788. *The Catalytic Oxidation of Rye-flour Arabinoxylan.*

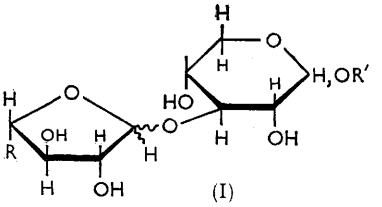
By G. O. Aspinall and I. M. Cairncross.

Catalytic oxidation of rye-flour arabinoxylan results in selective oxidation of some of the primary alcoholic groups with the formation of carboxylic acids. Graded hydrolysis of the oxidised polysaccharide affords an aldobiouronic acid, (3-D-xylose L-arabinofuranosid)uronic acid. The structural significance of this result is discussed.

Previous investigations[1,2] have shown that rye flour contains a water-soluble arabinoxylan in which a main chain of 1,4-linked β-D-xylopyranose residues carries terminal L-arabinofuranose residues as single-unit side-chains linked through position 3 of approximately every second xylose residue. Hitherto, the evidence for the mode of attachment of arabinose to xylose residues in the polysaccharide has been obtained, *indirectly*, by a comparison of the original polysaccharide and a degraded polysaccharide from which some of the acid-labile arabinofuranose residues have been removed by controlled acid-hydrolysis,[1] and, *directly*, by the isolation as a product of partial enzymic hydrolysis of the trisaccharide, *O*-L-arabinofuranosyl-*O*-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-D-xylose.[2] This paper describes the isolation of an aldobiouronic acid as a product of graded acid-hydrolysis after chemical modification of the polysaccharide. A preliminary account of these results has been published.[3]

The selective oxidation of primary alcoholic groups in simple hexopyranosides by gaseous oxygen in the presence of a platinum catalyst provides the simplest method for the formation of glycosiduronic acids.[4] This procedure has now been applied to the oxidation of a proportion of the primary alcoholic groups in the rye-flour arabinoxylan, which are found only in the terminal L-arabinofuranose residues. An acidic polysaccharide with a uronic acid content of 4% was isolated. Hydrolysis of the oxidised polysaccharide, followed by chromatographic separations of the products on charcoal–Celite and on filter sheets led to the isolation of an acidic disaccharide.

The acidic disaccharide was shown to be (3-D-xylose L-arabinofuranosid)uronic acid (I; R = CO_2H, R′ = H) by the following experiments. Reduction of the derived methyl ester methyl glycosides (I; R = CO_2Me, R′ = Me) furnished the methyl glycosides of the corresponding neutral disaccharide (I; R = CH_2·OH, R = Me), hydrolysis of which gave equal proportions of arabinose and xylose. From the mode of formation of the



(I)

....4 D-Xyl*p* 1....
3
|
L-Ara*f* 1

(II)

aldobiouronic acid it is clear that the disaccharide is a (xylose arabinofuranosid)uronic acid. Oxidation of the aldobiouronic acid with periodate gave 1·07 mol. of formaldehyde, whereas oxidation of the acidic disaccharide alcohol (from treatment of the reducing disaccharide with borohydride) gave 1·9 mol. of formaldehyde. This result is consistent only with the presence of a 3-substituted xylose derivative. Further evidence in support of the structure for the disaccharide came from observations that the methyl ester methyl glycosides (I; R = CO_2Me, R′ = Me) of the aldobiouronic acid consumed only 0·93 mol. of periodate, that the methyl glycosides of the neutral disaccharide (I; R = CH_2·OH

[1] Aspinall and Sturgeon, *J.*, 1957, 4469.
[2] Aspinall, Cairncross, Sturgeon, and Wilkie, *J.*, 1960, 3881.
[3] Aspinall, Cairncross, and Nicolson, *Proc. Chem. Soc.*, 1959, 270.
[4] Mehltretter, *Adv. Carbohydrate Chem.*, 1953, **8**, 231.

R′ = Me), likewise, consumed 1·03 mol. of periodate, and that hydrolysis of the periodate-oxidised methyl glycosides gave xylose, showing the xylose moiety to be unattacked by the reagent. These results point to the presence in the disaccharide derivative of an arabinofuranose residue joined to xylose by a 1,3-linkage.

The isolation of the aldobiouronic acid, (3-D-xylose L-arabinofuranosid)uronic acid, in this way, taken together with a previous knowledge of the structural units present in the rye-flour arabinoxylan,[1] shows that the terminal L-arabinofuranose residues in the polysaccharide are attached as single-unit side-chains to position 3 of D-xylopyranose residues in the 1,4-linked basal xylan chain. This conclusion is in agreement with previous results.[1,2] It is noteworthy that glycofuranosiduronic acid linkages in polysaccharides, like the commonly encountered glycopyranosiduronic acid linkages, are resistant to acid-hydrolysis. Terminal L-arabinofuranose residues are of frequent occurrence in plant polysaccharides, and this procedure provides a method whereby direct evidence may be obtained for the mode of linkage of these acid-labile groups to the adjacent sugar residues.

Experimental

Paper chromatography was carried out on Whatman Nos. 1 and 3MM papers with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer). The rye-flour arabinoxylan was isolated as described by Preece and Hobkirk [5] and digested with salivary α-amylase to remove traces of contaminating starch.

Oxidation of Rye-flour Arabinoxylan.—A number of preliminary experiments indicated that oxidations of polysaccharides, which were carried out under the general conditions described below, were slow and that even after long periods oxidation of the available primary alcoholic groups was incomplete. Under similar conditions oxidations [4] of simple sugar derivatives, *e.g.*, methyl α-D-mannopyranoside, were complete after 12—24 hr.

Solutions of polysaccharide (3 g.) in water (150 ml.) and of sodium hydrogen carbonate (0·5 g.) in water (100 ml.) were each shaken with platinum catalyst (Adams platinic oxide after reduction with hydrogen; 10 mg.) to remove possible catalyst poisons, the platinum was removed at the centrifuge, and the solutions were combined. Platinum catalyst (0·2 g.) was added to the solution and oxygen was bubbled through the stirred mixture held at 65° for 4 days. Platinum was removed from the cooled solution by centrifugation, the solution was concentrated, and the oxidised polysaccharide was precipitated by addition of ethanol (3 vol.) and freeze-dried from aqueous solution to give a white powder (2·5 g.) [Found: uronic anhydride (Kaye and Kent's method),[6] 4%].

The acidic polysaccharide (2·5 g.) was hydrolysed by N-sulphuric acid (50 ml.) at 100° for 4 hr. After removal of traces of insoluble material at the centrifuge, the hydrolysate was poured on a column of charcoal–Celite (1 : 1; 20 g.). Elution with water removed sulphuric acid and monosaccharides. Elution with water containing 5% of butan-2-one removed oligosaccharides, the eluate was shaken with di-n-octylmethylamine (5% v/v in chloroform) to remove traces of sulphuric acid and concentrated to a syrup which contained an acidic sugar (R_{xylose} 0·47 in solvent B) and traces of arabinose and xylose. Further fractionation on filter sheets with solvent B furnished the chromatographically pure acidic disaccharide (55 mg.).

Examination of the Acidic Disaccharide.—The acid (5 mg.) was dissolved in water (20 ml.), freshly prepared 0·3M-sodium metaperiodate (2 ml.) was added, and the mixture was made up to 25 ml. with water and kept at 35° for 24 hr. The formaldehyde formed was determined by Hough, Powell, and Woods's method,[7] and corresponded to 1·07 mol. The acid (5 mg.) was dissolved in water (5 ml.) containing potassium borohydride (15 mg.), and the solution was kept at room temperature overnight. The solution was neutralised with sulphuric acid, 0·3M-sodium metaperiodate (2 ml.) was added, and the mixture was made up to 25 ml. with water and kept at 35° for 24 hr. The formaldehyde formed corresponded to 1·9 mol.

The acid (40 mg.) was refluxed with methanolic 5% hydrogen chloride, and after neutralisation with silver carbonate gave methyl ester methyl glycosides (30 mg.) which consumed 0·93

[5] Preece and Hobkirk, *J. Inst. Brewing*, 1953, **59**, 385.
[6] Kaye and Kent, *J.*, 1953, 79.
[7] Hough, Powell, and Woods, *J.*, 1956, 4799.

mol. of periodate (spectrophotometric determination [8]) on oxidation. The methyl ester methyl glycosides (25 mg.) were dissolved in water (5 ml.) containing potassium borohydride (50 mg.), and the whole was kept at room temperature overnight. Excess of borohydride was destroyed by treatment with Amberlite resin IR-120(H), and the solution was completely de-ionised by shaking with cation- and anion-exchange resins and on evaporation afforded the methyl glycosides of a neutral disaccharide (18 mg.).

The methyl glycosides (5 mg.) of the neutral disaccharide were hydrolysed and quantitative estimation [9] after chromatographic separation showed xylose (46%) and arabinose (44%). The methyl glycosides (5 mg.) were oxidised with 0·105M-sodium metaperiodate (10 ml.) at 35° with the consumption of 1·03 mol. of reagent.[8] A further quantity of methyl glycosides (5 mg.) was oxidised with sodium metaperiodate solution, sodium ions were removed by passage through a column of Amberlite resin IR-120(H), periodate and iodate were removed by addition of barium hydroxide solution, and excess of barium ions was precipitated as barium carbonate on passage of carbon dioxide through the solution. The filtrate was acidified with sulphuric acid and the solution was heated to effect hydrolysis. Quantitative paper chromatography [9] of the hydrolysate showed the presence of xylose (0·90 mol. per mol. of methyl arabinosylxylosides).

The authors thank Professor E. L. Hirst, C.B.E., F.R.S., for his interest and advice, and Imperial Chemical Industries Limited, Central Agricultural Control, for financial support.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, March 29th,* 1960.]

[8] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[9] Flood, Hirst, and Jones, *J.*, 1948, 1679.