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Chemical Studies on the Oriental Plant Drugs.[1] XXXI. Sapogenins of the Roots of *Platycodon grandiflorum* A. De Candolle. (2). Structure of Platycodigenin[2]

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The structure of platycodigenin, a spogenin of the saponin of the root of *Platycodon grandiflorum* A. DC. has been shown to be 2β,3β,16α,23,24-pentahydroxyolean-12-en-28-oic acid (I) by the nuclear magnetic resonance and mass spectral analyses of platycodigenin and its derivatives. The bromolactone (IX) of I was prepared to determine the absolute structure by the X-ray crystallographic analysis.

In our preceding paper,[1] we reported the isolation of the sapogenins, platycodigenin (I) and polygalacic acid (II) from the roots of *Platycodon grandiflorum* A. DC. and discussed the configuration of the 16-hydroxyl group of the latter sapogenin (II) to revise it to α(axial). The present paper concerns the structure of platycodigenin (I).

Platycodigenin (I) $C_{30}H_{48}O_7$, mp 241—242°, $[\alpha]_D+25.3$ (pyridine), gives a positive Liebermann–Burchard reaction and tetranitromethane test. Infrared (IR) spectrum showed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1700 cm^{-1}) groups and the ultraviolet (UV) spectrum showed strong end-absorption. On treatment with diazomethane I afforded methyl ester (III), $C_{31}H_{50}O_7$, mp 246° (M^+ 534). Acetylation of the methyl ester (III) gave penta-acetate (IV). The IR spectrum of IV in carbon tetrachloride solution revealed the absence of hydroxyl group. The nuclear magnetic resonance (NMR) spectrum of IV indicated the presence of five tertiary methyl, one carbomethoxyl and five acetyl groups, suggesting that platycodigenin (I) is a pentahydroxy-monocarboxylic acid. In the low-field region IV exhibited a pair of AB-doublets (2H, δ 4.04, 4.26, $J=12$ Hz) and a broad singlet (2H, δ 4.55) indicating the presence of two primary acetoxylmethylene groups.

Table I. Nuclear Magnetic Resonance Spectral Data of IV, VII, and V

Compounds	-C-C$\underline{H}_3$	-OCOC$\underline{H}_3$	-COOC$\underline{H}_3$	-C$\underline{H}_2$OAc	2α-H	3α-H	16β-H	12-H
VII	0.74 (3H) 0.94 (3H) 0.99 (3H) 1.04 (3H) 1.22 (6H)	2.00 (3H) 2.06 (6H) 2.09 (3H)	3.64 (3H)	3.75 (2H, bs)	5.36[a]	4.92 (d, $J=4$)	5.65 (bs)	5.36[a]
IV	0.73 (3H) 0.93 (3H) 0.98 (3H) 1.22 (6H)	1.99 (3H) 2.05 (3H) 2.08 (9H)	3.63 (3H)	4.26, 4.04 (2H, ABd, $J=12$) 4.55 (2H, bs)	5.44[a]	5.05 (d, $J=4$)	5.67 (bs)	5.44[a]
V	0.71 (3H) 0.88 (3H) 0.94 (3H) 1.31 (6H)	2.01 (3H) 2.05 (6H)	3.58 (3H)	4.01, 4.21 (2H, ABd, $J=12.5$) 4.55, 4.74 (2H, AB d, $J=10$)	4.15[a]	4.96 (d, $J=3$)	4.49[a]	5.38 (bs)

The spectra were determined in $CDCl_3$ with tetramethylsilane as an internal standard at 100 MHz.
Unless otherwise indicated, all signals are singlets. In other case bs=broad singlet, d=doublet and AB d=AB type doublets and coupling constants J are given in Hz. Chemical shifts are on δ scale.
a) was overlapped.

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1) Part XXX: T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1945 (1972).
2) Preliminary report: T. Akiyama, Y. Iitaka, and O. Tanaka, *Tetrahedron Letters*, **1969**, 5577.
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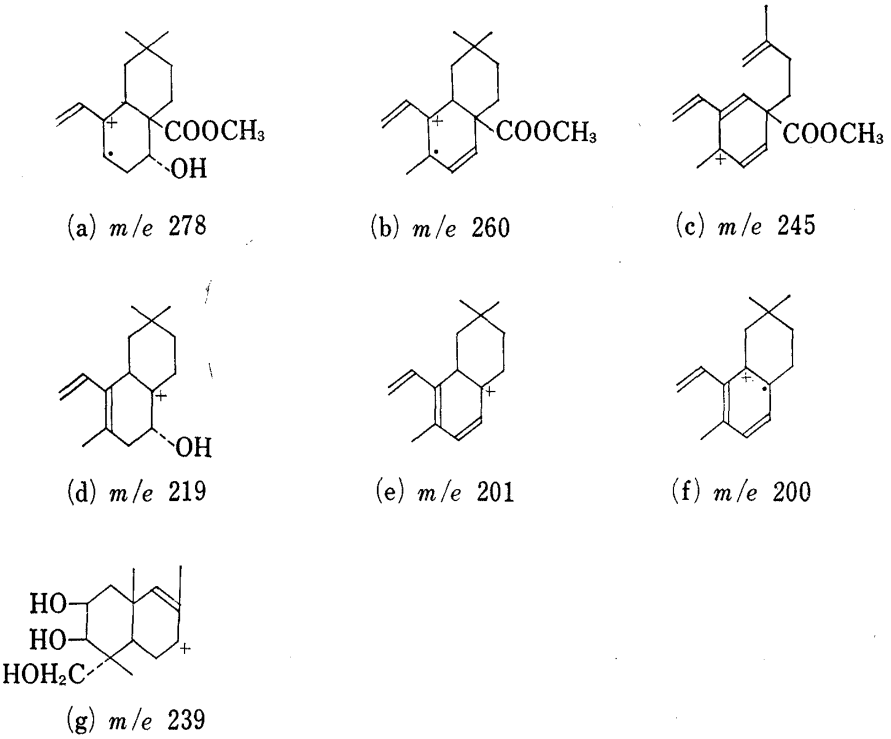
Table II. Mass Spectral Data of IV, VII, and V

VI		III		V		
m/e	Relative intensity	*m/e*	Relative intensity	*m/e*	Relative intensity	Assignment
518		534		660		M+
278	13	278	10	278	9.5	a
261	20	261	20	261	19	
260	85	260	86	260	68	b
		247	10	247	32	
245	16	245	16	245	20	c
239	18.5					g
		238	17			
219	14	219	15	219	15	d
		207	16			
203	19	203	21	203	20.5	
202	21	202	23	202	24.5	
201	100	201	100	201	100	e
200	23	200	24	200	24.5	f



(a) *m/e* 278 (b) *m/e* 260 (c) *m/e* 245

(d) *m/e* 219 (e) *m/e* 201 (f) *m/e* 200

(g) *m/e* 239

Chart 1

Comparative studies on NMR (Table I) and mass (Table II) spectra of the corresponding derivatives of platycodigenin (I) and polygalacic acid (II) clearly showed a close relationship between the structures of these sapogenins. As summarized in Table I, the NMR spectrum of methyl ester of penta-O-acetyl platycodigenin (IV) resembles very closely that of methyl tetra-O-acetyl polygalacate (VII), except one methyl signal at δ 1.04 observed in that of VII which is replaced by acetoxymethylene group in that of IV. Referring to the studies on the retro Diels-Alder cleavage of the olean-12-en type triterpenes,[4] the peaks of the mass spectrum of methyl polygalacate (VI) (Table II) at *m/e* 278, 260, 245, 219, 201 (base peak), and

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[4] H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963); J. Karliner and C. Djerassi, *J. Org. Chem.*, **31**, 1945 (1966).

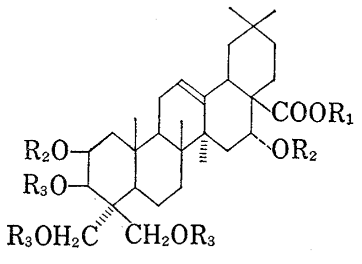
200 can be assigned to the fragments a—f (arising from D and E rings, respectively) (Chart 1). The mass spectrum of platycodigenin methyl ester (III) also showed all these peaks with the similar relative intensities to those of VI. However, it differs significantly from the spectrum of VI in the absence of the prominent peak at *m/e* 239 (g: arising from A and B rings) observed in the spectrum of VI. These evidences suggested that platycodigenin (I) is a member of olean-12-en type triterpenes and would be an analogue of polygalacic acid (II), having an additional primary hydroxyl group in A or B ring.

In order to determine the structure and the stereochemistry of platycodigenin (I), an X-ray crystallographic study was carried out using a derivative of this substance. As in the case of the known olean-12-en-28-oic acid type triterpenes,[5] platycodigenin (I) yielded a bromolactone (IX), mp 203—206°, by the action of Br_2-NaOAc in acetic acid.

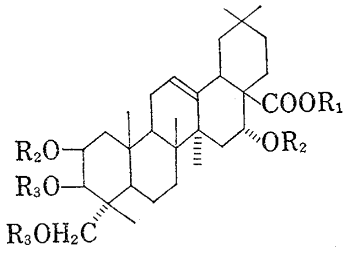
The X-ray crystallographic analysis of this bromolactone (IX) revealed the structure (IX) with its absolute configuration.[6] The structure of platycodigenin (I) has, therefore, been established as $2\beta,3\beta,16\alpha,23,24$-pentahydroxyolean-12-en-28-oic acid, which is the first example of the naturally occurring triterpene having geminal hydroxymethyl groups at $C_{(4)}$ in the A-ring.

During the determination of the crystal structure it was found that the crystal of IX contains one equivalent mole of benzene as the solvent of crystallization.

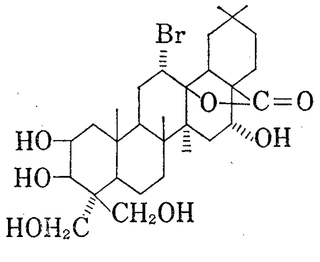
On standing in the air benzene was lost gradually from the crystal to form a white polycrystalline aggregate, though the external shape of the crystal was retained. The NMR spectrum of IX dried overnight at room temperature *in vacuo* was measured in d_6-DMSO solution to reveal the presence of a half equivalent mole of benzene. The analytical data could be interpreted in terms of hemi-benzene solvate. However, the density measured by the flotation method in a mixture of benzene and carbon tetrachloride (1.359 g. cm^{-3}) was in good agreement with the value calculated for $C_{30}H_{47}O_7Br \cdot C_6H_6$ (1.364 g. cm^{-3}). A distinct feature of crystal structure which is useful for the discussion of this observation has been



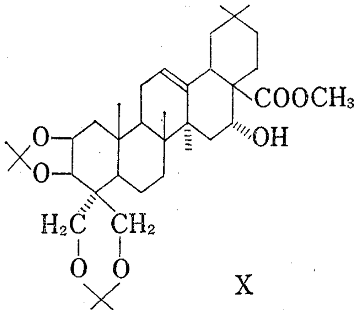
I: $R_1=R_2=R_3=H$; platycodigenin
III: $R_1=CH_3, R_2=R_3=H$
IV: $R_1=CH_3$, $R_2=R_3=Ac$
V: $R_1=CH_3$, $R_2=H$, $R_3=Ac$



II: $R_1=R_2=R_3=H$; polygalacic acid
VI: $R_1=CH_3$, $R_2=R_3=H$
VII: $R_1=CH_3$, $R_2=R_3=Ac$
VIII: $R_1=CH_3$, $R_2=H$, $R_3=Ac$



IX



X

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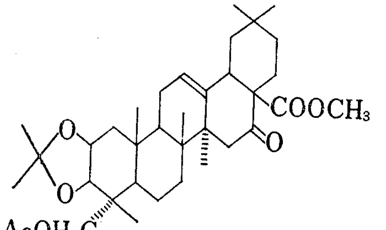
5) For example, E.J. Corey, and J.J. Ursprung, *J. Am. Chem. Soc.*, **78**, 183 (1956).
6) T. Akiyama, O. Tanaka, and Y. Iitaka, *Acta Cyrst.*, **B 26**, 163 (1970).

reported as well.⁶⁾ Partial acetylation of platycodigenin methyl ester (III) with acetic anhydride and pyridine under cooling gave an acetate (V), mp 189—190°.

The mass (Table II) and NMR (Table I) spectra were consistent with 3β,23,24-tri-O-acetyl platycodigenin methyl ester (V), which corresponds to methyl 3β,23-di-O-acetyl polygalacate (VIII).¹⁾

Treatment of the methyl ester (III) with acetone and *p*-toluenesulphonic acid yielded an amorphous isopropylidene derivative (X). The NMR spectrum (Table III) of X exhibited the presence of 9 C-CH_3 or O-CH_3 groups by the signals in δ 0.7—1.55 region, which suggests X is a diacetonide. By the comparative studies on the NMR spectra of the derivatives of platycodigenin (I) and polygalacic acid (II) the signals of X in the low field were assigned as shown in Table III. A distinct feature of the coupling pattern is the triplet nature of the signal assigned to 2α-proton.

The proton of 2,3-O,O-isopropylidene-23-O-acetyl-16-keto derivative (XI) of methyl polygalacate¹⁾ also showed the well separated signal⁷⁾ at δ 4.38 (1H, triplet, $J=7$ Hz). Examination of the molecular model of XI indicated the distortion of A-ring caused by the formation of the acetonide, which led to the dihedral angles H_α-C_1-C_2-H_α, H_β-C_1-C_2-H_α and H_α-C_2-C_3-H_α, approximately 30°, 90° and 30°, respectively (see Fig. 1). Application of Karplus' equation to these values afforded coupling constants, $J{=}_{2,3}7$ Hz, $J_{2,1\beta}=0$ Hz and $J_{2,1\beta}=$ 7 Hz, consistent with the experimental values. Considering this result, the triplet nature of the NMR signal due to 2α proton of X suggests the presence of 2,3-O,O-isopropylidene linkage. Consequently, the structure of X should be represented as 2β,3β,23,24-di-O,O-isopropylidene platycodigenin methyl ester. The structure of X was supported by the IR spectrum in carbon tetrachloride solution in which X exhibited strong bands as 1725, 1740 and 3620 cm^{-1}, showing no intramolecular hydrogen bonding between hydroxyl and carbonyl group.



XI

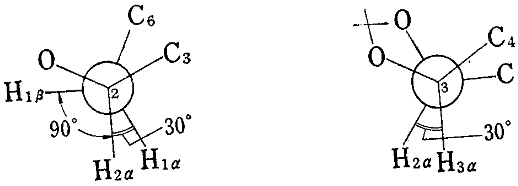


Fig. 1

Table III. Nuclear Magnetic Spectral Data of X

$-\dot{C}-C\underline{H}_3$ or $-O-C\underline{H}_3$	$-COOC\underline{H}_3$	$-C\underline{H}_2-O-$	2α-H	3α-H	16β-H	12-H
0.75 (3H)	3.61 (3H)	3.53 (2H bs)	4.35	4.02$^{a)}$	4.52	5.43
0.90 (3H)		3.89, 4.11$^{a)}$	(t, $J=6$)	(d, $J=6$)	(bs)	(bs)
0.97 (3H)		(2H, AB d, $J=12$)				
1.06 (3H)						
1.34 (3H)						
1.37 (9H)						
1.53 (3H)						

determined in $CDCl_3$ (δ ppm)
Unless otherwise indicated, all signals are singlets. In other case bs=broad singlet, d=doublet, ABd=AB type doublets and t=triplet, and coupling constants J are given in Hz.
a) was overlapped.

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7) In other derivatives of polygalacic acid, the overlapping of signal of 2α-proton with that of 16β-proton resulted in an ambiguous pattern of coupling.

After our preliminary communication was published, Kubota, *et al.* reported the evidences which suggested the structure (I) to platycodigenin.[8] Recently, they reported the structures of minor sapogenins, platycogenic acids A, B and C, isolated from the roots of *Platiycodon grandiflorum.*[9]

Experimental

Penta-O-acetyl Platycodigenin Methyl Ester (IV)——Platycodigenin methyl ester (III) (250 mg) was acetylated with acetic anhydride (2.5 ml) and pyridine (5 ml) at 40° for 2 days. The crude product was purified by chromatography over silica gel in chloroform to give amorphous pentaacetate (IV), which exhibited a single spot on TLC developed with $CHCl_3$–ether (20:1), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750 (C=O), no OH band.

Platycodigenin Bromolactone——A solution of platycodigenin (I) (250 mg) and NaOAc (1 g) in acetic acid (10 ml) was stirred during the dropwise addition of 3% bromine in acetic acid. The bromine was absorbed immediately and was remained in excess after the completion of the addition. The mixture was poured into aqueous sodium thiosulphate, and then extracted with ethyl acetate. The product obtained on evaporation of the solvent was crystallized from benzene–EtOH mixture to yield the bromolactone (IX), mp 203—206° (250 mg), $[\alpha]_D^{30}$ +45.9° (pyridine). *Anal.* Calcd. for $C_{30}H_{47}O_7Br$: C, 60.09; H, 7.90. Calcd. for $C_{30}H_{47}O_7Br \cdot C_6H_6$: C, 63.80; H, 7.88. Found: C, 60.79; H, 7.77; C, 61.01; H, 7.83. IR ν_{max}^{KBr} cm^{-1}: 1760 (γ-lactone).

Tri-O-acetyl Platycodigenin Methyl Ester (V)——Platycodigenin methyl ester (III) (200 mg) was acetylated with Ac_2O (4 ml) and pyridine (4 ml) under ice-cooling for 4 hr. The mixture was poured into water and the precipitates were collected by filtration, washed and dried. The product was chromatographed over silica gel (10 g) eluting with benzene–$CHCl_3$ (7:3—5:5). Crystallization from $CHCl_3$–hexane yielded triacetate (90 mg) (V), mp 189—190°, $[\alpha]_D^{15}$ +47.2° ($CHCl_3$). *Anal.* Calcd. for $C_{37}H_{56}O_{10}$: C, 67.25; H, 8.54. Found: C, 67.09; H, 8.49. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (-OH), 3540 (-OH), 1740 (C=O).

2β,3β,23,24-Di-O,O-isopropylideneplatycodigenin Methyl Ester (X)——A solution of III (100 mg), and *p*-toluenesulphonic acid monohydrate (20 mg) in dry acetone (20 ml) was stirred continuously at room temperature for 1 hr. After adding K_2CO_3 (20 mg) under cooling, the solution was concentrated into a syrup which was diluted with water. The white precipitates were collected by filtration, washed with water and dried. A benzene solution of the product was chromatographed over silica gel (10 g) eluted with benzene–$CHCl_3$ (7:3—6:4). The eluates gave an amorphous substance (X) on evaporation, which showed a single spot on TLC (solvent: $CHCl_3$–ether 20:1), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (-OH), 1740, 1725 (C=O).

Acknowledgement The authors are grateful to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University for the determination of mass spectra.

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8) T. Kubota and H. Kitatani, *Chem. Commun.*, **1969**, 190.
9) T. Kubota, H. Kitatani, and H. Hinoh, *Chem. Commun.*, **1969**, 1313.