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A NEW SPIROSTANOL GLYCOSIDE FROM *YUCCA ALOIFOLIA*

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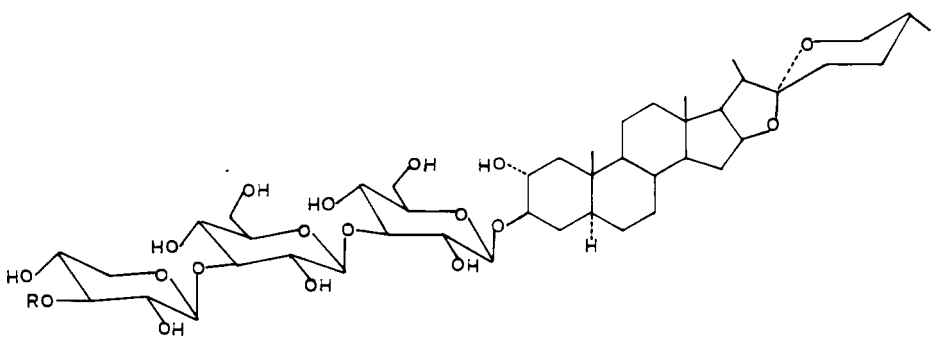
ABSTRACT.—From the EtOH extract of the rhizomes of *Yucca aloifolia* a new gitogenin-based glycoside has been isolated and characterized as 3-*O*-[β-D-xylopyranosyl(1↦3)-β-D-glucopyranosyl(1↦3)-β-D- xylopyranosyl(1↦3)-β-D-glucopyranosyl(1↦3)-β-D-glucopyranosyl]-25*R*,5α-spirostan-2α,3β-diol, using fdms, fabms, ^{1}H-nmr, ^{13}C-nmr, and chemical studies.

Yucca aloifolia L. (Agavaceae) plants exhibit oxytocic and anti-inflammatory properties (1). Previously, three steroidal saponins and their related sapogenins (2–5) have been reported from the rhizomes of this plant. We have also isolated two tigogenin-based glycosides (6) from the rhizomes. The isolation and characterization of a new gitogenin-based spirostanolic glycoside is reported here.

The saponin mixture obtained from the EtOH extract of the rhizomes, on repeated cc, afforded compound **1**, mp 268–270°, $[\alpha]_D$ −49.5° (MeOH). Acid hydrolysis of **1** with HCl/dioxane afforded an aglycone, mp 262–264°, ms *m/z* 432 $[M]^+$, ir 898>924 cm^{-1} (25*R* configuration), which was identified as gitogenin (mmp, co-tlc, and co-ir). The hydrolysate showed the presence of D-glucose and D-xylose in the ratio of 3:2 by colorimetric estimation (7). The fabms showed a pseudomolecular ion at *m/z* 1181 $[M-H]^-$ indicating its mol wt to be 1182. The peak at *m/z* 1049 $[M-H-132]^-$ corresponded to the loss of a terminal pentose unit, whereas other peaks at *m/z* 887 $[M-H-(132+162)]^-$, 755 $[M-H-(2\times132+162)]^-$, 593 $[M-H-2(132+162)]^-$, and 431 $[M-H-(2\times132+3\times162)]^-$ corresponded to the loss of pentosyl-hexose, dipentosyl-hexose, dipentosyl-dihexocyl, and dipentosyl-trihexosyl units, respectively. The results obtained from the fabms also indicated the sugar sequence. The permethyl ether of **1**, prepared by Hakomori's method (8), on methanolysis followed by hydrolysis produced methylated sugars identified as 2,3,4-tri-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose by pc, with the identities confirmed by direct comparison with authentic samples (9,10). The interglycosidation



1

R=β-D-xylopyranosyl(1↦3)-β-D-glucopyranosyl

was assigned by partial hydrolysis of **1** to afford prosapogenols I, II, III, and IV, in addition to gitogenin. On acidic hydrolysis, prosapogenols I and II gave D-glucose only, while III and IV gave D-glucose and D-xylose. These partial products, on permethylation followed by acidic hydrolysis, yielded 2,3,4,6-tetra-*O*-methyl-D-glucose from I, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose from II, 2,3,4-tri-*O*-methyl-D-xylose and 2,4,6-tri-*O*-methyl-D-glucose from III, and 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose from IV (pc).

The interglycosidation assignments were further confirmed by the chemical shifts of glycosylated carbon atoms in the ^{13}C-nmr spectrum. The C-3 of three glucose and one xylose units were observed at δ 87.09, 84.32, 86.99, and 81.13 ppm, respectively, revealing deshielding of ca. 9 ppm, 6 ppm, 8 ppm, and 4 ppm, respectively, for these carbon resonances; hence, C-3 is the glycosidation point of all the sugars. The ^{1}H-nmr spectrum showed five anomeric signals as doublets at δ 5.63 (J=7.3 Hz), 5.22 (J=7.9 Hz), 5.18 (J=7.7 Hz), 5.09 (J=7.3 Hz) and 4.92 (J=7.9 Hz) for anomeric protons of the three glucose and two xylose units, respectively. The chemical shifts and coupling constants of these signals suggested the β-anomeric configuration of the sugar moieties on comparison with reported values (11). On the basis of comparisons with other steroidal saponins (12,13), the point of attachment of sugar moieties in **1** appeared to be the 3β-hydroxyl rather than the 2α-hydroxyl group of gitogenin. This was also proved by the ^{13}C-nmr spectrum of **1**. The C-3 reported value δ 76.4 ppm (14) for gitogenin was observed at δ 79.21 ppm [shifted downfield by 4 ppm for this carbon resonance], whereas C-2 was observed at 70.37 ppm [shifted upfield 2.7 ppm for this carbon resonance], confirming that the C-3 of gitogenin was the point of attachment of the innermost sugar (glucose) moiety.

Therefore, **1** was characterized as 3-*O*-[β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl]-25*R*,5α-spirostan-2α,3β-diol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were recorded in a Boiteus microscopic apparatus and fabms (negative ion mode) in a JEOL JMS-HX 110 system using MeOH and glycerol as solvents. ^{1}H-nmr (60 MHz) and ^{13}C-nmr (25 Hz) spectra were taken in C_5D_5N with TMS as an internal standard. Cc was performed on Si gel Merck (60–120 mesh) and tlc on Kieselgel 60G, Merck; spots on tlc were visualized by spraying with 10% alcoholic H_2SO_4 followed by heating. Pc was carried out on a Whatman No. 1 paper using the descending mode and aniline hydrogen phthalate as developer. Colorimetric estimations were recorded on a Syntronic spectrocolorimeter. The following solvent systems were used: (A) $CHCl_3$-MeOH (70:30); (B) $CHCl_3$-MeOH-H_2O (65:40:10); (C) light petroleum ether (60–80°)-EtOAc (1:1); (D) C_6H_6-Me_2CO (4:1); (E) *n*-BuOH–HOAc–H_2O (4:1:5); (F) *n*-BuOH–EtOH–H_2O (5:1:4); (G) $CHCl_3$-MeOH (4:1).

ISOLATION OF SAPONIN.—The rhizomes (4 kg) were collected from Pauri in the month of July, and specimens were identified by Professor R.D. Gaur, Ethnobotanical Research Lab., H.N.B. Garhwal University, Srinagar, U.P.; a voucher specimen is available there. The extraction and isolation of the polar compound **1** (500 mg) followed the steps described previously (6).

COMPOUND **1**.—Crystallized from MeOH: mp 268–270°; $[\alpha]_D$ −49.5° ($CHCl_3$/MeOH), ir max cm^{-1} 3420, 1044 (OH), 862, 898, 924, 982 (intensity 898>924, 25*R* spiroketal); ^{1}H-nmr (in C_5D_5N) δ 5.63 (1H, d, J=7.3 Hz, H-1 glu), 5.22 (1H, d, J=7.9 Hz, H-1 glu′), 5.18 (1H, d, J=7.9 Hz, H-1 glu″), 5.09 (1H, d, J=7.4 Hz, H-1 xyl), 4.92 (1H, d, J=7.9 Hz, H-1 xyl′); ^{13}C-nmr (in C_5D_5N) aglycone carbons 45.57 (C-1), 70.37 (C-2), 79.21 (C-3), 34.00 (C-4), 44.58 (C-5), 28.10 (C-6), 32.13 (C-7), 34.58 (C-8), 54.56 (C-9), 36.86 (C-10), 21.43 (C-11), 40.06 (C-12), 40.78 (C-13), 56.32 (C-14), 31.83 (C-15), 80.63 (C-16), 62.98 (C-17), 16.63 (C-18), 14.41 (C-19), 41.99 (C-20), 15.02 (C-21), 109.24 (C-22), 32.22 (C-23), 29.27 (C-24), 30.61 (C-25), 66.87 (C-26), 17.33 (C-27), carbons of sugar moieties glu 105.4 (C-1), 74.80 (C-2), 87.09 (C-3), 71.4 (C-4), 78.3 (C-5), 62.5 (C-6), glu′ 104.4 (C-1),

75.04 (C-2), 84.32 (C-3), 70.73 (C-4), 77.74 (C-5), 62.37 (C-6), glu″ 103.27 (C-1), 75.38 (C-2), 81.13 (C-3), 70.79 (C-4), 77.93 (C-5), 62.90 (C-6), xyl 104.87 (C-1), 75.73 (C-2), 86.99 (C-3), 70.76 (C-4), 67.07 (C-5), xyl′ 106.15 (C-1), 77.93 (C-2), 78.10 (C-3), 72.56 (C-4), 67.25 (C-5); fdms *m/z* $[M+Na]^+$ 1205, fabms (negative ion mode) $[M-H]^-$ 1181, 1049, 887, 755, 593, 431. *Anal.* calcd for $C_{55}H_{92}O_{28}\cdot 2H_2O$, C 54.18, H 7.88; found C 53.90, H 7.75.

HYDROLYSIS OF **1**.—Compound **1** (20 mg) was refluxed with 2 N HCl-dioxane (1:1) (25 ml) on a boiling H_2O bath for 3 h to afford the aglycone gitogenin as colorless needles from MeOH: mp 262–264°; ir max (KBr) cm^{-1} 3400, 1044 (OH), 862, 898, 924, 982 (intensity 898>924, 25*R* spiroketal); eims *m/z* $[M]^+$ 432; identified by direct comparison (mmp, co-tlc, and co-ir) with an authentic sample. The neutralized and concentrated hydrolysate showed the presence of D-glucose and D-xylose (pc, solvent E, R_f values 0.23 and 0.28, respectively). The estimation of sugars was performed by the colorimetric estimation using a wavelength of 420 nm.

PERMETHYLATION OF **1**.—Compound **1** (150 mg) was permethylated with NaH and MeI by Hakomori's method, and complete methylation was checked by ir. The product was purified by cc (solvent C) to afford the permethyl ether (100 mg) of **1**.

METHANOLYSIS FOLLOWED BY HYDROLYSIS OF **1**.—The permethyl ether (75 mg) in 9% anhydrous HCl in MeOH (10 ml) was refluxed for 4 h, neutralized (Ag_2CO_3), and filtered to afford a mixture of methyl pyranosides of 2,3,4-tri-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose (pc, solvent F, R_{glu} values 0.94, 0.67, and 0.76, respectively).

PARTIAL HYDROLYSIS OF **1**.—Compound (150 mg) was partially hydrolyzed with 1 M HCl–*n*-BuOH (1:1, 50 ml) at 70° for 4 h. The *n*-BuOh phase was washed with H_2O and evaporated in vacuo. The residue on cc yielded gitogenin (5 mg) and prosapogenols I (20 mg), II (40 mg), III (35 mg), and IV (30 mg).

HYDROLYSIS OF PROSAPOGENOLS.—The prosapogenols I–IV (10 mg each) were separately hydrolyzed as above. The neutralized and concentrated hydrolysate of I and II gave D-glucose, while III and IV gave D-glucose and D-xylose (pc).

PERMETHYLATION FOLLOWED BY HYDROLYSIS OF PROSAPOGENOLS.—Prosapogenols I–IV (15 mg each, separately) on permethylation followed by hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose (R_{glu} 1.00) from I, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose from II (R_{glu} 1.00, 0.76), 2,3,4-tri-*O*-methyl-xylose, 2,4,6-tri-*O*-methyl-D-glucose (R_G 0.94, 0.76) from III, and 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-xylose, and 2,4,6-tri-*O*-methyl-D-glucose from IV (pc, solvent F).

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