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SPIROSTANOL GLYCOSIDE FROM FRUITS OF *ASPARAGUS OFFICINALIS*

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Key Word Index—*Asparagus officinalis*; Liliaceae; spirostanol glycoside; ^{13}C NMR–DEPT mode; 2D–heteronuclear shift correlation NMR spectrum; spermicidal potential.

Abstract—A spirostanol glycoside was isolated, together with some known compounds, from the methanolic extract of the fruits of *Asparagus officinalis* and characterized by chemical and spectral methods including ^{13}C NMR–DEPT and 2D–hetcor NMR spectra. The spirostanol glycoside caused 100% immobilization of human spermatozoa at 1.5% level.

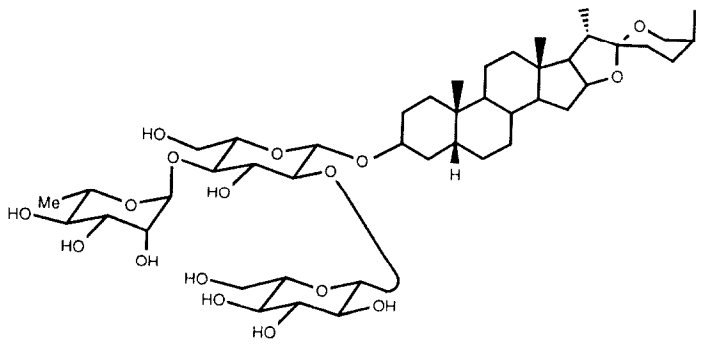
INTRODUCTION

Saponins have been described to have spermicidal potential [1]. In our search for other glycosides with this activity, we analysed the hitherto unexplored methanolic extract of the fruits of *Asparagus officinalis* to characterize a new spirostanol glycoside (**1**). It caused 100% immobilization of human spermatozoa when tested by established methods [2, 3] at 1.5% level. Previously a few sarsasapogenin glycosides have been reported from other parts of this plant [4, 5].

RESULTS AND DISCUSSION

Solvent partitions and CC of the methanolic extract of fruits gave a LB positive spirostanol glycoside **1** belonging to the (25*S*)-series (IR). FAB–mass spectrometry showed that compound **1** had an $[M+H]^+$ ion at *m/z* 887. Peaks at *m/z* 741 and 725 arose by the loss of deoxyhexose and hexose units, respectively, from the $[M+H]^+$ ion. Peaks at *m/z* 417 and 399 were suggestive of a saturated monohydroxy spirostane nucleus.

Acidic hydrolysis of **1** gave a genin, identified as sarsasapogenin [6], and glucose and rhamnose in a 2:1 ratio. The ^{13}C NMR spectrum of **1** (data in Experimental section) provided information about the points of attachment in the saccharide part. DEPT experiments with different values of θ and a linear combination of these spectra [7] led to a clean separation of all the 18 sugar CH, CH_2 and CH_3 signals. The presence of an upfield anomeric signal at δ 99.74 (C-1 of glucose substituted at C-2), two signals at 59.95 and 61.10 (both C-6 of two glucoses), the absence of a downfield signal [$\sim$ 84–86 ppm (C-3 OH of glucose glycosylated)] and the presence of C-2 and C-4 of glucose at 81.26 and 75.79 (downfield by $\sim$ 6 and 4.5 ppm, respectively [8]) eliminated C-3 and C-6, but established C-2 and C-4 of the inner glucose as the points of glycosidic linkages. These results were further supported by the permethylation studies of **1** and its partial products PS_1 and PS_2. Hydrolysis of **1a**, the permethylate of **1**, gave Wallenfels positive [9] 3,6-di-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,-tri-*O*-methyl-L-rhamnose [10]. A 2D heteronuclear shift correlation [7] carried out on **1**, differentiated between



1

C– and O– bound protons in the 1D ^{1}H NMR spectrum. δ values 100.18, 103.44 and 99.74 in F_2 domain (^{13}C) correlated with δ 4.70, 4.42 and 4.39 in F_1 domain (^{1}H) corresponding to the singlet and doublets of α–linked rhamnose and β–linked glucoses, respectively. Long-range heteronuclear shift correlation showed the linkages to be β-D-glucopyranosyl (1→2) and α-L-rhamnopyranosyl (1→4).

Thus, **1** was characterized as 3-*O*-{[α-L-rhamnopyranosyl (1→4)] [β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl}-(25*S*)-5β-spirostan-3β-ol.

EXPERIMENTAL

Mps: uncorr. FABMS: Kratos MS 50 instrument at 8 kV accelerating voltage. The atom beam was provided with an ion Tech FAB gun operating with Xe at 8 kV with a current of 30–40 μA; NMR spectra were recorded at 300 MHz, in DMSO with TMS as int. standard; CC: silica gel (BDH, 60–120 mesh); TLC: Kieselgel 60 G (Merck). The spots on TLC were visualized by spraying with 10% H_2SO_4 and in preparative work by H_2O; PC: Whatman no. 1 paper using a descending mode and aniline hydrogen phthalate as developer. Colorimetric estimation [11]. The following solvent systems were used: (A) $CHCl_3$–MeOH (9:1); (B) $CHCl_3$–MeOH–H_2O (13:3:2); (C) petrol (60–80)°–EtOAc (4:1); (D) C_6H_6–Me_2CO (4:1); (E) EtOAc–C_5H_5N–H_2O (10:4:3) and (F) *n*-BuOH–EtOH–H_2O (5:1:4).

Extraction and fractionation of the extract. Fruits of *Asparagus officinalis* (250 g), collected from Srinagar-Garhwal, U.P., were extracted with MeOH (4 × 1 l). The MeOH free residue was boiled with petrol (60–80)° and the soluble portion was concd. The petrol-insoluble residue was partitioned between *n*-BuOH and H_2O (1:1, 1 l).

Analysis of petrol soluble residue. This residue (9 g) was chromatographed over silica gel (solvent C) to isolate sitosterol [12] yamogenin [10] and another steroidal genin, mp 197–199°, $[\alpha]_D^{25}$ −76° ($CHCl_3$; *c* 1.5); IR ν_{max}^{KBr} cm^{-1}: 980, 920, 900, 855 (intensity 920 > 900, 25*S*–spiroketal); EIMS (probe) 70 eV, *m/z*: 416 [M]$^+$, 399, 139 (base peak), identified as sarsasapogenin.

Analysis of n-BuOH soluble residue. This material (22 g) on CC over silica gel (solvent A) gave sitosterol-β-D-glucoside [12] and compound **1** (200 mg).

Compound **1**. Colourless flakes from MeOH, mp 290–293°, $[\alpha]_D^{25}$ −66.0° (C_5H_5N; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 981, 922, 899, 855 (intensity 922 > 899, 25*S*-spiroketal). (Found: C, 59.98; H, 8.66. $C_{45}H_{74}O_{17}$ requires C, 60.92; H, 8.35%). ^{1}H NMR: δ 4.39 (1H, *d*, *J* = 7.5 Hz, H-1 of glu), 4.42 (1H, *d*, *J* = 7.3 Hz, H-1 of glu'), 4.70 (1H, *s*, H-1 of rha); full ^{13}C and ^{1}H assignments and experimental details are given in ref. [13].

Acidic hydrolysis of **1**. Compound **1** (30 mg) was refluxed with 2 M HCl–MeOH (1:1, 18 ml) on a boiling H_2O bath to afford the aglycone identified as sarsasapogenin as above. The neutralized (Ag_2CO_3) and concd hydrolysate showed the presence of rhamnose and glucose (R_f values 0.52 and 0.23, respectively, PC, solvent E).

Partial hydrolysis of **1**. Compound **1** (70 mg) was heated with 1 M HCl–*n*-BuOH (1:1, 18 ml) at 70° for 1 hr. The BuOH layer was washed with 5% $NaHCO_3$ and then with H_2O and conc *in vacuo* to afford a residue which was purified by prep. TLC (solvent B) to give sarsasapogenin (2 mg), PS_1 (20 mg) and PS_2 (16 mg).

Acidic hydrolysis of PS_1 *and* PS_2. Carried out as described for **1**.

Permethylation of **1**, PS_1 *and* PS_2. Compound **1** (15 mg), PS_1 and PS_2 (12 mg each) were separately permethylated with MeI (4 ml), Ag_2O (100 mg) in DMF (0.5 ml). Usual work-up gave syrups which were purified by prep. TLC (solvent D) to yield the permethylates **1a** (10 mg), PS_1a and PS_2a (8 mg each).

Hydrolysis of **1a**, PS_1*a and* PS_2*a*. Compounds **1a**, PS_1a and PS_2a (7 mg each) were separately refluxed with 1 M HCl–MeOH (1:1, 5 ml) for 2.5 hr. The neutralized and concd hydrolysate from **1a** contained Wallenfels positive 3,6-di-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose (R_G values 0.51, 1.0, 1.01, respectively, PC, solvent F). PS_1 a hydrolysate showed the last of these and a tri-*O*-methyl-D-glucose (R_G 0.84) whereas PS_2a gave tetramethyl-D-glucose and a tri-*O*-methyl-D-glucose (R_G 0.83).

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