[Chem. Pharm. Bull. 26(2) 674—677 (1978)]

UDC 547.918.02.08 : 581.192

Structures of Polygalacin-D and -D_2, Platycodin-D and -D_2, and Their Monoacetates, Saponins isolated from *Platycodon grandiflorum* A. DC., determined by Carbon-13 Nuclear Magnetic Resonance Spectroscopy[1]

The structure of new nine saponins isolated from *Platycodon grandiflorum* A. DC. have been elucidated by comparison of their ^{13}C nuclear magnetic resonance spectra with those of the known prosapogenins and that of platycodin-D (**1**). All the saponins proved to possess the same sugar chain as that of **1** at C-28 position. Eight of the nine (**2**, **3**, **5**, **6**, **8**, **9**, **11**, and **12**) have an O-acetyl group which was found to migrate easily and reversibly between 2-O- and 3-O-position of the rhamnose moiety in their sugar chain. The other one is an acetyl-free saponin named polygalacin-D_2 (**10**). Two new deacetylated saponins, platycodin-D_2 (**4**) and polygalacin-D (**7**), were prepared from **5** (**6**) and **8** (**9**), respectively.

keywords——oleanene-type saponins; *Platycodon grandiflorum*; C-13 NMR; C-13 partially-relaxed FT NMR; C-13 acetylation shifts; platycodin-D and -D_2; polygalacin-D and -D_2

Recently Shibata and coworkers[2] isolated platycodin-D (**1**) from *P. grandiflorum*, and elucidated the whole structure except for anomeric configuration of its arabinose moiety. We have also isolated ten pure saponins having *Rf* values higher than and around that of **1** from a hot-methanol extract of platycodi radix by repeated column chromatography and droplet counter current chromatography (see Table I).

Table I. Physical Properties of Platycodin-D (**1**) and -D_2 (**4**), Polygalacin-D (**7**) and -D_2 (**10**), and Their Monoacetates

No.	mp	*Rf*[a]	*Rf*[b]	$[\alpha]_D$[c]
1	228—237°	0.34	0.32	−30.5°
2	227—233°	0.46	0.41	−24.8°
3	227—231°	0.40	0.38	−39.8°
4[d]	227—235°	0.27	0.24	−27.9°
5	225—231°	0.38	0.32	−25.0°
6	224—232°	0.33	0.29	−35.3°
7[d]	221—226°	0.39	0.37	−41.5°
8	223—227°	0.50	0.47	−33.2°
9	219—225°	0.46	0.43	−41.2°
10	229—236°	0.32	0.30	−35.5°
11	229—235°	0.42	0.38	−32.0°
12	226—233°	0.37	0.35	−40.6°

a) Solvent, $CHCl_3$-MeOH-H_2O (15:10:2), double development.
b) Solvent, EtOAc-EtOH-H_2O (15:5:4), double development.
c) In MeOH at 23°.
d) Prepared by deacetylation.

Saponin **1** was considered to be platycodin-D[3] by comparison of their *Rf* values. In fact, on substructing the ^{13}C Fourier transform nuclear magnetic resonance (FT NMR) spectrum of 3-O-β-D-glucopyranosylplatycodigenin methyl ester (**13**)[4] from that of **1** taken in C_5D_5N

1) For the history of the research of components of the plant, see ref. 2) and 4).
2) A. Tada, Y. Kaneiwa, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **23**, 2965 (1975).
3) We sincerely thank Prof. J. Shoji of Showa University for a generous gift of an authentic sample of platycodin-D.
4) H. Ishii, K. Tori, T. Tozyo, and Y. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **26**, 671 (1978).

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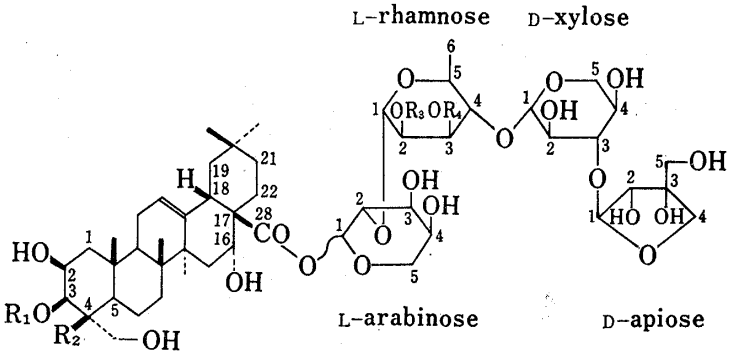
at 100°, we obtained a signal pattern reasonably due to the sugar chain at C-28 of platycodin-D, as shown in Table II. Signal positions of C-16—18, C-22, and C-28 are affected a little by a change from the OMe to O-sugar group at C-28, but other signals due to the 13 moiety remained unchanged. Thus, we tentatively assigned ^{13}C signals of the sugar chain in **1** as



	R_1	R_2	R_3	R_4	
1:	β-D-glucopyranosyl	CH_2OH	H	H	: platycodin-D
2:	β-D-glucopyranosyl	CH_2OH	Ac	H	
3:	β-D-glucopyranosyl	CH_2OH	H	Ac	
4:	β-laminaribiosyl	CH_2OH	H	H	: platycodin-D_2
5:	β-laminaribiosyl	CH_2OH	Ac	H	
6:	β-laminaribiosyl	CH_2OH	H	Ac	
7:	β-D-glucopyranosyl	Me	H	H	: polygalacin-D
8:	β-D-glucopyranosyl	Me	Ac	H	
9:	β-D-glucopyranosyl	Me	H	Ac	
10:	β-laminaribiosyl	Me	H	H	: polygalacin-D_2
11:	β-laminaribiosyl	Me	Ac	H	
12:	β-laminaribiosyl	Me	H	Ac	

Chart 1

shown in Table II in comparison with the data on ^{13}C chemical shifts of methyl arabinoside,[5,6] rhamnosides,[5] xylosides,[6,7] and apiosides,[8] and some O-acetylarabinosides,[6] on the following three very rough assumptions: (1) O-glycosylation of *sec*-OH except anomeric OH in a sugar causes a *ca.* +10 ppm downfield shifts of the α-carbon signal but little shifts of other signals;[9] (2) glycosidation shifts for a sugar, *i.e.*, signal shifts from methyl glycoside to *sec*-alcohol glycoside, are *ca.* −3 ppm for the anomeric carbon but less than 1 ppm for other carbons;[10] and (3) O-acetylation of *sec*-OH causes *ca.* +2 ppm downfield and *ca.* −3 ppm upfield shifts of the α- and β-carbon signals, respectively, but no shifts of other signals.[11] From the tentative assignments given here, L-arabinose moiety in **1** is suggested to have β-configuration at C-1.[5,6] The order of the signal recovery of the anomeric carbons in the sugar chain was found as arabinose>rhamnose>xylose>apiose in partially-relaxed FT NMR spectra[12] of **1**, being consistent[13] with the known sugar sequence of playtcodin-D.[2]

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TABLE II. Carbon-13 Chemical Shifts δ_C (± 0.1)[a] of Platycodin-D (**1**), Its Prosapogenin (**13**), Its Monoacetates (**2** and **3**), Its Peracetate (**17**), Polygalacin-D_2 (**10**), and Its Prosapogenin (**16**) in Pyridine-d_5 and Methanol-d_4 (in Parentheses)

No.	13, 16	1	2[c]	3[c]	17[d]	10[d]
Prosapogenin[b]						
C-16	74.5 (75.3)	74.1 (74.6)	74.1 (74.7)	74.1 (74.6)	76.2	74.2
C-17	49.6 (50.3)	50.1 (50.5)	50.1 (50.5)	50.1 (50.5)	48.8	50.1
C-18	41.7 (42.3)	41.8 (42.3)	41.7 (42.3)	41.7 (42.3)	41.5	41.6
C-22	32.1 (32.3)	31.3 (31.7)	31.3 (31.7)	31.3 (31.6)	31.0	31.3
C-28	177.7(179.5)	175.8(177.1)	175.9(177.1)	175.9(177.0)	174.1	175.8
Arabinose						
C-1		93.7 (94.2)	93.6 (94.2)	93.6 (94.0)	94.0	93.7
C-2		75.6 (75.8)	76.3 (76.2)	76.3 (76.4)	75.4	75.7
C-3		65.9 (66.9)[e]	65.8 (66.9)[e]	65.8 (66.7)	67.0	65.8
C-4		70.4 (71.5)	70.3 (71.5)	70.3 (71.5)	69.7	70.2
C-5		62.9 (64.1)	62.9 (64.1)	62.9 (64.1)	61.6	62.9
Rhamnose						
C-1		101.0(101.3)	98.4 (98.6)	101.6(101.4)	98.9	101.1
C-2		72.0 (72.2)	73.6 (73.9)	70.3 (70.1)	70.8	72.0
C-3		72.4 (72.4)	70.3 (70.5)	75.3 (75.3)	73.2[f]	72.5
C-4		83.7 (83.8)	83.4 (83.4)	77.6 (78.0)	77.2	83.7
C-5		68.6 (69.1)[f]	68.7 (69.1)[f]	68.7 (69.2)[e]	68.9	68.7
C-6		18.1 (18.1)	18.3 (18.1)	18.3 (18.4)	18.1	18.1
Xylose						
C-1		106.6(106.6)	106.4(106.4)	105.4(105.5)	101.6	106.6
C-2		75.0 (75.3)	75.0 (75.2)	75.0 (75.3)	72.1[e]	75.0
C-3		85.6 (86.0)	85.7 (85.9)	85.7 (85.5)	77.7	85.6
C-4		69.5 (70.0)[f]	69.5 (69.9)[f]	69.5 (70.1)[e]	70.8	69.5
C-5		66.8 (67.2)[e]	66.7 (67.2)[e]	66.7 (66.7)	63.0	66.8
Apiose						
C-1		111.2(111.2)	111.2(111.2)	111.2(111.2)	107.9	111.3
C-2		77.9 (78.1)	77.9 (78.0)	77.9 (78.0)	79.9	77.9
C-3		80.0 (80.4)	80.0 (80.3)	80.0 (80.4)	84.4	80.0
C-4		75.0 (75.3)	75.0 (75.2)	75.0 (75.3)	72.8[e,f]	75.0
C-5		65.7 (65.5)	65.8 (65.4)	65.8 (65.5)	63.7	65.8

a) ^{13}C NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer at 15.087 MHz in 8-mm spinning tubes with TMS as internal reference (δ_C 0); concentrations were about 0.1 mmol/cm^3. FT measurement conditions were as follows: spectral width, 3923 Hz; pulse flipping angle, *ca.* 36°; acquisition time, 0.6 sec; number of data points, 4820.
b) For the data on the other carbons, see ref. 4).
c) Data in pyridine-d_5 were taken from their 1:1 mixture spectra.
d) Data in methanol are not shown.
e, f) Assignments may be interchanged in each vertical column.

The ^{13}C spectrum of saponin **10**, named polygalacin-D_2, in C_5D_5N at 100° apparently showed that it consists of the spectrum of methyl 3-O-laminaribiosylpolygalacate (**16**), a prosapogenin methyl ester reported in the preceding paper,[4] and the signal pattern of the same sugar chain at C-28 of **1** (see Table II). Thus, the structure of polygalacin-D_2 has been elucidated to be shown by formula **10**.

The remaining eight saponins were found to have an O-acetyl group and not to be easily purified. Acetylsaponins **2** and **3**, **5** and **6**, **8** and **9**, and **11** and **12** were converted into each other in dilute alcoholic solutions (*ca.* 5 mg/ml) to give about 1:1 mixtures of them and then produce their parent deacetylated saponins after a long standing, as detected by thin-layer chromatography. Further, ^{13}C spectra of both **2** and **3** in C_5D_5N at 100° during one night were found to be the same one showing it a spectrum of 1:1 mixture of **2** and **3**. This is also the case with the other pairs. However, ^{13}C spectra of the acetylsaponins in CD_3OD

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(*ca.* 200 mg/ml) at 60° showed that they are pure compounds. The aceytl migration[14] seems to occur slowly in such concentrated alcoholic solutions.

As reported previously,[15] the acetylation shift rule[11] in ^{13}C NMR spectroscopy can easily point out the position of an O-acetyl group, if ^{13}C signals of the mother compound (deacetyl derivative) are fully assigned. Therefore, we compared the spectra of these saponins and the peracetate of **1** (**17**), examined the acetylation shifts (see underlined figures in Table II), and found that **2** and **3** are 2-O- and 3-O-acetyl derivatives, respectively, in the rhamnose moiety of **1**, and **11** and **12** are also 2-O- and 3-O-acetyl derivatives, respectively, in the rhamnose moiety of **10**. The spectra of **5** and **6** consist of that of 3-O-β-laminaribiosyl-platycodigenin methyl ester (**14**)[4] and of the sugar chain of **2** (and **11**) and **3** (and **12**), respectively, and the spectra of **8** and **9** consist of that of methyl 3-O-β-D-glucopyranosyl-polygalacate (**15**)[4] and of the sugar chain of **2** and **3**, respectively. Therefore, **5** and **8** were elucidated to be 2-O-acetyl derivatives in the rhamnose moiety of new saponins named platycodin-D_2 (**4**) and polygalacin-D (**7**) (see Table I), respectively; **6** and **9** also proved to be 3-O-acetyl derivatives in the rhamnose moiety of **4** and **7**, respectively.

Platycodin-D (**1**) and -D_2 (**4**), and polygalacin-D (**7**) and -D_2 (**10**) were respectively derived from **2** (**3**), **5** (**6**), **8** (**9**), and **11** (**12**) by deacetylation, and identified by the ^{13}C spectra. Furthermore, their corresponding prosapogenins **13**—**16** were obtained by respective alkali hydrolyses of **1**, **4**, **7**, and **10**, and identified by the ^{13}C spectra.

The discovery of easy reversible migration of an O-acetyl group between two adjacent positions in a sugar moiety in natural plant glycosides should be emphasized.

Acknowledgements We thank Drs. K. Takeda and S. Seo for their helpful discussion and advice, and wish to dedicate this paper to the memory of the late Dr. T. Kubota of this laboratory.

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Received November 18, 1977

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