Chem. Pharm. Bull. 14(1) 50~55 (1966)

UDC 582.59 : 581.19

9. Michiya Kimura, Masahiko Tohma, and Itsuo Yoshizawa :

Constituents of Convallaria. IV.*1 Isolation of Convallasaponin-A, -B, and -C.

(*Faculty of Pharmaceutical Sciences, Hokkaido University**2)

In previous papers[1,2] it has been shown on paper chromatography of crude extracts of the dried flowers of *Convallaria keisukei* MIQ., Japanese lily of the valley, that besides the known cardiac glycosides of convallaria more than ten spots could be detected by the Kedde reaction,[3] characteristic for the cardenolide. Convallatoxin and desglucocheirotoxin[2] were subsequently isolated and identified. Isolation of the other cardiac glycosides seemed to be somewhat difficult due to interferences with accompanied non-cardiac substances. The present paper describes the isolation of three such substances in fairly pure state and identifying them as new steroidsaponins.

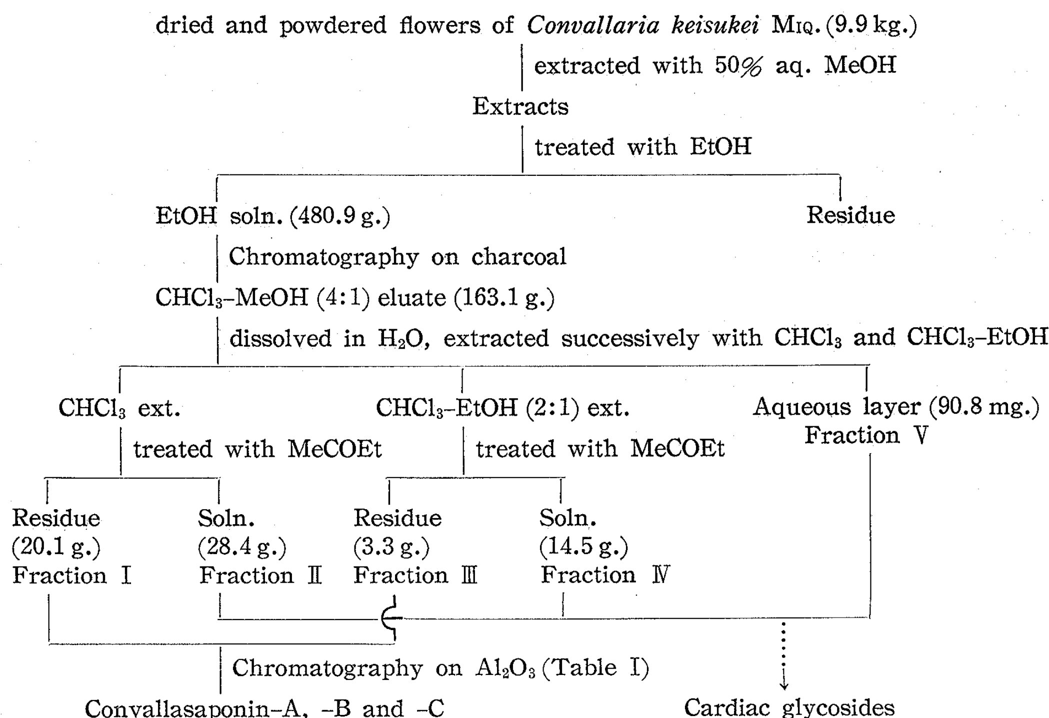

Fig. 1. Extraction and Isolation of Steroidsaponins

The method of extraction is shown in Fig. 1 : Extracts of the dried and powdered flowers with 50% aqueous methanol were treated again with 95% ethanol and the soluble portion was submitted to chromatography over charcoal. The pale yellow powder from the fraction eluted with chloroform-methanol (4:1) was dissolved in water and then shaken out with chloroform and chloroform-ethanol (2:1) mixture successively. While methyl ethyl ketone soluble portions (Fraction II and IV in Chart 1) of

*1 Part III : Yakugaku Zasshi, 84, 1227 (1964).

*2 Nishi-5-chome, Kita-12-jo, Sapporo (木村道也, 藤間貞彦, 吉沢逸雄).

1) M. Ishidate, M. Kimura, S. Tamikado, M. Kawata : Yakugaku Zasshi, **77**, 679 (1957).
2) M. Kimura, M. Tohma, T. Kikuchi : *Ibid*., **82**, 1320 (1962).
3) D.L. Kedde, Diss., Leyden (1946); cf. I.E. Bush, D.A.H. Taylor : Biochem. J., **52**, 643 (1952).

NII-Electronic Library Service

these extracts contained cardiac glycosides giving positive Kedde reaction, the residual portions (Fraction I and III) showed three spots on paper chromatogram, giving negative Kedde reaction but yellow color with cinnamic aldehyde and acetic anhydride-concentrated sulfuric acid reagent.[4] The latter portions (Fraction I and III) were chromatographed on alumina (Table I) and three crystals were isolated as colorless needles, all of which showed positive color tests for the steroid and saponin-like characters such as hemolytic and foaming effects.[5] Since these substances were recognized to be new steroidsaponins from the above-mentioned characters and by following studies, they were named convallasaponin-A, -B and -C respectively.

Table I. Alumina Chromatography of Fraction I and III

Fraction No.	Solvent	Vol. (L.)	Eluted product (g.)	Rf
1	$CHCl_3$	4	oil	—
2	$CHCl_3$-MeOH (94:6)	4	〃	—
3	〃 (90:10)	10	3.938	0.68
4	〃 (90:10)	2	0.542	0.68, 0.52
5	〃 (85:15)	12	3.209	0.52
6	〃 (70:30)	16	1.697	0.52
7	〃 (50:50)	6	0.366	0.52
8	MeOH	2	1.208	0.52, 0.34
9	〃	20	3.946	0.34
10	MeOH-AcOH (98:2)	8	1.493	0.34

Convallasaponin-A was corresponded to the formula $C_{32}H_{52}O_9$ from the elemental analysis, giving tetraacetate by drastic acetylation with acetic anhydride and pyridine on a steam bath. According to the detailed studies on the infrared spectra of steroidsapogenins[6] and steroidsaponins[7] by Wall, *et al.*, the spiroketal structure of side chain shows a characteristic spectrum for sapogenin having absorption maxima near 980, 920, 900 and 860 cm^{-1}, and comparison of intensities of bands at 920 and 900 cm^{-1} can be used to distinguish 25L (C_{25}-CH_3 : axial)-from 25D (C_{25}-CH_3 : equatorial)-saponin. The characteristic absorption bands of convallasaponin-A and its acetates were both observed at 980, 921, 898 and 850 cm^{-1}, and comparison of intensities of bands at 921 and 898 cm^{-1} indicated the 25L-configuration.

Hydrolysis of the saponin was carried out by 1*N* hydrochloric acid in 50% aqueous ethanol to give an aglycone as needles, $C_{27}H_{44}O_5$, m.p. 268~269°, which seemed to belong to the 25L-series on the basis of comparison of intensities of the infrared absorption bands at 922 and 897 cm^{-1} as described above. Acetylation of the sapogenin by the usual method and/or by further heating on a steam bath gave monoacetate and diacetate. The latter thus obtained showed infrared absorption bands at 3580 (OH) and 1739 (OAc) cm^{-1}, so that the original sapogenin seemed to be a triol having an unacetylable hydroxyl group. These data indicated that the aglycone is a new trihydroxy 25L-sapogenin, designated as convallagenin-A.

Convallasaponin-B was represented as the molecular formula $C_{32}H_{52}O_{10}$ affording pentaacetate. Hydrolysis of this glycoside under the conditions as described above for convallasaponin-A gave mainly an aglycone as colorless needles, $C_{27}H_{44}O_6$, m.p.

4) E. Heftmann, A.L. Hayden : J. Biol. Chem., **197**, 47 (1952).
5) E. Heftmann. E. Mosettig : "Biochemistry of Steroid," 48 (1960), Reinhold Publish. Corp., New York.
6) M.E. Wall, C.R. Eddy, M.L. McClennan, M.E. Klumpp : Anal. Chem., **24**, 1337 (1952); C.R. Eddy, M.E. Wall, M.K. Scott : *Ibid.*, **25**, 266 (1953).
7) E.S. Rothman, M.E. Wall, C.R. Eddy : J. Am. Chem. Soc., **74**, 4013 (1952).

NII-Electronic Library Service

277~278°, which yielded triacetate and amorphous diacetate by drastic acetylation. Infrared spectra showed that all of this saponin, its aglycone and their acetates belong to the 25L-series and the triacetate still contains a free hydroxyl group. The aglycone was, therefore, concluded to be a new tetrahydroxy 25L-sapogenin, designated as convallagenin-B.

Convallasaponin-C, $C_{44}H_{72}O_{16}$, was the sole 25D-steroidsaponin obtained in this study. The glycoside afforded octaacetate on acetylation with acetic anhydride-pyridine at room temperature, which no longer showed absorption bands corresponding to hydroxyl groups in the infrared spectrum. Upon acid hydrolysis the saponin yielded an aglycone as colorless needles, m.p. 242~243°, $C_{27}H_{44}O_4$, which gave diacetate under usual condition. From the results of the infrared spectra and the melting point on admixture this sapogenin was identified as isorhodeasapogenin isolated from *Rhodea japonica* (THUNB.) ROTH by Nawa[8] and from *Convallaria keisukei* MIQ. by Okanishi, *et al.*[9]

On the aqueous layers of the hydrolyzates of convallasaponin-A, -B and -C the color tests for sugars were carried out indicating that these saponins contained aldopentose and convallasaponin-C had additional methylpentose as shown in Table II.

TABLE II. Color Tests and Paperchromatography of Sugar Portions

	Sugar of convallasaponin			L-Arabinose	L-Rhamnose
	-A	-B	-C		
(1) Color test					
AgNO$_3$-NH$_3$	+	+	+	+	+
Fehling's	+	+	+	+	+
Aniline-phthalate[a]	pink	pink	brown	pink	brown
conc. HCl-Acetone[b]	—	—	red	—	red
(2) Paperchromatography (Rf)[c]					
Solvent					
AcOEt-Py-H$_2$O (2:1:2)	0.34	0.34	0.34, 0.58	0.34	0.58
iso-AmOH-Py-H$_2$O (1:1:1)	0.43	0.43	0.43, 0.63	0.43	0.63
BuOH-AcOH-H$_2$O (4:1:5)	0.21	0.21	0.21, 0.37	0.21	0.37

a) S.M. Partridge: Nature, **164**, 443 (1949).
b) L. Rosenthaler: Z. anal. Chem., **48**, 167 (1909).
c) Toyo Roshi No. 51, ascending method, detected by aniline-phthalate.

It was observed on paper chromatography that the sugar moieties of convallasaponin-A and -B are L-arabinose, while those of convallasaponin-C are L-arabinose and L-rhamnose.

No other steroidsaponin containing L-arabinose has ever been known except yononin (yonogenin-α-L-arabinose) and tokoronin (tokorogenin-α-L-arabinose) recently reported by Kawasaki, *et al.*[10] Although there appeared a few works[9,11] concerning steroidsapogenin in Convallaria species, investigation on the principal glycoside has not been extended beyond convallamarin by Voss and Vogt,[12] which was composed of convallamaretin, glucose and rhamnose. Details on the structures of the glycosides and their aglycones described in this paper will be presented in the forth coming papers.

8) H. Nawa: Yakugaku Zasshi, **73**, 1192 (1953); This Bulletin, **6**, 255 (1958).
9) T. Okanishi, A. Akahori, F. Yasuda: Ann. Repts. Shionogi Research Lab., **10**, 1407 (1960).
10) T. Kawasaki, T. Yamauchi: Yakugaku Zasshi, **83**, 757 (1963).
11) R. Tschesche, H. Schwarz, C. Snatzke: Chem. Ber., **94**, 1699 (1961).
12) W. Voss, G. Vogt: *Ibid.*, **69**, 2333 (1936).

NII-Electronic Library Service

Experimental

Extraction from *Convallaria keisukei* Miq.

Dried and powdered flowers (9.9 kg.) were extracted three times with 50% MeOH (30 L.) for 48 hr. at room temperature. After filtration, the extracts were concentrated under reduced pressure at 45~50° by adding a few drops of Toshiba Silicone TS-984E to give resinous residue. The ethanol extracts of this residue were evaporated to afford hygroscopic powder (480.9 g.) and then it was dissolved in water, followed by chromatography over charcoal (980 g.). After washing the column with MeOH, fractions eluted by $CHCl_3$-MeOH (4:1) were concentrated to give pale yellow powder (163.1 g.), aqueous solution (500 ml.) of which was shaken out successively with $CHCl_3$ (300 ml. × 4) and $CHCl_3$-EtOH (2:1) mixture (300 ml. × 4).

Each of these extracts was concentrated and treated with methyl ethyl ketone to give white powdered residues (Fraction I and III in Fig. 1), 20.1 g. and 3.3 g. respectively, showing negative Kedde reaction[3] but positive Liebermann-Burchard reaction. Paper chromatographic analysis using methyl ethyl ketone saturated with water as solvent revealed three yellow spots (Rf 0.68, 0.52 and 0.34) after spraying cinnamic aldehyde (1% in EtOH) and Ac_2O-conc. H_2SO_4 (12:1).[4] The methyl ethyl ketone soluble portions (Fraction II and IV) and the aqueous layer (Fraction V) gave positive Kedde reaction.

Isolation of convallasaponin-A, -B and -C: The portion insoluble in methyl ethyl ketone (Fraction I and III, 22.25 g.) was dissolved in $CHCl_3$ and submitted to chromatography on alumina (668 g.) giving results shown in Table I. Three glycosides isolated, convallasaponin-A, -B and -C, showed respective Rf values of 0.68, 0.52 and 0.34 on the paper chromatogram as described above.

Convallasaponin-A

The fraction No. 3 obtained by the chromatography (Table I) was recrystallized from MeOH-$CHCl_3$ to convallasaponin-A as colorless needles, m.p. 238~240° (decomp.), $[\alpha]_D^{22}$ −39.7° (c=0.527, $CHCl_3$). This glycoside gave a negative test for Kedde reagent, coloration changing from red to brown in Liebermann-Burchard reaction, pink color with $SbCl_3$, and yellow with cinnamic aldehyde-Ac_2O-conc. H_2SO_4. IR ν_{max}^{KBr} cm^{-1}: 3600~3200 (OH, broad), 980, 921>898, 850 (spiroketal side chain, 25 L). *Anal.* Calcd. for $C_{32}H_{52}O_9$: C, 66.18; H, 9.03. Found: C, 65.96; H, 8.97.

Acetylation of convallasaponin-A: A solution of convallasaponin-A (120 mg.) in a mixture of pyridine (2 ml.) and Ac_2O (1 ml.) was heated on a steam-bath for 2 hr. The solution was concentrated under reduced pressure and diluted with water to give a crude acetate (127 mg.) which was recrystallized from MeOH-$CHCl_3$ to convallasaponin-A tetraacetate as colorless needles, m.p. 274~276°, $[\alpha]_D^{22}$ −59.2° (c=0.374, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH, sharp), 1742 (OAc), 980, 921>898, 850 (25 L-spiroketal). *Anal.* Calcd. for $C_{40}H_{60}O_{13}$: C, 64.16; H, 8.08. Found: C, 64.02; H, 7.98.

Hydrolysis of convallasaponin-A: A mixture of convallasaponin-A (1.1 g.) and 1*N* HCl in 50% EtOH (110 ml.) was refluxed for 5 hr. on a water-bath and concentrated under reduced pressure to remove EtOH. The crude aglycone (890 mg.) precipitated was collected and washed with ether. The insoluble residue was recrystallized from MeOH-$CHCl_3$ to give convallagenin-A as colorless needles (580 mg.), m.p. 268~269°, $[\alpha]_D^{20}$ −28.0° (c=0.500, $CHCl_3$-MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500~3300 (OH, broad), 982, 922>897, 852 (25 L-spiroketal). *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.05; H, 9.70.

Acetylation of convallagenin-A: A solution of convallagenin-A (580 mg.) in a mixture of pyridine (10 ml.) and Ac_2O (10 ml.) was allowed to stand for 20 hr. at room temperature. The reaction mixture showed two spots of acetates (Rf 0.79 and 0.55) on thin-layer chromatogram using $CHCl_3$-acetone (9:1) as solvent. Although two spots were still retained after further heating on a steam-bath for 3 hr., the solution was concentrated under reduced pressure, diluted with water and extracted with ether. After successive washing the ether solution with 2*N* HCl, 2*N* Na_2CO_3 and water, it was dried over Na_2SO_4 and then evaporated. The residue (662 mg.) was chromatographed on alumina (20 g.). Elution with benzene afforded diacetate (163 mg.) which was recrystallized from MeOH as colorless plates, m.p. 208~210°, $[\alpha]_D^{20}$ −78.4° (c=0.535, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH, sharp), 1739 (OAc), 981, 919>897, 848 (25 L-spiroketal). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.87; H, 9.08. Found: C, 69.76; H, 8.97. Further elution with benzene-$CHCl_3$ (9:1) gave monoacetate (274 mg.) which was recrystallized from MeOH as colorless needles, m.p. 190~191°, $[\alpha]_D^{20}$ −77.9° (c=0.565, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH, broad), 1737 (OAc), 979, 918>901, 845 (25 L-spiroketal). *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.82; H, 9.52. Found: C, 70.98; H, 9.45.

Convallasaponin-B

The fractions Nos. 5~7 obtained by the chromatography described above (Table I) were recrystallized from MeOH-$CHCl_3$ to afford convallasaponin-B as colorless needles, m.p. 273~274°, $[\alpha]_D^{22}$ −55.7° (c=0.435, $CHCl_3$-MeOH). The glycoside gave similar color reactions to those for convallasaponin-A: Kedde, Liebermann-Burchard, $SbCl_3$ and cinnamic aldehyde-Ac_2O-conc. H_2SO_4 reactions. IR ν_{max}^{KBr} cm^{-1}: 3600~3200 (OH, broad), 980, 918>897, 854 (25 L-spiroketal). *Anal.* Calcd. for $C_{32}H_{52}O_{10}$: C, 64.40; H, 8.78. Found: C, 64.23; H, 8.88.

NII-Electronic Library Service

Acetylation of convallasaponin-B : A solution of convallasaponin-B (253 mg.) in a mixture of pyridine (2.5 ml.) and Ac_2O (3 ml.) was allowed to stand for 3 days at room temperature. The solution was concentrated under reduced pressure and diluted with water to give crude acetate (189 mg.) which was recrystallized from MeOH to convallasaponin-B pentaacetate as colorless plates, m.p. 183~184°, $[\alpha]_D^{22}$ −48.5° (c=0.548, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3580 (OH, sharp), 1739 (OAc), 980, 919>897, 854 (25 L-spiroketal). *Anal.* Calcd. for $C_{42}H_{62}O_{15}$: C, 62.51; H, 7.75. Found : C, 62.23; H, 7.61.

Hydrolysis of convallasaponin-B : A mixture of convallasaponin-B (301 mg.) and 1*N* HCl in 50% EtOH (36 ml.) was refluxed for 5 hr. on a water-bath and the reaction mixture was treated as described above on the hydrolysis of convallasaponin-A. The aglycone was recrystallized from MeOH-$CHCl_3$ as colorless needles (190 mg.), m.p. 277~278°, $[\alpha]_D^{21}$ −42.7° (c=1.03, $CHCl_3$-MeOH). IR ν_{max}^{KBr} cm^{-1} : 3570, 3500~3200 (OH, broad), 980, 920>897, 856 (25 L-spiroketal). *Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.78; H, 9.55. Found: C, 69.91; H, 9.48.

Acetylation of convallagenin-B : A solution of convallagenin-B (261 mg.) in a mixture of pyridine (3 ml.) and Ac_2O (2.6 ml.) was treated as described above on the acetylation of convallagenin-A. The resulting residue (279 mg.) which also showed two spots (Rf 0.58 and 0.30) on the thin-layer chromatography using $CHCl_3$-MeOH (9:1) as solvent, was chromatographed on alumina (8.4 g.). Elution with hexane-benzene (3:2) gave triacetate (168 mg.), colorless needles, m.p. 228~230° (from MeOH), $[\alpha]_D^{21}$ −46.5° (c=0.990, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3590 (OH, sharp), 1742 (OAc), 982, 920>898, 855 (25 L-spiroketal). *Anal.* Calcd. for $C_{33}H_{50}O_9$: C, 67.09; H, 8.53. Found : C, 67.01; H, 8.67. Further elution with hexane-benzene (1:4) gave an amorphous material (76 mg.) which was regarded as diacetate. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3460 (OH, broad), 1742 (OAc), 980, 922>898, 855 (25 L-spiroketal).

Convallasaponin-C

The fractions Nos. 9~10 obtained by the chromatography described above (Table I) were recrystallized from MeOH-$CHCl_3$ to convallasaponin-C as colorless needles, m.p. 218~221° (decomp.), $[\alpha]_D^{19}$ −89.7° (c=0.78, MeOH-$CHCl_3$). This glycoside also gave similar color reactions to those for convallasaponin-A and -B. IR ν_{max}^{KBr} cm^{-1} : 3500~3200 (OH, broad), 983, 918<900, 865 (25 D-spiroketal). *Anal.* Calcd. for $C_{44}H_{72}O_{16} \cdot H_2O$: C, 60.25; H, 8.50. Found : C, 60.08; H, 8.48.

Acetylation of convallasaponin-C : A solution of convallasaponin-C (250 mg.) in a mixture of pyridine (5 ml.) and Ac_2O (5ml.) was allowed to stand for 24 hr. at room temperature. The reaction mixture was treated in the usual way to give a crude acetate (284mg.) which was recrystallized from EtOH to convallasaponin-C octaacetate as colorless needles, m.p. 152~154°, $[\alpha]_D^{21}$ −34.7° (c=0.580, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1740 (OAc), 983, 918<898, 866 (25 D-spiroketal). *Anal.* Calcd. for $C_{60}H_{88}O_{24}$: C, 60.39; H, 7.43. Found : C, 60.39; H, 7.63.

Hydrolysis of convallasaponin-C : The glycoside (490 mg.) was hydrolyzed by the method similar to that for convallasaponin-A and the aglycone obtained was recrystallized from EtOH to colorless needles (194 mg.), m.p. 242~243°, $[\alpha]_D^{18}$ −70.8° (c=0.480, MeOH-$CHCl_3$), which was identical with the authentic isorhodeasapogenin in mixed melting point and comparison of the infrared spectrum. IR ν_{max}^{KBr} cm^{-1} : 3500~3400 (OH, broad), 982, 919<899, 865 (25 D-spiroketal). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found : C, 74.88; H, 10.22.

Acetylation of aglycone (Isorhodeasapogenin) : The aglycone obtained (15 mg.) was acetylated by the usual means with pyridine (2 ml.) and Ac_2O (2 ml.) to give diacetate (11 mg.) as colorless needles, m.p. 201~203° (EtOH), $[\alpha]_D^{23}$ −74.8° (c=1.015, $CHCl_3$-MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1732 (OAc), 982, 920<898, 868 (25 D-spiroketal). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found : C, 71.82; H, 9.38.

Sugar Moieties of Convallasaponin-A, -B and -C

Aqueous layers of hydrolyzates of the saponins described above were neutralized with Amberlite IR-4B and evaporatd under reduced pressure to give the respective sugar moieties as syrups. The results of color tests and paper chromatography of these syrups are listed in Table II.

The authors express their deep gratitude to Prof. S. Wakayama, Hokkaido Gakugei University and students of Toyoni and Tomioka Junior High Schools for collecting materials. They are also indebted to Dr. H. Nawa, Takeda Research Laboratories, and Dr. T. Okanishi, Shionogi Research Laboratories for generous gifts of precious authentic samples. Thanks are also due to Mrs. T. Tohma and Miss A. Maeda of the analytical laboratory of this Faculty for elemental analysis.

Summary

Three kinds of new steroidsaponin, convallasaponin-A, m.p. 238~240° (decomp.), $[\alpha]_D^{22}$ −39.7° ($CHCl_3$), convallasaponin-B, m.p. 273~274°, $[\alpha]_D^{22}$ −55.7° ($CHCl_3$-MeOH), and convallasaponin-C, m.p. 218~221° (decomp.), $[\alpha]_D^{19}$ −89.7° ($CHCl_3$-MeOH) were isolated

NII-Electronic Library Service

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.

(Received June 9, 1965)

[Chem. Pharm. Bull. 14(1) 55~61 (1966)]

UDC 582.59 : 581.19

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

(*Faculty of Pharmaceutical Sciences, Hokkaido University**2)

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

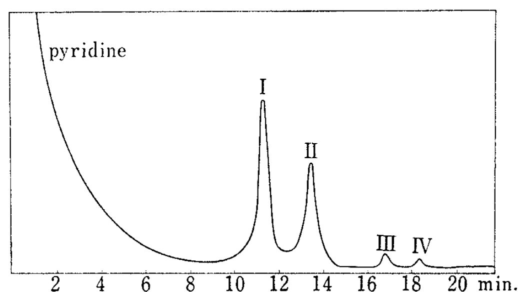

Fig. 1. Gas Chromatographic Separation of Sugar Trimethylsilyl Ethers

5% nitrile silicone (XF-1150) on Gas-chrom 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).
2) H. Nawa : Yakugaku Zasshi, **73**, 1192 (1953); This Bulletin, **6**, 255 (1958); T. Okanishi, A. Akahori, F. Yasuda : Ann. Repts. Shionogi Research Lab., **10**, 1407 (1960).
3) W.S. Reich : J. Biol. Chem., **33**, 1000 (1939).
4) G.E. Coleman, C.M. McColkey : J. Am. Chem. Soc., **65**, 1588 (1943).
5) C.C. Sweeley, R. Bentley, M. Makita, W.W. Wells : *Ibid.*, **85**, 2497 (1963).

NII-Electronic Library Service