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SPIROSTANOL GLYCOSIDES FROM *ASPARAGUS PLUMOSUS*

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

Abstract—Three spirostanol glycosides were isolated from a methanol extract of the leaves of *Asparagus plumosus* and characterized.

INTRODUCTION

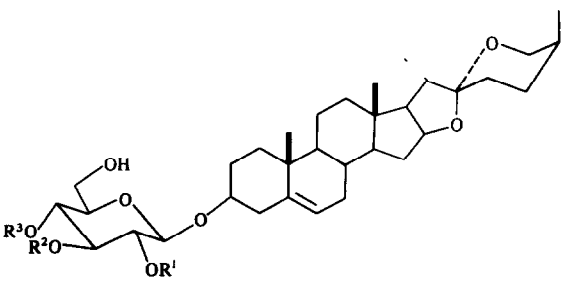
Asparagus plants find a wide use in medicine [1, 2]. The isolation and characterization of three new yamogenin-based glycosides from a methanol extract of the leaves of *Asparagus plumosus* are reported here.

RESULTS AND DISCUSSION

Column chromatography of the saponin mixture from a methanol extract of the leaves of *A. plumosus* gave compounds **1**, **2** and **3**, which were unsaturated spirostanol glycosides (IR). The results of field desorption (FD) mass spectrometry of underivatized **1**, **2** and **3** and of fast atom bombardment (FAB) mass spectrometry of **2** are presented in Table 1 and Fig. 1. The MW of **3** was 884, as concluded from the peaks at *m/z* 923 $[M+K]^+$, 907 $[M+Na]^+$ and 885 $[M+H]^+$. The peaks at *m/z* 739 $[M+H-146]^+$ and 723 $[M+H-162]^+$ correspond to the loss of terminal deoxyhexose (rhamnose) and hexose (glucose) units, respectively, whereas the peak at *m/z* 577 arises from the further loss of hexose from the former and deoxyhexose from the latter. The peak at *m/z* 415 is assigned to the mass of the protonated aglycone, thus indicating the sequence of sugars to be $\begin{matrix}\text{rha}\\ \text{glu}\end{matrix}\!\!>$glu–aglycone. Similarly, the cation complexes of **1** and **2** indicated their MWs to be 722 and 868, respectively. These spectra established the sequence of sugars in **1** and **2** to be rha–glu–aglycone and$(\text{rha})_2$glu–aglycone, respectively. The FAB mass spectra also supported these conclusions.

Hydrolysis of **1–3** with aqueous hydrochloric acid-dioxane gave yamogenin whereas with boiling ethanolic hydrochloric acid diosgenin was also obtained [3]. In the previous study treatment of **1–3** gave D-glucose and L-rhamnose in the ratios 1:1, 1:2 and 2:1, respectively (colorimetric estimations [4]). The terminal rhamnose in **1** is glycosylated at C-3 of the inner glucose, which in turn is linked with C-3 of yamogenin, as was proved by sodium periodate oxidation studies. Methanolysis studies of the permethyl ether (**1a**) of **1** also supported these results. Methanolysis of the permethyl ethers (**2a** and **3a**) of **2** and **3** gave methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside. Compound **2a** also gave the methyl pyranoside 4,6-di-*O*-methyl-D-glucose, and **3a** afforded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside together with 2,6-di-*O*-methyl-D-glucopyranoside. Both of these di-*O*-methyl sugars were decomposed by sodium periodate, confirming their identity as vicinal diols. The hydrolysis of the above methanolysis products of **2a** gave 2,3,4-tri-*O*-methyl-L-rhamnose and 4,6-di-*O*-methyl-D-glucose (pink colour with Wallenfel's reagent [5]). Compound **3a** afforded 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,6-di-*O*-methyl-D-glucose (negative to Wallenfel's reagent). Partial hydrolysis of **2** yielded three prosapogenins, PS_1, PS_2 (identical to **1**) and PS_3. PS_1 was found to be yamogenin monoglucoside and PS_3 permethylate on hydrolysis gave 3,4,6-tri-*O*-methyl-D-glucose (positive to Wallenfel's reagent) and 2,3,4-tri-*O*-methyl-L-rhamnose. These results prove that **2** was the rhamnoside of **1**. Thus, **1** and **2** were characterized as 3-*O*-[{α-L-rhamnopyranosyl (1 → 3)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-en-3β-ol and 3-*O*-[{α-L-rhamnopyranosyl (1 → 2)}{α-L-rhamnopyranosyl (1 → 3)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-en-3β-ol, respectively.

In order to determine the exact linkages of the monosaccharides in the sugar moiety of **3**, **3** was partially hydrolysed to afford PS_1, PS_4 and PS_5. Acid hydrolysis of PS_4 and PS_5 gave D-glucose; PS_4 also gave L-rhamnose. PS_5 on sodium periodate oxidation and hydrolysis gave D-glucose, and its permethylate on hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose. PS_4 permethylate on hydrolysis yielded 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose. Therefore, **3** was characterized as 3-*O*-[{β-D-glucopy-



1 $R^1 = R^3 = H$, $R^2 = \alpha$–L–rha (pyr)
2 $R^1 = R^2 = \alpha$–L–rha (pyr), $R^3 = H$
3 $R^1 = H$, $R^2 = \beta$–D–glu (pyr), $R^3 = \alpha$–L–rha (pyr)

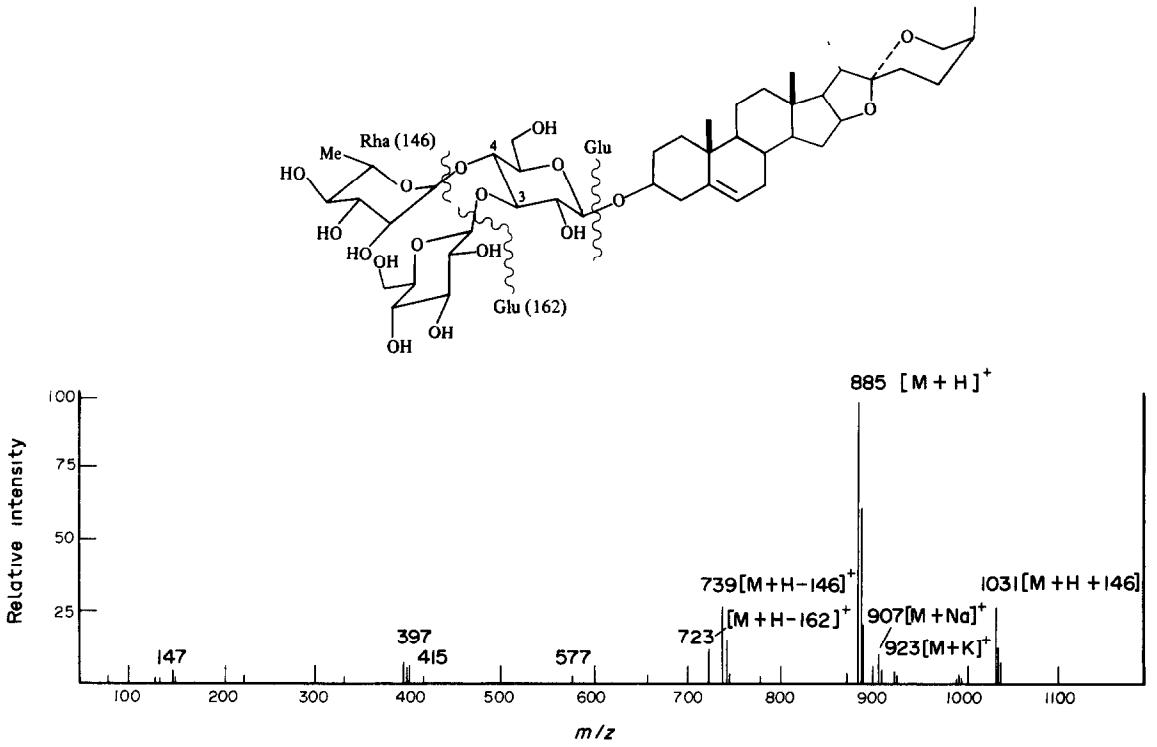


Fig 1 FD mass spectrum of compound 3.

Table 1. FD (A) and FAB (B) mass spectral data for compounds 1, 2 and 3

1	2		3
(A)	(A)	(B)	(A)
746 $[M+Na]^+$ (5.0)	1015 $[M+H+146]^+$ (11.4)	869 $[M+H]^+$ (0.57)	1031 $[M+H+146]^+$ (28.6)
723 $[M+H]^+$ (100.0)	891 $[M+Na]^+$ (5.6)	723 $[M+H-146]^+$ (0.32)	923 $[M+K]^+$ (4.9)
577 $[M+H-146]^+$ (34 1)	869 $[M+H]^+$ (100.0)	415 $[M+H-454]^+$ (3.1)	907 $[M+Na]^+$ (18.8)
415 $[M+H-308]^+$ (15.3)	868 $[M]^+$ (49.7)	139 [from genin] (44.0)	885 $[M+H]^+$ (100.0)
	850 $[M-H_2O]^+$ (4 5)	105 [from genin] (100.0)	739 $[M+H-146]^+$ (26.6)
	722 $[M-146]^+$ (32.5)		723 $[M+H-162]^+$ (12.2)
	576 $[M-292]^+$ (2.8)		577 $[M+H-308]^+$ (2.5)
	415 $[M+H-454]^+$ (24.1)		415 $[M+H-470]^+$ (2.0)

Assignments and relative intensities of peaks are shown in brackets and parentheses, respectively. The mass units which are lost corresponds to the following fragments: 146: rhamnose; 162: glucose; 292: two rhamnose; 308: rhamnose + glucose; 454: two rhamnose + glucose; 470: rhamnose + two glucose.

ranosyl (1 → 3)}{α-L-rhamnopyranosyl (1 → 4)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-en-3β-ol.

The identities of the above methylated sugars were also confirmed by direct comparison with corresponding authentic samples [6, 7]. The anomeric configurations in **1**–**3** were established by application of Klyne's rule [8].

EXPERIMENTAL

Mps were recorded in a Boetius microscopic apparatus. MS was on a JEOL JMS DX-300/JMA system (FD mode: E.H.C.: 22–23 mA; cathode voltage: −5 kV, accelerating voltage: 2 kV, ion multi. voltage: 2.5 kV; FAB mode: solvent: DMSO–glycerol, accelerating voltage: 2 kV; gas: Xe). CC was on silica gel (BDH; 60–120 mesh) and TLC on Kieselgel 60 G (Merck); spots were visualized by spraying with 10% alcoholic H_2SO_4 followed by heating. PC was carried out on Whatman No. 1 paper using the descending mode and aniline hydrogen phthalate as the developer Colorimetric estimations were recorded on a Syntronics Spectrocolorimeter Type 103. The following solvent systems were used: (A) $CHCl_3$–MeOH–H_2O (13:6:2); (B) C_6H_6–EtOAc (9:1); (C) C_6H_6–Me_2CO (7:3); (D) C_6H_6–Me_2CO (9 1); (E) *n*-

BuOH–HOAc–H_2O (4·1:5) and (F) *n*-BuOH–EtOH–H_2O (5:1:4).

Isolation of saponins. The leaves (2.5 kg), collected from Jeolikote (U.P.) in September, were air-dried and defatted with petrol in a Soxhlet. The solvent-free leaves were exhaustively extracted with 90% MeOH until the extracts became colourless. The conc. mass was shaken with $CHCl_3$ (3 × 1 l.) and filtered The residue was taken up in H_2O and extracted with *n*-BuOH (4 × 300 ml). The *n*-BuOH extracts after concn under red. pres. yielded a saponin mixture (9 g) which was chromatographed (solvent A) to afford **1** (500 mg) and a very complex mixture which on repeated CC yielded **2** (2 g), **3** (1 g) and a mixture of **4** and **5** (positive to Ehrlich reagent; characterization is in progress)

Compound **1** Colourless crystals, mp 230–231° (MeOH), $[\alpha]_D^{25}$ −103° ($CHCl_3$–MeOH; *c* 0.5); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650, 980, 920, 899 and 850 (intensity 920 > 899, 25*S*-spiroketal). (Found: C, 64.99; H, 8.28. $C_{39}H_{62}O_{12}$ requires: C, 64 82, H, 8.59%.) FDMS (*m/z*): 723 $[M+H]^+$

Compound **2**. Colourless flakes from MeOH, mp 295–296°, $[\alpha]_D^{25}$ −99° ($CHCl_3$–MeOH; *c* 0.9); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=C), 988, 921, 900, 855 (intensity 921 > 900, 25*S*-spiroketal). (Found: C, 61.80; H, 7.49. $C_{45}H_{72}O_{16}$ requires. C, 62 21; H, 8 29%.) FDMS and FABMS: see Table 1.

Compound **3**. Colourless needles from MeOH, mp 300–301°, $[\alpha]_D^{25}$ −94° ($CHCl_3$–MeOH, *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650, 980, 920, 898, 855 (intensity 920 > 898, 25*S*-spiroketal). (Found· C, 61.74; H, 8.24 $C_{45}H_{72}O_{17}$ requires· C, 61 86; H, 8.14%.) FDMS: see Table 1.

Acid hydrolysis of **1–3**. Compounds **1–3** (25 mg each) were hydrolysed with 2 M HCl–dioxane (1:1, 25 ml) on a boiling water bath for 3 hr to afford the aglycone (yamogenin): colourless needles (EtOAc), mp 201–202°, $[\alpha]_D^{25}$ −125° (lit. [9]: mp 201°, $[\alpha]_D^{20}$ −129°); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650, 981, 920, 900, 864 (intensity 920 > 900, 25 *S*-spiroketal). EIMS (probe) 70 eV, *m/z*: 414 $[M]^+$, 397, 355, 345, 342, 300 and 139. The neutralized and conc. aq. hydrolysate showed the presence of D-glucose and L-rhamnose (PC, solvent E, R_f values: 0.18 and 0.37, respectively) Estimation of sugars was performed by the method of ref [4] using the wavelength 420 nm

Hydrolysis of **2** *with ethanolic* HCl. Compound **2** (50 mg) was refluxed with 90% EtOH (15 ml) and 2 M HCl (10 ml) for 10 hr. The contents were poured into H_2O and the mixture was extracted with $CHCl_3$. The extract was washed with H_2O, 8% $NaHCO_3$ soln and dried over Na_2SO_4 The conc mass was purified by prep. TLC (solvent C) to afford yamogenin (5 mg) and diosgenin (10 mg), mp 205° (Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1660, 920, 900, 864 (intensity 900 > 920, 25*R*-spiroketal). Identity was confirmed by co-TLC and mmp

$NaIO_4$ *oxidation of* **1**. Compound **1** (10 mg) in H_2O (5 ml) was mixed with $NaIO_4$ (20 mg) and kept in the dark for 24 hr. Excess reagent was decomposed by ethylene glycol and hydrolysed by refluxing with 1M HCl (3 ml) to show the presence of D-glucose (PC, solvent E).

Permethylation of **1**, **2** *and* **3**. Compounds **1** (100 mg), **2** (500 mg) and **3** (400 mg) were separately methylated by Hakomori's method [10] to yield permethylates **1a**, **2a** and **3a**, purified by CC (solvent B).

Methanolysis followed by hydrolysis of **1a**. Compound **1a** (20 mg) in dry 10% HCl–MeOH (7 ml) was refluxed (4 hr), the solvent was removed, H_2O (5 ml) added and the mixture further warmed on a steam bath (3 hr). It was cooled and filtered The filtrate on concn showed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose and 2,4,6-tri-*O*-methyl-D-glucose (negative to Wallenfel's reagent) (PC, solvent F, R_G values: 1.01 and 0.76, respectively).

Compound **2a**. Mp 84–86°, $[\alpha]_D^{25}$ −97° ($CHCl_3$; *c* 0.9); IR ν_{max}^{KBr} cm^{-1}: no –OH, 1650, 980, 915, 900, 870. (Found: C, 65.13; H, 9.01. $C_{53}H_{88}O_{16}$ requires: C, 64.89; H, 8.98%.)

Methanolysis and hydrolysis of **2a**. Compound **2a** (100 mg) on methanolysis and usual work-up gave a mixture of methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose and 4,6-di-*O*-methyl-D-glucose (TLC, solvent D, R_fs: 0.84 and 0.19, respectively). A portion of the above mixture (2 ml) was subjected to $NaIO_4$ oxidation as for **1**. The mixture was checked on TLC (solvent D) to note that a spot corresponding to methyl dimethyl sugar had disappeared. Hydrolysis of this mixture of methyl pyranosides showed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose (R_G 1.01) and 4,6-di-*O*-methyl-D-glucose (R_G 0 46, pink colour with Wallenfel's reagent) (PC, solvent F)

Methanolysis followed by hydrolysis of **3a**. Compound **3a** (70 mg) was subjected to the title treatment as for **2a** to afford 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,6-di-*O*-methyl-D-glucose (PC, solvent F, R_G values. 1.01, 1.00 and 0.64, respectively). The methyl pyranoside of the last sugar disappeared on $NaIO_4$ oxidation and its hydrolysate was negative to Wallenfel's reagent

Partial hydrolysis of **2**. A soln of **2** (1 g) in 2 M HCl–MeOH (1:1, 30 ml) was refluxed on a steam bath (25 min), concd under red. pres., precipitated in cold H_2O and filtered The filtrate was neutralized with 5% $NaHCO_3$ and extracted with *n*-BuOH. The BuOH extract was concd under red. pres. and the two residues were mixed and then fractionated (CC, solvent A) to yield yamogenin (15 mg), diosgenin (4 mg), PS_1 (85 mg), PS_2 (195 mg) (identical to **1** by co-TLC, mmp and superimposable IR) and PS_3 (50 mg).

Hydrolysis of PS_1 *and* PS_3 *permethylates.* PS_1 (30 mg) and PS_3 (45 mg) were permethylated as above and hydrolysed as for **2a** PS_1 permethylate afforded 2,3,4,6-tetra-*O*-methyl-D-glucose whereas PS_3 permethylate gave 2,3,4-tri-*O*-methyl-L-rhamnose and 3,4,6-tri-*O*-methyl-D-glucose (pink colour with wallenfel's reagent) (PC, solvent F, R_G values: 1.00, 1 01 and 0.84, respectively).

Partial hydrolysis of **3**. Compound **3** (250 mg) on partial hydrolysis and usual work-up as in the case of **2** yielded PS_4 (40 mg) and PS_5 (35 mg) in addition to PS_1 and sapogenins. Acid hydrolysis of PS_4 and PS_5 (5 mg each) with 1M HCl–dioxane (5 ml) as above showed D-glucose; PS_4 also gave L-rhamnose (PC, solvent E, R_fs: 0 18 and 0.37, respectively).

$NaIO_4$ *oxidation of* PS_5. $NaIO_4$ oxidation followed by hydrolysis of PS_5 (5 mg) afforded the hydrolysate containing D-glucose (PC).

Hydrolysis of PS_4 *and* PS_5 *permethylates.* PS_4 and PS_5 (15 mg, each) were permethylated as above. PS_5 permethylate (10 mg) on hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose (PC, solvent F, R_G values: 1 00 and 0.76, respectively). On similar treatments, PS_4 permethylate gave 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose (PC, solvent F, R_G values: 1.01 and 0.83, respectively).

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