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**Letter**

# A New Sesquiterpenoid Glycoside from Rhizomes of *Atractylodes lancea*

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ABSTRACT

**Objective** To study the water-soluble chemical constituents from the rhizomes of *Atratylodes lancea*. **Methods** Two sesquiterpenoid glycosides were purified by column chromatography and their structures were determined by spectroscopic analysis. They were also evaluated for anti-inflammatory activity by determining the inhibitory activity on LPS-induced NO and $PGE_2$ generation in RAW 264.7 cell lines. **Results** Compound **1** was a new sesquiterpenoid glycoside, named as (*1S*,*4S*,*5R*,*7R*,*10S*)-4,11,14-trihydroxy-guai-3-one-11-*O*-β-*D*-glucopyranoside, but exhibited no appreciable activity. Compound **2** was atractyloside A and showed weak activity. **Conclusion** The hydroxyl group at C-10 of guaiol-type glycoside could be important for anti-inflammatory activity.

*Key words*

anti-inflammatory activity; *Atractylodes lancea*; guaiol-type glycoside; sesquiterpenoid glycoside



## 1. Introduction

The rhizome of *Atractylodes lancea* DC. (Compositae) is known as an important Chinese materia medica used for the treatment of rheumatic disease, functional dyspepsia and gastroparesis, night blindness, and influenza (Pharmacopoeia Committee of P. R. China, 2010). In recent decades, the constituents in the rhizomes of *A. lancea* have been investigated, and a number of sesquiterpenoids (Wang et al, 2008), polyacetylenes (Zhen et al, 2012; Ji et al, 2010; Chen et al, 2012), and essential oils (Duan et al, 2008) with non-polar constituents were reported. Furthermore, a series of sesquiterpenoid glycosides (Kitajima et al, 2003a; 2003b) have been paid attention to. Therefore, we report here the structure elucidation of two guaiol-type glycosides (Figure 1) from the water-soluble constituents of *A. lancea* and their anti-inflammatory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$) production in RAW 264.7 cell lines. Compound **1** is characterized as a new sesquiterpenoid glycoside, named as (*1S*,*4S*,*5R*,*7R*,*10S*)-4,11,14-trihydroxy-guai-3-one-11-*O*-β-*D*-glucopyranoside.

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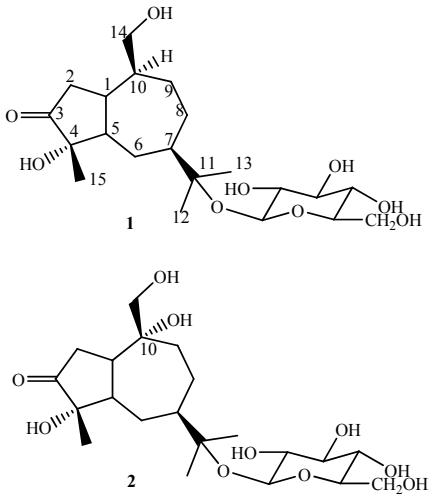

**Figure 1 Structures of compounds 1 and 2**

## 2. Materials and methods

### 2.1 General

Column chromatography (CC): silica gel (200−300 mesh) was from Qingdao Marine Chemical Plant (China); Sephadex LH-20 and ODS were purchased from Amersham Biosciences Inc. (USA) and YMC Co., Ltd. (Japan), respectively. Semi-preparative HPLC was carried out with Waters 600 Liquid Chromatograph with Alltech 2000 Esc ELSD Detector (110 °C, flow rate of the condensed air: 3.1 mL/min) and a Phenomenex Hydro-RP 80R HPLC column (200 mm × 40 mm, 4 μm). IR spectra were recorded on an Impact 400 (KBr) Spectrometer. NMR spectra were obtained with a Bruker 500 Spectrometer operating at 500 MHz for $^1$H-NMR and 125 MHz for $^{13}$C-NMR, respectively. Chemical shifts were reported in parts per million on the $\delta$ scale with TMS as internal standard. The optical rotations were measured on a Jasco P−1020 Optical Rotation Apparatus. HR-ESI-MS spectra were measured on Agilent 1100 LC/MSD TOF Mass Spectrometer. The CD spectrum was obtained on a Jasco 810 Spectropolarimeter. GC was carried out on a Shimadzu GC−2010 Gas Chromatograph, with a DB−1 capillary column (30 m × 0.25 mm) and an FID detector operated at 270 °C (column temperature: initial temperature of 150 °C for 2 min and rising with the rate of 5 °C /min to final 200 °C), 3.0 mL/min $N_2$ as carrier gas.

### 2.2 Plant materials

The fresh rhizomes of *Atractylodes lancea* (Thunb.) DC collected from Maoshan, Jiangsu province of China in July 2010 were taxonomically identified by Prof. Chang-qi Yuan. A voucher specimen was deposited in Nanjing Botanical Garden Mem. Sun Yat-Sen, China.

### 2.3 Extraction and isolation

The fresh rhizomes of *A. lancea* (84.5 kg) were extracted for three times with 95% EtOH at room temperature, and the EtOH extract was concentrated at 50 °C under vacuum condition to aqueous solution and successively extracted with EtOAc. The aqueous layer was passed through non-polar macroporous resin D-101 eluted with $H_2O$ and 30%, 50%, and 90% EtOH. The 50% EtOH eluate (127 g) was chromatographed over silica gel column (200−300 mesh, 1100 g, 150 cm × 9.0 cm) [$CHCl_3$-MeOH-$H_2O$ (15:1:0.05→10:1:0.1→7:3:0.5→2:1:0.5→MeOH)] to give seven fractions (Frs. A−G). Fr. D (15.3 g) was passed through an RP-18 column to give five fractions (Frs. D1−D5). Fr. D3 (110 mg) was subjected to a semi-preparative HPLC (MeOH:$H_2O$ 28:72, flow rate of 2 mL/min) to give compound **1** (30 mg). Fr. E (26.8 g) was applied to silica gel column to afford seven fractions (Frs. E1−E7). Fr. E5 (115 mg) was subjected to a semi-preparative HPLC (MeOH:$H_2O$ 25:75, flow rate of 2 mL/min) to give compound **2** (10.8 mg).

### 2.4 Acid hydrolysis for sugar analysis

Each glycoside (2 mg) was heated in 1 mL of 1 mol/L HCl (dioxane-$H_2O$, 1:1) at 80 °C for 3 h in a water bath. Dioxane was removed and the solution was extracted with EtOAc (1 mL × 3). The EtOAc portion was washed with water and separated from EtOAc. The combined aqueous solution of acid hydrolysis was neutralized by passing through an Amberlite MB−3 resin column eluted with $H_2O$, then concentrated and dried. The dried sugar mixture was dissolved in pyridine (0.5 mL), and then treated with HMDS (0.2 mL) and $(CH_3)_3SiCl$ (0.1 mL) at room temperature for 6 h. After centrifugation, the above fraction was analyzed by GC analysis with authentic monosaccharides.

## 3. Results

Compound **1** was obtained as a white amorphous powder. IR(KBr)$\nu_{max}$(cm$^{-1}$): 3445 (OH), 2940, 1715 (C=O), 1370 (-C-O-), 890. Its formula $C_{21}H_{36}O_9$ was deduced from HR-ESI-MS for the $[M + Na]^+$ ion peak at *m/z* 455.2230 (Calcd. for $[M + Na]^+$, 455.2257). The $^1$H-NMR spectrum of compound **1** (Table 1) displayed signals for three methyl groups linking to the quaternary carbons at $\delta_H$ 1.33 (3H, s), 1.45 (3H, s), 1.24 (3H, s) and for one hydroxyl group linking to the tertiary carbon at $\delta_H$ 3.68 (1H, dd, $J$ = 6.8, 10.5 Hz) and 3.81 (1H, dd, $J$ = 3.6, 10.0 Hz), which were characteristic of a guaiol sesquiterpenoid. The presence of one anomeric proton at $\delta_H$ 5.01 (1H, d, $J$ = 7.7 Hz) in the $^1$H-NMR spectrum (correlated to carbon signal at $\delta_C$ 98.7 in the HSQC spectrum) indicated the presence of one monosaccharide. The HSQC spectrum showed the presence of three methyls, four methylenes, four methines, two hydroxylated quaternary carbons, and one carbonyl carbon, in addition to the β-glycopyranosyl moiety. Acid hydrolysis and gas chromatographic analysis of the persilanizated derivative of sugar indicated that the sugar was *D*-glucopyranose. Compound **1** was supposed to be a 10-dehydroxylated derivative of compound **2** (Figure 1) through the comparison of

**Table 1 $^{1}$H-NMR (500 MHz), $^{13}$C-NMR (125 MHz), HMBC, and main ROESY spectra data for compound 1 ($\delta$, TMS, pyridine-$d_5$)**

| Position | $\delta_C$ | | $\delta_H$ (mult, *J*, Hz) | HMBC | ROESY |
|---|---|---|---|---|---|
| C-1 | 40.5 | H-1β | 1.87 (1H, dd, 10.2, 10.5) | C-5, 6, 9, 14 | H-14a, 14b, $H_3$-15 |
| C-2 | 42.4 | H-2β | 2.14 (1H, dd, 6.5, 19.1) | C-1, 3, 5 | |
| | | H-2α | 2.84 (1H, dd, 7.8, 19.1) | C-1, 3, 5 | |
| C-3 | 220.0 | | | | |
| C-4 | 80.6 | | | | |
| C-5 | 51.8 | H-5α | 2.18 (1H, br.dd, 11.0, 11.1) | C-1, 4, 6, 7, 15 | H-7, H-10 |
| C-6 | 29.0 | H-6β | 1.31(1H, br. dd, 12.6, 13.0) | C-7 | |
| | | H-6α | 2.80 (1H, br. d, 10.5) | C-1, 5, 7, 8 | |
| C-7 | 48.5 | H-7α | 2.09 (1H, br.d, 9.1, 9.8) | | H-5, H-10 |
| C-8 | 26.9 | H-8β | 1.75 (1H, ddd, 7.1, 12.2, 12.3) | C-7, 9 | |
| | | H-8α | 1.90 (1H, m) | | |
| C-9 | 28.9 | H-9β | 1.93 (1H, m) | C-8 | |
| | | H-9α | 1.98 (1H, m) | C-1, 8 | |
| C-10 | 48.9 | H-10α | 1.60 (1H, m) | | H-5, H-7 |
| C-11 | 80.9 | | | | |
| C-12 | 24.1 | $H_3$-12 | 1.33 (3H, s) | C-7, 11, 13 | |
| C-13 | 24.7 | $H_3$-13 | 1.45 (3H, s) | C-7, 11, 12 | |
| C-14 | 66.0 | H-14a | 3.68 (1H, dd, 6.8, 10.5) | C-1, 9, 10 | |
| | | H-14b | 3.81 (1H, dd, 3.6, 10.0) | C-1, 9, 10 | |
| C-15 | 18.6 | $H_3$-15β | 1.24 (3H, s) | C-3, C-4, C-5 | |
| Glc-1 | 98.7 | H-1′ | 5.01 (1H, d, 7.7) | C-11 | |
| Glc-2 | 75.4 | H-2′ | 3.93 (1H, t, 8.5) | C-G-3 | |
| Glc-3 | 78.8 | H-3′ | 4.20 (1H, t, 8.8) | C-G-2, C-G-4 | |
| Glc-4 | 71.7 | H-4′ | 4.14 (1H, t, 9.1) | C-G-3 | |
| Glc-5 | 78.2 | H-5′ | 3.86 (1H, m) | C-G-4 | |
| Glc-6 | 62.7 | H-6′a | 4.30 (1H, dd, 5.0, 11.8) | C-G-5, C-G-4 | |
| | | H-6′b | 4.45 (1H, br. d, 11.8) | C-G-4 | |

NMR data. The resonance of C-10 was shifted upfield to $\delta_C$ 48.9 from $\delta_C$ 85.3 of compound **2**, and the signals of H-14a and H-14b were coupling with an adjacent proton (Figure 2).

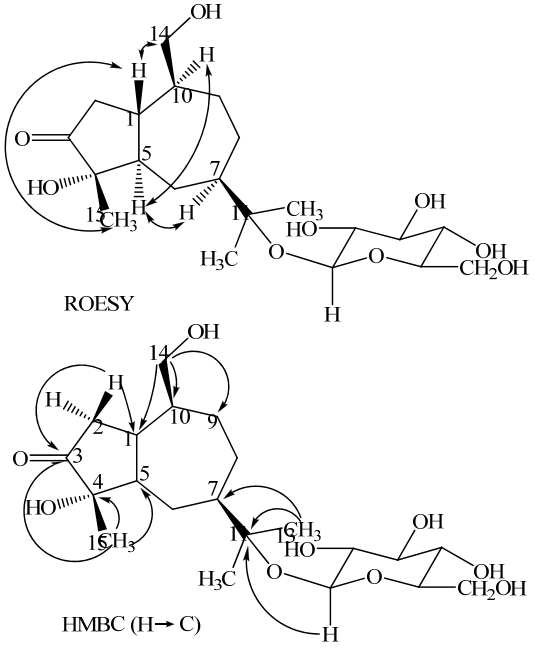


**Figure 2 Key NOE interactions observed in ROESY and selected HMBC (H→C) correlations of compound 1**

The aforementioned interpretation was further confirmed by the analysis on HMBC spectrum of compound **1** (Figure 1). In the HMBC experiment, long-range correlation between the following carbons and protons was observed: $H_3$-15 ($\delta$ 1.24, s) with C-3 ($\delta$ 220.0), C-4 ($\delta$ 80.6), and C-5 ($\delta$ 51.8); $H_3$-12 ($\delta$ 1.33, s), $H_3$-13 ($\delta$ 1.45, s) with C-7 ($\delta$ 48.5) and C-11 ($\delta$ 80.9); H-14a ($\delta$ 3.68, dd, *J* = 6.8 Hz, 10.5 Hz) with C-1 ($\delta$ 40.5), C-9 ($\delta$ 28.9), and C-10 ($\delta$ 48.9); H-2*β* ($\delta$ 2.14, dd, *J* = 6.5, 19.1 Hz) with C-1 ($\delta$ 40.5), C-3 ($\delta$ 220.0), and C-5 ($\delta$ 51.8); H-8β ($\delta$ 1.75, ddd, *J* = 7.1, 12.2, 12.3 Hz) with C-7 ($\delta$ 48.5) and C-9 ($\delta$ 28.9); H-6*α* ($\delta$ 2.80, br d, *J* = 10.5 Hz) with C-5 ($\delta$ 51.8), C-7 ($\delta$ 48.5), C-8 ($\delta$ 26.9), and C-1 ($\delta$ 40.5). The glycosyl group located at C-11 was confirmed by HMBC correlations of Glc H-1 ($\delta$ 5.01, d) with C-11 ($\delta$ 80.9). In addition, the observed NOE interactions between H-1 and $H_3$-15, H-14a, and H-14b; Between H-5 and H-7, H-10 in the ROESY spectrum suggested that the ring juncture was trans, and configuration of C-10-hydroxymethyl, C-4 methyl, and H-1 was β while that of H-5 and H-7 was α. Since compounds **1** and **2** showed the same positive cotton effect in the circular dichroism (CD) at 306 nm (compound **1**: $\Delta\varepsilon$ +17.33 *c* = 0.011 mol/L; compound **2**: $\Delta\varepsilon$ +2.03 *c* = 0.010 mol/L), they were considered to have the same configuration of C-4. Therefore, compound **1** was characterized as (*1S*,*4S*,*5R*,*7R*,*10S*)-4,11,14-trihydroxyguai-3-one 11-*O*-β-*D*-glucopyranoside.

Compound **2** was identified as atractyloside A, (*1S*,*4S*,*5R*,

*7R*,*10R*)-4,10,11,14-tetrahydroxyguai-3-one 11-*O*-β-*D*-glucopyranoside, which was isolated from *A. lancea* previously (Yahara et al, 1989). It was obtained as a white amorphous powder, mp 196−197 °C. The $^{13}$C-NMR spectrum (pyridine-$d_5$，125 MHz) of compound **2** displayed 21 signals: $\delta$ 44.2 (C-1), 37.0 (C-2), 225.9 (C-3), 77.4 (C-4), 47.8 (C-5), 31.0 (C-6), 50.0 (C-7)，23.9 (C-8)，36.3 (C-9)，85.3 (C-10)，83.8 (C-11), 25.5 (C-12), 25. 8 (C-13), 70.5 (C-14), 19.7 (C-15), 99.0 (C-1′), 76.0 (C-2′), 78.2 (C-3′), 72.5 (C-4′), 78.2 (C-5′), and 63.5 (C-6′).

Two sesquiterpenoid glycosides were tested for their anti-inflammatory activity by determining inhibitory activity on LPS induced NO and $PGE_2$ production in RAW 264.7 cell lines. Results showed that the inhibitory rates of NO were 6.58% (compound **1**), 6.47% (compound **2**), and 76.81% (indomethacin), and the inhibitory rates of $PGE_2$ were −8.95% (compound **1**), 17.75% (compound **2**) and 26.10% (indomethacin), respectively. So compound **2** showed weak activity, while compound **1** was inactive.

## 4. Discussion

Compound **1** is a new sesquiterpenoid glycoside and a 10-dehydroxylated derivative of compound **2**. Compound **2** has the weak inhibitory activity on LPS-induced NO and $PGE_2$ production in RAW 264.7 cell lines, and compound **1** is inactive. The hydroxyl group at C-10 of guaiol-type glycoside is suggested to be important for anti-inflammatory activity.

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