Recognition of Paleobiochemicals by a Combined Molecular Sulfur and Isotope Geochemical Approach

Math E. L. Kohnen,* Stefan Schouten, Jaap S. Sinninghe Damsté,† Jan W. de Leeuw, Dawn A. Merritt, J. M. Hayes

Study of organic matter in immature sediments from a Messinian evaporitic basin shows that consideration of structures, modes of occurrence, and carbon isotopic compositions of free and sulfur-bound carbon skeletons allow identification of biochemical precursors. Detailed information concerning biotic communities present during deposition of sediments can be retrieved in this way. Moreover, unprecedented biochemicals were recognized; these extend the horizon of biomarker geochemistry.

Examination of organic compounds preserved in sedimentary rocks has become an important approach to reconstruction of ancient environments (*1*, *2*). The molecular structures of many sedimentary hydrocarbons can be related to those of precursor lipids characteristic of specific organisms. Then, from knowledge of which organisms were present, aspects of the ancient environment can be deduced. Precise resolution of biological sources can, however, be difficult because structures of primary biosynthetic products are commonly altered by microbial reworking and chemical transformations. Many of the "biomarkers" are thus defunctionalized, sometimes obscuring their precursor origins. Before defunctionalization, however, lipids may react with inorganic sulfides (HS_x^-, $x = 1$ to 5) to form organic sulfur compounds (OSC) (*3*). These sulfurized biomarkers are less susceptible to microbial attack, and their structures provide clues about the nature and former positions of functional groups (*4*) and thus the paleoenvironment (*5*, *6*). Isotopic compositions of geolipids approximate those of their biological precursors, which are, in turn, determined by (i) the isotopic composition of the carbon assimilated by the organism and (ii) the biochemical processes involved in their synthesis (*7*). The isotopic composition therefore encodes information about the source organism and its habitat (*7*, *8*).

In this report we show that the combined application of organic-sulfur geochemistry and isotopic analysis can yield a further recovery of paleoenvironmental information. We tested this approach in five S-rich, immature sediments reflecting one evaporitic cycle in the Messinian Vena del Gesso Basin (northern Apennines, Italy) deposited 5.2 million years ago during the salinity crisis in the Mediterranean (*9*). The extracts of these sediments were separated into hydrocarbon, alkylthiophene, alkylsulfide, and polar fractions (*10*), the latter containing sulfide-linked macromolecular aggregates (*11*) and ether-linked lipids (*12*). All fractions were quantitatively analyzed by gas chromatography–mass spectrometry (GC-MS). Fractions from one marl sample were also analyzed by isotope-ratio-monitoring GC-MS in order to determine C isotopic compositions of individual compounds (*13*).

A few of the C skeletons were extracted as free hydrocarbons, the rest were recovered from sulfurized biomarkers. The latter group comprised compounds containing S-heterocyclic rings and having molecular weights (MW) up to ~500 daltons and aggregates (MW >500 daltons) of diverse carbon skeletons linked by sulfide bridges. Their formation most likely involved attack by inorganic sulfides on functionalized (mainly double bonds) biolipids to form a C–S bond (*4*, *14*). The reactive intermediate thus formed is stabilized by formation of a second C–S bond. If this second reaction is intramolecular, a cyclic product (*15*) is formed. This occurs only when two sites suitable for C–S bond formation are separated by fewer than four sp^3-hybridized C atoms (*4*). When this condition is not met, intermolecular reactions yield aggregates of C skeletons (*11*). Consequently, the positions and number of functional groups of the precursor biolipid control partitioning of the C skeleton among the various modes of occurrence.

For example, the dinosterane (**I**, Fig. 1) and di-aromatic carotenoid (**II**) C skeletons occur exclusively in the macromolecular fraction (Table 1). In the case of dinosterane, this occurs because the functionalized positions in both the putative precursor biolipid, dinosterol (**III**), and its early diagenetic product, dinosterene, are separated by many more than four methylene groups. The precursor of the diaromatic carotenoid skeleton (**II**) is a highly unsaturated pigment synthesized by photosynthetic bacteria (*16*). Within such polyenes, sites for attack by sulfur are numerous, and multiple reactions are possible. Formation of at least one intermolecular S-linkage is thus likely even though the reactive sites are separated by less than four methylene groups. Carbon skeletons **I** and **II** occur only as S-bound products, which indicates that their precursors were sulfurized much faster than they were hydrogenated. If **I** and **II** are representative, it follows that free hydrocarbons in immature sediments must never have contained functional groups providing sites for attack by sulfur. Consequently, the free hydrocarbons must have been biosynthesized as such.

Detailed study of structures of OSC allows resolution of multiple chemical fossils that have the same C skeleton but different origins. For example, the squalane C skeleton occurs in four forms (Table 1). These can be related to at least three precursors (Fig. 2): (i) the saturated hydrocarbon squalane (**A**); (ii) octahydrosqualene (**B**), a distinct biosynthetic product apparently of bacterial origin (*17*); and (iii) the prominent biosynthetic product, squalene (**C**). As with **II**, S-linked products derived from the polyene (**C**) are concentrated in the macromolecular fraction. In contrast, **B** is expected to stabilize S-linkages primarily through intramolecular cyclization, forming products like the thiane **D** and the thiophene **E**. The separate origins for the squalane C skeletons are established by their distinct C isotopic compositions (Table 1). The free hydrocarbon has the highest $\delta^{13}C$ value (*18*), and the two stereoisomers of the alkylthiane have the lowest values. It is not plausible that the different values derive from postdepositional isotopic fractionation. Sulfurization, for example, would fractionate C isotopes only at the positions participating in formation of C–S bonds; the fractionation required to explain the overall difference of 10 per mil (−31.6 versus −41.5 per mil) would exceed by orders of magnitude those consistent with any known isotope effects. Accordingly, separate biosynthetic origins are required for the identical C skeletons and are most logically related to differences in source organisms and their habitats.

Sources can be resolved in greater detail if isotopic analysis precedes desulfurization. For example, there are two different alkylthiophenes with the *n*-octadecane C skeleton. Desulfurization combines these C pools. As shown in Fig. 3A, however, when the isomeric alkylthiophenes are separated by GC and analyzed separately, the $\delta^{13}C$ value of 2-tetradecylthiophene is 13

M. E. L. Kohnen, S. Schouten, J. S. S. Damsté, J. W. de Leeuw, Organic Geochemistry Unit, Faculty of Chemical Technology and Materials Science, Delft University of Technology, De Vries van Heystplantsoen 2, 2628 RZ Delft, the Netherlands.
D. A. Merritt and J. M. Hayes, Biogeochemical Laboratories, Departments of Chemistry and Geology, Geology Building, Indiana University, Bloomington, IN 47405.

*Present address: Koninklijke/Shell Exploratie en Productie Laboratorium, Volmerlaan 6, 2288 GD Rijswijk, the Netherlands.
†To whom correspondence should be addressed.

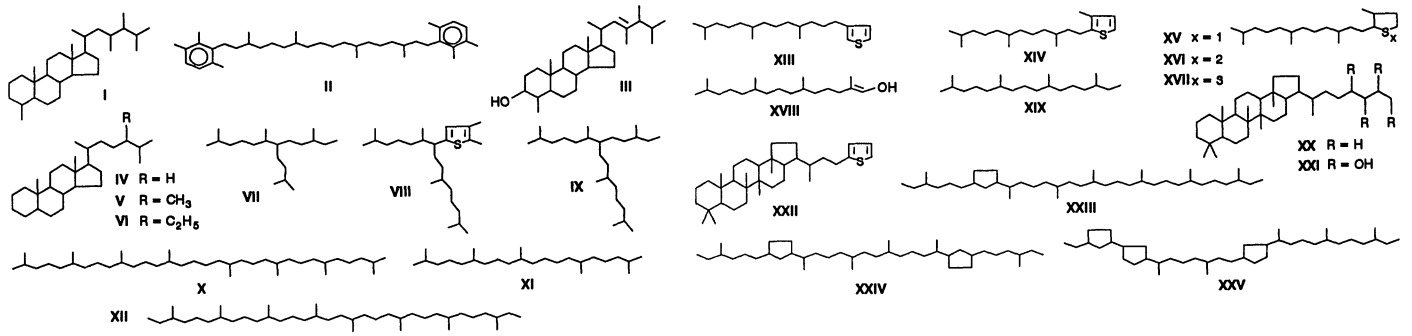


Fig. 1. Structures of compounds.

per mil greater than that of 2-methyl-5-tridecylthiophene. The precursors of these compounds must have differed in placement of double bonds or other functional groups, and their isotopic compositions require distinct biosynthetic sources. The other *n*-alkylthiophene clusters (Fig. 3A) show similar complexities and an interesting regularity: Enrichment in ^{13}C first shows up at C_{18} and involves a thiophene ring with a single C_{14}-alkyl substituent. Subsequent enrichments involve a series of thiophenes with a C_{14} *n*-alkyl substituent and an extending alkyl chain on the other side of the thiophene ring. Thus, at C_{19}, 2-methyl-5-tetradecylthiophene is enriched in ^{13}C; at C_{20}, 2-ethyl-5-tetradecylthiophene is strongly enriched in ^{13}C, and so forth. The differing levels of enrichment (−12.9 versus −17.9 versus −9.5 per mil, and so forth) can be associated with varying levels of dilution of a single family with a high $\delta^{13}C$ value, which must be −9.5 per mil or greater. These observations indicate that a series of *n*-alkyl lipids was present in the paleoenvironment, as depicted in Fig. 3B. This series of precursors is thought to have resulted from an unknown biochemical pathway involving chain elongation of C_{18} *n*-alkadienes.

Table 1. Mode of occurrence, abundance, and isotopic composition of C skeletons and their inferred precursor paleobiochemicals and source organisms. Carbon skeletons are identified in Figs. 1 and 2 and text. Carbon skeletons occur in the following modes: free hydrocarbons (free), alkylthiophene (TP), alkythiolane (TL), alkylthiane (TN), alkyldithiane (DT), alkyltrithiepane (TT), macromolecularly S-bound moiety (S-bd), and ether-bound moiety (E-bd). Isotopic analyses are average of two to six replicate measurements; standard deviation of measurements is typically less than 0.5 per mil and in all cases is less than 1.0 per mil. Abbreviations are gly, glycerol; di-ar, di-aromatic; der., derivatives; Meth. archbact., methanogenic archaebacteria; Chem., chemautotrophic; Heterotr. bact., heterotrophic bacteria; *Chrom., Chromatiaceae.*

C skeleton	Mode	Abundance (mg kg^{-1} extract)	$\delta^{13}C$ (per mil)	Paleobiochemical	Source organism
hentriacontane	Free	1900	−28.8	hentriacontane	Higher plants
	S-bd	560	−17.7	*n*-C_{31} (poly)ene	Blooming algae
C_{20}HBI (**VII**)	Free	200	−17.7	C_{20} (poly)ene HBI alkane	Blooming algae
C_{25} HBI (**IX**)	TP(**VIII**)	30	−27.3*	C_{25} HBI diene	Diatoms
	S-bd	120	−23.9	C_{25} HBI (poly)ene	Algae
phytane (**XIX**)	Free	90	−32.8	phytane	—
	TP(**XIII**)	200	−30.6	phytol	Algae/*Chrom.*
	TP(**XIV**)	290	−30.3	phytol	Algae/*Chrom.*
	TL(**XV**)	520	−30.2	phytol	Algae/*Chrom.*
	DT(**XVI**)	930	−30.1	phytol	Algae/*Chrom.*
	TT(**XVII**)	300	−30.8	phytol	Algae/*Chrom.*
	S-bd	6400	−30.5	phytol	Algae/*Chrom.*
PME (**XI**)	Free	270	−25.8	PME	Algae–meth. archbact.
squalane (**A**)	Free	40	−31.6	squalane	Chem. bacteria
	TP(**E**)	20	−36.6*	**B**‖	Chem. bacteria
	TN(**D**)†	440	−41.5	**B**	Chem. bacteria
			−40.4	**B**	Chem. bacteria
	S-bd	400	−33.2	squalene (**C**)	Chem. bacteria
lycopane (**X**)	Free	870	−25.3	lycopane	Algae
XII	E-bd	ND	−23.9	gly di- or tetraether	Meth. archbact.
XXIII	E-bd	ND	−21.1	gly tetraether	Meth. archbact.
XXIV	E-bd	ND	−20.5	gly tetraether	Meth. archbact.
XXV	E-bd	ND	−20.6	gly tetraether	Meth. archbact.
cholestane **IV**‡	S-bd	2000	−26.3§	cholesterol	Algae
dinosterane (**I**)	S-bd	510	−26.3	dinosterol (**III**)	Dinoflagellates
XX	TP(**XXII**)	90	−26.3*	hopanetetrol der.	Heterotr. bact.
	S-bd	460	−22.7	hopanetetrol der.	Cyanobacteria
II	S-bd	2400	−10.7	di-ar. carotenoid	*Chlorobiaceae*

*Isotope analyses were performed on desulfurized compound. †Two stereoisomers (*cis* and *trans*) were detected. ‡5α(H),14α(H),17α(H)-cholestane. §The macromolecularly S-bound 5β(H),14α(H),17α(H)-cholestane, and 24-ethylcholestanes are isotopically identical to the macromolecularly S-bound 5α(H),14α(H),17α(H)-cholestane carbon skeleton. ‖Also hexahydrosqualene.

Long-chain *n*-alkanes with odd C numbers are much more abundant than even C homologs in the range C_{25} to C_{31} [C preference index (*19*) = 6.3 in marl-2]. Such distributions are characteristic of *n*-alkanes biosynthesized by vascular plants (*20*), and the $\delta^{13}C$ values (−29.8 to −28.8 per mil for all C_{25} to C_{31} homologs) are consistent with derivation from land plants (*21*). Variations in the abundance of these compounds with depth (Fig. 4A) indicate variations in the contributions of terrigenous organic matter to the Vena del Gesso sediments.

Dinosterol (**III**) is biosynthesized exclusively by dinoflagellate algae (*2*, *22*). Therefore, the $\delta^{13}C$ value of the macromolecularly S-bound dinosterane C skeleton (Table 1) is an indication of the isotopic composition of polyisoprenoid lipids produced by dinoflagellates. Other eukaryotic algae utilizing the same C source (same isotopic composition, same concentration of dissolved CO_2) as the dinoflagellates are likely to have produced polyisoprenoid C skeletons with similar isotopic compositions. Because the $\delta^{13}C$ value for dinosterane is −26.3 per mil and polyisoprenoid lipids are commonly depleted in ^{13}C rela-

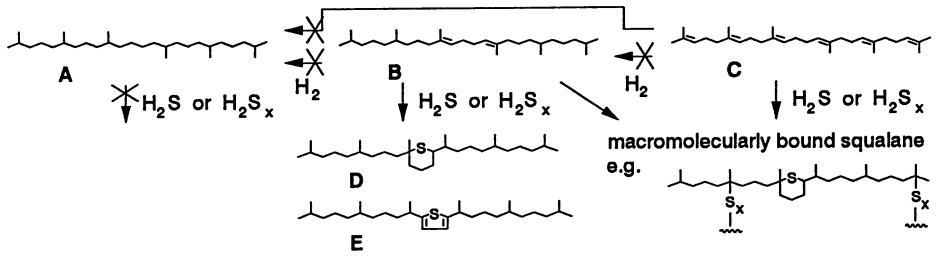


Fig. 2. Schematic rationale for the observed distribution of the squalane C skeleton in marl-2. Sulfur incorporation into compounds **B** and **C** will yield the OSC **D** and **E** and the macromolecularly S-bound squalane C skeleton. However, alkylthiophene **E** and alkylthiane **D** are isotopically distinct (Table 1), and thus an additional precursor has to be invoked. A hexahydrosqualene with the double bonds situated in the middle of the C skeleton (not shown) could be a suitable precursor substrate for the formation of alkylthiophene **D**. The depicted example of macromolecularly S-bound squalane is only consistent with S-incorporation into polyene **C**.

tive to total biomass by ~3 per mil (*23*), the estimated $\delta^{13}C$ value for one component of primary production in the Vena del Gesso paleoenvironment is near −23.3 per mil. Isotopic compositions of other steroidal carbon skeletons (**IV** to **VI**), which are also exclusively bound in the S-linked macromolecular aggregates, are consistent with this interpretation. Variations in the concentration of S-bound steranes (for example, dinosterane, Fig. 4B) likely reflect variations in the abundance of eukaryotic algae in the paleoenvironment.

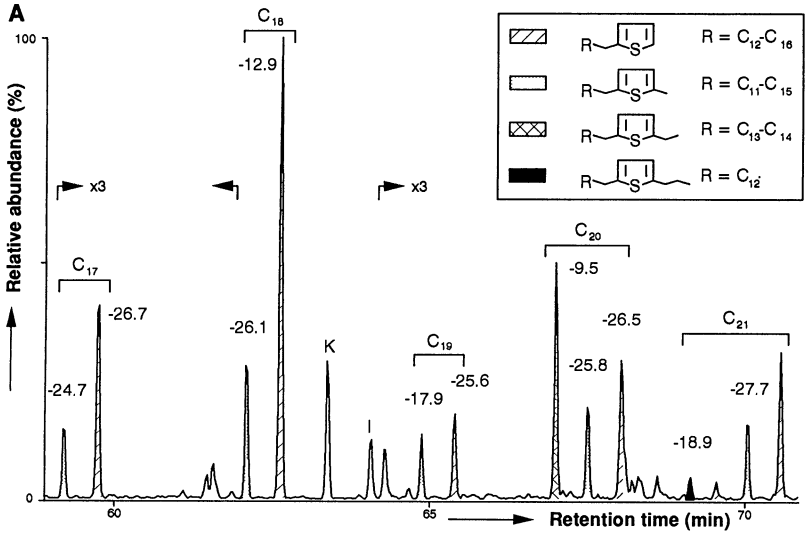
The macromolecularly S-bound n-C_{31} skeleton almost certainly also derives from an aquatic phototroph. Long-chain n-alkenes are abundant in the coccolithophorid *Emiliana huxleyi* (*24*) and in the (freshwater) green alga *Botryococcus braunii* (*25*), and such molecules should be bound in the macromolecular fraction. The $\delta^{13}C$ value of the n-C_{31} skeleton (−17.7 per mil) is, however, different from that of the steroidal C skeletons (Table 1). This difference suggests that isotopic compositions of primary materials in the environment may have varied significantly because of ecological [for example, blooming (*26*)] or physiological [for example, bicarbonate pumping (*21*)] factors. The C_{20} highly branched isoprenoid (HBI) alkane (**VII**), possibly derived from the green alga *Enteromorpha prolifera* (*27*), is similarly enriched in ^{13}C. A common biological source for precursors of the C_{20} HBI alkane and the macromolecularly S-bound n-C_{31} is suggested by this enrichment and by their similar concentration profiles in the marl sequence (Figs. 4, C and D). However, their concentration profiles in the stromatolite and the gypsum layers are distinct, and thus another biological source during the deposition of these layers has to be invoked for the n-C_{31} alkenes.

The C_{25} HBI alkylthiophene (**VIII**) is probably formed by S incorporation into a C_{25} HBI diene (*28*) biosynthesized by diatoms (*29*), as implied by its similar isotopic signature to that of the algal-derived steranes (Table 1). The macromolecularly S-bound C_{25} HBI skeleton (**IX**) has a different precursor as reflected by its different mode of occurrence and isotopic composition (Table 1). However, the similar depth profiles of **VIII** and S-bound **IX** suggest that the different source organisms had a comparable habitat.

It is not unusual for $\delta^{13}C$ values of primary products to vary significantly. Isotopic fractionation associated with photosynthetic C fixation can be influenced by the concentration of dissolved CO_2 (*21*) and rate of growth (*26*). Particular algae tend to be enriched in ^{13}C (for example, diatoms), and the range of values can exceed 10 per mil (*26*). Considering potential biological variability and the possibility of rapid growth resulting in drawdown of dissolved CO_2, we suggest that lipids derived from primary producers in the Vena del Gesso water column had $\delta^{13}C$ values of −26 to −17 per mil.

On structural and isotopic grounds, the C_{40} di-aromatic carotenoid skeleton (**II**; $\delta^{13}C$ = −10.7 per mil, Table 1) apparently was derived from photosynthetic green sulfur bacteria [*Chlorobiaceae* (*16*)]. These organisms fix C through the reverse tricarboxylic acid cycle and produce biomass strongly enriched in ^{13}C (*30*). These phototrophs are obligately anaerobes, and the paleoenvironment must therefore have included an anaerobic photic zone, at least during an interval of evaporite deposition (Fig. 4F). The similarity of the extreme $\delta^{13}C$ values of the di-aromatic carotenoid and the ^{13}C enriched series of n-alkylthiophenes (Table 1 and Fig. 3A) suggests that *Chlorobiaceae* also produce a series of n-alkyl lipids (Fig. 3B), which have been unknown in these organisms.

Lycopane (**X**) must represent a direct input because it occurs as a free hydrocarbon. The carotenoid lycopene or the lycopadiene, biosynthesized by *Botryoccoccus braunii* race L (*31*) and both commonly invoked as precursors for lycopane found in sediments are thus ruled out. The $\delta^{13}C$ value of lycopane falls in the range of values



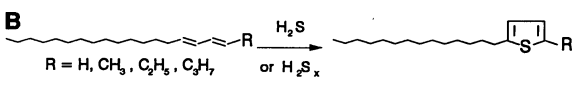


Fig. 3. (**A**) Partial mass chromatogram for *m/z* 266+280+296+308+322 of the alkylthiophene fraction from marl-2 exhibiting the distribution of the alkylthiophenes with n-alkyl C skeletons (C_{17} to C_{21}). The mean $\delta^{13}C$ values of the major isomers are depicted. A reliable isotopic composition of the major structural isomer in the cluster of alkylthiophenes possessing an n-C_{21} carbon skeleton could not be determined because of severe coelution. The peaks denoted with K and I represent alkylthiophenes possessing a phytane C skeleton. Their structures and isotopic compositions are presented in Fig. 1 and Table 1, respectively. (**B**) Hypothetical precursor for the isotopic heavy alkylthiophene series.

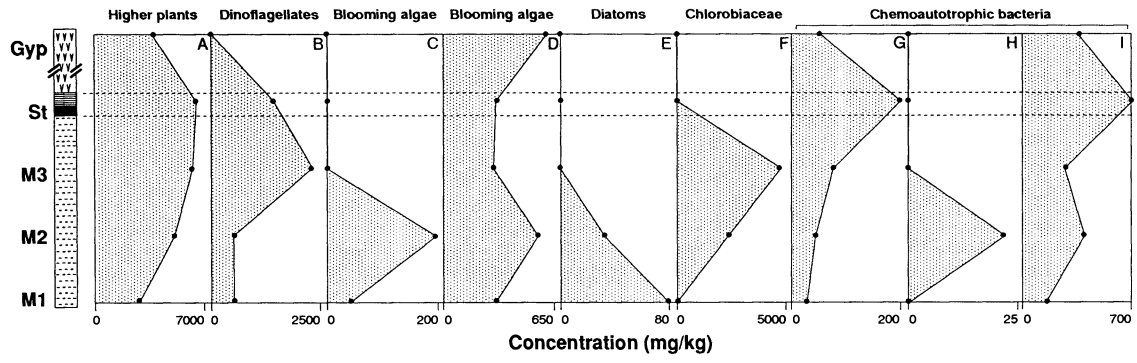


Fig. 4. Depth profiles in an evaporitic cycle of the Messinian Vena del Gesso Basin of: (**A**) $\Sigma(C_{25}$ to $C_{31})$ *n*-alkanes, (**B**) macromolecularly S-bound dinosterane, (**C**) C_{20} HBI alkane, (**D**) macromolecularly S-bound hentriacontane, (**E**) C_{25} HBI alkylthiophene, (**F**) macromolecularly S-bound di-aromatic carotenoid skeleton, (**G**) squalane, (**H**) alkylthiophene with squalane C skeleton (**E**, Fig. 2), (**I**) macromolecularly S-bound squalane skeleton. Gyp, gypsum; St, stromabolite layer; M, marl. Concentration is in milligrams of extract per kilograms.

of algal polyisoprenoids and is thus consistent with an algal derivation (*17*).

The acyclic polyisoprenoid C skeleton, 2,6,10,15,19-pentamethyleicosane (PME, **XI**) also occurs as the free hydrocarbon and is thus likely unaltered. It also has been reported in methanogenic and thermoacidophilic archeabacteria (*32*). Thus PME has been proposed as a marker for methanogenic activity in Recent and immature oceanic sediments (*33*). Although the $\delta^{13}C$ value of PME is close to that of the ether-linked C_{40} isoprenoids (**XII**) derived from methanogenic archaebacteria (see below), it is identical to that of the algal-derived lycopane. The frequently reported co-occurrence of PME and lycopane in both water columns and sediments (*17*, *33*) further supports an algal origin of PME.

The macromolecularly S-bound phytane C skeleton and the low-molecular-weight OSC possessing a phytane C skeleton (**XIII** to **XVII**) result from sulfur incorporation into phytol-derived phytadienes (*11*). Phytol (**XVIII**) is considered to be an excellent molecular marker of photosynthetic organisms. However, the isotopic composition of the S-bound phytane skeletons falls outside the range suggested for lipids of aerobic photoautotrophs and thus indicates that the S-bound phytanes must derive at least partially from other sources. Photosynthetic purple sulfur bacteria (*Chromatiaceae*) produce phytol side chains and often co-occur with *Chlorobiaceae*. On chemical grounds, phytane (**XIX**) occurring as the free hydrocarbon is not derived from phytadienes, an inference supported by its distinct isotopic composition (Table 1) and depth profile.

All of the precursors of the squalane (**A**, Fig. 2) C skeleton (see above and Table 1) have been identified in various strains of archaebacteria (*32*). In addition, squalene is the biosynthetic precursor for cyclic triterpenoids and is thus present in almost any prokaryote or eukaryote. Thus multiple sources are likely. The relatively low $\delta^{13}C$ values may suggest, at least in part, a derivation from chemoautotrophic bacteria because dissolved CO_2 at the oxic-anoxic boundary is often depleted in ^{13}C (*34*) and present in enhanced concentrations that lead to increased isotopic fractionation (*21*). The different depth profiles of the squalane C skeleton in its different modes (Fig. 4, G to I) also reflect multiple sources and, presumably, paleoenvironmental changes.

The C_{35} hopanoid skeleton (**XX**) is observed in two modes of occurrence, and a dual source has to be invoked. The precursors of these sulfurized lipids are the bacteriohopanetetrol (**XXI**) derivatives exclusively biosynthesized by heterotrophic bacteria and cyanobacteria (*35*). The isotopic composition of the C_{35} hopanoid thiophene (**XXII**) is the same as that of the sterane C skeletons (Table 1) and suggests that its precursor lipid is derived from heterotrophic bacteria consuming the primary photosynthate with a high conversion efficiency (*7*). Moreover, the abundance of the C_{35} hopanoid thiophene is 1/10 to 1/50 of that of the lipids derived from primary producers (Table 1), in accordance with organisms living one trophic level higher in the paleoenvironment. In contrast to the thiophene, the macromolecularly S-bound C_{35} hopane carbon skeleton has a $\delta^{13}C$ value 3.6 per mil higher than that of most of the lipids derived of aerobic photoautotrophic origin (Table 1). A distinct source, probably cyanobacterial, is required.

The ether-bound C_{40} isoprenoid hydrocarbons **XII** and **XXIII** to **XXV** originate from isoprenoid glycerol ethers, exclusively found as membrane constituents of archaebacteria (*36*). The $\delta^{13}C$ values (Table 1) indicate that the cyclized isoprenoids (**XXIII** to **XXV**) have a common source that is distinct from that of the acyclic C_{40} isoprenoid (**XII**). Ether-bound C_{40} isoprenoids possessing cyclopentane rings are widespread in thermophilic archaebacteria and occur also in nonthermophilic methanogenic archaebacteria (*36*, *37*). Glycerol ethers possessing acyclic C_{40} isoprenoid chains (**XII**) are reported in methanogenic and thermophilic archaebacteria (*36*). We propose that these ether-bound lipids originate from methanogens because derivation from thermophilic archaebacteria (that is, organisms living at 50° to 110°C) would be inconsistent with all the other data that point to a water temperature probably not exceeding 25°C. Although bacterial sulfate reduction must have been important in the paleoenvironment, some methanogenic activity cannot be excluded because certain methanogens can compete with sulfate reducers for specific substrates, such as methanol and methylated amines (*38*). One of these methanogens, *Methanosarcina barkeri*, indeed biosynthesizes glycerol ethers with cyclized C_{40} isoprenoid chains [**XXIII** to **XXV** (*37*)]. Because the hopanoids and acyclic isoprenoids are not strongly depleted in ^{13}C (< −50 per mil) cycling of methane was a less important process in the paleoenvironment (*8*).

The above demonstrates that isotopic compositions and modes of S-binding together provide valuable information regarding identities and sources of biolipid precursors of sedimentary biomarkers. Moreover, recognition of such paleobiochemicals may stimulate new studies on the modern biota and lead to improved views of the evolution of biosynthetic pathways.

REFERENCES AND NOTES

1. S. C. Brassell and G. Eglinton, in *Organic Marine Geochemistry*, M. L. Sohn, Ed. (American Chemical Society, Washington, DC, 1986), pp. 10–32; J. W. de Leeuw, *ibid.*, pp. 33–61.
2. J. K. Volkman, in *Lacustrine Petroleum Source Rocks*, A. J. Fleet, K. Kelts, M. R. Talbot, Eds. (Blackwell, Oxford, 1988), pp. 103–122.
3. J. S. Sinninghe Damsté and J. W. de Leeuw, *Org. Geochem.* **10**, 1077 (1990).
4. J. S. Sinninghe Damsté, W. I. C. Rijpstra, A. C. Kock-van Dalen, J. W. de Leeuw, P. A. Schenck, *Geochim. Cosmochim. Acta* **53**, 1343 (1989).
5. J. S. Sinninghe Damsté, M. E. L. Kohnen, J. W. de Leeuw, *Nature* **345**, 609 (1990).
6. M. E. L. Kohnen, J. S. Sinninghe Damsté, W. I. C. Rijpstra, J. W. de Leeuw, *Am. Chem. Soc. Symp.*

Ser. **429**, 444 (1990).
7. J. M. Hayes, K. H. Freeman, B. N. Popp, C. H. Hoham, *Org. Geochem.* **10**, 1115 (1990).
8. K. H. Freeman, J. M. Hayes, J. M. Trendel, P. Albrecht, *Nature* **343**, 254 (1990).
9. The sample set consists of three subsamples of the bituminous marl (marls 1 to 3), a sample from the stromatolitic limestone, and a sample from the massive gypsum from the same evaporitic cycle (Fig. 4). The geology of this basin is extensively described by G. B. Vai and F. R. Ricci Lucci [*Sedimentology* **24**, 211 (1977)].
10. The freeze-dried samples were powdered and Soxhlet extracted with methanol:CH_2Cl_2 (1:7.5 by volume for 40 hours) under nitrogen. Saturated hydrocarbon, alkylthiophene, alkylsulphide, and polar fractions were isolated from the extracts as in (*11*) and analyzed using GC and GC-MS after addition of internal standards prior to fractionation (*6*).
11. M. E. L. Kohnen, J. S. Sinninghe Damsté, A. C. Kock-van Dalen, J. W. de Leeuw, *Geochim. Cosmochim. Acta* **55**, 1375 (1991).
12. Portions of the polar, alkylthiophene, and alkylsulphide fractions were desulfurized with Raney Ni and subsequently hydrogenated as described previously (*14*). To release ether-bound carbon skeletons a portion (~35 mg) of the polar fraction of marl-2 was dissolved in a 57% HI aqueous solution and refluxed for 2 hours. The alkyliodides obtained were isolated [R. V. Panganamala, C. F. Sievert, D. G. Cornwell, *Chem. Phys. Lipids* **7**, 336 (1971)] and reduced with $LiAlH_4$ [modified after J. E. Johnson, R. H. Blizzard, H. W. Carhart, *J. Am. Chem. Soc.* **70**, 3664 (1948)].
13. The isotope-ratio-monitoring GC-MS system is described in (*7, 8*). A fused silica capillary column (50 m by 0.32 mm) coated with cross-linked methyl silicone gum (Ultra-1, film thickness 0.52 μm) was used with He as carrier gas to analyze the fractions of marl-2 (Fig. 4). In the case of the alkylthiophene and the alkylsulphide fraction both the untreated and desulfurized fractions were analyzed.
14. J. S. Sinninghe Damsté, W. I. C. Rijpstra, J. W. de Leeuw, P. A. Schenck, *Org. Geochem.* **13**, 593 (1988).
15. The S-containing heterocycles isolated from the Vena del Gesso sediments have five-, six-, and seven-membered rings containing four or five adjacent C atoms and one to three S atoms. These include thiophenes (C_4S ring, aromatic) and the saturated thiolanes (C_4S), thianes (C_5S), dithianes (C_4S_2), and trithiepanes (C_4S_3).
16. S. Liaaen-Jensen, in *Marine Natural Products*, D. J. Faulkner and W. H. Fenical, Eds. (Academic Press, New York, 1978), pp. 1–73; S. Liaaen-Jensen, in *Photosynthetic Bacteria*, R. K. Clayton and W. R. Sistrom, Eds. (Plenum, New York, 1978), pp. 233–247.
17. S. G. Wakeham, *Geochim. Cosmochim. Acta* **54**, 1325 (1990).
18. $\delta^{13}C = 10^3[(R_x - R_s)/R_s]$, where $R = {}^{13}C/{}^{12}C$, x designates sample, s designates PDB standard and $R_s = 0.0112372$.
19. E. E. Bray and E. D. Evans, *Geochim. Cosmochim. Acta* **22**, 2 (1961).
20. G. Eglinton, R. J. Hamilton in *Chemical Plant Taxonomy*, T. Swain, Ed. (Academic Press, New York, 1963), pp. 187–208.
21. B. N. Popp, R. Takigiku, J. M. Hayes, J. W. Louda, E. W. Baker, *Am. J. Sci.* **289**, 436 (1989).
22. J. J. Boon *et al.*, *Nature* **277**, 125 (1979).
23. P. Deines, in *Handbook of Environmental Isotope*, P. Fritz and J. C. Fontes, Eds. (Elsevier, Amsterdam, 1980), vol. 1A, pp. 329–406.
24. J. K. Volkman, G. Eglinton, E. D. S. Corner, T. E. Forsberg, *Phytochemistry* **19**, 2619 (1980).
25. P. Metzger, C. Berkaloff, E. Casavall, A. Coute, *ibid.* **24**, 2305 (1985).
26. W. G. Deuser, *Nature* **225**, 1069 (1970).
27. S. J. Rowland, D. A. Yon, C. A. Lewis, J. R. Maxwell, *Org. Geochem.* **8**, 207 (1985).
28. J. S. Sinninghe Damsté, E. R. van Koert, A. C. Kock-van Dalen, J. W. de Leeuw, P. A. Schenck, *Org. Geochem.* **14**, 555 (1989); M. E. L. Kohnen *et al.*, *Geochim. Cosmochim. Acta* **54**, 3053 (1990).
29. P. D. Nichols, J. K. Volkman, A. C. Palmisano, G. A. Smith, D. C. White, *J. Phycol.* **24**, 90 (1988).
30. L. Quandt, G. Gottschalk, H. Ziegler, W. Stichler, *FEMS Microbiol. Lett.* **1**, 125 (1977); R. Sirevag, B. B. Buchanan, J. A. Berry, J. H. Troughton, *Arch. Microbiol.* **112**, 35 (1977); R. E. Summons and T. G. Powell, *Geochim. Cosmochim. Acta* **51**, 557 (1987); *Nature* **319**, 763 (1986).
31. P. Metzger and E. Casadevalle, *Tetrahedron Lett.* **28**, 3931 (1987).
32. G. Holzer, J. Oro, T. G. Tornabene, *J. Chromatogr.* **186**, 795 (1979); T. G. Tornabene, T. A. Langworthy, G. Holzer, J. Oro, *J. Mol. Evol.* **13** (1979); J. B. Risatti, S. J. Rowland, D. A. Yon, J. R. Maxwell, *Org. Geochem.* **6**, 93 (1984).
33. S. C. Brassell, A. M. K. Wardroper, I. D. Thomson, J. R. Maxwell, G. Eglinton, *Nature* **290**, 693 (1981).
34. E. S. Deevey and M. S. Stuiver, *Limnol. Oceanogr.* **9**, 1 (1964); T. Torgerson, D. E. Hammond, W. B. Clarke, T.-H. Peng, *ibid.* **26**, 110 (1981).
35. G. Ourisson, P. Albrecht, M. Rohmer, *Pure Appl. Chem.* **51**, 709 (1979); G. Ourisson, P. Albrecht, M. Rohmer, *Trends Biochem. Sci.* **7**, 236 (1982).
36. M. De Rosa and A. Gambacorta, *Prog. Lipid Res.* **27**, 153 (1988).
37. M. De Rosa *et al.*, *Biochim. Biophys. Acta* **875**, 487 (1986).
38. R. S. Oremland, L. M. Marsh, S. Polcin, *Nature* **296**, 143 (1982).
39. Supported in part by the Netherlands Foundation for Earth Science Research (AWON) with financial aid from the Netherlands Organization for Scientific Research (NWO). We thank M. Baas, A. H. Knol-Kalkman, W. Pool, M. P. Ricci, and S. A. Studley for technical and analytical assistance. M.E.L.K. acknowledges the EAOG for a travel scholarship. Delft Organic Geochemistry Unit Contribution 251.

24 October 1991; accepted 3 February 1992

Contact Electrification and Adhesion Between Dissimilar Materials

Roger G. Horn and Douglas T. Smith

Simultaneous measurements of surface force and surface charge demonstrate strong attraction due to the spontaneous transfer of electrical charge from one smooth insulator (mica) to another (silica) as a result of simple, nonsliding contact in dry nitrogen. The measured surface charge densities are 5 to 20 millicoulombs per square meter after contact. The work required to separate the charged surfaces is typically 6 to 9 joules per square meter, comparable to the fracture energies of ionic-covalent materials. Observation of partial gas discharges when the surfaces are approximately 1 micrometer apart gives valuable insight into the charge separation processes underlying static electrical phenomena in general.

Contact electrification, manifest as static or triboelectricity, is a well-known effect whose consequences can be very irksome or very beneficial depending on the circumstances. Despite the familiarity and importance of this phenomenon, there is limited understanding of the fundamental mechanisms by which charge transfers from one insulator to another during contact and remains there as the materials are separated (*1, 2*).

Particles and surfaces are charged intentionally in such applications as photocopying, laser printing, electrostatic precipitation, and particle separation processes, which make use of electrostatic forces to promote adhesion. However, the notion that spontaneous charge transfer between materials in contact acts to increase adhesion between them has received little attention despite the efforts of Derjaguin *et al.* (*3*) and the work of Dickinson and coworkers (*4*) demonstrating that charge separation occurs during interfacial fracture.

We report an experimental method based on the use of a surface force apparatus (*5*) with in situ electrometers (*6*), which enables both surface forces and surface charges to be measured. Two smooth dissimilar insulating materials are brought into contact and separated; direct measurements are made of the charge transferred during contact and of the resulting electrostatic attractive force. These experiments demonstrate and quantify the attraction that results from contact electrification and show that the work required to separate two charged surfaces in a dry atmosphere is comparable to the work required to fracture the individual materials. This work of adhesion depends on how much excess charge remains on the surfaces after a series of discharges across the gap between the materials as they are separated through the micrometer range.

Two thin (1 to 10 μm), transparent solids are mounted as crossed cylinders of radius ≈20 mm in the surface force apparatus. For these experiments we used one sheet of atomically smooth mica and one of fused silica (*7*), prepared by a blown-bubble method that gives near-atomic smoothness (*8*). Solid-solid separation D is controlled and measured with subnanometer accuracy by interferometry (*9*) between silver coat-

Ceramics Division, National Institute of Standards and Technology, Gaithersburg, MD 20899.