New *seco*-Prezizaane-Type Sesquiterpenes, Jiadifenin with Neurotrophic Activity and 1,2-Dehydroneomajucin from *Illicium jiadifengpi*[⊥]

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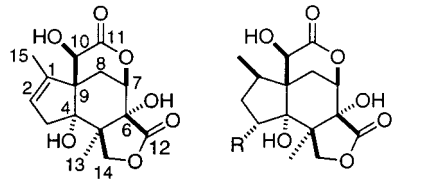
Two new *seco*-prezizaane-type sesquiterpenes, 1,2-dehydroneomajucin (**1**) and jiadifenin (**2**), were isolated from the methanol extract of the pericarps of *Illicium jiadifengpi*, indigenous to the southern part of China. Their structures were elucidated on the basis of NMR data. Compound **2**, which is an equilibrated mixture of the epimers **2a** and **2b** on the C-10 acetal carbon, is the first example of a majucin-type *seco*-prezizaane with an oxo-function at the C-10 position. The proposed structure for **2** was unambiguously confirmed by chemical conversion of the known sesquiterpene (2*S*)-hydroxy-3,4-dehydroneomajucin (**5**) to **2**. Compounds **2** and **5** were found to significantly promote neurite outgrowth in primary cultures of fetal rat cortical neurons at concentrations from 0.1 to 10 μM.

The genus *Illicium* is the only member of the family Illiciaceae and is an evergreen shrub or tree. About 40 species have been found disjunctively in eastern North America, Mexico, the West Indies, and eastern Asia. The highest concentration of species is in northern Myanmar and southern China, where nearly 35 species have been described.[1] From a chemical point of view, the *Illicium* species are interesting sources rich in biosynthetically unique *seco*-prezizaane-type sesquiterpenes, some of which exhibit neurotoxic and neurotrophic activities.[2−4] *seco*-Prezizaane-type sesquiterpenes can be categorized into four subgroups according to their carbon skeletons: anisatin-type,[5,6] pseudoanisatin-type,[7] majucin-type,[8] and minwanensin-type.[9] Recently, a rare cage-like *seco*-prezizaane-type sesquiterpene, cycloparvifloralone,[10,11] and tricyclic anislactones[12,13] and merrilactones[14,15] have been added as new subtypes. Hence, the diversity of *seco*-prezizaane-type sesquiterpenes and their fascinating biological activities have inspired us to continue chemical studies of the *Illicium* species.[16,17] As a result, two new majucin-type prezizaanes, **1** and **2**, named 1,2-dehydroneomajucin and jiadifenin, were isolated from the methanol extract of the pericarps of *Illicium jiadifengpi*, indigenous to China. Jiadifenin (**2**), in particular, not only is the first example of majucin-type sesquiterpenes with an oxo-function at the C-10 but also shows potent neurite outgrowth promoting activity in the primary cultures of rat cortical neurons. In this paper, we report the structure of new sesquiterpenes of **1** and **2** and the neurotrophic activity of **2** and **5**.

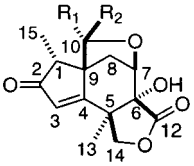
Results and Discussion

The methanol extract of the pericarps of *I. jiadifengpi* was fractionated by a combination of silica gel and reversed-phase RP-8 column chromatographies, resulting in the isolation of two new sesquiterpenes, 1,2-dehydroneomajucin (**1**) and jiadifenin (**2**), along with the previously known compounds, majucin (**3**),[8] neomajucin (**4**),[8] (2*S*)-hydroxy-3,4-dehydroneomajucin (**5**),[18] (1*S*)-2-oxo-3,4-dehydroneomajucin (**6**),[18] (1*R*)-2-oxo-3,4-dehydroneomajucin (**7**),[18] 2-oxoneomajucin (**8**),[18] and 2,3-dehydroneomajucin (**9**).[19]

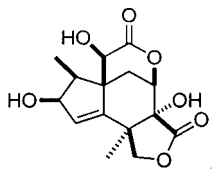


1

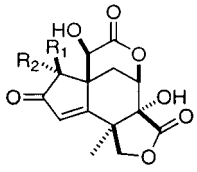
3: R = OH
4: R = H



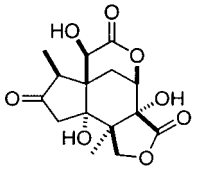
2a: R_1 = OH, R_2 = CO_2Me
2b: R_1 = CO_2Me, R_2 = OH



5



6: R_1 = Me, R_2 = H
7: R_1 = H, R_2 = Me



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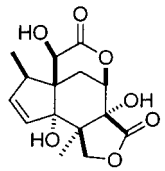

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Compound **1** had a M^+ peak at *m*/*z* 310.0537 in the high-resolution EIMS, corresponding to the molecular formula $C_{15}H_{18}O_7$. The NMR spectra of **1** were similar to those of neomajucin (**4**) except for the presence of a trisubstituted double bond [δ_H 5.68 (dt, J = 3.1, 2.7, 1.4 Hz, H-2); δ_C 141.1 (C-1) and 127.3 (C-2)]. The ^{1}H NMR spectrum of **1** showed low-shifted signals at δ_H 1.72 (dt, J = 2.7, 1.4 Hz) due to an olefinic methyl group, which coupled to the olefinic H-2

[⊥] Dedicated to Prof. Yoshinori Asakawa on the occasion of his 60th birthday.

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Table 1. ^{13}C and ^{1}H NMR Spectral Data (δ) of **2a** and **2b**[a]

	2a		**2b**	
position	δ_C	δ_H	δ_C	δ_H
1	42.9	2.93 q (7.7)	44.8	3.49 q (7.7)
2	208.9		209.8	
3	130.6	6.56 s	131.2	6.48 s
4	180.2		177.4	
5	45.2		44.8	
6	80.5		79.4	
7	80.9	5.03 d (6.3)	80.3	5.11 d (6.3)
8	31.4	2.55 d (12.3) 3.00 dd (12.3, 6.3)	31.6	2.60 d (11.8) 3.15 dd (11.8, 6.3)
9	60.2		60.2	
10	105.9		104.1	
11	171.5		169.2	
12	178.9		178.9	
13	23.2	1.67 s	23.1	1.62 s
14	76.0	4.19 d (8.5) 5.85 d (8.5)	75.3	4.15 d (9.1) 4.41 d (9.1)
15	13.6	1.22 (d, 7.7)	14.5	1.32 d (7.7)
OCH_3	52.7	3.66 s	52.0	3.54 s

[a] In C_5D_5N at 600 MHz; *J* values (Hz) in parentheses.

and the H-3 methylene resonating at δ_H 2.13 and 2.58. These spectral data disclosed the presence of a double bond at the C-1 and C-2 positions in **1**. These assignments were confirmed by HMBC correlations of 15-Me (δ_H 1.72) and H-3 (δ_H 2.13 and 2.58) to C-1 (δ_C 141.1), C-2 (δ_C 127.3), and C-9 (δ_C 58.4), respectively. The relative stereochemistry of **1**, which was elucidated on the basis of the NOESY, was identical to that of neomajucin (**4**). Thus, the structure of compound **1** was determined to be 1,2-dehydroneomajucin.

Jiadifenin (**2**) had the molecular formula $C_{16}H_{18}O_8$, determined by the high-resolution EIMS at *m/z* 338.1000, and its IR spectrum displayed absorptions due to a hydroxyl group at 3420 cm^{-1}, a γ-lactone moiety at 1745 cm^{-1}, and carbonyl groups at 1695 and 1659 cm^{-1}. The NMR spectra of **2**, however, contained a pair of well-separated signals, indicating a mixture of two compounds. Jiadifenin must be an inseparable equilibrated mixture of **2a** and **2b** since TLC and HPLC analyses of **2** are homogeneous in a variety of solvent systems. Therefore, each assignment of the well-separated NMR signals for **2a** and **2b** was readily made. The ^{1}H and ^{13}C NMR data (Table 1) of **2a** showed the presence of a tertiary methyl group (δ_H 1.67), a secondary methyl group [δ_H 1.22 (d, J = 7.7 Hz)], a conjugated ketone [δ_H 6.56 (s); δ_C 208.9 (C-2), 130.6 (C-3), and 180.2 (C-4)], an oxymethylene [δ_H 4.19 and 5.85 (each d, J = 8.5 Hz); δ_C 76.0 (C-14)], and an oxymethine [δ_H 5.03 (d, J = 6.3 Hz); δ_C 80.9 (C-7)], which was coupled to a methylene [δ_H 2.55 (d, J = 12.3 Hz) and 3.00 (dd, J = 12.3, 6.3 Hz); δ_C 31.4 (C-8)]. Additionally, the ^{1}H and ^{13}C NMR data for **2b** were summarized in Table 1. The aforementioned data also indicated that **2a** and **2b** were similar to a majucin-type sesquiterpene, (1*R*)-2-oxo-3,4-dehydroneomajucin (**7**).[8] All the spectral data of **2a** and **2b**, however, ruled out the presence of the δ-lactone ring characteristic of the majucin-type sesquiterpenes. The NMR data (Table 1) of **2a** and **2b** contained additional signals due to a methyl ester group (δ_H 3.66, δ_C 171.5 and 52.7 for **2a**; δ_H 3.54, δ_C 169.2 and 52.0 for **2b**) and an acetal carbon, which resonated at δ_C 105.9 (s) and 104.1 (s), respectively.

Routine analyses of $^{1}H-^{1}H$ COSY, HMQC, and HMBC of **2a** and **2b** as shown in Figure 1 were carried out, resulting in the same correlations in **2a** and **2b**. For convenience sake, the discussion is followed for **2a**. The acetal carbon at δ_C 105.9 showed HMBC correlations with both H-7 and H-8, thereby assignable to C-10. However,

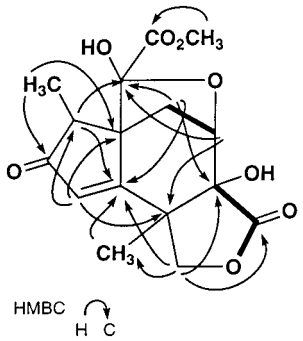


Figure 1. Representative HMBC correlations of **2a** and/or **2b**.

these HMBC data gave no help for the position of the methyl ester group. In consideration of the C-10 acetal carbon being quaternary, the remaining methyl ester group could only be placed at C-10. The above spectral data culminated in proposing the tentative structures of **2a** and **2b**, which included a five-membered acetal ring between the C-7 hydroxyl group and the oxidized C-10 hydroxyl group of majucin-type compound **6** or **7**. The equilibrium between **2a** and **2b** is therefore driven by opening of the acetal and reclosing in either the **2a** and **2b** form since formation of the more stable lactone as in **6** and **7** is blocked by a methyl ester group.

The relative stereochemistry of **2a** and **2b** were elucidated partly on the basis of NOESY and comparison of their chemical shift values for H-14 as shown in Figure 2. Namely, the NOESY could assign the Me-15 and Me-13 as α-configurations for both **2a** and **2b**, but gave no help to the stereochemistry on C-10. The chemical shift value of H-14β in **2a** was shifted into a lower field by ca. 1.41 ppm than that of **2b**, presumably due to the anisotropic effect of the C-11 ester carbonyl group. Therefore, the methyl ester group in **2a**, which is close to H-14β, was assigned a β-configuration, whereas **2b** has an α-oriented ester group. To confirm the structure and establish the absolute structure of **2**, **2** was synthesized from 2-hydroxy-3,4-dehydroneomajucin (**5**),[18] which is the main sesquiterpene isolated from this plant.

First of all, the modified Mosher's method[20] was applied to clarify the absolute configuration of **5**, which had not been determined. The $\Delta\delta(S-R)$ value as shown in Figure 3 enabled us to assign the C-2 configuration as *S*.

Next, the C-2 hydroxyl group of compound **5** was oxidized with Dess−Martin reagent to give a ketone **6** in 81% yield, which previously was obtained as a natural product from *Illicium majus*.[12] Treatment of **6** with DBU in benzene caused an epimerization at C-15, resulting in a thermodynamically more stable product **7**.[18] It is noted herein that the chemical conversion of **5** to **6** and **7** can assign the absolute configurations of **6** and **7** as (1*S*)-2-oxo-3,4-dehydroneomajucin and (1*R*)-2-oxo-3,4-dehydroneomajucin, respectively.

Compound **11** is anticipated to be produced if the C-10 hydroxyl group is oxidized to the ketone. Thus, **7** was subjected to Swern oxidation, giving rise to an unexpected oxetane **10**. This usual reaction was reasonably rationalized on the basis of the generally accepted mechanism of Swern oxidation as follows. A sulfoxonium intermediate **A** abstracts the more acidic H-1 than H-10, and then the formed carbanion attacks the oxygen of the sulfoxonium species in **B**, resulting in the formation of **10**. Dess−Martin reagent, however, could oxidize the C-10 hydroxyl group to the ketone, and a usual workup gave the expected acetal **11** in good yield. Adding methanol to the reaction mixture

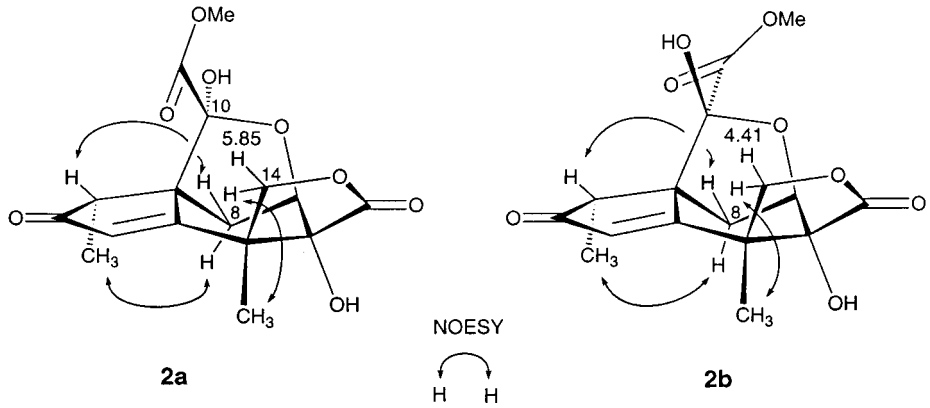


Figure 2. Representative NOESY correlations of **2a** and **2b**.

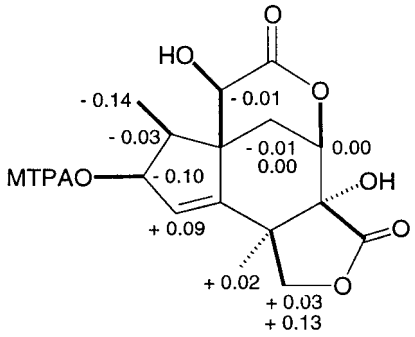


Figure 3. $\Delta\delta(S-R)$ values (ppm) for MTPA ester derivatives of **5**.

led to the direct formation of jiadefenin (**2**) as an equilibrated mixture, which was identical in all respects with the natural one.

In conclusion, we have established the absolute structure of jiadefenin by chemical conversion of **5**, the absolute configuration of which has been defined, to **2**.

Additionally, jiadefenin (**2**) and (2*S*)-hydroxy-3,4-dehydroneomajucin (**5**) were found to exhibit potent neurite outgrowth promoting activity in primary cultures of rat cortical neurons[21] in the range of concentration from 0.1 to 10 μM. As shown in Figure 4, the measurement of the longest axons extending from each cell body indicates that both **2** and **5** can promote neurite outgrowth more than bFGF, basic fibroblast growth factor. Thus, compounds **2** and **5** show potential as candidates of nonpeptide neurotrophic agents useful for treatment of neurodegenerative diseases.

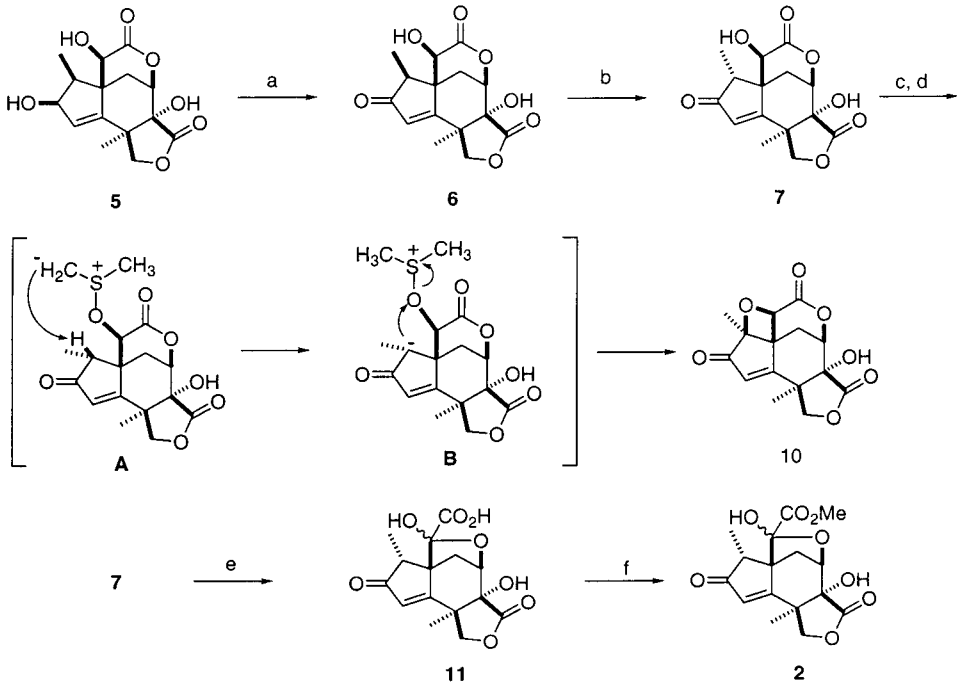
Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were measured on a Jasco FT-IR 5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity 600 or 400 instrument. Chemical shifts are given as δ (ppm) with TMS as internal standard. The MS were recorded on a JEOL AX-500 instrument. Column chromatography was carried out on Kiselgel 60 (70−230 mesh and 230−400 mesh).

Plant Material. The ripe fruits of *Illicium jiadifengpi* were collected in Yunnan, China, in September 1998, and a voucher specimen (94041) is deposited at Beijing University of Chinese Medicine.

Extraction and Isolation. The dried pericarps of *I. jiadifengpi* (3.5 kg) were powdered and extracted with MeOH

Scheme 1[a]



[a] Reagents and conditions: (a) Dess−Martin reagent, CH_2Cl_2, rt, 81%; (b) DBU, benzene, 80 °C, 74%; (c) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; (d) Et_3N, −78 °C to 0 °C, 34%; (e) Dess−Martin reagent, dioxane, rt; (f) MeOH, rt, 9%.

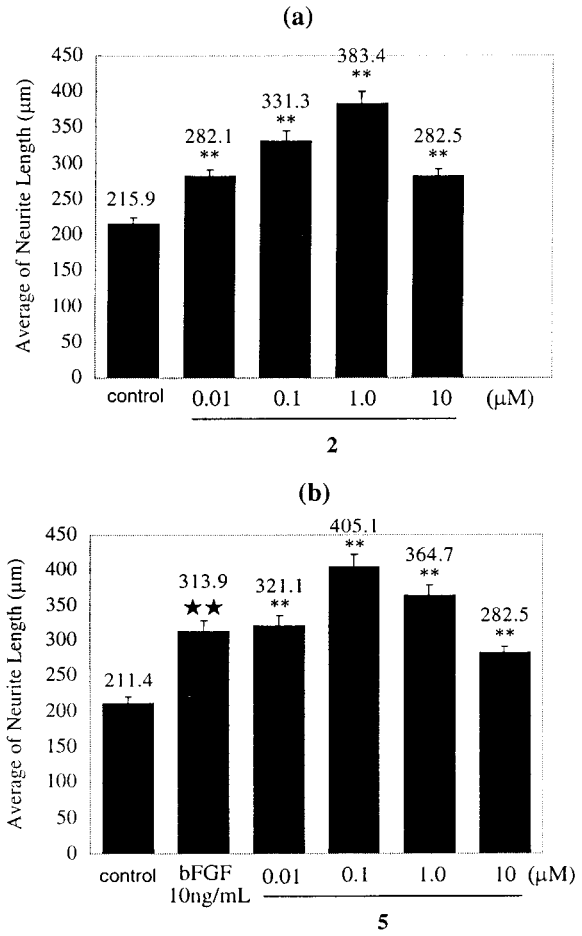


Figure 4. Enhancement of neurite outgrowth of rat cortical neurons by compounds **2** and **5** in primary culture in the presence of (a) jiadifenin (**2**); (b) (2*S*)-hydroxy-3,4-dehydroneomajucin (**5**). Data are represented as a mean + SE ($n = 80$). Student's t-test; ★★P <0.01 versus control. Dunnet's t-test; **$P < 0.01$ versus control.

at room temperature to give 1.5 kg of pale yellow extract. An aliquot of the MeOH extract (210 g) was divided into the methanol-soluble portion (173.5 g) and the methanol-insoluble portion (36.5 g). The methanol-soluble portion was chromatographed on a Si gel column (1.2 kg) eluting with a step gradient of CH_2Cl_2 (A: 100%), CH_2Cl_2−EtOAc (B: 3:1, C: 1:3), EtOAc (D: 100%), and EtOAc−MeOH (E: 3:1, F: 1:3) to yield six fractions (A−F).

Fractions C and D were first subjected to Si gel chromatography eluting with benzene−EtOAc (1:7) to give fractions 1−9. Fraction 6 (444 mg) was further chromatographed on a Si gel column eluting with $CHCl_3$−MeOH (3:7) to give jiadifenin (**2a** and **2b**) (5.0 mg) as an equilibrate mixture and the previously known sesquiterpenes (1*S*)-2-oxo-3,4-dehydroneomajucin (**6**) (52.8 mg), (1*R*)-2-oxo-3,4-dehydroneomajucin (**7**) (39.8 mg), and (2*S*)-hydroxy-3,4-dehydroneomajucin (**5**) (172 mg).

Fraction 9 (716 mg) was separated by reversed-phase HPLC (Cosmosil 5C18-AR-II, ϕ 10 × 250 mm) using MeOH−H_2O (4:6) as a solvent and finally purified by preparative TLC (EtOAc, 100%) to give 1,2-dehydroneomajucin (**1**) (4.6 mg) and the previously known sesquiterpene neomajucin (**4**) (5.0 mg).

1, 2-Dehydroneomajucin (1): amorphous solid; $[\alpha]^{20}_D$ −7.8° (*c* 0.16, EtOH); IR (film) ν_{max} 3490 (OH), 1778, and 1720 (C=O) cm^{-1}; HREIMS *m/z* 310.0537 (calcd for $C_{15}H_{28}O_7$, 310.0536); ^1H NMR (CD_3OD, 600 MHz) δ 1.31 (3H, s, H-13), 1.72 (3H, dt, $J = 1.4$, 1.4 Hz, H-15), 2.06 (1H, dd, $J = 14.5$, 3.0 Hz, H-8α), 2.13 (1H, ddq, $J = 14.8$, 2.7, 1.4 Hz, H-3), 2.58 (1H, ddq, $J = 14.8$, 3.1, 1.4 Hz, H-3), 2.87 (1H, dd, $J = 14.5$, 2.7 Hz, H-8β), 3.98 (1H, d, $J = 11.3$ Hz, H-14), 4.20 (1H, s, H-10), 4.45 (1H, d, $J = 11.3$ Hz, H-14), 4.59 (1H, dd, $J = 3.0$, 2.7 Hz, H-7), 5.68 (1H, ddq, $J = 3.1$, 2.7, 1.4 Hz, H-2); ^{13}C NMR (CD_3OD, 150 MHz) δ 177.8 (C-12), 174.4 (C-11), 141.1 (C-1), 127.3 (C-2), 83.8 (C-4), 81.2 (C-7), 80.1 (C-6), 73.4 (C-14), 71.3 (C-10), 58.4 (C-9), 47.4 (C-5), 40.0 (C-3), 25.7 (C-8), 20.9 (C-13), 12.3 (C-15).

Jiadifenin (2): colorless amorphous; $[\alpha]^{22}_D$ −152.9° (*c* 024, EtOH); IR (film) ν_{max} 3420 (OH), 1745, 1695, and 1659 (C=O) cm^{-1}; HREIMS *m/z* 338.1000 (calcd for $C_{16}H_{28}O_8$, 338.1010); ^1H and ^{13}C NMR, see Table 1.

***R*-MTPA Ester of (2*S*)-Hydroxy-3,4-dehydroneomajucin (5).** A solution of compound **5** (6.0 mg, 0.029 mmol) in dry dichloromethane (5.0 mL) was treated with (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6.0 mg), dicyclohexylcarbodiimide (7.0 mg), and 4-dimethylaminopyridine (2.0 mg) for 24 h at room temperature to give, after separation by preparative TLC (EtOAc, 100%), the 2-(−)-MTPA ester (1.7 mg): ^1H NMR (CD_3OD, 600 MHz) δ 1.17 (3H, d, $J = 7.4$ Hz, H-15), 1.38 (3H, s, H-13), 1.94 (1H, dd, $J = 14.5$, 1.8 Hz, H-8), 2.35 (1H, qd, $J = 7.4$, 7.1 Hz, H-1), 2.45 (1H, dd, $J = 14.5$, 4.0 Hz, H-8), 3.96 (1H, d, $J = 10.6$ Hz, H-14), 3.93 (1H, d, $J = 10.6$ Hz, H-14), 4.01 (1H, s, H-10), 4.59 (1H, dd, $J = 4.0$, 1.8 Hz, H-7), 5.68 (1H, dd, $J = 7.1$, 3.0 Hz, H-2), 6.19 (1H, d, $J = 3.3$ Hz, H-3).

***S*-MTPA Ester of (2*S*)-Hydroxy-3,4-dehydroneomajucin (5).** The (+)-MTPA ester (2.0 mg) of **5** was prepared from **5** (10.0 mg) (benzene as a solvent, stirred at 50 °C for 5 days) according to the similar procedure used for the preparation of the (−)-MTPA ester of **5**: ^1H NMR (CD_3OD, 600 MHz) δ 1.03 (3H, d, $J = 7.1$ Hz, H-15), 1.38 (3H, s, H-13), 1.93 (1H, dd, $J = 14.5$, 1.8 Hz, H-8), 2.32 (1H, qd, $J = 7.4$, 7.1 Hz, H-1), 2.44 (1H, dd, $J = 14.2$, 4.1 Hz, H-8), 3.99 (1H, d, $J = 10.6$ Hz, H-14), 4.07 (1H, d, $J = 10.6$ Hz, H-14), 4.08 (1H, s, H-10), 4.59 (1H, dd, $J = 4.5$, 1.8 Hz, H-7), 5.58 (1H, dd, $J = 7.4$, 3.2 Hz, H-2), 6.27 (1H, d, $J = 3.2$ Hz, H-3).

Conversion of (2*S*)-Hydroxy-3,4-dehydroneomajucin (5) to (1*R*)-2-Oxo-3,4-dehydroneomajucin (7). To a solution of **5** (9.0 mg, 0.029 mmol) in CH_2Cl_2 was added [1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one] periodinane (Dess−Martin reagent) (6.4 mg). After being stirred for 24 h at room temperature and being filtered, the solvent was evaporated in vacuo to leave a crude product, which was purified by column chromatography on Si gel eluting with $CHCl_3$-EtOAc (2:8) to give (1*S*)-2-oxo-3,4-dehydroneomajucin **(6)** (9.0 mg, 81.0%). To a solution of **6** (7.2 mg, 0.023 mmol) in benzene (8 mL) was added of 1,8-diazabicyclo [5,4,0]-7-undecene (0.2 mL), and the reaction mixture was refluxed for 2 days. Removal of the solvent in vacuo gave the residue, which was purified by reversed-phase HPLC (Cosmosil 5C18-AR-II, ϕ 10 × 250 mm) using MeOH−H_2O (1:9) as a solvent to afford (1*R*)-2-oxo-3,4-dehydroneomajucin (**7**) (5.4 mg, 74%). Compounds **6** and **7** were identical with data reported in the literature.[18]

Swern Oxidation of (1*R*)-2-Oxo-3, 4-dehydroneomajucin (7). To a solution of compound **7** (70.0 mg, 0.23 mmol) in CH_2Cl_2 (10 mL) were added oxalyl chloride (250 mL, 0.46 mmol) and DMSO (180 mL, 0.69 mmol), and the mixture was stirred at −78 °C for 1 h. After triethylamine (350 mL, 1.61 mmol) was added, the reaction mixture was stirred at 0 °C for 30 min. The reaction solution was diluted with saturated $NaHCO_3$ solution (60 mL), and then extracted with EtOAc. The organic layer was washed with saturated NaCl solution, dried over $MgSO_4$, and condensed in vacuo to give the crude product, which was purified by column chromatography on Si gel eluting with EtOAc (100%) to yield **10** (22.3 mg, 34%): $[\alpha]^{24}_D$ −7.9° (*c* 1.30, EtOH); IR (film) ν_{max} 3470 (OH), 1788 (C=O), and 1613 cm^{-1}; EIMS *m/z* 316 $[M]^+$; ^1H NMR (CD_3OD, 300 MHz): δ 1.46 (3H, s, H-13), 1.69 (3H, s, H-15), 2.39 and 2.89 (1H, dd, $J = 14.8$, 4.6 Hz and 14.8, 1.9 Hz, H-8), 3.89 and 4.09 (1H, d, $J = 10.6$ Hz), 4.45 (1H, s, H-10), 4.79 (1H, dd, $J = 4.6$ and 1.9 Hz, H-7), 6.50 (1H, s, H-3); ^{13}C NMR (CD_3OD, 75 MHz) δ 200.6 (C-2), 177.5 (C-4), 173.4 (C-11), 170.1 (C-12), 132.8 (C-3), 79.5 (C-10), 78.6 (C-6), 74.5 (C-14), 74.1 (C-1), 70.6 (C-7), 55.2 (C-9), 43.7 (C-5), 26.2 (C-8), 22.6 (C-13), 19.4 (C-15).

Dess−Martin Oxidation of (1*R*)-2-Oxo-3, 4-dehydroneomajucin (7). To a solution of **7** (31.0 mg, 0.1 mmol) in 1,4-dioxane (7 mL) was added Dess−Martin reagent (36.8 mg).

After being stirred at room temperature for 24 h, MeOH (5 mL) was added to this reaction mixture, and then stirring was continued for 5 min. After the solvent was evaporated in vacuo, the residue was purified by column chromatography on Si gel eluting with $CHCl_3$−EtOAc (1:3) to afford jiadifenin (**2**) (2.7 mg, 9%). This product was identical in all respects with natural jiadifenin.

In another instance, the reaction mixture was condensed in vacuo without adding MeOH, and purification by column chromatography on Si gel eluting with $CHCl_3$−EtOAc (1:3) afforded **11** (2.2 mg, 7%) as an inseparable mixture: $[\alpha]^{24}_D$ −72.7° (*c* 0.02, EtOH); IR (film) ν_{max} 3422 (OH), 1739, 1691, and 1656 (C=O) cm^{-1}; EIMS *m*/*z* 324 $[M]^+$; ^1H NMR (C_5D_5N, 600 MHz) δ 1.25 and 1.33 (3H, d, J = 7.5 Hz, H-15), 1.65 and 1.59 (3H, s, H-13), 2.56 and 2.62 (1H, d, J = 12.3 and 11.9 Hz, H-8), 2.94 and 3.44 (1H, q, J = 7.5 Hz, H-1), 2.99 and 3.14 (1H, dd, J = 12.3, 6.5 Hz and 11.9, 6.4 Hz, H-8), 4.15 and 4.45 (1H, d, J = 8.6 and 9.0 Hz, H-14), 5.08 (1H, d, J = 6.5 Hz, H-7), 5.90 and 5.90 (1H, d, J = 9.0 and 8.6 Hz, H-14), 6.50 and 6.60 (1H, s, H-3); ^{13}C NMR (CD_3OD, 150 MHz) δ 208.6 and 209.2 (C-2), 180.0 and 178.0 (C-4), 178.3 and 178.1 (C-12), 172.6 and 174.5 (C-11), 140.0 and 142.3 (C-3), 106.5 and 107.9 (C-10), 79.4 and 80.8 (C-6), 80.2 and 80.6 (C-7), 75.1 and 75.8 (C-14), 59.0 and 59.2 (C-9), 45.2 and 45.7 (C-5), 43.2 and 44.6 (C-1), 30.4 and 30.5 (C-8), 22.5 and 22.8 (C-13), 13.4 and 14.5 (C-15).

Neurotrophic Bioassay.[21] The neuronal cells are separated from the cerebral hemispheres of fetal 18 day SD rat (Japan SLC, Inc.) and suspended in 10% FAB/MEM, then seeded at 9000 cells/cm^2 into poly-L-lysine-coated 24-well culture plates. After 48 h, the medium is changed to a serum-free medium, Neurobasal medium (NBM) supplemented with B27, in the presence or absence of compounds at 0.1, 1, and 10 μM. After incubating for 5 days, the cells are fixed with 4% paraformaldehyde/PBS for anti-MAP-2 immunohistochemical stain. The neurite outgrowths affected by samples as an average of neurite length were analyzed under microscope. Eighty neurons which made network-formation less than three cells and were well-stained with anti-MAP-2 were selected for measurements from each sample. The length of the longest axon extended from the cell body was measurerd and calucu-lated by using Lumina Vjsion and MacSCOP software.

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References and Notes

(1) Richard, M. K.; Saunders, F. L. S. *Bot. J. Linnean Soc.* **1995**, *117*, 333−352.
(2) Niwa, H.; Yamada, K. *Pharmacia* **1991**, *27*, 924−927.
(3) Fukuyama, Y.; Shida, N.; Kodama, M. *Planta Med.* **1993**, *59*, 181−182.
(4) Fukuyama, Y.; Shida, N.; Kodama, M.; Chaki, H.; Yugami, T. *Chem. Pharm. Bull.* **1995**, *43*, 2270−2272.
(5) Fukuyama, Y.; Hata, Y.; Kodama, M. *Planta Med.* **1997**, *63*, 2199−2272.
(6) Lane, J. F.; Koch, W. T.; Leeds, N. S.; Gorin, G. *J. Am. Chem. Soc.* **1952**, *74*, 3211−3214.
(7) Yamada, K.; Takada, S.; Nakamura, S.; Hirata, Y. *Tetrahedron* **1968**, *24*, 199−229.
(8) Kouno, I.; Irie, H.; Kawano, N.; Katsube, Y. *Tetrahedron Lett.* **1983**, *24*, 771−772.
(9) Yang, C.-S.; Kouno, I.; Kawano, N.; Sato, S. *Tetrahedron Lett.* **1988**, *29*, 1165−1168.
(10) Wang, J.-L.; Yang, C.-S.; Yan, R.-N.; Yao, B.; Yang X.-B. *Zhongguo Yaoxue Zazhi* **1994**, *29*, 693−696.
(11) Schmidt, T. J. *J. Nat. Prod.* **1999**, *62*, 684−687.
(12) Huang, J.-M.; Yokoyama, R.; Yang, C.-S.; Fukuyama, Y. *J. Nat. Prod.* **2001**, *64*, 428−431.
(13) Kuno, I.; Mori, K.; Okamoto, S. *Chem. Pharm. Bull.* **1990**, *38*, 3060−3063.
(14) Huang, J. M.; Yang, C.-S.; Wang, H.; Wu, Q.-M.; Wang, J.-L.; Fukuyama, Y. *Chem. Pharm. Bull.* **1999**, *47*, 1749−1752.
(15) Huang, J. M.; Yokoyama, R.; Yang, C.-S.; Fukuyama, Y. *Terahedron Lett.* **2000**, *41*, 6111−6114.
(16) Huang, J. M.; Yang, C.-S.; Tanaka, M.; Fukuyama, Y. *Tetrahedron* **2001**, *57*, 4691−4698.
(17) Hunag, J.-M.; Nakade, K.; Kondo, M.; Yang, C.-S.; Fukuyama, Y. *Chem. Pharm. Bull.*, in press.
(18) Kouno, I.; Buba, N.; Hashimoto, M.; Kawano, N.; Takahashi, M.; Kaneto, H.; Yang, C.-S. *Chem. Pharm Bull.* **1990**, *38*, 422−425.
(19) Kouno, I.; Buba, N.; Hashimoto, M.; Takahashi, M.; Kawano, N.; Yang, C.-S.; Sato, Y. *Chem. Pharm Bull.* **1989**, *37*, 2448−2451.
(20) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *J. Am. Chem. Sos.* **1991**, *113*, 4092−4069.
(21) Brewer, G. J. *J. Neurosci. Res.* **1995**, *42*, 674−683.

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