## NOTES

# MICROBIAL PRODUCTS. III[1] EPI-DEOXYNEGAMYCIN FROM A *MICROMONOSPORA*

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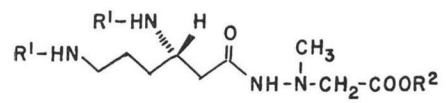
Negamycin is a hydrazide antibiotic previously described as a metabolite of a streptomycete[2] and similarly, epi-deoxynegamycin (**1**) was found as a product of *Streptomyces goshikiensis*.[3] Accordant with the apparent parsimony of *Micromonospora* in introducing hydroxyl groups into their metabolites, epi-deoxynegamycin has recently been discovered by us as a metabolite of *Micromonospora* strain X-14807.

Young colonies of strain X-14807 were flat and orange; later they became raised and convoluted developing an area of sporulation which varied from medium to dark brown. Formation of a white aerial mycelium was observed on starch-casein agar composed of (g/liter) soluble starch (10), casein (1), dipotassium hydrogen phosphate (0.5), magnesium sulfate (0.5), agar (15), and distilled water, with pH adjustment to 7.4 prior to sterilization.

Single spores along the sporulated hyphae were seen under the microscope. In whole-cell hydrolysates the presence of *meso*-diaminopimelic acid was demonstrated by standard chromatographic procedures. The above characteristics place the organism in the genus *Micromonospora*.

The strain grew well in most current actinomycete media, with optimum pH-range between 6 and 7, and no growth at pH 5 or below. The culture grew best between 30 and 36°C, and had a marginal tolerance to 3% sodium chloride. Carbohydrate-utilization tests indicated excellent growth on D-glucose and on L-arabinose. Growth on lactose, D-mannose, D-fructose and cellulose was better than on L-sorbose, glycerol and *meso*-inositol.

Strain X-14807 was cultivated in shaken flasks for 72 hours at 36°C in a medium containing (g/liter) tomato pomace dried solids (5.0, Seabord Supply), distillers dried solubles (5.0, Brown and Forman), O.M. peptone (5.0, Oscar Meyer), corn starch (20.0), calcium carbonate (1.0), and dipotassium hydrogen phosphate (1.0), with pH adjustment to 7.0 (NaOH) prior to sterilization. Fermentations were conducted in stirred (280 rpm) and aerated (0.57 $m^3$/min) tanks of 14 hl capacity at 36°C for 5 days using the same medium and 3% (v/v) inoculum.




**1**: $R^1 = R^2 = H$ (epi-deoxynegamycin)

**2**: $R^1 = COCH_3$, $R^2 = H$

**3**: $R^1 = COCH_3$, $R^2 = CH_3$

To isolate the antibiotic, the broth filtrate (1,300 liters) was adjusted to pH 2.0 (sulfuric acid), filtered, readjusted to pH 7.0 (ammonium hydroxide) and passed through a column of Amberlite IRC-50 ($NH_4^+$, 30 cm × 90 cm) at a rate of 3 liters/min. The column was washed with water and eluted with 1.0 M ammonium hydroxide. The biologically active, ammoniacal fraction, after concentration under reduced pressure to remove ammonia, was readjusted to pH 7.5, and chromatography on Amberlite IRC-50 ($NH_4^+$, 9 cm × 100 cm) was repeated. Concentration and freeze-drying of the active fraction gave crude **1** (14 g) as light-tan, amorphous powder of approximately 50% purity.

A solution of crude **1** (13 g) in water was adjusted to pH 3.5 (hydrochloric acid) and charged onto a column of Dowex 50W-X4 ($Na^+$, 200~400 mesh, 2.5 cm × 102 cm) which was equilibrated and developed with 0.13 M trisodium citrate solution with the pH adjusted to 4.50 (conc. hydrochloric acid). The active band was contained in the effluent volume 5.00~9.25 liters and was desalted by adsorption onto a column of Dowex 50W-X4 ($H^+$, 100~200 mesh,

2 liters), followed by aqueous washes and elution with 1 M ammonium hydroxide. Concentration and freeze-drying gave **1** (7 g) as a white, amorphous solid of approximately 95% purity, Rf 0.37 (tlc, silica gel; chloroform - methanol - conc. ammonium hydroxide - water, 1: 4: 2: 1, v/v), $[\alpha]_D^{25}$ +2° (*c* 1.0, water), $\delta_{D_2O}^{TMS}$ 1.9~2.4 (m, $CH_2CH_2$), 2.86 (m, C-$CH_2$-CO), 3.14 and 3.15 ($NCH_3$), 3.56 (t, N-$CH_2$, $J_{5,6}$=7 Hz), 3.85 (m, N-CH), and 3.92 (s, N-$CH_2$-CO). The observed minimum inhibitory concentration against *E. coli* ATCC 27856 of 0.125 mg/ml was comparable to the reported value for **1** and lays far below that of negamycin.[3]

The diacetyl derivative (**2**) was prepared by dropwise addition of acetic anhydride (0.5 ml) to a vigorously stirred solution of **1** (50 mg) and potassium hydrogen carbonate (375 mg) in water (1.5 ml) at 0°C. The mixture was stirred for 2 hours at room temperature and then desalted with Dowex 50W-X4 ($H^+$). The aqueous solution of **2** was evaporated and taken up in methanol. Addition of ether precipitated white, amorphous **2** (34 mg), $\delta_{D_2O}^{TMS}$ 2.05 (m, $CH_2CH_2$), 2.49 (s, 2 $CH_3CO$), 2.84 (m, C-$CH_2$-CO), 3.13 (s, N-$CH_3$), 3.67 (m, $CH_2N$), 3.93 (s, N-$CH_2$-CO), 4.64 (m, N-CH).

A solution of **2** (17 mg) in methanol (5 ml) was treated with ethereal diazomethane for 20 hours at 5°C. The solution was evaporated to dryness and the residue taken up in aqueous methanol. Unreacted **2** was removed through a column of Dowex 1-X2 ($HCO_3^-$, 2 ml). The evaporated column effluent gave a mass spectrum (FD) with the expected *m/e* 331 (M+1) and 353 (M+Na) for 3,6-bis-(acetylamino)hexanoic acid 2-(methoxycarbonylmethyl)-2-methyl-hydrazide(**3**).

In view of the low rotation of epi-deoxynegamycin[3] the assignment of chirality needed additional verification. Thus, **1** was hydrolyzed in 6 N hydrochloric acid at 100°C for 18 hours. The hydrolyzate was evaporated to dryness, redissolved in water and charged to a column of Dowex 50W-X4 ($H^+$, 100~200 mesh). The column was washed successively with water and 10% aqueous pyridine and finally eluted with 1 M ammonium hydroxide yielding amorphous $\beta$-lysine after concentration and freeze-drying of the ammoniacal fraction. The pH of the aqueous solution of $\beta$-lysine was adjusted with 4-[(4-hydroxyphenyl)azo]benzenesulfonic acid to 5.8. The solution was concentrated and allowed to crystallize first at room temperature and then at 5°C. Recrystallization from hot water gave needles of L-$\beta$-lysine 4-[(4-hydroxyphenyl) azo] benzenesulfonic acid salt, m.p. 220~230°C (dec).

*Anal.* Calcd for $C_{10}H_{14}N_2O_2 \cdot C_8H_{10}N_2O_4S$ (424.41): C 50.93, H 5.70, N 13.20.
Found: C 50.72, H 5.86, N 13.16

$[\alpha]_D$ +11.7° (*c* 0.5, $H_2O$); +12.7° (*c* 0.16, ethanol - water, 4: 1, v/v).

The $[\alpha]_D$ value of L-$\beta$-lysine, isolated from a hydrolyzate of geomycin[4] and streptolin[5] and obtained as the double salt with 4-[(4-hydroxyphenyl)azo]benzenesulfonic acid, was reported to be +5.5° (95% EtOH) and +6.5° (EtOH), respectively, so that the (S)-configuration of **1** was established.

## References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