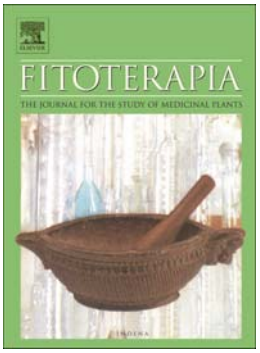

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Eight new eudesmane- and eremophilane-type sesquiterpenoids from *Atractylodes lancea*

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ABSTRACT

Phytochemical and pharmacological study on the rhizomes of *Atractylodes lancea* led to the identification of twenty-one compounds: six new eudesmane-type sesquiterpenoids (**1**−**6**), two new eremophilane-type sesquiterpenoids (**7**, **8**), and thirteen known compounds (**9**−**21**). These new compounds were elucidated using extensive spectroscopic analyses with experimental and calculated electronic circular dichroism (ECD) for the configurational assignments. Notably, this study was the first report on the isolation of two eremophilane-type sesquiterpenoids (**7**, **8**) from the genus *Atractylodes*. Compounds **5**, **7**, and **16** showed potent hepatoprotective activities against *N*-acetyl-*p*-aminophenol (APAP)-induced HepG2 cell injury at a concentration of 10 μM (bicyclol as the positive drug).

Keywords:

Atractylodes lancea
eudesmane
eremophilane
sesquiterpenoids
hepatoprotective activity

1. Introduction

Hepatic injury is the most common syndrome among all hepatic disorders.[1] Medicinal plants supply a rich source for screening live-protective ingredients. *Atractylodes lancea* (Thunb.) DC., which is a perennial herb known as "Cangzhu", has been reputed in Traditional Chinese Medicine for "strengthening spleen, removing cold, and improving eyesight".[2] Eudesmane- and guaiane-type sesquiterpenoids and polyacetylenes are considered characteristic phytochemicals.[3–11] A literature survey disclosed that the extract and chemical constituents from the rhizomes of *A. lancea* exhibit potent hepatoprotective effects.[12] In our search for hepatoprotective agents from *A. lancea*, six new eudesmane-type sesquiterpenoids (**1**−**6**), two new eremophilane-type sesquiterpenoids (**7**, **8**), and thirteen known compounds (**12**−**21**) were isolated using various column chromatographic methods. The structures were elucidated via 1D and 2D NMR spectroscopic analyses. The configurational assignments of these new compounds were established using ECD (electronic circular dichroism), whereas those of monosaccharide moieties were analysed by GC after the chiral derivatization. This study was the first report on the isolation of eremophilane-type sesquiterpenoids from genus *Atractylodes*. All isolated compounds were assayed for hepatoprotective activities against APAP-induced HepG2 cell injury (bicyclol as the positive contrast). The information in this paper will benefit subsequent phytochemical studies of genus *Atractylodes*.

2. Materials and methods

2.1. General experimental procedures

The specific rotations, UV, and ECD data were individually measured on JASCO P-2000, JASCO V-650, and JASCO J-815 spectrometers (JASCO, Easton, MD, U.S.A.). IR spectra were collected by a Nicolet 5700 instrument (Thermo Scientific, Waltham, MA, U.S.A.). NMR spectra were run on a Bruker 500 Hz spectrometer (Bruker-Biospin, Billerica, MA, U.S.A), and chemical shifts were given in δ (ppm) with DMSO-d_6 peaks as the reference.

HRESIMS data were collected using an Agilent 1100 series LC/MSD ESI/TOF instrument (Agilent Technologies, Waldbronn, Germany). GC analyses were performed on an Agilent 7890A system. HP-20 (Mitsubishi Chemical Corp., Tokyo, Japan), RP-18 (50 μm, YMC, Kyoto, Japan) and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) were used for chromatographic substrates. A Shimadzu LC-10AT system equipped with a SPD-10A detector and an YMC-Pack ODS-A column (250 × 20 mm, 5 μm, Kyoto, Japan) was used for reversed-phase preparative HPLC (P-HPLC). An Agilent 1260 series system equipped with an Apollo C_{18} column (250 × 4.6 mm, 5 μm, Grace Davison) was used for HPLC analyses.

2.2 Plant materials

The rhizomes of *A. lancea* were collected at Huanggang City (Hubei Province, China) in June 2014 and were identified by Prof. Lin Ma. A voucher specimen (ID-s-2596) was deposited in the herbarium at the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing 100050, China).

2.3 Extraction and isolation

The dried rhizomes of *A. lancea* (100 kg) were extracted thrice with 80% EtOH (*v*/*v*) under reflux condition for 2 h. The crude extract (25.6 kg) was suspended in 30 L distilled H_2O and separately partitioned with petroleum ether, EtOAc, and n-BuOH (three times each). The n-BuOH fraction (1.2 kg) was chromatographed on an HP-20 column and eluted with a step gradient of EtOH-H_2O (*v*/*v*) to provide five fractions: A (H_2O fraction, 824 g), B (15% EtOH fraction, 88.6 g), C (30% EtOH fraction, 106.4 g), D (50% EtOH fraction, 53.3 g), and E (95% EtOH fraction, 19.5 g). Fraction C (106.4 g) was chromatographed on an RP-18 and eluted at a gradient of MeOH-H_2O (0:100−100:0, *v*/*v*) to obtain fractions C1-C7 using HPLC analyses. Fraction C1 (30.5 g) was chromatographed on an LH-20 column using distilled H_2O to obtain 123 subfractions (Fr. C1.1−Fr. C1.123). Subtractions Fr. C1.49−Fr. C1.56 were further separated using P-HPLC, with 25% MeOH (*v*/*v*) to yield **11** (115 mg) and **14** (13 mg).

Similarly, the purification of subfractions Fr. C1.57−Fr. C1.95 using P-HPLC produced **10** (20 mg), **15** (78 mg), **16** (13 mg), **17** (8 mg), **18** (55 mg), **20** (33 mg) and **21** (11 mg). Fraction C2 (8.2 g) was chromatographed on an LH-20 column using distilled H_2O to yield 30 subfractions (Fr. C2.1−Fr. C2.30). These subfractions were purified using P-HPLC with a MeOH:H_2O ratio of 30:70 (*v*/*v*). Subfraction Fr. C2.8 produced **19** (162 mg), and Fr. C2.22−Fr. C2.27 gave **9** (4 mg). Fraction C3 (10.0 g) was eluted using distilled H_2O on an LH-20 column to yield 42 subfractions (Fr. C3.1−Fr. C3.42). Subfraction Fr. C3.4 was purified using P-HPLC with 30% MeOH (*v*/*v*) to give **12** (75 mg). Fraction C4 (10.2 g) was chromatographed on an LH-20 column using distilled H_2O to yield 32 subfractions (Fr. C4.1−Fr. C4.32). These subfractions were purified using P-HPLC with an MeOH:H_2O ratio of 30:70 (*v*/*v*). Fr. C4.2−Fr. C4.4 afforded **8** (6 mg). Fraction C6 (6.3 g) was chromatographed using an LH-20 column with distilled H_2O to produce 37 subfractions (Fr. C6.1−Fr. C6.37). Then, Fr. C6.11−Fr. C6.14 were purified using P-HPLC with 35% MeOH (*v*/*v*) to produce **4** (19 mg), **5** (9 mg), **6** (20 mg), and **7** (17 mg). Fraction C7 (9.7 g) was separated using LH-20 with distilled H_2O (subtractions Fr. C7.1−Fr. C7.35) and further purified using P-HPLC with MeOH:H_2O (40:60, *v*/*v*). Fr. C7.6−Fr. C7.7 yielded **2** (14 mg) and **3** (12 mg), Fr. C7.8−Fr. C7.12 produced **1** (84 mg), and Fr. C7.29−Fr. C7.32 produced **13** (5 mg).

(5*R*,7*R*,10*S*)-Isopterocarpolone-11-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (1). White amorphous powder; $[\alpha]_D^{20}$ +7.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.03) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (−2.96), 244 (+3.79), 327 (−0.51) nm; IR (KBr) ν_{max}: 3390, 2973, 2934, 2881, 1650, 1084, 1047 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 553.2619 $[M + Na]^+$ (calcd for $C_{26}H_{42}O_{11}Na$, 553.2625).

(5*R*,7*R*,10*S*)-6''-*O*-acetylatractyloside I (2). White amorphous powder; $[\alpha]_D^{20}$ +18.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 255 (3.93) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (−0.73), 258 (+2.19), 322 (−1.39) nm; IR (KBr) ν_{max}: 3403, 2932, 1737, 1659, 1074 cm^{-1}; ^1H and ^{13}C

NMR data see Table 1; HRESIMS *m/z* 641.2781 $[M + Na]^+$ (calcd for $C_{29}H_{46}O_{14}Na$, 641.2785).

(5*R*,7*R*,10*S*)-6'-*O*-acetylatractyloside I (3). White amorphous powder; $[\alpha]_D^{20}$ −10.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 254 (3.79) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 260 (+1.33), 324 (−0.85) nm; IR (KBr) ν_{max}: 3400, 2972, 2928, 1722, 1660, 1621, 1077, 1043 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 641.2775 $[M + Na]^+$ (calcd for $C_{29}H_{46}O_{14}Na$, 641.2785).

(5*R*,7*R*,10*S*)-3-Hydroxylisopterocarpolone-3-*O*-*β*-D-glucopyranoside (4). White amorphous powder; $[\alpha]_D^{20}$ +7.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 255 (3.78) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (−0.40), 257 (+2.00), 322 (−1.16) nm; IR (KBr) ν_{max}: 3374, 2969, 2936, 1660, 1616, 1076 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 413.2186 $[M - H]^-$ (calcd for $C_{21}H_{33}O_8$, 413.2175).

(2*S*,7*R*,10*S*)-3-Hydroxylcarissone-11-*O*-*β*-D-glucopyranoside (5). White amorphous powder; $[\alpha]_D^{20}$ −106.2 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.95) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 252 (−15.06), 317 (+1.29) nm; IR (KBr) ν_{max}: 3384, 2920, 1667, 1614, 1078, 1027 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 459.2238 $[M + COOH]^-$ (calcd for $C_{22}H_{35}O_{10}$, 459.2230).

(2*R*,7*R*,10*S*)-3-Hydroxylcarissone-11-*O*-*β*-D-glucopyranoside (6). White amorphous powder; $[\alpha]_D^{20}$ +63.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 250 (4.00) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 251 (+9.43), 316 (−1.03) nm; IR (KBr) ν_{max}: 3393, 2973, 2931, 1666, 1610, 1074, 1042 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 459.2237 $[M + COOH]^-$ (calcd for $C_{22}H_{35}O_{10}$, 459.2230).

(3*S*,4*R*,5*R*,7*R*)-3,11-Dihydroxy-11,12-dihydronootkatone-11-*O*-*β*-D-glucopyranoside (7). White amorphous powder; $[\alpha]_D^{20}$ −75.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.99) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 247 (−3.14), 306 (+0.10) nm; IR (KBr) ν_{max}: 3417, 2972,

2878, 1665, 1075, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; HRESIMS *m/z* 459.2234 [M + COOH]$^-$ (calcd for $C_{22}H_{35}O_{10}$, 459.2230).

(3*S*,4*R*,5*S*,7*R*)-3,4,11-Trihydroxy-11,12-dihydronootkatone-11-*O*-*β*-D-glucopyranoside (8). White amorphous powder; $[\alpha]_D^{20}$ −34.1 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.78) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 249 (−3.24), 313 (+1.06) nm; IR (KBr) ν_{max}: 3381, 2973, 2937, 1670, 1078, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; HRESIMS *m/z* 453.2104 [M + Na]$^+$ (calcd for $C_{21}H_{34}O_9Na$, 453.2101).

*2.4 Acid hydrolysis of compound **1***

Compound **1** (5 mg) was dissolved in 1 mol/L HCl-dioxane (1:1, 5 mL) and maintained at 60 °C for 6 h.[13,14] After drying in vacuum, the residue was partitioned in H_2O (5 mL) and extracted thrice with EtOAc (5 mL). The aqueous solution was evaporated in vacuum to obtain the monosaccharide residue. These monosaccharide residues were processed using a reported method.[15–17] The configurational assignments of apiose and glucose were established by comparing the retention times of their chiral derivatives with those of standard substances, which were prepared using the identical procedure (D-apiose 14.56 min, D-glucose 20.56 min).

2.5 Hepatoprotective activity assay

The standard MTT assay was used to assess the hepatoprotective activity. Human hepatoma cells (HepG2) were cultured in a DMEM medium, which was supplemented with 10% (*v*/*v*) foetal calf serum (FCS) and penicillin (100 U/mL)-streptomycin (100 μg/mL) solution, at 37 °C in a humidified atmosphere with 5% CO_2. The growing cells were seeded in 96-well plates and incubated for 12 h. Then, these cells were treated with APAP (8 mM) and various test samples (10 μM) and were further incubated for 48 h. Then, 100 μL of MTT solution (0.5 mg/mL) was added to each well after the medium was removed, and the solutions were incubated for an additional 4 h. The residuum was dissolved in 150 μL DMSO

after emptying the culture medium, and the absorbance was quantified at 570 nm using a microplate reader. Bicyclol was used as the positive contrast.

3. Results and discussion

Compound **1** was obtained as a white powder. An HRESIMS adduct ion at *m*/*z* 553.2619 $[M + Na]^+$ corresponds to a molecular formula of $C_{26}H_{42}O_{11}$, which suggests six degrees of unsaturation. The IR absorptions indicate the presence of hydroxyl (3390 cm^{-1}) and carbonyl (1650 cm^{-1}) groups. The ^1H NMR data (Table 1) show an olefinic proton at δ_H 5.76, four methyl protons at δ_H 0.76, 1.13, 1.17, and 1.87, and two anomeric protons at δ_H 4.30 and 4.79. The ^{13}C NMR data (Table 1) exhibit 26 carbons, eleven of which were attributed to a glucosyl moiety (δ_C 97.1, 73.6, 76.9, 70.3, 75.2, and 68.1) and an apiosyl moiety (δ_C 109.3, 75.9, 78.8, 73.2, and 63.2). The remaining 15 carbons, which are attributable to an α,*β*-unsaturated carbonyl moiety (δ_C 125.6, 164.2, and 198.1), an oxygenated tertiary carbon (δ_C 78.8), and other aliphatic carbons, reveal a skeleton of eudesmane-type sesquiterpenoid. The NMR spectroscopic data were similar to those of isopterocarpolone-11-*O*-*β*-D-glucopyranoside, except for an additional apiosyl moiety.[8] A key HMBC correlation (Figure 2) from the proton at δ_H 4.79 to the carbon at δ_C 68.1 suggests that the apiosyl moiety was located at Glc-C-6'. After the acid hydrolysis of **1**, D-glucose was determined using GC analysis after the chiral derivatization (20.57 min), whereas the *β*-configuration was deduced based on the coupling constant (7.5 Hz) of the anomeric proton. The *β*-D-configuration of apiose was established by the similar GC method (14.53 min) and ^{13}C NMR data.[18] Thus, compound **1** is elucidated as isopterocarpolone-11-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside. The ROESY correlations (Figure 3) of H-5 with H-1a, H-6a, H-7, and H-9b and that of H_3-15 with H-1b, H-6b, and H-9a indicate that the juncture of A- and B- rings is *trans*-configured, H-5 and CH_3-10 are axial, whereas C-7 hydroxyisopropyl is equatorial. According to Snatzke's rule for a cyclohexenone unit,[19,20] the negative ($\Delta\varepsilon = -0.51$) Cotton effect (CE) at 327 nm (Figure 4),

which is contributed by the n→π* electron transition of the α,*β*-unsaturated carbonyl moiety, unambiguously favours a 5*R*,7*R*,10*S* configuration.

Compound **2** shows similar IR absorptions to **1**, which can be assigned to hydroxyl (3403 cm^{-1}) and carbonyl (1659, 1737 cm^{-1}) groups. Its molecular formula $C_{29}H_{46}O_{14}$ with seven degrees of unsaturation was deduced based on an HRESIMS adduct ion at *m*/*z* 641.2781 $[M + Na]^+$. The preliminary inspections of the NMR data (Table 1) characterised the resonances as a eudesmane-type sesquiterpenoid. Except for an additional acetyl group (δ_H 1.95; δ_C 20.6, and 170.3), the 1D NMR data exhibit four methyl groups (δ_H 0.83, 1.12, 1.17, and 1.83), an α,*β*-unsaturated carbonyl moiety (δ_C 145.0, 150.1, and 193.7), two *β*-D-glucopyranosyl moieties, and an oxygenated tertiary carbon (δ_C 78.9), which are consistent with those of atractyloside I.[8] The HMBC spectrum demonstrates that the acetyl group is at Glc-C-6" via a long-range correlation (Figure 2) of H-6" (δ_H 3.96 and 4.23) with C-7" (δ_C 170.3). Therefore, the structure of **2** was defined as 6"-*O*-acetylatractyloside I. The ROESY correlations (Figure 3) of H-5 with H-1a, H-6a, H-7, and H-9b and that of H_3-15 with H-1b, H-6b, and H-9a suggest that the axial configuration of H-5 and CH_3-10, whereas C-7 hydroxyisopropyl was equatorial. The ECD spectrum (Figure 4) of **2** show negative CE sign at 322 nm ($\Delta\varepsilon = -1.39$), which supports that the absolute configuration is 5*R*,7*R*,10*S*.[19,20]

Compounds **3** and **2** have the identical molecular formula of $C_{29}H_{46}O_{14}$ and similar spectroscopic data, which suggests that their structures are highly similar. In the ^{13}C NMR spectrum, the deshielded resonance of Glc-C-6' at δ_C 63.7 indicates that the acetyl group (δ_H 1.96; δ_C 20.7 and 170.1) in **3** was substituted at Glc-C-6' instead of Glc-C-6". This assignment was verified with a key HMBC cross-peak from the protons at δ_H 3.94 and 4.25 to the carbon at δ_C 170.1. With the exception of this difference, other 1D NMR data of **3** and **2** were matched. Thus, compound **3** was elucidated as (5*R*,7*R*,10*S*)-6'-*O*-acetylatractyloside I, based on similar ROESY (Figure 3) and ECD data (Figure 4) to those of **2**.

The molecular formula $C_{21}H_{34}O_8$ of compound **4** was established using an HRESIMS quasi-molecular ion at *m*/*z* 413.2186 $[M - H]^-$. Its IR spectrum exhibits the absorption characteristic of hydroxyl (3374 cm^{-1}) and carbonyl (1660 cm^{-1}) groups. Detailed inspections of the 1D NMR data (Table 2) with that of **2** reveal the absence of a 6"-*O*-acetyl-*β*-D-glucosyl moiety in **4**, which is supported by the resonance of C-11 at δ_C 70.6 in **4**, instead of the deshielded resonance at δ_C 78.9 in **2**. In addition, their other NMR spectroscopic data are highly similar. Consequently, compound **4** was elucidated as 3-hydroxylisopterocarpolone-3-*O*-*β*-D-glucopyranoside. The consistent ROESY (Figure 3) and ECD data (Figure 4) with those of **2** suggest that compound **4** has the 5*R*,7*R*,10*S* configuration.

Compounds **5** and **6** were isolated as white amorphous powders with the identical molecular formula of $C_{21}H_{34}O_8$, which corresponds to the $[M + COOH]^-$ adduct ions at *m*/*z* 459.2238 and 459.2237, respectively. The 1D NMR data (Table 2) of these two compounds show an α,*β*-unsaturated carbonyl moiety (δ_C 124.4, 162.9, and 200.1 in **5**; δ_C 125.6, 162.7, and 200.1 in **6**), four methyl groups [δ_H 1.13 (3H), 1.18 (6H), and 1.71 (3H) in **5**; δ_H 1.18 (6H), 1.24 (3H), and 1.71 (3H) in **6**], and a *β*-D-glucopyranosyl moiety. The identical molecular formula and similar spectroscopic data imply that they are a pair of stereoisomers. In the HMBC experiments, the long-range correlations (Figure 2) of H-1 with C-3 and C-5, H-14 with C-3, C-4, and C-5, H-15 with C-1, C-9, and C-10, and H-1' with C-11 indicate a eudesmane-type sesquiterpenoid with a glucosyl unit at C-11. In association with HSQC and ^1H-^1H COSY (Figure 2) data, two spin systems [$C(1)H_2$-C(2)H and $C(6)H_2$-C(7)H-$C(8)H_2$-$C(9)H_2$] were established, and a hydroxyl group was determined to locate at C-2. Thus, the planar structure of **5** and **6** was identified as 3-hydroxylcarissone-11-*O*-*β*-D-glucopyranoside. To designate their absolute configurations, ROESY and ECD analyses were performed. For **5**, the correlations (Figure 3) of H-2 with H-9a, H-7 with H-6a, and H_3-15 with H-6b and H-9b suggest that H-2 and H-7 are on the same side of the eudesmane-ring, whereas CH_3-10 is on

the opposite side. For **6**, H_3-15 correlates with H-2 and H-6b, and H-7 correlates with H-6a, which suggests that H-2 and CH_3-10 are on the same side of the eudesmane-ring and opposite to H-7. Their ECD data (Figure 5) show opposite CE signs at approximately 315 nm ($\Delta\varepsilon$ = +1.29 in **5**; $\Delta\varepsilon$ = −1.03 in **6**), which were derived from the n→π* electron transition of the α,*β*-unsaturated carbonyl moiety. Based on Snatzke's rule for a cyclohexenone unit,[19,20] the positive CE of **5** enables the assignment of a 2*S*,7*R*,10*S* configuration, whereas the negative CE of **6** favours a 2*R*,7*R*,10*S* configuration. Their configurational assignments are also supported by ECD calculations (see supplementary data, S42), which was performed using an MMFF94 force field and the time-dependent density functional theory (TDDFT) at the B3LYP/6-31+G(d,p) level. The calculated ECD data of **5** and **6** (Figure 5) are consistent with their experimental data.

Compound **7** has a molecular formula of $C_{21}H_{34}O_8$ with five degrees of unsaturation, as deduced by the $[M + COOH]^-$ adduct ion at *m*/*z* 459.2234 in the HRESIMS and ^{13}C NMR data. Its IR spectrum exhibit absorptions of hydroxyl (3417 cm^{-1}) and carbonyl (1665 cm^{-1}) groups. The most obvious characteristic in the ^1H NMR spectrum is that the four methyl resonances distribute in a smaller range (δ_H 1.04−1.09) than those of **5** and **6**. Fifteen carbon resonances in the ^{13}C NMR spectrum are attributed to be an eremophilane-type sesquiterpenoid, except for a glucopyranosyl moiety.[21] H-14 and H-15 have long-range correlations with C-4 and C-5 in the HMBC spectrum, which indicates that CH_3-4 and CH_3-5 are substituted at the adjacent carbons. In combination with the HSQC data, the HMBC cross-peaks of H-1 with C-3, H-3 with C-5 and C-14, and H-1' with C-11 suggest that a hydroxyl group is at C-3 and that the *β*-D-glucopyranosyl moiety is connected at C-11. Therefore, **7** is elucidated as 3,11-dihydroxy-11,12-dihydronootkatone-11-*O*-*β*-D-glucopyranoside. The $^3J_{3,4}$ value (12.5 Hz) indicates a 3,4-*trans* configuration. The ROESY correlations (Figure 3) of H-3 with H_3-15 and H-4 with H-7 reveal that H-3 and CH_3-5 are on the same side of the

eremophilane-ring, whereas H-4 and H-7 are on the opposite side. The positive CE at 306 nm (Figure 6) reflects the absolute configuration as 3*S*,4*R*,5*R*,7*R*.[19,20] In addition, ECD calculation is performed using an MMFF94 force field and the TDDFT method at the B3LYP/6-31+G(d,p) level. The obtained theoretical data of the (3*S*,4*R*,5*R*,7*R*)-conformer is consistent with the experimental data for compound **7**. To the best of our knowledge, this study was the first report on the isolation of eremophilane-type sesquiterpenoid from the genus *Atractylodes*.

Compound **8** was shown to have a molecular formula of $C_{21}H_{34}O_9$ using an HRESIMS adduct ion at *m*/*z* 453.2104 $[M + Na]^+$, which indicates five degrees of unsaturation. The ^{13}C NMR data (Table 3) reveal an α,*β*-unsaturated carbonyl moiety at δ_C 122.4, 171.9, and 198.0, which is supported by an IR absorption at ν_{max} 1670 cm^{-1}. In the ^1H NMR spectrum, an olefinic proton at δ_H 5.78, an oxygenated proton at δ_H 4.20, four methyl protons at δ_H 0.98, 1.10, 1.11, and 1.25, a group of glucopyranosyl protons at δ_H 4.24, 2.88−3.13, 3.44, and 3.61, and other aliphatic resonances at 1.01−2.53 were observed. The comparisons of its 1D data (Table 3) with those of **7** indicate that **8** is also an eremophilane-type sesquiterpenoid glucopyranoside. An assigned ^1H-^1H spin system [C(6)H_2-C(7)H-C(8)H_2-C(9)H_2] (Figure 2) and the HMBC correlations (Figure 2) from H-1 with C-3 and C-5, H-3 with C-5 and C-14, H-14 with C-3, C-4, and C-5, H-15 with C-5, C-6, and C-10, and H-1' with C-11 determine the locations of two hydroxyl groups (C-3 and C-4) and the *β*-D-glucopyranosyl unit (C-11). Accordingly, **8** was identified as 3,4,11-trihydroxy-11,12-dihydronootkatone-11-*O*-*β*-D-glucopyranoside. The unambiguous ROESY correlations (Figure 3) of H-3 with H_3-15 and H_3-14 with H-7 suggest that H-3, OH-4, and CH_3-5 are on the same side of the eremophilane-ring, whereas CH_3-4 and H-7 are on the opposite side. The experimental ECD data of **8** is consistent with its calculated ECD data (Figure 6), which facilitates the assignment of a 3*S*,4*R*,5*S*,7*R* configuration for compound **8**.[19,20]

In addition, thirteen known compounds (**9**−**21**) were isolated from the rhizomes of *A. lancea*: (*E*)-isoconiferin (**9**),[22] syringin (**10**),[23] 4-*O*-caffeoylquinic acid (**11**),[24] (2*E*,8*R*)-decene-4,6-diyne-1,8-diol-8-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**12**),[25] (2*E*,8*E*,12*R*)-tetradecane-2,8-diene-4,6-diyne-1,12,14-triol-1-*O*-*β*-D-glucopyranoside (**13**).[26] cichoriin (**14**).[27] scopoletin-7-*O*-*β*-D-glucopyranoside (**15**),[28] scopoletin-7-O-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**16**),[29] scopoletin-7-O-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**17**),[30] isoscopoletin-6-O-*β*-D-glucopyranoside (**18**),[31] phenylmethanol 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**19**),[32] (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**20**),[33] and (7*E*)-sinapate-4-*O*-*β*-D-glucopyranoside (**21**).[34]

All compounds were assayed for hepatoprotective activities against APAP-induced HepG2 cell injury. As shown in Table 4, compared with the model group, compounds **5**, **7**, and **16** exhibited potent hepatoprotective activities with the cell survival rates of 34.6% ($p < 0.01$), 37.7% ($p < 0.001$), and 35.9% ($p < 0.001$) at a concentration of 10 μM (the positive drug bicyclol with 32.7%, $p < 0.01$).

Conflict of interest

Author's declares that there are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://///

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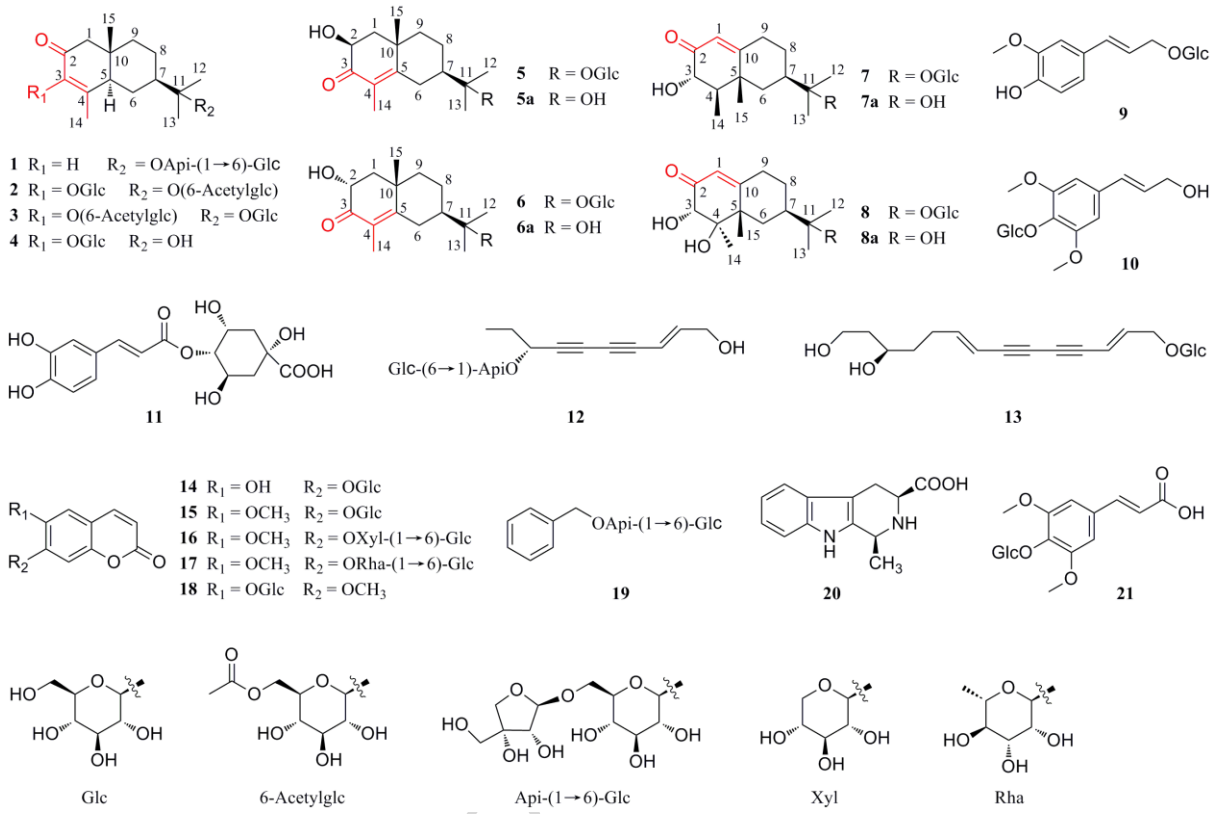

Fig. 1. The structures of compounds **1**–**21**.

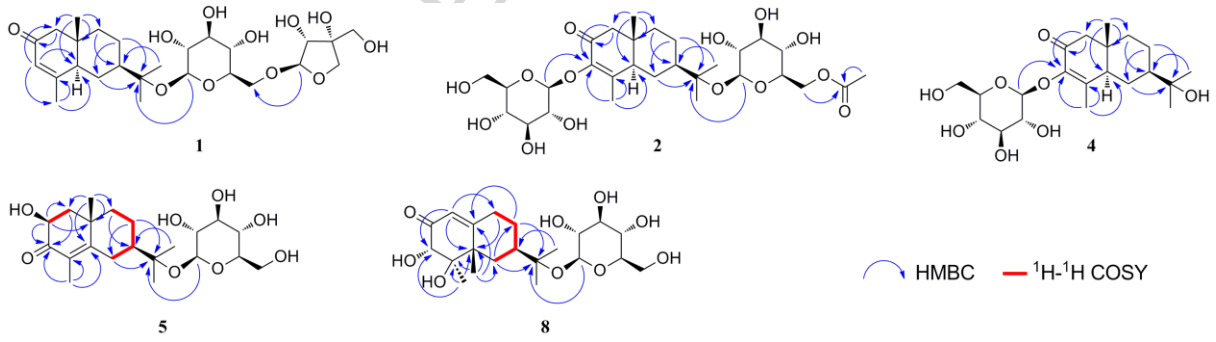

Fig. 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**, **2**, **4**, **5**, and **8**.

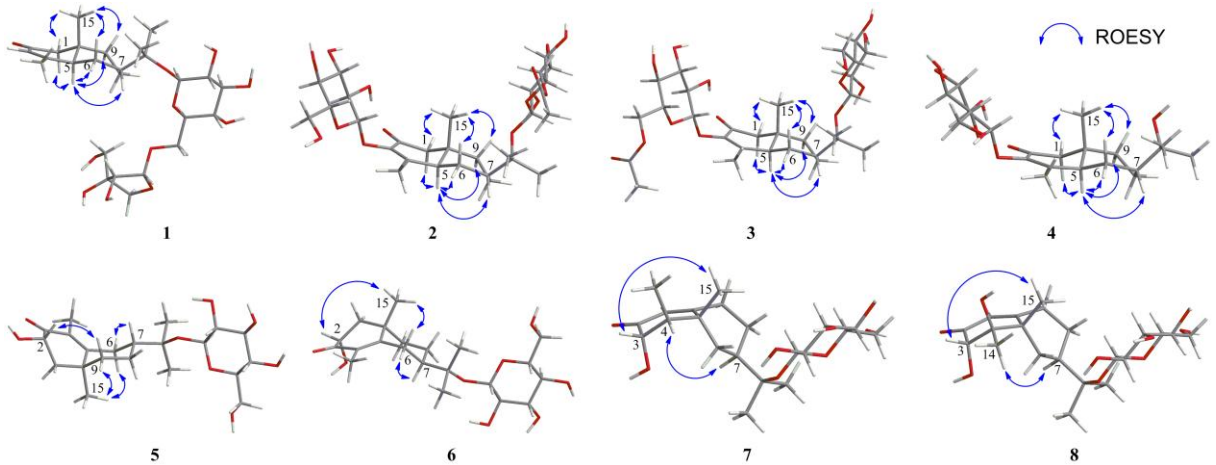

Fig. 3. Key ROESY correlations of compounds **1**−**8**.

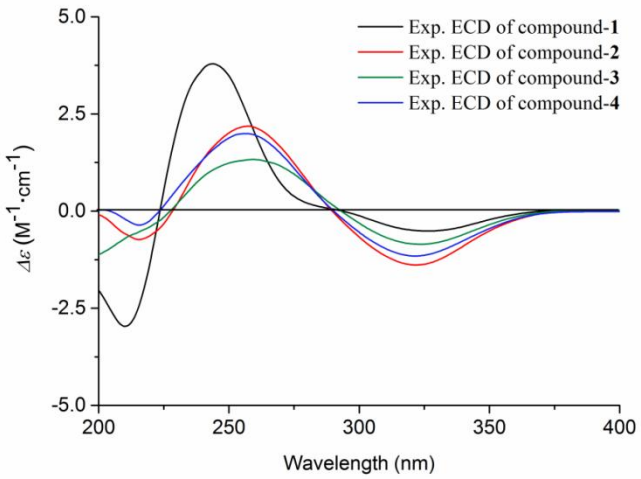

Fig. 4. Experimental ECD spectra of compounds **1**−**4**.

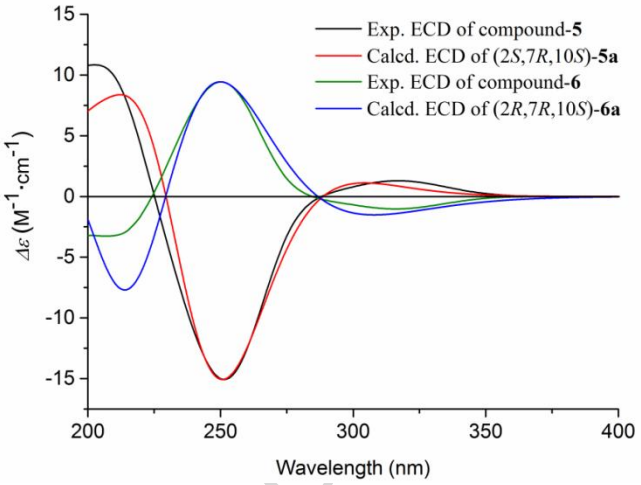

Fig. 5. Experimental and calculated ECD spectra of compounds **5** and **6**.

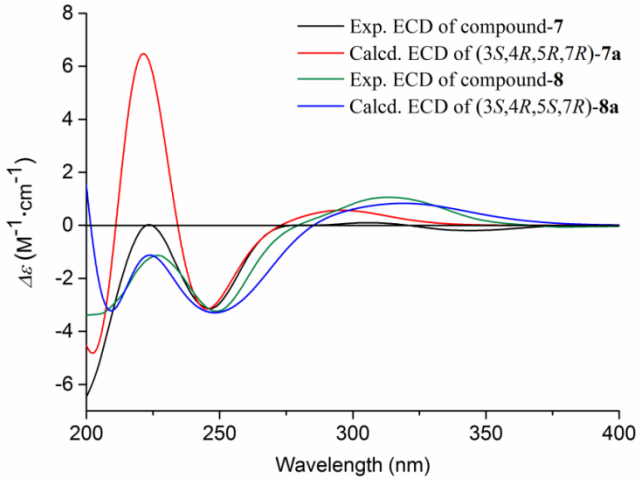

Fig. 6. Experimental and calculated ECD spectra of compounds **7** and **8**.

Table 1

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data (δ in ppm, J in Hz) for compounds **1**−**3** in DMSO-d_6

Position	1		Position	2		Position	3	
	δ_H	δ_C		δ_H	δ_C		δ_H	δ_C
1a	2.25, d (15.5)	53.9	1a	2.28, d (16.0)	53.8	1a	2.27, d (16.0)	53.8
1b	2.03, d (15.5)		1b	2.19, d (16.0)		1b	2.18, d (16.0)	
2		198.1	2		193.7	2		193.3
3	5.76, s	125.6	3		145.0	3		144.2
4		164.2	4		150.1	4		150.4
5	2.35, d (11.5)	46.9	5	2.41, d (12.0)	46.4	5	2.47, d (12.0)	46.2
6a	2.22, d (14.0)	23.1	6a	2.20, m	23.7	6a	2.21, m	23.9
6b	0.99, d (14.0)		6b	1.00, m		6b	0.98, m	
7	1.54, m	47.5	7	1.52, m	47.2	7	1.51, m	47.3
8a	1.57, m	21.7	8a	1.55, m	21.6	8a	1.58, m	21.4
8b	1.24, m		8b	1.23, m		8b	1.23, m	
9a	1.45, m	39.2	9a	1.45, m	39.0	9a	1.45, m	38.8
9b	1.35, m		9b	1.32, m		9b	1.31, m	
10		37.1	10		36.7	10		36.6
11		78.8	11		78.9	11		78.6
12	1.13, s	22.7	12	1.12, s	22.7	12	1.13, s	22.7
13	1.17, s	25.0	13	1.17, s	25.0	13	1.17, s	25.0
14	1.87, s	21.6	14	1.83, s	14.6	14	1.81, s	14.6
15	0.76, s	16.6	15	0.83, s	16.6	15	0.83, s	16.4
Glc-1'	4.30, d (7.5)	97.1	Glc-1'	4.51, d (7.5)	103.0	Glc-1'	4.60, d (7.5)	102.0
2'	2.91, t (8.5)	73.6	2'	3.11, overlap	74.3	2'	3.13, overlap	74.2
3'	3.14, t (8.5)	76.9	3'	3.16, t (8.5)	76.5	3'	3.19, overlap	76.1
4'	2.96, t (9.0)	70.3	4'	3.08, overlap	69.9	4'	3.04, overlap	70.2
5'	3.22, m	75.2	5'	3.01, overlap	77.2	5'	3.25, overlap	74.0
6'a	3.80, brd (11.0)	68.1	6'a	3.59, brd (11.5)	61.0	6'a	4.25, brd (11.5)	63.7
6'b	3.36, overlap		6'b	3.41, m		6'b	3.94, m	
Api-1"	4.79, d (3.0)	109.3	Glc-1"	4.35, d (7.5)	97.0	7'		170.1
2"	3.68, d (3.0)	75.9	2"	2.93, t (8.5)	73.6	8'	1.96, s	20.7
3"		78.8	3"	3.16, t (8.5)	76.6	Glc-1"	4.30, d (7.5)	97.1
4"a	3.83, d (9.5)	73.2	4"	3.02, overlap	70.2	2"	2.90, t (8.5)	73.7
4"b	3.56, d (9.5)		5"	3.32, overlap	73.3	3"	3.14, overlap	77.1
5"	3.31, d (11.0)	63.2	6"a	4.23, brd (11.5)	64.2	4"	3.04, overlap	70.2
			6"b	3.96, m		5"	3.05, overlap	76.6
			7"		170.3	6"a	3.62, m	61.3
			8"	1.95, s	20.6	6"b	3.38, m	

Table 2

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data (δ in ppm, J in Hz) for compounds **4**−**6** in DMSO-d_6

Position	4		5		6	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	2.32, d (16.5)	53.7	1.87, dd (6.0, 13.0)	43.8	1.91, dd (5.5, 12.5)	46.0
1b	2.18, d (16.5)		1.61, d (13.0)		1.51, d (12.5)	
2		193.8	4.06, dd (6.0, 13.0)	68.0	4.16, dd (5.5, 14.0)	68.2
3		144.9		200.1		200.1
4		149.9		124.4		125.6
5	2.45, d (11.5)	46.3		162.9		162.7
6a	1.98, m	24.2	2.75, d (12.5)	28.7	2.83, d (14.5)	28.3
6b	1.03, m		2.01, t (12.5)		1.85, overlap	
7	1.31, m	48.8	1.41, m	50.2	1.48, m	48.4
8a	1.56, m	21.3	1.83, m	22.1	1.87, m	21.9
8b	1.24, m		1.73, m		1.67, m	
9a	1.46, m	39.1	1.75, m	37.7	1.62, m	42.3
9b	1.30, m		1.56, m		1.26, m	
10		36.7		37.3		36.8
11		70.6		78.0		78.1
12	1.07, s	26.7	1.18, s	22.6	1.18, s	22.4
13	1.08, s	27.7	1.18, s	24.9	1.18, s	24.9
14	1.87, s	14.7	1.71, s	11.3	1.71, s	11.0
15	0.82, s	16.4	1.13, s	27.2	1.24, s	22.7
Glc-1'	4.53, d (7.5)	102.8	4.30, d (7.5)	97.2	4.30, d (7.5)	97.2
2'	3.11, overlap	74.3	2.90, t (8.5)	73.8	2.90, t (8.5)	73.8
3'	3.17, t (8.5)	76.5	3.14, t (8.5)	77.2	3.14, t (8.5)	77.1
4'	3.09, overlap	69.9	3.01, t (8.5)	70.4	3.02, t (8.5)	70.3
5'	3.01, overlap	77.2	3.04, m	76.6	3.04, m	76.5
6'a	3.59, brd (11.5)	61.0	3.61, dd (2.0, 11.5)	61.3	3.61, dd (2.0, 11.5)	61.3
6'b	3.41, m		3.37, m		3.38, m	

Table 3

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data (δ in ppm, J in Hz) for compounds **7** and **8** in DMSO-d_6

Position	**7**		**8**	
	δ_H	δ_C	δ_H	δ_C
1	5.76, s	122.8	5.78, s	122.4
2		198.8		198.0
3	3.81, d (12.5)	73.6	4.20, s	76.7
4	1.94, dq (6.5, 12.5)	42.6		78.7
5		41.4		45.9
6a	1.87, d (13.0)	34.8	2.21, brd (14.0)	34.0
6b	1.13, t (13.0)		1.01, brd (14.0)	
7	1.38, m	40.6	1.49, m	42.3
8a	1.65, m	25.4	1.77, m	24.2
8b	1.65, m		1.64, m	
9a	2.44, m	28.2	2.53, m	28.3
9b	2.18, m		2.29, m	
10		175.4		171.9
11		77.9		78.3
12	1.08, s	22.4	1.10, s	22.7
13	1.09, s	24.5	1.11, s	24.3
14	1.04, d (6.5)	11.8	0.98, s	17.1
15	1.06, s	20.2	1.25, s	23.9
Glc-1'	4.25, d (7.5)	97.1	4.24, d (7.5)	97.0
2'	2.86, t (8.5)	73.8	2.88, t (8.5)	73.7
3'	3.12, t (8.5)	77.0	3.13, t (8.5)	77.0
4'	2.98, t (8.5)	70.3	3.01, overlap	70.4
5'	3.03, m	76.6	3.04, overlap	76.6
6'a	3.61, brd (11.5)	61.2	3.61, brd (11.0)	61.2
6'b	3.37, overlap		3.44, overlap	

Table 4

Hepatoprotective activities against APAP-induced HepG2 cells injure

Compounds	Cell Survival Rates	
	(% of normal)	(% of model)
Normal	100.00 ± 5.94	—
Model	39.87 ± 3.11***	—
Bicyclol	52.87 ± 2.04$^{\#\#}$	32.7
5	53.67 ± 1.11$^{\#\#}$	34.6
7	54.90 ± 0.66$^{\#\#\#}$	37.7
14	43.75 ± 3.69$^{\#}$	9.8
16	54.16 ± 0.61$^{\#\#\#}$	35.9
17	52.85 ± 1.72$^{\#\#}$	32.6
18	52.81 ± 6.35$^{\#}$	32.5

[a)] Bicyclol as the positive drug; [b)] APAP (8 mM)-induced cells as the model group; [c)] Other compounds were tested at 10 μM; [d)] Results are expressed as the mean ± SD (n = 3); ***p < 0.001 (*vs* control group), $^{\#\#\#}p$ < 0.001, $^{\#\#}p$ < 0.01, $^{\#}p$ < 0.05 (*vs* model group).

GRAPHIC ABSTRACT