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The first examples of *seco*-prezizaane-type norsesquiterpenoids with neurotrophic activity from *Illicium jiadifengpi*

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ABSTRACT

Two *seco*-prezizaane-type sesquiterpenoids (2*R*)-hydroxy-norneomajucin (**1**) and jiadifenone (**2**) that represent the first examples of nor-type were isolated from the methanol extract of the pericarps of *Illicium jiadifengpi*. Their structures and the absolute configuration of **1** were established by the analysis of spectroscopic data and chemical conversion of (2*S*)-hydroxyneomajucin to **1**, respectively. In addition, compound **1** exhibited neurotrophic activity to significantly promote neurite outgrowth in the primary cultured rat cortical neurons at concentrations ranging from 1 to 10 μmol L^{-1}.

The family Illiciaceae consists of a single genus, *Illicium*, which contains 42 species. Over 70% of *Illicium* species are distributed in southwestern and eastern Asia. The fruit of *Illicium verum*, known as star-anise, is used for seasoning in Chinese and Southeast-Asian food.[1] On the other hand, Japanese star-anise, *Illicium anisatum*, is used to produce incense but its fruits contain a neurotoxic sesquiterpene, anisatin, which is a representative of *seco*-prezizaane-type sesquiterpenoids.[2] In the pursuit of non-peptide neurotrophic compounds in plants, we have already found a few interesting sesquiterpenoids with neurotrophic properties,[3] for example, merrilactone A,[4] and jiadifenin[5] and jiadifenolide,[6] from *Illicium merrillianum* and *Illicium jiadifengpi*, respectively. Thus, the *Illicium* plants have made us fascinate the search for neurotrophin-mimic natural products. We have continued our study on the chemical constituents of the pericarps of *I. jiadifengpi* collected in the South-Western China, resulting in the first isolation of two novel *seco*-prezizaane-type norsesquiterpenoids **1** and **2** named (2*R*)-hydroxy-norneomajucin and jiadifenone, respectively. In this Letter, we report the structures and the neurotrophic properties of **1** and **2** (Fig. 1).

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, leading to the isolation of new compounds **1** and **2** along with the previously known compounds, such as (2*S**)-hydroxyneomajucin (**3**),[7] (2*R**)-hydroxyneomajucin (**4**),[9] neomajucin (**5**),[8] majucin,[8] 1,2-epoxyneomajucin,[9] 2-oxo-3,4-dehydroxyneomajucin,[7] 2,3-dehydroxyneomajucin,[10] (2*S*)-hydroxy-3,4-dehydroneomajucin,[7] 1,2-dehydroneomajucin,[5] jiadifenoxolane B,[6] and pseudomajucin.[10]

Compound **1** has the molecular formula $C_{14}H_{18}O_7$, as deduced from high resolution (HR) EI-MS at *m*/*z* 298.1055 $[M]^+$. The IR spectrum displayed absorptions due to a hydroxy group at 3370 cm^{-1} and a γ-lactone moiety at 1771 cm^{-1}. The ^1H and ^{13}C NMR data of **1** (Table 1) indicated the presence of a tertiary methyl group (δ_H 1.22), a secondary methyl group [δ_H 1.04 (d, *J* = 7.6 Hz)], an oxymethylene [δ_H 3.92 and 4.09 (each d, *J* = 10.0 Hz); δ_C 75.4 (C-13)], two oxymethine [δ_H 4.66 (d, *J* = 5.6 Hz); δ_C 82.0 (C-7); δ_H 4.31 (dt, *J* = 7.8, 4.9 Hz); δ_C 73.0 (C-2)], and two methylene [δ_H 2.28 (dd, *J* = 12.2, 5.6 Hz), 2.75 (d, *J* = 12.2 Hz); δ_C 30.2 (C-8); δ_H 1.45 (dd,

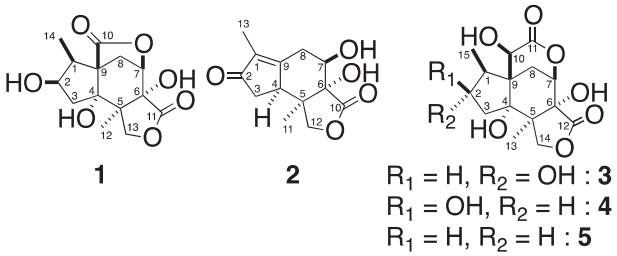

Figure 1. Structures of **1**–**5**.

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Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **1** and **2** in CD_3OD

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.60 (dq, 7.8, 7.6)	38.8		138.6
2	4.31 (dt, 7.8, 4.9)	73.0		210.2
3α	2.44 (dd, 14.7, 7.8)	43.7	2.53 (dd, 18.9, 6.7)	36.5
3β	1.45 (dd, 14.7, 4.9)		2.03 (ddq, 18.9, 2.7, 0.6)	
4		80.2	2.88 (dd, 6.7, 2.7)	44.4
5		47.5		47.9
6		76.2		79.5
7	4.66 (d, 5.6)	82.0	4.15 (dd, 3.0, 3.0)	81.1
8α	2.75 (d, 12.2)	30.2	2.70 (ddq, 13.7, 3.0, 1.4)	32.9
8β	2.28 (dd, 12.2, 5.6)		2.74 (dd, 13.7, 3.0)	
9		60.7		171.9
10		181.1		180.5
11		178.8	1.22 (3H, d, 0.8)	23.2
12α	1.22 (3H, s)	19.9	3.80 (d, 8.0)	72.2
12β			4.10 (dq, 8.0, 0.8)	
13α	3.92 (d, 10.0)	75.4	1.71 (3H, dd, 1.4, 0.6)	7.7
13β	4.09 (d, 10.0)	74.6		
14	1.04 (3H, d, 7.6)	7.7		

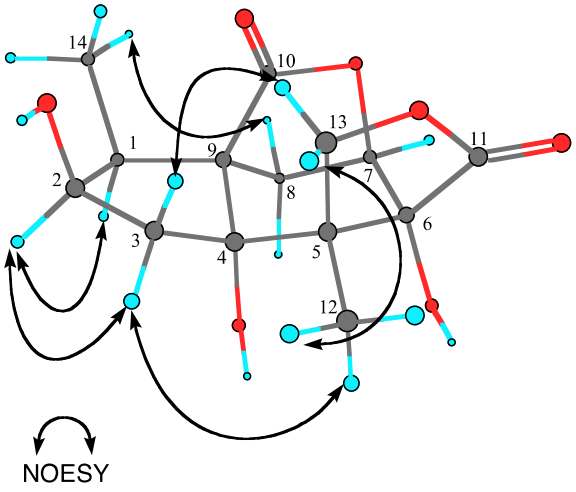


Figure 3. NOESY correlations of **1**.

J = 14.7, 4.9 Hz), 2.44 (dd, J = 14.7, 7.8 Hz); δ_C 43.7 (C-3)]. The aforementioned spectroscopic data indicated that **1** belongs to majucin-like *seco*-prezizaane-type sesquiterpenoids except for the absence of a δ-lactone ring, which is a characteristic of these sesquiterpenoids. Furthermore, the molecular formula indicated that the carbon number of **1** is one less than that of normal sesquiterpenoids, implying that **1** is a norsesquiterpenoid. Therefore, the spectroscopic data of **1** failed to refer to those of the previously known *seco*-prezizaane-type sesquiterpenoids. Extensive analyses of ^{1}H–^{1}H COSY, HMQC, and HMBC of **1** (Fig. 2) showed that **1** has the same A–C ring system as (2*R**)-hydroxyneomajucin (**4**). In addition, the ester carbon signal (δ_C 181.1) showed HMBC correlations with each H-7, H-8, and H-1, thereby allowing us to form a γ-lactone ring D between C-7 and C-9. Since the NMR signal corresponding to the C-11 position which commonly exists in all the majucin derivatives was found to be missing, **1** was assumed to be a nor-type of (2*R**)-hydroxyneomajucin (**4**).

The relative stereochemistry of **1** was elucidated on the basis of NOESY as shown in Figure 3. Namely, H-1/H-2, H-2/H-3α, and H-3α/H_3-12 indicated that the methyl group at C-14 and the hydroxy group at C-2 take β-configurations and the methyl group at C-12 is in α-configuration. In addition, both the methyl group at C-12 and hydroxy group at C-6 were assigned as α-configurations from the NOE correlation of H_3-14/H-8, and H-13β/H-3β and H-13α/H_3-12. On the basis of the aforementioned data, the structure of **1** was deduced to be represented as 11-nor-(2*R*)-hydroxyneomajucin, which is the first example of *seco*-prezizaane-type norsesquiterpenoid, and thus **1** was named as (2*R*)-hydroxy-norneomajucin.

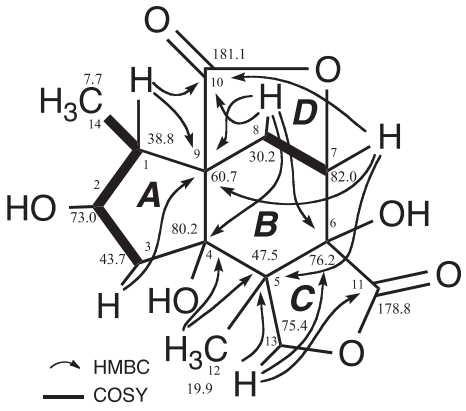
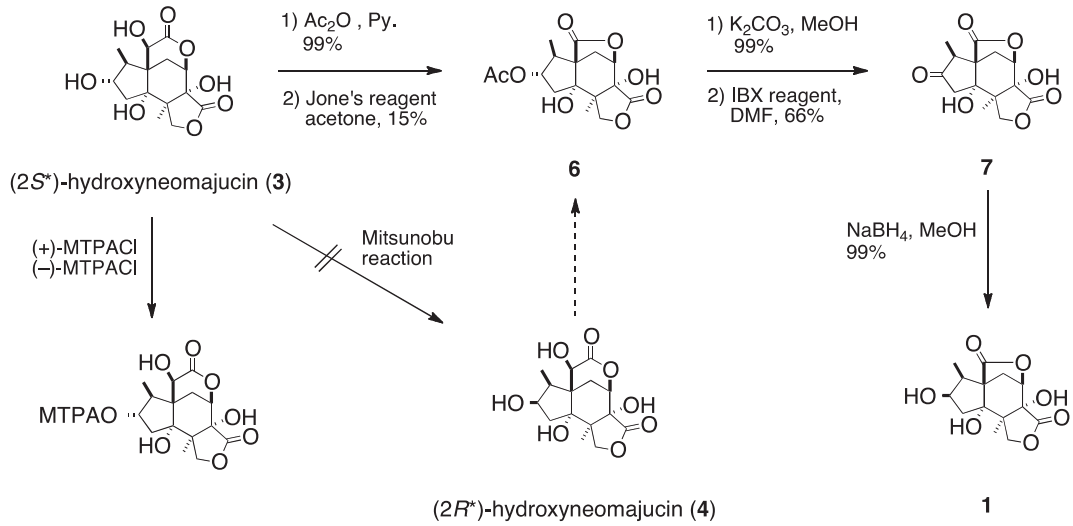


Figure 2. ^{13}C NMR data and HMBC correlations of **1**.

Next, we have decided to confirm the absolute structure of **1** by synthesizing from (2*R**)-hydroxyneomajucin (**4**). Previously, we have demonstrated that jiadifenolide can be obtained from neomajucine by oxidizing the C-10 hydroxy group.[6] Moreover, Danishefsky's group had succeeded in the total synthesis of (±)-jiadifenin by applying our oxidative method in the final step.[11] Following the previous protocol, the secondary hydroxy group at C-2 in **4** was first acetylated, but the reaction did not proceed presumably because of a steric hindrance. Therefore, (2*S**)-hydroxyneomajucin (**3**) was used for further elaboration. In the case of compound **3,** the secondary hydroxyl group at C-2 was readily acetylated, and then the subsequent Jone's oxidation gave rise to **6** as anticipated (Scheme 1). Hydrolysis of **6**, followed by the oxidation of the generated secondary alcohol at C-2 with IBX, gave rise to ketone **7**. Finally, the $NaBH_4$ reduction of **7** solely led to the alcohol, all the spectroscopic data of which were identical with those of compound **1**. This chemical conversion confirmed that the absolute configuration of **1** is the same as that of **3**. Next, the Kusumi's method[12] was applied to establish the absolute configuration of **3**, which has not yet been determined. The Δδ values as shown in Figure 4, enabled us to unambiguously assign the C-2 configuration in **3** and **4** as *S* and *R*, respectively. Accordingly, it was noted that the absolute configuration of **1** is the same as that of (2*S*)-hydroxyneomajucin (**3**).

Compound **2** has the molecular formula $C_{13}H_{16}O_5$, as deduced in the HR-EI-MS at *m*/*z* 252.1012 $[M]^+$, indicating 6 degrees of unsaturation. Its IR spectrum revealed the presence of a hydroxy group at 3417 cm^{-1}, an α,β-unsaturated carbonyl group at 1693 cm^{-1}, and a γ-lactone moiety at 1770 cm^{-1}. The ^{1}H NMR data of **2** (Table 1) showed a low-shifted signal at δ_H 1.71 (dd, J = 1.4, 0.6 Hz) due to an olefinic methyl group, which long-range coupled to the H-3β [δ_H 2.03 (ddq, J = 18.9, 2.7, 0.6 Hz)] and H-8α [δ_H 2.70 (ddq, J = 13.7, 3.0, 1.4 Hz)]. The NMR spectrum of **2** was similar to that of 2-oxo-3,4-dehydroxyneomajucin except for the absence of a δ-lactone ring.

Furthermore, the molecular formula of **2** indicated that the carbon number of **2** was two less than that of normal *seco*-prezizaane-type sesquiterpenoids. Analyses of ^{1}H–^{1}H COSY, HMQC, and HMBC experiments were carried out (Fig. 5). The HMBC correlations of H-3 and H-4/C-2 and C-9, as well as H_3-13/C-1 and C-2, revealed the presence of 2-methylcyclopent-2-enone moiety A. The other HMBC correlations showed that **2** has the same B–C ring system as 2-oxo-3,4-dehydroxyneomajucin. The additional HMBC correlations, as



Scheme 1. Chemical conversion of (2*S*)-hydroxyneomajucin (**3**) to **1**.

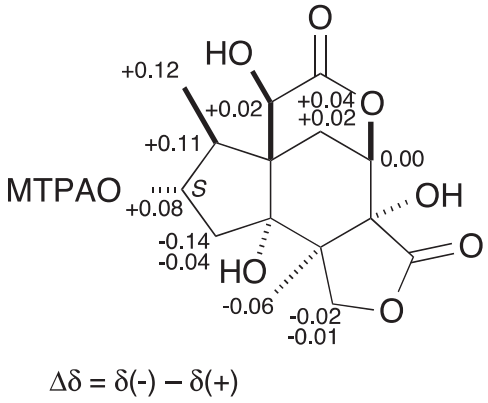


Figure 4. Δδ values (ppm) for MTPA ester derivatives of **3**.

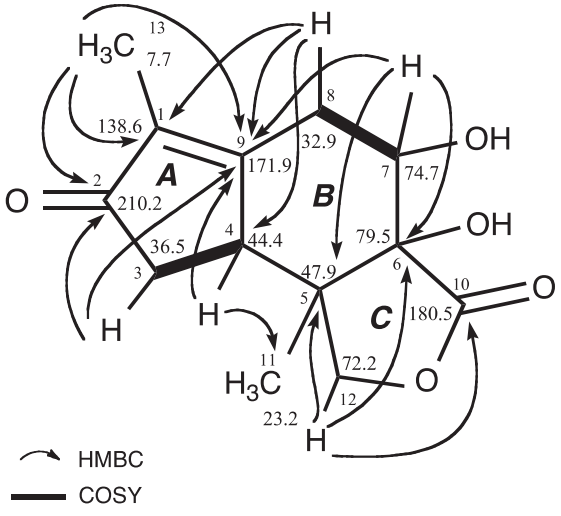


Figure 5. ^{13}C NMR data HMBC correlations of **2**.

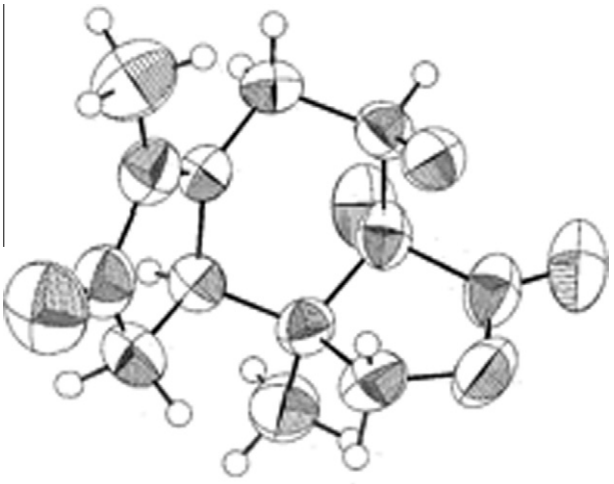
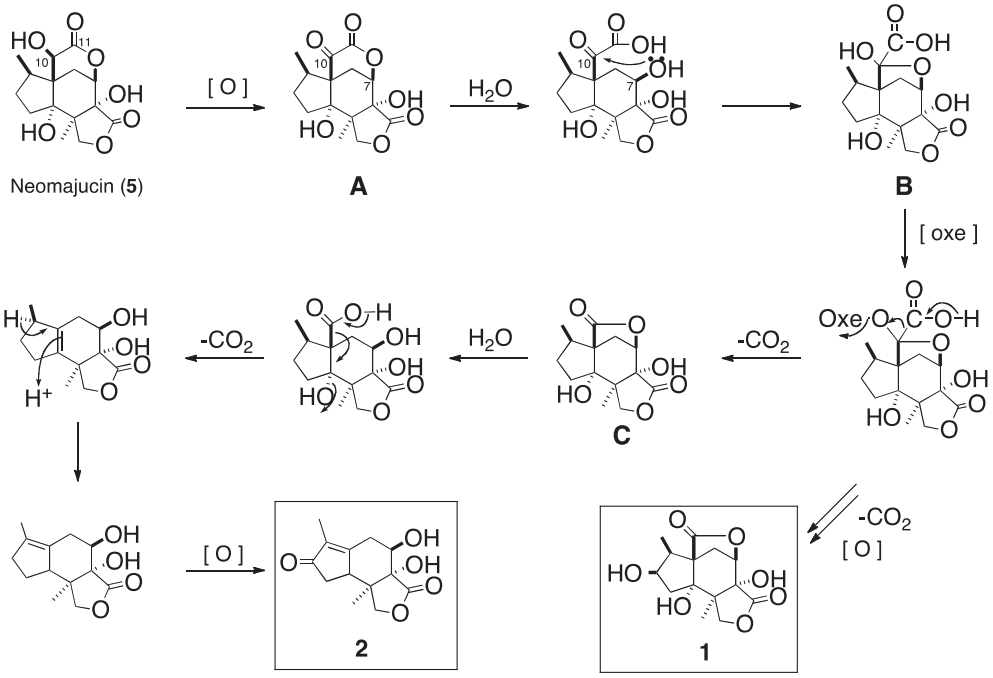


Figure 6. The X-ray crystallographic molecular structure of **2**.

shown in Figure 5, and the ^{13}C NMR chemical shift values were similar to those of majucin-type sesquiterpenoids, suggesting that **2** has the same structural core (B and C rings) as majucin-type sesquiterpenoids without the δ-lactone ring. Taking account for six degrees of unsaturation and the above spectroscopic data disclosed that the plane structure of **2** is tricyclic in structure as depicted in Figure 5. Fortunately, **2** gave single crystals suitable for X-ray crystallographic analysis. The X-ray crystallographic structure of **2**[13] as shown in Figure 6 demonstrates that the δ-lactone ring is not formed, thereby resulting in a tricyclic structure. The CD spectrum showed a negative Cotton effect ($\Delta\varepsilon$ −4.3) at 249 nm,[14] ensuring the absolute configuration of **2**. Thus, compound **2** named jiadifenone was determined to be a unique dinorsesquiterpenoid structure.

The plausible biosynthetic routes for **1** and **2** are postulated as follows: the C-10 hydroxy group in **5** is oxidized to a highly strained α-keto-δ-lactone **A**, which would be opened to an α-keto-carboxylic acid, and then the acetal formation between C-7 and C-10 gives rise to a less strained five-membered acetal intermediate **B**. The enzymatic decarboxylation of **B** would lead to **C**. Oxidation at C-2 in **C** would provide **1**, whereas the decarboxylation of **C** would lose both the C-10 carbon and the hydroxy group at C-4 and then the C-2 position would be oxidized again, leading to **2** (Scheme 2).

Compound **1** has been found to exhibit a significant neurotrophic activity, such as greatly promoting neurite outgrowth in the



Scheme 2. Plausible biosynthesis of **1** and **2**.

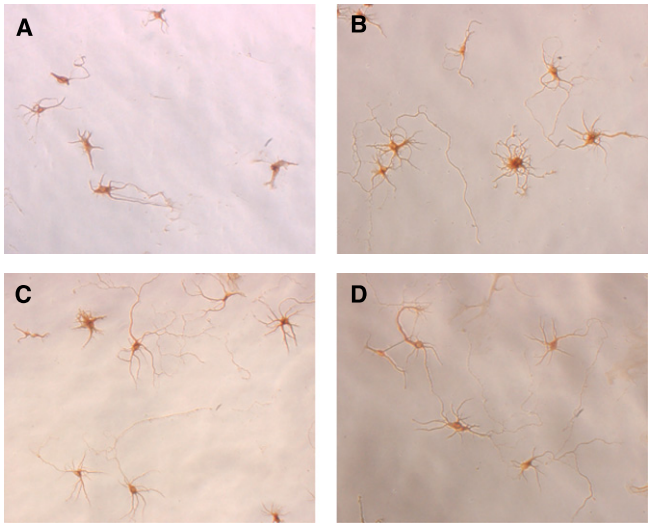


Figure 7. Neurite outgrowth-promoting the activity of **1** in the primary cultured rat cortical neurons. (**A**): morphology of neurons in control groups, (**B**): morphology of neurons in bFGF 10 ng/mL, (**C**): morphology of neurons in 10 μM of **1**, (**D**): morphology of neurons in 1 μM of **1**.

primary cultures of fetal rat cortical neurons[15] at concentrations ranging from 1 to 10 μmol/L as shown in Figure 7. However, compound **2** has lost most of the neurotrophic activity. In consideration of our previous studies, a few sesquiterpenoids with neurotrophic properties,[5,6] the majucine-type sesquiterpenoid structure is most likely to be responsible for its activity.

In conclusion, two novel *seco*-prezizaane-type sesquiterpenoids **1** and **2** were isolated from the pericarps of *I. jiadifengpi*. It should be emphasized that **1** and **2** are the first examples of *seco*-prezizaane-type norsesquiterpenoids. Moreover, compound **1** has been found to show neurite outgrowth-promoting activity in the primary cultured rat cortical neurons. The present study provides additional evidence that *seco*-prezizaane-type sesquiterpenoids exclusively occurring in the *Illicium* plants have great potential as lead compounds to develop non-peptide neurotrophic agents useful for the treatment of neurodegenerative diseases such as the Alzheimer's disease.[3,6]

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Supplementary data

Supplementary data (experimental procedures and characterization data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.107.

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