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Two novel immunosuppressive pregnane glycosides from the roots of *Stephanotis mucronata*

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Abstract—Two novel pregnane glycosides, together with one new aglycone, were isolated from the roots of *Stephanotis mucronata*. Their structures were determined by means of chemical evidence and extensive spectroscopic methods including 1D and 2D NMR. The two pregnane glycosides displayed significant immunosuppressive activities in vitro.

The dried roots and stems of *Stephanotis mucronata* (Blanco) Merr. (Asclepiadaceae) are used for the treatment of rheumatoid arthritis and relieve rheumatic aches in folk medicine in the region of southern China. The crude steroidal glycosides from the stems of *S. mucronata* were evaluated by murine anti-inflammation and adjuvant-induced arthritis experiments and showed activities (not reported). In previous paper, we reported the isolation and structural elucidation of 16 pregnane glycosides, mucronatosides A–J and stephanoside E from the stems[1,2] and stemucronatosides A–G from the roots[3,4] of *S. mucronata*, and their immunological activities. In a continuation of our interest in the biological active constituents, chemical studies[5] on $CHCl_3$-soluble material of the ethanolic extract from the roots of this plant were undertaken by screening with immunological tests in vitro[6] to lead to two novel pregnane glycosides. Their chemical structures were determined by acid hydrolysis and NMR spectra, including HMQC, HMBC, and ^1H–^1H COSY techniques. Compounds **1** and **2** displayed significant immunosuppressive activities in vitro by mice splenocyte proliferation assay.

Compound **1**, $[\alpha]_D^{20}$ −50.1 (*c* 0.1, MeOH), mp 183–185 °C, was isolated as an amorphous powder, and had positive Liebermann–Buchard and Keller–Kiliani reactions, suggesting the presence of a steroid skeleton and 2-deoxy sugar moieties in the molecule. Compound **1** showed an intense blue fluorescence in methanol solution, indicating the presence of an (*N*-methyl) anthraninoyl group.[7] In the IR spectra of **1**, there were absorption bands at 1728 (C=O) and strong absorption bands at 3425 (OH) and 1085 (C–O–C) cm^{-1}. Acid hydrolysis[8] of **1** afforded an aglycone (**3**), mp 157–159 °C, $[\alpha]_D^{20}$ −11.0 (*c* 0.1, MeOH). Compound **3** had a molecular formula $C_{31}H_{45}NO_8$ (582.3053 $[C_{31}H_{45}NO_8+Na]^+$, calcd 582.3037) on the basis of the HR-ESI-MS, ^{13}C NMR, DEPT, and quasimolecular ion peak at *m/z* 560.0 $[M+H]^+$, 582.0 $[M+Na]^+$ in the ESI-MS spectra. ^{13}C and ^1H NMR data[9] of **3** showed three methyls, eight methylenes, two methines, and two quaternary carbons, three oxygenated quaternary carbons, and three oxygenated methine carbons in the steroid nucleus, and two acyl substituents in the side chain. The acyl substituents in **3** were assigned as an acetyl group, which was determined from ^1H and ^{13}C NMR signals at δ 2.08 (s, H-2′), 171.5 (C=O), 22.2 (C-2′), and an (*N*-methyl) anthraniloyl group, which was characterized by the UV absorption bands at 222, 253, and 355 nm; the ^1H NMR proton signals at δ 6.70 (d, J = 8.5 Hz, H-3″), 7.38 (ddd, J = 8.5, 8.0, 1.5 Hz, H-4″), 6.53 (t, J = 7.5 Hz, H-5″), 8.30 (d, J = 8.0 Hz, H-6″), 2.76 (d, J = 5.0 Hz, *N*–CH_3); and the ^{13}C NMR carbon signals at δ 111.1 (C-1″), 152.9 (C-2″), 111.7 (C-3″), 135.4 (C-4″), 114.9 (C-5″), 132.8 (C-6″), 168.4 (C=O), 29.7 (*N*–CH_3). Comparison of ^{13}C and ^1H NMR data of **3** with those of stephanthraniline A indicated that **3** had a distinct similarity to stephanthraniline A,[10] except

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Table 1. NMR data of compound **1** (δ in parts per million, J in hertz, pyridine-d_5)

Position	^{13}C[a]	^{1}H[b]	HMBC	^{1}H–^{1}H COSY
Aglycone moieties				
1	37.8 (t)[c]			
2	29.5 (t)			
3	76.6 (d)	3.88, m	C-2, 5, Sa-C1	
4	34.4 (t)			
5	45.2 (d)			
6	25.1 (t)			
7	34.4 (t)			
8	76.0 (s)			
9	46.8 (d)			
10	36.4 (s)			
11	24.5 (t)			
12	74.7 (d)	5.16, dd (11.5, 4.5)	C-1′, 18	
13	57.2 (s)			
14	88.7 (s)			
15	33.5 (t)			
16	33.9 (t)			
17	87.8 (s)			
18	11.8 (q)	2.05, s	C-12, C-17	
19	12.9 (q)	1.14, s	C-5, C-9, C-10	
20	74.8 (d)	5.21, q (6.0)	C-7″, 21	H-21
21	15.9 (q)	1.55, d (5.5)	C-17, 20	H-20
12-*O*-Acetyl				
1′	171.3 (s)			
2′	22.0 (q)	2.11, s	C-1′	
20-*O*-(*N*-Methyl) anthraniloyl				
1″	110.8 (s)			
2″	152.6 (s)			
3″	111.5 (d)	6.74, d (8.5)	C-1″, 5″	H-4″
4″	135.1 (d)	7.43, ddd (8.5, 8.0, 1.5)	C-2″, 6″	H-5″
5″	114.7 (d)	6.57, t (7.5)	C-3″	H-4″, H-6″
6″	132.5 (d)	8.33, d (7.5)	C-2″, 4″, 7″	H-5″
7″	168.2 (s)			
8″	NCH_3:29.5 (q)	NCH_3:2.81, d (5.0)	C-2″	
Sugar moieties				
cym				
Sa-1	95.9 (d)	5.32, d (9.0)	C-3, Sa-C2	Sa-H2
Sa-2	37.0 (t)	2.23, m; 1.73, m	Sa-C1, C3	
Sa-3	78.2 (d)	4.10, m	Sa-C2, C4, OCH_3	Sa-H6
Sa-4	83.5 (d)	3.53, d (8.5)	Sb-C1, Sa-C5, C6, OCH_3	
Sa-5	69.1 (d)	4.28, m	Sa-C1, C6	
Sa-6	18.7 (q)	1.45, d (6.0)	Sa-C4, C5	
Sa-OCH_3	59.0 (q)	3.65, s	Sa-C3	
cym				
Sb-1	100.4 (d)	5.14, d (9.5)	Sa-C4, Sb-C2	Sb-H2
Sb-2	37.3 (t)	2.23, m; 1.80, m	Sb-C1, C3	
Sb-3	78.1 (d)	4.06, m	Sb-C2, C4, OCH_3	Sb-H6
Sb-4	83.1 (d)	3.92, d (9.0)	Sc-C1, Sb-C5, C6	
Sb-5	69.3 (d)	4.20, m	Sb-C1, C6	
Sb-6	18.5 (q)	1.59, d (6.0)	Sb-C4, C5	
Sb-OCH_3	58.9 (q)	3.58, s	Sb-C3	Sb-H3
allm				
Sc-1	104.4 (d)	5.17, d (7.5)	Sb-C4, Sc-C2	Sc-H2
Sc-2	75.0 (d)	3.97, m	Sc-C1, C3	Sc-H1
Sc-3	85.9 (d)	3.74, t (8.5)	Sc-C2, C4, OCH_3	
Sc-4	83.0 (d)	3.55, d (10.5)	Sd-C1, Sc-C5, C6	Sc-H2
Sc-5	72.0 (d)	3.80, m	Sc-C1, C6	
Sc-6	18.7 (q)	1.77, d (6.0)	Sc-C4, C5	
Sc-OCH_3	60.6 (q)	3.97, s	Sc-C3	Sc-H3
glc				
Sd-1	106.0 (d)	4.73, d (7.5)	Sc-C4, Sd-C5	Sd-H5
Sd-2	75.8 (d)	4.08, m	Sd-C1, C3	

(*continued on next page*)

Table 1 (*continued*)

Position	^{13}C[a]	^{1}H[b]	HMBC	^{1}H–^{1}H COSY
Sd-3	78.7 (d)	4.26, m	Sd-C1, C2, C4	
Sd-4	72.0 (d)	4.24, m	Sd-C3, C5	
Sd-5	78.1 (d)	4.05, m	Sd-C4, C6	
Sd-6	63.1 (t)	4.56, d (10.5); 4.38, dd (11.0, 5.0)	Sd-C4; Sd-C4, C5	

[a] 125 MHz.
[b] 500 MHz.
[c] Multiplicities by DEPT experiments in parentheses: s-quaternary, d-CH, t-CH_2, and q-CH_3 C-atoms.

for absence of the double bond between C-5 and C-6. The most significant differences in the ^{13}C NMR data between 5α-H and 5β-H steroid nucleus involve the resonance of C-5 and C-19. The chemical shifts of C-5 and C-19 of 5α-H steroid nucleus are at δ ~44.9 and 12.3, respectively, but those of C-5 and C-19 of 5β-H steroid nucleus appear at δ ~36.5 and 23.9, respectively.[11] The chemical shifts of C-5 and C-19 in compound **3** appear at δ 46.0 and 13.4, respectively, suggesting the α-configuration of hydrogen at C-5. Thus, the stereochemistry of A/B ring in compound **3** was *trans* fused. The location of acetyl and (*N*-methyl) anthraniloyl substituents was deduced by HMBC correlations between δ 168.4 [C-7″ of (*N*-methyl) anthraniloyl group] and δ 5.20 (H-20 of steroid nucleus), δ 171.5 (C-1′ of acetyl group) and δ 5.10 (H-12 of steroid nucleus), establishing that the (*N*-methyl) anthraniloyl group was located at C-20 and the acetyl group was located at C-12 in **3**. Thus, compound **3** was established as 12-*O*-acetyl-20-*O*-(*N*-methyl) anthraniloyl-5,6-dihydrosarcostin.

Compound **1** had a molecular formula $C_{58}H_{91}NO_{23}$ (1192.5914 $[C_{58}H_{91}NO_{23}+Na]^+$, calcd 1192.5874) on the basis of the HR-ESI-MS, ^{13}C NMR, DEPT, and quasimolecular ion peak at *m*/*z* 1192.6 $[M+Na]^+$ in the ESI-MS spectrum. The ^{13}C NMR and DEPT spectrum of **1** showed 58 carbon signals, which consisted of 11 methyl carbon signals, 11 methylene carbon ones, 27 methine carbon ones, and 9 quaternary carbon ones. The carbon and proton signals of **1** in the NMR spectra were assigned by extensive techniques including HMQC, HMBC, and ^{1}H–^{1}H COSY (Table 1). The ^{1}H and ^{13}C NMR spectra displayed four anomeric protons at δ 5.32 (d, J = 9.0 Hz), 5.14 (d, J = 9.5 Hz), 5.17 (d, J = 7.5 Hz), and 4.73 (1H, d, J = 7.5 Hz), and corresponding anomeric carbons at δ 95.9, 100.4, 104.4, and 106.0, respectively. The β-linkages of all the sugars were revealed by the coupling constants of the anomeric protons (7.5–9.5 Hz). There were three secondary methyl signals at δ 1.45 (d, J = 6.0 Hz), 1.59 (d, J = 6.0 Hz), 1.77 (d, J = 6.0 Hz), three methoxyl signals at δ 3.58, 3.65, 3.97 (each s) in the ^{1}H NMR spectra, and two methylene signals at δ 37.0 (Sa-C2), 37.3 (Sb-C2) in ^{13}C NMR spectra, suggesting the presence of two 2,6-dideoxy-3-*O*-methyl-ribo-hexose units, one 6-deoxy-3-*O*-methyl-ribo-hexose unit, and one hexose unit in the molecule. TLC chromatography of the hydrolyzate[8] revealed the existence of three kinds of monosaccharides, two of which were identified as glucose and cymarose by comparison with authentic samples. The HMBC and ^{1}H,^{1}H-COSY experiment allowed the sequential assignments of ^{13}C and ^{1}H resonances for the unidentified sugar as shown in Table 1, starting from the anomeric proton and carbon signal at δ 5.17 (d, J = 7.5 Hz) and 104.4. Those findings suggested that the unidentified sugar (detected on TLC) is 6-deoxy-3-*O*-methyl-β-D-allose (abbreviated as allm) on the basis of its ^{1}H NMR data together with ^{13}C NMR assignments in agreement with those in other compounds.[10] Further, a comparison of the chemical shifts of the anomeric protons of other compounds showed the anomeric proton signal of allm appears at lower field than 5.00 ppm, while that of thv (β-D-thevetose) appears at higher field than 5.00 ppm in pyridine-d_5.[10,12] This confirmed the assignment of the anomeric proton at δ 5.17 (d, J = 7.5 Hz) to allm.[10] Corroborative evidence from HMQC, HMBC, and ^{1}H, ^{1}H-COSY spectra, which led to the full ^{1}H and ^{13}C assignment of each sugar moiety (Table 1), and TLC chromatography of the hydrolysate showed that the four sugars were two β-cymarose, one 6-deoxy-3-*O*-methyl-β-allose, and one β-glucose, respectively. In the HMBC spectra, correlations were observed between the following protons and carbons: δ 5.32 (Sa-H1 of the β-cymaropyranose) and δ 77.6 (C-3 of the aglycone), δ 5.14 (Sb-H1 of the β-cymaropyranose) and δ 83.5 (Sa-C4 of the β-cymaropyranose), δ 5.17 (Sc-H1 of the 6-deoxy-3-*O*-methyl-β-allopyranose) and δ 83.1 (Sb-C4 of the β-cymaropyranose), δ 4.73 (Sc-H1 of the β-glucopyranose) and δ 83.0 (Sc-C4 of the 6-deoxy-3-*O*-methyl-β-allopyranose). Compared to those of **3** in ^{13}C NMR spectrum, the glycosylation shifts of the aglycone

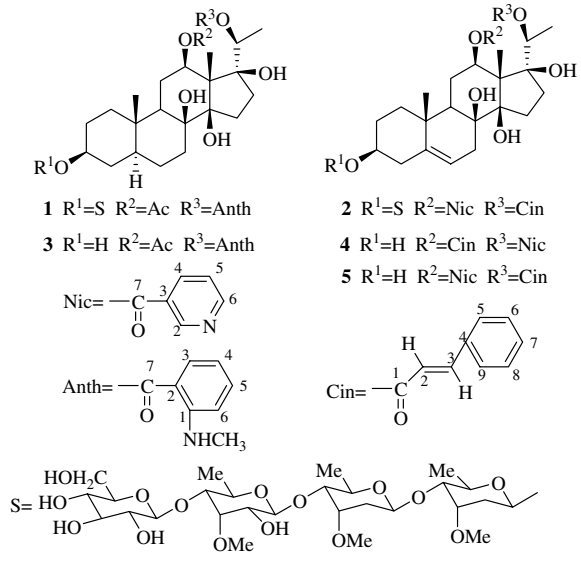

Figure 1. The structures of compounds **1**–**5**.

Table 2. NMR data of compound **2** (δ in parts per million, J in hertz, pyridine-d_5)

Position	^{13}C[a]	^{1}H[b]	HMBC	^{1}H–^{1}H COSY
Aglycone moieties				
1	39.2 (t)[c]			
2	29.8 (t)			
3	77.6 (d)	3.88, m	C-2, 5, Sa-C1	
4	38.7 (t)			
5	139.3 (s)			
6	119.3 (d)	5.40, br s	C-8, 10	
7	34.8 (t)			
8	74.3 (s)			
9	44.0 (d)			
10	37.2 (s)			
11	25.7 (t)			
12	74.6 (d)	5.36, dd (11.0, 5.0)	C-2′, 18	
13	57.1 (s)			
14	87.4 (s)			
15	33.6 (t)			
16	34.0 (t)			
17	88.9 (s)			
18	11.5 (q)	2.16, s	C-12, C-17	
19	18.0 (q)	1.32, s	C-5, C-9, C-10	
20	76.4 (d)	5.34, q (6.0)	C-1″, 21	H-21
21	15.3 (q)	1.60, d (6.0)	C-17, 20	H-20
12-*O*-Nicotinoyl				
2′	151.4 (d)	9.57, d (1.5)	C-3′, 4′,6′	
3′	126.9 (s)			
4′	137.4 (d)	8.35, d (7.5)	C-2′, 6′, 7′	H-5′
5′	123.8 (d)	7.24, dd (8.0, 5.0)	C-3′	H-6′
6′	153.8 (d)	8.87, dd (5.0, 1.0)	C-2′, 4′, 5′	H-4′
7′	164.7 (s)			
20-*O*-Cinnamoyl				
1″	166.8 (s)			
2″	120.2 (d)	6.71, d (13.0)	C-1″	H-3″
3″	144.1 (d)	7.88, d (16.0)	C-2″, 6″, 8″	H-2″
4″	134.8 (s)			
5″, 9″	129.3 (d)	7.39, m	C-4″, 7″	H-6″, 8″
6″, 8″	128.6 (d)	7.56, m	C-3″, 7″	
7″	130.6 (d)	7.36, d (3.5)	C-5″, 9″	
Sugar moieties				
cym				
Sa-1	96.3 (d)	5.31, d (10.0)	C-3, Sa-C2	Sa-H2
Sa-2	37.1 (t)	2.38, m; 1.77, m	Sa-C1, C3	
Sa-3	78.0 (d)	4.05, m	Sa-C2, C4, OCH_3	Sa-H6
Sa-4	82.8 (d)	3.54, d (9.0)	Sb-C1, Sa-C5, C6, OCH_3	
Sa-5	69.0 (d)	4.23, m	Sa-C1, C6	
Sa-6	18.2 (q)	1.41, d (6.0)	Sa-C4, C5	
Sa-OCH_3	58.8 (q)	3.60, s	Sa-C3	Sa-H3
cym				
Sb-1	100.4 (d)	5.13, d (8.0)	Sa-C4, Sb-C2	Sb-H2
Sb-2	36.9 (t)	2.36, m; 1.83, m	Sb-C1, C3	
Sb-3	77.9 (d)	4.06, m	Sb-C2, C4, OCH_3	Sb-H6
Sb-4	83.1 (d)	3.94, d (10.0)	Sc-C1, Sb-C5, C6	
Sb-5	69.3 (d)	4.21, m	Sb-C1, C6	
Sb-6	18.5 (q)	1.60, d (6.0)	Sb-C4, C5	
Sb-OCH_3	58.9 (q)	3.64, s	Sb-C3	Sb-H3
allm				
Sc-1	104.7 (d)	5.15, d (7.5)	Sb-C4, Sc-C2	Sc-H2
Sc-2	74.7 (d)	3.94, m	Sc-C1, C3	Sc-H1
Sc-3	85.9 (d)	3.75, t (8.5)	Sc-C2, C4, OCH_3	
Sc-4	83.3 (d)	3.52, d (10.5)	Sd-C1, Sc-C5, C6	
Sc-5	71.9 (d)	3.80, m	Sc-C1	
Sc-6	18.6 (q)	1.76, d (6.0)	Sc-C4, C5	Sc-H3
Sc-OCH_3	60.7 (q)	3.97, s	Sc-C3	

(*continued on next page*)

Table 2 (*continued*)

Position	^{13}C[a]	^{1}H[b]	HMBC	^{1}H–^{1}H COSY
glc				
Sd-1	105.9 (d)	4.74, d (7.5)	Sc-C4, Sd-C5	Sd-H5
Sd-2	75.8 (d)	4.06, m	Sd-C1, C3	
Sd-3	78.6 (d)	4.29, m	Sd-C1, C2, C4	
Sd-4	71.9 (d)	4.28, m	Sd-C3, C5	
Sd-5	78.3 (d)	4.02, m	Sd-C4, C6	
Sd-6	63.0 (t)	4.58, d (10.0);	Sd-C4	
		4.38, dd (11.5, 5.5)	Sd-C4, C5	

[a] 125 MHz.
[b] 500 MHz.
[c] Multiplicities by DEPT experiments in parentheses: s-quaternary, d-CH, t-CH_2, and q-CH_3 C-atoms.

portion in **1** were observed at C-3 (+5.7 ppm), C-2 (−2.7 ppm), and C-4 (−4.0 ppm), indicating that the oligosaccharide chain was attached to C-3 hydroxyl group of the aglycone in **1**.

Therefore, the structure of **1** can be established as 12-*O*-acetyl-20-*O*-(*N*-methyl) anthraniloyl-5,6-dihydrosarcostin 3-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (Fig. 1).

Compound **2**, $[\alpha]_D^{20}$ −23.1 (*c* 0.1, MeOH), mp 178–180 °C, was obtained as an amorphous powder, and showed positive Liebermann–Burchard and Keller–Kiliani reactions, indicating the presence of a steroid skeleton and 2-deoxy sugar moieties in the molecule. In the IR spectra of **2**, there were absorption bands at 1709 (C═O) and strong absorption bands at 3440 (OH) and 1081 (C–O–C) cm^{-1}. Compound **2** had a molecular formula $C_{63}H_{89}NO_{23}$ (1250.5670 $[C_{63}H_{89}NO_{23}+Na]^+$, calcd 1250.5718) on the basis of the HR-ESI-MS, ^{13}C NMR, DEPT, and quasimolecular ion peak at *m*/*z* 1250.1 $[M+Na]^+$ in the ESI-MS spectrum. The ^{13}C NMR and DEPT spectrum of **2** showed 63 carbon signals, which consisted of 9 methyl carbon signals, 10 methylene carbon ones, 33 methine carbon ones, and 11 quaternary carbon ones. The carbon and proton signals of **2** in the NMR spectra were assigned by extensive techniques including HMQC, HMBC, and ^{1}H–^{1}H COSY (Table 2). The ^{1}H, ^{13}C NMR and MS data revealed **2** had the same ester substituents as those of gagaminine (**4**).[13] The acyl substituents in the aglycone of **2** were assigned as a nicotinoyl group, which is determined from ^{1}H NMR signals at δ 9.57 (d, *J* = 1.5 Hz, H-2′), 8.35 (d, *J* = 7.5 Hz, H-4′), 7.24 (dd, *J* = 8.0, 5.0 Hz, H-5′), 8.87 (dd, *J* = 5.0, 1.0 Hz, H-6′), and ^{13}C NMR signals at δ 151.4 (C-2′), 126.9 (C-3′), 137.4 (C-4′), 123.8 (C-5′), 153.8 (C-6′), 164.7 (C-7′), and a cinnamoyl group, which is characterized by the UV absorption bands at 205.5, 217.5, and 280 nm; the ^{1}H NMR proton signals at δ 6.71 (d, *J* = 13.0 Hz, H-2″), 7.88 (d, *J* = 16.0 Hz, H-3″), 7.39 (m, H-5″, 9″), 7.56 (m, H-6″, 8″), 7.36 (d, *J* = 3.5 Hz, H-7″); and the ^{13}C NMR carbon signals at δ 166.8 (C-1″), 120.2 (C-2″), 144.1 (C-3″), 134.8 (C-4″), 129.3 (C-5″, 9″), 128.6 (C-6″, 8″), 130.6 (C-7″). The acyl groups were further confirmed by the molecular ion peak at *m*/*z* 1250.1 $[M+Na]^+$, and the fragment ion peak at *m*/*z* 1102.1 [M+Na–cinnamic acid]$^+$, 979.2 [M+Na–cinnamic acid–nicotinic acid]$^+$. The ^{1}H and ^{13}C NMR showed that the aglycone moiety of **2** was similar to that of gagaminine (**4**),[13] except for the major difference being the absence of signals for an OH group at C-3. In the HMBC spectrum, the carbonyl signal of the cinnamoyl group at δ 166.8 was correlated with the methine proton (H-20) at δ 5.34 (q, *J* = 6.0 Hz) on an oxygen-bearing carbon (C-20) at δ 76.4, establishing that the cinnamoyl group is located at C-20 in **2**, while the carbonyl signal of the nicotinoyl group at δ 164.7 was correlated with the methine proton (H-12) at δ 5.36 (dd, *J* = 11.0, 5.0 Hz) on an oxygen-bearing carbon (C-12) at δ 74.6, establishing that the nicotinoyl group is located at C-12. Thus, the aglycone of **2** was 12-*O*-nicotinoyl-20-*O*-cinnamoylsarcostin (isogagaminine, **5**).

Comparison of the ^{13}C and ^{1}H NMR spectra of **2** with those of **1** indicated that the sugar moieties were identical with those of **1**. In the HMBC spectra, correlations were observed between the following protons and carbons: δ 5.31 (Sa-H1 of the β-cymaropyranose) and δ 77.6 (C-3 of the aglycone), δ 5.13 (Sb-H1 of the β-cymaropyranose) and δ 82.8 (Sa-C4 of the β-cymaropyranose), δ 5.15 (Sc-H1 of the 6-deoxy-3-*O*-methyl-β-allopyranose) and δ 83.1 (Sb-C4 of the β-cymaropyranose), δ 4.74 (Sc-H1 of the β-glucopyranose) and δ 83.3 (Sc-C4 of the 6-deoxy-3-*O*-methyl-β-allopyranose). Thus, Compound **2** had the same sugar moieties and linkages as those of **1**.

Therefore, the structure of **2** can be established as 12-*O*-nicotinoyl-20-*O*-cinnamoyl sarcostin 3-*O*-β-D-glucoyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (Fig. 1).

The effects of **1** and **2** on mitogen-stimulated mice splenocyte proliferation in vitro are shown in Figures 2 and 3. Compounds **1** and **2** significantly inhibited the Con A- and LPS-stimulated mice splenocyte proliferations in a concentration-dependent manner. There were no significant differences in inhibitory effect on LPS-stimulated splenocyte proliferation between CsA (Cyclosporin A) and compound **1** at the concentration of 0.1 and 1 μg/ml, while the inhibitory effect of compounds **1** and **2** on Con A-stimulated splenocyte proliferation and compound 2 on LPS-stimulated splenocyte proliferation was less significant than that of CsA at the same concentration ($P < 0.05$).

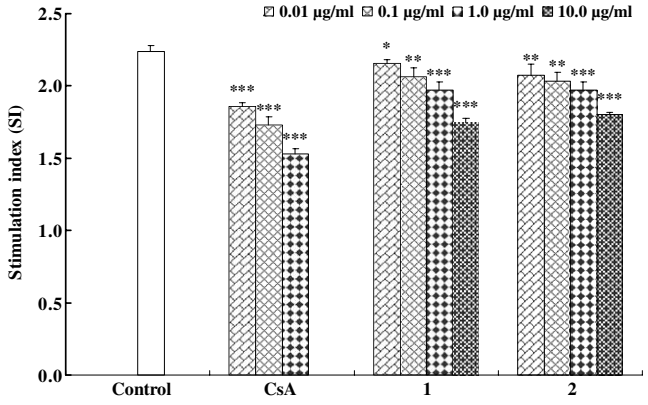

Figure 2. Effect of compounds **1** and **2** on Con A-stimulated splenocyte proliferation in vitro. Splenocytes were cultured with Con A (5 μg/mL) and the various concentrations of compounds **1** or **2** in RPMI 1640 medium for 48 h. Cellular proliferation was measured by the MTT method as described in the text and shown as a stimulation index. The values are presented as means ± SD ($n = 4$). Significant differences compared to control are designated as $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

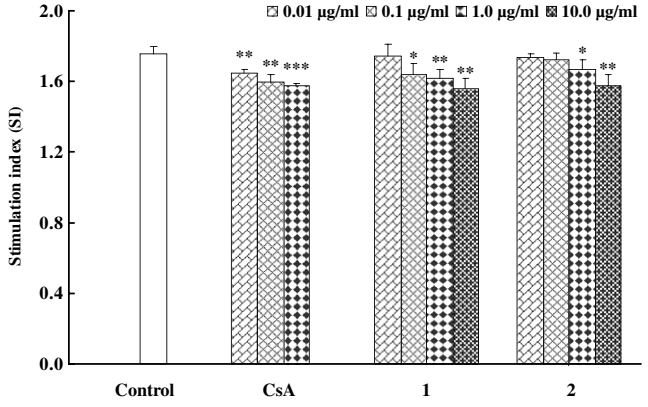

Figure 3. Effect of compounds **1** and **2** on LPS-stimulated splenocyte proliferation in vitro. Splenocytes were cultured with LPS (10 μg/mL) and the various concentrations of compounds **1** or **2** in RPMI 1640 medium for 48 h. Cellular proliferation was measured by the MTT method as described in the text and shown as a stimulation index. The values are presented as means ± SD ($n = 4$). Significant differences compared to control are designated as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

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References and notes

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5. The dried roots of *S. mucronata* (10 kg) were ground and extracted three times with 95% EtOH under reflux for 2 h. The extracts were evaporated in vacuum to give the EtOH extract. The ethanolic extract was partitioned with $CHCl_3$ under reflux, and a yellow residue (520 g) was given by concentration of the $CHCl_3$ extract. The residue was subjected to CC (silica gel, gradient $CHCl_3$/MeOH 100:0 → 2:1 v/v) to give 10 main fractions. Fr. 6 (50 g) was subjected CC (Rp-$_{18}$, MeOH/H_2O 1:1 v/v; then Sephadex LH-20, MeOH; and then HPLC, MeOH/H_2O 65:35 v/v) to afford **1** (92 mg), **2** (110 mg).
6. Mice splenocytes were seeded into four wells of a 96-well flat-bottomed microtiter plate (Nunc) at a cell density of 1×10^7 per mL in 100 μL complete medium where 100 μL of **1**–**2** (0.01 – 10 μg/mL), and concanavalin A (Con A, final concentration 5 μg/mL), lipopolysaccharide (LPS, final concentration 10 μg/mL), or medium were then added. The plate was incubated at 37 °C in a humid atmosphere with 5% CO_2. After 44 h, 50 μL of MTT solution (2 mg/mL) was added to each well and incubated for further 4 h. The microtiter plates were centrifuged ($1400 \times g$, 5 min) and the untransformed MTT was removed carefully by pipetting. To each well, 200 μL of a Me_2SO working solution (192 μL Me_2SO with 8 μL HCl 1 mol/L) was added, and the absorbance (A) was evaluated in an ELISA reader at 570 nm with a 630 nm reference after 15 min.
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8. Ten milliliters of 0.1 N H_2SO_4 was added to a solution of compound **1** (40 mg) in 10 mL MeOH, and solution was kept at 60 °C for 2 h. Reaction mixture was diluted with H_2O (20 mL) and concentrated to 30 mL. The solution was kept for 60 °C for further 1 h, then neutralized with aq satd $Ba(OH)_2$ and the precipitation was filtered off. The filtrate was concentrated to dryness and chromatographed on a column of silica gel with $CHCl_3$/MeOH (100:1 → 50:1) to afford **3** (14 mg). The sugar components in hydrolysate were identified by TLC comparison with authentic samples. The R_f values of D-cymarose were 0.45 with the solvent $CHCl_3$/MeOH (9:1), 0.31 with the solvent Me_2CO/petrol (2:3), and 0.50 with the solvent $CHCl_3$/C_2H_5OH (9:1). The R_f value of D-glucose was 0.15 with the solvent $CHCl_3$/MeOH/H_2O (4:3:1).
9. ^{13}C NMR (C_5D_5N, 125 MHz): 39.1 (C-1), 32.2 (C-2), 70.9 (C-3), 38.4 (C-4), 46.0 (C-5), 25.5 (C-6), 34.2 (C-7), 76.4 (C-8), 47.1 (C-9), 36.7 (C-10), 24.8 (C-11), 75.0 (C-12), 57.5 (C-13), 89.0 (C-14), 33.8 (C-15), 34.7 (C-16), 88.1 (C-17), 12.0 (C-18), 13.4 (C-19), 75.3 (C-20), 15.9 (C-21), 171.5 (C-1′), 22.2 (C-2′), 111.1 (C-1″), 152.9 (C-2″), 111.7 (C-3″), 135.4 (C-4″), 114.9 (C-5″), 132.8 (C-6″), 168.4 (C-7″), 29.7 (NCH_3). ^1H NMR (C_5D_5N, 500 MHz): δ 1.19 (3H, s, H-19), 2.03 (3H, s, H-18), 1.52 (3H, d, $J = 5.5$ Hz, H-21), 5.10 (1H, dd, $J = 10.5$, 4.0 Hz, H-12), 5.20 (1H, q, $J = 6.5$ Hz, H-20), 2.08 (3H, s, H-2′), 6.70 (1H, d, $J = 8.5$ Hz, H-3″), 7.38 (1H, ddd, $J = 8.5$, 8.0, 1.5 Hz, H-4″), 6.53 (1H, t, $J = 7.5$ Hz, H-5″), 8.30 (1H, t, $J = 8.0$ Hz, H-6″), 2.76 (3H, d, $J = 5.0$ Hz, NCH_3).
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