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New hexalactone derivatives and a pair of new oxaspiro-carbon epimeric glycosides from the fruits of *Illicium lanceolatum*

Yang-Lan Liu[1], Xiao-Jing Wang[1], Ru-Bing Wang, Mi Li, Wen-Rui Li, Jian-Pei Zhang, Xiu-Qi Bao, Dan Zhang, Shuang-Gang Ma*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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ABSTRACT

Five new compounds (**1–5**), including three hexalactone derivatives (**1–3**) and a pair of new oxaspiro-carbon epimeric glycosides (**4** and **5**), and six known compounds (**6–11**) were obtained from the fruits of *Illicium lanceolatum*. The structures of the new compounds were elucidated using extensive spectroscopic data. The absolute configurations of compounds **1–3** were determined by an analysis of their CD spectra. It was determined that compounds **4** and **5**, which are epimeric at C-5, possess the same 1-oxaspiro[4,5]decane-7*α*,8*α*,9*β*-triol moiety. Plausible biogenetic pathways for **4** and **5** derived from the key precursor shikimic acid were proposed. Compounds **1–11** were all assayed on monosodium glutamate-induced human neuroblastoma SH-SY5Y cell damage. The results demonstrated that compounds **4**, **5**, and **8–10** possess potential neuroprotective effects. The anti-inflammatory, antiviral, and cytotoxic activities of **1–11** were also evaluated.

1. Introduction

The genus *Illicium*, the sole genus currently classified in the family Illiciaceae, are widely distributed in southeastern Asia and America with more than 40 species [1]. In the phytochemical studies of those species, the unique *seco*-prezizaane sesquiterpenes and prenylated C_6—C_3 compounds were the focus [2,3]. Notably, the fruits of the plants of the genus *Illicium* are a rich source of (−)-shikimic acid possessing useful inherent carbon chirality and considered an excellent chiral precursor for biosynthesis and chemosynthesis (e.g., in Tamiflu) [4–6].

Illicium lanceolatum A. C. Smith is a folk medicinal plant with the Chinese name 'Mangcao' [7]. Its roots and root bark have historically been used as folk medicine to treat bruises, internal injuries, and rheumatoid osteoarthritis [8]. Previous investigations of the plant resulted in the isolation of menthane monoterpenes, germacrane sesquiterpenes, santalane sesquiterpenoids and tetranor sesquiterpenoids [9–11]. In our research on the bioactive compounds from the fruits of *I. lanceolatum*, five new compounds, including three hexalactone derivatives, (4*S*,6*R*)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-one (**1**, lanceolactone A), (4*S*,6*R*)-4-*O*-*β*-D-glucopyranosyl-4,6-dimethyltetrahydro-2*H*-pyran-2-one (**2**, lanceolactonoside A), and methyl 2-((2*S*,4*R*)-4-hydroxy-4-methyl-6-oxotetrahydro-2*H*-pyran-2-yl)acetate (**3**, lanceolactone B), and a pair of structurally unique epimeric1-oxaspiro[4,5]decane glycosides, 5*S**-(7*α*,8*α*,9*β*)-8-*O*-*β*-D-glucopyranosyl-7,9- dimethoxy-1-oxaspiro[4,5]dencane (**4**, spirolancinoside A) and 5*R**-(7*α*,8*α*,9*β*)-8-*O*-*β*-D-glucopyranosyl-7,9-dimethoxy-1-oxaspiro[4,5]decane (**5**, spirolancinoside B), together with six known compounds (**6–11**), have been isolated from the H_2O-soluble fraction of the 95% ethanol extract of the fruits of *I. lanceolatum*. Notably, compounds **4** and **5** were the first examples of 1-oxaspiro[4,5]decane glycosides and possess the same 1-oxaspiro[4,5]decane-7*α*,8*α*,9*β*-triol moiety, and they could be biosynthesized from the key precursor (-)-shikimic acid, which has been obtained on a kilogram scale from the H_2O-soluble fraction. Additionally, the neuroprotective, anti-inflammatory, antiviral, and cytotoxic activities of **1–11** were evaluated.

2. Materials and methods

2.1. General experimental procedures

The UV data were measured on a JASCO V-650 spectrophotometer, and the CD spectra were obtained on a JASCO J-815 spectrophotometer. Optical rotations were recorded on a Rudolph Autopol V automatic polarimeter at 20 °C. IR spectra were recorded on a Thermo

* Corresponding author.
E-mail address: mashuanggang@imm.ac.cn (S.-G. Ma).
[1] These authors contributed equally to this work.

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Table 1
^{1}H and ^{13}C NMR data in MeOH-d_4 for **1–3**.

Position	**1**[a]		**2**[b]		**3**[a]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	174.6		175.2		173.0	
3	45.2	Hα 2.69 d (16.0) Hβ 2.47 d (16.0)	42.7	Hα 2.82 d (15.6) Hβ 2.66 d (15.6)	44.3	2.54 br s (2H)
4	69.4		76.7		68.3	
5	45.9	Hα 2.07 dd (14.5, 4.0) Hβ 1.71 dd (14.5, 12.0)	45.2	Hα 2.15 dd (14.4, 3.6) Hβ 1.94 dd (14.4, 11.4)	41.1	Hα 1.94 dt (14.0, 3.0) Hβ 1.77 dd (14.0, 12.0)
6	75.1	4.45 m	75.0	4.50 m	75.4	5.07 dtd (12.0, 6.5, 3.0)
7	21.4	1.37 d (6.5)	21.3	1.38 d (6.6)	40.9	2.72 d (6.5)
8	29.4	1.35 s	26.3	1.45 s	172.2	
9					29.5	1.31 s
10					52.3	3.71 s
1′			98.5	4.46 d (7.8)		
2′			74.9	3.12 dd (9.0, 7.8)		
3′			78.1	3.35 dd (9.0, 9.0)		
4′			72.0	3.18 dd (10.2, 9.0)		
5′			77.8	3.29 dd (7.2, 2.4)		
6′			63.4	3.83 dd (10.0, 2.4) 3.56 dd (10.0, 7.2)		

[a] ^{1}H NMR data were measured at 500 MHz, ^{13}C NMR data were measured at 125 MHz.
[b] ^{1}H NMR data were measured at 600 MHz, ^{13}C NMR data were measured at 150 MHz.

Nicolet 5700 spectrometer through FT-IR Microscope Transmission. NMR spectra were obtained on a VNS-600 spectrometer for 1D NOE and a BRUKER-AV-III-500 spectrometer for ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, HSQC, HMBC, ROESY and TOCSY. Chemical shifts are given in δ (ppm) with solvent signals (CD_3OD: δ_H 3.31/ δ_C 49.0) as references. LC-MS and HRESIMS data were recorded on a Thermo Fisher Scientific Q-Exactive Focus Orbitrap LC-MS/MS spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a dual detector of Shimadzu SPD-20A and Shimadzu RID-20A, using a CAPCELL PAK C-18 column (10 mm × 250 mm, 5 μm). ODS (50 μm, YMC.GEL ODS-A-HG, Japan), Sephadex LH-20 (GE Healthcare, Sweden), polyamide (30 – 60 mesh, Sinopharm Chemical Reagent Co., China), macroporous resin (D101, Zhengzhou Qinshi Technology Co., China) and silica gel (160–200, 200–300 mesh, Qingdao Marine Chemical Factory, China) were used for column chromatography. $HSGF_{254}$ (Yantai Institute of Chemical Industry) was used for TLC. Snailase (Yuanye Biotechnology Co., Ltd., Shanghai, China) was used for hydrolysis.

2.2. Plant material

Fruits of *Illicium lanceolatum* A. C. Smith were collected from Guilin of Guangxi Province, China, in October 2012 and identified by Prof. Lin Ma (Institute of Materia Medica, Chinese Academy of Medical Sciences). A voucher specimen (ID-S-2832) was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

2.3. Extraction and isolation

The air-dried, pulverized fruits of *I. lanceolatum* (100 kg) were extracted three times with 95% EtOH, and the solvent was evaporated in vacuo to obtain 19.8 kg of residue. The residue was suspended in H_2O and extracted with EtOAc to yield an EtOAc-soluble part (3.2 kg) and a H_2O-soluble part (2.2 kg). The H_2O-soluble part was chromatographed on a D101 macroporous resin column, eluting with gradient EtOH-H_2O (0%, 30%, 60% and 95% EtOH). The 30% EtOH fraction (625 g) was subjected to a column of polyamide (6 L) with gradient elution using H_2O, 30% EtOH, 60% EtOH and 95% EtOH. Then, the H_2O fraction (400 g) was chromatographed on the D101 macroporous resin column (5 L) again, using the gradient EtOH-H_2O as a mobile phase. This 30% EtOH fraction (220 g) was separated on silica gel (3.5 kg) column chromatography using CH_2Cl_2-MeOH (30:1 to 1:1, with 5‰ HCOOH) to yield twenty-three fractions (G1–G23). Fraction G4 (1.31 g) was separated by column chromatography (CC) on silica (30 g) with CH_2Cl_2/MeOH (30:1) to afford seven subfractions, of which subfraction 5 (462 mg) was purified by HPLC using 15% CH_3CN-H_2O to yield **1** (50 mg, t_R = 13 min) and **3** (59 mg, t_R = 19 min). Fraction G12 (5 g) was separated by column chromatography on Sephadex LH-20 with MeOH and gave seven subfractions. Subfraction 5 (1.3 g) was purified by HPLC to yield **7** (7 mg, t_R = 26 min) with 17% CH_3CN-H_2O and **6** (662 mg, t_R = 15 min) with 13% CH_3CN-H_2O. Fraction G16 (21 g) was separated on a column of Sephadex LH-20 and eluted with CH_3OH to provide seven fractions. Fraction 3 (12.4 g) afforded five subfractions upon being passed through another Sephadex LH-20 column with H_2O as the mobile phase. Subfraction 1 (700 mg) was further purified by HPLC to yield **4** (21 mg, t_R = 35 min) and **5** (57 mg, t_R = 51 min) with 10% CH_3CN-H_2O (with 5‰ TFA) and **2** (31 mg, t_R = 21 min) with 5% CH_3CN-H_2O (with 5‰ TFA). Subfraction 2 (7.6 g) was subjected to an ODS column with a gradient of MeOH-H_2O (with 5‰ TFA) and then purified by HPLC to give **10** (34 mg, t_R = 46 min) and **11** (3 mg, t_R = 51 min) with 25% MeOH-H_2O (with 0.1% TFA), **9** (10 mg, t_R = 70 min) with 20% MeOH-H_2O (with 0.1% TFA) and **8** (20 mg, t_R = 41 min) with 30% MeOH-H_2O (with 0.1% TFA).

2.4. Lanceolactone A (1)

A colorless oil, [α] − 41.2 (*c* 0.94, MeOH); IR: ν_{max} 3422, 2979, 2935, 1725, 1458, 1384, 1256, 1084, 1024, 984 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−1.54) nm; ^{1}H NMR (500 MHz, Methanol-d_4) and ^{13}C NMR (125 MHz, Methanol-d_4) data, see Table 1; (+)-HRESIMS *m/z* 145.08586 $[M+H]^+$ (calcd for $C_7H_{13}O_3$, 145.08592).

2.5. Lanceolactonoside A (2)

A colorless oil, [α] − 15.2 (*c* 0.77, MeOH); IR: ν_{max} 3383, 2978, 2922, 1727, 1450, 1387, 1264, 1077, 1041, 865, 813 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (−1.03) nm; ^{1}H NMR (600 MHz, Methanol-d_4) and ^{13}C NMR (150 MHz, Methanol-d_4) data, see Table 1; (+)-HRESIMS *m/z* 329.12054 $[M+Na]^+$ (calcd for $C_{13}H_{22}O_8Na$, 329.12069).

Compound **2a**: Compound **2** (4 mg) was stirred in H_2O (500 μL) with snailase (20 mg) at 37 °C for 3 d, and then the hydrolysate was extracted with EtOAc to afford **2a** as a colorless oil; ^{1}H NMR (Methanol-d_4,

Table 2
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data in MeOH-d_4 for **4** and **5**.

Position	**4**		**5**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	67.2	3.78 t (6.6)	68.1	3.82 t (7.2)
3	26.8	2.00 m[b]	26.1	1.93 m[a]
4	37.7	H*a* 2.00 m[b]; H*b* 1.79 m[a]	39.5	1.80 m (2H)[a]
5	83.6		83.6	
6	37.8	H*a* 1.95 dd (12.0, 10.8)[a]; H*β* 1.75 m[a]	37.0	H*a* 1.92 dd (13.2, 9.6)[a]; H*β* 1.74 dd (13.2, 3.6)[a]
7	78.2	3.52 ddd (11.4, 4.2, 3.0)	77.2	3.76 m[b]
8	76.7	4.08 dd (4.2, 3.0)	78.1	4.07 dd (5.4, 3.0)
9	79.4	3.84 ddd (4.2, 4.2, 3.6)	79.6	3.74 m[b]
10	36.0	H*a* 1.88 dd (14.4, 3.6)[a]; H*b* 1.75 m[a]	36.4	H*a* 1.87 dd (14.4, 4.2)[a]; H*b* 1.72 dd (14.4, 4.2)[a]
11	57.6	3.36 s	57.5	3.38 s
12	56.8	3.39 s	57.0	3.40 s
1′	105.2	4.48 d (7.8)	104.5	4.47 d (7.8)
2′	75.5	3.21 dd (9.0, 7.8)	75.4	3.20 dd (9.6, 7.8)
3′	78.0	3.28 m	77.9	3.28 m
4′	71.6	3.26 m	71.6	3.27 m
5′	77.9	3.35 m	77.9	3.35 m
6′	62.8	H*a* 3.86 dd (12.0, 1.2); H*b* 3.65 m	62.8	H*a* 3.86 dd (12.0, 1.8); H*b* 3.66 m

[a] Assigned by TOCSY spectrum.
[b] Overlapped.

500 MHz) δ_H 4.47 (1H, m, H-6), 2.69 (1H, d, J = 16.0 Hz, H-3a), 2.47 (1H, d, J = 16.0 Hz, H-3b), 2.07 (1H, dd, J = 14.0, 3.5 Hz, H-5*α*), 1.71 (1H, dd, J = 14.0, 11.5 Hz, H-5*β*), 1.37 (3H, d, J = 6.0 Hz, H-7), 1.35 (3H, s, H-8); (+)-HRESIMS m/z 145.08585 $[M+H]^+$ (calcd for $C_7H_{13}O_3$, 145.08592).

2.6. Lanceolactone B (3)

A colorless oil, [*α*] + 10.0 (*c* 1.27, MeOH); IR: ν_{max} 3436, 2962.1, 1737, 1440, 1384, 1259, 1025 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 221 (−0.86) nm; ^{1}H NMR (500 MHz, Methanol-d_4) and ^{13}C NMR (125 MHz, Methanol-d_4) data, see Table 1; (+)-HRESIMS m/z 203.09138 $[M+H]^+$ (calcd for $C_9H_{15}O_5$, 203.09140).

2.7. Spirolancinoside A (4)

A colorless oil; [*α*] − 10.0 (*c* 4.01, MeOH); IR: ν_{max} 3385, 2930, 2885, 1679, 1452, 1365, 1085, 827 cm^{-1}; ^{1}H NMR (600 MHz, Methanol-d_4) and ^{13}C NMR (150 MHz, Methanol-d_4) data, see Table 2; (+)-HRESIMS m/z 401.17743 $[M+Na]^+$ (calcd for $C_{17}H_{30}O_9Na$, 401.17820).

2.8. Spirolancinoside B (5)

A colorless oil; [*α*] − 22.0 (*c* 0.81, MeOH); IR: ν_{max} 3393, 2925, 2878, 1679, 1450, 1365, 1076, 801 cm^{-1}; ^{1}H NMR (600 MHz, Methanol-d_4) and ^{13}C NMR (150 MHz, Methanol-d_4) data, see Table 2; (+)-HRESIMS m/z 401.17780 $[M+Na]^+$ (calcd for $C_{17}H_{30}O_9Na$, 401.17820).

2.9. Hydrolysis and determination of the absolute configuration of the sugar moieties of compounds 2, 4, and 5 [12]

Compound **2** (4 mg) was stirred in H_2O (500 μL) with snailase (20 mg, Yuanye Biotechnology Co., Ltd., Shanghai, China) at 37 °C for 3 d. Then, the solution was extracted with EtOAc five times, and MeOH was added into the aqueous layer for the precipitation of the snailase. After evaporation under reduced pressure and drying *in vacuo*, the aqueous layer yielded a monosaccharide residue.

The residue was dissolved in pyridine (2 mL), and L-cysteine methyl ester hydrochloride was added. The mixture was maintained at 60 °C for 2 h and evaporated and dried *in vacuo*. Then, the residue was reacted with *N*-trimethylsilylimidazole (for gas chromatography, 500 μL) at 60 °C for 2 h, and the solution was transferred into H_2O (2 mL) and extracted with *n*-hexane (2 mL) five times to give the trimethylsilylated derivative of the monosaccharide. A standard D-glucose derivative was prepared in a similar way. Finally, the derivative of the monosaccharide was analyzed by GC–MS and compared with the standard D-glucose derivative under the following conditions. Capillary column: SE-54 (30 m × 0.25 mm, i.d.); detection: FID; initial temperature: 200 °C; final temperature: 280 °C (10 °C/min) for 35 min; carrier: N_2 gas. The retention times of the monosaccharide derivative and standard D-glucose derivative were the same, 29.4 min. The monosaccharide absolute configurations of compounds **4** and **5** were determined in the same way.

2.10. Bioactivity assay

Neuroprotective Assay. Human neuroblastoma SH-SY5Y cells were cultured in 96-well plates at a concentration of 8×10^{-3} for 24 h. Then, mixtures of monosodium glutamate without and with the test compounds were added to the culture medium to give final concentrations of 120 mM and 10 μM, respectively. All the experiments were carried out in triplicate and incubated for 24 h. Then, 100 μL MTT (0.5 mg/mL) was added to each well, and the plates were incubated for 4 h at 37 °C. Finally, the absorbances at 570 nm were measured after adding 150 μL DMSO.

Protection rate % = $(OD_{test\ group} - OD_{model\ group})/(OD_{control\ group} - OD_{model\ group}) \times 100\%$

According to previously published methods, the cytotoxic [13], anti-inflammatory [14], and antiviral activities [15] of all compounds were assayed.

3. Results and discussion

Compound **1** was obtained as a colorless oil. Its molecular formula, $C_7H_{12}O_3$, was assigned based on the HRESIMS and NMR data. Its IR spectrum showed typical absorption bands for OH (3422 cm^{-1}) and carbonyl (1725 cm^{-1}) groups. The ^{13}C (Table 1) and HSQC NMR data displayed seven carbons, including a carbonyl (δ_C 174.6), a tertiary oxygenated carbon (δ_C 69.4), an oxygenated methine (δ_C 75.1), two methylenes (δ_C 45.9 and 45.2), and two methyls (δ_C 29.4 and 21.4). These ^{13}C NMR data were similar to those of 3,6-dimethyl-3-hydroxytetrahydro-2*H*-pyran-2-one [16]. A careful comparison of their NMR data revealed that a hydroxyl and a singlet methyl (δ_H 1.35) were both located at the C-4 of the hexalactone ring instead of C-3 similar to in the latter, which is supported by the HMBC correlations from H-8 to C-3, C-4, and C-5 in the HMBC spectrum. The planar structure of **1**, as shown in Fig. 1, was further confirmed by the key HMBC correlations from H-6 to C-2 and C-7 and from CH_3-7 to C-5 and C-6 (Fig. 2).

The relative configuration of **1** was determined by the NOE experiment, and the absolute configuration was elucidated by the circular dichroism (CD). The NOE correlation from H-6 to H-8 indicated that H-6 and CH_3-8 were on the same face of the six-membered lactone ring (Fig. 3). The CD spectrum showed a negative Cotton effect at 213 nm for the n → π* transition of the lactone group, indicating that the C-*β* of the lactone carbonyl was below the C-CO−O−C lactone plane (Fig. 4) based on Beecham's rules [17,18]. Based on the above mentioned relative configuration and the *α*-oriented C-*β* of the lactone ring, the absolute configuration of **1** was established as 4*S*,6*R*. Thus, the structure of compound **1** was assigned as (4*S*,6*R*)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-one, and it was named lanceolactone A.

Compound **2** was a colorless oil with the molecular formula $C_{13}H_{22}O_8$, as determined by the HRESIMS and NMR data. A glucopyranosyl anomeric proton δ_H 4.46 (1H, d, J = 7.8 Hz, H-1′) was observed in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR spectrum showed 13

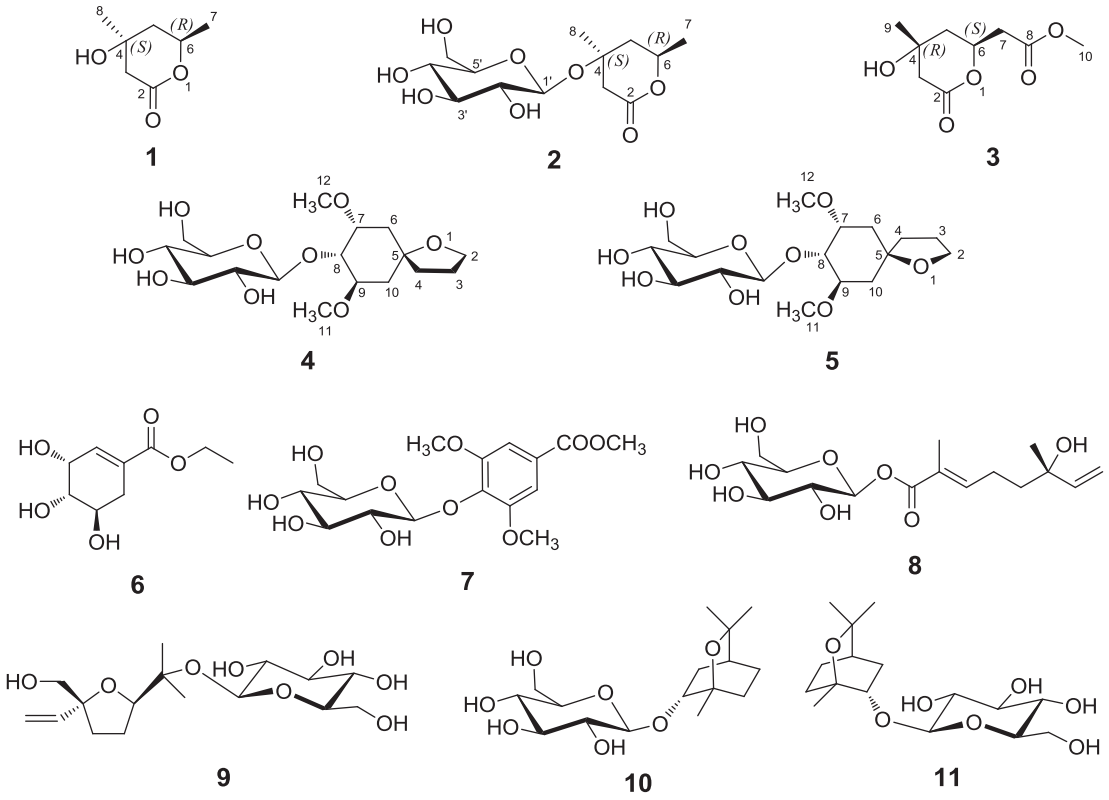


Fig. 1. Chemical structures of compounds **1–11**.

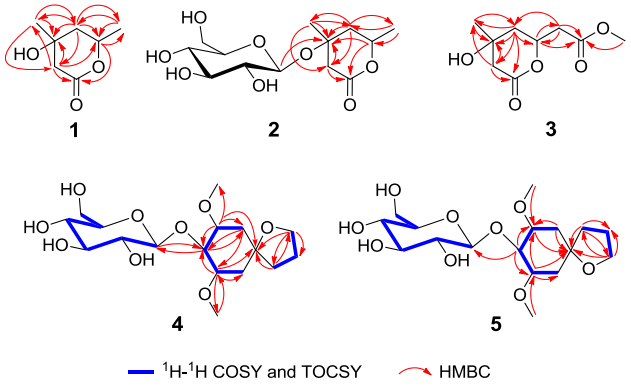


Fig. 2. Key HMBC correlations of compounds **1–5** and ^{1}H–^{1}H COSY and TOCSY of compounds **4** and **5**.

carbon signals, including six carbons (δ_C 98.5, 78.1, 77.8, 74.9, 72.0, 63.4) of a glucopyranosyl moiety (Table 1). Aside from the hydrogen and carbon signals of the glucose unit in the ^{1}H and ^{13}C NMR, the remaining NMR data of **2** were very similar to those of **1**, suggesting that **2** might be a glycoside of **1**. The aglycone (compound **2a**) and monosaccharide were obtained by snailase hydrolysis. The HRESIMS and ^{1}H NMR data of **2a** were the same as those of **1** (Supporting Information, Fig. S17, S18), and the monosaccharide was determined to be *β*-D-glucose by the coupling constant (J = 7.8 Hz) of anomeric proton together with the GC analysis of its trimethylsilyl L-cysteine derivative [12]. Additionally, in the HMBC spectrum, an HMBC correlation was observed from the glucose anomeric proton (δ_H 4.46) to the tertiary oxygenated carbon (δ_C 76.7), indicating that the *β*-D-glucose moiety was located at the C-4 of the lactone ring (Fig. 2). The absolute configuration (4*S*,6*R*) of **2** was determined by the NOE correlation from H-6 to H-8 and the negative Cotton effect at 212 nm in the CD spectrum, similar to in **1**. On the basis of these data, compound **2** was determined as (4*S*,6*R*)-4-*O*-*β*-D-glucopyranosyl-4,6-dimethyltetrahydro-2*H*-pyran-2-one and named lanceolactonoside A.

Compound **3** was isolated as a colorless oil. The molecular formula of **3** was determined as $C_9H_{14}O_5$ from HRESIMS and ^{13}C NMR data. A careful analysis of the ^{1}H and ^{13}C NMR data (Table 1) implied the similarity of the NMR data of **3** to those of **1**, and the differences between them were the disappearance of one doublet methyl signal (CH_3-7) along with the presence of a set of NMR signals of the 2-methoxy-2-oxoethyl group (CH_3—O—CO—CH_2) in **3** (Table 1). It was suggested that the 2-methoxy-2-oxoethyl group was located at the C-6 of the lactone ring, which is supported by the key HMBC correlations from H-7 to C-5, C-6, and C-8 and from CH_3-10 (δ_H 3.71) to C-8.

Due to no NOE correlations between CH_3-9 and H-6 or 2-methoxy-2-oxoethyl group were observed in the NOE difference experiment (in MeOH-d_4), a ROESY experiment on **3** was carried out in dry acetone-d_6. In the ROESY spectrum, the observed ROESY correlation from H-6 to 4-

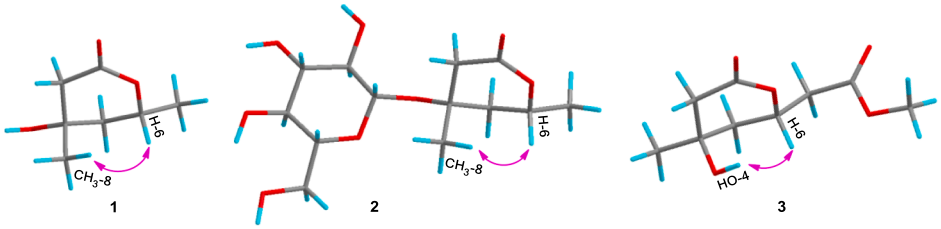
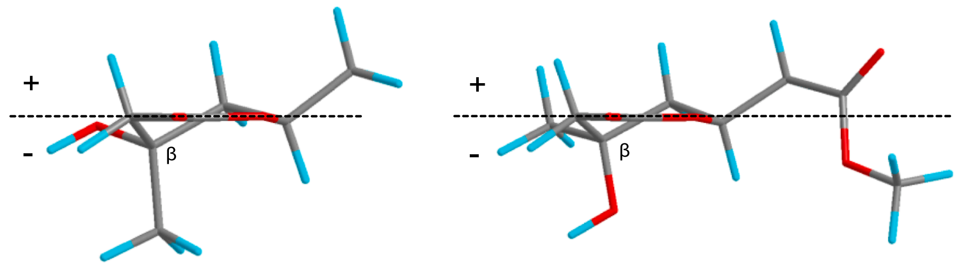


Fig. 3. Key NOE and ROESY correlations of compounds **1–3**.



1 3

Fig. 4. The Beecham's rules for lactones of compounds **1** and **3**.

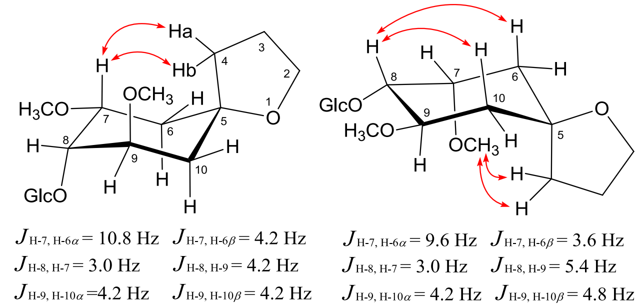
OH (Supporting Information, Fig. S26) revealed that 4-OH and H-6 were on the same side of the lactone ring (Fig. 3). Additionally, the negative Cotton effect at 220 nm indicated that C-*β* was below the C—CO—O—C lactone plane (Fig. 4). Therefore, the absolute configuration of **3** was established as 4*R*,6*S*. Thus, the structure of compound **3** was characterized as methyl 2-((2*S*,4*R*)-4-hydroxy-4-methyl-6-oxotetrahydro-2*H*-pyran-2-yl)acetate, and it was named lanceolactone B.

Compound **4** was obtained as a colorless oil. Its molecular formula, $C_{17}H_{30}O_9$ with 3 degrees of unsaturation, was determined from ^{13}C NMR data and the HRESIMS ion at *m/z* 401.17780 $[M+Na]^+$. The ^{1}H NMR spectrum of **4** indicated a *β*-glucopyranosyl anomeric proton at δ_H 4.48 (1H, d, *J* = 7.8 Hz), The ^{13}C NMR data (Table 2) showed 17 carbon signals, including six carbons ascribed to an *O*-glucose unit that was confirmed by 1D TOCSY, HSQC, and HMBC data (Supporting Information, Fig. S33). The monosaccharide, obtained by snailase hydrolysis, was determined to be *β*-D-glucose by GC analysis in comparison with the trimethylsilyl L-cysteine derivative of standard glucose [12].

Additionally, three oxymethine protons [δ_H 4.08 (dd, *J* = 4.2, 3.0 Hz, H-8), 3.84 (ddd, *J* = 4.2, 4.2, 4.2, H-9), 3.52 (ddd, *J* = 10.8, 4.2, 3.0, H-7)], one oxymethylene proton [δ_H 3.78 (t, *J* = 6.6 Hz, H-2)] and two oxymethyl protons [δ_H 3.39 (s, H-12), 3.36 (s, H-11)] were observed in the ^{1}H NMR spectrum. In the high-field region (δ_H 2.00–1.75), eight overlapped proton signals were unambiguously ascribed to four methylenes (δ_C 37.8, 37.7, 36.0, 26.8) by the aid of 1D TOCSY and HSQC data (Table 1). Additionally, from a careful analysis of the ^{1}H–^{1}H COSY and 1D TOCSY spectra, two spin systems [CH_2(2)—CH_2(3)—CH_2(4) and CH_2(6)—CH(7)—CH(8)—CH(9)—CH_2(10)] were established (Fig. 2). In the HMBC spectrum, the correlations from H-6/7/9 to the oxygenated quaternary carbon δ_C 83.6 (C-5) and from H-2/4 to C-5 suggested that **4** possesses a 1-oxaspiro[4,5]decane skeleton (Fig. 2). These data suggest that **4** is a 1-oxaspiro[4,5]decane glycosidation derivative. The HMBC correlations from H-8 to C-1′ and from H-1′ to C-8 indicate that the glucose moiety is located at C-8, and the correlations from CH_3-12 to C-7 and from CH_3-11 to C-9 reveal that the two oxymethyls are located at C-7 and C-9, respectively.

The relative configuration of hexatomic ring was determined based on the coupling constant of $^3J_{H\text{-}H}$ (Fig. 5). In the ^{1}H NMR spectrum, the large coupling constant between H-7 and H-6*α* (*J* = 10.8 Hz) and small coupling constant between H-7 and H-6*β* (*J* = 4.2 Hz) suggest that H-7 is facing in the axial direction. An analysis of the coupling constants of H-9 ($J_{H\text{-}9,\ H\text{-}10\alpha}$ = 4.2 Hz and $J_{H\text{-}9,\ H\text{-}10\beta}$ = 4.2 Hz) indicates that H-9 is located at the equatorial bond, Similarly, H-8 was determined to be on the equatorial bond by its two small coupling constants ($J_{H\text{-}8,\ H\text{-}7}$ = 3.0 Hz, $J_{H\text{-}8,\ H\text{-}9}$ = 4.2 Hz). These coupling constant data indicate that the six-member carbon ring is in the pseudochair conformation. Furthermore, the relative configuration of the spiro carbon (C-4) was determined by NOE experiments. The irradiation of H-7 enhanced H-4, indicating that C-4 and H-7 were on the same side of the hexatomic ring, as shown in Fig. 5. Thus, the structure of compound **4** was determined to be 5*S**-(7*α*,8*α*,9*β*)-8-*O*-*β*-D-glucopyranosyl-7,9-dimethoxy-1-oxaspiro[4,5]decane, and it was named spirolancinoside A.

Compound **5** has the same molecular formula as **4**, and the NMR data of **5** also resembled those of **4**. The structure was elucidated in the same manner as for **4**. A careful analysis of the 1D TOCSY, ^{1}H–^{1}H COSY, HSQC, and HMBC correlations allowed us to conclude that compounds **4** and **5** possessed the same plane structure (Fig. 2 and Supporting Information, Fig. S43). The relative configuration of the hexatomic ring was established to be the same as that of compound **4** by an analysis of these $^3J_{H\text{-}H}$ coupling constants, as shown in Fig. 5. A comparison of the $J_{H\text{-}8,\ H\text{-}7}$ and $J_{H\text{-}8,\ H\text{-}7}$ coupling constants and the chemical shift of C-8 of **5** with those of **4**, showing a larger coupling constant ($J_{H\text{-}8,\ H\text{-}9}$ = 5.4 Hz) and lower field chemical shift of C-8 (δ_C78.1) indicated that H-8 was in a pseudoaxial orientation, which was supported by the NOE correlations from H-8 to H-6 and H-10 (Supporting Information, Fig. S46). In the ROESY spectrum, the key correlation from CH_3-12 to H-4 suggested that the spiro carbon C-4 and 7-OCH_3 were on the same side of the hexatomic ring, as shown in Fig. 5. Thus, the structure of compound **5** was determined to be 5*R**-(7*α*,8*α*,9*β*)-8-*O*-*β*-D-glucopyranosyl-7,9-dimethoxy-1-oxaspiro[4,5]decane, and it was named spirolancinoside B.

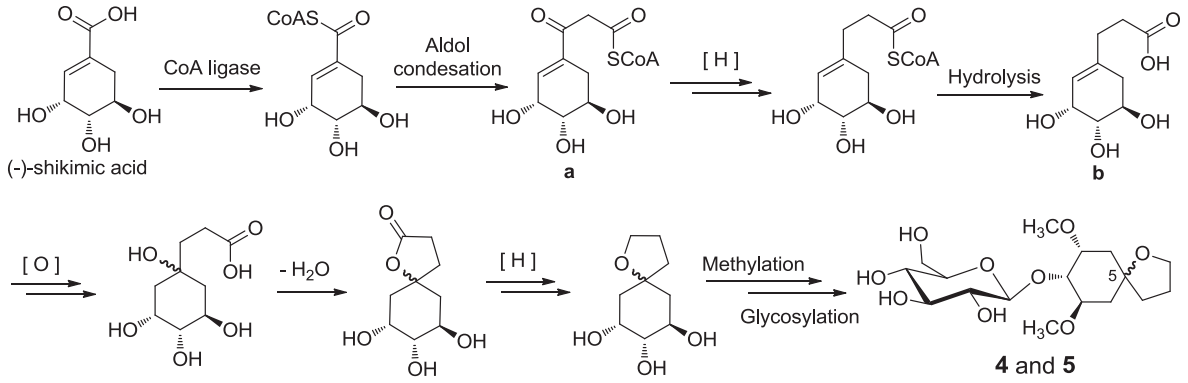


4 5

Fig.5. Key coupling constants of pseudochair conformation hexatomic ring and ROESY correlations of compounds **4** and **5**.

It is interesting that compounds **4** and **5**, which are epimeric at C-4, feature a 7*α*/8*α*/9*β*-oxygen-bearing substituted 1-oxaspiro[4,5]decane structural unit, which suggests that they are derived from a common biosynthesis precursor of naturally occurring (–)-shikimic acid, which was also isolated in large quantities from the fruits of this plant, and their proposed biosynthetic pathways are shown in Scheme 1. Activation of shikimic acid by CoA ligase and subsequent aldol condensation could lead to the formation of a β-keto ester **a**, following by reduction and hydrolysis to give a carboxylic acid **b**. Subsequently, **b** undergo oxidation, hydration, reduction, methylation, and glycosylation could produce a pair of unprecedented oxaspiro-carbon epimeric glycosides (**4** and **5**) [19].

In addition to the five new compounds (**1–5**), six known compounds were also isolated from the H_2O-soluble fraction (Fig. 1). They were identified as ethyl shikimate (**6**) [20], methyl syringate 4-*O*-*β*-D-glucopyranoside (**7**) [21], 2-*trans*-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid *β*-glucopyranosyl ester (**8**) [22], 10-hydroxy-*trans*-linalyl oxide 7-*O*-*β*-D-glucopyranoside (**9**) [23], (1*R*,2*R*,4*S*)-*trans*-2-hydroxy-1,8-cineole *β*-D-glucopyranoside (**10**), and (1*S*,2*S*,4*R*)-*trans*-2-hydroxy-1,8-cineole *β*-D-glucopyranoside (**11**) [24] by a comparison of their ^{1}H and ^{13}C



Scheme 1. Possible biosynthesis pathways of compounds **4** and **5**.

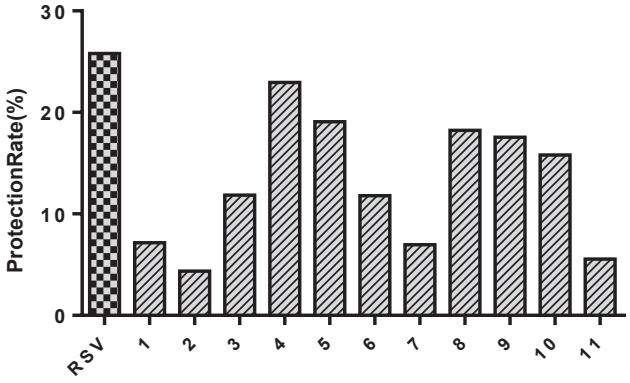


Fig. 6. Protection rate of resveratrol (RSV) and compounds **1–11** against monosodium glutamate-induced human neuroblastoma SH-SY5Y cells injury (at 10 μM).

NMR data with those in the literature. Among them, compounds **8–11** were monoterpene glycosides isolated from *I. lanceolatum* for the first time.

The neuroprotective, anti-inflammatory, antiviral, and cytotoxic activities of **1–11** were evaluated *in vitro*. Compounds **4**, **5** and **8–10** displayed neuroprotective effects against monosodium glutamate-induced injury in human neuroblastoma SH-SY5Y cells, with protection rates of 23.0, 19.1, 18.2, 17.7 and 15.8%, respectively at 10 μM (Fig. 6), and resveratrol was used as positive control with protection rate of 25.8% at 10 μM. However, none of the compounds showed cytotoxic, antiviral, or anti-inflammatory activities.

4. Conclusion

Five new compounds (**1–5**), including three hexalactone derivatives (**1–3**) and a pair of new oxaspiro-carbon epimeric glycosides (**4** and **5**), and six known compounds (**6–11**) were isolated from the fruits of *I. lanceolatum*. The configurations of the new compounds **1–5** were determined by extensive spectroscopic evidence and CD data. Compounds **4** and **5**, a pair of unusual oxaspiro-carbon epimeric glycosides, possess a 1-oxaspiro[4,5]decane-7α,8α,9β-triol moiety, and plausible biogenetic pathways for **4** and **5** from the naturally occurring precursor (−)-shikimic acid were proposed. Additionally, the isolated compounds were evaluated for their neuroprotective, anti-inflammatory, antiviral and cytotoxic activities. Compounds **4**, **5**, and **8–10** displayed potential neuroprotective effects at a concentration of 10 μM.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103113.

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