University of California

Ernest O. Lawrence Radiation Laboratory

ORGANIC GEOCHEMICAL STUDIES. II. THE DISTRIBUTION OF ALIPHATIC HYDROCARBONS IN ALGAE, BACTERIA, AND IN A RECENT LAKE SEDIMENT: A PRELIMINARY REPORT

Jerry Han, E. D. McCarthy, William Van Hoeven,
Melvin Calvin and W. H. Bradley

November 1967

Submitted to Proceedings of the National Academy of Sciences

UCRL-17975
Preprint

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

ORGANIC GEOCHEMICAL STUDIES. II. THE DISTRIBUTION OF ALIPHATIC HYDROCARBONS IN ALGAE, BACTERIA, AND IN A RECENT LAKE SEDIMENT: A PRELIMINARY REPORT

Jerry Han, E. D. McCarthy, William Van Hoeven,
Melvin Calvin and W. H. Bradley

November 1967

ORGANIC GEOCHEMICAL STUDIES. II. THE DISTRIBUTION OF ALIPHATIC HYDROCARBONS IN ALGAE, BACTERIA, AND IN A RECENT LAKE SEDIMENT: A PRELIMINARY REPORT

Jerry Han, E. D. McCarthy, William Van Hoeven, Melvin Calvin and W. H. Bradley

November 1967

Department of Chemistry, Space Sciences Laboratory, and Laboratory of Chemical Biodynamics, University of California, Berkeley; and U.S. Geological Survey, Washington, D. C.

The theory that algal oozes could give rise to oil shales is not a recent one.[1,2,3] Evidence for this theory rests on the finding that algae have less cellulose and a correspondingly greater proportion of lipids than most plant material. In addition, the contemporary alga Botyrococcus is present in microscopic remains in some organic oozes.[4] Since the algal ooze precursor theory rests primarily on geological and paleobotanical evidence, we have sought to complement this evidence by making a study of the constituents of various genera of algae at the molecular level and comparing them with the organic constituents isolated and identified in the algal ooze from a Florida lake.

We have analyzed the hydrocarbon constituents of four species of algae: the blue-greens, Nostoc and Anacystis, the green algae, Spirogyra and Chlorella.* The general extraction scheme is outlined below:

---

* Unlike the Nostoc, Anacystis and Chlorella, which were cultured in this laboratory, the Spirogyra sample was collected from the shallow water of Mud Lake, Florida, rinsed clean with lake water, and dried over several days and nights at room temperature.

25 grams algae (e.g., Chlorella)

↓ Soxhlet extraction, 12 hrs, 3:1 benzene-methanol

evaporate to dryness

↓

green residue (1.8 g)

↓

dissolve in heptane (10 ml)

residue (for fatty acid analysis)

soluble material

↓ column chromatography with neutral alumina

1. heptane eluate (8 mg) → combined gas chromatography-mass spectrometry analysis of aliphatic hydrocarbons
2. benzene eluate (16 mg)
3. methanol eluate (150 mg)

Table I represents the weight of total heptane eluate obtained as a percentage of the dry cell weight.

TABLE I

Algae	Total Heptane Eluate
Nostoc	0.035%
Anacystis	0.032%
Spirogyra	0.004%
Chlorella	0.032%

The subsequent analysis of the heptane eluate by combined capillary gas chromatography and mass spectrometry revealed the presence of a series of normal hydrocarbons ranging in carbon number from n-C_{15} to n-C_{20}, with the n-C_{17} hydrocarbon being the predominant member for each species. In addition to the normal hydrocarbons, a monounsaturated C_{17} hydrocarbon was isolated from Chlorella, and the branched alkanes, pristane and phytane, isolated from the Spirogyra sample. The relative abundance of the aliphatic hydrocarbons are shown in Table II. The heptane eluate from

(insert Table II here)

the Nostoc sample exhibits only two major components: The n-C_{17} hydrocarbon and a branched C_{18} alkane. This latter component was isolated in pure form by preparative gas chromatography and its mass spectrum is shown in Figure 1. It has been impossible to assign an unequivocal structure to this compound on the basis of mass spectrometry alone. The mass spectrum suggests that it is not related to any of the common hydrocarbon structures that have been characterized in biological sources and may have the structure 7,9-dimethylhexadecane. We are investigating the structure of this compound in order to establish it unequivocally.

An analogous study of the heptane eluate from bacteria indicated the presence of a similar series of hydrocarbons. The marked predominance of the n-C_{17} alkane in algae is less conspicuous in photosynthetic bacteria and does not occur in nonphotosynthetic bacteria and yeast. Furthermore, the higher molecular weight hydrocarbons (> C_{20}) constitute a smaller fraction of the total heptane eluate of the photosynthetic bacteria than of the nonphotosynthetic bacteria. The relative abundance of these hydrocarbons are shown in Table III.

(insert Table III here)

TABLE II

HYDROCARBONS FROM ALGAE

	BLUE-GREEN ALGAE		GREEN ALGAE	
	Nostoc	Anacystis	Spirogyra	Chlorella
n-C_{15}	0.42	28	--	0.7
n-C_{16}	0.42	3.4	6.7	0.4
Pristane	--	--	22	--
Δ-C_{17}	--	4.0	--	450
n-C_{17}	100	100	100	100
branched C_{18}	19.4	0.44	--	--
Phytane	--	--	15.5	--
n-C_{18}	0.5	--	58	0.3
n-C_{19}	0.4	--	62	0.1
n-C_{20}	0.4	--	22	--
higher MW hydrocarbons	no	no	less than 30% of total hydrocarbons	--
major component	n-C_{17}	n-C_{17}	n-C_{17}	Δ-C_{17}

Peak heights are relative to n-C_{17} peak taken as 100

-- indicates less than 0.5 (n-C_{17} = 100)

TABLE III

HYDROCARBONS FROM BACTERIA AND YEAST

	PHOTOSYNTHETIC BACTERIA		NONPHOTOSYNTHETIC BACTERIA		YEAST
	Rhodopseudomonas spheroides	Rhodospirillum rubrum	Micrococcus Lysodeikticus (Anaerobic B)	E. coli (Aerobic B)	
n-C_{15}	2	0.3	112	10	50
n-C_{16}	7	1.7	95	37	60
Pristane	22	3	55	--	
Δ-C_{17}	--	--	--	--	
n-C_{17}	100	100	100	100	100
branched C_{18}	--	--	--	--	
Phytane	3	--	21	--	
n-C_{18}	44	10	58	700	500
n-C_{19}	43	13	147	210	450
n-C_{20}	8.8	9	53	200	1000
higher MW hydrocarbons carbons	less than 5% of total hydro-carbons	less than 15% of total hydro-carbons	more than 50% of total hydro-carbons	more than 60% of total hydro-carbons	more than 60% of total hydro-carbons
major component	n-C_{17}	n-C_{17}	n-C_{19}	n-C_{18}	n-C_{20}

Peak heights are relative to n-C_{17} peak taken as 100

--- indicates less than 0.5 (n-C_{17} = 100)

Bradley[5] has reported the finding of a lake in Florida producing an organic ooze, predominantly algal in character, which he considers to represent the modern analogue of the precursors of rich oil shales, such as the Green River Shale. This organic ooze from Mud Lake, Florida, was analyzed for the aliphatic hydrocarbon content. A sample taken at a depth of two feet below the mud-water interface showed a predominance of n-alkanes in the higher molecular weight region, n-C_{20} to n-C_{33}, particularly the n-C_{27}, n-C_{29} alkane and n-C_{31} alkane, in contrast to our findings from the algae and the photosynthetic bacteria. A capillary gas chromatogram of the aliphatic hydrocarbons from the Mud Lake sample is shown in Figure 2. In addition to the aliphatic hydrocarbons, we have also characterized the carotenoids, β-carotene and xanthophyll, as the dominant pigment constituents of the Mud Lake extract. The identification of perhydro-β-carotene,[6] the fully saturated analogue of β-carotene, in the Green River Formation lends credence to the theory that the Mud Lake deposits may indeed be modern counterparts of rich oil shales, such as the Green River Formation, but at the post-Pleistocene period (the age of the Mud Lake sediment) the transformation of the chemical constituents has not taken place to any significant extent.

Studies carried out by other research groups[7,8,9] have shown the predominance of both n-C_{15} and n-C_{17} hydrocarbons in benthic algae. Oró's study[10] on the aliphatic hydrocarbons in marine and freshwater microorganisms has also shown the predominance of the n-C_{17} in the freshwater blue-green alga, <u>Anacystis nidulans</u>. The findings from our studies on both the blue-green algae and the green algae indicate that the n-C_{17} hydrocarbon is a major constituent of these algae also. The normal hydrocarbons of the rich oil shale from the Green River Formation exhibit a bimodal pattern with a maximum at n-C_{17} and at n-C_{29}, and are further characterized by a predominance of the odd-numbered hydrocarbons over the even-numbered hydrocarbons. The occurrence of the n-C_{17} alkane in the Green River Oil Shale is consistent with the theory that algae, <u>in part</u>, give rise to the organic material of rich oil shale. Although the Florida Mud Lake ooze at the mud-water interface and below for about six inches consists wholly of minute fecal pellets, which are made up exclusively of blue-green algae, there is no evidence for the n-C_{17} alkane being present as a major constituent of the Mud Lake sediment. The total heptane extract from this sediment exhibits a normal alkane distribution characteristic of a recent sediment.[11,12,13] The absence of the n-C_{17} alkane from this predominantly algal ooze remains unexplicable, unless it is a particularly suitable substrate for certain nonphotosynthetic bacteria which might convert it into hydrocarbons in the higher molecular weight region.

A study of the fatty acid content of the same group of organisms (cf. Table II and Table III) has also been carried out via conversion to the methyl esters. The distribution, as so far determined, indicates that the fatty acids of <u>all</u> the organism groups have their dominant molecules among the lower molecular weights (C_{16}-C_{18}), and, therefore, are not likely precursors for the heavier hydrocarbons which dominate the Mud Lake sediment. In fact, it would seem that they are not even precursors for the lighter group of hydrocarbons either by decarboxylation or reduction, a conclusion already hinted at by others.[7,10] On the other hand, Robinson[14] has indicated that lower molecular weight fatty acids may be precursors of the higher molecular weight alkanes.

The occurrence of higher molecular weight hydrocarbons in the Mud Lake sediment might indicate a contribution from higher plant material. There is very little evidence, however, for the theory that higher plants contributed significantly to the hydrocarbons of either the Green River Oil Shale or of the Mud Lake sediment. On the other hand, pollen and spores are found in abundance in the Green River Oil Shale but not extensively in the Mud Lake sediment. A preliminary analysis in this laboratory indicates the presence of $n-C_{23}$ and $n-C_{25}$ hydrocarbons as the major constituents of Ponderosa pine pollen. Nilsson et al.[15] have analyzed the constituents of pollen and have identified long-chain hydrocarbons, including the $n-C_{25}$, $n-C_{27}$ and $n-C_{29}$ alkanes. It is possible that wind-blown pollen contributed significantly to the hydrocarbons of higher molecular weight in these sediments. We are at present investigating this possibility.

This work was supported, in part, by the Natioanl Aeronautics and Space Administration, Grant NsG 101-61, and, in part, by the U.S. Atomic Energy Commission.

REFERENCES

1. Zalessky, M.D. Com. Geol. Bull. 33, 495 (1914); Zalessky, M.D. Soc. Paleont. Russie Annuaire, 1, 25 (1916).

2. Thiessen, R. U.S. Geol. Survey Prof. Paper, 132, 126 (1925).

3. White, D. "Treatise on Sedimentation" (ed. W.H. Twenhofel), pp. 289-304, 1926.

4. Blackburn, K.B. and Temperley, B.N. Trans. Roy. Soc. Edin. 58, 841 (1936).

5. Bradley, W.H. Geol. Soc. Amer. Bull. 77, 1333 (1966).

6. Murray, M.T.J., McCormick, A. and Eglinton, G. Science, 157, 1042 (1967).

7. Clark, Jr., R.C. and Blumer, M. Limnol. and Oceanog. 12, 79 (1967).

8. Clark, Jr., R.C., M.S. Thesis, Massachusetts Institute of Technology, 1966.

9. Heilbron, I.M., Parry, E.G. and Peters, R.F. Biochem. J. 29, 1376 (1935).

10. Oró, J., Tornabene, T.G., Nooner, D.W. and Gelpi, E. J. Bacteriol. 93, 1811 (1967).

11. Evans, E.D., Kenny, G.S., Meinschein, W.G. and Bray, E.E. Anal. Chem. 29, 1858 (1957).

12. Kvennolden, K.A. Bull. Amer. Assoc. Petrol. Geol. 46, 1643 (1962).

13. Stevens, N.P., Bray, E.E. and Evans, E.D., ibid., 40, 975 (1956).

14. Robinson, R. Nature, 214, 263 (1967).

15. Nilsson, M., Ryhage, R. and Von Sydow, E. Acta Chem. Scan. 11, 634 (1957).

FIGURE CAPTIONS

Figure 1 Mass spectrum of branched C_{18} alkane from Nostoc

Figure 2 Gas chromatograph of aliphatic hydrocarbons from Mud Lake algal ooze

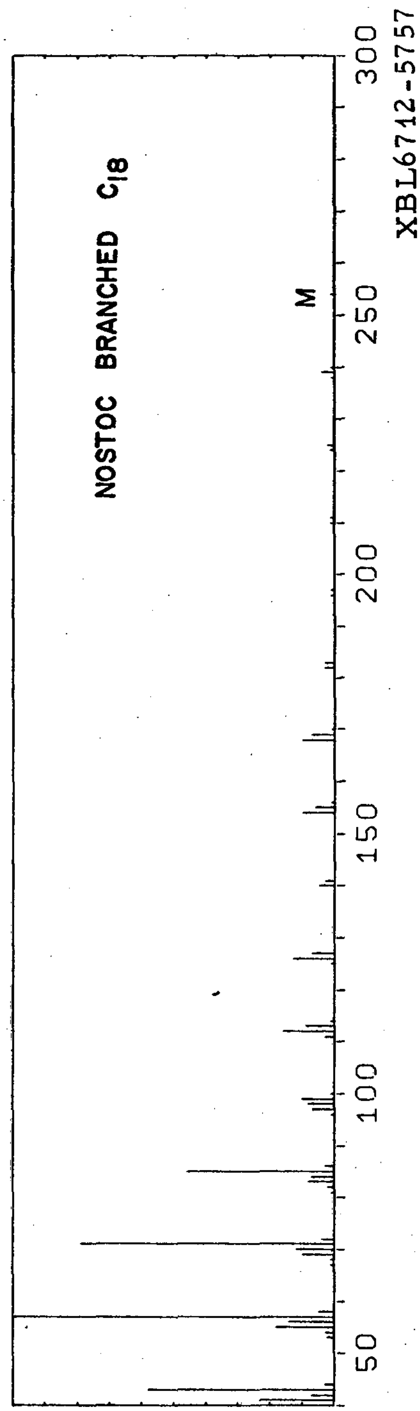

Fig. 1

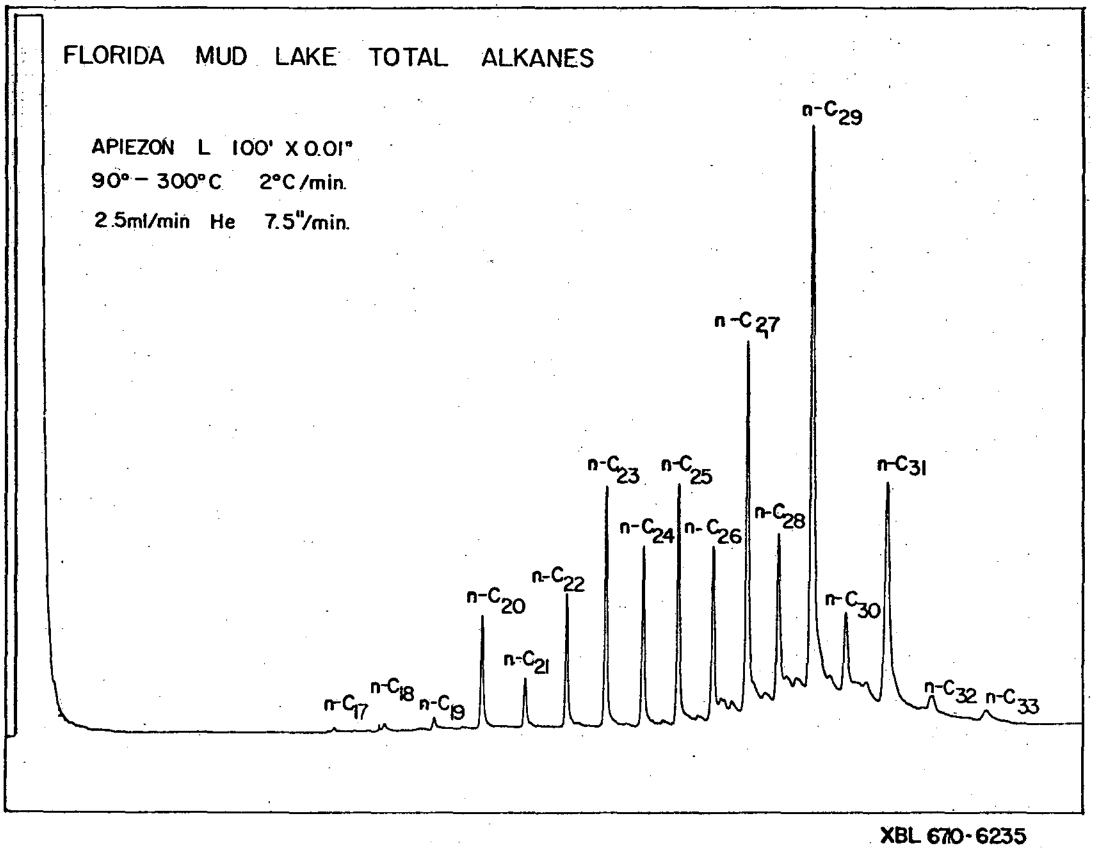
FLORIDA MUD LAKE TOTAL ALKANES
APIEZON L 100' X 0.01"
90° – 300°C 2°C/min.
2.5ml/min He 7.5"/min.
n-C29
n-C27
n-C23
n-C25
n-C31
n-C24
n-C26
n-C28
n-C22
n-C20
n-C30
n-C21
n-C18
n-C17
n-C19
n-C32
n-C33
XBL 670-6235

Fig. 2