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16-*EPI*-SILICINE, AN ALKALOID OF THE ERVATAMINE-TYPE FROM *PANDACA CADUCIFOLIA*

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Key Word Index *Pandaca caducifolia*; Apocynaceae; indole alkaloid; silicine; 16-*epi*-silicine; NMR.

Abstract—The structures of two ervatamine-type alkaloids previously isolated from *Pandaca caducifolia* are established as the known silicine and the new 16-*epi*-silicine after careful NMR analysis of the two compounds and their olefinic derivatives.

INTRODUCTION

Alkaloids of the ervatamine-type are well known to possess various pharmacological activities [1, 2]. Furthermore, structure–activity relationships have demonstrated that the junction between the C and D rings is critical for their biological activities [3]. In the course of our studies on *Ervatamia* species [4–7], we have carried out careful NMR analysis of several alkaloids with an ervatamine skeleton isolated from *E. malaccensis* [4, 5] and also completed previous NMR data of several known compounds belonging to this group. This was the opportunity to complete the structural elucidation of alkaloid **J** to be **1**, previously isolated from *Pandaca caducifolia* [8, 9]. This compound was found to be an isomer of alkaloid **K**, also present in this species, which was identified as silicine (**2**) by a comparison with an authentic sample. Silicine (**2**) has been isolated from *Rauwolfia discolor* [10] and occurs also in *Hazunta* ssp. [11–14]. A vobasinic structure was first proposed [10]. This was then revised to 16-demethoxycarbonyl-20-*epi*-ervatamine and fully established by *X*-ray analysis [15, 16]. We report herein on the structure of 16-*epi*-silicine (**1**) according to NMR data.

RESULTS AND DISCUSSION

Alkaloid **J** (**1**) and silicine (**2**) were isolated from the stem bark, root bark and leaves of *P. caducifolia* together with 10 other alkaloids whose structural determinations are reported elsewhere [9, 17]. In the case of **1**, if the configuration 15-β-H is fixed for biogenetical reasons [18, 19], the configurations at C-16 and C-20 still remain unknown. The structure and conformation of **1** were established by analysis of NMR data and comparison with those of silicine (**2**) as follows.

Signals in the ^{1}H NMR and ^{13}C NMR spectra of **1** and **2** (Tables 1 and 2) were unambigously assigned by 2-D experiments. Direct deduction of the junction geometry between the C and D rings of **1** from the H-15/H-16 coupling constant was impossible because of the complexity of their signals and overlapping with other resonances. Selective decoupling experiments and chemical shift variations induced by *N*-(4) oxidation were unsuccessfully tried to simplify these signals. The problem was solved by analysing the D ring protons signals of **1** and by comparison with those of silicine (**2**) which has a *cis*-C/D ring junction and an α-equatorial ethyl side-chain [16].

In both cases (**1** and **2**), the equatorial protons of methylenes CH_2-5 and CH_2-21 were identified by their long-range W-coupling. Their rather high down-field shift ($\Delta\delta$ = + 1 ppm) compared with the respective axial protons, H-5 and H-21, favours an axial orientation of the *N*-(4) doublet. Moreover, the large coupling constant H-5/H-16 (J = 10.8 Hz for **1** and 11.3 Hz for **2**) reveals an axial orientation of H-16. Therefore, the D ring of silicine (**2**) adopts, the same conformation in solution as in the solid state (a) [16], while two chair conformations (a and b) are still possible for compound **1** because of the unknown coupling constant between H-15 and H-16.

The axial position of the ethyl side-chain at C-20 of **1** (a1 or b1) was deduced from the small coupling constant H-20/H-21 (J = 2.2 Hz) by comparison with that of **2** (J = 11.4 Hz), in which the ethyl group is α-equatorial. Furthermore, the observation in **1** of nOes between the terminal CH_3-18 and H-21′ (eq), H-14, H-14′ and H-16, indicated a *trans*-junction between the C and D rings (b_1), and then an α axial ethyl chain configuration. Compound **1** is therefore the 16-epimer of silicine (**2**).

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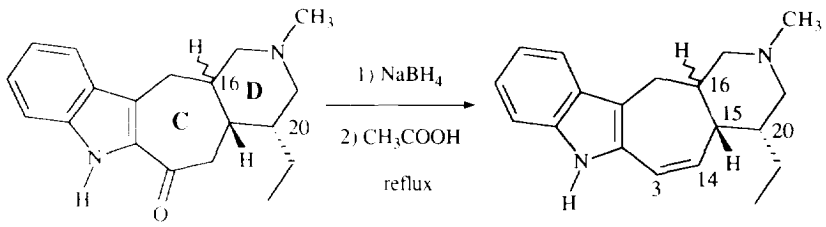
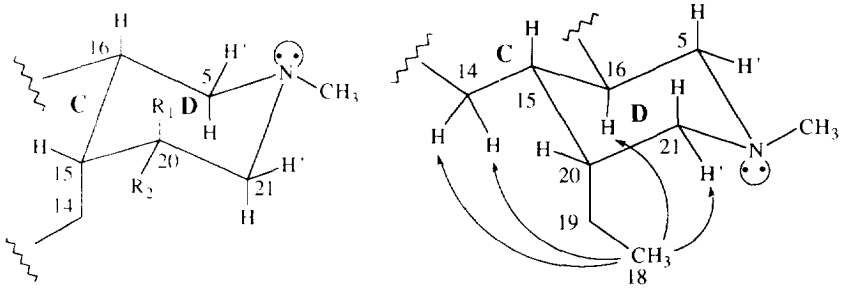


1 H-16α = 16-*epi*-silicine

2 H-16β = silicine

3 H-16α

4 H-16β



a (*cis*-junction)

a1 : $R_1 = CH_2 - CH_3$; $R_2 = H$

2 : $R_1 = H$; $R_2 = CH_2 - CH_3$

b (*trans*-junction)

b1 = 1

nOe interactions

To confirm this structure, we have compared NMR data of the olefinic derivatives **3** and **4** obtained from **1** and **2**, respectively. In **3**, the H-15 proton shows a H-15/H-16 coupling constant (J = 10.8 Hz) fully in agreement with the axial orientations of H-15 and H-16 and a C/D *trans*-junction. In **4**, H-15 appears as a broad singlet consistent with its equatorial orientation and, therefore, a C/D *cis*-junction.

Analysis of the ^{13}C NMR data of **1**–**4** shows that changing from the *cis*-series (**2** and **4**) to the *trans*-series (**1**–**3**) results in deshielding of C-5, C-6, C-7, C-14, C-15 and C-20. This can be interpreted by a more planar geometry of the molecule that releases the steric compression observed in the *cis*-compounds. Conversely, C-19 becomes shielded according to its change from the equatorial to the axial position. These observations fully agree with the proposed conformation of **1** as b.

It should be noted that the majority of ervatamine-type alkaloids belong to the *cis*-C/D series. In the *trans*-series, besides 16-*epi*-methuenine and its *N*-(4)-oxy derivative, alkaloid **1** appears to be the third example of this type. To the best of our knowledge, this compound has only been isolated from *P. caducifolia*.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts are reported in δ from TMS. 1-D (^{1}H and ^{13}C) and 2-D expts were performed using standard Bruker microprograms.

Plant material. Pandaca caducifolia Mgf. was collected at Sahafary, in the Diego Suarez region of northeast Madagascar. Sample was directly compared with holotype, Capuron 20179.

Extraction and isolation. Isolation of **1** and **2** from stem bark, root bark and leaves of *P. caducifolia* is reported in refs [8, 19].

16-epi-*Silicine* (**1**). Ceric-spray: greenish. Mp 153°. $[\alpha]_D$ + 20° ($CHCl_3$; *c* 1). UV λ_{max}^{MeOH} nm: 213, 238, 314. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2940, 2780, 1630, 1580, 1540, 1460, 1330. MS *m/z* (rel. int.): 296 [M]$^+$ (100), 267 (11), 253 (8), 138 (40), 130 (14), 124 (27), 98 (13). ^{1}H, ^{13}C NMR (COSY, NOESY, XHCORRD, COLOC): see Tables 1 and 2.

Silicine (**2**). Ceric-spray: greenish. Mp 95–102°. $[\alpha]_D$ − 20° ($CHCl_3$; *c* 1). UV λ_{max}^{MeOH} nm: 213, 238, 314. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280, 2920, 2780, 1630, 1560, 1520, 1450, 1320. MS *m/z* (rel. int.): 296 [M]$^+$ (64), 268 (5), 199 (10),

Table 1. ^{1}H NMR data (300 MHz) of compounds **1**–**4** (δ; (J = Hz)

H	1*	2*	3†	4†
3	—	—	6.26 *dd* (12.2, 2.8)	6.34 *dd* (12.3, 2.9)
5	1.78 *t* (10.8)	1.98 *t* (11.3)	1.65 *t* (11.3)	1.95 *t* (11.2)
5′	2.97 *ddd* (10.8, 3.6, 1.3)	2.95 *ddd* (11.3, 3.9, 1.1)	2.91 *ddd* (11.3, 3.5, 1.2)	2.38 *bd* (11.2)
6	2.83 *dd* (16.4, 6.5)	2.45 *dd* (15.1, 9.9)	2.39 *dd* (15.7, 10.3)	2.92 *dd* (16.5, 3.4)
6′	3.17 *dd* (16.4, 6.5)	3.42 *dd* (15.1, 6.9)	2.71 *dd* (15.7, 1.6)	3.01 *dd* (16.5, 4.7)
9	7.67 *bd* (8.1)	7.70 *bd* (7.0)	7.30 *bd* (7.0)	7.30 *bd* (7.1)
10	7.15 *ddd* (8.1, 7.3, 1.2)	7.16 *td* (7.0, 1.0)	6.89 *td* (7.0, 1.2)	6.87 *td* (7.1, 0.8)
11	7.34 *ddd* (8.1, 7.3, 1.2)	7.34 *td* (7.0, 1.0)	6.95 *td* (7.0, 1.2)	6.94 *td* (7.1, 0.8)
12	7.41 *bd* (8.1)	7.41 *bd* (7.0)	7.14 *bd* (7.0)	7.12 *bd* (7.1)
14–14′	2.82 *d* (5.7)	2.49–2.60 *m*	5.37 *dd* (12.2, 2.5)	5.43 *dd* (12.3, 1.0)
15	1.73–1.83 *m*	2.04–2.13 *m*	2.3 *ddt* (10.8, 4.0, 2.8)	2.75 *bs* (W 1/2:9)
16	2.18–2.32 *m*	2.23–2.60 *m*	1.83–1.94 *m*	2.26–2.38 *m*
18	0.97 *t* (7.4)	0.86 *t* (7.4)	0.78 *t* (7.4)	0.86 *t* (7.4)
19	1.33–1.47 *m*	1.28–1.47 *m*	1.20–1.40 *m*	1.22–1.36 *m*
19′	1.55–1.73 *m*	1.28–1.47 *m*	1.20–1.40 *m*	1.22–1.36 *m*
20	1.55–1.73 *m*	1.72–1.85 *m*	1.46–1.57 *m*	1.68–1.80 *m*
21	1.87 *ddd* (11.3, 2.2, 1.0)	1.60 *t* (11.4)	1.83–1.94 *m*	1.68–1.80 *m*
21′	2.92 *dt* (11.3, 1.7)	2.68 *bd* (11.4)	2.86 *dt* (11.3, 2.0)	2.53 *bd* (7.8)
N(4)CH$_3$	2.25 *s*	2.33 *s*	2.15 *s*	2.05 *s*
NH	9.15 *bs*	9.42 *bs*	‡	‡

*$CDCl_3$.
†$CDCl_3$ + CH_3OD (two drops).
‡NH exchanged.

Table 2. ^{13}C NMR data (75 MHz) of compounds **1**–**4** (δ; $CDCl_3$)

C	1	2*	3	4
2	132.6	132.4	131.9	132.3
3	194.4	195.2	119.1	121.3
5	63.5	58.6	63.9	56.1
6	27.2	25.8	29.5	27.7
7	123.1	121.6	113.2	110.4
8	127.5	127.1	128.4	128.5
9	120.5	120.6	118.0	117.5
10	120.0	120.0	119.3	118.5
11	126.1	126.2	121.6	121.5
12	112.3	112.2	110.6	110.3
13	136.7	136.8	135.2	135.5
14	46.9	35.4	134.5	126.1
15	42.0	35.4	49.8	41.9
16	35.9	35.4	32.9	37.1
18	12.8	11.4	13.0	10.9
19	18.8	23.7	19.6	23.5
20	43.1	41.1	43.3	42.5
21	57.8	57.3	57.7	56.4

*Data in agreement with ref. [12].

172 (4), 138 (30), 130 (16), 124 (100), 122 (8), 98 (18). ^{1}H, ^{13}C NMR (COSY, NOESY, XHCORRD, COLOC): see Tables 1 and 2.

Dehydroxy-16-epi-*silicinol* (**3**). To a soln of **1** (30 mg) in MeOH (2 ml), 10 mg of $NaBH_4$ was added slowly. The mixt. was stirred for 2 hr at room temp., then dil with H_2O and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over Na_2SO_4 and evapd under red. pres. The residue was dissolved in 1 ml HOAc and the soln refluxed for 3 hr. The soln was then basified with Na_2CO_3 and extracted with CH_2Cl_2. The organic layer was washed, dried and evapd to give a residue (15 mg; yield 53%) which contained **3** in a pure state. Ceric-spray: yellowish. UV λ_{max}^{MeOH} nm: 238, 307 sh, 316, 323 sh, 338 sh. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3220, 2960, 2920, 2860, 2780, 1650. MS *m/z* (rel. int.): 280 [M]$^+$ (100), 265 (16), 251 (16), 237 (13), 236 (13), 222 (16), 208 (16), 194 (14), 180 (19), 168 (12), 167 (12), 138 (30), 137 (16), 124 (10); ^{1}H, ^{13}C NMR (COSY, HMBC, HMQC): see Tables 1 and 2.

Dehydroxy silicinol (**4**). From **2** (30 mg), the same process gave **4** (16 mg) in a pure state. Ceric-spray: yellowish. UV λ_{max}^{MeOH} nm: 241, 310 sh, 318, 326 sh, 342 sh. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1630, 1460. MS *m/z* (rel. int.): 280

$[M]^+$ (79), 279 (14), 265 (3), 251 (8), 237 (5), 180 (11), 124 (100), 98 (23). ^1H, ^{13}C NMR (COSY, HMBC, HMQC): see Tables 1 and 2.

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