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STEROIDAL SAPONINS OF *ASPARAGUS ADSCENDENS*

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Key Word Index—*Asparagus adscendens*; Liliaceae; saponins; spirostanol glycosides; furostanol glycosides.

Abstract—From the methanol extract of the fruits of *Asparagus adscendens* sitosterol-β-D-glucoside, two spirostanol glycosides (asparanin A and B) and two furostanol glycosides (asparoside A and B) were isolated and characterized as 3-*O*-[β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol, 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-22α-methoxy-(25*S*)-5β-furostan-3β,26-diol and 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25*S*)-5β-furostan-3β,22α, 26-triol, respectively.

INTRODUCTION

Asparagus adscendens is known [1] for its medicinal utility, and sitosterol, sarsasapogenin and diosgenin have been reported [2] by us from the fruits of this plant. Two spirostanol glycosides and two furostanol glycosides are now reported from a methanol extract of the fruits.

RESULTS AND DISCUSSION

The methanol extract of the fruits afforded sitosterol-β-D-glucoside, asparanin A (**1**), asparanin B (**2**) and a mixture of asparoside A (**3**) and asparoside B (**4**). The configurations of the glycosidic bonds in **1–4** were established by ^{1}H NMR studies and by the application of Klyne's rule [3], which showed D-glucose-β and L-rhamnose-α linkages.

Compounds **1** and **2** showed spiroketal absorptions in their IR spectra whereas compounds **3** and **4** (Ehrlich reagent [4, 5] positive) did not. Therefore, **1** and **2** were spirostanol glycosides and **3** and **4** were furostanol glycosides. Acid hydrolysis of **1** and **2** afforded sarsasapogenin (mmp, co-TLC, MS and comparison of IR spectra) and D-glucose; **2** also gave L-rhamnose (in the ratio D-glucose–L-rhamnose, 2:1, GC of the aldetol acetates).

Compound **1**, mp 276–280°, $[\alpha]_D^{18}$ −61.5° (pyridine), was permethylated by Hakomori's method [6] to give **1a** whose mass spectrum showed an $[M]^+$ peak and other peaks corresponding to sarsasapogenin-diglucoside. Hydrolysis of **1a** gave 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and 3, 4, 6-tri-*O*-methyl-D-glucose (PC, pink colour with Wallenfel's reagent [7]). The identity of the latter sugar was also confirmed by the mass spectrum of its methyl pyranoside, which showed peaks in accordance with the expected pattern [8]. Thus, **1** was characterized as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol.

Compound **2**, mp 258–262°, $[\alpha]_D^{20}$ −64.5°, was permethylated to afford **2a**. The methanolysis of **2a** gave a mixture of methyl pyranosides of 2, 3, 4-tri-*O*-methyl-L-rhamnose, 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and 3, 6-di-*O*-methyl-D-glucose (GC). Hydrolysis of the above sugar mixture gave the corresponding methylated sugars (PC). 3, 6-Di-*O*-methyl-D-glucose was positive to Wallenfel's reagent. The mass spectral studies on **2a** also supported the above results indicating that D-glucose and L-rhamnose were the terminal sugars attached at C-2 and C-4 of another D-glucose molecule which is glycosidated at C-1 with C-3 of sarsasapogenin.

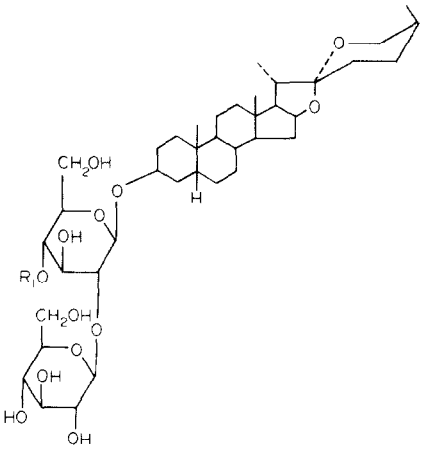
Partial hydrolysis of **2** afforded the prosapogenins PS_1, PS_2 (identical with saponin **1**) and PS_3, indicating that the saponin **1** structure is present as such in **2**. Hydrolysis of PS_1 and PS_3 furnished sarsasapogenin and D-glucose; PS_3 also provided L-rhamnose. Hydrolysis of PS_1 and PS_3 permethyl ethers afforded 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, and 2, 3, 4-tri-*O*-methyl-L-rhamnose and 2, 3, 6-tri-*O*-methyl-D-glucose (identity confirmed by mass spectrum of the methyl pyranoside) respectively. These results prove that **2** was a rhamnoside of **1** and it was characterized as 3-*O*-{[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol.

Compounds **3** and **4** were inseparable by CC and the ^{1}H NMR spectrum exhibited a methoxyl signal at δ 3.25 which was not observed on refluxing with dioxane–water. The above treatment gave **4**, while back transformation into **3** could be obtained by refluxing in dry methanol. All these results coupled with the IR spectra and 2-D TLC [9] suggested these compounds to be furostanol glycosides with **3** as the C-22 methoxy and **4** as the C-22 hydroxy derivatives. This was confirmed by subjecting **3** and **4** to oxidative degradation using the method of Tschesche *et al.* [10]

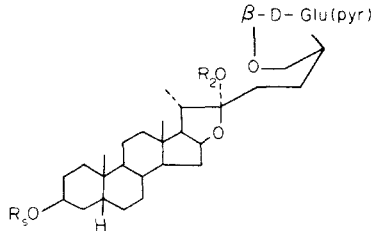
to isolate 3β-hydroxy-5β-pregn-16-en-20-one acetate, IR, 1724, 1662 cm^{-1} (characteristic of a Δ^{16}-20-keto compound [11]); MS (*m/z*): 358 $[M]^+$; UV λ_{max} 239 nm and δ-hydroxy-γ-methylvaleric acid ester glucoside tetraacetate (MS gave the expected pattern [12]). These results suggest that only one molecule of D-glucose was attached at C-26 of the furostanol skeleton. Enzymatic hydrolysis of **3** and **4** gave **2**, PS_3 and D-glucose formed by the elimination of D-glucose molecules from C-26 as well as from the main sugar chain at C-3. Compounds **3** and **4** were characterized as 3 - *O* - {[β - D - glucopyranosyl(1 → 2)][α - L - rhamnopyranosyl(1 → 4)] - β - D - glucopyranosyl} - 26 - *O* - (β - D - glucopyranosyl) - (25*S*) - 5β - furostan - 22α - methoxy - 3β, 26 - diol and 3 - *O* - {[β - D - glucopyranosyl(1 → 2)][α - L - rhamnopyranosyl(1 → 4)] - β - D - glucopyranosyl} - 26 - *O* - (β - D - glucopyranosyl) - (25*S*) - 5β - furostan - 3β, 22α, 26 - triol, respectively. It is probable that **3** is an artifact produced from **4** during the extraction of the plant material with methanol.

EXPERIMENTAL

All mps are uncorr. CC was carried out on Si gel (60–120 mesh) and TLC on Si gel G. Spots on TLC were developed with 10% H_2SO_4 followed by heating. PC was carried out on Whatman No. 1 paper using descending methods and aniline hydrogen phthalate as visualizing agent. The following solvent systems were employed: solvent A, $CHCl_3$–MeOH–H_2O (65:30:10); solvent B, $CHCl_3$–MeOH–H_2O (65:35:10); solvent C, C_6H_6–EtOAc (9:1); solvent D, C_6H_6–EtOAc (4:1); solvent E, *n*-BuOH–HOAc–H_2O (4:1:5); solvent F, *n*-BuOH–EtOH–H_2O (5:1:4); solvent G, C_6H_6–Me_2CO (10:1). GC of methyl methylated sugars: dual FID: column: 5% SE-30, 3 m × 2 mm, N_2 (40 ml/min.). Programmed from 150 to 190° at 4°/min.



1 R_1 = H
2 R_1 = α-L-Rha (pyr)



3 R_5 as in structure **2**, R_2 = Me
4 R_5 as in structure **2**, R_2 = H

Isolation of saponins. Almost mature fruits (2 kg, green in colour), collected from Kangra (H.P.), were well dried, powdered and defatted with petrol and the solvent free fruits were then exhaustively extracted with 90% aq. MeOH until the extractive became colourless. Evaporation of the solvent under red. pres. gave a crude saponin mixture (15.0 g) which was purified as usual for the isolation of saponins. The saponin mixture, thus obtained, was chromatographed (solvent A) to afford sitosterol-β-D-glucoside (800 mg), **1** (750 mg), **2** (2.5 g) and a mixture of **3** and **4** (3 g).

Sitosterol-β-D-glucoside. Mp 295–298° (MeOH), $[\alpha]_D^{20}$ – 41.5° (pyridine). Its identity was further confirmed by mmp, co-TLC and superimposable IR with an authentic sample.

Compound **1**. Mp 276–280° (decomp.), $[\alpha]_D^{18}$ – 61.5° (pyridine; *c* = 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 918, 898, 850 (intensity 918 > 898, 25*S* spiroketal). (Found: C, 61.90; H, 8.30. $C_{39}H_{64}O_{13}\cdot H_2O$ requires: C, 61.58; H, 8.77%.)

Compound **2**. Colourless needles from MeOH, mp 258–262° (decomp.), $[\alpha]_D^{20}$ – 64.5° (pyridine; *c* 1.5); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 918, 898, 852 (intensity 918 > 898, 25*S* spiroketal); ^1H NMR (C_5D_5N): δ 0.82 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 1.08 (3H, *d*, *J* = 6Hz, H-27), 1.15 (3H, *d*, *J* = 6 Hz, H-21), 1.65 (3H, *d*, *J* = 6 Hz, H-6 of rhamnose), 4.85, 5.43 (1H each, both *d*, *J* = 7 Hz, H-1 of two glucose units) and 5.88 (1H, *s*, H-1 of rhamnose). (Found: C, 58.33; H, 8.80. $C_{45}H_{74}O_{17}\cdot 2H_2O$ requires: C, 58.55; H, 8.52%.)

Compound **3**. The mixture of **3** and **4** (100 mg) was refluxed with dry MeOH (50 ml) for 6 hr. The soln was concd and cooled to afford **3** which was recrystallized from MeOH, mp 180–184° (decomp.), $[\alpha]_D^{20}$ – 53° (MeOH; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions; ^1H NMR (C_5D_5N): δ 3.25 (3H, *s*, OMe-22), 4.82 (1H, *d*, *J* = 7 Hz), 5.41 (1H, *d*, *J* = 7.1 Hz), 5.90 (1H, *s*). (Found: C, 57.50; H, 8.10. $C_{51}H_{88}O_{23}$ requires: C, 57.77; H, 8.14%.)

Compound **4**. Compounds **3** and **4** (100 mg) were refluxed with dioxane–H_2O (1:1, 15 ml) for 10 hr, concd, cooled and the solid deposited was recrystallized twice from Me_2CO–H_2O to give **4**, mp 170–178° (decomp.), $[\alpha]_D^{20}$ – 58° (pyridine; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions. (Found: C, 57.39; H, 8.70. $C_{50}H_{86}O_{23}$ requires: C, 57.41; H, 8.67%.)

Acid hydrolysis of **1** *and* **2**. Compounds **1** and **2** (100 mg) each were separately hydrolysed by refluxing with 7% H_2SO_4 (25 ml) for 4 hr, cooled and filtered to afford the aglycone (sarsasapogenin): colourless needles (MeOH), mp 198–199°, $[\alpha]_D^{20}$ – 74° ($CHCl_3$; *c* 1.0); (lit. [2]: mp 199°, $[\alpha]_D^{20}$ – 75°); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 981, 920, 900, 855 (intensity 920 > 900, 25*S* spiroketal); EIMS (probe) 70 eV, *m/z*: 416 $[M]^+$. The neutralized (Ag_2CO_3) and concd aq. hydrolysate of **1** showed D-glucose and of **2** showed L-rhamnose and D-glucose (PC, solvent E, R_fs 0.37 and 0.18 respectively). The hydrolysate of **2** was reduced with $NaBH_4$ (850 mg) for 4 hr and neutralized by passing through Dowex 50 (H^+). Boric acid was removed by co-distillation with MeOH and the product was acetylated (Ac_2O–pyridine, 1:1) 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, 1.3 m × 2.5 mm; N_2 40 ml/min, column temp. 180°. R_t (min): 4.5 (rhamnose) and 25.0 (glucose) in the ratio 1:2.

Permethylation of **1** *and* **2**. Compounds **1** (300 mg) and **2** (500 g) were separately permethylated by Hakomori's method to yield the permethylates (**1a** and **2a** respectively), which were purified by CC (solvent D).

Compound **1a**. Mp 87–89°, $[\alpha]_D^{18} - 68.5°$ ($CHCl_3$; *c* 1.20); IR ν_{max}^{KBr} cm^{-1}: no OH, 984, 920, 898, 851; EIMS (probe) 70 eV, *m/z*: 838 $[M]^+$, 603 [M − tetra-*O*-methyl glucose + H]$^+$, 399, 219, 187, 139, 101, 88, 75, 71, 55, 45. (Found: C, 65.50; H, 9.80. $C_{46}H_{78}O_{13}$ requires: C, 65.84; H, 9.37%.)

Methanolysis of **1a**. Compound **1a** (150 mg) in 1 N HCl–MeOH (15 ml) was refluxed (4 hr), neutralized (Ag_2CO_3), filtered and the filtrate after concn was subjected to prep. TLC (solvent G, I_2 as visualizing agent) to isolate methyl-3, 4, 6-tri-*O*-methyl-D-glucopyranoside, EIMS (probe) 70 eV, *m/z*: 205, 191, 173, 149, 141, 127, 102, 101, 99, 89, 88, 87, 75 (base peak), 74, 73, 59, 45. Hydrolysis of a portion of the above hydrolysate afforded 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and 3, 4, 6-tri-*O*-methyl-D-glucose (PC, solvent F, R_G values: 1.0 and 0.84 respectively). On spraying with Wallenfel's reagent the spot corresponding to R_G value 0.84 gave an intense pink colour.

Compound **2a**. Mp 93–96°, $[\alpha]_D^{20} - 72.0°$ ($CHCl_3$; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: no OH, 985, 920, 897, 850; EIMS (probe) 70 eV, *m/z*: 1012 $[M]^+$, 980 [M − MeOH]$^+$, 967 [M − CH_2OMe]$^+$, 807 [M − tri-*O*-methyl rhamnose + H]$^+$, 777 [M − tetra-*O*-methyl glucose + H]$^+$, 399, 255, 219, 189, 187, 139, 111, 88, 71, 55, 45.

Methanolysis of **2a**. Compound **2a** (100 mg) was methanolysed and worked-up as above to afford a mixture of methyl pyranosides of the following sugars: methyl-2, 3, 4-tri-*O*-methyl-L-rhamnose (GC R_t 1.90 min), 2.81, 3.15, 2, 3, 4, 6-tetra-*O*-methyl-D-glucose (2.81 and 3.15 min) and 3, 6-di-*O*-methyl-D-glucose (3.59 min). Hydrolysis of a portion of the above sugar mixture showed the following sugars on PC (solvent F): R_G: 1.01 (2, 3, 4-tri-*O*-methyl-L-rhamnose), 1.00 (2, 3, 4, 6-tetra-*O*-methyl-D-glucose), 0.51 (3, 6-di-*O*-methyl-D-glucose; pink colour with Wallenfel's reagent).

Partial hydrolysis of **2**. A soln of **2** (1.5 g) in 5% aq. HCl–MeOH (1:1, 50 ml) was heated under reflux for 45 min, concd under red. pres., H_2O (30 ml) added and the mixture filtered. The aq. filtrate was neutralized with 5% aq. KOH, extracted with *n*-BuOH (50 ml twice) and the organic phase was concd under red. pres. to dryness. The residue thus obtained was mixed with the above residue and separated by CC (solvent A) to give sarsasapogenin (50 mg), PS_1 (100 mg), PS_2 (asparanin A, 200 mg), PS_3 (350 mg) and **2** (100 mg).

PS_1. Mp 240–243° (decomp.), $[\alpha]_D^{20} - 59°$ (MeOH–$CHCl_3$; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3450(OH), 983, 919, 898, 855 (intensity 919 > 898, 25*S* spiroketal). (Found: C, 68.75; H, 9.25. $C_{33}H_{54}O_8$ calc. C, 68.48; H, 9.40%.)

PS_3. Mp 252–257° (MeOH), $[\alpha]_D^{20} - 61.5°$ (MeOH–$CHCl_3$; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3390–3500 (OH), 983, 920, 900, 856 (intensity 920 > 900, 25*S* spiroketal). (Found: C, 64.40; H, 8.60. $C_{39}H_{64}O_{12}\cdot H_2O$ requires: C, 63.05, H, 8.95%.)

Permethylation of PS_1 and PS_3. PS_1 (50 mg) and PS_3 (100 mg) were permethylated as before. Methanolysis followed by hydrolysis of PS_1 permethylate as usual afforded 2, 3, 4, 6-tetra-*O*-methyl-D-glucose (PC, R_G = 1.0, authentic sample run in parallel).

PS_3-permethylate. Mp 85–88°, $[\alpha]_D^{20} - 60.5°$ ($CHCl_3$; *c* 1.05); IR ν_{max}^{KBr} cm^{-1}: no OH, 980, 918, 900, 854; EIMS (probe) 70 eV, *m/z*: 808 $[M]^+$, 603 [M − tri-*O*-methyl rhamnose + H]$^+$, 399 [M − hexa-*O*-methyl rhamnosyl glucose + H]$^+$, 189, 139, 111, 101, 55, 45. (Found: C, 66.45; H, 9.30. $C_{45}H_{66}O_{12}$ requires: C, 66.83; H, 9.40%.)

Methanolysis followed by hydrolysis of PS_3-permethylate. PS_3-permethylate (100 mg) was hydrolysed and worked-up as above. PC (solvent F) of the conc. hydrolysate showed 2, 3, 4-tri-*O*-methyl-L-rhamnose (R_G = 1.01) and 2, 3, 6-tri-*O*-methyl-D-glucose (R_G = 0.83). Its methanolysis and usual work-up as above afforded 2, 3, 6-tri-*O*-methyl-D-glucopyranoside, EIMS (probe) 70 eV, *m/z*: 205, 173, 145, 111, 101, 88, 75, 73, 57, 45.

Periodate oxidation of **2**. Compound **2** (25 ml) in H_2O (10 ml) was mixed with $NaIO_4$ (250 mg) and the soln was kept in the dark for 48 hr. Ethylene glycol (1 ml) was added to decompose excess $NaIO_4$ and the soln was hydrolysed with 10% MeOH–HCl (45 min). It was filtered, the filtrate neutralized, concd and examined by PC (solvent E) to detect D-glucose (R_f = 0.18).

Enzymatic hydrolysis of **3** *and* **4**. Compounds **3** and **4** (50 mg) were taken up in H_2O (20 ml) and incubated with β-glucosidase. Toluene (2 ml) was added and the soln was kept for 36 hr at room temp. Compound **2** and PS_3 were detected on TLC (solvent B) and PC showed D-glucose (solvent E).

CrO_3 *oxidation of* **3** *and* **4**. Compounds **3** and **4** acetates (1 g prepared as usual) were taken up in Ac_2O (20 ml), refluxed (1 hr), cooled and H_2O (10 ml) added. The mixture was dried under red. pres. and to the residue was added HOAc (15 ml) and NaOAc (250 mg). To the mixture at 15° was added CrO_3 (800 mg) in 50% HOAc (15 ml) over 15 min with continuous stirring for 2 hr. The reaction mixture was diluted with H_2O (50 ml) and extracted with Et_2O. The Et_2O extract was evaporated to dryness and the residue taken up in *t*-BuOH (25 ml) and KOH (1.5 g) in H_2O (15 ml) added. It was stirred at 30° for 4 hr under N_2, N_2O (20 ml) added, *t*-BuOH removed and extracted with *n*-BuOH.

3β-Hydroxy-5β-pregn-16-en-20-one acetate. The *n*-BuOH extract was concd to dryness and the residue purified by CC (solvent A). The purified glycoside thus obtained was hydrolysed by refluxing with 5% HCl–toluene (20 ml) for 4 hr. The reaction mixture was cooled and the toluene phase separated, evaporated and acetylated as usual to get a solid, IR ν_{max}^{KBr} cm^{-1}: 1724, 1662, 958, 920, 895, 820; EIMS (probe), 70 eV, *m/z*: 358 $[M]^+$; UV λ_{max} 239 nm.

δ-Hydroxy-γ-methyl-valeric acid methyl ester glucoside acetate. The above aq. phase was adjusted to pH 3 with 2 N HCl, and extracted alternately with *n*-BuOH and $CHCl_3$. The aq. phase was neutralized with 2 N NaOH and evaporated. The residue was acetylated, worked-up as usual and treated with CH_2N_2 (15 ml) for 15 min. The reaction mixture was evaporated to yield a syrup, EIMS (probe), 70 eV, *m/z*: 331, 243, 242, 200, 169, 157, 145, 141, 140, 129, 115, 109, 103, 98, 97, 45.

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