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OLIGOFURO- AND SPIRO-STANOSIDES OF *ASPARAGUS ADSCENDENS*

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Key Word Index—*Asparagus adscendens*; Liliaceae; saponins; oligofurostanosides; spirostanosides.

Abstract—Two oligofurostanosides and two spirostanosides, isolated from a methanol extract of *Asparagus adscendens* (leaves), were characterized as 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-22α-methoxy-(25*S*)-furost-5-en-3β,26-diol (Adscendoside A), 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-furost-5-en-3β,22α,26-triol-(Adscendoside B), 3-*O*-[{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-(25*S*)-spirostan-5-en-3β-ol (Adscendin A) and 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-(25*S*)-spirostan-5-en-3β-ol (Adscendin B), respectively. Adscendin B and Adscendoside A are the artefacts of Adscendoside B formed through hydrolysis and methanol extraction respectively.

INTRODUCTION

Asparagus plants are widely used in medicine [1]. Isolation and characterization of four new spirostanosides and four oligofurostanosides from the fruits [2] and roots [3] of *Asparagus adscendens* have been published. Two new oligofurostanosides Adscendosides A (**3**) and B (**4**) and two spirostanosides Adscendins A (**1**) and B (**2**) are reported here from the methanol extract of the leaves of this plant.

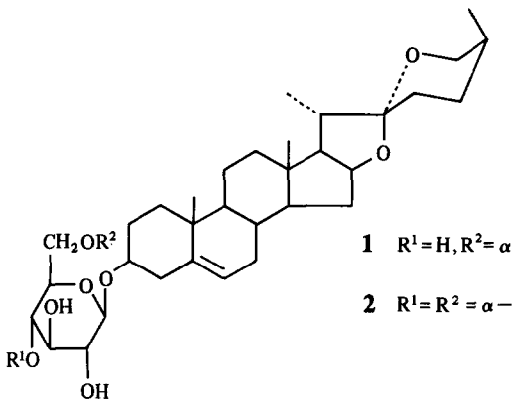
RESULTS AND DISCUSSION

Chromatography of the saponin mixture obtained from the methanol extract of the leaves of *A. adscendens* afforded **1**, **2** and an inseparable mixture of **3** and **4**. Compounds **1** and **2** gave all the usual tests and IR data for unsaturated spirostanosides, while compounds **3** and **4** gave an intense red colour with the Ehrlich reagent [4, 5] and other characteristic tests of oligofurostanosides.

The mixture of **3** and **4** on refluxing with dioxane–water was converted to TLC homogeneous compound **4**, $[\alpha]_D$ −92° (H_2O). By contrast, refluxing in dry methanol furnished TLC pure, non-crystallizable, compound **3**, $[\alpha]_D$ −89° (MeOH), which exhibited a methoxy signal at δ 3.25 in its ^1H NMR spectrum. Such transformations are known [6, 7] in oligofurostanosides. Therefore, the mixture of **3** and **4** was used as such for all studies. Enzymatic hydrolysis of **3** and **4** with β-glucosidase gave a saponin (identical by mmp and co-TLC with **2**) and D-glucose. Thus, to determine the structures of **3** and **4** the structure of their artefact **2** was determined first.

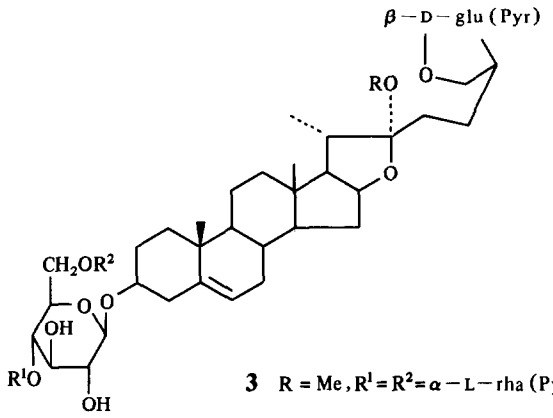
On acid hydrolysis **2** gave yamogenin (comparison of physical data with lit., IR and MS), D-glucose and L-rhamnose (1:2, GC). The permethylate **2a** of **2**, $C_{45}H_{72}O_{16}$, mp 244–246°, $[\alpha]_D^{20}$ −95° (prepared by Hakomori's method [8]) on methanolysis gave a mixture of methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3-di-*O*-methyl-glucose (2:1, GC) which showed that two rhamnose molecules are attached at two different carbon atoms (C-4 and C-6) of the glucose, whose C-1 is linked with C-3 of yamogenin. The identity of 2,3-di-*O*-methyl-D-glucose was further confirmed by its negative Wallenfels' reagent test [9] and resistance to periodate oxidation. The mass spectrum of **2a** showed peaks at 980 $[M]^+$, 775 $[M - \text{tri-}O\text{-methyl rhamnose} + H]^+$, 323 [rhamnose unit at C-6 of D-glucose [10]], 189 [terminal rhamnose]. These conclusions were also supported by the partial hydrolysis of **2** to isolate three prosapogenins PS_1, PS_2 (identical by mmp and co-TLC with **1**) and PS_3. Hydrolysis of PS_1, PS_2 and PS_3 furnished yamogenin and D-glucose; PS_2 and PS_3 also provided L-rhamnose. Hydrolysis of PS_2 permethyl ether (**1a**) afforded 2,3,4-tri-*O*-methyl-rhamnose and 2,3,4-tri-*O*-methyl-D-glucose (negative to Wallenfels' reagent). PS_3 permethyl ether on hydrolysis gave 2,3,4-tri-*O*-methyl-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose. The identity of the latter sugar was confirmed by direct comparison (co-PC) with an authentic sample [7]. The exact configurations at the glycosidic points in all the compounds were established by Klyne's rule [11]. The Field Desorption mass spectrum (FDMS) of **2** fully corraborated the above conclusions through the following peaks at *m/z* 907 $[M + K]^+$, i.e. $M^+ = 868$ [413 + 455], cationized cluster ion peak 891 $[M + Na]^+$ (base peak), 745 $[(M + Na + H) - 147]^+$, 761 $[(M + K + H) - 147]^+$, 614 $[(M + K + H) - 294]^+$ and 598 $[(M + Na + H) - 294]^+$, 575 $[(M + H) - 2 \text{ rhamnose}]^+$. The loss of two units of mass 147 and formation of ion peaks at *m/z* 745 and 598 clearly established the presence of two rhamnose units at the end of the sugar-chain and the ion peak at *m/z* 575 confirmed that one glucose molecule was attached to yamogenin. This data also confirmed the purity and molecular weight of this compound. From all these studies **1** and **2** were assigned the structures 3-*O*-[{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-(25*S*)-spirostan-5-en-3β-ol and 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-(25*S*)-spirostan-5-en-3β-ol, respectively.

In order to ascertain how many glucose molecules are removed in the transformation of **3** and **4** into **2**, the



1 $R^1 = H, R^2 = \alpha - L - rha(Pyr)$

2 $R^1 = R^2 = \alpha - L - rha(Pyr)$



3 $R = Me, R^1 = R^2 = \alpha - L - rha(Pyr)$

4 $R = H, R^1 = R^2 = \alpha - L - rha(Pyr)$

mixture of **3** and **4** was permethylated and methanolysed to yield the methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose (2:1:1, GC). These results showed the appearance of only one additional molecule of 2,3,4,6-tetra-*O*-methyl-D-glucose as compared to the methanolysis results of **2a**. This implies that the sugar chain present in **2** at C-3 of yamogenin is intact in both these compounds and the additional 2,3,4,6-tetra-*O*-methyl-D-glucose molecule arises from a glucose attached at C-26 of the furostane skeleton. This fact was further supported by oxidative degradation [7] of **3** and **4** to afford 3β-acetoxypregn-5,16-dien-20-one (IR, MS) and δ-hydroxy-γ-methyl-valeric acid-ester glucoside-tetraacetate, *m/z* 331 (tetra-*O*-acetyl glucopyranosyl ion) and other peaks in accordance with the expected pattern [12]. Its identity was also confirmed by direct comparison (co-TLC) with an authentic sample [7]. Therefore, **3** and **4** were assigned the structures 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-22α-methoxy-(25*S*)-furost-5-en-3β,26-diol, and 3-*O*-[{α-L-rhamnopyranosyl (1 → 4)}{α-L-rhamnopyranosyl (1 → 6)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-furost-5-en-3β-22α,26-triol, respectively.

EXPERIMENTAL

All mps are uncorr. CC was carried out on silica gel (60–120 mesh) and TLC on silica 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 the descending method and aniline hydrogen phthalate as visualizer. 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). GC of methyl methylated sugars: succinate polyester (10% H.P.) column, N_2, 30 ml/min. Oven temp. 175°.

Isolation of saponins. Asparagus adscendens Roxb. leaves (2 kg) were collected from Bilaspur (H.P.) in August, dried, coarsely powdered and defatted with petrol in a Soxhlet and solvent free powder was then exhaustively extracted with 90% aq. MeOH. Evaporation of the solvent under red. pres. gave a crude saponin mixture (14.0 g) which was purified as usual for the isolation of saponins. The saponin mixture, thus obtained, was chromatographed (solvent A) to afford Adscendin A (**1**, 800 mg), Adscendin B (**2**, 3 g) and a mixture of Adscendosides A and B (**3** and **4**, 4 g).

Compound **1**. Mp 219–221°, $[\alpha]_D^{20} - 102°$ (pyridine, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 915, 896 and 850 (intensity 915 > 896, 25*S*-spiroketal). (Found: C, 64.95; H, 8.30. $C_{39}H_{62}O_{12}$ requires: C, 64.82; H, 8.59%.)

Compound **2**. Colourless needles from MeOH, mp 244–246°, $[\alpha]_D^{20} - 95°$ (pyridine, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 915, 896, 850 (intensity 915 > 896, 25*S*-spiroketal); ^1H NMR (C_5D_5N): δ 4.82 (1H, *d*, *J* = 6 Hz), 4.98 (1H, *br s*), 5.23 (1H, *br s*). (Found: C, 61.85; H, 8.01. $C_{45}H_{72}O_{16}$ requires C, 62.21; H, 8.29%.)

Compound **3**. The mixture of **3** and **4** (1 g) was refluxed with dry MeOH (100 ml) for 6 hr. The soln was concd and cooled to afford **3**, TLC homogeneous (solvent B) amorphous solid, $[\alpha]_D^{20} - 89°$ (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* = 6 Hz) 4.98 (1H, *br s*), 5.10 (1H, *d*, *J* = 6 Hz), 5.25 (1H, *br s*).

(Found: C, 58.19; H, 7.75; $C_{52}H_{86}O_{22}$ requires: C, 58.85; H, 8.09%.)

Compound **4**. The mixture of **3** and **4** (900 mg) was refluxed with dioxane–H_2O (1:1, 50 ml) for 10 hr, concd, cooled to give TLC homogeneous compound **4** (solvent B) as an amorphous solid (not crystallizable), $[\alpha]_D^{20} - 92°$ (H_2O, *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH) no spiroketal absorptions. (Found: C, 57.25; H, 8.30; $C_{51}H_{84}O_{22}$ requires C, 58.39; H, 8.01%.)

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** was detected on TLC (solvent B) and PC showed D-glucose (solvent E).

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 2 hr, cooled and filtered to afford the aglycone (yamogenin); colourless needles (MeOH), mp 200–202°, $[\alpha]_D^{20} - 126°$ ($CHCl_3$; *c* 1.0); lit. [13, 14] mp 201°, $[\alpha]_D^{20} - 129°$; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 915, 896, 850 (intensity 915 > 896, 25*S*-spiroketal); EIMS (probe) 70 eV, *m/z*; 414 $[M^+]$, 355, 345, 342, 300, 285, 271, 139 (base peak). (Found: C, 78.19; H, 10.11; $C_{27}H_{42}O_3$ calc. for C, 78.26; H, 10.14%.) The neutralized (Ag_2CO_3) and concd aq. hydrolysate of **1** and **2** showed D-glucose and L-rhamnose (PC, solvent E, R_fs 0.18 and 0.37, respectively).

Permethylation of **1**, **2** *and a mixture of* **3** *and* **4**. Compounds **1** (350 mg), **2** (500 mg), **3** and **4** (500 mg) were separately permethylated by Hakomori's method to yield their permethylates (**1a**, **2a**, **3a** and **4a** respectively), which were purified by CC (solvent D).

Compound **1a**. Mp 58–59°, $[\alpha]_D^{18} - 97°$ ($CHCl_3$; *c* 1.3); IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 70 eV, *m/z*: 806 $[M]^+$, 601 $[M - \text{tri-}O\text{-methyl-rhamnose} + H]^+$, 397, 323, 189, 157, 139, 101, 88, 75, 71, 55, 45. (Found: C, 66.96; H, 9.12; $C_{45}H_{74}O_{12}$ requires C, 66.99; H, 9.18%.)

Methanolysis of **1a**. Compound **1a** (100 mg) in 1 N HCl–MeOH (1:1, 15 ml) was refluxed (4 hr), neutralized, filtered and the filtrate after concn was subjected to GC; RR_t (min): 0.80 (2,3,4-tri-*O*-methyl-L-rhamnopyranoside and 0.90 (2,3,4-tri-*O*-methyl-D-glucopyranoside), whose hydrolysis afforded 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-D-glucose (PC, solvent F, R_G values 1.01 and 0.85 respectively). Both these methylated sugars were negative to Wallenfels' reagent test.

Compound **2a**. Mp 81–83°, $[\alpha]_D^{20} - 93°$ ($CHCl_3$; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 70 eV, *m/z*: 980 $[M]^+$, 948 $[M - MeOH]^+$, 775 $[M - \text{tri-}O\text{-methyl-L-rhamnose} + H]^+$, 397, 323, 189 (terminal rhamnose) 157, 139, 101, 88. (Found: C, 64.75; H, 8.57; $C_{53}H_{88}O_{16}$ requires: C, 64.89; H, 8.98%.)

Methanolysis of **2a**. Compound **2a** (100 mg) was methanolysed and the products examined by GC to yield the methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose (RR_t 0.8 min) and 2,3-di-*O*-methyl-D-glucose (RR_t 1.37 min) as a 2:1 mixture, which on hydrolysis liberated sugars identified by PC (solvent F): R_G 1.01 (2,3,4-tri-*O*-methyl-L-rhamnose), 0.57 (2,3-di-*O*-methyl-D-glucose; no pink colour with Wallenfels' reagent). $NaIO_4$ oxidation (as above) of the above mixture of methylated sugars did not show the disappearance of any of the spots previously present.

Partial hydrolysis of **2**. A soln of **2** (1.5 g) in 5% aq. HCl was refluxed for 30 min, concd under red. pres., H_2O (30 ml) added and the ppt removed by filtration. The aq. filtrate was neutralized with 5% aq. KOH, extracted with *n*-BuOH (50 ml, × 2) and the *n*-butanol removed from the extract under vacuum. The resulting residue and the above ppt were together subjected to CC (solvent A) to yield yamogenin (50 mg), PS_1 (100 mg), PS_2 (250 mg), PS_3 (200 mg) and **2** (100 mg).

Permethylation of PS_1 *and* PS_3. PS_1 (50 mg) and PS_3 (100 mg) were permethylated, methanolysed and hydrolysed. 2,3,4,6-Tetra-*O*-methyl-D-glucose was obtained from PS_1 (co-PC, R_G = 1.0, solvent F); 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) were obtained from PS_3.

FDMS of **2**. *m/z*: 907 $[M+K]^+$ i.e., M^+ = 868 [413 + 455], 891 $[M + Na]^+$ (base peak), 745 $[(M + Na + H) - 147]^+$, 761 $[(M + K + H) - 147]^+$, 614 $[(M + K + H) - 294]^+$ and 598 $[(M + Na + H) - 294]^+$, 575 $[(M + H) - 294]^+$.

Periodate oxidation of **2**. Compound **2** (25 mg) 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), which did not show the presence of any monosaccharide.

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 were 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, 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, H_2O (20 ml) added, *t*-BuOH removed and extracted with *n*-BuOH.

3β-Acetoxy-pregn-5,16-dien-20-one. The *n*-BuOH extract was concd to dryness and the residue purified by CC (solvent A). The purified glycoside 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 yield a solid; IR ν_{max}^{KBr} cm^{-1}: 1724, 1662, 958, 920, 895, 820; EIMS (probe), 70 eV, *m/z*: 356 $[M]^+$; UV λ_{max}: 239 nm.

δ-Hydroxy-γ-methyl-valeric acid methyl ester-glucoside tetra acetate. The above aq. phase was adjusted to pH 3 with 2 N HCl, and extracted alternatively 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.

Methanolysis of **3a** *and* **4a**. The mixture of **3a** and **4a** (100 mg) was methanolysed and worked up as usual to afford a mixture of methylpyranosides of the following sugars: GC, RR_t (min): 0.80 (2,3,4-tri-*O*-methyl-L-rhamnose), 0.83 (2,3,4,6-tetra-*O*-methyl-D-glucose) and 1.37 (2,3-di-*O*-methyl-D-glucose) in the ratio 2:1:1.

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