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FATTY ACIDS IN SOME NOVA SCOTIAN MARINE SEAWEEDS: A SURVEY FOR OCTADECAPENTAENOIC AND OTHER BIOCHEMICALLY NOVEL FATTY ACIDS*

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The total fatty acids of 15 seaweeds from Nova Scotia were examined for unusual fatty acids by open-tubular gas-liquid chromatography. Several unknown fatty acids were detected, but the novel 3,6,9,12,15-octadecapentaenoic acid was not observed. Three species of Chlorophyceae showed cis-11-octadecenoic acid in proportions greater than the more common cis-9-octadecenoic isomer. The C_{20} acids with unsaturation in the Δ^5 position and in one or more of the Δ^{11}, Δ^{14}, or Δ^{17} positions were frequently observed in traces but were important (4%) in Ascophyllum nodosum. Agarum cribrosum, Porphyra leucosticta and A. nodosum showed unusual fatty acid features warranting further investigaton from a chemotaxonomic point of view.

INTRODUCTION

Seaweeds of numerous types are plentiful along the shores of Nova Scotia, but only a few species are exploited commercially, and then primarily for polysaccharides (Neish et al 1977). The recent discovery by Joseph (1975) of 18:5ω3 (all-cis 3,6,9,12,15-octadecapentaenoic acid)** as a major fatty acid in the unicellular alga Prorocentran minimum and later by Mayzaud et al (1976) in other dinoflagellates, was a development somewhat unexpected to lipid chemists. No precedent for a cis-ethylenic

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**A shorthand notation of chain length: number of ethylenic bonds will be used. This presumes that all bonds are cis, all are methylene-interrupted, and gives the position of the bond nearest to the terminal methyl group. The conventional system of numbering from the carboxyl group uses a Δ^2 notation and makes the same assumption unless otherwise indicated.

bond in the Δ^3 position of a polyunsaturated fatty acid existed. Although a precise biochemical pathway for the formation of this acid is not known, the simple pathway proposed by Joseph (1975) of chain shortening of 20:5ω3 (all-cis 5,8,11,14,17- eicosapentaenoic acid) suggested that 18:5ω3 might have been overlooked in seaweeds which are often rich in 20:5ω3. This report is of an examination of 15 common Nova Scotian seaweeds for 18:5ω3 and other novel marine fatty acids of possible commercial or scientific importance. Existing information of this type from the North Atlantic littoral (Chuecas & Riley 1966; Jamieson & Reid 1972b; Klenk et al 1963; Laur 1965; Wagner & Pohl 1965) is supplemented by details of isomers and other structures ascertained by open-tubular gas-liquid chromatographic technology. A brief report has appeared elsewhere (Ackman & McLachlan 1978).

MATERIALS and METHODS

Seaweeds were collected in various Nova Scotian localities in the autumn of 1977. The species, locations and dates are given in Table 1.

Table 1. Sample code, species, collection site, and date of collection.

Code No.	Species	Collection Site	Date
	Chlorophyceae		
20	Acrosiphonia arcta	Finck Cove, Hfx. Co.	15/2/77
19	Blidingia minima	Finck Cove, Hfx. Co.	15/2/77
	Phaeophyceae		
13	Agarum cribrosum	Paddy Head, Hfx. Co.	1/2/77
7	Alaria esculenta	Finck Cove, Hfx. Co.	6/12/77
3	Ascophyllum nodosum	Finck Cove, Hfx. Co.	29/11/77
4	Fucus vesiculosus	Finck Cove, Hfx. Co.	29/11/76
9	Laminaria digitata	Finck Cove, Hfx. Co.	6/12/77
15	Ralfsia fungiformis	Gulliver Cove, Digby Co.	7/2/77
	Rhodophyceae		
8	Chondrus crispus	Finck Cove, Hfx.Co.	6/12/76
17	Gigartina stellata	Gulliver Cove, Digby Co.	7/2/77
18	Halosaccion ramentaceum	Finck Cove, Hfx. Co.	15/2/77
10	Palmaria palmata	Finck Cove, Hfx. Co.	23/12/77
14	Polysiphonia lanosa	Paddy Head, Hfx. Co.	1/2/77
16	Porphyra leucosticta	Gulliver Cove, Digby Co.	7/2/77
11	Ptilota serrata	Finck Cove, Hfx. Co.	18/1/77

Species were separated, transported to the laboratory in plastic bags and ice, and frozen for storage prior to study, usually within a few weeks of collection. For analyses, samples were thawed slowly at room temperature and blotted free of seawater. All obvious foreign material was removed. The basic extraction technique employed was to

weigh 10 g of coarsely-chopped samples into a 250 ml round-bottomed standard-taper flask, add a solution of NaOH (0.5N) in MeOH (100 ml), and reflux under nitrogen for 2 h. After cooling, the solution was filtered through pre-rinsed (MeOH) coarse filter paper into a nitrogen-flushed 250 ml separating funnel containing distilled water (100 ml) and redistilled n-hexanes (50 ml). After shaking and allowing to clear, the bottom layer was drained into a second funnel (nitrogen-flush) containing n-hexanes (50 ml) and reextracted. The two n-hexane extracts of unsaponifiable materials were discarded. From the second extraction, the separated bottom layer was drained into a third funnel (nitrogen-flush) containing n-hexanes (50 ml), and concentrated HCl (approximately 5 ml) was added slowly with swirling of the separatory funnel. After allowing the contents to cool to room temperature under nitrogen, the mixture was vigorously shaken and checked for acidity. Any objectionable solids formed by acidification were removed by rapid filtration through coarse filter paper pre-rinsed with n-hexanes. Separation of the phases (overnight if convenient or necessary) was followed by a similar reextraction of the bottom aqueous phase. The combined n-hexanes phases recovered from the two extractions of acids were then washed (nitrogen) with distilled water in 50 ml lots until neutral. Any emulsion problems were controlled with a few drops of MeOH or of a saturated solution of Na_2SO_4. The final n-hexanes extract was dried over anhydrous Na_2SO_4 in a 125 ml erlenmeyer flask and filtered through pre-rinsed (hexanes) fine filter paper. The recovered fatty acids were converted to methyl esters with BF_3-MeOH (Morrison & Smith 1964), and one-third of the methyl esters was hydrogenated over platinum catalyst (Ackman et al 1967). In two cases, Ascophyllum nodosum and Fucus vesiculosus, 100 g lots of coarsely-chopped algae were blended with $CHCl_3$ (100 ml) and MeOH (50 ml) in a Sorvall Omni-mixer. The blades did not finely mince the algae as had been expected and the extraction was judged incomplete. The extracted total lipid was, however, recovered and saponified essentially as described above for whole algae. The fatty acids were recovered and converted to methyl esters. Gas-liquid chromatography used stainless-steel wall-coated columns, 46 m x 0.25 mm I.D., in a Perkin-Elmer 3920 apparatus with flame ionization detection. The liquid phases were SILAR-5CP (Applied Science Laboratories) and Apiezon-L. Respective operating temperatures were 170° and 180°C. The carrier gas was helium at a pressure of 3.5 $kgcm^{-2}$ (50 psig). The inlet-splitter system and manifold were operated at 225°C and output was recorded on a Honeywell 1 mV recorder fitted with a Disc Instruments Inc. ball and disc type integrator.

Peak areas were converted to weight percent through appropriate correction factors (Ackman et al 1967). Retention data and examples of chromatograms including 18:5ω3 will be found elsewhere (Ackman et al 1974; Mayzaud et al 1976).

RESULTS and DISCUSSION

The methanolic alkaline extraction of 10 g samples gave about the same yield of fatty acids (ca 0.02-0.05 g in most cases) as were recovered from the lipid extracted from the two 100 g samples with $CHCl_3$/MeOH. No obvious qualitative or quantitative difference in composition of

methyl esters was noted in the products from the two techniques and the alkali method is recommended as simpler and safer, and yielding ample ester for GLC study. The sole case of a poor yield from this procedure was of a Cladophora rupestris sample, but extraction of a second lot from the same sample gave the normal weight recovery of fatty acid. Pending further study the detailed fatty acid composition of this sample is omitted from this report.

Fatty acids found in most terrestrial plants extend from C_{14} to C_{18}, with a few families also including C_{20} and C_{22} acids, mostly monoethylenic. Aquatic plants (unicellular algae and seaweeds), especially marine species, afford a greater vareity of fatty acids than do most terrestrial plants (Hilditch & Williams 1964; Pohl & Wagner 1972; Wood 1974). All animals include polyethylenic fatty acids of the C_{20} and C_{22} chain lengths in their vital cellular membrane, and aquatic species also accumulate these in their depot fats (Ackman 1964; Hilditch & Williams 1964; Wagner & Pohl 1966). It is often convenient to classify plants and animals by the proportions of the three basic types of fatty acids, saturated, monoethylenic, and polyethylenic. Table II shows, however, that these totals are not especially different in the three divisions represented in the study.

The saturated fatty acids 14:0 and 16:0 are an interesting pair, as 16:0 is common and is the typical saturated fatty acid of terrestrial plants, whereas 14:0 is generally much less common (Hilditch & Williams 1964) except among certain families also containing 12:0 (lauric acid). As shown in Table II, 14:0 is relatively low in the two Chlorophyta listed (and in the unlisted C. rupestris), although Jamieson and Reid (1972b) reported appreciable 14:0 in C. rupestris (4.9%) and Cladophora albida (6.4%). Brush and Percival (1972) refer to 14:0 as "small" as galactolipids of Chlorophyta, and Hayashi et al (1974) give only 0.6% for Ulva pertusa, whereas Sato (1975) shows 3.6% for Codium fragile. The high proportion of 14:0 in the Phaeophyta, A. nodosum and F. vesiculosus, is confirmed by Jamieson and Reid (1972b), but the Rhodophyta, as well as some others of the Phaeophyta, also have moderately high levels of 14:0 except for Porphyra leucosticta. An apparent lack of saturated acid chain extensions (eg 14:0→16:0→18:0) in F. vesiculosus is also shown by the extremely low level of 18:0 (0.1%) compared to the percentages in the other algae. The exception (Table II) to the general importance of 14:0 is P. leucosticta, but a Porphyra sp studied by Sato et al (1974) had 14:0 at about 5% under some growth conditions but not others. Generalities are evidently difficult to sustain even with common fatty acids. Since odd-chain saturated acids are not reported in any other recent fatty acid study of North Atlantic seaweeds, except for 15:0 in some Chlorophyta examined by Jamieson and Reid (1972b), two are included in Table II. There is, however, no unusual proportion except in Agarum cribrosum (Laminarales). In this sample, 15:0 was accompanied by an equal amount of 15:1, presumably the Δ^9 (ie ω6) isomer, suggesting a basic involvement in the metabolism of 14:0 and/or 16:0. Since 18:0 was also high in A. cribrosum, it is not unexpected to find 17:0 and 17:1 (not shown) high in the same sample for similar reasons.

The monoethylenic acid 16:1ω7 (palmitoleic acid) is of special interest (Koroly & Corner 1976) because it may or may not be chain extended to 18:1ω7 (cis-vaccenic acid). The latter is not usually separated from 18:1ω9 (oleic acid) by packed-column gas-liquid chromatography. This separation is, however, readily achieved by open-tubular gas-liquid chromatography. Two examples, A. cribrosum (No. 13) and Acrosiphonia

Table II. Summary of fatty acid classes and of certain common saturated fatty acids in weight percent in 15 species of seaweeds

Fatty Acid	Acrosiphonia arcta	Blidingia minima
Σsaturated	35	21
Σmonoethylenic	13	20
Σpolyethylenic	59	59
14:0	1.2	0.5
16:0	33.4	20.0
18:0	0.2	0.1
15:0	0.2	0.1
17:0	0.2	<0.1

	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus
Σsaturated	24	34	25	18	27	27
Σmonoethylenic	26	27	23	11	25	10
Σpolyethylenic	50	39	52	71	48	63
14:0	1.9	5.4	5.4	5.5	12.4	21.5
16:0	19.9	23.7	19.8	12.4	15.0	5.5
18:0	0.5	1.2	0.7	0.7	0.3	<0.1
15:0	0.3	1.7	0.3	0.3	0.5	0.4
17:0	0.3	0.7	0.2	0.2	0.3	0.3

	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
Σsaturated	30	40	22	36	30	24	48
Σmonoethylenic	11	13	7	11	20	8	24
Σpolyethylenic	59	47	71	53	50	68	28
14:0	3.7	2.3	6.4	8.9	1.9	0.2	3.5
16:0	26.7	35.6	14.0	25.0	25.6	22.7	41.7
18:0	0.5	0.6	0.4	0.8	0.5	0.4	1.3
15:0	0.3	0.4	0.3	0.6	0.4	0.1	0.6
17:0	0.2	0.7	0.1	0.1	0.4	0.1	0.5

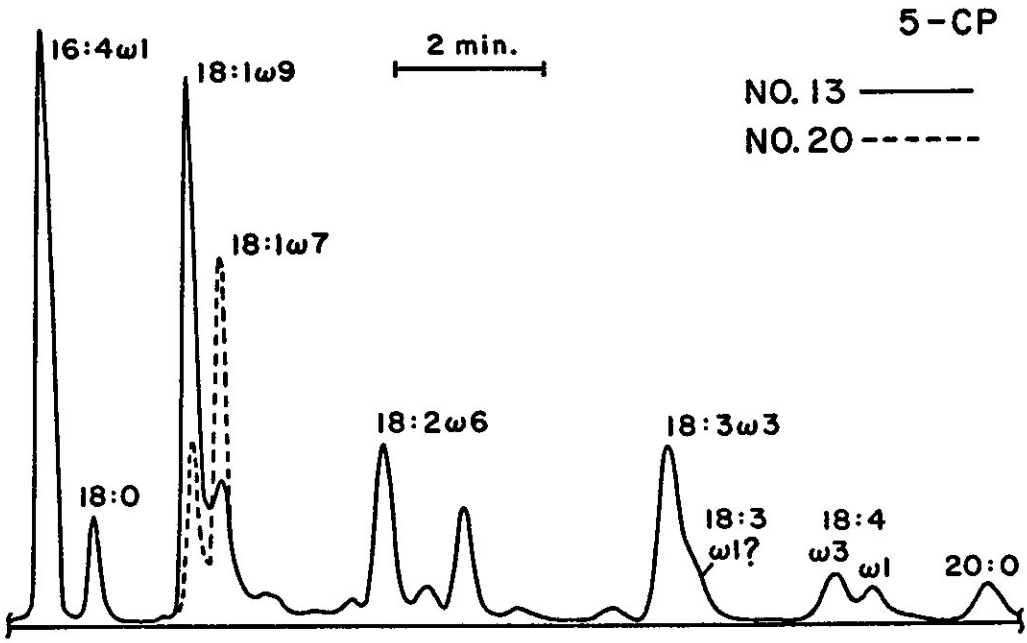

Fig 1. Part of the chart record from analysis by open-tubular gas-liquid chromatography on SILAR-5CP of the methyl esters of fatty acids from A. cribrosum (No. 13) compared with the 18:1 isomer sector of A. arcta (No. 20) to show differences in 18:1 isomer proportions. Note unknown shoulder following 18:3ω3 and small proportion of possible 18:4ω1. These are discussed in the text.

arcta (No. 20), are illustrated in Figure 1. In the one case there is a 6:1 ratio of ω9 to ω7, in the other the ratio is 0.6:1. The information provided by the separation of these two 18:1 isomers (Table III) is therefore novel. The dominance of 18:1ω7 over 18:1ω9 in the Chlorophyta is confirmed by the Cladophora data (not included) and separates this division from the Phaeophyta, although various Rhodophyta, especially Polysiphonia lanosa, also have high proportions of 18:1ω7 presumably derived from 16:1ω7. In view of possible contributions of macrophyte material to the local water column proposed by Mann (1972), these acids could be useful markers for biochemical processes, including local geochemistry (Volkman & Johns 1977) in the form of detritus fallout. Discussion of the longer chain (C_{20}, C_{22}) monoethylenic isomers is reserved for another report following further study. More than one isomer was observed in certain cases, but open-tubular gas-liquid chromatography can not separate certain isomers from each other (eg 22:1ω13 and 22:1ω11), although both can be separated from 22:1ω9 as shown by Ackman and Castell (1966).

The proportions among the C_{18} polyunsaturated acids (Table V) and C_{20} polyethylenic acids (Tables VI & VII) for Chondrus crispus, Gigartina stellata, Laminaria digitata, Alaria esculenta, A. nodosum, and F. vesiculosus are broadly in agreement with those reported by Jamieson and Reid (1972b). The absence (Table VII) of 22:5ω3 and 22:6ω3 in A. nodosum and F. vesiculosus (Fucales) differs from Jamieson and Reid's report (1972) of as much as 1.9% of 22:5ω3 in F. vesiculosis. Such chain extension in this class of macrophyte is not improbable as we noted 5.2%

Table III. Important and unusual isomers of C_{14}, C_{16}, and C_{18} monoethylenic fatty acids in weight percent

Isomers	Acrosiphonia arcta	Blidingia minima
16:1ω7	7.2	3.3
18:1ω9	1.3	0.1
18:1ω7	2.3	14.0
Trans 14:1ω11	<0.1	0.0
Trans 16:1ω13	0.8	2.5

	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus
16:1ω7	1.6	13.0	2.9	0.5	2.1	1.1
18:1ω9	22.1	5.9	18.9	6.9	15.9	6.9
18:1ω7	0.7	0.9	0.2	0.3	0.7	0.1
Trans 14:1ω11	0.3	0.8	0.2	0.5	0.0	0.0
Trans 16:1ω13	0.7	0.1	0.6	1.9	0.2	0.3

	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
16:1ω7	0.5	3.2	0.2	0.6	8.2	0.4	7.7
18:1ω9	6.2	6.0	3.0	3.6	1.7	2.0	9.4
18:1ω7	1.5	2.6	0.8	1.5	8.2	1.0	5.1
Trans 14:1ω11	0.1	0.0	0.1	0.1	0.0	<0.1	0.1
Trans 16:1ω13	0.9	0.4	1.8	1.8	0.9	1.9	0.1

Table IV. Isomers of C_{20} and C_{22}, and total 24:1 monoethylenic fatty acids, in weight percent

Isomers	Acrosiphonia arcta	Blidingia minima
20:1ω11	<0.1	0.0
20:1ω9	1.1	0.0
20:1ω7	<0.1	<0.1
22:1ω13 + 11	0.0	0.0
22:1ω9	0.3	0.0
22:1ω7	0.0	<0.1
24:1	<0.1	0.0

	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus
20:1ω11	0.1	0.1	<0.1	0.1	0.2	0.1
20:1ω9	0.1	<0.1	<0.1	0.1	0.1	0.0
20:1ω7	0.1	<0.1	<0.1	0.2	<0.1	<0.1
22:1ω13 + 11	0.0	<0.1	0.0	0.0	0.2	0.1
22:1ω9	0.0	<0.1	0.0	0.0	0.0	0.0
22:1ω7	0.0	0.0	0.0	0.0	0.0	0.0
24:1	0.0	0.0	0.0	0.0	0.4	0.1

	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
20:1ω11	<0.1	0.0	0.0	<0.1	<0.1	0.0	0.2
20:1ω9	<0.1	0.2	0.5	1.2	0.1	2.3	0.1
20:1ω7	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	0.0
22:1ω13 + 11	0.0	0.0	<0.1	0.1	0.1	<0.1	<0.1
22:1ω9	0.0	0.2	0.2	0.8	<0.1	<0.1	0.1
22:1ω7	0.0	0.0	<0.1	0.0	0.0	0.0	0.0
24:1	0.0	<0.1	<0.1	0.0	0.0	0.0	0.2

Table V. Common isomers of C_{18} polyethylenic fatty acids in weight percent

Isomers	Acrosiphonia arcta	Blidingia minima					
18:2ω6	4.0	3.0					
18:3ω6	0.5	0.4					
18:3ω3	17.4	17.0					
18:4ω3	11.4	15.7					
	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllium nodosum	Fucus vesiculosus	
18:2ω6	9.2	2.2	8.5	4.6	6.0	9.4	
18:3ω6	1.0	0.2	0.5	0.6	0.2	0.7	
18:3ω3	5.0	3.7*	6.3	10.6	5.1	10.4	
18:4ω3	7.2	1.2**	9.7	24.6	6.2	13.5	
	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
18:2ω6	2.4	1.1	0.6	0.6	2.2	1.4	1.2
18:3ω6	0.9	0.4	0.2	0.2	0.7	0.5	0.2
18:3ω3	1.9	3.1	1.0	0.9	1.0*	0.1	0.2*
18:4ω3	3.5	0.7	2.6	1.8	2.1	0.2	0.3

* Possible 18:3ω1 may accompany 18:3ω3 in A. cribrosum and P. lanosa; same or different component found in P. serrata.

** Accompanied by 0.8% tentatively identified 18:4ω1.

Table VI. Selected polyethylenic C_{20} and C_{22} fatty acids of the ω6 family in weight percent

Fatty Acid	Acrosiphonia arcta	Blidingia minima					
20:2ω6	0.1	<0.1					
20:3ω6	0.0	0.1					
20:4ω6	0.1	0.2					
22:4ω6	0.0	0.3					
	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus	
20:2ω6	0.2	0.1	0.1	0.1	1.8	0.2	
20:3ω6	1.3	0.1	0.4	0.4	0.3	0.7	
20:4ω6	9.7	7.2	11.4	13.6	13.3	12.6	
22:4ω6	<0.1	0.5	0.0	<0.1	0.1	0.1	
	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
20:2ω6	0.1	0.1	<0.1	0.1	0.2	1.1	0.3
20:3ω6	0.4	0.2	<0.1	0.1	0.5	2.2	<0.1
20:4ω6	23.9	14.3	0.9	0.9	3.1	6.3	3.0
22:4ω6	0.0	0.2	<0.1	0.1	0.0	0.0	<0.1

Table VII. Selected polyethylenic C_{20} and C_{22} fatty acids including the ω3 family in weight percent

Fatty Acid	Acrosiphonia arcta	Blidingia minima
20:3ω3	0.1	0.1
20:4ω3	0.7	0.3
20:5ω3	2.3	0.6
22:5ω3	0.6	0.7
22:6ω3	1.9	0.0

	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus
20:3ω3	0.1	0.1	<0.1	<0.1	0.4	0.1
20:4ω3	0.1	0.1	0.4	0.5	0.1	0.8
20:5ω3	14.3	4.6	13.9	15.7	9.7	14.3
22:5ω3	0.1	5.2	0.0	0.0	0.0	0.0
22:6ω3	0.1	0.0	0.0	0.0	0.0	0.0
Δ^5,11 20:2	0.0	<0.1	<0.1	<0.1	0.1	<0.1
Δ^5,11,14 20:3	<0.1	<0.1	<0.1	0.1	2.1	0.1
Δ^5,11,14,17 20:4	0.1	0.1	0.1	0.1	1.8	0.1

	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
20:3ω3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3
20:4ω3	0.1	0.1	0.1	0.1	0.1	0.2	<0.1
20:5ω3	24.9	24.3	63.7	45.5	36.4	56.0	21.6
22:5ω3	0.0	0.2	<0.1	0.0	0.0	0.1	0.0
22:6ω3	0.0	0.0	<0.1	0.0	0.0	0.0	0.0
Δ^5,11 20:2	0.0	0.0	0.0	<0.1	<0.1	0.0	0.0
Δ^5,11,14 20:3	0.1	0.0	0.0	0.1	<0.1	0.0	0.0
Δ^5,11,14,17 20:4	<0.1	0.0	0.0	0.2	0.1	<0.1	0.1

Table VIII. Selected tentatively identified polyethylenic C_{16} fatty acids, and totals of other similar acids, in weight percent

Fatty Acid	Acrosiphonia arcta	Blidingia minima
16:2ω6	1.0	0.6
16:3ω6	<0.1	<0.1
16:3ω3	3.0	1.8
16:4ω3	7.7	16.4
16:2ω7	0.0	0.1
16:2ω4	0.2	0.9
16:4ω1	0.2	0.1
Other*	0.0	0.3

	Ralfsia fungiformis	Agarum cribrosum	Laminaria digitata	Alaria esculenta	Ascophyllum nodosum	Fucus vesiculosus
16:2ω6	<0.1	0.0	<0.1	0.0	0.0	<0.1
16:3ω6	<0.1	0.3	<0.1	0.0	0.0	0.0
16:3ω3	<0.1	<0.1	<0.1	0.1	0.1	0.1
16:4ω3	0.1	0.0	<0.1	<0.1	<0.1	0.2
16:2ω7	<0.1	0.6	<0.1	0.1	0.0	<0.1
16:2ω4	0.0	3.3	0.0	0.0	0.0	0.0
16:4ω1	0.1	6.6	0.1	0.1	<0.1	0.1
Other*	<0.1	1.1	<0.1	0.1	0.0	<0.1

	Chondrus crispus	Gigartina stellata	Halosaccion ramentaceum	Palmaria palmata	Polysiphonia lanosa	Porphyra leucosticta	Ptilota serrata
16:2ω6	<0.1	<0.1	0.0	0.1	<0.1	0.0	0.0
16:3ω6	<0.1	0.0	<0.1	0.1	<0.1	<0.1	<0.1
16:3ω3	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1
16:4ω3	<0.1	1.1	<0.1	<0.1	<0.1	<0.1	<0.1
16:2ω7	0.0	<0.1	0.1	0.0	<0.1	<0.1	<0.0
16:2ω4	0.7	0.2	0.1	<0.1	2.9	0.0	0.0
16:4ω1	0.1	0.1	0.1	0.0	0.2	0.0	0.1
Other*	0.0	<0.1	2.1	2.9	<0.1	<0.1	0.0

* Mostly component of ECL (SILAR-5CP) of 16.63 important at 2.1% in H. ramentaceum and 2.9% in P. palmata, and possible 16:3ω1 in A. cribrosum.

of 22:5ω3 in A. cribrosum and traces (ca 0.1%) of 22:5ω3 and 22:6ω3 in Ralfsia fungiformis. Our work confirms the report of Jamieson and Reid (1972b) that A. nodosum, but not F. vesiculosus, is relatively rich in the unusual series of polyethylenic acids with isolated Δ^5 unsaturation. These occur in different forms in various terrestrial plant families (Fedeli & Tacini 1971; Jamieson & Reid 1972a; Pohl & Wagner 1972), as well as sponges (Morales & Litchfield 1976), shellfish, and other marine forms (Paradis & Ackman 1977). Traces of these acids also were found in the other Phaeophyta, and in the Rhodophyta, except for G. stellata and Halosaccion ramentaceum.

The emphasis on the "ω6" acids in algae (Table VI), especially 20:4ω6 (Nichols & Appleby 1969), is always a novelty to lipid chemists as this series of fatty acids, essential to terrestrial animals (Crawford et al 1976), is replaced by the "ω3" acids in most lipids of marine fish, and to some extent in freshwater fish (Ackman 1967).

The C_{16} polyethylenic fatty acids present an unusual analytical problem, owing to the possible coincidences with iso and anteiso 17:0, 17:1 etc. They also may include a peculiar series with "ω" values of 1, 4 and 7, found when 16:0 is subject to the desaturation processes intended to produce the polyethylenic C_{18} acids from 18:0. These C_{16} polyunsaturated acids are common in certain phytoplankters (Ackman 1964). In Table VIII are presented data based on tentative identifications of some of the important polyunsaturated C_{16} acids and a total for others assumed to be acids of this chain length. One with an ECL (equivalent chain length) of 17.00 on SILAR-5CP, probably 16:2ω4, was important in P. lanosa at 2.9%, in A. cribrosum at 3.3%, in Blidingia minima at 0.9% in C. crispus at 0.7% and in G. stellata and A. arcta at 0.2% each. In close proximity, at ECL 16.79 in SILAR-5CP, is the component listed in Table VIII as 16:2ω6. The A. cribrosum sample was also remarkable for the high proportion of the component tentatively identified throughout the study as 16:4ω1. (ECL 17.83 on SILAR-5CP and an ECL of 15.40 on AP-L). It is probably significant that this sample had the lowest level among the Phaeophyta (Table V) of the 18:4ω3 related as described by Ackman (1964), but this was accompanied by a relatively important component (Fig 1,2) thought to be 18:4ω1. The tentatively identified 16:4ω3 (ECL 17.63 on SILAR-5CP and 15.35 on AP-L) amounted to 1.1% in G. stellata, but otherwise was important only in the two samples of Chlorophyta. A C. rupestris sample (data not presented) included the component presumed to be 16:4ω3 at a moderately high proportion of unsaturation. Jamieson and Reid (1972b) list 16:4ω3 at 10 to 20% in three Chlorophyta and 7.2% in another. Our levels of 16:3ω3 for Chlorophyta are also similar to theirs at 2 to 3%. These authors have discussed the discrepancies between their work and earlier studies for fatty acids of all chain lengths. It is suspected that trace percentages of 16:4ω3 and 16:4ω1 could in some cases be 17:2 isomers.

One other interesting but unidentified component in various samples disappeared on hydrogenation and was therefore unsaturated and probably C_{16}. It had an ECL of 16.63 on SILAR-5CP and an ECL of 15.95 on AP-L. This was important only in Palmaria palmata at 2.9% (Table VIII), and in H. ramentaceum at 2.0%. Evidently further work detailing the structures of the expected C_{16} polyunsaturated acids and observed peaks in GLC is required, especially on newer type GLC phases of the cyanoalkyl types such as SILAR-5CP. There were various other unusual fatty acids noted in this study. In addition to the trans-3-hexadecenoic

(trans-16:1ω13) acid already reported for common North Atlantic algae at approximately the 1% level by Jamieson and Reid (1972b), the corresponding trans-3-tetradecenoic and some trans-2-hexadecenoic acids were suspected as trace components in some samples (Table III). There were also several occurrences of unsaturated materials giving iso-15:0 on hydrogenation, and known to be monoethylenic from their retention times on the two liquid phases studied. Such acids are more often associated with bacteria (Boon et al 1977; Weeks 1976) than plants and may not be actual algal fatty acids.

Despite the high proportion of 20:5ω3 in several species, especially in the Rhodophyta (see below) no significant amount (ie 0.1% or more) of 18:5ω3 could be found in any of the macrophytes examined. A trace component, possibly 18:5ω3, at (0.02%) in P. leucosticta was quantitatively the most likely candidate. Each mixture of methyl esters was examined critically for this component on the polar liquid phase SILAR-5CP and the non-polar Apiezon-L. To confirm 18:5ω3, matching component peaks would have to be found in analyses on both polar and non-polar columns. Often a number of components on Apiezon-L (ed Fig 2) quantitatively matched one or more components on SILAR-5CP (Fig 1) and confused the issue. The 18:5ω3 position on SILAR-5CP at approximately ECL 20.13 is not presented in Figure 1 as the attenuation employed did not show any component of the relative size of "A" of Figure 2.

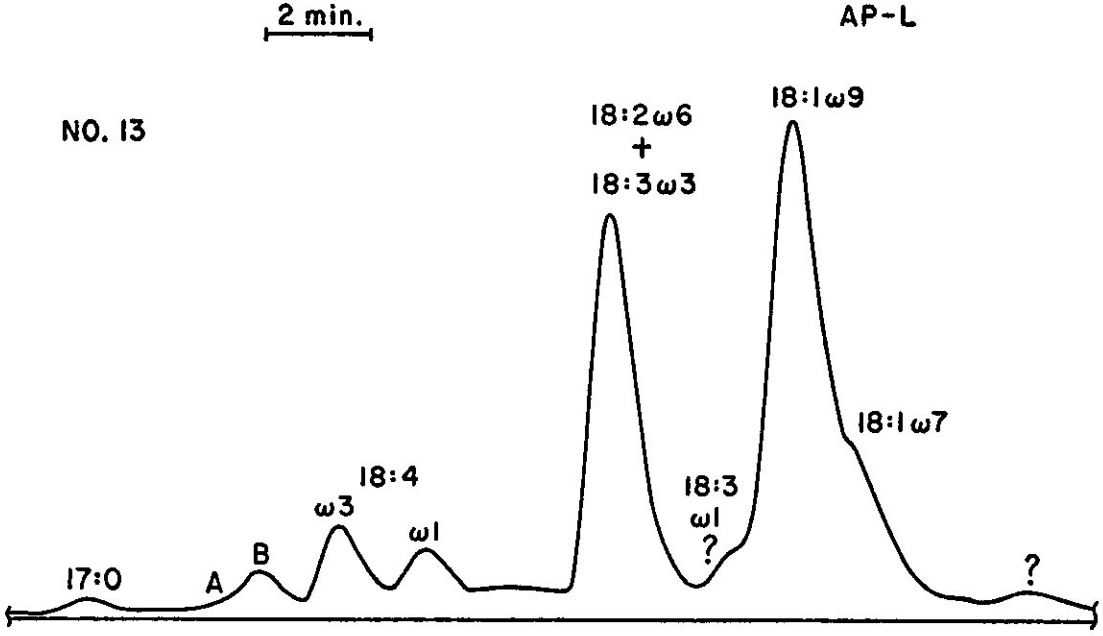

Fig 2. Part of the chart record from analysis by open-tubular gas-liquid chromatography on Apiezon-L of the methyl esters of fatty acids from A. cribrosum. Note two isomers of 18:4, shoulder for possible 18:3ω1, and minor component B. (See Fig 1 and text discussion).

The open-tubular gas-liquid chromatographic analyses resolve some questions and create others. In the analysis of A. cribrosum on the polar phase SILAR-5CP (Fig 1) a shoulder was observed following the 18:3ω3 peak. Another laboratory independently observed the same effect (M. Ikawa, in litt). Speculative calculations (Ackman & Hooper 1973a; 1973b; Ackman et al 1974) of retention time for various C_{18} polyunsaturated fatty acids indicate that 18:4ω6 would have an ECL value on SILAR-5CP of between 19.45 and 19.61 (estimated ECL for shoulder component is 19.50). On AP-L 18:4ω6 can be more closely calculated to have an ECL value of about 17.17, and a component "B" of the correct size is located in this position at ECL 17.18 (Fig 2). Alternatively, instead of a chain shortening product (20:4ω6→18:4ω6), chain elongation of 16:3ωl would be more probable (compare 16:4ωl→18:4ωl), and lead to a possible 18:3ωl (the shoulder so marked in Fig 2). Different proportions of a component following or trailing 18:3ω3 (SILAR-5CP analyses) also were seen in the mutually related (Ceramiales) P. serrata and P. lanosa of the Rhodophyceae. These appear to lack the 16:3ωl precursor for 18:3ωl, and are not especially rich in 20:4ω6. These algae may contain yet another unusual C_{18} fatty acid requiring further study.

It must be concluded that the chance of finding quantities of 3,6,9, 12,15-octadecapentanoic acid in macrophytes appears slim although several of the samples studied had high proportions of the related 20:5ω3. However, there is a wealth of detail in the fatty acids of Nova Scotian seaweeds which can be conveniently revealed by open-tubular gas-liquid chromatography for more detailed study. The levels of fatty acids in seaweeds are low (Hayashi et al 1974; Wagner & Pohl 1965) but when compared to the limited yields from cultures of unicellular algae (Ackman et al 1968; Collyer 1962; Ivanova & Popov 1972; Stoianova & Mladenova 1972) seaweeds offer immediate large-scale sources for recovery of specific fatty acids, for example the extremely pure 16:1ω7 (palmitoleic acid) of G. stellata or the virtually pure 18:1ω7 (cis-vaccenic acid) of B. minima.

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