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FATTY ACID COMPOSITION OF TEN MARINE ALGAE FROM AUSTRALIAN WATERS

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Key Word Index—Algae; Chlorophyta; Rhodophyta; Phaeophyta; fatty acids; *cis*-vaccenic acid; chemotaxonomy; food chain studies.

Abstract—Detailed fatty acid analyses are reported for ten benthic algae from Australian waters of which the data for *Cladophora fascicularis* (Chlorophyta); *Polysiphonia pungens*, *Porphyra* sp., *Centroceras clavatum* (Rhodophyta); *Hormosira banksii*, *Ralfsia* sp., and *Dictyota dichomota* (Phaeophyta) are presented for the first time. The analyses report the exact molecular structure of component acids which is essential for taxonomic and food chain studies. The acid 16:4ω3 could be taxonomically distinguishing for species of the Chlorophyta. The occurrence of *cis*-vaccenic acid (18:1ω7) in the algae reported here suggests a distribution in marine benthic algae which is wider than hitherto realised, with particular taxonomic importance for Chlorophyta species in which it occurs in high levels. *Corallina officinalis* was found to contain the non-methylene interrupted acids 20:2 and 22:2.

INTRODUCTION

Food chain studies with molluscs in our laboratory necessitated an evaluation of lipid data on relevant algal species in the literature. Such an assessment revealed that fatty acid distributions of a large number of algal species have been reported and reviewed, but in varying degrees of detail [1–4]. Whilst phytoplankton species have received extensive attention for total fatty acid content, the lipid composition of benthic algae, particularly of members of the Rhodophyta and Phaeophyta, has received relatively little attention. A particular weakness of the early literature on fatty acids of marine algae is that little or no emphasis has been placed on double bond positions for the unsaturated acids.

The total fatty acids of ten algae from Australian waters are reported in detail in this paper. Component fatty acids of *Cladophora fascicularis* (Chlorophyta), the red algae *Polysiphonia pungens*, *Porphyra* sp. and *Centroceras clavatum*, and the brown algae *Hormosira banksii* (peculiar to Australian and New Zealand waters), *Ralfsia* sp. and *Dictyota dichomota* are presented for the first time. The fatty acids of the green algae *Enteromorpha intestinalis* and *Ulva lactuca*, and *Corallina officinalis* (Rhodophyta) are included for comparative purposes. For each species, however, the exact molecular structure of component fatty acids, including those in trace amounts, are reported since such data are essential in order to identify distinguishing taxonomic features, and allow fatty acids to be used directly in food chain studies.

* Double bond positions (ω) are numbered from the methyl end of the fatty acid, all subsequent double bonds are methylene interrupted.

RESULTS AND DISCUSSION

Rhodophyta

The total fatty acids of the four Rhodophyta species (Table 1) exhibit a distribution characteristic of the phylum [4–6] with high relative levels of the acids 16:0, 20:5ω3* and 20:4ω6. A number of C_{18} polyunsaturated fatty acids (PUFAs) are present in significant amounts, whilst C_{16} and C_{22} PUFAs occur in trace levels only. Each Rhodophyta species examined contained significant levels of monounsaturated fatty acids (Table 1), including unexpectedly high levels of *cis*-vaccenic acid (18:1ω7). In one species, *Polysiphonia pungens* 18:1ω7 was the predominant monounsaturated fatty acid detected. Non-methylene interrupted (NMI) fatty acids 20:2 and 22:2 were identified in *Corallina officinalis*. Although NMI 20:3 fatty acids have been reported in a number of brown algae [5], non-methylene interrupted acids are rarely reported in algae. They do occur frequently in marine molluscs [7, 8] although the origin of NMI acids has been uncertain. Studies in our laboratory and the data reported here point to the fact that they originate in algae and consequently may pass through the marine food chains into higher trophic levels.

Phaeophyta

These brown algae (*Hormosira banksii*, *Ralfsia* sp. and *Dictyota dichomota*) were examined for fatty acid composition (Table 2). Despite the marked morphological differences which exist between these three species, they have similar fatty acid patterns. The predominant acids found in each species were 16:0, 18:1ω9, 20:4ω6 and 20:5ω3, which is in agreement with analyses of other representatives of this phylum [4, 5, 9]. Significant levels of 14:0, 18:2ω6, 18:3ω3 and 18:4ω3 were also present in each species. These C_{18} PUFAs are of greater abundance in these Phaeophyta species than the red algae

Table 1. Fatty acid composition of Rhodophyta species

Acid	Percentage of total fatty acids *Corallina officinalis**† (Aug. 1976)	*Porphyra* sp.‡ (Aug. 1977)	*Polysiphonia pungens* (Aug. 1977)	*Centroceras clavatum* (Nov. 1977)
12:0	tr	0.07	—	0.05
14:0	2.48	0.86	4.08	1.19
iso 15:0	0.12	tr	tr	0.05
anteiso 15:0	—	tr	tr	tr
15:0	0.34	0.26	0.16	0.15
iso 16:0	—	0.07	0.19	—
16:0	29:46	32:67	30:48	38:01
16:1ω9	tr	tr	tr	—
16:1ω7	2.18	1.14	3.01	4.94
16:1ω5	—	tr	tr	—
*trans*16:1ω13	1.20	1.60	1.16	0.74
16:2ω6	0.10	{ tr	{ tr	{ 0.65
16:2ω4	0.41			
16:3ω6	tr			
16:3ω4	0.70	{ tr	{ tr	{ tr
16:3ω3	0.13			
16:4ω3	0.94	tr	tr	tr
iso 17:0	—	tr	0.23	0.05
anteiso 17:0	—	0.08	0.26	0.08
17:0	tr	0.12	0.46	0.06
18:0	0.58	0.92	1.41	0.64
18:1ω9	3.53	2.89	2.04	4.32
18:1ω7	2.86	2.23	7.67	2.27
18:2ω6	1.73	{ 3.07	{ 5.38	{ 0.79
18:3ω3	2.35			
18:3ω6	0.48	1.52	1.89	0.92
18:4ω3	1.61			
19:0	—	1.67	0.19	—
20:0	0.09	tr	tr	0.23
20:1ω9	0.89	2.81	—	0.13
20:1ω7	tr	tr		
20:2ω6	0.44	0.81	0.16	0.17
20:3ω6	0.84	{ 3.98	{ 0.68	{ 0.47
20:3ω3	0.16			
20:4ω3	0.33	nd	nd	nd
20:4ω6	11.45	41.80	38.79	43.44
20:5ω3	32.10			
22:0	0.22	tr	0.13	0.06
22:1ω7§	0.63	0.44	0.06	0.19
22:4ω6	tr	0.37	0.17	0.06
22:5ω3	tr			
22:6ω3	tr	1.01	—	tr
Unidentified	0.77	0.77	0.20	0.30

tr Components less than 0.05%. nd Not determined.

* Fatty acid data includes analysis on BDS and SE30 capillary columns, other species analysed on SE30 column only.

† *C. officinalis* also contains 19:1 (0.18%), two 20:2 NMI (0.19%) and 22:2 NMI (0.34%).

‡ *Porphyra* sp. also contains 22:3ω6 (0.08%).

§ Other isomers present.

Table 2. Fatty acid composition of Phaeophyta species

Acid	Percentage of total fatty acids *Hormosira banksii**† (Jan. 1977)	*Ralfsia* sp. (Aug. 1976)	*Dictyota dichomota*‡ (Nov. 1977)
12:0	0.08	tr	0.09
14:0	3.43	7.17	10.32
iso 15:0	0.07	0.06	—
15:0	0.48	0.40	0.53
16:0	24·65	35.88	23.05
16:1ω9	0.36	0.16	—
16:1ω7	1.93	1.43	1.57
16:1ω5	tr	0.13	8.09
trans 16:1ω13	0.17	0.97	0.71
16:2ω6	tr	0.13	—
16:2ω4	0.16	0.34	—
16:3ω6	tr	0.05	—
16:3ω4	tr	0.09	—
16:3ω3	tr	0.19	—
16:4ω3	0.09	0.83	nd
17:0	0.21	tr	0.18
17:1ω8	0.27	tr	—
18:0	1.15	0.54	2.40
18:1ω9	18.69	14.92	22.67
18:1ω7	1.05	1.57	—
18:2ω6	6.39	3.80	{ 2.89
18:3ω3	7.98	6.70	
18:3ω6	0.34	0.48	{ 8.81
18:4ω3	3.80	5.87	
19:0	0.21	tr	0.53
20:0	0.19	0.35	0.80
20:1ω9	0.22	0.16	0.27
20:1ω7	—	0.13	—
20:2ω6	1.63	0.16	—
20:3ω6	4.11	0.76	3.11
20:3ω3	0.44	0.17	—
20:4ω6	12.98	6.83	{ 11.46
20:5ω3	6.64	8.69	
20:4ω3	1.57	0.88	tr
22:0	0.12	tr	tr
2:4ω6	0.12	tr	{ 1.60
22:5ω3	0.07	tr	
22:5ω6	tr	tr	—
22:6ω3	0.13	tr	—

tr Components less than 0.05%. nd Not determined.

* Fatty acid data includes analysis on BDS and SE30 capillary columns, other species analysed on SE30 column only.

† *H. banksii* also contains 13:0 (0.09%).

‡ *D. dichomota* also contains 18:1ω5 (0.53%) and 22:3ω6 (0.36%).

examined (Table 1). *cis*-Vaccenic acid was found in levels approximately 1% of the total fatty acids in *Ralfsia* and *Hormosira banksii* but was not detected in *D. dichomota*. However this last alga contained high levels of 16:1ω5 (8%), an uncommon acid in algal lipids with trace amounts of 18:1ω5. This last acid could be derived by chain elongation from the former.

Chlorophyta

The fatty acids obtained from *Enteromorpha intestinalis*, *Ulva lactuca* and *Cladophora fascicularis*, three representatives of the Chlorophyta, are given in Table 3. All three species have similar fatty acid patterns with four components, 16:0, 16:4ω3, 18:3ω3 and 18:4ω3 clearly predominating. The abundance of 18:3ω3 has been previously noted as a characteristic fatty acid of this phylum. The high level of 18:4ω3 found in *Ulva lactuca* and *Enteromorpha intestinalis* has been previously reported in these two algae [5], but the high levels of 16:4ω3 may prove taxonomically useful as it is detected in only trace amounts in the other phyla examined here. Analyses for these two species are given at two different collection dates (Table 3). Fatty acid composition in general is similar for both winter and spring; however, the relative level of 16:0 appears to increase between winter (August) and spring (November) whilst 16:4ω3 undergoes a dramatic reduction. An in-

Table 3. Fatty acid composition of Chlorophyta species

Acid	RRT*	Percentage of total fatty acids *Enteromorpha intestinalis* 1976†	*Enteromorpha intestinalis* 1977	*Ulva lactuca* 1976†	*Ulva lactuca* 1977	*Cladophora fascicularis* 1977
12:0	0.111	tr	tr	tr	tr	tr
14:0	0.229	0.50	0.36	0.46	0.62	9.76
iso 15:0	—	—	0.06	—	—	0.12
15:0	0.330	0.21	0.37	0.08	—	0.12
16:0	0.479	19.27	28.67	18.11	31.73	21.94
16:1ω9	0.524	0.44	tr	0.39	tr	1.62
16:1ω7	0.537	0.66	1.33	0.54	2.28	2.42
trans 16:1ω13	0.577	3.34	1.22	2.83	1.77	1.01
16:2ω6	0.633	0.78	nd	0.59	nd	tr
16:2ω4	0.681	tr		tr		
16:3ω6	0.707	0.09	tr	0.21		
16:3ω4	0.784	tr	3.81	—	1.10	0.75
16:3ω3	0.816	3.32		2.59		
16:4ω3	0.916	16.36	6.44	19.26	6.67	8.49
iso 17:0	—	—	0.06	—	—	0.12
18:0	1.00	0.15	0.23	0.07	0.29	0.23
18:1ω9	1.08	0.56	0.38	0.27	2.80	17.53
18:1ω7	1.11	6.12	13.15	5.17	15.03	5.04
18:2ω6	1.30	4.38	31.14	3.86	20.44	21.58
18:3ω3	1.66	19.94		20.61		
18:3ω3	1.47	0.82	6.10	0.83	13.26	0.72
18:4ω3	1.89	15.64		15.83		
20:0	2.07	0.07	0.06	0.06	0.13	—
20:1ω9	2.20	0.08	0.06	0.10	0.09	—
20:1ω7	2.26	0.06	—	0.06		—
20:2ω6	2.64	0.09	0.06	0.10	0.07	0.32
20:3ω6	2.92	0.40	1.45	0.27	0.73	0.59
20:3ω3	3.35	0.08		0.11		
20:4ω3	3.73	0.75	nd	0.94	nd	nd
20:4ω6	3.17	0.68	2.95	0.59	1.10	5.99
20:5ω3	4.05	1.34		1.74		
22:0	4.30	0.52	0.50	0.26	0.79	0.21
22:1ω7	4.69	0.10	tr	0.29	0.07	0.11
22:4ω6	6.40	0.58	1.52	0.41	2.07	1.31
22:5ω3	8.14	2.52		3.29		
22:5ω6	7.16	0.15	tr	0.06	tr	tr
22:6ω3	8.96	tr	tr	tr	tr	tr

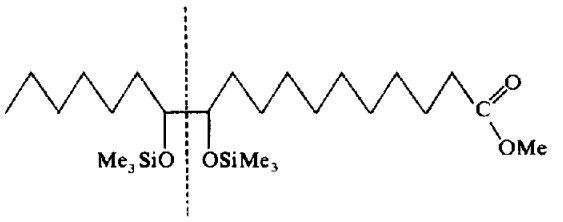
tr Components less than 0.05%. nd Not determined.

* Relative retention time measurements (18:0 = 1.00) from BDS capillary column at 170° for August 1976 analyses for *E. intestinalis* and *U. lactuca*.

† Fatty acid data include analysis on BDS and SE30 capillary columns, other samples analysed on the SE30 column only.

crease is also apparent for the relative level of *cis*-vaccenic acid between the winter and spring collections.

Double bond positions have been determined for all unsaturated fatty acids present in the algae studied. During this isomer analysis, unexpectedly high levels of *cis*-vaccenic acid (18:1ω7) were observed particularly in *U. lactuca* and *E. intestinalis*. Identification of *cis*-vaccenic acid was based on argentation TLC separation of the ω7 and ω9 esters and relative retention time measurements on a capillary BDS column [10] (Table 3). Unequivocal determination of the double bond position was achieved by modification of the double bond with OsO_4 and ultimate analysis of the diol produced as the disilyloxy methyl ester by GC–MS. The position of the original double bond is marked by high intensity mass



spectral fragment ion peaks generated as a result of the cleavage of the carbon bond between the two silyloxy groups [11]. Thus the 18:1ω7 disilyoxy methyl ester was characterised by the following fragment ions: *m/e* 287 $C_{15}H_{31}O_3Si$ [M-187], *m/e* 187 $C_{10}H_{23}OSi$ [M-287] and *m/e* 97 C_7H_{13} [M-367]. *cis*-Vaccenic acid has often been identified in marine organisms including molluscs [7, 8], fish [12] and crustacea [13]. However in all these organisms the ratio of oleic acid (18:1ω9) to *cis*-vaccenic acid is always greater than one, and often *cis*-vaccenic acid is only detected in trace amounts. Many reports of algal fatty acids do not indicate double bond positions for all unsaturated components [9, 14, 15]; those that do generally identify oleic acid (18:1ω9) as the predominant C_{18} monounsaturated fatty acid [3, 4] but fail to enumerate the structure of other isomers present. *cis*-Vaccenic acid has previously been identified in algae in only small quantities [2, 16], apart from the report for the unicellular alga *Isochrysis galbana*, in which 18:1ω7 is the dominant C_{18} unsaturated fatty acid, accounting for nearly 10% of the total fatty acids [17]. In a number of the algae reported in this paper, also, *cis*-vaccenic acid was found in relatively high levels. In the Rhodophyta species examined (Table 1), *cis*-vaccenic acid was detected in all four species and represented over 7% of the total fatty acids in *C. clavatum*. Although 18:1ω7 was identified in two Phaeophyta species (Table 2), it is the Chlorophyta species (Table 3) which contained the highest relative amounts. Considerable seasonal variation can be seen between the fatty acids for the two collections of *U. lactuca* and *E. intestinalis* (Table 3) but in all instances 18:1ω7 was the major monounsaturated fatty acid detected.

Jamieson and Reid [5] studied the fatty acids of five species of green algae including *U. lactuca* and *E. intestinalis* collected from Scotland. In these algae they reported the major 18:1 isomer as 18:1ω9, although the presence of other isomers was indicated. Our data accord with those of Ackman and McLachlan [18] who also find *cis*-vaccenic acid as the major 18:1 isomer in the Chlorophyta. Although *cis*-vaccenic acid is a common component of bacterial lipids [19], it has not previously been recognised as a major constituent of some algal fatty acids. It is presumably biosynthesized in algae by chain elongation of 16:1ω7 which can be a product of a Δ^9 desaturase [2]. The results reported here for 10 species of marine algae suggest that the abundance of *cis*-vaccenic acid in marine algae is greater then hitherto realised and that it has particular taxonomic value in Chlorophyta species.

EXPERIMENTAL

Samples of *Porphyra* were collected at San Remo, Victoria and *H. banksii* was found at Flinders, Victoria. All other algae were collected from an intertidal zone at Brighton, Victoria; they were identified by members of the Botany Department, Uni-

versity of Melbourne. Prior to extraction of lipids algae were washed thoroughly in distilled water to remove sand and adhering detritus.

Fatty acids were isolated using techniques previously described [7, 20]. Fatty acid methyl esters were separated by argentation TLC [silica GF_{254} plus 5% $AgNO_3$, developed in *n*-heptane–Et_2O–MeOH (90:10:1)] prior to GLC analysis on SE30 (SCOT) and BDS (WCOT) capillary columns. Components were identified by co-chromatography with authentic standards and relative retention time measurements [10]. Osmate esters produced by the reaction of OsO_4 with the fatty acid methyl esters, followed by treatment with H_2S and the appropriate work-up conditions were converted to the corresponding diol fatty acid methyl esters [21]. Disilyloxy fatty acid methyl esters were then produced by addition of *bis*-(trimethylsilyl) acetamide and analysed by GC–MS on a Perkin Elmer 270B at 70 eV.

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