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THE CAROTENOIDS OF BLUE-GREEN ALGAE—II.

THE CAROTENOIDS OF *APHANIZOMENON FLOS-AQUAE*

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Abstract—The carotenoid composition of *Aphanizomenon flos-aquae* has been re-examined in a quantitative manner. The epiphasic fraction comprised β-carotene (I), flavacin, aphanin and aphanicin. The last two were shown to be identical with echinenone (IV) and canthaxanthin (VI) respectively. The hypophasic fraction contained in addition to aphanizophyll small amounts of myxoxanthophyll. Aphanizophyll and myxoxanthophyll are different pigments.

INTRODUCTION

THE carotenoids of *A. flos-aquae* have previously been studied in a qualitative manner by Tischer,[1,2] who reported the presence of β-carotene (I) and four new pigments, designated flavacin, aphanin, aphanicin and aphanizophyll.

Karrer and Jucker[3] have suggested that flavacin (m.p. 155°) might be identical with mutatochrome (m.p. 163–164°) (II). It is remarkable that in spite of the visible light absorption data (and melting points) Goodwin[4] states that flavacin very probably is ζ-carotene (III).

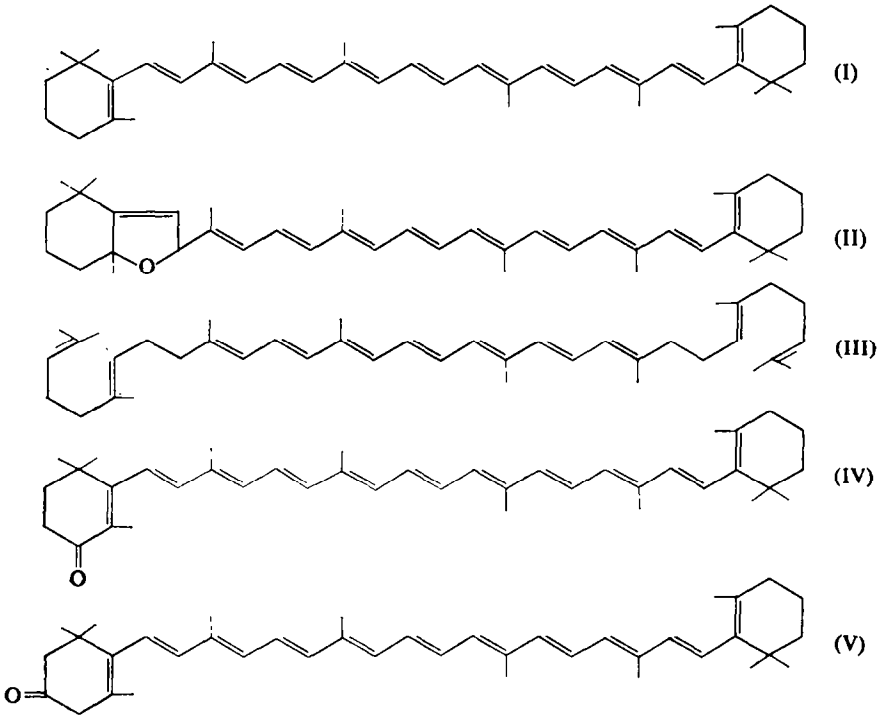
Although Tischer[1,5] claimed that aphanin contained an unconjugated carbonyl group, this pigment has been considered identical with myxoxanthin (echinenone) (IV) isolated from *Oscillatoria* spp.[6] With somewhat irrelevant objections Tischer[2,5] disagreed with this proposal and favoured the structure 3-keto-β-carotene (V) for aphanin. However, a direct comparison of aphanin and echinenone (IV) has never been undertaken.

On the basis of its biological vitamin A activity[7] and other chemical and physical data Tischer[2] considered aphanicin as a di-carotenoid of two aphanin molecules joined together by an ether bridge.

Aphanizophyll was described by Tischer[1,2] as a strongly polar carotenoid. The possible identity of aphanizophyll and myxoxanthophyll isolated from *Oscillatoria rubescens*[7,8] has been disputed,[1,10,11] but again a direct comparison has never been attempted.

[1] J. TISCHER, *Z. Physiol. Chem. Hoppe-Seyler's* **251**, 109 (1938).
[2] J. TISCHER, *Z. Physiol Chem. Hoppe-Seyler's* **260**, 257 (1939).
[3] P. KARRER and E. JUCKER, *Carotinoide*, p. 316. Verlag Birkhäuser, Basel (1948).
[4] T. W. GOODWIN, *The Comparative Biochemistry of the Carotenoids*, p. 137. Chapman & Hall, London (1952).
[5] J. TISCHER, *Z. Physiol. Chem. Hoppe-Seyler's* **311**, 140 (1958).
[6] T. W. GOODWIN and M. M. TAHA, *Biochem. J.* **47**, 513 (1951).
[7] A. SCHEUNERT and K. H. WAGNER, *Z. Physiol. Chem. Hoppe-Seyler's* **260**, 272 (1939).
[8] I. M. HEILBRON and B. LYTHGOE, *J. Chem. Soc.* 1376 (1936).
[9] P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* **27**, 1691 (1944).
[10] I. M. HEILBRON, *J. Chem. Soc.* 79 (1942).
[11] T. W. GOODWIN, *J. Gen. Microbiol.* **17**, 476 (1957).

On this background of diverging opinions and conflicting evidence it seemed desirable to carry out a re-examination of the carotenoids of *A. flos-aquae* using modern methods.



RESULTS AND DISCUSSION

The carotenoid composition of *A. flos-aquae*, as established in the present investigation is presented in Table 1. In agreement with the result of Tischer[1] β-carotene (I) was identified from its absorption spectrum in visible light and adsorptive properties, as directly compared with synthetic β-carotene.

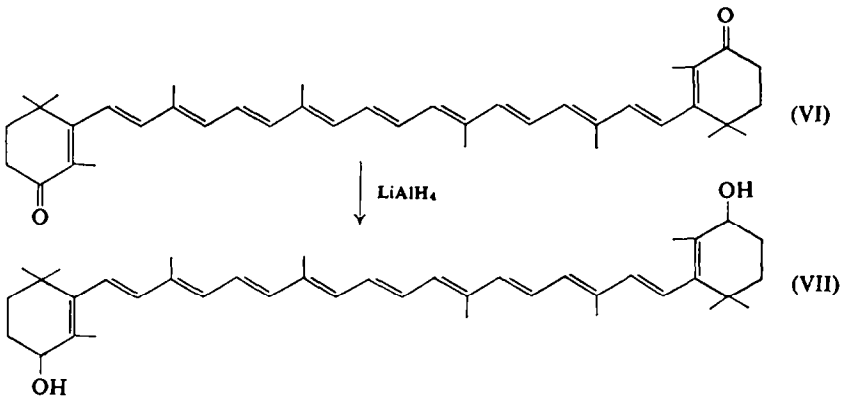
Flavacin was somewhat more strongly adsorbed than β-carotene on the alumina column, and a pale blue colour was observed on treatment of this pigment with hydrochloric acid in ether solution, as was expected for the furanoid structure (II). However, a direct comparison with mutatochrome (II) ought to be carried out.

Also present in the epiphasic fraction were two further components, the absorption spectra of which corresponded to aphanin and aphanicin isolated by Tischer.[1,2] The former carotenoid proved to be identical with synthetic echinenone (IV) as shown by visible light absorption spectra, co-chromatography tests and comparison of partition ratios and melting points. Our natural aphanin melted at 182°; Warren and Weedon cite 178–179°[12] and 186–187°[13] for synthetic echinenone. Also the i.r. spectrum, see Fig. 1, was very similar to that of synthetic echinenone. Hydride reduction of aphanin yielded a mono-ol, presumably identical with aphanol described by Tischer.[2] This mono-ol exhibited a β-carotene-type absorption spectrum and was chromatographically inseparable from isocryptoxanthin (4-hydroxy-β-carotene), prepared by *N*-bromosuccinimide treatment of β-carotene in acetic

[12] C. K. WARREN and B. C. L. WEEDON, *J. Chem. Soc.* 372 (1958).
[13] C. K. WARREN and B. C. L. WEEDON, *J. Chem. Soc.* 3986 (1958).

acid-containing chloroform according to the method of Karrer and Entschel.[14] The identity of aphanin and echinenone (IV) seems therefore to be settled.

Crystalline aphanicin, melting point 216–218°, was obtained. Warren and Weedon[13] reported 215° and Zeller *et al.*[15] 216–217° for synthetic canthaxanthin (VI). Aphanicin could not be chromatographically separated from synthetic VI, and the i.r. spectrum (Fig. 2) agreed well with that of synthetic canthaxanthin. Hydride reduction resulted in a diol, presumably identical with aphanicol obtained by Tischer[2] on reduction of aphanicin. This diol was spectroscopically (visible light) and chromatographically indistinguishable from synthetic isozeaxanthin (VII). On the basis of these data there can be no doubt that aphanicin is identical with canthaxanthin (VI).



Myxoxanthophyll was a minor constituent of the hypophasic fraction. Identity with myxoxanthophyll isolated from *Oscillatoria rubescens*[16] was shown by spectral (visible light) and adsorptive properties of the pigment itself, as well as the corresponding properties of the final acetate and the allylic dehydration product derived from myxoxanthophyll isolated from the two sources.

The major hypophasic carotenoid is considered to be aphanizophyll.[1,2] The absorption spectrum in visible light of aphanizophyll was similar to that of myxoxanthophyll, see Fig. 3. However, aphanizophyll had stronger adsorptive properties than myxoxanthophyll. The final acetate obtained on acetylation was also more strongly retained than myxoxanthophyll acetate. Aphanizophyll and myxoxanthophyll are therefore different pigments. The chemical structure of aphanizophyll will be further studied when more material becomes available, and the results will be published later.[17]

The systematic position of *A. flos-aquae* relative to the members of Oscillatoriaceae examined in the first report of this series, was given in that paper.[16] β-Carotene (I) and aphanin ≡ myxoxanthin ≡ echinenone (IV) were present in the Oscillatoriaceae spp. studied and in *A. flos-aquae*. The latter alga, however, lacks zeaxanthin and cryptoxanthin, but contains flavacin (II?) and aphanicin ≡ canthaxanthin (VI). The hypophasic carotenoids of *A. flos-aquae* comprised a relatively smaller amount of the total carotenoid. Oscillaxanthin

[14] P. KARRER and R. ENTSCHEL, *Helv. Chim. Acta* **41**, 402 (1958).
[15] P. ZELLER, F. BADER, H. LINDLAR, M. MONTAVON, P. MÜLLER, R. RÜEGG, G. RYSER, G. SAUCY, S. F. SCHAEREN, U. SCHWIETER, K. STRICKER, R. TAMM, P. ZÜRCHER and O. ISLER, *Helv. Chim. Acta* **42**, 841 (1959).
[16] S. HERTZBERG and S. LIAAEN JENSEN, *Phytochem.* **5**, 557 (1966).
[17] S. HERTZBERG and S. LIAAEN JENSEN, *Acta Chem. Scand.* To be published.

was absent, myxoxanthophyll was only a minor component and aphanizophyll was the major hypophasic carotenoid.

EXPERIMENTAL

Materials and methods were as described in the preceding paper.[16]

Biological material. Aphanizomenon flos-aquae, collected from a natural habitat in the Rødnessjøen Lake, Østfold, was kindly provided by the Norwegian Institute for Water Research, Oslo.

Carotenoid content. A total of 20·4 mg carotenoids or 0·12 per cent of the extracted algal residue, was isolated after saponification and column chromatography. The carotenoid composition is given in Table 1, and the identification of the individual components is described below in order of increasing adsorption.

TABLE 1. CAROTENOID COMPOSITION OF *A. flos-aquae*

Group	Carotenoid	In % of total carotenoid	
Epiphasic	β-Carotene (I)	32	86
	Flavacin (II?)	2	
	Aphanin ≡ echinenone (IV)	14	
	Aphanicin ≡ canthaxanthin (VI)	38	
Hypophasic	Myxoxanthophyll	1	14
	Aphanizophyll	13	

β-Carotene (I) required 5% ether–light petrol for elution from deactivated alumina. This pigment was spectroscopically (λ_{max} (425), 450 and 476 nm in light petrol) and chromatographically (R_f=0·25 on aluminium oxide paper; light petrol) indistinguishable from synthetic β-carotene.

Flavacin (II?) required 15% ether–light petrol for elution from deactivated alumina. Flavacin had λ_{max} in ether at 415, 428 and 452 nm, see Fig. 3. A pale blue colour was observed on treatment of flavacin in ether, with conc. HCl.

Aphanin ≡ echinenone (IV) required 20% ether–light petrol for elution from the alumina column. Crystalline aphanin, m.p. 182°, was obtained from acetone–light petrol. The crystalline specimen had λ_{max} at 455 nm (broad peak) in ether, R_f=0·81 on kieselguhr paper (2% acetone–light petrol) and partition ratio in light petrol–95% methanol, 94:6. The i.r. spectrum of aphanin is presented in Fig. 1, together with that of synthetic echinenone.

Lithium aluminium hydride reduction of aphanin (1·1 mg) in 4 ml dry ether gave 85 per cent pigment recovery. The product aphanol ≡ isocryptoxanthin (4-hydroxy-β-carotene) had λ_{max} at (425), 449 and 474 nm in ether and could not be separated from synthetic isocryptoxanthin on kieselguhr paper (R_f=0·69; 5% acetone–light petrol).

Aphanicin ≡ canthaxanthin (VI) required around 10% acetone–light petrol for elution from deactivated alumina. Crystalline aphanicin, m.p. 216–218°, was obtained from acetone–light petrol. The crystalline specimen had λ_{max} at 456 nm (broad peak) in ether, R_f=0·68 on kieselguhr paper (5% acetone–light petrol) and had in light petrol–95% methanol partition ratio 47:53. Synthetic canthaxanthin had partition ratio 50:50 in the latter system, superimposable absorption spectrum in visible light and could not be chromatographically

separated from aphanicin. The i.r. spectra of crystalline aphanicin and synthetic canthaxanthin are presented in Fig. 2.

Hydride reduction of aphanicin (0·94 mg) was carried out in like manner to the reduction of aphanin; pigment recovery 88%. The reduction product aphanicol ≡ isozeaxanthin (VII) could not be spectroscopically (visible light) or chromatographically (R_f = 0·42 on kieselguhr paper; 10% acetone–light petrol) separated from synthetic isozeaxanthin.

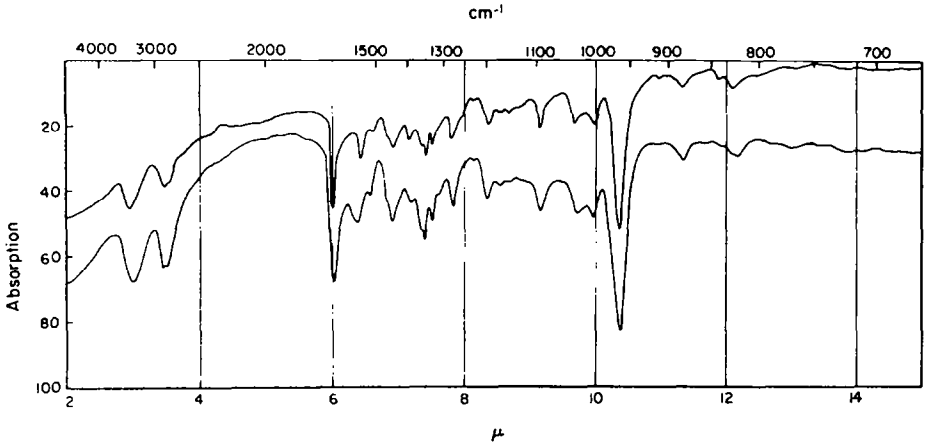


FIG. 1. INFRARED SPECTRA, MEASURED IN KBr, OF APHANIN (UPPER CURVE) AND SYNTHETIC ECHINENONE (LOWER CURVE).

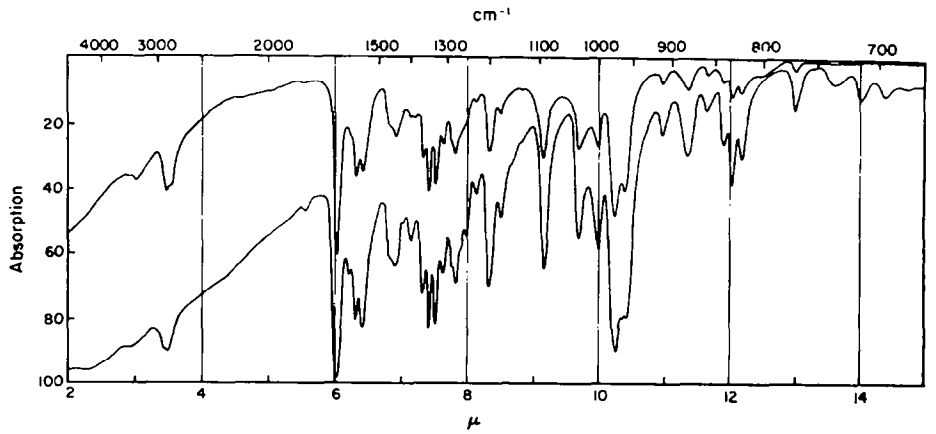


FIG. 2. INFRARED SPECTRA, MEASURED IN KBr, OF APHANICIN (UPPER CURVE) AND SYNTHETIC CANTHAXANTHIN (LOWER CURVE).

Myxoxanthophyll required 20% acetone–light petrol for elution from the cellulose column; R_f = 0·58 on kieselguhr paper (50% acetone–light petrol). No separation was obtained on co-chromatography with authentic myxoxanthophyll isolated from *Athrospira* sp.[16] This pigment (partly *cis*-isomerized) had λ_{max} in acetone at 362, 450, 474 and 504 nm.

Myxoxanthophyll acetate was prepared in the usual manner and had R_f = 0·50 on kieselguhr paper (10% acetone–light petrol); it could not be separated from authentic myxoxanthophyll acetate.

The allylic dehydration product obtained on treatment with acid chloroform had λ_{max} at (470), 490 and 520 nm in acetone, and could not be separated from the corresponding product obtained from authentic myxoxanthophyll (R_f=0·35 on aluminium oxide paper; 2% acetone–light petrol).

Aphanizophyll required 30–50% acetone–light petrol for elution from the cellulose column; R_f=0·42 on kieselguhr paper (50% acetone–light petrol). It was shown by co-chromatography tests to be more strongly adsorbed than myxoxanthophyll.

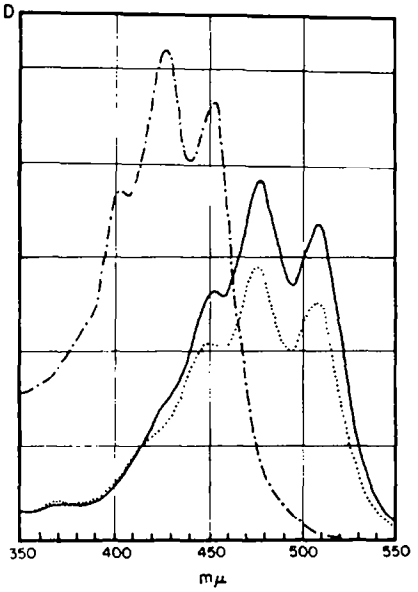


FIG. 3. ABSORPTION SPECTRA IN VISIBLE LIGHT.
— · — flavacin in ether;
——— *trans* myxoxanthophyll in acetone;
····· *trans* aphanizophyll in acetone.

Trans aphanizophyll had λ_{max} in light petrol at 350, 362, 446, 472 and 502 nm, % III/II[18]=64 and in acetone at 365, 450, 476 and 507 nm, % III/II=57 (see Fig. 3).

Aphanizophyll acetate was prepared in the usual manner. The acetate had R_f=0·40 on kieselguhr paper (10% acetone–light petrol) and was more strongly adsorbed than myxoxanthophyll acetate (R_f=0·55).

Acknowledgements—Synthetic β-carotene, echinenone, canthaxanthin and isozeaxanthin, used as reference samples, were gifts from Dr. O. Isler, Hoffmann-La Roche, Basel. This work was supported by a grant to S. L. J. from Fa. Hoffmann-La Roche, Basel, supporting fundamental research in the field of naturally occurring carotenoids. The grant was used for financial support of S. H. A grant from Norges Tekniske Høgskoles Fond to technical assistance is also gratefully acknowledged.

[18] S. LIAAEN JENSEN, *Kgl. Norske Videnskab. Selskabs Skrifter* No. 2 (1962).