A NEW ANTIBIOTIC, 
NEGAMYCIN

Sir:

After more than 20 years of screening studies, one rarely encounters new antibacterial antibiotics with low toxicity. However, negamycin was found by an ordinary screening method and is unique type of antibiotic.

Negamycin was obtained from culture filtrates of three strains of *Streptomyces*; strain No. M890-C2 was isolated from a soil sample collected at Myogisan, Gunma Prefecture in 1964, the strain MA91-M1 from Nojiriko, Nagano Prefecture in 1965 and strain MA104-M1 from Sakomachi, Tokushima Prefecture in 1965. These strains are very closely related to *S. purpeofuscus*. In a medium containing 2.0 % glucose, 2.0 % starch, 2.0 % soybean meal, 0.5 % dry yeast, 0.25 % NaCl, 0.35 % CaCO₃, 0.0005 % CuSO₄·5H₂O, 0.0005 % MnCl₂·4H₂O, 0.005 % ZnSO₄·7H₂O after shaking culture of the strain No. M890-C2 for 3~8 days negamycin accumulated in the culture liquid. It was adsorbed on a cation-exchange resin containing carboxyl or sulfonic acid group and eluted with dilute aqueous ammonia. Negamycin in the active eluate was adsorbed on a column of Dowex 1 X2 (OH⁻ form) and after washing with water, was eluted with dilute hydrochloric acid. After neutralization of the active eluate, evaporation yielded crude negamycin hydrochloride. An aqueous solution of the hydrochloride was passed through a column of Amberlite CG 50 (NH₄⁺ form) and the chromatograph was developed with 0.1 % ammonia water. Lyophilization of the active fraction yielded pure negamycin.

Negamycin is a colorless powder, m.p. 110~120°C (decomp.), [α]D² +2.5° (c 2, H₂O). The antibiotic is soluble in water, and practically insoluble in methanol, ethanol, butanol, ethyl acetate, butyl acetate, chloroform and benzene. It shows no ultraviolet absorption except end absorption. It gives positive ninhydrin, red tetrazolium and RYDON-SMITH reactions, and heating at 105°C for 6 hours in 6 N HCl gives more than three ninhydrin-positive degradation products. Under paper electrophoresis, 3,500 V for 15 minutes in formic acid-acetic acid-water (25 : 75 : 900), it moved 12 cm to cathode with Rm (L-alanine 1.0) of 1.4. Thus, negamycin has basic properties. Negamycin gives salts with picric acid and -hydroxyazobenzene-′-sulfonic acid. Negamycin -hydroxyazobenzene-′-sulfonate crystallizes as yellowish orange plates, m. p. 180~182°C (decomp.).

In the infrared spectrum of negamycin, carboxylate absorption is shown at 1590 cm⁻¹ and in that of the -hydroxyazobenzene-′-sulfonate the carboxyl band is shown at 1730 cm⁻¹. Treatment of negamycin with hydrochloric acid-methanol gives negamycin methyl ester hydrochloride which shows an ester carbonyl band at 1740 cm⁻¹. pK values of 3.55, 8.10, 9.75 were found by titration with an equivalent weight of 287. Thus, negamycin is an amphoteric compound containing a carboxyl and two basic groups. Elemental analyses of negamycin and its -hydroxyazobenzene-′-sulfonate are as follows:

Negamycin ; found :
C 40.63, H 7.90, N 21.11, O 29.70.
Calcd. for C₉H₁₈N₄O₄·H₂O :

Negamycin ′-hydroxyazobenzene-′-sulfonate ; found :
Calcd. for C₉H₁₈N₄O₄(C₁₂H₁₀N₂O₄S)₂·H₂O :

The molecular formula was derived from the high-resolution mass spectrum of di-N-acetylnegamycin methyl ester : calcd. mol. wt. for C₁₄H₂₆N₄O₆, 346.185; found, m/e 346.188±0.005.

On nutrient agar medium negamycin showed complete inhibition of test organisms at the following concentrations (the values in the parentheses are for partial inhibitions): *Staphylococcus aureus* FDA 209P, 50 µg/ml; *S. aureus* TERAIMA strain, 12.5 µg/ml; *Sarcina lutea* PCI 1001, 12.5 µg/ml (1.56 µg/ml); *Bacillus subtilis* NRRL B-558, 25 µg/ml (12.5 µg/ml); *Escherichia coli* K-12, 3.12 µg/ml (1.56 µg/ml); *E. coli* K-12 ML
1629 (carrying multiple resistant R factor),
6.25 µg/ml (3.12 µg/ml); E. coli NIHJ, 12.5
µg/ml (3.12 µg/ml); Shigella flexneri 1a
Ew 8, 12.5 µg/ml; Salmonella typhosa, 3.12
µg/ml; Proteus vulgaris OX 19, 6.25 µg/ml;
P. rettgeri GN 311, 12.5 µg/ml (6.25 µg/ml);
Serratia marcescens, 12.5 µg/ml (6.25 µg/ml);
Pseudomonas aeruginosa A3, 6.25 µg/ml;
Klebsiella pneumoniae PCI 602, 12.5 µg/ml
(6.25 µg/ml); Mycobacterium smegmatis
ATCC 607, 100 µg/ml. Fifteen strains of
Pseudomonas aeruginosa isolated from
patients were inhibited completely at 12.5–
50 µg/ml and partially at 6.25–25 µg/ml
when one loopful of their broth culture
was streaked on a nutrient agar medium
containing negamycin. However, when one
loopful of broth culture diluted 1,000 times
was streaked, the complete and the partial
inhibition were observed at half the above
concentrations. When 0.5 % peptone agar
was used instead of nutrient agar, the com-
plete and the partial inhibitions were
observed at lower concentrations as follows:
S. aureus FDA 209P, 1.56 µg/ml; E. coli
K-12, 1.56 µg/ml; E. coli K-12 ML 1629,
1.56 µg/ml (0.78 µg/ml); S. flexneri 1a Ew 8,
3.12 µg/ml; S. typhosa, <0.78 µg/ml; P. ret-
tgeri GN 311, 1.56 µg/ml; Pseudomonas
aeruginosa, 3.12–12.5 µg/ml; K. pneumo-
niae, 6.25 µg/ml (3.12 µg/ml). The growth
inhibitory effect was not reduced by serum.
E. coli K-12 was inhibited at 1.56–3.12
µg/ml when serum was added to 0.5 %
peptone water at 10–40%.

The intramuscular injection of 50 mg/kg
to a rabbit gave a serum concentration of
about 100 µg/ml at 1 hour after administra-
tion with 80 % excreted in the urine in
24 hours resulting in a high concentration
in urine, for instance 4480 µg/ml in urine
taken 1–2 hours after the injection.

Negamycin was effective in vivo against
infections of Pseudomonas aeruginosa No.
12, Klebsiella pneumoniae S-1802, Salmon-
ella typhosa 63 and Staphylococcus aureus
Smith S-424 in mice. The CD50 against
these infections was 4.4, 5.0, 2.5 and 12.5
mg/kg, respectively, when 10 MLD was
infected intraperitoneally and negamycin
was subcutaneously injected immediately
and 6 hours after the infection. The LD50
of negamycin to mice by intravenous injec-
tion was 400–500 mg/kg and daily intra-
peritoneal injection of 200 mg/kg for 10
days caused no toxicity.

As described above, negamycin is an
antibiotic with low toxicity and effective
in vivo against infections of Gram-negative
and Gram-positive bacteria. Especially, an
effect against resistant Gram-negative or-
ganisms including Pseudomonas was found. The
structure and the pharmacology will be
reported in other papers.

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