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Characteristic carotenoids in some phytobenthos species in the coastal area of the Adriatic Sea

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Abstract

The author investigated the presence of various carotenoids in some phytobenthos species (20 species representative of *Chlorophyta*, *Phaeophyta* and *Rhodophyta*) from the coast of the Adriatic Sea. The presence of following carotenoids has been determined: 1) in *Chlorophyta*: lycopene, α-, β-, γ-, ε-carotene, β-cryptoxanthin, lutein, lutein epoxide, zeaxanthin, antheraxanthin, neoxanthin, violaxanthin, siphonein and astaxanthin ester; 2) in *Phaeophyta*: α-, β-, γ-, ε-carotene, zeaxanthin, antheraxanthin, diatoxanthin, fucoxanthin, fucoxanthol, neoxanthin, violaxanthin and rhodoxanthin-like; 3) in *Rhodophyta*: α-, β-, γ-carotene, α-, β-cryptoxanthin, lutein, lutein epoxide, zeaxanthin, antheraxanthin, mutatoxanthin, fucoxanthin neoxanthin and violaxanthin. The total carotenoid content ranged from 1.197 (*Cystoseira corniculata*) to 16.748 mg g^{-1} dry weight (*Chaetomorpha aerea*).

Key words: *Chlorophyta*, *Phaeophyta*, *Rhodophyta*, *algae carotenoids*

INTRODUCTION

In recent years, interest has grown in the biologically active compounds formed by marine organisms. The main purpose of such investigations is to determine the amount of these substances and their application now and, above all, in the nearest and more distant future. Among such biologically active substances are the carotenoids which are to be found, quite frequently in large amounts, in marine organisms and which are produced by plant organisms (Liaaen-Jensen 1977, Wettern and Weber 1979). In studies on the amount of these substances in the various fresh-water and marine algae (Czeczuga 1979) their content in phytobenthos representatives in the Adriatic Sea which is rich in this type of algae was studied. In previous papers, data were published on the carotenoid content in some species from the North Sea (Czeczuga 1976), Black Sea (Czeczuga 1979) and Mediterranean Sea (Czeczuga 1975). It is felt that the findings presented in this paper will widen our knowledge of the subject.

MATERIALS AND METHODS

Six species of *Chlorophyta* (*Halimeda tuna* (Ellis et Sol.) Lam., *Acetabularia mediterranea* Lamour., *Codium dichotomum* (Huds.) Setsch., *Chaetomorpha aerea* (Dilw.) Kutz., *Derbesia lamourouxi* (J. Ag.) Sol. and *Ulva lactuca* L.), 9 species of *Phaeophyta* (*Dictyopteris polypodioides* (Desf.) Lamour., *Dictyota dichotoma* (Huds).) Lam., *Padina pavonia* (L.) Gail., *Cystoseira adriatica* Sauvag., *Cystoseira corniculata* Hauck., *Sargassum vulgare* J. Ag., *Colpomenia sinuosa* Derb. et Sol., *Fucus virsoides* J. Ag. and *Stilophora rhizoides* (Erht.) J. Ag.) and 5 *Rhodophyta* species (*Gelidium latifolium* (Grev.) Thur. u. Born., *Laurencia obtusa* (Huds.) Lam., *Digenea simplex* (Wulf.) J. Ag., *Nemalion helminthoides* (Velly) Batters and *Phyllophorea nervosa* (Dc.) Grev.) were investigated. The material was collected in August 1980 from the coastal area of the Adriatic Sea on Sipan Island near Dubrownik in Yugoslavia.

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out with 6% KOH in ethanol at a temperature of about 40° C for several hours in the dark in a nitrogen atmosphere.

Column and thin-layer chromatography, described in detail in a previous paper (Czeczuga 1974), were used for the separation of the various carotenoids. A glass column (Quickfit-England) approximately 1 cm in diameter and 15–20 cm in length, filled Al_2O_3, was used in column chromatography. The extract was passed through the column after which the different fractions were eluted with solvent systems. Silica gel was used for the thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot.

The pigments were identified by the following methods: a) behaviour upon column chromatography: b) absorption spectra of the pigment in various solvents were recorded by a Beckman spectrophotometer model 2400 DU; c) the partition characteristics of the carotenoid between hexane and 95% methanol; c) comparison of R_f on thin-layer chromatography with standard carotenoids (Firms Hoffman-La Roche and Co. Ltd., Basle Switzerland and Sigma Chemical Company, U.S.A.); e) the presence of allylic hydroxyl groups were determined by the acid chloroform test; f) the epoxide test.

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations were based on the extinction coefficient $E^{1\%}_{cm}$ at the wavelengths of maximum absorbance in petroleum ether of hexane (Davies 1976).

RESULTS

The carotenoids identified in the studied material are listed in Table 1 and their structure in Fig. 1.

As the data given in Table 2 show, 14 carotenoids were found to be presented in the green algae species under investigation. The most interesting findings were; lycopene in the thalli of *Chaetomorpha aerea* and *Ulva lactuca*, ε-carotene in *Halimeda tuna*, and γ-carotene in the thalli of *Ulva lactuca*. In addition, the *Codium dichotomum* thalli

Table 1

List of the carotenoids from the investigated materials

Carotenoid	Structure (see Fig. 1)	Semisystematic name
Lycopene	a — R — a	ψ,ψ-carotene
γ-Carotene	a — R — c	β,ψ-carotene
α-Carotene	b — R — c	β,ε-carotene
β-Carotene	c — R — c	β,β-carotene
ε-Carotene	b — R — b	ε,ε-carotene
α-Cryptoxanthin	b — R — e	β,ε-caroten-3′-ol
β-Cryptoxanthin	c — R — e	β,β-caroten-3-ol
Lutein	d — R — e	β,ε-carotene-3,3′-diol
Zeaxanthin	e — R — e	β,β-carotene-3,3′-diol
Lutein epoxide	d — R — f	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3′-diol
Antheraxanthin	e — R — f	5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol
Mutatoxanthin	e — R_1 — g	5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol
Neoxanthin	f — R_1 — i	5′,6′-epoxy-6,7-dihydro-5,6,5′,6′-tetrahydro-β,β-carotene--3,5-3′-triol
Violaxanthin	f — R — f	5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol
Fucoxanthin	h — R_2 — k	5,6-epoxy-3,3′,5′-trihydroxy-6,7′-didehydro-5,6,7,8,5′,6′--hexahydro-β,β-caroten-8-one 3′-acetate
Fucoxanthol	h — R_2 — i	5,6-epoxy-3,3′,5′-trihydroxy-6′,7′-didehydro-5,6,8,5′,6′-hexahydro-β,β-caroten-8-one
Astaxanthin	n — R — n	3,3′-dihydroxy-β,β-carotene-4,4′-dione
Diatoxanthin	e — R_1 — l	7,8-didehydro-β,β-carotene-3,3′-diol
Rhodoxanthin	m — R_4 — m	4′,5′-didehydro-4,5′-retro-β,β-carotene-3,3′-dione
Siphonein	d — R_3 — e	3,19,3′trihydroxy-7,8-dihydro-β,ε-caroten-8-one 19-laurate

Table 2

The carotenoid content in six species of *Chlorophyta* (% of total carotenoid)

Carotenoid	*Halimeda tuna*	*Acetabularia mediterranea*	*Codium dichotomum*	*Chaetomorpha aerea*	*Derbesia lamourouxi*	*Ulva lactuca*
Lycopene				5.6		17.3
α-Carotene				4.6	3.3	
β-Carotene	6.3	19.6				9.1
γ-Carotene						10.7
ε-Carotene	5.2					
β-Cryptoxanthin	10.4	50.0		8.6	55.5	
Lutein	11.9		38.0	10.1	10.6	23.1
Lutein epoxide	27.2	13.2		44.5	7.5	12.7
Zeaxanthin				8.6	4.6	19.5
Antheraxanthin	32.8					
Neoxanthin			23.5	8.6	8.5	
Violaxanthin	6.2	trace	19.1	9.4	10.0	7.6
Siphonein			19.4			
Astaxanthin ester		17.2				
Total content of carotenoids, mg per g dry weight	5.076	2.600	1.235	16.748	16.092	7.425

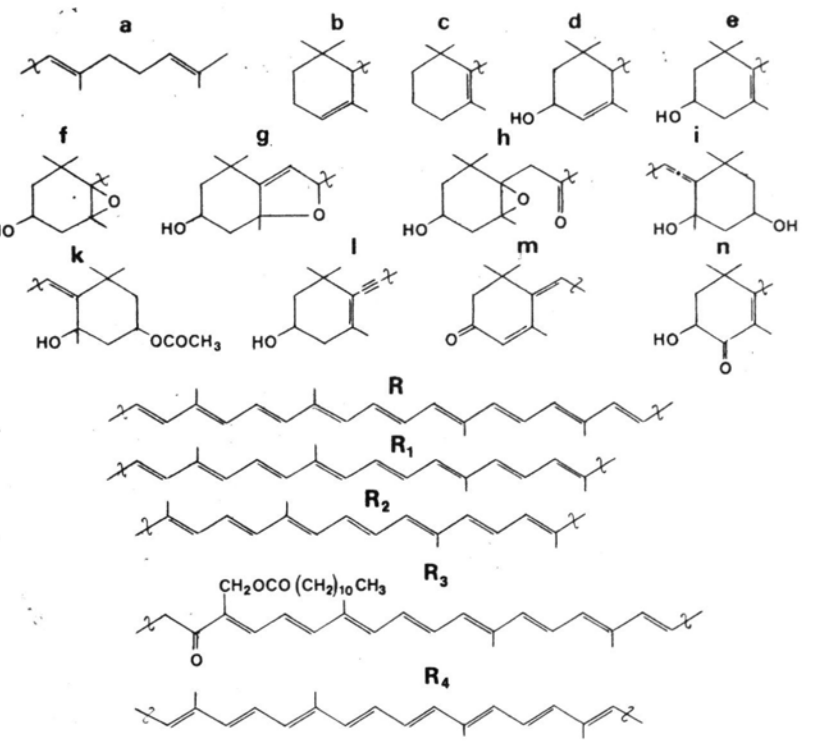

Fig. 1. Structural features of carotenoids from investigated materials (see Table 1)

Table 3

The carotenoid content in nine species of *Phaeophyta* (% of total carotenoid)

Carotenoid	*D. poly-polioides*	*D. dicho-toma*	*P. pavo-nia*	*C. corni-culata*	*S. vulgare*	*C. adriatica*	*C. sinuosa*	*F. virsoides*	*S. rhi-zoides*
α-Carotene							11.5	8.5	
β-Carotene		28.8	15.6	55.5	40.8	12.2		33.7	23.2
γ-Carotene							6.8		19.1
ε-Carotene		17.5					9.7		9.1
Zeaxanthin			24.9			73.0	22.3	27.6	17.7
Antheraxanthin	21.4				37.9		10.2	11.8	7.7
Diatoxanthin	31.7		26.2			4.0		6.8	23.2
Fucoxanthin	27.0	27.0	21.1		9.8		23.6		
Fucoxanthol	19.9	13.5		44.5	11.5				
Neoxanthin						5.2	8.2	8.5	
Violaxanthin		13.2	12.2				7.7	3.1	
Rhodoxanthin-like						5.6			
Total content of carotenoids, mg per g dry weight	3.024	2.963	5.125	1.197	1.526	2.690	6.261	10.666	6.052

Table 4

The carotenoid content in five species of *Rhodophyta* (% of total carotenoid)

Carotenoid	*Gelidium latifolium*	*Laurencia obtusa*	*Digenea simplex*	*Nemalion helminthoides*	*Phyllophora nervosa*
α-Carotene	7.8	4.7	8.6	trace	6.0
β-Carotene	10.3	16.3	11.8	6.9	trace
γ-Carotene		5.1	9.1		
α-Cryptoxanthin				4.7	
β-Cryptoxanthin	11.5	17.4	8.6	45.4	53.7
Lutein	46.3		8.6		
Lutein epoxide		5.4	19.0		21.5
Zeaxanthin	14.4	44.2	23.9	20.2	4.9
Antheraxanthin					7.6
Mutatoxanthin				11.5	
Fucoxanthin	9.7				
Neoxanthin			6.9	11.3	1.6
Violaxanthin		2.2	3.5		4.7
Total content of carotenoids, mg per g dry weight	9.110	7.266	4.862	6.967	8.765

were found to contain siphonein, and *Acetabularia mediterranea* — astaxanthin ester. The total carotenoid content ranged from 1.235 mg (*Codium dichotomum*) to 16.748 mg·g^{-1} of dry mass (*Chaetomorpha areae*).

In the brown algae species studied (Table 3), 12 carotenoids were determined of which the most worthy of note are γ-carotene (*Colpomenia sinuosa*, *Stilophora rhizoides*), ε-carotene (*Dictyota dichotoma*, *Colpomenia sinuosa*, *Stilophora rhizoides*) and a rhodoxanthin-like carotenoid in the thalli of *Cystoseira adriatica*. The total carotenoid content in the brown algae thalli varied between 1.197 mg (*Cystoseira corniculata*) to 10.666 mg·g^{-1} of dry mass (*Fucus virsoides*).

As can be seen in Table 4, in the thalli of the 5 red algae species, 13 carotenoids were identified. Of particular interest is the finding of γ-carotene in the *Laurencia obtusa* and *Digenea simplex* thalli, mutatoxanthin in *Nemalion helminthoides*, and fucoxanthin in *Gelidium latifolium*. The total carotenoid content in these red algae varied between 4.862 mg (*Digenea simplex*) and 9.110 mg·g^{-1} of dry mass (*Gelidium latifolium*).

DISCUSSION

Nearly all the carotenoids identified in the green algae studied had already been reported, with the exception of ε-carotene, in other species of that genera (Liaaen-Jensen 1977, Wettern and Weber 1979, Weber and Wettern 1980). ε-Carotene has, on the other hand, been found in the *Diatoma*, brown algae and cryptomonads (Wettern and Weber 1979, Weber and Wettern 1980); the finding of this carotenoid in *Halimeda tuna* is the second report of its presence in green algae. ε-Carotene was first found in green algae by Benson and Cobb (1981). Another interesting observation is that of the presence of astaxanthin ester in *Acetabularia mediterranea*. This carotenoid was found in the thalli of *Acetabularia mediterranea* by Kleinig and Egger (1967).

Almost all the carotenoids found in the brown algae from the Adriatic Sea had been noted previously in other brown algae species (Liaaen--Jensen 1977, Wettern and Weber 1979, Czeczuga 1979, Weber and Wettern 1980). Only two findings are of interest, that is, the presence of γ-carotene in two species and a rhodoxanthin-like carotenoid in the *Cystoseira adriatica* thalli. While γ-carotene has been previously noted in algae of other types (Wettern and Weber 1979, Weber and Wettern 1980), and in several green algae species, the finding of a rhodoxanthin-like carotenoids is probably the first report of this type of carotenoid in algae.

The investigations on the red algae species also resulted in two interesting findings; that of the presence of γ-carotene in two species and α-cryptoxanthin in the *Nemalion helminthoides* thalli. The former has been previously reported in blue-green algae and in green algae (Wettern and Weber 1979, Weber and Wettern 1980), and the latter quite frequently occurs in green algae (Liaaen-Jensen 1977) but rarely in red algae (Bjørnland 1983). α-Cryptoxanthin was found in red algae by Czeczuga (1976) in the thalli of *Porphyra leucostica* and in the *Ceramium rubrum* thalli by Bjornland and Aguilar-Martinez (1976). Peridinin has been reported in some red algae species of Australian waters (Arnesen et al. 1979) but was not found in our material.

Carotenoids together with chlorophylls constitute the photosynthetically active group of pigments in the majority of algae whereas in blue-green algae, red algae and cryptomonad algae phycobiliprotein pigments also belong to the above-mentioned group of pigments.

As we know, light is one of the factors limiting the occurrence of algae in the deeper parts of the littoral. For this reason, chromatic adaptation of algae to light conditions is characteristic; the content of some pigments of the photosynthetically active group undergoes changes. The deeper the water (less light), the greater the total content of photosynthetically active pigments, above all of chlorophyll b, is to be found in the algae of that environment (Yakohama and Misonon 1980, Falkowski et al. 1981, Perez-Bermudez et al. 1981, Rivkin et al. 1982). The composition of the carotenoids in the alga thalli may also change at greater depths. This applies chiefly to such carotenoids as siphonaxanthin and siphonein on the one hand and lutein on the other. As the studies of Yokohama (1981, 1982, 1983) and O'Kelly (1982) showed, in green algae species occurring at greater depths penetrated generally only by green rays, siphonaxanthin and siphonein dominate whereas in species from shallow waters, large amounts of lutein are found. In the case of the algae investigated in the present studies, the fact that all the species were collected from the shallow littoral zone seems to be the reason for the large amounts of lutein (pure and epoxide forms) found in the green algae species under study.

The total carotenoid content of the green algae, brown algae and red algae species studied as representatives of phytobenthos was similar to that noted in other alga species (Czeczuga 1979, Wettern and Weber 1979, Goodwin 1971, 1980).

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Karotenoidy charakterystyczne dla niektórych gatunków fitobentosu z przybrzeżnej strefy morza Adriatyckiego

Streszczenie

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów u 20 gatunków glonów bentosowych, przedstawicieli zielenic, brunatnic i krasnorostów zebranych w przybrzeżnej strefie morza Adriatyckiego. Stwierdzono występowanie następujących karotenoidów: 1) u 6 przedstawicieli zielenic: lykopen, α-, β-, γ-, ε-karoten, β-kryptoksantyna, luteina, epoksyd luteiny, zeaksantyna, anteraksantyna, neoksantyna, wiolaksantyna, sifoneina i formę estrową astaksantyny; 2) u 9 przedstawicieli brunatnic: α-, β-, γ-, ε-karoten, zeaksantyna, anteraksantyna, diatoksantyna, fukoksantyna, fukoksantol, neoksantyna, wiolaksantyna i ksantofil rodoksantyno-podobny; 3) u 5 przedstawicieli krasnorostów: α-, β-, γ-karoten, α-, β-kryptoksantyna, luteina, epoksyd luteiny, zeaksantyna, anteraksantyna, mutatoksantyna, fukoksantyna, neoksantyna i wiolaksantyna. Ogólna zawartość karotenoidów wahała się of 1,197 (*Cystoseira corniculata*) do 16,748 $mg \cdot g^{-1}$ suchej masy (*Chaetomorpha aerea*).