Phytochemistry, Vol. 26, No. 6, pp. 1789–1790, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

GITOGENIN-3-*O*-β-D-LAMINARIBIOSIDE FROM THE AERIAL PART OF *AGAVE CANTALA*

DHARAM C. JAIN

Central Institute of Medicinal and Aromatic Plants, Lucknow 226016, India

(*Revised received* 21 *May* 1986)

Key Word Index—*Agave cantala*; Agavacae; steroidal saponin; gitogenin-3-*O*-β-D-laminaribioside.

Abstract—A new steroidal saponin has been isolated from the aerial part of *Agave cantala* and characterized as gitogenin-3-*O*-β-D-glucopyranosyl (1 → 3)-β-D-glucopyranoside.

INTRODUCTION

Agave cantala Linn. is used in folk medicine in India. It exhibits piscicidal and anti-cancer properties [1, 2] and its leaves, fruits and rhizomes contain steroidal saponins [3–6]. This paper describes the isolation and characterization of a new steroidal saponin (**1**) from the aerial part of the plant.

RESULTS AND DISCUSSION

Compound **1** gave positive Liebermann–Burchard and Feigl's tests, but was negative to Ehrlich's reagent. Its IR spectrum showed the characteristic absorption bands for a 25*R* spirostane nucleus [7], the 25*R* stereochemistry of which was confirmed by ^{13}C NMR spectroscopy [8]. Acid hydrolysis of **1** gave the aglycone **2**, $C_{27}H_{44}O_4$ (M^+, *m/z* 432), which was identified as gitogenin by IR, MS, ^{1}H and ^{13}C NMR [9] and by direct comparison with an authentic sample. Acetylation of **2** gave the diacetate **3**, $C_{31}H_{48}O_6$ (M^+, *m/z*, 516; IR ν_{max}^{KBr} 1738 and 1242 cm^{-1}), the ^{1}H NMR spectrum of which showed the presence of a 2α- and a 3β-acetoxy group (3H, *s*, δ2.02 and 2.03; 1H, *m*, δ4.81; 1H, *m*, δ5.05). The only sugar in the aqueous hydrolysate was D-glucose (PC and GLC). These results, the presence of two anomeric C signals in the ^{13}C NMR spectrum of **1** and the elemental analysis, $C_{39}H_{54}D_{14}$, of **1** suggested that the new compound was a diglucoside of gitogenin.

Methanolysis of the permethylate (**4**) of **1**, prepared by Hakomori's method [10], gave two methylated sugars which were identified by GC as the methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose. The periodate oxidation results also indicated the absence of free vicinal hydroxyl groups in the inner glucose.

Comparison of the ^{13}C NMR signals of **1** (Table 1) with those of **2**, and methyl-β-D-laminaribioside [11] showed it to be a diglucoside with a 1 → 3 glycosidic linkage between the two glucose units. Thus the C-3 resonance of the inner glucose residue was shifted +9.0 ppm downfield whilst the C-2/C-4 resonance was shifted −2.3 ppm upfield compared to the equivalent signal of methyl-β-D-glucopyranoside [11]. The C-3 signals of the aglycone were shifted downfield, while the C-2 and C-4 signals were shifted upfield. These changes in chemical shifts are explained by the glycosidation shifts [12] of the 3-*O*-glucosidic structure. No shift for the C-1 signal was observed. Thus the absence of glycosidation at C-2 was confirmed. The nature of the sugar linkage in **1** was established as β by enzymatic hydrolysis and ^{13}C NMR data.

Based on the above data the structure of compound **1** has been established as gitogenin-3-*O*-β-D-glucopyranosyl (1 → 3)-β-D-glucopyranoside.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR (400 MHz) and ^{13}C NMR (80 MHz): $CDCl_3$ and pyridine-d_5, TMS as internal standard; TLC and CC: silica gel G (BDH) using the solvent system: *a*, $CHCl_3$–MeOH–H_2O 13:7:2; *b*, C_6H_6–Me_2CO 17:3; *c*, BuOH–C_5H_5N–H_2O (6:4:3); *d*, *n*-hexane–EtOAc (2:1); *e*, petrol–EtOAc (1:4). Spray reagents, 10% H_2SO_4 and aniline hydrogen phthalate; GC (sugars): dual FID column (6'), 3% OV-17 chromosorb-W, N_2 10 ml/min, column temp 125° (4 min hold) to 265° at 10°/min (GC-1) or 150° (2 min hold) to 275° at 10°/min (GC-2); DCCC (DCC-A apparatus by Tokyo Rikakikai, Tokyo, Japan): 250 glass tubes (400 × 2 mm, id.).

Isolation of saponin. The air dried aerial part (600 g) of the plant, collected from Kurukshetra (India), was extracted with MeOH at room temperature. The MeOH extract (46.82 g) was taken up in H_2O, defatted with *n*-hexane and extracted with BuOH. The BuOH extract (14 g) was chromatographed on silica gel (150 g), eluted with $CHCl_3$ followed by $CHCl_3$–MeOH (9:1) to give **1** (0.4 g) in nearly pure form. Further purification by DCCC [$CHCl_3$–MeOH–H_2O (7:13:8), descending mode, flow rate (13–15 ml/mn)], afforded pure compound **1** (0.25 g): colourless crystals (from MeOH), mp 235–238° (decomp), R_f 0.20 (system *a*), $[\alpha]_D$ −62.03° (MeOH, *c*, 1). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 981, 955, 920, 898, 865 (intensity 898 > 920 (25*R*) spiroketal) [found C, 62.61; H, 7.29; $C_{39}H_{54}O_{14}$ requires C, 62.73; H, 7.23]; ^{13}C NMR: Table 1.

Hydrolysis of compound **1**. Compound **1** (100 mg) was hydrolysed with 2 M HCl (5 ml) for 5 hr. The usual work-up afforded **2**, (57 mg) as colourless needles (from MeOH), mp 261–262°, R_f 0.25 (system *b*), $[\alpha]_D$ −80.6° ($CHCl_3$; *c*, 1). IR ν_{max}^{KBr} cm^{-1}: 981, 960, 920, 900, 865 (intensity 900 > 920 (25*R*, spiroketal); MS *m/z*: 432 $[M]^+$, 414, 373, 363, 360, 318, 303, 300, 289, 271, 139

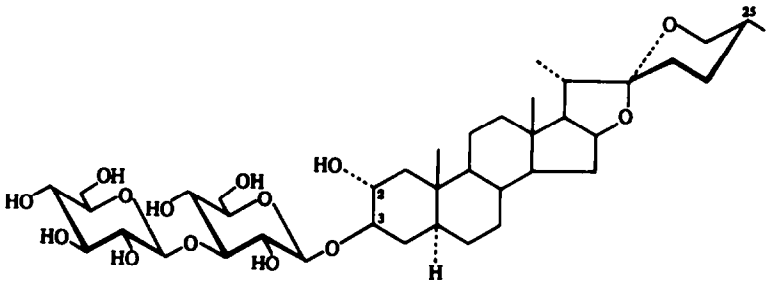

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Table 1. ^{13}C NMR data of compound 1 (pyridine-d_5 at room temperature)

C	1	C	1	C	1
1	45.7	15	32.3*	Glucose (inner)	
2	72.7	16	81.4	1	103.4
3	84.6	17	62.8	2	72.7
4	34.1	18	16.8	3	87.4
5	44.9	19	13.6	4	69.7
6	28.3	20	42.2	5	77.5
7	32.5*	21	15.2	6	62.5
8	34.9	22	109.5	Glucose (terminal)	
9	54.7	23	32.1*	1	104.9
10	37.2	24	29.5	2	75.0
11	21.7	25	30.8	3	78.4*
12	40.3	26	67.2	4	70.5
13	41.0	27	17.6	5	78.2*
14	56.6			6	63.0

*Assignments may be interchanged.

(base peak) and 115; ^{1}H NMR: δ0.76 (3H, *s*, C_{18}-Me), 0.78 (3H, *d*, $J = 6.5$; C_{27}-Me), 0.86 (3H, *s*, C_{19}-Me), 0.95 (3H, *d*, $J = 6$, C_{21}-Me), 3.2–3.6 (4H, *m*), 4.34 (1H, *q*, $J = 6.5$, C_{16}-H); ^{13}C NMR ($CDCl_3$); δ45.0, 73.0, 76.0, 35.4, 44.8, 27.5, 31.8, 34.2, 54.0, 37.4, 21.0, 39.8, 40.4, 56.0, 31.5, 80.5, 62.0, 16.0, 13.0, 41.5, 14.0, 109.0, 31.0, 28.6, 30.0, 66.7, 16.9.

Acetylation of 2. Acetate 3 was prepared from 2 with Ac_2O and C_5H_5N and crystallized from Me_2CO, mp 227° IR ν_{max}^{KBr} cm^{-1}: 1740, 1240 (acetate carbonyl); ^{1}H NMR: δ0.76 (3H, *s*, C_{18}-Me), 0.79 (3H, *d*, $J = 6.5$ Hz, C_{27}-Me), 0.95 (3H, *s*, C_{19}-Me), 0.97 (3H, *d*, $J = 6$ Hz, C_{21}-Me), 2.02 (3H, *s*, $OCOCH_3$), 2.03 (3H, *s*, $OCOCH_3$), 3.38 (1H, *t*, $J = 9$ Hz, 26 α-H), 3.46 (1H, *dd*, $J = 4$ Hz, 4 Hz, 26 β-H), 4.4 (1H, *q*, $J = 6$ Hz, C_{16}-H), 4.81 (1H, *m*, $W_{1/2} = 25$ Hz, 2α-H) and 5.05 (1H, *m*, $W_{1/2}$ 25 Hz, 3β-H); MS *m/z*: 516 $[M]^+$, 501 $[M - Me]^+$ 457, 447, 444, 387, 373, 343, 328, 281, 254 and 139.

The filtrate was neutralized with Ag_2CO_3, filtered and the filtrate concd and examined by PC (system *c*) and GC (Gc-1). The only sugar detected was glucose.

Methylation of 1. Compound 1 (100 mg) was permethylated with NaH and Me_3I by Hakomori's method. The product was purified by CC (system *e*) to afford permethylate 4 (60 mg); IR ν_{max}^{KBr} cm^{-1}: no OH.

Methanolysis of 4. Compound 4 was treated with 3% methanolic-HCl (10 ml). The neutralized (Ag_2CO_3) and concd, mass was examined by TLC (system *d*) and GC (GC-2), and two methylated sugars were detected and identified as the methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose.

Periodate treatment of 1. Compound 1 (20 mg) was taken up in H_2O (5 ml) and treated with 0.05 M $NaIO_4$ (4 ml) in the dark for 36 hr. After usual work up the product was acid hydrolysed, to give gitogenin as a precipitate. Analysis of the concd aq. filtrate by TLC (system *a*) showed the presence of glucose.

Enzymatic hydrolysis of 1. Compound 1 (25 mg) was dissolved in H_2O (3 ml) and emulsin (almond) added. The mixture was then incubated at 37° for 5 days. Analysis of the products by TLC (system *a* and *b*) showed the presence of gitogenin and glucose.

Acknowledgement—I thank Dr. Akhtar Husain, Director, CIMAP, Lucknow, for his interest and encouragement.

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