315. *The Degradation of Two Periodate-oxidised Arabinoxylans.*

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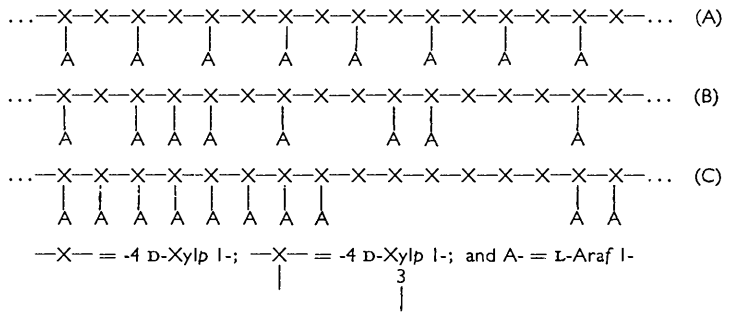
Reduction of periodate-oxidised rye-flour arabinoxylan, followed by mild acid hydrolysis, affords 2-*O*-β-D-xylopyranosylglycerol (I), *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol (II), and *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-xylopyranosyl-(1 → 2)-glycerol (III) in the molar ratio of 7·5 : 2·2 : 1. It is concluded that the L-arabinofuranose side-chains in rye-flour arabinoxylan are attached randomly along the main xylan chain. A similar degradation of barley-husk arabinoxylan furnishes the xylosylglycerol (I), the xylobiosylglycerol (II), and *O*-β-L-arabinofuranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol (VI). Isolation of the glycoside (VI), when taken together with previous structural evidence, shows that the 2-*O*-β-D-xylopyranosyl-L-arabinofuranose side-chains in the polysaccharide are directly linked to the main xylan chain.

Rye-flour arabinoxylan contains a main chain of 1,4′-linked β-D-xylopyranose residues in which, on the average, every second unit carries as a side-chain attached by a 1 → 3′-linkage a single L-arabinofuranose residue.[1] Hitherto it has not been known whether the side-chains are attached regularly to alternate xylose residues (A), randomly along the xylan chain (B), or whether blocks of xylose residues which carry side-chains are separated by corresponding blocks of unbranched xylose residues (C). These possible structures have been distinguished by the application of the elegant degradative procedure developed by Smith and his collaborators.[2] Since only those D-xylose residues which carry side-chains are resistant to oxidation by periodate, this procedure provides a method for assessing the distribution of branching points along the main chain.



Periodate-oxidised rye-flour arabinoxylan was reduced with potassium borohydride and the resulting polyalcohol was hydrolysed with cold dilute sulphuric acid. The hydrolysate contained three glycosidic components (I—III) in the molar ratio of 7·5 : 2·2 : 1, but no reducing sugars. The glycosides were fractionated by chromatography on a resin column [3] followed by partition chromatography on filter sheets. The structures of the three glycosides were established by the following experiments, the results of which were summarised in the Table. Hydrolysis of the glycosides (I—III) gave xylose and glycerol in the molar ratios of 1 : 1, 2 : 1, and 3 : 1, respectively. Periodate oxidation of the glycosides indicated that non-reducing xylopyranose end groups were present in the pyranose form and that the other xylose residues were cleaved by the reagent. Since no formaldehyde was formed on periodate oxidation it follows that the glycerol portions were 2-*O*-substituted.

[1] (*a*) Aspinall and Sturgeon, *J.*, 1957, 4469; (*b*) Aspinall, Cairncross, Sturgeon, and Wilkie, *J.*, 1960, 3881; (*c*) Aspinall and Cairncross, *J.*, 1960, 3998; (*d*) Aspinall, Greenwood, and Sturgeon, *J.*, 1961, 3667.

[2] Goldstein, Hay, Lewis, and Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.

[3] Jones and Wall, *Canad. J. Chem.*, 1960, **38**, 2290.

Further evidence for the presence of xylopyranose end groups in all the glycosides and of 1,4′-linked xylose residues in glycosides (II) and (III) was obtained by examination of the methanolysis products from the corresponding methylated derivatives by gas–liquid partition chromatography. As indicated in the Table components having the characteristic retention times of the methyl glycosides of 2,3,4-tri- and 2,3-di-*O*-methyl-D-xylose were recognised. Gas chromatography permits the recognition of 2,3,4-tri- and the various di-*O*-methylxylopyranoses, and 2,3,5-tri-*O*-methylarabinose by the retention times of the components of the equilibrium mixtures of methyl glycosides which are formed when the sugars are refluxed with methanolic hydrogen chloride. The optical rotations of the three glycosides were consistent with those of a polymer-homologous series in which each higher member contains an additional β-D-xylopyranose residue. It follows that the glycosides are 2-*O*-β-D-xylopyranosylglycerol (I), *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol (II), and *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-glycerol (III). In the course of the work 2-*O*-β-D-xylopyranosylglycerol (I) was synthesised by the condensation of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide with *cis*-1,3-*O*-benzylideneglycerol followed by removal of substituent groups.

β-D-Xylp I → 2 Glycerol (I)

β-D-Xylp I → 4 β-D-Xylp I → 2 Glycerol (II)

β-D-Xylp I → 4 β-D-Xylp I → 4 β-D-Xylp I → 2 Glycerol (III)

The isolation of the three glycosides (I—III), but of no products of higher molecular weight, from the degradation of rye-flour arabinoxylan indicates that L-arabinofuranose residues are attached to isolated and, less frequently, to two and three, but not more, continguous D-xylopyranose residues (as in structure B). The molar ratio in which the

TABLE 1.

Examination of glycerol glycosides.

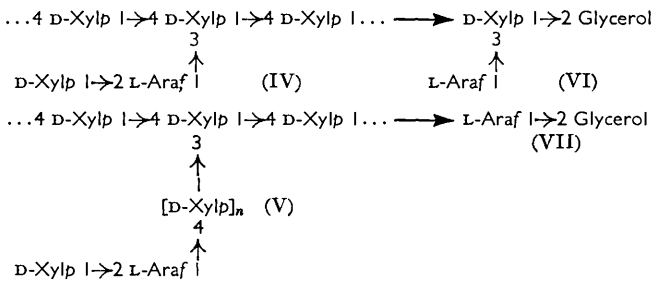
Glycoside and source *		Hydrolysis products (mol. prop.) Xyl	Ara	Glycerol	Periodate oxidation: Reagent consumed (mol. prop.)	Acid formed (mol. prop.)	Sugars formed on cleavage of methylated derivative
I	S				1·9	1·0	2,3,4-Me_3xylose
	R	0·9		1·0	1·9	1·0	2,3,4-Me_3xylose
	B	1·0		1·1	1·9	0·9	2,3,4-Me_3xylose
II	R	2·1		1·1	2·8	1·0	2,3,4-Me_3xylose + 2,3-Me_2xylose
	B	1·8		0·9	2·8	1·0	2,3,4-Me_3xylose + 2,3-Me_2xylose
III	R	2·8		1·1	3·8	1·1	2,3,4-Me_3xylose + 2,3-Me_2xylose
VI	B	0·9	0·9	1·1	0·8	Nil †	2,3,5-Me_3arabinose + 2,4-Me_2xylose

* S = synthesis; R = rye-flour arabinoxylan; and B = barley-husk arabinoxylan. † In this case xylose resídues remained unattacked; in the other cases no residue remained unattacked.

three glycosides were formed is consistent with a fairly random distribution of side-chains along the main xylan chain. The only previous case in which the arrangement of side-chains in a branched arabinoxylan has been assessed is that of the rather similar polysaccharide from wheat flour.[4] Ewald and Perlin [4] degraded the periodate-oxidised arabinoxylan by treatment with a limited amount of phenylhydrazine to give xylose, xylobiose, and a trace of xylotriose. The results of their studies on the wheat polysaccharide are qualitatively similar to those reported here for the rye polysaccharide.

[4] Ewald and Perlin, *Canad. J. Chem.*, 1959, **37**, 1254.

The xylan from barley-husks [5] is a rather less highly branched polysaccharide of the same general type in which the main chain carries three types of side-chain, single L-arabinofuranose and D-glucopyranosyluronic acid units, and longer side-chains terminated by 2-*O*-β-D-xylopyranosyl-L-arabinofuranose units. Previous studies on this polysaccharide did not show whether these disaccharide units were directly attached to the main chain (as in IV) or whether one or more D-xylose residues were interposed (as in V). In this polysaccharide both the non-terminal L-arabinofuranose residues and the D-xylopyranose residues which are present as branching points are resistant to oxidation. Degradation of the polysaccharide by Smith's procedure would lead to the formation of a disaccharide derivative (VI) from the structural unit (IV) which contains adjacent periodate-resistant L-arabinose and D-xylose residues, whereas the periodate-resistant L-arabinose residue in structural unit (V) would give rise to 2-*O*-L-arabinofuranosyl-glycerol (VII).



Periodate-oxidised barley-husk xylan was reduced with potassium borohydride and the resulting polyalcohol was hydrolysed with cold dilute sulphuric acid. The hydrolysate was fractionated by chromatography on charcoal–Celite followed by partition chromatography on filter sheets, and three glycosidic components were isolated. 2-*O*-β-D-Xylopyranosylglycerol (I) and *O*-β-D-xylopyranosyl-(1 ⟶ 4)-*O*-β-D-xylopyranosyl-(1 ⟶ 2)-glycerol (II) were characterised as described previously (see Table for summary of results). The third glycosidic component was characterised as *O*-β-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 2)-glycerol (VI) by the following observations. Hydrolysis of the glycoside gave arabinose, xylose, and glycerol in equimolecular proportions. The low consumption (<1 mol.) of reagent during periodate oxidation with the release of neither formic acid nor formaldehyde, together with the formation of xylose on hydrolysis of the oxidised glycoside, was consistent with the presence of a terminal arabinofuranose residue, a 1,3′-linked xylopyranose residue, and a 2-*O*-substituted glycerol portion. Further evidence for the nature of the sugar units was provided by gas chromatography of the methanolysis products of the methylated glycoside, which indicated components having the retention times of methyl glycosides of 2,3,5-tri-*O*-methylarabinose and 2,4-di-*O*-methylxylose. On the assumption that the polysaccharide contains only β-D-xylopyranose residues a comparison of the molecular rotations of 2-*O*-β-D-xylopyranosylglycerol ($[M]_D$ −80°) and of *O*-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 2)-glycerol ($[M]_D$ −82°) indicates that the periodate-resistant L-arabinofuranose residues in the polysaccharide have the β-configuration.

The isolation of the glycoside (VI) as the major arabinose-containing fragment from the degradation of barley-husk xylan together with the absence of 2-*O*-L-arabinofuranosyl-glycerol provides clear evidence that the 2-*O*-β-D-xylopyranosyl-L-arabinofuranose units are directly attached to the main xylan chain through position 3 of the xylose residue constituting the branching point (as in IV). Although the barley-husk arabinoxylan contains a smaller proportion of side-chains than the rye-flour polysaccharide, the isolation of xylobiosylglycerol (II) shows that in some regions of the basal xylan chain side-chains are attached to contiguous xylose residues.

[5] Aspinall and Ferrier, *J.*, 1957, 4188.

Experimental

Paper chromatography was carried out on Whatman nos. 1 and 3MM papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer). Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas–liquid partition chromatography of the methyl glycosides of methylated sugars was carried out in a Pye argon chromatograph according to the procedure of Bishop and Cooper [6] (see also accompanying paper [7]). Separations were carried out on a column (120 × 0·5 cm.) of 15% by weight of butane-1,4-diol succinate polyester [6] on acid-washed Celite at 150° at gas flow rates of 80—100 ml./min. In all cases the methyl glycosides of reference compounds have been formed from sugars which have been characterised independently. Retention times (T) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Rye-flour arabinoxylan was sample B which had been used in an earlier investigation.[1d] Barley-husk arabinoxylan (from Carlsberg variety, harvested in 1959) was isolated as described previously.[5] The essential structural similarity of this sample of polysaccharide to that used in the earlier work [5] was indicated by the preparation of the methylated polysaccharide (0·32 g.) (Found: OMe, 38·5%) from a sample (0·5 g.) of the new batch of arabinoxylan. Gas-chromatographic examination of the methanolysis products from the two samples of methylated polysaccharide showed that the same cleavage products were formed and the presence of methyl glycosides of the following sugars was indicated in each case; 2,3,5-tri- (T 0·52, 0·69) and 3,5-di-*O*-methylarabinose (T 1·08, 2·81), and 2,3,4-tri- (T 0·42, 0·55) and 2,3-di-*O*-methylxylose (T 1·52, 1·62, 1·89). In the conditions used the retention times of methyl glycosides of mono-*O*-methylpentoses and tri-*O*-methylglucuronic acid were too great to permit identification.

Degradation of Rye-flour Arabinoxylan.—Arabinoxylan (4·4 g.) was oxidised with 0·15M-sodium metaperiodate solution (400 ml.) for 54 hr. (uptake of reagent was constant and corresponded to the consumption of 0·67 mole of reagent per pentose residue) and the excess of reagent was destroyed by the addition of ethylene glycol. The solution was shaken with Amberlite resin IR-120(H) to remove sodium ions and iodic acid was neutralised with barium hydroxide and barium carbonate. The filtered solution was treated with potassium borohydride (3·0 g.) at room temperature for 20 hr., the excess of hydride was destroyed, and potassium ions were removed by shaking the solution with Amberlite resin IR-120(H); the solution was then concentrated, methanol being added to facilitate removal of boric acid as methyl borate. The residue was treated with N-sulphuric acid (200 ml.) at room temperature for 3 hr., neutralised with barium hydroxide and barium carbonate, concentrated, and poured into ethanol (5 vol.). The slight precipitate which separated was hydrolysed with N-sulphuric acid for a further 2 hr., and the solution was worked up in the same way, but no precipitate was formed when the mixture was poured into ethanol. The combined solutions were concentrated to a syrup (4·1 g.). Chromatography of the syrup in solvent A indicated the presence of glycerol, glycollaldehyde, and three components (subsequently shown to be glycosides) having R_{xylose} 1·00, 0·62, and 0·35. Quantitative paper chromatography with the phenol–sulphuric acid reagent [8] showed that the glycosides were present in the molar ratio of 7·5 : 2·2 : 1·0.

The three glycosidic components were isolated in pure form after elution of the syrup (3·6 g.) with water from a column (100 × 2·8 cm.) of Dowex resin 50 WX3 (Ba^{2+}, 200—400 mesh), followed, where necessary, by fractionation on filter sheets with solvent A. Glycoside I, R_{xylose} 1·00 (indistinguishable from synthetic 2-*O*-β-D-xylopyranosylglycerol in solvents A, B, and C), had $[\alpha]_D$ −34·4° (*c* 2·0); glycoside II, R_{xylose} 0·62, had $[\alpha]_D$ −49° (*c* 1·0); and glycoside III, R_{xylose} 0·35, had $[\alpha]_D$ −59° (*c* 1·0).

Examination of Glycosides.—Glycosides (*ca.* 30 mg.) were hydrolysed with N-sulphuric acid on the boiling-water bath for 6 hr., and, after addition of L-rhamnose as an internal reference and neutralisation with barium carbonate, the hydrolysate was separated chromatographically by using solvent B. After elution from chromatograms, reducing sugars were estimated by the phenol–sulphuric acid reagent,[8] and glycerol was estimated by oxidation with sodium metaperiodate and determination of the formaldehyde formed by the chromotropic acid reagent.[9]

Samples (3—4 mg.) of glycosides were methylated in *NN*-dimethylformamide with methyl

[6] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[7] Aspinall, preceding paper.
[8] Dubois, Gillies, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.
[9] McFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

iodide and silver oxide according to the procedure of Kuhn *et al.*[10] The methylated glycosides were boiled with methanolic 3% hydrogen chloride for 12 hr. and the methanolysis products were examined by gas–liquid partition chromatography. The methyl glycosides of the methylated sugars quoted in Table 1 were characterised by comparison of their relative retention times with those of methyl glycosides of authentic sugars (see Table 2). In addition, gas chromatography of the methanolysis products of all the methylated glycosides showed the presence of a component of low relative retention time (*T* 0·12) which was probably 1,3-di-*O*-methylglycerol.

The glycosides (10—20 mg.) were oxidised with 0·1M-sodium metaperiodate solution at room temperature. Aliquot parts were withdrawn, ethylene glycol was added, and acid released during the oxidation was titrated with 0·01N-sodium hydroxide. The consumption of oxidant was measured spectrophotometrically [11] by the withdrawal of samples and dilution to suitable

TABLE 2.

Gas chromatography of methyl glycosides of methylated sugars.

Sugar	Relative retention times (*T*) of methyl glycosides	Methanolysis products of methylated glycosides				
		Synth *	I	II	III	IV
2,3,5-Tri-*O*-methyl-L-arabinose	0·51s					+
	0·69w					
2,3,4-Tri-*O*-methyl-D-xylose	0·41m	+	+	+	+	
	0·55s					
2,3-Di-*O*-methyl-D-xylose	1·49m					
	1·61w			+	++	
	1·87s					
2,4-Di-*O*-methyl-D-xylose	1·52m					+
	2·12m					
3,4-Di-*O*-methyl-D-xylose	1·32m					
	1·63s					

Synth = Synthetic 2-*O*-β-D-xylopyranosylglycerol.

concentrations. Other samples were tested with the chromotropic acid reagent. When oxidation was complete the remaining solutions were deionised by shaking them with Amberlite resins IR-120(H) and IR-4B(OH), and concentrated to syrups which were hydrolysed with N-sulphuric acid on the boiling-water bath for 6 hr. The hydrolysates were examined chromatographically for the presence of reducing sugars.

Degradation of Barley-husk Arabinoxylan.—Arabinoxylan (20 g.) was oxidised with 0·5M-periodic acid (2 l.) at 5° for 30 hr. (uptake of reagent was constant and corresponded to the consumption of 0·94 mole of reagent per pentose residue), the excess of reagent was destroyed by ethylene glycol, and iodic acid was neutralised with barium hydroxide and barium carbonate. The filtered solution was deionised, concentrated (to 1 l.), and treated with potassium borohydride (10 g.) for 16 hr. The resulting solution was treated alternately with cation- and anion-exchangers, and the remaining boric acid was removed as methyl borate by evaporation with methanol. The residue was treated with N-sulphuric acid (500 ml.) for 5 hr., neutralised with barium carbonate, filtered, deionised, and concentrated to a syrup (18·8 g.). The syrup was adsorbed on charcoal–Celite (1 : 1; 28 × 4 cm.); elution with water (1 l.) gave fraction 1 (17·4 g.) which contained glycollaldehyde, glycerol, ethylene glycol, and traces of xylose and arabinose and was not examined further. The column was then eluted with a gradient of water containing 0·0—30% of ethanol to give fractions 2—8, which were examined chromatographically in solvents A and B together with their hydrolysis products. Fraction 2 (206 mg.) contained glycerol, glycoside I, and traces of arabinose and xylose. Fraction 3 (164 mg.) contained glycerol and glycoside I. Fraction 4 (96 mg.) contained glycosides I and VI. Fraction 5 (130 mg.) contained glycoside VI, a trace of glycoside I, and an unidentified xylose-containing oligosaccharide (R_{xylose} 0·34 in solvent A). Fraction 6 (94 mg.) contained glycoside II and the above-mentioned oligosaccharide (R_{xylose} 0·34). Fraction 7 (74 mg.) contained glycoside II, xylobiose (R_{xylose} 0·64), and an oligosaccharide (R_{xylose} 0·55) which gave xylose and arabinose on hydrolysis. Fraction 8 (58 mg.) contained two oligosaccharides (R_{xylose} 0·55, 0·19) and gave xylose and arabinose on hydrolysis. Fractions 3, 4, and 6 were fractionated on filter sheets

[10] Kuhn, Trischmann, and Löw, *Angew. Chem.*, 1955, **67**, 32.
[11] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

with solvents A and B, to give chromatographically pure samples of glycoside I, R_{xylose} 1·0 in solvent A and $[\alpha]_D$ −32° (*c* 2·0), glycoside II, R_{xylose} 0·62 and $[\alpha]_D$ −50° (*c* 0·5), and glycoside VI, R_{xylose} 0·75 and $[\alpha]_D$ −22·4° (*c* 1·0). The three glycosides were examined by the methods described previously.

2-*O*-β-D-*Xylopyranosylglycerol*.—*cis*-1,3-*O*-Benzylideneglycerol (25 g.), freshly prepared silver carbonate (50 g.), and anhydrous calcium sulphate (100 g.) were shaken in benzene (300 ml.) overnight. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (37·4 g.) in benzene (300 ml.) was added slowly with stirring, and the mixture was shaken in the dark for 3 days with occasional release of carbon dioxide. The mixture was finally refluxed for 15 min., filtered, and concentrated. The residue was dissolved in benzene and chromatographed on alumina (P. Spence and Sons, Ltd.; shaken with acetic acid, washed free from acid, and dried at 260°). The desired product, 1,3-O-*benzylidene*-2-O-(2,3,4-*tri*-O-*acetyl*-β-D-*xylopyranosyl*)*glycerol* (1·6 g.) was eluted with light petroleum (b. p. 60—80°)–benzene (3 : 2), and after recrystallisation from light petroleum (b. p. 40—60°)–ethanol (2 : 1) had m. p. 183—184° and $[\alpha]_D$ −33·8° (*c* 1·0 in $CHCl_3$) (Found: C, 57·6; H, 6·0. $C_{21}H_{26}O_{11}$ requires C, 57·5; H, 5·9%). This substance (1·3 g.) was treated with sodium methoxide (0·8 g.) in methanol (40 ml.) for 18 hr., and the resulting solution was deionised with cation- and anion-exchangers, and concentrated to a syrup which crystallised. Recrystallisation from ethanol–ether (2 : 1) furnished 1,3-*O*-benzylidene-2-*O*-β-D-xylopyranosylglycerol (0·86 g.), m. p. 132—134°, $[\alpha]_D$ −41·4° (*c* 1·0). The *O*-benzylidene compound (0·8 g.) in ethanol (50 ml.) was shaken in hydrogen at atmospheric pressure over 10% palladium–charcoal (0·8 g.) for 48 hr. Filtration and concentration afforded the syrupy 2-*O*-β-D-xylopyranosylglycerol, $[\alpha]_D$ −36° (*c* 2·0) (Charlson, Gorin, and Perlin [12] give $[\alpha]_D$ −37°).

The synthetic 2-*O*-β-D-xylopyranosylglycerol was analysed by methylation and by periodate oxidation as described previously. The compound (20 mg.) was shaken with benzaldehyde (1 ml.) and powdered fused zinc chloride (0·1 g.) for 24 hr. The mixture was poured into a mixture of light petroleum (b. p. 40—60°) (5 ml.) and water (5 ml.) which was shaken for 5 min. The aqueous layer was separated, filtered, washed with light petroleum, and concentrated to give 1,3-*O*-benzylidene-2-*O*-β-D-xylopyranosylglycerol, which after recrystallisation from methanol–ether (2 : 1) had m. p. and mixed m. p. 130—132° and $[\alpha]_D$ −40·8° (*c* 0·5).

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[12] Charlson, Gorin, and Perlin, *Canad. J. Chem.*, 1957, **35**, 365.