FULL PAPER

Two New 3,4-*seco*-Cycloartane Triterpenes from *Gardenia sootepensis*

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Two new 3,4-*seco*-cycloartane triterpenes, named sootepin F (**1**) and sootepin G (**2**), together with two known compounds, coronalolide methyl ester (**3**) and sootepin D (**4**), were isolated from the leaves and twigs of *Gardenia sootepensis*. Their structures were elucidated on the basis of 1D- and 2D-NMR experiments, including HMBC, HSQC, ^{1}H,^{1}H-COSY, and ROESY, as well as HR-MS.

Introduction. – The genus *Gardenia* (family Rubiaceae) comprises about 250 species, and most of them are growing in the tropical and subtropical area of the eastern hemisphere. Five species and one variant of *Gardenia* are found in China [1]. *Gardenia sootepensis* is a medicinal plant widely grown in Xishuangbanna, Yunnan, P. R. China, and used in folk medicine for treating diseases, such as blood congestion and swelling [2].

The *Gardenia* plants, such as *G. sootepensis*, *G. aubryi*, *G. obtusifolia*, and *G. tubifera*, have proven to be a rich source of 3,4-*seco*-cycloartane triterpenes [3 – 11]. Such compounds often have biological activities, such as cytotoxic and anti-HIV-1 effects [3][5 – 7][12]. As part of our ongoing program on the discovery of anticancer agents, two new 3,4-*seco*-cycloartane triterpenes, sootepins F and G (**1** and **2**) and two known compounds (*Fig. 1*) were isolated and identified from the leaves and twigs of *G. sootepensis*. Herein, we report the isolation and structure elucidation of these compounds.

Results and Discussion. – Sootepin F (**1**) was obtained as colorless oil, and was shown to possess the molecular formula $C_{31}H_{46}O_5$ by HR-EI-MS (*m/z* 498.3351 (M^+; calc. 498.3345)), indicating nine degrees of unsaturation. The IR spectrum showed absorption bands for methyl ester (1737 cm^{-1}), γ-lactone (1768 cm^{-1}), and α,β-unsaturated aldehyde (1685 cm^{-1}), respectively. The ^{1}H-NMR spectrum of **1** (*Table*) exhibited a pair of *doublet*s at δ(H) 0.52 (*d*, $J = 4.2$, 1 H) and 0.17 (*d*, $J = 4.2$, 1 H), characteristic of CH_2(19) H-atoms of the cyclopropane ring in a cycloartane

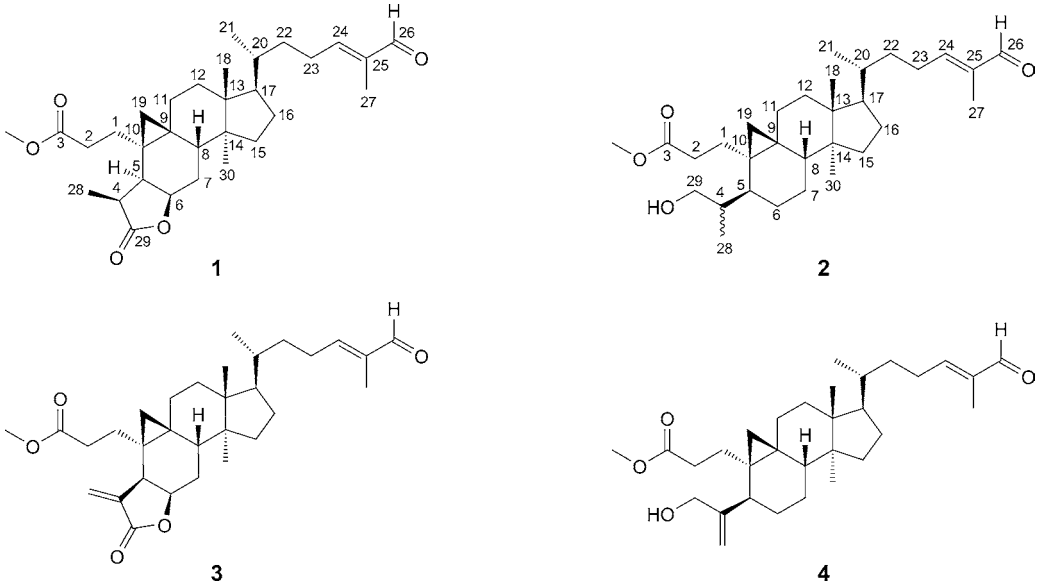

Fig. 1. *Structures of Compounds* **1** – **4**

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Table. *[1]H- and [13]C-NMR Data* (in $CDCl_3$) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position	Sootepin F (**1**)[a])		Sootepin G (**2**)[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.99 – 1.90 (*m*), 1.25 (overlapped)	31.8 (*t*)	2.22 – 2.12 (*m*), 1.37 – 1.28 (*m*)	28.2 (*t*)
2	2.34 – 2.29 (*m*), 2.25 – 2.19 (*m*)	31.3 (*t*)	2.50 – 2.42 (*m*), 2.28 – 2.17 (*m*)	31.8 (*t*)
3		173.6 (*s*)		175.0 (*s*)
4	2.80 – 2.72 (*m*)	40.6 (*d*)	2.13 – 2.02 (*m*)	37.1 (*d*)
5	2.50 (*t*, *J* = 6.5)	38.1 (*d*)	1.45 – 1.36 (*m*)	36.2 (*d*)
6	4.56 – 4.50 (*m*)	76.9 (*d*)	1.47 – 1.36 (*m*), 0.81 – 0.70 (*m*)	21.1 (*t*)
7	1.70 (overlapped), 1.35 – 1.30 (*m*)	27.5 (*t*)	2.12 – 2.03 (*m*), 1.20 – 1.11 (*m*)	27.1 (*t*)
8	1.59 – 1.51 (*m*)	38.1 (*d*)	1.44 – 1.35 (*m*)	48.5 (*d*)
9		22.6 (*s*)		21.4 (*s*)
10		23.2 (*s*)		27.3 (*s*)
11	2.34 – 2.27 (*m*), 2.12 – 2.06 (*m*)	26.0 (*t*)	1.29 – 1.20 (*m*), 1.04 – 0.92 (*m*)	25.3 (*t*)
12	1.59 – 1.51 (*m*)	32.9 (*t*)	1.67 – 1.57 (*m*)	33.2 (*t*)
13		45.2 (*s*)		45.3 (*s*)
14		48.2 (*s*)		49.1 (*s*)
15	1.26 – 1.19 (*m*)	35.8 (*t*)	1.32 – 1.22 (*m*)	35.9 (*t*)
16	1.87 – 1.76 (*m*), 1.26 – 1.17 (*m*)	28.1 (*t*)	1.93 – 1.80 (*m*), 1.28 – 1.20 (*m*)	28.3 (*t*)
17	1.58 – 1.45 (*m*)	52.4 (*d*)	1.62 – 1.50 (*m*)	52.3 (*d*)
18	0.84 (*s*)	18.9 (*q*)	0.92 (*s*)	18.5 (*q*)
19	0.52 (*d*, *J* = 4.2), 0.17 (*d*, *J* = 4.2)	30.7 (*t*)	0.58 (br. *s*), 0.36 (br. *s*),	30.4 (*t*)
20	1.41 – 1.31 (*m*)	35.8 (*d*)	1.96 – 1.85 (*m*)	35.8 (*d*)
21	0.80 (*d*, *J* = 6.2)	17.9 (*q*)	0.88 (*s*)	18.2 (*q*)
22	1.56 – 1.47 (*m*), 1.17 – 1.05 (*m*)	34.6 (*t*)	1.62 – 1.51 (*m*), 1.22 – 1.12 (*m*)	34.9 (*t*)
23	2.22 – 2.13 (*m*), 1.04 – 0.94 (*m*)	25.9 (*t*)	2.41 – 2.33 (*m*), 2.28 – 2.19 (*m*)	26.2 (*t*)
24	6.39 (*t*, *J* = 7.1)	155.5 (*d*)	6.46 (br. *s*)	155.9 (*d*)
25		138.9 (*s*)		139.2 (*s*)
26	9.27 (*s*)	195.3 (*d*)	9.35 (*s*)	195.7 (*d*)
27	1.62 (*s*)	9.1 (*q*)	1.71 (*s*)	9.3 (*q*)
28	1.25 (*d*, *J* = 7.2)	9.5 (*q*)	0.78 (*d*, *J* = 5.3)	12.0 (*q*)
29		180.3 (*s*)	3.44 (*d*, *J* = 6.0)	67.1 (*t*)
30	0.85 (*s*)	19.7 (*q*)	0.88 (*s*)	19.6 (*q*)
MeO	3.54 (*s*)	51.7 (*q*)	3.62 (*s*)	51.8 (*q*)

[a]) At 400 and 100 MHz, resp. [b]) At 600 and 150 MHz, resp.

triterpene [11]. The signal at δ(H) 9.27 (*s*, 1 H) and 3.54 (*s*, 3 H) confirmed the presence of an aldehyde and of a MeO group, respectively. The ^{13}C-NMR spectra data showed 31 C-atoms, including five Me, a MeO, ten CH_2, eight CH C-atoms, and seven C_q-atoms (*Table*). Taking into account the nine degrees of unsaturation and comparison of the NMR data with those of coronalolide methyl ester (**3**) reported in the literature, led to the conclusion that **1** was a 3,4-*seco*-cycloartane triterpene, with an extremely similar structure to that of **3**, the only differences occurring at C(4) and C(28) [3]. The C(4)=C(28) bond in **3** was replaced by Me(28)–CH(4) in **1** (*Fig. 1*). A *doublet* at δ(H) 1.25 (*d*, *J* = 7.2, 3 H) was ascribed to this Me(28) group in the γ-lactone ring, and the signal of H–C(α) appeared at δ(H) 2.80 – 2.72 (*m*, 1 H), the H–C(β) and H–C(γ) in the γ-lactone unit showed up at 2.50 (*t*, *J* = 6.5, 1 H) and at 4.56 – 4.50 (*m*, 1 H), respectively. Assignments of the ^{1}H- and ^{13}C-signals as shown in the *Table* were carried out on the basis of 2D-NMR data and by direct comparison of the chemical shifts with those of similar compounds reported in the literature [3]. The HMBCs from H–C(4), H–C(5) and H–C(28) to C(29) and the COSY correlations between H–C(28) and H–C(4), between H–C(4) and H–C(5) and between H–C(5) and H–C(6) suggested that the γ-lactone ring was attached to C(5) and C(6) (*Fig. 2*). The HMBCs from H–C(26) to C(25), C(27), and C(24) and from H–C(24) to C(25), C(26), and C(27) confirmed the presence of a conjugate system including one aldehyde C-atom and two olefinic C-atoms in the side chain. In addition, another series key HMBCs from H–C(1), H–C(2), and MeO to C(3) suggested that the MeO group was linked to C(3). Thus, the constitutional formula of **1** was established as shown in *Fig. 1*.

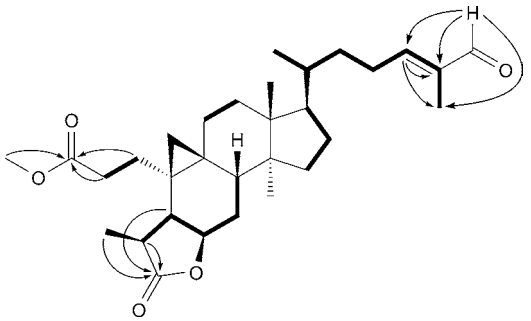

Fig. 2. *Key [1]H,[1]H-COSY* (—) *and HMB* (H → C) *correlations for* **1**

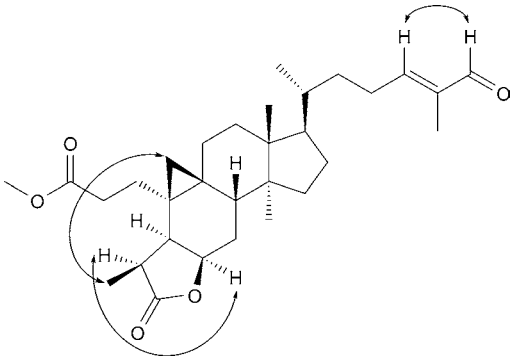

Fig. 3. *Key ROESY* (H ↔ H) *correlations for* **1**

The relative configuration of **1** was established on the basis of its ROESY spectrum (*Fig. 3*), whose correlations between H–C(4) and H–C(6), and between H–C(28) and H–C(19) indicated that the Me group at C(28) has β-orientation, same as C(19), but the H-atoms of H–C(4), H–C(5), and H–C(6) are positioned on the other side of the molecular plane.

Sootepin G (**2**) was also obtained as colorless oil. The molecular formula was established as $C_{31}H_{50}O_4$ according to the HR-EI-MS at *m*/*z* 486.3702 (M^+; calc. 486.3709), indicating seven degrees of unsaturation. The IR spectrum showed absorptions at 3448, 1738, 1688, and 1641 cm^{-1}, accounting for one OH group, one C=O group, one α,β-unsaturated aldehyde, and a C=C bond. The ^{1}H-NMR spectrum of **2** exhibited a characteristic pair of broad *singlet*s at δ(H) 0.58 and 0.36, suggesting the presence of CH_2(19) of the cyclopropane ring of a cycloartane triterpene [11]. A *singlet* at δ(H) 9.35 (*s*, 1 H) was assigned to the aldehyde group and a broad *singlet* at δ(H) 6.46 (br. *s*, H–C(24)) confirmed the presence of an α,β-unsaturated aldehyde. A signal for the MeO group was observed at δ(H) 3.62 (*s*, 3 H). The ^{13}C-NMR spectrum of **2** revealed the presence of six C_q-atoms, seven CH, twelve CH_2, and six Me groups. Two olefinic C-atoms were observed at δ(C) 155.9 and 139.2, together with an aldehyde C-atom at δ(C) 195.7. The signals at δ(C) 175.0 and 51.8 were assigned to a methyl ester. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of sootepin D (**4**) revealed them to be very similar. The only difference was the appearance of a signal due to a Me group at δ(C) 12.0, coupled in the HSQC spectrum to a newly appearing Me group at δ(H) 0.78 (*d*, $J = 5.3$, 3 H), while a pair of terminal olefinic C-atom signals at δ(C) 152.4 and 110.2 had disappeared. The relative configuration of **2** was established by its ROESY spectrum, comparison of H-atom coupling constants and other spectra data of **2** with those of **4**. The configuration at C(4) could not be established so far. All of the remaining signals have been confirmed by HMBC, ^{1}H,^{1}H-COSY, and ROESY spectra. Thus, the structure of **2** was determined as shown in *Fig. 1*.

The structures of the two known compounds were determined as coronalolide methyl ester (**3**) and sootepin D (**4**) by comparison of their spectra data with those reported in the literature [3][5].

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Experimental Part

General. TLC: silica-gel *G* plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Marine Chemical Co., Ltd.*). Optical rotation: *Horiba-SEAP-300* spectropolarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* sectrometer, KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* and *DRX-500* instruments; at 400 and 100 MHz, resp., and 500 and 125 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. FAB-MS: *VG AutoSpec-3000*; in *m*/*z*. HR-ESI-MS: *API Qstar-Pulsar LC/TOF* mass spectrometers; in *m*/*z*.

Plant Material. The leaves and twigs of *G. sootepensis* were collected in Xishuangbanna, Yunnan, P. R. China, in November 2009 and identified by Prof. *Yu-Min Shui*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 331081) was deposited with the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried leaves and twigs (14 kg) of *G. sootepensis* were extracted with MeOH at r.t. (4 × 40 l). The extracts were combined and concentrated, and the residue was suspended in H_2O, and then successively partitioned with petroleum ether (PE), AcOEt, and BuOH, resp. The AcOEt-soluble extract (767 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100 : 0 → 70 : 30) to afford seven fractions: *Frs. 1 – 7*. *Fr. 2* was passed through CC (SiO_2; $CHCl_3$/ AcOEt 30 : 1 → 10 : 1) to afford five fractions: *Frs. 2.1 – 2.5*. *Fr. 2.2* was subjected to CC (SiO_2; PE/AcOEt 10 : 1 → 3 : 1) to afford **1** (126 mg). Compounds **2** (12 mg), **3** (80 mg), and **4** (470 mg) were obtained from *Fr. 2.3* after repeated CC (SiO_2; $CHCl_3$/AcOEt 30 : 1 → 10 : 1) and *RP-18* (MeOH/H_2O 70 : 30 → 100 : 0).

Sootepin F (= *Methyl 3-[(1a*S*,3a*R*,4*R*,6a*S*,6b*S*,7a*R*,10*S*,10a*S*, 10b*R*)-Dodecahydro-3a,6a,10-trimethyl-4-[(5*E*)-6-methyl-7-oxohept-5-en-2-yl]-9-oxo-1*H*-cyclopenta[7,8]cyclopropa[4,4a]naphtho[2,3-*b*]furan-10b(2*H*)-yl]propanoate*; **1**). Colorless oil. UV ($CHCl_3$): 240.8 (3.91). $[\alpha]_D^{24.5} = +89.8$ ($c = 0.80$, $CHCl_3$). IR (KBr): 3448, 2946, 2877, 1768, 1737, 1685, 1644, 1171, 957, 755. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS: 521 ([M + Na]$^+$). HR-EI-MS (pos.): 498.3351 (M^+, $C_{31}H_{46}O_5^+$; calc. 498.3345).

Sootepin G (= *Methyl 3-[(1*R*,3a*S*,3b*S*,6S,6a*R*,7a*S*,9a*R*)-Decahydro-6-(1-hydroxypropan-2-yl)-3a,9a-dimethyl-1-[(5*E*)-6-methyl-7-oxohept-5-en-2-yl]-1*H*-cyclopenta[a]cyclopropa[e]naphthalen-6a(7*H*)-yl]propanoate*; **2**). Colorless oil. UV ($CHCl_3$): 241.4 (4.07). $[\alpha]_D^{24.6} = +36.8$ ($c = 0.34$, $CHCl_3$). IR (KBr): 3448, 2936, 2873, 1738, 1688, 1641, 1377, 1170, 1035. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 486 (M^+). HR-EI-MS (pos.): 486.3702 (M^+, $C_{31}H_{50}O_4^+$; calc. 486.3709).

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