

Lawrence Berkeley National Laboratory

LBL Publications

Title

Hydrocarbon Constituents of Iceland Leaf Fossil

Permalink

<https://escholarship.org/uc/item/06f180gt>

Authors

Han, Jerry

Calvin, Melvin

Publication Date

1970-10-01

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

HYDROCARBON CONSTITUENTS OF ICELAND
LEAF FOSSIL

Jerry Han and Melvin Calvin

October 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

*This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545*

48
LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-20157
C. 2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Hydrocarbon Constituents of Iceland Leaf Fossil

Jerry Han and Melvin Calvin
Department of Chemistry, Space Sciences Laboratory and
Laboratory of Chemical Biodynamics, University of California
Berkeley, California 94720

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 Hoeven 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^o to 300^oC at 2^oC/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^oC 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^oC. 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 terpene
B	262	$C_{19}H_{34}$	tricyclic terpene
C	328	$C_{24}H_{40}$	two degree of unsat. tricyclic terpene
D	318	$C_{23}H_{42}$	tricyclic terpene

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-C₁₇. This result together with n-C₂₇ and n-C₂₉ 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 phytyl 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 C₁₅ to C₂₀ 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 gibberillins. 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.

Acknowledgements---We thank Dr. J.A. Wolfe, U.S. Geological Survey for supplies of Iceland Leaf Fossils. The work described was supported in part by the National Aeronautics and Space Administration Grant No. 05-003-003 and in part by the U.S. Atomic Energy Commission.

References

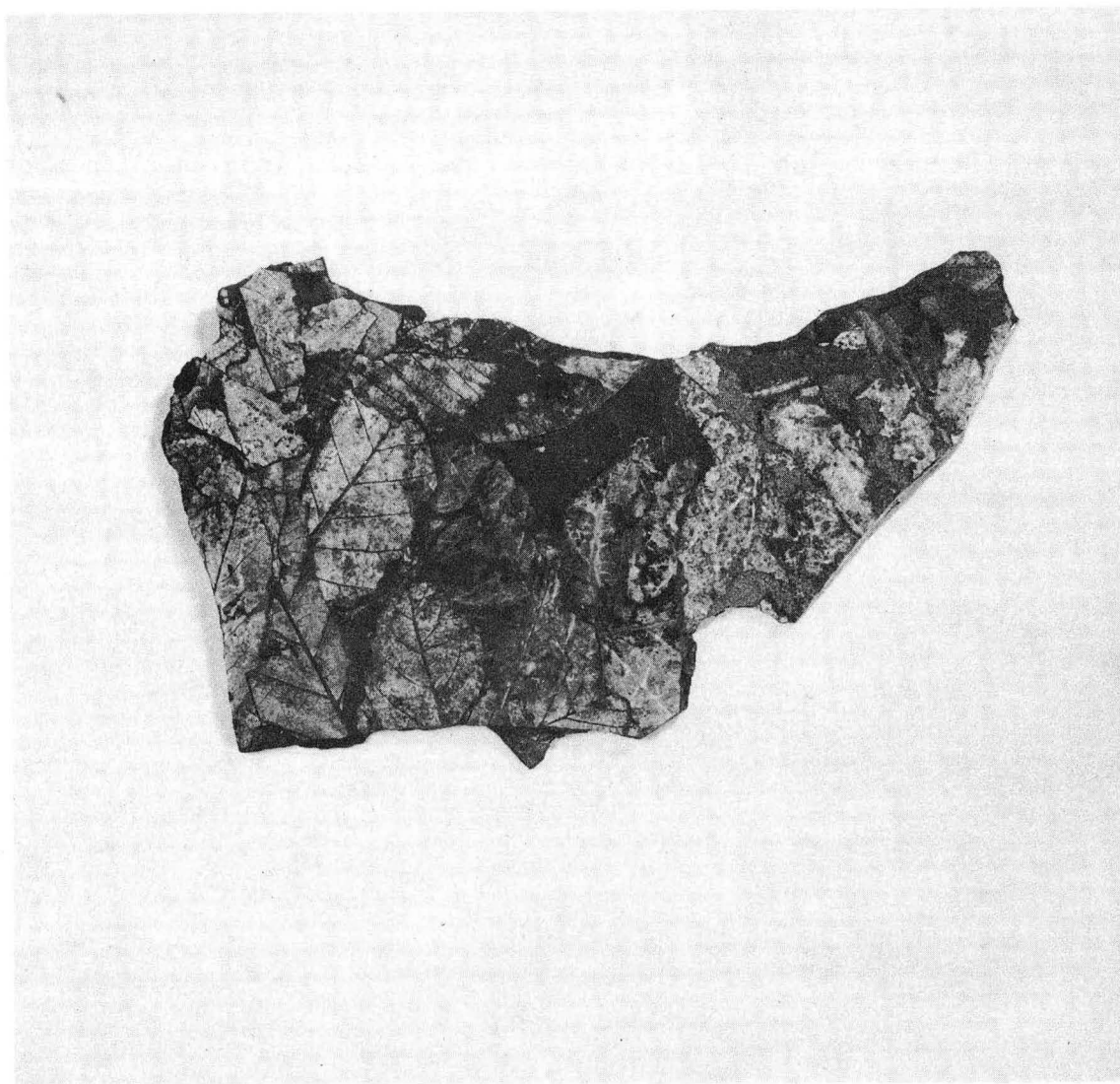
- BENDORAITIS J.G., BROWN B.L. and HEPNER L.S. (1963) Isolation and identification of isoprenoids in petroleum. World Petroleum Congress, Frankfurt/Main, Germany, June 19-26.
- BENDORAITIS J.G., BROWN G.L. and HEPNER L.S. (1962) Isoprenoid hydrocarbons in petroleum. *Anal. Chem.* 34, 49-53.
- BRAY E.E., and EVANS E.D. (1965) Hydrocarbons in non-reservoir-rock source beds, *Bull. Am. Asso. Petro. Geo.* 49, 248-257.
- BURLINGAME A.L., HAUG P., BELSKY T., and CALVIN M., (1965) Occurrence of biogenic steranes and pentacyclic triterpanes in an Eocene shale and in an early pre-cambrian shale, *Proc. Natl. Acad. Sci.* 54, 1406-1412.
- EGLINTON G. and HAMILTON R.J. (1963) *The Distribution of Alkanes Chemical Plant Taxonomy* Ed. Swain T., Acad. Press, London and New York 187-217.
- EINARSSON T. (1963) Some chapters of the Tertiary History of Iceland, North Atlantic Biota and Their History, Editors, A. Love and D. Love, The MacMillan Co., N.Y.
- HAN J. and CALVIN M. (1969a) Occurrence of fatty acids and aliphatic Hydrocarbons in a 3.4 billion-year-old sediment, *Nature* 224, 576-577.
- HAN J. and CALVIN M. (1969b) Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments, *Proc. Natl. Acad. Sci.* 64, 436-443.
- HAN J., MCCARTHY E.D., VanHOEVEN, CALVIN M., and BRADLEY W.H. (1968) Organic Geochemical Studies II A preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria, and in a recent lake sediment, *Natl. Acad. Sci.* 59, 29-33.
- HENDERSON W., WOLLRAB V., and EGLINTON G. (1969) Identification of Steranes and Triterpanes from a geological Source by Capillary Gas Liquid Chromatography and Mass Spectrometry, *Advances in Organic Geochemistry 1968*, (I. Havenaar and P.A. Schenck, Eds.) Pergamon - Vieweg, Braunschweig, 181-207.

- HODGSON G.W., HITCHON B., TAGUCHI K., BAKER B.L. and PEAKE E. (1968) Geochemistry of porphyrins, chlorins and polycyclic aromatics in solids, sediments and sedimentary rocks. *Geochim. Cosmochim. Acta* 32, 737-772.
- HOERING T.C. and ABELSON P.H. (1964) Chemicals from Nonesuch Shale, Carnegie Inst., Wash. Y.B. 63, 262-269.
- JOHNS R.B., BELSKY T., MCCARTHY E.D., BURLINGAME A.L., HAUG P., SCHNOES H.K., RICHTER W., and CALVIN M. (1966) The organic geochemistry of ancient sediments - Part II, *Geochim. et Cosmochim. Acta* 30, 1191-1222.
- KVENVOLDEN K.A. (1967) Normal fatty acids in sediments, *J. Am. Oil Chem. Soc.* 44, 628-636.
- MEINSCHEN W.G., BARGHOORN E.S. and SCHOPF J.W. (1965) Paleobiology of a Precambrian shale. *Science* 148, 461-472.
- MORRIS, L.J. (1962) Separation of Higher Fatty Acids Isomers and Vinyllogues by Thin-Layer Chromatography. *Chemistry & Industry* 1238.
- MURPHY M.T.J., MCCORMICK A., and EGLINTON G. (1967) Perhydro- β -carioten in the Green River Shale, *Science* 157, 1040-1042.
- O'CONNOR J.G., BURROW F.H. and NORRIS M.S. (1962) Determination of Normal Paraffins in C_{20} to C_{32} paraffin waxes by molecular sieve adsorption. Molecular weight distribution by gas-liquid chromatography. *Anal. Chem.* 34, 82-85.
- ORÓ J., NOONER D.W., ZLTKIS A., WIKSTRÖM S.A. and BARGHOORN E.S. (1965) Hydrocarbons of biological origin in sediments about 2 billion years old, *Science* 148, 77-79.
- PFLUG, H.D. (1959) Sporenbilder aus Island und ihre stratigraphische deutung. *Neues Jahrb. Geol. Paläontolog. Abh.* 107, 141-172.
- SCHOPF J.W., KVENVOLDEN K.A. and BARGHOORN E.S. (1968) Amino acids in Precambrian sediments: an assay. *Proc. Nat. Acad. Sci.* 59, 639-646.
- STRANSKY K., STREIBL M., and HEROUT V. (1966) On natural waxes VI. Distribution of wax hydrocarbons in plants at different evolutionary levels, *Coll. Czeh. Chem. Commun.* 32, 3213-3220.

STRANSKY K. and STREIBL M. (1969) On natural waxes XII Composition of hydrocarbons in morphologically different plant parts, Coll. Czech. Chem. Commun. 34, 103-117.

VanHOEVEN W., MAXWELL J.R. and CALVIN M. (1969) Fatty acids and hydrocarbons as evidence of life processes in ancient sediments and crude oil, Geochim. et Cosmochim. Acta 33, 877-881.

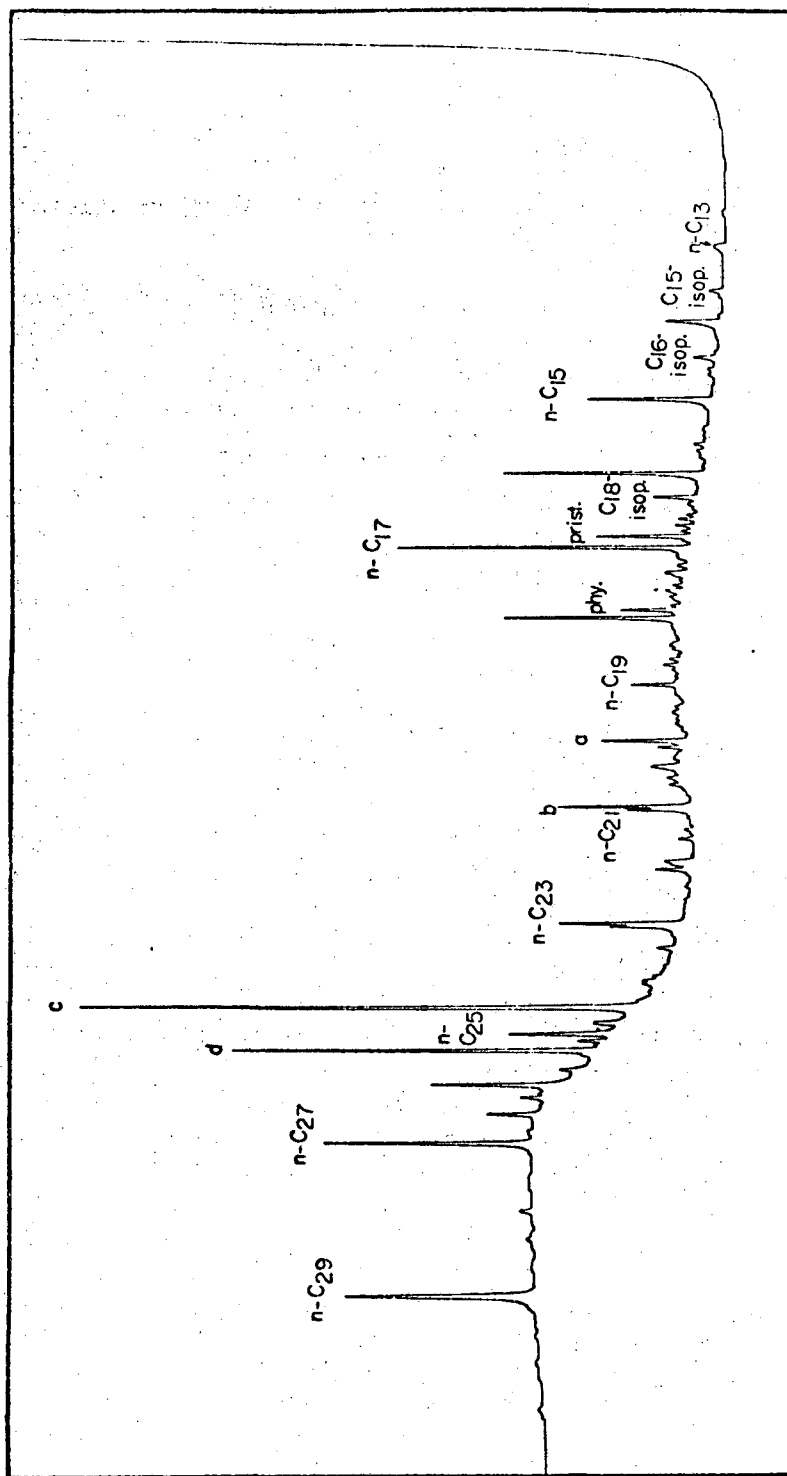
Figure 1 A small slab of Iceland leaf fossil, 20cm long and 12cm wide.



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.



XBL709-5732

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.

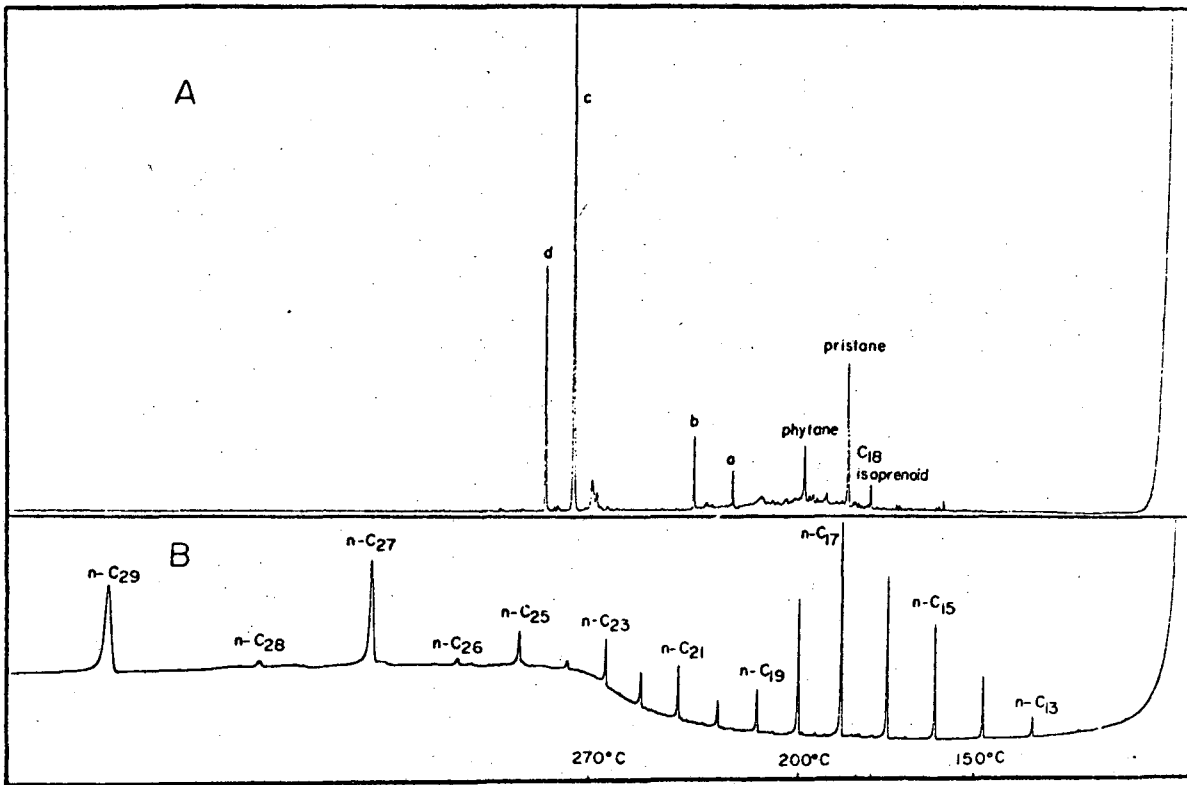
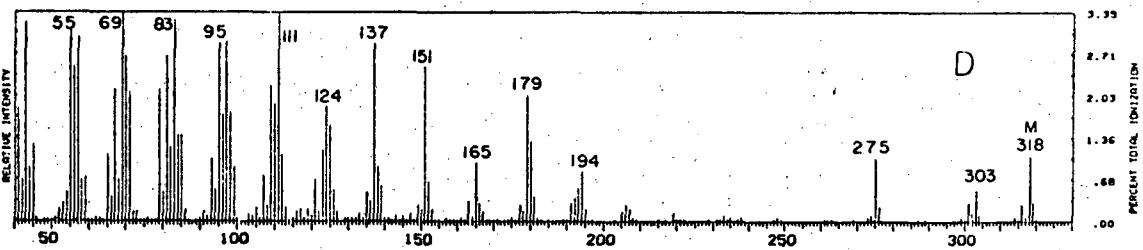
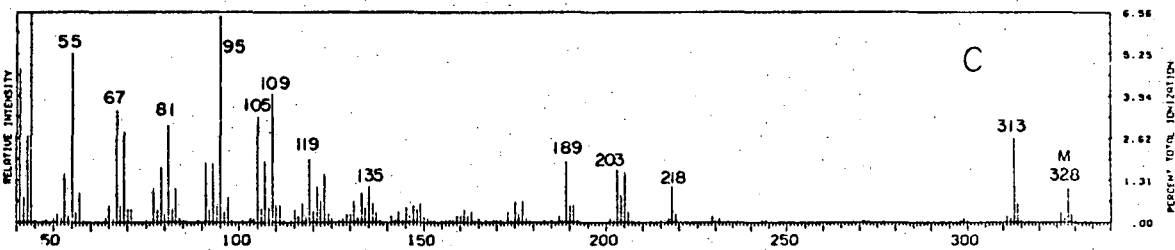
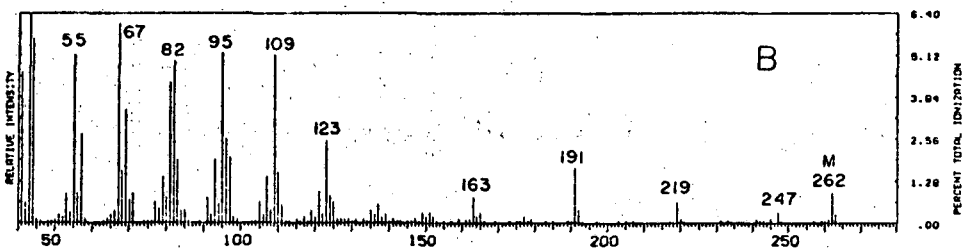
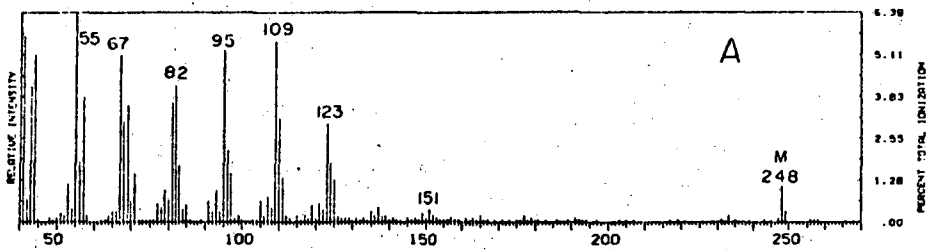


Fig. 4 The mass spectra of the major components in fig. 3A Spectra A,B,C, & D are corresponded to peak a,b,c, and d, respectively. They were taken as the components were eluted from a 100 ft. x 0.01 in. I.D. Apiezon L capillary gas chromatographic column. The compounds were ionized by electron impact 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.



LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or*
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.*

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720