Steroidal glycosides of *Tribulus terrestris* Linn.

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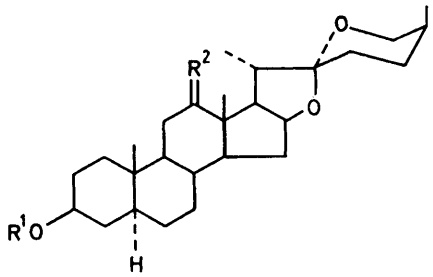
Besides β-sitosterol-β-D-glucoside and dioscin, two new steroidal glycosides, neohecogenin glucoside and tribulosin, isolated from the aerial part of *Tribulus terrestris* Linn. were respectively shown to be neohecogenin-3-*O*-β-D-glucopyranoside (2) and neotigogenin-3-*O*-β-D-xylopyranosyl(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-galactopyranoside (7).

THE plant *Tribulus terrestris* Linn. (Zygophyllaceae) is used in folk medicine in India. The reported medicinal properties of the plant are manifold.[1-5] Considerable phytochemical works on the plant have been done and the isolation of two carboline alkaloids,[6-7] several steroidal sapogenins,[8-10] the steroidal saponins [11-13] dioscin, gracillin, kikuba saponin, and protodioscin, and three flavonoid glycosides [14] have been reported. However, no detailed chemical investigation appears to have been done on *T. terrestris* growing in India, particularly on saponins. The present communication reports the isolation and characterization of two new steroidal glycosides.

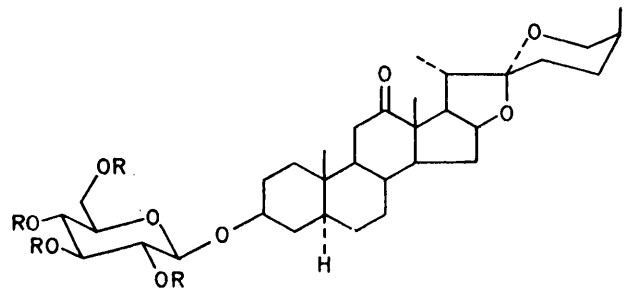
RESULTS AND DISCUSSION

The ethanolic extract of the overground part of the plant, on repeated chromatographic purification, gave four steroidal glycosides A—D. Glycoside A (1), which liberated β-sitosterol and D-glucose on hydrolysis, was found to be identical with β-sitosterol-β-D-glucoside in all respects. Glycoside B (2) gave positive Liebermann–Burchard and Molisch tests, but was negative to the Ehrlich reagent. Its i.r. spectrum was indicative of a spirostanol glycoside. The greater intensity of the band at 920 cm^{-1} than that at 900 cm^{-1} revealed that the genin belongs to the neo-series.[15] On acid hydrolysis (2) yielded a genin (3), and glucose as the only sugar constituent. The identity of (3) as neohecogenin was established by the following observations. The mass spectrum was very similar to that of hecogenin. However, the i.r. spectrum showed bands characteristic of a neo-compound. The genin (3) was then converted into its acetate (4) in the usual way. The ^1H n.m.r. spectrum of (4) showed 26-H_2 signals as a doublet at δ 3.30 and a doublet of doublets centred at δ 3.98, characteristic of a 25*S* configuration.[16,17] Finally, (4) was found to be identical with neohecogenin acetate, prepared from hecogenin by Wall's method.[18] This is the first example of the isolation of neohecogenin from a natural source other than *Agave sisalana*.[19-21] The permethylated glycoside B (5), prepared by Hakomori's method,[22] furnished on acid hydrolysis neohecogenin and 2,3,4,6-tetra-*O*-methyl-D-glucose. The ^1H n.m.r. spectrum of (5) exhibited a signal at δ 4.35 (1 H, d, *J* 6 Hz) assignable to the C-1-H of glucose, suggesting the β configuration (in C 1 form) at the anomeric centre of the glucose. The anomeric configuration was further confirmed by the application of Klyne's rule of molecular rotation.[23] Thus glycoside B is neohecogenin-3-*O*-β-D-glucopyranoside (2).

Glycoside C (6) did not respond to the Ehrlich test and on hydrolysis it yielded diosgenin as the aglycone and D-glucose and L-rhamnose as the carbohydrate portions.



(3) R^1 = H, R^2 = O
(4) R^1 = Ac, R^2 = O
(8) R^1 = H, R^2 = H_2
(9) R^1 = Ac, R^2 = H_2



(2) R = H
(5) R = Me

It was finally identified as dioscin by direct comparison with an authentic sample.[24]

Glycoside D, although homogeneous by t.l.c., turned out to be a mixture of two compounds by h.p.l.c., which could not be separated by repeated column chromatography. A pure glycoside designated as tribulosin (7)

was, however, obtained by repeated crystallization from 90% ethanol. The other component was a very minor one and could not be isolated in a pure state.

The usual colour tests indicated (7) to be a spirostanol glycoside. Its i.r. spectrum revealed that the genin has the 25*S* configuration. Acid hydrolysis of (7) resulted in the formation of an aglycone (8), and monosaccharides identified as D-glucose, D-galactose, D-xylose and L-rhamnose. Purified (8) showed in its ^{1}H n.m.r. spectrum signals due to 26-H_2 at δ 3.27 and 3.94, the pattern and position being characteristic of 25*S* spirostanes. The ^{13}C n.m.r. spectrum of (8) was identical with that of neotigogenin, and not with that of sarsasapogenin.[25] Moreover, the aglycone acetate (9) was identical to neotigogenin acetate prepared from diosgenin by catalytic hydrogenation followed by epimerisation at C-25.[18]

The molecular weight of tribulosin (7) was successfully determined by field-desorption mass spectrometry [26-29] (F.D.-m.s.) which is a powerful tool not only in molecular weight determination, but also in the investigation of structures of underivatised oligoglycosides. The F.D. spectrum of (7) exhibited ion peaks at *m/e* 1 189 and 1 173 formed by cationization of the molecule with $[K]^+$ and $[Na]^+$, respectively. The intensity of the $[M + K]^+$ ion peak was smaller than that due to $[M + Na]^+$. Although under the condition of F.D.-m.s. the molecular ion could not be detected, the appearance of the $[M + Na]^+$ and $[M + K]^+$ ion peaks helped in the determination of the molecular weight of (7). The peaks at *m/e* 1 057 and 1 041 are attributed to the fragment ions $[(M + K + H) - A]^+$ and $[(M + Na + H) - A]^+$, formed by the loss of a xylosyl residue from the potassium and sodium cation complexes, respectively, by protonation-induced cleavage of the glycosidic bond.[29] The intensities and assignments of different fragment ion peaks are shown in the Figure. The doubly charged $[M + 2Na]^{++}$ and $[(M + 2Na + H) - A]^{++}$ ions at *m/e* 598 and 532, respectively, further supported the assignment of the molecular weight. The peaks at *m/e* 1 305 and 1 321 presumably originate from a second high molecular weight compound which is present in the sample at a very low concentration. The formation of different ion peaks (Figure) indicated that xylose and rhamnose units are present at terminal positions. However, the sequence of glucose and galactose in (7) could not be ascertained by F.D.-m.s. (because the two fragments have the same molecular weight), and this was therefore ascertained by partial hydrolysis studies.

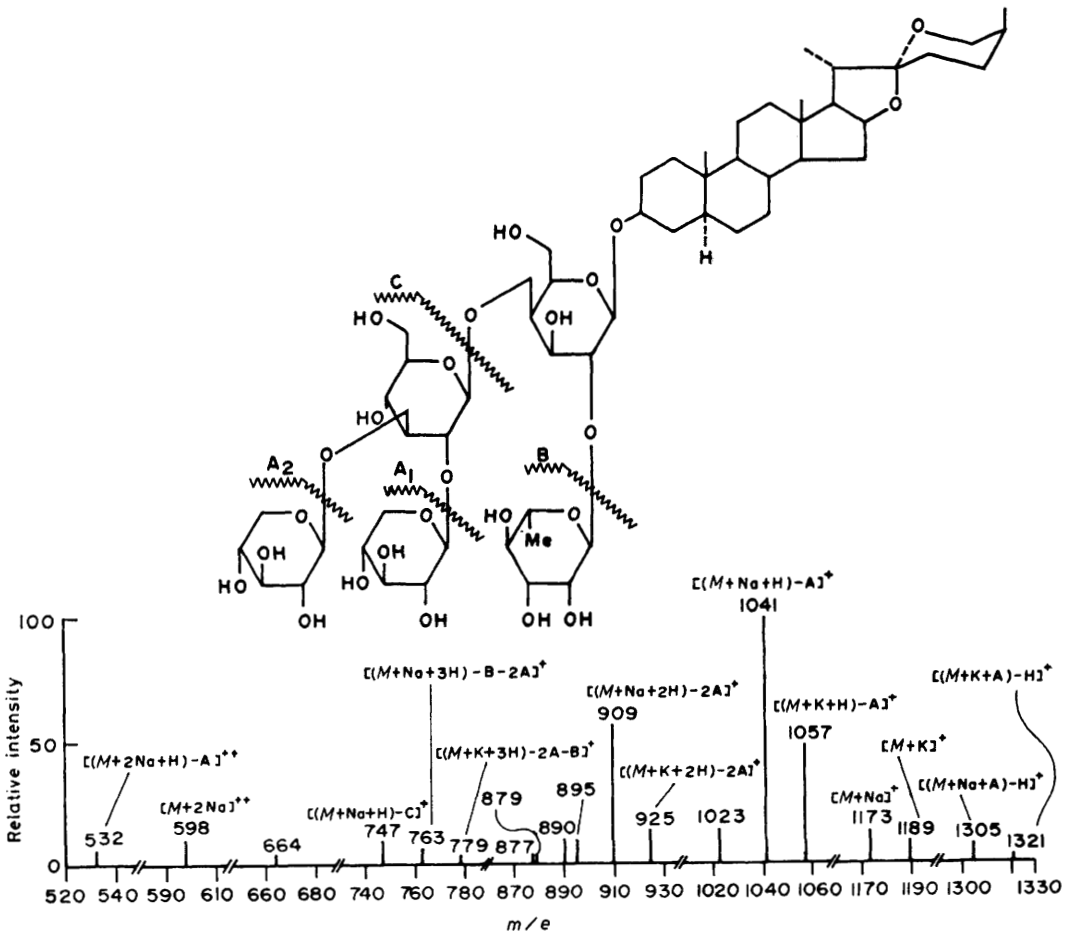


FIGURE Field-desorption mass spectrum of tribulosin (7) showing characteristic cleavages in the molecule (emitter heating current 18.5 mA)

When (7) was subjected to partial hydrolysis with 0.75M H_2SO_4 in EtOH on a steam-bath for 20 min, a mixture of prosapogenins was obtained from which only two prosapogenins, PS-1 (10) and PS-2 (11) could be isolated in a pure state. Compound (10) on hydrolysis yielded neotigogenin as the aglycone and D-galactose as the carbohydrate portion, whereas (11) furnished two monosaccharides, D-galactose and D-glucose. Consequently, it followed that in (7) galactose is directly linked to neotigogenin and glucose is linked to galactose. Moreover, (7), on treatment with sodium metaperiodate followed by acid hydrolysis, afforded only glucose and galactose as the sugar constituents indicating that the sugar units in (7) are inter-linked such that glucose and galactose do not have free vicinal hydroxyls. Thus the foregoing F.D.-m.s. and chemical studies suggested the following three possible monosaccharide sequences in the glycone portion of (7).

(a) S–gal–glc[3]–xyl
|
rha–xyl

(b) S–gal–glc(–xyl, –xyl)
|
rha

(c) S–gal–glc[3]–xyl–xyl
|
rha

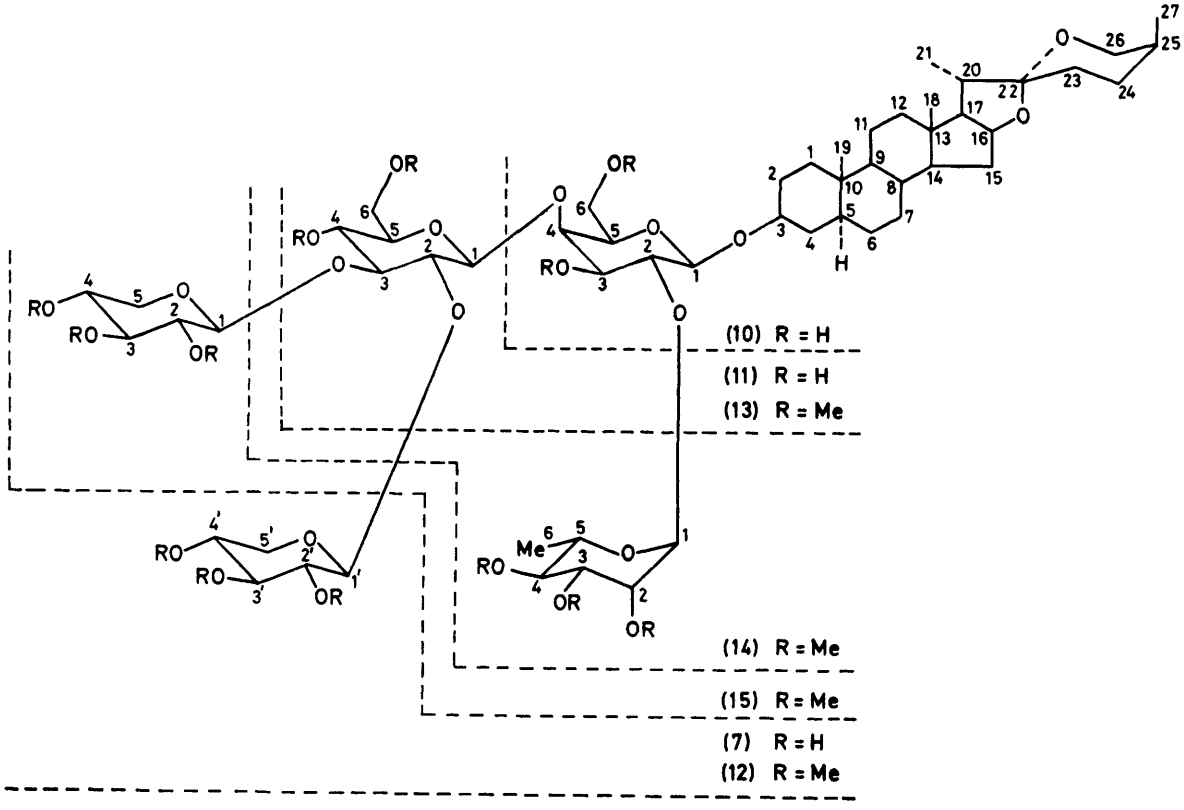
S = neotigogenin

The tribulosin permethylate (12), prepared using NaH–MeI in hexamethylphosphoramide, displayed in its F.D.-m.s. intense peaks at *m/e* 1 332 and 1 355, ascribable to the molecular ion and the ion formed by cationization of the neutral molecule with Na^+, respectively. On methanolysis (12) liberated methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 3,6-di-*O*-methyl-D-galactopyranoside, and methyl 4,6-di-*O*-methyl-D-glucopyranoside. Consequently the monosaccharide sequence in the sugar moiety of (7) must be (b), which itself represents two isomers, (b_1) and (b_2)

(b_1) S–gal[4]–glc(2-xyl, 3-xyl)
|[2]
rha

(b_2) S–gal[2]–glc(2-xyl, 3-xyl)
|[4]
rha

Compound (7) was partially hydrolysed with 2M HCl in Bu^nOH at 70 °C for 3 h to give a mixture of prosapogenins, which was permethylated and subjected to p.l.c. to yield four permethylates, A_1—D_1. Permethylate D_1 was identical to (12). The ^1H n.m.r. spectrum of permethylate A_1 (13) displayed signals at δ 4.32 (1 H, d, *J* 6 Hz) and 4.53 (1 H, d, *J* 7 Hz) assignable to two anomeric protons, indicating a β-orientation (C-1 conformation) for both. On methanolysis (13) yielded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,6-tri-*O*-methyl-D-galactopyranoside. The



formation of the latter compound demonstrated that (7) has the linkage sequence shown in b_1. In the ^{1}H n.m.r. spectrum of permethylate B_1 (14) signals ascribable to three anomeric protons were observed at δ 4.36 (1 H, d, *J* 6 Hz), 4.52 (1 H, d, *J* 7 Hz), and 5.22 (1 H, br s), which indicated β, β, and α linkages, respectively. Methanolysis of (14) liberated methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, and methyl 3,6-di-*O*-methyl-D-galactopyranoside. The ^{1}H n.m.r. spectrum of permethylate C_1 (15) exhibited signals ascribable to four anomeric protons at δ 4.32 (1 H, d, *J* 6 Hz), 4.46 (1 H, d, *J* 7 Hz), 4.75 (1 H, d, *J* 7 Hz), and 5.15 (1 H, br s), which indicated the presence of three β linkages and one α linkage. On methanolysis (15) yielded methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside, and methyl 3,6-di-*O*-methyl-D-galactopyranoside.

The ^{1}H n.m.r. spectrum of (12) showed signals assignable to five anomeric protons at δ 4.33 (1 H, d, *J* 6 Hz), 4.49 (1 H, d, *J* 7 Hz), 4.81 and 4.90 (each 1 H, d, *J* 7 Hz), and 5.15 (1 H, br s), indicating the presence of four β linkages and one α linkage.

Based on the foregoing evidence the structure of tribulosin has been established as neotigogenin 3-*O*-β-D-xylopyranosyl(1 → 2)-[β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside (7).

The ^{13}C n.m.r. spectrum of tribulosin (7) also supports the structure shown. The spectrum was recorded in [$^2H_5$]pyridine. The ^{13}C chemical shifts of methyl β-D-galactopyranoside, methyl β-D-glucopyranoside, methyl α-L-rhamnopyranoside, and methyl β-D-xylopyranoside in [$^2H_5$]pyridine are available,[30,31] and the ^{13}C signals of neotigogenin were assigned by comparison with the reported data.[25] Assignments of the signals of (7) (Table) were made by comparison with those of the sapogenin and sugar moieties, using known chemical-shift rules [32] and glycosylation shifts.[31,33-35]

^{13}C Chemical shifts δ_C (±0.1) of neotigogenin (8) ($CDCl_3$) and tribulosin (7) (C_5D_5N)

Carbon	(8)	(7)	Carbon	(7)
1	36.9	37.3	ga-1	100.2
2	31.4	29.9	ga-2	81.1
3	71.1	78.5	ga-3	70.5 [c]
4	38.1	34.5	ga-4	76.8 [e]
5	44.3	44.8	ga-5	75.5
6	28.6	29.0	ga-6	62.9
7	32.2	32.5	g-1	104.7 [a]
8	35.1	35.4	g-2	78.4
9	54.3	54.6	g-3	87.6
10	35.5	36.0	g-4	72.1 [b]
11	21.1	21.3	g-5	76.3 [e]
12	40.0	40.2	g-6	62.9
13	40.5	40.8	xy-1	105.1 [a]
14	56.2	56.5	xy-2	74.9
15	31.7	32.1	xy-3	77.3 [d]
16	80.8	81.1	xy-4	70.2 [c]
17	61.9	60.4	xy-5	67.0
18	16.5	16.5	xy-1′	105.0 [a]
19	12.4	12.4	xy-2′	74.9
20	42.1	42.5	xy-3′	77.4 [d]
21	14.3	14.7	xy-4′	70.5
22	109.5	109.4	xy-5′	67.0
23	27.0	27.6	r-1	101.6
24	25.9 [a]	26.2 [a]	r-2	72.5 [b]
25	25.8 [a]	26.5 [a]	r-3	72.1 [b]
26	65.0	65.1	r-4	73.9
27	16.0	16.3	r-5	69.1
			r-6	18.3

[a-e] Signals may be interchanged in each vertical column. ga = galactose, g = glucose, xy = xylose, r = rhamnose

EXPERIMENTAL

M.p.s are uncorrected. T.l.c. was carried out on silica gel G (B.D.H.) using the solvent systems: (A) benzene–$CHCl_3$–EtOAc (1 : 2 : 2); (B) water (2 ml) was added to $CHCl_3$ (60 ml); MeOH was then added till the solution became clear. Paper chromatography for sugars was done on Whatman paper No. 1 using the solvent systems (C) Bu^nOH–C_5H_5N–H_2O (6 : 4 : 3); (D) Bu^nOH–EtOH–H_2O (4 : 1 : 5, upper layer); other solvent systems are detailed in the text. A saturated solution of aniline oxalate in water was used as staining agent. G.l.c. was performed on a Hewlett–Packard model 5730 A instrument using the following columns: (*i*) ECNSS-M, 3% on Gas Chrome Q at 190 °C for alditol acetates; and (*ii*) OV-225 on Gas Chrome Q at 195 °C for partially methylated alditol acetates. G.l.c. of the methylated methyl monosaccharides was run on a JEOL-JGC-1100 with flame-ionisation detector, column, 1.5% butane-1,4-diol succinate polyester in glass tube (4 mm × 1.2 m); carrier gas N_2, 2 kg cm^{-2}. T.l.c. of the permethylate was performed on DC-alufolien Kieselgel F_{254} (Merck). Optical rotations were measured on a Perkin-Elmer automatic polarimeter; i.r. spectra were recorded in Nujol mulls on a Perkin-Elmer model 177 instrument. ^{1}H N.m.r. spectra were recorded either on a Varian (90 MHz) or on JEOL PS-100 (100 MHz) and FX-100 (99.6 MHz) instruments, in $CDCl_3$ or C_5D_5N. ^{13}C N.m.r. spectra were recorded on a JEOL FX-100 Fourier-transform spectrometer operating at 25.05 MHz, in $CDCl_3$ or C_5D_5N with tetramethylsilane as internal standard. Electron-impact mass spectra were recorded on a Hitachi model RMU-6L or a JEOL JMS 01SG mass spectrometer. Field-desorption mass spectra were recorded on a JEOL D-300 instrument with data system JMA-2000 electronically, with scan times 13–21 s/decade. Field-desorption emitters used in all experiments, were prepared by high-temperature activation of 10-μm diameter tungsten wire. F.D. emitters with an average length of 30 μm for the carbon microneedles were used as standards. The ionization efficiency and the adjustment of the F.D. emitter were determined by means of the acetone peak at *m/e* 58 in the field-ionization mode. All F.D. spectra were produced at an ion-source pressure of 3 × 10^{-7} Torr, and an ion-source temperature of 60—70 °C; the ion-source potentials were +2 or +3 kV for the field anode and −5 kV for the slotted cathode plate. The samples were desorbed by direct heating (emitter heating current) without emission control. Methanol was used as solvent for the compounds. In general 1 × 10^{-5} g was applied as sample to the standard emitter *via* the syringe technique.

Isolation of Steroidal Glycosides.—The air-dried and powdered aerial part of *T. terrestris* (2 kg) was successively extracted with light petroleum, chloroform, and 90% ethanol. The ethanolic extract, on removal of the solvent under reduced pressure, yielded a viscous dark brown mass (130 g). Part of this extract (60 g) was chromatographed on silica gel (900 g). Graded elution was effected with

benzene, followed by benzene–chloroform and chloroform–methanol. A total of 105 fractions of 250 ml each were collected and mixed on the basis of t.l.c.

β-*Sitosterol Glucoside* (1).—Fractions 22—30, eluted with chloroform–methanol (95 : 5) were mixed (0.55 g), purified by re-chromatography, and crystallized from chroform–methanol to yield colourless crystals of (1), m.p. 286—288 °C (decomp.), $[\alpha]_D$ −39° (pyridine) (Found: C, 72.4; H, 10.8. Calc. for $C_{35}H_{60}O_6$: C, 72.87; H, 10.48%).

Neohecogenin Glucoside (2).—Fractions 31—46, eluted with chloroform–methanol (93 : 7), were collected (0.8 g) and re-chromatographed yielding a colourless solid (0.2 g), which was crystallized from methanol to afford colourless micro-needles of (2), m.p. 280—282 °C; $[\alpha]_D$ −14° (pyridine); $\nu_{max.}$ 3 400 (OH), 1 700, 987, 920, 900, and 866 cm^{-1} (spiroacetal absorptions) (Found: C, 67.0; H, 8.8; $C_{33}H_{52}O_9$ requires C, 66.86; H, 8.84%).

Hydrolysis of Neohecogenin Glucoside (2).—Compound (2) (50 mg) was boiled with 5% (H_2SO_4 in MeOH (10 ml) for 6 h and worked up in the usual way; the sapogenol (3) was crystallized from MeOH to give colourless prisms (28 mg), m.p. 245—246 °C; $[\alpha]_D$ −4.5° ($CHCl_3$); $\nu_{max.}$ 3 400 (br), 1 703 (CO), 987, 920, 900, and 866 cm^{-1} (spiroketal absorptions); *m/e* 430 (M^+, 22%), 415 (M^+ − Me, 6), 412 (M^+ − H_2O, 5), 402 (M^+ − CO, 12), 371 (14), 358 (20), 343 (12), 316 (40), 298 (15), 273 (30), 139 (100), and 126 (50). Acetylation of (3) by heating with Ac_2O–pyridine (1 : 1) gave (4) as colourless needles (from EtOAc), m.p. 228—232 °C; $[\alpha]_D$ −11° ($CHCl_3$); $\nu_{max.}$ 1 702 (CO), 1 740, 1 243 (acetate CO), 980, 920, 900, and 868 cm^{-1}; δ ($CDCl_3$, 90 MHz) 0.92 (3 H, s, 19-Me), 1.05 (3 H, s, 18-Me), 1.0 (3 H, d, *J* 6 Hz, 21-Me), 0.90 (3 H, d, *J* 7 Hz, 27-Me), 1.97 (3 H, s, OCOMe), 4.18 (1 H, m, C-16-H), 3.30 (d, *J* 10 Hz, C-26-H), and 3.98 (dd, *J* 11, 3 Hz, C-26-H′) (Found: C, 73.7; H, 9.65. Calc. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38%).

The filtrate from the above hydrolysate was neutralised with $BaCO_3$ and filtered. The filtrate was concentrated under reduced pressure and the residue was tested for carbohydrates; only D-glucose was identified by paper chromatography using solvent system C, by comparison with an authentic sample.

Permethylation of (2) *and Hydrolysis*.—Compound (2) (150 mg) was completely methylated by the Hakomari method. Usual work-up followed by purification by p.l.c. provided (5) (120 mg) (no hydroxy-absorption in the i.r. spectrum). The permethylate (5) (80 mg) was hydrolysed on refluxing with 5% HCl in MeOH (15 ml) for 3 h. The reaction mixture was cooled, evaporated to dryness *in vacuo*, diluted with water, and filtered. The filtrate was neutralised with Ag_2CO_3 and filtered. The filtrate after concentration was tested for carbohydrates by paper chromatography (solvent D). Only one spot, corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose, could be identified using an authentic sample. A portion of the concentrated filtrate was reduced with $NaBH_4$ and worked up in the usual manner. The residue was acetylated with Ac_2O–pyridine (1 : 1) at water-bath temperature for 1 h, dried *in vacuo*, purified by chromatography over silica gel, and subjected to g.l.c. analysis using column (*ii*). Only one peak corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucitol acetate, was obtained.

Isolation of Dioscin (6).—Elution of the column with $CHCl_3$–MeOH (85 : 15) yielded a solid which on further purification by chromatography, followed by crystallization from MeOH, afforded micro-needles of dioscin (6) (110 mg), m.p. 286—287 °C; $[\alpha]_D$ −104° (MeOH) (Found: C, 61.8; H, 8.6. Calc. for $C_{45}H_{72}O_{16}$: C, 62.19; H, 8.35%).

Isolation of Tribulosin (7).—The fractions eluted with $CHCl_3$–MeOH (80 : 20) yielded a residue which, on crystallization from 90% ethanol, provided a crystalline compound (315 mg). This appeared to be homogeneous by t.l.c. but was found to be a mixture by h.p.l.c. On repeated crystallization from 90% ethanol it afforded colourless crystals (single peak in h.p.l.c.) of tribulosin (7) (180 mg), m.p. >300 °C; $[\alpha]_D$ −61° (pyridine); $\nu_{max.}$ 3 300 (br), 980, 920, 900, and 865 cm^{-1} (peak at 920 more intense than that at 900) (Found: C, 57.8; H, 7.4. Calc. for $C_{55}H_{90}O_{25}$: C, 57.38; H, 7.88%).

Hydrolysis of (7).—Compound (7) (80 mg) was hydrolysed with 2.5M HCl in EtOH (25 ml) at water-bath temperature for 5 h. The usual work-up gave the neotigogenin (8), which was crystallized from methanol as colourless needles (25 mg), m.p. 192—194 °C; $[\alpha]_D$ −65° ($CHCl_3$); $\nu_{max.}$ 3 300 (OH), 1 660, 985, 920, 900, and 850 cm^{-1} (the peak at 920 was more intense than that at 900); *m/e* 416 (M^+, 6%), 401 (M^+ − Me, 3), 398 (M^+ − H_2O, 4), 357 (4), 347 (10), 344 (15), 302 (34), 287 (24), 273 (56), 255 (7), 176 (15), 161 (10), 147 (7), 139 (100), and 115 (36); δ ($CDCl_3$, 100 MHz), 0.75 (3 H, s, 18-Me), 0.82 (3 H, s, 19-Me), 0.98 (3 H, d, *J* 6 Hz, 27-Me), 1.07 (3 H, d, *J* 7 Hz, 21-Me), 3.27 (1 H, d, *J* 11 Hz, 26-ax-H), 3.94 (1 H, dd, *J* 11, 2.5 Hz, 26-eq-H), 3.56 (1 H, m, $W_{\frac{1}{2}}$, 24 Hz, 3-H), and 4.38 (1 H, m, 16-H). Acetylation of (8) (12 mg) with Ac_2O–pyridine (1 : 1) yielded the acetate (9) (10 mg), which crystallised from acetone as colourless plates, m.p. 177—179 °C; $[\alpha]_D$ −70° ($CHCl_3$); $\nu_{max.}$ 1 735, 1 240 (acetate CO), 985, 918, and 860 cm^{-1} (918 more intense than 900); δ ($CDCl_3$, 100 MHz) 0.75 (3 H, s, 18-Me), 0.82 (3 H, s, 19-Me), and 4.66 (1 H, m, $W_{\frac{1}{2}}$ 24 Hz, 3-H) (Found: C, 76.1; H, 10.0. Calc. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11%).

The filtrate from the hydrolysate was worked up and tested for carbohydrates by paper chromatography using solvent system C. D-Glucose, D-galactose, D-xylose, and L-rhamnose were identified using authentic specimens. The presence of these monosaccharides was also confirmed by g.l.c. of the carbohydrate mixture after preparation of their alditol acetates using column (*i*).

Methylation of (7) *followed by Methanolysis*.—Compound (7) (5 mg) in hexamethylphosphoramide (1 ml) was treated with NaH (30 mg) and MeI (1 ml) at room temperature for 3 h. The reaction mixture was extracted with ether, the extract evaporated, and the residue chromatographed on silica gel eluting with EtOAc–hexane (1 : 2) to give compound (12) as a white powder, m.p. 143 °C (no hydroxy-absorption in the i.r. spectrum); F.D.-m.s. (emitter current 18.5 mA), *m/e* 1 332 $[M]^+$ and 1 355 $[M + Na]^+$; δ ($CDCl_3$, 99.60 MHz) 0.75 (3 H, s, 18-Me), 0.80 (3 H, s, 19-Me), 4.33 (1 H, d, *J* 6 Hz, C-1-H of glucose unit), 4.49 (1 H, d, *J* 7 Hz, C-1-H of galactose unit), 4.81, 4.90 (each 1 H, d, *J* 7 Hz, C-1-H of xylose units), and 5.15 (1 H, br s, C-1-H of rhamnose unit).

A solution of (12) (2 mg) in anhydrous 2M HCl–MeOH (0.2 ml) was heated at 60 °C for 2 h. To the reaction mixture were added molecular sieve (4 Å, 0.3 g) and a small amount of acetone and the mixture was subjected to g.l.c. Four peaks were detected and identified as those of the methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-rhamnose (R_t 1.8 min, column temperature 150 °C), 2,3,4-tri-*O*-methyl-α-D-xylose (R_t 2.4 min, 150 °C), 4,6-di-*O*-methyl-α-D-glucose (R_t 7.6 min, 169 °C), and 3,6-di-*O*-methyl-α-D-galactose (R_t 8.8 min, 169 °C), by comparison with authentic samples.[36]

PS-1 (10) *and PS*-2 (11).—Compound (7) (50 mg) was hydrolysed with 0.75M H_2SO_4 in ethanol (5 ml) on a steam-bath for 20 min. The solution was cooled, diluted with water (50 ml), and the precipitates were collected by filtration and then subjected to p.l.c. (solvent system $CHCl_3$–MeOH, 3 : 1). Thus (10) (3 mg) and (11) (3.5 mg) could be isolated in a pure state. Compound (10) crystallized from $CHCl_3$–MeOH as needles, m.p. 278—280 °C (decomp.), $[\alpha]_D$ −54° (EtOH). On acid hydrolysis it provided neotigogenin and D-galactose (Found: C, 68.6; H, 9.1; $C_{33}H_{54}O_8$ requires C, 68.48; H, 9.40%).

Compound (11) crystallized from acetone–methanol as micro-needles, m.p. 272—274 °C (decomp.). On acid hydrolysis it yielded neotigogenin, D-galactose, and D-glucose (Found: C, 62.9; H, 9.5; $C_{39}H_{64}O_{13}$ requires C, 63.22; H, 8.71%).

Periodate Oxidation of (7) *and Hydrolysis*.—Compound (7) (10 mg) and a solution of sodium metaperiodate (10%; 3 ml) were left in the dark at room temperature for seven days. Work-up as usual and hydrolysis of the residue with 2M HCl (3 ml) and tested for carbohydrates by g.l.c. Only D-glucose and D-galactose were identified.

Partial Hydrolysis of (7) *and Methylation of the Prosapogenin Mixture followed by their Separation*.—Compound (7) (15 mg) in 2M HCl (1 ml) and Bu^nOH (1 ml) was heated at 70 °C for 3 h. To the reaction mixture was added n-BuOH (30 ml); acid and monosaccharides in the Bu^nOH layer were removed by washing with water, and Bu^nOH was removed under reduced pressure to give a prosapogenin mixture (8.5 mg). This was methylated in the same way as for (7) to give a resinous substance (9.5 mg) which was subjected to p.l.c. [silica gel, eluant, benzene–acetone (4 : 1)] to give four fractions. Each fraction was further purified by p.l.c. to give the pure permethylates A_1 (2.2 mg, R_F 0.51), B_1 (1.3 mg, R_F 0.33), C_1 (0.5 mg, R_F 0.27), and D_1 (1.5 mg, R_F 0.21). Permethylate D_1 was identical with (12).

Permethylate A_1 (13). ^1H N.m.r. ($CDCl_3$, 99.60 MHz at δ 0.76 (3 H, s, 18-Me), 0.81 (3 H, s, 19-Me), 4.32 (1 H, d, *J* 6 Hz, C-1-H of glucose unit), and 4.53 (1 H, d, *J* 7 Hz, C-1-H of galactose unit). On methanolysis it furnished methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (R_t 3.7 min, 150 °C) and methyl 2,3,6,-tri-*O*-methyl-α-D-galactopyranoside (R_t 8.1 min, 150 °C).

Permethylate B_1 (14). ^1H N.m.r. ($CDCl_3$, 99.60 MHz) at δ 4.36 (1 H, d, *J* 6 Hz, C-1-H of glucose unit), 4.52 (1 H, d, *J* 7 Hz, C-1-H of galactose unit), and 5.22 (1 H, br s, C-1-H of rhamnose unit). Compound (14) on methanolysis gave methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside (R_t 1.8 min, 150 °C), methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (R_t 3.7 min, 150 °C), and methyl 3,6-di-*O*-methyl-α-D-galactopyranoside (R_t 8.8, 169 °C).

Permethylate C_1 (15). ^1H N.m.r. ($CDCl_3$, 99.60 MHz) at δ 4.32 (1 H, d, *J* 6 Hz, C-1-H of glucose unit), 4.46 (1 H, d, *J* 7 Hz, C-1-H of galactose unit), 4.75 (1 H, d, *J* 7 Hz, C-1-H of xylose unit), and 5.15 (1 H, br s, C-1-H of rhamnose unit). This on methanolysis yielded methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside (R_t 1.8 min, 150 °C), methyl 2,3,4-tri-*O*-methyl-α-D-xylopyranoside (R_t 2.4 min, 150 °C), methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside (R_t 2.5 min, 169 °C), and methyl 3,6-di-*O*-methyl-α-D-galactopyranoside (R_t 8.8 min, 169 °C).

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