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A SPIROSTANOL GLYCOSIDE FROM *AGAVE CANTALA*

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Abstract—A new spirostanol glycoside, cantalasaponin-3, isolated from the methanolic extract of the rhizomes of *Agave cantala*, has been characterized.

INTRODUCTION

Agave species have been used for medicinal purposes and various saponins have been reported from *A. cantala* Roxb. [1]. This communication deals with the structure elucidation of cantalasaponin-3 (**2**) isolated from the rhizomes of this plant.

RESULTS AND DISCUSSION

Saponin **2**, a 25*R*-spirostane derivative (IR) was found to have an M_r of 1034, as indicated from the pseudomolecular ions at m/z 1073, 1057 and 1035 corresponding to $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$ ions, respectively, in its FD-mass spectrum. The peaks at m/z 925/903 and at 895/873 arise from the loss of terminal pentose and hexose, respectively, from $[M+Na]^+/[M+H]^+$ ions.

Acidic hydrolysis of **2** gave tigogenin, and D-galactose, D-glucose and D-xylose in the ratio 1:2:1.

The interglycosidic linkages in **2** were established by means of ^{13}C NMR spectroscopy. ^{13}C chemical shifts of methyl pyranosides of β-D-galactose, β-D-glucose and β-D-xylose in pyridine-d_5 [2–4] and those of tigogenin [5] are available and the signals in **2** were assigned by the application of glycosylation shifts [2, 3]. In the ^{13}C INEPT spectrum, by setting the delay time Δ as 3/4J [6], CH and Me signals were in phase, CH_2 out of phase, and quaternary carbons and carbons of the solvent were absent. In the ^{1}H decoupled mode the signals in the sugar region of **2** and **1** [1], the 12-oxo analogue of **2**, were almost superimposable. This observation was further supported when the permethylation products of **2** and its partial hydrolysis product, PS_2, gave methylated sugars identical to those obtained after permethylation of **1** and PS_3 [1], respectively.

The anomeric linkages were deduced as β from the ^{1}H NMR spectrum of **2** and by the application of Klyne's rule [7].

Thus, **2** was characterized as 3-*O*-[{β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl(1 → 2)}{β-D-xylopyranosyl(1 → 4)}-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-3β-ol, a 12-deoxo analogue of **1** [1]. This provides an example of the co-occurrence of hecogenin and tigogenin glycosides with identical sugar chains.

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EXPERIMENTAL

Almost all the instrumentation techniques were as described in ref. [1]. ^{13}C INEPT and ^{1}H decoupled, ^{1}H NMR spectra were recorded on a JEOL FX-100 Fourier-transform spectrometer operating at 25/100 MHz. Isolation of **2** and the solvent systems used for the TLC examination of sugars and methylated sugars are detailed in ref. [1]. The compounds on TLC were visualized with 10% ethanolic H_2SO_4 and on prep. TLC by spraying with H_2O.

Compound **2**. Colourless plates (130 mg) from aq. EtOH, mp 298–302°, $[\alpha]_D^{15-17}$ −54.8° (C_5H_5N; *c* 1.29). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 985, 925, 900, 870 (intensity 900 > 925, 25*R*-spiroketal); FDMS, *m/z* (rel. int.): 1073 $[M+K]^+$ (10.1), 1057 $[M+Na]^+$ (100.0), 1035 $[M+H]^+$ (11.9), 925 $[M+Na-132]^+$ (27.8), 903 $[M+H-132]^+$ (23.0), 895 $[M+Na-162]^+$ (10.5), 873 $[M+H-162]^+$ (10.5), 741 $[M+H-132-162]^+$ (3.8), 579 $[M+H-132-162-162]^+$ (4.3), 399 $[genin+H-H_2O]^+$ (7.2), 163 $[hex+H-H_2O]^+$ (8.3), 133 $[xyl+H-H_2O]^+$ (14.0); ^{1}H NMR: δ0.67 (6H, *m*, 18-Me, 27-Me), 0.82 (3H, *s*, 19-Me), 1.12 (3H, *d*, *J* = 7 Hz, 21-Me), 4.85 (1H, *d*, *J* = 7.1 Hz, H-1 of glu), 5.20 (1H, *d*, *J* = 7.3 Hz, H-1 of glu), 5.26 (1H, *d*, *J* = 7 Hz, H-1 of gal), 5.61 (1H, *br s*, H-1 of xyl); ^{13}C NMR: aglycone: δ37.2, 29.9, 78.6, 34.8, 44.6, 29.0, 32.4, 35.3, 54.4, 35.8, 21.3, 40.1, 40.8, 56.4, 31.8, 81.1, 63.0, 16.6, 12.3, 42.0, 15.0, 109.2, 32.1, 28.9, 30.6, 66.9, 17.3 (C-1–C-27); sugar moiety: δ102.4, 81.1, 73.1, 79.9, 76.2, 60.2 (galactosyl C-1–C-6), 104.9, 70.7, 86.8, 70.4, 77.6, 62.4[a] (glucosyl C-1–C-6), 104.9, 75.5, 78.6, 71.0, 78.6, 63.0[a] (glucosyl C-1′–C-6′), 104.9, 75.1, 77.4, 70.7, 67.3 (xylosyl C-1–C-5). (Found: C, 58.19; H, 7.99. $C_{50}H_{82}O_{22}$ requires C, 58.03; H, 7.93%.)

Acidic hydrolysis of **2**. Compound **2** (15 mg) was refluxed with 2 M HCl–MeOH (1:1, 8 ml) on a boiling water bath for 3 hr to afford the aglycone (tigogenin), mp 203–205°, $[\alpha]_D^{20}$ −64° ($CHCl_3$; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 980, 920, 900, 864 (intensity 900 > 920); EIMS: *m/z* 416 $[M]^+$. The neutralized and conc. aq. hydrolysate contained D-galactose, D-glucose and D-xylose (PC). Sugars were estimated by colorimetry using a wavelength of 420 nm.

Partial hydrolysis of **2**. Compound **2** (70 mg) in 1 M HCl–*n*-BuOH (1:1, 20 ml) was heated at 70° for 2 hr. The BuOH layer was washed with 5% $NaHCO_3$ and then with H_2O and concd *in vacuo* to afford a residue, which was purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 13:4:2) to give tigogenin (2.5 mg), PS_1 (6 mg) and PS_2 (20 mg).

PS_1. Colourless plates from MeOH, mp 232–235°, $[\alpha]_D^{20}$ −38.0° (C_5H_5N; *c* 1.0). PS_1 (4 mg) on hydrolysis gave D-galactose and was found to be identical with tigogenin-D-galactoside [8] (IR).

Permethylation of **2** *and* PS_2. Compounds **2** and PS_2 (14 mg each) were separately permethylated with MeI (3 ml) and Ag_2O (200 mg) in DMF (0.5 ml). Usual work-up gave syrups which were purified by prep. TLC (C_6H_6–Me_2CO, 4:1) to yield **2a** (7 mg) and PS_2a (6 mg).

Hydrolysis of **2a** *and* PS_2a. Compound **2a** and PS_2a (6 mg) each) were separately refluxed with 1 M HCl–MeOH (1:1, 5 ml) for 3.5 hr and 2 hr, respectively. The neutralized and concd hydrolysate from **2a** contained 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose and Wallenfel's positive 3,6-di-*O*-methyl-D-galactose, all identical to those in the hydrolysate of **1a**. PS_2a gave methylated sugars identical to those in the hydrolysate of the permethylate of PS_3 [1].

[a]^{13}C NMR data: [a]Assignments are interchangeable between carbons marked with similar sign.

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