A NEW PIERICIDIN RHAMNOSIDE, 3'-RHAMNOPIERICIDIN A_1

Sir:

In the course of our screening program for new antibiotics from microorganisms, we have isolated a new piericidin group antibiotic named 3'-rhamnopiericidin A_1 (SN-198-C) from the culture broth of *Streptomyces* sp. SN-198. We report here the isolation, structure, and biological properties of this antibiotic.

The organism was isolated from a soil sample collected in Ishibashi-machi, Tochigi Prefecture, Japan and taxonomic studies indicated that it belonged to the genus *Streptomyces*. The strain was cultured in a 30-liter jar fermenter at 27°C in 18 liters of medium (glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, NaCl 0.2%, and K_2HPO_4 0.005%). The fermentation broth was filtered and the mycelium was extracted with 80% acetone. After removal of acetone, the extract was combined with the culture filtrate and the mixture was extracted with ethyl acetate. After drying over Na_2SO_4 (anhydrous), the ethyl acetate extract was concentrated *in vacuo* to an oily residue (27.3 g). The oily residue was chromatographed on a silica gel column (3 × 40 cm) with chloroform - methanol (100 : 0→98 : 2→96 : 4→94 : 6). The major compound produced by SN-198 was piericidin A_1 (SN-198-E)[1,2] which was eluted with chloroform - methanol (98 : 2) before 3'-rhamnopiericidin A_1. 3'-Rhamnopiericidin A_1 fraction (94 : 6) was concentrated *in vacuo* to dryness (280 mg). After dissolving in a small amount of methanol, it was loaded onto a Sephadex LH-20 column (2.7 × 86 cm) using methanol as the eluent. Final purification was carried out by preparative HPLC using Nucleosil $5C_{18}$ (20 × 250 mm) with 85% methanol. After concentration and lyophilization, it gave 3'-rhamnopiericidin A_1 (12 mg) as a white powder.

3'-Rhamnopiericidin A_1 is a white amorphous powder which gradually changes to an oily substance above 82°C: $[\alpha]_D^{25}$ −44.0° (*c* 0.1, MeOH); molecular formula $C_{31}H_{47}NO_8$; FAB-MS *m/z* 562 $(M+H)^+$, 398 $((M-164)+H)^+$; elementary analysis, *Anal* Calcd for $C_{31}H_{47}NO_8 \cdot \frac{1}{2}H_2O$: C 65.26, H 8.42, N 2.46. Found: C 64.82, H 8.30, N 2.40. UV λ_{max}^{MeOH} nm (ε) 231 (39,300), 237 (37,700), 276 (7,200); IR ν_{max} (KBr) cm^{-1} 3420, 2930, 1580, 1470, 1400, 1120, 1060, 970.

3'-Rhamnopiericidin A_1 is soluble in methanol, ethanol, acetone, ethyl acetate, chloroform, but not soluble in water and *n*-hexane. The UV spectrum of 3'-rhamnopiericidin A_1 was very similar to that of piericidin A_1. Determination of the structure of 3'-rhamnopiericidin A_1 was achieved by comparison of the ^1H and ^{13}C NMR spectra with those of piericidin A_1. The ^1H NMR spectrum of 3'-rhamnopiericidin A_1 is shown in Fig. 1. It is very

Fig. 1. ^1H NMR spectrum of 3'-rhamnopiericidin A_1 ($CDCl_3$, 500 MHz).

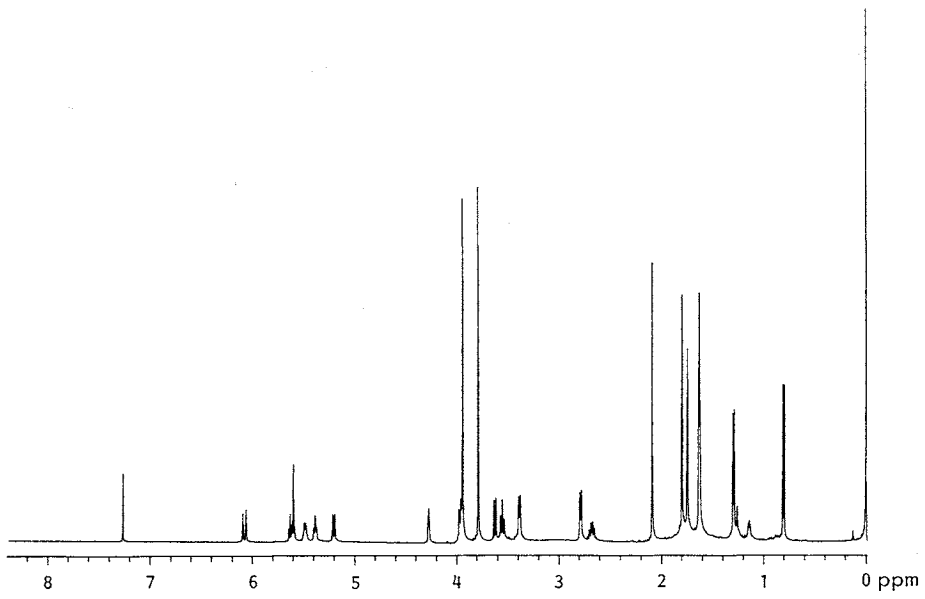


Table 1. Assignments of ^{13}C NMR spectra of piericidin A_1 and 3′-rhamnopiericidin A_1 (22.5 MHz in $CDCl_3$).

	Piericidin A_1 (SN-198-E)[a]	3′-Rhamnopiericidin A_1 (SN-198-C)[b]
C-1	34.3	34.7 t
C-2	122.2	122.1 d
C-3	134.6	135.0 s
C-4	43.0	43.0 t
C-5	126.6	126.6 d
C-6	135.7	135.7 d
C-7	135.7	135.0 s
C-8	133.0	133.2 d
C-9	36.9	36.9 d
C-10	82.7	82.8 d
C-11	135.8	136.0 s
C-12	123.2	123.5 d
C-13	13.0	13.1 q
C-14	10.5	10.6 q
C-15	17.3	17.4 q
C-16	16.5	16.6 q
C-17	13.0	13.2 q
C-1′	150.7	151.1 s
C-2′	111.9	117.4 s
C-3′	154.0	155.8 s
C-4′	127.8	133.2 s
C-5′	153.5	154.7 s
C-6′	10.4	10.6 q
C-7′	60.4	60.5 q
C-8′	52.9	53.3 q
C-1″	—	101.8 d
C-2″	—	70.9 d
C-3″	—	71.7 d
C-4″	—	73.1 d
C-5″	—	69.8 d
C-6″	—	17.5 q

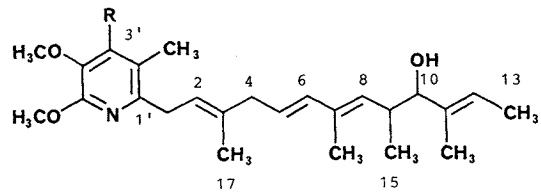
[a] Assignments were based on comparison with the literature[2].

[b] Assignments were based on ^1H-^1H COSY and ^{13}C-^1H COSY spectra.

similar to that of piericidin A_1, except for an additional five protons including one characteristic anomeric proton at δ 5.60 (overlapped with 5-H) and one methyl proton. Assignments for the ^{13}C NMR spectra of piericidin A_1 and 3′-rhamnopiericidin A_1 are shown in Table 1. In the ^{13}C NMR spectrum of 3′-rhamnopiericidin A_1, six new signals derived from rhamnoside (C-1″ ~ C-6″) are observed in comparison with that of piericidin A_1.

On acid hydrolysis with 5 N HCl at 60°C for 30 minutes, 3′-rhamnopiericidin A_1 afforded rhamnose identified by GC-MS. In the ^{13}C NMR spectrum of 3′-rhamnopiericidin A_1, C-2′ (δ 117.4) and C-4′ (δ 133.2) show significant down-field shifts (about 5 ppm) and C-3′ (δ 155.8) show a slight down-field shift. The down-field shifts are similar to the case of glucopiericidin B (piericidin A_1, 3′-*O*-D-glucoside)[3] and indicate that C-3′ of 3′-rhamnopiericidin A_1 is substituted with rhamnose. Other signals were identical with those of piericidin A_1. From the results described above, the structure of 3′-rhamnopiericidin A_1 is determined as piericidin A_1, 3′-*O*-rhamnoside as shown in Fig. 2.

Fig. 2. Structure of 3′-rhamnopiericidin A_1.



Piericidin A_1 R = OH

3′-Rhamnopiericidin A_1 R =

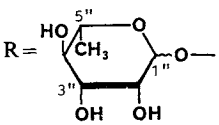


Table 2. Antimicrobial spectra of 3′-rhamnopiericidin A_1 and piericidin A_1.

Test organisms	Diameter of inhibition zone (mm)[a]	
	3′-Rhamnopiericidin A_1	Piericidin A_1
Pseudomonas aeruginosa N-10 L-form	14	12
Xanthomonas oryzae IFO 3312	14	35
X. citri IFO 3781	0	(23)[b]
Botrytis cinerea IFO 5365	0	(19)[b]
Alternaria mali IFO 8984	0	(21)[b]
Pyricularia oryzae IFO 5994	19	31
Trichophyton rubrum	0	15
Chlorella vulgaris	0	41

[a] Paper disks (diameter, 8 mm) were used containing 20 μg of the antibiotic.

[b] Partial inhibition.

3'-Rhamnopiericidin A_1 showed toxicity to HeLa and KB cells in culture. IC_{50} were 2.8 and 0.74 μg/ml, respectively. It also showed antibacterial activity against Gram-negative bacteria and fungi, but activity was less than that of piericidin A_1 (Table 2).

Several piericidin antibiotics, piericidins A[4] and B[5], $A_1 \sim A_4$, $B_1 \sim B_4$, $C_1 \sim C_4$ and $D_1 \sim D_4$[1,2], and piericidin glucoside antibiotics, glucopiericidins A and B[3], glucopiericidinols A_1 and A_2[6] have been isolated. Glucopiericidins A, B, and glucopiericidinols A_1, A_2 are reported to contain D-glucose. However, 3'-rhamnopiericidin A_1 containing rhamnose is clearly different from these antibiotics. Other substances produced by SN-198 are now under investigation.

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KEN-ICHI KIMURA
SHŌJI NAKAYAMA
NOBORU NAKAJIMA
MAKOTO YOSHIHAMA
NOBUO MIYATA
GOSEI KAWANISHI

Research Institute of Life Science,
Snow Brand Milk Products Co., Ltd.,
Ishibashi-machi, Shimotsuga-gun,
Tochigi 329-05, Japan

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