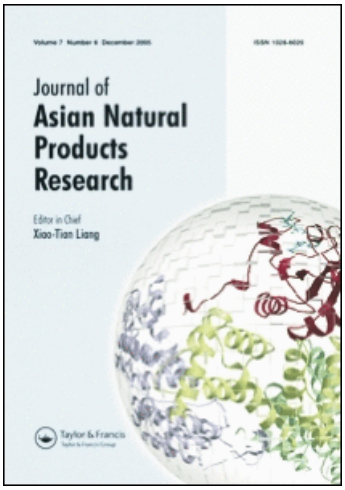

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Three new compounds from the roots of *Saposhnikovia divaricata*

Jie Kang[a]; Lei Zhou[a]; Jing-Hao Sun[a]; Min Ye[a]; Jian Han[a]; Bao-Rong Wang[a]; De-An Guo[a]
[a] The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China

To cite this Article Kang, Jie , Zhou, Lei , Sun, Jing-Hao , Ye, Min , Han, Jian , Wang, Bao-Rong and Guo, De-An(2008) 'Three new compounds from the roots of *Saposhnikovia divaricata*', Journal of Asian Natural Products Research, 10: 10, 971 — 976
To link to this Article: DOI: 10.1080/10286020802217556
URL: http://dx.doi.org/10.1080/10286020802217556

Journal of Asian Natural Products Research
Vol. 10, No. 10, October 2008, 971–976

Three new compounds from the roots of *Saposhnikovia divaricata*

Jie Kang, Lei Zhou, Jing-Hao Sun, Min Ye, Jian Han, Bao-Rong Wang and De-An Guo*

The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China

(*Received 25 February 2008; final version received 4 May 2008*)

From the roots of *Saposhnikovia divaricata*, three new compounds, divaricataesters A (**1**), B (**2**), and C (**3**) were isolated, along with three known compounds, cimifugin (**4**), (3*S*)-2,2-dimethyl-3,5-dihydroxy-8-hydroxymethyl-3,4-dihydro-2H,6H-benzo[1,2-b:5,4-b′]dipyran-6-one (**5**) and 5-hydroxymethyl-furfurol (**6**). Their structures were established by spectral analysis and comparison with the reported data in literatures.

Keywords: *Saposhnikovia divaricata*; divaricataester A; divaricataester B; divaricataester C

1. Introduction

The genus *Saposhnikovia* (Umbelliferae) comprises the sole species, *Saposhnikovia divaricata*, which was mainly distributed in eastern Siberia and Northern Asia [1]. The roots of *S. divaricata*, named 'Fang feng' in Chinese, is a famous traditional Chinese medicine (TCM), which was used for the treatment of rheumatism, headache, convulsion, and nerve paralysis in Chinese clinics [1,2]. Earlier phytochemical investigations showed that 'Fang feng' contained various types of compounds, including chromones, coumarins, alkaalkyne, and polysaccharides, etc [2–7]. Our present research on the roots of *S. divaricata* resulted in the isolation of three new compounds, a 2-furoylmethyl amino acid derivative, divaricataester A (**1**), a linear dihydrofurochromone, divaricataester B (**2**), and a benzofuran glycoside, divaricataester C (**3**), along with three known compounds, cimifugin (**4**) (Figure 1) [8], (3*S*)-2,2-dimethyl-3,5-dihydroxy-8-hydroxymethyl-3,4-dihydro- 2H,6H-benzo[1,2-b:5,4-b′]dipyran-6-one (**5**) [9] and 5-hydroxymethyl-furfurol (**6**) [10]. Among them, compounds **5** and **6** were isolated from the genus for the first time.

2. Results and discussion

Compound **1** was obtained as brown powder. Its molecular formula was determined to be $C_{13}H_{15}NO_5$ based on its HRESIMS at *m*/*z* 266.1024 $[M + H]^+$. The UV spectrum of **1** displayed absorption maximum at 272 nm, showing the probable presence of conjugated carbonyl moiety. IR absorption bands at 2934, 1737, 1686, 1468, 1257, and 1028 cm^{-1} indicated the presence of alkyl, carbonyl, aromatic ring and ester groups, respectively. The ^1H NMR spectrum showed furan protons at δ 7.77 (1H, d, $J = 1.2$ Hz), 7.37 (1H, d, $J = 3.6$ Hz), and 6.62 (1H, dd, $J = 3.6$, 1.2 Hz); two geminal coupling protons at δ 4.91 (1H, d, $J = 18.0$ Hz) and 4.38 (1H, d, $J = 18.0$ Hz); ethoxy protons at δ 4.14 (2H, q, $J = 7.2$ Hz) and 1.19 (3H, t, $J = 7.2$ Hz); and other aliphatic protons at δ 4.37 (1H, m), 2.45 (2H, m), and 2.12 (2H, m). In the heteronuclear multiple bond correlation (HMBC) spectrum (Figure 2), correlations of H-1′/C-2, C-5, C-2′ indicated that C-2′

*Corresponding author. Email: gda@bjmu.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802217556
http://www.informaworld.com

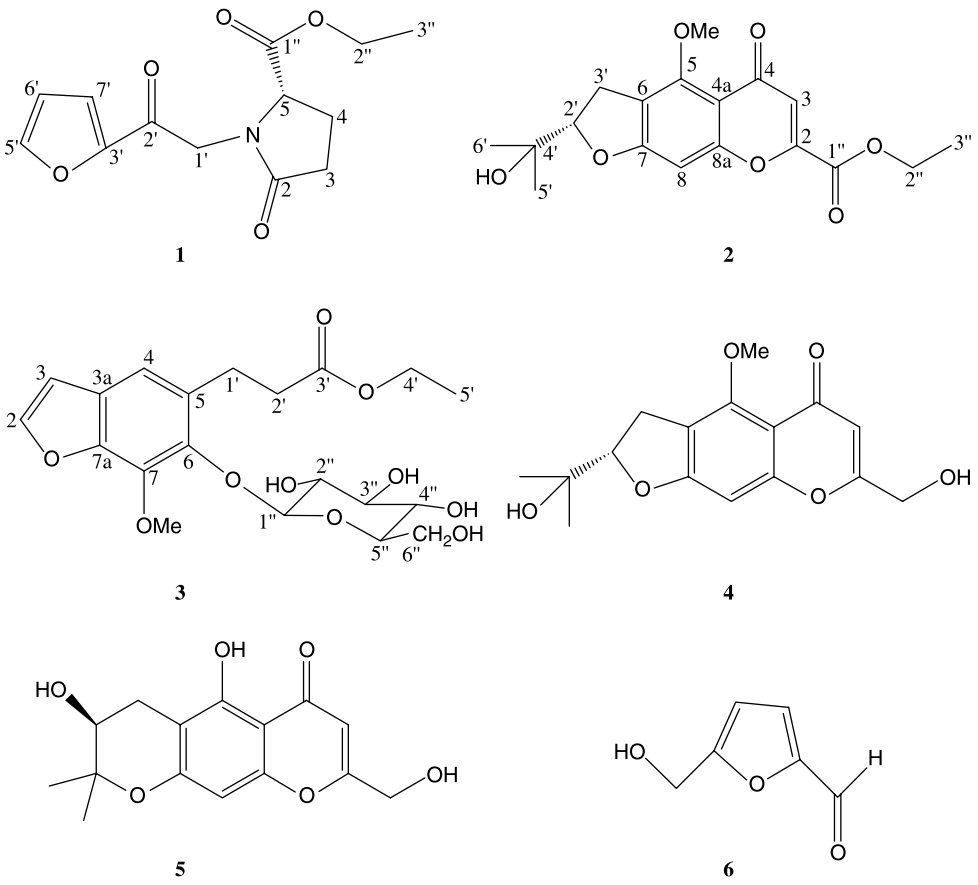

Figure 1. The structures of compounds **1**–**6**.

of a furoyl group and the $N-1$ of 5-ethoxycarbonyl-2-pyrrolidone were connected by a methylene (C-1′). The corresponding free acid of compound **1**, 2-furoylmethyl pyrrolidone carboxylic acid, has been detected in acid hydrolysate from the stored orange juice by HPLC/ESIMS [11]. Since the chiral carbon of the corresponding free acid of compound **1** was in *S*-configuration, C-5 of **1** was also postulated to be *S*-configuration. This new compound was named as divaricataester A. This is the first time to isolate furoylmethyl amino acid derivative from natural sources.

Compound **2** was obtained as brown powder. The HRESIMS of **2** gave an ion peak at *m*/*z* 349.1283 $[M + H]^+$, corresponding to a molecular formula of $C_{18}H_{20}O_7$. The UV spectrum of **2** showed absorption maxima at 218, 243, and 314 nm, indicating the presence of an aromatic ring and conjugated carbonyl groups. The IR spectrum displayed absorption bands at 3407, 2928, 1736, 1651, 1251, and 1095 cm^{-1}, which proved the existence of hydroxyl, alkyl, carbonyl, aromatic ring and ester groups, respectively. The ^1H and ^{13}C NMR spectral data of **2** were very similar to those of cimifugin [8], except for the substitution group in C-2 position. The HMBC cross-peaks of H-2″/C-1″, 3″, and H-3/C-1″ confirmed that the ethoxycarbonyl group was attached to C-2. The CD spectrum of **2** showed a negative Cotton at 294 nm and a positive Cotton at 246 nm analogous to that of (*S*)-(+)-cimifugin (see 3.3.4). Moreover, the optical rotation values

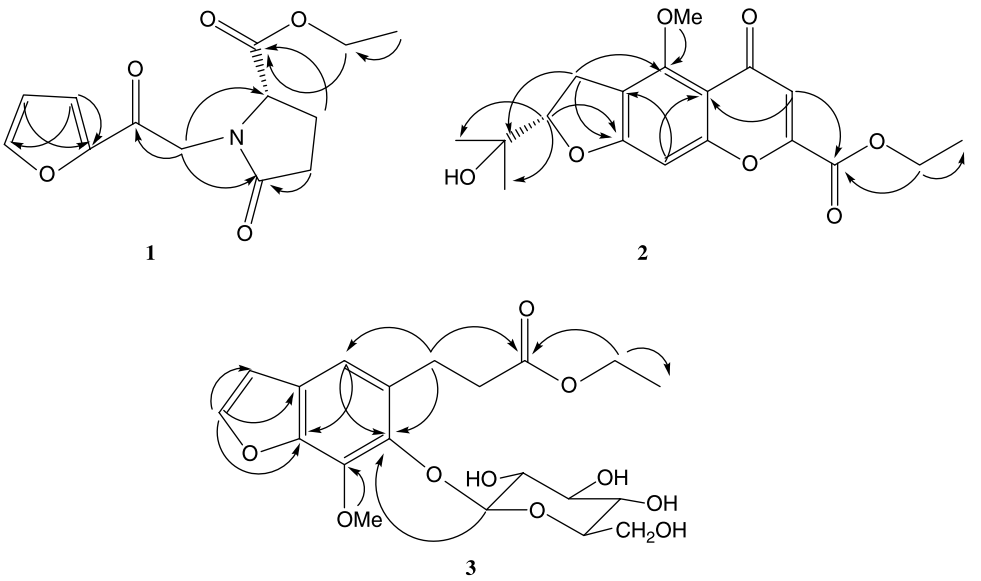

Figure 2. The key HMBC correlations of compounds **1**–**3**.

of the two compounds were both positive [8]. Therefore, C-2′ of compound **2** was in the *S*-configuration. Thus, compound **2** was named as divaricataester B, which is a new compound. Compound **2** and its corresponding free acid are both new compounds. In addition, among the natural linear dihydrofurochromones, it was rare that a carbonyl group was attached to C-2.

Compound **3** was obtained as brown powder. Its molecular formula was determined to be $C_{20}H_{26}O_{10}$ based on the HRESIMS $[M + Na]^+$ ion at *m/z* 449.1421. The UV spectrum of **3** exhibited absorption maxima at 211 and 249 nm, suggesting the presence of an aromatic ring. IR absorption bands at 3414, 2928, 1725, 1623, 1468, and 1075 cm^{-1} could be attributed to hydroxyl, alkyl, carbonyl, aromatic ring and ester groups, respectively. The ^1H NMR spectrum of **3** showed the presence of two furan protons at δ 7.64 (1H, d, $J = 2.4$ Hz) and 6.68 (1H, d, $J = 2.4$ Hz), one aromatic proton at δ 7.03 (1H, s) and methoxy protons at δ 4.07 (3H, s). In addition, the spectrum showed the signals of an ethoxy group at δ 4.03 (2H, q, $J = 7.2$ Hz) and 1.15 (3H, t, $J = 7.2$ Hz), and the signals of an ethylene group at δ 3.03 (2H, m) and 2.65 (2H, m). In the HMBC spectrum, correlations of H-1′/C-4, 6, 3′ and H-4′/C-3′, 5′ revealed the ethylene group linked to the aromatic ring and ethoxycarbonyl group. The correlation between the signals at δ 5.04 (1H, d, $J = 7.2$ Hz, glu-1″) and 143.3 (C-6) suggested that a sugar moiety was located at C-6 position of aglycone. The methoxy group at C-7 position was confirmed by the correlation between the signals at δ 4.07 (3H, s, 7-OCH_3) and 137.8 (C-7). The anomeric proton signal at δ 5.04 (1H, d, $J = 7.2$ Hz, H-1″) in the ^1H NMR spectrum and six sugar carbon signals at δ 104.1 (C-1″), 74.7 (C-2″), 76.8 (C-3″), 70.4 (C-4″), 77.1 (C-5″), and 61.4 (C-6″) determined that the sugar was β-D-glucose, which was also confirmed by acid hydrolysis and subsequent TLC analysis [12]. Compound **3** was a new compound, assigned as divaricataester C. And the benzofuran glycoside compound was reported from the genus of *Saposhnikovia* for the first time. However, its corresponding free acid, cnidioside B, has been isolated from the ripe fruit of *Cnidium monnieri* [7].

The three new ethyl esters compounds that were isolated from the 95% EtOH extract were not artificial products, because their

molecular ions were all detected in the MeOH extract of roots of *S. divaricata* by HPLC/ESIMS^n.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a Perkin-Elmer 341LC digital polarimeter. UV spectra were recorded on a Varian CARY-300 spectrometer (Melbourne, AU). CD spectra were recorded using a JASCO J-810 spectropolarimeter (Tokyo, Japan) in MeOH at 25°C. IR spectra were recorded on a Nicolet NEXUS-470 spectrometer (Madison, WI, USA). ^{1}H NMR and ^{13}C NMR spectra were run on a Varian system-600 spectrometer (Palo Alto, CA, USA). HRESIMS spectra were performed on a Bruker APEX IV FT-MS spectrometer (Billerica, MA, USA). Sephadex LH-20 (GE Healthcare Bio-Sciences Corporation, formerly Amersham Pharmacia Biotechnology Incorporation, NJ, USA) and silica gel (160–200, 60–100 mesh; Branch of Qingdao Haiyang Chemical Plant, Qingdao, China) were used for column chromatography and silica gel GF-254 (Branch of Qingdao Haiyang Chemical Plant, Qingdao, China) for TLC. HPLC separations were conducted on a TSP 100 pump system equipped with a TSP 100 UV detector (Thermo, San Jose, CA, USA), and with an Agilent Zorbax SB-C_{18} column (5 μm, 250 × 9.4 mm). For HPLC/ESIMS^n analysis, a Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) was connected to the Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) *via* an ESI interface, using an Agilent Zorbax Extend-C_{18} column (5 μm, 250 × 4.6 mm), operated at 25°C. Compounds were detected by UV radiation at 254 nm.

3.2 Plant material

The roots of *S. divaricata* were purchased from Tong Ren Tang drugstore of Beijing. A voucher specimen has been deposited at the Herbarium (No. 20060910) of School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China.

3.3 Extraction and isolation

The air-dried and powdered roots of *S. divaricata* (3.5 kg) were extracted with 95% EtOH three times by reflux. The solvent was evaporated *in vacuo*. Then the residue (700 g) was subjected to chromatography over silica gel column (60–100 mesh) and eluted with $CHCl_3$, EtOAc, CH_3COCH_3, and MeOH, successively. The $CHCl_3$ fraction (75 g) was then chromatographied on silica gel column (160–200 mesh) using petroleum ether–acetone as gradient eluent to provide 17 fractions. The fraction 15 (F-1-15 2 g) was subjected to Sephadex LH-20 column (MeOH) to give subfractions 1–5. The EtOAc fraction (70 g) was also subjected to silica gel column chromatography using $CHCl_3$—MeOH as gradient eluent to provide 14 fractions. From fractions 4, 5, and 7 (F-2-4, 5, 7), compounds **6**, **4**, and **5** were obtained, respectively. The fraction 8 (F-2-8 3 g) was subjected to chromatography over Sephadex LH-20 (MeOH) to give subfractions 1–7. The subfraction 2 (F-1-15-2) was subjected to semi-preparative reversed-phase HPLC (flow rate = 2.0 ml min^{-1}) with MeOH—H_2O (55:45) to afford 3.0 mg of **1** (t_R = 9.0 min) and 4.0 mg of **2** (t_R = 20.0 min). The subfraction 3 (F-2-8-3) was subjected to semi-preparative reversed-phase HPLC (flow rate = 2.0 ml min^{-1}) with MeOH—H_2O (52:48) to afford 6.0 mg of **3** (t_R = 20 min).

3.3.1 Divaricataester A (*1*)

Brown powder. $[\alpha]_D^{25}$ −30.48 (*c* 0.11, MeOH). UV (MeOH) λ_{max} (nm) (log ε) 272 (3.89). IR (KBr) ν_{max} (cm^{-1}): 2934, 1737, 1686, 1468, 1257, 1028. The ^{1}H and ^{13}C NMR spectral data (see Table 1). HRESIMS: *m/z* 266.1024 $[M + H]^+$ (calcd for $C_{13}H_{16}NO_5$, 266.1023).

3.3.2 Divaricataester B (*2*)

Brown powder. $[\alpha]_D^{25}$ +32.2 (*c* 0.09, MeOH). UV (MeOH) λ_{max} (nm) (log ε) 314 (3.61), 243 (sh) (3.83), 218 (4.19). IR (KBr) ν_{max} (cm^{-1}): 3407, 2928, 1736, 1651, 1251, 1095. CD (MeOH) $\Delta\varepsilon_{294nm}$ −4.202, $\Delta\varepsilon_{246nm}$ +10.805.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3** (in CD_3OD).

	1			**2**			**3**	
No.	^{1}H	^{13}C	No.	^{1}H	^{13}C	No.	^{1}H	^{13}C
2		178.6	2		152.3	2	7.64 d, $J = 2.4$ Hz	145.5
3	2.45 m (2H)	30.1	3	6.78 s	116.0	3	6.68 d, $J = 2.4$ Hz	106.4
4	2.12 m (2H)	23.8	4		179.1	4	7.03 s	114.6
5	4.37 m	61.9	5		157.1	5		131.1
1′	4.91 d, $J = 18.0$ Hz	49.0	6		119.2	6		143.3
	4.38 d, $J = 18.0$ Hz		7		168.0	7		137.8
2′		184.3	8	6.66 s	94.8	3a		125.9
3′		152.4	4a		113.3	7a		145.6
5′	7.77 d, $J = 1.2$ Hz	149.1	8a		161.0	7-OCH_3	4.07 s (3H)	60.3
6′	6.62 dd, $J = 3.6$, 1.2 Hz	113.6	5-OCH_3	3.90 s (3H)	61.1	1′	3.03 m (2H)	26.4
7′	7.37 d, $J = 3.6$ Hz	119.6	2′	4.73 t, $J = 7.8$ Hz	92.9	2′	2.65 m (2H)	34.9
1″		173.0	3′	3.31 t, $J = 7.8$ Hz	28.8	3′		174.5
2″	4.14 q, $J = 7.2$ Hz (2H)	62.7	4′		72.2	4′	4.03 q, $J = 7.2$ Hz (2H)	60.2
3″	1.19 t, $J = 7.2$ Hz (3H)	14.3	5′	1.18 s (3H)	25.4	5′	1.15 t, $J = 7.2$ Hz (3H)	13.3
			6′	1.25 s (3H)	25.4	Glu		
			1″		161.6	1″	5.04 d, $J = 7.2$ Hz	104.1
			2″	4.37 q, $J = 7.2$ Hz (2H)	64.0	2″	3.42 m	74.7
			3″	1.35 t, $J = 7.2$ Hz (3H)	14.3	3″	3.40 m	76.8
						4″	3.33 m	70.4
						5″	3.16 m	77.1
						6″	3.60 dd, $J = 12.0$, 6.6 Hz, 3.71 dd, $J = 12.0$, 2.4 Hz	61.4

The ^{1}H and ^{13}C NMR spectral data (see Table 1). HRESIMS: *m/z* 349.1283 $[M + H]^+$ (calcd for $C_{18}H_{21}O_7$, 349.1282).

*3.3.3 Divaricataester C (**3**)*

Brown powder. $[\alpha]_D^{25}$ −8.2 (*c* 0.11, MeOH). UV (MeOH) λ_{max} (nm) (log ε) 211 (4.06), 249 (3.63). IR (KBr) ν_{max} (cm^{-1}): 3414, 2928, 1725, 1623, 1468, and 1075. The ^{1}H and ^{13}C NMR spectral data (see Table 1). HRESIMS: *m/z* 449.1421 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_{10}Na$, 449.1418).

*3.3.4 (S)-(+)-Cimifugin (**4**)*

CD (MeOH) $\Delta\varepsilon_{285nm}$ −3.953, $\Delta\varepsilon_{248nm}$ +4.533.

Acknowledgements

We acknowledge the Cultivation Fund of the Key Scientific and Technical Innovation Project (No. 104218) and the Program for Changjiang Scholar and Innovative Team in University (No. 985-2-063-112) from Ministry of Education of China of financial support of this work.

References

[1] G.X. Pei, P.L. Da, and L.Y. Shi, *New Edited Record of TCM*, Vol. 1, (China Chemical Industry Press, Beijing, 2002), p. 465.
[2] J.H. Wang and Z.C. Lou, *Chin. Pharm. J.* **27**, 323 (1992).
[3] D.A. Guo, Z.A. Liu, and Z.C. Lou, *J. Chin. Pharm. Sci.* **1**, 81 (1992).
[4] Y.L. Gao, J. *Shanxi Med. Univ.* **35**, 216 (2004).
[5] K. Baba, Y. Yoneda, M. Kozawa, E. Fujita, N.H. Wang, and C.Q. Yuan, *Shoyakugaku Zasshi* **43**, 216 (1989).
[6] K. Baba, X.Y. Qing, M. Taniguchi, M. Kozawa, and E. Fujita, *Shoyakugaku Zasshi* **45**, 167 (1991).
[7] S. Yahara, C. Sugimura, and T. Nohara, *Shoyakugaku Zasshi* **47**, 74 (1993).
[8] H. Sasaki, H. Taguchi, T. Endo, and I. Yosioka, *Chem. Pharm. Bull.* **30**, 3555 (1982).
[9] K. Baba, K. Hata, Y. Kimura, Y. Matsuyama, and M. Kozawa, *Chem. Pharm. Bull.* **29**, 2565 (1981).
[10] D.Q. Yu and Y.S. Yang, *Handbook of Analytical Chemistry*, Vol. 7, (China Chemical Industry Press, Beijing, 1999), p. 113.
[11] M.D.D. Castillo, M.L. Sanz, M.J.V. Arana, and N. Corzo, *Food Chem.* **79**, 261 (2002).
[12] P.P. Zhao, B.M. Li, and L.Y. He, *Acta Pharmacol. Sin.* **22**, 70 (1987).