Phytochemistry, 1970, Vol. 9, pp. 613 to 617. Pergamon Press. Printed in England.

FATTY ACIDS OF GEOCHEMICAL SIGNIFICANCE IN MICROSCOPIC ALGAE*

H. SCHNEIDER, E. GELPI,*† E. O. BENNETT and J. ORÓ

Departments of Biophysical Sciences and Biology, University of Houston, Houston, Texas 77004, U.S.A.

(*Received* 18 *April* 1969, *in revised form* 20 *August* 1969)

Abstract—Fatty acids ranging from 14:0 to 18:4 were found in eleven different species of green and blue-green algae. In general, the unsaturated C_{18} fatty acids predominate in all the species of green algae analyzed while with some exceptions the C_{16} saturated acids are predominant in the blue-green algae. By the nature of their gas chromatographic patterns, there appears to be no direct correlation between the fatty acids and the hydrocarbons within the same species of algae. The fatty acids are distributed in a narrower molecular weight range (C_{14}–C_{18}) than the hydrocarbons (C_{15}–C_{33}) and do not show any significant qualitative variations between species. All assignments of structural identities are supported by gas chromatographic and mass spectrometric data.

INTRODUCTION

DIFFERENT extant forms of ancient algae have been shown to contain hydrocarbon distributions similar to those found in sediments (see previous paper[1] for references). These same algae usually contain considerably greater amounts of fatty acids than aliphatic hydrocarbons. However, contrary to the hydrocarbons, these fatty acids are without exception more restricted in their molecular weight range.[2–5]

The geological significance of fatty acids in general has been discussed,[4,6] as has been their possible relationship to hydrocarbon production in sediments and in petroleum.[6,9] Algal fatty acids in particular, whether derived from either prokaryots or eukaryots, do not differ much in their distribution or relative amounts. It has been shown that changing environmental conditions will alter the fatty acid distribution of micro-organisms markedly;[9,10] however, these environmental changes do not seem to affect the molecular weight range.

Although the fatty acid compositions of several blue-green algae are rather simple in terms of their molecular structure, variations among different species have been noted.

* Part II in the series "Lipids of Geochemical Significance in Microscopic Algae", For Part I see *Phytochem.* 9, 603 (1970).

† Present address: Space Sciences Laboratory, University of California, Berkeley, California 94720.

[1] E. GELPI, H. SCHNEIDER, J. MANN and J. ORÓ, *Phytochem.* **9**, 603 (1970).
[2] R. W. HOLTON, H. H. BLECKER and T. S. STEVENS, *Science* **160**, 545 (1968).
[3] J. ORÓ, T. G. TORNABENE, D. W. NOONER and E. GELPI, *J. Bacteriol.* **93**, 1811 (1967).
[4] P. L. PARKER, C. VAN BAALEN and L. MAURER, *Science* **155**, 707 (1967).
[5] H. SCHLENK, H. K. MANGOLD, J. L. GELLERMAN, W. E. LINK, R. A. MORRISSETTE, R. T. HOLMAN and HAYES, *J. Am. Oil Chem. Soc.* **37**, 547 (1960).
[6] K. A. KVENVOLDEN, *J. Am. Oil Chem. Soc.* **44**, 628 (1967).
[7] J. E. COOPER and E. E. BRAY, *Geochim. Cosmochim. Acta* **27**, 1113 (1963).
[8] J. HAN, E. D. MCCARTHY, W. VAN HOEVEN, M. CALVIN and W. H. BRADLEY, *Proc. Nat* 29 (1968).
[9] R. W. HOLTON, H. H. BLECKER and M. ONORE, *Phytochem.* **3**, 595 (1964).
[10] T. G. TORNABENE, E. O. BENNET and J. ORÓ, *J. Bacteriol.* **94**, 344 (1967).

Also, a certain degree of correlation has been shown to exist between the degree of unsaturation of fatty acids in blue-green algae and their morphological complexity.[2] However, no such correlations apply to the aliphatic hydrocarbons.

Well-characterized fossil algae have been shown to be present in relatively large quantities in certain recent sediments.[11–13] Likewise, ancient sediments have been shown to contain different microscopic bodies whose morphology is analogous to that of several present-day species of blue-green and green algae.[14, 15] To better understand the possible relationship of fatty acids to hydrocarbons as well as the origin of some of the lipids in sediments, the fatty acids of contemporary counterparts of several fossil algae have been studied in our laboratory.

RESULTS

Table 1 gives the fatty acid ranges of the algae analyzed (see Table 1 in previous paper[1] for the sediments in which the fossil forms are found). The widest range is found in the green

TABLE 1. FATTY ACIDS FROM CONTEMPORARY REPRESENTATIVES OF ALGAE FOUND IN SEDIMENTS*

Organism	Fatty acid Me esters	
	Range	Major
Coelastrum microsporum	14:0–18:4	18:1
Chlorella pyrenoidosa	14:0–18:3	18:3
Scenedesmus quadricauda	16:0–18:3	18:3
Tetraedron sp.	16:1–18:4	18:1
Anacystis cyanea	15:0–18:3	16:0, 16:1
A. nidulans	14:0–18:1	16:0, 16:1
Spirulina platensis	14:0–18:3	16:0
Lyngbya aestuarrii	16:0–18:3	16:0
Nostoc sp.	14:0–18:3	16:0
Chroococcus turgidus	16:0–18:3	16:0, 18:3
Anacystis montana	16:0–18:3	18:1

* Refer to Table 2 in the preceeding paper for classification by genera and occurrence in sediments.

algae *Coelastrum microsporum*, 14:0 to 18:4. All of the algae analyzed fall within this range. Table 1 also shows the predominant fatty acids of each organism. In general, the green algae have a C_{18} unsaturate as their major fatty acid, while all of the blue-green algae, except for *Anacystis montana*, have either a 16:0 or 16:1 as their major component. The relative percentage composition for each individual fatty acid is given in Table 2.

All patterns appear similar and are in close agreement with other data on micro-organisms presented in the literature.[2–5, 8, 9, 16] Fatty acid distributions of a number of cyanophycean algae have been reported.[2, 4, 8] The major variations in the fatty acid patterns relate to the

[11] W. H. BRADLEY, *Bull. Torrey Botan. Club* **56**, 421 (1929).
[12] A. TRAVERSE, *Micropaleontology* **1**, No. 4, 343 (1955).
[13] K. R. NEWMAN, *The Mountain Geol.* **2**, 79 (1966).
[14] E. S. BARGHOORN and J. W. SCHOPF, *Science* **152**, 758 (1966).
[15] A. E. J. ENGEL, B. NAGY, L. A. NAGY, C. G. ENGEL, G. O. KREMP and C. M. DREW, *Science* **161**, 1005 (1968).
[16] T. G. TORNABENE, E. GELPI and J. ORÓ, *J. Bacteriol.* **94**, 333 (1967).

relative amounts and degree of saturation. The identities of all the fatty acid methyl esters reported in Tables 1 and 2 have also been confirmed by mass spectrometry. Their mass spectrometric fragmentation patterns have already been discussed in the literature.[3,16] Considered as a whole, the C_{16} acids appear to predominate in some species of blue-green algae, according to the computed ratio of total C_{16}/C_{18} fatty acids given in Table 2. The variations are great, however. From the relatively very high value for *A. nidulans* to the even and low values for *Lyngbya aestauri, Chroococcus turgidus* and *A. montana.* Conversely, the ratio of C_{16}/C_{18} fatty acids in the green algae (Table 2), shows without exception the C_{18} fatty acids to be predominant.

TABLE 2. RELATIVE PERCENT FATTY ACID COMPOSITION OF SEVERAL MICROSCOPIC ALGAE*

	Green algae				Blue-green algae						
Fatty acid Me esters	Chlor. pyr.	Tetr. sp.	Coel. micro.	Scen. quad.	Ana. nid.	Ana. cyan.	Spir. plat.	Nost. sp.	Lyng. aest.	Chroo. turg.	Ana. mont.
14:0	1·6		0·7		3·0		1·9	2·3			
14:1					4·0						
15:0						3·0					
16:0	21·4	18·3	13·1	13·0	30·7	23·0	45·0	36·3	25·8	28·2	21·3
16:1	2·0	2·6	1·3	4·0	33·3	20·0	6·0	10·0	15·4	12·3	3·1
16:2	5·8	2·7	4·6					1·0	3·2	3·8	
17:0					1·2						
17:1							2·0				
18:0		1·7			0·4	2·0		3·0			
18:1†	16·7	33·5	30·0	18·0	3·6	7·0	10·1	19·8	8·3	7·3	25·3
18:1‡			7·0	14·0							
18:2	18·7	11·1	13·0	7·0		10·0	16·0	8·0	16·8	16·4	17·8
18:3	26·4	18·3	16·0	29·0		5·0	13·0	6·5	18·2	25·7	19·4
18:4		6·3	7·0								
Total C_{16}/C_{18}	0·47	0·33	0·26	0·25	16·0	1·92	1·31	1·27	1·02	0·92	0·39

See Table 1 for species.
* Differences to 100% made up by unidentified compounds.
† 9-*cis*-Octadecenoic acid (oleic).
‡ 9-*trans*-Octadecenoic acid (elaidic).

All of the algae except *A. nidulans* have a ratio of saturated to unsaturated C_{16} in which the saturated form predominates, while the reverse is true for the C_{18} acids. Also, the octadecanoic acids show somewhat larger amounts and variety of polyunsaturated forms in all of the algae analyzed, with the exception again of *A. nidulans*.

Both *Scenedesmus quadricauda* and *Coelastrum microsporum* contain two components with different retention times, that show the mass spectral characteristics of a mono unsaturated C_{18} acid. By comparing the gas chromatographic peak retention time with that of the authentic *cis* and *trans* isomers of 9-octadecenoid acid (oleic and elaidic) they were respectively identified as *cis* and *trans* $C_{18:1}$ isomers. A similar situation applies to the mono unsaturated C_{16} acid in the same two species of algae. Only two of the algae analyzed, both green, showed detectable amounts of tetraunsaturated C_{18} fatty acids as seen in (Table 2).

DISCUSSION

The data obtained on algae so far does not suggest a direct biosynthetic relationship between hydrocarbons and fatty acids.[3,8] Rather, if the hydrocarbons in the algal cells arise from the regular fatty acids, a variety of complex intermediate processes must be involved.

For instance, the marked predominance of the n-C_{17} hydrocarbon (see first paper[1]) could be a point in favor of either of two direct pathways; elongation of the C_{16} fatty acids or decarboxylation of the C_{18} acids. However, depending on the prevailing mechanism, one would also expect to see substantial amounts of pentadecane, if the C_{16} fatty acid were to undergo decarboxylation, or nonadecane if the C_{18} acid were to elongate. The absence of significant amounts of these two hydrocarbons implies a more complex mechanism than simple elongation or decarboxylation of corresponding fatty acids. The same would apply to the high molecular weight hydrocarbons found in some species of algae,[1,17,18] since their corresponding fatty acids are absent. In this case, the mechanisms proposed for the condensation of two fatty acids involving the participation of alcohols and ketones or aldehydes[19,20] as intermediates do not seem to explain the facts either. An alternative mechanism would be that of the elongation–decarboxylation complex recently proposed by Kolattukudy.[21]

Considering the fatty acid distributions, any attempt made to correlate the variations in these distributions to phylogenetic positions is in our opinion still premature. Our patterns do not show significant and consistent variations between species or in some cases genera (Tables 1 and 2).

Among the fatty acid patterns there are considerable differences in the degree of saturation of the C_{16} and C_{18} fatty acids and, as shown by recent literature, changes in growth parameters such as temperature can lead to shifts in the degree of saturation of fatty acids.[9,22] However, in our case all cultures were grown under controlled conditions which would tend to minimize any shifts in degree of saturation. In addition, phylogenetic interpretation of fatty acid patterns should correlate with observed variations in hydrocarbon distributions. We cannot at present decifer any meaningful correspondence along the two lines of evidence. The only marked difference we have noted that could be related to phylogenetic position or morphological complexity is the total C_{16}/C_{18} ratio which shows (Table 2) the blue-green (prokaryots) to have a predominance of C_{16} fatty acids and the greens (eukaryots) to have a predominance of C_{18} fatty acids. If this were really an indication of morphological complexity the low value for *Anacystis montana* (Table 2) would seem to indicate that this organism is a transitional form[16] between the blue-green and green algae.

In relation to their geochemistry, the specific involvement of algal fatty acids in sediments does not seem to be as well substantiated as that of the aliphatic hydrocarbons (cf. first paper[1]). However, fatty acids are among the most abundant components of living systems and in general have been often regarded as the possible precursors of petroleum and sedimentary hydrocarbons.[6,7]

Normal fatty acids are present in recent as well as Precambrian sediments and considering the large number of algae implicated in these sediments, the data presented here would

[17] E. GELPI, J. ORÓ, H. J. SCHNEIDER and E. O. BENNET, *Science* **161**, 700 (1968).
[18] G. W. PATTERSON, *J. Phycol.* **3**, 22 (1967).
[19] A. C. CHIBNALL and S. H. PIPER, *Biochem. J.* **28**, 2209 (1934).
[20] P. W. ALBRO and J. C. DITTMER, *Biochem. J.* **8**, 394 (1969).
[21] P. E. KOLATTUKUDY, *Science* **159**, 498 (1968).
[22] H. SCHNEIDER, M. S. Thesis, University of Houston, Houston, Texas (1967).

certainly suggest that algae must have contributed in one way or another to the fatty acid distributions found in our lithosphere.[6, 23, 24] However, it is hard to evaluate the extent of their contributions.

The absence of the unsaturated acids in sediments as opposed to their abundance in living algae may be explained by their lower stability to a reducing geological environment. Likewise, the appearance of significant amounts of odd carbon numbered normal fatty acids with geological time, which parallels the appearance of even carbon numbered hydrocarbons, can also be related to diagenetic changes of the originally deposited algal debris.

On the other hand, the fatty acid distributions of contemporary algae fail to account for the presence in sediments of branched-chain acids[23–25] and of acids higher than C_{18}.[6, 7, 23, 24] They do not account either for the presence of odd fatty acids in recent muds and soils. Isoprenoid fatty acids, which have been reported in recent sediments,[23, 26, 27] have not been detected in the algae studied in our laboratory. This, however, would not be unexpected since they most likely arise from slow diagenetic degradation of the algal chlorophylls. On the other hand there is one report in the literature on the presence of significant amounts of pristane in a green alga.[8]

Consideration of the contributions of bacteria and higher plants to the fatty acid patterns observed in Tertiary and Cambrian sediments would fill the gap left by algae. Concerning the Precambrian, the scarce data available in the literature appears to support the role of algae as their direct source, except for the C_{20} and C_{22} acids.[6]

In conclusion, it seems that the correlation of algal fatty acids with the normal fatty acids in sediments, contrary to the hydrocarbons, is less apparent, although nevertheless valid in the C_{12}–C_{20} range.

EXPERIMENTAL

The growth parameters, source, and harvesting of the algae are given in the preceding paper. Sample preparations are performed in the same manner as described in Paper I,[1] except that the methanol fraction containing free fatty acids, glycerides and other lipids was taken to dryness and methyl esters were produced by treatment with BF_3.[28] The fatty acid methyl ester fraction was concentrated by evaporation under N_2 and analyzed by GLC, using a Barber–Coleman 5000 gas chromatograph equipped with a hydrogen flame-ionization detector; and combined gas chromatography–mass spectrometry (LKB-9000 Gas Chromatograph–mass spectrometer).

Separation of the fatty acid methyl esters was achieved on a stainless-steel column (9·6 m × 0·3 cm, i.d.) packed with 15% EGS on 80/100 Chromosorb W AW, N_2 pressure of 703 g/cm^2, battery setting of ×1 and attenuation of 10. The GLC conditions were designed specifically to detect any fatty acids in the C_{12}–C_{30} range. Dry weights of algae extracted ranged from 0·29 to 1·0 g.

Acknowledgements—We thank Dr. J. Mann for the preparation and harvesting of the algal cultures. This work was supported by NASA Grants: NGR 44–005–020 and NGR 44–005–002.

[23] A. L. Burlingame, P. A. Haug, H. K. Schnoes and B. R. Simoneit, 4th International Meeting Org. Geochem, Amsterdam, Sept. 16–18, 1968.
[24] A. L. Burlingame, P. C. Wszolek and B. R. Simoneit, 4th International Meeting Org. Geochem., Amsterdam, Sept. 16–18, 1968.
[25] R. F. Leo and P. L. Parker, *Science* **152**, 649 (1966).
[26] M. Blumer and W. J. Cooper, *Science* **158**, 1463 (1967).
[27] G. Eglington, A. G. Douglas, Jr., Maxwell, J. N. Ramsay and S. Stallberg-Stenhagen, *Science* **153**, 1133 (1966).
[28] W. R. Morrison and L. M. Smith, *J. Lipid Res.* **5**, 600 (1964).