# 3'-O-α-D-FOROSAMINYL-(+)-GRISEUSIN A FROM *Streptomyces griseus*

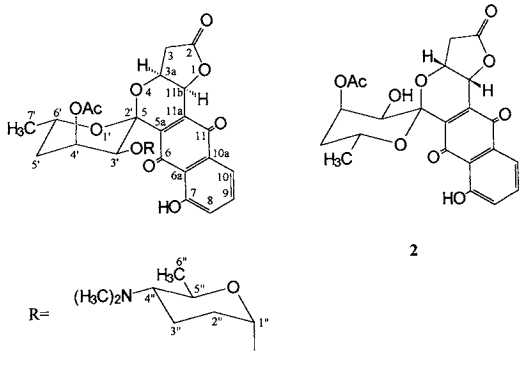
Sir:

In the course of our screening for new antibiotics, we have isolated a new member of naphthoquinone antibiotics (**1**, Fig. 1), from the culture broth of the strain MJ361-48F3. The antibiotic is active against Gram-positive bacteria including methicillin-resistant strain of *Staphylococcus aureus* (MRSA). In this communication, we report the production, isolation, physico-chemical properties, structure elucidation and biological properties of **1**.

The producing microorganism was isolated from a soil sample collected at Nemuro-shi, Hokkaido, Japan, which was classified as *Streptomyces griseus*. The slant culture of the producing organism was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of seed medium consisting of glycerol 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, yeast extract (Wako Chem.) 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The seed culture was carried out at 27°C for 2 days on a rotary shaker. Two ml of the seed culture was transferred to a 500-ml Sakaguchi flask containing 125 ml of a producing medium which was composed of glycerol 2.0%, dextrin 1.0%, Bacto-Soytone 0.5%, yeast extract 0.25%, $(NH_4)_2SO_4$ 0.1% and $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The fermentation was carried out at 27°C for 2 days.

The antibiotic was extracted from the broth filtrate with BuOAc at pH 7.8. The active substance was transferred to acidic water (pH 2.0) and re-extracted with BuOAc at pH 7.0. Its further purification was performed by using Sephadex LH-20 column, silica gel column and centrifugal partition chromatography as shown in Fig. 2. The active substance was monitored by antibacterial activity against *Bacillus stearothermophilus* during the purification process.

Fig. 1. Structures of compound **1** and griseusin A (**2**).




1 2

Physico-chemical properties of **1** are summarized in Table 1. Compound **1** was obtained as an orange powder. The molecular formula was established as $C_{30}H_{35}NO_{11}$ by HRFAB-MS (Found: *m/z* 586.2297 $(M+H)^+$, Calcd for $C_{30}H_{36}NO_{11}$: *m/z* 586.2288) and NMR spectral analysis. The UV-VIS spectra suggested the presence of 5-hydroxy-1,4-naphthoquinone chromophore. The IR spectrum of **1** showed absorption bands attributed to γ-lactone (1798 $cm^{-1}$), ester (1738 $cm^{-1}$), quinone (1669 $cm^{-1}$) and hydrogen-bonded quinone carbonyl (1649 $cm^{-1}$). The IR and UV-VIS spectra suggested that compound **1** was related to naphthoquinone antibiotics having γ-lactone moiety such as lactoquinomycin[1], kalafungin[2] and griseusin A[3].

In the $^1H$ NMR spectrum, *N,N*-dimethyl protons (δ 1.92, 6H, s), C-methyl protons (δ 1.24, 3H, d and δ 0.58, 3H, d), acetyl methyl protons (δ 2.08, 3H, s) and an anomeric proton (δ 4.82, 1H, br s) were observed. The presence of two oxygens-bearing

Fig. 2. Isolation of compound **1**.

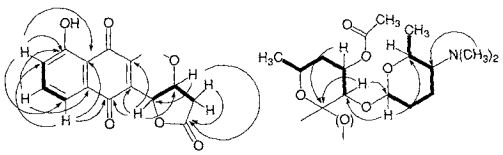
Broth filtrate (pH 7.8, 28.4 liters)
↓
BuOAc extract (pH 7, 14.2 liters)
↓
Water extract (pH 2, 7.0 liters)
↓
BuOAc extract (pH 7, 3.5 liters)
↓
Crude oil (322 mg)
↓
Sephadex LH-20 column chromatography (100 ml)
eluted with EtOAc - MeOH (4 : 1)
↓
Centrifugal partition chromatography
hexane - acetonitrile (1 : 1)
↓
Silica gel column chromatography (20 ml)
eluted with $CHCl_3$ - MeOH (50 : 1)
↓
Centrifugal partition chromatography
EtOAc - $H_2O$ - AcOH (500 : 3000 : 1)
↓
EtOAc extract (pH 7)
↓
Compound **1** (70.0 mg, yield 49.3%)

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

| | |
|---|---|
| Appearance | Orange powder |
| MP | 135~140°C (dec) |
| $[\alpha]_D^{24}$ | +190° (*c* 0.21, $CHCl_3$) |
| Molecular formula | $C_{30}H_{35}NO_{11}$ |
| FAB-MS (*m/z*) | 586 $(M+H)^+$ |
| HRFAB-MS (*m/z*) | |
| Found: | 586.2297 |
| Calcd for $C_{30}H_{36}NO_{11}$: | 586.2288 |
| UV $\lambda_{max}$ nm (ε) | |
| MeOH | 213 (32,000), 246 (9,660), 434 (3,300) |
| 0.1 M HCl-MeOH | 213 (31,600), 246 (9,650), 434 (3,300) |
| 0.1 M NaOH-MeOH | 215 (24,900), 276 (6,480), 451 (2,190), 556 (2,680) |
| IR $\nu_{max}$ (KBr) $cm^{-1}$ | 3438, 2970, 2934, 2874, 1798, 1738, 1669, 1649, 1620, 1456, 1364, 1285, 1255, 1229, 1200, 1173, 1140, 1100, 1078, 1064, 1055, 1035, 1019, 993, 982 |
| Rf value on silica gel TLC | 0.66 ($CHCl_3$-MeOH, 10:1, Art5715, Merck) |
| High voltage paper electrophoresis[a] (Rm) | 0.62 (Ala 1.00) |
| Solubility | Soluble: Acidic water, MeOH, acetone, EtOAc, $CHCl_3$<br>Insoluble: Basic water, *n*-hexane |

[a] Formic acid-AcOH-$H_2O$ (1:3:36), 3,000 V, 20 minutes.

Fig. 3. Partial structures of **1**.



— $^1H$-$^1H$ COSY
⌒ HMBC

quarternary carbon, $>C<^{O}_{O}$, was indicated by the $^{13}C$ signal at $\delta_C$ 96.1 in the $^{13}C$ NMR and DEPT spectra. The analyses of the $^1H$-$^1H$ COSY and HMBC spectra of **1** revealed the two partial structures (Fig. 3), which resembled to those of griseusin A except for the presence of a terminal deoxysugar moiety. The NMR data of the terminal deoxysugar moiety of **1** in Table 2 is consistent with that of forosamine which is contained in spiramycins[4].

The acid hydrolysis of **1** with a sulfonic acid resin, Amberlist 15 (Rohm and Haas), in 0.01 N HCl (50°C, 5 hours) gave two compounds, an aglycone [FAB-MS; *m/z* 444 $(M-H)^-$] and the deoxysugar [FAB-MS; *m/z* 159 $(M+H)^+$]. The aglycone moiety obtained from the acid hydrolysate of **1** was identified as griseusin A (**2**) by comparison of their $^1H$ NMR spectra, mass spectra and Rf values on a silica gel TLC (Art5715, Merck, $CHCl_3$-MeOH, 10:1, Rf 0.87). The deoxysugar was identified as forosamine by comparison of their $^{13}C$ NMR

Table 2. NMR data for compound **1** in $CDCl_3$.

| Position No. | $\delta_C$ (100 MHz) | $\delta_H$[a] (500 MHz) |
|---|---|---|
| 2 | 173.8 | |
| 3 | 37.1 | 2.71 (1H, d, 17.1),<br>2.98 (1H, dd, 4.6, 17.1) |
| 3a | 65.7 | 4.72 (1H, dd, 2.4, 4.3) |
| 5, 2′ | 96.1 | — |
| 5a | 143.7 | — |
| 6 | 187.2 | — |
| 6a | 115.3 | — |
| 7 | 162.3 | — |
| 8 | 125.6 | 7.32 (1H, dd, 1.8, 7.9) |
| 9 | 137.1 | 7.65 (1H, t, 7.9) |
| 10 | 119.8 | 7.68 (1H, dd, 1.8, 7.9) |
| 10a | 131.0 | — |
| 11 | 181.8 | — |
| 11a | 138.2 | — |
| 11b | 68.2 | 5.26 (1H, d, 2.7) |
| 3′ | 69.7 | 4.90 (1H, d, 3.6) |
| 4′ | 64.6 | 5.59 (1H, q, 3.6) |
| 4′-OCOC*H*$_3$ | 21.1 | 2.08 (3H, s) |
| 4′-O*C*OCH$_3$ | 170.4 | — |
| 5′ | 36.0 | ~1.91 (2H, m) |
| 6′ | 63.0 | 4.37 (1H, m) |
| 7′ | 20.6 | 1.24 (3H, d, 6.4) |
| 1″ | 93.7 | 4.82 (1H, br s) |
| 2″ | 29.7 | 1.47 (1H, m),<br>1.61 (1H, m) |
| 3″ | 13.6 | 1.47 (2H, m) |
| 4″ | 65.7 | 1.95 (1H, m) |
| 4″-N(CH$_3$)$_2$ | 40.3 | 1.92 (6H, s) |
| 5″ | 68.0 | 3.19 (1H, dq, 6.4, 9.8) |
| 6″ | 18.2 | 0.58 (3H, d, 6.4) |

[a] $\delta$ ppm from TMS (integration, multiplicity, *J* value in Hz).

Table 3. Antimicrobial activities of **1** on Mueller-Hinton agar.

| Test organism | MIC (μg/ml) |
|---|---|
| *Staphylococcus aureus* Smith | 0.39 |
| *S. aureus* No. 5 (MRSA) | 0.78 |
| *S. aureus* No. 17 (MRSA) | 1.56 |
| *Micrococcus luteus* PCI 1001 | 0.39 |
| *Bacillus subtilis* PCI 219 | 0.39 |
| *B. stearotherophilus* IFO 13737[a] | 1.56 |
| *Corynebacterium bovis* 1810 | 0.78 |
| *Escherichia coli* K-12 | >100 |
| *Shigella dysenteriae* JS11910 | 25 |
| *Salmonella typhi* T-63 | 100 |
| *Proteus vulgaris* OX19 | 100 |
| *Serratia marcescens* | >100 |
| *Pseudomonas aeruginosa* A3 | 100 |
| *Klebsiella pneumoniae* PCI 602 | 100 |
| *Mycobacterium smegmatis* ATCC 607 | >100 |
| *Candida albicans* 3147 | >100 |

[a] 50°C, 18 hours.

spectral data[5] and Rf values on silica gel TLC.

In the HMBC spectrum of **1**, cross peaks between 3′-H and C-1″, and between 1″-H and C-3′ were observed. In the $^1$H NMR spectrum, the multiplicity of 1″-H was a broad singlet indicating an equatorial proton. These data revealed that C-1″ of forosamine was linked to C-3′ of the aglycone through an α-glycosidic bond.

The optical rotation and CD spectrum* of the aglycone were opposite to those of griseusin A [aglycone of **1**, $[\alpha]_D^{23}$ +140° (*c* 0.11, $CHCl_3$), CD (MeOH) $\theta_{290}$ −7600, $\theta_{350}$ 0, $\theta_{390}$ +1100, $\theta_{460}$ +2500; griseusin **A** (**2**), $[\alpha]_D^{23}$ −140° (*c* 0.17, $CHCl_3$), CD (MeOH) $\theta_{290}$ +5700, $\theta_{350}$ 0, $\theta_{390}$ −1400, $\theta_{460}$ −2600]. Therefore, the aglycone was the antipode of griseusin A. By comparison of the optical rotational value of the deoxysugar $[[\alpha]_D^{23}$ +69° (*c* 0.12, MeOH)] with literature data[4] $[[\alpha]_D^{23}$ +83.9° (*c* 1.0, MeOH)], the sugar was found to be the D-series.

From the above mentioned results, the structure of **1** was proposed to be 3′-*O*-α-D-forosaminyl-(+)-griseusin A.

Antimicrobial activities of **1** are shown in Table 3. Compound **1** showed antimicrobial activities against Gram-positive bacteria including MRSA. The MICs of **1** were 0.39~1.56 μg/ml. The acute toxicity of **1** ($LD_{50}$ in mice) was 12.5 mg/kg with iv administration.

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* CD spectra were measured on a JASCO J-20 automatic recording spectropolarimeter.