Three New Saponins from the Leaves of *Hovenia dulcis* (Rhamnaceae)

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The carbohydrate sequences of three new saponins, saponin C_2 (1a), D (2a), and G (3a), obtained from the leaves of *Hovenia dulcis* Thunb. (Rhamnaceae), have been identified, mainly by ^{13}C n.m.r. spectroscopy, and the full molecular structures of these saponins have been assigned as 3-*O*-(2-*O*-α-L-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-α-L-arabinopyranosyl)jujubogenin, 3-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-20-*O*-α-L-rhamnopyranosyljujubogenin, and 3-*O*-β-D-glucopyranosyl-20-*O*-α-L-rhamnopyranosyljujubogenin, respectively, from additional chemical and spectroscopic evidence.

PREVIOUS papers [1,2] have described the isolation and the chemical studies of saponins obtained from the root bark of *Hovenia dulcis* Thunb.[3] (Rhamnaceae) (the Japanese name is 'kenponashi,' and the seeds are used as a folk medicine). This paper reports the isolation of ten saponins and the structural identification of three new saponins, C_2 (1a), D (2a), and G (3a), from the methanolic extracts of the leaves of *Hovenia dulcis* Thunb., the corresponding sapogenin of which is jujubogenin (5).[4]

The crude glycoside fraction from the methanolic extracts was purified by a combination of droplet counter-current chromatography and silica-gel column chromatography to afford the saponins A_1, A_2, B, C_1, C_2, D, E, F, G, and H.

On acidic hydrolysis, the glycoside C_2 (1a), $C_{47}H_{76}O_{17}$, afforded a secondary sapogenin, ebelin lactone (4), and arabinose, rhamnose, and glucose as the sugar components (molar ratio 1 : 1 : 1). Methylation of compound (1a) by the Hakomori method [5] yielded the nona-*O*-methyl ether (1b) and the octa-*O*-methyl ether (1c). The ^1H n.m.r. spectrum of compound (1b) exhibits three sets of anomeric proton signals at δ 4.39 (1 H, d, *J* 7 Hz), 4.58 (1 H, d, *J* 5 Hz), and 5.20 (1 H, br s), assigned, respectively, to β-D-glucopyranose ($^4C_1$ conformation), α-L-arabinopyranose ($^1C_4$ conformation), and α-L-rhamnopyranose ($^1C_4$ conformation). The ^1H n.m.r. spectrum of compound (1b) exhibits an extra signal for the tertiary OMe group at δ 3.18 (3 H, s). This indicates that all the carbohydrate moieties of compound (1a) are linked to the genin *via* the C-3 hydroxy-group. On methanolysis, the nona-*O*-methyl ether (1b) yielded the α- and β-methylpyranosides of 2,3,4-tri-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylglucose, and 4-mono-*O*-methylarabinose, which were identified with authentic samples by g.l.c. Finally, saponin C_2 (1a) was identified with prosapogenin-III derived from the known saponin, jujuboside A (7),[6] by the action of naringinase. Furthermore, prosapogenin-III was identified with the zizyphus saponin-II [7] isolated from *Zizyphi fructus*. Thus, the structure of saponin C_2 (1a) was determined to be 3-*O*-(2-*O*-α-L-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-α-L-arabinopyranosyl)jujubogenin.

On acidic hydrolysis, the glycoside D (2a), $C_{48}H_{78}O_{17}$, gave ebelin lactone (4) and L-rhamnose and D-glucose as the sugar components (molar ratio 2 : 1). Complete methylation of compound (2a) by the Hakomori method yielded the nona-*O*-methyl derivative (2b). The ^1H n.m.r. spectrum of compound (2b) exhibits the signals of three anomeric protons at δ 4.34 (1 H, d, *J* 7 Hz), 5.32 (1 H, br s), and 5.34 (1 H, br s), assigned, respectively, to α-L-rhamnopyranose ($^1C_4$ conformation) and β-D-glucopyranose ($^4C_1$ conformation). This conformational analysis is supported by application of Klyne's rule [8] (Table 1). The methanolysis product of compound (2b)

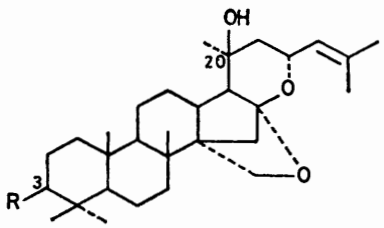
TABLE 1

Values of molecular rotation

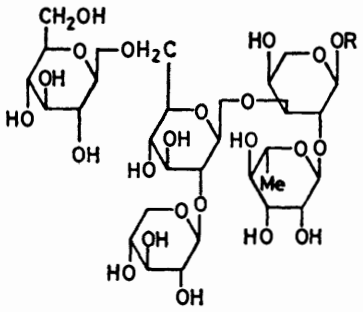
Saponins	$[\alpha]_D$/°	$[M]_D$/° × 10^{-2}	$-\Delta[M]_D$/° × 10^{-2}
(5)	−36.0	−169.9	−88.3
(3a)	−33.1	−258.2	
(2a)	−48.6	−450.0	−191.8
Methyl β-D-glucopyranoside		−66	
Methyl α-D-glucopyranoside		+309	
Methyl α-L-rhamnopyranoside		−111	
Methyl β-L-rhamnopyranoside		+170	

gave two kinds of methylated sugar. These were identified by g.l.c. as the α- and β-methylpyranosides of 3,4,6-tri-*O*-methylglucose and 2,3,4-tri-*O*-methylrhamnose. Furthermore, the ^1H n.m.r. spectrum of compound (2b) exhibited no extra signal assignable to the tertiary OMe group. This indicated that one of the rhamnose moieties is linked to jujubogenin (5) at the C-20 hydroxy-group. In a previous paper,[9] we have discussed the elucidation of the dammarane-type saponins by ^{13}C n.m.r. spectroscopy. This work enabled the assignments of the saponins D (2a) and G (3a) as C-3 and C-20 glycosides, since the C-3 signal, observed as a doublet in the off-resonance decoupled spectra, was at δ_C 88.7 p.p.m. in the spectra of both saponins, and at δ_C 78.0 p.p.m. in those of the corresponding aglycone. On the other hand, a downfield glycosylation shift of *ca.* 10 p.p.m. has been observed at C-20 for dammarane-type saponins.[10] The C-20 signal of saponin D (2a) was observed as a downfield rhamnosylation shift of *ca.* 7 p.p.m. One of the rhamnosyl anomeric carbons was observed at δ_C 96.3 as an upfield shift of *ca.* 6 p.p.m. On the basis of these data, the structure of saponin D (2a) was elucidated as 3-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-20-*O*-α-L-rhamnopyranosyljujubogenin.

On acidic hydrolysis, the glycoside G (3a), $C_{42}H_{68}O_{13}$, afforded ebelin lactone (4) and L-rhamnose and D-glucose

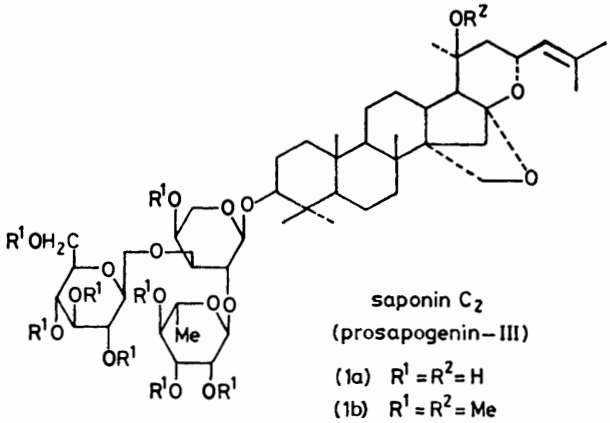


Jujubogenin (5) R = OH



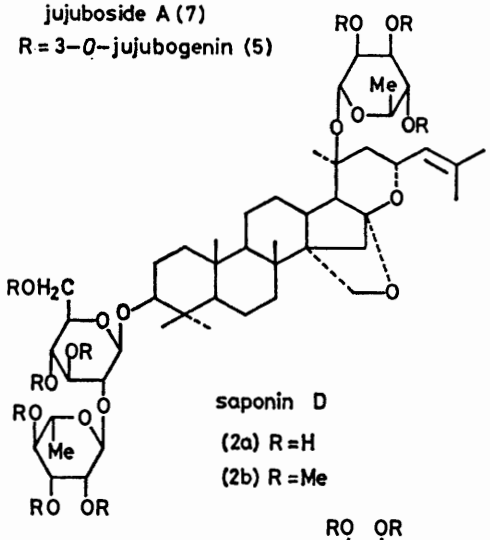
jujuboside A (7)
R = 3-*O*-jujubogenin (5)

naringinase



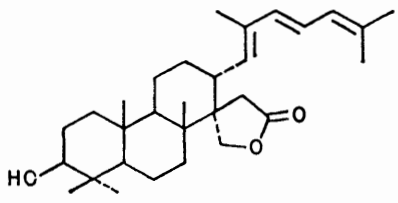
saponin C_2
(prosapogenin-III)
(1a) $R^1 = R^2 = H$
(1b) $R^1 = R^2 = Me$
(1c) $R^1 = Me$, $R^2 = H$

H^+



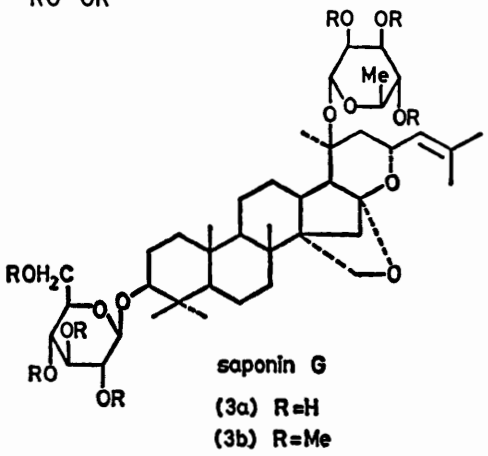
saponin D
(2a) R = H
(2b) R = Me

H^+



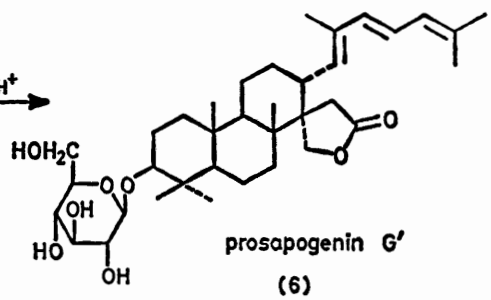
ebelin lactone (4)

H^+



saponin G
(3a) R = H
(3b) R = Me

mild H^+



prosapogenin G'
(6)

as the sugar components (molar ratio 1 : 1). Methylation of compound (3a) by the Hakomori method afforded the hepta-*O*-methyl derivative (3b). The ^1H n.m.r. spectrum of compound (3b) showed the signals of two anomeric protons at δ 4.25 (1 H, d, *J* 7 Hz) and 5.12 (1 H, br s), assigned, respectively, to α-L-rhamnopyranose ($^1C_4$ conformation) and β-D-glucopyranose ($^4C_1$ conformation). This conformational analysis was supported by application of Klyne's rule (Table 1). Methanolysis products of compound (3b) were identified by g.l.c. as the α- and β-methylpyranosides of 2,3,4,6-tetra-*O*-methylglucose and 2,3,4-tri-*O*-methylrhamnose. This indicates that the rhamnose or glucose moiety of compound (3a) is linked to jujubogenin (5) at the C-20 hydroxy-group. The ^{13}C n.m.r. spectrum of compound (3a) was compared with that of saponin D (2a) and jujubogenin (5). The assignment of the C-20 signal was carried out by observing the rhamnosylation shift [a downfield shift of δ_C +7.1 p.p.m. (δ 68.5 ⟶ 75.6)]. The rhamnosyl anomeric carbon signal was observed at δ_C 96.3 with an upfield shift of *ca.* 6 p.p.m. Thus, the rhamnose moiety of saponin G (3a) was supposed to combine at the C-20 hydroxy-group of jujubogenin (5). On the other hand, saponin G (3a) was partially hydrolysed in 5% HCl–MeOH to give prosapogenin G′ (6). Prosapogenin G′ (6) exhibits a strong u.v. absorption at λ 280 nm and the absorption of a five-membered ring lactone at 1 773 cm^{-1} in the i.r. spectrum. These spectral data are very similar to those of ebelin lactone (4). On acidic hydrolysis prosapogenin G′ (6) afforded ebelin lactone (4) and D-glucose as the sugar component. The ^{13}C n.m.r. spectrum of compound (6) indicated the existence of glycosidic bond between β-D-glucose and C-3 of ebelin lactone (4); saponin G (3a) is identified consequently as 3-*O*-β-D-glucopyranosyl-20-*O*-α-L-rhamnopyranosyljujubogenin.

Other saponins (E and H), obtained from the same material, in which the sapogenin moieties are different from jujubogenin are under investigation.

EXPERIMENTAL

M.p.s were measured with a Yanagimoto microapparatus, u.v. spectra were taken for solutions in methanol, optical rotation for solutions in methanol, and n.m.r. spectra (^{13}C data given in Table 2) for solutions in deuteriopyridine or deuteriochloroform. Droplet counter-current chromatography (d.c.c.) was carried out on home-made d.c.c. apparatus equipped with 500 columns (1.65 × 600 mm) by the ascending process [moving phase, upper layer; stationary phase, lower layer of $CHCl_3$–MeOH–H_2O (35 : 65 : 40)].

Isolation of Saponins C_2 (1a), *D* (2a), *G* (3a), *E, and H.*—The dried leaves of *Hovenia dulcis* Thunb. (1 kg) were extracted with methanol. The extracts were diluted with water and then extracted with ether. The aqueous layer was extracted with n-butanol and washed with 1% aqueous KOH. From the butanol layer crude saponin was obtained (yield *ca.* 0.7%). Crude saponins (5 g) were subjected to d.c.c. In this work the *glycoside* C_2 (1a) (110 mg) was obtained as needles, m.p. 281—283 °C, $[\alpha]_D$ −27.7° (*c* 0.5) (Found: C, 59.3; H, 8.5. $C_{47}H_{76}O_{17}\cdot 2H_2O$ requires C, 59.5; H, 8.5%). The *glycoside D* (2a) (200 mg) was obtained as needles, m.p. 270—272 °C, $[\alpha]_D$ −48.6° (*c* 0.28) (Found: C, 58.85; H, 8.65. $C_{48}H_{78}O_{17}\cdot 3H_2O$, requires C, 58.75; H, 8.65%). The *glycoside G* (3a) (200 mg) was obtained as needles, m.p. 257—259 °C, $[\alpha]_D$ −33.1° (*c* 0.35) (Found: C, 61.1; H, 8.85. $C_{42}H_{68}O_{13}\cdot 2.5H_2O$ requires C, 61.05; H, 8.9%). On the other hand, the glycoside-E and H fractions were chromatographed on silica gel to afford the pure glycosides E (500 mg) and H (50 mg). The glycoside E was obtained as needles, m.p. 167—169.5 °C, $[\alpha]_D$ −26.5° (*c* 0.32); $\nu_{max.}$ (KBr) 3 400 and 1 768 cm^{-1}; $\lambda_{max.}$ no absorption above 210 nm (yield: 10% of the crude saponin). The glycoside H was obtained as needles, m.p. 171—172 °C, $[\alpha]_D$ −14.3° (*c* 0.35); $\nu_{max.}$ (KBr) 3 400 and 1 768 cm^{-1}; $\lambda_{max.}$ no absorption above 210 nm (yield: 1% of the crude saponin).

Acidic Hydrolysis of Saponins.—Saponin C_2 (1a) (3 mg) was dissolved in dioxan (1 ml), 2M H_2SO_4 (2 ml), and water (1 ml) and heated under reflux for 5 h. The solution was diluted with water and extracted with ether. From this layer ebelin lactone (4) was obtained, which was identical with an authentic sample in all respects.[2] The aqueous layer of the hydrolysate was neutralized with ion-exchange resin (IR-45) and evaporated. Trimethylsilylation followed by g.l.c. [2% OV-1 on Chromosorb W (60—80 mesh) (3 mm × 2 m); column temperature 160 °C; N_2 flow-rate 70 ml min^{-1}] showed the presence of arabinose, glucose, and rhamnose in the ratio 1 : 1 : 1. In the case of saponins D (2a) and G (3a), glucose and rhamnose were identified in the ratios 1 : 2 and 1 : 1, respectively.

Permethylation of Saponins by the Hakomori Method.[5]—To a stirred solution of saponin C_2 (1a) (80 mg) in dimethyl sulphoxide (10 ml) under argon was added a solution (20 ml) of methylsulphinyl methanide and the mixture was stirred at room temperature for 5 h. Methyl iodide (10 ml) was then added and the reaction was continued for 24 h. The mixture was poured into water and extracted with chloroform. The organic layer was washed with water and evaporated. The residue was chromatographed on silica gel [n-hexane–acetone (6 : 1) as eluant] to give the nona-*O*-methyl derivative (1b) (21 mg) and octa-*O*-methyl derivative (1c) (30 mg). The *nona-O-methyl derivative* (1b) was recrystallized from methanol as needles, m.p. 222—223 °C; δ ($CDCl_3$) 1.23 (3 H, d, *J* 6 Hz), 3.18 (3 H, s, 20*S*-OMe), 4.39 (1 H, d, *J* 7 Hz), 4.58 (1 H, d, *J* 5 Hz), and 5.20 (1 H, br s) (Found: C, 63.9; H, 9.35. $C_{56}H_{94}O_{17}\cdot 0.5\ H_2O$ requires C, 64.15; H, 9.15%). The *octa-O-methyl derivative* (1c) was recrystallized from methanol as needles, m.p. 134—136 °C; δ ($CDCl_3$) 1.23 (3 H, d, *J* 6 Hz), 4.39 (1 H, d, *J* 7 Hz), 4.58 (1 H, d, *J* 5 Hz), and 5.19 (1 H, br s) (Found: C, 62.65; H, 8.9. $C_{55}H_{92}O_{17}\cdot H_2O$ requires C, 62.25; H, 9.1%).

Saponin D (2a) (100 mg) was methylated and the product worked up as for compound (1a) to give the *nona-O-methyl derivative* (2b) (16 mg) as needles, m.p. 115—117 °C; no OH i.r. absorption; δ ($CDCl_3$) 1.23 (3 H, d, *J* 6 Hz), 4.34 (1 H, d, *J* 7 Hz), 5.32 (1 H, br s), and 5.34 (1 H, br s) (Found: C, 64.6; H, 9.1. $C_{57}H_{96}O_{17}$ requires C, 65.0; H, 9.2%).

Saponin G (3a) (100 mg) was methylated and the product worked up as for compound (1a) to give the *hepta-O-methyl derivative* (3b) (18 mg) as a syrup; no OH i.r. absorption; δ ($CDCl_3$) 1.23 (3 H, d, *J* 6 Hz), 4.34 (1 H, d, *J* 7 Hz), and 5.12 (1 H, br s) (Found: C, 66.75; H, 9.5. $C_{49}H_{82}O_{13}$ requires C, 66.95; H, 9.4%).

Methanolysis of the Permethylated Saponins.—A solution of the sample (5—10 mg) in 5% HCl–MeOH was refluxed

TABLE 2

^{13}C Chemical shifts (δ in p.p.m. from Me_4Si; solvent C_5D_5N) [a]

	C [c]	(5)	(1a)	Pro-III [b]		(2a)	(3a)	(6)	(4)
	1	38.9	39.0	38.9		38.9	38.6	38.5	38.7
	2	27.9	26.7	26.8		26.8	26.5	26.6	28.0
	3	78.0	88.1	88.2		88.7	88.7	88.6	77.8
	4	39.5	39.7	39.7		39.6	39.6	39.5	39.4
	5	56.0	56.3	56.3		56.2	56.0	55.3	55.2
	6	18.4	18.3	18.3		18.8	18.8	18.1	18.1
	7	36.1	36.0	36.0		35.6	35.6	34.5	34.5
	8	37.6	37.3	37.3		37.0	37.0	40.3	40.2
	9	53.0	53.0	53.0		52.7	52.7	52.6	52.7
	10	37.6	37.3	37.3		37.0	37.0	36.9	37.3
	11	21.7	21.8	21.8		21.5	21.5	20.1	20.1
	12	28.6	28.6	28.6		27.9	28.1	28.9	28.6
	13	37.0	37.5	37.5		37.2	37.2	39.5	39.5
	14	53.7	53.7	53.7		53.7	53.7	52.0	52.0
	15	37.0	37.1	37.0		37.0	37.0	34.9	35.0
	16	110.6	110.6	110.5		109.9	109.8	176.9	176.7
	17	53.7	53.9	53.9		54.8	54.8	131.3 [d]	131.6 [d]
	18	18.4	18.3	18.3		18.8	18.8	18.2	18.3
	19	16.3	16.4	16.4		16.4	16.3	16.0	16.1
	20	68.6	68.5	68.5		75.7	75.6	135.4 [e]	135.2 [e]
	21	30.0	30.0	30.1		24.3	24.3	13.2	13.3
	22	45.2	45.5	45.5		40.9	40.9	134.8 [d]	134.9 [d]
	23	68.7	68.5	68.5		68.2	68.2	124.5 [d]	124.7 [d]
	24	126.8	127.0	127.1		126.4	126.4	126.3 [d]	126.3 [d]
	25	134.3	134.2	134.0		134.6	134.6	137.1 [e]	137.0
	26	25.8	25.5	25.5		25.6	25.5	26.1	26.1
	27	18.8	18.9	18.9		19.0	19.1	18.2	18.3
	28	28.6	28.0	28.0		27.9	28.1	28.1	28.6
	29	16.3	16.8	16.8		16.8	16.7	16.8	16.2
	30	65.9	65.8	65.8		65.7	65.7	69.7	69.6
Ara	1		104.7	104.7		105.3	106.6	106.9	
	2		74.9	74.9		79.6	75.6	75.7	
	3		82.2	82.2	C-3 Glc	77.7	78.6	78.7	
	4		68.1	68.1		72.2	71.8	71.9	
	5		64.9	64.8		78.0	78.1	78.3	
	6					62.9	63.0	63.2	
Glc	1		104.7	104.7		101.5			
	2		74.9	74.9		72.2			
	3		78.2	78.2	C-3 Rham	72.2			
	4		71.5	71.5		74.0			
	5		78.5	78.5		69.4			
	6		62.6	62.7		18.6			
Rham	1		101.9	101.8		96.3	96.3		
	2		72.4	72.4		72.4	72.6		
	3		72.4	72.4	C-20 Rham	73.5	73.5		
	4		73.9	73.9		73.5	73.5		
	5		70.0	70.0		71.1	71.1		
	6		18.6	18.6		18.6	18.6		

[a] ^{13}C N.m.r. spectra were recorded on a JEOL Model JNM–FX–100 spectrometer using [$^2H_5$]pyridine solutions, containing trimethylsilane as an internal reference, in 5 mm spinning tubes at room temperature. [b] Pro-III = prosapogenin-III. [c] Ara = arabinopyranosyl, Glc = glucopyranosyl, Rham = rhamnopyranosyl. [d,e] Assignments may be reversed in each vertical column.

for 2 h and evaporated to afford a sample for g.l.c. [conditions for g.l.c.: 10% DEGS on Chromosorb W (3 mm × 2 m); column temperature 160 °C].

(a) In the sample from compound (1c), α- and β-methylpyranosides of 2,3,4,6-tetra-*O*-methylglucose, 2,3,4-tri-*O*-methylrhamnose, and 4-mono-*O*-methylarabinose were identified.

(b) In the sample from compounds (2b) and (3b), α- and β-methylpyranosides of 2,3,4-tri-*O*-methylrhamnose and 3,4,6-tri-*O*-methylglucose were detected.

Hydrolysis of Jujuboside A (7) *with Crude Enzymes.*—Jujuboside A (7) (1 g) in distilled water (500 ml) was incubated with commercial naringinase (1.5 g) at 38 °C for 10 d and the hydrolysate was extracted with n-butanol saturated with water. The organic layer was concentrated to dryness and subjected to d.c.c. with solvent system [moving phase, upper layer; stationary phase, lower layer of $CHCl_3$-MeOH–H_2O (35 : 65 : 40)] to give crystalline prosapogenin-III (16 mg). Saponin C_2 (1a) was identified with the above prosapogenin-III by ^{13}C n.m.r. spectroscopy.

Partial Hydrolysis of Saponin G (3a).—Saponin G (3a) (100 mg) was dissolved in 5% HCl–MeOH (5 ml) and stirred at room temperature for 1 h. The mixture was diluted with water and extracted with n-butanol. The organic layer was washed and evaporated to dryness and the residue was chromatographed on silica gel [MeOH–$CHCl_3$ (1 : 10) as eluant]. Further purification (preparative t.l.c.) gave compound (6) (9 mg) 3-*O*-(β-D-glucopyranosyl)ebelin lactone (prosapogenin G′) as needles, m.p. 190.5—192 °C; $\nu_{max.}$ 1 773 cm^{-1}, $\lambda_{max.}$ 280 nm (Found: C, 66.05; H, 9.1. $C_{36}H_{56}O_8 \cdot 2H_2O$ requires C, 66.25; H, 9.25%). Compound (6) was hydrolysed in the usual way. From the organic layer compound (4) was obtained, which was identified with an authentic sample of ebelin lactone in all respects [2] (t.l.c., i.r., and ^{13}C n.m.r.), and D-glucose was detected by g.l.c.

[0/1973 *Received*, 23*rd December*, 1980]

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