Notes

3,3′-NEOTREHALOSADIAMINE (BMY-28251)†, A NEW AMINOSUGAR ANTIBIOTIC

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In our screening program designed to obtain novel aminoglycoside antibiotics using an aminoglycoside hypersensitive strain Kp-126[1], a bacterial strain No. K169-B91 was found to produce a new antibiotic designated as BU-2797 (later called BMY-28251). The structure of the new antibiotic was determined to be 3,3′-diamino-3,3′-dideoxy-α,β-trehalose, designated as 3,3′-neotrehalosadiamine. The present paper describes the production, isolation, properties and structure determination of the antibiotic (hereafter called BMY-28251 for simplicity).

The producing organism, strain K169-B91, was isolated from a soil sample collected in Peru. It is an aerobic, Gram-positive, spore-forming rod bacterium classified as belonging to the genus *Bacillus*. Further studies on the morphological, cultural and physiological characteristics of strain K169-B91 revealed that the strain should be classified as *Bacillus pumilus* Meyer and Gottheil 1901. Thus the strain was named *B. pumilus* K169-B91. BMY-28251 was produced by shaken fermentation at 28°C using a medium consisting of soybean meal, 3%; corn starch, 2%; $CaCO_3$ 1% and $MgSO_4 \cdot 7H_2O$, 0.33%. Antibiotic production was monitored by paper disc assay with Kp-126 and reached a maximum of 700 μg/ml after 42 hours. The harvested broth (10 liters) was clarified using a continuous centrifuge. The mycelial cake was extracted with MeOH and the extract concd to an aq solution. The broth supernate and the concentrate of the methanolic extract were combined, adjusted to pH 7.0 and applied on a column of Amberlite IRC-50 (NH_4^+ - H^+, 7: 3). The column was developed with H_2O, 0.1 N NH_4OH, 0.5 N NH_4OH and 1 N NH_4OH, successively. The active fractions were collected and concd *in vacuo* to yield a semi-pure solid which was further chromatographed on Amberlite CG-50 (NH_4^+) to afford a homogeneous preparation of BMY-28251 (3.6 g).

The physico-chemical properties of BMY-

Table 1. Physico-chemical properties of BMY-28251.

Nature	White amorphous powder
MP	116~119°C (dec)
$[\alpha]_D^{24}$	+36° (*c* 0.4, H_2O)
Elemental analysis	
Calcd for $C_{12}H_{24}N_2O_9$:	C 42.35, H 7.11, N 8.23
Found:	C 42.54, H 7.43, N 8.60
EI-MS (*m/z*)	340 (molecular ion)
pKa′	7.90 (in H_2O)
Color reactions Positive	Ninhydrin, anthrone
Negative	Tollens', $FeCl_3$
UV (MeOH)	End absorption
IR (KBr) cm^{-1}	3500~3200, 1620, 1440, 1325, 1080, 1030
^1H NMR δ in ppm	3.2~4.2 (12H, m), 4.78 (1H, d, J=8.0 Hz),
(60 MHz, in D_2O, pD=2.0)	5.33 (1H, d, J=3.5 Hz)
^{13}C NMR in δ ppm	54.7 (d), 58.6 (d), 61.7 (t), 61.8 (t), 70.6 (d), 70.7 (d), 72.2 (d),
(20 MHz, in D_2O, pD≧11.0)	72.9 (d), 75.6 (d), 77.8 (d), 93.0 (d), 98.3 (d)

† Originally called BU-2797.

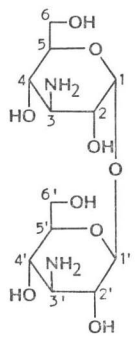
Table 2. Cup assay of BMY-28251 and trehalosamine.

Organism	Medium[a]	Inhibition zone[b] (mm) at 1 mg/ml	
		BMY-28251	Trehalosamine
Staphylococcus aureus 209P	NA	21w	27w
Bacillus subtilis PCI-219 (pH 6.0)	NA	—	20w
B. subtilis PCI-219 (pH 8.0)	NA	18w	23
Klebsiella pneumoniae No. 22-3083	NA	—	—
K. pneumoniae No. 126	NA	19w	—
K. pneumoniae No. 126	No. 1003	23	21w

[a] NA: Nutrient agar.
No. 1003: Peptone 10 g, meat extract 5 g and agar 12 g/liter.
[b] w: Hazy inhibition zone.
—: No inhibition zone.

28251 summarized in Table 1 suggested a disaccharide structure containing two amino functions. On acetylation with acetic anhydride and pyridine, BMY-28251 afforded an octaacetyl derivative which exhibited the molecular ion at m/z 676 and strong fragment ions at 616 ($M^+ -$ AcOH), 556 ($M^+ -2$AcOH), 482 (556 $-$ $AcOCH_3$) and 330 (oxonium ion of aminohexose). BMY-28251 was heated under reflux for 40 hours with methanolic hydrogen chloride (1.5 N). The reaction mixture was chromatographed on Amberlite CG-50 (NH_4^+) to isolate the α- and β-methyl glycosides (**Ia** and **Ib** respectively) of the sugar which were purified by Sephadex LH-20 chromatography. **Ia** and **Ib** exhibited $[\alpha]_D^{24}$ $+130°$ (c 0.4, H_2O) and $[\alpha]_D^{29}$ $-28°$ (c 0.4, H_2O), respectively. In the ^1H NMR spectra in D_2O, the anomeric proton of **Ia** appeared as a doublet (J=3.5 Hz) at δ 4.75 ppm while that of **Ib** resonated at higher field with a larger coupling constant (δ 4.41 ppm, d, J=7.5 Hz). They were identified as methyl 3-amino-3-deoxy-α-D-glucopyranoside (**Ia**)[2] and its β-anomer (**Ib**)[3]. Since no fragment other than **Ia** and **Ib** was found in the acid hydrolysate, BMY-28251 should be composed of two mol of 3-amino-3-deoxy-D-glucose. The fact that BMY-28251 was negative to Tollens' reaction indicated an 1-1 glycoside structure for the antibiotic. The anomeric protons observed at δ 5.33 ppm (J= 3.5 Hz) and 4.78 ppm (J=8.0 Hz) are assignable to those of α and β-glycoside, respectively. In the ^{13}C NMR spectrum, the carbon signals assigned to C-2 (δ 72.9 ppm), C-2′ (75.6), C-4′ (70.7) and C-4 (70.6) underwent β-shift (*ca.* 4~5 ppm) upon acidification. As in the case of 3-trehalosamine, the C-1 and C-1′ carbon signals of BMY-28251 appeared at rather high field.

Scheme 1.



Thus, BMY-28251 was determined to be 3-amino-3-deoxy-α-D-glucopyranosyl 3-amino-3-deoxy-β-D-glucopyranoside (3,3′-diamino-3,3′-dideoxy-α,β-trehalose).

BMY-28251 exhibited hazy inhibition zones on nutrient agar plates seeded with *Staphylococcus aureus* 209P, *Bacillus subtilis* PCI-219 (pH 8.0) and *Klebsiella pneumoniae* No. 126 (Kp-126) by the cylinder-plate assay method. Clear inhibition zones were obtained on a Kp-126 plate using No. 1003 medium (Table 2). These test organisms were not inhibited using the agar dilution method at 100 μg/ml of BMY-28251. Trehalosamine[4], which is structually related to BMY-28251, also showed antibacterial activity only by the cylinder-plate assay method although its activity profile was somewhat different from that of BMY-28251 (Table 2). Unlike the case of trehalosamine, the activity of BMY-28251 was not antagonized by trehalose. BMY-28251 was nontoxic in mice at 400 mg/kg, i.v.

Several 1-1 glycoside linked aminodisaccharide antibiotics have been reported including trehalosamine, 3-trehalosamine[5], 4-trehalosamine[6] and mannosyl glucosaminide[7]. They are all

α,α-glycosides of an amino sugar and a neutral sugar. BMY-28251 is the first example of α,β-glycoside of two amino sugars. Since the α,β-trehalose has been named neotrehalose, we propose to call the BMY-28251-type antibiotics neotrehalosadiamines.

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