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HYDROCARBONS OF GEOCHEMICAL SIGNIFICANCE IN MICROSCOPIC ALGAE*

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Abstract—Recent investigations have implicated a correlation between the hydrocarbon composition of algae and that found in geological sediments. This report presents information on hydrocarbon production by twelve species of algae, morphologically similar to fossil forms, including *Anacystis cyanea*, *A. montana*, *Spirulina platensis*, *Lyngbya aestuarii*, *Chroococcus turgidus*, *Chlorella pyrenoidosa*, *Coelastrum microsporum*, *Tetraedron* sp. and *Scenedesmus quadricauda*, along with confirmatory data on *Anacystis nidulans*, *Nostoc* sp. and *Botryococcus braunii* which have previously been reported. The normal hydrocarbon range is from C_{15} to C_{19} in most species, and n-C_{17} is predominant in all cultures. However, a few of the species show a bimodal distribution of aliphatic hydrocarbons with maxima at C_{17} and C_{27}, with significant amounts of C_{23}, C_{27}, and C_{29} straight-chain olefins and paraffins. Similar bimodal distributions of saturated hydrocarbons have been observed in both tertiary and Precambrian sediments, supporting the interpretation of biological origin. The production of methyl-substituted alkanes by blue-green algae and a tentative identification of a triterpene from three species is also reported. Squalene is the only isoprenoid reported.

INTRODUCTION

THE GEOLOGICAL formations of the earth contain morphological (fossil structures of ancient organisms) and molecular (chemical fossils) records of ancient life. The study of these records can provide an insight into the origin and evolutionary history of the first living organisms.

Although fossils have been observed since Greek times, they were not studied in a systematic manner until the science of paleontology came into being around the eighteenth century. Paleontologists were able to find well-defined morphological remains[1-3] within a geological period that started about 600 million years ago, at the start of the Precambrian. Later micropaleontologists and paleobiologists have been able to extend this period well into the Precambrian with the identification of microscopic-sized remains of simple morphological entities.[4,5] Fossilized algae have been reported present in both recent and ancient sediments.[1-9] Precambrian deposits have been shown to contain a number of cyanophycean (blue-green) algae. These organisms, closely related to bacteria, are characterized by

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[1] M. H. KAHN, *Micropaleontology* **8**, 385 (1962).
[2] P. H. ABELSON, *Scient. Am.* **195**, 83 (1956).
[3] W. H. BRADLEY, *Bull. Torrey Botan. Club* **56**, 421 (1929).
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[5] A. E. J. ENGEL, B. NAGY, L. A. NAGY, C. G. ENGEL, G. O. KREMP and C. M. DREW, *Science* **161**, 1005 (1968).
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[7] K. MATHUR, *Sci. Cult.* **30**, 607 (1963).
[8] P. ECHLIN, *Scient. Am.* **214**, 75 (1966).
[9] K. R. NEWMAN, *The Mountain Geol.* **2**, 79 (1966).

prokaryotic cells and have, therefore, been designated as one of the earliest forms of terrestrial life.[4, 8, 10] Tertiary sediments, including the Green River shale, the Waltman shale and the olive-green shales belonging to the Sabathu series of Himachal Pradesh, India,[7] contain fossils of more advanced eukaryotic algae.

Moderate amounts of aliphatic hydrocarbons and fatty acids have been shown to be among the lipids of several species of contemporary algae.[10-16] Also, the organic geochemical studies carried out on a number of sediments from the Precambrian[17-21] and more recent tertiary sediments[17] have revealed the existence of small amounts of hydrocarbons and fatty acids trapped within the rock matrix. Thus to better complement the chemical and morphological criteria we must gain a good knowledge of the hydrocarbon and lipid composition of the contemporary forms of the ancient microflora, found by the paleobiologist in ancient as well as in recent sediments. As an integral phase of these studies[11-14, 17-21] the present report discusses the analysis and distribution of the hydrocarbons produced by twelve contemporary relatives of these fossilized algae.

RESULTS

Tables 1 and 2 summarize the data for the hydrocarbon composition of the twelve species of algae analyzed. The hydrocarbon compositions in both tables show that the n-C_{17} alkane is a predominant constituent of most of the algae.[11-14, 16] Ten of the twelve species have n-C_{17} as a major hydrocarbon and four show high molecular weight hydrocarbons as a major component. All identifications of the hydrocarbons reported here have been verified by mass spectrometry.

The results of the gas chromatographic–mass spectrometric analyses show some rather unusual features such as the presence of 2-, 7- and 8-monomethyl alkanes; the high molecular weight monoenes, dienes, and trienes previously reported[14] and the presence in three of the blue-green algae of an isomer of squalene with the structural characteristics of a polycyclic triterpene. Squalene has also been identified in the benzene fraction of two of these algae (Table 2).

The mass spectra of the two mixtures of monomethyl alkanes are shown in Fig. 1B and C. The presence of a small amount of an equimolecular mixture of 7-methyl and 8-methylheptadecane in *Nostoc* has already been reported by Han *et al.*[22] Their compound gave a mass spectrum consistent with a 7,9-dimethyl hexadecane structure[13] (major fragments at C_8, C_9, C_{11}, C_{12} and parent ion at C_{18}). However, the synthesis of the pure diastereoisomers

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TABLE 1. HYDROCARBONS FROM CONTEMPORARY REPRESENTATIVES OF ALGAE FOUND IN SEDIMENTS

Division†	Organism	Sediment	Alkane Hydrocarbons	
			Range	Major
Chlorophycophyta	*Coelastrum microsporum*	Green River	C_{17}	C_{17}
	Chlorella pyrenoidosa	Bitter Springs	C_{17}	ΔC_{17}
	Scenedesmus quadricauda	—	C_{17}–ΔC_{27}	C_{17}, ΔC_{27}
	Tetraedron sp.	Green River	C_{15}–C_{27}	C_{17}, C_{23}
Cyanophycophyta	*Anacystis cyanea*	Bitter Springs	C_{17}, $7MeC_{17}$, $8MeC_{17}$	C_{17}
	Anacystis nidulans	—	C_{15}–C_{18}	C_{17}
	Spirulina platensis	Bitter Springs	C_{15}–C_{17}	C_{17}
	Lyngbya aestuarii	Bitter Springs	C_{15}–C_{18}*	C_{17}
	Nostoc sp.	—	C_{15}–C_{18}*	C_{17}
	Chroococcus turgidus	Green River	C_{16}–C_{19}*	C_{17}*
	Anacystis montana	Bitter Springs	C_{17}–C_{29}	ΔC_{25}, ΔC_{27}
Chrysophycophyta	*Botryococcus braunii*‡	Tertiary	ΔC_{17}–$2\Delta C_{33}$	$2\Delta C_{29}$, $2\Delta C_{31}$

* Compound with mass spectrometric fragmentation pattern corresponding to a polycyclic triterpenoid with empirical formula $C_{30}H_{50}$.

† Chlorophycophyta: Green algae. Cyanophycophyta: Blue-green algae. Chrysophycophyta: Golden brown alga.

‡ Some authors classify this organism in the Chlorophycophyta.

TABLE 2. RELATIVE PERCENT HYDROCARBON COMPOSITION OF SEVERAL MICROSCOPIC ALGAE†

	Chlorophycophyta				Cyanophycophyta							Chryso-phycophyta
Hydrocarbon	Coel. micro.	Chlor. pyr.	Scen. quad.	Tetr. sp.	Ana. cyan.	Ana. nid.	Spir. plat.	Lyng. aest.	Nost. sp.	Chroo. turg.	Ana. mont.	Boty. brau.
C_{15}				1		23	10	2	3			
C_{16}						8	20	6	4	3		
$6Me + 7MeC_{16}$										1·0		
C_{17}	100	18·5	26	30	87	44	70	35	48	32	11·5	
ΔC_{17}		76·9	0·6			20	<1					1·5
$7Me + 8MeC_{17}$					13			38	27	22		
C_{18}						2		1·4		3		
$2MeC_{18}$										<1		
C_{19}			6·8							2		
2Δ-C_{21}											8·9	
C_{23}				40								
ΔC_{23}											8·0	0·14
C_{24}				2·6								
C_{25}				20·0								
Δ-C_{25}											14·6	0·65
Δ-C_{26}											3·8	
C_{27}				5·9								
Δ-C_{27}			43·2								34·7	
$2\Delta C_{27}$											2·8	11·1
2Δ-C_{29}												50·4
3Δ-C_{29}												5·5
2Δ-C_{31}												27·9
2Δ-C_{33}												2·0
*								16	10	38		
Squalene‡										‡		‡

* Compound with mass spectrometric fragmentation pattern corresponding to a polycyclic triterpenoid structure with empirical formula $C_{30}H_{50}$.

† Differences to 100 made up by unidentified compounds. Species as in Table 1.

‡ In benzene fraction.

of 7,9-dimethyl hexadecane followed by capillary coinjection techniques[23] showed that this was not the proper structure. A synthetic mixture of the two methyl heptadecanes demonstrated that the single chromatographic peak actually consisted of a mixture of the two alkanes.

We have shown that this is also the case in some of our samples by a different technique. Although these alkanes were not well separated by GLC columns, repetitive scans of the chromatographic peak in the ascending and descending slopes showed very clearly that we were not dealing with a single compound but with a mixture of two isomeric C_{18} alkanes.

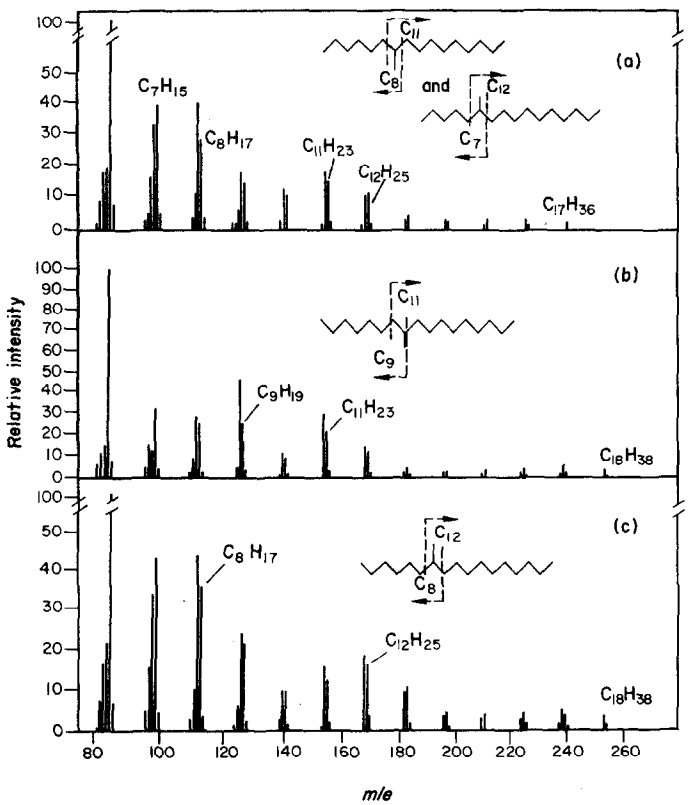

FIG. 1. MASS SPECTRA OF METHYL ALKANES OF *Chroococcus turgidus*.
Taken as they emerged from a 155 m × 0·076 mm I.D. capillary gas chromatographic column coated with Igepal Co-990 (see text for details).

The fragments at C_9 and C_{11} predominate over the C_8 and C_{12} fragments in Fig. 1B, which is representative of a mass spectrum taken on the upward slope. On the other hand, the C_8 and C_{12} fragments were found to show an increase in relative intensity along the downward slope of the GC peak, Fig. 1C. The mass spectral pattern of Fig. 1B is consistent with that of a mixture of 8-methyl and 7-methyl heptadecanes in which the first predominates. The same is true of the pattern in Fig. 1C but with the second compound predominating. Also, the relatively high intensity of the C_7 and C_{13} ions in Fig. 1C appears to indicate the presence of smaller amounts of the 6-methyl heptadecane.

[23] J. HAN, E. D. MCCARTHY, M. CALVIN and M. H. BENN, *J. Chem. Soc.* (C) 2785 (1968).

The order of elution of these methyl alkanes would be, first the 8-methyl heptadecane followed by the 7-methyl heptadecane and the 6-methyl heptadecane. This order is consistent with the apparent distribution of these compounds within the GLC peak. Also, their retention times correspond to those of the monomethylated compounds. The dimethyl substituted isomers being more volatile would be eluted in front of them.

All this also applies to the mass spectrometric pattern shown in Fig. 1A, but in this case repetitive scanning of the peak was not possible due to its small concentration in the sample.

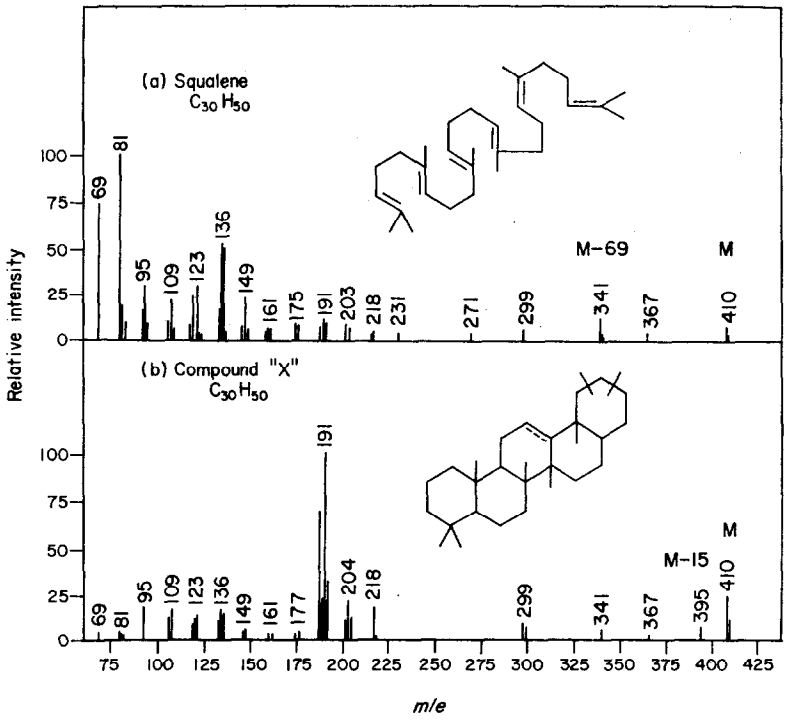

FIG. 2. MASS SPECTRA OF SQUALENE AND COMPOUND X IN *Chroococcus turgidus*.

Taken as they emerged from a 1·7 m × 3 mm I.D. glass column packed with OV-1 (see text for details).

The partial mass spectrum of the compound identified as squalene is shown in Fig. 2A. Aside from the molecular ion at *m/e* 410 ($C_{30}H_{50}$) the most characteristic features are, the base peak at *m/e* 81, the relatively intense doublet at *m/e* 136 and 137, and the M-69 and M-43 fragments.[24]

Of particular interest is the mass spectrum of compound X (Fig. 2B) which shows a fragmentation pattern consistent with that of a polycyclic triterpenoid structure of empirical formula $C_{30}H_{50}$. The base peak at *m/e* 191 is a characteristic fragment in the mass spectra of pentacyclic triterpenes.[25] This is also supported by other ions, especially those at *m/e* 218,

[24] T. G. TORNABENE, M. KATES, E. GELPI and J. ORÓ, *J. Lipid Res.* **10**, 294 (1969).
[25] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

204, 205, 136, 123, 109. The strong molecular ion at 410 also reflects the higher stability inherent in any polycyclic structure. Presumably the structure of this compound might be closely related to that of gammacerane. Hydrogenation of the double bond and comparison of the mass spectra of the hydrogenated product with that of authentic gammacerane should prove whether this is the case. The position of the double bond is only tentatively indicated in Fig. 2B. Its location within the molecule might have a significant influence on the fragmentation pattern since for instance in $\Delta^{13(18)}$-oleanene and $\Delta^{12(13)}$-oleanene, the base peak in the spectra changes from *m/e* 205 to *m/e* 218. The position of the methyl substitutents (Fig. 2B) for ring E is also doubtful at present.

In terms of the hydrocarbon distribution the algae examined can be separated into two different groups without regard to the phylogenetic classification: those with low molecular weight hydrocarbons only and those showing significant amounts of high molecular weight hydrocarbons (Tables 1 and 2).

The hydrocarbon compositions of all the algae investigated in our laboratory are given in Table 2. *Coelastrum microsporum* and *Chlorella pyrenoidosa* both show the C_{17} hydrocarbon either saturated or unsaturated as the only major component. In the case of *Chlorella*, traces of a C_{17} diene and a C_{18} monoene were also identified from their respective mass spectra. *Spirulina platensis* contains *n*-C_{17} alkane as the major component. Traces of the C_{17} olefin have also been detected and the *n*-C_{15} and *n*-C_{16} alkanes are particularly abundant, comprising 30 per cent of the total hydrocarbon fraction. *Anacystis nidulans*[11] shows a very similar pattern. *A. cyanea* also contains *n*-C_{17} as the major hydrocarbon; the smaller component which accounts for 13 per cent of the total aliphatic hydrocarbons has been identified as a mixture of the 7-methyl, and 8-methyl heptadecanes. *Pediastrum* sp. which is not included in Table 2 has also been analyzed. Its hydrocarbon pattern is similar to that of *Coelastrum microsporum*. The major components are *n*-heptadecane and *n*-heptadecene. However, there are also minor high molecular weight components whose identities have not been studied by mass spectrometry at this time.

The hydrocarbon patterns for *Lyngbya aestuarii*, *Nostoc* sp. and *Chroococcus turgidus* exhibit a great deal of similarity in their distributions. All three of these cyanophycean algae have an *n*-C_{17} along with substantial amounts of the mixture of 7-methyl and 8-methyl heptadecanes and of compound X. In addition, *C. turgidus* shows trace amounts of an isoC_{19} alkane and possibly of the corresponding 6-methyl plus 7-methyl hexadecane mixture (see Fig. 1). Squalene was also found (Fig. 2A) but its relative concentration has not been calculated.

A particularly interesting observation made in this study is the relatively large amounts of long-chain hydrocarbons[14, 26] in a number of the organisms examined. *Scenedesmus quadricauda*, besides the typical *n*-C_{17} peak, shows a prominent peak corresponding to a C_{27} monoene. Smaller amounts of the C_{19} paraffin and other hydrocarbons in the C_{16}–C_{31} range also seem to be present but, with the exception of the C_{19}, their structures have not been verified mass spectrometrically. *Tetraedron* sp. similarly displays the common *n*-C_{17} plus a sharp distribution of higher hydrocarbons which peaks at the *n*-C_{23} alkane. The cyanophycean alga *Anacystis montana*[14] and the chrysophycean alga *Botryococcus braunii*[14] show a predominance of odd/even carbon chains with two well-defined distributions of medium and high molecular weight hydrocarbons. *A. montana* shows maxima at C_{17} and C_{27} and *B. braunii* at C_{17} and C_{29}.

[26] G. W. PATTERSON, *J. Phycol.* 3, 22 (1967). High molecular weight hydrocarbons have also been reported by this author in autotrophically grown *Chlorella vulgaris*.

DISCUSSION

Phylogenetic Considerations

There has been a tendency in the literature[14, 15, 27] to correlate molecular complexity with evolutionary development, with more complex molecules representing advanced phylogeny. Such interpretations must be considered suspect, however, when one takes into account the inconsistencies in distributions found in the hydrocarbons. If one considers the prokaryotic cyanophycean algae *Anacystis cyanea, Spirulina platensis, Lyngbya aestuarii* and *A. montana*, the first three exhibit simple hydrocarbon distribution whereas *A. montana* gives a pattern similar to higher plants.[14] This would imply that *A. montana* is a transitional organism. However, *Chlorella pyrenoidosa*, an eukaryotic chlorophycean alga, displays a very simple hydrocarbon pattern, as do the chlorophycean algae *Coelastrum microsporum, S. obliquus* and *Pediastrum* sp. One is hard pressed to make phylogenetic conclusions solely on the basis of hydrocarbon distribution in the light of such discrepancies. Phylogenetic comparisons appear to be justified only within a limited taxonomic grouping.

Geochemical Significance

The cyanophycean algae *A. cyanea, S. platensis, L. aestuarii* and *A. montana* have been implicated in Precambrian sediments. Their hydrocarbon distribution ranges from the *n*-C_{17} in *A. cyanea* to the bimodal distribution of medium and high molecular weight hydrocarbons as seen in *A. montana*.[14] The possibility of these organisms contributing to the complex patterns of normal and isomeric alkanes found in Precambrian sediments has been discussed.[14] Such a possibility is particularly attractive in view of the paleobiological evidence surrounding the presence of ancient counterparts of these organisms in Precambrian sediments.[4, 5] Furthermore, the proposed biological origin of these hydrocarbons is reinforced by the presence of isoprenoid hydrocarbons in the sediments which can be easily explained as the diagenetic products of algal pigments of carotenoid and xanthopyll nature and of algal chlorophylls.

Along the same line the green algae *C. microsporum, Tetraedron* sp. and *Pediastrum* sp., the blue-green alga *Chroococcus turgidus* and the golden brown algae *Botryococcus braunii* have all been implicated in tertiary sediments.[3, 6, 7, 9] Again, these species represent a range in complexity from the single *n*-C_{17} component in *Coelastrum microsporum* and *Pediastrum* sp. to the more complex distributions in *Tetraedron* sp. and *B. braunii*.*

The aliphatic hydrocarbon distribution of one of these tertiary sediments, the Green River shale, displays a pattern which can be easily correlated to the hydrocarbon pattern of *B. braunii*.[14] Although this alga is not listed among the species found in this particular shale[3] it has more recently been observed in swamp sediments of the lowest parts of the Green River formation.[9] Likewise, *Tetraedron* sp. with its distribution of high molecular weight alkanes could also play a major role in the deposition of sedimentary hydrocarbons. The marked odd/even predominance typical of the algal distributions finds its counterpart in the hydrocarbon distributions of recent sediments.[17] The gradual disappearance of such a predominance with the increasing age of the sediment (Precambrian sediments) has been explained as

* After this paper was submitted we learned of the recent work on *Botryococcus braunii* by A. C. BROWN, B. A. KNIGHT and E. CONWAY, *Phytochem.* **8**, 543 (1969). As stated in their paper their results are in close agreement with our previous report.[14]

[27] T. W. GOODWIN, Proc. of the Fifth Inter. Congress of Biochemistry, Moscow, p. 307 (1961).

a consequence of the longer time allowed for diagenetic changes of the originally deposited organic matter.[14]

The attempted morphological and geochemical correlations, based before almost exclusively on the nature and distributions of the normal hydrocarbons, can be developed now along new lines of evidence. As our knowledge of the algal lipids increases it is becoming clear that the extreme morphological simplicity exhibited by most species of algae is not readily reflected in the simplicity of their lipid constituents. However, it should be pointed out that culturing conditions can play a significant role in the observed distributions of algal lipids,[10, 26] especially in heterotrophic micro-organisms.

In any case, the results of the present study seem to indicate that these algae are capable of biosynthesizing a more complex array of organic compounds than was previously thought. Examples are the methylalkanes, the squalene and the triterpenoid structure indicated on Tables 1 and 2. Recent literature has also shown the presence of steroids in various species of algae.[28, 29]

Although it has been found in very small amounts, it is important to evaluate the presence of the isoC$_{19}$ hydrocarbon in algae against the widespread occurrence of iso and anteiso alkanes in petroleum crudes, oil shales, and Precambrian sediments.

Our positive identification of squalene in two species of algae, coupled with the recent reports on the identification of steroids[29] in blue-greens, demonstrates that this particular channel of the isoprenoid biosynthetic pathways is active in the contemporary algae. With this in mind, the possible presence of polycyclic triterpenes in some species of algae would not be too surprising. Thus, the triterpenoid structure tentatively identified in three blue-green algae (compound X) could be directly derived from squalene. Squalene has not been reported in sediments but there is tentative gas chromatographic evidence of the presence of its fully saturated counterpart, squalane, in petroleum.[30] Also, polycyclic triterpanes, as well as steranes, have been found in petroleum, oil shales, and sediments.[31] Again reductive diagenesis could account for the saturation of squalane to squalane, the steranes to steranes and the triterpenes to triterpanes.

Concerning the mixture of 7-methyl and 8-methyl heptadecanes, their presence in algal extracts constitutes a unique example of their occurrence in nature. This mixture, first detected by Han *et al.*,[13] in an extract of *Nostoc* sp., was reported as a minor component not amounting to more than 19 per cent of the heptadecane. However, in our case (see Table 2) the same mixture accounts for a substantial proportion of the total amount of hydrocarbons in this genus. To establish a comparison, the culture of *Nostoc* sp. grown in our laboratory contains an amount of the two, possibly three, methyl alkanes close to 50 per cent of the C$_{17}$ peak. This variation could simply reflect, as indicated before, different growth parameters.[26] At present it is hard to attach any geochemical significance to these internally methyl substituted hydrocarbons since there are no reports of their presence in geological samples. However, from our particular experience in the high resolution GLC of hydrocarbon mixtures, we feel that the lack of reports may simply be a consequence of the difficulties involved in their proper isolation and identification. Petroleum crudes have been shown to contain methyl alkanes ranging progressively from 2-methyl to the more internally substituted 5-methyl

[28] R. C. REITZ and J. G. HAMILTON, *Comp. Biochem. Physiol.* **25**, 401 (1968).
[29] N. J. DESOUZA and W. R. NES, *Science* **162**, 363 (1968).
[30] I. R. HILLS and E. V. WHITEHEAD, Int. Meeting Org. Geochem., London, Sept. 26–28, 1966.
[31] I. R. HILLS and E. V. WHITEHEAD, Symp. on Hydrocarbons from Living Organisms and Recent Sediments. Div. Petroleum Chem. ACS, Atlantic City, Sept. 8–13, 1968.

alkanes.[31] This is also true in meteorite extracts,[32] Precambrian sediments[33] and Fischer Tropsch products.[34]

Considering now the overall pattern of all of the organisms analyzed, perhaps the most salient feature common to all of them is the high amounts of the n-C_{17} hydrocarbon (see Table 2). It would be expected that in general the contribution of algae to the hydrocarbons in geological samples would be most strongly felt in the C_{17} content of these samples. Although with time, and due to its higher vapor pressure relative to the heavier hydrocarbons, C_{17} would be preferentially lost. Thus the amount of C_{17} and other lighter hydrocarbons would decrease with time in relation to the higher molecular weight hydrocarbons. Eventually this could lead to a shift of the distribution mode towards the latter.

The data presented here clearly shows that algae of different taxonomic classifications produce significant amounts of paraffins and olefins in both the medium and high molecular weight ranges, and that some contain methyl branched hydrocarbons as well as acyclic and cyclic triterpenes. The majority of these organisms have been implicated in ancient and/or recent sediments.

EXPERIMENTAL

Unigal cultures were obtained from culture collections at the University of Indiana or Cambridge University. Verification of all cultures was made using phase microscopy. Two media were selected to provide for optimal growth. *Botryococcus braunii* and all cyanophycean algae were grown in a modification of Kratz and Myers media.[35] Chlorophycean algae were grown in a modification of the *Scenedesmus* medium of Trainor and Hilton.[36] The composition of these media is listed in Table 3. Each organism was grown by aseptic transfer into 200 ml batch cultures in 38 × 300 mm test-tubes. Each test-tube contained a bubbling tube connected to a manifold system which provided for aeration by 1% CO_2 in air at an approximate rate of 25 ml/min tube. The aeration system employed cotton filtration at several levels to eliminate contamination and introduction of extraneous materials. Illumination was provided by 30 lux cool white fluorescent light banks placed at a distance of 10 cm from the test-tubes. Growth runs were conducted in thermostatted water baths, with Cyanophycean algae grown at 35 ± 0·5° and other cultures at 25 ± 0·5°. Duration of growth, depending upon species and size of the inoculum, varied between 1 and 2 weeks. Sufficient replicate samples were run to provide for 0·5 to 1·0 g dry weight of cells. Second-stage microscopic examination was performed at the end of the growth runs, verifying typical morphology and pigmentation as well as a bacterial level of contamination between trace and void. Algal cultures were harvested by centrifugation at 10,000 *g* for 10 min. The algal pellet was dried over P_2O_5 under vacuum and the dry weight determined.

Dried cells were extracted with 50 ml hot benzene–$CHCl_3$ (3:1 v/v) for 40 min with constant stirring. The extract was subsequently evaporated to near dryness at 40° under purified N_2. Fractionation of the residue was conducted on a column of heat-activated (410° for 24 hr) silica gel (1 × 18 cm) prewashed with 30 ml n-heptane. Three fractions were extracted: n-heptane fraction containing aliphatic hydrocarbons; benzene fraction containing aromatic compounds; and MeOH fractions containing free fatty acids, glycerides, and other lipids.

The n-heptane or aliphatic hydrocarbon fraction was concentrated by evaporation under N_2 and analyzed by GLC using a Barber–Colman 5000 gas chromatograph equipped with a hydrogen flame-ionization detector, and combined gas chromatography–mass spectrometry. Two different gas chromatographic systems were employed for the separation of hydrocarbons: (*a*) separation on a glass column (1·7 m × 3 mm I.D.) packed with 1% OV-1 (methyl silicone) on 100–120 mesh Gas Chrom G, N_2 pressure of 703 g/cm², battery setting of $x1$ and attenuation of 10 which allowed a relatively fast scan of high molecular weight compounds and (*b*) separation on a stainless-steel capillary column (155 m × 0·076 mm I.D.) coated with Igepal CO-990, N_2 pressure of 933 g/cm², battery setting of $x1$ and attenuation of 10, which gave the resolution necessary to achieve a satisfactory separation of closely related isomers.

The gas chromatographic–mass spectrometric analyses were carried out on an LKB 9000 gas chromatograph–mass spectrometer. The same columns described above were used for the combination analyses. Dried

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[34] E. Gelpi, Ph.D. Dissertation, University of Houston, Houston, Texas (1968).
[35] W. A. Kratz and J. Myers, *Am. J. Botany* **42**, 282 (1955).
[36] F. Trainor and R. Hitton, *Bull. Torrey Botan. Club* **90** 407 (1963).

cell weights extracted ranged from 0·2 to 10 g, aliphatic hydrocarbon content ranged from 52 ppm in *Anacystis nidulans* to 3000 ppm in *B. braunii*.

TABLE 3. MEDIA

A. Kratz and Myers media

Component	Concentration (g/l.)
KNO_3	4·0
K_2HPO_4	1·0
$MgSO_4.7H_2O$	0·25
Na citrate	0·165
$Ca(NO_3)_2.4H_2O$	0·025
$Fe_2(SO_4)_3.6H_2O$	0·004
Plus 1 ml Hutner's A-5 microelement solution pH adjusted to 7·6	

B. Scenedesmus medium

Component	Concentration (g/l.)
NH_4NO_3	0·3
$MgSO_4.7H_2O$	0·3
K_2HPO_4	0·1
KH_2PO_4	0·1
$CaCl_2$	0·04
$FeCl_3.6H_2O$	0·01
Na citrate	0·5
Plus 1 ml Hutner's A-5 microelement solution pH adjusted to 7·2	

C. Hutner's A-5 microelement solution

Component	Concentration (g/l.)
H_3BO_3	2·86
$MnCl_2.4H_2O$	1·81
$ZnSO_4.7H_2O$	0·222
$CuSO_4.5H_2O$	0·079
MoO_3 (85%)	0·0177