Communications to the editor

MINOSAMINOMYCIN, A NEW ANTIBIOTIC CONTAINING *MYO*-INOSAMINE

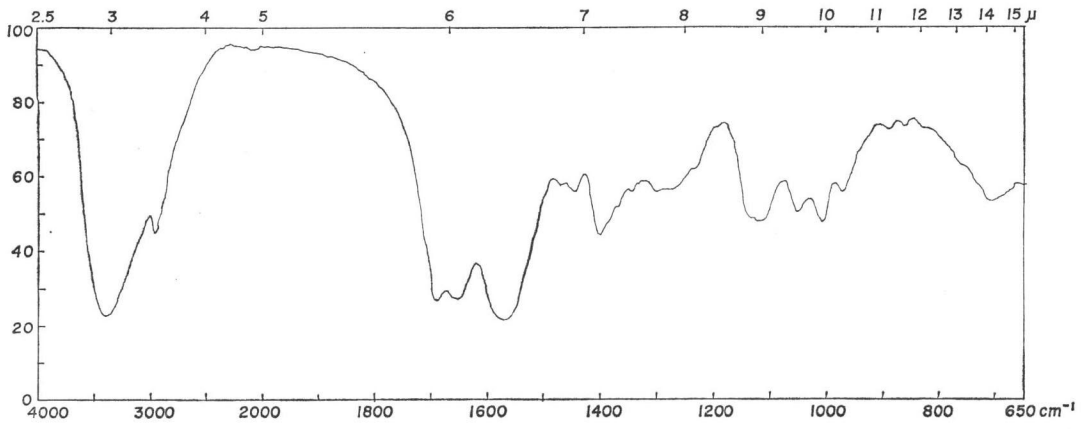
Sir:

A new antibiotic, minosaminomycin, containing a *myo*-inosamine has been isolated from the culture broth of *Streptomyces* No. MA514-Al which is very closely related to *Actinomyces aureomonopodiales*[1].

The antibiotic was produced by shaking culture of the strain at 30°C for 4 days in a medium containing 2.0 % glucose, 2.0 % starch, 2.0% soybean meal, 0.5% dry yeast, 0.25% NaCl, 0.32 % $CaCO_3$, 0.0005 % $CuSO_4 \cdot 5H_2O$, 0.0005 % $MnCl_2 \cdot 4H_2O$ and 0.005 % $ZnSO_4 \cdot 7H_2O$ (adjusted to pH 7.4). The broth (pH 8.4) containing 40 mcg/ml of the antibiotic determined by the usual cylinder plate method against *Mycobacterium smegmatis* ATCC 607, was harvested and filtered. The antibiotic in the filtrate was adsorbed on a column of Amberlite IRC 50 (70 % Na^+ form) and eluted with 1 N hydrochloric acid. The antibiotic in the eluate was neutralized with Amberlite IR 45 (OH^- form) and adsorbed on a column of activated carbon. After washing with water and 0.05 N hydrochloric acid, it was eluted with 0.05 N hydrochloric acid in 50 % methanol. The active eluate was neutralized with Amberlite IR 45 (OH^- form) and concentrated under reduced pressure. The antibiotic in the concentrated solution was readsorbed on a column of Amberlite CG 50 (70 % NH_4^+ form) and eluted with 0.3 % ammonia. The active eluate was concentrated to dryness yielding the almost completely purified antibiotic in 68 % yield. Further purification was accomplished by column chromatography of cellulose powder (Whatman CF 11) using butanol - pyridine - acetic acid - water (6 : 4 : 1 : 3 in volume) as a developing solvent. The antibiotic was also adsorbed on an anion exchanger, Dowex 1×2 (OH^- form) and eluted with 0.5 N hydrochloric acid.

The antibiotic is an amphoteric colorless powder melting over the wide range of 225~260°C with decomposition. $[\alpha]_D^{22}+30°$ (*c* 1.0, water). Anal. calcd. for $C_{25}H_{46}N_8O_{10} \cdot 2H_2O$: C 45.85, H 7.70, N 17.12, mol. wt. 654.714. Found: C 45.40, H 7.83, N 17.10, titration equivalent 654. The pKa′ values are 2.9, 6.2, 8.1 and >12. It shows no ultraviolet absorption except end absorption. The IR spectrum is represented in Fig. 1. The PMR spectrum in D_2O (tetramethylsilane as the external reference) shows signals at δ 1.38 (dd, 6H), 1.72 (d, 3H), 1.9~2.2 (3H), 2.35 (2H), 2.55 (2H), 3.2~4.9 (13H) and 5.45 ppm (d, 1H). The compound gives positive ninhydrin, RYDON-SMITH and pentacyanoaquoferriate reactions, and negative SAKAGUCHI, diacetyl and red tetrazolium reactions. It is soluble in water, but almost insoluble in organic solvents. Under high-voltage paper electrophoresis, 3,000 V for 20 minutes in formic acid - acetic acid - water (25 : 75 : 900 in volume), it moves 10.6 cm to cathode

Fig. 1. The IR spectrum of minosaminomycin in KBr



with an Rm (relative mobility against alanine) of 1.21. On thin-layer chromatography using Silica gel G (E. Merck), the antibiotic gives a single spot at Rf 0.13 wtih butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume) and at Rf 0.08 with butanol-pyridine-acetic acid-water (6:4:1:3 in volume).

By acid hydrolysis of minosaminomycin (502 mg) with 6 N hydrochloric acid at 100°C for 5 hours, an aminocyclitol hydrochloride was obtained in the form of coloreless needles in good yield (140 mg), mp 201~203°C, $[\alpha]_D^{26}$ −9.5° (*c* 6.2, water). Anal. calcd. for $C_6H_{13}NO_5 \cdot HCl \cdot \frac{1}{2}H_2O$: C 32.08, H 6.73, N 6.24, Cl 15.78, mol. wt. 224.651. Found: C 31.78, H 6.65, N 6.33, Cl 15.04, titration equivalent 211. The pKa′ value is 8.1. The carbon-13 NMR spectrum of the hydrochloride in D_2O shows six signals at δ 54.0, 68.7, 69.6, 71.8, 72.3 and 74.9 ppm from tetramethylsilane. The free base of the aminocyclitol was prepared from the hydrochloride by Amberlite CG 50 chromatography followed by elution with 0.1% ammonia, mp 207~212°C (dec), $[\alpha]_D^{28}$ −3.9° (*c* 4.3, water). The PMR spectrum of the hexaacetyl derivative (mp 212~214°C, *m/e* 431) was identical with that of hexaacetyl-DL-*myo*-inosamine-1 reported by LICHTENTHALER[2,3]. Therefore, the aminocyclitol here obtained was identified to be (−)-isomer of *myo*-inosamine-1*. It is the first finding of this compound in natural products. By application of the TACu method[5], the aminocyclitol showed positive contribution ($\Delta[M]_{436(TACu)}$ +767°) and the absolute structure was determined to be (−)-1D-1-amino-1-deoxy-*myo*-inositol (L-*myo*-inosamine-1).

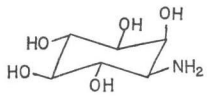


Table 1. The antimicrobial spectrum of minosaminomycin

Organisms	Minimum inhibitory concentrations (mcg/ml)
Staphylococcus aureus FDA 209P	>100
Staphylococcus aureus SMITH	>100
Staphylococcus aureus TERAJIMA	>100
Micrococcus flavus FDA 16	50
Sarcina lutea PCI 1001	>100
Bacillus anthracis	>100
Bacillus subtilis NRRL B-558	>100
Escherichia coli NIHJ	100
Escherichia coli K-12	>100
Escherichia coli K-12 ML 1629	>100
Shigella dysenteriae JS 11910	50
Shigella flexneri 4b JS 11811	>100
Shigella sonnei JS 11746	100
Salmonella typhosa T-63	50
Salmonella enteritidis 1891	100
Proteus vulgaris OX 19	50
Klebsiella pneumoniae PCI 602	100
Pseudomonas aeruginosa A3	>100
Pseudomonas aeruginosa No. 12	>100
Mycobacterium smegmatis ATCC 607	1.56
Mycobacterium phlei	6.25
Candida albicans 3147	>100

The antimicrobial spectrum of the antibiotic tested by the agar dilution method is represented in Table 1, showing that it is active against mycobacteria, and weakly active against the other bacteria. The minimum inhibitory concentration against *Mycobacterium tuberculosis* $H_{37}Rv$ in KIRCHNER's semiliquid medium with 10% horse serum was 16 mcg/ml. Acute intravenous LD_{50} of the antibiotic in mice was 50~100 mg/kg.

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* The (+)-isomer of *myo*-inosamine-1 (dp 200~205°C, $[\alpha]_D$ +3.8°) and its hexaacetate (mp 206~207°C) were described by ANGYAL and ANDERSON[4].

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