PHENOLIC COMPOUNDS FROM *Ranunculus chinensis*

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Ranunchinesin A, a new phenolic compound, and four known phenolic compounds were isolated from Ranunculus chinensis *Bge. The structure of the new compound was elucidated by spectral methods.*

Keywords: *Ranunculus chinensis* Bge., ranunchinesin A, phenolic compounds, Ranunculaceae.

Ranunculus chinensis Bge., distributed widely in China, is a perennial plant of the Ranunculus genus (Ranunculaceae) with a height of 20 cm to 70 cm and yellow flowers. It has been used as Chinese folk medicine for the treatment of acute and chronic hepatitis and peritoneal dropsy [1]. Flavonoids [2, 3], alkaloids [4], triterpene saponins [5], and lactones such as ranunculin and protoanemonin [6] were isolated by previous phytochemical studies of this genus. Recently, we reported six flavonoid glycosides from the aerial parts of the titled plant [7]. Further investigation on the extracts of *Ranunculus chinensis* Bge. resulted in the isolation of a new phenolic compound, ranunchinesin A (**1**), and four known phenolic compounds, oresbiusin A (**2**) [8], ternatoside B (**3**) [9], *p*-hydroxybenzoic acid (**4**) [10], and protocatechuic acid (**5**) [11]. Their structures were elucidated by spectral methods.

Compound **1** was obtained as a light yellow gum, $[\alpha]_D^{22}$ +5.6° (*c* 0.445, MeOH). The IR bands at 3396, 1737, 1606, 1525, and 1446 cm^{-1} revealed the presence of hydroxyl, carbonyl, and aromatic ring groups. The molecular formula was determined as $C_{12}H_{16}O_7$, with five degrees of unsaturation, on the basis of the mass spectrum and NMR spectral data, and further confirmed by the HR-EI-MS molecular ion peak at *m/z* 272.0887. The EI-MS spectrum gave a fragment ion peak at *m/z* 61, suggesting the existence of a glyceryl group. The ^{1}H NMR spectrum displayed signals for a 1,2,4-trisubstituted aromatic ring (δ 6.68 (1H, d, J = 1.8 Hz), 6.66 (1H, d, J = 8.1 Hz), and 6.56 (1H, dd, J = 8.0, 1.8Hz)), a methylene (δ 2.96 (1H, dd, J = 14.0, 5.1 Hz) and 2.82 (1H, dd, J = 14.0, 7.5 Hz)), an oxybearing methine (δ 4.35 (1H, dd, J = 7.5, 5.1 Hz)), and a glyceryl group (δ 4.21 (2H, m), 3.82 (1H, m), and 3.53 (2H, d, J = 5.7 Hz)). In the ^{13}C NMR spectrum, six sp^2 carbon signals at δ 130.4, 118.1, 146.5, 145.5, 116.7, and 122.4 were assigned to the aromatic ring. The remaining signals at δ 175.8, 73.9, 41.6, and 67.5, 71.5, and 64.4 were attributed to the carbons of carbonyl, carbinol, methylene, and the glyceryl groups, respectively. The ^{1}H and ^{13}C NMR spectra of **1** were closely similar to those of **2** except for the glyceroyl group instead of the methoxyl of **2**, indicating **1** to be 3-(3′, 4′-dihydroxyphenyl) lactate glycerol ester, named ranunchinesin A. The structure of **1** was further confirmed by HMBC correlations of H-4 with C-1, C-5, and C-6, and of H-3 with C-1, C-2, C-1′, C-2′, and C-6′ (Fig. 1).

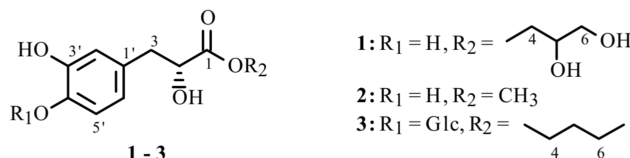

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TABLE 1. ^1H NMR (300 MHz) and ^{13}C NMR (100 MHz) Spectral Data of **1–3** (CD_3OD, TMS, δ, ppm)

C atom	δ_C			δ_H (mult. J/Hz)		
	1	**2**	**3**	**1**	**2**	**3**
1	175.8	176.4	176.0			
2	73.9	73.8	73.7	4.35 (dd, J = 7.5, 5.1)	4.29 (dd, J = 7.4, 5.2)	4.32 (dd, J = 7.2, 5.4)
3	41.6	41.6	41.5	2.96 (dd, J = 14.1, 5.1) 2.82 (dd, J = 14.1, 7.5)	2.91 (dd, J = 14.0, 5.2) 2.79 (dd, J = 14.0, 7.6)	2.96 (dd, J = 13.5, 5.7) 2.86 (dd, J = 13.5, 7.2)
4	67.5	52.7	66.7	4.21 (m)	3.68 (s)	4.11 (t, J = 6.6)
5	71.5		31.9	3.82 (m)		1.62 (m)
6	64.4		20.6	3.53 (d, J = 5.7)		1.37 (m)
7			15.1			0.95 (t, J = 7.5)
1′	130.4	130.3	130.2			
2′	118.1	118.1	118.3	6.68 (d, J = 1.8)	6.65 (d, J = 2.4)	6.74 (d, J = 2.1)
3′	146.5	146.6	146.5			
4′	145.5	145.6	147.9			
5′	116.7	116.6	116.4	6.66 (d, J = 8.1)	6.67 (d, J = 8.0)	7.11 (d, J = 8.1)
6′	122.4	122.3	122.2	6.56 (dd, J = 8.1, 1.8)	6.53 (dd, J = 8.0, 2.0)	6.66 (dd, J = 8.1, 2.0)
1″			104.1			4.71 (d, J = 7.5)
2″			73.9			3.47–3.38 (m)
3″			76.5			3.47–3.38 (m)
4″			69.4			3.47–3.38 (m)
5″			77.3			3.47–3.38 (m)
6″			60.9			3.90 (d, J = 11.8) 3.74 (dd, J = 12.0, 4.8)

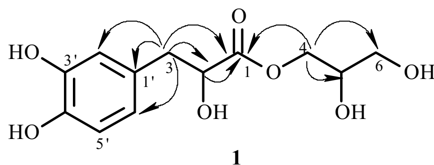

Fig. 1. Significant HMBC (H to C) correlations of **1**.

EXPERIMENTAL

General Procedures. ^1H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker-DRX-300 or Bruker-DRX-400 spectrometers; chemical shifts (δ) were expressed in ppm with TMS as reference. IR spectra were measured on a Nicolet-Magna-750-FTIR spectrometer, KBr pellets, in cm^{-1}. ESI-MS and HR-ESI-MS were run on LCQ-Deca and Q-Tof Ultima mass spectrometers, respectively. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Silica gel (200–300 or 400 mesh; Qingdao Haiyang Co., China), ODS-A gel (Greenherbs Science & Technology Development Co., Ltd., Beijing, China), D-1400 macroporous resin (Yangzhou Pharmaceutical Factory, China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography. Semi-preparative HPLC was run on a Varian SD-1 Deliver System pump, equipped with a Varian D-320 UV detector, and a column Kromasil 100-5-C18 (10×250 mm). Thin-layer chromatography was performed on glass-backed plates coated with silica gel GF 254. Fractions were monitored with TLC, and spots were visualized by spraying 10% alc. H_2SO_4 as well as 5% vanillin followed by heating at 105°C for 4 min.

Plant Material and Extraction and Isolation. The aerial parts of *Ranunculus chinensis* Bge. were collected in March, 2005, from Dali of Yunnan Province, P. R. China and was authenticated by Dr. J. Huang of our Institute. A voucher specimen (No. 20050303) was deposited at the Herbarium of the Shanghai Institute of Materia Medica. The aerial parts of *Ranunculus chinensis* Bge. (13 kg) were extracted with 95% ethanol (50 L) three times by maceration for 48 h. The solvent was evaporated under reduced pressure, and the residue (800 g) was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble part (160 g) was subjected to column chromatography over silica gel (2 kg), eluting with a mixture of chloroform and methanol in an order of increasing polarity to give 8 fractions (frs.1–8).

Fractions 3 and 4 were further chromatographed to afford **4** (19 mg) and **5** (21 mg), respectively. Fraction 5 was separated over silica gel column, eluting with chloroform–methanol (60:1, 40:1, 20:1, 10:1, 5:1, v/v, each 3 L) to give six subfractions (frs. 5A-5E). Fraction 5B was further purified over silica gel and Sephadex LH-20 columns to afford **2** (90 mg). Repeated purification of fr. 5D over silica gel and Sephadex LH-20 columns yielded **1** (30 mg). The *n*-BuOH–soluble part (250 g) was subjected to column chromatography (macroporous resin (i.d. 10 × 80 cm), EtOH–H_2O (v/v) 0:100, 10:90, 30:70, 50:50, 70:30, 95:5): frs. A–F. Fraction C (30% EtOH, 30 g) was separated by column chromatography (SiO_2, $CHCl_3$–MeOH 100:0→0:100) to give 10 fractions (frs. C1–C10). Fraction C2 was repeatedly column chromatographed over silica gel, Sephadex LH-20, and ODS gel to give a subfraction whose main component was **3**. The subfraction was purified by semi-preparative HPLC separation (30% MeOH, 3 mL/min, t_R = 21.6 min) to yield **3** (5 mg).

Ranunchinesin A (**1**). Light yellow gum, $[\alpha]_D^{22}$ +5.6° (*c* 0.445, MeOH); IR (KBr, ν, cm^{-1}): 3396, 2956, 1737, 1606, 1525, 1446; ^{1}H NMR and ^{13}C NMR are shown in Table 1; EI-MS *m/z* (%): 272 [M^+] (15), 254 (4), 163 (6), 153 (8), 137 (3), 123 (100), 61 (55); ESI-MS *m/z*: 295 $[M+Na]^+$, 271 $[M–H]^–$, 543 $[2M–H]^–$, HR-EI-MS *m/z*: 272.0887.

Oresbiusin A (2). Light yellow gum, $[\alpha]_D^{22}$ +7.9° (*c* 0.410, MeOH); IR (KBr, ν, cm^{-1}): 3402, 2943, 1725, 1601, 1520, 1450; ^{1}H NMR and ^{13}C NMR are shown in Table 1; EI-MS *m/z* (%): 212 [M^+] (16), 194 (4), 163 (3), 153 (3), 137 (3), 123 (100); ESI-MS *m/z*: 235 $[M+Na]^+$, 211 $[M–H]^–$, 423 $[2M–H]^–$.

Ternatoside B (3). White amorphous powder, $[\alpha]_D^{22}$ –20.3° (*c* 0.460, MeOH); IR (KBr, ν, cm^{-1}): 3415, 2940, 1721, 1597, 1519, 1432; ^{1}H NMR and ^{13}C NMR are shown in Table 1; ESI-MS *m/z*: 439 $[M+Na]^+$, 415 $[M–H]^–$, 831 $[2M–H]^–$, 253 $[M–H–162]^–$.

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