840. *The Constitution of Barley Husk Hemicellulose.*

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

Barley husk hemicellulose, containing a small proportion (*ca.* 4%) of glucuronic acid residues, gave on hydrolysis xylose and arabinose in the ratio of 6 : 1. 2-*O*-D-Xylopyranosyl-L-arabinose was identified amongst the products of mild acid hydrolysis. Hydrolysis of the methylated polysaccharide afforded 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 3 : 5-di-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)-xylose in the approximate molar ratio 1 : 1 : 2 : 14 : 3 : 1. It is concluded from these and other experiments that the polysaccharide is composed of chains of 1 : 4-linked β-D-xylopyranose residues to which are attached side-chains of L-arabofuranose and 2-*O*-D-xylopyranosyl-L-arabofuranose residues through position 3, and glucopyranuronic acid residues through position 2. A small degree of branching in the backbone of D-xylose residues is indicated.

Most lignified tissues contain polysaccharides composed mainly of D-xylose residues. The xylans from various land plants, so far examined, are similar in possessing backbones of 1 : 4-linked β-D-xylopyranose units, but differ in the nature and number of sugar units attached as side-chains.[1] In continuation of structural investigations of the polysaccharide components of barley carried out in these laboratories [2] we have now examined the hemicellulose of barley husk.

Barley husks, separated from the grain, were extracted with benzene–ethanol to remove

[1] Hirst, *J.*, 1955, 2974; Aspinall and Schwarz, *Ann. Reports*, 1955, **52**, 261.
[2] Percival and McWilliam, *J.*, 1951, 2259; Aspinall and Telfer, *J.*, 1954, 3519; Aspinall, Hirst, and McArthur, *J.*, 1955, 3075.

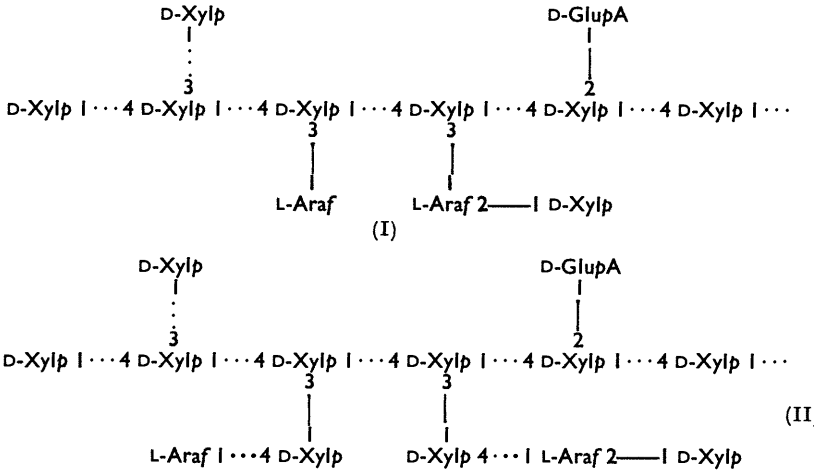
fats and colouring materials and with hot water to remove adhering starch. Extraction of the husks with cold aqueous sodium hydroxide afforded a hemicellulose containing about *ca.* 4% of uronic acid residues. This on hydrolysis gave xylose and arabinose in the ratio of 6 : 1, together with small quantities of acidic oligosaccharides and traces of glucose and galactose. Various attempts to fractionate the polysaccharide failed. A resistant acidic fraction was isolated after vigorous hydrolysis of the polysaccharide; reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis, gave xylose and glucose with traces of 4-*O*-methylglucose, indicating the presence in the polysaccharide of residues of glucuronic acid (a small proportion as the 4-methyl ether).

The hemicellulose was fully methylated; hydrolysis of the derivative afforded the following, characterised by the formation of crystalline derivatives: 2 : 3 : 4-tri-*O*-methyl-D-xylose (2 parts), 2 : 3-di-*O*-methyl-D-xylose (14 parts), 2-*O*-methyl-D-xylose (3 parts), and 3 : 5-di-*O*-methyl-L-arabinose (1 part). In addition, 2 : 3 : 5-tri-*O*-methyl-L-arabinose (1 part) and an acidic fraction (1 part) were isolated. The arabinose methyl ether was identified by chromatographic mobility and by yielding arabinose on demethylation, optical rotations of mixtures of this sugar and 2 : 3 : 4-tri-*O*-methyl-D-xylose indicating the sugar to be a derivative of L-arabinose. Chromatography of the acidic fraction showed the presence of at least two components, the minor one travelling on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The acidic fraction was converted into the methyl ester methyl glycoside, reduction of which followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose in the ratio of 1·2 : 1 together with a trace of 2 : 3-di-*O*-methylxylose. A portion of the reduced acidic fraction was remethylated and after hydrolysis yielded 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose. Although insufficient of the acidic fraction was available for the complete characterisation of its hydrolysis products, these observations show that the major component was 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)xylose. It follows from the methylation studies that this polysaccharide contains chains of 1 : 4-linked β-D-xylopyranose units to which are attached at least three types of side-chain, glucuronic acid units directly linked to xylose through position 2, side-chains terminated by L-arabofuranose units and linked to the backbone through position 3 of xylose, and side-chains terminated by D-xylopyranose units and linked to the backbone through position 3 of xylose. It is not possible to decide on the present evidence whether the small amount of D-xylose also isolated from the hydrolysis of the methylated polysaccharide is of structural significance, arising from a double-branching point, or whether the sugar results from incomplete methylation of the polysaccharide or demethylation during hydrolysis.

Evidence for the mode of attachment of the non-terminal L-arabofuranose residues follows from the isolation of 2-*O*-D-xylopyranosyl-L-arabinose from the products of mild acid hydrolysis of the polysaccharide. This disaccharide, isolated as the trihydrate, although differing slightly in physical constants from those reported by Whistler and McGilvray,[3] gave an *X*-ray powder photograph identical with that of an authentic sample kindly provided by Dr. D. I. McGilvray. It is probable that the xylopyranose residue of the disaccharide is derived from a non-reducing end-group in the polysaccharide since the disaccharide was released under relatively mild conditions of hydrolysis and no evidence was found for the presence in the hydrolysate of higher oligosaccharides containing arabinose residues. Although it is not possible to advance a unique structure for this hemicellulose, structures (I) and (II) are consistent with the results so far presented. The following evidence, however, shows that at least some terminal L-arabofuranose residues are linked directly to the backbone of D-xylose residues as in structure (I). Hydrolysis of the periodate-oxidised polysaccharide indicated the presence in the polysaccharide of

[3] Whistler and McGilvray, *J. Amer. Chem. Soc.*, 1955, **77**, 2212; see also Whistler and Corbett, *ibid.*, p. 3822.

xylose (21 ± 1·5%) and arabinose (6 ± 0·5%) residues unattacked by periodate. Controlled acid hydrolysis of the polysaccharide under mild conditions resulted in selective hydrolysis of some arabofuranosyl linkages with the formation of a degraded poly-



[Sugar residues joined by dotted lines may be linked either directly or through a chain of 1 : 4-linked D-xylopyranose residues].

saccharide. It was not possible, however, to remove all the arabinose residues without extensive hydrolysis of xylopyranosyl linkages. Hydrolysis of the periodate-oxidised degraded polysaccharide indicated the presence therein of xylose (16 ± 1·5%) and arabinose (5 ± 0·5%) residues unattacked by periodate. The decrease in xylose residues unattacked by periodate after selective removal of arabofuranose residues would be expected on the basis of structure (I), whereas no such decrease would result from the removal of arabofuranose residues in structure (II). The proportion of arabinose residues in the degraded polysaccharide, which are unattacked by periodate, indicates that the non-terminal arabofuranosyl linkages are more resistant to acid hydrolysis than terminal arabofuranosyl linkages. Indeed, under the conditions used in the isolation of 2-*O*-D-xylopyranosyl-L-arabinose, the disaccharide was accompanied by appreciable quantities of xylose-containing oligosaccharides. It is not possible, therefore, on the present evidence to decide whether these disaccharide side-chains are also attached directly to the xylan backbone.

A molecular-weight determination by the isothermal-distillation method (by courtesy of Drs. C. T. Greenwood and W. N. Broatch) gave a value of 10,500 ± 500 (degree of polymerisation, 66 ± 3) for the methylated polysaccharide. The methylation analysis indicated the presence of some six non-reducing D-xylopyranose end-groups per molecule; three such end-groups, as shown by the isolation of 2-*O*-D-xylopyranosyl-L-arabinose on partial acid hydrolysis of the original polysaccharide and by the quantity of 3 : 5-di-*O*-methyl-L-arabinose formed on hydrolysis of the methylated polysaccharide, must be linked to the non-terminal arabinose residues. The presence in the molecule, therefore, of three non-reducing D-xylopyranose end-groups linked to other xylose residues indicates some branching in the backbone of D-xylose residues. From the quantity of 2-*O*-methyl-D-xylose formed on hydrolysis of the methylated polysaccharide it is clear that these branch points must involve 1 : 3-linkages.

These results show that this barley husk hemicellulose contains many of the structural features encountered in other polysaccharides of the xylan group.[1] Here, as in the wood xylans and as in several xylans isolated from the Gramineae, glucuronic acid (either unsubstituted or as its 4-methyl ether) is found linked directly to position 2 of a xylose

residue in the backbone of the molecule. Again, terminal non-reducing L-arabofuranose residues, linked to the main chain through $C_{(3)}$ of the xylose residue, are commonly present in polysaccharides of this group. A novel feature of this xylan is the occurrence of non-terminal L-arabofuranose units found in the 2-*O*-D-xylopyranosyl-L-arabofuranose grouping. This disaccharide has been isolated previously from the partial acid hydrolysis of corn cob hemicellulose B;[3] since cleavage of the disaccharide was effected under mild conditions it is probable that in the latter case also this grouping is attached to the backbone of the molecule through an arabofuranosyl linkage. In the barley husk hemicellulose some branching in the backbone of xylose residues is clearly indicated. In the case of several other polysaccharides of this group, however, definite proof of the presence or absence of such branching must await the development of more precise methods of structural analysis.

In the course of the investigation the husk hemicellulose was isolated in a similar way from the corresponding sample of malted barley. A preliminary examination showed that the polysaccharide was qualitatively similar to that isolated from the original barley; thus the same sugar units were present, and chromatography showed that mild acid hydrolysis released arabinose and 2-*O*-xylopyranosylarabinose. Small quantitative differences were shown between the two polysaccharides, *e.g.*, the hemicellulose from the malted barley gave rather less formic acid on periodate oxidation than the barley hemicellulose. The somewhat higher glucosan content in the malted barley polysaccharide probably arose from incomplete removal of starchy polysaccharides since treatment with salivary α-amylase resulted in the formation of glucose and maltose. It is probable, therefore, that only minor changes have taken place in the barley husk hemicellulose during malting.

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); (*D*) butan-1-ol–formic acid–water (500 : 115 : 385; upper layer), (*E*) ethyl acetate–pyridine–water (10 : 4 : 3).

Isolation of Barley Husk Hemicellulose.—Barley (Carlsberg variety, harvested in 1953) (10 kg.) in batches (80 g.) was passed through an automatic polishing machine. The husks separated in this manner (*ca.* 10% of the grain) were contaminated with a large amount of starchy powder which was removed by means of a 20 mesh sieve, leaving behind the husk (470 g.) The husks were extracted for 24 hr. with boiling benzene–ethanol (2 : 1) in order to inactivate enzymes and to remove fats and colouring materials. Further extractions with cold water, hot water (75—80°), and with 0·01N-sodium hydroxide (under nitrogen) resulted in complete removal of starch and of water-soluble polysaccharides. The hemicellulose was extracted in the following manner: husks (80 g.) were extracted four times with N-sodium hydroxide (750 ml.), each extraction being carried out for 16 hr. in an atmosphere of nitrogen. The extracts were acidified with glacial acetic acid to pH 4—5 and the polysaccharide was precipitated by addition of acetone (0·8 vol.). The total yield (34 g.) of hemicellulose represented 0·34% by weight of the original barley or 7·2% of the weight of the husk. The polysaccharide had $[\alpha]_D^{16}$ −102° (*c* 0·5 in 0·5N-sodium hydroxide), and chromatographic examination of the hydrolysate by Flood, Hirst, and Jones's[4] method with solvent *A* showed the presence of xylose (67%), arabinose (11%), glucose (2%), and galactose (1%) [Found: ash, 1·2; lignin, 7·8; uronic anhydride (by decarboxylation), 3·7; OMe, 1·2%]. Attempts to fractionate the polysaccharide by precipitation of the copper complex and by precipitation from aqueous solution with ammonium sulphate failed to yield components significantly different in composition.

The polysaccharide (10 g.) was hydrolysed with 2N-sulphuric acid (150 ml.) for 4 hr. at 100°. The solution was neutralised with barium carbonate, the filtrate was concentrated to 50 ml.,

[4] Flood, Hirst, and Jones, *J.*, 1948, 1679.

barium ions were removed by treatment with Amberlite resin IR-100(H), and the solution was concentrated. The resulting syrup was fractionated on acid-washed charcoal–celite (1 : 1, w/w) (60 × 8 cm.), elution with water yielding monosaccharides and elution with ethyl methyl ketone–water (5 : 95, v/v) yielding acidic oligosaccharides together with some neutral sugars (50 mg.). Further fractionation on filter sheets with solvent *A* afforded an acidic oligosaccharide (17 mg.) and a trace of an unidentified sugar. The acidic component was converted into the methyl ester methyl glycoside, reduced with potassium borohydride, and hydrolysed, chromatography showing approximately equal amounts of xylose and glucose, and a trace of 4-*O*-methylglucose.

Methylation of Barley Husk Hemicellulose.—The polysaccharide (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide. The product (6·5 g.) was fractionated by precipitation from chloroform with light petroleum (b. p. 60—80°), giving two main fractions: 1, precipitated with 3 vol. of light petroleum {4·2 g.; $[\alpha]_D$ −92° ($CHCl_3$); OMe, 38·7%}, and 2, precipitated with 4 vol. of light petroleum {1·6 g.; $[\alpha]_D$ −95° ($CHCl_3$); OMe, 38·7%}; these fractions were combined and were used in subsequent experiments.

Hydrolysis of Methylated Hemicellulose and Separation of Methylated Sugars.—The methylated polysaccharide (4·4 g.) was hydrolysed successively with boiling methanolic 3% hydrogen chloride (500 ml.) for 2·5 hr. and with 0·5N-hydrochloric acid (500 ml.) at 100° for 4·5 hr. A small quantity (0·12 g.) of insoluble material was separated at this stage; further hydrolysis yielded no detectable sugars [Found: OMe, 31·0; lignin, 80%]. Evaporation after neutralisation with silver carbonate yielded a syrup (4·3 g.), which was treated (in aqueous solution) with barium carbonate (0·2 g.). The mixture of methylated sugars (4·02 g.) was fractionated on cellulose (90 × 3 cm.) by elution with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, butan-1-ol partly saturated with water, and with water to give nine fractions.

Fraction 1. The sugar (34 mg.) travelled on the chromatogram in solvent *B* at the same rate as 2 : 3 : 5-tri-*O*-methyl-L-arabinose and/or 2 : 3 : 4-tri-*O*-methyl-D-xylose. Demethylation showed only arabinose, and chromatography in solvent *C* showed only tri-*O*-methylarabinose (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Attempts to prepare crystalline derivatives failed.

Fractions 2 *and* 3. Chromatography showed that both these fractions (79 and 270 mg.) contained mixtures of 2 : 3 : 5-tri-*O*-methylarabinose and 2 : 3 : 4-tri-*O*-methylxylose. Calculations from the observed optical rotations, $[\alpha]_D$ −31° and +9° (H_2O), indicated that fractions 2 and 3 contained 86% and 19% respectively, of 2 : 3 : 5-tri-*O*-methyl-L-arabinose {2 : 3 : 5-tri-*O*-methyl-L-arabinose has $[\alpha]_D$ −39·5° (H_2O), and 2 : 3 : 4-tri-*O*-methyl-D-xylose has $[\alpha]_D$ +20° (H_2O)}.

Fraction 4. The sugar (80 mg.) travelled on the chromatogram in solvents *B* and *C* at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-xylose and slowly crystallised (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). After recrystallisation from acetone–light petroleum the sugar had m. p. and mixed m. p. 87—89° and $[\alpha]_D$ +64·5° (2 min.) ⟶ +20° (80 min., const.) (*c* 0·6 in H_2O). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 100—101°.

Fraction 5. The syrup (141 mg.) had $[\alpha]_D$ −16° (*c* 0·6 in H_2O) (Found: OMe, 35·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) and on demethylation yielded arabinose. Its rate of movement on the chromatogram in solvent *B* was the same as that of 2 : 5-di-*O*-methyl-L-arabinose, but the brown coloration (and yellow fluorescence in ultraviolet light) with aniline oxalate differed markedly from the grey coloration (and pink fluorescence in ultraviolet light) given by the 2 : 5-isomer. The sugar was further distinguished from the 2 : 5-dimethyl ether by its considerably greater ionophoretic mobility. The sugar was identified as 3 : 5-di-*O*-methyl-L-arabinose by conversion into 3 : 5-di-*O*-methyl-L-arabonolactone, m. p. 68—71°, which gave an *X*-ray powder photograph (by courtesy of Dr. C. A. Beevers) identical with that of an authentic specimen, and into 3 : 5-di-*O*-methyl-L-arabonamide, identified by its m. p. and mixed m. p. 144·5—145° and by its *X*-ray powder photograph.

Fraction 6. The syrup (2·113 g.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 81—83° and $[\alpha]_D^{17}$ −20·6° (4 min.) ⟶ +24·9° (65 min., const.) (*c* 1·2 in H_2O) (Found: OMe, 35·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The identity of the sugar was confirmed by its conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine,

m. p. and mixed m. p. 123—124°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 133—134°.

Fraction 7. The syrup (412 mg.) slowly yielded a crystalline sugar together with a small quantity of syrup. The crystalline material was ionophoretically pure 2-*O*-methylxylose; the syrupy material contained mainly the 2-methyl ether with a trace of the 3-methyl ether. Recrystallisation afforded 2-*O*-methyl-β-D-xylose, m. p. and mixed m. p. 133—134°, $[\alpha]_D^{17}$ $-5{\cdot}6° \longrightarrow +34{\cdot}4°$ (90 min., const.) (*c* 1·4 in H_2O) (Found: OMe, 19·1. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The derived 2-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 125—126°.

Fraction 8. The sugar (34 mg.) had m. p. 140—142° and mixed m. p. (with D-xylose) 141—144°, and gave an *X*-ray powder photograph identical with that of D-xylose.

Fraction 9. The fraction (259 mg.) obtained by elution of the cellulose with water was purified by solution in hot methanol, and after removal of barium ions by treatment with Amberlite resin IR-120(H) yielded a syrup (154 mg.), $[\alpha]_D^{17}$ +96° (H_2O) (Found: OMe, 29·6. Calc. for a tetra-*O*-methylaldobiouronic acid, $C_{15}H_{26}O_{11}$: OMe, 32·4%). Chromatography in solvent *D* showed two components, the minor and faster-moving one travelling at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. On further hydrolysis the slower-moving component decreased in amount with the formation of more tri-*O*-methylglucuronic acid and of a mono-*O*-methylxylose. Some of the acidic fraction (50 mg.) was converted into the methyl ester methyl glycoside by refluxing it with methanolic 1% hydrogen chloride (25 ml.) for 6 hr. The product was dissolved in dry ether (25 ml.), and lithium aluminium hydride (50 mg.) was added slowly during 3 hr. to the boiling solution. Water was added to the cooled solution to destroy excess of hydride, and the mixture was acidified with sulphuric acid and extracted with chloroform. Hydrolysis of the extract with N-sulphuric acid for 5 hr. at 100° yielded two main components *a* and *b* together with a trace of 2 : 3-di-*O*-methylxylose. Quantitative determination showed that fractions *a* and *b* were present in the ratio of 1 : 1·2. Fraction *a* travelled on the chromatogram in solvent *B* at the same rate as 2- and/or 3-*O*-methyl-D-xylose; paper ionophoresis showed that the fraction was mainly 3-*O*-methylxylose with only a trace of the 2-methyl ether. Fraction *b* travelled on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucose and gave glucose on demethylation.

Another portion of the acidic fraction (30 mg.) was converted into the methyl ester methyl glycoside and reduced with lithium aluminium hydride as described previously. The reduction product was methylated with methyl iodide and silver oxide and the methylated disaccharide was hydrolysed with 2N-hydrochloric acid for 4 hr. at 100° yielding two sugars *c* and *d*. In solvent *B* sugar *c* travelled on the chromatogram slightly more slowly than 2 : 3-di-*O*-methyl-D-xylose and at a rate corresponding to 3 : 4-di-*O*-methylxylose. The ionophoretic mobility of sugar *c* was considerably greater than that of 2 : 3-di-*O*-methylxylose as would be expected for the 3 : 4-dimethyl ether. Sugar *d* was chromatographically indistinguishable from 2 : 3 : 4 : 6-tetra-*O*-methylglucose; a small sample was obtained crystalline, m. p. 76—84°

Partial Acid Hydrolysis of Hemicellulose and Isolation of 2-O-D-*Xylopyranosyl*-L-*arabinose.*—Barley husk hemicellulose (13 g.) was heated in aqueous 0·02N-oxalic acid (1·3 l.) at 96° for 3·5 hr. The cooled solution was neutralised with calcium carbonate, filtered, concentrated, and poured into ethanol (10 vol.) to precipitate material of high molecular weight. The supernatant liquid was concentrated to a syrup (*ca.* 3 g.) which was fractionated on charcoal–celite (1 : 1, w/w/) (25 × 4 cm.), elution with water giving monosaccharides (1·7 g.), and elution with ethanol–water (5 : 95) giving fractions (i) (0·39 g.) and (ii) (0·145 g.). Fraction (i) contained a major component with R_{xylose} 0·67 in solvent *E* and a minor component with R_{xylose} 0·59, travelling at the same rate as xylobiose. Fraction (ii) contained the same two components (mainly xylobiose) together with traces of slower-moving oligosaccharides having R_{xylose} 0·40, 0·28, and 0·14. The main component from fraction (i) crystallised readily and after several recrystallisations from ethanol–water had m. p. 97—98°, $[\alpha]_D^{19}$ $+53{\cdot}7° \text{ (6 min.)} \longrightarrow +33{\cdot}2°$ (2 hr., const.) (*c* 1·3 in H_2O) (Found: C, 36·1; H, 7·0. Calc. for $C_{10}H_{18}O_9,3H_2O$: C, 35·7; H, 7·2%). A molecular-weight determination from a single crystal *X*-ray photograph and a density measurement (kindly carried out by Mr. H. W. Erhlich) gave a value of 333 ± 6 (calc. for a disaccharide trihydrate, 336). Hydrolysis of the sugar gave xylose and arabinose, but after oxidation with bromine water hydrolysis gave only xylose. The sugar was chromatographically indistinguishable from and gave an *X*-ray powder photograph identical with that of 2-*O*-D-xylopyranosyl-L-arabinose.

Periodate Oxidation of Hemicellulose.—Hemicellulose (269 mg.) was dissolved in potassium chloride solution (0·56M; 60 ml.), and sodium metaperiodate (0·2M; 20 ml.) was added. The formic acid released, estimated by Anderson, Greenwood, and Hirst's method,[5] reached a constant value of 0·10 mole per pentose residue after 7 days. Oxidation of the polysaccharide with sodium metaperiodate solution indicated a consumption of periodate of 0·85 mole per pentose residue (constant after 2 days).

Estimation of Sugar Residues Unattacked by Periodate.—The polysaccharide (0·5 g.) in sodium metaperiodate solution (0·2M, 25 ml.) was set aside for 54 hr. Periodate and iodate ions were precipitated by the addition of barium chloride solution and the resulting solution was dialysed against distilled water for 3 days; concentration and precipitation with acetone afforded the periodate-oxidised polysaccharide (0·25 g.). Hydrolysis of this material and chromatographic examination of the hydrolysate,[4] using galactose as reference sugar, showed the presence of xylose (21 $\pm$ 1·5%) and arabinose (6 $\pm$ 0·5%).

The hemicellulose (0·8 g.) was heated with aqueous oxalic acid (0·02N; 80 ml.) at 96° for 1 hr. (these represent optimun conditions for the removal of arabinose residues without extensive degradation of the molecule). The cooled solution was neutralised with calcium carbonate, and the centrifugate was poured into acetone (10 vol.) to precipitate degraded polysaccharide. The mother liquor was concentrated and sugars (0·057 g.) were extracted with boiling methanol. Quantitative chromatography showed the presence in the methanol extract of arabinose and xylose in the ratio of 11 : 1 together with traces of xylosylarabinose and other oligosaccharides. The degraded hemicellulose was converted into the corresponding periodate-oxidised polysaccharide, hydrolysis of which afforded xylose (16 $\pm$ 1·5%) and arabinose (5 $\pm$ 0·5%) (these figures are expressed as percentages of the original hemicellulose).

Husk Hemicellulose from Malted Barley.—Malted barley (Carlsberg variety, harvested in 1953) (700 g.) was given a rapid treatment in an automatic milling machine, and most of the husks were removed from the fragmented grain particles. The husks (63 g.) were extracted in the manner described previously except that four extractions with hot water for 8 hr. each were required to remove the adhering starch. Alkaline extraction afforded hemicellulose (6·2 g.), which was further extracted with boiling ethanol–water (4 : 1) to ensure complete removal of contaminating sugars. The hemicellulose had $[\alpha]_D$ −81° (*c* 0·5 in 0·5N-NaOH) and gave on hydrolysis xylose, arabinose, and glucose in the ratio of 76 : 16 : 8. Digestion of a 1% solution of the polysaccharide with salivary α-amylase in phosphate–citrate buffer at pH 6·8 at 35° followed by chromatographic examination of the hydrolysate showed that maltose and glucose were formed. Partial acid hydrolysis of the polysaccharide yielded arabinose and xylopyranosylarabinose, together with traces of xylose and xylose-containing oligosaccharides. A sample of the hemicellulose was oxidised with potassium metaperiodate, and the formic acid released [5] reached a constant value of 0·09 mole per pentose residue after 11 days.

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice throughout the investigation. This work was carried out under the auspices of the Brewing Industry Research Foundation, to whom the authors are indebted for the award of a scholarship to one of them (R. J. F.). Thanks are also expressed to the Rockefeller Foundation and the Earl of Moray Endowment for grants.

Department of Chemistry,
The University of Edinburgh. [*Received, March 27th*, 1957.]

[5] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.