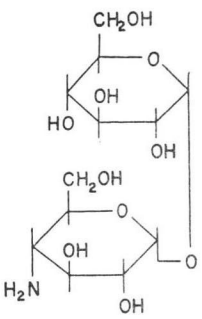
4-AMINO-4-DEOXY-α, α-TREHALOSE, A NEW METABOLITE OF A STREPTOMYCES

Sir:

In the course of our screening of new antibiotics, a new metabolite (**I**) which had a weakly antibacterial activity was isolated from a cultured broth of a *Streptomyces*. This strain (MD 303-SF 1) was isolated from a soil collected in Hokkaido, Japan, and was related to *Streptomyces cirratus*[1,2]. The structure of **I** was determined to be 4-amino-4-deoxy-α, α-trehalose (4-trehalosamine), a positional isomer of trehalosamine: 2-amino-2-deoxy-α, α-trehalose. In this communication, the production, isolation, properties and structural determination of 4-trehalosamine are reported.

A medium containing 1.5% starch, 1.5% glucose, 2.0% corn steep liquor, 0.3% yeast extract, 0.3% NaCl, 0.3% $CaCO_3$, 0.005% $ZnSO_4 \cdot 7H_2O$, 0.0005% $CuSO_4 \cdot 5H_2O$ and 0.0005% $MnCl_2 \cdot 4H_2O$ was used for the production of **I**. The maximum production was obtained at fourth day of shaking culture at 27°C. The filtrate showed an antibacterial activity of 0.78 mg/ml of **I**. Five liters of the filtrate was treated with 150 g of charcoal, and the adsorbed material was eluted with 80% aqueous methanol. The eluate was dried to give 6.9 g of the crude powder (purity 42%). It was purified by Amberlite CG-50 (NH_4^+) column chromatography developed with 0.05 N NH_4OH. Thus, 2.1 g of purified **I** was obtained.



4-Trehalosamine

Compound **I** was a colorless powder, m. p. 140°C, $[\alpha]_D$ +179° (*c* 0.5, H_2O). Found: C, 40.11; H, 6.94; N, 3.94; O, 47.53. Calcd, for $C_{12}H_{23}NO_{10} \cdot H_2O$: C, 40.11; H, 7.01; N, 3.90; O, 48.98. There is no UV absorption except end absorption. The potentiometric titration showed the presence of a basic function of pKá 6.8 and the titration equivalent was 380 (Calcd. for $C_{12}H_{23}NO_{10} \cdot H_2O$; 359). It was positive in a ninhydrin reaction.

The NMR spectrum (in D_2O, external TMS reference) showed the presence of 1 proton at δ 5.67 (doublet, J=3.5 Hz), 1 proton at 5.63 (doublet, J=3.6 Hz), 11 protons at 3.8~4.5 and 1 proton at 3.24 (multiplet). The IR spectrum showed broad and strong absorptions at 980~1140 cm^{-1}, but there was no absorption in the carbonyl region. Treatment of **I** with acetic anhydride and pyridine gave the octa-acetate (*m/e* 678.2262. Calcd. for $C_{28}H_{40}NO_8$: 678.2251). The above results suggested that **I** is a disaccharide composed of a hexose and a hexosamine. Compound **I** did not show reducing property, wich was suggesting a 1, 1-glycoside.

Compound **I** was refluxed in methanol with Amberlyst 15[3], a marcoreticular and strongly acidic ion-exchange resin suitable for non-aqueous reaction. The resulting methyl glycoside of the hexosamine was adsorbed on the resin and while the methyl glycoside of the hexose remained in solution.

The NMR spectrum and thin-layer chromatography of the filtrate material indicated that it should be the anomeric mixture of methyl glucopyranosides. The dried material of the filtrate was treated by a Dowex 1×2 (OH^-) column developed with water to separate the anomers. The α-anomer was eluted faster than the β-anomer. The α-anomer, m. p. 165~166°C, $[\alpha]_D^{30}$ +152° (*c* 1.3 in H_2O), was confirmed to be metyl-α-D-glucopyranoside by direct comparison with authentic material.

The methyl glycoside of the hexosamine was isolated from the ion-exchange resin by elution with a dilute ammonia. The dried eluate was acetylated and then subjected to silica gel column chromatography developed with chloroform and methanol (50 : 1) to separate the anomers. The major component was eluted faster. It was crystallized with chloroform and *n*-hexane, m. p. 146°C, $[\alpha]_D^{25}$ +176° (*c* 0.2, $CHCl_3$), Found: C, 50.03; H, 6.41; N, 3.94. Calcd. for $C_{15}H_{23}NO_9$ (MW. 361): C, 49.86; H, 6.42; N, 3.88. MS. *m/e* 330(M-31). The NMR spectrum taken in $CDCl_3$ solution indicated that it should be methyl-4-acetamido-4-deoxy-2, 3, 6-tri-O-acetyl-α-D-glucopyranoside. [δ 1.93, 2.03. 2.09 and 2.10 (4 Ac), 3.40 (OMe), 4.99 (1-CH, J=3.6 Hz), 4.92 (2-CH, J=3.6, 8.5), 5.32 (3-CH, J=8.5, 9.0), 4.21 (4-CH, J=9.0, 9.0, 11.0), 3.81 (5-CH, J=4.0, 4.0, 11.0), 4.22 (6-CH_2, J=4.0), 5.75 (N-H, J=9.0)].

The minor component, the β-anomer, was

crystallized with chloroform and *n*-hexane, m.p. 188°C, $[\alpha]_D^{30}+21.8°$ (*c* 0.46, $CHCl_3$). It was synthesized from methyl-4-azido-4-deoxy-2,3,6-tri-O-benzoyl-β-D-glucopyranoside, which was kindly supplied by Dr. TSUCHIYA, Keio University, by a series of derivations: reduction, debenzoylation and acetylation. The synthetic and natural products were identical with respect to their IR and NMR spectra, optical rotation and mixed melting point.

The configuration of the 1,1-glycosidic linkage of **I** was assigned to be α and α from the coupling constants of the anomeric protons (J=3.5 at δ 5.67 and J=3.6 at δ 5.63). Thus, the structure of **I** was determined to be 4-amino-4-deoxy-α, α-trehalose (4-trehalosamine).

4-Trehalosamine showed weakly antibacterial activity against some bacterial species by the cup assay method (Table 1). However, it was inactive against such bacteria at 200 μg/ml by the agar dilution method. 4-Trehalosamine was inactive against *Mycobacterium smegmatis* ATCC 607 by cup assay and agar dilution methods, while trehalosamine was active at 6.25 μg/ml by agar dilution method. The antibacterial activity of 4-trehalosamine against *Bacillus subtilis* and *Escherichia coli* shown by the cup assay method was not diminished by addition of equal amount of trehalose. 4-Trehalosamine did not show any toxicity to mouse at a dose of 625 mg/kg by intravenous administration.

Recently, S. HANESSIAN *et al.*[4] reported the synthesis of 6-trehalosamine, which was also inactive against *Mycobacterium tuberculosis* at 200 μg/ml.

Table 1. Diameter of inhibition zone of 4-trehalosamine by cup assay method

	2 mg/ml	1 mg/ml	0.5 mg/ml
E. coli NIHJ	25.8 mm	22.5 mm	19.5 mm
E. coli K-12	28.0	23.5	19.3
Kleb. pneumoniae	(18.0)*	(15.3)	trace
B. subtilis PCI 219	21.3	16.0	12.8

* Partial inhibition.

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