Communications to the Editor

ISOLATION OF 3,3′-NEOTREHALOSADIAMINE (BMY-28251) FROM A BUTIROSIN-PRODUCING ORGANISM

Sir:

The aminoglycoside hypersensitive mutant of *Klebsiella pneumoniae* (Kp-126) was isolated and used for a specific detection of new aminoglycoside-type antibiotics[1]. From the fermentation broth of a butirosin-producing strain, 3,3′-neotrehalosadiamine (BMY-28251) was detected as a minor component specifically active against strain Kp-126. This communication describes the isolation and identification of BMY-28251 from *Bacillus circulans* YQW-B6[2].

The medium FR8 containing soluble starch 2%, glucose 0.2%, soybean meal 3%, $MgSO_4 \cdot 7H_2O$ 0.3% and $CaCO_3$ 1% was used for the production of BMY-28251.

A well-grown agar slant of strain YQW-B6 was inoculated to 100 ml of the heart infusion broth (Difco) in 500-ml Erlenmeyer flask and incubated at 32°C for 24 hours on a rotary shaker (180 rpm) to obtain seed culture. Two ml of the culture was transferred into 100 ml of medium FR8 in 500-ml flask which was shaken at 28°C for 4 days.

The broth filtrate (2 liters) was adsorbed onto a column of Amberlite IRC-50 (70% NH_4^+ form). The column was washed with H_2O and then eluted with 1 N NH_4OH. Active eluates were combined and concentrated *in vacuo* to afford hygroscopic solid (2.8 g) as a total active fraction. A part of the solid (150 mg) was dissolved in a small amount of H_2O and applied to a column (1.0 cm × 37 cm) of Amberlite CG-50 (NH_4^+ form). The column was developed stepwise at a flow rate of 0.25 ml/minute with H_2O (fraction Nos. 1~10), 0.15 N NH_4OH (fraction Nos. 11~30) and 0.5 N NH_4OH (fraction Nos. 31~80). The antibiotic activity in each 5 ml of the elute was monitored by the paper disc-agar diffusion method using Kp-126 and *Bacillus subtilis* PCI 219 (Bs-1) as test organisms. As shown in Fig. 1, peak I, designated as component X, was highly active against Kp-126 but only weakly against Bs-1. The other two peaks showed comparable activity against Kp-126 and Bs-1. Peak III

Fig. 1. The chromatography of the total active fraction of YQW-B6 on a column of Amberlite CG-50 (NH_4^+ form).
●: Kp-126, ○: Bs-1.

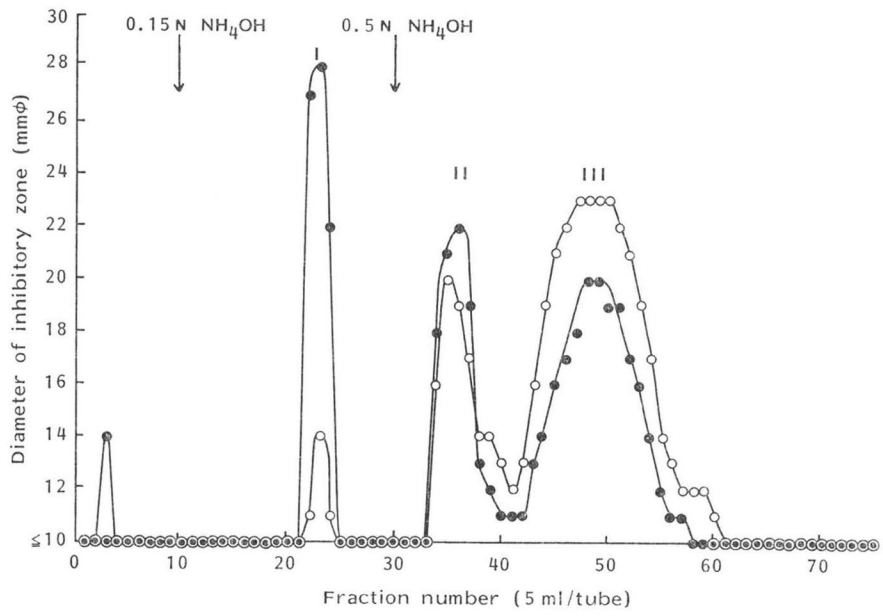

Fig. 2. NMR spectrum of component X in D_2O (pD 3.0).

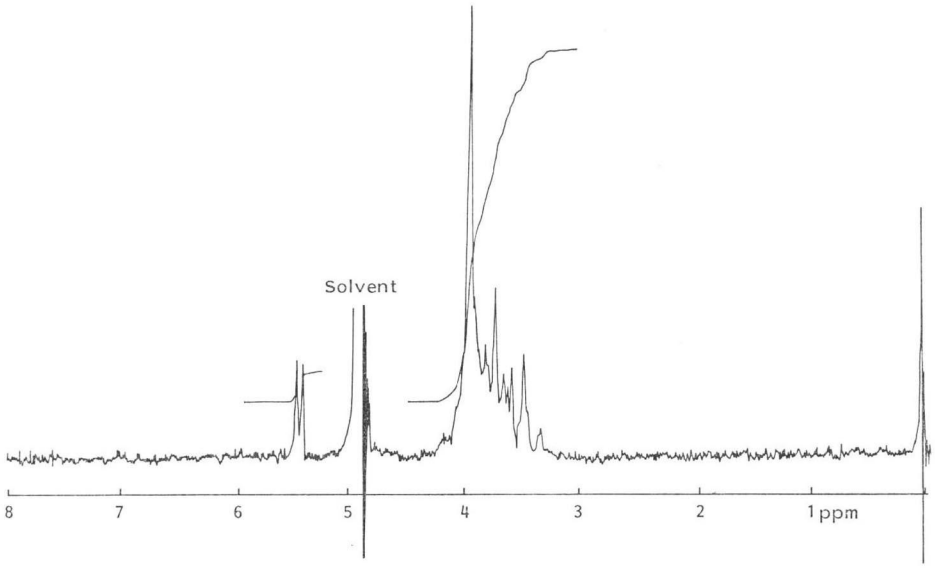

Table 1. Rf values of antibiotic components produced by strain YQW-B6 on TLC.

Component	Rf value		
	S-110*	S-102**	S-101***
X	0.56	0.64	0.55
Butirosin A	0.15	0.07	0.05
Xylostatin	0.35	0.07	0.05
Neamine	0.43	0.07	0.05

* Silica gel: $CHCl_3$ - MeOH - 28% NH_4OH - H_2O (1 : 4 : 2 : 1).
** Silica gel: 10% aq $AcONH_4$ - MeOH (1 : 1).
*** Silica gel: PrOH - pyridine - AcOH - H_2O (15 : 10 : 3 : 12).

was identified as butirosin A by TLC analysis and peak II contained several antibacterial components.

For further characterization of component X, the remainder of the total active fraction (2.5 g) was purified by the procedure described above to afford 688 mg of semi-pure solid (purity: *ca.* 30%), which was further chromatographed on Amberlite CG-50 (NH_4^+ form) to give a pure sample of component X (165 mg) as a white amorphous solid.

The component X gave positive reactions with ninhydrin and anthrone reagents, but was negative with Tollens reaction. The Rf values on silica gel TLC of component X are shown in Table 1 compared with those of butirosin and related compounds. Component X had the $[\alpha]_D^{27}$ value of +33° (*c* 1.0, H_2O), and showed only end absorption in the UV region and a carbohydrate-type IR spectrum. In the NMR spectrum in DCl/D_2O (Fig. 2), a doublet anomeric proton (5.35 ppm, J=3.5 Hz) and 11~12 methine protons adjacent to amino- and/or hydroxyl-groups (3.2~4.2 ppm) were observed.

From the results and direct TLC comparison with the authentic sample, component X was identified as 3,3′-diamino-3,3′-dideoxy-α,β-trehalose (BMY-28251), which was also found in the fermentation broth of *Bacillus pumilus* K169-B91 by using the aminoglycoside hypersensitive strain Kp-126[3].

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