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Chemical Studies on the Oriental Plant Drugs. XXX.[1] Sapogenins of the Roots of *Platycodon grandiflorum* A. De Candolle. (1). Isolation of the Sapogenins and the Stereochemistry of Polygalacic Acid[2]

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From the saponins of the roots of *Platycodon grandiflorum* A. DC. polygalacic acid and a new triterpene acid, platycodigenin, were obtained as main sapogenins. The structure of polygalacic acid formerly proposed by Rondest *et al.*, was amended to be 2β, 3β, 16α, 23-tetrahydroxy-olean-12-en-28-oic acid.

The saponin and sapogenin of a Chinese drug "Jiegeng" (桔梗, Japanese name: Kikyo), the roots of *Platycodon grandiflorum A. DC.* (Campanulaceae) were studied earlier by Tsujimoto[4] and later by Yamaguchi, *et al.*,[5] but little has been known about their structures.

The crude saponins were separated from the methanolic extracts of the roots as shown in Chart 1.[6] Refluxing with dil. H_2SO_4 in aqueous ethanol, the crude saponins afforded a complex mixture of sapogenins, which were separated by column chromatography on silica gel to give two crystalline compounds as major sapogenins along with other several minor products.

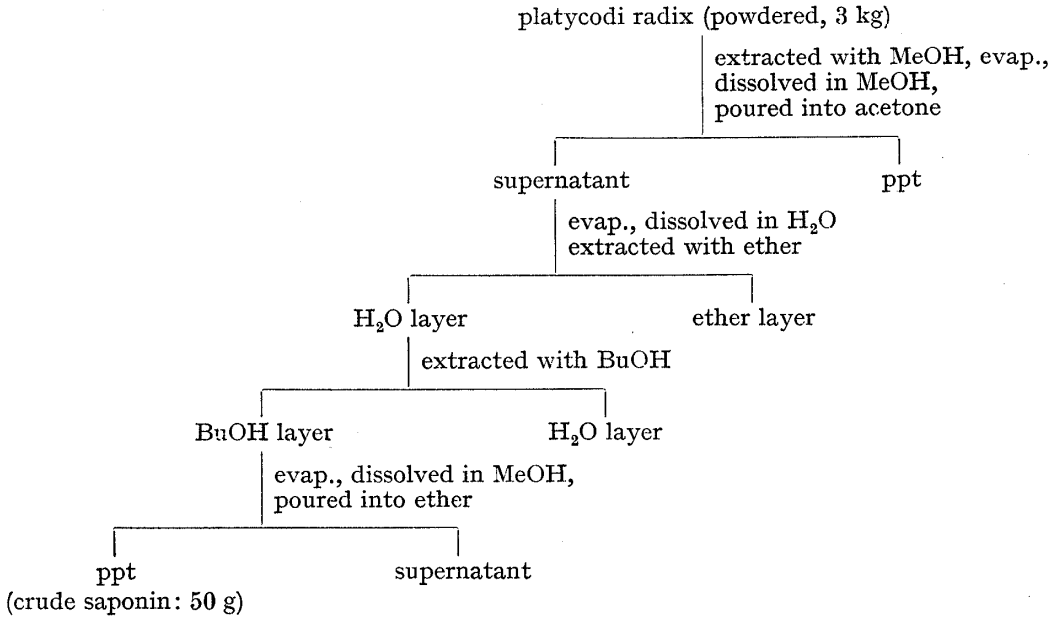


Chart 1. Extraction of Crude Saponin from Playcodi Radix

1) Part XXIX: T, Ohsawa, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, (1890) (1972).
2) Preliminary report: T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 2300 (1968).
3) *a*) Location: *Hongo, Bunkyo-ku, Tokyo*; *b*) Present address: *Institute of Pharmaceutical Sciences, Medical School, Hiroshima University, Hiroshima.*
4) M. Tsujimoto, *J. Agric. Chem. Soc. Japan*, **16**, 613 (1940) and references cited therein.
5) K. Yamaguchi, M. Ito, M. Nishimoto, and S. Natori, *Shoyakugaku Zasshi*, **18**, 12 (1964).
6) The physiological activities of these crude saponins have been studied by Takagi, *et al.*

The main sapogenin, $C_{30}H_{48}O_7$, mp 241—242°, $[\alpha]_D$+35.3° (I) afforded a methyl ester, $C_{31}H_{50}O_7$, mp 246° (II) by treatment with diazomethane. In view of the similarity of the analytical data and the physical constants, this sapogenin (I) should be identical with platycodigenin, mp 241—242°, $[\alpha]_D$+59.45° (EtOH), $C_{30}H_{48}O_7$, isolated from the same plant by Tsujimoto (in 1939),[4] though the direct comparison is impossible at present time. As reported preliminarily,[7] the structure of this sapogenin, platycodigenin (I), has been established to be 2β,3β,16α,23,24-pentahydroxyolean-12-en-28-oic acid. The experimental details of this study will be described in the subsequent paper.

Another major sapogenin, $C_{30}H_{48}O_6$, mp 308°, $[\alpha]_D$+45.0° (III) afforded a methyl ester, mp 245.0—245.5°, (IV) and a methyl ester tetra-acetate, mp 175—175.5°, (V). This sapogenin (III) was found to be identical with polygalacic acid which was previously isolated from *Polygala paenea L.* by Rondest and Polonsky.[8] The identity was established by mixed melting point determination of V with authentic methyl tetra-O-acetyl polygalacate and the comparisons of thin-layer chromatograms and infrared (IR) spectra of III and V with those of the respective authentic samples.

The structure of polygalacic acid (III) has been proposed by Rondest and Polonsky[8] to be VI possessing 16-β hydroxyl group. In the present study, acetylation of IV with acetic anhydride and pyridine at 0° gave an acetate (VII) mp 128—132°. The nuclear magnetic resonance (NMR) spectrum of VII showed the presence of two acetyl groups as shown in Table I. In the low field region VII exhibited a quartet-like signal at δ 4.25 assigned to 2α-proton, and a triplet-like signal at δ 4.54 which should be assigned to the proton at C-16.

Table I. Nuclear Magnetic Resonance Spectral Data of VII, VIII, IX, XIV

Compounds	C-CH_3 or O-CH_3	-$OCOCH_3$	-$COOCH_3$	-CH_2OR	2α-H	3α-H	16β-H	12-H
VII	0.79 (3H) 0.94 (3H) 1.02 (3H) 1.12 (3H) 1.34 (3H) 1.38 (3H)	2.08 (3H) 2.13 (3H)	3.62 (3H)	R=Ac 3.70 3.86 (ABd, J=12)	4.25 (bs)	4.93 (d, J=3)	4.54 (bs)	5.42 (bs)
VIII	0.73 (3H) 0.88 (3H) 0.90 (3H) 0.98 (3H) 1.17 (3H) 1.33 (6H) 1.48 (3H)		3.60 (3H)	R=H 3.38 (bs)	4.40[a] (t, J=7)	4.18 (d, J=7)	4.51 (bs)	5.42 (bs)
IX	0.73 (3H) 0.93 (6H) 0.98 (3H) 1.17 (3H) 1.34 (6H) 1.48 (3H)	2.06 (3H)	3.60 (3H)	R=Ac 3.69 4.01 (AB d, J=12)	4.40[a] (t, J=6)	4.09 (d, J=6)	4.52 (bs)	5.42 (bs)
XIV	0.84 (3H) 0.87 (3H) 0.92 (6H) 1.17 (6H) 1.33 (3H) 1.48 (3H)	2.06 (3H)	3.65 (3H)	R=Ac 3.70 3.97 (AB d, J=14)	4.38 (t, J=7)	4.09 (d, J=7)		5.57 (bs)

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

7) T. Akiyama, Y. Iitaka, and O. Tanaka, *Tetrahedron Letters*, **1968**, 5577.
8) J. Rondest and J. Polonsky, *Bull. Soc. Chim. France*, **1963**, 1253.

The chemical shifts showed a good agreement with the fact that hydroxyl groups of IV at C-2 and C-16 were not acetylated under the conditions described as above. The coupling feature of signal assigned to the proton at C-16 (triplet-like, $W_{1/2}$=4 Hz) clearly revealed[9] the axial configuration of the hydroxyl group of polygalacic acid at C-16.

Table II. Mass Spectrum of IX

m/e	Relative intensity	Assignment
600		M+
278	9	a
261	15	
260	80	b
245	28	c
219	20	d
204	25	
203	42.5	
202	29	
201	100	e
200	47	f

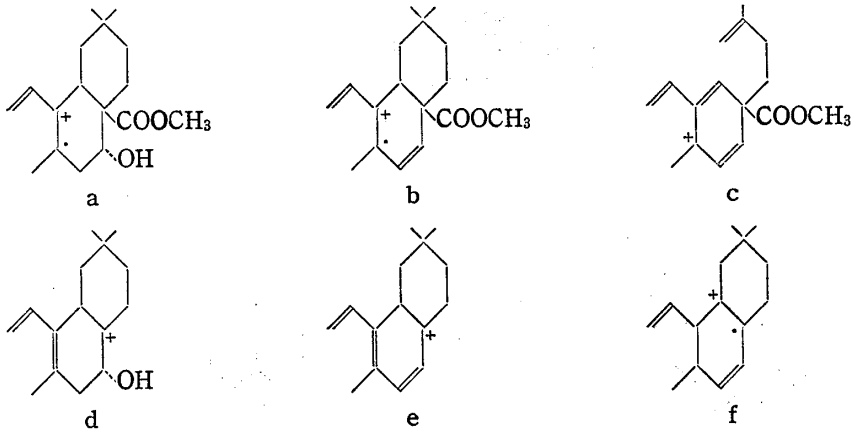
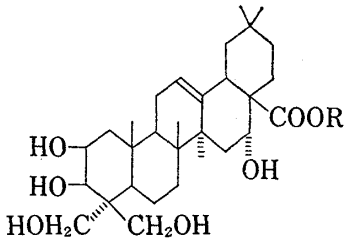


Chart 2

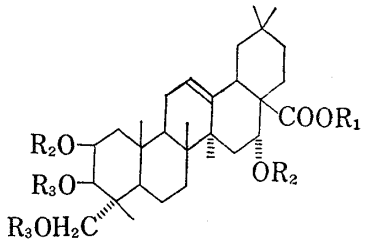
The α-configuration of the 16-hydroxyl group of III was also supported by IR spectrum of a 23-O-acetyl 2β,3β-O,O-isopropylidene derivative (IX), mp 190—194°, which was prepared as follows: the treatment of methyl polygalacate (IV) with acetone and *p*-toluenesulphonic acid gave the 2β,3β-O,O-isopropylidene derivative (VIII), mp 196—200°, which had previously been prepared by Rondest, *et al.*[8] Partial acetylation of VIII with acetic anhydride and pyridine under cooling gave 23-O-acetyl-2β,3β-O,O-isopropylidene derivative (IX), mp 190—194°. The structures of VIII and IX were confirmed by the NMR and mass spectra (Table I and Table II). The IR spectrum of methyl cochalate (X) in carbon tetrachloride solution exhibited the bands at 3630 and 1740 cm^{-1} due to free hydroxyl and carbomethoxy groups respectively, as well as the intense bands at 3540 and 1705 cm^{-1} suggesting the presence of intramolecular hydrogen bonding between hydroxy group at C-16 and carbomethoxy group at C-17.[10] However, the IR spectrum of IX in carbon tetrachloride solution exhibited the intense bands at 3630 and 1740 cm^{-1}, showing no hydrogen bonding between hydroxyl and carbomethoxy groups.

9) In other derivatives of polygalacic acid, the overlapping of signals makes difficult the observation of $W_{1/2}$ of signals due to the protons at C-16.
10) *cf.* A.R.H. Cole and G.T.A. Müller, *J. Chem. Soc.*, **1959**, 1224.

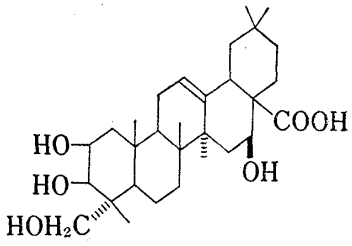
It has been known that the reduction of the 16-ketone of olean-12-ene type triterpenes afforded mainly 16α(axial) hydroxyl groups, while sodium borohydride reduction of methyl diketoechinocystate (XI) prepared from methyl cochalate (X), possessing 16β hydroxyl group, afforded methyl echinocystate (XII) having 16α hydroxyl group. The previous assignment of the configuration of the hydroxyl group at C-16 of III was based on the fact that the 16-keto-23-aldehyde derivative (XIII) prepared from IV through methyl 2,3-O,O-isopropylidene-polygalacate (VIII) did not afford, on sodium borohydride reduction followed by acid treatment, the starting material, methyl polygalacate (IV). However, the purity and the structure of this reaction product, mp 233—235°, were not fully descirbed.[8]



I: R=H; platycodigenin
II: R=CH_3

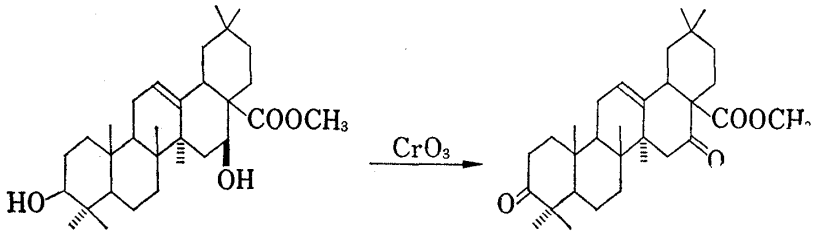


III: R_1=H, R_2=R_3=H; polygalacic acid (proposed by present authors)
IV: R_1=CH_3, R_2=R_3=H
V: R_1=CH_3, R_2=R_3=Ac
VII: R_1=CH_3, R_2=H, R_3=Ac



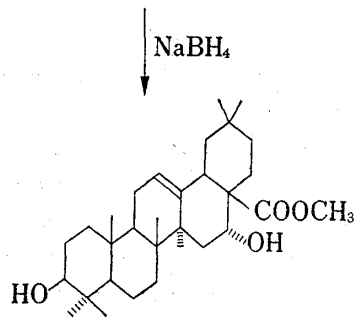
VI: polygalacic acid (proposed by Rondest, *et al.*)

Chart 3



X: methyl cochalate

XI: methyl diketoechinocystate



XII: methyl echinocystate

Chart 4

In order to re-examine the stereochemistry by the reduction of the 16-keto group, methyl 23-O-acetyl-2β,3β-O,O-isopropylidene-polygalacate (IX) was oxidized with chromic acid to give 16-keto derivative (XIV), amorphous, but homogeneous by the thin-layer chromatography (TLC). The structure of XIV was confirmed by IR and NMR (Table I) spectra and optical rotatory dispersion and circular dichroism curves with negative Cotton effect as being

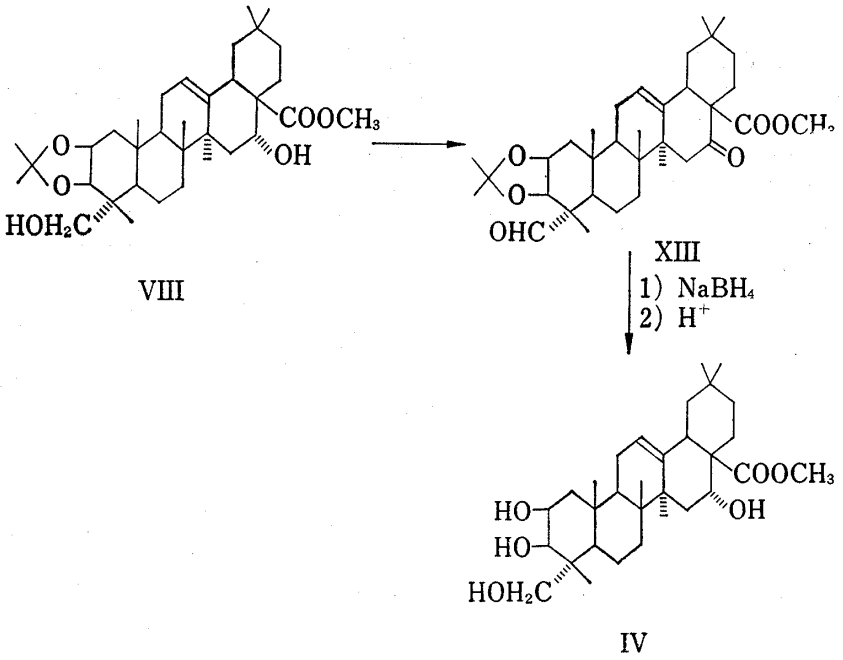


Chart 5

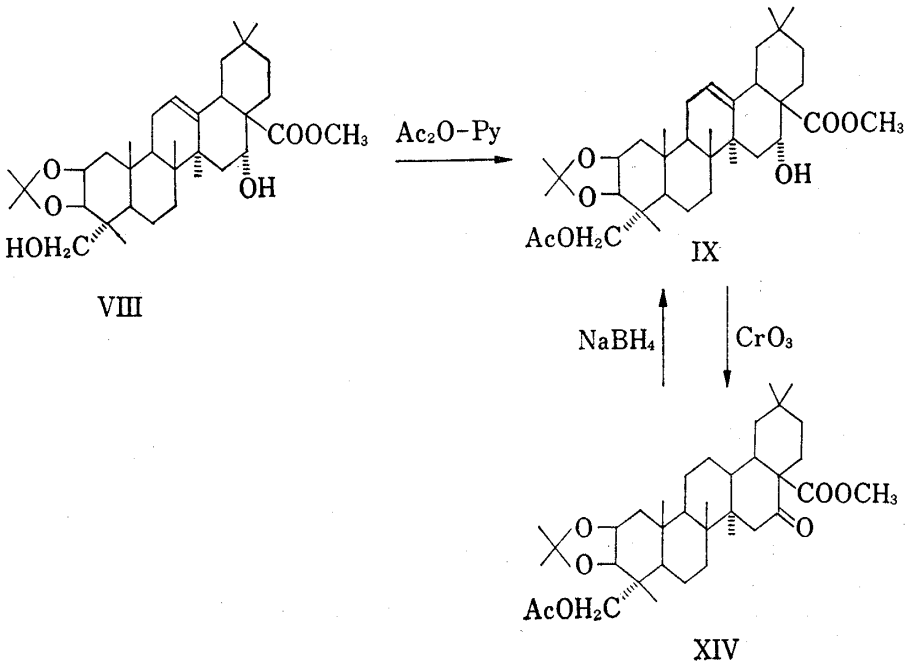


Chart 6

characteristic of the 16-keto group of triterpenes of this type.[11] The reduction of 16-keto derivative (XIV) with sodium borohydride reproduced IX along with a small amount of deacetylation product (VIII). Consequently, the configuration of the C-16 hydroxyl group of polygalacic acid (III) has now been revised to be α (axial). Shortly before our preliminaly report[2] was published, Kubota and Kitatani[12] reported the isolation of polygalacic acid from the same plant source and amendment of the configuration of the 16-hydroxyl group to be α by the chemical correlation of III with quillaic acid (3β,16α,23-trihydroxy-olean-12-en-28-oic acid).

11) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).
12) T. Kubota and H. Kitatani, *Chem. Commun.*, **1968**, 1005.

Experimental

Melting points were determined in a Yanagimoto melting point apparatus and are uncorrected. Unless otherwise stated, NMR spectra were measured in $CDCl_3$ at 100 Hz. and recorded in δ-values with TMS as the internal references. Optical rotations were measured with a Yanagimoto Photo-magenetic polarimeter Model OR-20. IR spectra were measured on a Japan Spectroscopic Co. Model DS-402G spectrometer.

Isolation of the Saponin——The dried, cut roots (3 kg) of *Platycodon grandiflorum* were extracted several times with methanol (total 20 liters) under reflux. The methanol solution was concentrated in vacuo to 2 liters and poured into acetone (10 liters) to remove an acetone-insoluble portion to give syrupy precipitates which were removed by decantation. The supernatant was evaporated *in vacuo* to give a dark brown residue, which was dissolved in water (2 liters). The aqueous solution was extracted with ether (2.5 liters) and butanol (saturated with water) (5 liters), successively. The butanol layer was evaporated *in vacuo* to afford a dark brown residue, which was dissolved in MeOH (200 ml). The MeOH solution was poured into ether (3 liters) and the resulted precipitates were collected by filtration, washed with ether and dried to give the crude samponins as brownish powder (yield: 50 g) which has the property of foaming in aqueous solution.

Isolation of Triterpenoids——To the solution of the crude saponins (90 g) in EtOH (1 liter) was added 8% H_2SO_4 (1 liter) and the mixture was refluxed for 15 hr. The solution was concentrated to 1 liter under reduced pressure, and the precipitates formed were collected by filtration, washed with water and dried to give a brown solid (28 g), which was chromatographed over 1 kg of silica gel using ether saturated with water as the developing solvent. The early fractions were combined and evaporated to give a brown residue, which was washed with MeOH several times to give a colourless solid (1.2 g), which was almost homogenous by the thin-layer chromatogram (solvent, benzene: EtOH (9:1)). Analytical sample, mp 308°, $[\alpha]_D^{25}$ +45.0° (pyridine), was obtained by repeated recrystallization from aq. EtOH. This compound was found to be identical with polygalacic acid (III) by comparing their IR spectra and TLC. *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.37; H, 9.61. Found: C, 71.13; H, 9.66.

The later fractions were worked-up in the similar way to give practically pure platycodigenin (I) (3.1 g). The analytical sample, mp 241—242°, $[\alpha]_D^{25}$ +35.3 (pyridine) was obtained by repeated recrystallization with aqueous EtOH. *Anal.* Calcd. for $C_{30}H_{48}O_7 \cdot 1/2\ H_2O$: C, 67.99; H, 9.35. Found: C, 68.24; H, 9.29. IR ν_{max}^{KBr} cm^{-1}: —3400 (-OH), —1700 (>C=O).

Methyl Ester of Platycodigenin (II)——A solution of platycodigenin (I) (100 mg) in tetrahydrofurane (5 ml) was treated with ethereal diazomethane. On evaporation of the solvent, the mixture gave colourless oily residue, which was crystallized from equeous EtOH to yield methyl ester of platycodigenin (70 mg), mp 243—246°. The analytical sample melted at 246°, $[\alpha]_D^{25}$ +45.0° (pyridine). *Anal.* Calcd. for $C_{31}H_{50}O_7$: C, 69.63; H, 9.43. Found: C, 69.35; H, 9.39; C, 69.65; H, 9.49. IR ν_{max}^{KBr} cm^{-1}: —3400, 1710.

Methyl Polygalacate (IV)——A solution of polygalacic acid (III) (100 mg) obtained from the roots of *Platycodon grandiflorum* in tetrahydrofurane (5 ml) was treated with ethereal diazomethane to yield methyl polygalacate (34 mg) mp 245—245.5° (from aq. EtOH) $[\alpha]_D^{25}$ +47.3 (EtOH). IR ν_{max}^{KBr} cm^{-1}: —3400, —1705.

Methyl Tetra-O-acetyl Polygalacate (V)——Acetylation of methyl polygalacate (IV) (100 mg) with acetic anhydride (1 ml) and pyridine (2 ml) mixture at 40°, 2 days, produced methyl tetra-O-acetyl polygalacate. anhydride (1 ml) and pyridine (2 ml) mixture at 30°, 2 days, produced methyl *tetra*-O-acetyl polygalacte. Repeated recrystallization from MeOH yielded the pure sample mp 175—175.5°, $[\alpha]_D$ +6.3° (EtOH). The identification with the authentic sample was achieved by mixed melting point and comparisons of the infrared spectra (KBr Tab.) and the thin-layer chromatograms (solvent: chloroform–ether 10:1). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750 (>C=O), No -OH.

Methyl Di-O-acetyl Polygalacate (VII)——Methyl polygalacate (IV) (200 mg) was dissolved in acetic anhydride (2 ml)–pyridine (4 ml) previously cooled to 0° and the mixture was kept at the temperature for 4 hr. The mixture was poured into ice-water and the resulted colourless precipitates were collected by filtration, washed with water and dried. Chromatography in benzene–chloroform (10:0—8:2) over 5 g of silica gel gave a solid, which was crystallized from chloroform–hexane to yield 130 mg of diacetate (VII), mp 128—132°, $[\alpha]_D$ +66.2 ($CHCl_3$). *Anal.* Calcd. for $C_{35}H_{54}O_8$: C, 67.93; H, 8.80. Found: 68.00; H, 8.83. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (-OH), 1740 (>C=O).

2β,3β-O,O-Isopropylidene Derivative of Methyl Polygalacate (VIII)——A solution of IV (300 mg) and *p*-toluenesulphonic acid monohydrate (60 mg) in dry acetone (60 ml) was allowed to stand at room temperature for 1 hr. After addition of K_2CO_3 (60 mg) under cooling, the solution was evaporated *in vacuo* and then diluted with water. The precipitates formed were collected by filtration, dried and crystallized from ether–hexane to yield. VIII, 185 mg, mp 196—200°, $[\alpha]_D^{24}$ +58.7 (EtOH). IR ν_{max}^{KBr} cm^{-1}: 3425 (-OH) 1720 (>C=O).

Partial Acetylation of VIII——The acetonide (VIII) (170 mg) was dissolved in acetic anhydride (5 ml)–pyridine (5 ml) previously cooled at −15——10° and the solution was kept at the temperature for 5 hr. The mixture was poured into ice-water and the colourless precipitates were collected by filtration, washed and dried. Crystallization from methanol gave 145 mg of IX as colourless needles, mp 184—187°. The analytical sample of IX melted at 190—194°. $[\alpha]_D^{25}$ +65.6 ($CHCl_3$). *Anal.* Calcd. for $C_{36}H_{56}O_7 \cdot 1/2\ H_2O$: C,

71.00; H, 9.43. Found: C, 71.17; H, 9.52: C, 70.80; H, 9.29. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3630 (-OH), 1740 (>C=O).

Pyridine-Chromium Trioxide Complex Oxidation of IX——A solution of IX (70 mg) in pyridine (2 ml) was added to an ice-cooled suspension of pyridine (3 ml)–CrO_3 (70 mg) complex and allowed to stand at room temperature for 18 hr. The mixture was poured into ice-water and the precipitates were extracted with ether. After washing with diluted hydrochloric acid and water, the ethereal layer was evaporated to give a yellow gum, which was chromatographed over 2 g of silica gel. The elution with benzene–chloroform (8:2) gave XIV, which was failed to be obtained in a crystalline form but showed a single spot on a TLC (solvent: chloroform). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745, 1715 (C=O), No OH band. ORD (MeOH) (nm) M: 244 (792), 274 (1148), 319 (678).

$NaBH_4$ Reduction of XIV——A solution of XIV (25 mg) and sodium borohydride (25 mg) in 1.7 ml of MeOH and 0.3 ml of water was left at room temperature for 4 hr. The precipitates formed were collected by filtration, dissolved in ether. The ethereal layer was washed with water and evaporated. The residue was chromatographed on 0.5 g of silica gel to give 3 mg of IX, which was no depression in melting point on admixture with the authentic sample and the IR spectra were identical. TLC showed the presence of a small amount of VIII.

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