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Hydrocarbon Constituents of Iceland Leaf Fossil

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Abstract---The hydrocarbon content of leaf fossils from Iceland has been investigated by gas chromatography-mass spectrometry. The distribution patterns of normal hydrocarbons, branched hydrocarbons, and cyclic hydrocarbons are compared to those of present-day living organisms. The diagenetic pathways of these hydrocarbons are discussed.

Introduction

Organic molecules derived from dead organisms as a result of diagenesis are important in organic geochemistry. Those molecules result from three processes: anaerobic bacterial transformation, thermal decomposition or thermal cracking, and condensation. It is suggested that, given sufficient time, all organic and biological matter is eventually reduced either to condensates resembling kerogen and graphite, or to thermostable paraffinic hydrocarbons. Temperature and pressure are the major factors for the degradation and condensation reactions.

The association of organic matter with sediments has been studied for the past decade. Several classes of organic compounds have been reported, such as alkanes (Oro 1965, Bray 1965, Johns 1966, and Han 1969a), fatty acids (Kvenvolden 1967, Hoering 1964, and Van Hoesen 1969), amino acids (Schopf 1968) and porphyrins (Meinschein 1965, and Hodgson 1968). The occurrence of matter other than hydrocarbons may serve as an indication that diagenesis of these molecules has

not reached its final stages. The hydrocarbons, which are the stable biological markers, have probably received the most attention. Some firm correlation of hydrocarbons between the present-day living organisms and the fossils permits a systematic search for chemical evidence of diagenesis over long periods of geological time.

A large slab of leaf fossil (Fig. 1) from Iceland was received from Dr. J.A. Wolfe, U.S. Geological Survey. It is found that the leaf fossils are piled very closely together and sandstones scattered around the slab. The sample is collected from Brjánslaekur, northwest peninsula, Iceland. The fossil is on the middle of a number of horizons in the plateau (a Tertiary flora of Hoffell and Tjörnes type, ranging perhaps from Upper Miocene to Upper Pliocene) $10\sim 15 \times 10^6$ years, a mild-temperature climate is indicated (Pflug, 1959). The fossils seem to belong to willows, birch, and alder (T. Einarsson, 1963), which are embedded in clay and consist of numerous leaves and a small part of wood chips, apparently carried by a sluggish river and buried in its muddy bottom.

Experimental

After the outside surface of the rock was removed by a clean water-cooled diamond saw, the larger rock segment was wrapped in aluminum foil and fragmented using a hammer. The chips were collected and washed in a flask containing a mixture of benzene and methanol. The chips were dried in an oven and pulverized by a disc mill. The pulverized powder was less than 120 mesh in size. The powder was extracted three times with 3:1 benzene/methanol using an ultrasonic vibrator. After centrifugation, the supernatant solution

was removed, yielding the "total extract."

An alumina column (3 ft x 0.5 in.) was prepared from 150g of activated neutral aluminum oxide and washed with 200ml of n-heptane. The total extract was mixed with 2g aluminum oxide and transferred to the top of the column, after which it was eluted with 150ml of n-heptane. The "total hydrocarbons" represented in this fraction show minimal u.v. absorption at 270m μ . The normal alkanes were separated from branched-cyclic hydrocarbons with a 5 \AA molecular sieve (O'Connor et al., 1962). A 50:1 weight ratio of sieve to sample was used. The sieve and sample were placed in a round-bottomed flask and refluxed in dry isooctane for two days. After centrifugation, the solution containing the branched-cyclic fraction was removed. The sieve was thoroughly extracted with hot isooctane for several hours and the washings were added to the branched-cyclic fraction. The isooctane-washed sieve, which contained the normal paraffins, was treated with a mixture of 20% HF and benzene for one hour. After separation of the layers, the benzene solution of n-alkanes was filtered, and the solvent was evaporated.

All fractions were analyzed on Aerograph Model 204 gas chromatograph equipped with a capillary column which was coated in this laboratory. The gas chromatograms of "total hydrocarbons," "branched-cyclic hydrocarbons" and "normal hydrocarbons" are shown in Figures 2, 3A, and 3B, respectively. Coinjections of pristane and phytane were made, the locations of these stands coincide with the peaks labeled on Figure 3A.

All mass spectra were taken using a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer. The gas chromatographic

oven temperature was programmed from 90° to 300°C at 2°C/min with a helium flow rate of 2.5ml/min. The effluent from the capillary column was split into two parts, 1.5ml/min going to the flame ionization detector and 1ml/min going through a 1 ft x 0.002 in. heated stainless steel tube at 220°C to the ion source of the mass spectrometer. These spectra were determined at an ionizing voltage of 70eV and an ionizing current of 50μA. The temperature of the ion source was 200°C. Each spectrum was scanned in three seconds. The spectra were recorded on an oscillograph recorder.

Results

The weight of the total heptane eluate contained 85ppm of the sample weight. The n-alkanes, representing 50ppm of leaf fossil, range from C₁₂ to C₂₉ (Fig. 3B). Identifications were made by mass spectral analyses and by coinjections with standard samples on a capillary gas chromatograph. There was no significant predominance of odd over even carbon numbered n-paraffins in the range from C₁₂ to C₁₉. However, the ratio of odd over even carbon-numbered alkanes increased for higher molecular weights, and it showed a marked odd/even preference with n-C₂₇ and n-C₂₉ the most abundant hydrocarbons.

The mass spectra of the major components in Fig. 3A's peaks, a,b,c and d are shown in Fig. 4A, B, C and D, respectively. Their molecular weights and formulas are listed in Table I.

Table I

| Compound | Mol. Wt. | Formula | |
|----------|----------|----------------|---|
| A | 248 | $C_{18}H_{32}$ | tricyclic terpane |
| B | 262 | $C_{19}H_{34}$ | tricyclic terpane |
| C | 328 | $C_{24}H_{40}$ | two degree of unsat. tricyclic terpane |
| D | 318 | $C_{23}H_{42}$ | tricyclic terpane |

It has been impossible to assign unequivocal structures to these compounds on the basis of mass spectrometry alone. The mass spectrum suggests that these substances are not related to any of the common cyclic hydrocarbon structures such as steranes, triterpanes, and tetraterpanes that have been characterized in sediments (Burlingame et al., 1965, Murphy et al., 1967, Henderson et al., 1969) and may have the structures of a tricyclic series related to diterpanes, and triterpanes. We are investigating the structures of these compounds in order to establish them unequivocally. We already know from their behavior on silver nitrate thin layer chromatography (Morris, 1962) that A, B, and D do not contain any double bonds, while C does. From the molecular weight of C, we deduce two degrees of unsaturation.

Since the leaf fossils were identified as willows, birch, and alder, we were interested in comparing the hydrocarbon constituents between modern day living organism and the fossils. We examined five species of leaves: the Willows Salix Spp. (with long, slender, tapering foliage with prominent basal stipules, flowers in catkins, and cottony winged seeds); Salix matsudana (its rounded crown of twisted, spiralling branches may reach 30' in height and 20' in diameter. The narrow 3" leaves have sharply toothed margins, are

whitish on the underside and curled or twisted); the birch Betula papyrifera (the ovate leaf blades on slightly hairy stems, are 4" long by half as broad with doubly serrated margins and flattened or heart-shaped bases); Betula verrucosa (European white birch); and the alder, Alnus oregona (its 3" to 6" leaves are coarsely toothed and have rolled edges.) The outline of the extraction scheme was described elsewhere (Han et al., 1968). Table II shows the relative abundance of the individual hydrocarbons in total heptane eluate (given as % of total hydrocarbon present).

Table II

| | n-C ₂₃ | n-C ₂₅ | n-C ₂₇ | n-C ₂₉ | n-C ₃₁ |
|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| <u>Salix spp.</u> | - | - | 40 | 59 | - |
| <u>Salix matsudana</u> | 1 | 10 | 60 | 25 | 2 |
| <u>Betula papyrifera</u> | 1 | 7 | 40 | 47 | 5 |
| <u>Betula verrucosa</u> | - | 3 | 46 | 50 | 1 |
| <u>Alnus oregona</u> | 1 | 2 | 50 | 42 | 4 |

The analysis of the heptane fraction, revealed the presence of a series of odd numbered normal hydrocarbons, from n-C₂₃ to n-C₃₁ with the n-C₂₇ and n-C₂₉ hydrocarbon predominant for each species and the absence of the aforementioned tricyclic hydrocarbons. In addition, neither the even numbered hydrocarbons nor the lower molecular weight alkanes have been found in significant amount. In each case the weight of total heptane eluate obtained 0.02-0.05% of the dry cell weight.

Discussion

Normal paraffins are among the most stable of all biogenic organic compounds and are thought to be diagnostic of biologically produced organic matter which can be derived from the decarboxylation of fatty acids. The $n\text{-C}_{27}$ and $n\text{-C}_{29}$ are the dominant hydrocarbons in the present-day leaves of willows, birch, and alder. The Iceland leaf fossils have two peak maxima in the normal alkane fraction, one at $n\text{-C}_{17}$ another one at $n\text{-C}_{27}$ and $n\text{-C}_{29}$. It appears that the high molecular weight of odd-numbered paraffins, $n\text{-C}_{27}$ and $n\text{-C}_{29}$ are contributed by higher plants. These materials may well be preserved over long periods of geological time without changing their structure.

The distribution pattern of normal alkanes in higher organized plants has been always characterized by the occurrence of higher hydrocarbons (from C_{23} to C_{35} , Eglinton et al., 1963) with clearly predominating odd-numbered homologues. This view, based especially on the work of Stransky and co-workers (1966, 1969), has been commonly accepted to be universally valid. Some of the earlier microorganism studied (Han et al., 1969b) contain clearly more lower alkanes (lower than $n\text{-C}_{23}$). The $n\text{-C}_{17}$ hydrocarbon is predominant in algae, and its dominance is decreased in photosynthetic bacteria. Normal heptadecane intensity is not outstanding in non-photosynthetic bacteria and is generally absent in higher plants. In all cases, the odd over even carbon-numbered n -paraffins is approximately 1.0 in aerobic and anaerobic bacteria, while the value is 1.0 to 5.0 in algae when the predominant component $n\text{-C}_{17}$ is excluded.

The gas chromatogram of the lower part of normal alkane fraction of leaf fossil (Fig. 3B) shows a smooth distribution with a maximum at $n\text{-C}_{17}$. This result together with $n\text{-C}_{27}$ and $n\text{-C}_{29}$ alkanes found in higher range, could be interpreted that the higher plants (willows, birch, and alder) and microorganisms (algae and bacteria) have played equally important roles in normal hydrocarbon production in this fossilized rock.

Isoprenoid hydrocarbons are absent in algae and willows, etc., but the chlorophylls are present in algae and leaves. Isoprenoid hydrocarbons are considered to be the diagenetic products of the phytol side chain of the chlorophyll molecule (Bendoraitis *et al.*, 1962, 1963). It is suggested that the initial degradation process has to take place by attack at the alcohol end. This could then be followed by reducing and thermal cracking mechanisms to give the C_{15} to C_{20} isoprenoid hydrocarbons found in the short period of geological time.

Tricyclic diterpenes have been found in present-day biological source materials, particularly the resin acids and gibberellins. The present hydrocarbons must surely have a biological origin for precursor. It would seem reasonable to assume that they represent the products of diagenetic alteration of tricyclic terpenoids in this Iceland fossil. As such, this series of new biological markers seems to be significant, and an understanding of their detailed structure and origin could further illuminate the problem of biochemical and plant evolution and also increase our knowledge of diagenetic process in sediments.

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Figure 1 A small slab of Iceland leaf fossil, 20cm long and 12cm wide.



XBB 698-5355

Fig. 1

Fig. 2 Gas chromatographic separation of total aliphatic hydrocarbons of Iceland leaf fossil. A 100 ft. x 0.01 in. I.D. stainless steel column coated with Apiezon L was used. Helium pressure, 20 psi; flow rate 2.5ml/min; no split. An aerograph 204 apparatus equipped with a flame ionization detector was used. Temperature was programmed at 2°C/min. from 90°C to 290°C and was held isothermally at 290°C.

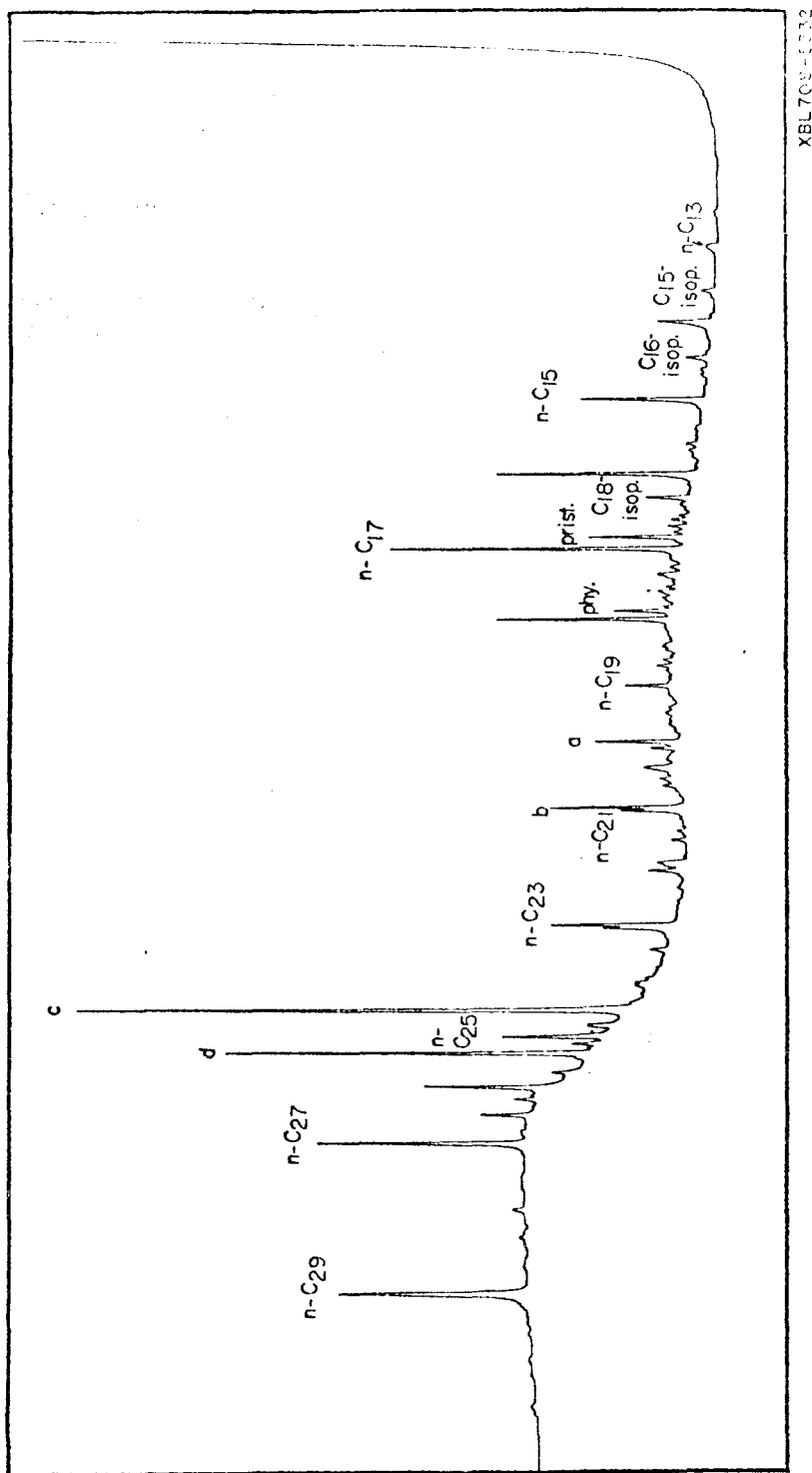
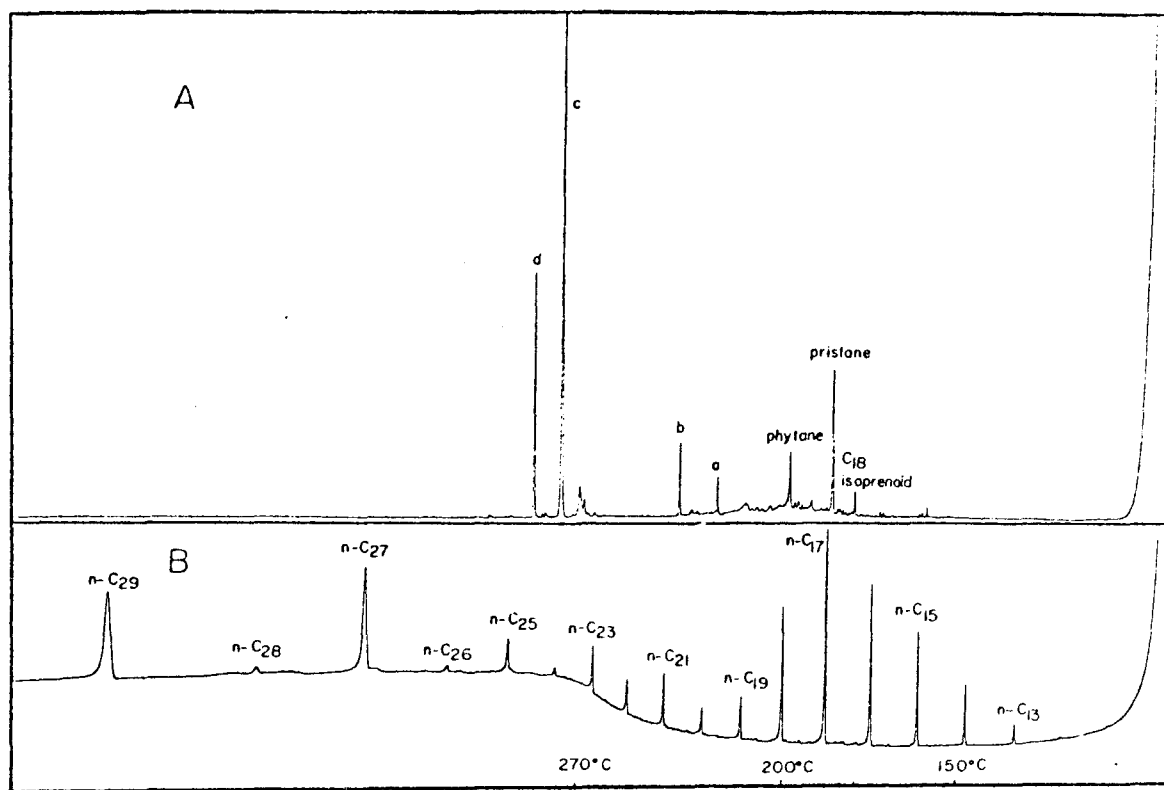
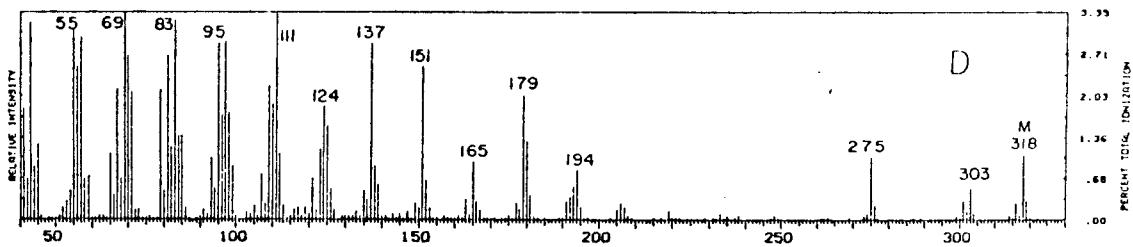
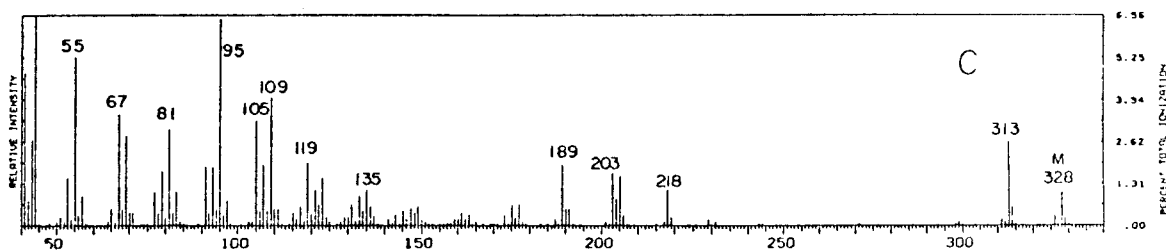
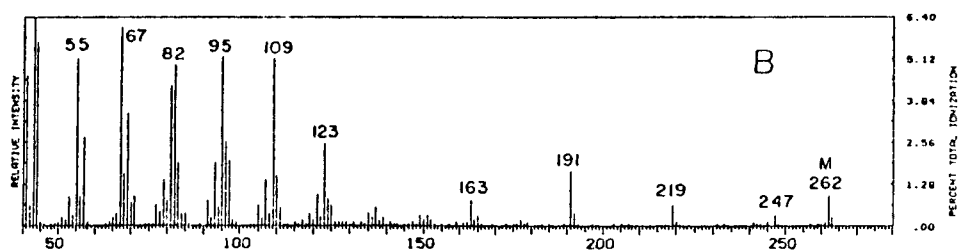
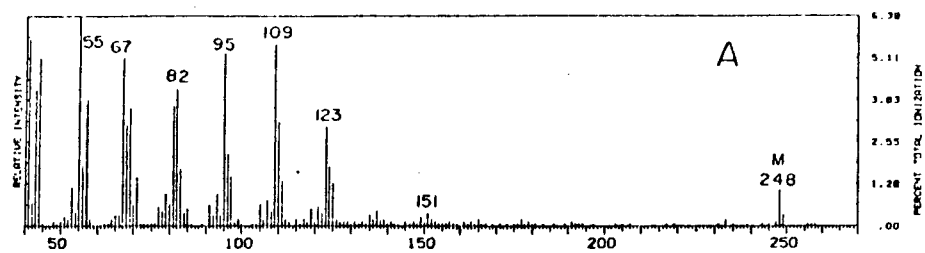


Fig. 3 The top figure (A) shows the gas chromatogram of branched and cyclic hydrocarbon fraction and the bottom figure shows the normal hydrocarbon fraction from Iceland leaf fossil. All the conditions are described in fig. 2 except the temperature was programmed from 90°C to 270°C and was held isothermally at 270°C.



XBL 708-5333

Fig. 4 The mass spectra of the major components in fig. 3A Spectra A,B,C, & D are corresponded to peak a,b,c, and d, respectively. They were taken as the components were eluted from a 100 ft. x 0.01 in. I.D. Apiezon L capillary gas chromatographic column. The compounds were ionized by electron impact at 70 ev, the ion source temperature at 200°C, and an ionizing current of 50 μ A. Each peak was scanned within the range of 10-600 mass units in 3 sec. on an A.E.I. MS-12 mass spectrometer.



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