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NEW CAROTENOID GLYCOSIDES FROM *OSCILLATORIA LIMOSA**

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Abstract—The carotenoid composition of *Oscillatoria limosa* has been studied in a quantitative manner. In addition to β-carotene, cryptoxanthin, echinenone, canthaxanthin and a zeaxanthin-like carotenoid, three new glycosidic carotenoids were encountered. These were assigned the gross structures myxol-2′-*O*-methyl-methylpentoside (I), oscillol-2,2′-di-(*O*-methyl-methylpentoside) (IX) and 4-keto-myxol-2′-methylpentoside (XIII) on the basis of chemical and spectral evidence. The mass spectra of the acetylated glycosides were particularly informative.

INTRODUCTION

THE IDENTITY and structures of a number of carotenoids of blue–green algae have been described in previous papers in the present series.[1–5] Healey[6] has recently examined the carotenoid composition of other blue–green algae, and has demonstrated the presence of further hypophasic carotenoids of undetermined structure in *Phormidium* species. The present paper represents another contribution to the distribution and structure of carotenoids of blue–green algae.

RESULTS AND DISCUSSION

The carotenoid composition of one batch of *Oscillatoria limosa* was studied in a quantitative manner. The total lipid content was 16 per cent and the carotenoid content 0·14 per cent of the dry weight. The epiphasic carotenoids consisted of β-carotene (17 per cent of total carotenoid), cryptoxanthin (1 per cent), echinenone (23 per cent) and canthaxanthin (7 per cent), and the hypophasic fraction contained a zeaxanthin-like carotenoid (22 per cent), P476 (27 per cent), P483 (1 per cent) and P496 (9 per cent). In the present work emphasis was placed on the structure determination of the strongly polar xanthophylls P476, P483 and P496. Exceptionally high amounts of non-carotenoid contaminants prevented the isolation of the pure, crystalline xanthophylls. However, their chemical reactions and the mass spectra of the acetylated derivatives permit plausible structural assignments.

P476 (I) is a myxoxanthophyll-like glycoside with the aglycone II, for which the trivial a name *myxol* is suggested, in common with myxoxanthophyll. Whereas myxoxanthophyll is myxol-2′-rhamnoside (III)[4], P476 is evidently a myxol-2′-*O*-methyl-methylpentoside (I), Fig. 1.

* Part VI in the series "Carotenoids of Blue–Green Algae"; for Part V see *Phytochem.* **8**, 1281 (1969).

[1] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **5**, 557 (1966).
[2] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **5**, 565 (1966).
[3] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **6**, 1119 (1967).
[4] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1259 (1969).
[5] S. HERTZBERG and S. LIAAEN-JENSEN, *Phytochem.* **8**, 1281 (1969).
[6] F. P. HEALEY, *J. Phycol.* **4**, 126 (1968).

Judged from the visible spectra identical chromophores were present in I and III. P476 (I) was stable towards alkali treatment and $NaBH_4$ reduction, and thus did not contain ester, keto or aldehyde groups. Adsorptive properties and the large increase in R_f on acetylation indicated the presence of several hydroxy groups. The acetate (IV) gave a mono-trimethylsilyl ether (V), thus demonstrating the presence of one tertiary hydroxy group in I. The failure of oxidation with *p*-chloranil showed that allylic hydroxy substituents were absent. Treatment of P476 (I) with HCl–$CHCl_3$ gave a non-polar elimination product (VI) which appeared to be the same as that obtained[4] from myxoxanthophyll (III), and indicated an allylic ether substituent. Moreover P476 (I), like myxoxanthophyll (III), gave saproxanthin (VII) and

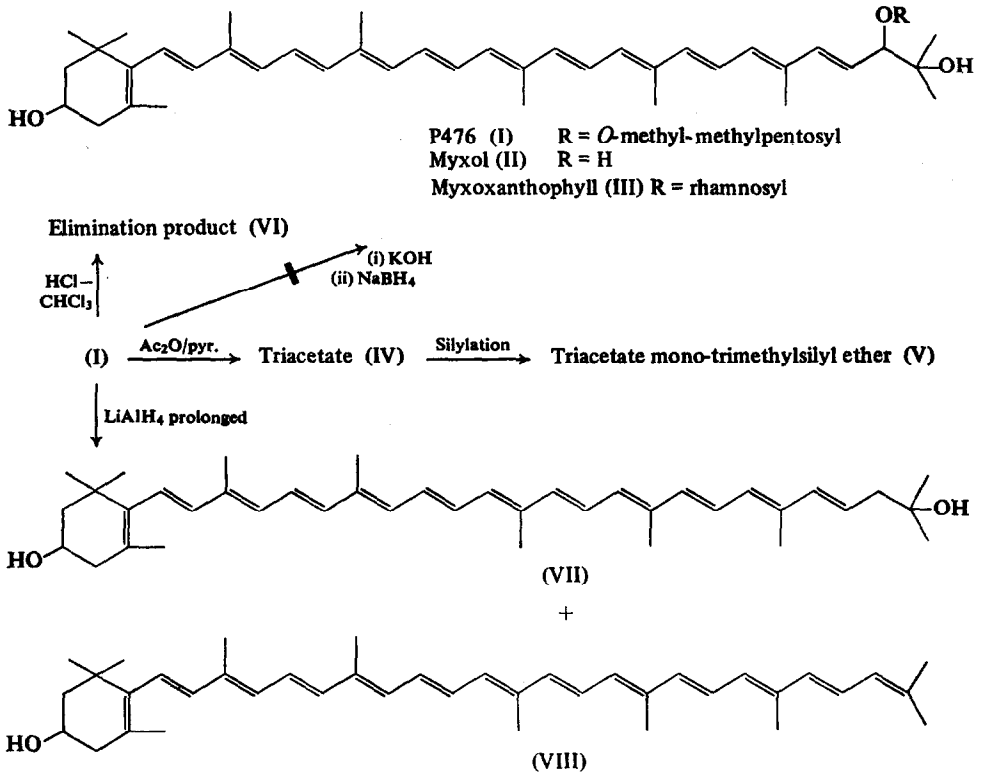
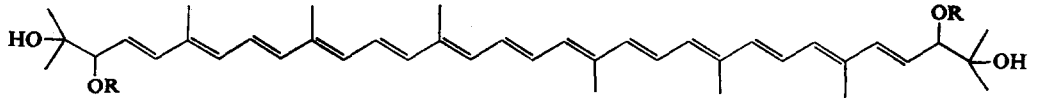


FIG. 1. CHEMICAL REACTIONS OF P476 = MYXOL-2′-*O*-METHYL-METHYLPENTOSIDE (I).

anhydro-saproxanthin (VIII) on prolonged treatment with $LiAlH_4$, and this strongly supported the presence of a common aglycone. The further structural assignment follows from mass spectral data. P476 (M = 744) was less polar than myxoxanthophyll (M = 730) and the mass spectrum of P476 acetate (M = 870) demonstrated the presence of three secondary (or primary) hydroxyl groups in P476 (I) as opposed to four in myxoxanthophyll (III). Support for the *O*-methyl-methylpentoside formulation in P476 (I) is derived from the mass spectrum of the acetate (IV) which exhibits a prominent peak at *m/e* 245 ($C_{11}H_{17}O_6$) for the expected oxonium ion[7] and *m/e* 153 and 111 ions derived from this (Fig. 4) in principle according to the scheme

[7] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products*, Vol. 2, Chapter 27, Holden-Day, San Francisco (1964).

of Biemann *et al.*[8,9] Both P476 (I) and the triacetate (IV) show M-92 and M-106 ions and I an M-158 ion, confirming the assignment of the molecular ion. P476 (I) also shows *a*, *b*, *c* and *d* ions and ions ascribed to the loss of 106 mass units from *b*, *c* and *d*. Possible mechanisms for the formation of these ions are given in Fig. 5. In the absence of metastable peaks, it is not possible to decide whether *b* and *d* are formed by the concerted processes shown or through *a*, although the latter seems less likely on account of the fact that *a* is not observed for the acetylated glycosides (IV, XII and XIV) discussed below. In the spectrum of the triacetate (IV) *b*, *c*, *d*, *d*-60 and *d*-106 ions were observed. The spectra of both I and IV showed M-58 ions.[10] Chemical and spectral data are hence in full accord with structure I for the main carotenoid glycoside of *O. limosa*. The relationship between our pigment I and unknown 1 of Healey[6] from *Phormidium fragile* is not known.

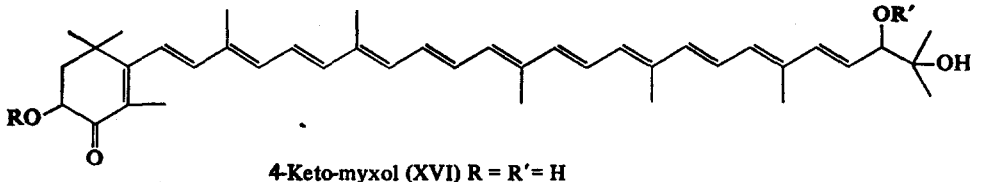


P496 (IX) R = *O*-methyl-methylpentosyl
Oscillaxanthin (X) R = rhamnosyl
Oscillol (XI) R = H

FIG. 2. GLYCOSIDES OF OSCILLOL.

P496 (IX) is an oscillaxanthin-like carotenoid less polar than the dirhamnoside oscillaxanthin (X)[5] but with the same tetraol aglycone (XI), for which the trivial name *oscillol* is suggested for convenience, Fig. 2.

The visible absorption spectra indicate identical chromophores in IX and X. P496 acetate (XII) gave a mono- and subsequently a di-(trimethylsilyl) ether on silylation, demonstrating the presence of two tertiary hydroxy groups in P496 (IX). The further structural assignment of P496 as an oscillol-2,2'-di-(*O*-methyl-methylpentoside) (IX) follows from the mass spectrum of P496 acetate (XII). The assignment of the molecular ion of the acetate (M = 1088) is confirmed by the occurrence of a stronger M-106 ion. The *O*-methyl-methylpentose moiety is supported by *m/e* 245, 153 and 111 ions (Fig. 5). Ions *b*, *c*, *b*-106 and *d*-106 (Fig. 5) support the suggested structure.



4-Keto-myxol (XVI) R = R' = H
P483 (XIII) R = H, R' = methylpentosyl
P483 tetraacetate (XIV) R = Ac, R' = triacetylmethylpentosyl

FIG. 3. GLYCOSIDE OF 4-KETO-OSCILLOL.

The third glycoside P483 (XIII) was present in trace quantities and was separated from P496 (IX) only after acetylation. The visible spectrum corresponded to that of the glucoside

[8] K. BIEMANN, D. C. DE JONGH and H. K. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).
[9] A. J. AASEN, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.*, in press.
[10] C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **23**, 727 (1969).

4-keto-phleixanthophyll.[11] Brief hydride reduction of the acetate (XIV) caused a hypsochromic shift of 7 nm in acetone, and the visible spectrum of the reduction product (XV) corresponded to that of P476 (I). XV was more polar than I. The acetate (XIV) exhibited the molecular ion at *m/e* 912, and M-58, M-92 and M-58-106 ions were prominent. Ions corresponding to *b*, *c*, *d* and *d*-106 (Fig. 5) were observed at *m/e* values 14 mass units higher than for P476 triacetate (IV), which together with visible spectral data support the formulation of P483 aglycone as 4-keto-myxol (XVI). The required formulation of R (Fig. 3) in the acetate (XIV) as triacetylmethylpentosyl to account for M = 912 was confirmed by ions at *m/e* 273 ($C_{12}H_{17}O_7$), 153 and 111 (Fig. 4).

Myxoxanthophyll (III) was recently shown to be a mixed glycoside in which myxol (II) was predominantly (*ca.* 90 per cent) bound to rhamnose and partly (10 per cent) to a hexose.[4] A relatively prominent ion at *m/e* 245 (Fig. 4) for XIV, and weak ions at *m/e* 331 (tetraacetylhexose oxonium ion) for IV, XII and XIV would indicate that a similar situation

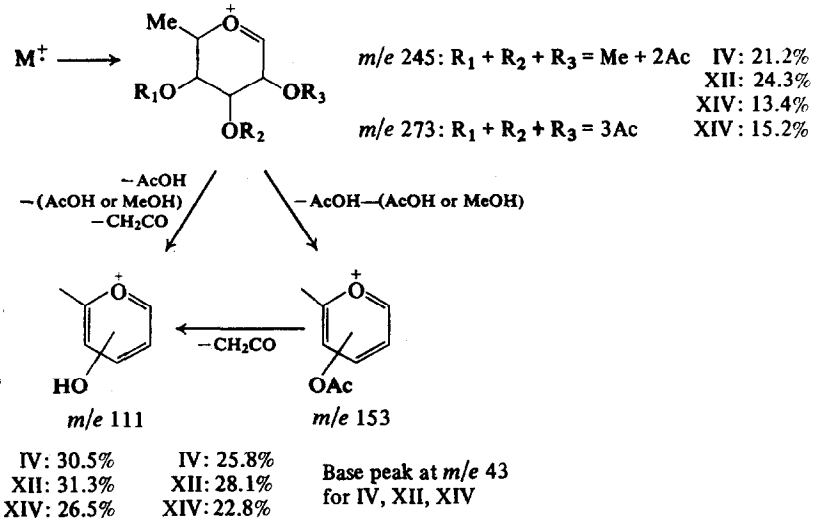


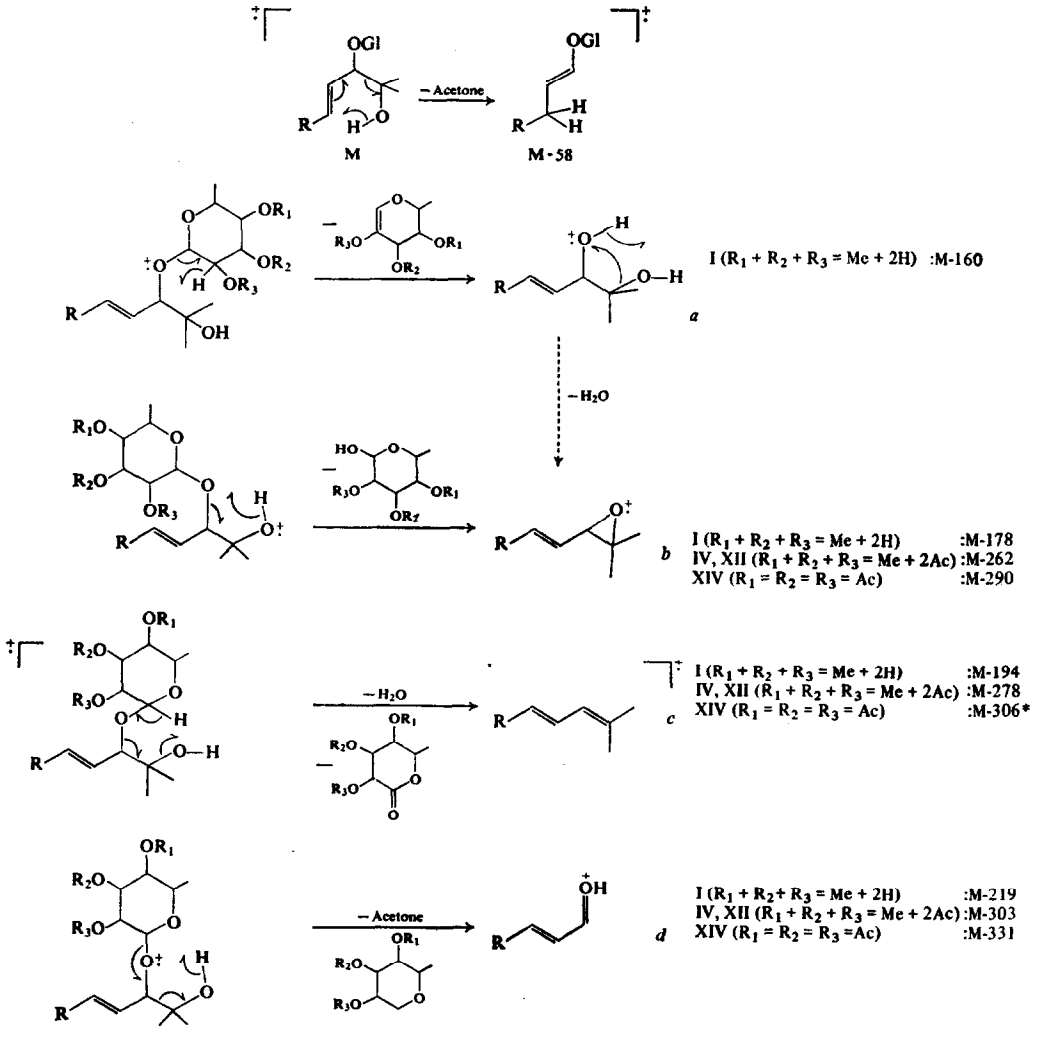
FIG. 4. OXONIUM IONS DERIVED FROM *O. limosa* GLYCOSIDES.

existed for the glycosides studied here. However, no molecular ions in support of mixed glycosides were observed in these cases.

In conclusion, glycosidic carotenoids comprise *ca.* 37 per cent of the total carotenoids of *O. limosa*. The aglycones are identical with or closely related to those of myxoxanthophyll[4] and oscillaxanthin,[5] and differences are mainly restricted to the sugar moiety. Material was not available to allow identification of the sugars formed after glycoside hydrolysis. Since carotenoid rhamnosides occur in other *Oscillatoria* spp.[3–5] the methylpentose of P483 (XIII) may be rhamnose. Concerning P476 (I) and P496 (IX), *O*-methyl-methylpentoses are well known; for example, L-acofriose which is 3-*O*-methyl-L-rhamnose.[12]

Carotenoid glycosides have so far been found in higher plants, blue–green algae and non-photosynthetic bacteria.[13] Their general distribution, localization and function are not yet established.

[11] S. HERTZBERG and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **21**, 15 (1967).
[12] H. MUHR, A. HUNGER and P. REICHSTEIN, *Helv. Chim. Acta* **37**, 403 (1954).
[13] S. LIAAEN-JENSEN, *J. Pure Applied Chem.*, in press.



*Observed only in combination with other losses

FIG. 5. END GROUP FRAGMENTATIONS OF THE GLYCOSIDE I AND THE ACETYLATED GLYCOSIDES IV, XII AND XIV.

EXPERIMENTAL

Isolation

Materials and methods were as reported elsewhere.[4,14] The mass spectra were recorded on an MS 902 instrument using the direct inlet system.

Oscillatoria limosa was harvested at the bank of the Nidelv river, near Marienborg, Trondheim, in August 1965 and October 1966, and stored frozen. The isolation procedure earlier described[1,4,5] was used. Hypophasic carotenoids were not saponified. Batch 1 contained 45 g algae (dry wt. basis), ether-soluble lipids 16 per cent of dry wt. and total carotenoid 64·1 mg or 0·14 per cent of dry wt. In batch 2 only the hypophasic carotenoids (121 mg) were examined.

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

TABLE 1. ABSORPTIVE PROPERTIES IN VISIBLE LIGHT AND ADSORPTIVE DATA FOR VARIOUS GLYCOSIDIC CAROTENOIDS AND SOME DERIVATIVES THEREOF

Carotenoid	λ_{max} (nm) in acetone			Required eluent: Cellulose column (%)	Required eluent: Alumina grade 2 (%)	R_f kieselguhr paper: 2%*	5%*	10%*	20%*	30%†	50%†	2%§
P476 (I)	450	476	508	10*						0·71		
Myxoxanthophyll (III)	450	478	510							0·52		
Myxoxanthophyll (III) tetraacetate								0·55				
P476 triacetate (IV)	450	477	509	15‡	20*		0·17	0·55				
P476 triacetate mono-TMS ether (V)						0·42						
P476 elimination product (VI)	460	488	519		6‡	0·39						
Saproxanthin (VII)	450	476	506		40†		0·09	0·37				
Saproxanthin (VII) acetate	450	476	506				0·29					
Anhydro-saproxanthin (VIII)	460	488	520		10†		0·25	0·59				
P496 (IX)	469	496	530	>30†							0·74	0·98
Oscillaxanthin (X)	470	499	534									0·59
P496 acetate (XII)	469	498	532	70‡	50*		0·01	0·15	0·54			
P496 acetate (XII) mono-TMS ether							0·18					
P496 acetate (XII) di-TMS ether							0·54					
P483 acetate (XIV)		483	(510)	50‡					0·79			
KOH-treated P483 acetate		483	(510)							0·45	0·70	
$LiAlH_4$-reduced P483 acetate (XV)	450	474	506								0·66	

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

Epiphasic Carotenoids

The individual components of the epiphasic fraction (29 per cent of total carotenoid) were identified after column chromatography on deactivated alumina from visible absorption spectra and R_f's on kieselguhr and aluminium oxide papers determined by co-chromatography with authentic samples: β-carotene (17 per cent of total carotenoid), cryptoxanthin (1 per cent), echinenone (23 per cent) and canthaxanthin (7 per cent).

Hypophasic Carotenoids

These were chromatographed on cellulose columns. A zeaxanthin-like carotenoid (22 per cent) was not identified because of oily contaminants. Mixed fractions, submitted to acetylation, followed by chromatography on deactivated alumina, gave the respective acetates. Spectral and chromatographic properties of P476, P483 and P496 and their derivatives are compiled in Table 1. Further reference to these data is not made.

P476 = myxol-2′-*O*-methyl-methylpentoside (I). I co-precipitated with non-carotenoid substance as a crude concentrate, m.p. 100–115°, methyl signals at τ-values ($CDCl_3$) 8·03, 8·10, 8·30, 8·72, 8·80 and 8·92; hydroxyl absorption at ν_{max} 3400, 1070, 1038; *m/e* 744 (M), M-58, M-92, M-106, M-158, M-160, M-178, M-194, M-219, M-158–92, M-178–106, M-194–106, M-21–106.

I was stable towards standard saponification conditions, reduction with $NaBH_4$ and oxidation with *p*-chloranil. Treatment with HCl–$CHCl_3$[4] gave a major product (VI) which could not be acetylated and was spectrophotometrically and chromatographically indistinguishable from the main elimination product obtained in the same manner from myxoxanthophyll (III). Treatment of I with excess $LiAlH_4$ in ether for 30 min gave saproxanthin (VII) and anhydro-saproxanthin (VIII) in ratio 4:1. VII had partition ratio 70:30 in petroleum ether/85% methanol, was chromatographically different from plectaniaxanthin[15] and gave saproxanthin (VII) acetate on acetylation.

Acetylation provided P476 acetate (IV), which partly hydrolysed on deactivated alumina columns, and was therefore reacetylated and chromatographed on cellulose. IV could not be separated from myxoxanthophyll (III) acetate in kieselguhr paper. Precipitated IV had *m/e* 870 (M), M-42, M-58, M-60, M-92, M-106, M-117, M-165, M-117-60-60, M-262, M-278, M-303, M-331, M-303-60, (331), 245 (found, 245·1018, calc. for $C_{11}H_{17}O_6$, 245·1025), 153, 111, 43.

P496 = oscillol-2,2′-di-(*O*-methyl-methylpentoside) (IX). Chlorophyll and oily contaminants interfered with the separation on cellulose columns. The recovery of IX after saponification was variable and IX was best isolated after acetylation as P496 acetate (XII) as IV above. Precipitated XII had *m/e* 1088 (M), M-2, M-106, M-262, M-278, M-278-2, M-262-106, M-303-106, (331), 245 (found, 245·1018; calc. for $C_{11}H_{17}O_6$, 245·1025), 153, 111, 43.

The formation of a mono- and di-(trimethylsilyl) ether during silylation of XII was followed by paper chromatography.

P483 = 4-keto-myxol-2′-methylpentoside (XIII). P483 was characterized as the acetate (XIV), adsorbed below XII on cellulose columns. XIV crystallized sparingly as needles from petroleum ether: *m/e* 912 (M), M-16, M-2-42, M-58, M-60, M-92, M-106, M-117, M-106-58, M-106-60, M-290, M-2-306, M-331, M-2-92-306, (331), 273 (found, 273·0962; calc. for $C_{12}H_{17}O_7$, 273·0974), 245 (found, 245·1018; calc. for $C_{11}H_{17}O_6$, 245·1025), 153, 111, 43.

$LiAlH_4$-reduction of XIV gave XV, different from alkali-treated P483 acetate.

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