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

III. A NEW COMPONENT, NANAOMYCIN C, AND BIOLOGICAL ACTIVITIES OF NANAOMYCIN DERIVATIVES

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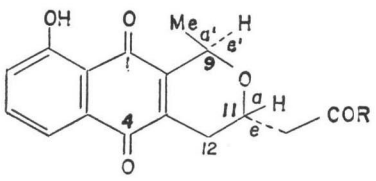
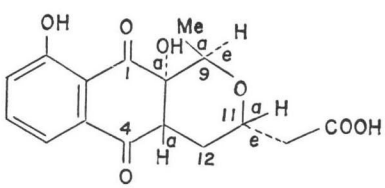
A new component, nanaomycin C, has been isolated from the culture medium of *Streptomyces rosa* var. *notoensis*, which had been found to produce nanaomycins A and B. Nanaomycin C is an amide of nanaomycin A. Biological activities of nanaomycin C and several derivatives of nanaomycin A are also shown. Acetylnanaomycin A exhibits as strong activities against Gram-positive bacteria, fungi and *Mycoplasma gallisepticum* as nanaomycin A.

It has already been reported by the present authors that nanaomycins A and B, which inhibit mycoplasma, fungi and Gram-positive bacteria, are produced by *Streptomyces rosa* var. *notoensis*,[1,2] and that their structures are **I** and **III**, respectively[3]. Further investigation led to the discovery of a new component, which was designated as nanaomycin C, from the culture filtrate of the same organism.

The present paper deals with the isolation, characterization, biological properties of nanaomycin C. In addition, antimicrobial activities of several derivatives of nanaomycin A are also described.



I: R=OH (Nanaomycin A)
II: R=NH_2 (Nanaomycin C)



III (Nanaomycin B)

Production of Nanaomycin C

For the production of nanaomycin C, *Streptomyces rosa* var. *notoensis* (FERM-P No. 2209) was used. The characteristics of the strain has been reported in the previous paper[2].

The production was performed with the organism according to the method for the produc-

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tion of nanaomycins A and B[2]. The amount of nanaomycin C accumulated at 4th day was smaller than that of nanaomycin A or B.

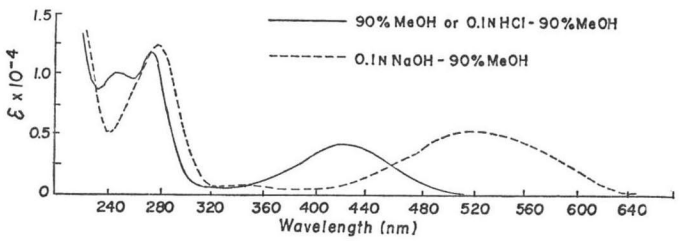
Isolation of Nanaomycin C

Culture broth (20 liters) obtained by incubation of *Streptomyces rosa* var. *notoensis* in a 30-liter jar fermentor, was used as a starting material for the isolation of nanaomycin C. After the culture supernatant was adjusted to pH 2.0 with 6 N hydrochloric acid, nanaomycins A, B and C were extracted with butyl acetate. Nanaomycins A and B were then transferred into 1 % sodium bicarbonate solution from the extract, while nanaomycin C was remained in the butyl acetate layer. A crude powder (1.3 g) of nanaomycin C was obtained by evaporating the solvent layer dried with sodium sulfate (anhydrous). The crude powder was chromatographed on a column of silica gel No. 923 (Davison Chemical Co.) with chloroform-methanol. Nanaomycin C was eluted with chloroform-methanol (50:1). The fractions containing nanaomycin C were combined and concentrated under reduced pressure to dryness. Orange needles of nanaomycin C were obtained from an ethyl acetate solution of the powder. The crystals were recrystallized from an ethyl acetate solution: yield 35 mg; mp 222~224°C (decomp.).

Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.78; H, 4.64; N, 5.02
Found: C, 63.46; H, 4.50; N, 4.89

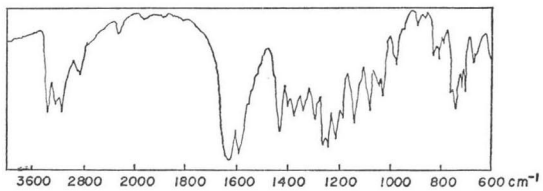
UV λ_{max}^{MeOH}nm(ε): 248(10,100), 274(12,400), 424(4,610). $[\alpha]_D^{26}$: −2° (*c* 0.5, dioxane). Mass M^+ (*m/e*): 301.092. The UV and IR spectra are shown in Figs. 1 and 2, respectively. Nanaomycin

Fig. 1. UV-spectra of nanaomycin C



C is soluble in methanol, ethanol, ethyl acetate, chloroform and acetone, but insoluble in water, *n*-hexane and petroleum ether. It gave positive reactions to ferric chloride, 2,4-dinitrophenyl hydrazine and formaldehyde-*O*-dinitrobenzene reagents, but negative reactions to ninhydrin and EHLRICH reagents. Results of paper electrophoresis indicated nanaomycin C to be a neutral substance. Rf value of nanaomycin C on silica gel thin-layer chromatography using chloroform-methanol (10:1, v/v) was 0.35.

Fig. 2. Infrared spectrum of nanaomycin C (KBr)



Structure of Nanaomycin C

Results of the elementary and mass spectral (M^+ *m/e*: 301.092) analyses gave the molecular

formula of $C_{16}H_{15}NO_5$. The ultraviolet spectra of nanaomycin C in both neutral and basic media (Fig. 1) showed a striking resemblance to those of nanaomycin A[2] suggesting that a juglone moiety was also present in nanaomycin C.

Fig. 3. NMR-spectrum of nanaomycin C in $CDCl_3$

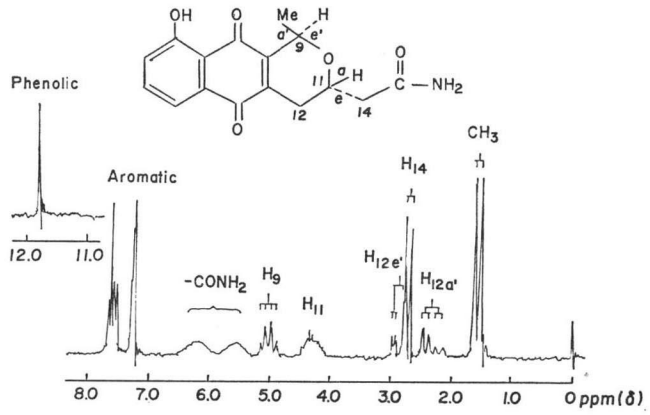


Table 1. Antimicrobial spectrum of nanaomycin C

Test organism	Medium*	Minimal inhibitory concentration (μg/ml)
Staphylococcus aureus FDA 209P	N	6.3
S. aureus FDA 209P (JC-1)	N	3.1
Bacillus subtilis PCI 219	N	6.3
Sarcina lutea PCI 1001	N	25
Mycobacterium smegmatis ATCC 607	N	50
Escherichia coli NIHJ	N	100
E. coli NIHJ (JC-2)	N	>100
Klebsiella pneumoniae PCI 602	N	>100
Salmonella typhimurium	N	>100
Shigella flexneri	N	>100
Pseudomonas aeruginosa	N	>100
Candida albicans	P	>100
Saccharomyces sake	P	>100
Aspergillus niger	P	>100
Trichophyton interdigitale	P	100
Sclerotinia cinerea	P	100
Mycoplasma gallisepticum KP-13	E	12.5
M. gallisepticum S-6	E	6.3
M. gallisepticum 333P (spiramycin resistant)	E	3.1
M. gallinarum	E	12.5
M. iners	E	50
M. pneumoniae	E	6.3

* Abbreviations used: N, nutrient agar (pH 7.0, 2 days, 37°C); P, potato agar (pH 6.4, 4 days, 27°C); E, Eiken PPLO agar (pH 7.8, 8 days, 37°C).

From the fact that nanaomycin C is neutral and contains a nitrogen atom, it was speculated that nanaomycin C was an amide of nanaomycin A. This was confirmed by the results of NMR spectrometry. NMR spectrum of nanaomycin C in deuterium chloroform (Fig. 3) was almost identical with that of nanaomycin A[8] except for the absence of a signal of a carboxylic acid and the appearance of broad signals of an,amide (δ 5.36 and δ 6.00). The infrared absorption at 3400 cm^{-1} and the peak at *m/e* 284 ($M^+ - NH_3$) in the mass spectrum also proved the presence of amide moiety in nanaomycin C.

Thus, the structure of nanaomycin C was determined to be **II**.

Biological Activities

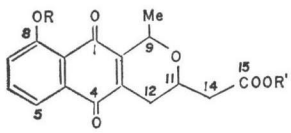
The antimicrobial spectra of nanaomycin C is shown in Table 1. This test was conducted by agar dilution method using nutrient agar for bacteria, potato agar for fungi, and Eiken PPLO agar for mycoplasmas.

Nanaomycin C inhibits mainly Gram-positive bacteria and mycoplasmas. Nanaomycin C exerts as strong activity against Gram-positive bacteria as nanaomycin A, but weaker activity against fungi and mycoplasmas than nanaomycin A.

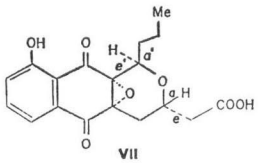
Biological Activities of Derivatives of Nanaomycin A

Antimicrobial spectra of several derivatives of nanaomycin A synthesized to elucidate the structure[8] are shown in Table 2. Structures of these derivatives are shown below.

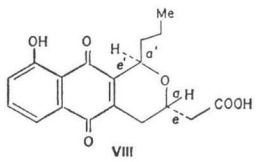
Acetylnanaomycin A (**IV**) exhibits as strong activities against Gram-positive bacteria, fungi, and *Mycoplasma gallisepticum* as nanaomycin A, while methyl ester of nanaomycin A exhibits less activity, and methyl ester of *O*-methylnanaomycin A hardly exhibits activity at the range of the concentrations tested.



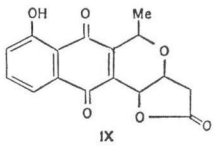
I: R = R' = H (Nanaomycin A)
IV: R = Ac, R' = H
V: R = H, R' = Me
VI: R = Me, R' = Me



VII



VIII



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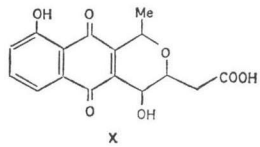

X

Discussion

From the above data, it was found that *Streptomyces rosa* var. *notoensis* producing nanaomycins A and B also produced another new component, nanaomycin C (**II**). Nanaomycin C is an amide of nanaomycin A, and is a new compound.

Results of antimicrobial activities of nanaomycins and their derivatives indicated that the phenolic hydroxyl and carboxylic groups were important for antimicrobial activities.

The acetylation of the phenolic hydroxyl group does not reduce the activity at all, while the *O*-methylation almost completely abolished the activity. This seems to indicate that the

Table 2. Antimicrobial spectra of some derivatives of nanaomycin A

Test organism	Medium	Minimum inhibitory concentration (μ/ml)			
		I	IV	V	VI
Staphylococcus aureus FDA 209P	N	3.9	1.6	12.5	100
S. aureus FDA 209P (JC-1)	N	2.0	0.8	25	>100
S. aureus FS 1227 (PC-R)	N	1.6	0.8	25	>100
S. aureus KB 61 (R-TC, EM)	N	1.6	0.8	12.5	>100
S. aureus KB 64 (R-TC, EM)	N	0.8	0.4	12.5	>100
Bacillus subtilis PCI 219	N	6.3	3.1	25	>100
B. cereus T	N	12.5	25	25	>100
Sarcina lutea PCI 1001	N	1.6	1.6	25	>100
Corynebacterium paurometabolum	N	12.5	6.3	25	>100
Mycobacterium smegmatis ATCC 607	N	100	50	12.5	>100
Aerobacter aerogenes IAM 1183	N	>100	>100	>100	>100
Proteus vulgaris IFO 3167	N	50	50	>100	>100
P. mirabilis	N	>100	>100	>100	>100
Escherichia coli NIHJ (JC-2)	N	>100	>100	>100	>100
Salmonella typhimurium	N	100	50	>100	>100
Shigella sonnei E 33	N	100	100	>100	>100
Pseudomonas aeruginosa P-3	N	>100	>100	>100	>100
Candida albicans	P	50	25	>100	>100
Saccharomyces sake	P	12.5	12.5	50	>100
Piricularia oryzae	P	0.8	0.8	12.5	>100
Aspergillus niger ATCC 6275	P	25	25	>100	>100
A. fumigatus IAM 2162	P	6.3	6.3	100	>100
Microsporum gypseum 704	P	0.8	0.4	1.6	>100
Trichophyton asteroides	P	0.8	0.8	12.5	>100
T. ferrugineum	P	0.8	0.8	12.5	>100
T. interdigitale	P	1.6	1.6	12.5	>100
T. mentagrophytes	P	<0.2	0.4	6.3	100
T. pedis 804	P	0.8	0.8	12.5	>100
T. purpureum	P	0.4	1.6	12.5	100
T. roseum	P	<0.2	0.4	0.8	100
T. rubrum	P	<0.2	<0.2	<0.2	>100
T. schoenleini	P	<0.2	<0.2	<0.2	—
T. violaceum	P	3.1	0.8	12.5	>100

* Abbreviations used: N, nutrient agar (pH 7.0, 2 days, 37°C); P, potato agar (pH 6.4, 4 days, 27°C); R, resistant strain; PC, penicillin; TC, tetracycline; EM, erythromycin.

Table 3. Antimycoplasma activities of some derivatives of nanaomycin A[4]

Test organism	Medium*	Inhibitory zone (mm)			
		I	IV	V	VI
Mycoplasma gallisepticum KP-13	E	28.7	28.5	28.5	none
Acholeplasma laidlawii (A)	H	none	none	none	none

* Abbreviations used: E, Eiken PPLO agar (pH 7.8, 8 days, 37°C); H, Hokken PPLO agar (pH 7.8, 8 days, 37°C).

phenolic hydroxyl group plays a vital role at the molecular level for antimicrobial activity, and **IV** exhibits the activity after the easy deacetylation, while demethylation of **VI** hardly occurs.

It is interesting that the phenolic hydroxyl group is also contained in all the antibiotics which are analogous to nanaomycins, for example, frenolicin[5] (**VII**), deoxyfrenolicin[6] (**VIII**), kalafungin[7] (**IX**) and kalamycinic acid[8] (**X**).

Esterification and amidation of the carboxylic group of nanaomycin A were found to reduce the activity somewhat, and the activity against Gram-positive bacteria follows the order **I** (-COOH) **II** (-$CONH_2$) **V** (-COOMe). This and the fact that kalafungin has strong activity seem to suggest that the carbonyl group at the position 15 is required for the antimicrobial activity.

References

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