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Steroid Glycosides in *Paris polyphylla* Sm.

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Steroid glycosides in dried rhizomes of *Paris polyphylla* Sm. obtained in a market of Katmandu, Nepal, were investigated.

Diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-β-D-glucopyranoside (I), mp 276—278° (decomp.), $[\alpha]_D$ −133.0°, diosgenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (V), mp 203—206° (decomp.), $[\alpha]_D$ −153.2°, and pregna-5,16-dien-3β-ol-20-one 3-O-β-chacotrioside (IX), mp 260—262° (decomp.), $[\alpha]_D$ −72.2°, together with dioscin (diosgenin 3-O-β-chacotrioside) (VII), were isolated and characterized.

I is the first diosgenin glycoside to have a L-arabinose unit and that in the furanose type, and V is a new tetraglycoside which could be regarded as a probable parent saponin of co-existing VII. IX attracts a particular attention in that the sugar moiety and the aglycone are respectively identical with and closely related to those of VII.

Co-occurrence of the proto-type compounds of I and VII was also suggested.

The rhizomes of Himalayan *Paris polyphylla* Sm. (Liliaceae) are said to be used as a folk medicine in some parts of the area and called "*Satuwa*" in Nepal.[2] Dutt and his collaborators[3] obtained from those of Nepal two glycosides, crystalline and amorphous, similar to unidentified glycosides, paridin and paristyphnin, found in European *Paris quadrifolia* L.[4] Singh and his co-workers[5] isolated from the roots a diosgenin monoglucoside, mp 274°, and Wei-Kuang Huang[6] reported that, of six species of *Paris* in Yunnan, all contained diosgenin, three yielded pennogenin and the rhizomes of *Paris dunniana var. oligophylla* contained dioscin and a crystalline saponin, mp 254—256°.

As a part of study on the medicinal plants in Eastern Himalaya a chemical investigation on the dried rhizomes[7] of the title plant commercially available in a market of Katmandu, Nepal, was carried out, and this is to report isolation and characterization of two new diosgenin glycosides (I, V) accompanied by dioscin (VII) and of a new pregnane glycoside (IX).

The methanol extractives of plant material were treated and fractionated as shown in Chart 1 to give four compounds, tentatively named Pa—d, all as thin-layer chromatographically (TLC) pure crystals.

Pa (I), mp 276—278° (decomp.), $[\alpha]_D$ −133.0°, showed on an infrared (IR) spectrum the absorptions of 25D-spiroketal side chain[8] and polyhydroxy groups, and was negative to the Ehrlich reagent[9] suggesting that it is a 25D-spirostanol glycoside. On acid hydrolysis it

1) Location: *1276 Katakasu, Fukuoka.*
2) Private communications from Prof. O. Tanaka and from Dr. H. Kanai through Dr. U. Sankawa.
3) *a*) R.N. Chopra, R.L. Badhwar and G. Ghosh, "Poisonous Plants of India," Vol. II, Indian Council of Agricultural Research, New Delhi, 1965, p. 870; *b*) A.T. Dutt, N.R. Chatterjee, S. Ghosh, and R.N. Chopra, *Arch. Pharm.*, **276**, 343 (1938).
4) Lliteratures cited in 3b).
5) A. Singh, S.N. Srivastava and L.D. Kapoor, *Indian J. Chem.*, **4**, 460 (1966) [*C.A.*, **66**, 35388 (1967)].
6) Wei-Kuang Huang, *Yao Hsueh Hsueh Pao*, **12**, 657 (1965) [*C.A.*, **64**, 11553 g (1966)].
7) Furnished by Prof. O. Tanaka.
8) E.S. Rothman, M.E. Wall and C.R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952).
9) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968).

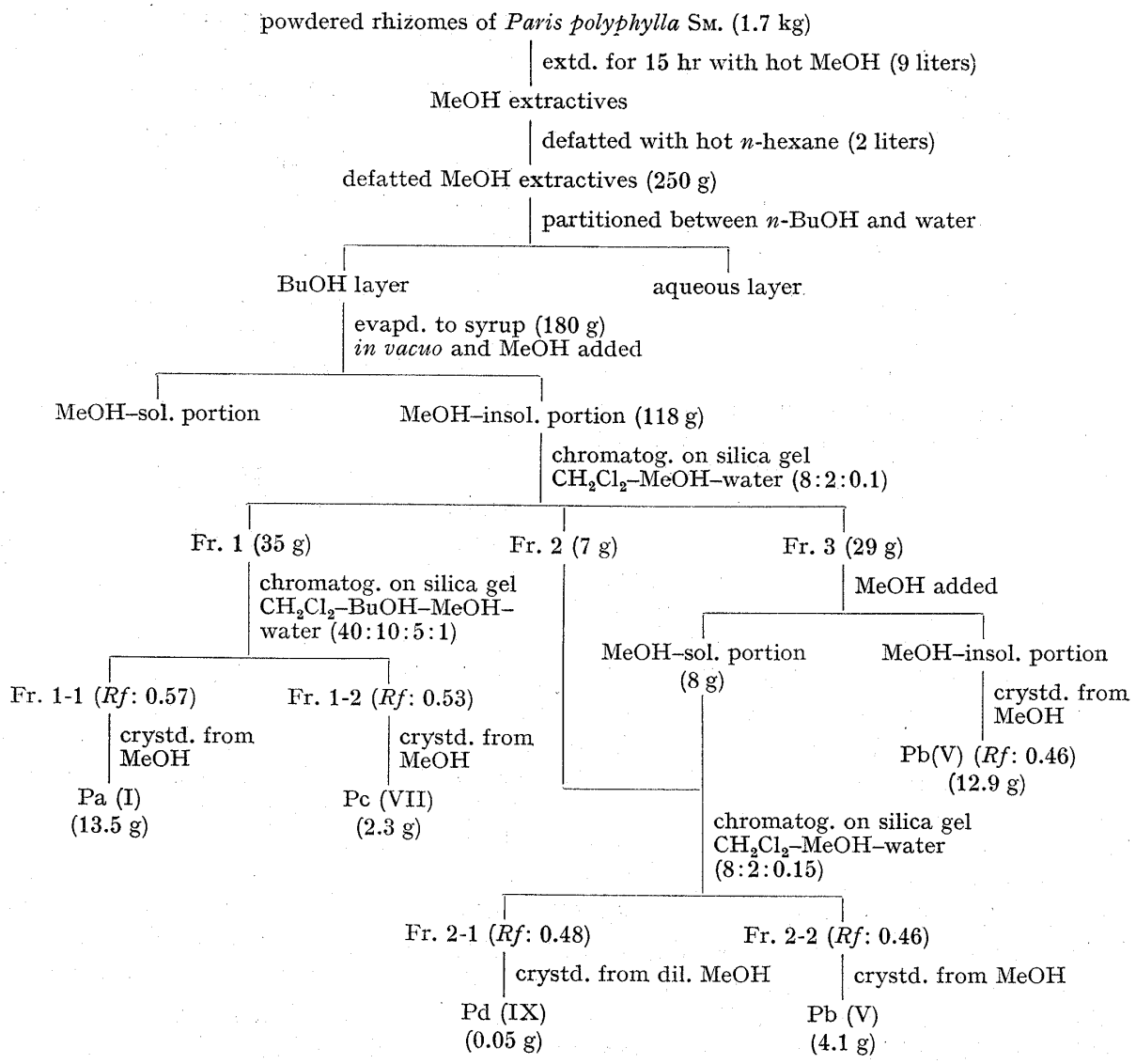


Chart 1

yielded diosgenin, glucose, rhamnose and arabinose. The permethylate (II), mp 162—163°, prepared by the Hakomori method,[10] exhibited on its mass spectrum the molecular ion at *m/e* 966 and the peaks at *m/e* 189 and 175 but not at 219, which are ascribable to the fragments originated from the permethylated terminal methylpentose, pentose and hexose residues, respectively.[11] The above data indicate that I is a diosgenin triglycoside having rhamnose and arabinose units combined with one glucose residue. The methanolysis products of II were examined by gas–liquid chromatography (GLC) to show the existence of methyl glycosides of 2,3,4-tri-O-methylrhamnopyranose, 2,3,5-tri-O-methylarabinofuranose and 3,6-di-O-methylglucopyranose, and they were acid hydrolyzed and subsequently separated on a silica gel column to give 2,3,4-tri-O-methyl-L-rhamnose, 2,3,5-tri-O-methyl-L-arabinose and 3,6-di-O-methyl-D-glucose. Therefore the sugar moiety of I is either L-arabinofuranosyl-(1→2)-[L-rhamnopyranosyl-(1→4)]-D-glucopyranose or L-rhamnopyranosyl-(1→2)-[L-arabinofuranosyl-(1→4)]-D-glucopyranose. The former is ruled out because a mild acid hydrolysis of

10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
11) 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.

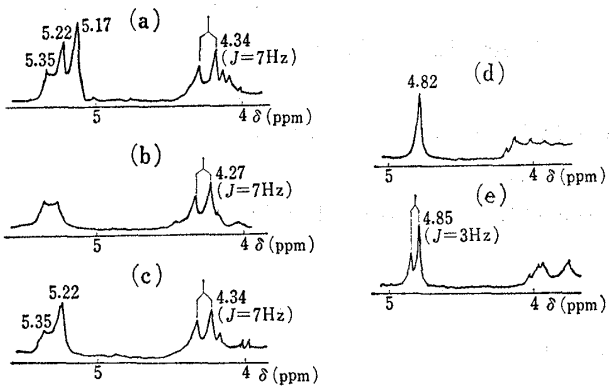


Fig. 1. NMR Spectra (in $CDCl_3$, 60 MHz) of II (a), the Permethylates of III (b) and IV (c) and Synthetic Methyl 2,3,5-Tri-O-methyl-α- and β-L-arabinofuranosides (d and e)

I yielded two glycosides which were identified as trillin (diosgenin 3-O-β-D-glucopyranoside)[12] (III) and prosapogenin A of dioscin (diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside)[12,13] (IV). Molecular rotation difference (−284°) between I and IV suggests[14] the α-configuration of L-arabinofuranose unit ($[M]_D$ of methyl α- and β-L-arabinofuranosides,[15] −210° and +194°, respectively) and it was confirmed by comparison (Fig. 1) of the anomeric proton signals on the nuclear magnetic resonance (NMR) spectra of II, the permethylates of III and IV and of synthetic methyl 2,3,5-tri-O-methyl-α- and β-L-arabinofuranosides. Consequently I is assigned the structure, diosgenin-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-β-D-glucopyranoside.

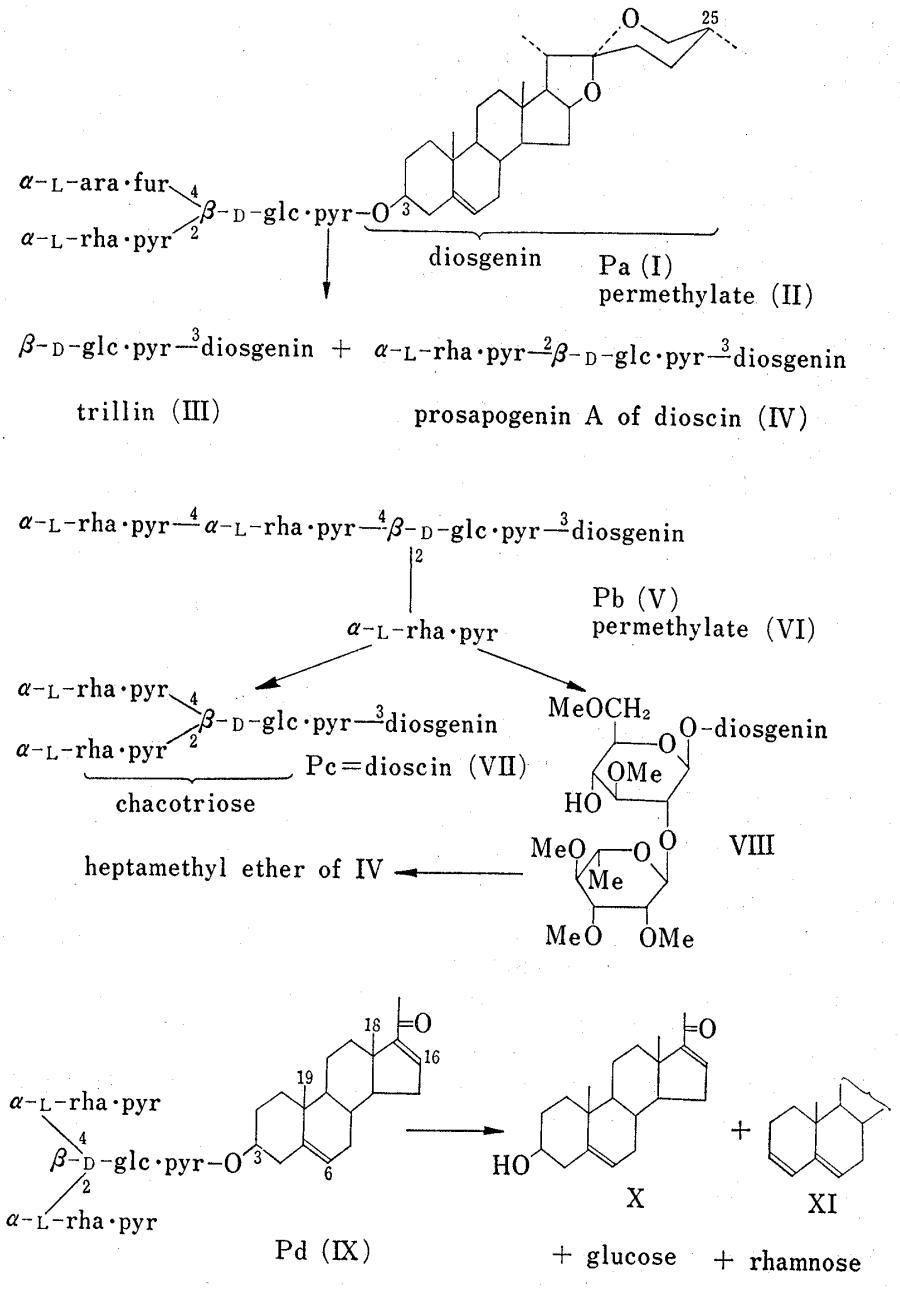
Pb (V), mp 203—206° (decomp.), $[\alpha]_D$ −113.4°, was supposed in the same way as in I to be 25D-spirostanol glycoside, and an acid hydrolysis yielded diosgenin, D-glucose and L-rhamnose. The permethylate (VI), mp 138—140°, gave a mass spectrum on which the molecular ion and the peaks due to permethylated terminal methylpentose and methylpentosyl-methylpentose were observed at *m/e* 1154, 189 and 363, respectively. The methanolysis products of VI showed on GLC three peaks identical with those of methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 2,3-di-O-methyl-rhamnose and 3,6-di-O-methyl-glucose. From these results V is considered to have another mole of L-rhamnopyranose combined with one of two terminal rhamnose units of dioscin (diosgenin β-chacotrioside)[12] (VII). A partial acid hydrolysis of V gave prosapogenin, mp 277—279°, (decomp.), which was identical with Pc, mp 277—278° (decomp.), also obtained from crude drug, and they were proved to be nothing but VII by direct comparison with an authentic specimen. When VI was acid hydrolyzed in a mild condition a few compounds were provided, and the main (VIII) was successfully isolated with the aid of preparative thin-layer chromatography. VIII, mp 186—187°, $[\alpha]_D$ −98.8°, showed on an IR spectrum the hydroxy absorptions, and on a mass spectrum the molecular ion at *m/e* 792 and the fragment originated from permethylated terminal methylpentose residue at *m/e* 189. On methylation it gave a product identical with the per (hepta) methyl ether of IV. These data indicate that VIII is the hexamethyl ether of IV and the free hydroxy group is located at C-4 of glucose, where the rhamnosyl-rhamnose residue is linked to form V. The molecular rotation difference (−252°) between V and VII suggests the α-conjugation to VII of the third L-rhamnopyranosyl residue.

Thus the structure of V is considered as diosgenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside.

Pd (IX), mp 260—262° (decomp.), $[\alpha]_D$ −72.2°, showed on an IR spectrum the absorptions of enone system and hydroxy groups but none of a spiroketal side chain. Hydrolysis with sulfuric acid in 50% acetone[16] yielded two kinds of water-insoluble product (X and XI)

12) *a*) T. Tsukamoto, T. Kawasaki and T. Yamauchi, *Pharm. Bull.* (Japan), **4**, 35 (1965); *b*) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).
13) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1070 (1968).
14) W. Klyne, *Biochem. J.*, **47**, xli (1950).
15) J. Staněk, "The Monosaccharides," Academic Press, New York and London, 1963, pp. 275, 305.
16) Hydrolysis with HCl in MeOH provided at least three kinds of water-insoluble product. Two of them were identical on TLC with X and XI, and the third one was assumed to be 16-methoxy-pregn-5-en-3β-ol-20-one.

along with glucose and rhamnose. X was presumed to be pregna-5,16-dien-3β-ol-20-one on the basis of its analytical and mass spectral data ($C_{21}H_{30}O_2$, M^+, *m/e* 314), IR (hydroxy and enone), ultraviolet (UV) (enone) and NMR spectra (18- and 19-methyl groups of steroid, an acetyl side chain and two vinyl protons), and it was found actually to be pregna-5,16-dien-3β-ol-20-one by identification with the sample synthesized from diosgenin according to a modified Marker method.[17] Another product XI, showing on a mass spectrum the molecular ion at *m/e* 296 ($C_{21}H_{28}O^+$), has, in addition to enone grouping, a conjugated diene but no hydroxy group in the molecule. Accordingly XI is safely regarded as pregna-3,5,16-trien-20-one, an artifact formed secondarily from X during hydrolysis,[18] and IX is considered to be a glycoside composed of X, glucose and rhamnose. On a mass spectrum of IX permethylate (XII) the molecular ion and the fragment due to permethylated terminal methylpentose re-



17) D.H. Gould, H. Staeudle and E.B. Hershberg, *J. Am. Chem. Soc.*, **74**, 3685 (1952).
18) T. Tsukamoto, T. Kawasaki, T. Yamauchi and Y. Shimauchi, *Pharm. Bull.* (Tokyo), **5**, 492 (1957); T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **7**, 343 (1959).

sidue were observed at *m/e* 880 and 189, respectively, indicating that the sugar moiety is a trisacchride consisting of one mole of glucose and two moles of rhammose, one or both of which is located at terminal. Methanolysis of XII and examination of the products by GLC showed the presence of only two methylated sugars, methyl pyranosides of 2,3,4-tri-O-methylrhamnose and 3,6-di-O-methylglucose. Therefore the two rhamnose residues should be combined with the hydroxy groups at C-2 and C-4 of the glucose unit to form a branched-chain trisaccharide such as chacotriose, the sugar moiety of VII, and IX seems probably to be pregna-5,16-dien-3β-ol-20-one 3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (3-O-β-chacotrioside). The structure was unequivocally corraborated by direct comparison with the sample[19] prepared from 26-O-β-D-glucopyranosyl 22-methoxyfurost-5-ene-3β,26-diol 3-O-β-chacotrioside.[9]

The methanol- and butanol-soluble portion of defatted methanol extractives (Chart 1) was separated through a silica gel column to give an amorphous but thin-layer chromatographically homogeneous compound (XIII). It was positive to the Ehrlich reagent[9] and a hydrolysate with almond emulsin showed on TLC two spots identical with those of I and VII. The results suggest that XIII is a mixture of the furostanol bisglycosides (proto-type compounds)[9] corresponding to I and VII.

The diosgenin glycosides so far obtained in pure state and characterized in detail are, except one, the lycotetraoside containing glucose, galactose and xylose,[20] the triglycosides having rhamnose and glucose or their corresponding prosapogenins.[12,13,21] I is the first example to have an L-arabinose unit and that in the furanose type, and V is a new tetraglycoside which could be regarded as a probable parent saponin of co-existing VII.

Pregn-5-en-3β-ol-20-one and 5α-pregnan-3β-ol-20-one were found as their unidentified glycosides in Uzara root,[22] the mono- and triglucosides and two diglucosides of the former were isolated from *Nerium odorum*,[23] and X was obtained from *Solanum vespertilio*.[24] IX attracts a particular attention in that the sugar moiety and the aglycone are respectively identical with and closely related (chemically and possibly biogenetically) to those of VII.

Most likely co-existence of the proto-type compounds of I and VII is also noted.

Experimental

Melting points were determined on a micro melting point apparatus (an air-bath type) and are uncorrected. Optical rotations were measured with JASCO DIP-SL automatic polarimeter at 20—25° unless otherwise specified. IR and UV spectra were obtained with a JASCO IR-G and a Shimadzu SV50-A spectrometers, respectively, and NMR spectra were taken at 60 MHz on a JEOL-C-60H spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard (s, singlet; d, doublet; m, multiplet). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 4.6 and 6.4 kV, an ionizing potential of 30 eV and a source temperature of 180—230°. GLC was run on a Yanagimoto GCG 550F with flame ionization detector using glass column (4 mm×1.7 m) packed with 5% 1,4-butanediol succinate on Chromosorb W (60—80 mesh): for trimethyl ethers of arabinose and rhamnose, column temp. 108°, N_2 73 ml/min; for dimethyl ethers of glucose, column temp. 198°, N_2 55 ml/min; for methyl ethers of rhamnose, column temp. 178°, N_2 60 ml/min. PPC was conducted on Tōyō Roshi No. 50 in double ascending method using upper layer of *n*-BuOH–pyridine–water (6: 2: 3) +pyridine (1) as solvent and aniline hydrogen phthalate as visualizing agent. TLC was performed on Kieselgel G nach Stahl (Merck) using $CHCl_3$–MeOH–water (7: 3: 0.5) (solv. 1) and *n*-hexane–AcOEt (1: 1) (solv. 2) as solvents and 10% H_2SO_4 (spraying followed by heating) as detector. Column chromatography was carried out with

19) Presented at the Kyushu Branch Meeting of Pharmaceutical Society of Japan, Kumamoto, May 1968 (to be published).
20) Y. Mōri and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 224 (1973).
21) T. Tsukamoto and T. Kawasaki, *Pharm. Bull.* (Japan), **4**, 104 (1956).
22) R. Tschesche and G. Snatzke, *Ann.*, **636**, 105 (1960).
23) T. Yamauchi, M. Hara and K. Mihashi, *Phytochemistry*, **11**, 3345 (1972).
24) A.G. Gonzarez, C. Garcia Francisco, R. Freire Barreira and E. Suarez Lopez, *An. Quim.*, **67**, 433 (1971) [*C.A.*, **75**, 115896 (1971)].

Kieselgel (0.05—0.2 mm) (Merck) in thirty to fifty times quantity of the material unless otherwise indicated.

Extraction and Isolation of Steroid Glycoside——Commercial dried rhizomes (purchased in a market of Katmandu)[7] were powdered and treated as shown in Chart 1. Each fraction was examined by thin-layer chromatography (TLC).

Pa (I)——Colorless needles, mp 276—278° (decomp.), $[\alpha]_D$ −133.0° (c=0.56, MeOH) ($[M]_D$ −1136°). IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 982, 920, 898, 866 (intensity 898>920, 25D-spiroketal side chain). Ehrlich test: negative. *Anal.* Calcd. for $C_{44}H_{72}O_{16}\cdot H_2O$: C, 60.53; H, 8.31. Found: C, 60.48; H, 8.39.

I (140 mg) was refluxed with 3N H_2SO_4 in 50% EtOH (20 ml) for 2 hr. The reaction mixture was treated in a usual way, water-insoluble product was crystallized from MeOH to give diosgenin, mp 198—200° (alone and on admixture with an authentic sample), $[\alpha]_D$ −125° (c=0.58, $CHCl_3$) and the sugar fraction was examined by paper partition chromatography (PPC) to show the presence of glucose, rhamnose and arabinose.

Permethylate (II) of I——I (1.2 g) was methylated by the Hakomori method[10] to give II (720 mg) as colorless needles (from MeOH), mp 162—163°, $[\alpha]_D$ −107.0° (c=1.02, $CHCl_3$), no hydroxy absorptions on IR spectrum. Mass spectrum: *m/e* 966 ($C_{52}H_{86}O_{16}^+$, M^+), 189 ($C_9H_{17}O_4^+$), 175 ($C_8H_{15}O_4^+$). NMR ($CDCl_3$): 5.22 (1H, broad singlet), 5.17 (1H, s), 4.34 (1H, d, J=7 Hz) (Fig. 1). *Anal.* Calcd. for $C_{52}H_{86}O_{16}$: C, 64.57; H, 8.96. Found: C, 64.55; H, 8.82.

II (1.2 g) was methanolyzed on refluxing with 1N HCl in MeOH (20 ml) for 1.5 hr, worked up as usual and the methylated sugar portion was examined by GLC. Four peaks were detected and identified with those of methyl glycosides of 2,3,4-tri-O-methyl-α-L-rhamnopyranose,[25] 2,3,5-tri-O-methyl-α- and β-L-arabinofuranoses[26] and 3,6-di-O-methyl-α-D-glucopyranose[25] by comparison with their authentic samples and the reference compounds, methyl glycosides of 2,3,4-tri-O-methyl-α- and β-L-arabinopyranoses,[26] 2,3-, 2,4-, 3,4- and 4,6-di-O-methyl-α-D-glucopyranoses.[25]

The methylated sugar portion (140 mg) was hydrolyzed with 1N HCl for 2 hr, the hydrolysate was neutralized with Amberlite A-400, evaporated *in vacuo* and the residue was chromatographed on silica gel (45 g) (solvent, CH_2Cl_2–MeOH–water 10: 2: 0.1→8: 2: 0.2) to give three compounds, colorless crystals (from benzene), mp 78—80°, $[\alpha]_D^{14}$ +126.8° $\xrightarrow{2\text{ hr}}$ +149.2° (c=0.43, water), a syrup, $[\alpha]_D^{14}$ +24.2° $\xrightarrow{2\text{ hr}}$ +25.4° (c=0.83, water) (anilide, colorless needles (from pet. ether), mp 112—114°, $[\alpha]_D^{14}$ +126.9° (c=0.38, EtOH)), and colorless crystals (from AcOEt), mp 113—115°, $[\alpha]_D^{14}$ +100.2° $\xrightarrow{5\text{ hr}}$ +59.8° (c=0.81, water). They were identical, in their melting points and optical rotations, respectively with those of 2,3,5-tri-O-methyl-L-arabinose,[15] 2,3,4-tri-O-methyl-L-rhamnose[12b] and 3,6-di-O-methyl-D-glucose[12b] appearing in the literatures.

Partial Hydrolysis of I——I (150 mg) in 0.4N H_2SO_4 in 50% EtOH (10 ml) was refluxed for 1 hr. The mixture was diluted with water (20 ml), the precipitates (110 mg) were collected and chromatographed on silica gel (31 g) with CH_2Cl_2–MeOH–water (8: 2: 0.1) as solvent to give two compounds. The major product was crystallized from MeOH to yield colorless fine needles, mp 238—243° (decomp.), $[\alpha]_D$ −118.2°, (c=0.5, MeOH) ($[M]_D$ −852.0°), *Rf* on TLC 0.63 (solv. 1), (peracetate: colorless needles (from MeOH), mp 206—209°, $[\alpha]_D$ −60.5°, (c=0.63, $CHCl_3$)), which was identified as prosapogenin A of dioscin (IV) by comparison (mixed melting point, co-chromatography on TLC) with authentic samples[27] (IV, mp 242—246° (decomp.), $[\alpha]_D$ −121.6° (c=0.66, MeOH), −108.3° (c=0.97, pyridine), *Rf* 0.63, IV peracetate, mp 204—207°, $[\alpha]_D$ −62.5° (c=1.09, $CHCl_3$)) (prosapogenin B of dioscin[13] run in parallel, *Rf* 0.69). The minor product was obtained as a minute amount of white powder (*Rf* 0.81) and identified with trillin (III) on TLC.

Pb (V)——Colorless needles, mp 203—206° (decomp.), $[\alpha]_D$ −113.4° (c=0.57, MeOH) ($[M]_D$ −1150°),

25) H. Okabe, N. Koshito, K. Tanaka and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2394 (1971).

26) Methyl 2,3,5-tri-O-methyl-α- and β-L-arabinofuranosides and 2,3,4-tri-O-methyl-α- and β-pyranosides were prepared on methylation by the Purdie method of the four methyl L-arabinosides, which were obtained by separation of their mixture yielded on usual methyl glycosidation on silica gel column using AcOEt–MeOH–water (50: 10: 1). The physical constants are as follows (those reported in the literature[15] are given in parentheses). Methyl 2,3,5-tri-O-methyl-L-arabinofuranoside: α-, $[\alpha]_D$ −83.9° (c=8.70, $CHCl_3$); β-, $[\alpha]_D$ +112.1° (c=3.39, $CHCl_3$). 2,3,4-Tri-O-methyl pyranoside: α-, mp 41—44° $[\alpha]_D$ +45.8° (c=1.12, water) (mp 46—48°, $[\alpha]_D$ +46.1°): β-, mp 45°, $[\alpha]_D$ +234.2° (c=1.03, water) (mp 44—46°, $[\alpha]_D$ +250°). Methyl L-arabinofuranoside: α-, mp 50—51°, $[\alpha]_D$ −127.5° (c=1.1, water) (mp 52°, $[\alpha]_D$ −128°): β-, mp 57°, $[\alpha]_D$ +85.5° (c=2.99, MeOH) (mp 58°, $[\alpha]_D$ +118°). Methyl pyranoside: α-, mp 127—129°, $[\alpha]_D$ +19.1° (c=1.10, water) (mp 131°, $[\alpha]_D$ +17.3°); β-, mp 168—170°, $[\alpha]_D$ +226.4° (c=1.62, water) (mp 169°, $[\alpha]_D$ +245.6°).

27) A rhamnosyl glucoside of diosgenin isolated from the underground parts of *Trillium Kamtschaticum* PALL. showed the same *Rf* value on TLC with that of IV[12,13] from dioscin and the structure was corroborated as IV on the basis of chemical and spectral data (presented at the 14th Symposium on the Chemistry of Natural Products, Fukuoka, October, 1970; to be published). The optical rotations[12] of IV and its peracetate and the melting point[13] of the permethylate reported earlier should be corrected as given in the text.

$-136.2°$ ($c=0.89$, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH), 980, 915, 900, 863 (intensity 900>915, 25D-spiroketal side chain). Ehrlich test: negative. *Anal.* Calcd. for $C_{51}H_{82}O_{20}\cdot 2H_2O$: C, 57.39; H, 8.12. Found: C, 57.14; H, 8.16. V (800 mg) was hydrolyzed and the products were examined in the same way as in I. Diosgenin was obtained as the aglycone and glucose and rhamnose were detected on PPC in the sugar portion. The sugar portion was chromatographed on cellulose powder (100—200 mesh, Tōyō) by using MeOH–AcOEt (3: 1) as a solvent to yield D-glucose, $[\alpha]_D$ $+56.5°$ ($c=1.11$, water, after 3 hr), and L-rhamnose, $[\alpha]_D$ $+8.2°$ ($c=1.02$, water, after 3 hr).

Permethylate (VI) of V——V was methylated as I to give VI as colorless needles (from MeOH), mp 138—140°, $[\alpha]_D$ $-109.8°$ ($c=0.54$, $CHCl_3$), no hydroxy absorptions on IR spectrum. Mass spectrum: *m/e* 1154 ($C_{61}H_{102}O_{20}^+$, M^+), 363 ($C_{17}H_{31}O_8^+$), 189 ($C_9H_{17}O_4^+$). NMR ($CDCl_3$): 4.40 (1H, d, $J=7$ Hz), 5.03 (1H, broad singlet), 5.21 (2H, broad singlet). *Anal.* Calcd. for $C_{61}H_{102}O_{20}$: C, 63.41; H, 8.91. Found: C, 63.26; H, 8.89. VI was subjected to methanolysis in the same way as II and the products were examined by GLC. Three peaks were detected and identified with those of methyl pyranosides[25] of 3,6-di-O-methyl-α-D-glucose, 2,3-di-O-methyl- and 2,3,4-tri-O-methyl-α-L-rhamnoses by comparison with the authentic samples including methyl 2,4- and 3,4-di-O-methyl-α-L-rhamnopyranosides.

Partial Hydrolysis of V——V (180 mg) was refluxed with 0.2N H_2SO_4 in 50% EtOH (15 ml) for 2 hr, water-insoluble product was crystallized from dil. MeOH to yield colorless needles (42 mg), mp 276—279° (decomp.) $[\alpha]_D$ $-103.4°$ ($c=0.89$, MeOH) ($[M]_D$ $-898°$). Identified as dioscin (VII) by direct comparison (mixed melting point, IR, co-chromatography on TLC) with an authentic sample.

Partial Hydrolysis of VI——VI (125 mg) in 0.5N HCl in MeOH (20 ml) was refluxed for 0.5 hr. The reaction mixture was passed through an Amberlite A-400 column, evaporated and water was added. The precipitates were collected and subjected to preparative TLC (plate, 20×20 cm; Kiesegel F (254+266) (Merck), 4 g; solvent, $CHCl_3$–MeOH (95: 5); visualized under UV light) and the main product (VIII) was separated and crystallized from MeOH to give colorless needles (25 mg), mp 186—187°, $[\alpha]_D$ $-98.8°$ ($c=0.61$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH), 1030—1150 (OCH_3). Mass spectrum: *m/e* 792 ($C_{44}H_{72}O_{12}^+$, M^+), 189 ($C_9H_{17}O_4^+$). VIII (20 mg) in $CHCl_3$ (3 ml), methyl iodide (5 ml) and silver oxide (1 g) were stirred at room temperature for 3 hr. Silver salt was removed, the solvent evaporated and the residue was crystallized from MeOH to give a compound, mp 167°, $[\alpha]_D$ $-94.0°$ ($c=0.83$, $CHCl_3$), *Rf* 0.43 (solv. 2), which was identified with the heptamethyl ether of IV,[27] mp 167°, $[\alpha]_D$ $-92.3°$ ($c=1.10$, $CHCl_3$), *Rf* 0.43, by comparison of their melting points (alone and on admixture), IR and NMR spectra and *Rf* values on TLC (heptamethyl ether of prosapogenin B of dioscin[13] run in parallel, *Rf* 0.46).

Pc (VII)——Colorless needles, mp 277—279° (decomp.), $[\alpha]_D$ $-115.0°$ ($c=0.56$, MeOH). Identified with a prosapogenin of V and dioscin (VII) by direct comparison.

Pd (IX)——Colorless plates, mp 260—262° (decomp.), $[\alpha]_D$ $-72.2°$ ($c=0.49$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1645, 1587 (enone), no spiroketal absorptions. UV λ_{max}^{EtOH} nm: 239 ($\varepsilon=7.16\times10^3$). NMR ($CD_3OD$): 0.93 (3H, s, 18-C<u>H</u>$_3$), 1.08 (3H, s, 19-C<u>H</u>$_3$), 2.27 (3H, s, C<u>H</u>$_3$CO-), 4.57 (1H, d, $J=6$ Hz), 5.28 (2H, broad singlet), 5.45 (1H, m, vinyl proton at C-6), 6.99 (1H, m, vinyl proton at C-16). *Anal.* Calcd. for $C_{39}H_{60}O_{15}\cdot 1/2H_2O$: C, 60.28; H, 7.86. Found: C, 60.26: H, 8.05. Identified as pregna-5,16-dien-3β-ol-20-one 3-O-β-chacotrioside (IX) by comparison of the melting point, IR, NMR spectra and *Rf* value on TLC with those of an authentic sample, mp 259—262° (decomp.), $[\alpha]_D$ $-79.2°$ ($c=0.71$, MeOH), prepared[19] from methyl protodioscin.[9]

Hydrolysis of IX——IX (120 mg) was refluxed with 1N H_2SO_4 in 50% acetone for 4 hr. The precipitates formed on cooling were collected by filtration and hydrolyzed as above for another 1.5 hr. A solid deposited on cooling was filtered off, the filtrate was combined with that of the first hydrolysis, and worked up as usual to give a syrup, which showed on PPC the spots of glucose and rhamnose. A solid was placed on a silica gel column and eluted with *n*-hexane–AcOEt (3: 1) to give two compounds (X and XI).

X was obtained as colorless plates (from MeOH) (16 mg), mp 203—204°, $[\alpha]_D$ $-35.9°$ ($c=0.43$, $CHCl_3$) and showed the following spectra. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1650, 1580 (enone). UV λ_{max}^{EtOH} nm: 239 ($\varepsilon=1.83\times10^4$). NMR ($CDCl_3$): 0.93 (3H, s, 18-C<u>H</u>$_3$), 1.05 (3H, s, 19-C<u>H</u>$_3$), 2.28 (3H, s, C<u>H</u>$_3$CO-), 5.35 (1H, m, vinyl proton at C-6), 6.75 (1H, m, vinyl proton at C-16). *Anal.* Calcd. for $C_{21}H_{30}O_2$: C, 80.25; H, 9.55. Found: C, 79.98; H, 9.48. X was identified (mixed mp, IR, NMR, TLC) with pregna-5,16-dien-3β-ol-20-one, mp 201—204°, $[\alpha]_D$ $-37.5°$ ($c=1.0$, $CHCl_3$), prepared from diosgenin acetate by a modified Marker method.[17]

Another product XI was obtained as a small amount of white powder, mp 141—143°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1580 (enone), no hydroxy absorptions, UV λ_{max}^{EtOH} nm: 244 ($\varepsilon=1.45\times10^4$), 236 ($\varepsilon=2.05\times10^4$), 229 ($\varepsilon=1.96\times10^4$) (enone and conjugated diene).

Permethylate (XII) of IX——Permethylation of IX as I and subsequent purification of the product by chromatography on silica gel (solvent, *n*-hexane–AcOEt (1: 2)) gave XII as a white powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650, 1580 (enone), no hydroxy absorptions. Mass Spectrum: *m/e* 880 ($C_{47}H_{76}O_{15}^+$, M^+), 189 ($C_9H_{17}O_4^+$).

Methanolysis of XII and GLC examination of the products were carried out in the same way as in II. Two peaks were observed and identified with those of methyl pyranosides[25] of 3,6-di-O-methyl-α-D-glucose and 2,3,4-tri-O-methyl-α-L-rhamnose.

Ehrlich-positive Compound (XIII)——The MeOH- and BuOH-soluble portion of defatted MeOH extractives (Chart 1) was separated on a silica gel column by using CH_2Cl_2–MeOH–water (7 : 3 : 0.3) as a solvent into two fractions. While the less polar one contained I, V, VII, and IX, the other (XIII) seemed thin-layer chromatographically homogenous, but could not be crystallized. XIII was positive to the Ehrlich reagent[9] and incubated with almond emulsin[28] at 37° for 12 hr to yield a mixture of two compounds identical on TLC with I and VII.

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