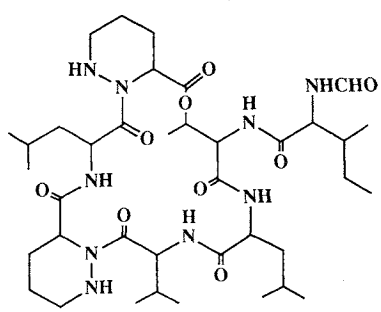
DEPSIDOMYCIN, A NEW IMMUNOMODULATING ANTIBIOTIC

Sir:

In the course of a screening program for new antibiotics, we isolated a new depsipeptide antibiotic, depsidomycin (**1**) from the cultured broth of *Streptomyces lavendofoliae* MI951-62F2. It is primarily active against Gram-positive microorganisms and has immunosuppressive activity. Now we report the fermentation, isolation, physico-chemical properties, biological activities and the structure determination of depsidomycin.

The producing strain MI951-62F2 was cultured at 27°C for 48 hours on a rotary shaker (180 rpm) in a 500-ml baffled Erlenmeyer flask containing 110 ml of seed medium (adjusted at pH 7.4 before sterilization) consisting of Bacto Soytone 1.0%, galactose 2.0%, corn steep liquor 0.5%, dextrin 2.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$, 0.2%. The seed culture, thus obtained, was inoculated into 500-ml baffled Erlenmeyer flasks each containing 110 ml of producing medium consisting of potato starch 3.0%, glucose 2.0%, soybean meal 2.0%, yeast extract 0.5%, NaCl 0.25%, $CaCO_3$ 0.3%, $CuSO_4 \cdot 5H_2O$ 0.005%, $MnCl_2 \cdot 4H_2O$ 0.005% and $ZnSO_4 \cdot 7H_2O$ 0.05% (pH 7.4), and cultured for 48 hours at 27°C on a rotary shaker (180 rpm). The cultured broth (6 liters) was centrifuged to separate the mycelium. The mycelial cake was extracted with MeOH (1.5 liters). The extract was concentrated under reduced pressure to remove organic solvent. The residue was combined with the supernatant and extracted with BuOAc (6 liters). The extract was concentrated and the dried residue (450 mg) was subjected to a silica gel (25 g) column chromatography. The active fraction was eluted with toluene - acetone (2 : 1) and concentrated under reduced pressure. The crude powder (181 mg), thus obtained, was then chromatographed on a Sephadex LH-20 column (200 ml) and eluted with MeOH. The active eluate was concentrated and the residue was crystallized from acetone - hexane to give colorless crystals of depsidomycin (170.5 mg).

Fig. 1. The structure of depsidomycin (**1**).



The physico-chemical properties of **1** were listed in Table 1. The molecular formula $C_{38}H_{65}N_9O_9$ was determined by SI-MS spectrum (*m/z* 792 $(M+H)^+$) and elemental analysis (calcd for $C_{38}H_{65}N_9O_9$: C 57.63, H 8.27, N 15.92, O 18.18, found: C 57.62, H 8.45, N 16.05, O 18.23). The IR spectrum of **1** showed the ester and amide carbonyl at 1745 and 1650 cm^{-1}, respectively. Acid hydrolysis (6 M HCl, 110°C, 24 hours) of **1** gave valine, threonine and leucine which were identified by amino acid analyzer (L-8500 Hitachi Co., Ltd.). Mild alkaline methanolysis (Na_2CO_3 in MeOH, room temperature, 4 hours) of **1** gave methyl ester **2** (SI-MS: *m/z* 824 $(M+H)^+$).

^1H and ^{13}C NMR spectra of **1** (Table 2) were studied as follows: 1 mol of valine and threonine and 2 mol of leucine residues were easily assigned. One mol of isoleucine and 2 mol of piperazic acid residues which have been found in monamycins[1], azinothricin[2] or A83586C[3] were assigned by ^1H-^1H COSY and ^1H-detected heteronuclear multiple-bond correlation (HMBC) spectra of **1** as illustrated in Fig. 2. The isoleucine residue, which was not identified by amino acid analyzer was supposed to be alloisoleucine.

The sequence of amino acid residues for **1** was established from HMBC spectral data of **1** and **2** as follows (Fig. 3). The amide protons and amide carbonyl carbons from formyl group at the *N*-terminal to valine through threonine and leucine (Leu-1) residues were connected in sequence by the HMBC spectrum of **1**. The spectrum indicated also the sequence of piperazic acid (Pip-1) and leucine

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

Appearance	White crystals
SI-MS (*m/z*)	792 $(M+H)^+$
Molecular formula	$C_{38}H_{65}N_9O_9$
Elemental analysis	Calcd: C 57.63, H 8.27, N 15.92, O 18.18. Found: C 57.62, H 8.45, N 16.05, O 18.23.
MP (dec)	272~274°C
$[\alpha]_D^{27}$ (*c* 1.0, MeOH)	−59.7°
IR ν_{max} cm^{-1}	3320, 2960, 2950, 1745, 1650, 1530, 1390
UV λ_{max}^{MeOH} nm (ε)	End absorption
Solubility	Soluble in MeOH, EtOAc, $CHCl_3$ Insoluble in H_2O, *n*-hexane

Table 2. ^1H and ^{13}C NMR chemical shifts[a] of **1** in acetone-d_6.

Assignemnt		^{13}C (100 MHz)	^1H (400 MHz)
Leu-2	CO	175.4 s	
Val	CO	175.3 s	
Leu-1	CO	174.8 s	
Ileu	CO	171.1 s	
Pip-2	CO	169.3 s	
Pip-1	CO	167.9 s	
Thr	CO	167.4 s	
CHO		161.8 d	8.27 (1H, br s)[b]
Thr	β	71.4 d	4.90 (1H, dq, 3.7, 6.4)
Val	α	56.3 d	5.20 (1H, dd, 6.1, 10.3)
Thr	α	55.9 d	4.50 (1H, dd, 3.7, 6.4)
Ileu	α	55.5 d	4.76 (1H, dd, 4.6, 9.3)
Pip-1	α	53.1 d	5.11 (1H, dd, 1.7, 5.4)
Pip-2	α	51.6 d	5.16 (1H, dd, 2.0, 4.9)
Leu-1	α	51.2 d	4.97 (1H, m)
Leu-2	α	49.6 d	5.40 (1H, m)
Pip-1	δ	48.0 t	2.77, 3.12 each (1H, m)
Pip-2	δ	47.8 t	2.85, 3.14 each (1H, m)
Leu-2	β	41.4 t	1.69, 1.95 each (1H, m)
Leu-1	β	41.1 t	1.68, 2.17 each (1H, m)
Ileu	β	38.5 d	1.92 (1H, m)
Val	β	29.4 d	1.98 (1H, m)
Ileu	γ	27.0 t	1.19, 1.42 each (1H, m)
Leu-2	γ	26.8 d	1.78 (1H, m)
Leu-1	γ	25.9 d	1.70 (1H, m)
Pip-1	β	25.1 t	1.55, 2.57 each (1H, m)
Leu-1	CH_3	24.1 q	0.91 (3H, d, 6.8)
Leu-2	CH_3	23.8 q	0.93 (3H, d, 6.6)
Pip-2	β	23.4 t	1.86, 2.19 each (1H, m)
Pip-1	γ	23.0 t	1.40, 1.55 each (1H, m)
Pip-2	γ	21.4 t	1.55, 1.86 each (1H, m)
Leu-2	CH_3	21.3 q	1.02 (3H, d, 6.6)
Leu-1	CH_3	20.8 q	0.89 (3H, d, 6.8)
Val	CH_3	20.1 q	1.05 (3H, d, 6.6)
Val	CH_3	19.9 q	0.97 (3H, d, 6.6)
Ileu	β-CH_3	14.7 q	0.87 (3H, d, 6.8)
Thr	CH_3	13.9 q	1.17 (3H, d, 6.4)
Ileu	CH_3	12.1 q	0.89 (3H, t, 7.3)
Thr	NH		7.69 (1H, d, 6.4)
Leu-2	NH		7.65 (1H, d, 9.8)
Val	NH		7.56 (1H, d, 6.1)
Ileu	NH		7.52 (1H, d, 9.3)
Leu-1	NH		7.10 (1H, d, 9.8)
Pip-2	NH		4.88 (1H, dd, 2.4, 12.0)
Pip-1	NH		3.94 (1H, dd, 2.0, 12.9)

[a] δ values (ppm) relative to TMS=0.
[b] Proton signal multiplicity and coupling constant (J=Hz).

(Leu-2) residues. The *C*-terminal amino acid residue was determined to be piperazic acid (Pip-2) by long range coupling between methoxy protons in ester group and carbonyl carbon of Pip-2 in the HMBC spectrum of **2**. The spectrum also showed the amino proton of Pip-2 coupled with carbonyl carbon of Leu-2.

This result was confirmed by the SI-MS spectrum of **2** (Fig. 4). The fragment ion peaks appeared at *m/z* 145, 258, 370, 469, 582, 683, 796 ($C^+ + 2$; *C*-terminal fragments) and *m/z* 243, 356, 455, 567, 680 (N^+, *N*-terminal fragments).

The HPLC analysis of the acid hydrolysate of **1** was carried out by use of a chiral recognition column, Crownpak CR (Daisel Chemical Industries, Ltd., Tokyo) and aq $HClO_4$ (pH 2) as a mobile phase at both 0 and 24°C. It indicated the presence of D-valine, L-threonine, L-leucine, D-leucine and L-alloisoleucine. The configuration of piperazic acid remains to be elucidated. The structural studies, including stereochemistry, will be reported later.

As shown in Table 3, **1** is selectively active against *Micrococcus luteus* FDA 16, *M. luteus* IFO 3333 and *Mycobacterium vaccae* ATCC 15483. The acute toxicity LD_{50} in mice was >100 mg/kg by intraperitoneal injection. **1** has immunosuppressive activity as follows.

Table 3. The antibacterial activity of **1**.

Test organisms	MIC (μg/ml)
Staphylococcus aureus FDA 209P	> 50
S. aureus Smith	>100
Micrococcus luteus FDA 16	3.12
M. luteus IFO 3333	1.56
M. luteus PCI 1001	>100
Bacillus anthracis	> 50
B. subtilis PCI 219	>100
Corynebacterium bovis 1810	1.56
Escherichia coli NIHJ	>100
E. coli K-12	>100
Mycobacterium vaccae ATCC 15483	3.12

Influence of **1** on lectin-induced blastogenesis was examined. Spleen cells from CDF_1 mice (female, 12 weeks old) were cultured in RPMI-1640 containing 1% fetal calf serum (FCS) and concanavalin A (Con A, 0.5 μg/ml) or lipopolysaccharide (LPS, 2 μg/ml) for 3 days in 5% CO_2 in air. [^{3}H]Thymidine ([^{3}H]TdR) was added 16 hours before cell harvest. The effect was determined by measuring the incorporation of [^{3}H]TdR into the cultured cells. Triplicate determinations were made. **1** inhibited LPS-induced blastogenesis at 25 μg/ml but not Con A-induced blastogenesis even at 100 μg/ml. According to the method described previously[4], the effect of **1** on mixed lymphocyte culture (MLC) reaction was examined. Spleen cells taken from WKY rats used as the responder were mixed with stimulator spleen cells taken from F344 rats the stimulator cells had been previously incubated with 50 μg/ml of mitomycin C (Kyowa Hakko Kogyo Co., Ltd.,

Fig. 2. Amino acid subunits for **1**.

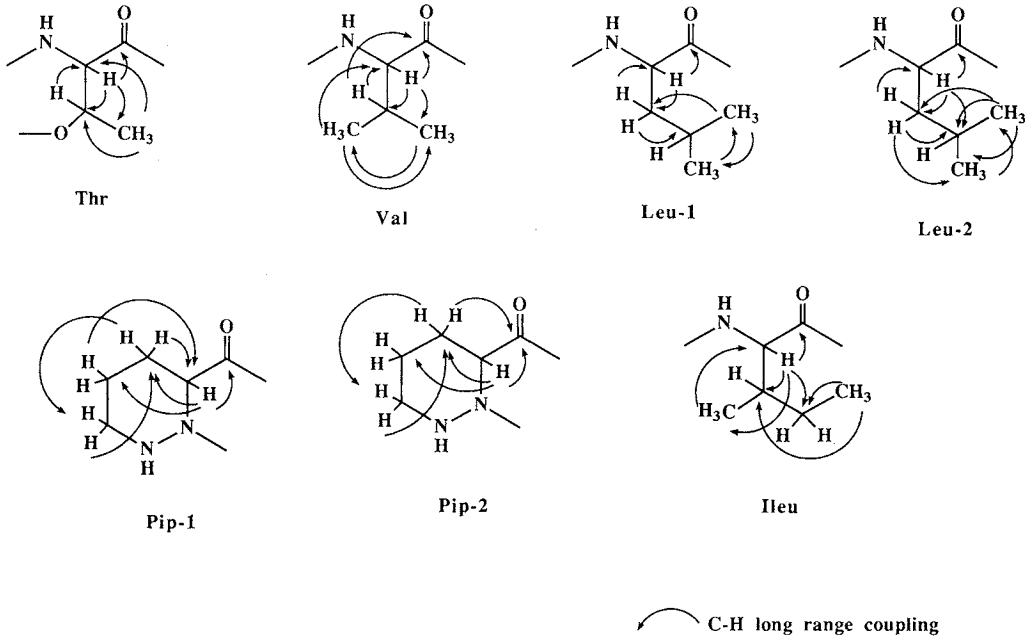


Fig. 3. The sequence of amino acid residues for **1** and **2**.

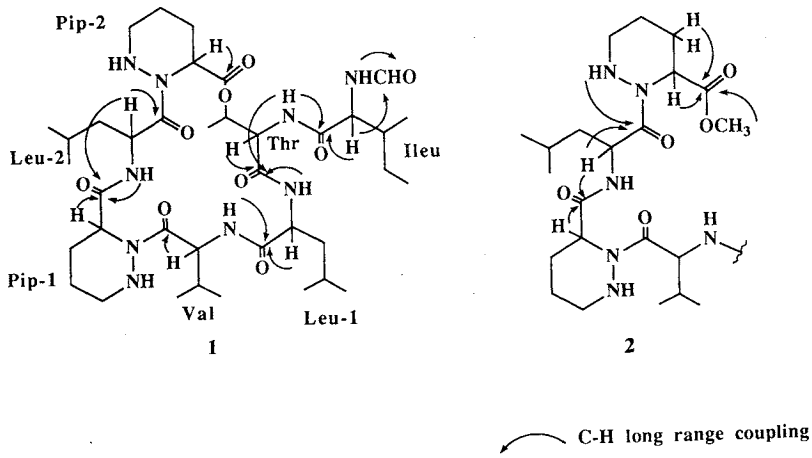
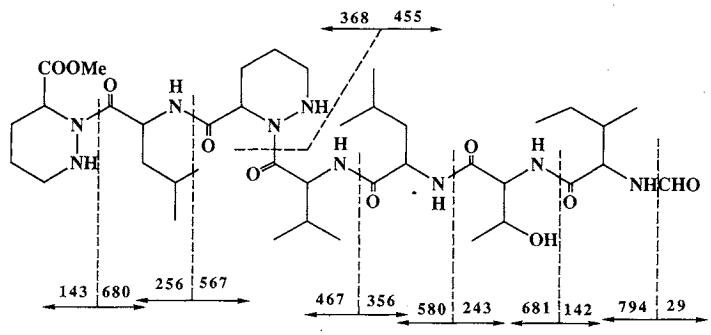


Fig. 4. SI-MS fragmentation of **2**.



Tokyo) at 37°C for 20 minutes. The mixed spleen cells were cultured with or without drugs in medium containing 5% FCS (Gibco, Grand Island, N.Y.) at 37°C for 5 days in 5% CO_2 in air and [^{3}H]TdR was added 16 hours before the assay. MLC reaction was determined by measuring the incorporation of [^{3}H]TdR into the cultured cells. **1** inhibited MLC reaction, and IC_{50} was at 1.6 μg/ml.

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