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SHORT COMMUNICATION

OCCURRENCE OF ANTHERAXANTHIN IN TWO RHODOPHYCEAE *ACANTHOPHORA SPICIFERA* AND *GRACILARIA LICHENOIDES*

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Abstract—The carotenoids in two Rhodophyceae, *Acanthophora spicifera* and *Gracilaria lichenoides* were examined. β-Carotene and its xanthophyll derivatives accounted for approximately 78 per cent of the total pigments in both organisms. Antheraxanthin, mono-epoxy zeaxanthin, which has not been reported previously in the Rhodophyceae, was a major fraction in both algae.

EPOXY carotenoids have been found in many algal classes[1-4] but not in the Cyanophyceae[1] and Rhodophyceae,[5,6] although in the latter tentative identification of neoxanthin has been reported.[4] Our interest in the biosynthesis and function of epoxy xanthophylls prompted examinations of the carotenoids in two Rhodophyceae commonly found in Hawaii, *Acanthophora spicifera* and *Gracilaria lichenoides*. *A. spicifera* is a relatively recent invader of Hawaii's marine flora[7] and *G. lichenoides* is an edible alga known locally as "ogo." We report herein that antheraxanthin, an epoxy carotenoid, was the major carotenoid in both organisms.

The algae were collected from reef flats near Waikiki. They were washed with tap water, inactivated in 10 volumes w/v of boiling 1% KOH-methanol solution and stored at −18°. This procedure extracted almost all of the carotenoids and further extraction was not required. The next day, the extracts were decanted and saponified in 25% KOH-methanol solution for ½ hr in the dark with constant stirring. After saponification the pigments were washed into peroxide-free ethyl ether, evaporated to dryness and chromatographed on Micro-Cel C with 13% acetone-light petroleum (b.p. 30–60°).[8] The carotene fraction was rechromatographed on magnesium oxide:Hyflo Super-Cel 1:2 w/w with 5% acetone-light petroleum. Lutein and zeaxanthin were separated by rechromatography on magnesium oxide:Hyflo Super-Cel with 25% acetone-light petroleum.[9] Both column and thin layer chromatographic methods were used. The pigments were identified by their relative chromatographic positions,[8,9]

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spectra,[10] M_{50} value,[11] acid catalyzed isomerization of 5,6 epoxides,[10] and comparisons with reference samples. The reference pigments were α- and β-carotene from carrot roots, β-cryptoxanthin from papaya,[12] and lutein, zeaxanthin and antheraxanthin from spinach leaves.[13]

TABLE 1. CAROTENOIDS IN *Gracilaria lichenoides*

Fraction	Per cent composition	M_{50}	Number of epoxide groups	Absorption maxima in ethyl ether, nm	Identification
1·1	24·1	—	0	(424), 448, 476	β-carotene
1·2	Trace	—	–	—	—
1·3	10·8	99·3	0	(420), 446, 472	β-cryptoxanthin
1·4	Trace	—	–	—	—
2	19·0	80·0	0	(425), 450, 477	Zeaxanthin
3	24·6	74·0	1	(422), 445, 473	Antheraxanthin
4	4·0	79·5	0	(424), 448, 475	—
5	8·4	—	0	(420), 446, 470	—
6	9·1	—	0	(417), 445, 468	—

(*a*) Fractions 1·1–1·4 were from Micro-Cel C fraction 1 rechromatographed on magnesium oxide: Hyflo Super-Cel.
(*b*) The total carotenoid yield was 362 μg/100 g wet cells.

TABLE 2. CAROTENOIDS IN *Acanthophora spicifera*

Fraction	Per cent composition	M_{50}	Number of epoxide groups	Absorption maxima in ethyl ether, nm	Identification
1·1	1·0	—	—	—	—
1·2	23·3	—	0	(424), 450, 476	β-carotene
1·3	3·8	99·5	0	(420), 447, 475	β-cryptoxanthin
1·4	3·3	—	—	—	—
2	19·2	82·0	0	(422), 447, 475	Zeaxanthin, with trace lutein
3	32·2	74·0	1	(420), 445, 473	Antheraxanthin
4–10	17·2	—	—	—	—

(*a*) Fractions 1·1–1·4 were Micro-Cel C fraction 1 rechromatographed on magnesium oxide: Hyflo Super-Cel.
(*b*) Rechromatography of fraction 2 on magnesium oxide: Hyflo Super-Cel showed the presence of a small amount of lutein.
(*c*) Fractions 4–10 were well resolved bands of relatively low concentration but they were eluted and estimated as a single fraction.
(*d*) The total carotenoid yield was 705 μg/100 g wet cells.

Typical results for *G. lichenoides* and *A. spicifera* are shown in Tables 1 and 2, respectively. As seen in Table 1, the pigments in *G. lichenoides* were resolved into 9 fractions of which 4 were of the β-carotene series and accounted for approximately 78 per cent of the total carotenoids. Most significantly, lutein was not detectable, and antheraxanthin, a mono-

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epoxide was a major component. Rechromatography of the lutein-zeaxanthin fraction from Micro-Cel C on magnesium oxide showed that lutein was not present in *G. lichenoides*. Table 2 shows that the pigment composition of *A. spicifera* was more complex. Approximately 13 fractions were obtained but most were relatively minor fractions constituting 5 per cent or less of the total carotenoids. Although more complex, the major fractions in *A. spicifera* were of the β-carotene series, accounting for about 78 per cent of the total carotenoids, and antheraxanthin was the major xanthophyll. However, as was not the case with *G. lichenoides*, lutein was found in trace amounts in the lutein-zeaxanthin fraction. Violaxanthin, neoxanthin, and α-carotene were not detected in *G. lichenoides* or *A. spicifera*.

The identification of antheraxanthin in both *G. lichenoides* and *A. spicifera* firmly establishes the occurrence of epoxy xanthophylls in the Rhodophyceae subclass Florideae. It is significant that antheraxanthin was a major component in both organisms examined since this pigment had not been detected in any of the large number of Rhodophyceae examined previously.[4–6] Although the present results could be entirely due to coincidence, it is also possible that antheraxanthin is more widely distributed in the Rhodophyceae but has escaped detection because of its similarity to lutein in spectrum and chromatographic behavior.

Based on morphology and pigment content, the Rhodophyceae can be considered just above the Cyanophyceae in the phylogenetic scale.[15,16] The apparent lack of epoxy xanthophylls in Cyanophyceae[1] and the occurrence of mono-epoxy xanthophyll in the subclass Florideae but apparently not in the subclass Bangiodeae[17] of the Rhodophyceae suggests that biosynthetic competence for epoxidation evolved some time during the development of the Rhodophyceae. Also it is interesting that besides in the Rhodophyceae, antheraxanthin has been found to be a major carotenoid in organisms from three other classes of algae, *Euglenophyceae*,[14] *Xanthophyceae*[2] and *Chloromonadophyceae*.[3]

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