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CANTALASAPONIN-1, A NOVEL SPIROSTANOL BISDESMOSIDE FROM *AGAVE CANTALA*

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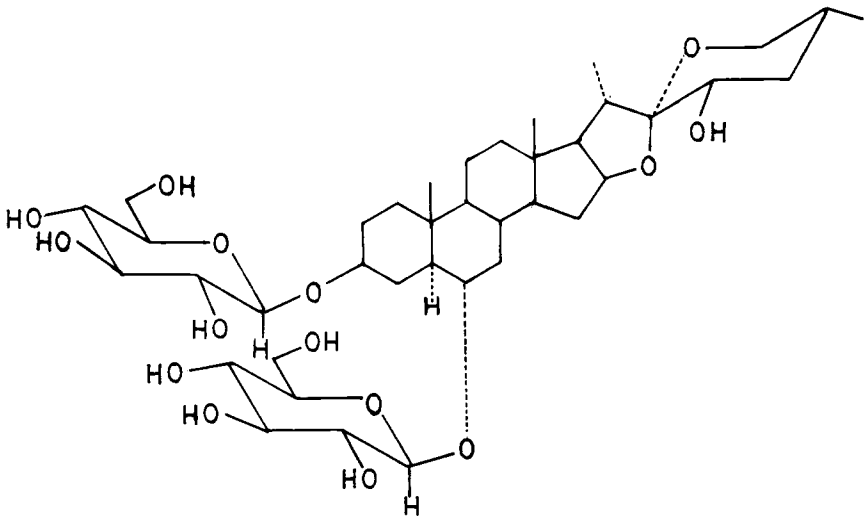
ABSTRACT.—Cantalasaponin-1 (**1**), a novel spirostanol bisdesmoside (the first hongguanggenin-based glycoside), has been isolated from the methanolic extract of the rhizomes of *Agave cantala* and characterized as 3,6-di-*O*-[β-D-glucopyranosyl]-(25 *R*)-spirostan-3β,6α,23α-triol with the help of fdms, fabms, eims, ^{1}H nmr and ^{13}C nmr.

Agave plants exhibit piscicidal (1) and anticancer (2) properties. Two hecogenin-based glycosides, one each from the leaves (3) and fruits (4), and a chlorogenin diglucoside (5) from the leaves of *Agave cantala* Roxb. have been reported. We have previously reported (6) steroidal sapogenins from the EtOAc extract, and here the isolation and characterization of cantalasaponin-1 (**1**), a new spirostanol bisdesmoside (the first hongguanggenin-based glycoside) from the methanolic extract of the rhizomes of this plant is described; **1** has been found to possess cytotoxicity against JTC-26 (originating from human cervical carcinoma) (7).

RESULTS AND DISCUSSION

A very complex saponin mixture obtained from the methanolic extract on repeated cc afforded compound **1** which showed characteristic absorptions of the 25 *R* spirostane nucleus in its ir spectrum. 25 *R* stereochemistry is further confirmed by its ^{1}H-nmr spectrum (8). The results of fdms and fabms (data tabulated in Table 1) of underivatized **1** are complementary to each other, the former providing specific information in the higher and the latter in the lower mass region. The molecular weight of **1** is 772, as evidenced by the appearance of pseudomolecular ions in these spectra at *m/z* 811, 795 (base peak in fdms), and 773 corresponding to $[M+K]^+$, $[M+Na]^+$, and $[M+H]^+$, respectively, and a doubly charged ion at 409 $[M+2Na]^{++}$ in fdms. The peaks at *m/z* 611



1

TABLE 1. Fdms and Fabms Data of **1**[a]

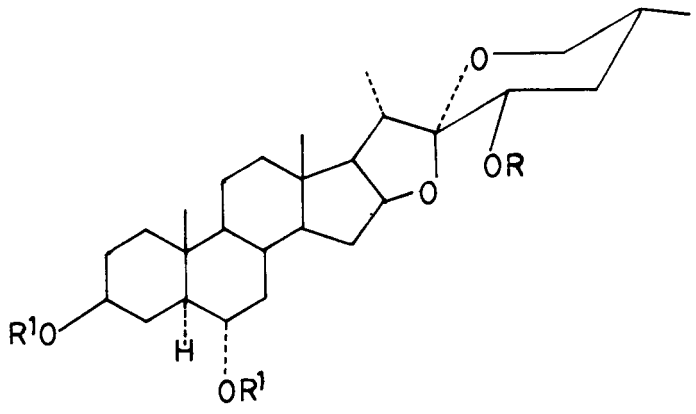
Fdms		Fabms	Fabms (contd.)
811[b]	$[M+K]^+$ (9.58)	811 $[M+K]^+$ (0.34)	395[c] (12.25)
795	$[M+Na]^+$ (100.00)	795 $[M+Na]^+$ (2.71)	363 (1.86)
777	$[M-H_2O+Na]^+$ (2.69)	773 $[M+H]^+$ (0.58)	345 (5.3)
775	$[M-2H_2O+K]^+$ (2.72)	772 $[M]^+$ (0.68)	327 (13.3)
773	$[M+H]^+$ (11.37)	755 $[M-H_2O+H]^+$ (2.08)	271 (33.36)
772	$[M]^+$ (3.02)	611 $[M-162+H]^+$ (1.34)	253 (33.92)
754	$[M-H_2O]^+$ (4.01)	593 $[M-162-H_2O+H]^+$ (3.44)	155 (21.82)
736	$[M-2H_2O]^+$ (2.15)	575 $[M-162-2H_2O+H]^+$ (1.76)	142 (23.25)
611	$[M-162+H]^+$ (3.65)	449 $[M-(2x162)+H]^+$ (2.07)	131 (63.50)
409	$[M+2Na]^{++}$ (4.42)	431 $[M-(2x162)-H_2O+H]^+$ (6.42)	113 (23.14)
163	$[hexose-H_2O+H]^+$ (3.23)	413 $[M-(2x162)-2H_2O+H]^+$ (16.16)	105[c] (100.00)

[a]Assignments of the peaks are shown in brackets and the relative intensities, in parentheses.
[b]*m/z*.
[c]Fragments 395 to 105 mass units in fabms arise from the genin.

$[M-162\ (hexose-H_2O)+H]^+$ and 163 confirm the terminal hexose. The peak at *m/z* 449 $[M-(2\times162)+H]^+$ in fabms corresponds to the loss of the two hexose residues and those at *m/z* 448, 395, 327, 271, 253, 155, 142, and 131 indicate the aglycone to be a saturated trihydroxyspirostane with one of the hydroxyl groups present in ring F.

Acidic hydrolysis of **1** afforded the aglycone **2**, $C_{27}H_{44}O_5$, identified as hongguanggenin (9) by ms, co-tlc, mmp, and superimposable ir. ^{13}C-nmr data (Table 2) of **2** is reported for the first time. The assignment of the signals has been done by comparison with the reported data for chlorogenin (10). The appearance of the C-23 signal downfield by 36.4 ppm, relative to an unsubstituted C-23, is attributed to the hydroxylation shift (11). The aqueous hydrolysate showed the presence of D-glucose only (co-pc).

The methanolysis of the permethylate **1a** of **1**, prepared by Hakomori's method (12), gave the aglycone, **3**, which in ms showed the molecular ion at *m/z* 462 and other peaks at 363, 345, 327, 289, 271, and 253 (similar to those recorded in the ms of **2**). The peaks at *m/z* 169 and 145 indicate **3** to be the 23-*O*-methyl derivative of **2**. Hydrolysis of **1a** gave tetra-*O*-methyl-D-glucose only (pc, authentic sample run in parallel). The above results confirm that the C-3 and C-6 hydroxyl groups of **2** are



1a R=Me, R^1=tetra-*O*-methyl-β-D-glu (pyr)
2 R=R^1=H
3 R=Me, R^1=H

glycosylated, each by one molecule of glucose in **1**. Assignment of ^{13}C-nmr signals of **1** (Table 2) has been done by comparison with the ^{13}C-nmr signals of **2** and of methyl-β-D-glucopyranoside (13, 14). Application of the known glycosylation shift rules (14) reveals the characteristic signal shifts at α- and β-positions of the -OH groups which are

TABLE 2. ^{13}C-Chemical Shifts (δ in ppm) of **1** and **2**.

Carbon Atom	Compound		
	2	**1**	
1	38.0	37.5	
2	32.3	29.8	(−2.5)[c]
3	71.0	79.9	(+8.9)
4	33.6	28.5	(−5.1)
5	52.7	50.8	(−1.9)
6	68.6	77.0	(+8.4)
7	42.8	41.4[b]	(−1.4)
8	34.2	33.9	
9	54.3	53.8	
10	36.6	36.7	
11	21.4	21.2	
12	40.5	40.4	
13	41.4	41.3[b]	
14	56.4	56.3	
15	31.7	31.7	
16	81.6	81.6	
17	62.6	62.5	
18	16.8[b]	16.9	
19	13.7	13.3	
20	38.7	38.8	
21	14.7	14.7	
22	117.7	111.6	
23	67.4	67.4	
24	35.8	35.7	
25	32.1	32.0	
26	66.0	65.9	
27	16.9[b]	16.9	
g-1		106.2[a]	
g-2		75.4	
g-3		77.9	
g-4		71.7	
g-5		78.5	
g-6		63.1	
g-1′		101.6[a]	
g-2′		75.6	
g-3′		78.0	
g-4′		71.7	
g-5′		78.3	
g-6′		62.5	

[a]Assignments are interchangeable between the corresponding carbons in the two glucose residues.

[b]Assignments may be interchanged in the vertical columns between the carbons marked with similar sign.

[c]Values in parentheses denote downfield (upfield) shifts as compared to the aglycone alcohol.

glycosylated. The C-3 and C-6 (α carbon) signals of the aglycone alcohol are shifted downfield by 8.9 and 8.4 ppm, respectively, while C-2 and C-4 signals shift upfield by 2.5 and 5.1 ppm; C-5 and C-7 upfield by 1.9 and 1.4 ppm, respectively. No shift for C-23, C-22, and C-24 signals is observed. Thus, non-glycosylation at C-23 is amply confirmed.

The nature of the sugar linkages in **1** is established as β- by the application of Klyne's rule (15) and by nmr data. The ^{1}H-nmr spectrum displays two doublets (J=7.8 Hz) at δ 4.84 and 5.08 ppm (two C_1-H of glucose units). The ^{13}C-nmr signals of C-3/C-5 of the two sugar moieties occurred at δ values 77.9/78.5 and 77.8/78.3 ppm as expected (14) for β-linked glucose.

Thus, cantalasaponin-1 (**1**) is characterized as 3,6-di-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-spirostan-3β,6α,23α-triol.

EXPERIMENTAL

GENERAL.—Mps were recorded in a Boetius microscopic melting point apparatus. Mass spectra: JEOL JMS DX-300/JMA-3500 (fd-mode: E.H.C.: 22~23 mA, cathode V.: -5kV, accel. V.: 2kV, ion multi. V.: 2.5kV; fab-mode: solv.: DMSO-glycerol, accel. V.: 2kV, gas: Xe; ei-mode: ionizing V. 70eV, accel. V.: 3kV). Nmr spectra: JEOL PS-100 and JEOL FX-100 spectrometer (100 MHz for ^{1}H nmr and 25 MHz for ^{13}C nmr, solvent C_5D_5N, δ values from TMS). Optical rotations: JASCO DIP-SL automatic polarimeter. Cc: Si gel (60-120 mesh, BDH) [i] and Lobar Lichroprep Rp-8 (Merck, size B) [ii]. Tlc: Kieselgel 60 (Merck) [iii] and DC-Alufolien Kieselgel 60 F_{254} (Merck) [iv]. The spots on tlc were detected by spraying with 10% H_2SO_4 and in preparative tlc by spraying with H_2O-Pc: Whatman No. 1 filter paper, aniline hydrogen phthalate as visualizer. Following solvent systems were used: A. $CHCl_3$-MeOH-H_2O (65:35:10); B. MeOH-H_2O (9:1); C. Light petroleum, (60-80)°-EtOAc (2:8); D. C_6H_6-Me_2CO (9:1); E. C_6H_6-Me_2CO (1:1); F. C_5H_5N-EtOAc-H_2O (4:10:3); G. *n*-BuOH-EtOH-H_2O (5:1:4).

ISOLATION.—The rhizomes (6 kg) of the wild-growing *A. cantala* were collected in the month of September from Srinagar, U.P., and specimens were identified by FRI, Dehradun, U.P. (a voucher specimen is available there). The material was dried, powdered, and extracted with EtOAc, and the solvent free mass was exhaustively extracted with MeOH until the extractives became colorless. The concentrated extract was partitioned between H_2O and *n*-BuOH (1:1, 1 liter, 4 times) to isolate (16) the saponin fraction. This fraction (5 gm), purified first over [i] (solvent A) and finally over [ii] (solvent B) to afford **1** (380 mg). The fractions were monitored by [iv].

SAPONIN **1**.—Colorless needles (EtOH), mp 243-245°, $[\alpha]^{15\text{-}17}$D −51.5° (C_5H_5N, c 2.2), ir ν max (KBr) cm^{-1} 3400 (OH), 977, 928, 900, 870 (intensity 900>928, 25 *R* spiroketal); ^{1}H nmr δ 0.64 (3H, s, 18-CH_3), 0.74 (3H, d, 27-CH_3), 0.96 (3H, s, 19-CH_3), 1.18 (3H, d, 21-CH_3), 4.84 and 5.08 (1H each, both d, J=7.8 Hz, C_1-H of two glucose units); ^{13}C-nmr data (Table 2); (found: C, 60.47; H, 8.35. $C_{39}H_{64}O_{15}$ requires C, 60.62; H, 8.29%).

HYDROLYSIS OF **1**.—Compound **1** (130 mg) was hydrolyzed by refluxing with 5% HCl (15 ml) for 3.5 h on a steam bath and cooled. The crude product was removed by filtration and purified by preparative tlc [iii] (solvent E) to afford **2** (25 mg), crystallized from MeOH as colorless needles, mp 235-236°, $[\alpha]^{20}$D −42° ($CHCl_3$-MeOH; c 1.0) [lit. (9) mp 234-237°, [α] D −42.2°]; ir ν max (KBr) cm^{-1} 3500 (OH), 973, 929, 900, 870 (intensity 900>929, 25 *R* spiroketal); eims *m/z* (rel. int.) 448 $[M]^+$ (1.4), 431 $[M\text{-}H_2O+H]^+$ (1.7), 430 $[M\text{-}H_2O]^+$ (1.3), 415 $[M\text{-}H_2O\text{-}CH_3]^+$ (1.6), 413 $[M\text{-}2H_2O+H]^+$ (7.5), 397 $[M\text{-}2H_2O\text{-}CH_3]^+$ (1.77), 395 $[M\text{-}3H_2O+H]^+$ (4.9), 363 (72.5), 345 (100.0), 327 (85.5), 289 (55.7), 271 (86.4), 253 (70.0), 155 (6.4), 142 (21.6), 131 (14.0), 113 (17.5). ^{13}C-nmr data (Table 2).

The aqueous hydrolysate showed the presence of D-glucose only (co-pc, solvent F, Rf 0.23).

COMPOUND **1a**.—Compound **1** (180 mg) was permethylated with NaH and CH_3I (12), and the product was purified by cc (solvent C) to afford **1a** (140 mg), ir ν max (KBr) cm^{-1} no OH; mp 111-113°.

METHANOLYSIS OF **1a**.—Compound **1a** (115 mg) was methanolyzed with 1N HCl-MeOH (1:1, 14 ml). The neutralized (Ag_2CO_3) and concentrated mass was subjected to preparative tlc (solvent D) to afford **3** (10 mg), amorphous solid, eims *m/z* (rel. int.) 462 $[M]^+$ (11.6), 430 $[M\text{-}MeOH]^+$ (0.27), 363 (5.81), 361 (20.1), 345 (3.3), 327 (2.7), 318 (0.89), 303 (0.87), 289 (4.13), 271 (3.39), 253 (2.15), 169 (1.7), 147 (2.78), 145 (2.69), 58 (100.0).

Compound **1a** (15 mg) was hydrolyzed with 5% HCl for 3 h and filtered. The filtrate was neutralized (Ag_2CO_3), filtered, and concentrated under reduced pressure to show the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose only (pc, solvent G, R_G 1.0, authentic sample run in parallel (16)).

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