22. Takeo Tsukamoto and Toshio Kawasaki: Saponins of Japanese Dioscoreaceae. VI.[1] The Structure of Gracillin. (1).

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Gracillin is a new diosgenin glycoside which was present together with dioscin in the water-insoluble saponins obtained from the rhizome of *Dioscorea gracillima* Miq.[2,3,4]

Gracillin had a little lower Rf value than that of dioscin on a paper chromatogram[3] suggesting its more hydrophilic property, and it was hydrolyzed to diosgenin, D-glucose, and L-rhamonse on boiling with 2*N* hydrochloric acid in 50% ethanol for 5 hours,[4] indicating that, similar to dioscin,[1] it had an oligosaccharide moiety composed of the foregoing two sugars, which is combined with the hydroxyl group at C(3β) of diosgenin.

The above data and the analytical figures suggest several possible molecular formulae for gracillin as shown in Table I.

Table I. Possible Molecular Formulae

	(1) Diosg.+1Glu. +2Rham.		(2) Diosg.+2Glu. +1Rham.		(3) Diosg.+1Glu. +3Rham.		(4) Diosg.+3Glu. +1Rham.		(5) Diosg.+2Glu. +2Rham.	
	Glycoside-$3H_2O$	Octaacetate-H_2O	Glycoside-$2H_2O$	Nonaacetate	Glycoside-$2H_2O$	Decaacetate	Glycoside	Dodecaacetate	Glycoside-H_2O	Undecaacetate
	$C_{45}H_{72}O_{16}\cdot 3H_2O$	$C_{61}H_{88}O_{24}\cdot H_2O$	$C_{45}H_{72}O_{17}\cdot 2H_2O$	$C_{63}H_{90}O_{26}$	$C_{51}H_{82}O_{20}\cdot 2H_2O$	$C_{71}H_{102}O_{30}$	$C_{51}H_{82}O_{22}$	$C_{75}H_{106}O_{34}$	$C_{51}H_{82}O_{21}\cdot H_2O$	$C_{73}H_{104}O_{32}$
C%	58.55	59.89	58.68	59.89	58.27	59.40	58.49	58.05	58.38	58.70
H%	8.52	7.42	8.32	7.18	8.25	7.16	7.89	6.89	8.07	7.02
Ac%		28.2		30.7		30.0		33.3		31.7
Diosgenin yield %	44.92		45.01		39.44		39.59		39.52	
Molecular weight	923.1		921.1		1051.2		1047.2		1049.2	

Diosg.: diosgenin Glu.: glucose Rham.: rhamnose

By paper chromatographical examination of the hydrolyzed products of gracillin, it was indicated that under the above-mentioned conditions or on boiling with 4*N* hydrochloric acid in 50% ethanol-benzene mixture for 5 hours[5] the saponin is co mpletely hydrolyzed to give diosgenin, glucose, and rhamnose, while under the milder conditions partial hydrolysis occurs to yield two prosapogenins, tentatively disignated as prosapogenins A and C (Table II).

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1) Part V. T. Tsukamoto, T. Kawasaki, T. Yamauchi: This Bulletin, **4**, 35(1956).
2) T. Tsukamoto, T. Kawasaki, A. Naraki, T. Yamauchi: J. Pharm. Soc. Japan, **74**, 984(1954) (C. A., **49**, 1282(1955)).
3) T. Tsukamoto, T. Kawasaki, A. Naraki, T. Yamauchi: *Ibid.*, **74**, 1097(1954)(C. A., **49**, 2032 (1955)).
4) T. Tsukamoto, T. Kawasaki: *Ibid.*, **74**, 1127(1954)(C. A., **49**, 2032(1955)).
5) E. S. Rothman, M. E. Wall, H. A. Walens: J. Am. Chem. Soc., **74**, 5791(1952).

Table II. Hydrolyses of Gracillin and Paper Chromatographic Examinations of their Products

Hydrolytic Agent	Acid	HCl			H_2SO_4					
	Solvent	50% EtOH : benzene(1:1) [a]	50% EtOH	94% EtOH	dioxane: water(1:3)	80% EtOH				
	Concn. (*N*)	4	2	1/5	1	1	1	1/2	1/5	1/10
Refluxing Time (hr.)		5	5	1	3	3	1	1	1	1
Non-sugar Substance	Diosgenin (96~97) [b]	+	+	−	?	+	−	−	−	−
	Prosapogenin C (86~91)	−	−	+	±	++	+	±	?	?
	Prosapogenin A (50~56)	−	−	+	±	++	++	+	+	±
	Gracillin (16~22)	−	−	+	++	?	+	+	+	+
Sugar	Oligosaccharide	−	−	−	−	−	−	−	−	−
	Glucose (16~19)	++	++	+	+	++	±	+	±	?
	Rhamnose (34~38)	+	+	++	++	+	+	++	+	+

a) According to the method of E. S. Rothman, *et al.*[5]
b) Figures in parentheses indicate the Rf values (×100).
Rf values of reference compounds chromatographed in parallel with the test meterials: Dioscin 30~37, prosapogenin A of dioscin 53~59, prosapogenin C of dioscin (trillin) 86~90.

Quantitative analyses of the products formed by complete hydolysis were then undertaken and the yields of diosgenin from gracillin, determined gravimetrically, agreed approximately with those calculated from the molecular formulae (1) and (2) in Table I, while paper chromatographic separation followed by colorimetric estimation of each sugar in the hydrolyzate showed that glucose and rhamnose existed in a molar ratio of 2 : 1 (Table III).

Table III. Separatory Estimation of Glucose and Rhamnose by the Method of E. Borel, *et al.*

Test material	Conditon of Hydrolysis	Spot (cc.)	Found G (mg.)	R (mg.)	Molar Ratio G:R
Gracillin 105.8 mg.	2*N* HCl in 50% EtOH (10 cc.), 5 hrs.	0.03	0.286	0.135	1.93 : 1
		0.04	0.462	0.195	2.16 : 1
G 25.4 mg.+R 14.5 mg. (1.58 : 1)*	2N HCl in 50% EtOH (10 cc.), 3.5 hrs.	0.05	0.285	0.140	1.86 : 1

* Molar Ratio (G:R) G : glucose R : rhamnose

Therefore, gracillin might have a molecular formula (2), consisting of one mole each of diosgenin and L-rhamnose and two moles of D-glucose.

The same combination of sugars (two moles of D-glucose and one of L-rhamnose) has been reported to occur in three other glycosides, parillin,[6] gluco-convalloside,[7] and a bitter glycoside from oats.[8]

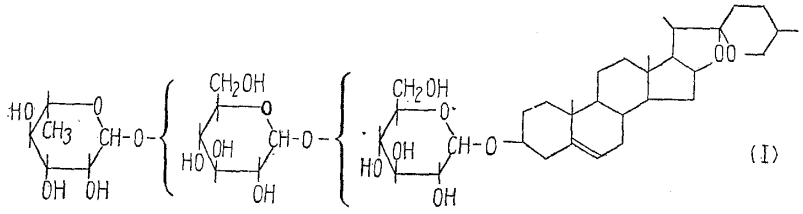
Of the two prosapogenins, C and A, the former had the Rf value agreeing with that of trillin (diosgenin monoglucoside[1]) and the latter had a little lower Rf than that of prosapogenin A of dioscin (diosgenin rhamnosidoglucoside).[1] Thus, it seems likely that prosapogenin C is a monoglucoside and A a glucosidoglucoside.** This presumption, having been supported further by the fact that rhamnose is predominant in the sugar portions of the partial hydrolyzates (Table II), was finally confirmed by examination of each purified prosapogenin.

6) A. W. Van der Haar : Rec. trav. chim., **48**, 726(1929).
7) R. Tschesche, F. Seehofer : Ber., **87**, 1108(1954).
8) M. Rohrlich, G. Train : Zeitschrif. Lebensm.-Untersuch. u-Forsch., **99**, 346(1954)(C. A. **49**, 2573(1955)).

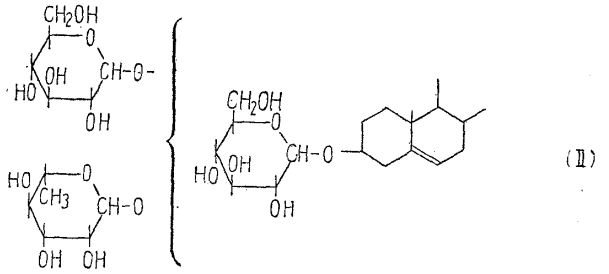
** Trillarin, a glycoside obtained from *Trillium erectum*, m.p. 200°(uncorr.) (air-dried crystals), 211°(oven-dried crystals), $[\alpha]_D^{22}$: −116.3°, has been reported to be a diosgenin diglucoside. D. C. Grove, G. L. Jenkins, M. R. Thompson : J. Am. Pharm. Assoc., **27**, 457(1938); R. E. Marker, J. Krueger : J. Am. Chem. Soc., **62**, 2548(1940).

Furthermore, since gracillin did not reduce Fehling's reagent,[4] it is apparent that the C_1-position of all the sugar components should take part in forming linkages.

Consequently, assuming the pyranose type, which is the most common ring present in the glycosides, for all the sugars, we wish to propose for gracillin either the partial structure (I) or (II).



(I)



(II)

Though the linear one (I) seems rather probable since gracillin was partially hydrolyzed to only two prosapogenins, the branched-chain structure (II), in which the rhamnose linkage might be far easier to split than that between two glucose units, cannot forever be excluded.***

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Experimental****

The paper chromatographic procedure for examination of non-sugar substances (saponin, prosapogenin, and sapogenin) was according to that described in the preceding paper[1] (at 18~23°) and the paper chromatograms of sugars were developed by BuOH:AcOH:H_2O(4:1:5) on the Tōyō Roshi No. 50 (ascending, 14~20 hrs., at 10~24°) and sprayed with aniline hydrogen phthalate solution. In all cases, reference specimens were chromatographed in parallel with test materials. A relative amount of each substance detected on the paper chromatogram was represented as +, ±, − etc., by visual comparison of the intensity and the area of the spot.

Gracillin—The procedures of isolation and purification, and the properties were described in Parts II,[2] III,[3] and IV[4] of this series. m.p. 290~293°(decomp.)(Kofler); $[\alpha]_D^{14}$: −88°(c=0.308, in pyridine); Rf: 0.18 (dioscin, 0.35). *Anal.* Found: C, 58.88, 58.70; H, 8.17, 7.87.

*** α-Solanin, the solanidine glycoside which was proved by R. Kuhn and I. Löw to have the branched-chain trisaccharide moiety, solatriose (-D-galactose<L-rhamnose / D-glucose), affords merely two prosapogenins, a galactoside and a glucosidogalactoside, owing to the great ease of hydrolysis of the rhamnose unit. Both α-chaconin, another solanidine glycoside, and solamargin, a solasodine glycoside, were suggested by the same authors possibly to have the branched-chain trisaccharide moiety, chacotriose (-D-glucose<L-rhamnose / L-rhamnose), though they respectively yielded only two prosapogenins, a glucoside and a rhamnosidoglucoside. cf. R. Kuhn, I. Löw: Ber., 88, 289(1955); R. Kuhn, I. Löw, H. Trischmann: *Ibid.*, 88, 1492(1955).

**** Specimens for microanalyses were dried over P_2O_5 in 2-mm. vacuum at room temperature for more than a week. All melting points are uncorrected unless otherwise noted.

Gracillin Acetate—Prepared by the method described in Part IV.[4] m.p. 204°(Kofler); $[\alpha]_D^{13}$: −47°(c=0.402, in $CHCl_3$). *Anal.* Found: C, 59.52, 59.35; H, 7.02, 7.03; CH_3CO, 30.53, 31.10 (Kuhn-Roth method).

Hydrolysis of Gracillin (Table II)—Gracillin (30~100 mg.) was refluxed with a hydrolytic agent, 1 cc./10 mg. of sample. The hydrolyzate was treated as reported in the preceding paper,[1] then the sugars and non-sugar substances formed were examined by paper chromatography.

Assay of Diosgenin and Separatory Estimation of Glucose and Rhamnose in the Complete Hydrolyzate—Gracillin ((1) 105.8 mg., (2) 49.7 mg.) was boiled for 5 hrs. with (1) 2*N* HCl in 50% EtOH(10 cc.) or (2) 4*N* HCl in 50% EtOH(2.5 cc.)-benzene (2.5 cc.) mixture.[5] After the hydrolysis, diosgenin was assayed gravimetrically by the procedure described before,[1] and each sugar was first separated by paper chromatography then estimated by the method of Borel, *et al.* Yield of diosgenin: (1) 46.9 mg.(44.3%), (2) 22.1 mg.(44.5%). Molecular weight of gracillin calculated from the yield: 931.7, 935.9. The results of sugar estimation are given in Table III.

Prosapogenin C—A mixture (250 mg.) of non-sugar substances, obtained by partial hydrolysis of gracillin (330 mg.) with *N* H_2SO_4 in 80% EtOH (33 cc.) for 3 hrs., was dissolved in $CHCl_3$(20 cc.), passed through an alumina column (Brockmann Al_2O_3: 3.6 g., 54×10 mm.), eluted successively with $CHCl_3$, $CHCl_3$-MeOH mixture, MeOH, and dil. MeOH, and each fraction was examined by paper chromatography (Table IV). Fractions No. 4~5 were combined (75 mg.) and acetylated by allowing to stand 2 days with Ac_2O (0.8 cc.) and pyridine (0.8 cc.). The reaction mixture was poured into ice-water, the separated crude acetate was collected, washed with water, and crystallized from MeOH. Recrystallization gave pure prosapogenin C acetate, colorless fine needles, m.p. 205~206° (in capillary, corr.), m.p. 202°(Kofler); $[\alpha]_D^{11}$: −83°(c=0.46, in $CHCl_3$). Mixed m.p. with trillin acetate,[1] m.p. 206~207° (in capillary, corr.), m.p. 203~205°(Kofler), $[\alpha]_D^{30}$: −77°(c=0.61, in $CHCl_3$), was undepressed. *Anal.* Calcd. for $C_{41}H_{60}O_{12}$(Diosgenin monoglucoside tetraacetate): C, 66.11; H, 8.12. Found: C, 65.79; H, 7.97.

TABLE IV. Liquid Chromatographic Separation of Prosapogenins

Fr. No.	1	2	3	4	5	6	7	8	9
Solvent (cc.)	$CHCl_3$ (80)	$CHCl_3$: MeOH 50:1 (80)	$CHCl_3$: MeOH 30:1 (90)	$CHCl_3$: MeOH 20:1(250)	$CHCl_3$: MeOH 10:1(260)	$CHCl_3$: MeOH 5:1 (60)	$CHCl_3$: MeOH 1:1 (330)	MeOH (170)	MeOH: H_2O 5:1 (60)
Residue (mg.)	35	trace	trace	40	35	trace	80	15	30
Diosgenin (96~97)*	+	+	+	−	−	−	−	−	−
Prosapgenin C (86~88)	−	−	−	+	+	±	±	±	±
Prosapgenin A (50~52)	−	−	−	−	−	+	++	++	++
Gracillin (16)	−	−	−	−	−	−	−	?	±

* Rf values (×100)

The acetate (40 mg.) was saponified on boiling with 5% KOH in MeOH(6 cc.) for 45 mins. Water was added to the reaction mixture, insoluble prosapogenin C was collected, washed with water, dried *in vacuo* over H_2SO_4, and recrystallized twice from MeOH to colorless fine needles, m.p. 259°(decomp.)(Kofler), $[\alpha]_D^{11}$: −89°(c=0.47, in dioxane). Admixture with trillin,[1] m.p. 262~264°(decomp.)(Kofler); $[\alpha]_D^{30}$: −91°(c=0.47, in dioxane), gave no m.p. depression. Rf 0.88 (trillin, 0.87). *Anal.* Calcd. for $C_{33}H_{52}O_8 \cdot 1\frac{1}{2} H_2O$ (Diosgenin monoglucoside+1½ H_2O): C, 65.64; H, 9.18. Found: C, 65.95; H, 9.07.

Prosapogenin C (10 mg.) was refluxed for 3 hrs. with 1*N* H_2SO_4 in 80% EtOH (1 cc.) and the hydrolysis products were examined by paper chromatography. Non-sugar substances, Rf 0.88 (+), 0.97(+)(trillin 0.87, diosgenin 0.97). Sugar, Rf 0.22(glucose 0.22).

Prosapogenin (A)—Fraction No. 7 (80 mg.) in the afore-mentioned liquid chromatography (Table IV) was let stand 2 days with 0.8 cc. each of pyridine and Ac_2O, the reaction mixture was poured into water, the deposited white substance was collected, washed with water, and crystallized from MeOH. Further recrystallization gave pure prosapogenin (A) acetate as white lustrous crystals (colorless, fine and thin plates), m.p. 236~237°(Kofler); $[\alpha]_D^{11}$: −132°(c=0.37, in $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{76}O_{20} \cdot H_2O$ (Diosgenin glucosidoglucoside heptaacetate+H_2O): C, 60.56; H, 7.48. Found: C, 60.38; H, 7.72.

The acetate (40 mg.) was deacetylated on boiling for 1 hr. with 5% KOH in MeOH (5 cc.) and the regenerated prosapogenin A was recrystallized twice from MeOH to colorless fine prisms, m.p. 260~264°(decomp.)(Kofler); $[\alpha]_D^{12}$: −112°(c=0.25, in pyridine). Rf 0.55 (prosapogenin (A) of dioscin 0.58, trillin 0.87). *Anal.* Calcd. for $C_{39}H_{62}O_{13} \cdot \frac{1}{2} H_2O$ (Diosgenin glucosidoglucoside+½ H_2O): C, 62.63; H, 8.49. Found: C, 62.60; H, 9.01.

Prosapogenin A (10 mg.) was partially hydrolyzed on refluxing for 1 hr. with 1*N* H_2SO_4 in 80% EtOH (1 cc.) and the products were examined. Non-sugar substances, Rf 0.54(+), 0.87(+), 0.96(±)(prosapogenin A of gracillin 0.54, prosapogenin A of dioscin 0.57, trillin 0.87, diosgenin

0.97). Sugar, Rf 0.22 (glucose 0.22).

Summary

Gracillin, a new diosgenin glycoside which was present together with dioscin in the water-insoluble saponins obtained from the rhizome of *Dioscorea gracillima* Miq., was proved to be composed of one mole each of diosgenin and of L-rhamnose and two of D-glucose. It afforded two prosapogenins C and A on partial hydrolysis, both of which were isolated and proved to be trillin (diosgenin monoglucoside) and glucosidoglucoside, respectively. Two possible formulae (I) and (II) were proposed for gracillin.

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23. Morizo Ishidate and Masahisa Yoshida: The Cleavage of Camphor Ring. IV. 5-Dehydrosantenic Acid.

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In the previous paper[1] it was shown that *d-trans*-7-hydroxy-π-apocamphor (7-hydroxy-α-santenone)(I) is hardly existent as such and converts spontaneously under ring cleavage between C_1 and C_7 to 1-methyl-4-acetylcyclohexan-2-one (II), whereas *d-trans*-7-hydroxy-π-apoborneol actually exists. Since the various hydroxycamphors, viz. 3-, 4-, 5-, and 6-hydroxycamphor, are all fairly stable compounds, the unstable character of (I) seemed to be due to the particular electronic property of C_7 atom caused by the strain of bicyclopentanone ring.

Now, in order to confirm this assumption and to compare with the monocyclic derivative, preparation of 5-hydroxy-π-apocamphoric acid (5-hydroxy-*d*-santenic acid) (VIII)** was attempted.

The starting meterial, 5-amino-α-santenic acid (VII), was synthesized by the following process. Isoketopinic acid (III) was oxidized with selenium dioxide to the quinone (IV). 7-Carbamyl-*trans*-π-apocamphoquinone (V) was further oxidized with hydrogen peroxide in alkaline medium to the corresponding dicarboxylic acid (VI), which was then subjected to Hofmann degradation to give 5-amino-α-santenic acid (VII), as its hydrochloride of m.p. 247°. If isoketopinoyl amide (IX) was treated with selenium dioxide in acetic anhydride, 7-cyano-π-apocamphoquinone (X) was mainly produced.

Diazotization of 5-amino-α-santenic acid (VII) resulted in a nitrogen-free dicarboxylic acid, $C_9H_{12}O_4$, m.p. 181°. The acid readily consumed hydrogen bromide and its infrared absorption spectrum (Fig. 1 A) proved to have no hydroxyl group, but to exhibit characteristic bands[2] at 6.06~6.10 and 11.00~11.24 μ (>C=CH_2). This indicates that the acid should have a structure of dehydrosantenic acid.

Among the possible dehydrosantenic acids, two optically inactive isomers, 3-

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1) Parts I and II; M. Yoshida: This Bulletin, **3**, 215, 219(1955); Part III, M. Ishidate, M. Yoshida: *Ibid.*, **4**, 43(1956).

** *trans*-π-Apocamphor and *trans*-π-apocamphoric acid would be synonymous with α-santenone and α-santenic acid, respectively.

2) D. Barnard, *et al.*: J. Chem. Soc., **1950**, 915; L. Ruzicka, *et al.*: Helv. Chim. Acta, **32**, 2125 (1949).