Immunomodulating Steroidal Glycosides from the Roots of *Stephanotis mucronata*

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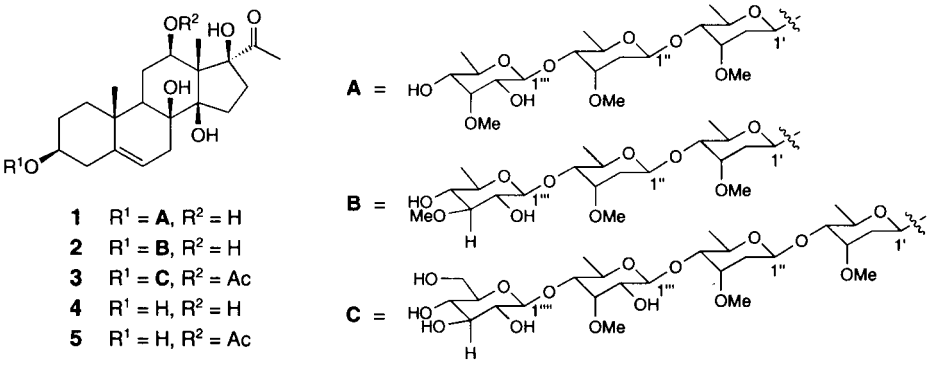
Guided by *in vitro* immunological tests, three immunomodulating steroidal glycosides, stemucronatosides A (**1**), B (**2**), and C (**3**), were isolated from the roots of *Stephanotis mucronata*. On the basis of chemical evidence and extensive spectroscopic methods including 1D and 2D NMR, their structures were determined as 12-*O*-deacetylmetaplexigenin 3-[*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside], 12-*O*-deacetylmetaplexigenin 3-[*O*-β-D-thevetopyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside], and metaplexigenin 3-[*O*-β-D-glucopyranosyl-(1→4)-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-*O*-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside], respectively. These compounds showed immunomodulating activities *in vitro*.

Introduction. – The plants beonging to the Asclepiadaceae family are reported to be rich in pregnane and cardiac glycosides [1][2]. In recent years, the pregnanes and their glycosides have been shown to possess antitumor [3][4], antiepilepsy [5], and antifertility activities [6]. The dried roots of *Stephanotis mucronata* (BLANCO) MEER. (Asclepiadaceae) are used for the treatment of rheumatoid arthritis and rheumatic aches in Chinese folk medicine. We previously reported the isolation and structural elucidation of three pregnane glycosides, mucronatoside A and B and stephanoside E, from the stems of *S. mucronata* [7]. To obtain biological pregnane glycosides, chemical studies of the $CHCl_3$-soluble extract from the roots of this plant were undertaken by screening with immunological tests *in vitro*, and we obtained three novel pregnane oligoglycosides named stemucronatosides A (**1**), B (**2**), and C (**3**).



1 R^1 = **A**, R^2 = H
2 R^1 = **B**, R^2 = H
3 R^1 = **C**, R^2 = Ac
4 R^1 = H, R^2 = H
5 R^1 = H, R^2 = Ac

Results and Discussion. – The EtOH extract of the roots of *Stephanotis mucronata* was extracted with $CHCl_3$. The $CHCl_3$-soluble portion was subsequently separated by column chromatography (silica gel, reversed-phase silica gel, and *Sephadex HL-20*) to provide the three compounds **1**–**3**. Each of the isolates was subjected to detailed spectroscopic analysis to establish their chemical structures.

Stemucronatosides A (**1**) was isolated as an amorphous powder that showed positive *Liebermann–Buchard* and *Keller–Kiliani* reactions indicating the presence of a steroidal and 2-deoxysugar(s) moieties in the molecule. The EI-MS showed the quasimolecular ion at *m/z* 851.5 ($[M+Na]^+$) and 867.4 ($[M+K]^+$), in agreement with the molecular formula $C_{42}H_{68}O_{16}$, which was supported by the ^{13}C-NMR and DEPT spectrum. The EI-MS also displayed other prominent fragments at *m/z* 691.3 ($[M+Na-160]^+$), 547.2 ($[M+Na-160-144]^+$). The IR spectrum of **1** showed OH (3510 cm^{-1}), C=O (1690 cm^{-1}), olefinic (1646 cm^{-1}), and C–O–C (1080 cm^{-1}) groups. The assignments of the ^{1}H- and ^{13}C-NMR signals of **1** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Tables 1* and *2*). On the basis of its spectroscopic data, comparison with those of compound **4**, and the results of acid hydrolysis, compound **1** was established as 12-*O*-deacetylmetaplexigenin 3-[*O*-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*O*-*β*-D-cymaropyranosyl-(1 → 4)-*β*-D-cymaropyranoside][1]).

The ^{13}C-NMR and DEPT spectra (125 MHz, (D_5)pyridine) of **1** allowed the attribution of 42 C-signals to 9 Me, 9 CH_2, 17 CH, and 7 quaternary C-atoms. The ^{1}H- (500 MHz) and ^{13}C-NMR data for the aglycone moiety of **1** were similar to those of 12-*O*-deacetylmetaplexigenin (**4**) [8], the major difference being the absence of signals for an OH group at C(3). The only other difference in the ^{13}C-NMR data between **1** and **4** occurred for the atoms C(2), C(3), and C(4): C(2) and C(4) of **1** were shifted upfield by 2.8 and 4.3 ppm, respectively, and C(3) of **1** was shifted downfield by 5.9 ppm in comparison with the corresponding signals of **4**. The NMR (^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC) spectral data of compound **1** showed that it contained three anomeric C-signals at *δ* 96.0, 100.0, and 103.8, correlating with the anomeric protons at *δ* 5.31, 5.14, and 5.16, respectively, which indicated that there were three sugar units in compound **1**. Thus, compound **1** was believed to be a 12-*O*-deacetylmetaplexigenin 3-*O*-trioside.

Mild acid hydrolysis of **1** afforded 12-*O*-deacetylmetaplexigenin (**4**), D-cymarose (=2,6-dideoxy-3-*O*-methyl-D-*ribo*-hexose), and an unidentified sugar (on TLC). The HMBC and ^{1}H,^{1}H-COSY experiment allowed the sequential assignments of the *δ*(C) and *δ*(H) for the unidentified sugar as shown in *Tables 1* and *2*, starting from the anomeric proton and C-signal at *δ* 5.16 (*d*, *J* = 9.5 Hz) and 103.8. Those findings suggested that the unidentified sugar (detected by TLC) is 6-deoxy-3-*O*-methyl-*β*-D-allose (abbreviated as AllMe) on the basis of its ^{1}H-NMR data and ^{13}C-NMR assignments in agreement with those of similar compounds [9]. Further, a comparison of the chemical shifts of the anomeric protons of other compounds showed that the anomeric-proton signal of AllMe appears at lower field than *δ*(H) 5.00, while that of Thv (*β*-D-thevetose = 6-deoxy-3-*O*-methyl-D-glucose) appears at higher field than *δ*(H) 5.00 in (D_5)pyridine [9][10]. This confirmed the assignment of the anomeric-proton *d* at *δ* 5.16 to AllMe [10]. The anomeric proton signals due to two cymarose units were observed at *δ* 5.31 and 5.14 (each *d*, *J* = 9.5 Hz, 1 H) in the ^{1}H-NMR spectrum of **1**, which indicated that cymarose is of *β*-D-configuration as judged from the chemical shifts and coupling constants [11]. The chemical shifts for C(2′) (*δ* 36.9) and C(2″) (*δ* 36.5) of the two cymarose units of **1** showed that both have *β*-D configuration [12].

As regards the sugar linkage, the following long-range correlations were observed in the HMBC spectrum: C(1′) of the *β*-D-cymaropyranose (*δ* 96.0) and H–C(3) of the aglycone (*δ* 3.90, *m*), C(1″) of the *β*-D-cymaropyranose (*δ* 100.0) and H–C(4′) of the *β*-D-cymaropyranose (*δ* 3.50, *dd*, *J* = 9.5, 2.5 Hz), and C(1‴) of the 6-deoxy-3-*O*-methyl-*β*-D-allopyranose (*δ* 103.8) and H–C(4″) of the *β*-D-cymaropyranose (*δ* 3.57, *dd*, *J* = 9.5, 2.5 Hz). Consequently, the sugar sequence was established as *O*-6-deoxy-3-*O*-methyl-D-allosyl-(1 → 4)-*O*-D-cymarosyl-(1 → 4)-D-cymaroside attached at C(3) of the aglycone.

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

Table 1. *^{13}C-NMR Data* ((D$_5$)pyridine) *of Compounds* **1**–**5**. δ in ppm, J in Hz[a]).

C	**1**	**2**	**3**	**4**	**5**
C(1)	38.6 (*t*)	38.4 (*t*)	38.9 (*t*)	39.0 (*t*)	39.0 (*t*)
C(2)	29.1 (*t*)	29.4 (*t*)	29.8 (*t*)	31.9 (*t*)	31.8 (*t*)
C(3)	77.3 (*d*)	77.2 (*d*)	77.6 (*d*)	71.4 (*d*)	71.3 (*d*)
C(4)	38.9 (*t*)	38.8 (*t*)	39.2 (*t*)	43.2 (*t*)	43.1 (*t*)
C(5)	138.9 (*s*)	138.8 (*s*)	139.2 (*s*)	140.1 (*s*)	140.1 (*s*)
C(6)	119.1 (*d*)	119.0 (*d*)	119.2 (*d*)	118.6 (*d*)	118.3 (*d*)
C(7)	33.8 (*t*)	33.7 (*t*)	33.7 (*t*)	34.0 (*t*)	33.6 (*t*)
C(8)	73.9 (*s*)	73.7 (*s*)	74.2 (*s*)	74.2 (*s*)	74.2 (*s*)
C(9)	44.5 (*d*)	44.4 (*d*)	44.4 (*d*)	44.8 (*d*)	44.3 (*d*)
C(10)	37.0 (*s*)	36.7 (*s*)	37.3 (*s*)	37.2 (*s*)	37.2 (*s*)
C(11)	29.5 (*t*)	28.9 (*t*)	24.8 (*t*)	29.3 (*t*)	24.7 (*t*)
C(12)	68.5 (*d*)	68.4 (*d*)	73.5 (*d*)	68.8 (*d*)	73.4 (*d*)
C(13)	60.0 (*s*)	59.9 (*s*)	57.9 (*s*)	60.2 (*s*)	57.7 (*s*)
C(14)	88.9 (*s*)	88.8 (*s*)	89.4 (*s*)	89.2 (*s*)	89.3 (*s*)
C(15)	34.7 (*t*)	34.5 (*t*)	34.7 (*t*)	34.9 (*t*)	34.5 (*t*)
C(16)	32.4 (*t*)	32.3 (*t*)	32.8 (*t*)	32.6 (*t*)	32.6 (*t*)
C(17)	92.2 (*s*)	92.0 (*s*)	92.4 (*s*)	92.4 (*s*)	92.2 (*s*)
C(18)	9.0 (*q*)	8.9 (*q*)	10.4 (*q*)	9.2 (*q*)	10.2 (*q*)
C(19)	18.2 (*q*)	17.8 (*q*)	18.1 (*q*)	18.3 (*q*)	18.1 (*q*)
C(20)	209.2 (*s*)	209.1 (*s*)	210.2 (*s*)	209.4 (*s*)	210.0 (*s*)
C(21)	27.5 (*q*)	27.4 (*q*)	27.6 (*q*)	27.7 (*q*)	27.4 (*q*)
Me*C*OO–C(12)	–	–	169.9 (*s*)	–	169.7 (*s*)
*Me*COO–C(12)	–	–	20.8 (*q*)	–	20.6 (*q*)
Cym[1] C(1′)	96.0 (*d*)	95.8 (*d*)	96.4 (*d*)		
C(2′)	36.9 (*t*)	36.4 (*t*)	37.2 (*t*)		
C(3′)	77.7 (*d*)	77.6 (*d*)	77.9 (*d*)		
C(4′)	82.8 (*d*)	82.9 (*d*)	83.3 (*d*)		
C(5′)	68.9 (*d*)	68.8 (*d*)	69.2 (*d*)		
C(6′)	18.0 (*q*)	18.0 (*q*)	18.6 (*q*)		
MeO	58.5 (*q*)	58.4 (*q*)	58.8 (*q*)		
Cym[2] C(1″)	100.0 (*d*)	99.9 (*d*)	100.4 (*d*)		
C(2″)	36.5 (*t*)	36.8 (*t*)	37.0 (*t*)		
C(3″)	77.6 (*d*)	77.5 (*d*)	78.0 (*d*)		
C(4″)	83.0 (*d*)	82.5 (*d*)	82.9 (*d*)		
C(5″)	68.6 (*d*)	68.4 (*d*)	69.0 (*d*)		
C(6″)	18.3 (*q*)	18.0 (*q*)	18.5 (*q*)		
MeO	58.4 (*q*)	58.3 (*q*)	58.9 (*q*)		
Carb[3]	AllMe	Thv	AllMe		
C(1‴)	103.8 (*d*)	105.7 (*d*)	104.8 (*d*)		
C(2‴)	74.0 (*d*)	75.3 (*d*)	74.7 (*d*)		
C(3‴)	83.6 (*d*)	87.3 (*d*)	85.8 (*d*)		
C(4‴)	72.7 (*d*)	74.6 (*d*)	83.1 (*d*)		
C(5‴)	70.3 (*d*)	72.2 (*d*)	71.8 (*d*)		
C(6‴)	18.3 (*q*)	18.1 (*q*)	18.6 (*q*)		
MeO	61.8 (*q*)	61.0 (*q*)	60.6 (*q*)		
Glc[4] C(1⁗)			106.0 (*d*)		
C(2⁗)			75.8 (*d*)		
C(3⁗)			78.6 (*d*)		
C(4⁗)			71.9 (*d*)		
C(5⁗)			78.1 (*d*)		
C(6⁗)			63.0 (*t*)		

[a]) ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HMQC, and HMBC data were obtained at 500 and 125 MHz at room temperature, respectively. Multiplicities by DEPT experiments.

Table 2. *^{1}H-NMR Data* ((D$_5$)pyridine) *of Compounds* **1**–**5**. δ in ppm, J in Hz.

H	**1**	**2**	**3**	**4**	**5**
H–C(3)	3.90 (*m*)	3.87 (*m*)	3.92 (*m*)	3.93 (*m*)	3.91 (*m*)
H–C(6)	5.36 (br. *s*)	5.37 (br. *s*)	5.33 (br. *s*)	5.42 (br. *s*)	5.35 (br. *s*)
H–C(12)	3.97 (*dd*, *J* = 11.5, 4.0)	3.96 (*m*)	4.99 (*dd*, *J* = 11.5, 4.0)	3.98 (*dd*, *J* = 11.5, 4.0)	5.00 (*dd*, *J* = 11.5, 4.0)
Me(18)	2.04 (*s*)	2.02 (*s*)	1.94 (*s*)	2.04 (*s*)	1.97 (*s*)
Me(19)	1.41 (*s*)	1.41 (*s*)	1.35 (*s*)	1.49 (*s*)	1.43 (*s*);
Me(21)	2.66 (*s*)	2.66 (*s*)	2.50 (*s*)	2.68 (*s*)	2.51 (*s*)
AcO–C(12)	–	–	2.09 (*s*)	–	2.10 (*s*)
Cym[1] H–C(1′)	5.31 (*d*, *J* = 9.5)	5.30 (*d*, *J* = 10)	5.30 (*d*, *J* = 9.5)		
H–C(3′)	4.07 (*m*)	4.09 (*m*)	4.04 (*m*)		
H–C(4′)	3.50 (*dd*, *J* = 9.5, 2.5)	3.50 (*dd*, *J* = 9.5, 2.5)	3.52 (*dd*, *J* = 10.0, 2.5)		
H–C(5′)	4.22 (*m*)	4.23 (*dq*, *J* = 9.5, 6.5)	4.22 (*m*)		
Me(6′)	1.35 (*d*, *J* = 7.0)	1.39 (*d*, *J* = 6.0)	1.40 (*d*, *J* = 6.0)		
MeO	3.61 (*s*)	3.63 (*s*)	3.63 (*s*)		
Cym[2] H–C(1″)	5.14 (*d*, *J* = 9.5)	5.13 (*d*, *J* = 10)	5.14 (*d*, *J* = 10.0)		
H–C(3″)	4.10 (*m*)	4.08 (*m*)	4.09 (*m*)		
H–C(4″)	3.57 (*dd*, *J* = 9.5, 2.5)	3.60 (*dd*, *J* = 10.0, 2.0)	3.85 (*dd*, *J* = 11.5, 5.0)		
H–C(5″)	4.23 (*m*)	4.25 (*dq*, *J* = 9.5, 6.5)	4.19 (*m*)		
Me(6″)	1.57 (*d*, *J* = 6.5)	1.63 (*d*, *J* = 6.0)	1.82 (*d*, *J* = 6.0)		
MeO	3.60 (*s*)	3.58 (*s*)	3.59 (*s*)		
Carb[3]	AllMe	Thv	AllMe		
H–C(1‴)	5.16 (*d*, *J* = 9.5)	4.80 (*d*, *J* = 10)	5.17 (*dd*, *J* = 10.5, 2.5)		
H–C(2‴)	3.93 (*m*)	3.95 (*m*)	3.87 (*t*, *J* = 9.0)		
H–C(3‴)	4.10 (*m*)	3.63 (*m*)	3.75 (*t*, *J* = 8.0)		
H–C(4‴)	3.64 (*m*)	3.65 (*m*)	3.55 (*dd*, *J* = 9.5, 2.5)		
H–C(5‴)	4.18 (*m*)	3.76 (*dq*, *J* = 8.5, 6.0)	3.79 (*dq*, *J* = 6.0, 3.0)		
Me(6‴)	1.52 (*d*, *J* = 6.5)	1.62 (*d*, *J* = 6.0)	1.59 (*d*, *J* = 6.0)		
MeO	3.88 (*s*)	3.93 (*s*)	3.96 (*s*)		
Glc[4] H–C(1⁗)			4.73 (*d*, *J* = 7.5)		
H–C(2⁗)			3.83 (*m*)		
H–C(3⁗)			4.28 (*m*)		
H–C(4⁗)			4.22 (*m*)		
H–C(5⁗)			4.09 (*m*)		
CH$_2$ (6⁗)			4.38 (*m*), 4.55 (*d*, *J* = 7.5)		

Stemucronatosides B (**2**) was isolated as an amorphous powder, and showed positive *Liebermann–Buchard* and *Keller–Kiliani* reactions indicating the presence of a steroidal and 2-deoxysugar(s) moieties in the molecule. It has the same molecular formula, $C_{42}H_{68}O_{16}$, as **1**, as established by quasimolecular ion peak at *m*/*z* 851.6 ($[M+Na]^+$) in the EI-MS, the ^{1}H- and ^{13}C-NMR spectra, and the ^{1}H-detected HMQC experiment. The EI-MS of **2** also displayed other prominent fragments at *m*/*z* 867.4 ($[M+K]^+$), 691.3 ($[M+Na-160]^+$), and 547.2 ($[M+Na-160-144]^+$). Its IR spectrum showed OH (3510 cm^{-1}), C=O (1690 cm^{-1}), olefinic (1646 cm^{-1}), and C–O–C (1085 cm^{-1}) groups. The assignments of the ^{1}H- and ^{13}C-NMR signals of **2** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Tables 1* and *2*). On the basis of its spectroscopic data, comparison with those of compound **1**, and the results of acid hydrolysis, compound **2** was identified as 12-*O*-deacetylmetaplexigenin 3-[*O*-β-D-thevetopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside].

The ^{13}C-NMR and DEPT spectra (125 MHz, (D_5)pyridine) of compound **2** allowed the attribution of 42 C-signals to 9 Me, 9 CH_2, 17 CH, and 7 quaternary C-atoms. The ^{1}H-(500 MHz) and ^{13}C-NMR data for the aglycone moiety of **2** were similar to those for the aglycone moiety of **1**, indicating that **2** should also be 12-*O*-deacetylmetaplexigenin 3-*O*-trioside. The NMR (^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC) data of **2** showed that it contained three anomeric C-signals at δ 95.8, 99.9, and 105.7, correlating with anomeric protons at δ 5.30, 5.13, and 4.80 (each *d*, $J = 10$ Hz, 1 H), respectively, which indicated the presence of three sugar units in **2**.

On mild acid hydrolysis, compound **2** gave 12-*O*-deacetylmetaplexigenin (**4**), D-cymarose, and an unidentified sugar (on TLC). The HMBC and ^{1}H,^{1}H COSY experiments allowed the sequential assignments of the δ(C) and δ(H) for the unidentified sugar as shown in *Tables 1* and *2*, starting from the anomeric proton and C-signal at δ 4.80 (*d*, $J = 10.0$ Hz) and 105.7. Those findings suggested that the unidentified sugar (detected on TLC) was β-D-thevetose because its NMR data were similar to those in other compounds [9]. As the ^{1}H-NMR spectrum of **2** exhibited three MeO *s* at 3.58, 3.63, and 3.93, the sugar moiety of **2** consisted of two cymarose and one thevetose units. The coupling constant of each sugar indicated that these sugars had β-D-glycosidic linkages. In the HMBC spectrum, significant correlations were observed between H–C(1′) of the β-D-cymaropyranose (δ 5.30, *d* $J = 10$ Hz) and C(3) of the aglycone (δ 77.2), H–C(1″) of the β-D-cymaropyranose (δ 5.13, *d*, $J =$ 10 Hz) and C(4′) of the β-D-cymaropyranose (δ 82.9), and H–C(1‴) of the β-D-thevetopyranose (δ 4.80, *d*, $J =$ 10 Hz) and C(4″) of the β-D-cymaropyranose (δ 82.5), establishing the sugar sequence *O*-D-thevetosyl-(1 → 4)-*O*-D-cymarosyl-(1 → 4)-*O*-D-cymaroside attached at C(3) of the aglycone.

Stemucronatosides C (**3**) was isolated as an amorphous powder that showed positive *Liebermann–Buchard* and *Keller–Kiliani* reactions indicating the presence of a steroidal and 2-deoxysugar moieties in the molecule. It has the molecular formula $C_{50}H_{80}O_{22}$ as deduced from the EI-MS (*m*/*z* at 1055.6 ($[M + Na]^+$)) and ^{13}C-NMR data. The EI-MS of **3** exhibited other prominent fragment-ion peaks at *m*/*z* 995.5 ($[M + Na - 60]^+$) and 833.4 ($[M + Na - 60 - 162]^+$). The IR spectrum showed OH (3510), C=O (1690 cm^{-1}), olefinic (1646 cm^{-1}), and C–O–C (1080) groups. The assignments of the ^{1}H- and ^{13}C-NMR signals of **3** were successfully carried out with ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Tables 1* and *2*). On the basis of spectroscopic data, comparison with those of compound **5**, and the results of acid hydrolysis, the structure of **3** was assigned as metaplexigenin 3-[*O*-β-D-glucopyranosyl-(1 → 4)-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside].

The ^{13}C-NMR and DEPT spectra (125 MHz,(D_5)pyridine) of **3** allowed the attribution of 50 C-signals to 10 Me, 10 CH_2, 22 CH, and 8 quaternary C-atoms. The ^{1}H- (500 MHz) and ^{13}C-NMR data for the aglycone moiety of **3** were similar to those of metaplexigenin (**5**), the major difference being the absence of signals for an OH group at C(3). The only other difference in the ^{13}C-NMR data between **3** and **5** occurred for the atoms C(2), C(3), and C(4): C(2) and C(4) of **3** were shifted upfield by 2.0 and 3.9 ppm, respectively, and C(3) of **3** was shifted downfield by 6.3 ppm in comparison with the corresponding signals of **5**. The NMR (^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC) data of **3** showed that it contained four anomeric C-signals at δ 96.4, 100.4, 104.8, and 106.0, correlating with anomeric protons at δ 5.30 (*d*, $J = 9.5$ Hz), 5.14 (*d*, $J = 10.0$ Hz), 5.17 (*dd*, $J = 10.5$, 2.5 Hz), and 4.73 (*d*, $J = 7.5$ Hz), respectively, which indicated the presence of four sugar units in **3**. Thus, compound **3** was believed to be a metaplexigenin 3-*O*-tetraside.

Acid hydrolysis of **3** afforded metaplexigenin (**5**), cymarose, allomethylose (=6-deoxyallose), and glucose as the aglycone and the sugar moieties. ^{13}C-NMR Comparison of **3** with **2** showed a glycosylation shift of +8.5 ppm for C(4) of 6-deoxy-3-*O*-methylallose in **3** [13][14], indicating that the 4-*O* should be glucosylated. The coupling constant of each sugar moiety indicated that β-D-glycosidic linkages were present. The sugar sequence of **3** was confirmed by the HMBC spectrum, which showed prominent cross-peaks for H–C(1′) of the β-D-cymaropyranose (δ 5.30, *d*, $J = 9.5$ Hz) to C(3) of the aglycone (δ 77.6), H–C(1″) of the β-D-cymaropyranose (δ 5.14, *d*, $J = 10.0$ Hz) to C(4′) of the β-D-cymaropyranose (δ 83.3), H–C(1‴) of the 6-deoxy-3-*O*-methyl-β-D-allopyranose (δ 5.17, *dd*, $J = 10.5$, 2.5 Hz) to C(4″) of the β-D-cymaropyranose (δ 82.9), and H–C(1⁗) of the β-D-glucopyranose (δ 4.73, *d*, $J = 7.5$ Hz) to C(4‴) of the 6-deoxy-3-*O*-methyl-β-D-allopyranose (δ 83.1). Thus, the sugar sequence was established as *O*-D-glucosyl-(1 → 4)-*O*-D-6-deoxy-3-*O*-methyl-D-allosyl-(1 → 4)-*O*-D-cymarosyl-(1 → 4)-D-cymaroside attached at C(3) of the aglycone.

The immunomodulating activities of compounds **1**–**3** were determined *in vitro* against concanavalin-A- and lipopolysaccharide-induced (Con-A- and LPS-induced) proliferation of mice splenocytes by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay [15] and shown in *Table 3*. Compounds **1**–**3** significantly enhanced the Con-A- and LPS-induced mice splenocyte proliferation at the concentrations of 0.01–100.0 μg/ml. The concentration-effect proliferation relationship seems to be bell-shaped.

Table 3. *Effect of Three Compounds on* in vitro *Mitogen-Induced Mice Splenocyte Proliferation*[a])

Concentration [μg/ml]	Mitogen	**1**	**2**	**3**
0.00	ConA		2.047 ± 0.058	
0.01		2.540 ± 0.041***	2.240 ± 0.066**	2.412 ± 0.124**
0.10		2.602 ± 0.091***	2.566 ± 0.035***	2.671 ± 0.098***
1.00		2.681 ± 0.040***	2.672 ± 0.030***	2.534 ± 0.053***
10.0		2.600 ± 0.032***	2.835 ± 0.085***	2.387 ± 0.102**
100.0		2.566 ± 0.042***	2.656 ± 0.125***	2.285 ± 0.068**
0.00	LPS		1.542 ± 0.059	
0.01		1.859 ± 0.046***	1.763 ± 0.077**	1.793 ± 0.053**
0.10		1.939 ± 0.078***	1.954 ± 0.065***	1.822 ± 0.045***
1.00		1.955 ± 0.039***	1.962 ± 0.054***	1.799 ± 0.045***
10.0		1.910 ± 0.042***	1.877 ± 0.040***	1.725 ± 0.037**
100.0		1.652 ± 0.038*	1.830 ± 0.075**	1.708 ± 0.028**

[a]) Splenocytes were cultured with the various concentrations of these compounds and Con A (final concentration 5 μg/ml) or LPS (final concentration 10 μg/ml) 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 ± standard error ($n = 4$). Significant differences with 0 μg/ml were designated as *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$).

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Experimental Part

General. TLC: precoated silica gel *60 F_{254}* plates and *Rp C_{28}* (*Merck*); detection by spraying with 10% H_2SO_4 followed by heating. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao*), *Rp-C_{18}* silica gel (40–63 μm, *Merck*), and *Sephadex LH-20* (*Pharmacia*). IR Spectra: KBr pellets; *Perkin-Elmer-577* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR, DEPT, ^1H,^1H-COSY, HMQC and HMBC Spectra: *Bruker-DRX-500* instrument; at 500 (^1H) and 125 MHz (^{13}C); $SiMe_4$ as internal standard in (D_5)pyridine. EI-MS: *Bruker-Esquire-3000^{plus}* mass spectrometer.

Plant Material. The roots of *Stephanotis mucronata* were obtained from Yueqing, Zhejiang province, China. A voucher specimen (No. 200309) was identified by Prof. *Zhang Zhi-Guo* and deposited in the Laboratory of Natural Products Chemistry, Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou, China.

Extraction and Isolation Procedures. The dried roots of *Stephanotis mucronata* (10 kg) were ground and extracted three times with 95% EtOH under reflux for 2 h. The extracts were evaporated. This EtOH extract was extracted with $CHCl_3$ under reflux, and a yellow residue (520 g) was obtained on evaporation of the $CHCl_3$ extract. The residue was subjected to CC (silica gel, gradient $CHCl_3$/MeOH 100 : 0 → 2 : 1): 10 main fractions. *Fr. 3* (12 g) was subjected to CC (*Rp-C_{18}*, MeOH/H_2O 1 : 1; then *Sephadex LH-20*, MeOH): **1** (170 mg). *Fr. 4* (9 g) was subjected to CC (*Rp-C_{18}* and *Sephadex LH-20*): **2** (713 mg). *Fr. 6* (25 g) was separated by CC (*RP-C_{18}* and *Sephadex LH-20*): **3** (245 mg).

Stemucronatosides A (*=(3β,12β,14β,17α)-3-{[*O*-6-Deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1 → 4)-*O*-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl]oxy}-3,8,12,14,17-pentahydroxypregn-5-en-20-one*; **1**): Amorphous powder. IR (KBr): 3510, 1690, 1646, 1080. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (pos.): 851.5 ($[M+Na]^+$), 867.4 ($[M+K]^+$), 691.3 ($[M+Na\text{-AllMe}]^+$), 547.2 ($[M+Na-AllMe\text{-}Cym]^+$).

Stemucronatosides B (*=(3β,12β,14β,17α)-3-{[*O*-6-Deoxy-3-*O*-methyl-β-*D*-glucopyranosyl-(1 → 4)-*O*-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl]oxy}-3,8,12,14,17-pentahydroxypregn-5-en-20-one*; **2**): Amorphous powder. IR (KBr): 3510, 1690, 1646, 1085. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (pos.): 851.6 ($[M+Na]^+$), 867.4 ($[M+K]^+$), 691.3 ($[M+Na-Thv]^+$), 547.2 ($[M+Na-Thv-Cym]^+$).

Stemucronatosides C (*=(3β,12β,14β,17α)-12-(Acetyloxy)-3-{[*O*-β-*D*-glucopyranosyl-(1 → 4)-*O*-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1 → 4)-*O*-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-ribo-hexopyranosyl]oxy}-3,8,14,17-tetrahydroxypregn-5-en-20-one*; **3**): Amorphous powder. IR (KBr): 3510, 1690, 1646, 1080. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (pos.): 1055.6 ($[M+Na]^+$),995.5 ($[M+Na-MeCOOH]^+$), 833.4 ($[M+Na-MeCOOH-Glc]^+$).

Acidic Hydrolysis of Glycosides **1**–**3**. To a soln. of each compound (30 mg) in MeOH (10 ml) was added 0.1N H_2SO_4 (10 ml). The soln. was kept at 60° for 2 h, then diluted with H_2O (20 ml), and concentrated to 30 ml. The soln. was kept at 60° for a further hour and then neutralized with sat. aq. $Ba(OH)_2$ soln. The precipitation was filtered off, the filtrate evaporated, and the residue subjected to CC (silica gel, $CHCl_3$/MeOH 100 : 1 → 50 : 1): **4** (12 mg and 10.5 mg from **1** and **2**, resp.) or **5** (15 mg from **3**). The sugar components in each hydrolysate were identified by TLC comparison with authentic samples: R_f of D-cymarose 0.42 ($CHCl_3$/MeOH 9 : 1) and 0.35 (Me_2CO/petroleum ether 2 : 3).

12-O-Deacetylmetaplexigenin (*=(3β,12β,14β,17α)-3,8,12,14,17-Pentahydroxypregn-5-en-20-one*; **4**): Colorless needles. IR (KBr): 3510, 1690. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (pos.): 403.1 ($[M+Na]^+$).

Metaplexigenin (*=(3β,12β,14β,17α)-12-(Acetyloxy)-3,8,14,17-tetrahydroxypregn-5-en-20-one*; **5**): Colorless needles. IR (KBr): 3510, 1690. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (pos.): 445.1 ($[M+Na]^+$).

Splenocyte Proliferation Assay. Single-cell suspensions were prepared as previously described [15]. Splenocytes were seeded into four wells of a 96-well flat-bottom microtiter plate (*Nunc*) at a cell density of 1×10^7 per l in 100 µl of complete medium where 100 µl of **1**–**3** (0.01–100 µg/ml), and Con A (final concentration 5 mg·l^{-1}), LPS (final concentration 10 mg·l^{-1}), or medium were then added. The plate was incubated at 37° in a humid atmosphere with 5% CO_2. After 44 h, 50 µl of MTT solution (2 g·l^{-1}) was added to each well and incubated for 4 h. The microtiter plates were centrifuged (1400 × *g*, 5 min), and the untransformed MTT was removed carefully by pipetting. To each well, 200 µl of a Me_2SO working soln. (192 µl of Me_2SO with 8 µl of HCl 1 mol·l^{-1}) 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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