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THE CAROTENOIDS OF BLUE-GREEN ALGAE*

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Abstract—The carotenoid compositions of *Phormidium persicinum*, *P. luridum*, *P. faveolarum* and *Anabaena flos-aquae* have been studied, both quantitatively and qualitatively. β-Carotene is the major carotenoid in all species. The xanthophylls comprise zeaxanthin, echinenone, canthaxanthin and the furanoid mutatochrome. *Phormidium persicinum* lacks glycosidic carotenoids. Myxoxanthophyll (myxol-2′-rhamnoside) and a 4-keto-myxol-2′-methylpentoside (tentatively 4-keto-myxoxanthophyll) are present in the other species. These distribution patterns are compared with those observed in other blue-green algae and some correlations with taxonomy are apparent.

INTRODUCTION

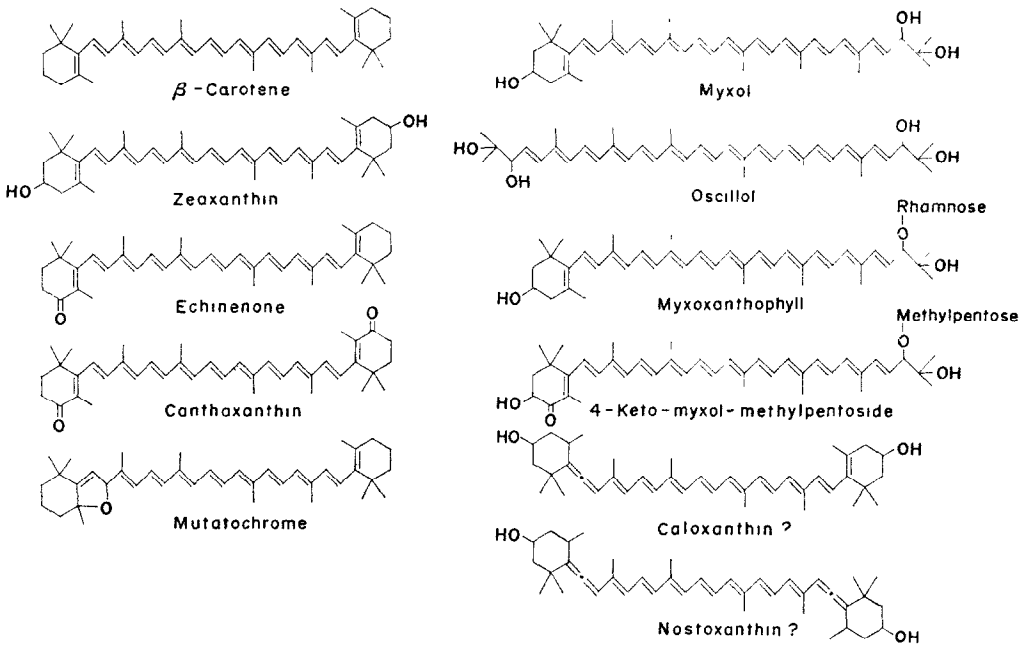
IN PREVIOUS papers,[1-6] we have studied the carotenoid composition of *Oscillatoria rubescens*,[1] *O. agardhii*,[3] *O. limosa*,[6] an *Arthrospira* sp.[1,4,5] and *Aphanizomenon flos-aquae*[2] by modern methods.

Aphanin has been identified as echinenone,[2] aphanicin as canthaxanthin,[2] and flavacin as mutatochrome.[3] A minor carotenoid with the probable structure 4-keto-3′-hydroxy-β-carotene has been encountered.[1,3] Structures have been assigned to myxoxanthophyll (myxol-2′-rhamnoside)[4] of *Arthrospira* origin, oscillaxanthin (oscillol-2,2′-dirhamnoside)[5] of the same origin, and to further new glycosidic carotenoids from *O. limosa*, myxol-2′-*o*-methyl-methylpentoside, oscillol-2,2′-di(*o*-methyl)-methylpentoside and 4-keto-myxol-2′-methylpentoside.[6] The trivial names myxol and oscillol have been suggested for the two common aglycones.[6] It has been demonstrated that myxoxanthophyll and aphanizophyll are different pigments,[2] but the structure of aphanizophyll is not yet established.

When an *o*-methyl-methylpentose and a methylpentose are glycosidically bound to the same aglycone, this results in readily separable glycosides.[4-6] Rhamnose and an undefined hexose, however, attached to the same aglycone give glycosides not separable in the systems used, even as acetates.[4] Co-chromatography tests and electronic spectra are consequently not sufficient criteria of identity, and mass spectra and glycoside hydrolysis, for identification of the sugar component, are desirable.

* Part VII in the series "Carotenoids of Blue-Green Algae". For Part VI, see *Phytochem.* **9**, 629 (1970).

[1] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **5**, 557 (1966).
[2] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **5**, 565 (1966).
[3] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **6**, 1119 (1967).
[4] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1259 (1969).
[5] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1281 (1969).
[6] G. W. FRANCIS, S. HERTZBERG, K. ANDERSEN and S. LIAAEN-JENSEN, *Phytochem.* **9** ,629 (1970).



Healey[7] has studied the carotenoid composition of *Anabaena variabilis*, *Phormidium ectocarpi*, *P. fragile* and *P. persicinum* and did not observe glycosidic carotenoids in any of the *Phormidium* spp. (Table 2).

More recently Stransky and Hager[8] reported on the carotenoid composition of fourteen species of blue–green algae (Table 2), and suggested structures for two new allenic carotenoids, caloxanthin and nostoxanthin. It should be noted that the electronic spectra reported for caloxanthin and nostoxanthin are not compatible with the structures proposed.

TABLE 1. CAROTENOID COMPOSITION OF FOUR BLUE–GREEN ALGAE

Carotenoid	Per cent of total carotenoid			
	Anabaena flos-aquae	*Phormidium persicinum*	*Phormidium faveolarum*	*Phormidium luridium*
β-Carotene	63	69	48	64
Mutatochrome	—	4	1	—
Canthaxanthin	12	—	3	1
Zeaxanthin	—	19	3	11
Trihydroxy-β-carotene		7		
Myxoxanthophyll	4		8	5
4-Keto-myxol-2′-methylpentoside	8		4	
	88 + 12	100	88 + 12	95

[7] F. P. HEALEY, *J. Phycol.* **4**, 126 (1968).
[8] H. STRANSKY and A. HAGER, *Arch. Mikrobiol.* **72**, 84 (1970).

The present investigation was undertaken to gain further information about the distribution, particularly of the furanoid mutatochrome and glycosidic carotenoids in blue-green algae, and to check the variation in the sugar component of such glycosidic carotenoids.

RESULTS AND DISCUSSION

In the present study, cultures of *Phormidium persicinum*, *P. faveolarum*, *P. luridum* (all of order Nostocales, family Oscillatoriaceae)[9] and *Anabaena flos-aquae* (order Nostocales, family Nostocaceae)[9] were grown under conditions described below. The carotenoid composition of each alga was analyzed quantitatively (Table 1).

In agreement with the findings of Healey,[7] no glycosidic carotenoids were encountered in *Phormidium persicinum*. The major components, β-carotene and zeaxanthin, were identified by their electronic, IR and mass spectra and by co-chromatography with authentic samples. Mutatochrome and echinenone were identified by their electronic spectra and by co-chromatography with authentic samples. Mass spectral evidence revealed that 'Unknown 1' of Healey[7] is a triol with hydroxy groups which are easily acetylated. The triol exhibited its molecular ion at *m/e* 584 (corresponding to $C_{40}H_{56}O_3$), confirmed by M-18, M-18-18, M-92, M-106 and M-158 ions.[10] The mass spectrum of the final acetate showed *m/e* 710 (M, corresponding to $C_{40}H_{53}(CH_3COO)_3$). Neither spectrum revealed characteristics in-chain cleavages permitting conclusions to be drawn as to the location of the hydroxy groups in the triol. The partition ratio[7] and electronic spectrum are compatible with a trihydroxy-β-carotene. The IR spectrum supported this assignment with absorption at 1040 cm^{-1} for non-allylic hydroxyl in a β-ring.[11]

Phormidium faveolarum contained the same epiphasic carotenoids as *P. persicinum*. β-Carotene and zeaxanthin were identified on the basis of co-chromatography with authentic samples, electronic, IR and mass spectra, whereas echinenone, canthaxanthin and mutatochrome were identified by the two former criteria only.

The hypophasic fraction comprised myxoxanthophyll and a 4-keto-myxol-2'-methyl pentoside, probably 4-keto-myxoxanthophyll. These glycosides were only separable as acetates. Myxoxanthophyll was identified from its electronic spectrum and co-chromatography of the free glycoside and acetylated derivative with authentic myxoxanthophyll (*Arthrospira*) and myxoxanthophyll acetate. The mass spectrum of the acetate exhibited *m/e* 898 (M), M-106, 331(?), 273. The second glycoside, an α-ketol, was transformed to the corresponding diosphenol by saponification. Its acetate exhibited a molecular ion at *m/e* 910 (M), M-42, M-58, M-92, M-106, 273, and co-chromatographed with the corresponding enol acetate of *Anabaena flos-aquae* origin. *Phormidium faveolarum* was previously studied by Stransky and Hager[8] (Table 2) and quantitatively our findings are in agreement, except that they identified cryptoxanthin (3-hydroxy-β-carotene) but no flavacin and 4-keto-myxol glycoside.

Crystalline β-carotene, m.p. 180°, was obtained from *P. luridum*. The identification was based on co-chromatography with an authentic sample, electronic, IR and mass spectra. Echinenone was identified by the same criteria; canthaxanthin and zeaxanthin from electronic spectra and co-chromatography alone. The electronic spectrum of the hypophasic carotenoid(s) corresponded to that of myxoxanthophyll and, on co-chromatography, no separation was achieved from myxoxanthophyll. Admixture with a 4-keto-myxol glycoside or a myxol-2'-hexoside would not have been detected by these methods.

[9] R. A. LEWIN, *Physiology and Biochemistry of Algae*, Academic Press, New York (1962).
[10] C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **23**, 717 (1969).
[11] C. BODEA, E. NICOARA, V. TAMAS and H. MANTSCH, *Ann.* **666**, 189 (1963).

TABLE 2. CAROTENOID COMPOSITION (IN PER CENT OF TOTAL CAROTENOID

		Order	Nostocales												
		Family	Oscillatoriaceae												
		Species	*Oscillatoria rubescens*	*O. agardhii*	*O. amoena*	*O. tenuis*	*O. limosa*	*Arthrospira* sp.	*Phormidium ectocarpi*	*P. percicnum*		*P. faveolarum*		*P. luridum*	*Microcoleus paludosus*	*Microleus vaginatus*
Most characteristic structural feature	CAROTENOID Designation	Reference	1	3	12	8	6	1	7	7	*	*	8	*	8	13
Carotene	β-carotene		29	35	+	65	17	27	42	49	69	48	30	64	72	63
Furanoid	Mutatochrome (≡ flavacin)		—	1	—	—	—	—	—	—	4	1	—	—	—	—
OH, C=O containing Xantho-phylls	Cryptoxanthin		4	4	+	—	1	2	—	—	—	—	7	—	—	—
	Zeaxanthin		8	9	+	—	22	15	50	47	19	3	5	11	—	9
	Isozeaxanthin (4,4′-dihydroxy-β-carotene)		—	—	—	—	—	—	tr.	tr	—	—	—	—	9	—
	Echinenone (≡ aphanin ≡ myxoxanthin)		19	8	+	17	23	3	tr.	tr.	1	33	42	19	10	6
	Canthaxanthin (≡ aphanicin)		—	—	—	5	7	—	—	—	—	3	6	1	—	—
	4-keto-3′-hydroxy-β-carotene		1	1	—	—	—	2	—	—	—	—	—	—	—	—
	Trihydroxy-β-carotene		—	—	—	—	—	—	8	4	7	—	—	—	—	—
	Unknown 2		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Unknown 3		—	—	—	—	—	—	—	tr.	—	—	—	—	—	—
Allenic?	Caloxanthin		—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Nostoxanthin		—	—	—	—	—	—	—	—	—	—	—	—	—	—
Glycosides	Myxoxanthophyll (myxol-2′-rhamnoside)		30	33	+	12	—	46	—	—	—	8	29	5	10	22
	Myxol-2′-*o*-methyl-methylpentoside		—	—	—	—	27	—	—	—	—	—	—	—	—	—
	4-keto-myxol-2′-methyl-pentoside (4-keto-myxoxanthophyll?)		—	—	—	—	1	—	—	—	—	4	—	?	—	—
	Aphanizophyll (4-hydroxy-myxoxanthophyll?)		—	—	—	2	—	—	—	—	—	—	—	—	—	—
	Oscillaxanthin (oscillol-2,2′-dirhamnoside)		10	10	+	—	—	5	—	—	—	—	—	—	—	—
	Oscillol-2,2′-di(*o*-methyl)-methylpentoside		—	—	—	—	9	—	—	—	—	—	—	—	—	—

		Nostocaceae										Scytonemataceae	Stigonematales: Rivulariaceae	Stigonematales: Masticogladaceae	Chroococcales: Chroococcaceae				
Aphanizomenon flos-aquae	*Anabaena flos-aquae*	*A. cylindrica*		*A. variabilis*			*A. aerulosa oscillatorioides*	*Cylindrospermum*	*Nostoc* sp.	*N. commune*	*Tolypothrix tenuis*	*Calothrix parietina*	*Masticogladus*	*Coccochloris elabens*	*Anacystis nidulans*	*Synechococcus elongatus*	*Microcystus aeruginosa*	*Merismopedia punctata*
2	*	13	8	13	7	8	8	13	13	8	8	8	13	13	8	8	8	8
32	63	54	26	34	50	38	25	50	28	39	18	22	42	42	25	22	38	44
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1	1
—	tr.	tr.	—	4	—	4	22	tr.	tr.	10	2	16	8	tr.	39	60	11	14
—	—	—	—	—	—	—	—	—	—	5	—	6	—	—	—	—	2	3
14	13	19	35	28	20	41	18	25	53	17	40	23	39	16	—	2	27	16
38	12	—	8	—	10	—	2	—	—	7	16	4	—	—	—	—	4	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	tr.	1	—
—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	8	—	12	—	—	25	2	—	—
—	—	—	—	—	—	—	—	—	—	7	—	4	—	—	10	1	—	—
13	4	17	32	33	20	18	31	25	19	8	23	13	11	42	—	4	31	19
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	2	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Anabaena flos-aquae also contained β-carotene (co-chromatography, electronic and mass spectrum) as the major carotenoid. Canthaxanthin was identified by the same criteria; echinenone by co-chromatography tests with authentic echinenone and electronic spectrum. It was expected that aphanizophyll would be encountered in this alga, since its systematic position is rather close to *Aphanizomenon flos-aquae*. Consequently, the hypophasic carotenoids were studied in more detail. No aphanizophyll was present. The glycosidic carotenoids comprised myxoxanthophyll and 4-keto-myxol-2′-methylpentoside, separable only as acetates.

The mass spectrum of myxoxanthophyll tetraacetate showed *m/e* 898 (M), M-58, M-60, M-92, M-106, M-60-58, M-158, M-106-58, M-106-60, 273, 171, 153 and agreed with the mass spectrum of myxol-2′-rhamnoside tetraacetate (*Arthrospira* sp.). Electronic and IR spectra were also in agreement and the two acetates could not be separated by circular paper chromatography. Both acetates gave the same mono (trimethylsilyl) ether judged by co-chromatography. Prolonged treatment of myxoxanthophyll (*Anabaena flos-aquae*) tetraacetate with $LiAlH_4$ gave 3,4-dehydrorubixanthin and saproxanthin in further support of the myxol-2′-glycoside structure.[4]

The acetate of 4-keto-myxol-2′-methylpentoside exhibited the same electronic spectrum and could not be separated from the acetate of 4-keto-myxol-2′-methylpentoside of *O. limosa* origin.[6] The mass spectrum showed *m/e* 912 (M), M-60, M-106, M-106-58, M-290, M-331, 273, 213, 171, 153 and corresponded to that of the reference compound above.

Saponification of the above acetate (R_f 0·63), followed by re-acetylation gave a different, more strongly adsorbed (R_f 0·55) enol acetate, in accordance with the α-ketol → diosphenol transformation by alkali in the presence of traces of oxygen. Prolonged hydride reduction of the former acetate gave several products, a major one with torulene chromophore and R_f compatible with 3,4-dihydroxytorulene and an aphanizophyll-like product, more strongly adsorbed than myxoxanthophyll. These data support the 4-keto-myxol-2′-methylpentoside structure. Sufficient material was not available for the exact identification of the methylpentoside involved after glycoside hydrolysis.

DISCUSSION

For convenience the carotenoid composition of blue–green algae studied by modern techniques is compiled in Table 2. Some conclusions may be drawn: β-Carotene is invariably present and frequently a major carotenoid, as previously anticipated.[13] Echinenone is nearly always found. Zeaxanthin is also found in most blue–green algae studied. Canthaxanthin is less regularly present. Other minor xanthophylls, usually with keto groups in 4- and hydroxy groups in 3-position in β rings are encountered.

The furanoid mutatochrome is sometimes a trace carotenoid, and because of its low concentration may have been overlooked in some studies. The occurrence of mutatochrome in blue–green algae is interesting, particularly with regard to the hypothesis that epoxidic/furanoid carotenoids should be present in all oxygen-evolving photosynthetic organisms.[14] The assumed allenic caloxanthin and nostoxanthin[8] do not appear to be widely distributed. These carotenoids should be further investigated and their possible identity with our 'trihydroxy-β-carotene' and 'Unknown 3' of Healey[7] ascertained.

Glycosidic carotenoids are present in most blue–green algae studied; secondary, allylic

[12] J. TISCHER, *Hoppe-Seyler's Z. physiol Chem.* **311,** 140 (1958).
[13] T. W. GOODWIN, *J. Gen. Microbiol.* **17,** 467 (1957).
[14] G. D. DOROUGH and M. CALVIN, *J Am. Chem. Soc.* **73,** 2362 (1957)

2′-glycosides of myxol, 4-keto-myxol and of oscillol are found. The strongly hypophasic fraction containing the glycosidic carotenoids have in most cases not been thoroughly studied. Methylpentose (rhamnose) is the sugar component hitherto most frequently ncountered in the carotenoid glycosides of blue–green algae.[4–6]

Blue–green algae have, relative to other organisms, a rather characteristic carotenoid composition. In particular the carotenoid glycosides encountered are specific to blue–green algae. Sufficient variation to assist species identification on a carotenoid basis seems to occur in many cases, although the effect of cultural conditions are not yet well known.[8] However, there is no specific carotenoid pattern for distinguishing the different orders or families within the Cyanophyta.

EXPERIMENTAL

Biological material. Large-scale culture of the algae[15] was at 25° in 180-l. polyethylene drums. All freshwater species were grown in D medium[16] and *Phormidium persicinum* was grown in an enriched artificial seawater medium.[17] Yields were about 300–600 g/160 l. after 10–14 days of growth. The algae were harvested, frozen, lyophilized, and stored at 4° before use.

Materials and methods. Reagents and solvents,[1] instruments used,[1,6] extraction, saponification and chromatographic procedures,[1,18] general methods, acetylation and silylation procedures[18] were as described elsewhere. Prolonged hydride reduction of glycosidic carotenoids was performed by the usual procedure.[5,6]

The carotenoids were extracted with acetone–MeOH and separated into epiphasic and hypophasic carotenoids on partition between petroleum ether and 80% MeOH in water. The epiphasic carotenoid mixture was saponified in the usual manner and the carotenoids separated by chromatography on Woelm neutral alumina activity grade 2. The hypophasic carotenoids were chromatographed on cellulose columns. In addition circular chromatography on kieselguhr papers was extensively used for separations on the micro scale.

Chromatographic and absorption (visible light, IR) data for the native carotenoids and characteristic derivatives thereof are given in earlier papers of this series.[1–6]

Phormidium persicinum: Lyophilized algae (30 g) provided 13·4 mg carotenoids, corresponding to 0·05% of the acetone extracted residue. *P. faveolarum:* Lyophilized algae (50 g) gave 18·5 mg carotenoids, corresponding to 0·04% of the acetone extracted residue. *P. luridum:* Lyophilized algae (50 g) gave 30 mg carotenoids, corresponding to 0·06% of the acetone extracted residue. *Anabaena flos-aquae:* Lyophilized algae (240 g) provided 86 mg carotenoids, corresponding to 0·03% of the acetone extracted residue.

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[15] H. W. Siegelman and R. R. L. Guillard, in *Methods in Enzymology* (edited by A. San Pietro), Vol. 23, p. 110. Academic Press, New York (1971).
[16] W. A. Kratz and J. Myers, *Am. J. Bot.* **42**, 282 (1955).
[17] I. J. Pintner and L. Provasoli, *J. Gen. Micriobol.* **18**, 190 (1958).
[18] A. J. Aasen and S. Liaaen-Jensen, *Acta Chem. Scand.* **20**, 1970 (1966).