NANAOMYCINS PRODUCTION BY
A FRENOLICIN B PRODUCING
STRAIN

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We have reported that Streptomyces roseofulvus
AM-3867 produced two anti-mycoplasmal an-
tibiotics, antibiotic AM-38671 (deoxyfrenolicin)
as a main component and antibiotic AM-3867
II (frenolicin B) as a minor one3). Recently,
frenolicin B was found to be highly active against
Eimeria tenella infection in chicks2).

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 nanao-
mycins III4) and A4), respectively. It is the first
finding that two types of benzoisochromane-
quinone antibiotics with different configurations
at C-1 and C-3 positions are produced by the
same strain. In the present paper, we describc
the isolation and identification of the additional
components III and IV, and the structure deter-
mination of component III (nanaomycin βA)
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
broth (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 50 liters per minutes and agita-
tion of 180 rpm.

The composition of the seed medium was
glucose 1.0%, starch 2.0%, yeast extract 0.5%,
Polypepton 0.5% and CaCO3 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%, KH2PO4 0.2%, MgSO4·7H2O 0.1% and
Mg(P04)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 Me2CO
(9:1). The four active fractions against Achole-
plasma laidlawii were collected, concd and crystal-
lized 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 13C
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 physico-
chemical properties: [α]D25 = -1.2 (c 1.0 CHCl3);
IR νmax (KBr) 3350, 1645, 1615 cm⁻¹; UV λmax
nm (log ε) 248 (3.70), 273 (3.80), 422 (3.48); 1H
NMR (CDCl3) δ 1.59 (3H, d, J = 6.6 Hz, CH3),
1.92 (2H, m, H-12), 2.55 (2H, ABX, JAB=19.4
Hz, JAx=10.1 Hz, Jbx=3.3 Hz), 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-11), 7.24 (1H, m, H-6), 7.61
(2H, m, H-5 and H-7). The molecular formula,
C16H1605, 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 13C NMR
spectrum shown in Table 1 resembles that of 4
except for the large upfield shift of 60.4 ppm
corresponding to the carboxylic acid of 4 was obser-
vied. 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 con-
sistent with those of 4. Furthermore, treatment
of the carboxylic acid with \( \text{Ag}_2\text{O} \) 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 kala-fungin (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 configurations 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\(^5\), and are produced by different *Streptomyces* strains, *Streptomyces roseofulvus* 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


3) IWAI, Y.; K. KIMURA, Y. TAKAHASHI, K. HINOTOZAWA, H. SHIMIZU, T. 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

