[Chem. Pharm. Bull. 23(11)2965—2972(1975)]

UDC 547.918.02.05 : 581.192

Studies on the Saponins of the Root of *Platycodon grandiflorum* A. De Candolle. I. Isolation and the Structure of Platycodin-D*

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(Received April 3, 1975)

The chemical structure of platycodin-D, $C_{57}H_{92}O_{28}$, $[\alpha]_D^{21}-32.3°$ (MeOH), which was isolated from platycodi radix (root of *Platycodon grandiflorum* A. DC.) was elucidated as I on the basis of physical data of platycodin-D, its derivatives and degradation products.

It should be noted that platycodin-D is the first example of saponin containing apiose in the sugar moiety.

The saponin and sapogenin of a Chinese drug "Jieseng" (桔梗, Japanese name: Kikyo), the roots of *Platycodon grandiflorum* A. DC. (Campanulaceae) were studied earlier by Tsujimoto[2] and later by Yamaguchi, *et al.*,[3] but little was known about their structures.

Recently Kubota, *et al.*[4] and Shibata, *et al.*[5] have reported the isolation and the structure elucidation of major sapogenin, polygalacic acid and platycodigenin, independently. Furthermore, Kubota, *et al.* reported the structures of minor sapogenins, platycogenic acid A, B and C, isolated from the roots of *Platycodon grandiflorum.*[4c] Elyakov, *et al.*[6] have reported the isolation of one of the saponins, named platycodiside C, from the same source. Platycodiside C has been suggested to be composed of platycodigenin and the carbohydrate part (glucose: xylose: rhamnose: arabinose=2: 1: 1: 1), but the complete structure has not been established. The present paper describes the isolation and the structure elucidation of the main saponin named platycodin-D (I). The conclusion obtained in our study is quite different from that of Elyakov, *et al.*, but the physical properties of both saponins resemble each other as described in experimental part.

The roots of *Platycodon grandiflorum* A. De Candolle were extracted with hot methanol and the extract was fractionated by the method reported by Akiyama, *et al.*[5a] as shown in Chart 1. The thin-layer chromatograms (TLC) of crude saponin on Silica gel H are shown in Fig. 1 and nine saponins were named platycodin-A to I in order of increasing polarity. The saponin mixture was separated by chromatography over silica gel using chloroform–methanol–water (65: 35: 10, the lower phase), and the platycodin-D rich fraction was rechromatographed over silica gel using *n*-butanol–ethyl acetate–water (4: 1: 2, the upper layer) to obtain a chromatographically pure saponin, platycodin-D.

Platycodin-D (I), $C_{57}H_{92}O_{28}$, $[\alpha]_D^{21}$ $-32.3°$ (in methanol), forms a white powder from ethanol–ethyl acetate, whose infrared (IR) spectrum indicates the presence of many hydroxyl groups (3500—3300 cm^{-1}) and an ester group (1725 cm^{-1}).

* Dedicated to the memory of Prof. Eiji Ochiai.

1) Location: *a) Hatanodai 1-5-8, Shinagawa-ku, Tokyo; b) Hongo, Tokyo.*

2) M. Tsujimoto, *J. Agric. Chem. Soc. Japan*, **16**, 613 (1940) and reference cited therein.

3) K. Yamaguchi, M. Ito, M. Nishimoto, and S. Natori, *Shoyakugaku Zasshi*, **18**, 12 (1964).

4) *a)* T. Kubota and H. Kitatani, *Chem. Commun.*, **1968**, 1005; *b)* T. Kubota and H. Kitatani, *Chem. Commun.*, **1969**, 190; *c)* T. Kubota, H. Kiatatani, and H. Hinoh, *Chem. Commun.*, **1969**, 1313.

5) *a)* T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1945 (1972); *b)* T. Akiyama, Y. Iitaka, and O. Tanaka, *Tetrahedron Letters*, **1969**, 5577; *c)* T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1952 (1972); *d)* T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1957 (1972).

6) G.B. Elyakov and N.G. Aladjina, *Tetrahedron Letters*, **1972**, 3651.

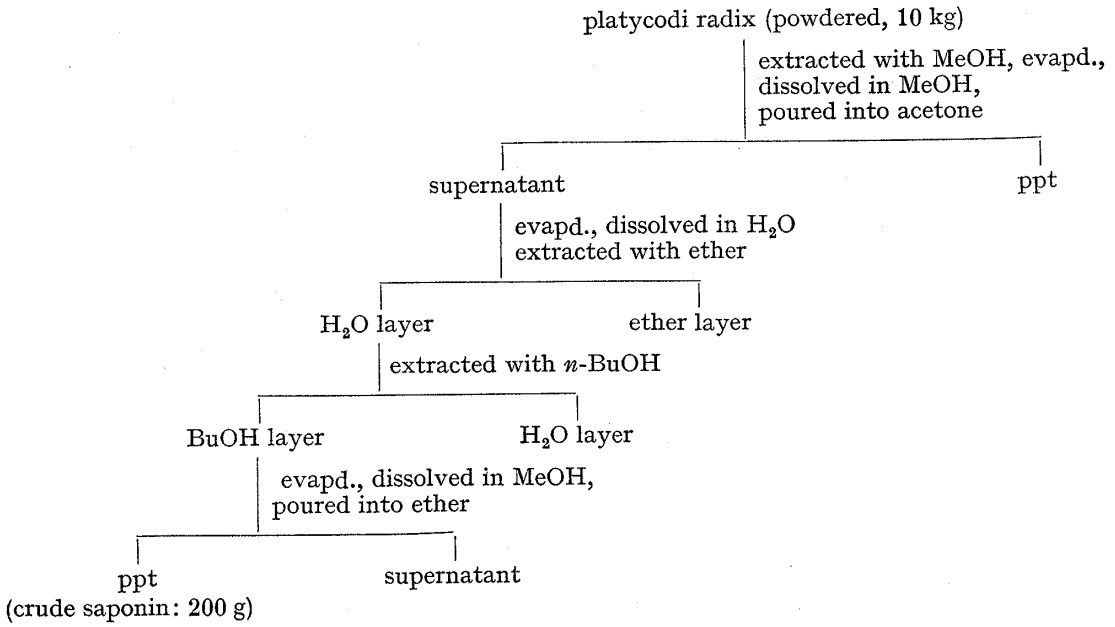


Chart 1. Extraction of Crude Saponin from Platycodi Radix

After hydrolysis of I with 4N hydrogen chloride–dioxane–benzene (3:1:2), the hydrolysate in aqueous layer was examined by partition paper chromatography (PPC) and gas liquid chromatography (GLC) to prove the occurrence of glucose, arabinose, rhamnose, xylose and apiose,[7] while TLC of the organic layer showed the presence of several kinds of aglycones. As was pointed out by Elyakov, *et al.*,[6] hydrolysis of I by the condition using hydrogen chloride is unsuitable to obtain a single aglycone, so that the hydrolysis of I with 8% sulfuric acid and ethyl alcohol (1:1 v/v) was carried out to afford platycodigenin (III), $C_{30}H_{48}O_7$, colorless needles, mp 241—243°, which was identified with the authentic sample.[5] In this case, the occurrence of some by-products was also detected by TLC. To establish the genuine aglycone, I was degradated by Smith oxidation and only platycodigenin (III) was obtained. Consequently, I was suggested to be a glycoside consisting of platycodigenin, glucose, arabinose, rhamnose, xylose and apiose. The presence of apiose in the sugar moiety of platycodin-D is noted, whose identification will be described later.

Hydrolysis of I with 5% potassium hydroxide in ethanol afforded a prosapogenin, $C_{36}H_{58}O_{12}$, mp 282—285° (decomp.), colorless needles, $[\alpha]_D^{22}$ +29.6° (in pyridine), (IV), which was identified with the authentic sample of 3-O-β-glucopyranosyl platycodigenin reported by Akiyama, *et al.*[5d] by a mixed fusion, IR spectrum and TLC.

I was methylated by the Hakomori's method to afford per-O-methyl ether of I, $C_{74}H_{126}O_{28}$, $[\alpha]_D^{18}$ −50.5° (in chloroform), (V), which shows no hydroxyl absorption band in the IR spectrum. The NMR spectrum of V shows the presence of six methyl groups (δ=0.75 (s., C$\underline{H}_3$), 0.87 (s., C$\underline{H}_3$), 0.96 (s., C$\underline{H}_3$), 1.22—1.26 (broad s., 3×C$\underline{H}_3$), seventeen O-methyl groups (δ=3.19—3.65), five anomeric protons (δ=4.28 (d., J=7 Hz), 4.62 (d., J=8 Hz), 4.99 (broad s.), 5.48 (d., J=2 Hz), 5.86 (broad s.)) and an olefinic proton (δ=5.40 (broad s.)).

On methanolysis with 3N hydrogen chloride in dried methanol V gave methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3-di-O-methyl-L-rhamnopyranoside, methyl 2,4-di-O-methyl-D-xylopyranoside, methyl 3,4-di-O-methyl-L-arabinopyranoside and methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI). The O-methylated monosaccharides described

7) J. Stanek, M. Cerny, J. Kocourek, and J. Pacak, "The Monosaccharides," Academic Press, New York and London, 1963, p. 83.
8) D.H. Ball, F.H. Bissett, I.L. Klundt, and L. Long, Jr., *Carbohyd. Res.*, **17**, 165 (1971); J. Medicino and R. Hanna, *J. Biol. Chem.*, **245**, 6113 (1970).

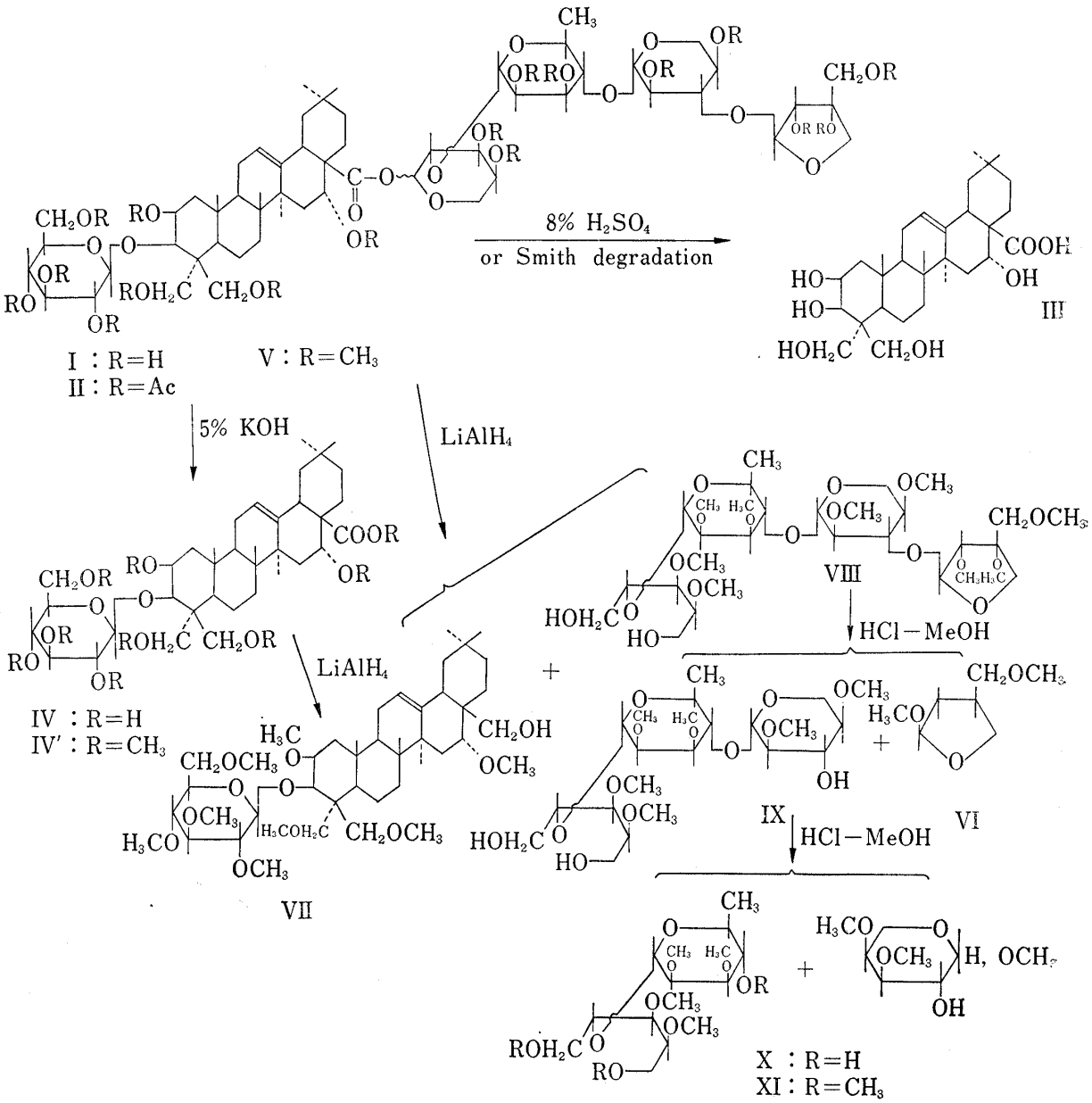
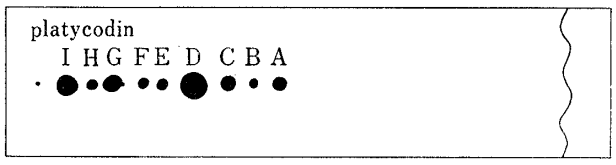


Chart 2



solvent: $CHCl_3$: MeOH : H_2O=65 : 35 : 10
the lower phase
plate : Kieselgel H
color reagent: 10% H_2SO_4

Fig. 1. Thin-layer Chromatogram of Crude Saponin

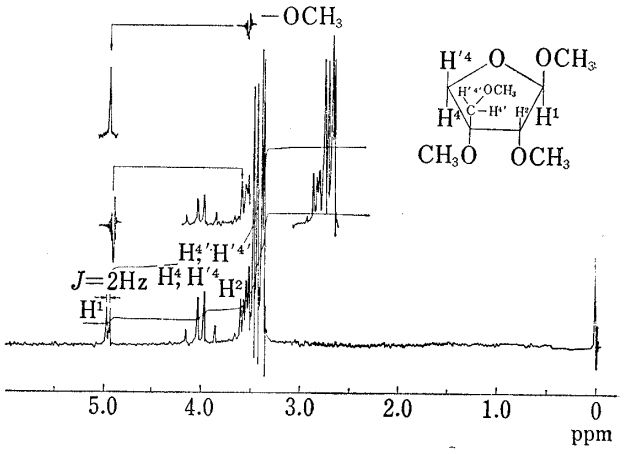


Fig. 2. NMR Spectrum of Compound VI (Per-O-methylapiose) (in $CDCl_3$)

above were isolated by column chromatography and identified by TLC and GLC with the authentic samples. Furthermore, each methyl glycoside was hydrolyzed with aqueous 1N sulfuric acid, and the purified O-methylsugars were examined their optical properties. Compound VI, syrup, $[\alpha]_D^{25}$ −72.9° (in chloroform), shows no hydroxyl absorption in the IR spectrum, while the nuclear magnetic resonance (NMR) spectrum reveals the signals of four O-methyl groups (δ=3.36, 3.38, 3.42, 3.46), two tertiary methylene groups adjacent to oxygen function (δ=3.52 (*ca.* 1H) and 3.55 (*ca.* 1H) partially overlapped with other signals; δ=3.91 (1H, d., J=10 Hz) and 4.09 (1H, d., J=10 Hz)), one anomeric proton (δ=4.97 (d., J=2 Hz) and one methine proton (δ=3.58 (d., J=2 Hz)) coupled with anomeric proton. Based on the physical properties, VI was assumed to be methyl 2,3,5-tri-O-methylapiofuranoside[8] and identified by comparing with an authentic sample prepared from apiin, a flavonoid glycoside of pasely.[9]

On reduction with lithium aluminium hydride in ether, V afforded compound VII, $C_{44}H_{76}O_{11}$, $[\alpha]_D^{21}$ +21.1° (in chloroform), from the ether extract of the reaction mixture, and compound VIII, $C_{30}H_{56}O_{17}$, syrup, $[\alpha]_D^{21}$ −81.8° (in chloroform), from the chloroform extract. The NMR spectrum of VII shows the presence of five C-methyl groups, eight O-methyl groups, one anomeric proton and one vinyl proton, while the IR spectrum indicates the absence of carbonyl group in this compound. Methanolysis of VII with 3N hydrogen chloride in dried methanol gave methyl 2,3,4,6-tetra-O-methylglucopyranoside and an aglycone. Taking account of the foregoing alkaline degradation of I affording platycodigenin-3-O-β-D-glucopyranoside, VII was deduced to be 2β,16α,23,24-tetramethoxyolean-12-ene-3β,28-diol (3)-O-(tetra-O-methyl)-β-D-glucopyranoside, and identified with a synthetic sample prepared from per-O-methylplatycodigenin-3-O-β-D-glucopyranoside.[5d] Consequently, the oligosaccharide moiety of I was suggested to be linked to 28-carboxyl group of platycodigenin in ester form.

The another product, VIII, shows the presence of one secondary methyl group (δ=1.28, 3H (d., J=6 Hz), nine O-methyl groups (δ=3.38—3.58) and three anomeric protons (δ=4.62 1H (d., J=8 Hz), 4.98 1H (broad s.), 5.46 (1H (d., J=2 Hz)) in the NMR spectrum. On methanolysis with 0.2N hydrogen chloride in methanol VIII gave methyl tri-O-methyl-D-apiofuranoside and compound IX, $C_{22}H_{42}O_{13}$, syrup, $[\alpha]_D^{25}$ −55.8° (in chloroform). The NMR spectrum of IX shows the presence of one secondary methyl group (δ=1.28 (d., J=6 Hz)), six O-methyl groups (δ=3.41—3.57) and two anomeric protons (δ=4.64 1H (d., J=8 Hz), 4.98 1H (broad s.)). On methanolysis with 3N hydrogen chloride in methanol IX gave methyl 2,3-di-O-methyl-L-rhamnopyranoside, methyl 2,4-di-O-methyl-D-xylopyranoside and 3,4-di-O-methyl-L-arabitol, while methanolysis with 0.5N hydrogen chloride in methanol at room temperature afforded a partially methanolyzed product, compound X, $C_{15}H_{30}O_9$, syrup, $[\alpha]_D^{30}$ −24.0° (in chloroform). The NMR spectrum of X shows the presence of one secondary methyl group (δ=1.30 (d., J=6 Hz), four O-methyl groups (δ=3.38—3.46) and one anomeric proton (δ=4.98, broad s.). X was methylated by the Hakomori's method to form compound XI, $C_{18}H_{36}O_9$, $[\alpha]_D^{27}$ −34.6° (in chloroform), syrup, which shows the presence of one secondary methyl group (δ=1.24 (d., J=6 Hz), seven O-methyl groups (δ=3.26—3.48) and one anomeric proton (δ=4.92 (broad s.)). XI was methanolyzed with 2N hydrogen chloride in methanol to afford methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and 1,3,4,5-tetra-O-methyl-L-arabitol, $C_9H_{20}O_5$, $[\alpha]_D^{28}$ +15.4° (in chloroform), syrup.

Therefore the structure of VIII is established to be 2,3,5-tri-O-methyl-D-apiofuranosyl(1→3)-2,4-di-O-methyl-D-xylopyranosyl(1→4)-2,3-di-O-methyl-L-rhamnopyranosyl(1→2)-3,4-di-O-methyl-L-arabitol. The configuration of each sugar was deduced as follows. Molecular rotation difference (−194°) between VIII and IX suggests[10] the β-configuration of 2,3,5-tri-O-methyl-D-apiofuranose unit ($[M]_D$ of methyl 2,3,5-tri-O-methyl-α- and β-D-apiofuranoside[8]

9) R.K. Hulyalker, J.K.N. Jones and M.B. Perry, *Canad. J. Chem.*, **43**, 2085 (1965).
10) W. Klyne, *Biochem. J.*, **47**, xli (1950).

+239° and −163°, respectively), while the configuration of 2,4-di-O-methyl-D-xylopyranose is revealed to be β-form by the coupling constant (J=8 Hz) of anomeric proton signal in NMR spectrum of VIII, and molecular rotation difference (−202°) between IX and X supports the β-configuration of 2,4-di-O-methyl-D-xylopyranose unit ($[M]_D$ of methyl 2,4-di-O-methyl-β-D-xylopyranoside, −158°[11]). Furthermore, molecular rotation difference (−169°) between XI and 1,3,4,5-tetra-O-methyl-L-arabitol suggests the α-configuration of 2,3,4-tri-O-methyl-L-rhamnopyranose unit ($[M]_D$ of methyl 2,3,4-tri-O-methyl-α-[12a] and β-L-rhamnopyranoside,[12b] −33.2° and +233.2°, respectively).

Finally, the configuration of L-arabinose has not been determined because the signal of the anomeric proton of arabinose in the NMR spectrum shows a broad signal, and prosapogenin composed of platycodigenin and L-arabinose has not been obtained. The enzymatic hydrolysis of I to obtain this prosapogenin is under progress.

It should be noted that platycodin-D, a main saponin of *Platycodon grandiflorum* A. DC. is the first example of saponin containing apiose as a sugar component. The study on the structure of other platycodins will be reported in the near future.

Table I. Assignment of Configuration of Sugars

Apiose→xylose	NMR anomeric H	δ 5.46	J=2 Hz	β
	$[M]_D$·VIII−$[M]_D$·IX		−194°[a]	β
Xylose→rhamnose	NMR anomeric H	δ 4.62	J=8 Hz	β
	$[M]_D$·IX−$[M]_D$·X		−202°[b]	β
Rhamnose→arabinose	NMR anomeric H	δ 4.98	bs	α
	$[M]_D$·XI−$[M]_D$·TMA[c]		−169°[d]	α

a) methyl 2,3,5-tri-O-methyl-α-D-apiofuranoside $[M]_D$ +239°
methyl 2,3,5-tri-O-methyl-β-D-apiofuranoside $[M]_D$ −163°
b) methyl 2,4-di-O-methyl-β-D-xylopyranoside $[M]_D$ −158°
c) TMA=1,3,4,5-tetra-O-methyl-L-arabitol
d) methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside $[M]_D$ − 33.2°
methyl 2,3,4-tri-O-methyl-β-L-rhamnopyranoside $[M]_D$ +233.2°

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR absorption spectra were obtained with a Hitachi Model EPI-2. NMR spectra were measured with a Hitachi Model R-22 High Resolution NMR spectrometer (90 MHz) with tetramethylsilane (TMS) as an internal standard. Gas chromatography was run on a Hitachi Model K-53 with a hydrogen flame ionization detector.

Material——The dried root of *Platycodon grandiflorum* A. De Candolle cultivated at Kemigawa Pharmaceutical Botanical Garden attached to the Faculty of Pharmaceutical Sciences, University of Tokyo was used.

Extraction and Isolation of Platycodin-D (I)——According to the method of Akiyama and Shibata[5a] 10 kg of crushed material was extracted with MeOH and the extract was fractionated as shown in Chart 1 to afford the crude saponin (yield: *ca*: 200 g). The crude saponin was examined by TLC on Silica gel H and revealed to consist of more than eight saponins named platycodin-A to I in order of increasing polarity (Fig. 1). The crude saponin was chromatographed over 1 kg of silica gel using $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase) as the developing solvent. The platycodin-D rich fraction was rechromatographed over silica gel using $CHCl_3$–MeOH–H_2O (7: 3: 1 the lower phase) to afford an almost pure platycodin-D (I) (yield, 0.20% on dried root basis).

Properties of I——Analytical sample, a white powder, (mp 230—233° (decomp.)), $[\alpha]_D^{21}$ −32.3° (c=1.55 MeOH), (*cf.* platycodiside C: mp 216—221°, $[\alpha]_D^{20}$ −34.6°),[6] was obtained by repeated reprecipitation from EtOH–AcOEt. *Anal.* Calcd. for $C_{57}H_{92}O_{28}\cdot 3/2H_2O$: C, 54.68; H, 7.59. Found: C, 54.51; H, 7.31. IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 1725 (COOR).

I Heptadecaacetate (II)——I (0.2 g) was dissolved in pyridine (5 ml) and Ac_2O (3 ml), and the solution was allowed to stand for 72 hr at room temperature. The reaction mixture was worked up as usual and the product was purified by reprecipitation from EtOH to give II as a white powder, (mp 161—163°), $[\alpha]_D^{21}$ −39.9°

11) G.J. Robertson and T.H. Speedie, *J. Chem. Soc.*, **1934**, 824.
12) *a*) T. Purdie and C.R. Young, *J. Chem. Soc.*, **1906**, 1194; *b*) W.N. Haworth, E.L. Hirst, and E.J. Miller, *J. Chem. Soc.*, **1929**, 2469.

(c=1.9 $CHCl_3$). (*cf.* platycodiside C acetate: mp 147—149°, $[\alpha]_D^{21}$ −40.8°).[6] *Anal.* Calcd. for $C_{91}H_{126}O_{45}$: C, 56.34; H, 6.55. Found: C, 56.21; H, 6.55. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1740—1725 (COOR).

Hydrolysis of I with 4N HCl in Aqueous Dioxane——A solution of I (10 mg) in 4N HCl (3 ml)–dioxane (1 ml)–benzene (2 ml) was refluxed on the water bath for 4 hr. After cooling the reaction mixture was diluted with water (20 ml) and extracted with benzene (10 ml×3) and then with *n*-BuOH saturated with water. The organic layers were combined and evaporated *in vacuo*. The residue was examined by TLC. TLC (plate: Silica gel H; solvent: $CHCl_3$: MeOH: H_2O=65: 35: 10 the lower phase) *Rf* 0.82, 0.70 (platycodigenin), 0.62, 0.53, 0.41 (prosapogenin). The aqueous layer was neutralized with Ag_2CO_3 and evaporated *in vacuo*. The residue was found to be a mixture of glucose, arabinose, xylose, apiose and rhamnose by PPC and GLC. PPC: paper, Toyo Roshi No. 51; solvent, *n*-BuOH–AcOH–H_2O (4: 1: 5, the upper layer); color reagent, aniline hydrogen phthalate; *Rf* 0.08 (glucose), 0.13 (arabinose), 0.16 (xylose), 0.24 (apiose), 0.29 (rhamnose). GLC: column 5% SE-52 on Chromosorb W, 3 mm×2 m; column temperature 150°; injection temperature 250°; carrier gas N_2 flow 1.0 kg/cm²; sample TMS derivatives; t_R (min) 4.8, 5.5 (apiose), 5.5, 6.3 (arabinose), 5.7, 7.6 (rhamnose), 8.2, 10.0 (xylose), 17.4, 26.2 (glucose).

Hydrolysis of I with 4% H_2SO_4 in Aqueous EtOH——To the solution of I (100 mg) in EtOH (5 ml) was added 8% H_2SO_4 (5 ml) and the mixture was refluxed for 10 hr. The solution was concentrated to 5 ml under reduced pressure, and the precipitates formed were collected by filtration, washed with water and dried to give a brown solid which was examined by TLC. TLC (plate: Silica gel H; solvent: $CHCl_3$: MeOH: H_2O=7: 3: 1, the lower phase) *Rf* 0.13 (prosapogenin), 0.28 (minor), 0.31 (platycodigenin), 0.48 (minor). A brown solid was chromatographed over silica gel using AcOEt (saturated with water): MeOH=20: 1 as the developing solvent to afford a colorless solid. The analytical sample, mp 241—243°, $[\alpha]_D^{21}$ +36.6° (c= 1.5 pyridine) was obtained by repeated recrystallization from aqueous EtOH. *Anal.* Calcd. for $C_{30}H_{48}O_7 \cdot 1/2H_2O$: C, 67.99; H, 9.35. Found: C, 68.24; H, 9.29. This compound was found to be identical with platycodigenin (III) by comparing their IR spectra and TLC, and by a mixed fusion.

Degradation of I by Smith Oxidation——To the solution of I (500 mg) in 95% MeOH (150 ml) was added $NaIO_4$ (1.5 g). The solution was stirred for 24 hr at 2°. The formed precipitate was filtered off and the filtrate was concentrated *in vacuo*. To the concentrated solution was added water and the solution was extracted with *n*-BuOH saturated with water. The BuOH solution was washed with water and evaporated *in vacuo*. The residue (450 mg) was dissolved in 95% MeOH (50 ml) and $NaBH_4$ (350 mg) was added. After the solution was stirred for 2 hr at room temperature, the reaction mixture was neutralized with 5% AcOH and evaporated *in vacuo*. The residue was suspended in water and extracted with *n*-BuOH saturated with water. The BuOH solution was washed with water and evaporated *in vacuo* to afford the residue (200 mg) which was hydrolyzed with 0.05N H_2SO_4 in 50% MeOH (30 ml) by heating on a water bath for 30 min. The reaction mixture was diluted with water and evaporated *in vacuo*. The residual aqueous solution was extracted with *n*-BuOH saturated with water and the BuOH solution was washed with water and evaporated to dryness. The residue (130 mg) was repeatedly recrystallized from EtOH to afford colorless needles, mp 241—243°, which was identical with III by comparing their IR spectra and TLC and by a mixed fusion.

Hydrolysis of I with Potassium Hydroxide——A solution of I (200 mg) in 10% KOH (10 ml) and EtOH (10 ml) was heated under N_2 gas flow on a water bath for 8 hr. The reaction mixture was cooled at room temperature and neutralized with HCl. The neutral solution was extracted several times with *n*-BuOH and the organic layers were combined, washed with water and then evaporated to dryness. The residue (80 mg) was repeatedly recrystallized from EtOH to give colorless needles, mp 282—285°, $[\alpha]_D^{22}$ +29.6° (c=1.55 pyridine). *Anal.* Calcd. for $C_{36}H_{58}O_{12} \cdot H_2O$: C, 61.69; H, 8.63. Found: C, 61.70; H, 8.35. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH), which was found to be identical with the prosapogenin (platycodigenin 3-β-D-glucopyranoside)[5b] by comparing their IR spectra and TLC and by a mixed fusion.

Permethylation of I——According to the Hakomori's method, NaH (600 mg) was stirred with dimethylsulfoxide (DMSO 10 ml) at 70° for 45 min under N_2 gas flow. To this reagent I (600 mg) in DMSO (10 ml) was added and the mixture was stirred for 20 min at room temperature under N_2 gas flow. CH_3I (5 ml) was added and the reaction mixture was allowed to stand at room temperature for 6 hr with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated to afford a syrup (700 mg). The residue was chromatographed over 100 g of silica gel using *n*-hexane: acetone=2: 1 to afford a homogeneous per-O-methylplatycodin-D (V), which was repeatedly precipitated from *n*-hexane to give a white powder, (mp 120—123°), $[\alpha]_D^{18}$ −50.5° (c=2.0 $CHCl_3$). *Anal.* Calcd. for $C_{74}H_{126}O_{28}$: C, 60.74; H, 8.64. Found: C, 61.00; H, 8.64. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1725 (COOR). NMR (in $CDCl_3$) δ: 0.75 (3H, s., C<u>H</u>$_3$), 0.87 (3H, s., C<u>H</u>$_3$), 0.96 (3H, s., C<u>H</u>$_3$), 1.22—1.26 (3H×3, C<u>H</u>$_3$×3), 3.19—3.65 (3H×17, OC<u>H</u>$_3$×17), 4.28 (1H, d., J=7 Hz, anomeric <u>H</u>), 4.62 (1H, d., J=8 Hz, anomeric <u>H</u>), 4.99 (1H, broad s., anomeric <u>H</u>), 5.40 (1H, broad s., –CH=C<), 5.48 (1H, d., J=2 Hz, anomeric <u>H</u>), 5.86 (1H, broad s., anomeric <u>H</u>).

Methanolysis of V——A solution of V (200 mg) in methanolic 3N HCl (20 ml) was refluxed for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was dissolved in water. The water insoluble product was filtered off and the filtrate was evaporated *in vacuo* to afford a syrup which was fractionated by chromatography over silica gel using benzene–acetone

(4: 1—2: 1 gradient). The each O-methylsugar was identified with the authentic samples by TLC and GLC. TLC: i) solvent, AcOEt; *Rf* 0.46 (methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI)), 0.46, 0.44 (methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside), 0.32, 0.29 (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 0.22, 0.18 (methyl 2,4-di-O-methyl-D-xylopyranoside), 0.11, 0.07 (methyl 3,4-di-O-methyl-L-arabinopyranoside). ii) solvent, benzene–acetone (2: 1); *Rf* 0.47 (methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI)), 0.51, 0.42 (methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside), 0.27, 0.18 (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 0.27, 0.18 (methyl 2,4-di-O-methyl-D-xylopyranoside), 0.11, 0.08 (methyl 3,4-di-O-methyl-L-arabinopyranoside). GLC: column 5% NPGS on Chromosorb w, 3 mm×2 m; column temperature 150°; injection temperature 200°; carrier gas N_2 flow 0.8 kg/cm²; t_R (min) 2.8, 3.6 (methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI)), 6.4, 9.0 (methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside), 5.6, 7.8 (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 7.8, 9.0 (methyl 2,4-di-O-methyl-D-xylopyranoside), 9.2, 9.8 (methyl 3,4-di-O-methyl-L-arabinopyranoside).

A solution of each methyl O-methylglycoside was heated with aqueous 1N H_2SO_4 on a boiling water bath for 5 hr. After cooling at room temperature each solution was neutralized with Amberlite MB-3 and evaporated *in vacuo*. The residue was purified by preparative TLC (plate, Silica gel H; solvent, hexane: acetone=2: 1) and each O-methylsugar was examined optical property. *Rf* 0.14, $[\alpha]_D^{23}$ +120.0° (*c*=0.55 H_2O) (3,4-di-O-methyl-L-arabinose, lit.[13] $[\alpha]_D$ +104°, +116°, +125° in H_2O); *Rf* 0.26, $[\alpha]_D^{23}$ +21.0° (*c*=1.95 H_2O) (2,4-di-O-methyl-D-xylose, lit.[14] $[\alpha]_D$ +22°, +23° in H_2O); *Rf* 0.31, $[\alpha]_D^{23}$ +44.3° (*c*=1.85 H_2O) (2,3-di-O-methyl-L-rhamnose, lit.[15] $[\alpha]_D$ +47.6°, 42.5° in H_2O).

Reduction of V with Lithium Aluminium Hydride——To a solution of V (250 mg) in absolute ether (20 ml) was added 150 mg of $LiAlH_4$ and the mixture was refluxed for 2 hr. After the excess $LiAlH_4$ was decomposed with ethyl acetate the reaction mixture was poured into a large amount of water and the aqueous solution was extracted with ether and then with $CHCl_3$. The ether solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by repeated precipitation from *n*-hexane to afford a white powder, VII, (mp 91—93°), $[\alpha]_D^{21}$ +21.1° (*c*=1.8 $CHCl_3$). *Anal.* Calcd. for $C_{44}H_{76}O_{11}$: C, 67.67; H, 9.74. Found: C, 67.52; H, 9.45. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (nil). NMR (in $CDCl_3$) δ: 0.88 (3H, s., C$\underline{H}_3$), 0.92 (3H, s., C$\underline{H}_3$), 1.26 (3H×2, C$\underline{H}_3$×2), 1.29 (3H, s., C$\underline{H}_3$), 3.20—3.64 (3H×8, OC$\underline{H}_3$×8), 4.29 (1H, d., *J*=7 Hz, anomeric H), 5.26 (1H, broad s., –CH=C<). The compound was identified with 2β,16α,23,24-tetramethoxyolean-12-ene-3β,28-diol (3)-O-(tetra-O-methyl)-β-D-glucopyranoside derived from per-O-methyl-platycodigenin-3-O-β-D-glucopyranoside by comparing their melting points, TLC, IR spectra and NMR spectra.

The chloroform solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed over silica gel using AcOEt (saturated with water): MeOH=20: 1 to afford a syrup, VIII, $[\alpha]_D^{21}$ −81.8° (*c*=2.2 $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{56}O_{17}$: C, 52.33; H, 8.14. Found: C, 52.20; H, 8.04. NMR (in $CDCl_3$) δ: 1.28 (3H, d., *J*=6 Hz, C$\underline{H}_3$), 3.83—3.58 (3H×9, OC$\underline{H}_3$×9), 4.62 (1H, d., *J*= 8 Hz, anomeric H), 4.98 (1H, broad s., anomeric H), 5.46 (1H, d., *J*=2 Hz, anomeric H).

Partial Methanolysis of VIII——The solution of VIII (120 mg) in methanolic 0.2N HCl (12 ml) was allowed to stand for 48 hr at room temperature. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC (solvent: AcOEt saturated with water) to show the main three spots (*Rf* 0.03, 0.08, 0.71). The product corresponding to *Rf* 0.71 was isolated by chromatography over silica gel using AcOEt to afford methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI), a syrup, $[\alpha]_D^{25}$ −72.9° (*c*=1.7 $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil), NMR (in $CDCl_3$) δ: 3.36 (3H, s., OC$\underline{H}_3$), 3.38 (3H, s., OC$\underline{H}_3$), 3.42 (3H, s., OC$\underline{H}_3$), 3.46 (3H, s., OC$\underline{H}_3$), 3.52 and 3.55 (2H, C_4'-H,H', partially overlaped with OC$\underline{H}_3$ and C_2–H signals), 3.58 (1H, d., *J*=2 Hz, C_2–H), 3.91 (1H, d., *J*=10 Hz, C_4–H'), 4.09 (1H, d., *J*=10 Hz, C_4–H), 4.97 (1H, d., *J*=2 Hz, anomeric H). Methyl 2,3,5-tri-O-methyl-D-apiofuranoside (VI) was identified with an authentic sample obtained from per-O-methylapiin by comparing TLC and NMR spectra.

The products corresponding to *Rf* 0.03 and 0.08 were isolated by chromatography using AcOEt saturated with water to give VIII (30 mg) and another oligosaccharide (IX) (55 mg). IX was a syrup, $[\alpha]_D^{25}$ −55.8° (*c*=1.8 $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{42}O_{13}$: C, 51.35; H, 8.23. Found: C, 51.40; H, 8.18. NMR (in $CDCl_3$) δ: 1.28 (3H, d., *J*=6 Hz, C$\underline{H}_3$), 3.41—3.57 (3H×6, OC$\underline{H}_3$×6), 4.64 (1H, d., *J*=8 Hz, anomeric H), 4.98 (1H, broad s., anomeric H).

Methanolysis of IX——IX was refluxed with methanolic 3N HCl for 1 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC. TLC (solvent: AcOEt saturated with water) *Rf* 0.46, 0.22 (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 0.34 (methyl 2,4-di-O-methyl-D-xylopyranoside), 0.06 (3,4-di-O-methyl-L-arabitol).

Partial Methanolysis of IX——The solution of IX (53 mg) in methanolic 0.5N HCl (5 ml) was allowed to stand for 72 hr at room temperature. The reaction mixture was treated as described above. The residue was examined by TLC (solvent: AcOEt saturated with water) to show the main three spots (*Rf* 0.03 (IX),

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0.18 (X), 0.34 (methyl 2,4-di-O-methyl-D-xylopyranoside)). X was isolated by chromatography over silica gel using AcOEt saturated with water as a syrup, $[\alpha]_D^{30}$ −24.0° (c=1.0 $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{30}O_9$: C, 50.83; H, 8.53. Found: C, 51.16; H, 8.26. NMR (in $CDCl_3$) δ: 1.30 (3H, d., J=6 Hz), 3.38—3.46 (3H×4, $OC\underline{H}_3$×4), 4.98 (1H, broad s., anomeric H).

Methylation of X by the Hakomori's Method——According to the Hakomori's method, X was methylated and the reaction mixture was treated as usual. The product was examined by TLC (solvent: AcOEt saturated with water) to show the presence of per-O-methylated X (XI) (Rf 0.46) which was isolated by chromatography over silica gel using AcOEt saturated with water to afford a syrup, $[\alpha]_D^{27}$ −34.6° (c=0.62 $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{36}O_9$: C, 54.53; H, 9.15. Found: C, 54.33; H, 8.68. NMR (in $CDCl_3$) δ: 1.24 (3H, d., J=6 Hz, $C\underline{H}_3$), 3.26—3.48 (3H×7, OCH_3×7), 4.92 (1H, broad s., anometic H).

Methanolysis of XI——XI was refluxed with methanolic 2N HCl for 1 hr. The reaction mixture was treated as usual and the product was purified by chromatography over silica gel using AcOEt to afford methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and 1,3,4,5-tetra-O-methyl-L-arabitol. TLC (solvent: *n*-hexane: acetone=2: 1): Rf 0.48, 0.38 (methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside), 0.18 (1,3,4,5-tetra-O-methyl-L-arabitol). 1,3,4,5-Tetra-O-methyl-L-arabitol is a syrup, $[\alpha]_D^{28}$ +15.4° (c=0.65, $CHCl_3$).

Acknowledgement The authors express their gratitude to Dr. M. Goto, Chemical Research Laboratories, Takeda Chemical Industries, Ltd. for his kind supply of the materials. Thanks are also due to the members of Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analyses and NMR spectral measurement. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education which is gratefully acknowledged.