# Two New-Skeleton Compounds from *Sarcandra glabra*

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Two new-skeleton compounds, sarcaboside A (**1**) together with its artifact sarcaboside B (**2**), were isolated from the whole plant of *Sarcandra glabra*. Their structures were elucidated on the basis of extensive spectroscopic analysis as well as HR-ESI-MS$^n$ study.

**Introduction.** – *Sarcandra glabra* (THUNB.) NAKAI (Chloranthaceae) is a traditional Chinese medicine (TCM), which has been frequently used to treat acute influenza, pharyngolaryngitis, thrombocytopenia, pneumonia, cellulites, appendicitis, shigellosis, leukoderma vitiligo, abscess, cancer, *etc.* [1]. Phenolic acids, flavonoids, coumarins, and sesquiterpenoids, *etc*. have been isolated from this plant [2][3]. In this study, we report the isolation and structure elucidation of two new-skeleton compounds, named sarcaboside A and sarcaboside B (**1** and **2**, resp.; *Fig. 1*) by extensive spectroscopic analysis as well as HR-ESI-MS$^n$ study.

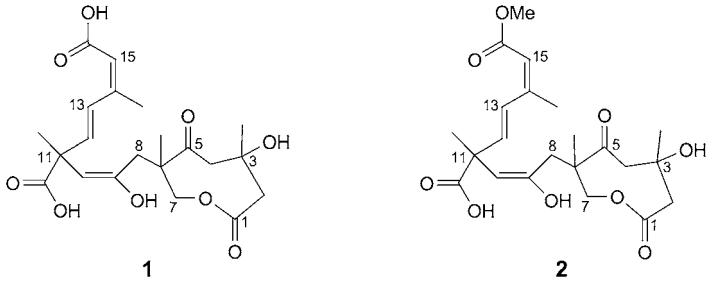


Fig. 1. *Structures of compounds* **1** *and* **2**

**Result and Discussion.** – Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS exhibited a quasimolecular ion at $m/z$ 423.1643 ($[M-H]^-$), corresponding to the molecular formula $C_{21}H_{28}O_9$ and eight degrees of unsaturation. Its UV spectra showed maximal absorption at 263 nm, suggesting a conjugated moiety. The $^1$H-NMR spectrum displayed signals of four Me groups at $\delta$(H) 1.10, 1.35, 1.95 and 2.05, and the $^{13}$C-NMR spectrum exhibited the corresponding C-atom signals at $\delta$(C) 20.3, 28.0, 19.6 and 21.3, respectively (*Table*). The $^1$H-NMR spectrum also showed two vicinally coupling olefinic H-atom signals at $\delta$(H) 6.22 (H–C(12)) and 7.79

(H–C(13))[1]) and two single olefinic H-atom signals at $\delta$(H) 5.99 (H–C(10)) and 5.79 (H–C(15)), corresponding to the olefinic C-atom signals at $\delta$(C) 137.0, 130.0, 128.4, and 120.5, respectively. These data suggested the presence of three C=C bonds, together with four C=O groups ($\delta$(C) 172.8, 199.7, 165.9 and 169.8), indicating a monocycle for the one unsaturation degree left.

Table. *$^1$H- and $^{13}$C-NMR Data* (500 and 125 MHz, resp.; $CD_3OD$) *of Compounds* **1** *and* **2**. $\delta$ in ppm, $J$ in Hz. For atom numbering, see *Fig. 1*.

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| 1 | | 172.8 | | 172.7 |
| 2 | 2.67 (*s*, 2 H) | 46.3 | 2.66 (*s*, 2 H) | 46.3 |
| 3 | | 70.9 | | 70.8 |
| 3-Me | 1.35 (*s*, 3 H) | 28.0 | 1.35 (*s*, 3 H) | 28.0 |
| 4 | 2.62 (*s*, 2 H) | 46.3 | 2.66 (*s*, 2 H) | 46.1 |
| 5 | | 199.7 | | 199.7 |
| 6 | | 46.5 | | 46.4 |
| 6-Me | 1.10 (*s*, 3 H) | 20.3 | 1.09 (*s*, 3 H) | 20.2 |
| 7$\alpha$ | 4.15 (*d*, $J$ = 15.0, 1 H) | 68.9 | 4.14 (*d*, $J$ = 15.0, 1 H) | 68.8 |
| 7$\beta$ | 4.28 (*d*, $J$ = 15.0, 1 H) | | 4.27 (*d*, $J$ = 15.0, 1 H) | |
| 8$\alpha$ | 2.45 (*d*, $J$ = 20.0, 1 H) | 45.0 | 2.44 (*d*, $J$ = 20.0, 1 H) | 45.0 |
| 8$\beta$ | 2.54 (*d*, $J$ = 20.0, 1 H) | | 2.52 (*d*, $J$ = 20.0, 1 H) | |
| 9 | | – | | – |
| 10 | 5.99 (*s*, 1 H) | 128.4 | 5.98 (*s*, 1 H) | 128.3 |
| 11 | | 79.7 | | 79.7 |
| 11-Me | 1.95 (*s*, 3 H) | 19.6 | 1.94 (*s*, 3 H) | 19.7 |
| 11-COOH | | 165.9 | | 166.0 |
| 12 | 6.22 (*d*, $J$ = 15.0, 1 H) | 137.0 | 6.18 (*d*, $J$ = 15.0, 1 H) | 136.5 |
| 13 | 7.79 (*d*, $J$ = 15.0, 1 H) | 130.0 | 7.75 (*d*, $J$ = 15.0, 1 H) | 130.2 |
| 14 | | 150.6 | | 149.0 |
| 14-Me | 2.05 (*s*, 3 H) | 21.3 | 2.03 (*s*, 3 H) | 21.3 |
| 15 | 5.79 (*s*, 1 H) | 120.5 | 5.80 (*s*, 1 H) | 120.8 |
| 15-COO | | 169.8 | | 173.2 |
| 15-COO*Me* | | | 3.67 (*s*, 2 H) | 52.1 |

The $^1$H- and $^{13}$C-NMR data of **1** were further assigned with the help of a DEPT spectrum, and HMQC and HMB correlations. The DEPT spectrum showed signals of four CH groups at $\delta$(C) 137.0, 130.0, 128.4, 120.5, and of four Me groups at $\delta$(C) 28.0, 20.3, 19.6, 21.3, which confirmed the above H- and C-atom assignments. Besides, four $CH_2$ signals at $\delta$(C) 68.9, 45.0, 46.3 (2×) could also be observed. The HMBC correlation of 14-Me ($\delta$(H) 2.03) with the olefinic C-atoms C(13), C(14), and C(15) indicated the presence of two conjugated C=C bonds substituted with a Me group at C(14) (*Fig. 2*). The HMBC correlations H–C(12)/C(11), 11-COOH, and C(14); H–C(13)/C(11), and 11-Me/C(11) and 11-COOH, indicated a quaternary C-atom substituted with a Me group, and that a COOH-containing moiety was connected with the C(12)=C(13) bond. Besides, another single olefinic H-atom (H–C(10), $\delta$(H) 5.98)

[1]) Atom numbering as indicated in *Fig. 1*. For systematic names, see the *Exper. Part.*

showed HMBC correlation with C(11), 11-Me, and C(8), suggesting that a trisubstituted C=C bond was located between C(11) and C(8). The HMBC correlations H–C(8)/C(10), C(5), C(6), and C(7); and 6-Me/C(5), C(6), and C(7), confirmed the location of these fragments as shown in *Fig. 1*. Two $CH_2$ *singlet*s corresponding to H–C(2) and H–C(4) showed long-range correlations with C(1) and C(3), and C(6), C(3), and 3-Me, respectively, suggesting that a fragment of $C_5H_8O_3$ underwent cyclization to form an eight-membered ring between C(5) and C(7) accounting for the one degree of unsaturation left. The positions C(15) and C(9) can only be substituted with COOH and OH, respectively, according to the determined molecular formula. However, the chemical shift of C(9) could not be observed, even when the tested amount of **1** was 30 mg. The HR-ESI-MS$^n$ analysis for the proposed fragment pathways of **1** is outlined in *Fig. 3*, which further confirmed the deduced structure. The configurations of C(14)=C(15) and C(9)=C(10) were deduced from the NOESY spectrum. The NOESY correlations (*Fig. 2*) H–C(15) ($\delta$(H) 5.79)/14-Me ($\delta$(H) 2.05) allowed us to assign the (*Z*)-configuration to C(14)=C(15). Additionally, NOESY correlations H–C(10) ($\delta$(H) 5.99)/11-Me ($\delta$(H) 1.95), H–C(12) ($\delta$(H) 6.22)/H–C(8) ($\delta$(H) 2.45), and H–C(12)/6-Me ($\delta$(H) 1.10) could also be observed, evidencing the (*E*)-configuration for the C(9)=C(10) bond. The (*E*)-configuration of the C(12)=C(13) bond was easily deduced from the characteristic coupling constant ($J$ = 15.0 Hz) of the two olefinic H-atoms. Thus the structure of compound **1** was elucidated as (2*Z*,4*E*)-6-[(1*E*)-2-hydroxy-3-(6-hydroxy-3,6-dimethyl-4,8-dioxooxocan-3-yl)prop-1-en-1-yl]-3,6-dimethylhepta-2,4-dienedioic acid, named sarcaboside A.

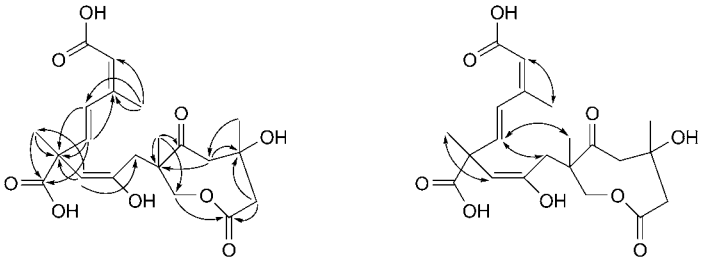


Fig. 2. *Key HMBC* (H → C) and *NOESY* (H ↔ H) *correlations of compound* **1**

Compound **2** was also obtained as a white amorphous powder with the molecular formula $C_{22}H_{30}O_9$ deduced from HR-ESI-MS (*m*/*z* 437.1812 ([$M-H$]$^-$)). A comparison of the $^1$H- and $^{13}$C-NMR data (*Table*) of **2** with those of **1** revealed that they were almost identical, except for the signal of a MeO group at $\delta$(H) 3.67 (*s*, 3 H), together with the corresponding C-atom signal at $\delta$(C) 52.0 indicating that a COOH group was methylated. This evidence was further confirmed by the downfield shift of the 15-COO C-atom signal by *ca.* 3 ppm and the HMBC correlation 15-COO*Me*/15-CO. Since the parent ion of compound **2** could not be detected by LC/MS analysis of the extract of *Sarcandra glabra* [4], **2** be an artifact of **1** formed during the purification process. Thus, compound **2** was determined as (3*E*,5*Z*)-2-[(1*E*)-2-hydroxy-3-(6-hydroxy-3,6-dimethyl-4,8-dioxooxocan-3-yl)prop-1-en-1-yl]-7-methoxy-2,5-dimethyl-7-oxohepta-3,5-dienoic acid, named sarcaboside B.

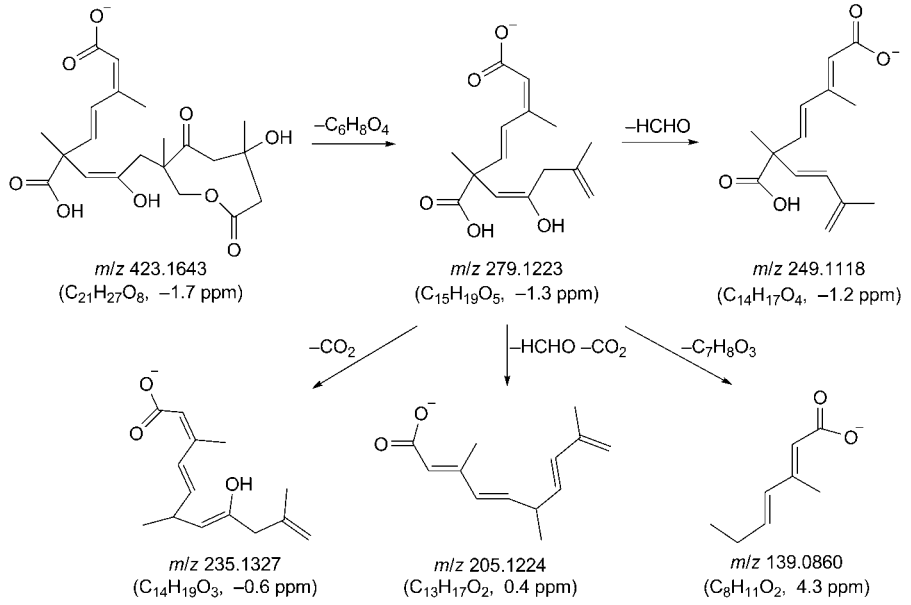


Fig. 3. *Proposed fragmentation pathway for compound* **1**

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## Experimental Part

*General.* Column chromatograpy (CC): silica gel ($SiO_2$, 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, China) and *Sephadex LH-20* (*Sigma*). TLC: Pre-coated silical gel $F_{254}$ plates ($SiO_2$, *Qingdao Haiyang Chemical Co., Ltd.*), detection at 254 nm and by spraying with 10% $H_2SO_4$ in EtOH, followed by heating. Semi-prep. HPLC: *Agilent-1200* apparatus; PDA detector at 260 nm; *Zorbax-SB-C-18* column (9.4 × 250 mm, 5 $\mu$m; *Agilent*). Optical rotations: *Jasco P-1020* digital polarimeter. UV Spectra: *Shimadzu-UV-2450* spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. $^1$H- and $^{13}$C-NMR spectra: *Bruker-AM-500* instrument at 500 and 125 MHz, resp.; in $CD_3OD$; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, $J$ in Hz. HR-ESI-MS: *LTQ Orbitrap XL* mass spectrometer (*Thermo Scientific*); in *m/z* (rel. %).

*Plant Material.* The whole plant of *Sarcandra glabra* from Sichuan Province was supplied by *Kangmei Pharmaceutical Co., Ltd*. A voucher specimen (No. 20081110) was deposited with the Center for Laboratory, Second Affiliated Hospital, Guangzhou University of Traditional Chinese Medicine.

*Extraction and Isolation.* The whole dried plants (6.7 kg) were extracted with 70% EtOH (4 × 50 l), under reflux for a total of 6 h, and the combined extracts were concentrated, and, after evaporation of EtOH *in vacuo*, the residue was suspended in $H_2O$, and partitioned successively with AcOEt and BuOH of the same volume three times. The BuOH extract (65 g) was subjected to CC ($SiO_2$ (500 g, 100–200 mesh); $CH_2Cl_2$/MeOH 15 : 1 → 10 : 3 → 10 : 3 → 1 : 1 → 0 : 1) to provide 33 fractions. *Fr. 3* was subjected to CC (*Sephadex LH-20*; MeOH). The subfraction was purified by prep. HPLC (MeOH/$H_2O$ 45 : 55) to afford compound **1** (33 mg) and **2** (9 mg).

*Sarcaboside A* (= *(2Z,4E)-6-[(1E)-2-Hydroxy-3-(6-hydroxy-3,6-dimethyl-4,8-dioxooxocan-3-yl)-prop-1-en-1-yl]-3,6-dimethylhepta-2,4-dienedioic Acid*; **1**). White amorphous powder (MeOH/$H_2O$). $[\alpha]_D^{25} = +24.5°$ ($c = 0.33$, MeOH). UV (MeOH): 263 (2.2). $^1$H- and $^{13}$C-NMR: *Table*. HR-ESI-MS:

423.1643 ($[M-H]^-$, $C_{21}H_{27}O_9^-$; calc. 423.1655). HR-ESI-MS$^2$: 279.1223 (100). HR-ESI-MS$^3$ (423 → 279): 249.1118 (25), 235.1327 (20), 205.1224 (28), 139.0860 (100).

*Sarcaboside B* (=*(3*E*,5*Z*)-2-[(1*E*)-2-Hydroxy-3-(6-hydroxy-3,6-dimethyl-4,8-dioxooxocan-3-yl)-prop-1-en-1-yl]-7-methoxy-2,5-dimethyl-7-oxohepta-3,5-dienoic Acid*; **2**). White amorphous powder (MeOH/$H_2O$). UV (MeOH): 249 (2.4). $^1$H- and $^{13}$C-NMR: *Table*. HR-ESI-MS: 437.1812 ($[M-H]^-$, $C_{22}H_{29}O_9^-$; calc. 437.1812).

## REFERENCES

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