# ISOLATION AND STRUCTURE DETERMINATION OF A NOVEL PHOSPHATIDYLINOSITOL TURNOVER INHIBITOR, PIERICIDIN $B_1$ *N*-OXIDE

Sir:

Piericidins are insecticidal compounds isolated from mycelia of *Streptomyces mobaraensis*[1,2] and *Streptomyces pactum*[3,4]. They are toxic to several species of insects, aphids, and mites. Piericidin A has been shown to block electron transport between NADH dehydrogenase and coenzyme Q[5]. The structures of the piericidin group have been elucidated. Members of this group are piericidins $A_n$, $B_n$, $C_n$ and $D_n$ (n=1, 2, 3 and 4)[3,4]. In the course of our screening program to find inhibitors of phosphatidylinositol turnover, we have isolated a novel antibiotic, piericidin $B_1$ *N*-oxide.

For the production of piericidin $B_1$ *N*-oxide the *Streptomyces* strain MJ288-OF3 was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of seed medium consisting of sucrose 4.0%, soybean meal 2.5%, NaCl 0.25%, $CaCO_3$ 0.32%, $CuSO_4 \cdot 5H_2O$ 0.0005%, $MnCl_2 \cdot 4H_2O$ 0.0005%, and $ZnSO_4 \cdot 7H_2O$ 0.0005% (pH 7.4). The seed culture was incubated for 3 days at 28°C on a rotary shaker (180 rpm). Two ml of the culture was then transferred to another 500-ml Erlenmeyer flask, this one containing 110 ml of fermentation medium whose composition was equivalent to that of the seed medium. The fermentation was carried out for 4 days at 28°C on a rotary shaker (180 rpm). Morphological and physiological studies revealed that the strain MJ288-OF3 resembled *Streptomyces aburaviensis*.

The fermentation broth (6 liters) was filtered, and the mycelia were extracted with acetone. After removal of the acetone, the extract was combined with the filtrate; and the mixture was next extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* to give an oily matter (2.3 g), which was mixed with silica gel and applied to a silica gel (50 g) column. The column was washed with $CHCl_3$ and eluted with a mixture of $CHCl_3$ and MeOH (10:1). After evaporation to dryness, the residue (350 mg) was partitioned in a solvent system $CHCl_3$ - MeOH - $H_2O$ (5:6:4) by centrifugal partition chromatography (CPC, Sanki Engineering Co. Ltd.), in which the lower portion was stationary. The partition coefficient in this system was 0.11. The combined active fractions were chromatographed on Sephadex LH-20 (200 ml) with EtOAc. The crude material (90 mg) was further purified by preparative HPLC using a Nucleosil $_5C_{18}$ column (30 × 250 mm) with 80% MeOH. By these steps, 53 mg of piericidin $B_1$ *N*-oxide was obtained from the 6 liters of fermentation broth.

Piericidin $B_1$ *N*-oxide is pale yellow oil and soluble in MeOH, EtOAc, and $CHCl_3$ but insoluble in water. The UV spectra showed maxima at 226 (ε 34,130), 238 sh (ε 29,680), 246 sh (ε 20,780), and 267 nm (ε 7,570) in MeOH; at 212 (ε 35,020), 238 (ε 24,960), 246 sh (ε 20,780), and 275 nm (ε 5,030) in 0.1 N HCl - MeOH; and at 238 sh (ε 33,240), 246 sh (ε 22,300), and 276 nm (ε 11,130) in 0.1 N NaOH - MeOH. The IR spectrum ($CHCl_3$) showed absorption at 3530, 3000, 2950, 2890 (sh), 2850, 1620, 1520, 1480, 1470, 1380, 1360, 1310, 1290, 1200, 1170, 1130, 1110, 1080, 1020, 980, 930, 880, and 840 $cm^{-1}$. Piericidin $B_1$ *N*-oxide was assigned a molecular formula of $C_{26}H_{39}NO_5$ based on the HRFAB mass spectrum (*m/z* 446.2897 $(M+H)^+$), and $^1H$ and $^{13}C$ NMR spectra. The $[\alpha]_D^{25}$ was −4.5° (*c* 0.2, MeOH).

The UV spectrum of piericidin $B_1$ *N*-oxide resembled that of piericidin $B_1$[2]. The structure of piericidin $B_1$ *N*-oxide was determined by comparison of its $^1H$ and $^{13}C$ NMR spectra with those of piericidin $B_1$ (a kind gift from Dr. S. YOSHIDA, the Institute of Physical and Chemical Research, Wako)[6]. The results of $^1H$ and $^{13}C$ NMR of piericidin $B_1$ *N*-oxide are compiled in Table 1.

As shown in Table 1, the $^1H$ and $^{13}C$ chemical shifts of this antibiotic are similar to those of piericidin $B_1$[6] in terms of side chains (C-2 ~ C-18). In contrast, the chemical shifts of C-1 and the chromophore (C-1′ ~ C-8′) significantly differ from those of piericidin $B_1$, suggesting that their chromophore structures are different. By mass spectral analysis the antibiotic was shown to have one more oxygen than piericidin $B_1$, and so we presumed the molecule to be piericidin $B_1$ *N*-oxide. To confirm this structure we chemically reduced the compound to piericidin $B_1$. A mixture of piericidin $B_1$ *N*-oxide (10.7 mg) and zinc powder (100 mg) in $CH_3COOH$ (1 ml) was stirred at 40°C for 2 hours and then cooled to room temperature. The reaction mixture was diluted with distilled water (50 ml) and extracted with EtOAc (25 ml × 2 times). The EtOAc layer was successively washed with aqueous $NaHCO_3$ (50 ml) and distilled water (25 ml × 2 times). The organic extract was concentrated *in vacuo* and subjected to HPLC purification. Preparative HPLC was carried out on a Nucleosil $_5C_{18}$

Table 1. $^{1}H^{a}$ and $^{13}C^{b}$ NMR data of piericidin $B_1$ *N*-oxide in $CDCl_3$-*d*.

| Position | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1 | 3.65 d ($J_{1,2}$=7.0 Hz) | 27.1 t |
| 2 | 5.18 t | 118.9 d |
| 3 | | 136.7 s |
| 4 | 2.73 d ($J_{4,5}$=6.4 Hz) | 43.2 t |
| 5 | 5.45 m ($J_{5,6}$=16.0 Hz) | 124.8 d |
| 6 | 6.05 d | 136.6 d |
| 7 | | 133.3 s |
| 8 | 5.28 d ($J_{8,9}$=8.4 Hz) | 135.2 d |
| 9 | 2.62 m ($J_{9,10}$=9.2 Hz) | 35.4 d |
| 10 | 3.16 d | 92.7 d |
| 11 | | 134.0 s |
| 12 | 5.41 q ($J_{12,13}$=6.2 Hz) | 124.3 d |
| 13 | 1.64 d | 13.0 q |
| 14 | 1.74 s | 16.5 q |
| 15 | 1.71 s | 12.9 q |
| 16 | 0.76 d ($J_{9,16}$=6.8 Hz) | 17.7 q |
| 17 | 1.52 s | 10.4 q |
| 18 | 3.11 s | 56.2 q |
| 1′ | | 145.7 s |
| 2′ | | 117.5 s |
| 3′ | | 159.0 s |
| 4′ | | 135.0 s |
| 5′ | | 151.5 s |
| 6′ | 2.11 s | 11.3 q |
| 7′ | 3.72 s | 61.0 q |
| 8′ | 3.98 s | 60.9 q |

[a] $^{1}H$ Chemical shifts (ppm), signal multiplicities, and coupling constants (*J* in Hz) in parentheses at 400 MHz.

[b] $^{13}C$ Chemical shifts (ppm) and signal multiplicites at 100 MHz.

column (8×300 mm) with 95% MeOH. After concentration, 4.5 mg of the reduced material was obtained. The reduced substance was identical to piericidin $B_1$[6] by FAB-MS and $^{1}H$ NMR analyses. Absolute configurations of C-9 and C-10 in piericidin $B_1$ *N*-oxide were assigned to be *S*-*S* since the optical rotation of piericidin $B_1$ *N*-oxide (−4.5°) is similar to that of piericidin $B_1$ (−6.5°)[2]. Thus, we have concluded the structure of piericidin $B_1$ *N*-oxide to be as shown in Fig. 1.

Phosphatidylinositol turnover was assayed by epidermal growth factor (EGF)-stimulated incorporation of *myo*-[$^{3}$H]inositol into phospholipids of A431 cells[7]. Previously, we isolated two inhibitors of this turnover, psi-tectorigenin[7] ($IC_{50}$, 1 μg/ml) and inostamycin[8] ($IC_{50}$, 0.5 μg/ml) from *Nocardiopsis* and *Streptomyces*, respectively. Piericidin $B_1$ *N*-oxide inhibited the phosphatidylinositol turnover with an $IC_{50}$ of 1.2 μg/ml. Piericidin $B_1$ showed weaker inhibitory activity toward phosphatidylinositol turnover ($IC_{50}$, 5.0 μg/ml) than the *N*-oxide.

Fig. 1. Structure of piericidin $B_1$ *N*-oxide.

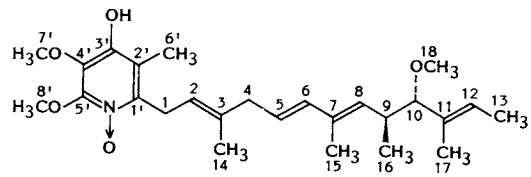


Table 2. Antimicrobial activities of piericidin $B_1$ *N*-oxide and piericidin $B_1$ in agar dilution assay.

| Test organisms | MIC (μg/ml) | |
|---|---|---|
| | Piericidin $B_1$ *N*-oxide | Piericidin $B_1$ |
| *Staphylococcus aureus* Smith | 50 | >100 |
| *Micrococcus luteus* FDA 16 | 12.5 | >100 |
| *Bacillus anthracis* | 25 | 100 |
| *Corynebacterium bovis* 1810 | 12.5 | >100 |
| *Escherichia coli* NIHJ | 12.5 | >100 |
| *Shigella dysenteriae* JS11910 | 6.3 | >100 |
| *Salmonella typhi* T-63 | >100 | >100 |
| *Proteus vulgaris* OX 19 | >100 | >100 |
| *Serratia marcescens* | >100 | >100 |
| *Pseudomonas aeruginosa* A3 | 12.5 | >100 |
| *Klebsiella pneumoniae* PCI 602 | >100 | >100 |
| *Mycobacterium smegmatis* ATCC 607 | 50 | >100 |
| *Candida albicans* 3147 | 25 | >100 |
| *Saccharomyces cerevisiae* F-7 | >100 | >100 |
| *Cryptococcus neoformans* F-10 | 12.5 | >100 |
| *Cochliobolus miyabeanus* | 100 | >100 |
| *Pyricularia oryzae* | 12.5 | >100 |
| *Pellicularia sasakii* | 3.1 | 100 |
| *Xanthomonas citri* | 3.1 | >100 |
| *Trichophyton asteroides* 429 | 3.1 | >100 |
| *Aspergillus niger* F-16 | >100 | >100 |

Antimicrobial activities of piericidin $B_1$ *N*-oxide and piericidin $B_1$ are summarized in Table 2. Piericidin $B_1$ *N*-oxide showed antibacterial activity against Gram-positive and Gram-negative bacteria and fungi, while piericidin $B_1$ did not.

Thus, piericidin $B_1$ *N*-oxide is a new member of the piericidin family, having both antibacterial and phosphatidylinositol turnover-inhibiting activities.

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HIROSHI NISHIOKA
TSUTOMU SAWA
KUNIO ISSHIKI
YOSHIKAZU TAKAHASHI

HIROSHI NAGANAWA
NAOKO MATSUDA
SEIKO HATTORI
MASA HAMADA
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

KAZUO UMEZAWA*

Department of Applied Chemistry,
Faculty of Science and Technology,
Keio University,
3-14-1 Hiyoshi, Kohoku-ku,
Yokohama 223, Japan

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