Note

# NANAOMYCINS PRODUCTION BY A FRENOLICIN B PRODUCING STRAIN

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We have reported that *Streptomyces roseofulvus* AM-3867 produced two anti-mycoplasmal antibiotics, antibiotic AM-3867 I (deoxyfrenolicin) as a main component and antibiotic AM-3867 II (frenolicin B) as a minor one[1]. Recently, frenolicin B was found to be highly active against *Eimeria tenella* infection in chicks[2].

In the course of frenolicin B production, two additional minor components, antibiotic AM-3867 III and IV, were isolated from the culture broth of *S. roseofulvus* AM-3867. Antibiotics AM-3867 III and IV were identical with nanaomycins $\beta$A[3] and A[4], respectively. It is the first finding that two types of benzoisochromanequinone antibiotics with different configurations at C-1 and C-3 positions are produced by the same strain. In the present paper, we describe the isolation and identification of the additional components III and IV, and the structure determination of component III (nanaomycin $\beta$A) which has not been reported as yet.

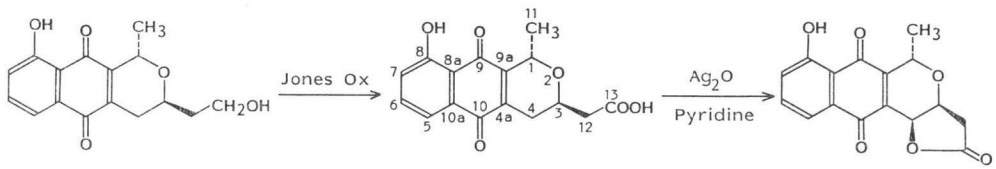
A stock culture of *S. roseofulvus* AM-3867 was inoculated into 100 ml of a seed medium in 500-ml Erlenmeyer flasks and incubated at 25°C on a rotary shaker. A 48-hour culture (1.2 liters) was transferred to 60 liters of the seed medium in a 100-liter fermentor and the culture was cultivated at 27°C for 24 hours with aeration of 50 liters per minute and agitation of 250 rpm. Twenty five liters of the second seed culture was transferred to 500 liters of a production medium in a 1,000-liter fermentor at 27°C for 138 hours with aeration of 500 liters per minutes and agitation of 180 rpm.

The composition of the seed medium was glucose 1.0%, starch 2.0%, yeast extract 0.5%, Polypepton 0.5% and $CaCO_3$ 0.4% (pH 7.0 before sterilization). The production medium consisted of glucose 4.0%, corn steep liquor 3.0%, beet molases 4.0%, yeast extract 1.0%, soybean oil 3.0%, $KH_2PO_4$ 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1% and $Mg(PO_4)_2$ 0.3% (pH 7.5 before sterilization).

The broth filtrate (160 liters) was extracted with EtOAc. The organic layer was concd *in vacuo* to dryness to give an oily material (36.6 g). The crude material was chromatographed on silica gel with a solvent mixture of *n*-hexane and $Me_2CO$ (9 : 1). The four active fractions against *Acholeplasma laidlawii* were collected, concd and crystallized to yield antibiotics AM-3867 II (34 mg), III (15 mg), IV (17 mg) and I (22 g), respectively.

The spectral data (UV, IR, Mass, $^1H$ and $^{13}C$ NMR and optical rotation) of components I, II and IV were consistent with those of the authentic samples of deoxyfrenolicin (**1**), frenolicin B (**2**) and nanaomycin A (**4**).

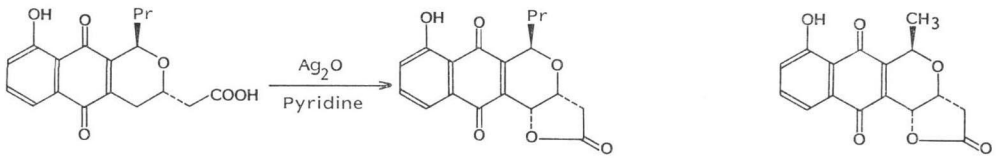
Component III had the following physicochemical properties: $[\alpha]_D^{20}$ −1.2 (*c* 1.0 $CHCl_3$); IR $\nu_{max}$ (KBr) 3350, 1645, 1615 $cm^{-1}$; UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$) 248 (3.70), 273 (3.80), 422 (3.48); $^1H$ NMR ($CDCl_3$) $\delta$ 1.59 (3H, d, $J$=6.6 Hz, $CH_3$), 1.92 (2H, m, H-12), 2.55 (2H, ABX, $J_{AB}$=19.4 Hz, $J_{AX}$=10.1 Hz, $J_{BX}$=3.3 Hz, H-4), 3.87 (2H, t, $J$=5.5 Hz, H-13), 4.11 (1H, m, H-3), 5.03 (1H, br q, $J$=6.6 Hz, H-1), 7.24 (1H, m, H-6), 7.61 (2H, m, H-5 and H-7). The molecular formula, $C_{16}H_{16}O_5$, was assigned to **3** on the basis of its high resolution mass spectrum ($M^+$, 288.0981, calcd 288.0997). Elemental analysis showed the same carbon number as in **4**. The $^{13}C$ NMR spectrum shown in Table 1 resembles that of **4** except for the large upfield shift of 60.4 ppm for C-13 compared with that of **4** (175.7 ppm). In the IR spectrum, no absorption at 1710 $cm^{-1}$ corresponding to the carboxylic acid of **4** was observed. These observations support structure **3** for the component III. To confirm the stereochemistry of the pyrane ring, the alcohol **3** was oxidized by Jones reagent to yield the carboxylic acid, whose spectral data including optical rotation were consistent with those of **4**. Furthermore, treatment




Nanaomycin βA (**3**)
MP 149 ~ 150°C
$[\alpha]_D^{25}$ −1.2° (*c* 1, $CHCl_3$)

Nanaomycin A (**4**)
MP 178 ~ 180°C
$[\alpha]_D^{26}$ −27.5° (*c* 1, MeOH)

Nanaomycin D (**5**)
MP 170 ~ 173°C
$[\alpha]_D^{20}$ −278° (*c* 0.44, MeOH)




Deoxyfrenolicin (**1**)
MP 173 ~ 175°C
$[\alpha]_D^{25}$ +112° (*c* 1, MeOH)

Frenolicin B (**2**)
MP 170 ~ 171°C
$[\alpha]_D^{20}$ +215° (*c* 1, $CHCl_3$)

Kalafungin (**6**)
MP 163 ~ 166°C
$[\alpha]_D^{25}$ +159° (*c* 1, $CHCl_3$)

Table 1. $^{13}C$ Chemical shifts data on nanaomycin A (**4**) and nanaomycin βA (**3**) in $CDCl_3$.

| Carbon No. | 4 | 3 |
|---|---|---|
| 1 | 67.2 | 67.0 |
| 3 | 63.2 | 65.8 |
| 4 | 27.8 | 28.4 |
| 4a | 141.9 | 142.8 |
| 5 | 119.0 | 119.1 |
| 6 | 136.1 | 136.3 |
| 7 | 124.3 | 124.4 |
| 8 | 161.4 | 161.6 |
| 8a | 114.6 | 114.8 |
| 9 | 188.1 | 188.5 |
| 9a | 146.1 | 146.3 |
| 10 | 182.7 | 183.1 |
| 10a | 131.6 | 131.9 |
| 11 | 19.4 | 19.7 |
| 12 | 40.3 | 37.8 |
| 13 | 175.7 | 60.4 |

of the carboxylic acid with $Ag_2O$ in pyridine gave nanaomycin D (**5**).

The results of these transformation clarified unambiguously that the configurations at all chiral centers of **3** and **4** are opposite to those of deoxyfrenolicin (**1**), frenolicin B (**2**) and kalafungin (**6**)[5].

It has been known that benzoisochromanequinone antibiotics produced by *Streptomyces* strains can be classified into two types, kalafungin type and nanaomycin type on the basis of the absolute configulations at C-1 and C-3.

Kalafungin, frenolicin, deoxyfrenolicin, frenolicin B and medermycin belong to kalafungin type which has 1*R* and 3*R* configurations. On the other hand, nanaomycin type, such as nanaomycins, antibiotic OM-173 and griseusins, has 1*S* and 3*S* configurations. Nanaomycin D and kalafungin are enantiomers of each other[6], and are produced by different *Streptomyces* strains, *Streptomyces rosa* var. *notoensis* and *Streptomyces tanashiensis* Kala, respectively.

The present paper shows that *S. roseofulvus* AM-3867 produces two types of benzoisochromanequinone antibiotics. To our knowledge, this is the first report that two types of benzoisochromanequinone antibiotics are produced by single strain.

## References

1) Iwai, Y.; A. Kōra, Y. Takahashi, T. Hayashi, J. Awaya, R. Masuma, R. Ōiwa & S. Ōmura: Production of deoxyfrenolicin and a new antibiotic, frenolicin B by *Streptomyces roseofulvus* strain AM-3867. J. Antibiotics 31: 959~965, 1978

2) Ōmura, S.; K. Tsuzuki, Y. Iwai, M. Kishi, S. Watanabe & H. Shimizu: Anticoccidial activity of frenolicin B and its derivatives. J. Antibiotics 38: 1447~1448, 1985

3) Iwai, Y.; K. Kimura, Y. Takahashi, K. Hinotozawa, H. Shimizu, H. Tanaka & S. Ōmura: OM-173, new nanaomycin-type antibiotics produced by a strain of *Streptomyces*. Taxonomy,

production, isolation and biological properties. J. Antibiotics 36: 1268~1274, 1983

4) 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

5) HOEKSEMA, H. & W. C. KRUEGER: Kalafungin. II. Chemical transformations and the absolute configuration. J. Antibiotics 29: 704~709, 1976

6) ŌMURA, S.; H. TANAKA, Y. OKADA & H. MARUMO: Isolation and structure of nanaomycin D, an enantiomer of the antibiotic kalafungin. J. Chem. Soc. Chem. Commun. 1976: 320~321, 1976