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SOLANOPUBAMINE, A STEROIDAL ALKALOID FROM *SOLANUM PUBESCENS*

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Key Word Index—*Solanum pubescens*; Solanaceae; steroidal alkaloid; 3β-amino-5α,22α*H*,25β*H*-solanidan-23β-ol.

Abstract—Aerial parts of *Solanum pubescens* yielded a new steroidal alkaloid, solanopubamine, the structure of which was elucidated as 3β-amino-5α,22α*H*,25β*H*-solanidan-23β-ol by ^{13}C NMR, ^{1}H NMR, IR, mass spectral analysis and chemical degradation methods.

INTRODUCTION

In our continued chemical examination of the taxon *Solanum pubescens* Willd. [1–3] we isolated a novel steroidal alkaloid, solanopubamine, from the aerial parts. The alkaloid has the molecular formula $C_{27}H_{46}N_2O$ ($[M]^+$, *m/z* 414) as reported in this paper and its structure has been elucidated as 3β-amino-5α,22α*H*,25β*H*-solanidan-23β-ol(3-deoxy-3β-amino-5α,6-dihydroleptinidine, **1**).

RESULTS AND DISCUSSION

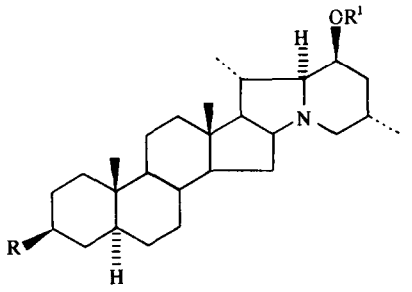
The mass spectrum of **1** showed diagnostic fragmentations [4, 5] at *m/z* 166 (100%) and 220 (24%) for a solanidane skeleton with a hydroxyl in either rings E or F. Furthermore, the mass spectral fragments at *m/z* 370 (5%) and 343 (6%) fixed the hydroxyl on C-23 [6]. The ions at *m/z* 56 (9%) and 82 (8%) indicated the presence of a 3-amino group [4], accounting for the second nitrogen, one being in the indolizidine moiety.

The ^{1}H NMR spectrum of **1** at 270 MHz in deuterochloroform plus deuteromethanol showed two tertiary methyl groups at δ0.85 (3H, *s*) and 0.89 (3H, *s*) and two secondary methyl groups at δ0.98 (3H, *d*, $J = 6.5$ Hz) and 1.20 (3H, *d*, $J = 7$ Hz) which were allocated to Me-18, Me-19, Me-21 and Me-27, respectively.

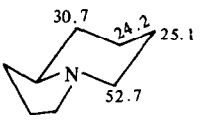
Solanopubamine (**1**) formed a diacetate, **2** ($C_{31}H_{50}N_2O_3$). Its 200 MHz ^{1}H NMR spectrum in deuterochloroform indicated two acetyl signals at δ1.95 and 2.02. The protons due to the 3*N*-acetyl group appeared as a broad doublet at δ5.28 (1H, $J = 8$ Hz) and a singlet at δ1.95 (3H) and were assigned to NH and $NHCOCH_3$. The 23 *O*-acetyl signal appeared at δ2.02.

The broad low-field signal at δ3.04 (1H, $W_{1/2} = 18$ Hz) in **1** which shifted on acetylation to δ3.75 was allocated to the proton on C-3 bearing the amino group [7] and assigned an axial orientation of H-3 from the $W_{1/2}$ values.

The characteristic intramolecularly hydrogen bonded hydroxyl absorption band at ν_{max}^{KBr} cm^{-1}: 3530 [8] in the IR spectrum of **1** remained unchanged in dilution experiments. The hydroxyl was allocated to C-23, as that was the only available position for a secondary hydroxyl to form an intramolecular hydrogen bond with the indolizidine nitrogen lone pair. The signal at δ3.83 (1H, $W_{1/2} = 7$ Hz) in **1** and δ5.02 in its acetate, **2**, were assigned to the proton on C-23 bearing the hydroxyl group. The $W_{1/2}$ value of 7 Hz for H-23 indicated an axial orientation of the OH-23



1 R = NH_2, R^1 = H
2 R = NHAc, R^1 = Ac
3 R = OH, R^1 = H



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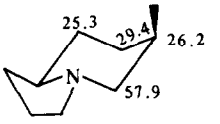

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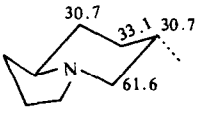

10

group [6]. The absence of any other signal beyond δ3.00 except for the protons at C-3 and C-23 indicated that both the protons at C-16 and C-22 must be *trans* to the lone pair of electrons on the nitrogen as in other solanidanes [6].

The presence of the Bohlmann band at ν_{max}^{KBr} cm^{-1}: 2750 in the IR spectrum of **1** supported the *trans*-indolizidine fusion of the E and F rings similar to that of solanidine. The signal at δ2.77 (*d*, J = 11 Hz) was assigned to the equatorial H-26 by analogy with that of solanogantamine [6].

The ^{13}C NMR assignment of solanopubamine (**1**) (Table 1) was based on the data of 3β-amino-5α-cholestane (**4**) [9], jurubidine (**5**) [9], indolizidine (**6**) [10] and demissidine (**7**) [11]. The chemical shift values for the A–C ring carbons in **1** were similar to that of **4** and **5** and supported the allocation of the amino group to C-3 and assigns the stereochemistry of rings A–C as all *trans*. A comparison of the D–F ring signals of **1** with those of **6** and **7** indicated that the observed $\Delta\delta$ values within the reported range [9, 12] are in excellent agreement and supported the axial orientation of OH-23 in **1**. The α-(23) and β-(22 and 24) carbons moved downfield by $\Delta\delta$ +36.4 (δ65.7), +4.4 (δ79.1) and +2.8 (δ33.9), respectively. The γ-(20 and 25) carbons showed an upfield shift of $\Delta\delta$ −5.8 (δ30.9) and −3.3 (δ28.0), respectively.

A study of the effect of the methyl substituent of indolizidine as of cyclohexane was found to be useful in assigning the orientation of the methyl group attached to C-25. These effects were calculated using the $\Delta\delta$ values from cyclohexane to methylcyclohexane [13]. From the calculated values for **9** and **10**, C-25 was expected to resonate at δ26.2 for axial orientation and at δ30.7 for equatorial orientation of the Me-27 group.

The chemical shift due to C-25 appeared at δ28.0 ($\Delta\delta$ −2.7). The upfield shift from the calculated value can be attributed to the γ-effect experienced by this carbon due to the OH-23 group [12, 13] ($\Delta\delta$ is −3.3 when compared to the demissidine C-25 signal at δ31.3). Hence, the orientation of the Me-27 group in **1** was assigned as equatorial. As the expected chemical shift value must be further upfield from the calculated δ26.2 for an axial methyl group, this possibility was ruled out.

The equatorial orientation was further supported by deamination of **1**. Nitrous acid deamination of **1** produced a diol as the major product which was found to be dihydroleptinidine (**3**) [14] from mp 221–222°, $[\alpha]_D$ +29.7° ($CHCl_3$; *c* 0.39), IR ν_{max}^{KBr} cm^{-1}: 3600, 3500, 2750, 833 and ^{13}C NMR spectral data (Table 1). The formation of **3** supported the orientation of the OH-23 and Me-27 groups as β-axial and α-equatorial, respectively.

Solanopubamine was found to be a stereoisomer of solanogantamine [6, 15] and their non-identity was proved by direct comparison with an authentic sample by mmp and co-TLC. The deamination products from these two compounds were also found to be different (Table 2).

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR spectra were measured at 270, 100, 99.5 and 199.5 MHz, ^{13}C NMR at 25 and 50 MHz.

Table 1. ^{13}C NMR data of compounds **1** and **3**–**7**

Carbon No.	**1** (deutero-pyridine)	**3** (deutero-chloroform)	**4** [9]	**5** [9]	**6** [10]	**7** [11]
1	37.5	37.1	37.8	37.7	—	37.1
2	32.2	31.6	32.6	32.3	—	31.6
3	50.9	71.4	51.2	50.9	—	71.3
4	40.1	38.3	39.6	40.1	—	38.3
5	45.1	45.0	45.7	45.5	—	45.0
6	28.7	28.7	28.9	28.6	—	28.8
7	31.5	32.3	32.3	31.7	—	32.3
8	35.3	35.4	35.6	35.2	—	35.4
9	54.3	54.5	54.6	54.5	—	54.6
10	35.7	35.6	35.6	35.6	—	35.6
11	21.0	21.1	21.2	21.0	—	21.1
12	36.9	39.6	40.2	37.6	—	40.2
13	41.4	41.5	42.7	40.6	—	40.6
14	57.4	57.5	56.4	56.4	—	57.4
15	27.5	31.6	24.3	30.9	—	33.5
16	69.8	69.6	28.3	80.9	53.9	69.0
17	62.5	62.2	56.6	62.1	20.3	63.3
18	17.1	16.8	12.1	16.5	—	17.1
19	12.2	12.4	12.4	12.3	—	12.4
20	30.9	30.7	35.9	42.2	30.1	36.7
21	18.7	18.9	18.7	14.3	—	18.3
22	79.1	79.0	36.3	109.7	64.1	74.7
23	65.7	67.0	23.9	26.0	30.7	29.3
24	33.9	37.1	39.4	25.8	24.2	31.1
25	28.0	26.9	28.1	27.1	25.1	31.3
26	58.7	58.7	22.9	65.1	52.7	60.2
27	21.9	22.4	22.9	16.2	—	19.5

Table 2. Physical constants of solanopubamine (1) and solanogantamine, their deamination products and dihydroleptinidine

Compound	Mp	$[\alpha]_D$	(solvent)	Ref.
Solanopubamine (1)	263°	+30.5°	(methanol)	—
Deamination product (3)	221–222°	+29.7°	(chloroform)	—
Dihydroleptinidine	221–224°	+31.3°	(chloroform)	[14]
Solanogantamine	180°	+35°	(chloroform)	[6]
Deamination product	215–216°	+40.4°	(chloroform)	[6]

Leaves and stems of *S. pubescens* Willd. were collected at Nagarjuna Sagar in Andhra Pradesh in Feb. 1982. Air dried, powdered material (5 kg) was extracted with *n*-hexane and MeOH successively.

The concd MeOH extract was separated into phenolic and non-phenolic parts with neutral lead acetate. The non-phenolic part, after de-leading, was refluxed with 10% HCl at 100° for 2 hr, cooled, basified with 10% NaOH soln to pH 8, allowed to stand at room temp., filtered and washed with H_2O. The brown residue was chromatographed on a column of silica gel using C_6H_6, C_6H_6–Me_2CO as solvents. The benzene–Me_2CO (1 + 1) fraction yielded **1**. It was recrystallized from Me_2CO–MeOH to yield white needles, mp 263°. (Found: C, 78.3; H, 11.0; N, 6.60. $C_{27}H_{46}N_2O$ requires: C, 78.2; H, 11.8 and N, 6.7%.) $[\alpha]_D$ +30.5° (MeOH); IR ν_{max}^{KBr} cm^{-1}: 3530 (m), 3075 (m), 2950 (s), 2900 (s), 2850 (m), 2820 (m), 2750 (m), 1530 (m), 1460 (m), 1400 (s), 1330 (s), 1240 (w), 1170 (w), 1150 (w), 1030 (m), 835 (m) and 825 (m); ^1H NMR (270 MHz, $CDCl_3 + CD_3OD$): δ0.85 (3H, *s*), 0.89 (3H, *s*), 0.98 (3H, *d*, J = 6.5 Hz); 1.20 (3H, *d*, J = 7 Hz), 2.77 (1H, *d*, J = 11 Hz), 2.86 (1H, $W_{1/2}$ = 18 Hz), 3.04 (1H, $W_{1/2}$ = 18 Hz), 3.83 (1H, $W_{1/2}$ = 7 Hz); MS *m/z* (rel. int.): 414 $[M]^+$ (27.1), 413 (7.1), 399 (4.9), 396 (1.8), 370 (4.8), 343 (5.5), 220 (24), 166 (100), 82 (8.3), 56 (9.0).

Acetylation of **1**. To a soln of 40 mg **1** in 1 ml C_5H_5N, 0.5 ml Ac_2O was added and kept at room temp. for 40 hr. Excess reagent was removed under red. pres. and recrystallized from MeOH to yield 40 mg white needles, mp 232°. (Found: C, 74.72; H, 9.97; N, 5.56. $C_{31}H_{50}N_2O_3$ requires: C, 74.69; H, 10.00; N, 5.60%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200, 1720, 1680, 1620, 1240; ^1H NMR (200 MHz, $CDCl_3$): δ0.79 (3H, *s*), 0.89 (3H, *s*); 0.93 (3H, *d*, J = 6.3 Hz), 1.13 (3H, *d*, J = 6.8 Hz), 1.95 (3H, *s*), 2.02 (3H, *s*), 2.65 (1H, $W_{1/2}$ = 18 Hz); 2.68 (1H, *d*, J = 11 Hz), 3.75 (1H, $W_{1/2}$ = 20 Hz), 5.02 (1H, $W_{1/2}$ = 8 Hz), 5.28 (1H, *d*, J = 8 Hz).

Nitrous acid deamination of **1**. To a soln of 20 mg **1** in 2 ml 50% HOAc, 200 mg $NaNO_2$ was added and kept at room temp. for 18 hr. The reaction mixture was diluted with H_2O, basified with NH_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O and dried over Na_2SO_4. The major product **3**, was separated by prep. TLC using C_6H_6–Me_2CO (9:1) (R_f 0.32) and recrystallized from Me_2CO to yield 10 mg white plates, mp 221–222°; $[\alpha]_D^{22}$ +29.7° ($CHCl_3$; *c* 0.39); IR ν_{max}^{KBr} cm^{-1}: 3600, 3500, 2750, 833.

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