Short Communication

**A Novel Nonreducing Disaccharide,
O- β -D-Xylopyranosyl-(1 $\rightarrow$ 1')- β -D-
Xylopyranose, Produced from
Xylobiose by Transxylosylation
with *Aspergillus niger* β -Xylosidase**

Tsuneo YASUI, Hideki KIZAWA,
Yoshie MASADA and Hirofumi SHINOYAMA

*Institute of Applied Biochemistry,
University of Tsukuba, Tsukuba,
Ibaraki 305, Japan*

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β -Xylosidases, which hydrolyze oligosaccharides and xylosides with a β -xylosyl linkage, also catalyze transxylosyl reactions. The β -xylosidase from *Aspergillus niger* IFO 6662 has a strong transxylosylation that has been used for the synthesis of alkyl β -xylosides.¹⁾

In this study we describe the production of a nonreducing disaccharide consisting of xylose, using β -(1 $\rightarrow$ 4)-xylobiose (xylobiose) as substrate, by transxylosylation with *A. niger* β -xylosidase. This is the first isolation of the nonreducing xylobiose.

β -Xylosidase from *A. niger* IFO 6662, prepared as described previously,¹⁾ was concentrated by ultrafiltration before use. β -Xylosidase activity was measured using phenyl β -D-xyloside as a substrate, as described previously.¹⁾

For the transxylosyl reaction, a reaction mixture containing 2.0 g xylobiose and 260 units of *A. niger* β -xylosidase in 80 ml of 0.1 M acetate buffer (pH 4.4) was incubated at 50°C for 1.5 hr. After the mixture was heated in a boiling water bath for 10 min to inactivate the enzyme, the proteinaceous precipitate was removed by ultrafiltration, and the filtrate was concentrated to 15 ml under reduced pressure. The syrup thus obtained was adsorbed and chromatographed on a charcoal column. Elution was first done with water to remove a

hydrolysis product, xylose (fraction P). Then the column was eluted with 5% (v/v) ethanol and last with 30% ethanol to wash out the remaining sugars. The total sugar content in each fraction was estimated as xylose by the phenol-sulfuric acid method.²⁾

As shown in Fig. 1, the 5% ethanol fractions were separated into two peaks (P-1, P-2). Sugars contained in the fractions of P, P-1, and P-2 were analyzed by thin layer chromatography (TLC) on silica gel plates, using the solvent system of acetone-H₂O-chloroform-methanol (8:0.5:1:1, v/v). After development, sugars were detected by spraying with 20% sulfuric acid in methanol, or *p*-anisidine hydrochloric acid solution. As shown in Fig. 2, the spot of P-1 (saccharide O-1) could be only detected by 20% sulfuric acid in methanol, but not by *p*-anisidine hydrochloric acid solution, suggesting it to be a nonreducing sugar. The spots of peak P and P-2 (saccharide O, O-2) were thought to be xylose and β -(1 $\rightarrow$ 4)-xylobiose, respectively. The fractions which

Fig. 1. Elution Pattern of Charcoal Column Chromatography of Reaction Products.

The reaction mixture contained 2.0 g β -(1 $\rightarrow$ 4)-xylobiose and 260 units of *A. niger* β -xylosidase in 80 ml of 0.1 M acetate buffer (pH 4.4). After incubating at 50°C for 1.5 hr, the reaction mixture was heated in a boiling water bath for 10 min, concentrated to 15 ml under reduced pressure, and then adsorbed on activated charcoal column (Wako Jun Yaku Co., 2.6 $\times$ 38 cm). The successive elutions were done with distilled water and 5% and 30% (v/v) ethanol. The size of each fraction was 15 ml and the flow rate of eluent was 50 ml/hr.

Fig. 2. TLC of Transxylosylation Products Isolated by Charcoal Column Chromatography.

(a) Sprayed with 20% sulfuric acid in methanol; and (b) sprayed with *p*-anisidine hydrochloric acid solution. S, standard xylooligosaccharides; X1, xylose; X2, β -(1 $\rightarrow$ 4)-xylobiose; X3, β -(1 $\rightarrow$ 4)-xylotriose.

corresponded to one spot of this nonreducing sugar on TLC were collected and concentrated under reduced pressure. The yield of the saccharide O-1 was approximately 20 mg.

Next, structural analysis of this nonreducing sugar was done by methylation and $^1\text{H-NMR}$ as follows: Saccharide O-1 was methylated by the method of Ciucanu and Kerek,³⁾ and hydrolyzed for 2 hr with 10% (v/v) trifluoroacetic acid (TFA) at 100°C, and the methylated sugars were converted into their alditol acetates after reduction with sodium borohydride. The resultant alditol acetates were analyzed by gas liquid chromatography (GLC).⁴⁾ A hydrolysate of methylated O-1 gave only 1,5-diacetyl-2,3,4-tri-*O*-methyl-D-xylitol, indicating that saccharide O-1 was a xylodisaccharide having a (1 $\rightarrow$ 1')-linkage between the two xylosyl residues.

The $^1\text{H-NMR}$ spectrum for a solution in deuterium oxide (internal standard of 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid, sodium salt (TSP)) was recorded at 200 MHz with a super-conduction nuclear magnetic resonance instrument AC 200 (Nihon Bruker Co., Ltd.). This signal supports the deduction that both the two D-xylose moieties have the β -

configuration. In the spectrum, no signals due to α -anomeric protons were observed.

In addition, the degree of polymerization (DP)⁵⁾ and its specific rotation gave 2 and $[\alpha]_{\text{D}}^{25} - 72.3$ ($c=0.75$, H_2O), respectively. An acid hydrolysate of the saccharide consisted of only xylose. From these data, the saccharide O-1 was identified as *O*- β -D-xylopyranosyl-(1 $\rightarrow$ 1')- β -D-xylopyranose. This saccharide was thought to be produced by a transxylosyl reaction from xylobiose toward the C-1 position of xylose, the hydrolysis product.

We succeeded in the isolation of a new xylodisaccharide with a (1 $\rightarrow$ 1')-linkage by the charcoal column chromatography and its detection on TLC by both 20% sulfuric acid in methanol and *p*-anisidine hydrochloric acid solution.

On the other hand, as the transxylosylation products in this study, besides the products as described above, xylodisaccharides and xylotrisaccharides with dissimilar linkages had also been observed on TLC in the shoulder peak after P-2 and the 30% ethanol fractions (data not shown). Probably *A. niger* β -xylosidase has a broad specificity for the positions transferred toward the hydroxyl groups of a xylosyl acceptor.

Closer structural analysis and identification of the other transfer products are now in progress.

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References

- 1) H. Shinoyama, Y. Kamiyama and T. Yasui, *Agric. Biol. Chem.*, **52**, 2197 (1988).
- 2) M. Dubois, R. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
- 3) I. Ciucanu and F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).
- 4) I. Kusakabe, Y. Kamiyama and T. Yasui, *Nippon Nōgeikagaku Kaishi*, **51**, 167 (1977).
- 5) T. E. Timell, *Svensk Papperstid.*, **63**, 668 (1960).