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Germacrane and *m*-Menthane from *Illicium lanceolatum*

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Abstract: Three new germacrane sesquiterpenes and a new *m*-menthane monoterpene were isolated together with four known compounds from the pericarp of *Illicium lanceolatum*, an adulterant to star anise (*Illicium verum*). All compounds were isolated from *Illicium* plants for the first time. The absolute stereochemistry of all germacrane and *m*-menthane was established by a combination of NMR and the modified Mosher's ester method. The biological activity was evaluated on SH-SY5Y neuroblastoma cell line. (1*S*,5*R*,7*R*)-1,5-Dihydroxygermacra-4(15),10(14),11(12)-triene (at 62.5 μM) and (1*R*,5*R*,7*R*)-1,5-dihydroxygermacra-4(15),10(14),11(12)-triene (at 15.6 μM) promoted the

proliferation of SH-SY5Y by 36.2% and 45.8%, respectively, after 48 h incubation, indicating potential neurotrophic activity.

Keywords: *Illicium lanceolatum*; Illiciaceae; germacrane sesquiterpenes; *m*-menthane monoterpene; absolute stereochemistry; proliferative promotion; SH-SY5Y

1. Introduction

The genus *Illicium* L. (Illiciaceae) consists of *ca.* 40 species that form one of the earliest evolutionary branches of the angiosperms [1]. This small taxon is represented by evergreen trees and shrubs disjunctively distributed in North America, Mexico, Peru, the West Indies and eastern Asia, with the highest concentration of species found in northern Myanmar and southern China [1,2]. The most well-known member of this genus is probably *Illicium verum*. It serves as the source material of shikimic acid in the production of oseltamivir (Tamiflu) [3], and its ripe pericarps (known as star anise) are widely used as a spice in many countries in Asia, in particular, China, India, and Vietnam [4]. *I. verum* also has a long history of medicinal applications in China [5]. In Mexico and the southwestern United States, its fruits are used to make herbal tea to alleviate colic of babies and stomach aches [4,6]. However, in recent years, intoxication cases related to the culinary and medicinal use of star anise have been reported, associating with neurological effects such as seizures, vomiting, jitteriness, rapid eye movement, and even death [7–9]. Follow-up investigations indicated that most, if not all, of the adverse effects were caused by adulterated toxic *Illicium* plants. Phytochemical and biological studies pointed to *seco*-prezizaane sesquiterpenes (such as anisatin and neoanisatin) to be the toxic ingredients [10–12]. The toxicological mechanism was elucidated to be a picrotoxin-like, non-competitive antagonism to the γ -aminobutyric acid (GABA) receptor [13–16]. However, systematic studies of the structure-toxicity relationship are limited [17–19]. To safeguard the use of star anise and its products, studies on adulterant species of *Illicium* is warranted.

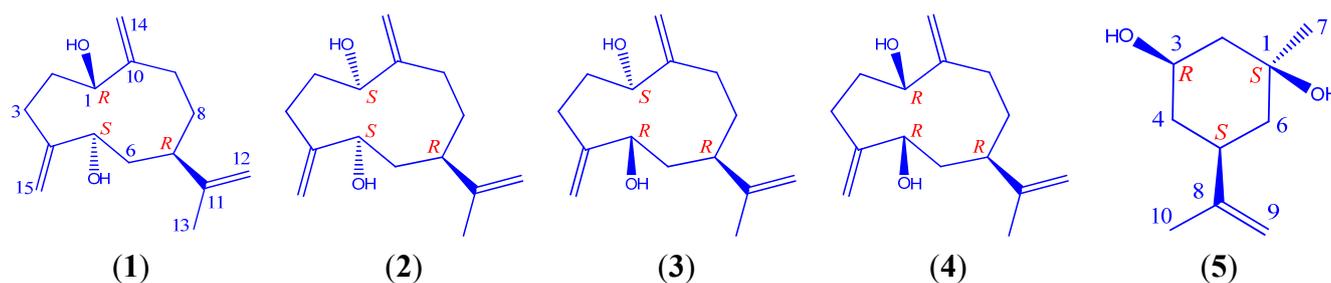
Apart from the potential toxicity, some ingredients of *Illicium* plants are known to display neurotrophic properties. Among others, jiadifenin, jiadifenolide, illicinin A, and 4-allyl-2,6-dimethoxy-3-(3-methylbut-2-enyl)phenol have been reported to promote neurite outgrowth in primary cultures of fetal rat cortical neurons [20–23]. *Illicium* plants are thus considered a potential source of neurotrophin-like natural products.

We are interested in constructing a library of secondary metabolites of *Illicium* plants, to identify toxic components on one hand, and search for neurotrophin-mimic natural products on the other. Several *Illicium* species are being investigated in our group. As part of the studies, *I. lanceolatum*, a toxic adulterant of Chinese star anise, was investigated for its chemical composition. This paper reports the structures of four germacrane sesquiterpenes (including three new structures), a new *m*-menthane monoterpene, and three other known compounds, and their biological activities in the SH-SY5Y neuroblastoma cell line.

2. Results and Discussion

From the pericarps of *I. lanceolatum*, repeated open column (silica gel, RP-18, MCI, and Sephadex LH-20) and semi-preparative chromatographic separations resulted in the purification of four germacrane sesquiterpenes **1–4**, and a *m*-menthane monoterpene **5** (Figure 1), together with three other known compounds **6–8**. Germacrane **4** was a known compound, but its absolute stereochemistry was newly established in the present work.

Figure 1. Structures of compounds **1–5**.



Compound **1** was obtained as a white amorphous powder. A molecular formula of $C_{15}H_{24}O_2$ was determined based on the HR-ESI-MS result at m/z 219.1733 $[M-H_2O+H]^+$ (calcd. 219.1743), indicating four degrees of unsaturation. The 1H -, ^{13}C - and DEPT-NMR spectra (Tables 1 and 2) indicated the presence of one methyl, eight methylenes (including three olefinic ones), three methines (including two oxygenated groups), and three quaternary olefinic carbons. The presence of three double bonds accounted for three degrees of unsaturation, the remaining one was therefore deduced arising from a ring structure in the molecule.

In the 1H - 1H COSY spectrum, signals at δ_H 1.98 and 2.10 (*m*, H-2) displayed correlations with signals at δ_H 4.24 (dd, $J = 5.2, 10.5$ Hz, H-1) and δ_H 2.29 (*m*, H-3), respectively, suggesting the presence of structural fragment **1a** (Figure 2). The correlations of δ_H 1.83 (*m*, H-6) with δ_H 4.36 (dd, $J = 3.9, 7.0$ Hz, H-5) and δ_H 2.68 (*m*, H-7), and that of δ_H 1.68 (*m*, H-8) with δ_H 2.68 (*m*, H-7) and δ_H 2.04 (*m*, H-9), led to the establishment of fragment **1b** (Figure 2). In the HMBC (Figure 3), the cross peaks between δ_H 1.76 (*s*, H-13) and δ_C 39.7 (CH, C-7), δ_C 151.3 (C, C-11) and δ_C 110.3 (CH₂, C-12) suggested a partial structure **1c** (Figure 2). The connection of **1a** and **1c** via two exocyclic double bonds was established based on the following evidence: long-range correlations between δ_H 5.16 (*s*, H-14) and 77.2 (CH, C-1) and 29.8 (CH₂, C-9); as well as those between δ_H 5.13/5.02 (both *s*, H-15) and 72.5 (CH, C-5) and 29.2 (CH₂, C-3). All available evidence led to the planar structure **1d** (Figure 2), belonging to germacrane sesquiterpene.

To determine the absolute configuration of C-1 and C-5, the modified Mosher ester procedure was employed [24,25]. Thus, treatment with (*R*)- and (*S*)-MTPA chlorides led to esterification of 1-OH and 5-OH, affording (*S*)- and (*R*)-MTPA derivatives, respectively. The 1H -NMR chemical shift differences ($\Delta\delta_{S,R}$) were observed (**1**, Figure 4). The absolute configuration of C-1 and C-5 were consequently determined to be *R* and *S*, respectively. In the NOESY spectrum, δ_H 2.68 (*m*, H-7) correlated with δ_H 4.24 (dd, $J = 5.2, 10.5$ Hz, H-1), suggesting the *R*-configuration of C-7. Thus, **1** was identified to be (1*R*,5*S*,7*R*)-1,5-dihydroxygermacra-4(15),10(14),11(12)-triene. To the best of our knowledge, it is the first time a germacrane sesquiterpene is isolated from *Illicium* plants.

Table 1. ^{13}C -NMR spectroscopic data for compounds 1–4 (CD_3OD , 100 MHz).

No.	1 (mult.)	2 (mult.)	3 (mult.)	4 (mult.)
1	77.2 (C)	70.6 (C)	74.1 (C)	75.3 (C)
2	33.9 (CH_2)	33.5 (CH_2)	34.4 (CH_2)	33.5 (CH_2)
3	29.2 (CH_2)	26.7 (CH_2)	27.0 (CH_2)	25.8 (CH_2)
4	152.7 (C)	152.2 (C)	153.6 (C)	151.6 (C)
5	72.5 (CH)	74.6 (CH)	76.7 (CH)	77.3 (CH)
6	41.1 (CH_2)	40.6 (CH_2)	40.1 (CH_2)	37.8 (CH_2)
7	39.7 (CH)	40.8 (CH)	42.1 (CH)	42.2 (CH)
8	30.4 (CH_2)	30.1 (CH_2)	31.5 (CH_2)	33.4 (CH_2)
9	29.8 (CH_2)	35.2 (CH_2)	32.1 (CH_2)	31.4 (CH_2)
10	150.6 (C)	152.1 (C)	151.7 (C)	150.6 (C)
11	151.3 (C)	150.7 (C)	150.4 (C)	150.5 (C)
12	110.3 (CH_2)	110.5 (CH_2)	110.4 (CH_2)	110.5 (CH_2)
13	20.1 (CH_3)	20.5 (CH_3)	19.0 (CH_3)	19.4 (CH_3)
14	116.9 (CH_2)	113.8 (CH_2)	114.3 (CH_2)	114.4 (CH_2)
15	112.4 (CH_2)	111.4 (CH_2)	112.8 (CH_2)	114.9 (CH_2)

Table 2. ^1H -NMR Spectroscopic Data for Compounds 1–4 (CD_3OD , 400 MHz).

No.	1 [mult., J (Hz)]	2 [mult., J (Hz)]	3 [mult., J (Hz)]	4 [mult., J (Hz)]
1	4.24 dd (5.2, 10.5)	4.23 dd (4.5, 9.6)	4.11 dd (4.2, 10.4)	4.16 dd (4.1, 9.6)
2	2.10 m; 1.98 m	2.22 m; 1.95 m	2.25 m; 1.82 m	2.09 m; 1.56 m
3	2.29 m	2.38 dd (4.0, 11.4); 2.07 m	2.11 (overlap)	2.32 dd (4.0, 14.2); 2.07 m
5	4.36 dd (3.9, 7.0)	4.31 dd (3.5, 5.9)	3.88 t (7.8)	3.92 dd (3.9, 11.4)
6	1.83 m	1.91 ddd (2.3, 6.4, 14.2); 1.69 (overlap)	1.67 (overlap)	1.85 ddd (2.7, 11.3, 14.0); 1.59 m
7	2.68 m	2.42 m	2.11 (overlap)	2.17 m
8	1.68 m	1.74 m; 1.53 m	1.61 m	1.96 m; 1.64 m
9	2.45 m; 2.04 m	2.20 m; 2.13 dd (3.0, 11.2)	2.14 (overlap)	2.42 m; 2.05 m
12	4.77 s	4.73 s; 4.69 s	4.66 br s	4.69 s; 4.68 s
13	1.76 s	1.70 s	1.67 s	1.69 s
14	5.16 s; 4.95 s	5.19 s; 5.03 s	5.24 s; 5.02 s	5.18 s; 5.02 s
15	5.13 s; 5.02 s	5.05 s; 4.98 s	5.12 s; 5.05 s	5.02 br s

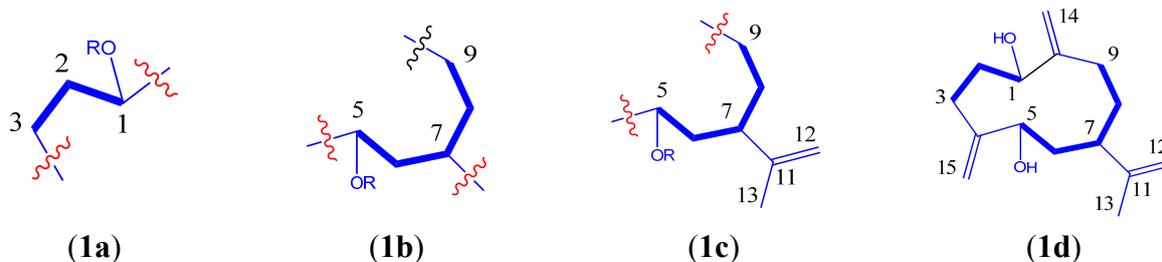
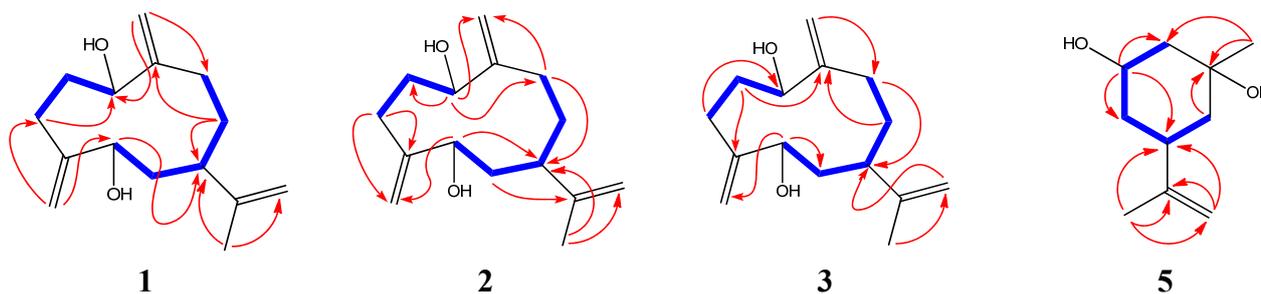
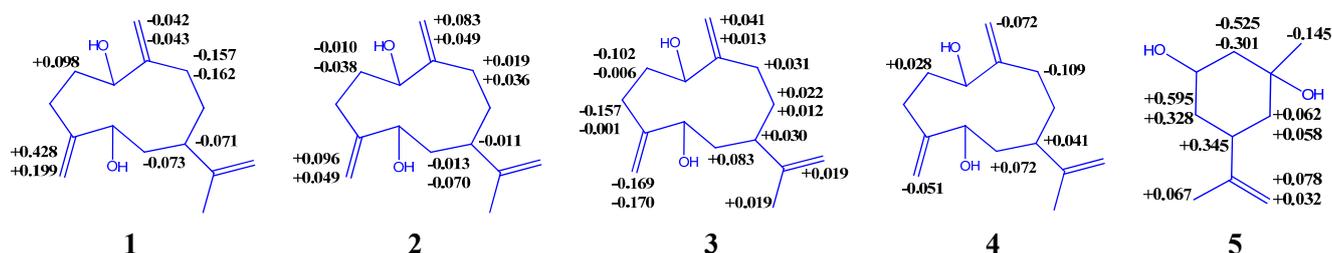
Figure 2. Partial structures of 1.

Figure 3. Selected ^1H - ^1H COSY (—) and HMBC (→) correlations of compounds 1–3, 5.**Figure 4.** $\Delta\delta_{S-R}$ values of MTPA esters of 1–5.

Compounds **2**, **3**, and **4** were stereoisomers of **1**. By using the same strategies as in the structural elucidation of **1**, compounds **2**, **3**, and **4** were identified to be (1*S*,5*S*,7*R*)-1,5-dihydroxygermacra-4(15),10(14),11(12)-triene, (1*S*,5*R*,7*R*)-1,5-dihydroxygermacra-4(15),10(14),11(12)-triene, and (1*R*,5*R*,7*R*)-1,5-dihydroxygermacra-4(15),10(14),11(12)-triene (Figures 1 and 4), respectively. In the NOESY spectrum of **2**, δ_{H} 1.70 (s, H-13) correlated with δ_{H} 4.31 (dd, $J = 3.5, 5.9$, H-5), indicating the α -orientation of H-7. For compound **3**, due to the unresolved overlap of the H-7 signal in CD_3OD , NOESY spectrum was further acquired in CDCl_3 for the assignment of relative configuration of H-7. The α -orientation of H-7 was suggested by the NOESY correlation between H-7 and 5α -H. While the α -orientation of H-7 in **4** was suggested by the NOESY correlation from δ_{H} 2.17 (m, H-7) to both δ_{H} 4.16 (dd, $J = 4.1, 9.6$, H-1) and δ_{H} 3.92 (dd, $J = 3.9, 11.4$, H-5). Compound **4** is a known structure, previously reported from *Gonospermum elegans* but with undetermined absolute configuration [26].

Compound **5** was obtained as a white amorphous powder. A molecular formula of $\text{C}_{10}\text{H}_{18}\text{O}_2$ was determined based on the HRESIMS result at m/z 193.1202 [$\text{M} + \text{Na}]^+$ (calcd. 193.1199), indicating two degrees of unsaturation. The ^1H -, ^{13}C - and DEPT-NMR spectra (Table 3) revealed the presence of two methyls, four methylenes (including one olefinic ones), two methines (including one oxygenated group), and two quaternary carbons (including an olefinic one). All proton signals were assignable based on the gHSQC experiment (Table 3). The ^1H - ^1H COSY spectrum displayed correlations between δ_{H} 1.72 (H-2) and δ_{H} 3.60 (d, $J = 3.16$ Hz, H-3), between δ_{H} 3.60 (H-3) and δ_{H} 1.91 (ddd, $J = 2.8, 11.6, 14.0$ Hz, H-4a) and δ_{H} 1.63 (dt, $J = 3.4, 13.8$ Hz, H-4b), between δ_{H} 1.91 (H-4a) and δ_{H} 2.23 (m, H-5), between δ_{H} 2.23 (H-5) and δ_{H} 1.53 (H-6), leading to the connections of between C_2 - C_3 - C_4 - C_5 - C_6 (Figures 1 and 3). The HMBC correlations between δ_{H} 1.69 (s, 10-H) and δ_{C} 37.4 (C-5), δ_{C} 109.0 (C-9), and δ_{C} 149.2 (C-8), as well as correlations between δ_{H} 4.70 (br s, H-9) and δ_{C} 21.1 (C-10), δ_{C} 37.4 (C-5), and δ_{C} 149.2 (C-8), suggested that C-9 and C-10 were connected to C-5 via C-8 (Figures 1 and 3). The remaining part of the structure was established based on the following long-range correlations: δ_{H} 1.53 (H-6)/ δ_{C} 71.4 (C-1); δ_{H} 1.23 (s, H-7)/ δ_{C} 71.4 (C-1) and δ_{C} 33.7 (C-2). Two hydroxyl groups were

assigned to C-1 and C-3, respectively. In the NOESY spectrum, δ_{H} 2.23 (m, H-5) displayed correlations with δ_{H} 3.60 (d, $J = 3.2$ Hz, H-3), and δ_{H} 1.23 (s, H-7), indicating the relative stereochemistry of 1 β ,3 β -dihydroxy-(5 α H)-*m*-menth-8-ene. To determine the absolute stereochemistry, modified Mosher's ester procedure was carried out. Due to the steric hindrance on C-1, only 3-OH was esterified by (*R*)- and (*S*)-MTPA chlorides into (*S*)- and (*R*)-MTPA derivatives. The absolute configuration of C-3 was finally deduced to be *R* based on the proton shift difference between (*S*)- and (*R*)-MTPA derivatives (**5**, Figure 4), indicating a 3 α -H. The 1*S*- and 5*S*-configuration were then assignable based on the NOESY results mentioned above. Consequently, **5** was determined to be (1*S*,3*R*,5*S*)-1,3-dihydroxy-*m*-menth-8-ene.

Table 3. ^{13}C - and ^1H -NMR spectroscopic data for compound **5** (CDCl_3) *.

No.	δ_{C} (mult.)	δ_{H} [mult., J (Hz)]
1	71.4 (C)	-
2	33.7 (CH_2)	1.72 (partial overlap); 1.49 (partial overlap)
3	73.8 (CH)	3.60 d (3.2)
4	34.0 (CH_2)	1.91 ddd (2.8, 11.6, 14.0); 1.63 dt (3.4, 13.8)
5	37.4 (CH)	2.23 m
6	26.1 (CH_2)	1.53 (partial overlap)
7	26.5 (CH_3)	1.23 s
8	149.2 (C)	-
9	109.0 (CH_2)	4.70 br s
10	21.1 (CH_3)	1.69 s

* 100 MHz for ^{13}C and 400 MHz for ^1H , respectively.

Compounds **6**, **7**, and **8** were identified to be 3-hydroxyocta-1,5*E*-dien-7-one [27], 2-(4-methylphenyl)-1,2-propanediol [28], and *trans*-3,4,5-trimethoxycinnamic alcohol [29], respectively, based on the interpretation of their NMR spectroscopic data and comparison with reported data. **6**, **7**, and **8** were isolated from *Illicium* plants for the first time.

Compounds **3** and **4** exhibited proliferative activity in SH-SY5Y cells at concentrations of 0.49 μM –125 μM . Compounds **3** (at 62.5 μM) and **4** (at 15.6 μM) could promote proliferation by 36.2% and 45.8% after 48-h incubation, respectively. Compounds **5**–**8** were inactive; **6** and **8** displayed cytotoxicity at the concentrations above 50 μM . Due to the scarcity of **1** and **2**, they were not tested for the bioactivity. Efforts to obtain additional crops of these compounds are in progress.

3. Experimental

3.1. General

The FT-IR spectra (KBr) were recorded using a Thermo FT-IR Nicolet 5700. Optical rotations at sodium D line were measured with a Perkin-Elmer 241 digital polarimeter using quartz cell with a path length of 100 mm at room temperature. Concentrations (c) are given in g/100 mL. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-400 or AVANCE-400 spectrometer running at 400 MHz for ^1H and 100 MHz for ^{13}C , respectively. All chemical shifts were quoted on the δ scale in ppm using residual solvent as the internal standard (CDCl_3 : 7.24 ppm for ^1H -NMR,

77.0 ppm for ^{13}C -NMR; CD_3OD : 3.30 ppm for ^1H -NMR, 49.0 ppm for ^{13}C -NMR; $\text{C}_5\text{D}_5\text{N}$: 8.71 ppm, 7.55 ppm, 7.19 ppm for ^1H -NMR, 149.9 ppm, 135.5 ppm, 123.5 ppm for ^{13}C -NMR). Coupling constants (J) are reported in Hz. The following abbreviations are used to indicate the multiplicity: s = singlet, d = doublet, t = triple, dd = double doublet, dt = double triplet, br = broad. Thin layer chromatography (TLC) was carried out using Merck aluminium backed sheets coated with 60F254 silica gel or 60F254 RP-silica gel. Visualization of the plates was achieved by using a UV lamp ($\lambda_{\text{max}} = 254 \text{ nm}$), and spraying a mixture of 2% *p*-hydroxybenzaldehyde methanolic solution and 5% sulphuric acid ethanolic solution (10:1, v/v) followed by heating. Open column chromatography was carried out on columns packed with silica gel, RP silica gel (C_{18}) (Macherey-Nagel GmbH & Co. KG, Düren, Germany), MCI gel CPH 20 (Supelco, Sigma-Aldrich, Bellefonte, PA, USA), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). For HPLC purification, a C_{18} semi-preparative HPLC column (Phenomenex C_{18} column, $250 \times 10 \text{ mm}$, $5 \mu\text{m}$) and a Shimadzu UFLC system were used; the UV detection wavelength and flow rate were set at $\text{UV}_{210\text{nm}}$ and 4 mL/min , respectively. A Shimadzu UFLC XR system coupled with a LCMS-2020 liquid chromatography mass spectrometer was used for sample analysis. All solvents used were analytical or HPLC grade. HRESIMS were measured on a Shimadzu LCMS-IT-TOF Mass Spectrometry.

3.2. Plant Material

The pericarps of *Illicium lanceolatum* A. C. Smith. were collected from An-hui Province, China, in October 2011, and were identified by one of the authors (Jin-Ao Duan). A voucher specimen was deposited in the Department of Medicinal Chemistry and Pharmacognosy (UICMCP001-Ilan-P), College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA.

3.3. Extraction and Isolation

The dried pericarps of *Illicium lanceolatum* (1 kg) were powdered and extracted by percolation with methanol (MeOH, 15 L), yielding 300 g of extract. The extract was applied to a silica gel flash column eluted with mixtures of petroleum ether (PE)-ethyl acetate (EA) (100:0 \rightarrow 50:50), followed by dichloromethane (DCM)-MeOH (90:10, 85:15, and 0:100), to yield 40 fractions (A_1 – A_{40}). The fractions after A_{35} (A_{35} : elute of DCM-MeOH, 85:15) contained plenty of shikimic acid. Fraction A_{17} was further fractionated into 23 fractions (B_1 – B_{23}) on a silica gel column eluted with mixtures of PE-EA (70:30 and 60:40). B_{11} was subjected to RP-18 chromatography eluted with aqueous MeOH (20% and 100%) to yield 15 fractions (C_1 – C_{15}). Compound **6** (6.1 mg) was purified from fractions of C_4 and C_5 by Sephadex LH-20 chromatography (eluted with 30% aqueous MeOH). Fractions of C_8 – C_{14} were combined and subjected to Sephadex LH-20 chromatography (eluted with 30% aqueous MeOH) to obtain a mixture ($>100 \text{ mg}$) of compounds **5** and **7**. An aliquot of the mixture was purified by semi-preparative HPLC using 30% aqueous acetonitrile (AcCN) as mobile phase to yield compounds **5** ($>50 \text{ mg}$), and **7** (5 mg). Fractions A_{20} – A_{22} were further fractionated into 25 subfractions (D_1 – D_{25}) on a MCI column eluted with aqueous methanol (10% \rightarrow 100%). D_{14} and D_{15} were subjected to Sephadex LH-20 chromatography (eluted by MeOH) to yield 20 fractions (E_1 – E_{20}). Compound **8** (18 mg) was purified from E_{13} – E_{15} by semi-preparative HPLC separation (35% aqueous AcCN). E_7 and E_8 were further subjected to a silica gel column eluted with mixtures of PE-EA

(80:0 → 65:35) to yield 32 fractions (F₁–F₃₂). There were three main components in fractions of F₁₃–F₁₆. They were purified by semi-preparative HPLC chromatography (35% aqueous MeOH) to yield compounds **1** (2.5 mg), **2** (2.2 mg). Compounds **3** (15 mg) and **4** (50 mg) were purified from F₁₇–F₂₆ by semi-preparative HPLC chromatography (35% aqueous MeOH).

3.4. Spectral Data

(1*R*,5*S*,7*R*)-1,5-Dihydroxygermacra-4(15),10(14),11(12)-triene (**1**). White amorphous powder. IR (cm⁻¹): 3377, 2989, 1703, 1576, 1405, 1081. $[\alpha]_D^{20}$ -3° (*c* 0.12, MeOH). ¹³C- and ¹H-NMR spectroscopic data (CD₃OD): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 219.1733 [M–H₂O+H]⁺ (calcd. for C₁₅H₂₃O, 219.1743), *m/z* 201.1639 [M–2H₂O+H]⁺.

(1*S*,5*S*,7*R*)-1,5-Dihydroxygermacra-4(15),10(14),11(12)-triene (**2**). Colorless oil. IR (cm⁻¹): 3359, 2928, 1641, 1439, 1015, 895. $[\alpha]_D^{20}$ -7° (*c* 0.14, MeOH). ¹³C- and ¹H-NMR spectroscopic data (CD₃OD): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 219.1735 [M–H₂O+H]⁺ (calcd. for C₁₅H₂₃O, 219.1743), 201.1630 [M–2H₂O+H]⁺.

(1*S*,5*R*,7*R*)-1,5-Dihydroxygermacra-4(15),10(14),11(12)-triene (**3**). White amorphous powder. IR (cm⁻¹): 3260, 2928, 1644, 1434, 1020, 905. $[\alpha]_D^{20}$ -4° (*c* 0.17, MeOH). ¹³C- and ¹H-NMR spectroscopic data (CD₃OD): see Tables 1 and 2, respectively. ¹H spectroscopic data (CDCl₃, 400 Hz): δ_H 5.24 (1H, s, H-14a), 5.03 (1H, s, H-14b); 5.16 (1H, s, H-15a), 5.06 (1H, s, H-15b); 4.65 (1H, s, H-12a), 4.64 (1H, s, H-12b); 4.16 (1H, dd, *J* = 4.3, 10.2 Hz, H-1); 3.95 (1H, dd, *J* = 5.0, 10.7 Hz, H-5); 2.27 (1H, m, H-2a), 1.83 (1H, m, H-2b); 2.13 (4H, overlapped, H-3 and H-9); 2.06 (1H, t, *J* = 5.7 Hz, H-7); 1.73 (1H, t, *J* = 5.2 Hz, H-6a), 1.59 (1H, partially overlapped, H-6b); 1.58 (2H, partially overlapped, H-8a); 1.65 (3H, s, H-13). HR-ESI-MS *m/z* 219.1734 [M–H₂O+H]⁺ (calcd. for C₁₅H₂₃O, 219.1743), 201.1642 [M–2H₂O+H]⁺, 237.1820 [M+H]⁺.

(1*R*,5*R*,7*R*)-1,5-Dihydroxygermacra-4(15),10(14),11(12)-triene (**4**). Colorless oil. IR (cm⁻¹): 3368, 2927, 1642, 1449, 1012, 893. $[\alpha]_D^{20}$ +11° (*c* 0.16, MeOH). ¹³C- and ¹H-NMR spectroscopic data (CD₃OD): see Tables 1 and 2, respectively. HR-ESI-MS *m/z* 219.1740 [M–H₂O+H]⁺ (calcd. for C₁₅H₂₃O, 219.1743), 201.1650 [M–2H₂O+H]⁺.

(1*S*,3*R*,5*S*)-1,3-Dihydroxy-*m*-menth-8-ene (**5**). White amorphous powder. ¹³C- and ¹H-NMR spectroscopic data (CDCl₃): see Table 3. HR-ESI-MS *m/z* 193.1202 [M+Na]⁺ (calcd. for C₁₀H₁₈O₂Na, 193.1199).

3.5. Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of **1–5**

In these experiments, (*R*)- and (*S*)-MTPA chloride was used to react with each compound to yield its (*S*)- and (*R*)-MTPA derivatives, respectively [25]. Two aliquots of compound (0.5–1.0 mg each) were transferred into two NMR tubes and dried overnight in a desiccator with P₂O₅ inside. After successive addition of 6 μL of (*R*)- or (*S*)-MTPA chloride and 600 μL of pyridine-*d*₅, the NMR tubes were sealed immediately, and shaken vigorously. The tubes were then kept in desiccator overnight until the reaction was complete [30]. The ¹H-NMR spectra of the final (*R*)- and (*S*)-MTPA derivatives were recorded, and the chemical shifts were assigned based on the ¹H-¹H COSY NMR experiments. In

case that signals could not be unambiguously assigned, gHSQC and gHMBC experiments were carried out. The $\Delta\delta_{S-R}$ values were calculated [24,25].

3.6. In Vitro Assay on SH-SY5Y

The SH-SY5Y cells were maintained in the Opti-MEM with 10% FBS, 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin. The cells (5×10^4 or 1×10^5 /well in 1 mL growth medium) were incubated with various concentrations of compounds in 24-well culture plates. After 48-h incubation, 20 μL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/mL in PBS) were added to the each well. The supernatant were removed after further 4 h incubation. The formazan in each well were dissolved in 300 μL isopropanol with 4 mM HCl and 0.1% Nondet P-40. The absorbance was read at 590 nm with a reference filter of 620 nm by using microplate reader (Infinite M200 Pro, Tecan, San Jose, CA, USA). The cells without treatment were as vehicle control. The cells were treated by corresponding concentration of DMSO as control. The percentage of growth promotion was calculated using the following formula: % cell promotion = $100 \times (\text{OD}_{590\text{nm test compound}} - \text{OD}_{590\text{nm control}}) / \text{OD}_{590\text{nm control}}$. Results were expressed as the mean of at least three independent experiments.

4. Conclusions

Three new germacrane sesquiterpenes **1–3**, a new *m*-menthane monoterpene **5**, together with a known germacrane sesquiterpene **4**, and three other known compounds **6–8** have been identified from *I. lanceolatum*. All the compounds were isolated from *Illicium* plants for the first time. Absolute stereochemistry for germacranes and *m*-menthane were established. Compounds **3** and **4** exhibited proliferative activity on the SH-SY5Y cell lines, indicating potential neurotrophic activity. Further biological evaluation on primary cultures of fetal rat cortical neurons is under planning.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/19/4/4326/s1>.

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Author Contributions

Ming Zhao carried out the isolation, purification, and structural elucidation of natural products, and prepared the manuscript. Xianming Zhang contributed to the bioassay test. Karina M. Szymulanska-Ramamurthy and Zhiqi Yin helped in modified Mosher's ester reactions and

interpretation. Yan Wang, Min Huang, and Tanja Gödecke were responsible for the physical data collection and NMR data acquisition. Jin-Ao Duan collected and authenticated the plant materials. Chun-Tao Che supervised the whole research project.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compound **3**, **4**, and **5–8** are available from the authors.

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