New Phenolic Constituents from the Stems of *Spatholobus suberectus*

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Three new phenolic compounds, 5-*O*-(β-apiosyl-(1 → 2)-*O*-β-xylopyranosyl)gentisic acid (**1**), 1-*O*-(β-apiosyl-(1 → 6)-*O*-β-glucopyranosyl)-3-*O*-methylphloroglucinol (**2**), and 15-*O*-(α-rhamnopyranosyl)-aloe-emodin (**3**), together with the known compound aloe emodin (**4**), were isolated from the stems of *Spatholobus suberectus*. Their structures were characterized by chemical and spectroscopic methods. The absolute configurations of the sugar units were not determined.

1. Introduction. – *Spatholobus suberectus* (Fabaceae) is known to produce red, juice-like cock blood when its bark is broken. Therefore, in traditional Chinese medicine (TCM), it is called '*ji xue teng*', which means 'cock-blood stems'. The drug is said to augment the proliferation of colony-forming-units granulocyte-macrophages (CFU-GM), increase the peripheral white blood-cells (WBC), the bone marrow mononuclear cells (BMC) quantity, and the granulopoiesis divisional index [1]. It may not only irritate hematopoiesis, but also exert reconstructive action to marrow micro-environment [2]. *S. suberectus* has been reported to contain flavonoids, emodins, triterpenoids, and steroids [3].

In the present work, we report the isolation and characterization of three new phenolic compounds (**1**–**3**) and of one known constituent, aloe-emodin (**4**), from *S. suberectus*. Their structures were mainly elucidated on the basis of spectroscopic and chemical methods.

2. Results and Discussion. – Compound **1** was obtained as a colorless powder. Its molecular formula was assigned as $C_{17}H_{22}O_{12}$, on the basis of the $[M-H]^-$ ion peak at *m*/*z* 417.1064 (calc. 417.1033) in the HR-ESI mass spectrum. The ^{13}C-NMR and HMQC spectra of **1** (*Table 1*) showed the presence of 17 C-atoms, including one COOH, three CH_2, nine CH, and four quaternary C-atoms. Assignment of the aglycone moiety was achieved by comparison of ^{1}H- and ^{13}C-NMR data with those of gentisic acid 5-*O*-β-D-xylopyranoside reported by *Fayos et al.* [4a]. The NMR spectra showed that **1** also contained an apiose (Api) and a xylose (Xyl) moiety [4]. In the ^{1}H-NMR spectrum, anomeric H-atoms appeared at δ(H) 5.40 (*d*, *J* = 2.4 Hz, H–C(1″)) and 4.97 (*d*, *J* = 6.5 Hz, H–C(1′)), indicating an Api and a β-Xyl moiety, respectively.

The Xyl residue was attached to the aglycone at C(5), as confirmed by the HMBC correlation between H–C(1′) at δ(H) 4.97 and C(5) at δ(C) 149.0 (*Figure*). The HMBC cross-peak between H–C(2′) at δ(H) 3.60–3.62 and C(1″) at δ(C) 109.7 indicated that

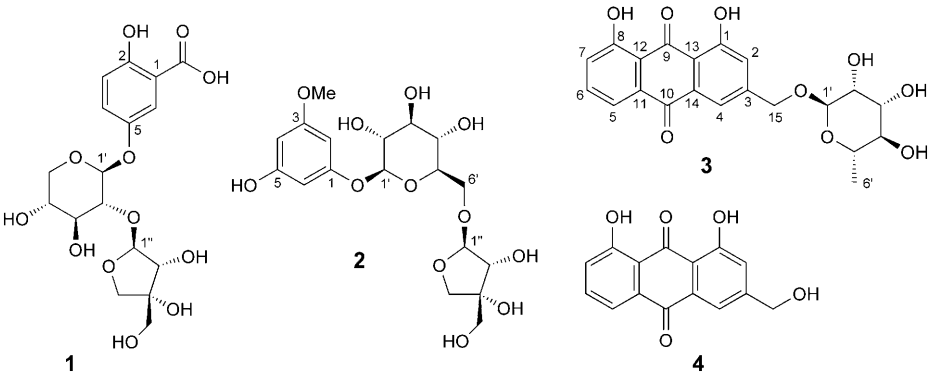

Table 1. *^{1}H and ^{13}C-NMR Data of* **1** *and* **2**. At 400 MHz in D_2O; δ in ppm, J in Hz[a]).

Atom[b])	**1**		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	118.6		160.9	
C(2) or H–C(2)	155.6		97.7	6.10 (*d*, *J* = 2.2)
H–C(3) or C(3)	117.5	6.91 (*d*, *J* = 8.9)	163.3	
H–C(4)	123.4	7.18 (*dd*, *J* = 8.9, 1.7)	98.6	6.05 (*d*, *J* = 2.2)
C(5)	149.0		159.9	
H–C(6)	118.1	7.53 (*d*, *J* = 1.6)	99.4	6.09 (*d*, *J* = 2.2)
1-COOH	174.6		–	
3-MeO	–		57.8	3.79 (*s*)
H–C(1′)	101.1	4.97 (*d*, *J* = 6.5)	102.5	4.86 (*d*, *J* = 7.4)
H–C(2′)	78.8	3.60–3.62 (*m*)	75.2	3.57 (*dd*, *J* = 7.7, 8.9)
H–C(3′)	75.9	3.60–3.66 (*m*)	77.9	3.62 (*t*, *J* = 8.9)
H–C(4′)	69.3	3.67–3.74 (*m*)	72.0	3.52 (*t*, *J* = 8.7, 8.4)
H–C(5′) or CH_2(5′)	65.4	3.41 (*t*), 3.98 (*dd*)	77.3	3.73–3.82 (*m*)
CH_2(6′)	–		69.9	4.07 (*dd*, *J* = 5.4, 14.3), 3.76 (*dd*, *J* = 5.7, 14.3)
H–C(1″)	109.7	5.40 (*d*, *J* = 2.4)	111.5	4.90 (*d*, *J* = 2.4)
H–C(2″)	77.4	4.05 (*d*, *J* = 2.4)	79.1	4.0 (*d*, *J* = 2.5)
C(3″)	79.8		81.7	
CH_2(4″)	74.1	3.87 (*d*, *J* = 10.1) 4.02 (*d*, *J* = 10.1)	76.0	4.03 (*d*, *J* = 10.2), 3.89 (*d*, *J* = 10.2)
CH_2(5″)	64.3	3.62 (*s*)	66.1	3.79 (*s*)

[a]) In case of overlapping signals, no multiplicities are given. [b]) Arbitrary numbering.

the Api unit was connected to C(2′) of the Xyl moiety, which was confirmed by a downfield shift of C(2′) by 5.6 ppm compared to the corresponding resonance in gentisic acid

5-*O*-*β*-D-xylopyranoside [4a]. Therefore, compound **1** was identified as 5-*O*-[*β*-apiosyl-(1 → 2)-*O*-*β*-xylopyranosyl]gentisic acid[1]).

Compound **2** was isolated as a brown-yellow powder. Its molecular formula was determined as $C_{18}H_{26}O_{12}$ by HR-ESI-MS, the $[M+H]^+$ ion peak being observed at *m*/*z* 435.1796 (calc. 435.1503). The ^{13}C-NMR and HMQC spectra of **2** showed the presence of 18 C-atoms, including one MeO, three CH_2, ten CH, and four quaternary C-atoms. Complete assignment of all ^{1}H- and ^{13}C-NMR resonances of the aglycone portion was achieved by comparison with the NMR data of 1-*O*-(*β*-D-glucopyranosyl)-3-*O*-methylphloroglucinol reported by *Sakar et al.* [5]. The NMR spectra showed that **2** included an Api and a *β*-glucose (Glc) moiety [6]. The ^{1}H-NMR spectrum showed the anomeric resonances at *δ*(H) 4.90 (*d*, *J* = 2.4 Hz, H–C(1″)) and 4.86 (*d*, *J* = 7.4 Hz, H–C(1′)). The HMBC spectrum showed a correlation between H–C(1′) of Glc and C(1) at *δ*(C) 160.9 of the aglycone, which indicated that the Glc unit was bonded to the aglycone. The HMBC cross-peak between H–C(1″) and C(6′) at *δ*(C) 69.9 showed that the Api residue was attached in 6′-position to Glc, as corroborated by a downfield shift of C(6′) by 7.3 ppm relative to the corresponding resonance in 1-*O*-(*β*-D-glucopyranosyl)-3-*O*-methylphloroglucinol [5]. On the basis of the above data, compound **2** was, thus, identified as 1-*O*-(*β*-apiosyl-(1 → 6)-*O*-*β*-glucopyranosyl)-3-*O*-methylphloroglucinol[1]).

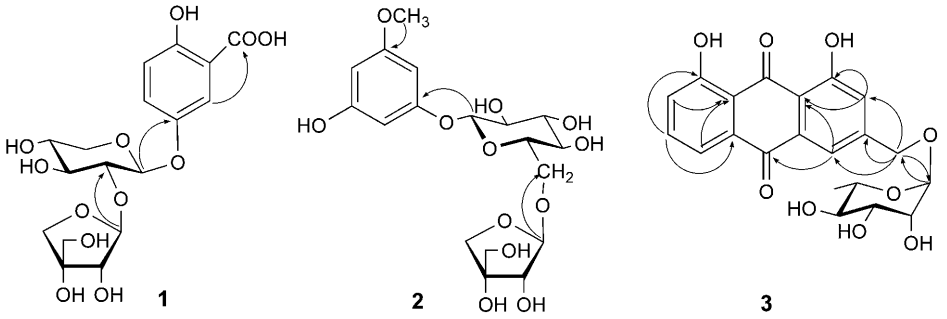

Figure. *Key HMBC correlations for* **1**–**3**

Compound **3** was obtained as a yellow powder. HR-ESI-MS showed the $[M+Na]^+$ ion peak at *m*/*z* 439.1005 (calc. 439.1005), corresponding to the molecular formula $C_{21}H_{20}O_9$. The IR spectrum of **3** showed absorption bands at 3392, 1670, and 1629 cm^{-1}, suggesting the presence of OH, and both free and H-bonded C=O functions. The UV spectrum indicated the presence of an anthraquinone moiety, with characteristic signals at λ_{max} 428, 285, 255, and 226 nm [7]. The ^{1}H- and ^{13}C-NMR spectra of the aglycone of **3** were consistent with those of aloe emodin (**4**) reported by *Danielsen* and *Aksnes* [8]. The ^{1}H- and ^{13}C-NMR spectra of **3** also exhibited a series of sugar signals at *δ*(H) 1.14–3.72 (*δ*(C) 17.9–70.4), the anomeric H-atom resonating at *δ*(H) 4.69 (*d*, *J* = 1.3 Hz; *δ*(C) 100.0). By means of HMQC, HMBC, and ^{1}H,^{1}H-COSY experiments,

[1]) For systematic names, see the *Exper. Part.*

Table 2. *^{1}H and ^{13}C-NMR Data of* **3** *and* **4**. At 400 MHz in (D_6)DMSO; δ in ppm, J in Hz.

Atom[a])	**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	161.5	–	161.6	–
H–C(2)	121.8	7.28 (*d*, *J* = 1.0)	120.6	7.3 (*s*)
C(3)	148.8	–	153.7	–
H–C(4)	117.7	7.64 (*d*, *J* = 1.0)	117.1	7.65 (*s*)
H–C(5)	119.4	7.69 (*dd*, *J* = 7.5, 0.8)	119.3	7.7 (*dd*, *J* = 7.7, 1.0)
H–C(6)	137.5	7.79 (*dd*, *J* = 7.8, 8.1)	137.3	7.8 (*dd*, *J* = 8.2, 7.7)
H–C(7)	124.5	7.36 (*dd*, *J* = 8.2, 0.8)	124.4	7.4 (*dd*, *J* = 7.2, 1.0)
C(8)	161.4	–	161.3	–
C(9)	191.7	–	191.6	–
C(10)	181.4	–	181.4	–
C(11)	133.3	–	133.1	–
C(12)	115.9	–	115.8	–
C(13)	115.0	–	114.4	–
C(14)	133.3	–	133.3	–
CH_2(15)	66.9	4.72 (*d*, *J* = 14.1), 4.57 (*d*, *J* = 14.1)	62.0	4.62 (*s*)
H–C(1′)	100.0	4.69 (*d*, *J* = 1.3)	–	–
H–C(2′)	70.4	3.72 (*dd*, *J* = 3.0, 1.4)	–	–
H–C(3′)	70.8	3.50 (*dd*, *J* = 9.4, 3.3)	–	–
H–C(4′)	71.9	3.21 (*d*, *J* = 9.4, 9.3)	–	–
H–C(5′)	69.0	3.42 (*dq*, *J* = 9.3, 6.3)	–	–
Me(6′)	17.9	1.14 (*d*, *J* = 6.4)	–	–

[a]) Arbitrary numbering.

these signals could be assigned to a rhamnose (Rha) moiety [9]. The configuration of the anomeric center of Rha was concluded to be α, based on the characteristic ^{13}C-NMR chemical shifts of C(3′) (δ(C) 70.8) and C(5′) (69.0). The HMBC cross-peak of **3** between CH_2(15) of the aglycone and C(1′) of Rha indicated that the sugar unit was bonded to C(15) (*Figure*), as corroborated by a change in chemical shift from 62.0 to 66.9 ppm for C(15), when compared to **4**. Finally, acid hydrolysis of **3** with 2N aqueous HCl for 4 h at 90° produced **4** and Rha (identified by co-TLC). So, compound **3** was identified as 15-*O*-(α-rhamnopyranosyl)aloe-emodin[1]).

Aloe emodin (**4**) was obtained in the form of red-brown needles. This compound had been isolated before from *Aloe* and *Rheum* species [10–13], but not from *S. suberectus*.

Experimental Part

General. Reverse-phase column chromatography (CC): *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries Co., Ltd.*), *HW-40F* (30–60 μm; *Tosoh Co., Ltd.*). TLC: silica gel GF_{254}; visualization under UV light, with I_2 vapor, or by spraying with anisaldehyde/H_2SO_4. UV Spectra: *Shimadzu UV-2450*

spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi 275-50* spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR, COSY, HMQC, and HMBC Spectra: *Bruker DRX-400* spectrometer; δ in ppm, J in Hz. ESI-MS: *Finnigan LCQ-DECA* spectrometer; in m/z.

Plant Material. The stems of *S. suberectus* were collected from Guangxi Province, P. R. China, and were identified by *Yang He-Ming*. A voucher specimen was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Science.

Extraction and Isolation. The air-dried stems of *S. suberectus* (5.0 kg) were powdered and extracted three times with 70% (*v*/*v*) aq. acetone at r.t. for 3 d each. After removal of the acetone in vacuum, the suspended residue was discarded by centrifugation. The aq. soln. was subjected to CC (*MCI* gel; MeOH/H_2O gradient). The fraction eluted with H_2O was resubjected to CC (*HW-40F*; H_2O) to afford **1** (82 mg) and **2** (154 mg). The original fraction eluted with 40% aq. MeOH was subjected to CC (*HW-40F*; 20% aq. MeOH) to provide **3** (35 mg). Finally, the original fraction eluted with 100% MeOH afforded **4** (2130 mg).

Acidic Hydrolyses. Each sample (1 mg) was treated with 2N aq. HCl at 90° for 4 h. The mixture was neutralized with $NaHCO_3$, and extracted with BuOH. The H_2O of the aq. portion was removed under reduced pressure, and the residue was extracted with pyridine. Then, the soln. was analyzed by TLC, co-eluting with authentic monosaccharide samples.

5-O-(β-Apiosyl-(1 → 2)-O-β-xylopyranosyl)gentisic Acid (*=5-[(2-O-β-Apiosyl-β-xylopyranosyl)-oxy]-2-hydroxybenzoic acid*; **1**). Colorless, amorphous powder. UV (MeOH): 314 (3.50), 230 (3.78). IR (KBr): 3388, 2883, 1631, 1579, 1490, 1446. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (neg.): 417 ($[M-H]^-$), 153 ($[M-C_{10}H_{17}O_8]^-$), 152 ($[M-H-C_{10}H_{17}O_8]^-$), 108 ($[M-H-C_{10}H_{17}O_8-COOH]^-$). HR-ESI-MS (neg.): 417.1064 ($[M-H]^-$, $C_{17}H_{21}O_{12}^-$; calc. 417.1033).

1-O-(β-Apiosyl-(1 → 6)-O-β-glucopyranosyl)-3-O-methylphloroglucinol (*=3-[(6-O-β-Apiosyl-β-glucopyranosyl)oxy]-6-methoxyphenol*; **2**). Brown-yellow, amorphous powder. UV (MeOH): 267 (3.1), 225 (3.9). IR (KBr): 3396, 2933, 1606, 1460, 827. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 435.1796 ($[M+H]^+$, $C_{18}H_{27}O_{12}^+$; calc. 435.1503).

15-O-(α-Rhamnopyranosyl)aloe-emodin (*=1,8-Dihydroxy-3-{[(α-rhamnopyranosyl)oxy]methyl}anthracene-9,10-dione*; **3**). Yellow, amorphous powder. UV (MeOH): 428 (3.74), 285 (3.63), 255 (3.98), 226 (4.36). IR (KBr): 3392, 1670, 1629. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS (pos.): 439.1005 ($[M+Na]^+$, $C_{21}H_{20}NaO_9^+$; calc. 439.1005).

Aloe Emodin (**4**). Red-brown needles. UV (MeOH): 430, 286, 255, 225. IR (KBr): 3330, 1676, 1627, 1573. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 270 (100), 252 (4), 241 (90), 224 (10), 213 (12), 139 (18).

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