

THE STRUCTURE OF MYXOXANTHOPHYLL*

SISSEL HERTZBERG and SYNNOVE LIAAEN-JENSEN

Organic Chemistry Laboratories, Norway Institute of Technology, Trondheim, Norway

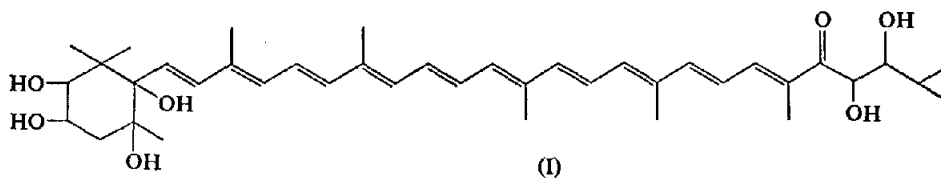
(Received 17 December 1968)

Abstract—The structure of myxoxanthophyll, the characteristic xanthophyll of blue-green algae, has been investigated by chemical and physical methods including NMR and mass spectra. The evidence presented suggests that chromatographically homogeneous myxoxanthophyll is a mixed (1',2'-dihydro-3',4'-didehydro-3,1'-dihydroxy- γ -caroten-2'-yl)-glycoside (IVc and IVd)† in which rhamnose is the dominant sugar moiety and a hexose a minor component.

INTRODUCTION

MYXOXANTHOPHYLL, the characteristic xanthophyll of blue-green algae,¹⁻⁵ was first isolated by Heilbron and Lythgoe⁶ from *Oscillatoria rubescens*. Absorption spectra in visible light, melting point (169–170°), a hypophasic partition behaviour and molecular composition $C_{40}H_{56}O_7$ were reported. The elementary analysis was supported by results obtained by Karrer and Rutschmann.⁷ On catalytic hydrogenation their myxoxanthophyll (m.p. 180°) consumed 11 moles of hydrogen. Acetylation provided a tetraacetate, m.p. 131–132°, $C_{48}H_{64}O_{11}$, determined by combustion analysis. Determination of active hydrogen indicated the presence of two tertiary hydroxy groups in the tetraacetate. On the basis of the absorption spectrum in visible light the seventh oxygen function was considered to be a carbonyl group in spite of lack of response to Meerwein-Ponndorf reduction. Structure I for myxoxanthophyll was considered by Karrer and Rutschmann.⁷

A possible identity of myxoxanthophyll and aphanizophyll⁸ was for some time disputed,^{6, 7, 9, 10} but these compounds were subsequently found to be different.¹¹

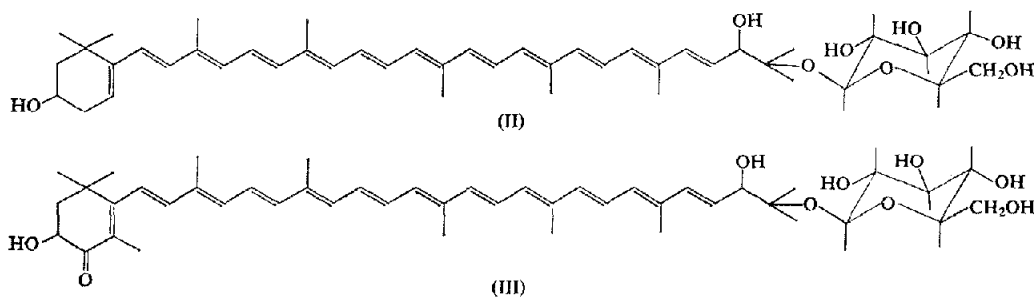


* Part IV in the series "The Carotenoids of Blue-green Algae"; for Part III see *Phytochem.* 6, 1119 (1967).

† The didehydro designation is used throughout this paper in accordance with the new IUPAC rules of carotenoid nomenclature soon to be published.

- ¹ I. M. HEILBRON, *J. Chem. Soc.* 79 (1942).
- ² T. W. GOODWIN, *J. Gen. Microbiol.* 17, 467 (1957).
- ³ J. TISCHER, *Hoppe-Seyler's Z. Physiol. Chem.* 311, 140 (1958).
- ⁴ H. H. STRAIN, *Ann. Priestley Lectures* (1958); *Chem. Abstr.* 52, 13890f (1958).
- ⁵ S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* 5, 557 (1966).
- ⁶ I. M. HEILBRON and B. LYTHGOE, *J. Chem. Soc.* 1376 (1936).
- ⁷ P. KARRER and J. RUTSCHMANN, *Helv. Chim. Acta* 27, 1691 (1944).
- ⁸ J. TISCHER, *Hoppe-Seyler's Z. Physiol. Chem.* 251, 109 (1938).
- ⁹ J. TISCHER, *Hoppe-Seyler's Z. Physiol. Chem.* 260, 257 (1939).
- ¹⁰ H. KYLIN, *Kgl. Fysiogr. Sällskap Lund Förh.* 9, 213 (1939).
- ¹¹ S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* 5, 565 (1966).

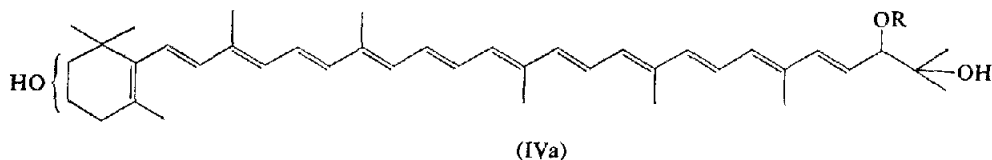
From a strain of *Mycobacterium phlei* Schlegel¹² isolated a carotenoid presumed to be myxoxanthophyll. The bacterial carotenoid was found to be different from myxoxanthophyll⁵ and was later shown to be a mixture of the tertiary glucosides phleixanthophyll (II) and 4-keto-phleixanthophyll (III).¹³



In the present work a reinvestigation of the chemical structure of myxoxanthophyll has been carried out by modern methods.

RESULTS AND DISCUSSION

Myxoxanthophyll (IV) was obtained as red needles, m.p. 173°, from an *Arthrospira* sp. A direct comparison between myxoxanthophyll from this blue-green algae and authentic myxoxanthophyll from *Oscillatoria rubescens* has been reported earlier.⁵ The absorption spectrum in visible light (Fig. 1) corresponded to that of phleixanthophyll (II). During acetylation under standard conditions four intermediates were isolated (see Fig. 2), indicating the presence of at least three primary or secondary hydroxy groups in myxoxanthophyll. None of these appeared to be in a position allylic to the polyene chain, since attempted oxidations with *p*-chloranil,¹⁴ 2,3-dichloro-5,6-dicyanobenzoquinone¹⁵ or nickel peroxide¹⁶ failed. The peracetate (V) gave a monotrimethylsilyl ether (VI), demonstrating the presence of one tertiary hydroxy group in the peracetate (V). Dehydration of V could, however, not be achieved with POCl₃ in pyridine.¹⁷ Treatment of myxoxanthophyll (IV) with acidified CHCl₃¹⁸ resulted in the formation of non-polar elimination products with prolonged chromophore (see below). This evidence pointed towards the partial structure IVa for myxoxanthophyll.



¹² H. G. SCHLEGEL, *Arch. Mikrobiol.* **31**, 231 (1958).

¹³ S. HERTZBERG and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **21**, 15 (1967).

¹⁴ S. LIAAEN-JENSEN, *Acta Chem. Scand.* **19**, 1166 (1965).

¹⁵ E. R. H. JONES, B. E. LOWE and G. LOWE, *J. Chem. Soc.* 1476 (1964).

¹⁶ S. LIAAEN-JENSEN and S. HERTZBERG, *Acta Chem. Scand.* **20**, 703 (1966).

¹⁷ J. D. SURMATHIS and A. OFNER, *J. Org. Chem.* **28**, 2735 (1963).

¹⁸ P. KARRER and E. LEUMANN, *Helv. Chim. Acta* **34**, 445 (1951).

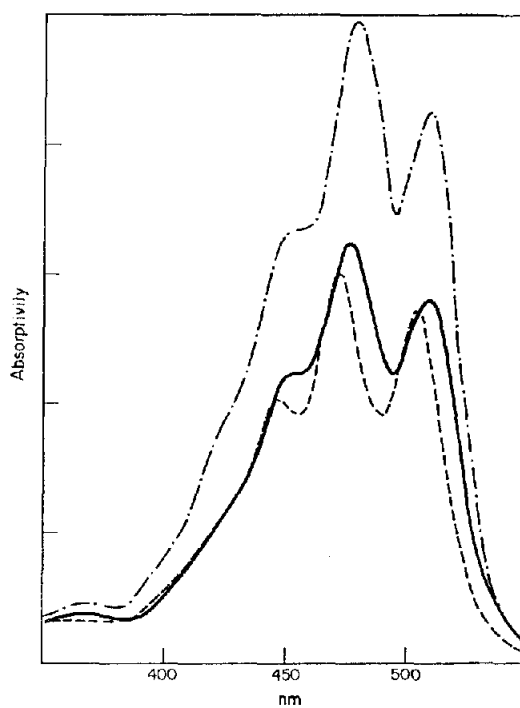


FIG. 1. ABSORPTION SPECTRA IN ACETONE OF: — MYXOXANTHOPHYLL (IV), — · — PHLEI-XANTHOPHYLL (II), - - - LYCOPENE.

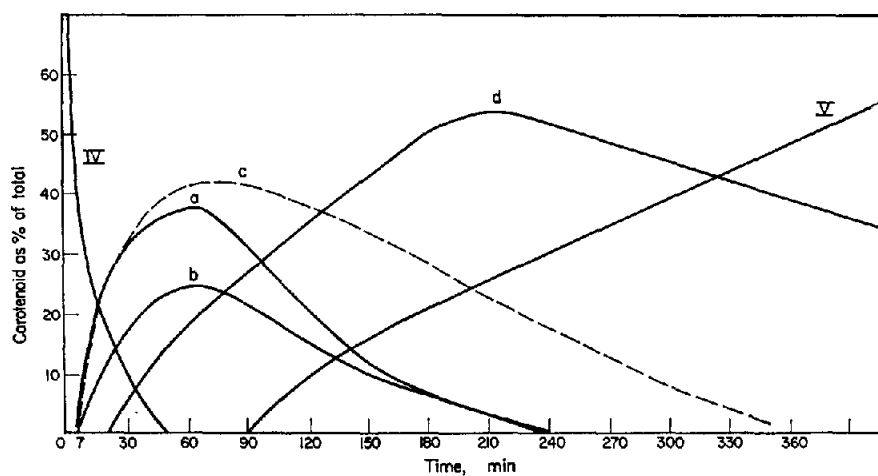
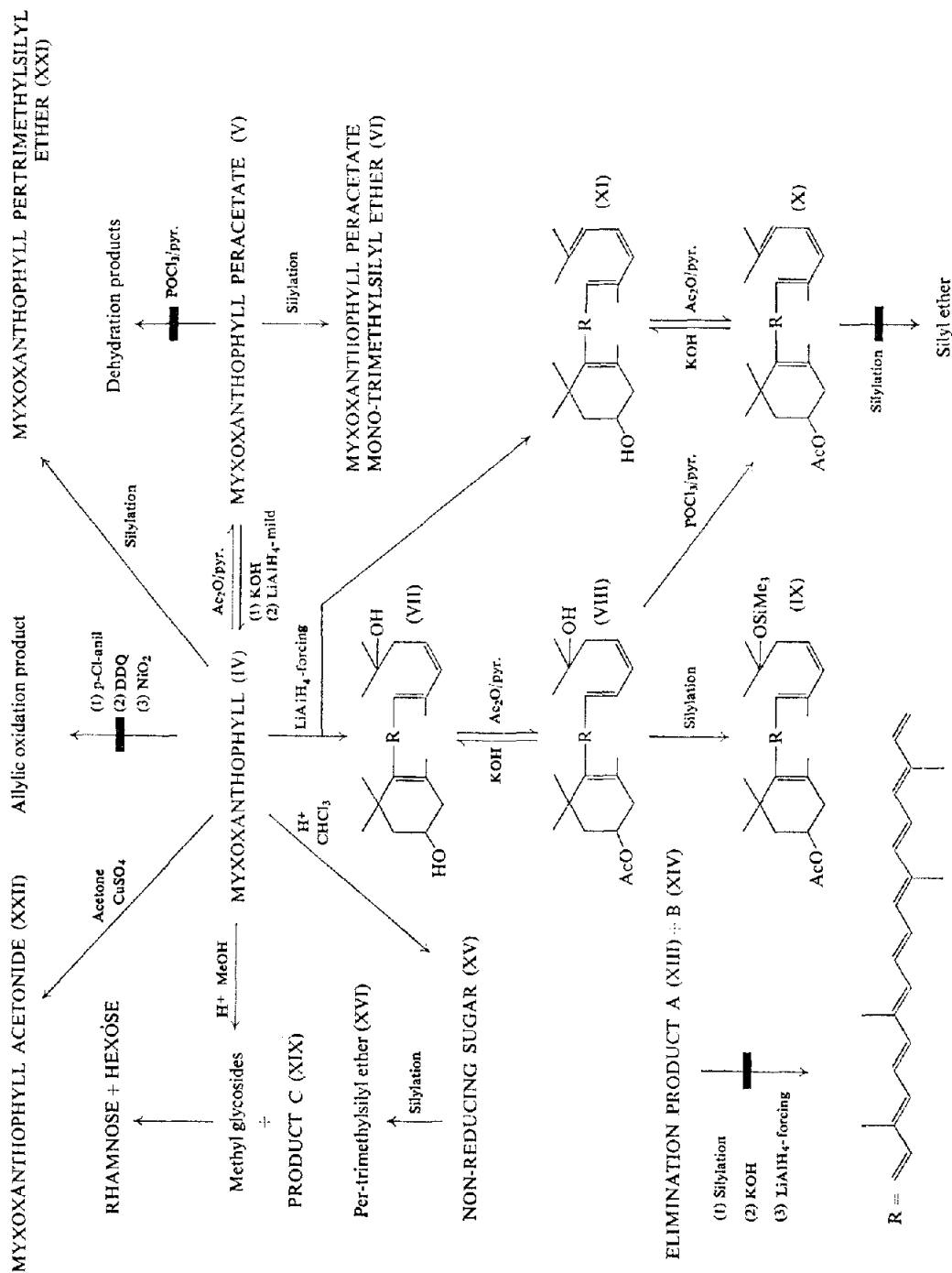


FIG. 2. THE COURSE OF ACETYLATION OF MYXOXANTHOPHYLL (IV).
For further description of intermediary acetates a-d see Table 6.



Myxoxanthophyll was resistant to mild hydride reduction, but treatment with excess LiAlH_4 gave in moderate yield two products. The more polar product was identified as saproxanthin (VII).¹⁹ Identification was based on stereomutation studies, partition behaviour, visible spectra and adsorptive properties of the reduction product (VII), and various properties of its acetate (VIII), the silyl ether (IX) of the acetate (VIII), the dehydration product (X) of the acetate (VIII) and its hydrolysis product (XI) (Fig. 3). The derivatives

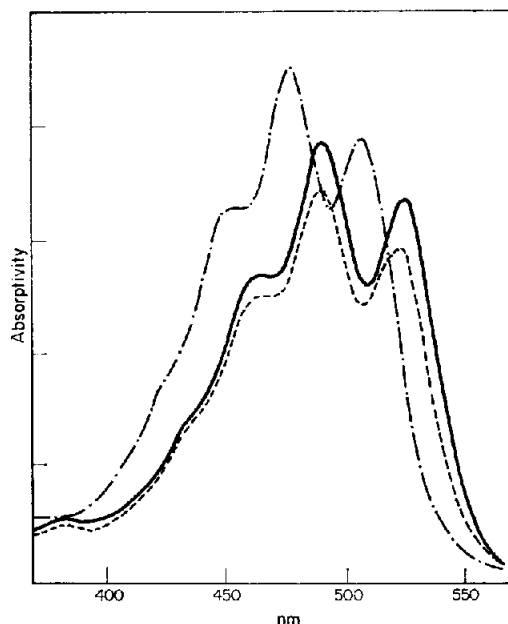


FIG. 3. ABSORPTION SPECTRA IN ACETONE OF VARIOUS DERIVATIVES OF MYXOXANTHOPHYLL (IV): — SAPROXANTHIN (VII), ---- 3',4'-DIDEHYDRORUBIXANTHIN (XI), —·— ELIMINATION PRODUCT A (XII).

VIII–XI of saproxanthin (VII) were available from a previous investigation.¹⁹ The possibility that the hydroxyl group in the β -ring is in the alternative 2-position in saproxanthin (VII), and consequently also in our reduction product, has been discussed elsewhere.¹⁹ Our product (VII) differed from synthetic 1',2'-dihydro-1',2'-dihydroxytorulene²⁰ (= plectanixanthin²¹). The second reduction product was identified as 3',4'-didehydrorubixanthin (XI), judged by absorption spectra (Fig. 3) and adsorptive properties of the reduction product (XI) itself and its acetate (X), as well as failure to form a trimethylsilyl ether of the acetate (X), as compared with the properties of corresponding derivatives of saproxanthin (VII).¹⁹ The course of the hydride reduction will be discussed elsewhere.²²

Effort was devoted towards the identification of the main elimination product A obtained on acid CHCl_3 treatment of myxoxanthophyll (IV). Its polarity was indicative of a hydro-

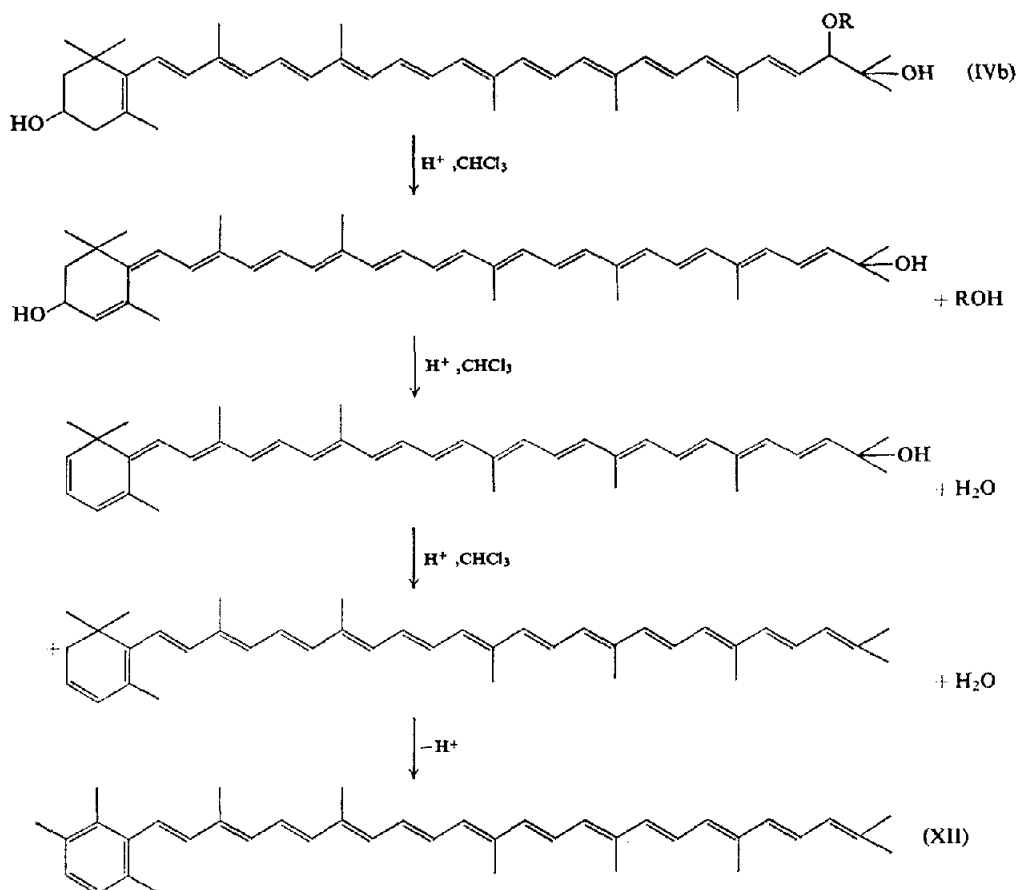
¹⁹ A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 811 (1966).

²⁰ U. SCHWIETER and O. ISLER, unpublished results.

²¹ N. ARPIN and S. LIAAEN-JENSEN, *Phytochem.* **6**, 995 (1967).

²² S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1281 (1969).

carbon, and the visible spectrum corresponded to that of torulene. However, torulene was chromatographically less strongly adsorbed. If structure IVb is accepted for myxoxanthophyll, then aromatization might occur according to the scheme below:



3',4'-Didehydrochlorobactene (XII) for comparison was prepared by treatment of chlorobactene²² with *N*-bromosuccinimide. The result of standard micro tests including stereomutation behaviour (see Table 1 and Fig. 3) supported this identification. However, the product could not be obtained in satisfactory purity and quantity. The i.r. spectrum (absorption at 800 cm^{-1} for two adjacent aromatic hydrogens; assignment dubious due to some silicon grease contaminant) and also the mass spectrum of a sample containing elimination products A (XIII) and B (XIV) lent some support to the aromatic character (peaks at 530 (M), M-92, M-106, *m/e* 133), but the latter did not reveal the corresponding hydrogen transfers in the fractionation pattern predicted on the basis of the mass spectra of chlorobactene and 3,4,3',4'-tetradehydrolycopene.²⁴

Similarities in adsorptive properties, chemical behaviour and i.r. spectra of myxoxanthophyll (IV, Fig. 4) and phleixanthophyll (II)¹³ suggested that IV was a glycoside. Whereas glucose (in unknown yield) was isolated on short treatment of phleixanthophyll (II) with

²³ S. LIAAEN-JENSEN, E. HEGGE and L. M. JACKMAN, *Acta Chem. Scand.* **18**, 1703 (1964).

²⁴ C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **23**, (1969), in press.

TABLE 1. PROPERTIES OF THE IODINE CATALYSED EQUILIBRIUM MIXTURES OF THE ELIMINATION PRODUCT (XIII) OF MYXOXANTHOPHYLL (IV) AND 3',4'-DIDYDROCHLOROBACTENE (XII)

Carotenoid	Member of the stereoisomeric set	R_f^*			λ_{max} in acetone (in nm)	% of total carotenoid
		S & S No. 288 paper 2% acetone†	S & S No. 287 paper 1% acetone			
Elimination product (XIII)	<i>Trans</i>	0.34	0.30		463	40
	Neo a	0.41			491	
	Neo b	0.46	0.42		(460) 483	35
	Neo c	0.57			(510)	
3',4'-Didydrochlorobactene (XII)	Neo d	0.63	0.63		380	25
					480	
	<i>Trans</i>	0.34	0.30		(465) 491	47
	Neo a	0.41			524	
	Neo b		0.42		(460) 483	30
	Neo c	0.57			510	
	Neo d	0.63	0.63		380 (458) 480	23
					(510)	

* Determined by co-chromatography.

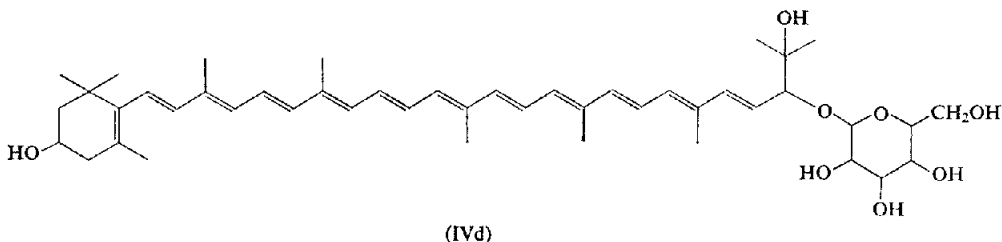
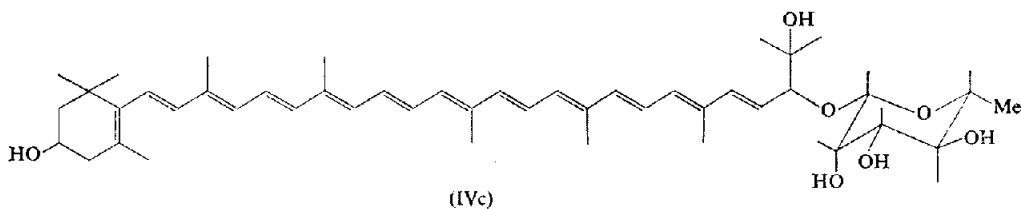
† In petroleum ether.

0.03 N HCl gas in CHCl_3 ,¹³ reducing sugars were not found in the aqueous hypophase following such treatment of myxoxanthophyll (IV). Paper chromatography disclosed the presence of a non-reducing sugar derivative (XV) with R_f (Table 5) in various systems corresponding to the methyl pentitols rhamnitols (XV) or fucitol, prepared by hydride reduction of the corresponding sugars. The per-trimethylsilyl ether (XVII) of the hydrolysis product was submitted to gas chromatography. No separation from rhamnitols penta-trimethylsilyl ether (XVIII) was obtained, whereas our silylated product was well separated from the per-trimethylsilyl ethers of fucitol, fucose and rhamnose (Table 6).

However, treatment of myxoxanthophyll under conditions for glycoside hydrolysis (0.15 N HCl in MeOH for 20 hr) resulted, after subsequent hydrolysis of the methyl glycosides formed, in the isolation of two reducing sugars by paper chromatography (Table 7). A major spot (estimated roughly to be 90 per cent of total) had R_f corresponding to rhamnose and a minor spot had the R_f of glucose.

The glycoside hydrolysis proceeded at a considerably lower rate than the HCl- CHCl_3 reaction, and the products differed. This result is not unexpected. Although the mechanism of the elimination reaction is not established, it is considered to involve bond cleavage of the allylic carotenoid-*O* (as ether or hydroxyl) bond,^{25, 26} whereas glycoside hydrolysis implies cleavage of the sugar-*O* bond,²⁷ subsequently referred to as the glycosidic linkage. However, in our case neither of the products in the elimination reaction is unequivocally identified, nor the ultimate carotenoid product in the glycoside hydrolysis, Product C (XIX) (the strong acid is expected to react with the aglycone).

The chemical evidence discussed favours structure IVb for myxoxanthophyll. The ease of elimination of the sugar moiety in the HCl- CHCl_3 reaction (cf. phleixanthophyll (II) *contra* 2'-didehydrophleixanthophyll¹¹), resistance towards dehydration with POCl_3 of the peracetate (V) and result of forcing hydride reduction all support an allylic ether substituent in 2'-position. Disregarding the fate of the sugar residue in the acid CHCl_3 treatment, the chemical data further suggested that myxoxanthophyll is a mixed glycoside of the rhamnoside IVc (drawn as β -L-rhamnoside although the stereochemistry is unknown) and IVd.



²⁵ L. ZECHMEISTER, *Fortschr. Chemie Org. Naturstoffe* **15**, 31 (1958).

²⁶ B. C. L. WEEDON, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), Ch. 3, Academic Press, London (1965).

²⁷ J. FERRIER, L. R. HATTON and W. G. OVEREND, *Carbohydr. Res.* **8**, 56 (1968).

Further spectroscopic data will now be considered. The i.r. spectrum of myxoxanthophyll (IV, Fig. 4) bears much resemblance to that of phleixanthophyll (II),¹³ but exhibits absorption at 1042 cm^{-1} for non-allylic, secondary hydroxy²⁸ and is lacking the bands at 1020 and 1100 cm^{-1} attributed to the 2'-hydroxy group of phleixanthophyll (II). Absorptions at 1140 and 905 cm^{-1} for tertiary hydroxyl²⁹ are seen particularly for the peracetate (V, Fig. 4). The i.r. spectrum further confirms the absence of carbonyl and aryl groups.

The NMR spectrum of myxoxanthophyll peracetate (V) is presented in Fig. 5 together with that of synthetic plectanixanthin (1',2'-dihydroxy-1',2'-dihydro-3',4'-didehydro- γ -carotene). The NMR spectrum confirms the structure assigned to the aglycone in IVc, d.

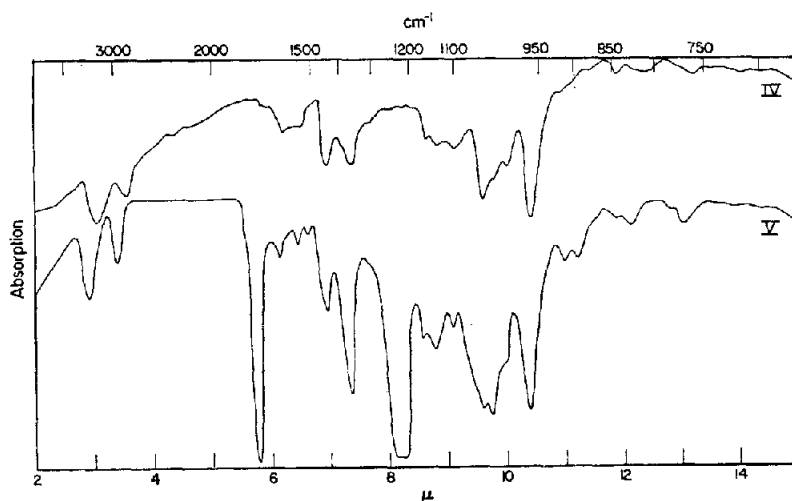


FIG. 4. I.R. SPECTRA OF MYXOXANTHOPHYLL (IV) AND MYXOXANTHOPHYLL PERACETATE (V) IN KBr.

The acetate (V) exhibited characteristic signals at 8.01 (in-chain methyl), 8.06 (in-chain/end-of-chain methyl) and at 8.77 and 8.82τ . The two latter signals are characteristic of geminal dimethyl groups at a tertiary carbon with a tertiary oxygen substituent and are ascribed to the $16', 17'$ -methyl groups. The different chemical shift of these geminal dimethyl groups support the presence of a substituent in 2'-position, since only one signal is observed in the case of rhodopin (100 Mc/sec)³⁰ and spirilloxanthin,³¹ whereas two signals are seen at 8.77 and 8.83τ in synthetic plectanixanthin. Magnetic non-equivalence of such geminal dimethyl groups appears to be a general result of molecular asymmetry,^{32, 33} caused by the asymmetric 2'-carbon.

The methyl groups attached to the β -ring of myxoxanthophyll peracetate (V) cause signals at 8.26 (3H), and 8.86 and 8.90τ in good agreement with the NMR spectrum of zeaxanthin diacetate which exhibited corresponding signals at 8.28 , 8.85 and 8.90τ .³¹ This

²⁸ C. BODEA, E. NICOARA, V. TAMAS and H. MANTSCH, *Ann.* **666**, 189 (1963).

²⁹ S. LIAAEN-JENSEN, *Kgl. Norske Videnskabers Selskabs Skr.* No. 8 (1962).

³⁰ L. RYVARDEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **18**, 643 (1964).

³¹ L. M. JACKMAN, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 110, Pergamon, Oxford (1962).

³² N. S. BOWMAN, D. E. RICE and B. R. SWITZER, *J. Am. Chem. Soc.* **87**, 4477 (1965).

³³ M. L. MARTIN and G. J. MARTIN, *Bull. Soc. Chim. Fr.* 2117 (1966).

is taken to support the presence of a 3-acetoxy substituent in the β -ring, cf. Ref. 34. The signals at 8.72 and 9.1 τ are caused by a contaminant derived from reagents used in the isolation process. A detailed comparison of the olefinic region (3-ca. 4.5 τ) in the spectra of IV and XII favours identical polyene systems in the two compounds. The resonance for the 2-methylene group is seen in the 8.5 τ region. The expected doublet for the 4-methylene in the

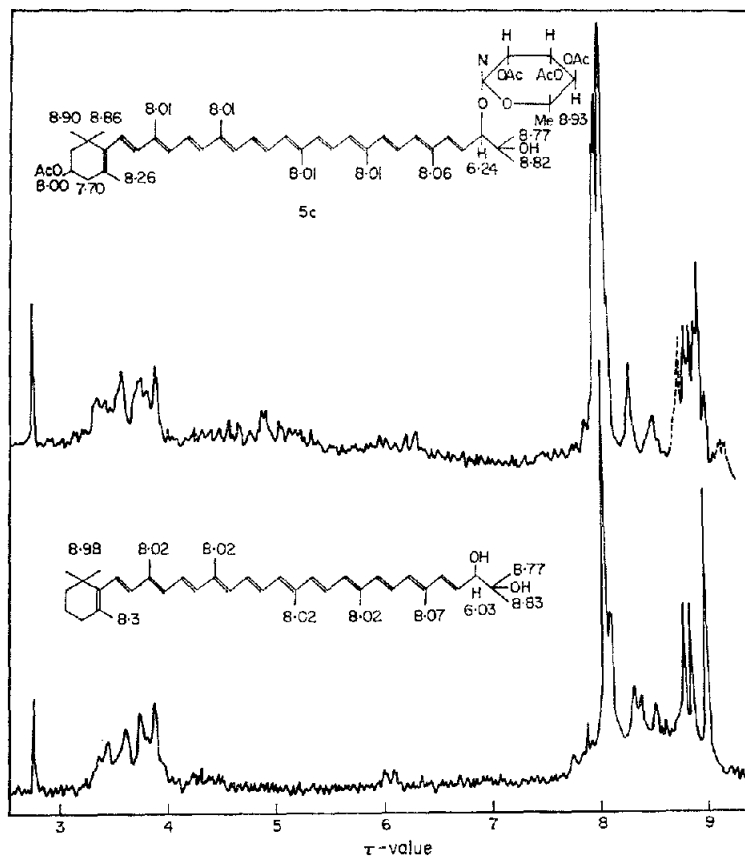


FIG. 5. PROTON MAGNETIC RESONANCE SPECTRA IN CDCl_3 AT 100 Mc/sec OF MYXOXANTHOPHYLL PERACETATE (V) AND SYNTHETIC PLECTANIXANTHIN.

β -ring at ca. 7.7 τ ($J=7$ cps)^{34,35} appears here as a double doublet at 7.70 τ . The multiplet expected below 6.1 τ , cf. Ref. 34 for the methine proton at C_3 cannot be distinguished. A doublet at 6.02 τ ($J=8$ cps) in the synthetic diol is attributed to the allylic 2'-methine proton, and a corresponding doublet is found for myxoxanthophyll at 6.24 τ ($J=8$ cps).

The integral of the 7.90–8.10 τ complex corresponds to 27 protons and hence by difference indicates the presence of four acetate groups in IV. Since the acetoxy methyl group of zeaxanthin caused a signal at 8.00 τ ,³⁴ the remaining acetoxy signals at 7.94, 7.96 and 7.98 τ

³⁴ N. ARPIN and S. LIAAEN-JENSEN, *Phytochem.* 7, 839 (1968).

³⁵ A. K. MALLAMS, E. S. WRIGHT, B. C. L. WEEDON, D. J. CHAPMAN, F. T. HAXO, T. W. GOODWIN and D. M. THOMAS, *Chem. Commun.* 301 (1967).

are ascribed to three acetoxy groups in the sugar moiety. For triacetoxy- β -methylrhamnoside the acetoxy signals were located at 7.83, 7.97 and 8.01 τ .³⁶ The methyl group in the rhamnoside residue of IVc is seen as a doublet at 8.93 τ ($J=7$ cps), the 8.90 τ signal being superimposed on that of one of the geminal dimethyl groups of the β -ring; Bohlmann *et al.*³⁷ state 8.86 τ ($J=6.4$ cps) and Hemmer *et al.*³⁶ 8.72 τ ($J=6.8$ cps) for other acetylated rhamnosides. The remaining signals in the 4.5–6.2 τ region are ascribed to the ring protons in the rhamnoside. The spectrum does not allow a detailed analysis in this region, although there is gross similarity with that of triacetoxy- β -L-methylrhamnoside.³⁶

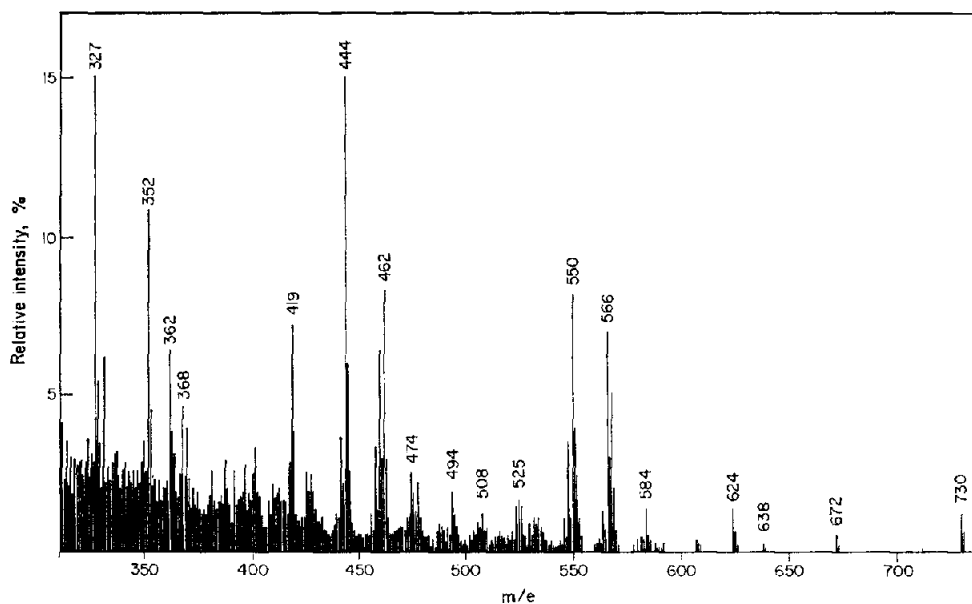


FIG. 6. MASS SPECTRUM OF MYXOXANTHOPHYLL.

In summary the NMR spectrum of myxoxanthophyll peracetate (V) supports a tetra-acetate formulation and its main features appear to be in satisfactory agreement with structure IVc for myxoxanthophyll.

The absence of significant resonances in the 5.7 τ region, expected for the $>\text{CH}-\text{CH}_2\text{OAc}$ group in IVd³⁸ and the intensity of the methyl doublet of the rhamnoside moiety relative to that of the 18-methyl group of the β -ring, does not reveal detectable amounts of IVd in the sample examined.

The mass spectra of myxoxanthophyll (IV) and myxoxanthophyll peracetate (V) are given in Figs. 6 and 7. The diagnostically important fragmentations on electron impact are indicated below.

As expected from previous experience²⁴ the mass spectra of IV and V are dominated by losses from the polyene chain and the aliphatic end-group.

³⁶ E. HEMMER and S. LIAAEN-JENSEN, to be published.

³⁷ F. BOHLMANN, K.-M. RODE and E. WALDAU, *Chem. Ber.* 1915 (1967).

³⁸ T. D. INCH, J. R. PLIMMER and H. G. FLETCHER, *J. Am. Chem. Soc.* 31, 1825 (1966).

Myxoxanthophyll has a low intensity molecular ion peak at m/e 730-4852 ($C_{46}H_{66}O_7$) in agreement with structure IVc. The characteristic losses of 92 and 106 mass units from the polyene chain are observed.^{24, 39, 40} Loss of 58 mass units (acetone), in agreement with the fragmentation previously observed for plectanixanthin,²⁴ confirms the formulation of the aliphatic end-group. A peak at m/e 566-4114 ($C_{40}H_{54}O_2$) corresponding to a loss of $C_6H_{12}O_5$ demonstrates cleavage of the carotenoid ether bond with hydrogen transfer to the sugar moiety, whereas a peak at (M-146) corresponds to cleavage of the glycosidic linkage with hydrogen transfer in the opposite direction. A peak at M-236 is interpreted as caused by

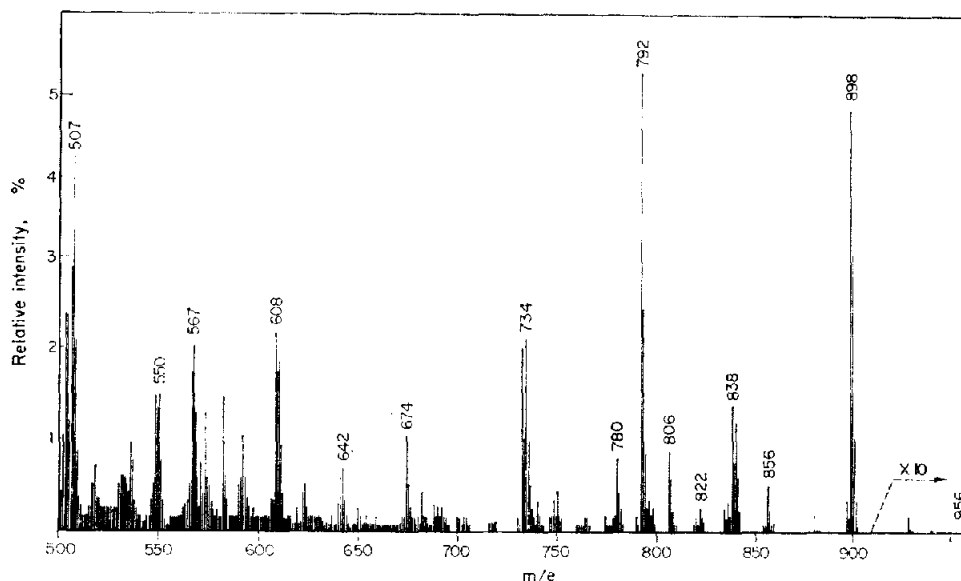


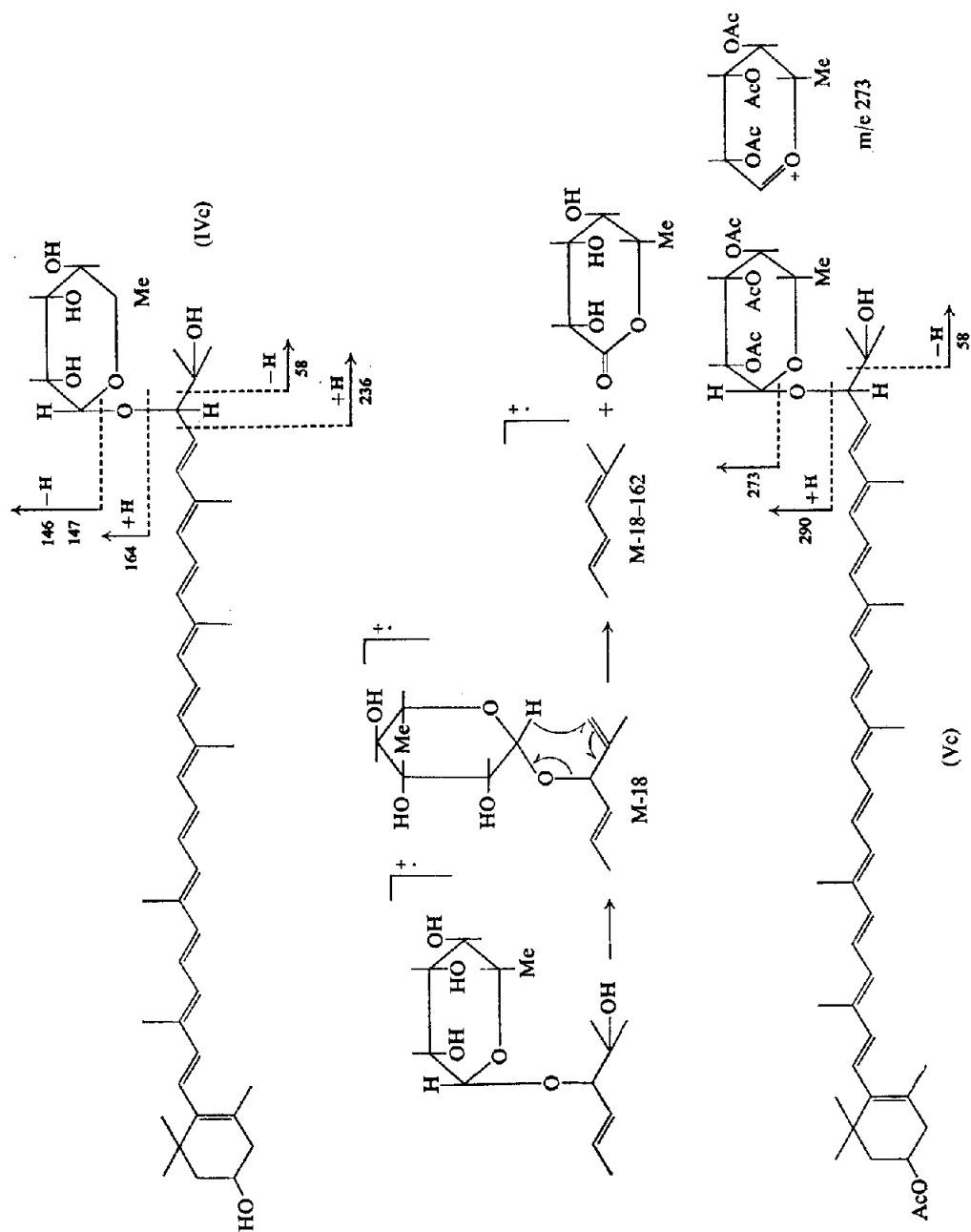
FIG. 7. MASS SPECTRUM OF MYXOXANTHOPHYLL PERACETATE.

The spectrum was not recorded below m/e 110. Intensities are given relative to m/e 153.

cleavage α to the ether linkage, again with hydrogen transfer. A peak at m/e 550-4164 ($C_{40}H_{54}O$) corresponding to the loss of $C_6H_{12}O_6$ from the molecular ion may be considered as due to a two-step loss of H_2O and $C_6H_{10}O_5$, in the latter case accompanied by hydrogen transfer to the carotenoid ion. Weak peaks for M-18, M-36 and M- H_2O - C_8H_{10} (m/e 606-3917; $C_{38}H_{56}O_6$) are also observed. Multiple losses involving 18 (water), 58 (acetone), 92 (toluene) and 106 (xylene) mass units and various sugar fragments are also seen (M-254 = M-236-18 (from the β -ring), M-256 = M-164-92, M-270 = M-164-106 M-286 = M-18-162-106). When cleavage of the glycosidic linkage is preceded by loss of acetone, it occurs without hydrogen transfer, peaks at m/e 525 (M-58-147), m/e 419 (M-58-147-106) and m/e 327 (M-58-147-106-92). In all fragmentations discussed for myxoxanthophyll the charge is retained on the carotenoid residue.

³⁹ U. SCHWIETER, H. R. BOLLGER, L. H. CHOPARD-DIT-JEAN, G. ENGLERT, M. KOFLER, A. KÖNIG, C. PLANTA, R. RÜEGG, W. VETTER and O. ISLER, *Chimia* **19**, 294 (1965).

⁴⁰ C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **22**, 1054 (1968).



The mass spectrum of myxoxanthophyll peracetate is dominated by peaks caused by the rhamnoside Vc, but also demonstrates the presence of Vd, the acetylated hexose glycoside.

Myxoxanthophyll tetraacetate (Vc) shows a strong molecular ion peak at m/e 898·5221 ($C_{54}H_{74}O_{11}$). The assignment is supported by losses of toluene (M-92, m/e 806), xylene (M-106, m/e 792·4465 = $C_{46}H_{64}O_{11}$) and acetone (M-58, m/e 840; metastable peak at m/e 786) and multiple losses (m/e 734 = (M-106)-58; metastable peak at m/e 680, and m/e 642 = M-106-58-92). Losses of 42 (ketene) and 60 (acetic acid) mass units from the molecular ion and peaks at m/e 780 = (M-58)-60 (metastable peak at m/e 724), m/e 732 = M-106-60 and m/e 674 = M-106-58-60, confirm the presence of acetoxy group(s). As with the free pentaol (IVc) cleavage of the glycosidic linkage is observed combined with losses of acetone: m/e 567 = M-58-273, m/e 507 = M-58-273-60 (acetic acid from the second end group), m/e 461 = M-58-273-106, m/e 401 = M-273-106-60 (acetic acid from the second end group). Corresponding cleavage of the ether linkage (with hydrogen transfer) is demonstrated by peaks at m/e 608 (M-290), m/e 550 (M-290-58), m/e 548 (M-290-60) (acetic acid from the second end-group) and m/e 502 (M-290-106). The data given above further support the location of one acetoxy group in the aglycone. A strong peak at m/e 273 reveals that on cleavage of the glycosidic linkage the charge is mainly retained on the sugar moiety. This is in agreement with previous findings^{41, 42} that sugar acetates undergo primary fragmentation with formation of oxonium ions.

Evidence for the presence of the pentaacetate of IVd in myxoxanthophyll peracetate will now be considered. A very low intensity peak at m/e 956 (corresponding to $C_{48}H_{68}O_9$) is ascribed to the molecular ion. The assignment is supported by peaks at m/e 864 (M-92), m/e 850 (M-106) and m/e 822 (M-42-92). A stronger peak at m/e 331 for the tetraacetoxy-hexose oxonium ion further confirms the hexose component of IVc. The apparently higher relative stability of the molecular ions of the peracetates than of the free alcohols explains why the hexose component is more readily revealed in the spectrum of the peracetate. However, a very weak peak is also found at m/e 746 for myxoxanthophyll (IV) itself corresponding to the molecular ion of IVd ($C_{46}H_{66}O_8$). Judged by the relative intensities of (i) the m/e 956 and 898 peaks and (ii) the m/e 331 and 273 peaks in myxoxanthophyll peracetate (Vc + Vd) originating from two entirely different isolations, a 2–10 per cent presence of IVd in myxoxanthophyll is indicated.

In conclusion, the spectroscopic evidence supports the formulation of myxoxanthophyll as a mixed glycoside (IVc, d) in which rhamnose is the dominant sugar component (ca. 90 per cent) and a hexose the minor component.

EXPERIMENTAL

Materials and Methods

When not stated to the contrary these were as recently summarized.⁴³ Raman spectra were recorded on a Cary Model 18 spectrometer. Gas chromatography was carried out on an Aerograph HyFy A-600-B gas chromatograph. Paper chromatography of carotenoids was partly performed on Schleicher & Schüll No. 597 paper. Pigment recoveries were spectrophotometrically determined using $E_{1\%}^{1\text{cm}} = 2000$ at λ_{max} when not otherwise stated. Dry peroxide-free tetrahydrofuran was prepared by filtration through an alumina (highly activated) column followed by distillation over $LiAlH_4$. Adsorptive properties of the carotenoids described are given in Table 2.

⁴¹ K. BIEMANN, D. C. DEJONGH and H. K. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).

⁴² I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 837 (1968).

⁴³ A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 1970 (1966).

TABLE 2. CHROMATOGRAPHIC PROPERTIES OF VARIOUS DERIVATIVES OF MYXOXANTHOPHYLL (IV)

Carotenoid	Required eluent from		R_f				
			Cellulose column		Alumina activity grade 2		
					1%*	2%* 5%* 7%* 10%* 20%* 30%†	S & S No. 287 paper S & S No. 288 (2%)*
Myxoxanthophyll per-trimethylsilyl ether (XXI)							597 (30%)*
Elimination product (XIII)							288 (2%)*
Saproxanthin acetate trimethylsilyl ether (IX)	1% acetone-petrol ether	10% ether-petrol ether	0.30				
3',4'-Dehydrorubixanthin acetate (X)		10-20% ether-petrol ether		0.53	0.55		
Acetylated hydrolysis product (XX)						0.70	
Myxoxanthophyll peracetate mono-trimethylsilyl ether (VI)					0.42		0.82
Saproxanthin acetate (VIII)					0.32		0.73
Hydrolysis product (XIX)		20% acetone-petrol ether				0.38	0.60
3',4'-Didehydrorubixanthin (XI)							0.57
Myxoxanthophyll peracetate (V)							0.50
Saproxanthin (VII)		0.5-1% methanol-ether					0.42
Myxoxanthophyll acetoneide (XXII)							0.83
Myxoxanthophyll (IV)	25-30% acetone-petrol ether						0.67
						0.52	0.71

* Acetone in petroleum ether.

† Acetone in benzene.

Biological Material

An *Arthrospira* sp., collected from Lake Aranguadi, near Debre Zeit, Ethiopia, was used.

Pigment Extraction

The dried algae (254 g) was extracted at room temperature with acetone-methanol (15 l., 7:3). The extract was concentrated to 3 l., and the carotenoids transferred to ether after addition of 5% aqueous NaCl. The ether extract was concentrated to dryness, and the carotenoids partitioned between petroleum ether-85% aqueous methanol (4 l.), cf. Ref. 5. The hypophasic carotenoids comprised 270 mg or 0.11 per cent of the dried algae. From two separate isolations some 100 mg of hypophasic carotenoids were isolated.

Chromatography

The hypophasic carotenoid mixture was chromatographed on a cellulose column. Myxoxanthophyll (IV, ca. 80 per cent of total) was not completely separated from oscillaxanthin (ca. 20 per cent of total). Mixed fractions were acetylated and the peracetates separated by rapid chromatography on deactivated alumina. Separation of IV and oscillaxanthin was also achieved on columns of magnesium silicate. Myxoxanthophyll (IV) was not effected by alkali treatment (see below), and was in most cases treated with 5% methanolic KOH-solution prior to rechromatography on cellulose.

Myxoxanthophyll (IV)

IV was obtained as red needles, forming aggregates, from acetone-petroleum ether; yield ca. 70 mg, m.p. 173°. Karrer and Rutschmann⁷ give m.p. 182° and Heilbron and Lythgoe⁶ m.p. 169-170°. It was insoluble in petroleum ether and exhibited increasing solubility properties in benzene, ether, acetone, methanol, chloroform and pyridine. Its adsorptive properties are given in Table 2. For elution from magnesium silicate columns it required 15% methanol in acetone. It has λ_{\max} in acetone at (450), 478 and 510 nm, % III/II²⁹ = 57, % D_B/D_H²⁹ = 12 (see Fig. 1); in CHCl₃ at (460), 488 and 522 nm, % III/II = 50, % D_B/D_H = 11; in benzene at 462, 488 and 522 nm, % III/II = 51, % D_B/D_H = 16, and in pyridine at (465), 493 and 528 nm, % III/II = 50, % D_B/D_H = 11. In acetone $E_{1\%}^{1\text{cm}}$ = 2160 (ϵ = 158000) at λ_{\max} compared with ϵ = 174900 for β -apo-2'-carotenyl acetate.⁴⁴ I.r. spectrum of IV in KBr is given in Fig. 4; characteristic absorptions were at 3280, 2950, 1610, 1440, 1370, 1355, 1155, 1135, 1092, 1030, 1000, 962, and 840 cm⁻¹. The NMR spectrum (10 mg) in CDCl₃ (0.2 ml) at 60 Mc/sec showed characteristic signals at 8.93 (sharp, *gem.* methyl in β -ring), 8.83 (broad, *gem.* methyl at *tert.* hydroxyl), 8.28 (sharp, 18-methyl in β -ring), and 8.05 τ (sharp, in-chain methyl). Solid IV exhibited weak Raman bands at 1515 and 1155 cm⁻¹, imposed on a broad band with maximum at 750 cm⁻¹. The mass spectrum (Fig. 6) showed prominent peaks at *m/e* 730.4852 \pm 4.4 m.m.u. (calc. for M = C₄₆H₆₆O₇ 730.4808), 712 (M-18), 694 (M-36), 672 (M-58), 638 (M-92), 624.4011 \pm 1.5 m.m.u. (calc. for C₃₈H₅₆O₇ 624.4026), 608 (M-122), 606.3917 \pm 0.3 m.m.u. (calc. for C₃₈H₅₄O₆ 606.3920), 584 (M-146), 566.4114 \pm 1.0 m.m.u. (calc. for C₄₀H₅₄O₂ 566.4124), 550.4164 \pm 1.1 m.m.u. (calc. for C₄₀H₅₂O 550.4175), 525 (M-205), 508 (M-222), 494 (M-236), 478 (M-252), 476 (M-254), 474 (M-256), 462 (M-268), 460 (M-270), 444 (M-286), 419 (4-311) and 327 (M-403).

IV was entirely hypophasic in partition between petroleum ether and 60% aqueous methanol. The iodine catalysed equilibrium mixture of IV in benzene had abs. max. at 373, 460, 485 and 519 nm, % III/II = 30, % D_B/D_H = 25. No distinct separation of *cis-trans* isomers was achieved by paper chromatography. IV was recovered unchanged on treatment with 5% KOH for 2 hr in ether-methanol. IV was unaffected on treatment under standard conditions with (a) acetone and anhydrous CuSO₄⁴⁵ (b) *p*-chloranil in benzene-ethanol¹⁴ in the presence of I₂ and (c) 2,3-dichloro-5,6-dicyanobenzoquinone.⁴⁶

IV (0.5 mg) in ether-benzene (4 ml, 1:1) was completely bleached within 5 min when treated with nickel peroxide¹⁶ (5 mg; available oxygen 2.41 \cdot 10⁻³ g atom/g nickel peroxide determined by titration). IV (0.5 mg) was silylated in the usual manner. The final product (XXI) exhibited the same absorption spectrum in visible light as IV; for R_f see Table 2.

Myxoxanthophyll Peracetate (V)

The course of acetylation of IV (1 mg) in dry pyridine (4 ml) with Ac₂O (0.2 ml) was followed by paper chromatography, and by spectrophotometric determination of the products eluted from the paper chromatograms (Fig. 2 and Table 3). The peracetate (V) was prepared by similar acetylation of IV (20 mg) for 22 hr; pigment recovery was 70 per cent. Further purification of V was achieved by rapid chromatography on deactivated alumina (Table 2). V crystallized as red needles from acetone-petroleum ether, yield ca. 10 mg, m.p. 126-130°. Karrer and Rutschmann⁷ reported m.p. 131-132°. Its adsorptive properties are given in

⁴⁴ R. RÜEGG, M. MONTAVON, G. RYSER, G. SAUCY, U. SCHWIETER and O. ISLER, *Helv. Chim. Acta* **42**, 854 (1959).

⁴⁵ W. H. TALLENT, *J. Org. Chem.* **29**, 2756 (1964).

⁴⁶ A. P. LEFTWICK and B. C. L. WEEDON, *Acta Chem. Scand.* **20**, 1195 (1966).

Table 2. It was readily soluble in CHCl_3 and ether. Its λ_{max} in acetone corresponded completely to that of IV, $E_1^{1\%} = 1680$ ($\epsilon = 158000$) at 478 nm (cf. Fig. 1). The i.r. spectrum in KBr pellet (0.36 mg) is given in Fig. 4. Characteristic absorptions were at 3450, 2980, 1725, 1628, 1550, 1490, 1355, 1225, 1215, 1155, 1138, 1098, 1042, 1026, 962, 911, 892 and 822 cm^{-1} .

The NMR-spectrum of V (4 mg) in CDCl_3 is presented in Fig. 5, signals being at 3.33, 3.38, 3.54, 3.71 3.76, 3.86, (3.90), 4.00, 4.12, 4.20, 4.36, 4.43 (ca. 15 H); 5.96, 6.03 (ca. 1 H); 6.20, 6.28 (ca. 1 H); 7.70 (double doublet); 7.94, 7.96, 7.98, 8.01, 8.06 (ca. 27 H); 8.26 (ca. 3 H); 8.72 (impurity), 8.77, 8.82, 8.86, 8.90 and 8.96 τ . For comparison the spectrum of synthetic plectanixanthin²⁰ was recorded (Fig. 5); signals were at 3.40, 3.56, 3.70, (3.76), 3.85, 3.90, 4.18, 4.26, 4.34, 4.42 (ca. 16 H); 6.03 (ca. 1 H, doublet $J = 8$ cps); 8.02, 8.07 (ca. 15 H); 8.30, 8.37 (ca. 3 H); 8.77, 8.83 (ca. 6 H) and 8.98 (ca. 6 H) τ .

The Raman spectrum of V (1 mg) showed weak absorption at 1000, 1150 and 1515 cm^{-1} and broad intense band at 950 cm^{-1} .

The mass spectrum is given in Fig. 7. In the higher mass region the following peaks were ascribed to Vc: m/e 898-5221 ± 1.0 m.m.u. (calc. for $\text{C}_{54}\text{H}_{74}\text{O}_{11}$ 898-5321), 856 (M-42), 840 (M-58), 838 (M-60), 806 (M-92), 792-4463 ± 1.7 m.m.u. (calc. for $\text{C}_{46}\text{H}_{64}\text{O}_{11}$ 792-4448), 780 (M-118), 734 (M-164), 732 (M-166), 674 (M-224), 642 (M-256), 608 (M-290), 567 (M-331), 550 (M-340), 548 (M-350), 507 (M-391), 503 (M-396), 461 (M-437),

TABLE 3. INTERMEDIATES IN THE ACETYLATION OF MYXOXANTHOPHYLL (IV) TO ITS PERACETATE (V)

Acetylated product	R_f kieselguhr paper*		Partition ratio		Tentative assignment based on IVc, Vc
	20% acetone†	10% acetone	Petrol. ether/70% CH_3OH	Petrol. ether/85% CH_3OH	
a	0.19		0:100		} Monoacetates
b	0.36		10:90		
c	0.57	0.12		2:98	Diacetates
d	0.77	0.28		24:76	Triacetates
V	0.92	0.50		84:16	Tetraacetate

* Schleicher & Schüll No. 287.

† In petroleum ether.

401 (M-497) 273 and 213 (273-60). Metastable peaks were observed for m/e 898 \rightarrow 840 at 786, m/e 792 \rightarrow 734 at 680 and m/e 840 \rightarrow 780 at 724. These peaks were ascribed to Vd: m/e 956 (M), 914 (M-42), 864 (M-92), 856 (M-100), 850 (M-106), 822 (M-134) and 331. Peaks at m/e 940, 928, 633 and 610 were not identified.

In petroleum ether/95% methanol the partition ratio of V was 22:78. V (ca. 0.2 mg) in ether (5 ml) and glacial acetic acid (0.2 ml) remained unaltered after 18 hr (no 5,6-epoxide group). Formic acid (10 drops) was added and the mixture kept for 2 days; no furanoid rearrangement occurred. V (1 mg) was silylated in the usual manner, pigment recovery was 85 per cent. The trimethyl silyl ether (VI) had the same absorption spectrum as V, for R_f see Table 2. V (0.13 mg) in dry pyridine (5 ml) was treated with POCl_3 (0.05 ml) at 50° for 1½ hr; pigment recovery was 58 per cent but no product less polar than V were obtained. On treatment with alkali or with LiAlH_4 in tetrahydrofuran for 5 min, V was converted completely to IV.

Reduction of Myxoxanthophyll Peracetate with Excess LiAlH_4

In two separate experiments V (2 and 8 mg) in dry tetrahydrofuran (15 and 60 ml) was treated with LiAlH_4 (2 and 20 mg) for 1½ hr, resulting in pigment recoveries of 58 and 25 per cent. Saproxanthin (VII) constituted 66 and 86 per cent respectively of the recovered carotenoid, which had λ_{max} at (365), 453, 478, and 508 nm in acetone (Fig. 3). The partition ratio of VII in petroleum ether/85% methanol was 22:78; previously reported for saproxanthin 24:76,¹⁹ and for plectanixanthin (XII) 90:10.²¹ The product was inseparable when co-chromatographed (kieselguhr paper or aluminium oxide paper) with authentic saproxanthin,¹⁹ whereas separation from synthetic plectanixanthin was achieved on aluminium oxide paper (see Table 2). The result of iodine catalysed isomerization of the product and authentic saproxanthin (VII) is given in Table 4. The reduction product (VII) gave an acetate (VIII) which was inseparable from authentic saproxanthin acetate on kieselguhr paper. The acetate (VIII) of the reduction product was saponified to saproxanthin (VII), and in a separate experiment converted to its trimethylsilyl ether (IX). The saproxanthin acetate (VIII, 0.15 mg) derived from myxoxanthophyll was dehydrated with POCl_3 in pyridine with a pigment recovery of 75 per cent.

The reaction mixture contained 65 per cent of a product, 3',4'-didehydrorubixanthin acetate (IX), further converted by saponification in 5% methanolic KOH to 3',4'-didehydrorubixanthin (XI). Authentic saproxanthin acetate (VIII) was treated in like manner, and the 3',4'-didehydrorubixanthin (XI) thus prepared was spectrophotometrically and chromatographically (Table 2) indistinguishable from XI derived from myxoxanthophyll.

The second product obtained on vigorous hydride reduction of myxoxanthophyll peracetate, obtained in 14 and 34 per cent yield respectively of the recovered carotenoid, was identified as 3',4'-didehydrorubixanthin (XI). Absorption maxima were at 460, 487, and 518 nm in acetone (see Fig. 3) as for torulene, recorded for comparison. No separation of this product and authentic 3',4'-didehydrorubixanthin (XI), derived from saproxanthin¹⁹ was obtained either on kieselguhr or aluminium oxide paper. The product (XI, 0.01 mg) was acetylated; pigment recovery was 80 per cent. The resulting acetate (X) was inseparable from X derived from saproxanthin above. Silylation of the acetate derived from myxoxanthophyll appeared to be negative.

Treatment of Myxoxanthophyll with Acidified CHCl₃

The reaction⁴⁷ was carried out in ethanol-free CHCl₃ containing dry HCl gas, using a final acid concentration of 0.03 N. Seven experiments employing IV (1–10 mg) gave corresponding results and in general a pigment recovery of ca. 60 per cent. A bathochromic colour change occurred immediately, and the reaction was interrupted in the usual manner after 5–15 min by distribution between ether and water. The epiphasic carotenoid solution was washed with aqueous NaHCO₃ solution and the carotenoids submitted to chromatography on deactivated alumina.

Elimination product A (XIII) was the exclusive or major product: for adsorptive properties see Table 2. This product (XIII) had λ_{\max} in acetone at 463, 491 and 524 nm, % III/II = 58 and % D_B/D_{II} = 14 (see Fig. 3). In one of the larger-scale preparations XIII was obtained mixed with a white contaminant as red flakes, m.p. unsharp 170°, from ether-methanol. Separation from the white impurity was not achieved by several recrystallizations, treatment with alkali or with Ac₂O in pyridine, or by rechromatography on deactivated alumina and calcium hydroxide. The i.r. spectrum in KBr resembled that of chlorobactene,²³ but revealed some silicone grease contaminant. From a second preparation crystalline XIII, m.p. 155–160°, was obtained in ca. 9:1 mixture with XIV (see below) after purification on a cellulose column. The mass spectrum of this sample exhibited peaks in the higher mass region at 568 (M₁), 532 (M₂?), 530 (M₃?), 476 (M₁-92), 462 (M₁-106), 438 (M₃-92), 424 (M₃-106), 412, 410 (M₁-158), 399 (M₃-131), 396 (M₃-134), 383 (M₃-147), 372 (M₃-158), 368, 357 (M₃-173), 344 (M₃-186), 331 (M₃-199), 317 (M₃-213), 363, 289 (M₃-241) and a strong peak at *m/e* 133 (60 per cent of base peak). XIII was unaffected by silylation under standard conditions or by treatment in dry ether with LiAlH₄ for 6 hr. The absorption spectrum in visible light was in complete agreement with that of torulene, recorded for comparison. However, torulene (*R_f* 0.44 on kieselguhr paper; 1% acetone-petroleum ether) was less polar. 3,4-Didehydrotorulene, obtained from phleixanthophyll (II)¹³ (abs. max. (470), 493, and 523 nm in acetone) exhibited less spectral fine structure and was slightly less strongly adsorbed (*R_f* 0.40 on kieselguhr paper; 1% acetone in petroleum ether). 3',4'-Didehydrochlorobactene (XII) was prepared for comparison by treatment of chlorobactene²³ (11.2 mg) with *N*-bromosuccinimide (3.3 mg) in CCl₄ (6 ml) for 24 hr;⁴⁶ pigment recovery was 48 per cent. The reaction mixture contained XII (8 per cent), further purified by alkali treatment, rechromatography and paper chromatography. The result of separate iodine catalysed isomerization in petroleum ether of *trans* XIII and *trans* XII is presented in Table 1.

In three of the larger-scale experiments small amounts of Product B (XIV), slightly more polar (*R_f* 0.24 on aluminium oxide paper; 2% acetone in petroleum ether) than XIII was isolated. This product was not separated from XIII on alumina or cellulose columns, and did not belong to the XIII stereoisomeric set, as shown by iodine catalysed isomerization. The minor product had abs. max. at 490 and (520) nm in acetone and exhibited little spectral fine-structure.

The aqueous hypophase after acid treatment of IV was neutralized to pH 7 by 0.1 N NaOH or silver carbonate. The extract was concentrated to dryness *in vacuo*, the residue treated with warm ethanol and filtered. An aliquot of the ethanol extract gave no typical Molisch test on treatment with α -naphthol-ethanol-sulphuric acid.⁴⁸ Paper and thin-layer chromatography in the systems listed in Table 5 gave no evidence for the presence of reducing sugars; the *R_f* value of the hydrolysis product corresponded to that of rhamnitol (XVI) and fucitol.

Rhamnitol (XVI) and fucitol for comparison were prepared by NaBH₄ reduction of the corresponding methylpentoses according to Abdel-Akker *et al.*⁵² When tested in System 2 (Table 5) together with rhamnose,

⁴⁷ R. ENTSCHER and P. KARRER, *Helv. Chim. Acta* **41**, 402 (1958).

⁴⁸ M. E. EVERETT, *Medical Biochemistry*, 2nd edition, p. 277, Hoeber, New York (1948).

⁴⁹ J. S. FISCHER and E. J. PERCTVAL, *J. Chem. Soc.* 266 (1957).

⁵⁰ E. STAHL, *Dünnschicht-Chromatographie*, Springer, Berlin (1962).

⁵¹ J. A. CIFORELLI and F. SMITH, *Anal. Chem.* **26**, 1137 (1954).

⁵² M. ABDEL-AKKER, J. K. HAMILTON and F. SMITH, *J. Am. Chem. Soc.* **73**, 4691 (1951).

TABLE 4. COMPOSITION OF THE IODINE CATALYSED EQUILIBRIUM MIXTURE OF AUTHENTIC SAPROXANTHIN (VII) AND SAPROXANTHIN (VII) DERIVED FROM MYXOXANTHOPHYLL (IV)

Origin	Member of the stereo-isomeric set	R_f^* Schleicher & Schüll No. 287 paper		λ_{\max} in acetone (in nm)		% of total carotenoid
		10% acetone†	20% acetone			
Authentic ¹⁹	<i>Trans</i>	0.42	0.70	(350)	473	68
	Neo A	0.48	0.77	(350)	466	32
Myxoxanthophyll (IV)	<i>Trans</i>	0.42	0.70	(350)	474	71
	Neo A	0.48	0.77	(350)	468	29

* Determined by co-chromatography.

† In petroleum ether.

the aniline-perphthalic acid reagent⁵⁰ gave a brown spot for rhamnose only, whereas the NaIO₄-benzidine reagent caused white spots at identical *R_f* for rhamnose and rhamnitrol.

The hydrolysis product (XV) was further examined by gas chromatography of its per-trimethylsilyl ether (XVII). For reference purposes various trimethylsilyl ethers were prepared according to Hedgley and Overend⁵³ and Sweeley *et al.*⁵⁴ In Experiment 1 the crude hydrolysis product was silylated according to these procedures. In Experiment 2 the hydrolysis product was first submitted to a paper-chromatographic purification in System 5, Table 5. The vertical edges of the paper were sprayed with NaIO₄-benzidine reagent for localization of the hydrolysis product, which was eluted with water-alcohol and silylated as above.

TABLE 5. CHROMATOGRAPHIC DATA PERTAINING TO THE IDENTIFICATION OF THE ELIMINATION PRODUCT (XV) OF MYXOXANTHOPHYLL (IV)

Compound	System 1 (<i>R_f</i> _{glucose})	System 2 (<i>R_f</i>)	System 3 (<i>R_f</i>)	System 4 (<i>R_f</i>)
Mannose	—	0.34	0.53	0.58
Galactose	0.89	0.35	—	—
Glucose	1.00	0.37	—	—
Xylose	1.42	0.43	—	—
Fucose	—	0.44	0.60	0.61
Rhamnose	—	0.54	0.63	0.67
Hydrolysate	—	0.52	0.68	—
Mannitol	—	0.34	0.55	—
Galactitol	—	0.35	—	—
Fucitol	—	—	0.68	—
Rhamnitrol	—	—	0.68	—

System 1:⁴⁹ Schleicher & Schüll No. 2043B paper; ascending method; butanol-pyridine-water (1:5:3); development with aniline-trichloroacetic acid (2.5 per cent in acetic acid at 100°; brown spots for reducing sugars.

System 2:⁵⁰ TLC on alumina/kieselgel G (1:1); *n*-butanol-acetic acid-water (6:3:1); development with NaIO₄-benzidine reagent;⁵¹ white spots on a blue background for reducing sugars and sugar alcohols.

System 3: Schleicher & Schüll No. 2043B paper; isopropanol-acetic acid-water (3:1:1);⁵⁴ development with NaIO₄-benzidine reagent.⁵¹

System 4: As system 3; development with aniline-phthalic acid reagent⁵⁰ at 100°; brown spots for reducing sugars in visible light, white fluorescence in u.v.-light.

Retention times for the various silyl ethers are listed in Table 6. On co-chromatography the main component of the per-silylated hydrolysate (XVII) could not be separated from rhamnitrol penta-trimethylsilyl ether (XVIII). A minor peak (*ca.* 17 per cent of the main peak) was not identified, but differed vastly from that of rhamnose tetra-trimethylsilyl ether, *cf.* Table 6.

Glycoside Hydrolysis of Myxoxanthophyll

IV (5.4 mg) in methanol (5 ml) was treated at room temperature with methanol acidified with HCl gas (3 N) to give a final acid concentration of 0.15 N. Paper chromatography revealed after 5 hr a *ca.* 50 per cent and after 20 hr a *ca.* 90 per cent conversion to less polar, coloured products. The reaction was interrupted after 20 hr by addition of methanol and evaporation in a vacuum desiccator over KOH. The methyl glycosides were hydrolysed by treating the evaporated residue with 0.04 N aqueous polystyrene-sulphonic acid (0.2 ml) for

⁵³ E. J. HEDGLEY and W. C. OVEREND, *Chem. & Ind.* 378 (1960).

⁵⁴ C. C. SWEeley, R. BENTLEY, M. MAKITA and W. W. WELLS, *J. Am. Chem. Soc.* 85, 2497 (1963).

TABLE 6. RETENTION TIMES ON GAS CHROMATOGRAPHY OF THE PER-TRIMETHYLSILYL ETHERS OF SOME REDUCING SUGARS AND SUGAR ALCOHOLS ON SE-30 COLUMNS

Per-trimethylsilyl ether of	Series 1. 2% SE-30 (150°)	Series 2. 20% SE-30 (188°)
Rhamnose	4' 24"	
Fucose	5' 0"	
Fucitol	6' 15"	2' 0"
Rhamnitol (XVI)	(7' 48") 8' 42"	2' 30"
Hydrolysate	(4' 20") (7' 42") 8' 38"	2' 30"
Rhamnitol + hydrolysate	(7' 42") 8' 42"	2' 30"
Fucitol + hydrolysate		2' 15" 2' 36"
Mannitol		4' 42"

Minor peaks are given in parentheses.

2 hr at 100°. ⁵⁶ Authentic methyl galactoside and methyl rhamnoside, treated in like manner for comparison, were completely hydrolysed by this procedure. The filtrate was submitted to paper chromatography in systems 3 and 5. The result is summarized in Table 7.

TABLE 7. CHROMATOGRAPHIC DATA OF THE SUGAR RESIDUE AFTER GLYCOSIDIC HYDROLYSIS OF MYXOXANTHOPHYLL (IV)

Compound	System 3 (R_f)	System 5 ($R_{f\text{rhamnose}}$)
Galactose	0.47	0.56
Glucose	—	0.60
Fucose	0.60	0.83
Rhamnose	0.67	1.00
Hydrolysate	0.66 (major) 0.51 (minor)	0.98 Too weak

System 3: See Table 5.

System 5: As system 4, using benzene:
butanol:pyridin:water, 1:5:3:3.

In a separate experiment using IV (3 mg), the hydrolysis was interrupted after 5 hr by transfer to ether. The ether extract was neutralized with aqueous NaHCO₃ solution; pigment recovery was 48 per cent. The major product (XIX), purified by chromatography on an alumina column and kieselguhr paper (see Table 2), had torulene-like spectrum (cf. XI, Fig. 3) with abs. max. in acetone at (365), (380), 462, 489 and 522 nm, % D_B/D_{II} = 14, % III/II = 65, yield 0.5 mg. 3',4'-Didehydrorubixanthin (XI) for direct comparison was not available at this stage. XIX (0.3 mg) was acetylated and paper chromatography revealed complete conversion to a product (XX) (Table 2), XX (0.2 mg) submitted to standard silylation conditions gave no silyl ether.

⁵⁵ H. O. BOUVENG and B. LINDBERG, *Adv. Carbohydrate Chem.* 15, 53 (1960).

⁵⁶ T. J. PAINTER, *Chem. & Ind.* 1214 (1960).

Acknowledgements—The biological material was most kindly collected by Dr. R. M. Baxter, University College, Haile Sellassie I University, Addis Ababa. We are greatly indebted to Dr. J. Feeney, Varian Ass., London, Dr. J. R. Chapman, Associated Electrical Industries, Manchester, Dr. C. Enzell and G. Francis, Swedish Tobacco Co., Stockholm, Dr. H. S. Haber, Cary Instruments, Palo Alto, and Fa. Hoffmann-La Roche, Basel, for their spectroscopic services and to Dr. O. Isler, Hoffmann-La Roche, for synthetic plectanixanthin. S. H. was supported by a grant from Hoffmann-La Roche to S. L. J. for fundamental research in the field of naturally occurring carotenoids. A grant from *Norges tekniske høyskoles fond* for technical assistance is also gratefully acknowledged. Dr. M. Kelly kindly made linguistic corrections to the manuscript.