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

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The structural elucidation of seven related oxypregnane-oligoglycosides stephanosides K (1), L (2), M (3), N (4), O (5), P (6), and Q (7) from the fresh stem of *Stephanotis lutchuensis* var. japonica (Asclepiadaceae) was achieved through on a detailed study of their high-field ^{1}H- and ^{13}C-NMR spectra. The results show that all the sugars are β (1→4)-linked. The aglycones of stephanosides K, M, and O, and stephanosides L, N, and P were identified as 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl, and 12-*O*-cinnamoyl-20-*O*-nicotinoyl derivatives of (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol, respectively. The aglycone of stephanoside Q was 12-*O*-cinnamoyldeacetylmetaplexigenin (kidjoranin).

Key words *Stephanotis lutchuensis* var. *japonica*; Asclepiadaceae; oxypregnane-oligoglycoside; 5α-hydroxypregnane; stephanoside; 2-deoxysugar

In a previous paper,[1)] we have reported the isolation and structural elucidation of ten oxypregnane-oligoglycosides, stephanosides A—J, from the stem of *Stephanotis lutchuensis* var. *japonica* (Asclepiadaceae). The present paper describes the isolation and full structural elucidation of seven additional new oxypregnane-oligoglycosides named stephanosides K—Q (1—7).

The EtOH extract obtained from the fresh stem 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 reversed-phase adsorbent provided compounds named stephanosides K (1), L (2), M (3), N (4), O (5), P (6), and Q (7). The results of ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H–^{13}C COSY, total correlation spectroscopy (TOCSY), heteronuclear multiple-bond correlation (HMBC) and rotating Overhauser enhancement spectroscopy (ROESY) experiments allowed us to determine the structures of 1—7.

The IR spectra of 1, 3, and 5 showed carbonyl absorption bands at 1710 cm^{-1} and strong absorption bands at 3480 and 1170 cm^{-1} suggestive of oligoglycosidic structure. The ^{1}H- and ^{13}C-NMR spectra of 1, 3, and 5 exhibited signals characteristic of an oxypregnane-oligoglycoside containing two or three 2-deoxyhexoses and one hexose, as well as one (*E*)-cinnamic acid and one (*E*)-2-methyl-2-butenoic acid (tiglic acid) as acyl moieties (Tables 1—3). On mild acid hydrolysis, 1, 3, and 5 gave the common prosapogenin 8.

Prosapogenin 8 has the molecular formula $C_{35}H_{46}O_9$ based on high-resolution CI-MS. On alkaline hydrolysis, 8 afforded (*E*)-cinnamic acid and (*E*)-2-methyl-2-butenoic acid, which were identical with authentic samples. The ^{13}C-NMR (distortionless enhancement by polarization transfer, DEPT) and ^{1}H-NMR spectra of 8 indicated the presence of three methyl carbons, six methylene carbons, one methine carbon, two quaternary carbons, three oxygen-bearing methine carbons, and four oxygen-bearing quaternary carbons due to the aglycone. The ^{1}H–^{1}H COSY and TOCSY spectra of 8 revealed isolated spin systems (H-1-4, H-6-7, H-9-12, H-15-16, H-20-21) (Fig. 1). The gross structure of 8 was determined by analysis of NMR data, including ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC (Fig. 1) and ROESY experiments, and by referring to the data for stephanosides A—J.[1)] Thus, the aglycone of 8 was shown to be pregn-6-ene-3,5,8,12,14,17,20-heptaol. The relative stereochemistry of 8 was elucidated by means of a ROESY experiment (in dimethyl sulfoxide (DMSO)-d_6) (Fig. 2) and from the coupling constants. The nuclear Overhauser effect (NOEs) between H_α-1 at δ 1.65 and H-3 at δ 3.95, and H_α-1 at δ 1.65 and C_5-OH at δ 4.61 indicated that the A ring is in chair form and the hydroxyl group in C-3 is β-oriented. On the basis of the NOE between H_α-1 at δ 1.65 and H-9 at δ 1.84, the trans A/B ring junction was assigned to 8. The NOEs between H_3-18 at δ 1.44 and C_8-OH at δ 3.91, H_3-18 at δ 1.44 and C_{14}-OH at δ 5.06, and H_3-18 at δ 1.44 and C_{17}-OH at δ 5.08 established all of the OH orientations as β. The α-oriented hydrogen at C_{12} was confirmed by NOE between H-12 and H-9. The remaining chiral center, C-20, must have the *S* configuration (assuming the absolute structure as in Fig. 2), as judged from the NOEs between H-12 at δ 4.69 and H-20 at δ 4.40, H-20 at δ 4.40 and H_α-16 at δ 1.88, and H_3-21 at δ 1.08 and H_β-16 at δ 1.72. Based on the above evidence, the fundamental structure of the prosapogenin 8 has been established as (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol.

The locations of the cinnamoyl group and (*E*)-2-methyl-2-butenoyl group in 8 have been determined by means of an HMBC experiment (in DMSO-d_6) (Fig. 1). In the HMBC spectrum, the carbonyl carbon signal of the cinnamoyl group at δ 165.4 was correlated with the methine proton (H-12) at δ 4.69 on an oxygen-bearing carbon (C-12) at δ 74.4, and that of the (*E*)-2-methyl-2-butenoyl group at δ 165.7 was correlated with the methine proton (H-20) at δ 4.40 on an oxygen-bearing carbon (C-20) at δ 73.2, establishing that in 8 the cinnamoyl group is located at *O*-12 and the (*E*)-2-methyl-2-butenoyl group at *O*-20. Thus, the structure of the prosapogenin 8 has been established as 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β, 17β, 20-heptaol.

Stephanoside K (1) has the molecular formula $C_{56}H_{82}O_{18}$ based on elemental analysis. On acid hydrolysis, 1 afford-

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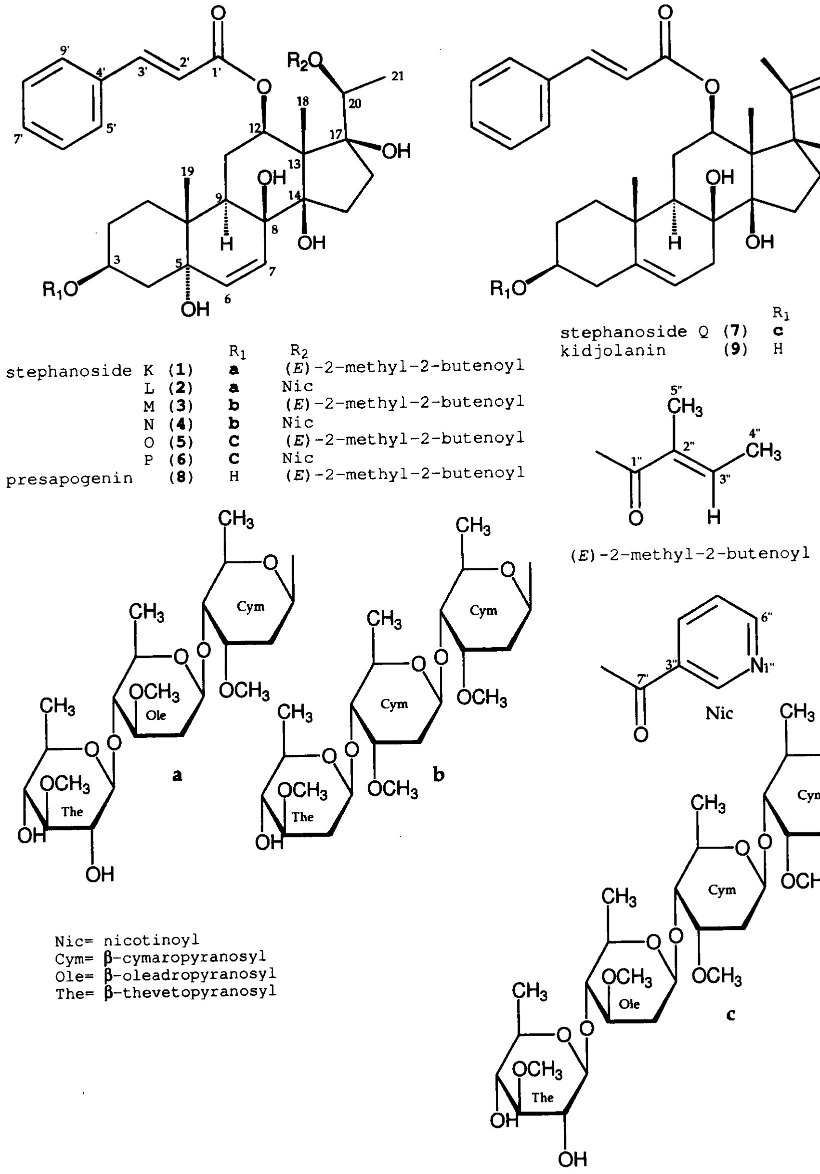

		R_1	R_2
stephanoside K	(**1**)	**a**	(*E*)-2-methyl-2-butenoyl
L	(**2**)	**a**	Nic
M	(**3**)	**b**	(*E*)-2-methyl-2-butenoyl
N	(**4**)	**b**	Nic
O	(**5**)	**c**	(*E*)-2-methyl-2-butenoyl
P	(**6**)	**c**	Nic
presapogenin	(**8**)	H	(*E*)-2-methyl-2-butenoyl

		R_1
stephanoside Q	(**7**)	**c**
kidjolanin	(**9**)	H

(*E*)-2-methyl-2-butenoyl

Nic= nicotinoyl
Cym= β-cymaropyranosyl
Ole= β-oleadropyranosyl
The= β-thevetopyranosyl

Chart 1

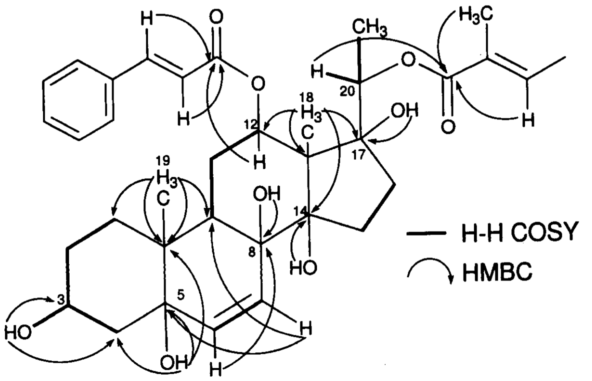

Fig. 1. Significant Correlations Observed in HMBC of the Prosapogenin (**8**)

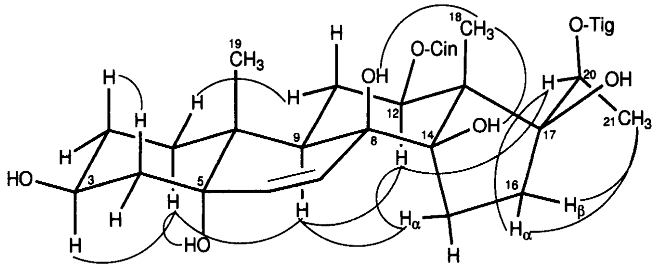

Fig. 2. Significant NOEs of the Prosapogenin (**8**)

ed cymarose, oleandrose and thevetose. The anomeric proton signals due to cymarose, oleandrose and thevetose were observed in the ^{1}H-NMR spectrum of **1** at δ 5.15 and 4.68 (each 1H, dd, $J=9.5$, 1.5 Hz) and δ 4.95 (1H, d, $J=8.0$ Hz), which indicated that all the glycosidic linkages are of β-configuration, as judged from the coupling constants.[2,3] In the ^{13}C-NMR spectrum of **1**, three anomeric carbon signals were observed at δ 97.8 (C-1′), 101.9 (C-1″) and 104.2 (C-1‴).

The location of the carbohydrate moiety attached to the aglycone was determined from the fact that glycosylation shifts[4] were observed in the ^{13}C-NMR spectrum of **1** for the C-2 (−1.9 ppm), C-3 (+8.3 ppm), and C-4 (−1.9 ppm) signals as compared with those of the prosapogenin **8**. As regards the sugar linkage, the followig long-range correlations were observed in the HMBC

Table 1. ^{13}C-NMR Data for the Aglycone Parts of Stephanosides K (**1**), L (**2**), M (**3**), N (**4**), O (**5**), P (**6**) and Q (**7**), the Prosapogenin (**8**) and Kidjoranin (**9**) (in Pyridine-d_5)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**8**[a]	**9**
1	27.8	27.8	27.7	27.7	27.6	27.7	39.2	27.0	26.0	39.2
2	26.7	26.8	26.6	26.7	26.6	26.6	30.0	28.6	27.0	32.0
3	75.0	75.0	74.9	75.0	75.0	74.8	77.9	66.7	65.4	71.6
4	39.1	39.3	39.1	39.2	39.1	39.1	39.4	41.0	39.8	43.3
5	75.0	75.0	74.9	74.9	74.8	74.9	139.4	75.6	74.4	140.3
6	136.7	136.9	136.7	136.8	136.7	136.2	119.2	136.9	135.4	118.5
7	127.5	127.6	127.4	127.3	127.4	127.3	35.0	127.1	125.8	34.8
8	74.1	74.3	74.1	74.1	74.0	74.1	74.5	74.1	72.6	74.4
9	36.7	36.9	36.9	36.8	36.8	36.7	44.7	36.4	34.7	44.6
10	39.7	39.8	39.7	39.7	39.7	39.7	37.6	39.9	38.5	37.4
11	23.8	23.8	23.8	23.8	23.7	23.7	25.2	23.8	22.4	25.1
12	75.8	75.8	75.8	75.8	75.8	75.8	73.9	75.7	74.4	73.7
13	58.1	58.3	58.1	58.2	58.0	58.3	58.3	58.1	56.7	58.1
14	88.3	88.5	88.3	88.4	88.2	88.3	89.7	88.3	87.0	89.5
15	33.3	33.2	33.3	33.2	33.2	33.2	33.2	33.2	32.0	33.1
16	34.4	34.5	34.3	34.4	34.3	34.3	34.1	34.4	33.4	33.9
17	87.9	87.6	87.8	87.6	87.8	87.6	92.6	87.8	86.5	92.4
18	12.5	12.6	12.5	12.5	12.4	12.5	11.0	12.4	11.5	10.7
19	21.7	21.7	21.6	21.6	21.6	21.6	18.4	21.8	21.0	18.4
20	74.6	75.9	74.5	75.9	74.5	75.9	210.5	74.5	73.2	209.9
21	15.6	15.8	15.7	15.7	15.6	15.7	28.0	15.6	14.9	27.7
Cinnamoyl moiety										
1′	166.9	166.8	166.8	166.8	166.8	166.8	166.2	166.8	165.4	165.9
2′	120.5	120.1	120.5	120.1	120.5	120.1	119.5	120.5	119.1	119.2
3′	143.9	144.4	143.9	144.3	143.8	144.2	145.3	143.8	143.0	145.0
4′	135.1	134.9	135.1	134.8	135.2	134.8	135.1	135.1	134.1	135.1
5′, 9′	128.7	128.7	128.6	128.6	128.5	128.6	128.8	128.5	128.0	128.6
6′, 8′	129.4	129.5	129.5	129.4	129.2	129.3	129.6	129.2	128.8	129.3
7′	130.7	130.9	130.6	130.8	130.5	130.7	131.0	130.5	130.2	130.6
Nictinoyl or (*E*)-2-methyl-2-butenoyl moiety										
1″	166.9		166.8		166.8			166.8	165.7	
2″	129.5	151.1	129.6	151.4	129.5	151.4		129.5	128.3	
3″	138.0	127.1	137.8	127.0	137.7	126.9		137.7	137.4	
4″	14.3	137.7	14.2	137.6	14.1	137.5		14.1	14.0	
5″	12.4	123.6	12.3	123.6	12.2	123.6		12.2	11.7	
6″		154.0		153.8		153.9				
7″		164.8		164.8		164.8				

a) Measured in DMSO-d_6.

spectrum: δ 97.8 [C-1′ of the β-cymaropyranosyl] and δ 4.16 (1H, m) [H-3 of the aglycone], δ 101.9 [C-1″ of the β-oleandropyranosyl] and δ 3.43 (1H, dd, J=9.5, 2.5 Hz) [H-4′ of the β-cymaropyranosyl], and δ 104.2 [C-1‴ of the β-thevetopyranosyl] and δ 3.66 (1H, dd, J=9.5, 9.0 Hz) [H-4″ of the β-oleandropyranosyl]. Further, in a ROESY experiment, NOEs were observed between H-3 of the aglycone at δ 4.16 and the H-1′ signal of the β-cymaropyranosyl at δ 5.15, H-4′ of the β-cymaropyranosyl at δ 3.43 and the H-1″ signal of the β-oleandropyranosyl at δ 4.68, and H-4″ of the β-oleandropyranosyl at δ 3.66 and the H-1‴ signal of the β-thevetopyranosyl at δ 4.95. Based on the above evidence, the structure of **1** has been established as 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-thevetopyranosyl(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranoside.

Stephanoside M (**3**) provided the same molecular formula, $C_{56}H_{82}O_{18}$, as that of **1**, and afforded cymarose and thevetose as the sugar moieties on acid hydrolysis. The ^{1}H-NMR spectrum of **3** showed three anomeric proton signals at δ 5.16 and 5.09 (each 1H, dd, J=9.5, 1.5 Hz), and 4.96 (d, J=8.0 Hz). The coupling patterns indicated all glycosidic linkages to have β-configuration. The sugar linkage at C-3 was determined by the HMBC experiment. Cross peaks between H-3 of the aglycone at δ 4.16 and C-1′ of the inner cymarose δ 97.8, H-4′ of the inner cymarose at δ 3.43 and C-1″ of the outer cymarose at δ 100.4, and H-4″ of the outer cymarose at δ 3.57 and C-1‴ of the thevetose at δ 106.2 were observed. Consequently, the sugar sequence was established as thevetose (1→4) cymarose (1→4) cymarose. Based on the above information, the structure of **3** has been established as 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-thevetopyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranoside.

Stephanoside O (**5**) has the molecular formula $C_{63}H_{94}O_{21}$, $C_7H_{12}O_3$ more than that of **1** and afforded cymarose, oleandrose and thevetose as the sugar moieties on acid hydrolysis. The ^{1}H-NMR spectrum of **5** indicated four anomeric proton signals at δ 4.86, 5.09, and 5.16 (each 1H, dd, J=9.5, 1.5 Hz) and δ 4.96 (1H, d, J=8.0 Hz) and the coupling constants show all glycosidic linkages to have β-orientation. The sugar linkages at C-3 were determined by means of an HMBC experiment in the same way as

Table 2. ^{1}H-NMR Data for the Aglycone and Ester Parts of Stephanosides K (**1**), L (**2**), M (**3**), N (**4**), O (**5**), P (**6**), and Q (**7**), and for the Prosapogenin (**8**) (in Pyridine-d_5)

	1, 3, 5	**2, 4, 6**	**7**
Aglycone moiety			
3	4.16 (1H, m)	4.18 (1H, m)	3.86 (1H, m)
6	5.92 (1H, d, J=10.5 Hz)	5.93 (1H, d, J=10.5 Hz)	5.36 (1H, m)
7	6.22 (1H, d, J=10.5 Hz)	6.23 (1H, d, J=10.5 Hz)	
12	5.34 (1H, dd, J=10.5, 4.5 Hz)	5.36 (1H, dd, J=11.0, 4.5 Hz)	5.22 (1H, dd, J=11.0, 4.5 Hz)
18	2.13 (3H, s)	2.15 (3H, s)	2.06 (3H, s)
19	1.54 (3H, s)	1.52 (3H, s)	1.42 (3H, s)
20	5.10 (1H, q, J=6.0 Hz)	5.27 (1H, q, J=6.5 Hz)	—
21	1.46 (3H, d, J=6.0 Hz)	1.56 (3H, d, J=6.5 Hz)	2.52 (3H, s)
Cinnamoyl moiety			
2′	6.71 (1H, d, J=16.0 Hz)	6.53 (1H, d, J=16.0 Hz)	6.82 (1H, d, J=15.5 Hz)
3′	7.90 (1H, d, J=16.0 Hz)	7.78 (1H, d, J=16.0 Hz)	8.01 (1H, d, J=15.5 Hz)
5′, 9′	7.63 (2H, dd, J=7.0, 1.5 Hz)	7.40 (2H, dd, J=7.0, 1.5 Hz)	7.64 (2H, m)
6′, 8′	7.39 (2H, t, J=7.0 Hz)	7.35 (2H, t, J=7.0 Hz)	7.38 (2H, m)
7′	7.36 (1H, dt, J=7.0, 1.5 Hz)	7.34 (1H, dt, J=7.0, 1.5 Hz)	7.38 (1H, m)
(E)-2-Methyl-2-butenoyl moiety		Nicotinoyl moiety	
3″	6.97 (1H, q, J=8.0 Hz)	2″ 8.29 (1H, ddd, J=8.0, 2.0, 1.5 Hz)	
4″	1.49 (3H, d, J=8.0 Hz)	5″ 7.15 (1H, dd, J=8.0, 5.0 Hz)	
5″	1.77 (3H, s)	6″ 8.79 (1H, dd, J=5.0, 1.5 Hz)	

	8	**8**[a)]
Aglycone moiety		
3	4.30 (1H, m)	3.95 (1H, m)
6	5.97 (1H, d, J=10.5 Hz)	5.39 (1H, d, J=10.5 Hz)
7	6.23 (1H, d, J=10.5 Hz)	5.60 (1H, d, J=10.5 Hz)
12	5.38 (1H, dd, J=10.5, 3.5 Hz)	4.69 (1H, dd, J=10.5, 4.6 Hz)
18	2.15 (3H, s)	1.44 (3H, s)
19	1.60 (3H, s)	0.89 (3H, s)
20	5.14 (1H, q, J=6.0 Hz)	4.40 (1H, q, J=6.0 Hz)
21	1.50 (3H, d, J=6.0 Hz)	1.08 (3H, d, J=6.0 Hz)
Cinnamoyl moiety		
2′	6.73 (1H, d, J=16.0 Hz)	6.20 (1H, d, J=16.0 Hz)
3′	7.90 (1H, d, J=16.0 Hz)	7.40 (1H, d, J=16.0 Hz)
5′, 9′	7.63 (2H, dd, J=7.0, 1.5 Hz)	7.56 (2H, dd, J=7.5, 1.5 Hz)
6′, 8′	7.38 (2H, dd, J=7.0, 6.5 Hz)	7.41 (2H, m)
7′	7.36 (1H, ddd, J=7.0, 6.5, 1.5 Hz)	7.41 (1H, m)
(E)-2-Methyl-2-butenoyl moiety		
3″	6.97 (1H, q, J=6.5 Hz)	6.74 (1H, q, J=7.0 Hz)
4″	1.48 (3H, d, J=6.5 Hz)	1.62 (3H, d, J=7.0 Hz)
5″	1.78 (3H, s)	1.55 (3H, s)

a) Measured in DMSO-d_6. Five signals due to OH groups at δ 3.91 s (8-OH), 4.61 s (5-OH), 5.03 d (J=4.0 Hz, 3-OH), 5.06 s (14-OH), 5.08 s (17-OH).

in the case of **1**. The anomeric proton signals at δ 5.16 (inner cymarose), 5.09 (outer cymarose), 4.86 (oleandrose) and δ 4.96 (thevetose) showed long-range correlations with the ^{13}C signals at δ 75.0 (C-3), 83.3 (C-4′ of inner cymarose), 83.1 (C-4″ of outer cymarose), and 83.3 (C-4‴ of oleandrose), respectively in the HMBC spectrum. Consequently, the structure of **5** has been established as 12-O-cinnamoyl-20-O-(E)-2-methyl-2-butenoyl(20S)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-O-β-thevetopyranosyl(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranoside.

The IR spectra of **2**, **4**, and **6** showed carbonyl absorption bands at 1715 cm^{-1} and strong absorption bands at 3455 and 1165 cm^{-1} suggestive of oligoglycosidic structure. The ^{1}H- and ^{13}C-NMR spectra of **2**, **4**, and **6** exhibited signals due to the same aglycone as in the case of **1**, containing two or three 2-deoxyhexoses and one hexose, and one (E)-cinnamic acid and one nicotinic acid as acyl moieties (Tables 1—3). On alkaline hydrolysis, **2**, **4**, and **6** afforded (E)-cinnamic acid and nicotinic acid, which were identical with authentic samples. In the HMBC spectrum, the carbonyl carbon signal of the cinnamoyl group at δ 166.8 was correlated with the methine proton (H-12) at δ 5.36 on an oxygen-bearing carbon (C-12) at δ 75.8, and that of the nicotinoyl group at δ 164.8 was correlated with the methine proton (H-20) at δ 5.27 on an oxygen-bearing carbon (C-20) at δ 75.9, establishing that in **2**, **4**, and **6** the cinnamoyl group is located at O-12 and the nicotinoyl group at O-20.

Stephanoside L (**2**) showed a positive FAB ion at m/z 1120 $(M+K)^+$, indicating its molecular weight to be 1081, and gave cymarose, oleandrose and thevetose on acid hydrolysis. By comparison of the ^{1}H- and ^{13}C-NMR data for **2** with those for **1**, **2** was determined to have the same aglycone and the same sugar sequence in its oligosaccharide moiety as those of **1**, which carries its sugar moiety on the C-3 hydroxyl group of the aglycone. Thus, the structure of stephanoside L (**2**) has been established as 12-O-cinnamoyl-20-O-nicotinoyl(20S)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-O-β-thevetopyranosyl-(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranoside.

Stephanoside N (**4**) has the same molecular formula, $C_{57}H_{79}NO_{19}$, as that of **2**, and afforded cymarose and thevetose as the sugar moieties on acid hydrolysis. The proton and the carbon signals in the NMR spectrum of **4** were superimposable on those of **3** except for the acyl moieties, indicating that C-3 of **4** bears the same sugar sequence as that of **3**. Hence, the structure of **4** has been established as 12-O-cinnamoyl-20-O-nicotinoyl(20S)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-O-β-thevetopyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranoside.

Stephanoside P (**6**) has the molecular formula, $C_{64}H_{91}NO_{22}$, and afforded cymarose and oleandrose and thevetose as the sugar moieties on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **6** indicated that the oligosaccharide moiety of **6** is identical with that of **5** and is attached to the C-3 hydroxyl group of the aglycone. Hence, the structure of **6** has been established as 12-O-cinnamoyl-20-O-nicotinoyl(20S)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-O-β-thevetopyranosyl(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranoside.

Stephanoside Q (**7**) showed a positive FAB ion at m/z

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

No.	a		b		c	
Sugar-1	Cym		Cym		Cym	
1′	97.8	5.15 dd (9.5, 1.5 Hz)	97.8	5.16 dd (9.5, 1.5 Hz)	97.9	5.16 dd (9.5, 1.5 Hz)
2′	36.8	1.90 m, 2.31 m	36.8	1.89 m, 2.31 m	37.1	1.90 m, 2.31 m
3′	77.8	3.93 dd (2.5, 1.5 Hz)	77.9	4.00 dd (2.5, 1.5 Hz)	78.1	*ca.* 4.02 m
4′	83.2	3.43 dd (9.5, 2.5 Hz)	83.0	3.43 dd (9.5, 2.5 Hz)	83.3	3.42 dd (9.5, 2.5 Hz)
5′	69.1	4.15 dq (9.5, 6.0 Hz)	69.2	4.16 dq (9.5, 6.5 Hz)	69.3	4.14 dq (9.5, 6.5 Hz)
6′	18.6	1.38 d (6.0 Hz)	18.5	1.34 d (6.5 Hz)	18.6	1.38 d (6.0 Hz)
O-Me	58.9	3.56 s	58.8	3.55 s	59.0	3.56
Sugar-2	Ole		Cym		Ole	
1″	101.9	4.68 dd (9.5, 1.5 Hz)	100.4	5.09 dd (9.5, 1.5 Hz)	100.6	5.09 dd (9.5, 1.5 Hz)
2″	37.6	1.81 m, 2.48 m	36.9	1.81 m, 2.30 m	36.9	1.82 m, 2.31 m
3″	79.2	*ca.* 3.59 m	78.2	4.04 dd (3.0, 1.5 Hz)	77.7	*ca.* 4.02 m
4″	83.1	3.66 dd (9.5, 9.0 Hz)	83.1	3.57 dd (9.5, 3.0 Hz)	83.1	3.45 dd (9.5, 2.5 Hz)
5″	72.1	*ca.* 3.57 m	69.4	4.20 dq (9.5, 6.0 Hz)	69.1	4.16 dq (9.5, 6.0 Hz)
6″	19.0	1.69 d (6.0 Hz)	18.6	1.58 d (6.0 Hz)	18.7	1.37 d (6.0 Hz)
O-Me	57.3	3.52 s	58.8	3.55 s	59.1	3.56 s
Sugar-3	The		The		Ole	
1‴	104.2	4.95 d (8.0 Hz)	106.2	4.96 d (8.0 Hz)	102.1	4.86 dd (9.5, 1.5 Hz)
2‴	75.3	3.92 t (8.0 Hz)	75.1	3.92 t (8.0 Hz)	37.7	1.80 m, 2.48 m
3‴	88.2	3.62 t (8.0 Hz)	87.8	*ca.* 3.61 m	79.4	3.59 dd (9.5, 3.0 Hz)
4‴	76.0	3.63 dd (8.5, 8.0 Hz)	75.9	*ca.* 3.61 m	83.3	3.67 dd (9.5, 9.0 Hz)
5‴	72.9	3.73 dq (8.5, 6.0 Hz)	72.8	3.73 dq (8.5, 6.0 Hz)	72.2	*ca.* 3.58 m
6‴	18.6	1.59 d (6.0 Hz)	18.6	1.58 d (6.0 Hz)	19.1	1.71 d (6.0 Hz)
O-Me	61.0	3.90 s	61.1	3.90 s	57.5	3.53 s
Sugar-4					The	
1⁗					104.3	4.96 d (8.1 Hz)
2⁗					75.4	3.93 t (8.0 Hz)
3⁗					88.2	3.61 t (8.0 Hz)
4⁗					76.2	3.63 dd (8.5, 8.0 Hz)
5⁗					73.0	3.74 dq (8.5, 6.0 Hz)
6⁗					18.7	1.60 d (6.0 Hz)
O-Me					61.2	3.90 s

Cym = β-cymaropyranosyl. Ole = β-oleadropyranosyl. The = β-thevetopyranosyl.

1141 for $[M+K]^+$ suggesting the molecular formula $C_{58}H_{86}O_{20}$. The ^{1}H- and ^{13}C-NMR spectra of **7** exhibited signals characteristic of a polyoxypregnane-oligoglycoside containing three 2-deoxyhexoses and one hexose, and one (*E*)-cinnamic acid. On mild acid hydrolysis, **7** afforded kidjoranin (**9**),[5] and cymarose, oleandrose and thevetose and also provided (*E*)-cinnamic acid on alkaline hydrolysis. The carbon signals due to the sugar moieties at C-3 are superimposable on those of **5**, indicating that **7** has the same sugar moiety at C-3. Hence, the structure of **7** has been established as 12-*O*-cinnamoyl pregn-5-en-20-one-3β,8β,12β,14β,17β-pentaol (kidjoranin) 3-*O*-β-thevetopyranosyl(1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranoside.

As far as we know, this is the first time that oxypregnane derivatives having a 5-hydroxy-6-ene structure have been obtained from a natural source. We attempted to isolate the aglycone of stephanosides K—P but without success, because the prosapogenin (**8**) gave several dehydro products on neutralization using Amberlyst **15** after alkaline hydrolysis.

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 Varian UNITY 200 and 600 spectrometers in C_5D_5N solution using trimethylsilyl (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, 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 The 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 mm × 20 mm, CH_3CN : H_2O = 45 : 55—40 : 60) to afford stephanosides K (**1**, 30 mg), L (**2**, 80 mg), M (**3**, 200 mg), N (**4**, 130 mg), O (**5**, 580 mg), P (**6**, 320 mg), and Q (**7**, 120 mg).

Stephanoside K (**1**): mp 150—152 °C, $[\alpha]_D^{20}$ +88.2° (c = 1.4, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1170. UV (EtOH) nm log ε: 206 (4.23), 216 (4.26), 280 (4.15). Positive FAB-MS *m/z*: 1081 $[M+K]^+$. *Anal.* Calcd for $C_{56}H_{82}O_{18} \cdot 2H_2O$: C, 62.30; H, 8.04. Found: C, 62.34; H, 8.02. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside L (**2**): mp 142—144 °C, $[\alpha]_D^{20}$ +80.2° (c = 3.8, $CHCl_3$). IR (film) cm^{-1}: 3460, 1715, 1640, 1165. UV (EtOH) nm log ε: 206 (4.29), 217.5 (4.34), 262.5 (4.20), 280 (4.20). Positive FAB-MS *m/z*: 1120 $[M+K]^+$. *Anal.* Calcd for $C_{57}H_{79}NO_{19} \cdot H_2O$: C, 62.23; H, 7.42; N, 1.27. Found: C, 62.22; H, 7.34; N, 1.30. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside M (**3**): mp 172—174 °C, $[\alpha]_D^{20}$ +83.7° (c = 1.7, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1170. UV (EtOH) nm log ε: 206 (4.24), 216.5 (4.27), 280 (4.16). Positive FAB-MS *m/z*: 1081 $[M+K]^+$. *Anal.* Calcd for $C_{56}H_{82}O_{18} \cdot 5/2H_2O$: C, 61.80; H, 8.06. Found: C, 61.85; H, 7.76. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside N (**4**): mp 148—150 °C, $[\alpha]_D^{20}$ +64.2° (c = 0.4, $CHCl_3$).

IR (film) cm^{-1}: 3455, 1715, 1640, 1165. UV (EtOH) nm logε: 206.5 (4.21), 217.5 (4.24), 280 (4.13). Positive FAB-MS *m/z*: 1120 $[M+K]^+$. *Anal.* Calcd for $C_{57}H_{79}NO_{19} \cdot 3H_2O$: C, 60.25; H, 7.54; N, 1.23. Found: C, 60.49; H, 7.63; N, 1.02. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside O (**5**): mp 147—149 °C, $[\alpha]_D^{20}$ +72.5° (c=3.8, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1170. UV (EtOH) nm logε: 205.5 (4.27), 216.5 (4.28), 280 (4.17). Positive FAB-MS *m/z*: 1125 $[M+K]^+$. *Anal.* Calcd for $C_{63}H_{94}O_{21} \cdot H_2O$: C, 62.77; H, 8.03. Found: C, 62.53; H, 7.90. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside P (**6**): mp 152—154 °C, $[\alpha]_D^{20}$ +96.0° (c=2.3, $CHCl_3$). IR (film) cm^{-1}: 3460, 1715, 1640, 1165. UV (EtOH) nm logε: 206 (4.34), 217.5 (4.37), 281 (4.26). Positive FAB-MS *m/z*: 1264 $[M+K]^+$. *Anal.* Calcd for $C_{64}H_{91}NO_{22} \cdot 1/2\,H_2O$: C, 62.23; H, 7.51; N, 1.13. Found: C, 62.26; H, 7.59; N, 1.33. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Stephanoside Q (**7**): mp 191—193 °C, $[\alpha]_D^{20}$ +88.2° (c=1.4, $CHCl_3$). IR (film) cm^{-1}: 3480, 1715, 1640, 1170. UV (EtOH) nm logε: 206 (4.23), 217 (4.20), 281 (4.30). Positive FAB-MS *m/z*: 1141 $[M+K]^+$. *Anal.* Calcd for $C_{58}H_{86}O_{20} \cdot 2H_2O$: C, 61.14; H, 7.96. Found: C, 61.25; H, 7.78. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Mild Acid Hydrolysis of Stephanoside O (5) A solution of **5** (95 mg) in 3 ml of MeOH was treated 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 (20 mg). The CH_2Cl_2 extract (20 mg) was separated by HPLC (YMC-ODS, 250 mm × 20 mm, $CH_3CN:H_2O=45:55$—$40:60$) to afford the prosapogenin (**8**, 10 mg): A white amorphous powder $[\alpha]_D^{20}$ +134° (c=1.0, MeOH). IR (film) cm^{-1}: 3480, 1710, 1640, 1170. UV (EtOH) nm logε: 206 (4.24), 216.5 (4.27), 280 (4.16). Positive FAB-MS *m/z*: 649 $[M+K]^+$. High-resolution CI-MS *m/z*: 611.3235 (Calcd for $C_{35}H_{46}O_9+H^+$ 611.3220). ^{1}H-NMR and ^{13}C-NMR: Tables 1—3. The H_2O phase was neutralized with Amberlite IR-45 and evaporated under reduced pressure to give the sugar portion. Cymarose, oleandrose and thevetose 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 K (1) and M (3) Each compound (20 mg) was hydrolyzed in the same way as **5**. The prosapogenin (**8**, *ca.* 2 mg) was obtained from each CH_2Cl_2 phase. Each of the H_2O phases was analyzed in the same way as described for **5**. Cymarose, oleandrose and thevetose were detected from **1**, and cymarose and thevetose from **3**.

Alkaline Hydrolysis of Prosapogenin (8) The prosapogenin (**8**, 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 Amberlyst **15**. The methanol eluate gave (E)-cinnamic acid and (E)-2-methyl-2-butenoic acid, which were confirmed by comparison of ^{1}H-NMR data and by co-HPLC with authentic samples.

Alkaline Hydrolysis of Stephanosides L (2), N (4), and O (6) for Analysis of the Acyl Moiety Each compound (8—10 mg) was hydrolyzed in the same way as **8**. The methanol eluate gave (E)-cinnamic acid and nicotinic acid, which were confirmed by comparison of ^{1}H-NMR data and by co-HPLC with authentic samples.

Mild Acidic Hydrolysis of Stephanosides L (2), N (4), and O (6) Each compound (2 mg) was treated in the same way as **5**. The H_2O phases gave the following sugars. Cymarose, oleandrose and thevetose were detected from **2** and **6**, and cymarose and thevetose from **4**.

Mild Acidic Hydrolysis of Stephanoside Q (7) Compound **7** (40 mg) was treated in the same way as **1**. The CH_2Cl_2 phase gave the prosapogenin (**9**, *ca.* 10 mg). Compound **9**, mp 145—147 °C, $[\alpha]_D^{20}$ +60.2° (c=2.2, $CHCl_3$) was identified as kidjoranin (lit.[5] mp 147—149 °C, $[\alpha]_D^{20}$ +63.2° ($CHCl_3$)). CI-MS *m/z*: 511 $[M+H]^+$. High-resolution CI-MS *m/z*: 511.2610 (Calcd for $C_{35}H_{46}O_9+H^+$ 511.2615). ^{1}H-NMR (C_5D_5N) δ: 1.42 (3H, s, H-19), 2.06 (3H, s, H-18), 2.52 (3H, s, H-21), 3.86 (1H, m, H-3), 5.22 (1H, dd, J=10.5, 3.5 Hz, H-12), 5.36 (1H, m, H-6), 6.82 (1H, d, J=15.5 Hz, H-2″ of cin), 7.38 (3H, m, H-6′, 7′, 8′ of cin), 7.64 (2H, m, H-5′, 9′ of cin). The H_2O phase gave cymarose, oleandrose, and thevetose.

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