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Hydrocarbons in Benthic Algae from the Eastern Gulf of Mexico

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Abstract

Thirty-six samples of benthic algae were collected from the continental shelf along the eastern Gulf of Mexico. The algae contain 82.8 ± 143 ppm aliphatic hydrocarbons by dry weight and 11.8 ± 22.7 ppm aromatic and polyolefinic hydrocarbons. The aliphatic constituents of red algae are composed almost entirely of n-C_{17} (70 to 95% of aliphatic weight); green algae have varying amounts of n-alkanes in the range of n-C_{15} to n-C_{19}, with homologous series of odd carbon number n-alkenes. Phytadienes occur as high as 740 ppm dry weight in the green algae and do not appear to be related to time or place of collection or to presence of any petroleum pollutants. The single brown algal species contains predominantly n-C_{15}. About 30% of the samples have hydrocarbons resembling degraded petroleum residues, but no petroleum pollution of recent origin was detected in any specimen.

Introduction

Hydrocarbons constitute a minor but ubiquitous component of all marine organisms. Their chemical stability and wide structural differences make hydrocarbons good environmental indicators. With release of fossil fuels into the marine environment, it is increasingly important to differentiate biogenic and fossil fuel hydrocarbons in order to accurately assess the extent of oil pollution. Knowledge of the natural hydrocarbon background is limited and must be expanded to include indicator species where possible. Various organisms (Clark and Finley, 1973; Clark *et al.*, 1973; Ehrhardt and Heinemann, 1974; Mayo *et al.*, 1974; Farrington and Medeiros, 1975; Teal, 1976) have been used successfully in this capacity, and Berner *et al.* (1976) have suggested several indicator species suitable in various coastal areas. This work presents data on the hydrocarbons in benthic algae from the warmer waters of the Gulf of Mexico and evaluates their value as petroleum pollution monitors.

Materials and Methods

Thirty-six samples of benthic algae were collected from the northeastern Gulf of Mexico continental shelf during 3 periods: (1) June, 1975; (2) October, 1975; (3) January, 1976 (Table 1). Transect I extends east-west from Fort Myers, Florida, USA, in water depths of 36 m. Transect II sites lie off Tampa, Florida in water depths of 30 to 60 m. Transect III begins at a point midway between Tampa and St. Marks, Florida in depths of 24 m extending to 42 m. Transect IV extends in a west-southwest direction from St. Marks, Florida with average water depths of 36 m. Transect V stations lie southwest of Panama City, Florida in average water depths of 36 m. A Transect VI, east-southeast of Pascagoula, Mississippi, failed to yield any algal specimens. Collections were made primarily by SCUBA divers, but some collections were made by dredge and trawl. The algae were sorted and identified, placed in acid-washed glass containers and frozen immediately. All precautions were taken to minimize hydrocarbon contamination during collection and sorting. The samples remained frozen until analysis.

The algae were thawed and washed with distilled water, homogenized in methanol using a stainless steel Waring blender, and then extracted in a Soxhlet apparatus with methanol-benzene (1:1) for 12 h. The methanol-benzene was removed and replaced with fresh benzene. Extraction

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Table 1. Benthic algae. Algal taxa and collection stations

Transect and Sample No.	Location	Species	Class
June 1975			
Transect I			
IA-A+B-6	26°25.5'N; 82°59.5'W	*Halimeda* sp.	Chlorophyceae
IA-A+B-7	26°25.5'N; 82°59.5'W	*Rhodymenia* sp.	Rhodophyceae
IA-A+B-9	26°25.5'N; 82°59.5'W	*Cystodictyon pavonium*	Chlorophyceae
Transect II			
IIA-A+B-1	27°50'N; 83°31'W	*Laurencia corallopsis,*	Rhodophyceae
		Gracilaria cylindrica +	Rhodophyceae
		G. blodgettii	Rhodophyceae
IIA-A+B-2	27°50'N; 83°31'W	*Gracilaria mammilaris*	Rhodophyceae
IIA-A+B-3	27°50'N; 83°31'W	*Eucheuma* sp.	Rhodophyceae
062-A-17	27°49'N; 55°90'W	*Caulerpa sertularoides*	Chlorophyceae
Transect III			
IIIA-A+B-2	28°29'N; 84°21'W	*Codium* sp.[a]	Chlorophyceae
047-A-3	28°34'N; 84°20'12"W	*Codium repens*	Chlorophyceae
047-A-5a	28°34'N; 84°20'12"W	*Halimeda discoidea*	Chlorophyceae
146-B-1	28°41'N; 84°24'W	*Kallymenia perforata* +	Rhodophyceae
		Dictyota dichotoma	Phyaeophyceae
147-B-5	28°40'N; 84°13'W	*Halimeda discoidea*	Chlorophyceae
247-A-2	28°36'16"N; 84°15'40"W	*Codium repens*	Chlorophyceae
251-B-25	28°33'N; 84°16'W	*Halimeda discoidea*	Chlorophyceae
October 1975			
Transect II			
IIA-A-12	27°50'N; 83°31'W	*Halymenia* sp.	Rhodophyceae
062-A-5	27°49'55"N; 83°31'10"W	*Caulerpa sertularoides*	Chlorophyceae
064-A-3	27°50'N; 83°25'W	*Gracilaria blodgetti* +	Rhodophyceae
		G. compressa	Rhodophyceae
064-B-3	27°50'N; 83°25'W	*Gracilaria blodgetti*	Rhodophyceae
Transect III			
IIIA-A-10	28°29'N; 84°21'W	*Caulerpa* sp.	Chlorophyceae
047-A-5b	28°34'N; 84°20'12"W	*Halimeda discoidea*	Chlorophyceae
146-B-14	28°41'N; 84°23'40"W	*Dictyota dichotoma*	Phyaeophyceae
147-A-2a	28°38'18"N; 84°13'54"W	*Halimeda discoidea*	Chlorophyceae
151-A-3	28°32'07"N; 84°18'24"W	*Laurencia intricata*	Rhodophyceae
247-A-4	28°36'16"N; 84°15'40"W	*Codium repens*	Chlorophyceae
251-A-3	28°32'40"N; 84°16'03"W	*Halimeda discoidea*	Chlorophyceae
Transect IV			
IVA-A-6	29°04'N; 85°14'W	*Codium* sp.[a]	Chlorophyceae
Transect V			
VA-A-12	29°50'N; 86°05.5'W	*Pryssonnelia rubra*	Rhodophyceae
January 1976			
Transect II			
IIA-A-17	27°50'N; 83°31'W	*Codium* sp.[a]	Chlorophyceae
062-A-1	27°49'55"N; 83°31'10"W	*Codium isthmocladium*	Chlorophyceae
064-A-9	27°50'N; 83°25'W	*Eucheuma isiforme*	Rhodophyceae
Transect III			
047-A-25	28°34'N; 84°20'12"W	*Codium caroliniarum*	Chlorophyceae
146-A-1	28°41'N; 84°23'40"W	*Codium caroliniarum*	Chlorophyceae
147-A-2b	28°38'18"N; 84°13'54"W	*Halimeda discoidea*	Chlorophyceae
151-A-1	28°32'07"N; 84°18'24"W	*Codium caroliniarum*	Chlorophyceae
247-A-27	28°36'16"N; 84°15'40"W	*Codium caroliniarum*	Chlorophyceae
251-A-10	28°32'40"N; 84°16'03"W	*Codium caroliniarum*	Chlorophyceae

[a] Not necessarily the same species.

continued in the Soxhlet apparatus for 12 additional hours. The extracts were combined and washed with acidic water (pH 4). An aliquot of the lipid was dried and weighed; the remainder was saponified by refluxing overnight with 0.5 N KOH-MeOH. The nonsaponifiable fraction obtained by benzene extraction of the methanolic solution was fractionated on a glass column packed with activity I silica gel beneath activity I neutral alumina (2:1 by volume). The column packing to sample ratio was always greater than 50:1. Two column volumes of hexane eluted the aliphatic hydrocarbon fraction; two column volumes of benzene eluted the aromatic (and polyolefinic) hydrocarbons. The two fractions were dried and analysed on a Perkin-Elmer Model 990 gas chromatograph using 2 m x 3.2 mm stainless steel columns packed with 4% FFAP (free fatty acid phase) and 3% SE-30 (a silicone gum rubber) on Gas-Chrom Z, 80 to 100 mesh (U.S. Standard Series). Data synthesis was performed with a Perkin-Elmer PEP-1 data system. Tentative identification of peaks was by comparison of retention times on both GLC (gas-liquid chromatography) phases with those of standards of

Table 2. Benthic algae. Chromatographic parameters. Tr/Cl: Transect and algal class (R: red, G: green, B: brown). Weights were determined from gas chromatographic data, using standards to interpolate response factors. Major *n*-alkanes are denoted by carbon number, *n*-alkenes by carbon number and number of double bonds. Listed are peaks with largest concentration, followed by all comprising at least 5% of largest peak. Monoalkenes are those whose gas chromatographic retention times match those of authentic *n*-alkenes with terminal unsaturation. Concentrations in ppm dry weight are given in parentheses, nd: not detected. KI: Kovats Index

Sample No.	Tr/Cl	Hydrocarbon classes (ppm dry weight)			Aliphatic components (ppm dry weight)		
		Aliphatics	Aromatics	*n*-Alkanes	Major alkanes, and alkenes	Phytadienes, KI 1922,1953, 1979,2004	Homologous *n*-alkene group, KI 1712,1914, 2116,2320,2524
June 1975							
IA-A+B-6	I/G	3.8	1.0	2.5	17(1.8),19(0.24)	nd	nd
IA-A+B-7	I/R	35.0	11.3	30.0	17(27.0),19:1(0.75)	2.0	nd
IA-A+B-8	I/G	68.0	8.7	35.0	17(32.0)	1.3	nd
IIA-A+B-1	II/R	38.0	12.0	25.0	17(19.8),15(0.72)	8.5	nd
IIA-A+B-2	II/R	171.0	8.0	130.0	17(112),15(4.3)	23.0	nd
IIA-A+B-3	II/R	50.0	7.0	19.0	17(17.5)	29.0	nd
062-A-17	II/G	153.0	11.5	95.0	19(46.3),17(38.0), 19:1(6.3),17:1(4.2)	nd	44.9
IIIA-A+B-2	III/G	21.0	1.1	16.1	17(13.4),19(1.39), 17:1(0.56)	nd	nd
047-A-3	III/G	51.0	1.1	39.0	17(35.0),19(2.6)	nd	nd
047-A-5a	III/G	96.0	6.7	84.0	17(46.0),19(21.0), 23(5.1),15(2.9)	nd	2.9
146-B-1	III/R+B	143.0	14.0	133.0	17(95.0),15(33.0)	nd	nd
147-B-5	III/G	72.0	11.6	62.0	17(25.0),19(10.0), 23(2.9)	nd	nd
247-A-2	III/G	44.0	5.0	38.0	17(34.0),19(2.3)	3.0	2.9
251-B-25	III/G	31.0	4.3	26.0	17(15.9),19(7.3), 23(0.87)	1.5	nd
October 1975							
IIA-A-12	II/R	43.0	5.7	40.0	17(35.0)	nd	nd
062-A-5	II/G	31.0	11.5	79.7	19(11.7),17(5.1), 19:1(1.39)	nd	14.4
064-A-3	II/R	61.0	13.6	56.0	17(49.0)	nd	nd
064-B-3	II/R	66.0	0.9	64.0	17(62.0)	2.0	nd
IIIA-A-10	III/G	850.0	26.0	42.0	17(22.0),21(3.0), 23(1.82)	740.0	18.6
047-A-5b	III/G	80.0	10.4	49.0	17(37.0),23(3.6), 19(2.4)	23.0	15.4
146-B-14	III/B	330.0	140.0	220.0	15(198.0),19(13.6), 17(4.9)	nd	nd
147-A-2a	III/G	22.0	4.0	19.0	17(17.7),19(1.72), 17:1(1.71)	nd	10.0
151-A-3	III/R	20.0	50.0	15.0	17(12.3),17:1(0.67)	nd	nd
247-A-4	III/G	92.0	10.1	75.0	17(62.0),19(6.4)	2.6	nd
251-A-3	III/G	39.0	2.5	26.0	17(19.8),19(2.2), 23(1.9)	nd	9.9
IVA-A-6	IV/G	33.0	2.6	16.7	17(14.0),19(1.41)	14.5	nd
VA-A-12	V/R	6.5	3.0	5.3	17(4.8),17:1(0.47)	nd	nd
January 1976							
IIA-A-17	II/G	4.2	5.3	3.1	17(2.6)	nd	nd
062-A-1	II/G	4.4	3.3	3.4	17(2.8),19(0.15)	0.5	nd
064-A-9	II/R	36.0	2.7	34.0	17(34.0)	0.3	nd
047-A-25	III/G	30.0	3.5	18.4	17(16.6),19(0.96)	10.0	nd
146-A-1	III/G	80.0	10.3	47.0	17(42.0),19(2.5), 15(1.16)	5.0	nd
147-A-2b	III/G	39.0	9.3	24.0	17(22.0),19(6.4), 23(2.1)	nd	2.0
151-A-1	III/G	67.0	6.3	57.0	17(51.0),19(3.1), 15(0.50)	3.0	nd
247-A-27	III/G	41.0	3.1	24.0	17(20.0),19(1.11)	2.5	nd
251-A-10	III/G	28.0	1.3	24.0	17(22.0),19(1.23), 23(0.24),15(0.21)	1.8	nd

n-alkanes, pristane, phytane, *n*-alkenes and aromatic compounds. The identification techniques failed to yield identities of components of the aromatic fraction, which were usually at very low concentrations. Certain gas chromatographic parameters were derived by computing various ratios of aliphatic hydrocarbon constituents for use in diagnosing petroleum pollution. The use of parameters dependent upon pristane and phytane were of little use because of their low concentrations. The presence of highly degraded petroleum in the algal specimens was indicated by the presence of large amounts of unresolved ma-

Table 3. Benthic algae. Parameters of pollution interest. Tr/Cl: Transect and algal class (R: red, G: green, B: brown). Presence of high molecular weight *n*-alkanes of carbon preference index = 1 (i.e., Σodds/Σevens = 1), with a large unresolved envelope, was the primary factor indicating pollution in these algae. nm: not measured

Sample	Tr/Cl	Pristane / *n*-C17	Phytane / *n*-C18	odd / even C≤20	odd / even C≥21	ΣC≤20 / ΣC≥21	Indications of Pollution[b]
June 1975							
IA-A+B-6	I/G	0.011	0.0	27.0	3.0	10.9	No
IA-A+B-7	I/R	0.0	0.0	150.0	1.0	51.0	Very slightly, if at all
IA-A+B-8	I/G	0.0	0.0	nm	0.9	15.0	Very slightly
IIA-A+B-1	II/R	0.005	2.5	44.0	1.0	7.9	Definitely, considerable HMW unresolved material
IIA-A+B-2	II/R	0.005	1.4	nm	0.9	11.5	Yes
IIA-A+B-3	II/R	0.003	0.5	106.0	0.9	19.2	Very slightly, if at all
062-A-17	II/G	0.003	0.0	20.0	2.0	37.0	Yes
IIIA-A+B-2	III/G	0.010	0.0	9.8	1.4	19.8	?
047-A-3	III/G	0.002	0.0	39.0	3.0	105.0	No
047-A-5a	III/G	0.003	0.6	86.0	5.5	9.6	No
146-B-1	III/R+B	0.002	0.0	?	1.5	107.0	Very slightly, if at all
147-B-5	III/G	0.004	0.7	78.1	1.4	3.0	Definitely, considerable HMW unresolved material
247-A-2	III/G	0.001	0.5	73.0	0.8	103.0	No
251-B-25	III/G	0.002	0.0	73.0	5.8	16.3	No
October 1975							
IIA-A-12	II/R	0.003	0.8	60.0	1.0	16.5	Yes
062-A-5	II/G	0.0	0.0	64.0	2.8	14.4	No
064-A-3	II/R	0.003	0.0	46.0	1.6	13.7	Very slightly, if at all
064-B-3	II/R	0.002	0.0	156.0	0.5	84.0	Very slightly, if at all
III-A-A-10	III/G	0.040	0.2	7.4	1.1	4.2	?
047-A-5b	III/G	0.001	0.0	11.9	7.1	12.2	No
146-B-14	III/B	0.101	0.0	260.0	0.8	703.0	No
147-A-2a	III/G	0.004	nm	78.0	7.4	10.5	No
151-A-3	III/R	0.0	0.0	72.0	nm	32.0	No
247-A-4	III/G	0.002	0.0	67.0	1.3	22.0	Very definitely, HMW unresolved material
251-A-3	III/G	0.003	nm	81.0	nm	7.5	No
IVA-A-6	IV/G	0.004	0.0	40.0	1.5	50.0	Very slightly, if at all
VA-A-12	V/R	0.017	0.0	28.0	1.3	18.4	No
January 1976							
IIA-A-17	II/G	0.017	nm	22.0	2.4	13.9	No
062-A-1	II/G	0.014	0.4	36.0	1.3	16.2	No
064-A-9	II/R	nm	nm	nm	nm	Very large	No
047-A-25	III/G	0.002	0.0	42.0	1.7	74.0	No
146-A-1	III/G	0.002	0.0	46.0	nm	28.0	Definitely, considerable unresolved HMW material
147-A-2b	III/G	0.004	0.0	61.0	4.7	7.8	?
151-A-1	III/G	0.001	0.0	61.0	0.5	53.0	?
247-A-27	III/G	0.005	0.3	51.0	0.9	12.6	Definitely, considerable unresolved HMW material
251-A-10	III/G	0.002	0.0	75.0	2.4	44.0	No

terial and also the presence of high molecular weight (carbon number >20) *n*-alkanes with a predominance of neither odd nor even carbon number alkanes.

The compounds were hydrogenated for further characterization. Hydrogen was bubbled at atmospheric pressure into aliquots of all samples suspected of containing olefinic material. PtO_2 was added to the sample in hexane and the sample stirred for 20 min under hydrogen taking care to maintain the initial 1 ml volume of hexane. The sample was then rechromatographed. Normal alkane identities were confirmed if retention times agreed with those of standards on FFAP and SE-30. Shifts in retention times after hydrogenation on both columns also further characterized those hydrocarbons having double bonds.

Peaks that were suspected of being phytadienes were characterized in two samples (*Caulerpa* sp. and *Eucheuma* sp.) containing an abundance of the suspected materials. Untreated lipid extracts of these two algae were analyzed directly and after hydrogenation by gas chromatography, bypassing saponification and adsorption chromatography. As further proof of identification of phytadienes, the assumption was made that chlorophyll represents at most ca. 2% dry weight of some algae and that phytol (the suggested precursor of phytadienes) represents ca. 33% of chlorophyll. A quantity of purified phytol equivalent to 50 times that which would occur in the plant tissue used in the previous experiment was chromatographed on fully activated alumina and silica gel. The phytadienes resulting in the aliphatic fraction were quantitated by gas chromatography.

Results

The predominant hydrocarbon material in these benthic algae is aliphatic (Table 2). In some cases (e.g. Sample Nos. IA-A+B-7, IIA-A+B-1, 062-A-5, 146-B-14, VA-A-12) the aromatics constitute as much as one-third of the weight of aliphatics. However hydrogenation of some of these aromatic fractions indicates that much of this material may be polyolefinic hydrocarbon material. The vast majority of the aliphatic components consists of *n*-alkanes occurring in the range of C_{15} to C_{23}, with constituents evident which are occasionally as high as C_{32} or as low as C_{13} *n*-alkanes. With few exceptions, the values for aliphatic, aromatic and *n*-alkanes fall respectively within one order of magnitude. The *Caulerpa* sp. from Transect III, October 1975 (Sample No. IIIA-A-10) has an unusually high aliphatic weight, but the *n*-alkane concentration is about average for the remaining species. Only the *Dichtyota dichotoma* from the same transect and sampling period (Sample 146-B-14) contains grossly elevated values of all three parameters, surpassing most species by a factor of 10.

There are simple distributions of algal aliphatic hydrocarbons (Table 2). Either *n*-C_{15}, *n*-C_{17} or *n*-C_{19} predominates in every instance. In addition to these low molecular weight saturated materials, there is a lesser but not insignificant contribution of the *n*-alkenes $C_{17:1}$ and $C_{19:1}$ and some higher molecular weight (*n*-C_{23+}) alkane material.

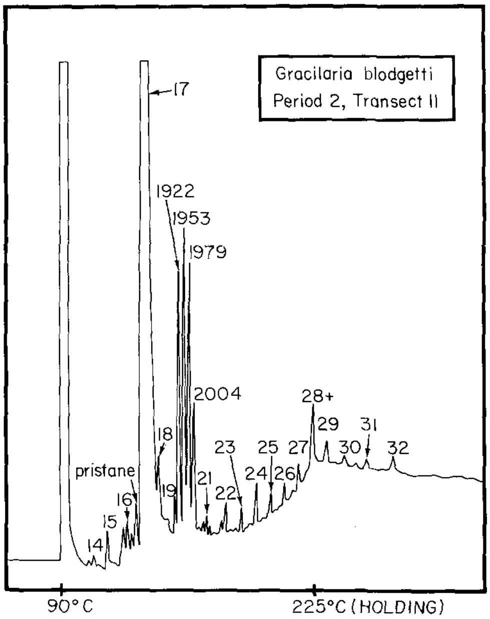

Fig. 1. *Gracillaria blodgetti*. Gas chromatogram of aliphatic hydrocarbons containing 4 phytadiene peaks labeled 1922, 1953, 1979 and 2004 in addition to *n*-C_{17} and high molecular weight *n*-alkanes. Gas chromatographic conditions valid for this and all following figures: 4% FFAP on 80 to 100 mesh Gas-Chrom Z, programmed for 90° to 225°C at 4 C° per min, with isothermal hold at 225°C

Pristane and phytane occur in very low concentrations in a great number of specimens as can be seen in the pristane/*n*-C_{17} and phytane/*n*-C_{18} ratios (Table 3). Possibly related biochemically to the occurrence of phytane in certain of the algae is the presence of 4 peaks between Kovats Indices (KI) of ca. 1900 and 2000 in the aliphatic chromatograms of many of the algal species (typified by *Gracilaria blodgetti* in Fig. 1) which were identified as phytadienes. Blumer and Thomas (1965) report that the 4 phytadienes elute gas chromatographically from Carbowax between KI 1900 and 2000 and can be hydrogenated to phytane. This was the basis for our identification of phytadienes in the benthic algae. FFAP, being a modified Carbowax, should give results similar to Carbowax. In the *Caulerpa* sp. (Sample No. IIIA-A-10) and *Eucheuma* sp. (Sample No. IIA-A+B-3) used to verify the presence of phytadienes, the 4 suspect peaks were in the lipid extracts, but disappeared with a simultaneous increase in the amount of phytane after the raw lipid was hydrogenated, in agreement with results obtained with the aliphatic hydrocarbon fractions. Chromatograms of *Eucheuma* sp. aliphatic hydrocarbon fractions and lipid aliquots before and after hydrogenation are shown in Figs. 2 and 3, respectively. Quantitative data from this experiment are shown in Table 4 with data from a similar experiment involving phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, which is part of the chlorophyll molecule and is considered to be the precursor of 4 phytadienes (3, 7, 11, 15-tetramethylhexadecadiene, double-bond positions shown in Blumer and Thomas, 1965) and phytane (2, 6, 10, 4-tetramethylhexadecane). The phytol dehydration procedure yielded about 30 ppm (µg phytadienes g^{-1} phytol), far less than in many algae (Table 2). The retention indices match those of the 4 suspect compounds, further confirming the identity of the phytadienes in the benthic algae.

There was a homologous series of *n*-alkenes eluting just after the odd car-

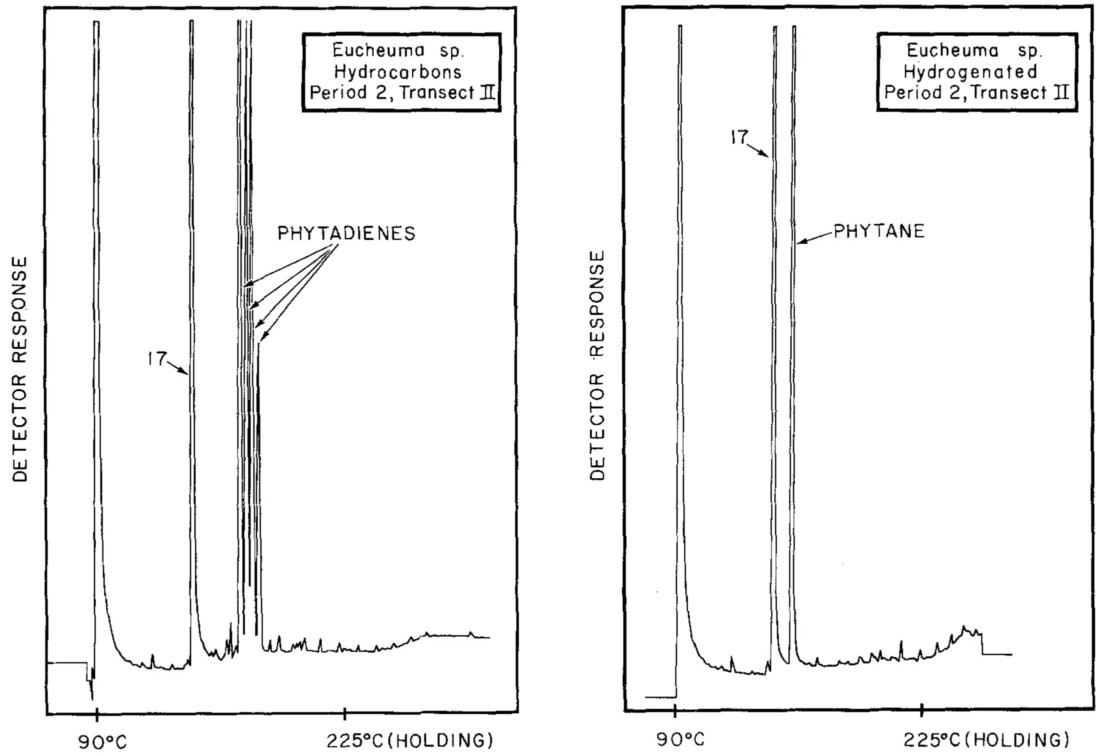

Fig. 2. *Eucheuma* sp. Gas chromatograms of aliphatic hydrocarbons before and after hydrogenation which converts the 4 phytadienes to phytane

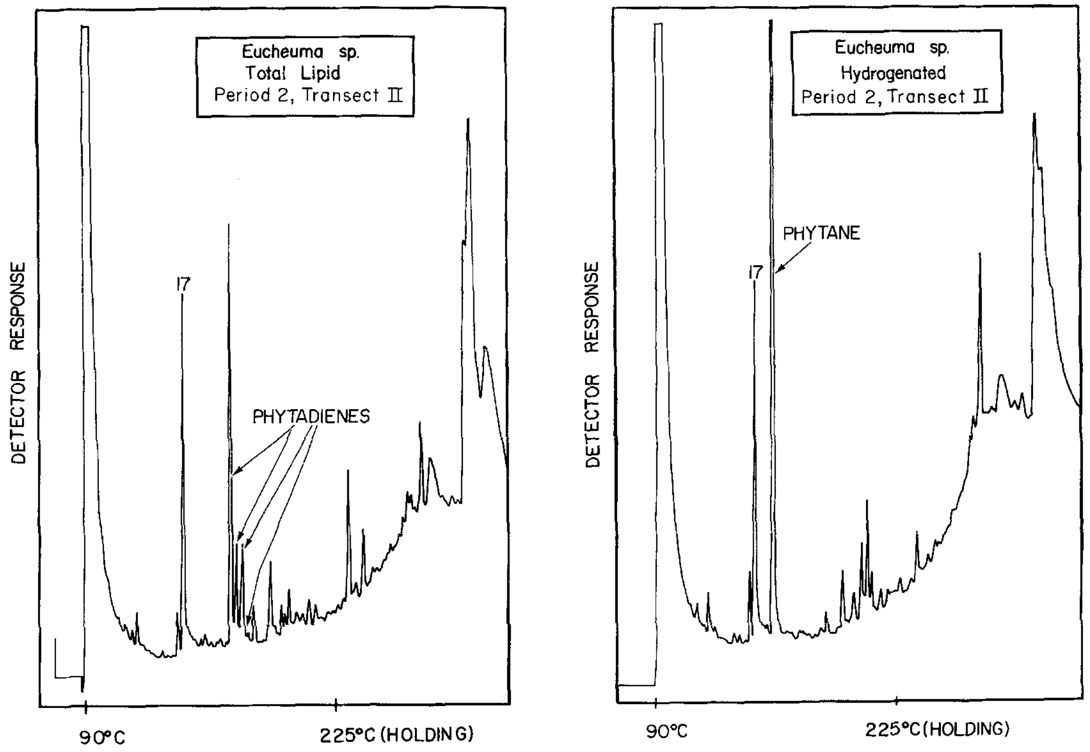

Fig. 3. *Eucheuma* sp. Gas chromatograms of lipid extract before and after hydrogenation with the 4 phytadienes clearly in evidence

bon number *n*-alkanes on FFAP with KIs of ca. 1712, 1914, 2116, 2320 and 2524 (Table 2). In some samples (notably Sample No. 062-A-17) these unsaturates constitute a sizeable fraction of the total aliphatics. Although upon hydrogenation the peaks disappear and increase the adjacent *n*-alkane peak area by equivalent amounts, they are not 1-*n*-alkenes which elute at higher KIs. Accompanying this homologous series are two and possibly three lesser series peaking at the C_{19} and C_{21} homologues. These series elute approximately 45, 65 and 95 Kovats units higher than the saturated homologues on FFAP.

Table 4. Phytadienes in algal extracts

Sample	Total µg of phytadienes[a]	Total µg of phytane after hydrogenation
Caulerpa sp.		
IIIA-A-10 lipid[b]	74	208
IIIA-10 aliphatics[b]	288	301
Eucheuma sp.		
IIA-A+B-3 lipid[b]	193	247
IIA-A+B-3 aliphatics[b]	136	96
Phytol treated with alumina-silica gel[c]	22	12.6

[a] Phytadienes are the four compounds with KI of ca. 1922, 1953, 1979 and 2004.

[b] Weights are calculated to represent the same initial weight of alga.

[c] Phytol treated was 50X greater than would normally be found in the weight of algae analyzed for this experiment.

A large hump of unresolved complex material (UCM) is apparent in several of the chromatograms (e.g. *Codium caroliniarum* Fig. 4). Accompanying the UCM in most cases is a suite of high molecular weight *n*-alkanes with no pronounced odd/even preference. The ratio of HMW (high molecular weight) alkanes to LMW (low molecular weight) alkanes and the ratio of odd/even members in each fraction are given in Table 3. In all specimens where the HMW fraction constitutes a major portion of the *n*-alkanes, this suite occurs with little preference for odd-carbon number. In sharp contrast, the odd: even ratio in low molecular weight *n*-alkanes is invariably very high. At least 30% of the samples collected contained both the suite of HMW alkanes and some unresolved complex mixture.

Discussion

There was less diversity in hydrocarbon distribution in the representatives of Rhodophyte than the Chlorophyte and Phaeophyte. Although some pristane, phytane and phytadienes were noted, the gas chromatograms of the red algae are dominated by the *n*-alkane, C_{17}, constituting 70 to 95% of the total aliphatic fraction. Clark and Blumer (1967) and Youngblood *et al.* (1971) also noted a predomi-

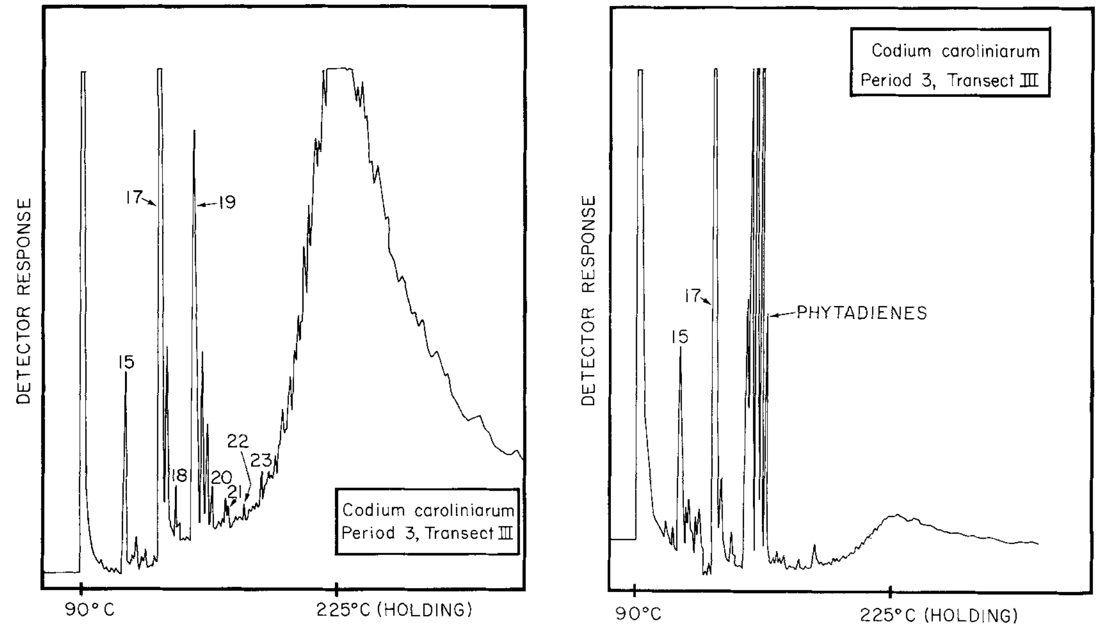

Fig. 4. *Codium caroliniarum*. Gas chromatogram of aliphatic hydrocarbons from 2 specimens collected at same location during same sampling period; 1 specimen (on left) contains abundant unresolved complex material (UCM) underlying high molecular weight *n*-alkanes, with no preference for odd or even carbon numbers

nance of either n-C_{15}, n-C_{17} or n-C_{19} in benthic algae. No other n-alkane or n-alkene in the range of C_{15} to C_{19} occurred at a concentration even 10% that of C_{17}. Another characteristic of these red algae is the presence of quantities of HMW n-alkane material with an absence of any odd carbon number dominance which could be the remnants of petroleum n-alkanes also of no odd/even preference (Petrović and Vitorović, 1976). Youngblood *et al.* (1971) also noted a suite of n-alkanes in benthic algae outside the range of C_{14} to C_{19} but did not explore the possibility of a petroleum source. Although the impact of oil pollution is usually detected in the lower molecular weight range of the aliphatic materials, severely weathered petroleum products or tarry residues will produce a more complex suite of aliphatics, resulting primarily in a high molecular weight unresolved complex mixture. The UCM, consisting of cyclic and branched materials used as an indicator of petroleum pollution (Blumer and Sass, 1972; Zafiriou, 1973; Farrington *et al.*, 1976; Teal, 1976), resists microbial degradation more effectively than the n-alkane assemblage and so tends to remain in polluted sediments and organisms after the n-alkanes have dissipated (ZoBell, 1969; Blumer *et al.*, 1973; Lee, 1976). The Rhodophyta samples contain HMW n-alkanes accompanied by little of the UCM, thereby indicating a non-petroleum and probably a biogenic source for these hydrocarbons. It has been suggested that epiphytic bacteria may be responsible for HMW n-alkanes. In a study of *Sargassum* this source of the HMW material occurring in that alga was dismissed (Burns and Teal, 1973). The epiphytic organisms probably cannot be entirely discarded as a possible contributor.

The aliphatic hydrocarbons of Chlorophyta consist primarily of the n-alkanes from n-C_{15} to n-C_{23}, marked by a very complex assortment of hydrocarbon distributions. The predominant n-alkane in *Caulerpa sertularoides* (Sample Nos. 062-A-17 and 062-A-5) is n-C_{19}, in contrast to the more common predominance of n-C_{17} in other species of red and green algae. Even where n-C_{19} does not occur in the highest concentrations, it and other low molecular weight alkanes and alkenes represent a large fraction of the total aliphatic hydrocarbons. Both pristane and phytane are detectable in all specimens. The occurrence of pristane is not unusual in plants and has been noted in other algal species (Clark and Blumer, 1967; Youngblood *et al.*, 1971). Of rarer occurrence in plants is phytane, which has been identified in extreme trace quantities in a few algal samples (notably Burns and Teal, 1973). Youngblood *et al.* (1971) did not detect phytane in any of the algae collected in the Cape Cod area. The presence of phytane does not necessarily signal the presence of petroleum contamination in algal specimens; however, it is one of the many indicators used to detect petroleum hydrocarbons.

Nearly all of the green algal samples contain quantities of phytadienes, ranging to as high as 740 ppm by weight in one *Caulerpa* sp. (Sample No. IIIA-10). Blumer and Thomas (1965) and Blumer *et al.* (1969) have suggested that phytadienes are present in some zooplankton and fish, but may occur as artifacts from any number of laboratory procedures commonly associated with hydrocarbon analysis. Since the precursor of phytadienes is assumed to be phytol, part of the chlorophyll molecule, their creation by saponification and/or adsorption chromatography would seem feasible in algal extracts. However, the appearance of the phytadienes in both original extracts and in extracts saponified and subjected to adsorption chromatography indicates clearly that phytadienes do not occur in algal extracts merely as laboratory artifacts. The amount of phytadienes occurring as artifacts from phytol dehydration would in most cases be insignificant and probably could not account for the levels of phytadienes found. Phytadienes might also result from decomposition of plant tissue. As only live, healthy specimens were collected, this seems unlikely. The presence of these phytadienes may be related biochemically to the presence of phytane, but this relationship is not apparent when one looks at the levels of phytadienes versus levels of phytane. They both occur in most specimens, but there are instances where either appears alone. If chemical stress were responsible for the presence of the phytadienes, one might expect a relationship between phytadienes and petroleum-like hydrocarbons; no strong relationship was found. The production of phytadienes does not appear to be seasonally affected; the phytadienes are almost always present, although varying in concentration.

The homologous series of odd carbon number n-alkenes with KIs of 1712 to 2524 occur exclusively in the green algal specimens, and may be the polyolefins reported by Youngblood *et al.* (1971), although occurring in the present samples in much lower concentrations. Besides the phytadienes, the 1-n-alkenes, $C_{17:1}$ and $C_{19:1}$ are among the major aliphatic components in many of the green algal species. The *Codium* spp. are perhaps unique among the green algae in containing so little unsaturated hydrocarbons, excluding phytadienes. *Codium fragile* also has a very low quantity of unsaturated hydrocarbons (Youngblood *et al.*, 1971) compared to other green algal species.

Sufficient numbers of *Codium* spp. were collected to evaluate variability among closely related species. Both *C. repens* (Sample Nos. 047-A-3, 247-A-2 and 247-A-4) and *C. caroliniarum* (Sample Nos. 047-A-25, 146-A-1, 151-A-1, 247-A-27 and 251-A-10) produce almost identical hydrocarbon chromatograms, each having a large n-C_{15} with n-C_{17} dominant. *C. isthmocladium* (Sample No. 062-A-1) and the three unidentified *Codium* species (Sample Nos. IIIA-A+B-2, IVA-A-6 and IIA-A-17) contain only traces of n-C_{15}. Thus, the major hydrocarbon constituents probably are too variable to permit their use as a taxonomic aid much below the class level of algae.

Intraseasonal variability for the green alga *Halimeda discoidea* (Sample Nos. 047-A-5a, 147-B-5, 047-A-5b, 147-A-2a, 251-A-3 and 147-A-2b) is as great as interseasonal variability both in hydrocarbon levels and distribution. Overall hydrocarbon distributions in the red and green algae change little with season.

The brown algae contain a hydrocarbon distribution that easily sets them apart from the green and red algae. The predominance of n-C_{15} in *Dictyota dichotoma* occurs in other brown algae (Youngblood *et al.*, 1971; Burns and Teal, 1973; Lytle and Lytle, unpublished data). Neither sample of *D. dichotoma* (Sample Nos. 146-B-14 and 146-B-1) produce high molecular weight hydrocarbons or unsaturated hydrocarbons.

Hydrocarbon materials indicative of highly degraded petroleum occur almost randomly, and in some specimens but not others of the same species from the same sample location and period. These petroleum residues are probably entrapped tar particles which have been found throughout the Gulf of Mexico (Horn *et al.*, 1970; Butler *et al.*, 1973; Jeffrey *et al.*, 1974; McGowan *et al.*, 1974; Butler and Harris, 1975; Wade *et al.*, 1976). Although most larger tar particles occur in surface algal samples, micro-fine particles may occur throughout the water column and could account for these algal samples having tarry residues. Teal (1976) noted the incorporation of relatively undegraded tar particles by benthic organisms. The residues from the algae would appear to lie at some midpoint in degradation between pelagic tars found in the Gulf of Mexico with little UCM material and much n-alkane material and abyssal tars with mostly UCM indicating a high degree of degradation (Jeffrey *et al.*, 1974). The intermediate depth of algal collections tends to support this contention. No petroleum pollution of recent derivation was found in these algal specimens, and none has been found in the sediments off the coast of Florida where these specimens were collected (Gearing *et al.*, 1976).

The only trend in levels of highly degraded petroleum pollution that might be significant occurred along Transect II off Tampa, Florida. During Collection Period 1, all specimens contained these petroleum residues; during Period 2, 75% showed pollution, and during Period 3, none. This seeming decline was coincident with the migration of the loop current, which might be transporting various Mississippi River-derived and other materials across the shelf at this transect, further from the Florida coastline (Maul, 1977).

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