7. *The Acid-catalysed Reversion of* D-*Xylose.*

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Treatment of D-xylose with 6N-hydrochloric acid has yielded a mixture of oligosaccharides in addition to the starting material. Five disaccharides were isolated, three of them crystalline, namely, xylobiose, *O*-α-D-xylopyranosyl α-D-xylopyranoside, and 3-*O*-α-D-xylopyranosyl-D-xylose. The other two disaccharides were the 2- and the 4-*O*-isomer of the last-named sugar.

As in the case of L-arabinose (preceding paper), D-xylose is converted by 6N-hydrochloric acid into a mixture of xylose and xylose-containing oligosaccharides. The complex mixture was fractionated by chromatography on charcoal and cellulose columns. Three crystalline disaccharides were isolated and identified: (1) xylobiose, (2) *O*-α-D-xylopyranosyl α-D-xylopyranoside, and (3) 3-*O*-α-D-xylopyranosyl-D-xylose. In addition, evidence was obtained for the presence of (4) 4- and (5) 2-*O*-α-D-xylopyranosyl-D-xylose.

Xylobiose, which has been obtained previously on hydrolysis of xylan,[1] was characterised as the crystalline sugar and its phenylosazone.

Disaccharide (2) was a non-reducing sugar which could not be detected on paper chromatograms with the *p*-anisidine hydrochloride spray [2] but reduced the silver nitrate spray reagent of Trevelyan *et al.*[3] This sugar crystallised along with sugar (3), but was separated from it after the mixture had been reduced and then acetylated. Oxidation of sugar (2) with lead tetra-acetate (4 moles) yielded 2 moles of formic acid per mole of sugar, proving the presence of two unsubstituted xylopyranose residue in the disaccharide. The molecular rotation agreed well with that calculated for an α : α-linked xylobiose of the trehalose type by Hudson's rule.[4]

The crystalline disaccharide (3) was oxidised with the consumption of 3 mols. of lead tetra-acetate, and concomitant formation of 1 mol. of formic acid. No formaldehyde was produced. In the non-catalysed oxidation of the disaccharide, 1 mol. of oxidant was consumed rapidly within five minutes, and further oxidation was very slow.[5] Hydrolysis of the oxidised product gave equimolecular quantities of D-xylose and D-threose. Previous

[1] Whistler and Tu, *J. Amer. Chem. Soc.*, 1951, **73**, 1389; Whistler, Bachrach, and Tu, *ibid.*, 1952, **74**, 3059.
[2] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[3] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[4] Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66; 1916, **38**, 1566.
[5] Charlson and Perlin, *Canad. J. Chem.*, 1956, **34**, 1200.

experiments [6] have shown that these are the results expected from the oxidation of a 1 ⟶ 3-linked dipentoside. Calculations of molecular rotation [4] indicated that the two xylose residues are joined by an α-glycosidic linkage. Reduction of the sugar gave a xylitol derivative which, on oxidation with metaperiodate at pH 8, gave 2·5 mols. of formaldehyde. This result confirmed the presence of a 1 ⟶ 3 linkage in the disaccharide; 1 ⟶ 2, 1 ⟶ 4, and 1 ⟶ 5-linked disaccharides would liberate only 1 mol. of formaldehyde under these conditions, since they are not readily overoxidised.[7]

Identification of the fourth and the fifth sugar is not so conclusive, since they were obtained as an inseparable syrup. Methylation of the mixture of sugars gave a hexamethyl derivative which, on hydrolysis, yielded crystalline 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-D-xylose. The syrup remaining after crystallisation of the 2 : 3-di-*O*-methyl-D-xylose was oxidised, and crystalline 3 : 4-di-*O*-methyl-D-xylonolactone [8] was obtained from the product. These results show that the xylose residues were probably linked through positions 4 and 2 respectively in the two disaccharides. Since the syrupy mixture of disaccharides was chromatographically distinct from xylobiose (4-*O*-β-D-xylopyranosyl-D-xylose), it was inferred that the 1 ⟶ 4-linked component contained an α-linkage. Further, the optical rotation of the syrup was such that both components probably contained α-glycosidic linkages, and that the xylose units were probably in the pyranose form. Experience has shown that xylofuranosides move faster on paper chromatograms than the corresponding pyranosides. Since these two sugars move more slowly on chromatograms than does the disaccharide (2), which is a xylopyranose derivative, it is inferred that they contain pyranose residues only.

These results indicate that the isolation of α-linked xylose-containing disaccharides on hydrolysis of a xylose-containing polymer should be treated with reserve. If more than one such disaccharide is detected in the hydrolysate, they may be reversion products. On the other hand, the formation of one disaccharide only is indication that it is not an artefact.

Experimental

Paper chromatography was carried out by the descending method [9] on Whatman No. 1 filter paper, the following solvent systems being used: (*a*) butanol-1-ol–ethanol–water (3 : 1 : 1), (*b*) butan-1-ol–pyridine–water (10 : 3 : 3), (*c*) ethyl acetate–acetic acid–water (9 : 2 : 2), and (*d*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) (all v/v). The positions of the sugars on the chromatograms were determined by spraying them either with silver nitrate in acetone followed by sodium hydroxide in ethanol [2] or with *p*-anisidine hydrochloride in butan-1-ol.[2] The rates of movement of the sugars are quoted relative to that of galactose (R_{gal}) or rhamnose (R_{rh}) on the same chromatogram. Optical rotations were determined on aqueous solutions at 23° ± 3° and are equilibrium values unless otherwise stated. Solvents were removed under reduced pressure.

Formation of Oligosaccharides.—To D-xylose (80 g.), dissolved in water (80 ml.), was added concentrated hydrochloric acid (80 ml.). After 6 days, no further change in the composition of the reversion mixture could be detected on paper chromatograms. The dark solution was filtered through " Celite " and stirred for 24 hr. with Amberlite resin IR-4B. The last traces of acid were removed by passing the solution down a column of this resin. Concentration of the neutral effluent afforded a syrup which consisted of xylose and a highly complex mixture of oligosaccharides (as detected by chromatography with solvents *a*, *b*, *c*, and *d*.)

Fractionation of the Oligosaccharides.—The syrup, dissolved in a little water, was applied to the top of a column of charcoal (Darco G60) and Celite (1 : 1; w/w) of 12·2 cm. diameter and of 13 cm. depth. The column was eluted with water and then with increasing concentrations of ethanol in water,[10] and the fractionation was followed paper-chromatographically.

[6] Perlin, *Analyt. Chem.*, 1955, **27**, 396.
[7] Hough and Perry, *Chem. and Ind.*, 1956, 768.
[8] James and Smith, *J.*, 1945, 739.
[9] Partridge, *Biochem. J.*, 1948, **42**, 238.
[10] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.

The first fraction (A) (41·5 g.) consisted of D-xylose. The next four fractions (B, 1·0 g.; C, 10·8 g.; D, 0·9 g.; and E, 1·8 g.) contained oligosaccharides.

Fraction (C) contained sugars having R_F values in the range expected for pentose-containing disaccharides only. On paper chromatograms, developed in solvent *b*, and sprayed with the *p*-anisidine hydrochloride reagent, three major spots, α, β, and γ, were apparent, having R_{gal} values of 1·25, 1·2, and 0·75, respectively. Fraction C was therefore refractionated on cellulose columns, with butan-1-ol–water (15 : 1 v/v) as the mobile phase. In this way, three sub-fractions, C_1, C_2, and C_3 were obtained, which, on paper chromatograms sprayed with the *p*-anisidine hydrochloride reagent, gave single spots corresponding to α, β, and γ respectively.

Separation of Fraction C_1 into Reducing and Non-reducing Components.—When chromatograms of fraction C_1 were sprayed with the silver nitrate reagent, another spot was observed in addition to that detected by the *p*-anisidine hydrochloride reagent; it had R_{gal} 1·15 (solvent *b*). On storage, fraction C_1 crystallised in part, and it was divided into crystalline and non-crystalline portions (C_1' and C_1'' respectively). By means of paper chromatography, with both spray reagents, it was shown that the crystalline fraction C_1' was a reducing sugar, and that the syrup C_1'' was enriched with the non-reducing component.

In an attempt to obtain the non-reducing sugar in a pure condition, fraction C_1'' (0·36 g.) was acetylated with anhydrous sodium acetate (0·36 g.) and acetic anhydride (4·0 ml.) at 80—100° for 4 hr. The yellow mixture was poured into water, and the resulting crystalline acetates were recrystallised from methanol. The product had m. p. 180—240°, indicating that no fractionation of acetates had occurred. Further recrystallisation did not alter the m. p. (Found: C, 49·5; H, 5·8; OAc, 49·6. Calc. for $C_{22}H_{30}O_{15}$: C, 49·4; H, 5·7; OAc, 48·3%).

Fraction C_1'' (0·51 g.) was dissolved in water and reduced with sodium amalgam, the pH being kept at 7—9 by addition of acetic acid. The non-reducing solution was then freeze-dried, and the residue acetylated with pyridine and acetic anhydride. The resulting mixture of acetates crystallised in part; the crystalline portion, recrystallised from ethanol, had m. p. 242—250° and $[\alpha]_D$ +159° (*c* 1·25 in $CHCl_3$) (Found: C, 49·8; H, 5·7; OAc, 49·7. $C_{22}H_{30}O_{15}$ requires C, 49·4; H, 5·7; OAc, 48·3%).

This crystalline *acetate* was de-acetylated catalytically. A crystalline *sugar* was obtained which, after recrystallisation from moist methanol, had m. p. 269—272°, $[\alpha]_D$ +210° (*c* 1·0) (Found: C, 42·7; H, 6·4. $C_{10}H_{18}O_9$ requires C, 42·6: H, 6·4%). Hydrolysis of this material gave xylose only. Oxidation with lead tetra-acetate resulted in the uptake of 4 mols. of oxidant and the liberation of 2 moles of formic acid per mole of sugar, but no formaldehyde (chromotropic acid test).[11]

3-O-α-D-*Xylopyranosyl*-D-*xylose.*—After recrystallisation from methanol, fraction C_1' had m. p. 178° and $[\alpha]_D$ +106° (5 min.), +118° (75 min., const.; *c* 1·0). The compound moved as one spot on paper chromatograms and had R_{gal} 1·05 (solvent *a*) and 1·25 (solvent *b*).

The disaccharide was oxidised by lead tetra-acetate in 5 hr. with the consumption of 3 mols. of lead tetra-acetate and formation of 1 mol. of formic acid, but no formaldehyde. In the non-catalysed oxidation (no potassium acetate) of the disaccharide,[5] 1 mol. of oxidant was consumed in 5 min. and, after hydrolysis of the oxidised compound, xylose and threose were detected chromatographically.

The crystalline disaccharide (10 ml.) was reduced in water with potassium borohydride.[12] The solution was neutralised with acetic acid and de-ionised with Amberlite resins IR-120 and IR-4B. Concentration then gave a syrup (8 mg.) which moved as one spot on paper chromatograms (R_{gal} 1·0, solvent *a*). It was not detectable with the *p*-anisidine hydrochloride spray. A portion of the syrup was oxidised with periodate at pH 8 and the yield of formaldehyde was determined colorimetrically (chromotropic acid test): 1·29 (3 min.); 1·61 (8 min.); 2·24 (20 min.); 2·42 (50 min.); 2·53 (350 min.); 2·45 equivs. (500 min.).

Identification of Two Disaccharides in Fraction C_2 as 4- *and* 2-O-α-D-*Xylopyranosyl*-D-*xylose.*—Fraction C_2, a syrup, had $[\alpha]_D$ +77° (*c* 7·1) and did not yield a crystalline osazone. It behaved as a single compound on paper chromatograms. A portion (0·50 g.) was methylated with methyl sulphate and alkali in the usual way. The product (0·39 g.) had n_D^{20} 1·4627 and crystallised in part. The crystals were separated and, after recrystallisation from *n*-hexane,

[11] Boyd and Logan, *J. Biol. Chem.*, 1942, **146**, 279.
[12] Wolfrom and Wood, *J. Amer. Chem. Soc.*, 1951, **73**, 2933; Abdel-Akher, Hamilton, and Smith, *ibid.*, p. 4691.

the *ether* had m. p. 109°, $[\alpha]_D$ +50° (*c* 1·86 in MeOH) (Found: C, 52·5; H, 8·1; OMe, 50·5. $C_{16}H_{30}O_9$ requires C, 52·5; H, 8·3; OMe, 50·8%).

A portion (85 mg.) of the ether was hydrolysed by N-sulphuric acid at 90° for 5 hr. and the component sugars were separated on sheets of filter paper (solvent *a*). The faster-moving sugar (30 mg.) was identified as 2 : 3 : 4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 86—88°. The slower-moving compound (25 mg.) crystallised and had m. p. 82—85°, not depressed on admixture with 2 : 3-di-*O*-methyl-D-xylose, m. p. 86—88°. The identity was confirmed by *X*-ray powder photography.

The syrup remaining after the removal of the crystalline methylated disaccharide had $[\alpha]_D$ +74° (*c* 3·5 in MeOH). A portion was hydrolysed by N-sulphuric acid at 100° for 2 hr. and the syrupy reducing sugars were isolated and chromatographed. The faster-moving component was identified as 2 : 3 : 4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 85—87°, $[\alpha]_D$ +42° (7 min.) ⟶ +18° (80 min., constant). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-α-D-xylopyranosylamine had m. p. and mixed m. p. 103—105°.

The slower-moving component did not crystallise. It moved as one spot on paper chromatograms in all solvents used and had the same rate of movement as 2 : 3-di-*O*-methyl-D-xylose. It gave a brownish-red colour with the *p*-anisidine hydrochloride spray, whereas the 2 : 3- and 2 : 4-di-*O*-methyl derivatives both gave deep pink-red colours. The syrup was oxidised with bromine water, and the methylated xylonic acid was converted into the lactone by heating it at 90° under reduced pressure for 30 min. The lactone readily crystallised and, after recrystallisation from ether–*n*-hexane, had m. p. 68°, not depressed on admixture with 3 : 4-di-*O*-methyl-D-xylonolactone.

4-O-β-D-*Xylopyranosyl*-D-*xylose*.—Fraction C_3 crystallised and was recrystallised twice from moist ethanol. The crystals had m. p. 195—197°, undepressed on admixture with xylobiose obtained from aspen wood sawdust. On chromatograms, the two sugars were indistinguishable. They had $[\alpha]_D$ −40° (3 min.) ⟶ −27° (1 hr., constant; *c* 2·5). Both had the same *X*-ray powder photograph. The derived phenylosazone had m. p. and mixed m. p. 210—213° (decomp.).

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