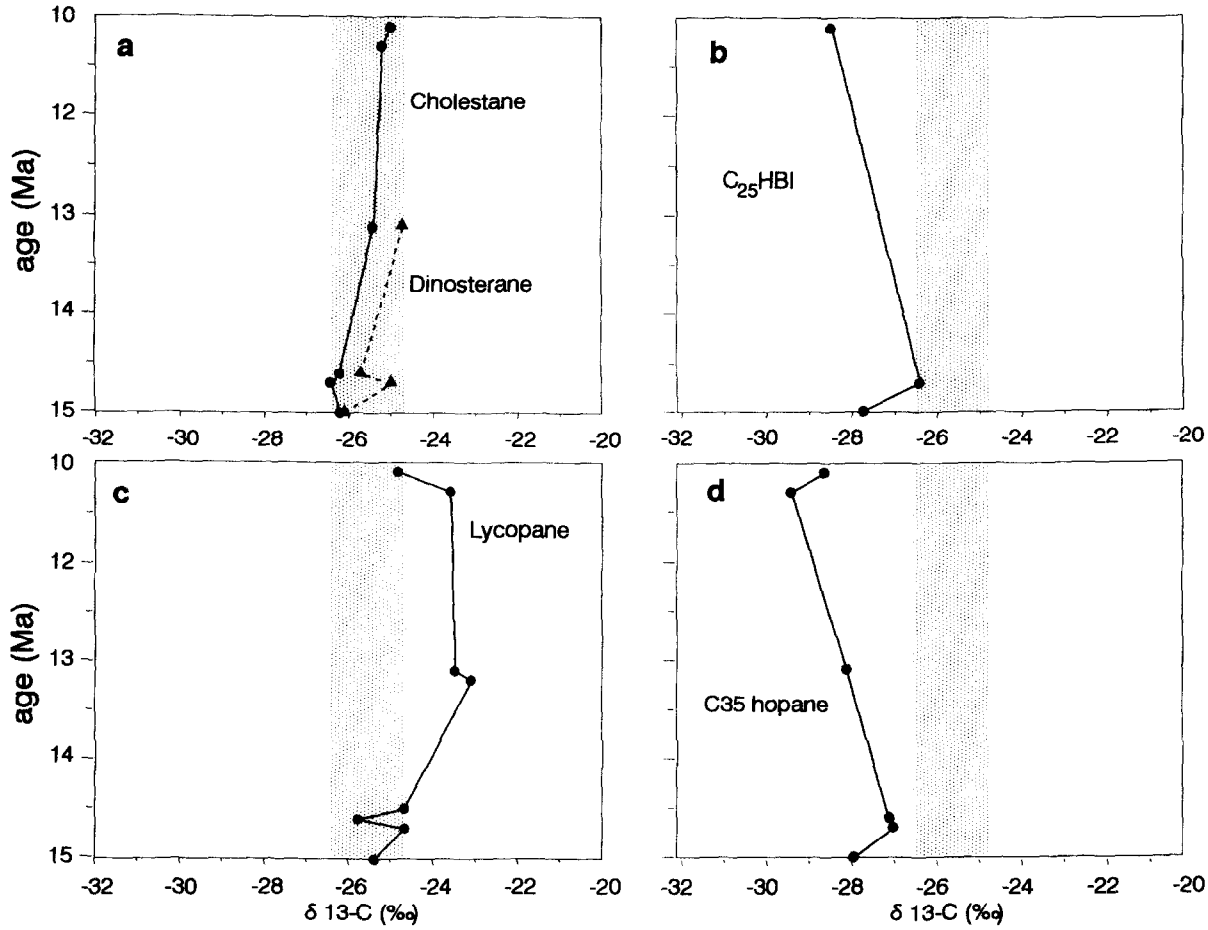


Fig. 9. Stable carbon isotope profiles of selected compounds (a: S-bound 5α-cholestane and S-bound dinosterane, b: S C_{25} HBI, c: free lycopane, and d: S-bound 17β,21β(H)-pentakishomohopane) in Monterey samples from the Pismo basin vs. time (Ma). The stippled area indicates the range of carbon isotopic compositions of S-bound 5α-cholestane and S-bound dinosterane.

tional environment of the Pismo basin, then the enrichment in ^{13}C may be explained by different average growth rates and/or cell size of the lycopane-synthesizing photoautotrophs, though it could also reflect the different time period in which the lycopane was predominantly biosynthesized.

The C_{35} hopane (**II**) is likely derived from cyanobacteria which can occupy a wider range of depths in the water column than algae (Olson et al., 1990). The isotopic composition of C_{35} hopanes is ~1‰ depleted compared to the algal signal in SB-1 to SB-3 (15.0–14.7 Ma) but up to 4‰ in SB-13 (11 Ma; Fig. 9d). Recently, it has become clear that bacteriohopanepolyols in specific eubacteria are biosynthesized from different carbon sources than steroids are in eukaryotes (Rohmer et al., 1993; Rohmer and Bisseret, 1994). Kenig et al. (1995) estimated that if cyanobacteria have a similar biosynthetic pathway for biosynthesizing bacteriohopanepolyols as these eubacteria, cyanobacterial C_{35} hopanoids should be enriched compared to algal steranes if the cyanobacteria and algae are living under similar conditions (i.e., temperature, pCO_2, etc.). A ^{13}C-depletion in C_{35} hopanes from cyanobacteria is therefore an indication for different habitats of cyanobacteria compared to algae. Since the C_{35} hopane in the Pismo basin sediments is depleted by several permil compared to the steranes, the cyanobacteria probably lived under slightly higher pCO_2 and/or lower temperatures than the algae. Since the C_{35} hopane carbon skeleton is biosynthesized by numerous cyanobacteria (Rohmer et al., 1991), its ^{13}C-content represents an average of the cyanobacterial community, and variations in growth rates and cell sizes are averaged out. Furthermore, on average, the cyanobacteria, like *Prochlorococcus*, have very small cell sizes (Vaulot et al., 1995), thus severely diminishing the influence of growth rate on the biological carbon isotopic fractionation (B. Popp, pers. commun.). Thus, the increase in the difference between cholestane and C_{35} hopane during deposition of younger sediments may be attributed to environmental factors such as an increased temperature gradient in the photic zone during deposition of SB-6, SB-13, and SB-18 (13–11 Ma) as suggested by Schoell et al. (1994a). This is consistent with data obtained from oxygen isotope measurements on benthic and planktonic foraminifera showing that temperatures in the upper part of the photic zone

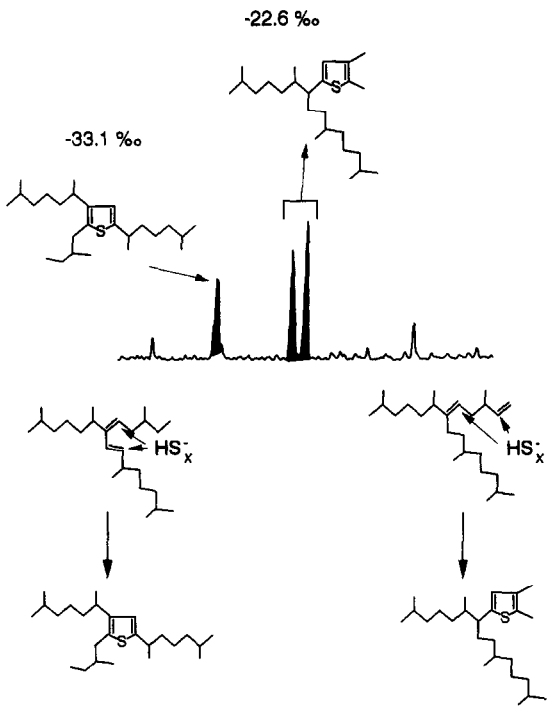


Fig. 10. Partial FID-trace of the A2-fraction of sample SB-18 showing the C_{25} HBI thiophene isomers and their carbon isotopic compositions (top) and possible precursors for the thiophene isomers (bottom; after Sinninghe Damsté et al., 1989).

were rather constant in contrast to bottom waters (e.g., Gasperi and Kennett, 1993). Furthermore, this is also in agreement with data of Monterey sediments from the Santa Barbara-Ventura basin (Schoell et al., 1994a).

S-bound phytane has an isotopic composition similar to that of the steranes (Table 5; Fig. 11a) which are derived from dinoflagellates or other algae. This indicates that S-bound phytane is predominantly derived from chlorophyll biosynthesized by these organisms. Since S-bound phytane is not depleted in ^{13}C compared to the cholestane value, it can be inferred that the input of phytane derived from chlorophyll of cyanobacteria is probably low. Free phytane is depleted in ^{13}C by several permil compared to the algal signal (Table 5; Fig. 11a). This suggests that these compounds are, for a substantial part, derived from other sources than chlorophyll produced by algae. Alternatively, it could be suggested that this pool of phytane was derived from chlorophyll of different algae which primarily lived in time periods where the sulfurization process was somehow not active. This seems, however, unlikely since the precursor steranes, which were biosynthesized continuously during deposition of the sediment, are almost only present in a S-bound form and not as free steranes.

The acyclic and cyclic biphytanyls (**XIV–XVII**) isolated after HI-treatment of the residual polar fraction are characteristic biomarkers for Archaeabacteria (de Rosa and Gambacorta, 1988). Their presence in the marine water column (Hoefs et al., 1997) and marine sediments (Chappe et al., 1982; Kohnen et al., 1992a) provides evidence for the presence of pelagic Archaea which occurs widespread in oceans (cf. Fuhrman et al., 1992; DeLong et al., 1994). Their ^{13}C-contents are several permil higher than those of cholestane (Fig. 11b). Hoefs et al. (1997) suggested that the relative ^{13}C-enrichment may be due to the fact that the compounds are derived from pelagic Archaea living on reduced C_1-substrates supplied by bacteria-fermenting sugars and proteins of degrading algae. Another archaeal compound, 2,6,10,14,18-pentamethylicosane (**XIII**; de Rosa and Gambacorta, 1988), probably derived from methanogenic archaea is also present in low amounts. The $\delta^{13}C$-value of this bacterial marker is ~4‰ lighter than that of cholestane and ~8‰ lighter than those of the cyclic biphytanes. Methanogens living on, for instance, pore water CO_2 or those living in the guts of zooplankton on reduced C_1-substrates may be the source for this compound.

Dinorhopane (**VI**) varies in isotopic composition between −24.5 and −28.8‰ (Fig. 11c). Previous studies at Naples Beach and in Monterey oils suggested that this compound is derived from bacteria living in the pore waters of sediments and that these bacteria utilize pore water CO_2 as their carbon source (Schoell et al., 1992; Schouten et al., 1997b). The isotopic composition of this CO_2 is depending on the intensities of sulfate reduction and methanogenesis (Blair and Carter, 1992), thus leading to variable isotopic compositions in lipids biosynthesized by sedimentary organisms living on porewater CO_2.

Bacteriohopanepolyol derivatives, the precursors for extended hopanes, are biosynthesized by heterotrophic bacteria, methanotrophic bacteria, chemoautotrophic bacteria, and cyanobacteria (Rohmer et al., 1991). Based on its isotopic composition S-bound pentakishomohopane (**II**) has been attributed to cyanobacteria since it is slightly depleted in ^{13}C compared to the algal signal in the Santa Barbara-Ventura basin (Schoell et al., 1994a; Schouten et al., 1997b) and Pismo basin. The free 17β,21β(H)-homohopane (**VII**) has an isotopic composition enriched by 1–3‰ compared to the algal signal (Fig. 11d). The difference in biosynthetic pathways of steroids and bacteriohopanepolyols theoretically results in an ~1.8‰ enrichment of the latter compared to the former as suggested by Kenig et al. (1995). This means that there is a residual enrichment of 0–1‰ left after subtracting the enrichment introduced by biosynthetic pathways. Biomass from heterotrophic organisms tends to be somewhat enriched (up to 1.5‰) in ^{13}C compared to their carbon source (De Niro and Epstein, 1977; Hayes, 1993; Kenig et al., 1994). This suggests a heterotrophic bacterial origin for the homohopane since this heterotrophic enrichment falls in the range of the residual enrichment of the C_{31} hopane.

The carbon isotopic compositions of the free n-alkanes are shown in Fig. 12. It is striking that all n-alkanes from the Shell Beach samples examined fall into a fairly narrow range of −27 to −31‰. There is no particular pattern of depletion in ^{13}C with increasing carbon number, as observed for some terrestrial derived n-alkanes (Rieley et al., 1993; Schouten et al., 1997b), and the carbon preference index is also low, indicating only minor terrestrial influences (Eglinton and Hamilton, 1963). Algal sources may be envisaged for these n-alkanes. The isotopic compositions of n-alkanes

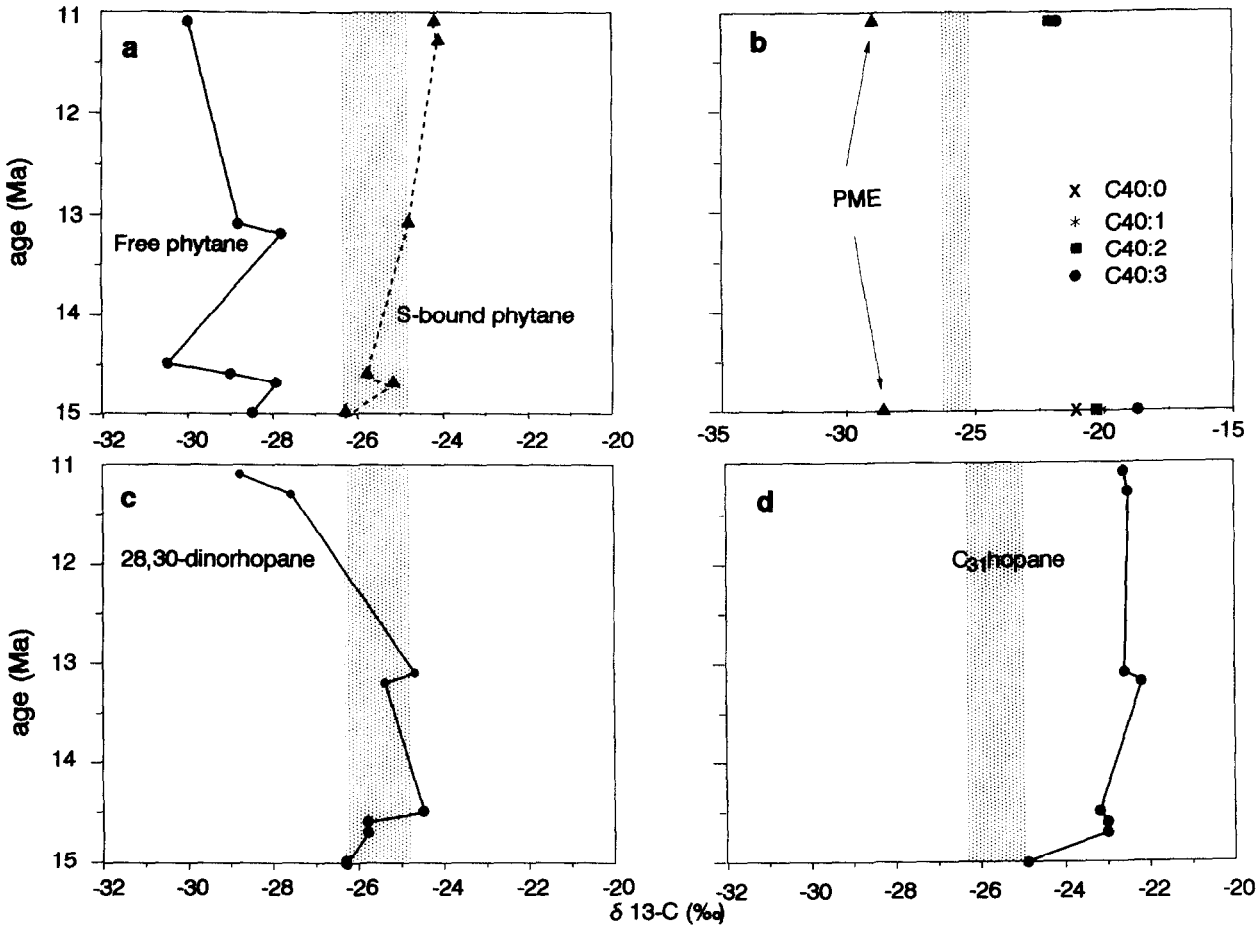


Fig. 11. Stable carbon isotopic compositions of selected compounds (a: S-bound phytane and free phytane, b: ether-bound acyclic and cyclic biphytanes and free 2,6,10,14,18-pentamethylicosane, c: 28,30-dinorhopane, and d: 17β,21β(H)-homohopane) in Monterey samples from the Pismo basin vs. time (Ma). The stippled area indicates the range of carbon isotopic compositions of S-bound 5α-cholestane and S-bound dinosterane.

derived from the algae which biosynthesized cholestane or dinosterane can be estimated by subtracting 1–2‰ from the average isotopic composition of these cyclic isoprenoids, assuming that straight chain compounds are somewhat more depleted in ^{13}C compared to isoprenoids (Hayes, 1993). The estimated δ-values range from −27 to −28‰, which is close to the range of values for the free n-alkanes. The remaining differences may be attributed to a minor terrestrial input which is likely to be more depleted in ^{13}C (Schouten et al., 1997b).

The isotopic compositions of the S-bound n-alkanes which could be determined are shown in Fig. 13. Their range (−24 to −30‰, excluding n-C_{25}) is larger than that of the free n-alkanes, indicating several sources for these n-alkanes. Their isotopic compositions are similar to the estimated value of algal n-alkanes indicating that they are also predominantly algal-derived. The C_{25} n-alkane is anomalously depleted by ~5‰ in ^{13}C compared to the other S-bound n-alkanes. Reinspection of the mass spectrum of the chromatographic peak did not reveal unusual fragments from coeluting compounds which may have been adducted by the molecular sieves. Thus, it is likely that the C_{25} n-alkane itself is depleted in ^{13}C by ~5‰ and is at least partly sourced by another organism. Interestingly, the thiophenes present in the Shell Beach sediments are dominated by one C_{25} 2-alkylthiophene isomer (and in SB-18, also by a C_{25} alkylthiophene) with a linear carbon skeleton (Fig. 13). ten Haven et al. (1990) also reported the predominance of these thiophenes in Cenozoic sediments from high productive depositional environments and attributed this to specific precursor lipids. Isotopic measurements of the C_{25} 2-alkylthiophene in SB-18 and SB-1 indeed confirm that this thiophene has depleted $\delta^{13}C$-values of ~33 and −36‰, respectively, which fits well with the isotopic compositions of the n-C_{25} alkane in the desulfurized polar fractions. Part of the precursor lipid for this thiophene may thus also be macromolecularly S-bound and may thus be the cause for the negative ^{13}C-value. The precursors for these sulfur compounds are in all probability compounds with straight chain n-C_{25} and n-C_{27} carbon skeletons possessing functionalities (e.g., double bonds or more likely aldehydes) at terminal positions. Indeed, Gelpi et al. (1968) and Gelin et al. (pers. commun.) reported the

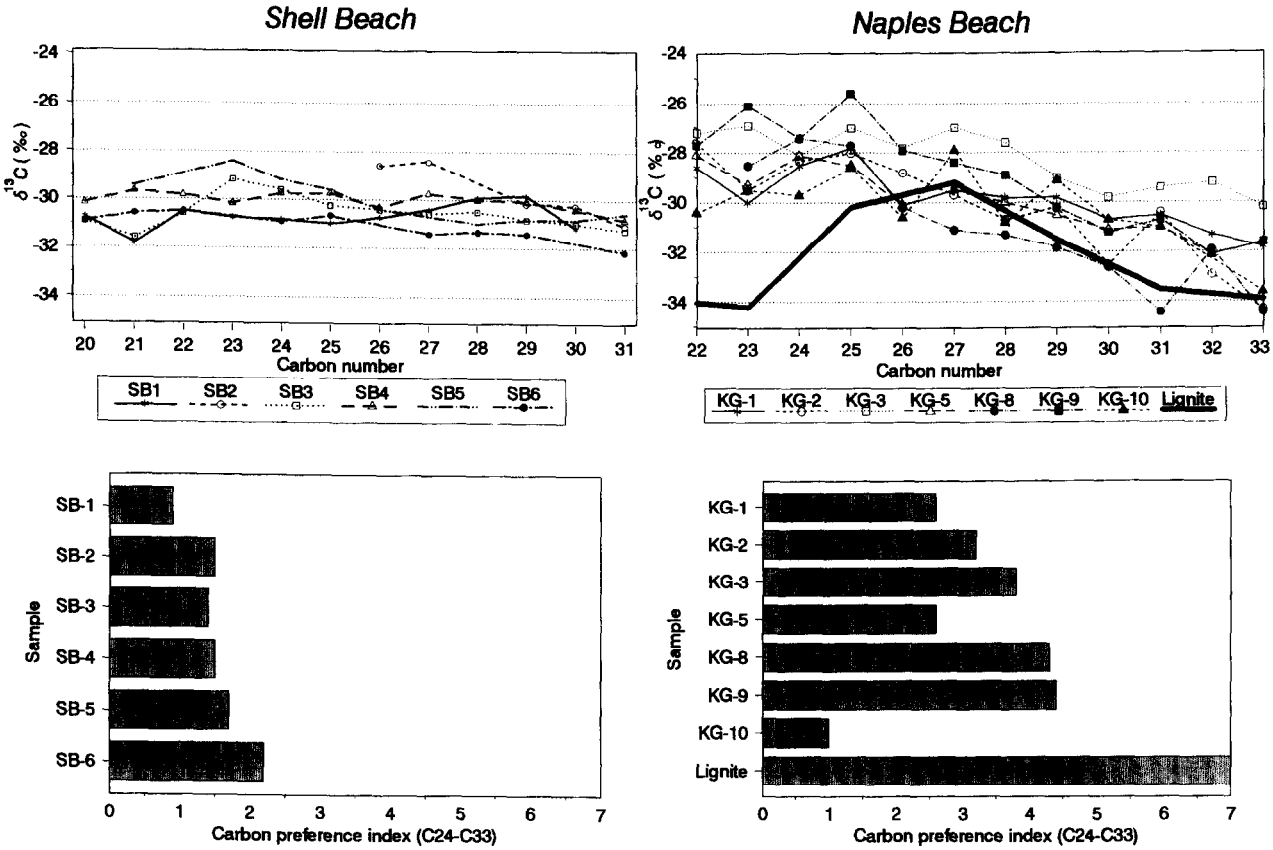


Fig. 12. Stable carbon isotope compositions and carbon preference index of free *n*-alkanes in samples from the Pismo basin (left) and Santa Barbara-Ventura basin (right). Standard deviations (not shown) are typically 0.7‰ or less.

presence of alkenes, alkadienes, and alkatrienes with 25, 27, and 29 carbon atoms in particular microalgae. The light isotopic compositions of these thiophenes may be explained by a similar hypothesis put forward for the C_{25} HBI thiophenes: only specific algal species biosynthesized the functionalized precursors and had on average low growth rates and/or small cell sizes compared to the rest of the algal community.

5. COMPARISON WITH SANTA BARBARA-VENTURA BASIN

A comparison of molecular data from the Pismo basin with those from the Santa Barbara-Ventura basin (Schouten et al., 1997a,b) reveals interesting differences in the depositional environments of both basins. The free hydrocarbon fraction of Pismo basin samples contains the C_{20} HBI (**V**; absent in Santa Barbara-Ventura basin samples), almost no steranes (present in small amounts in Santa Barbara-Ventura basin samples), and very low amounts of 25,28,30-trinorhopane (**IX**; present and sometimes very abundant in Santa Barbara-Ventura basin samples). The desulfurized polar fractions of Santa Barbara-Ventura basin samples are dominated by phytane; whereas in Pismo basin samples steranes (**Ia–c**) and the C_{35} hopane (**II**) are dominating. Furthermore, compounds with carbon skeletons related to dinosterane (**XIIa**) are virtually absent in Santa Barbara-Ventura basin samples, in contrast to Pismo basin samples which suggest a relatively higher abundance of dinoflagellates in the depositional environment of the Pismo basin.

The isotopic compositions of cholestane at both sites are very similar (-25.7 ± 0.7‰ and -25.6 ± 0.7‰ in the Santa Barbara-Ventura basin and Pismo basin, respectively; Fig. 14a). Following the palaeoenvironmental interpretation for the Santa Barbara-Ventura basin (Schoell et al., 1994a), we infer that similar conditions prevailed at both sites in the upper part of the photic zone. The C_{35} hopane isotope profile in the sediments from the Pismo basin is also similar to that from the Santa Barbara-Ventura basin (Fig. 14b). Schoell et al. (1994a) used the difference between cholestane and the C_{35} hopane in the Santa Barbara-Ventura basin as an indicator for the temperature gradient in the photic zone. The increase in difference in carbon isotopic compositions of the two molecules during deposition of younger sediments was attributed to an influx of cool, CO_2-rich bottom waters, as evidenced by benthic foram data from the Miocene Pacific ocean (Gaspari and Kennett, 1993). The similar differences in isotopic compositions between cholestane and the C_{35} hopane in the same time span thus indicates similar condi-

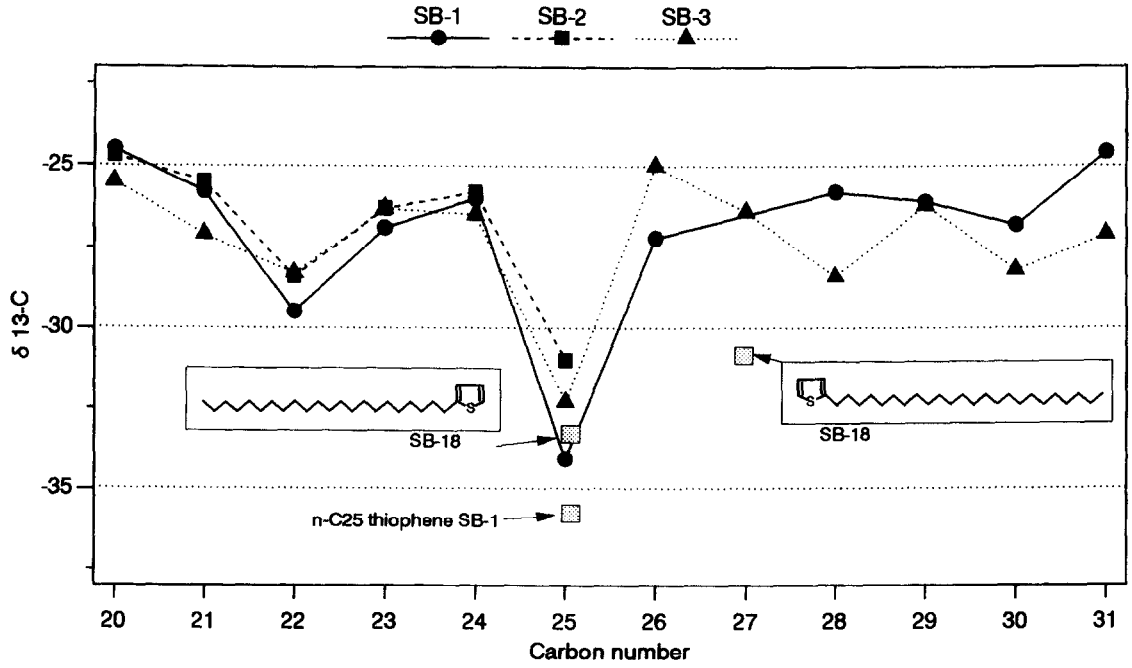


Fig. 13. Stable carbon isotope composition of S-bound n-alkanes in selected samples from the Pismo basin (Shell Beach). Standard deviations (not shown) are typically 0.7‰ or less. Filled rectangles show the isotopic compositions of thiophenes with C_{25} and C_{27} linear carbon skeletons.

tions of the photic zone in the Pismo basin and that of the Santa Barbara-Ventura basin. Our results confirm that this phenomenon was not a local feature but a general trend in the depositional environment of the Monterey Formation and of the Miocene in general.

The isotopic profile of dinorhopane (**VI**) of sediments from the Pismo basin is different from that recorded in the Santa Barbara-Ventura basin (Fig. 14c). Both profiles show a shift towards depleted values compared to cholestane but apparently in different time spans, although the limited number of samples prohibits any firm conclusions. The isotopic composition of dinorhopane has been proposed to reflect the ^{13}C-value of pore water CO_2 (Schouten et al., 1997), which leads us to conclude that the Pismo basin and Santa Barbara-Ventura basin pore water carbonate systems were different.

The isotopic compositions of compounds with C_{25} HBI carbon skeletons (**X**) in Pismo basin samples differ from those of these compounds in the Santa Barbara-Ventura basin samples since the Pismo basin samples contain skeletons with both depleted (7‰) and enriched (4‰) $\delta^{13}C$-values compared to cholestane in contrast to the Santa Barbara-Ventura basin samples which only contain C_{25} HBI carbon skeletons predominantly enriched by ~4‰ compared to the steranes (Fig. 14d). This indicates that in the depositional environments of the Santa Barbara-Ventura and Pismo basins, different species of HBI-synthesizing diatoms may have been present with different physiological behaviour (e.g., growth rates and/or cell sizes) and also different blooming periods.

The n-alkanes in the Santa Barbara-Ventura basin samples have a characteristic terrestrial fingerprint; the n-alkanes with more than C_{25} carbon atoms are depleted in ^{13}C with increasing carbon number. The free n-alkanes in the Pismo basin samples do not show this trend. Furthermore, the carbon-preference-index of free n-alkanes in Pismo basin samples (ca. 1.5; Fig. 12) is also much lower than in the Santa Barbara-Ventura basin samples (ca. 4; Fig. 12). We conclude that the terrestrial contribution of free n-alkanes to the Pismo basin samples is low compared to Santa Barbara-Ventura basin samples. The S-bound n-alkanes are also different. In some Santa Barbara-Ventura basin samples an odd-over-even carbon number predominance could be observed, which we attribute to a small contribution of terrestrially derived n-alkanes. Since this pattern is not observed in the Pismo basin samples, it is concluded that there is little or no terrestrially derived S-bound n-alkanes in Pismo basin samples.

6. CONCLUSION

A 15–11 Ma section of the Miocene Monterey Formation in the Pismo basin, composed of a calcareous phosphatic and a siliceous facies, was investigated. S-bound steranes present in the polar fractions have carbon isotopic compositions of −25 to −27‰ indicating stable environmental conditions, averaged over ca. 2000 years, for the upper part of the photic zone during deposition. The CPI and ^{13}C-values of free and S-bound n-alkanes indicated that they mainly originate from marine organisms and not from terrestrial sources as in Santa Barbara-Ventura basin samples, indicating terrestrial input was relatively lower. S-bound pentakishomohopane, probably derived from cyanobacteria living at

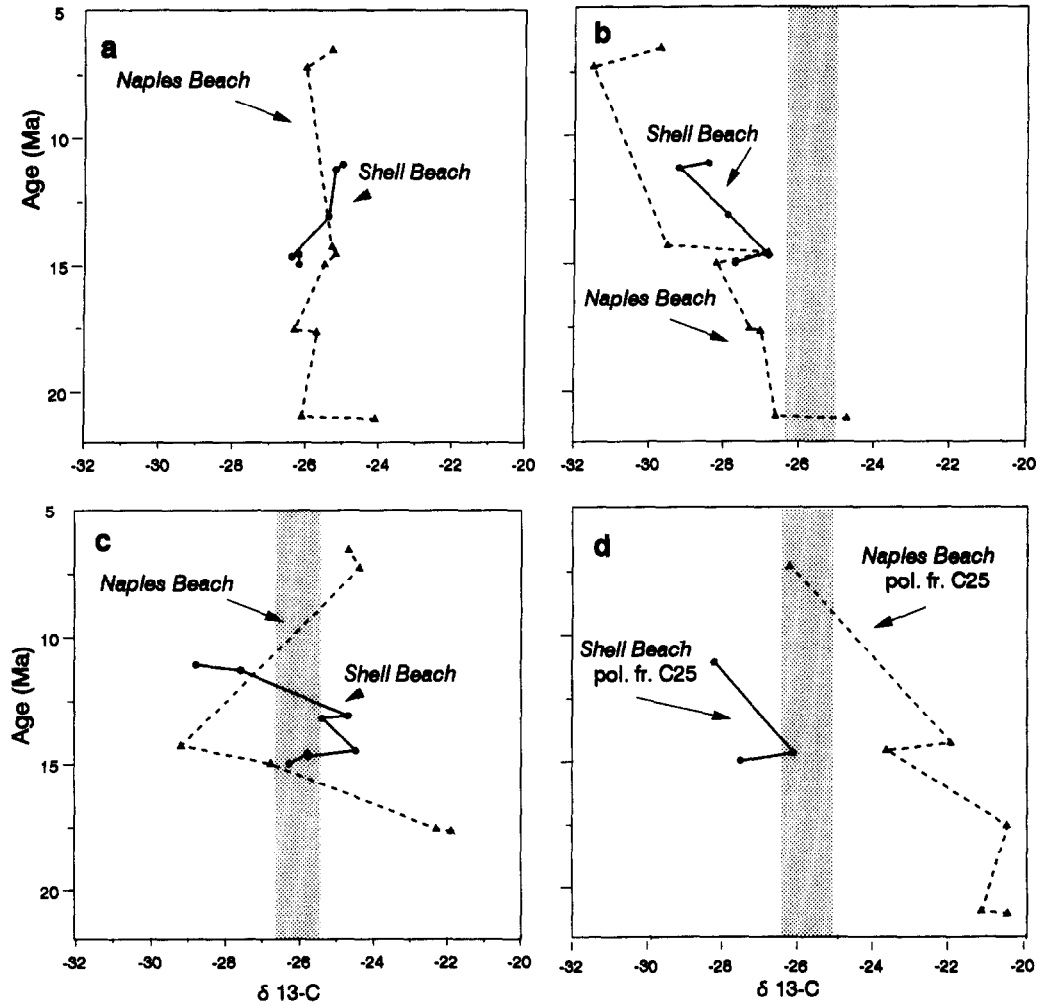


Fig. 14. Stable carbon isotope profiles of selected compounds (a: S-bound 5α-cholestane, b: S-bound 17β,21β(H)-pentakishomohopane, c: free 28,30-dinorhopane, and d: S-bound C_{25} HBI and free C_{20} HBI) in Monterey sediments from the Pismo basin and Santa Barbara-Ventura basin. The stippled area indicates the range of carbon isotopic compositions of S-bound 5α-cholestane.

the bottom of the photic zone, is ca. 1 to 4‰ depleted compared to the S-bound steranes, which is similar to Middle Miocene carbon isotopic data obtained from Monterey sediments at the Santa Barbara-Ventura basin. This indicates similar average conditions of the water bodies of the depositional basins. Highly branched isoprenoids have different isotopic compositions compared to each other in these Miocene sediments, indicating the presence of different diatom species and/or special growth conditions during different time periods in the Pismo basin compared to the Santa Barbara-Ventura basin. Lycopane is enriched in ^{13}C by 1–3‰ compared to the steranes, indicating other sources then a photoautotrophic source or photoautotrophs with different physiological behaviour or different bloom periods. The isotopic composition of dinorhopane varies differently in the two depositional settings and it is proposed that this reflects differences in ^{13}C-contents of pore water CO_2. Free 17β,21β(H)-homohopane is enriched by 1–4‰ in ^{13}C compared to other hopanes and steranes. Specific bacteria, possibly heterotrophs, may have been the organisms producing the precursor of this compound.

Acknowledgments—The Chevron Petroleum Technology Company is acknowledged for financial support under contract LH-2691. This study was partly supported by a PIONIER grant to JSSD by the Netherlands Organization for Scientific Research (NWO). The Shell International Petroleum Maatschappij BV is thanked for the financial support for the irm-GC-MS facility at Texel. We thank W. Pool, M. Dekker, and M. Stauffer for analytical assistance in the NIOZ and Chevron laboratories. Drs. B. Popp, T. Filley, S. Wakeham, and an anonymous reviewer are thanked for their constructive comments to improve the manuscript. This is Netherlands Institute for Sea Research contribution no. 3132.

Editorial handling: S. Wakeham

REFERENCES

Adam P. (1991) Nouvelles structures organo-soufrées d'intérêt géochimique: aspects moléculaires et macromoléculaires. Ph.D. dissertation. Univ. Strasbourg.

Blair N. E. and Carter W. D. (1992) The carbon isotope geochemistry of acetate from a methanogenic marine sediment. *Geochim. Cosmochim. Acta* **56,** 1247–1257.

Chappe B., Albrecht P., and Michaelis W. (1982) Polar lipids of archaebacteria in sediments and petroleum. *Science* **217,** 65–66.

Collister J. W., Summons R. E., Lichtfouse E., and Hayes J. M. (1992) An isotopic biogeochemical study of the Green River oil shale. In *Advances in Organic Geochemistry 1991* (ed. C. B. Eckhardt et al.); *Org. Geochem.* **19,** 265–276.

DeLong E. F., Ying Wu K., Prezeling B. B., and Jovine R. V. M. (1994) High abundance of Archaea in Antartic marine picoplankton. *Nature* **371,** 695–697.

De Niro M. J. and Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197,** 261–263.

Eglinton G. and Hamilton R. J. (1963) The distribution of alkanes. In *Chemical Plant Taxonomy* (ed. T. Swain), 187–208. Academic Press.

Francois R., Altabet M. A., Goericke R., McCorkle D. C., Brunet C., and Poisson A. (1993) Changes in the $\delta^{13}C$ of surface water particulate organic matter across the subtropical convergence in the S. W. Indian Ocean. *Global Biogeochem. Cycles* **6,** 627–644.

Freeman K. H., Hayes J. M., Trendel J. M., and Albrecht P. (1990) Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature* **343,** 254–256.

Freeman K. H., Wakeham S. G., and Hayes J. M. (1994) Predictive isotopic biogeochemistry: Hydrocarbons from anoxic marine basins. *Org. Geochem.* **21,** 629–643.

Fry B. D. and Wainwright S. C. (1991) Diatom sources of ^{13}C-rich carbon in marine food webs. *Mar. Ecol. Prog. Ser.* **76,** 149–157.

Fuhrman J. A., McCallum K., and Davis A. A. (1992) Novel major archaebacterial group from marine plankton. *Nature* **356,** 148.

Gasperi J. T. and Kennett J. P. (1993) Vertical thermal structure evolution of Miocene surface waters: Western equatorial Pacific DSDP Site 289. *Mar. Micropaleont.* **22,** 235–254.

Gelpi E., Oro J., Schneider H. J., and Bennet E. O. (1968) Olefins of high molecular weight in two microscopic algae. *Science* **161,** 700–702.

Goad L. J. and Withers N. (1982) Identification of 27-nor-(24R)-24-methyl-cholesta-5,22-dien-3β-ol and brassicasterol as the major sterols of the marine dinoflagellate *Gymnodinium simplex*. *Lipids* **17,** 853–858.

Goericke R., Montoya J. P., and Fry B. (1994) Physiology of isotopic fractionation in algae and cyanobacteria. In *Stable Isotopes in Ecology and Environmental Science* (ed. K. Lajtha and R. H. Michener), pp. 187–221. Blackwell.

Hartgers W. A., Lopez J. F., de Las Heras F. X. C., and Grimalt J. O. (1995) Sulphur-bonding in recent environments. I. Lipid byproducts from Ni_2B desulphurization. In *Organic Geochemistry: Developments and applications to energy, climate, environment and human history* (ed. J. O. Grimalt and C. Dorronsoro), pp. 1050–1052. A.I.G.O.A., San Sebastian.

ten Haven H. L., Rullkötter J., Sinninghe Damsté J. S., and de Leeuw J. W. (1990) Distribution of organic sulfur compounds in Mesozoic and Cenozoic sediments from the Atlantic and Pacific Oceans and the Gulf of California. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symp. Ser.* **429,** pp. 613–632.

Hayes J. M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* **113,** 111–125.

Hayes J. M., Freeman K. H., Popp B. N., and Hoham C. H. (1990) Compound-specific isotope analysis: A novel tool for reconstruction of ancient biogeochemical processes. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16,** 1115–1128.

Hird S. J. and Rowland S. J. (1995) An investigation of the sources and seasonal variations of highly branched isoprenoid hydrocarbons in intertidal sediments of the Tamar estuary, UK. *Mar. Environ. Res.* **40,** 423–437.

Hoefs M. J. L., Schouten S., de Leeuw J. W., King L., Wakeham S. G., and Sinninghe Damsté J. S. (1997) Ether lipids of pelagic archaea in the marine water column. *Appl. Environ. Microbiol.* (submitted).

Kenig F., Hayes J. M., Popp B. N., and Summons R. E. (1994) Isotopic biogeochemistry of the Oxford Clay Formation (Jurassic), UK. *J. Geol. Soc.* **151,** 139–152.

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

Kohnen M. E. L., Sinninghe Damsté J. S., Rijpstra W. I. C., and de Leeuw J. W. (1990) Alkylthiophenes as sensitive indicators of paleoenvironmental changes: A study of a Cretaceous oil shale from Jordan. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. D. White); *ACS Symposium Series* **42,** 444–485.

Kohnen M. E. L., Sinninghe Damsté J. S., Kock-van Dalen A. C., and de Leeuw J. W. (1991) Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. *Geochim. Cosmochim. Acta* **55,** 1375–1394.

Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merrit D., and Hayes J. M. (1992a) Improved recognition of paleobiochemicals by a combined molecular sulfur and isotope geochemical approach. *Science* **256,** 358–362.

Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merrit D., and Hayes J. M. (1992b) The combined application of organic sulphur and isotope geochemistry to assess multiple sources of palaeobiochemicals with identical carbon skeletons. In *Advances in Organic Geochemistry 1991* (ed. C. B. Eckhardt et al.); *Org. Geochem.* **19,** 403–420.

Laws E. A., Popp B. N., Bigidare R. R., Kennicutt M. C., and Macko S. A. (1995) Dependence of phytoplankton isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental results. *Geochim. Cosmochim. Acta* **59,** 1131–1138.

Merrit D. A. and Hayes J. M. (1994) Factors controlling precision and accuracy in isotope-ratio-monotoring mass spectrometry. *Anal. Chem.* **66,** 2336–2347.

Meunier-Christmann C. (1988) Geochimie organique de phosphates et schistes bitumineux Marocains: Etude du processus de phosphatogenese. Ph.D. dissertation. Univ. Strasbourg.

Moldowan J. M., Lee C. Y., Watt D. S., Jeganathan A., Slougui N. E., and Gallegos E. J. (1991) Analysis and occurrence of C_{26}-steranes in petroleum and source rocks. *Geochim. Cosmochim. Acta* **55,** 1065–1081.

O'Connor J. G., Burrow F. H., and Norris M. S. (1962) Determination of normal paraffins in C_{20} to C_{30} paraffin waxes by molecular sieve adsorption. *Anal. Chem.* **34,** 82–85.

Olson R. O., Chrisholm S. W., Zettler E. R., Altabet M., and Dusenberry J. (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep Sea Res.* **37,** 1033.

Omarzai S. K. (1992) Monterey Formation of California at Shell Beach (Pismo Basin): Its lithofacies, paleomagnetism, age, and origin. In *Sequence Stratigraphy in Fine-Grained Rocks: Examples from the Monterey Formation* (ed. J. R. Schwalbach and K. M. Bohacs), pp. 47–67. Pacific Section SEPM, Tempo Offset, Santa Fe Springs.

Prahl F. G., Pinto L. A., and Sparrow M. A. (1996) Phytane from chemolytic analysis of modern marine sediments: A product of desulfurization or not? *Geochim. Cosmochim. Acta* **60,** 1065–1073.

Raven J. A., Johnston A. M., Newman J. R., and Scrimgeour C. M. (1994) Inorganic carbon acquisition by aquatic photolithotrophs of the Dighty Burn, Angus, U.K.: Uses and limitations of the natural abundance measurements of carbon isotopes. *New Phyt.* **127,** 271–286.

Rieley G., Collister J. W., Stern B., and Eglinton G. (1993) Gas chromatography/isotope ratio mass spectrometry of leaf wax *n*-alkanes from plants of differing carbon dioxide metabolisms. *Rap. Comm. Mass. Spectr.* **7,** 488–491.

Rohmer M. and Bisseret P. (1994) Hopanoid and other polyterpen-

oid biosynthesis in Eubacteria. In *Isopentoids and Other Natural Products* (ed. W. Nes); *ACS Symp. Ser.* **562,** pp. 31–43.

Rohmer M., Bisseret P., and Neunlist S. (1991) The hopanoids; prokaryotic triterpenoids and precursors of ubiquitous molecular fossils. In *Biological Markers in Sediments and Petroleum* (ed. J. M. Moldowan et al.), pp. 1–17. Prentice Hall.

Rohmer M., Knani M., Simonin P., Sutter B., and Sahm H. (1993) Isoprenoid biosynthesis in bacteria: A novel pathway for the early steps leading to isopentyl diphosphate. *Biochem. J.* **295,** 517–524.

de Rosa M. and Gambacorta A. (1988) The lipids of archaebacteria. *Prog. Lipid Res.* **27,** 153–175.

Rowland S. J., Yon D. A., Lewis C. A., and Maxwell J. R. (1985) Occurrence of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane and related hydrocarbons in the green alga *Enteromorpha prolifera* and sediments. *Org. Geochem.* **8,** 207–213.

Schaeffer P., Reiss C., and Albrecht P. (1995) Geochemical study of macromolecular organic matter from sulfur-rich sediments of evaporitic origin (Messinian of Sicily) by chemical degradations. *Org. Geochem.* **23,** 567–582.

Schoell M., McCaffrey M. A., Fago F. J., and Moldowan J. M. (1992) Carbon isotopic composition of 28,30-bisnorhopanes and other biological markers in a Monterey crude oil. *Geochim. Cosmochim. Acta* **56,** 1391–1399.

Schoell M., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., and Summons R. E. (1994a) A molecular organic carbon isotopic record of Miocene climate changes. *Science* **263,** 1122–1125.

Schoell M., Hwang R. J., Carlson R. M. K., and Welton J. E. (1994b) Carbon isotopic composition of individual biomarkers in gilsonites (Utah). *Org. Geochem.* **21,** 673–684.

Schouten S., Pavlovic D., Sinninghe Damsté J. S., and de Leeuw J. W. (1993) Nickel boride: An improved desulphurizing agent for sulphur-rich geomacromolecules in polar and asphaltene fractions. *Org. Geochem.* **20,** 901–909.

Schouten S., Schoell M., Sinninghe Damsté J. S., and de Leeuw J. W. (1994) A novel sterane, 27-nor-24-methylcholestane, in sediments. *Geochim. Cosmochim. Acta* **58,** 3741–3745.

Schouten S., Sinninghe Damsté J. S., Schoell M., and de Leeuw J. W. (1995) The effect of hydrosulphurization on the carbon isotopic compositions of free and sulphur-bound lipids. *Geochim. Cosmochim. Acta* **59,** 1605–1609.

Schouten S., de Loureiro M. R. B., Sinninghe Damsté J. S., and de Leeuw J. W. (1997a) Molecular biogeochemistry of Monterey sediments (Naples Beach, USA) I: Distributions of hydrocarbons and organic sulphur compounds. In *CMOGS-volume* (ed. C. M. Isaacs and J. Rullkötter). Columbia University Press (in press).

Schouten S., Schoell M., Sinninghe Damsté J. S., Summons R. E., and de Leeuw J. W. (1997b) Molecular biogeochemistry of Monterey sediments (Naples Beach, USA) II: Carbon isotopic compositions of free and sulphur-bound carbon skeletons. In *CMOGS-volume* (ed. C. M. Isaacs and J. Rullkötter). Columbia University Press (in press).

Sinninghe Damsté J. S., van Koert E. R., Kock-van Dalen A. C., de Leeuw J. W., and Schenck P. A. (1989) Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils. *Org. Geochem.* **14,** 555–567.

Summons R. E., Volkman J. K., and Boreham C. J. (1987) Dinosterane and other steroidal hydrocarbons of dinoflagellate origin in sediments and petroleum. *Geochim. Cosmochim. Acta* **51,** 3075–3082.

Summons R. E., Barrow R. A., Capon R. J., Hope J. M., and Stranger C. (1993) The structure of a new C_{25} isoprenoid alkene biomarker from diatomaceous microbial communities. *Australian J. Chem.* **46,** 907–915.

Summons R. E., Jahnke L. L., and Roksandic Z. (1994) Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers. *Geochim. Cosmochim. Acta* **58,** 2853–2863.

Suzuki N., Sampei Y., and Koga O. (1993) Norcholestane in Miocene Onnagawa siliceous sediments, Japan. *Geochim. Cosmochim. Acta* **57,** 4539–4545.

Takahashi K., Wada E., and Sakamoto M. (1991) Relationship between carbon isotope discrimination and the specific growth rate of green alga *Chlamydomonas reinahardtii*. *Jpn. J. Limnol.* **52,** 105–112.

Vaulot D., Marie D., Olson R. J., and Chrisholm S. W. (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. *Science* **268,** 1480–1482.

Volkman J. K. (1986) A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.* **9,** 83–99.

Volkman J. K., Barret S. M., Dunstan G. A., and Jeffrey S. W. (1993) Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. *Org. Geochem.* **20,** 7–16.

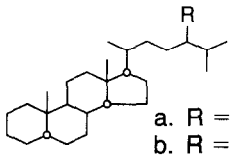
Volkman J. K., Barnett S. M., and Dunstan G. A. (1994) C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org. Geochem.* **21,** 407–413.

Wakeham S. G., Freeman K. H., Pease T. K., and Hayes J. M. (1993) A photoautotrophic source for lycopane in marine water columns. *Geochim. Cosmochim. Acta* **57,** 159–166.

West N., Alexander R., and Kagi R. I. (1990) The use of silicalite for the rapid isolation of branched and cylic alkane fractions of petroleum. *Org. Geochem.* **15,** 499–501.

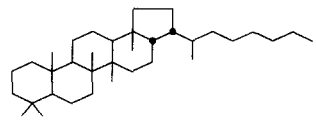
APPENDIX

I

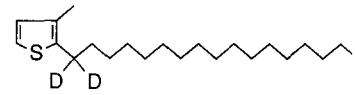


a. R = H
b. R = Me
c. R = Et

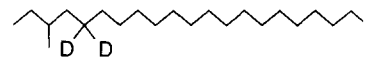
II



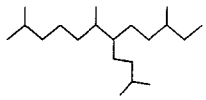
III



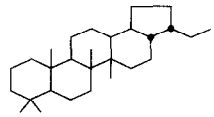
IV



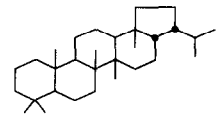
V



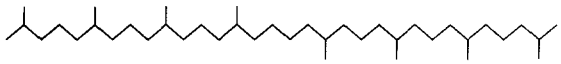
VI



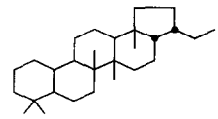
VII



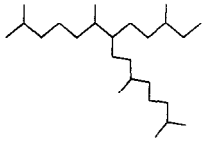
VIII



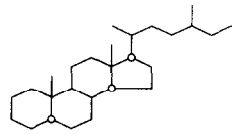
IX



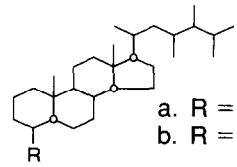
X



XI

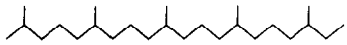


XII

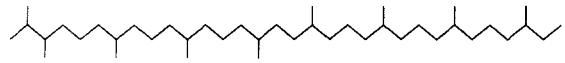


a. R = H
b. R = Me

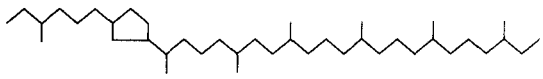
XIII



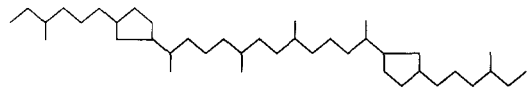
XIV



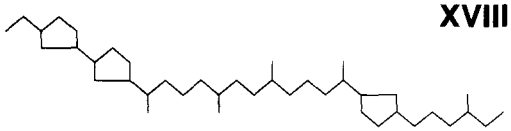
XV



XVI



XVII



XVIII