

## A New Antimicrobial Antibiotic from *Actinoplanes capillaceus* sp. K95-5561<sup>T</sup>

AKIKO FUKAMI, TOMONORI NAKAMURA,  
KANAKO KAWAGUCHI, MUN-CHUAL RHO,  
ATSUKO MATSUMOTO, YOKO TAKAHASHI,  
KAZURO SHIOMI, MASAHIKO HAYASHI,  
KANKI KOMIYAMA and SATOSHI ŌMURA\*

The Kitasato Institute,  
5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

(Received for publication March 24, 2000)

In our ongoing search for novel biologically active metabolites from rare actinomycetes, an antibiotic 2-hydroxyethyl-3-methyl-1,4-naphthoquinone (**1**, Fig. 1) was isolated from a new species named *Actinoplanes capillaceus*. This is the first report that **1** was isolated from natural source. Herein, we report the fermentation, isolation and biological activity of **1**.

### Materials and Methods

#### A Medium of Culturing K95-5561<sup>T</sup>

The culture medium contained 2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract and 0.4% CaCO<sub>3</sub> (pH 7.0 before sterilization).

#### Spectroscopic Studies

UV spectrum was recorded on a Hitachi U-2000 spectrophotometer. IR spectrum was recorded on a Horiba FT-210 FT-IR spectrometer. FAB-MS spectra were recorded on JMS-DX300 and JMS-AX505 HA mass spectrometers. NMR spectra were obtained on a Varian UNITY INOVA 600 MHz NMR Spectrometer Systems.

#### Antimicrobial Activity<sup>1)</sup>

Antimicrobial activity was tested using 14 species of microorganisms. Six mm paper discs containing 10 μg of sample were placed upon seeded agar plates which were incubated for 24~48 hours at 27°C or 37°C. Then antimicrobial activity was determined by the diameter of inhibitory zone.

### Results and Discussion

A motile actinomycete strain K95-5561<sup>T</sup> was isolated from a soil sample collected in Sayama City, Saitama, Japan by the chemotactic methods using xylose<sup>2)</sup>. The strain produced bell shaped sporangia with hairy surfaces, which released motile spores. It contained *meso*- and 3-hydroxy-DAP, galactose, arabinose and xylose in the whole-cell hydrolysates, and contained MK-9(H<sub>4</sub>) as a predominant menaquinone, and MK-9(H<sub>6</sub>) and MK-10(H<sub>4</sub>) as minor components. Based on these taxonomic properties, strain K95-5561<sup>T</sup> was considered to belong to the genus *Actinoplanes*, and was classified as a new species named *Actinoplanes capillaceus*. This strain has been deposited at Japan Collection of Microorganisms and the Institute for Fermentation, Osaka under accession number 10268<sup>T</sup> and 16408<sup>T</sup>, respectively. Detailed taxonomic study of the strain K95-5561<sup>T</sup> will be reported elsewhere<sup>3)</sup>.

A stock culture of the producing organism was inoculated into two 500-ml Erlenmeyer flasks containing 100 ml of culturing medium. The flasks were incubated at 27°C for 72 hours on a rotary shaker (210 rpm). Then, 5 ml portions of the growth were transferred into thirty 500-ml Erlenmeyer flasks containing 100 ml of the same medium. The flasks were incubated at 27°C for 72 hours on a rotary shaker, and 3 liters of the resulting culture was transferred into a 90-liter jar fermentor containing 60 liters of the same medium as described above. The fermentation was carried out at 27°C for 6 days using an agitation rate of 210 rpm and an aeration rate of 60 liters per minute.

The fermentation broth of *Actinoplanes capillaceus* K95-5561<sup>T</sup> (60 liters) was extracted with the same volume of EtOAc, and the EtOAc layer was dried up *in vacuo* to yield a brown oil (7.4 g). This brown oil was subjected to a silica

Fig. 1. Structure of **1**.

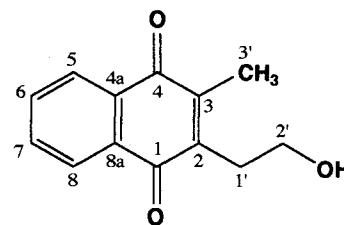


Table 1. Physico-chemical properties of **1**.

Appearance	Orange-yellow plate
Melting point (°C)	115-118
Molecular formula	C <sub>13</sub> H <sub>12</sub> O <sub>3</sub>
Molecular weight	217 (M+H) <sup>+</sup> , 239 (M+Na) <sup>+</sup>
HR FAB-MS (m/z)	Found 217.0878 (C <sub>13</sub> H <sub>13</sub> O <sub>3</sub> ) Calcd. 217.0865
UV λ <sub>max</sub> nm (log ε) (MeOH)	205 (4.05), 246 (4.13), 263 (4.06), 333 (3.38)
IR ν <sub>max</sub> (MeOH) cm <sup>-1</sup>	3321, 1660
<sup>1</sup> H NMR (600 MHz) (δ from TMS in CDCl <sub>3</sub> )	δ 8.09 (2H, m, 5-H, 6-H), 7.71 (2H, m, 6-H, 7-H), 3.85 (2H, t, J=6.3, 2-H <sub>2</sub> ), 2.97 (2H, t, J=6.3, 1'- H <sub>2</sub> ), 2.25 (3H, s, 3'-H <sub>3</sub> )
<sup>13</sup> C NMR (125 MHz) (δ from TMS in CDCl <sub>3</sub> )	δ 185.51 (C-1), 184.99 (C-4), 145.15 (C-3), 143.82 (C-2), 133.55 (C-7), 133.44 (C-6), 132.18 (C-8a), 132.03 (C-4a), 126.36 (C-8), 126.34 (C-5), 61.55 (C-2'), 30.62 (C-1'), 12.96 (C-3')

Table 2. Antimicrobial spectrum of **1**.

Test organisms	Diameter of inhibition zone (mm)
<i>Bacillus subtilis</i>	11
<i>Staphylococcus aureus</i>	-
<i>Micrococcus luteus</i>	-
<i>Mycobacterium smegmatis</i>	-
<i>Escherichia coli</i>	12
<i>Pseudomonas aeruginosa</i>	-
<i>Xanthomonas campestris</i> pv. <i>oryzae</i>	-
<i>Bacteroides fragilis</i>	-
<i>Acholeplasma laidlawii</i>	-
<i>Pyricularia oryzae</i>	-
<i>Aspergillus niger</i>	-
<i>Mucor racemosus</i>	-
<i>Candida albicans</i>	-
<i>Saccharomyces cerevisiae</i>	12

Samples (10 μg) were applied on 6 mm paper discs. Values are diameters (mm) of inhibitory zones.

gel column (70~240 mesh) using CHCl<sub>3</sub>-acetone-EtOAc as the developing solvent. The fractions eluted with CHCl<sub>3</sub>-acetone (9:1) were rechromatographed on a silica gel column (230~400 mesh) with CHCl<sub>3</sub>-MeOH (100:1). Final isolation was performed using a preparative HPLC (Senshu Pak Pegasil-B ODS, i.d. 20×250 mm; detection, UV at 210 nm; flow rate, 7 ml/minute; solvent system,

CH<sub>3</sub>CN-H<sub>2</sub>O, 40:60 v/v) to afford pure orange-yellow plates of **1** (3.0 mg).

Physico-chemical properties of **1** are summarized in Table 1. These data were well consistent with that of synthetic compound, 2-hydroxyethyl-3-methyl-1,4-naphthoquinone, a intramolecular benzannulation product of siloxycarbene complex<sup>3</sup>. This is the first report that **1**

was produced by a microorganism. To date no biological activities has been reported for **1**.

Some naphthoquinone derivatives possess antimicrobial activities to Gram-positive bacteria, such as deoxylapachol<sup>4)</sup> and alnumycin<sup>5)</sup>. Compound **1** showed antimicrobial activity on *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae* as shown in Table 2. Detailed investigations on other biological activities are now underway.

#### Acknowledgments

This study was supported in part by a Grant-in Aid from the Ministry of Education, Science, Sports and Culture, government of Japan and funds from the Japan Keirin Association.

#### References

- 1) YAMADA, H.; K. SHIOMI, Q. XU, T. NAGAI, M. SHIBATA, I. OYA, Y. TAKAHASHI & S. ŌMURA: New glycosidase inhibitors, panosialins D and wD produced by *Streptomyces* sp. OH-5186. *J. Antibiotics* 48: 205~210, 1995
- 2) HAYAKAWA, M.; T. TAMURA & H. NOMURA: Selective isolation of *Actinoplanes* and *Dactylosporangium* from soil by using  $\gamma$ -collidine as the chemoattractant. *J. Ferment. Bioeng.* 72: 426~432, 1992
- 3) MATSUMOTO, A.; Y. TAKAHASHI, T. KUDO, A. SEINO, Y. IWAI & S. OMURA: *Actinoplanes capillanceus* sp. nov., a new species of the genus *Actinoplanes*. *Antonie van Leeuwenhoek*, submitted
- 4) GROSS, M. F. & M. G. FINN: Intramolecular benzannulation of chromium siloxycarbene complexes: regiochemical control and the "xenochemical effect" of alkyne additives. *J. Am. Chem. Soc.* 116: 10921~10933, 1994
- 5) PERRY, N. B.; J. W. BLUNT & M. H. G. MUNRO: A cytotoxic and antifungal 1,4-naphthoquinone and related compound from a New Zealand brown alga, *Landsburgia quercifolia*. *J. Natl. Prod.* 54: 978~985, 1991
- 6) BIEBER, B.; J. NÜSKE, M. RITZAU & U. GRÄFE: Alnumycin a new naphthoquinone antibiotic produced by an endophytic *Streptomyces* sp. *J. Antibiotics* 51: 381~382, 1998