HETEROCYCLES, Vol. 68, No. 1, 2006, pp. 111 – 123. © The Japan Institute of Heterocyclic Chemistry
Received, 11th October, 2005, Accepted, 7th December, 2005, Published online, 9th December, 2005. COM-05-10589

SHIMALACTONES, NEURITOGENIC POLYKETIDES FROM A MARINE-DERIVED FUNGUS *EMERICELLA VARIECOLOR* GF10

Hong Wei, Takuya Itoh, Naoyuki Kotoku, and Motomasa Kobayashi*

Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka 1-6, Suita, Osaka 565-0871, Japan. e-mail: kobayasi@phs.osaka-u.ac.jp

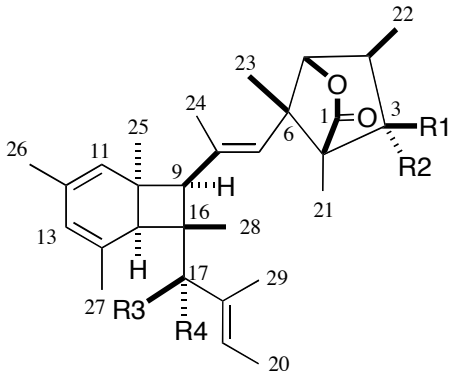
Abstract – Shimalactone B (**2**), a novel polyketide having bicyclo[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units, was isolated as a minor constituent from a cultured marine-derived fungus of *Emericella variecolor* GF10. The chemical structure of **2** and the structural relationship between shimalactones A and B (**2**) were determined by detailed NMR and CD spectroscopic analyses and chemical derivatization. Shimalactones A and B (**2**) induced neuritogenesis against neuroblastoma Neuro 2A cells at 10 μg/mL concentration.

INTRODUCTION

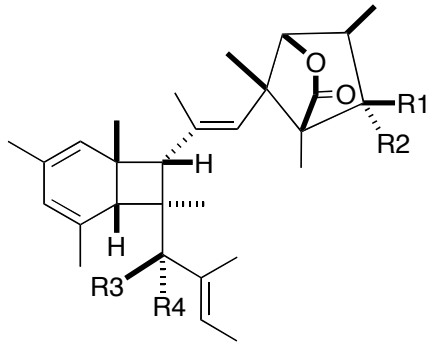
In recent years, marine microorganisms have been paid much attention as a significant source in the search for new drug leads.[1-3] As a part of our ongoing search for new biologically active substances from marine microorganisms, we previously reported the isolation of ophiobolins-type cytotoxic sesterterpenes[4] and a novel polyketide named shimalactone A (**1**) [5] from the marine-derived fungus, *Emericella variecolor* GF10. Shimalactone A (**1**) possessed a unique carbon skeleton such as bicyclo[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units, of which the stereostructure was deduced from 2D NMR and X-Ray crystallographic analyses of **1** and its derivatives. Continued study on the extract of *E. variecolor* GF10 has led us to isolation of shimalactone B (**2**) as a minor constituent. Details of the structure elucidation of shimalactone B (**2**) are presented here.

RESULTS AND DISCUSSION

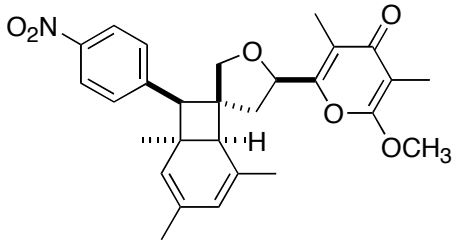
The fungus strain of *Emericella variecolor* GF10 was cultured for 2 weeks at 30 ºC on a solid rice-based medium prepared with artificial seawater. The MeOH extract was fractionated by silica gel column chromatography and HPLC to obtain a new polyketide named shimalactone B (**2**) together with shimalactone A (**1**).



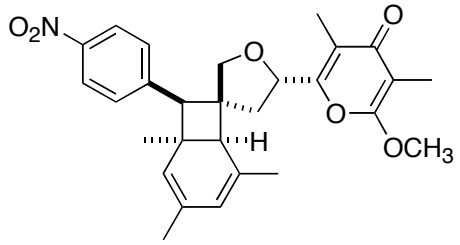
1: R^1, R^2 = O, R^3 = OH, R^4 = H
3: R^1, R^2 = O, R^3, R^4 = O
5: R^1 = R^4 = H, R^2 = R^3 = OH
7: R^2 = R^4 = H, R^1 = R^3 = OH



2: R^1, R^2 = O, R^3 = OH, R^4 = H
4: R^1, R^2 = O, R^3, R^4 = O
6: R^1 = R^4 = H, R^2 = R^3 = OH
8: R^2 = R^4 = H, R^1 = R^3 = OH
11: R^1, R^2 = O, R^3 = $OCOC_6H_5$, R^4 = H
12a: R^1, R^2 = O, R^3 = O(*R*)-MTPA, R^4 = H
12b: R^1, R^2 = O, R^3 = O(*S*)-MTPA, R^4 = H



SNF4435C (**16**)



SNF4435C (**17**)

Shimalactone B (**2**) was obtained as a colorless oil. Its molecular formula $C_{29}H_{40}O_4$ was determined by HRFABMS and NMR spectral analysis. The ^{1}H NMR spectrum of **2** indicated the presence of eight singlet-methyl protons (δ_H 1.77, 1.66, 1.57, 1.50, 1.32, 1.15, 1.13, 0.98), two doublet-methyl protons (δ_H 1.58, 1.21), and nine methine protons (δ_H 5.46, 5.42, 5.28, 5.12, 4.76, 3.66, 3.03, 2.77, 2.68), which were closely similar to those of **1**.[5] A careful analysis of its 2D NMR (COSY, HMQC, HMBC, NOESY) spectral data resulted in the conclusion that compound (**2**) has the same planar structure as **1** (Figure 1 and Table 1). The *E*-configuration for both trisubstituted olefins was deduced by the presence of the NOE correlations between H_3-23 (δ_H 1.32, s) and H_3-24 (δ_H 1.77, s), H_3-20 (δ_H 1.58, d, J = 6.6 Hz) and H_3-29 (δ_H 1.50, s), and the absence of the NOE between H-7 (δ_H 5.28, s) and H_3-24 (Figure 2).

The strong NOE correlations among H-9 (δ_H 3.03, s), H-15 (δ_H 2.68, s), and H_3-25 (δ_H 1.13, s) indicated that the bicyclo[4.2.0]octadiene unit in **2** has the same relative stereochemistry as that of **1** (Figure 2). Then, it was suggested that compound (**2**) might be a stereo-isomer of **1** at the C-17 secondary hydroxy function, since slight differences of the NMR spectra were observed at C-9 (**1:** δ_H 2.68 s, δ_C 60.7; **2:** δ_H 3.03 s, δ_C 58.8), C-15 (**1:** δ_H 2.39 s, δ_C 51.7; **2:** δ_H 2.68 s, δ_C 47.5), C-16 (**1:** δ_C 49.6; **2:** δ_C 48.6), C-17 (**1:**

δ_H 3.84 s, δ_C 86.3; **2:** δ_H 3.66 s, δ_C 85.4), C-18 (**1:** δ_C 136.5; **2:** δ_C 136.2), C-29 (**1:** δ_H 1.55 s, δ_C 14.4; **2:** δ_H 1.50 s, δ_C 12.6)-signals. The Dess-Martin periodinane treatment of **1** and **2** afforded 17-keto derivatives (**3**) and (**4**), respectively. Although the NMR spectra of **3** and **4** were also highly similar, **3** and **4** were not identical by HPLC analysis. Then, an intensive study was required to elucidate the stereostructure of **2**.

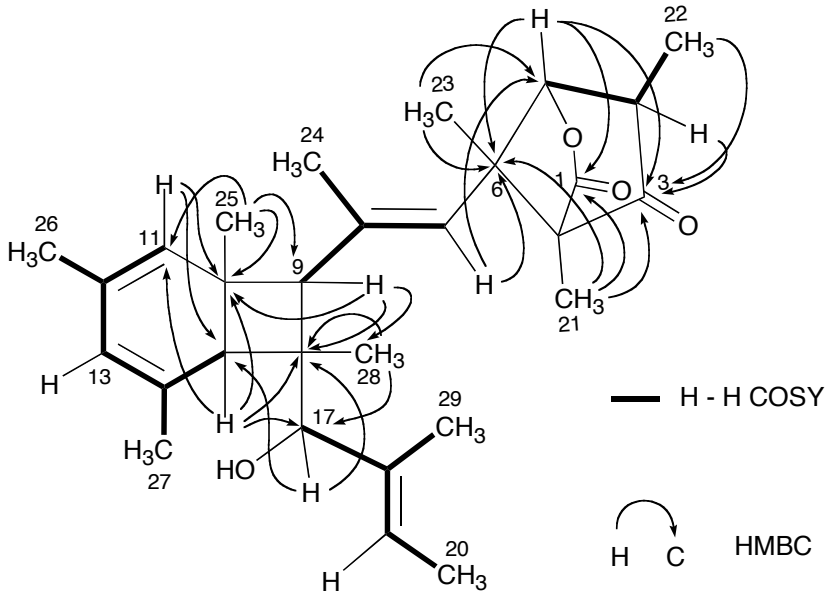


Figure 1. COSY and key HMBC correlations for shimalactone B (**2**)

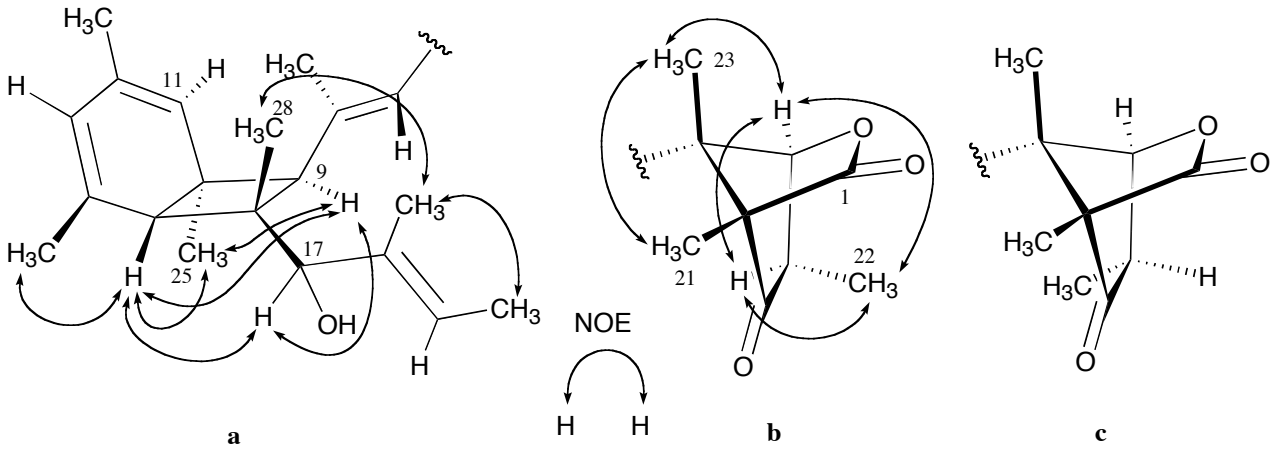


Figure 2. Key NOE correlations of shimalactone B (**2**).

Firstly, the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in **2** was proposed as **b** or **c** in Figure 2 on the basis of its NOE correlations. Compound (**2**) was treated with $NaBH_4$ in Et_2O to give two reduced products (**6**) and (**8**) (Figure 3). Comparison of the ^1H NMR spectra of **6** and **8** with **5** and **7**,

which were produced from **1** by the same $NaBH_4$ treatment,[5] revealed that the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in **6** and **8** were identical with those of **5** and **7**, respectively. Then, the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in **2** was clarified to be the same as that of **1**.

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectral data for shimalactone B (**2**) in $CDCl_3$.

No.	^{13}C δ_C	^1H δ_H (mult., *J* (Hz))	HMBC correlations [a)]
1	171.8		
2	58.2		
3	207.6		
4	44.0	2.77 (1H, qd, 7.4, 1.9)	C-3, C-22
5	85.4	5.12 (1H, d, 1.9)	C-1, C-3, C-6
6	70.1		
7	123.3	5.28 (1H, s)	C-5, C-6, C-9
8	139.3		
9	58.8	3.03 (1H, s)	C-7, C-8, C-10, C-16, C-24, C-25, C-28
10	41.1		
11	123.7	4.76 (1H, s)	C-10, C-13, C-15
12	130.0		
13	123.5	5.46 (1H, s)	C-11, C-15
14	132.4		
15	47.5	2.68 (1H, s)	C-10, C-11, C-14, C-16, C-17, C-25
16	48.6		
17	85.4	3.66 (1H, s)	C-9, C-15, C-16, C-18, C-19
18	136.2		
19	123.7	5.42 (1H, q, 6.6)	C-17, C-20, C-29
20	13.0	1.58 (3H, d, 6.6)	C-18, C-19
21	4.7	1.15 (3H, s)	C-1, C-3, C-6
22	11.6	1.21 (3H, d, 7.4)	C-3, C-4, C-5
23	16.5	1.32 (3H, s)	C-2, C-5, C-6, C-7
24	20.2	1.77 (3H, s)	C-7, C-8, C-9
25	31.0	1.13 (3H, s)	C-9, C-10, C-11, C-15
26	22.0	1.66 (3H, s)	C-11, C-12, C-13
27	23.5	1.57 (3H, s)	C-14, C-15
28	15.5	0.98 (3H, s)	C-15, C-16, C-17
29	12.6	1.50 (3H, s)	C-18, C-19

a) C coupled with H.

Secondly, the CD spectra of **1** and **2** showed a complex and different pattern from each other (Figures 4, 5). Compounds (**7**) and (**8**) lacking a 5-membered ketone chromophore also showed complex and different CD spectra, respectively. The differential CD spectum of **1** and **7** showed a strong negative maximum at 231 nm and a weak negative maximum at 284 nm (Figure 4), while that of **2** and **8** showed a strong negative maximum at 245 nm and a weak positive maximum at 288 nm (Figure 5). The CD maxima at around 240 nm of the differential CD spectra were presumed to come mainly from the interaction between the five-membered ketone and the lactone carbonyl chromophores, and the CD maxima at around 285 nm were presumed to come from the interaction between the five-membered

ketone and the homoannular diene chromophores. Based on the above information, we tentatively presumed that the absolute configuration of the oxabicyclo[2.2.1]heptane unit of **2** is the same as that of **1**, while the absolute configuration of the bicyclo[4.2.0]octadiene unit of **2** might be different from that of **1**.

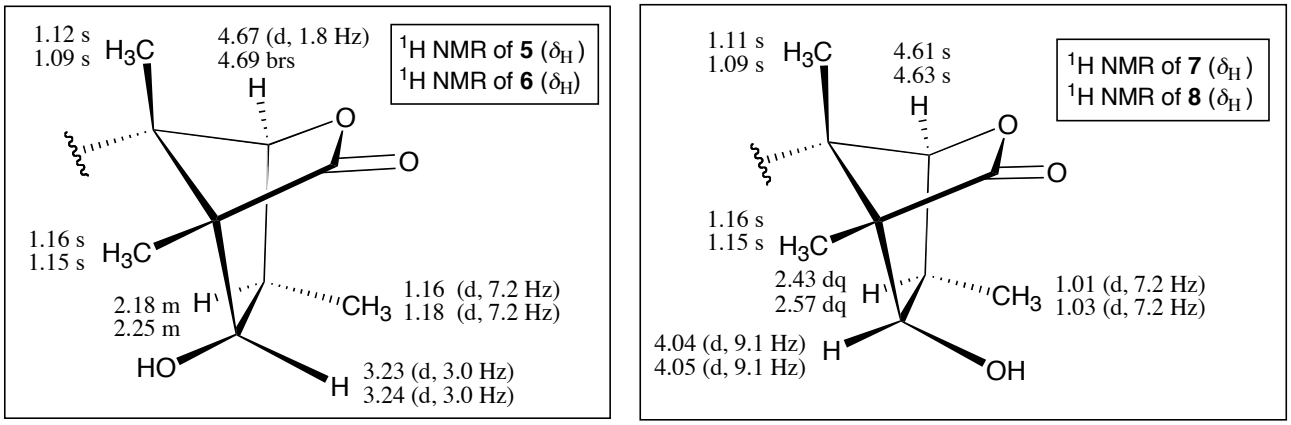


Figure 3. ^{1}H NMR spectral data for the oxabicyclo[2.2.1]heptane units in **5**, **6**, **7** and **8**

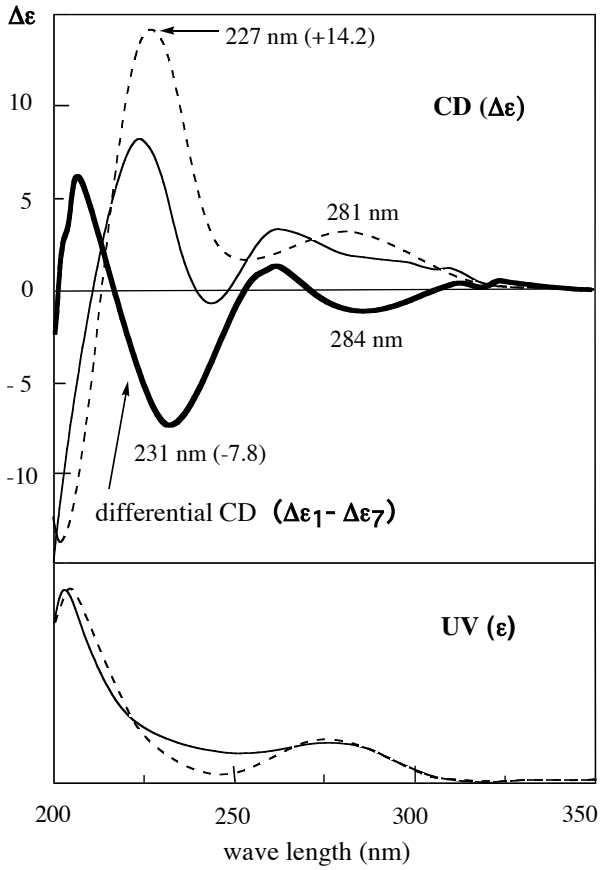


Figure 4. CD and UV spectra of shimalactone A (**1**, solid line) and **7** (dotted line) and differential CD spectrum (bold line) of **1** and **7** in methanol

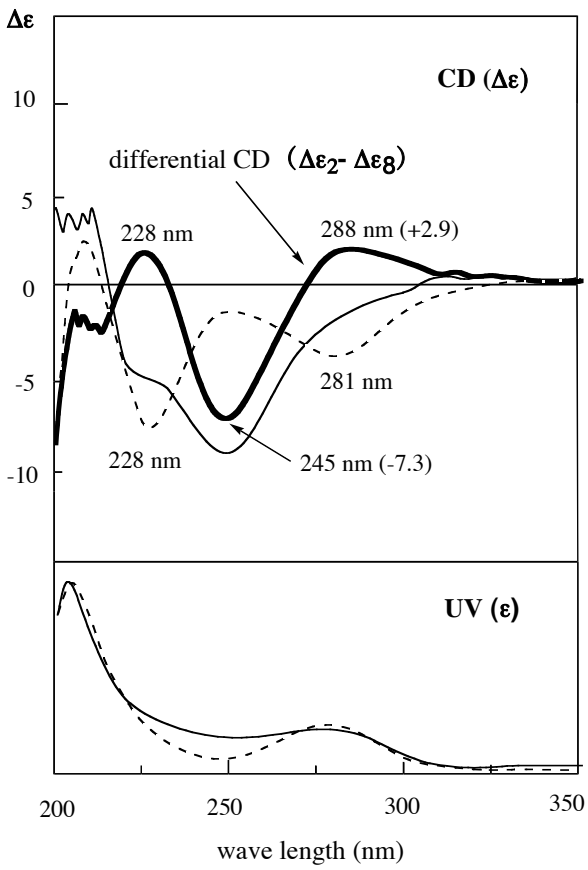


Figure 5. CD and UV spectra of shimalactone B (**2**, solid line) and **8** (dotted line) and differential CD spectrum (bold line) of **2** and **8** in methanol

Thirdly, the ozonolysis followed by 2,4-dinitrophenylhydrazine treatment of **1** and **2** respectively afforded hydrazone derivatives (**9** and **10**), which were found by spectral analysis to be identical including the optical rotation (Figure 6). This result confirmed that the absolute configuration for the oxabicyclo[2.2.1]heptane unit of **2** was the same as that of **1**. Then, the absolute configuration of the bicyclo[4.2.0]octadiene unit of **2** should be opposite to that of **1**, since **3** and **4** are different.

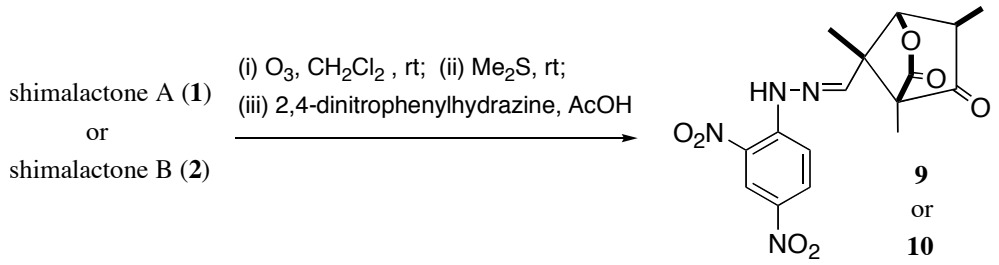


Figure 6. Hydrazone derivatives prepared from shimalactones A (**1**) and B (**2**)

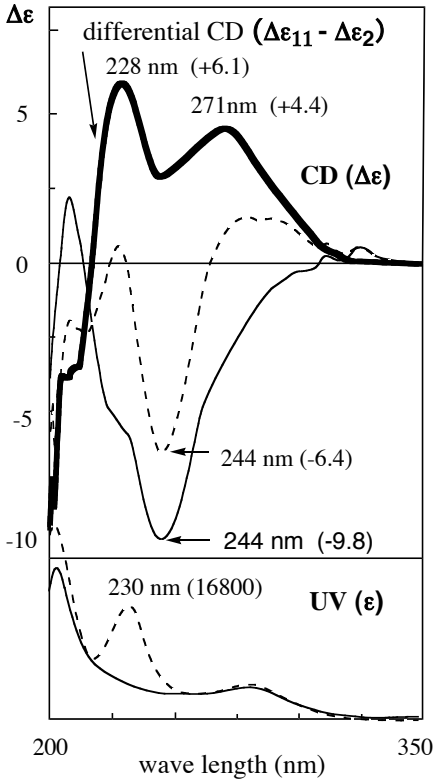
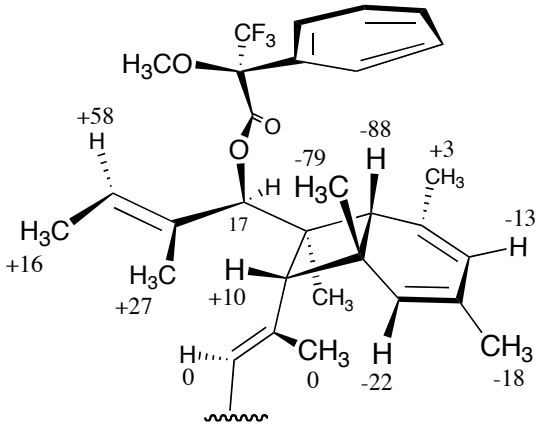


Figure 7. CD and UV spectra of shimalactone B (**2**, solid line) and **11** (dotted line) and differential CD spectrum (bold line) of **11** and **2** in methanol



12b: (*S*)-MTPA ester

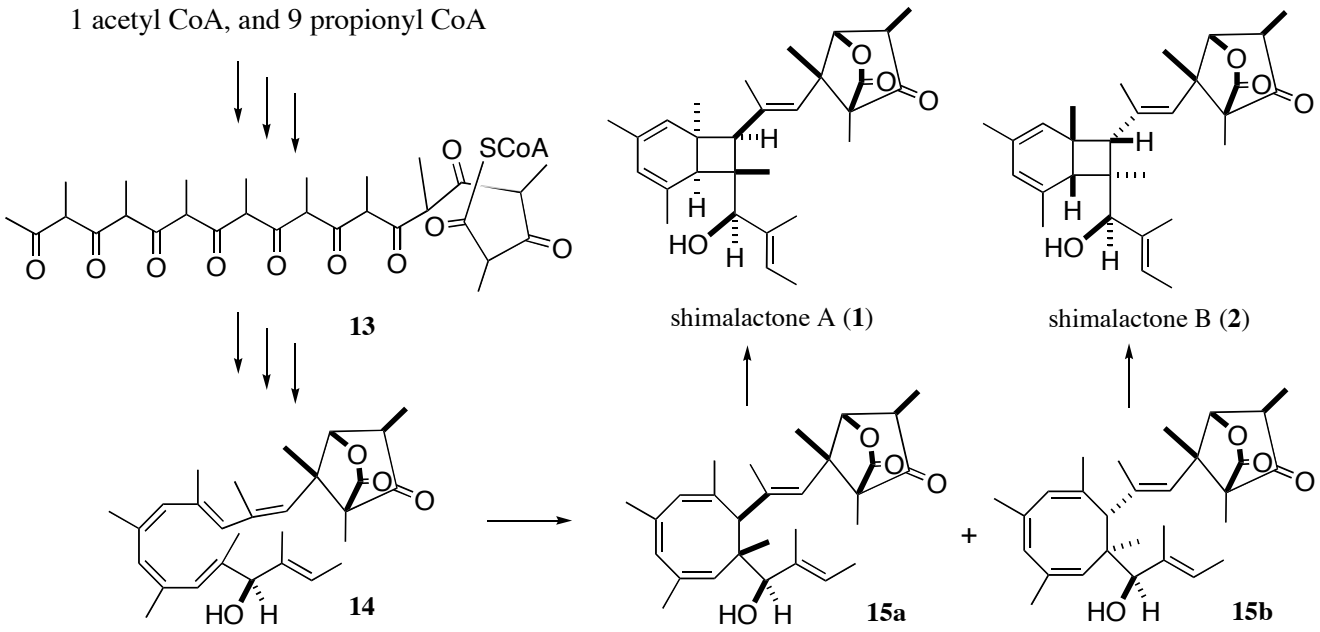
$\Delta\delta = \delta_{(S)\text{-MTPA ester}} - \delta_{(R)\text{-MTPA ester}}$ (Hz)

Figure 8. Application of modified Mosher's method to shimalactone B (**2**)

To determine the absolute configuration at the C-17 secondary hydroxy group in **2**, the pyridine solution of **2** was treated with benzoyl chloride and 4-(dimethylamino)pyridine (DMAP) at 80 °C to give the

benzoate (**11**). The differential CD spectrum of **11** and **2** showed two positive maxima at 228 nm ($\Delta\varepsilon$ = + 6.1) and 271 nm (+4.4). The CD maximum at 228 nm can be attributed to the interaction (plus chirality) between the benzoate and the Δ^{18} olefin chromophores, suggesting the *R* configuration at C-17 in **11**.[5,6] On the other hand, the CD maximum at 271 nm, which was not observed in the differential CD spectrum between **1** and its benzoate derivative, is presumed to come from the interaction between the benzoate and the homoannular diene chromophores in **11** (Figure 7).

The absolute configuration at C-17 in **2** was further confirmed by application of the modified Mosher's method.[7] Thus, a pyridine solution of **2** was treated with DMAP, (*S*)-(+)- or (*R*)-(-)-methoxy(trifluoromethyl)phenylacetyl chloride [(*S*)- or (*R*)-MTPA chloride] at 80 ºC to obtain two Mosher esters (**12a**) and (**12b**). The detailed NMR spectral analyses of the 17-MTPA esters (**12a**) and (**12b**) supported that the absolute configuration at C-17 in **2** is *R* (Figure 8). Consequently, it was clarified that shimalactone B (**2**) is an enantiomer of the bicyclo[4.2.0]octadiene unit in shimalactone A (**1**).



Scheme 1. Proposed biosynthesis of shimalactones

Shimalactones A (**1**) and B (**2**) are polyketides having two bicyclic ring systems (bicyclo[4.2.0]octadiene unit and oxabicyclo[2.2.1]heptane unit). Recently, two similar polyketides, SNF4435C (**16**) and SNF4435D (**17**), having a bicyclo[4.2.0]octadiene unit have been found as immunosuppressive substances from the cultured broth of *Streptomyces spectabilis*.[8] The biomimetic synthesis of the bicyclo[4.2.0]octadiene unit has been also achieved from an all-*trans* tetraene precursor through a tandem double electrocyclization.[9,10] Shimalactones A (**1**) and B (**2**) are presumed to be biosynthesized from an acetate and nine propionates. Thus, a polyene lactone (**14**) is formed from a precursor (**13**). Then,

conrotatory 8π-electrocyclization of the common precursor (**14**) affords cyclooctatrienes (**15a**) and (**15b**), which are stereoselectively converted to **1** and **2** by disrotatory 6π-electrocyclization (Scheme 1).[9,10] The absolute configurations of SNF4435C (**16**) and SNF4435D (**17**) have not yet been elucidated. On the basis of the structure-relationship of shimalactones A and B, SNF4435C (**16**) and SNF4435D (**17**) might be enantiomers of the bicyclo[4.2.0]octadiene unit.

Shimalactones A (**1**) and B (**2**) induced neuritogenesis in neuroblastoma Neuro 2A cells at the concentration of 10 μg/mL. At the higher concentration of 20 μg/mL, they were cytotoxic against Neuro 2A cells.

EXPERIMENTAL

Optical rotations were measured using a JASCO DIP-370 polarimeter. UV spectra were performed with a Shimazu UV-2425 spectrophotometer. CD spectra were recorded on a JASCO J-720 spectrometer (L = 1 mm). IR spectra were recorded on a JASCO FT/IR-5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity Inova 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer. HRFABMS spectra were obtained on a JEOL JMS SX-102 mass spectrometer. Silica gel (Merck 60-230 mesh) and precoated thin-layer chromatography (TLC) plates (Merck, Kiesel gel, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC were detected by spraying $1\%Ce(SO_4)_2/10\%H_2SO_4$ with subsequent heating. Artificial seawater was prepared by Aquamarine (Yashima Pure Chemical Co. LTD, Japan).

Fungus material, culture conditions, and extraction. The *Emericella variecolor* GF10 strain was separated from the marine sediment collected from a depth of 70 m off Gokasyo Gulf, Mie Prefecture, Japan, in 2002 and deposited in our laboratory.[4] The GF10 strain was cultured in the MG medium (malt extract: 20 g, glucose: 20 g, bact peptone: 1 g, artificial seawater: 1000 mL) for 5 days at 30 ºC. Then, the broth of the strain was inoculated into rice solid medium (rice: 25 g, artificial seawater: 50 mL, in a 500 mL flask) and cultured under static conditions for 2 weeks at 30 ºC. The culture was extracted with acetone and a mixed solvent (EtOAc-MeOH-acetone= 1:2:4), and then the organic solvent was combined and evaporated under reduced pressure to give an extract. The extract was partitioned into a 2-butanone-H_2O mixture, and the 2-butanone-soluble portion (12.0 g) was further partitioned into an *n*-hexane-90% aq. MeOH mixture to furnish a MeOH extract (5.0 g).

Isolation of shimalactone B (2). The MeOH extract (5.0 g) of the solid culture (75 g x 40) was fractionated by SiO_2 column chromatography (*n*-hexane-EtOAc) to give seven fractions (A-G). Fraction B (460 mg) was then subjected to SiO_2 column chromatography ($CHCl_3$-MeOH) to afford four fractions (B-1 to B-4). Fraction B-3 (183 mg) was separated by reversed phase HPLC (Cosmosil 5C18-AR,

MeOH-H_2O = 80:20) to obtain three fractions B-3.1, B-3.2 and B-3.3. Fraction B-3.3 (112 mg) was further purified by HPLC (Cosmosil 5SL, *n*-hexane-EtOAc = 88:12) to furnish shimalactone A (**1**, 77 mg) and shimalactone B (**2**, 25 mg).

Shimalactone B (2) was obtained as colorless oil: $[\alpha]^{23}_D$ –48° (*c* 1.50, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.15), 281 (3.57) nm; CD (MeOH) $\Delta\varepsilon_{207}$ +2.1, $\Delta\varepsilon_{213}$ 0, $\Delta\varepsilon_{225}$ -5.2 (sh), $\Delta\varepsilon_{244}$ -9.8, $\Delta\varepsilon_{307}$ 0, $\Delta\varepsilon_{324}$ 0.5; IR (KBr) ν_{max} 3510, 2975, 1795, 1750 cm^{-1}; ^1H and ^{13}C NMR, see Table 4; FABMS *m/z* 475 $[M+Na]^+$; HRFABMS *m/z* 475.2815 (calcd for $C_{29}H_{40}O_4Na$, 475.2824).

Dess-Martin oxidation of shimalactones A (1) and B (2). A CH_2Cl_2 (1.5 mL) solution of **1** (6 mg) was treated with Dess-Martin periodinane (15 mg) and the mixture was stirred for 1 h at rt. The reaction mixture was poured into 10 mL of saturated aq. $NaHCO_3$ solution then extracted with EtOAc. The EtOAc phase was washed with brine and dried over anhydrous $MgSO_4$. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by SiO_2 gel chromatography (eluted with *n*-hexane-EtOAc = 1 : 1) to give **3** (6 mg). Treatment of **2** (6 mg) by the same procedure as described above afforded **4** (5 mg). When they were analyzed by HPLC (Cosmosil 5SL, 10 mm x 250 mm; mobile phase, *n*-hexane-EtOAc = 5:1; flow rate, 4.0 mL/min), **3** (t_R 8.2 min) and **4** (t_R 8.6 min) displayed different retention time.

3: colorless amorphous; ^1H NMR ($CDCl_3$, 600 MHz) δ_H 6.20 (1H, q, *J* = 6.9 Hz, H-19), 5.55 (1H, s, H-13), 5.38 (1H, s, 7-H), 5.18 (1H, d, *J* = 1.9 Hz, H-5), 4.72 (1H, s, H-11), 3.03 (1H, s, H-15), 2.83 (1H, s, H-9), 2.65 (1H, dq, *J* = 1.9, 7.1 Hz, H-4), 1.82 (3H, s, H_3-24), 1.77 (3H, d, *J* = 6.9 Hz, H_3-20), 1.74 (3H, s, H_3-29), 1.72 (3H, s, H_3-27), 1.69 (3H, s, H_3-26), 1.41 (3H, s, H_3-23), 1.30 (3H, s, H_3-28), 1.25 (3H, d, *J* = 7.1 Hz, H_3-22), 1.18 (3H, s, H_3-21), 1.04 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 207.2 (C, C-3), 206.3 (C, C-17), 171.4 (C, C-1), 137.6 (C, C-8), 136.9 (CH, C-19), 134.7 (C, C-18), 132.7 (C, C-14), 130.0 (C, C-12), 126.0 (C, C-7), 123.9 (CH, C-13), 123.7 (CH, C-11), 84.7 (CH, C-5), 70.0 (C, C-6), 61.6 (CH, C-9), 58.2 (C, C-2), 55.2 (C, C-16), 49.6 (CH, C-15), 44.2 (CH, C-4), 41.6 (C, C-10), 30.9 (CH_3, C-25), 22.8 (CH_3, C-27), 22.1 (CH_3, C-26), 20.8 (CH_3, C-24), 18.1 (CH_3, C-28), 16.9 (CH_3, C-23), 14.5 (CH_3, C-20), 12.0 (CH_3, C-29), 11.6 (CH_3, C-22), 4.7 (CH_3, C-21). FABMS *m/z* 473 $[M+Na]^+$; HRFABMS *m/z* 473.2675 (calcd for $C_{29}H_{38}O_4Na$, 473.2668).

4: colorless amorphous; ^1H NMR ($CDCl_3$, 600 MHz) δ_H 6.31 (1H, q, *J* = 6.9 Hz, 19-H), 5.54 (1H, s, H-13), 5.50 (1H, s, H-7), 5.17 (1H, d, *J* = 1.9 Hz, H-5), 4.60 (1H, s, H-11), 3.02 (1H, s, H-15), 2.80 (1H, s, H-9), 2.75 (1H, dq, *J* = 1.9, 7.1 Hz, H-4), 1.80 (3H, s, H_3-24), 1.77 (3H, d, *J* = 6.9 Hz, H_3-20), 1.74 (3H, s, H_3-29), 1.72 (3H, s, H_3-27), 1.69 (3H, s, H_3-26), 1.40 (3H, s, H_3-23), 1.34 (3H, s, H_3-28), 1.26 (3H, d, *J* =

7.1 Hz, H_3-22), 1.18 (3H, s, H_3-21), 1.06 (3H, s, H_3-25); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 207.0 (C, C-3), 206.3 (C, C-17), 171.3 (C, C-1), 137.5 (C, C-8), 137.0 (CH, C-19), 134.7 (C, C-18), 132.8 (C, C-14), 130.6 (C, C-12), 125.7 (CH, C-7), 124.1 (CH, C-13), 123.2 (CH, C-11), 85.1 (CH, C-5), 70.0 (C, C-6), 61.0 (CH, C-9), 58.3 (C, C-2), 55.0 (C, C-16), 49.6 (CH, C-15), 44.2 (CH, C-4), 41.8 (C, C-10), 31.1 (CH_3, C-25), 22.8 (CH_3, C-27), 21.9 (CH_3, C-26), 21.6 (CH_3, C-24), 18.0 (CH_3, C-28), 16.9 (CH_3, C-23), 14.5 (CH_3, C-20), 12.0 (CH_3, C-29), 11.6 (CH_3, C-22), 4.7 (CH_3, C-21); FABMS *m/z* 473 $[M+Na]^+$; HRFABMS *m/z* 473.2657 (calcd for $C_{29}H_{38}O_4Na$, 473.2668).

Reduction of shimalactones A (1) and B (2). An Et_2O (10 mL) solution of **1** (21 mg) was treated with $NaBH_4$ (5 mg) for 4 days at rt. The reaction mixture was filtered, and the filtrate was purified by HPLC (Cosmosil 5SL, *n*-hexane-EtOAc= 4:1) to give **5** (9 mg) and **7** (7 mg).[5] The treatment of **2** (20 mg) with the same procedure afforded **6** (9 mg) and **8** (7 mg).

6: colorless amorphous; ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H 5.51 (1H, s, H-7), 5.50 (1H, q, J = 6.7 Hz, H-19), 5.45 (1H, s, H-13), 4.92 (1H, s, H-11), 4.69 (1H, brs, H-5), 3.89 (1H, s, H-17), 3.24 (1H, d, J = 3.0 Hz, H-3), 2.90 (1H, s, H-9), 2.47 (1H, s, H-15), 2.25 (1H, m, H-4), 1.75 (3H, s, H_3-24), 1.66 (3H, s, H_3-26), 1.57 (3H, d, J = 6.7 Hz, H_3-20), 1.56 (3H, s, H_3-29), 1.48 (3H, s, H_3-27), 1.18 (3H, d, J = 7.2 Hz, H_3-22), 1.15 (3H, s, H_3-21), 1.12 (3H, s, H_3-25), 1.09 (3H, s, H_3-23), 1.08 (3H, s, H_3-28); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 178.3 (C, C-1), 136.8 (C, C-8), 135.8 (C, C-18), 132.4 (C, C-14), 129.2 (C, C-12), 127.3 (CH, C-7), 124.3 (CH, C-11), 124.2 (CH, C-19), 123.7 (CH, C-13), 88.4 (CH, C-5), 88.3 (CH, C-17), 80.0 (CH, C-3), 64.8 (CH, C-9), 59.9 (C, C-6), 56.2 (C, C-2), 50.2 (C, C-16), 48.4 (CH, C-15), 45.0 (CH, C-4), 41.3 (C, C-10), 31.6 (CH_3, C-25), 23.5 (CH_3, C-27), 22.0 (CH_3, C-26), 18.5 (CH_3, C-24), 16.4 (CH_3, C-23), 14.5 (CH_3, C-22), 14.2 (CH_3, C-28), 13.3 (CH_3, C-29), 13.0 (CH_3, C-20), 6.5 (CH_3, C-21); FABMS *m/z* 477 $[M+Na]^+$; HRFABMS *m/z* 477.2960 (calcd for $C_{29}H_{42}O_4Na$, 477.2981).

8: colorless amorphous; UV (MeOH) λ_{max} (log ε) 202 (4.20), 281 (3.60) nm; CD (MeOH) $\Delta\varepsilon_{202}$ 3.5, $\Delta\varepsilon_{215}$ 0, $\Delta\varepsilon_{228}$ -7.8, $\Delta\varepsilon_{248}$ -1.7, $\Delta\varepsilon_{281}$ -3.5; ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H 5.47 (1H, s, H-13), 5.42 (1H, q, J = 6.7 Hz, H-19), 5.31 (1H, s, H-7), 4.85 (1H, s, H-11), 4.63 (1H, s, H-5), 4.05 (1H, d, J = 9.1 Hz, H-3), 3.66 (1H, s, H-17), 3.04 (1H, s, H-9), 2.71 (1H, s, H-15), 2.57 (1H, dq, J = 9.1, 7.2 Hz, H-4), 1.66 (3H, s, H_3-24), 1.65 (3H, s, H_3-26), 1.58 (3H, d, J = 6.7 Hz, H_3-20), 1.57 (3H, s, H_3-27), 1.51 (3H, s, H_3-29), 1.15 (3H, s, H_3-21), 1.13 (3H, s, H_3-25), 1.09 (3H, s, H_3-23), 1.07 (3H, s, H_3-28), 1.03 (3H, d, J = 7.2 Hz, H_3-22); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 177.0 (C, C-1), 137.2 (C, C-8), 136.3 (C, C-18), 132.4 (C, C-14), 129.7 (C, C-12), 124.5 (CH, C-7), 124.1 (CH, C-11), 123.6 (CH, C-19), 123.5 (CH, C-13), 87.0 (CH, C-5), 85.3 (CH, C-17), 74.8 (CH, C-3), 61.0 (C, C-6), 58.7 (CH, C-9), 54.3 (C, C-2), 48.8 (C, C-16), 47.4 (CH, C-15), 41.1 (C, C-10), 36.2 (CH, C-4), 30.8 (CH_3, C-25), 23.5 (CH_3, C-27), 22.1 (CH_3, C-26), 19.5

(CH_3, C-24), 16.2 (CH_3, C-23), 16.0 (CH_3, C-28), 13.0 (CH_3, C-20), 12.5 (CH_3, C-29), 9.1 (CH_3, C-22), 7.9 (CH_3, C-21); FABMS *m/z* 477 $[M+Na]^+$; HRFABMS *m/z* 477.2999 (calcd for $C_{29}H_{42}O_4Na$, 477.2981).

Preparation of Hydrazone Derivatives 9 and 10. A CH_2Cl_2 (10 mL) solution of shimalactone A (**1**, 16 mg) was treated with ozone (O_3) for 20 min at rt, and then $(CH_3)_2S$ (0.1 mL) was added. After stirring for 2 h at rt, the mixture was treated with 2,4-dinitrophenylhydrazine (20 mg) and AcOH (0.1 mL) and further stirred for 1 h at rt. The reaction mixture was poured into saturated aq. $NaHCO_3$ solution and then extracted twice with EtOAc. The EtOAc phase was washed with brine and dried over anhydrous $MgSO_4$. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by SiO_2 gel chromatography (*n*-hexane-EtOAc = 1 : 1) to give **9** (7 mg). Shimalactone B (**2**, 12 mg) was treated by the same procedure to afford **10** (4 mg).

9: yellow amorphous; $[\alpha]^{20}_D$ –85° (*c* 0.51, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H 11.10 (1H, s), 9.10 (1H, d, *J* = 2.3 Hz), 8.40 (1H, dd, *J* = 9.5, 2.3 Hz), 7.61 (1H, d, *J* = 9.5 Hz), 7.35 (1H, s), 4.92 (1H, d, *J* = 2.2 Hz), 2.72 (1H, dq, *J* = 2.2, 7.2 Hz), 1.53 (3H, s), 1.37 (3H, s), 1.26 (3H, d, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 205.4, 170.6, 145.6 (C x 2), 144.3, 130.7, 123.1, 116.5, 83.0, 68.4, 60.4, 59.5, 43.9, 15.9, 11.4, 5.9; FABMS *m/z* 399 $[M+Na]^+$; HRFABMS *m/z* 399.0932 (calcd for $C_{16}H_{16}N_4O_7Na$, 399.0917).

10: yellow amorphous; $[\alpha]^{20}_D$ -84° (*c* 0.28, MeOH).

Benzoylation of shimalactone B (2). A solution of **2** (5 mg) in pyridine (1.0 mL) was treated with benzoyl chloride (0.1 mL) and DMAP (2.0 mg) and the mixture was stirred for 2 h at 80 ºC. The reaction was quenched by saturated aq. NH_4Cl and extracted with EtOAc. After being washed with saturated aq. NH_4Cl, saturated aq. $NaHCO_3$, and brine in sequence, the EtOAc layer was dried over $MgSO_4$ and evaporated *in vacuo* to afford a crude product. The crude product was purified by reversed phase HPLC (Cosmosil 5C18-AR, MeOH-H_2O = 80:20) to obtain **11** (5 mg).

11: colorless oil; IR (KBr) ν_{max} 2970, 1795, 1750, 1720 cm^{-1}; UV (MeOH) λ_{max} (log ε) 201 (4.39), 230 (4.22), 280 (3.62); CD (MeOH) $\Delta\varepsilon_{213}$ -2.5, $\Delta\varepsilon_{224}$ 0, $\Delta\varepsilon_{227}$ 0.6, $\Delta\varepsilon_{230}$ 0, $\Delta\varepsilon_{244}$ -6.4, $\Delta\varepsilon_{264}$ 0, $\Delta\varepsilon_{280}$ 1.5; ^{1}H NMR (CD_3OD, 600 MHz) δ_H 8.04 (2H, d, *J* = 7.7 Hz, H-2', 6'), 7.62 (1H, t, *J* = 7.7 Hz, H-4'), 7.50 (2H, t, *J* = 7.7 Hz, H-3', 5'), 5.65 (1H, q, *J* = 6.9 Hz, H-19), 5.50 (1H, s, H-13), 5.29 (1H, s, H-5), 5.27 (1H, s, H-7), 5.25 (1H, s, H-17), 4.85 (1H, s, H-11), 3.04 (1H, s, H-9), 2.77 (1H, dq, *J* = 2.0, 7.4 Hz, H-4), 2.50 (1H, s, H-15), 1.72 (3H, s, H_3-24), 1.64 (6H, s, H_3-26, 29), 1.61 (3H, d, *J* = 6.9 Hz, H_3-20), 1.55 (3H, s, H_3-27), 1.19 (3H, s, H_3-28), 1.14 (3H, s, H_3-25), 1.11 (3H, s, H_3-23), 1.11 (3H, d, *J* = 7.4 Hz, H_3-22), 1.03

(3H, s, H_3-21); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.03 (2H, dd, J = 7.4, 1.0 Hz, H-2', 6'), 7.56 (1H, td, J = 7.4, 1.0 Hz, H-4'), 7.44 (2H, t, J = 7.4 Hz, H-3', 5'), 5.63 (1H, q, J = 6.6 Hz, H-19), 5.47 (1H, s, H-13), 5.28 (1H, s, H-7), 5.22 (1H, s, H-17), 5.04 (1H, d, J = 1.8 Hz, H-5), 4.74 (1H, s, H-11), 2.97 (1H, s, H-9), 2.71 (1H, dq, J = 1.8, 7.4 Hz, H-4), 2.47 (1H, s, H-15), 1.66 (3H, s, H_3-24), 1.64 (3H, s, H_3-26), 1.60 (3H, s, H_3-29), 1.57 (3H, d, J = 6.6 Hz, H_3-20), 1.53 (3H, s, H_3-27), 1.15 (3H, s, H_3-28), 1.15 (3H, d, J = 7.4 Hz, H_3-22), 1.14 (3H, s, H_3-25), 1.12 (3H, s, H_3-23), 1.07 (3H, s, H_3-21); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 207.4 (C, C-3), 171.6 (C, C-1), 165.5 (C, C-7'), 138.5 (C, C-8), 133.1 (CH, C-4'), 132.1 (C, C-18), 131.5 (C, C-14), 130.6 (C, C-12), 130.6 (C, C-1'), 129.5 (CH, C-2', 6'), 128.5 (CH, C-3', 5'), 126.1 (CH, C-19), 124.2 (CH, C-13), 123.2 (CH, C-11), 122.9 (CH, C-7), 87.6 (CH, C-17), 85.3 (CH, C-5), 70.1 (C, C-6), 61.3 (CH, C-9), 58.1 (C, C-2), 48.7 (CH, C-15), 48.6 (C, C-16), 44.0 (CH, C-4), 41.4 (C, C-10), 31.6 (CH_3, C-25), 23.4 (CH_3, C-27), 21.9 (CH_3, C-26), 20.3 (CH_3, C-24), 16.1 (CH_3, C-23), 15.2 (CH_3, C-28), 13.5 (CH_3, C-29), 13.1 (CH_3, C-20), 11.5 (CH_3, C-22), 4.8 (CH_3, C-21); FABMS *m/z* 579 $[M+Na]^+$; HRFABMS *m/z* 579.3066 $[M+Na]^+$; (calcd for $C_{36}H_{44}O_5Na$, 579.3086).

Preparation of (*R*)- or (*S*)-MTPA ester of shimalactone B (2). A dried pyridine (1 mL) solution of 2 (5 mg) was treated with (*S*)-MTPA chloride (10 μL), DMAP (2 mg). The reaction mixture was stirred for 2 h at 80 ºC. The reaction was quenched by 5% HCl and the whole was extracted with EtOAc. The EtOAc phase was washed by saturated aq. $NaHCO_3$, brine and then dried over $MgSO_4$. After evaporation of the solvent, the resulting residue was purified by SiO_2 gel column (*n*-hexane-EtOAc = 4:1) to afford the (*R*)-MTPA ester **12a** (4.5 mg). A dried pyridine (1 mL) solution of **2** (5 mg) was similarly treated with (*R*)-MTPA chloride (10 μL), and DMAP (2.0 mg) to afford the (*S*)-MTPA ester **12b** (4 mg).

12a: ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H 7.51 (2H, d, J = 7.2 Hz, 2', 6'-H), 7.40 (3H, m, H-3', 4', 5'), 5.48 (1H, s, H-13), 5.45 (1H, q, J = 6.6 Hz, H-19), 5.17 (1H, s, H-7), 5.08 (1H, d, J = 2.2 Hz, H-5), 4.85 (1H, s, H-17), 4.71 (1H, s, H-11), 3.49 (3H, s, OCH_3), 2.89 (1H, s, H-9), 2.71 (1H, dq, J = 2.2, 7.2 Hz, H-4), 2.37 (1H, s, H-15), 1.68 (3H, s, H_3-24), 1.65 (3H, s, H_3-26), 1.57 (3H, d, J = 6.6 Hz, H_3-20), 1.54 (3H, s, H_3-27), 1.42 (3H, s, H_3-29), 1.26 (3H, s, H_3-23), 1.19 (3H, d, J = 7.2 Hz, H_3-22), 1.11 (3H, s, H_3-21), 1.05 (3H, s, H_3-28), 0.96 (3H, s, H_3-25); FABMS *m/z* 691 $[M+Na]^+$; HRFABMS *m/z* 691.3251 (calcd for $C_{39}H_{47}O_6F_3Na$, 691.3222).

12b: ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H: 7.51 (2H, d, J = 7.2 Hz, H-2', 6'), 7.38 (3H, m, H-3', 4', 5'), 5.54 (1H, q, J = 6.6 Hz, H-19), 5.46 (1H, s, H-13), 5.17 (1H, s, H-7), 5.08 (1H, d, J = 2.2 Hz, H-5), 4.94 (1H, s, H-17), 4.67 (1H, s, H-11), 3.55 (3H, s, OCH_3), 2.91 (1H, s, H-9), 2.71 (1H, dq, J = 2.2, 7.2 Hz, H-4), 2.22 (1H, s, H-15), 1.68 (3H, s, H_3-24), 1.63 (3H, s, H_3-26), 1.59 (3H, d, J = 6.6 Hz, H_3-20), 1.55 (3H, s, H_3-27), 1.46 (3H, s, H_3-29), 1.28 (3H, s, H_3-23), 1.20 (3H, d, J = 7.2 Hz, H_3-22), 1.12 (3H, s, H_3-21), 1.02

(3H, s, H_3-28), 0.83 (3H, s, H_3-25); FABMS *m/z* 691 $[M+Na]^+$; HRFABMS *m/z* 691.3207 (calcd for $C_{39}H_{47}O_6F_3Na$, 691.3222).

Assay for neuritogenic activity against Neuro 2A cells. Neuro 2A cells were grown in Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum (FBS). The cells were kept in incubator at 37 °C with 5% CO_2. The cells were plated on 24-well plates at a density of 2 x 10^4 per well with 1 mL of culture medium. After 24 h cultivation, the medium was exchanged with fresh medium, and the testing sample as 10 μL of EtOH solution was added to each well. After 24 h incubation, morphological changes in the cells were observed under microscope.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Nobuyuki Harada, Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, for the helpful discussion about CD analysis. We are grateful to the Takeda Science Foundation and the Ministry of Education, Culture, Sports, Science, and Technology of Japan for financial support.

REFERENCES

1. J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote, and M. R. Prinsep, *Nat. Prod. Rep.*, 2005, **22**, 15.
2. P. R. Jensen and W. Fenical, 'Drug from the Sea', ed. by N. Fusetani, Karger, Basel, 2000, pp. 6-29.
3. F. Pietra, *Nat. Prod. Rep.*, 1997, **14**, 453.
4. H. Wei, T. Itoh, M. Kinoshita, Y. Nakai, M. Kurotaki, and M. Kobayashi, *Tetrahedron,* 2004, **60**, 6015.
5. H. Wei, T. Itoh, M. Kinoshita, N. Kotoku, S. Aoki, and M. Kobayashi, *Tetrahedron,* 2005, **61**, 8054.
6. N. C. Gonnella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, *J. Am. Chem. Soc.,* 1982, **104**, 3775.
7. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.,* 1991, **113**, 4092.
8. K. Takahashi, E. Tsuda, and K. Kurosawa, *J. Antibiot.*, 2001, **54**, 548.
9. J. E. Moses, J. E. Baldwin, R. Marquez, and R. M. Adilington, *Org. Lett.,* 2002, **4**, 3731.
10. C. M. Beaudry and D. Trauner, *Org. Lett.,* 2002, **4**, 2221.