Three New Cycloartane Triterpene Glycosides from *Souliea vaginata*

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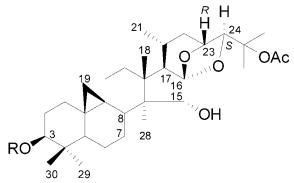
Three new cycloartane triterpene glycosides, soulieosides A (1), B (2), and C (3), were isolated from the rhizomes of *Souliea vaginata*, and their structures were elucidated on the basis of extensive NMR experiments and chemical methods. Soulieosides A—C were assigned as 25-*O*-acetylcimigenol-3-*O*-β-D-(2-acetyl)xylopyranoside (1), 24-*O*-acetyl-isodahurinol-3-*O*-β-D-(2-acetyl)xylopyranoside (2) and 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-3β,23,24-trihydroxy-9,19-cyclolanostane-3-*O*-β-D-(4-acetyl)xylopyranoside (3), respectively.

Key words *Souliea vaginata*; cycloartane; triterpene glycoside

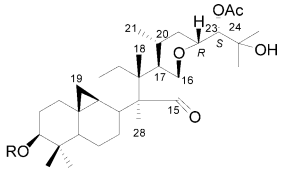
Souliea vaginata (MAXIM.) FRANCH. (Ranunculaceae) is widely distributed in the southwest and northwest of the People's Republic of China. As a well-known Chinese folk medicine, it possesses anti-inflammatory analgesic functions. Its rhizomes or the whole plant are used to treat conjunctivitis, stomatitis, pharyngitis, enteritis, and diarrhea.[1)] Previous phytochemical investigations have resulted in the isolation of 9,19-cyclolanostane triterpene glycosides from this plant.[2,3)] In the present work, three new cycloartane triterpene glycosides were isolated from the ethanol extract of the rhizomes of this plant, soulieosides A (**1**), B (**2**), and C (**3**), together with the known xylosides, 25-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside (**4**), 24-*O*-acetylisodahurinol-3-*O*-β-D-xylopyranoside (**5**), cimiaceroside B (**6**). This paper deals with the isolation and structural elucidation of the compounds **1**—**3**.

Results and Discussion

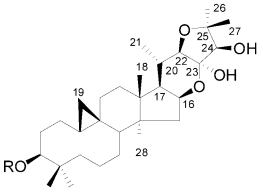
Compound **1** was obtained as white amorphous powder and exhibited a positive Libermann–Burchard reaction. Its IR spectrum showed absorbtions of a broad hydroxyl band (3469 cm^{-1}) and a carbonyl group (1738 cm^{-1}). The positive-ion FAB-MS and HR-FAB-MS showed a ion peaks at *m*/*z* 705 $[M+H]^+$ and *m*/*z* 727.4004 $[M+Na]^+$ (Calcd 727.4033), indicating a molecular formula of $C_{39}H_{60}O_{11}$. The ^{1}H-NMR spectrum (Table 1) showed the presence of cyclopropane methylene groups at δ 0.23 and 0.46 (1H each, d, J=4.0 Hz), two acetyl methyl groups at δ 1.95 and 2.14, a secondary methyl group and six tertiary methyl groups at δ 0.84—1.67. The ^{13}C-NMR spectrum of **1** (Table 1) showed methylene carbon of cyclopropane ring at δ 30.8 (C-19), methine carbons at δ 88.7 (C-3), 86.7 (C-24), 80.2 (C-15), and 71.7 (C-23), two quaternary carbons at δ 112.4 (C-16) and 83.1 (C-25), and two carbonyl groups at δ 170.2 and 170.0. The spectral data of **1** showed a very close similarity to those of **4**[6)] and also suggested the configurations at C-23, C-24, and C-15 are the same in those two compounds. The sugar was identified as D-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 4.82, 1H, d, J=8.0 Hz). Furthermore, compared with those of **4**, a significant difference in the ^{1}H-NMR spectrum of the sugar moiety was methine proton that appeared at δ 5.56 instead of at δ 4.02. In addition, the ^{13}C-NMR signal due to C-1′ showed an upfield shift from δ 107.5 to 104.7, the signal for C-2′ shifted from δ 75.5 to 75.6, and the signal due to C-3′ exhibited an upfield shift from δ 78.6 to 76.3. These shifts could be explained by acetylation at C-2 of the xylose unit. This result was also supported by the HMBC correlation between the H-2′ signal and the carbonyl group signal at δ 170.0. Therefore, the structure of **1** was assigned as 25-*O*-acetylcimigenol-3-*O*-β-D-(2-acetyl)xylopyranoside, and has been named soulieoside A.



1.R=2'-O-acetylxylose(p)
4.R=xylose(p)



2.R=2'-O-acetylxylose(p)
5.R=xylose(p)



3.R=4'-O-acetylxylose(p)
6.R=xylose(p)

Compound **2** was isolated as a white amorphous powder and exhibited a positive Libermann–Burchard reaction. In the IR spectrum of **2**, absorption bands for hydroxy (3479 cm^{-1}) and carbonyl groups (1743 cm^{-1}) were observed, and a molecular formula of $C_{39}H_{60}O_{11}$ was deduced from the positive HR-FAB-MS, which showed a molecular peak at *m*/*z* 727.4059 $[M+Na]^+$ (Calcd 727.4033). On the basis of a comparison of the NMR spectral data with those of **5**,[7)] **2** could be assigned as a close derivative of **5**. The sugar was identified with the same method as compound **1**. The C-24 configuration of **2** was elucidated as *S* by comparison of the coupling constants of H-24 (δ 5.29, 1H, d, J=2.5 Hz) with those of dahurinyl diacetate (J=9 Hz) and isodahurinyl diacetate (J=2 Hz).[4)] Acetylation at C-2′ was identified with the same procedure as that employed for compound **1**. This result was also supported by the HMBC correlation between the H-2′ signal and the carbonyl group signal at δ 170.0. In the HMBC spectrum, significant correlations were observed between C-15 and H-16, C-15 and the 28-methyl group; between C-23 and H-16; and between the acetyl carbonyl group

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

Position	**1**[a)]		**2**[a)]		**3**[b)]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.20 m, 1.51 m	32.2	1.14 m, 1.53 m	32.3	1.26 m, 1.57 m	32.1
2	1.87 m, 2.26 m	30.0	1.83 m, 2.25 m	30.0	1.90 m, 2.28 m	30.0
3	3.38 dd (4.0, 11.5)	88.7	3.36 dd (4.5, 12.0)	88.6	3.46 dd (4.5, 12.0)	88.4
4		41.0		41.0		41.3
5	1.30 m	47.4	1.35 m	47.3	1.29 m	47.5
6	0.70 q (11.5), 1.51 m	21.1	0.60 q (11.0), 1.47 m	20.8	0.70 q (12.5), 1.53 m	20.9
7	1.13 m, 2.04 m	26.3	1.04 m, 1.66 m	25.9	1.04 m, 1.29 m	26.2
8	1.64 m	48.7	1.25 m	43.6	1.58 m	47.5
9		20.1		20.1		20.0
10		26.4		27.0		26.5
11	1.13 m, 2.04 m	26.5	1.02 m, 2.23 m	26.1	1.06 m, 1.89 m	26.3
12	1.53 m, 1.62 m	34.0	1.48 m, 1.69 m	31.1	1.54 m	33.4
13		41.8		40.0		46.8
14		47.2		55.1		45.2
15	4.27 s	80.2		213.8	1.61 m, 1.91 m	43.3
16		112.4	3.78 d (11.6)	84.3	4.96 br s	72.4
17	1.44 d (11.0)	59.4	1.55 m	52.4	1.58 m	52.3
18	1.08 s	19.5	1.07 s	20.3	1.20 s	20.6
19	0.23 d (4.0), 0.46 d (4.0)	30.8	0.21 d (3.5), 0.43 d (3.5)	31.4	0.18 d (4.0), 0.45 d (4.0)	30.1
20	1.57 m	23.9	1.80 m	33.3	2.28 m	34.7
21	0.84 d (6.5)	19.5	0.90 d (6.0)	21.0	1.22 d (6.5)	17.5
22	0.94 m, 2.26 m	37.9	1.48 m, 1.73 m	38.8	3.89 d (10.5)	86.9
23	4.58 d (9.0)	71.7	4.24 m	79.1		106.0
24	4.11 s	86.7	5.29 d (2.5)	79.8	4.17 s	83.3
25		83.1		72.1		83.6
26	1.65 s	22.3	1.59 s	26.8	1.76 s	27.7
27	1.67 s	23.4	1.59 s	28.4	1.68 s	24.8
28	1.13 s	11.8	0.92 s	17.6	0.84 s	19.6
29	1.20 s	25.4	1.15 s	25.4	1.32 s	25.7
30	0.97 s	15.2	1.00 s	15.2	1.01 s	15.3
1′	4.82 d (8.0)	104.7	4.80 d (8.0)	104.7	4.86 d (7.5)	107.3
2′	5.56 t (8.0)	75.6	5.53 t (8.0)	75.7	4.05 t (8.0)	75.7
3′	4.16 t (8.0)	76.3	4.16 t (8.0)	76.3	4.26 t (8.0)	75.0
4′	4.20 m	71.4	4.22 m	71.4	5.41 ddd (5.5, 8.5, 11.0)	73.2
5′	3.68 t (10.5), 4.31 dd (4.5, 10.5)	67.1	3.68 t (11.5), 4.29 dd (5.0, 11.5)	67.2	3.60 t (10.5), 4.33 dd (5.5, 11.0)	63.2
25-$COCH_3$	1.95 s	170.2, 21.5				
24-$COCH_3$			2.13	171.0, 20.0		
2′-$COCH_3$	2.14 s	170.0, 21.3	2.14	170.0, 21.3		
4′-$COCH_3$					1.95 s	170.6, 20.9

a) Singnals were assigned by HMQC, HMBC and ^{1}H–^{1}H COSY. *b*) Singnals were assigned by HMQC, HMBC, NOESY and ^{1}H–^{1}H COSY.

and H-24. On the basis of these data, the structure of **2** was elucidated as 24-*O*-acetyl-isodahurinol-3-*O*-β-D-(2-acetyl)-xylopyranoside, and has been named soulieoside B.

Compound **3** 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 HR-FAB-MS, showing a $[M+Na]^+$ ion peak at m/z 685.3874 (Calcd 685.3928). The IR spectrum showed strong hydroxyl bands absorption at 3444 cm^{-1} and carbonyl band absorption at 1747 cm^{-1}. The sugar was identified with the same method as compound **1**. The spectral data of **3** showed a very close similarity to those of **6**.[5)] Acetylation at C-4′ was identified by C-4′ acetylation shift with the same procedure as that employed for compound **1**. This result was also supported by the HMBC correlation between the H-4′ signal and the carbonyl group signal at δ 170.6. The relative stereochemistry of **3** was determined on the basis of NOESY experiments, the coupling constants of the protons, and comparison of the ^{1}H- and ^{13}C-NMR data with those of **6**. 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, and H-3/CH_3-29. Because the chemical shifts of position 16, 20, 22, 23, and 24 were identical with those of **6**, compound **3** should be determined to have the same configuration at these positions. Therefore, **3** was identified as 20(*S*),22(*R*),23(*S*),24(*R*)-16β:23;22:25-diepoxy-3β,23,24-trihydroxy-9,19-cyclolanostane-3-*O*-β-D-(4-acetyl)xylopyranoside, and has been named soulieoside C.

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 spectrometer. 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 FAB-MS and HR-FAB-MS. 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 Devel-

opment, 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 whole plant of *Souliea vaginata* (5.0 kg) was extracted two times with 95% EtOH for 2 h under reflux, and then extracted two times with 50% EtOH 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 Fr. 1—8. Fraction 6 (8 g) was isolated by repeated LPLC over Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (4 : 5 : 0–4 : 5 : 1), to give a1—a8. Fraction a1 (220 mg) was isolated by repeated LPLC over Silica gel 60H, eluting with $CHCl_3$–MeOH (97 : 3), to afford **1** (60 mg) and **4** (100 mg). Fraction a4 (800 mg) was purified by repeated LPLC over Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (70 : 30 : 5), to afford **3** (260 mg) and **6** (10 mg). Fraction a8 (1 g) was purified by repeated LPLC over Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (65 : 35 : 7), to afford **2** (20 mg) and **5** (350 mg).

Soulieoside A (**1**): White amorphous powder, mp 150—152 °C (MeOH), $[\alpha]_D^{20}$ +22.0° (*c*=0.05, $CHCl_3$–CH_3OH, 1 : 1); IR (KBr) ν_{max} cm^{-1}: 3469, 1738; ^{1}H- and ^{13}C-NMR data, see Table 1; positive-ion FAB-MS *m*/*z* 705 $[M+H]^+$, 513, 469, 453, 435, 175, 157 (100); positive-ion HR-FAB-MS *m*/*z* 727.4004 $[M+Na]^+$ (Calcd 727.4033).

Soulieoside B (**2**): White amorphous powder, mp 157—160 °C (MeOH), $[\alpha]_D^{20}$ +14.0° (*c*=0.05, $CHCl_3$–CH_3OH, 1 : 1); IR (KBr) ν_{max} cm^{-1}: 3479, 1743; ^{1}H- and ^{13}C-NMR data, see Table 1; positive-ion FAB-MS *m*/*z* 705 $[M+H]^+$, 687, 513, 175, 157 (100); positive-ion HR-FAB-MS *m*/*z* 727.4059 $[M+Na]^+$ (Calcd 727.4033).

Soulieoside C (**3**): Colorless needles, mp 237—239 °C (MeOH), $[\alpha]_D^{20}$ −8.6° (*c*=0.07, $CHCl_3$–CH_3OH, 1 : 1); IR (KBr) ν_{max} cm^{-1}: 3444, 1747; ^{1}H- and ^{13}C-NMR data, see Table 1; positive-ion FAB-MS *m*/*z* 663 $[M+H]^+$, 645, 471, 453, 185 (100), 175; positive-ion HR-FAB-MS *m*/*z* 685.3874 $[M+Na]^+$ (Calcd 685.3928).

25-*O*-Acetylcimigenol-3-*O*-β-D-xylopyranoside (**4**): White amorphous powder, mp 223—224 °C (MeOH); IR, ^{1}H- and ^{13}C-NMR data consistent with literature values[6]; positive-ion FAB-MS *m*/*z* 663 $[M+H]^+$.

24-*O*-Acetylisodahurinol-3-*O*-β-D-xylopyranoside (**5**): Colorless needles, mp 220—222 °C (MeOH); IR, ^{1}H- and ^{13}C-NMR data consistent with literature values[7]; positive-ion FAB-MS *m*/*z* 663 $[M+H]^+$.

Cimiaceroside B (**6**): White amorphous powder, mp 239—241 °C (MeOH); IR, ^{1}H- and ^{13}C-NMR data consistent with literature values[5]; positive-ion FAB-MS *m*/*z* 602 $[M+H-H_2O]^+$.

Acid Hydrolysis of 1—3 Compounds **1**—**3** (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 D-xylose by HPLC [solvent, CH_3OH–H_2O (2 : 8); column, Nova pack C_{18} (30 cm×3.9 mm, 4 μm); flow rate, 0.8 ml/min; detector, ELSD], when compared with authentic samples.

Alkaline Treatment of 1—5 Compounds **1**—**5** (each 12 mg) were treated with 2.5% KOH–MeOH solution (10 ml) at 80 °C for 3 h, respectively. After neutralization with 0.4 N HCl, 20 ml water was added to the mixture, and the whole were extracted with aqueous saturated *n*-BuOH, respectively. Removal of the solvent in vacuum yielded a product, which was purified by LPLC [Silica gel 60H, petroleum ether–EtOAc–MeOH (65 : 35 : 7)], to get **1a** (5 mg), **2a** (6.5 mg), **3a** (4.2 mg), **4a** (5 mg), **5a** (5.5 mg). Compounds **1a** and **4a**, **2a** and **5a**, **3a** and **6** were showed to be identical, respectively, by mixed mp. Determination and IR (KBr) and by TLC [$CHCl_3$–MeOH (92 : 8), petroleum ether–EtOAc–MeOH (65 : 35 : 10), benzene–EtOH (88 : 12)].

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