Notes

ISOLATION OF 1,5-DIDEOXY-1,5-IMINO-D-MANNITOL FROM CULTURE BROTH OF *STREPTOMYCES* SPECIES

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(Received for publication February 26, 1988)

Many kinds of polyhydroxylated alkaloids which structurally resemble monosaccharides, such as nojirimycin[1~5], moranoline[6,7], nojirimycin B[8], galactostatin[9], fagomine[10], 2*R*,5*R*-dihydroxymethyl-3*R*,4*R*-dihydroxypyrrolidine (DMDP)[11], 3,4-dihydroxy-2-hydroxymethylpyrrolidine[12], (2*R*,3*S*)-2-hydroxymethyl-3-hydroxypyrrolidine[13], swainsonine[14] and castanospermine[15] have been found in a variety of organisms, including higher plants. These compounds have been the focus of intensive investigations because of their interesting biological activities.

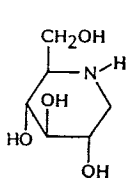
1,5-Dideoxy-1,5-imino-D-mannitol (**2**) is a potent mannosidase inhibitor which blocks conversion of high-mannose to complex oligosaccharides[16,17].

2 was first isolated from the seed of *Lonchocarpus sericeus*[18], but the production in the culture broth of *Streptomyces* species has not been established.

In the present paper, we describe the isolation of **2** from the culture broth of *Streptomyces lavendulae* GC-148 which has been already reported as a high-yielding strain of moranoline (**1**)[7].

The strain was cultivated on a rotary shaker for 3 days at 27°C in 500-ml Erlenmeyer flasks containing 100 ml of a seed medium consisting of soluble starch 8%, soy bean meal 1%, yeast extract 1%, NaCl 0.5%, $NaNO_3$ 0.2%, KCl 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 7.2). This seed culture (300 ml) was transferred to a 30-liter jar fermentor (B. E. Marubishi Co., Ltd., MSJ-U3) containing 15 liters of medium which was the same as the seed medium described above. The fermentation was carried out at 27°C at an agitation speed of 300 rpm and an aeration rate of 20 liters/minute. After 11 days cultivation, the culture broth was filtered to remove the mycelium. The filtrate (12.9 liters) was applied to a column of Dowex 50WX2 (H^+) (1,000 ml). After washing sufficiently with distilled water, **2** was desorbed with 1 N aqueous ammonia. After the eluate was concentrated under reduced pressure, the solution was applied to a column of Diaion SA-11A(OH^-) (500 ml) and **2** was developed with distilled water. The eluate was concentrated under reduced pressure, and the solution was applied again to a column of Dowex 50WX2(H^+) (300 ml). After washing with distilled water, **2** was chromatographed with 0.5 N aqueous ammonia. Fractions containing **2** were evaporated and dried *in vacuo*. The residue was dissolved in methanol and the solution was applied to a column of Sephadex LH-20 (200 ml) and developed with methanol. This procedure (Sephadex column chromatography) was repeated three times. Fractions containing **2** were evaporated, dried *in vacuo* and recrystallized from methanol to give **2** (140 mg).

The physico-chemical properties of **2** were as follows: MP 187~188°C; $[\alpha]_D^{24}$ −45.5° (*c* 1.02, H_2O). *Anal* Calcd for $C_6H_{13}NO_4$: C 44.17, H 8.08, N 8.63. Found: C 44.16, H 8.03, N 8.58. The mass spectrum showed peaks at *m/z* 163



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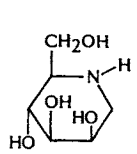

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(M^+) and 164 ($M^+ +1$). The ^1H and ^{13}C NMR spectra were recorded using a Varian XL-200 spectrometer at 200 and 50 MHz respectively. ^1H NMR (in D_2O, 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS) as internal standard) δ 2.45 (1H, m), 2.73 (1H, dd, J=1.5 and 14.0 Hz), 2.98 (1H, dd, J=2.9 and 14.0 Hz), 3.50~3.64 (2H, m), 3.75 (2H, d, J=3.8 Hz), 3.97 (1H, m); ^{13}C NMR (in D_2O, CH_3OH as internal standard) δ 49.1 (CH_2), 61.3 (CH), 61.6 (CH_2), 69.2 (CH), 70.1 (CH), 75.4 (CH). ^1H NMR (in D_2O with DCl, DSS as internal standard) δ 2.87 (1H, m), 3.04 (1H, dd, J=1.5 and 14.0 Hz), 3.25 (1H, dd, J=2.9 and 14.0 Hz), 3.60~3.95 (4H, m), 4.14 (1H, m); ^{13}C NMR (in D_2O with DCl, CH_3OH as internal standard) δ 48.4 (CH_2), 58.9 (CH), 61.2 (CH_2), 66.5 (CH). 66.7 (CH), 73.2 (CH).

These data were in good agreement with those of the literature[18]. Furthermore, the structure of the title compound isolated from the culture broth of *S. lavendulae* GC-148 was confirmed by comparing the physico-chemical data with **2** which was synthesized by the method of BÖSHAGEN *et al.*[19].

Acknowledgment

We thank Nippon Shinyaku Co., Ltd. and Takara Shuzo Co., Ltd. for granting permission for this publication.

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