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THE STRUCTURE OF OSCILLAXANTHIN*

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Dedicated to Professor Dr. Paul Karrer on his 80th Birthday

Abstract—The chemical structure of oscillaxanthin has been investigated by modern methods including NMR and mass spectrometry and glycoside hydrolysis. The evidence obtained supports the tridecaene octa-ol structure 1,1′-dihydroxy-2,2′-di-β-L-rhamnosyl-1,2,1′,2′-tetrahydro-3,4,3′,4′-tetradehydrolycopene (I).

INTRODUCTION

CAROTENOID glycosides have been considered rare, but an increasing number are now being discovered from organisms of different phylogenetic levels.[1–3] The structure of the first carotenoid glycoside known, crocin, a digentiobiose ester of the dicarboxylic acid crocetin,[1] was elucidated by Karrer and co-workers in 1927–1933. Oscillaxanthin was also first isolated in 1944 by the same school,[4] but the techniques available at that time did not allow the structure determination of this minor carotenoid.

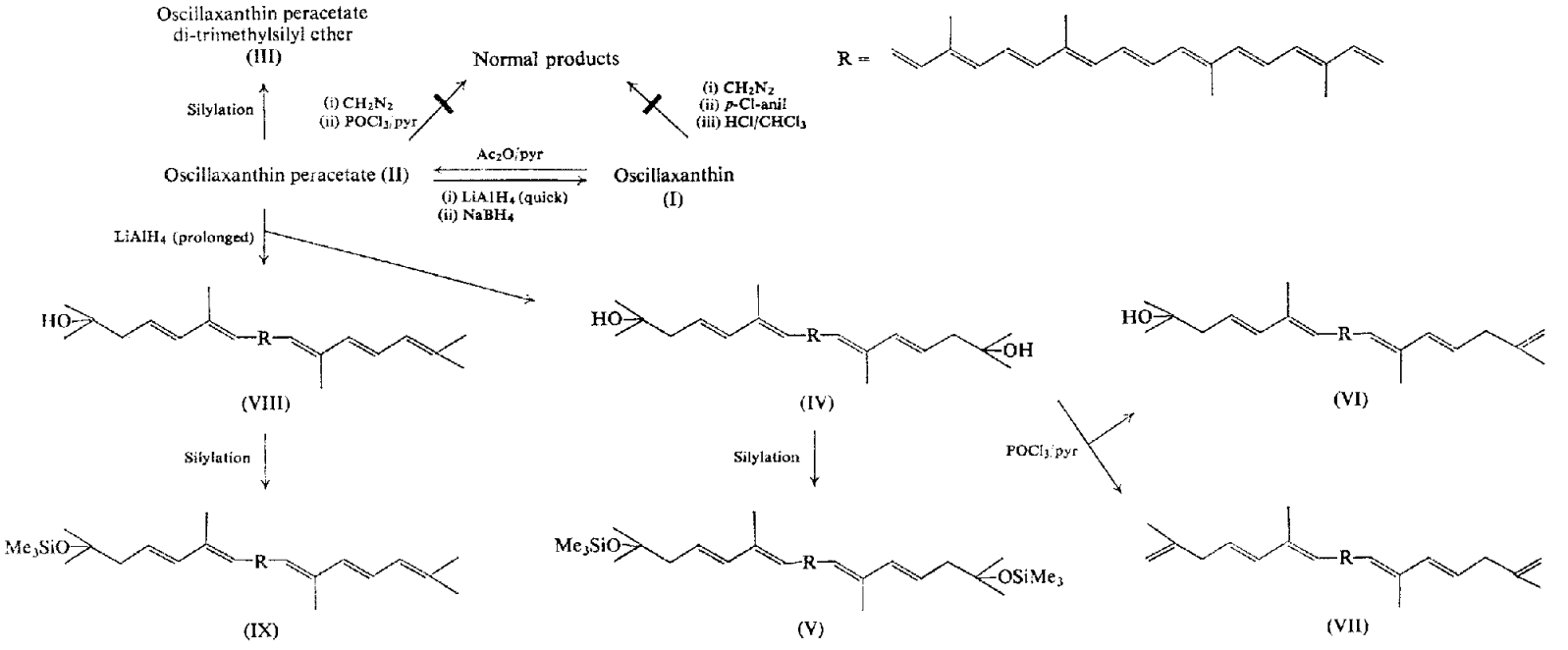
Oscillaxanthin was isolated by Karrer and Rutschmann[4] from *Oscillatoria rubescens*. It could only be obtained in an impure state and was described as an acidic pigment whose properties were unchanged after alkali treatment. Absorption maxima in visible light, absorptive and solubility properties were reported. A compound with similar properties was later isolated from *O. amoena*[5] and an *Arthrospira* sp.[6] In the latter case the identification was supported by identity in R_f-values of the native pigment and its peracetate with authentic oscillaxanthin (*O. rubescens*) and oscillaxanthin peracetate respectively. Further data about the chemistry of oscillaxanthin have not been reported.

RESULTS AND DISCUSSION

Oscillaxanthin (I) was isolated from the *Arthrospira* sp. previously examined.[6] It constituted *ca.* 5 per cent of the total carotenoid. Oscillaxanthin was obtained in the pure state only as the peracetate (II); blue-black needles m.p. 123°, yield *ca.* 4 mg. The absorption spectrum of oscillaxanthin peracetate (II) in visible light (Fig. 1) corresponds to those of 3,4-didehydrolycopene and spirilloxanthin, suggesting an aliphatic tridecaene chromophore.

* Part V in the series "Carotenoids of blue-green algae"; for Part IV see *Phytochem.* **8**, 1259 (1969).

[1] P. KARRER and K. MIKI, *Helv. Chim. Acta* **12**, 985 (1929).
[2] S. HERTZBERG and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **21**, 15 (1967).
[3] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1259 (1969).
[4] P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* **27**, 1691 (1944).
siol. Chem. **311**, 140 (1958).
S. LIAAEN-JENSEN, *Phytochem.* **5**, 557 (1966).


Oscillaxanthin peracetate di-trimethylsilyl ether (III)
Normal products
R =
Silylation
(i) CH_2N_2 (ii) $POCl_3$/pyr
(i) CH_2N_2 (ii) p-Cl-anil (iii) HCl/$CHCl_3$
Ac_2O/pyr
Oscillaxanthin peracetate (II)
Oscillaxanthin (I)
(i) $LiAlH_4$ (quick) (ii) $NaBH_4$
$LiAlH_4$ (prolonged)
HO
R
OH
(VIII)
(IV)
(VI)
Silylation
Silylation
$POCl_3$/pyr
Me_3SiO
$OSiMe_3$
(IX)
(V)
(VII)

The course of acetylation was followed (Fig. 2). Five intermediary acetates were isolated, demonstrating the presence of three or more secondary or primary hydroxyl groups in

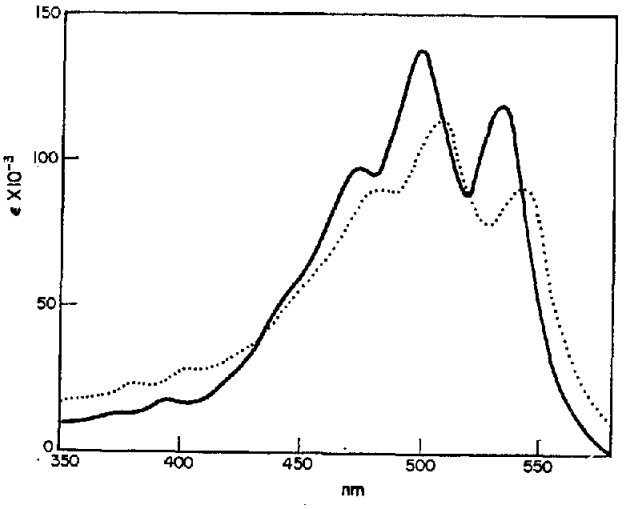


FIG. 1. ABSORPTION SPECTRA IN VISIBLE LIGHT RECORDED IN ACETONE OF ——— *trans* OSCILLAXANTHIN PERACETATE (II) AND ····· *trans* 1-HYDROXY-1,2-DIHYDRO-3,4,3′,4′-TETRADEHYDROLYCOPENE (IX). EXTINCTION VALUES VALID FOR II ONLY.

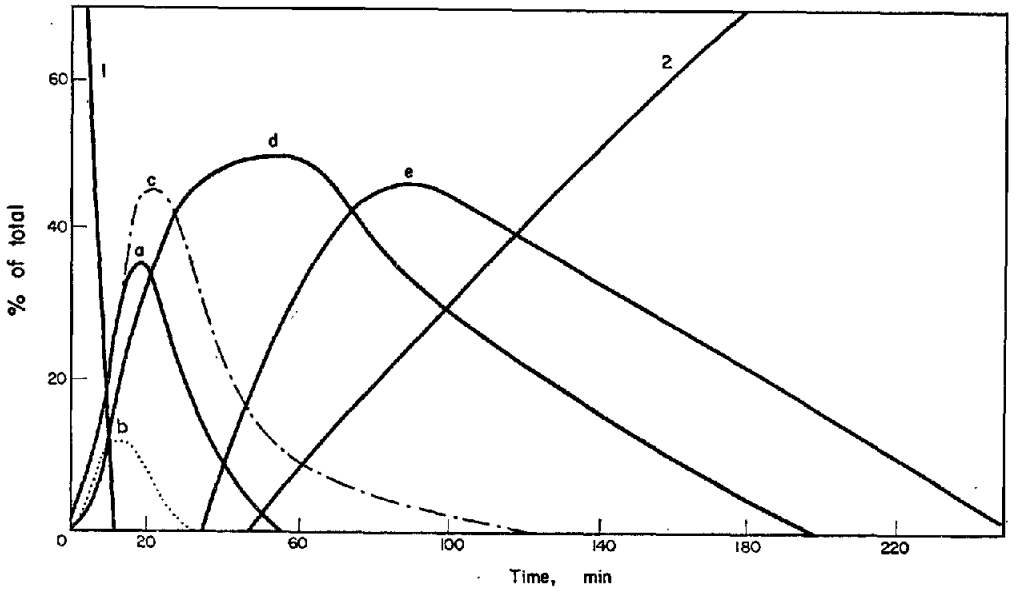


FIG. 2. THE COURSE OF ACETYLATION OF OSCILLAXANTHIN (I) TO ITS PERACETATE (II) UNDER CONDITIONS SPECIFIED IN EXPERIMENTAL PART; *a–e* REPRESENT INTERMEDIARY ACETATES.

oscillaxanthin (I). None of these appeared to be in an allylic position to the polyene chain, since attempted oxidation with *p*-chloranil[7] or allylic dehydration with acid chloroform[8]

[7] S. LIAAEN-JENSEN, *Acta Chem. Scand.* **19**, 1166 (1965).
[8] P. KARRER and E. LEUMANN, *Helv. Chim. Acta* **34**, 445 (1951).

failed. On silylation,[9] the peracetate (II) provided a mono- and a di-trimethylsilyl ether (III), demonstrating the presence of two tertiary hydroxy groups in II and I. The tertiary hydroxy groups were not in positions allowing dehydration of the peracetate (II) with $POCl_3$.[10] The total number of hydroxyl groups in oscillaxanthin (I) should therefore be no less than five, which is supported by its strongly hypophasic character and adsorptivity.

Mild treatment of oscillaxanthin peracetate (II) with $LiAlH_4$ gave oscillaxanthin, demonstrating the absence of enolizable carbonyl functions in I. Moreover, neither oxcillaxanthin, nor its peracetate, gave any products with diazomethane, and the adsorptive properties of the peracetate are not compatible with the presence of a carboxyl group in I. We assume that the large number of hydroxyl groups in oscillaxanthin is responsible for its strongly polar character which led others[1] to consider it to be an acidic carotenoid. The structural implications of the effect of alkali is less clear. Whereas Karrer and Rutschmann[4] recovered oscillaxanthin after saponification, in our experience the predominant products were of shorter chromophore. Hence alkali treatment of oscillaxanthin and its peracetate was avoided.

TABLE 1. COMPOSITION OF THE IODINE-CATALYSED EQUILIBRIUM MIXTURES OF SYNTHETIC 1,1′-DIHYDROXY-1,2,1′,2′-TETRAHYDRO-3,4,3′,4′-TETRADEHYDROLYCOPENE (IV) AND IV OF MATERIAL DERIVED FROM OSCILLAXANTHIN

Origin	Member of the stereoisomeric set	R_f on kieselguhr paper 20% acetone in petrol. ether	λ_{max} (in nm) in acetone	% III/II	% D_R/D_{II}	% Of total
Synthetic[8]	*trans*	0·58	386 467, 495, 528	52	17	58
	neo A	0·64				
	neo B	0·77	368,386 (460), 485, 517			42
	neo C	0·86				
Natural	*trans*	0·58	385 467, 493, 527	43	21	59
	neo A	0·64				
	neo B	0·77	368,387 (460), 484, 515			41
	neo C	0·86				

Whereas quick, standard treatment of oscillaxanthin peracetate with $LiAlH_4$ gave oscillaxanthin, prolonged treatment gave two less polar products, the major one being 1,1′-dihydroxy-1,2,1′,2′-tetrahydro-3,4,3′,4′-tetradehydrolycopene (IV). The identification of IV was based on direct comparison of stereomutation behaviour including relative abundance, spectral properties in visible light, and adsorptive properties of the main stereoisomers with that of synthetic IV[11] (Table 1), as well as identity in R_fs of the corresponding di-trimethylsilyl ethers (V). Dehydration of the major reduction product and the synthetic diol (IV) with $POCl_3$ in pyridine[10] in neither case gave products with prolonged chromophore. However, both compounds gave in low yield two products with spectral and adsorptive properties compatible with the mono-ol VI and the hydrocarbon VII. The course of the dehydration will be discussed elsewhere.

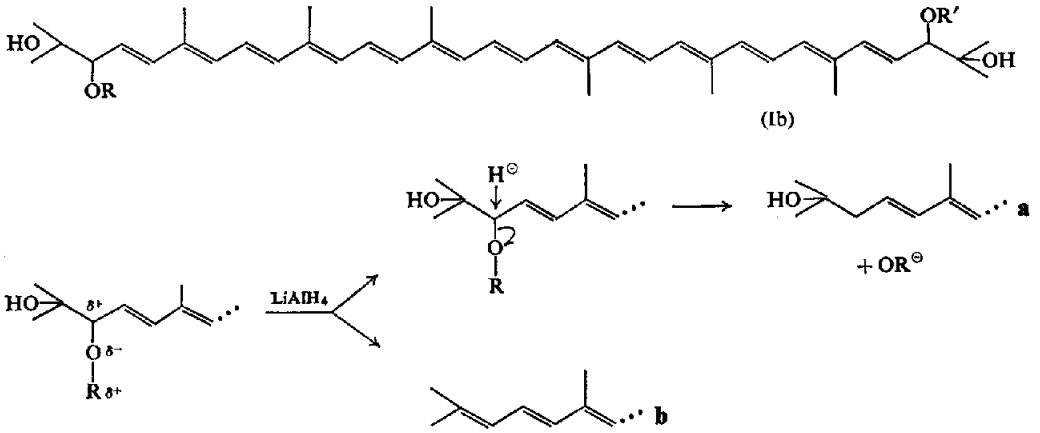
The absorption spectrum of the second product obtained on prolonged hydride reduction of oscillaxanthin peracetate was shifted towards longer wavelengths (Fig. 1). This product gave a trimethylsilyl ether on silylation and no acetate on acetylation. The tetradecaene

[9] A. MCCORMICK and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 1989 (1966).
[10] J. D. SURMATIS and A. OFNER, *J. Org. Chem.* **28**, 2735 (1963).
[11] D. F. SCHNEIDER and B. C. L. WEEDON, *J. Chem. Soc. C* 1686 (1967).

structure VIII (previously undescribed chromophore) is considered for this second product and IX for its trimethylsilyl ether.

Previous experience [12–15] has revealed that allylic ethers react with $LiAlH_4$ under forcing conditions to give rise to free alcohols and elimination products. On the basis of the partial structure Ib for oscillaxanthin the present findings are in agreement with the reduction data obtained for myxoxanthophyll.[3] Hydride attack at 2-position, followed by expulsion of OR^- could account for the formation of the end-group *a* present twice in the diol IV and once in the mono-ol VIII. The mechanism of the elimination reaction leading to *b* is less readily explained, but may be related to the one giving olefins from epoxides.

Support for the partial structure Ib for oscillaxanthin was obtained from i.r. evidence (Fig. 3). The peracetate (II) exhibits characteristic absorption at 1140 and 910 cm^{-1} for tertiary hydroxyl.[16] A striking similarity between the spectra of myxoxanthophyll peracetate and oscillaxanthin peracetate in the 9–10 μ region together with the acetylation evidence indicated a glycosidic structure. Hydrolysis of oscillaxanthin by the method used for myxoxanthophyll[3] led to identification by paper chromatography of rhamnose.



The NMR spectrum of oscillaxanthin peracetate is given in Fig. 4, including signal assignments, cf.[3] Presence of in-chain methyl (τ 8·00) and in-chain/end-of-chain methyl signals (τ 8·05), absence of end-of-chain methyl signals and the integral of the olefinic proton signals (τ 3–4, 4, *ca.* 19 H, calc. 20 H) supported the symmetrical location of the tridecaene chromophore. A doublet at τ 6·23 (J=8 cps, *ca.* 2 H) and absence of allylic methylene at τ 7·70 supported oxygen substituents in 2,2′-positions. The intensity of the methyl doublet at τ 8·93 (J=6·2 cps, 6 H) relative to the *gem.* dimethyl signals of the aglycone at τ 8·77, 8·81 (*ca.* 13 H, calc. 12 H), indicated a dirhamnoside. The required hexa-acetate formulation was confirmed by the integral of the 8 τ complex including acetate methyl signals at τ 7·92, 7·95, and 7·98 and chain-methyl signals (*ca.* 33 H, calc. 36 H) and the ring proton complex at τ 4·5–6·1 (*ca.* 8 H, calc. 8 H).

[12] E. C. Grob and W. Siekmann, *Helv. Chim. Acta* **48**, 1199 (1965).
[13] L. Cholnoky, K. Gyørgyfy, J. Szabolcs and B. C. L. Weedon, *Chem. Res. Commun.* **13**, 404 (1966).
[14] B. P. Schimmer and N. I. Krinsky, *Biochemistry* **5**, 3649 (1966).
[15] S. Hertzberg and S. Liaaen-Jensen, *Phytochem.* **6**, 1119 (1967).
[16] S. Liaaen-Jensen, *Kgl. Norske Videnskab. Selskabs Skrifter* 8 (1962).

The upper part of the mass spectrum of oscillaxanthin peracetate is given in Fig. 5. Peaks above *m/e* 1068 were not recorded. Likely losses were observed from the strong peak

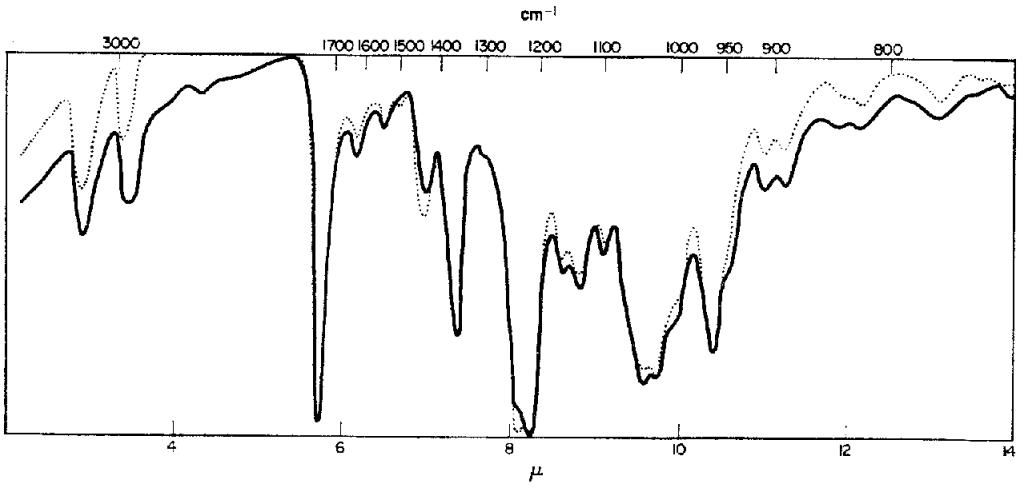


FIG. 3. I.r. SPECTRA (KBr) OF ——— OSCILLAXANTHIN PERACETATE (II) AND ····· MYXOXANTHOPHYLL PERACETATE.[3]

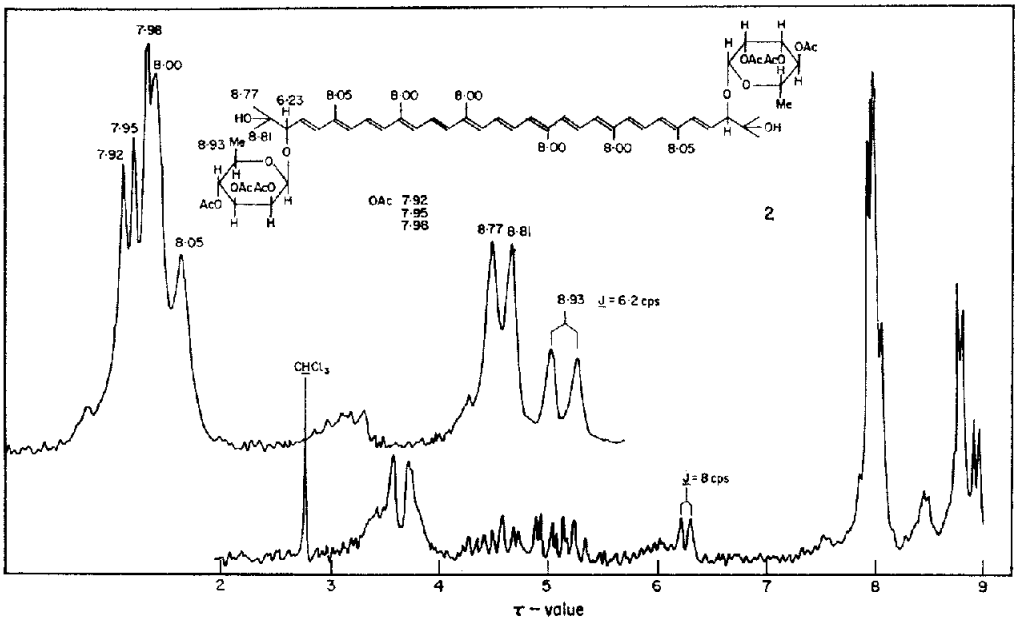


FIG. 4. PROTON MAGNETIC RESONANCE SPECTRUM ($CDCl_3$) OF OSCILLAXANTHIN HEXAACETATE (II).

at *m/e* 1038, which for a while was considered to be the molecular ion. However, on the assumption of oscillaxanthin being the dirhamnoside, (I) the peak at *m/e* 1038 represents the M-106 ion. The intensity ratio 0·24 of the M-92 and M-106 peaks is then more in accord-

ance with the ratios previously recorded for a tridecaene chromophore[17] than if *m/e* 1038 represented the molecular ion (then giving intensity ratio 0·60 for the above peaks). Moreover, the fragmentation pattern observed on the basis of M = 1144 ($C_{64}H_{88}O_{18}$) is in good agreement with the dirhamnoside formulation of oscillaxanthin peracetate. In addition to the common losses of 92 and 106 mass units (and multiple losses involving these entities), the main fragmentations observed are indicated below.

There is a strong resemblance between the fragmentation pattern of oscillaxanthin hexacetate and the cleavages previously observed for the rhamnoside myxoxanthophyll and

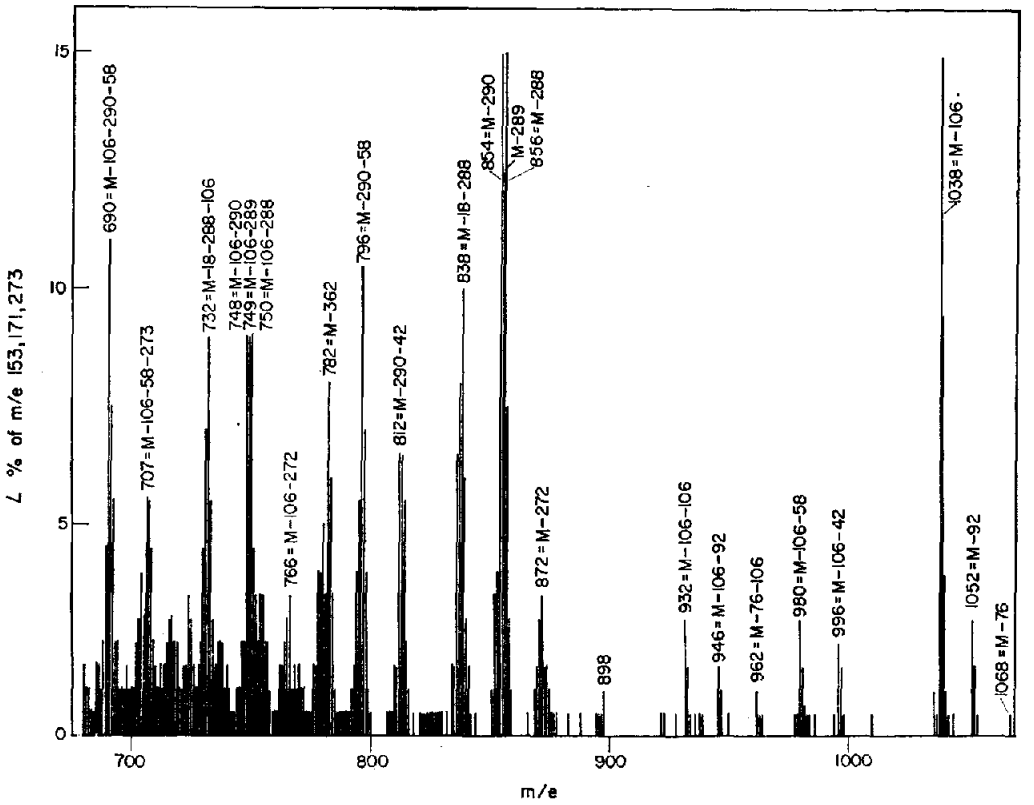
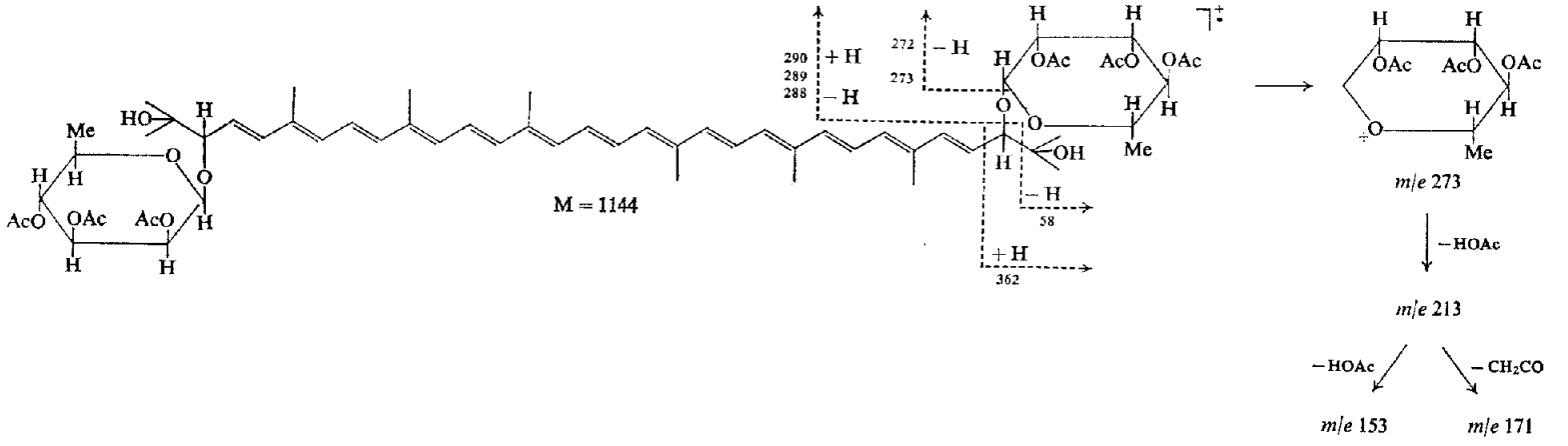


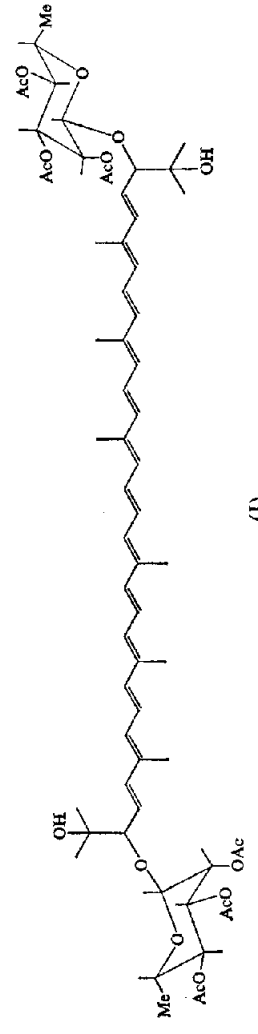
FIG. 5. MASS SPECTRUM OF OSCILLAXANTHIN HEXAACETATE (II).

its tetraacetate.[3] Rupture of the sugar-O ether bond is seen from peaks at M-272, M-272-106, and M-273-58-106, that is with or without hydrogen transfer to the sugar moiety with charge retention on the aglycone. A very strong peak at *m/e* 273 indicates that in cleavage of this bond oxonium ion formation is preferred, cf.[18]

Cleavage of the aglycone-O ether bond is observed without and with hydrogen transfer in both directions (peaks at M-288, M-289, and M-290). These fragmentations are also seen in multiple losses with xylene (M-288-106, M-289-106, and M-290-106). The loss of 290 mass units, the only one of these observed in the case of myxoxanthophyll peracetate,[3] is also recognized in multiple losses with 58 (acetone) and 42 (ketene, derived from the second

[17] C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **22**, 1054 (1968).
[18] K. BIEMANN, D. C. DEJONGH and K. H. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).


M = 1144
290
289
288
+ H
− H
272
273
− H
+ H
362
58
m/e 273
−HOAc
m/e 213
−HOAc
−CH2CO
m/e 153
m/e 171
−H2O
M-18
M-18-288



(I)

acetylated end-group) and 106 mass units; peaks at M-290-42, M-290-58, M-106-290, M-106-290-58. Loss of ketene is also evident from a M-42-106 peak.

A peak at M-306 could be considered the result of a two-step loss of water and 288 mass units, cf. myxoxanthophyll,[3] as indicated in the scheme above. Finally loss of acetone (M-18-58, M-106-58, M-106-92-58, M-18-58-106-106, M-290-58), M-106-58-273, M-106-290-58) and of 362 mass units, resulting from cleavage of the 2,3 bond with hydrogen transfer to the smaller fragment supports the formulation of the aliphatic end-group. A weak peak at *m/e* 898 could be caused by a slight contamination with myxoxanthophyll tetraacetate (M = 898).[3] Otherwise all prominent peaks in the region including the 500 upper mass units of the spectrum are readily accounted for by the dirhamnoside formulation, II.

In the lower mass region (recorded to *m/e* 140) strong peaks were observed at *m/e* 273, 13, 171, and 153. The three latter peaks can all be accounted for by losses of acetic acid and ketene from the *m/e* 273 oxonium ion analogous to the fragmentations discussed by Biemann *et al.*[18] In summary all prominent peaks in the mass spectrum of oscillaxanthin peracetate support the hexaacetate formulation of a dirhamnoside (II).

The evidence discussed is therefore in complete agreement with structure I for oscillaxanthin.

By analogy with other naturally occurring rhamnosides[19] oscillaxanthin is most likely an L-rhamnoside. The chemical shift positions and coupling constants in the NMR-spectra of triacetoxy methyl α- and β-L-rhamnoside[20] and of the glycosidic moiety of oscillaxanthin peracetate (II) are best accommodated with oscillaxanthin being a di-β-L-rhamnoside, with four heavy equatorial substituents including the polyene chain.

Myxoxanthophyll, isolated from the same *Arthrospira* sp. was recently shown to be a mixed monoglycoside in which rhamnose was the dominant sugar with a hexose as a minor component. No hexoside was detected in the mass spectrum of oscillaxanthin peracetate. However, after the glycoside hydrolysis of another sample of oscillaxanthin, originating from cells of a different harvest, a weak spot with R_f similar to glucose was detected.

EXPERIMENTAL

Materials and Methods

These were as reported elsewhere.[3,21] The *Arthrospira* sp.[3,6] was used. Pigment extraction and chromatography have already been described.[3] Saponification was not included in the purification procedure. Oscillaxanthin (I) was isolated after column chromatography on cellulose powder. The peracetate (II) was obtained after quick column chromatography on deactivated alumina of an acetylated mixture of oscillaxanthin and myxoxanthophyll.

Oscillaxanthin (I)

Red-black needles of I were obtained from pyridine-petroleum ether, yield *ca.* 2 mg of indefinite melting point (> 130°). Crystalline I was readily soluble in pyridine, moderately soluble in tetrahydrofuran, chloroform, methanol and acetone and insoluble in ether, benzene and petroleum ether. The adsorptive properties are given in Table 2. I was completely hypophasic when partitioned between petroleum ether and 60% aqueous methanol. The absorption spectra in visible light corresponded to those of the peracetate (II), see below and Fig. 1. In pyridine–methanol (1:9) $E^{1\%}_{1cm} = 750$ ($\epsilon = 66{,}900$) at 490 nm, compared with $\epsilon = 142{,}000$ for spirilloxanthin at λ_{max} in benzene.[22] I in KBr exhibited i.r. maxima at 3325 (OH); 2950 (CH); 1735 (impurity), 1665, 1650, 1630, 1550, 1530; 1450 (CH_2); 1360 (CH_3), 1220, 1052, 1035; 965 (*trans* disubst. double bonds), 908 and 820 (*trans* trisubst. double bonds) cm^{-1}.

[19] L. F. FIESER and M. FIESER, *Organic Chemistry*, 2nd ed., Ch. 15, Reinhold, New York (1950).
[20] E. HEMMER and S. LIAAEN-JENSEN, to be published.
[21] A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 1970 (1966).
[22] M. S. BARBER, L. M. JACKMAN, P. S. MANCHAND and B. C. L. WEEDON, *J. Chem. Soc. C* 2166 (1966).

TABLE 2. ADSORPTIVE PROPERTIES OF OSCILLAXANTHIN AND ITS DERIVATIVES

Carotenoid	Required eluent from: Cellulose powder	Required eluent from: Neutral alumina activity grade 2	R_f on kieselguhr paper: 5%†	10%†	20%†	30%‡	2%§
Oscillaxanthin (I)	50–100% acetone*						0·58
Oscillaxanthin acetate *a*						0·29	
b						0·55	
c						0·75	
d					0·12		
e					0·28		
Oscillaxanthin peracetate (II)		25–40% acetone*		0·11	0·41		
Oscillaxanthin peracetate mono-trimethylsilyl ether				0·56			
Oscillaxanthin peracetate di-trimethylsilyl ether (III)				0·31			
1,1′-Dihydroxy-1,2,1′,2′-tetradehydrolycopene (IV)		1% methanol in ether			0·58		
IV Di-trimethylsilyl ether (V)			0·73				
1-Hydroxy-1,2,1′,2′-tetrahydro-3,4,1′,16′,3′,4′-hexadehydrolycopene (VI)?		50% ether* or 10% acetone*		0·45			
1,2,1′,2′-Tetrahydro-1,16,3,4,1′,16′,3′,4′-octadehydrolycopene (VII)?		10–30% ether*		0·75			
1-Hydroxy-1,2-dihydro-3,4,3′,4′-tetradehydrolycopene (VIII)		100% ether		0·22			
VIII Trimethylsilyl ether (IX)				1·00			

* In petroleum ether.
† Acetone in petroleum ether.
‡ Acetone in benzene.
§ Methanol in acetone.

Alkali treatment. I (*ca.* 0·3 mg) was treated with 10% KOH in methanol (5 ml) for 1 hr. The product was readily transferred to ether-$CHCl_3$ on addition of aqueous NaCl. The recovered pigment had λ_{max} at 370, 390, 420, 469, (490) and (520) nm in methanol and $R_f = 0$ on kieselguhr paper (2% methanol in acetone).

Attempts at allylic oxidation, elimination and esterification. I (0·23 mg) was treated with *p*-chloranil for 18 hr in the usual manner; pigment recovery was 62 per cent. No allylic oxidation products were obtained. I (0·3 mg) was treated with 0·03 N HCl–$CHCl_3$ for 5 min; pigment recovery was 60 per cent. The reaction mixture exhibited λ_{max} at 495 nm in ether, contained unreacted I, more yellow products and a trace of a product ($R_f = 1·0$ on kieselguhr paper; 30% acetone in benzene) with similar colour to I. I (1·7 mg) in methanol was recovered unchanged, after treatment with CH_2N_2 in ether for 1·5 hr at room temperature and at −20° over night.

Glycoside hydrolysis. I (*ca.* 2 mg, obtained from a second isolation) was hydrolysed in methanol as described for myxoxanthophyll.[3] The methyl glycoside obtained was further hydrolysed to reducing sugar.[3] Paper chromatography in system 5[3] gave the following result: hydrolysate major spot $R_{galactose}$ 1·68 and minor spot $R_{galactose}$ 1·09; rhamnose $R_{galactose}$ 1·75; fucose $R_{galactose}$ 1·46. In a separate experiment found for glucose $R_{galactose}$ 1·07.

Oscillaxanthin Peracetate (II)

Preparation. The course of acetylation at room temperature of I (0·3 mg) in dry pyridine (3 ml) with acetic anhydride (0·3 ml) was followed by paper chromatography and spectrophotometric determination of the eluted products, see Fig. 2 and Table 2. The peracetate (II) was prepared by acetylation in the standard manner over night; pigment recoveries were *ca.* 60 per cent. It was obtained as blue-black needles from acetone-petroleum ether; yield *ca.* 4 mg, m.p. 123°.

Properties. II was more strongly adsorbed (Table 2) than 1,1′-dihydroxy-2,2′-diacetoxy-1,2,1′,2′-tetrahydro-3,4,3′,4′-tetradehydro-lycopene (R_f 0·70 on kieselguhr paper; 20% acetone in petroleum ether),

prepared by hydride reduction followed by acetylation of synthetic phillipsiaxanthin.[23,24] Partition ratio in petroleum ether/85% methanol was 16:84. II had λ_{max} in acetone at 390, 470, 499 ($\epsilon = 137{,}900$) and 534 nm, % III/II = 60, % D_B/D_{II} = 15 (Fig. 1) and in $CHCl_3$ at 397, 480, 510 and 548 nm, % III/II = 60, % D_B/D_{II} = 20. For comparison synthetic spirilloxanthin had λ_{max} (380), 397, 480, 510 and 548 nm, % III/II = 67 and % D_B/D_{II} = 14 in $CHCl_3$ and synthetic 3,4-didehydrolycopene λ_{max} 373, 390, 470, 499 and 533 nm, % III/II = 61 and % D_B/D_{II} = 13 in acetone.

In its i.r. spectrum in KBr (Fig. 3), II had max. at 3460 (OH); 2930 (CH); 1750 (acetate); 1635, 1545 (conj. double bonds); 1440 (CH_2); 1370 (CH_3); 1245, 1220 (acetate), 1163; 1135 (*tert.* OH); 1105, 1050; 1030 (acetate); 965 (*trans* disubst. double bonds), 910 and 820 (*trans* trisubst. double bonds) cm^{-1}. The NMR spectrum of II (2 mg) in $CDCl_3$ at 100 Mc/sec is given in Fig. 4. An expanded spectrum of the ring-proton region of the rhamnose residue led to the following assignments, *cf.*[20]: τ 4·52 broad singlet (H-1, ax., $J_{1-2} = ca.$ 0cps), τ 5·20 broad singlet (H-2, $J_{1-2} = ca.$ 0cps, $J_{2-3} = ca.$ 0cps), τ 4·86 doublet (H-3, $J_{2-3} = ca.$ 0cps, J_{3-4} = 4 cps (ax. ax.)), τ 5·06 double doublet (H-4, J_{3-4} = 4 cps (ax. ax.), J_{4-5} = 9·5 cps (ax. ax.)), τ 9·01 double quartet (H-5, J_{4-5} = 9·5 cps (ax. ax.), J_{5-Me} = 6·5 cps). II exhibited weak Raman absorption at 1000, 1155 and 1515 cm^{-1} and a broad, intense band at 950 cm^{-1}. Its mass spectrum is given in Fig. 5.

Attempts at esterification and dehydration. II (1·4 mg) was recovered unchanged after treatment with CH_2N_2 in ether for 1·5 hr at room temperature, than at −20° over night; pigment recovery was 90 per cent. II (0·1 and 0·2 mg in two separate experiments) in dry pyridine (5 ml) was treated with $POCl_3$ (0·05 ml) for 1·5 hr at 50°. The recovery of epiphasic products after transfer to ether on admixture with aqueous NaCl-solution was 20 and 60 per cent. The recovered pigment contained II and strongly polar products.

Oscillaxanthin peracetate di-trimethylsilyl ether (III). The course of silylation of II (0·3 and 1 mg) in dry pyridine (1 ml) with hexamethyldisilazane (0·4 ml) and trimethylchlorosilane (0·2 ml) was followed by paper chromatography. After 3 min a monoether and a diether (III) were formed. The two products were eluted and submitted separately to further silylation, whereupon the monoether was quantitatively converted to the diether (III), which gave no further products.

Alkali treatment. II (0·1 mg) was treated with 5% KOH in methanol for 30 min; pigment recovery was 30%. The reaction mixture contained some oscillaxanthin but mainly orange-yellow, strongly-adsorbed products (R_f 0·33–0·69 on paper; 50% acetone in petroleum ether).

Reductions. II (0·8 mg) in ethanol (5 ml) was treated with $NaBH_4$ (20 mg) for 2 hr; pigment recovery after transfer to ether was 90 per cent. The reaction mixture contained I adsorbed to white inorganic material from which it was not readily separated. Quick reduction of II (0·1 mg) in dry ether with a filtered (glass wool) suspension of $LiAlH_4$ in dry ether for 5 min gave I, again strongly adsorbed to inorganic reaction products. Prolonged hydride reduction of II (0·8 and 1·1 mg) in dry, peroxide-free tetrahydrofuran was carried out with a weak suspension of $LiAlH_4$ in tetrahydrofuran for 20 and 3 hr; pigment recovery was 14 and 15 per cent. The reaction mixture in each experiment comprised IV (*ca.* 55 per cent) and VIII (*ca.* 35 per cent).

1,1′-*Dihydroxy*-1,2,1′,2′-*tetrahydro*-3,4,3′,4′-*tetradehydrolycopene* (IV). The *trans* isomer had λ_{max} at 392, 471, 500 and 532 nm in acetone (cf. Fig. 1); for adsorptive properties see Table 2. The result of separate iodine catalysed isomerization in benzene of this product and synthetic IV is given in Table 1. No separation of the corresponding stereoisomers was obtained during co-chromatography. Samples of IV, one synthetic and one prepared from II, were silylated in the usual manner for 1 hr, and gave the same per-trimethylsilyl ether (V), with unchanged absorption spectrum in visible light; for R_fs see Table 2. Treatment of the product (IV, 0·05 mg) in dry pyridine (5 ml) with $POCl_3$ (0·05 ml) for 50 min at 50° gave 48 per cent pigment recovery. Two products (VI and VII) comprising 70 and 30 per cent respectively of the recovered pigment exhibited unchanged spectral properties in visible light and were less strongly adsorbed than IV (Table 2). In a parallel experiment synthetic IV (0·34 mg) was treated likewise; pigment recovery was 41 per cent. The two main products (40 and 60 per cent respectively of total) corresponded to those tentatively identified as VI and VII above.

1-*Hydroxy*-1,2-*dihydro*-3,4,3′,4′-*tetradehydro-lycopene* (VIII). The *trans* isomer of the second product (VIII) had λ_{max} in acetone at 380, 400, 480, 508 and 542 nm (Fig. 1); for adsorptive properties see Table 2. On co-chromatography synthetic 3,4,3′,4′-tetradehydrolycopene was less polar. Small scale standard acetylation appeared negative, whereas silylation gave a product considered as IX.

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