Cereal Gums. Part I. The Methylation of Barley Glucosans.

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

[Reprint Order No. 5440.]

Two samples of water-soluble lævorotatory glucosan from barley grain have been shown to be composed solely of D-glucose residues. Hydrolysis of the methylated polysaccharides gave 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucoses. It is concluded that these barley glucosans contain unbranched chains of β-D-glucopyranose residues with approximately equal proportions of 1 : 3- and 1 : 4-linkages.

The cereal gums are the non-starchy water-soluble polysaccharides found in cereal grains (for a review see Preece, *Proc. Eur. Brew. Conv., Brighton*, 1951, 213). Hydrolysis of various cereal gums has shown that residues of D-glucose, D-xylose, and L-arabinose are always present and sometimes in addition smaller amounts of D-galactose and D-mannose. Structural investigations so far carried out have been concerned mainly with fractions rich in pentosan isolated from wheat. Ford and Peat (*J.*, 1941, 856) isolated from wheat grain a water-soluble polysaccharide associated with β-amylase and showed that a highly branched molecule containing L-arabofuranose, D-xylopyranose and D-galactopyranose residues was present. More recently, Perlin (*Cereal Chem.*, 1951, **28**, 370, 382) has shown wheat flours to contain a mixture of water-soluble pentosans and hexosans, the pentosan being an araboxylan containing L-arabofuranose residues attached as side-chains to a main chain of 1 : 4-linked β-D-xylopyranose residues.

A considerable advance in understanding the nature and properties of these water-soluble gums has been made recently by Preece and Mackenzie (*J. Inst. Brewing*, 1952, **58**, 353) who have succeeded in preparing a lævorotatory glucosan free from pentosan by fractionation of barley extracts. Barley grain, previously extracted with boiling 80% aqueous ethanol to remove free sugars and oligosaccharides and to inactivate enzymes, was extracted with water at 40° and the aqueous extracts were fractionated by the addition of ammonium sulphate. A sample of barley glucosan prepared under these mild conditions was kindly placed at our disposal for structural investigations by Professor I. A. Preece and Dr. K. G. Mackenzie of the Heriot-Watt College, Edinburgh. We were also provided with a sample of glucosan isolated from barley extracts modified during the preparation by digestion with an enzymically active barley extract. These glucosans are of great importance in malting as Preece and Mackenzie (*loc. cit.*) have shown that barley enzymes, during germination, produce a rapid hydrolysis so that little of these polysaccharides survives in malt.

Both samples of glucosan (from unmodified and from modified barley) were lævorotatory ($[\alpha]_D^{15}$ −12·5° and −13° respectively in H_2O) and gave on hydrolysis only glucose (96—97%). Hydrolysis of the corresponding methylated glucosans gave mixtures of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucose together with small quantities of an unidentified di-*O*-methylglucose (probably arising from undermethylation of the polysaccharides and/or demethylation during hydrolysis). Both tri-*O*-methyl-D-glucoses were obtained crystalline and the 2 : 4 : 6-isomer was also converted into its aniline derivative. No evidence was obtained for the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in the hydrolysate of the methylated unmodified glucosan, but chromatographic evidence suggested that a small quantity (<0·5%) of this sugar was present in the hydrolysate of the methylated modified glucosan. The proportions of the 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucoses were estimated by following the changes in optical rotation in cold methanolic hydrogen chloride of the hydrolysates from both methylated glucosans (cf. Granichstädten and Percival, *J.*, 1943, 54) : the contributions of minor components being neglected, the two isomers were found to be present in practically equal amounts.

Molecular-weight determinations by the isothermal-distillation method and by osmotic-pressure measurements (by the courtesy of Mr. W. N. Broatch and Dr. C. T. Greenwood) gave values of *ca.* 20,000 (degree of polymerisation *ca.* 100) for the methylated glucosan

from unmodified barley. It is difficult, therefore, to understand the complete absence of tetra-*O*-methyl-D-glucose arising from a non-reducing end-group unless a loop structure is postulated for the polysaccharide. However, in the light of our knowledge of the structure of other polysaccharides, such a loop appears unlikely, although it cannot be excluded. Although some evidence was obtained for the presence of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose in the hydrolysate of the methylated glucosan from modified barley, the quantity indicated was considerably smaller than would be expected from a linear molecule of similar size. In all other respects, the two samples of glucosan were closely similar. An ultracentrifugal examination of the glucosan from unmodified barley (by the courtesy of Dr. C. T. Greenwood) indicated the presence of only one component and thus suggested the presence of a single polysaccharide containing 1 : 3- and 1 : 4-linked β-D-glucopyranose residues rather than a mixture of two molecular species.

During the present investigation the preliminary results of structural investigations of the water-soluble polysaccharides of barley grain have been published elsewhere. Gilles, Meredith, and Smith (*Cereal Chem.*, 1952, **29**, 314) showed that the aqueous extract of barley flour (barley gum) gave glucose, xylose, and arabinose on hydrolysis, but did not attempt the isolation of individual components. Fractionation of methylated barley gum gave three components: (*a*) a methylated araboxylan (cf. Perlin, *loc. cit.*); (*b*) a methylated poly-α-glucosan, similar to methylated starch; and (*c*) a methylated poly-β-glucosan ($[\alpha]_D$ −9° in acetone). These workers, however, isolated only 2 : 3 : 6-tri-*O*-methyl-D-glucose from the hydrolysis of the methylated poly-β-glucosan and concluded that this component was probably structurally related to cellulose. It appears more likely that this component was similar to our methylated polysaccharides.

The present investigation indicates that barley glucosans contain unbranched chains of β-D-glucopyranose residues containing approximately equal numbers of 1 : 3- and 1 : 4-linkages. These polysaccharides are therefore structurally similar to lichenin (Meyer and Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751; Chanda and Hirst, unpublished work). It is interesting that the enzyme lichenase is found in the seeds of most plants (Karrer and his co-workers, *Helv. Chim. Acta*, 1924, **7**, 144, 159, 916) and that the isolation of lichenin from oat seeds has been claimed (Morris, *J. Biol. Chem.*, 1942, **142**, 881). Preece and Mackenzie's study (*J. Inst. Brewing*, 1952, **58**, 457) of the distribution of pentosans and hexosans in the water-soluble gums of the common cereals (including oats) suggests that the so-called lichenin from oats is similar to the glucosans of barley. Further investigations will be necessary before it can be decided whether these glucosans are truly linear in structure and whether the 1 : 3- and 1 : 4-linkages are regularly or randomly distributed.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems: (*a*) ethyl acetate–acetic acid–water (3 : 1 : 3; v/v; top layer); (*b*) butan-1-ol–ethanol–water (4 : 1 : 5; v/v; top layer); (*c*) benzene–ethanol–water (169 : 47 : 15; v/v; top layer clarified with ethanol).

Glucosan from unmodified barley

The polysaccharide was prepared, and kindly made available to us, by Professor I. A. Preece and Dr. K. G. Mackenzie (see *J. Inst. Brew.*, 1952, **58**, 353). It had $[\alpha]_D^{15}$ −12·5° (*c*, 1·0 in H_2O) and chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1569) in solvent (*a*) showed the presence of glucose (97%) only.

Methylation of the Glucosan.—The glucosan (6·5 g.) was methylated five times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature and once with methyl iodide and silver oxide, and the product (6·8 g.) (Found: OMe, 45·0%) isolated by dissolution in chloroform. Fractionation was effected by refluxing chloroform–light petroleum (b. p. 60—80°) mixtures of different compositions. Two main fractions were obtained and these were combined for subsequent work:

Fraction	% of $CHCl_3$ in solvent	$[\alpha]_D^{17}$ (*c*, 1·0 in $CHCl_3$)	OMe, %	Wt. (g.)
1	20	−5·0°	44·8	2·5
2	25	−5·5	45·0	2·1

Hydrolysis of Methylated Glucosan.—The methylated glucosan (4·1 g.) was refluxed with methanolic 1% hydrogen chloride (200 c.c.) for 6 hr. (constant rotation). Then the solution was neutralised with silver carbonate and concentrated, and the resultant syrup was hydrolysed on the water-bath with 2% hydrochloric acid (160 c.c.) for 3 hr. (constant rotation). After neutralisation with silver carbonate the aqueous solution was concentrated to a syrup (4·27 g.). Chromatographic examination in solvent (*b*) showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucoses and a trace of a di-*O*-methylglucose.

Separation of Methylated Sugars and Examination of Fractions.—The syrup (3·79 g.) was fractionated on cellulose (70 × 3 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, as eluant, to give five fractions.

Fraction 1. The syrup (0·808 g.) was non-reducing. A sample was further hydrolysed and chromatographic examination of the hydrolysate showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucoses.

Fraction 2. Chromatographic examination of the syrup (0·188 g.) showed the presence of 2 : 3 : 6-tri-*O*-methylglucose together with a small quantity of a substance travelling faster on the chromatogram. Separation on filter sheets with solvent (*c*) gave fractions 2*a* (0·152 g.) and 2*b* (0·030 g.). Fraction 2*a* crystallised and after two recrystallisations from dry ether had m. p. 120—122° (unchanged on admixture with authentic 2 : 3 : 6-tri-*O*-methyl-D-glucose, but depressed on admixture with authentic 2 : 4 : 6-tri-*O*-methyl-D-glucose) and $[\alpha]_D^{16}$ +70° (equil.) (*c*, 1·0 in H_2O) (Found : C, 48·5; H, 7·9; OMe, 41·2. Calc. for $C_9H_{18}O_6$: C, 41·9; H, 8·1; OMe, 41·9%).

Fraction 3. Chromatographic examination of the syrup (2·847 g.) showed 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose. Fractions 1, 2*b*, and 3 were combined and rehydrolysed to give a syrup (3·51 g.), which had $[\alpha]_D^{16}$ +70° ⟶ +18° (*c*, 1·9 in methanolic 1% hydrogen chloride) and showed only 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose on the chromatogram.

Fraction 4. The syrup (0·238 g.) crystallised and after two recrystallisations from dry ether had m. p. and mixed m. p. (with authentic 2 : 4 : 6-tri-*O*-methyl-D-glucose) 120—122° and $[\alpha]_D^{17}$ +72° (equil.) (*c*, 1·5 in H_2O) (Found : C, 48·6; H, 7·8; OMe, 41·4. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·1; OMe, 41·9%). The derived 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. 144—145° (from ethanol–light petroleum) and 162—164° (from ethyl acetate).

Fraction 5. The syrup (14 mg.), which showed a mixture of tri-, di-, and mono-*O*-methylglucose on the chromatogram, was not examined further.

Estimation of the Relative Proportions of Tri-O-methylglucoses.—A sample (*ca.* 300 mg.) of the hydrolysate of the methylated glucosan was rehydrolysed with 2% hydrochloric acid (30 c.c.) for 7 hr. on the water-bath. After neutralisation of the hydrolysate with silver carbonate, concentration of the solution gave a syrup which showed $[\alpha]_D^{18}$ +61° ⟶ +12° (*c*, 1·32 in methanolic 1% hydrogen chloride). A synthetic mixture of 2 : 3 : 6- (46%) and 2 : 4 : 6-tri-*O*-methyl-D-glucose (54%) showed $[\alpha]_D$ +71° ⟶ +18° (*c*, 1·0 in methanolic 1% hydrogen chloride).

Glucosan from modified barley

The glucosan had $[\alpha]_D^{15}$ −13° (*c*, 0·88 in H_2O) and chromatographic examination of the hydrolysate (Hirst and Jones, *loc. cit.*) in solvent (*a*) showed the presence of glucose (96%) only.

Methylation of the Glucosan.—The glucosan (4·5 g.) was methylated six times with methyl sulphate and sodium hydroxide solution under nitrogen at room temperature, and twice with methyl iodide and silver oxide. The product (3·7 g.) (OMe, 45·0%), $[\alpha]_D^{16}$ −5·3° (*c*, 1·0 in $CHCl_3$), was purified by precipitation from chloroform solution with light petroleum (b. p. 40—60°).

Hydrolysis of Methylated Glucosan.—The methylated glucosan (3·0 g.) was refluxed with methanolic 1% hydrogen chloride (175 c.c.) for 7 hr. (constant rotation). After neutralisation with silver carbonate the residual syrup was hydrolysed on the water-bath with 2% hydrochloric acid (150 c.c.) for 10 hr., the hydrolysate was neutralised with silver carbobate and the solution was taken to dryness to give a syrup (2·96 g.). Paper chromatographic examination in solvents (*b*) and (*c*) showed the presence of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose together with traces of tetra-*O*-methylglucose and a di-*O*-methylglucose.

Separation of Methylated Sugars and Examination of Fractions.—The syrup (2·68 g.) was fractionated on cellulose (70 × 3 cm.) with light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water, as eluant, to give six fractions.

Fraction 1. Chromatographic examination of the syrup (12 mg.) showed the presence of 2 : 3 : 4 : 6-tetra-*O*-methylglucose. The syrup, however, did not crystallise when seeded with authentic tetra-*O*-methyl-D-glucose and chromatographic examination after further hydrolysis

showed that it also contained an approximately equal amount of methyl 2 : 3 : 6-tri-*O*-methyl-D-glucoside.

Fraction 2. The syrup (0·41 g.) was non-reducing and was rehydrolysed with 2% hydrochloric acid (20 c.c.). The syrupy hydrolysate (0·40 g.) was examined chromatographically and shown to contain a mixture of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose.

Fraction 3. The syrup (1·15 g.) was chromatographically pure and crystallised. Two recrystallisations from dry ether gave 2 : 3 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 122—123°, $[\alpha]_D^{17}$ +71° (equil.) (*c*, 1·1 in H_2O) (Found: OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 4. The syrup (0·24 g.) was shown chromatographically to contain a mixture of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methylglucose.

Fraction 5. The syrup (0·78 g.) crystallised and chromatographic examination showed the presence of 2 : 4 : 6-tri-*O*-methylglucose together with some 2 : 3 : 6-tri-*O*-methylglucose. Three recrystallisations from dry ether gave 2 : 4 : 6-tri-*O*-methyl-D-glucose, m. p. and mixed m. p. 120—122°, $[\alpha]_D^{15}$ +73° (equil.) (*c*, 2·0 in H_2O). The identity of the sugars was confirmed by conversion into 2 : 4 : 6-tri-*O*-methyl-*N*-phenyl-D-glucosylamine, m. p. and mixed m. p. 162—164°.

Fraction 6. After separation of fraction 5 the column was eluted with butanol, partly saturated with water, to give a syrup (11 mg.) which travelled on the chromatogram at the rate of a di-*O*-methylglucose.

Estimation of the Relative Proportions of Tri-O-methylglucoses.—A sample (*ca.* 100 mg.) of the hydrolysate of the methylated glucosan was rehydrolysed with 2% hydrochloric acid (15 c.c.) for 4 hr. on the water-bath. After neutralisation of the hydrolysate with silver carbonate, concentration gave a syrup which showed $[\alpha]_D^{16}$ +61° ⟶ +15° (*c*, 0·87 in methanolic 1% hydrogen chloride).

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice, Professor I. A. Preece and Dr. K. G. Mackenzie for the supply of barley glucosans, and the University of Edinburgh for the award of a Post-graduate Studentship to one of them (R. G. J. T.).

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, June 3rd,* 1954.]