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Novel Pentacyclic *seco*-Prezizaane-Type Sesquiterpenoids with Neurotrophic Properties from *Illicium jiadifengpi*

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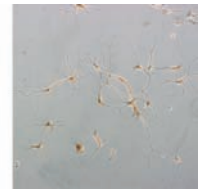
ABSTRACT

Jiadifenolide



Three novel *seco*-prezizaane-type sesquiterpenoids, jiadifenolide (1), jiadifenoxolane A (2), and jiadifenoxolane B (3), were isolated from the pericarps of *Illicium jiadifengpi*. Their pentacyclic cage structures were determined by 2D-NMR methods, chemical conversion, and single-crystal X-ray analysis. Compounds 1 and 2 strongly promote neurite outgrowth in primary cultured rat cortical neurons at concentrations ranging from 0.01 to 10 μM.

Neurotrophic factors (neurotrophins) are recognized as important regulatory substances in the nervous system.[1] Neurotrophins have been selected as candidates for a current therapeutic strategy aimed at controlling the loss of nerve function in the brains of Alzheimer's disease patients. However, they cannot persist in the body for a long period and also cannot cross the brain−blood barrier because they are high molecular peptides. To address this issue, considerable efforts have been made to find small molecules that mimic the properties of neurotrophins. As part of our continuing studies on neurotrophic factor-like compounds in *Illicium* species,[2] we investigated the chemical constituents of the pericarps of *Illicium jiadifengpi* collected in southwestern China, which resulted in the isolation of three novel *seco*-prezizaane-type sesquiterpenoids **1**, **2**, and **3**, named jiadifenolide and jiadifenoxolanes A and B, respectively. In this paper, we report the structures and the neurotrophic properties of compounds **1**−**3**.

The MeOH extract of the dried pericarps of *I. jiadifengpi* was fractionated by silica gel and Sephadex LH-20 column chromatographies and finally purified by reverse-phase HPLC, which led to the isolation of the new compounds **1**, **2**, and **3** along with the previously known compounds, neomajucin (**4**),[3] majucin (**5**),[3] 2-oxoneomajucin,[4] 1,2-epoxyneomajucin,[5] 2-oxo-3,4-dehydroxyneomajucin,[4] and 2,3-dehydroxyneomajucin.[6]

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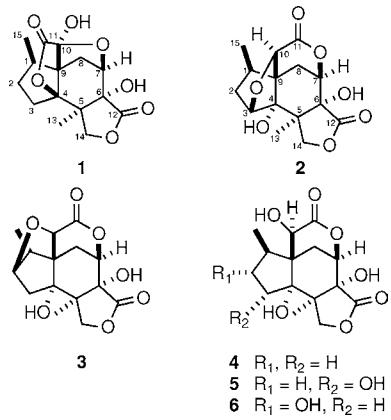


Figure 1. *seco*-Prezizanne-type sesquiterpenoids isolated from *Illicium jiadifengpi*.

Jiadifenolide (**1**) has the molecular formula $C_{15}H_{18}O_7$, as deduced from high-resolution (HR) CI-MS at *m*/*z* 311 [M + H]$^+$. The IR spectrum displayed absorptions due to a hydroxy group at 3393 cm^{-1} and a γ-lactone moiety at 1772 cm^{-1}. The ^{1}H and ^{13}C NMR data of **1** showed the presence of a tertiary methyl group (δ_H 1.22), a secondary methyl group [δ_H 1.20 (d, J = 7.1 Hz)], an oxymethylene [δ_H 3.79 and 4.60 (each d, J = 9.3 Hz); δ_C 74.6 (C-14)], and an oxymethine [δ_H 4.41 (d, J = 5.8 Hz); δ_C 79.1 (C-7)], which was coupled to a methylene [δ_H 2.09 (d, J = 12.9 Hz), 2.46 (d, J = 12.9, 5.8 Hz); δ_C 32.1 (C-8)]. The aforementioned data indicated that **1** is a majucin-type *seco*-prezizaane, except for the absence of the δ-lactone ring characteristic of the majucin-type sesquiterpenoids. The ^{13}C NMR spectrum of **1** contained additional signals due to an ester carbonyl group (δ_C 173.6) and an acetal carbon resonated at δ_C 101.9. ^{1}H−^{1}H COSY, HMQC, and HMBC analyses of **1** (Figure 1) showed that **1** had the same A−C rings as neomajucin (**4**). The acetal carbon signal showed HMBC correlations with both H-7 and H-8, thereby allowing us to form the five-membered acetal ring D between C-7 and C-9. However, the HMBC gave no help for the position of the remaining ester group. In consideration of the C-10 acetal carbon being quaternary, the ester carbonyl may bond to C-10 in the same manner as jiadifenin, which was previously isolated from the title plant.[7] Moreover, taking the 7 degrees of unsaturation and low field ^{13}C NMR chemical shift (δ_C 97.4) of the C-4 oxygenated carbon into consideration, an ester linkage between C-4 and C-11 was made to form a γ-lactone ring.

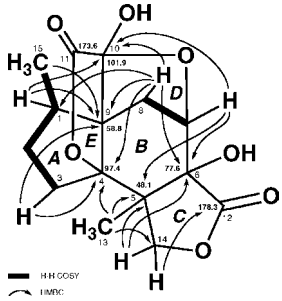


Figure 2. HMBC correlations of **1**.

Fortunately, **1** gave single crystals suitable for X-ray crystallographic analysis. The X-ray crystallographic structure of **1**[8] as shown in Figure 3 demonstrates that a γ-lactone ring is closed between C-4 and C-11. Thus, jiadifenolide was determined to be a unique pentacyclic cagelike structure as **1**.

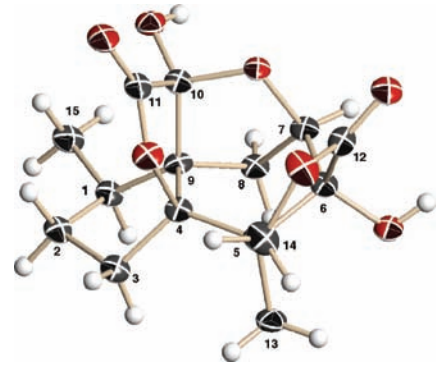
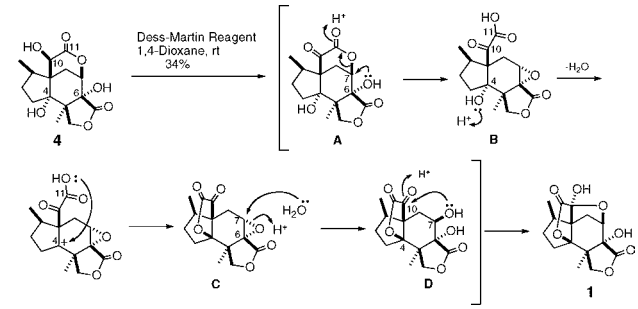


Figure 3. X-ray crystallographic structure of **1**.

Previously, we demonstrated that jiadifenin could be converted from (1*R*)-2-oxo-3,4-dehydroneomajucin by oxidizing the C-10 hydroxy group.[7] Danishefsky was succeeded in the total synthesis of (±)-jiadifenin by applying our oxidative method to the final step.[9] Additionally, we envisaged that jiadifenolide (**1**) would be derived from neomajucin (**4**), which is the main sesquiterpene isolated from the title plant, by following the oxidative procedure used for the synthesis of jiadifenin. We were pleased to find that the Dess−Martin oxidation of **4** gave rise to **1** in a straightforward fashion (Scheme 1). This result indicated that the absolute configuration

Scheme 1. Plausible Mechanism for Oxidative Conversion of **4** to **1**



of **1** was assigned as the same as that of **4**. This one-step conversion can be reasonably rationalized under acidic conditions as follows. The C-10 hydroxy group in **4** was oxidized to the unstable α-keto-δ-lactone **A**, which was presumably opened by an intramolecular attack from the C-6 hydroxy group on the C-7 position to give rise to the epoxide intermediate **B**. Dehydration then generated a stable cation at C-4, which was attacked from up-side by the C-11 carboxylic acid, leading to the α-keto-γ-lactone **C**. The epoxide in **C** was then opened again to give the diol **D**. Finally, the acetal formation between C-7 and C-10 provided the desired jiadifenolide (**1**). This proposed mechanism can explain the inversion of the configuration on C-4, but a question remains of whether the epoxide intermediates **B** and **C** occur in this oxidative procedure.

Jiadifenoxolane A (**2**) has the molecular formula $C_{15}H_{18}O_7$ as deduced by HR-EIMS at *m*/*z* 310.1060 $[M]^+$, indicating 7 degrees of unsaturation. The ^{1}H NMR spectrum of **2** was similar to those of majucin (**5**) except for the observation of one proton signal being shifted upfield [δ_H 1.22 (dd, J = 13.0, 5.4 Hz)]. This spectral similarity and one-increased unsaturation suggested that **2** has the structure of majucin with one extra ring. This was supported by 2D-NMR experiments (Figure 4). The

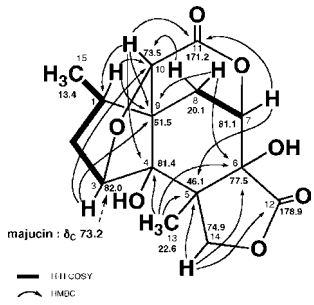


Figure 4. HMBC correlations of **2**.

HMBC correlation of H-3 to the C-10 allowed us to connect C-3 and C-10 through an ether bond, resulting in the formation of an oxolane ring. This tentative structure is consistent with 7 degrees of unsaturation and explains why the C-3 signal is shifted up to δ_C 82.0. Thus, the above spectral data culminated in the proposal of the pentacyclic plane structure **2**. The relative stereochemistry of **2** was elucidated on the basis of NOESY and a comparison of the chemical shift values for H-2 as shown in Figure 5. Namely, the chemical shift value of H-2β in **2** was shifted higher upfield by 0.47 ppm than that of **5** due to the anisotropic effect of the tetrahydropyran ring. Additionally, the H_3-15 and the H_3-13 methyl groups were

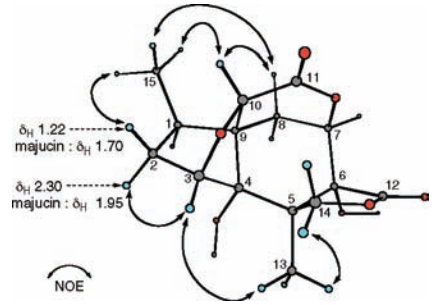


Figure 5. NOESY correlations of **2**.

assigned as β and α on the basis of NOESY (Figure 5), respectively. On the basis of the aforementioned spectra data, the structure of jiadifenoxolane A was elucidated as **2**.

Jiadifenoxolane B (**3**) has the molecular formula $C_{15}H_{18}O_7$, which was established by HR-EIMS at *m*/*z* 310 $[M]^+$. The IR spectrum displayed absorptions ascribable to a hydroxy group (3440 cm^{-1}) and γ-lactone (1768 cm^{-1}) and δ-lactone (1752 cm^{-1}) moieties. The ^{1}H and ^{13}C NMR data of **3** were similar to those of (2*S**)-hydroxyneomajucin (**6**)[4] except for the chemical shift values of C-2 and C-10 which were shifted lower downfield by ca. 4−5 ppm than those of **6**. ^{1}H−^{1}H-COSY, HMQC, and HMBC analyses of **3** as shown in Figure 6 led to the proposal of another pentacyclic plane structure

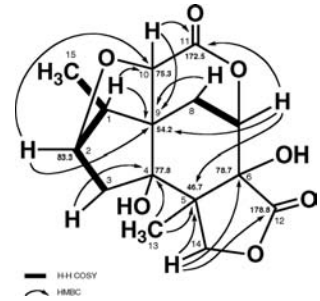


Figure 6. HMBC correlations of **3**.

3 in which an ether bond was closed between C-2 and C-10. The relative stereochemistry of **3** was assigned as the same as that of **6**. Thus, the structure of jiadifenoxolane B was represented as **3**.

Jiadifenolide (**1**) and jiadifenoxolane A (**2**)[10] were found to significantly potentiate neurite outgrowth in the primary cultured rat cortical neurons[11] at the range of concentration from 0.01 to 10 μM as shown in Figure 7. Quantitative analysis of the length of the longest neurite extending from each cell body was also performed. The mean neurite lengths

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(8) Crystallographic Data for **1** have been deposited at the Cambridge Crystallographic Data Center (Deposition number CCDC-745403).

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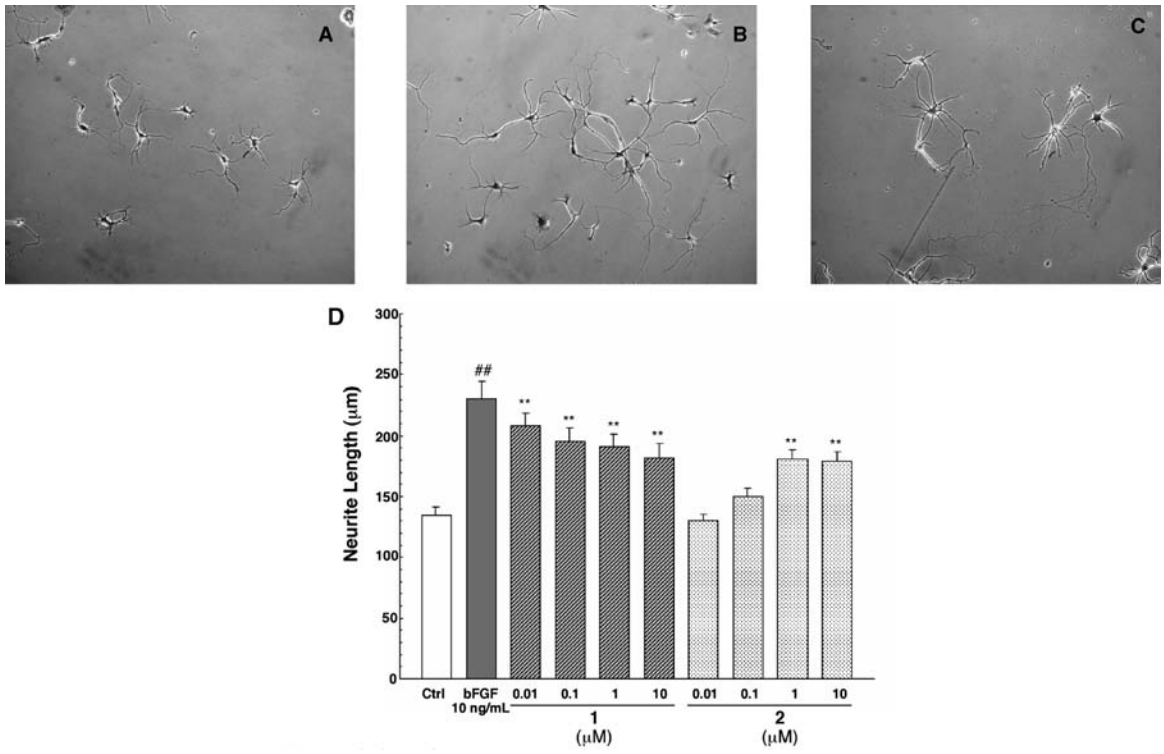

Figure 7. Neurite outgrowth-promoting activity of **1** and **2** in primary cultured rat cortical neurons. (A) Morphology of neurons in the control groups. (B) Morphology of neurons in 0.01 μM **1**. (C) Morphology of neurons in 10 μM **2**. (D) Quantitative analysis of neurite outgrowth. In each group, the mean length of the primary dendrite-like processes of 63 neurons was measured. Data are expressed as means ± SE. The differences between groups were tested with Dunnett's *t* test. ##, $P < 0.01$, **, $P < 0.01$ vs control according to Dunnett's *t* test.

of **1** and **2** were longer than that of the control. It should be noted that **1** exhibits more potent activity at as low concentrations as 0.01 μM. A dose of **1** lower than 0.01 μM resulted in a tendency toward reduced promotion of neurite outgrowth (data not shown). The present study implied that majucin-type sesquiterpenoids are most likely to be a druglike chemical group suitable for showing neurite outgrowth-promoting activity.

In conclusion, three novel *seco*-prezizaane-type sesquiterpenoids **1**−**3** were isolated from the pericarps of *Illicium jiadifengpi*. Their structures are unique pentacyclic sesquitepenoids with cagelike structures. To the best of our knowledge, jiadifenolide (**1**) is the first example of a *seco*-prezizaane-type sesquiterpenoid with a γ-lactone formed between the C-11 carbonyl and the C-4 hydroxy group. Moreover, compounds **1** and **2** have been found to show neurite outgrowth-promoting activity in the primary cultured rat cortical neurons. In particular, jiadifenolide (**1**), which shows potent neurotrophic activity at a low dose, has a great potential as a lead compound of non-peptide neurotrophic agent useful for the treatment of neurodegenerative diseases such as Alzheimer's disease.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra for **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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