The Structures of Bioactive Cyclodepsipeptides, Beauveriolides I and II, Metabolites of Entomopathogenic Fungi *Beauveria* sp.

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(Received May 17, 1993)

Among the cyclodepsipeptides, beauveriolides I (**1**) and II (**2**) have been isolated from the mycelium of *Beauveria* sp., and their stereostructures were elucidated by spectral analyses coupled with syntheses of the corresponding 3-hydroxyoctanoic acid derivatives. Beauveriolide I (**1**) exhibited moderate insecticidal activities against *Spodoptera litura* and *Callosobruchus chinensis*.

Cyclodepsipeptides have been produced as secondary metabolites by some bacteria, actinomycetes and fungi. Although their essential biological functions are not clearly understood,[1,2] many congeners of this family have been known to show a wide range of bioactivities. During a search for bioactive substances produced by entomopathogenic fungi, two cyclodepsipeptides named beauveriolides I (**1**) and II (**2**) were isolated from the mycelium of the strain *Beauveria* sp.,[3,4] In this present paper we describe the isolation and stereostructures of these cyclodepsipeptides, along with their biological activity.

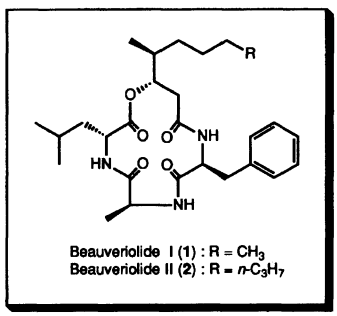
Results and Discussion

The aqueous medium prepared by extracting pupae powder of silkworm with 2% glucose-containing water was inoculated with a suspension of the mycelium of *Beauveria* sp. (unidentified) in sterilized water, and incubated with shaking at 24 °C for 3 d. After filtration, the combined extracts were concentrated and subjected to silica-gel column chromatography. The fraction eluted earlier was further separated by HPLC to give rise to beauveriolide I (**1**) as colorless needles in 7.0% yield. Beauveriolide II (**2**) was also obtained from a less polar fraction than **1** as colorless needles in 3.5% yield (Fig. 1).

The high resolution FAB mass spectrum (positive) of beauveriolide I (**1**) exhibited the molecular formula $C_{27}H_{42}N_3O_5$ [m/z 488.3111 (M+H)]. The IR absorption bands at 3300, 1680, 1640, and 1540 cm^{-1} indicated the presence of a peptide structure of **1**. The ^1H NMR ($CDCl_3$) spectrum of **1** reveals the presence of a mono-substituted aromatic ring, three NH protons (δ=6.25, 6.30, and 7.38), two methylenes (δ=2.38, 2.47, 3.20, and 3.37), five methyls [δ=0.87 (6H), 0.94 (6H), and 1.37 (3H)], and three protons couple with NH protons (δ=4.06, 4.35, and 4.63). On the basis of the ^1H NMR spectrum coupled with decoupling experiments, beauveriolide I (**1**) has three amino acids (alanine, phenylalanine, and leucine). Furthermore, the observation of four signals (δ=170.1, 171.7, 171.8, and 172.0) in the ^{13}C NMR spectrum suggested the presence of three peptide bonds and one ester group. In light of the decoupling experiments, particularly, the remaining NMR signals other than those due to the three amino acids, exhibit the partial structure [**A**] (Fig. 2).

Additionally, the ninhydrin test was negative in beauveriolide I (**1**); **1** was thus considered to have a cyclodepsipeptide structure comprising three amino acids and one β-hydroxylic acid. In order to determine the sequence of these acids, **1** was treated with K_2CO_3 in MeOH to cleave the cyclodepsipeptide ring at only an ester part, resulting in the formation of the corresponding methyl ester (**3**), whose EI mass spectrum shows several significant peaks at m/z 519 (M), 375, 304, 276, 120, and 91, indicating the sequence of these amino and β-hydroxylic acids (Fig. 3).

Furthermore, **1** was subjected to acid hydrolysis; the product was partitioned between water and ether. The aqueous layer provided equimolar amounts of L-alanine, L-phenylalanine, and D-leucine, which were detected by HPLC using the chiral pack column. From the ether layer, methyl 3-hydroxy-4-methyloctanoate (**4**) was obtained, and its stereostructure, including the absolute configuration, was unambiguously determined by a comparison of the ^1H and ^{13}C NMR spectral data and the optical rotations with the octanoate derivatives



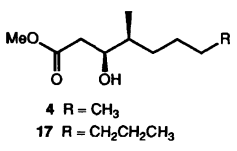
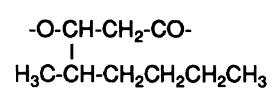


Fig. 1. Structures of beauveriolides I (**1**) and II (**2**), and the corresponding methyl esters (**4** and **17**).



[A]

Fig. 2. Partial structure [**A**] of **1**.

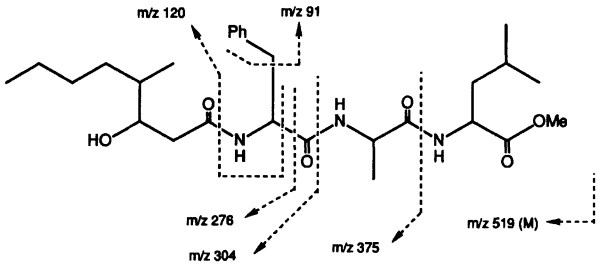


Fig. 3. Structure of the methyl ester derivative (**3**) and its fragmentation of the EI mass spectrum.

(**10** and **16**) synthesized in optically active forms.

As can be seen in Scheme 1, the synthetic process was initiated by a conversion of the known alcohol (**5**)[5] to **6** in five steps involving one-carbon elongation and a reductive ring-opening of the acetal. Compound **6** in hand was submitted to Swern oxidation, followed by a Wittig reaction to yield approximately a 1:1 mixture of the corresponding olefinic isomers (**7**), in which the required carbon chain had already been set up. Selective catalytic hydrogenation effected the saturation of the olefinic bond without any removal of the benzyl group, yielding **8**. After a cleavage of the silyl protective group with the fluoride anion, the resulting alcohol was submitted to Swern oxidation, followed by $KMnO_4$ oxidation,[6] and methylation to **9**. Finally, the hydrogenolysis of **9** yielded the (3*R*,4*S*)-hydroxy methyl ester (**10**). Additionally, (3*S*,4*S*)-hydroxy methyl ester (**16**) was also synthesized from **7** as follows.

The olefinic mixture (**7**) was submitted to exhaustive hydrogenation to give **11**. Inversion of the hydroxyl group was achieved by mesylation, followed by an S_N2 reaction employing CsOAc[7] to give the expected acetate (**12**). After alkaline hydrolysis of **12**, the glycol obtained was reacted with benzaldehyde dimethyl acetal to furnish the benzylidene acetal (**13**), which was reacted with diisobutylaluminum hydride (DIBAL), leading to **14**. Compound **14** was treated with essentially the same procedure as in the case of **10** to produce the desired ester (**16**) through the benzyl ether (**15**). A comparison of the spectroscopic data and optical rotations of the newly synthesized **10** and **16** with those of **4** {$[\alpha]_D$ −19° (**4**); +23° (**10**); −44° (**16**)} indicated that the natural methyl ester (**4**) has the 3*S*,4*S*-configuration. The difference in the optical rotation values between **4** and **16** is probably due to an insufficient sample amount of **4** causing an experimental error.

Beauveriolide II (**2**) has the molecular formula $C_{29}H_{45}N_3O_5$ [*m/z* 516.3446 (M+H)]. The ^1H and ^{13}C NMR spectral data of **2** coincided with those of beauveriolide I (**1**) with the exception of two more methylenes in the hydrocarbon region of those of **2**. From these observations, **2** was assumed to have 3-hydroxy-4-methyldecanoic acid instead of the (3*S*,4*S*)-4-methyloctanoic acid of **1**. In fact, upon an acidic treatment under the same conditions as in the case of beauveriolide I (**1**), **2** afforded L-phenylalnine, L-alanine, and D-leucine, along with methyl 3-hydroxy-4-methyldecanoate {**17**, $[\alpha]_D$ −9.6°}. In addition to the close similarity of the ^1H NMR chemical shifts of **17** to **4**, rather than **10** bearing the 3*R*,4*S*-configuration [H-2: δ=2.47 (**17**); 2.47 (**4**); 2.41 and 2.49 (**10**), H-3: δ=3.94 (**17**); 3.94 (**4**); 3.87 (**10**)], the same sign of the optical rotations of both compounds suggested that **17** has the same stereochemistry (3*S*,4*S*) at the C_3–C_4 part as that of **4**. Accordingly, the structure of beauveriolide II (**2**) has been proposed to be as shown in Fig. 1, although the absolute configuration of the 3-hydroxydecanoic acid residue has not been directly proved.

As discussed above, spectroscopic and chemical evidence coupled with the stereoselective synthesis of **10** and **16** provided a determination of the stereostructures of beauveriolides I and II (**1** and **2**); their structures are quite similar to those of beauverolides H and I[8] bearing 3-hydroxynonanoic acid and 3-hydroxyundecanoic acid as the non-amino acid moieties.

As depicted in Tables 1 and 2, although no remarkable antimicrobial activities were detected, **1** exhibited ca. 20% insecticidal activity against *Spodoptera litura* at the 10 μg/body level. Additionally, 40% activity was observed against females of *Callosobruchus chinensis*, whereas the males suffered no damage.

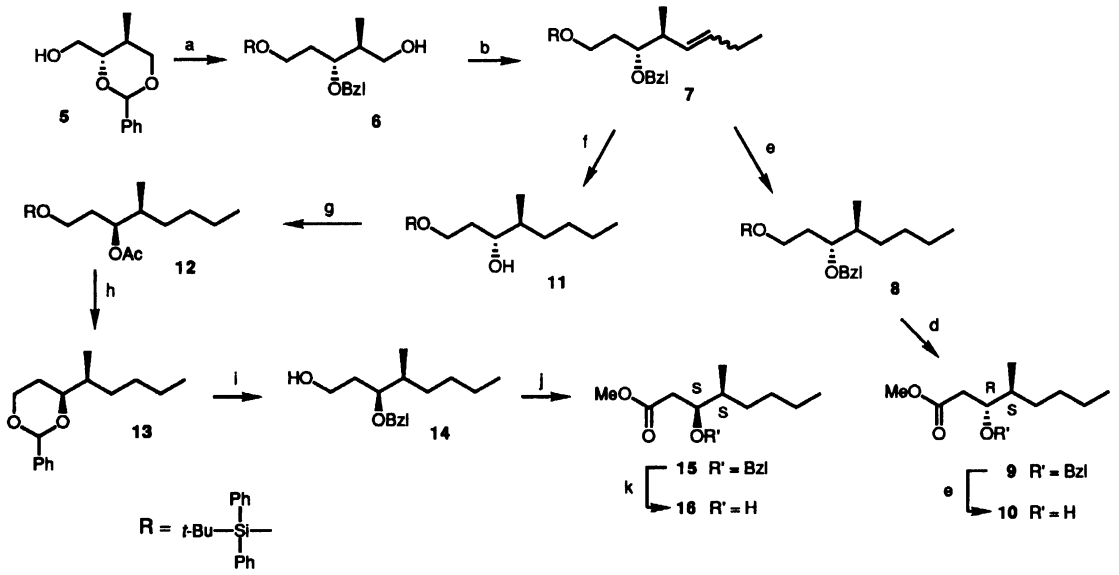
Table 1. Antimicrobial Activities of Beauveriolide I (**1**)

Organisms	MIC (μg ml^{-1})
S. aureus 209P JC-1	>100
St. faecalis CN-478	>100
P. mirabilis TU-1698	>100
C. freundii TL-12	>100
E. coli KC-14	50
E. coli ATCC 27166	50
B. subtilus ATCC 6633	50

Table 2. Insecticidal Activities of Beauveriolide I (**1**) against *Spodoptera litura* (larva) and *Callosobruchus chinensis*

Dose (μg/body)	Sample	Survival	Agony	Death
Spodoptera litura (larva)				
10[a]	15	12	—	3
1[a]	15	14	—	1
Blank[b]	15	15	—	0
Callosobruchus chinensis				
10[c]	10(male)	9	1	0
	10(female)	5	1	4
Blank[d]	10(male)	9	1	0
	10(female)	7	2	1

a) Injected as a DMSO solution (0.4 μl). b) DMSO (0.4 μl) was used. c) Injected as a MeOH–$CHCl_3$ solution (1/1, 0.5 μl). d) MeOH–$CHCl_3$ (1/1, 0.5 μl) was used.



a. i) Swern Oxid.; ii) $Ph_3P{=}CH_2$ (73% in two steps); iii) 9-BBN, then NaOH, H_2O_2 (86%); iv) TBDPSCl, Imd. (99%); v) DIBAL (94%). b. i) Swern Oxid. (100%); ii) $PH_3P{=}CHCH_2CH_3$ (50%). c. H_2 / 10% Pd on carbon (98%). d. i) *n*-Bu_4NF (98%); ii) Swern Oxid. (90%); iii) $KMnO_4$; iv) $TMSCHN_2$ (70% in two steps). e. H_2 / Pd black (100%). f. H_2 / Pd black (73%). g. i) MsCl, pyr. (97%); ii) CsOAc, 18-Crown-6 / PhH, refluxing temp. (85%). h. i) KOH; ii) $PhCH(OMe)_2$, cat. TsOH (88% in two steps). i. DIBAL (87%). j. i) Swern Oxid. (89%); ii) $KMnO_4$; iii) $TMSCHN_2$ (62% in two steps). l. H_2/ Pd black. (100%).

Scheme 1.

Experimental

All of the melting points were obtained on a Mitamura Riken melting-point apparatus and are uncorrected. The IR spectra were recorded on a JASCO Model A-202 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL FX-90 A or a JEOL JNM GX-400 NMR spectrometer in a deuteriochloroform ($CDCl_3$) solution using tetramethylsilane as an internal standard, unless otherwise stated. High-resolution mass spectra were obtained on a Hitachi M-80 GC-MS spectrometer operating at the ionization energy (70 eV). Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 F254, E. Merck A. G., Germany) using UV light and/or 5% molybdophosphoric acid in ethanol for detection. Katayama silica-gel (K 070) was used for column chromatography.

Isolation of Beauveriolides I and II (1 and 2). An aqueous medium prepared by extracting pupae powder (40 g) of silkworm with glucose (20 g) - containing water (1 dm^3) was inoculated with a suspension of the mycelium of *Beauveria* sp. (unidentified) in a sterilized water, and incubated with shaking at 24 °C for 3 d. After filtration, the filtrate was evaporated to give a wet mixture (600 g), which was extracted with acetone (1 dm^3); the extracts were concentrated in vacuo to give a crude oil (9.89 g). A 52 mg portion of the oil was submitted to silica-gel column chromatography using a gradient solvent system of MeOH–$CHCl_3$ (5→100%). Further HPLC (Unisil Pack Type 5C18-250R×2, $CHCl_3$–MeOH=50 : 1) separation gave rise to beauveriolide I (**1**, 3.64 mg), and beauveriolide II (**2**, 1.67 mg).

1: as colorless needles mp 244—246 °C (from MeOH); $[\alpha]_D^{23}$ −25° [*c* 0.36, $CHCl_3$–MeOH (4 : 1)]; IR (film) 3300, 1725, 1680, 1640, and 1540 cm^{-1}; ^{1}H NMR δ=0.87 (6H, complex), 0.94 (6H, complex, overlapped with 1H signal), 1.37 (3H, d, J=6 Hz, overlapped with 8H signal), 2.10 (1H, m), 2.38 (1H, dd, J=14.8, 9.9 Hz), 2.47 (1H, dd, J=14.8, 4.7 Hz), 3.20 (1H, dd, J=13.6, 7.3 Hz), 3.37 (1H, dd, J=13.6, 8.8 Hz), 4.06 (1H, m), 4.35 (1H, m), 4.63 (1H, m), 5.05 (1H, m), 6.25 (1H, broad s), 6.30 (1H, broad d, J=8 Hz), 7.15—7.30 (5H, complex), and 7.38 (1H, broad d, J=8 Hz); ^{13}C NMR (CD_3OD–$CDCl_3$=1 : 4) δ=14.1, 15.6, 22.3, 22.5, 23.1, 25.2, 25.2, 29.7, 30.9, 36.2, 36.4, 36.8, 41.8, 50.3, 53.1, 57.6, 76.3, 126.9, 128.7, 129.7, 138.1, 170.1, 171.7, 171.8, and 172.0. Found: *m/z* 488.3111. Calcd for $C_{27}H_{42}N_3O_5$: M+H, 488.3124. **2**: as colorless needles mp 245—247 °C (from MeOH); $[\alpha]_D^{22}$ −21° [*c* 0.17, $CHCl_3$–MeOH (4 : 1)]; IR (film) 3300, 1725, 1680, 1635, and 1535 cm^{-1}; ^{1}H NMR δ (CD_3OD–$CDCl_3$=1 : 4)=0.89 (6H, complex), 0.91 (6H, complex), 1.05 (1H, m), 1.2—1.5 (12H, complex), 1.56 (3H, complex), 2.14 (1H, m), 2.49 (2H, complex), 3.00 (1H, dd, J=7.8, 13.7 Hz), 3.10 (1H, dd, J=8.3, 13.7 Hz), 3.92 (1H, m), 4.25 (1H, t, J=8 Hz), 4.63 (1H, m), 4.97 (1H, m), 6.97 (1H, d, J=10 Hz), and 7.18—7.34 (7H, complex); ^{13}C NMR (CD_3OD–$CDCl_3$=1 : 4) δ=13.7, 14.7, 15.2, 21.7, 22.0, 22.3, 24.8, 26.9, 29.3, 30.8, 31.5, 35.2, 35.4, 35.7, 41.0, 49.2, 52.4, 56.7, 76.0, 122.8, 128.3, 128.8, 136.2, 169.7, 171.2, 171.4, and 171.8. Found: *m/z* 516.3446. Calcd for $C_{29}H_{46}N_3O_5$: M+H, 516.3437.

Lactone Cleavage Reactions of 1 under Basic Conditions. A mixture of **1** (9 mg) and K_2CO_3 (10 mg)

in MeOH (10 ml) was kept at room temperature for 1 d. After removing the solvent, separation by preparative TLC ($CHCl_3$–acetone–AcOH=32:8:1) provided the corresponding methyl ester (**3**, 2.2 mg), which was directly submitted to EI mass spectrometry. Found: m/z 519.3306; 376.2366; 304.1937; 276.1971; 120.0778; 91.0542. Calcd for $C_{28}H_{45}N_3O_6$: M, 519.3306; $C_{21}H_{32}N_2O_4$: 376.2360; $C_{18}H_{26}NO_3$: 304.1911; $C_{17}H_{26}NO_2$: 276.1962; $C_8H_{10}N$: 120.0812; C_7H_7: 91.0547.

Acid Hydrolysis of 1 and 2. A 10 mg portion of **1** in a mixture of 6 M HCl (6 ml, 1 M=1 mol dm^{-3}) and dioxane (2 ml) was heated at 110 °C for 1 d in a sealed tube. The mixture was partitioned between ether and H_2O. The aqueous layer was evaporated, and the residue was subjected to HPLC (Fuji Davison ODS, MeOH–H_2O=1:9) to give a mixture of the corresponding amino acids, which were identified by HPLC (CROWNPAC CRA, pH 1.5 aq $HClO_4$ as a solvent). The organic layer was concentrated in vacuo, and the residue was treated with diazomethane in MeOH (2 ml) until a yellow color remained. After evaporation, the residue was purified by preparative TLC ($CHCl_3$–MeOH=15:1) to give methyl (3*S*,4*S*)-3-hydroxy-4-methyloctanoate (**4**, 2.7 mg): $[\alpha]_D^{20}$ −19° (*c* 0.13, $CHCl_3$); IR (film) 3400 and 1730 cm^{-1}; ^1H NMR δ=0.90 (3H, t, J=6.8 Hz), 0.91 (3H, d, J=6.8 Hz), 1.15 (1H, m), 1.30 (4H, complex), 1.50 (2H, complex), 2.47 (2H, complex), 3.72 (3H, s), and 3.94 (1H, m); ^{13}C NMR δ=14.0, 14.2, 22.9, 29.4, 32.4, 38.0, 38.6, 52.0, 71.2, and 173.9.

According to essentially the same procedure, **2** (3.7 mg) gave the amino acids and methyl (3*S*,4*S*)-3-hydroxy-4-methyloctanoate (**17**, 1.1 mg,): $[\alpha]_D^{20}$ −9.6° (*c* 0.30, $CHCl_3$); IR (film) 3500 and 1740 cm^{-1}; ^1H NMR δ=0.88 (3H, t, J=7.2 Hz), 0.91 (3H, d, J=6.8 Hz), 1.16 (1H, m), 1.2—1.6 (10H, complex), 2.47 (2H, complex), 3.72 (3H, s), and 3.94 (1H, dt, J=4.6, 6.8 Hz); ^{13}C NMR δ=14.1, 14.2, 22.6, 27.2, 29.5, 31.8, 32.7, 38.0, 38.6, 51.8, 71.2, and 173.9.

(2*S*,3*R*)-3-Benzyloxy-5-(*t*-butyl)diphenylsiloxy-2-methyl-1-pentanol (6). To a mixture of oxalyl chloride (0.77 ml) and DMSO (1.04 ml) in CH_2Cl_2 (30 ml) at −50 °C was added (4*R*,5*S*)-5-methyl-2-phenyl-1,3-dioxane-4-methanol (**5**, 1.22 g)[5] in CH_2Cl_2 (5 ml). After 20 min, Et_3N (4.1 ml) was added, and the reaction mixture was stirred at the same temperature for 45 min, then partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was passed through a short column of silica-gel (hexane–EtOAc=3:1) to give a crude oil, which was dissolved in THF (5 ml). The THF solution was added to a reddish solution of triphenylmethylenephosphorane in THF (30 ml) at 0 °C prepared from methyltriphenylphosphonium bromide (3.3 g) and 1.65 M *n*-BuLi (5.3 ml). The stirred mixture was gradually warmed to room temperature. After being reacted overnight, the resulting suspension was diluted with aq NH_4Cl, and extracted with ether. The ethereal solution was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. Silica-gel chromatographic purification (hexane–EtOAc=20:1) of the residue provided the olefinic product (0.87 g).

To an ice-cooled solution of the olefin (895 mg) was added 9-BBN (15.1 ml); the mixture was stirred overnight. After the addition of a 3 M NaOH solution (4 ml) and 30% H_2O_2 (4 ml), the mixture was stirred for 3 h; $NaHSO_3$ was then added. The reaction solution was partitioned between EtOAc and H_2O, and the organic extracts were washed with sat. aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was purified by a silica-gel column (hexane–EtOAc=3:1) to give the corresponding alcohol (842 mg).

A mixture of the alcohol (781 mg), *t*-butyldiphenylsilyl chloride (TBDPSCl) (1.1 ml), and imidazole (570 mg) in DMF (20 ml) was stirred at room temperature for 3 d. The reaction mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was chromatographed on a silica-gel column (hexane–EtOAc=20:1) to yield the silyl ether (1.59 g): $[\alpha]_D^{21}$ +38° (*c* 0.84, $CHCl_3$); IR (film) 1580 cm^{-1}; ^1H NMR δ=0.78 (3H, d, J=7 Hz), 1.06 (9H, s), 1.5—2.1 (3H, complex), 3.49 (1H, t, J=11.4 Hz), 3.62 (1H, dd, J=4, 9 Hz), 3.8—3.9 (2H, complex), 4.11 (1H, dd, J=5, 11.5 Hz), 5.44 (1H, s), 7.35 (10H, complex), and 7.70 (5H, complex); ^{13}C NMR δ=12.4, 19.2, 26.9, 33.9, 35.6, 59.6, 73.1, 79.6, 100.9, 126.0, 127.59, 127.62, 128.1, 128.5, 129.50, 129.54, 133.90, 133.96, 135.5, and 138.8.

To a solution of the silyl ether (578 mg) in $PhCH_3$ (12.5 ml) at −60 °C was added 0.99 M DIBAL in $PhCH_3$ (3.8 ml). After being stirred at the same temperature for 5 h, 3.8 ml of 0.99 M DIBAL in $PhCH_3$ was further added, and the stirring was continued for 2 h; 2 M HCl was then added. The resulting mixture was partitioned between EtOAc and H_2O, and the organic extracts were washed with sat. aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was purified by silica-gel column chromatography (hexane–EtOAc=20:1→1:1) to give **6** (157 mg) along with the starting material (387 mg). **6**: $[\alpha]_D^{20}$ −6.9° (*c* 1.0, $CHCl_3$); IR (film) 3450 and 1590 cm^{-1}; ^1H NMR δ=0.92 (3H, d, J=7 Hz), 1.06 (9H, s), 1.85 (3H, complex), 3.5—3.9 (5H, complex), 4.40 (1H, d, J=11.4 Hz), 4.55 (1H, d, J=11.4 Hz), 7.40 (10H, complex), and 7.70 (5H, complex); ^{13}C NMR δ=13.9, 19.2, 26.9, 34.4, 38.5, 60.4, 66.2, 72.2, 80.9, 127.7, 127.8, 128.4, 129.6, 133.8, 135.6, and 138.3. Found: m/z 463.2656. Calcd for $C_{29}H_{39}O_3Si$: M+H, 463.2665.

(5*S*,6*R*)-6-Benzyloxy-8-(*t*-butyl)diphenylsiloxy-5-methyl-3-octene (7). To a mixture of oxalyl chloride (0.08 ml) and DMSO (0.12 ml) in CH_2Cl_2 (3.4 ml) at −50 °C was added **6** (157 mg) in CH_2Cl_2 (2 ml). After 15 min, Et_3N (0.33 ml) was added, and the stirring mixture was gradually warmed to room temperature during 1 h. The resulting suspension was diluted with H_2O, and extracted with EtOAc. The EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC (hexane–EtOAc=5:1) to give the corresponding aldehyde (127 mg) and unchanged **6** (39 mg).

To a stirring mixture of propyltriphenylphosphonium bromide (289 mg) and 1.65 M *n*-BuLi in hexane (0.49 ml) in THF (2.6 ml) at 0 °C was added the aldehyde (124 mg) in THF (2 ml). After being reacted overnight at the same temperature, the reaction was quenched by addition of aq NH_4Cl. The resulting mixture was partitioned between EtOAc and H_2O; the organic layer was then washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was chromatographed on a silica-gel column (hexane–EtOAc=30:1) to give **7** (66 mg) which was used for the

next step without further separation of the olefinic isomers: ^{1}H NMR δ=3.55 (0.5H, m), 3.80 (0.5H, m), and 5.30 (1H, complex). Found: m/z 429.2221. Calcd for $C_{28}H_{33}O_2Si$: M−C_4H_8, 429.2247.

(3*R*,4*S*)-3-Benzyloxy-1-(*t*-butyl)diphenylsiloxy-4-methyloctane (8). A solution of **7** (157 mg) in EtOAc (5 ml) containing catalytic amounts of 10% Pd on carbon was stirred at room temperature for 5.5 h under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to give **8** as a colorless oil (154 mg): IR (film) 1590 cm^{-1}; ^{1}H NMR δ=0.86 (3H, d, J=7 Hz), 0.90 (3H, t, J=7 Hz), 1.05 (9H, s), 1.3 (5H, complex), 1.5—1.8 (4H, complex), 3.57 (1H, m), 3.80 (2H, complex), 4.37 (1H, d, J=11.2 Hz), 4.52 (1H, d, J=11.2 Hz), 7.25 (4H, complex), 7.40 (7H, complex), and 7.65 (4H, complex); ^{13}C NMR δ= 14.1, 14.3, 19.2, 23.0, 26.9, 29.8, 32.7, 34.9, 60.9, 71.4, 79.4, 127.3, 127.6, 127.7, 128.2, 129.5, 134.0, 135.6, and 139.2. Found: m/z 432.2493. Calcd for $C_{28}H_{36}O_2Si$: M−C_4H_8, 432.2483.

Methyl (3*R*,4*S*)-3-Benzyloxy-4-methyloctanoate (9). To a solution of **8** (154 mg) in THF (3 ml) was added 1 M n-Bu_4NF in THF (0.64 ml), and the solution was kept at room temperature for 3.5 h. The reaction mixture was partitioned between EtOAc and H_2O, and the organic extracts were washed with brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was purified by preparative TLC (hexane–EtOAc=3:1) to give the corresponding alcohol (77 mg).

To a mixture of oxalyl chloride (0.08 ml) and DMSO (0.1 ml) in CH_2Cl_2 (1.5 ml) at −63 °C was added the alcohol (36 mg) in CH_2Cl_2 (2 ml). After 10 min, Et_3N (0.24 ml) was added, and the stirring mixture was gradually warmed to room temperature during 40 min. The resulting suspension was diluted with H_2O, and extracted with EtOAc. The EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC (hexane–EtOAc=8:1) to give the corresponding aldehyde (32 mg).

A mixture of the aldehyde (23 mg) and $KMnO_4$ (16 mg) in t-BuOH (0.5 ml) containing pH 7 phosphate buffer (0.5 ml) was stirred at room temperature for 15 min. After addition of aq $NaHSO_3$, the resulting mixture was partitioned between ether and H_2O, and the ethereal extracts were washed with brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was dissolved in MeOH (0.5 ml), and 90% $TMSCHN_2$ in hexane[9] was added until the reaction solution kept a yellow color. After concentration in vacuo, the residue was purified by a silica-gel column (PhH–EtOAc=100:1) to give **9** (18 mg) as an oil: IR (film) 1740 cm^{-1}; ^{1}H NMR δ=0.90 (3H, t, J=7 Hz, overlapped with 3H signal), 1.10 (1H, m), 1.15—1.4 (4H, complex), 1.52 (1H, m), 1.71 (1H, m), 2.46 (1H, dd, J=4.4, 15.1 Hz), 2.57 (1H, dd, J=8.3, 15.1 Hz), 3.67 (3H, s), 3.84 (1H, m), 4.54 (2H, s), and 7.31 (5H, complex); ^{13}C NMR δ=14.1, 15.0, 22.9, 29.7, 31.8, 36.3, 36.8, 51.6, 72.2, 80.0, 127.4, 127.6, 128.2, 138.8, and 172.8. Found: m/z 278.1832. Calcd for $C_{17}H_{26}O_3$: M, 278.1880.

Methyl (3*R*,4*S*)-3-Hydroxy-4-methyloctanoate (10). A solution of **9** (15 mg) in MeOH (1 ml) in the presence of Pd black was stirred overnight under a hydrogen atmosphere. After filtration, the filtrate was evaporated, and the residue was purified by a silica-gel column (PhH–EtOAc=50:1) to give **10** (9.8 mg) as an oil and recovered **9** (1.9 mg). **10**: $[\alpha]_D^{22}$ +23° (c 0.62, $CHCl_3$); IR (film) 3500 and 1740 cm^{-1}; ^{1}H NMR δ=0.89 (3H, d, J=6.8 Hz), 0.90 (3H, t, J=6.8 Hz), 1.1—1.3 (5H, complex), 1.46 (1H, m), 1.60 (1H, m), 2.41 (1H, dd, J=9.5, 16.2 Hz), 2.49 (1H, dd, J=2.9, 16.2 Hz), 3.71 (3H, s), and 3.87 (1H, ddd, J=2.9, 6.2, 9.5 Hz); ^{13}C NMR δ=14.0, 14.9, 22.9, 29.3, 32.0, 37.6, 38.12, 51.8, 71.8, and 174.0.

(3*R*,4*S*)-1-(*t*-Butyl)diphenylsiloxy-4-methyl-3-octanol (11). A solution of **7** (410 mg) in MeOH (9 ml) containing catalytic amounts of Pd black was stirred overnight under a hydrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to give a residue which was purified by preparative TLC (two times developments, hexane–EtOAc=50:1 and 30:1) to give **11** (245 mg) as an oil: $[\alpha]_D^{20}$ −7.5° (c 1.0, $CHCl_3$); IR (film) 3500 and 1585 cm^{-1}; ^{1}H NMR δ=0.85 (6H, complex), 1.05 (9H, s), 1.1—1.7 (10H, complex), 3.5—3.9 (3H, complex), 7.25 (6H, complex), and 7.70 (4H, complex); ^{13}C NMR δ=14.1, 14.9, 19.0, 23.0, 26.8, 29.6, 32.0, 34.4, 38.7, 63.9, 75.7, 127.8, 129.8, 133.1, and 135.6. Found: m/z 399.2676. Calcd for $C_{25}H_{39}O_2Si$: M+H, 399.2716.

(3*S*,4*S*)-3-Acetoxy-1-(*t*-butyl)diphenylsiloxy-4-methyloctane (12). A mixture of **11** (214 mg), methanesulfonyl chloride (MsCl) (0.47 ml) and pyridine (2.7 ml) in CH_2Cl_2 (2.7 ml) was kept at room temperature overnight. The reaction mixture was partitioned between EtOAc and H_2O, and the organic extracts were washed with sat. aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was purified by a silica-gel column (hexane–EtOAc=30:1) to yield 250 mg of the corresponding mesylate.

A mixture of the mesylate (245 mg) and CsOAc (543 mg) in benzene (20 ml) containing catalytic amounts of 18-Crown-6 was heated at refluxing temperature for 2 d. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was purified by preparative TLC (hexane–EtOAc=50:1 and 30:1) to give **12** (192 mg) as an oil: $[\alpha]_D^{21}$ −16° (c 1.0, $CHCl_3$); IR (film) 1735 cm^{-1}; ^{1}H NMR δ=0.85 (6H, complex), 1.04 (9H, s), 1.2—1.6 (7H, complex), 1.76 (2H, complex), 1.95 (3H, s), 3.64 (2H, t, J=6.5 Hz), 5.00 (1H, m), 7.35 (6H, complex), and 7.60 (4H, complex); ^{13}C NMR δ=14.0, 14.6, 19.1, 21.1, 22.9, 26.8, 29.4, 32.2, 34.0, 36.2, 60.7, 74.4, 127.6, 129.5, 133.7, 135.6, and 170.6. Found: m/z 383.2046. Calcd for $C_{23}H_{31}O_3Si$: M−C_4H_9, 383.2041.

(4*S*)-4-[(1*R*)-1-Methylpentyl]-2-phenyl-1,3-dioxane (13). A solution of **12** (192 mg) in MeOH (4 ml) containing 5 M KOH (0.5 ml) and dioxane (0.5 ml) was kept at room temperature for 3 d. After neutralization by the addition of 1 M HCl, the reaction mixture was partitioned between EtOAc and H_2O, and the organic extracts were washed with brine, dried over anhydrous Na_2SO_4, then evaporated. A mixture of the residue and benzaldehyde dimethyl acetal (10 ml) in CH_2Cl_2 (4.5 ml) in the presence of catalytic amounts of p-TsOH was stirred at room temperature for 2.5 h. The reaction mixture was diluted with EtOAc, washed with sat. aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated. Purification using preparative TLC (hexane–EtOAc=30:1, two times developments) provided **13** (93 mg). **13**: $[\alpha]_D^{21}$ −20° (c 1.0, $CHCl_3$); IR (film) 1620 cm^{-1};

^{1}H NMR δ=0.99 (3H, d, J=6.5 Hz, overlapped with 3H, signal), 1.4—2.1 (9H, complex), 3.65 (1H, m), 3.99 (1H, dd, J=3, 11.4 Hz), 4.26 (1H, broad dd, J=3, 11.4 Hz), 5.49 (1H, s), and 7.40 (5H, complex); ^{13}C NMR δ=14.1, 15.0, 23.0, 28.3, 29.3, 31.8, 37.8, 67.3, 81.1, 101.1, 126.0, 128.1, 128.5, and 132.8. Found: m/z 248.1750. Calcd for $C_{16}H_{24}O_2$: M, 248.1774.

(3*S*,4*S*)-3-Benzyloxy-4-methyl-1-octanol (14). To a stirring solution of **13** (84 mg) in $PhCH_3$ (3.5 ml) at −52 °C was added 1.5 M DIBAL in $PhCH_3$ (0.68 ml), and the reaction mixture was gradually warmed to room temperature during 3 h. After addition of 1 M HCl, the resulting mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with sat. aq $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was separated by preparative TLC (hexane–EtOAc=5:1) to give **14** (73 mg) and the starting material (1 mg). **14**: $[\alpha]_D^{18}$ −61° (c 1.0, $CHCl_3$); IR (film) 3400 cm^{-1}; ^{1}H NMR δ=0.90 (3H, d, J=6.6 Hz, overlapped with 3H, signal), 1.2—1.9 (10H, complex), 3.51 (1H, m), 3.74 (2H, t, J=6 Hz), 4.45 (1H, d, J=11.4 Hz), 4.64 (1H, d, J=11.4 Hz), and 7.33 (5H, complex); ^{13}C NMR δ=14.1, 15.9, 23.0, 29.9, 30.9, 32.1, 35.0, 61.4, 71.5, 83.1, 127.7, 127.8, 128.4, and 138.5. Found: m/z 250.1915. Calcd for $C_{16}H_{26}O_2$: M, 250.1931.

Methyl (3*S*,4*S*)-3-Benzyloxy-4-methyloctanoate (15). To a mixture of oxalyl chloride (0.1 ml) and DMSO (0.13 ml) in CH_2Cl_2 (2 ml) at −72 °C was added **14** (47 mg) in CH_2Cl_2 (47 mg, 2 ml). After 10 min, Et_3N (0.32 ml) was added, and the stirring mixture was gradually warmed to room temperature during 45 min. The resulting suspension was diluted with H_2O, and extracted with EtOAc. The EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by preparative TLC (hexane–EtOAc=8:1) to give the corresponding aldehyde (42 mg).

A mixture of the aldehyde (39 mg) and $KMnO_4$ (25 mg), in t-BuOH (0.8 ml) containing pH 7 phosphate buffer (0.8 ml) was stirred at room temperature for 25 min. After addition of aq $NaHSO_3$, the resulting mixture was partitioned between ether and H_2O, and the ethereal extracts were washed with brine, dried over anhydrous Na_2SO_4, then evaporated. The residue was dissolved in MeOH (0.5 ml), and 90% $TMSCHN_2$ in hexane was added until the reaction solution kept yellow color. After concentration, the residue was purified by a silica-gel column (hexane–EtOAc=10:1) to give the corresponding methyl ester (**15**, 27 mg). $[\alpha]_D^{20}$ −35° (c 1.0, $CHCl_3$); IR (film) 1740 cm^{-1}; ^{1}H NMR δ=0.95 (6H, complex), 1.1—1.6 (7H, complex), 2.5 (2H, complex), 3.66 (3H, s), 3.75 (1H, m), 4.53 (2H, s), and 7.30 (5H, s); ^{13}C NMR δ=14.1, 15.0, 22.9, 29.7, 31.8, 36.3, 36.8, 51.6, 72.2, 80.0, 127.4, 127.6, 128.2, 138.8, and 172.8. Found: m/z 263.1619. Calcd for $C_{16}H_{23}O_3$: M−CH_3, 263.1645.

Methyl (3*S*, 4*S*)-3-Hydroxy-4-methyloctanoate (16). A solution of **15** (19 mg) in MeOH (1 ml) in the presence of Pd black was stirred for 2.5 h under hydrogen atmosphere. After filtration, the filtrate was evaporated to give **16** (14 mg) as an oil: $[\alpha]_D^{20}$ −44° (c 0.98, $CHCl_3$). The spectral data (^{1}H and ^{13}C) were superimposable to those of **4**.

The authors wish to thank both Nippon Kayaku Co., Ltd. and Ajinomoto Co., Inc. for measurements of the FAB mass spectra. Regarding the bioassay of **1**, we are indebted to Sumitomo Chemical Co., Ltd.

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