Phytochemistry, Vol 22, No 5, pp 1241–1244, 1983
Printed in Great Britain

0031-9422/83/051241-04$03 00/0

OLIGOFUROSTANOSIDES FROM *SOLANUM NIGRUM*

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(*Revised received* 13 *September* 1982)

Key Word Index—*Solanum nigrum*, Solanaceae, saponins, spirostanol glycosides; furostanol glycosides.

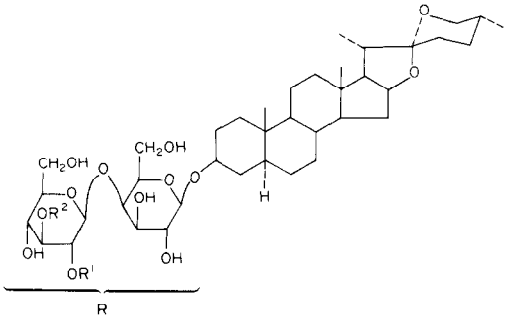
Abstract—One spirostanol glycoside and two furostanol glycosides have been isolated from a methanol extract of the stems and roots of *Solanum nigrum* and identified as 3-*O*-(β-lycotetraosyl)-(25*R*)-5α-spirostan-3β-ol (uttronin A), 3-*O*-(β-lycotetraosyl)-26-*O*-(β-D-glucopyranosyl)-(25*R*)-22α-methoxy-5α-furostane-3β,26-diol(uttrosideA) and 3-*O*-(β-lycotetraosyl)-26-*O*-(β-D-glucopyranosyl)-(25*R*)-5α-furostane-3β,22α,26-triol (uttroside B).

INTRODUCTION

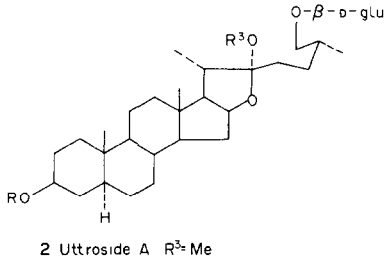
Solanum nigrum (local name 'Makoi') grows wild in India and is reported [1, 2] to be useful for treatment of piles, gonorrhoea, inflammatory swellings and chronic cirrhosis of the liver and spleen Different parts of this plant have been reported [3–9] to contain the steroidal alkaloid glycosides solasonine, α- and β-solamargine, α- and β-solanigrine, as well as the steroidal sapogenins tigogenin and diosgenin. However, no chemical work has been done on the saponin content of this plant.

RESULTS AND DISCUSSION

The crude saponin mixture obtained from a methanol extract of the stems and roots of this plant gave uttronin A (**1**) with typical spiroketal absorptions in the IR spectrum of a spirostanol glycoside. Also isolated were uttrosides A (**2**) and B (**3**) shown to be oligofurostanosides [10] which exhibited no spiroketal absorptions in their IR spectra and were positive to Ehrlich reagent [11] Acid hydrolysis of **1**–**3** furnished only tigogenin (mmp, co-TLC, mass spectrum and superimposable IR spectrum with an authentic sample [9]). The aqueous hydrolysate of **1** contained D-galactose, D-glucose and D-xylose in the ratio of 1·2·1 while that of **2** and **3** contained these sugars in the ratio of 1·3:1 (GC of the alditol acetates). The type of linkages in the carbohydrate moieties were established by NMR and application of Klyne's rule [12].



R¹ = β-D-glu(pyr)-, R² = β-D-xyl(pyr)-
1 Uttronin A



2 Uttroside A R³ = Me
3 Uttroside B R³ = H

In order to determine the structure of the carbohydrate moiety of **1**, it was permethylated by Hakomori's method [13] to yield the permethylate (**1a**) which in its mass spectrum showed peaks at *m/z* 1202 $[M]^+$, 1011 $[M - tri\text{-}O\text{-methylxylose} + H]^+$, 967 $[M - tetra\text{-}O\text{-methylglucose} + H]^+$, 219 and 175 (terminal glucose and xylose units). On methanolysis followed by hydrolysis **1a** gave a mixture of methylated sugars identified as 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,6-tri-*O*-methyl-D-galactose and 4,6-di-*O*-methyl-D-glucose. The latter two sugar derivatives were identified by comparison of their constants with the respective literature values. 4,6-Di-*O*-methyl-D-glucose also gave a pink colour with Wallenfels' reagent [14] These studies indicate that in **1** D-glucose and D-xylose are the terminal sugars attached through another D-glucose and D-galactose to the C-3 hydroxyl of tigogenin.

In order to determine the exact linkages of the monosaccharides in the sugar moiety of **1**, it was partially hydrolysed to afford tigogenin, and three new saponins designated as PS_1, PS_2 and PS_3. Acid hydrolysis of these three saponins gave tigogenin as the aglycone, the aqueous hydrolysate of PS_1 contained D-galactose, while that of PS_2 and PS_3 contained D-glucose and D-galactose All these saponins were permethylated PS_1 permethylate on hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose and tigogenin. Thus, PS_1 was characterized as 3-*O*-(β-D-galactopyranosyl)-(25*R*)-5α-spirostan-3β-ol. This result showed that in **1** the first sugar attached to tigogenin was D-galactose.

PS_2 permethylate on hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose, showing that the terminal glucose in PS_2 is attached at C-4 of galactose which is in turn glycosylated with tigogenin.

PS_2 was, therefore, shown to be 3-*O*-[β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-3β-ol

PS_3 permethylate on hydrolysis gave a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose The identity of 3,4,6-tri-*O*-methyl-D-glucose was further confirmed by its positive response with Wallenfels' reagent and the mass spectrum of its methyl pyranoside which was in accordance to the expected spectrum [15] Thus, PS_3 was shown to be 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-3β-ol

Based on the above studies **1** was characterized as 3-*O*-{[β-D-glucopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl}-(25*R*)-5α-spirostan-3β-ol The above structure was confirmed by its periodate oxidation followed by hydrolysis to give D-glucose From the above structure it is clear that the carbohydrate moiety of **1** is identical with β-lycotetraose, the sugar moiety of the steroid alkaloid glycoside, α-tomatine [3] Therefore, the structure of **1** may also be written as 3-*O*-(β-lycotetraosyl)-(25*R*)-5α-spirostan-3β-ol

Uttrosides A and B could not be separated by CC but after refluxing the mixture with dry methanol, TLC homogeneous uttroside A (**2**) resulted as a colourless solid. Contrast refluxing in acetone–water afforded uttroside B (**3**) All these results, coupled with their IR spectra and their positive test with Ehrlich's reagent, indicated these compounds to be oligofurostanosides [10] This was further confirmed by Marker's oxidative degradation using Tschesche's method [10] The degradation products were identified as 3β-acetoxy-5α-pregn-16-en-20-one [IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1724, 1662 (characteristic of Δ^{16}-20-keto [16]), MS *m/z* 358 $[M]^+$] and δ-hydroxy-γ-methyl-*n*-valeric acid methyl ester glucoside tetra-acetate [MS *m/z*: 331 (tetra-*O*-acetyl glucopyranosyl ion) and other peaks in accordance with the expected fragmentation pattern [18]]. The former product corresponds to a glucose unit joined at C-26 Sodium borohydride reduction of the mixture of **2** and **3** followed by acid hydrolysis gave dihydrotigogenin, mp 163–167°, MS *m/z* 418 $[M]^+$ (identity confirmed by direct comparison with an authentic sample [17]

Enzymatic hydrolysis of the mixture of **2** and **3** afforded **1** and D-glucose This showed that the carbohydrate moiety of **1** was present in these compounds Thus, **2** and **3** were characterized as 3-*O*-(β-lycotetraosyl)-26-*O*-(β-D-glucopyranosyl)-(25*R*)-22α-methoxy-5α-furostane-3β, 26-diol and 3-*O*-(β-lycotetraosyl)-26-*O*-(β-D-glucopyranosyl)-(25*R*)-5α-furostane-3β, 22α,26-triol, respectively

EXPERIMENTAL

Mps (uncorr.) were determined in open capillaries in an electrothermal mp apparatus CC was carried out using Si gel (60–120 mesh, BDH) Spots on TLC (Si gel) were visualized by 10% H_2SO_4 and Ehrlich reagent and on PC (Whatman No 1) by aniline hydrogen phthalate and triphenyl tetrazolium chloride (Wallenfels' reagent) The following solvent systems were employed (A) $CHCl_3$–MeOH–H_2O (65 30 10), (B) $CHCl_3$–MeOH–H_2O (65 35 10), (C) C_6H_6–EtOAc (9 1), (D) C_6H_6–EtOAc (4 1), (F) *n*-BuOH–HOAc–H_2O (4 1 5), (G) *n*-BuOH–EtOH–H_2O (5 1 4), (H) C_6H_6–Me_2CO (10 1) GC of methyl methylated sugars employed a dual FID, column 3 m × 2 mm, 5% SE-30, N_2 (40 ml/min), programmed from 150 to 190° at 8°/min

Isolation of saponins Well-dried and finely powdered roots and stems (3 kg), collected from Simla (H P.) were defatted with petrol in a Soxhlet and the solvent free powder was likewise extracted with MeOH until the extract became colourless Removal of the solvent from the extract gave a viscous mass (8 g) which was purified as usual for the isolation of saponins The purified mass (5 5 g) thus obtained was repeatedly chromatographed (solvent A) to give uttronin A (**1**) (2 2 g) and a mixture of uttroside A (**2**) and uttroside B (**3**) (1 5 g)

Uttronin A (**1**) Crystallized from MeOH, mp 241–245°, $[\alpha]_D^{20}$ −51.0° (pyridine, *c* 1 5), IR ν_{max}^{KBr} cm^{-1} 3300–3540 (OH), 982, 925, 905, 870 (intensity 905 > 925, 25*R*-spiroketal), ^1H NMR (DMSO-d_6) δ 0 72 (3H, s, Me-18), 0 78 (3H, s, Me-19), 5 05, 5 12, 5 68 (1H each, all *d*, *J* = 6 Hz, anomeric H, 3 glc) and 5 28 (1H, *d*, *J* = 7 Hz, anomeric H) (Found C, 58 90, H, 7 85 $C_{50}H_{82}O_{22}$ requires C, 58 02, H, 7 93%)

Hydrolysis of **1**–**3** Compound **1** and a mixture of **2** and **3** (100 mg, each) were separately hydrolysed by refluxing with 7% H_2SO_4 (25 ml) for 4 hr on a steam bath and were then cooled and filtered to afford the aglycone (tigogenin) Colourless needles (MeOH), mp 202–204 (lit [9] mp 206 208°, $[\alpha]_D^{20}$ −65 ($CHCl_3$, *c* 1 0), yellow colour with Sannié's reagent and no colour with tetranitromethane, IR ν_{max}^{KBr} cm^{-1} 3300–3540 (OH), 985, 925, 905, 865 (intensity 905 > 925, 25*R*-spiroketal), MS (*m/z*) 416 $[M]^+$ The neutralized (Ag_2CO_3) and concd aq hydrolysates showed the presence of D-galactose, D-glucose and D-xylose (PC, solvent F, R_fs 0 15, 0 18 and 0 27, respectively)

The above filtrate of **1** was reduced with $NaBH_4$ (750 mg) for 4 hr and neutralized by passing through Dowex 50 (H^+) H_3BO_3 was removed by co-distillation with MeOH and the product was acetylated with Ac_2O–pyridine (1 1, 10 ml) at 100° for 5 min, diluted with H_2O, evaporated to dryness, dissolved in $CHCl_3$ and analysed by GC (column 10% silicone VC-W 982 on Chrom Q, 80–120 mesh, 1 3 m × 2 5 mm, column temp 180°, carrier gas N_2, flow rate 40 ml/min) R_t (min) 8 2 (xylose), 22 4 (galactose), 24 0 (glucose) in the ratio 1 1 2

Similarly the **2** and **3** hydrolysates contained D-galactose, D-glucose and D-xylose in the ratio 1 3 1

Partial hydrolysis of **1** Compound **1** (1 5 g) in 5% aq H_2SO_4–MeOH (150 ml) was refluxed on a steam bath for 40 min, MeOH was removed and H_2O (25 ml) added The aq soln was extracted with *n*-BuOH, concd and chromatographed (solvent A) to yield tigogenin, PS_1 (100 mg), PS_2 (200 mg) and PS_3 (300 mg) PS_1 mp 230–235 (dec., MeOH), $[\alpha]_D^{20}$ −38 2 ($CHCl_3$–MeOH), IR ν_{max}^{KBr} cm^{-1} 3390–3450 (OH), 982, 918, 898, 855 (intensity 898 > 918, 25*R*-spiroketal) (Found C, 68 00, H, 9 30 $C_{33}H_{54}O_8$ calcd C, 68 51, H, 9 34%) PS_2 mp 220–225°, $[\alpha]_D^{20}$ −42 8 ($CHCl_3$–MeOH), IR ν_{max}^{KBr} cm^{-1} 3410–3490 (OH), 985, 920, 900, 865 (intensity 900 > 920, 25*R*-spiroketal) (Found C, 63 00, H, 8 70 $C_{39}H_{64}O_{13}$ calcd. C, 63 24, H, 8 65%) PS_3 mp 230–235 (dec.), $[\alpha]_D^{20}$ −45 0° ($CHCl_3$–MeOH), IR ν_{max}^{KBr} cm^{-1} 3450 (OH), 985, 923, 900, 867 (intensity 900 > 923, 25*R*-spiroketal) (Found C, 59 80, H, 8 15 $C_{45}H_{74}O_{18}$ calcd C, 59 86, H, 8 20%)

Hydrolysis of PS_1, PS_2 *and* PS_3 Each (50 mg) were separately hydrolysed as above The aglycone was identified as tigogenin (mmp co-TLC) The aq hydrolysate of PS_1 contained D-galactose and those of PS_2 and PS_3 contained D-galactose and D-glucose (PC, solvent F, R_fs 0 15 and 0 18, respectively)

Permethylation of **1**, PS_1, PS_2 *and* PS_3 Compound **1** (400 mg), PS_1 (50 mg), PS_2 (100 mg) and PS_3 (200 mg) were separately permethylated by Hakomori's method [13] and after usual work-up the products were purified by CC (solvent C) to give the permethylates

Uttronin A permethylate (**1a**). Crystallized from Et_2O–petrol, mp 89–91°; $[\alpha]_D^{20}$ – 43° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: no OH, 982, 920, 898, 856; EIMS (probe) 70 eV, *m/z*: 1202 $[M]^+$, 1011, 967, 583, 399, 219, 187, 175, 143, 139, 111, 101, 88, 75, 55. (Found: C, 61.70; H, 8.55%. $C_{62}H_{106}O_{22}$ requires C, 61.89, H, 8.82%.)

Methanolysis followed by hydrolysis of **1a**. A soln of **1a** (250 mg) in dry 10% HCl–MeOH (50 ml) was refluxed on a water bath (5 hr), the MeOH evaporated, H_2O (20 ml) was added and the mixture heated for 3 hr on a water bath. The ppt was filtered and the aq. hydrolysate was neutralized (Ag_2CO_3), filtered and the filtrate concd. PC of the concd filtrate showed the presence of 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 4,6-di-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose (solvent G, R_G values 0.93, 1.00, 0.46 and 0.71, respectively). The identity of 4,6-di-*O*-methyl-D-glucose was revealed by spraying the chromatogram with triphenyl tetrazolium chloride to give a pink spot (R_G 0.46).

The identities of 2,3,4-tri-*O*-methyl-D-galactose and 4,6-di-*O*-methyl-D-glucose were further confirmed by their isolation by prep. TLC (solvent D, I_2 as visualizing agent), physical constants and direct comparison (co-TLC) with an authentic sample [17].

2,3,6-*Tri-O-methyl-D-galactose*. $[\alpha]_D^{20}$ + 80° (H_2O, *c* 1.0) [lit. [17] $[\alpha]_D$ + 80° (H_2O; *c* 0.5), not crystallizable]

4,6-*Di-O-methyl-D-glucose*. Mp 158–161° (EtOAc), $[\alpha]_D^{20}$ + 104° → + 64° (H_2O, *c* 1.0) [lit. [17]: mp 159–162° (HOAc), $[\alpha]_D$ + 104° → + 64° (H_2O, *c* 0.5)].

$NaIO_4$ *oxidation of* **1**. Compound **1** (20 mg) was dispersed in H_2O–MeOH (1.1, 10 ml) and $NaIO_4$ (25 mg) was added. The reaction mixture was kept in the dark at room temp. (48 hr) and ethylene glycol added to destroy excess $NaIO_4$. It was filtered, washed with H_2O and the ppt was acid hydrolysed followed by the usual work-up which showed the presence of D-glucose only (PC, solvent F, R_f 0.18).

Methanolysis of PS$_3$ *permethylate*. PS_3 permethylate (100 mg) was methanolized as before, cooled, filtered and the filtrate was neutralized (Ag_2CO_3) and filtered. The filtrate was concd and subjected to prep. TLC (solvent system H, I_2 as visualizing agent) to isolate methyl-3,4,6-tri-*O*-methyl-β-D-glucopyranoside, EIMS (*m/z*): 205, 191, 173, 149, 141, 127, 102, 101, 99, 89, 88, 87, 75 (base peak), 74, 71, 59 and 45.

Methanolysis followed by hydrolysis of PS$_1$, *PS*$_2$ *and PS*$_3$ *permethylates*. The permethylates PS_1, PS_2 and PS_3 (50 mg, each) were methanolized and hydrolysed as before. The aglycone ppt was identified as tigogenin. PS_1 hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-galactose (PC, R_G 0.88, solvent G); PS_2 hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose (PC, R_G 1.00 and 0.71, respectively) and PS_3 hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-galactose (R_G 1.00, 0.84 and 0.71, respectively). Spraying the paper with Wallenfels' reagent revealed a pink coloured spot for 3,4,6-tri-*O*-methyl-D-glucose.

Uttroside A (**2**). The mixture of **2** and **3** (50 mg) was boiled with dry MeOH (20 ml) for 4 hr. It was crystallized from MeOH to yield colourless **2**, mp 220–225° (dec.); $[\alpha]_D^{20}$ – 49° (MeOH, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions. (Found: C, 54.65, H, 8.30. $C_{57}H_{96}O_{28}.H_2O$ requires. C, 54.89; H, 7.81%.)

Uttroside B (**3**). The mixture of **2** and **3** (50 mg) was refluxed with Me_2CO–H_2O (4:1, 10 ml) for 12 hr and the soln concd. The solid deposited was collected and recrystallized from Me_2CO–H_2O to yield **3**, mp 210–215° (dec.), $[\alpha]_D^{20}$ – 46° (pyridine, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400–3450 (OH), no spiroketal absorptions. (Found: C, 54.90, H, 8.10. $C_{56}H_{94}O_{28}.H_2O$ requires. C, 54.55; H, 7.79%.)

Enzymatic hydrolysis of **2** *and* **3**. The mixture of **2** and **3** (50 mg) was taken-up in H_2O (10 ml), β-glucosidase (5 mg) was added and the aq. layer covered with toluene. The reaction mixture was kept at room temp. for 48 hr. TLC (solvent B) and PC (solvent F) showed the formation of **1**, saponin PS_3 and D-glucose.

$NaBH_4$ *reduction of* **2** *and* **3**. To a mixture of **2** and **3** (50 mg) in H_2O was added $NaBH_4$ (500 mg) and the mixture kept at room temp. for 24 hr. The resulting soln was hydrolysed with 3 N HCl (50 ml) on a boiling water bath for 6 hr, extracted with Et_2O and the solvent removed from the extract to give a sapogenin mixture. It was chromatographed (solvent C) to afford tigogenin with dihydrotigogenin. Dihydrotigogenin: colourless needles (MeOH), mp 163–167°; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions, EIMS (probe) 70 eV, *m/z*. 418 $[M]^+$, 400, 344, 341, 313, 273, 255 and 144. Identity confirmed by direct comparison mmp and co-TLC with an authentic sample [17].

CrO_3 *oxidation of* **2** *and* **3**. Compounds **2** and **3** (650 mg) were kept in pyridine–Ac_2O (1·1, 50 ml) for 48 hr at room temp. The reaction mixture was poured into ice-cooled H_2O (100 ml), filtered and the ppt, after pyridine was removed by repeated washing with H_2O, was dried. The acetates (700 mg) thus formed in Ac_2O (20 ml) were refluxed for 1 hr. After cooling, H_2O (10 ml) was added to the mixture which was then evaporated to dryness. To the residue was added HOAc (15 ml) and NaOAc (300 mg). The mixture was cooled to 15° and cold (5–10°) CrO_3 (500 mg) in 50% HOAc (5 ml) was added. The reaction mixture was stirred for 3 hr at room temp., diluted with H_2O (50 ml) and extracted with Et_2O. The Et_2O extract was washed with H_2O and evaporated. To the residue (1 g) in *t*-BuOH (25 ml) was added KOH (1.5 g) in H_2O (5 ml) and the mixture was stirred at 30° for 4 hr under N_2. Afterwards H_2O (25 ml) was added, *t*-BuOH was removed and the remaining mass extracted with *n*-BuOH. The *n*-BuOH extract was evaporated and the residue was hydrolysed by refluxing with 1 N HCl–toluene (30 ml) for 3 hr. After cooling the toluene phase was separated, evaporated and the acetate of the product was prepared as usual to give 3β-acetoxy-5α-pregn-16-en-20-one, mp 162–163°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1724, 1662, 956, 918, 895, 820; EIMS (probe) 70 eV, *m/z* 358 $[M]^+$; UV λ_{max} nm: 239 (lit. [19]: mp 162°). The aq. phase adjusted to pH 3 with 2 N HCl was extracted with *n*-BuOH and $CHCl_3$ alternately. The aq. phase was then neutralized with 2 N NaOH and evaporated. The residue was acetylated (Ac_2O–pyridine, 1.1) and worked up as usual and treated with ethereal CH_2N_2 (15 ml) for 15 min. The reaction mixture was evaporated to give a syrup of δ-hydroxy-γ-methyl-*n*-valeric acid methyl ester glucoside tetra-acetate, EIMS (probe) 70 eV, *m/z* (rel. int.). 331 (0.65), 242 (0.8), 200 (1.0), 169 (13.0), 145 (1.0), 129 (77), 115 (32), 97 (1.5).

Acknowledgements—Thanks are due to Professor Dr. G. Snatzke, University of Bochum (West Germany) and Dr. G. Eckhard, University of Bonn (West Germany) for mass spectra. O.P.S. thanks the UGC for a fellowship and the University of Garhwal, Srinagar (U.P.) for granting study leave.

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