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Note

Enzymatic Synthesis of Hydroquinone β-Xyloside from Xylooligosaccharides

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β-Xylosidase of *Aspergillus pulverulentus* catalyzes a transxylosylation reaction from xylooligosaccharides to acceptor hydroquinone. Two transfer products were purified and their structures were examined. These were identified as hydroquinone β-(1→4)-xyloside and hydroquinone 4-*O*-(β-xylosyl)-β-xyloside. The hydrolysis of these glycosides apparently took place accompanying the synthesis of the transfer products.

The transxylosylation reaction is an enzymatic process in which a certain xyloside is converted into a new glycoside in the presence of a suitable acceptor.[1] With the transfer reaction, various alkyl β-xylosides were effectively synthesized from xylobiose by *Aspergillus niger* β-xylosidase.[2] By application of the reaction with *Aspergillus pulverulentus* β-xylosidase, some transfer products were detected from reaction mixtures containing xylooligosaccharides and phenolic compounds.[3] This enzyme was then used for the transxylosylation from xylobiose and xylooligosaccharides to hydroquinone, where a new glycoside, hydroquinone β-xyloside, was similar to arbutin. Arbutin is a monoglucoside of hydroquinone, a potent suppressor in the synthesis of human skin melanin without having any apparent side effects.[4] By this transxylosylation reaction two transfer products, hydroquinone β-(1→4)-xyloside (HQX) and hydroquinone 4-*O*-(β-xylosyl)-β-xyloside (HQXB) were synthesized and the products were purified and their structures analyzed. This paper describes the synthesis of these new glycosides by the application of the transxyloxylation reaction using a commerical product, Pectinase G containing a β-xylosidase obtained from *Asp. pulverulentus*.

β-Xylosidase activity was assayed using phenyl β-D-xyloside[2] as its substrate. Pectinase G was purchased from Amano Co. A xylooligosaccharide mixture (containing xylobiose 42.3, xylotriose 37.2, and xylotetraose *etc.* 20.4%) was from Towa Chemical Co. Hydroquinone and phenyl β-D-xyloside were from Nacalai Chemicals Co. The upper space of a test tube was replaced with nitrogen gas and the mixture containing 8.1 g of xylooligosaccharides (XO), 10 g of hydroquinone (HQ), and 186 units of crude β-xylosidase in 0.1 M sodium acetate buffer, pH 4.0 (200 ml) was incubated at 40°C for 3 h. To improve the separation of the transfer products (TPs) from the hydrolysis products (xylose (X) and XO) by a charcoal column, the reaction mixture was treated with aniline acetate at 80°C for 3 h,[5] cooled and filtered through Toyo filter paper No. 2. Colored substances were removed by extraction with diethyl ether and the remaining solvent in water layer was removed by evaporation. Approximately 10 ml of the concentrate of reaction mixture was put onto a charcoal column (2.5 × 20 cm). Elution was done with a linear gradient of ethanol (10–40%) at a flow rate of 60 ml/h. Fractions containing transfer products were put onto a silica gel column (2.6 × 38 cm). Elution was done with ethyl acetate–acetic acid–water, 3 : 1 : 1 at aflow rate of 60 ml/h. Two peaks of sugars were collected separately, evaporated, and further purified through a column (2.5 × 20 cm) of Biogel P-2 equilibrated with water. Five-ml fractions were collected at flow rate of 48 ml/h.

TLC was done on a Kiesel Gel 60 plate (Merck), developed with the same solvent system used in silica gel column chromatography. The sugars on the plate were detected by heating at 140–150°C for 5 min after spraying with sulfuric acid–methanol (1 : 1, v/v). The reaction products were measured by high performance liquid chromatography (HPLC) with a Waters Associates M-45 solvent delivery system and detector, a Waters Differential Refractometer R401, under the following conditions: column, Shodex Sugar SP 0810 (Pb^{2+}) (8 mm × 30 cm); solvent, double distilled water; flow rate, 0.5 ml/min; temperature, 80°C.

The amounts of TPs were estimated according to a calibration curve prepared by using a ratio of peak area of arbutin to inositol (internal standard). Total sugars were measured by the phenol-sulfuric acid method[6] using xylose as a standard. The optical rotation of the products were measured with a model DIP-141 (Japan Spectroscopic Co.) digital polarimeter. Melting points were measured on a hot stage apparatus (Mitamurariken Kogyo Co.). Nuclear magnetic resonance (^{13}C NMR) spectra of the samples in D_2O were recorded at 67.8 MHz with JEOL *Ex* 270 spectrometer operating in the proton-complete decoupled mode. The chemical shift (δ) were given in ppm values with DSS (δ 0.0) as an internal standard.

To identify the structures of the transfer products (TPs), TP-1, TP-2, and TP-3 were isolated as shown in Fig. 1. The TPs, which are non-reducing sugars, could be discriminated from the hydrolysis products by their higher R_f values and by the spots after

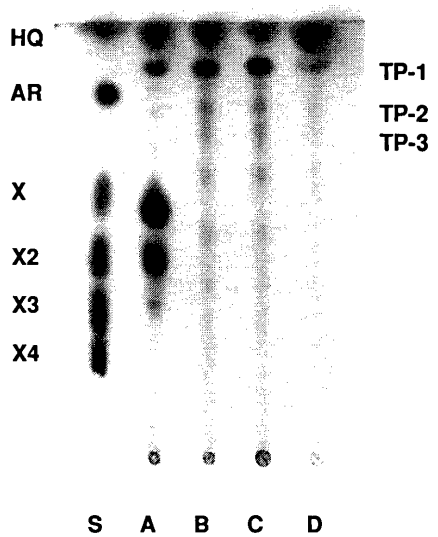

Fig. 1. Thin Layer Chromatogram of Reaction Products from Xylooligosaccharides at Step of Processing.

The reaction conditions were described in the text S, standards (HQ, hydroquinone; AR, arbutin; X, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose); TP-1–TP-3, transfer products. A, before aniline treatment; B, after aniline treatment; C, after diethyl ether extraction (water layer); D, see legend of C (ether layer).

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Table ^{13}C NMR Chemical Shift of Transfer Products and Related Compounds in D_2O

Compound	Residue or group	Chemical shifts (δ in ppm[a])					
		C-1	C-2	C-3	C-4	C-5	C-6
TP-1	β-Xyl(1→4)-	104.7	75.6	78.2	71.8	67.9	
	HQ	152.8	118.9	121.3	154.1	121.3	118.9
TP-2	β-Xyl(1→4)-	104.6	75.5	78.3	71.9	67.9	
	β-Xyl(1→4)-	104.6	75.5	76.3	78.9	65.7	
	HQ	152.8	119.0	121.3	154.1	121.3	119.0
Hydroquinone		151.7	119.1	119.1	151.7	119.1	119.1
Xylose[c]	β-Xyl	99.3	76.7	78.5	71.9	67.9	
Xylobiose[c]	β-Xyl(1→4)-	104.5	75.4	78.3	71.9	67.9	
	β-Xyl	99.2	76.6[b]	76.7[b]	79.1	65.7	

[a] DSS (δ 0.0) as internal standard.
[b] The assignments may be reversed.
[c] The signals were assigned in accordance with refs. 8 and 9.

aniline treatment, by which XO were changed to aniline derivatives[7] while the TPs remained unchanged. The aniline derivatives of the xylosaccharides were adsorbed onto the activated carbon column more strongly than the TPs. They were retained in the column until the TPs were completely eluted with a linear gradient of ethanol concentration. Consequently, unlike TP-3, TP-1 (224 mg) and TP-2 (162 mg) were obtained in a homogeneous state as detected by TLC and HPLC after purification on the silica gel and the Biogel P-2 column chromatographies.

Chemical shifts of the ^{13}C NMR of the two TPs and their related compounds are given in Table. The TP-1 was obtained as a white-creamy powder by freeze-drying, $[\alpha]_D -44.1°$ ($c=0.8$, H_2O) and mp 183–184°C. In the NMR spectra, the TP-1 contained four carbon signals at δ 67.9, 71.8, 75.6, and 78.2, which were non-substituted by C-4 of the hydroquinone moiety, while the carbon signal at 104.7 can be assigned as C-1 of a xylosyl unit bound to the C-4 of the hydroquinone moiety, indicating that the TP-1 must be a mono β-(1→4) xyloside. The upfield shift (1.3 ppm) for C-4 from C-1 in hydroquinone group of the TP-1 also support the idea of the occurrence of the linkage at this carbon. The TP-2 was obtained as a white powder, $[\alpha]_D -41.7°$ ($c=0.4$, H_2O) and mp 135–136°C. In contrast to the TP-1, the TP-2 contained 7 signals at δ 65.7, 67.9, 71.9, 75.5, 76.3, 78.3, and 78.9, which were non-substituted by C-4 of the hydroquinone group, while a carbon signal at δ 104.6 can be assigned as C-1 of the two β-D-xylosyl unit bound to the C-4 of xylose and C-4 of the hydroquinone moiety, indicating that the TP-2 consisted of two xylose residues and one hydroquinone moiety bound with β-xylosidic linkages. Furthermore it was recognized that the final hydrolysis products of the TP-1 and TP-2 were X and HQ. Thus from the spectra of ^{13}C NMR and hydrolysis of the two TPs, it was concluded that TP-1 is hydroquinone β-(1→4)-xylopyranoside (HQX) while the TP-2 is hydroquinone 4-*O*-(β-xylopyranosyl)-β-xylopyranoside or hydroquinone β-(1→4)-xylobioside (HQXB).

During the transxylosylation reaction on xylobiose, the formation of transfer and hydrolysis products were analyzed by HPLC (Fig. 2). As the reaction progressed, xylobiose was rapidly converted into HQX and X. The HQX was produced in the initial stage of the reaction and then decreased in 3 h of reaction. The yield of HQX was 19.3 mol% based on xylobiose from the initial 177 mM of xylobiose. Although a trace of the HQXB was synthesized later than the HQX, it was gradually reduced as the reaction time was prolonged. A trace of xylotriose was also found during the reaction.

To investigate the effects of donor xylobiose concentrations on

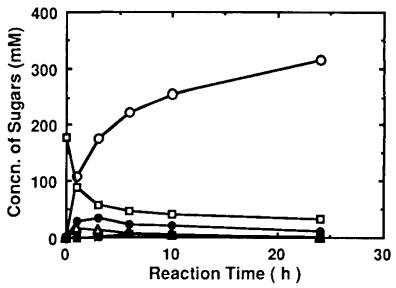

Fig. 2. Course of Formation of Hydroquinone β-Xyloside.

The reaction mixture (2 ml) consisted of β-xylosidase (2.4 U), xylobiose (177 mM), and hydroquinone (454 mM) was incubated at 40°C, pH 4.0. Samples were collected at intervals and analyzed by HPLC. ○, xylose; □, xylobiose; △, xylotriose; ●, hydroquinone β-xyloside (HQX); ■, hydroquinone β-xylobioside (HQXB).

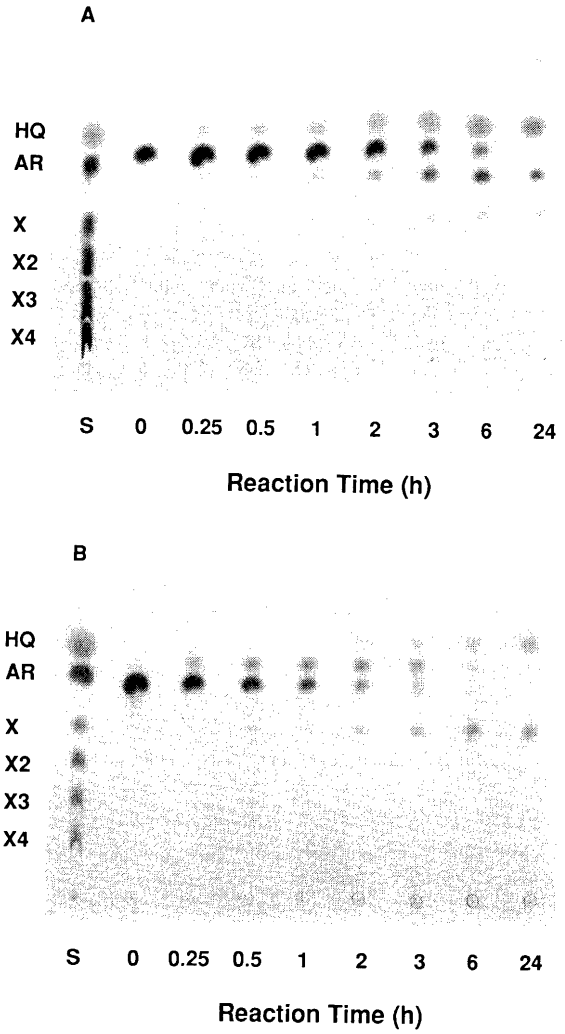

Fig. 3. Thin-layer Chromatogram of Hydrolyzates of HQX and HQXB by *Asp. pulverulentus* β-Xylosidase.

Reaction mixture (1.0 ml) containing HQX (A) and or HQXB (B) (10 mg/ml) in 0.1 M sodium acetate buffer (pH 4.0), as substrate, and β-xylosidase (0.1 U/ml) was incubated at 40°C for an appropriate time. Samples (20 μl) were collected at intervals and analyzed by TLC. S, standards (HQ, hydroquinone; AR, arbutin; X, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose).

the formation of HQX, the enzyme was incubated with various concentrations of xylobiose at 40°C. Since the HQ shows a low solubility (below 550 mM) in the buffer, in the presence of 454 mM of HQ, the yields of HQX expressed in mol% that resulted from 106, 177, and 301 mM of xylobiose were 25, 19, and 15%, respectively. To increase the yield of transfer products, some

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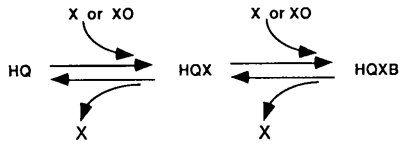
further investigations are required to find suitable conditions for the reaction such as reaction in organic solvents.

Susceptibility of the HQX and the HQXB to the *Asp. pulverulentus* β-xylosidase were examined. The formation of X and HQ were observed (Fig. 3). The most likely explanation is that HQ was liberated directly from HQX, while, the hydrolysis of the HQX apparently caused the synthesis of the HQXB, following a liberation of X and HQ (Fig. 3A). In contrast, the preference for hydrolysis rather than synthesis reaction was observed when the HQXB was used as the substrate. At the initial stage of the reaction, the HQX was formed from the HQXB and this was followed by a hydrolysis reaction which resulted in the liberation of X and HQ nearly at the same time (Fig. 3B). The liberation of HQ from HQX was more rapid than that from HQXB. On the basis of analysis on transfer- and hydrolysis reaction, the synthesis of HQX and HQXB may be represented as follows,



However, a detailed further investigation on that mechanism is necessary.

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