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SPIROSTANOL SAPONINS FROM *YUCCA ALOIFOLIA* RHIZOMES

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Abstract—From the rhizomes of *Yucca aloifolia* two spirostanol saponins (A and B) have been isolated and characterized as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-25*R*-5α-spirostane-3β-ol and 3-*O*-[β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl]-25*R*-5α-spirostane-3β-ol by FDMS, FABMS, ^{13}C and ^{1}H NMR and by chemical evidence

INTRODUCTION

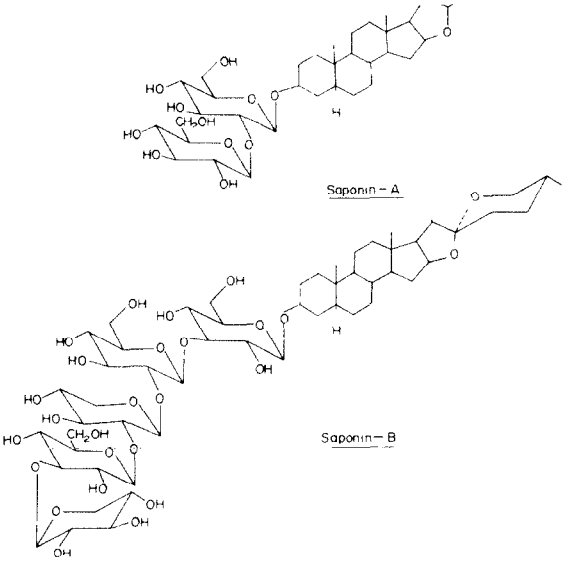
Three steroidal saponins [1–3] and their related sapogenins [1, 4] have been reported from the fresh leaves of *Yucca aloifolia*. An ethanolic extract of the rhizomes of *Y. aloifolia* was found to possess anti-inflammatory and oxytocic activity. It was fractionated to yield two new spirostanol tigogenin glycosides and two gitogenin based saponins which are yet to be characterized. Characterization of new tigogenin based saponins is presented in this paper.

RESULTS AND DISCUSSION

The saponins obtained from the 90% aq. ethanolic extract on repeated CC, showed characteristic absorptions of the 25*R*-spirostane nucleus in its IR [5]. This was further confirmed by the ^{1}H NMR spectra [6].

On acidic hydrolysis saponin A yielded an aglycone identified as tigogenin MS (*m/z*) 416 $[M]^+$, co-TLC, mmp and superimposable IR. The aqueous hydrolysate showed the presence of D-glucose only (PC). The FDMS showed pseudomolecular ions at *m/z* 763 $[M+Na]^+$ and 741 $[M+H]^+$ indicating its M_r to be 740. The peaks at *m/z* 601 $[M+Na-162]^+$ and 439 $[M+Na-2\times162]^+$ arose by the loss of terminal hexose and dihexosyl units, respectively. The permethylate prepared by Hakomori's method [7] on acidic hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-glucose and a Wallenfel's positive [8] sugar 3,4,6-tri-*O*-methyl-D-glucose (PC, identity was confirmed by direct comparison with authentic samples [9]). The type of linkages at glycosidic points was confirmed by the application of Klyne's rule [10]. Thus, it was characterized as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-25*R*-5α-spirostane-3β-ol.

Saponin B after acidic hydrolysis afforded tigogenin, D-glucose and D-xylose in the molecular ratio of 3:2 (colorimetric estimation [11]). This was corroborated by the occurrence of only five signals in the anomeric region of the ^{13}C NMR spectrum (δ 102.47, 104.00, 104.93, 104.93 and 106.22). The FABMS (negative ion mode) showed a molecular ion at *m/z* 1165 $[M-H]^-$ indicating a M_r of 1166. Peaks at *m/z* 1033 $[M-H-132]^-$, 871 $[M-H-(132+162)]^-$, 739 $[M-H-(2\times132+162)]^-$, 579 $[M-H-2(132+162)]^-$ and 415 $[M-H-(2\times132+3\times162)]^-$ indicated the sugar sequence to be pentosyl-hexosyl-pentosyl-hexosyl-hexosyl-tigogenin. Interglycosidation points were elucidated by the permethylation [7] studies. The permethylate on hydrolysis afforded 2,3,4-tri-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-xylose and 3,4,6-tri-*O*-methyl-D-glucose. The latter two sugars gave an intense pink colour with Wallenfel's reagent, indicating monosaccharides unsubstituted in the C-2 position. These sugars were identified by direct comparison with authentic samples [9, 12]. Partial hydrolysis of B afforded tigogenin and four prosapogenols called PS_1, PS_2, PS_3 and PS_4. Further confirmation of interglycosidation was obtained by the hydrolysis of the permethylates of the above prosapogenols. The above studies showed that the terminal xylose was attached at C-3 of a glucose moiety which was glycosidated at C-2 of another xylose. In turn this xylose was linked at C-2 of another glucose molecule which was attached at C-3 to the innermost glucose forming the



oligosaccharide moiety The innermost glucose was glycosidated at C-3 of tigogenin The ^{13}C NMR spectrum of B also corroborated these results, which was made by comparison with the reported values of tigogenin [13] and sugars [14]. In the ^{13}C NMR spectrum the C-2 and C-3 signals of the glucose and xylose units showed downfield glycosidation shifts of ~6, ~10, ~10 and ~4 ppm, respectively

The nature of the sugar linkages was established as β-by the ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum showed five doublets at δ5.58 (J=7.3 Hz), 5.20 (J =7.7 Hz), 5.16 (J=7.7 Hz), 5.10 (J=7.3 Hz) and 4.98 (J =7.7 Hz) for three C_1-H of the glucose and two C_1-H of the xylose units, respectively Thus, saponin **B** was characterized as 3-*O*-[β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl (1→2)-β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl]-25*R*-5α-spirostane-3β-ol

EXPERIMENTAL

CC. over silica gel (Merck), the spots on TLC were detected by spraying with 10% H_2SO_4 PC on Whatman no 1 filter paper, aniline hydrogenphthalate as visualizer FABMS (neg. ion mode) MeOH and glycerol as solvent, ^1H NMR (60 MHz) and ^{13}C NMR (25 MHz) spectra taken in pyridine-d_5 and chemical shifts are given with TMS as int standard

Plant material The rhizomes of *Yucca aloifolia* were collected from Pauri (Garhwal, U P) in July and the identity confirmed by the Department of Botany, University of Garhwal, Srinagar, U P

Extraction and isolation. The air-dried and powdered rhizomes (3 kg) were defatted with petrol (60–80°) and the residue extracted with hot EtOH (90%) Removal of the solvent by evapn under red pres yielded a brown viscous mass (500 g) This extract (20 g) was chromatographed over silica gel, using $CHCl_3$–MeOH (9 1) as eluant Gradient elution by increasing the quantity of MeOH afforded four compounds named saponin A–D Characterization of gitogenin based saponins C and D is in progress.

Saponin A Crystallized from MeOH as needles, mp 265–268°, $[\alpha]_D$ −52.3° (MeOH), IR 3400 (OH), 980, 928, 900 and 875 cm^{-1} (intensity 875 >900 cm^{-1}, 25*R*-stereochemistry), EIMS 416 $[M]^+$, 139, FDMS (*m/z*) 763 $[M+Na]^+$, 741 $[M+H]^+$, 601 $[M+H-162]^+$, 439 $[M+Na-2\times162]^+$, 415 $[M+Na-(2\times162+23)]^+$, 399, 347, 344, 272, 139 (base peak)

Saponin B Crystallized from MeOH as needles, mp 270–272°, $[\alpha]_D$ −57.2° (MeOH); IR ν_{max} cm^{-1} 3350 (OH), 986, 915, 890, 880 (intensity 890 >915, 25*R*, spiroketal), FABMS *m/z* 1165, 1033, 871, 739, 577, 415, 139, ^1H NMR δ5.58 (1H, *d*, J=7.3 Hz), 5.20 (1H, *d*, J=7.7 Hz), 5.16 (*d*, J=7.7 Hz), 5.10 (1H, *d*, J =7.3 Hz), 4.98 (1H, *d*, J=7.7 Hz) [anomeric protons], 0.64 (3H, *s*, 18-Me), 0.70 (3H, *s*, 27-Me), 0.82 (3H, *s*, 19-Me), 1.14 (3H, *s*, 21-Me), ^{13}C NMR (C_1–C_2, carbons of aglycone part) δ37.19, 29.91, 77.45, 34.80, 44.65, 28.93, 32.41, 35.25, 54.40, 35.82, 21.28, 40.15, 40.79, 56.45, 32.15, 81.15, 63.04, 16.62, 12.31, 42.00, 15.04, 109.24, 31.83, 29.28, 30.61, 66.88, 17.34, (carbons of sugar moieties) glucopyranosyl 104.00, 75.14, 86.72, 70.44, 78.30, 60.69, glu′ 104.93, 80.76, 75.52, 70.41, 78.38, 62.15, glu″ 104.93, 75.14, 86.76, 70.81, 77.79, 62.97, xylopyranosyl 106.22, 79.67, 75.45, 70.8, 67.17, xyl 102.00, 73.76, 77.61, 69.16, 67.30

Acid hydrolysis of saponins A and B Saponins A and B (30 mg, each) were separately refluxed with 2 M HCl–MeOH (1 1, 5 ml) on a boiling water bath for 3 hr to afford the aglycone, colourless needles, mp 203–205 (ref [12] mp 203–205°), MS *m/z* 416 $[M]^+$, identified as tigogenin by direct comparison (co-TLC, co-IR, mmp) with authentic sample [12]. The neutralized (Ag_2CO_3) and concd hydrolysate of A showed D-glucose whereas that of B showed D-glucose and D-xylose in the ratio of 3 2 [PC, *n*-BuOH–AcOH–H_2O, (4 1 5), R_f 0.23 and 0.37, respectively]

Permethylation of saponins A and B Saponins A and B (200 mg, each) were separately permethylated with NaH and MeI by the method of [7] and the products purified by CC (C_6H_6–EtOAc, 9 1) to afford permethyl ether A_1 and B_1 (100 mg), IR ν_{max}^{KBr} cm^{-1} no-OH

Methanolysis followed by hydrolysis of A_1 and B_1 The permethylates A_1 and B_1 (30 mg, each) in dry HCl–MeOH (15 ml) were separately refluxed (4 hr), neutralized (Ag_2CO_3) and filtered to give a mixture of methylpyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-glucose from A_1 whereas B_1 afforded 2,3,4-tri-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-xylose and 3,4,6-tri-*O*-methyl-D-glucose, (PC, *n*-BuOH–EtOH–H_2O, 5 1 4) 3,4,6-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose gave a pink colour with Wallenfel's reagent

Partial hydrolysis of saponin B Saponin B (500 mg) in 1 M HCl–BuOH (1 1, 25 ml) was heated, washed with H_2O and evapd to dryness *in vacuo* The residue on CC ($CHCl_3$–MeOH, 4 1) yielded tigogenin (6 mg) and prosapogenols PS_1 (50 mg), PS_2 (40 mg) PS_3 (40 mg) and PS_4 (55 mg)

Acidic hydrolysis of the prosapogenols The prosapogenols PS_1, PS_2, PS_3 and PS_4 (5 mg, each) were separately hydrolysed as above The neutralized and concd hydrolysate of PS_1 gave D-glucose whereas PS_2, PS_3 and PS_4 gave D-glucose and D-xylose (PC)

Permethylation followed by hydrolysis of prosapogenols PS_1, PS_2, PS_3 and PS_4 (10 mg each) were separately permethylated as above Hydrolysis of permethylates showed 2,3,4,6-tetra-*O*-methyl-D-glucose from PS_1, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose from PS_2, 2,3,4-tri-*O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose from PS_3 and 2,3,4,6-tetra-D-methyl-*O*-glucose, 3,4-di *O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose from PS_4 (PC)

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