New Piericidin Antibiotics, 7-Demethylpiericidin A_1 and 7-Demethyl-3'-rhamnopiericidin A_1

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In the course of our screening program for new antitumor antibiotics, we have reported a new piericidin, 3'-rhamnopiericidin A_1 (SN-198-C (**4**)) isolated from the culture broth of *Streptomyces* sp. SN-198[1,2]. Recently, two new piericidin family of antibiotics, SN-198-D (**1**) and SN-198-B (**2**), were purified from fermentation broth of the same strain described above. This report is concerned with the isolation, structures and biological properties of these novel antibiotics.

The producing microorganism was incubated at 27°C for 96 hours in a 30-liter jar fermentor containing 18 liters of medium composed of 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%, adjusted to pH 6.8. Active principles were extracted with ethyl acetate from the filtered broth and aqueous acetone extract of the mycelium. Activity was traced by cytotoxity against KB cell and growth inhibition against some bacteria. Purification of the active substances was carried out according to the procedure shown in Fig. 1. Finally, SN-198-D (**1**) (28 mg) was obtained as yellowish oil and SN-198-B (**2**) (4.2 mg) as a white amorphous powder by preparative HPLC using ODS column. During the isolation procedure, piericidin A_1[3] (SN-198-E (**3**), 412 mg) and 3'-rhamnopiericidin A_1 (SN-198-C (**4**), 12 mg) were separated in pure form.

The UV and IR spectra of SN-198-D (**1**) and SN-198-B (**2**) showed close similarity to those of piericidin A_1 (**3**) and 3'-rhamnopiericidin A_1 (**4**), respectively. FAB-MS spectrum of SN-198-D (**1**) indicated a $(M+H)^+$ ion at m/z 402, which confirmed the molecular weight of 401. The molecular formula was determined by HRFAB-MS

Fig. 1. Isolation procedure of SN-198 compounds.

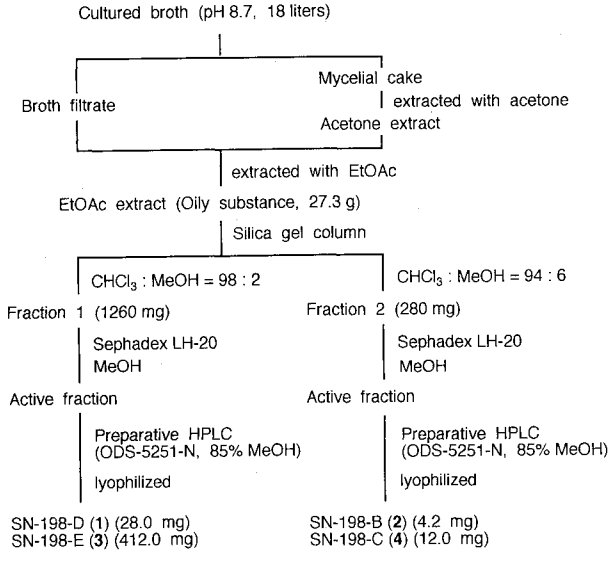
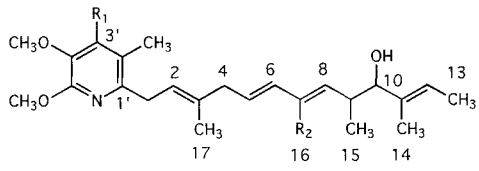


Fig. 2. Structures of SN-198 compounds.



Compounds	R_1	R_2
SN-198-D (**1**) (7 - Demethylpiericidin A_1)	OH	H
SN-198-B (**2**) (7 - Demethyl - 3' - rhamnopiericidin A_1)	Rham	H
SN-198-E (**3**) (Piericidin A_1)	OH	CH_3
SN-198-C (**4**) (3' - Rhamnopiericidin A_1)	Rham	CH_3

Rham : Rhamnose

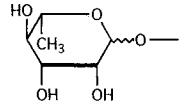


Table 1. ^1H NMR chemical shifts of SN-198 compounds (500 MHz, $CDCl_3$).

	SN-198-D (**1**)	B (**2**)	E (**3**)	C (**4**)
1	3.36 (d)	3.38 (d)	3.37 (d)	3.38 (d)
2	5.38 (t)	5.35 (t)	5.41 (t)	5.38 (t)
4	2.76 (d)	2.76 (d)	2.78 (d)	2.79 (d)
5	5.62 (dt)	5.63 (dt)	5.60 (dt)	5.61 (dt)
6	6.05 (dd)	6.03 (dd)	6.09 (d)	6.08 (d)
7	6.15 (dd)	6.15 (dd)	—	—
8	5.47 (m)*	5.47 (m)*	5.21 (d)	5.20 (d)
9	2.31 (m)	2.30 (m)	2.68 (m)	2.68 (m)
10	3.62 (d)	3.62 (d)	3.63 (d)	3.62 (d)
12	5.47 (m)*	5.47 (m)*	5.47 (q)	5.48 (q)
13	1.62 (d)	1.63 (d)	1.62 (d)	1.62 (d)
14	1.60 (s)	1.60 (s)	1.63 (s)	1.63 (s)
15	0.86 (d)	0.85 (d)	0.81 (d)	0.80 (d)
16	—	—	1.75 (s)	1.74 (s)
17	1.74 (s)	1.73 (s)	1.80 (s)	1.80 (s)
6'	2.08 (s)	2.08 (s)	2.09 (s)	2.09 (s)
7'	3.85 (s)	3.79 (s)	3.83 (s)	3.79 (s)
8'	3.94 (s)	3.95 (s)*	3.94 (s)	3.94 (s)*
1''	—	5.61 (d)	—	5.60 (d)
2''	—	4.28 (dd)	—	4.27 (dd)
3''	—	3.97 (dd)	—	3.97 (dd)
4''	—	3.55 (t)	—	3.55 (t)
5''	—	3.94*	—	3.94*
6''	—	1.30 (d)	—	1.29 (d)

* Signals are overlapped.

Table 2. Antimicrobial activities of SN-198 compounds.

Microorganism	Inhibition zone (mm)*			
	SN-198-D (**1**)	B (**2**)	E (**3**)	C (**4**)
Pseudomonas aeruginosa N-10 L-form	10.7	13.4	10.3	13.4
Salmonella typhimurium SL1102	0	0	0	0
Bacillus subtilis rec$^+$	0	0	0	0
Bacillus subtilis rec$^-$	0	0	0	0
Mycobacterium phlei IFO 3158	0	0	0	0
Xanthomonas oryzae IFO 3312	26.9	14.5	29.8	19.4
Xanthomonas citri IFO 3781	0	0	0	0
Pyricularia oryzae IFO 5994	29.7	21.6	27.9	21.8
Botrytis cinerea IFO 5365	22.6**	15.2**	23.7**	16.7**

* Paper disc (diameter, 8 mm) were used containing 40 μg of the compounds.
** Partial inhibition.

as $C_{24}H_{35}NO_4$ (Found: $(M+H)^+$, m/z 402.2658; Calcd: $(M+H)^+$, m/z 402.2644), which was CH_2 smaller than the formula of piericidin A_1 (**3**).

In the ^1H and ^{13}C NMR spectra of SN-198-D (**1**), most of the signals were observed at the same chemical shifts as in those of piericidin A_1 (**3**). However, a signal at δ_H 1.75 (3H, s, C7-CH_3) observed in the ^1H NMR spectrum of piericidin A_1 (**3**) disappeared in that of SN-198-D (**1**). On the other hand, a signal at δ_H 6.15 (1H, dd, H-7), which was not existed in the ^1H NMR spectrum of piericidin A_1 (**3**), was exhibited in that of SN-198-D (**1**). Correlation of the proton with the signals at δ_H 6.05 (1H, dd, H-6), 6.15 (1H, dd, H-7) and δ_H 5.47 (1H, m, H-8 overlapped with H-12) was established by analysis of the ^1H-^1H COSY spectrum of SN-198-D (**1**). Besides, in the ^{13}C NMR spectrum of SN-198-D (**1**), a methyl signal, which was present at δ_C 13.2 (q, C7-CH_3) in piericidin A_1 (**3**), was not observed. These data unequivocally showed that SN-198-D (**1**) was 7-demethylpiericidin A_1 (Fig. 2). Proton chemical shifts of SN-198-D (**1**) are listed in Table 1.

FAB-MS spectrum of SN-198-B (**2**) gave a $(M+H)^+$ ion at m/z 548 and precise mass of the ion by HRFAB-MS was m/z 548.3220 (Calcd. for $C_{30}H_{46}NO_8$: m/z 548.3224). Thus, the molecular formula of SN-198-B (**2**) was determined as $C_{30}H_{45}NO_8$ (MW 547). The resemblance in the ^1H and ^{13}C NMR spectra of SN-198-B (**2**) and 3′-rhamnopiericidin A_1 (**4**) indicated that SN-198-B (**2**) has a rhamnose moiety in the molecule. The difference of CH_2 in the molecular formulae of both compounds suggested that the relationship between two compounds was the same as that between SN-198-D (**1**) and piericidin A_1 (**3**). Absence of a proton signal at δ_H 1.74 (3H, s, C7-CH_3) and a carbon signal at δ_C 13.2 (q, C7-CH_3) and presence of a proton signal at δ_H 6.15 (1H, dd, H-7) in the NMR spectra of SN-198-B (**2**) showed the discrepancy in the structures between SN-198-B (**2**) and 3′-rhamnopiericidin A_1 (**4**). Attached position of rhamnose in SN-198-B (**2**) was concluded to be 3′-oxygen by down field shift of 5 ppm in the chemical shift of C-2′ (δ_C 117.4) compared with that of SN-198-D (δ_C 112.0). From these results, it was confirmed that SN-198-B (**2**) was 7-demethyl-3′-rhamnopiericidin A_1 (Fig. 2). The structure of SN-198-B (**2**) was supported by the correlation of protons by analysis of ^1H-^1H COSY spectrum. Proton chemical shifts of SN-198-B (**2**) are shown in Table 1. Though a number of piericidin family of antibiotics have been discovered[3~12], this is the first report on C7-demethyl piericidin.

Table 3. Cytotoxic activities of SN-198 compounds.

Cell	IC_{50} (μg/ml)*			
	SN-198-D (**1**)	B (**2**)	E (**3**)	C (**4**)
KB	11.0	4.5	8.9	3.8
K562	>12.5	2.9	>12.5	4.0

* MTT assay.

Antimicrobial and cytotoxic activities of the piericidin compounds were shown in Tables 2 and 3. It was interesting that SN-198-B (**2**) and C (**4**) which have sugar moiety showed lower antimicrobial activity against some fungi and gram negative bacteria than SN-198-D (**1**) and E (**3**) which have no sugar moiety (Table 2). On the contrary, SN-198-B (**2**) and C (**4**) showed stronger cytotoxicity against epidermoid carcinoma KB and chronic myelogenous leukemia K562 cells (Table 3). The difference in characteristics of activity may depend on the drug permeability against cell membrane. Methyl moiety at C-7 did not affect biological activities so much (Table 2 and 3).

Besides insecticidal, antimicrobial and cytotoxic activities, piericidin antibiotics showed many kinds of activities such as inhibition of antibody formation[7] and PI turnover[11,12], and antitumor activity[9,13]. We are now interested in the activity of piericidin rhamnoside and its demethyl type against such new targets.

References

1) KIMURA, K.; S. NAKAYAMA, N. NAKAJIMA, M. YOSHIHAMA, N. MIYATA & G. KAWANISHI: A new piericidin rhamnoside, 3'-rhamnopiericidin A_1. J. Antibiotics 43: 1341~1343, 1990
2) KIMURA, K.; M. YOSHIHAMA & N. MIYATA: Studies on 3'-rhamnopiericidin A_1. Taxonomy, fermentation, isolation, structure elucidation and biological characteristics. Yukijirushi nyūgyou kenkyūsyo houkoku No. 98: 21~34, 1992
3) TAMURA, S.; N. TAKAHASHI, S. MIYAMOTO, R. MORI, S. SUZUKI & J. NAGATSU: Isolation and physiological activities of piericidin A, a natural insecticide produced by *Streptomyces*. Agric. Biol. Chem. 27: 576~582, 1963
4) TAKAHASHI, N.; A. SUZUKI, Y. KIMURA, S. MIYAMOTO, S. TAMURA, T. MITSUI & J. FUKAMI: Isolation, structure and physiological activities of piericidin B, natural insecticide produced by a *Streptomyces*. Agric. Biol. Chem. 32: 1115~1122, 1968
5) YOSHIDA, S.; K. YONEYAMA, S. SHIRAISHI, A. WATANABE & N. TAKAHASHI: Isolation and physical properties of new piericidins produced by *Streptomyces pactum*. Agric. Biol. Chem. 41: 849~853, 1977
6) YOSHIDA, S.; K. YONEYAMA, S. SHIRAISHI, A. WATANABE & N. TAKAHASHI: Chemical structures of new piericidins produced by *Streptomyces pactum*. Agric. Biol. Chem. 41: 855~862, 1977
7) MATSUMOTO, M.; K. MOGI, K. NAGAOKA, S. ISHIZEKI, R. KAWAHARA & T. NAKASHIMA: New piericidin glucosides, glucopiericidins A and B. J. Antibiotics 40: 149~156, 1987
8) FUNAYAMA, S.; M. ISHIBASHI, Y. ANRAKU, M. MIYAUCHI, H. MORI, K. KOMIYAMA & S. Ōmura: Novel cytocidal antibiotics, glucopiericidinols A_1 and A_2. Taxonomy, fermentation, isolation, structure elucidation and biological characteristics. J. Antibiotics 42: 1734~1740, 1989
9) IWASAKI, H.; K. KAMISANGO, H. KUBONIWA, H. SASAKI & S. MATSUBARA: 3'-Deoxytalopiericidin A_1, a novel analog of antitumor antibiotics from oligotroph. J. Antibiotics 43: 451~452, 1990
10) MORI, H.; S. FUNAYAMA, Y. SUDO, K. KOMIYAMA & S. ŌMURA: A new antibiotic 13-hydroxyglucopiericidin A. Isolation, structure elucidation and biological characteristics. J. Antibiotics 43: 1329~1331, 1990
11) NISHIOKA, H.; T. SAWA, K. ISSHIKI, Y. TAKAHASHI, H. NAGANAWA, N. MATSUDA, S. HATTORI, M. HAMADA & T. TAKEUCHI: Isolation and structure determination of a novel phosphatidylinositol turnover inhibitor, piericidin B_1 *N*-oxide. J. Antibiotics 44: 1283~1285, 1991
12) NISHIOKA, H.; T. SAWA, Y. TAKAHASHI, H. NAGANAWA, M. HAMADA & T. TAKEUCHI: Isolation and structure determination of novel phosphatidylinositol turnover inhibitors, piericidin B_5 and piericidin B_5 *N*-oxide, from *Streptomyces* sp. J. Antibiotics 46: 564~568, 1993
13) NISHIOKA, H.; M. IMOTO, T. IMAOKA, T. SAWA, T. TAKEUCHI & K. UMEZAWA: Antitumor effect of piericidin B_1 *N*-oxide. J. Antibiotics 47: 447~452, 1994