Sesquiterpenes from the Roots of *Illicium jiadifengpi*

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Key words
- *Illicium jiadifengpi*
- Illiciaceae
- *seco*-prezizaane sesquiterpene
- allo-cedrane sesquiterpene glycoside
- acorane sesquiterpene

Abstract

Two new sesquiterpenes (**1, 2**) and two new sesquiterpene glycosides (**3, 4**) with a *seco*-prezizaane skeleton, three new allo-cedrane sesquiterpene glycosides (**5**–**7**), and a new acorane sesquiterpene (**8**) as well as 15 known analogues were isolated from the roots of *Illicium jiadifengpi* used for the treatment of rheumatoid arthritis. The structures of these compounds were elucidated by extensive spectroscopic analysis and chemical methods. The absolute configurations of compounds **5**–**7** were confirmed by CD experiments. The configuration of compound **8** was assigned by single-crystal X-ray crystallographic analysis and CD experiments. Compounds **4, 6, 7, 14**, and **23** showed moderate antiviral activities against Coxsackie virus B3, and all compounds were inactive when evaluated for their cytotoxic activities against five human tumor cell lines and neuroprotection.

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Introduction

Illicium jiadifengpi B.N. Chang (Illiciaceae) is distributed mainly in South China. Its cortex and root bark have been used in Chinese folk medicine for the treatment of rheumatoid arthritis. However, many people have been seriously poisoned by its use [1]. This threat of poisoning by *I. jiadifengpi* prompted us to investigate the bioactive substances from the plant so as to evaluate its efficacy and toxicity. In our previous research on the roots of *I. jiadifengpi*, we isolated and determined the structure of 19 diterpenes from the EtOAc extract; some of them displayed significant activities against Coxsackie virus B [2]. Our continuing examination of the root extract has resulted in the characterization of eight new sesquiterpenes (**1**–**8**). In addition, 15 known sesquiterpenes were characterized: majucin (**9**) [3], neomajucin (**10**) [4], (2*R*)-2-hydroxyneomajucin (**11**) [4], (2*S**)-hydroxyneomajucin (**12**) [5], (2*R**)-hydroxy-3,4-dehydroxyneomajucin (**13**) [5], 2-oxoneomajucin (**14**) [5], 2-oxo-3,4-dehydroxyneomajucin (**15**) [5], (1*R**)-2-oxo-3,4-dehydroxyneomajucin (**16**) [5], pseudomajucin (**17**) [6], 7-*O*-*β*-D-glucopyranosylpseudomajucin (**18**) [6], abscisic acid (**19**) [7], roseoside (**20**) [8], 1*β*,4*β*,7*α*-trihydroxyeudesmane (**21**) [9], cadinane-4*β*,5*α*,10*β*-triol (**22**) [10], and bullatantriol (**23**) [9]. The following describes the isolation and structure elucidation of the eight new sesquiterpenes (**1**–**8**) (**Fig. 1**). The antiviral activities against Coxsackie virus B3, cytotoxic activities against five human tumor cell lines, and neuroprotective activities of the 23 compounds are also stated.

Results and Discussion

Compound **1** was isolated as colorless crystals. Its molecular formula was determined to be $C_{22}H_{26}O_7$ from the HR-ESIMS and NMR data. A detailed NMR analysis indicated that **1** was a benzoyl-substituted pseudomajucin sesquiterpene and that it was structurally similar to the known compound 2*β*-benzoyloxypseudomajucin [11], except for the presence of an additional hydroxyl group at C-6. The HMBC correlations from H_3-12 (δ_H 1.24)/H_3-13 (δ_H 1.00) to C-6 (δ_C 79.1) supported the above deduction. The ROESY spectrum showed cross-peaks between H_3-15 and H-10a/H-3*β* and between H-2 and H-1/H-3*α*, which indicated that CH_3-15 and the benzoyloxy group were *β*-oriented. The ROESY correlations of H_3-12 with

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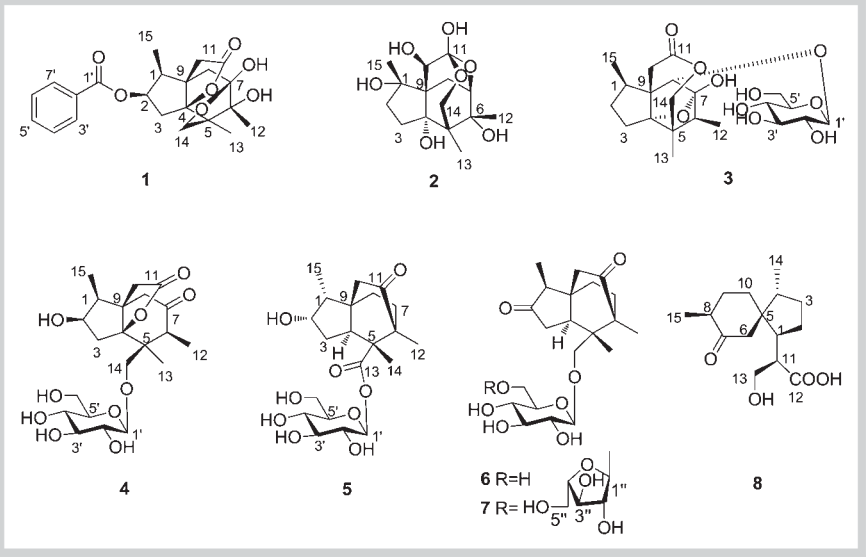

Fig. 1 Structures of compounds **1**–**8**.

H-14b and of H-14a with H-10b confirmed the *β*-orientation of CH_3-12 and the *α*-orientation of CH_3-13. Therefore, **1** was elucidated as 2*β*-benzoyloxy-6*α*-hydroxypseudomajucin.

Compound **2** was obtained as colorless crystals, and the molecular formula was determined to be $C_{15}H_{24}O_7$ by its HR-ESIMS. Its ^{1}H and ^{13}C NMR data (**Tables 1** and **2**) were very similar to those of (11)7,14-ortholactone-3*α*-hydroxyfloridanolide [12], except that the position of the hydroxyl group is located at C-1 in **2** instead of at C-3. This finding was confirmed by the HMBC correlations from H-10 (δ_H 4.45)/H_3-15 (δ_H 1.63) to C-1 (δ_C 82.3). Moreover, the ROESY spectrum showed a correlation between H_3-15 and H-10, which confirmed the *α*-orientation of the hydroxyl group at C-1. Thus, **2** was assigned as (11)7,14-ortholactone-1*α*-hydroxyfloridanolide.

Compound **3** was isolated as a white powder, and its molecular formula was determined to be $C_{21}H_{32}O_{10}$ by HR-ESIMS. A comparison of the NMR data of **3** with those of cycloparviflorolide [13] revealed that both shared a pseudoanisatin sesquiterpene skeleton. The main differences were the absence of the hydroxyl groups at C-6 and C-10 and the presence of an oxygen-bearing substitute at C-8. In addition, there was a glucopyranosyl moiety (δ_C 105.2, C-1′; δ_H 5.00, H-1′) connected to C-8 in **3**. The observed HMBC correlations from H_3-12 (δ_H 1.28)/H_3-13 (δ_H 0.90) to C-6 (δ_C 49.1), H-6 (δ_H 2.26)/H-1′ to C-8 (δ_C 81.3), and H-8 (δ_H 4.32) to C-10 (δ_C 32.1) supported the above deduction. In the ROESY spectrum, the correlations between H-8/H_3-12 and H-10a indicated the *β*-orientations of H-8 and CH_3-12. The large coupling constant (8.0 Hz) of the anomeric proton revealed that glucose was in the *β*-configuration, and the D-configuration of the moiety was established by GC analysis [14]. Thus, compound **3** was elucidated as 8-*O*-*β*-D-glucopyranosyl-8*α*-hydroxy-6,10-dideoxycycloparviflorolide.

Compound **4** was obtained as a white powder. The molecular formula of **4** was established as $C_{21}H_{32}O_{10}$ by the HR-ESIMS and NMR data. The ^{13}C NMR data (**Table 2**) showed twenty-one carbon signals, of which fifteen were assigned to the aglycone and six to the glucopyranosyl moiety. A comparison of the NMR data of **4** with those of 2,14-diacetylpseudomajucinone [6] revealed that they possessed the same skeleton. The difference was the presence of a glucopyranosyl moiety at C-14 of the skeleton. This was confirmed by the HMBC correlation from H-1′ to C-14 (δ_C 69.5). The glucose was deduced to be in the *β*-configuration from its large coupling constant (8.0 Hz) of the anomeric proton, and it was confirmed to be in the D-configuration by acid hydrolysis and GC analysis of the derivatized sugar [14]. Thus, compound **4** was deduced to be 14-*O*-*β*-D-glucopyranosylpseudomajucinone.

Compound **5** was obtained as a white powder. The molecular formula $C_{21}H_{32}O_9$ of **5** was indicated by the HR-ESIMS and NMR data. The NMR data suggested that **5** was a sesquiterpene glycoside. The NMR data of its aglycone showed a close resemblance to those of allo-cedrol [15], indicating that both possessed the allocedrane skeleton. The HMBC correlations from H_3-15/H-4 (δ_H 3.62) to C-2 (δ_C 73.9) suggested an additional hydroxyl group at C-2. In addition, the HMBC correlations from H_2-7/H_2-10/H_3-12 to C-11 (δ_C 213.4) and from H-4/H_3-14 to C-13 (δ_C 174.9) revealed that there was a ketone group and an ester carbonyl located at C-11 and C-13, respectively. The linkage of the sugar unit at C-13 was supported by the HMBC correlation from H-1′ to C-13. In the ROESY spectrum, correlations between H-2/H_3-14 and H-10a and between H-4 and H-7*α*/H-8*α*/H_3-15 indicated that H-2 and CH_3-14 were *β*-oriented and that H-4 and CH_3-15 were *α*-oriented. Based on the ^{1}H and ^{13}C NMR data, the sugar unit was identified as a *β*-glucopyranosyl unit. The configuration of the glucose unit was determined to be D after hydrolysis and GC analysis [14]. Furthermore, the absolute configuration for the aglycone of **5** was determined by analysis of its CD spectrum (Supporting Information, **Fig. 39S**). The negative Cotton effect at 295 nm ($\Delta\varepsilon$ −2.57) indicated that the absolute configuration of the aglycone was 1*R*, 2*S*, 4*S*, 5*R*, 6*R*, and 9*S* based on the octant rule for cyclohexanone [16]. Therefore, **5** was determined to be (1*R*, 2*S*, 4*S*, 5*R*, 6*R*, 9*S*)-2-hydroxy-11-oxoallo-cedra-13-oic acid 13-*O*-*β*-D-glucopyranosyl ester.

Compound **6** was isolated as a white powder. The molecular formula $C_{21}H_{32}O_8$ was established by the HR-ESIMS and NMR data. A comparison of the NMR data of **6** (**Tables 1** and **2**) with those of **5** indicated that the hydroxyl group at C-2 and the ester carboxyl group at C-13 were substituted by ketocarboxyl and hydroxymethyl, respectively. These substitutions were confirmed by the HMBC correlations from H-1/H-3/H_3-15 to C-2 (δ_C 217.2) and from H-4/H_3-14 to C-13 (δ_C 76.7). The glucose connected to C-13 was established by the HMBC correlation from H-1′ (δ_H 4.62) to C-13. The ROESY correlations between H_3-15/H_3-14 and

Table 1 ^{1}H NMR data for compounds **1**–**8**.

No.	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	7[c]	8[a]
1	2.57 m		3.16 m	1.74 m	1.72[d] m	1.98 q (7.2)	1.94 m	2.12 m
2α	5.35 t (4.5)	2.35 m	1.27 m	4.26 m				1.87[d] m
2β		2.55 m	1.84[d] m		4.46 m			1.29[d] m
3α	2.99 dd (15.0, 4.5)	2.04 m	1.84[d] m	2.21 dd (14.5, 3.5)	1.96 br dd (12.5, 5.0)	2.21[d] d (10.6)	2.23 m	1.87[d] m
3β	2.18 d (15.0)	2.89 m	1.84[d] m	2.38 d (14.5)	1.75[d] dd (12.5, 7.0)	2.21[d] d (10.6)	2.14 m	1.66[d] m
4					3.62 dd (13.0, 6.5)	2.41 t (10.6)	2.41 t (9.5)	1.64[d] m
6			2.26 q (7.5)	2.30 m				2.67 d (9.0) 2.00 d (9.0)
7		4.19 br s			2.91 m 1.29 m	1.17[d] m 1.64 m	1.14[d] m 1.64 m	
8α	2.10 d (13.5)	3.11 br d (13.5)		3.50 d (18.0)	1.88 m	1.17[d] m	1.14[d] m	2.40 m
8β	1.99 d (13.5)	1.91 br d (13.5)	4.32 s	2.61 d (18.0)	1.32 m	1.17[d] m	1.14[d] m	
9								1.93 m 1.28[d] m
10 a	3.02 d (19.5)	4.45 s	3.11 d (14.5)	3.23 d (18.0)	2.42 dd (19.0, 2.5)	2.49 d (18.6)	2.46 d (18.0)	2.02 m
10 b	2.76 d (19.5)		2.73 d (14.5)	2.69 d (18.0)	2.12 dd (19.0, 1.0)	2.19[d] d (18.6)	2.17 d (18.0)	1.56 m
11								2.29 m
12	1.24 s	1.80 s	1.28 d (7.5)	1.26 d (7.0)	1.31 s	0.99 s	0.99 s	
13	1.00 s	1.28 s	0.90 s	0.87 s		3.79 d (9.0) 3.19 d (9.0)	3.84 d (9.0) 3.20 d (9.0)	3.65 br s 3.65 br s
14a	3.77 d (9.5)	4.61 d (12.5)	4.27[d] m	4.26[d] m	1.25 s	0.75 s	0.76 s	0.78 d (6.5)
14b	3.50 d (9.5)	3.63 d (12.5)	4.00[d] m	3.52 d (9.0)				
15	1.00 d (7.5)	1.63 s	1.04 d (6.5)	1.07 d (6.5)	1.06 d (7.5)	0.90 d (7.2)	0.87 d (6.5)	0.89 d (4.0)
1′			5.00 d (8.0)	4.64 d (8.0)	6.33 d (8.0)	4.62 d (7.8)	4.57 d (7.5)	
2′			4.05[d] t (8.5)	3.86 t (8.0)	4.15 t (9.0)	3.88 t (7.8)	3.83 t (9.0)	
3′	7.90 d (8.5)		4.18 t (9.0)	4.13 t (9.0)	4.25 t (9.0)	4.19 t (9.0)	4.14 t (9.0)	
4′	7.38 t (9.0)		4.10 t (9.0)	4.15 t (9.0)	4.27 t (9.0)	4.16 t (9.0)	4.00 t (9.0)	
5′	7.52 t (7.5)		3.91 m	3.79 m	4.00 m	3.92 br t (6.0)	4.02 t (9.0)	
6′a	7.38 t (9.0)		4.55 br d (10.0)	4.41 br d (11.5)	4.40 dd (12.0, 2.5)	4.56 dd (11.4,1.8)	4.72[d] m (overlapped)	
6′b			4.31[d] m	4.29[d] m	4.30 dd (12.0, 2.5)	4.34 dd (11.4, 4.5)	4.16[d] m (overlapped)	
7′	7.90 d (8.5)							
1′′							5.68 d (1.0)	
2′′							4.83 m	
3′′							4.76 t (6.0)	
4′′							4.68 m	
5′′							a 4.28 dd (12.0, 3.5) b 4.14 dd (12.0, 5.0)	

Data were recorded at [a] 500 MHz in CD_3OD; [b] 500 MHz in pyridine-d_5; [c] 600 MHz in pyridine-d_5. [d] Signals overlapped

H_2-10 and between H-4 and H-1/H_2-13 revealed the β-orientations of CH_3-14 and CH_3-15 and the α-orientation of H-4. The CD spectrum (Supporting Information, **Fig. 50S**) of **6** showed a negative Cotton effect at 293 nm ($\Delta\varepsilon$ −5.58), which was stronger than that of **5** at the same mol concentration, indicating that the cyclohexanone and cyclopentanone moieties could contribute to the Cotton effect. However, the additional contribution of the cyclopentanone moiety showed no impact on the assignment of the 1*S*, 4*S*, 5*R*, 6*R*, and 9*S* configurations for **6**. Thus, **6** was elucidated as (1*S*,4*S*,5*R*,6*R*,9*S*)-13-*O*-β-D-glucopyranosyl-13-hydroxyallo-cedra-2,11-dione.

Compound **7** was obtained as a white powder with the molecular formula $C_{26}H_{40}O_{12}$ based on the HR-ESIMS and NMR data. The NMR spectra of **7** (**Tables 1** and **2**) were similar to those of **6**, except for the presence of an α-L-arabinofuranosyl unit at the C-6′ of the glucose. This observation was confirmed by 2D NMR experiments and GC analysis of the hydrolysate of **7** [14]. In the HMBC spectrum, the correlation from H-1′′ (δ_H 5.68) to C-6′ (δ_C 68.1) confirmed the location of the arabinose moiety. Thus, compound **7** was assigned as (1*S*, 4*S*, 5*R*, 6*R*, 9*S*)-13-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranosyl-13-hydroxyallo-cedra-2,11-dione.

Compound **8** was obtained as colorless needles. Its molecular formula was determined to be $C_{15}H_{24}O_4$ from the HR-ESIMS and NMR data. The IR absorption bands at 3436, 1736, and 1702 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities.

Table 2 ^{13}C NMR data for compounds **1**–**8**.

No.	1[a]	2[b]	3[b]	4[b]	5[b]	6[c]	7[b]	8[a]
1	52.5	82.3	37.8	52.1	45.5	53.3	53.3	44.7
2	80.0	41.3	30.0	72.7	73.9	217.2	217.2	27.8
3	43.8	32.8	23.7	44.8	35.1	36.8	36.7	29.2
4	103.7	91.1	92.8	97.7	45.9	44.2	44.0	39.3
5	51.5	48.2	44.5	45.9	48.3	40.7	40.6	54.3
6	79.1	77.5	49.1	47.1	51.0	48.8	48.7	47.3
7	107.0	78.7	107.9	211.1	28.4	27.8	27.6	216.3
8	50.2	27.7	81.3	51.9	30.1	19.5	19.5	45.9
9	49.6	53.5	56.6	49.7	44.6	43.0	42.8	33.9
10	41.6	75.4	32.1	41.8	47.7	49.2	49.1	31.6
11	179.2	113.6	174.6	178.2	213.4	218.5	218.5	52.5
12	17.9	21.8	9.3	9.1	15.4	14.3	14.3	ND[d]
13	12.7	15.0	24.1	20.0	174.9	76.7	76.6	65.4
14	71.7	67.9	69.3	69.5	17.9	15.7	15.7	17.2
15	10.1	23.1	15.8	9.8	10.2	8.1	8.0	14.7
1′	167.6		105.2	103.8	96.7	105.1	105.7	
2′	131.2		75.3	75.0	74.2	75.6	75.4	
3′	130.7		78.2	78.5	78.8	77.8	77.6	
4′	129.5		71.3	70.9	71.0	71.6	71.7	
5′	134.2		78.8	78.2	79.5	78.7	77.0	
6′	129.5		62.9	62.4	62.2	62.8	68.1	
7′	130.7							
1′′							110.1	
2′′							83.2	
3′′							78.6	
4′′							86.0	
5′′							62.6	

Data were recorded at [a] 125 MHz in CD_3OD; [b] 125 MHz in pyridine-d_5; [c] 150 MHz in pyridine-d_5. [d] Signal not detected

Analysis of the 1D and 2D NMR data revealed that **8** was a sesquiterpene. In the ^1H-^1H COSY spectrum, the correlations H-1/H_2-2/H_2-3/H-4/H_3-14 and H_3-15/H-8/H_2-9/H_2-10 demonstrated the presence of vicinal coupling systems. However, the absence of definitive NMR signals prevented a determination of the structure. We were fortunate to obtain small crystals of **8**. The X-ray assignment of the planar structure and the relative configuration are shown in **Fig. 2**. The negative Cotton effect at 294 nm ($\Delta\varepsilon$ − 0.32) in the CD spectrum (Supporting Information, **Fig. 67S**) indicated that the absolute configuration of **8** was 1*S*, 4*R*, 5*S*, 8*S*, and 11*R* according to the $n\rightarrow\pi^*$ transition of cyclohexanone [16]. Thus, compound **8** was deduced to be 1*S*-(2*R*-hydroxymethylacetoxy)-4*R*, 8*S*-dimethyl spiro[4.5]dec-7-one [17].

To determine the efficacy and toxicity of this plant, the isolated compounds were tested against five human tumor cell lines [18]. All of them were found to be inactive. However, this result did not mean that the plant was not toxic as our previous investigations [2] showed high cytotoxicity against Vero cells by some of the compounds isolated from the plant. Further, compound **10** was found to exhibit picrotoxin-like toxicity [19]. Based on the reported literature [2,20], the isolates were also evaluated for possible antiviral (against Coxsackie virus B3 [21]) and neuroprotective activities [22]. Compounds **4, 6, 7, 14**, and **23** showed activities against Coxsackie virus B3 with IC_{50} values of 40.50, 66.67, 91.07, 27.14, and 9.86 µmol/mL, respectively, and SI values of 2.20–8.71 (the positive control, ribavirin, IC_{50} 1.25 µmol/mL, SI 6.84). However, none of the compounds showed neuroprotective activity.

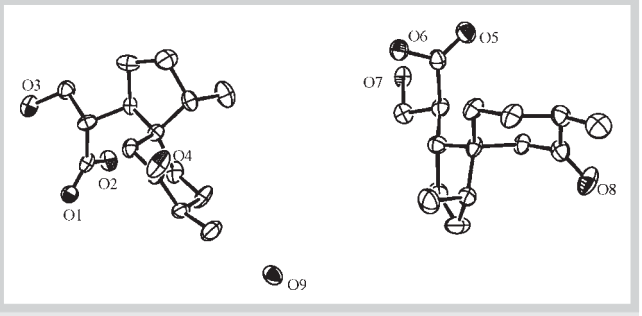

Fig. 2 X-ray crystal structure of compound **8**.

Materials and Methods

General experimental procedures

The melting points were measured on an XT5B (Beijing Keyi Electric Light Instrument Co., Ltd) melting instrument and were uncorrected. The optical rotations were measured on a JASCO P-2000 automatic polarimeter. The UV spectra were recorded on a JASCO V-650 spectrophotometer. The CD spectra were obtained on a JASCO J-815 spectropolarimeter. The IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrophotometer (FT-IR Microscope Transmission). The NMR spectra were collected on an INOVA-500 or a Mercury-300 spectrometer in pyridine-d_5, acetone-d_6, or MeOH-d_4 with solvent peaks as references. The ESIMS data were collected on an Agilent 1100 Series LC/MSD Trap/SL (Turbo Ionspray source) spectrometer. The HR-

ESIMS data were measured using an Agilent 6250 Accurate-Mass Q-TOF LC/MS spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc.), Sephadex LH-20 (Pharmacia), polyamide (30–60 mesh; Jiangsu Linjiang Chemical Reagents Factory), and ODS (50 µm; YMC) were used for column chromatography. Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A or an RID detector using a YMC-Pack ODS-A column (250 × 50 mm, 5 µm). TLC was carried out with precoated silica gel GF_{254} glass plates (Qingdao Marine Chemical Inc.). The spots were visualized under UV light or by spraying with 10% H_2SO_4 acid in EtOH followed by heating. The GC analysis was conducted on an Agilent 7890A instrument.

Plant material

The roots from *I. jiadifengpi* samples were collected in Guangxi Province, China in August 2009 and were identified by Prof. Songji Wei of the Guangxi Traditional Medical College. A voucher specimen (No. 21976) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and isolation

Roots of *I. jiadifengpi* (10 kg) were extracted three times with 95% EtOH (50 L × 3) under reflux. The residue (900 g) obtained on evaporation of the solvent was absorbed onto kieselguhr and extracted successively with petroleum ether, EtOAc, and MeOH. The EtOAc extract (47 g) yielded five fractions (Y1–Y5) when chromatographed on silica gel column (80 × 8 cm, 1000 g) with petroleum ether/acetone (100 : 0 to 100 : 25) and CH_2Cl_2/MeOH (100 : 4 to 100 : 25) as eluents.

Fraction Y3 (14.0 g) yielded five fractions (Y3-1–Y3–5) when chromatographed on silica gel column (80 × 4 cm, 500 g) with petroleum ether/acetone (6 : 1, 10 L) as the eluent. Fraction Y3–4 (3.2 g) yielded six subfractions (Y3–4–1–Y3–4–6) when chromatographed on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (45 : 55, 1.5 L; 50 : 50, 1.5 L; 55 : 45, 2 L; 60 : 40, 2 L; 65 : 35, 2 L; 70 : 30, 3 L; 75 : 25, 1 L; 80 : 20, 1 L; 90 : 10, 1 L; and 100 : 0, 3 L) as the eluent. Y3–4–2 (0.45 g) yielded compound **19** (30 mg, t_R = 20 min) when chromatographed on Sephadex LH-20 (120 × 2.5 cm, 100 g) and then on preparative HPLC with 30% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. Fraction Y3–5 (6.5 g) yielded seven subfractions (Y3–5–1–Y3–5–7) when chromatographed on Sephadex LH-20 (100 × 4 cm, 300 g) with CH_2Cl_2 (5 L) and then on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (20 : 80, 2 L; 25 : 75, 2 L; 30 : 70, 2 L; 35 : 65, 3 L; 40 : 60, 3 L; 45 : 55, 3 L; 50 : 50, 3 L; 55 : 45, 2 L; 60 : 40, 1.5 L; 65 : 35, 1.5 L; and 70 : 30, 1.5 L) as the eluent. Fraction Y3–5–2 (0.6 g) yielded compound **10** (400 mg, t_R = 45 min) when chromatographed on preparative HPLC with 20% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. Fraction Y3–5–3 (80 mg) yielded compound **21** (32 mg) when chromatographed on silica gel column (40 × 1.5 cm, 200–300 mesh, 15 g) with CH_2Cl_2/CH_3OH (10 : 1, 0.5 L) as eluent. Y3–5–4 (0.23 g) yielded compound **22** (20 mg) when chromatographed on Sephadex LH-20 with CH_2Cl_2/CH_3OH (10 : 1) as the eluent.

Fraction Y4 (7.5 g) yielded ten fractions (Y4-1–Y4-10) when chromatographed on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (5 : 95, 1 L; 10 : 90, 2 L; 15 : 85, 2 L; 20 : 80, 3 L; 25 : 75, 3 L; 30 : 70, 3 L; 35 : 65, 3 L; 40 : 60, 2 L; 45 : 55, 2 L; 50 : 50, 2 L; 55 : 45, 2 L; 60 : 40, 1.5 L; 65 : 35, 1.5 L; and 70 : 30, 1 L) as the eluent. Y4-1 (0.15 g) yielded compound **8** (32 mg) when chromatographed on Sephadex LH-20 with CH_2Cl_2/CH_3OH (10 : 1, 2 L) as the eluent. Y4-3 (1.11 g) yielded six subfractions (Y4-3–1–Y4-3–6) when chromatographed on Sephadex LH-20 with CH_2Cl_2/CH_3OH (10 : 1, 2 L) as the eluent. Y4-3–2 (0.3 g) yielded compounds **14** (17 mg, t_R = 15 min), **15** (56 mg, t_R = 35 min), **16** (10 mg, t_R = 45 min), and **13** (60 mg, t_R = 48 min) when chromatographed on preparative HPLC with 5% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. Y4-3–5 (0.05 g) was repeatedly crystallized with CH_2Cl_2/CH_3OH (1 : 1, v/v) to yield compound **9** (40 mg). Y4-4 (0.45 g) yielded two fractions (Y4-4–1–Y4-4–2) when chromatographed on Sephadex LH-20 with CH_2Cl_2/CH_3OH (1 : 1, 0.5 L) as eluent. Y4-4–1 (0.15 g) yielded compound **17** (45 mg) when repeatedly crystallized with CH_2Cl_2/CH_3OH (1 : 1, v/v). Fraction Y4–9 (0.7 g) yielded nine fractions (Y4–9–1–Y4–9–9) when chromatographed on Sephadex LH-20 column with 50% MeOH/H_2O (2 L) as the eluent. Y4–9–4 (0.09 g) yielded compound **1** (10 mg, t_R = 44 min) when chromatographed on preparative HPLC with CH_3CN/0.03% TFA-H_2O (35 : 65, v/v, 7 mL/min) as the eluent.

Fraction Y5 (4.7 g) yielded six subfractions (Y5-1–Y5–6) when chromatographed on Sephadex LH-20 and then on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (5 : 95, 1 L; 10 : 90, 1 L; 15 : 85, 1.5 L; 20 : 80, 3 L; 25 : 75, 3 L; 30 : 70, 3 L; 35 : 65, 3 L; 45 : 55, 2 L; 50 : 50, 2 L; 55 : 45, 2 L; 60 : 40, 1.5 L; 65 : 35, 1.5 L; 70 : 30, 1 L; 80 : 20, 1 L; and 90 : 10, 1 L) as the eluent. Y5-1 (0.25 g) yielded two fractions (Y5-1–1–Y5-1–2) when chromatographed on Sephadex LH-20 with CH_2Cl_2/CH_3OH (1 : 1, 0.3 L) as the eluent. Y5-1–1 (0.2 g) yielded compounds **11** (87 mg, t_R = 37 min) and **12** (13 mg, t_R = 44 min) when chromatographed on preparative HPLC with CH_3CN/0.03% TFA-H_2O (5 : 95, v/v, 7 mL/min) as the eluent. Y5-2 (0.07 g) was further purified with a Sephadex LH-20 and was repeatedly crystallized with CH_2Cl_2/CH_3OH (1 : 1, v/v) to yield compound **2** (23 mg). Compound **23** (23 mg) was obtained by repeated chromatography on Sephadex LH-20 from Y-5-5 (0.07 g).

The CH_3OH fraction (540 g) yielded three fractions when chromatographed on a D101 macroporous adsorbent resin column (120 × 15 cm, 2500 g) with H_2O (50 L), 50% EtOH (100 L), and 95% EtOH (50 L) as eluents. The 50% fraction (200 g) yielded six fractions (M1–M6) when chromatographed on silica gel column (150 × 10 cm, 2000 g) with CH_2Cl_2/CH_3OH (100 : 0 to 100 : 50) as the eluent.

M4 (7.0 g) yielded twenty fractions (M4-1–M4-20) when chromatographed on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (10 : 90, 1 L; 15 : 85, 1.5 L; 20 : 80, 1.5 L; 25 : 75, 1.5 L; 30 : 70, 1.5 L; 35 : 65, 1.5 L; 40 : 60, 1.5 L; 45 : 55, 3 L; 50 : 50, 3 L; 55 : 45, 3 L; 60 : 40, 2 L; 65 : 35, 1.5 L; and 70 : 30, 1 L) as the eluent. M4-13 (0.16 g) yielded compound **3** (15 mg, t_R = 33 min) when chromatographed on Sephadex LH-20 and on preparative HPLC with 15% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. M4-17 (0.58 g) yielded seven fractions (M4-17–1–M4-17–7) when chromatographed on Sephadex LH-20 with 30% CH_3OH/H_2O (3 L) as the eluent. M4-17–1 (0.08 g) yielded compound **6** (10 mg, t_R = 39 min) when chromatographed on preparative HPLC with 20% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent.

M5 (32 g) yielded two fractions when chromatographed on polyamide column (100 × 8 cm, 500 g) with H_2O (12 L) and 95% EtOH (8 L) as eluents. The H_2O fraction (15 g) yielded eighteen fractions (M5-1–M5-18) when chromatographed on ODS column (40 × 3 cm, 340 g) with an MeOH/H_2O gradient (3 : 97, 2 L; 7 : 93, 2 L; 10 : 90, 3 L; 5 : 85, 3 L; 20 : 80, 2 L; 25 : 75, 2 L; 30 : 70, 2 L; and 40 : 60, 1 L) as the eluent. M5-5 (0.9 g) yielded seven fractions (M5-5–1–M5-5–7) when chromatographed on Sephadex LH-20 with 10% CH_3OH/H_2O (1 L) as the eluent. M5-5–2 (0.32 g) yielded

compounds **18** (99 mg, t_R = 20 min) and **4** (28 mg, t_R = 37 min) when chromatographed on preparative HPLC with CH_3CN/0.03% TFA-H_2O (12 : 88, v/v, 7 mL/min) as the eluent. M5–6 (0.8 g) yielded four fractions (M5–6–1–M5–6–4) when chromatographed on Sephadex LH-20 with 10% CH_3OH/H_2O (1 L) as the eluent. M5–6–1 (0.14 g) yielded compound **20** (28 mg, t_R = 35 min) when chromatographed on preparative HPLC with 12% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. M5–9 (0.9 g) yielded six fractions (M5–9–1–M5–9–6) when chromatographed on Sephadex LH-20 with 10% CH_3OH/H_2O (1.5 L) as the eluent. M5–9–2 (0.45 g) yielded compound **5** (158 mg, t_R = 83 min) when chromatographed on preparative HPLC with 12.5% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent. M5-13 (0.65 g) yielded nine fractions (M5-13–1–M5-13–9) when chromatographed on Sephadex LH-20 with 10% CH_3OH/H_2O (2 L) as the eluent. M5-13–4 (0.1 g) yielded compound **7** (16 mg, t_R = 39 min) when chromatographed on preparative HPLC with 17.5% CH_3CN/0.03% TFA-H_2O (7 mL/min) as the eluent.

The purities of the isolated compounds ranged from 95 to 99.5% as determined by HPLC.

2β-Benzoyloxy-6α-hydroxypseudomajucin (**1**): colorless crystals, mp 198–200 °C; $[\alpha]_D^{20}$ − 67.2 (*c* 0.063, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 230 (4.02), 273 (2.86) nm; IR ν_{max} 3498, 3441, 2984, 1713, 1600, 1451, 1299, and 1279 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 403.1760 $[M + H]^+$ (calcd. for $C_{22}H_{27}O_7$, 403.1751), *m/z* 425.1580 $[M + Na]^+$ (calcd. for $C_{22}H_{26}O_7Na$, 425.1571).

(11)7,14-Ortholactone-1α-hydroxyfloridanolide (**2**): colorless crystals, mp 260 °C; $[\alpha]_D^{20}$ − 3.5 (*c* 0.062, CH_3OH); IR ν_{max} 3393, 3304, 1721, 1459, 1383, 1353, and 798 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 339.1408 $[M + Na]^+$ (calcd. for $C_{15}H_{24}O_7Na$, 339.1414).

8-O-β-D-Glucopyranosyl-8α-hydroxy-6,10-dideoxycycloparviflorolide (**3**): white powder, $[\alpha]_D^{20}$ − 28.1 (*c* 0.059, CH_3OH); IR ν_{max} 3414, 2964, 2877, 1710, 1677, and 1076 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 467.1886 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_{10}Na$, 467.1888).

14-O-β-D-Glucopyranosylpseudomajucinone (**4**): white powder, $[\alpha]_D^{20}$ − 2.7 (*c* 0.056, CH_3OH); IR ν_{max} 3427, 3317, 2900, 1739, 1691, and 1077 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 467.1887 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_{10}Na$, 467.1888).

(1R,2S,4S,5R,6R,9S)-2-Hydroxy-11-oxoallo-cedra-13-oic acid 13-O-β-D-glucopyranosyl ester (**5**): white powder, $[\alpha]_D^{20}$ + 3.5 (*c* 0.059, CH_3OH); CD (CH_3OH) 293 ($\Delta\varepsilon$ − 2.57) nm; IR ν_{max} 3377, 2935, 1717, and 1070 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 451.1943 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_9Na$, 451.1939).

(1S,4S,5R,6R,9S)-13-O-β-D-Glucopyranosyl-13-hydroxyallo-cedra-2,11-dione (**6**): white powder, $[\alpha]_D^{20}$ − 108.5 (*c* 0.055, CH_3OH); CD (CH_3OH) 293 ($\Delta\varepsilon$ − 5.58) nm; IR ν_{max} 3449, 2935, 1733, 1702, 1414, 1081, 1050, and 1018 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 413.2165 $[M + H]^+$ (calcd. for $C_{21}H_{33}O_8$, 413.217),*m/z* 435.1994 $[M + Na]^+$ (calcd. for $C_{21}H_{32}O_8Na$, 435.1989).

(1S,4S,5R,6R,9S)-13-O-α-L-Arabinofuranosyl-(1→6)-β-D-glucopyranosyl-13-hydroxyallo-cedra-2,11-dione (**7**): white powder, $[\alpha]_D^{20}$ − 113.3 (*c* 0.060, CH_3OH); CD (CH_3OH) 293 ($\Delta\varepsilon$ − 3.54) nm; IR ν_{max} 3415, 2933, 1731, 1704, 1074, and 1049 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; positive HR-ESIMS *m/z* 567.2415 $[M + Na]^+$ (calcd. for $C_{26}H_{40}O_{12}Na$, 567.2412).

1S-(2R-Hydroxymethylacetoxy)-4R,8S-dimethyl spiro[4.5]dec-7-one (**8**): colorless needles, mp 148–150 °C; $[\alpha]_D^{20}$ − 36.6 (*c* 0.083, CH_3OH); CD (CH_3OH) 293 ($\Delta\varepsilon$ − 0.32) nm; IR ν_{max} 3436, 3233, 2966, 2938, 1736, 1702, 1458, 1290, 1049, and 1017 cm^{-1}; ^1H NMR and ^{13}C NMR, see **Tables 1** and **2**; negative HR-ESIMS *m/z* 267.1586 $[M - H]^-$ (calcd. for $C_{15}H_{23}O_4$, 267.1602).

X-ray crystallographic analysis of compound **8**. $C_{30}H_{50}O_9$, M = 554.70, size 0.30 × 0.30 × 0.20 mm^3, orthorhombic, space group P2(1)2(1)2(1); a = 6.6776(13) Å, b = 19.663(4) Å, c = 23.491 (5) Å, $\alpha = \beta = \gamma = 90°$, V = 3084.4(11) $Å^3$, T = 293(2) K, Z = 4, ρ_{calcd} = 1.195 Mg/m^3, μ (Mo Ka) = 0.087 mm^{-1}. The total number of reflections measured was 3977, of which 1344 reflections were observed ($|F| \geq 2\sigma|F|$), R1 = 0.0637, *w*R2 = 0.1416. The crystal structure of **8** was solved by direct methods (SHELXS), expanded using difference Fourier techniques and refined by the program and full-matrix least-squares calculations. The crystallographic data for the structure of **8** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 893804). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.

Acid hydrolysis and determination of the absolute configuration of the monosaccharides [14]

Compound **7** (2 mg) was dissolved in 2 M HCl–H_2O (3 mL) and heated at 70 °C for 12 h. The reaction mixture was extracted with EtOAc. The aqueous layer was evaporated under vacuum, diluted repeatedly with H_2O and evaporated *in vacuo* to furnish a neutral residue. The residue was dissolved in anhydrous pyridine (1 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h, and after evaporation *in vacuo* to dryness, 0.2 mL of *N*-trimethylsilylimidazole was added. The mixture was kept at 60 °C for another 2 h. The reaction mixture was partitioned between *n*-hexane and H_2O (2 mL each). The *n*-hexane extract was analyzed by GC under the following conditions: capillary column, HP-5 (30 m × 0.25 mm, with a 0.25 µm film; Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 100 °C for 2 min, then raised to 280 °C at 10 °C/min, final temperature maintained for 10 min; and carrier, N_2 gas. From the acid hydrolysate of **7**, D-glucose and L-arabinose were confirmed by comparing the retention times of their derivatives with those of authentic sugars derivatized in a similar way, which showed retention times of 14.85 and 11.32 min, respectively. The constituent sugars of compounds **3**–**6** were identified by the same method as **7**.

Anti-coxsackie virus B3 activity assay

The antiviral activities against Coxsackie virus B3 in African green monkey kidney cells (Vero cells) were determined using a cytopathogenic effect (CPE) assay according to the literature [21]. Ribavirin (Hubei Keyi Pharmaceutic Co., Ltd.; 95% purity) was used as the positive control.

Cytotoxicity assay

Compounds **1**–**23** were tested for cytotoxicity against HT-29 (human colon cancer cell line), HePG2 (human hepatoma cancer cell line), BGC-823 (human gastric cancer cell line), A549 (human lung epithelial cell line), and A375 (human amelanotic melanoma cell line) by means of the MTT method as described in the literature [18]. Camptothecin (Sigma; 95% purity) was used as the positive control.

Neuroprotective activity assay

The cell culture and the cell viability assay were performed according to previously reported procedures [22]. NGF (Sigma, purity > 97%) was used as the positive control.

Supporting information

Original spectra for compounds **1**–**8** are available as Supporting Information.

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Conflict of Interest

None of the authors have conflicts of interest in this study.

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