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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).

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).

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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} ($\Delta\varepsilon$)

Table 1
^1H 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	