NEOTHRAMYCINS A AND B, NEW ANTITUMOR ANTIBIOTICS

Sir:

Two new antibiotics, neothramycins A and B have been isolated from culture broth of *Streptomyces* No. MC916-C4, a strain belonging to the group C strains[1] of cycloheximide-producing *Streptomyces*.

Neothramycins A and B were produced when grown in aerated culture at 28°C in a medium containing 2.0% glucose, 2.0% glycerol, 1.2% soybean meal, 1.0% cotton seed meal, 0.32% $CaCO_3$, 0.5% NaCl and 0.0005% $MnCl_2 \cdot 4H_2O$ (adjusted to pH 6.8 with 5 N NaOH). The fermentation was stopped after 4 days and the fermented broth (pH 6.5, 80~800 mcg/ml of neothramycins) was filtered. Concentrations of neothramycins were determined by the usual paper disk-plate method against *Staphylococcus aureus* SMITH using pure neothramycin A as an assay standard.

These antibiotics in the filtrate were adsorbed on activated carbon and eluted with 50% aqueous acetone at pH 8.0 (adjusted with aqueous ammonia) and also by extraction from the filtrate with an equal volume of *n*-butanol. The eluate or the butanol extract is concentrated to dryness yielding a brownish crude powder. The crude powder was subjectd to column chromatography on Sephadex LH-20 using methanol as developing agent. The eluate containing neothramycins is concentrated to dryness and the residue is chromatographed on a column of silica gel (Mallinkrodt, CC-7) with a mixture of chloroform and ethanol (30:1, v/v) as eluent. In this chromatography, neothramycin A (3.8% yield from the broth filtrate) is eluted first and thereafter neothramycin B (3.4% yield) appears. The low yields are due to their lability properties in solution, especially in alcohols, chloroform, *etc.* For further purification, this chromatographic technique is repeated. These purification steps should be operated in a cold room (5°C).

Neothramycin A is obtained as a colorless amorphous powder melting over the wide range of 132~147°C with decomposition. $[\alpha]_D^{26}$ +272° (*c* 0.52, dioxane). Anal. calcd. for $C_{13}H_{14}N_2O_4 \cdot 1/2H_2O$: C 57.56, H 5.57, N 10.33, O 26.54, mol. wt. 271.27. Found: C 57.46, H 5.76, N 9.84, O 26.94, mol. wt. 250~300 (BARGER-AKIYA method in methanol).

The molecular formula can be shown by the high-resolution MS spectrum (calcd. mol. wt. for $C_{13}H_{14}N_2O_4$, 262.0952; found *m/e* 262.0934). It shows the following maxima in UV spectra: at 223 (ε 22,400), 240 (sh), 265 (7,600) and 318 nm (4,100) in 90% aqueous methanol, at 223 (ε 23,200), 240 (sh.), 265 (7,600) and 320 nm (3,640) in 0.1 N HCl in 90% aqueous methanol, and at 228 (ε 16,700), 254 (14,800), 291 (11,100) and 324 nm (10,800) in 0.1 N NaOH in 90% aqueous methanol. The IR spectrum is represented in Fig. 1. The PMR chemical shifts are shown in Table 1.

The properties of neothramycin B is very similar to those of neothramycin A. Neothramycin B is a colorless amorphous powder,

Table 1. PMR chemical shifts of neothramycins and their methyl derivatives

Proton	Neothramycin A	Neothramycin B	Methylneothramycin A	Methylneothramycin B
CH_2x2	1.7~2.5	1.7~2.5	1.8~2.6	1.8~2.3
OCH_3			3.28 s	3.44 s
CH	3.80 m	3.78 m	3.72 m	3.80 dd
arom. OCH_3	3.90 s	3.88 s	3.90 s	3.88 s
OH	5.00 d	5.10 d		
CH	5.69 dd	5.78 m	5.56 d	5.35 dd
arom. H	6.70 s	6.69 s	6.75 s	6.64 s
arom. H	7.43 s	7.40 s	7.48 s	7.36 s
CH	7.62 d	7.70 d	7.73 d	7.54 d
phenol OH	8.00 s	7.98 s	8.04 s	7.94 s

Chemical shifts, δ(ppm) were measured in deuterodioxane using TMS as the internal reference.

Fig. 1. The IR spectrum of neothramycin A in KBr.

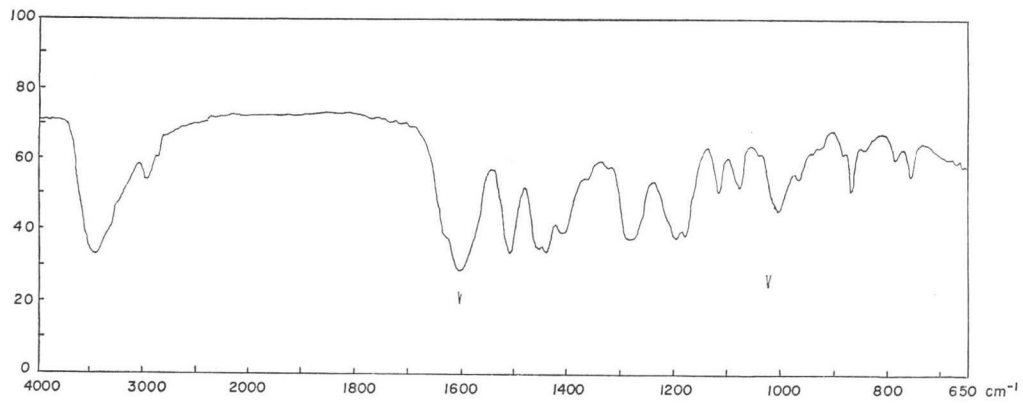
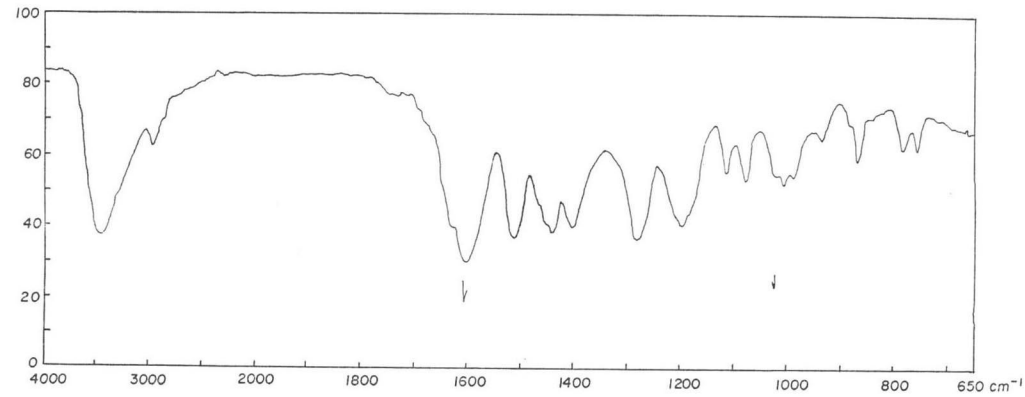


Fig. 2. The IR spectrum of neothramycin B in KBr.



mp. 144~151°C (dec.); $[\alpha]_D^{25}$ +314° (*c* 0.48, dioxane). Anal. calcd. for $C_{13}H_{14}N_2O_4 \cdot 1/2H_2O$: C 57.56, H 5.57, N 10.33, O 26.54. Found: C 57.00, H 5.58, N 9.75, O 27.67. MS, *m/e* 262.0939. It shows UV maxima at 224 (ε 24,200), 240 (sh.), 265 (sh.) and 318 nm (4,380) in 90% aqueous methanol, at 224 (ε 26,200), 240 (sh.), 265 (sh.) and 320 nm (4,080) in 0.1 N HCl in 90% aqueous methanol, and at 228 (ε 20,900), 254 (19,000), 291 (11,900) and 324 nm (12,200) in 0.1 N NaOH in 90% aqueous methanol. The IR spectrum and PMR data are shown in Fig. 2 and Table 1, respectively.

Both neothramycins A and B give positive RYDON-SMITH, red tetrazolium, fast blue B, BRADY and ninhydrin (weak brownish yellow) reactions, and negative SAKAGUCHI, pentacyanoaquoferriate and EHRLICH reactions. They are soluble in methanol, butanol, ethyl acetate, acetone, dioxane, chloroform, dimethylformamide and dimethylsulfoxide, and almost insoluble or insoluble in benzene, *n*-hexane, ethyl ether and water. Neothramycins A and B can be separated by thin-layer chromatography using Silica gel G (Merck, Art. 5715) with chloroform-methanol (10:1, v/v) as developing solvent. Neothramycin A has Rf 0.57 and B Rf 0.50.

Neothramycins A and B are unstable in 50% aqueous ethanol at pH 2.5 and their activities are reduced to 25% and 22%, respectively, at room temperature for 16 hours. In 50% aqueous ethanol at pH 6.5 or pH 8.0 at room temperature for 16 hours, 80~90% activity of neothramycin A and 70~80% activity of neothramycin B remained. However, an equilibrium conversion of neothramycin A to B or B to A is shown by thin-layer chromatographic analysis. Neothramycin A or B is easily converted to a mixture of methylneothramycins A (Rf 0.71 on silica gel

thin-layer chromatogram with chloroform-methanol, 10:1, v/v) and B (Rf 0.61) in anhydrous methanol at room temperature for 16 hours. Methylneothramycin A is crystallized from a mixture of acetone and benzene, colorless microcrystals, mp 137~140°C (dec.); $[\alpha]_D^{26} + 640°$ (*c* 0.24, dioxane), MS, *m/e* 276.1089 (calcd. mol. wt. for $C_{14}H_{16}N_2O_4$, 276.1108). Methylneothramycin B is obtained as a colorless powder, mp 61~69°C (dec.); $[\alpha]_D^{26} + 778°$ (*c* 0.22, dioxane), MS, *m/e* 276.1071. UV spectra of methylneothramycins are similar to those of neothramycins and the PMR chemical shifts are shown in Table 1. Mild hydrolysis of methylneothramycin A or B in 0.01 N HCl-dioxane (1:1, v/v) at room temperature for 1 hour followed by column chromatography on silica gel gives neothramycins A and B in a good yield.

We conclude from these data that neothramycins A and B are interconvertible isomers and that they belong to the anthramycin group antibiotics possessing a benzodiazepine structure. They may be distinguished from anthramycin,[2] dextrochrysin[3] and sibiromycin[4] by their UV spectra. UV spectra of tomaymycin[5] and neothramycins are very similar, but they are different in their molecular formulae and other spectra. As shown in Table 1, the PMR spectra of neothramycins A and B are almost similar, but the signal of a methine proton at δ 5.69 in neothramycin A is different from that at δ 5.78 in neothramycin B. It is suggested that the structures of neothramycins A and B are different in configuration of this methine carbon. The structural studies on neothramycins will be presented elsewhere.

Neothramycins A and B have weak activities against some bacteria and fungi as shown in Table 2. A marked prolongation in the survival period of mice implanted with the mouse leukemia L-1210 cells has been observed after treatment with neothramycin A or B intraperitoneally, as shown in Table 3. In the treatment with daily intraperitoneal doses of 25~100 mcg of neothramycin A or B per mouse for 10 days, more than 200% of prolongation in the survival period of mice inoculated with EHRLICH ascites carcinoma cells were observed. Neothramycins A and B also inhibited multiplications of YOSHIDA

Table 2. The antimicrobial spectra of neothramycins

Test organisms	Minimum inhibitory concentrations (mcg/ml)	
	Neothramycin A	Neothramycin B
Staphylococcus aureus SMITH	50	100
Staphylococcus aureus FDA 209P	>100	>100
Bacillus subtilis PCI 219	100	>100
Klebsiella pneumoniae PCI 602	50	100
Escherichia coli NIHJ	100	100
Escherichia coli K-12	100	100
Escherichia coli W677	50	100
Escherichia coli JR66/W677	100	>100
Pseudomonas aeruginosa No. 12	>100	>100
Aeromonas salmonecida ATCC14174	25	50
Vibrio anguillarum NCBM 6	50	100
Xanthomonas citri	>100	>100
Xanthomonas oryzae	50	100
Saccharomyces cerevisiae	50	>100
Candida albicans 3147	>100	>100
Aspergillus niger	100	>100
Piricularia oryzae	50	>100

Bacteria were incubated on nutrient agar plates at 37°C for 17 hours and fungi on nutrient agar plates containing 1% glucose at 27°C for 40 hours.

Table 3. Prolongation rates in the survival period of mice with L-1210 by treatments of neothramycins

Dosage (mcg/mouse/day for 10 days)	Prolongation rate (%)	
	Neothramycin A	Neothramycin B
300	death	death
150	200	192
75	167	154
37.5	154	128
18.7	122	103

rat sarcoma cells and C3H cells transformed by SV40 *in* tissue cultures. Acute LD_{50} of neothramycin A or B in mice was 20~30 mg/kg by the intravenous injection and 20~30 mg/kg by the intraperitoneal injection.

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