PREPARATION OF 3-AMINO-3-DEOXY DERIVATIVES OF TREHALOSE AND SUCROSE AND THEIR ACTIVITIES

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The 3-keto derivatives of trehalose and sucrose respectively were prepared by a one-step enzymatic route using D-glucoside 3-dehydrogenase from *Flavobacterium saccharophilum*. Reductive amination of 3-ketotrehalose with ammonium acetate and sodium cyanoborohydride gave three compounds, 3-amino-3-deoxy-α-D-allopyranosyl α-D-glucopyranoside, 3-amino-3-deoxy-α-D-glucopyranosyl α-D-glucopyranoside (3-trehalosamine) and 3-amino-3-deoxy-α-D-mannopyranosyl α-D-glucopyranoside. From the reductive amination of 3-ketosucrose, 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside and 3-amino-3-deoxy-α-D-glucopyranosyl β-D-fructofuranoside were obtained. The antibiotic and glycohydrolase inhibitory activities of these 3-amino-3-deoxy derivatives were determined.

D-Glucoside 3-dehydrogenase (EC 1.1.99.13) was first found in *Agrobacterium tumefaciens*, the agent for crown gall disease of several plants. This enzyme converts some hexopyranosides to the corresponding 3-keto compounds[1~4]. The purification and characterization of this enzyme was reported by some researchers[5~7]. However, the D-glucoside 3-dehydrogenase of *A. tumefaciens* is an inducible enzyme and its activity rapidly decays on cessation of growth on cultivation. This feature limits the application of the bacteria as biosynthetic agents.

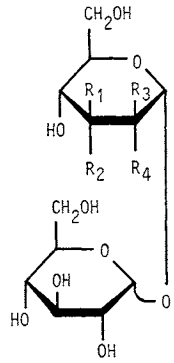
We recently found the D-glucoside 3-dehydrogenase in *Flavobacterium saccharophilum* which is involved in the degradation of the antifungal agents, validamycins, as the trigger enzyme in the C-N bond cleavage of validoxylamine A[8~10]. The D-glucoside 3-dehydrogenase in *F. saccharophilum* is a constitutive enzyme and approximately 80% of its activity is contained in the membrane fraction of this organism.

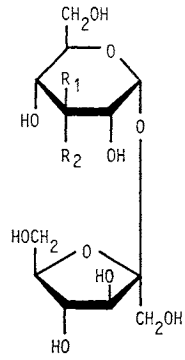
This paper describes the preparation of 3-ketotrehalose and 3-ketosucrose using a membrane fraction of *F. saccharophilum*, the reductive amination of these keto compounds with ammonium acetate and sodium cyanoborohydride, and the catalytic hydrogenation of the oximes obtained from the keto compounds. The antibiotic and glycohydrolase inhibitory activities of the 3-amino-3-deoxy disaccharides are also reported.

Preparation of 3-Ketotrehalose and 3-Ketosucrose

3-Ketotrehalose and 3-ketosucrose were prepared by the one-step enzymatic route using a membrane fraction of *F. saccharophilum*. The resulting incubation mixture of a substrate (1 g) and a membrane fraction was centrifuged and the supernatant was applied to a column of activated carbon and eluted with 50% MeOH. The concentrate of the eluate was then applied to a column of Dowex 50W-X8 (Ca^{2+}). The column was developed with water to give 3-ketotrehalose (550 mg) or 3-ketosucrose (470 mg). The structure of the 3-keto compounds were confirmed by ^{1}H and ^{13}C NMR data.

Fig. 1. Structures of 3-amino-3-deoxy disaccharides.





T-I	$R_1=R_3=H$	$R_2=NH_2$	$R_4=OH$	S-I	$R_1=H$	$R_2=NH_2$
T-II	$R_1=NH_2$	$R_2=R_3=H$	$R_4=OH$	S-II	$R_1=NH_2$	$R_2=H$
T-III	$R_1=NH_2$	$R_2=R_4=H$	$R_3=OH$			

Reductive Amination of 3-Keto Compounds

The reductive amination of 3-ketotrehalose (1 g) with ammonium acetate and sodium cyanoborohydride in MeOH gave a mixture of compounds T-I, T-II and T-III. The mixture was purified by the column chromatography on activated carbon and Amberlite CG-50 (NH_4^+). The succeeding chromatography on Dowex 1-X2 (OH^-) with water gave homogeneous compounds T-I (150 mg), T-II (65 mg) and T-III (28 mg), respectively. The reductive amination of 3-ketosucrose yielded compounds S-I and S-II, which were separated from the reaction mixture by column chromatography on CM-Sephadex C-25 (NH_4^+). Compounds S-I and S-II were isolated as compound S-I hydrochloride (160 mg) and compound S-II hydrochloride (20 mg) by Dowex 1-X2 (OH^-) chromatography with 0.2 N hydrochloric acid because compounds S-I and S-II were rigidly retained on a Dowex 1-X2 (OH^-) column.

Catalytic hydrogenation of the oxime obtained by treatment of 3-ketotrehalose (500 mg) with hydroxylamine in MeOH stereoselectively formed compound T-I (240 mg). In the case of 3-ketosucrose, the desired 3-amino-3-deoxy disaccharide was not obtained using similar techniques because the glycosidic bond of the oxime derivative was cleaved by catalytic hydrogenation.

Structure Determination of 3-Amino-3-deoxy Derivatives

Compound T-I or S-I was heated under reflux with Amberlyst 15 in MeOH for 40 hours. The hexosamine moiety was adsorbed on the ion-exchange resin and eluted from the resin with concentrated NH_4OH - MeOH (14 : 86). The eluate was chromatographed on CM-Sephadex C-25 (NH_4^+) and Dowex 1-X2 (OH^-) to give two products. They were identified as methyl 3-amino-3-deoxy-α-D-allopyranoside and 3-amino-1,6-anhydro-3-deoxy-β-D-allopyranose by direct comparison of their ^1H, ^{13}C NMR spectra and GC-MS analysis with those of authentic samples. Thus, the structures of compounds T-I and S-I were determined to be 3-amino-3-deoxy-α-D-allopyranosyl α-D-glucopyranoside and 3-amino-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside, respectively. In the same manner, the structures of compounds T-II and S-II were confirmed to be 3-amino-3-deoxy-α-D-glucopyranosyl α-D-glucopyranoside (3-trehalosamine)[11,12] and 3-amino-3-deoxy-α-D-glucopyranosyl β-D-fructofuranoside (designated as 3-sucrosamine), respectively, since the methanolysis of these compounds

gave methyl 3-amino-3-deoxy-α-D-glucopyranoside and its β-anomer. The structure of compound T-III was determined to be 3-amino-3-deoxy-α-D-mannopyranosyl α-D-glucopyranoside since its methanolysis gave only methyl 3-amino-3-deoxy-α-D-mannopyranoside.

Biological Properties

2-Trehalosamine[13], 3-trehalosamine[12], 4-trehalosamine[14] and mannosyl glucosaminide[15] have so far been reported as naturally occurring 1-1 linked aminodisaccharide antibiotics. They are all α,α-glycosides of an aminosugar and a neutral sugar. Recently, the only example of α,β-glycoside of two aminosugars, 3,3′-neotrehalosadiamine, has been reported[16]. The synthetic 3,3′-diamino-3,3′-dideoxy disaccharide, 3-amino-3-deoxy-α-D-mannopyranosyl 3-amino-3-deoxy-α-D-mannopyranoside[17], possesses a remarkable inhibitory activity against *Mycobacterium tuberculosis* and *Mycobacterium avium*, whereas the synthetic 6-trehalosamine[18] is inactive.

The prepared 3-amino-3-deoxy disaccharides, except compound S-I, showed weak antibacterial activity against some bacterial species by the cylinder-plate assay method (Table 1). In particular, it is noteworthy that 3-sucrosamine (S-II) is no less active against *Staphylococcus aureus* and *Bacillus subtilis* than 3-trehalosamine (T-II). Compounds T-I and T-III showed weak antibacterial activity against *Escherichia coli*, while 3-trehalosamine and 3-sucrosamine were inactive.

Table 1. Cylinder-plate assay of 3-amino-3-deoxy disaccharides.

Organism	Inhibition zone (mm) at 1 mg/ml				
	Compounds				
	T-I	T-II	T-III	S-I	S-II
Staphylococcus aureus FDA 209P	—	27	—	—	23
Bacillus subtilis PCI 219	15	16	11	—	23
Mycobacterium smegmatis ATCC 607	—	—	—	—	—
Escherichia coli K-12	19	—	11	—	—
Pseudomonas aeruginosa IFO 1313	—	—	—	—	—
Saccharomyces cerevisiae IFO 0209	—	—	—	—	—
Candida albicans IFO 0583	—	—	—	—	—

—: No inhibition zone.

Table 2. Inhibitory activity of 3-amino-3-deoxy disaccharides against glycohydrolases.

Enzyme (origin)	IC_{50} (M)					
	Compounds					2-Trehalosamine
	T-I	T-II	T-III	S-I	S-II	
Invertase						
Porcine	—	—	—	3.1×10^{-4}	—	—
Baker's yeast	—	—	—	5.6×10^{-4}	—	—
Candida utilis	—	—	—	7.8×10^{-4}	—	—
Maltase						
Porcine	—	—	—	—	—	—
Baker's yeast	—	—	—	—	—	—
Trehalase						
Porcine	—	—	—	—	—	—
Baker's yeast	—	—	—	—	—	7.2×10^{-5}
Spodoptera litura	—	—	—	—	7.4×10^{-4}	—

—: No activity at 10^{-3} M.

The 3-amino-3-deoxy disaccharides prepared from trehalose showed no inhibitory activity against any glycohydrolases tested. Compounds S-I and S-II showed weak inhibitory activity against various invertases and an insect trehalase, respectively (Table 2).

Experimental

MP's were determined with a Yamato MP-21 apparatus and are uncorrected. Optical rotations were measured with a Jasco digital polarimeter DIP-4. ^{1}H NMR spectra were recorded with a Jeol JNM-FX100 spectrometer (at 100 MHz). Chemical shifts are reported in ppm from internal sodium 3-(trimethylsilyl)propionate (TSP, in D_2O) or TMS (in $CDCl_3$ or C_6D_6). ^{13}C NMR spectra were recorded with Jeol JNM-FX100 spectrometer at 25.0 MHz. The trimethylsilyl derivatives of hexosamines were analyzed with GC-MS (Jeol JMS-DX300 JMA-DA5000). TLC was performed on precoated Kieselgel F_{254} plates (Merck) with PrOH - AcOH - H_2O (4 : 1 : 1), unless otherwise specified.

3-Aminohexoses

Methyl 3-amino-3-deoxy-β-D-glucopyranoside and methyl 3-amino-3-deoxy-α-D-mannopyranoside were purchased from Lucerna-Chem AG.; 3-amino-3-deoxy-D-glucose from Calbiochem-Behring Corp.; and 3-acetamido-3-deoxy-D-allopyranose from Sigma Chemical Company. Methyl 3-amino-3-deoxy-α-D-glucopyranoside[19] and methyl 3-amino-3-deoxy-β-D-glucopyranoside were prepared by methanolysis of 3-amino-3-deoxy-D-glucose. 3-Amino-1,6-anhydro-3-deoxy-β-D-allopyranose and methyl 3-amino-3-deoxy-α-D-allopyranoside were prepared by methanolysis of 3-acetamide-3-deoxy-D-allopyranose.

GC-MS Analysis of Aminohexose Trimethylsilyl Derivatives

Approxymately 1 mg of the aminohexoses in a silicone-capped small tube were dissolved into 100 μl of pyridine. Bis(trimethylsilyl)acetamide 100 μl and trimethylchlorosilane 50 μl were added. The tube was heated at 75°C for 20 minutes. Each sample was analyzed on a glass column (0.26× 200 cm) with 7% silicone OV-17 on Chromosorb W AW DMCS. The reaction was performed at 175°C. The GC-MS analysis of aminohexose *N,O*-trimethylsilyl derivatives resulted in the following retention times (t_R) and *m/z* peaks;

3-Amino-1,6-anhydro-3-deoxy-β-D-allopyranose: t_R 4.4 minutes, *m/z* 377 $(M)^+$.
Methyl 3-amino-3-deoxy-α-D-allopyranoside: t_R 6.7 minutes, *m/z* 481 $(M)^+$.
Methyl 3-amino-3-deoxy-α-D-glucopyranoside: t_R 11.1 minutes, *m/z* 481 $(M)^+$.
Methyl 3-amino-3-deoxy-β-D-glucopyranoside: t_R 10.2 minutes, *m/z* 481 $(M)^+$.
Methyl 3-amino-3-deoxy-α-D-mannopyranoside: t_R 6.2 minutes, *m/z* 481 $(M)^+$.

Preparation of 3-Ketotrehalose and 3-Ketosucrose

A mixture of trehalose (1 g) or sucrose (1 g) and the membrane fraction[8,9] obtained from 150 g wet cells of *F. saccharophilum* in 0.05 M acetate buffer (pH 5.5, 100 ml) containing 1 mM EDTA were incubated at 37°C for 9 hours with shaking. The incubation mixture was centrifuged and the supernatant was applied to a column of activated carbon (200 ml) and eluted with 50% MeOH. The concentrate was then applied to a column of Dowex 50W-X8 (Ca^{2+}, 450 ml). The column was developed with water to give 3-ketotrehalose (550 mg) or 3-ketosucrose (470 mg).

α-D-Glucopyranosyl α-D-*ribo*-3-Hexosulopyranoside (3-Ketotrehalose): Rf 0.43; $[\alpha]_D^{20}$ +147.1° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 3.32~4.16 (9H), 4.43 (1H, dd, J=1.5 and 9.5 Hz, 4-H), 4.68 (1H, dd, J=1.5 and 4.6 Hz, 2-H), 5.17 (1H, d, J=3.2 Hz, 1′-H), 5.56 (1H, d, J=4.6 Hz, 1-H); ^{13}C NMR (D_2O) δ 63.2 (t, C-6, C-6′), 72.3 (d, C-4′), 73.7 (d, C-2′), 74.4 (d, C-5′), 75.1 (d, C-4, C-3′), 76.6 (d, C-2), 78.0 (d, C-5), 96.5 (d, C-1′), 99.0 (d, C-1), 209.8 (s, C-3).

β-D-Fructofranosyl α-D-*ribo*-3-Hexosulopyranoside (3-Ketosucrose): Rf 0.46; $[\alpha]_D^{20}$ +55.0° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 3.63~4.27 (10H), 4.45 (1H, dd, J=1.5 and 9.5 Hz, 4-H), 4.61 (1H, dd, J= 1.5 and 4.6 Hz, 2-H), 5.79 (1H, d, J=4.6 Hz, 1-H); ^{13}C NMR (D_2O) δ 63.0 (t, C-6), 63.9 (t, C-1′), 65.1 (t, C-6′), 74.1 (d, C-4), 76.6 (d, C-2, C-4′), 78.2 (d, C-5), 78.9 (d, C-3′), 84.2 (d, C-5′), 97.7 (d, C-1), 106.8 (s, C-2′), 210.2 (s, C-3).

Reductive Amination of 3-Keto Compounds

Method A: To a solution of 3-ketotrehalose (1 g) and CH_3COONH_4 (3 g) in MeOH (20 ml), $NaBH_3CN$ (1.5 g) and HCl (2 N, 0.6 ml) were added, and the solution was stirred for 16 hours at room temperature. The mixture was concentrated, dissolved in water (200 ml), and then applied to a column of activated carbon (200 ml). The column was washed with water and eluted with 50% MeOH. The eluate was concentrated and chromatographed on a column of Amberlite CG-50 (NH_4^+, 100 ml) with 0.1 N NH_4OH. Finally, the eluate was chromatographed on a column of Dowex 1-X2 (OH^-, 170 ml) with water to give homogeneous compounds T-I (150 mg), T-II (65 mg) and T-III (28 mg), respectively.

Compound T-I: Rf 0.33; $[\alpha]_D^{20}$ +184.0° (*c* 0.5, H_2O); ^1H NMR (D_2O) δ 5.14 (1H, d, J=3.4 Hz, anomeric proton), 5.18 (1H, d, J=3.4 Hz, anomeric proton); ^{13}C NMR (D_2O, pD 2.0) δ 57.1 (d, C-3), 63.0 (t, C-6), 63.2 (t, C-6′), 64.8 (d, C-4), 66.5 (d, C-2), 70.2 (d, C-5), 72.1 (d, C-4′), 73.1 (d, C-2′), 75.1 (d, C-5′), 75.5 (d, C-3′), 95.8 (d, C-1), 96.4 (d, C-1′).

Compound T-II: Rf 0.30; $[\alpha]_D^{20}$ +207.6° (*c* 0.5, H_2O) (literature 12, +161° (*c* 13.7, H_2O)); ^1H NMR (D_2O) δ 5.15 (1H, d, J=3.7 Hz, anomeric proton), 5.19 (1H, d, J=3.7 Hz, anomeric proton); ^{13}C NMR (D_2O, pD 1.7) δ 57.6 (d, C-3), 62.6 (t, C-6), 63.3 (t, C-6′), 68.6 (d, C-4), 70.4 (d, C-2), 72.4 (d, C-4′), 73.7 (d, C-2′), 74.7 (d, C-5), 75.0 (d, C-5′), 75.2 (d, C-3′), 95.0 (d, C-1), 96.2 (d, C-1′).

Compound T-III: Rf 0.30; $[\alpha]_D^{20}$ +145.0° (*c* 0.5, H_2O); ^1H NMR (D_2O) δ 5.08 (1H, d, J=1.7 Hz, anomeric proton), 5.19 (1H, d, J=3.4 Hz, anomeric proton); ^{13}C NMR (D_2O, pD 1.9) δ 55.8 (d, C-3), 63.0 (t, C-6), 63.3 (t, C-6′), 65.9 (d, C-2), 69.5 (d, C-4), 72.3 (d, C-4′), 73.5 (d, C-2′), 75.3 (d, C-5, C-5′), 75.6 (d, C-3′), 96.3 (d, C-1′), 96.7 (d, C-1).

3-Ketosucrose (1 g) was treated with the same manner mentioned above. The reaction mixture was purified on a column of activated carbon (200 ml) and Amberlite CG-50 (NH_4^+, 100 ml). The eluate from Amberlite CG-50 was chromatographed on a CM-Sephadex C-25 (NH_4^+, 100 ml) with 0.02 N NH_4OH to resolve two fractions. Each fraction was chromatographed on a column of Dowex 1-X2 (OH^-, 50 ml). The column was washed with water and then eluted with 0.2 N HCl. The eluate was concentrated and then lyophilized to give compound S-I hydrochloride (160 mg) or compound S-II hydrochloride (20 mg).

Compound S-I Hydrochloride: Rf 0.40; $[\alpha]_D^{20}$ +41.1° (*c* 0.5, H_2O); ^1H NMR (D_2O) δ 5.46 (1H, d, J=3.4 Hz, anomeric proton); ^{13}C NMR (D_2O) δ 57.2 (d, C-3), 62.7 (t, C-6), 64.5 (d, C-4), 64.5 (t, C-1′), 66.4 (d, C-2), 70.4 (d, C-5), 76.4 (d, C-4′), 79.4 (d, C-3′), 84.3 (d, C-5′), 94.3 (d, C-1), 106.9 (s, C-2′).

Compound S-II Hydrochloride: Rf 0.36; $[\alpha]_D^{20}$ +173.0° (*c* 0.5, H_2O); ^1H NMR (D_2O) δ 5.45 (1H, d, J=3.7 Hz, anomeric proton); ^{13}C NMR (D_2O) δ 57.7 (d, C-3), 62.3 (t, C-6), 64.1 (t, C-1′), 65.1 (t, C-6′), 68.3 (d, C-4), 70.5 (d, C-2), 75.0 (d, C-5), 76.7 (d, C-4′), 79.2 (d, C-3′), 84.2 (d, C-5′), 93.9 (d, C-1), 106.6 (s, C-2′).

Method B: A solution of 3-ketotrehalose (500 mg) and hydroxylamine hydrochloride (200 mg) in MeOH was stirred at room temperature for 3 hours. The reaction mixture was hydrogenated in the presence of PtO_2 at atmospheric pressure for 6 hours. The filtrate was concentrated and chromatographed on a column of Dowex 1-X2 (OH^-, 200 ml). The column was eluted with water to give compound T-I (240 mg) as the sole product.

In the case of 3-ketosucrose, the desired 3-amino-3-deoxy derivative was not formed using the same method mentioned above.

Assay of Glycohydrolase Inhibitory Activity

Invertase (*Candida utilis* and baker's yeast) and maltase (baker's yeast) were purchased from Sigma Chemical Company. Invertase, maltase and trehalase from procine sources were prepared from small intestine mucosa according to the method of DAHLQVIST[20]. The trehalases of baker's yeast[21] and insect[22] (*Spodoptera litura*) were prepared as described in the literature. The inhibitory activity was determined by incubating an enzyme solution (125 μl) with a 0.5-M substrate solution (50 μl) and an inhibitor solution (200 μl) in 0.05 M phosphate buffer (pH 6.0, in a final volume of 500 μl) at 37°C for 15 minutes and by measuring the amount of released D-glucose by the glucose oxidase

method using the commercially available Glucose B-Test Wako kit (Wako Pure Chemical Industries, Ltd.).

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