Structure and Neurotrophic Activity of *seco*-Prezizaane-Type Sesquiterpenes from *Illicium merrillianum*

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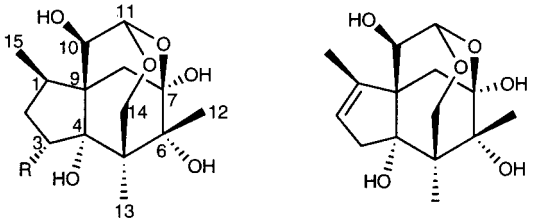
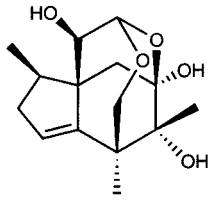
An extract of the pericarps of *Illicium merrillianum* has yielded four new sesquiterpenes: 3α-hydroxycycloparvifloralone (**1**), 1,2-dehydrocycloparvifloralone (**2**), (11) 7,14-ortholactone-3α-hydroxy-floridanolide (**3**), and 11-*O*-debenzoyltashironin (**4**) along with cycloparvifloralone (**5**), merrillianone (**6**), and tashironin (**7**). The structures of **1**−**4** were determined on the basis of spectroscopic analyses. 11-*O*-Debenzoyltashironin (**4**) showed neurotrophic activity in primary culture of rat cortical neurons at 0.1−10 μM. However, cycloparvifloralone-type sesquiterpenes (**1**, **2**, **5**, and **6**) and tashironin (**7**) had no neurotrophic activity at these concentrations.

Species of the genus *Illicium* are distinctive in producing biosynthetically unique *seco*-prezizaane-type sesquiterpenes, which can be further categorized into four subgroups: anisatin-type,[1] pseudoanisatin-type,[2] majucin-type,[3] and minwanensin-type.[4] Recently, a cage-like *seco*-prezizaane-type sesquiterpene, cycloparvifloralone, isolated from North America *Illicium* species, *I. parviflorum* and *I. floridanum*, has been added as a new subtype.[5] Among eastern Asia species, only *I. merrillianum* has been found to produce these types of sesquiterpenes to date.[6] Merrilactone A, isolated from *I. merrillianum*, can promote neurite outgrowth in the rat cortical neuronal culture.[7] These structurally unique sesquiterpenes and their interesting biological activities encouraged us to extensively investigate chemical components of *I. merrillianum*, thus resulting in the isolation of new cycloparvifloralone-type sesquiterpenes **1**−**3** and 11-*O*-debenzoyltashironin (**4**) In this paper, we report the structure elucidation and neurotrophic activity of these compounds.



1 R=OH
5 R=H

2



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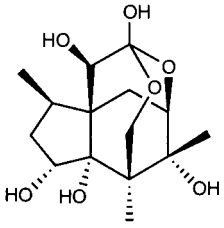

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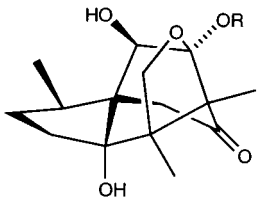

4 R=H
7 R=Bz

Results and Discussion

The methanol extract of the pericarps of *Illicium merrillianum* A. C. Smith (Illiciaceae) was subjected to a variety of chromatographic methods to give compounds **1**−**4** along with the previously known sesquiterpenes anisatin,[1] cycloparvifloralone (**5**),[5] merrilianone (**6**),[6] pseudomajucin,[9] cycloparviflorolide and parviflorolide,[5] anislactone A, anislactone B,[10] 7-*O*-acetylanislactone B,[6] and tashironin (**7**).[8]

The molecular formula of **1** was established as $C_{15}H_{24}O_7$ by high-resolution FABMS. ^{1}H NMR and ^{1}H−^{1}H COSY spectra of **1** revealed the presence of two tertiary methyl groups (CH_3-12 and CH_3-13) and a secondary methyl group that made up a CH_3(15)−CH(1)−CH_2(2)−CH(3)−O partial unit, and a CH(10)−CH(11) moiety corresponding to two low-field doublet signals with coupling constant 5.5 Hz, one isolated methylene group (CH_2-8), and one isolated oxygen-bearing methylene group (CH_2-14). The ^{13}C NMR (Table 1) and IR data indicated the absence of carbonyl groups in the molecule, but indicated the presence of an acetal carbon (C-11, δ_C 97) and a hemiacetal carbon (C-7, δ_C 99). The data were similar to that of cycloparvifloralone (**5**)[5] except for the downfield-shifted signal (δ_H 4.69) for H-3. Thus, **1** was deduced to be the 3-hydroxy derivative of **5**. This was confirmed by data obtained from HMQC and HMBC spectra. The NOESY correlations (Figure 1) between H-15 and H-10, H-8β and H-10, and H-14 and H-12 suggested that the CH_3-12, CH_3-15, and the hydroxyl group at C-10 took a β-orientation. The hydroxyl group at C-3 was assigned to the α-configuration based on the NOESY correlation between H-3 and H-14.

The high-resolution FABMS of **2** indicated the molecular formula $C_{15}H_{22}O_6$. The ^{1}H and ^{13}C NMR showed signals for CH(10)−CH(11), two isolated methylene groups (CH_2-8 and CH_2-14), and two tertiary methyls (CH_3-12 and CH_3-

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Table 1. ^{13}C NMR Spectral Data (δ) of **1**−**4**

C	**1**[a]	**2**[b]	**3**[b]	**4**[c]
1	38.5	139.2	38.5	38.0
2	42.8	127.4	42.7	30.7
3	72.9	40.4	72.8	34.3
4	81.4	89.9	87.8	85.1
5	47.5	47.0	48.4	54.9
6	87.5	81.7	78.5	60.1
7	99.4	99.7	79.0	211.1
8	37.7	35.3	31.4	43.7
9	52.5	59.6	51.2	52.3
10	70.6	70.0	75.4	76.9
11	97.6	97.0	113.5	106.4
12	15.8	16.5	20.9	8.3
13	15.7	15.8	15.3	14.5
14	68.6	68.6	67.8	73.2
15	13.5	12.6	13.5	13.7

[a] Recorded in CD_3OD at 125 MHz. [b] Recorded in CD_3OD at 150 MHz. [c] Recorded in $CDCl_3$ at 150 MHz

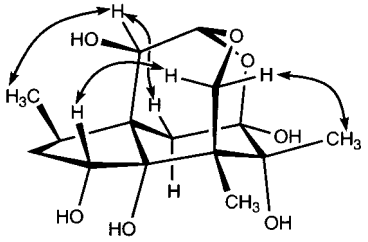

Figure 1. The representative NOESY correlations of **1**.

13) as well as signals of four quaternary carbons (C-4, C-5, C-6, and C-7) that were almost superimposable upon those of **5**. However, judging from one more degree of unsaturation in **2** and from the NMR data, a trisubstituted double bond must be present in **2**. Moreover, H-15 showed HMBC correlations to both of the olefinic carbons, supporting the position of the double bond. The relative configurations of the hydroxyl group at C-10 and methyl group at C-6 were assigned to the same β-orientation as in **1** according to the NOESY spectrum of **2**.

The spectral data for **3** ($C_{15}H_{24}O_7$) resembled those of **1**. The five-membered ring was also present in **3** by analyses of 1D NMR and various 2D NMR data. Comparing the ^1H NMR data of **3** and **1**, the following differences were found: the signal of H-8 at δ_H 1.58 and 2.28 was changed to a double doublet; the doublet signal of H-10 at δ_H 3.67 became a singlet. In addition to these differences, the DEPT and ^{13}C NMR shift values for C-7 (δ_C 79.0) and C-11 (δ_C 113.5) indicated that C-7 was an oxygen-bearing methine and C-11 was an ortho ester carbon. Additional evidence for a C-11 ortho ester was obtained by the HMBC correlations from C-11 to H-7, H-10, and H-14 (Figure. 2). The relative configuration of **3** was the same as that of **1** according to NOESY correlations.

Compound **4** had the molecular formula $C_{15}H_{22}O_5$ (HREIMS). The ^1H and ^{13}C NMR data showed the presence of two tertiary methyl groups, one secondary methyl group which was involved in a $CH_3(15)-CH(1)-CH_2(2)-CH_2(3)$ moiety, an isolated methylene group, an oxygen-bearing isolated methylene group, an oxygen-bearing methine group, and six quaternary carbons. The presence of hydroxyl groups and a ketone group were deduced from IR absorption (3433, 1705 cm^{-1}) and ^{13}C NMR data. The presence of a ketone group and the absence of a lactone group suggested that **4** had a carbon skeleton similar to tashironin (**7**), which was previously isolated from *I. tashironin*.[8] However, **4** lacked a benzoyl group at C-11 in **7**. In fact, the signal of C-11 in **4** was shifted to δ_C 106.4 in

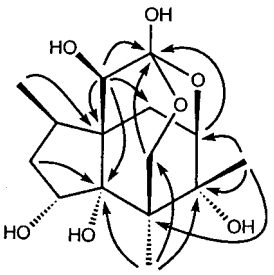

Figure 2. Key HMBC correlations of **3**.

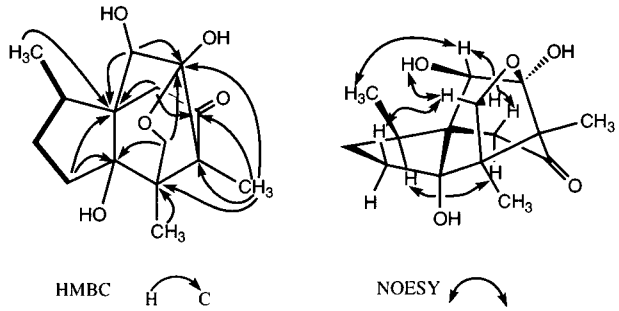

Figure 3. Representative HMBC and NOESY correlations of **4**.

comparison with δ_C 110.2 for C-11 in **7**. The HMBC correlations as shown in Figure 3 confirmed this planar structure. The relative stereochemistry of **4** was deduced to be the same as **7** by NOESY experiment (Figure 3). Thus, **4** was a 11-*O*-debenzoyl derivative of **7**. It should be emphasized that **4** has a tricarbocyclic skeleton corresponding to a key intermediate in the biogenesis of *seco*-prezizaane-type sesquiterpenes isolated from *Illicium* plants.[8]

We have found that **4** can promote neurite outgrowth in primary neuronal cultures in the range of concentration from 0.1 to 10 μM (Figure 4). However, **1**, **2**, **5**, **6**, and **7** exhibited no efficacy at the same concentrations.

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 were 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 Kieselgel 60 (70−230 mesh and 230−400 mesh) and Sephadex LH-20.

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

Extraction and Isolation. The dried pericarps of *I. merrillianum* (3.7 kg) were powdered and extracted with MeOH at room temperature to give 1 kg of pale yellow extract. The extract (430 g) was chromatographed on 400 g of silica gel (70−230 mesh) eluted successively with CH_2Cl_2, CH_2Cl_2/EtOAc (9:1, 1:1), EtOAc, EtOAc/MeOH (7:3), and MeOH to yield seven fractions (A−G).

Fraction E was first subjected to chromatography on Sephadex LH-20 to give fractions 1−4. Fraction 2 was further separated by column chromatography on silica gel (*n*-hexane/MeOH = 1:4) to afford fractions 5−14. Anisatin[1] (20 mg) was obtained from fraction 7. Fraction 8 (430 mg) included anislactone B[10] (258 mg) and cycloparvifloralone[5] (162 mg). Fractions 9 (264 mg) and 10 (210 mg) were a mixture of cycloparvifloralone and **1**. Pure **1** (529 mg) was obtained from fractions 11 and 12. Pseudomajucin[9] (595 mg) was crystallized

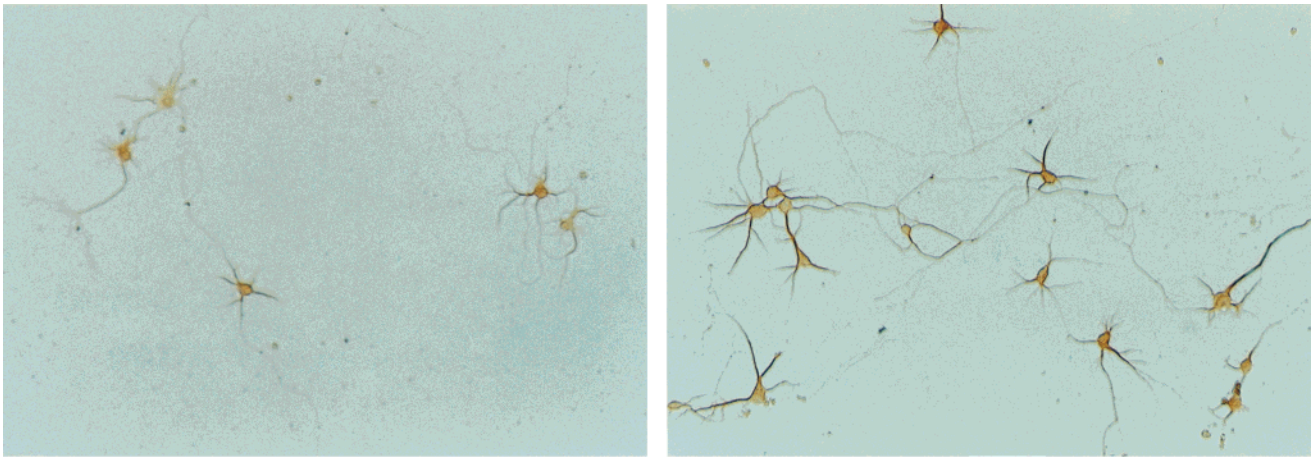

Figure 4. Neurotrophic effect of 11-*O*-debenzoyltashironin (**4**) in rat cortical neuronal culture: (a) control culture treated with 0.5% EtOH; (b) culture treated with **4** (0.1 μM).

from fraction 13. Fraction 14 was separated by reversed-phase column chromatography to provide fractions 15−18. Compound **2** (6 mg) was obtained from fraction 15 by reversed-phase HPLC (Cosmosil $5C_{18}$-AR-II, MeOH/H_2O = 1:4).

Fraction D was chromatographed on Sephadex LH-20 to give fractions 19−24. Fractions 25−28 were obtained from fraction 21 by column chromatography on silica gel (*n*-hexane/EtOAc = 1:3). Fraction 26 was subjected to TLC on RP-8 (MeOH/MeCN/H_2O = 1:1:4) to give merrilianone[6] (1 mg), anislactone A (1 mg), anislactone B[10] (4 mg), 7-*O*-acetylanislactone B[6] (1 mg), and a mixture of cycloparviflorolide and parviflorolide[5] (3 mg). The main compound of fraction 27 (500 mg) was cycloparvifloralone. Fraction 28 gave **1** (9 mg), **3** (2 mg), and 1α-hydroxy-3-dehydroxypseudoanisatin (4 mg) by TLC on RP-8 (MeOH/MeCN/H_2O = 1:1:4).

Fraction B was first separated by column chromatography on silica gel to give seven fractions. The last second fraction was further purified by Sephadex LH-20 chromatography, PTLC on silica gel and RP-8, to give **4** (3 mg). Compound **7** was also isolated from fraction B as described in a previous paper.[11]

3α-Hydroxycycloparvifloralone (1): colorless amorphous powder; $[\alpha]^{19}_D$ +11° (*c* 1.35, CH_3OH); IR (film) ν_{max} 3501 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 5.02 (1H, d, *J* = 5.5 Hz, H-11), 4.69 (1H, dd, *J* = 9.9, 4.7 Hz, H-3), 4.04 (1H, d, *J* = 12.4 Hz, H-14), 3.80 (1H, d, *J* = 5.5 Hz, H-10), 3.44 (1H, d, *J* = 12.4 Hz, H-14), 2.26 (1H, qdd, *J* = 11.0, 8.8, 7.3 Hz, H-1), 2.21 (1H, d, *J* = 13.8 Hz, H-8), 1.92 (1H, ddd, *J* = 12.8, 9.9, 8.8 Hz, H-2), 1.64 (1H, ddd, *J* = 12.8, 11.0, 4.7 Hz, H-2), 1.45 (1H, d, *J* = 13.8 Hz, H-8), 1.29 (1H, s, H-12), 1.12 (1H, s, H-13), 0.96 (1H, d, *J* = 7.3 Hz, H-15); ^{13}C NMR, see Table 1; HRFABMS *m/z* 339.1425 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_7Na$: 339.1420).

1,2-Dehydroxycycloparvifloralone (2): colorless amorphous powder; $[\alpha]^{22}_D$ +14° (*c* 1.77, CH_3OH); IR (film) ν_{max} 3312, 1645 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 5.64 (1H, qdd, *J* = 2.0, 1.6, 1.4 Hz, H-2), 5.14 (1H, d, *J* = 5.5 Hz, H-11), 4.06 (1H, d, *J* = 12.4 Hz, H-14), 3.78 (1H, d, *J* = 5.5 Hz, H-10), 3.44 (1H, d, *J* = 12.4 Hz, H-14), 2.80 (1H, qdd, *J* = 14.8, 2.2, 2.0 Hz, H-3), 2.59 (1H, d, *J* = 12.9 Hz, H-8), 1.92 (1H, brd, *J* = 14.8 Hz, H-3), 1.63 (1H, ddd, *J* = 2.0, 2.0, 1.4 Hz, H-15), 1.60 (1H, d, *J* = 12.9 Hz, H-8), 1.36 (1H, s, H-12), 1.07 (1H, s, H-13); ^{13}C NMR, see Table 1; HRCIMS *m/z* 299.1481 (calcd for $C_{15}H_{23}O_6$, 299.1494); CIMS *m/z* (rel int) 299 (2), 281 (15), 263 (26), 178 (100), 133 (41).

(11) 7,14-Ortholactone-3α-hydroxyfloridanolide (3): colorless amorphous powder; $[\alpha]^{20}_D$ −9° (*c* 0.40, CH_3OH); IR (film) ν_{max} 3412 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 4.78 (1H, dd, *J* = 9.6, 4.5 Hz, H-3), 4.22 (1H, d, *J* = 12.6 Hz, H-14), 3.67 (1H, s, H-10), 3.56 (1H, dd, *J* = 3.4, 2.3 Hz, H-7), 3.39 (1H, d, *J* = 12.6 Hz, H-14), 2.27 (1H, qdd, *J* = 10.7, 9.0, 7.1 Hz, H-1), 2.28 (1H, d, *J* = 13.6, 2.3 Hz, H-8), 1.94 (1H, ddd, *J* = 13.0, 9.6, 9.0 Hz, H-2), 1.63 (1H, ddd, *J* = 13.0, 10.7, 4.5 Hz, H-2), 1.58 (1H, d, *J* = 13.6, 3.4 Hz, H-8), 1.40 (1H, s, H-12), 1.08 (1H, s, H-13), 0.95 (1H, d, *J* = 7.1 Hz, H-15); ^{13}C NMR, see Tables 1; HRFABMS *m/z* 339.1413 (calcd for $C_{15}H_{24}O_7Na$, 339.1420); EIMS *m/z* (rel int) 298 (10), 280 (25), 163 (76).

11-*O*-Debenzoyltashironin (4): colorless solid; $[\alpha]^{22}_D$ −65° (*c* 0.72, $CHCl_3$); IR (film) ν_{max} 3433, 1705 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.96 (1H, d, *J* = 9.6 Hz, H-14), 3.81 (1H, d, *J* = 5.4 Hz, H-10), 3.76 (1H, d, *J* = 9.6 Hz, H-14), 3.12 (1H, d, *J* = 5.4 Hz, OH-10), 2.86 (1H, s, OH-11), 2.56 (1H, d, *J* = 18.6 Hz, H-8), 2.26 (1H, qdd, *J* = 10.2, 9.0, 7.2 Hz, H-1), 2.28 (1H, ddd, *J* = 12.0, 12.0, 6.6 Hz, H-3), 2.08 (1H, dddd, *J* = 12.6, 10.2, 9.0, 6.6 Hz, H-2), 2.00 (1H, d, *J* = 18.6 Hz, H-8), 1.72 (1H, dddd, *J* = 12.6, 12.0, 9.0, 3.0 Hz, H-2), 1.54 (1H, ddd, *J* = 12.0, 9.0, 3.0 Hz, H-3), 1.22 (1H, d, *J* = 7.2 Hz, H-15), 1.12 (1H, s, H-12), 0.98 (1H, s, H-13); ^{13}C NMR, see Table 1; HREIMS *m/z* 282.1468 $[M]^+$ (calcd for $C_{15}H_{22}O_5$, 282.1467); EIMS *m/z* 282 (11), 264 (22), 235 (20), 189 (50), 113 (100).

Neurotrophic Bioassay.[12] Neuronal cells were separated from the cerebral hemispheres of a fetal 18 day SD rat (Japan SLC, Inc.) and suspended in 10% FAB/MEM, then seeded at 12 000 cells/cm^2 into poly-L-lysine-coated 24-well culture plates. After 48 h, the medium was changed to a serum-free medium, neurobasal medium (NBM) supplemented with B27, in the presence or absence of the compounds at 0.1, 1, and 10 μM. After incubation for 5 days, the cells were fixed with 4% paraformaldehyde/PBS for anti-MAP-2 immunohistochemical stain. The neurite outgrowths affected by samples were analyzed under a microscope and photographs taken with 200 magnifications (Figure 4).

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