PRODUCTION OF ENANTIOMER OF NANAOMYCIN A BY *NOCARDIA*

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(Received for publication March 3, 1989)

Nanaomycins (NNM's)[1,2], kalafungin[3], OM-173[4] and frenolicins[5,6] are members of a family of benzoisochromanquinone antibiotics. These antibiotics exhibit significant antimicrobial activities. Among these antibiotics, NNM-D and kalafungin are enantiomers with each other[7]. The relationship of NNM-A and (+) NNM-A corresponds to that of NNM-D and kalafungin. NNM-A, NNM-D and kalafungin have been isolated from natural sources. (+) NNM-A has been prepared by enantiodivergent total syntheses[8] but has not been reported as a natural product to date. We now report the isolation of (+) NNM-A from a fermentation broth of *Nocardia* species.

The producing organism, YS-02931K, was isolated from a soil sample collected at Kohama Island, Okinawa Prefecture, Japan. The strain cultivated on the standard International Streptomyces Project (ISP) media produces substrate mycelia which slightly show fragmentation after several days. It forms no aerial mycelium. Sporangia, screlotia and motile elements are not found. This strain grows well at 24 and 45°C. Melanoid pigment, amylase and gelatinase are produced. On the basis of chemo-taxonomic studies, the cell wall hydrolysates of this strain contains *meso*-diaminopimeric acid, galactose and arabinose. Thus, it appears that the cell wall of this strain is type IV A[9]. The morphological and physiological studies as well as chemo-taxonomic studies suggest the genus *Nocardia* as the possible genus of this organism. Definitive taxonomic studies are in progress and will be published later.

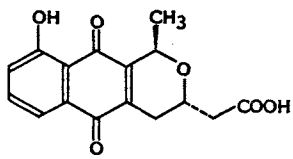
A stock culture of strain YS-02931K was inoculated into 500-ml Erlenmeyer flasks containing 60 ml of a seed medium consisting of dextrin 2.0%, glucose 0.5%, Polypepton 0.5%, yeast extract 0.5%, corn steep liquor 0.5%, brain heart infusion 0.52% and $CaCO_3$ 0.2% (pH 8.0 before sterilization). After incubation at 27°C for 72 hours on a rotary shaker, the seed culture (750 ml) was transferred to 25 liters of the production medium in a 30-liter jar fermenter and the culture was cultivated at 27°C for 72 hours with aeration of 20 liters/minute and agitation of 100 rpm. The composition of a production medium was potato starch 3.0%, soybean meal 1.5%, corn steep liquor 0.5%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.3% and $CoCl_2 \cdot 6H_2O$ 0.002% (pH 7.1 before sterilization). The production of antibiotic 2931-α and β was monitored by TLC using Silica gel 60 F_{254} plates (Merck, Art. No. 5715) with $CHCl_3$ - MeOH (7:1). The antibiotics were detected by bioautography using *Bacillus subtilis* ATCC 6633 as a test organism.

The culture filtrate of strain YS-02931K was adjusted to pH 3 and extracted with EtOAc. The organic layer was extracted with 1% sodium bicarbonate solution and the aqueous layer was reextracted with EtOAc at pH 3. The organic layer was concentrated *in vacuo* to give an oily material (1 g). The crude material was chromatographed on a column of silica gel with $CHCl_3$ - MeOH (100:1). Two main active peaks were eluted separately from the column. The fractions of the first active peak were combined and concentrated to yield 2931-α (40 mg). The fractions of the second active peak were combined and concentrated to give a crude powder of 2931-β (50 mg). The crude powder was purified by preparative TLC using $CHCl_3$ - MeOH (7:1). Antibiotic 2931-β was isolated as a yellow powder (30 mg) in pure form.

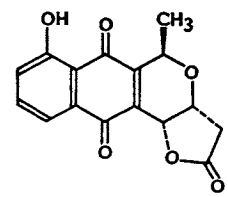
The spectral data (UV, IR, mass, ^1H and ^{13}C NMR and optical rotation) of 2931-α was consistent with those of the authentic sample of kalafungin.

Antibiotic 2931-β showed the following physico-chemical properties: UV λ_{max}^{MeOH} nm (ε) 250 (9,800), 274 (12,000), 423 (4,050); IR ν_{max}^{KBr} cm^{-1} 1710, 1645, 1615; ^1H NMR (100 MHz, $CDCl_3$) δ 1.59 (3H, d, J=6.8 Hz), 2.35 (1H, ddd, J=2.0, 10.1 and 19.3 Hz), 2.72 (2H, d, J=6.3 Hz), 2.87 (1H, dd, J=3.1 and 19.3 Hz), 4.34 (1H, m), 5.05 (1H, dq, J=6.8 and 2.0 Hz), 7.15~7.75 (3H, m), 9.20 (1H, br), 11.99 (1H, s); ^{13}C NMR (25.1 MHz, $CDCl_3$) δ 19.3, 27.8, 40.3, 63.3, 67.4, 114.6, 119.1, 124.4, 131.5, 136.1, 141.9, 146.1, 161.4, 175.6, 182.7, 188.1; electron impact MS 302

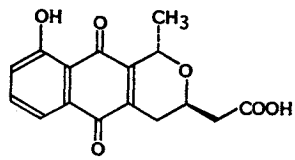
Fig. 1. Structures of (+) NNM-A and related antibiotics.



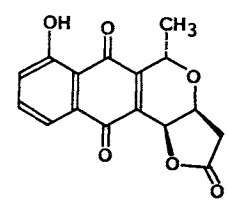
(+) NNM-A



Kalafungin



NNM-A



NNM-D

(M^+); *Anal* Found: C 63.39, H 4.48, N 0, Calcd for $C_{16}H_{14}O_6$: C 63.57, H 4.66. These properties coincided with those of NNM-A, but the direction of the specific rotation of 2931-β, $[\alpha]_D^{25}$ +26° (*c* 1.0, MeOH), was opposite to that of NNM-A, $[\alpha]_D^{26}$ −27.5° (*c* 1.0, MeOH).

To confirm the stereochemistry of the pyran ring, treatment of 2931-β with Ag_2O in pyridine gave kalafungin. The result of this transformation shows that the configurations at all chiral centers of the antibiotic is opposite to those of NNM-A. The antibiotic has the 1*R* and 3*R* configurations, like kalafungin, and was identified as (+) NNM-A (Fig. 1).

(+) NNM-A exhibits antimicrobial activity against mainly fungi and Gram-positive bacteria. The antimicrobial spectrum of (+) NNM-A is similar to that of NNM-A.

Studies of the biosynthetic relationship of the NNMs using a bioconversion method with cerulenin have revealed that the biosynthetic sequence is: NNM-D→NNM-A→NNM-E→NNM-B[10]. We speculate that kalafungin is a direct precursor of (+) NNM-A.

Acknowledgments

We thank Prof. SATOSHI ŌMURA, Kitasato University, and The Kitasato Institute for a gift of nanaomycin A. We are grateful to the staff of the Physico-analysis Center for measurement of spectra and useful suggestions.

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