Steroidal Glycosides from the Fresh Stem of *Stephanotis lutchuensis* var. *japonica* (Asclepiadaceae). Chemical Structures of Stephanosides A—J

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The structures of ten related oxypregnane-oligoglycosides stephanosides A (1), B (2), C (3), D (4), E (5), F (6), G (7), H (8), I (9), and J (10) from the fresh stem of *Stephanotis lutchuensis* var. *japonica* (Asclepiadaceae) were elucidated on the basis of a detailed study of their high-field ^{1}H- and ^{13}C-NMR spectra. All the sugars are β (1→4)-linked and the aglycones are 12, 20 bis-*O*-acyl or 12-*O*-acyl derivatives of sarcostin.

Key words *Stephanotis lutchuensis* var. *japonica*; Asclepiadaceae; oxypregnane-oligoglycoside; stephanoside; stephanthraniline A; (*E* and *Z*)-cinnamic acid

In the previous paper,[1] we reported the isolation and structural elucidation of five oleanane glycosides named sitakisosides VI—X as antisweet principles from the saponin fraction of the fresh stem of *Stephanotis* (*S.*) *lutchuensis* var. *japonica* (Asclepiadaceae). Further research on this plant afforded ten oxypregnane-oligoglycosides. Previous phytochemical studies[2] on pregnane glycosides of this plant have been limited to the structural elucidation of the acid hydrolysates of the extract. The present paper describes the isolation and full structural elucidation of ten new oxypregnane-oligoglycosides named stephanosides A—J (**1**—**10**).

The EtOH extract obtained from the fresh stems of *S. lutchuensis* var. *japonica* (8.5 kg) was partitioned into an ethyl acetate–water mixture. Separation of the ethyl acetate-soluble portion by silica gel column chromatography and subsequent HPLC on a reversed-phase adsorbent provided compounds named stephanosides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), H (**8**), I (**9**), and J (**10**). ^{1}H–^{1}H Correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H–^{13}C COSY, total correlation spectroscopy (TCOSY), heteronuclear multiple-bond correlation (HMBC) and rotating Overhauser enhancement spectroscopy (ROESY) experiments led to the determination of the complete structures of **1**—**10**, and the structure revision of stephanthraniline A.

Stephanosides A—E (**1**—**5**) show an intense blue fluorescence in methanol solution, indicating the presence of an *N*-methylanthraniloyl group.[1] The IR spectra of **1**—**5** showed absorption bands at 1730, 1680, 1240—1245 cm^{-1} and strong absorption bands at 3445 and 1160—1170 cm^{-1} suggestive of oligoglycosidic structure. On mild acid hydrolysis, **1**—**5** gave the prosapogenin (**11**), mp 160—162 °C, $[\alpha]_D^{20}$ +10.0° (c=2.9, $CHCl_3$), $C_{31}H_{43}NO_8$ [chemical ionization (CI-MS) m/z 558 $[M+H]^+$]. The prosapogenin **11** was identical with stephanthraniline A isolated from the acid hydrolysis products of the crude glycosides of *S. japonica*, for which the structure **11′** has been proposed by Mitsuhashi *et al.*, by comparing the physical data with the literature values.[2] Our NMR data, however, suggested that the structure should be revised to **11**. Formula **11′** was solely based on the chemical shift values of the carbons and protons at C-12 and C-20 by referring to data for related compounds. In the HMBC spectrum, we found that the carbonyl signal of the acetyl group at δ 171.5 was correlated with the signal of a methine proton (H-12) at δ 5.15 on an oxygen-bearing carbon (C-12) at δ 74.7, and that of the *N*-methylanthraniloyl group at δ 168.3 was correlated with the the signal of methine proton (H-20) at δ 5.20 on an oxygen-bearing carbon (C-20) at δ 75.0, establishing that in **11**, the acetyl group is located at *O*-12 and the *N*-methylanthraniloyl group at *O*-20. Thus, the structure of the stephanthraniline A should be revised to 12-*O*-acetyl-20-*O*-*N*-methylanthraniloyl sarcostin. It is not teasible to assign the position of each of the acyl moieties based only on the values of acylation shift.

Stephanoside E (**5**) has the molecular formula $C_{52}H_{79}NO_{18}$ based on elemental analysis. On acid hydrolysis, **5** afforded cymarose and an unidentified sugar on TLC. The ^{1}H–^{1}H COSY experiments allowed the sequential assignments of the ^{1}H resonances for the unidentified sugar as shown in Table 3, starting from the anomeric proton signal at δ 4.77 (d, J=8.0 Hz). Furthermore, a long-range correlation was observed between methoxy protons at δ 3.89 (s) and the C-3‴ carbon at δ 87.8 in the HMBC spectrum of **5**. Those findings suggested that the unidentified sugar is β-thevetose.[3] The anomeric proton signals due to two cymaroses were observed at δ 5.11 and 5.26 (each 1H, dd, J=9.5, 1.5 Hz) in the ^{1}H-NMR spectrum of **5**, which indicated that cymarose is of β-configuration, as judged from the coupling constants.[4] Also, in the ^{13}C-NMR spectrum of **5**, three anomeric carbon signals were observed at δ 96.4 (C-1′), 100.4 (C-1″) and 106.2 (C-1‴). The chemical shift values for C-2′ (δ 37.2) and C-2″ (δ 36.9) of the two cymaroses in **5** showed that both have β-D configuration.[5]

A ^{13}C-NMR spectral comparison of **5** with **11** showed glycosylation shifts[6] for the C-2 (−2.0 ppm) and C-3 (+5.9 ppm) signals, demonstrating the sugar linkages to be at C-3-OH. For the sugar linkage, long-range correlations were observed as follows in the HMBC spectrum, δ 96.4 [C-1′ of the β-cymaropyranosyl] and δ 3.82 (1H, m) [H-3 of the aglycone], δ 100.4 [C-1″ of the outer β-cymaropyranosyl] and δ 3.48 (1H, dd, J=9.5, 2.5 Hz) [H-4′ of the inner β-cymaropyranosyl], and δ 106.2 [C-1‴ of the β-thevetopyranosyl] and δ 3.61 (1H, dd, J=9.5, 2.5 Hz) [H-4″ of the β-cymaropyranosyl]. Further, in the

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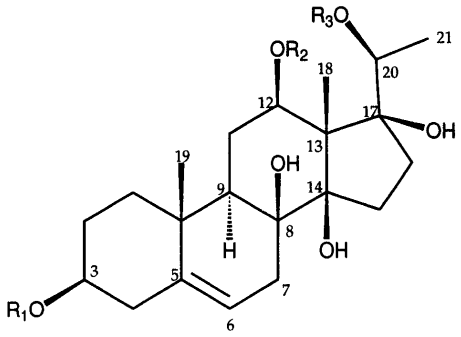
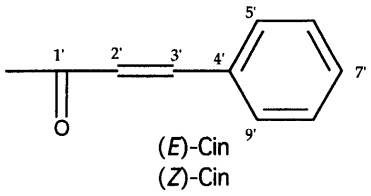
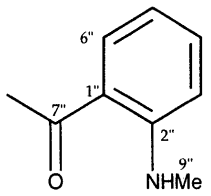
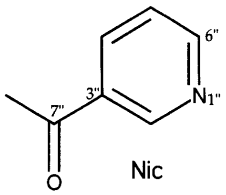
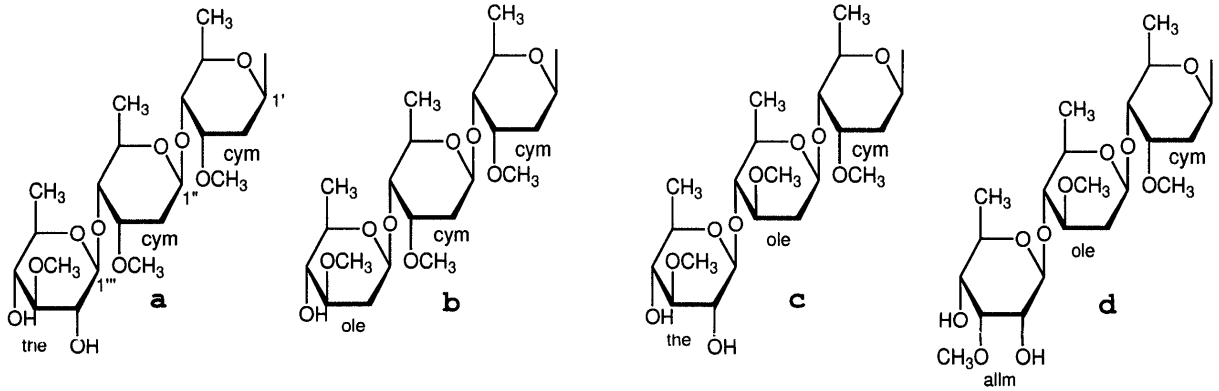








			R_1	R_2	R_3
Stephanoside	A	(**1**)	e	Ac	Anth
	B	(**2**)	d	Ac	Anth
	C	(**3**)	c	Ac	Anth
	D	(**4**)	b	Ac	Anth
	E	(**5**)	a	Ac	Anth
	F	(**6**)	e	(*E*)-Cin	Nic
	G	(**7**)	c	(*E*)-Cin	Nic
	H	(**8**)	a	(*E*)-Cin	Nic
	I	(**9**)	e	(*E*)-Cin	H
	J	(**10**)	f	(*Z*)-Cin	H
Prosapogenin		(**11**)	H	Ac	Anth
Stephanthraniline A		(**11'**)	H	Anth	Ac



(*E*)-Cin: (*E*)-Cinnamoyl
(*Z*)-Cin: (*Z*)-Cinnamoyl
Anth : *N*-Methylanthraniloyl
Nic : Nicotinoyl
Ac : Acetyl
cym : D-cymarose
ole : D-olenadrose
the : D-thevetose
allm : D-allomethylose

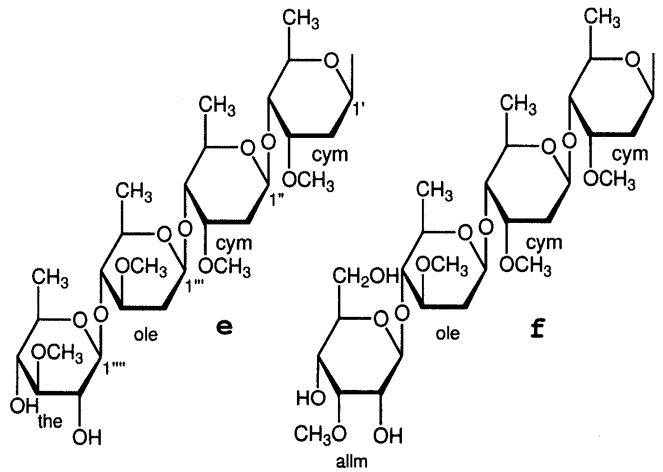


Chart 1

ROESY experiment, nuclear Overhauser effects (NOEs) were observed between H-3 of the aglycone at δ 3.82 and the H-1′ of the inner β-cymaropyranosyl at δ 5.26, H-4′ of the inner β-cymaropyranosyl at δ 3.48 and the H-1″ of the outer β-cymaropyranosyl at δ 5.11, and H-4″ of the β-cymaropyranosyl at δ 3.61 and the H-1‴ of the β-thevetopyranosyl at δ 4.77. Based on the above evidence, the structure of **5** has been established as 12-*O*-acetyl-20-

Table 1. ^{13}C-NMR Data for the Aglycone Parts of Stephanosides A-J (**1**—**10**) and Presapogenin (**11**) (in Pyridine-d_5)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	38.8	38.8	38.9	38.8	38.8	38.8	38.8	38.8	39.0	39.0	39.1
2	29.9	30.0	30.0	29.8	30.0	29.9	29.9	30.0	30.0	30.0	32.0
3	77.7	77.7	77.8	77.6	77.6	77.6	77.7	77.6	77.8	77.8	71.7
4	39.3	39.3	39.3	39.3	39.2	39.3	39.3	39.3	39.4	39.4	43.3
5	139.3	139.3	139.3	139.2	139.2	139.3	139.3	139.3	139.2	139.2	140.2
6	119.4	119.4	119.5	119.4	119.4	119.3	119.4	119.3	119.7	119.7	118.8
7	34.9	34.9	35.0	34.9	34.8	34.9	34.9	34.9	35.2	35.2	34.9
8	74.3	74.3	74.4	74.3	74.2	74.3	74.3	74.3	74.3	74.2	74.4
9	44.1	44.1	44.1	44.0	44.0	44.1	44.1	44.1	44.2	44.2	44.7
10	37.3	37.3	37.3	37.2	37.2	37.3	37.3	37.3	37.4	37.3	37.3
11	25.6	25.6	25.7	25.6	25.6	25.7	25.7	25.7	25.8	25.8	25.7
12	74.6	74.6	74.7	74.5	74.5	74.6	74.6	74.6	74.9	74.9	74.7
13	56.9	56.9	57.0	56.9	56.9	57.1	57.1	57.1	57.0	57.0	57.0
14	88.9	88.9	89.0	88.9	88.9	88.9	89.0	88.9	89.0	89.0	89.0
15	33.7	33.8	33.8	33.7	33.7	33.8	33.7	33.7	33.0	33.0	33.8
16	33.9	33.9	34.0	33.9	33.9	34.1	33.9	34.1	34.4	34.4	34.0
17	87.6	87.6	87.7	87.6	87.6	87.6	87.6	87.5	88.7	88.7	87.7
18	11.3	11.3	11.4	11.3	11.3	11.5	11.3	11.5	11.9	11.9	11.4
19	18.1	18.1	18.1	18.0	18.0	18.1	18.1	18.1	18.3	18.3	18.3
20	74.9	74.9	75.0	74.9	74.9	76.4	76.4	76.4	71.1	71.1	75.0
21	15.6	15.6	15.6	15.6	15.6	15.4	15.4	15.4	19.5	19.5	15.7
12-*O*-Acetyl moieties or cinnamoyl moieties											
1′	171.4	171.3	171.5	171.3	171.9	166.7	166.8	166.7	167.1	167.1	171.5
2′	22.0	22.0	22.1	22.0	22.0	120.2	120.2	120.2	119.7	121.0	22.1
3′						144.1	144.1	144.1	145.4	144.0	
4′						134.8	134.6	134.9	135.1	135.1	
5′						128.4	128.6	128.6	128.8	130.7	
6′						129.3	129.3	129.8	129.3	128.6	
7′						130.6	130.6	130.6	130.7	129.7	
8′						129.3	129.3	129.3	129.3	128.6	
9′						128.4	128.6	128.6	128.8	130.7	
20-*O*-*N*-Methylanthraniloyl moieties or nicotinoyl moieties											
1″	111.0	111.0	111.0	111.0	110.9						111.0
2″	152.6	152.6	152.7	152.6	152.6	151.4	151.4	151.4			152.7
3″	111.5	111.5	111.6	111.5	111.5	126.9	127.0	126.9			111.6
4″	135.1	135.1	135.1	135.1	135.1	137.4	137.4	137.4			135.1
5″	114.8	114.7	114.8	114.7	114.7	123.6	123.6	123.6			114.8
6″	132.6	132.6	132.7	132.6	132.6	153.8	153.8	153.8			132.7
7″	168.2	168.2	168.3	168.2	168.2	164.7	164.7	164.7			168.3
CH_3	29.6	29.6	29.7	29.5	29.5						29.6

Table 2. ^{1}H-NMR Data for the Aglycone and Ester Parts of Stephanosides A—J (**1**—**10**) (in Pyridine-d_5)

1—5		**6, 7, 8**		**9**		**10**	
Aglycone moiety		Aglycone moiety		Aglycone moiety		Aglycone moiety	
3	3.82 (1H, m)	3	3.85 (1H, m)	3	3.84 (1H, m)	3	3.82 (1H, m)
6	5.32 (1H, m)	6	5.32 (1H, m)	6	5.36 (1H, m)	6	5.36 (1H, m)
12	5.14 (1H, dd, J=11.5, 4.5 Hz)	12	5.34 (1H, dd, J=11.5, 4.5 Hz)	12	5.19 (1H, dd, J=11.5, 4.5 Hz)	12	5.19 (1H, dd, J=11.5, 4.5 Hz)
18	2.02 (3H, s)	18	2.14 (3H, s)	18	2.15 (3H, s)	18	1.99 (3H, s)
19	1.30 (3H, s)	19	1.30 (3H, s)	19	1.44 (3H, s)	19	1.38 (3H, s)
20	5.17 (1H, q, J=6.5 Hz)	20	5.10 (1H, q, J=6.5 Hz)	20	4.06 (1H, q, J=6.5 Hz)	20	4.06 (1H, q, J=6.5 Hz)
21	1.56 (3H, d, J=6.5 Hz)	21	1.58 (3H, d, J=6.5 Hz)	21	1.46 (3H, d, J=6.5 Hz)	21	1.39 (3H, d, J=6.5 Hz)
Acetyl moiety		(*E*)-Cinnamoyl moiety		(*E*)-Cinnamoyl moiety		(*Z*)-Cinnamoyl moiety	
	2.10 (3H, s)	2′	6.54 (1H, d, J=16.0 Hz)	2′	6.96 (1H, d, J=16.0 Hz)	2′	6.40 (1H, d, J=12.5 Hz)
		3′	7.85 (1H, d, J=16.0 Hz)	3′	8.18 (1H, d, J=16.0 Hz)	3′	7.02 (1H, d, J=12.5 Hz)
		5′,9′	*ca.* 7.43 (2H, m)	5′,9′	*ca.* 7.52 (2H, m)	5′,9′	7.91 (2H, d, J=7.5 Hz)
		6′,8′	*ca.* 7.38 (2H, m)	6′,8′	*ca.* 7.29 (2H, m)	6′,8′	7.37 (2H, t, J=7.5 Hz)
		7′	*ca.* 7.38 (1H, m)	7′	*ca.* 7.30 (1H, m)	7′	7.30 (1H, t, J=7.5 Hz)
N-Methyl anthraniloyl		Nicotinoyl moiety					
2″		2″	9.55 (1H, d, J=2.0 Hz)				
3″	6.72 (1H, d, J=8.5 Hz)						
4″	7.43 (1H, ddd, J=8.5, 8.0, 1.5 Hz)	4″	8.35 (1H, ddd, J=8.0, 2.0, 1.5 Hz)				
5″	6.59 (1H, t, J=8.0 Hz)	5″	7.24 (1H, dd, J=8.0, 4.5 Hz)				
6″	8.35 (1H, dd, J=8.0, 1.5 Hz)	6″	8.85 (1H, dd, J=4.5, 1.5 Hz)				
8″	8.10 (1H, q, J=5.0 Hz)						
9″	2.74 (3H, d, J=5.0 Hz)						

Table 3. NMR Data for Sugar Moieties (in Pyridine-d_5)

No.	a		b		c	
Sugar-1		cym		cym		cym
1′	96.4	5.26 dd (9.5, 1.5 Hz)	96.4	5.26 dd (9.5, 1.5 Hz)	96.5	5.25 dd (9.5, 1.5 Hz)
2′	37.2	1.90 m, 2.31 m	37.2	1.90 m, 2.31 m	37.3	1.89 m, 2.31 m
3′	78.1	4.06 dd (2.5, 1.5 Hz)	77.8	4.06 dd (2.5, 1.5 Hz)	78.0	3.92 dd (2.5, 1.5 Hz)
4′	83.1	3.48 dd (9.5, 2.5 Hz)	83.1	3.49 dd (9.5, 2.5 Hz)	83.5	3.52 dd (9.5, 2.5 Hz)
5′	69.3	4.20 dq (9.5, 6.5 Hz)	69.0	4.18 dq (9.5, 6.0 Hz)	69.1	4.24 dq (9.5, 6.5 Hz)
6′	18.6	1.38 d (6.5 Hz)	18.6	1.39 d (6.0 Hz)	18.7	1.45 d (6.5 Hz)
O-Me	58.8	3.61 s	58.9	3.62 s	59.0	3.59 s
Sugar-2		cym		cym		ole
1″	100.4	5.11 dd (9.5, 1.5 Hz)	100.5	5.09 dd (9.5, 1.5 Hz)	102.2	4.73 d (8.0 Hz)
2″	36.9	1.82 m, 2.31 m	37.2	1.81 m, 2.30 m	37.7	1.82 m, 2.50 m
3″	78.0	4.07 m	78.0	4.10 dd (2.5, 1.5 Hz)	79.3	*ca.* 3.60 m
4″	83.4	3.61 dd (9.5, 2.5 Hz)	83.4	3.51 dd (9.5, 2.5 Hz)	83.2	*ca.* 3.60 m
5″	69.0	4.22 dq (9.5, 6.0 Hz)	69.0	4.21 dq (9.5, 6.0 Hz)	72.1	*ca.* 3.56 m
6″	18.6	1.58 d (6.0 Hz)	18.6	1.42 d (6.0 Hz)	19.0	1.72 d (6.0 Hz)
O-Me	58.9	3.56 s	58.9	3.57 s	57.4	3.54 s
Sugar-3		the		ole		the
1‴	106.2	4.77 d (8.0 Hz)	102.2	4.77 dd (9.5, 1.5 Hz)	104.2	4.97 d (8.0 Hz)
2‴	75.1	3.92 t (8.0 Hz)	37.0	1.80 m, 2.48 m	75.3	3.92 t (8.0 Hz)
3‴	87.8	3.62 t (8.0 Hz)	81.4	3.59 dd (9.5, 3.0 Hz)	88.2	3.63 t (8.0 Hz)
4‴	75.8	3.61 dd (8.5, 8.0 Hz)	76.2	3.62 dd (9.5, 9.0 Hz)	76.1	3.62 dd (8.5, 8.0 Hz)
5‴	72.8	3.73 dq (8.5, 6.0 Hz)	72.9	*ca.* 3.58 m	72.9	3.74 dq (8.5, 6.0 Hz)
6‴	18.6	1.59 d (6.0 Hz)	18.7	1.57 d (6.0 Hz)	18.8	1.61 d (6.0 Hz)
O-Me	61.1	3.89 s	57.1	3.47 s	61.1	3.92 s

No.	d		e		f	
Sugar-1		cym		cym		cym
1′	96.4	5.25 dd (9.5, 1.5 Hz)	96.4	5.27 dd (9.5, 1.5 Hz)	96.5	5.28 dd (9.5, 1.5 Hz)
2′	37.3	1.90 m, 2.30 m	37.2	1.90 m, 2.31 m	37.2	1.80 m, 2.30 m
3′	77.9	4.03 dd (2.5, 1.5 Hz)	78.1	4.09 dd (2.5, 1.5 Hz)	77.8	4.00 dd (2.5, 1.5 Hz)
4′	83.5	3.47 dd (9.5, 2.5 Hz)	83.4	3.52 dd (9.5, 2.5 Hz)	83.2	3.42 dd (9.5, 2.5 Hz)
5′	69.0	4.22 dq (9.5, 6.5 Hz)	69.1	4.22 dq (9.5, 6.5 Hz)	69.2	4.15 dq (9.5, 6.0 Hz)
6′	18.7	1.42 d (6.5 Hz)	18.6	1.39 d (6.5 Hz)	18.6	1.39 d (6.0 Hz)
O-Me	58.9	3.55 g	58.9	3.62 s	58.9	3.62 s
Sugar-2		ole		cym		cym
1″	101.9	4.67 dd (9.5, 1.5 Hz)	100.5	5.12 dd (9.5, 1.5 Hz)	100.5	5.12 dd (9.5, 1.5 Hz)
2″	37.5	1.82 m, 2.50 m	37.0	1.82 m, 2.31 m	37.0	1.81 m, 2.31 m
3″	79.3	*ca.* 3.57 m	77.8	4.02 dd (2.5, 1.5 Hz)	78.0	4.05 dd (2.5, 1.5 Hz)
4″	82.8	3.59 dd (9.5, 9.0 Hz)	83.4	3.44 dd (9.5, 2.5 Hz)	83.4	3.46 dd (9.5, 2.5 Hz)
5″	72.0	*ca.* 3.53 m	69.0	4.17 dq (9.5, 6.5 Hz)	69.0	4.20 dq (9.5, 6.5 Hz)
6″	19.0	1.64 d (6.0 Hz)	18.6	1.39 d (6.0 Hz)	18.7	1.39 d (6.0 Hz)
O-Me	57.2	3.52 s	59.0	3.58 s	59.0	3.55 s
Sugar-3		allm		ole		ole
1‴	102.2	5.29 d (8.0 Hz)	101.9	4.70 dd (10.0, 1.5 Hz)	101.8	4.67 dd (9.5, 1.5 Hz)
2‴	73.2	3.88 dd (8.0, 3.0 Hz)	37.6	1.80 m, 2.48 m	37.6	*ca.* 1.75 m, 2.28 m
3‴	84.0	4.08 t (3.0 Hz)	79.3	*ca.* 3.61 m	79.2	3.58 dd (9.5, 3.0 Hz)
4‴	74.6	3.61 dd (8.5, 3.0 Hz)	83.2	3.64 t (9.0 Hz)	82.8	3.62 dd (9.5, 9.0 Hz)
5‴	71.0	4.16 dq (8.5, 6.0 Hz)	72.1	*ca.* 3.59 m	72.0	3.60 dq (9.0, 6.0 Hz)
6‴	18.7	1.55 d (6.0 Hz)	18.9	1.71 d (6.0 Hz)	19.0	1.64 d (6.0 Hz)
O-Me	62.1	3.83 s	57.3	3.53 s	57.4	3.53 s
Sugar-4				the		allm
1⁗			104.2	4.96 d (8.0 Hz)	102.2	5.31 d (8.0 Hz)
2⁗			75.3	3.91 t (8.0 Hz)	73.2	3.95 dd (8.0, 3.0 Hz)
3⁗			88.2	3.63 t (8.0 Hz)	84.0	4.05 dd (8.0, 3.0 Hz)
4⁗			76.1	3.62 t (8.0 Hz)	74.6	3.60 dd (8.0, 3.0 Hz)
5⁗			72.9	3.74 dq (8.5, 6.0 Hz)	71.0	4.16 dq (8.0, 6.5 Hz)
6⁗			18.6	1.60 d (6.0 Hz)	18.7	1.56 d (6.5 Hz)
O-Me			61.0	3.90 s	62.1	3.85 s

Abbreviation: allm = β-D-allomethylopyranosyl, cym = β-D-cymaropyranosyl, ole = β-D-oleadropyranosyl, the = β-D-thevetopyranosyl.

O-(*N*-methyl)anthraniloyl sarcostin 3-*O*-β-D-thevetopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside D (**4**) has the molecular formula $C_{52}H_{79}NO_{17}$ [positive FAB ion at *m/z* 1028 $[M+K]^+$], *i.e.*, one oxygen atom less than that of **5**. On acid hydrolysis, **4** afforded cymarose and oleandrose as the sugar moieties. In the ^{1}H-NMR spectrum of **4**, three anomeric proton signals were observed at δ 5.26, 5.09 and 4.77 (each 1H, dd, *J* = 9.5, 1.5 Hz), which indicated all the

glycosidic linkages to have β-orientation. The sugar linkage at C-3 was determined by the HMBC experiment. Cross peaks between H-3 of the aglycone at δ 3.82 and C-1′ of the inner cymarose at δ 96.4, H-4′ of the inner cymarose at δ 3.49 and C-1″ of the outer cymarose at δ 100.5, and H-4″ of the outer cymarose at δ 3.51 and C-1‴ of the oleandrose at δ 102.2 were observed. Consequently, the sugar sequence was established as D-oleandrose-(1→4)-D-cymarose-(1→4)-D-cymarose. Based on the above information, the structure of **4** has been established as 12-*O*-acetyl-20-*O*-(*N*-methyl)anthraniloyl sarcostin 3-*O*-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside C (**3**) has the same molecular formula, $C_{52}H_{79}NO_{18}$, as that of **5**, and afforded cymarose, oleandrose and thevetose as the sugar moieties on acid hydrolysis. The ^{1}H-NMR spectrum of **3** showed three anomeric proton signals at δ 5.25 (1H, dd, J=9.5, 1.5 Hz), δ 4.73 (1H, d, J=8.0 Hz) and δ 4.97 (1H, d, J=8.1 Hz). The coupling patterns indicated all glycosidic linkages to have β-configuration. The sugar linkages at C-3 were determined by means of an HMBC experiment in the same way as for **5**. The anomeric proton signals at δ 5.25 (cymarose), 4.73 (oleandrose) and δ 4.97 (thevetose) showed long-range correlations with the ^{13}C signals at δ 77.8 (C-3), 83.5 (C-4′ of cymarose), and δ 83.2 (C-4″ of oleandrose), respectively in the HMBC spectrum. Consequently, the structure of **3** has been established as 12-*O*-acetyl-20-*O*-(*N*-methyl)anthraniloyl sarcostin 3-*O*-β-D-thevetopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside B (**2**) has the molecular formula $C_{52}H_{79}NO_{18}$, [negative FAB-MS ion at m/z 1004 $[M-H]^-$], as that of **3**, and afforded cymarose, oleandrose and an unidentified sugar (on TLC) as the sugar moieties after acid hydrolysis. ^{1}H–^{1}H COSY experiments allowed the sequential assignments of the ^{1}H resonances for the unidentified sugar as shown in Table 3, starting from the anomeric proton signal at δ 5.29 (d, J=8.0 Hz). Furthermore, a long-range correlation was observed between methoxy protons at δ 3.83 (s) and the C-3‴ carbon at δ 84.0 in the HMBC spectrum of **2**. Those findings suggested that the unidentified sugar (detected on TLC) is β-3-*O*-methyl-6-deoxyallose (abbreviated as allomethylose).[7] The anomeric proton signals due to oleandrose and cymarose were observed in the ^{1}H-NMR spectrum of **2** at δ 4.67 and 5.25 (1H each, dd, J=9.5, 1.5 Hz), which indicated that oleandrose and cymarose have β-configuration.

The sugar linkages at C-3 were determined by means of an HMBC experiment in the same way as for **5**. The anomeric proton signals at δ 5.25 (cymarose), 4.67 (oleandrose) and δ 5.29 (allomethylose) showed long-range correlations with the ^{13}C signals at δ 77.7 (C-3), 83.5 (C-4′ of cymarose) and δ 82.8 (C-4″ of oleandrose), respectively in the HMBC spectrum. Based on the above information, the structure of **2** has been established as 12-*O*-acetyl-20-*O*-(*N*-methyl)anthraniloyl sarcostin 3-*O*-β-D-(3-*O*-methyl-6-deoxoallopyranosyl)(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside A (**1**) has the molecular formula, $C_{59}H_{91}NO_{21}$, and affords cymarose and oleandrose and thevetose as the sugar moieties on acid hydrolysis. In the ^{1}H-NMR spectrum of **1**, four anomeric proton signals were observed at δ 5.27 and 5.12 (each 1H, dd, J=9.5, 1.5 Hz), 4.70 (1H dd, J=10.0, 1.5 Hz) and δ 4.96 (1H, d, J=8.0 Hz), whose coupling constants indicated all the glycosidic linkages to have β-orientation. For the sugar linkage, long-range correlations were observed as follows in the HMBC spectrum, δ 96.4 [C-1′ of the β-cymaropyranosyl] and δ 3.82 (1H, m) [H-3 of aglycone], δ 100.5 [C-1″ of outer β-cymaropyranosyl] and δ 3.52 (1H, dd, J=9.5, 2.5 Hz) [H-4′ of inner β-cymaropyranosyl], δ 101.9 [C-1‴ of the β-oleandropyranosyl] and δ 3.44 (1H, dd, J=9.5, 2.5 Hz) [H-4″ of outer β-cymaropyranosyl], and δ 104.2 [C-1⁗ of β-thevetopyranosyl] and δ 3.64 (1H, t, J=9 Hz) [H-4‴ of β-oleandropyranosyl]. Consequently, the structure of **1** has been established as 12-*O*-acetyl-20-*O*-(*N*-methyl)anthraniloyl sarcostin 3-*O*-β-D-thevetopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

The ^{1}H-NMR and ^{13}C-NMR spectra of stephanosides F-H (**6**—**8**) suggested that **6**—**8** were composed of 1 mol each of (*E*)-cinnamic acid, nicotinic acid and sarcostin, except for sugar moieties. On alkaline hydrolysis, **6**—**8** gave (*E*)-cinnamic acid and nicotinic acid as the acyl groups. In the HMBC spectrum, the carbonyl carbon signal of the cinnamoyl group at δ 166.7 was correlated with the signal of the methine proton (H-12) at δ 5.34 on an oxygen-bearing carbon (C-12) at δ 74.6, and that of the nicotinoyl group at δ 164.7 was correlated with that of the methine proton (H-20) at δ 5.10 on an oxygen-bearing carbon (C-20) at δ 76.4, establishing that in **6**—**8**, the cinnamoyl group is located at *O*-12 and the nicotinoyl group at *O*-20.

Stephanoside F (**6**) has the molecular formula $C_{64}H_{91}NO_{21}$, [negative FAB-MS ion at m/z 1208 $[M-H]^-$] and afforded cymarose, oleandrose and thevetose as the sugar moieties on acid hydrolysis. By comparison of the ^{1}H- and ^{13}C-NMR data for **6** with those of for **1**, it was shown that **6** possesses the same sugar sequence in the oligosaccharide moiety as that of **1** and the moiety is attached to the C-3 hydroxyl group of the aglycone. Thus, the structure of **6** has been established as 12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin 3-*O*-β-D-thevetopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside G (**7**) has the molecular formula $C_{57}H_{79}NO_{18}$ [positive FAB-MS ion at m/z 1104 for $[M+K]^+$], and afforded cymarose, oleandrose and thevetose as the sugar moieties on acid hydrolysis. The carbon signals due to the sugar moieties were superimposable on those of **3**, indicating that the sugar linkages are the same. Consequently, the structure of **7** has been established as 12-*O*-cinnamoyl-20-*O*-nicotinoyl sarcostin 3-*O*-β-D-thevetopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranoside.

Stephanoside H (**8**) has the same molecular formula, $C_{57}H_{79}O_{18}$, as **7**, and afforded cymarose and thevetose as the sugar moieties on acid hydrolysis. The ^{1}H- and ^{13}C-NMR signals due to the aglycone and sugar moieties of **8** were almost superimposable on those of **5** and differed

only in those due to the acyl moieties linked with *O*-12 and *O*-20. Hence, the structure of **8** has been established as 12-*O*-cinnamoyl-20-*O*-nicotinoyl sarcostin 3-*O*-*β*-D-thevetopyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranoside.

Stephanoside I (**9**) has the molecular formula, $C_{58}H_{88}O_{20}$, and afford cymarose, oleandrose and thevetose as the sugar moieties on acid hydrolysis. Alkaline hydrolysis of **9** furnished (*E*)-cinnamic acid. A ^{1}H- and ^{13}C-NMR spectral comparison of **9** with **6** disclosed C_{20} [−0.94 ppm (H-20), −5.3 ppm (C-20)] as the deacylation site in the former. The carbon signals due to the sugar moieties were superimposable on those of **6**, indicating that the sugar moieties are the same. Therefore, the structure of **9** has been established as 12-*O*-cinnamoyl sarcostin 3-*O*-*β*-D-thevetopyranosyl(1→4)-*β*-D-oleandropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranoside.

Stephanoside J (**10**) has the same molecular formula, $C_{58}H_{88}O_{20}$, as **9** and afforded allomethylose, cymarose and oleandrose as the sugar moieties on acid hydrolysis. The carbon signals due to the aglycone in the ^{13}C-NMR spectrum of **10** were shown to be superimposable on those of **9**, suggesting that **10** is a 12-*O*-acyl-3-*O*-glycoside of sarcostin. The ^{1}H-NMR spectrum of **10** showed signals assignable to a (*Z*)-cinnamoyl group at *δ* 6.40 and 7.02 (each 1H, d, *J*=12.5 Hz, H-2′, H-3′). Further, a correlation peak was observed between the H-12 at *δ* 5.19 (dd, *J*=11.5, 4.0 Hz) and carbonyl carbon (C-1′) at *δ* 167.1 in the HMBC spectrum, establishing that the (*Z*)-cinnamoyl group is located at *O*-12. The ^{1}H-NMR spectrum of **10** showed four anomeric proton signals at *δ* 5.28, 5.12 and 4.67 (each 1H dd, *J*=9.5, 1.5 Hz) and *δ* 5.31 (1H, d, *J*=8.0 Hz), whose coupling constants indicated the *β*-orientation for all glycosidic linkages. The sugar linkages at C-3 were determined by means of an HMBC experiment in the same way as for **5**. The anomeric proton signals at *δ* 5.28 (inner cymarose), 5.12 (outer cymarose), 4.67 (oleandrose) and *δ* 5.31 (allomethylose) showed long-range correlations with the ^{13}C signals at *δ* 77.8 (C-3), 83.2 (C-4′ of inner cymarose), 83.4 (C-4″ of outer cymarose), and *δ* 82.8 (C-4‴ of oleandrose), respectively, in the HMBC spectrum. Hence, the structure of **10** has been established as 12-*O*-(*E*)-cinnamoyl sarcostin 3-*O*-*β*-D-(3-*O*-methyl-6-deoxyallopyranosyl)(1→4)-*β*-D-oleandropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranoside. Recently, Yoshikawa *et al.* reported that *E*-senegasaponins a and b and their *Z*-isomers isolated from Senegae Radix were isomerized in aqueous methanolic solution on standing.[8] However, we could not obtain such an isomer from the extract.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. IR and UV spectra were measured with JASCO FT/IR-5300 and Shimadzu UV-160 instruments. NMR spectra were recorded on a Varian UNITY 200 or 600 spectrometer in C_5D_5N solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, distortionless enhancement by polarization transfer (DEPT), HMBC (512×1024 data matrix size, 128 scans, recycle delay=1.16 s), TOCSY and ROESY. Coupling constants (*J* values) are given in Hz. The FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as the matrix) were measured on a JEOL JMS-PX303 mass spectrometer. For column chromatography, Kiesel gel 60 (230—400 mesh, Merck), and for TLC, silica gel 60F-254 (Merck) were used. HPLC was carried out with a Waters ALC/GPC 244 instrument.

Isolation of Saponins Fresh stems (8.5 kg) of *S. lutchuensis* var. *japonica* collected in Tokushima prefecture in June, 1993, were extracted with absolute EtOH at room temperature for 3 weeks and the solvent was evaporated off under reduced pressure to give the EtOH extract (540 g). The ethanolic extract was partitioned between H_2O and EtOAc. The EtOAc phase was evaporated under reduced pressure to afford the EtOAc extract (80 g). The EtOAc extract (40 g) was separated by column chromatography (CH_2Cl_2 : MeOH = 100 : 0—25 : 2) and HPLC (YMC-ODS, 250×20 mm, CH_3CN : H_2O = 45 : 55—40 : 60) to afford stephanosides A (**1**, 500 mg), B (**2**, 370 mg), C (**3**, 165 mg), D (**4**, 30 mg), E (**5**, 230 mg), F (**6**, 450 mg), G (**7**, 100 mg), H (**8**, 100 mg), I (**9**, 560 mg), and J (**10**, 40 mg).

Stephanoside A (**1**): mp 143—145 °C, $[\alpha]_D^{20}$ +12.0° (*c*=1.9, $CHCl_3$). IR (film): 3445, 1730, 1715, 1580, 1245, 1100 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 206 (4.05), 222 (4.17), 257 (3.74), 279 (3.61), 354 (3.38). Negative FAB-MS *m/z*: 1148 $[M-H]^-$. *Anal.* Calcd for $C_{59}H_{91}NO_{21}\cdot 1/2H_2O$: C, 61.12; H, 8.00; N, 1.21. Found: C, 61.26; H, 8.29; N, 1.33. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside B (**2**): mp 140—142 °C, $[\alpha]_D^{20}$ +2.0° (*c*=3.3, $CHCl_3$). IR (film): 3445, 1730, 1680, 1580, 1240, 1160 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 221 (5.32), 255 (4.96), 280 (4.17), 350 (4.65). Negative FAB-MS *m/z*: 1004 $[M-H]^-$. *Anal.* Calcd for $C_{52}H_{79}NO_{21}\cdot 1/2H_2O$: C, 61.97; H, 7.85; N, 1.37. Found: C, 61.72; H, 8.14; N, 1.50. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside C (**3**): mp 151—153 °C, $[\alpha]_D^{20}$ +5.9° (*c*=1.5, $CHCl_3$). IR (film): 3445, 1730, 1680, 1580, 1245, 1165 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 220 (5.33), 255 (4.80), 281 (4.20), 351 (4.68). Positive FAB-MS *m/z*: 1044 $[M+K]^+$. *Anal.* Calcd for $C_{52}H_{79}NO_{18}\cdot 1/2H_2O$: C, 61.52; H, 7.94; N, 1.38. Found: C, 61.49; H, 8.10; N, 1.15. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside D (**4**): mp 148—150 °C, $[\alpha]_D^{20}$ −48.8° (*c*=1.0, $CHCl_3$). IR (film): 3445, 1730, 1685, 1240, 1170 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 221 (5.20), 256 (4.75), 280 (4.20), 352 (4.77). Positive FAB-MS *m/z*: 1028 $[M+K]^+$, 1012 $[M+Na]^+$, 990 $[M+H]^+$. *Anal.* Calcd for $C_{52}H_{79}NO_{17}\cdot H_2O$: C, 61.95; H, 8.01; N, 1.34. Found: C, 61.85; H, 7.76; N, 1.50. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside E (**5**): mp 153—155 °C, $[\alpha]_D^{20}$ −4.8° (*c*=3.8, $CHCl_3$). IR (film): 3445, 1730, 1685, 1580, 1240, 1165 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 220 (5.32), 256 (4.76), 280 (4.25), 350 (4.75). Positive FAB-MS *m/z*: 1044 $[M+K]^+$, 1028 $[M+Na]^+$, 1006 $[M+H]^+$. *Anal.* Calcd for $C_{52}H_{79}NO_{18}\cdot 3/2H_2O$: C, 60.45; H, 8.00; N, 1.36. Found: C, 60.19; H, 7.79; N, 1.18. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside F (**6**): mp 152—154 °C, $[\alpha]_D^{20}$ +90.8° (*c*=2.5, $CHCl_3$). IR (film): 3460, 1715, 1635, 1595, 1100 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 217 (4.30), 281 (4.22). Negative FAB-MS *m/z*: 1208 $[M-H]^-$. *Anal.* Calcd for $C_{64}H_{91}NO_{21}\cdot H_2O$: C, 62.58; H, 7.63; N, 1.14. Found: C, 62.79; H, 7.62; N, 1.30. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside G (**7**): mp 150—152 °C, $[\alpha]_D^{20}$ +60.6° (*c*=1.1, $CHCl_3$). IR (film): 3445, 1715, 1635, 1560, 1100 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 206 (4.18), 218 (4.23), 280 (4.11). Positive FAB-MS *m/z*: 1104 $[M+K]^+$, 1066 $[M+H]^+$. *Anal.* Calcd for $C_{57}H_{79}NO_{18}\cdot H_2O$: C, 63.14; H, 7.53; N, 1.29. Found: C, 63.25; H, 7.50; N, 1.20. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside H (**8**): mp 160—162 °C, $[\alpha]_D^{20}$ +92.0° (*c*=1.1, $CHCl_3$). IR (film): 3445, 1715, 1635, 1595, 1560, 1100 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 206 (4.18), 217 (4.30), 280 (4.23). Positive FAB-MS *m/z*: 1104 $[M+K]^+$, 1066 $[M+H]^+$. *Anal.* Calcd for $C_{57}H_{79}NO_{18}\cdot 3H_2O$: C, 62.00; H, 7.76; N, 1.27. Found: C, 62.26; H, 7.39; N, 1.50. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside I (**9**): mp 145—147 °C, $[\alpha]_D^{20}$ +13.6° (*c*=3.7, $CHCl_3$). IR (film): 3460, 1705, 1635, 1580, 1120 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 205 (4.18), 217 (4.18), 279 (4.33). Positive FAB-MS *m/z*: 1143 $[M+K]^+$. *Anal.* Calcd for $C_{58}H_{88}O_{20}\cdot 1/2H_2O$: C, 62.52; H, 8.05. Found: C, 62.34; H, 8.02. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside J (**10**): mp 125—127 °C, $[\alpha]_D^{20}$ +21.0° (*c*=1.9, $CHCl_3$). IR (film): 3450, 1705, 1635, 1585, 1120 cm^{-1}. UV λ_{max}^{EtOH} nm (log *ε*): 200 (4.50), 276 (4.11). Positive FAB-MS *m/z*: 1143 $[M+K]^+$. *Anal.* Calcd for $C_{58}H_{88}O_{20}\cdot H_2O$: C, 61.25; H, 7.98; N, 1.23. Found: C, 61.00; H, 8.00; N, 1.30. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Mild Acidic Hydrolysis of Stephanoside A (1) A solution of **1** (95 mg) in 3 ml of MeOH was hydrolyzed with 1 ml of 1% H_2SO_4 with stirring at 60 °C for 45 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 phase was evaporated under reduced pressure to afford the CH_2Cl_2 extract (40 mg). The CH_2Cl_2 extract (40 mg) was separated by HPLC (YMC-ODS, 250 × 20 mm, CH_3CN : H_2O = 45 : 55—40 : 60) to afford the prosapogenin (**11**, 15 mg): mp 160—162 °C, $[\alpha]_D^{20}$ +10.0° (c = 2.9, $CHCl_3$). IR (film): 3375, 1730, 1705, 1675, 1240, 1160, 1130, 1080 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 203 (4.27), 222 (4.50), 254 (4.04), 354 (3.83). CI-MS m/z: 558 $[M+H]^+$. High-resolution CI-MS m/z: 558.3050 (Calcd for $C_{31}H_{43}NO_8+H^+$: 558.3065). ^{1}H-NMR δ: 1.37 (3H, s, H-19), 1.55 (3H, d, J = 6.0 Hz, H-21), 2.04 (3H, s, H-18), 2.12 (3H, s, Ac), 2.80 (3H, d, J = 5.0 Hz, N-Me), 3.88 (1H, m, H-3), 5.15 (1H, dd, J = 11.0, 4.5 Hz, H-12), 5.18 (1H, q, J = 6.5 Hz, H-20), 5.35 (1H, m, H-6), 6.60 (1H, dd, J = 8.0, 8.0 Hz, H-5″ of anth.), 6.75 (1H, d, J = 8.0 Hz, H-3″ of anth.), 7.44 (1H, ddd, J = 8.0, 8.0, 1.5 Hz, H-4″ of anth.), 8.12 (1H, q, J = 5.0 Hz, NH of anth.), 8.36 (1H, dd, J = 8.0, 1.5 Hz, H-6″ of anth.). ^{13}C-NMR: Table 1. The H_2O phase was neutralized with Amberlite IRA-45 and evaporated under reduced pressure to give the sugar portion. Oleandrose and cymarose were identified by comparison with authentic samples on TLC with solvents 1 ($CHCl_3$: MeOH = 15 : 1) and 2 (EtOAc : MeOH = 9 : 1).

Mild Acidic Hydrolysis of Stephanosides B—E (2—5) A solution of each compound (20 mg) was hydrolyzed in the same way as **1**. The prosapogenin (**11**, *ca.* 2 mg) was obtained from each CH_2Cl_2 phase. Each H_2O phase was analyzed in the same way as described for **1**. Cymarose, oleandrose and allomethylose were detected from the hydrolysate of **2**. Cymarose, oleandrose and thevetose were detected from that of **3**. Cymarose and oleandrose were detected from that of **4**. Cymarose and thevetose was detected from that of **5**.

Alkaline Hydrolysis of Prosapogenin (11) The prosapogenin (**11**, 10 mg) was heated in 28% sodium methylate and methanol (1 : 1) at 37 °C for 2 h. The reaction mixture was diluted with H_2O and passed through a column of Amberlite IR-120B on Mitsubishi Diaion HP-20. The methanol eluate gave sarcostin (**12**, 6 mg), mp 260—262 °C. CI-MS m/z: 383 $(M+H)^+$ and methyl *N*-methylanthranilate (1.5 mg).[1)]

Acidic Hydrolysis of Stephanosides F—J (6—10) for Analysis of the Sugar Moiety A solution of each compound (2—3 mg) was treated in the same way as described for **1**. Cymarose, oleandrose and thevetose were detected from the hydrolysate of **6**, **7** and **9**. Cymarose and thevetose were detected from that of **8**. Cymarose, oleandrose and allomethylose were detected from that of **10**.

Alkaline Hydrolysis of 6—9 for Analysis of Acyl Moiety A solution of each compound (2—3 mg) was treated in the same way as **11**. Compounds **6**, **7** and **8** gave (*E*)-cinnamic acid (J'sphero ODS-H-80, 25% CH_3CN, t_R 14.8 min) and nicotinic acid (Daisogel SP-120-5-ODS-Bp, 3 mM $HClO_4$, t_R 4.5 min). Compound **9** gave (*E*)-cinnamic acid.

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