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 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% CaCO₃, 0.5% NaCl and 0.0005% MnCl₂·4H₂O (adjusted to pH 6.8 with 5 N NaOH). The fermentation was stopped after 4 days and the fermented broth (pH 6.5, 8000 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 subjected 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. [α]D +272° (c 0.52, dioxane). Anal. calcd. for C₁₃H₂₀N₂O₄·1/2H₂O: C 57.56, H 5.57, N 10.33, O 26.54, mol. wt. 262.54. 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 C₁₃H₂₁N₂O₄, 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

<table>
<thead>
<tr>
<th>Proton</th>
<th>Neothramycin A</th>
<th>Neothramycin B</th>
<th>Methylneothramycin A</th>
<th>Methylneothramycin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂X2</td>
<td>1.7~2.5</td>
<td>1.7~2.5</td>
<td>1.8~2.6</td>
<td>1.8~2.3</td>
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<tr>
<td>OCH₃</td>
<td></td>
<td></td>
<td>3.28 s</td>
<td>3.44 s</td>
</tr>
<tr>
<td>CH</td>
<td>3.80 m</td>
<td>3.78 m</td>
<td>3.72 m</td>
<td>3.80 dd</td>
</tr>
<tr>
<td>arom. OCH₃</td>
<td>3.90 s</td>
<td>3.88 s</td>
<td>3.90 s</td>
<td>3.88 s</td>
</tr>
<tr>
<td>OH</td>
<td>5.00 d</td>
<td>5.10 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>5.69 dd</td>
<td>5.78 m</td>
<td>5.56 d</td>
<td>5.35 dd</td>
</tr>
<tr>
<td>arom. H</td>
<td>6.70 s</td>
<td>6.69 s</td>
<td>6.75 s</td>
<td>6.64 s</td>
</tr>
<tr>
<td>arom. H</td>
<td>7.43 s</td>
<td>7.40 s</td>
<td>7.48 s</td>
<td>7.36 s</td>
</tr>
<tr>
<td>CH</td>
<td>7.62 d</td>
<td>7.70 d</td>
<td>7.73 d</td>
<td>7.54 d</td>
</tr>
<tr>
<td>phenol OH</td>
<td>8.00 s</td>
<td>7.98 s</td>
<td>8.04 s</td>
<td>7.94 s</td>
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</table>

Chemical shifts, δ(ppm) were measured in deuterodioxane using TMS as the internal reference.
mp. 144 ~ 151 °C (dec.); [α]D 314° (c 0.48, dioxane). Anal. calcd. for C13H11N3O4·1/2H2O:
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 318 nm
(4,380) 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, penta-
cyanoaquoferriate 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 crystal-
lized from a mixture of acetone and benzene,
colorless microcrystals, mp 137-140°C (dec.);
$[\alpha]_D^{26} + 640^\circ$ (c 0.24, dioxane), MS, $m/e$
276.1089 (calcd. mol. wt. for $C_{14}H_{20}N_2O_4$, 276.1108). Methylneothramycin B is obtained
as a colorless powder, mp 61-69°C (dec.);
$[\alpha]_D^{26} + 778^\circ$ (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 neothra-
mycins A and B in a good yield.

We conclude from these data that neothra-
mycins 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,$^3$ dextrochrysin,$^3$ and sibiroma-
ycin$^5$ by their UV spectra. UV spectra of
tomaymycin$^3$ and neothramycins are very
similar, but they are different in their mole-
cular 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 $\delta$ 5.69 in neothramycin
A is different from that at $\delta$ 5.78 in neothra-
mycin 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>
<thead>
<tr>
<th>Test organisms</th>
<th>Minimum inhibitory concentrations (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neothramycin A</td>
</tr>
<tr>
<td>Staphylococcus aureus SMITH</td>
<td>50</td>
</tr>
<tr>
<td>Staphylococcus aureus FDA 209P</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bacillus subtilis PCI 219</td>
<td>100</td>
</tr>
<tr>
<td>Klebsiella pneumoniae PCI 602</td>
<td>50</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia coli K-12</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia coli W677</td>
<td>50</td>
</tr>
<tr>
<td>Escherichia coli JR66/W677</td>
<td>100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa No. 12</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Aeromonas salmonicida ATCC14174</td>
<td>25</td>
</tr>
<tr>
<td>Vibrio anguillarum NCBM 6</td>
<td>50</td>
</tr>
<tr>
<td>Xanthomonas citri</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Xanthomonas oryzae</td>
<td>50</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>50</td>
</tr>
<tr>
<td>Candida albicans 3147</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>100</td>
</tr>
<tr>
<td>Pithicarlia oryzae</td>
<td>50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Dosage (mcg/mouse/day for 10 days)</th>
<th>Prolongation rate (%)</th>
<th>Neothramycin A</th>
<th>Neothramycin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>death</td>
<td>200</td>
<td>192</td>
</tr>
<tr>
<td>150</td>
<td>death</td>
<td>167</td>
<td>154</td>
</tr>
<tr>
<td>75</td>
<td>154</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>37.5</td>
<td>122</td>
<td>103</td>
<td></td>
</tr>
</tbody>
</table>

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TOMIO TAKEUCHI
MASASHI MIYAMOTO
MASAAKI ISHIZUKA
HIROSHI NAGANAWA

References