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The quest for evidence of life processes, past or present, in extra-terrestrial environments is of current scientific concern. Isoprenoid alkanes have been proposed as "biological markers" since their high degree of structural specificity is indicative of biogenesis.[1] We have continued our search for these and other markers in ancient sediments not only with the objective of determining by a chemical approach whether organic compounds being identified in meteorites[2] could be of biological origin but also to trace, if possible, the course of the earliest stages of organic evolution. In our initial invesigations of a one-billion year old sediment, the Nonesuch Shale, pristane and phytane which are found in contemporary animals were isolated and identified spectroscopically.[1] Thus, such isoprenoids have formed a basis for the extension in scope of our researches to sediments varying in age and geologic type.

We report here our results from the analysis of (a) the Soudan Iron Formation of Minnesota, the age of which has been dated isotopically at not less than two and a half billion years[3a] and therefore extends beyond the age limit of rocks in which morphological remains have been

accepted as identified[4]; (b) the Antrim Shale, considered late Devonian in age (ca. 265 x 10^6 years); and (c) the San Joaquin Oil which is approximately 30 million years old. Whether or not the extractable hydrocarbons we have found in the Soudan were deposited at the time of formation of the sediment, or were added at a later time by migration from elsewhere, remains a question. Conclusions concerning the age of the biosynthetic mechanisms which produced them are subject to reinterpretation in the light of a more certain answer to this question as discussed below.

Continuing the approach of actual isolation and identification by physical techniques,[1] the hydrocarbon fraction from the Soudan Shale[5] was isolated and purified by procedures previously detailed.[1,6] An hydrocarbon fraction was first separated from the extractables by alumina chromatography and from this "total oil" sample the straight chain hydrocarbons were isolated, uncontaminated by isomers, by occlusion in 5 Å molecular sieves. The branched and cyclic hydrocarbon fraction which remained was examined by GLC techniques. The isoprenoid components were separated on a GLC column (10' x 1/4") using 1% SE-30 as liquid phase and further purified on successive columns (25' x 1/4") of liquid phases of 2-1/2" 7-ring metapolyphenylether and 2-1/2" tetracyanoethylatedpentaerythritol. The structures of the collected samples were determined by mass spectrometry. In addition to the isoprenoids $C_{18}H_{38}$, $C_{19}H_{40}$ (pristane) and $C_{20}H_{42}$ (phytane) previously recognized[1] in the Nonesuch samples, the presence of the $C_{21}H_{44}$ isoprenoid was established in the Soudan by its isolation and identification from its mass spectrometric fragmentation pattern.

A sample of Antrim Shale from Midland County, Michigan,[6] and a sample of San Joaquin Oil[6] were analyzed and from both samples C_{18}, C_{19} (pristane) and C_{20} (phytane) isoprenoids were identified. These "biological markers" which are found in a variety of contemporary animals have thus been shown to be present in samples ranging in age to greater than 2.5×10^9 years.

From the Soudan Shale the isoprenoids have been isolated in the approximate proportions of C_{18}:C_{19}:C_{20}:C_{21} as 3:5:3:1 and from the Antrim Shale C_{18}:C_{19}:C_{20} as 5:3:2.5. Some further quantitative trends are shown in Table 1.

A second homologous series, the normal hydrocarbons, provides another basis for comparison. Fig. 1 shows the relative distribution of the n-paraffins found in some marine sediments[3b] and in the non-marine Green River Shale.[3b,7] Any predominance of odd over even hydrocarbons is very slight in most of these samples. In the Soudan, although the carbon number nominally runs from C_{13} to C_{32}, 98% of the n-hydrocarbons are represented by n-C_{15} to n-C_{20} inclusively. This contraction in the distribution of the n-hydrocarbons provides the most noteable difference in appearance of the oil fractions from the Soudan and younger sediments. We believe that this is a real difference and not an apparent one due to any selective movement of the n-hydrocarbons within the rock because of the occurrence not only of the higher molecular weight members of this homology, but also of a substantial fraction of higher molecular weight branched and cyclic hydrocarbons which in their distribution pattern show no evidence of any preferential movement in the sediment. Rather, the GLC "fingerprint" pattern of the branched and cyclic hydrocarbons suggests a complexity comparable to that found in the Nonesuch Shale.

Table 1

PERCENTAGE COMPOSITIONS

Samples	Antrim Shale	Nonesuch Oil	Soudan Shale
Total carbon in shale	8.8		3.0
% of total carbon extrac table	ca.4.5		ca.1.5
Pristane in branched/cyclic fraction	0.3	1.2	1.3
Phytane in branched/cyclic fraction	0.2	0.6	0.7

The problem of contamination of the sediment by movement into or out of the rock since deposition of the hydrocarbon constituents is a continuing one. Compaction of a sediment after initial deposition is known to occur very rapidly in the initial stages,[8] but the great age of the Soudan could allow this initial period to be spread over several hundreds of millions of years and still require it to be in a compacted state for by far the greatest period of its existence. Migration of hydrocarbons from outside sources into compacted sediment may be possible, but normally only in oil-producing areas,[9] which is not the physical environment of the Soudan formation as known today.[3a]

One method of attempting to resolve this difficulty of contemporary nature of the hydrocarbon fraction and the rock is the use of C^{13}/C^{12} ratios. Dr. T. Hoering has informed us of his examination of a sample of the Soudan Iron Formation stratigraphically related to our own sample,[3b,10] in which a significant difference was found between the C^{13}/C^{12} ratios of the extractable and non-extractable carbon. This might be interpreted as an indication of separate origin of the extractable and non-extractable carbon. We have accordingly examined the hydrocarbon released from our powdered sample by digestion in hydrofluoric acid, after previously exhaustively solvent-extracting the powder with benzene-methanol. The straight chain hydrocarbon fraction of this second extract displays the same restricted GLC appearance as that obtained from the initial benzene-methanol extractions. This suggests that the hydrocarbons are evenly distributed throughout the sediment. Further, it is difficult to interpret our

other GLC distribution patterns in terms of any previous hydrocarbon fractionation which might well be expected on the basis of migration since deposition. For the present we regard the evidence for the hydrocarbon being indigenous to the sediment as sufficient for the basis of the arguments outlined here.

A proper evaluation of the data being obtained requires some insight into the diagenetic processes acting since deposition of the sediment. Accordingly, a more complete study of the extractable hydrocarbon fractions has been initiated. From the Nonesuch Seep Oil, components of two more homologous series have now been isolated and identified. The 2-methyl -pentadecane, -hexadecane and -heptadecane (isoparaffin series) together with the 3-methyl isomers (anteisoparrafin series) were isolated and their structures determined from the characteristic mass spectrometric fragmentation patterns. The isoparaffinic hydrocarbons have been isolated from rose petal wax,[11] and certain plants.[12] Members of both series have been isolated from a California naphtha,[13] tobacco leaf wax,[14,15] refined paraffin wax,[16] wool wax,[7] and Cuban sugar cane wax.[18] An anteisoparaffin has also been isolated from the American Cockroach.[19] The synthesis of these hydrocarbons may be understood in terms of known biological mechanisms and represent further examples of organic compounds common to both Precambrian sediments and contemporary plants and animals.

Table 2 lists the constituents which we have identified up to date. If these molecules are as old as the rocks, we have thus shortened the time available for the generation of the complex biosynthetic sequences which give rise to these specific hydrocarbons (polyisoprenoids) to less than two billion years. They provide a not unreasonable basis

Table 2

HYDROCARBONS IDENTIFIED

Sample	Age (years)	Normals (range)	Isoprenoids
Soudan Shale	2.5×10^9	C_{13} to C_{32}	C_{18}, C_{19}, C_{20} C_{21}
Nonesuch Shale+	1×10^9	C_{12} to C_{35}	C_{16}, C_{19}
Nonesuch Oil*,+	1×10^9	C_{12} to C_{34}	C_{15}, C_{16}, C_{19} C_{20}
Calcite Inclusion+ in Nonesuch Shale	1×10^9	C_{11} to C_{35}	C_{18}, C_{19}, C_{20}
Antrim Shale	265×10^6	C_{13} to C_{35}	C_{18}, C_{19} C_{20}
San Joaquin Oil	30×10^6	C_{10} to C_{33}	C_{18}, C_{19}, C_{20}

* Members of two further homologies are also present, viz., 2-methyl and 3-methyl penta, hexa and heptadecanes.

\+ Cited in references 1 and 6

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for believing that indigenous hydrocarbons of Precambrian sediments are indeed of biological origin but, equally importantly, that the use of "biological markers" will prove a valid approach in evaluating the significance of the hydrocarbons isolated from meteorites with respect to the question of their biological or abiological origin.

Summary

Isoprenoid hydrocarbons including pristane and phytane, which are found in contemporary animals and are synthesized by known biological pathways, have been isolated from a two and a half billion year old sediment, the Soudan Iron Formation of Minnesota, as well as the Antrim Shale and San Joaquin Oil of much younger ages. These chemical markers can thus be traced back in time well into the Precambrian era, suggesting a biological origin for the hydrocarbons found in these ancient shales. This evidence is further strengthened by isolation of two new homologies of singly branched paraffins, also found in contemporary plants, from the Nonesuch Seep Oil.

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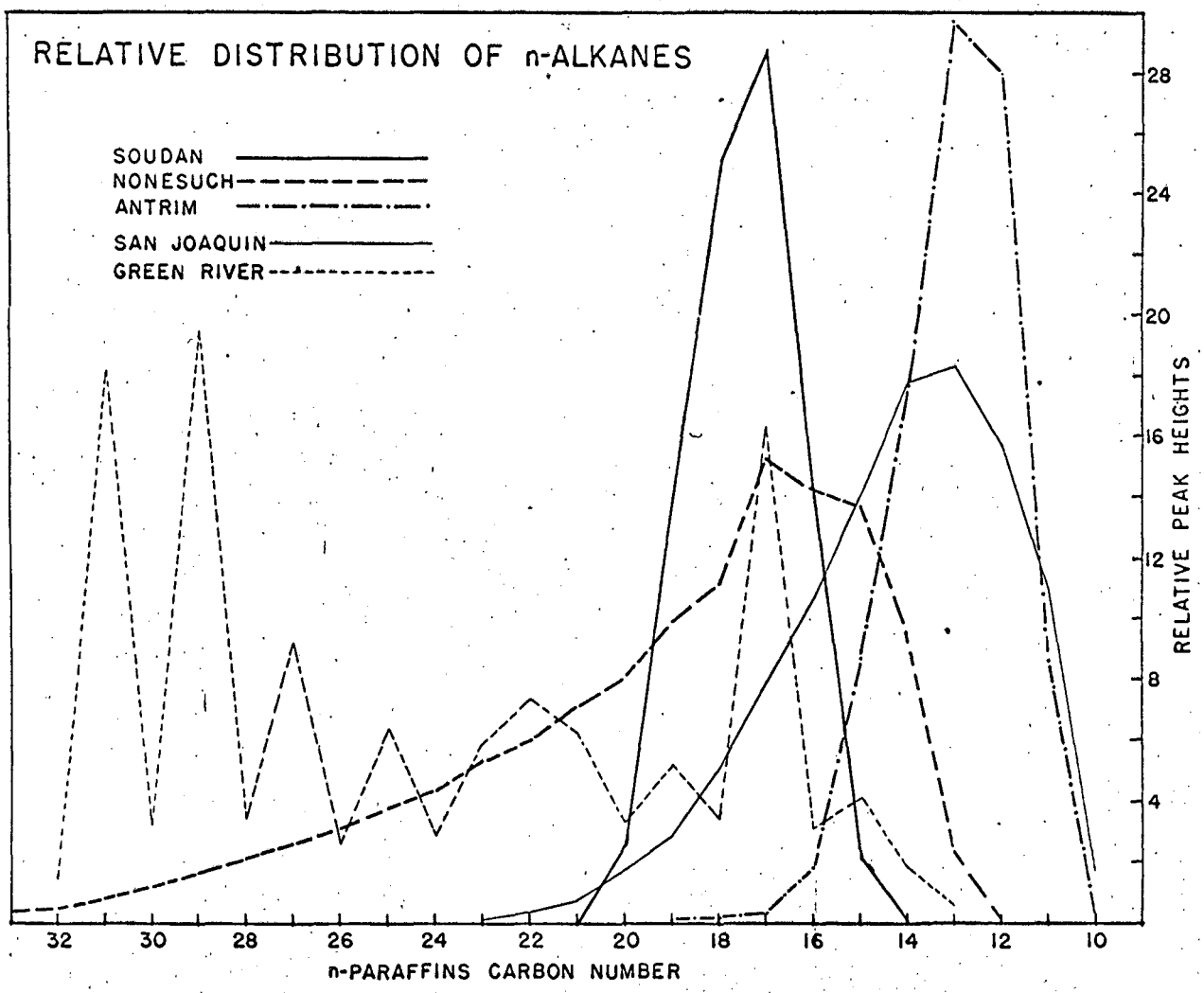
RELATIVE DISTRIBUTION OF n-ALKANES
SOUDAN
NONESUCH
ANTRIM
SAN JOAQUIN
GREEN RIVER
32
30
28
26
24
22
20
18
16
14
12
10
n-PARAFFINS CARBON NUMBER
4
8
12
16
20
24
28
RELATIVE PEAK HEIGHTS

MUB-5195