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Chemical Studies on the Oriental Plant Drugs. XXXII.[1] Sapogenins from the Roots of *Platycodon grandiflorum* A. De Candolle. (3). The Structure of a Prosapogenin, 3-O-β-Glucosylplatycodigenin

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A prosapogenin was obtained by the acid hydrolysis of saponin mixture isolated from the roots of *Platycodon grandiflorum* A. De Candolle.

The structure of the prosapogenin was established as 3-O-glucosylplatycodigenin. The same prosapogenin was also afforded by the hydrolysis of the saponin with alkali.

In our preceding paper,[1] we reported the structural elucidation of platycodigenin (I), the sapogenin isolated from the roots of *Platycodon grandiflorum* A. De Candolle. The present paper describes the structure of a prosapogenin obtained by the partial hydrolysis of the saponin.

The thin-layer chromatogram (TLC) of the crude hydrolysate of the saponin mixture indicated the presence of a significant amount of the more polar substances than platycodigenin (I), polygalacic acid (II) and some other sapogenins. The polar substances were produced in a good yield under milder conditions of acid hydrolysis, and separated by column chromatography to give compound (III), $C_{36}H_{58}O_{12}$, mp 274—275°, $[\alpha]_D^{20}$+30.1°. It showed strong end absorption in ultraviolet (UV) spectrum, and the absorptions indicating the presence of a carbonyl 1690 cm^{-1} and a hydroxyl 3400 cm^{-1} in the infrared (IR) spectrum. The nuclear magnetic resonance (NMR) spectrum of III revealed the presence of five tertiary methyl groups as shown in Table I. Methylation of III with diazomethane gave an amorphous methyl ester (IV).

In the NMR spectrum IV exhibited the presence of one carbomethoxyl group along with five tertiary methyl groups (Table I). Further treatment of prosapogenin (III) with diluted sulphuric acid-ethanol gave platycodigenin (I)[1] in a good yield.

The water soluble fraction of the reaction mixture was subjected to trimethylsilylation and analyzed by gas liquid chromatography (GLC) to show the presence of glucose. Furthermore, the sugar fraction was reduced with sodium borohydride to yield the corresponding cyclitol, which was identified to be sorbitol by means of GLC in the form of its tetrafluoroacetate.[3]

Table I. Nuclear Magnetic Resonance Spectral Data

	III	IV
-C-CH_3	0.96 (3H), 0.98 (3H), 1.12 (3H), 1.37 (3H) 1.66 (3H)	0.92 (3H), 1.00 (3H), 1.09 (3H), 1.53 (3H), 1.72 (3H)
-$COOCH_3$		3.69 (3H)

The spectra were determined in pyridine-d_5 with tetramethyl silane as an internal standard at 100 MHz. Chemical shifts are on the δ scale. All signals are singlets.

1) Part XXXI: T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1952 (1972).
2) Location: *Bunkyo-ku, Tokyo, Japan*; *a*) Present address: *Institute of Pharmaceutical Sciences, Medical School, Hiroshima University, Hiroshima.*
3) T. Imanari, Y. Arakawa, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1967 (1969).

The prosapogenin (III) afforded permethyl ether methyl ester (V) on repeated methylation by Hakomori's procedure[4] followed by purification with column chromatography. The IR spectrum of V in carbon tetrachloride solution showed the absence of hydroxyl group. The sugar moiety of the prosapogenin (III) was proved to be a monosaccharide since nine OCH_3 signals were observed in the NMR spectrum of V (Table II). On methanolysis of permethyl ether methyl ester (V), methyl tetra-O-methyl glucoside was afforded, which was detected by GLC. The β-glucoside linkage was defined by the coupling constant and chemical shift of the signal due to the anomeric proton (Table II).[5] All the above evidences showed that the prosapogenin (III) is mono-O-glucoside of platycodigenin (I).

Table II. Nuclear Magnetic Resonance Spectral Data (at 100 MHz) of Methyl Ester of Octa-O-methyl Prosapogenin (V)

	-C-C<u>H</u>$_3$	-O-C<u>H</u>$_3$	anomeric H	12-H
in $CDCl_3$	0.72 (3H) 0.89 (3H) 0.97 (3H) 1.28 (6H)	3.22 (3H), 3.38 (3H) 3.32 (3H), 3.53 (3H) 3.34 (3H), 3.61 (3H) 3.36 (3H), 3.65 (6H)	4.30 (d, J=7)	5.36 (bs)
in pyridine-d_5	0.89 (3H) 0.94 (3H) 1.02 (3H) 1.44 (3H) 1.51 (3H)	3.31 (6H), 3.56 (3H) 3.40 (3H), 3.65 (3H) 3.43 (3H), 3.68 (3H) 3.47 (3H), 3.72 (3H)	4.62 (d, J=7)	5.52 (bs)

Unless otherwise indicated, all signals are singlets. In other case bs=broad singlet and d=doublet and coupling constants J are given in Hz.

The position of linkage between platycodigenin (I) and glucose was determined as follows: The prosapogenin (III) afforded platycodigenin (I) by Smith's degradation[6] or by its de Mayo's modification.[7] This disclosed that one of the hydroxyl groups of the α-glycol

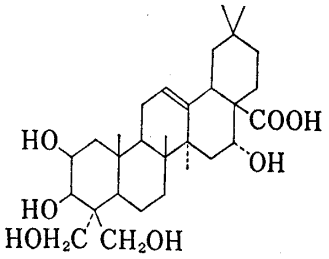
Table III. Nuclear Magnetic Resonance Spectral Data of VI, VII, and VIII

Compounds	C-C<u>H</u>$_3$	-OCOC<u>H</u>$_3$	-O-C<u>H</u>$_3$	-C<u>H</u>$_2$-O-	2α-H	3α-H	16β-H	12-H
VI	0.75 (3H) 0.92 (3H) 1.00 (3H) 1.26 (3H) 1.31 (3H)		3.22 (3H) 3.32 (3H) 3.38 (6H) 3.40 (3H) 3.62 (3H)	3.50, 4.15 (2H, AB d, J=10)	3.78 (bs)		3.96 (bs)	5.38 (bs)
VII	0.74 (3H) 0.92 (3H) 0.98 (3H) 1.25 (3H) 1.35 (3H)		3.22 (3H) 3.37 (6H) 3.39 (3H) 3.63 (3H)	3.48, 4.13 (2H, AB d, J=10)	3.77 (bs)	3.30 (d, J=4)	4.53 (bs)	5.42 (bs)
VIII	0.72 (3H) 0.88 (3H) 0.95 (3H) 1.23 (3H) 1.27 (3H)	2.10 (3H)	3.23 (3H) 3.30 (9H) 3.59 (3H)	3.47, 4.02[a] (2H, AB d, J=10)	3.68 (bs)	5.08 (d, J=4)	3.93[a] (bs)	5.34 (bs)

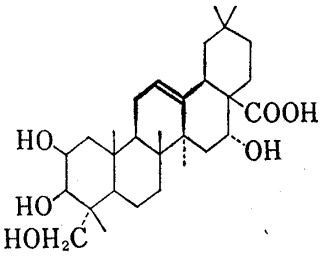
The spectra were determined in $CDCl_3$. Unless otherwise indicated, all signals are singlets.
In other case bs=broad singlet, d=doublet and AB d=AB type doublets and coupling constants J are given in Hz.
a) was overlapped.

4) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
5) J.M. van der Veen, *J. Org. Chem.*, **28**, 564 (1963).
6) F. Smith and A.M. Unran, *Chem. & Ind.*, **1959**, 881; I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, "Methods in Carbohydrate Chemistry" ed R.L. Whistler, Vol. V. Academic Press, N.Y. & London, 1956, p. 361.
7) J.J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).

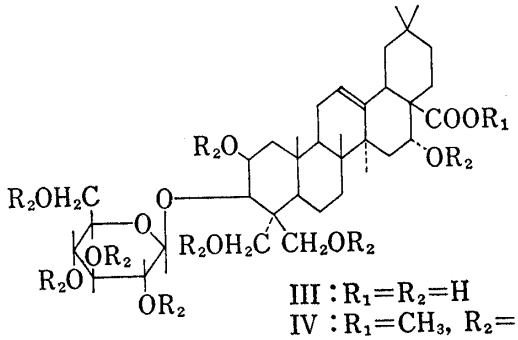
system of platycodigenin (I) at $C_{(2)}$ and $C_{(3)}$ should be protected by the glucoside likage. Methylation of platycodigenin (I) by Hakomori's procedure gave two kinds of O-methyl ether methyl ester, $C_{36}H_{60}O_7$, mp 187—188° (VI) and amorphous one (VII). In the IR spectrum in carbon tetrachloride solution VI showed the absence of hydroxyl group. The NMR spectrum of VI (Table III) exhibited six OCH_3 signals. Consequently, VI is 2β,3β,16α,23,24-penta-O-methylplatycodigenin. The NMR spectrum of VII (Table III) was compared with that of VI to find that a triplet-like signal attributed to 16β-proton in VI was shifted to the down field while five OCH_3 signals appeared. The IR spectrum of VII in carbon tetrachloride solution exhibited the presence of an isolated hydroxyl group. Therefore, this compound was formulated to be tetra-O-methylplatycodigenin methyl ester (VII).



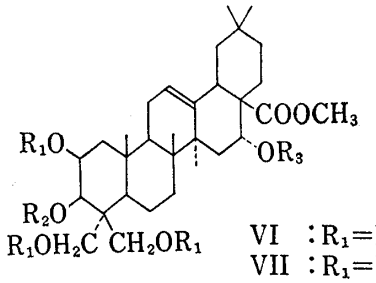
I: platycodigenin



II: polygalacic acid



III: $R_1=R_2=H$
IV: $R_1=CH_3$, $R_2=H$
V: $R_1=R_2=CH_3$



VI: $R_1=R_2=R_3=CH_3$
VII: $R_1=R_2=CH_3$, $R_3=H$
VIII: $R_1=R_3=CH_3$, $R_2=Ac$

Permethyl ether of prosapogenin methyl ester (V) was hydrolyzed by refluxing with dil. sulphuric acid-ethanol followed by acetylation to afford O-acetyl-tetra-O-methyl platycodigenin methyl ester (VIII), $C_{37}H_{60}O_8$, mp 163—164°. The NMR spectrum of VIII (Table III) exhibited the presence of five OCH_3 groups along with one acetoxymethyl group. VIII exhibited 3α-proton in the down-field (δ 5.08, doublet, $J=4$ Hz), while VI and VII showed doublet signals in the region of δ 3.0—3.5. Therefore, VIII has been proved to be 3β-O-acetyl-2β, 16α,23,24-tetra-O-methylplatycodigenin methyl ester. These evidences have led to formulate the prosapogenin as 3-O-β-glucosyl platycodigenin (III).

It should be noted that the prosapogenin (III) is also formed from the crude saponin on treatment with alkali indicating that the saponins of this plant contain those possessing ester linkage to combine the prosapogenin (III) with other part of the molecule.

Experimental

Isolation of Prosapogenin (III)——A solution of the saponin of *Platycodon grandiflorum* root (70 g) in EtOH (840 ml)–8% H_2SO_4 (840 ml) was refluxed on a boiling water bath. After 1 hr the solution was concentrated to a half volume, neutralized with Na_2CO_3, and extracted with BuOH (5 times; total 2.5 liter). The BuOH layer was washed with water, evaporated *in vacuo* and dried to yield a brown residue, which was chromatographed over silica gel (800 g) using ethyl acetate saturated with water as the developing solvent. The later part of eluates was combined and evaporated in vacuo to give a brown residue. Repeated chromatography of the residue on silica gel (200 g) eluted with CH_2Cl_2–EtOH (94:6—9:1) afforded colourless solid, which was crystallized from EtOH to give prosapogenin (III), mp 272°, yield: 830 mg.

The analytical sample of III was obtained by recrystallization from EtOH mp 274—275°, $[\alpha]_D^{20}$ +30.1° (pyridine). *Anal.* Calcd. for $C_{36}H_{58}O_{12}\cdot H_2O$: C, 61.70; H, 8.64. Found: C, 61.74; H, 8.57. C, 61.31; H, 8.50.

Prosapogenin Methyl Ester (IV)——A solution of prosapogenin (III) in dry tetrahydrofurane was treated with excess CH_2N_2 in ether. Evaporation of the solvent gave a colourless solid which was purified by column chromatography on silica gel using CH_2Cl_2–EtOH as the developing solvent. The methyl ester (IV) could not be obtained in crystalline form, but showed a single spot on a TLC (solvent: $CHCl_3$–MeOH–H_2O (70:20:3)).

Hydrolysis of Prosapogenin (III)——(i) A solution of III (200 mg) in EtOH (10 ml) and 8% aq. H_2SO_4 (10 ml) was heated on a boiling water-bath for 10 hr. The solvent was removed under reduced pressure to obtain precipitates which were collected by filtration, washed with water and dried to give crude sapogenin. The sapogenin was purified by silica gel column chromatography using a mixed solvent, CH_2Cl_2–EtOH. Crystallization from aq. EtOH gave platycodigenin (75 mg) as colourless needles, mp 242—244°, which was identified with the authentic sample by the comparisons of melting point, IR spectra and TLC. Platycodigenin obtained as above was methylated with CH_2N_2 to identified with the authentic sample of the methyl ester.

(ii) A solution of prosapogenin (III) in 3 ml of 1 N HCl (dioxane–H_2O) (1:3)) was heated on a boiling water-bath. After 5 hr the mixture was neutralized by stirring with ion exchange resin (Amberlite IR-4B (OH^-)) and evaporated to dryness. The paper chromatography of the residue (solvent: BuOH–AcOH–H_2O (4:1:5)). Colour reagent: Aniline hydrogenphthalate showed the presence of D-glucose only, which was confirmed also by GLC. 1) Trimethylsilyl ether was prepared by treatment with hexamethyldisilazane (0.2 ml) and trimethylsilyl chloride (0.2 ml) in pyridine solution. After 15 min an aliquot was used for GLC analysis to prove the presence of D-glucose penta-trimethylsilyl ether only. Conditions of GLC: Column: SE 30; column temperature: 162°, carrier gas: N_2: detector: flame ionization detector. 2) To an aqueous solution (1 ml) of the residue described above 1% $NaBH_4$ in water (1 ml) was added. The solution was allowed to stand for 30 min at room temperature. The excess of $NaBH_4$ was decomposed by the addition of Amberlite CG-120 (0.5 ml). The resin was removed by filtration and the filtrate was evaporated to dryness. A small amount of MeOH was added to the residue and evaporated to remove the borate as the methylate. After the repetition of the final treatment three times, the residue was dried *in vacuo*. The sample was treated with trifluoroacetic anhydride (0.1 ml) in ethyl acetate (0.1 ml) for 30 min at room temperature, and the reaction mixture was charged directly to the gas chromatograph. A single peak of sorbitol hexafluoroacetate was given under the following conditions of GLC. Column: 2% XF-1105; column temperature: 145°; carrier gas: N_2; detector: flame ionization detector.

Methylation of Prosapogenin (III)——NaH (50%) (200 mg) was washed with *n*-hexane and warmed with dimethylsulphoxide (10 ml) at 60—65° for 1 hr. To this reagent was added a solution of prosapogenin (III) (100 mg) in dimethylsulphoxide (5 ml), and the mixture was stirred for 2 hr at room temperature. CH_3I (2 ml) was then added under ice cooling, and the reaction mixture was allowed to stand at room temperature for 1 hr. After dilution with water, the mixture was extracted with ether, and the organic layer was washed with water, dried and concentrated to dryness. The residue was methylated 2 times under the same conditions as above, and the product was chromatographed on silica gel to give a colourless solid, which was not obtained in a crystalline form, but exhibited a single spot on TLC developed with benzene–EtOH (50:1).

Methanolysis of Prosapogenin Permethyl Ether Methyl Ester (V)——The prosapogenin permethyl ether methyl ester (V) (20 mg) was heated with 5% HCl–MeOH (2 ml) in a sealed tube on a boiling water-bath for 6 hr. The solution was neutralized by passing through a column of Amberlite IR-4B and evaporated to dryness. The presence of methyl 2,3,4,6-tetramethyl-D-glucopyranoside in this residue was shown by GLC. Conditions: Column: SE-30; column temperature: 110°; carrier gas: N_2; Detector: flame ionization detector.

Hydrolysis of Prosapogenin (III) under Mild Conditions——Prosapogenin (III) (20 mg) was dissolved in aqueous EtOH (1:1) (1.5 ml) and the solution of $NaIO_4$ (25 mg) in water (0.5 ml) was added under ice cooling, and the mixture was stirred at room temperature overngiht. The reaction mixture was extracted with BuOH and the BuOH layer was washed subsequently with $NaHCO_3$ and water. On evaporation an alcoholic substance was obtained.

1) A half amount of the alcoholic substance was dissolved in EtOH (1 ml) and $NaBH_4$ (5 mg) was added. The mixture was stirred at room temperature overnight, and then the excess of $NaBH_4$ was decomposed by the addition of AcOH. After adding water the reaction mixture was extracted with BuOH, and the BuOH layer was washed with water. The alcoholic product obtained on evaporation of organic solvent was dissolved in EtOH (2 ml) in which 0.3 N H_2SO_4 (1 ml) was added. The mixture was stirred at room temperature over-night and then extracted with ethyl acetate. The organic layer was washed with water and evaporated to dryness. The TLC of the residue showed that the main product is platycodigenin. 2) The product obtained by the oxidation of prosapogenin (III) with $NaIO_4$ was dissolved in EtOH (2 ml) in which 10% KOH solution (2 ml) was added. The mixture was heated for 1.5 hr under stirring in N_2 atmosphere. To the reaction mixture water was added and neutralized with 2 N HCl, and then extracted

with BuOH. Evaporation of BuOH gave colourless residue. The main part of the residue was proved to be platycodigenin by TLC.

Methylation of Platycodigenin (I)——Platycodigenin (I) (200 mg) was methylated with NaH (50%) (400 mg) and CH_3I (4 ml) in dimethylsulphoxide in the same way as described for methylation of prosapogenin. The product was separated by column chromatography (silica gel (20 g)) eluting with benzene–$CHCl_3$ (85:15—8:2). The earlier part of the eluate afforded crystalline permethylplatycodigenin methyl ester (VI) (from MeOH–acetone). Yield: 25 mg. An analytical sample was prepared by recrystallization from MeOH to give mp 187—188°, $[\alpha]_D^{15}$ +59.1° ($CHCl_3$). *Anal.* Calcd. for $C_{36}H_{60}O_7$: C, 71.48; H, 10.00. Found: C, 71.27; H, 10.00. The later part of eluate gave amorphous 2β,3β,23,24-tetra-O-methylplatycodigenin methyl ester (VII). Yield: 70 mg, which showed a single spot on TLC (solvent: benzene–EtOH 50:1).

Hydrolysis of Platycodigenin Methyl Ester (V)——Platycodigenin permethyl ether methyl ester (V) (300 mg) was refluxed with 3% H_2SO_4 in aq. EtOH (1:1 (60 ml)). The reaction mixture was concentrated to a half volume and extracted with ether. The ethereal layer was washed with water, dried and evaporated to give a colurless solid. The product was purified by passing through a column of silica gel. The eluates gave a colurless solid on evaporation, which was dried *in vacuo* and acetylated in the usual method. The product was purified by chromatography on silica gel. Recrystallization from MeOH afforded VIII, 18 mg, mp 163—164°, $[\alpha]_D^{15}$ +52.5° ($CHCl_3$). *Anal.* Calcd. for $C_{37}H_{60}O_8$: C, 70.22; H, 9.56. Found: C, 70.40; H, 9.54.

Isolation of Prosapogenin (III) by the Alkaline Hydrolysis of Saponin——To a solution of crude saponin (10 g) of *Platycodon glandiflorum* in aq. EtOH (1:1 (240 ml)) KOH (12 g) was added, and the mixture was refluxed on a boiling water-bath for 8 hr. The reaction mixture was neutralized by adding 2 N HCl and extracted with BuOH. The BuOH layer was washed with water, evaporated and dried. The residue was purified by column chromatography over silica gel (300 g) using CH_2Cl_2–EtOH (93:7) as the developing solvent. Repeated chromatography followed by the recrystallization from EtOH afforded prosapogenin (III), mp 276°, $[\alpha]_D^{15}$ +29.7° (pyridine), which was proved to be identical with the prosapogenin (III) obtained by the acid hydrolysis by the comparisons of mp, $[\alpha]_D$, and IR spectra, as well as by the X-ray powder diffractometry.

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