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Chlorophyll *a* and Carotenoids of Five Isolates of the Red Alga *Antithamnion plumula*

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Key Word Index – *Antithamnion plumula*; Rhodophyceae; chlorophyll; carotenoids; α-cryptoxanthin; neoxanthin; chemosystematics.

Abstract – Female plants of five isolates (including two varieties) of the red alga *Antithamnion plumula* were cultured monoalgally and analysed for chlorophylls and carotenoids. The relative amounts of the individual pigments were similar for the five isolates. All isolates contained chlorophyll *a*, β,β-carotene, β,ε-carotene, α-cryptoxanthin and lutein. Allylic methylation of α-cryptoxanthin indicated a 3′-position of the hydroxyl group. An earlier tentative identification of neoxanthin in *A. plumula* could not be confirmed.

Introduction

The distribution of carotenoids within the Phycophyta [1–3] provides a powerful taxonomic tool on the class level. Among the major characteristics of algal carotenoid pigmentation in chemosystematic considerations are the presence or absence of β,ε-carotene derivatives and of the acetylenic bond, and the identity of the structurally most elaborated xanthophyll. With very few exceptions these features are constant within each class of the Phycophyta.

Generally, the carotenoids of the red algae are biochemically simple, the most elaborate being zeaxanthin and lutein in the β,β- and β,ε-carotene biosynthetic chain, respectively [4]. A few representatives, however, have been reported to deviate from this general picture (for references, see [4]). Most outstanding is the reported occurrence of neoxanthin (incompletely identified) in cultured material of *Nemalion helminthoides* (= *N. multifidum*) and *Antithamnion plumula* [5]. By the possession of both secondary and tertiary hydroxyl groups, an epoxide group and an allenic bond neoxanthin is structurally far more complex than lutein and zeaxanthin (Fig. 1). In addition, neoxanthin is among the structurally most elaborated carotenoids within the green algal classes Euglenophyceae, Prasinophyceae and Chlorophyceae and has been reported as a minor constituent in several representatives of the Chromophyta. For chemosystematic reasons it therefore seemed justified to make a re-investigation of these two algae. The results for *N. helminthoides* have been published earlier [4], the results for *A. plumula* are reported here.

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Results and discussion

Cultured material of five isolates of *A. plumula* was analysed qualitatively and quantitatively for chlorophylls and carotenoids. Isolates *1, 2* and *3* belonged to var. *plumula* while isolates *4* and *5* belonged to var. *bebbii*. The amounts of chlorophyll *a* and total carotenoids were calculated from the UV–visible spectra of the total extracts; the amounts of the individual carotenoids from UV–visible spectra of TLC-pure samples. The carotenoids of isolates *1, 2, 3* and *5* were characterized by UV–visible spectrophotometry and co-chromatography with authentic samples on two different TLC systems (see Experimental). The carotenoids of isolate *4* were further characterized by ^{1}H NMR, MS, simple chemical derivatizations and MS of the reaction products. β,β-Carotene, β,ε-carotene and lutein of isolate *4* were obtained in the crystalline state. The results are shown in Table 1.

The pigment level of the five isolates varied considerably (Table 1). This variation may not be correlated with differences in culture conditions, culture density or time of harvest during the LD

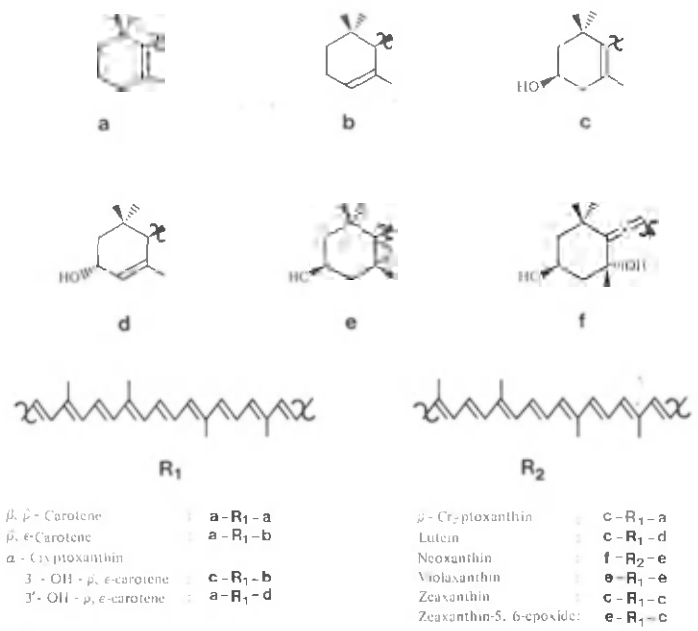

FIG. 1. CAROTENOID STRUCTURES. The chirality of the red alga carotenoids has been assumed to be the same as in other biological material [20]. This assumption is valid for the red alga carotenoids examined for chiroptic properties up to the present (Bjørnland, Borch and Liaaen-Jensen, to be published).

photoperiod (see Experimental). The different pigment levels do not necessarily reflect variations in chloroplast properties among the isolates, but may equally well be ascribed to differences in cell-wall thickness, degree of ramification and so forth. The relative amounts of the individual pigments, however, were comparable for all five isolates.

Chlorophyll *a* was the only chlorophyll present. Chlorophyll *d*, the occurrence of which in red algae is disputed [6, 7], could not be detected.

The carotenoids were structurally simple. *A.*

TABLE 1. CHLOROPHYLL *a* AND CAROTENOIDS OF FIVE ISOLATES OF *ANTITHAMNION PLUMULA*

	Isolate					UV–visible †	
	1	*2*	*3*	*4*	*5*	Absorption maxima ‡	III/II
	(mg/g*)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(nm)	(%)
Chlorophyll *a* §	21.1	6.03	6.47	8.86	4.53	383, 412, 431 578, 615, 663	
β,β-Carotene	0.13 (6)	0.09 (8)	0.11 (6)	0.22 (8)	0.12 (8)	(429), 450, 478	40
β,ε-Carotene	0.24 (11)	0.14 (12)	0.31 (18)	0.41 (15)	0.20 (14)	421, 444, 474	71
α-Cryptoxanthin	0.10 (4)	0.06 (5)	0.06 (4)	0.14 (5)	0.06 (4)	420, 443, 472	57
Lutein	1.81 (79)	0.89 (75)	1.23 (72)	1.94 (72)	1.07 (74)	420, 444, 473	75
Total carotenoids §	7.39	2.30	2.61	3.26	1.93		
Total carotenoids § / Chlorophyll *a* §	0.35	0.38	0.40	0.37	0.43		

*mg/g dry wt; % of total carotenoids in parentheses.

† Data given for isolate *4*.

‡ Solvent: light petroleum (Me_2CO for chlorophyll *a*).

§ Calculated from the UV–visible spectrum of the total extract.

plumula possessed the capacity to synthesize both β- and ε-rings, but the biochemical elaboration above the bicyclic carotene level was restricted to the establishment of the allylic and non-allylic OH structure elements in the 3- and 3'-positions. The carotenoid composition was further characterized (compare ref. [8]) by the dominance of β,ε-carotenoids.

Carotenoids termed α-cryptoxanthin have previously been isolated from the red algae *Ceramium elegans* [9], *C. rubrum* [4], *Lenormandia prolifera* [10] and *Porphyra leucosticta* [11]. Accordingly, its occurrence is not restricted to the Amansia group or the Rhodomelacea [10], but is probably a frequent minor constituent in red algae with β,ε-carotenoids dominating. The position of the OH group has not been firmly established in the cited works. The α-cryptoxanthins reported may accordingly be structurally different. Due to the positive allylic methylation test in the present work, the OH group in α-cryptoxanthin from *A. plumula* is tentatively assigned the 3'-position. More conclusive data on the structure of α-cryptoxanthins from Rhodophyceae will be presented in a recently concluded work on the chirality of red alga carotenoids (Bjørnland, Borch and Liaaen-Jensen, to be published). α-Cryptoxanthin from *A. plumula* co-chromatographed with α-cryptoxanthin from naturally occurring *C. rubrum* both on TLP-I and TLP-II (see Experimental). The latter pigment has previously been detected also in monoalgally cultured material [4]. The "unknown" from *A. plumula* in ref. [5] is probably identical with α-cryptoxanthin.

The identity of red alga carotenoids long relied mainly on UV–visible spectrophotometric and chromatographic properties and has only recently [4, 12, 13] been confirmed by modern methods such as MS. Lutein, the main xanthophyll of the majority of red algae, has been further characterized in the present work. Notably the ^{1}H NMR spectrum provided data consistent with the expected end-groups **c** and **d** (Fig. 1). The presence of one allylic and one non-allylic primary/secondary OH group was further confirmed by selective methylation of the allylic OH group and subsequent acetylation of the lutein-3'-methyl ether.

Neoxanthin, as well as its probable biosynthetic precursors violaxanthin, zeaxanthin-5,6-epoxide, zeaxanthin and β-cryptoxanthin, was absent. The absence of neoxanthin contradicted earlier data [5]. Unfortunately, the original culture used in [5] is no longer available (Fries, personal communication). However, both *A. plumula* in ref. [5] and *A. plumula* var. *bebbii* (isolate *5*) in the present investigation have been isolated from the Kristineberg area on the Swedish West Coast.

The absence of neoxanthin in the biological material investigated may not be ascribed to the presence of female plants only. An extract of a less-defined gross sample of monoalgally cultured *A. plumula* consisting of several geographical isolates and all stages in the life cycles did not contain any trace of neoxanthin. Contrary to the earlier investigation [5], the five isolates accordingly all possessed the biochemically simple carotenoid pigmentation encountered in the majority of red algae.

Experimental

Biological material. Five isolates of *Antithamnion plumula* (Ellis) Thur. in Le Jol. were obtained from the culture collection of the Section of Marine Botany, University of Oslo. The specimens were collected at the following localities: isolate *1*: Fulehuk in the Oslofjord; isolate *2*: Espegrend at Bergen; isolate *3*: Hoftøy in the Oslofjord; isolate *4*: Hvaler in the Oslofjord; isolate *5*: Kristineberg at the Swedish West Coast. Isolates *1*, *2* and *3* belonged to var. *plumula*, while isolates *4* and *5* belonged to var. *bebbii* (Reinsch) J. Feldm. In culture the growth of both male plants and tetrasporophytes was soon retarded by the profuse formation of male gonidia and tetraspores, respectively. For this reason only female plants were selected for the culture work.

Culture conditions. All five isolates were cultured monoalgally at 18° in polystyrene dishes (Heger Plastics A/S, type 10100) under identical conditions. The light sources were Philips fluorescent tubes (TL/55). The light intensity was 8.6 $\mu E/m^2/s$ as measured with a LI-188 integrating quantum photometer fitted with a LI-190s cosinus sensor (Lambda Instr. Corp.), and the LD photoperiod was 14:10. Each culture dish contained 140 ml of the enriched sea water medium IMR [14]. The salinity was 30‰ and the medium was renewed every second week. The number of culture dishes, duration of the cultivation period (days) and harvested amount (g lipid-extracted dry-wt) were as follows: isolate *1*: 2 dishes, 222 days, 0.42 g; isolate *2*: 1 dish, 276 days, 0.25 g; isolate *3*: 16 dishes, 310 days, 2.52 g; isolate *4*: 24 dishes, 290 days, 3.02 g; isolate *5*: 8 dishes, 368 days, 1.64 g. The algae were harvested 3–3.5 h after the onset of the diurnal light phase.

Physical and chemical methods. Fresh algal material was extracted with Me_2CO followed by Me_2CO–MeOH (7:3). The extracts were saponified with 5% KOH in MeOH (20°, 12 h) and the individual carotenoids isolated by a two-step TLC procedure. The carotenoids were separated into carotenes, monohydroxyxanthophylls and dihydroxyxanthophylls by TLC on Si gel G–$CaCO_3$ (1:1) (TLP-I) [4]. The three fractions obtained were further separated into their β,β- and β,ε-

carotene derivatives by TLC on Si gel G–$Ca(OH)_2$–MgO–$CaSO_4$ (10:4:3:1) (TLP-II) [4]. The developing solvent was light petroleum with varying amounts of Me_2CO and *iso*-PrOH.

The amounts of chlorophyll *a* and total carotenoids were calculated from the UV–visible spectrum of the total extract; the amounts of the individual carotenoids from the UV-visible spectrum of TLC-pure samples. $E^{1\%}_{1cm}$ (solvent: Me_2CO): chlorophyll *a*: 881.5 [15]; total carotenoids: 2260 (calculated for a β,ε-chromophoric system at 476–478 nm); TLC-pure carotenoids: 2500.

The identity of the pigments was established by UV–visible spectrophotometry and by co-chromatography with authentic samples on paper Schleicher & Schüll No. 287 (chlorophyll *a*) [16], TLP-I (chlorophyll *a* and carotenoids) and TLP-II (carotenoids). The identity of the carotenoids of isolate *4* was further ascertained by MS (70 eV, 190°) [17], ^{1}H NMR-FT (100 MHz in $CDCl_3$ with TMS) [17], acetylation with $(MeCO)_2O$ in dry pyridine [18] and allylic methylation with HCl–MeOH in $CHCl_3$ [19].

Authentic pigments. β,β-Carotene and β,ε-carotene: *Daucus carota;* α-cryptoxanthin: *Ceramium rubrum;* β-cryptoxanthin and zeaxanthin: calyx of *Physalis alkekengi;* lutein: *Medicago sativa;* violaxanthin: petals of *Viola tricolor;* neoxanthin and chlorophyll *a*: *Hordeum vulgare.*

Chemical and physical data

. *Chlorophyll a.* UV–visible: λ_{max} (nm) in Me_2CO: 383, 412, 431, 578, 615 and 663.

β,β-Carotene. UV–visible (cryst.): λ_{max} (nm) in light petroleum: (429), 450 and 478; III/II (%) = 40. MS: *m/z* (rel. int.): 536 (M^+) (22%), 444 (4) and 430 (3).

β,ε-Carotene. UV–visible (cryst.): λ_{max} (nm) in light petroleum: 421, 444 and 474; III/II (%) = 71. MS: *m/z* (rel. int): 536 (M^+) (35%), 444 (4) and 430 (6).

α-Cryptoxanthin. UV–visible: λ_{max} (nm) in light petroleum: 420, 443 and 472; III/II (%) = 57. MS: *m/z* (rel. int.): 552 (M^+) (100%), 534 (91), 496 (0.3), 460 (6), 446 (1), 442 (9) and 428 (2). Acetylation yielded a monoacetate: MS: *m/z* (rel. int.): 594 (M^+) (100%), 534 (34), 502 (10), 488 (3), 442 (8) and 428 (2). Allylic methylation yielded a monomethyl ether: MS: *m/z* (rel. int.): 566 (M^+) (62%), 553 (19), 534 (8), 474 (7), 460 (1) and 442 (1).

Lutein. UV–visible (cryst.): λ_{max} (nm) in light petroleum: 420, 444 and 473; III/II (%) = 75. MS: *m/z* (rel. int.): 568 (M^+) (96%), 550 (50), 532 (1), 476 (6), 462 (3), 458 (3), 444 (1) and 430 (2). ^{1}H NMR (δ-values): 0.85 (Me-1'), 1.00 (Me-1'), 1.08 (Me-1,1), 1.63 (Me-5'), 1.73 (Me-5), 1.91 (Me-9') and 1.97 (Me-9,13,13'). Allylic methylation gave lutein-3'-methyl ether in 83% yield: MS *m/z* (rel. int.): 582 (M^+) (100%), 564 (0.3), 550 (7), 526 (0.6), 490 (7), 476 (2), 458 (1) and 444 (0.4). Acetylation of lutein-3'-methyl ether yielded a monoacetate: MS: *m/z* (rel. int.): 624 (M^+) (100%), 592 (7), 568 (0.2), 564 (3), 532 (5), 518 (2), 500 (0.4), 486 (0.4), 472 (6) and 458 (0.6).

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