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ACULEAMINE, A SOLANOCAPSINE-TYPE STEROIDAL ALKALOID FROM *SOLANUM ACULEATUM**

F. COLL, A. PREISS†, M. BASTERECHEA, L. KUTSCHABSKY‡, D. L. MOLA, K. SCHREIBER† and G. ADAM†

University of Havana, Cuba; †Institute for Plant Biochemistry, Academy of Sciences of the GDR, 4010 Halle/Saale, German Democratic Republic; ‡Central Institute of Molecular Biology, Academy of Sciences of the GDR, 1115 Berlin, German Democratic Republic

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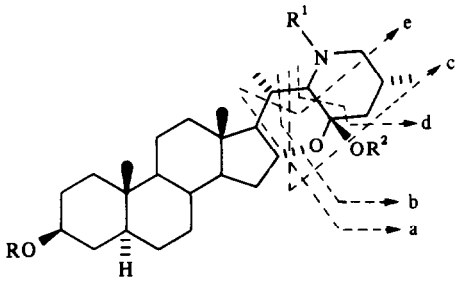
Key Word Index—*Solanum aculeatum*; Solanaceae; roots; steroidal alkaloids; solanocapsine-type alkaloids; aculeamine; X-ray analysis.

Abstract—A new solanocapsine-type alkaloid named aculeamine has been isolated from roots of *Solanum aculeatum* and its structure elucidated by physical methods including X-ray analysis as 22,26-epimino-22β-methoxy-16α,23-epoxy-5α,22αH,25βH-cholestane-3β-ol. The corresponding 23β-ethoxy compound was also isolated as an artefact.

INTRODUCTION

Steroidal alkaloids of the solanocapsine group are rather scarce in the plant kingdom [2]. In a previous paper [1] we reported on the new member 3-desamino-3β-hydroxy-solanocapsine (**1**) from roots of *Solanum aculeatum* Jacq., an endemic species from Cuba. The present communication deals with the isolation and structure of another new solanocapsine-type alkaloid named aculeamine, which was isolated from the same plant source and established as **2** by spectral data, X-ray analysis and partial synthesis. Furthermore, the corresponding ethoxy compound **5** was isolated as an artefact.



	R	R^1	R^2
1	H	H	H
2	H	H	Me
3	Ac	Ac	Me
4	H	NO	Me
5	H	H	Et
6	Ac	Ac	Et
7	H	NO	Et

*Part 112 in the series "*Solanum* Alkaloids". For part 111 see ref. [1].

RESULTS AND DISCUSSION

Acid hydrolysis (N HCl–EtOH) of the glycosidic mixture obtained in the methanol extracts of dried roots followed by silica gel chromatography yielded a mixture of the two alkamines **2** and **5** which were separated on a $AgNO_3$-impregnated silica gel column. The alkaloid **2** has the elemental composition $C_{28}H_{47}NO_3$ ($[M]^+$ found 445.3569; calc. 445.3556) and shows in the IR spectrum hydroxyl absorption at 3300–3400 cm^{-1}. The high resolution EI mass spectrum exhibits a fragment ion at *m/z* 413 due to the loss of methanol from the $[M]^+$ which suggests the presence of a methoxyl group. The important solanocapsine-type fragments [3] at *m/z* 112 (c), 84 (d) and 70 (e), derived from rings E/F, are the same as observed for **1** [1]. On the other hand the intense ions at *m/z* (a, bp) and 144 (b) appear 14 mass units higher than the corresponding key ions of **1** due to the replacement of the 23-hydroxy function by methoxyl.

The 200 MHz ^1H NMR spectrum of **2** is similar to that of **1** [1] but shows an additional singlet at δ 3.18 ppm for a methoxyl group. In the ^{13}C NMR spectrum of **2** signal assignments were carried out by means of the SFORD spectrum and comparison with the data of **1** [1] (Table 1). The chemical shifts values of the ring A, B, C and D carbon atoms (excepting C-15) were in good agreement ($\Delta\delta \leqslant 0.3$ ppm) with the corresponding data of **1**. The signals C-20 to C-27 in the spectrum of **2** correspond to those of **1** but are shifted due to the methylation of the 23β-hydroxyl group. Thus, C-23 is shifted slightly downfield whereas C-24 especially suffered a remarkable high-field shift presumably because of the γ-effect of the methoxy carbon atom [4].

Acetylation of **2** with acetic anhydride–pyridine (24 hr at 20°) yielded the *O,N*-diacetate **3** with IR absorption at 1640 and 1735 cm^{-1} for tertiary amide and *O*-acetyl, respectively. Nitrosation with nitrous acid furnished the *N*-nitroso derivative **4** which showed the same ORD curve as reported for *N*-nitroso-3-desamino-3β-hydroxy-solanocapsine [5].

All these data suggested the alkaloid aculeamine as 22,26-epimino-23β-methoxy-16α,23-epoxy-5α,22αH,25βH-

Table 1. ^{13}C Chemical shift data of **1**, **2** and **5** [50.33 MHz, δ values (ppm) measured from the central solvent line ($CDCl_3$) and calculated to TMS: $\delta_{TMS} = \delta_{CDCl_3} + 77.0$ ppm]

Carbon	1	2	5	Carbon	1	2	5
1	36.8	36.8	36.7	15	28.4†	27.8	28.2
2	31.5‡	31.4‡	31.4‡	16	74.5	74.6	73.9
3	71.3	71.2	71.2	17	60.4	60.1	60.3
4	38.2	38.1	38.1	18	13.6	13.6	13.6
5	45.0	44.9	44.9	19	12.3	12.3	12.3
6	28.6*	28.5	28.6	20	33.0	32.0	32.9
7	31.8‡	31.7‡	31.8*	21	15.1	15.0	15.4
8	34.9	34.8	34.9	22	68.8	67.2	68.7
9	54.8	54.7	54.8	23	96.0	96.7	98.4
10	35.6	35.6	35.5	24	46.2	37.9	40.2
11	20.5	20.4	20.5	25	30.0	25.7	30.8
12	39.2	39.0	39.2	26	55.0	51.8	54.0
13	41.8	42.1	41.9	27	18.7	18.1	18.6
14	54.8	54.9	54.8	28	—	46.7	54.3
				29	—	—	15.4

*,‡ Values bearing the same superscript may be interchanged

cholestane-3β-ol (**2**). This structure was independently. confirmed by X-ray analysis of a single crystal of **2** using direct methods [6]. Crystal data: hexagonal (from acetone–water), space group $P6_1$; unit cell $a = b = 16.706, c = 17.056$ Å; $a = \beta = 90°, \gamma = 120°$; $Z = 6$; $D_x = 1.0759\,cm^{-1}$; $R = 0.079$. The molecular structure is shown in Fig. 1. Finally, aculeamine **2** was synthesized from **1** by treatment with HCl gas/methanol via an elimination/addition reaction sequence similar as described earlier [7] for solanocapsine.

The alkamine **5** has the elemental composition $C_{29}H_{49}NO_3$ ($[M]^+$ found 459.3724; calc. 459.3712) and shows IR absorption at 3350–3500 cm^{-1} for hydroxyl. The high resolution EI mass spectrum displayed a fragment ion at *m/z* 413 due to the loss of one molecule of ethanol. Solanocapsine-type fragments [3] at *m/z* 112 (c), 84 (d) and 70 (e) together with the important key ions at *m/z* 185 (a) and 158 (b), which appeared 14 mass units higher than found for **2**, suggested structure **5** with a 23β-ethoxyl function. Corresponding to this the 200 MHz ^1H NMR spectrum of **5** exhibited additional signals for an ethyl group with a triplet (3H, $J = 7$ Hz) at δ 1.18 for the methyl protons and a multiplet at 3.35 for the two diastereotopic methylene protons (AB-system determined by a decoupling experiment). Also the ^{13}C NMR spectrum is in good agreement with structure **5**. The signal assignment was done by the SFORD technique and comparison with the data for **1** and **2** (Table 1).

The alkamine **5** was further characterized by its *O,N*-diacetyl derivative **6** and *N*-nitroso compound **7**, the latter one showing again the characteristic negative Cotton effect for *N*-nitroso derivatives of the solanocapsine type [5]. Compound **5** was shown to be an artefact produced from **2** during acid hydrolysis of the glycosidic mixture with boiling ethanolic HCl. Thus, upon extraction of the plant material with *iso*-propanol instead of methanol followed by hydrolysis with *iso*-propanol–HCl only the alkaloid **2** but not **5** could be detected.

EXPERIMENTAL

Mps are corr. Optical rotations were measured in $CHCl_3$ and IR spectra in Nujol. UV and ORD were determined in MeOH. High resolution EIMS were recorded at 70 eV: EAMS at 16 eV. NMR were determined in $CDCl_3$. *S. aceuleatum* Jacq. was collected in Guantanamo (Cuba) and identified by M. Sc. A. Arecedes. A voucher specimen is kept in the herbarium of the National Botanical Garden of Cuba, Havana.

Isolation. Dried and powdered roots (500 g) were extracted successively with $CHCl_3$ and with MeOH in a Soxhlet. The MeOH soln was concd to dryness under red. pres., the residue dissolved in 20% HOAc and extracted with C_6H_6–Et_2O to remove pigments. The aq. layer was made alkalined with NH_3, the glycosidic mixture extracted with EtOH and the obtained soln concd to dryness *in vacuo*. The residue was refluxed with 1 N EtOH–HCl (500 ml) for 2.5 hr and poured into H_2O. Alkalization with NH_3, extraction with $CHCl_3$–EtOH (19:1) and evaporation of organic phase gave a residue which was chromatographed over silica gel (Merck). The progress of the separation was followed by TLC on $AgNO_3$ impregnated silica gel plates [8] ($CHCl_3$–MeOH, 9:1). Elution with $CHCl_3$–MeOH

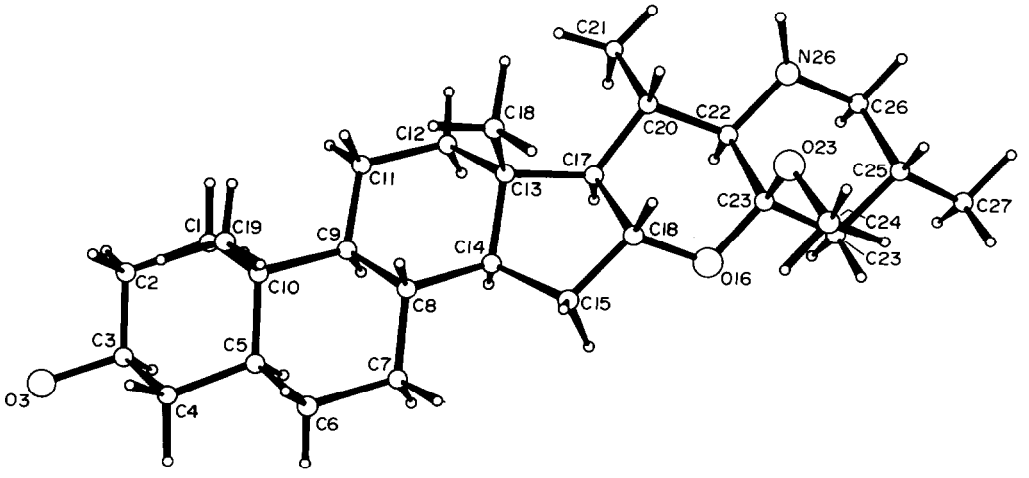


Fig. 1. Molecular structure of aculeamine (**2**).

(19:1) gave 250 mg of crystalline **2** + **5** of mp 167–178°. A second column chromatography on silica gel impregnated with 20% $AgNO_3$ [9] upon elution with $CHCl_3$–EtOAc (3:2) yielded 135 mg (0.008% BDM) aculeamine (**2**). Needles (Me_2CO–H_2O) mp 205–207°, $[\alpha]_D^{24}$ + 50.8° (*c* 0.9), R_f 0.50. ^{1}H NMR: δ 0.72, 0.76 (*s* × 2, H_3-18 and H_3-19), 0.95, 1.19 (*d* × 2, *J* = 6.5 Hz, H_3-21 and H_3-27), 3.18 (*s*, OMe), 3.56 (*m* × 2, H-3α, H-22), 4.09 (*m*, H-16β); EIMS *m/z* (rel. int.): 445 $[M]^+$ (8), 430 $[M-Me]^+$ (15), 413 $[M-MeOH]^+$ (10), 344 (7.5), 171 $[a]^+$ (100), 144 $[b]^+$ (13), 112 $[c]^+$ (24), 84 $[d]^+$, 70 $[e]^+$ (48).

Elution of the $AgNO_3$ impregnated silica gel column with $CHCl_3$–EtOAc (1:1) yielded 85 mg (0.005% BDM) of alkamine **5**. Needles (Me_2CO–H_2O) mp 183–185°, $[\alpha]_D^{23}$ + 45.2° (*c* 0.4), R_f 0.45. ^{1}H NMR: δ 0.72, 0.78 (*s* × 2, H_3-18 and H_3-19), 0.81, 0.97 (*d* × 2, *J* = 6.5 Hz, H_3-21 and H_3-27), 1.18 (*t*, *J* = 7.4 Hz, H_3-29), 3.01 (*m*, H-22), 3.37 (*m*, H_2-28), 3.56 (*m*, H-3α), 4.08 (*m*, H-16β). EIMS *m/z* (rel. int.): 459 $[M]^+$ (7), 430 $[M-C_2H_5]^+$ (36), 413 $[M-EtOH]^+$ (14), 344 (9), 185 $[a]^+$ (100), 158 $[b]^+$ (14), 139 (8), 112 $[c]^+$ (5), 84 $[d]^+$ (10), 70 $[e]^+$ (32). When in the above described extraction, hydrolysis and separation procedures MeOH and EtOH was substituted by *iso*-PrOH only aculeamine **2** could be isolated.

O,N-*Diacetylaculeamine* (**3**). A soln of **2** (20 mg) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) at room temp for 24 hr and worked up as usual. Amorphous (12 mg), $[\alpha]_D^{25}$ − 31.2° (*c* 0.4); IR γ_{max} cm^{-1}: 1735 (OAc), 1640 (>N–Ac), 1240 (OAc). EAMS *m/z* (rel. int.): 529 $[M]^+$ (51), 511 $[M-H_2O]^+$ (18), 486 $[M-Ac]^+$ (8), 469 $[M-AcOH]^+$ (15), 213 $[a]^+$ (100), 198 (71), 186 $[b]^+$ (48), 154 $[c]^+$ (68), 126 (31).

N-*Nitrosoaculeamine* (**4**). To a soln of **2** (20 mg) in 1 ml HOAc satd aq. $NaNO_2$ soln (5 ml) was added dropwise with stirring at 0°. After extraction with $CHCl_3$ the organic phase was washed with 0.5 N NaOH, 0.5 N HCl and H_2O and dried over $NaSO_4$. Evaporation *in vacuo* gave a residue which was crystallized from Me_2CO–H_2O. Needles (9 mg) mp 197–198° (dec), $[\alpha]_D^{24}$ + 153.1° (*c* 0.32). UV: λ_{max} nm (ε): 365 (100), 242 (4300). ORD (*c* 1): $[\phi]_{396}$ − 1420°; $[\phi]_{346}$ + 9480° (a-109).

Aculeamine (**2**) *from* **1**. To a soln of **1** (30 mg) in MeOH (15 ml) a cold stream of dry HCl gas was bubbled until no more **1** was detected by TLC. Dilution with H_2O and alkalization with aq. NH_3 yielded a product, which was chromatographed on silica gel (15 g). Elution with $CHCl_3$–MeOH (9:1) furnished 14 mg (47%) of a white solid which crystallized from Me_2CO–H_2O, needles mp 206–207° and $[\alpha]_D^{25}$ + 51.6° (*c* 0.5) identical in every aspect with **2** from *S. aculeatum*.

Diacetyl derivative (**6**). Acetylation of 15 mg **5** as described for **2** yielded 8 mg amorphous **6**; $[\alpha]_D^{24}$ − 18.1° (*c* 0.3). IR ν_{max} cm^{-1}: 1725 (OAc), 1650 (>N–Ac). EAMS *m/z* (rel. int.): 543 $[M]^+$ (50), 528 $[M-Me]^+$ (35), 514 $[M-C_2H_5]^+$ (18), 497 $[M-EtOH]^+$ (74), 472 (22), 473 $[M-AcOH]^+$ (17), 227 $[a]^+$ (100), 212 (68), 200 $[b]^+$ (42), 154 $[c]^+$ (38).

N-*Nitroso derivative* (**7**). Nitrosation of 15 mg **5** as described for **2** yielded after recrystallization from Me_2CO–H_2O 7 mg **7** as needles mp 176° (dec) and $[\alpha]_D^{25}$ + 141.3° (*c* 0.35). UV: λ_{max} nm (ε): 368 (100), 242 (4200). ORD (*c* 0.85): $[\phi]_{396}$ − 995° $[\phi]_{346}$ + 8530° (a-95).

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