900. *Cereal Gums. Part II.* The Constitution of an Araboxylan from Rye Flour.*

By G. O. Aspinall and R. J. Sturgeon.

A water-soluble polysaccharide isolated from rye flour gave on hydrolysis xylose (60%), arabinose (29%), and glucose (5%). From hydrolysis of the methylated polysaccharide, controlled acid hydrolysis of the polysaccharide, and quantitative estimation of xylose residues unattacked by periodate in the original and degraded polysaccharides it is concluded that this highly-branched araboxylan contains chains of 1 : 4-linked β-D-xylopyranose residues with approximately every second xylose residue carrying a terminal L-arabofuranose residue linked through position 3.

The water-soluble gums isolated from cereal grains contain residues of D-glucose, D-xylose, and L-arabinose in varying proportions. Hexosan-rich and pentosan-rich fractions may be obtained either by graded precipitation from aqueous solution by ammonium sulphate [1,2] or by fractional precipitation of the derived acetylated polysaccharides.[3,4] The β-glucans from barley [5] and oats [6] are similar to lichenin [7] in structure in containing chains of 1 : 3- and 1 : 4-linked D-glucopyranose residues. The pentosan from wheat flour has been studied by Perlin [3] and by Montgomery and Smith.[4] The L-arabinose residues are present exclusively as non-reducing end-groups in the furanose form, and it is probable that these units are directly attached to a backbone of 1 : 4-linked β-D-xylopyranose residues. It has been shown by Preece and Hobkirk [2] that the main component of the water-soluble gum fraction from rye flour is an araboxylan of similar composition to the polysaccharide from wheat flour. We are very grateful to Professor I. A. Preece for kindly placing at our disposal a quantity of the rye araboxylan for structural investigation, the results of which are described in this paper.

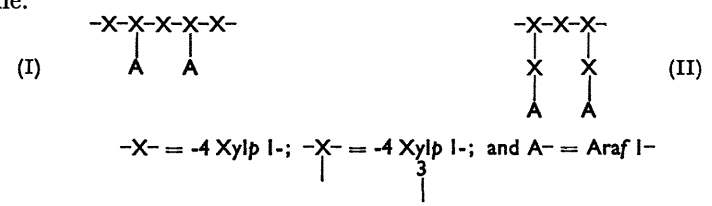
The polysaccharide had a high negative rotation ($[\alpha]_D$ −107° in N-NaOH) and yielded on hydrolysis xylose (60%), arabinose (29%), and glucose (5·5%). Hydrolysis of the derived methylated polysaccharide afforded the following sugars, characterised by crystalline derivatives: 2 : 3 : 5-tri-*O*-methyl-L-arabinose (30%), 2 : 3-di-*O*-methyl-D-xylose (36%), 2-*O*-methyl-D-xylose (31%), and D-xylose (2·5%). In addition, chromatography showed traces of 2 : 3 : 4-tri-*O*-methylxylose, tri-*O*-methylglucose, and 3-*O*-methylxylose. These results indicate the presence in the polysaccharide of chains of 1 : 4-linked D-xylose residues with branching mainly through position 3. All the side-chains are terminated by L-arabofuranose residues, this being the sole mode of linkage of the arabinose residues. It is not certain whether the small amount of D-xylose isolated from the hydrolysis of the

* Part I, *J.*, 1954, 3519.

[1] Preece and Mackenzie, *J. Inst. Brewing*, 1952, **58**, 353, 457.
[2] Preece and Hobkirk, *ibid.*, 1953, **59**, 385.
[3] Perlin, *Cereal Chem.*, 1951, **28**, 370, 382.
[4] Montgomery and Smith, *J. Amer. Chem. Soc.*, 1955, **77**, 3325.
[5] Aspinall and Telfer, *J.*, 1954, 3519.
[6] Acker, Diemair, and Samhammer, *Z. Lebensm.-Untersuch.*, 1955, **100**, 180; **102**, 225.
[7] Chanda, Hirst, and Manners, *J.*, 1957, 1951.

methylated polysaccharide represents some double branching points or whether the sugar arises from incomplete methylation of the polysaccharide or demethylation during hydrolysis. It is probable that the glucose residues present in the polysaccharide and the tri-*O*-methylglucose isolated on hydrolysis of the methylated polysaccharide arise from a contaminating glucan since no methyl ethers of glucose could be detected in the hydrolysate of another fraction of methylated polysaccharide.

On the basis of the methylation results two probable structures (I and II) may be put forward for the repeating unit of the polysaccharide. The following results provide evidence in favour of structure (I). Hydrolysis of the periodate-oxidised polysaccharide indicated the presence in the polysaccharide of xylose (24—25%) residues unattacked by periodate. This value is slightly lower than would be expected if all the arabinose residues were attached to singly branched xylose residues (*ca.* 29%). Controlled hydrolysis of the polysaccharide caused selective cleavage of some of the arabofuranosyl linkages with the formation of a degraded polysaccharide, giving on hydrolysis xylose (60%) and arabinose (10%) (these and subsequent values are expressed as percentages of the undegraded polysaccharide). Hydrolysis of the periodate-oxidised degraded polysaccharide indicated the presence therein of xylose (8%) residues unattacked by periodate. The reduction (*ca.* 16—17%) in xylose residues unattacked by periodate accompanying the controlled degradation of the polysaccharide corresponds approximately to the decrease (*ca.* 19%) in arabinose residues. This result would be expected on the basis of structure (I) only, and shows that the majority, at least, of the L-arabofuranosyl residues must be attached directly to position 3 of β-D-xylopyranose residues present in the essentially linear backbone of the molecule.



The araboxylan from rye flour is in many respects similar to the araboxylan from wheat flour,[3,4] notably in that the L-arabofuranose units, present only as end-groups, are attached directly to the backbone of 1 : 4-linked β-D-xylopyranose units. The main structural difference between the two polysaccharides lies in the mode of attachment of some of the arabofuranose residues. In the rye pentosan the majority at least of the arabinose residues are linked through $C_{(3)}$ of singly branched xylose residues, whereas in the wheat pentosan an appreciable proportion of arabinose residues are also linked through $C_{(2)}$ of doubly branched xylose residues. It is noteworthy that terminal L-arabofuranose units linked to $C_{(3)}$ of 1 : 4-linked β-D-xylopyranose units are commonly found in xylans from lignified tissues, especially of the *Gramineae*.[8]

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) benzene–ethanol–water (169 : 47 : 15, upper layer).

Rye araboxylan had $[\alpha]_D^{18}$ −107° (*c* 1·2 in N-NaOH); a sample was hydrolysed with N-sulphuric acid for 4 hr. at 100°, and chromatographic examination [9] of the hydrolysate in solvent A then showed the presence of xylose (60%), arabinose (29%), and glucose (5·5%).

Methylation of Rye Araboxylan.—The polysaccharide (2 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide. The product (1·8 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—80°), to give a main fraction (1·33 g.), soluble in chloroform–light petroleum

[8] Hirst, *J.*, 1955, 2974; Aspinall and Schwarz, *Ann. Reports*, 1955, **52**, 261.
[9] Flood, Hirst, and Jones, *J.*, 1948, 1679.

(3 : 7), which had $[\alpha]_D^{18}$ −113° (*c* 0·5 in $CHCl_3$) and was used in subsequent experiments (Found: OMe, 38·8%). Chromatography of the hydrolysate in solvent B showed tri-, di-, and mono-*O*-methylpentoses, together with smaller quantities of xylose and tri-*O*-methylglucose. A minor fraction (0·28 g.) of the methylated polysaccharide, soluble in chloroform–light petroleum (4 : 6), had $[\alpha]_D^{18}$ −121° (*c* 0·5 in $CHCl_3$), and chromatography of the hydrolysate showed the same pentose derivatives but no tri-*O*-methylglucose.

Hydrolysis of Methylated Araboxylan and Separation of Methylated Sugars.—The methylated polysaccharide (1·3 g.) was kept in N-hydrochloric acid (250 ml.) at 30° for 6 days, and the solution was then heated at 100° for 6 hr. After neutralisation with silver carbonate, concentration gave a syrupy mixture of sugars. The syrup (1·06 g.) was fractionated on cellulose (60 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water as eluant to give five fractions.

Fraction 1. The syrup (273 mg.) had $[\alpha]_D^{18}$ −36·3° (*c* 0·45 in H_2O) (Found: OMe, 46·4. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Chromatography in solvent C showed 2 : 3 : 5-tri-*O*-methylarabinose and a trace of 2 : 3 : 4-tri-*O*-methylxylose, and demethylation gave arabinose and a trace of xylose. The major component was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 130—132°.

Fraction 2. The syrup (105 mg.) had $[\alpha]_D^{18}$ +24·2° (*c* 0·44 in H_2O) (Found: OMe, 35·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), and chromatography in solvent B showed 2 : 3-di-*O*-methylxylose and a small amount of tri-*O*-methylglucose. The syrup crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 76—78°. The derived 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 121—122°. Approximate calculation from optical rotation indicated the presence in the fraction of 102 mg. of di-*O*-methyl-D-xylose and 3 mg. of tri-*O*-methyl-D-glucose.

Fraction 3. The chromatographically pure sugar (199 mg.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose, and had m. p. and mixed m. p. 80—81° and $[\alpha]_D^{18}$ −20·1° ⟶ +22·6° (equil.) (*c* 0·35 in H_2O) (Found: OMe, 34·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The aniline derivative had m. p. and mixed m. p. 121—123°.

Fraction 4. The crystalline sugar (237 mg.), after recrystallisation from methanol–water, had m. p. and mixed m. p. (with 2-*O*-methyl-β-D-xylose) 130°, and $[\alpha]_D^{18}$ −9·5° ⟶ +35° (equil.) (*c* 0·75 in H_2O) (Found: OMe, 18·7. Calc. for $C_6H_{12}O_5$: OMe, 18·8%). Ionophoretic examination of the mother-liquors showed that a small amount of the 3-methyl ether was also present.

Fraction 5. The syrup (17 mg.) travelled on the chromatogram at the same rate as D-xylose, had $[\alpha]_D^{18}$ +18° (*c* 0·75 in H_2O), and was characterised by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 208—209°.

Estimation of Sugar Residues Unattacked by Periodate.—The polysaccharide (352 mg.) was dissolved in water (10 ml.), sodium metaperiodate (792 mg.) was added, and the solution set aside in the dark for 4 days. Excess of barium chloride solution was added, insoluble barium salts were filtered off, and the filtrate was dialysed for 3 days. Concentration of the solution to small volume and addition of acetone (10 vol.) precipitated the periodate-oxidised polysaccharide (190 mg.). Hydrolysis of this material with N-sulphuric acid for 4 hr. at 100° and chromatographic examination [9] of the hydrolysate, using galactose as reference sugar, showed the presence of xylose (24%).

The polysaccharide (502 mg.) was dissolved in 0·01N-oxalic acid (50 ml.) and heated on the boiling-water bath for 1·5 hr. Ethanol (5 vol.) was added to the cooled solution, and degraded polysaccharide (387 mg.) was precipitated. Chromatography of the supernatant liquor showed only arabinose. Hydrolysis of the degraded polysaccharide afforded xylose (60%) and arabinose (10%) (these and the subsequent value are expressed as percentages of the undegraded polysaccharide). The degraded polysaccharide was converted into the corresponding periodate-oxidised polysaccharide, hydrolysis of which with N-sulphuric acid for 4 hr. at 100°, followed by chromatography [9] of the hydrolysate, showed xylose (8%).

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice, Professor I. A. Preece for the supply of rye araboxylan, the Department of Scientific and Industrial Research for the award of a Maintenance Allowance (to R. J. S.), and the Rockefeller Foundation and Imperial Chemical Industries Limited for grants.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, June 24th,* 1957.]