Three Novel Sesquiterpene Glycosides of *Sarcandra glabra*

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Three new sesquiterpene glycosides, 8β,9β-epoxy-4α-hydroxy-5αH-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (1, sarcaglaboside F), 4α-hydroxy-5α,8βH-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (2, sarcaglaboside G), 4α-hydroxy-5α,8βH-eudesman-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (3, sarcaglaboside H), together with five known compounds, chloranoside A (4), sarcaglaboside C (5), dihydrovomifoliol-*O*-β-D-glucopyranoside (6), 9-hydroxy-heterogorgiolide (7) and chloranthalactone B (8), were isolated from *Sarcandra glabra* Thumb. Nakai. The structures and relative configurations of three new compounds were determined on the basis of their spectroscopic data and chemical evidence.

Key words *Sarcandra glabra*; sesquiterpene; sarcaglaboside

Sarcandra glabra Thumb. Nakai (*Chloranthus glaber* Thunb. Makino), distributed mainly in the south of Asia, was widely used as an antibacterial and antitumor herb in China.[1] Previous phytochemical studies indicated that sesquiterpenes were characteristic constituents for *S. glabra*.[2—4] Our interest in identification of sesquiterpene constituents prompted us to conduct a detailed chemical investigation into the *S. glabra*. In our research, two new lindenane sesquiterpene glycosides **1**, **2** and a new eudesmanolide sesquiterpene glycoside **3**, together with five known sesquiterpenes and sesquiterpene glycosides (**4**—**8**), have been isolated and structurally characterized.

The 95% EtOH extract of the whole plant of *S. glabra* afforded three new compounds named sarcaglabosides F—H. Their structures were established by means of spectroscopic and chemical methods as 8β,9β-epoxy-4α-hydroxy-5αH-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (**1**), 4α-hydroxy-5α,8βH-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (**2**) and 4α-hydroxy-5α,8βH-eudesman-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside (**3**), respectively. The five known compounds were subsequently identified as chloranoside A (**4**),[4] sarcaglaboside C (**5**),[5] dihydrovomifoliol-*O*-β-D-glucopyranoside (**6**),[6] 9-hydroxy-heterogorgiolide (**7**)[7] and chloranthalactone B (**8**)[2] by comparison of their spectral data (UV, IR, NMR and MS) with those reported previously. Compounds **6** and **7** were isolated from *S. glabra* for the first time.

Compound **1** was obtained as a white amorphous powder. HR-ESI-MS and ESI-MS of **1** gave a molecular ion peak at *m*/*z* 463.1575 $[M+Na]^+$ and a quasimolecular ion peak at *m*/*z* 463 $[M+Na]^+$ in the positive-ion mode, respectively. In conjunction with the analysis of ^{1}H- and ^{13}C-NMR spectra, the molecular formula of **1** was deduced as $C_{21}H_{28}O_{10}$. UV (λ_{max} at 229 nm) spectrum and absorption bands at 3407,

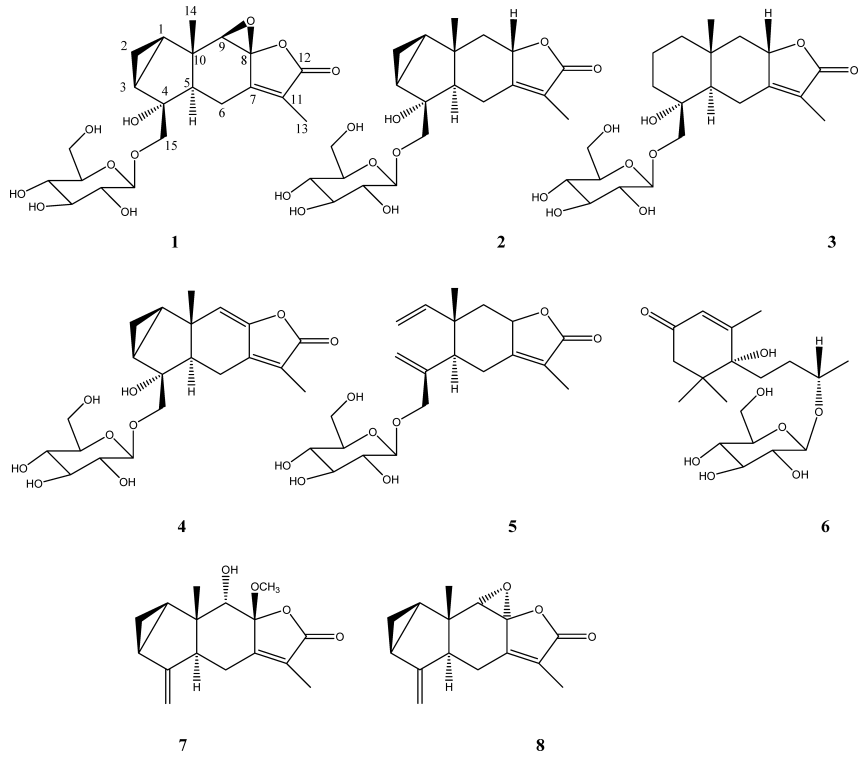

Fig. 1. Structures of Compounds **1**—**8**

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Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data for Compounds **1**, **2** and **3** (δ in ppm, J in Hz, in CD_3OD)[a]

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	1.47, m	25.0	1.39, dd (3.5, 4.5)	28.3	1.19, m	41.5
1β					1.56, m	
2α	0.71, m	10.9	0.68, m	12.2	1.56, m	20.3
2β	0.90, m		0.98, m		1.64, m	
3α	1.59, m	30.7	1.49, m	31.0	1.18, m	38.3
3β					2.15, m	
4		80.2		80.0		74.3
5	3.47, dd (7.0, 13.0)	58.7	2.09, dd (2.5, 14.0)	69.9	1.37, dd (3.5, 14.0)	55.5
6α	2.74, m	24.1	2.30, t (14.0)	26.0	2.42, t (14.0)	24.0
6β	2.74, m		2.84, dd (2.5, 14.0)		3.17, dd (3.5, 14.0)	
7		163.4		166.5		166.4
8		92.9	5.09, t (7.5)	82.0	4.90, t (6.0)	79.9
9α	3.12, s	50.9	1.42, m	49.0[b]	1.00, dd (6.0, 10.5)	52.2
9β			2.50, dd (7.5, 11.5)		2.19, m	
10		43.5		40.8		36.7
11		125.7		121.7		119.9
12		173.9		177.6		177.4
13	1.72, 3H, s	8.6	1.78, 3H, s	8.7	1.79, 3H, s	8.0
14	0.75, 3H, s	21.3	1.02, 3H, s	19.0	1.12, 3H, s	19.4
15α	3.65, d (10.5)	73.5	3.62, d (10.5)	73.6	3.69, d (13.5)	73.0
15β	4.02, d (10.5)		3.92, d (10.5)		4.07, d (13.5)	
1′	4.32, d (8.0)	104.7	4.30, d (7.5)	105.0	4.25, d (8.0)	105.1
2′	3.24, m	75.4	3.21, m	75.8	3.22, m	75.2
3′	3.36,[b] m	78.2	3.27, m	78.6	3.36,[b] m	77.9
4′	3.29,[b] m	71.9	3.26, m	72.2	3.27, m	71.7
5′	3.38, t (8.5)	77.9	3.37, t (9.0)	78.4	3.28, m	78.1
6′	3.90, dd (2.0, 12.0)	63.0	3.66, dd (1.5, 11.5)	63.3	3.66, m	62.8
	3.64, dd (2.0, 12.0)		3.87, dd (1.5, 11.5)		3.87, dd (1.5, 13.5)	

a) All spectra were recorded at 500 MHz for ^{1}H and at 125 MHz for ^{13}C. *b*) Overlapped with other signal.

1747 and 1662 cm^{-1} in its IR spectrum exhibited the presence of hydroxyl and α,β-unsaturated butyrolactone ring groups, respectively. It could be determined by the following reason that **1** was a sesquiterpene glycoside. The ^{1}H-NMR spectrum (Table 1) of **1** displayed one signal for an anomeric proton (δ 4.32, d, J=8.0 Hz). The ^{13}C-NMR spectrum (Table 1) contained 21 signals, of which five oxygenated carbon signals resonated in the region 60—80 ppm and a methine carbon signal was at δ 104.7. The sugar moiety was confirmed as β-D-glucose ($[\alpha]_D^{20}$ +46.3°, c=0.019, H_2O) by acid hydrolysis and comparison with an authentic D-glucose, and from the coupling constant (J=8.0 Hz) of the anomeric proton. The ^{1}H- and ^{13}C-NMR spectroscopic data of **1** resembled those of chloranoside A,[4] which showed that **1** was a β-D-glycoside of lindenane sesquiterpene. In **1** an epoxy bridge was deduced to be at C-8 and C-9, which was supported by the chemical shifts of C-8 and C-9 correspondent with those of chloranthalactone F.[1] The ^{1}H- and ^{13}C-NMR data of **1** was completely assigned with the help of heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum correlation (HMQC), and presented in Table 1. The key HMBC correlations of **1** were showed in Fig. 2. The relative configuration was suggested by correlations observed in the nuclear Overhauser effect spectroscopy (NOESY) spectrum. H-1 gave NOESY correlations to H-9 and H-3, which indicated that the epoxy bridge was located on the β-oriented. The epoxy bridge and methylene groups of C-2 and C-15 would then be β-oriented, for H_β-2 and H_β-15 gave NOESY correlations to H_3-14 (Fig. 3). Therefore, **1** was deduced to possess a *trans*-A/B ring junction. The cyclopropane ring, the epoxy bridge, C-14 methyl and C-15 methylene groups were on the β-oriented. Thus, the structure of **1** was elucidated as 8β,9β-epoxy-4α-hydroxy-5αH-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside and named sarcaglaboside F.

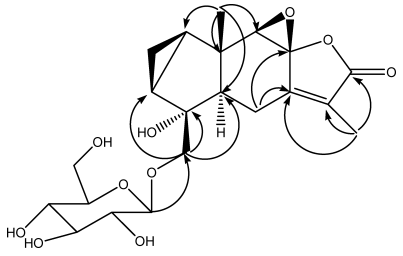

Fig. 2. Key HMBC Correlations of **1**

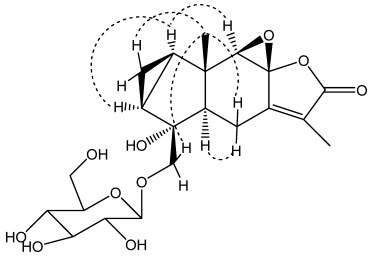

Fig. 3. Key NOESY Correlations of **1**

Compound **2** was obtained as a white amorphous powder that determined as $C_{21}H_{30}O_9$ by HR-ESI-MS (*m/z* 449.1772, $[M+Na]^+$). The presence of hydroxyl and α,β-unsaturated

butyrolactone ring groups was suggested by its IR and UV spectra. The ^{1}H- and ^{13}C-NMR spectroscopic data (Table 1) of **2** was similar to **1**, which suggested **2** was a lindenane sesquiterpene glycoside. However, in **2** there were one methylene (δ_H 1.42, δ 2.50 and δ_C 49.0) and one oxgented methine (δ_H 5.09 and δ_C 82.0) groups instead of the epoxy bridge group (δ_H 3.12, δ_C 92.9 and δ 50.9) in **1**. Accordingly, there was no epoxy bridge group in **2**. The sugar moiety was confirmed as β-D-glucose ($[\alpha]_D^{20}$ +46.6°, c=0.021, H_2O) by acid hydrolysis and comparison with an authentic D-glucose, and from the coupling constant of the anomeric proton (δ 4.30, d, J=7.5 Hz). The ^{1}H- and ^{13}C-NMR data of **2** was completely assigned with the help of HMBC and HMQC correlations and presented in Table 1. The relative configuration of **2** was determined by the NOESY correlations between H-5/H-1 and H_α-6; H-8/H_3-14; H_3-14/H_β-2, 6 and 15. Consequently, **2** was deduced to possess a *trans*-A/B ring junction. The cyclopropane ring, H-8, C-14 methyl and C-15 methylene groups were β-oriented. The structure of **2** was elucidated as 4α-hydroxy-5α,8β*H*-lindan-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside and named sarcaglaboside G.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{21}H_{32}O_9$ by HR-ESI-MS (*m*/*z* 451.1939 $[M+Na]^+$). A close comparison of the UV, IR, ^{1}H- and ^{13}C-NMR (Table 1) spectroscopic data of **3** with **2** showed that their data was almost identical. The difference between **3** and **2** was that in **3** there were just five methylene carbons in the upfield region of the ^{13}C-NMR spectrum. Therefore, **3** was demonstrated to be a eudesmanolide sesquiterpene glycoside. The sugar moiety was determined as β-D-glucose ($[\alpha]_D^{20}$ +46.6°, c=0.020, H_2O) by acid hydrolysis and comparison with an authentic D-glucose, and from the coupling constant of an anomeric proton (δ 4.25, d, J=8.0 Hz). The ^{1}H- and ^{13}C-NMR data of **3** was completely assigned with the help of HMBC and HMQC correlations and presented in Table 1. The relative configuration of **3** was determined by the NOESY experiment. The NOESY correlations between H-5/H_α-3, 6 and 9 and H-14/H_β-6, 9, 15 and H-8 confirmed that **3** possessed a *trans*-A/B ring junction, while C-14 methyl, C-15 methylene and H-8 were β-oriented. Thus, the structure of **3** was elucidated as 4α-hydroxy-5α,8β*H*-eudesman-7(11)-en-8α,12-olide-15-*O*-β-D-glucopyranoside and named sarcaglaboside H.

Experimental

General Procedure Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2550 UV–vis spectrophotometer. IR spectra for KBr were recorded on a Shimadzu FTIR-8400S infrared spectrometer. HR-ESI-MS spectra were recorded on a LTQ Orbitrap XL mass spectrometer (Thermo Scientific, America). ^{1}H-, ^{13}C- and 2D-NMR spectra were recorded by Bruker-AM-500 instrument at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. Absorbents for column chromatography were silica gel (100—200 and 200—300 mesh, Qingdao Marine Chemistry Ltd., P. R. China), Sephadex LH-20 (20—100 μ, Pharmacia). Silica gel GF_{254} plates (Yantai Marine Chemical Co., Ltd., P. R. China) were used for thin-layer chromatography. Preparative HPLC was performed on a LUMTECH instrument with a UV detector at 230 nm and using a C_{30} column (Develosil rpaqueous Packed Φ 20×100 mm, Nomura Chemistry Ltd., Japan).

Plant Material The whole plant of *Sarcandra glabra* was collected from Xiushui in Jiangxi Province, P. R. China, in May 2006, and authenticated by professor Ce-ming Tan, Jiujiang Institute of Forest Plants, Jiangxi, P. R. China. A living voucher (No. CSH2006058018) was deposited at the Herbarium of the Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, P. R. China.

Extraction and Isolation The whole air-dried plant (5 kg) was reduced to a coarse powder and refluxed with acetone and 95% EtOH three times, respectively. The acetone extract was evaporated to dryness under reduced pressure. The residue (102.2 g) was chromatographed on a silica gel column with a gradient of petroleum ether–EtOAC (1 : 0→0 : 1, v/v) to afford sixteen fractions (Fractions A—P), pooled by common TLC characteristics. Fraction C was separated by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1, v/v) to afford **7** (5 mg) and **8** (20 mg). The EtOH extract was dried to afford the residue (236.5 g) by evaporation under reduced pressure. The residue was subjected to silica gel column chromatography eluted with a gradient of $CHCl_3$–MeOH (10 : 0→0 : 10, v/v) to afford 36 fractions. Fraction 26 (21.5 g) was subjected to a silica gel column with a gradient of $CHCl_3$–MeOH (20 : 1→1 : 1, v/v) to give 25 fractions (F_1—F_{25}). Fraction F_{18} was separated by preparative HPLC with MeOH–H_2O (3 : 7, v/v) to give **1** (2 mg, 3.0 ml/min, t_R=16.3 min) and **4** (200 mg, 3.0 ml/min, t_R=73.0 min). Fraction F_{19} was subjected to preparative HPLC eluted with MeOH–H_2O (26 : 74, v/v) to give **6** (5 mg, 5 ml/min, t_R=28.4 min), **2** (3 mg, 5 ml/min, t_R=67.4 min) and **3** (5 mg, 5 ml/min, t_R=92.9 min). Fraction F_{20} was purified by preparative HPLC eluted with MeOH–H_2O (33.5 : 66.5, v/v) to give **5** (4 mg, 6 ml/min, t_R=64.6 min).

Compound **1**: White amorphous powder. $[\alpha]_D^{20}$ −76.7° (c=0.030, MeOH). UV λ_{max} (MeOH) nm (log ε): 229 (3.92). IR (KBr) cm^{-1}: 3407, 3390, 3010, 2931, 1747, 1662, 1423, 1384, 1328, 1101, 1078, 1031. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): shown in Table 1. HR-EI-MS *m*/*z*: 463.1575 $[M+Na]^+$ (Calcd for $C_{21}H_{28}O_{10}Na$: 463.1580).

Compound **2**: White amorphous powder. $[\alpha]_D^{20}$ +42.8° (c=0.063, MeOH). UV λ_{max} (MeOH) nm (log ε): 218 (4.07). IR (KBr) cm^{-1}: 3400, 3010, 2929, 1735, 1676, 1436, 1388, 1330, 1203, 1078, 1033. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): shown in Table 1. HR-EI-MS *m*/*z*: 449.1772 $[M+Na]^+$ (Calcd for $C_{21}H_{30}O_9Na$: 449.1782).

Compound **3**: White amorphous powder. $[\alpha]_D^{20}$ +82.6° (c=0.060, MeOH). UV λ_{max} (MeOH) nm (log ε): 220 (4.11). IR (KBr) cm^{-1}: 3400, 2931, 1735, 1679, 1450, 1419, 1388, 1328, 1203, 1078, 1031. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): shown in Table 1. HR-EI-MS *m*/*z*: 451.1939 $[M+Na]^+$ (Calcd for $C_{21}H_{32}O_9Na$: 451.1944).

Acid Hydrolysis of 1, 2 and 3 Compound **1** (1 mg) in 2.0 mol/l HCl–MeOH was heated at 90 °C for 4 h. After cooling, the mixture was extracted with $CHCl_3$ (3 ml). The water layer was neutralized with 8% NaOH and concentrated to afford a pure sugar (0.24 mg). The sugar was confirmed as D-glucose by comparison with an authentic sample on TLC [silica-gel, EtOAc–MeOH–H_2O–AcOH (6.5 : 2.0 : 1.5 : 1.5), *Rf*=0.42] and by measuring its optical rotation ($[\alpha]_D^{20}$ +46.3°, c=0.019, H_2O). Compounds **2** and **3** were hydrolyzed to give D-glucose (0.27 mg and 0.25 mg, respectively) by the same method.

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