

the fiber state. The combination of these and other approaches will be needed to determine the details of how proteins can function as fibers.

Commercial application of spider silk fibers will depend on the availability of a sufficient amount of material. Clearly the most likely source will be to use microorganisms to produce these proteins, which can then be spun into fibers. Our efforts in this direction have shown that it is feasible to produce these proteins in bacteria, but the yields have been very low. With this

hurdle surmounted the key factor will become cost. At this point it is likely the first applications will be in medical products where cost is less of a factor, although other high-technology applications may be cost effective as well.

Although it is unlikely that either a complete understanding of the mechanism of spider silk strength and elasticity or commercial production of spider silk proteins will be achieved in the short term, the mystery of spider silk is unraveling.

Hopanoids. 1. Geohopanoids: The Most Abundant Natural Products on Earth?

GUY OURISSON* and PIERRE ALBRECHT

Laboratoire de Chimie des Substances Naturelles, CNRS, Université Louis Pasteur, F-67084 Strasbourg, France

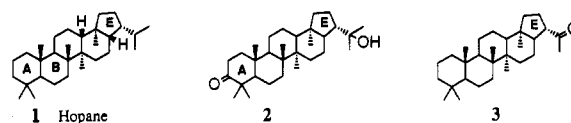
Received January 29, 1992

Introduction

This Account introduces the reader to a most abundant family of complex organic substances on Earth and to their significance in geochemistry, as well as in fields from petroleum exploration to archaeology. These "geohopanoids" are molecular fossils, whose identification has exposed their precursors, the "biohopanoids", a novel family of bacterial lipids presented in the following Account in this issue.

Hopane, the name of the parent hydrocarbon 1, is derived neither from that of one of the cardinal virtues nor from that of *Humulus lupulus* (though "hops" is indeed a slang name of hopanoids used by American petroleum geochemists). It recalls rather the lesser 18th century British botanist John Hope, in whose honor his colleague Roxbury called a genus of huge Burmese trees *Hopea*, lavish producers of a resin used in the varnish trade called "dammar"; in 1955, at the British Museum, Mills and Werner isolated from such a *Hopea* dammar

the hydroxy ketone 2, which they called "hydroxy-hopanone".¹



Since then, C₃₀ (or C₂₉) derivatives of hopane 1 have been found in scattered plants, some in higher plants (then, always with an oxygen function at C-3) or, without such a function, some in lichens and many ferns; these "phytohopanoids" have remained rare and few, and constitute only one of the many minor families of plant triterpenes present in secretions, barks, and cuticles.

It was therefore a major surprise some 20 years ago, soon after we initiated our study of sedimentary organic substances,² that Ted Whitehead, at British Petroleum, would identify hopane derivatives in a crude oil and we in several sediments.³ The surprise became exhilaration when we realized that we were dealing with a major finding, as geohopanoids are very varied (more than 200 structures identified so far), more abundant globally than any other group of natural products, ubiquitous in sediments, useful in many ways, and biologically important. The study of organic geochemical problems in the Strasbourg group and elsewhere has provided many important results, but the "hopanoid story" remains probably the most extraordinary one.

The Prehistory of Geohopanoids

Our identification of these geohopanoids was markedly eased by our unplanned preparedness. Firstly, with Takeoshi Takahashi, we had studied the "gurjun" balsams of *Dipterocarpus*, trees closely allied to *Hopea* and containing polyterpenes closely related to those of

Guy Ourisson, born in 1926, was educated in Paris (Ecole Normale Supérieure; Dr.Sc., G. Dupont, 1954) and at Harvard University (Ph.D., L. F. Fieser, 1952). In 1955, he was appointed to the University of Strasbourg, where he has since been a Professor. From 1971 to 1975, he was Founding President of the new Université Louis Pasteur. From 1985 to 1989, he was Director of the CNRS Institut de Chimie des Substances Naturelles in Gif-sur-Yvette, France. G.O. has received prizes from the French, Belgian, British, American, and German Chemical Societies, as well as the Rousset Prize which he shared with P. A., the Heinrich Wieland Award, and the A. von Humboldt Forschungspreis. He is an Honorary Member of the Chemical Societies of Belgium, Great Britain, and Switzerland and a Foreign Member of the Science Academies of Sweden, Denmark, India, Rhineland-Westphalia, Luxembourg, Serbia, Leopoldina (Halle), and Academia Europaea; he is also the Foreign Secretary of the French Académie des Sciences. P.A. has twice received the Best Paper Award (once shared with G.O.) and the Alfred Treibs Award from the American Geochemical Society and prizes from CNRS and the French Chemical Society. Along with structural studies on many natural products, G.O. has launched a variety of new lines of research in Strasbourg, always at the interface between chemistry and biology or other fields and often pursued by his former students.

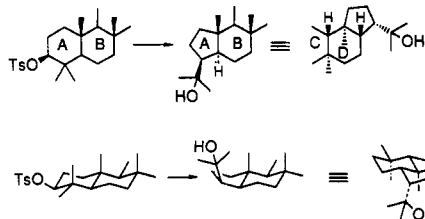
Pierre Albrecht, born in 1941, was educated in Strasbourg and obtained his doctorate in 1969 in G.O.'s group, in which he initiated geochemical studies. After a postdoctoral year at the University of California at Berkeley with W. G. Dauben, he returned to Strasbourg where he runs the Organic Geochemistry Laboratory. A Research Director in the CNRS, he has succeeded G.O. as Director of the Laboratoire de Chimie Organique des Substances Naturelles, a federation of teams of researchers at Université Louis Pasteur, associated with CNRS.

(1) Mills, J. S.; Werner, A. E. *J. Chem. Soc.* 1955, 3132-3140.

(2) Albrecht, P.; Ourisson, G. *Geochim. Cosmochim. Acta* 1969, 227-230. Albrecht, P.; Ourisson, G. *Angew. Chem., Int. Ed. Engl.* 1971, 41, 209-225.

(3) Whitehead, E. V. *Chem. Ind.* 1971, 1116-1118. Ensminger, A.; Albrecht, P.; Ourisson, G.; Kimble, B. J.; Maxwell, J. R.; Eglinton, G. *Tetrahedron Lett.* 1972, 3861-3864.

dammars.⁴ We had therefore very early been made aware of hopane derivatives. Secondly, Jean-François Biellmann had studied the stereochemistry of the A-ring contraction resulting from the solvolysis of triterpenic β -mesylates and had, for the first time, completely elucidated the steric course at all centers of a Wagner-Meerwein rearrangement.⁵ The A-nor/BC part of the products is identical with the E/DC part of hopane, and a chiroptical comparison of Mills' and our products led us to deduce stereostructure 1 for the former, a conclusion confirmed later.⁶ Thirdly, during



his Ph.D. work, Jean-Marie Lehn had participated in the structure determination of a triterpene isolated by Giancarlo Berti from the fern adiantone; we had shown it to be the C₂₉ norhopane ketone 3 by NMR and mass spectroscopy, chemical transformations, and chiroptical comparison of derivatives with Biellmann's samples.⁷ This work left us with reference samples, which proved to be most useful once convergent indications suggested the presence of hopane derivatives in sedimentary extracts. While we were of course in no way looking for hopanoids in sediments (but only for what was there), we were therefore unusually well prepared to identify them, with the rigorous criteria of natural products chemistry, when they came our way.

It soon became evident, both in Strasbourg and in Geoffrey Eglinton's group in Bristol, that geohopanoids were not only present in some sediments but *constantly* detectable in any sediment: marine or terrestrial, young or old (from soils or muds only a few years old to shales at least 1.5×10^9 yrs old), rich or poor (in crude oils or coals, in oil shales, in dry shales, in peats, in limestones, etc.), from all parts of the world.⁸ This ubiquitous presence is shared by no other family of complex organic substances and it makes them extremely abundant globally, even though their average content in any particular sediment is low: from the thousands of analyses run by ourselves and many others, one can estimate this average content at about 0.1% of the total *extractable* organic matter, which itself represents about one-tenth of the total organic C content of the sediments. This average organic C content (excluding methane) is itself ca. 2% of the total mass of sediments, i.e., 2% of about 5×10^{17} tons. The total amount of geohopanoids is therefore on the order of 10^{12} tons. This also happens to be the order of magnitude esti-

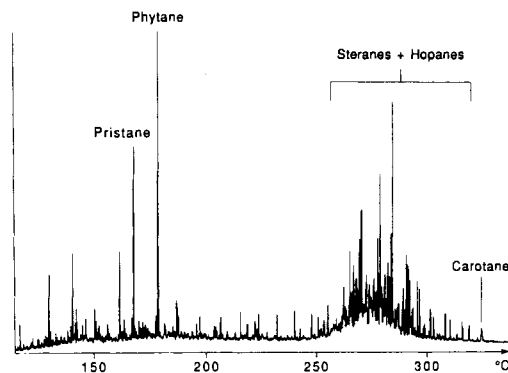


Figure 1. Typical gas chromatogram of the branched and cyclic alkane fraction obtained from a crude oil on a capillary column.

ated for the total mass of organic C in *all* presently living organisms! Of course, such global estimates are hazardous, but even an error of 1 order of magnitude would not alter the conclusion that no other family of natural products can approach hopanoids in their global mass (only methane may well be more abundant).⁹ We shall see that their importance does not lie only in their total mass.

Isolation and Structural Identification of Geohopanoids

The standard procedure for the study of organic components of a sediment is to use successive solvent extraction (often with mixtures, for instance, of toluene/methanol), adsorption chromatography (selection by polarity), and molecular sieving (selection by molecular shape).⁸ Each subfraction still gives very complex gas chromatograms; for instance, a typical capillary GC trace of the saturated branched and cyclic hydrocarbon fraction displays a few hundred peaks (Figure 1). In these, a series constantly reoccurs, recognized by its retention time and fragmentation pattern in MS to correspond to the hopanoid hydrocarbons 4–6, 1, 7, 8–12, C₂₇H₄₆, C₂₈H₄₈ (usually minor), C₂₉H₅₀, C₃₀H₅₂, C₃₁H₅₄ (two peaks), and C₃₂H₅₆ to at most C₃₅H₆₂ (each with two peaks progressively better resolved). All of these hydrocarbons undergo, on electron impact MS, a highly favored fragmentation in ring C, giving a base peak at *m/e* 191 (rings A and B); this is accompanied by intense peaks corresponding to rings D and E plus the side chain at 149 for 4, 163 for 5 (less abundant as it implies two side chain C–C cleavages),^{8,10} 177 for 6, 191 only for hopane (1) itself, 205 for 7, ..., up to 261 for 12. Hydrocarbons 4–8 and 12 were identified by direct comparison (retention times on several columns, MS fragmentations) with authentic samples obtained by obvious modifications of the various epimers at C-17 and C-21, themselves resulting from unrelated work of Corbett⁶ and from Biellmann's and Lehn's chemistry on Mills' hydroxyhopanone. The structures of 9–11 were deduced by interpolation. In a typical immature sediment, young or old, these substances have the 17 β ,21 β H configuration of hopane 1 itself, but in an ancient mature sediment or in a crude oil, they usually belong predominately to the 17 α ,21 β H series, with the

(4) Cosserrat, L.; Ourisson, G.; Takahashi, T. *Chem. Ind.* 1956, 190–191.

(5) Biellmann, J. F.; Ourisson, G. *Bull. Soc. Chim. Fr.* 1962, 331–336.

(6) (a) Schaffner, K.; Cagliotti, L.; Arigoni, D.; Jeger, O. *Helv. Chim. Acta* 1958, 41, 152–159. (b) Corbett, R. E.; Heng, C. K. *J. Chem. Soc. C* 1971, 1885–1888.

(7) Berti, G.; Bottari, F.; Marsili, A.; Lehn, J. M.; Witz, P.; Ourisson, G. *Tetrahedron Lett.* 1963, 1283–1287.

(8) Ensminger, A.; Van Dorsselaer, A.; Spyckerelle, C.; Albrecht, P.; Ourisson, G. *Adv. Org. Chem.* 1973 1974, 245–260. Van Dorsselaer, A.; Ensminger, A.; Spyckerelle, C.; Dastillung, M.; Sieskind, O.; Arpino, P.; Albrecht, P.; Ourisson, G.; Brooks, P. W.; Gaskell, S. J.; Kimble, B. J.; Philp, R. P.; Maxwell, J. R.; Eglinton, G. *Tetrahedron Lett.* 1974, 1349–1352. Kimble, B. J.; Maxwell, J. R.; Philp, R. P.; Eglinton, G.; Albrecht, P.; Ensminger, A.; Arpino, P.; Ourisson, G. *Geochim. Cosmochim. Acta* 1974, 38, 1165–1181.

(9) Ourisson, G.; Albrecht, P.; Rohmer, M. *Sci. Am.* 1984, 251 [2] (Aug), 44–51.

(10) Seifert, W. K.; Moldowan, J. M.; Smith, G. W.; Whitehead, E. V. *Nature* 1978, 271, 436–437. Moldowan, J. M.; Fago, F. J.; Carlson, R. M. K.; Young, D. C.; van Duyn, G.; Clardy, J.; Schoell, M.; Pillingier, C. T.; Watt, D. S. *Geochim. Cosmochim. Acta* 1991, 55, 3333–3353.

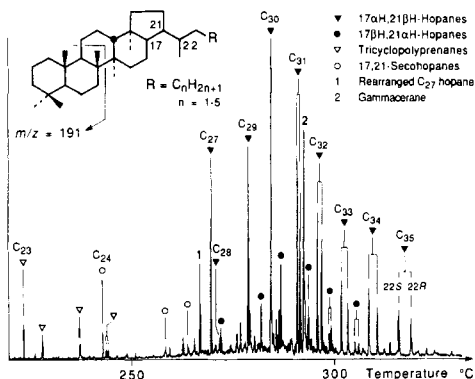
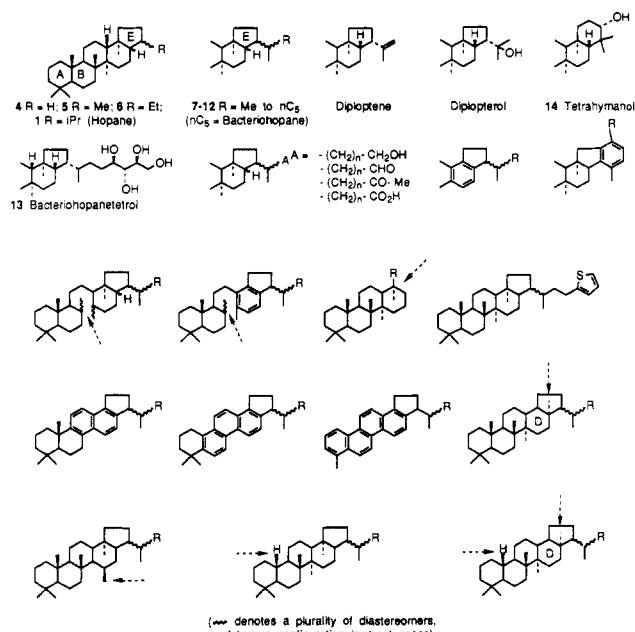


Figure 2. Mass fragmentogram m/e 191 showing selectively the distribution of polycyclic terpanes in the fraction of Figure 1.

Chart I Geohopanooids of Established Structure



more stable *cis*-hydrindan arrangement of rings D/E.^{8,11}

The intensity of the 191 peak is such that a 191 fragmentogram, representing the ion current at m/e 191 measured during the chromatographic flow, amounts to a "hopanogram" (Figure 2). We shall see below that these hopanograms must of course be used carefully: there are "hidden" geohopanooids, and some other geoterpenoids show up in a 191 hopanogram.

All of the geohopanooids represented in Chart I have been fully identified (including their *R* or *S* configuration at C-22, often for both diastereomers), usually by direct comparison with natural, semisynthetic, or totally synthetic samples (in very few cases, by interpolation) and for several key representatives by X-ray crystallography. Most result from the work of the Strasbourg group,¹² a few have been added to the list by Seifert and

(11) Van Dorsselaer, A.; Albrecht, P.; Ourisson, G. *Bull. Soc. Chim. Fr.* 1977, 165-170.

(12) Ourisson, G.; Albrecht, P.; Rohmer, M. *Pure Appl. Chem.* 1979, 51, 709-729 and references therein. Dastillung, M.; Albrecht, P.; Ourisson, G. *J. Chem. Res.* 1980, (S) 166-167; (M) 2325-2352. *Idem. Ibid.* 1980 (S) 168-169; (M) 2353-2374 and references therein. For later references on individual structures of geohopanooids, the search in *Chemical Abstracts* is run under "Gammacer...", with the prefix "A'-neo"; in *Science Citation Index*, it is searched under "hopa*", "hope*", "norhop*", etc., with the EXPAND command to eliminate the obvious noise generated by "hope", "Hopen Island", etc. We thank Dr. R. E. Wolff for his help with these searches.

Moldowan¹⁰ and Rullkötter.¹³

Of the geohopanooids of this chart, only four have been found outside sediments in living organisms: diploptene, diplopterol, the tetrol 13, and tetrahymanol 14 (a "quasi-hopanooid"). All of the others must be transformation products; we shall see below the significance of the fact that the D/E hydrindan junction of the first three (17 β ,21 β H) is that of hopane, i.e., *trans* and unstable.

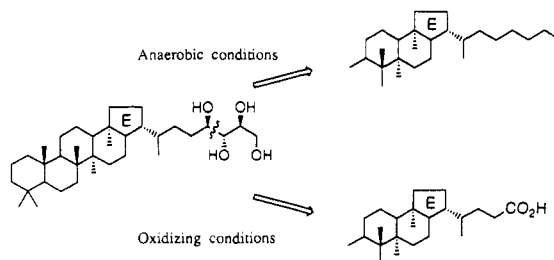
Geohopanooids as Geochemical Markers^{8,9,14}

The ubiquity of geohopanooids, their relatively easy detection, and their derivation from a limited number of precursors presenting isomerizable sites make them very useful geochemical markers at several levels of sophistication.

The most simplistic use of hopanooids is to rely on their ubiquity and on the fact that two unrelated sediments usually contain different sets of geohopanooids. They can therefore be used to correlate oils and sediments by "fingerprinting" them, without paying much attention to the particular molecular structures involved. Thus, when the "hopanograms" of a crude oil and of a presumed source rock are very different, the suspected origin can be dismissed because enough data have shown that migration of an oil is usually not accompanied by important changes in the composition of the geohopanooid fraction.^{9,10,15} Similar conclusions can of course be confirmed (and should be) from the consideration of other groups of geochemical constituents (for instance sterane derivatives, which are very often present).¹⁶

More informatively, one can use the structural details now recognized among geohopanooids to relate them in a rational way with the conditions of sedimentation transformation in the sediment by "maturation", and later biodegradation.^{15,16} We shall describe these factors in terms of the four precursors mentioned in the chart, even though the tetrol 13 is probably not the only C₃₅ precursor involved (see following Account in this issue).

A first major difference can be noted between sediments deposited under anaerobic or aerated conditions (attested by the geology of the site). Under anaerobic conditions, reduction prevails: tetrahymanol is reduced to the parent hydrocarbon, gammacerane, and the distribution of the geohopanooid hydrocarbons extends up to C₃₅. When deposition has taken place under



(13) Rullkötter, J.; Wendisch, D. *Geochim. Cosmochim. Acta* 1982, 46, 1545-1553.

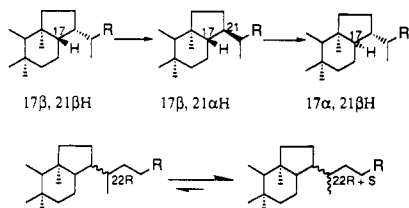
(14) Tissot, B. P.; Welte, D. H. *Petroleum Formation and Occurrence*, 2nd ed.; Springer Verlag: Berlin, 1984; p 699. Rullkötter, J. *Nachr. Chem. Tech. Lab.* 1984, 32, 418-423.

(15) (a) Seifert, W. K.; Moldowan, J. M. *Geochim. Cosmochim. Acta* 1981, 45, 783-794. (b) Rohmer, M.; Dastillung, M.; Ourisson, G. *Naturwissenschaften* 1980, 67, 456-457.

(16) Rubinstein, I.; Sieskind, O.; Albrecht, P. *J. Chem. Soc., Perkin Trans. 1* 1975, 1833-1836. Ensminger, A.; Joly, G.; Albrecht, P. *Tetrahedron Lett.* 1978, 1575-1578. Mackenzie, A. S.; Brassell, S. C.; Eglinton, G.; Maxwell, J. R. *Science* 1982, 217, 491-503.

obviously well-aerated conditions, oxidation takes over and the C₃₂ carboxylic acid becomes a major component, whereas the hydrocarbon fraction does not extend significantly beyond the C₃₁ homolog that results from decarboxylation of the C₃₂ acid (just like the odd-numbered *n*-alkanes predominate in young sediments, where they presumably arise by decarboxylation of the higher, even-numbered *n*-carboxylic acids, in vivo or "in petra"). Extrapolation from the attested cases to geologically uncertain ones reverses the roles of the criteria: the hopanogram then becomes a key indicator of anoxic or oxidative deposition.

Another type of information is deduced from the thermodynamic instability of the precursors of geohopanoids at positions 17 and 21, diastereomerically homogeneous at C-22 (22*R*).^{11,17} The configurations at the first two centers can be deduced from their retention times (by comparison with authentic standards) and easily recognized from the relative intensities of the two mass spectral peaks resulting from the C-ring cleavage mentioned above. One can thus observe that young, immature sediments contain 17β,21βH hopanoids (termed "ββ"), but older ones, especially those which have been buried deep enough to have had a "thermal history", are mostly of the more stable 17α,21βH series and, to a lesser extent, the 17β,21αH series.¹¹ We have also checked that very moderate



heating of hydrocarbons in the presence of a suitable clay can bring about this type of epimerization.^{11,18} The ratio of diastereomers can then be used as an indicator of maturation: "ββ hops" are less mature than "βα hops", which are themselves less mature than "αβ hops". This is a useful indicator in oil exploration. Similarly, the 22*S*/22*R* ratio rises from zero toward ca. 3:2 as maturity increases and can be used as an indicator of maturation.¹⁷

Geohopanoids also provide a clear indication of the bacterial degradation which occurs when a crude oil is exposed to water and air in surface seeps or natural reservoirs. Biodegradation ensues and selectively destroys most of the components, although the highly branched polyterpanes, and in particular the quite insoluble hopanoids, are affected only in extreme cases.¹⁸

Another type of information is rapidly becoming important as it adds apparently independent data: the isotopic composition (¹³C/¹²C ratio) has been introduced and is being put to excellent use by John Hayes to add one further dimension to hopanograms by providing more precise indications on the origin of their various components.¹⁹

(17) Seifert, W. K.; Moldowan, J. M. *Adv. Org. Geochem.* 1979 1980, 229-239. Ensminger, A.; Albrecht, P.; Ourisson, G.; Tissot, B. *Adv. Org. Geochem.* 1979 1980, 45-52.

(18) Seifert, W. K.; Moldowan, J. M. *Geochim. Cosmochim. Acta* 1979, 43, 111-126.

(19) Freeman, K. H.; Hayes, J. M.; Trendel, J. M.; Albrecht, P. *Nature* 1990, 343, 254-256. Kohnen, M. E. L.; Schouten, S.; Sinnighe Damsté, J. S.; de Leeuw, J. W.; Merritt, D. A.; Hayes, J. M. *Science* 1992, 256, 358-362.

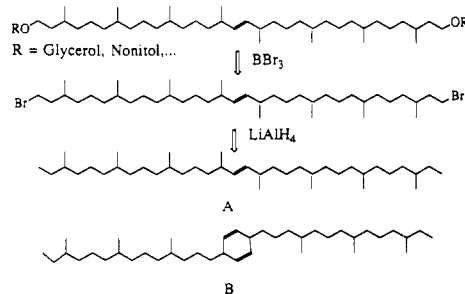
The geohopanoid picture is of course not the only one available for these purposes; for instance, it is very often possible instead to use, or in a complementary way, as indicators several other families of markers: the steranes, the rearranged steranes (diasteranes) and their aromatic and heterocyclic derivatives, indicative of an algal, marine origin very frequently present in sediments,¹⁶ or those derived from the higher plant triterpenes, indicative of a terrestrial origin,²⁰ etc.

Geohopanoids and Archaeology

A series of remarkable studies have made use of the fingerprinting available through the analyses of hopanoids (and of steranes/diasteranes) to identify the origin of bitumens used in ancient Egypt in embalming mummies (from the Dead Sea)^{21a} or in Mesopotamia to seal the stone slabs of Nebuchadnezzar's great processional esplanade in Babylon (from some well-identified natural sources).^{21b}

"Hidden" Hopanoids and Related Orphan Molecular Fossils

All of the geohopanoids mentioned so far have been isolated from extracts of sediments or from petroleum. We have however mentioned that most (ca. 90%) of the organic carbon of sediments is insoluble. This "kerogen" is a highly heterogeneous polymer, defined not structurally but operationally: the kerogen is what remains from a sediment after its soluble organic constituents have been exhaustively extracted and its minerals have been solubilized by successive HCl-HF treatments (under specified conditions). It has been possible to dissect some kerogens (or the related petroleum asphaltenes) by reactions selectively cleaving some functional groups and by analyzing the resulting low molecular weight fragments. Thus, Michaelis had isolated from a kerogen, by ether cleavage with boron tribromide followed by reduction of the resulting bromides, long chain polyterpanes, which were recognized only later as originating from the lipids of archaeobacteria.²² He even isolated from the same kero-



A, B: Sedimentary Hydrocarbons; B: Precursor still unknown

gen, by reductive cleavage, bacteriohopanetetrol itself.²³ In a similar way, by selectively oxidizing the aromatic rings of asphaltenes, hopanecarboxylic acids (as well as steranecarboxylic acids) have been isolated, which

(20) Corbet, B.; Albrecht, P.; Ourisson, G. *J. Am. Chem. Soc.* 1980, 102, 1171-1173.

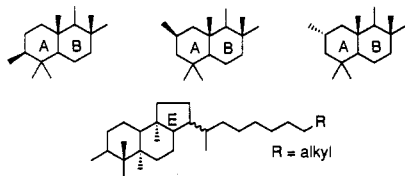
(21) (a) Rullkötter, J.; Nissenbaum, A. *Naturwissenschaften* 1988, 75, 618-621. Connan, J.; Dessort, D. *C. R. Acad. Sci. Paris* 1991, 312 II, 1445-1452. (b) Connan, J. *Bull. Cent. Rech. Explor. Prod. Elf-Aquitaine* 1989, 12, 759-787.

(22) Michaelis, W.; Albrecht, P. *Naturwissenschaften* 1979, 66, 420-421. Chappe, B.; Michaelis, W.; Albrecht, P.; Ourisson, G. *Naturwissenschaften* 1979, 66, 522-523. Moldowan, J. M.; Seifert, W. K. *Science* 1979, 204, 169-171.

(23) Mycke, B.; Narjes, F.; Michaelis, W. *Nature* 1987, 326, 179-181.

represent a very minor part of the organic matrix but obviously demonstrate that the distribution of geohopanooids extends beyond the soluble fractions.²⁴ Still another source of "hidden" hopanooids lies in the sulfur-rich macromolecular fractions of petroleum: their desulfuration with Raney nickel liberates in particular hopane derivatives.²⁵ One does not yet know the quantitative significance of these findings.

Other extensions are known from some sediments and concern homologous hopanooids giving MS fragmentation patterns different from the classical ones: there are some geohopanooids methylated at positions 2 and 3 that are therefore cleaved by electron impact MS to a fragment 205 for rings A and B, not 191 as in all the other hopanooids,²⁶ and other geohopanooids bearing a side chain longer than that of bacteriohopane and described only once¹³ or with some of the angular methyl groups rearranged or absent.²⁷



Further variants of geohopanooids in ring A and the side-chain

The last reference to the characteristic peak at 191 should not lead to the conclusion that anything giving such a fragmentation must be a hopanooid. In fact, another family of tricyclic polyterpanes has been found which has the same A/B/C arrangement as hopane. The presence of fully substituted carbon atoms at C-8 and C-14 triggers the MS cleavage of the 8(14) bond,

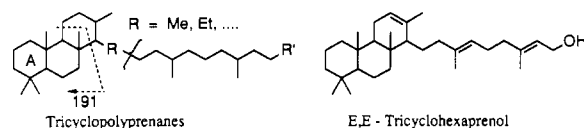
(24) Trifilieff, S.; Sieskind, O.; Albrecht, P. In *Biological Markers in Sediments and Petroleum*; Moldowan, J. M., Albrecht, P., Philp, R. P., Eds.; Prentice Hall: Englewood Cliffs, NJ 1992; pp 350-369.

(25) Adam, P.; Schmid, J. C.; Mycke, B.; Strazielle, C.; Connan, J.; Huc, A.; Riva, A.; Albrecht, P. *Geochim. Cosmochim. Acta*, in press.

(26) Summons, R. E.; Jahnke, L. L. *Geochim. Cosmochim. Acta* 1990, 54, 247-251.

(27) Moldowan, J. M.; Seifert, W. K.; Arnold, E.; Clardy, J. *Geochim. Cosmochim. Acta* 1984, 48, 1651-1661. Trendel, J. M.; Guilhem, J.; Crisp, P.; Repeta, D.; Connan, J.; Albrecht, P. *J. Chem. Soc., Chem. Commun.* 1990, 424-425.

leading to the 191 peak for the AB rings (which explains their presence in the "hopanogram" of Figure 1). However, their skeleton is clearly different, as it derives from a regular polyterpene not from the symmetrical squalene.²⁸ These tricyclopolyterpanes may derive from a triterpene of a novel type still unknown in living organisms, tricyclohexaprenol; even though the two *E,E* and *Z,Z* isomers of this potential precursor have now been synthesized,²⁹ the tricyclopolyterpanes are still orphan molecular fossils, and the synthetic samples serve only as robot portraits of one of their putative parents. Similarly, most remarkable 3-alkylsteranes were recently found in petroleum (alkyl = C₁-C₅), whose precursors might be still unknown steroids bearing at C-3 side chain resembling that of bacteriohopanetetrol.³⁰



Conclusion

The discovery of a novel class of natural products of major importance (at least weightwise and albeit underground) is an exceptional event. It was made even stranger by the absence, at the time of their discovery, of any obvious precursor. The following Account in this issue will show how the puzzle of their origin was resolved by the still more unexpected discovery of a major novel microbial lipid class previously unsuspected, a discovery triggered by the previous recognition of the structures of their molecular fossils, a most unusual sequence.

(28) Aquino Neto, F. R.; Restle, A.; Connan, J.; Albrecht, P.; Ourisson, G. *Tetrahedron Lett.* 1982, 23, 2027-2030.

(29) Corey, E. J.; Burk, R. M. *Tetrahedron Lett.* 1987, 28, 6413-6416. Heissler, D.; Ladenburger, C. *Tetrahedron* 1988, 44, 2513-2621. Heissler, D.; Jenn, T.; Nagano, H. *Tetrahedron Lett.* 1991, 47, 7587-7590. Ladenburger, C.; Heissler, D. *J. Chem. Res. S* 1991, 330. For some differently cyclized derivatives of tetra-, penta-, and hexaprenols isolated from sponges, see: Isaacs, S.; Kashman, Y. *Tetrahedron Lett.* 1992, 33, 2227-2230.

(30) Dahl, J.; Moldowan, J. M.; McCaffrey, M. A.; Lipton, P. A. *Nature* 1992, 355, 154-157.