The principle of glycosyl transfer from uridine diphosphate sugars to suitable acceptors mediated by enzymes from various sources has now become well established (1, 2). Although Neish (3), and Slater and Beevers (4), on the basis of theoretical considerations, suggested that uridine diphosphate pentoses are possible precursors in the synthesis of plant pentosans, no experimental evidence of an enzymatic transfer of pentose units to any acceptor has hitherto been submitted. In this communication evidence is presented to the effect that an enzyme preparation obtained from asparagus shoots is capable of catalyzing the transfer of xylosyl units from uridine diphosphate D-xylose to xylo-oligosaccharides.

EXPERIMENTAL

Substrates—UDP-glucuronic acid1 labeled with C14 in the uronic acid moiety was prepared enzymatically from UDP-glucose labeled with C14 in the glucose moiety (2).

UDP-pentose labeled with C14 in the pentose moiety was obtained by decarboxylation of UDP-gluconic acid by an asparagus particulate preparation (5) and purified by electrophoresis, first at pH 3.6 and then at pH 5.8. This UDP-pentose consisted of a mixture of 67 per cent UDP-xylose and 33 per cent UDP-arabinose. Treatment of the UDP-pentose with nucleotide pyrophosphatase (6), followed by electrophoretic separation at pH 3.6, resulted in a mixture of arabinose l-phosphate and xylose l-phosphate.

Members of the homologous series of β-1,4-linked xylene-oligosaccharides of degree of polymerization ranging from 2 to 6 were kindly given to us by Dr. R. L. Whistler, Purdue University.

Enzyme Preparation—A soluble asparagus extract was prepared by a method similar to that previously described for mung beans (2). Asparagus shoots (Asparagus officinalis, purchased from a local grocer), 100 gm., were blended with 70 ml. of 0.01 M sodium and potassium phosphate buffer at pH 7.0. The homogenate obtained after filtering the slurry through cheese-cloth was centrifuged at 20,000 x g for 30 minutes, and the precipitate was suspended in 0.065 ml. of 0.08 M Tris buffer, pH 7.0. The subsequent liquid was centrifuged at 20,000 x g for 30 minutes, and the precipitate was suspended in 0.5 ml. of 0.1 M Tris, pH 7.0. The particulate preparation so obtained was vigorously shaken with an equal volume of a 1 per cent solution of digitonin and centrifuged at 20,000 x g for 30 minutes. When the supernatant liquid was examined, UDP-gluconic acid decarboxylase (5), and xylosyl transferase activities were found to be present. No diminution of activity of transferase was observed after the preparation had been kept at −10° for periods up to 2 weeks. This enzyme preparation contained 8 mg. of protein per ml.

Analytical Methods—Paper electrophoresis was carried out in three different buffer solutions: 0.1 M ammonium formate, pH 3.6, 0.2 M ammonium acetate, pH 5.8, and 0.1 M sodium tetaborate, pH 9.2 (2). Paper chromatography of oligosaccharides was performed by radial development with either p-propanol-ethyl acetate-water, 7:1:2, or water-saturated phenol. Mono- saccharides were chromatographed two-dimensionally on Whatman No. 1 paper with the use of water-saturated phenol in the first dimension and butanol-acetic acid-water (52:13:35) in the second. Sugars were detected with p-anisidine phosphate spray reagent (1 gm. of p-anisidine phosphate in 140 ml. of 70 per cent ethanol). Radioactive substances were located on paper and counted as previously described (2).

All enzymatic reactions and acid hydrolyses were carried out in capillary tubes of 1.2 to 1.5 mm. diameter (7).

RESULTS

Formation of Radioactive Oligosaccharides—It was observed in preliminary experiments that incubation of C14-labeled UDP-pentose in the presence of the soluble enzyme preparation and a β-1,4-linked xylene-oligosaccharide resulted in an increase in incorporation of the radioactivity into the neutral components over that found when the oligosaccharide was omitted or replaced by xylose. This observation was therefore followed up with more precise experiments.

UDP-pentose (0.84 x 10−2 μmoles; 2.7 x 104 c.p.m.) and D-xylose, or UDP-pentose and a xylo-oligosaccharide (1.0 pmole) were incubated with enzyme preparations (0.4 mg. of protein) in 0.005 ml. of 0.05 M Tris buffer, pH 7. After 3 hours at 37° the reaction mixtures were separated electrophoretically at pH 3.6, at 35 volts per cm. for 2 hours. Upon examination of the area on which neutral compounds appeared, it was found that 4 per cent of the total radioactivity resided in that area of the mixture to which D-xylose was added, and 12 to 15 per cent in those mixtures to which a xylo-oligosaccharide was added.

The neutral compounds were eluted from the electrophoretograms and subjected to circular chromatography in propanol-ethyl acetate-water. Free radioactive pentose was found in all cases. Its presence was probably the result of enzymatic hydrolysis of UDP-pentose. However, wherever a xylo-oligosaccharide had been added, a new radioactive band appeared.

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Identification of Radioactive Oligosaccharides—The new radioactive substances were eluted and identified by (a) chromatographic and electrophoretic comparison with the authentic β-1,4-linked xylo-oligosaccharides and (b) examination of the products of acid hydrolysis.

Cocromatography and coelectrophoresis of each of the isolated radioactive compounds with the authentic member of the xylo-oligosaccharide homologous series containing one more pentose unit than the initial xylo-oligosaccharide acceptor showed that they were identical. Total acid hydrolysis of the radioactive compounds (1 N HCl at 100° for 60 minutes) yielded xylose as the sole radioactive product. Partial hydrolysis in 0.1 N HCl at 100° for 15 minutes of radioactive oligosaccharide which had been mixed with a xylo-oligosaccharide of, presumably, the same degree of polymerization, produced in each case xylose as well as a series of radioactive oligosaccharides of lower degree of polymerization. Each of these radioactive oligosaccharides cochromatographed with a member of the β-1,4-linked xylo-oligosaccharide series formed by partial hydrolysis of the non-radioactive carrier. It was thus shown that reaction mixtures originally containing a series of xylo-oligosaccharides ranging in degree of polymerization from 3 to 5, when incubated with the enzyme, formed oligosaccharides of the same homologous series ranging in degree of polymerization from 3 to 6.

Primer Requirements—In order for the transfer to take place, the concentration of xylo-oligosaccharide acceptor required was high. With xylose trisaccharide a concentration of $2 \times 10^{-2} \text{M}$ was required to effect half-maximal incorporation of xylose. Similar concentrations of the di-, tetra- and penta-xylo-oligosaccharides were found to be required for transfer of a xylosyl residue from the UDP-xylose.

In the preparations described above, about $1 \times 10^{-3} \mu\text{ mole}$ of xylose was transferred to the primer. Since $1 \mu\text{ mole}$ of primer was originally present, the concentration of the new oligosaccharide formed was about $10^3$ times less. In view of the fact that the affinity of the enzyme for the acceptor was of the same order of magnitude for all the oligosaccharides tested, such an amount would be insufficient to allow the newly formed oligosaccharide to successfully compete with the original acceptor. This explains why only one-step transfers were observed.

Formation of Radioactive Ethanol-Soluble Substance from UDP-xylose—In addition to catalyzing the transfer of xylosyl residues to xylo-oligosaccharide acceptors, the asparagus particulate preparation catalyzed the incorporation of approximately 10 per cent of the C$^3$H$^4$ UDP-xylose radioactivity into a new radioactive compound. This compound was electrophoretically immobile and could not be eluted from paper with water, but was soluble in boiling 95 per cent ethanol.

The ethanol-soluble radioactive material was prepared by incubating at 37° UDP-xylose (1.15 $\times 10^{-2} \mu\text{ moles}; 3.5 \times 10^4$ c.p.m.), Tris buffer, pH 7 (50 μmoles), and asparagus particulate preparation representing 10 gm. of fresh asparagus in a total volume of 0.075 ml. After 1 hour the particulate material was washed repeatedly by suspension in water and centrifugation until no radioactivity could be detected in the washings. The radioactive particulate material so obtained was suspended in 42 per cent HCl at $-16^\circ$ and held at 0° for 3 hours. The acid hydrolysate was then concentrated to dryness in a vacuum desiccator. When this material was dissolved in water and chromatographed, xylose was found to be the only radioactive product present.

These findings suggest that a xylosyl transfer from UDP-xylose to a hydrophobic aglycone acceptor occurred. The nature of this aglycone is now under investigation.

DISCUSSION

The enzymatic transfer of one xylosyl residue from UDP-xylose to β-1,4-linked xylo-oligosaccharide acceptors resulted in the formation of members of the 1,4-linked xylo-oligosaccharide series. Hence, the transfer must have been effected at the C-4 position of the terminal xylose unit of the acceptor molecule. Whether the bond formed is of α or β type has not been determined.

The fact that the affinity of the transferring enzyme was essentially the same for each of the xylo-oligosaccharide acceptors accounts for the observed one-step reaction. Since the formation of xylan would require a multi-step reaction, synthesis of this polysaccharide in vivo by this enzyme system would not be expected to occur. However, it is possible that xylan is synthesized by the same enzyme, but that a primer of higher molecular weight is required. Another possibility is that this enzyme is part of a system involved in the synthesis of a more complex polysaccharide, such as a hemicellulose, consisting of D-xylose, uronic acids, and monosaccharides other than D-xylose, and that a heterologous primer is necessary. Finally, it may well be that an enzyme similar to the xylosyl transferring system which forms an ethanol-soluble xylside in asparagus is responsible for the synthesis of the xylsides occurring in plants, such as digitonin, ruberythric acid, and tomatin. In this connection, it is of interest that Cardini and Yamaha (8) demonstrated the formation of some naturally occurring glucosides by transfer of glucose from UDP-glucos to various phenols, catalyzed by a wheat germ extract.

SUMMARY

A soluble enzyme preparation from asparagus shoots contains an enzymatic system capable of catalyzing the transfer of D-xylose from uridine diphosphate D-xylose to β-1,4-linked xylo-oligosaccharides, ranging in degree of polymerization from 2 to 5. This single-step transfer reaction results in the production of 1,4-linked oligosaccharides of degree of polymerization ranging from 3 to 6, each containing one more D-xylose residue than the acceptor.

Acknowledgment—We gratefully acknowledge the able technical assistance of Miss Sofia Moszkowski.

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