MICROBIAL EPIMERIZATION OF 1-DEOXYNOJIRIMYCIN TO 1-DEOXYMANNOJIRIMYCIN

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As part of a program concerned with the utilization of unusual carbohydrates by soil bacteria to produce useful bioactive compounds, we reported that the isolation of bacteria able to utilize "pseudo-D-glucose" in which the ring-oxygen of D-glucose is replaced by a methylene group.[1] A strain No. 19-1 of *Agrobacterium* sp. oxidizes pseudo-β-D-glucose at 2-OH group. A β-elimination and reduction occurs to form a 4-deoxy derivative.

Interestingly, with 1-deoxyglucose as a substrate, the epimerization of the 2-position occurred without the β-elimination. It is thought that this reaction is thought to occure by oxidation to a 2-keto compound followed by reduction to give an epimeric derivative.

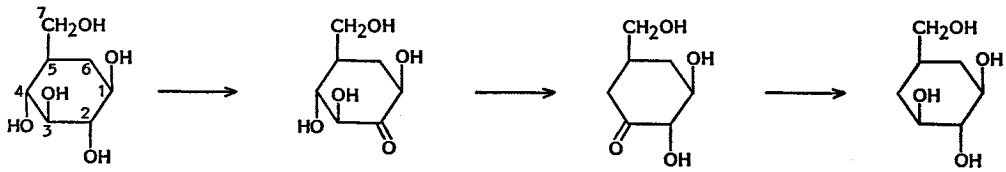
Based on these results we attempted to epimerize 1-deoxynojirimycin to produce 1-deoxymannojirimycin, a potent mannosidase inhibitor which blocks conversion of high-mannose to complex saccharide on *N*-asparagine-linked glucan biosynthesis.[2~4] The data reported describes the microbial conversion of 1-deoxynojirimycin to the manno-form and its glycosidase inhibitory activity.

The strain was cultivated on a rotary shaker for 24 hours at 27°C in 500-ml Erlenmeyer flasks containing the nutrient broth (peptone 1.5%, meat extract 0.5%, NaCl 0.5%, K_2HPO_4 0.5%, pH 7.0). The cells were collected by centrifugation, washed with 50 mM phosphate buffer (pH 6.5) and suspended in the same buffer. 1-Deoxynojirimycin (190 mg) was added to the cell suspension (10 g/100 ml) and incubated at 27°C for 18 hours with shaking. The reaction process was monitored by the gas-liquid chromatography (TMS derivatives, 5% Silicone SE-30 on Chromosorb, 180°C) and a typical chromatogram is shown in Fig. 2. The formation of conversion product in the reaction mixture is shown in Fig. 3.

The reaction mixture was filtered and passed through a column of activated carbon (50 ml), which was eluted with 50% EtOH (150 ml). After evaporation, the residue was chromatographed on a column of Dowex 1-X2 (OH^- form, 1.8×40 cm, 50 ml) using water as a solvent. The differential refractometer was used for the elution and each fraction was checked by gas-liquid chromatography. Repeated separation and recrystallization from methanol yielded pure conversion product, 1-deoxymannojirimycin (140 mg).

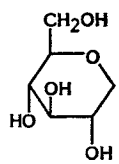
The physico-chemical properties of the product were as follows: $[\alpha]_D^{21}$ −38.6° (*c* 1.0, H_2O),

Scheme 1.

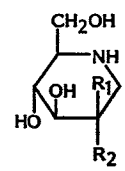


Pseudo-β-D-glucose

Fig. 1. Structures of 1-deoxy-D-glucose, 1-deoxynojirimycin and 1-deoxymannojirimycin.



1-Deoxy-D-glucose



1-Deoxynojirimycin R_1=H R_2=OH
1-Deoxymannojirimycin R_1=OH R_2=H

Fig. 2. Gas-liquid chromatography of the conversion product and 1-deoxynojirimycin (TMS-derivatives).

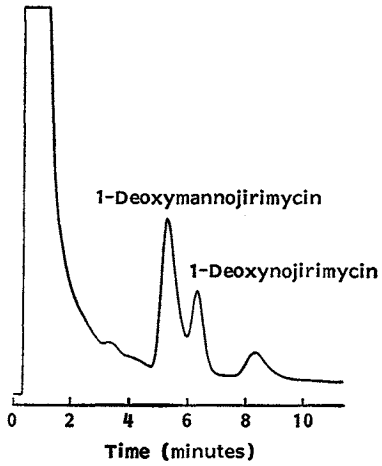
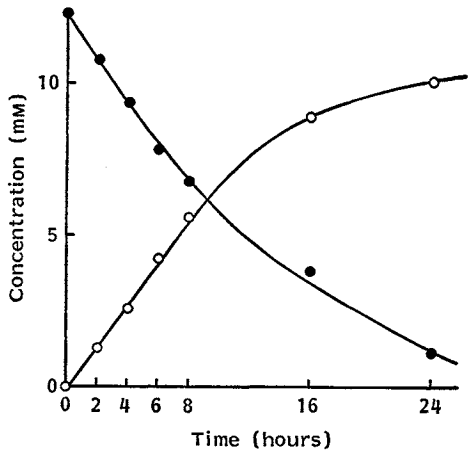


Fig. 3. Microbial conversion of 1-deoxynojirimycin (●) to 1-deoxymannojirimycin (○) by *Agrobacterium* sp.



$-10.1°$ (*c* 1.0, pH 5.0, HCl); *Anal* Calcd for $C_6H_{13}NO_4$: C 44.08, H 8.12, N 8.38. Found: C 44.16, H 8.03, N 8.58. The MS (70 eV) showed peaks at *m/z* 163 (M^+) and 164 ($M^+ +1$). The ^1H and ^{13}C NMR spectra were recorded using a Jeol FX-100 spectrometer at 100 and 25 MHz, respectively. ^1H NMR (in D_2O with DCl, 3-(trimethylsilyl)-1-propionic acid sodium salt (TSP) as internal standard) δ 3.17 (m, J= 9.8, 6.2 and 3.7 Hz), 3.20 (dd, J=13.7 and 1.7 Hz), 3.48 (dd, J=13.7 and 2.9 Hz), 3.72 (dd, J=9.4 and 2.9 Hz), 3.87 (dd, J=12.6 and 6.2 Hz), 3.90 (t, J=9.6 Hz), 4.05 (dd, J=12.6 and 3.7 Hz), 4.27 (dt, J=2.9, 2.9 and 1.7 Hz); ^{13}C NMR (in D_2O with DCl, TSP as internal standard) δ 50.1 (CH_2N), 60.6 (CHN), 62.9 (CH_2O), 68.3 (CH), 68.4 (CH), 74.9 (CH).

The 1-deoxymannojirimycin produced was tested for α-glycosidase inhibitory activity against commercially available jack bean α-mannosidase (Sigma M 7257) and green bean α-galactosidase (Sigma G 8507) with 5 mM *p*-nitrophenyl-D-α-glycosides as substrate. The molar concentration required to give 50% inhibition (IC_{50} (M)) against α-mannosidase and α-galactosidase was 1.9×10^{-4} and 3.1×10^{-4}, respectively. These values are very low compared with 1-deoxynojirimycin, which shows no inhibitory activity at 1 mM. These results were good agreement with those of 1-deoxymannojirimycin reported in the literatures.[3,5]

This study gives a new convenient method to produce 1-deoxymannojirimycin by the microbial conversion.

References

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