DISTRIBUTION OF *n*–PARAFFINS IN MARINE ORGANISMS AND SEDIMENT[1]

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ABSTRACT

Twelve species of benthic algae from the northeast coast of the United States, three species of planktonic algae grown in the laboratory, a pelagic alga, a sample of mixed phytoplankton and zooplankton, and a recent marine sediment were analyzed for their normal paraffin distribution from $C_{14}H_{30}$ to $C_{32}H_{66}$.

Normal paraffins occurred in all samples. Benthic and planktonic algae and the mixed plankton sample exhibited only a slight odd-carbon predominance. All algae showed a major maximum at *n*-$C_{15}H_{32}$ or *n*-$C_{17}H_{36}$, a minimum between *n*-$C_{18}H_{38}$ and *n*-$C_{21}H_{44}$ and a secondary maximum between *n*-$C_{27}H_{56}$ and *n*-$C_{30}H_{62}$. In all these features, the algae and the plankton differed from recent marine sediments. This suggests that the normal paraffins of recent marine sediments are largely derived from sources other than the organisms studied.

Differences in the hydrocarbon distribution patterns of various classes of benthic algae may be of taxonomic value. Pristane occurs in several benthic and planktonic algae; phytane, if present, occurs at a concentration too low to be detected by the method used.

INTRODUCTION

Straight-chain saturated hydrocarbons (*n*-alkane or *n*-paraffins, C_nH_{2n+2}) are minor but ever-present compounds in the marine environment. They occur in marine organisms, in sediments, and in the seawater itself. Two principal sources contribute hydrocarbons to the marine environment: fossil fuels and recent organisms.

Fossil hydrocarbons of ancient sediments and petroleum are introduced into the sea by pollution, by submarine oil seeps, and by expulsion of interstitial solutions from sediments. The hydrocarbon distribution in petroleum and products derived from fossil fuels is well documented. Fossil hydrocarbon mixtures show little or no odd-carbon predominance (Cooper and Bray 1963; Eglinton et al. 1965; Oró et al. 1965; Robinson, Cummins, and Dinneen 1965).

Our knowledge of the recent biogenic hydrocarbons is limited. Selected land plants have been investigated systematically for their straight-chain hydrocarbon content. These hydrocarbons, usually found in the waxy coating of stems, leaves, flowers, or fruit, cover the range to at least *n*-dohexacontane ($C_{62}H_{126}$) (Kranz et al. 1960; Waldron et al. 1961; Eglinton et al. 1962; Capella et al. 1963; Wollrab, Streible, and Sǒrm 1965). Odd-carbon paraffins predominate in most terrestrial plants although occasionally an even predominance has been reported (Stevenson 1961; Cocker and Shaw 1963).

The content and distribution of hydrocarbons in recent sediments has been investigated in connection with the problem

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of the origin of petroleum. Recent sediments exhibit a pronounced predominance of odd-carbon normal paraffins (Bray and Evans 1961). This presumably reflects the composition of organisms that contribute their organic matter to the sediments. However, the specific organisms responsible for this odd-carbon predominance have not yet been identified.

The hydrocarbons of benthic marine invertebrates and of fishes (Lambertsen and Holman 1963; Ciereszko, Attaway, and Koons 1963; Pasby, Cooper, and Hood 1964; Koons, Jamieson, and Ciereszko 1965) have been studied in greater detail than those of marine algae (Heilbron, Parry, and Phipers 1935) and of zooplankton (Blumer 1965). These latter two types of organisms, representing the bulk of the marine biomass, are of special interest as potential sources of a large fraction of the marine hydrocarbons. In the marine environment, paraffins are relatively stable; for this reason their residence time in the ocean should exceed that of most nutrients. As compounds that are not easily metabolized, paraffins may pass unaltered through the marine food chain. Thus, all marine organisms potentially contribute hydrocarbons from various sources to the sediments and eventually to petroleum.

Here we report the normal paraffin analyses of 12 species of northeast Atlantic intertidal zone seaweeds, one species of free-drifting pelagic seaweed, three species of cultured planktonic algae, a mixed marine plankton sample, and a recent sediment.

METHODS AND MATERIALS

Samples

Samples were stored at –20C if not analyzed immediately (for details of collection and storage *see* Clark 1966*a*). The benthic seaweed samples were *Fucus* sp. collected November 1965 at Little Harbor, Woods Hole, Massachusetts; *Polysiphonia* sp., *Chondrus crispus* (Stackhouse), *Fucus* sp., *Agarum cribrosum* (Mertens) Bory, *Laminaria digitata* (L.) Lamouroux and *Chaetomorpha linum* (Müller) Kützing collected February 1966 at Jaffrey Point, New Castle Island, New Hampshire; *Corallina officinalis* L., *Rhodymenia palmata* (L.) Greville (epiphytic on *Ascophyllum)* and *Ascophyllum nodosum* (L.) Le Jolis collected March 1966 at Jaffrey Point; and *Chondrus crispus* and *Fucus* sp. collected March 1966 at Falmouth, Massachusetts. The pelagic seaweed, *Sargassum* sp., was collected in the summer of 1963 in the Sargasso Sea north of Bermuda.

Planktonic algae which cannot be collected easily as pure species or in unpolluted form from the ocean were grown in 1963 in an artificial medium excluding hydrocarbon contamination (*Syracosphaera carterae, Skeletonema costatum* and a cryptophycean). Details of culturing procedures are described by Clark (1966*a*).

The mixed marine phytoplankton and zooplankton sample was collected March 1966 at Tarpaulin Cove, Naushon Island, Massachusetts, with a ¾-m No. 2 nylon net. About two-thirds of its bulk was phytoplankton. The sediment sample was collected March 1966 with a pipe dredge in 6–9 m of water at Tarpaulin Cove, Massachusetts. For carbonate and organic carbon analysis, the sample was dried at 140C and ground to 200 mesh.

Solvents and reagents

All solvents were distilled through a packed 50 × 3-cm glass column. After every distillation the still was steam-cleaned by distilling 500 ml of water; this removes accumulated high-boiling (n-C_{24} to n-C_{30} range) contaminants from the column surfaces. The solvents, except pentane, were then redistilled through the same column.

Methanol, anhydrous (Mallinckrodt), after two distillations contained less than 2.5×10^{-6} g/liter of n-octacosane and less than 6×10^{-7} g/liter of n-tetracosane.

Pentane, 99 mol %, pure grade (Phillips Petroleum Co.) was distilled, then stored 12 hr over 5-Å molecular sieves (0.16-cm pellets, Linde Division, Union Carbide Corp.). The second distillate through a steam-cleaned 115 × 2-cm packed glass column contained less than 2×10^{-6} g/liter of n-octacosane.

The reference compounds used were: *n*-tetradecane, *n*-hexadecane, *n*-octadecane, and *n*-eicosane (Matheson, Coleman, and Bell); *n*-pentadecane and *n*-heptadecane (Aldrich Chemical Co.); *n*-tetracosane, *n*-octacosane, and *n*-dotriacontane (Humphrey-Wilkinson). Pristane (2,6,10,14-tetramethylpentadecane, Fisher Scientific Co.) was purified by frontal elution from silica gel.

Extraction

All-glass Soxhlet extraction apparatus was used throughout. The large seaweeds were extracted without thimbles. The sediment and cultured planktonic algae were extracted in thimbles that had been cleaned by extraction for 72 hr in refluxing methanol-benzene with frequent solvent changes. Blanks were run for the entire procedure and blank corrections (smaller than 6×10^{-7} g per thimble for *n*-hexacosane, the major contaminant) were applied where necessary.

The samples were extracted with methanol-benzene (1 : 1 v/v) for 10–20 hr; extraction continued with fresh solvent for an additional 24–36 hr (total solvent volume: 250–300 ml). The extract was transferred to a 500-ml separatory funnel, and the flask was rinsed with 25 ml of tap water and 25 ml of *n*-pentane into the separatory funnel. The aqueous phase from the first washing was re-extracted with 25 ml of fresh *n*-pentane and discarded. The two organic phases were combined. The organic solvent was then removed using a rotary evaporator at 15C and 100 mm Hg to near dryness and the concentrate transferred in *n*-pentane (3–5 ml) to a weighed vial. The extract was stored under refrigeration. The extracted residues were air-dried at room temperature.

Chromatography

Saturated hydrocarbons were isolated by chromatography on silica gel (Davison Grade 922, 200 mesh; Davison Chemical Co., Baltimore) activated at 220C and then partially deactivated by adding 5% (w/w) tap water. The silica gel was packed (100 ml of silica gel/1 g of extract) into a chromatographic column (25 mm ID) having a Teflon stopcock.

Before sample application, the column was washed with *n*-pentane (400 ml). The extract, dissolved in 4–6 ml of pentane, was transferred to the column. Five 50-ml fractions of pentane eluent were collected at a flow rate of 0.5–1.0 ml/min; the solvent was evaporated first to near dryness on the rotary evaporator, and then the evaporation was completed in a weighed vial under a stream of filtered, water-pumped nitrogen.

In some cases polar compounds broke through the adsorbent together with the hydrocarbons. In these instances the samples were rechromatographed on 75 ml of silica gel packed beneath 25 ml of alumina (activated at 220C, Al-0102-P; Harshaw Chemical Co., Cleveland, Ohio).

Sulfur removal

The sediment sample contained free sulfur. This was removed after chromatography on silica gel by percolation through an active copper column (Blumer 1957).

Gas chromatography

The equipment consisted of a Model 600 Aerograph with a hydrogen flame detector, a linear temperature programmer (Model 325, Wilkens Instrument and Research; Walnut Creek, Calif.), an automatic attenuator (Model 50, F & M Scientific Corp.; Avondale, Pa.) and a 1 mv recorder. Operating conditions were as follows: injection temperature 200C, nitrogen flow 21–24 ml/min, hydrogen flow 17 ml/min.

Chromosorb G (80/100 mesh, acid washed, dichlorodimethylsilane treated; Johns-Manville) was used in 1.8-m × 0.3-cm ID stainless steel columns. The nonpolar stationary phase was Dow Corning RTV 502 silicone rubber (1.5% by weight, based on silicone rubber after removal of filler by centrifugation in benzene), coated from chloroform solution and conditioned (Wotiz and Chatteraj 1964). Plate efficiencies varied from 600 to 1,200 plates/m (*n*-tetracosane at 200C with retention time ca. 40 min). The silicone rubber column does not

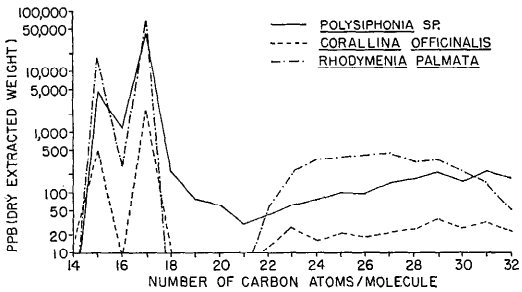

FIG. 1. Normal alkane content of red algae.

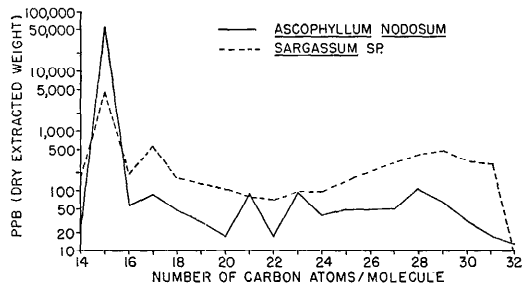

FIG. 3. Normal alkane content of brown algae.

sufficiently resolve *n*-heptadecane from pristane, therefore more polar columns were used. These were Carbowax 20M (3.5% wt) and FFAP (35% wt; Wilkens Instrument and Research), both coated from chloroform.

Samples were injected in CS_2 onto a cooled silicone rubber column (60C) with a microsyringe and programmed from 100 to 320C at 3C/min. Occasional steam-cleaning by injection of 0.025 ml of distilled water at 250C prolongs the useful column life.

Chromatograms of all samples were repeated after the addition of internal standards for calculation of retention indices. The paraffins in the five fractions from the silica gel chromatography were quantitatively determined from peak areas (peak height times the width at half height). The sums of the blanks were subtracted from the individual paraffin quantities. The blank correction typically did not exceed 10% of the sample value, often being negligible; occasionally, in samples with low hydrocarbon content, it would be as high as 30%.

Pristane was positively identified by trapping in a Dry Ice-chilled capillary tube those components emerging from the Carbowax column with a retention index (Kováts 1958) of 1,670. These were rechromatographed on silicone rubber and FFAP columns and produced peaks at retention indices corresponding exactly to those of authentic pristane.

RESULTS

The analytical data are summarized in Table 1. Individual paraffin concentrations from C_{14} to C_{32} are plotted in Figs. 1–7.

The red algae (Rhodophyceae) contained large concentrations of *n*-C_{15} and *n*-C_{17} (Figs. 1 and 2). The hydrocarbon concentration passes through a minimum near *n*-C_{21} and increases to a secondary maximum in the *n*-C_{27} region. In addition to the odd-carbon predominance at *n*-C_{15} and *n*-C_{17}, there was a minor predominance in the *n*-C_{20} to *n*-C_{32} region for *C. officinalis* (CPI =

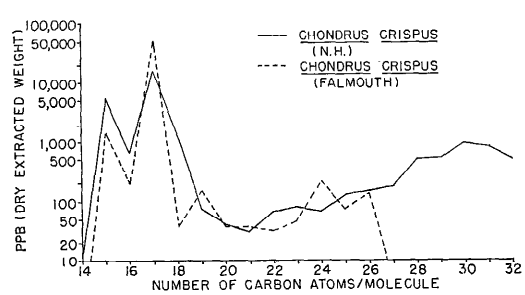

FIG. 2. Normal alkane content of red algae (*Chondrus*).

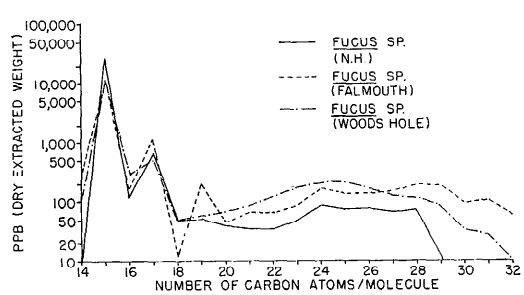

FIG. 4. Normal alkane content of brown algae (*Fucus*).

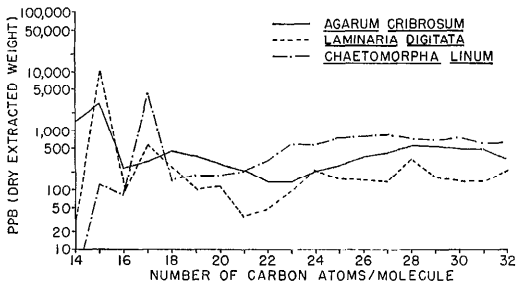

FIG. 5. Normal alkane content of brown and green algae.

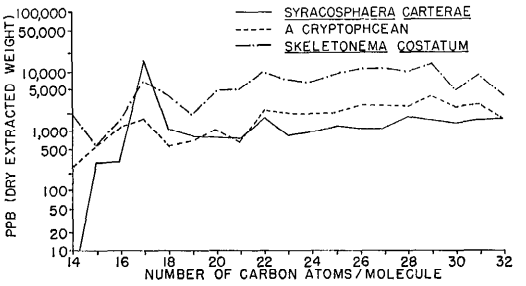

FIG. 6. Normal alkane content of planktonic algae.

1.3) and *Polysiphonia* (CPI = 1.2). It was smaller in *R. palmata* (CPI = 1.1) and *C. crispus* has an even-carbon predominance (CPI = 0.9 for the New Hampshire sample and 0.4 for the Falmouth sample). The two *C. crispus* samples, although collected at the same period in early spring, showed external differences. The Falmouth sample was more loosely branched and very light in color, especially on the branch tips, while the New Hampshire sample was dark purple and compact.

Normal pentadecane was the predominant paraffin in the Fucales (brown algae). It accounts for more than 94% of the normal paraffin of *Fucus* and *A. nodosum* (Figs. 3 and 4); *n*-heptadecane is the next most important paraffin. *Sargassum*, the pelagic brown alga, contained less *n*-C_{15} relative to the other normal paraffins, but its CPI was the highest of the family (CPI = 1.5). *A. nodosum*, but not *Fucus*, had an odd-carbon predominance; the *Fucus* sample from New Hampshire had a slight even-carbon predominance. There was a minimum near *n*-C_{18} to *n*-C_{21} with a moderate maximum near *n*-C_{25} to *n*-C_{28}. The Laminariales, another family of brown algae, resemble the Fucales, but their *n*-C_{15} predominance was less pronounced (Fig. 5). The minimum occurred at a higher carbon number (near *n*-C_{21}) and the secondary maximum was shifted to *n*-C_{28}. The *A. cribrosum* showed no odd-carbon predominance in the *n*-C_{20} to *n*-C_{32} region (CPI = 1.0), while *L. digitata* had a slight even-carbon predominance (CPI = 0.7).

The only green seaweed (Chlorophyceae) studied, *C. linum*, had a high content of *n*-heptadecane (35% of the paraffins), a minimum at *n*-C_{18}, a secondary maximum at *n*-C_{27} and no odd-carbon predominance (Fig. 5).

The cultured planktonic algae and the benthic seaweeds (Fig. 6) had different distributions of normal paraffins. While each cultured species had a slight predominance of *n*-C_{17} over the adjacent homologs, it was the major normal paraffin only in the *S. carterae* (45% of the paraffins). There was a slight odd-carbon predominance in all three algae with a slight maximum near *n*-C_{29}.

Pristane and *n*-heptadecane were predominant in the mixed phytoplankton and zooplankton sample. The pristane content (Fig. 7) was the difference between the total C_{17} and the normal C_{17} content. There was a slight odd-carbon predominance (CPI = 1.2) in the *n*-C_{20} to *n*-C_{32} region. No peak at the retention index of phytane (1,786, silicone rubber) was observed in this sample in any of the algae. It is estimated that phytane would have been detected if the ratio of phytane to *n*-C_{18} had been larger than 1 : 1,000.

The recent marine sediment had the marked odd-carbon predominance (CPI = 4.0) usually associated with recent sediments. Neither *n*-C_{15} nor *n*-C_{17} predominated over the other odd-carbon homologs. The sample had a calcium carbonate content of 3.18%, an organic carbon content of 1.1%, and a sulfur content of at least 250 ppm.

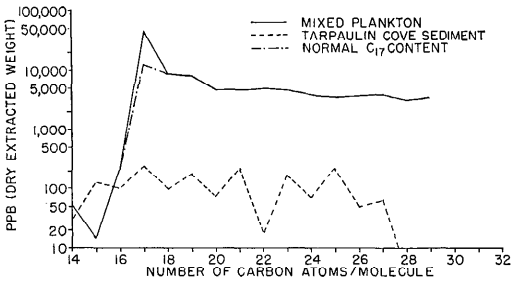

FIG. 7. Normal alkane content of sediment and plankton. Pristane is abundant in zooplankton. The upper curve therefore gives the value for total hydrocarbons eluted at C_{17} and the corrected *n*-heptadecane content.

CONCLUSIONS

All 16 marine algae analyzed contained saturated straight-chain hydrocarbons. The cultured planktonic algae contained much more extractable material (12 to 48% of the dry extracted weight) than the benthic algae (0.67 to 7.5% by weight). The hydrocarbon content of both groups was similar (34–120 ppm in planktonic and 10–120 ppm in benthic algae). *Sargassum* contained relatively little extractable material (1.5% by weight) and its normal hydrocarbon content was low (8 ppm). Unicellular algae use low-density compounds for flotation whereas some of the larger benthic and pelagic algae use gas bladders. It is difficult to compare the hydrocarbon contents reported here to those of land plants because of the great diversity of terrestrial plants and of the limited number of analyses in existence (Clark 1966*b*).

The analysis of a recent sediment from Tarpaulin Cove confirmed the predominance of odd-carbon number paraffins in an area for which no analyses were available. The odd-carbon predominance (CPI = 4.0) was well within the range (CPI = 2.4 to 5.5) found in other regions (Cooper and Bray 1963). The source for the odd-carbon predominance in the sedimentary organic material has to be sought in terrestrial detritus, in organisms living in the water column, on the surface of the sediment, or within the sediment.

Planktonic algae grown in the laboratory and mixed marine plankton from Tarpaulin Cove had only a slight odd-carbon predominance (CPI = 1.2). A similar, insignificant odd-carbon predominance has also been found in marine zooplankton, predominantly calanoid species of the Gulf of Maine (Blumer, unpublished data). Further, the odd-carbon predominances of pelagic seaweed (CPI = 1.5) or of benthic algae (CPI = 0.4 to 1.4) are minor compared to that in the sediments.

Thus, the odd-carbon predominance of recent sediment does not reflect the hydrocarbon composition of planktonic or benthic algae. Rather, it resembles that found in many land plants, for instance in cane grass, CPI = 4.2 (Kranz et al. 1961); maize, 5.1, and barley, 7.2 (Cooper and Bray 1963); sugar cane, 7.3 (Kranz et al. 1960); oats, 9.2 (Cooper and Bray 1963); tree leaves (*Aeonium virgineum*) II and Italian rye grass, 36 (Eglinton et al. 1962). This suggests that the source of a large fraction of sedimentary paraffins is not marine planktonic or benthic algae, but it may be organisms indigenous to the sediment or in terrestrial plant detritus.

The benthic algae contain high concentrations of *n*-pentadecane and *n*-heptadecane. Further, all algae analyzed had a definite minimum in their normal paraffin content between C_{19} and C_{21} and a secondary maximum near C_{27} to C_{30}. These characteristics are not encountered in the recent marine sediment, an additional indication that the benthic and planktonic algae are not the major sources for the sedimentary normal paraffins.

Different genera of algae have different hydrocarbon distributions, especially in the C_{15}–C_{17} molecular weight range. The benthic algae can be grouped into two categories: in the red and green algae, *n*-heptadecane predominates (36 to 95% of the paraffins) while in the brown algae, *n*-pentadecane predominates (to 98% of the normal paraffins). Only one of the planktonic algae (*S. carterae*) had a moderate *n*-C_{17} predominance.

The red and brown algae analyzed were collected close to each other. The predomi-

TABLE 1. *Analytical data for organisms and sediment examined*

	Organic extract ppm*	Total *n*-paraffins ppm*	Major *n*-paraffin: Carbon number	Major *n*-paraffin: Total paraffins (%)	CPI†	Pristane : *n*-C_{17}
			Benthic and pelagic algae			
Chlorophyceae						
Chaetomorpha linum (N.H.)	28,000	12.7	C_{17}	35.5	1.0	1:130
Rhodophyceae						
Corallina officinalis (N.H.)	—	—	C_{17}	76.3	1.3	<1:1,300
Polysiphonia sp. (N.H.)	5,600	54.9	C_{17}	86.1	1.2	<1:4,500
Rhodymenia palmata (N.H.)	6,700	93.8	C_{17}	79.0	1.1	<1:1,000
Chondrus crispus (N.H.)	14,000	28.2	C_{17}	57.7	0.9	<1:2,000
Chondrus crispus (Falmouth)	8,400	54.4	C_{17}	95.2	0.4	<1:3,300
Phaeophyceae						
Fucales						
Ascophyllum nodosum (N.H.)	40,500	57.1	C_{15}	98.4	1.5	<1:285
Fucus sp. (N.H.)	41,000	28.3	C_{15}	94.7	1.4	<1:5,000
Fucus sp. (Woods Hole)	69,700	110.3	C_{15}	96.9	1.1	1:34
Fucus sp. (Falmouth)	75,000	122.5	C_{15}	97.9	1.0	<1:10,000
Sargassum sp. (Sargasso Sea)	15,000	8.4	C_{15}	54.9	0.8	<1:5,000
Laminariales						
Agarum cribrosum (N.H.)	29,000	10.1	C_{15}	28.3	1.0	1:20
Laminaria digitata (N.H.)	49,000	13.2	C_{15}	77.1	0.7	1:14
			Planktonic algae			
Chrysophyceae						
Syracosphaera carterae (culture)	120,000	33.9	C_{17}	45.5	1.1	1:2.2
Bacillariophyceae						
Skeletonema costatum (culture)	480,000	120.7	C_{20}	10.5	1.2	1:1.6
Cryptophyceae						
Undetermined cryptomonad (culture)	305,000	33.5	C_{20}	11.3	1.1	1:5
Recent sediment (Tarpaulin Cove, Mass.)	2,800	1.68	*n*-C_{17} *n*-C_{25}	14.0 14.0	4.0	1:3.5
Mixed marine plankton (Tarpaulin Cove, Mass.)	150,000	102.0	Pristane *n*-C_{17}	21.0 9.0	1.2	1:0.41

* Weights in ppm based on the sum of the weights of the extracted dry residue plus the organic extract.
† The Carbon preference index (CPI) is calculated over the range *n*-C_{20} to *n*-C_{32} (Cooper and Bray 1963):

$$\mathrm{CPI} = \tfrac{1}{2}\left[\frac{\sum_{n=21}^{n=31} \mathrm{HC}_{odd}}{\sum_{n=22}^{n=32} \mathrm{HC}_{even}} + \frac{\sum_{n=21}^{n=31} \mathrm{HC}_{odd}}{\sum_{n=20}^{n=30} \mathrm{HC}_{even}}\right].$$

nance of a different single paraffin in them, therefore, reflects a taxonomic difference rather than the environment. If this chemical difference between algae can be confirmed in specimens from other areas, it may have important taxonomic implications. It could be especially useful in a class like the Rhodophyta (red algae) where complete agreement about the taxonomy has not been reached.

The two samples of *C. crispus* and *Fucus* (collected at the same location in Falmouth) had a fourfold predominance of *n*-C_{19} over any of the other algae analyzed. This deviation in the normal paraffin distribution of *C. crispus* and *Fucus* from other localities is at present unexplained; it may be due to the environment.

The origin of the normal paraffins in benthic algae is uncertain. It is possible that

the algae concentrate paraffins from seawater, although it is more likely that they are biochemical products. The presence of normal paraffins in the planktonic algae grown under clean conditions in the laboratory supports this assumption. It has been suggested that paraffins result from the decarboxylation of the saturated normal fatty acids (Cooper and Bray 1963). The high concentrations of *n*-pentadecane and *n*-heptadecane in benthic algae are not easily explained on this basis as they do not exhibit the necessary predominance of normal hexadecanoic and octadecanoic acids (Klenk et al. 1963).

Isoprenoid hydrocarbons, especially phytadienes, pristane, and related C_{19} mono-, di- and triolefins, are abundant in the marine environment (Blumer, Mullin, and Thomas 1964; Blumer and Thomas 1965*a*, *b*; Blumer 1965). Pristane occurs in recent sediments (Blumer and Snyder 1965) and both pristane and phytane have been isolated from petroleum (Bendoraitis, Brown, and Hepner 1962) and from oil shales (Cummins and Robinson 1963) as well as from Precambrian sediments (Meinschein 1965; Oró et al. 1965). It is believed that zooplankton convert ingested phytol to pristane and that this is the principal source of the pristane found in other marine animals (Blumer, Mullin, and Thomas 1964) and at least a partial source of sedimentary pristane. The sedimentary phytane may well be formed by hydrogenation from the biogenic phytadienes (Blumer and Thomas 1965*a*).

A preliminary report by Oró and Nooner (1966) suggests the presence of pristane and phytane in marine algal mats. If confirmed, this finding may have important implications for the biogeochemistry of isoprenoids. The analyses reported here are in partial agreement with Oró and Nooner. Pristane was not detected in the red algae, in *A. nodosum*, nor in the *Fucus* collected in the spring of 1966 (Falmouth and New Hampshire), but it was present in all the other algae. Its identification is based on the appearance of a peak at the proper retention index on silicone rubber and FFAP columns after initial separation on a Carbowax 20M column. Relative to *n*-C_{17}, the cultured planktonic algae appear to contain one to two orders of magnitude more pristane than benthic algae (*see* Table 1). Among the benthic algae, the Laminariales have the largest pristane content. With the exception of one *Fucus* sample (Woods Hole), the benthic algae contain less than 1%, in most cases less than 0.1%, pristane relative to *n*-C_{17}.

In contrast to the results of Oró and Nooner, phytane was not found in the algal specimens or in the marine sediment sample. It is estimated that phytane would have been detected at a level two orders of magnitude lower than that of pristane. Pristane, phytane, and other hydrocarbons are found in appreciable quantities in commercial reagent-grade solvents (Blumer and Snyder 1965); extreme caution is required to eliminate contamination of samples by these fossil isoprenoids.

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