



## VULCANINE, A $\beta$ -CARBOLINE ALKALOID FROM *HAPLOPHYLLUM VULCANICUM*

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**Key Word Index**—*Haplophyllum vulcanicum*; Rutaceae;  $\beta$ -carboline alkaloid; vulcanine.

**Abstract**—The isolation of a novel  $\beta$ -carboline alkaloid, vulcanine (1-(2-methyl-1-propenyl)- $\beta$ -carboline) from *Haplophyllum vulcanicum* marks the first occurrence of this skeleton in the genus. Copyright © 1996 Elsevier Science Ltd

### INTRODUCTION

Members of the Rutaceae are known to contain alkaloids that almost exclusively possess the quinoline and furoquinoline rings, while only a few species of diverse genera have been shown to contain simple  $\beta$ -carboline alkaloids [1, 2]. In the course of a phytochemical investigation of *Haplophyllum vulcanicum* growing in Turkey, we had occasion to investigate the contents of a fraction obtained by elution of a basic ethanolic extract through a silica gel column ( $\text{CHCl}_3$ –MeOH, 97.5:2.5). This fraction, further purified by CC followed by preparative TLC, yielded a novel optically inactive  $\beta$ -carboline alkaloid, vulcanine (**1**),  $\text{C}_{15}\text{H}_{14}\text{N}_2$  ( $M_r$  222).

### RESULTS AND DISCUSSION

The structure of **1** was indicated by its low  $M_r$ , deduced from the EI [3] and CI mass spectra and also by the  $^{13}\text{C}$  NMR spectrum, which accounted for a total of 15 carbons. The UV spectrum (MeOH) displayed maxima at 212, 239, 260sh, 292, 345sh and 355 nm ( $\log \epsilon$  4.58, 4.70, 4.43, 4.36, 3.98, 4.01) characteristic of a  $\beta$ -carboline chromophore.

The  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ , 600 MHz) displayed signals for two relatively high-field Me groups and a total of eight protons in the aromatic region. Of the latter, two  $d$  at  $\delta$  8.46 and 7.84 ( $J = 5.3$  Hz) clearly belong to H-3 and H-4 of the  $\beta$ -carboline skeleton, while the  $\text{D}_2\text{O}$ -exchangeable broad  $s$  at  $\delta$  9.22 is characteristic of H-9. Four signals at  $\delta$  8.12 (H-5), 7.53 (H-7), 7.52 (H-8) and 7.28 (H-6) interrelated by spin-decoupling,  $^1\text{H}$ – $^1\text{H}$  COSY and  $^1\text{H}$  TOCSY experiments, verified the unsubstituted nature of the carbocyclic ring. The complex pattern resulting from the second-

order splitting of the latter three signals have been scrutinized by the  $^1\text{H}$  line-shape simulation of the aromatic region using the PANIC program, which afforded a pattern that is strikingly similar to that of the original spectrum.

The remaining high-frequency proton resonating as a  $t$  at  $\delta$  6.63 ( $J$  1.2 Hz) and the two Me groups at  $s$  1.93 ( $d$ ,  $J = 1.2$  Hz) and 2.00 ( $d$ ,  $J = 1.0$  Hz) belong to the substituent at C-1. Spin-decoupling and  $^1\text{H}$ – $^1\text{H}$  COSY experiments indicated a long-range interaction between the olefinic proton and the Me groups. The geminal disposition of the Me groups on a quaternary  $sp^2$  carbon was evident from their relatively high field resonances and the nonequivalence of their chemical shifts. HSQC, HMBC,  $^{13}\text{C}$ -INADEQUATE experiments provided efficient proof that the exact identity of the fragment at C-1 was a 2-methylpropenyl moiety. Unambiguous assignments of the carbon chemical shifts (Fig. 1) were achieved by HSQC, HMBC and  $^{13}\text{C}$ -INADEQUATE experiments. Final confirmation of the structure was obtained by X-ray crystallographic analysis of the hydrochloride salt [4].

Catalytic hydrogenation of **1** resulted in the saturation of the double bond in the side-chain to furnish **2**, which resisted further hydrogenation en route to the tetrahydro- $\beta$ -carboline analogue.

Despite their quite widespread distribution among plants, simple  $\beta$ -carboline alkaloids have been reported so far only in a very limited number of Rutaceous plants. The present study is the first report of the occurrence of a simple  $\beta$ -carboline in a *Haplophyllum* species.

### EXPERIMENTAL

**General.**  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and 2D correlation spectra: Bruker AMX 600. MS: EIMS 70 eV; CIMS,  $\text{NH}_3$ .

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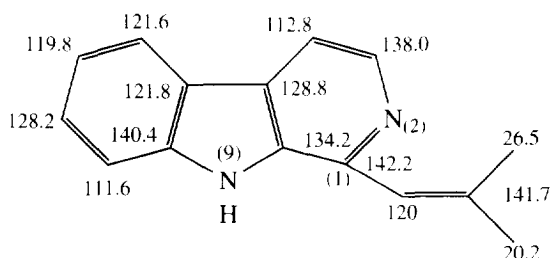


Fig. 1. Assignment of carbon chemical shifts in compound **1**.

**Plant material.** *Haplophyllum vulcanicum* Boiss. et Heldr. was collected from Nigde, Ulukisla, Turkey, in June 1994. A voucher specimen, No 1177, is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

**Extraction and isolation.** Dried and powdered total plant material (40 kg) was extracted with EtOH (800 l) at room temp. to furnish a crude alcoholic extract, which was then dissolved in 5% aq. HCl (20 l) and filtered. The acidic soln was made alkaline with 10% aq.  $\text{NH}_4\text{OH}$  and then extracted with  $\text{CHCl}_3$ . Evapn of the organic solvent furnished 67.14 g of a crude basic extract. During preliminary separation of the components through a column packed with Kieselgel 60 (70–230 mesh ASTM, Merck) using  $\text{CHCl}_3$  gradually enriched with MeOH, elution with 2.5% MeOH in  $\text{CHCl}_3$  afforded a fr. (14.92 g), which was further fractionated by CC on Kieselgel 60H (Merck) using benzene–EtOAc–MeOH– $\text{NH}_4\text{OH}$  (200:20:20:1). Subsequent prep. TLC of one of the early frs (176.7 mg) on ready-made silica gel plates (0.25 mm, Merck) using cyclohexane– $\text{CHCl}_3$ – $\text{Et}_2\text{NH}$  (7:2:1) afforded 44.8 mg of pure vulcanine (**1**), mp (**1**·HCl) 103°. IR  $\nu_{\text{max}}^{\text{CHCl}_3}$ : 1655, 1630, 1570, 1495, 1455, 1425, 1380, 1320, 1235  $\text{cm}^{-1}$ .  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz): Fig. 1 EI-MS  $m/z$  (rel. int.): 222 (100,  $[\text{M}]^+$ ), 221 (16), 208 (11), 207 (69), 206 (31), 205 (13), 182 (14), 103 (24).

**X-ray analysis of 1.**  $\text{C}_{15}\text{H}_{15}\text{N}_2^+\text{Cl}^- \cdot 3\text{H}_2\text{O}$ .  $M_r = 312.80$  g  $\text{mol}^{-1}$ . Yellow prisms, triclinic, space group  $PT$ ,  $a = 9.340(2)$ ,  $b = 12.038(2)$ ,  $c = 7.262(2)$  Å,  $\alpha = 98.66(2)^\circ$ ,  $\beta = 95.62(2)^\circ$ ,  $\gamma = 82.43(2)^\circ$ ,  $V = 797.6(3)$  Å $^3$ ,  $Z = 2$ ,  $D_x = 1.302$  g  $\text{cm}^{-3}$ ,  $m = 0.250$  mm $^{-1}$ ,  $T = 173$  K. Rigaku AFC-5R diffractometer.  $\text{MoK}_\alpha$  radiation,  $\lambda = 0.71069$  Å, empirical absorption correction from azimuthal scans, structure solved by direct methods (SHELXS-80) [4] and refined with TEXSAN (Molecular Structure Corporation, the Woodlands, Texas, 1989). Of the 3893 measured reflections ( $2\theta < 55^\circ$ ), 3668 were unique and 3130 reflections  $|I| >$

$2\sigma(I)$  were used for the least-squares refinement of  $F$  of 274 parameters. Final  $R = 0.0354$ ,  $R_w = 0.0365$ ,  $\text{GoF} = 2.171$ ,  $\Delta_{\text{max}}/s = 0.0007$ ,  $\Delta r_{\text{max}} = 0.27$  e Å $^{-3}$ . Crystal data and atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

**Catalytic hydrogenation of 1.** Compound **1** (45 mg) in 100 ml MeOH was subjected to catalytical hydrogenation using 100 mg Pd/C (5%) for 4 hr at 3.5 bar. The crude product was filtered (Celite), washed with MeOH and purified by prep. TLC to yield 32 mg of amorphous **2**. IR  $\nu_{\text{max}}^{\text{CHCl}_3}$ : 1630, 1570, 1495, 1455, 1425, 1380, 1320, 1235  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  8.23 (1H,  $d$ ,  $J = 5.6$  Hz, H-3), 8.01 (1H,  $d$ ,  $J = 7.9$  Hz, H-5), 7.79 (1H,  $d$ ,  $J = 5.5$  Hz, H-4), 7.56 (1H,  $d$ ,  $J = 8.3$  Hz, H-8), 7.43 (1H,  $dd$ ,  $J = 7.6, 0.8$  Hz, H-7), 7.18 (1H,  $dd$ ,  $J = 8.1, 6.7$  Hz, H-6), 3.06 (2H,  $d$ ,  $J = 7.4$  Hz, H-1'), 2.28 (1H,  $m$ , H-2'), 0.84 (3H,  $s$ , Me), 0.82 (3H,  $s$ , Me);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  145.1, 140.3, 138.1, 134.7, 128.7, 128.2, 121.9, 121.7, 120.0, 112.8, 111.6, 43.2, 28.7, 22.7. EI-MS  $m/z$  (rel. int.): 224 (13,  $[\text{M}]^+$ ), 223 (9), 209 (9), 183 (14), 182 (100), 181 (11), 154 (8), 149 (6).

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