

SHORT COMMUNICATION

THREE NEW CAROTENOIDS ISOLATED FROM ALGAE

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DURING an investigation of the carotenoids of some flagellates of the algal class Cryptophyceae^{1,2} three apparently new xanthophylls were encountered. Since carotenoid composition may provide important clues as to the taxonomy of the group, and as the results are in some respects at variance with the work of others (cf. Ref. 3), preliminary results concerning their identity are communicated below.

The flagellates investigated were *Cryptomonas ovata* var. *palustris*, *Rhodomonas* Strain D3 and *Hemiselmis virescens* (Droop's Strain). They were obtained as axenic cultures from Dr. L. Provasoli and cultured as previously described.^{1,2} The carotenoids were extracted from freshly harvested cells in ethanol and passed into hexane-diethyl ether after removal of the chlorophylls by saponification. Preparatory to crystallization the carotenoids were fractionated and purified by successive chromatography on columns of CaCO₃-Ca(OH)₂-diatomaceous earth (2:2:1 w/w) with 0-20% acetone in benzene; microcel C (Johns Manville) with 10-80% diethyl ether in petroleum ether and on ZnCO₃-diatomaceous earth (3:1 w/w) with 0-25% acetone in petroleum ether. Mixed chromatography and the determination of *R_f* values were performed on thin-layer chromatograms of silica gel G-Al₂O₃ (1:1 w/w) developed with 20, 30 or 35% acetone in hexane. Absorption spectra were recorded on a Cary model 14 spectrophotometer. Melting points were determined on a Berl block and are given as corrected values. Diatoxanthin was prepared from *Isochrysis galbana* by similar chromatographic procedures after initial chromatography on silica gel (0-15% *n*-propanol in hexane) to remove the abundant quantities of fucoxanthin.

All three cryptomonads possessed the same strongly adsorbed major xanthophyll comprising 75-90 per cent of the total carotenoids. This new xanthophyll for which the name alloxanthin is proposed was first noted in *Cryptomonas ovata* var. *palustris* by Haxo and Fork⁴ who commented on its similarity to zeaxanthin and diatoxanthin. Allen *et al.*³ examined two strains of cryptomonads, including *Hemiselmis virescens*, and demonstrated that the major xanthophyll was not zeaxanthin, and consequently inferred that it was diatoxanthin, although no direct comparison with diatoxanthin was made. Alloxanthin was crystallized from pyridine-water and recrystallized three times from acetone-petroleum ether. The crystals appear as rosettes of small needles. The spectra and adsorptive properties

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¹ D. J. CHAPMAN and F. T. HAXO, *Plant Cell Physiol.* **4**, 56 (1963).

² D. J. CHAPMAN, Ph.D., dissertation. University of California, San Diego (1965).

³ M. B. ALLEN, L. FRIES, T. W. GOODWIN and D. M. THOMAS, *J. Gen. Microbiol.* **34**, 259 (1964).

⁴ F. T. HAXO and D. C. FORK, *Nature* **184**, 1051 (1959).

of alloxanthin, zeaxanthin and diatoxanthin are very similar (Table 1). Non-identity of zeaxanthin (R_f 0.48) and alloxanthin (R_f 0.42) has been confirmed by mixed chromatography on a silica gel-alumina plate with 35% acetone in hexane. Attempts to separate diatoxanthin and alloxanthin by mixed chromatography, employing a variety of different solvent-adsorbent systems, were only partially successful.

Non-identity of these two xanthophylls was established by a comparison of the spectral changes that occur upon photoisomerization (in the presence of iodine: 3/4 per cent of carotenoid by weight) of the all-trans carotenoid to yield their respective *cis-trans* equilibrium mixture.^{1,5} The distinct spectral differences (Table 1) that occur between the two xanthophylls reflect the even greater differences that appear in the quantitative and qualitative distribution patterns of the individual isomers in the equilibrium mixture.

TABLE 1. ABSORPTION SPECTRA MAXIMA AND MINIMA (IN PARENTHESES) AND SPECTRAL CHARACTERISTICS* OF ALLOXANTHIN, MONADOXANTHIN, CROCOXANTHIN, DIATOXANTHIN, LUTEIN AND MONO-HYDROXY α -CAROTENES

	λ_{\max} (m μ in hexane)					$\% \frac{D_B}{D_{II}}$	$\% \frac{III}{II}$	m.p.
Alloxanthin (<i>trans</i>)	480	(467)	451			6	50	186-188
Iodine equilibrium	467	(455)	439			11	45	
Diatoxanthin (<i>trans</i>)	479	(469)	450			5	48	201
Iodine equilibrium	470	(460)	442			17	31	
Monadoxanthin	475	(461)	445	(427)	422	8	72	165
Lutein	474	(460)	445	(425)	420	6	74	193 ⁶
Crocoxanthin	475	(462)	445	(427)	422	7	69	163-165
Zeinoxanthin ⁷	474	(463)	445	(428)	422		77	174-176
α -Cryptoxanthin ⁸	475	(462)	445	(427)	422		69	184
4-Hydroxy- α -carotene ⁹	474	—	445	—	421		—	177-178

* The absorption profile can be described quantitatively by use of the terms

$$\% \frac{D_B}{D_{II}} \text{ and } \% \frac{III}{II}.$$

For a description of these terms, see Ref. 5.

In *Rhodomonas* D3, a yellow fraction comprising 15 per cent of the total carotenoids, develops immediately in front of alloxanthin. The name proposed for this xanthophyll, which is spectrally and adsorptively very similar to lutein, (Table 1) is monadoxanthin. This xanthophyll was crystallized as small rectangular plates by the same method employed for alloxanthin and diatoxanthin. Non-identity with lutein (isolated from *Spinacea oleracea*) was established by mixed chromatography on silica gel-alumina plates with 30% acetone in hexane. Lutein (R_f 0.41) moves ahead of monadoxanthin (R_f 0.38). Failure to obtain a

⁵ S. L. JENSEN, *Kgl. Norsk. Vidensk. Selsk. Skr. Nr.* 8 (1962).

⁶ P. KARRER and E. JUCKER, *Carotenoids*. Elsevier, Amsterdam (1950).

⁷ E. N. PETZOLD and F. W. QUACKENBUSH, *Arch. Biochem. Biophys.* **86**, 163 (1960).

⁸ L. CHOLNOKY, J. SZABOLCS and E. NAGY, *Ann. Chem.* **616**, 207 (1958).

⁹ W. V. BUSH and L. ZECHMEISTER, *J. Am. Chem. Soc.* **80**, 2991, 1958.

positive test (appearance of a blue color in HCl-methanol) for the presence of epoxide groups¹⁰ eliminated possible identity with similar epoxy carotenoids.

All three cryptomonads contained a weakly adsorbed xanthophyll, representing 5–15 per cent of the total carotenoids, with spectral and adsorptive properties very similar to those encountered in the monohydroxy α -carotenes (Table 1). This carotenoid appears to be new and the name crocoxanthin is proposed. Crocoxanthin was crystallized thrice from benzene-methanol as large rectangular plates. This carotenoid, is of a non-epoxide nature. Non-identity of 4-OH- α -carotene (R_f 0.77) α -cryptoxanthin (R_f 0.61), and crocoxanthin (R_f 0.57) was determined by their separability on silica gel-alumina plates developed with 25% acetone in hexane. Although no direct comparison has been possible with zeinoxanthin,⁷ non-identity may be presumed on the basis of differences in melting points (crocoxanthin 163–165°, zeinoxanthin 174–176°) and absorption characteristics in the “cis-peak” region. In this region (~ 320 – 340 m μ) isomerized crocoxanthin possesses a distinct double peak, a feature that is not characteristic of a mono-hydroxy α -carotene type chromophore.

More detailed results of this investigation and studies on the structures of these xanthophylls will be published elsewhere, as will the implications of this work in the taxonomy of the Cryptophyceae.

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¹⁰ A. L. CURL and G. F. BAILEY, *J. Agr. Food Chem.* **9**, 405, 1961.