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Constituents of Convallaria. XI.[1] On the Structure of Convallasaponin-D

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The structure of the steroidal saponin, convallasaponin-D,[3] $C_{50}H_{82}O_{21}$, mp 264—265°, $[\alpha]_D^{21}$: −66°, which was isolated from the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley, was studied and elucidated as rhodeasapogenin (1)-β-D-glucopyranosido, (3)-α-L-rhamnopyranosyl ($1_{rha}\rightarrow 2_{xyl}$)-β-D-xylopyranosyl ($1_{xyl}\rightarrow 3_{rha}$)-α-L-rhamnopyranoside.

In the previous paper[3] of this series, it was reported that three new steroid saponins, glucoconvallasaponin-A, -B, and convallasaponin-D were obtained from the aqueous layer in extraction of the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley (Suzuran), and the chemical constitutions were determined on the two of the former glycosides. The present paper describes the structure elucidation of convallasaponin-D (I).

Hydrolysis of I with 1 N hydrochloric acid in 50% ethanol on reflux for five hours afforded rhodeasapogenin (II),[4] 25L,5β-spirostan-1β,3β-diol, and three kinds of sugars[5] D-xylose, D-glucose, and L-rhamnose. The molar ratio of the sugar portion was determined as 1:1:2 by using of gas chromatography[6] and I, therefore, should have the molecular formula, $C_{50}H_{82}O_{21}$, which was supported by the isolation of four kinds of prosapogenins obtained from the mild hydrolysis of I as described later. Hydrolysis of I by β-glucosidase in Takadiastase gave the prosapogenin (III), $C_{44}H_{72}O_{16}$, which further on acid hydrolysis gave II, D-xylose, and L-rhamnose, the molar ratio of the sugar portion being 1:2.[6] As the permethylate (IX) of

Table I. Chromatography of Partial Hydrolyzates of Prosapogenin III

Fraction	Solvent	Vol. (liter)	Weight (mg)	*Rf*[a]			
1	$CHCl_3$	0.2	23	0.75			
2	$CHCl_3$–MeOH (95:5)	0.2	28		0.35		
3	$CHCl_3$–MeOH (95:5)	0.1	2		0.35	0.27	
4	$CHCl_3$–MeOH (95:5)	0.1	8			0.27	
5	$CHCl_3$–MeOH (90:10)	0.3	6			0.27	
6	$CHCl_3$–MeOH (80:20)	0.2	8			0.27	
7	$CHCl_3$–MeOH (80:20)	0.1	3			0.27	0.10
8	$CHCl_3$–MeOH (50:50)	0.2	9				0.10

a) *Rf*-values in thin-layer chromatography were determined using Wakogel B-5 (Wako Pure Chem. Co., Tokyo) and CH_2Cl_2–MeOH–$HCONH_2$ (80:10:1) as adsorbent and solvent, respectively. Detection was made by 5% H_2SO_4 with heating at 120°. Spots were identified as follows:
0.75: rhodeasapogenin 0.35: prosapogenin VII
0.27: prosapogenin VIII 0.10: prosapogenin III

1) Part X: *Chem. Pharm. Bull.* (Tokyo), **16**, 25 (1968).
2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo.*
3) M. Kimura, M. Tohma, I. Yoshizawa, and H. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **16**, 25 (1968).
4) H. Nawa, *Yakugaku Zasshi*, **73**, 1192 (1953); *Chem. Pharm. Bull.* (Tokyo), **6**, 255 (1958). T. Okanishi, A. Akahori, and F. Yasuda, *Ann. Reports Shionogi Res. Lab.*, **10**, 1407 (1960).
5) Their azoates were identified as described in the experimental part.
6) M. Kimura, M. Tohma, Y. Hattori, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 613 (1968).

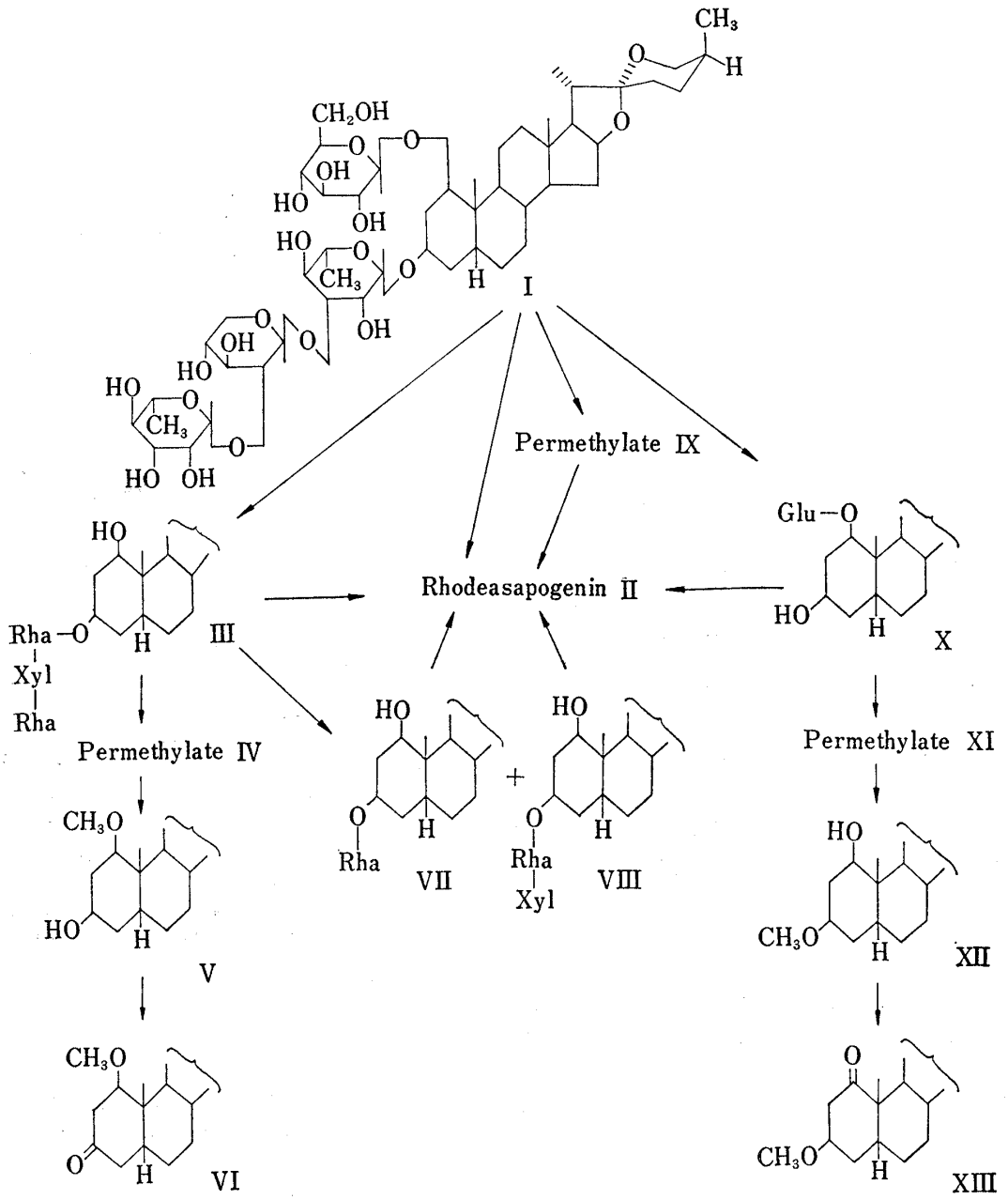

Chart 1

I gave also II on acid hydrolysis, the two hydroxyl groups of this aglycone were assumed to be offered for the glycosidic linkages and D-glucose was suspected to be linked directly with the aglycone.

On acid hydrolysis the permethylate (IV) of III gave rhodeasapogenin mono methylate (V), which was oxidized with chromium trioxide in acetic acid to give a ketone (VI) showing a strong absorption band at 1718 cm^{-1} due to the 3-keto group in A/B-*cis* junction.[7] Optical rotatory dispersion curve of VI revealed a weakly negative Cotton effect with the molecular amplitude of −28.0 (Fig. 1) indicating the presence of 3-keto group.[8] From these results the sugar moiety of III was concluded to be linked with the aglycone at the hydroxyl group

7) R.N. Jones, P. Humphries, and K. Dobriner, *J. Am. Chem. Soc.*, **72**, 956 (1950); **70**, 2024 (1948). R.N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954). R.N. Jones, D.A. Ramsey, D.S. Keir, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 80 (1952).

8) W. Moffit, R.B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).

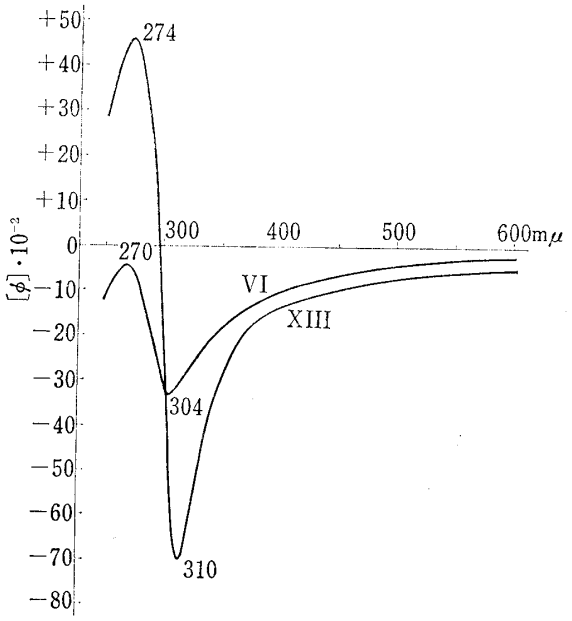

Fig. 1. Optical Rotatory Dispersion Curves of Ketones (VI) and (XIII) in Methanol

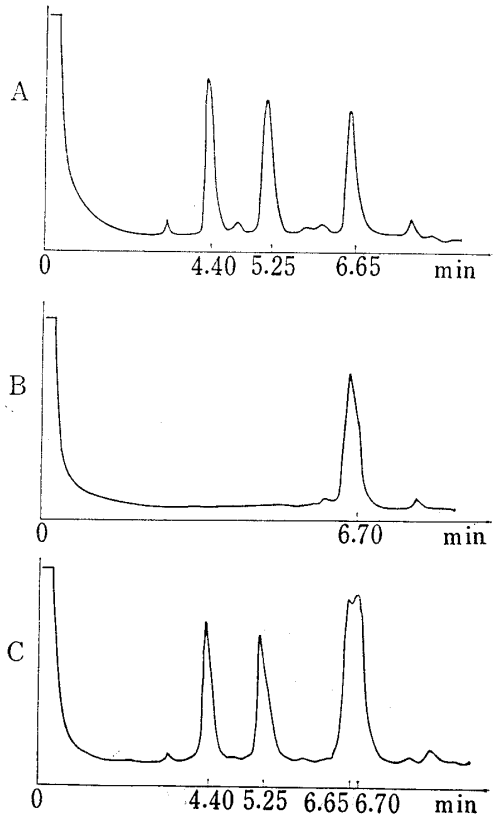

Fig. 2. Gas Chromatographic Analysis of Partially Methylated Sugars from Permethylates IV (A), XI (B), and IX (C)

1.5% SE-30 on chromosorb W, 190°, 30 ml N_2/min
2,3,4-tri-O-methyl-L-rhamnopyranose (4.40 min)
2,4-di-O-methyl-L-rhamnopyranose (5.25 min)
3,4-di-O-methyl-D-xylopyranose (6.65 min)
2,3,4,6-tetra-O-methyl-D-glucopyranose (6.70 min)

of C–3, not of C–1. Partial hydrolysis of III with 1 N hydrochloric acid in 50% ethanol on reflux for one hour gave two major products, the prosapogenin (VII), $C_{33}H_{54}O_8$, and the prosapogenin (VIII), $C_{38}H_{62}O_{12}$, which were separated by alumina chromatography (Table I). On further acid hydrolysis the former gave II as well as L–rhamnose and the latter gave the same aglycone together with L–rhamnose and D–xylose, the molar ratio of the sugars being 1:1.[6] Therefore, it was clear that L–rhamnose should be linked directly with the aglycone on one hand and the two sugars, L–rhamnose and D–xylose, on the other hand. The fully methylated prosapogenin (IV) gave on acid hydrolysis three kinds of partially methylated sugars, which were identified as 2,3,4–tri–O–methyl–L–rhamnopyranose, 2,4–di–O–methyl–L–rhamnopyranose, and 3,4–di–O–methyl–D–xylopyranose by comparing with the authentic specimens on thin–layer chromatography and gas chromatograpy (Fig. 2). The molar ratio of these sugars was determined roughly as 1:1:1 by using gas chromatography. Thus the sugar portion of III may be arranged in such a straight chain as $rha_1 \rightarrow {}_2xyl_1 \rightarrow {}_3rha_1 \rightarrow C_3$–OH (aglycone).

Hydrolysis of I with 1 N hydrochloric acid on reflux for two hours gave the prosapogenin (X), $C_{33}H_{54}O_9$, as a major product, which on further acid hydrolysis gave II and D–glucose. The permethylate (XI) of X gave on acid hydrolysis rhodeasapogenin monomethylate (XII), the isomer of V, together with 2,3,4,6–tetra–O–methyl–D–glucopyranose (Fgi. 2). Oxidation of XII with chromium trioxide in acetic acid gave a ketone (XIII) showing a strong absorption band at 1701 cm^{-1} due to 1–keto group.[9] Optical rotatory dispersion curve of XIII showed a highly negative Cotton effect with the molecular amplitude of −116.5 (Fig. 1) indicating the presence of 1–keto group.[8] D–glucose may consequently be attached to the aglycone at the hydroxyl group of C–1. By applying the Klyne rule[10] to I and its prosapogenins, the

9) F. Sollmann and Ch. Tamm, *Helv. Chim. Acta*, **39**, 1340 (1956).
10) W. Klyne, *Biochem. J.*, **47**, xli (1950).

Table II. Chromatography of Partial Hydrolyzates of Convallasaponin–D

Fraction	Solvent	Vol. (liter)	Weight (mg)	*Rf*[a]				
1	$CHCl_3$	3	403	0.75				
2	$CHCl_3$–MeOH (95:5)	3	101	0.75	0.30			
3	$CHCl_3$–MeOH (95:5)	6	801		0.30			
4	$CHCl_3$–MeOH (95:5)	2	25		0.30	0.25		
5	$CHCl_3$–MeOH (90:10)	4	270		0.30	0.25	0.20	
6	$CHCl_3$–MeOH (70:30)	3	135			0.25	0.20	0.10
7	$CHCl_3$–MeOH (50:50)	7	301			0.25	0.20	0.10
8	MeOH	2	120				0.20	0.10

a) *Rf*–values in thin–layer chromatography were determined using Wakogel B–5 and CH_2Cl_2–MeOH–$HCONH_2$ (80:20:1) as adsorbent and solvent, respectively. Detection was made by 5% H_2SO_4 with heating.

glycosidic linkages were likely to be in α–form for L–rhamnose and β–form for D–glucose and D–xylose (Table III). The structure of convallasaponin–D (I) may, therefore, be defined as rhodeasapogenin (1)–β–D–glucopyranosido, (3)–α–L–rhamnopyranosyl($1_{rha}\rightarrow2_{xyl}$)–β–D–xylopyranosyl($1_{xyl}\rightarrow3_{rha}$)–α–L–rhamnopyranoside.

Only isorhodeasapogenin was obtained as an aglycone from convallasaponin–C[11] and rhodeasapogenin (II) was also the sole one from convallasaponin–D (I) in this paper. Both of these isomeric aglycones may reasonably be considered to be naturally present in the plant body as different glycosides since no structural change has been observed in the spiroketal side chain on the experimental hydrolysis conditions.[12] As in the case of glucoconvallasaponin–B,[3] two sugar chains are encountered in I; one is bound to the hydroxyl group at C–1 and the other to the one at C–3 of the aglycone. These novel types of saponin are interesting from the points of view of the chemicl structure and particularly of the biogenesis.

Table III. Molecular Rotation Differences

	$[\alpha]_D$(°C)	$[M]_D$(°C)	$\Delta[M]_D$(°C)
Rhodeasapogenin (II)	−72	−311	−129
Prosapogenin (VII)	−76	−440	−123
Prosapogenin (VIII)	−75	−563	−181
Prosapogenin (III)	−87	−744	+ 73
Convallasaponin–D (I)	−66	−671	
Rhodeasapogenin (II)	−72	−311	+ 13
Prosapogenin (X)	−50	−298	

α–Me–L–rhamnopyranoside: $[M]_D$ −111°; β–Me–L–rhamnopyranoside: $[M]_D$ +170°
α–Me–D–glucopyranoside: $[M]_D$ +307°; β–Me–D–glucopyranoside: $[M]_D$ − 63°
α–Me–D–xylopyranoside: $[M]_D$ +249°; β–Me–D–xylopyranoside: $[M]_D$ −107°

Experimental

Enzymatic Hydrolysis of Convallasaponin–D (I)——The saponin (1.52 g) was dissolved in water (300 ml) and allowed to stand with takadiastase preparation (3.1 g) for 2 days at 34° under the presence of toluene (1 ml). After the incubation mixture was concentrated to *ca.* 100 ml at below 50° *in vacuo*, EtOH (500 ml)

11) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 55 (1966).
12) E.S. Rothman, M.E. Wall, and C.R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952). M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952). C.R. Eddy, M.E. Wall, and M.K. Scott, *ibid.*, **25**, 266 (1953).

was added. The enzyme precipitated was filtered off through Hyfro-Super Cel and the filtrate was again concentrated to *ca.* 100 ml and extracted with $CHCl_3$–BuOH (2:1). The extract (1.35 g) was chromatographed on alumina (40 g) and the fraction eluted with MeOH–$CHCl_3$ (3:2) gave the white powders (1.19 g) which were crystallized from aq.EtOH to give fine colourless needles (III), mp 223—227°. $[\alpha]_D^{21}$: −87° (c=0.71, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH, broad), 982, 917>897, 849 (25 L-spiroketal). *Anal.* Calcd. for $C_{44}H_{72}O_{16}$: C, 61.66; H, 8.47. Found: C, 61.51; H, 8.39.

Acetylation of Prosapogenin (III)——By the usual means III (75 mg) was acetylated in pyridine (2 ml) and Ac_2O (2 ml) to give a crude acetate (61 mg) which was recrystallized from aq.MeOH to fine colourless needles, mp 183°. $[\alpha]_D^{21}$: −88° (c=0.61, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1740 (AcO). *Anal.* Calcd. for $C_{60}H_{88}O_{24}$: C, 60.39; H, 7.43. Found: C, 60.53; H, 7.36.

Acid Hydrolysis of III——After III (110 mg) was refluxed with 1 N HCl in EtOH–H_2O (1:1) on a water bath for 5 hr., the aglycone obtained was recrystallized from MeOH–$CHCl_3$ to fine colourless needles, mp 291—293°, identical with the authentic rhodeasapogenin in the mixed melting point and the comparison of the infrared spectra. $[\alpha]_D^{19}$: −70° (c=0.68, $CHCl_3$–MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH, broad), 984, 915 >894, 850 (25L-spiroketal). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.09; H, 10.38.

Permethylation of III——Under nitrogen stream III (1.28 g) was dissolved in DMSO (50 ml) with stirring for 30 min at room temperature and the amount of NaH roughly equivalent to the hydroxyl content of the prosapogenin was added. After the mixture was stirred under nitrogen stream for another 30 min, an excess of MeI was added and it was stirred further for 30 min. The reaction mixture was diluted with water (300 ml), extracted with $CHCl_3$, washed with water, and evaporated *in vacuo*. The residue was taken up in 100 ml, of ether and washed with water to remove a trace of DMSO. Evaporation of ether gave a crystalline residue. The same treatment was further repeated (twice) until no hydroxyl absorption band was found on IR spectrum. The completely methylated product (1.18 g) was recrystallized from *n*-hexane to give fine colourless needles (IV), mp 174—176°. $[\alpha]_D^{20}$: −79° (c=0.87, $CHCl_3$). *Anal.* Calcd. for $C_{52}H_{88}O_{16}$: C, 64.44; H, 9.15. Found: C, 64.18; H, 9.07.

Hydrolysis of Methylated Prosapogenin (IV)——After IV (1.00 g) was refluxed in 3.3% HCl/MeOH (100 ml) on a water bath for 3 hr, water (50 ml) was added and the reaction mixture was refluxed for another 3 hr. Methanol was evaporated and the precipitate formed was filtered, dissolved in $CHCl_3$, washed with water, dried over Na_2SO_4. Evaporation of $CHCl_3$ gave a residue (350 mg), which was submitted to chromatography on alumina. From the fraction eluted with *n*-hexane–benzene white powder (322 mg) was obtained, which was recrystallized from *n*-hexane–$CHCl_3$ to give fine colourless needles (V), mp 235—237.5°. $[\alpha]_D^{19}$: −67° (c=0.68, MeOH). *Anal.* Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.09; H, 10.14.

An aqueous layer of the hydrolysation mixture was neutralized with Amberlite IR-4B and concentrated to dryness under reduced pressure to give a yellow syrup (560 mg) which was found to contain three kinds of partially methylated sugars. These were identified as 2,3,4-tri-O-Me-L-rhamnopyranose, 2,4-di-O-Me-L-rhamnopyranose, and 3,4-di-O-Me-D-xylopyranose by thin-layer and gas chromatography (Fig. 2). TLC (Wako gel; solvent system: ether–toluene (2:1); detection: with 5% H_2SO_4 under heat at 120° for 5 min): Rf=0.80, 0.68 and 0.31 (agreed closely with those obtained with authentic 2,3,4-tri-O-Me-L-rhamnopyranose 2,4-di-O-Me-L-rhamnopyranose and 3,4-di-O-Me-D-xylopyranose, respectively). GLC: t_R (min)=4.40 (2,3,4-tri-O-Me-L-rhamnopyranose; authentic specimen: 4.39), t_R=5.25 (2,4-di-O-Me-L-rhamnopyranose; authentic specimen: 5.27), t_R=6.65 (3,4-di-O-Me-D-xylopyranose; authentic specimen: 6.65).

Oxidation of V with CrO_3——To a solution of V (82 mg) in AcOH (10 ml) CrO_3 (98 mg) and one drop of water were added, and the mixture was left for 48 hr at room temperature. After adding 10 ml of MeOH, one drop of conc.H_2SO_4, and small amount of water to the reaction mixture, excess MeOH was evaporated and the precipitate was extracted with ether, washed with water, with 5% aq. Na_2CO_3, again with water, and dried over Na_2SO_4. Evaporation of ether gave a residue (73 mg) which was recrystallized from acetone to give fine colourless needles (VI), mp 198—200°. $[\alpha]_D^{23}$: −26° (c=0.47, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 1717 (C=O).ORD (Fig. 1): a=−28.0 (c=0.16, MeOH). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.36; H, 9.75.

Partial Hydrolysis of III——A mixture of hydrolyzate (89 mg) obtained by refluxing III with 1 N HCl/EtOH (1:1, 10 ml) on a water bath for 1 hr, was dissolved in $CHCl_3$ and submitted to chromatography on alumina using the solvent system of $CHCl_3$ with increasing MeOH content. Each eluate was evaporated to dryness and examined by thin-layer chromatography. Two kinds of prosapogenin were obtained as shown in Table I.

Prosapogenin (VII)——The residue of Fraction 2 (Table I) was recrystallized from MeOH–$CHCl_3$ to give fine colourless needles, mp 266—268°. $[\alpha]_D^{21}$: −76° (c=0.47, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_8$: C, 68.48; H, 9.40. Found: C, 68.39; H, 9.28. Acetate: fine colourless needles, mp 236—237° (prepared by the usual means and recrystallized from aq. MeOH). $[\alpha]_D^{20}$: −69° (c=0.44, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{62}O_{12}$: C, 65.92; H, 8.36. Found: C, 66.10; H, 8.12.

Hydrolysis of VII——With 1 N HCl in aq. EtOH (1:1) VII (9 mg) was refluxed to give rhodeasapogenin and L-rhamnose, which were detected and identified by thin-layer and paper chromatography. TLC (Wako gel; solvent system: Me_2CO–$CHCl_3$ (1:9); detection: with 5% H_2SO_4 at 120° for 5 min): Rf=0.40 (rhodea-

sapogenin). PPC (solvent system: AcOEt–pyridine–H_2O (2:1:2:); detection: with aniniline hydrogenphthalate at 120° for 5 min): Rf=0.51 (L-rhamnose).

Prosapogenin (VIII)——The residue of Fractions 4—6 (Table I) was recrystallized from MeOH–$CHCl_3$ to give fine colourless needles, mp 249—252.5°. $[\alpha]_D^{21}$: −75°(c=0.49, MeOH–$CHCl_3$). *Anal.* Calcd. for $C_{38}H_{62}O_{12}$: C, 64.20; H, 8.79. Found: C, 63.94; H, 8.97. The acetate prepared by usual means was recrystallized from aq. MeOH to give fine colourless needles, mp 199—201°. $[\alpha]_D^{19}$: −73° (c=0.46, $CHCl_3$). *Anal.* Calcd. for $C_{50}H_{74}O_{18}$: C, 62.35; H, 7.74. Found: C, 62.68; H, 7.53.

Hydrolysis of VIII——In a similar way to VII, VIII (5.4 mg) was hydrolyzed to give rhodeasapogenin, L-rhamnose, and D-xylose which were detected and identified by thin-layer or paper chromatography. The ratio of sugar portion has been determined as 1 : 1 by gas chromatography in the previous paper.[6] TLC (Wako gel; solvent system: Me_2CO–$CHCl_3$ (1:9); detection: with 5% H_2SO_4 at 120° for 5 min) Rf=0.39 (rhodeasapogenin). PPC (solvent system: AcOEt–pyridine–H_2O (2:1:2); detection: with aniline hydrogenphthalate at 120° for 5 min): Rf=0.50 (L-rhamnose), 0.39 (D-xylose).

Partial Hydrolysis of Convallasaponin-D (I)——A mixture of I (4.1 g) and 0.1 N HCl (400 ml) was refluxed on a water bath for 2 hr. The precipitates formed were filtered, dissolved in $CHCl_3$, washed with 5% aq. Na_2CO_3, then with water dried over Na_2SO_4, and evaporated to give a residue (2.3 g). The hydrolyzate was submitted to chromatography on alumina (Table II) and the fraction eluted with MeOH–$CHCl_3$ (5:95) gave white crystalline powder (801 mg) which was recrystallized from MeOH–$CHCl_3$ to colourless fine needles (X), mp 236—238.5°. $[\alpha]_D^{21}$: −50° (c=0.62, MeOH–$CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_9$: C, 66.64; H, 9.15. Found: C, 66.81; H, 9.06 The acetate prepared by the usual method was recrystallized from aq. EtOH to give colourless fine needles, mp 219—221°. $[\alpha]_D^{17}$: −48° (c=0.57, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1743 (AcO). *Anal.* Calcd. for $C_{43}H_{64}O_{14}$: C, 64.16; H, 8.02. Found: C, 64.49; H, 7.88.

Hydrolysis of X——In a similar way to VII, X (84 mg) was hydrolyzed to give rhodeasapogenin (46 mg), mp 280—283°, which was identical with the authentic specimen in mixed melting point as well as infrared spectrum. An aqueous layer of the hydrolyzation mixture was neutralyzed with Amberlite IR-4B and concentrated solution was submitted to paper chromatography for the identification of sugar. PPC (solvent system: AcOEt–pyridine–H_2O (2:1:2); detection: aniline hydrogenphthalate at 120° for 5 min): Rf=0.27 (D-glucose).

Permethylation of X——In a similar way to III, X (480 mg) was completely methylated to give a product having no absorption band due to hydroxyl group in infrared spectrum. The permethylate formed was submitted to chromatography on alumina and the fraction eluted with *n*-hexane–benzene (1:1) gave white residue (390 mg) which was recrystallized from *n*-hexane to give fine colourless needles (XI), mp 174—175.5°. $[\alpha]_D^{17}$: −51° (c=0.49, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{64}O_8$: C, 68.64; H, 9.70. Found: C, 68.30; H, 9.91.

Hydrolysis of XI——After XI (310 mg) was refluxed in 3.3% HCl/MeOH (30 ml) on a water bath for 3 hr, water (30 ml) was added and the reaction mixture was refluxed for another 3 hr. Methanol was evaporated and the precipitate was filtered, dissolved in $CHCl_3$, washed with 5% aq. Na_2CO_3, then with water, dried over Na_2SO_4. Chloroform was evaporated and the residue (350 mg) was submitted to chromatography on alumina. The fractions eluted with *n*-hexane–benzene (1:1) and (2:1) gave white crystalline powders (322 mg) which was recrystallized from *n*-hexane–$CHCl_3$ to give fine colourless needles (XII), mp 235—237.5°. $[\alpha]_D^{19}$: −67° (c=0.68, MeOH). *Anal.* Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.09; H, 10.14.

An aqueous layer of the hydrolyzation mixture was neutralized with Amberlite IR-4B and concentrated under reduced pressure to give a syrup (560 mg) which was identified as 2,3,4,6-tetra-O-Me-D-glucopyranose by thin-layer and gas chromatography. TLC (Wako gel; solvent system: ether–toluene (2:1); detection: with 5% H_2SO_4 at 120° for 5 min.): Rf=0.44. GLC (Fig. 2): t_R=6.70 min (authentic specimen: 6.68).

Oxidation of XII——A mixture of XII (76 mg), AcOH (8 ml), CrO_3 (80 mg), and a drop of water was left for 48 hr at room temperature. After adding MeOH (10 ml) and one drop of conc. H_2SO_4 to a reaction mixture, MeOH was evaporated off and the precipitates were extracted with ether, washed with water, then with 5% aq. Na_2CO_3, again with water, dried over Na_2SO_4. Evaporation of ether gave a residue which was treated with alumina and recrystallized to fine colourless needles (XIII), mp 265.5—268°. $[\alpha]_D^{23}$: −98° (c=0.26, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1701 (C=O). ORD (Fig. 1): a=−116.5 (c=0.25, MeOH). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.43; H, 9.68.

Permethylation of I——The saponin (3.05 g) was completely methylated by a similar method for III to give the permethylate (2.90 g) which was submitted to chromatography on alumina. The fraction eluted with *n*-hexane–benzene gave residue (2.71 g) which was recrystallized from *n*-hexane to give fine colourless needles (IX), mp 143—144.5°. $[\alpha]_D^{17}$: −63° (c=0.57, MeOH). *Anal.* Calcd. for $C_{61}H_{104}O_{21}$: C, 62.43; H, 8.93. Found: C, 62.50; H, 8.78.

Hydrolysis of IX——After IX (450 mg) was refluxed in 3.3% HCl/MeOH (50 ml) on a water bath for 3 hr, water (50 ml) was added and the reaction mixture was refluxed again for another 3 hr. Evapolation of MeOH gave the precipitates which were filtered, washed with water giving white powders (159 mg), and recrystallized from MeOH–$CHCl_3$ to fine colourless needles, mp 280—281.5°, identical with the authentic rhodeasapogenin in melting point as well as infrared spectrum.

An aqueous layer of the hydrolyzation mixture was neutralized with Amberlite IR-4B and concentrated *in vacuo* to give a syrup (241 mg) which was submitted to gas chromatography for the identification of partial-

ly methylated sugars (Fig. 2): t_R=4.39 min (2,3,4-tri-O-Me-L-rhamnopyranose), 5.25 (2,4-di-O-Me-L-rhamnopyranose), 6.65 (3,4-di-O-Me-D-xylopyranose), 6.70 (2,3,4,6-tetra-O-Me-D-glucopyranose).

Azoyl Derivatives of Sugars——1) Azoylation: A mixture of dry pyridine (10 ml, distilled over BaO) and finely powdered azoyl chloride (1.2 g) was shaken for 1 hr at room temperature. The dried sugar portion (129 mg) obtained in the complete hydrolysis of I was added to the mixture. After mechanical shaking for 2 days at room temperature, absolute MeOH (1 ml, dried over BaO and distilled) was added to the solution which was then shaken for another 2 days at room temperature. Solids separated were collected, dissolved in $CHCl_3$, precipitated with absolute EtOH, collected again and washed with absolute EtOH. Red powders (dried over conc. H_2SO_4 *in vacuo*, 511 mg) obtained were dissolved in *n*-hexane–benzene–$CHCl_3$ (1:1:1) and submitted to chromatography on silica gel giving three coloured fractions, Nos. 1,2, and 3.

2) L-rhamnose tetraazoate: The residue of the fraction No. 1 was recrystallized from benzene–$CHCl_3$ to fine orange needles (184 mg), mp 156—157°, $[\alpha]_D^{18}$: +897° (c=0.98, pyridine), identical with the synthetic specimen in the mixed melting point. *Anal.* Calcd. for $C_{58}H_{44}O_9N_8$: C, 69.87; H, 4.45; N, 11.24. Found: C, 69.64; H, 4.50; N, 11.01.

3) D-xylose tetraazoate: The residue of the fraction No. 2 was recrystallized from benzene–$CHCl_3$ to fine reddish needles, mp 152°, $[\alpha]_D^{18}$: +233° (c=0.78, pyridine), identified by the direct comparison with authentic specimen. *Anal.* Calcd. for $C_{57}H_{42}O_9N_8$: C, 69.64; H, 4.31; N, 11.40. Found: C, 69.31; H, 4.19; N, 11.28.

4) D-glucose pentaazoate: The residue of the fraction No. 3 was recrystallized from benzene–$CHCl_3$ to fine orange needles, mp 251°, $[\alpha]_D^{18}$: −42° (c=0.69, pyridine), identified by the direct comparison with synthetic specimen. *Anal.* Calcd. for $C_{71}H_{52}O_{11}N_{10}$: C, 69.82; H, 4.29; N, 10.47. Found: C, 69.49; H, 4.13; N, 10.63.

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