[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY NATIONAL RESEARCH LABORATORIES]

Structure of a Trisaccharide from Wheat Straw Xylan[1,2]

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A trisaccharide containing two D-xylose units and one L-arabinose unit was produced in 3.36% yield from wheat straw xylan by hydrolysis with an enzyme isolated from the mold, *Myrothecium verrucaria*. Oxidation of the trisaccharide by periodate and identification of hydrolysis products of its methyl ether showed the structure to be *O*-(α or β)-L-arabofuranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose. Characterization of this compound constitutes proof that L-arabofuranose units are an integral part of the wheat straw hemicellulose.

Xylans isolated from various plants usually yield some arabinose on hydrolysis, and it has been uncertain whether this arabinose is an integral part of the xylan molecule or a component of an admixed araban.[3] Hydrolysis of the methyl ether of wheat straw xylan has shown that arabinose is present in the molecule as single furanoside units attached along a chain of β,1→4-xylopyranoside units.[4,5] However, final proof that wheat straw xylan contained arabo-xylan molecules required isolation, from a hydrolysate of wheat straw xylan, of oligosaccharides in which arabinose and xylose units were combined. Acids were unsuitable hydrolytic agents for this purpose since they cleave the arabofuranoside linkages more readily than the pyranoside linkages between the xylose units. A purified cellulolytic enzyme preparation from the mold *Myrothecium verrucaria*[6] was found to hydrolyze linear chains of β,1→4-xylopyranoside units and hydrolysis of wheat straw xylan[4] by this enzyme produced a series of oligosaccharides in which xylose and arabinose were combined.[7] No evidence of oligosaccharide synthesis was found when the enzyme was incubated, under the conditions of hydrolysis, with mixtures of xylose and arabinose. This report is concerned with the determination of the structure of a trisaccharide formed in this enzymolysis.

The trisaccharide was isolated by successive chromatographic fractionations on charcoal columns[8] and large sheets of filter paper.[9] The product was chromatographically homogeneous in four solvent systems but did not crystallize nor did it yield a crystalline acetate. The molecular weight, determined by oxidation of the reducing group by alkaline iodine,[10] corresponded with the calculated value for a trisaccharide containing three pentose residues. Acid hydrolysis of the trisaccharide yielded two moles of D-xylose and 1 mole of L-arabinose, with small amounts of xylobiose being evident before hydrolysis was complete. After the trisaccharide was oxidized, hydrolysis of the resulting acid produced equal amounts of D-xylose and L-arabinose thus showing that the terminal reducing end in the molecule was D-xylose. These observations taken with the results of methylation experiments indicated that the trisaccharide consisted of an L-arabofuranoside unit joined to one of the available positions of a xylobiose molecule. When the trisaccharide was oxidized by periodate, 3.08 moles of oxidant was consumed with the concurrent release of 1.91 moles of formic acid. When the oxidized trisaccharide was hydrolyzed, chromatographic detection of xylose in the hydrolysate showed that at least one xylose unit of the trisaccharide was linked so that it could not be oxidized by periodate. Figure 1 is a xylobiose molecule showing all the possible positions for attachment of an L-arabofuranoside unit and Table I gives the theoretical periodate consumptions and formic acid productions for the resulting trisaccharides. It was evident that for the trisaccharide to give the observed periodate oxidation data the L-arabofuranoside unit could only be joined to C-3 of the non-reducing xylose unit in xylobiose.

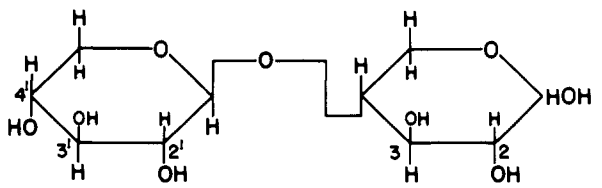

Fig. 1.—Positions available for attachment of an L-arabofuranoside unit to D-xylobiose.

TABLE I

THEORETICAL RESULTS FOR PERIODATE OXIDATION OF A TRISACCHARIDE IN WHICH AN L-ARABOFURANOSIDE UNIT IS JOINED TO ONE OF THE INDICATED POSITIONS (FIG. 1) OF A D-XYLOBIOSE MOLECULE

Position	Periodate consumed, moles	Formic acid produced, moles
2	3	1
3	4	2
2′	4	2
3′	3	2
4′	4	2

The trisaccharide was methylated and hydrolysis of its methyl ether yielded approximately equimolar quantities of 2,3-di-*O*-methyl-D-xylopyranose, 2,4-di-*O*-methyl-D-xylopyranose and 2,3,5-tri-*O*-methyl-L-arabofuranose. Identification of these methylated sugars by chromatographic behavior, specific rotations and isolation of crystalline derivatives confirmed the location of the glycosidic bonds pre-

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dicted by periodate oxidation. Making the reasonable assumption that the 1→4-glycosidic linkage between the two D-xylose units was in the β-configuration as were the linkages of the D-xylose units in the parent polysaccharide,[4] an assumption that was strengthened by the negative rotation (−19.3°) of the trisaccharide, then the trisaccharide was *O*-(α or β)-L-arabofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose.

Experimental

Paper Chromatography.—Separations were carried out at 25 ± 3° by the descending method[11] on Whatman No. 1 filter paper using one of the following solvent systems in the volume ratios indicated: (A) pyridine–ethyl acetate–water (1:2:2), (B) 1-butanol–pyridine–water (6:4:3), (C) 1-butanol–pyridine–water (5:5:3), (D) N-amyl alcohol–pyridine–water (7:7:6), (E) 1-butanol–ethanol–water (5:1:4). Sugars were detected on the papers by silver nitrate[12] and aniline oxalate[13] sprays.

Enzymic Hydrolysis of Wheat Straw Xylan.—Wheat straw xylan[4] (8.00 g. in 400 ml. of buffer, *p*H 6.0) was hydrolyzed by an enzyme preparation (11.6 mg.) from the mold *Myrothecium verrucaria*,[6] and oligosaccharides were isolated from the hydrolysate as previously described.[7] Incubation of an equimolar mixture of D-xylose and L-arabinose with the enzyme under the same conditions yielded no oligosaccharides.

Characterization of Trisaccharide.—The trisaccharide (269.2 mg., 3.36% of the weight of the xylan) was a cream-colored powder, $[\alpha]^{25}D$ −19.3° (*c* 1.0 in water), showing only one spot on chromatography in solvents A, B, C or D. Its R_x value (movement relative to that of xylose) in solvent A was 0.46. *Anal.* Calcd. for $C_{15}H_{26}O_{13}$: mol. wt., 414. Found: mol. wt. (by the hypoiodite method[10]), 407, 396.

Hydrolysis of the trisaccharide (10 mg.) by heating at 97° with 0.05 *N* hydrochloric acid (1 ml.) was followed chromatographically in solvent A using D-xylose, L-arabinose and D-xylobiose as reference compounds. After 15 min. hydrolysis L-arabinose was detectable on the chromatograms, together with D-xylobiose (R_x 0.61), the original trisaccharide (R_x 0.46) and a trace of D-xylose (R_x 1.0). The spots attributed to the oligosaccharides gradually disappeared as hydrolysis progressed and after 1.5 hr. only L-arabinose and D-xylose were present. The ratio of D-xylose to L-arabinose in the trisaccharide was 2:1 as shown by quantitative paper chromatography[9] (solvent A) of the 1.5-hr. hydrolysate. The trisaccharide (10 mg.) in water (1 ml.) was oxidized by bromine (0.1 ml.) for 36 hours. The presence of equal parts of D-xylose and L-arabinose in a hydrolysate of the oxidized trisaccharide was demonstrated by quantitative paper chromatography (solvent A) using the spot area method.[14]

Samples (2.19 mg.) of the trisaccharide were oxidized by sodium metaperiodate at *p*H 5.7 and 18° using the Warburg respirometer technique developed by Perlin.[15] After 46 hours reaction, formic acid production (measured by carbon dioxide evolved) became constant at 1.91 moles/mole of trisaccharide and the amount of periodate consumed was 3.08 moles/mole of trisaccharide. These results were an average of good checks from four identical oxidations. Inorganic ions were removed from the four combined oxidations by Amberlite IR 120 and Dowex-2 (carbonate form). The ion-free solution, concentrated to 1 ml., was acidified with concd. hydrochloric acid (3 drops) and heated at 97° for 2 hr. Chromatography (solvent A) of the hydrolysate showed the presence of D-xylose.

The trisaccharide (239 mg.) was acetylated by heating under reflux at 97° for 2.5 hr., with anhydrous sodium acetate (400 mg.) and acetic anhydride (15 ml.). The acetate (320 mg.), isolated by extraction with benzene after evaporation of the acetic anhydride, could not be crystallized and was not examined further. The trisaccharide acetate (320 mg.) was dissolved in acetone (10 ml.) and the solution was cooled to 0° before adding dimethyl sulfate (9 ml.). Methylation was then carried out by the dropwise addition of 30% (w./w.) sodium hydroxide (18 ml.) over a 2-hr. period with vigorous stirring. The solution was stirred for a further 12 hr. during which it was allowed to come to room temperature. Sodium hydroxide (30%, 18 ml.) was then added followed by dimethyl sulfate (9 ml.), drop by drop, over 8 hr. The mixture was stirred for a further 18 hr. after which the latter methylation was repeated once more. Following this third methylation the mixture was neutralized (*p*H 6.5) with 10% sulfuric acid and extracted continuously with chloroform until no more product could be removed. The extracts were dried over anhydrous sodium sulfate and evaporated to yield a sirup (130 mg.) which was methylated twice by Purdie reagents (silver oxide 0.7 g., and methyl iodide, 12 ml. for each methylation). The sirupy trisaccharide methyl ether (135 mg.) showed no hydroxyl groups in its infrared spectra.

Anal. Calcd. for $C_{23}H_{42}O_{13}$: OMe, 47.1. Found: OMe, 46.0.

The fully methylated trisaccharide was dissolved in 5% methanolic hydrogen chloride (10 ml.), and the solution ($[\alpha]^{25}D$ −13.3°) was refluxed for 6 hr. The solution became colored so that polarimetric readings could not be made. Methanol was evaporated at room temperature by a stream of air and *N* hydrochloric acid (10 ml.) was added to the residue. This mixture was then heated for 6 hr. under reflux on a boiling water-bath to hydrolyze the methyl glycosides. Acid was removed by Dowex-2 (carbonate form) and the neutral solution was examined by paper chromatography (solvent E). The three components found were chromatographically indistinguishable from known samples of 2,3-di-*O*-methyl-D-xylopyranose, 2,4-di-*O*-methyl-D-xylopyranose and 2,3,5-tri-*O*-methyl-L-arabofuranose run on the same paper. Separation of this mixture by chromatography on large (11.5″ × 19.5″) sheets of Whatman No. 1 yielded: (a) 2,3-di-*O*-methyl-D-xylopyranose (26.1 mg., 0.146 millimole), $[\alpha]^{25}D$ +24.2° (*c* 1.0 in water), reported[16] $[\alpha]^{15}D$ +23° (*c* 1.0 in water); (b) 2,4-di-O-methyl-D-xylopyranose (22.7 mg., 0.128 millimole), $[\alpha]^{25}D$ +21.8° (*c* 0.8 in water), reported[17] $[\alpha]^{20}D$ +22° (*c* 1.0 in water); (c) 2,3,5-tri-*O*-methyl-L-arabofuranose (24.5 mg., 0.128 millimole), $[\alpha]^{25}D$ ±0° (*c* 1.23 in water), reported[18] $[\alpha]^{25}D$ 0° (water).

Fraction a was heated under reflux for 1.5 hr. in ethanol (1 ml.) to which aniline (20 mg.) had been added. The solution was evaporated to a sirup from which excess aniline and decomposition products were extracted by ether. Recrystallization of the residue from ethyl acetate yielded 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine, m.p. 124–125°, unchanged on admixture with an authentic sample of the same derivative having m.p. 125–126°.

Fraction b was crystallized from ethyl acetate and identified as 2,4-di-*O*-methyl-D-xylopyranose by its m.p. of 110–111°, unchanged when mixed with a known sample.

Fraction c, in water (0.75 ml.), was oxidized by bromine (0.2 ml.) at room temperature in the absence of light for 60 hr. Bromine was removed by aeration and the solution was neutralized by silver carbonate. Silver ions were precipitated as the sulfide and the mixture was evaporated to dryness. The residue was extracted with ethanol and anhydrous ammonia was passed through the extract for 15 min. The ammoniacal solution was kept at room temperature for 2 days after which evaporation left the crystalline amide of 2,3,5-tri-*O*-methyl-L-arabonic acid.[19] Recrystallized from methanol–petroleum ether, this compound had m.p. and mixed m.p. 136–137°.

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