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STEROIDAL SAPONINS OF *ASPARAGUS CURILLUS*

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

Abstract—Three spirostanol and two furostanol glycosides were isolated from a methanol extract of the roots of *Asparagus curillus* and characterized as 3-*O*-[α-L-arabinopyranosyl (1→4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, 3-*O*-[{α-L-rhamnopyranosyl (1→2)} {α-L-arabinopyranosyl (1→4)}-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, 3-*O*-[{β-D-glucopyranosyl (1→2)} {α-L-arabinopyranosyl (1→4)}-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, 3-*O*-[{β-D-glucopyranosyl (1→2)} {α-L-arabinopyranosyl (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-arabinopyranosyl (1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-5β-furostan-3β, 22α, 26-triol respectively.

INTRODUCTION

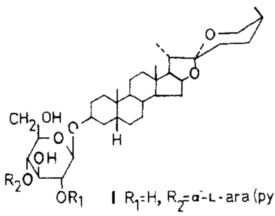
Asparagus plants are widely used in medicines [1]. Isolation and characterization of two new spirostanol glycosides and two furostanol glycosides (Oligofurostanosides) [2–5] have been reported [6] by us from the fruits of *Asparagus curillus*. Three new spirostanol glycosides and two new oligofurostanosides are now reported from a methanol extract of the roots of this plant.

RESULTS AND DISCUSSION

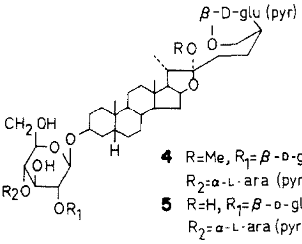
CC of the saponin mixture obtained from a methanol extract of the roots of this plant gave compounds **1–3** and a mixture of **4** and **5**. Compounds **1–3** were spirostanol glycosides (IR), and **4** and **5** were furostanol glycosides (positive to Ehrlich reagent [7, 8]). The exact configurations of the sugar linkages in compounds **1–3** were established by the application of Klyne's rule [9] which showed D-glucose-β, L-arabinose-α- and L-rhamnose-α-linkages. Acid hydrolysis of **1–3** gave sarsasapogenin and D-glucose and L-arabinose; **2** also gave L-rhamnose. The permethyl ether (**3a**) of **3** ($C_{44}H_{72}O_{17}$; mp 208–210°; $[\alpha]_D^{23} - 48°$, pyridine, $c = 0.5$), prepared by Hakomori's method [10], on methanolysis gave a mixture of methyl pyranosides of 2, 3, 4-tri-*O*-methyl-L-arabinose, 2, 3, 4, 6-tetra-*O*-methyl-D-glucose and 3, 6-di-*O*-methyl-D-glucose (GC). Hydrolysis of the above methyl pyranosides mixture showed corresponding methylated sugars (PC). 3, 6-Di-*O*-methyl-D-glucose was positive to Wallenfel's reagent [11]. The mass spectrum of **3a** showed peaks at *m*/*z* 998 $[M]^+$, 807 $[M - \text{tri-}O\text{-methyl glucose} + H]^+$, 763 $[M - \text{tetra-}O\text{-methyl glucose} + H]^+$, 219 and 175 (terminal glucose and arabinose units respectively). Methanolysis of the permethyl ether (**2a**) of **2** ($C_{44}H_{72}O_{16}$; mp 223–225°; $[\alpha]_D^{23} - 57°$, pyridine), gave a mixture of methyl pyranosides of 2, 3, 4-tri-*O*-methyl-L-rhamnose in addition to 2, 3, 4-tri-*O*-methyl-L-arabinose and 3, 6-di-*O*-methyl-D-glucose which are also obtained from methanolysis of **3a**. The mass spectrum of **2a** supported the above methanolysis studies. All the above studies indicated that in compounds **2** and **3** sarsasapogenin was glycosidated with C-1 of D-glucose which was further attached with a molecule of L-arabinose. Compounds **2** and **3** differed in the fact that another D-glucose formed a branching on the central D-glucose in **3**, whereas L-rhamnose caused branching in **2**.

To determine the exact sugar linkages, **2** and **3** were subjected to partial hydrolysis. Partial hydrolysis of **2** and **3** afforded the prosapogenins PS_1 and PS_2 (identical with saponin, **1**); **2** also gave PS_4 and **3** gave PS_3. Hydrolysis of PS_1 and PS_2 furnished sarsasapogenin and D-glucose; PS_2 also provided L-arabinose, Hydrolysis of the permethylethers of PS_1 and PS_2 afforded 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, and 2, 3, 4-tri-*O*-methyl-L-arabinose and 2, 3, 6-tri-*O*-methyl-D-glucose, respectively. This showed that saponin **1** has the structure 3-*O*-[α-L-arabinopyranosyl (1→4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, which was confirmed by mass spectral studies on its permethyl ether. PS_3 and PS_4 on hydrolysis furnished sarsasapogenin and D-glucose; PS_4 also gave L-rhamnose. Hydrolysis of PS_3 and PS_4 permethyl ethers provided 3, 4, 6-tri-*O*-methyl-D-glucose in addition to 2, 3, 4, 6-tetra-*O*-methyl-D-glucose from PS_3 permethylate and 2, 3, 4-tri-*O*-methyl-L-rhamnose from PS_4 permethylate. The mass spectral studies on PS_3 and PS_4 permethyl ethers also supported the methanolysis results. These acid hydrolysis studies on **2** and **3** showed that they were respectively the rhamnoside and glucoside of **1**.

From all the above studies **2** and **3** were characterized as 3-*O*-[{α-L-rhamnopyranosyl (1→2)} {α-L-arabinopyranosyl (1→4)}-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol and 3-*O*-[{β-D-glucopyranosyl (1→2)} {α-L-arabino-



1 R_1=H, R_2=α-L-ara (pyr)
2 R_1=α-L-rha (pyr)
R_2=α-L-ara (pyr)
3 R_1=β-D-glu (pyr)
R_2=α-L-ara (pyr)



4 R=Me, R_1=β-D-glu (pyr)
R_2=α-L-ara (pyr)
5 R=H, R_1=β-D-glu (pyr)
R_2=α-L-ara (pyr)

pyranosyl (1→4)} - β - D - glucopyranosyl] - (25*S*) - 5β - spirostan - 3β - ol, respectively.

Compounds **4** and **5** (Ehrlich reagent positive) could not be separated by CC and the ^{1}H NMR spectrum exhibited a methoxy signal at δ 3.25 which was not observed after refluxing with Me_2CO-H_2O. Refluxing with Me_2CO-H_2O, yielded a TLC homogeneous compound **5**, as an amorphous solid, $[\alpha]_D^{23}$ $-51°$ (H_2O, $c = 1.0$). By contrast refluxing the above mixture with dry MeOH afforded compound **4**, $[\alpha]_D^{23} - 47°$ (MeOH, $c = 0.5$). All these results, coupled with IR spectra and two-dimensional TLC results, confirmed these compounds to be oligofurostanosides [3–5], with **4** as 22-OMe and **5** as 22-OH derivatives. The furostanolic nature of **4** and **5** were further established by oxidative decomposition [12–14] of **4** and **5** to afford 3β - acetoxy - 5β - pregn - 16 - en - 20 - one (IR, MS and UV) and β - hydroxy - γ - methyl valeric acid-ester glucoside tetracetate, mass spectral peak at *m/z* 331 (tetra-*O*-acetyl glucopyranosyl ion) and other peaks in accordance with the expected pattern [17], which confirm that only one molecule of D-glucose was attached at position C-26. Enzymatic hydrolysis of **4** and **5** with β-glucosidase furnished two degradation products found to be identical with compounds **1** and **3**, which were formed by the cleaving of the terminal glucose molecules from C-26 as well as the main sugar chain. Therefore, compounds **4** and **5** were characterized as 3 - *O* - [{β - D - glucopyranosyl (1→2)} {α - L - arabinopyranosyl (1→4)} - β - D - glucopyransoyl] - 26 - *O* - [β - D - glucopyranosyl] - 22α - methoxy - (25*S*), 5β - furostan - 3β, 26 - diol and 3 - *O* - [{β - D - glucopyranosyl (1→2)} {α - L - arabinopyranosyl (1→4)} - β - D - glucopyranosyl] - 26 - *O* - [β - D - glucopyranosyl] - (25*S*), 5β - furostan - 3β, 22α, 26-triol, respectively.

EXPERIMENTAL

Mps (uncorr.) were determined in open capillaries in an electrothermal melting point apparatus. CC was carried out using Si gel (60–120 mesh, BDH) and TLC was on Si gel G (BDH). Elutions were carried out by different solvents in the order of increasing polarity and homogeneity of fractions was tested on TLC. Spots on TLC were visualized by 10% alcoholic H_2SO_4 and Ehrlich reagent, followed by heating and on PC (Whatman No. 1) by aniline hydrogen phthalate and triphenyl tetrazolium chloride (Wallenfel's reagent) by the descending method. 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 F, *n*-BuOH-HOAc-H_2O (4 : 1 : 5); solvent G, *n*-BuOH-EtOH-H_2O (5 : 1 : 4); solvent H, C_6H_6-Me_2CO (10 : 1). GC of methylated sugars: dual FID; column: 5% SE-30, 3 m × 2 mm, N_2 (40 ml/min), Programmed from 150 to 190° at 8°/min.

Isolation of saponins. The roots (10 kg) of *Asparagus curillus* Buch-Ham were collected from Pauri (U.P.) in December, dried, coarsely powdered and defatted with petrol in a Soxhlet. The recovered solvent-free powder was likewise extracted with MeOH until the extract became colourless. Removal of the solvent from the extract gave a brown residue which was purified as usual for the isolation of saponins. The purified mass (15 g) thus obtained was repeatedly chromatographed (solvent A) to give compound **1** (1 g), **2** (2.5 g), **3** (4 g) and a mixture of **4** and **5** (3.5 g).

Compound **1**. Crystallized from MeOH, mp 221–223°, $[\alpha]_D^{20} - 50°$ ($CHCl_3$-MeOH, $c = 0.8$); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 990, 920, 898, 850 (intensity 920 > 898, 25*S*-spiroketal). (Found: C, 64.50; H, 8.50. $C_{38}H_{62}O_2$ requires C, 64.23; H, 8.23%.)

Compound **2**. Mp 223–225°, $[\alpha]_D^{20} - 57°$ (pyridine, $c = 1.0$); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 988, 920, 898, 850 (intensity 920 > 898, 25*S*-spiroketal). (Found: C, 61.75; H, 8.50. $C_{44}H_{72}O_{16}$ requires C, 61.68; H, 8.41%.)

Compound **3**. Crystallized from MeOH as colourless needles, mp 205–210°, $[\alpha]_D^{20} - 49°$ ($CHCl_3$-MeOH, $c = 0.95$). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 980, 922, 900, 865 (intensity 922 > 900, 25*S*-spiroketal). (Found C, 60.15; H, 8.10 $C_{44}H_{72}O_{17}$ requires C, 60.55; H, 8.26%.)

Acid hydrolysis of the saponins **1**–**3**. The glycosides (100 mg, each) were separately hydrolysed by refluxing with 7% H_2SO_4 (20 ml) for 3 hr on a steam-bath to afford the aglycone (sarsasapogenin); colourless needles (EtOH), mp 198–199°, $[\alpha]_D^{20} - 74°$ ($CHCl_3$, $c = 1.0$) (lit. [16]: mp 199°, $[\alpha]_D^{20} - 75°$); IR ν_{max}^{KBr} cm^{-1}: 981, 920, 900, 855 (intensity 920 > 900, 25*S*-spiroketal); EIMS (probe) 70 eV, *m/z*; 416 $[M]^+$. (Found: C, 77.78; H, 10.75. Calc. for $C_{27}H_{44}O_3$: C, 77.84; H, 10.64%.) The neutralized (Ag_2CO_3) and concentrated aq. hydrolysates showed the presence of the following sugars. Compound **1**: L-arabinose and D-glucose; compound **2**: L-rhamnose, L-arabinose and D-glucose; compound **3**; L-arabinose and D-glucose [PC, solvent F, D-glucose (R_f 0.18), L-arabinose (R_f 0.21), L-rhamnose (R_f 0.37)].

Permethylation of **1**, **2** *and* **3**. Compound **1** (150 mg) and compounds **2** and **3** (300 mg, each) were separately permethylated by Hakomori's method to yield the permethylates **1a** (120 mg), **2a** (250 mg) and **3a** (270 mg) respectively. These were purified by CC (solvent C). **1a**: crystallized from Et_2O-petrol, mp 78–80°, $[\alpha]_D^{20} - 53°$ ($CHCl_3$, $c = 0.5$); IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 70 eV, *m/z* (rel. int.); 794 $[M]^+$ (0.1), 603 (0.3), 399 (50), 175 (85), 143 (100), 139 (40), 101 (55), 88 (80). (Found: C, 66.67; H, 9.40. $C_{44}H_{74}O_{12}$ requires C, 66.50; H, 9.32%.)

Methanolysis of **1a**. Compound **1a** (100 mg) in 1 N HCl-MeOH (10 ml) was refluxed (4 hr), neutralized (Ag_2CO_3), filtered and the filtrate was concentrated. It was subjected to prep. TLC using I_2 as visualizing agent (solvent H), to yield methyl - 2, 3, 6 - tri - *O* - methyl - D - glucopyranoside;

EIMS (probe) 70 eV, m/z (rel. int.): 205 (0.1), 173 (3.7), 145 (1.0), 111 (1.3), 101 (40), 88 (100), 75 (50), 73 (15), 57 (10), 45 (10). Hydrolysis of a portion of the above filtrate with 5% H_2SO_4 as usual, showed the presence of 2, 3, 6- tri - *O* - methyl - D - glucose (R_G 0.82) (PC, solvent G). Compound **3a**: mp 88–91° (Et_2O–petrol), $[\alpha]_D^{20} - 56°$ ($CHCl_3$, $c = 1.0$). IR ν_{max}^{KBr}: no OH; EIMS (probe) 70 eV, m/z (rel. int.): 998 $[M]^+$ (0.05), 807 (0.1), 777 (0.05), 763 (0.1), 219 (30), 187 (100), 175 (40), 143 (35), 139 (25), 111 (55), 88 (40), 75 (25), 45 (13). (Found: C, 63.65; H, 9.20. $C_{53}H_{90}O_{17}$ requires C, 63.73; H, 9.08%.)

Methanolysis of **3a**. Compound **3a** (150 mg) on methanolysis and usual work-up was subjected to GLC; RR_t (min): 2.72 (methyl - 2, 3, 4 - tri - *O* - methyl - L - arabinopyranoside), 3.23 (methyl - 2, 3, 4, 6 - tetra - *O* - methyl - D - glucopyranoside) and 3.69 (methyl - 3, 6 - di - *O* - methyl - D - glucopyranoside). Usual hydrolysis of the above mixture of methyl pyranosides of methyl sugars showed the following sugars: 2, 3, 4, 6 - tetra - *O* - methyl - D - glucose (R_G 1.0), 2, 3, 4 - tri - *O* - methyl - L - arabinose (R_G 0.76) and 3, 6 - di - *O* - methyl - D - glucose (R_G 0.51, pink colour with Wallenfel's reagent) (PC, solvent G). Compound **2a**: mp 90–93° (Et_2O–petrol), $[\alpha]_D^{20} - 61.5°$ ($CHCl_3$, $c = 1.0$); IR ν_{max}^{KBr}: no OH; EIMS (probe) 70 eV, m/z (rel. int.): 968 $[M]^+$, 777 (0.07), 763 (0.04), 399 (50), 189 (100), 175 (45), 143 (50), 101 (44), 88 (85), 75 (40). (Found: C, 64.40; H, 9.0 $C_{52}H_{88}O_{16}$ requires C, 64.46; H, 9.09%.)

Methanolysis of **2a**. Compound **2a** (150 mg) on methanolysis and usual work-up was examined by GLC; RR_t (min): 1.90 (methyl - 2, 3, 4 - tri - *O* - methyl - L - rhamnopyranoside), 2.72 (methyl - 2, 3, 4 - tri - *O* - methyl - L - arabinopyranoside) and 3.69 (3, 6 - di - *O* - methyl - D - glucopyranoside).

Partial hydrolysis of **3**. Compound **3** (2 g) and 1% H_2SO_4 in MeOH (150 ml) were kept for 4 days at room temp. MeOH was removed and H_2O (25 ml) was added. The aq. soln was extracted with *n*-BuOH, concentrated and chromatographed (solvent A) whereupon sarsasapogenin (50 mg) and three prosapogenins PS_1 (200 mg), PS_2 (250 mg) and PS_3 (300 mg) were obtained.

PS_1 permethylate. Obtained as above. On hydrolysis it released 2, 3, 4, 6 - tetra - *O* - methyl - D - glucose (R_G 1.0, solvent G).

PS_3 permethylate. Obtained as above, mp 87–89° (MeOH–H_2O) $[\alpha]_D^{20} - 68.1°$ ($CHCl_3$, $c = 1.0$); IR ν_{max}^{KBr}: no OH; EIMS (probe) 70 eV, m/z (rel. int.): 838 $[M]^+$ (0.03), 603 (0.1), 399 (55), 219 (28), 187 (100), 139 (30), 101 (45), 88 (85), 75 (30). (Found: C, 65.50; H, 9.80. $C_{46}H_{78}O_{13}$ requires: C, 65.87; H, 9.31%.) On hydrolysis it released 2, 3, 4, 6 - tetra - *O* - methyl - D - glucose (R_G 1.0), and 3, 4, 6 - tri - *O* - methyl - D - glucose (R_G 0.84; pink colour with Wallenfel's reagent) (PC, solvent G).

Partial hydrolysis of **2**. Compound **2** (1 g) on partial hydrolysis as above afforded sarsasapogenin (30 mg), PS_1 (150 mg), PS_2 (200 mg) and PS_4 (300 mg).

PS_4 permethylate. PS_4 (150 mg) was permethylated and worked up as above. Mp 82–84°; $[\alpha]_D^{20} - 60.5°$ ($CHCl_3$, $c = 1.0$); IR ν_{max}^{KBr}: no OH; EIMS (probe) 70 eV, m/z (rel. int.): 808 $[M]^+$ (0.05), 603 (0.07), 399 (50), 189 (100), 139 (28), 101 (48). (Found: C, 66.81; H, 9.30. $C_{45}H_{76}O_{12}$ requires: C, 66.83; H, 9.41%.) On hydrolysis it released 2, 3, 4 - tri - *O* - methyl - L - rhamnose (R_G 1.01) and 3, 4, 6 - tri - *O* - methyl - D - glucose (R_G 0.84, pink colour with Wallenfel's reagent) (PC, solvent G).

Compound **4**. The mixture of **4** and **5** (150 mg) was refluxed with dry MeOH (50 ml) for 6 hr, the soln concentrated and cooled to get TLC homogeneous (solvent A), amorphous solid (could not be crystallized), $[\alpha]_D^{20} - 47°$ (MeOH, $c = 0.5$) IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions; ^{1}H NMR (C_5D_5N); δ 3.25 (3H, C-22-OMe), 4.75 (1H, *d*, $J = 7.3$ Hz), 5.00 (1H, *d*, $J = 7.0$ Hz) 6.65 (1H, *br s*). (Found: C, 57.20; H, 7.95. $C_{51}H_{86}O_{23}$ requires: C, 57.41; H, 8.07%.)

Compound **5**. Compounds **4** and **5** (175 mg) were refluxed with aq. Me_2CO (1 : 2, 15 ml) for 10 hr, concentrated and cooled to give TLC homogeneous compound **5** (solvent A) as an amorphous solid, $[\alpha]_D^{20} - 51°$ (H_2O, $c = 1.0$); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), no spiroketal absorptions. (Found: C, 56.95; H, 8.05. $C_{50}H_{84}O_{23}$ requires C, 57.03; H, 7.98%.)

Oxidative decomposition of **4** *and* **5**. Compounds **4**- and **5**-acetates (1 g) in Ac_2O (15 ml) was refluxed for 1 hr. After cooling, H_2O (10 ml) was added to the reaction mixture which was then evapd to dryness to give a syrup. To this was added HOAc (15 ml) and NaOAc (300 mg). The mixture was cooled to 15° and CrO_3 (500 mg) in 50% HOAc (5 ml) was added during 15 min with continuous stirring which was continued for 1 hr at room temp. The mixture was then diluted with H_2O (50 ml) and extracted with Et_2O. The Et_2O extract was washed with H_2O before the extract was dried and then evapd. To the residue (1 g), in *t*-BuOH (25 ml) was added KOH (1.5 g) in H_2O (5 ml) and stirred at 30° for 4 hr under N_2. Afterwards H_2O (25 ml) was added, *t*-BuOH was removed and extracted with *n*-BuOH.

5β-Pregn-16-en-3-ol-20-one acetate. The *n*-BuOH extract was evapd and the residue was purified by CC (solvent A). The TLC pure glycoside was hydrolysed by refluxing with 1N HCl–toluene (25 ml) for 3 hr. After cooling the toluene phase was separated, evapd and the acetate of the product was prepared as usual, purified by CC (solvent D) to get a solid, IR ν_{max}^{KBr} cm^{-1}: 1724, 1662 (characteristic of Δ^{16}-20 ketone [15], 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. The aq. phase adjusted at pH 3 with 2 N HCl was extracted with *n*-BuOH and $CHCl_3$ alternatively. The aq. phase was then neutralized with 2 N NaOH and evapd. The residue was acetylated (Ac_2O–pyridine, 1 : 1) and worked up as usual and treated with CH_2N_2 soln (15 ml) for 15 min. The reaction mixture was evapd to give a syrup purified by CC (solvent C) to afford a TLC pure compound (as a colourless syrup), EIMS (probe) 70 eV, m/z (rel. int.): 331 (0.7), 242 (0.8), 200 (1.0), 169 (13.0), 145 (1.0), 129 (77), 115 (32), 97 (1.5).

Enzymatic hydrolysis of **4** *and* **5**. The mixture of **4** and **5** (50 mg) in H_2O (5 ml) was incubated with β-glucosidase (Sigma) at 37° for 7 hr. The hydrolysate on usual work-up for saponins, afforded compounds **1** and **3**. The aq. phase showed the presence of D-glucose (PC, solvent F, R_f 0.18).

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