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Molecular and bulk isotopic analyses of organic matter in marls of the Mulhouse Basin (Tertiary, Alsace, France)

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Abstract—Contents of ^{13}C in kerogens and carbonates in 21 samples from a core of the MAX borehole, Mulhouse Evaporite Basin, range from −27.3 to −23.5 and −3.7 to −1.8‰ vs PDB, respectively. Organic nitrogen in the same samples is enriched in ^{15}N relative to atmospheric N_2 by 12.2–15.7‰. Hydrogen indices and δ values for kerogens vary systematically with facies, averaging 493 mg HC/g C_{org} and −25.7‰ in the most saline facies (dominated by inputs from aquatic sources) and 267 mg HC/g C_{org} and −23.7‰ in the least saline facies (50/50 aquatic/terrigenous). Values of δ were measured for individual aliphatic hydrocarbons from three samples representing three different organic facies. For all samples, terrigenous inputs were unusually rich in ^{13}C, the estimated δ value for bulk terrigenous debris, apparently derived partly from CAM plants, being −22.5‰. In the most saline facies, isotopic evidence indicates the mixing of ^{13}C-depleted products of photosynthetic bacteria with ^{13}C-enriched products of halotolerant eukaryotic algae. At lower salinities, a change in the producer community is marked by a decrease in the ^{13}C content of algal lipids. The content of ^{13}C in algal lipids increases in the least saline facies, due either to succession of different organisms or to decreased concentrations of dissolved CO_2.

Key words—Carbon isotopes, organic facies, evaporites, algol lipids, kerogens, paleoenvironment

INTRODUCTION

The Eocene–Oligocene Mulhouse Potash Basin (Alsace, France) contains a thick series of evaporitic sediments interbedded with marl horizons. The intercalated marls are organic-rich, with TOC contents varying from 1 to 6% (Blanc-Valleron, 1990; Blanc-Valleron *et al.*, 1991; Gely *et al.*, 1991; Betts *et al.*, 1991). Throughout the sequence, evidence of micro- or macro-bioturbation is absent, indicating that decomposition of organic matter by benthic animals was minimal (Blanc-Valleron, 1990; Blanc-Valleron *et al.*, 1991, Gely *et al.*, 1991; Hofmann, 1992). Initial studies of the organic matter from one of these cycles indicated the presence of three distinct types based on bulk properties of kerogens and composition of bitumens (Blanc-Valleron *et al.*, 1991; Gely *et al.*, 1991). Facies A is richest in organic matter, having the highest hydrogen index (HI) and the highest H/C ratio in kerogen. Concentrations of organic matter are lowest in facies C and the type found there has the lowest HI and the lowest H/C ratio in its kerogen. Characteristics of facies B are intermediate. Palynologic studies indicate that the organic matter in facies A and B is predominantly of aquatic origin (95 and 90%, respectively) whereas, in facies C, the contribution of terrestrial organic material is said to be up to 50% (Blanc-Valleron *et al.*, 1991).

Facies A is characterized by a bimodal distribution of *n*-alkanes with modes at C_{16}–C_{18} and C_{24}; isoprenoids from C_{18} to C_{25}, with the most abundant being pristane and phytane; a group of iso-, anteiso-, and alkylcycloalkanes; and cyclic compounds dominated by A-ring-methyl steranes, normal steranes, and diasteranes. Facies B is marked by a distribution of *n*-alkanes maximizing at C_{15}–C_{17}; isoprenoids from C_{18} to C_{25}, with pristane and phytane dominant; iso-, anteiso-, and alkylcycloalkanes; and cyclic compounds dominated by steranes and diasteranes. Facies C is characterized by *n*-alkanes maximizing at C_{25}–C_{33}, with a strong odd/even predominance; isoprenoids extending from C_{18} to C_{25}; abundant iso-, anteiso-, and alkylcycloalkanes and cyclic compounds dominated by steranes, hopanes and diasteranes. More recently, results obtained by multivariate analyses of abundances of biomarkers indicated that the characteristics of the organic matter range between those of two end members (Keely *et al.*, 1993) corresponding to organic facies A and C, and that "facies B" is a point on the A → C continuum.

The variations between facies A, B, and C suggest that factors in addition to changes in proportions of aquatic and terrestrial contributions have been important in controlling the composition of the organic matter. These could include variations in the chemistry of the water column, relative preservation of

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organic matter, and changes in the aquatic biological assemblages. To investigate these alternatives, we have undertaken bulk isotopic analyses of the kerogens (carbon and nitrogen) and carbonates (carbon and oxygen), and molecular carbon isotopic analyses of individual aliphatic hydrocarbons in samples representing the different organic facies.

METHODS

Twenty-one sediment samples from a core (MAX Borehole; Gely *et al.*, 1991) were demineralized using 1N HCl and 40% HF for the preparation of kerogens. Kerogens free of solvent extractable materials were obtained by ultrasonic extraction using dichloromethane. Concentrations of total organic carbon (TOC) were determined using a LECO apparatus. Elemental compositions of kerogens and Rock-Eval parameters were determined ATX Inc., France, and at the IFP, respectively. Bulk isotopic compositions of carbonates (carbon and oxygen) and organic matter (carbon and nitrogen) were analyzed using standard methods and corrections of Craig (1957), Bremner (1965), and Mariotti and Letolle (1978). The carbon, oxygen and nitrogen isotopic results are reported as permit (‰) deviations relative to the international isotopic standards, PDB (Pee Dee Belemnite) for carbon and oxygen, and atmospheric N_2 for nitrogen;

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1]10^3$$

where $R = {}^{13}C/{}^{12}C$, ${}^{18}O/{}^{16}O$, or ${}^{15}N/{}^{14}N$. Precisions of isotopic results were calculated from duplicate analyses. The standard deviation of the mean calculated for duplicate samples was ± 0.09‰ for carbon and ± 0.11‰ for oxygen of carbonates, ± 0.21‰ for organic carbon, and ± 0.25‰ for organic nitrogen.

For the molecular organic geochemical portion of this study, marl samples from facies A and B were collected from the Mulhouse potash mine, whereas the sample from facies C was subsampled from the Max Borehole core. The samples representing facies A and B were taken from the upper and lower portions of bed S, whereas that representative of facies C was collected in the upper portion of bed Ti. Corresponding depths are indicated in the lithostratigraphic column shown in Fig. 1.

Bitumens were obtained by extraction with dichloromethane and a saturated hydrocarbon fraction was isolated. *n*-Alkanes were separated from the total standard-hydrocarbon fraction by urea adduction. Saturated hydrocarbons were analyzed by gas chromatography and by GCMS as reported elsewhere (Sinninghe Damsté *et al.*, 1993b). Carbon isotopic ratios of individual compounds from the urea-adducted *n*-alkane fractions and nonadducted branched/cyclic fractions were analyzed at Indiana University using an improved method of isotope-ratio-monitoring gas chromatography–mass spectroscopy (irmGCMS, Hayes *et al.*, 1990; Freeman *et al.*, 1990). The standard deviations of the mean calculated for duplicate and triplicate irmGCMS analyses ranged between 0.2 and 1.2‰ and averaged 0.42‰.

RESULTS AND DISCUSSION

Elemental, molecular, and isotopic compositions

Results of analyses of bulk samples are graphically summarized in Fig. 1 and compiled in Table 1. Abundances and isotopic compositions of *n*-alkanes and acyclic isoprenoids in the three samples chosen as representative of facies A, B, and C are listed in Table 2 and graphically summarized in Fig. 2. Chromatograms summarizing the abundances of steranes and hopanes in the three different extracts are shown in Fig. 3 The identifications and, where available, isotopic compositions of the compounds represented by the numbered peaks are given in Table 3.

Sources of organic matter

Palynological studies have indicated that facies C may contain a maximum of 50% terrigenous debris while facies A and B contain only 5 and 10%, respectively (Blanc-Valleron *et al.*, 1991). These estimates are based on density separation whereby the technique utilized may exhibit a slight bias toward recovery of terrigenous constituents (Bertrand, personal communication). Reported contents of terrigenous debris are, therefore, probably maxima. Earlier investigations of extractable hydrocarbons have supported the conclusion that terrigenous debris is more prevalent in facies C (Blanc-Valleron *et al.*, 1991; Gely *et al.*, 1991).

Compounds for which isotopic analyses are available include at least three distinct indicators of the sources of primary organic material. First, long-chain *n*-alkanes derived partly from vascular plant waxes appear in extracts from all three facies. They are most prominent in the extract from facies C (CPI = 2.73) and their δ value there is well defined at ~ -28.5‰. As shown in Fig. 2, somewhat lighter values are observed for these compounds in facies A and B. Long-chain *n*-alkanes with even carbon numbers are relatively more abundant in those extracts (CPI = 0.95), indicating the presence of an additional source that must contribute more strongly at the even-carbon numbered components. Indeed, δ values of even-carbon species are more negative, indicating that the second source is depleted in ${}^{13}C$ relative to the plant wax components. Studies of cultured algae and cyanobacteria (Gelpi *et al.*, 1970) and of marine lipids in the Southern Ocean, where diatoms are dominant (Nichols *et al.*, 1988), have shown that significant amounts of long-chain alkanes can be produced by aquatic organisms. In the Mulhouse Basin, in addition to *n*-alkanes contributed from such an aquatic origin, *n*-alkanes may have been contributed as a result of diagenetic transformations of functional-

ized, predominantly even-carbon-numbered precursors of the same aquatic origin. Such aquatic inputs could account for the observed distribution of *n*-alkanes in facies A and B as well as for the varying isotopic compositions of longer-chain *n*-alkanes. Accordingly, the δ value of the plant wax component is probably constant across all three facies. If, in vascular plants, wax alkanes are depleted in ^{13}C relative to total plant biomass by ~6‰ (Galimov and Shirinskiy, 1975; Rieley *et al.*, 1992) the related terrigenous input would have a δ value of −22.5‰. Typically, remains of higher plants (e.g. lignites and coals) of Eocene age have δ values around −27‰ (Arthur *et al.*, 1985; Dean *et al.*, 1986; Freeman *et al.*, 1990). The δ value estimated here thus indicates significant enrichment in ^{13}C. Palynological reports refer to a "Mediterranean climate with a long dry season" (Schuler, 1988). Plants adopted to dry environments may therefore have been prominent. Although the earliest isotopic and fossil evidence for C_4 photosynthesis dates only from the Pliocene (Nambudiri *et al.*, 1978), it is possible that C_4 plants may have evolved as early as the late Jurassic (Brown and Smith, 1972), and there is phylogenetic evidence that CAM photosynthesis has much earlier origins (Taylor and Hickey, 1992). It is therefore possible that the high "terrigenous δ value" estimated here reflects inputs from such species, although no isotopic evidence indicating input of C_4 plants in Eocene–Oligocene sediments has been previously published. Mechanisms of transport and preservation are likely to discriminate between waxes and bulk debris (e.g. Prahl, 1985). Relationships between the long-chain *n*-alkanes and the bulk terrigenous component present in these facies are therefore uncertain but, for the moment, the alkane-based δ value provides the best view of terrigenous inputs.

Second, extracts from all three facies contain steranes presumably derived from the algae that would have been important aquatic primary producers. In facies A, the δ values of the most abundant steranes are near −26‰. Since algal lipids are depleted in ^{13}C relative to total biomass by 3–4‰ (Abelson and Hoering, 1961), inputs of aquatic eukaryotic (sterol-bearing) algal debris to facies A probably had δ values near −22.5‰. Steranes in samples from facies B and C have different δ values, indicating that the isotopic compositions of eukaryotic algal biomass varied, dropping $\approx$ −26‰ in facies C and to $\approx$ −28‰ in facies B.

Third, extracts from all three facies contain *n*-heptadecane, the *n*-alkane most commonly produced by both eukaryotic and prokaryotic algae (Gelpi *et al.*, 1970). If *n*-alkyl carbon skeletons are slightly depleted in ^{13}C relative to polyisoprenoid carbon skeletons (Hayes, 1994), δ values reconstructed for aquatic inputs on the basis of n-C_{17} would be −24.7, −30, and −27.2‰ for facies A, B, and C, respectively. Differences between n-C_{17}-based and sterane-based biomass estimates for facies C are small. Either prokaryotes and eukaryotes were not isotopically distinct or prokaryotic contributions were not large. In facies A and B, however, the *n*-alkane-based estimates fall 2‰ below the sterane-based estimate, suggesting that ^{13}C-depleted cyanobacterial contributions may have been significant.

A fourth category of compounds—the acyclic isoprenoids, in particular phytane and pristane—is probably also related to primary producers in the Mulhouse paleoenvironment. The possible sources of these carbon skeletons are numerous and diverse and are discussed below.

Having no firm basis for further refinement of estimates of the isotopic composition of inputs from aquatic sources, rough averages of the sterane- and n-C_{17}-based values can be adapted (weighting the estimates to favor the n-C_{17} values, since that product can derive from both prokaryotic and eukaryotic species). In order to recognize the lack of agreement between indicators, but not to express any rigorously defined uncertainty, these values can be noted as -24 ± 1, -29 ± 1, and -26.8 ± 0.5‰ for aquatic inputs to facies A, B, and C, respectively. Inclusion of terrigenous inputs represented by the plant waxes then yields weighted-average primary δ values of -24 ± 1‰ for facies A (if 95/5 aquatic/terrestrial, as suggested by the palynological analyses), -28.4 ± 1‰ for facies B (90/10), and −24.6‰ for facies C (50/50). These compare to observed kerogen δ values of −25.7, −25 and −23.8‰, respectively. With some risk of oversimplifying (additional factors are considered below), it thus appears that kerogens of facies A contain an additional ^{13}C-depleted component, that those of facies B contain an additional ^{13}C-enriched component, and that the isotopic composition of kerogens in facies C can be accounted for with only minor additions of a ^{13}C-enriched component.

Reconstruction of paleoenvironmental conditions

Anaerobic conditions in photic zone. The isotopic data include several paleoenvironmental clues that can be integrated with relatively firm evidence from other investigations. Specifically, the marked depletion of ^{13}C in aquatic products at the A → B facies transition and the presence in facies A of pristane and phytane strongly depleted in ^{13}C (Table 2) can be correlated with evidence for the existence of an anaerobic photic zone and changing salinity levels in the paleoenvironment. Sediments from facies A and B contain porphyrins with extended alkyl substituents (Keely and Maxwell, 1993). These must derive from bacteriochlorophylls *c* and *d*, which are products of green photosynthetic bacteria. The presence of these obligately anaerobic photoautotrophs indicates that anaerobic conditions within the water column reached upward into the photic zone at least intermittently. Notably, however, the sediments do not contain trimethylphenyl isoprenoids (Summons and Powell, 1987) and the kerogen pyrolyzates do

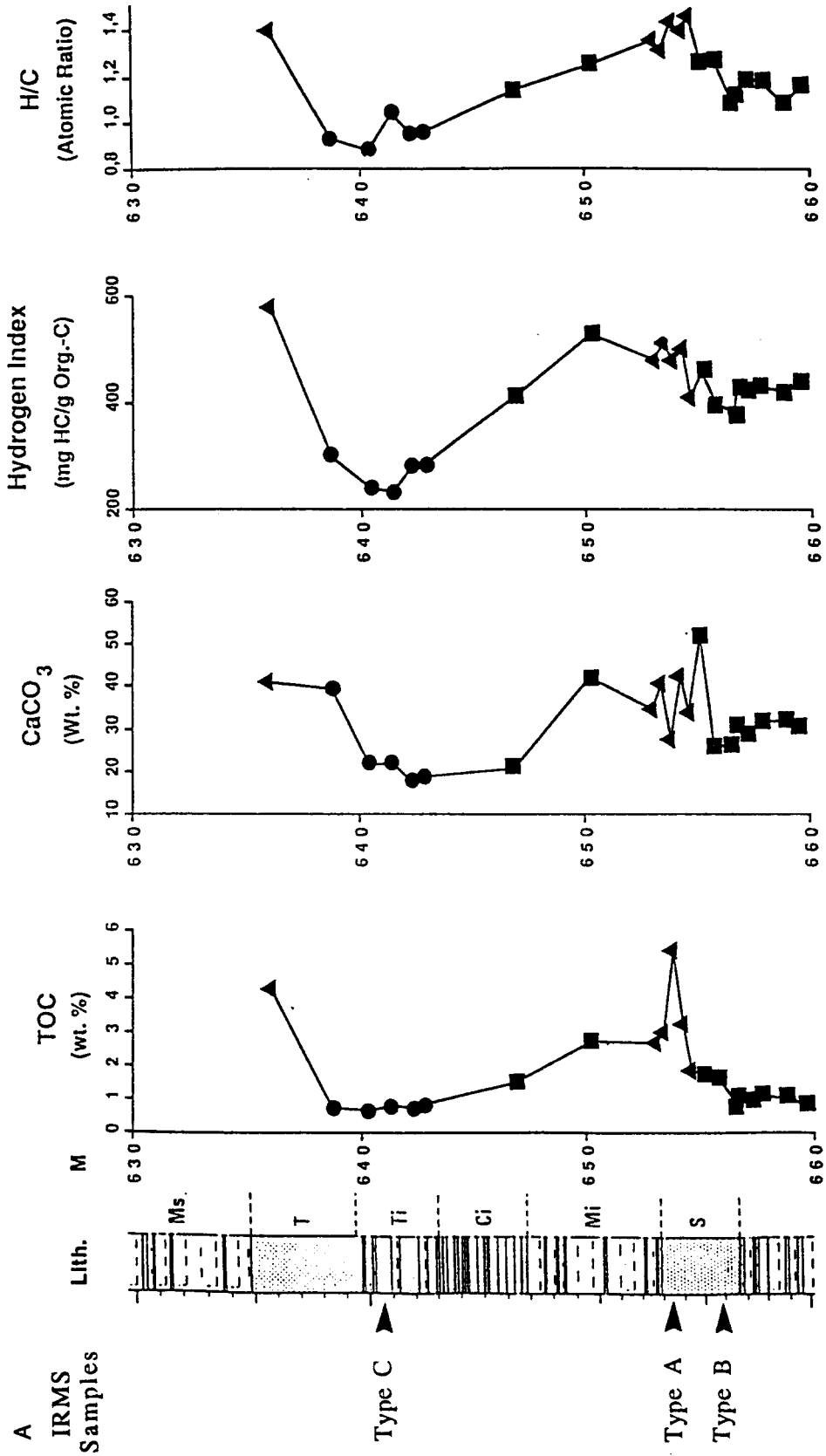
A
IRMS Samples
Type C
Type A
Type B
Lith.
Ms
T
Ti
Ci
Mi
S
M
630
640
650
660
TOC (wt. %)
0 1 2 3 4 5 6
CaCO3 (Wt. %)
10 20 30 40 50 60
Hydrogen Index (mg HC/g Org.-C)
200 400 600
H/C (Atomic Ratio)
0,8 1,0 1,2 1,4

Fig. 1(A)

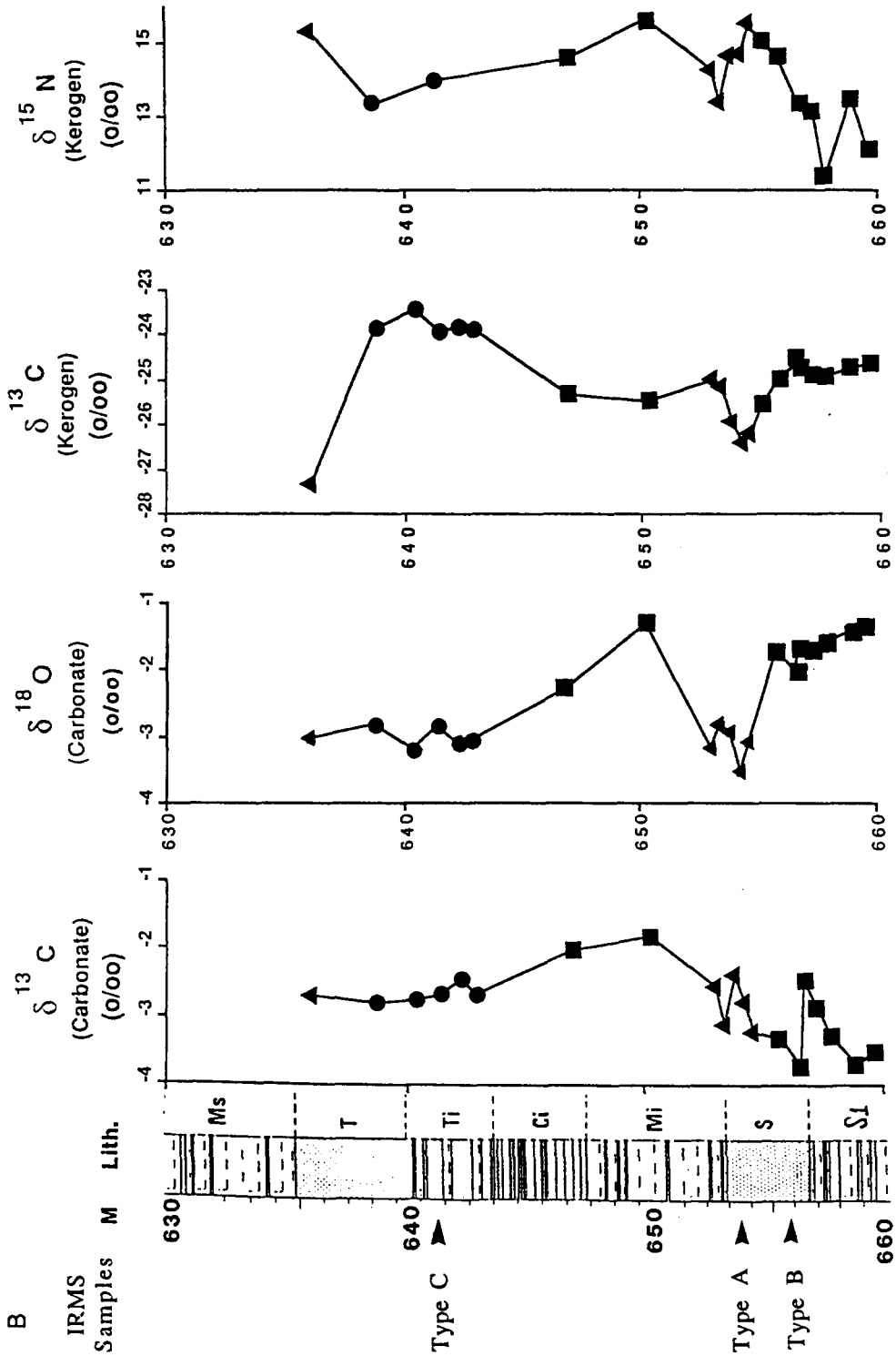

Fig. 1. (A) Lithostratigraphy (with indication of beds from which samples were taken for compound-specific isotopic analyses), total organic carbon (wt %), carbonate concentration (wt %), hydrogen indices, and atomic H/C ratios of isolated kerogens for 21 samples from the MAX Borehole. The sedimentary units comprise organic-matter-containig marls interbedded weith potassium salts (Ci), halite-rich beds with clayey-anhydrite intercalations of a few cm to several tens of cm (Si, Mi, and Ti), and clayey-anhydrite alterations with 1–10 cm-thick marly and anhydrite-rich layers (S and T). Triangles represent facies A, squares represent facies B, and circles represent facies C. (B) Lithostratigraphy (with indication of beds from which samples were taken for compound-specific isotopic analyses) and isotopic compositions of carbonates and kerogens from the same samples noted in Fig. 1(A).

Table 1. Chemical and isotopic composition of samples

Depth (m)	Organic facies	Sed. zone	Carbonates Conc. (wt % C)	Carbonates $\delta^{13}C$ (‰ vs PDB)	Carbonates $\delta^{18}O$ (‰ vs PDB)	Kerogens Conc. (wt % C)	Kerogens Hi (mg HC/g C_{org})	Kerogens H/C	Kerogens $\delta^{13}C$ (‰ vs PDB)	Kerogens $\delta^{15}N$ (% vs AIR)
636.00	A	T	4.9	−2.7	−3.0	4.26	578	1.39	−27.3	15.4
638.75	C	T	4.7	−2.8	−2.8	0.74	298	0.93	−23.9	13.3
640.42	C	Ti	2.6	−2.7	−3.2	0.66	241	0.88	−23.5	ND
641.41	C	Ti	2.7	−2.7	−2.9	0.82	232	1.04	−23.9	14.0
642.30	C	Ti	2.1	−2.4	−3.1	0.76	281	0.95	−23.8	ND
642.83	C	Ti	2.3	−2.7	−3.1	0.84	285	0.96	−23.9	ND
646.85	B	Ci	2.5	−2.0	−2.3	1.49	412	1.14	−25.3	14.6
650.24	B	Mi	5.1	−1.8	−1.3	2.70	527	1.25	−25.5	15.7
653.00	A	S	4.2	−2.5	−3.2	2.64	480	1.35	−25.0	14.3
653.40	A	S	4.9	−3.1	−2.8	2.93	512	1.31	−25.1	13.4
653.80	A	S	3.3	−2.4	−2.9	5.40	478	1.43	−25.9	14.7
654.24	A	S	5.1	−2.8	−3.5	3.20	498	1.39	−26.4	14.7
654.60	A	S	4.1	−3.2	−3.1	1.87	410	1.45	−26.2	15.5
655.15	B	S	6.3	ND	ND	1.75	456	1.27	−25.6	15.0
655.80	B	S	3.1	−3.3	−1.7	1.67	399	1.26	−25.0	14.7
656.59	B	S	3.2	−3.7	−2.0	0.88	380	1.09	−24.6	ND
656.75	B	Sl	3.7	−2.5	−1.7	1.14	431	1.12	−24.8	13.3
657.30	B	Sl	3.4	−2.9	−1.7	1.03	425	1.19	−24.9	13.1
657.85	B	Sl	3.8	−3.3	−1.6	1.09	427	1.18	−24.9	11.3
658.88	B	Sl	3.8	−3.7	−1.4	1.01	422	1.08	−24.8	13.5
659.57	B	Sl	3.7	−3.5	−1.4	0.83	434	1.16	−24.7	12.2

not include 1,2,3,4-tetramethylbenzene (Sinninghe Damsté *et al.*, 1993a), both expected to derive from pigments produced by Chlorobiaceae (representative of green photosynthetic bacteria). Organic matter enriched in ^{13}C due to production by organisms fixing carbon via the reverse-TCA cycle (an additional characteristic of green photosynthetic bacteria) is also not evident. It appears, therefore, that the green photosynthetic bacteria, though present, did not flourish in the paleoenvironment.

Given the evidence for the existence of an anaerobic photic zone, it is then logical to consider the presence of alternative anaerobic phototrophs, specifically the purple photosynthetic bacteria. The carbon skeletons produced by these organisms are not structurally unique, but their presence in the Mulhouse community is probably indicated by the low δ values found for the pristane and phytane extracted from the facies-A sample (Table 2). The purple photosynthetic bacteria (for example, the Chromatiaceae) utilize the Calvin Cycle for fixation

Table 2. Carbon isotopic compositions of *n*-alkanes and of acyclic isoprenoids

Compound	$\delta^{13}C$ (‰ vs PDB) Facies A	Facies B	Facies C
n-C_{15}	−31.0	−33.5	
n-C_{16}	−32.2	−33.9	
n-C_{17}	−31.7	−34.0	−31.2
n-C_{18}	−31.4	−33.2	−30.7
n-C_{19}	−30.3	−32.5	−27.1
n-C_{20}	−29.6	−31.8	−29.1
n-C_{21}	−30.2	−30.8	−29.6
n-C_{22}	−31.4	−30.6	−28.6
n-C_{23}	−32.5	−30.2	−28.7
n-C_{24}	−31.7	−30.9	−28.8
n-C_{25}	−31.8	−30.1	−28.0
n-C_{26}	−31.5	−32.0	−28.9
n-C_{27}	−29.1	−29.3	−28.1
n-C_{28}	−30.1	−30.2	−28.2
n-C_{29}	−29.0	−29.3	−28.3
n-C_{30}		−31.2	−28.2
n-C_{31}		−29.5	−28.7
n-C_{32}		−31.3	−28.5
n-C_{33}		−29.5	−27.5
nor-Pristane	−31.6	−27.7	−24.6
Pristane	−33.1	−29.1	−27.1
Phytane	−33.2	−26.6	−28.3
Isopr.-C_{21}	−27.7	−27.2	−25.4
Isopr.-C_{22}	−27.3	−26.0	−24.1
Isopr.-C_{23}	−29.2	−25.3	−23.7
Isopr.-C_{24}	−29.9	−26.1	−24.0
Isopr.-C_{25}	−28.3	−24.3	−24.6

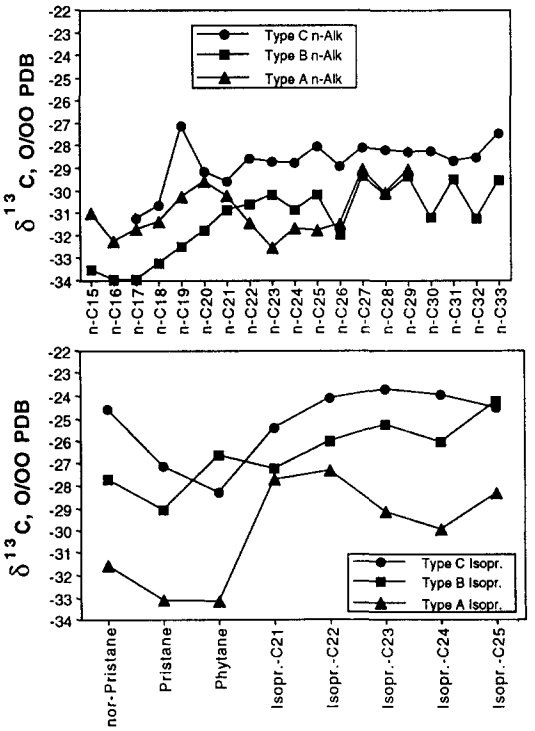

Fig. 2. Carbon isotopic compositions of individual *n*-alkanes and of acyclic isoprenoids from extracts of organic facies A, B, and C.

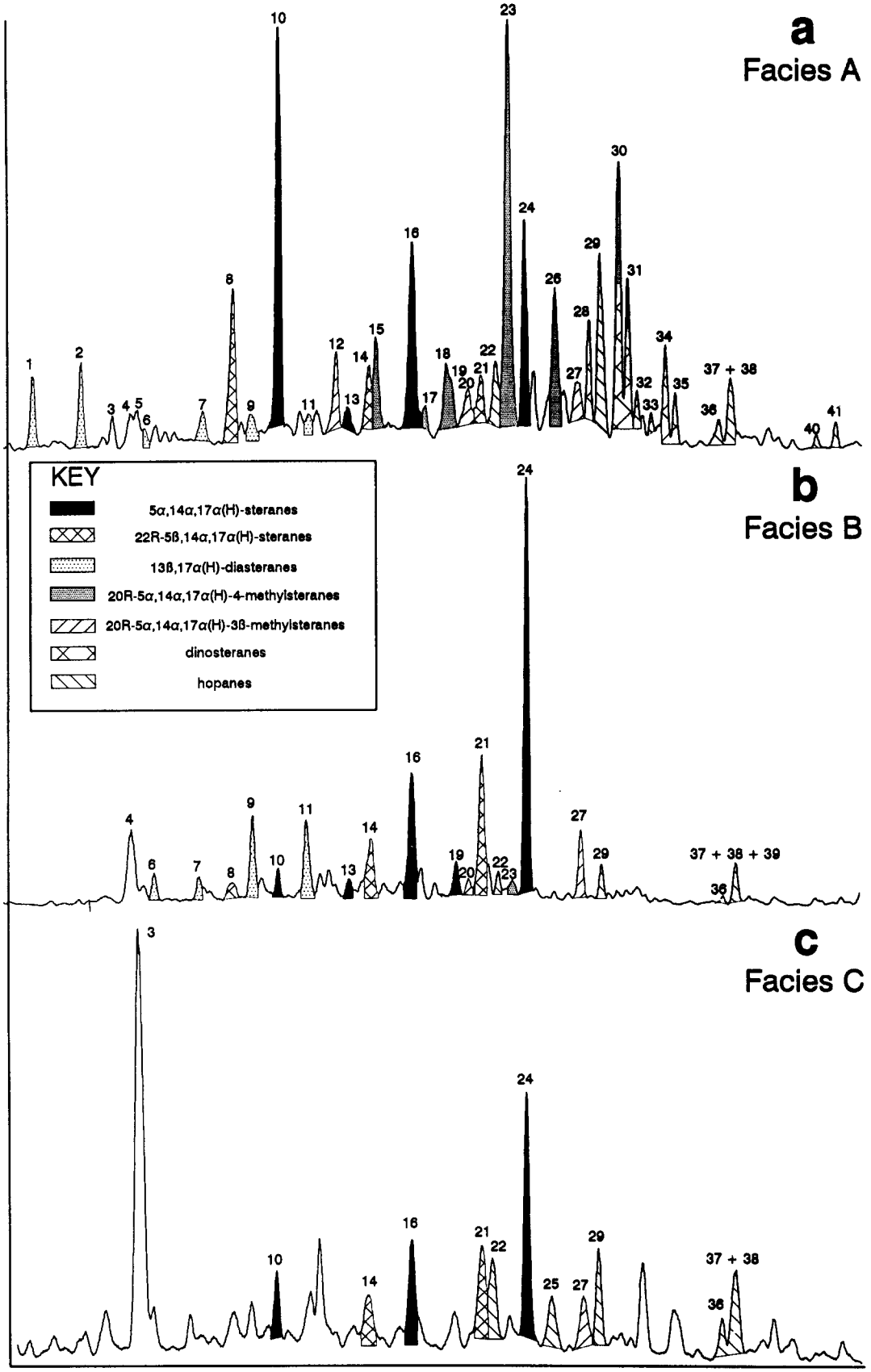

Fig. 3. Chromatograms indicating the distributions of steranes and hopanes from extracts of organic facies A, B, and C. Identities and, where possible, isotopic compositions of compounds represented by the numbered peaks are summarized in Table 3.

Table 3. Steranes and triterpanes identified and their stable carbon isotopic composition

	Compound	$\delta^{13}C$ (‰ vs PDB) Facies A	Facies B	Facies C
1	20*S*-13β,17α (H)-diacholestane	−27.3	ND	ND
2	20*R*-13β,17α (H)-diacholestane	−26.4	ND	ND
3	20*S*-13α,17β (H)-diacholestane			
4	squalane	ND	ND	−26.5
5	20*R*-13α,17β (H)-diacholestane			
6	20*S*-13β,17α (H)-24-methyldiacholestane			
7	20*R*-13β,17α (H)-24-methyldiacholestane			
8	20*R*-5β,14α,17α (H)-cholestane	−24.4	ND	ND
9	20*S*-13β,17α (H)-24-ethyldiacholestane	ND	−32.5	ND
10	20*R*-5α,14α,17α (H)-cholestane	−26.3	ND	ND
11	20*R*-13β,17α (H)-24-ethyldiacholestane	ND	−32.3	ND
12	20*R*-5α,14α,17α(*H*)-3β-methylcholestane			
13	20*S*-5α,14α,17α (H)-245-methylcholestane			
14	20*R*-5β,14α,17α (H)-24-methylcholestane			
15	20*R*-5α,14α,17α (H)-4α-methylcholestane	−21.1	ND	ND
16	20*R*-5α,14α,17α (H)-24-methylcholestane	−27.4	−29.6	−30.2
17	20*R*-5α,14α,17α (H)-4β-methylcholestane			
18	20*R*-5β,14α,17α (H)-4α,24-dimethylcholestane			
19	20*S*-5α,14α,17α (H)-24-ethylcholestane			
20	20*R*-5α,14α,17α (H)-3β,24-dimethylcholestane			
21	20*R*-5β,14α,17α (H)-24-ethylcholestane	ND	−31.9	−30.4
22	17α,21β (H)-norhopane	ND	ND	−30.4
23	20*R*-5α,14α,17α (H)-4α,24-dimethylcholestane	−25.7	ND	ND
24	20*R*-5α,14α,17α (H)-24-ethylcholestane	−27.8	−31.2	−28.2
25	17β,21α (H)-norhopane	ND	ND	−31.6
26	20*R*-5α,14α,17α (H)-4β,24-dimethylcholestane			
27	20*R*-5α,14α,17α (H)-3β-methyl-24-ethylcholestane			
28	20*R*-5α,14α,17α (H)-4α,23,24-trimethylcholestane			
29	17α,21β (H)-hopane	ND	ND	ND
30	20*R*-5α,14α,17α (H)-4α-methyl-24-ethylcholestane and 20*R*-5α,14α,17α (H)-4α,23,24-trimethylcholestane	−26.5	ND	ND
31	20*R*-5α,14α,17α (H)-4α,23,24-trimethylcholestane	−25.7	ND	ND
32	20*R*-5α,14α,17α (H)-4β,23,24-trimethylcholestane			
33	20*R*-5α,14α,17α (H)-4β,23,24-trimethylcholestane			
34	20*R*-5α,14α,17α (H)-4β,23,24-trimethylcholestane			
35	20*R*-5α,14α,17α (H)-4β,23,24-trimethylcholestane			
36	22*S*-17α,21β (H)-homohopane	ND	ND	−29.0
37	gammacerane			
38	22*R*-17α,21β (H)-homohopane			
39	20*R*-5α,14α,17α (H)-3β,24-diethylcholestane			
40	22*S*-17α,21β (H)-bishomohopane			
41	22*R*-17α,21β (H)-bishomohopane			

of carbon and thus impose isotopic fractionations like those characteristic of oxygenic algae. However, their habitats often contain dissolved inorganic carbon that is depleted in ^{13}C and/or high concentrations of dissolved CO_2. Due to these environmental factors, their products are often depleted in ^{13}C relative to those of other Calvin-cycle autotrophs. In the preceding section, it was estimated that algal products in facies A had $\delta \approx -24‰$. Isoprenoids deriving from algal products would thus be expected to have δ values near −28‰. This is observed for the acyclic C_{21}–C_{25} species, but the pristane and phytane are depleted by an additional 5‰ (Table 2). Since at least some inputs of C_{19} and C_{20} isoprenoid carbon skeletons with $\delta \approx -28$ must have been present, it follows that pristane and phytane precursors with $\delta < -33‰$ are required. These were apparently provided by bacteriochlorophylls *a* and *b*, which are produced by the purple photosynthetic bacteria and which contain ester-linked phytol and geranyl geraniol. A role for purple photosynthetic bacteria as a source of ^{13}C-depleted phytane was also suggested by Kohnen *et al.* (1992) in their examination of sulfur-bound biomarkers in sediments from the Messinian Veno del Gesso evaporite basin.

Paleosalinities, changes in producer community. Stratification of the water column (allowing development of anaerobic conditions within the photic zone and accumulation below the chemocline of high concentrations of inorganic carbon depleted in ^{13}C) would have been favored by development of gradients in salinity. Significantly, Sinninghe Damsté *et al.* (1993b) have shown that distributions of isoprenoid chromans suggest the occurrence of strong gradients, with the highest photic-zone paleosalinity occurring during deposition of facies A. The chroman ratio in samples from facies B is distinctly higher, indicating a decrease in salinity in the photic zone relative to facies A. The increased chroman ratio is correlated with a decrease in the ^{13}C content of algal products. The isotopic shift is not likely to be due to a decrease in the ^{13}C content of the carbon source: (i) there is no evidence for secular change, either in the global record of marine carbonates at this time or in the δ values of carbonate minerals in the Mulhouse sediments (Table 1); (ii) an apparent decrease in

photic-zone salinity suggests, if anything, increased separation of the producer community from the portion of the water column likely to contain ^{13}C-depleted inorganic carbon. The decrease in δ values of primary organic carbon may therefore indicate an increase in concentrations of dissolved CO_2 and/or a change in the isotope effects associated with photosynthetic fixation of CO_2. The latter could be associated with a change in the producer community. Such a change is, in fact, directly indicated by the changing chroman ratio. It is the most likely cause of the observed isotopic variations since the alternative—an increase in $[CO_2(aq)]$—would require that the facies-A community had been existing at a CO_2 minimum within the water column, somehow sustaining that condition between a high-CO_2 atmosphere (Freman and Hayes, 1992) and lower, anaerobic portions of the photic zone that were probably rich in dissolved CO_2. It is not known exactly *why* the "high-salinity organisms", whatever their identity, would have had a lower isotope effect and thus produced organic material with higher δ values. However, in terms of the two-step model for fractionation of isotopes during photosynthetic fixation of carbon (Farquhar *et al.*, 1982), reduction of the isotope effect would indicate greater control of the overall rate by the process of mass transport. The isotopic shift may therefore be associated with the maximal control that organisms adapted to high-salinity environments must exert over all inputs and outputs of water and solutes.

Further evidence for stratification in the water column is likely provided by the observed enrichment of ^{15}N in kerogens from these sediments (Table 1). Velinsky *et al.* (1991) have shown that dissolved ammonium is strongly enriched in ^{15}N at oxyclines in modern stratified environments (the Black Sea, Saanich Inlet, and Framvaren Fjord), and Collister and Hayes (1991) found that kerogens from the strongly stratified Green River lacustrine system were also enriched in ^{15}N. The enrichments stem from isotope effects associated with uptake, oxidation, and reduction of N species within the water column and are characteristic of systems in which high concentrations of NH_4^+ develop in anaerobic bottom waters.

The requirement for an additional, ^{13}C-rich input to account for the δ value of facies-B kerogens is probably met by the producers of the C_{21}–C_{25} acyclic isoprenoids. Circumstantial evidence for such a significant contribution is also provided by the abundant isoprenoids, including C_{21}–C_{25} components, in the pyrolysates of kerogen from facies B (Sinninghe Damsté *et al.*, 1993a). Values of δ for these compounds in facies B exceed those of coexisting steranes by almost 6‰ and would be associated with biomass-C having δ values near −22‰. Significant inputs from this source could pull kerogen values up from the expected −28.5‰ toward the observed −25‰ and could account for the elevation of pristane δ values relative to those of the steranes.

The absence of compounds strongly depleted in ^{13}C indicates that recycling of methane was not an important process in the paleoenvironment. With intermittent flooding of the basin by waters of *relatively* low salinity (marine and riverine waters would have had lower salinities than did the bottom-water brine; Hofmann, 1992; Hofmann *et al.*, 1993), dissolution of pre-existing evaporite minerals would have generated abundant concentrations of dissolved sulfate in the water column and in sediment pore waters, thereby limiting methane production. The widespread occurrence of pyrite framboids supports these assertions (Hofmann, 1992).

Facies C. The chroman ratio of facies C points to a photic zone with normal marine or fresh water (Sinninghe Damsté *et al.*, 1993b). In facies C, δ values of all isoprenoids (steranes, pristane and phytane, and C_{21}–C_{25} acyclic species) increase by about 2‰. This general enrichment of ^{13}C in aquatic indicators is plausibly associated with a decrease in the concentration of dissolved CO_2, that change being due to a more complete separation of the producer community from the anaerobic portion of the water column.

It was concluded in the preceding discussion of sources that the overall mass balance suggested a "minor addition of a ^{13}C-enriched component" to the facies-C kerogen, and this is likely represented by the producers of the C_{21}–C_{25} acyclic isoprenoids, which were not taken into account in that discussion.

The nature of ultimately preserved TOC

Tissot and Welte (1984) have suggested that terrigenous organic residues in marine sediments typically have HI values near 200 mg HC/g C_{org}. Using this value, the palynologically assessed marine/terrestrial ratios, and the HI values of *total* organic matter in these sediments, the HI values of the aquatic components can be estimated by difference (e.g. if the bulk HI were 400 and the marine/terrestrial ratio were 50/50, the implied HI of the marine component would be 600). Results indicate that the aquatic organic material has an average HI values of 560, 490, and 365 mg HC/g C_{org} for facies A, B, and C, respectively. These variations cannot be ascribed to differences in thermal maturation or benthic reworking. They must reflect variations in the nature of the aquatic input, due either to preferential degradation (or selective preservation) during biological reworking of primary inputs (Demaison and Morre, 1980; Tissot and Welte, 1984; Jones, 1989; Hollander *et al.*, 1990) or to varying content of lipids in the initial inputs.

Here, hydrogen richness varies only slightly between facies A and B and is not low in either case. During the deposition of facies A and B, a stratified water column characterized by high salinity, low oxygen bottom waters would be the ideal environment to enhance the preservation of sinking organic matter resulting in the comparatively higher HI values (i.e. selective preservation of lipid components).

Environmental conditions affecting the reworking of primary inputs were apparently similar, as was suggested by multivariate analyses of biomarker distributions and concentrations (Keely *et al.*, 1993). The lower HI value estimated for the aquatic component in facies C may indeed reflect an increase in the degree of heterotrophic reworking (i.e. preferential degradation of lipid components) in a less stratified water column characterized by normal marine to fresh water salinities and bottom waters which are oxygenated. The concentration of hopanes, produced solely by prokaryotes, is highest in facies C. This would suggest that, during the deposition of facies C, bacterial production (i.e. cyanobacteria) and bacterial reworking of primary material were particularly significant and partly responsible for decreasing the hydrocarbon richness of the kerogens. The δ values of the hopanes range from -29 to -31.5‰ and are very similar to those of the steranes, suggesting that prokaryotes and eukaryotes were residing in approximately the same habitat. In addition, it has been documented that various primary producers have slightly varying H/C ratios which decrease from dinoflagellates to diatoms to coccoliths (Pelet, 1983). Consequently, organisms with a lower H/C ratio (and lower HI value) could have contributed more to the total organic carbon and could, in part, account for the lower HI values of aquatic material in facies C.

CONCLUSIONS

1. The δ value of terrestrial vascular plants contributing terrigenous debris to the Mulhouse sediments was near -22.5‰ vs PDB. This value, unusually elevated in comparison with most estimates of δ values of Eocene vascular plants, is consistent with placement of the basin in an arid environment in which CAM plants would be favored. Mixture of ^{13}C-enriched debris from such plants with lighter contributions from C_3 plants could easily produce average δ values near -22.5‰.

2. Contents of ^{13}C in aquatic inputs varied significantly, from near -24‰ in facies A, to approx. -29‰ in facies B, and -27‰ in facies C. These variations are due mainly to changes in the producer community. In facies A, the δ value of aquatic biomass represents a combination of ^{13}C-depleted inputs (relative to the -24‰ average for algal biomass) from purple photosynthetic bacteria and ^{13}C-enriched inputs of products of the organism(s) producing the C_{21}–C_{25} acyclic isoprenoids. In facies B, the δ value of products of eukaryotic algae was distinctly lower than that in facies A, apparently due mainly to displacement of halotolerant organisms by more normal species, and isotopic evidence for photosynthetic bacterial inputs is lacking. In facies C, a further change in the producer community is indicated by changes in biomarker distributions. That change may explain the increase in aquatic δ_{org}, but it is also possible that concentrations of dissolved CO_2 decreased (thus decreasing the isotopic fractionation) due to a deepening of the chemocline.

3. Although the presence of porphyrins with extended alkyl substituents indicates that green photosynthetic bacteria were present in the Mulhouse community, the absence of abundant products enriched in ^{13}C (a characteristic of carbon fixed by the reverse-TCA cycle) indicates that their quantitative contribution to the accumulated organic matter was relatively small.

4. Isotopic evidence suggests accumulation of ^{13}C-depleted dissolved inorganic carbon in waters below the mixed layer during deposition of facies A.

5. The observed enrichment of ^{15}N in kerogens throughout the sequence suggests persistent stratification of the water column.

6. Estimated hydrogen indices of aquatic components are lower in facies C, indicating either (i) increased oxidative reworking of primary inputs (i.e. preferential degradation of lipid components) or (ii) lower hydrogen richness in the primary inputs. Either of these effects could be associated with increased bacterial or cyanobacterial inputs, and these are indicated by an increase in the hopane content.

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