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**HYDROCARBONS OF BIOLOGICAL ORIGIN FROM  
A ONE-BILLION YEAR OLD SEDIMENT**

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HYDROCARBONS OF BIOLOGICAL ORIGIN FROM  
A ONE-BILLION YEAR OLD SEDIMENT

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Abstract

The isoprenoid hydrocarbons, phytane ( $C_{20}H_{42}$ ) and pristane ( $C_{19}H_{40}$ ) are present in the oil seeping from the Pre-Cambrian Nonesuch Formation at the White Pine Mine, Michigan. Gas-liquid chromatography and mass spectrometry provide the isolation and identification procedures.

Two experimental approaches currently bear on the origin of terrestrial life and the time of its first appearance. The "primitive atmosphere" experiments (1) demonstrate that a wide variety of small molecules of biological significance can be formed in the laboratory from mixtures of extremely simple substances, such as methane, ammonia and water. The geological approach utilizes the record in the ancient sediments which is written in the form of shapes attributable to fossil organisms and, more recently, in the chemical nature of the imprisoned organic matter (2,3,4). Earlier than about 700 million years ago the morphological remains are scanty and commonly of microscopic dimensions and are difficult to relate conclusively to living things (5). A firm correlation between the morphological evidence and the organic matter present in the same rock would permit a systematic search for chemical evidence of early life in the ancient sediments. Certain classes of compound, such as the alkanes (6), the long-chain fatty acids (7) and the porphyrin pigments (8), show promise as biological markers since they are evidently stable for long periods of time under geologic conditions. These compounds are truly valid as biological markers only insofar as they cannot be synthesized in significant proportions by abiogenic means. For this reason, therefore, "primitive atmosphere" experiments play an important role. The range of compounds based on the isoprenoid subunit is particularly attractive, for here we have a high degree of structural specificity coupled with a

widespread distribution in nature. Thus pristane (2,6,10,14-tetramethylpentadecane) and other isoprenoid hydrocarbons have been isolated from crude petroleums of moderate ages (Mesozoic and Paleozoic) in concentrations vastly greater than those anticipated for individual branched alkanes in a thermally-derived mixture (9). Pristane is a known constituent of living things [inter alia zooplankton (10), fish and whale oils (11) wool wax (12), and marine sponges (13)], but the original source of the mineralized material may be the phytol portion of chlorophyll degraded, either biogenically or abiogenically (9,14). There is every prospect, therefore, that the isoprenoid hydrocarbons, and the related alcohols and acids, will be useful biological markers.

We now report the isolation and identification of phytane (2,6,10,14-tetramethylhexadecane) and pristane in the oil which seeps in small quantities from the base of the Pre-Cambrian Nonesuch Shale formation in Michigan. This rock (15) is late Keweenawan and is in the region of 1 billion ( $10^9$ ) years old. The identification of these hydrocarbons augurs well for the extension of the approach to even older Pre-Cambrian formations.

We established the conditions required for the isolation by processing suitable model mixtures. Thus, Linde 5 Å molecular sieve (1.6 mm pellets, predried at 200°C and  $10^{-3}$  mm and used on a 20:1 by weight basis with the alkanes) quantitatively removed (16) the normal isomers from a benzene solution of tobacco wax alkanes (17), the analysis being performed by gas-liquid chromatography at 230°C (3% SE-30 silicone gum on Gas Chrom Z, 100-120

mesh, 170  $\mu$  x 3 mm). Even after prolonged (up to 72 hrs) reflux, the iso- and anteiso- alkanes ( $C_{25}$ - $C_{35}$ ) were quantitatively retained in the solvent and the necessary solvent washings. A second sieving treatment of the hydrocarbons was found to be unnecessary. The same fractionation quantitatively removed n-heptacosane from a mixture containing cholestane, pristane and squalane. In each case the n-alkanes were readily recovered from the sieve by heating in n-hexane or by dissolution of the sieve in dilute HF.

In another experiment an oil shale (18) from the Green River Formation (Eocene age, ca.  $40 \times 10^6$  yrs) at Rifle, Colorado was extracted for several hours with n-hexane under reflux and the extract placed on a prewashed activated alumina column (2-44  $\mu$  particle size). The initial hexane eluate contained only the alkane fraction which was then subjected to the sieving process, followed by gas-liquid chromatographic analysis (3% SE-30 column, programmed from 100-300°C at 6°C per minute). The distribution of the n-alkanes closely paralleled that reported for this shale by Cummins and Robinson (18) with the marked dominance of the odd-carbon number alkanes, especially  $C_{27}$ ,  $C_{29}$  and  $C_{31}$ , so characteristic of most plants (19) and relatively young sediments (6). Again, we confirm these workers' prior findings (18) that the lower molecular weight range of the branched and cyclic alkane fraction is mainly composed of phytane, pristane and other terpenoids, since gas-liquid chromatographic fractions collected in capillary tubes from 6 mm columns displayed the appropriate

mass spectral characteristics (20). Thus, the biological history of this rock from the Cenozoic era is evident from both the very uneven distribution of the n-alkanes and from the presence of large proportions of isoprenoid alkanes.

We treated a small sample (4.1 g) of the black viscous oil which oozes from the Pre-Cambrian Nonesuch Shale (15) using the above procedures and found that the members of the n-alkane series range from C<sub>11</sub> to about C<sub>35</sub>. The distribution reaches a maximum around C<sub>19</sub> and there is a very slight, but quite definite, predominance of odd-numbered members. The branched-cyclic fraction (2.5 g), which still contains small amounts of aromatic hydrocarbon, is very complex, but is characterized by several prominent peaks in the region corresponding in retention time to that between n-C<sub>14</sub> and n-C<sub>18</sub> on the silicone gum column. We collected amounts of the order of a milligram for each of these peaks by successive sampling on a 6 mm column and then rechromatographed these at 90°C upon a strongly polar substrate [5% tetra(cyanoethyl)-pentaerythritol on Gas-Chrom RA, 80-100 mesh, 170 cm x 6 mm] which provides a particularly effective separation. Two of the fractions so obtained when chromatographed on a variety of substrates [Apiezon 'L', fluorosilicone QF-1, silicone gum SE-30, Carbowax-20M and tetra(cyanoethyl)-pentaerythritol] gave single peaks of the same retention times as phytane and pristane. These identifications were then confirmed by direct mass spectral comparison (20).

Mass spectral data on incompletely separated fractions show that other compounds with isoprenoid skeletons are present, and

work is continuing with these fractions, which may represent, in part, geologic breakdown products from the more abundant phytane and pristane. Capillary column studies (Apiezon 'L', 45 m x 0.25 mm at 170°C) of cuts taken from the packed columns show that the branched fraction is an extremely complex mixture; even so, the phytane and pristane comprise approximately 0.6% and 1.2%, respectively, of the branched-cyclic fraction.

If one accepts the presence of these hydrocarbons as evidence of former life, there remains the question of the relationship between the oil and the rock. We believe that the oil is indigenous to the rock since we have found an almost identical pattern for the normal and the branched-cyclic alkane fractions (ca. 3 mg total) isolated from carefully washed (water, HF and benzene-methanol) and pulverized Nonesuch Shale (18 g) taken from the "marked bed", depth 160 m, situated above the stratum from which the oil had been collected. No method for the dating of ancient organic matter exists as yet, so that some doubt must remain as to the precise age of this oil; however, the geologic evidence (15) favors the viewpoint that the organic matter and the associated copper are sedimentary in origin.

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  20. Mass spectra were determined on a modified CEC 21-103C mass  
spectrometer (for details of modifications and performance,  
see F. C. Walls and A. L. Burlingame, Anal. Chem. in prepara-  
tion) equipped with heated glass inlet system operated at  
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