U.D.C. 547.918 : 582.572.7

9. Takeo Tsukamoto, Toshio Kawasaki, and Tatsuo Yamauchi : Saponins of Japanese Dioscoreaceae. V.* On the Structure of Dioscin. (1)

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu***)

Dioscin is a water-insoluble, crystalline saponin which was isolated from the rhizome of *Dioscorea Tokoro* Makino, first in 1904 by J. Honda,[1] later, in 1936, by T. Tsukamoto and Y. Ueno.[2] As for its molecular formula, Honda proposed $C_{24}H_{38}O_9 \cdot 3H_2O$, and Tsukamoto, *et al.* suggested $(C_{20}H_{34}O_8)_x$, obtaining an aglycone, diosgenin, $C_{27}H_{42}O_3$, and two unidentified sugars on acid hydrolysis. Shortly after this, the structure of diosgenin was established as Δ^5-22a-spirosten-3β-ol.[3] Recently, studies on the acid or enzymatic hydrolysis of "dioscin" obtained from *Dioscorea composita* or from an unidentified Dioscorea species, were reported.[4~6] The structure of dioscin, however, has remained still unknown.

In the previous papers,[7,8] we reported that it was isolated also from other three domestic Dioscorea species and that dioscin crystallized from ethanol, as well as the specimen of Tsukamoto, *et al.*, being yet impure, could be purified with the aid of a chromatography. The present communication deals with its partial structure.

Hydrolysis of dioscin on boiling with 5% hydrochloric acid in 50% ethanol for 3.5 hours afforded D-glucose, L-rhamnose, and the aglycone, diosgenin.[7] As described by the American workers[5,6,9,10] against the view of Marker and Lopez,[11] and as shown

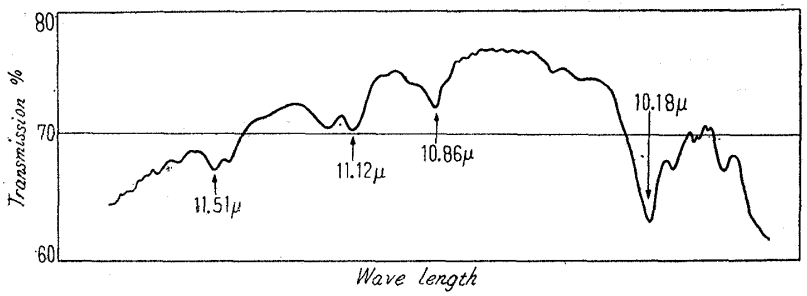


Fig. 1. Infrared Spectrum of Dioscin (in Nujol)

* Part IV. J. Pharm. Soc. Japan, **74**, 1127(1954) (C. A., **49**, 2032(1955)).
** Katakasu, Fukuoka (塚本赳夫, 川崎敏男, 山内辰郎).

1) J. Honda : Arch. exptl. Pharmakol. Path., **51**, 211(1904).
2) T. Tsukamoto, Y. Ueno : J. Pharm. Soc. Japan, **56**, 802(1936) (C. A., **32**, 7470(1938)).
3) T. Tsukamoto, *et al.* : *Ibid.*, **56**, 931(1936); *Ibid.*, **57**, 283(1937); R. E. Marker, T. Tsukamoto, D. L. Turner; J. Am. Chem. Soc., **62**, 2525(1940). Nomenclature : cf. G. Rosenkranz, C. Djerassi : Nature, **166**, 104(1950). Recently the configuration at C(25) has been defined by introducing "25a" for the diosgenin side-chain (related to D-glyceraldehyde). V. H. T. James : Chemistry & Industry, **1953**, 1388; J. Chem. Soc., **1955**, 637; I. Scheer, R. B. Kostic, E. Mosettig : J. Am. Chem. Soc., **77**, 641(1955); C. Djerassi, J. Fishman : *Ibid.*, **77**, 4291(1955).
4) E. S. Rothman, M. E. Wall, H. A. Walens: J. Am. Chem. Soc., **74**, 5791(1952).
5) M. M. Krider, M. E. Wall : *Ibid.*, **74**, 3201(1952).
6) M. M. Krider, M. E. Wall : *Ibid.*, **76**, 2938(1954).
7) T. Tsukamoto, T. Kawasaki, A. Naraki, T. Yamauchi : J. Pharm. Soc. Japan, **74**, 984(1954) (C. A., **49**, 1282(1955)).
8) T. Tsukamoto, T, Kawasaki, A. Naraki, T. Yamauchi : *Ibid.*, **74**, 1097(1954) (C. A., **49**, 2032 (1955)).
9) E. S. Rothman, M. E. Wall, C. R. Eddy : J. Am. Chem. Soc., **74**, 4013(1952).
10) M. E. Wall, S. Serota, L. P. Witnauer : *Ibid.*, **77**, 3086(1955).
11) R. E. Marker, J. Lopez : *Ibid.*, **69**, 2389(1947).

by the infrared absorption spectrum of dioscin (Fig. 1),*1 the aglycone portion suffers no structural change on hydrolysis under the above-mentioned experimental conditions and should be diosgenin itself. Since diosgenin has only one hydroxyl group at C (3β), glucose and rhamnose then should form an oligosaccharide combined at that position. The analytical data of dioscin and its acetate agreed with those calculated from the molecular formulae shown in Table I, but molecular weight determinations by the usual methods gave no reliable results.

Table I. Possible Molecular Formulae

	(1) 1 Glucose +1 Rhamnose		(2) 1 Glucose +2 Rhamnose		(3) 2 Glucose +1 Rhamnose		(4) 1 Glucose +3 Rhamnose	
	Glycoside $+3H_2O$	Hexa-acetate	Glycoside $+1.5H_2O$	Octaacetate $+H_2O$	Glycoside $+H_2O$	Nona-acetate	Glycoside	Deca-acetate
	$C_{39}H_{62}O_{12}\cdot 3H_2O$	$C_{51}H_{74}O_{18}$	$C_{45}H_{72}O_{16}\cdot 1.5H_2O$	$C_{61}H_{88}O_{24}\cdot H_2O$	$C_{45}H_{72}O_{17}\cdot H_2O$	$C_{63}H_{90}O_{26}$	$C_{51}H_{82}O_{20}$	$C_{71}H_{102}O_{30}$
C%	60.28	62.81	60.31	59.89	59.85	59.89	60.34	59.40
H%	8.82	7.65	8.44	7.42	8.26	7.18	8.14	7.16
Ac%		26.5		28.2		30.7		30.0
M.W.	777		896		903		1015	
Diosgenin Yield %	53.4		46.3		45.9		40.8	
Total Sugar Yield %*	43.9		56.0		58.0		65.2	
Rhamnose Yield %	21.1		36.6		18.3		48.5	

* Calcd. as glucose (rhamnose was converted into glucose).

Of primary requisite were the conditions for complete or partial hydrolysis of dioscin which could be used either in assaying diosgenin and sugars for the determination of the molecular weight and the number of sugars, or in clarifying the number and the order of combination of the components. Acid hydrolysis under various conditions were then carried out, followed by paper chromatographic examinations of their products. The results, summarized in Table II, showed that complete hydrolysis occurred not only on boiling with $4N$ hydrochloric acid in 50% ethanol–benzene mixture for 5 hours[4] but also with $2N$ hydrochloric acid in 50% ethanol for 3 hours,*2 and that under milder conditions various degrees of partial hydrolysis occurred, yielding two major amounts of prosapogenin (A) and (C), along with a minor prosapogenin (B), temporarily designated as such in the order of their Rf values on the paper chromatogram (order of Rf values : dioscin<A<B<C<diosgenin). On the basis of the above results, dioscin was completely hydrolyzed, followed by estimation of diosgenin. The yields, and the molecular weights calculated therefrom, suggested that the molecular formula and the number of the component monosaccharides of dioscin might be (2) or (3) given in Table I. A separatory estimation of sugars according to the Borel-Hostettler-Deuel method[12] in the complete hydro-

*1 Four absorption bands at 11.51, 11.12, 10.86, and 10.18 μ and a stronger intensity of the 11.12 μ band than that at 10.18 μ are characteristic for closed-ring isospiroketal side-chain of steroidal sapogenins. cf. 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).

*2 E. S. Rothman, *et al.*[4] described that yields of diosgenin on hydrolysis of "dioscin" isolated from rhizomes of *Dioscorea composita* with $2N$ and $4N$ HCl for 3 hrs. were 70% and 91%, respectively, of that with $4N$ HCl for 4~5 hrs. (100%).

It was also reported by M. M. Krider, *et al.*[6] that "dioscin" (original plant being undescribed) was hydrolyzed to diosgenin in 40% yield (based on dioscin). These "dioscin" are likely to be different from the currently described dioscin in their sugar moieties.

12) E. Borel, F. Hostettler, H. Deuel : Helv. Chim. Acta, **35**, 115(1952).

Table II. Hydrolysis of Dioscin

Acid	HCl										H_2SO_4											H_3PO_4		HO-Ac
Concn. (N) in 50% EtOH	a) 12%	b) 4	2	2	2	1	1/2	1/5	1/10	1/100	2	2	2	2	1	c) 1	1	1	1/2	1/5	1/10	2	2	d) 20%
Time (hr.)	3	5	22	3	1	1	1	1	1	7	20	7	3	1	3	3	1	1/2	1	1	1	1	3	3
Yield of Water-Insoluble products %		48.2 49.4	51.0	47.6 47.9	47 46	63 63	67	76	95							63 62	74 70							
Diosgenin (96~97)[e]	+	+	+	+	+	±	?	−	−	−	+	+	+	+	±	±	±	−	−	−	−	−	−	−
Pro-s.g. (C) (Synth. Trillin) (88~90)	−	−	−	−	?	+	+	+	−	?	−	?	+	+	+	+	+	+	+	?	−	−	±	−
Pro-s.g. (B) (72~75)	−	−	−	−	−	−	±	±	?	±	−	−	−	−	−	−	±	±	±	±	?	?	±	−
Pre-s.g. (A) (55~60)	−	−	−	−	−	−	+	+	±	+	−	−	−	−	−	−	+	+	+	+	+	+	+	−
Dioscin (33~39)	−	−	−	−	−	−	+	+	+	‡	−	−	−	−	−	−	±	+	+	‡	‡	‡	‡	+
Oligosaccharide		−		−	−	−	−	−	−				−	−	−	−	−	−	−	−	−			
Glucose ((a)15~19 (b)31~36)		+		+	+	±	±	?	−				+	+	+	±	±	−	−	−	−			
Rhamnose ((a)32~38 (b)46~52)		+		+	+	+	+	+	+				+	+	+	+	+	+	+	+	+			

a) In water, 3 cc./10 mg. of dioscin.
b) Method of E. S. Rothman, *et al.*,[4] 5 cc. each of $4N$ HCl in 50% EtOH and of benzene per 100 mg. of dioscin.
c) In dioxane-water (1:3) mixture.
d) In water.
e) Figures in parentheses indicate Rf values (×100) on the paper chromatograms which were developed for water-insoluble products by benzene : BuOH : water (10 : 4 : 5) at 22~25° and for sugars by BuOH : AcOH : water (4 : 1 : 5) at 16~21° (a) and by BuOH:pyridin:water (3 : 2 : 1.5) at 14~22° (b).

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

Test Material	Condition of Hydrolysis	Spot (cc.)	Found G (mg.)	Found R (mg.)	Molar Ratio G:R
Dioscin 100.0 mg.	$2N$ HCl in 50% EtOH 10 cc., 3 hr.	0.04	0.175	0.305	1 : 1.91
		0.05	0.187	0.380	1 : 2.23
〃 〃	〃	0.05	0.160	0.258	1 : 1.78
		0.06	0.207	0.335	1 : 1.78
〃 99.8 mg.	〃	0.06	0.190	0.335	1 : 2.03
		0.08	0.267	0.510	1 : 2.12
〃 100.9 mg.	$4N$ HCl in 50% EtOH 5 cc., benzene 5 cc., 5 hrs.	0.05	0.120	0.254	1 : 2.11
		0.06	0.160	0.310	1 : 1.94
G. 13.1 mg.+R. 24.4 mg. (1 : 2.03)	$2N$ HCl in 50% EtOH 10 cc., 3 hrs.	0.03	0.100	0.180	1 : 1.98
		0.05	0.175	0.272	1 : 1.71
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
G. 13.4 mg.+R. 24.6 mg. (1 : 1.96)	$4N$ HCl in 50% EtOH 5 cc., benzene 5 cc., 5 hrs.	0.12	0.260	0.433	1 : 1.83

Figures in parentheses indicate the molar ratio of glucose to rhamnose.
G : glucose, R : rhamnose.

lyzate showed that glucose and rhamnose were present in the molar ratio of 1:2, suggesting the formula (2) for dioscin (Table III). The total amount of sugars given by complete hydrolysis could not be determined, since it was found in a preliminary experiment that the amount of glucose as well as rhamnose was underestimated by the Fehling-Lehmann-Schoorl method when boiled with aqueous ethanolic hydrochloric or sulfuric acid, probably due to the loss of their reducing powers. However, glucose and rhamnose showed practically no loss on boiling with $1N$ sulfuric acid in

dilute dioxane for 3 hours, on which dioscin was hydrolyzed to prosapogenin (C) and rhamnose together with minor amounts of diosgenin and glucose, and no loss of glucose was caused by boiling with 1*N* hydrochloric acid in dilute dioxane for 3 hours, by which prosapogenin (C) was found to suffer complete hydrolysis, yielding diosgenin and glucose (Tables II and IV). Dioscin was then completely hydrolyzed in two steps

Table IV. Estimations of Glucose and Rhamnose by the Fehling-Lehmann-Schoorl Method after Boiling with Acid

Acid (cc.) in 50% EtOH		2*N* HCl (10)	*N* HCl (10)	2*N* H_2SO_4 (10)	*N* H_2SO_4 (10)	*N* H_2SO_4 (5)	*N* H_2SO_4[a] (5)	*N* H_2SO_4[b] (5)	*N* H_2SO_4[c] (10~15)	*N* HCl[c] (10~15)
Boil. Time (hr.)		3	3	3	3	1	1	1	3~3.5	3
Glucose (mg.)	Original	53.8	54.0	52.8	35.3	33.8			20.1	21.0, 21.0
	Found	28.1	28.4	29.1	24.7	29.4			19.5	20.5, 20.8
Rhamnose (mg.)	Original	55.8			47.8	47.7	46.1	46.0	45.9, 55.7, 44.1	50.1
	Found	25.8			18.5	24.2	36.4	45.5	43.1, 52.4, 41.9	45.9

a) In 18% EtOH b) In water c) In dioxane-water (1:3) mixture

and the yield of diosgenin and the total amount of sugars were determined. The values agreed with those calculated from the molecular formula (2) in Table I. Moreover, an estimation of rhamnose by the Tollens method in a dioscin molecule also gave a figure supporting the above results.

It follows, therefore, that dioscin should have a molecular formula $C_{45}H_{72}O_{16}\cdot 1\frac{1}{2}H_2O$, consisting of one mole each of diosgenin and of D-glucose and two of L-rhamnose.

According to the results of partial hydrolyses (Table II), it seemed probable that glucose was joined to diosgenin, since the Rf value of prosapogenin (C) agreed well with that of trillin (diosgenin-monoglucoside) synthesized by the Marker method and no glucose was detected except when diosgenin was found in the dioscin hydrolyzate. This fact was proved by the identification of prosapogenin (C) with synthetic trillin by comparisons of the Rf values and of the optical rotations, as well as by mixed fusions of the glycosides and of their acetates. Furthermore, the prosapogenin (A) was proved to be L-rhamnosido-D-glucoside by the analytical data and by the fact that it was partially hydrolyzed to prosapogenin (C) and rhamnose, not yielding any prosapogenin (B).

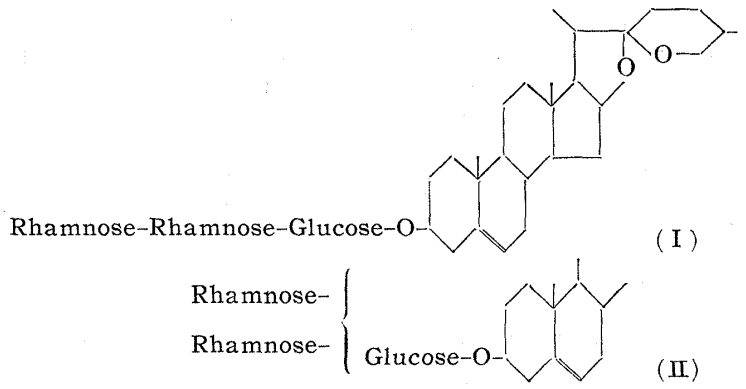
Consequently, dioscin may be represented in a partial structural formula (I) or (II),*3 in both of which all the sugar components should be joined through their potential aldehyde groups since dioscin does not reduce the Fehling reagent.[7]

*3 Two rhamnose linkages are supposed to be β from the relative ease of their splitting, while though prosapogenin (C) was identified with trillin (according to Marker, *et al.*,[13] α-glucoside) and the glucose linkage was not split off so easily as those of rhamnose suggesting that glucose was joined through α, the molecular rotation of trillin compared with that of diosgenin is likely to stand against this suggestion. cf. W. Klyne: Biochem. J.(London), **46**, xxii(1950); *Ibid.*, **47**, xli(1950). It may be due to the relative difficulty of splitting of the glucose linkage that no oligosaccharide was detected in the partial hydrolyzates (Table II).[14],*4 Enzymatic hydrolysis of pure dioscin failed on account of its low solubility in water or in 10% EtOH.

*4 α-Solanin, another solanidine glycoside, was proved to have a branched-chain trisaccharide, solatriose $\left(\text{D-galactose}<^{\text{L-rhamnose}}_{\text{D-glucose}}\right)$, as its sugar moiety. On partial hydrolysis it yields only two prosapogenins on account of the great ease of hydrolysis of the rhamnose unit. α-Chaconin also affords two prosapogenins. cf. R. Kuhn, I. Löw: Angew. Chem., **66**, 639(1954).

13) R. E. Marker, J. Krueger: J. Am. Chem. Soc., **62**, 3349(1940).

14) cf. R. Kuhn, I. Löw: Ber., **86**, 1027(1953).



Structural Formula of Dioscin

The linear structure (I) cannot explain the formation of prosapogenin (B) except by alloting to the prosapogenin (B) a new compound possibly originated from an impurity in dioscin, while the branched-chain one (II) explains by assuming a rhamnosido-glucoside of diosgenin for (B) differing from prosapogenin (A). Of the two possible structures, the latter (II) seems rather probable because not only prosapogenin (A) but also a mixture of (B) and (C) was partially hydrolyzed to (C) and rhamnose. Definite proof, however, is still lacking.

The same combination of sugars (two moles of L-rhamnose and one of D-glucose) occurs in three other glycosides, convallamarin, α-chaconin, and solamargin. Though the structure of the first has not been established,[15] that of the second was suggested by Kuhn and Löw[16] possibly to have a branched-chain trisaccharide moiety,*4 chacotriose, attached to the aglycone solanidine with its glucose unit, and the third was reported by Briggs, Brooker, *et al.*[17,18] to be a L-rhamnosido-L-rhamnosido-D-glucoside of solasodine,*5 while according to Kuhn and Löw[16] it might also have the same sugar moiety as that of α-chaconine.

We are indebted to the members of Asahi Kasei Co. for the determination of the infrared spectra. The microanalyses were carried out by Mr. T. Hattori of the microanalytical laboratory of this Institute and a part of the paper chromatographic examinations was carried out by Mr. Y. Shimauchi of this laboratory, to all of whom our thanks are due. This work was financed in part by a Grant in Aid for Scientific Research from the Ministry of Education, to which our thanks are also due.

Experimental

Dioscin—Isolated chiefly from *D. nipponica* Makino, purified, and dried over P_2O_5 in 2-mm. vacuum at 110~120° for 30 hrs. or at room temperature for more than a week.*6 The procedures of isolation and purification and the properties were described in Parts II[7] and III[8] of this series. m.p. 275~277°(decomp.)(Kofler. uncorr.); $[\alpha]_D^{13}$: −115°(c=0.373, in EtOH). *Anal.* Found: C, 60.19, 59.65, 60.39, 60.34; H, 8.50, 8.36, 8.71, 8.71.

Dioscin Acetate—Prepared by the method reported in Part II,[7] m.p. 143~145°(Kofler, uncorr), $[\alpha]_D^{12}$: −46°(c=0.392, in $CHCl_3$). *Anal.**7 Found: C, 59.76, 60.22, 60.16, 60.17, 60.08; H, 7.16, 7.44,

*5 Solasodine is very closely related in structure to diosgenin. cf. L. H. Briggs, *et al.*: J. Chem. Soc., **1959**, 3013; F. C. Uhle: J. Am. Chem. Soc., **76**, 4245(1954); Y. Sato, H. G. Latham, Jr.: *Ibid.*, **75**, 6067(1953).

*6 Dioscin dried over H_2SO_4 or P_2O_5 in 3-mm. vacuum for 24 hrs. at room temperature, of which analytical figures (C, 57.41; H, 8.53) were reported in Part II,[7] gave on complete hydrolysis diosgenin in 46.7% yield (1.7765 g.→0.8294 g.). It (432.1 mg.) lost its crystal water (22.7 mg, 5.25%) on drying to constant weight (in 2-mm. vacuum, at room temperature, or over P_2O_5 for 5 days). Calcd. for $C_{45}H_{72}O_{16}\cdot 4H_2O$: C, 57.43; H, 8.57; diosgenin (yield), 44.1; $2.5H_2O$, 4.8.

15) W. Voss, G. Vogt: Ber., **69**, 2333(1936).
16) R. Kuhn, I. Löw: *Ibid.*, 88, 289(1955).
17) L. H. Briggs, E. G. Brooker, W. E. Harvey, A. L. Odell: J. Chem. Soc., **1952**, 3587.
18) L. H. Briggs, L. C. Vining: *Ibid.*, **1953**, 2811; L. H. Briggs, E. G. Brooker: *Ibid*,. **1953**, 2833.

7.26, 7.23, 7.01; CH_3CO(Kuhn-Roth method), 28.34.

Paper Chromatography—The method for examination of non-sugar substances (saponin, prosapogenin, sapogenin) used in this work was as described in Part III,[8] using benzene:BuOH:water (10:4:5) as a developing solvent and $SbCl_3$ in $CHCl_3$ as a spray reagent, at 18~25°. Paper chromatograms of sugars, unless specified, were developed by (a) BuOH:AcOH:water (4:1:5)(ascending, 14 hrs., at 11~23°), and (b) by BuOH:pyridine:water (3:2:1.5)[19](ascending, 19 hrs. at 14~22°), in both of which the filter paper and spray reagent employed were Toyo Roshi No. 50 and aniline hydrogen phthalate, respectively. In all examinations appropriate known 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.

Hydrolysis of Dioscin (Table II)—Dioscin (30~100 mg.) was refluxed with a hydrolytic agent, 1 cc. per 10 mg. of sample. Water was added to the hydrolyzate and the water-insoluble products were separated by filtration or centrifugation, washed with water, dried *in vacuo*, and extracted with hot $CHCl_3$-MeOH mixture. The solution was evaporated to dryness and the residue was examined by paper chromatography. The Rf value of prosapogenin (C) agreed well with that of a reference compound, synthetic trillin.[8] The aqueous solution was deionized by passage through a column of ion exchange resin (Amberlite IR-4B) or neutralized with $BaCO_3$ (for H_2SO_4) or with Ag_2CO_3 followed by treatment with H_2S (for HCl) and evaporated *in vacuo* to dryness. The residue was dissolved in a small amount of MeOH or water and paper chromatographically tested for non-sugar products or sugars. The former was not detected.

Assay of Diosgenin and Separatory Estimation of Glucose and Rhamnose in the Complete Hydrolyzates—Dioscin (about 100 mg.) was boiled with 10 cc. of 2*N* HCl in 50% EtOH for 3 hrs. (or 22 hrs.). To the reaction mixture, water was added and deposited diosgenin was collected on a tared, sintered-glass filter or extracted with ether (benzene), washed with water, and the ether (benzene) solution was placed in a tared flask and evaporated to dryness. Dioscin was also hydrolyzed by the method of Rothman, *et al.*[4] and treated as above. Yields of diosgenin are given in Table II. Molecular weight of dioscin calculated therefrom: 860, 839, 813, 871, 866. The aqueous solution was deionized by Amberlite IR-4B, evaporated *in vacuo*, and the sugar mixture was diluted with water to a constant volume in 2-cc. measuring flask. The test solution (0.04~0.08 cc.) was spotted on a filter paper (Toyo Roshi No. 3) and developed for 24 hrs. with BuOH:AcOH:H_2O (4:1:5) mixture. After this, according to the procedure of Borel, *et al.*[12]*8 glucose and rhamnose were separatory estimated. As a reference experiment, mixtures of known amounts of glucose and rhamnose were similarly treated and estimated. The results are shown in Table III.

Preliminary Experiments for Total Sugar Estimation—(1) Known amount of glucose or rhamnose was boiled with acid hydrolytic agent, 30 cc. of water added, and the solution was neutralized with 1*N* NaOH (indicator: phenolphthalein). To the test solution, Fehling reagent (20 cc.) was added and total sugar was assayed by the Fehling-Lehmann-Schoorl method. The results are given in Table IV.

(2) A mixture (30 mg.) of prosapogenin (C) and a minor amount of diosgenin, which was obtained on hydrolysis of dioscin with 1*N* H_2SO_4 in dioxane-water (1:3) for 3 hrs., was refluxed for 3 hrs. with 3 cc. of (a) 1*N* H_2SO_4 in 50% EtOH, (b) 1*N* HCl in 50% EtOH, (c) 1*N* H_2SO_4 in 70% EtOH (for 2 hrs.) or (d) 1*N* HCl in dioxane-water (1:3). The reaction mixture was treated in the same way as above and the products were examined. With (b) or (d): Non-sugar substance, Rf 0.97 (diosgenin, 0.97); sugar, Rf 0.17 (solvent (a), glucose, 0.16). With (a) or (c): Non-sugar substance, Rf 0.97, 0.90 (diosgenin 0.97, prosapogenin (C) 0.89); sugar, Rf 0.17 (solvent (a), glucose 0.16).

Complete Hydrolysis in Two Steps; Total Sugar Estimation and Assay of Diosgenin—Dioscin, (a) 151.5 mg., (b) 153.0 mg., was boiled with 15 cc. of 1*N* H_2SO_4 in dioxane-water (1:3) for 3 hrs. (accompanied by foaming). The reaction mixture was cooled in ice water, 10 cc. of water was added, and the water-insoluble product was collected and washed with 20 cc. of water. The sugars in the filtrate were estimated using the method described above. Yield of reducing sugars (as glucose): (a) 64.6 mg., (b) 65.1 mg. The water-insoluble product was dried, extracted with $CHCl_3$-

*7 Specimens for microanalyses in this research were dried over P_2O_5 in 2-mm. vacuum at room temperature for more than a week.

*8 Shimadzu Photoelectric Colorimeter A. K. A. 5D (Filter: 500 mμ) was used in place of Beckman Model DU Spectrophotometer. Concentration-extinction curves of glucose and of rhamnose were taken, which were shown to be linear in the range of 0.1~0.5 mg./10 cc. of each sugar.

19) A. Jeanes, C. S. Wise, R. J. Dimler: Anal. Chem., **23**, 415(1951).

MeOH mixture, and the solution was evaporated to dryness. Yield : (a) 95.6 mg., (b) 94.7 mg. The dried residue was further refluxed with 1 *N* HCl in dioxane-water (1:3) (10 cc.) for 3 hrs. (accompanied by foaming at the begining), then diosgenin and glucose obtained were estimated. Yield, glucose, (a) 21.8 mg., (b) 20.2 mg.; total reducing sugars (as glucose), (a) 86.4 mg. (57.0%), (b) 85.3 mg. (55.7%); diosgenin (a) 74.9 mg. (49.4%), (b) 75.0 mg. (49.0%).

Estimation of Rhamnose by the Tollens Method—Dioscin (102.6 mg.) was completely hydrolyzed (cf. Table I) with 12% HCl in water (100 cc.) by the Tollens method affording 19.0 mg. of methylfurfurol phloroglucide (dried over P_2O_5 in 4-mm. vacuum at 100° to constant weight). Yield of rhamnose : 40.7 mg. (39.7%).

Prosapogenin (C)—A mixture (220 mg.) of prosapogenin (C) and diosgenin, which was obtained on hydrolysis of dioscin with 1*N* HCl in 50% EtOH for 1 hr., was dissolved in $CHCl_3$ (30 cc.), passed through an alumina column (Brockmann Al_2O_3 : 3 g., 39×10 mm.), and successively eluted with $CHCl_3$ (120 cc.), $CHCl_3$:MeOH (20:1) (160 cc), and $CHCl_3$:MeOH (10:1) (300 cc.). Evaporation of the first eluate gave 80 mg. of residue which was identified as diosgenin. The second and the third eluates gave residues 50 mg. and 70 mg., respectively, both showing one spot (Rf 0.88) on a paper chromatogram. Prosapogenin (C) thus obtained (170 mg.) was dissolved in pyridine (1.6 cc.) and Ac_2O (1.6 cc.) was added. The solution was allowed to stand for 24 hrs. and poured into ice water. The acetate was collected, washed with water, air dried, and crystallized from MeOH. Yield : 185 mg. Colorless fine needles, m.p. 203~205° (Kofler, uncorr.), m.p. 206~207° (in capillary, corr.); $[\alpha]_D^{30}$: −77° (c=0.61, in $CHCl_3$). A mixed m.p. with synthetic trillin acetate, m.p. 205° (Kofler, uncorr.),*9 $[\alpha]_D^{12}$: −72° (c=0.403, in $CHCl_3$), was undepressed. *Anal.* Calcd. for $C_{41}H_{60}O_{12}$ (Diosgenin monoglucoside tetraacetate) : C, 66.11; H, 8.12; CH_3CO, 23.11. Found : C, 65.62; H, 8.18; CH_3CO, 23.09, 23.00.

The acetate (20 mg.) was refluxed with 5% KOH in MeOH (3 cc.) for 45 mins., the reaction mixture was diluted with water, and the deposited substance was collected, washed, air-dried, and extracted with hot MeOH. On concentration and cooling, prosapogenin (C) separated out as a white gelatinous substance which was recrystallized from MeOH to colorless fine needles, m.p. 262~264° (decomp.) (Kofler, uncorr.), m.p. 250~255° (decomp.) (in capillary, uncorr.), $[\alpha]_D^{30}$: −91° (c=0.47, in dioxane) (diosgenin, $[\alpha]_D^{30}$: 111° (c=0.46, in dioxane)), Rf 0.86 (synth. trillin, 0.86). A mixed fusion with synth. trillin, m.p. 260~262° (decomp.) (Kofler, uncorr.), $[\alpha]_D^{30}$: −89° (c=0.46, in dioxane), gave no m.p. depression. *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, 66.01; H, 9.28.

Prosapogenin (A)—A mixture (740 mg.) obtained on hydrolysis of dioscin with 1*N* H_2SO_4 in 50% EtOH for 1 hr. was dissolved in $CHCl_3$-MeOH (50:1) (50 cc.) and passed through an alumina column (Brockmann Al_2O_3:10 g., 100×12 mm.) previously saturated with $CHCl_3$. Elution was made with a $CHCl_3$-MeOH mixture of increasing MeOH content up to 100% and next with MeOH-water (10:1 and 5:1). Each eluate was evaporated to dryness and examined by paper chormatography. Fractions No. 7~8 were combined and subjected to the second similar chromatography and its fraction No. 4 was further separated by the third one (Table V). A mixture (160 mg.) of prosapogenin (A) and a minute amount of dioscin was allowed to stand for 24 hrs. with 1.6 cc. each of pyridine and of Ac_2O. The reaction mixture was poured into ice water, and the product was collected, washed with water, dried, and crystallized from MeOH. Yield : 130 mg. Further recrystallization gave pure prosapogenin (A) acetate, as colorless fine needles, m.p. 207~209° (Kofler, uncorr.), m.p. 208~210° (in capillary, corr.) (mixed m.p. with trillin acetate gave depression). $[\alpha]_D^{10}$: −35° (c=0.428, in $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{74}O_{18}$ (Diosgenin rhamnosido-glucoside hexaacetate) : C, 62.81; H, 7.65. Found : C, 62.77; H, 7.58.

The acetate (20 mg.) was boiled with 5% KOH in MeOH (2 cc.) for 45 mins. and prosapogenin (A) regenerated was crystallized from MeOH to fine white needles (or plates), m.p. ca. 230~245° (decomp.)*10 (Kofler, uncorr.); $[\alpha]_D^{10}$: −155° (c=0.234, in dioxane); Rf 0.54. *Anal.* Calcd. for $C_{39}H_{62}O_{12} \cdot H_2O$ (Diosgenin rhamnosido-glucoside +H_2O) : C, 63.22; H, 8.71. Found : C, 63.19; H, 8.65.

Prosapogenin (A) (10 mg.) was refluxed with 0.5*N* H_2SO_4 in 50% EtOH (1 cc.) for 1 hr. and the products were examined by paper chromatography. Non-sugar substance : Rf 0.57 (±), 0.89 (+), 0.97 (?)*11 (dioscin 0.30, prosapogenin (A) 0.60, (B) 0.77, (C) 0.89, diosgenin 0.97). Sugar : Rf

*9 m.p. 190~193° (Kofler, uncorr.) and m.p. 197~201° (in capillary, corr.), reported in Part III,[8] are hereby corrected as 203~205° and 205~207°, respectively.

*10 Being pure paper chromatographically and its acetate having a sharp melting point, this showed no sharp and no constant melting point.

*11 Being only trace in these examinations, diosgenin was fairly distinctly detected by the paper chromatography for sapogenin.[20] Solvent : petr. ether : toluene : EtOH : water (40:5:1:9), at 18~19°. Toyo Roshi No. 50. Rf : 0 (++), 0.62 (±) (diosgenin 0.63).

20) T. Tsukamoto, T. Kawasaki : J. Pharm. Soc. Japan, **74**, 72 (1954) (C. A., **48**, 5440 (1954)).

TABLE V. Liquid Chromatography of Prosapogenin Mixture

(1)

Fr. No.	1	2	3	4	5	6	7	8
Solvent	$CHCl_3$-MeOH 50:1	30:1	20:1	15:1 10:1	5:1	2:1	1:1	MeOH MeOH-H_2O
Residue (mg.)	70	10	70	125	60	55	75	260
Paper Chromatogram: Diosgenin	+	+						
Pro-s.g.(C)		±	+	‡	‡	+	±	±
Pro-s.g.(B)				±	±	±	?	
Pro-s.g.(A)						±	+	+
Dioscin						±	±	±

(2)

Fr. No.	1	2	3	4	5	6
Solvent	$CHCl_3$-MeOH 50:1	20:1	10:1	5:1	1:1	MeOH MeOH-H_2O
Residue (mg.)	—	15	15	110	110	65
Paper Chromatogram: Diosgenin						
Pro-s.g.(C)		+	+	±	?	?
Pro-s.g.(B)			±	?		
Pro-s.g.(A)			±	+	+	+
Dioscin			±	±	±	?

(3)

Fr. No.	1	2	3	4	5
Solvent	$CHCl_3$-MeOH 50:1	20:1	10:1	1:1	MeOH MeOH-H_2O
Residue (mg.)	—	trace	trace	50	40
Paper Chromatogram: Diosgenin					
Pro-s.g.(C)		+	±		
Pro-s.g.(B)		±	±		
Pro-s.g.(A)		?	+	+	+
Dioscin			+	±	±

(solvent (a)); 0.22(±), 0.39(+) (glucose 0.23, rhamnose 0.39).

Prosapogenin (B)—A mixture of prosapogenin (B) and (C) obtained during the isolation of prosapogenin (A) (Fr. 4~5, Table V, (1)) was further chromatographed on Al_2O_3 column, but (B) could not be isolated. This mixture (20 mg.) was boiled with 0.5*N* H_2SO_4 in 50% EtOH (2 cc.) for 1 hr. and the products were exmined. Non-sugar substance : Rf 0.90(+), 0.97(?)*[11] (original mixture 0.69(+), 0.89(+), trillin 0.89). Sugar : Rf (solvent (a)) 0.15(±), 0.35(+) (glucose 0.15, rhamnose 0.36).

Summary

Dioscin, a steroidal saponin obtained from some Japanese Dioscoreaceae plants, was proved to be composed of one mole each of diosgenin and of D-glucose and two of L-rhamnose. Of the three prosapogenins, (A), (B), and (C), formed by its partial hydrolysis, (C) and (A) were isolated and proved to be a diosgenin glucoside and a rhamnosido-glucoside, respectively. Two possible formulae (I) and (II) were proposed for dioscin, of which the latter, assuming a rhamnosido-glucoside for prosapogenin (B), seemed rather probable.

(Received November 25, 1955)