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DIGITALIS SAPONINS—IV[1]

STRUCTURE OF F-GITONIN

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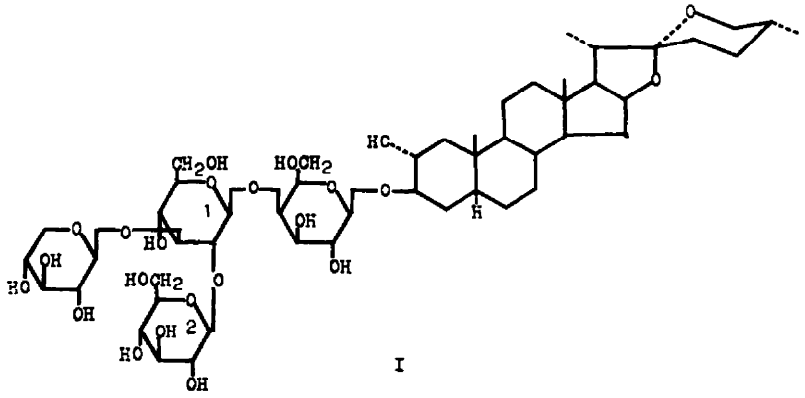
Abstract—F-gitonin, a saponin from the leaves of *Digitalis purpurea* L., is shown to be O-β-D-glucopyranosyl-(1 → 2 glc 1)-O-β-D-xylopyranosyl-(1 → 3 glc 1)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-galactopyranosyl 25D, 5α-spirostane-2α, 3β-diol (gitogenin β-lycotetraoside) (I) and the conjugation of lycotetraose with 3β-hydroxyl group of gitogenin is definitely proved.

F-GITONIN is one of the leaf saponins of *Digitalis purpurea* L. which was isolated[2] in a pure form from the fraction precipitated by cholesterol from the butanol extracts left after the manufacture of cardiac glycosides. It is a gitogenin tetraglycoside[2] of which the sugar composition, two moles of D-glucose (glc) and one mole each of D-galactose (gal) and D-xylose (xyl), is different from that (2 gal + glc + xyl) of gitonin,[3] the gitogenin tetraglycoside, in *Digitalis purpurea* seeds.[4] In the present paper, the structure elucidation of F-gitonin which leads to the assignment of the structure I has been described.



I

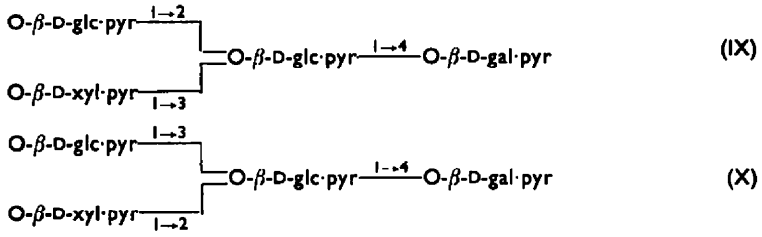
The IR spectrum of F-gitonin indicates the presence of the iso-spiroketal ring system[5] and hence the sugar moiety must be combined with the hydroxyl group(s) at C-2 or/and C-3 of gitogenin.

F-gitonin permethylate prepared by repeated methylation by the Kuhn method[6] gives, on hydrolysis, an aglycone $C_{28}H_{46}O_4$ (II), m.p. 222–225°, $[\alpha]_D$ −118°, and a

[1] Part III: V. Kawasaki, I. Nishioka, T. Yamauchi, K. Miyahara and M. Enbutsu, to be published.
[2] T. Kawasaki and I. Nishioka, to be published.
[2a] R. Tschesche and G. Wulff, *Chem. Ber.* **94**, 2019 (1961);
[2b] R. Tschesche and G. Wulff, *Tetrahedron* **19**, 621 (1963);
[2c] T. Kawasaki and I. Nishioka, to be published.
[4] R. Tschesche and G. Balle [*Tetrahedron* **19**, 2323 (1963)] have reported that lanatigonin I (tigogenin + 2 glc + 2 gal + xyl) from the seeds of *Digitalis lanata* Ehrh. is not identical with tigonin from the leaves of the same plant.
[5] Cf. E. S. Rothman, M. E. Wall and C. R. Eddy, *J. Amer. Chem. Soc.* **74**, 4013 (1952).

mixture of methylated sugars. Compound II on oxidation with chromium trioxide in acetic acid yields a monoketone $C_{28}H_{44}O_4$ (III), m.p. 189°, $[\alpha]_D$ −16°, IR 1735 cm^{-1} and after acetylation with acetic anhydride–pyridine affords a monoacetate $C_{30}H_{48}O_5$ (IV), m.p. 206–207·5°, $[\alpha]_D$ −135°. The above results indicate that (II) is gitogenin monomethylether and that the four monosaccharides of F-gitonin form a tetrasaccharide which is combined with the hydroxyl group at C-2 or C-3 of gitogenin. The sugar portion of the hydrolysate was fractionated by chromatography on cellulose powder and on carbon-celite (1 : 1) mixture to give 2,3,4-tri-O-methyl-α-D-xylopyranose (V), 2,3,4,6-tetra-O-methyl-α-D-glucopyranose (VI), 4,6-di-O-methyl-α-D-glucopyranose (VII) and a syrup, b.p. 120° (bath temp) at 0·001 mm, n_D^{21} 1·4682, which was characterized as 2,3,6-tri-O-methyl-D-galactopyranose (VIII) by oxidation with bromine to the laevo rotatory five-membered lactone $C_9H_{16}O_6$, m.p. 98–100°, $[\alpha]_D$ −27°, IR 1785 cm^{-1}.

Therefore the tetrasaccharide portion of F-gitonin should have a branched-chain structure and is represented by either formula IX or X, in which the four monosaccharides of the D-series are assumed all to be β-linked by application of the Klyne rule.[7] When F-gitonin is hydrolysed under mild conditions and the products examined by



paper chromatography, the results show that the terminal xylose is released with greater ease than glucose at another terminal and only one of the two possible triglycosides is produced together with di- and mono- glycosides. The partial hydrolysate containing three prosapogenins on chromatography on alumina yields a galactoside, m.p. 253–255° (dec), a glucosyl-galactoside, m.p. 272–274° (dec) and a glucosyl-glucosyl-galactoside (XI), m.p. 225–230° (dec). Permethylation of XI followed by hydrolysis and chromatographic separation of the resultant methylated sugars provides VI, VIII and a methylated monosaccharide (XII). Comparison of the R_f value (0·70) of XII with those of VI (0·85), VII (0·48) and VIII (0·64) on a paper chromatogram suggests XII to be the tri-O-methyl-D-glucopyranose and the positive colour reaction with the Wallenfels' reagent[8] and the osazone formation prove it to have the free hydroxyl group at C-2. Thus XII is regarded as 3,4,6-tri-O-methyl-D-glucopyranose, XI as O-D-glucopyranosyl-(1 → 2)-O-D-glucopyranosyl-(1 → 4)-D-galactopyranoside and the sugar moiety of F-gitonin is formulated as O-β-D-glucopyranosyl-(1 → 2_{glcl})-O-β-D-xylopyranosyl-(1 → 3_{glcl})-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranose (IX), which is lycotetraose, the sugar portion of tomatine and demissine.[9,10]

[6] R. Kuhn, I. Löw and H. Trischmann, *Chem. Ber.* **88**, 1492, 1690 (1955).

[7] W. Klyne, *Biochem. J.* **47**, X1i (1950).

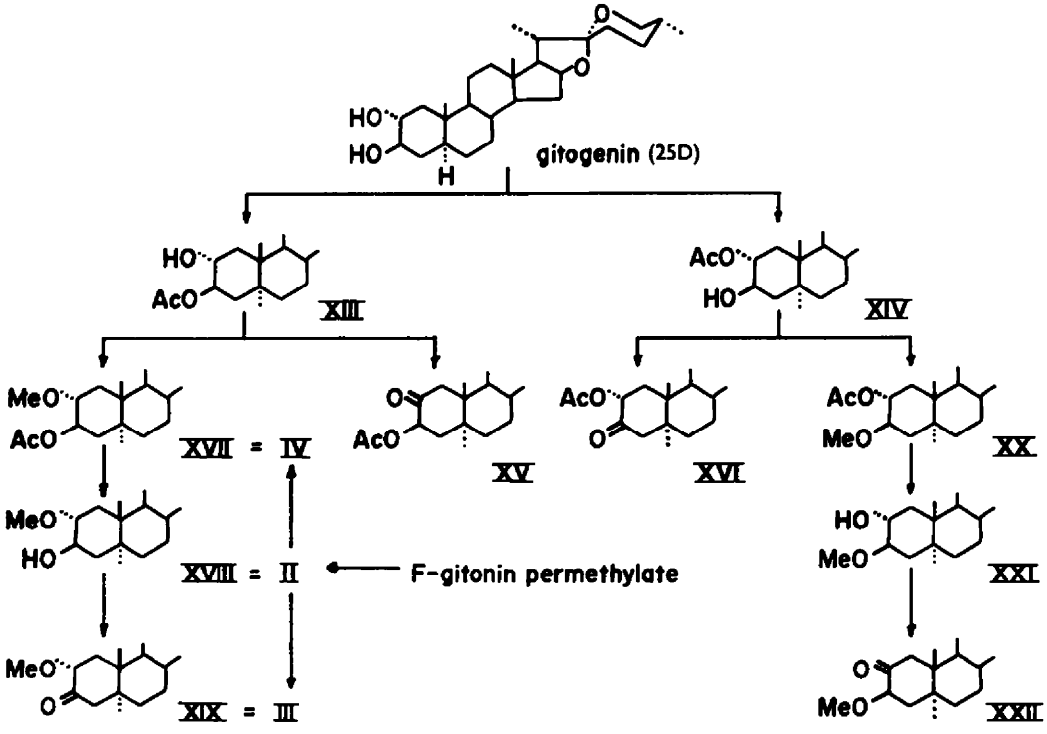
[8] K. Wallenfels, *Naturwiss.* **37**, 491 (1950).

[9] R. Kuhn, I. Löw and H. Trischmann, *Chem. Ber.* **90**, 203 (1957).

[10] Dioscin, a diosgenin glycoside from *Dioscorea Tokoro* Makino [T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull. Tokyo*, **10**, 703 (1962)], has the same branched-chain trisaccharide (chacotriose) moiety as that of α-chaconine[6] and β-solamarine [P. M. Boll, *Acta Chem. Scand.* **17**, 1852 (1963)].

Based on a comparison with other steroidal glycosides, the point of attachment of lycotetraose in F-gitonin, appears to be the 3β-hydroxyl rather than the 2α-hydroxyl group of gitogenin. As definite proof for the site of a sugar linkage to a spirostane with more than two hydroxyl groups is still lacking,[11] the synthesis of two isomeric gitogenin monomethylethers and the comparison with the aglycone (II) of F-gitonin permethylate was undertaken.

When gitogenin is heated with acetic acid two isomeric monoacetates ($C_{29}H_{46}O_5$) are formed and the individual isomers, m.p. 189–193°, $[\alpha]_D$ −89°, (XIII) and m.p. 227–231°, $[\alpha]_D$ −90° (XIV), may be separated by chromatography on silica gel or by fractional recrystallization. Upon oxidation with chromium trioxide, XIII gives an acetoxy ketone $C_{29}H_{44}O_5$, m.p. 237–239°, $[\alpha]_D$ −12° (XV), while XIV affords an isomer, m.p. 225–232°, $[\alpha]_D$ −43° (XVI). It is known[12] that the rotatory dispersion spectra of 3β-acetoxy-2-one and 2α-acetoxy-3-one of 5α-cholestane series show similar positive Cotton curves (peak 305 mμ, through 270–265 mμ, in methanol) but that the amplitude of the former curve is much larger than that of the latter. The isomeric acetoxy ketones, (XV) and (XVI), of the 5α-spirostane series exhibit the O.R.D. curves shown in Fig. 1 and by analogy with the 5α-cholestane series they are regarded as 3β-acetoxy-2-one and 2α-acetoxy-3-one, respectively, and hence XIII is considered to be gitogenin 3-acetate and XIV to be gitogenin 2-acetate. On methylation with methyl iodide and



[11] Digitonin[3b] and lanadigalonin I (Ref. cited[4]) are the only two glycosides of such spirostane of which structures are established and in either case the attachment of sugar component to the 3β-hydroxyl group of aglycone is assumed on the basis of an analogy.

[12] K. L. Williamson and W. S. Johnson, *J. Amer. Chem. Soc.* **83**, 4623 (1961); H. B. Henbest, D. N. Jones and G. P. Slater, *J. Chem. Soc.* 4472 (1961).

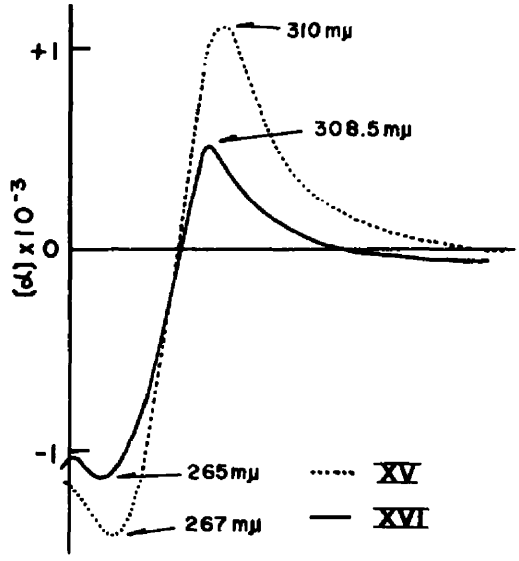


FIG. 1. Rotatory dispersion spectra of XV and XVI in MeOH

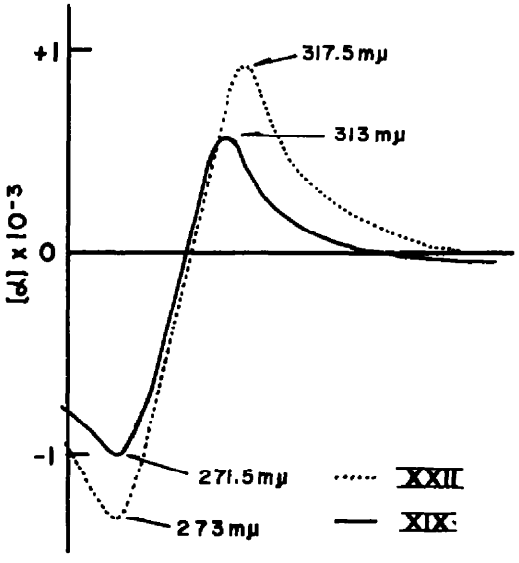


FIG. 2. Rotatory dispersion spectra of XIX and XXII in MeOH

silver oxide in dry benzene followed by hydrolysis and subsequent oxidation, XIII is converted to 2-methylether 3-acetate $C_{30}H_{48}O_5$ (XVII), m.p. 205–206°, $[\alpha]_D$ −127°, 2-methylether $C_{28}H_{46}O_4$ (XVIII), m.p. 221–224°, $[\alpha]_D$ −111°, and 2-methylether 3-one $C_{28}H_{44}O_4$ (XIX), m.p. 177–188°, $[\alpha]_D$ −18°, O.R.D. (Fig. 2), whereas XIV provides 3-methylether 2-acetate (XX), m.p. 194–198°, $[\alpha]_D$ −132°, 3-methylether (XXI), m.p. 234–235°, $[\alpha]_D$ −111° and 3-methylether 2-one (XXII), m.p. 170–185°, $[\alpha]_D$ −9°, O.R.D. (Fig. 2). The aglycone (II) of F-gitonin permethylate and its derivatives, (III

and IV), are identical with XVIII, XIX and XVII respectively by direct comparisons (mixed m.p., IR and co-chromatography).

The conjugation of the lycotetraose residue with the 3β-hydroxyl group of gitogenin to form F-gitonin has since been verified.

In conclusion the complete structure of F-gitonin is defined as O-β-D-glucopyranosyl-(1 $\rightarrow$ 2_{glc1})-O-β-D-xylopyranosyl-(1 $\rightarrow$ 3_{glc1})-O-β-D-glucopyranosyl-(1 $\rightarrow$ 4)-O-β-D-galactopyranosyl-(1 $\rightarrow$ 3_{gltog})-25^{13}_{D},5α-spirostane-2α,3β-diol (gitogenin 3-β-lycotetraoside) (I).

EXPERIMENTAL

All m.ps were taken on a Kofler block and are uncorrected; rotations were determined in $CHCl_3$ unless otherwise stated; IR spectra were obtained in KBr disks with an IR recording spectrophotometer, Koken Model DS-201; O.R.D. curves were measured in methanol solution using Rudolph Recording Spectropolarimeter. R_f values of sapogenin and its derivatives were determined by thin layer chromatography on silica gel G (Merck) using cyclohexane–ethyl acetate (2:1) mixture as a solvent and 10% H_2SO_4 (spraying followed by heating) as a staining agent. R_f values of methylated sugars were taken on paper chromatograms (Tōyō Roshi No. 50, solvent: butanol–ethanol–water–NH_4OH (40:10:49:1), spray reagent: aniline hydrogenphthalate).

F-gitonin

This was obtained by enzymatic hydrolysis of the saponin fraction precipitated by cholesterol of *Digitalis purpurea* leaves, purified by chromatography on silica gel or by repeated recrystallization and identified with authentic sample;[1,2] thin layer chromatographically[14] pure, m.p. 251–255° (dec), $[\alpha]_D^{28}$ − 66° (*c*, 0·50 in pyridine); ν_{max} 862, 898, 924 and 982 cm^{-1} (intensity 898 > 924).

Permethylation of F-*gitonin*

According to the Khun method,[6] F-gitonin (2 g) in dimethylformamide (30 ml) was methylated with Ag_2O (7 g) and methyliodide (5 ml) at 25–30° for 48 hr. The product was methylated again in the same way and the procedure was repeated 4 times. A syrup (1·34 g) thus obtained was chromatographed on alumina (20 g; solvent; benzene and benzene–methylene chloride (19:1) to give F-gitonin permethylate (1·17 g), a viscous syrup revealing a single spot on thin layer chromatogram[14] and almost no hydroxyl absorption in the IR spectrum.

Hydrolysis of F-*gitonin permethylate*

The above permethylate (1·16 g) was refluxed with 7% HCl in methanol (70 ml) for 6·5 hr, the solvent was evaporated *in vacuo* and the residue heated with 1N HCl (60 ml) on a water bath for 4 hr. The precipitate (aglycone, 390 mg) was filtered off and the solution deionized and evaporated *in vacuo* in an usual manner to afford a syrup (sugar fraction, 600 mg).

Aglycone (II)

The crude aglycone obtained in 2 runs were combined (730 mg) and chromatographed on alumina (15 g). The fraction (450 mg) of benzene–methylene chloride (10:1) was further purified by chromatography on Florisil (45 g) in benzene–methylene chloride (1:1 and 1:2) mixture to give a solid (450 mg) which was recrystallized from acetone to provide pure II, prisms, m.p. 222–225°, $[\alpha]_D^{18}$ −118°(*c*, 0·58). R_f 0·44; ν_{max} 864, 901, 926 and 981 cm^{-1} (intensity 901 > 926). (Found: C, 75·44; H, 10·51. $C_{28}H_{46}O_4$ requires: C, 75·29; H, 10·38%).

[13] R. Tschesche, G. Wulff and G. Balle [*Tetrahedron* **18**, 959 (1962)] have reported that in *Digitalis purpurea* seeds isosapogenins (25D series) are accompanied by considerable amounts of the corresponding neo-sapogenins (25L). The sapogenin fraction obtained by refluxing F-gitonin with 2N HCl in 50% ethanol for 2 hr was examined in the manner described by Tschesche *et al.* (Ref. cited above) and the content of neo-gitogenin in the fraction was presumed to be about 3%. Since digitogenin (25D) diacetate is also reported (Ref. cited above) to be partly (about 5%) epimerized to neo-digitogenin (25L) under a similar condition as above, it is highly probable that the small amount of neo-gitogenin had been produced from gitogenin while refluxing with acid, and F-gitonin is not considered to be contaminated with neo-gitogenin lycotetraoside.

[14] T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull., Tokyo* **11**, 1546 (1963).

Chromium trioxide oxidation of aglycone (II) *to monoketone* (III)

Compound II (72 mg) in acetic acid (5 ml) was stirred with CrO_3 (40 mg) in 80% acetic acid (0·2 ml) at room temp for 1·5 hr. The solution was treated with $NaHSO_3$aq, diluted with water (50 ml), extracted with ether and the extract washed successively with water, $NaHCO_3$aq and water, dried and evaporated. Recrystallization of the residue (67 mg) from acetone furnished III as needles, m.p. 177–189°, $[\alpha]_D^{13}$ −16° (*c*, 0·53), R_f 0·64; ν_{max} 1735 cm^{-1}. (Found: C, 75·60; H, 9·91. $C_{28}H_{44}O_4$ requires: C, 75·63; H, 9·97%).

Acetylation of aglycone (II) *to monoacetate* (IV)

Compound II (20 mg) was left to stand with pyridine (1·6 ml) and acetic anhydride (0·4 ml) at room temp for 24 hr. The product was recrystallized from methanol to provide IV as prisms, m.p. 206–207·5°, $[\alpha]_D^{20}$ −135° (*c*, 0·20), R_f 0·79; ν_{max} 1750 and 1242 cm^{-1}. (Found: C, 73·49; H, 10·13. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%).

Fractionation of methylated sugars

The sugar fraction (600 mg) of the hydrolysate of permethylated F-gitonin was placed on a cellulose powder column (30 × 200 mm) and eluted with hexane–butanol (3:2) mixture saturated with water: Fraction 1, 250 mg (R_f 0·83 ~ 0·80); Fraction 2, 10 mg (0·83, 0·63); Fraction 3, 110 mg (0·63); Fraction 4, trace (0·63, 0·46); Fraction 5, 120 mg (0·47). The first fractions obtained in 2 runs were combined (410 mg) and chromatographed on carbon–celite (1:1) mixture (10 g) using 5% methylethyl ketone in water: Fr 1′, 100 mg (R_f 0·78); Fr 2′, 20 mg (0·78, 0·82); Fr 3′, 100 mg (0·85).

2,3,4-*Tri*-O-*methyl*-α-D-*xylopyranose* (V). Fr 1′ was crystallized from hexane to afford V as prisms, m.p. 88–90°, $[\alpha]_D^{11}$ +54 → +19° (*c*, 1·86 in water). (Found: C, 49·84; H, 8·52. Calc. for $C_8H_{16}O_5$: C, 49·99; H, 8·39%). Mixed m.p. with synthetic sample (m.p. 85–87°, R_f 0·78) showed no depression and the co-chromatography gave a single spot.

2,3,4,6-*Tetra*-O-*methyl*-α-D-*glucopyranose* (VI). Fr 3′ was purified by crystallization from hexane followed by distillation to give a solid, b.p. 100° (bath temp) at 0·02 mm, which was recrystallized from hexane to afford VI as needles, m.p. 88–94°, $[\alpha]_D^{13}$ +90 → +85° (*c*, 1·96 in water). (Found: C, 50·48; H, 8·45. Calc. for $C_{10}H_{20}O_6$: C, 50·83; H, 8·53%). Identified with a synthetic sample (m.p. 90–93°, R_f 0·85) by mixed m.p. and co-chromatography.

4,6-*Di*-O-*methyl*-α-D-*glucopyranose* (VII). Fr 5 was crystallized from ethyl acetate to give VII as needles, m.p. 158–162°, $[\alpha]_D^{25}$ +132 → +78° (*c*, 2·12 in water). (Found: C, 46·22; H, 7·79. Calc. for $C_8H_{16}O_6$: C, 46·15; H, 7·75%). Identified with an authentic sample[15] (m.p. 155–162°, R_f 0·48) by mixed m.p. and co-chromatography.

2,3,6-*Tri*-O-*methyl*-D-*galactopyranose* (VIII). Fr 3 was purified by distillation to give VIII as a syrup, b.p. 120° (bath temp) at 0·001 mm, n_D^{31} 1·4682, $[\alpha]_D^{9}$ + 79° (*c*, 1·3 in water). Lit[9]: b.p. 115 ~ 125° (bath temp) at 0·001 mm, n_D^{22} 1·4698. The syrup (130 mg) in water (5 ml) was oxidized with Br_2 (0·1 ml) at room temp for 6 days, excess Br_2 was removed by air bubbling, the solution was treated with Ag_2CO_3 and filtered. The filtrate was passed through a column (10 × 50 mm) of ion-exchange resin (Amberlite IR-120), the water eluate was evaporated *in vacuo* and extracted with ether. Crystallization of the ether-soluble substance from ether–hexane mixture provided needles, m.p. 98–100°, $[\alpha]_D^{18}$ −27° (*c*, 0·74); ν_{max} 1785 cm^{-1}. Lit[9]: 2,3,6-tri-O-methyl-D-galactofuranolactone, m.p. 98–99°, $[\alpha]_D^{28}$ −28° (Found: C, 48·81; H, 7·38. Calc. for $C_9H_{16}O_6$: C, 49·08; H, 7·32%).

Partial hydrolysis of F-*gitonin*

F-gitonin (20 mg) was refluxed with an hydrolytic agent (4 ml) for 1 hr. The hydrolysate was worked up and examined by paper chromatography in the same manner as described before.[16] The results are listed in Table 1. Taking into account the structure, IX or X, of the sugar moiety of F-gitonin and comparing the R_f values of prosapogenins with those of reference compounds, prosapogenin A, B and C were regarded as tri-, di- and mono-glycoside, respectively.

[15] Provided by Prof. R. Kuhn.

[16] T. Tsukamoto and T. Kawasaki, *Chem. Pharm. Bull., Tokyo* **4**, 35, 104 (1956).

F-gitonin (2 g) was refluxed with 1N HCl in 50% ethanol (200 ml) for 1 hr, EtOH removed *in vacuo*, water added and the precipitates collected by filtration. The water insoluble product (900 mg) was placed on an alumina column (Woelm, grade IV, 30 g) and eluted successively with chloroform–methanol (30:1 → 1:1) mixture and butanol saturated with water: Fraction 1, 200 mg (R_f 0·98); Fr 2, 30 mg (0·84); Fr 3, 20 mg (0·83, 0·31); Fr 4, 50 mg (0·30); Fr 5, 170 mg (0·28, 0·06); Fr 6, 300 mg (0·07); Fr 7, 100 mg (0·06, 0).

Monoglycoside (*gitogenin* D-*galactoside*). Fr 2 was recrystallized from methanol to give prisms, m.p. 253–255° (dec). Hydrolysis with 2N HCl in 50% ethanol for 2 hr gave gitogenin and D-galactose (examined by thin layer and paper chromatography, respectively).

TABLE 1. PARTIAL HYDROLYSIS OF F-GITONIN

Hydrolytic Agent	Product							
	F-gitonin R_f 0*	Prosapogenin A R_f 0·07	Prosapogenin B R_f 0·30	Prosapogenin C R_f 0·84	Gitogenin R_f 0·98	xyl	glc	gal
0·2N HCl in 50% EtOH	±	±	··	−	−	+	−	−
0·5N HCl in 50% EtOH	±	+	±	±	+	±	+	+
1N HCl in 50% EtOH	··	+	±	+	+	+	+	+

* R_f values were determined by paper chromatography on Tōyō Roshi No. 50 using benzene–butanol–water (10:4:5) (ascending method, at 10–20°, spray reagent: $SbCl_3$ in chloroform and anisaldehyde in ethanol). Reference compounds run in parallel and their R_f values: trillin (diosgenin + glc)[16] 0·88, Prosapogenin A of gracillin (diosgenin + 2 glc)[16] 0·51, gracillin (diosgenin + rhamnose + 2 glc)[16] 0·15.

Diglycoside (*gitogenin* D-*glucosyl*-D-*galactoside*). Fr 4 was recrystallized from methanol affording a white powder, m.p. 272–274° (dec). Acid hydrolysis as above yielded gitogenin, D-glucose and D-galactose.

Triglycoside (*gitogenin* D-*glucosyl*-D-*glucosyl*-D-*galactoside*) (XI). Recrystallization of Fr 6 from butanol saturated with water provided a crystalline solid, m.p. 225–230° (dec). Acid hydrolysis gave gitogenin, glucose and galactose.

Permethylation of triglycoside (XI) *and hydrolysis of the product*

As described above for F-gitonin and its permethylate, (XI; 500 mg) was methylated repeatedly and the product (250 mg; a syrup, showing a negligible hydroxyl absorption in the IR spectrum) was hydrolysed. The methylated sugar fraction (120 mg) of the hydrolysate was separated into 4 fractions by chromatography on a cellulose powder column (18 × 200 mm) using hexane–butanol (3:2) mixture saturated with water: Fr 1, 30 mg (R_f 0·85); Fr 2, 20 mg (0·70); Fr 3, 20 mg (0·70, 0·64); Fr 4, 20 mg (0·63). Fr 1 and 4 were respectively identified with VI (R_f 0·85) and VIII (R_f 0·63) by co-chromatography.

3,4,6-*Tri*-O-*methyl*-D-*glucopyranose* (XII). Fr 2, a syrup, could not be crystallized, but was considered to be XII on the basis of its R_f value (0·70) in comparison with those (0·85, 0·48, 0·63) of VI, VII and VIII chromatographed in parallel and pink colouration with triphenyltetrazolium chloride reagent[8]. The syrup (about 20 mg) in water (1 ml) was heated with phenylhydrazine hydrochloride (100 mg) and AcONa (70 mg) for 2·5 hr. The product (a brownish yellow resin) was washed

with ice water and crystallized from 50% ethanol and from pet. ether (b.p. 35 ~ 36°) to give the phenylosazone of XII, yellow needles, m.p. 75–79°. Lit:[17] m.p. 75–80° (monohydrate).

Partial acetylation of gitogenin

Gitogenin (200 mg) in 95% acetic acid (10 ml) was heated at 100° for 12 hr. The reaction mixture was diluted with water (100 ml) and the precipitates (195 mg) were collected by filtration. Chromatography of the crude product (100 mg) on silica gel (50 g) using hexane–ethyl acetate (3:1) mixture provided 5 fractions: Fr 1, 14 mg (R_f 0·76); Fr 2, 28 mg (0·43); Fr 3, 9 mg (0·43, 0·39); Fr 4, 30 mg (0·39); Fr 5, 12 mg (0·12) (gitogenin diacetate R_f 0·76, gitogenin 0·12).

Gitogenin 3-acetate (XIII). Fr 2 was recrystallized from hexane to give XIII, needles, m.p. 189–193°, $[\alpha]_D^{16}$ −89° (*c*, 0·52); ν_{max} 3559, 1721, 1242 and 1031 cm^{-1}. (Found: C, 73·34; H, 9·93. $C_{29}H_{46}O_5$ requires: C, 73·38; H, 9·77%).

Gitogenin 2-acetate (XIV). Recrystallization of Fr 4 from hexane gave XIV as prisms, m.p. 227–231°, $[\alpha]_D^{17}$ −90° (*c*, 0·57); ν_{max} 3584, 1730, 1247 and 1027 cm^{-1}. (Found: C, 73·29; H, 9·82. $C_{29}H_{46}O_5$ requires: C, 73·38; H, 9·77%).

In another experiment, gitogenin (2·7 g) in acetic acid (b.p. 118°; 200 ml) was heated at 90° for 18 hr, the reaction mixture was concentrated *in vacuo* to about ½ volume and diluted with water (200 ml). The water-insoluble product (2·77 g) was collected by filtration and chromatographed on alumina (100 g): Fr 1 (benzene), 377 mg (R_f 0·76); Fr 2[benzene–chloroform (1:1)], 1517 mg (0·43, 0·39); Fr 3 (chloroform), 762 mg (0·12). Fractional recrystallization of Fr 2 gave XIII (463 mg) and XIV (529 mg).

Oxidation of gitogenin monoacetates (XIII *and* XIV)

25 D, 5α-*Spirostan*-3β-*ol*-2-*one* 3-*acetate* (XV). To the solution of XIII (51 mg) in acetic acid–acetone (1:9) mixture (10 ml) CrO_3 (50 mg) was added portionwise and stirred at 20° for 6 hr. Excess CrO_3 was decomposed with $NaHSO_3$aq, the reaction mixture diluted with water (300 ml) and a water-insoluble solid (46 mg) collected by filtration. The crude product was purified by recrystallization from hexane to provide XV (31 mg) as needles, m.p. 237–239°, $[\alpha]_D^{16}$ −12° (*c*, 0·44), R_f 0·71; ν_{max} 1757, 1730 and 1242 cm^{-1}; O.R.D. (Fig. 1), [α] peak (310 mμ) + 1050°, [α] trough (267 mμ) − 1450° (*c*, 0·066). (Found: C, 73·58; H, 9·40. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%).

25 D, 5α-*Spirostan*-2α-*ol*-3-*one* 2-*acetate* (XVI). Compound XIV (60 mg) in acetone (10 ml) was oxidized with CrO_3 (30 mg) at 20° for 4 hr. Being treated with $NaHSO_3$aq and diluted with water (50 ml), the reaction mixture was extracted with ether. The ether solution was washed with water, dried and evaporated to a solid (60 mg). Recrystallization of the residue from methanol gave XVI (47 mg) as needles, m.p. 225–232°, $[\alpha]_D^{16}$ −43° (*c*, 0·50), R_f 0·73; ν_{max} 1751, 1733, 1244 and 1233 cm^{-1}; O.R.D. (Fig. 1), [α] peak (308·5 mμ) +420°, [α] trough (265 mμ) − 1130° (*c*, 0·066). (Found: C, 73·67; H, 9·44. $C_{29}H_{44}O_5$ requires: C, 73·69; H, 9·38%).

Methylation of gitogenin monoacetates (XIII *and* XIV)

Gitogenin 2-methylether 3-acetate (XVII). Compound XIII (122 mg) in anhydrous benzene (7 ml) was stirred with methyl iodide (1 g) and Ag_2O (1 g) at room temp for 62 hr. Precipitates were filtered off, the benzene solution evaporated to dryness and the residue (115 mg) recrystallized from acetone to afford XVII (104 mg) as needles, m.p. 204–206°, $[\alpha]_D^{11}$ −127° (*c*, 0·55), R_f 0·79; ν_{max} 1750 and 1242 cm^{-1}. (Found: C, 73·55; H, 9·96. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%). Mixed m.p. with IV showed no depression, the IR spectra were superimposable and co-chromatography gave single spot.

Gitogenin 3-methylether 2-acetate (XX). Compound XIV (180 mg) was methylated as above affording a crude methylether (179 mg), which was recrystallized from methanol and from acetone to give XX (115 mg) as plates, m.p. 194–198°, $[\alpha]_D^{15}$ −132° (*c*, 0·43), R_f 0·81; ν_{max} 1739 and 1245 cm^{-1}. (Found: C, 73·82; H, 10·05. $C_{30}H_{48}O_5$ requires: C, 73·73; H, 9·90%).

Saponification of gitogenin monomethylether monacetates (XVII *and* XX)

Gitogenin 2-methylether (XVIII). Compound XVII (64 mg) in 5% KOH in MeOH (15 ml) was refluxed for 1 hr. A crude product (58 mg) was recrystallized from methanol and from ethyl acetate

[17] R. M. Horowitz and B. Gentili, *Tetrahedron* **19**, 773 (1963); W. N. Haworth and A. Learner, *J. Chem. Soc.* 619 (1928).

to give XVIII (39 mg) as prisms, m.p. 221–224°, $[\alpha]_D^{15}$ −111° (*c*, 0·65), R_f 0·44. (Found: C, 75·28; H, 10·34. $C_{28}H_{46}O_4$ requires: C, 75·29; H, 10·38%). It was found to be identical with II by direct comparisons (mixed m.p., IR, co-chromatography).

Gitogenin 3-methylether (XXI). Saponification of XX (81 mg) as above followed by recrystallization of the product (72 mg) from acetone furnished XXI (65 mg) as prisms, m.p. 234–235°, $[\alpha]_D^{15}$ −111° (*c*, 0·60), R_f 0·48. (Found: C, 75·21; H, 10·37. $C_{28}H_{46}O_4$ requires: C, 75·29; H, 10·38%).

Oxidation of gitogenin monomethyl ethers (XVIII *and* XXI)

25D, *5α-Spirostan-2α-ol-3-one 2-methylether* (XIX). Compound XVIII (30 mg) was oxidized as described for III. Recrystallization of the product (26 mg) from acetone gave XIX (15 mg) as needles, m.p. 177–188°, $[\alpha]_D^{15}$ −18° (*c*, 0·42), R_f 0·64; ν_{max} 1735 cm^{-1}; O.R.D. (Fig. 2), [α] peak (313 mμ) +483°, [α] trough (271·5 mμ) −963° (*c*, 0·298). (Found: C, 75·78; H, 9·97. $C_{28}H_{44}O_4$ requires: C, 75·63; H, 9·97%). It was identified as III by direct comparisons (mixed m.p., IR, co-chromatography).

25D, *5α-Spirostan-3β-ol-2-one 3-methylether* (XXII). Compound XXI (54 mg) was oxidized as above and the product (49 mg) was recrystallized from methanol to give XXII (39 mg) as prisms, m.p. 176–185°, $[\alpha]_D^{15}$ −9° (*c*, 0·64), R_f 0·61; ν_{max} 1733 cm^{-1}; O.R.D. (Fig 2), [α] peak (317·5 mμ) +805°, [α] trough (273 mμ) −1233° (*c*, 0·326). (Found: C, 75·70; H, 9·88. $C_{28}H_{44}O_4$ requires: C, 75·63; H, 9·97%).

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