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Novel Spirocyclic Trichothecanes, Spirotenuipesine A and B, Isolated from Entomopathogenic Fungus, *Paecilomyces tenuipes*

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Entomopathogenic fungi forming fruiting bodies have been employed as tonics and antitussives from ancient times. *Paecilomyces tenuipes*, which is also called *Isaria japonica*, is a very popular entomopathogenic fungus and is often considered a health food in northeast Asian countries such as China, Korea, and Japan. We cultivated the fruiting bodies of *Paecilomyces tenuipes*. Among the large-scale cultivations, fruiting body grown in barley grain contained two novel spirocyclic trichothecane derivatives, spirotenuipesine A (**1**) and B (**2**), and known trichothecane mycotoxins. Compounds **1** and **2** showed potent activity in neurotrophic factor biosynthesis in glial cells. The isolation of these compounds indicated that *P. tenuipes* is a promising source for producing various biologically active substances including trichothecanes. It is noteworthy that trichothecane mycotoxins are present in *Paecilomyces tenuipes*, which is typically used in medicinal health food.

Introduction

A group of entomopathogenic fungi that form fruiting bodies mainly belong to *Cordyceps*, *Paecilomyces*, and *Hirsutella* genera and are known as caterpillar fungi. In Japanese they are called "Toh-Chu-Kasou", which translates as winter worm and summer grass. These fungi are parasites on the pupae of various insects. Mycelia grow in the intestine and fruiting bodies are formed from the body. Some of these fungi have been employed as tonics and antitussives from ancient times.[1] Some compounds, which were isolated from a culture broth or mycelium of *Cordyceps* and *Paecilomyces* species, have been reported such as ISP-I (myriocin),[2] an immunosuppressant from *Isaria sinclairii* (which is a synonym of *Paecilomyces cicadae*), cordycepin (3′-deoxyadenosine),[3] an antibiotic from *Cordyceps militaris*, and cordyanhydride A and B,[4] maleic anhydride derivatives with unique skeletons from *Cordyceps pseudomilitaris*.

Paecilomyces tenuipes, also called *Isaria japonica*, is a very popular entomopathogenic fungi and is a common health food in northeast Asian countries such as China, Korea, and Japan. Although phytochemists have investigated the chemical components and biological activity, there are no scientific reports on the fruiting bodies. We undertook a large-scale cultivation of the fruiting body from this fungus and investigated the secondary metabolites. This paper describes the isolation of two novel spirocyclic trichothecanes, spirotenuipesine A (**1**) and B (**2**), and known trichotecane mycotoxins from a methanol extract. Their potency in neurotrophic factor biosynthesis in glial cells is also described.

Results and Discussion

Isolation. *Paecilomyces tenuipes* was cultivated on a medium composed of barley grain and brewers' yeast. The cultured fruiting bodies (6.8 kg) were extracted three times with methanol at room temperature to yield the extract (2.1 kg). The ethyl acetate soluble fraction (159 g) of the extract was separated by repeated column chromatography over silica gel and ODS to yield a novel trichothecane **1** (80 mg) and six conventional trichothecenes (**3**−**8**). An especially large amount of **7** (22.1 g) was isolated. In the same manner, a novel compound **2**

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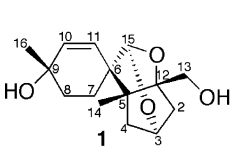
TABLE 1. ^{13}C and ^{1}H NMR Spectral Data of Spirotenuipesine A (1) and B (2)[a]

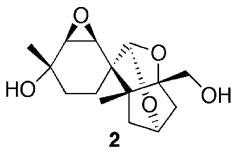
	spirotenuipesine A (**1**)		spirotenuipesine B (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
2α	43.1	2.22 (1H, ddd, *J* = 11.6, 3.6, 1.3 Hz)	43.1	2.17 (1H, br dd, *J* = 11.2, 3.7 Hz)
β		1.48 (1H, dq, *J* = 11.6, 1.3 Hz)		1.46 (1H, br dd, *J* = 11.2, 1.5 Hz)
3	76.1	4.44 (1H, quint, *J* = 1.3 Hz)	75.7	4.44 (1H, t, *J* = 1.5 Hz)
4α	40.8	2.36 (1H, ddd, *J* = 12.9, 3.6, 1.3 Hz)	41.1	2.27 (1H, ddd, *J* = 12.7, 3.7, 1.5 Hz)
β		1.19 (1H, br d, *J* = 12.9 Hz)		1.16 (1H, br d, *J* = 12.7 Hz)
5	49.1		49.5	
6	52.9		48.3	
7α	24.4	2.10 (1H, dddd, *J* = 13.6, 4.5, 3.2, 1.7 Hz)	23.8	1.84 (1H, dtd, *J* = 13.8, 3.6, 1.7 Hz)
β		1.50 (1H, td, *J* = 13.6, 3.4 Hz)		1.26 (1H, td, *J* = 13.8, 3.6 Hz)
8α	37.0	1.68 (1H, td, *J* = 13.6, 3.2 Hz)	31.8	1.60 (1H, td, *J* = 13.8, 3.6 Hz)
β		1.87 (1H, dddd, *J* = 13.6, 4.5, 3.4, 1.7 Hz)		1.48 (1H, dtd, *J* = 13.8, 3.6, 1.2 Hz)
9	69.2		69.6	
10	137.0	5.70 (1H, dd, *J* = 10.5, 1.7 Hz)	60.8	3.07 (1H, dd, *J* = 4.0, 1.2 Hz)
11	130.5	5.46 (1H, dd, *J* = 10.5, 1.7 Hz)	59.4	3.47 (1H, dd, *J* = 4.0, 1.7 Hz)
12	90.0		89.9	
13α	64.8	3.82 (1H, d, *J* = 12.1 Hz)	63.9	3.85 (1H, d, *J* = 12.7 Hz)
β		3.71 (1H, d, *J* = 12.1 Hz)		3.78 (1H, d, *J* = 12.7 Hz)
14	15.7	1.01 (3H, s)	15.2	1.22 (3H, s)
15	102.9	4.99 (1H, s)	101.2	5.22 (1H, s)
16	27.5	1.26 (3H, s)	22.3	1.30 (3H, s)

[a] 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.

(42 mg) and a known trichothecene (**9**) were afforded from the *n*-butanol soluble fraction (365 g) of the extract.



1



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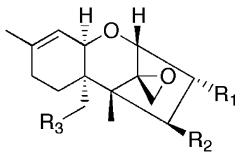


3 : R_1=OAc, R_2=OAc, R_3=OAc
4 : R_1=OAc, R_2=OAc, R_3=OH
5 : R_1=OH, R_2=OAc, R_3=OAc
6 : R_1=OAc, R_2=H, R_3=OH
7 : R_1=OH, R_2=OAc, R_3=OH
8 : R_1=OH, R_2=H, R_3=OH
9 : R_1=OH, R_2=OH, R_3=OH

Structure Elucidation. HREI-MS (*m*/*z* 266.1528 $[M]^+$), ^{1}H, and ^{13}C NMR spectra indicated that the molecular formula of **1** was $C_{15}H_{22}O_4$. The ^{13}C NMR spectrum of **1** showed the presence of two olefinic, an acetal, two oxygenated quaternary, an oxymethine, an oxymethylene, two quaternary, four methylene, and two methyl carbons (Table 1). $^{1}H-^{1}H$ COSY revealed that C-2−C-3−C-4, C-7−C-8, and C-10−C-11 were connected. The methyl protons at C-16 were correlated to three carbons (C-8, -9, and -10) and the five protons at C-7, -8, -10, -11, and -15 were correlated with the quaternary carbon (C-6) in the HMBC spectrum, which suggests a substituted cyclohexene ring (Figure 1A). The methyl proton at C-14 coupled with three carbons (C-4, -5, and -12) and the two protons at C-2 and -13 were correlated to the quaternary carbon (C-5), which implied a substituted cyclopentane ring (Figure 1B). The correlations between peaks H-7−C-5, H-15−C-5, and H-14−C-6 indicated that the partial structures A and B were connected through the C-5−C-6 bond. The intramolecular acetal moiety was deduced by the correlational peaks of methine proton at C-15 with the two carbons at C-3 and C-12 in the HMBC spectrum. Therefore, Figure 1C depicts the planar structure of **1** for a trichothecane sesquiterpenoid.

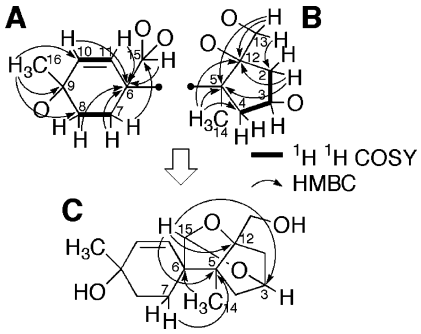


FIGURE 1. Planar structure of spirotenuipesine A (**1**).

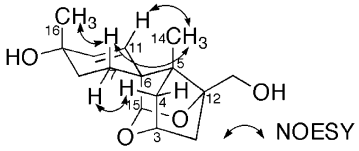
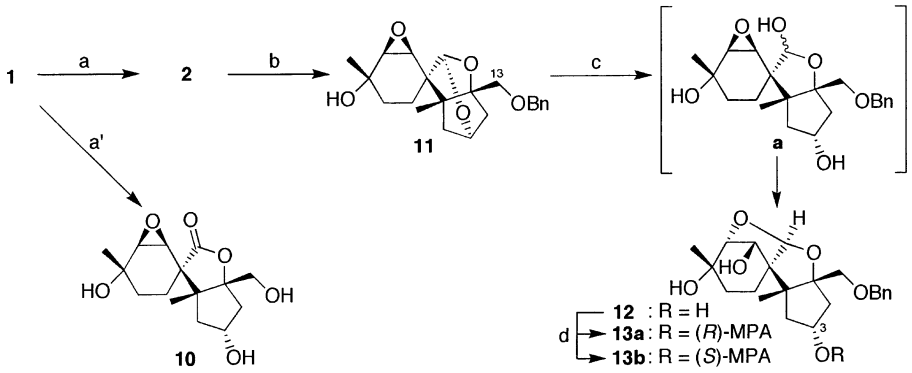


FIGURE 2. Relative structure of spirotenuipesine A (**1**).

The cross-peak between H-7*β* and H-16 in the NOESY spectrum indicated that the configuration of the methyl group at position 16 was axial in the cyclohexene ring. The methyl group at position 14 faced the same direction as C-16 because of the NOEs between H-7*β* and H-14. The correlational peak between H-4α and H-7α determined the relative configuration of C-5 and C-6, and the configuration of the other stereocenters at C-3, C-12, and C-15 was fixed by steric constraints of the caged structure (Figure 2). Structural rigidity and distortion of the spiro and tricyclo rings in **1** allowed for the observation of W-couplings between H-2α−H-4α (J = 1.3 Hz), H-7α−H-11 (J = 1.7 Hz), and H-8*β*−H-10 (J = 1.3 Hz).

The HREIMS of **2** (*m*/*z* 282.1470 $[M]^+$) gave a molecular formula, $C_{15}H_{22}O_5$, that differs from that of **1** by one oxygen atom. The ^{1}H NMR spectrum of **2** was nearly identical with that of **1**, though **2** lacked the olefinic proton signals (δ_H 5.70 and 5.46) which were replaced by two other signals (δ_H 3.47 and 3.07). The correspond-

SCHEME 1[a]



[a] Reagents and conditions: (a) Oxone, CH_2Cl_2−MeOH−phosphate buffer (pH 9.2)−acetone (1:4:2:0.3), 0 °C; (a′) Oxone, CH_2Cl_2−MeOH−phosphate buffer (pH 9.2)−acetone (1:4:2:0.3), rt; (b) benzyl bromide, sodium hydride, THF, 0 °C; (c) 10% HCl−MeOH, rt; (d) (*R*)- or (*S*)-α-methoxyphenylacetic acid, EDCI·HCl, DMAP, CH_2Cl_2, 0 °C.

ing signals in the ^{13}C NMR spectrum of **2** were at δ_C 59.4 and 60.8. In view of the above findings, we concluded that **2** has an epoxy ring between positions 10 and 11 (Table 1). This is supported by the fact that the epoxidation of **1** with dimethyldioxirane, which was prepared from acetone and Oxone in situ at 0 °C, gives a high yield of **2** (synthetic $[\alpha]^{25}_D$ +4.6 (*c* 0.252, $CHCl_3$), natural $[\alpha]^{25}_D$ +5.5 (*c* 0.832, $CHCl_3$)) (Scheme 1), although the reaction at room temperature yields the overoxidized product (**10**). The cross-peak between H-11 and H-15 in the NOESY spectrum revealed that the stereochemistry of the epoxide was *β*. Approach of dimethyldioxirane on **1** thus occurs from the less hindered *β* face of the ring. This fact was supported by transacetalization at C-15 under acidic conditions described later.

Determination of Absolute Configuration. To determine the absolute configurations of **1** and **2**, the following transformations were conducted (Scheme 1). 13-*O*-Benzyl ether (**11**) was prepared by treating **2** with benzyl bromide. Under acidic conditions, epoxide opening and transacetalization yielded a sole product **12**. The *β*-configuration of the epoxide between C-10 and C-11 was substantiated by this reaction via hemiacetal intermediate **a** in addition to the NOE data mentioned above. Esterification of **12** with (*R*)-α-methoxyphenylacetic acid[5] in the presence of EDCI and DMAP yielded only 3-*O*-(*R*)-α-methoxyphenylacetate (**13a**). In a similar manner, 3-*O*-(*S*)-α-methoxyphenylacetate (**13b**) was afforded. The $\Delta\delta_{RS}$ value of each proton was calculated from the difference in chemical shifts between **13a** and **13b** (Figure 3). Then, the structure of **12** was fitted into the proposed model of α-methoxyphenylacetate[5] in accordance with the sign of $\Delta\delta_{RS}$ to give the absolute configuration of **12** as 3*R*, 5*R*, 6*S*, 9*S*, 10*R*, 11*S*, 12*R*, and 15*S*. Therefore, the absolute configurations of **1** and **2** were based on **12** and determined to be (3*R*, 5*R*, 6*R*, 9*S*, 12*R*, 15*R*) and (3*R*, 5*R*, 6*S*, 9*S*, 12*R*, 15*R*), respectively.

Biological Activities. To investigate the biological effects of **1** and **2**, 1321N1 human astrocytoma cells (glial cell line) were incubated for 2 days with each compound. Then rat pheochromocytoma (PC-12) cells were cultivated for 2 days in the conditioned 1321N1 culture medium. The culture medium has been shown to contain neurotrophic factors synthesized in 1321N1 cells, which promote the differentiation of PC-12 cells.[6] The culture medium conditioned with 1 *μ*M **1** or **2** and 100 nM phorbol 12-myristate 13-acetate (PMA), which is an activator of neurotrophic factor biosynthesis, enhanced the extension of neurite outgrowth of PC-12 cells (Figure 4). **1** and **2** caused a concentration-dependent differentiation of PC-12 cells through the biosynthesis of nerotrophic factors from 1321N1 cells (Figure 5). These results indicate that **1** and **2** biosynthesize and release neurotrophic factors from 1321N1 cells and the released neurotrophic factors promote neuronal differentiation of PC-12 cells. Therefore, **1** and **2** are active in neurotrophic factor biosynthesis, suggesting that both may be lead compounds in drug synthesis for serious neuronal disorders such as Alzheimer's disease.

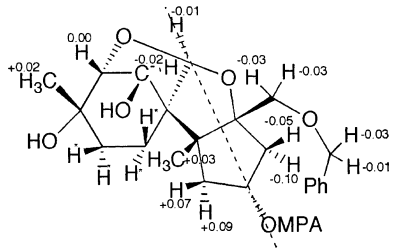


FIGURE 3. $\Delta\delta^{RS}$ values for (*R*)-MPA ester (**13a**) and (*S*)-MPA ester (**13b**).

Conclusion

Although a number of trichothecane-type sesquiterpenoids have been isolated as fungal metabolites produced by various species of *Fusarium*, *Myrothecium*, *Trichoderma*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*,[8] **1** and **2** are uniquely cyclized trichothecanes containing spirocyclic and tricyclo ring systems. It is speculated that an analogue of trichotriol (**17**) and FS-2 (**18**) biosynthesized **1** and **2**.[9] Numerous biological studies

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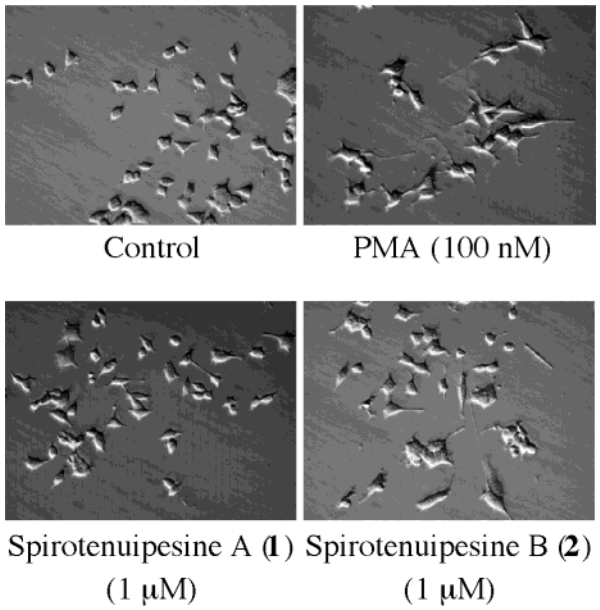


FIGURE 4. Glial cell-mediated morphological change of PC-12 cells by spirotenuipesine A (**1**) and B (**2**). PC-12 cells were incubated for 2 days in conditioned medium of 1321N1 human astrocytoma cells (glial cells) with or without 1 μM **1** or **2**, or with 100 nM PMA for 2 days. Phase contrast microscopies of PC-12 cells are shown.

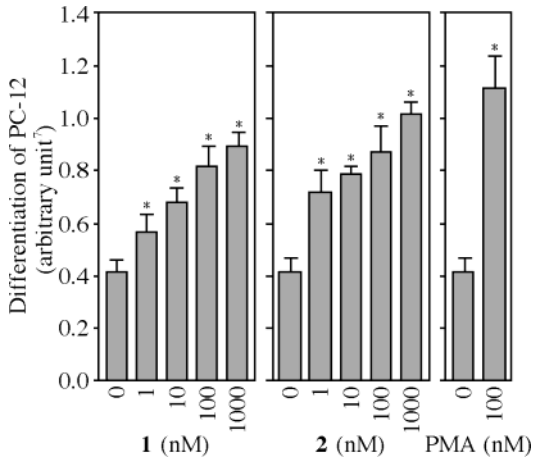
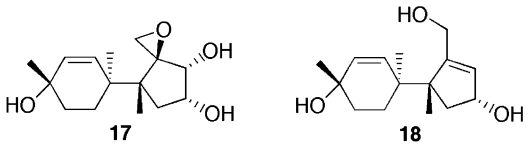


FIGURE 5. Effects of spirotenuipesine A (**1**) and B (**2**) on glial cell-mediated morphological changes of PC-12 cells. PC-12 cells were incubated for 2 days in a conditioned medium of 1321N1 human astrocytoma cells (glial cells) with varying concentrations of **1** or **2**, or PMA for 2 days. The differentiation of PC-12 cells was evaluated as previously described.[7] Each column represents the mean with SE of three or four experiments. Significant difference without drug (*P < 0.05).



on trichothecanes have focused on their antibacterial, antifungal, insecticidal, and cytostatic properties, especially phytotoxicity, but our study demonstrated that **1** and **2** show activity in neurotrophic factor biosynthesis. This biological activity of trichothecanes and *P. tenuipes* is a promising source for producing various biologically active compounds including trichothecanes.

Recently, two groups also reported the isolation of 4β-acetoxyscirpene-3,15-diol (**7**) from the fruiting body of *P. tenuipes* and its apoptosis-inducing activities.[10,11] This study also demonstrated that the fruiting body of *P. tenuipes* cultured on barley grain produced a large amount of **7**. This is noteworthy since *P. tenuipes* cultivated on media that contain grains were typically used in medcinal health foods.

Experimental Section

Organism and Culture Conditions. The strain MH19912 of *P. tenuipes* (synonym of *Isaria japonica*) was isolated at Yamanashi Prefecture, Japan, and preserved at Hokuto Corporation (Nagano, Japan). This strain was cultured on a medium composed of barley grain, brewers' yeast, and distilled water (weight ratio 57:9:120). Millet grain instead of barley grain or only living silkworm was also used in the medium. This medium was poured into a plastic bag, autoclaved at 120 °C for 40 min, and inoculated with a mycelial agar disk (5 mm in diameter) of this strain precultured on the potato dextrose agar medium. The culture was incubated at 25 °C for 21 days. After mycelia grew sufficiently, the surface of the culture was scratched and the culture was transferred to the condition for formation of fruiting bodies (synnemata), at 20 °C, 90% relative humidity, with a lighting cycle of 15 min light and 45 min dark, for 21 days. After cultivation, the fruiting bodies were harvested and dried.

Isolation of 1 and 2. The cultured fruiting bodies (dry weight 6.79 kg) of *P. tenuipes* were extracted three times with methanol (90 L) at room temperature to give the extract (2.06 kg). This extract was partitioned with ethyl acetate and water to yield ethyl acetate solubles (159 g) and a water layer, which was extracted with *n*-butanol three times to afford *n*-butanol solubles (365 g). The ethyl acetate solubles were chromatographed over SiO_2, and the column eluted with *n*-hexanes−ethyl acetate mixtures with increasing polarity. The ethyl acetate eluent (4.00 g) was further chromatographed over SiO_2 with chloroform−methanol (39:1) and then ODS with water−methanol (1:99) to give spirotenuipesine A (**1**) (80 mg). The *n*-butanol solubles were chromatographed over SiO_2, and the column was eluted with ethyl acetate−methanol mixtures with increasing polarity. Ethyl acetate eluent (5.36 g) was further chromatographed over ODS with water−methanol (1:99) and then SiO_2 with chloroform−methanol (19:1) to give spirotenuipesine B (**2**) (42 mg). **1** ((3*R*,5*R*,6*R*,9*S*,12*R*,15*R*)-7′-hydroxymethyl-3′,4-dimethylspiro[cyclohex-2-ene-1,2′-[8,9]-dioxatricyclo[3.3.1.0^{3,7}]nonan]-4-ol): colorless oil; $[\alpha]^{25}_D$ +32.8 (*c* 0.417, $CHCl_3$); ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EI-MS *m/z* 266 $[M]^+$, 248, 220, 189, 172, 145, 43 (base); HREI-MS *m/z* 266.1528 (266.1518 calcd for $C_{15}H_{22}O_4$). **2** ((3*R*,5*R*,6*S*,9*S*,12*R*,15*R*)-2,3-epoxy-7′-hydroxymethyl-3′,4-dimethylspiro[cyclohexane-1,2′-[8,9]dioxatricyclo[3.3.1.0^{3,7}]nonan]-4-ol): colorless oil; $[\alpha]^{25}_D$ +5.5 (*c* 0.832, $CHCl_3$); ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EI-MS *m/z* 282 $[M]^+$, 264, 206, 187, 227, 43 (base); HREI-MS *m/z* 282.1470 (282.1467 calcd for $C_{15}H_{22}O_5$).

Conversion of 1 into 2. To a solution of **1** (2.7 mg, 10.1 μmol) in dichloromethane−methanol−phosphate buffer (pH 9.2) (1:4:2) (1.5 mL) was added acetone (30 μL) and 0.25 M Oxone solution in water (0.5 mL) at 0 °C. After being stirred for 6 h, this mixture was poured into water (5 mL) and extracted with 1-butanol (10 mL) three times. The organic

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layer was evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (9:1) to afford **2** (2.6 mg, 9.2 μmol, 91%). Synthetic **2**: $[\alpha]^{25}_D$ +4.6 (*c* 0.252, $CHCl_3$); other spectral data were identical with those of the natural **2**.

(3*R*,5*R*,6*S*,9*S*,12*R*)-2,3-Epoxy-5-hydroxymethyl-1′,4-dimethyl-3′-oxospiro[cyclohexane-1,2′-[4]oxa-bicyclo[3.3.0]octane]-4,7′-diol (10). To a solution of **1** (12.6 mg, 44.7 μmol) in dichloromethane−methanol−phosphate buffer (pH 9.2) (1:4:2) (1.5 mL) was added acetone (30 μL) and a 0.25 M Oxone solution in water (0.6 mL) at room temperature. After being stirred for 3 h, this mixture was poured into water (5 mL), then extracted with 1-butanol (10 mL) three times. The organic layer was evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (9:1) to afford **10** (4.8 mg, 16.0 μmol, 36%). **10**: colorless oil; $[\alpha]^{25}_D$ +19.9 (*c* 0.483, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 4.28 (1H, m), 4.02 (1H, d, J = 3.8 Hz), 3.79 (1H, d, J = 12.7 Hz), 3.70 (1H, d, J = 12.7 Hz), 3.03 (1H, d, J = 3.8 Hz), 2.11 (1H, ddd, J = 14.8, 6.4, 1.2 Hz), 2.06−2.12 (1H, m), 1.98 (1H, ddd, J = 14.4, 9.4, 3.3 Hz), 1.93 (1H, ddd, J = 14.8, 5.2, 1.4 Hz), 1.87 (1H, ddd, J = 13.8, 9.1, 3.3 Hz), 1.78 (1H, ddd, J = 13.4, 5.1, 1.2 Hz), 1.53 (1H, ddd, J = 14.4, 9.1, 3.4 Hz), 1.39 (1H, ddd, J = 13.8, 9.4, 3.4 Hz), 1.31 (3H, s), 1.30 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 179.5, 96.7, 70.9, 68.3, 64.4, 60.2, 58.2, 53.3, 49.5, 48.2, 45.1, 32.2, 26.0, 24.4, 19.1; EI-MS *m*/*z* 298 $[M]^+$, 267, 249, 228, 43 (base); HREI-MS *m*/*z* 298.1415 (298.1416 calcd for $C_{15}H_{22}O_6$).

(3*R*,5*R*,6*S*,9*S*,12*R*,15*R*)-2,3-Epoxy-7′-benzyloxymethyl-3′,4-dimethylspiro[cyclohexane-1,2′-[8,9]dioxa-tricyclo-[3.3.1.0^{3,7}]nonan]-4-ol (11). To a solution of **2** (10.0 mg, 35.4 μmol) in THF (1 mL) was added sodium hydride (3.6 mg) and benzyl bromide (100 μL) at 0 °C. After being stirred for 5 h, the mixture was poured into water (5 mL) and extracted with ethyl acetate three times. The organic layer was dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed over silica gel eluted by *n*-hexane−chloroform (1:9) to afford **11** (8.7 mg, 23.4 μmol, 66%). **11**: colorless oil; $[\alpha]^{25}_D$ +6.9 (*c* 0.434, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25−7.36 (5H, m), 5.21 (1H, s), 4.63 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 12.0 Hz), 4.42 (1H, br s), 3.73 (1H, d, J = 10.9 Hz), 3.65 (1H, d, J = 10.9 Hz), 3.50 (1H, dd, J = 3.9, 1.6 Hz), 3.01 (1H, dd, J = 3.9, 1.1 Hz), 2.24 (1H, br d, J = 12.6 Hz), 2.18 (1H, br dd, J = 11.4, 2.8 Hz), 1.83 (1H, dtd, J = 13.7, 3.7, 1.6 Hz), 1.77 (1H, br s), 1.60 (1H, td, J = 13.7, 3.7 Hz), 1.54 (1H, br d, J = 11.4 Hz), 1.47 (1H, dtd, J = 13.7, 3.7, 1.1 Hz), 1.29 (3H, s), 1.24 (1H, td, J = 13.7, 3.7 Hz), 1.20 (3H, s), 1.15 (1H, br d, J = 12.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 128.3 (2C), 127.6 (2C), 127.6, 101.2, 89.1, 75.7, 73.9, 71.2, 69.8, 60.5, 59.6, 49.8, 48.5, 43.4, 41.2, 32.1, 23.8, 22.4, 15.5; EI-MS *m*/*z* 372 $[M]^+$, 354, 91 (base), 43; HREI-MS *m*/*z* 372.1942 (372.1947 calcd for $C_{22}H_{28}O_5$).

(3*R*,5*R*,6*S*,9*S*,10*R*,11*S*,12*R*,15*S*)-5-Benzyloxymethyl-9,-13-dimethyl-2,4-dioxa-tetracyclo[8.3.1.0^{3,10}.0^{5,9}]tetradecane-7,13,14-triol (12). Benzyl ether **11** (2.0 mg, 5.4 μmol) was dissolved in 10% hydrogen chloride containing methanol (0.5 mL). This solution was stirred for 7 h at room temperature, refluxed for 2.5 h, and evaporated. The residue was chromatographed over silica gel eluted by chloroform−methanol (49:1) to afford **12** (0.7 mg, 2.0 μmol, 37%). **12**: colorless oil; $[\alpha]^{25}_D$ +312 (*c* 0.120, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20−7.30 (5H, m), 5.59 (1H, s), 5.24 (1H, dd, J = 5.5, 1.7 Hz), 4.62 (1H, d, J = 11.8 Hz), 4.50 (1H, d, J = 11.8 Hz), 4.15 (1H, br s), 4.00 (1H, d, J = 5.5 Hz), 3.57 (1H, d, J = 10.7 Hz), 3.42 (1H, d, J = 10.7 Hz), 2.15 (1H, ddd, J = 14.0, 6.6, 1.9 Hz), 1.91−2.03 (2H, m), 1.90 (1H, dd, J = 12.5, 8.6 Hz), 1.79 (1H, dd, J = 14.0, 8.5 Hz), 1.71 (1H, ddd, J = 12.5, 5.4, 1.9 Hz), 1.55−1.65 (2H, m), 1.37 (3H, s), 1.20 (3H, s); EI-MS *m*/*z* 373 $[M - OH]^+$, 355, 217, 91 (base), 43; HREI-MS *m*/*z* 373.1998 (373.2016 calcd for $C_{22}H_{29}O_5$).

(*R*)- and (*S*)-α-Methoxyphenylacetate of 12 (13a and 13b). To a solution of **12** (0.7 mg, 2.0 μmol) in dichloromethane (1 mL) was added triethylamine (20 μL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.1 mg), (*R*)-α-methoxyphenylacetic acid (0.9 mg), and DMAP (0.4 mg) at 0 °C. After being stirred for 6 h, the mixture was poured into 0.25 M hydrochloric acid (5 mL), then extracted with ethyl acetate three times. The organic layer was collected and evaporated. The residue was chromatographed over silica gel eluted by chloroform to afford (*R*)-MPA ester **13a** (0.5 mg, 1.0 μmol, 50%). In the same manner, (*S*)-MPA ester (**13b**) (0.6 mg, 1.2 μmol, 29%) was synthesized from **12** (1.5 mg, 4.0 μmol). **13a**: colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25−7.39 (10H, m), 5.35 (1H, s), 5.22 (1H, dd, J = 5.5, 1.5 Hz), 4.95−5.01 (1H, m), 4.70 (1H, s), 4.59 (1H, d, J = 11.9 Hz), 4.46 (1H, d, J = 11.9 Hz), 3.95 (1H, d, J = 5.5 Hz), 3.51 (1H, d, J = 10.8 Hz), 3.38 (1H, d, J = 10.8 Hz), 3.37 (3H, s), 2.14 (1H, ddd, J = 14.6, 6.8, 1.2 Hz), 2.02 (1H, br dd, J = 13.6, 7.5 Hz), 1.90−1.99 (2H, m), 1.78 (1H, dd, J = 14.6, 6.9 Hz), 1.75−1.79 (1H, m), 1.48−1.53 (2H, m), 1.35 (3H, s), 1.19 (3H, s); EI-MS *m*/*z* 521 $[M - OH]^+$, 199, 121 (base), 91; HREI-MS *m*/*z* 521.2540 (521.2542 calcd for $C_{31}H_{37}O_7$). **13b**: colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26−7.43 (10H, m), 5.36 (1H, s), 5.24 (1H, dd, J = 5.5, 1.7 Hz), 4.99−5.04 (1H, m), 4.70 (1H, s), 4.60 (1H, d, J = 11.9 Hz), 4.49 (1H, d, J = 11.9 Hz), 3.95 (1H, d, J = 5.5 Hz), 3.54 (1H, d, J = 10.7 Hz), 3.36 (1H, d, J = 10.7 Hz), 3.36 (3H, s), 2.19 (1H, ddd, J = 14.3, 6.8, 1.2 Hz), 1.93 (1H, dd, J = 13.7, 7.1 Hz), 1.86−1.90 (1H, m), 1.83−1.91 (2H, m), 1.70 (1H, br dd, J = 13.7, 6.0 Hz), 1.32−1.43 (2H, m), 1.33 (3H, s), 1.16 (3H, s); EI-MS *m*/*z* 521 $[M - OH]^+$, 199, 121 (base), 91; HREI-MS *m*/*z* 521.2543 (521.2542 calcd for $C_{31}H_{37}O_7$).

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Supporting Information Available: NMR spectra of new compounds such as spirotenuipesine A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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