# MICROBIAL SEMI-SYNTHESIS OF AMINOGLYCOSIDIC ANTIBIOTICS BY MUTANTS OF *S. RIBOSIDIFICUS* AND *S. KANAMYCETICUS*

Sir:

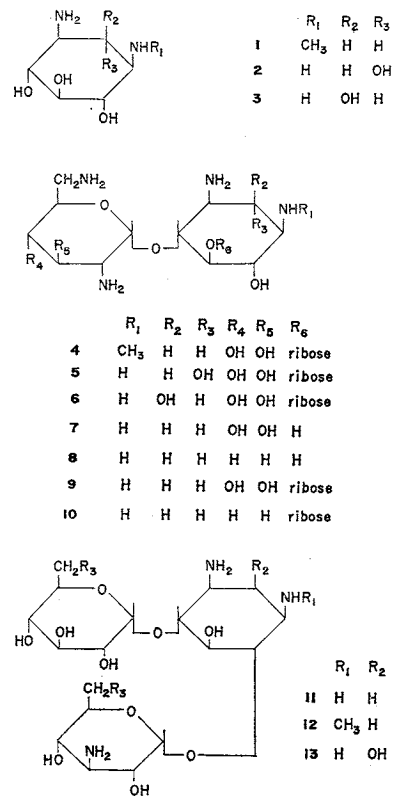
RINEHART Jr. *et al.*[1] reported biosynthesis of aminoglycosidic antibiotics by a mutant of *Streptomyces fradiae*. This mutant produced four new neomycin analogs, hybrimycin $A_1$, $A_2$, $B_1$ and $B_2$ in media containing streptamine and epi-streptamine.

During studies on the biosynthesis of aminoglycosidic antibiotics, we have also isolated deoxystreptamine-negative mutants of *Streptomyces ribosidificus*[2] and *Streptomyces kanamyceticus*[3].

The deoxystreptamine-negative strain of *S. ribosidificus* (named AF-1 strain) produced new ribostamycin[4] (SF-733) analogs by the addition of deoxystreptamine analogs or a neamine analog to the culture medium. Deoxystreptamine analogs examined were 1-N-methyl deoxystreptamine* (**1**), streptamine (**2**), *myo*-inosadiamine-1, 3** (2-epi-streptamine)[5] (**3**), streptidine, N-monoacetyl deoxystreptamine[6], N,N′-diacetyl deoxystreptamine and N, N′-dimethyl deoxystreptamine*. Among them, **1**, **2** and **3** were utilized by AF-1 strain to produce new bioactive ribostamycins, **4**, **5** and **6**, but no evidence was obtained on the incorporation of the other aminocyclitols to ribostamycins. Addition of neamine (7) and 3′, 4′-dideoxyneamine[7] (**8**) in the cultured broth resulted in the biosynthesis of ribostamycin (**9**) and 3′, 4′-dideoxy ribostamycin[8] (**10**), respectively. These ribostamycin analogs were isolated from each culture broth by column chromatography of Amberlite IRC-50 ($Na^+$ type) and Amberlite CG-50($NH_4^+$ type) resins developed with dilute ammonia.

1-N-Methyl ribostamycin (**4**) showed m. p. 239°C (dec.), $[\alpha]_D^{23}$+34.6 (*c* 1.0, $H_2O$), $R_{Rm}$=1.1 (relative Rf value against ribostamycin) on silica-gel TLC developed with chloroform-butanol-ethanol-17 % ammonia (2 : 4 : 5 : 5). It had one-fourth the bioactivity (against *Bacillus subtilis*) of ribostamycin. The NMR spectrum of **4** in $D_2O$ exhibited N-methyl signal at $\delta$ 2.6. The mass spectrum of N-acetyl-O-trimethysilyl-4 showed $M^+$ at *m/e* 1,063, and the peaks indicating aminocyclotol moiety were shifted 14 mass units higher than those of ribostamycin.

4-O-(2,6-Diamino-2,6-dideoxy-$\alpha$-D-glucopyranosyl) 5-O-($\beta$-D-ribofuranosyl) streptamine (2-hydroxy ribostamycin) (**5**) showed m. p. 244°C (dec.), $[\alpha]_D^{27}$+36.1 (*c* 0.72, $H_2O$), $R_{Rm}$=0.92 (TLC), and had one tenth the bioactivity of ribostamycin. The mass spectrum of N-acetyl-O-trimethylsilyl-**5** showed $M^+$ at *m/e* 1,142 and




* These compounds were kindly supplied by Dr. S. KONDO, Institute of Microbial Chemistry.

** *myo*-Inosadiamine-1, 3 was kindly supplied by Prof. T. SUAMI, Keio University.

the peaks indicating aminocyclitol moiety were shifted 88 mass units higher than those of ribostamycin. This increment corresponded to an extra O-trimethylsilyl group in **5**.

4-O-(2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl) 5-O-(β-D-ribofuranosyl) epi-streptamine(2-epi-hydroxy ribostamycin) (**6**) showed m.p. 235°C (dec.), $[\alpha]_D^{27}$+37 (*c* 1.0, $H_2O$), $R_{Rm}$=0.88 (TLC). It had less than one tenth the bioactivity compared with ribostamycin. The mass spectrum of N-acetyl-O-trimethylsilyl-**6** was the same as that of **5**.

3′, 4′-Dideoxy ribostamycin (**10**) showed m.p. 234~236°C (dec.), $[\alpha]_D^{24}$+53 (*c* 1.0, $H_2O$). Compound **10** showed stronger activity than ribostamycin against *Pseudomonas aeruginosa* and kanamycin-ribostamycin resistant *Escherichia coli*, in accord with the results already reported[8]. The mass spectrum of N-acetyl-O-trimethylsilyl-**10** showed $M^+$ at *m/e* 878, and a strong peak at *m/e* 213 due to N-acetyl-2′, 3′, 4′, 6′-tetradeoxy-2′, 6′-diaminoglucose moiety.

In case of the deoxystreptamine-negative mutant of *S. kanamyceticus* which produced kanamycin (**11**), two new kanamycin analogs were obtained by the addition of two deoxystreptamine analogs, 1-N-methyl deoxystreptamine (**1**) and *myo*-inosadiamine-1, 3 (**3**). Isolation and purification procedure of these new kanamycin analogs were the same as described above for ribostamycin. However, when the structure of these antibiotics were examined by NMR and acid hydrolysis, it was found that these compounds were not the expected products, *i.e.* 1-N-methyl kanamycin and 2-epi-hydroxy kanamycin, but 4-O-(α-D-glucopyranosyl) 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl) 1-N-methyl-2-deoxystreptamine (6′-deamino-6′-hydroxy-1-N-methyl kanamycin) (**12**) and 4-O-(α-D-glucopyranosyl) 6-O-(3-amino-3-deoxy-α-D-glucopyranosyl) 2-epi-streptamine (**13**). Addition of the other deoxystreptamine analogs such as streptamine, N, N′-diacetyl deoxystreptamine and N, N′-dimethyl deoxystreptamine gave no bioactive substance.

Compound **12** showed m.p. 250°C (dec.) $[\alpha]_D^{27}$ +81.5 (*c* 1.0, $H_2O$). Acid hydrolysate (6N HCl, 100°C 45 min.) of **12** gave three spots on PPC corresponding to 3-aminoglucose, N-methyl deoxystreptamine and glucose, developed with butanol-pyridine-acetic acid-water (6 : 4 : 1 : 3). The NMR spectrum of **12** in $D_2O$ exhibited N-methyl signal at $\delta$ 2.6.

Compound **13** showed m.p. 248°C (dec.), $[\alpha]_D^{27}$ +113 (*c* 1.0, $H_2O$) and acid hydrolysate of **13** gave 3-aminoglucose, inosadiamine and glucose on PPC. Compounds **12** and **13** exhibited weak bioactivity.

Detailed procedure on fermentation, isolation, physico-chemical properties and structure determination will be published in another paper.

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