Saponins from Roots of *Platycodon grandiflorum*. Part 1. Structure of Prosapogenins [1]

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Seven new prosapogenins have been isolated as their methyl esters [(1), (2), and (4)—(8)] from the roots of *Platycodon grandiflorum* A. DC., along with the known 3-*O*-β-D-glucopyranosylplatycodigenin methyl ester (3). The structures of the seven compounds were elucidated using ^{13}C and ^{1}H n.m.r. spectroscopy.

THE roots of *Platycodon grandiflorum* A. DC. (Chinese drug, ' Jiegeng '; Japanese name, ' Kikyo ') are used as an expectorant in traditional oriental medicine. Several sapogenins have thus far been isolated from the drug and their structures determined independently by Shibata's school [2,3] and Kubota *et al.*[4-6] However, reports on the structure of their glycosides from this plant are limited to those on a main saponin, platycodin D,[7] and its prosapogenin, 3-*O*-β-D-glucopyranosylplatycodigenin.[8]

Recently, Yamasaki *et al.*[9] and we [10] reported the utility of ^{13}C n.m.r. spectroscopy in the structure elucidation of saikosaponins, glycosides of oleanene-type triterpenes. Since the sapogenins obtained from *P. grandiflorum* are also triterpenes of this type,[2-6] we undertook the determination of the whole structure of saponins other than platycodin D using ^{13}C n.m.r. spectroscopy. As the first step, we describe here the structure of prosapogenins prepared by alkaline hydrolysis of a crude saponin mixture.

RESULTS AND DISCUSSION

The plant material was extracted with methanol and the extract was partitioned between butan-1-ol and water. The organic layer was concentrated and the residue obtained was washed with ether to remove the oily components, giving a crude saponin mixture. The mixture was hydrolysed with aqueous potassium hydroxide to give a mixture of prosapogenins, which was divided into four fractions by silica gel chromatography. The fractions were separately methylated with diazomethane and further chromatographed to give eight prosapogenin methyl esters (1)—(8). Their physical properties are listed in Table 1.

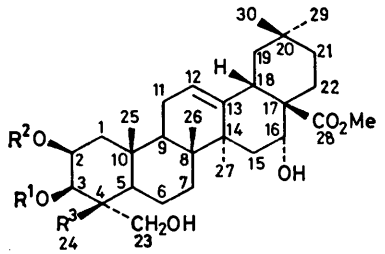
TABLE 1

Physical properties of prosapogenin methyl esters (1)—(8)

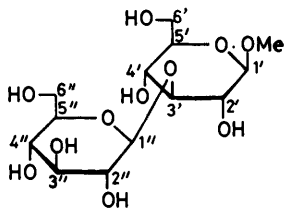
Compd.	M.p. (°C)	$[\alpha]_D$ (°) (MeOH)	Formula	R_F *
(1)	269—278°	+18.3	$C_{37}H_{60}O_{11}$	0.43
(2)	244—248°	+16.7	$C_{43}H_{70}O_{16}$	0.29
(3)	213—219°	+29.1	$C_{37}H_{60}O_{12}$	0.36
(4)	231—237°	+20.9	$C_{43}H_{70}O_{17}$	0.24
(5)	209—218°	+23.9	$C_{43}H_{70}O_{17}$	0.21
(6)	225—228°	+44.0	$C_{37}H_{58}O_{12}$	0.44
(7)	254—257°	+54.5	$C_{38}H_{60}O_{13}$	0.48
(8)	194—203°	+29.6	$C_{39}H_{62}O_{13}$	0.32

* Pre-coated t.l.c. plates of Kieselgel 60 F_{254} (Merck), solvent $CHCl_3$–MeOH–H_2O (30 : 10 : 1), double development.

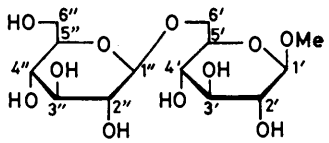
The ^{1}H n.m.r. spectra at 100 MHz were first examined for these compounds (1)—(8), and the known methyl esters (9)—(12) derived from sapogenins of this plant, polygalacic acid,[2,4] platycodigenin,[3,5] platycogenic acid



	R^1	R^2	R^3
(1)	β-D-glucopyranosyl	H	Me
(2)	β-laminaribiosyl (Lam)	H	Me
(3)	β-D-glucopyranosyl	H	CH_2OH
(4)	β-laminaribiosyl (Lam)	H	CH_2OH
(5)	β-gentiobiosyl (Gen)	H	CH_2OH
(6)	β-D-glucopyranosyl	└CO┘	
(7)	β-D-glucopyranosyl	H	CO_2Me
(8)	β-D-glucopyranosyl	CH	CO_2Me
(9)	H	H	Me
(10)	H	H	CH_2OH
(11)	H	└CO┘	
(12)	H	H	CO_2Me



(14)
Methyl β-laminaribioside



(15)
Methyl β-gentiobioside

A lactone,[6] and platycogenic acid A,[6] in [$^2H_5$]pyridine, together with their peracetates (1a)—(11a) † in [^2H]-chloroform (Table 2). The examination showed that the spectra of (1) and (2) have six angular methyl signals and

† Peracetate (12a) was not prepared because there was insufficient compound (12).

resemble that of methyl polygalacate (9), that the spectra of (3)—(5) have five angular methyl singals and resemble that of platycodigenin methyl ester (10), that the spectrum of (6) has five angular methyl signals and resembles that of platycogenic acid A lactone methyl ester (11), and that the spectrum of (7) has five angular methyl and two methoxy-signals and resembles that of dimethyl platycogenate A (12). The spectrum of (8) showed five angular methyl and three methoxy-signals. These results suggested that the aglycones of (1) and (2), (3)—(5), (6), and (7) are (9), (10), (11), and (12), respectively.

A triplet appeared at δ 5.50—5.56 ($W_{\frac{1}{2}}$ 6 Hz) in compounds (1)—(6) and (9)—(11) and was ascribed to H-12 from the signal pattern and the small chemical shift on acetylation. Another triplet at δ 4.95—5.00 ($W_{\frac{1}{2}}$ 7 Hz) and a quartet at δ 4.77—4.96 ($W_{\frac{1}{2}}$ 8 Hz) [except that in lactone (6)] were also ascribed to H-16β and H-2α, respectively, from the splitting pattern and downfield shifts of 0.5—0.7 p.p.m. on acetylation.

The H-3α signal (doublet, J 4 Hz) at δ 4.22—4.59 in aglycones (9)—(11) shifted to δ 4.94—5.05 on acetylation, showing that the 3β-hydroxy-group had been acetylated. On the other hand, the corresponding signal in prosapogenins, assignable only in the acetates (1a)—(6a) at δ 3.47—3.72, appeared at considerably higher fields than those of H-2α and H-16β, suggesting that the 3β-*O*-position in compounds (1)—(6) did not undergo acetylation, *i.e.* it had been occupied by a glycosyl group.

Next, complete ^{1}H-decoupled ^{13}C n.m.r. spectra of (1)—(12) in [$^2H_5$]pyridine at 100 °C and those of (1a)—(11a) in [^{2}H]chloroform at 80 °C were measured and compared with each other. The ^{13}C signals of sapogenins (9)—(12) and their acetates (9a)—(11a) were assigned by using known data on a number of oleanene-type triterpenoids,[11] known chemical-shift rules,[11] various ^{1}H-decoupling techniques,[11] and chemical-shift comparisons from compound to compound. Thus, we found that the signal positions of the aglycone moieties of compounds (1)—(7) agree well with those of the corresponding aglycones (9)—(12) assigned above, except for some signals originating from the A-ring carbons (Table 3).

The C-3 signals of prosapogenins (1)—(7) were shifted downfield by 8.6—12.4 p.p.m. from those of the corresponding aglycones (9)—(12), while the C-2 signals were shifted upfield by 1.2—2.9 p.p.m. These chemical-shift changes are explained by the glycosidation shifts[12] of the 3-*O*-glycoside structure, confirming the suggestion from the ^{1}H n.m.r. data. The C-4 signals showed a small shift on glycosidation, because the C-4 carbons are quarternary.[12] The chemical-shift changes of C-23 from (1) and (2) to (9), and those of C-24 from (3)—(5) to (10), may result from changes in the interaction and conformation of the hydroxy-groups situated around the 3-*O*-glycosidic linkages. The acetylation shifts from (1)—(11) to (1a)—(11a), respectively, agree with those expected by the rules.[13]

The ^{13}C spectrum of (8) resembled that of (7) except for an additional methoxy-signal. The C-2 signal of (8) was shifted downfield by 10.5 p.p.m. from that of (7), whereas the C-1 signal was shifted upfield by 2.0 p.p.m.

TABLE 2

^{1}H N.m.r. chemical-shift data of compounds (1)—(12) in [$^2H_5$]pyridine and of (1a)—(11a) in [^{2}H]chloroform (in parentheses)

Hydrogen	(9) (9a)	(1) (1a)	(2) (2a)	(10) (10a)	(3) (3a)	(4) (4a)	(5) (5a)	(11) (11a)	(6) (6a)	(12)	(7) (7a)	(8) (8a)
H-2α	4.51 (5.42)	4.78 (5.32)	4.77 (5.34)	4.60 (5.41)	4.95 (5.38)	4.96 (5.35)	4.85 (5.43)	4.95 (4.78)	5.18 (4.68)	4.95	*a* (4.46)	*a* (*a*)
H-3α	4.22 (4.94)	*a* (3.58)	*a* (3.47)	4.37 (5.04)	*a* (3.72)	*a* (3.54)	*a* (3.58)	4.59 (5.05)	*a* (3.60)	*a*	*a* (*a*)	*a* (*a*)
H-12	5.56 (5.43)	5.56 (5.40)	5.56 (5.41)	5.56 (5.44)	5.56 (5.40)	5.54 (5.41)	5.53 (5.43)	5.51 (5.40)	5.50 (5.41)	5.56	5.54 (5.41)	5.58 (5.42)
H-16β	4.97 (5.67)	4.98 (5.67)	5.00 (5.64)	4.97 (5.67)	4.97 (5.65)	4.96 (5.65)	4.95 (5.65)	4.97 (5.65)	4.95 (5.67)	4.99	*a* (5.66)	4.95 (5.64)
H-24	1.35 (1.05)	1.36 (0.97)	1.35 (0.95)	*a* (*a*)	*a* (*a*)	*a* (*a*)	*a* (*a*)					
H-25	1.64 (1.26)	1.62 (1.14)	1.61 (1.13)	1.67 (1.26)	1.54 (1.15)	1.54 (1.16)	1.47 (1.18)	1.42 (1.19)	1.35 (1.18)	1.49	1.40 (1.10)	1.43 (1.15)
H-26	0.97 (0.77)	0.97 (0.73)	0.95 (0.72)	0.95 (0.75)	0.92 (0.72)	0.92 (0.73)	0.91 (0.73)	0.89 (0.73)	0.86 (0.72)	0.97	0.91 (0.76)	0.97 (0.75)
H-27	1.72 (1.26)	1.75 (1.22)	1.75 (1.22)	1.72 (1.26)	1.72 (1.22)	1.72 (1.21)	1.72 (1.21)	1.68 (1.27)	1.67 (1.28)	1.72	1.73 (1.24)	1.73 (1.22)
H-29	1.08 (1.00)	1.10 (0.99)	1.10 (0.98)	1.08 (0.99)	1.09 (0.99)	1.09 (0.99)	1.07 (0.99)	1.08 (0.98)	1.08 (0.99)	1.09	1.09 (0.99)	1.08 (0.98)
H-30	0.98 (0.95)	0.98 (0.94)	1.00 (0.93)	1.00 (0.94)	1.00 (0.94)	1.00 (0.93)	0.99 (0.93)	0.99 (0.93)	1.00 (0.94)	1.00	1.01 (0.93)	1.00 (0.93)
2-OMe												3.66 *b* (3.37)
24-OMe										3.70 *b*	3.59 (3.65)	3.61 *b* (3.59)
28-OMe	3.68 (3.63)	3.67 (3.63)	3.68 (3.62)	3.67 (3.63)	3.68 (3.63)	3.67 (3.63)	3.67 (3.63)	3.65 (3.61)	3.67 (3.63)	3.69 *b*	3.68 (3.62)	3.68 *b* (3.62)
COMe	(2.00) (2.05 ×2) (2.09)	(2.00 ×2) (2.03 ×2) (2.09 ×3)	(1.97) (2.01 ×3) (2.07) (2.08) (2.10) (2.15)	(1.99) (2.03) (2.08 ×3)	(1.98) (2.00) (2.03 ×3) (2.08 ×2) (2.12)	(1.95) (1.98) (1.99 ×2) (2.00) (2.02) (2.04) (2.06 ×2) (2.11) (2.13)	(1.95 ×2) (1.97) (2.01 ×2) (2.03) (2.06 ×5)	(2.04) (2.07) (2.08)	(1.96 ×2) (1.99) (2.06) (2.07) (2.08)		(1.99) (2.00 ×2) (2.01) (2.09 ×2) (2.12)	(1.99) (2.02 ×2) (2.06) (2.07) (2.12)

a Not determined. *b* Assignments may be interchanged in each vertical column.

TABLE 3

^{13}C N.m.r. chemical-shift data of compounds (1)—(12) in [$^2H_5$]pyridine and of (1a)—(11a) in [^{2}H]chloroform (in parentheses)

Carbon	(9) (9a)	(1) (1a)	(2) (2a)	(10) (10a)	(3) (3a)	(4) (4a)	(5) (5a)	(11) (11a)	(6) (6a)	(12)	(7) (7a)	(8) (8a)
C-1	45.0	44.2	44.2	44.6	45.0	44.9	45.1	41.2	41.6	46.3	45.8	43.8
	(41.7)	(41.7)	(41.8)	(41.8)	(42.0)	(42.0)	(42.4)	(40.7)	(41.2)		(41.9)	(43.4)
C-2	71.6	70.3	70.4	71.6	69.3	69.4	68.5	84.0	82.8	71.0	69.8	80.3
	(69.6)	(72.2)	(72.1)	(69.3)	(72.1)	(72.1)	(72.2)	(80.8)	(82.0)		(70.7)	(79.9)
C-3	74.1	83.8	83.7	75.3	86.4	86.3	87.7	81.2	89.8	75.3	84.4	85.3
	(72.0)	(80.7)	(80.3)	(71.9)	(80.6)	(79.9)	(80.8)	(81.5)	(89.2)		(79.2)	(83.7)
C-4	42.3	42.8	42.9	46.6	47.7	47.7	46.5	54.2	53.9	54.8	56.2	55.2
	(40.1)	(41.5)	(41.5)	(43.7)	(45.4)	(45.5)	(45.6)	(50.5)	(51.2)		(53.6)	(52.8)
C-5	49.1	48.3	48.2	48.8	48.0	48.0	48.1	51.4	52.0	50.3	50.2	50.5
	(47.4)	(48.0)	(48.0)	(47.6)	(48.5)	(48.5)	(48.7)	(51.8)	(52.1)		(49.0)	(50.5) *
C-6	18.6	18.3	18.3	19.1	19.6	19.5	19.6	19.4	19.4	20.5	20.4	20.6
	(17.7)	(18.0)	(18.0)	(19.1)	(19.6)	(19.7)	(19.7)	(19.0)	(19.2)		(20.1)	(20.0)
C-7	33.4	33.4	33.4	33.6	33.7	33.7	33.7	33.2	33.5	33.7	33.6	34.0
	(32.6)	(32.9)	(32.9)	(33.1)	(33.5)	(33.5)	(33.6)	(32.9)	(33.1)		(33.5)	(33.7)
C-8	40.3	40.3	40.3	40.0	40.5	40.4	40.6	40.1	40.4	40.2	40.2	40.5
	(39.6)	(39.9)	(39.9)	(39.6)	(39.9)	(39.9)	(39.9)	(39.9)	(40.2)		(40.2)	(40.2)
C-9	47.9	47.8	47.9	47.9	47.9	47.9	47.8	48.1	48.3	47.5	47.5	47.7
	(47.8)	(48.0)	(48.0)	(48.1)	(48.0)	(48.0)	(48.1)	(47.8)	(48.3)		(47.8) *	(48.1)
C-10	37.4	37.2	37.2	37.1	37.6	37.5	38.0	37.6	37.8	37.5	37.4	38.0
	(36.7)	(36.6)	(36.6)	(36.7)	(36.7)	(36.6)	(36.6)	(37.4)	(37.6)		(37.0)	(37.6)
C-11	24.1	24.1	24.1	24.1	24.2	24.2	24.1	24.6	24.6	24.3	24.4	24.5
	(23.4)	(23.7)	(23.7)	(23.5)	(23.8)	(23.7)	(23.6)	(24.1)	(24.4)		(24.0)	(24.0)
C-12	122.9	123.0	123.0	122.7	123.0	123.0	123.1	121.9	122.1	122.9	122.9	123.0
	(123.4)	(123.7)	(123.7)	(123.2)	(123.5)	(123.5)	(123.8)	(122.7)	(122.9)		(123.8)	(124.0)
C-13	144.6	144.6	144.6	144.4	144.5	144.5	144.4	145.1	145.1	144.6	144.6	144.7
	(142.1)	(142.4)	(142.3)	(142.1)	(142.3)	(142.3)	(142.1)	(142.7)	(143.0)		(142.3)	(142.4)
C-14	42.3	42.3	42.3	42.0	42.3	42.3	42.4	42.7	42.3	42.4	42.4	42.5
	(41.2)	(41.6)	(41.6)	(41.1)	(41.4)	(41.4)	(41.4)	(41.2)	(41.4)		(41.6)	(41.6)
C-15	36.1	36.1	36.1	35.9	36.1	36.1	36.1	35.8	36.0	36.1	36.1	36.2
	(32.1)	(32.4)	(32.4)	(32.1)	(32.4)	(32.4)	(32.3)	(32.0)	(32.3)		(32.4)	(32.5)
C-16	74.5	74.5	74.5	74.3	74.5	74.5	74.4	74.2	74.3	74.5	74.5	74.6
	(76.2)	(76.3)	(76.4)	(76.1)	(76.3)	(76.4)	(76.3)	(76.1)	(76.2)		(76.4)	(76.4)
C-17	49.5	49.5	49.5	49.1	49.6	49.5	49.6	49.0	49.4	49.6	49.6	49.7
	(47.6)	(47.7)	(47.7)	(47.6)	(48.0)	(48.0)	(48.0)	(47.5)	(47.9)		(48.1) *	(48.1)
C-18	41.6	41.6	41.6	41.3	41.7	41.7	41.8	41.2	41.6	41.7	41.7	41.8
	(40.4)	(40.8)	(40.8)	(40.4)	(40.8)	(40.8)	(40.8)	(40.3)	(40.7)		(41.8)	(41.0)
C-19	47.1	47.1	47.1	46.9	47.1	47.1	47.1	46.9	47.0	47.2	47.2	47.2
	(46.1)	(46.4)	(46.4)	(46.1)	(46.4)	(46.4)	(46.6)	(46.1)	(46.4)		(46.4)	(46.5)
C-20	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.9
	(30.4)	(30.5)	(30.5)	(30.4)	(30.5)	(30.5)	(30.5)	(30.4)	(30.5)		(30.5)	(30.5)
C-21	36.1	36.1	36.1	35.9	36.1	36.1	36.1	35.8	36.0	36.1	36.1	36.2
	(35.1)	(35.3)	(35.3)	(35.1)	(35.3)	(35.3)	(35.3)	(35.0)	(35.2)		(35.4)	(35.4)
C-22	32.1	32.1	32.2	32.3	32.1	32.1	32.1	32.4	32.0	32.1	32.1	32.1
	(31.0)	(31.0)	(31.0)	(31.0)	(31.0)	(31.0)	(31.0)	(31.0)	(31.0)		(31.1)	(31.1)
C-23	69.3	66.5	66.2	64.3	63.8	63.8	63.6	57.8	57.4	66.5	64.4	64.1
	(65.5)	(66.2)	(66.2)	(62.8)	(63.4)	(63.3)	(63.0)	(58.8)	(59.6)		(64.9)	(64.2)
C-24	14.2	14.8	14.8	64.7	66.3	65.7	67.7	178.7	177.6	176.9	175.5	173.6
	(13.9)	(13.7)	(13.7)	(63.9)	(64.0)	(64.0)	(63.9)	(174.9)	(174.7)		(170.4)	(171.2)
C-25	17.4	17.4	17.4	17.2	18.1	18.0	18.7	17.8	18.0	16.3	15.7	16.7
	(16.6)	(16.6)	(16.5)	(16.4)	(16.5)	(16.4)	(16.4)	(16.8)	(16.9)		(16.8)	(16.7)
C-26	17.4	17.4	17.4	17.3	17.4	17.4	17.4	17.5	17.3	17.3	17.3	17.5
	(17.0)	(17.2)	(17.2)	(16.7)	(16.9)	(16.9)	(16.9)	(17.4)	(17.6)		(17.2)	(17.2)
C-27	27.3	27.3	27.3	27.1	27.2	27.2	27.2	27.2	27.3	27.2	27.2	27.3
	(26.3)	(26.4)	(26.4)	(26.3)	(26.4)	(26.3)	(26.3)	(26.7)	(26.8)		(26.4)	(26.5)
C-28	177.8	177.6	177.8	177.7	177.7	177.8	177.7	177.7	177.6	177.8	177.7	177.8
	(176.0)	(176.0)	(176.0)	(175.9)	(176.0)	(176.0)	(176.0)	(175.9)	(175.9)		(176.0)	(176.0)
C-29	33.1	33.1	33.1	33.1	33.1	33.0	33.1	33.2	33.0	33.1	33.1	33.1
	(33.2)	(33.1)	(33.1)	(33.1)	(33.1)	(33.1)	(33.2)	(33.1)	(33.1)		(33.1)	(33.1)
C-30	24.9	24.9	25.0	24.7	24.9	24.9	24.9	24.6	24.9	25.0	25.0	25.0
	(24.2)	(24.4)	(24.4)	(24.2)	(24.4)	(24.4)	(24.4)	(24.2)	(24.4)		(24.4)	(24.5)
C-1′		105.2	105.6		106.0	105.5	106.0		105.2		106.3	106.6
		(102.2)	(102.4)		(102.2)	(102.2)	(102.8)		(101.5)		(102.0)	(103.0)
C-2′		75.5	74.2		75.2	74.1	75.0		75.1		75.3	75.7
		(71.5)	(71.6)		(71.2)	(71.4) *	(72.2)		(71.9)		(71.8)	(72.1)
C-3′		78.6	88.8		78.6	88.7	78.0		78.2		78.6	78.5
		(73.3)	(78.9)		(73.3)	(78.8)	(74.5)		(72.9)		(73.3)	(73.2)
C-4′		72.0	70.0		72.0	70.0	72.1 *		71.7		72.1	72.3
		(68.9)	(68.8)		(68.9)	(68.9)	(68.1)		(68.9)		(69.1)	(69.5)
C-5′		78.0	77.7		78.2	77.9	76·0		78.2		78.2	78.1
		(72.0)	(73.6)		(72.1)	(73.4)	(73.3)		(72.2)		(72.2)	(72.1)
C-6′		63.0	62.7		62.9	62.6	70.4		62.9		63.1	63.4
		(62.1)	(62.2)		(62.0)	(62.2)	(69.4)		(62.1)		(62.2)	(62.4)
C-1″			105.0			105.5	104.8					
			(101.1)			(101.1)	(100.8)					
C-2″			75.4			75.4	75.3					
			(71.6)			(71.5) *	(71.8)					
C-3″			78.4			78.4	78.7					
			(73.4)			(73.4)	(73.3)					
C-4″			72.0			71.9	71.9 *					
			(68.8)			(68.9)	(69.0)					
C-5″			78.2			78.2	78.0					
			(72.0)			(72.1)	(72.2)					
C-6″			62.8			62.8	63.0					
			(62.2)			(62.2)	(62.2)					
2-OMe												59.0
												(58.9)
24-OMe										51.2	51.2	50.5
											(51.2)	(50.9) *
28-OMe	51.5	51.5	51.6	51.6	51.5	51.6	51.5	51.8	51.6	51.6	51.6	51.5
	(52.1)	(52.0)	(52.0)	(52.1)	(52.0)	(52.0)	(52.0)	(52.2)	(52.1)		(52.0)	(52.0)
OCO*Me*	(20.8 × 2)	(20.5 × 4)	(26.5 × 6)	(20.7)	(20.5 × 4)	(20.5 × 6)	(20.5 × 6)	(20.7 × 2)	(20.4 × 3)		(20.5 × 4)	(20.4 × 4)
	(21.2)	(20.8)	(20.9 × 2)	(20.9 × 2)	(20.8 × 2)	(20.9 × 3)	(20.8 × 3)	(22.0)	(20.6 × 2)		(20.8)	(20.7)
	(22.0)	(21.3)	(21.2)	(21.2)	(21.2)	(21.2)	(21.4)		(21.8)		(21.1)	(21.8)
		(21.8)	(21.9)	(21.9)	(21.9)	(21.9)	(21.8)				(21.9)	
OCOMe	(169.8)	(169.2 × 2)	(168.9)	(169.8 × 2)	(169.2 × 2)	(168.8)	(168.8)	(169.9)	(169.1 × 2)		(169.1)	(169.0)
	(170.0)	(169.6)	(169.1 × 3)	(170.0)	(169.6)	(169.0 × 3)	(169.3 × 3)	(170.1 × 2)	(169.6)		(169.2)	(169.2)
	(170.3)	(169.9)	(169.7)	(170.3)	(169.8)	(169.2 × 2)	(169.6)		(169.7)		(169.7)	(169.6 × 2)
	(170.7)	(170.1 × 2)	(169.9)	(170.5)	(170.1 × 2)	(169.7 × 4)	(169.7)		(170.0)		(169.9)	(170.0 × 2)
		(170.4)	(170.1)		(170.3)	(170.2)	(170.0 × 3)		(170.2)		(170.0)	
			(170.3 × 2)		(170.4)	(170.5)	(170.2)				(170.1)	
			(170.5)				(170.4)				(170.4)	

* Assignments may be interchanged in each vertical column.

These facts indicate that (8) is the 2-*O*-methyl derivative of (7).

Features of the ^{13}C signals due to the sugar moieties suggested that the sugar attached to (1), (3), (6), (7), and (8) is glucose, and those attached to (2), (4), and (5) are glucobioses. Therefore, the spectra of (1)—(8) were compared with those of methyl β-D-glucopyranoside (13) and some methyl glucobiosides, *e.g.*, methyl β-laminaribioside (14) and methyl β-gentiobioside (15), in [$^2H_5$]-pyridine (Table 4). The C-1′ signals of (1), (3), (6), (7),

TABLE 4

^{13}C N.m.r. chemical-shift data of compounds (13)—(15) in [$^2H_5$]pyridine and of (13a)—(15a) in [^2H]chloroform (in parentheses)

Carbon	(13) (13a)	(14) (14a)	(15) (15a)
C-1	105.4 (101.8)	105.5 (101.8)	105.3 (101.8)
C-2	75.0 (71.9)	73.8 (73.1)	74.9 (71.8)
C-3	78.4 (73.4)	88.7 (79.1)	78.3 (73.8)
C-4	72.0 (69.3)	70.2 (69.0)	71.9 (69.2)
C-5	78.0 (72.3)	77.7 (72.5)	76.9 (73.4) *
C-6	63.0 (62.5)	62.8 (62.8)	70.2 (69.8)
C-1′		105.0 (101.1)	105.0 (101.1)
C-2′		75.4 (71.7)	75.0 (71.8)
C-3′		78.3 (73.4)	78.3 (73.3) *
C-4′		71.9 (69.2)	72.1 (68.5)
C-5′		78.2 (72.3)	77.9 (72.5)
C-6′		62.8 (62.3)	63.1 (62.4)
OMe	56.6 (56.5)	56.5 (56.3)	56.7 (56.7)
CO*Me*	(20.4 ×4)	(20.5 ×6) (20.9)	(20.5 ×7)
*C*OMe	(169.0) (169.2)	(168.8) (169.2 ×3)	(169.1) (169.3)
	(169.9) (170.2)	(170.1) (170.2)	(169.5) (170.0 ×3)
		(170.4)	(170.3)

* Assignments may be interchanged in each column.

and (8) were shifted by −0.2 to +1.2 p.p.m., and the C-2′ signals shifted slightly, whereas the other signal positions were in good agreement with the corresponding signals in (13). These glycosidation shifts [12] together with the shifts of C-2, C-3, and C-4 signals in the aglycone moiety indicated that the five compounds have the β-D-glucopyranosyl group. Similar inspection of the glycosidation shifts on (2), (4), and (5) showed that the former two are β-laminaribiosides, whereas the latter is a β-gentiobioside.

Aglycones (9)—(12) and D-glucose composing prosapogenins (1)—(7) were also identified chemically by acid hydrolyses (see Experimental section).

Thus, we concluded that the prosapogenin methyl esters (1)—(8) are the methyl esters of 3-*O*-β-D-glucopyranosyl- and 3-*O*-β-laminaribiosyl-polygalacic acids, 3-*O*-β-D-glucopyranosyl-, 3-*O*-β-laminaribiosyl-, and 3-*O*-β-gentiobiosyl-platycodigenins, 3-*O*-β-D-glucopyranosyl-platycogenic acid A lactone, 3-*O*-β-D-glucopyranosyl-platycogenic acid A, and 2-*O*-methyl-3-*O*-β-D-glucopyranosylplatycogenic acid A, respectively.

EXPERIMENTAL

M.p.s were measured with a Yanagimoto micro-apparatus. Silica gel used for column chromatography was Kieselgel 60 (Merck). Optical rotations were measured on solutions in methanol. ^1H N.m.r. spectra were taken with a Varian XL-100-12A, HA-100, or A-60A spectrometer using [$^2H_5$]-pyridine solutions for (1)—(12) and [^2H]chloroform solutions for (1a)—(11a) containing tetramethylsilane as internal reference. ^{13}C N.m.r. spectra were recorded on a Varian NV-14 Fourier-transform n.m.r. spectrometer at 15.087 MHz using [$^2H_5$]pyridine solutions for (1)—(15) and [^2H]-chloroform solutions for (1a)—(11a) and (13a)—(15a), with tetramethylsilane as internal reference in 8-mm spinning tubes at elevated temperatures [100 °C for (1)—(15) and 80 °C for (1a)—(11a) and (13a)—(15a) to prevent line broadening].[14] Typical Fourier-transform measurement conditions were as follows: spectral width, 3923 Hz; acquisition time, 0.6 s; pulse width, 10—20 μs (pulse flipping angle, 15—30°); number of data points, 4 820; number of transitions, 10 000—100 000. Accuracies of δ_H and δ_C are ±0.02 and ±0.1 p.p.m., respectively.

Extraction and Fractionation of Prosapogenins.—Dried and cut roots of *P. grandiflorum* (2 kg), purchased from Tochimoto-Tenkaido Co., Ltd., Osaka, were extracted with refluxing methanol (5 × 3 l) for 1 h. Material from the methanolic extract (341 g) was partitioned with a butan-1-ol–water system five times and the organic layer was evaporated *in vacuo*. The resulting residue (43.6 g) was triturated with ether (300 ml) to remove oily components (22.7 g), leaving a crude saponin mixture as a pale yellow powder (18.8 g).

The crude saponin mixture (10 g) was dissolved in 5% aqueous potassium hydroxide (150 ml) and kept at 90 °C for 10 h. The reaction mixture was acidified (pH 3) with dilute hydrochloric acid and extracted with butan-1-ol (4 × 50 ml). The organic layer was washed and evaporated *in vacuo* to give a prosapogenin mixture (5.8 g). The mixture was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (6 : 4 : 0.8). Fractions having similar t.l.c. properties were combined to give, in the order of increasing polarity, four larger fractions: A (262 mg), B (1.861 g), C (1.342 g), and D (1.784 g).

Methyl 3-O-β-D-*Glucopyranosylpolygalacate* (1).—Fraction A (262 mg) was treated with ethereal diazomethane as in the standard procedure and the product (304 mg) was chromatographed on silica gel (100 g) with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to give a fraction rich in (1), which was similarly chromatographed twice to yield *compound* (1) (162 mg) as a white powder, m.p. 269—279 °C (from methanol–ether), $[\alpha]_D^{22}$ +18.3° (*c* 1.05); $\nu_{max.}$ (KBr) 3 400 (br) and 1 715 cm^{-1} (Found: C, 62.5; H, 8.7. $C_{37}H_{60}O_{11}\cdot 1.5H_2O$ requires C, 62.78; H, 8.97%).

Acetylation of (1) (90 mg) with acetic anhydride (0.75 ml) in pyridine (1 ml) at 90 °C for 15 h gave a product (116 mg), which was purified by chromatography on silica gel (30 g) with $CHCl_3$–acetone (9 : 1) giving the *hepta-acetate* (1a) as a white powder (98 mg), m.p. 148—152 °C (from ether–n-pentane), $[\alpha]_D^{22}$ +5.9° (*c* 0.98) (Found: C, 62.55; H, 7.7. $C_{51}H_{74}O_{18}$ requires C, 62.81; H, 7.65%).

Acid Hydrolysis of the Methyl Ester (1).—Compound (1) (100 mg) was refluxed with 5% sulphuric acid in 50% ethanol (20 ml) for 6 h. The solution was diluted with water and extracted with ethyl acetate. The extract (67 mg) was purified by chromatography on silica gel (20 g) with $CHCl_3$–MeOH–H_2O (95 : 5 : 0.5), giving needles (55 mg) identical to those of methyl polygalacate (9) [4] (i.r. spectrum and mixed m.p.). The aqueous layer was filtered through a column of Dowex 1 × 8 resin and evaporated. The

residue was confirmed to be glucose by t.l.c. comparisons with an authentic sample.

Methyl 3-O-β-*Laminaribiosylpolygalacate* (2).—Fraction B (1.861 g) was methylated with diazomethane and the product was chromatographed on silica gel (150 g). The main eluate with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) gave a fraction rich in (3) and the tail eluate rich in (2). The latter fraction was purified by further chromatography and yielded *compound* (2) as a white powder (278 mg), m.p. 244—248 °C (from methanol–ether), $[\alpha]_D^{22}$ +16.7° (*c* 1.0); $\nu_{max.}$ (KBr) 3 400 (br) and 1 715 cm^{-1} (Found: C, 59.95; H, 8.35. $C_{43}H_{70}O_{16}\cdot H_2O$ requires C, 59.98; H, 8.43%).

Compound (2) was acetylated as described above and gave a product (145 mg) which was chromatographed on silica gel (50 g) with benzene–ethyl acetate (1 : 1) to yield the *deca-acetate* (2a) as a white powder, m.p. 156—160 °C (from ether–n-pentane), $[\alpha]_D^{23}$ −10.7° (*c* 1.0) (Found: C, 60.0; H, 7.3. $C_{63}H_{90}O_{26}$ requires C, 59.89; H, 7.18%).

Hydrolysis of (2) (100 mg) with 5% sulphuric acid was carried out as described for (1) and gave methyl polygalacate (9) [4] (21 mg) (mixed m.p. and i.r. spectrum) and glucose (t.l.c.).

3-O-β-D-*Glucopyranosylplatycodigenin Methyl Ester* (3).—The fraction rich in (3) described above was submitted to repeated chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) giving compound (3) [8] (875 mg) as a white powder, m.p. 213—219 °C (from methanol–ether), $[\alpha]_D^{22}$ +29.1° (*c* 1.02); $\nu_{max.}$ (KBr) 3 400(br) and 1 715 cm^{-1} (Found: C, 61.9; H, 8.45. Calc. for $C_{37}H_{60}O_{12}\cdot H_2O$: C, 62.16; H, 8.74%).

Acetylation of (3) in the manner described for (1) gave a crude product (117 mg), which when purified by chromatography yielded the *octa-acetate* (3a) as a white powder (98 mg), m.p. 139—144 °C (Found: C, 61.4; H, 7.45. $C_{53}H_{76}O_{20}$ requires C, 61.61; H, 7.42%).

Compound (3) (100 mg) was hydrolysed as described for (1), giving platycodigenin methyl ester (10) [5] (43 mg) (mixed m.p. and i.r. spectrum) and glucose (t.l.c.).

3-O-β-*Laminaribiosylplatycodigenin Methyl Ester* (4).—Fraction C (1.342 g) was esterified with diazomethane and the product was chromatographed on silica gel (200 g). Elution with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.3) afforded successively fractions rich in (4) and (5). The former fraction was purified by repeated chromatography with the same solvent system and gave *compound* (4) (427 mg) as a white powder, m.p. 231—237 °C (from methanol–ether), $[\alpha]_D^{22}$ +20.9° (*c* 1.02); $\nu_{max.}$ (KBr) 3 400(br) and 1 715 cm^{-1} (Found: C, 57.65; H, 8.15. $C_{43}H_{70}O_{17}\cdot 2H_2O$ requires C, 57.70; H, 8.33%).

Acetylation of (4) (80 mg) in the manner described above gave the *undeca-acetate* (4a) (104 mg) as a white powder, m.p. 155—158 °C (from ether–n-pentane), $[\alpha]_D^{21}$ −3.7° (*c* 1.02) (Found: C, 58.75; H, 7.0. $C_{65}H_{92}O_{28}$ requires C, 59.08; H, 7.02%).

Hydrolysis of (4) (100 mg) with 5% sulphuric acid gave platycodigenin methyl ester (10) [5] (33 mg) (mixed m.p. and i.r. spectrum) and glucose (t.l.c.).

3-O-β-*Gentiobiosylplatycodigenin Methyl Ester* (5).—The fraction rich in (5) described above was purified by repeated chromatography to yield *compound* (5) (199 mg) as a white powder, m.p. 209–218 °C (from methanol–ether), $[\alpha]_D^{22}$ +23.9° (*c* 1.03); $\nu_{max.}$ (KBr) 3 400(br) and 1 715 cm^{-1} (Found: C, 56.8; H, 8.2 $C_{43}H_{70}O_{17}\cdot 3H_2O$ requires C, 56.56; H, 8.39%).

Acetylation of (5) (70 mg) gave the *undeca-acetate* (5a) (96 mg) as a white powder, m.p. 148—151 °C (from ether–n-pentane), $[\alpha]_D^{21}$ +4.3° (*c* 1.0) (Found: C, 59.0; H, 7.15. $C_{65}H_{92}O_{28}$ requires C, 59.08; H, 7.02%).

Hydrolysis of (5) (100 mg) with 5% sulphuric acid gave platycodigenin methyl ester (10) [5] (26 mg) (mixed m.p. and i.r. spectrum) and glucose (t.l.c.).

3-O-β-D-*Glucopyranosylplaticogenic Acid A Lactone Methyl Ester* (6).—Fraction D (1.784 g) was treated with diazomethane and the product was repeatedly chromatographed on silica gel (200 g) with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2), yielding successively (7)-rich, (6)-rich, and (8)-rich fractions. The (6)-rich fraction (233 mg) was rechromatographed on silica gel (50 g) with EtOAc–EtOH–H_2O (9 : 1 : 0.5) to give *compound* (6) (182 mg) as a white powder, m.p. 225—228 °C (from methanol–ether), $[\alpha]_D^{22}$ +44.0° (*c* 1.0); $\nu_{max.}$ (KBr) 3 425(br), 1 765, and 1 720 cm^{-1} (Found: C, 63.05; H, 8.35. $C_{37}H_{56}O_{12}\cdot 0.5H_2O$ requires C, 63.32; H, 8.19%).

Acetylation of (6) (80 mg) gave the *hexa-acetate* (6a) (116 mg), m.p. 149—152 °C (from ether–pentane), $[\alpha]_D^{24}$ +22.3° (*c* 0.98) (Found: C, 62.25; H, 7.3. $C_{49}H_{68}O_{18}$ requires C, 62.27; H, 7.25%).

Compound (6) (90 mg) was refluxed with 5% sulphuric acid in 50% ethanol (20 ml) for 25 h. The reaction mixture was worked up as usual and gave platycogenic acid A lactone methyl ester (11) [6] (45 mg) (mixed m.p. and i.r. spectrum) and glucose (t.l.c.).

Dimethyl 3-O-β-D-*Glucopyranosylplatycogenate A* (7).—The (7)-rich fraction (98 mg) was re-chromatographed on silica gel (30 g) with EtOAc–EtOH–H_2O (9 : 1 : 0.5) and gave *compound* (7) (69 mg) as a white powder, m.p. 254—257 °C (from methanol–ether), $[\alpha]_D^{22}$ +54.5° (*c* 1.0); ν_{max} (KBr) 3 450(br), 1 735, and 1 715 cm^{-1} (Found: C, 60.45; H, 8.35. $C_{38}H_{60}O_{13}\cdot 1.5H_2O$ requires C, 60.70; H, 8.45%).

Acetylation of (7) (70 mg) gave the *hepta-acetate* (7a) (88 mg) as a white powder, m.p. 139—143 °C (from ether–pentane), $[\alpha]_D^{24}$ +2.7° (*c* 0.96) (Found: C, 61.45; H, 7.2. $C_{52}H_{74}O_{20}$ requires C, 61.28; H, 7.32%).

Acid Hydrolysis of Compound (7).—Compound (7) (100 mg) was hydrolysed with 5% sulphuric acid as described for (1). The ethyl acetate-soluble portion (78 mg) was chromatographed on silica gel (50 g) and eluted with $CHCl_3$–MeOH–H_2O (95 : 5 : 0.5). The head eluate gave *dimethyl platycogenate A* (12) (36 mg) as a white powder, m.p. 147—150 °C (from ether–n-pentane), $[\alpha]_D^{20}$ +78.4° (*c* 1.0); $\nu_{max.}$ (Nujol) 3 430(br) and 1 715(br) cm^{-1} (Found: C, 66.8; H, 8.85. $C_{32}H_{50}O_8\cdot 0.67H_2O$ requires C, 66.87; H, 9.00%). This was identified with an authentic sample prepared by methylation of platycogenic acid A [6] (mixed m.p. and i.r. spectrum). The tail eluate from the same solvent system gave the lactonized compound (11) (31 mg) (mixed m.p. and i.r. spectrum). The aqueous layer showed the presence of glucose (t.l.c.).

Dimethyl 2-O-*Methyl*-3-O-β-D-*glucopyranosylplatycogenate A* (8).—The (8)-rich fraction (75 mg) was purified by chromatography on silica gel (30 g). Elution with EtOAc–EtOH–H_2O (9 : 1 : 0.5) gave *compound* (8) (47 mg) as a white powder, m.p. 194—203 °C (from methanol–ether), $[\alpha]_D^{23}$ +29.6° (*c* 1.03); $\nu_{max.}$ (KBr) 3 400(br) and 1 725(br) cm^{-1} (Found: C, 59.7; H, 8.35. $C_{39}H_{62}O_{13}\cdot 2.5H_2O$ requires C, 59.75; H, 8.62%).

Acetylation of (8) (80 mg) gave the *hexa-acetate* (8a) as a white powder (97 mg), m.p. 134—139 °C (from ether–pentane), $[\alpha]_D^{22}$ −6.1° (*c* 1.02) (Found: C, 61.45; H, 7.55. $C_{51}H_{74}O_{19}$ requires: C, 61.80; H, 7.53%).

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