Phytochemistry, Vol 23, No 7, pp 1421-1423, 1984
Printed in Great Britain

0031-9422/84 $3 00+0 00

OCCURRENCE OF 2-HYDROXY ACIDS IN MICROALGAE

GENKI I MATSUMOTO, MAKOTO SHIOYA and HIDEYUKI NAGASHIMA*

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Fukazawa 2-1-1, Setagaya-ku, Tokyo 158, Japan, *Department of Biology, Faculty of Science, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo 162, Japan

(*Received* 8 *June* 1983)

Key Word Index—Microalgae, Chlorophyta, Rhodophyta, Cyanophyta, 2-hydroxy acids, chemotaxonomy, geochemistry

Abstract—2-Hydroxy acids were believed to be absent in algae until this study, in which the analysis of microalgae belonging to Chlorophyta (*Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa*), Rhodophyta (*Cyanidium caldarium* M-8 and *Cyanidium caldarium* RK-1) and Cyanophyta (*Anbaena variabilis, Anacystis nidulans, Oscillatoria* species and *Phormidium foveolarum*) is reported 2-Hydroxy acids with carbon chain lengths of C_{16}–C_{26} were found in all the algal samples studied, ranging in concentrations from 4 0 to 320 μg/g dry alga. The dominant constituents are 2-hydroxyhexadecanoic, 2-hydroxynonadecanoic, 2-hydroxyhexacosanoic and a branched 2-hydroxy-C_{19} acid The distribution patterns of the acids differed significantly among the algal samples Hence 2-hydroxy acids may be useful for the classification of algal species as well as being an important source of 2-hydroxy acids in the natural environment

INTRODUCTION

2- and 3-hydroxy acids occur in a wide variety of micro-organisms, such as bacteria, actinomycetes, fungi and yeasts, animal tissue and plants, and they are often intermediates in the α- and β-oxidation pathways of fatty acid degradation [1–14] However, they had not been found in algae, except for certain Cyanophyta [15], until we recently found normal and branched (*iso* and/or *anteiso*) 3-hydroxy acids with carbon chain lengths of C_8–C_{28} in eight species of microalgae belonging to Chlorophyta, Rhodophyta and Cyanophyta [16] We report here the occurrence of normal and branched 2-hydroxy acids in some mesophiles of Chlorophyta and Cyanophyta, and in thermophiles of Rhodophyta, and discuss their taxonomical and geochemical significance

RESULTS AND DISCUSSION

Normal 2-hydroxy acids with carbon chain lengths of C_{16}–C_{26} were found in all the algal samples studied (Table 1), a branched 2-hydroxy acid was detected only in *Oscillatoria* species Their mass spectra had all the characteristic peaks of 2-hydroxy acid trimethylsilyloxy ether methyl esters at m/z $[M-15]^+$, $[M-59]^+$, 159, 129, 103, 89 and 73 (Table 2) [3, 10, 11] The concentrations of the acids ranging from 4 0 to 320 μg/g dry alga were considerably lower than those of 3-hydroxy acids in the same algal samples (36–2300 μg/g) [16] 2-Hydroxyhexadecanoic acid was the main component in Chlorophyta (*Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa*) and Cyanophyta (*Anabaena variabilis, Anacystis nidulans* and *Phormidium foveolarum*) *Anacystis* is unique because it contains only the C_{16} acid. Thermal Rhodophyta (*Cyanidium caldarium* strains M-8 and RK-1) and Cyanophyta (*Oscillatoria* species) contained abundantly 2-hydroxyhexacosanoic, 2-hydroxynonadecanoic and branched 2-hydroxy C_{19} acids, respectively Even/odd carbon ratios for normal 2-hydroxy acids show that the even-carbon numbers are more abundant than the odd ones (> 9 6) in the algae except for *Cyanidium* RK-1 (1 2, Table 1) The low even/odd ratio for *Cyanidium* RK-1 is consistent with that of normal alkanoic acids (unpublished results)

The ratios of higher (nC_{20}–nC_{26}) to lower (nC_{16}–nC_{19}) MW 2-hydroxy acids differed considerably among the algal species tested (Table 1) *Cyanidium* M-8 shows the highest value (6 1), while *Anabaena* and *Anacystis* show zero

Morphological studies and carbohydrate analysis of *Cyanidium* have shown that *Cyanidium* strain M-8 may belong to a different genus (*Chroococcidiopsis*) [17–19] The major hydroxy acid constituents of *Cyanidium* RK-1 and M-8 are considerably different from each other, in addition to the marked differences of the even/odd and higher/lower ratios (Table 1) These results also support the idea that *Cyanidium* M-8 is different at the genus level from *Cyanidium* RK-1

Of special interest is the abundance of 2-hydroxyhexacosanoic acid in *Chlamydomonas* and *Cyanidium* M-8 Normal C_{26} alkanoic and/or alkenoic acids were not found in the fatty acid fractions from the algal materials (unpublished results) Our results indicate that 2-hydroxyhexacosanoic acid does not derive from α-oxidation of the corresponding alkanoic and/or alkenoic acids, and thus suggest that some synthetic pathway of long-chain 2-hydroxy acids occurs exclusively in the microalgae

2-Hydroxy acids have been found in contemporary lacustrine and marine sediments [3, 4, 10, 11, 13, 14] They are believed to be derived from micro-organisms except for microalgae, in which they have not been found Our results suggest that microalgae are one of the important sources of 2-hydroxy acids, as well as 3-hydroxy acids, in the natural environment [16]

Table 1 2-Hydroxy acids found in microalgae

Alga	Conc (μg/g dry alga)	Even/ odd*	Higher/ lower†	Composition (%)											
				nC_{16}	nC_{17}	nC_{18}	nC_{19}	nC_{20}	nC_{21}	nC_{22}	nC_{23}	nC_{24}	nC_{25}	nC_{26}	brC_{19}‡
Chlorophyta															
Chlamydomonas reinhardtii	48	99	0 79	54 6	—	1 3	—	—	—	1 0	—	3 4	1 0	38 7	—
Chlorella pyrenoidosa	10	66	0 27	78 9	—	—	—	—	—	2 0	0 5	14 6	1 0	3 0	—
Rhodophyta															
Cyanidium caldarium M-8§	73	13	6 1	2 5	0 1	9 4	2 1	33 5	0 5	3 0	0 4	2 6	4 3	41 6	—
Cyanidium caldarium RK-1	320	1 2	0 86	4 5	1 0	8 1	40 3	36 0	1 4	0 4	0 1	1 4	3 3	3 5	—
Cyanophyta															
Anabaena variabilis	4 0	large	0 0	95 0	—	5 0	—	—	—	—	—	—	—	—	—
Anacystis nidulans	13	large	0 0	100	—	—	—	—	—	—	—	—	—	—	
Oscillatoria sp	58	9 6	0 35	5 9	0 5	8 2	0 5	1 0	0 3	1 1	0 4	1 7	0 2	0 4	79 8
Phormidium foveolarum	6 3	11	0 11	85 8	2 6	1 9	—	1 2	—	0 7	1 9	1 6	3 7	0 6	—

*The ratio of even/odd carbon numbers of normal 2-hydroxy acids (nC_{16}–nC_{26})

†Higher (nC_{20}–nC_{26})/lower (nC_{16}–nC_{19}) ratio

‡Branched acid

§This strain is named *Chroococcidiopsis* species in refs [17–19]

—, Quantity less than twice that of the blank

Table 2 Typical mass spectra data of 2-hydroxy acid trimethylsilyloxy ether methyl esters found in microalgae

	Fragment ion m/z, relative abundance (%)						
Compound	73	89	103	129	159	$[M-59]^+$	$[M-15]^+$
nC_{16}*	100	59	23	16	19	47	51
nC_{17}†	+	+	+	+	+	+	+
nC_{18}	100	61	35	23	19	92	46
nC_{19}	100	36	25	25	14	74	28
nC_{20}	100	47	32	24	15	76	26
nC_{21}†	+	+	+	+	+	+	+
nC_{22}	95	46	34	26	16	100	42
nC_{23}†	+	+	+	+	+	+	+
nC_{24}	100	41	33	27	17	95	36
nC_{25}	89	38	28	32	13	100	37
nC_{26}	100	41	29	31	14	94	38
brC_{19}	74	30	22	13	9	100	40

*Mixture of 2- and 3-hydroxy acids.

†Although their intensities were weak, the mass spectra showed characteristic peaks of 2-hydroxy acid trimethylsilyloxy ether methyl esters

+, Fragment ions were present

EXPERIMENTAL

Chlamydomonas reinhardtii C-8 and *Chlorella pyrenoidosa* C-28 (Chlorophyta), *Cyanidium caldarium* M-8 and RK-1 (tentatively included in the Rhodophyta), and *Anabaena variabilis* M-3, *Anacystis nidulans* M-6 and *Phormidium foveolarum* M-43 (Cyanophyta) were obtained by laboratory axenic cultures *Oscillatoria* sp (Cyanophyta) was collected from a natural pond Algal sources and culture methods were as reported previously [16–18]

The extraction procedures and some analytical methods have been described elsewhere [16] Briefly, wet alga was directly saponified with 0 5 M KOH–MeOH soln After acidification, hydroxy acids were extracted with EtOAc The extracts were chromatographed on a silica gel column (180 × 5 mm i d, 100 mesh, 5% H_2O). Hydroxy acids were eluted with C_6H_6–EtOAc (1 1) after the elution of hydrocarbons, fatty acids and alcohols Hydroxy acid fractions were methylated with BF_3–MeOH soln and then examined by silica gel TLC developed with hexane–EtOAc–HOAc (65 35 1). 2-Hydroxy acids (R_f 0 78–0 69) were scraped, extracted with C_6H_6–MeOH (3 2), and trimethylsilylated with *N,O*-bis(trimethylsilyl)acetamide–MeCN soln

2-Hydroxy acid TMSi Me esters were analysed by GC/EIMS [16] using a glass column (2 m × 3 mm i d) packed with 1% OV-1 on Chromosorb W AW DMCS or a GC/MS system equipped with a 0 3 mm i d × 25 m fused silica capillary column coated with SE 54 Oven temp was programmed from 70° (maintained for 0 5 min) to 130° at 30°/min and then to 300° at 8°/min Temps of injector and ion source were 300° and 200°, respectively The flow rate of the carrier gas (He) was 1 5 ml/min, splitless mode The ionization energy was 70 eV

2-Hydroxy acids were identified by the comparison of R_t and MS with those of authentic specimens and published lit, and were quantified by the peak ht on the mass chromatogram carried at m/z $[M-59]^+$, which is a characteristic peak of 2-TMSi Me esters (Table 2, [3, 10, 11]) In order to check the analytical reliability, spiked expts of authentic 2-hydroxyhexadecanoic acid showed recoveries of 66% (s d 8 6%) Analysis of blank culture media indicated that possible contaminants of 2-hydroxy acids were each less than 0 2 μg per culture

Acknowledgements—We are grateful to the Geochemical Laboratory, Technology Research Center, Japan National Oil Corporation, for providing the opportunity to use their GC/MS system, and Professor T Hanya for his encouragement

REFERENCES

1 Stodola, F H, Deinema, M H and Spencer, J F T (1967) *Bacteriol Rev* **31**, 194
2 Fulco, A J (1967) *J Biol Chem* **242**, 3608
3 Eglinton, G, Hunneman, D H and Douraghi-Zadeh, K (1968) *Tetrahedron* **24**, 5929
4 Hunneman, D H and Eglinton, G (1969) in *Advances in Organic Geochemistry 1968* (Schenck, P A and Havenaar, I, eds), pp 157–165 Pergamon Press, Oxford
5 Hancock, I C, Humphreys, G O and Meadow, P M (1970) *Biochim Biophys Acta* **202**, 389
6 Yano, I, Furukawa, Y and Kusunose, M (1971) *Biochim Biophys Acta* **239**, 513
7 Ferguson, K A, Conner, R L, Mallory, F B and Mallory, C W (1972) *Biochim Biophys Acta* **270**, 111
8 Moss, C W, Samuels, S B, Liddle, J and McKinney, R M (1973) *J Bacteriol* **114**, 1018
9 Yano, I, Ohno, Y, Masui, M, Kato, K, Yabuuchi, E and Ohyama, A (1976) *Lipids* **11**, 685
10 Boon, J J, DeLange, F, Schuyl, P J W, De Leeuw, J W and Schenck, P A (1977) in *Advances in Organic Geochemistry 1975* (Campos, R and Goñi, J, eds.), pp 255–272 Enadimsa, Madrid
11 Cardoso, J N and Eglinton, G (1977) in *Advances in Organic Geochemistry 1975* (Campos, R and Goñi, J, eds), pp 273–287 Enadimsa, Madrid
12 Bryn, K and Rietschel, E T (1978) *Eur J Biochem* **86**, 311
13 Volkman, J K, Johns, R B, Gillan, F T and Perry, G J (1980) *Geochim Cosmochim Acta* **44**, 1133
14 Cranwell, P A (1981) *Geochim Cosmochim Acta* **45**, 547
15 Weckesser, J, Katz, A, Drews, G, Mayer, H and Fromme, I (1974) *J Bacteriol* **120**, 672
16 Matsumoto, G I and Nagashima, H, *Geochim. Cosmochim. Acta* (submitted).
17 Nagashima, H and Fukuda, I (1981) *Phytochemistry* **20**, 439
18 Nagashima, H and Fukuda, I (1981) *Jpn J Phycol* **29**, 237
19 Nagashima, H and Fukuda, I (1983) *Phytochemistry* **22**, 1949