Journal of Natural Products
Vol. 48, No. 3, pp. 390-394, May-Jun 1985

NEW FUROSTANOL GLYCOSIDES FROM *ASPARAGUS PLUMOSUS* LEAVES

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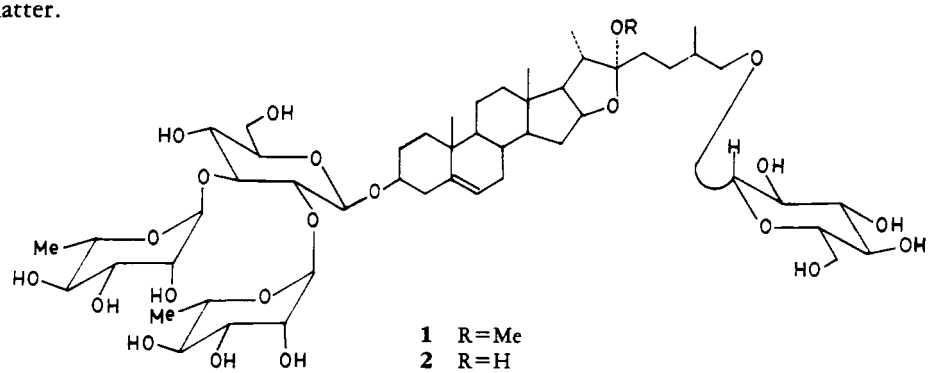
ABSTRACT.—Two new furostanol glycosides have been isolated from the MeOH extract of the leaves of *Asparagus plumosus* and characterized as 3-*O*-[{α-L-rhamnopyranosyl(1↦2)}{α-L-rhamnopyranosyl(1↦3)}-β-D-glucopyranosyl], 26-*O*-[β-D-glucopyranosyl]-(25- *S*)-22α-methoxy-furost-5-en-3β,26-diol (**1**), and its corresponding 22-hydroxy analog (**2**). Both were inactive against the snails *Biomphalaria glabrata*.

Asparagus plants find wide medicinal application (1), and we have reported molluscicidal spirostanol glycosides (2) from the MeOH extract of the leaves of *Asparagus plumosus* Baker (3). The isolation, characterization, and the results of the molluscicidal bioassay of two new furostanol glycosides are being reported here.

RESULTS AND DISCUSSION

The *n*-BuOH fraction of the methanolic extract of the leaves of *A. plumosus* on cc yielded the compounds previously identified (3) as 3-*O*-[α-L-rhamnopyranosyl(1↦3)-β-D-glucopyranosyl]-(25 *S*)-spirost-5-en-3β-ol (compound A), 3-*O*-[{α-L-rhamnopyranosyl(1↦2)}{α-L-rhamnopyranosyl(1↦3)}-β-D-glucopyranosyl]-(25 *S*)-spirost-5-en-3β-ol (compound B), and 3-*O*-[{α-L-rhamnopyranosyl(1↦4)}{β-D-glucopyranosyl(1↦3)}-β-D-glucopyranosyl]-(25 *S*)-spirost-5-en-3β-ol (compound C), and a mixture of **1** and **2**. The mixture exhibited a pink color with Ehrlich reagent (4) and lacked typical spiroketal absorptions in the ir. The mixture of **1** and **2** on boiling with dry MeOH yielded tlc homogeneous **1**, mp 171-173°, $[\alpha]^{27}D$ −78° (MeOH); while refluxing with Me_2CO and H_2O formed **2**, mp 193-195°, $[\alpha]^{27}D$ −81°. All the above studies showed **1** and **2** to be furostanolic in nature.

Compound **1** in fdms and fabms (data in Table 1; Figures 1 and 2) displayed feeble pseudomolecular ions at *m/z* 1085 $[M+Na]^+$ and 1063 $[M+H]^+$ indicating its molecular weight to be 1062. The loss of a MeOH molecule from these two ions was reflected in the doublet of peaks at *m/z* 1053/1031. The peaks at *m/z* 885 $[(M+H)-MeOH-146]^+$ and 869 $[(M+H)-MeOH-162]^+$ arose by the loss of terminal methyl pentose and hexose, respectively. The simultaneous loss of MeOH and two methyl pentoses and a methyl pentose and a hexose residue was concluded from the most intense ion recorded at *m/z* 739 and at 722. The peak at *m/z* 576 was ascribable to the ion formed by the loss of a hexose residue from the former and methyl pentose from the latter.



1 R=Me
2 R=H

TABLE 1. Fdms and Fabms Data of **1**[a]

Fdms	Fabms
1085 $[M+Na]^+$ (0.55)	1085 $[M+Na]^+$ (0.26)
1063 $[M+H]^+$ (5.45)	1053 $[(M+Na)-MeOH]^+$ (0.30)
1053 $[(M+Na)-MeOH]^+$ (1.65)	739 $[(M+H)-MeOH-292]^+$ (0.21)
1031 $[(M+H)-MeOH]^+$ (37.7)	631 $[(M+Na)-454]^+$ (0.19)
885 $[(M+H)-MeOH-146]^+$ (64.2)	609 $[(M+H)-454]^+$ (0.24)
869 $[(M+H)-MeOH-162]^+$ (40.7)	599 $[(M+Na)-MeOH-454]^+$ (0.31)
771 $[(M+H)-292]^+$ (6.99)	285 (1.0)[c]
754 $[(M+H)-308]^+$ (6.17)	271 (1.1)[c]
739 $[(M+H)-MeOH-292]^+$ (100.0)	267 (1.3)[c]
722 $[M-MeOH-308]^+$ (56.6)	253 (5.0)[c]
577 $[(M+2H)-MeOH-454]^+$ (50.9)	
576 $[(M+H)-MeOH-454)^+$ (59.0)	
414 $[M-MeOH-616]^+$ (6.71)	
163 $[glu+H-H_2O]^+$ (6.20)	
147 $[rha+H-H_2O]^+$ (22.3)	
128 $[rha-2H_2O]^+$ (23.1)	

[a] *m/z*.

[b] Assignments and the relative intensities of the peaks are shown in brackets. The mass units which are lost, correspond to the following fragments: 146: rhamnose; 292: two rhamnose; 454: two rhamnose+glucose; 162: glucose; 308: rhamnose+glucose; 616: two rhamnose+two glucose.

[c] The fragments 285, 271, 267 and 253 arise from the genin.

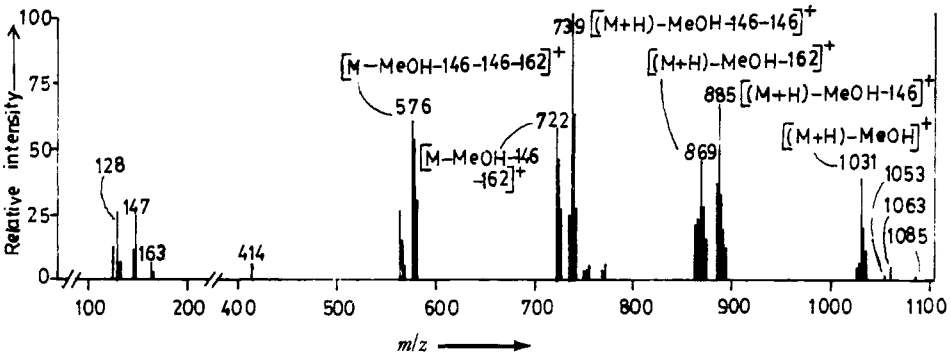


FIGURE 1.

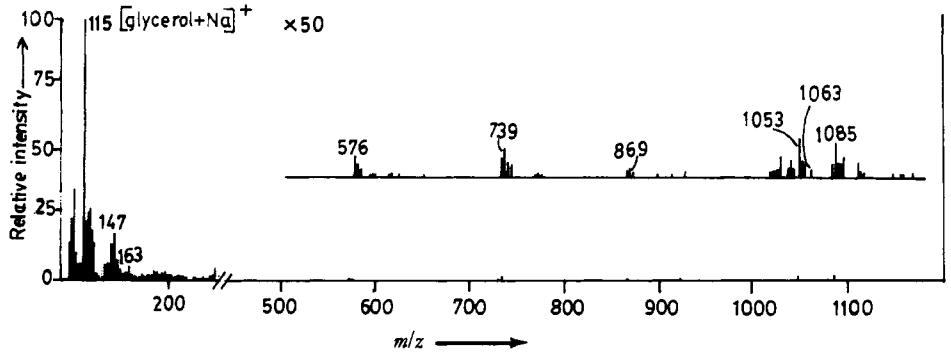


FIGURE 2.

Acid hydrolysis of **1** and **2** with HCl-dioxane gave an aglycone, mp 201-202°, ms *m/z* 414 $[M]^+$, ir 921>899 cm^{-1} (25 *S* configuration) which was identified as yamogenin (mmp, co-tlc, and co-ir). The hydrolysate showed the presence of glucose and rhamnose in the ratio 1:1 [colorimetric estimation (5)]. The permethyl ether of **1**, prepared by Hakomori's method (6), on methanolysis produced methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose, and 4,6-di-*O*-methyl-D-glucose ($NaIO_4$ decomposable). The acid hydrolysis of the above sugars afforded 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose, and a Wallenfel's positive spot (7) corresponding to 4,6-di-*O*-methyl-D-glucose (pc, identities also confirmed by direct comparison with authentic samples). These studies established vicinal branching in the inner glucose of the oligosaccharide moiety at positions 2 and 3. Enzymatic hydrolysis with β-glucosidase produced a spirostanol glycoside, mp 295-296°, $[\alpha]^{27}D$ −97°; ir 3400 (OH), 1650 (>C=C<), 921, 900 cm^{-1}; fdms *m/z* 869 $[M+H]^+$, found identical to compound B (3) (mmp, co-tlc and co-ir). The aqueous phase showed D-glucose only (pc). These results suggest **1** and **2** to be prototype saponins of compound B.

The presence of glucose at C-26 and the furostanolic nature of **1** and **2** was further supported by Marker's oxidation using Tschesche's method (8). The decomposition products were identified as pregn-5,16-dien-3β-ol-20-one [co-ir, co-tlc, and comparison with authentic sample (9)] and δ-hydroxy-γ-methyl valeric acid methyl ester glucoside tetraacetate, ms *m/z* 331 (tetra-*O*-acetyl glucopyranosyl ion).

Thus, **1** and **2** were characterized as 3-*O*-[{α-L-rhamnopyranosyl(1→2)}{α-L-rhamnopyranosyl(1→3)}-β-D-glucopyranosyl], 26-*O*-[β-D-glucopyranosyl]-(25 *S*)-22α-methoxy-furost-5-en-3β,26-diol and its 22 hydroxy analog, respectively.

Compounds **1** and **2** were found to be inactive against the snails *Biomphalaria glabrata*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were recorded in a 'Boetius' microscopic apparatus, ms in a JEOL JMS DX-300 system (fd mode, E.H.C., 22-23 mA; cathode V, -5 kV; accel. V, 2 kV; ion. multi. V, 2.5 kV; fab mode, solvent, DMSO-glycerol; gas, Xe). Cc was performed on Si gel, BDH (60-120 mesh) and tlc on Kieselgel 60 G, Merck; spots were visualized by spraying with 10% ethanolic H_2SO_4 followed by heating. Pc was performed on Whatman No. 1 paper using the descending mode and aniline hydrogen phthalate as developer. Colorimetric estimations were recorded on Syntronics Spectrocolorimeter Type 103. The following solvent systems were used: A) $CHCl_3$-MeOH-H_2O (65:30:10), B) C_6H_6-EtOAc (9:1), C) C_6H_6-Me_2CO (9:1), D) *n*-BuOH-HOAc-H_2O (4:1:5), E) *n*-BuOH-EtOH-H_2O (5:1:4), and F) $CHCl_3$-MeOH (95:5).

ISOLATION OF SAPONINS.—The leaves (5 kg), collected from Jeolikote (U.P.) in the month of Sept. and were authenticated by FRI, DDN, were air-dried and defatted with petrol in a Soxhlet extractor. The solvent free leaves were exhaustively extracted with 90% MeOH until the extractives became colorless. The concentrated mass was shaken with $CHCl_3$ (1 liter×3) and filtered. The residue was taken up in H_2O and extracted with *n*-BuOH (4×300 ml). The *n*-BuOH extracts, after concentrating at reduced pressure, yielded a saponin mixture (17 g) which was chromatographed (solvent A) to afford a mixture of **1** and **2** (7 g) in addition to compound A (1 g), compound B (3.8 g), and compound C (2.5 g) (3).

COMPOUND **1**.—The mixture of **1** and **2** (2 g) was refluxed on a water bath with dry MeOH (330 ml) for 24 h. The solution was concentrated and cooled, recrystallized from MeOH to give **1** (1.2 g), mp 171-173°, $[\alpha]^{27}D$ −78° (MeOH, c=0.9), ir ν max (KBr) cm^{-1} 3400 (OH), no spiroketal absorptions, fdms and fabms data in Table 1. (Found: C, 57.94; H, 7.82. $C_{52}H_{86}O_{22}$ requires C, 58.75; H, 8.09%).

COMPOUND **2**.—The mixture of **1** and **2** (500 mg) was refluxed in aqueous Me_2CO (1:2, 20 ml) for 40 h and cooled to obtain **2** (270 mg), mp 193-195°, $[\alpha]^{27}D$ −81° (H_2O, c=1.0), ir ν max (KBr) cm^{-1} 3400 (OH), no spiroketal absorptions. (Found: C, 57.52; H, 7.75. $C_{51}H_{84}O_{22}$ requires C, 58.39; H, 8.01%).

HYDROLYSIS OF **1** AND **2**.—Compounds **1** and **2** (20 mg, each) were separately refluxed with 2 N

HCl-dioxane (1:1, 30 ml) on a boiling water bath for 3 h to afford the aglycone (yamogenin): colorless needles, mp 201°, $[\alpha]^{20}D$ −129° ($CHCl_3$, c=0.9), ir ν max (KBr) cm^{-1} 3400 (OH), 1650 (>C=C<), 981, 920, 900, 854 (intensity 920>900, 25 *S* spiroketal); ms *m/z* 414 $[M]^+$, 397, 355, 345, 342, 300, and 139. The neutralized and concentrated aqueous hydrolysate showed the presence of D-glucose and L-rhamnose (pc, solvent D, Rf values: 0.18 and 0.37, respectively). The estimation of sugars was performed by the colorimetric methods (5) using a wavelength of 420 nm.

PERMETHYLATION OF **1**.—Compound **1** (200 mg) was permethylated by Hakomori's method. The reaction afforded the crude permethyl ether, purified by cc on Si gel, solvent B, to obtain a tlc homogeneous syrup, permethyl ether of **1** (45 mg), ir: no -OH.

METHANOLYSIS OF THE PERMETHYL ETHER OF **1**.—The permethyl ether of **1** (30 mg) in dry HCl-MeOH (15 ml) was refluxed (5 h), neutralized, and filtered to give a mixture of methyl pyranosides of 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 4,6-di-*O*-methyl-D-glucose (tlc, solvent C, Rfs, 0.84, 0.78, and 0.20, respectively). A portion of the above mixture was mixed with $NaIO_4$ (10 mg) and kept in the dark for 30 h. Excess of the reagent was decomposed by ethylene glycol, and the product was hydrolyzed by refluxing with 2 N HCl. The mixture was checked on tlc (solvent C) and disappearance of methyl-4,6-di-*O*-methyl-D-glucopyranoside was noted. Hydrolysis of the methanolyzed product showed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose (R_G 1.01), 2,3,4,6-tetra-*O*-methyl-D-glucose (R_G 1.00), and 4,6-di-*O*-methyl-D-glucose (R_G 0.46, pink color with Wallenfel's reagent) [pc, solvent E].

ENZYMATIC HYDROLYSIS OF **1** AND **2**.—The mixture of **1** and **2** (50 mg) in NaOAc-AcOH buffer (pH 4.8-5.0) was incubated with β-glucosidase (50 mg) at 37° for 24 h. The precipitate was collected by filtration, dried, and crystallized from MeOH to afford a compound as colorless flakes, mp 295-297°; $[\alpha]^{27}D$ −97° ($CHCl_3$-MeOH, c=0.95); ir ν max (KBr) cm^{-1} 3400 (OH), 1650 (>C=C<), 921, 900; fdms *m/z* 869 $[M+H]^+$; found identical to compound B (3) (mmp, co-tlc and co-ir). The aqueous filtrate on concentration was subjected to pc (solvent D) to show the presence of D-glucose only.

MARKER'S DEGRADATION OF **1**.—Compound **1** (600 mg) was dissolved in pyridine and Ac_2O (1:1, 50 ml), and the solution was heated at 35° with stirring under dry conditions for 4 h and then kept at room temperature for 48 h. The solvent was removed, and the concentrated mass was purified by cc, solvent F, to afford the peracetate of **1**, white powder, ir: no -OH. The peracetate (400 mg) was dissolved in glacial HOAc (15 ml) and refluxed for 1 h. The reaction mixture was then dried in vacuo. The residue was taken up in 80% HOAc (18 ml), and a solution of CrO_3 (45 mg) in 80% HOAc (1.5 ml) was added to the solution over 30 min with stirring (temperature kept below 20°). The reaction mixture was stirred for 4 h at room temperature and excess of CrO_3 was decomposed by adding MeOH (1 ml). It was diluted with H_2O, and, on usual workup, a residue (250 mg) was obtained. This was refluxed with 5% K_2CO_3 in iPrOH (15 ml) for 1.5 h, diluted with H_2O, and extracted with *n*-BuOH. The concentrated *n*-BuOH extract was hydrolysed by heating with 2 N HCl-dioxane (15 ml) and extracted with $CHCl_3$. This, on removal of solvent under reduced pressure, gave a crude mass purified by cc over Si gel, to afford 3β-hydroxypregna-5,16-dien-20-one (5 mg) mp, 211-213°, ir ν max (KBr) cm^{-1} 3400 (OH), 1656, 1580 (enone). The identity was further confirmed by direct comparison with an authentic sample. The alkaline aqueous layer was adjusted to pH 5.5 with HOAc and extracted with *n*-BuOH saturated with water. The *n*-BuOH solution was evaporated to dryness. The residue was acetylated as usual and treated with CH_2N_2 (3% CH_2N_2 in Et_2O) for 20 min. The reaction mixture was evaporated to give a syrup, purified by cc (solvent C). A tlc homogeneous colorless syrup was obtained, ms: *m/z* 331, 243, 242, 200, 169, 157, 147, 141, 140, 129, 115, 109, 103, 98, 97 in accordance with the expected pattern (10).

MOLLUSCICIDAL TEST.—Compounds **1** and **2** were submitted to the molluscicidal bioassay against the snails *Biomphalaria glabrata* by the method reported earlier (11).

ACKNOWLEDGMENTS

Thanks are due to Prof. T. Kawasaki and Prof. K. Miyahara, Kyushu University, Fukuoka, Japan, for mass spectra. We are thankful to Prof. J. Shoji, Tokyo, Japan, for an authentic sample of 3β-hydroxypregna-5,16-dien-20-one and the Council of Scientific and Industrial Research, New Delhi, India, for grants. Authentication of the plant material done by FRI, DDN, is gratefully acknowledged (voucher specimen is available with them).

This work is a part of a Ph.D. thesis of GP to be submitted in Garhwal University, Srinagar, U.P.

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Received 13 August 1984