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THE CAROTENOIDS OF BLUE-GREEN ALGAE—III.*

A COMPARATIVE STUDY OF MUTATOCHROME AND FLAVACIN

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Abstract—Flavacin, obtained in small quantity from *Aphanizomenon flos-aquae* and *Oscillatoria agardhii* has been directly compared with mutatochrome (III), synthesized by monoperphthalic acid oxidation of β-carotene (I) to β-carotene-monoepoxide (II) and subsequent acid rearrangement. The results favour identity, although the available evidence does not rule out stereochemical differences. Complex metal hydride reduction of mutatochrome (III) (and flavacin) resulted in hydrogenolytic opening of the oxide ring. The main product 5-hydroxy-5,6-dihydro-β-carotene (IV) was dehydrated by phosphorous oxychloride to α-carotene (V).

INTRODUCTION

FROM the epiphasic carotenoid fraction of *Aphanizomenon flos-aquae* Tischer[1] isolated a minor carotenoid, which he designated flavacin and partly characterized. Karrer and Jucker[2] have pointed out that flavacin might be identical with mutatochrome (III). The improbable suggestion that flavacin may be ζ-carotene has also been put forward.[3,4]

In the present investigation physical and chemical properties of flavacin have been directly compared with those of mutatochrome (III) prepared from β-carotene (I).

RESULTS AND DISCUSSION

Mutatochrome (III) was synthesized by monoperphthalic acid oxidation of β-carotene (I) to β-carotene-monoepoxide (II), according to the method of Euler, Karrer and Walker,[5] followed by complete rearrangement to the corresponding furanoid oxide (III) by further acid treatment.[2] The i.r. spectrum appeared identical with that reported by Bodea, Nicoara and Salontai.[6]

Cholnoky *et al.*[7] have recently claimed that i.r. irradiation of an ethereal solution of the furanoid oxides of β-carotene and lithium aluminium hydride regenerates the parent carotene, β-carotene (I). In our experience the formation of β-carotene (I) on hydride reduction of mutatochrome (III) is not dependent on i.r. light, and occurs only when a large excess of lithium aluminium hydride is employed. Under the latter condition there was only low pigment recovery. Hydride reduction of mutatochrome (III), under mild conditions, gave no carotenes in i.r. light or darkness. But the products A and B with α-carotene-type electronic spectra were produced; see Table 1.

* Part II. *Phytochem.* **5**, 565 (1966).

[1] J. TISCHER, *Z. physiol. Chem.* **251**, 257 (1938).
[2] P. KARRER and E. JUCKER, *Carotinoide*. Birkhäuser, Basel (1948).
[3] T. W. GOODWIN, *The Comparative Biochemistry of the Carotenoids*, p. 137. Chapman and Hall, London (1952).
[4] S. HERTZBERG and S. LIAAEN JENSEN, *Phytochem.* **5**, 565 (1966).
[5] H. v. EULER, P. KARRER and O. WALKER, *Helv. Chim. Acta* **15**, 1507 (1932).
[6] C. BODEA, E. NICOARA and T. SALONTAI, *Ann. Chem.* **648**, 147 (1961).
[7] L. CHOLNOKY, K. GYÖRGYFY, J. SZABOLCS and B. C. L. WEEDON, *Chem. Res. Commun.* **13**, 404 (1966).

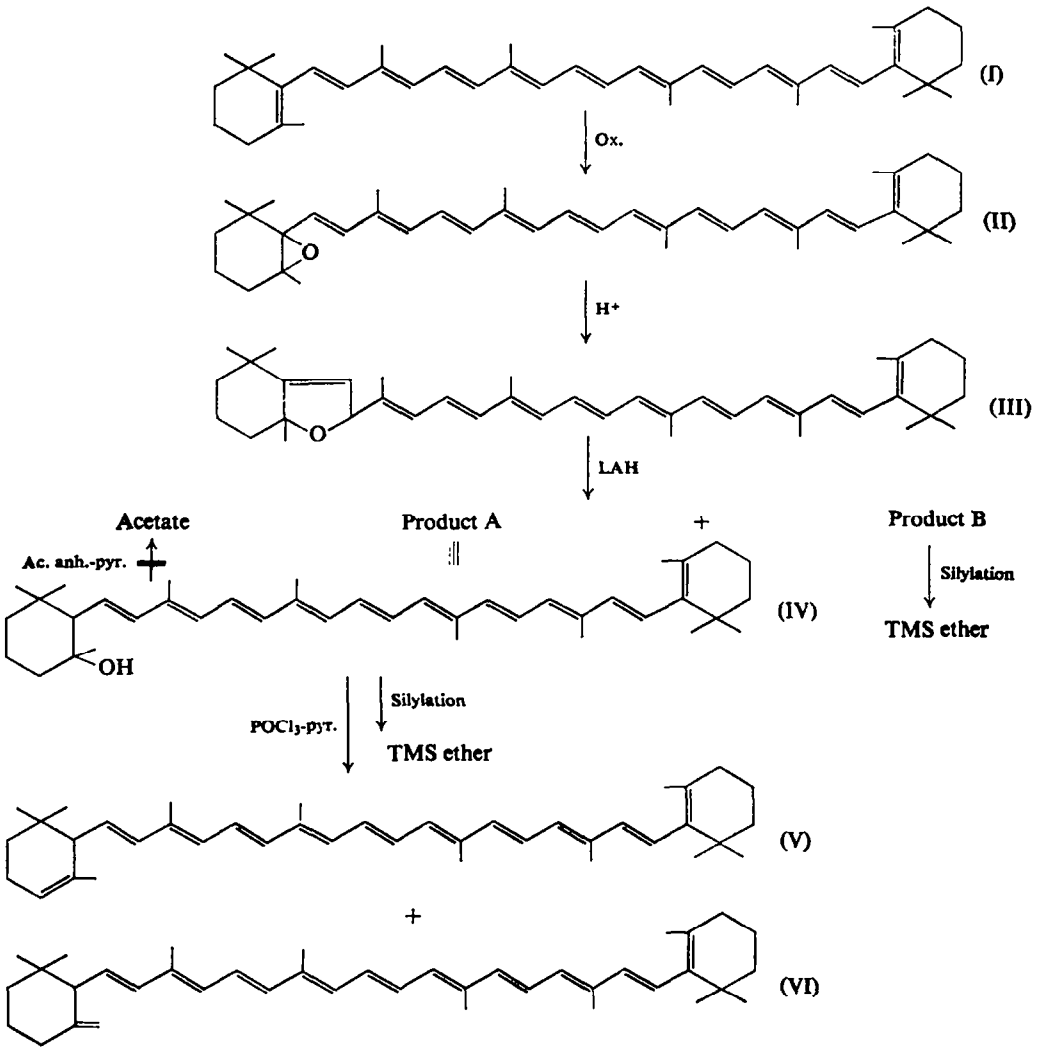


TABLE 1. PROPERTIES OF PRODUCTS OBTAINED ON MILD HYDRIDE REDUCTION OF FLAVACIN AND MUTATOCHROME (III)

Origin	Product	Stereoisomer*	Abs. max. in nm in ether	% Of recovered pigment	R_f-value on kieselguhr paper 2% acetone†
Flavacin	A	*Trans* (60)	(420) 443 471	90	0·55
		Neo A (40)	(418) 438 465		0·64
	B			trace?	
Mutatochrome (III)	A	*Trans* (50)	(420) 443 472	60	0·55
		Neo A (50)	(418) 439 465		0·64
	B	*Trans*	(423) 443 473	20	0·29

* Isolated after iodine-catalysed isomerization. Values in parenthesis give percentage of total at isomerization equilibrium.

† In light petroleum; R_f-values determined by co-chromatography.

Both products (A and B) exhibited adsorptive properties characteristic of mono-ols. They gave no acetates on acetylation but each gave a trimethylsilyl ether on silylation.[8] Products A and B therefore each contain a tertiary hydroxyl group. The main reduction product (A) was dehydrated with phosphorous oxychloride[9] to give a minor product tentatively identified as (VI) and a major dehydration product identical with α-carotene (V) isolated from carrots,[10] see Table 2. It was thus inferred that the main reduction product of mutatochrome (III) was 5-hydroxy-5,6-dihydro-β-carotene (IV). The formation of (IV) could be the result of attack by the hydride ion (formally considered as H^-) at C_8 in (III) to give (VII), followed by isomerization of the exocyclic 6,7 double bond into conjugation. However, since no intermediate alcohol corresponding to (VII) was detected, an alternative attack by the hydride ion at C_6 leading by electron shift directly to (IV) appears more plausible. It may be mentioned that Grob and Siekmann[11] on hydride reduction of β-carotene-monoepoxide (II) also obtained 5-hydroxy-5,6-dihydro-β-carotene (IV) as a major product.

TABLE 2. PROPERTIES OF IODINE-CATALYSED EQUILIBRIUM MIXTURES OF α-CAROTENE (V) AND THE MAIN DEHYDRATION PRODUCT OBTAINED FROM A (IV)

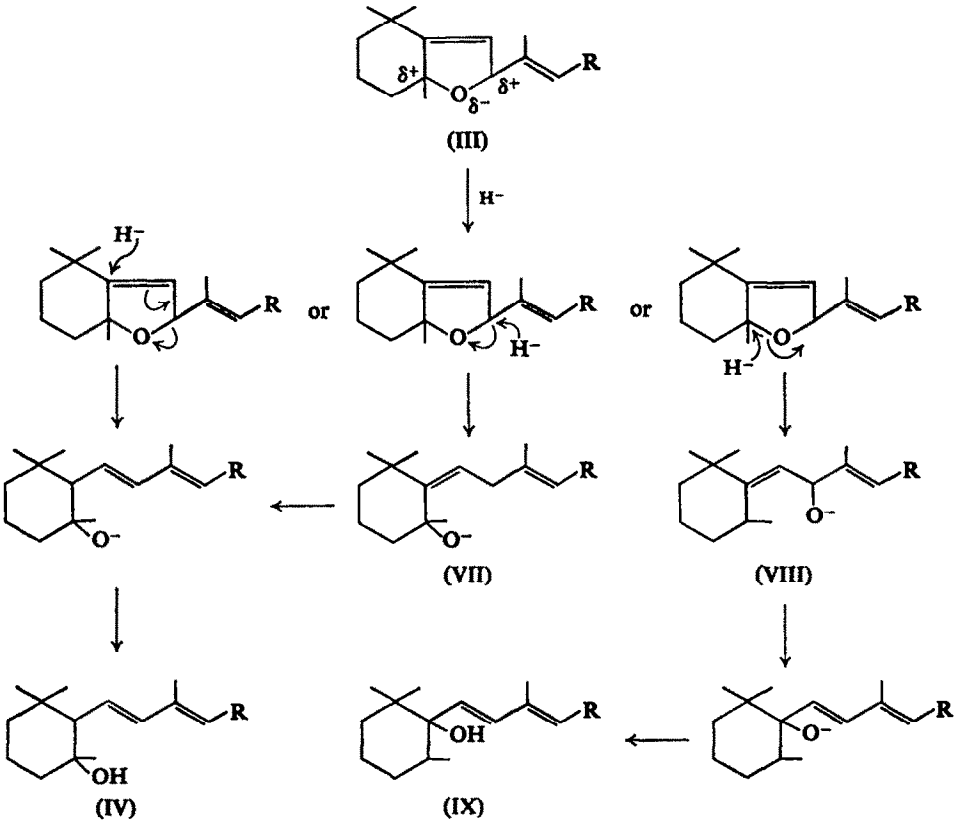
Origin	Member of the set	R_f-value*	In ether			Approximate relative ratio
			Abs. max. in nm	% III/II	% D_B/D_{II}	
Carrots	Neo A	0·72	330 (415) 438 463	0	34	3
	Trans	0·52	(330) 421 444 473	55	10	7
	Neo U	0·31				trace
Dehydration product from A (IV)	Neo A	0·72	330 (416) 439 463	0	32	3
	Trans	0·52	(330) 422 445 474	51	11	7
	Neo U	0·31				trace

* On unactivated Schleicher and Schüll No. 288 paper (aluminium oxide containing); light petroleum. R_f-values determined by co-chromatography.

Structure (IX) may be considered for Product B. However, the allylic rearrangement of (VIII) to (IX) would be expected to require acidic conditions. Since azafrin gives a monotrimethylsilyl ether only,[8] one might expect (IX) to be resistant towards silylation. Moreover, neither Product A (IV) nor B gave any allylic dehydration products with acid chloroform.[12] Nor was a product with properties compatible with the unrearranged alcohol (VIII) isolated on hydride reduction of (III). This is consistent with observations that such reduction of trisubstituted 1,2-epoxides preferentially leads to tertiary alcohols.[13]

Although our mutatochrome (III) appeared homogeneous in all chromotographic systems tried, it is reasonable to expect, from the mode of preparation, that the synthetic sample of mutatochrome should be an epimeric mixture.[14] Product B may therefore also possibly be represented by formula (IV), but with a different configuration at C_5 and/or C_6 than in Product A.

[8] A. MCCORMICK and S. LIAAEN JENSEN, *Acta Chem. Scand.* **20**, 1989 (1966).
[9] J. SURMATIS and A. OFNER, *J. Org. Chem.* **28**, 2735 (1963).
[10] R. KUHN and E. LEDERER, *Chem. Ber.* **64**, 1349 (1931).
[11] E. C. GROB and W. SIEKMANN, *Helv. Chim. Acta* **48**, 1199 (1965).
[12] P. KARRER and E. LEUMANN, *Helv. Chim. Acta* **34**, 445 (1951).
[13] N. G. GAYLORD, *Reduction with Complex Metal Hydrides*, p. 646. Interscience, New York (1956).
[14] M. C. BARBER, J. B. DAVIS, L. M. JACKMAN and B. C. L. WEEDON, *J. Chem. Soc.* 2870 (1960).



Flavacin was isolated from *Aphanizomenon flos-aquae* as previously described.[4] It was also obtained in small quantity from *Oscillatoria agardhii*. The amount of flavacin isolated was insufficient for crystallization.

Natural flavacin proved to have an electronic spectrum identical with that of mutatochrome (III) (see Fig. 1) and the two compounds exhibited identical adsorptive properties. Separate, iodine-catalysed stereoisomerization of the two samples caused the same spectral effects in visible light; see Table 3. In neither case was a satisfactory chromatographic separation from a neo A and a neo U isomer obtained. Hydride reduction of flavacin, under mild conditions, gave a major product with electronic spectrum and chromatographic properties identical with those of Product A (IV) above; see Table 1. From this evidence it may seem justified to conclude that flavacin is identical with mutatochrome (III). Mutatochrome is a common carotenoid of many fruits,[15,16] but is the only carotenoid oxide which has so far been isolated from blue–green algae. However, no conclusions can yet be drawn concerning the configuration of flavacin, citroxanthin[15] and mutatochrome.

If the dehydration of 5-hydroxy-5,6-dihydro-β-carotene (IV) occurred by a E_1 mechanism, β-carotene (I) would, according to Saytzeff's rule, be expected to be the major dehydration product. Assuming that the elimination proceeded by a diaxial *trans* E_2-reaction,[17,18] the

[15] P. KARRER and E. JUCKER, *Helv. Chim. Acta* **30**, 536 (1947).
[16] A. L. CURL, *Food Res.* **22**, 63 (1957).
[17] G. ROSENKRANZ, O. MANCERA and F. SONDHEIMER, *J. Am. Chem. Soc.* **76**, 2227 (1954).
[18] C. W. SHOPPEE and G. H. R. SUMMERS, *J. Chem. Soc.* 1786 (1952).

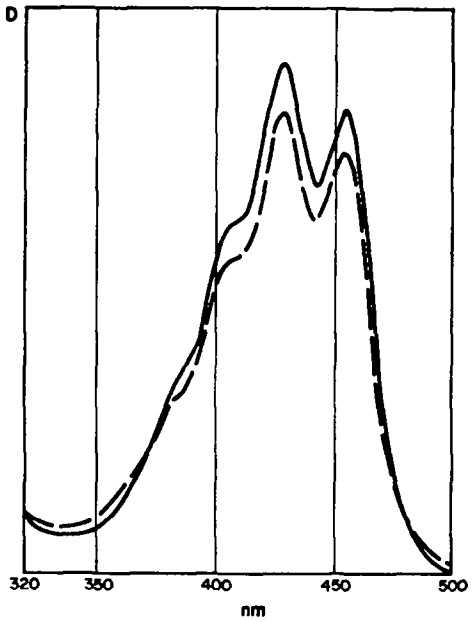


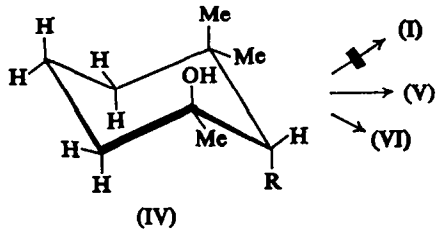
FIG. 1. ELECTRONIC SPECTRA IN ETHER OF —— FLAVACIN AND --- MUTATOCHROME (III).

TABLE 3. DIRECT COMPARISON OF MUTATOCHROME (III) AND FLAVACIN

Sample	Member of stereoisomeric set	R_f-value†	Spectral properties: Abs. max. in nm	% III/II	% D_B/D_{II}
Mutatochrome (III)	*Trans*	0·54	(407) 428 454‡	63	—
	Equilibrium mixture*	0·52–0·56	310 (402) 423 449§	42	17
Flavacin	*Trans*	0·54	(407) 428 453‡	64	—
	Equilibrium mixture*	0·52–0·56	310 (402) 423 449§	46	15

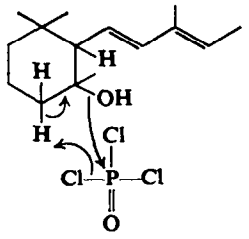
* After iodine catalysis.
† Unactivated Schleicher and Schüll No. 288 paper; 1% acetone–petroleum ether.
‡ In ether.
§ In light petroleum.

remarkable preferential formation of α-carotene (V) could be accounted for provided that (IV) had a relative configuration as indicated below. Although γ-branched, the necessity of formulating the heavy polyene chain (R) as an axial substituent seems unattractive. An alternative, not necessarily coplanar, concerted elimination as depicted below could, because of



(IV)

steric hindrance, also account for the preferential formation of α-carotene (V). However, in no case may the complete relative configuration of mutatochrome (III) and flavacin (III) be deduced from the stereochemistry of the reduction product (IV).



(IV) ⟶ (V)+HCl+PO(OH)Cl_2

EXPERIMENTAL

Materials and Methods

Reagents, solvents, instruments and paper-chromatographic methods used have been described in an earlier paper in this series.[19] Column chromatography was carried out on Woelm neutral alumina; activity grade 2.[20] Melting points were measured in evacuated capillary tubes on a Berl block and are uncorrected. The terms in which spectral characteristics are described have been defined elsewhere.[21] Iodine-catalysed isomerization studies were carried out as previously described.[21] Acetylation was effected by acetic anhydride in pyridine in the usual manner.[21] The methods for silylation[8] and for dehydration with $POCl_3$ in pyridine[9] have been reported elsewhere. The procedure recommended by Entschel and Karrer[22] for allylic dehydration with dry hydrochloric acid in chloroform was used.

Synthesis of mutatochrome (III). The procedure of Euler *et al.* was used. To β-carotene (I; 536 mg, $1 \cdot 10^{-6}$ moles) in ether (100 ml) was added an ethereal solution (62 ml) of monoperphthalic acid (corresponding to $1 \cdot 5.10^{-6}$ moles active oxygen) prepared by H_2O_2 oxidation of phthalic acid.[23] The mixture was stirred mechanically for 1 hr at room temperature, the reaction course being followed by periodical paper-chromatographic analysis. The pigments were isolated in the usual manner and chromatographed on an alumina column; pigment recovery was 22 per cent after chromatography. The result is presented in Table 4.

TABLE 4. RESULT OF PERPHTHALIC ACID OXIDATION OF β-CAROTENE (I)

Required eluant from Al_2O_3 activity grade 2	Abs. max. in nm in petroleum ether	% Of total carotenoid	Tentative identification
Light petroleum	(425) 449 476	11	β-Carotene (I)
1–2% Ether*	(420) 442 470	18	β-Carotene-monoepoxide (II)
5–7% Ether	409 428 452	21	Mutatochrome (III)
7–10% Ether	417 439 470	28	β-Carotene-diepoxide
10% Ether	398 421 499	22	β-Carotene-monoepoxide-monofuranoxide

* In light petroleum.

The β-carotene-monoepoxide (II) was treated with 0·04 N HCl in chloroform for 5 min. The resulting mutatochrome (III) was isolated, chromatographed on alumina as above, and combined with III originally obtained.

Chromatographically pure mutatochrome crystallized from ether-methanol as organge-red flakes; m.p. 147° (Euler *et al.*[5] state 178° and Bodea *et al.*[6] 162°); abs. max. (418), 428 ($E^{1\%}_{1cm}$ = 2260) and 436 nm in ether; characteristic i.r. absorption (KBr-pellet) 1068 (furanoid ether), 1000 and 962 (*trans* disubst. double bonds) cm^{-1}. Adsorptive and stereomutation behaviour are given in Tables 3 and 4.

[19] S. HERTZBERG and S. LIAAEN JENSEN, *Phytochem.* **5**, 557 (1966).
[20] H. BROCKMANN and H. SCHODDER, *Chem. Ber.* **74**, 73 (1941).
[21] S. LIAAEN JENSEN, *Kgl. Norske Videnskab. Selskabs Skrifter* No. 8 (1962).
[22] R. ENTSCHEL and P. KARRER, *Helv. Chim. Acta* **41**, 402 (1958).
[23] H. BÖHME, *Org. Syn.* **3**, 619 (1955).

Hydride reduction of mutatochrome (III). (*a*) Strong conditions. A characteristic experiment was as follows: Mutatochrome (2·9 mg) in dry ether (5 ml) was treated with $LiAlH_4$ (510 mg) in darkness at room temperature. The reaction was followed by periodical paper-chromatographic examination. After 3 hr, 25 per cent of the reaction mixture comprised more polar products but no conversion to β-carotene (I) was observed. After 5 hr, 40 per cent of the reaction mixture consisted of more polar products and 5 per cent of β-carotene. After 24 hr a pigment recovery of 16 per cent was recorded. β-Carotene (I) accounted for 30 per cent, Product A (IV) 53 per cent and Product B (see below) 17 per cent of the recovered carotenoid.

(*b*) Mild conditions. The reaction was carried out in (i) darkness, (ii) light of 750–1150 nm or (iii) visible + i.r. light. The same products in the same relative amounts, and pigment recoveries of 60–80 per cent were obtained in all cases. A characteristic experiment of type (i) was as follows: To mutatochrome (III, 0·98 mg) in dry, peroxide-free ether (2 ml) a filtered solution of ethereal $LiAlH_4$ (1·5 ml saturated, 0·83 M,[13] solution; containing *ca.* 5 mg $LiAlH_4$) was added. After 3 hr the pigments were isolated in the usual manner and chromatographed on alumina; total pigment recovery was 82 per cent. No β-carotene (I) was present. Two products (A and B) accounted for 60 and 20 per cent respectively of the recovered carotenoid. Their properties are described in Table 1 and below.

Product B required 20–30% ether in light petroleum for elution from deactivated alumina, exhibited the same spectrum as Product A (cf. Fig. 2), gave a trimethylsilyl ether (R_f=0·98 on kieselguhr paper; 2% acetone in light petroleum), and showed a response towards standard treatment with acid chloroform similar to that of Product A below.

Product A (IV). This product required 50% ether–5% acetone in light petroleum for elution from the alumina column. The spectrum is given in Fig. 2. No acetate was produced under conditions for acetylation. Silylation furnished a trimethylsilyl ether (R_f=0·94 on kieselguhr paper; light petroleum). Standard treatment with acid chloroform produced a green colour but no allylic dehydration products.

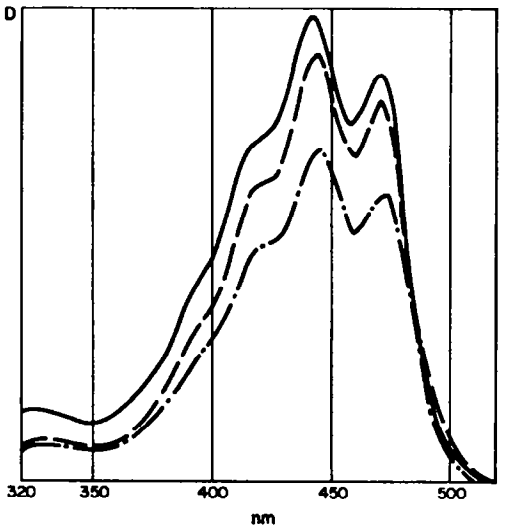


FIG. 2. ELECTRONIC SPECTRA IN ETHER OF —— PRODUCT A (IV) FROM MUTATOCHROME, ---- PRODUCT A (IV) FROM FLAVACIN AND -·- α-CAROTENE (V).

Dehydration of Product A (IV) to α-carotene (V). The dehydration was carried out with $POCl_3$ in pyridine in the usual manner; pigment recovery was 70 per cent. Chromatography on alumina revealed the presence of unreacted IV (24 per cent of total) and a carotene fraction (76 per cent) requiring an eluent of 3% ether in light petroleum. The latter fraction was resolved on aluminium oxide paper (light petroleum) into α-carotene (V; 70 per cent), abs. max. 330, 420, 443 and 470 nm in ether, R_f=0·33, and a minor product (VI?; 30% per cent with the same electronic spectrum and having an R_f=0·15.

α-Carotene (VI), for comparison, was isolated from carrots by ether–light petroleum extraction of a homogenate. The pigments, epiphasic to 95% aqueous methanol–light petroleum, were purified by saponification and column chromatography on calcium hydroxide. α-Carotene crystallized as red needles from light petroleum; m.p. 176–180° (Tscharner *et al.*[24] reported 178° and Kuhn and Lederer[10] 180°); abs. max. (420),

[24] C. TSCHARNER, C. H. EUGSTER and P. KARRER, *Helv. Chim. Acta* **40**, 1676 (1957).

443 and 472 nm in ether (see Fig. 2). α-Carotene was less strongly adsorbed on calcium hydroxide than β-carotene (I).

Data obtained by direct comparison of natural α-carotene (I) and the main dehydration product of A (V) are given in Table 2.

β-Carotene (I), not present in the dehydration mixture, showed an intermediate adsorptivity between the two dehydration products. Judging from isomerization tests the minor product was not a *cis* isomer of β-carotene.

Natural flavacin. The isolation has been described previously.[4] Flavacin was also isolated by the same procedure from *Oscillatoria agardhii*, harvested from Lake Aarungen, Aas, Norway. The latter algae contained 0·13 per cent carotenoid of the dry weight, and its carotenoid composition was as follows (in per cent of total): Myxoxanthophyll 33%, oscillaxanthin 10%, zeaxanthin 9%, 4-keto-3′-hydroxy-β-carotene 0·5%, cryptoxanthin 4%, echinenone 8%, flavacin 0·7% and β-carotene 35%. A total of 0·2 mg of flavacin was available for the present study.

Spectral data in visible light of flavacin, determined in direct comparison with mutatochrome (III), are given in Fig. 1 and Table 3. After iodine-catalysed stereoisomerization of each sample, a neo A and a neo U isomer could be distinguished, but could not be satisfactorily separated from the *trans* zone on aluminium oxide paper, see Table 3.

Flavacin was reduced with lithium aluminium hydride under mild conditions as described for mutatochrome above. The result is presented in Table 1.

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