NANAOMYCINS A AND B*, NEW ANTIBIOTICS PRODUCED BY A STRAIN OF *STREPTOMYCES*

Sir:

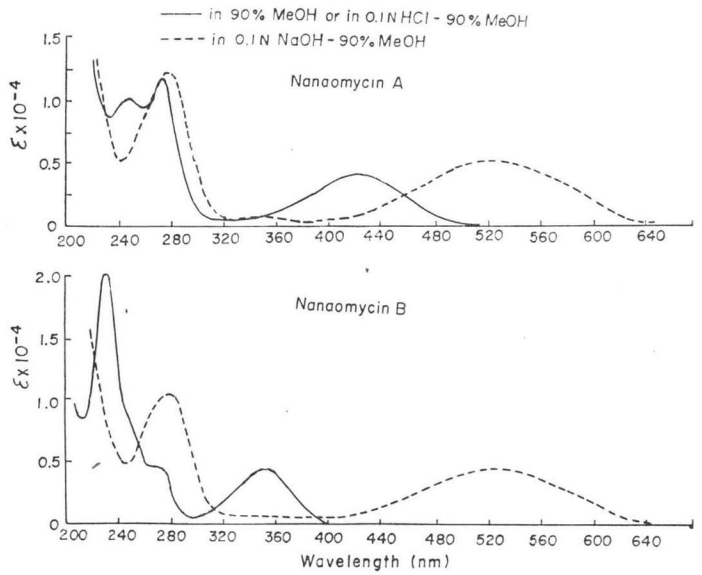
In the course of our research for new antibiotics produced by actinomycetes, new antibiotics, nanaomycins A and B, effective especially against mycoplasmas and fungi were obtained from the cultured broth of a strain which had been isolated from a soil sample collected at the Noto Peninsula and designated *Streptomyces rosa* var. *notoensis*.

Fermentation was carried out using a 400-liter tank fermentor containing 200 liters of a medium for 4 days at 27°C. The composition of the medium was 2% glycerol, 2% soy bean meal and 0.3% sodium chloride (pH 7.0 before sterilization). The culture supernatant was adjusted to pH 2.0 with hydrochloric acid and extracted with butyl acetate. The antibiotics contained in the extract were then back-extracted into 1% sodium bicarbonate solution, and then extracted again with ethyl acetate from the water layer after adjusting to pH 2.0 with hydrochloric acid. A crude powder (10.9 g) of nanaomycins was obtained by evaporating the solvent layer. The crude powder was chromatographed on a column of silica gel No. 923 (Davison Chemical Co.) with benzene-ethyl acetate (4 : 1, v/v). Two main active peaks (nanaomycins A and B) were eluted separately from the column.

The fractions of the first active peak were combined and concentrated under reduced pressure to dryness. Orange needles of nanaomycin A were obtained from an ethanol solution of the powder: yield 317 mg; mp 178~180°C. *Anal.* Found: C, 63.35; H, 4.47; N, 0. Calcd. for $C_{16}H_{14}O_6$: C, 63.57; H, 4.66; N, 0%. UV λ_{max}^{MeOH} nm(ε): 250 (9850), 274 (12,200), 423 (4040). $[\alpha]_D^{26}$: −27.5° (*c* 1.0, MeOH). Mass M^+ (*m/e*): 302.07904. The UV and IR spectra are shown in Figs. 1 and 2, respectively. The molecular formula, $C_{16}H_{14}O_6$, was assigned to the compound from elementary analysis and its mass spectrum.

The fractions of the second active peak were combined and concentrated under reduced pressure to give a powder of nanaomycin B (4.5 g). A purer powder of nanaomycin B was obtained by rechromatography: yield,

Fig. 1. UV spectra of nanaomycins A and B



* Named rosanomycins when presented at the 94th Annual Meeting of the Pharmaceutical Society of Japan, Apr. 6, 1974 (Sendai) and the 47th Annual Meeting of Japanese Society for Bacteriology, Apr. 2, 1974 (Kyoto).

2.7 g. *Anal.* Found: C, 59.70; H, 4.90; N, 0. Calcd. for $C_{16}H_{16}O_7$: C, 59.99; H, 5.03; N, 0 %. UV λ_{max}^{MeOH} nm(ε): 231 (20,800), 248 (8700, sh), 269 (5030, sh), 352 (4970). $[\alpha]_D^{26}$: −74.5° (*c* 1.0, MeOH). Mass M^+ (*m/e*): 320.08961. The UV and IR spectra are shown in Figs. 1

Fig. 2. IR spectra of nanaomycins A and B (KBr)

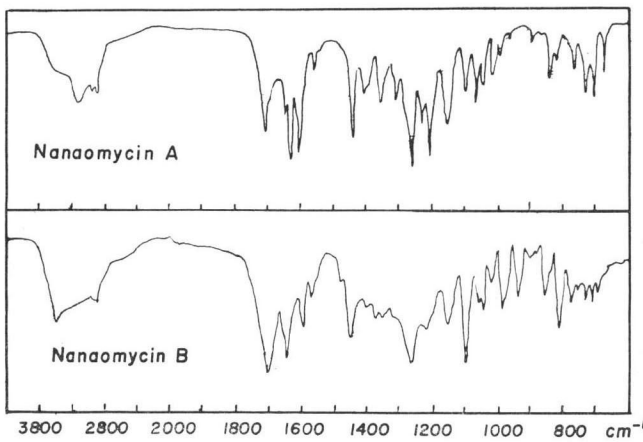


Table 1. Antimicrobial spectra of nanaomycins A and B

Test organism*1	Minimal inhibitory concentration (μg/ml)	
	A	B
Bacillus subtilis PCI 219*2	7.8	7.8
Staphylococcus aureus FDA 209 P*2	3.9	3.9
Sarcina lutea PCI 1001*2	2.0	2.0
Escherichia coli NIHJ*2	31.3	15.6
Escherichia coli NIHJ JC-2*2	250	500
*Klebsiella pneumoniae*2	31.3	31.3
*Salmonella typhimurium*2	62.5	62.5
*Shigella flexneri*2	31.3	62.5
*Pseudomonas aeruginosa*2	500	>500
Mycobacterium smegmatis ATCC 607*2	62.5	125
*Candida albicans*3	31.3	31.3
*Aspergillus niger*3	62.5	62.5
*Saccharomyces sake*3	31.3	62.5
*Piricularia oryzae*3	7.8	15.6
*Trychophyton interdigitale*3	1.0	3.9
*Trychophyton rubrum*3	<0.1	3.1
*Trychophyton ferrugineum*3	1.56	12.5
*Microsporum gypseum*3	0.78	12.5
Mycoplasma gallisepticum KP-13*4	0.05	0.1
Mycoplasma gallisepticum S-6*4	0.05	0.1
*Mycoplasma pneumoniae*4	0.013	0.05

*1: Agar dilution method.
*2: Nutrient agar, 2 days, 37°C.
*3: Potato agar, 4 days, 27°C.
*4: Eiken PPLO agar, 3 days, 37°C.

and 2, respectively. The molecular formula, $C_{16}H_{16}O_7$, was assigned to nanaomycin B from elementary analysis and its mass spectrum.

Nanaomycins A and B are soluble in methanol, ethanol, ethyl acetate, chloroform, acetone and petroleum ether. They gave positive reactions to ferric choride, 2, 4-dinitrophenylhydrazine and formaldehyde-*O*-dinitrobenzene reagents, but negative reactions to ninhydrin, SAKAGUCHI, EHLRICH, FEHLING, MOLISCH and DRAGENDORFF reagents. R_f values of nanaomycins A and B on Silica gel thin-layer chromatography using chloroform - methanol (5 : 1, v/v), were 0.25 and 0.15, respectively. Nanaomycin B converts rapidly to nanaomycin A in alkaline solution at room temperature.

From the above data, it was concluded that nanaomycins A and B are new antibiotics having a quinone group and are closely-related.

The antimicrobial spectra of nanaomycins A and B by the agar dilution method are shown in Table 1. Nanaomycins A and B inhibit mainly mycoplasmas, fungi and gram-positive bacteria. The acute toxicities (LD_{50}, ip) of nanaomycins A and B in mice are 28.2 and 169 mg/kg, respectively.

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