that vitamin K_3 is excreted in urine faster and that the tissue incorporation is low compared with the other two homologs.

It is also interesting that vitamin K is incorporated into the tissues such as spleen, lymph node, and bone marrow which are responsible for lymphatic cell generation, but the relatively high radioactivity found in lymph node 1 hr after the administration may be related to the lymphatic absorption of these vitamins.[9]

The autoradiogram presented a good advantage for finding the radioactivity in intestinal mucosa, lymph node, and brown fat in which determination of radioactivity by combustion method had some technical difficulties.

9) R. Blomstrand and L. Forsgren, *Internat. Z. Vit. forschung*, **38** 45 (1968); Y. Aso, K. Mishima, T. Arisaka, and H. Kitagawa, Abstracts of paper, 1st Sym. on Drug Metabolism and Action, November 14 to 15, 1969 in Chiba, Japan.

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A New Diosgenin Glycoside, Aspidistrin, from *Aspidistra elatior* Blume

Yūjirō Mōri[1a] and Toshio Kawasaki[1b]

Faculty of Pharmaceutical Sciences, Fukuoka University[1a] and Faculty of Pharmaceutical Sciences, Kyushu University[1b]

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The air-dried underground parts of *Aspidistra elatior* Blume (Liliaceae) (Japanese name, haran) have been known in Japan as a folk medicine,[2] for instance as expectorant, diuretic and tonic. Concerning their steroidal constituents Takeda and his coworkers reported[3] diosgenin, a sapogenin (markogenin –), a phytosterol and an unidentified amorphous saponin in the material collected in February.

This paper describes the isolation of a new diosgenin glycoside named aspidistrin (I) and the characterisation as 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (3-O-β-lycotetraoside).

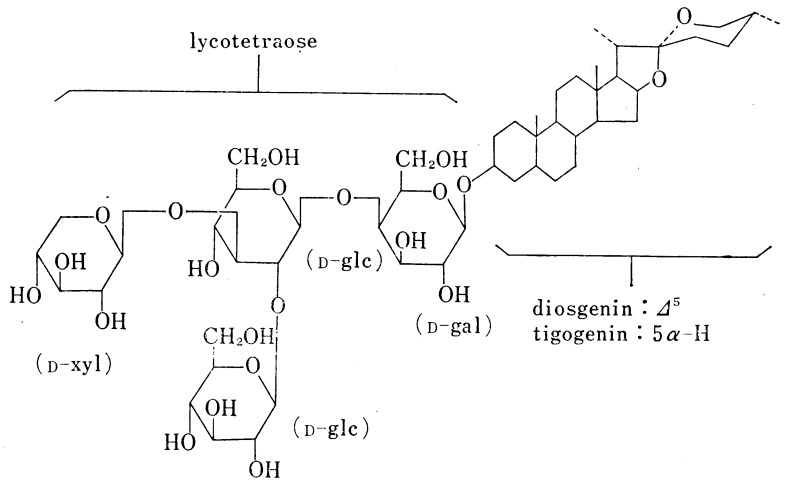
An examination of the underground parts collected in April for the steroidal constituents showed the existence of β-sitosterol and stigmasterol in a free state or as esters and of diosgenin along with a trace amount of unknown compound in the acid hydrolysate of the glycoside fraction. Therefore the major glycosidal steroidal compounds in the plants were thought to be the glycosides of diosgenin or the corresponding furostanol derivatives.[4]

Extraction and separation of the glycosides in conventional way as shown in Chart 1 gave a fraction (Fr. 4) which afforded on recrystallization a pure compound (I) as colorless needles, mp 265—267° (decomp.), $[\alpha]_D$ −68°. I was accompanied in the Fraction A by several more polar compounds which were positive to the Ehrlich reagent and presumed to be furostanol bisglycosides.[4] The Fraction C consisting of the Ehrlich positive compounds was

1) Location: *a) Nanakuma, Fukuoka; b) Katakasu, Fukuoka.*
2) T. Kariyone and K. Kimura (eds.), "Dictionary of Japanese Medicinal Plants," Hirokawa Publishing Co., Tokyo, 1963, p. 296.
3) K. Takeda, T. Okanishi, and A. Shimaoka, *Yakugaku Zasshi*, **76**, 445 (1956).
4) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968).

incubated with cellulase to give a product which showed on thin-layer chromatogram (TLC) almost single spot. It was purified through chromatography and recrystallization to yield colorless needles, mp 265—267° (decomp.), $[\alpha]_D$ −66°, which were identical in all respects with I.

I gave on complete acid hydrolysis one mole each of diosgenin, galactose and xylose together with two moles of glucose. It showed on the infrared (IR) spectrum the characteristic absorptions[5] of 25D-spirostane and was negative to the Ehrlich reagent[4] and active against a fungi *Piricularia orizae*.[6] Accordingly[4,7] I is considered to be a tetraglycoside of diosgenin and not the corresponding furostanol bisglycoside (proto-type compound) which yields diosgenin secondarily during acid hydrolysis. Furthermore, since the composition of the sugar moiety is just same as that of desgalactotigonin (II)[8] (tigogenin 3-O-β-lycotetraoside) and the peracetate of I, mp 114—118°, $[\alpha]_D$ −49.5°, showed on the mass spectrum the peaks[9] originated from the acetylated terminal pentose (*m/e* 259) and hexose (*m/e* 331) residues and a branched trisaccharide unit (pentose-(hexose-)-hexose) (*m/e* 835), I is assumed to be diosgenin 3-O-lycotetraoside. When I was hydrogenated over palladium charcoal in 60% alcohol dihydroaspidistrin (III), mp 280—283° (decomp.), $[\alpha]_D$ −64°, was provided. III was negative in the tetranitromethane test[10] and showed the IR absorptions due to 25D-spiroketal side chain. Hydrolysis of III gave galactose, xylose and glucose in a molar ratio of 1:1:2 and an aglycone which was identified as tigogenin. Therefore III is the tigogenin tetraglycoside corresponding to I. Comparisons of the melting points, alone and on admixture, optical



aspidistrin (I) : Δ^5
desgalactotigonin (II) = dihydroaspidistrin (III): 5α-H

5) E.S. Rothman, M.E. Wall, and C.R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952).
6) S. Imai, S. Fujioka, E. Murata, M. Goto, T. Kawasaki, and T. Yamauchi, *Ann. Rept. Takeda Res. Lab.*, **26**, 76 (1967).
7) R. Tschesche, *Kagaku no Ryōiki*, **25**, 571 (1971); H.D. Woitke, J.P. Kayser, and K. Hiller, *Pharmazie*, **25**, 133 (1970).
8) *a*) T. Kawasaki and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **12**, 1311 (1964); *b*) K. Miyahara, Y. Ida, and T. Kawasaki, *ibid.*, **20**, 2506 (1972).
9) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, pp. 203—227; T. Kawasaki, T. Komori, Y. Ida, Y. Inatsu, K. Miyahara, and T. Nohara, Preprints, International Conference on Mass Spectroscopy, Kyoto, September, 1969, p. 221.
10) T. Momose, "Yuki Teisei Bunseki," Hirokawa Publishing Co., Tokyo, 1967, p. 247.

rotations, IR, NMR, and mass spectra, and *Rf* values on TLC of III and/or its peracetate, mp 128—130°, $[\alpha]_D$ −37.1°, with those of II, mp 284—286° (decomp.), $[\alpha]_D$ −64°, and/or its peracetate, mp 129—130.5°, $[\alpha]_D$ −36.3°, respectively, showed the complete identity of III with II.

Consequently the structure of I, diosgenin 3-O-β-lycotetraoside, is established.

Except one, α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→3)]-β-D-glucopyranoside, recently isolated from *Paris polyphylla* Sm.,[11] all the diosgenin glycosides so far reported have the sugar moiety consisting of the glucose and rhamnose units and I is a new diosgenin glycoside having a tetrasaccharide part which contains galactose and xylose as well as glucose and is found in some solanum alkaloids (tomatine and demissine)[12] and leaf saponins of *Digitalis purpurea* L. (II[8] and F-gitonin[13]). Existence of the furostanol bisglycosides corresponding to I and possibly to its parent saponins having additional monosaccharide unit (s) combined with the sugar moiety of I is suggested and the isolation of them is under investigation.

Experimental[14]

Examination of the Steroidal Constituents——Chipped and air-dried underground parts (500 g) of *Aspidistra elatior* collected in Kagawa prefecture[15] during April were extracted and fractionated in the same way as reported previouly.[16] Benzene soluble part (7 g) of MeOH extractives (150 g) was saponified and in the unsaponifiable fraction β-sitosterol and stigmasterol but no steroidal sapogenin were detected. Defatted MeOH extractives were hydrolyzed with 2N HCl in 50% EtOH for 3 hr and a crude sapogenin mixture (10 g) showing the predominant existence of diosgenin was purified by chromatography over Al_2O_3 (Wakō, 200 g) to give diosgenin, mp 201°, $[\alpha]_D$ −125° (c=0.2, chloroform).

Isolation of Aspidistrin (I)——The procedure is shown in Chart 1. Fr. 4 in chromatography of Fr. B was crystallized from BuOH saturated with water to give I as colorless needles, mp 265—267° (decomp.), $[\alpha]_D$ −68° (c=1.08, pyridine). IR ν_{max} cm^{-1}: 980, 923 <900, 866. *Anal.* Calcd. for $C_{50}H_{80}O_{22}\cdot 3H_2O$: C, 55.24; H, 7.97. Found: C, 55.32; H, 7.87. Negative to the Ehrlich reagent,[4] active against *Piricularia orizae*.[6] I was acetylated with Ac_2O–pyridine (1:1) on a boiling water-bath[8b] to give the peracetate as a white powder (from ether–hexane), mp 114—118°, $[\alpha]_D$ −49.5° (c=0.85, chloroform). Mass Spectrum m/e: 835 ($C_{35}H_{47}O_{23}^+$), 397 ($C_{27}H_{41}O_2^+$), 396 ($C_{27}H_{40}O^+$), 331 ($C_{11}H_{19}O_9^+$), 259 ($C_{11}H_{15}O_7^+$).

Enzymatic Hydrolysis of a Mixture of the More Polar Saponins——Fr. C (15 g) (*Rf*, 0.30 (major), 0.26, 0.13, 0.07 (Ehrlich +); aspidistrin, 0.46, on TLC[4,16]) was treated with cellulase[17] (3 g) in water (1.5 liter) at 37° for 72 hr. The hydrolysate was shaken with BuOH saturated with water and the extractives (3.25 g) was chromatographed over silica gel (Kantō, 100—200 mesh, 400 g) using $CHCl_3$–MeOH–water (7:3:1) as solvent to give a homogeneous fraction (2.4 g). It was crystallized from BuOH saturated with water to yield colorless needles, mp 265—267° (decomp.), $[\alpha]_D$ −66° (c=1.02, pyridine), which were identified with I by mixed melting point determination and by comparisons of their *Rf* values on TLC and IR spectra.

Qualitative and Quantitative Determinations of the Components of I——Hydrolysis of I and determinations of the components were carried out in the same way as previously reported.[16] The aglycone (yield, 37.3%) was identified with diosgenin and the component monosaccharides were shown to be glucose, galactose and xylose in a molar ratio of 2.03: 0.98: 1.00 (total sugar yield, 62.8%, estimated as glucose) (Calcd. for diosgenin lycotetraoside, aglycone, 38.9%; total sugar, 61.1%).

11) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), submitted.
12) R. Kuhn, I. Löw, and H. Trishmann, *Chem. Ber.*, **90**, 203 (1957).
13) T. Kawasaki, I. Nishioka, T. Komori, T. Yamauchi, and K. Miyahara, *Tetrahedron*, **21**, 299 (1965).
14) Melting points were taken on a Kofler block and are uncorrected. Optical rotations were measured at 21—26° with a Yanagimoto Polarimeter OR-20. IR spectra were obtained in KBr disks with an IR spectrometer, Hitachi Model EPI-G 3. Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 4.4 kV, an ionizing potential of 75 eV and a source temperature of 200°. NMR spectra were taken at 60 MHz on a JEOL-C-60H spectrometer in $CDCl_3$ solution.
15) Supplied by Mr. Y. Nakai, to whom the authors are grateful.
16) T. Kawasaki, I. Nishioka, T. Tsukamoto, and K. Mihashi, *Yakugaku Zasshi*, **86**, 673 (1966).
17) Commercial preparation ("meicellase", Meiji Seika Co., Ltd.) consisting of mainly cellulase, collobiase, xylanase, amylase, lipase and protease. Kindly furnished by Dr. Hara of Meiji Seika Co., Ltd., to whom the authors thank.

Hydrogenation of I to Dihydroaspidistrin (III)——I (500 mg) in 60% EtOH (200 ml) was shaken with 5% Pd/C (200 mg) under H_2 at room temperature. The product was crystallized from dil. MeOH to give III as colorless needles (390 mg), mp 280—283° (decomp.), $[\alpha]_D$ −64° (c=1.01, pyridine). IR ν_{max} cm^{-1}: 982, 922<900, 866. Tetranitromethane test:[12] negative. Complete acid hydrolysis gave tigogenin, mp 204°, identified with an authentic sample, and glucose, galactose and xylose in a ratio of 2.3:1.2:1.0. Peracetate of III prepared in the same way as in I was crystallized from ether–hexane to give a white powder, mp 128—130°, $[\alpha]_D$ −37.1° (c=1.16, chloroform). Mass Spectrum m/e: 835 ($C_{35}H_{47}O_{23}^+$), 399 ($C_{27}H_{43}O_2^+$), 331 ($C_{14}H_{19}O_9^+$), 259 ($C_{11}H_{15}O_7^+$). III and its peracetate were identified with desgalactotigonin (II), mp 284—286° (decomp.), $[\alpha]_D$ −64.0° (c=0.5, pyridine)[8a] and its peracetate, mp 129—130.5°, $[\alpha]_D$ −36.3° (c=1.02, chloroform),[8b] respectively, by mixed melting point determination and by comparisons of their IR, NMR (acetate) and mass spectra (acetate) and *Rf* values on TLC.

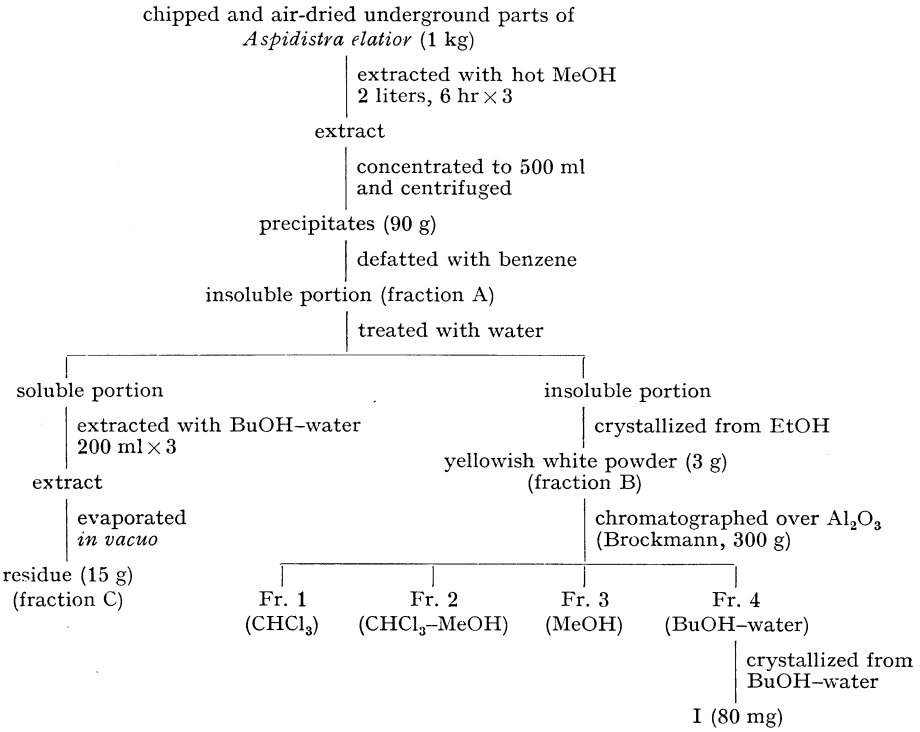


Chart 1

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