770. *A Trisaccharide from the Enzymic Degradation of Two Arabinoxylans.*

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The hydrolysis of arabinoxylans from rye flour and cocksfoot grass by a commercial enzyme preparation in the presence of arabonolactone furnishes a series of oligosaccharides, one of which has been identified as *O*-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 4)-D-xylose. The characterisation of this trisaccharide establishes the mode of attachment of arabinose to xylose residues in the polysaccharides.

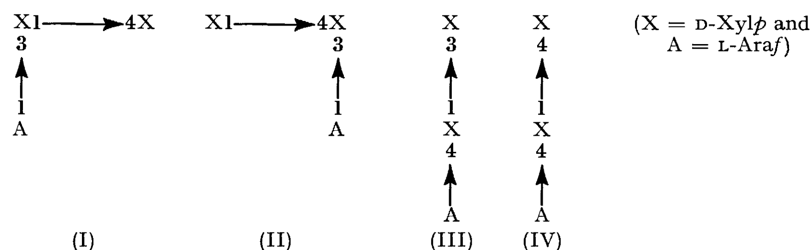
MANY polysaccharides of the xylan group contain terminal non-reducing L-arabinofuranosyl residues.[1] Where evidence is available it has been shown that these residues are attached directly as single-unit side-chains to xylose residues in the 1,4-linked main chain by 1,3-linkages (A), rather than by 1,4-linkages to xylose residues in the side-chains (B). In the majority of such cases, however, the evidence is indirect and is based on a comparison of the original polysaccharide and a degraded polysaccharide from which some of the acid-labile arabinofuranosyl residues have been removed by controlled acid-hydrolysis. In the case of wheat-straw arabinoxylan, Bishop and Whitaker [2] have isolated a series of oligosaccharides containing both arabinose and xylose residues as partial hydrolysis products using an enzyme preparation from the mould *Myrothecium verrucaria*. One of the components was shown to be *O*-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 4)-D-xylose,[3] and proof of its structure provided direct evidence in favour of (A) as a partial structure for the parent polysaccharide. This paper describes the isolation and characterisation of the same trisaccharide from the enzymic degradation of the arabinoxylans from rye flour [4] and cocksfoot grass.[5]

The preliminary examination of a number of enzyme preparations [6] showed that arabinoxylans were degraded by them to the constituent sugars, and that only xylobiose

[1] For a review see Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 429.
[2] Bishop and Whitaker, *Chem. and Ind.*, 1955, 119.
[3] Bishop, *J. Amer. Chem. Soc.*, 1956, **78**, 2840.
[4] Aspinall and Sturgeon, *J.*, 1957, 4469.
[5] Aspinall and Cairncross, preceding paper.
[6] Wilkie, Ph.D. Thesis, Edinburgh, 1955.

and its polymer homologues could be detected as products of incomplete hydrolysis: Conchie and Levvy [7] have shown that the glycosidase activities of certain enzymes are inhibited in the presence of aldonolactones of the corresponding configuration. When the arabinoxylans from rye flour and cocksfoot grass were degraded by a commercial enzyme preparation in the presence of 0·1M-L-arabonolactone a second series of oligosaccharides, containing both xylose and arabinose residues, was detected chromatographically amongst the products of partial acid-hydrolysis. When the enzyme digests were performed on a larger scale sufficient of a trisaccharide containing both arabinose and xylose residues was isolated from each of the polysaccharide hydrolysates for structural investigation.

The trisaccharide on acid-hydrolysis gave xylose and arabinose in the proportion of 2 : 1. From a knowledge of the structural units in the two polysaccharides, based on earlier investigations,[4,5] the following possible structures (I—IV) may be considered for the trisaccharide.



Trisaccharide	Partial hydrol. product	Degradn. by alkali	Periodate consumed (mol.)	Residues unattacked by periodate
I	Xylobiose	isoS.	3	Xylose (1 mol.)
II	Xylobiose	Unknown acid	4	None
III	Rhodymenabiose	metaS.	3—4	None
IV	Xylobiose	isoS.	4	None
Trisaccharide from arabinoxylans	Xylobiose	isoS.	3	Xylose (1 mol.)

(isoS. = isosaccharinolactone and metaS. = metasaccharinolactone)

The above results (with both samples of trisaccharide) may be compared with those to be expected from trisaccharides with structures (I—IV) (see Table) and clearly indicate structure (I) for the isolated trisaccharide. Mild acid-hydrolysis furnished arabinose and xylobiose (4-*O*-β-D-xylopyranosyl-D-xylose), which is readily distinguished from rhodymenabiose (3-*O*-β-D-xylopyranosyl-D-xylose).[8] Treatment of the trisaccharide with alkali afforded isosaccharinolactone,[9] and only on prolonged degradation was a metasaccharinolactone detected. On reaction with periodate the trisaccharide consumed 3 mol. of reagent, and hydrolysis of the oxidised trisaccharide showed that one xylose residue had been unattacked by the reagent. Further evidence that the terminal arabinose residue in the trisaccharide is joined to the adjacent xylose residue by a 1,3-linkage came from methylation studies. The trisaccharide was reduced to the corresponding glycitol with potassium borohydride, and hydrolysis of the methylated glycitol afforded 2,3,5-tri-*O*-methyl-L-arabinose and 2,4-di-*O*-methyl-D-xylose as the only reducing sugars.

The possibility that the trisaccharide was an artefact of enzymic synthesis seems unlikely in view of the short period of incubation and of the low carbohydrate concentration in the digest. The latter factor would favour hydrolysis rather than enzymic transfer of arabinofuranosyl residues to a xylobiose acceptor. In the absence of a suitable arabinofuranosyl donor as substrate, it was not possible to rule out this type of reaction.

[7] Conchie and Levvy, *Biochem. J.*, 1957, **65**, 389.
[8] Howard, *Biochem. J.*, 1957, **67**, 643.
[9] Aspinall, Carter, and Los, *J.*, 1956, 4807.

However, a control experiment showed that, in the conditions employed during the enzymic hydrolysis of the polysaccharides, free arabinose was not transferred to xylobiose or xylotriose. These results, taken together with a previous knowledge of the main structural features of the two parent polysaccharides, show that the trisaccharide, isolated as a product of partial enzymic hydrolysis, is *O*-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 4)-D-xylose. It follows that the majority, at least, of arabinofuranosyl end groups in both polysaccharides are attached as in (A) as single-unit side-chains to the basal xylan chains.

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) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer). Enzymic digests were carried out using " Hemicellulase " (L. Light and Co. Ltd.).

Enzymic Degradation of Cocksfoot Grass Xylan.—Digests containing xylan (*ca.* 20 mg.) in water (5 ml.), enzyme (10 mg.) in water (3 ml.), acetate buffer (0·05M; 10 ml.; pH 5), and toluene (2 ml.) were prepared in (*a*) water (25 ml.), (*b*) 0·01M-L-arabonolactone (25 ml.), and (*c*) 0·1M-L-arabonolactone (25 ml.), and where necessary the pH was brought to 5 by the addition of aqueous sodium hydroxide. The digests were incubated at 37°, and samples (3 ml.) were withdrawn at intervals. Samples were heated on a boiling-water bath for 3 min. to inactivate th enzyme, the cooled solutions were poured on columns (3 × 1 cm.) of charcoal–Celite, and the columns were eluted with water to remove most of the monosaccharides, inorganic material, and where present arabonolactone, and with water containing 5% and 15% of ethanol to remove the last traces of monosaccharides together with oligosaccharides. The eluates with water containing 5% and 15% of ethanol were examined chromatographically in solvent A.

(*a*) Samples withdrawn after 2, 4, 6, 8, and 10 hr. all showed xylose, arabinose, glucose, and galactose, and a series of oligosaccharides with similar chromatographic mobilities to those of xylobiose (R_{xylose} 0·60), xylotriose (R_{xylose} 0·30) and xylotetraose (R_{xylose} 0·14).

(*b*) A sample withdrawn after 16 hr. showed traces of two oligosaccharides (R_{xylose} 0·66 and 0·40) in addition to monosaccharides and xylobiose and its polymer homologues. These oligosaccharides were no longer present in the sample withdrawn after 24 hr.

(*c*) Samples withdrawn after 2, 4, 6, 8, and 10 hr. showed monosaccharides, xylobiose, and its polymer homologues, and a second series of oligosaccharides (R_{xylose} 0·66, 0·40, and 0·18), which was present in greatest amount after 4 hr.

Xylan (5 g.) was dispersed in water (500 ml.) by shaking overnight, and 3N-sodium hydroxide (10 ml.) was added to ensure complete dissolution. After 1 hr. the solution was neutralised to pH 5 by the addition of glacial acetic acid, and L-arabonolactone (25 g.) in water (250 ml.) and acetate buffer (0·05M; 500 ml.; pH 5) were added. The solution was heated to 35°, enzyme (500 mg.) was added, and the solution was incubated at 35° for 4 hr. The solution was heated on the boiling-water bath to inactivate the enzyme, and the cooled solution was poured into acetone (1 vol.). Precipitated polysaccharide was removed at the centrifuge, and the supernatant liquid was evaporated under reduced pressure to remove acetone. The remaining solution was poured on charcoal–Celite (1 : 1; 50 g.), and elution of the column with water removed arabonolactone, monosaccharides, and inorganic ions. Elution of the column with water containing varying amounts of ethanol failed to afford any satisfactory resolution of oligosaccharides, and the column was eluted with water containing 25% of ethanol to remove remaining oligosaccharides. Fractions containing oligosaccharides were evaporated under reduced pressure to remove ethanol, electrodialysed to remove inorganic ions, and concentrated. The residual syrup was separated on filter sheets with solvent A, to give oligosaccharides (R_{xylose} 0·60, 0·30, and 0·14), which gave only xylose on hydrolysis, and oligosaccharides (R_{xylose} 0·66, 0·40, and 0·18) which gave arabinose and xylose on hydrolysis. The trisaccharide (70 mg.) (R_{xylose} 0·40) was isolated in sufficient amount for more detailed investigation.

Examination of Trisaccharide.—The chromatographically pure sugar had $[\alpha]_D$ −14·9° (*c* 0·5 in H_2O), and chromatographic examination of the hydrolysate in solvent A followed by quantitative estimation [10] showed the presence of xylose (66%) and arabinose (33%). The

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

sugar (5 mg.) was heated in 0·01N-oxalic acid (2 ml.) at 100° and samples were withdrawn at intervals for chromatographic examination in solvent A. After 1 hr. arabinose, xylobiose, and unchanged trisaccharide were found, and after longer periods the trisaccharide disappeared and small amounts of xylose appeared. The sugar (5 mg.) was oxidised with 0·143M-sodium metaperiodate (10 ml.) for 4 days at room temperature (in a parallel experiment the consumption of periodate, measured spectrophotometrically,[11] corresponded to 2·9 moles per mole of trisaccharide). Sodium ions were removed with Amberlite resin IR-120(H), and iodate and periodate ions with an excess of barium hydroxide. Excess of barium hydroxide was precipitated as carbonate by passage of carbon dioxide through the solution. The oxidised trisaccharide was hydrolysed and quantitative chromatography [10] with L-rhamnose as reference sugar showed the presence of xylose (0·92 mole per mole of trisaccharide). The sugar (5 ml.) was dissolved in oxygen-free N-sodium hydroxide (5 ml.). Withdrawal of a sample after 56 hr., followed by passage through a column of Amberlite resin IR-120(H) and chromatography in solvent B, showed xyloisosaccharinolactone.[9]

Enzymic Degradation of Rye Flour Xylan and Examination of a Trisaccharide.—Rye-flour xylan (2 g.) was dissolved in water (150 ml.) by shaking overnight; 0·05M-acetate buffer (100 ml.; pH 4·5), L-arabonolactone (20 g.), and enzyme (700 mg.) in water (50 ml.) were added and the solution was incubated at 35° for 3 hr. The solution was heated at 100° for 5 min. to inactivate the enzyme, and after cooling was poured into ethanol (1 vol.). The mixture of protein and degraded polysaccharide was removed at the centrifuge, and the supernatant liquid was concentrated under reduced pressure to remove ethanol and poured on a column of charcoal–Celite (1 : 1; 20 g.). The column was washed with water until free from monosaccharides and then with water containing increasing amounts of ethanol to remove oligosaccharides. The fraction eluted with water containing 10% of ethanol contained a chromatographically pure trisaccharide (70 mg.), R_{xylose} 0·40 in solvent A, which was present in sufficient amount for more detailed investigation. Other fractions contained oligosaccharides with chromatographic mobilities similar to those of xylobiose (R_{xylose} 0·60) and xylotriose (R_{xylose} 0·30), and a disaccharide (R_{xylose} 0·66) which gave arabinose and xylose on hydrolysis.

The trisaccharide had $[\alpha]_D$ −15·3° (*c* 0·67 in H_2O), and chromatographic examination of the hydrolysate in solvent A followed by quantitative estimation [10] showed the presence of xylose (65%) and arabinose (32%). Hydrolysis of the trisaccharide with 0·01N-oxalic acid at 100° resulted in the rapid release of arabinose with the formation of xylobiose. The sugar (5 mg.) was oxidised with 0·143M-sodium metaperiodate (10 ml.) at room temperature (in a parallel experiment the consumption of periodate, measured spectrophotometrically,[11] corresponded to 3·1 moles per mole of trisaccharide). Sodium ions were removed with Amberlite resin IR-120(H), and iodate and periodate ions with an excess of barium hydroxide. Excess of barium hydroxide was precipitated as carbonate by passage of carbon dioxide through the solution. The oxidised trisaccharide was hydrolysed and quantitative chromatography,[10] with L-rhamnose as reference sugar, showed the presence of xylose (0·90 mole per mole of trisaccharide). The sugar (5 mg.) was dissolved in oxygen-free 0·95N-sodium hydroxide (5 ml.) and kept at room temperature. Withdrawal of a sample after 48 hr., followed by passage through a column of Amberlite resin IR-120(H) and chromatography showed xyloisosaccharinolactone.[9] After 96 hr. a trace of a xylometasaccharinolactone was also found.

Rye-flour xylan (100 mg.) in water (5 ml.) and 0·05M-acetate buffer (5 ml.; pH 4·5) was added to enzyme (30 mg.) in water (30 ml.), and the solution was incubated at 35° for 3 hr. The solution was heated at 100° for 5 min. to inactivate the enzyme, and after cooling was poured on charcoal–Celite (1 : 1; 10 g.). The column was eluted with water to remove monosaccharides and inorganic ions, and with water containing increasing amounts of ethanol to remove oligosaccharides. Chromatography of the eluates showed xylose, arabinose, xylobiose, and xylotriose, but no arabinose-containing oligosaccharides.

Methylation of the Trisaccharide Alcohol.—The trisaccharide (70 mg.) was treated with potassium borohydride (30 mg.) in water (2 ml.) for 3 hr. at room temperature, and excess of hydride was destroyed and potassium ions removed by shaking with Amberlite resin IR-120(H). The resulting solution was treated twice with methyl sulphate and sodium hydroxide, and the methylated glycitol (*ca.* 70 mg.) was isolated by extraction of the reaction mixture with chloroform. Hydrolysis of the methylated glycitol with N-sulphuric acid at 100° for 3 hr.,

[11] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

followed by neutralisation with barium carbonate and chromatographic separation of the hydrolysate with solvent B, gave two fractions containing reducing sugars. Fraction 1 (17 mg.) had R_G 0·96 in solvent B and was characterised 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. 135°. Fraction 2 (15 mg.), R_G 0·70 in solvent B, crystallised and had m. p. and mixed m. p. (with 2,4-di-*O*-methyl-D-xylose) 114°.

Test for the Formation of the Trisaccharide as a Product of Enzymic Synthesis.—Enzyme (40 mg.) in water (2 ml.) was added to a solution of L-arabinose (50 mg.), xylobiose (100 mg.), and xylotriose (100 mg.) in water (5 ml.) and 0·05M-acetate buffer (5 ml.; pH 4·5) containing L-arabonolactone (2 g.), and the solution was incubated at 35° for 3 hr. The products were worked up in the usual way and chromatography showed xylose and those sugars originally present but no arabinose-containing oligosaccharides.

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