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A molecular isotopic study of ^{13}C-enriched organic matter in evaporitic deposits: recognition of CO_2-limited ecosystems

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Abstract

Biomarkers were analysed in a surface sediment from La Trinitat saltern ponds and a Miocene marl from the Northern Appenines. Both are characterised by enriched $\delta^{13}C$ values for bulk organic matter and for biomarkers, i.e. heavier than −20‰. Exceptions are long-chain C_{22}–C_{34} *n*-alkanes and C_{16}–C_{20} fatty acids which have values typically lighter than −20‰, suggesting that they are not derived from aquatic organisms but from surrounding terrestrial vegetation. The enriched $\delta^{13}C$ values for biomarkers of different algae and bacteria suggest that the local (microbial mat) ecosystem may have been CO_2-limited.

Keywords: Lipids; Fatty acids; CO_2; Stable carbon isotope ratios; Microbial mats; Hypersaline; Evaporites

1. Introduction

Hypersaline ecosystems have been the subject of many geochemical studies (e.g. Friedman and Krumbein, 1985) since they are considered recent analogues for ancient evaporitic sediments such as the Zechstein salts and Italian Messinian evaporites. Also, based on the formation of stromatolite-like structures in some of the microbial mats present in hypersaline systems, they are thought to resemble the earliest life forms on Earth which are archived in the geological records as Precambrian stromatolites (e.g. Knoll, 1985).

Microbial mats in hypersaline systems are known, sometimes, to have relatively high $\delta^{13}C$ TOC-values of up to −5‰ (e.g. Schidlowski et al., 1984; des Marais et al., 1989; Lazar and Eeres, 1992; Kenig et al., 1994; Trichet et al., in press). Hypersalinity itself has been suggested as the cause for these heavy isotope values in microbial mats since at high salinities and temperatures $[CO_2]_{aq}$-concentrations are low due to precipitation of $CaCO_3$ (Schidlowski et al., 1984). Further research has shown, however, that this cannot be the only cause since no consistent trends are observed between isotopic composition of microbial mats living at different salinities (des Marais et al., 1989; Schidlowski et al., 1994). It was suggested that the high productivity typically encountered in microbial mats is also responsible for the decreased fractionation of ^{13}C. Thus, the combination of high growth rates and low CO_2 availability causes most of the hypersaline ecosystem to be CO_2-limited and enriched in ^{13}C. This is seemingly in contrast with

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the depleted ^{13}C-values reported for organic matter in Precambrian stromatolites (Schidlowski et al., 1984; des Marais et al., 1989). However, it is argued that during these times CO_2-concentrations were far higher and thus not limiting in microbial mat systems (Schidlowski et al., 1994).

Reports on the stable carbon isotopic compositions of organic compounds in CO_2-limited microbial mats are scarce. Kenig et al. (1994) reported the presence of isotopically enriched branched alkanes, derived from insect waxes, in microbial mats of the Abu Dhabi lagoon-sabkha. Moers et al. (1993) reported the ^{13}C-contents of sugars of the same microbial mats to be similarly enriched in ^{13}C. Here we report stable carbon isotopic compositions of alkanes, fatty acids and alcohols extracted from recently deposited organic matter derived from a microbial mat system in the La Trinitat saltern pond (NE Spain; Grimalt et al., 1992; Villanueva et at., 1994). These results are compared to a possible Miocene fossil analogue, i.e. the organic matter from a marl layer and a gypsum layer in an evaporitic deposit in the Northern Apennines. These layers are thought to contain mat-like structures that were deposited in a shallow hypersaline lagoon (Vai and Ricchi Lucci, 1977). Both the organic matter in the saltern pond sediment and the Northern Apennines marl are also relatively enriched in ^{13}C ($\delta^{13}C_{TOC} = -15.0‰$ and $-18.0‰$, respectively). The results demonstrate that compound-specific isotope analysis, in contrast to bulk isotopic analysis, can identify CO_2-limited ecosystems that exist presently and have existed in the past.

2. Experimental

2.1. Geological setting

Extracts analysed for this study were obtained from sediments from the calcite pond of a solar saltern (La Trinitat) and a bituminous marl and gypsum layer from the Perticara basin in the Northern Apennines, respectively. The solar saltern ponds of La Trinitat are located in the Ebro Delta (Catalonia, NE Spain; Fig. 1A) and consist of a series of calcite, gypsum and halite ponds interconnected by sluices. Samples were taken from the calcite ponds, containing water in a salinity range of 70–100 g/l and consist of a 0–10 cm section of the cyanobacterial mat. The molecular characteristics of microbial mats and their microbial biota have been described elsewhere (Boon et al., 1983; Barbé et al., 1990; de Wit and Grimalt, 1992; van Gemerden, 1993; Hartgers et al., 1996, 1998). Samples from the Northern Apennines were collected in the Perticara basin (Northern Italy; Fig. 1B), which is an evaporitic deposit consisting of bituminous marl layers, stromatolite layers and thick (up to 40 m) gypsum layers (ten Haven et al., 1985).

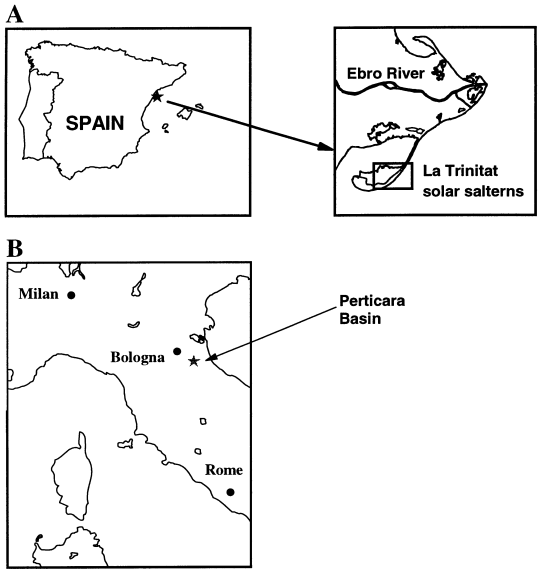

Fig. 1. Maps showing the location of (A) La Trinitat solar saltern ponds (NE Spain) and (B) the Petricara Basin (Northern Italy).

2.2. Extraction and fractionation

Extraction of sediments from La Trinitat and subsequent fractionation of the extract into an apolar fraction, polar fraction and fatty acid fraction has been described previously (Hartgers et al., 1996). Prior to isotopic measurements of the fatty acids, they were methylated using BF_3/methanol. The extract of the marl layers was fractionated according to the procedure described by Kohnen et al. (1992) yielding a saturated hydrocarbon fraction, a chroman fraction and a polar fraction. The saturated hydrocarbon fraction of the gypsum layer was isolated from the extract as previously reported by ten Haven et al. (1985). The polar fractions (ca 10 mg) were reduced using nickel boride (Schouten et al., 1993) for La Trinitat samples. Approximately 500 mg of anhydrous $NiCl_2$ and 500 mg of $NaBH_4$ were used for reduction. These amounts are around five times higher than described (Schouten et al., 1993) and were used in order to improve the yields. Raney nickel (Sinninghe Damsté et al., 1990) was used as the desulfurisation technique for the polar fractions isolated from the marl sample.

2.3. Argentatious thin layer chromatography

Aliquots (ca 10 mg) of the methylated fatty acid fractions from La Trinitat were further separated by argentatious thin layer chromatography (Ag^+-TLC) using toluene as developer. The $AgNO_3$-impregnated silica plates (Merck; 20×20 cm; thickness 0.25 mm) were prepared by dipping them in a solution of 10% $AgNO_3$ in CH_3CN for 4 min, drying at 70°C for 30 min and

subsequent activation at 110°C for 20 min. The silver-loading of the plates is approx. 23%, as determined by weighing the TLC-plate before and after impregnation. Four fractions (F1, $R_f = 0.87–1.00$; F2, $R_f = 0.47–0.87$; F3, $R_f = 0.14–0.47$; F4, $R_f = 0.00–0.14$) were scraped from the TLC plate and ultrasonically extracted with ethyl acetate (×3).

2.4. Derivatisation of fatty acids to oxazolines

To determine the position of the double bond(s) in unsaturated fatty acids, their corresponding oxazolines were prepared by a modification of the method described by Zhang et al. (1988). Typically, 50 μg of the fatty acid mixture was mixed with 250 μl (234 mg) of 2-amino-2-methylpropanol, flushed with N_2, in a screw-capped Pyrex™ test tube and heated at 180°C for 10 h. After cooling, water was added to the reaction mixture and the derivatised fatty acids were extracted with *n*-hexane (×3). The location of the double bond(s) is revealed by a mass separation of 12 amu instead of the regular 14 amu in the homologous ion series of the mass spectra (Zhang et al., 1988).

2.5. Instrumental analysis

Gas chromatography (GC) was performed using a Carlo Erba 5300 instrument equipped with a splitless injector and a FID detector. A fused silica capillary column (30×0.25 min i.d.) coated with DB-5 (J&W Scientific; film thickness 0.25 μm) was used with hydrogen as carrier gas (50 cm/s). The samples (in ethyl acetate) were injected at 70°C and the oven temperature was subsequently raised to 130°C at 10°C/min and then at 4°C to 320°C, and held there for 30 min. The injection was in the splitless mode (hot needle technique), keeping the split valve closed for 35 s. Injector and detector temperatures were respectively 300 and 330°C.

Gas chromatography–mass spectrometry (GC MS) was performed with a Fisons MD800 instrument. The gas chromatograph was equipped with a fused silica capillary column (30 m×0.25 mm i.d.) coated with HP-5MS (Hewlett-Packard; film thickness 0.25 μm). Helium was used as carrier gas. The oven temperature was programmed from 70°C to 130°C at 10°C/min and subsequently at 4°C/min to a final temperature of 310°C, and held there for 30 min. Injection conditions (300°C) were

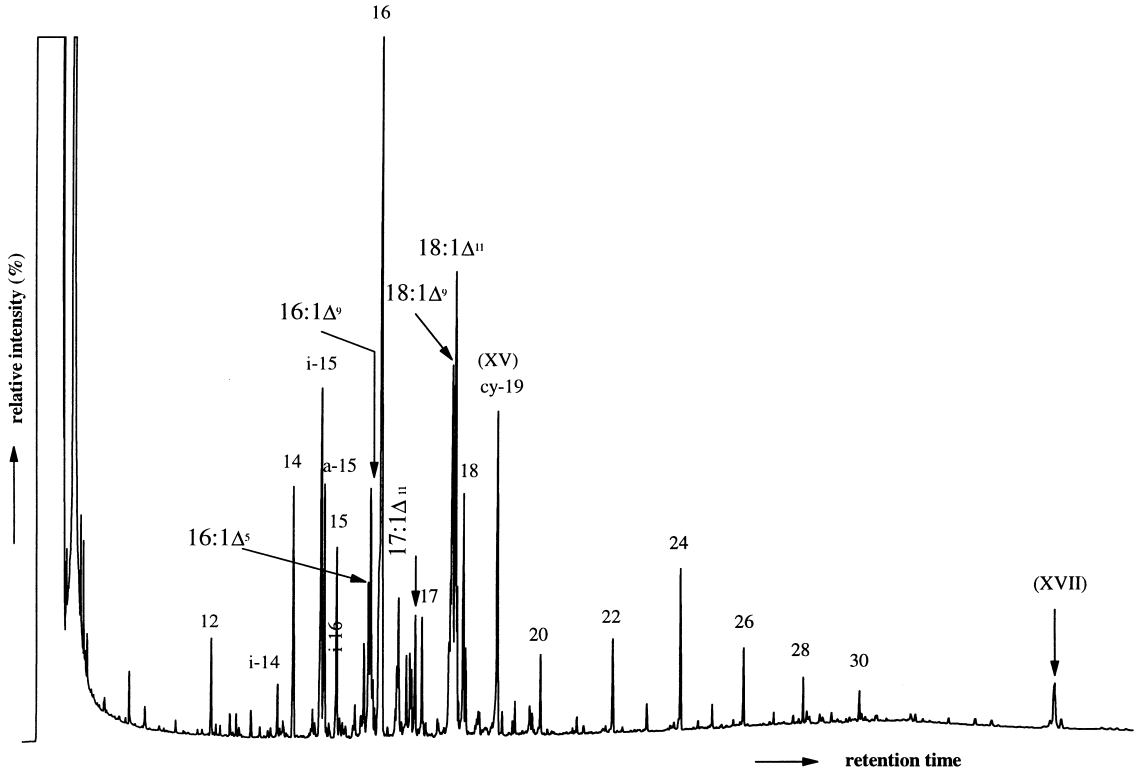

Fig. 2. Gas chromatogram of the fatty acid fraction (methylated and silylated) isolated from the extract of La Trinitat. Numbers indicate carbon chain length of the free acid. Key: a-15 = *anteiso* pentadecanoic acid; i-15 = *iso* pentadecanoic acid; cy-19 = methyleneoctadecanoic acid (see Barbé et al., 1990). Double bond positions of unsaturated fatty acids were determined by derivatisation to their corresponding 4,4-dimethyloxazolines.

as described above. Mass spectra were acquired in the electron ionisation mode (70 eV) scanning from 50 to 700 mass units with a cycle time of 1 s.

For isotopic analysis, samples were analysed on a Delta C irmGC–MS system and conditions for analysing the hydrocarbon, polar and desulfurised polar fractions were identical to those described by Schouten et al. (1998). The stable carbon isotopic values of the individual fatty acids were determined using a capillary column (20 m×0.18 mm i.d.) coated with DB-23 (J&W Scientific; film thickness 0.20 μm), enabling a base-line separation of the individual fatty acids. The stable carbon isotope compositions are reported in the delta notation against the PDB ^{13}C standard. The $\delta^{13}C$ values of alcohols were corrected for the isotopic contribution of the trimethylsilyl group which was determined from the stable carbon isotope values measured for 1-hexadecanol and its silylated counterpart. Likewise, the isotopic contribution of the methyl group in fatty acids was corrected by determining the $\delta^{13}C$ values (triplicate determinations) of dodecanoic acid and its methyl ester.

3. Results

3.1. Recent sediment: La Trinitat

The composition of the hydrocarbon, polar and reduced polar fractions have been described previously (Hartgers et al., 1996, 1998). Briefly, the apolar fraction contains high amounts of odd-carbon-numbered *n*-alkanes, saturated and unsaturated C_{20}, C_{25} and C_{30} highly branched isoprenoids (I, II, III), mono-unsaturated C_{17} and C_{18} alkenes, cholest-2-ene (IV) and diploptene (V). The polar fraction contains high amounts of phytol (VI), even carbon numbered alkan-1-ols in the C_{20}–C_{28} range and small amounts of C_{37} and C_{38} alkenones (VII). Upon treatment with Ni_2B the phytol is partially converted to phytane (VIII; Hartgers et al., 1996) which is the major compound in the reduced polar fraction. In addition, small amounts of 5α-cholestane (IX) and C_{32} 17β,21β(H)-hopane (X) were detected.

The distributions of total fatty acids in La Trinitat sediments are shown in Fig. 2. The mixtures comprise both free and ester-bound components released after

Table 1
Stable carbon isotope data of compounds from sediment extract of La Trinitat

Fraction/compound	$\delta^{13}C$ (‰)	Fraction/compound	$\delta^{13}C$ (‰)
Apolar fraction		*i*-C_{16}	−20.6±0.8
$C_{17:1}$ *n*-alkene	−21.9±0.6	*n*-C_{16}	−22.8±0.4
$C_{18:1}$ *n*-alkene	−25.8±0.1	Branched C_{17}	−22.5±0.3
Phytane	−22.0±0.3	*i*-C_{17}	−19.9±1.0
$C_{25:2}$HBI	−14.2±0.5	*a*-C_{17}	−20.9±0.8
C_{30} poly-unsat. HBI's	−17.5±0.3	*n*-C_{17}	−18.3±0.2
C_{27} *n*-alkane	−17.5±0.3	cy-C_{19}	−21.7±0.8
C_{29} *n*-alkane	−28.0±0.4	cy-C_{19}	−19.2±0.5
C_{31} *n*-alkane	−30.3±0.1	*n*-C_{20}	−24.3±0.9
C_{33} *n*-alkane	−30.2±0.2	*n*-C_{21}	−22.5±0.9
		n-C_{22}	−22.9±0.1
Polar fraction		*n*-C_{23}	−25.3±0.8
Phytol	−16.3±0.2	*n*-C_{24}	−21.2±0.5
C_{16} alkan-1-ol	−18.2±0.3	*n*-C_{25}	−27.3±0.3
C_{17} alkan-1-ol	−21.1±0.1	*n*-C_{26}	−26.5±0.4
C_{22} alkan-1-ol	−23.4±0.1	*n*-C_{28}	−26.9±0.9
C_{24} alkan-1-ol	−24.1±0.1	Bishomohopanoic acid	−24.9±0.4
C_{26} alkan-1-ol	−27.8±0.6		
C_{38} alkenones (avg.)	−21.4±1.1	*Fatty acids TLC F2*	
		n-$C_{16:1}\Delta^5$	−23.9±0.1
Reduced polar fraction		*n*-$C_{16:1}\Delta^9$	−18.6±0.2
Phytane	−16.5±0.1	*n*-$C_{17:1}\Delta^9$	−16.6±0.3
5α-cholestane	−24.5±0.7	*n*-$C_{17:1}\Delta^{11}$	−16.3±1.0
C_{32}-hopane	−17.9±0.5	*n*-$C_{18:1}\Delta^9$	−22.4±0.3
		n-$C_{18:1}\Delta^{11}$	−19.0±0.3
Fatty acids TLC-Fl		*n*-$C_{20:1}$	−25.2±0.7
n-C_{14}	−20.2±0.5	*n*-$C_{20:1}$	−20.3±0.2
i-C_{15}	−18.6±0.6		
a-C_{15}	−18.5±0.8		
n-C_{15}	−15.3±0.4		

saponification and the distribution is characterised by even-carbon-numbered alkanoic acids showing a bimodal distribution ranging from C_{12} to C_{18} and C_{20} to C_{30}. Furthermore, high amounts of monounsaturated fatty acids are present with 9-hexadecenoic acid (XI), 9-octadecenoic acid (XII) and 11-octadecenoic acid (XIII) dominating. Smaller amounts of C_{14}–C_{16} *iso* fatty acids (XIV) and a high amount of a methylene octadecanoic acid (XV) are also present. Finally, small amounts of a C_{32} hopanoic acid (XVI) were detected.

Isotopic analysis revealed that most aquatically derived compounds have δ^{13}C-values heavier than −22‰ (Table 1). For example, highly branched isoprenoids (derived from diatoms; Volkman et al., 1994), phytol (derived from the chlorophylls of photoautotrophic organisms) and hopanes (derived from bacteria; Rohmer et al., 1984) have δ^{13}C-values between −14‰–−18‰. Slightly more depleted in ^{13}C are 5α-cholestane (derived from algae; Volkman, 1986) and C_{38} alkenones (derived from haptophyte algae; Volkman et al., 1980), i.e. −24.5‰ and −21.4‰, respectively. Straight-chain compounds show an increasing depletion with carbon number (Fig. 3) and, especially at higher carbon numbers ($>C_{23}$), are isotopically significantly different from other, mostly aquatically derived, biomarkers. This strongly suggests that higher-carbon-number (i.e. $>C_{23}$) *n*-alkanes, fatty acids and alkan-1-ols are derived from terrestrial sources. Most of the short-chain (C_{16}–C_{20}) unsaturated fatty acids, probably derived from bacterial sources, also have δ^{13}C-values between −16‰ and −22‰.

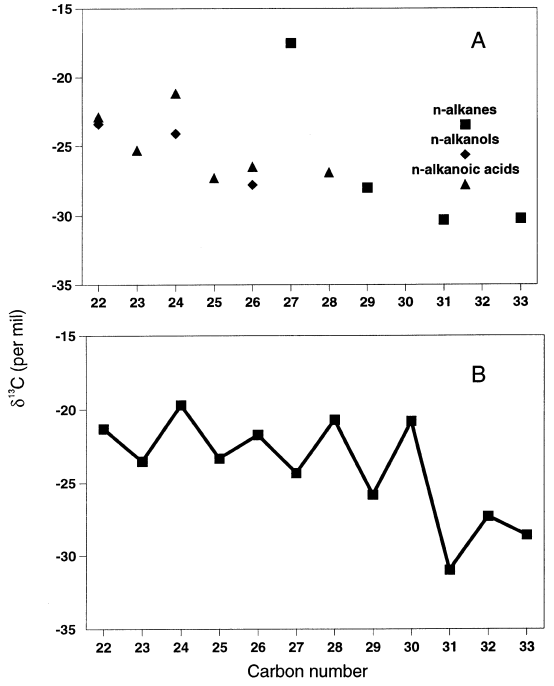

Fig. 3. Graphs showing the ^{13}C-contents of higher carbon number straight-chain lipids in (a) the fractions of La Trinitat and (b) Northern Apennines marl sample.

3.2. Miocene analogue: Northern Apennines marl

Fig. 4 shows gas chromatograms of the saturated hydrocarbon distribution from the Northern Apennines marl and the hydrocarbons released after desulfurisation of the polar fraction. The compound distributions show close similarities to those described by ten Haven et al. (1985) and by Sinninghe Damsté et al. (1990) although for the present study a different marl layer has been extracted. The hydrocarbon fraction of the marl sample contains substantial amounts of pristane (XVII), phytane (VIII), 5α- and 5β-cholestane (IX), 2,4,6,10,18-pentamethylicosane (XVIII) and a range of *n*-alkanes with the C_{25}–C_{33} homologs with an odd-over-even carbon number predominance. The desulfurised polar fraction also contains substantial amounts of phytane (VII), 5α-cholestane (IX) and a range of *n*-alkanes but also (partially) sulfurised β-carotane (XIX) and squalane (XX). A so-called chroman fraction was isolated using Ag^+-TLC and contains predominantly monomethylated and dimethylated chromans (XXI, XXII; Sinninghe Damsté et al., 1987). The saturated hydrocarbon fraction from a gypsum layer, previously described by ten Haven et al. (1985), contains predominantly phytane (VII), *n*-alkanes, 5α- and 5β-cholestane (IX) and pregnane (XXIII).

Table 2 shows the δ^{13}C-values of the compounds that could be determined in the different fractions of the sediments from the Northern Apennines. It is striking that most of the δ^{13}C-values in the marl and in the gypsum layer are heavier than −20‰. For instance, sulfur-bound phytane and β-carotane (derived from photoautotrophs), cholestane (derived from algae or zooplankton) and chromans (derived from unknown autotrophs; Sinninghe Damsté et al., 1987) have δ^{13}C-values between −16‰ and −19‰. Exceptions are the free C_{19} to C_{33} *n*-alkanes which are more depleted in ^{13}C with increasing carbon number (Fig. 3b). In addition, a "zigzag" pattern of the ^{13}C-contents of *n*-alkanes is observed, i.e. the odd-carbon numbered *n*-alkanes are more depleted in ^{13}C than the even-carbon-numbered *n*-alkanes. In combination with their odd-over-even carbon number predominance this strongly suggest that the odd-carbon-numbered *n*-alkanes are predominantly terrestrially derived, similarly to the *n*-alkanes in La Trinitat.

4. Discussion

Molecular stable carbon isotope analysis of both the recently deposited evaporitic sediment and the Miocene marl from the Northern Apennines reveal the same

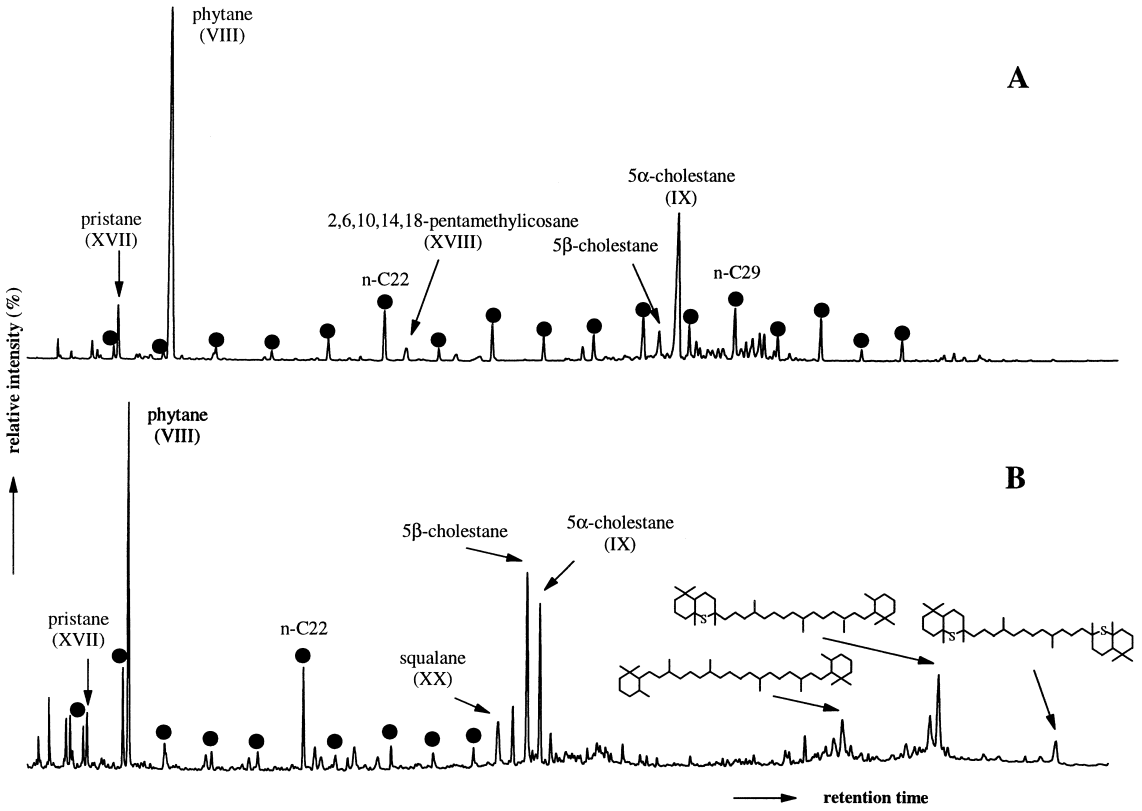

Fig. 4. Gas chromatograms of lipid fractions of the extract of Northern Apennines marl sample from the Petricara basin (Northern Italy). (A) Hydrocarbon fraction and (B) hydrocarbons obtained after Raney nickel desulfurisation of the polar fraction. Dots indicate *n*-alkanes.

Table 2
Carbon isotope data of fractions from sediment extracts of the Northern Apennines marl and gypsum

Fraction/compound	$\delta^{13}C$ (‰)	Fraction/compound	$\delta^{13}C$ (‰)
Free hydrocarbons marl		Monomethylated chroman	−17.9±0.2
C_{19} *n*-alkane	−19.6±0.6	Dimethylated chroman	−18.6±0.3
C_{20} *n*-alkane	−22.2±1.6		
C_{21} *n*-alkane	−21.5±1.2	*Desulfurised polar fraction marl*	
C_{22} *n*-alkane	−21.3±0.7	Pristane	−17.5±0.9
C_{23} *n*-alkane	−23.5±0.5	Phytane	−16.3±0.8
C_{24} *n*-alkane	−19.7±0.9	5α-Cholestane	−17.8±0.1
C_{25} *n*-alkane	−23.3±0.7	5β-Cholestane	−17.7±0.1
C_{26} *n*-alkane	−21.7±0.8	β-Carotane	−19.2±0.9
C_{27} *n*-alkane	−24.3±0.2		
C_{28} *n*-alkane	−20.7±0.7	*Free hydrocarbons gypsum*	
C_{29} *n*-alkane	−25.8±0.9	C_{19} *n*-alkane	−22.4±0.7
C_{30} *n*-alkane	−20.8±0.9	C_{20} *n*-alkane	−26.8±0.3
C_{31} *n*-alkane	−31.0±1.3	C_{21} *n*-alkane	−20.3±0.1
C_{32} *n*-alkane	−27.3±0.3	C_{22} *n*-alkane	−20.7±0.2
C_{33} *n*-alkane	−28.6±0.2	C_{25} *n*-alkane	−26.6±0.8
Pristane	−17.2±0.1	C_{26} *n*-alkane	−23.7±0.5
Phytane	−16.5±0.4	Phytane	−17.0±0.1
5α-Cholestane	−16.6±1.8	Pregnane	−18.3±0.1
5β-Cholestane	−17.7±0.3	Cholestanes (avg.)	−18.7±1.4
2,6,10,14,18-PMI	−16.2±0.2	C_{32} hopane	−23.4±0.2

Table 3
Carbon isotope data of phytane from sediment extracts of evaporitic sediments

Setting	Mode of binding	$\delta^{13}C$ phytane (‰)
Vena del Gesso (Italy), gypsum layer	S-bound	−28.9
Dead Sea marls (Israel)[a]	S-bound	−28.2
Jianghan Basin (China)[b]	S-bound	−25.0 to −29.4
Mulhouse marl layers (France)[c]	Free	−26.6 to −33.2

[a] Grice et al. (1998a).
[b] Grice et al. (1998b).
[c] Hollander et al. (1993).

pattern: ^{13}C-contents derived from aquatic autotrophs are relatively enriched in ^{13}C, i.e. mostly heavier than −20‰. This pattern is not typical for all ^{13}C-enriched sedimentary organic matter derived from microbial mats. For instance, Summons et al. (1996) and van der Meer et al. (2000) have shown that hot spring microbial mats which are enriched in ^{13}C (−14‰ and −17‰) have a diverse range of isotopic compositions for extracted lipids of aquatic origin (from −8‰ for green non sulfur bacteria to −37‰ for cyanobacteria). The observed pattern in the Northern Apennines marl sample is also not typical for organic matter in evaporitic deposits. Reported data for free and sulfur-bound phytane in a number of evaporitic sediments (Table 3) show no $\delta^{13}C$-values heavier than −20‰ as observed for the samples studied here.

The data reported here for La Trinitat and Northern Apennines marl are unique in the sense that most aquatic biomarkers derived from multiple sources are enriched in ^{13}C and not only bulk organic matter. Since lipids of most organisms are depleted in ^{13}C (up to 10‰; e.g. Hayes, 1993; Schouten et al., 1998) this suggest that the cell materials of the source organisms of the aquatic biomarkers are even more enriched in ^{13}C. If we assume that the isotopic composition of dissolved CO_2 was not extraordinarily high in these systems (e.g. des Marais et al., 1989) then the ^{13}C-enriched biomarkers can be best explained by a CO_2-limited ecosystem since this can lead to a decreased fractionation of ^{13}C in most autotrophic organisms (Popp et al., 1998). This limitation may be through a combined effect of limited CO_2-availability and high growth rates (e.g. des Marais et al., 1989; Schidlowski et al., 1994; Popp et al., 1998). Indeed, the highly ^{13}C-enriched stable carbon isotopic compositions of the alkenones are similar to those observed for alkenones in upwelling zones of the coasts of Peru, Oman and Pakistan (Bidigare et al., 1997; Schouten et al. 2000) where haptophyte algae grow at high rates. In addition to growth rates, light limitation may possibly also decrease isotopic fractionation in organisms independent of growth rate (Riebesell et al., 2000). However, although light intensity is diminishing fast with depth in dense microbial mat systems, it is unlikely that light is a limiting factor for *all* phototrophic organisms in microbial mat systems.

5. Conclusions

Our results demonstrate that compound-specific isotope analysis of aquatic biomarkers is potentially able to recognise CO_2-limited ecosystems and those that occurred in the past. In contrast, bulk carbon isotopic analysis will be less reliable since this may predominantly reflect the isotopic compositions of specific organisms with a large contribution to the organic matter of the sediment and thus not reflect the whole ecosystem. Compound specific isotope analysis of ^{13}C-enriched organic matter in hypersaline deposits can thus aid in the recognition of CO_2-limited (microbial mat) ecosystems of the past.

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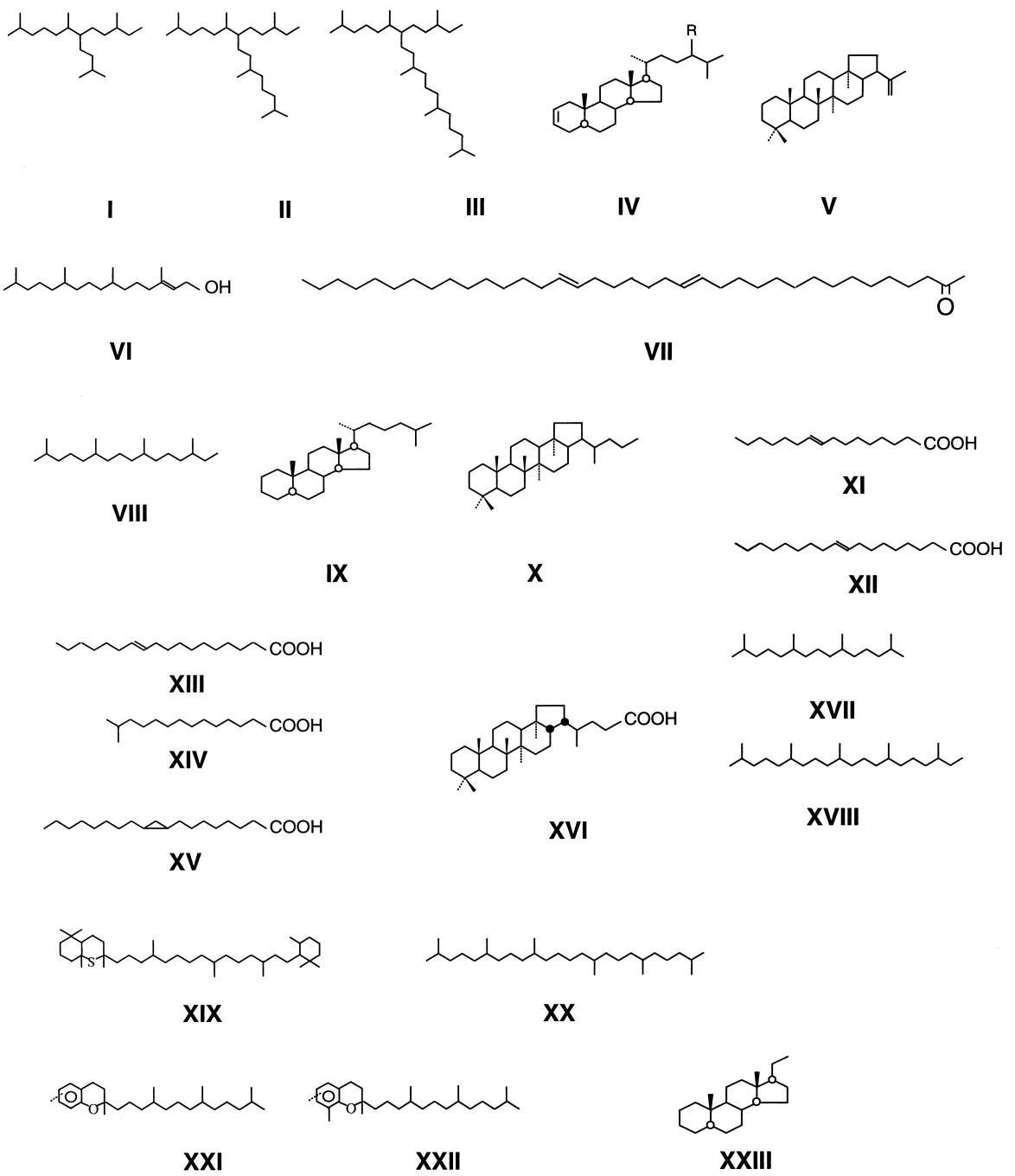
Appendix



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