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HYDROCARBONS IN ALASKAN MARINE INTERTIDAL ALGAE

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Key Word Index—Chlorophyta; Phaeophyta; Rhodophyta; benthic algae; hydrocarbons; pristane; phytane.

INTRODUCTION

There are several reasons for studying the hydrocarbon composition of marine benthic algae: to investigate chemotaxonomic relationships; to provide information related to possible petroleum contamination; and to identify components for use in studies of biogeochemical cycling. In view of this, it is surprising how few reports of hydrocarbons in macrophytic algae have appeared. An extensive group of plants from the coastal waters of the northeastern U.S.A. has been analysed [1–3]. However, only four plants collected in the Pacific and the Gulf of Mexico have been examined [4–6].

Here we report on the determination of hydrocarbons in 11 species of benthic algae collected at 11 locations within lower Cook Inlet, Alaska. These analyses augment the previous work with Pacific plants and allow comparisons between Atlantic and Pacific taxa.

RESULTS AND DISCUSSION

Cook Inlet is a large tidal estuary in south-central Alaska in which ice formation and scour occur in many intertidal areas during winter but air temperatures frequently exceed 20° in summer. Several of the genera and species whose hydrocarbons have been determined for New England [1–3] also occur in Cook Inlet. Tables 1 and 2 show the saturated and olefinic hydrocarbons found in the algae analysed. Table 2 also gives the sampling locations and dates.

Enteromorpha linza was the only member of Chlorophyta examined: its hydrocarbons were dominated by heptadecene (17:1) which was 79% of the total. Heptadecene was not detected in any of the other algae analysed in this study. In other studies [2, 3] heptadecene has been found to be dominant only in the genera *Enteromorpha* and *Ulva*, both members of the Ulotrichales. Among the saturated hydrocarbons of *E. linza*, pentadecane (C_{15}) was the most abundant being 8% of the total hydrocarbons.

Four species from Rhodophyta were examined: *Constantinea subulifera*, *Halosaccion* sp., *Halosaccion glandiforme*, and *Palmeria palmata*. In each of these, the most abundant single compound was heptadecane (C_{17}) which ranged from 59 to 99% of the total hydrocarbons in all but one of samples analysed. The second sample of *C. subulifera* contained only 20% heptadecane, but possessed a substantial amount of exogenous hydrocarbons (see below).

Six species of Phaeophyta were analysed: *Agarum cribosum*, *Alaria* sp., *Cymathere triplicata*, *Desmarestia aculeata*, *Fucus distichus*, and *Laminaria saccharina*. The

Table 1. Saturated hydrocarbons in marine benthic algae

Sample No.	Species	Hydrocarbons (μg/g dry wt)* 14	15	16	17	Pr	18	Ph	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Total sat.
1.	*Agarum cribosum*	0.54	5.5		0.21					0.06	0.07		0.07										8.3
2.	*Alaria* sp.		19.8		0.56																		40.1
3.	*Constantinea subulifera*				13.1				0.38		0.26	0.17	0.48	0.19	0.48	0.21	0.97	0.26	1.6		1.7	0.27	24.7
4.	*† „ „		0.10	0.04	35.4		0.05		0.39	0.04	0.05		0.04										36.4
5.	*Cymathere triplicata*	0.79	29.6																				30.1
6.	*Desmarestia aculeata*		0.85		0.57																		1.4
7.	„ „		0.05		0.16																		0.21
8.	*Enteromorpha linza*	0.82	10.2	t	t						0.73		0.73		1.63		0.63		0.33				15.1
9.	*Fucus distichus*		22.6																				22.6
10.	„ „		594																				594
11.	„ „		308																				308
12.	„ „		78.3																				78.3
13.	„ „		36.4																				36.4
14.	„ „		296																				296
15.	„ „		32.5																				32.5
16.	„ „		22.7	0.42	1.1	0.79	1.7	0.80	3.4	5.7	4.1	3.1	1.7	1.2	0.97	0.85	0.99	0.80					153
17.	*† „ „		3.9	0.08	0.29	0.21	0.56	0.21	0.90	0.93	0.64												7.9
18.	„ „	0.10	12.7	0.36	0.67	0.35	0.37	0.18	0.48	0.36	0.34		0.13	0.07	0.08	0.05	0.08	0.03	0.05		0.06		40.1
19.	*† „ „		9.5																				10.8
20.	„ „		28.5																				28.5
21.	„ „		22.7																				22.7
22.	„ „		18.6																				18.6
23.	„ „		23.3									0.12	0.38	0.65	0.98	1.2	1.7	1.5	2.3	1.3	1.8	0.72	36.0
24.	„ „		29.2																				29.2
25.	*Halosaccion* sp.	0.24	14.7	0.42	75.0								0.07		0.14		0.53						92.7
26.	*Halosaccion glandiforme*		t		1.8						0.28						0.16						2.2
27.	*Laminaria saccharina*		18.5																				18.9
28.	„ „		36.4																				36.6
29.	*Palmeria palmata*		0.06	0.07	26.7																		26.8

* 14, etc. = carbon length; Pr = pristane; Ph = phytane.
† Washed (see text).

Table 2. Olefinic hydrocarbons in marine benthic algae

Sample No.	Species	Date of collection	Location	Hydrocarbons (μg/g dry wt)* 14:1	17:1	19:3	19:4	19:5	21:5	21:6	Sq	Total unsat.	% H_2O
1.	*Agarum cribosum*	5 May 77	Anchor Point	0.46					1.0	6.2	1.5	10.8	80.9
2.	*Alaria* sp.	5 May 77	Anchor Point						46.7	19.9	1.8	50.3	86.6
3.	*Constantinea sublifera*	5 May 77	Anchor Point			1.4					1.1	42.4	79.2
4.	*† „ „	5 May 77	Anchor Point		0.16						2.4	3.1	93.3
5.	*Cymathere triplicata*	22 June 77	Koyuktolik Bay						9.8	5.4	1.3	16.9	77.8
6.	*Desmarestia aculeata*	7 May 77	Diamond Gulch					3.2		51.3	14.1	68.6	82.5
7.	„ „	6 May 77	Homer Spit (NW)					5.3		31.2	2.6	39.1	81.1
8.	*Enteromorpha linza*	4 May 77	Coal Bay		107					2.8	4.4	120	79.8
9.	*Fucus distichus*	25 June 77	Cottonwood Bay				0.99			109	54.1	165	86.3
10.	„ „	7 May 77	Diamond Gulch							2810	584	3420	79.4
11.	„ „	7 May 77	Diamond Gulch				t			1520	335	1860	79.4
12.	„ „	22 June 77	Koyuktolik Bay				2.0			155	70.2	229	82.1
13.	„ „	26 June 77	Douglas River				1.6			90.6	8.2	109	86.1
14.	„ „	4 May 77	Coal Bay				17.2			1050	127	1210	89.3
15.	„ „	4 May 77	Coal Bay							199	19.0	139	89.3
16.	„ „	6 May 77	Homer Spit (End)					3.0		112	98.9	277	79.1
17.	*† „ „	6 May 77	Homer Spit (End)							30.7	19.0	69.8	93.6
18.	„ „	2 May 78	Homer Spit (End)							116	40.2	158	79.3
19.	*† „ „	2 May 78	Homer Spit (End)							51.7	12.0	63.7	86.6
20.	„ „	25 June 77	Iniskin Bay				1.6			85.2	10.4	97.9	80.4
21.	„ „	23 June 77	Kasitsna Bay				0.68			49.2	50.3	123	80.2
22.	„ „	6 May 77	Homer Split (NW)							21.0	12.5	33.7	54.6
23.	„ „	6 May 77	Homer Spit (NW)							27.4	19.1	46.9	54.6
24.	„ „	26 June 77	Oil Bay				1.1			9.3	6.6	17.1	91.8
25.	*Halosaccion* sp.	26 June 77	Douglas River					0.43		4.0	4.0	9.6	82.4
26.	*Halosaccion glandiforme*	26 June 77	Douglas River					0.03		0.44	0.38	0.85	77.3
27.	*Laminaria saccharina*	7 May 77	Diamond Gulch							32.1	1.8	33.9	82.7
28.	„ „	6 May 77	Homer Spit (NW)							25.1	1.7	26.9	86.9
29.	*Palmeria palmata*	5 May 77	Anchor Point					0.05			0.24	0.29	74.9

* 14:1, etc. = carbon length and unsaturation; Sq = squalene.
† Washed (see text).

chief saturated hydrocarbon of these algae was pentadecane which was present in all specimens examined and was the only saturated hydrocarbon in several samples of *F. distichus*. The only exception to this was *D. aculeata* in which pentadecane and heptadecane were present in comparable amounts. Heneicosahexaene (21:6) was also present in all of the brown algae analysed, often as the most abundant hydrocarbon. This compound was present only in minor amounts, if at all, in the green and red algae. Heneicosapentaene (21:5) was also present in *A. cribosum*, *Alaria* sp., and *C. triplicata*. These two polyolefins have previously been reported in numerous marine algae [2–4, 7].

Samples of *D. aculeata*, *F. distichus* and *L. saccharina* were each collected at more than one location (Table 2). The results for these samples show a considerable degree of intraspecific qualitative similarity but substantial quantitative variation. Some of this variability may be related to the collection sites. However, the primary cause is probably differences in plant age or vigor since young rapidly growing plants and tissues have been shown to have higher hydrocarbon concentrations than mature ones [3]. Thus replicate analyses of *F. distichus* from Diamond Gulch (samples 10 and 11), Coal Bay (samples 14 and 15) and Homer Spit (NW) (samples 22 and 23) each show levels of variation similar to that between *F. distichus* samples from different locations (Tables 1 and 2).

Squalene was identified in the hydrocarbon fraction of every specimen examined in this study. This compound was reported among the hydrocarbons of *Macrocystis pyrifera* [4] but not in any of the Atlantic algae examined by Blumer and his co-workers [1–3]. However, this apparent difference is probably attributable to a difference in the preparation procedure. In Blumer's laboratory, the eluate from column chromatography was collected only before the breakthrough of the first pigmented band [2] which probably left squalene on the column. The chromatography procedures used in this study, on the other hand, eluted a considerable amount of pigment and, obviously, squalene.

It seems quite clear that the hydrocarbons discussed so far are products of endogenous biosynthesis of the algae. However, hydrocarbons which appear to be exogenous in origin also need to be considered. For example, of the sixteen collections of *Fucus distichus* examined, only sample 23, contains a predominance of odd carbon chain lengths. While similar arrays have been observed previously in *Fucus* and other algae [1, 2], other materials from the marine environment have been found to contain similar components: water collected from the surface microlayer [8] and mixed zooplankton [9]. Based on this apparently sporadic occurrence, we suspect that the source of these hydrocarbons may be bacterial. This suspicion is reinforced by the finding of quite similar arrays of hydrocarbons in some bacterial cultures [10].

Two other samples of *F. distichus* (16 and 18) and one of *Constantinea subulifera* (3) showed saturated hydrocarbons that might have been associated with exogenous coatings of the plants. To examine this possibility, additional plant material from those collections was rinsed copiously with distilled water and re-analyzed (samples 4, 17 and 19). In each case several components were reduced or removed. Sample 3, a specimen of *Constantinea subulifera*, showed normal hydrocarbons from nonadecane (C_{19}) through dotiacontane (C_{32}) with a marked dominance of odd carbon chain lengths. Also present in the unwashed *C. subulifera* were two terpenoid hydrocarbons: retene, 9.9 μg/g and simonellite, 6.7 μg/g. Both of these compounds, as well as the odd chain length normal alkanes, are associated with terrigenous plant materials [11]. These compounds are characteristic of

detrital coal and intertidal muds of the Anchor Point–Homer area. Their reduction or removal in the corresponding washed specimen (sample 4) indicates that most of this material was probably external. The apparent 3-fold increase in heptadecane concentration in *C. subulifera* can be attributed to variation between plants or among the tissues of a single plant discussed above. Samples 16 and 18, both specimens of *Fucus distichus* collected at the end of Homer Spit near a boat harbor, contained a suite of normal hydrocarbons from hexadecane (C_{16}) through at least octacosane (C_{28}), the acyclic isoprenoids pristane (Pr) and phytane (Ph) and substantial complex unresolved mixtures. Together these characteristics strongly suggest petroleum pollution, probably as fuel from the adjacent boat harbor. Rinsing these *F. distichus* with distilled water removed most of the petroleum hydrocarbons (samples 17 and 19) indicating that little if any of this material was actually present in the plants.

From the analyses reported here in comparison with previous studies we conclude that although hydrocarbon compositions of marine macrophytes reflect phylogenetic relationships, intraspecific variation largely obscures any chemotaxonomic relationships which may exist. In addition unwashed algae may contain exogenous hydrocarbons from microbial, detrital or pollution sources.

EXPERIMENTAL

Most of the algal samples were collected in the spring of 1977; the remainder in the following year. All of the collection localities (Table 2) can be found on National Ocean Survey Chart No. 8554 (U.S. Department of Commerce). Samples were frozen in glass containers and returned to the laboratory for analysis.

Whole algae were thawed, identified, diced and, together with a chrysene internal standard, refluxed for 24 hr in 4 N KOH and hexane (3:1). The cooled extracts were partitioned into hexane, dried (Na_2SO_4) and concd for column chromatography over Si gel (deactivated with 5% H_2O) which was eluted first with hexane and then with 20% dichloromethane in hexane. Each fraction was analysed by GLC (f.i.d.) using a 50 m × 0.7 mm SCOT column with OV-101 together with a smaller amount of Carbowax 20M. Temp. programming from 70 to 270° at 8°/min was used. Quantification was accomplished using a digital integrator. Quantitative results were corrected for % recovery and are expressed as μg/g dry wt basis. Peak identifications were made by use of external standards and by GC–MS (Hewlett Packard model 5930/5933).

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