Hydrocarbons of Biological Origin in Sediments about Two Billion Years Old

Abstract. *Normal paraffins in the C_{16} to C_{32} range and the saturated isoprenoid hydrocarbons, pristane and phytane, have been found in chert from the Gunflint iron formation (1.9×10^9 years old) of the north shore of Lake Superior. The distribution of* n-*alkanes shows two maxima, one at about C_{18} to C_{19} and the other at about C_{22} with a minimum occurring at C_{20} to C_{21}. No predominance of odd- to even-carbon-number alkanes is observed within the C_{16} to C_{32} range. The results agree with micropaleontological observations made on the Gunflint chert and provide a chemical characterization of Precambrian life existing about two eons ago.*

Chert from the Gunflint iron formation is perhaps the oldest Precambrian sedimentary rock known containing, in a three-dimensional matrix, well-preserved microfossils with the morphology of algae and simple fungi. Other microfossils found less frequently in the chert differ morphologically from all known existing plant and animal microorganisms (*1–3*). The organic residues of these microfossils yield small amounts of aliphatic and aromatic hydrocarbons (*2, 3*), and they contain hydrocarbons with 20 or more carbon atoms (*2*). The large abundance of microfossils resembling blue-green algae (*1–3*) suggests that chlorophyll degradation products such as phytane and pristane could also be present in the organic residues.

On the basis of these observations and suggestions we have analyzed the Gunflint chert for high molecular weight paraffins with the aim of establishing their identity, determining their molecular weight distribution, and finding out if hydrocarbons of definitely biological origin such as phytane and pristane are present (*4*).

A specimen of solid, black chert (No. C 6353 from the collection of E. S. Barghoorn) weighing approximately 150 g was cut and broken into several major pieces. Several thin sections were prepared from one of these pieces and examined microscopically. The most abundant microfossils appeared to be blue-green algae (*5*), an observation in agreement with those of Barghoorn and associates (*1–3*). The paraffinic hydrocarbons from the chert were analyzed by gas chromatography and by a combination of gas chromatography and mass spectrometry (*6, 7*). Only the necessary additional information pertaining to the treatment of the chert samples and extraction of the organic matter is described here. The organic matter was extracted from the chert, in an all-glass Soxhlet-type apparatus, with a mixture of benzene and methanol (3:1) (*8*) after the sample had been either demineralized with hydrofluoric acid (*9*) or pulverized (*10*). After the extraction, the organic residue was separated on a silica-gel column into four fractions (*10*). Only the first fraction which was eluted from the column with *n*-heptane and contained the paraffinic hydrocarbons was used in the analysis. The other fractions (the carbon tetrachloride, benzene, and methanol eluates) were retained for subsequent analysis.

Three samples of chert were treated as follows. Chert sample No. 1 (22.9 g) was demineralized, without prior removal of possible surface contamination, and the residue (230 mg) was extracted. Chert sample No. 2 (22.8 g) was pulverized, without prior removal of possible surface contamination and extracted. Chert sample No. 3 (10.8 g) was taken from an inside piece to eliminate contamination (*11*), pulverized, and extracted.

The residues from the *n*-heptane eluates, which approximated 0.1 mg hydrocarbon from a 20-g sample of

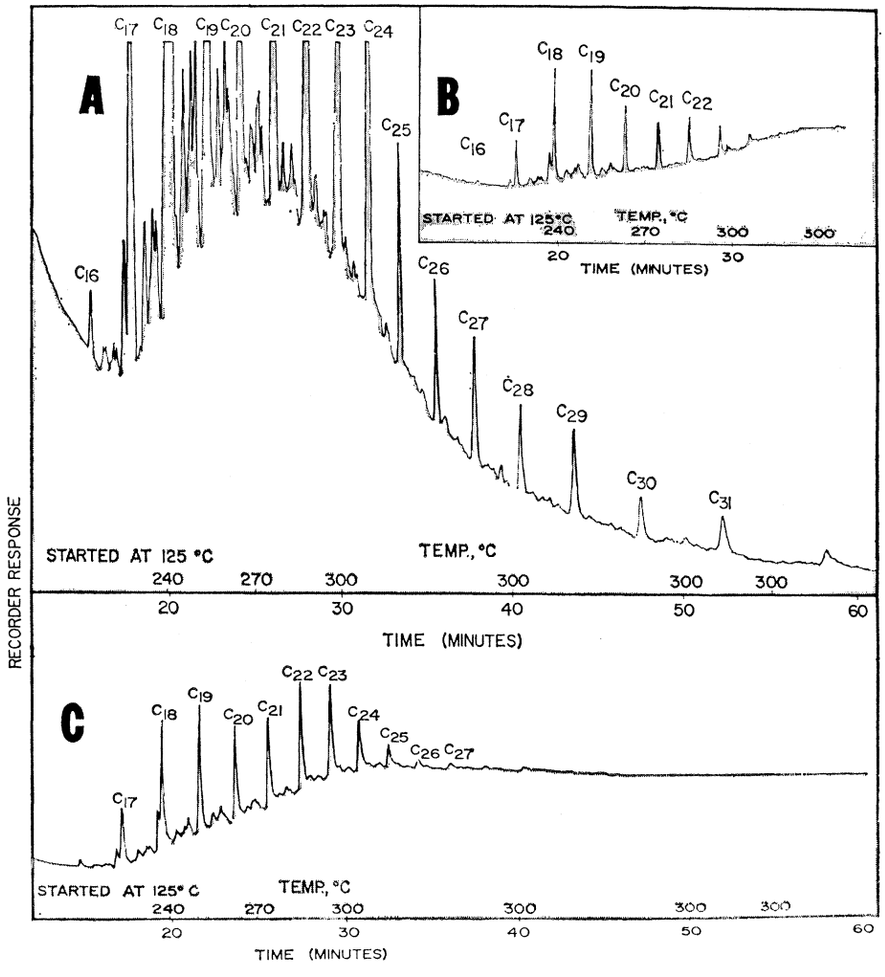

Fig. 1. Gas chromatographic separation of alkanes from the Gunflint chert. Nitrogen pressure, 2550 g/cm². Programmed from 125° to 300°C at 5.8°C per minute. Small split. Barber-Colman Series 5000 apparatus equipped with flame detector. *A*, Chert sample No. 1 (residue from HF treatment). About one-half of the *n*-heptane eluate was injected. Attenuation, 30. *B*, Same as *A* except about one-tenth of the hydrocarbons was injected. Attenuation, 100. *C*, Chert sample No. 2 (untreated with HF, pulverized). About one-sixth of the *n*-heptane eluate was injected. Attenuation, 300.

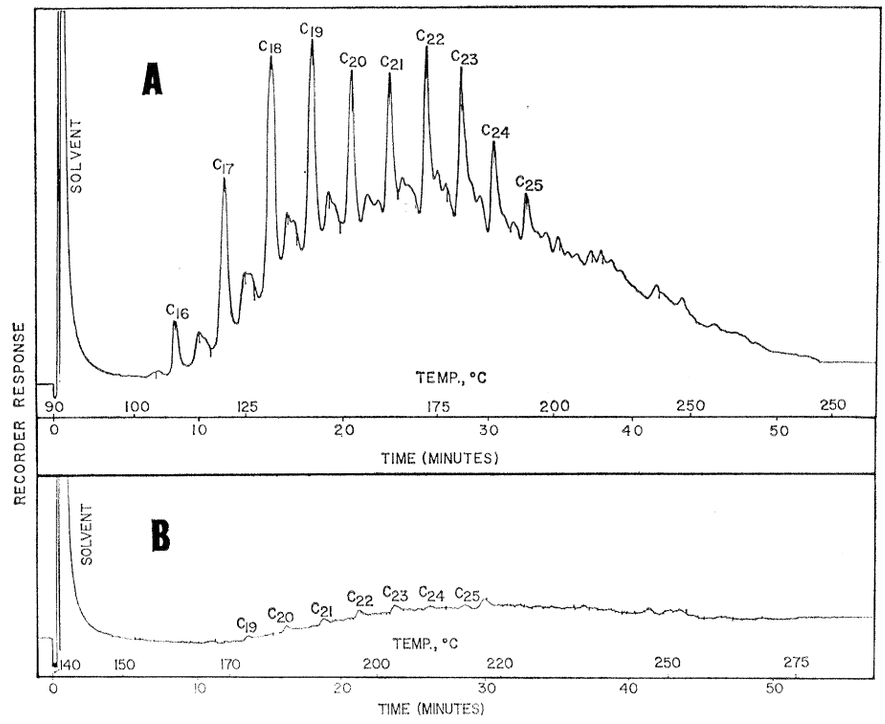

Fig. 2. Gas chromatographic separation of alkanes from the Gunflint chert with a packed column (F-60). Glass tubing, 1.8 m × 4 mm, containing 1 percent F-60 silicone oil (*12*) on 80 to 100 mesh acid washed silanized Gas Chrom P. Helium pressure, 1400 g/cm^2. No split. Mass spectrometer-gas chromatograph combination (*6*). *A*, Chert sample No. 2 (untreated with HF, pulverized). About one-third of the *n*-heptane eluate was injected. Attenuation, 100. Programmed from 90° to 250°C at approximately 3.5°C per minute. *B*, Surface washings from chert. About one-half of the *n*-heptane eluate was injected. Attenuation, 30. Programmed from 140° to 275°C at approximately 2.5°C per minute.

chert, were dissolved in benzene and samples were taken for chromatography (Figs. 1, 2, and 3). The chromatograms shown in Fig. 1 were obtained with an 0.03-cm capillary column coated with Apiezon L (*12*). Figure 1*A* shows a chromatogram of the alkanes in sample No. 1 which was demineralized by hydrofluoric acid. As judged by the retention times of individual hydrocarbons and hydrocarbon mixtures of known identity the major peaks in this and subsequent chromatograms corresponded to normal alkanes. The *n*-alkanes shown in Fig. 1*A* have 16 to 32 carbon atoms. The large sample taken and the high sensitivity of the test permitted detection of high-molecular-weight alkanes which are only present in relatively small amounts. Although only *n*-alkanes up to C_{32} are shown, small peaks for *n*-$C_{33}H_{68}$ and *n*-$C_{34}H_{70}$ were also observed in the chromatogram. This chromatogram also shows that many, probably isomeric, hydrocarbons are present which contribute to the base line hump and form the small incompletely resolved peaks between the normal alkanes. The peak immediately preceding that of the C_{17} *n*-alkane corresponds to pristane as judged by its retention time. The phytane peak which would immediately precede the C_{18} *n*-alkane peak is off scale and can not be seen in Fig. 1*A*, but its presence is obvious in Fig. 1*B*, where the chromatogram was made from a smaller sample of the same benzene solution.

Figure 1*C* shows a chromatogram of the paraffinic hydrocarbons obtained from chert sample No. 2. The distribution of hydrocarbons observed in the aforementioned two different treatments (demineralization and pulverization) is essentially the same. Some of the small differences may be caused by loss of the more volatile hydrocarbons during the evaporation procedure. The hydrocarbons appear to be distributed in two major groups of *n*-alkanes, one with a maximum at C_{18} to C_{19} and the other at C_{22} showing a minimum at C_{20} to C_{21} (*13*). This distribution can be interpreted as indicating that the hydrocarbons were derived from at least two major biosynthetic pathways or two major classes of organisms. No predominance of alkanes with an odd number of carbon atoms (C-odd) over those with an even number of carbon atoms (C-even) is observed in these hydrocarbons. Although, at first, this may appear strange for hydrocarbons of biological origin, the nonpreponderance of C-odd to C-even has been shown in the alkanes from a number of living and fossilized organisms (*14*).

Figure 2*A* shows the analysis of chert sample No. 2 on a packed column attached to an Atlas CH4 mass spectrometer (*6*, *15*). Confirmation of the identification of the C_{16} through C_{25} *n*-alkanes was afforded by the mass spectra of the individual components as they emerged from the column. The short lines at the top of the peaks indicate where the mass spectra were taken. The column used did not resolve pristane and phytane,

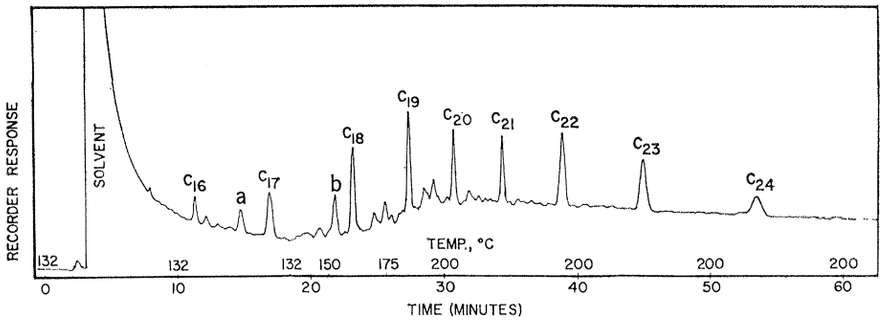

Fig. 3. Gas chromatographic separation of alkanes from the Gunflint chert with a capillary column (Polysev). Stainless steel tubing, 75 m × 0.08 cm, coated with Polysev (*12*). Nitrogen pressure, 700 g/cm^2. Isothermal at 132°C for 18 minutes, then programmed at 5.8°C/min at 200°C. Small split. Barber-Colman Series 5000 apparatus equipped with a flame detector. Chert sample No. 3 (untreated with HF; pulverized inside sample). About one-tenth of the *n*-heptane eluate was injected. Attenuation, 30.

but, with standard samples, these two hydrocarbons had retention times essentially identical to *n*-heptadecane and *n*-octadecane respectively. Evidence for the presence of pristane and phytane was obtained since the mass spectra for the peaks which gave cracking patterns of C_{17} and C_{18} *n*-alkanes also had small maxima at m/e (mass/charge) = 183 ($C_{13}H_{27}^+$). This indicated that the peaks consisted primarily of the *n*-alkane with a small admixture of pristane in peak C_{17} and phytane in peak C_{18}.

To check the effects of possible surface contamination (brought about by handling or by contemporary organisms) on the results shown in Figs. 1 and 2*A*, a large sample of chert was thoroughly washed with a total of 50 ml of benzene-methanol (3:1) mixture. The solution was then handled exactly as the chert extracts, and the alkane fraction was chromatographed. Figure 2*B* shows that contamination accounts for less than 1 percent of the hydrocarbons recovered from the chert (*16*).

The inside piece of chert (sample No. 3) was washed with benzene-methanol (3:1) before being pulverized, and the extract was chromatographed in a capillary column coated with Polysev (*12*) which very efficiently separates pristane, phytane, and other isomers from the *n*-alkanes (Fig. 3). This chromatogram also shows the bimodal distribution of alkanes characteristic of other samples from the Gunflint chert. It also gives convincing evidence for the presence of pristane and phytane because peaks *a* and *b* have retention times identical to that of pristane and phytane standards. The paraffinic hydrocarbons from the inside piece of chert were also analyzed on another capillary—stainless steel (0.03 cm × 90 m)—coated with Carbowax 20M terminated with terephthalic acid (*12*)—and programmed from 125° to 200°C at the rate of 5°C per minute. This system resolves pristane and phytane from the other alkanes. Again peaks with retention times corresponding to these two isoprenoid hydrocarbons were observed.

The carotenoid pigments and the phytol portion of the chlorophyll molecule have been mentioned as possible precursors of the isoprenoid hydrocarbons found in petroleum (*17*). To these may be added the substituted napthoquinones and tocopherols (vitamins E and K, respectively), ubiquinones and plastoquinones. Because the organic material of the chert is derived for the most part from algae we believe that the pristane and phytane in this sediment came from the phytyl alcohol of chlorophyll. Conceivably these two saturated isoprenoid hydrocarbons could also have been derived from the other possible precursors (substituted quinones) by diagenetic processes. However, if these biological quinones were present in sufficient quantities to produce pristane and phytane, then higher homologs of these branched compounds should also have been detected. The same reasoning can be used to show that these isoprenoid hydrocarbons could not have been produced abiotically. Independent evidence for the biological origin of the Gunflint organic matter has been provided recently by measurement of the C^{13}/C^{12} ratio of this matter and its comparison with the C^{13}/C^{12} ratio of the carbonate fraction in the same rock (*3*). These studies have shown that the degree of C^{13} depletion of the Gunflint organic compounds is similar to that found in contemporary organic compounds produced by photosynthesis (*3*).

From the foregoing chromatographic and mass spectrometric studies, we conclude that (i) the Gunflint chert contains pristane and phytane, (ii) the chemical evidence of life (presence of pristane and phytane) correlates with the morphological evidence provided by fossilized organisms, and (iii) the Gunflint chert also contains normal paraffinic hydrocarbons ranging approximately from C_{16} to C_{32}, the distribution being bimodal but showing no predominance of C-odd over C-even alkanes. As a corollary of (iii) and of other recent observations (*14*) it should be added that an odd- to even-carbon-number preference in the distribution of normal alkanes may be a sufficient but is not a necessary indication of biological origin.

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References and Notes

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2. E. S. Barghoorn and S. A. Tyler, *Ann. N.Y. Acad. Sci.* **108**, 451 (1963).
3. ———, *Science* **147**, 563 (1965).
4. Evidence for the presence of phytane and pristane in the one-billion-year-old Precambrian Nonesuch formation has been presented by G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame, M. Calvin, *Science* **145**, 263 (1964), and by W. G. Meinschein, E. S. Barghoorn, J. W. Schopf, *ibid.*, p. 262.
5. A number of spore-like bodies and a few structures of more complex morphology were also observed. These results are being prepared for publication.
6. R. Ryhage, *Anal. Chem.* **36**, 759 (1964).
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8. W. G. Meinschein, Quarterly Rept., contract No. NASw-508, 1 April 1964; W. G. Meinschein and G. L. Kenny, *Anal. Chem.* **29**, 1153 (1957).
9. We thank R. Louden for demineralizing the chert with hydrofluoric acid.
10. The chert was pulverized in a Carver press test cylinder. Material that passed through a 100-mesh sieve was extracted.
11. A piece of the chert was sawed and ground with Carborundum tools until at least 1 cm thickness of all the surface rock had been removed.
12. Apiezon L (a high temperature grease) and Polysev [*m*-bis *m*-(phenoxyphenoxy)-phenoxybenzene] were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania. The F-60 silicone oil was obtained from Dow Corning Corporation, Midland, Michigan. Carbowax 20M (terminated with terephthalic acid) was obtained from Wilkens Instrument and Research, Inc., Walnut Creek, California.
13. The maximum at C_{18} to C_{19} may be caused in part by the evaporation of the more volatile hydrocarbons during the isolation procedure.
14. J. Oró, D. W. Nooner, S. A. Wikström, unpublished; W. G. Meinschein, Quarterly Rept., contract No. NASw-508, 1 July 1964; B. Pasby, B. S. Cooper, D. W. Hood, Abstracts of papers presented at the 1964 Annual Meetings of the Geological Society of America, 19–21 November 1964, at Miami Beach, Florida, p. 149; N. P. Stevens, E. E. Bray, E. D. Evans in *Habitat of Oil*, L. G. Weeks, Ed. (American Association of Petroleum Geologists, Tulsa, Oklahoma, 1958), p. 779; L. S. Ciereszko, D. H. Attaway, M. A. Wolf, Petroleum Research Fund, 8th Annual Report (1963), p. 33.
15. We thank E. C. Horning, Lipid Research Center, Department of Biochemistry, Baylor University College of Medicine, Houston, Texas, for making the gas chromatograph-mass spectrometer combination available to us.
16. The small peaks in Fig. 2*B* correspond to *n*-alkanes showing a unimodal distribution with maximum at about C_{23}. This can be observed more clearly by injecting a much larger sample of the paraffinic fraction obtained from the chert surface washings. It is not yet certain whether these traces of alkanes are derived from the lipids of human hands or from other sources.
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18. This work was presented at the meeting of the Meteoritical Society, Arizona State University, Tempe, 30 October 1964, and the Group for the Analysis of Carbon Compounds in Carbonaceous Chondrites, Washington, D.C. We thank Dr. W. G. Meinschein for a sample of paraffins containing pristane, phytane, and squalane; Dr. M. Blumer for a sample of phytane and P. Simmonds for valuable assistance with the gas chromatographic analyses. Supported in part by NASA grant, NsG-257-62.

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