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### Chemical Constituents from the roots of *Ranunculus ternatus*

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## Chemical Constituents from the roots of *Ranunculus ternatus*

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Two new glycosides named as ternatoside A (**1**) and ternatoside B (**2**), with four known constituents sternbin (**3**), methylparaben (**4**), 4-O-D-glucopyranosyl-p-coumaric acid (**5**) and linocaffein (**6**) were isolated from the roots of *Ranunculus ternatus*. The structures of new compounds were determined by 1D and 2D NMR, MS techniques, and chemical methods.

**Keywords:** *Ranunculus ternatus*; Ternatoside A; Ternatoside B

### 1. Introduction

*Ranunculus ternatus* Thunb. is a plant of genus *Ranunculus* used for the treatment of tuberculosis [1]. Some fatty acid esters and  $\gamma$ -keto- $\delta$ -valerolactone have been isolated from this plant [2,3]. In this paper, we reported the isolation and structural elucidation of two new glycosides, ternatoside A (**1**) and B (**2**), with other four known constituents sternbin (**3**), methylparaben (**4**), 4-O-D-glucopyranosyl-p-coumaric acid (**5**) and linocaffein (**6**). The structures were identified on the basis of spectroscopic and chemical methods.

### 2. Results and discussion

Compound **1** was obtained as a colorless gum, and gave positive result to Molish test. In the positive and negative ESIMS, it showed quasi-molecular ion peaks at  $m/z$  373.2  $[M + Na]^+$ , 189.2  $[M - 162 + H]^+$  and 349.3  $[M - H]^-$ , respectively. The molecular formula  $C_{15}H_{26}O_9$  was determined by HRFABMS and  $^{13}C$ NMR. Glucose was detected after the acid hydrolysis and compared with authentic sample on TLC. The  $^1H$ ,  $^{13}C$ NMR and HMQC spectra indicated that this compound possesses three methylenes, one O-butyl, one carbonyl of ketone, one carbonyl of ester, and one glucosyl group (table 1). The structure of **1** was determined on the basis of  $^1H$ - $^1H$ COSEY and HMBC spectra. In HMBC spectrum of **1**,

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Table 1. NMR spectral data of **1** ( $^1\text{H}$ , 500 MHz;  $^{13}\text{C}$ , 125 MHz;  $\delta$ ppm, J Hz, in  $\text{DMSO}-d_6$ ).

No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$	No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1		172.9	9	0.87(t, 3H, 7.5)	14.0
2	2.48(t, 2H, 6.5)	27.7	1'	4.91(d, 1H, 7.5)	103.3
3	2.78(t, 2H, 6.5)	34.1	2'	3.04(t, 1H, 7.5)	74.0
4		207.9	3'	3.17(t, 1H, 7.5)	77.2
5	4.30, 4.22(d, 2H, 17.0)	73.7	4'	3.07(t, 1H, 7.5)	70.7
6	3.99(t, 2H, 7.5)	64.2	5'	3.25(m, 1H, 7.0)	77.5
7	1.52(m, 2H, 7.5)	30.8	6'	3.99, 3.66(dd, 2H, 13.5, 7.0)	61.7
8	1.31(m, 2H, 7.5)	19.1			

$^{13}\text{C}$ - $^1\text{H}$  long-range correlations were found between H-2, H-3, H-6 and C-1; H-2, H-3, H-5 and C-4; H-5 and C-3; The anomeric proton of glucosyl group at  $\delta$  4.91 (d, 1H,  $J = 7.5$  Hz) was correlated to C-5 of aglycone (figure 1). In  $^1\text{H}$ - $^1\text{H}$ COSY spectrum of **1**, correlations were found between H-2 and H-3; H-6 and H-7; H-7 and H-8; H-8 and H-9. The anomeric configuration of the glucose was deduced to be  $\beta$ -anomer from the coupling constant of the anomeric proton. Thus compound **1** was elucidated as 4-carbonyl-( $O$ - $\beta$ -D-glucopyranosyl)-pentanoic acid-1- $O$ -butyl ester, named as ternatoside A.

Compound **2** was obtained as a brown gum, and gave positive result to Molish test. In the positive and negative ESIMS, it showed quasi-molecular ion peaks at  $m/z$  439.2  $[\text{M} + \text{Na}]^+$ , 255.2  $[\text{M} - 162 + 1]^+$  and 415.3  $[\text{M} - \text{H}]^-$ , respectively. Its molecular formula  $\text{C}_{19}\text{H}_{28}\text{O}_{10}$  was established by HRFABMS and  $^{13}\text{C}$ NMR. Glucose was detected after the acid hydrolysis and compared with authentic sample on TLC. The  $^1\text{H}$ ,  $^{13}\text{C}$ NMR and HMQC spectra indicated that this compound possesses 19 carbon signals including one methylene, one methine, one phenyl, one  $O$ -butyl, one glucosyl group, one carbonyl of ester groups (table 2). In the HMBC of compound **2**,  $^{13}\text{C}$ - $^1\text{H}$  long-range correlations were found between H-2, H-3, H-4 and C-1; H-2, H-2', H-6' and C-3; H-2, H-3, H-2', H-5', H-6' and C-1'; H-2', H-5', H-6' and C-4'; The anomeric proton of glucosyl group at  $\delta$  4.61(d, 1H,  $J = 7.5$  Hz) was correlated to C-4' of phenyl (figure 1). In  $^1\text{H}$ - $^1\text{H}$ COSY spectrum of **2**, correlations were found between H-2 and H-3; H-5' and H-6'; H-4 and H-5; H-5 and H-6; H-6 and H-7. The anomeric configuration of the glucose was deduced to be  $\beta$ -anomer from the coupling constant of the anomeric proton. A comparison of the  $^{13}\text{C}$ NMR spectra of **2** with those of the known ( $R$ )-3-[3-hydroxy-4-( $O$ - $\beta$ -D-glucopyranosyl)-phenyl]-2-hydroxypropanoic acid [4], showed very similar  $\delta$  value, except for C-1 of **2** up-shifted for 10.90 ppm. Combining with the aid of HMBC correlation, it suggested that the carboxy of **2** have been esterified by butanol. Optical rotation of **2** was also compared with the known ( $R$ )-3-[3-hydroxy-4-( $O$ - $\beta$ -D-glucopyranosyl)-phenyl]-2-hydroxypropanoic acid, indicating compound **2** has a ( $R$ )-form. Therefore, compound **2** was elucidated as ( $R$ )-3-[3-hydroxy-4-( $O$ - $\beta$ -D-glucopyranosyl)-phenyl]-2-hydroxypropanoic acid butyl ester, named as ternatoside B.

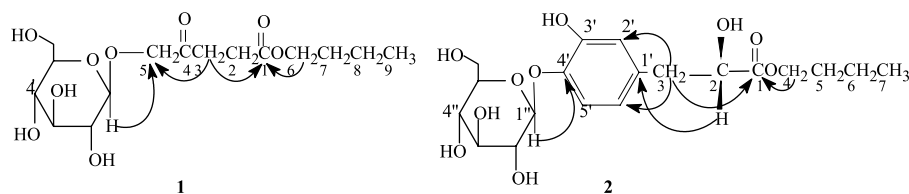
Figure 1. Structures and Key HMBC correlations of **1** and **2**.

Table 2. NMR spectral data of **2** ( $^1\text{H}$ , 500 MHz;  $^{13}\text{C}$ , 125 MHz;  $\delta$ ppm, J Hz, in  $\text{DMSO-}d_6$ ).

No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$	No.	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1		174.3	4'		146.0
2	4.16 (t, 1H, 6.0)	72.0	5'	6.67(d, 1H, 6.5)	116.1
3	2.81,2.69(dd, 2H, 14.5,6.0)	40.0	6'	6.69(dd, 1H, 6.5, 2.5)	124.4
4	3.96(t, 2H, 7.0)	64.3	1''	4.61(d, 1H, 7.5)	103.2
5	1.49(m, 2H, 7.0)	30.8	2''	3.27(t, 1H, 7.5)	74.0
6	1.26(m, 2H, 7.0)	19.2	3''	3.25(t, 1H, 7.5)	76.6
7	0.89(t, 3H, 7.0)	14.2	4''	3.18(t, 1H, 7.5)	70.3
1'		129.0	5''	3.29(m, 1H, 6.5)	77.8
2'	6.95(d, 1H, 2.5)	118.7	6''	3.72,3.63(dd, 2H, 11.7,6.5)	61.4
3'		145.5			

### 3. Experimental

#### 3.1 General experiment procedures

Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. UV spectra were measured by a Waters-2996 detector. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured on a Bruker AM-500 (500 MHz) instrument. FABMS were obtained on a Zabspec E spectrometer; ESIMS were obtained on an Esquire-LC00054 spectrometer. For column chromatography, silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used. TLC and HPTLC (silica gel GF<sub>254</sub> precoated plates, Qingdao Haiyang Chemical Co.) detection was obtained by spraying 10%  $\text{H}_2\text{SO}_4$  followed by heating.

#### 3.2 Plant material

The *Ranunculus ternatus* was collected from Henan province of China in October 2002, and identified by Dr. Xue-Sen Wen, School of Pharmaceutical Sciences, Shandong University. A voucher specimen is deposited in Department of Chinese Medicine Sciences & Engineering, Zhejiang University.

#### 3.3 Extraction and isolation

The dried powdered plant materials (10 kg) were refluxed with 70% EtOH twice, after removal of the solvent by evaporation, the combined extracts were partitioned between  $\text{H}_2\text{O}$  and petroleum ether,  $\text{CHCl}_3$ , EtOAc and *n*-BuOH, successively. The EtOAc extract (170 g) was chromatographed on Si gel column, eluting with  $\text{CHCl}_3$ -MeOH (MeOH contain 5%  $\text{H}_2\text{O}$ ) from 100 to 50:50 in a gradient manner divided into 15 fractions. Fraction 3( $\text{CHCl}_3$ -MeOH 95:5) (0.7 g) was separated on Si gel column, using petroleum ether-EtOAc (87:13) as eluent to afford **3**(26 mg), fraction 5( $\text{CHCl}_3$ -MeOH 90:10) (2.3 g) was separated on Si gel column, using petroleum ether-EtOAc (82:18) as eluent to afford **4**(280 mg), Fraction 8( $\text{CHCl}_3$ -MeOH 80:20) (13.0 g) was separated on Si gel column, using  $\text{CHCl}_3$ -MeOH (MeOH contain 5%  $\text{H}_2\text{O}$ ) (86:14) as eluent to afford **5** (19 mg) and **6**(35 mg), Fraction 11( $\text{CHCl}_3$ /MeOH 70:30) (7.7 g) was separated on Si gel column, using  $\text{CHCl}_3$ -MeOH (MeOH contain 5%  $\text{H}_2\text{O}$ ) (77:23) as eluent to afford **1** (22 mg) and **2** (16 mg).

**3.3.1 Compound 1.** Colorless gum (MeOH),  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz) and  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz) see table 1; positive ESIMS  $m/z$  373.2  $[\text{M} + \text{Na}]^+$ , 189.2  $[\text{M} - 162 + \text{H}]^+$ ; negative ESIMS  $m/z$  349.3  $[\text{M} - \text{H}]^-$ ; HRFABMS  $m/z$  373.1426  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{15}\text{H}_{26}\text{O}_9\text{Na}$ , 373.1475).

**3.3.2 Compound 2.** Brown gum (MeOH),  $[\alpha]_{\text{D}}^{20} - 41.20$  (c 0.50, MeOH);  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz) and  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz) see table 2; positive ESIMS  $m/z$  439.2  $[\text{M} + \text{Na}]^+$ , 255.2  $[\text{M} - 162 + \text{H}]^+$ ; negative ESIMS  $m/z$  415.3  $[\text{M} - \text{H}]^-$ ; HRFABMS  $m/z$  439.1551  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{19}\text{H}_{28}\text{O}_{10}\text{Na}$ , 439.1580).

**3.3.3 Compound 3.** Yellow needle crystal ( $\text{CH}_3\text{Cl}$ ), mp 221–223°C, positive ESIMS  $m/z$  325.3  $[\text{M} + \text{Na}]^+$ , 303.3  $[\text{M} + \text{H}]^+$ . By comparison of NMR and UV data with those of the literature value [5], it was identified as sternbin (**3**).

**3.3.4 Compound 4.** White needle crystal ( $\text{CH}_3\text{Cl}$ ), mp 129–131°C, IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$  3500, 3200, 2960, 2870, 1740, 1700, 1680, 1480, 1320  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz)  $\delta$  9.62 (1H, s, COOH), 7.22 (2H, d,  $J = 6.3$  Hz, H-2, 6), 6.57 (2H, d,  $J = 6.3$  Hz, H-3, 5), 4.64 (3H, s, Me);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta$  177.7(COO), 157.2(C-1), 152.8(C-4), 122.0(C-2, 6), 111.9 (C-3, 5), 64.6(Me); positive ESIMS  $m/z$  175.2  $[\text{M} + \text{Na}]^+$ , 153.1  $[\text{M} + \text{H}]^+$ . The above data revealed compound **4** was methylparaben.

**3.3.5 Compound 5.** Brown gum (MeOH), IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$  3400, 3300, 2850, 1770, 1690, 1670, 1320, 1200, 1030  $\text{cm}^{-1}$ ; positive ESIMS  $m/z$  349.3  $[\text{M} + \text{Na}]^+$ , 327.4  $[\text{M} + \text{H}]^+$ . By comparison of NMR data with those of the literature value [6], it was identified as 4-O-D-glucopyranosyl-p-coumaric acid (**5**).

**3.3.6 Compound 6.** Brown gum (MeOH), IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$  3450, 3280, 2860, 1770, 1690, 1670, 1330, 1210, 1020  $\text{cm}^{-1}$ ; positive ESIMS  $m/z$  355.3  $[\text{M} + \text{Na}]^+$ , 343.3  $[\text{M} + \text{H}]^+$ . By comparison of NMR data with the literature value [7], it was identified as linocaffein (**6**).

**3.3.7 Acid hydrolysis of 1–2 and 5–6.** Compounds of **1–2** and **5–6** (each 5 mg) were refluxed with 5% HCl in MeOH (10 mL) for 5 h, each mixture was diluted with  $\text{H}_2\text{O}$ , neutralized with  $\text{Na}_2\text{CO}_3$ . The neutral hydrolysate revealed the presence of glucose by HPTLC [ $\text{CH}_3\text{Cl}-\text{MeOH}-\text{H}_2\text{O}$  (65:35:10) lower layer] when compared with authentic sample (Sigma).

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