Note

Isolation of 2-Amino-2-deoxy-D-mannitol from Culture Broth of *Streptomyces* species

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Numerous papers have been published on bacterial polysaccharides containing amino sugars or aminouronic acids, and on oligosaccharides containing amino sugars in glycoproteins, glycosaminoglycans and glycolipids. In one of these papers,¹⁾ the title compound (2-amino-2-deoxy-D-mannitol, **3**) was reported as its acetate, but only for the purpose of analyzing the 2-aminoaldohexoses of bacterial heteroglycans.

However, no work has been reported on 2-amino-2-deoxy-D-mannitol of microbial origin.

Since we reported the production of moranoline (**1**) by *Streptomyces* sp.,²⁾ we have been studying a more advanced fermentation to improve the productivity of **1** for industrial purposes. A few by-products, which were water soluble, basic and of low molecular weight, were observed

in the culture broth. These by-products interfered with the purification of **1**, and we have been attempting to elucidate them. One of these by-products was revealed to be 1,5-dideoxy-1,5-imino-D-mannitol (1-deoxymannojirimycin, **2**).³⁾

In the present paper, we describe the isolation of another by-product (**3**) from the culture broth of *Streptomyces lavendulae* GC-148, a producing strain of **1** and **2**.

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 8% soluble starch, 1% soybean meal, 1% yeast extract, 0.5% NaCl, 0.2% NaNO₃, 0.05% KCl and 0.05% MgSO₄·7H₂O (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 l of the same medium as that just described. The fermentation was carried out at 27°C at an agitation speed of 300 rpm and an aeration rate of 20 l/min for 11 days. The broth filtrate (12.9 l) was applied to a 1000-ml column of Dowex 50W×2 (H⁺). After washing sufficiently with distilled water, 2-amino-2-deoxy-D-mannitol was desorbed with 1 N aqueous ammonia. The fractions containing 2-amino-2-deoxy-D-mannitol were analyzed by a 655 high-performance liquid chromatograph (Hitachi Ltd.) using a solvent system of acetonitrile–water (70:30, a column of Nucleosil 5NH₂ (5 μm, 4mm i.d.×25 cm) and an ERC-7510 RI detector (Erma Optical Works Ltd.). After the eluate had been concentrated under reduced pressure, the solution was applied to a 500-ml column of Diaion SA-11A (OH⁻), and the compound was developed with distilled water. The eluate was concentrated under reduced pressure and kept at 5°C to give crystals. The crystals were collected and recrystallized from the solvent (water–ethanol=20:80) to give 1 g of the compound.

The physico-chemical properties of the compound were as follows: mp 161~163°C, $[\alpha]_D^{25} + 4.0^\circ$ (c 1.0, H₂O). Anal. Calcd. for C₆H₁₅NO₅: C, 39.77; H, 8.34; N, 7.73. Found: C, 39.62; H, 8.17; N, 7.81. Mass spectra were obtained by a Hitachi RMU-6MG mass spectrometer operating at 20 eV. The mass spectrum showed peaks at *m/z* 182 (*M*⁺+1, 4.1%) and 183 (*M*⁺+2, 0.5%). The ¹H NMR and ¹³C NMR spectra were recorded on a Varian XL-200 spectrometer at 200 and 50 MHz, respectively. ¹H NMR (in D₂O, DSS as internal standard) δ in ppm: 2.98~3.12 (1H, m), 3.56~3.90 (7H, m). ¹³C NMR (in D₂O, methanol as internal standard) δ in ppm: 53.84 (CH), 64.05 (CH₂), 64.31 (CH₂), 70.73 (CH), 71.29 (CH), 71.85 (CH).

These data suggested that the isolated compound was an aminohexitol. Therefore, 2-amino-2-deoxy-D-glucitol, 2-amino-2-deoxy-D-galactitol and 2-amino-2-deoxy-D-mannitol were prepared by sodium borohydride reduction and consecutive *N*-deacetylation of *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetyl-D-mannosamine according to the method of Griminn.⁴⁾ Among these aminohexitols, the physico-chemical data of 2-amino-2-deoxy-D-mannitol were identical with those of

Fig. 1. Structure of Moranoline (**1**), 1,5-Dideoxy-1,5-imino-D-mannitol (**2**) and 2-Amino-2-deoxy-D-mannitol (**3**).

the natural compound. The structure of the compound was thus established as 2-amino-2-deoxy-D-mannitol (**3**).

S. lavendulae NL-476, a natural isolate of strain GC-148, was cultivated on a rotary shaker for 7 days at 27°C in 125-ml Erlenmeyer flasks containing 40 ml of a basal medium possessing same composition as the seed medium already described, and the same basal medium supplemented with **3** at a level of 0.5% or 1%, the amount of **1** being determined by HPLC as described before.²⁾ The yields of **1** in the basal medium, and in the basal medium supplemented with **3** at a level of 0.5% and 1% were 3601, 4969 and 6181 µg/ml, respectively. This result suggests that **3** is closely involved in the biosynthesis of **1**.

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