from the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley. Upon hydrolysis convallasaponin-A and -B gave new steroidsapogenins, convallagenin-A, m.p. 268~269°, $[\alpha]_D^{20}$ −28.0° ($CHCl_3$-MeOH) and convallagenin-B, m.p. 277~278°, $[\alpha]_D^{21}$ −42.7° ($CHCl_3$-MeOH) respectively, together with L-arabinose as the sugar moiety. Convallasaponin-C afforded isorhodeasapogenin, L-arabinose and L-rhamnose.

It is likely that convallagenin-A and -B belong to tri- and tetrahydroxy-25L-steroidsapogenin respectively, having one unacetylable hydroxyl group in each molecule.

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10. Michiya Kimura, Masahiko Tohma, and Itsuo Yoshizawa : Constituents of Convallaria. V.*1 On the Structure of Convallasaponin-C.

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In the previous paper[1] of this series, it was reported that three new steroidsaponins, convallasaponin-A, -B and -C were obtained from the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley. The present paper describes the structure elucidation of convallasaponin-C (I) which gave octaacetate. On saponification, the acetate regenerated the original saponin (I) revealing that convallasaponin-C could be regarded as a pure saponin.

Hydrolysis of convallasaponin-C with 2*N* hydrochloric acid in 50% ethanol for 6 hours afforded L-arabinose, L-rhamnose and aglycone, isorhodeasapogenin (25D-5β-spirostane-1β,3β-diol).[1,2] The sugar moiety thus obtained was treated with *p*-phenylazobenzoyl chloride (azoyl chloride)[3,4] to give a mixture of *p*-phenylazobenzoyl derivatives, from which tetraazoates of L-arabinose and of L-rhamnose were obtained in the molar ratio of 1:2 by chromatographic method using silicic acid. Furthermore, according to the Sweeley's procedure[5] the sugar moiety was treated in pyridine with hexamethyldisilazane and trimethylchlorosilane to afford a mixture of trimethylsilyl ethers which was submitted to gas chromatography (Fig. 1) : the molar ratio

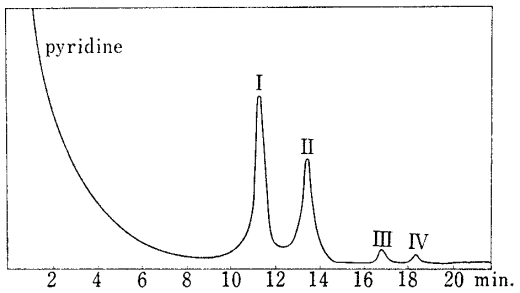

Fig. 1. Gas Chromatographic Separation of Sugar Trimethylsilyl Ethers

5% nitrile silicone (XF-1150) on Gaschrom P, 120°, 30 ml. N_2/min.

I : α-L-rhamnose (11.34)
II : α-L-arabinose (13.77)
III : β-L-rhamnose (17.00)
IV : β-L-arabinose (18.44)

*1 Part IV : This Bulletin, **14**, 50 (1966).
*2 Nishi-5-chome, Kita-12-jo, Sapporo (木村道也, 藤間貞彦, 吉沢逸雄).

1) M. Kimura, M. Tohma, I. Yoshizawa : This Bulletin, **14**, 50 (1966).
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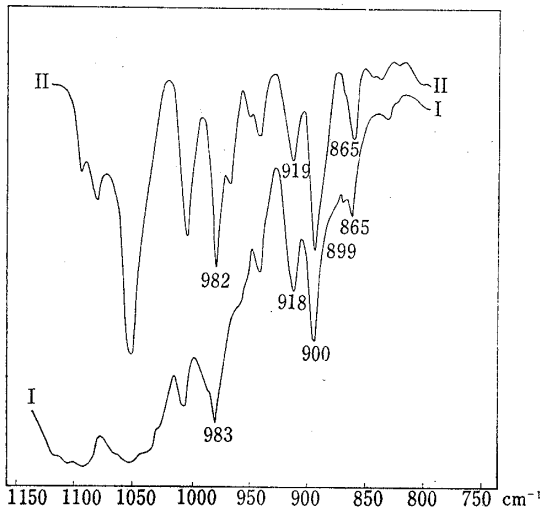

Fig. 2. Infrared Absorption Spectra of Convallasaponin-C and Isorhodeasapogenin

I : convallasaponin-C (KBr)
II : isorhodeasapogenin (KBr)

of these two sugars was roughly determined also as 1:2. Consisting of one mole of each of isorhodeasapogenin and L-arabinose and two moles of L-rhamnose, convallasaponin-C has, therefore, the molecular formula $C_{44}H_{72}O_{16}$, which is also supported by elemental analysis of the saponin itself and its acetate and further by the isolation of two prosapogenins after mild hydrolysis of the saponin described later (Table I).

As described by Wall, *et al.*[6] against the view of Marker and Lopez[7] and as previously shown by the infrared absorption spectrum of convallasaponin-C[1] (Fig. 2), the aglycone, isorhodeasapogenin, suffered no structural change on hydrolysis under experimental conditions described later. Since isorhodeasapogenin has two hydroxyl groups at C_1 and C_3, the position of glycosidic linkage was determined. Convallasaponin-C was completely methylated by the Kuhn's method[8] using methyliodide and silveroxide in dimethylformamide to yield permethylate (II) which on hydrolysis gave isorhodeasapogenin monomethylate (III) (Chart 1). Oxidation of III with chromium trioxide in acetic acid gave a ketone (IV)

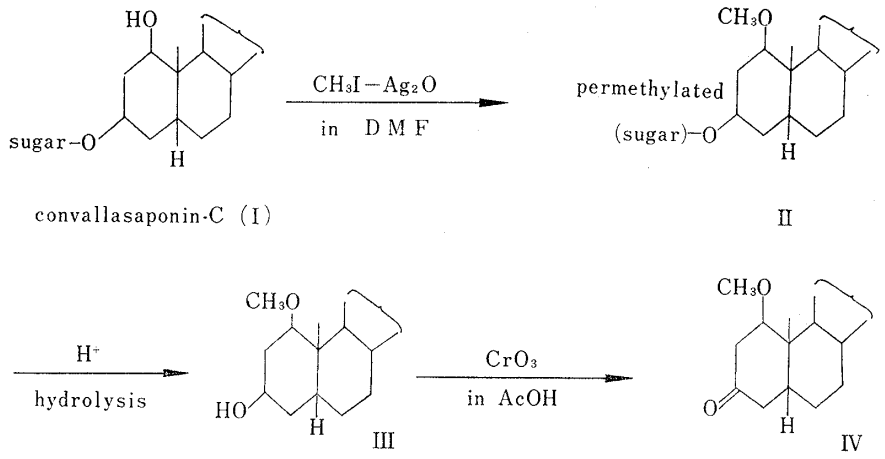

Chart 1.

whose infrared spectrum showed strong band at 1719 cm^{-1} corresponding to the 3-keto group in A/B-*cis* junction. Jones, *et al.*[9] reported that many 3-keto steroids of A/B-*cis* junction show the carbonyl stretching absorption between 1714~1719 cm^{-1}, which occurs at the region of lower wave numbers between 1704~1709 cm^{-1} in the case of

6) M.E. Wall, M.L. McClennan, C.R. Eddy, M.E. Klumpp : Anal. Chem., **24**, 1337 (1952); C.R. Eddy, M.E. Wall, M.K. Scott : *Ibid.*, **25**, 266 (1953).
7) M.F. Marker, J. Lopez : J. Am. Chem. Soc., **69**, 2389 (1947).
8) R. Kuhn : Angew. Chem., **67**, 32 (1955).
9) R.N. Jones, P. Humphries, K. Dobriner : J. Am. Chem. Soc., **72**, 956 (1950); *Ibid.*, **70**, 2024 (1948); R.N. Jones, F. Herling : J. Org. Chem., **19**, 1252 (1954); R.N. Jones, D.A. Ramsey, D.S. Keir, K. Dobriner : J. Am. Chem. Soc., **74**, 80 (1952).

1-keto steroids of this type.[10] Optical rotatory dispersion curve of Ⅳ showed a weakly negative Cotton effect with the molecular amplitude of −29 (Fig. 3). According to the study of W. Moffitt, *et al.*[11] both 1-keto and 3-keto steroids of A/B-*cis* junction show negative Cotton effect, the former having greater values of molecular amplitude than those of the latter; namely, 1-ketone : −90～−136 and 3-ketone : −23～−27. These results revealed that the ketone (Ⅳ) is a 3-keto steroid and the sugar moiety of the saponin (I) should, therefore, be linked with aglycone at the hydroxyl group of C_3, not of C_1.

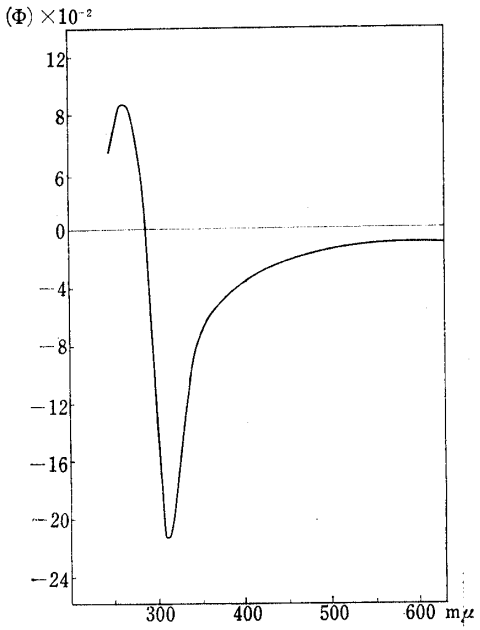

Fig. 3. Optical Rotatory Dispersion Curve of Ketone (Ⅳ)

Acid hydrolysis of convallasaponin-C under various conditions was carried out, followed by thin layer chromatographic examination of the products (Table I and Ⅱ) : it was shown that complete hydrolysis occured only on boiling with 1*N* hydrochloric acid in 50% ethanol for 4 hours and that under milder conditions partial hydrolysis occured, yielding two major products, prosapogenin-I and prosapogenin-Ⅱ. The former gave isorhodeasapogenin and L-arabinose on further acid hydrolysis. The latter gave the same aglycone together with L-arabinose and L-rhamnose. Consequently it is clear that the aglycone should be

Table I. Hydrolysis of Convallasaponin-C

1) Effect of Acid Concentration

(in boiling 50%-EtOH for 6 hr.)

	HCl							H_2SO_4				
	2*N*	1*N*	*N*/2	*N*/5	*N*/10	*N*/50	*N*/100	1*N*	*N*/2	*N*/5	*N*/10	*N*/50
Convallasaponin-C	−	−	+	+	++	+++	+++	−	+	+	+	++
Aglycone	+++	+++	++	++	+	−	−	+++	+++	++	++	+
Prosapogenin-I	−	−	++	+++	++	−	−	−	+	+++	+++	+
Prosapogenin-Ⅱ	−	−	++	++	++	−	−	−	+	++	+++	++
L-Arabinose	+++	+++	+	+	−	−	−	+++	++	++	+	+
L-Rhamnose	+++	+++	+++	++	+	−	−	+++	+++	++	++	+

2) Effect of Time

(with 1*N* HCl in 50%-EtOH)

Time (hr.)	0.5	1	2	3	4	6	8
Convallasaponin-C	+++	++	++	+	−	−	−
Aglycone	+	++	+++	+++	+++	+++	+++
Prosapogenin-I	+	+++	++	+	+	−	−
Prosapogenin-Ⅱ	++	++	+	+	−	−	−
L-Arabinose	+	+	++	++	+++	+++	+++
L-Rhamnose	+	+++	+++	+++	+++	+++	+++

10) F. Sollmann, Ch. Tamm : Helv., **39**, 1340 (1956).
11) W. Moffitt, R. B. Woodward, A. Moscowitz, R. S. Wklyne, C. Djerassi : J. Am. Chem. Soc., **83**, 4013 (1961).

Table II. Chromatographic Separation of Partial Hydrolyzate of Convallasaponin-C

Fraction	Solvent	Vol. (L.)	Weight (mg.)	Rf
1	$CHCl_3$	3.0	220	0.88
2	$CHCl_3$ –2%MeOH	1.8	117	0.88, 0.42
3	〃 –5% 〃	1.5	560	0.42
4	〃 –10% 〃	1.0	100	0.42
5	〃 –20% 〃	1.1	15	0.42, 0.24
6	〃 –30% 〃	1.5	159	0.24
7	〃 –50% 〃	0.5	29	0.24, 0.00
8	MeOH			0.00

Rf-values in thin-layer chromatography were determined using Wako-Gel B-5 and 2% MeOH-$CHCl_3$ as adsorbent and solvent respectively. Detection was made by 5% H_2SO_4 with heating. Spots were identified as follows:

0.88: isorhodeasapogenin
0.42: prosapogenin-I
0.24: prosapogenin-II
0.00: convallasaponin-C

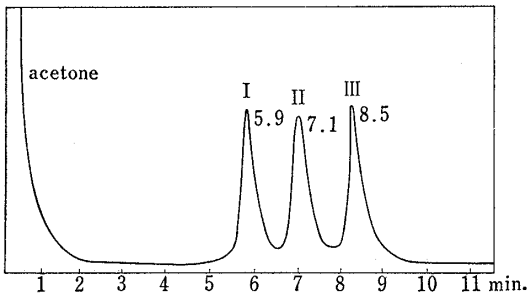

Fig. 4. Gas Chromatographic Separation of Partially Methylated Sugars

I: 2,3,4-tri-O-methyl-L-rhamnopyranose
II: 2,4-di-O-methyl-L-rhamnopyranose
III: 3,4-di-O-methyl-L-arabopyranose

1.5% SE-30 on chromosorb W, 180°, 30 ml. N_2/min.

linked directly with L-arabinose, not with L-rhamnose. Upon acid hydrolysis the fully methylated convallasaponin-C (II) gave three methylated sugars which could be detected by gas chromatography as well as paper and thin-layer chromatography. The molecular ratio of these methylated sugars was determined roughly as 1:1:1 (Fig. 4). These methylated sugars were separated on column chromatography over celite into three partially methylated sugars which were identified as 3,4-di-O-methyl-L-arabopyranose, 2,4-di-O-methyl-L-rhamnopyranose and 2,3,4-tri-O-methyl-L-rhamnopyranose respectively, by means of the direct comparisons with authentic specimens (Table III). Since convallasaponin-C did not reduce Fehling's solution, all of the sugar components should be linked through their potential aldehyde groups. By applying Klyne rule on the cardiac glycoside[12] Kawasaki, *et al.*[13] have determined the structure of

Table III. Chromatographic Separation of Methylated Sugars

Fraction	Solvent	Vol. (L.)	Weight (mg.)	Rf
1	H_2O	0.6	20	0.45
2	2% EtOH–H_2O	1.0	28	0.45
3	5% 〃 – 〃	0.8	16	0.45, 0.68
4	10% 〃 – 〃	1.4	61	0.68
5	15% 〃 – 〃	1.5	13	0.68
6	20% 〃 – 〃	1.5	16	0.68, 0.80
7	50% 〃 – 〃	1.0	34	0.80
8	EtOH	0.5	32	0.80

Rf-values in thin-layer chromatography were determined using Wako-Gel B-5 and ether-toluene (2:1) as adsorbent and solvent respectively. Detection was made by 5% H_2SO_4 with heating. Spots were identified as follows:

0.45: 3,4-di-O-methyl-L-arabinose
0.68: 2,4-di-O-methyl-L-rhamnose
0.80: 2,3,4-tri-O-methyl-L-rhamnose

12) W. Klyne: Biochem. J., **47**, xli (1950).
13) T. Kawasaki, T. Yamauchi: This Bulletin, **10**, 703 (1962).

Table IV. Comparison of Molecular Rotation

	α_D (°C)	M_D (°C)	M_D (°C)
Isorhodeasapogenin	−71	−307	+ 61
Prosapogenin-I	−37	−246	−373
Prosapogenin-II	−87	−619	−145
Convallasaponin-C	−89	−764	
α-Methyl-L-arabopyranoside	: M_D = + 29		
β-Methyl-L-arabopyranoside	: +403		
α-Methyl-L-rhamnopyranoside	: −111		
β-Methyl-L-rhamnopyranoside	: +170		

Optical rotations were determined in MeOH-$CHCl_3$.

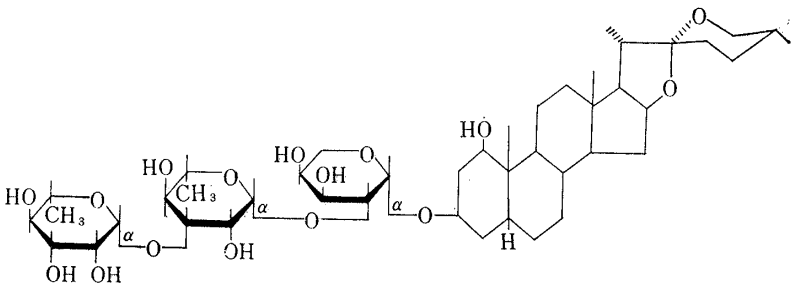

Chart 2. Structure of Convallasaponin-C

a steroid saponin, dioscin. As each sugar of convallasaponin-C belongs to L-series, all of the glycosidic linkages were similarly presumed to be in α-form (Table IV). From the results described above and the analysis of the molecular rotation differences between isorhodeasapogenin, prosapogenin-I, -II and the original saponin (I), a conclusion was drawn that convallasaponin-C might reasonably be formulated as isorhodeasapogenin (3) α-L-rhamnopyranosyl (1→3 rham.)-α-L-rhamnopyranosyl (1→2 arab.)-α-L-arabopyranoside.

Experimental

Saponification of Convallasaponin-C Octaacetate——The octaacetate[1] (120 mg.) was boiled with 5% KOH-MeOH (15 ml.) for 2 hr., diluted with water and extracted with BuOH. Crystallization of the BuOH-extract from MeOH gave convallasaponin-C[1] (78 mg.), m.p. 217~221°.

Hydrolysis of Convallasaponin-C (I)——i) General procedure : I (20~30 mg.) was refluxed with 5 ml. of the hydrolytic agent.[1] Water was added to the hydrolyzate and the insoluble products were separated by filtration, washed with water, dried *in vacuo*. This was examined by thin-layer chromatography using 2% MeOH-$CHCl_3$ as the solvent. Effects of acid concentration and period of time for hydrolysis are summerized in Table I.

ii) Partial hydrolysis of I : A mixture of hydrolyzate (1.7 g.) obtained on hydrolysis of I (3.0 g.) with 1*N* H_2SO_4 in 50% EtOH for 1 hr. with refluxing, was dissolved in $CHCl_3$ and submitted to chromatography on alumina (50 g.) previously saturated with $CHCl_3$-benzene (1:1). Elution was made by $CHCl_3$-MeOH mixture with increasing MeOH content. Each eluate was evaporated to dryness and examined by thin-layer chromatography. Two kinds of prosapogenin were obtained as summerized in Table II.

Prosapogenin-I——The residue of Fraction 3 (Table II) was crystallized from MeOH-$CHCl_3$ into colorless fine needles, m.p. 246~248°, $[\alpha]_D^{19}$ −37° (c=0.48, MeOH-$CHCl_3$). *Anal.* Calcd. for $C_{32}H_{52}O_8$: C, 68.05; H, 9.28. Found : C, 68.14; H, 9.24. The acetate was prepared by the usual method and crystallized from aqueous alcohol to give colorless fine needles, m.p. 117~121°, $[\alpha]_D^{19}$ −35.7° (c=0.35, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{60}O_{12}$: C, 65.55; H, 8.25. Found : C, 65.32; H, 8.16.

Hydrolysis of Prosapogenin-I : The sapogenin (120 mg.) was refluxed with 1*N* HCl in 50% EtOH (15 ml.). The hydrolyzate was treated as described above on the hydrolysis of I giving isorhodeasapogenin (72 mg.), m.p. 237~242°. L-Arabinose was detected by aniline-phthalate reagent on paper chromatogram (Toyo-Roshi No. 51; AcOEt-pyridine-H_2O=2:1:2; Rf : 0.34).

Prosapogenin-II——The residue of Fraction 5 (Table II) was crystallized from MeOH-$CHCl_3$ to colorless fine needles, m.p. 277~281°, $[\alpha]_D^{19}$ −87°(c=0.85, MeOH-$CHCl_3$). *Anal.* Calcd. for $C_{38}H_{62}O_{12}$: C, 64.20; H, 8.79. Found: C, 64.53; H, 8.95. The acetate was prepared by the usual method and crystallized from aqueous alcohol to colorless fine needles, m.p. 118~121°, $[\alpha]_D^{21}$ −70.7°(c=0.70, $CHCl_3$). *Anal.* Calcd. for $C_{50}H_{74}O_{18}$: C, 62.35; H, 7.74. Found: C, 62.26; H, 7.79.

Hydrolysis of prosapogenin-II: The sapogenin (140 mg.) was refluxed with 1*N* HCl in 50% EtOH to give the aglycone (81 mg.), isorhodeasapogenin, m.p. 240~243°. L-Arabinose and L-rhamnose were detected and identified by paper chromatography.

Azoyl Derivatives of Sugars——i) Azoylation: Finely powdered azoyl chloride (1.0 g.) was added to pyridine (20 ml.; distilled over BaO) and cooled to −15°. The mixture was shaken for 30 min. at the same temperatur and the dried sugar portion (100 mg.) obtained after complete hydrolysis of convallasaponin-C[1] was added. After keeping for 4 days at 20° with frequent shaking, the mixture was cooled again to −15° and 1 ml. of absolute methanol (dried over BaO and distilled) was added. It was then kept for 3 hr. at the same temperature followed by frequent shaking for 12 hr. at room temperature. Solid separated was collected, dissolved in $CHCl_3$, precipitated with absolute EtOH, collected again and washed with absolute EtOH. The red powders thus obtained were dried under reduced pressure over conc. H_2SO_4. Yield: 390 mg. Azoyl derivatives thus obtained were dissolved in a mixture of benzene-hexane-$CHCl_3$ (1:1:1) and submitted to chromatography on silica gel giving two colored fractions (No. 1 and 2).

ii) L-Rhamnose tetraazoate: The residue of fraction No. 1 thus obtained was crystallized from $CHCl_3$ into fine orange needles (215 mg.), m.p. 158°, $[\alpha]_D^{21}$ +917°(c=0.61, pyridine). *Anal.* Calcd. for $C_{58}H_{44}O_9N_8$: C, 69.87; H, 4.45; N, 11.24. Found: C, 69.53; H, 4.25; N, 11.43. This was identified as L-rhamnose tetraazoate by direct comparison with the synthetic specimen.

iii) L-Arabinose tetraazoate: The residue of fraction No. 2 described above was crystallized from MeOH-$CHCl_3$ into reddish fine needles (100 mg.), m.p. 261°, $[\alpha]_D^{21}$ +620°(c=0.52, pyridine). *Anal.* Calcd. for $C_{57}H_{42}O_9N_8$: C, 69.64; H, 4.31; N, 11.40. Found: C, 69.44; H, 4.38; N, 11.69. This was identified as L-arabinose tetraazoate by direct comparison with the synthetic specimen.

Permethylale (II) of Convallasaponin-C——i) Preparation: To convallasaponin-C (3.0 g.) in dimethylformamide (100 ml.) were added Ag_2O (20 g.) and CH_3I (20 ml.) and the mixture was kept for 90 hr. under stirring. After the precipitates were filtered off, the filtrate was shaken with additionl Ag_2O (15 g.) and CH_3I (15 ml.) for 90 hr. and filtered again. The same treatment was further repeated until no absorption of hydroxyl group was found in the infrared spectrum of the product. Crude convallasaponin-C permethylate thus obtained was dissolved in $CHCl_3$, washed with 1% KCN and then with water, dried over Na_2SO_4, and evaporated. The residue (3.2 g.) was submitted to chromatography on alumina using benzene as a solvent, followed by crystallization from EtOH-benzene, affording the permethylate as white powder, m.p. 78~80° (decomp.), $[\alpha]_D^{20}$ −71°(c=0.94, MeOH). *Anal.* Calcd. for $C_{52}H_{88}O_{16}$: C, 64.43; H, 9.15. Found: C, 64.52; H, 9.28.

ii) Hydrolysis of II: II (1.8 g.) was hydrolyzed with boiling 1*N* HCl in 50% EtOH for 6 hr. The reaction mixture was concentrated under reduced pressure and then water was added to give precipitates which were filtered off, washed with water, and dried *in vacuo* over conc. H_2SO_4. Yield: 740 mg. The aqueous solution was neutralyzed with Ag_2CO_3 and concentrated to a syrup (670 mg.).

Isorhodeasapogenin Monomethylate (III)——The aglycone portion obtained by the hydrolysis of II described above was crystallized from MeOH-$CHCl_3$ to colorless fine needles, m.p. 230~232°, $[\alpha]_D^{19}$ −90.9° (c=0.22, MeOH-$CHCl_3$). *Anal.* Calcd. for $C_{28}H_{46}O_4$: C, 75.27; H, 10.38. Found: C, 75.17; H, 10.20.

Oxidation of III——CrO_3 (60 mg.) and one drop of water were added to a solution of III (55 mg.) in AcOH (6 ml.), and the mixture was then left for 48 hr. at room temperature. After adding 5 ml. of MeOH and one drop of conc. H_2SO_4 small amount of water was added to the reaction mixture, followed by extraction with ether. The extract was washed with water, 5% Na_2CO_3 and again with water, then dried over Na_2SO_4 and evaporated. The residue (41 mg.) was crystallized from acetone to colorless needles (IV), m.p. 211~213°, $[\alpha]_D^{23}$ −13.7°(c=0.37, MeOH-$CHCl_3$), ORD: Peak, 260 mμ; λ_0, 287 mμ; Trough, 310 mμ (Fig. 3), IR $\nu_{max}^{CHCl_3}$: 1719 cm^{-1}(C=O). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.61; H, 9.97. Found: C, 75.51; H, 10.08.

Partially Methylated Derivatives of Sugars——The sugar syrup obtained by the hydrolysis of II was examined by thin-layer chromatography (TLC) as well as paper chromatography (PPC) and three spots of partially methylated sugars were detected. PPC (Toyo-Roshi No. 51; 20 hr. at 20~25°; BuOH-EtOH-H_2O=5:1:4; detected by aniline-phthalate): Rf 0.82, 0.75, and 0.57. TLC (Wako-Gel; ether-toluene=2:1; detected by H_2SO_4): Rf 0.80, 0.68, and 0.45.

i) Isolation: The sugar syrup (290 mg.) mentioned above was submitted to column chromatography using charcoal (previously treated with conc. HCl)-celite (10 g.:10 g.). Elution was made with H_2O-EtOH mixture of increasing EtOH content up to 100% (Table III). Each fraction was evaporated and examined by TLC.

ii) 3,4-Di-O-methyl-L-arabopyranose : Fraction 1 and 2 (Table Ⅲ) gave a syrup, $[\alpha]_D^{19}$ +120°(c=0.50, H_2O). *Anal.* Calcd. for $C_7H_{14}O_5$: C, 47.18; H, 7.92. Found : C, 47.01; H, 7.89. Identification as 3,4-di-O-methyl-L-arabopyranose was made by comparing with synthetic specimen in PPC and TLC.

iii) 2,4-Di-O-methyl-L-rhamnopyranose : The residues from Fraction 4 and 5 (Table Ⅲ) were treated with petroleum ether into colorless powder, m.p. 83°, $[\alpha]_D^{19}$ +9.0°(c=0.58, EtOH). *Anal.* Calcd. for $C_8H_{16}O_5$: C, 49.99; H, 8.39. Found : C, 50.12; H, 8.21. This was identified as 2,4-di-O-methyl-L-rhamnopyranose in PPC and TLC by comparing with synthetic specimen.

iv) 2,3,4-Tri-O-methyl-L-rhamnopyranose : Fraction 7 and 8 (Table Ⅲ) gave a syrup, $[\alpha]_D^{19}$ +19° (c=0.42, $CHCl_3$). *Anal.* Calcd. for $C_9H_{18}O_5$: C, 52.41; H, 8.80. Found : C, 52.16; H, 8.63. Identification as 2,3,4-tri-O-methyl-L-rhamnopyranose was made in comparison with synthetic specimen in PPC and TLC.

Gas Chromatography of Sugar Portion——A Shimazu GC-IB Gas Chromatograph equipped with a hydrogen flame detector was used throughout the work. The column was a stainless steel 225 cm. × 4 mm. in diameter packed with Gas-Chrom P coated with 5% nitrile silicone (XF-1150) as liquid phase for free sugars (Fig. 1) or packed with Chromosorb W coated with 1.5% SE-30 for partially methylated sugars (Fig. 4). Nitrogen was used as a carrier gas at a flow rate of 30 ml. per minute. Trimethylsilylated samples described below were dissolved in hexane and 4 μl. of the solution was introduced.

Trimethylsilylation of sugar portion : Aqueous portion of hydrolyzate was neutralyzed (Ag_2CO_3 for HCl and $BaCO_3$ for H_2SO_4) and concentrated to a syrup which was then dried over P_2O_5 at 70～80° for 90 hr. Sugar mixture thus obtained (2.5 mg.) was dissolved in absolute pyridine (1 ml.) and hexamethyldisilazane (0.2 ml.) and trimethylsilyl chloride (0.1 ml.) were added followed by shaking for 5 min. The reaction mixture was then evaporated under reduced pressure on water bath to colorless residue which was dissolved in hexane (1.0 ml.) for gas chromatography.

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Summary

Convallasaponin-C (I) from *Convallaria keisukei* Miq. was completely hydrolyzed affording isorhodeasapogenin (25D-5β-spirostane-1β,3β-diol), L-arabinose and L-rhamnose. The molar ratio of these sugars was found to be 1:2 on the basis of separating their tetraazoates prepared from the sugar moiety obtained after hydrolysis. This ratio was also indicated by gas chromatography (Fig. 1). Prosapogenin-I and prosapogenin-Ⅱ were obtained by the partial hydrolysis of I. The former was further hydrolyzed into isorhodeasapogenin and L-arabinose which were also obtained with the additional sugar component, L-rhamnose, from prosapogenin-Ⅱ.

Hydrolysis of the permethylate (Ⅱ), fully methylated I, afforded isorhodeasapogenin monomethylate (Ⅲ) which was oxidized to C_3-keto derivative (Ⅳ) by chromium trioxide in acetic acid. Hydrolysis of Ⅱ afforded three partially methylated sugars, 3,4-di-O-methyl-L-arabopyranose, 2,4-di-O-methyl-L-rhamnopyranose and 2,3,4-tri-O-methyl-L-rhamnopyranose in a molar ratio of 1:1:1 (Fig. 4).

Analysis of the molecular rotation differences (Table Ⅳ) between isorhodeasapogenin, prosapogenin-I, -Ⅱ and I would indicate that the glycosidic linkages in these substances are all in the α-form. Consequently the structure of convallasaponin-C might reasonably be formulated as isorhodeasapogenin (3) α-L-rhamnopyranosyl (1→3 rham.)-α-L-rhamnopyranosyl (1→2 arab.)-α-L-arabopyranoside (Chart 2).

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