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A NEW MOLLUSCICIDAL SPIROSTANOL GLYCOSIDE OF *YUCCA ALOIFOLIA*

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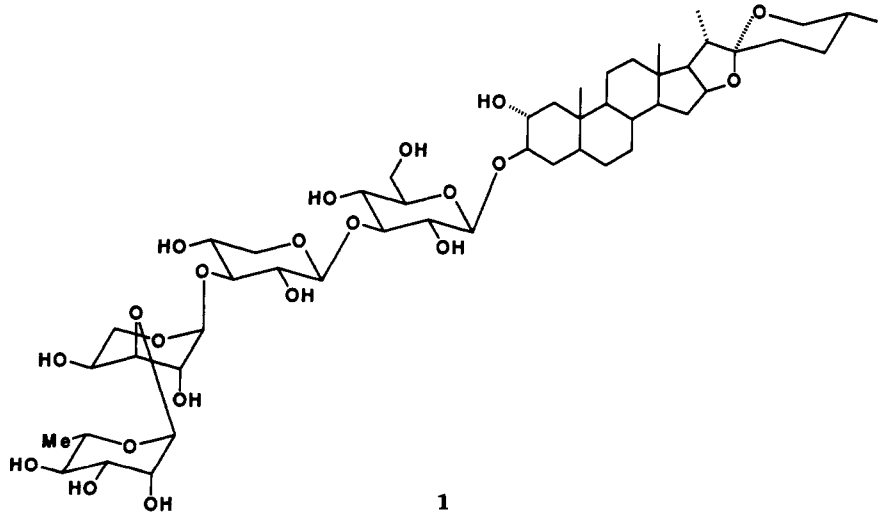
ABSTRACT.—A new molluscicidal spirostanol glycoside has been isolated from an EtOH extract of the inflorescence of *Yucca aloifolia* and characterized as 3-*O*-[α-L-rhamnopyranosyl (1↦3)-α-L-arabinopyranosyl(1↦3)-β-D-xylopyranosyl(1↦3)-β-D-glucopyranosyl]-25*R*,5α-spirostan-2α,3β-diol [**1**] with the help of fabms and ^{1}H- and ^{13}C-nmr spectra.

Yucca aloifolia L. (Agavaceae) is an ornamental plant, and many steroidal sapogenins have been reported from it (1–3). A literature survey showed that no phytochemical work has been reported from the inflorescence of this plant. Here, we report a new spirostanol glycoside from the EtOH extract of the inflorescence of this plant.

Repeated cc of the saponin mixture obtained from the inflorescence of *Y. aloifolia* afforded compound **1**. Its ir spectrum showed absorptions at 981, 926, 901, and 864 cm^{-1} (spiroketal moiety), with the absorption at 901 cm^{-1} being of greater intensity than at 926 cm^{-1} which is characteristic of the 25*R* stereochemistry (4). Acidic hydrolysis furnished gitogenin and D-glucose, D-xylose, L-arabinose, and L-rhamnose (pc). A mol wt of 1004 was established by the appearance of $[M]^+$ in the fabms, and peaks at 858 $[M - 146(\text{deoxyhexose} - H_2O)]^+$, 726 $[M - (146 + 132)]^+$, 594 $[M - (146 + 132 + 132)]^+$, and 432 $[M - (146 + 132 + 132 + 162)]^+$ indicated the sugar sequence to be deoxyhexosyl-pentosyl-pentosyl-hexosyl-gitogenin. Interglycosidation points were elucidated by the permethylation studies. The permethylate was obtained by Hakomori's method (5) and on hydrolysis afforded 2,3,4-tri-*O*-methyl-L-rhamnose, 2,4-di-*O*-methyl-L-arabinose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose. Partial hydrolysis of **1** afforded gitogenin and prosapogenins PS_1, PS_2, and PS_3. Confirmation of the glycoside sequence was obtained by the hydrolysis of permethylates of these prosapogenins. The permethylate of PS_1 on hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose; PS_2 afforded 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4,-tri-*O*-methyl-D-



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xylose; while PS_3 gave 2,4,6-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-L-arabinose. The conclusions of the chemical studies were corroborated by the chemical shifts of glycosidated carbon atoms in the ^{13}C-nmr spectrum. C-3 of the inner glucosyl unit was observed at δ 84.7, revealing deshielding of ~6.6 ppm from its reported value of 78.1 ppm for methyl-β-D-glucoside (6), showing it to be the point of glycosidation. Similarly, C-3 of xylosyl and arabinosyl units were observed at δ 82.7 and δ 80.1 ppm, revealing deshielding of ~4.6 ppm and ~5.7 ppm, respectively, from the reported values of δ 78.1 and δ 74.4 ppm for these carbon resonances for methyl-β-D-xylopyranoside (6) and methyl-α-L-arabinopyranoside (6), respectively. These studies showed that C-3 of these monosaccharides was the point of glycosidation. The type of linkages at glycosidic points was confirmed by the application of Klyne's rule (7) and nmr data. The ^{1}H-nmr spectrum showed three doublets at δ 5.68 [J = 7.2 Hz, H-1 of glucose (8)], 5.31 [J = 8 Hz, H-1 of xylose (8)], and 5.09 [J = 7.2 Hz, H-1 of arabinose (9)] and a singlet at δ 4.68 [H-1 of rhamnose (10)]. The chemical shifts and coupling constants of these signals proved the type of linkages at glycosidation points to be β for D-glucose and D-xylose and α for L-arabinose and L-rhamnose units. Based on a comparison with other steroidal saponins (11,12), the point of attachment of the sugar moiety in compound **1** appeared to be 3β-hydroxyl rather than the 2α-hydroxyl group of gitogenin. The C-3 carbon resonance of gitogenin appeared at δ 79.2 ppm (shifted downfield ~3 ppm as compared to the value of the genin), confirming it to be the point of attachment. Assignment of ^{13}C-nmr signals was made by comparison with the reported data of gitogenin (13) and the sugars (6,14). Thus, compound **1** was characterized as 3-*O*-[α-L-rhamnopyranosyl(1↦3)-α-L-arabinopyranosyl(1↦3)-β-D-xylopyranosyl(1↦3)-β-D-glucopyranosyl]-25*R*,5α-spirostan-2α,3β-diol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Mp's were recorded with Boetius microscopic apparatus. Fabms was obtained in negative ion mode. ^{1}H and ^{13}C nmr were recorded at 400 MHz and 100 MHz, respectively, in CD_3OD using TMS as an internal standard. Cc was performed on Si gel (Merck). The spots on tlc were visualized by spraying with 7% alcoholic H_2SO_4 followed by heating. Paper chromatography was carried out on Whatman No. 1 paper using descending mode and developed with aniline hydrogen phthalate. The following chromatographic solvent systems were used for tlc, pc, and cc: (A) $CHCl_3$-MeOH (85:15), (B) EtOAc-C_5D_5N-H_2O (10:4:3), (C) $CHCl_3$-MeOH (95:5), (D) petroleum ether (60–80°)-EtOAc (10:1), (E) C_6H_6-EtOAc (10:1), (F) *n*-BuOH–EtOH–H_2O (5:1:4).

ISOLATION.—Inflorescences (3 kg) of *Y. aloifolia* were collected in the month of August from Pauri, Uttar Pradesh, and specimens were identified by the Ethnomedicinal Plant Identification Laboratory, Department of Botany, H.N.B. Garhwal University, Srinagar, India, where a voucher specimen is deposited. The material was air-dried, coarsely powdered, and extracted exhaustively with aqueous EtOH. Repeated cc afforded compound **1** (solvent A).

COMPOUND **1**.—Colorless needles (300 mg) from MeOH, mp 372–375°, ir cm^{-1} 981, 926, 901, 864 (intensity 901>926, 25*R*-spiroketal); eims 432, 359, 318, 289; fabms 1004, 858, 726, 594, 432; ^{1}H nmr 0.66 (s), 0.72 (s), 0.84 (s), 1.04 (s) (each -Me), 4.68 (s, H-1 rhamnose), 5.09 (d, J = 7.2 Hz, H-1 xylose), 5.31 (d, J = 8 Hz, H-1 glucose), 5.68 (d, J = 7.2 Hz, H-1 arabinose) ppm; ^{13}C nmr aglycone (C-1 to C-27) 45.5, 70.4, 79.2, 36.2, 44.2, 27.5, 31.9, 33.9, 54.2, 37.0, 21.2, 40.0, 40.6, 56.3, 31.2, 80.3, 61.9, 17.0, 13.2, 41.2, 14.9, 108.9, 31.5, 29.1, 30.1, 66.3, 16.9; sugar moiety 103.0[a], 74.1[b], 84.7[c], 70.9[d], 76.7, 62.6 (glucosyl C-1 to C-6) 101.3[a], 73.7[b], 80.1[c], 69.1[d], 65.8 (arabinosyl C-1 to C-5) 103.0[a], 73.4[b], 82.7[c], 70.6[d], 68.1 (xylosyl C-1 to C-5) 102.8[a], 71.9, 72.7, 75.9, 69.4, 17.7 (rhamnosyl C-1 to C-6) (values with the same superscript may be interchanged).

ACIDIC HYDROLYSIS.—Compound **1** (50 mg) was refluxed with 2 M HCl-EtOH (1:1) (10 ml) on a steam bath for 4 h to afford the aglycone (gitogenin) as colorless needles (MeOH): mp 269–270°, ir cm^{-1} 981, 901, 926, 868 (intensity 901>926, 25*R*-spiroketal); ms [M]$^+$ 432. The neutralized (Ag_2CO_3) and concentrated hydrolysate showed the presence of D-glucose, D-

xylose, L-arabinose, and L-rhamnose (pc; solvent B; R_f 0.23, 0.37, 0.34, 0.38, respectively; authentic samples run in parallel). Sugars were estimated by the method of Mishra and Mohan Rao (15), using the wavelength of 420 nm.

PARTIAL HYDROLYSIS OF **1**.—Compound **1** (150 mg) in 1 M HCl–*n*-BuOH (1:1) (25 ml) was heated at 70° for 3 h. The *n*-BuOH layer was washed with H_2O and evaporated to dryness in vacuo. The residue on cc (solvent C) yielded gitogenin (7 mg), PS_1 (10 mg), PS_2 (30 mg), and PS_3 (30 mg).

Acidic hydrolysis of PS_1, PS_2, and PS_3.—PS_1, PS_2, and PS_3 (4 mg each) were hydrolyzed as above. The neutralized and concentrated hydrolysate from PS_1 gave D-glucose, PS_2 showed D-glucose and D-xylose, while PS_3 contained D-glucose, D-xylose, and L-arabinose (pc, solvent B).

*Permethylation of compound **1**, PS_1, PS_2, and PS_3.*—Compound **1** (100 mg), PS_1, PS_2, PS_3 (10 mg each) were separately permethylated by Hakomori's method. The permethylate of **1** was purified by cc (solvent D) while those of PS_1, PS_2, and PS_3 were purified by preparative tlc (solvent E, visualizing agent H_2O).

*Hydrolysis of PS_1, PS_2, PS_3, and Compound **1** permethylates.*—The permethylates (5 mg each) were separately refluxed with 1 M HCl-MeOH (1:1) (6 ml). The neutralized and concentrated hydrolysate from **1** showed 2,3,4-tri-*O*-methyl-L-rhamnose, 2,4-di-*O*-methyl-L-arabinose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose (pc; solvent F; R_G values 1.01, 0.64, 0.66, and 0.76, respectively). Permethylate of PS_1 afforded 2,3,4,6-tetra-*O*-methyl-D-glucose (pc; solvent F, R_G 1.00), PS_2 permethylate gave 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose (pc; solvent F; R_G 0.76 and 0.94, respectively), while PS_3 permethylate yielded 2,4,6-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-L-arabinose (pc; solvent F; R_G 0.76, 0.66, and 0.95, respectively). The identity of these sugars was confirmed by direct comparison with authentic samples (10, 16–18).

Compound **1** was submitted to molluscicidal bioassay against the snails *Biomphalaria glabrata* and found to exhibit 100% mortality at 10 ppm concentration using the method of Hostettmann *et al.* (19).

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