

HETEROCYCLES, Vol. 81, No. 12, 2010, pp. 2793 - 2806. © The Japan Institute of Heterocyclic Chemistry
Received, 1st September, 2010, Accepted, 21st October, 2010, Published online, 29th October, 2010
DOI: 10.3987/COM-10-12058

EIGHT NEW OLEANANE-TYPE TRITERPENOID SAPONINS FROM PLATYCODON ROOT¹

Motonori Fukumura,^a Daigo Iwasaki,^a Yasuaki Hirai,^a Yumiko Hori,^a Kazuo
Toriizuka,^a Peter T. M. Kenny,^b Yoshiyuki Kuchino,^{a,c} and Yoshiteru Ida^{a,*}

^a School of Pharmacy, Showa University, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo
142-8555, Japan; ^b School of Chemical Sciences, Dublin City University, Dublin 9,
Ireland; ^c Yokohama College of Pharmacy, 601, Matano-cho, Totsuka-ku,
Yokohama 245-0066, Japan; E-mail: y.ida@hamayaku.ac.jp

Abstract – Eight new triterpenoid saponins, platyconic acids B (**1**), C (**2**), D (**3**),
E (**4**), platycodins J (**5**), K (**6**), L (**7**) and platycosaponin A (**8**), were isolated from
Platycodon Root, together with twelve known compounds, and they were
characterized on the basis of their spectroscopic and chemical data.

INTRODUCTION

The Platycodon Root, the dried root of *Platycodon grandiflorum* A. DC., is one of the most important crude drugs in kampo medicine, and has been used as an antiphlogistic, antitussive and expectorant agent. As for the constituents of Platycodon Root (dried *Platycodon grandiflorum* roots), platycodins D (**9**), A (**10**), and C (**11**) were the first triterpenoidal saponins isolated from the Platycodon Root in our laboratory 35 years ago.^{2,3} Thereafter, a number of oleanane-type triterpenoidal saponins were further isolated namely: deapioplatycodin D (**12**), platycodin D₂ (**13**), 2''-O-acetylplatycodin D₂ (**14**), 3''-O-acetylplatycodin D₂, platycodin D₃ (**15**), deapioplatycodin D₃, polygalacin D, 2''-O-acetylpolygalacin D (**17**), 3''-O-acetylpolygalacin D, polygalacin D₂, 2''-O-acetylpolygalacin D₂, 3''-O-acetylpolygalacin D₂, methyl platyconate A (= platyconic acid A methyl ester), methyl 2-O-methylplatyconate A, platyconic acid A lactone, platycosides A, B, C, D, E (**16**) and F, platycosides G1, G2 and G3 (**18**), deapioplatycoside E, platycosides H, K and L, M1, M2 and M3, 16-oxo-platycodin D and platyconic acid A (**21**).⁴⁻¹³

It has recently been shown that platycodins D (**9**), A (**10**) and C (**11**) exhibited cytotoxicity against human cancer cells.¹⁴ In order to continue the studies for the estimation of their cytotoxicity against human

cancer cells, we commenced a phytochemical survey again on the plant to furnish the desired saponins, and isolated eight new saponins as well as twelve known compounds. This paper describes the isolation and characterization of eight new triterpenoid saponins.

RESULTS AND DISCUSSION

Commercial Platycodon Root (1.5 kg) was extracted with 70% aqueous methanol, and the extract, after concentration, was subjected to column chromatography over silica gel and octadecylsilanized silica gel (ODS) repeatedly, to give twenty compounds (see experimental).

Twelve of the compounds were identified by comparison of their physical and spectral data with those already reported: platycodins D (**9**, 101 mg),^{2,4} A (**10**, 426 mg),^{3,4} C (**11**, 314 mg),^{3,4} deapioplatycodin D (**12**, 18 mg),⁴ platycodin D₂ (**13**, 59 mg),⁴ 2''-O-acetylplatycodin D₂ (**14**, 39 mg),⁴ platycodin D₃ (**15**, 1.5 g),⁴ platycoside E (**16**, 3.5 g),⁶ 2''-O-acetylpolygalacin D (**17**, 94 mg),⁴ platycoside G3 (**18**, 52 mg),⁸ lobetyolin (**19**, 213 mg)¹⁵ and hexyl 6-O- α -L-arabinofuranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (**20**, 26 mg).¹⁶ The remaining eight compounds were found to be new triterpenoid saponins and they were named as follows after similarities of their structural features to the known compounds: platyconic acids B (**1**, 501 mg), C (**2**, 28 mg), D (**3**, 16 mg), E (**4**, 31 mg), platycodins J (**5**, 75 mg), K (**6**, 104 mg), L (**7**, 300 mg) and platycosaponin A (**8**, 46 mg), respectively (Figure 1).

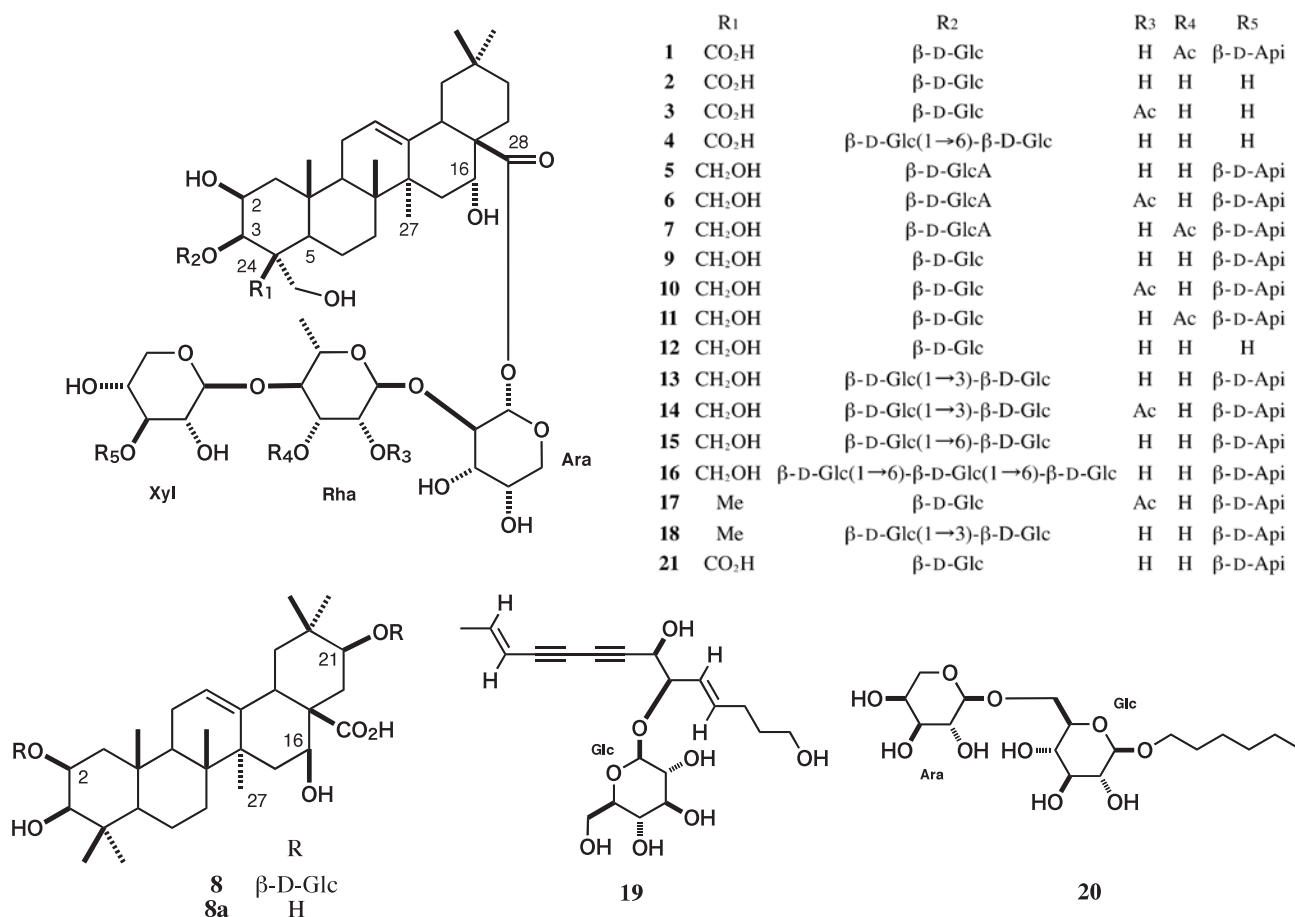


Figure 1. Structures of **1-21**

Table 1. ^{13}C NMR spectral data for **1-8** (125 MHz in $\text{C}_5\text{D}_5\text{N}$)

Pos.	1	2	3	4	5	6	7	8	Pos.	1	2	3	4	5	6	7	8
1	46.1	46.0	46.0	45.9	45.7	45.7	45.6	45.3	3-sugar (inner)								2-Glc
2	70.4	70.4	70.4	70.2	70.4	70.4	70.4	83.8	1	107.2	107.2	107.2	107.3	106.7	106.7	106.7	106.9
3	81.9	81.8	81.8	82.6	86.4	86.4	86.3	79.2	2	75.8	75.8	75.8	75.7	75.5	75.5	75.5	76.3
4	57.2	57.2	57.2	57.1	48.7	48.7	48.7	39.4	3	79.1	79.1	79.1	78.9	78.5	78.6	78.5	79.4
5	49.7	49.6	49.6	49.6	47.4	47.3	47.3	56.1	4	72.0	72.0	72.0	72.1	73.8	73.8	73.8	71.9
6	20.5	20.6	20.5	20.5	19.7	19.7	19.6	19.0	5	79.0	79.1	79.0	77.5	77.8	77.8	77.9	79.3
7	33.8	33.8	33.8	33.8	34.1	34.1	34.1	33.6	6	63.1	63.1	63.2	71.1	173.4	173.4	173.2	63.3
8	40.6	40.5	40.5	40.5	40.8	40.9	40.8	40.3	3-sugar (terminal)								21-Glc
9	48.0	48.0	48.0	47.9	48.2	48.2	48.2	48.0	1	-	-	-	106.0	-	-	-	108.6
10	37.5	37.5	37.5	37.5	37.8	37.8	37.8	37.5	2	-	-	-	75.6	-	-	-	76.5
11	24.8	24.8	24.8	24.8	24.6	24.7	24.6	24.5	3	-	-	-	79.1	-	-	-	79.4
12	123.4	123.3	123.3	123.3	123.6	123.6	123.6	124.2	4	-	-	-	72.2	-	-	-	73.0
13	145.0	145.0	145.0	144.9	144.8	144.8	144.7	143.1	5	-	-	-	78.9	-	-	-	79.3
14	42.7	42.7	42.7	42.7	42.8	42.8	42.7	45.1	6	-	-	-	63.1	-	-	-	64.4
15	36.5	36.5	36.5	36.5	36.5	36.6	36.6	38.3	28-Ara								
16	74.5	74.5	74.5	74.5	74.4	74.4	74.4	66.6	1	93.7	94.1	94.0	94.1	94.1	94.0	93.8	-
17	50.2	50.0	50.0	50.0	50.1	50.2	50.1	53.1	2	76.8	75.8	76.1	75.8	75.9	76.5	76.9	-
18	41.7	41.7	41.8	41.7	41.9	41.9	41.8	43.9	3	69.7	70.7	71.0	70.6	70.9	71.2	70.4	-
19	47.5	47.6	47.6	47.6	47.5	47.5	47.5	47.6	4	65.9	66.7	66.8	66.6	66.9	67.0	66.4	-
20	31.5	31.4	31.4	31.4	31.4	31.4	31.4	37.1	5	62.7	63.7	64.0	63.6	64.0	64.1	63.4	-
21	36.5	36.5	36.5	36.5	36.6	36.5	36.5	81.9	Rha								
22	32.6	32.6	32.6	32.6	32.7	32.6	32.6	35.1	1	101.9	101.7	98.6	101.7	101.8	98.7	102.0	-
23	63.1	63.1	63.2	63.4	64.1	64.2	64.2	18.6	2	70.4	72.5	73.9	72.5	72.4	73.9	70.4	-
24	176.9	176.9	176.8	176.6	65.9	65.9	65.9	30.5	3	75.9	73.2	70.7	73.2	73.2	70.8	75.9	-
25	16.2	16.2	16.2	16.2	18.5	18.6	18.5	16.7	4	77.8	83.9	83.2	83.9	84.3	83.5	78.0	-
26	18.0	18.0	18.0	18.0	18.1	18.1	18.1	17.8	5	69.3	69.1	68.9	69.1	69.0	68.9	69.3	-
27	27.7	27.6	27.6	27.6	27.6	27.6	27.6	27.4	6	19.0	18.9	18.7	18.9	18.9	18.7	19.1	-
28	176.5	176.4	176.4	176.4	176.5	176.5	176.5	180.6	$\text{CH}_3\text{CO-}$								
29	33.8	33.8	33.7	33.8	33.7	33.7	33.7	29.3	$\text{CH}_3\text{CO-}$								
30	25.3	25.2	25.2	25.3	25.2	25.2	25.2	18.9	Xyl								
									1	106.3	107.2	107.0	107.3	107.2	106.9	106.4	-
									2	74.8	76.5	76.5	76.5	75.7	75.6	74.8	-
									3	84.8	79.0	79.0	79.0	85.3	85.3	84.9	-
									4	70.0	71.5	71.5	71.5	69.9	69.9	70.0	-
									5	67.4	67.9	67.9	67.9	67.4	67.5	67.3	-
									Api								
									1	111.8	-	-	-	111.7	111.7	111.7	-
									2	78.2	-	-	-	78.3	78.3	78.2	-
									3	81.0	-	-	-	81.0	81.0	81.0	-
									4	75.6	-	-	-	75.7	75.7	75.6	-
									5	66.0	-	-	-	65.9	65.9	65.9	-

Platyconic acid B (**1**) was isolated as a white amorphous powder, and exhibited a pseudomolecular ion $[\text{M}+\text{H}]^+$ at m/z 1281.5729 in the HR-FAB MS corresponding to the molecular formula of $\text{C}_{59}\text{H}_{92}\text{O}_{30}$, and characteristic absorption bands at 3434 and 1736 cm^{-1} due to hydroxyl and ester carbonyl groups were present in the IR spectrum. The ^{13}C NMR spectrum of **1** showed 59 signals, five of which were assignable to anomeric carbons suggesting that **1** has five sugar units (Table 1). The ^1H NMR spectrum of **1** indicated a signal due to an acetyl group at δ 2.09 (Table 3). Acid hydrolysis of **1** with 1 M hydrochloric acid, afforded D-glucose (D-Glc), L-arabinose (L-Ara), L-rhamnose (L-Rha), D-xylose

Table 2. ¹H NMR spectral data for aglycone of **1-8** (500 MHz in C₇D₈N)

Pos.	1	2	3	4	5	6	7	8
1	1.37* 2.32*	1.35* 2.31*	1.32* 2.28*	1.54 (brd, 11.5) 2.43*	1.57* 2.10*	1.55* 2.09*	1.57* 2.12*	1.27* 2.44 (brd, 12.6)
2	5.09 (brd, 2.3)	5.09 (brs)	5.01 (brs)	5.29 (brd, 2.9)	4.81 (brs)	4.80 (brs)	4.83 (brs)	4.35 (brd, 5.2)
3	4.69 (brs)	4.68 (d, 4.0)	4.60 (brs)	4.63*	4.62 (brs)	4.60 (brs)	4.64 (brs)	4.43 (brd, 3.5)
4	-	-	-	-	-	-	-	-
5	2.05*	2.04*	1.93 (brd, 10.9)	1.97*	1.94*	1.92*	1.95*	0.94 (brd, 11.5)
6	2.05* 2.32*	2.04* 2.31*	2.28* 2.00*	1.97* 2.28*	1.51* 1.93*	1.59* 1.92*	1.63* 1.95*	1.46* 1.57*
7	1.43 (d, 12.0) 1.70*	1.49 (d, 12.0) 1.76*	1.50 (brd, 12.0) 1.68*	1.47 (brd, 12.6) 1.69*	1.51* 1.73*	1.50* 1.72*	1.48* 1.73*	1.33* 1.52*
8	-	-	-	-	-	-	-	-
9	1.85 (dd, 6.9, 10.3)	1.83 (dd, 6.3, 10.9)	1.78*	1.82 (dd, 6.9, 10.9)	1.94*	1.92*	1.95*	1.57*
10	-	-	-	-	-	-	-	-
11	2.07* 2.07*	2.06* 2.06*	2.02* 2.11*	2.08* 2.08*	2.03* 2.09*	2.02* 2.11*	2.03* 2.11*	1.79* 1.91*
12	5.63 (dd, 3.5, 3.5)	5.63 (brs)	5.61 (brs)	5.58 (brs)	5.62 (dd, 2.9, 2.9)	5.61 (dd, 2.9, 2.9)	5.61 (dd, 2.9, 2.9)	5.51 (dd, 2.9, 2.9)
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	1.70* 2.32*	1.76* 2.31*	1.78* 2.27*	1.78* 2.30*	1.80 (brd, 13.8) 2.30*	1.81* 2.26*	1.74* 2.31*	1.70 (dd, 2.3, 12.0) 2.29 (dd, 12.0, 12.0)
16	5.23 (brs)	5.25 (brs)	5.23 (brs)	5.26*	5.25*	5.22*	5.25 (brs)	4.61*
17	-	-	-	-	-	-	-	-
18	3.59 (dd, 4.0, 13.7)	3.56 (dd, 4.0, 13.7)	3.54*	3.57 (dd, 4.0, 13.8)	3.56 (dd, 3.5, 13.8)	3.53 (dd, 4.0, 13.8)	3.56 (dd, 4.6, 13.8)	3.58 (dd, 4.0, 13.8)
19	1.37* 2.76 (dd, 13.2, 13.2)	1.35* 2.76 (dd, 13.7, 13.7)	1.33* 2.74 (dd, 13.2, 13.7)	1.36* 2.76 (dd, 13.8, 13.8)	1.34 (dd, 3.5, 13.8) 2.75 (dd, 13.8, 13.8)	1.33 (dd, 4.0, 13.8) 2.73 (dd, 13.8, 13.8)	1.34 (dd, 4.6, 13.8) 2.75 (dd, 13.8, 13.8)	1.46* 2.01 (dd, 13.8, 13.8)
20	-	-	-	-	-	-	-	-
21	1.29* 2.40 (ddd, 4.6, 12.6, 12.6)	1.27* 2.41 (ddd, 4.6, 12.6, 12.6)	1.27* 2.40 (ddd, 4.0, 12.6, 12.6)	1.30* 2.43*	1.27* 2.40 (ddd, 4.6, 12.6, 12.6)	1.26* 2.39 (ddd, 4.0, 12.6, 12.6)	1.27* 2.40 (ddd, 4.6, 12.6, 12.6)	4.16* -
22	2.20 (ddd, 4.6, 12.6, 12.6) 2.32* 4.68*	2.18 (ddd, 4.6, 12.6, 12.6) 2.31* 4.67*	2.16* 2.34 (ddd, 4.0, 12.6, 12.6) 4.61 (d, 10.9)	2.19 (ddd, 4.6, 12.6, 12.6) 2.30* 4.63*	2.17 (ddd, 4.6, 12.6, 12.6) 2.33* 4.06*	2.16 (ddd, 4.0, 12.6, 12.6) 2.32* 4.05*	2.18 (ddd, 4.6, 12.6, 12.6) 2.31* 4.09*	2.15 (dd, 12.6, 12.6) 3.49 (dd, 2.9, 12.6) 1.22 (3H, s)
23	4.97*	4.96 (d, 10.9)	4.88 (d, 10.9)	4.96 (d, 10.9)	5.03 (d, 10.3)	5.02 (d, 9.8)	5.08 (d, 10.9)	-
24	-	-	-	-	4.19* 4.19*	4.23* 4.23*	4.21* 4.21*	1.35 (3H, s) -
25	1.63 (3H, s)	1.61 (3H, s)	1.58 (3H, s)	1.62 (3H, s)	1.49 (3H, s)	1.48 (3H, s)	1.50 (3H, s)	1.37 (3H, s)
26	1.14 (3H, s)	1.14 (3H, s)	1.12 (3H, s)	1.13 (3H, s)	1.11 (3H, s)	1.16 (3H, s)	1.12 (3H, s)	1.02 (3H, s)
27	1.77 (3H, s)	1.77 (3H, s)	1.75 (3H, s)	1.71 (3H, s)	1.76 (3H, s)	1.75 (3H, s)	1.76 (3H, s)	1.26 (3H, s)
28	-	-	-	-	-	-	-	-
29	1.00 (3H, s)	0.99 (3H, s)	0.98 (3H, s)	1.03 (3H, s)	0.98 (3H, s)	0.97 (3H, s)	0.99 (3H, s)	1.18 (3H, s)
30	1.16 (3H, s)	1.12 (3H, s)	1.11 (3H, s)	1.16 (3H, s)	1.13 (3H, s)	1.11 (3H, s)	1.13 (3H, s)	1.31 (3H, s)

*:Overlapped signal

(D-Xyl), D-apiose (D-Api) and an aglycone (**1a**). The molecular formula of **1a** was determined to be C₃₀H₄₄O₇ by the HR-FAB MS showing an [M+H]⁺ peak at *m/z* 517.3135. The IR spectrum of **1a** exhibited absorption bands at 3447, 1772 and 1750 cm⁻¹ due to hydroxyl, lactone and ester carbonyl groups respectively. The ¹³C NMR spectrum of **1a** showed thirty signals similar to those of platycodigenin (**9a**),⁵ the aglycone of platycodin D (**9**), except for a lactone carbonyl carbon signal (δ 179.4) present in **1a** instead of the C-24 carbonyl carbon signal (δ 65.1) in **9a** (Table 1). The carbon signal at δ 179.4 showed cross peaks with the H-2 and H-5 signals in the HMBC spectrum. Therefore, **1a** was characterized as platycogenic acid A lactone, which was thought to be formed from platycogenic acid A (2β,3β,16α,23-tetrahydroxyolean-12-ene-24,28-dioic acid) (**1b**)¹⁸ under acidic conditions. The genuine aglycone of **1** must be platycogenic acid A (**1b**), since **1** showed no signal due to the lactone carbonyl group both in the IR and ¹³C NMR spectra.

As for the sugar moieties of **1**, their carbon signals were similar to those in platycodin C (**11**), indicating that the sugar moieties of **1** are the same as those of platycodin C (**11**). This was confirmed by HMBC experiments on **1**, in which cross peaks were observed between Glc H-1/aglycone C-3, Ara H-1/aglycone C-28, Rha H-1/Ara C-2, Xyl H-1/Rha C-4, Api H-1/Xyl C-3 and Rha H-3/acetyl carbonyl carbon (Table

Table 3. ¹H NMR spectral data for sugar moiety of **1-8** (500 MHz in C₅D₅N)

Pos.	1	2	3	4	5	6	7	8
3-sugar (inner)								2-Glc
1	5.36 (d, 7.4)	5.36 (d, 8.0)	5.29*	5.26*	5.25*	5.24 (d, 7.4)	5.29 (d, 7.4)	5.08 (d, 7.1)
2	3.93*	3.99*	4.05*	3.97*	4.06*	4.05*	4.09*	4.01*
3	4.18*	4.16*	4.21*	4.16*	4.22*	4.23*	4.21*	4.20 (dd, 9.2, 9.2)
4	4.18*	4.16*	4.19*	4.12*	4.50*	4.51*	4.53*	4.14*
5	4.08*	3.99*	3.95*	3.97*	4.56*	4.55 (d, 9.8)	4.59 (d, 9.2)	3.90*
6	4.33*	4.33 (dd, 5.7, 12.6)	4.37*	4.12*	-	-	-	4.34 (dd, 5.7, 12.0)
	4.51*	4.52*	4.49*	4.85 (dd, 3.4, 9.2)	-	-	-	4.51 (brd, 9.8)
3-sugar (terminal)								21-Glc
1	-	-	-	5.03 (d, 8.0)	-	-	-	5.04 (d, 7.5)
2	-	-	-	4.06*	-	-	-	4.09*
3	-	-	-	4.26*	-	-	-	4.27 (dd, 9.2, 9.2)
4	-	-	-	4.26*	-	-	-	4.01*
5	-	-	-	3.91*	-	-	-	4.11*
6	-	-	-	4.37 (dd, 5.2, 11.5)	-	-	-	4.14*
			-	4.51*				4.61 (brd, 10.4)
28-Ara								
1	6.55 (brs)	6.47 (d, 2.9)	6.36 (d, 2.9)	6.48 (brs)	6.44 (d, 3.5)	6.37 (d, 3.5)	6.49 (d, 1.7)	-
2	4.49*	4.57*	4.54*	4.55*	4.56*	4.51*	4.52*	-
3	4.59*	4.52*	4.49*	4.55*	4.50*	4.47*	4.52*	-
4	4.58*	4.41*	4.37*	4.43*	4.39*	4.37*	4.42*	-
5	3.98*	3.95 (dd, 4.0, 10.9)	3.93 (dd, 3.5, 10.9)	3.97*	3.94 (dd, 3.5, 10.9)	3.91 (dd, 3.5, 10.9)	3.94*	-
	4.59*	4.52*	4.49*	4.51*	4.50*	4.47*	4.52*	-
Rha								
1	5.60 (brs)	5.79 (brs)	5.71 (brs)	5.78 (brs)	5.80 (brs)	5.68 (brs)	5.69 (brs)	-
2	4.69*	4.57*	5.79 (dd, 1.2, 3.5)	4.58*	4.59*	5.77 (dd, 1.2, 3.4)	4.77*	-
3	5.76 (dd, 2.9, 9.2)	4.61 (dd, 2.9, 8.6)	4.71 (dd, 3.5, 9.2)	4.63*	4.50*	4.66 (dd, 3.4, 9.8)	5.79 (dd, 2.9, 9.8)	-
4	4.51*	4.41*	4.28 (dd, 9.2, 9.2)	4.41*	4.39*	4.23 (dd, 9.8, 9.8)	4.53*	-
5	4.38*	4.41*	4.39*	4.43*	4.39*	4.37 (dd, 6.3, 9.8)	4.42*	-
6	1.68 (3H, d, 5.8)	1.72 (3H, d, 5.8)	1.71 (d, 6.3)	1.74 (3H, d, 5.8)	1.71 (3H, d, 5.7)	1.70 (3H, d, 6.3)	1.71 (3H, d, 6.3)	-
CH ₃ CO-	2.09 (3H, s)	-	1.99 (3H, s)	-	-	2.01 (3H, s)	2.09 (3H, s)	-
Xyl								
1	4.96 (d, 8.0)	5.20 (d, 7.5)	5.29*	5.21 (d, 7.4)	5.08 (d, 6.9)	5.18 (d, 6.9)	4.97 (d, 8.0)	-
2	3.90 (dd, 8.0, 8.0)	4.07*	4.05*	4.06*	4.06*	4.05*	3.94 (dd, 8.0, 8.0)	-
3	4.08*	4.07*	4.05*	4.06*	4.06*	4.05*	4.09*	-
4	4.08*	4.18*	4.25*	4.26*	4.06*	4.05*	4.09*	-
5	3.47 (dd, 10.3, 10.3Hz)	3.48 (dd, 10.9, 10.9)	3.54*	3.50 (dd, 10.3, 10.3)	3.38 (dd, 9.2, 11.5)	3.44 (dd, 9.2, 11.5)	3.47 (dd, 9.2, 11.5)	-
	4.18*	4.22 (dd, 5.2, 10.9)	4.27*	4.25*	4.19*	4.23*	4.20*	-
Api								
1	6.29 (d, 1.7)	-	-	-	6.24 (d, 2.3)	6.19 (d, 2.3)	6.31 (d, 2.3)	-
2	4.82 (d, 2.3)	-	-	-	4.85 (d, 2.3)	4.83 (d, 2.3)	4.86 (d, 2.3)	-
3	-	-	-	-	-	-	-	-
4	4.34 (d, 9.8)	-	-	-	4.34 (d, 9.2)	4.32 (d, 9.2)	4.34 (d, 9.7)	-
	4.76 (d, 9.6)	-	-	-	4.77 (d, 9.2)	4.74 (d, 9.2)	4.78 (d, 9.7)	-
5	4.18 (2H, s)	-	-	-	4.19 (2H, s)	4.17 (2H, s)	4.19 (2H, s)	-

*:Overlapped signal

1 – 3). Therefore, the structure of platyconic acid B (**1**) was determined as platycogenic acid A 3-*O*-β-D-glucopyranosido-28-*O*-β-D-apiofuranosyl(1→3)-β-D-xylopyranosyl(1→4)-[3-*O*-acetyl-α-L-rhamnopyranosyl](1→2)-α-L-arabinopyranoside, which is the corresponding 3''-*O*-acetate of platyconic acid A (**21**).¹

Platyconic acid C (**2**), a white amorphous powder, displayed an [M+H]⁺ peak at *m/z* 1107.5215 corresponding to the molecular formula of C₅₂H₈₂O₂₅ in the HR-FAB MS, and absorption bands at 3422 and 1736 cm⁻¹ due to hydroxyl and ester groups were present in the IR spectrum. The ¹³C NMR

spectrum of **2** showed 52 carbon signals including those due to Glc, Ara, Rha, Xyl and platycogenic acid A (**1b**) (Table 1). On acid hydrolysis, **2** afforded D-Glc, L-Ara, L-Rha, D-Xyl and **1a** instead of **1b** as in the case of **1**. The HMBC spectrum of **2** showed cross peaks between Glc H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2 and Xyl H-1/Rha C-4 (Table 1 - 3). The structure of platyconic acid C (**2**) was formulated as platycogenic acid A 3-*O*- β -D-glucopyranosido-28-*O*- β -D-xylopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platyconic acid D (**3**), a white amorphous powder, exhibited an $[M+H]^+$ peak at m/z 1149.5334 in the HR-FAB MS corresponding to the molecular formula of $C_{54}H_{84}O_{26}$, which is 42 mass unit (C_2H_2O) higher than that of **2**. The IR spectrum of **3** exhibited absorption bands at 3422 and 1734 cm^{-1} due to hydroxyl and ester groups respectively. The 1H NMR spectrum indicated an acetyl signal at δ 1.99 (Table 3). The ^{13}C NMR spectroscopic features of **3** resembled those of **2**, except for the appearance of acetyl signals (δ 21.4 and 171.0) (Table 1). On acid hydrolysis, **3** afforded D-Glc, L-Ara, L-Rha, D-Xyl and **1a**. In addition, the cross peak was observed between Rha H-2/the acetyl carbonyl carbon (δ 171.0) in the HMBC spectrum, suggesting that the acetyl group is located at the Rha C-2. Thus, the structure of platyconic acid D (**3**) was determined as platycogenic acid A 3-*O*- β -D-glucopyranosido-28-*O*- β -D-xylopyranosyl(1 $\rightarrow$ 4)-[2-*O*-acetyl- α -L-rhamnopyranosyl](1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platyconic acid E (**4**), a white amorphous powder, showed an $[M+H]^+$ peak at m/z 1269.5837 in its HR-FAB MS corresponding to the molecular formula of $C_{58}H_{92}O_{30}$, 162 mass unit ($C_6H_{10}O_5$) larger than that of **2**. The IR spectrum of **4** exhibited characteristic absorption bands at 3409 and 1736 cm^{-1} due to hydroxyl and ester groups respectively. On acid hydrolysis **4** afforded D-Glc, L-Ara, L-Rha, D-Xyl and **1a**, as in the case of **2** and **3**. The ^{13}C NMR spectrum of **4** showed 58 signals, the features of the spectrum resembled that of **2**, except for the appearance of six signals due to another D-glucopyranosyl moiety. The 46 signals were ascribable to those of platycogenic acid A 28-*O*- β -D-xylopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside (Table 1), while the other twelve signals were assignable to those of β -gentiobiosyl moiety was observed in the ^{13}C NMR spectrum of **15**.⁴ The correlation peaks between Glc H-1/Glc C-6 (the terminal Glc H-1 and the inner Glc C-6) and Glc H-1/C-3 were observed in the HMBC spectrum of **4**, indicating the presence of the β -gentiobiosyl moiety at the C-3 position. This Platyconic acid E (**4**) was therefore characterized as platycogenic acid A 3-*O*- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosido-28-*O*- β -D-xylopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platycodin J (**5**), a white amorphous powder, exhibited an $[M+H]^+$ peak at m/z 1239.5626 in its HR-FAB

MS corresponding to the molecular formula of $C_{57}H_{90}O_{29}$. The IR spectrum of **5** displayed absorption bands at 3398 and 1738 cm^{-1} due to hydroxyl and ester groups respectively. The ^{13}C NMR spectrum of **5** displayed 57 signals including five anomeric carbon signals (Table 1). On acid hydrolysis, **5** gave D-glucuronic acid (D-GluA), L-Ara, L-Rha, D-Xyl, D-Api and **9a** as its aglycone. The 51 carbon signals of 57 in **5** were identical with those due to platycodigenin 28-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 3)- β -D-xylopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyl. As for the location of GluA, the HMBC correlations were observed GluA H-1/C-3, indicating that GluA is located at the C-3 of the aglycone. The structure of platycodin J (**5**) was therefore formulated as platycodigenin 3-*O*- β -D-glucuronopyranosido-28-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 3)- β -D-xylopyranosyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platycodin K (**6**), a white amorphous powder, showed an $[\text{M}+\text{H}]^+$ peak at m/z 1281.5768 in its HR-FAB MS corresponding to the molecular formula of $C_{59}H_{92}O_{30}$, which is 42 mass unit ($\text{C}_2\text{H}_2\text{O}$) higher than that of **5**. The IR spectrum of **6** exhibited absorption bands at 3416 and 1735 cm^{-1} due to hydroxyl and ester groups respectively. The ^1H NMR spectrum of **6** indicated an acetyl signal at δ 2.01 (Table 3). The ^{13}C NMR spectroscopic features of **6** were similar to that of **5**, except for the appearance of two signals due to the acetyl group in **6** (Table 1). The HMBC correlation was observed between Rha H-2/acetyl carbonyl carbon, indicating that the acetoxyl group is located at the Rha C-2 position. Thus, platycodin K (**6**) was shown to be platycodigenin 3-*O*- β -D-glucuronopyranosido-28-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 3)- β -D-xylopyranosyl(1 $\rightarrow$ 4)-[2-*O*-acetyl- α -L-rhamnopyranosyl](1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platycodin L (**7**), a white amorphous powder, showed an $[\text{M}+\text{H}]^+$ peak at m/z 1281.5787 in its HR-FAB MS corresponding to the molecular formula of $C_{59}H_{92}O_{30}$ as in the case of **6**. The IR spectrum of **7** exhibited characteristic absorptions at 3408 and 1730 cm^{-1} due to hydroxyl and ester carbonyl groups, respectively, and the ^1H NMR spectrum showed an acetyl signal at δ 2.09 (Table 3). The ^{13}C NMR spectroscopic features of **7** were similar to that of **6**, except for the chemical shifts of the Rha C-1, C-2, C-3 and C-4 (Table 1). The HMBC correlation was observed between Rha H-3/acetyl carbonyl carbon, indicating that the acetoxyl group is located at the Rha C-3 position. The structure of platycodin L (**7**) was therefore determined as platycodigenin 3-*O*- β -D-glucuronopyranosido-28-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 3)- β -D-xylopyranosyl(1 $\rightarrow$ 4)-[3-*O*-acetyl- α -L-rhamnopyranosyl](1 $\rightarrow$ 2)- α -L-arabinopyranoside.

Platycosaponin A (**8**), a white amorphous powder, showed an $[\text{M}+\text{H}]^+$ peak at m/z 829.4631 in its HR-FAB MS corresponding to the molecular formula of $C_{42}H_{68}O_{16}$. The IR spectrum exhibited an absorption band due to hydroxyl groups at 3433 cm^{-1} . The ^{13}C NMR spectrum of **8** showed 42 signals

including two anomeric carbon signals (Table 1). On acid hydrolysis with 1 M hydrochloric acid **8** afforded D-Glc and an aglycone (**8a**). The aglycone (**8a**) exhibited an $[M+H]^+$ peak at m/z 505.3492 in its HR-FAB MS corresponding to the molecular formula $C_{30}H_{49}O_6$. The IR spectrum of **8a** exhibited absorption bands due to hydroxyl and ester groups at 3433 and 1737 cm^{-1} , respectively. Its NMR spectrum showed the presence of a carboxyl (δ_C 180.8), a trisubstituted double bond (δ_C 123.6, 143.4; δ_H 5.61), four oxygenated methines (δ_C 66.5, 71.9, 72.3, 78.8; δ_H 4.69, 4.42, 4.22, 3.45) and seven *t*-methyls (3H singlets at δ_H 1.11, 1.28, 1.30, 1.35, 1.38, 1.39, 1.52), suggesting **8a** to be a trihydroxy-oleanolic acid (Table 1 - 3). The detailed NMR analysis led us to the conclusion that **8a** is identical with 2 β ,3 β ,16 β ,21 β -tetrahydroxyolean-12-en-28-oic acid, i.e. platycogenic acid C, found from Platycodon Root, previously characterized by Kubota et al.,¹⁸ although the evidence described in their report was not good enough for the complete characterization. The partial structure of the A-ring was demonstrated by the following facts: the 1H - 1H COSY correlations between H₂-1/H-2 and H-2/H-3 and the HMBC correlations between H₃-23/C-3, H₃-24/C-3, H₃-23/C-4, H₃-24/C-4 and H₃-10/C-1; the *J* values of H-1, H-2 and H-3 signals included no diaxial coupling. While, the 16 β ,21 β -dihydroxy structure was deduced by the following data: the 1H - 1H COSY correlations between H₂-15/H-16 and H₂-22/H-21; the HMBC correlations between H₂-15/C-13, H₂-22/C-18, H-16/C-28, H₃-29/C-21, H₃-30/C-21 and H₂-22/C-28; the H-16 and H-21 signals showed a diaxial coupling, 12.0 and 12.1 Hz, respectively. Thus, platycogenic acid C (**8a**) was shown to be 2 β ,3 β ,16 β ,21 β -tetrahydroxyolean-12-en-28-oic acid.

The location of two glucosyl moieties were determined to be at C-2 and C-21, since HMBC correlations were observed between Glc H-1/C-2 and Glc' H-1/C-21. Consequently, platycosaponin A (**8**) was determined to be platycogenic acid C 2-*O*- β -D-glucopyranosido-21-*O*- β -D-glucopyranoside.

Twenty compounds including eight new triterpenoid saponins (**1** - **8**) have been isolated from Platycodon Root, and their structures elucidated by spectroscopic methods. This is the first isolation of platycogenic acid A glycosides (**1** - **4**) in free acid form,¹ although they were isolated as methyl ester and/or lactone form by Ishii et al.⁴ In addition, **5** - **7** are the first saponins containing D-glucuronic acid in their sugar moieties from Platycodon Root, and **8** is the first glycoside of platycogenic acid C.

EXPERIMENTAL

General Procedure Optical rotations were obtained on a JASCO P-1020 polarimeter. IR spectra were measured on a JASCO FT/IR-410 spectrometer. NMR spectra were recorded on a JEOL JMN LA-500 and a JEOL JMN ECA-500 spectrometers (500 MHz for 1H , 125 MHz for ^{13}C), and the chemical shifts were given in a ppm scale from TMS used as an internal standard. The signals were assigned by means of DEPT and 2D NMR techniques (1H - 1H COSY, HMQC, HMBC). Fast atom bombardment

mass spectra were obtained on a JEOL JMS-700 spectrometer and the matrix employed was 3-nitrobenzyl alcohol. HPLC was carried out on TSK-gel amide-80 column (4.6 x 250 mm) or COSMOSIL Sugar-D (nacalai tesque, 4.6 mm x 250 mm) with a RI-8020 (TOSOH Ltd.) and a OR-2090 Plus detector (JASCO Ltd.) for the D/L determination of monosaccharides. TLC was performed on precoated silica gel 60 F₂₅₄ or RP-18 WF₂₅₄ plates (Merck) with detection achieved by spraying with 10% H₂SO₄ followed by heating. Column chromatography (cc) was performed on silica gel 60 (spherical, 40~50um, Kanto Chemical Co. Ltd.) or ODS (chromatorex DM-1020T, Fuji-Silysia Co. Ltd.).

Extraction and Isolation Commercial Platycodon Root (1.5 Kg, Uchida Wakanyaku Co., Lot. No. 402204: imported from Hubei Province in China) was powdered and percolated with 70% MeOH (9.2 L) at room temperature; with the resulting 70% MeOH extract (688 g) concentrated in vacuo at 40 °C. The residual syrup was subjected to ODS cc with water containing increasing amounts of methanol to give 8 fractions: Frs. A (H₂O, 590 g), B (30% MeOH, 5.1 g), C (30% MeOH, 1.3 g), D (50% MeOH, 9.7 g), E (50% MeOH, 5.1 g), F (50% MeOH, 7.1 g), G (70% MeOH, 0.6 g) and H (MeOH, 4.2 g).

Fr. C was applied to silica gel cc [CHCl₃-MeOH-H₂O (6:4:1)] to afford five fractions: Frs. C-1 (23 mg), C-2 (331 mg), C-3 (395 mg), C-4 (269 mg) and C-5 (174 mg). Fr. C-2 was chromatographed on ODS cc (45%MeOH) to give three fractions: Frs. C-2-1 (98 mg), C-2-2 (132 mg) and C-2-3 (101 mg). Fr. C-2-1 was applied to silica gel cc [CHCl₃-MeOH-H₂O (78:22:2)] to give **20** (26 mg). Frs. C-2-2 and C-2-3 were applied to silica gel cc [CHCl₃-MeOH-H₂O (80:20:2 and 70:30:5, respectively)] to give **19** (24 mg), **6** (40 mg), respectively. Fr. C-3 was applied to silica gel cc [CHCl₃-MeOH-H₂O (65:35:7)] to give **6** (64 mg) and **7** (197 mg). Fr. C-4 was applied to silica gel cc [CHCl₃-MeOH-H₂O (65:35:7)] followed by an ODS cc (53% MeOH) to give **5** (75 mg).

Fr. D was applied to ODS cc (54% MeOH) to give six fractions: Frs. D-1 (0.49 g), D-2 (4.0 g), D-3 (1.2 g), D-4 (2.6 g), D-5 (1.2 g) and D-6 (0.62 g). Fr. D-2 was chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (56:64:10)] to give **7** (103 mg), **16** (3.5 g) and **19** (189 mg). Fr. D-4 was repeatedly chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (6:4:1 to 64:36:8)] to give **1** (159 mg), **2** (28 mg), **3** (16 mg), **4** (31 mg) and **15** (1.5 g). Fr. D-5 was repeatedly chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give **1** (342 mg) and **8** (46 mg).

Fr. E was applied to silica gel cc [CHCl₃-MeOH-H₂O (6:4:1)] to give five fractions: Frs. E-1 (2.1 g), E-2 (1.8 g), E-3 (0.87 g), E-4 (0.93 g) and E-5 (0.19 g). Fr. E-1 was applied to silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give three fractions: Frs. E-1-1 (0.15 g), E-1-2 (1.3 g) and E-1-3 (0.13 g). Fr. E-1-2 was repeatedly chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give **9** (36 mg), **10** (276 mg), **11** (42 mg) and **17** (25 mg). Fr. E-2 was chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give **14** (39 mg). Fr. E-4 was applied to silica gel cc [CHCl₃-MeOH-H₂O (62:38:9)] to give **15** (33 mg) and **18** (52 mg).

Fr. F was applied to a medium pressure ODS cc (57% MeOH) to give six fractions: Frs. F-1 (0.28 g), F-2 (1.2 g), F-3 (4.4 g), F-4 (1.0 g), F-5 (0.15 g) and F-6 (0.30 g). Fr. F-2 was applied to silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give four fractions: Frs. F-2-1 (381 mg), F-2-2 (623 mg), F-2-3 (77 mg) and F-2-4 (77 mg). Fr. F-2-2 was chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (64:36:8)] to give **11** (272 mg) and **12** (18 mg). Frs. F-2-3 and F-2-4 were individually chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (62:38:9)] to give **9** (65 mg) and **13** (59 mg), respectively. Frs. F-3 and F-5 were individually chromatographed on silica gel cc [CHCl₃-MeOH-H₂O (67:33:7)] to give **10** (150 mg) and **17** (69 mg), respectively.

Platyconic acid B (1) A white amorphous powder, $[\alpha]_D^{25}$ -20.7 (MeOH, *c* 0.91). Positive FAB-MS *m/z*: 1281.5729 ([M+H]⁺, C₅₉H₉₃O₃₀: 1281.5752). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3434, 2925, 2854, 1736, 1655, 1074, 1045. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: H-3/C-24, H-5/C-24, Glc H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Rha H-3/acetyl carbonyl carbon, Xyl H-1/Rha C-4, Api H-1/Xyl C-3.

Platyconic acid C (2) White amorphous powder, $[\alpha]_D^{25}$ -7.8 (pyridine, *c* 1.28). Positive FAB-MS *m/z*: 1107.5215 ([M+H]⁺, C₅₂H₈₃O₂₅: 1107.5223). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3422, 2925, 2853, 1736, 1655, 1078, 1043. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: H-3/C-24, H-5/C-24, Glc H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Xyl H-1/Rha C-4.

Platyconic acid D (3) White amorphous powder, $[\alpha]_D^{25}$ +4.5 (pyridine, *c* 0.94). Positive FAB-MS *m/z*: 1149.5334 ([M+H]⁺, C₅₄H₈₅O₂₆: 1149.5329). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3422, 2925, 2853, 1734, 1655, 1076, 1043. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: H-3/C-24, H-5/C-24, H-23/C-24, Glc H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Rha H-2/acetyl carbonyl carbon, Xyl H-1/Rha C-4.

Platyconic acid E (4) White amorphous powder, $[\alpha]_D^{25}$ -13.4 (MeOH, *c* 0.64). Positive FAB-MS *m/z*: 1269.5837 ([M+H]⁺, C₅₈H₉₃O₃₀: 1269.5752). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3409, 2926, 2856, 1736, 1655, 1075, 1043. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: H-3/C-24, H-5/C-24, Glc H-1/C-3, Glc' H-1/Glc C-6, Ara H-1/C-28, Rha H-1/Ara C-2, Xyl H-1/Rha C-4.

Platycodin J (5) White amorphous powder, $[\alpha]_D^{25}$ -33.8 (MeOH, *c* 1.02). Positive FAB-MS *m/z*: 1239.5626 ([M+H]⁺, C₅₇H₉₁O₂₉: 1239.5646). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3398, 2926, 2854, 1738, 1614, 1077, 1034. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: GluA H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Xyl H-1/Rha C-4, Api H-1/Xyl C-3.

Platycodin K (6) White amorphous powder, $[\alpha]_D^{25}$ -29.9 (MeOH, *c* 0.91). Positive FAB-MS *m/z*: 1281.5768 ([M+H]⁺, C₅₉H₉₃O₃₀: 1281.5752). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3416, 2932, 1735, 1636, 1044. ¹H and ¹³C NMR: Table 1 - 3. HMBC correlations: GluA H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Rha H-2/acetyl carbonyl carbon, Xyl H-1/Rha C-4, Api H-1/Xyl C-3.

Platycodin L (7) White amorphous powder, $[\alpha]_D^{25}$ -37.5 (MeOH, *c* 1.06). Positive FAB-MS *m/z*: 1281.5787 ([M+H]⁺, C₅₉H₉₃O₃₀: 1281.5752). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3408, 2926, 2858, 1730, 1635, 1041. ¹H

and ^{13}C NMR: Table 1 - 3. HMBC correlations: GluA H-1/C-3, Ara H-1/C-28, Rha H-1/Ara C-2, Rha H-3/acetyl carbonyl carbon, Xyl H-1/Rha C-4, Api H-1/Xyl C-3.

Platycosaponin A (8) White amorphous powder, $[\alpha]_{\text{D}}^{24} +33.4$ (MeOH, c 0.59). Positive FAB-MS m/z : 829.4631 ($[\text{M}+\text{H}]^+$, $\text{C}_{42}\text{H}_{69}\text{O}_{16}$: 861.4586). IR $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3433, 2925, 2855, 1736, 1655, 1048. ^1H and ^{13}C NMR: Table 1 - 3. HMBC correlations: Glc H-1/C-2, Glc' H-1/C-21.

Platycogenic acid A lactone (1a) White amorphous powder, $[\alpha]_{\text{D}}^{25} +32.9$ (pyridine, c 0.21). Positive FAB-MS m/z : 517.3135 ($[\text{M}+\text{H}]^+$, $\text{C}_{30}\text{H}_{45}\text{O}_7$: 517.3165). IR $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3447, 2925, 2854, 1772, 1750, 1655, 1036. ^{13}C NMR (pyridine- d_5) δ : 18.0 (C-25), 18.6 (C-26), 20.0 (C-6), 25.1 (C-11), 25.2 (C-30), 27.8 (C-27), 31.6 (C-20), 33.4 (C-22), 33.8 (C-29), 34.0 (C-7), 36.5 (C-15), 36.7 (C-21), 38.2 (C-10), 40.7 (C-8), 41.7 (C-1), 42.0 (C-18), 42.8 (C-14), 47.7 (C-19), 48.7 (C-9), 49.3 (C-17), 52.0 (C-5), 54.9 (C-4), 58.2 (C-23), 75.1 (C-16), 81.7 (C-3), 84.7 (C-2), 122.4 (C-12), 146.2 (C-13), 179.4 (C-24), 180.5 (C-28). ^1H NMR (pyridine- d_5) δ : 1.03 (3H, s, H_3 -29), 1.04 (3H, s, H_3 -26), 1.17 (3H, s, H_3 -30), 1.33 (1H, overlapped, H-21a), 1.37 (1H, overlapped, H-7a), 1.37 (1H, dd, $J=4.6, 12.6$ Hz, H-19a), 1.40 (3H, s, H_3 -25), 1.45 (1H, br. d, $J=14.3$ Hz, H-1a), 1.66 (1H, dd, $J=2.3, 14.9$ Hz, H-15a), 1.70 (1H, ddd, $J=2.9, 12.1, 12.1$ Hz, H-6a), 1.77 (3H, s, H_3 -27), 1.81 (1H, ddd, $J=2.9, 12.1, 12.1$ Hz, H-7b), 2.02 (1H, overlapped, H-11a), 2.07 (1H, overlapped, H-9), 2.08 (1H, dd, $J=4.6, 14.3$ Hz, H-1b), 2.08 (1H, overlapped, H-11b), 2.19 (1H, brd, $J=12.1$ Hz, H-5), 2.27 (2H, overlapped, H-6b and H-22a), 2.36 (1H, dd, $J=3.4, 14.9$ Hz, H-15b), 2.41 (1H, dd, $J=2.9, 4.6, 14.3$ Hz, H-22b), 2.51 (1H, dd, $J=4.6, 12.6, 12.6$ Hz, H-21b), 2.80 (1H, dd, $J=12.6, 14.3$ Hz, H-19b), 3.60 (1H, dd, $J=4.6, 14.3$ Hz, H-18), 4.43 (1H, d, $J=10.9$ Hz, H-23a), 4.63 (1H, brs, H-3), 4.75 (1H, d, $J=10.9$ Hz, H-23b), 4.98 (1H, brd, $J=4.6$ Hz, H-2), 5.22, (1H, brs, H-16), 5.62 (1H, dd, $J=2.9, 2.9$ Hz, H-12). HMBC correlations: H-2/C-24, H-3/C-24, H-5/C-24.

Platycogenic acid C (8a) White amorphous powder, $[\alpha]_{\text{D}}^{24} + 58.8$ (pyridine, c 0.59), $+ 47.6$ (EtOH, c 0.62). Positive FAB-MS m/z : 505.3492 ($[\text{M}+\text{H}]^+$, $\text{C}_{30}\text{H}_{49}\text{O}_6$: 505.3529). IR $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3433, 2925, 2855, 1737, 1655, 1049. ^{13}C NMR (pyridine- d_5) δ : 17.1 (C-25), 18.0 (C-26), 18.5 (C-30), 18.7 (C-24), 19.1 (C-6), 24.5 (C-11), 27.4 (C-27), 30.3 (C-29), 30.7 (C-23), 33.8 (C-7), 36.4 (C-22), 37.2 (C-20), 37.8 (C-10), 38.9 (C-15), 39.3 (C-4), 40.4 (C-8), 44.2 (C-18), 45.1 (C-14), 45.4 (C-1), 47.6 (C-19), 48.2 (C-9), 53.1 (C-17), 56.4 (C-5), 66.5 (C-16), 71.9 (C-2), 72.3 (C-21), 78.8 (C-3), 123.6 (C-12), 143.4 (C-13), 180.8 (C-28). ^1H NMR (pyridine- d_5) δ : 1.01 (1H, brd, $J=10.9$ Hz, H-5), 1.11 (3H, s, H_3 -26), 1.24 (1H, dd, $J=4.1, 13.8$ Hz, H-1a), 1.28 (3H, s, H_3 -23), 1.30 (3H, s, H_3 -29), 1.35 (3H, s, H_3 -30), 1.38 (3H, s, H_3 -24), 1.39 (3H, s, H_3 -27), 1.39 (1H, overlapped, H-7a), 1.52 (1H, dd, $J=4.6, 13.8$ Hz, H-19a), 1.52 (3H, s, H_3 -25), 1.57 (2H, overlapped, H-6a and H-7b), 1.66 (2H, overlapped, H-6b and H-9), 1.82 (1H, dd, $J=4.6, 13.2$ Hz, H-15a), 2.06 (1H, overlapped, H-11a), 2.14 (1H, overlapped, H-11b), 2.12 (1H, dd, $J=13.8, 14.3$ Hz, H-19b), 2.22 (1H, dd, $J=12.1, 13.2$ Hz, H-22a), 2.32 (1H, dd, $J=2.9, 13.8$ Hz, H-1b),

2.34 (1H, dd, $J=12.0, 13.2$ Hz, H-15b), 3.25 (1H, dd, $J=4.6, 13.2$ Hz, H-22b), 3.45 (1H, d, $J=4.1$ Hz, H-3), 3.66 (1H, dd, $J=4.6, 14.3$ Hz, H-18), 4.22 (1H, dd, $J=4.6, 12.1$ Hz, H-21), 4.42 (1H, ddd, $J=2.9, 4.1, 4.1$ Hz, H-2), 4.69 (1H, dd, $J=4.6, 12.0$ Hz, H-16), 5.61 (1H, dd, $J=3.5, 3.5$ Hz, H-12).

Platycodigenin (9a) White amorphous powder, $[\alpha]_D^{25} +18.4$ (pyridine, c 0.35). Positive FAB-MS m/z : 521.3456 ($[M+H]^+$, $C_{30}H_{49}O_7$: 521.3478). IR $\nu_{max}^{KBr} cm^{-1}$: 3423, 2925, 2854, 1736, 1675, 1636, 1040. ^{13}C NMR (pyridine- d_5) δ : 17.9 (C-25), 18.0 (C-26), 19.5 (C-6), 24.7 (C-11), 25.3 (C-30), 27.8 (C-27), 31.5 (C-20), 33.3 (C-22), 33.8 (C-29), 34.3 (C-7), 36.6 (C-15), 36.7 (C-21), 37.7 (C-10), 40.6 (C-8), 42.0 (C-18), 42.7 (C-14), 45.2 (C-1), 47.2 (C-4), 47.7 (C-19), 48.6 (C-9), 49.1 (C-5), 49.4 (C-17), 64.6 (C-23), 65.1 (C-24), 72.3 (C-2), 75.2 (C-16), 75.7 (C-3), 123.0 (C-12), 145.6 (C-13), 180.7 (C-28).

Acid hydrolysis of 1 **1** (22.5 mg) was dissolved in 1 ml of 1 M HCl (1,4-dioxane: $H_2O=1:1$) and heated at 100 °C for 2 h. The reaction mixture was diluted with H_2O and passed through Sep-pack ODS (H_2O and MeOH) to afford the H_2O eluate and the MeOH eluate. The MeOH eluate (14.1 mg) was chromatographed on silica gel cc [$CHCl_3$ -MeOH- H_2O (9:1:0.08)] to afford platycogenic acid A lactone (**1a**, 6.3 mg).¹⁸ The H_2O fraction was analyzed by HPLC under the following conditions: column, TSK-GEL Amido-80 (Tosoh, 4.6 mm x 250 mm); solvent, 100 mM triethylamine in 75% MeCN; flow rate, 0.5 ml/min; detector, RI-8012 and OR-2090 plus. The constituents of the H_2O fraction were determined to be a mixture of D-Glc (t_R 23.6 min, positive), L-Ara (t_R 18.2 min, positive), L-Rha (t_R 14.7 min, negative), D-Xyl (t_R 17.2 min, positive), D-Api (t_R 14.1 min, positive), by comparison with authentic samples.

Acid hydrolysis of 2, 3 and 4 Compounds **2**, **3** and **4** (5 mg each) were individually hydrolyzed in a similar way as in the case of **1**, and **1a** was obtained from the MeOH fraction and D-Glc, L-Ara, L-Rha, D-Xyl were detected in the H_2O fraction.

Acid hydrolysis of 5 Compound **5** (20.0 mg) was hydrolyzed in a similar fashion to **1**, and afforded a MeOH eluate (6.8 mg) and an H_2O eluate. The MeOH eluate was chromatographed on silica gel cc [$CHCl_3$ -MeOH (10:1)] to afford platycodigenin (**9a** 4.9 mg) identified on the basis of the data with those already reported.⁵ The H_2O fraction was analyzed by HPLC under the following conditions: column, COSMOSIL Sugar-D; solvent, MeCN-20 mM phosphate buffer (pH=7.0) (7:3); flow rate, 1.0 ml/min; detector, RI-8012 and OR-2090 plus. Consequently, the constituents of H_2O fraction were clarified to be a mixture of D-GluA (t_R 10.4 min, positive), L-Ara (t_R 6.3 min, positive), L-Rha (t_R 5.3 min, negative), D-Xyl (t_R 6.1 min, positive), D-Api (t_R 5.4 min, positive), by compared with authentic samples.

Acid hydrolysis of 6 and 7 Compounds **6** and **7** (5 mg each) were hydrolyzed in a similar way as in the case of **5**, and afforded **9a** from the MeOH fractions and D-GluA, L-Ara, L-Rha, D-Xyl, D-Api from the H_2O fraction, this was determined as described for **5**.

Acid hydrolysis of 8 Compound **8** (20.7 mg) was hydrolyzed in a similar way to **1**, and afforded a

MeOH eluate and an H₂O eluate. The MeOH eluate was chromatographed on silica gel cc [CHCl₃-MeOH (9:1:0.08)] to afford platycogenic acid C (**8a**, 7.5 mg), identified on the basis of the physical data already reported.¹⁸ The H₂O fraction was analyzed with similar way to **1**, and afforded D-Glc.

ACKNOWLEDGEMENTS

We express our appreciation to Dr. J. Kitajima of Showa Pharmaceutical University for measurement of mass spectra, also to Ms. K. Shiohara and Y. Odanaka of Showa University for the measurements of MS and NMR spectra, respectively.

REFERENCES

1. M. Fukumura, D. Iwasaki, Y. Hirai, Y. Hori, Y. Kuchino, and Y. Ida, The 125th Annual Meeting of the Pharmaceutical Society of Japan (Tokyo, 2005), abstract paper 4, p. 143.
2. A. Tada, Y. Kaneiwa, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.*, 1975, **23**, 2965.
3. T. Konishi, A. Tada, J. Shoji, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.*, 1978, **26**, 668.
4. H. Ishii, K. Tori, T. Tozyo, and Y. Yoshimura, *J. Chem. Soc., Perkin. Trans. 1*, 1984, 661.
5. T. Nikaido, K. Koike, K. Mitsunaga, and T. Saeki, *Nat. Med.*, 1998, **52**, 54.
6. T. Nikaido, K. Koike, K. Mitsunaga, and T. Saeki, *Chem. Pharm. Bull.*, 1999, **47**, 903.
7. K. Mitsunaga, K. Koike, M. Koshikawa, H. Takeuchi, T. Saeki, and T. Nikaido, *Nat. Med.*, 2000, **54**, 148.
8. Z. He, C. Qiao, Q. Han, Y. Wang, W. Ye, and H. Xu, *Tetrahedron*, 2005, **61**, 2211.
9. Y. S. Kim, J. S. Kim, S. U. Choi, H. S. Lee, S. H. Roh, Y. C. Jeong, Y. K. Kim, and S. Y. Ryu, *Planta Med.*, 2005, **71**, 556.
10. W. W. Fu, N. Shimizu, D. Q. Dou, T. Takeda, R. Fu, Y. H. Pei, and Y. J. Chen, *Chem. Pharm. Bull.*, 2006, **54**, 557.
11. W. W. Fu, N. Shimizu, T. Takeda, D. Q. Dou, B. Chen, Y. H. Pei, and Y. J. Chen, *Chem. Pharm. Bull.*, 2006, **54**, 1285.
12. W. Li, L. Xiang, J. Zhang, Y. N. Zheng, L. K. Han, and M. Saito, *Chinese Chem. Lett.*, 2007, **18**, 306.
13. Y. H. Choi, D.S. Yoo, C. W. Choi, M. R. Cha, Y. S. Kim, H. S. Lee, K. R. Lee, and S. Y. Ryu, *Molecules*, 2008, **13**, 2871.
14. Y. Hirai, M. Fukumura, Y. Kuchino, and Y. Ida, The 125th Annual Meeting of the Pharmaceutical Society of Japan (Tokyo, 2005), abstract paper 4, p. 143.
15. M. Yuda, K. Ohtani, K. Mizutani, R. Kasai, O. Tanaka, M. Jia, Y. Ling, X. Pu, and Y. Saruwatari, *Phytochemistry*, 1990, **29**, 1989.

16. W. Schwab and P. Schreier, *J. Agric. Food Chem.*, 1990, **38**, 757.
17. F. R. Melek, T. Miyase, N. S. Ghaly, and M. Nabil, *Phytochemistry*, 2007, **68**, 1261.
18. T. Kubota, H. Kitatani, and H. Hinoh, *Chem. Commun.*, 1969, 1313.