Bassiatin, a New Platelet Aggregation Inhibitor Produced by *Beauveria bassiana* K-717

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A new platelet aggregation inhibitor, bassiatin, was isolated from the cultured broth of *Beauveria bassiana* which had been isolated from a soil sample collected in Yunnan province, China. The structure of bassiatin was determined to be (3*S*,6*R*)-4-methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione by NMR analysis, X-ray crystallographic analysis and chemical synthesis. Bassiatin inhibited ADP-induced aggregation of rabbit platelets with the IC_{50} being 1.9×10^{-4} M.

In the course of our screening program for new platelet aggregation inhibitors from microbial extracts, we isolated a new compound designated bassiatin from the cultured broth of *Beauveria bassiana* K-717. This paper describes the taxonomy of the producing strain and the isolation, structure determination and biological activity of bassiatin.

Results

Taxonomy

Strain No. K-717 was isolated from a soil sample collected in Yunnan province, China. Morphological properties were examined after incubation at 21°C for 14 days on 1/3 oatmeal agar (Difco 0552-01-1). This organism grew moderately, attaining a diameter of 41~44 cm on 1/3 oatmeal agar after two weeks at 21°C, and formed powdery to felt-like pale yellow colonies. Clear droplets exuded onto the surface of the colonies. The conidiogenous cells arose directly from vegetative hyphae or through lateral swollen cells which were globose to clavate in shape. Basal parts of the conidiogenous cells inflated to globular shapes to accommodate flask shape, 2.8~12.0 μm in length, and 2.2~5.0 μm in width. In producing conidia, the fertile tips of the conidiogenous cells elongated sympodually, 5.0~15.0 μm in length to 1.0 μm in width.

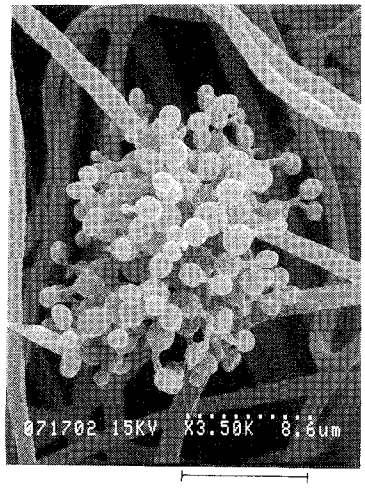
The conidia which were produced sympodually were hyaline in color, smooth-walled, mostly globose to subglobose, rarely ellipsoidal in shape, and 2.8~5.0 × 2.2~5.0 μm in size. Chlamydospores were absent. No teleomorph was observed in this strain. The scanning electron micrograph of K-717 is shown in Fig. 1.

From the characteristics described above, strain No. K-717 resembled *Beauveria bassiana* (Bals.) Vuill. 1912[1]. Therefore, we have designated this fungus as *Beauveria bassiana* K-717, and deposited it in the National Institute of Bioscience and Human-Technology (formerly the Fermentation Research Institute), Agency of Industrial Science and Technology, Japan, as FERM P-13085.

Fermentation

The growth of *Beauveria bassiana* K-717 on a mature slant culture was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of sterile medium composed of glucose 2%, yeast extract 0.2%, polypepton 0.5%,

Fig. 1. The scanning electron micrograph of K-717.
Bar represents 8.6 μm.



KH_2PO_4 0.1% and $MgSO_4$ 0.05% (pH 7.0) which was cultured at 26°C for 120 hours on a rotary shaker with 7-cm throw at 200 rpm.

Fig. 2. Isolation procedure for bassiatin and beauvericin.

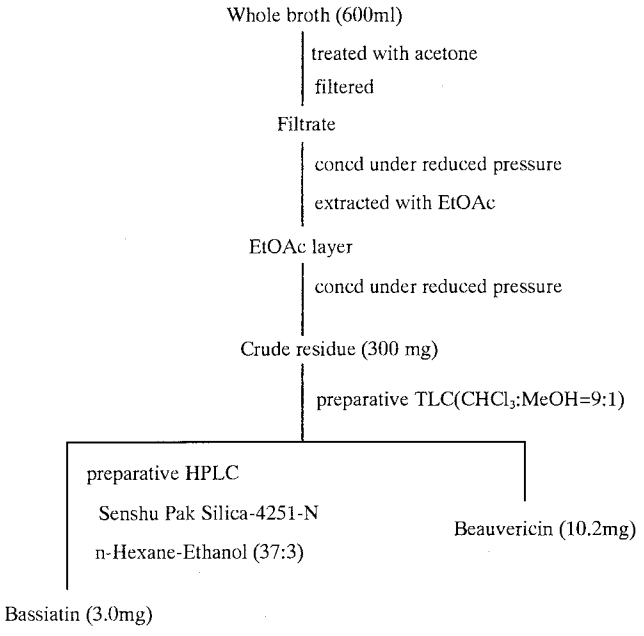


Table 1. Physico-chemical properties of bassiatin.

Appearance	Colorless crystal
$[\alpha]^{25}_D$(c=0.024,$CHCl_3$)	+181.05°
MP	143-148°C
EIMS	261$(M)^+$
HR-EIMS(m/z)	
calcd for $C_{15}H_{19}NO_3$:	261.1366
found:	261.1359
UVλ^{MeOH}_{max} nm(ε)	208(5800)
IR(KBr)cm-1	2970,1749,1654,1498,1409
	1365,1340,1257,1041,769,709

Isolation

The isolation scheme is shown in Fig. 2. The cultured broth (0.6 liters) was treated with acetone (0.6 liters) and filtered. The filtrate was concentrated *in vacuo*, and the resulting aqueous solution was extracted with ethyl acetate (0.6 liters). The organic layer was concentrated to dryness under reduced pressure. The residue (300 mg) was dissolved in $CHCl_3$ and subjected to preparative TLC with $CHCl_3$ - MeOH (9 : 1) as a developing solvent to give two active compounds. One of them was identified as beauvericin[2,3] from NMR spectroscopic studies.

The other compound was further purified by preparative HPLC with a silica gel column (Senshu Pak Silica 10 i.d. × 250 mm) using n-hexane - ethanol (37 : 3), 4.5 ml/minute.

Crystallization from EtOAc - n-hexane gave bassiatin (3 mg) as colorless crystals.

Structure Determination

The physico-chemical properties of bassiatin are summarized in Table 1. The molecular formula was established as $C_{15}H_{19}NO_3$ on the basis of HR-EIMS. In the IR spectrum the absorption at 1668, 1748 cm^{-1} showed the presence of amide and ester groups.

The ^1H NMR spectrum is shown in Fig. 3. The ^1H and ^{13}C NMR data are shown in Table 2. In the ^1H NMR spectrum, signals of two methyl protons (δ_H 0.82 (3H, d, J=7.1 Hz), 0.74 (3H, d, J=6.7 Hz)), one *N*-methyl protons (δ_H 3.00 (3H, s)), three methine protons (δ_H 4.38 (1H, t, J=4.5 Hz), 2.98 (1H, d, J=2.2 Hz), 2.29 (1H, m)) and one pair of methylene protons (δ_H 3.26 (1H, dd, J=14.0, 4.1 Hz), 3.17 (1H, dd, J=14.0, 4.5 Hz)) and five aromatic protons (δ_H 7.27~7.33 (3H, m), 7.09~7.13 (2H, m)) were observed. The ^1H-^1H COSY spectrum deduced the partial structures A and B (Fig. 4).

Partial structure B was further supported by observing the following cross peaks; 10-H→C-12 (C-16), 3-H→C-11, 12-H (16-H)→C-10, *N*-CH_3→C-3 and C-5, 3-H→

Fig. 3. ^1H NMR spectrum of bassiatin ($CDCl_3$, 400 MHz).

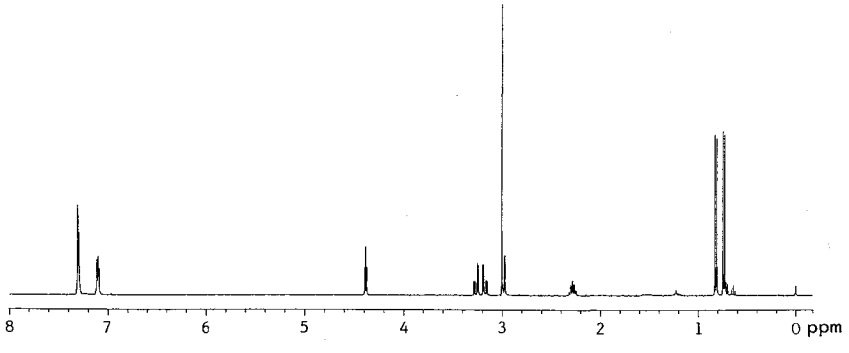


Table 2. ^1H and ^{13}C NMR chemical shifts[a] of bassiatin in $CDCl_3$.

	δ_C	δ_H
C-2	167.3 (s)	
C-3	62.8 (d)	4.38 (1H, t, J=4.5Hz)
C-5	165.5 (s)	
C-6	81.3 (d)	2.98 (1H, d, J=2.2Hz)
C-7	29.7 (d)	2.29 (1H, m)
C-8	18.6 (q)	0.82 (3H, d, J=7.1Hz)
C-9	15.1 (q)	0.74 (3H, d, J=6.7Hz)
C-10	37.1 (t)	3.26 (1H, dd, J=14.0, 4.1Hz) 3.17 (1H, dd, J=14.0, 4.5Hz)
C-11	134.1 (s)	
C-12, 16	129.8 (d)	7.09~7.13 (2H, m)
C-13, 15	129.2 (d)	7.27~7.33 (2H, m)
C-14	128.2 (d)	7.27~7.33 (1H, m)
N-CH_3	32.4 (q)	3.00 (3H, s)

[a] δ_C and δ_H values in ppm from $(CH_3)_4Si$ at 100 and 400 MHz, respectively.

Fig. 4. Partial structures of bassiatin.

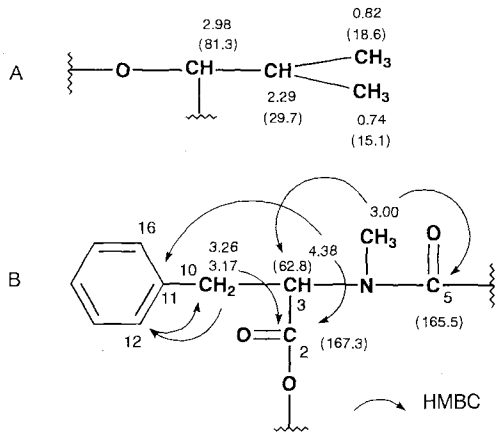


Fig. 5. Structure of bassiatin.

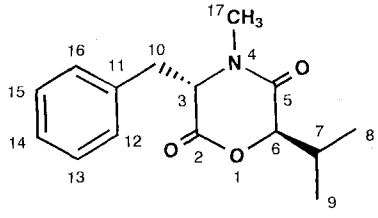
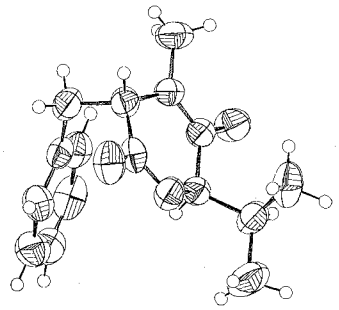


Fig. 6. A perspective view of bassiatin.



C-2 and 10-H→C-2. Thus the structure of bassiatin was elucidated as 4-methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione, consisting of one mole each of *N*-methylphenylalanine and 2-hydroxy-3-methylbutyric acid.

The relative stereochemistry of bassiatin was determined by X-ray crystallographic analysis. A single crystal having an approximate dimension of 0.50×0.50×0.40 mm was obtained from EtOAc-n-hexane and used for X-ray crystallographic analysis. The molecular structure of bassiatin is shown in Fig. 6, indicating that the relative stereochemistries at C-3 and C-6 could be either *S*/*R* or *R*/*S*. In the ^1H NMR spectrum, the signal of δ_H 2.98 assigned to 6-H is unusually in high field as an oxymethine proton neighboring with the 5-carbonyl group. Judging from the conformation of bassiatin, the unusual chemical shift of 6-H is the result of a strong shielding effect from the benzyl group at C-3.

To determine the absolute configuration of **1**, we synthesized (3*S*,6*R*)- and (3*R*,6*S*)-isomers by combination of D- and L-phenylalanine, with commercially available (+)-(*S*)- and (±)-2-hydroxy-3-methylbutyric acid (HMBA) (Scheme 1).

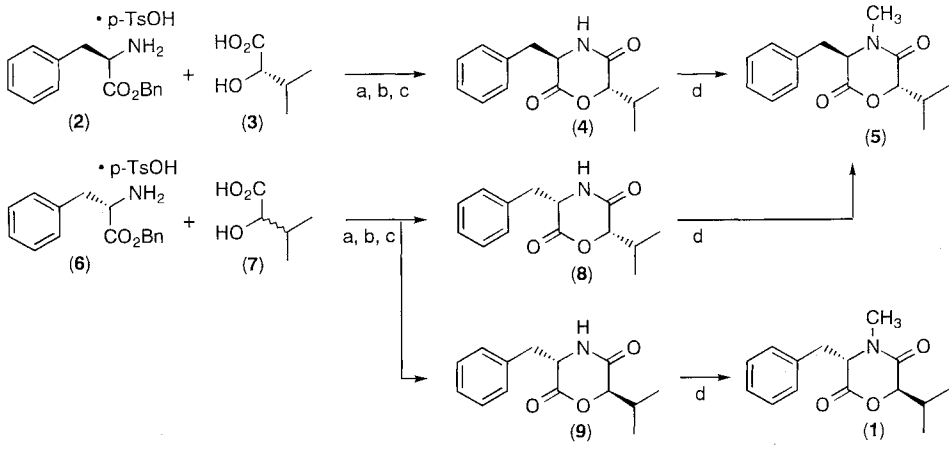
Coupling of D-phenylalanine benzyl ester·p-TsOH (**2**) and (+)-(*S*)-HMBA (**3**) with *N*-hydroxysuccinimide (HOSu) and water soluble carbodiimide hydrochloride (WSC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) in THF, catalytic hydrogenation of the benzyl ester in ethanol and lactonization with p-TsOH in benzene gave **4** (54% from **2**). Methylation of **4** with methyl iodide and sodium hydride in DMF gave the (3*R*,6*S*)-isomer **5** (57%).

Compounds **8** and **9** were synthesized from L-phenylalanine benzyl ester·p-TsOH (**6**) and (±)-HMBA (**7**) and were separated. The ^1H and ^{13}C NMR spectra of **9** were the same as those of **4**, indicating that the stereochemistry at C-3 and C-6 was *S*/*R*. Methylation of **9** gave the (3*S*,6*R*)-isomer **1** (58%).

The ^1H and ^{13}C NMR spectra of bassiatin were the same as those of **1** and **5**. Judging from the optical rotations of **1** ($[\alpha]_D^{29}$ +166° (*c* 1.0, $CHCl_3$)), **5** ($[\alpha]_D^{29}$ −167° (*c* 1.0, $CHCl_3$)) and bassiatin ($[\alpha]_D^{25}$ +181° (*c* 0.024, $CHCl_3$)), the structure of bassiatin was determined to be (3*S*,6*R*)-4-methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione.

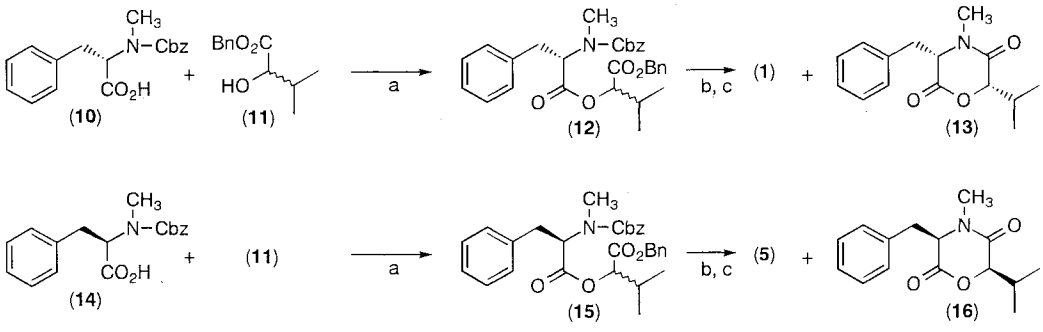
From the view point of structure-activity relationships, the remaining two stereoisomers were synthesized.

Scheme 1.



Reagents: (a) HOSu, WSC·HCl, NMM in THF. (b) H_2/Pd-C in EtOH. (c) p-TsOH·H_2O in Benzene. (d) MeI, NaH in DMF.

Scheme 2.



Reagents: (a) DMAP, DCC in CH_2Cl_2. (b) H_2/Pd-C in EtOH. (c) HOSu, WSC·HCl in DMF. Cbz=Carbobenzoxy.

Methylation of the (3*S*,6*S*)-isomer **8**, described above, gave the (3*R*,6*S*)-isomer **5** due to the epimerization at C-3 (Scheme 1).

When *N*-methylphenylalanine benzyl ester is used as a starting material, the coupling yield was very low. The (3*S*,6*S*)- (**13**) and (3*R*,6*R*)-isomers (**16**) were obtained from *N*-carbobenzoxy-*N*-methylphenylalanine (**10** and **14**) and (±)-HMBA benzyl ester (**11**) through esterification, catalytic hydrogenation, and intramolecular coupling (Scheme 2).

Biological Activity

Platelet aggregation test was performed according to BORN *et al.*[4] The inhibitory activities (IC_{50}) of bassiatin against rabbit platelet aggregation induced by ADP, collagen and arachidonic acid were 1.9×10^{-4} M, 3.8×10^{-4} M and 3.8×10^{-4} M, respectively. These values indicate that bassiatin is slightly less active than adenosine in inhibiting platelet aggregation. The stereoisomers of bassiatin, **5**, **13**, and **16** did not show activity in this assay. Beauvericin inhibited ADP induced platelet aggregation with an IC_{50} of 6.4×10^{-5} M.

Discussion

We isolated bassiatin, a new platelet aggregation inhibitor, from *Beauveria bassiana* K-717. Beauvericin[2,3], a known depsipeptide antibiotic, was also produced by the same strain. Both compounds showed the inhibitory activities against platelet aggregation. Bassiatin and beauvericin consist of *N*-methyl-L-phenylalanine and (−)-(*R*)-2-hydroxy-3-methylbutyric acid and bassiatin has the minimum unit of them.

Recently lateritin was isolated from *Gibberella lateritum* by ENDO *et al.*[5] as an inhibitor of acyl-CoA:cholesterol acyltransferase (ACAT), and the structure of bassiatin presented above, was proposed for

lateritin but without definition of the stereochemistry. The ^1H and ^{13}C NMR spectrum of bassiatin are quite different from those of lateritin, especially, the chemical shifts of 3-H and 6-H. (Bassiatin ; 4.39 ppm and 2.98 ppm, respectively, *cf*, lateritin ; 5.51 ppm and 4.89 ppm[5].) The physico-chemical properties of the other three stereoisomers, **5**, **13**, **16**, were also different from those of lateritin, bringing into question the proposed structure for lateritin. Neither bassiatin nor any of its stereoisomers exhibit any inhibitory activity against rabbit intestine ACAT at concentrations up to 10 μM.

Experimental

1) X-ray Crystallographic Analysis of Bassiatin:

Crystal Data

$C_{15}H_{19}NO_3$, $Mw=261.30$, orthorhombic, $a=10.839(3)$, $b=13.134(3)$, $c=10.010(3)$ Å, $V=1425.1(6)$ Å^3, space group $P2_12_12_1$, $Z=4$, $Dc=1.22\,g\,cm^{-3}$, μ(Cu-Kα)$=6.06\,cm^{-1}$, $F(000)=560$. Lattice parameters were determined from 20 reflections with $55°<2\theta<60°$.

Data Collection and Processing

A transparent colorless prism crystal ($0.50\times0.50\times0.40$ mm) was mounted on a Mac Science MXC18 diffractometer. Intensity data were measured using graphite-monochromated Cu-Kα radiation, $\lambda=1.54178$ Å. The three standard reflections showed no significant deterioration. The intensities were corrected for Lp factors.

Within the range of $2\theta\leq130°$, 1432 reflections were measured and 1364 unique reflections were used for structure solution.

Structure Analysis and Refinement

The structure of bassiatin was solved by direct methods using the program SHELX86[6] and refined by the full-matrix least-squares method. All H atom positions were located from a difference Fourier synthesis. All non-hydrogen atoms were refined anisotropically, and H atoms isotropically. Final *R* and *Rw* values were 0.030 and 0.050, respectively. The source of scattering factor data was derived from the International Tables for X-ray Crystallography (1974)[7].

2) Synthesis of Bassiatin and Related Compounds:

(3*R*,6*S*)-6-(1-Methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**4**)

N-Methylmorpholine (NMM) (1.29 ml, 11.7 mmol), HOSu (1.35 g, 11.7 mmol) and WSC·HCl (2.24 g, 11.7 mmol) were added to a solution of **2** (5.00 g, 11.7 mmol) and **3** (1.38 g, 11.7 mmol) in THF (100 ml) at 0°C, and the mixture was stirred at room temperature for 17 hours. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$-acetone, 20:1) to give a coupling compound (2.89 g, 69%). A solution of the coupling compound (2.89 g, 8.13 mmol) in EtOH (50 ml) was stirred with 5% Pd-C (480 mg) under an atmosphere of H_2 at room temperature for 4 hours. The mixture was filtered, and concentrated *in vacuo*. The residue was crystallized from EtOAc-n-hexane to give the carboxylic acid (2.14 g, 99%), which was then refluxed with p-TsOH·H_2O (230 mg, 1.21 mmol) in benzene (40 ml) for 4 hours. The mixture was poured into satd $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized from EtOAc-n-hexane to give **4** (1.58 g, 79%) as colorless crystals. MP 164~165°C; $[\alpha]_D^{23}$ −45.4° (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) 3202, 1757, 1684, 1348, 1330, 1239, 1032, 986, 741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, $J=6.9$ Hz, 9-H), 1.00 (3H, d, $J=6.9$ Hz, 8-H), 2.32 (1H, m, 7-H), 3.15 (1H, dd, $J=13.9$ and 7.2 Hz, 10-H), 3.28 (1H, dd, $J=13.9$ and 4.2 Hz, 10-H), 3.89 (1H, d, $J=3.4$ Hz, 6-H), 4.45 (1H, ddd, $J=7.2$, 4.2 and 2.1 Hz, 3-H), 6.82 (1H, d, $J=2.1$ Hz, 4-H), 7.20~7.27 (2H, m, 12- and 16-H), 7.32~7.39 (3H, m, 13~15-H); EI-MS *m/z* 247 $(M)^+$.

Anal Calcd for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66.
Found: C 67.97, H 6.95, N 5.71.

(3*R*,6*S*)-4-Methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**5**)

60% NaH oily dispersion (30 mg, 0.75 mmol) was added to a solution of **4** (124 mg, 501 μmol) and methyl iodide (125 μl, 2.00 mmol) in DMF (2 ml) at 0°C, and the reaction mixture was stirred at room temperature for 15 minutes. The mixture was poured into brine and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Column chromatography on silica gel ($CHCl_3$-acetone, 50:1) of the residue and recrystallization from EtOAc-n-hexane gave **5** (74.2 mg, 57%) as colorless crystals. MP 143~148°C; $[\alpha]_D^{29}$ −167° (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) 2970, 1749, 1654, 1498, 1409, 1365, 1340, 1257, 1041, 769, 709 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.77 (3H, d, $J=6.9$ Hz, 9-H), 0.85 (3H, d, $J=6.9$ Hz, 8-H), 2.31 (1H, m, 7-H), 3.01 (1H, d, $J=2.3$ Hz, 6-H), 3.02 (3H, s, NCH_3), 3.20 (1H, dd, $J=14.0$ and 4.3 Hz, 10-H), 3.29 (1H, dd, $J=14.0$ and 4.3 Hz, 10-H), 4.41 (1H, t, $J=4.3$ Hz, 3-H), 7.09~7.18 (2H, m, 12- and 16-H), 7.30~7.36 (3H, m, 13~15-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2 (q, C-9), 18.6 (q, C-8), 29.7 (d, C-7), 32.4 (q, NCH_3), 37.1 (t, C-10), 62.8 (d, C-3), 81.3 (d, C-6), 128.2 (d, C-14), 129.2 (d, C-13 and 15), 129.8 (d, C-12 and 16), 134.2 (s, C-11), 165.5 (s, C-5), 167.3 (s, C-2); EI-MS *m/z* 261 $(M)^+$.

(3*S*,6*R*)-6-(1-Methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**8**), (3*S*,6*S*)-6-(1-Methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**9**)

A mixture of diastereomers, prepared from L-phenylalanine benzyl ester·p-TsOH and (±)-HMBA in a similar manner to that described for **4**, was separated by column chromatography on silica gel, LiChroprep Si 60 (E. Merck) (n-hexane - EtOAc, 2 : 1) to give **8** (42%) and **9** (40%).

8: MP 131~132°C; $[\alpha]_D^{23}$ −185° (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) 3272, 2966, 1745, 1708, 1364, 1336, 1195, 1180, 1000, 749, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, d, J=6.9 Hz, 9-H), 1.11 (3H, d, J=6.9 Hz, 8-H), 2.39 (1H, m, 7-H), 2.96 (1H, dd, J=14.2 and 9.8 Hz, 10-H), 3.50 (1H, dd, J=14.2 and 3.8 Hz, 10-H), 4.40 (1H, dd, J=9.8 and 3.8 Hz, 3-H), 4.62 (1H, d, J=3.2 Hz, 6-H), 6.06 (1H, s, 4-H), 7.19~7.25 (2H, m, 12- and 16-H), 7.30~7.41 (3H, m, 13~15-H); EI-MS *m/z* 247 $(M)^+$.

Anal Calcd for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66.
Found: C 67.92, H 6.95, N 5.72.

9: $[\alpha]_D^{27}$ +47.7° (*c* 1.0, $CHCl_3$). (Other physico-chemical properties were the same as **4**.)

(3*S*,6*R*)-4-Methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**1**)

This compound was prepared by the methylation of **9** in a manner similar to that described for **5** in 58% yield as colorless crystals. $[\alpha]_D^{29}$ +166° (*c* 1.0, $CHCl_3$).

Anal Calcd for $C_{15}H_{19}NO_3$: C 68.94, H 7.33, N 5.36.
Found: C 68.88, H 7.34, N 5.32.

(Other physico-chemical properties were the same as **5**.)

N-Benzyloxycarbonyl-*N*-methyl-L-[(1-benzyloxycarbonyl-2-methyl)propyl] phenylalanate (**12**)

A solution of **10** (700 mg, 2.24 mmol) and **11** (512 mg, 2.46 mmol) in CH_2Cl_2 (15 ml) was stirred with 4,4-dimethylaminopyridine (DMAP, 27.4 mg, 224 μmol) and 1,3-dicyclohexylcarbodiimide (DCC, 693 mg, 3.36 mmol) at 0°C for an hour. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*.

The residue was purified by column chromatography on silica gel (n-hexane - EtOAc, 12 : 1) to give the ester **12** (903 mg, 80%) as an oil. FAB-MS *m/z* 504 $(M+H)^+$.

(3*S*,6*S*)-4-Methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dion (**13**) and compound **1**

A solution of **12** (564 mg, 1.12 mmol) in EtOH (12 ml) was stirred with 5% Pd-C (170 mg) under an atmosphere of H_2 at room temperature for 8 hours. The mixture was filtered, and concentrated *in vacuo*. The crude product was stirred with HOSu (258 mg, 2.24 mmol) and WSC·HCl (429 mg, 2.24 mmol) in DMF (12 ml) at room temperature for 16 hours. The mixture was poured into brine and extracted with EtOAc. The organic layer was washed with 1 N HCl, satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Column chromatography on silica gel ($CHCl_3$) of the residue gave **1** (58 mg, 20%) as colorless crystals and **13** (38 mg, 13%) as an oil.

13: $[\alpha]_D^{29}$ −24.3° (*c* 1.0, $CHCl_3$); IR ν_{max} (neat) 2968, 1746, 1666, 1497, 1456, 1377,1252, 1044, 756, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (3H, d, J=6.8 Hz, 9-H), 0.86 (3H, d, J=6.8 Hz, 8-H), 1.20 (1H, m, 7-H), 2.98 (3H, s, NCH_3), 3.28 (1H, dd, J=14.2 and 4.9 Hz, 10-H), 3.34 (1H, dd, J=14.2 and 4.9 Hz, 10-H), 4.35 (1H, d, J=6.8 Hz, 6-H), 4.40 (1H, t, J=4.9 Hz, 3-H), 7.12~7.18 (2H, m, 12- and 16-H), 7.24~7.36 (3H, m, 13~15-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.5 (q, C-9), 18.9 (q, C-8), 32.4 (d, C-7), 32.9 (q, NCH_3), 38.1 (t, C-10), 61.9 (d, C-3), 83.8 (d, C-6), 127.8 (d, C-14), 129.1 (d, C-13 and 15), 129.8 (d, C-12 and 16), 134.8 (s, C-11), 164.6 (s, C-5), 165.9 (s, C-2); EI-MS *m/z* 261 $(M)^+$.

N-Benzyloxycarbonyl-*N*-methyl-D-[(1-benzyloxycarbonyl-2-methyl)propyl] phenylalanate (**15**)

This compound was prepared from **14** and **11** in a manner similar to that described for **12** in 78% yield.

(3*R*,6*R*)-4-Methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (**16**) and compound **5**

Compounds **16** and **5** were prepared in 20 and 19% yields, respectively.

16: $[\alpha]_D^{29}$ +23.0° (*c* 1.0, $CHCl_3$). (Other physico-chemical properties were the same as **13**.)

References

1) De Hoog, G. S.: Studies in Mycology. 1: pp. 1~41, CBS, 1972
2) Hamill, R. L.; C. E. Higgens & M. Gorman: The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*. Tetrahedron Letters 49: 4255~4258, 1969
3) Ovchinnicov, Yu. A.; V. T. Ivanov & I. I. Mikhaleva: The synthesis and some properties of beauvericin. Tetrahedron Letters 2: 159~162, 1971
4) Born, G. V. R. & M. J. Cross: The aggregation of blood platelets. J. Physiol. 168: 178~195, 1968
5) Hasumi, K.; C. Shinohara, T. Iwanaga & A. Endo: Lateritin, a new inhibitor of acyl-CoA : Cholesterol acyltransferase produced by *Gibberella lateritum* IFO 7188. J. Antibiotics 46: 1782~1787, 1993
6) Sheldrick, G. M.: SHELX86, Program for Crystal Structure Determination. University of Göttingen, Germany, 1986
7) International Tables for X-Ray Crystallography. Vol. IV. Birmingham, Kynoch Press, 1974