Two New Flavan-Flavanones from *Sarcandra hainanensis*

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Chemical study of the whole plants of *Sarcandra hainanensis* yielded two new biflavonoids with a flavan-fla-vanone skeleton, sarcandrone C (**1**), D (**2**) and 6 known compounds (**3—8**). Structures were elucidated on the basis of NMR spectroscopic methods.

Key words   biflavonoid; flavan-flavanone; *Sarcandra hainanensis*; Chloranthaceae; NMR

The genus *Sarcandra* (family Chloranthaceae) of three species mainly grows in South and Southeast Asia. Two species of them, *S. glabra* (Thunb) Nakai and *S. hainanensis* (Pei) Swamy et Bailey, are distributed in south of China.[1] *S. hainanensis* is an endemic shrub or tree to China. It was used to invigorate blood circulation and eliminate blood-stasis in folk medicine.[2] Previous chemical studies of *Sarcandra* species have led to the isolation of chalcones and sesquiter-penes.[3—6] As a continuous study on the chemical con-stituents of *S. hainanensis*,[6,7] two new flavan-flavanones (**1**, **2**, Fig. 1) together with 6 known compounds (**3—8**) were iso-lated.

Results and Discussion

Compound **1** was obtained as colorless powder. The nega-tive ion high resolution-electrospray ionization (HR-ESI)-MS of **1** rise a [M−H]⁻ peak at *m/z* 553.1859 corresponding to $C_{33}H_{30}O_8$. In according with the molecular formula, 33 carbon signals in the ^{13}C-NMR spectrum of **1** were catego-rized by distortionless enhancement by polarization transfer (DEPT) experiments as one carbonyl, twelve sp^2 methines, twelve sp^2 quaternary, three methoxyls, two sp^3 methylenes and three sp^3 methines carbons. Apart from the three methoxyls, the remaining 30 carbons comprising its scaffold as two C_6–C_3–C_6 units with four benzene rings suggested that compound **1** was a flavanoid dimer.

The characteristic signals of a flavan moiety as a AB₂C

coupling system at δ_H 5.09, 2.32, 2.07 and 4.67 were ob-served in the ^{1}H-NMR spectrum of **1** (Table 1). Another spin system at δ_H 5.49 and 2.91 suggested a flavanone terminal unit. This flavanone was further supported by the signal at δ_C 189.7 (C-4, F) of **1** (Table 1). From the data discussed above, **1** was considered to be a flavan moiety joined to a flavanone nucleus.

The positions of the interflavan linkages were deduced from the heteronuclear multiple bond connectivity (HMBC) experiment. HMBC correlation peaks of the signal at δ_H 4.67 (H-4, C) with δ_C 162.4 (C-7, D), 109.9 (C-8, D) and 161.5 (C-9, D) combined with the HMBC correlation at δ_H 2.07 (H-3a, C) with δ_C 109.9 (C-8, D) suggested a C-4 (C)–C-8 (D) linkage between the units of flavan and flavanone of **1**.

In the ^{1}H-NMR spectrum of **1**, a multiplet at δ_H 7.41—7.30 (10H, m) and two singlets at δ_H 6.20 (1H, s), 6.03 (1H, s) appeared in aromatic region, these information indicated that **1** has two pentasubstituted and two monosubstituted ben-zene rings (Table 1). The HMBC experiment of **1** confirmed and extended these deductions. The HMBC correlations of the signal at δ_H 6.20 (C-6, A) with the signals at δ_C 154.7 (C-5, A), 149.8 (C-7, A), 129.3 (C-8, A) and 100.8 (C-10, A/C) illustrated this proton was connected to C-6 (A) (Fig. 2). While the HMBC correlations of the signal at δ_H 6.03 (C-6, D) to the signals at δ_C 160.5 (C-5, D), 162.4 (C-7, D), 109.9 (C-8, D) and 106.3 (C-10, D/F) implied this unsubsti-tuted proton of ring D was at C-6 (D) (Fig. 2). Additionally, the ^{1}H-NMR spectrum of **1** exhibited resonances of three methoxyl singlets at δ_H 3.87 (3H, s), 3.86 (3H, s) and 3.64 (3H, s). The HMBC correlations of these three singlets con-firmed the location of the methoxyl groups as C-5 (D), C-8 (A) and C-5 (A), respectively. These deductions were further verified by the nuclear Overhauser effects (NOEs) between 5-OCH₃ (D) and H-6 (D) together with the NOEs between 5-OCH₃ (A) and H-6 (A) (Fig. 3).

The relative stereochemistry of compound **1** could be de-duced by the coupling constants of the spin systems in its ^{1}H-NMR spectrum and the NOEs in its nuclear Overhauser ef-fect spectroscopy (NOESY) spectrum. $J_{2,3a(C)}=11.0$ Hz and $J_{2,3e(C)}=2.0$ Hz indicated H-2 retain as an axial bond. The equatorial orientation of H-4 was confirmed by its coupling constant of $J_{4,3a(C)}=5.5$ Hz and $J_{4,3e(C)}=2.0$ Hz.[8,9] Both H-3a (C) and H-3e (C) showed connectivity to H-2 (C) and H-4
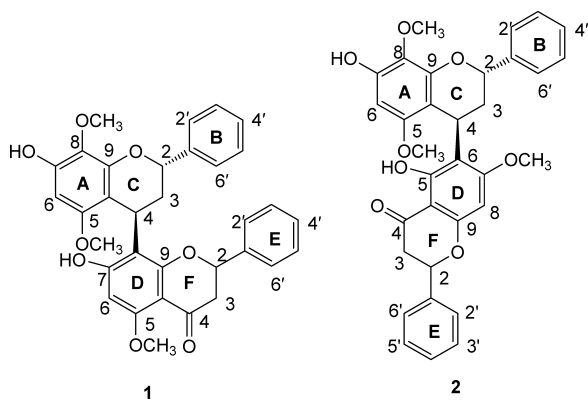

Fig. 1.   Structures of Compounds **1** and **2**

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** in CDCl$_3$ (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position		**1**		**2**	
Ring	Carbon	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
A/C	2	75.8	5.09, dd (11.0, 2.0)	75.8	5.08, dd (11.0, 2.0)
	3	37.3	2.32, dt (14.0, 2.0),	37.5	2.32, dt (13.5, 2.0)
			2.07, ddd (14.0, 11.0, 5.5)		2.07, ddd (13.5, 11.0, 6.0)
	4	27.4	4.67, dd (5.5, 2.0)	27.4	4.72, dd (6.0, 2.0)
	5	154.7		154.7	
	6	91.3	6.20, s	91.3	6.21, s
	7	149.8		149.8	
	8	129.3		129.3	
	9	148.9		149.0	
	10	100.8		100.8	
B	1′	140.8		140.7	
	2′, 6′	126.1	7.41—7.30, m	126.1	7.41—7.30, m
	3′, 5′	128.5		128.5	
	4′	128.0		128.0	
D/F	2	79.1	5.49, dd (13.0, 3.5)	78.6	5.46, dd (12.0, 3.0)
	3	45.9	2.91, m	45.1	3.01, m
	4 (C=O)	189.7		189.5	
	5	160.5		162.2	
	6	94.5	6.03, s	109.9	
	7	162.4		160.5	
	8	109.9		94.4	6.01, s
	9	161.5		161.2	
	10	106.3		106.4	
E	1′	139.2		138.9	
	2′, 6′	125.5	7.41—7.30, m	125.6	7.41—7.30, m
	3′, 5′	128.7		128.7	
	4′	128.3		128.3	
OMe	5(A)-OCH$_3$	56.1	3.64, s	56.1	3.69, s
	8(A)-OCH$_3$	61.4	3.86, s	61.4	3.86, s
	5(D)-OCH$_3$	56.0	3.87, s		
	7(D)-OCH$_3$			56.0	3.87, s
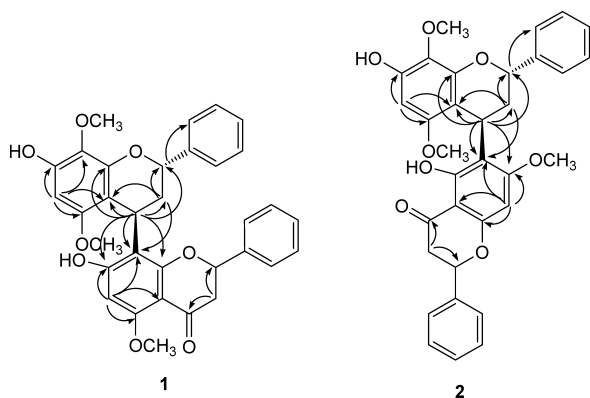
Fig. 2. Key HMBC Correlations (H→C) of **1** and **2**
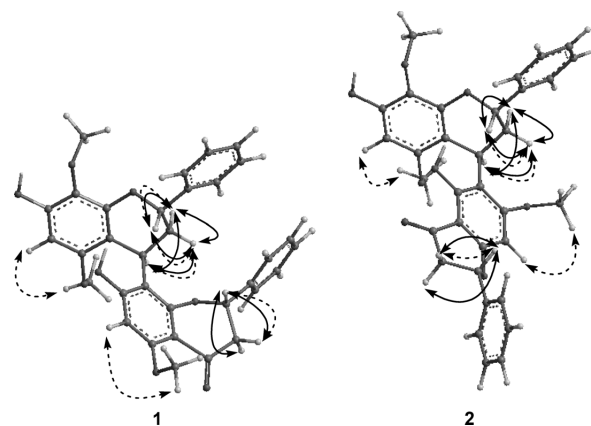
Fig. 3. Calculated Conformation by MM2 for Compounds **1** and **2**

(C) in ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of compound **1**. However, no NOE was observed between H-3a (C) and H-4 (C) while H-3e (C) exhibited NOEs to both H-2 and H-4 (Fig. 3). These results were in agreement with their relative stereochemistry. A *S* absolute configuration of compound **1** at C-4 (ring C) was determined by a negative Cotton effect of λ_{max} ($\Delta\varepsilon$) 214.5 (-2.192×10^4) nm and a positive Cotton effect of λ_{max} ($\Delta\varepsilon$) 209.0 (2.037×10^4) nm.[10] Therefore, it was presumed that compound **1** process a 2*S*,4*S* (C-ring) absolute configuration. Therefore, the structure of compound **1** was established as (2*S*,4*S*)-7-hydroxy-5,8-dimethoxyflavan(4→8)-7-hydroxy-5-methoxy-flavanone and it was

named as sarcandrone C (Fig. 1).

Compounds **2** possessed a molecular formula of C$_{33}$H$_{30}$O$_8$ as derived from HR-ESI-MS at *m/z* 553.1859 ([M−H]$^-$), which was exactly the same as compound **1**. With the structure of **1** in hand, the structure of compound **2** was easily deduced by its HMBC and NOESY data as well as comparison of the NMR data of **1** and **2**.

The ^{1}H- and ^{13}C-NMR data of **2** resembled those of **1**, suggesting a similar structural relationship. The ^{13}C-NMR spectrum of **2** differed from that of **1** by some shifted carbon signals. The deshielding shifted carbon signals at δ_C 45.1, 78.6,

94.4, 138.9, 161.2 and 189.5 were attributed to C-3 (F), C-2 (F), C-8 (D), C-1′ (E), C-9 (D/F) and C-4 (F), on the other hand, shielding shifted carbon signals at δ 37.5 and 106.4 were assigned to C-3 (C) and C-10 (D/F), respectively (Table 1). The distinctions mentioned above suggested that compound **2** differed from **1** by its interflavan linkage as C-4 (C)–C-6 (D) and the substitutions of ring D. The position of linkage in the flavanone moiety of ring D at C-6 was confirmed by the HMBC correlations of δ_H 6.01 (H-8, D) to δ_C 109.9 (C-6, D), 160.5 (C-7, D), 161.2 (C-9, D/F) and 106.4 (C-10, D/F) (Fig. 2). It is worthy to be mentioned that the location of the methoxyl group at C-7 (D) could be further defined by the NOESY spectrum of **2**, which showed correlation between δ_H 6.01 (H-8, D) and 3.87 (OMe-7, D) (Fig. 3).

The coupling constant of AB$_2$C spin systems in ring C [$J_{2,3a(C)}$=11.0 Hz; $J_{3a,4(C)}$=6.0 Hz] of **2** was similar to that of **1**. It suggested that **2** had an identical relative configuration as **1** had. The NOEs in the NOESY spectrum of **2** could confirm its relative configuration.[8,9] Different stereochemistry of **2** from that of **1** mainly reflected on the NOEs of H-2 (C) and H-4 (C). These two protons of **2** displayed weak NOEs to H-3e but no NOEs to H-3a. It might be caused by the weakness of the signals or difference of the dihedral angle between H-2 (C) and H-3a (C) (Fig. 3). And it could be deduced **2** also had a 2S,4S (ring C) absolute configuration from its Cotton effects [λ_{max} ($\Delta\varepsilon$) 216.5 (-3.140×10^4), 209.0 (2.730×10^4) nm].[10] Herein, the structure of compound **2** could be defined as (2S,4S)-7-hydroxy-5,8-dimethoxyflavan-(4→6)-5-hydroxy-7-methoxy-flavanone and it was named as sarcandrone D (Fig. 1).

By comparison with the NMR data reported in literatures,[11—13] the 6 known compounds were identified as 7-hydroxy-5,6-dimethoxyflavanone (**3**), 7-hydroxy-5-methoxyflavanone (**4**), naringenin-4′,7-dimethyl ether (**5**), kaempferol (**6**), 3,4′,5,7-tetrahydroxyflavanone-3-O-glucoside (**7**) and kaempferol 3-O-glucoside (**8**).

Experimental

General Experimental Procedures UV spectra were measured on a Shimadzu UV-2550 UV–visible spectrophotometer. Optical rotations were made on a Perkin-Elmer 341 polarimeter at room temperature. CD spectra were measured on a JASCO J-725 spectropolarimeter in MeOH. IR spectra were measured on a NEXUS-470 FTIR spectrophotometer. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR spectra were measured on a Bruker Avance DRX-500 spectrometer. Chemical shifts were referenced to the residual solvent signal (CDCl$_3$: δ_H 7.26, δ_C 77.0). HR-ESI-MS spectra were performed on APEX Q-FT-MS/MS spectrometer. All solvents used were of analytical grade (Beijing Chemical Plant, Beijing, People's Republic of China). Silica gel (300—400 mesh, Qingdao Marine Chemical Plant, Qingdao, People's Republic of China), C$_{18}$ reversed-phase silica gel (150—200 mesh, Merck), Sephadex LH-20 gel (pharmacia) and MCI gel (CHP20P, 75—150 μM, Mitsubishi Chemical Industries Ltd.) were used for column chromatography, and precoated silica gel GF$_{254}$ plates (Qingdao Marine Chemical Plant) were used for TLC. Chem 3D ultra 8.0 was used for calculating MM2.

Plant Material The whole plants of *Sarcandra hainanensis* were collected in Baishan couty, Hainan province, China, in April 2007 and were authenticated by Professor Pei-Gen Xiao of Institute of Medicinal Plant Development, Chinese Academy of Medicinal Science. A voucher specimen (SH070706) has been deposited in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College.

Extraction and Isolation The air dried, chipped whole plants of *S. hainanensis* (29 kg) were exhaustively extracted with 95% ethanol under reflux for twice (210 l×2). The extracts were evaporated to yield 2928 g of green residue. The EtOH extract was suspended in water saturated with

NaCl and then extracted with petroleum ether, CH$_2$Cl$_2$, EtOAc and *n*-BuOH in order.

The CH$_2$Cl$_2$-soluble portion was evaporated to leave a residue (720.3 g). A portion (611.0 g) of it was subjected to CC of silica gel (200—300 mesh, 3000 g). Five fractions (C1 to C5) were collected according to TLC. Fr. C3 was further chromatographed on silica gel column with a gradient of CH$_2$Cl$_2$–EtOAc with increasing polarity to give 15 subfractions (C3-1 to C3-15). Fr. C3-9 (8.0 g) was subjected to a CC (silica gel 200—300 mesh, 250 g) eluting with petroleum ether–EtOAc (5 : 2—1 : 2) to give 8 subfractions (C3-9-1 to C3-9-8). C3-9-3 was subjected to a column of Sephadex LH-20 eluting with CHCl$_3$–MeOH (2 : 3) to get compounds **3** (5.6 mg) and **4** (2.0 mg). C3-9-6 was chromatographed over a column chromatography (silica gel 200—300 mesh, 170 g) eluting with petroleum CHCl$_3$–MeOH (1 : 0—10 : 1) to give 4 subfractions (C3-9-6-1 to C3-9-6-4). C3-9-6-2 was further chromatographed on silica gel column with a gradient of EtOAc–MeOH (1 : 0—30 : 1) to get 3 subfractions. Purification of C3-9-6-2-2 on a Sephadex LH 20 eluted with CHCl$_3$–MeOH (2 : 3) yeilded compound **5** (5.2 mg). C3-9-7 was chromatographed over a dry column chromatography (silica gel 200—300 mesh, 250 g) eluting with petroleum ether–EtOAc (2 : 1) to give 3 subfractions (C3-9-7-1 to C3-9-7-3). Fr. C3-9-7-2 was purified by a column of Sephadex LH 20 eluted with CHCl$_3$–MeOH (2 : 3) and MeOH–H$_2$O (7 : 3) repeatedly to afford compounds **1** (10.3 mg) and **2** (3.5 mg).

The EtOAc-soluble portion was separated into neutral (En) and acidic (Ea) parts by extracting with NaHCO$_3$ aqueous solution and acidifying with HCl. Fr. Ea was subjected to a ODS column eluting with MeOH–H$_2$O (35 : 65—100 : 0) to afford Ea 1—Ea 8. Ea 5 was chromatograpied by a column of Sephadex LH 20 (MeOH : H$_2$O 7 : 3). Eleven subfractions were collected basing on TLC. Ea 5-11 was further purified by Sephadex LH 20 chromatography to afford compound **6** (10.4 mg). Fr. En (17.6 g) was subjected to a MCI HP20 column chromatography and 9 subfractions (En 1—En 9) were collected. En 2 (2.6 g) was submitted to a column of silica gel and eluted with CHCl$_3$–MeOH–H$_2$O (20 : 1 : 0.1—1 : 1 : 0.1) to furnish En 2-1—En 2-16. En 2-16 was chromatographed on a column of Sephadex LH 20 eluting with CHCl$_3$–MeOH (2 : 3) to get En 2-16-1—En 2-16-5. En 2-16-2 and En 2-16-4 were purified by Sephadex LH 20 column chromatography (CHCl$_3$–MeOH 2 : 3 and MeOH–H$_2$O 6 : 4) to yield compound **7** (43.6 mg) and **8** (8.9 mg), respectively.

Sarcandrone C (**1**): Colorless powder (MeOH); $[\alpha]_D^{20}$ +46 (c=8.60×10^{-3}, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.73), 289 (3.63) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 291.0 (1.632×10^4), 240.0 (2.862×10^4), 214.5 (-2.192×10^4), 209.0 (2.037×10^4); IR (KBr) ν_{max} (cm^{-1}) 3392, 2920, 2842, 1647, 1597, 1497, 1462, 1448, 1412, 1285, 1107, 1030, 698; ^{1}H- and ^{13}C-NMR data see Table 1; HR-ESI-MS m/z 553.1859 (Calcd for C$_{33}$H$_{30}$O$_8$ 553.1868, [M−H]$^-$).

Sarcandrone D (**2**): Colorless powder (MeOH); $[\alpha]_D^{20}$ −37 (c=8.00×10^{-3}, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.15), 289 (4.02) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 286.5 (2.190×10^4), 240.5 (-2.034×10^4), 216.5 (-3.140×10^4), 209.0 (2.730×10^4); IR (KBr) ν_{max} (cm^{-1}) 3421, 2956, 2852, 1641, 1596, 1498, 1463, 1448, 1412, 1285, 1107, 1030, 698; ^{1}H- and ^{13}C-NMR data see Table 1; HR-ESI-MS m/z 553.1859 (Calcd for C$_{33}$H$_{30}$O$_8$ 553.1868, [M−H]$^-$).

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