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Constituents of Convallaria. X.[1] Structures of Convallasaponin-A, -B, and Their Glucosides

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The structures of the steroidal saponins, convallasaponin-A (II), -B (IX), glucoconvallasaponin-A (I) and -B (VIII), which were isolated from the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley, were studied and they were elucidated as convallagenin-A-(3)-α-L-arabopyranoside, convallagenin-B-(5)-α-L-arabopyranoside, convallagenin-A-(3)-β-D-glucopyranosyl (1→2_{arab})-α-L-arabopyranoside, convallagenin-B-(3)-β-D-glucoypranosido, (5)-α-L-arabopyranoside, respectively.

In the previous paper[3] of this series, it was reported that three new steroidal saponins, convallasaponin-A, -B and -C, were isolated from the chloroform and chloroform-ethanol (2:1) extracts of the flowers of *Convallaria keisukei* Miq. (SUZURAN), Japanese lily of the valley. The structures of two new steroidal sapogenins, convallagenin-A[4] from convallasaponin-A as well as convallagenin-B[5] from convallasaponin-B, and of convallasaponin-C[6] were established as 25L,5β-spirostan-1β,3β,5β-triol, 25L,5β-spirostan-1β,3β,4β,5β-tetrol, and isorhodeasapogenin (3)-α-L-rhamnopyranosyl (1→3_{rham})-α-L-rhamnopyranosyl (1→2_{arab})-α-L-arabopyranoside, respectively. The present paper describes the isolation and the structure elucidation of three new steroidal saponins in the aqueous layer (Fraction V) shown in Fig. 1 of the previous paper.[3]

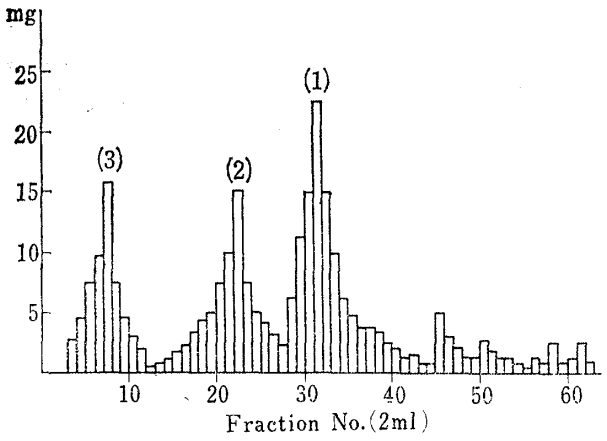

Fig. 1. Separation of Saponins by Gel Filtration

Condition : Sephadex G-15
Column : 13 mm×800 mm
V_o=45 ml, V_1=90 ml, V_t=150 ml
Rate : 10 ml/hr
Sample : 150 mg

The aqueous layer (90.8 g)[3] was roughly separated in eight fractions by alumina chromatography as summarized in Table I. From the fractions 2 and 3, convallatoxol, a cardiac glycoside, was obtained.[7] The methanol and aqueous fractions, 7 and 8, contained three major saponins together with minor components which gave yellow color with cinnamic aldehyde and acetic anhydride-concentrated sulfuric acid reagent, characteristic for steroidal saponins.[8] The separation of these substances was successfully carried out by the gel filtration

1) Part IX: *Chem. Pharm. Bull.* (Tokyo), in press; Communication, *ibid.*, **15**, 129 (1967).
2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo.*
3) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 50 (1966).
4) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 1204 (1967).
5) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 1713 (1967).
6) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 55 (1966).
7) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 226 (1967).
8) C. Sannie, S. Heitz, and H. Lapin, *Compt. rend.*, **233**, 1670 (1951).

method using Sephadex G-15, and three main peaks appeared as shown in Fig. 1, giving pure crystals. Since these saponins thus obtained were recognized to be new compounds from the following studies, they were named glucoconvallasaponin-A (from peak-1), glucoconvallasaponin-B (from peak-2), and convallasaponin-D (from peak-3), respectively.

Table I. Alumina Chromatography of the Fraction V[3]

Fraction	Solvent	Vol. (liter)	Weight (g)
1	MeOH–$CHCl_3$ (10:90)	10	6.78 (oil)
2	MeOH–$CHCl_3$ (15:85)	15	21.28
3	MeOH–$CHCl_3$ (20:80)	12	3.55
4	MeOH–$CHCl_3$ (30:70)	6	2.38
5	MeOH–$CHCl_3$ (40:60)	11	2.70
6	MeOH–$CHCl_3$ (60:40)	16	5.08
7	MeOH	29	11.20
8	H_2O	30	24.24

Convallasaponin-A and Glucoconvallasaponin-A

Upon hydrolysis using β-glucosidase or takadiastase, glucoconvallasaponin-A (I) gave convallasaponin-A (II), $C_{32}H_{52}O_9$. Hydrolysis of I with 1N hydrochloric acid in 50% ethanol for 6 hours afforded L-arabinose, D-glucose, and the aglycone, convallagenin-A,[4] $C_{27}H_{44}O_5$, (III). The molar ratio of the sugar portions was determined as 1:1 by gas chromatography, according to the method reported in the previous paper.[9] Consisting of each one mole of aglycone, L-arabinose and D-glucose, glucoconvallasaponin-A has, therefore, molecular formula $C_{38}H_{62}O_{14}$, that was also supported by elemental analysis of the saponin and its acetate. When this saponin was completely methylated in dimethyl sulfoxide with sodium hydride and methyl iodide according to Hakomori's method,[10] the permethylate (V) was obtained, which showed a sharp absorption band at 3585 cm^{-1} due to the tertiary hydroxyl group in the infrared spectrum; under this condition C-5 angular hydroxyl group was not methylated. On acid hydrolysis, both V and convallasaponin-A-methylate (IV) gave convallagenin-A-monomethylate (VI) which on oxidation with chromium trioxide in acetic

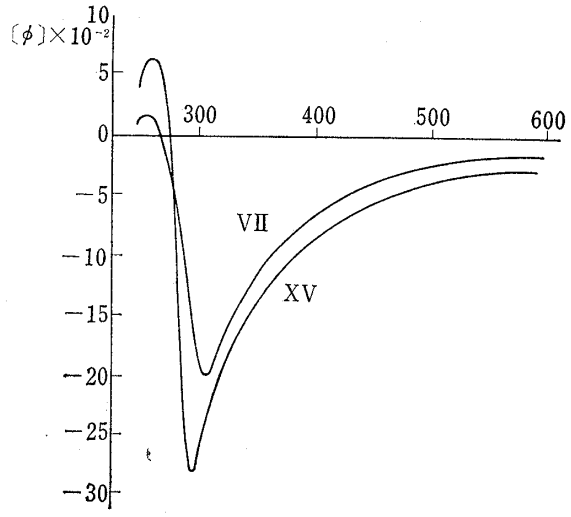

Fig. 2. Optical Rotatory Dispersion Curves of the Ketones (VII) and (XV)

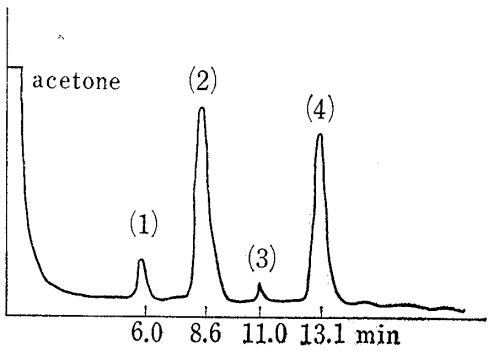

Fig 3. Gas Chromatographic Separation of Partially Methylated Sugars from Glucoconvallasaponin-A Permethylate

(1), (2): 2,3,4,6-tetra-O-methyl-D-glucopyranose
(3), (4): 3,4-di-O-methyl-L-arabopyranose
1.5% SE-30 on chromosorb W, 190°, 30 ml N_2/min

9) M. Kimura, Y. Hattori, I. Yoshizawa, and M. Tohma, *Chem. Pharm. Bull.* (Tokyo), in press.
10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

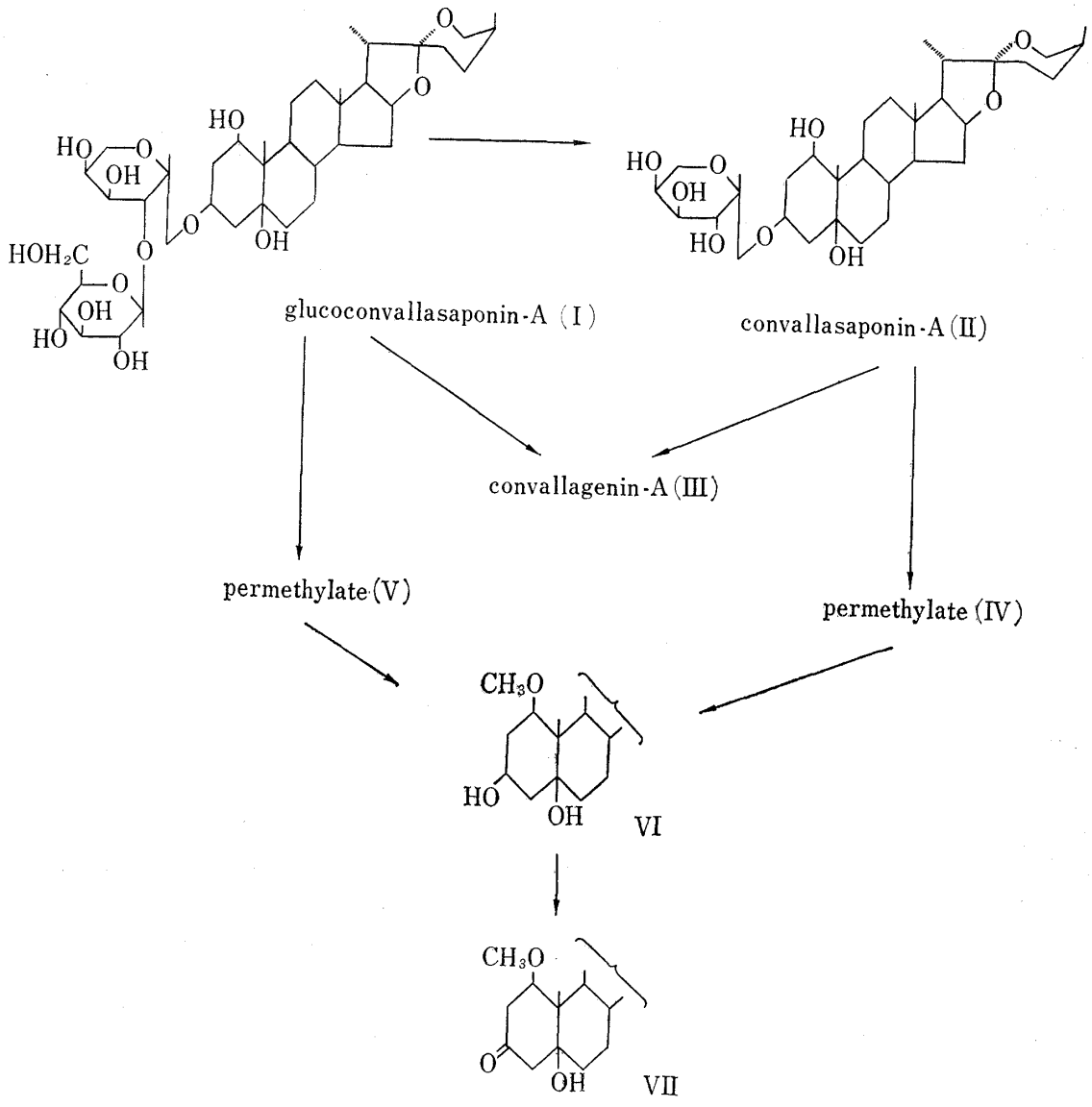

Chart 1

acid gave a ketone (VII) showing an absorption band at 1717 cm^{-1} due to the 3-keto group.[11] Optical rotatory dispersion curve of VII showed a weakly negative Cotton effect with the molecular amplitude of −24.5 as shown in Fig. 2.[12] These results indicated that VII is a 3-keto steroid and that the sugar moiety of glucoconvallasaponin-A as well as convallasaponin-A should be linked with aglycone through the hydroxyl group of C-3, not of C-1, of C-5.

On acid hydrolysis, V gave two partially methylated sugars, 3,4-di-O-methyl-L-arabopyranose and 2,3,4,6-tetra-O-methyl-D-glucopyranose, which were identified by gas chromatography as shown in Fig. 3. Peaks (1) and (2) in the Figure were identical with those of the authentic 2,3,4,6-tetra-O-methyl-D-glucopyranose. The relative retention time of peak (4) was 2.18 in relation to the tetramethyl-D-glucose as 1.00, indicating the presence

11) Many 3-keto steroids of A/B-*cis* junction show the carbonyl streching absorption between 1714—1719 cm^{-1} which appears at the region of lower wave numbers between 1704—1709 cm^{-1} in the case of 1-keto steroids having A/B-*cis* juncture (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.C. Keir and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 80 (1952)).

12) Both 1-keto and 3-keto steroids of A/B-*cis* junction show negative Cotton effect, the former having greater value (−90— −130) of molecular amplitude than those (−23— −27) of the latter (W. Moffitt, R.B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961)).

of 3,4-di-O-methyl-L-arabinose.[13] Therefore, these sugars should be arranged as $gluc_1 \rightarrow_2 arab_1 \rightarrow$C—3-OH (aglycone). According to the Klyne rule,[14] if it is permitted to be applicable in this case, each glycosidic linkage is likely to be in α-form for L-arabinose and β-form for D-glucose as indicated in Table II; the latter form is also supported by the result that this saponin is hydrolyzed by β-glucosidase. A conclusion was thus drawn that convallasaponin-A (II) and glucoconvallasaponin-A (I) may be formulated as convallagenin-A-(3)-α-L-arabopyranoside and convallagenin-A-(3)-β-D-glucopyranosyl $(1\rightarrow 2_{arab})$-α-L-arabopyranoside, respectively.

Table II. Molecular Rotation

	$[\alpha]_D$	M_D	ΔM_D
convallagenin-A	−28°	−126°	
convallasaponin-A	−40°	−232°	−106°
glucoconvallasaponin-A	−41°	−305°	− 73°
convallagenin-B	−43°	−199°	
convallasaponin-B	−56°	−334°	−135°
glucoconvallasaponin-B	−35°	−266°	+ 68°

α-Me-L-arabopyranoside: $M_D = +29°$ β-Me-L-arabopyranoside: $M_D = +403°$
α-Me-D-glucopyranoside: $M_D = +307°$ β-Me-D-glucopyranoside: $M_D = -63°$

Convallasaponin-B and Glucoconvallasaponin-B

Elemental analysis of glucoconvallasaponin-B (VIII) and its acetate gave the formula, $C_{38}H_{62}O_{15}$, to VIII and it was also supported by the facts that on acid hydrolysis it gave convallagenin-B,[5] $C_{27}H_{44}O_6$, (X) and two kinds of sugar, L-arabinose and D-glucose; the molar ratio of the sugars was determined as 1:1 by gas chromatography.[9] The saponin was hydrolyzed also by β-glucosidase or takadiastase to give convallasaponin-B,[3] $C_{32}H_{52}O_{10}$, (IX). On acid hydrolysis, convallasaponin-B-permethylate (XI) gave 1,3,4-trimethyl-convallagenin-B (XII) having no acetylable and no oxidizable hydroxyl group but showing a sharp absorption band at 3585 cm^{-1} due to the tertiary hydroxyl group. The trimethylate (XII) was identical with the authentic specimen obtained by the methylation of X. The acid hydrolysis of glucoconvallasaponin-B-permethylate (XIII), on the other hand, gave a product (XIV) which could be further methylated to XII. Therefore, D-glucose of glucoconvallasaponin-B is not linked to L-arabinose, but should be directly attached to the aglycone.

On oxidation with chromium trioxide in acetic acid, XIV gave a ketone (XV), exhibiting a strong carbonyl absorption band at 1733 cm^{-1} that shifted to a larger extent from 1717 cm^{-1} of the parent ketone, 1-methyl-3-oxoconvallagenin-A (VII), under the influence of C-4-equatorial methoxyl group adjacent to the carbonyl group. In 5α-cholestan-2- and -3-ones the equatorial methoxyl group of this kind was reported to rise the carbonyl absorption frequency, contrary to the axial one which does not have a significant effect.[15] It was also the case in 5β-spirostan series: 2β-methoxy (equatorial)-25D-5β-spirostan-3-one[16] showed an absorption band at 1732 cm^{-1} contrary to that at 1718 cm^{-1} of 25D-5β-spirostan-3-one and the isomeric 3α-methoxy (equatorial)-25D-5β-spirostan-2-one gave the value of 1733 cm^{-1} in contrast to that of 1719 cm^{-1} of 25D-5β-spirostan-2-one. The similar effect was also considered on the acetoxyl group.[17] Optical rotatory dispersion curve of XV showed a

13) B.O. Aspinall, *J. Chem. Soc.*, **1963**, 1676.
14) W. Klyne, *Biochem. J.*, **47**, xli (1950).
15) S.S. Stradling and D.S. Tarbell, *J. Chem. Soc.*, **1964**, 1170.
16) T. Kawasaki, I. Nishioka, T. Komori, T. Yamauchi, and K. Miyahara, *Tetrahedron*, **21**, 299 (1965).
17) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).

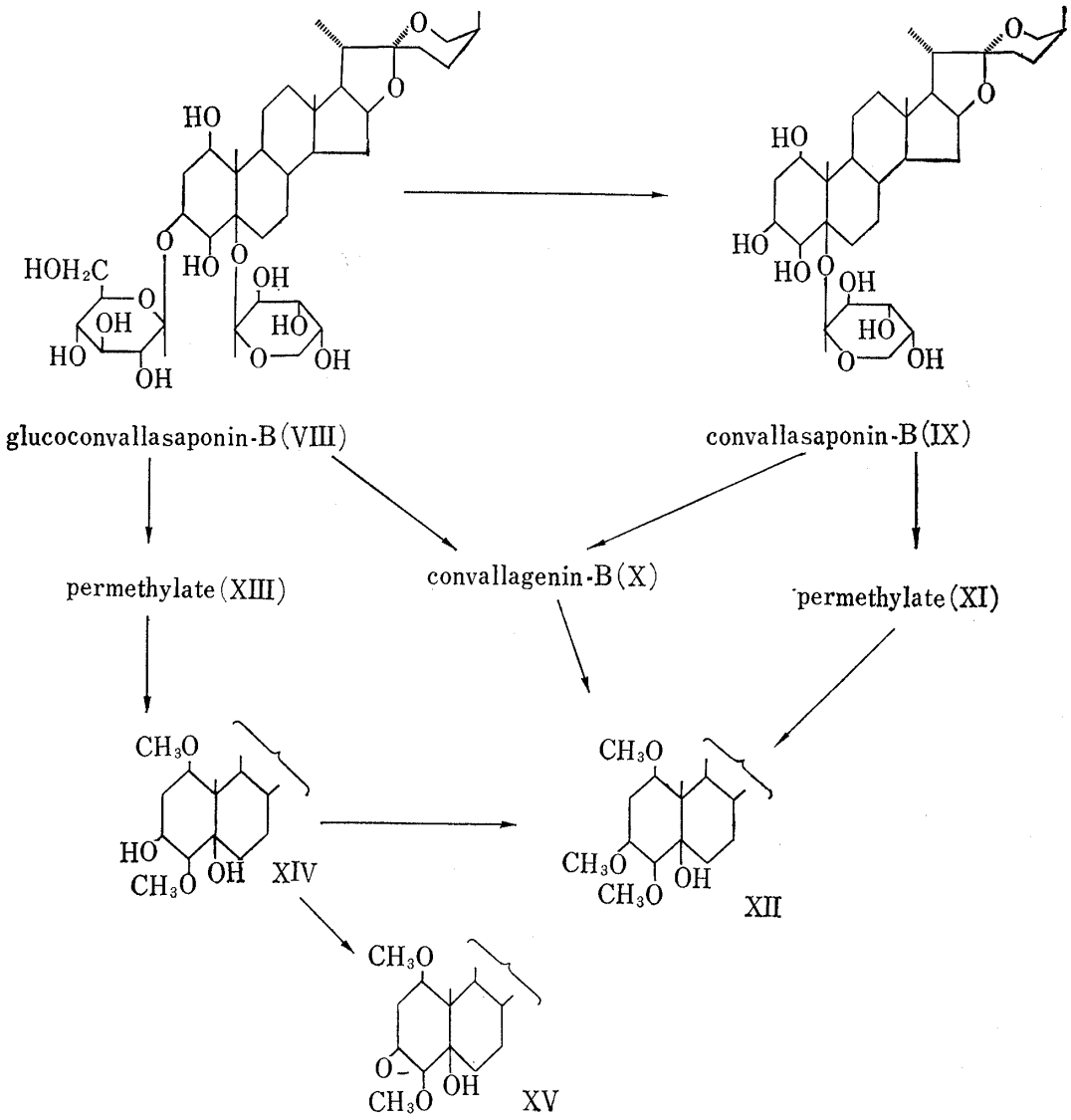

Chart 2

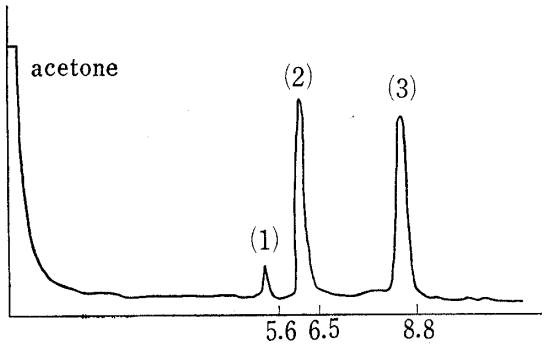

Fig. 4. Gas Chromatographic Separation of Partially Methylated Sugars from Glucoconvallasaponin-B Permethylate

(1), (2): 2,3,4-tri-O-methyl-L-arabopyranose
(3): 2,3,4,6-tetra-O-methyl-D-glucopyranose
1.5% SE-30 on chromosorb W, 190°, 30 ml N_2/min

weakly negative Cotton effect with the molecular amplitude of −33,[18] slightly larger than that (−24.5) of the parent ketone (VII). If the carbonyl group of this compound is not located at C-3 but at C-1 or C-4 of the aglycone, the former should have a larger negative Cotton effect with molecular amplitude of −90— −130 and the latter a weakly positive one (*ca.* +3).[12] These results revealed that the ketone is a 3-keto steroid and the D-glucose of VIII should be linked with the aglycone through the hydroxyl group of C-3. Therefore, the two sugar components of this saponin should be separately bound to the aglycone, D-glucose to C-3 and L-arabinose to C-5 hydro-

18) The amplitude of the dispersion curves of the equatorial methoxyl group adjacent to the ketone are higher than those of the axial group.

xyl groups of X, respectively. This conclusion was also supported by the result that on acid hydrolysis the permethylate (XIII) gave two terminally methylated sugars, 2,3,4-tri-O-methyl-L-arabopyranose and 2,3,4,6-tetra-O-methyl-D-glucopyranose, which were identified with the authentic specimens by gas chromatography as shown in Fig. 4. From the molecular rotation data in Table II, the Klyne rule indicated that L-arabinose is linked in the α-form and D-glucose in the β-one, though there has been no evidence for applying this rule to the glycosidic linkage at the angular position of steroid nucleus. These linkages of L-arabinose in several saponins from Convallaria and others[19] have been all shown to be also in the α-form. Thus, convallasaponin-B and glucoconvallasaponin-B may be formulated as convallagenin-B (5)-α-L-arabopyranoside and convallagenin-B (3)-β-D-glucopyranosido (5)-α-L-arabopyranoside, respectively.

Recently Kawasaki, *et al.*[17] reported the structure of yononin, yonogenin-α-L-arabopyranoside, in which the sugar moiety is attached to the hydroxyl group at C-2, not at C-3 in the ordinary steroidal saponins. Although bufotoxin[20] is considered without any definitive experimental evidence to have suberyl-arginate group at C-14, VIII and IX are the first steroidal glycosides shown to have the sugar moiety attached to the angular position of C-5. No other saponin having sugar moieties at the different positions whithin a steroidal nucleus has also ever been found than VIII; recently Kochetkov, *et al.*[19] studied on the triterpenoid saponins haveing the acyl-glycosidic linkage at C-17 in addition to the usual glycosidic one at C-3.

Hydrolysis of convallasaponin-D gave rhodeasapogenin, 25L-5β-spirostan-1β,3β-diol,[21] and three kinds of sugar which were detected and identified by paper and gas chromatography as D-glucose, D-xylose, and L-rhamnose. Details on the structure of this glycoside will be presented in the next paper.

Experimental

Methylation of Convallasaponins

Methylation of convallasaponin-A (II)——A mixture of II (740 mg) and DMSO (50 ml) was stirred for 30 min at room temperature under nitrogen atmosphere. The amount of NaH roughly equivalent to the hydroxyl group contents of the saponin was added to the solution which was also stirred under N_2 atmosphere for another 30 min and an excess of MeI (15 ml) was then added with further stirring for 30 min. After the reaction mixture was diluted with water (300 ml), the methylated product was extracted with $CHCl_3$, washed with water and evaporated *in vacuo*. The residue was dissolved in ether (100 ml) and washed with water to remove a trace of DMSO. Evaporation of the ether solution left crystalline residue. The same treatment was further repeated three times. The product thus obtained (690 mg) was purified by Al_2O_3 chromatography. From the *n*-hexane-benzene (1:1) eluate IV (463 mg) was obtained, which was crystallized from *n*-hexane to give fine needles, mp 204.5—206.5°, $[\alpha]_D^{21}$: $-41°$($c=0.44$ in $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3585 (*tert*-OH, sharp). *Anal.* Calcd. for $C_{36}H_{60}O_9$: C, 67.89; H, 9.50. Found: C, 67.68; H, 9.63.

Methylation of convallasaponin-B (IX)——The saponin (2.0 g) was methylated completely (three times) as in the case of II to give XI (1.74 g) which was purified by Al_2O_3 chromatography. Benzene-*n*-hexane (1:1) eluate gave crystalline powder (1.68 g) which was recrystallized from *n*-hexane to fine colorless needles, mp 215.5—216°, $[\alpha]_D^{21}$: $-60°$($c=0.78$ in $CHCl_3$), no hydroxyl absorption band in the infrared spectrum. *Anal.* Calcd. for $C_{38}H_{64}O_{10}$: C, 67.03; H, 9.47. Found: C, 67.24; H, 9.39.

Hydrolysis of Methylated Convallasaponins

Hydrolysis of convallasaponin-A permethylate (IV)——After IV (340 mg) was refluxed in 3.3% HCl/MeOH (30 ml) on a water bath for 6 hr, water (15 ml) was added and refluxed further for 3 hr MeOH was evaporated and the precipitate was filtered, dissolved in $CHCl_3$, washed with 10% $KHCO_3$, then with water, dried over Na_2SO_4 and evaporated to give residue (204 mg) which was purified by Al_2O_3 chromatography.

19) N.K. Kochetkov, A.J. Khorlin, and J.S. Ordov, *Tetrahedron Lett.*, **1963**, 477; N.K. Kochetkov and A.J. Khorlin, *Arzneimitt. Forsch.*, **16**, 101 (1966).
20) H.R. Urscheler, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **38**, 883 (1955).
21) H. Nawa, *Yakugaku Zasshi*, **73**, 1192 (1953); *Chem. Pharm. Bull.* (Tokyo), **6**, 255 (1958); T. Okanishi, A. Akahori, and F. Yasuda, *Ann. Repts. Shionogi Res. Lab.*, **10**, 1407 (1960).

From benzene–$CHCl_3$ (2:1) eluate the monomethylate (VI) (186 mg) was obtained, which was crystallized from benzene–hexane to fine needles, mp 203—206°, $[\alpha]_D^{20}$: −69°(c=0.51 in $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.51; H, 9.94.

By a mixture of pyridine (2 ml) and Ac_2O (2 ml) VI (24 mg) was acetylated to the monoacetate (19 mg) which was recrystallized from aq. EtOH to give fine colorless needles, mp 199—201°, $[\alpha]_D^{19}$: −71°(c=0.34 in $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.10; H, 9.40.

Hydrolysis of convallasaponin–B permethylate (XI)——After XI (350 mg) was refluxed in 3.3% HCl/MeOH (30 ml) on a water bath for 3 hr, water (30 ml) was added to the reaction mixture and refluxed further for 3 hr. Evaporation of MeOH gave the precipitate which was dissolved in $CHCl_3$, washed with 5% Na_2CO_3, then with water, dried over Na_2SO_4, evaporated to syrup (214 mg), and purified by alumina chromatography. Benzene–$CHCl_3$ (9:1) eluate gave XII (186 mg), mp 241°(n-hexane), $[\alpha]_D^{21}$: −54°(c=0.84 in $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3585 (*tert*–OH, sharp); not acetylated by usual method, even on reflux, not oxidized by CrO_3 in AcOH as well as by Kiliani reagent at least for 1 hr, and identical with the authentic specimen (XII) obtained by the method described below, in the mixed melting point and comparison of the infrared spectrum. *Anal.* Calcd. for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 71.19; H, 9.80.

Preparation of convallagenin–B trimethylate (XII)——By the similar method to that for convallasponin–B, X (141 mg) was methylated to give XII (87 mg), mp 240—241°, $[\alpha]_D^{21}$: −54.5°(c=0.74 in $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3585 (*tert*–OH, sharp); not acetylated and oxidized by the usual methods. *Anal.* Calcd. for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 71.10; H, 9.89.

Isolation of Glucoconvallasaponins

Glucoconvallasaponin–A (I)——The fraction of peak–1 obtained by the gel filtration (Fig. 1) was recrystallized from MeOH–H_2O to colorless fine needles, mp 213°, $[\alpha]_D^{18}$: −41°(c=0.87 in MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3600—3200 (OH, broad), 981, 921>897, 850 (25L–spiroketal). *Anal.* Calcd. for $C_{38}H_{62}O_{14}$: C, 61.43; H, 8.42. Found: C, 61.37; H, 8.42.

A mixture of this saponin (34 mg), pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand for 2 days at room temperature and concentrated *in vacuo*. Chloroform solution of the residue was washed with 5% $NaHCO_3$, water, N/2 HCl, again with water, dried over Na_2SO_4, and evaporated to give a crude acetate (28 mg) which was recrystallized from aq. MeOH to fine colorless needles, mp 246—247°, $[\alpha]_D^{18}$: −58°(c=0.43 in $CHCl_3$). *Anal.* Calcd. for $C_{52}H_{76}O_{21}$: C, 60.21; H, 7.38. Found: C, 60.07; H, 7.36.

Glucoconvallasaponin–B (VIII)——The fraction of peak–2 obtained by the gel filtration (Fig. 1) gave fine colorless needles, mp 221—222.5°(aq. MeOH), $[\alpha]_D^{18}$: −35°(c=0.69 in MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3600−3200 (OH, broad), 980, 920>895, 850 (25L–spiroketal). *Anal.* Calcd. for $C_{38}H_{62}O_{15}$: C, 60.13; H, 8.23. Found: C, 60.18; H, 8.11. A mixture of the saponin (47 mg), pyridine (3 ml) and Ac_2O (3 ml) was allowed to stand for 2 days at room temperature, evaporated *in vacuo* and the residue was dissolved in $CHCl_3$. The organic layer was washed with 5% $NaHCO_3$, water, N/2 HCl, again with water, dried over Na_2SO_4 and evaporated to give crude acetate (34 mg) which was recrsytallized from aq. MeOH to fine colorless needles, mp 176—178°, $[\alpha]_D^{18}$: −29°(c=0.39 in $CHCl_3$). *Anal.* Calcd. for $C_{56}H_{80}O_{24}$: C, 59.14; H, 7.09. Found: C, 59.29: H, 7.11.

Hydrolysis of Glucoconvallasaponins

Acid hydrolysis——i) Glucoconvallasaponin–A (120 mg) was refluxed in 1N HCl–50% EtOH to give an aglycone (68 mg) which was recrystallized from MeOH–$CHCl_3$ to fine colorless needles, mp 267—268°, $[\alpha]_D^{22}$: −28°(c=0.48 in $CHCl_3$–MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH, broad), 981, 920>898 851, (25L–spiroketal); identical with the authentic convallagenin–A in the mixed melting point and comparison of the infrared spectrum.

ii) Glucoconvallasaponin–B (74 mg) in 1N HCl–50% EtOH (6 ml) was refluxed on a water bath for 6 hr and concentrated under reduced pressure. Water (10 ml) was added and the precipitate formed was extracted with $CHCl_3$. The organic layer was washed with 5% $NaHCO_3$, water, dried over Na_2SO_4 and evaporated to give the aglycone (31 mg) which was purified by Al_2O_3 chromatography. White crystalline powder (26 mg) from benzene–$CHCl_3$ (1:9) eluate was recrystallized from MeOH–$CHCl_3$ to fine colorless needles, mp 276—278°, $[\alpha]_D^{21}$: −43°(c=0.74 in $CHCl_3$–MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3600—3200 (OH, broad), 981, 919>898, 854 (25L–spiroketal); identical with the authentic convallagenin–B in the mixed melting point and comparison of the infrared spectrum.

Enzymatic hydrolysis——i) Water (20 ml) was added to the methanolic solution (1 ml) of I (102 mg) and MeOH was then evaporated off. After takadiastase (200 mg) and toluene (1 ml) was added, the solution was allowed to stand for 48 hr at 34°. The incubation mixture was concentrated to *ca.* 5 ml *in vacuo* at the temperature below 50° and EtOH (20 ml) was added to precipitate the enzyme. The solution was filtered off by Hyflo–Super Cel. The filtrate was also evaporated to a few ml and water (10 ml) was added, extracted with $CHCl_3$ and evaporated to give white powder (68 mg) which was purified by Al_2O_3 chromatography. White powder (59 mg) obtained from 5% MeOH—$CHCl_3$ eluate was recrystallized from MeOH–$CHCl_3$ to fine colorless needles, mp 240°, $[\alpha]_D^{22}$: −40°(c=0.63 in MeOH–$CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3600—3200 (OH, broad), 980, 920>899, 850 (25L–spiroketal); identical with the authentic convallasaponin–A in the mixed melting point and comparison of the infrared specturm. The hydrolysis using β–glucosidase (Sigma) gave the same result.

ii) Glucoconvallasaponin–B (136 mg) was dissolved in a few ml of MeOH and diluted with water (30 ml). After MeOH was evaporated with warming, takadiastase (300 mg) and toluene (1 ml) was added to this solution and allowed to stand for 2 days at 34°. The incubation mixture was concentrated to *ca.* 5 ml under reduced pressure at the temperature below 50° and EtOH (20 ml) was added. The enzyme precipitated was filtered off with Hyflo–Super Cel, the filtrate was evaporated to a few ml, water (10 ml) was added, and extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to give crude prosapogenin (71 mg). The hydrolysate obtained was purified by Al_2O_3 chromatography and 10% MeOH–$CHCl_3$ eluate gave white powder (64 mg) which was crystallized from MeOH–$CHCl_3$ to fine colorless needles, mp 269—271°, $[\alpha]_D^{21}$: −56°(c=0.53 in MeOH–$CHCl_3$); identical with IX in the mixed melting point and comparison of the infrared spectrum. The hydrolysis using β–glucosidase (Sigma) gave the same result.

Hydrolysis of Glucoconvallasaponins Permethylates

Methylation of glucoconvallasaponins——i) Glucoconvallasaponin–A (640 mg) was methylated in DMSO (50 ml) with NaH (400 mg) and CH_3I (20 ml); the procedure was repeated three times. A syrup obtained (541 mg) showed a *tert*–OH absorption band at 3590 cm^{-1} in the infrared spectrum. The permethylate (V) was purified by Al_2O_3 chromatography to give white powder (483 mg) from *n*–hexane–benzene (1:1) eluate, which was recrystallized from MeOH to fine colorless needles, mp 179°, $[\alpha]_D^{22}$: −37°(c=0.43 in $CHCl_3$). *Anal.* Calcd. for $C_{45}H_{76}O_{14}$: C, 64.27; H, 9.10. Found: C, 64.37; H, 8.94.

ii) Glucoconvallasaponin–B (710 mg) was methylated in DMSO (50 ml) with NaH (500 mg) and CH_3I (20 ml) and the procedure was repeated three times until no hydroxyl absorption band was found in infrared spectrum. A syrup (540 mg) obtained was purified by Al_2O_3 chromatography and *n*–hexane eluate gave fine colorless needles, mp 194—194.5°, $[\alpha]_D^{21}$: −29°(c=0.43 in $CHCl_3$). *Anal.* Calcd. for $C_{47}H_{80}O_{15}$: C, 63.77; H, 9.10. Found: C, 63.59; H, 8.84.

Hydrolysis——i) After V (420 mg) was refluxed in 3.3% HCl/MeOH on a water bath for 3 hr, water (30 ml) was added and refluxed for another 3 hr. Methanol was evaporated *in vacuo* and the precipitate formed was dissolved in $CHCl_3$. The organic layer was washed with 5% $NaHCO_3$, with water, dried over Na_2SO_4, and evapotared to give a syrup (268 mg) which was purified by Al_2O_3 chromatography. Methylated aglycon (194 mg) obtained from benzene–$CHCl_3$ (2:1) eluate was crystallized from benzene–*n*–hexane to fine colorless needles, mp 204—205.5°, $[\alpha]_D^{20}$: −66°(c=0.44 in $CHCl_3$); identical with the authentic convallagenin–A monomethylate (VI) obtained from IV in the mixed melting point and comparison of the infrared spectrum.

ii) In the similar way to that of V, XIII (460 mg) was hydrolyzed to the aglycone (264 mg) which was chromatographed by an Al_2O_3 column. White powder obtained from *n*–hexane eluate was recrystallized from MeOH to fine colorless needles of convallagenin–B dimethylate (XIV), mp 175—176°, $[\alpha]_D^{21}$: −60° (c=0.66 in $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3590 (*tert*–OH, sharp), 3400—3200 (OH, broad). *Anal.* Calcd. for $C_{29}H_{48}O_6$: C, 70.69; H, 9.82. Found: C, 70.82; H, 10.07. Using a mixture of pyridine (2 ml) and Ac_2O (2 ml) XIV (40 mg) was acetylated at room temperature to the monoacetate which was recrystallized from aq. MeOH to fine colorless needles, mp 173°, $[\alpha]_D^{21}$: −53°(c=0.32 in $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3585 (*tert*–OH, sharp), 1740 (AcO). *Anal.* Calcd. for $C_{31}H_{50}O_7$: C, 69.63; H, 9.43. Found: C, 70.03; H, 9.61.

By the method mentioned above, XIV (104 mg) was further methylated to convallagenin–B trimethylate (78 mg), mp 221—224°; identical with the authentic specimen (XII) in the mixed melting point and comparison of the infrared spectrum.

Oxidation of Partially Methylated Convallagenins

A mixture of VI (103 mg), AcOH (10 ml), CrO_3 (105 mg) and one drop of water was left for 48 hr at room temperature. After 10 ml of water and one drop of conc. H_2SO_4 were added, the reaction mixture was extracted with ether. The organic layer was washed with water, 10% Na_2CO_3, again with water, dried over Na_2SO_4, and evaporated. The residue (84 mg) was chromatographed using an Al_2O_3 column to give crystalline powder (63 mg) from benzene–$CHCl_3$ (4:1) eluate, which was recrystallized from EtOH to fine colorless needles of VII, mp 240—242°, $[\alpha]_D^{19}$: −23°(c=0.17 in MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3590 (*tert*–OH, sharp), 1717 (C=O), O.R.D. (Fig. 2): peak (252 mμ), λ_0 (265 mμ), trough (304 mμ). *Anal.* Calcd. for $C_{28}H_{44}O_5$: C, 73.00; H, 9.63. Found: C, 73.14; H, 9.56.

A mixture of XIV (50 mg), AcOH (5 ml), CrO_3 (50 mg) and one drop of water was left for 40 hr at room temperature. After adding of 5 ml of MeOH and one drop of conc. H_2SO_4 the reaction mixture was extracted with ether and the organic layer was washed with water, 5% Na_2CO_3, again with water, dried over Na_2SO_4, and evaporated to give a residue (39 mg) which was crystallized from acetone to colorless fine needles of XV, mp 215°, $[\alpha]_{589}^{19}$: −11.2°(c=0.16 in MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3590 (*tert*–OH, sharp), 1733 (C=O), O.R.D. (Fig. 2): peak (264 mμ), λ_0 (274 mμ), trough (299 mμ). *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.82; H, 9.31.

Partially Methylated Sugars

The aqueous layers after hydrolysis of the permethylated saponins were neutralyzed by Amberlite IR–4B and concentrated *in vacuo* to give the syrups which were identified by using thin–layer chromatography (Wako gel, ether–toluene (2:1), detected by spraying 5% H_2SO_4 and heating at 120° for 5 min) and gas chromatography (1.5% SE–30 on Gaschrom P, N_2: 30 ml/min, 190°) as shown in Fig. 3 and 4.

The syrup (81 mg) obtained from IV was found to contain 2,3,4-tri-O-methyl-L-arabopyranose (*Rf*: 0.43; t_R in min: 6.4).

The syrup (103 mg) obtained from V was found to contain 3,4-di-O-methyl-L-arabopyranose (*Rf*: 0.23; t_R in min: 11.0 and 13.1) and 2,3,4,6-tetra-O-methyl-D-glucopyranose (*Rf*: 0.39; t_R in min: 8.6 and 6.0)

The syrup (71 mg) obtained from XI was found to contain 2,3,4-tri-O-methyl-L-arabopyranose (*Rf*: 0.43; t_R: 6.3).

The syrup (161 mg) obtained from XIII was found to contain 2,3,4-tri-O-methyl-L-arabopyranose (*Rf*: 0.43; t_R: 6.5 and 5.6) and 2,3,4,6-tetra-O-methyl-D-glucopyroanse (*Rf*: 0.39; t_R: 8.8).

Convallasaponin-D

The fraction of peak-3 obtained by gel filtration (Fig. 1) gave fine colorless needles, mp 264—265° (aq. EtOH), $[\alpha]_D^{21}$: −66°(c=0.68 in MeOH), IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH, broad), 986, 915>893, 851 (25L-spiroketal). *Anal.* Calcd. for $C_{50}H_{82}O_{21}\cdot H_2O$: C, 57.89; H, 8.16. Found: C, 57.75; H, 8.11. The saponin (43 mg) was acetylated by the usual method to give crude acetate (36 mg) which was recrystallized from aq. MeOH to fine colorless needles, mp 183°, $[\alpha]_D^{21}$: −68°(c=0.59 in $CHCl_3$). *Anal.* Calcd. for $C_{72}H_{104}O_{32}$: C, 58.37; H, 7.08. Found: C, 58.18; H, 6.99.

A mixture of the saponin (530 mg) and 1 N HCl (30 ml) was heated on a water bath for 6 hr and the crude aglycone precipitated was dissolved in $CHCl_3$. The organic layer was washed with water, 5% Na_2CO_3, again with water, dried over Na_2SO_4, and evaporated. The residue (173 mg) was purified by Al_2O_3 chromatography and white powder (135 mg) was obtained from the benzene–$CHCl_3$ (1:1) and $CHCl_3$ eluates, which was recrystallized from MeOH–$CHCl_3$ to fine colorless needles, mp 287—291°, $[\alpha]_D^{20}$: −72°(c=0.83 in MeOH–$CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH, broad), 984, 916>894, 850 (25L-spiroketal): identical with the authentic rhodeasapogenin (25L-5β-spirostan-1β,3β-diol) in the mixed melting point and the comparison of the infrared spectrum. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.77; H, 10.22.

The aqueous layer after hydrolysis was neutralized by Amberlite IR-4B and concentrated *in vacuo* to an yellow syrup which was found to contain three kinds of sugar, L-rhamnose, D-xylose, and D-glucose, by paper chromatography (AcOEt–pyridine–H_2O=2:1:2).

The aglycone (38 mg) was acetylated with a mixture of pyridine (4ml) and Ac_2O (4 ml) for 2 days at room temperature. The solution was concentrated under reduced pressure and diluted with ice-water to give the crude acetate. The chloroform solution of the acetate was washed with 5% Na_2CO_3, then with water, dried over Na_2SO_4, and evaporated to white powder (34 mg) which was recrystallized from aq. EtOH to fine colorless needles, mp 186°, $[\alpha]_D^{20}$: −71°(c=0.48 in $CHCl_3$); identical with the authentic rhodeasapogenin diacetate[16] in the mixed melting point and comparison of the infrared spectrum. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.94; H, 9.34.

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