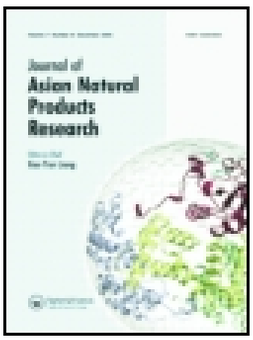

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A new sesquiterpene lactone glycoside and a new quinic acid methyl ester from *Patrinia villosa*

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ABSTRACT

A new sesquiterpene lactone glycoside (**1**) and a new quinic acid methyl ester (**2**) were isolated from *Patrinia villosa*, together with another two known compounds chlorogenic acid *n*-butyl ester (**3**), 3, 4-di-*O*-caffeoylquinic acid methyl ester (**4**). Their structures were established using 1D/2D-NMR spectroscopy, mass spectrometry, and comparing with spectroscopic data reported in the literature.

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KEYWORDS
Patrinia villosa; sesquiterpene lactone glycoside; quinic acid methyl ester; NMR spectroscopy

1. Introduction

Patrinia, a genus of about 20 species [1], was recorded as a traditional medicinal in *ShenNongBenCaoJing* [2]. It has been used for the treatment of inflammation, wound healing, ascetics, and abdominal pain after childbirth for hundreds of years [3]. So far, researches on *Patrinia* were mainly focused on *Patrinia scabiosaefolia* Fisch in China, whereas both pharmaceutical and phytochemical studies on *Patrinia villosa* were barely reported. In order to study the chemical constituents of *P. villosa*, we examined the *n*-butanol extract from this plant and isolated a new sesquiterpene lactone glycoside (**1**), a new quinic acid methyl ester (**2**), along with two known compounds chlorogenic acid *n*-butyl ester (**3**), 3, 4-di-*O*-caffeoyl quinic acid methyl ester (**4**). Herein, we describe their isolation and structure characterization.

2. Results and discussion

Compound **1** was obtained as a yellow gum, with $[\alpha]_D^{20}$ + 29.7 (*c* 0.3, MeOH), and its molecular formula was determined to be $C_{29}H_{38}O_{11}$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) in the positive ion mode, which showed a pseudomolecular ion peak at *m/z* 585.2311. The IR spectrum showed absorption bands for OH groups (3435 cm^{-1}), methyl groups (2960, 2874 cm^{-1}), carbonyl group (1736 cm^{-1}), and a benzyl

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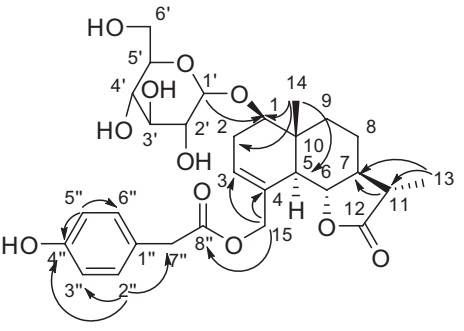
Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (400 MHz for ^{1}H and100 MHz for ^{13}C, DMSO).

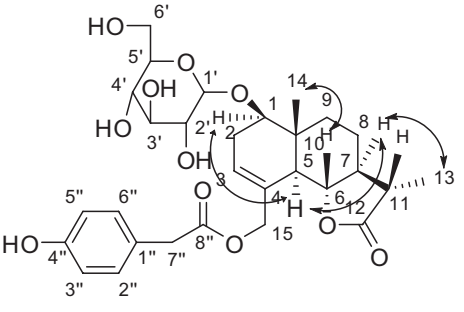
	1			**2**	
No.	δ_H (*J* Hz)	δ_C	No.	δ_H (*J* Hz)	δ_C
1	3.69 (dd, *J* = 6.0, 9.6 Hz)	48.8	1		72.6
2	1.95 (dd, *J* = 9.6, 18.6 Hz) 2.49~2.51 (m) [a]	28.9	2	1.99~2.01 (m)	34.7
3	5.67 (brs)	126.6	3	5.23~5.27 (m)	68.0
4		131.4	4	4.96 (dd, *J* = 3.6, 8.4 Hz)	70.2
5	2.27 (d, *J* = 12.6 Hz)	48.3	5	5.16~5.18 (m)	67.6
6	3.68 (t, *J* = 12.6 Hz)	79.6	6	1.97 (dd, *J* = 3.6, 14.4 Hz) 2.17 (dd, *J* = 5.6, 14.4 Hz)	36.4
7	1.49 (dddd, *J* = 7.2, 12.6, 3.0, 12.6 Hz)	52.2	7		173.6
8	1.35 (dddd, *J* = 3.0, 12.6, 3.6, 12.0 Hz) 1.69~1.71 (m)	22.0	8-OCH_3	3.64 (s)	52.0
9	1.20 (dddd, *J* = 6.6, 12.6, 3.6, 12.6 Hz) 1.89~1.91 (m)	34.2	1′[a]		124.0
10		40.9	2′[b]	6.94 (d, *J* = 8.4 Hz)	130.3
11	2.27 (dq, *J* = 6.6, 7.2 Hz)	39.4	3′[c]	6.67 (d, *J* = 8.4 Hz)	115.0
12		178.7	4′[d]		156.2
13	1.05 (d, *J* = 6.6 Hz)	12.3	5′[e]	6.94 (d, *J* = 8.4 Hz)	130.3
14	.77 (s)	11.7	6′[f]	6.67 (d, *J* = 8.4 Hz)	115.0
15	4.42 (s)	66.6	7′[g]	3.30 (brs)[a]	39.3
1′	4.17 (d, *J* = 7.6 Hz)	99.9	8′[h]		170.4
2′	2.90 (dd, *J* = 7.6, 8.4 Hz)	73.5	1″[a]		124.0
3′	3.00 (dd, *J* = 9.0, 8.4 Hz)	70.3	2″[b]	7.00 (d, *J* = 8.4 Hz)	130.2
4′	3.13 (dd, *J* = 8.4, 9.0 Hz)	76.9	3″[c]	6.68 (d, *J* = 8.4 Hz)	115.1
5′	3.69 (ddd, *J* = 1.8, 6.0, 9.0 Hz)	78.3	4″[d]		156.3
6′	3.41 (dd, *J* = 6.0, 11.4 Hz) 3.64 (dd, *J* = 1.8, 11.4 Hz)	61.3	5″e	7.00 (d, *J* = 8.4 Hz)	130.2
1″		124.6	6″[f]	6.68 (d, *J* = 8.4 Hz)	115.1
2″	7.00 (d, *J* = 8.4 Hz)	130.4	7″[g]	3.36 (brs)[a]	39.4
3″	6.67 (d, *J* = 8.4 Hz)	115.0	8″[h]		170.5
4″		156.1	1‴[a]		124.3
5″	6.67 (d, *J* = 8.4 Hz)	115.0	2‴[b]	7.04 (d, *J* = 8.4 Hz)	130.3
6″	7.00 (d, *J* = 8.4 Hz)	130.4	3‴[c]	6.70 (d, *J* = 8.4 Hz)	115.2
7″	3.49 (brs)	40.4	4‴[d]		156.3
8″		170.8	5‴[e]	7.04 (d, *J* = 8.4 Hz)	115.2
4″-OH	9.30 (brs)		6‴[f]	6.70 (d, *J* = 8.4 Hz)	130.3
			7‴[g]	3.40 (brs)[a]	39.5
			8‴[h]		170.9
			4′-OH[a]	9.30 (brs)	
			4″-OH[b]	9.33 (brs)	
			4‴-OH[c]	9.33 (brs)	

[a]Overlapped with other signals.
[a–h]Signals with the same column are interchangeable.

group (1636, 2922, 2852 cm^{-1}). The extensive analysis of ^{1}H-NMR, ^{13}C-NMR, and HSQC spectra revealed the assignment of 29 carbons, including 2 methyl groups (*δ* 12.3 and 11.7), 4 methylene groups (*δ* 28.9, 22.0, 34.2, and 40.4), 2 oxygen-substituted methylene groups (*δ* 66.6 and 61.3), 15 methine groups (*δ* 48.8, 126.6, 48.3, 79.6, 52.2, 39.4, 99.9, 73.5, 70.3, 76.9, 78.3, 130.4, 115.0, 115.0, and 130.4), 1 aliphatic quaternary carbon (*δ* 40.9), 2 aromatic quaternary carbons (*δ* 124.6 and 156.1), 1 olefinic quaternary carbon (*δ* 131.4), and 2 carbonyl quaternary carbons (*δ* 178.7 and 170.8) (Table 1). Typical signals for *β*-D-glucopyranoside were readily recognized from the NMR spectra, and the existence of a D-glucosyl moiety was further confirmed after acid hydrolysis of **1**. In addition, the ^{1}H NMR spectrum (Table 1) revealed the presence of *p*-hydroxyphenylacetyl moiety [*δ* 3.49 (2H, brs); *δ* 7.00 (2H, d, *J* = 8.4 Hz); *δ* 6.67 (2H, d, *J* = 8.4 Hz) [4]. The remaining signals was similar to those of



(a)

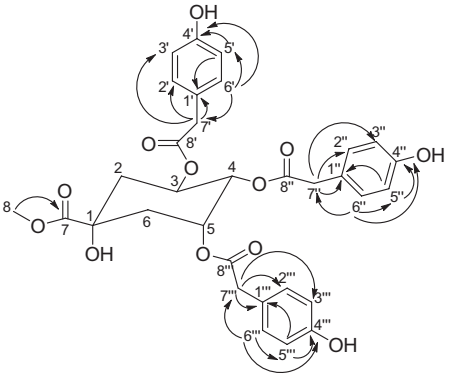


(b)

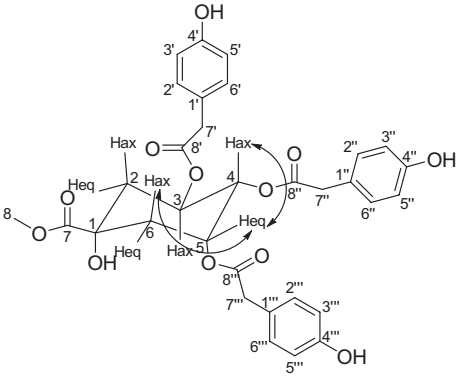
Figure 1. Key HMBC and NOESY correlations of compound **1**.

the known eudesmanolide [4,5]. Besides, long-range correlations in HMBC spectrum (Figure 1 (a)) from H-15 (δ 4.42) to C-8″ (δ 170.8), C-3 (δ 126.6), and C-4 (δ 131.4) confirmed the *p*-hydroxyphenyl acetyl moiety was located at C-15 of the aglycone. Meanwhile the attachment of β-D-glucose to C-1 of eudesmanolide was deduced from the long-range correlation from H-1′ to C-1 in the HMBC spectrum. The large coupling constants for H-1 with H-2 ($J_{1,2}$ = 9.6 Hz), H-5 with H-6 ($J_{5,6}$ = 12.6 Hz), H-6 with H-7 ($J_{6,7}$ = 12.6 Hz) allowed the assignment of the relative stereochemistry for H-1 as α-oriented and that of the lactone group at C-6 and C-7 as *trans* (6β, 7α). In the NOESY experiment (Figure 1 (b)), the cross-peaks between H-5α and H-7α, H-6β and H-14β indicated that the A/B ring was *trans*-fused. In the ^{13}C NMR spectrum, the carbon signal of a methyl group at δ 12.3 is typical for those of eudesmanolides with an α methyl group at C-11 [4], which was further confirmed by NOESY experiment (Figure 1 (b)), giving cross-peaks between H-7α and H-13, H-9α. So, the structure of **1** was characterized as 1β-*O*-β-D-glucopyranosyl-15-*O*-(*p*-hydroxylphenylacetyl)-5α, 6βH-eudesma-3-en-12, 6α-olide.

Compound **2** was obtained as a light brown gum, with $[\alpha]_D^{20}$ − 39.6 (*c* 0.3, MeOH). The molecular formula $C_{32}H_{32}O_{12}$ was indicated by HR-ESI-MS through $[M+Na]^+$ ion peak at *m/z* 631.1802. The IR spectrum of **2** displayed strong absorption bands at 3434, 2919, 1734, 1616, 1516, 1449 cm^{-1}, indicating the presence of hydroxyl, methylene, ester, and phenyl groups, respectively. The ^{1}H-NMR data (Table 1) revealed one methoxyl signal at δ 3.64 (3H, brs, H-8), two methylene signals at δ 1.97 (1H, dd, *J* = 3.6, 14.4 Hz)/2.17 (1H, dd, *J* = 5.6, 14.4 Hz) and δ 1.99~2.01 (2H, m); three oxygen-substituted methine signals at δ 5.23~5.27



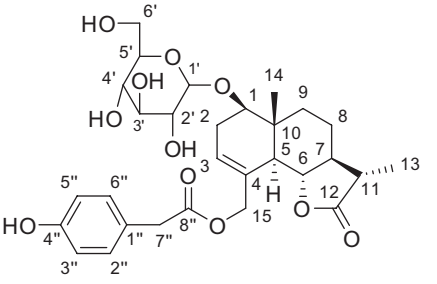
(a)



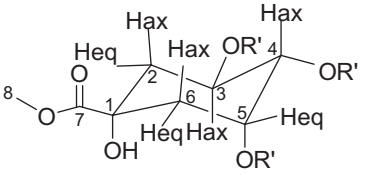
(b)

Figure 2. Key HMBC and NOESY correlations of compound **2**.

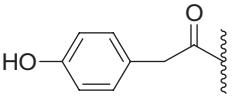
(1H, m), δ 4.96 (1H, dd, J = 3.6, 8.4 Hz), and δ 5.16~5.18 (1H, m), three pairs of aromatic protons at δ 6.67, 6.68, 6.70, 6.94, 7.00, and 7.04 (2H, d, J = 8.4 Hz), three methylene protons at δ 3.30, 3.36, and 3.40 (2H, s), combined with the long-range correlations from H-7′ to C-1′ (H-7″ to C-1″ and H-7‴ to C-1‴) in the HMBC spectrum (Figure 2 (a)). All those signals confirmed the presence of three same *p*-hydroxylphenylacetyl moiety. Meanwhile, the correlation from H-8 (δ 3.64) to C-7 (δ 173.6) was also observed in the HMBC spectrum, indicating the attachment of 8-OCH_3 to C-7. All remaining NMR data of **2** were almost identical to those of the known compound 3, 4, 5-tri-*O*-galloyquinic acid ethyl ester [6,7]. Thus, **2** was characterized as 3, 4, 5-tri-*O*-*p*-hydroxylphenylacetylquinic acid methyl ester. The relative stereochemistry was established from chemical shifts, *J*-coupling (Table 1) and NOESY data (Figure. 2 (b)). Among them, NOESY correlations between H-4 and H-5, H-5 and H-6ax, suggested that the 3-(4-*p*-hydroxylphenylacetyl) groups were equatorially orientated on the cyclohexane ring. The downfield shifts of the axial protons H-2 eq and H-6 eq, relative to H-2 ax and H-6 ax, were considered to be the results of deshielding effect by the carboxyl at C-1 [8]. However, the equatorial nature of the carboxyl was only tentatively assigned as the 3-(4-*p*-hydroxylphenylacetyl) moiety may have anisotropic effects as well. The large coupling constant $J_{3,4}$ = 8.4 Hz confirmed their diaxial orientation, whereas



1

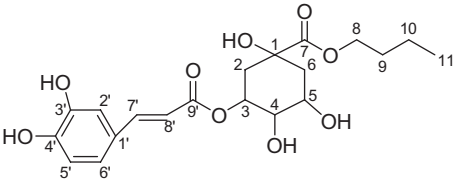


quinic acid methyl ester moiety

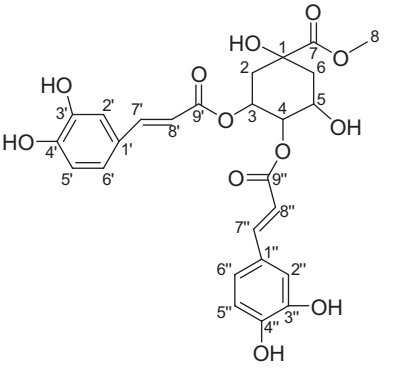


R′= *p*-hydroxylphenylacetyl moiety

2



3



4

Figure 3. The structures of compounds **1–4.**

the smaller coupling constant ($J_{4,5}$ = 3.6 Hz) was the characteristic of an axial – equatorial splitting [9]. Consequently, the structure of **2** was established as shown in Figure 3.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined using a WZZ-2A (Shanghai Base Solid Instrument Co., Ltd., Shanghai, China). UV spectra were recorded on a Shimadzu-2201 (Kyoto, Japan). The IR spectrum was obtained from a Bruker IFS-55 spectrophotometer (Karlsruhe, Germany) using KBr pellet. HR-ESI-MS data were measured on a Micro-mass Autospec-UntimaE TOF mass spectrophotometer (Waters, Massachusetts, USA). 1D- and 2D-NMR spectra were run on a Bruker AVANCE-400/-600 spectrometer (Karlsruhe, Germany). Column chromatography was performed on Silica gel G (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), flash C_{18} (Bonna-Agela Technologies incorporated company, Tianjin, China) and Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA) columns. Thin-layer chromatography (TLC) was carried out using Silica gel GF254 (Qingdao Haiyang Chemical Factory, Qingdao, China) plates. Analytical HPLC was carried out on a Shimadzu LC-10AT (Kyoto, Japan) liquid chromatography and preparative HPLC separation was performed on a YMC-Pack ODS-A column (10 × 250 mm, 5 m; YMC-Pack, Kyoto, Japan), equipped with a Shimadzu LC-8A pump (Kyoto, Japan) and a Shimadzu SPD-10A UV–vis detector (Kyoto, Japan).

3.2. Plant material

Patrinia villosa Juss was purchased from Anguo City Yu Yan Fang Chinese Herbal Medicine Co., Ltd. and was identified as *Patrinia villosa*. A voucher specimen was identified by Prof. Jincai Lu of Shenyang Pharmaceutical University and has been deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University (NO.20140713).

3.3. Extraction and isolation

The dried whole plant of *Patrinia villosa* (15 kg) was extracted three times with hot 95% EtOH (each 2 h) and the combined solution evaporated to dryness by a vacuum rotary evaporator to afford a syrup (1400 g). The crude extract was successively partitioned with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol to yield four layers of extracts. The *n*-butanol extract (349.2 g) was divided into five parts Fr. 1–Fr. 5 by flash C_{18} column eluted with a stepwise gradient mixture of MeOH/H_2O (20:80, 40:60, 60:40, 80:20, 100:0). Fr.4 (39.5 g) was separated by silica gel column under gradient CH_2Cl_2/MeOH (100:0–0:100), to give seven sub-fractions Frs 4.1–4.7. Fr 4.4 (8.0 g) was purified via Sephadex LH-20 eluting with MeOH, to afford 11 fractions (Fr.4.4.1–Fr.E4.4.11). Fr.4.4.6 (271.9 mg) was purified by semi-preparative HPLC eluting with 45% MeOH-H_2O to yield compound **3** (23.3 mg, t_R 76.4 min) and compound **2** (26.0 mg, t_R 98.1 min), compound **4** (30.9 mg, t_R 46.0 min) was obtained from Fr. 4.4.6 by semi-preparative HPLC eluting with 41% MeOH–H_2O as the eluent. Fr. 4.4.8 was purified by semi-preparative HPLC eluting with 37% MeOH–H_2O to yield compound **1** (16.3 mg, t_R 62.5 min).

3.3.1. 1β-O-β-D-Glucopyranosyl-15-O-(p-hydroxylphenylacetyl)-5α, 6βH-eudesma-3-en-12, 6α-olide (1)

Yellow gum; $[\alpha]_D^{20}$ + 29.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 202 (0.55), 224 (0.34) and 278 (0.09) nm; IR (KBr) υ_{max} 3435, 2960, 2922, 2852, 2874, 1736, 1636, 1517, 1457, 1384, 1263, 1164, 1077, 990, 839, 722 and 619 cm^{-1}; ^1H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS: *m/z* 585.2311 $[M+Na]^+$ (calcd for $C_{29}H_{38}O_{11}Na$, 585.2312).

3.3.2. 3,4,5-Tri-O-p-hydroxylphenylacetylquinic acid methyl ester (2)

Light brown gum; $[\alpha]_D^{20}$ − 39.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 203 (0.54), 224 (0.45) and 278 (0.13) nm; IR (KBr) υ_{max} 3434, 2919, 2851, 1734, 1616, 1516, 1449, 1384, 1219, 1129, 1023, 879, 825, 803, 619, and 522 cm^{-1}; ^1H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS: *m/z* 631.1802 $[M + Na]^+$ (calcd for $C_{32}H_{32}O_{12}Na$, 631.1791).

3.4. Acid hydrolysis of compound 1

Compound **1** (4 mg) dissolved in MeOH (3 ml) and aqueous H_2SO_4 (2 mol/L, 3 ml) was heated at 90 °C under refluxing for 3 h. After cooling, the reaction mixture was neutralized with aqueous saturated $Ba(OH)_2$ and the precipitates were filtered off, then partitioned between H_2O and $CHCl_3$. The water phase was concentrated, and subjected to TLC using $CHCl_3$/MeOH/H_2O (7:3:0.5) as the mobile phase, and analyzed with authentic sample D-glucose. The absolute configuration of glucose was confirmed as D-glucose by measuring its optical rotation ($[\alpha]_D^{20}$ + 32.2 (*c* 0.06, MeOH).

Disclosure statement

No potential conflict of interest was reported by the authors.

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