HETEROCYCLES, Vol. 65, No. 6, 2005, pp. 1409 - 1414
Received, 22nd December, 2004, Accepted, 11th April, 2005, Published online, 12th April, 2005

TWO NEW CYCLOARTANE TRITERPENE GLYCOSIDES AND A NEW ALKALOID FROM *SOULIEA VAGINATA*

Liang Zhou,[a] **Jun-Shan Yang**,*[a] **Xia Wu**,[a] **Jian-Hua Zou**,[b] **Xu-Dong Xu,**[a] and **Guang-Zhong Tu**[c]

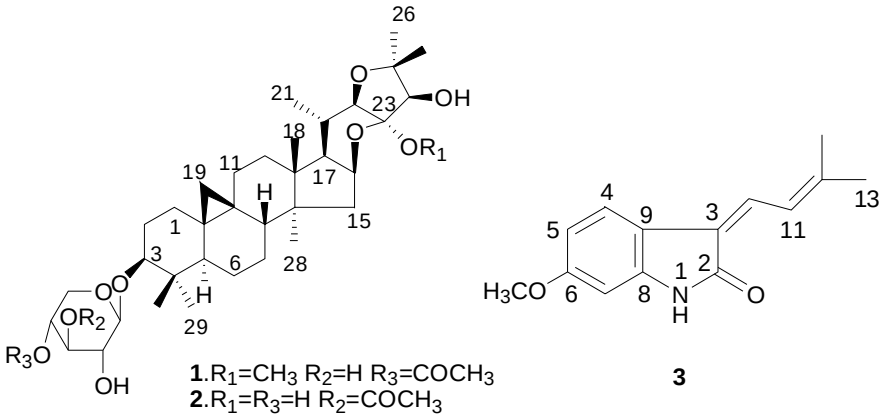
[a]Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, P. R. China
[b]Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China
[c]Beijing Institute of Microchemistry Research, Beijing 100060, P. R. China
Tel:+86-10-62899739,Fax:+86-10-62898425,E-mail:zhouliang_phar@hotmail.com and Junshanyang@hotmail.com

Abstract –Chemical investigation on the rhizome of *Souliea vaginata* resulted in the isolation of two new cycloartane triterpene glycosides and a new alkaloid, soulieosides D and E, and soulieotine (**1**—**3**), and their structures were elucidated on the basis of extensive NMR spectral experiments and chemical methods as (20*S**,22*R**,23*S**,24*R**)-16*β*:23;22:25-diepoxy-3*β*,24-dihydroxy-23-methoxy-9,19-cyclolanostane-3-*O*-*β*-D-(4-acetyl)xylopyranoside (**1**), (20*S**,22*R**,23*S**,24*R**)-16*β*:23;22:25-diepoxy-3*β*,23,24-trihydroxy-9,19-cyclolanostane-3-*O*-*β*-D-(3-acetyl)xylopyranoside (**2**), *E*-3-(3′-methyl-2′-butenylidene)-6-methoxy-2-indolinone (**3**).

INTRODUCTION

Souliea vaginata (Maxim.) Franch. (Ranunculaceae) is widely distributed in the southwest and northwest of the People's Republic of China. As a well-known Chinese folk medicine, it possesses anti-inflammatory analgesic functions.[1] Its rhizomes or the whole plant are used to treat conjunctivitis, stomatitis, pharyngitis, enteritis, and diarrhea.[1] Previous phytochemical investigations have resulted in the isolation of 9,19-cyclolanostane triterpene glycosides from the rhizomes of this plant.[2, 3] Our previous studies on the chemical constituents of the plant has resulted in the isolation of a variety of new cyclolanostane triterpene glycosides.[4] This paper describes the isolation and structural elucidation of the

new saponins, soulieosides D (**1**) and E (**2**), and new alkaloid soulieotine (**3**) which have been isolated from the rhizomes of this plant. The structures of the new compounds were determined by chemical methods and spectroscopic analysis, especially NMR spectroscopy.



1.R_1=CH_3 R_2=H R_3=$COCH_3$
2.R_1=R_3=H R_2=$COCH_3$

3

Figure. 1 Structure of Compounds (**1—3**)

RESULTS AND DISCUSSION

Compound (**1**) was isolated as colorless needles and exhibited a positive Libermann-Burchard reaction. Its molecular formula was determined as $C_{38}H_{60}O_{10}$ from the positive HRFABMS, showing a $[M+Na]^+$ ion peak at *m/z* 699.4086 (calcd 699.4084), corresponding to nine degrees of unsaturation. The IR spectrum showed strong hydroxyl absorption at 3444 cm^{-1} and carbonyl absorption at 1738 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectrum showed the presence of partial structures, 3β-hydroxypropanyl (H-3: δ_H 3.48, C-3: δ_C 88.5; H-2: δ_H 1.92, 2.27, C-2: δ_C 30.0; H-1: δ_H 1.27, 1.56, C-1: δ_C 32.1), a 9,9,10,10-tetra-substituted cyclopropane (H-19: δ_H 0.17, 0.43, C-19: δ_C 30.2; C-9: δ_C 19.7; C-10: δ_C 26.6), six tertiary methyl groups containing two deshielding methyl groups (H-18: δ_H 1.14, C-18: δ_C 20.5; H-26: δ_H 1.51, C-26: δ_C 27.1; H-27: δ_H 1.61, C-27: δ_C 24.5; H-28: δ_H 0.84, C-28: δ_C 19.6; H-29: δ_H 1.32, C-29: δ_C 25.7; H-30: δ_H 1.01, C-30: δ_C 15.4), an acetyl group (δ_H 1.96, δ_C 170.6, 20.9), a methoxyl group (δ_H 3.35, δ_C 49.5), 16β:23;22:25-diepoxy groups (H-16: δ_H 4.40, C-16: δ_C 72.5; H-22: δ_H 3.65, C-22: δ_C 86.3; C-23: δ_C 109.4; C-25: δ_C 83.4), and signals of sugar moiety. On the basis of a comparison of the NMR spectral data with those of cimiaceroside B,[5] **1** could be assigned as a close derivative of cimiaceroside B. The sugar was identified as xylose by acid hydrolysis followed by HPLC analysis with an authentic sample and its configuration was elucidated as β according to the coupling constants of H-1′ (δ 4.86, 1H, d, J = 7.5 Hz). Furthermore, compared with those of cimiaceroside B, a significant difference in the ^{1}H-NMR spectrum of the sugar moiety was that 4′-H appeared at δ 5.40 (1H, ddd, J = 5.0, 9.5, 10.5 Hz) instead of at δ 4.20. In addition, the ^{13}C-NMR signal for C-4′ shifted from δ 71.3 to 73.2, the signals due to C-3′, C-5′ showed upfield shift from δ 78.6, 67.1 to 75.0, 63.2 respectively. These shifts could be explained by acetylation at C-4 of the xylose unit. Methoxy could be assigned at C-23 by the signal due to

C-23 shifting from δ 106.1 to 109.4. These results were also supported by the HMBC correlation between H-4′ and carbonyl group signal at δ 170.6, the methoxy and C-23. In the NOESY spectrum, important correlations were observed between CH_3-18/H-20, H-22/CH_3-21, H-22/CH_3-26, H-24/CH_3-26, CH_3-28/H-16, H-16/OCH_3 and H-3/CH_3-29. Because the chemical shifts of position 16, 20, 22, 23, and 24 were identical with those of cimiaceroside B, compound (**1**) should be determined to have the same configuration at these positions. Therefore, **1** was identified as (20*S**,22*R**,23*S**,24*R**)-16β:23;22:25-diepoxy-3β,24-dihydroxy-23-methoxy-9,19-cyclolanostane-3-*O*-β-D-(4-acetyl)xylopyranoside, and has been named soulieoside D.

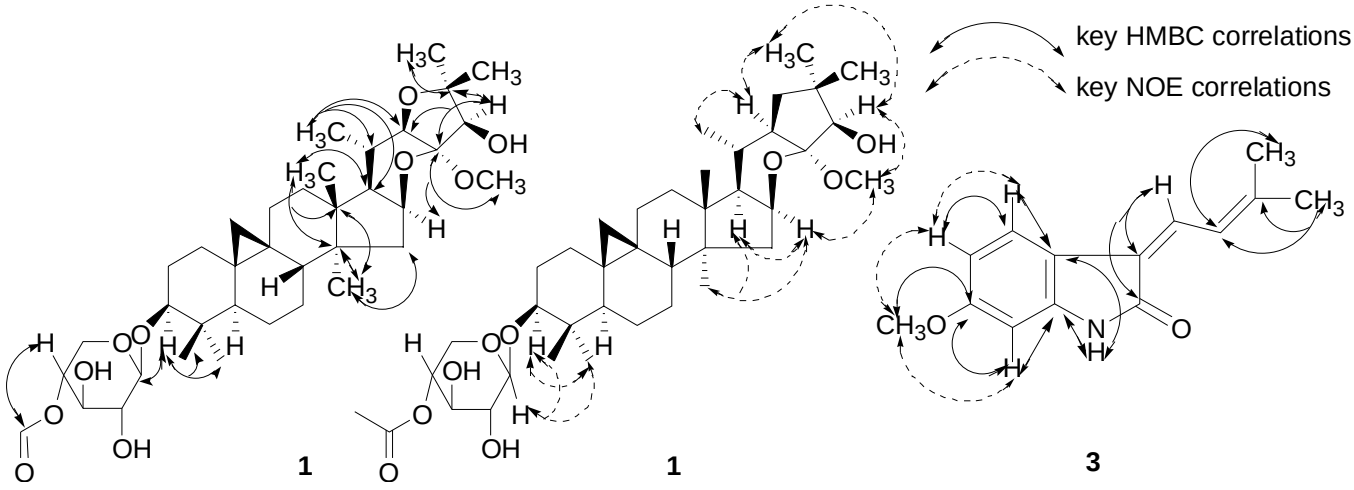


Figure. 2 Key HMBC and NOE Correlations of Compounds (**1**) and (**3**)

Compound (**2**) was isolated as colorless needles and exhibited a positive Libermann-Burchard reaction. Its molecular formula was determined as $C_{37}H_{58}O_{10}$ from the positive HRFABMS, showing a $[M+Na]^+$ ion peak at *m/z* 685.3926 (calcd 685.3928). On the basis of a comparison of the NMR spectral data with those of cimiaceroside B,[5] compound (**2**) could be assigned as a close derivative of cimiaceroside B except for the presence of acetyl group, whose position was determined by acetylation shift and HMBC experiment. Because the chemical shifts of positions 16, 20, 22, 23 and 24 were identical with those of cimiaceroside B,[5] **2** should have the same configurations at these positions. Following the same procedure as that employed for compound (**1**), all the data, including correlations in the HMBC cpectrum, proved compound (**2**) to be (20*S**,22*R**,23*S**,24*R**)-16β:23;22:25-diepoxy-3β,23,24-trihydroxy-9,19-cyclolanostane-3-*O*-β-D-(3-acetyl)xylopyranoside, which has been named soulieoside E.

Compound (**3**) was isolated as red needles and Its molecular formula was determined as $C_{14}H_{15}NO_2$ from the HREIMS, showing a M^+ ion peak at *m/z* 229.1081 (calcd 229.1103). **3** gave no reaction with Dragendorff's test solution. The IR spectrum of **3** suggested the presence of NH and lactam carbonyl groups as well as an aromatic ring. The ^{1}H-NMR spectrum of **3** showed signals which were assigned to $=C(CH_3)_2$ (δ 1.98, 2.03), =CH–CH= (δ 6.82, 7.32, d, J = 12.5 Hz), NH (δ 10.40, s), and OCH_3 (δ 3.80, s). The ^{1}H-NMR spectrum also showed the presence of a 1,2,4-trisubstituted benzene rings. The ^{13}C-NMR

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of **1, 2** in Pyridine-d_5 and **3** in DMSO-d_6

	1		**2**		**3**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.27,1.56	32.1	1.28, 1.55	32.1		
2	1.92, 2.27	30.0	1.92, 2.30	29.9		169.2
3	3.48 dd (4.0, 11.5)	88.5	3.45 dd (4.5,12.0)	88.7		123.8
4		41.3		41.2	6.93 d (7.0)	121.9
5	1.28	47.4	1.30	47.4	7.36 dd (2.5, 7.0)	116.3
6	0.66, 1.55	20.9	0.66, 1.53	20.9		143.6
7	1.05, 1.26	26.2	1.04, 1.28	26.3	6.92 d (2.5)	118.8
8	1.56	47.4	1.54	47.4		122.9
9		19.7		19.8		130.5
10		26.6		26.6	7.32 d (12.5)	130.9
11	1.11, 1.93	26.4	1.10, 1.93	26.3	6.82 d (12.5)	120.8
12	1.54 (2H)	33.4	1.55 (2H)	33.5		151.9
13		46.7		46.9	2.03 s	26.9
14		45.2		45.3	1.98 s	18.7
15	1.58, 1.90	42.7	1.59, 1.89	43.3		
16	4.40	72.5	4.95	72.4		
17	1.56	51.6	1.57	52.4		
18	1.14 s	20.5	1.19 s	20.6		
19	0.17 d (4.0), 0.43 d (4.0)	30.2	0.17 d (4.0), 0.45 d (4.0)	30.1		
20	2.16	34.3	2.24	34.7		
21	1.15 d (6.5)	17.5	1.20 d (6.5)	17.5		
22	3.65 d (10.5)	86.3	3.88 d (11)	86.9		
23		109.4		106.0		
24	4.11 s	76.6	4.17 s	83.3		
25		83.4		83.6		
26	1.51 s	27.1	1.74 s	27.7		
27	1.61 s	24.5	1.61 s	24.8		
28	0.84 s	19.6	0.83 s	19.6		
29	1.32 s	25.7	1.26 s	25.7		
30	1.01 s	15.4	0.98 s	15.3		
1′	4.86 d (7.5)	107.3	4.83 d (7.5)	107.1		
2′	4.04 t (8.0)	75.7	4.04 t (8.0)	73.1		
3′	4.26 t (8.0)	75.0	5.71 t (8.0)	79.3		
4′	5.40 ddd (5.0, 9.5, 10.5)	73.2	4.22 m	69.2		
5′	3.59 t (10.5), 4.33 dd (5.5, 10.5)	63.2	3.71t (11.0), 4.32 dd (5.0, 11.5)	66.7		
3′-$COCH_3$			1.97	170.8, 21.1		
4′-$COCH_3$	1.96 s	170.6, 20.9				
OCH_3	3.35 s	49.5			3.80 s	55.7

spectrum of **3** suggested carbonyl carbon of lactam at δ 169.2, four olefinic carbons at δ 123.8, 130.9,120.8, 151.9, a methoxy carbon at δ 55.7, and a group of carbon due to benzene ring. Compoud (**3**)

was assigned to be a close derivative of *E*-3-(3′-methyl-2′-butenylidene)-2-indolinone by a comparison of the NMR spectral data with those of the latter.[6,7] Methoxy group was located at C-6 by HMBC and NOE experiment. Therefore, **3** was confirmed as *E*-3-(3′-methyl-2′-butenylidene)-6-methoxy-2-indolinone.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. NMR spectra were measured in pyridine-d_5 on a Bruker AM-500 spectrometer, using TMS as internal standard. NMR experiments included the ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY pulse sequences. Coupling constants (*J* values) are given in Hz. An Autospec-Ultima ETOF spectrometer was used to record the FABMS and HRFABMS. Silica gel 60H (400–500 mesh) and silica gel GF_{254} sheets (0.20–0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material. The whole plant of *Souliea vaginata* was collected at Qing Mountain, Ganshu Province, People's Republic of China, in August 2002, and identified by Dr. Si-bao Chen, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (XC-03-0824) is deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. The air-dried and pulverized rhizomes of *Souliea vaginata* (5.0 kg) was extracted two times with 95% EtOH (5000 mL) for 2 h under reflux, and then extracted two times with 50% EtOH (5000 mL) for 2 h under reflux. After combination and removal of solvent, the residue (1.2 kg) was suspended in water (3000 mL) and partitioned successively with petroleum ether (3000 mL), $CHCl_3$ (3000 mL), and *n*-BuOH (3000 mL). The $CHCl_3$-soluble fraction (400 g) was subjected to low-pressure column chromatography (LPLC) on silica gel 60H (400-500 mesh). Gradient elution with $CHCl_3$-MeOH (10:0-7:3), gave four fractions, A (30 g), B (21 g), C (37 g), and D (60 g).

Fraction A was isolated by repeated LPLC over silica gel 60H, eluting with petroleum ether-EtOAc-MeOH (9:1:0–8:2:0–7:2:0.5–4:5:1) and $CHCl_3$-MeOH (10:0–9:1) to afford Frs. 1~8. Frs. 3 (3 g) was treated by repeated LPLC over silica gel 60H, eluting with $CHCl_3$-MeOH (99:1–95:5), to give a1~a8. Fr. a3 (70 mg) was treated by repeated LPLC over silica gel 60H, eluting with petroleum ether-EtOAc-MeOH (70:30:3), to afford **1** (5 mg). Fr. a6 (300 mg) was purified by repeated LPLC over silica gel 60H, eluting with petroleum ether-EtOAc-MeOH (70:30:5), to afford **2** (52 mg). Fr. a1 (700 mg) was treated by Sephadex LH-20, eluting with $CHCl_3$-MeOH (1:1), to afford **3** (70 mg).

Soulieoside D (1): colorless needles, mp 176-177 °C (MeOH), $[\alpha]_D^{20}$ –8.6° (*c* 0.07, $CHCl_3$-CH_3OH, 1:1);

IR (KBr) ν_{max} cm^{-1}: 3444 , 1738; ^1H NMR and ^{13}C NMR, see Table 1; positive-ion FABMS *m/z* 677 $[M+H]^+$, 645, 487, 471, 453, 433; positive-ion HRFABMS *m/z* 699.4086 $[M+Na]^+$ (calcd 699.4084).

Soulieoside E (2): colorless needles, mp 240-242 °C (MeOH), $[\alpha]_D^{20}$ –6.7° (*c* 0.03, $CHCl_3$-CH_3OH, 1:1); IR (KBr) ν_{max} cm^{-1}: 3421 , 1728, 1456, 1379, 1248, 1041; ^1H NMR and ^{13}C NMR, see Table 1; positive-ion FABMS *m/z* 663 $[M+H]^+$, 645, 471, 453; positive-ion HRFABMS *m/z* 685.3926 $[M+Na]^+$ (calcd 685.3928).

Soulieotine (3): red needles, mp 201-203 °C (MeOH), $[\alpha]_D^{20}$ +1.4° (*c* 0.07, $CHCl_3$-CH_3OH, 1:1); IR (KBr) ν_{max} cm^{-1}: 3100 , 1712, 1620, 1600; UV $\lambda_{max}^{CH_3OH}$ (log ε): 210 (4.40), 262 (3.94), 282 (3.09) nm; ^1H NMR and ^{13}C NMR, see Table 1; EIMS *m/z* (%) 229 (53), 228 (30), 214 (82), 199 (22), 44 (100); HREIMS *m/z* 229.1081 M^+(calcd 229.1103).

Acid Hydrolysis of 1 and 2. Compounds (**1**) and (**2**) (each 2 mg) were refluxed with 10% HCl in 75% EtOH (3 mL) for 6 h. Each reaction mixture was diluted with H_2O, and neutralized with Ag_2CO_3. The neutral hydrolysate revealed the presence of xylose by HPLC [solvent, CH_3OH–H_2O (2:8); column, Nova pack C_{18} (30cm×3.9mm, 4μm); flow rate, 0.8mL/min; detector, ELSD], when compared with authentic samples.

ACKNOWLEDGEMENTS

The authors thank the *National Natural Science Foundation of People Republic of China* for financial support (No. 20432030).

REFERENCES

1. Institute of Botany, Chinese Academy of Sciences & Institute of Materia Medica, Chinese Academy of Medical Sciences, 'Flora Republicae Popularis Sinicae', Science Press, Beijing, 1979, Vol. 27, p. 91.
2. I. Takao, S. Nobuko, N. Masahiro, and P. G. Xiao, *Phytochemistry*, 1985, **24**, 1329.
3. N. Sakurai, T. Goto, M. Nagai, T. Inoue, and P. G. Xiao, *Heterocycles*, 1990, **30**, 897.
4. L. Zhou, J. S. Yang, J. H. Zou, and G. Z. Tu, *Chem. Pharm. Bull.*, 2004, **52**, 622.
5. A. Kusano, M. Takahira, M. Shibano, T. Miyase, T. Okuyama, and G. Kusano, *Heterocycles*, 1998, **48**, 1003.
6. K. Baba, M. Kozawa, K. Hata, T. Ishida, and M. Inoue, *Chem. Pharm. Bull.*, 1981, **29**, 2182.
7. J. X. Li, S. Katota, M. Hattori, S. Yoshimachi, M. Shiro, N. Oogami, H. Mizuno, and T. Namba, *Chem. Pharm. Bull.*, 1993, **41**, 832.