

STUDY OF THE LIPID FRACTIONS OF SOME
ADRIATIC SEA ALGAE

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Abstract. Preliminary examination of the lipid fractions of two Adriatic Sea algae, Cystoseira barbata and Fucus virsoides, indicated that pentadecane and heptadecane were the main n-alkanes for the former while pentadecane was the dominant one for the latter. Palmitic acid was the principal fatty acid, comprising 70% in both.

In the initial portion of a joint program directed toward a chemical study of Adriatic Sea flora and fauna we examined some of the more common organisms found in the Bay of Kotor, Yugoslavia. Part of this work concerned the lipids of the octocoral Eunicella stricta and hexacoral Cladocora cespitosa (Š. Djarmati et al., 1978; M. Šaban et al., 1978). In this report we wish to describe preliminary results on the lipids of the algae, Cystoseira barbata and Fucus virsoides.

Investigations with Cystoseira barbata from the Mediterranean and Black Seas have been limited. Seasonal variations in the fat levels of C. barbata have been examined by Alimov (1960, 1963) while the overall amounts of some inorganic ions, alginic acid, mannitol, protein cellulose, and laminaran have been studied by a number of workers for C. barbata and other species (Abdel-Fattah and Hussein, 1970; Pellegrini and Pellegrini, 1971, 1972; Guven et al., 1976). Reports indicating palmitic acid is the major fatty acid present in C. barbata (Stoianova-Ivanova, 1972) and fucosterol, the major sterol (Guyen and Bergisadi, 1973; Francisco et al., 1977) have also appeared.

Similar information has been reported for the Fucus, primarily F. serratus and F. vesiculosus. For example, Gulbrand (1941) determined the amounts of alginic acid, sugars and proteins present in these two species, while Barashkov et al. (1966) considered the seasonal variation in fat content, sugars, ash, nitrogen, and iodine. Clark and

Blumer (1967) examined the paraffins for three northeastern U.S. Fucus spp. and found 95-98% pentadecane. In an extensive study of F. vesiculosus Halsall and Hills (1971) noted the presence of fucosterol, phytol, C₁₁-C₁₆ alkanols, C₁₀-C₃₅ paraffins, and various other hydrocarbons including pentaene and hexaene isomers of heneicosane. In the case of the fatty acids, Klenk et al. (1963) found the principal ones in three Fucus spp. to be palmitic acid (25-26%), a C₁₈ monounsaturated acid (16-19%), and a C₂₀ polyunsaturated acid (10-11%). For F. serratus and F. vesiculosus Chuecas and Riley (1966) noted some stearic acid, traces of C₈ and C₁₈ saturated acids, and considerable amounts of hexadecenoic, octadecenoic, and octadecadienoic acids. Other lipid research with these algae focused upon the overall lipid content and proportion of saponifiable and unsaponifiable lipids removed by nonpolar and polar solvents (Paquot et al., 1970) and upon the amounts of galactolipids (Radunz, 1968), phospholipids and sulfated alcohols (Pham Quang Liem and Laur, 1974, 1976a, 1976b).

EXPERIMENTAL

Preparation of Samples. Air-dried Cystoseira barbata (1.40 kg) collected from the Bay of Kotor, Yugoslavia, was extracted with hexane for 12 hr. in a Soxhlet apparatus. The hexane-soluble material (2.9 g) was chromatographed on 80 g of silica gel (Merck, particle size 0.063-0.20 mm) by eluting with hexane-benzene (1:1), benzene, and benzene-ethyl acetate (various ratios). The initial fractions yielded a semi-solid waxy material (0.6 g) which had ν_{\max} 728 and 717 cm⁻¹ indicative of -(CH₂)_x-vibrations. Preliminary GLC-MS analyses performed with an LKB 2091 system indicated the major components were n-alkanes, C₁₅H₃₂ and C₁₇H₃₆.

Elution with mixtures of benzene-ethyl acetate gave a solid which was recrystallized from acetone-hexane to mp 61-62°C; ν_{\max} 1700, 728, and 717 cm⁻¹. The crystalline material and resultant mother liquor were methylated with ethereal diazomethane.

Dried Fucus virsoides (210 g), also collected from the Bay of Kotor, was extracted with hexane for 12 hr. in a Soxhlet apparatus. The hexane residue (3.9 g) was chromatographed on 110 g of silica gel as above. Hydrocarbon and fatty acid fractions were obtained as the column was eluted. The latter material was methylated as before for GLC analysis.

Gas Liquid Chromatography Conditions. GLC analyses were performed on a Varian-Aerograph Series 2700 instrument with a flame ionization detector. It was coupled to an Autolab System IV computing integrator. The hydrocarbon fractions were determined on a 1/8 inch by 5 ft. column of OV-101 (1.5%) on 100/120 HP Chrom G with a temperature program of 4°C/min. from 95-240°C. Compound identifications were accomplished by coinjection of and correlation with hydrocarbon standards (Applied Science Lab) and extrapolation of retention times. Some signals were verified by combined GC-MS analysis. Alkenes were determined by chromatography of brominated samples.

The methylated fatty acids were analyzed on a 1/8 inch by 7 ft. column of 15% diethylene glycol succinate (DEGS) on 80/100 WAW by programming from 150-185°C at 4 °C/min. The components were differentiated by comparison with several standards.

DISCUSSION

Several observations can be made from the amounts of alkanes (Table I) and fatty acids (Table II) found in the two Adriatic Sea algae *Cystoseira barbata* and *Fucus virsoides*. In the case of the alkanes there seems to be no definite alternating pattern of odd and even alkanes with the odd-numbered alkanes predominating as noted in terrestrial plants (Douglas and Eglinton, 1966; Sorensen *et al.*, 1978). Instead, only two odd-numbered alkanes are present in the highest quantity with the rest within a 1-6% range. For *C. barbata* these two

TABLE I. *n*-Alkane Composition (%) of Adriatic Algae*

C. barbata

Carbon No.	14	15	16	17	18	19	20	21	22	23
		10.4	3.2	13.0	1.1		1.5	1.3	1.7	2.4
Carbon No.	24	25	26	27	28	29	30	31	32	Alkenes**
	3.2	6.2	5.6	5.7	5.3	4.9	4.2	3.5	2.4	20.2

F. virsoides

Carbon No.	14	15	16	17	18	19	20	21	22	23
	2.2	42.9	5.9		3.2	1.1	1.6		1.8	2.0
Carbon No.	24	25	26	27	28	29	30	31	32	Alkenes**
	2.3	16.4	1.9	1.4						13.7

* Materials present in less than 1% not indicated.

** Alkenes distributed in 5-6 peaks generally C₁₆-C₂₆.

are C₁₅ and C₁₇ and amount to 10.4 and 13.0%, respectively, values not dramatically different than the others. *F. virsoides*, though, does have C₁₅ in 42.9% and C₂₅ in 16.4% which are significantly higher. This latter result for C₁₅ is consistent with data reported for several brown algae from the Cape Cod area (28-98%; Clark and Blumer, 1967; Youngblood and Blumer, 1973) and for the giant kelp *Macrocystis pyrifera* (68-92%; Rossi *et al.*, 1978). Interestingly, pentadecane seems to be the major alkane, in general, for brown algae while other algae, such as the red, green, blue-green, etc., have heptadecane, nonadecane, and nonacosane present as the main alkanes with some consistency (Weete, 1976). The very low or negligible amounts of C₁₈-C₂₂ alkanes

of C. barbata and F. virsoides also parallel the results of other brown algae.

TABLE II. Fatty Acid Composition (%) of Certain Adriatic Algae*

Material	Carbon No.: Double Bond Amount							
	14:0	15:0	16:0	16:1	17:0	18:0	18:1	20:0
<u>C. barbata</u> (crystallized acids)	5.7	1.5	68.8			12.3	11.7	
<u>C. barbata</u> (mother liquor)	6.0	1.4	66.2	9.3		3.2	11.1	
<u>F. virsoides</u>	1.0		67.0		1.5	9.4	14.4	2.4

* Materials present in less than 1% not indicated.

The fatty acid results between the two algae under study and data from other studies of algae are quite similar. In both palmitic acid comprises nearly 70% of the fractions with stearic, octadecenoic, and/or hexadecenoic acids as the second highest (9-14%). Similar data were reported by Stoianova-Ivanova (1972) for C. barbata although the percentage of unsaturated acids was higher. On the other hand several major differences between F. virsoides and other Fucus spp. (Klenk et al., 1963; Chuecas and Riley, 1966) are apparent. For instance, the amount of palmitic acid (67%) is much higher than in the previous reports (25%). Also, the C₂₀ present in F. virsoides is absent in the others while the C₁₆ and C₂₂ unsaturated acids present in the other species were not noted in F. virsoides. In other brown algae palmitic acid is the main fatty acid although the amounts are again quite lower and variable (13-28%), and the unsaturated acids are present in more variety and in much higher amounts (Weete, 1976).

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