STRUCTURE OF NANAOMYCIN E, A NEW NANAOMYCIN

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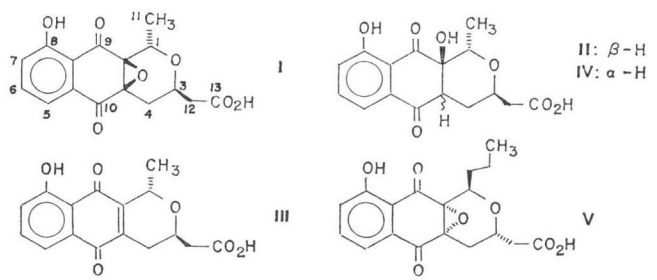
A new component, nanaomycin E, has been isolated from the culture broth of *Streptomyces rosa* var. *notoensis*, which had been found to produce nanaomycins A, B, C and D. Nanaomycin E was an epoxy derivative of nanaomycin A and was converted into nanaomycin A and 4a-*epi*-nanaomycin B by treatment with sodium hydrosulfite in an acidic aqueous solution. 4a-*epi*-Nanaomycin B was quantitatively converted into nanaomycin A under alkaline conditions.

We have previously reported the structures and biological characteristics of nanaomycins[1~5], which are effective against mycoplasma, fungi and Gram-positive bacteria. Further investigations of the fermentation of *Streptomyces rosa* var. *notoensis* revealed that another new active component, nanaomycin E (**I**) was produced. This paper describes the structure and the conversion of this compound into 4a-*epi*-nanaomycin B (**II**) and nanaomycin A (**III**).

Production and Isolation of Nanaomycin E

For the production of nanaomycin E (**I**), *Streptomyces rosa* var. *notoensis* (FERM-P No. 2209) was used. The seed culture for the production of nanaomycins was obtained according to the method reported in the previous paper[2]. Fermentation was carried out using a 30-liter jar fermentor containing 18 liters of a medium for 3 days at 27°C. The composition of the medium was 2% glucose, 1% meat extract, 0.5% sodium chloride and 0.3% calcium carbonate (pH 7.0 before autoclaving).

Culture broth (50 liters), obtained by incubation of *Streptomyces rosa* var. *notoensis* in three 30-liter jar fermentors, was used for the isolation of nanaomycin E (**I**). The culture broth, after the pH was adjusted to 4.5 with sulfuric acid, was extracted with ethyl acetate (50 liters). After separation of the organic layer, the crude powder of nanaomycins, obtained by evaporation of the organic layer, was chromatographed on a column of silica gel (2 liters, Kanto Chemical



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Co.) using a mixture of benzene and ethyl acetate (10: 1). The fractions containing nanaomycin E (**I**) were combined and concentrated *in vacuo* to dryness to give a yellowish brown powder (8.8 g). Concentration of a solution of the crude powder in 1 liter of 10% aqueous methanol afforded orange crystals of **I**, which were collected (7.9 g) and recrystallized from *n*-hexane - dichloromethane to yield orange plates (7.3 g), mp 172~174°C.

Anal. Found: C, 60.14; H, 4.34%.
Calcd. for $C_{16}H_{14}O_7$: C, 60.38; H, 4.43%.

Nanaomycin E (**I**) is soluble in methanol, ethanol, acetone and ethyl acetate and insoluble in *n*-hexane and petroleum ether. It was positive to a ferric chloride test. On silica gel thin-layer chromatography using chloroform - methanol (5: 1) **I** showed a yellow fluorescent spot with Rf of 0.45.

Structure of Nanaomycin E

Nanaomycin E (**I**) has the following physical properties:

$[\alpha]_D^{24}$ +89.0° (*c* 0.95, MeOH); IR (KBr) 3345 (νOH), 1728 (carboxylic acid νCO), 1683, 1653 cm^{-1} (quinone νCO); UV (MeOH) 236 (log ϵ 4.38), 280^{sh} (3.66), 364 nm (3.87); ^{13}C NMR* ($CDCl_3$–CD_3OD) δ 15.4 (C11), 24.9 (C12), 40.8 (C4), 61.5 (C3), 62.4 (C4a), 63.5 (C9a), 65.5 (C1), 114.5 (C8a), 119.7 (C5), 124.0 (C7), 131.7 (C10a), 137.3 (C6), 161.9 (C8), 173.0 (C13), 190.0 (C10), 195.9 ppm (C9); ^1H NMR ($CDCl_3$) δ 1.64 (d, J=6.8 Hz, CH_3), 1.94 (dd, J=15.4, 11.5 Hz, H4β), 2.61 (d, J=6.4 Hz, H12×2), 2.92 (dd, J=15.4, 4.7 Hz, H4α), 4.15 (m, H3), 4.77 (q, J=6.8 Hz, H1), 7.2~7.8 ppm (m, aromatic protons).

The molecular formula of **I**, $C_{16}H_{14}O_7$, was determined on the basis of its high resolution mass spectrum (M^+, 318.0760, calcd. 318.0739) and elemental analysis, showing that **I** had one more oxygen atom than nanaomycin A (**III**). In the IR spectrum of **I**, absorptions at 1683 and 1653 cm^{-1} due to the quinone carbonyls are shifted to higher wave numbers than those of **III** (1645, 1615 cm^{-1}). The ^{13}C NMR spectrum of **I** shows the large upfield shifts of 79.5 and 82.6 ppm for C4a and C9a, respectively, compared with those of **III** (141.9, 146.1 ppm). These spectra suggest that **I** had a 2,3-*epoxy*-1,4-naphthoquinone moiety. The fragmentations (*m/e* 303, 285, 229) and UV spectrum of **I** are similar to those of frenolicin (**V**)[8], and ^1H NMR spectrum of **I** also resembles that of **V** excepting the chemical shifts of methyl and methine protons at C1. The structure **I** for nanaomycin E was supported by these data and chemical evidence described below.

Conversion of Nanaomycin E into Nanaomycin A and 4a-*epi*-Nanaomycin B

Treatment of **I** with sodium hydrosulfite in 0.1 N aqueous sodium hydroxide gave **III**, which was identical with an authentic sample (IR and optical rotation), in 87% yield. A similar reaction in 0.02 N hydrochloric acid (room temperature, 1.5 hours) gave, after silica gel column chromatography, **II** and **III** in 63% and 35% yields, respectively. Compound **II** was quantitatively converted into **III** under alkaline conditions, and gave a mixture of **III** and nanaomycin B (**IV**), an epimer of **II**, by heating at its melting point of 160°C.

4a-*epi*-Nanaomycin B (**II**) is soluble in methanol, ethanol, acetone and ethyl acetate, and displays a positive ferric chloride test. The Rf value of **II** on silica gel thin-layer chromatography

* The assignments of C5 and C7 are determined according to those of the corresponding carbons of juglone derivatives[6,7].

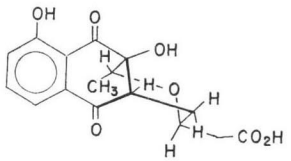
using chloroform - methanol (5: 1) was 0.35. A pale yellow powder of **II** was obtained by crystallization from *n*-hexane - dichloromethane, mp 158~161°C.

Anal. Found: C, 59.76; H, 5.03%.
Calcd. for $C_{16}H_{16}O_7$: C, 60.00; H, 5.04%.

4a-*epi*-Nanaomycin B (**II**) has the following physical properties: $[\alpha]_D^{20}$ −109.1° (*c* 1.08, MeOH); IR (KBr) 3490, 3200 (νOH), 1723 (carboxylic acid νCO), 1691, 1637 cm^{-1} (quinone νCO); UV (MeOH) 233 (log ε 4.35), 248^{sh} (4.04), 267^{sh} (3.70), 353 nm (3.74); ^{13}C NMR ($CDCl_3$–CD_3OD) δ 16.3 (C11), 24.9 (C12), 40.5 (C4), 50.3 (C4a), 64.1 (C3), 76.5 (C1), 77.1 (C9a), 116.7 (C8a), 118.5 (C5), 124.5 (C7), 136.1 (C10a), 137.9 (C6), 162.0 (C8), 173.4 (C13), 194. 2 (C10), 204.4 ppm (C9); ^1H NMR ($CDCl_3$–CD_3OD) δ 0.96 (d, J=7.2 Hz, CH_3), 1.92 (ddd, J=13.2, 10.2, 4.4 Hz, H4β), 2.53 (ddd, J=13.2, 2, 2 Hz, H4α), 2.55 (d, J=6.2 Hz, H12×2), 3.28 (dd, J=4.4, 2 Hz, H4a), 3.96 (q, J=7.2 Hz, H1), 4.27 (m, H3), 7.2~7.8 ppm (m, aromatic protons).

The molecular formula of **II**, $C_{16}H_{16}O_7$, was determined by its mass spectrum (M^+, *m/e* 320) and elemental analysis. The transformation of **II** into **III** through loss of H_2O was also found to occur in the mass spectrometer at 180°C. The UV and ^{13}C NMR spectra of **II** resemble those of **IV**. In its ^1H NMR spectrum, upfield shifts of approximately 0.45 ppm for both of the Cl methyl and methine protons were observed in comparison with those of **IV**. These large differences of the chemical shifts suggest that the methyl and methine protons are affected by the shielding of the aromatic and carbonyl groups. The above data, along with decoupling experiments and a DREIDING model lead to a suggested conformation of **II** as shown in Fig. 1.

Fig. 1. The conformation of 4a-*epi*-nanaomycin B (**II**).



Biological Activity

The antimicrobial spectra of nanaomycin E (**I**) and 4a-*epi*-nanaomycin B (**II**) are given in Table 1. Antimicrobial activities of I are weaker than those of nanaomycin A. The acute toxicities (LD_{50}, i.p.) of **I** and **II** in mice are 60 and 82.5 mg/kg, respectively.

Table 1. Antimicrobial spectra of nanaomycin E (**I**) and 4a-*epi*-nanaomycin B (**II**).

Test organisms	Minimal inhibitory concentration (μg/ml)*	
	I	**II**
Bacillus cereus	12.5	12.5
Staphylococcus aureus FDA 209P	25	0.78
Pseudomonas aeruginosa	>100	100
Candida albicans	>100	25
Trichophyton mentagrophytes	50	1.56

* Estimated using an agar dilution technique.

Discussion

Streptomyces rosa var. *notoensis*, when grown in shake culture in a medium containing glucose instead of glycerol as carbon source, produced a new component, nanaomycin E (**I**). The antibiotic is an epoxy derivative of nanaomycin A (**III**), and is converted into **III** and 4a-*epi*-nanaomycin B (**II**) by treatment with sodium hydrosulfite.

Nanaomycin E (**I**) may be a precursor of nanaomycin B (**IV**).

From the biological point of view, it is of interest that the configuration at all chiral centers of **I** are opposite to those of frenolicin (**V**). This kind of relationship is also found between nanaomycin D and kalafungin[5].

References

1) ŌMURA, S.; H. TANAKA, Y. KOYAMA, R. ŌIWA, M. KATAGIRI, J. AWAYA, T. NAGAI & T. HATA:

Nanaomycins A and B, new antibiotics produced by a strain of *Streptomyces*. J. Antibiotics 27: 363~365, 1974

2) TANAKA, H.; Y. KOYAMA, J. AWAYA, H. MARUMO, R. ŌIWA, M. KATAGIRI, T. NAGAI & S. ŌMURA: Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. I. Taxonomy, isolation, characterization and biological properties. J. Antibiotics 28: 860~867, 1975
3) TANAKA, H.; Y. KOYAMA, T. NAGAI, H. MARUMO & S. ŌMURA: Nanaomycins, new antibiotics by a strain of *Streptomyces*. II. Structure and biosynthesis. J. Antibiotics 28: 868~875, 1975
4) TANAKA, H.; H. MARUMO, T. NAGAI, M. OKADA, K. TANIGUCHI & S. ŌMURA: Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. III. A new component, nanaomycin C, and biological activities of nanaomycin derivatives. J. Antibiotics 28: 925~930, 1975
5) ŌMURA, S.; H. TANAKA, Y. OKADA & H. MARUMO: Isolation and structure of nanaomycin D, an enantiomer of the antibiotic kalafungin. J. Chem. Soc., Chem. Comm. 1976: 320~321, 1976
6) KOBAYASHI, M.; Y. TERUI, K. TORI & N. TSUJI: Carbon-13 NMR spectra of juglone, naphthazarin and their derivatives. Tetrahed. Lett. 1976: 619~620, 1976
7) TSUJI, N.; M. KOBAYASHI, Y. TERUI & K. TORI: The structures of griseusins A and B, new isochromanquinone antibiotics. Tetrahedron 32: 2207~2210, 1976
8) ELLESTAD, G. A.; M. P. KUNSTMANN, H. A. WHALEY & E. L. PATTERSON: The structure of frenolicin. J. Am. Chem. Soc. 90: 1325~1332, 1968