Phytochemistry, Vol. 23, No. 9, pp 2057–2058, 1984
Printed in Great Britain

0031–9422/84 $3.00 + 0.00

HAVANINE—A STEROIDAL ALKALOID GLYCOSIDE FROM *SOLANUM HAVANENSE**

M. BASTERECHEA,† A. PREISS,‡ F. COLL,† D. VOIGT,‡ J. L. MOLA† and G. ADAM‡

†University of Havana, Havana, Cuba; ‡Institute for Plant Biochemistry, Academy of Sciences of the G.D.R., Halle/S., German Democratic Republic

(*Received* 9 *December* 1983)

Key Word Index—*Solanum havanense*; Solanaceae; steroidal glycoalkaloids; 22,26-epiminocholestane-type alkaloids, havanine; (25*S*)-*O*(3)-β-D-glucopyranosyl-16α-acetoxy-22,26-epiminocholesta-5,22(*N*)-dien-3β-ol; ^{13}C NMR.

Abstract—A new steroidal alkaloid glycoside named havanine has been isolated from the leaves of *Solanum havanense* and its structure elucidated by spectral data as (25*S*)-*O*(3)-β-D-glucopyranosyl-16α-acetoxy-22,26-epiminocholesta-5,22(*N*)-dien-3β-ol.

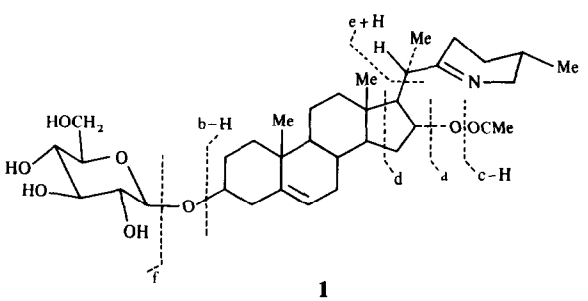
INTRODUCTION

From *Solanum havanense* only the steroidal alkaloids tomatidenol and etioline have so far been isolated after acidic hydrolysis of a glycosidic fraction [1]. We now report the isolation and structure elucidation of a new steroidal glycoalkaloid named havanine, which was isolated from this plant and established as **1** by spectral data.

RESULTS AND DISCUSSION

Al_2O_3 chromatography of the glycosidic mixture obtained from the ethanol extracts yielded 0.12% of the new glycoside, mp 186–187°. Its IR spectrum (KBr) showed the presence of *O*-acetyl (1260, 1735 cm^{-1}), an azomethine function (1660 cm^{-1}) as well as hydroxyl groups (3420 cm^{-1}). Acidic hydrolysis of **1** yielded etioline and glucose, the latter being detected by GC of the trimethylsilyl derivative.

The EI mass spectrum of **1** showed no molecular ion but an $[M-H_2O]^+$ peak (found: 599.3808; calculated for $C_{35}H_{53}NO_7$: 599.3822) besides typical ions at *m/z* 557.3758 (calculated for $[M-MeCOOH]^+$ 557.3716; $[a+H]^+$), 395.3201 ($[M-glucose-CH_2CO]^+$; calculated 395.3188; $[b-H, c-H]^+$) and 378.3111 ($[M-glucose-MeCOO]^+$; calculated 378.3161; $[a+b]^+$). Two fragments at *m/z* 163.1339 (calculated 163.1361; $C_{11}H_{17}N$; [d, $a+H]^+$) and 125.1222 (calculated 125.1204; $C_8H_{15}N$; [e $+H]^+$) are typical for the ring D fragmentation and the $\Delta^{22(N)}$-unsaturated side chain moiety [2] of **1**, respectively. The ion at *m/z* 162 $[f]^+$ indicates the sugar moiety [3]. In the FAB mass spectrum (positive mode) the peak of highest mass number is observed at *m/z* 618 ($[M+H]^+$) besides fragments at *m/z* 558, 454, 438, 392, 378 and 125 indicating losses as discussed above for the EI spectrum and the sugar fragments *m/z* 180, 179 and 162, respectively.



1

The monoglycosidic nature of **1** was also confirmed by its ^{13}C NMR data (Table 1), in which all 35 carbon atoms were assigned by means of SFORD and APT spectra and comparison with the published data of related compounds. The shift values of the ring A and B carbon atoms were in agreement with the corresponding data of the aglycone of cholesteryl-β-D-glucopyranoside [4] although the signals for the sugar moiety were shifted to higher field due to solvent effects. Much better correspondence was achieved when **1** was measured in pyridine-d_5. The shift values for C-11 to C-27 (except for C-19) corresponded very well to those of the ring C, D and F carbon atoms of *O,O'*-diacetyl-25-isosolafloridine ($\Delta\delta \leq 0.3$) [5]. These correlations indicated clearly that the glucosyl function is located at the 3β-position, whereas the acetoxy group is present at 16α.

In agreement with such a structure, the 200 MHz ^{1}H NMR spectrum of **1** showed two singlets (3H each) at δ 0.76 and 0.97 (H_3-18 and H_3-19), two doublets (3H each, J = 7.0 Hz) at δ 0.89 and 1.08 (H_3-27 eq. and H_3-21), a singlet (3H) at δ 1.99 for the acetyl group, an unresolved triplet at δ 4.93 (H-16β) and a broad singlet at δ 5.32 for the vinylic H-6. A doublet at δ 4.39 (J = 7.5 Hz) for the anomeric H-1 sugar proton indicated the β-configuration of the glucosidic linkage [6].

All these data suggested the glycoalkaloid havanine to be (25*S*)-*O*(3)-β-D-glucopyranosyl-16α-acetoxy-22,26-epiminocholesta-5,22(*N*)-dien-3β-ol (**1**). Other 16α-acetoxy-

*Part 114 in the series "*Solanum* Alkaloids". For Part 113 see Ripperger, H., Schreiber, K. and Symon, D., *Pharmazie* (in press)

Table 1. ^{13}C NMR spectral data of havanine (1) recorded at 50.33 MHz in $CDCl_3$*

Carbon		Carbon		Carbon	
1	37.2 (37.4)	13	43.4	25	27.5
2	29.6 (30.2)	14	53.9	26	56.9
3	79.2† (78.6)	15	34.6	27	19.5‡
4	38.8 (39.3)	16	79.4†	$\underline{C}H_3$–CO	21.6
5	140.4 (141.0)	17	58.8	Me–$\underline{C}$O	170.3
6	121.8 (121.5)	18	13.3	1′	101.2 (102.7)
7	31.8 (32.0)	19	19.3‡	2′	73.4 (75 3)
8	31 2 (31 3)	20	45.8	3′	76.4§(78.4)
9	49.9 (50.2)	21	17 3	4′	69 8 (71.8)
10	36.7 (36.9)	22	173.6	5′	75.5 (78.2)
11	20.8	23	26.9	6′	61.9 (62.9)
12	39.8	24	28.1		

*δ Values were measured from the central solvent line and calculated to TMS; δ values in parentheses were measured in C_5D_5N.

†,‡Values bearing the same superscript may be interchanged.

§Overlapped by a solvent line from $CDCl_3$.

lated epiminocholestane-type steroidal alkaloids are solaphyllidine from *Solanum hypomalacophyllum* [7] and muldamine from *Veratrum californicum* [8].

EXPERIMENTAL

S. havanense Jacq. was collected in Caimito, Havana, Cuba and identified by M. Sc. A. Areces. A voucher specimen has been deposited at the herbarium of the National Botanical Garden of Havana, Cuba.

Isolation. Dried and powdered leaves (250 g) were extracted with $CHCl_3$ in a Soxhlet and the residual plant material was then macerated with 95% EtOH for 48 hr at room temp. After filteration, the plant material was extracted 3 × with 95% EtOH for 5 hr at room temp. The combined EtOH extracts were concd to dryness under red pres. and the residue was dissolved in 20% HOAc and extracted 3 × with C_6H_6–Et_2O (1:1) to remove pigments. The acid phase was made alkaline with NH_3 and the precipitated glycosidic mixture (32.4 g) chromatographed on Al_2O_3 (Brockmann, grade II). The progress of the separation was followed by TLC on silica gel (Merck) ($CHCl_3$–MeOH, 17:3). Elution with $CHCl_3$–MeOH (17:3) gave 296 mg havanine (1). Crystals (MeOH), mp 186–187°, $[\alpha]_D^{25}$ −110.8° (MeOH; *c* 1). EIMS 70 eV *m/z* (rel. int.): 599 (6), 557 (64), 437 (1), 423 (1), 395 (30), 378 (26), 270 (2), 204 (6), 163 (89), 162 (100), 125 (15). FABMS positive mode *m/z* (rel. int.): 618 (48), 558 (13), 454 (8), 438 (19), 392 (11), 378 (26), 125 (100). Further spectral data in the text.

Hydrolysis. 1 (100 mg) was refluxed for 5 hr with 10 ml 1.5 M HCl, cooled, poured into an equal vol. of H_2O and made alkaline with NH_3. The ppt. was collected by centrifugation and recrystallized from Me_2CO–MeOH to give etioline, mp 164–165°, $[\alpha]_D^{24}$ −5.5° ($CHCl_3$; *c* 0.8) (lit. [1] mp 153–156°, $[\alpha]_D^{20}$ −4.2°) identical to an authentic specimen.

The aq soln was neutralized with Amberlite IR-4B and concd to dryness under red. pres. 10 mg of the residue was dissolved in 1 ml dry pyridine and treated with 0.2 ml HMDS and 1 ml TMCS. The TMSi derivative was shown to be identical to authentic tetra TMSi glucose by GC analysis [FID detector, N_2 (60 ml/min), glass column packed with 3% OV-1 on Chromosorb W].

Acknowledgements—We thank M. Sc. A. Areces from the National Botanical Garden of Cuba, Havana, for his help in collecting plant material; Dr. W. Schade from the Central Institute for Microbiology and Experimental Therapy of the Academy of Sciences of the G.D.R., Jena, for the high resolution MS; and Dr. G. Dube from the Central Institute of Physical Chemistry of the Academy of Sciences of the G.D.R., Berlin, for the FABMS.

REFERENCES

1 Ripperger, H. (1977) *Pharmazie* **32**, 537
2. Ripperger, H. and Schreiber, K. (1982) in *The Alkaloids* (Manske, R. H. F. and Rodrigo, R. G. A., eds.), Vol. XIX, p. 97. Academic Press, New York.
3. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 207. Holden-Day, San Francisco.
4. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
5. Bird, G. J., Collins, D. J., Eastword, F. W. and Exner, R H. (1979) *Aust. J Chem* **32**, 787.
6. Heguchi, R., Miyahara, K. and Kawasaki, T. (1972) *Chem. Pharm. Bull. (Tokyo)* **20**, 1935.
7. Usubillaga, A., Seelkopf, C., Karle, I. L., Daly, J. W. and Witkop, B. (1970) *J Am. Chem. Soc.* **92**, 700.
8. Gaffield, W., Wong, R. Y., Lundin, R. E. and Keeler, R. F. (1982) *Phytochemistry* **21**, 2397