Hepatoprotective Sesquiterpene Glycosides from *Sarcandra glabra*

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Six new sesquiterpene glycosides, compounds **1**−**6**, with eudesmanolide, elemanolide, lindenane, and germacranolide sesquiterpene aglycons, along with one known compound, chloranoside A (**7**), have been isolated from the whole plant of *Sarcandra glabra*. The structures of **1**−**6** were elucidated by chemical and spectroscopic methods including ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY NMR experiments. In addition, compounds **1**−**7** showed pronounced hepatoprotective activities against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells.

Sarcandra glabra (Thunb.) Nakai [*syn. Chloranthus glaber* (Thunb.) Makino] (Chloranthaceae) grows in the southern parts of mainland China and Japan and in southeastern Asia and is cultivated in Japan as an ornamental plant. The whole plant is a renowned traditional Chinese medicine used for its antibacterial and antitumor effects.[1] Several cycloeudesmanes, dihydrochalcones, triterpenoid saponins, and other flavonoids were isolated previously from this species.[1−6] While searching for hepatoprotective compounds from natural sources using rat hepatic epithelial stem-like cells (WB-F344) with D-galactosamine as a screening system, we found that the 70% ethanolic extract of *S. glabra* showed significant hepatoprotective activity. In an investigation of *S. glabra*, six new sesquiterpene glycosides, compounds **1**−**6**, along with the known chloranoside A (**7**), have been isolated and structurally characterized. The structures of compounds **1**−**6** were established through detailed analysis of their spectroscopic data and chemical evidence. D-Galactosamine-induced toxicity in WB-F344 was evaluated for compounds **1**−**7**.

Results and Discussion

Compound **1** was obtained as a white amorphous powder. The HREIMS and ESIMS of **1** exhibited a molecular ion peak at *m*/*z* 410.1935 and a quasimolecular ion peak at *m*/*z* 433 $[M + Na]^+$, respectively, corresponding to the molecular formula $C_{21}H_{30}O_8$. The presence of hydroxyl and α,*β*-unsaturated lactone groups were indicated by its IR (ν_{max} at 3425 and 1739 cm^{-1}, respectively) and UV (λ_{max} at 228 nm) spectra.[7,8] Preliminary inspection of the ^{1}H NMR spectrum (Table 1) of **1** led to the identification of the following representative signals: two methyl groups at δ_H 0.93 and 1.74, one terminal double bond at δ_H 4.63 (1H, d, J = 1.5 Hz) and 4.85 (1H, d, J = 1.5 Hz) for H-15,[7,8] and an anomeric proton at δ_H 4.35 (1H, d, J = 7.5 Hz). The ^{13}C NMR spectrum (Table 2) displayed 21 signals, of which five resonated in the region 60−80 ppm, and a methine signal at δ_C 105.6 suggested the presence of an anomeric carbon of a hexose residue. All of the data mentioned above was used in support of the assignment of compound **1** as a sesquiterpene glycoside.[9] The sugar moiety was determined as D-glucose ($[\alpha]^{20}_D$ +46.3, *c* 0.38, H_2O) by acid hydrolysis, by comparison with an authentic sample, and from the coupling constant of the anomeric proton. The signals observed at δ_C 8.3 (C-13), 79.0 (C-8), 120.1 (C-11), 163.3 (C-7), and 174.8 (C-12) were characteristic of a 3-methyl-5-hydrofuran-2-one functional moiety.[8] The glycosidic site was established unambiguously by a HMBC NMR experiment in which a long-range correlation between H-1′ (δ_H 4.35) and C-1 (δ_C 84.1) was apparent. In the HMBC spectrum of **1**, other ^{1}H−^{13}C long-range correlation signals were found for H-1/C-3, C-5, C-9, C-10, C-14, C-1′; H-5/C-4, C-6, C-7, C-10, C-14, C-15; H-8/C-7, C-9, C-11; H-14/C-1, C-5, C-9, C-10; and H-15/C-3, C-4, C-5, which enabled establishment of the planar structure of **1**. The aglycon of **1** was thus deduced to possess a eudesmanolide-type sesquiterpene skeleton. The NOESY correlations between H-1/H-14, H-8/H-14, H-5/H-6α, H-1/H-1′, and H-14/H-2*β* indicated that CH_3-14, H-1, and H-8 had *β*-configurations, while H-5 and the hydroxy at C-1 were α-oriented. On the basis of the above observations, **1** was assigned as 1*β*,5α,8*βH*-eudesman-4(15),7(11)-dien-8α,12-olide-1-*O*-*β*-D-glucopyranoside. This is a new sesquiterpene glycoside and has been assigned the trivial name sarcaglaboside A.

Compound **2** was obtained as a white amorphous powder with a molecular formula of $C_{21}H_{28}O_8$, as determined by HRFABMS (*m*/*z* 431.1656, $[M + Na]^+$) and NMR analysis. Its UV and IR spectra were characteristic of an α,*β*-unsaturated lactone.[7,8] The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **2** were similar to those of **1**, apart from signals for one double bond (δ_H 5.90, 6.17 and δ_C 128.4, 132.1) instead of two methylenes at C-2 and C-3 as in **1**. Thus, the difference in the molecular formula between **1** and **2** was due to two protons, which indicated the presence of another double bond in addition to the one between C-7 and C-11. In the HMBC spectrum, the clear correlations of one olefinic proton at δ_H 5.90 (H-2) with C-1, C-4, and C-10 and another olefinic proton at δ_H 6.17 (H-3) with C-1, C-4, C-5, and C-15 confirmed that the second double bond was at C-2 and C-3. The ^{1}H NMR coupling constant value of $J_{2,3}$ (10.0 Hz) favored a *Z*-geometry of the 2,3-double bond. Consequently, the structure of **2** was identified as 1*β*,5α,8*βH*-eudesman-2,4(15),7(11)-trien-8α,12-olide-1-*O*-*β*-D-glucopyranoside and has been given the trivial name sarcaglaboside B.

Compound **3** was obtained as a white amorphous powder. Its IR spectrum showed absorption bands of hydroxyl groups (3433 cm^{-1}), an α,*β*-unsaturated lactone (1747 cm^{-1}), and terminal olefinic bonds (916 and 847 cm^{-1}). The ^{13}C NMR spectrum (Table 2) exhibited 21 carbon signals including five quaternary carbons, eight methines, six methylenes, and two methyls. In the ESIMS, a quasimolecular ion peak was observed at *m*/*z* 433 $[M + Na]^+$. Its molecular formula was established as $C_{21}H_{30}O_8$, on the basis of the above information, which was supported by HRFABMS, which gave a quasimolecular ion peak at *m*/*z* 433.1851 $[M + Na]^+$ (calcd 433.1838). The ^{1}H NMR spectrum (Table 1) exhibited five olefinic proton signals at δ_H 5.77 (H-1), 4.94 (H-2a), 4.92 (H-2b), 5.34 (H-3a), and 5.00 (H-3b). The ^{13}C NMR spectrum also showed the corresponding carbon signals at δ_C 148.2 (C-1), 112.6 (C-2), 115.4

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Chart 1

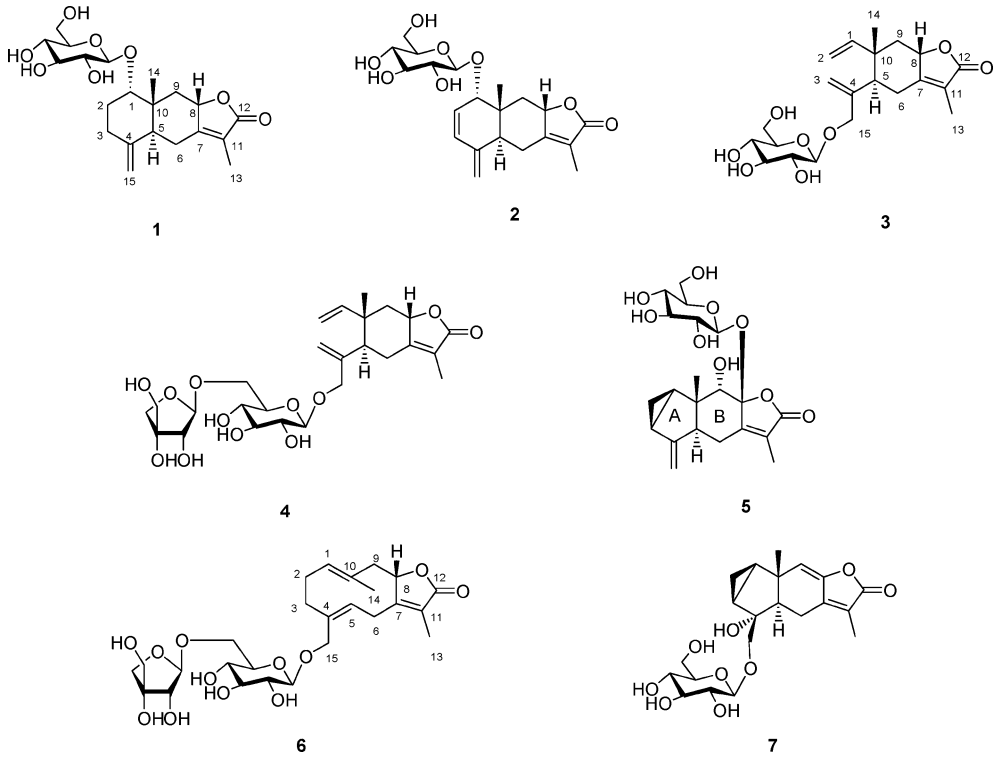

Table 1. ^{1}H (500 MHz) NMR Data of Compounds **1**−**6** (**1** in CD_3COCD_3 and **2**−**6** in CD_3OD)

position	1	2	3	4	5	6
1	3.52 brs	3.65 d (5.5)	5.77 dd (17.5, 11.0)	5.76 dd (17.5, 11.0)	1.80[a] m	4.88 dd (11.5, 4.0)
2	2.08 (α) m	5.90 dd (9.5, 5.5)	4.92 d (17.0)	4.94 d (17.5)	0.78 (α) m	2.05 (α) t (12.0)
	1.80 (*β*) ddd (2.5, 5.0, 14.0)		4.94 d (11.0)	4.95 d (11.0)	0.92 (*β*) m	2.13 (*β*) m
3	2.12[a] (α) m	6.17 d (10.0)	5.00 brs	5.00 brs	1.87 m	1.80 (α) m
	2.42[a] (*β*) m		5.34 brs	5.34 brs		2.56 (*β*) brd (12.0)
5	2.45[a] m	2.53 dd (10.0, 2.5)	2.28 dd (13.0, 4.0)	2.26 dd (13.5, 4.0)	2.90 dd (12.0, 2.0)	4.54 d (10)
6	2.77 (α) dd (12.5, 2.5)	3.12 (α) dd (14.0, 4.5)	2.74 (α) dd (13.5, 4.0)	2.78 (α) dd (13.5, 4.0)	2.59 (α) dd (13.0, 2.0)	3.19[a] (α) m
	2.38[a] (*β*) t (13.0)	2.32 (*β*) t (13.0)	2.69 (*β*) t (13.5)	2.66 (*β*) t (13.5)	2.42 (*β*) t (13.0)	3.44 (*β*) brd (15.0)
8	4.91 dd (10.5, 7.5)	4.95 dd (11.0, 6.5)	4.92[a] m	4.92[a] m		5.07 dd (10.5, 3.5)
9	2.04[a] (α) m	2.02 (α) t (12.0)	1.30 (α) t (12.0)	1.30 (α) t (12.0)	4.07 s	2.05 (α) t (12.0)
	2.12[a] (*β*) m	2.08 (*β*) dd (12.5, 6.5)	2.08 (*β*) dd (12.0, 6.5)	2.08 (*β*) dd (12.0, 6.5)		3.00 (*β*) brd (14.0)
13	1.74 s	1.77 s	1.74 s	1.75 s	1.82 s	1.78 s
14	0.93 s	0.87s	1.15 s	1.15 s	0.96 s	1.44 s
15	4.63 d (1.5)	5.04 brs	4.02 d (12.5)	4.02 d (13.0)	4.78 brs	3.89 d (11.5)
	4.85 d (1.5)		4.23 d (13.0)	4.19 d (13.0)	4.95 brs	4.49 d (11.5)
Glc-1	4.35 d (7.5)	4.27 d (7.5)	4.19 d (7.5)	4.17 d (8.0)	4.13 d (8.0)	4.24 d (8.0)
Glc-2	3.18 m	3.07 t (8.5)	3.14 t (8.0)	3.14 t (8.5)	3.21[a] m	3.13 t (8.5)
Glc-3	3.36[a] m	3.28[a] m	3.28[a] m	3.28 d (8.0)	3.28[a] m	3.29[a] m
Glc-4	3.29[a] m	3.20[a] m	3.21[a] m	3.18 t (8.5)	3.23[a] m	3.20[a] m
Glc-5	3.29[a] m	3.20[a] m	3.18[a] m	3.32 m	3.00 m	3.36 m
Glc-6	3.63 m	3.60 dd (11.5, 5.5)	3.54 dd (11.5, 5.5)	3.54 dd (10.0, 8.5)	3.55 dd (12.0, 6.0)	3.56 m
	3.80 m	3.81 dd (11.5, 2.0)	3.92 dd (12.0, 2.0)	3.92 dd (8.5, 1.5)	3.75 dd (12.0, 2.0)	3.92 dd (7.0, 1.5)
Api-1				4.93 d (3.0)		4.98 d (2.5)
Api-2				3.83 d (2.5)		3.82 d (2.0)
Api-4				3.69 d (10.0)		3.68 d (9.5)
				3.91 d (9.5)		3.90 d (9.5)
Api-5				3.50 brs		3.51 brs

[a] Overlapped with other signals.

(C-3), and 147.3 (C-4). Signals at δ_C 8.1 (C-13), 79.8 (C-8), 120.6 (C-11), 165.2 (C-7), and 177.3 (C-12) confirmed the presence of a 3-methyl-5-hydrofuran-2-one functional moiety.[7,8] Acid hydrolysis of **3** yielded D-glucose as the sugar moiety, as determined by TLC with an authentic sample. The ^{1}H NMR spectrum revealed an anomeric proton signal at δ_H 4.19 (1H, d, J = 7.5 Hz) and indicated the sugar unit to be a *β*-glycosidic linkage to the aglycon. In the HMBC spectrum, these fragments were connected by the correlations of H-8/C-10; H-5/C-4; H-14/C-1, C-5, C-9, C-10; and H-15/C-3, C-4, C-5, C-1′. Therefore, compound **3** was deduced to possess an aglycon of an elemanolide-type sesquiterpene.[10] In the NOESY spectrum of **3**, correlation signals were found between H-8 and H-9*β*, H-14; H-5 and H-6α, 9α; H-14 and H-6*β*, H-9*β*; and H-3b and H-6*β*. The structure of sarcaglaboside C (**3**) was proposed as 5α,8*β*H-eleman-1,3,7(11)-trien-8α,12-olide-15-*O*-*β*-D-glucopyranoside.

Compound **4** was obtained as a white amorphous powder, and its HRFABMS gave a quasimolecular ion peak at *m*/*z* 565.2284

Table 2. ^{13}C (125 MHz) NMR Data of Compounds **1**−**6** (**1** in CD_3COCD_3 and **2**−**6** in CD_3OD)

position	1	2	3	4	5	6
1	84.1, CH	81.8, CH	148.2, CH	148.1, CH	25.3, CH	131.3, CH
2	29.0, CH_2	128.4, CH	112.6, CH_2	112.7, CH_2	16.6, CH_2	27.1, CH_2
3	31.7, CH_2	132.1, CH	115.4, CH_2	115.6, CH_2	23.9, CH	35.9, CH_2
4	150.0, C	145.8, C	147.3, C	147.2, C	152.7, C	134.0, C
5	43.9, CH	40.6, CH	48.8, CH	48.8, CH	58.6, CH	129.6, CH
6	25.7, CH_2	25.9, CH_2	29.3, CH_2	29.3, CH_2	25.1, CH_2	27.8, CH_2
7	163.3, C	164.6, C	165.2, C	165.4, C	161.1, C	165.5, C
8	79.0, CH	80.6, CH	79.8, CH	79.8, CH	108.5, C	84.5, CH
9	41.8, CH_2	41.8, CH_2	47.0, CH_2	47.0, CH_2	76.7, CH	48.1, CH_2
10	42.5, C	40.2, C	42.0, C	42.0, C	43.9, C	134.0, C
11	120.1, C	120.8, C	120.6, C	120.5, C	125.8, C	126.8, C
12	174.8, C	177.4, C	177.3, C	177.3, C	174.1, C	176.1, C
13	8.3, CH_3	8.1, CH_3	8.1, CH_3	8.2, CH_3	8.3, CH_3	8.8, CH_3
14	17.1, CH_3	17.5, CH_3	16.7, CH_3	16.7, CH_3	17.3, CH_3	16.7, CH_3
15	107.0, CH_2	113.8, CH_2	74.7, CH_2	75.0, CH_2	106.3, CH_2	68.2, CH_2
Glc-1	105.6, CH	105.8, CH	104.3, CH	104.3, CH	97.8, CH	104.2, CH
Glc-2	75.3, CH	75.5, CH	75.2, CH	75.1, CH	74.4, CH	75.1, CH
Glc-3	78.1, CH	78.1, CH	78.2, CH	78.1, CH	78.2, CH	78.2, CH
Glc-4	71.7, CH	71.7, CH	71.8, CH	71.8, CH	71.6, CH	71.8, CH
Glc-5	77.3, CH	78.0, CH	78.0, CH	77.0, CH	78.5, CH	77.1, CH
Glc-6	63.0, CH_2	62.8, CH_2	62.8, CH_2	68.8, CH_2	62.6, CH_2	68.6, CH_2
Api-1				111.0, CH		111.0, CH
Api-2				78.0, CH		78.0, CH
Api-3				80.5, C		80.5, C
Api-4				75.0, CH_2		75.0, CH_2
Api-5				65.5, CH_2		65.6, CH_2

$[M + Na]^+$, for $C_{26}H_{38}O_{12}$ (calcd 565.2261). Its IR spectra showed characteristic peaks for an α,β-unsaturated lactone (1738 cm^{-1}), hydroxyl groups (3432 cm^{-1}), and terminal olefinic bonds (920 and 823 cm^{-1}). Acid hydrolysis of **4** yielded D-glucose and D-apiose ($[\alpha]^{20}_D$ +4.3, *c* 0.22, H_2O), shown by TLC with authentic samples. The ^{13}C NMR spectrum (Table 2) exhibited 26 carbon signals including six quaternary carbons, 10 methines, eight methylenes, and two methyls. The ^1H NMR spectrum of **4** revealed a second anomeric proton signal at δ_H 4.93 (1H, d, $J = 3.0$ Hz) in addition to the anomeric proton signal of glucose at δ_H 4.17 (1H, d, $J = 8.0$ Hz). The ^{13}C NMR data of **4** were similar to those of **3**, except for a set of signals belonging to an apiose moiety. Analysis of the HMQC and HMBC spectra of **4** also led to the proposal of an elemanolide-type skeleton similar to that of **3**. The difference lay in the diagnostic change in the glucose unit from the data obtained for **3** and indicated that the apiose was attached to C-6′. The anomeric proton (δ_H 4.93) of the apiose moiety gave a three-bond correlation to the C-6′ signal (δ_C 68.8) in the HMBC spectrum. The presence of a β-D-apiofuranosyl unit in **4** was inferred from the chemical shift of the apiosyl anomeric carbon, which resonated at δ_C 111.0 ppm.[11,12] Therefore, compound **4** was elucidated as 5α,8β*H*-eleman-1,3,7(11)-trien-8α,12-olide-15-*O*-[β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside] and was given the trivial name sarcaglaboside D.

The molecular formula of **5** was calculated as $C_{21}H_{28}O_9$ from its HRFABMS and ESIMS data. The presence of hydroxyl and α,β-unsaturated lactone groups was indicated from the IR spectrum (ν_{max} at 3402 and 1759 cm^{-1}, respectively). In the ^1H NMR spectrum, the four correlated proton signals at δ_H 0.78, 0.92, 1.80, and 1.87 indicated the presence of a three-membered ring system, and two singlets corresponding to the 13- and 14-methyl groups were observed at δ_H 1.82 and 0.96, respectively. Signals at δ_H 2.90, 2.42, and 2.59 were assigned to the C-5 methine and C-6 methylene protons, respectively, and the C-15 olefinic protons were observed as two singlets at δ_H 4.78 and 4.95. The downfield shifts of C-9 (δ_H 4.07) revealed the presence of a hydroxyl group at this position. Besides these signals, the presence of six complex signals at δ_H 3.0−4.0 and an anomeric proton (δ_H 4.13, d, $J = 8.0$ Hz) suggested that **5** is a β-glycoside of a lindane sesquiterpene, having an uncommon linear 3−5−6 ring system, like chloranosides A (**7**) and B.[3] From the acid hydrolysis of **5** to give D-glucose and comparison of the ^{13}C NMR data with those of chloranthalactone E,[13,14] **5** could be assigned as an *O*-β-glucoside of chloranthalactone E. In the HMBC spectrum, these fragments were connected by correlations of H-5/C-4, C-10; H-6/C-5, C-7, C-8, C-10, C-11; H-9/C-5, C-7, C-8, C-10, C-14; H-14/C-1, C-2, C-5, C-9, C-10; and H-15/C-3, C-5. The attachment of glucose to the hydroxy at C-8 was deduced by the long-range coupling between H-1′ and C-8. The relative configuration of **5** was established according to its NOESY spectrum, in which correlation signals between H-5/H-1, H-6α; H-9/H-14; and H-6β/H-14 suggested a *trans*-A/B ring junction, with the cyclopropane ring in the same orientation as the C-14 methyl group and a β-orientation of H-9. Another correlation in the NOESY spectrum was found between H-6α and the H_3-13 methyl signal, but not for H-6β and H-13 (Figure 1), supporting the *syn*-relationship of the C-14 methyl and the 8-*O*-glucoside substituents.[15] Hence, compound **5** (chloranthalactone E 8-*O*-β-D-glucopyranoside) was assigned as 8β,9α-dihydroxy-5α,9β*H*-lindan-4(15),7(13)-dien-8α,12-olide-8β-*O*-β-D-glucopyranoside.

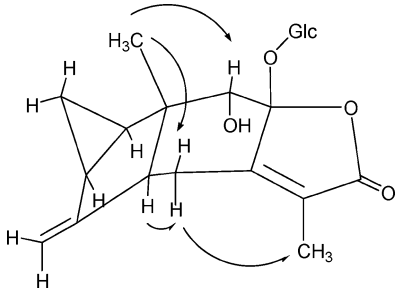

Figure 1. Key NOESY correlations for compound **5**.

Compound **6** was obtained as a white amorphous powder. HRFABMS of **6** gave a quasimolecular ion peak at *m/z* 565.2258 $[M + Na]^+$ for $C_{26}H_{38}O_{12}$ (calcd 565.2261). Its IR spectra showed peaks for an α,β-unsturated lactone (1739 cm^{-1}) and hydroxyl groups (3408 cm^{-1}). Acid hydrolysis of **6** yielded D-glucose and D-apiose by TLC comparison with the authentic samples. Two anomeric proton signals at δ_H 4.24 (1H, d, $J = 8.0$ Hz) and 4.98 (1H, d, $J = 2.5$ Hz) were assigned to β-glucose and β-apiose, respectively.[11,12] The ^{13}C NMR spectrum (Table 2) exhibited 26 carbon signals including six quaternary carbons, 10 methines, eight methylenes, and two methyls. Analysis of the $^1H-^1H$ COSY and HMQC spectra of **6** enabled the deduction of the fragments C-1,

Table 3. Hepatoprotective Effects of Compounds **1**−**7** against D-Galactosamine-Induced Toxicity in WB-F344 Cells[a]

compound	cell survival rate (% of normal)	inhibition (% of control)
normal	100.0 ± 9.3	
control	26.4 ± 0.7	
bicyclol[b]	46.6 ± 8.6*	27.4
1	47.5 ± 5.4**	28.7
2	74.9 ± 9.8**	65.9
3	53.0 ± 7.3**	36.1
4	46.3 ± 4.1**	27.0
5	45.5 ± 1.6**	26.0
6	42.4 ± 4.2**	21.7
7	54.5 ± 3.4*	38.2

[a] Results are expressed as means ± SD ($n = 3$; for normal and control, $n = 6$); *$p < 0.05$, **$p < 0.01$, significantly different from control by Student's *t*-test. [b] Positive control substance.

C-2, C-3; C-5, C-6; and C-8, C-9. In the HMBC spectrum of **6**, ^{1}H−^{13}C long-range correlation signals were found for H-14/C-1, C-9, C-10; H-5/C-3, C-7, C-15; H-6/C-4, C-5, C-7, C-8, C-11; and H-9/C-1, C-7, C-8, C-10, C-14, which enabled the establishment of a germacranolide-type sesquiterpene skeleton for the aglycon of **6**. The configurations of the two double bonds were determined mainly from the ^{13}C NMR chemical shifts of C-2, C-3, C-6, and C-9. In their ^{13}C NMR spectra, allylic methylene signals usually resonate at higher field in the *cis* isomer than in the *trans* isomer. In the case of germacranolide-type sesquiterpenes, having a *trans* 1,10-double bond, the C-9 methylene carbon usually resonates below 30 ppm. On the other hand, the C-2 carbon atom is unaffected whether the 1,10-double bond is *trans* or not.[16] This rule is also adaptable to 4,5-double bonds; so when the 4,5-double bond is *trans*, the C-3 carbon signal is observed at lower than 30 ppm and the C-6 carbon atom is unaffected.[16] The C-9 and C-3 carbon signals were both observed below 30 ppm, so that **6** has a 1,10-*trans*-4,5-*trans*-germacranolide-type skeleton. The NOESY correlations between H-1/H-5, H-8/H-9β, and H-8/H-14 indicated that H-8 is in the β-configuration. This aglycon was the same as that reported for glechomanolide.[17,18] The glycosidic sites were established by the HMBC experiment in which long-range correlations were observed between H-1′ (δ_H 4.24) and C-15 (δ_C 68.2) and between H-1″ (δ_H 4.98) and C-6′ (δ_C 68.6). Therefore, the linkage of two sugar units was β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside. Consequently, compound **6** was elucidated as (1*E*,4*Z*)-8β*H*-germacra-1,4,7(11)-trien-8α,12-olide-15-*O*-[β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside] and was given the trivial name sarcaglaboside E.

Hepatoprotective activities against D-galactosamine-induced toxicity of the six new compounds (**1**−**6**) and one known compound (**7**) obtained from *S. glabra* were examined in WB-F344 cells. All these sesquiterpene glycosides were found to show an inhibitory activity at 10^{-4} M in vitro, without any obvious cytotoxic effects (Table 3). The hepataprotective activities of **1**, **2**, **3**, and **7** were more potent than that of bicyclol, a drug showing potent hepatoprotective activity.[19] This is the first report of hepatoprotective activity from a *Sarcandra* species.

Experimental Section

General Experimental Procedures. The optical rotations were determined on a Perkin-Elmer digital polarimeter. UV spectra were taken on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on an IMPACT 400 spectrometer as KBr pellets. The ^{1}H, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were run on an INOVA-500 FT spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using solvent peaks as references. HREIMS and HRFABMS were performed on an Autospec Ultima-Tof mass spectrometer. ESIMS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Reversed-phase HPLC was carried out on a Shimadzu LC-6AD instrument using a SPD-10A detector. A reversed-phase C_{18} column (YMC-Pack ODS-A ϕ 20 × 250 mm, 10 μm) was employed. Column chromatography was carried out on macroporous resin D101 (26−60 mesh, Tianjin Haiguang Chemistry Company, Tianjin, People's Republic of China), polyamide (60−90 mesh, Linjiang Chemistry Company, Changzhou, People's Republic of China), silica gel (100−200, 200−300 mesh, Qingdao Marine Chemistry Company, Qingdao, People's Republic of China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Kieselgel 60 F_{254} silica gel plates (Merck, Germany) were used for analytical TLC.

Plant Material. The plant was collected in Dayu County of Jiangxi Province in July 2004 and indentified by Professor Yong-Ming Luo of the Faculty of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, People's Republic of China. A voucher specimen (No. 20040718) is deposited at the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China.

Extraction and Isolation. The whole air-dried plant (3.0 kg) of *Sarcandra glabra* was reduced to a coarse powder and refluxed with 70% EtOH three times. After evaporation of ethanol in vacuo, the aqueous residue was diluted with water. The filtrate was separated on macroporous resin D101 using water and 30%, 70%, and 95% EtOH−water in sequence to afford four fractions (parts A_1−A_4). Part A_3 (61.8 g) was subjected to passage over polyamide by elution with water and 30%, 60%, and 95% EtOH−water in sequence to give fractions B_1 (10.4 g), B_2 (11.3 g), B_3 (15.4 g), and B_4 (4.7 g). Fraction B_1 (10.4 g) was subjected to column chromatography on silica gel with $CHCl_3$−MeOH (0:1−1:1) to afford 14 fractions (F1−F14). Fraction F11 (0.9 g) was separated on a Sephadex LH-20 column, using petroleum ether−$CHCl_3$−MeOH (5:5:1) for elution, to produce eight fractions (F11-1 to F11-8). Subfraction F11-6 (0.2 g) was passed over a RP-18 column with MeOH−water (35−50%) and finally purified by RP-HPLC eluted with 18% CH_3CN in water to give sarcaglabosides A (**1**, 57 mg, 5 mL/min, t_R = 131.7 min), B (**2**, 10 mg, 5 mL/min, t_R = 117.6 min), and C (**3**, 5 mg, 5 mL/min, t_R = 96.6 min) and chloranthalactone E 8-*O*-β-D-glucopyranoside (**5**, 5 mg, 5 mL/min, t_R = 125.5 min). Fraction F12 (0.5 g) was chromatographed over a RP-18 column, eluted with 33% MeOH in water, to afford cloranoside A (23 mg). Fraction F13 (1.5 g) was separated on a Sephadex LH-20 column using MeOH for elution to produce seven fractions (F13-1 to F13-7). Subfraction F13-4 (0.2 g) was passed over a RP-18 column with MeOH−water (35−55%) and finally purified by RP-HPLC, eluted with 17% CH_3CN in water, to give sarcaglabosides D (**4**, 10 mg, 5 mL/min, t_R = 93.4 min) and E (**6**, 12 mg, 5 mL/min, t_R = 70.7 min).

Sarcaglaboside A (1): white amorphous powder; $[\alpha]^{20}_D$ +150.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.99) nm; IR (KBr) ν_{max} 3425, 2931, 1739, 1645, 1439, 1344, 1163, 1078, 1034, 889 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz), see Table 1; ^{13}C NMR (CD_3COCD_3, 125 MHz), see Table 2; ESIMS *m/z* 433 [M + Na]$^+$; HREIMS *m/z* 410.1935 [M]$^+$ (calcd for $C_{21}H_{30}O_8$, 410.1941).

Sarcaglaboside B (2): white amorphous powder; $[\alpha]^{20}_D$ +190.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.28) nm; IR (KBr) ν_{max} 3369, 2924, 1743, 1684, 1385, 1219, 1076, 1036, 1016, 897 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS *m/z* 431 [M + Na]$^+$; HRFABMS *m/z* 431.1656 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_8$, 431.1682).

Sarcaglaboside C (3): white amorphous powder; $[\alpha]^{20}_D$ +40.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.78) nm; IR (KBr) ν_{max} 3433, 2924, 1747, 1682, 1415, 1344, 1223, 1078, 1032, 916, 847 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS *m/z* 433 [M + Na]$^+$; HRFABMS *m/z* 433.1851 [M + Na]$^+$ (calcd for $C_{21}H_{30}O_8$, 433.1838).

Sarcaglaboside D (4): white amorphous powder; $[\alpha]^{20}_D$ +10.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.88) nm; IR (KBr) ν_{max} 3432, 2925, 1738, 1682, 1417, 1383, 1051, 920, 823 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS *m/z* 565 [M + Na]$^+$; HRFABMS *m/z* 565.2284 [M + Na]$^+$ (calcd for $C_{26}H_{38}O_{12}$, 565.2261).

Chloranthalactone E 8-*O*-β-D-glucopyranoside (5): white amorphous powder; $[\alpha]^{20}_D$ +31.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.90) nm; IR (KBr) ν_{max} 3402, 2924, 1759, 1662, 1616, 1385, 1250, 1076, 1034, 1001, 958, 918 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS *m/z* 447 [M + Na]$^+$; HRFABMS *m/z* 447.1641 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_9$, 447.1631).

Sarcaglaboside E (6): white amorphous powder; $[\alpha]^{20}_D$ +18.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.91) nm; IR (KBr) ν_{max} 3408, 2925, 1739, 1653, 1448, 1385, 1051, 1011 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2; ESIMS *m/z* 565 $[M + Na]^+$; HRFABMS *m/z* 565.2258 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_{12}$, 565.2261).

Acid Hydrolysis of 1−6. Compound **1** (10 mg) dissolved in 2 N HCl was heated at 90 °C on a waterbath for 2 h. After cooling, the reaction mixture was extracted with $CHCl_3$. The aqueous layer was neutralized with 8% NaOH and concentrated under reduced pressure. The residue was separated on a silica gel column (1 g), eluted with CH_3Cl_3−MeOH−water (7:3:0.5), to afford a pure sugar (2.0 mg). The sugar was confirmed as D-glucose by comparison with an authentic sample on TLC (EtOAc−MeOH−H_2O, 4:1:0.1, R_f 0.16) and by measuring its optical rotation ($[\alpha]^{20}_D$ +46.3).

Solutions of compounds **2**, **3**, and **5** (3 mg each) in 2 N HCl were heated for 2 h. After cooling, each mixture was neutralized with 8% NaOH and partitioned between $CHCl_3$ and H_2O, respectively. The H_2O-soluble phase was concentrated and examined by TLC (EtOAc−MeOH−H_2O, 4:1:0.1, R_f 0.16) in comparison with authentic D-glucose.

Compounds **4** and **6** (8 mg each) were treated as described for **1** to give a sugar (0.5 mg) and D-glucose (0.6 mg). The second sugar was confirmed as D-apiose by comparison with an authentic sample on TLC (EtOAc−MeOH−H_2O, 4:1:0.1, R_f 0.45) and measuring its optical rotation ($[\alpha]^{20}_D$ +4.3).

Protective Effect on Cytotoxicity Induced by D-Galactosamine in WB-F344 Cells. The hepatoprotective effects of compounds **1**−**7** were determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[20,21] in WB-F344 cells, with some modification. Each cell suspension of 1×10^4 cells in 200 μL of Dulbecco's modified Eagles medium containing fetal calf serum (3%), penicillin (100 units/mL), and streptomycin (100 μg/mL) was planted in a 96-well microplate and precultured for 24 h at 37 °C under a 5% CO_2 atmosphere. Fresh medium (200 μL) containing bicyclol and test samples was added, and the cells were cultured for 1 h. Then, the cultured cells were exposed to 40 mM D-galactosamine for 24 h. Cytotoxic effects of test samples were measured simultaneously in the absence of D-galactosamine. The medium was changed into a fresh one containing 0.5 mg/mL MTT. After 3.5 h incubation, the medium was removed and 150 μL of dimethyl sulfoxide was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm. Inhibition (%) was obstained by the following formula:

$$\text{inhibition (\%)} = [(OD_{(sample)} - OD_{(control)})/(OD_{(normal)} - OD_{(control)})] \times 100$$

Statistical Analysis. All values were expressed as ± SD. The Student's *t*-test for unpaired observations between normal or control and tested samples was carried out to identify statistical differences; *p* values less than 0.05 were considered as significantly different.

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