124. *Cereal Gums. Part III.* The Constitution of an Araboxylan from Barley Flour.*

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A water-soluble polysaccharide from barley flour gave xylose (59%), arabinose (37%), and glucose (4%) on hydrolysis. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-tri-*O*-methyl-L-arabinose (3 parts), 2 : 3-di-*O*-methyl-D-xylose (3 parts), a mixture (1 part) of 2- and 3-*O*-methyl-D-xylose, and D-xylose (1 part). It is concluded that the highly branched barley araboxylan contains chains of 1 : 4-linked β-D-xylopyranose residues to some of which terminal L-arabofuranose residues are attached through positions 2 and 3.

Barley gum, isolated from the grain by aqueous extraction, is a mixture of polysaccharides containing residues of glucose, xylose, and arabinose.[1] Preece and Mackenzie [1] isolated the major component, a β-glucan, by fractional precipitation from aqueous solution by addition of ammonium sulphate. It was shown in Part I [2] that this polysaccharide contains unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages. Other fractions from barley gum gave mainly xylose and arabinose on hydrolysis, but a pure pentosan was not obtained by this method. An indication that the barley pentosan is similar to that found in wheat flour [3] was obtained by Gilles, Meredith, and Smith [4] who isolated three components on fractionation of methylated barley gum: (*a*) a methylated araboxylan; (*b*) a methylated α-glucan; and (*c*) a methylated β-glucan. Hydrolysis of the methylated araboxylan gave 2 : 3 : 5-tri-*O*-methylarabinose (1 part), 2 : 3-di-*O*-methylxylose (12 parts), 2-*O*-methylxylose (4 parts), and xylose (2 parts). As part of a series of structural investigations of the polysaccharide components of barley,[2,5,6] we now report a more detailed study of the barley-flour araboxylan.

Barley gum, isolated from the flour by aqueous extraction at 40°,[1] was fractionated by addition of ammonium sulphate to the aqueous solution. Fractions rich in pentosan, but still containing appreciable quantities of β-glucan, were precipitated at high concentrations of ammonium sulphate. Acetylation of the mixture of polysaccharides, followed by fractional precipitation of the acetates from acetone solution by the addition of light

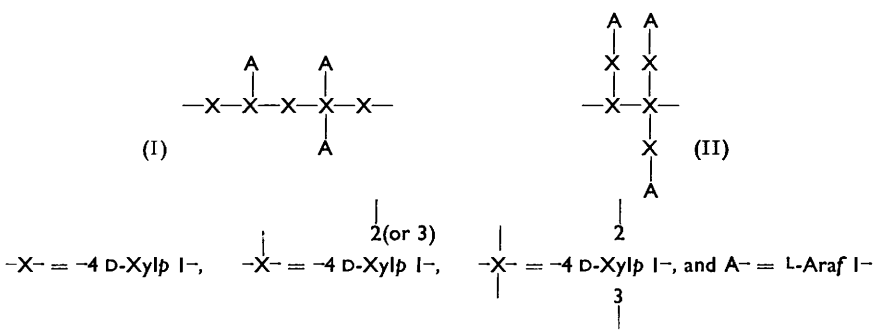
* Part II, *J.*, 1957, 4469.

[1] Preece and Mackenzie, *J. Inst. Brewing*, 1952, **58**, 353, 457.
[2] Perlin, *Cereal Chem.*, 1951, **28**, 370, 382.
[3] Montgomery and Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 2834, 3325.
[4] Aspinall and Telfer, *J.*, 1954, 3519.
[5] McWilliam and Percival, *J.*, 1951, 2259; Aspinall, Hirst, and McArthur, *J.*, 1955, 3075.
[6] Aspinall and Ferrier, *J.*, 1957, 4188.

petroleum, afforded substantially pure acetylated araboxylan. Hydrolysis of a sample of pentosan, regenerated from the acetate, gave xylose (59%), arabinose (37%), and glucose (4%).

The acetylated araboxylan was simultaneously deacetylated and methylated. Hydrolysis of a sample of the methylated polysaccharide and quantitative paper chromatography of the hydrolysate [7] showed the presence of tri-*O*-methyl- (41%), di-*O*-methyl- (36%), mono-*O*-methyl- (10%), and unsubstituted (13%) pentose. The mixture of methylated sugars obtained on hydrolysis of the methylated polysaccharide was fractionated on cellulose to give 2 : 3 : 5-tri-*O*-methyl-L-arabinose (39%), 2 : 3-di-*O*-methyl-D-xylose (36%), and D-xylose (14%), characterised as crystalline derivatives, and a mixture of mono-*O*-methylxyloses (11%). Paper ionophoresis showed that the mono-*O*-methylxylose fraction contained both the 2- and the 3-methyl ether, and the optical rotation of the syrupy mixture of sugars showed these to be present in the approximate ratio of 2 : 1. A portion of the mixture was converted into the corresponding mixture of methyl pyranosides, and the consumption of periodate, 0·76 mol., indicated that the 2- and the 3-methyl ether were present in the ratio of 3 : 1.

Several possible structures for the repeating unit of the polysaccharide may be put forward on the basis of these results, (I) and (II) being typical. In structure (I) the backbone of D-xylopyranose residues is linear and the L-arabofuranose residues are attached as single unit side-chains. In structure (II), on the other hand, the backbone of xylose residues is branched and the longer side-chains are terminated by arabofuranose residues. The following results provide evidence in favour of structure (I). The methylated araboxylan was heated with dilute aqueous methanolic hydrogen chloride under controlled conditions. The degraded methylated polysaccharide was separated from methylated sugars formed during the mild hydrolysis, and chromatographic examination of the sugars showed that about a quarter of the 2 : 3 : 5-tri-*O*-methylarabinose had been selectively removed, together with only a trace of di-*O*-methylxylose. The degraded methylated polysaccharide was remethylated and hydrolysed; quantitative estimation showed that 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose were present in the hydrolysate in the ratio of 11 : 1. For an araboxylan of structure (I) mild hydrolysis of the methylated polysaccharide followed by remethylation would cause no significant increase in xylose end-groups. On the other hand, with an araboxylan of structure (II), these operations would yield a methylated degraded polysaccharide with an increased number of xylose end-groups, each terminal arabinose removed giving rise to a terminal xylose residue. Thus, removal of approximately one quarter of the arabinose residues



followed by remethylation would result in the formation of methylated degraded araboxylan containing arabinose and xylose end-groups in the proportion of 3 : 1. It follows from the observed ratio of arabinose to xylose end-groups of 11 : 1 that the majority of arabinose residues in the barley araboxylan must be attached directly to the backbone of xylose residues as in structure (I).

[7] Hirst, Hough, and Jones, *J.*, 1949, 928.

It is clear from these results that the barley-flour araboxylan is similar to other polysaccharides of the xylan group [8] in containing chains of 1 : 4-linked β-D-xylopyranose residues to which are attached single-unit L-arabofuranose side-chains. The polysaccharide resembles most closely the araboxylans from wheat [3] and rye [9] flour, differing only in that some of the singly-branched xylose residues carry arabinose residues attached to $C_{(2)}$ whereas in other xylans arabinose residues are linked to $C_{(2)}$ only in the case of doubly-branched xylose residues carrying a substituent at $C_{(3)}$ also. It is of particular interest that within the general structural pattern common to the various xylans from land plants [8] the araboxylans from cereal grains form a group of substances which differ from the typical xylans of lignified tissues in containing a higher proportion of arabinose but no glucuronic acid residues often found in the latter group. Barley-flour araboxylan and barley-husk hemicellulose [6] provide an example of polysaccharides from the same plant which differ in detailed chemical structure and probably also in biological function.

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5); (C) benzene–ethanol–water (169 : 47 : 15). Paper ionophoresis was in borate buffer at pH 10. Optical rotations were observed at 18° ± 2°. Methylated sugars were demethylated with hydrobromic acid.[10]

Extraction and Fractionation of Barley Gum.—Barley (Carlsberg variety, harvested in 1953) was passed through an automatic polishing machine to remove husks, and the grain was ground to a fine flour. Barley flour (8 kg.), previously extracted with boiling ethanol–water (4 : 1) to inactivate enzymes and remove soluble sugars, waxes, and colouring matter, was extracted with water at 40° according to Preece and Mackenzie's [1] procedure and gave barley gum (14 g.; 0·18% of the grain). Precipitation of the gum from aqueous solution by the addition of ammonium sulphate afforded fractions (1) (8 g.; mainly glucan precipitated with 20% ammonium sulphate) and (2) (5·7 g.; precipitated with 30% and 40% ammonium sulphate). Fraction (2) had $[\alpha]_D$ −125° ± 5° (*c*, 0·2 in N-sodium hydroxide) and quantitative chromatography of the hydrolysate [11] showed xylose (49%), arabinose (32%), and glucose (19%). Fraction (2) (5 g.) was dispersed in formamide (65 ml.), and pyridine (195 ml.) was added and then acetic anhydride (100 ml.) slowly with stirring during 4 hr. The mixture was stirred for 2 days at room temperature and poured into water (2 l.) to give acetylated polysaccharide (6·4 g.), which was exhaustively extracted with acetone leaving a residue (0·21 g.). Addition of light petroleum (b. p. 60—80°; 250 ml.) to the acetone extract (500 ml.) gave acetate (A) (3·8 g.), $[\alpha]_D$ −107° (*c*, 1·0 in pyridine) [Found: OAc, 34·3%], and concentration of the supernatant liquid gave acetate (B) (2·2 g.), $[\alpha]_D$ −39° (*c* 1·0 in pyridine) [Found: OAc, 35·5%]. A sample of acetate (A) (0·5 g.) was deacetylated by Zemplen and Pacsu's [12] method to give polysaccharide (0·266 g.), $[\alpha]_D$ −104° (*c* 0·6 in N-sodium hydroxide), hydrolysis and quantitative chromatography [11] showing xylose (59%), arabinose (37%), and glucose (4%). A sample of acetate (B) was hydrolysed directly and quantitative chromatography showed xylose (29%), arabinose (27%), and glucose (44%).

Methylation.—Acetylated polysaccharide (A) (2·9 g.) was methylated by successive treatments with methyl sulphate and sodium hydroxide, and fractional precipitation of the product from chloroform by the addition of light petroleum (b. p. 60—80°) gave fractions (C) (1·177 g.), $[\alpha]_D$ −140° (*c* 0·5 in chloroform) (Found: OMe, 37·3%), and (D) (0·412 g.), $[\alpha]_D$ −134° (*c* 0·5 in chloroform) (Found: OMe, 37·7%). Samples of (C) and (D) were hydrolysed, and chromatography showed tri-*O*-methylarabinose, di- and mono-*O*-methylxylose, and xylose; small amounts of tri-*O*-methylglucose were also detected in the hydrolysate from (D), but not in this experiment in that from (C). Further methylation of fraction (C) gave methylated araboxylan

[8] Hirst, *J.*, 1955, 2974; Aspinall and Schwarz, *Ann. Reports*, 1955, **52**, 261; D. C. C. Smith, *ibid.*, 1956, **53**, 257.
[9] Aspinall and Sturgeon, *J.*, 1957, 4469.
[10] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[11] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[12] Zemplen and Pacsu, *Ber.*, 1929, **62**, 1613.

(0·94 g.), $[\alpha]_D$ −137° (*c* 0·6 in chloroform) (Found: OMe, 38·2%). Hydrolysis of a sample of methylated araboxylan, and quantitative chromatography [7] of the hydrolysate showed the relative amounts of pentose sugars to be: tri-*O*-methylarabinose, 4·1 parts, di-*O*-methylxylose, 3·6 parts, mono-*O*-methylxylose, 1 part, and xylose 1·3 parts.

Hydrolysis of Methylated Araboxylan and Separation of Methylated Sugars.—Methylated araboxylan (0·9 g.) was hydrolysed successively with boiling methanolic 3% hydrogen chloride (100 ml.) for 3·5 hr. and 0·5N-hydrochloric acid (100 ml.) at 100° for 3 hr. After neutralisation with silver carbonate, concentration gave a syrupy mixture of methylated sugars (0·72 g.) which was fractionated on cellulose (40 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, and butan-1-ol partly saturated with water, as eluants, to give eight fractions.

Fraction 1. The syrup (197 mg.) had $[\alpha]_D$ −35° (*c* 0·5 in water) (Found: OMe, 47·9. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), and was chromatographically indistinguishable from 2 : 3 : 5-tri-*O*-methyl-L-arabinose in solvents B and C. Demethylation gave arabinose. The sugar was characterised by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 133—135°.

Fraction 2. Chromatography in solvent B showed the syrup (28 mg.) to contain 2 : 3 : 5-tri-*O*-methylarabinose and tri-*O*-methylglucose in the approximate ratio of 3 : 1. Chromatography in solvent C showed that a trace of 2 : 3 : 4-tri-*O*-methylxylose was also present. Demethylation gave arabinose, glucose, and a trace of xylose.

Fraction 3. Chromatography in solvent B showed the syrup (43 mg.) to contain tri-*O*-methylglucose and 2 : 3-di-*O*-methylxylose. The optical rotation ($[\alpha]_D$ +44°, in water) was consistent with the presence of tri-*O*-methylglucose (2 : 3 : 6- and 2 : 4 : 6-trimethyl ethers have $[\alpha]_D$ +70°, in water [13]) and 2 : 3-di-*O*-methyl-D-xylose ($[\alpha]_D$ +23° in water [15]) in the ratio 1 : 1·2.

Fraction 4. The chromatographically pure syrup (154 mg.) had $[\alpha]_D$ +25° (*c* 0·7 in water) (Found: OMe, 33·5. Calc. for $C_7H_{14}O_5$: OMe, 35·0%), and gave xylose on demethylation. The sugar was characterised as 2 : 3-di-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 123—124°.

Fraction 5. The syrup (45 mg.) travelled on the chromatogram in solvent B at the same rate as 2- and 3-*O*-methyl-D-xylose, and ionophoresis showed both methyl ethers to be present (Found: OMe, 18·6. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Demethylation gave xylose. The optical rotation ($[\alpha]_D$ +29°, in water) was consistent with the presence of 2-*O*-methyl-D-xylose ($[\alpha]_D$ +17°) [15] and 3-*O*-methyl-D-xylose ($[\alpha]_D$ +35°) [16] in the ratio 2 : 1. The syrupy mixture of methyl pyranosides, prepared by refluxing the syrup with dry methanolic hydrogen chloride, consumed 0·76 mol. of periodate (spectrophotometric determination [17]).

Fraction 6. The syrup (9 mg.) contained an unknown sugar *a* (R_G 0·30 in solvent B) and xylose in the approximate ratio of 2 : 1. Hydrolysis of sugar *a* gave di- and mono-*O*-methylxylose in about equal amounts. In subsequent calculations 3 mg. of each were added to the quantities of xylose, mono-, and di-*O*-methylxylose isolated in pure fractions.

Fraction 7. Chromatographically pure D-xylose (45 mg.) crystallised and had m. p. and mixed m. p. 139—142°, $[\alpha]_D$ +22° (equil.) (in water).

Fraction 8. Chromatography showed the syrup (23 mg.) to contain xylose and a number of slower-moving components. The syrup was hydrolysed and quantitative estimation indicated the presence in the mixture of residues of xylose (13 mg.) and mono- (4 mg.) and di-*O*-methylxylose (6 mg.). In subsequent calculations these quantities were added to the appropriate fractions.

The quantities of sugars isolated from the hydrolysis of the methylated araboxylan indicate their presence in the following molar percentages: 2 : 3 : 5-tri-*O*-methyl-L-arabihose (39%), 2 : 3-di-*O*-methyl-D-xylose (36%), mono-*O*-methyl-D-xylose (11%), and D-xylose (14%).

Partial Hydrolysis of Methylated Araboxylan.—Methylated araboxylan (fraction D, 56 mg.) was dissolved in methanol (5 ml.), 0·2N-hydrochloric acid (5 ml.) was added, and the mixture was refluxed for 4 hr. The solution was neutralised with Amberlite resin IR-4B, filtered, and freeze-dried. The residue was exhaustively extracted with boiling light petroleum

[13] See Bourne and Peat, *Adv. Carbohydrate Chem.*, 1950, **5**, 145.
[14] Chanda, Percival, and Percival, *J.*, 1952, 260.
[15] Percival and Willox, *J.*, 1949, 1608.
[16] White, *J. Amer. Chem. Soc.*, 1953, **75**, 257, 4692.
[17] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

(b. p. 60—65°), and chromatographic examination of the extract (4·5 mg.) showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3-di-*O*-methylxylose. The residual methylated polysaccharide was remethylated with methyl iodide and silver oxide and yielded methylated degraded araboxylan (36 mg.) (Found: OMe, 38·9%). The methylated polysaccharide was hydrolysed as described previously, the tri-*O*-methylpentose fraction was resolved chromatographically by solvent C, and quantitative estimation by Pridham's [18] method showed 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose to be present in the ratio of 11 : 1. On treatment with *p*-anisidine hydrochloride the sugars gave products having absorption maxima at 390 and 500 mμ, respectively, and it was shown that at these wavelengths and for weights of sugar from 10—40 μg. the absorption was directly proportional to the quantity of sugar.

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[18] Pridham, *Analyt. Chem.*, 1956, **28**, 1967.