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Constituents of Convallaria. XII.[1] Convallasaponin-E: Diosgenin Triarabinoside

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The structure of a steroidal saponin, convallasaponin-E, $C_{42}H_{66}O_{15}$, mp 213—217°, $[\alpha]_D^{18}$ −149°, which was isolated from the flowers of *Convallaria keisukei* Miq., Japanese lily of the valley, was studied and elucidated as diosgenin-3-*α*-L-arabopyranosyl(1—2)-*α*-L-arabopyranosyl(1—2)-*α*-L-arabopyranoside.

Recently, Kawasaki, *et al.* reported yononin (yonogenin-L-arabinoside)[3a,b] and tokoronin (tokorogenin-L-arabinoside)[3a,c,d] from *Dioscorea Tokoro* Makino, as the first spirostanol glycosides having L-arabinose. The authors have also studied on the steroidal saponins: convallasaponin-A[4a,b] (I), -B[4a,b,c] (II), -C[4a,d] (III), -D[4c,e] (IV), glucoconvallasaponin-A[4b] (V), and -B[4b,c] (VI), from *Convallaria keisukei* Miq. With the exception of IV these spirostanol glycosides are monoarabinosides (yononin, tokoronin, I, and II) or those having additional sugar moieties (III, V, and VI). The present paper describes the isolation and the structure of a new type of saponin having three moles of L-arabinose as a sole sugar component.

The Fraction-5 obtained from the alumina chromatography of the aqueous layer that has been described in the previous paper[4b] was rechromatographed on Celite–Florisil (Table I) and a substance was isolated as colorless needles, mp 213—217°, $[\alpha]_D^{18}$ −149°, $C_{42}H_{66}O_{15}$. From the following studies, this was recognized as a new saponin and was named convallasaponin-E (VII).

Table I. Chromatographic Separation of Fraction 5[4b]

Fraction No.	Solvent	Vol. (liter)	Weight (mg)	*Rf* on TLC [a]
1	MeOH–$CHCl_3$(20:80)	8	214	0.79, 0.61, 0.55
2	MeOH–$CHCl_3$(25:75)	10	421	0.61, 0.55, 0.48
3	MeOH–$CHCl_3$(30:70)	12	381	0.48, 0.44
4	MeOH–$CHCl_3$(40:60)	16	581	0.44
5	MeOH–$CHCl_3$(50:50)	8	386	0.44, 0.40, 0.32
6	MeOH–$CHCl_3$(70:30)	10	210	0.40, 0.32
7	MeOH	8	224	0.32, 0.20, 0.12
8	H_2O	7	101	0.20, 0.12, 0.03

a) solvent system: MeOH–$CHCl_3$ (30:70); detection of spots: by heating at 120° for 5 min after spraying 5% aq. H_2SO_4; adsorbent: Wako Gel B-5

1) Part XI: M. Kimura, M. Tohama, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 1228 (1968).
2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo.*
3) *a*) T. Kawasaki and T. Yamauchi, *Yakugaku Zasshi*, **83**, 757 (1963); *b*) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965); *c*) T. Kawasaki, *XIth Pacific Science Congress at Tokyo*, 1966; *d*) T. Kawasaki and K. Miyahara, 87th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1967.
4) *a*) M. Kimura, M. Tohma and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 50 (1966); *b*) *Idem, ibid.*, **16**, 25 (1968); *c*) *Idem, ibid.*, **15**, 129 (1967); *d*) *Idem, ibid.*, **14**, 55 (1966); *e*) *Idem, ibid.*, 16, 1228 (1968).

Hydrolysis of VII with 1 N hydrochloric acid in 50% aqueous ethanol gave an aglycone (VIII), mp 204—206°, $[\alpha]_D^{18}$ −120°, $C_{27}H_{42}O_3$, which was identified as diosgenin[5] by mixed melting point and infrared spectral comparison with the authentic specimen. From the aqueous layer of the hydrolyzate, L-arabinose was detected by the paperchromatography and the molar number of the sugar portion was determined as three by the gas liquid chromatography that was reported in the previous paper.[6] Consisting of one mole of diosgenin and three moles of L-arabinose, convallasaponin-E has, therefore, the molecular formula $C_{42}H_{66}O_{15}$ which was also supported by elemental analysis of VII as well as its acetate and by the isolation of two kinds of prosapogenins obtained under mild acid hydrolysis of VII as described below. Methylation of VII by Hakomori's method[7] using sodium hydride and methyl iodide in dimethylsulfoxide gave convallasaponin-E heptamethylate (IX), mp 119°, $[\alpha]_D^{20}$ −132°, $C_{49}H_{80}O_{15}$. Hydrolysis of IX gave VIII and two kinds of partially methylated L-arabinose, 2,3,4-tri- and 3,4-di-O-methyl-L-arabopyranose. The molar ratio of these methylated sugars was determined as 1:2 by the gas liquid chromatography (Fig. 1).

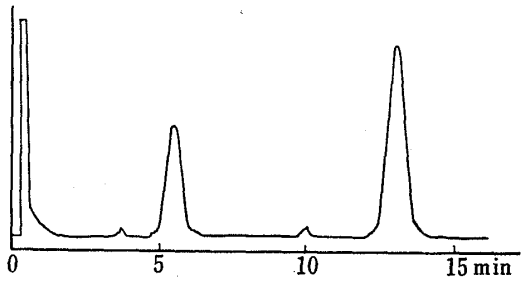


Fig. 1. Gas Chromatography of partially Methylated Sugars

1.5% SE-30 on Chromosorb-W, column temp.: 170°, N_2: 30 ml/min
5.6 min: 2,3,4-tri-O-methyl-L-arabopyranose
13.1 min: 3,4-di-O-methyl-L-arabopyranose

Mild acid hydrolysis of VII gave two kinds of prosapogenin-E-I (X), mp 201—202°, $[\alpha]_D^{18}$ −95°, $C_{32}H_{50}O_7$, and -E-II (XI) mp 232°, $[\alpha]_D^{18}$ −132°, $C_{37}H_{58}O_{11}$ (Table II). On further

Table II. Alumina Chromatography of Prosapogenins

Fraction No.	Solvent	Vol. (ml)	Weight (mg)	*Rf* on TLC[a]		
1	$CHCl_3$	300	29	0.75[b]		
2	$CHCl_3$–MeOH (90:10)	20	5	0.75,	0.55	
3	$CHCl_3$–MeOH (90:10)	780	35		0.55[c]	
4	$CHCl_3$–MeOH (80:20)	1200	1		0.55,	0.30
5	$CHCl_3$–MeOH (70:30)	200	1		0.55,	0.30
6	$CHCl_3$–MeOH (70:30)	1000	36			0.30[d]
7	$CHCl_3$–MeOH (50:50)	200	2			0.30, 0.23
8	$CHCl_3$–MeOH (50:50)	1000	27			0.23[e]

a) using Wako Gel B-5, developed by MeOH–$CHCl_3$ (1:9) and detected by heating after spraying 10% H_2SO_4
b) diosgenin (VIII) *c*) prosapogenin-E-I (X) *d*) prosapogenin-E-II (XI) *e*) convallasaponin-E (VII)

Table III. Molecular Rotation Differences

	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Diosgenin (VIII)	−120	−497	
			− 22
Prosapogenin (X)	− 95	−519	
			−376
Prosapogenin (XI)	−132	−895	
			−312
Convallasaponin–E (VII)	−149	−1207	

methyl α-L-arabopyranoside: $[M]_D = +29$ methyl β-L-arabopyranoside: $[M]_D = +403$

5) *a*) R.E. Marker, T. Tsukamoto, and D.L. Turner, *J. Am. Chem. Soc.*, **62**, 2525 (1940); *b*) T. Tsukamoto, Y. Ueno, and J. Ohta, *Yakugaku Zasshi*, **56**, 931 (1936); *c*) *Idem*, *ibid.*, **57**, 283 (1937).
6) M. Kimura, Y. Hattori, I. Yoshizawa, and M. Tohma, *Chem. Pharm. Bull.* (Tokyo), **16**, 613 (1968).
7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

acid hydrolysis, the former gave VIII as well as one mole of L-arabinose and the latter gave the same aglycone together with two moles of the same sugar. These facts may indicate that convallasaponin-E have a straight-chain structure and is represented by the formula: L-ara$_1$–$_2$L-ara$_1$–$_2$L-ara$_1$–aglycone, in which three monosaccharides of L-series are all assumed to be in the α-form by an application of the Klyne rule[8] (Table III).

The structure of convallasaponin-E (VII) may consequently be defined as diosgenin-3-O-α-L-arabopyranosyl (1—2)-α-L-arabopyranosyl (1—2)-α-L-arabopyranoside (Fig. 2). The

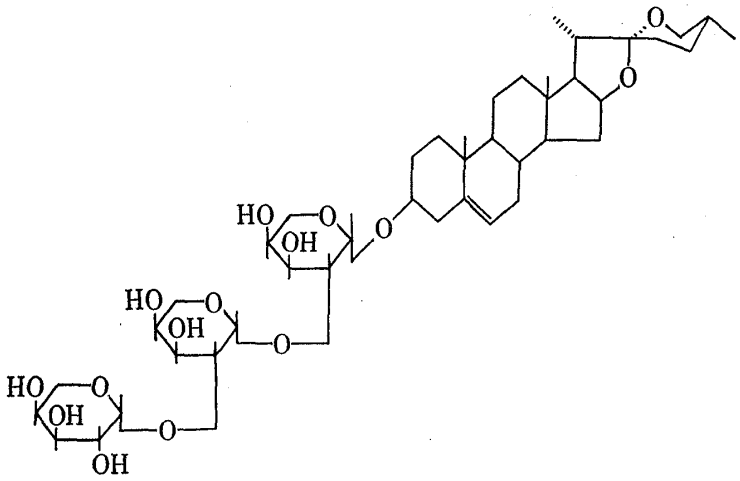
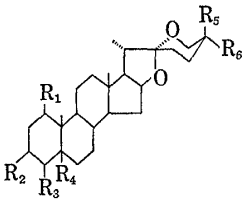


Fig. 2. Structure of Convallasaponin–E

Table IV. Steroidal Saponins from *Convallaria keisukei* Miq.



	R_1	R_2	R_3	R_4	R_5	R_6
Convallasaponin–A (I)	OH	O–$_1$ara	H	OH	CH_3	H
Convallasaponin–B (II)	OH	OH	OH	O–$_1$ara	CH_3	H
Convallasaponin–C (III)	OH	O–$_1$ara$_2$–$_1$rha$_3$–$_1$rha	H	H	H	CH_3
Convallasaponin–D(IV)	O–$_1$glu	O–$_1$rha$_3$–$_1$xyl$_2$–$_1$rha	H	H	CH_3	H
Convallasaponin–E (VII)	H	O–$_1$ara$_2$–$_1$ara$_2$–$_1$ara	H	Δ	H	CH_3
Glucoconvallasaponin–A (V)	OH	O–$_1$ara$_2$–$_1$glu	H	OH	CH_3	H
Glucoconvallasaponin–B (VI)	OH	O–$_1$glu	OH	O–$_1$ara	CH_3	H

steroidal saponins isolated from Convallaria are summarized in the Table IV and it is of quite interest in view of the chemistry and biogenetics of the saponins that some regularities concerning the monosaccharide linkages in these saponins would be infered from the Table; that is, arabinose and xylose are to be linked with the subsequent sugar through the hydroxyl group of C-2 and rhamnose is through that of C-3.

Experimental

Isolation of Convallasaponin-E (VII)——The powder (2.70 g) obtained from the fraction-5 as described in the previous paper[4b] was dissolved in 20% MeOH/$CHCl_3$ (30 ml) and was submitted to chromatography

8) W. Klyne, *Biochem. J.*, **47**, xli (1950).

on Celite–Florisil (1:2, 81 g) as shown in Table I. Fraction-4 gave a colorless powder (581 mg) which was recrystallized from aq. MeOH to afford colorless needles, mp 213—217°, $[\alpha]_D^{18}$ −149° (c=0.44, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3600—3200 (OH, broad). *Anal.* Calcd. for $C_{42}H_{66}O_{15}$: C, 62.19; H, 8.21. Found: C, 62.50; H, 8.33.

Acetylation of VII——A solution of VII (29 mg) in a mixture of pyridine (1 ml) and Ac_2O (1 ml) was allowed to stand for 24 hr at room temperature. The reaction mixture was treated in the usual way to give the crude product (27 mg) which was recrystallized from aq.EtOH to colorless needles of convallasaponin-E heptaacetate, mp 191—192°, $[\alpha]_D^{18}$ −139° (c=0.51, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1741 (OAc). *Anal.* Calcd. for $C_{56}H_{82}O_{22}$: C, 60.19; H, 7.41. Found: C, 59.99; H, 7.50.

Hydrolysis of VII——A mixture of VII (104 mg) and 1N HCl in 50% EtOH (10 ml) was refluxed for 5 hr on a water-bath and concentrated under reduced pressure to remove EtOH. The crude aglycone (51 mg) precipitated was collected, washed with water, and recrystallized from MeOH to give colorless fine needles, mp 204—206°, $[\alpha]_D^{18}$ −120° (c=0.94, MeOH), which was identical with the authentic diosgenin (VIII) by mixed melting point and infrared spectral comparison. *Anal.* Calcd. for $C_{27}H_{42}O_3 \cdot \frac{1}{2}H_2O$: C, 76.55; H, 10.23. Found: C, 76.83; H, 10.31.

Acetylation of VIII——In a similar way to the case of VII, the aglycone (VIII, 29 mg) was acetylated to give the crude product (31 mg), which was recrystallized from aq. EtOH to fine needles, mp 199.5—200°. No depression was observed in the mixed melting point with the authentic diosgenin acetate. *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.07; H, 9.89.

Permethylation of VII——A mixture of VII (121 mg) and dimethylsulfoxide (20 ml) was stirred for 30 min at room temperature under nitrogen stream. The amount of NaH roughly equivalent to the hydroxyl content of VII was added to the solution which was further stirred under N_2 stream for another 30 min and then excess of MeI (20 ml) was added. After the reaction mixture was diluted with water (50 ml), the methylated product was extracted with $CHCl_3$, washed with water to remove a trace of DMSO. Evaporation of $CHCl_3$ left crystalline residue. The same treatment was repeated three times until no absorption band of hydroxyl group was found in IR spectrum. The product thus obtained (101 mg) was recrystallized from hexane to give colorless fine needles of convallasaponin-E heptamethylate (IX), mp 119°, $[\alpha]_D^{20}$ −132° (c=0.44, $CHCl_3$). *Anal.* Calcd. for $C_{49}H_{80}O_{15}$: C, 65.74; H, 7.44. Found: C, 65.49; H, 7.34.

Hydrolysis of IX——After the permethylate (IX, 43 mg) was refluxed in 5.5% HCl/MeOH (5 ml) on a water bath for 4 hr, water (5 ml) was added and refluxed again for 3 hr. Methanol 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 the residue (17 mg) which was identified as diosgenin by thin layer chromatography.

The aqueous layer was neutralized by Amberlite IR-4B and concentrated *in vacuo* to the syrup (18 mg) which was found to contain two kinds of partially methylated sugars, 2,3,4,-tri-(*Rf* 0.43) and 3,4-di-O-methyl-L-arabopyranose (*Rf* 0.23) by using thin layer chromatography (Wakogel B-5, developed with ether–toluene=2:1, detected by spraying 10% H_2SO_4 and subsequent heating). The sugar portion was also submitted to gas liquid chromatography as shown in Fig. 1.

Partial Hydrolysis of VII——A mixture of hydrolyzate (150 mg) obtained by refluxing VII (256 mg) with 1N HCl-50% MeOH (40 ml) for 90 min was dissolved in $CHCl_3$ and submitted to chromatography on alumina (5 g). Each eluate was evaporated to dryness and examined by thin-layer chromatography. Two kinds of prosapogenin (X and XI) were obtained as shown in Table II.

Prosapogenin-E-I (X)——The residue of fraction-3 was crystallized from MeOH to colorless fine needles, mp 201—202°, $[\alpha]_D^{18}$ −95° (c=0.32, $CHCl_3$-MeOH). *Anal.* Calcd. for $C_{32}H_{50}O_7$: C, 70.30; H, 9.22. Found C, 70.54; H, 9.18. Hydrolysis of X (4 mg) afforded diosgenin and one mole of L–arabinose which was determined by gas liquid chromatography.[6]

Prosapogenin-E-II (XI)——The residue of Fraction-6 was crystallized from $CHCl_3$–MeOH to give fine colorless needles, mp 232°, $[\alpha]_D^{18}$ −132° (c=0.43, MeOH–$CHCl_3$). *Anal.* Calcd. for $C_{37}H_{58}O_{11}$: C, 65.46; H, 8.62. Found: C, 65.45; H, 8.60. Hydrolysis of XI (3 mg) gave diosgenin and two mole of L–arabinose.

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