

6-Acetyl-N-methyl-dihydrodecarine, a New Alkaloid from *Zanthoxylum riedelianum*

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Um novo alcalóide benzofenantridínico, 6-acetonil-N-metil-diidrodecarina foi isolado das raízes de *Zanthoxylum riedelianum* juntamente com lupeol, 6-acetonildiidroquelerytrina e 6-acetonildiidroavicina. As estruturas dessas substâncias foram estabelecidas com base na análise dos dados espectrométricos de IV, EM e RMN incluindo experimentos 2D.

A new benzophenanthridine alkaloid, 6-acetyl-N-methyl-dihydrodecarine was isolated from *Zanthoxylum riedelianum* roots together with lupeol, 6-acetyldihydrochelerythrine and 6-acetyldihydroavicine. The structures were established from the IR, MS and NMR spectral data, including 2D-NMR experiments.

Keywords: *Zanthoxylum riedelianum*, Rutaceae, benzophenanthridine alkaloids

Introduction

The *Zanthoxylum* genus (Rutaceae) is composed by more than 200 species and largely distributed around the world.¹ Chemically, this genus is characterized by alkaloids,²⁻⁷ coumarins,^{5,6,8} lignans,^{4,9,10} amides^{11,12} and terpenes.^{5,6,13,14} Ongoing studies have shown that *Zanthoxylum* exhibit a range of biological activities such as antichagas,² tripanocidal,⁹ antiplasmodial,⁷ anti-HIV¹³ and antiinflammatory,^{8,10} as well as anti-helminthic.¹² *Z. riedelianum* is used in folk medicine as a decoction against different types of inflammations, rheumatism and skin stains.¹⁵ Previous works reported the identification of terpenes from the essential oil¹⁴ and lignans from the leaves and the stem bark.¹⁰ In this work we report the isolation and structural elucidation of a novel benzophenanthridine alkaloid, namely, 6-acetyl-N-methyl-dihydrodecarine (**1**), together with two known alkaloids 6-acetyldihydrochelerythrine (**2**) and 6-acetyldihydroavicine (**3**) from the roots of *Z. riedelianum*.

Experimental

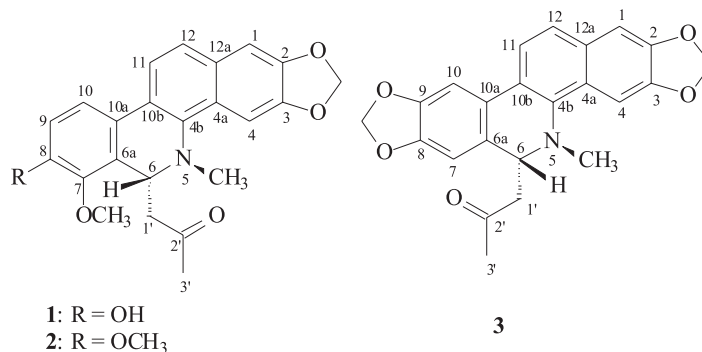
General procedures

Melting points were uncorrected. IR spectra were recorded on FTIR-Bomem-MB/100 model spectrophotometer using NaCl film. NMR spectra in CDCl₃, were recorded on Bruker ARX-400 (400 MHz for ¹H and 100 MHz for ¹³C); Bruker AC-200 (200 MHz for ¹H and 50 MHz for ¹³C) and Varian-Mercury 300 (300 MHz for ¹H and 75 MHz for ¹³C) spectrometers, using tetramethylsilane (TMS) as internal standard. Electron Ionization Mass Spectra (ESI-MS) was undertaken employing a Quatro LC-Micromass UK model spectrometer. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. CC: silica gel (Merck 70-230 mesh ASTM); TLC: silica gel G 60 and silica gel 60 PF₂₅₄ (Merck) were used to analyze the fractions collected from column chromatography (CC) with visualization by UV (254 and 366 nm), Dragendorff's reagent and exposure to iodine vapor.

Plant material

Zanthoxylum riedelianum (Engl.) was collected in Rio Manso Highway, km 22, Chapada dos Guimarães, Mato

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Grosso State, Brazil. A voucher specimen (No. 24.080) was deposited at Universidade Federal de Mato Grosso Central Herbarium.

Extraction and isolation

Dried roots (3.0 kg) were powdered and extracted with hexane and methanol by maceration at room temperature. The macerates were concentrated under reduced pressure to yield the extracts A (21.5 g) and B (200.5 g) from hexane and methanol, respectively. The extract A was partitioned with hexane, dichloromethane, ethyl acetate and methanol. Solvents were removed under reduced pressure and the dichloromethane residue (8.2 g) was submitted to column chromatography, carried out in a gradient system from hexane, dichloromethane, ethyl acetate, acetone and methanol as mobile phase. The 174 collected fractions were reunited in 30 fractions. Fraction 10 (1.0 g) afforded the triterpene lupeol (170.0 mg, mp 162.5-164.2 °C). Fraction 25 (200.0 mg) was submitted to preparative TLC with dichloromethane-methanol (2:8), affording the alkaloid 6-acetyldihydrochelerythrine (**2**, 30.0 mg, mp 171.6-173.0 °C).

The extract B was partitioned successively with hexane, dichloromethane, ethyl acetate and methanol. The dichloromethane residue (530.0 mg) was submitted to column chromatography performed in a gradient system with hexane, dichloromethane, acetone and methanol as mobile phase. The 243 collected fractions were reunited in 33 fractions after TLC comparison. Fraction 4 (190.0 mg) was submitted to preparative TLC eluting with dichloromethane-methanol (1:9), affording the alkaloids 6-acetyl-N-methyl-dihydrodecarine (**1**, 60.0 mg, mp 186-188 °C) and 6-acetyldihydroavicine (**3**, 57.0 mg, mp 184-185 °C).

6-Acetyl-N-methyl-dihydrodecarine, (**1**)

Brown amorphous solid. $[\alpha]_D^{21.5^\circ}$ -5.625 (CHCl₃; conc. 0.014 g mL⁻¹). IR (NaCl film) ν_{\max} /cm⁻¹: 3396, 1708, 1615,

1610, 1516, 1425, 1296, 1239. ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) (Table 1). ESIMS/MS: *m/z* (rel. int.) 391 [M+H]⁺.

Table 1. ¹H (400 MHz) and ¹³C-NMR (100 MHz) spectral data of **1** and **3**, in CDCl₃

C	1		3	
	δ_c	δ_H (m, J in Hz)	δ_c	δ_H (m, J in Hz)
1	104.4	7.10 (s)	107.7	6.82 (s)
2	148.5		148.2	
3	147.5		147.6*	
4	100.5	7.51 (s)	103.6	7.29 (s)
4a	123.9		124.8	
4b	138.8		131.0	
6	54.8	5.0 (dd, 3.5, 10.9)	60.2	4.54 (dd, 5.7, 8.7)
6a	123.3		123.5	
7	151.3		100.5	7.54 (s)
8	144.9		147.2	
9	115.1	7.01 (d, 8.4)	147.6*	
10	119.7	7.54 (d, 8.4)	104.3	7.11 (s)
10a	131.0		123.9	
10b	127.5		127.1	
11	120.0	7.72 (d, 8.5)	119.8	7.64 (d, 8.5)
12	124.7	7.51 (d, 8.5)	124.8	7.51 (d, 8.5)
12a	127.2		128.3	
1' (-CH ₂ CO)	46.5	2.25 (dd, 3.5, 15.2) and 2.66 (dd, 10.9, 15.2)	48.2	2.33 (dd, 5.7, 15.7) and 2.60 (dd, 8.7, 15.7)
2' (-COCH ₃)	207.6		207.7	
3' (-COCH ₃)	31.5	2.02 (s)	31.4	1.97 (s)
OCH ₂ O	101.1	6.05 (s)	101.1 and 101.9	6.10 (d, 2.5) and 6.0 (d, 2.5)
7-OCH ₃	61.9	3.95 (s)		
8-OH		5.30 (s)		
N-CH ₃	42.4	2.65 (s)	42.2	2.60 (s)

*Signals overlapped.

Results and Discussion

The well known natural compounds lupeol,¹⁶ 6-acetyldihydrochelerythrine (**2**)¹⁷ and 6-acetyldihydroavicine (**3**)¹⁸ were identified mainly by ¹H and ¹³C NMR spectral analyses, comparing with previous literature data.¹⁶⁻¹⁸

Compound **1** presented a positive test with Dragendorff's reagent, indicating it to be an alkaloid. The IR spectrum presented bands at 3396 (ν_{OH}), 1708 ($\nu_{\text{C=O}}$), 1615, 1516 cm^{-1} attributed to aromatic rings. The positive ESI-mass spectrum of **1** showed a *quasi*-molecular ion at m/z 391 [M+H]⁺ pointing out to a molecular formula C₂₃H₂₁NO₅. The ¹H NMR spectrum of **1**, exhibited signals corresponding to six aromatic hydrogen, characteristic of a benzophenanthridine system.¹⁸ Accordingly, the aromatic region from compound (**1**) ¹H NMR spectrum exhibited signals of two pairs of one-hydrogen doublets (δ_{H} 7.01 (H-9) and 7.54 (H-10); 7.72 (H-11) and 7.51 (H-12)) and two one-hydrogen singlets (δ_{H} 7.10 (H-1) and 7.51 (H-4)) indicating the presence of four aromatic hydrogens in *ortho* position and two isolated hydrogens. The ¹H NMR spectrum also displayed signals of methylenedioxy group at δ_{H} 6.05 (2H, AB system), hydroxyl group at δ_{H} 5.30 (s, 1H), a methoxyl group at δ_{H} 3.95 (s, 3H) and the *N*-methyl group at δ_{H} 2.65 (s, 3H). In addition to the benzophenanthridine signals, the presence of an acetylonyl group at C-6 was indicated by a methyl singlet at δ_{H} 2.02 and the AMX system with δ_{H} 2.25 (J 3.5 and 15.2 Hz) and 2.66 (J 10.9 and 15.2 Hz), respectively, and δ_{H} 5.0 (J 3.5 and 10.9 Hz), corresponding to the coupling constants between H-6 and the acetylonyl methylene hydrogens in the ¹H NMR and ¹H, ¹H-COSY spectra. The ¹³C NMR spectrum also confirmed the acetylonyl moiety with signals at 207.6 (C=O), 31.5 (COCH₃), 46.5 ($-\underline{\text{C}}\underline{\text{H}}_2\text{CO}$). Through the chemical shifts observed in the ¹³C, ¹H- δ SQC spectrum, it was possible the attribution of each carbon and its respective hydrogen (Table 1). The cross peaks observed in the HMBC spectrum showed long-range couplings from H-6 (δ_{H} 5.0) and H-3' (δ_{H} 2.02) with C-2' (δ_{C} 207.6), confirming the connection of the acetylonyl moiety with C-6. Further correlations were observed between OCH₃ (δ_{H} 3.95) with C-7 (δ_{C} 151.3), as well as H-9 (δ_{H} 7.01) and H-10 (δ_{H} 7.54) with C-8 (δ_{C} 144.9) and C-10 (δ_{C} 119.7), indicating that position 8 is substituted; finally, *N*-CH₃ (δ_{H} 2.65) with C-6 (δ_{C} 54.8). The absence of the methoxyl group in C-7 moves away the effect of the oxygen atom in C-6, justifying its largest chemical shift (δ_{C} 60.2) in compound **3**, when compared with the correspondent δ_{C} 54.8, attributed to C-6 in compound **1**; thus revealing $\Delta_{\text{C}} = 54.9-60.2 = -5.3$ ppm as γ effect. Therefore, the

structure of **1** was established as 6-acetylonyl-*N*-methyl-dihydrodecarine, a decarine derivative.¹⁸ Previous publications,^{17,18} however, reported the isolation of the unstable parent alkaloids avicine and nitidine. Although acetone derivatives of avicine and nitidine have been reported in the literature,^{19,21} as well as acetone adducts of two other benzophenanthridine alkaloids,^{22,23} it is controversial, however, whether those acetone derivatives are really present on the plant extract or were isolated as artifacts, due to the greater stability of the acetone adducts as compared to the parent alkaloids avicine and nitidine.

Acknowledgments

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Supplementary Information

Supplementary data of alkaloids structures as **1** and **2D** ¹H and ¹³C NMR spectra are available free of charge at <http://jbcbs.sbj.org.br>, as PDF file.

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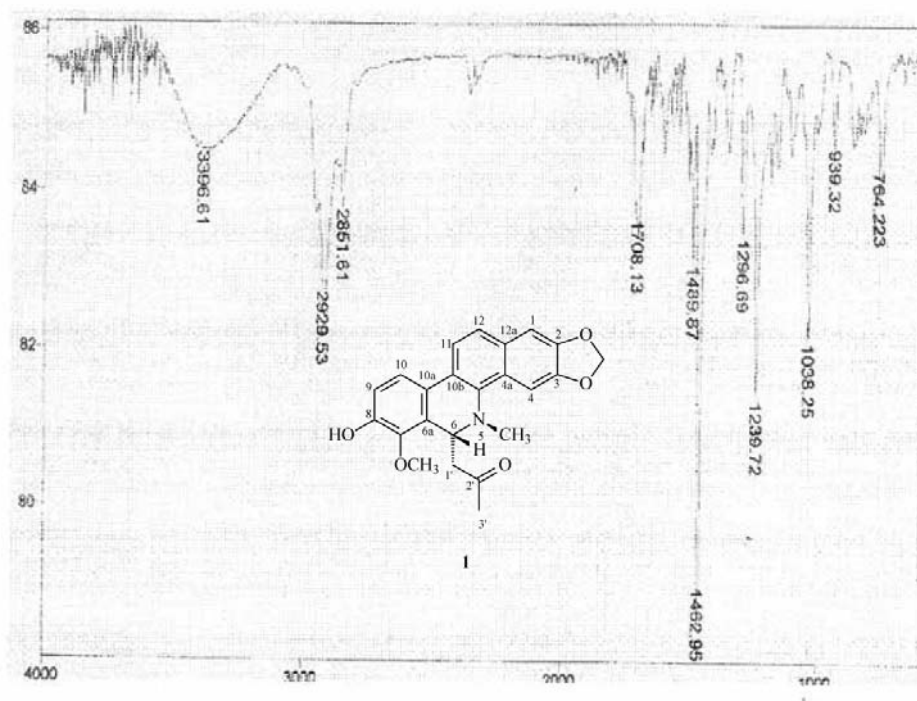


Figure S1. IR spectrum (NaCl film) of **1** (6-acetyl-N-methyl-dihydrodecarine).

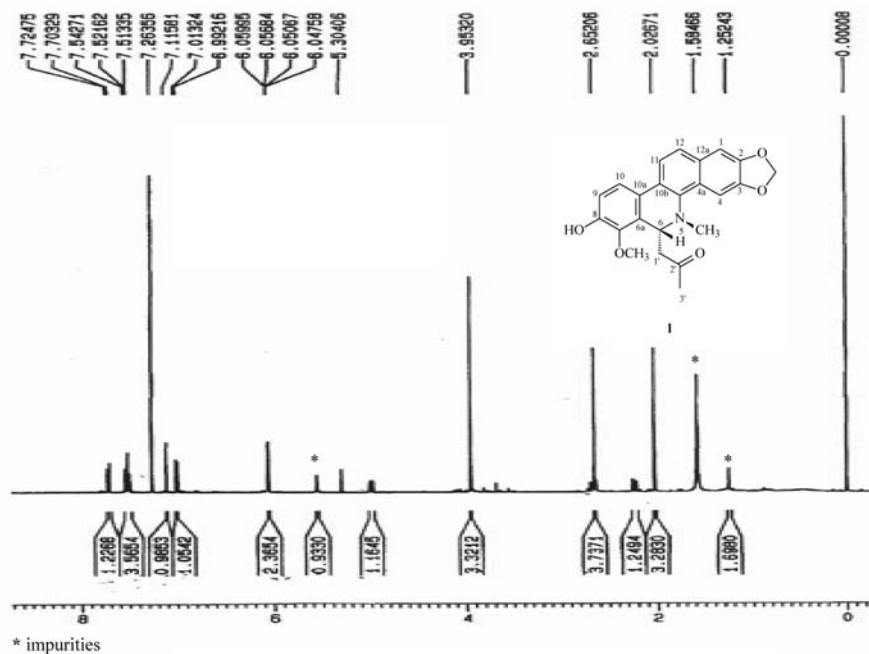


Figure S2. ^1H NMR spectrum (400 MHz, CDCl_3) of **1** (6-acetyl-N-methyl-dihydrodecarine).

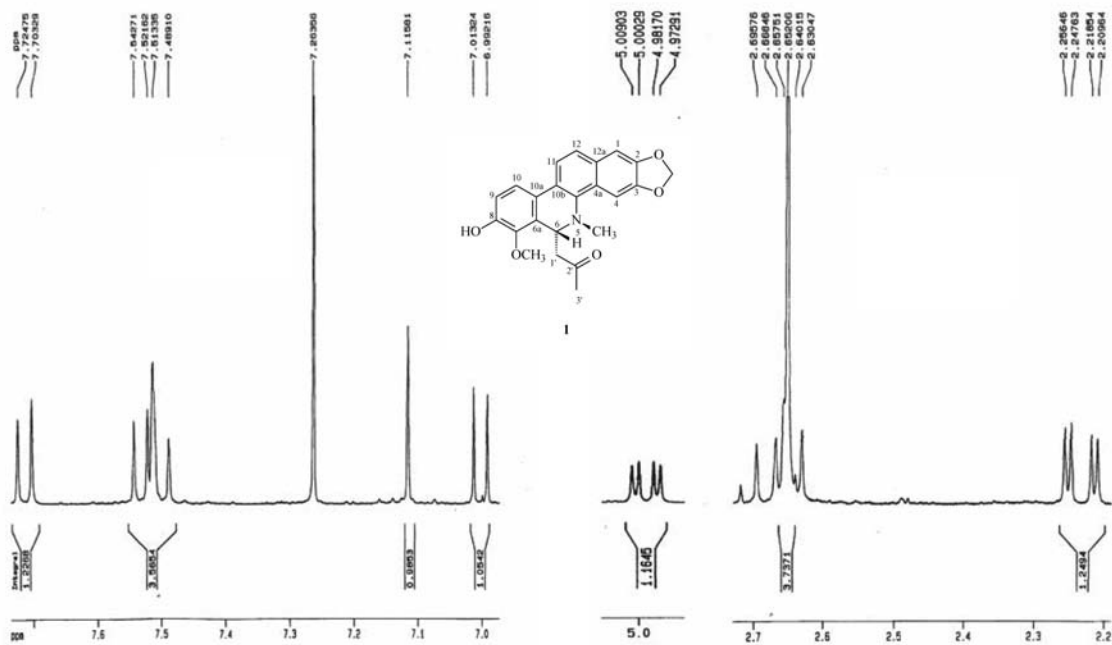


Figure S3. Expansions of the ^1H NMR spectrum (400 MHz, CDCl_3) of **1** (6-acetyl-N-methyl-dihydrodecarine).

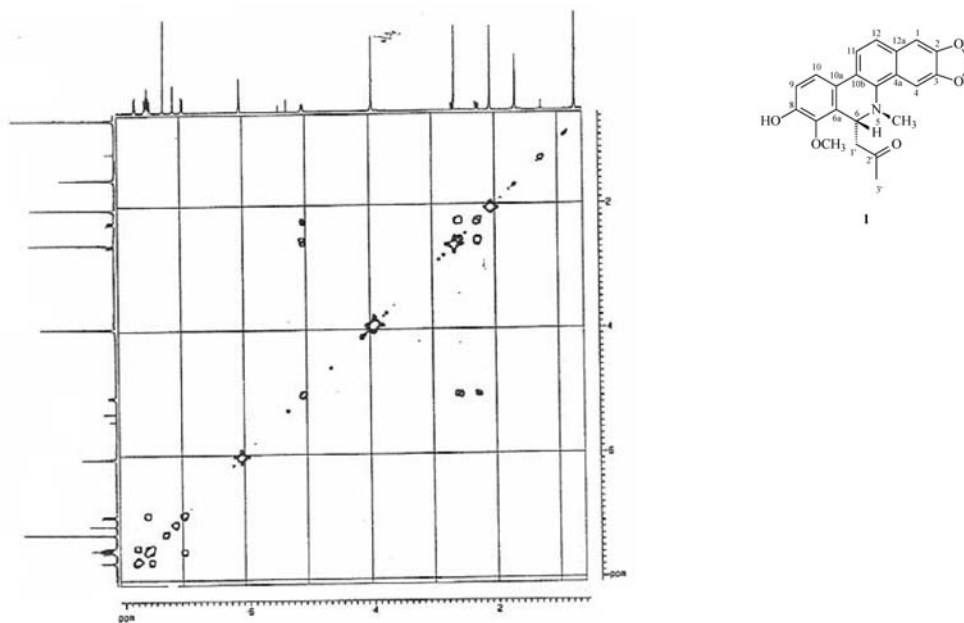


Figure S4. $^1\text{H}, ^1\text{H}$, COSY spectrum (400 MHz, CDCl_3) of **1** (6-acetyl-*N*-methyl-dihydrodecarine).

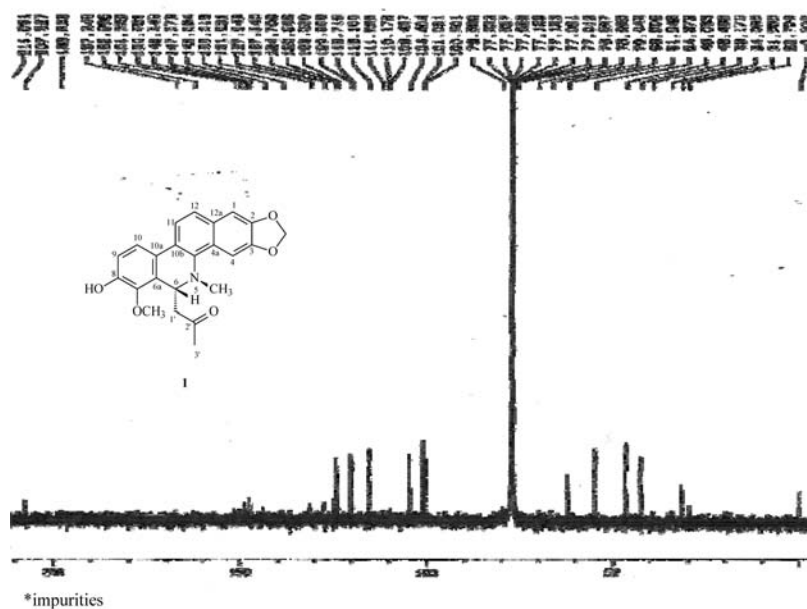


Figure S5. ^{13}C NMR spectrum (100 MHz, CDCl_3) of **1** (6-acetyl-*N*-methyl-dihydrodecarine).

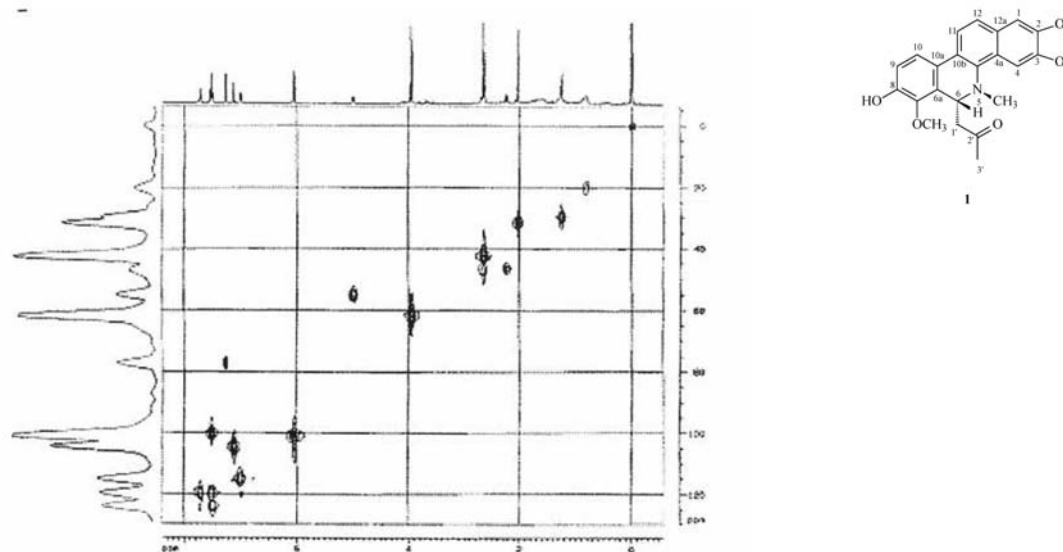


Figure S6. gHSQC spectrum (100 MHz, CDCl₃) of **1** (6-acetyl-*N*-methyl-dihydrodecarine).

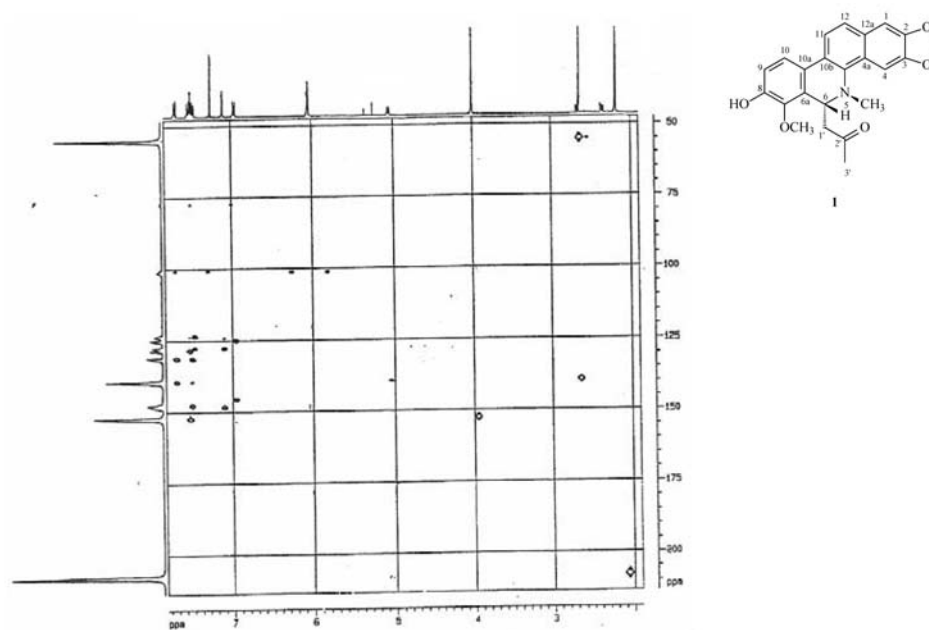


Figure S7. HMBC spectrum (100 MHz, CDCl₃) of **1** (6-acetyl-*N*-methyl-dihydrodecarine).

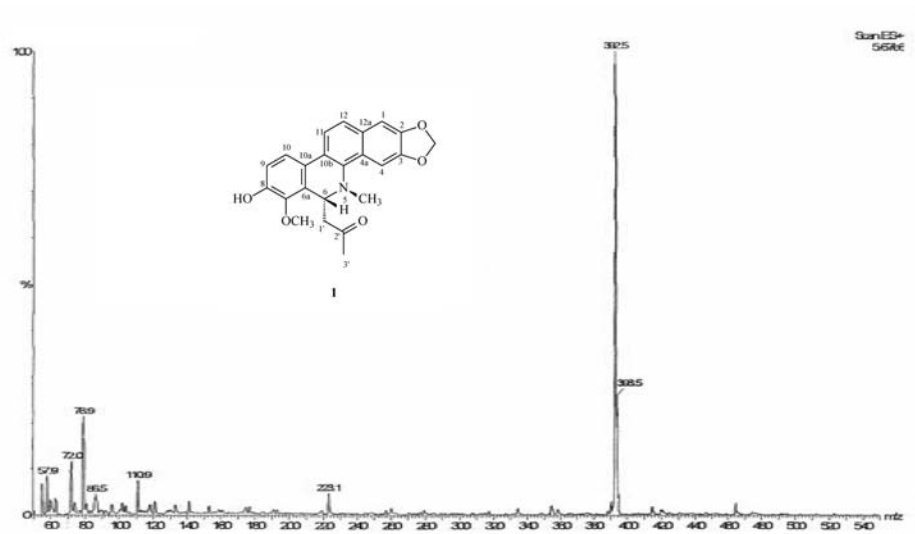


Figure S8. EIMS (+) of 1 (6-acetyl-N-methyl-dihydrodecarine).

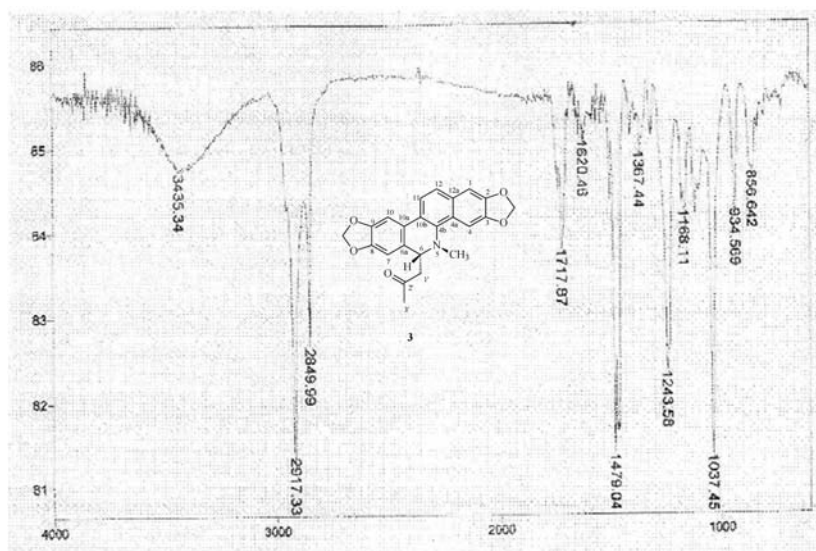


Figure S9. IR spectrum (NaCl film) of 3 (6-acetyldihydroavicine).

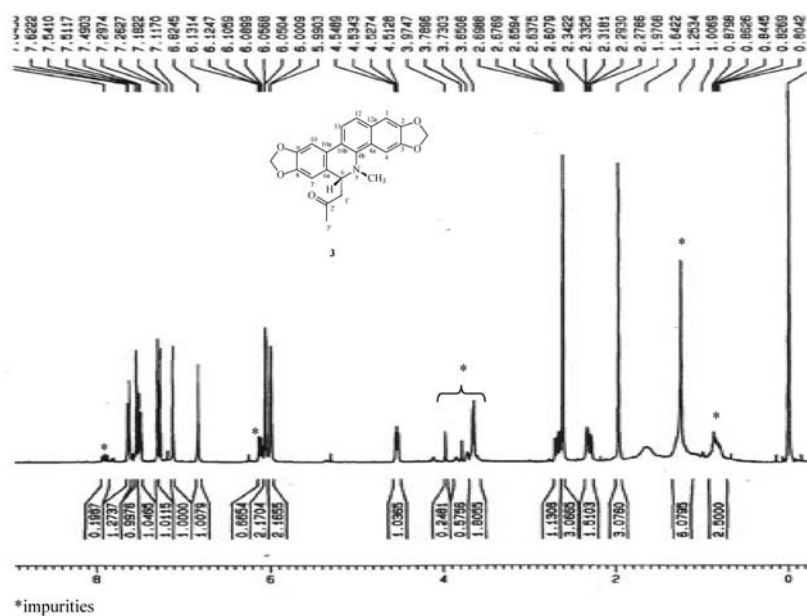


Figure S10. ^1H NMR spectrum (400 MHz, CDCl_3) of **3** (6-acetyldihydroavicine).

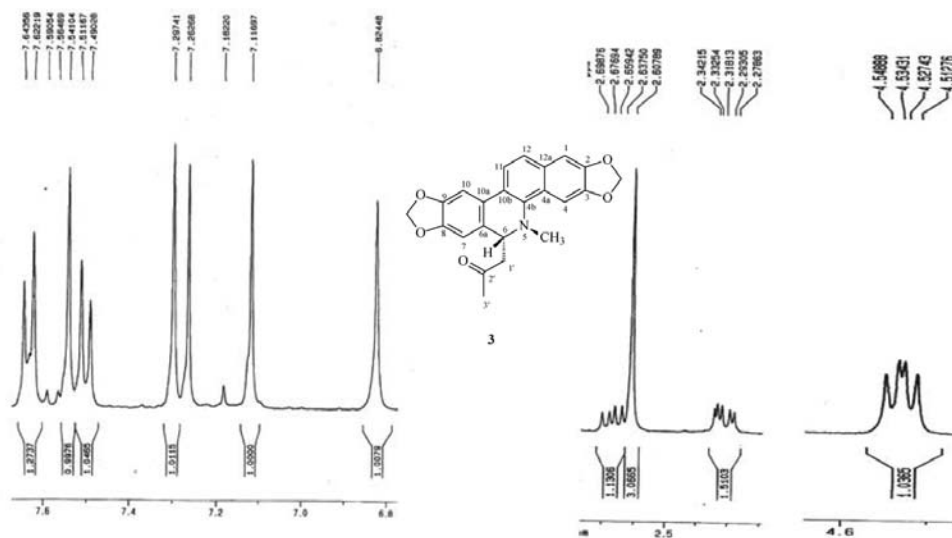


Figure S11. Expansions of the ^1H NMR spectrum (400 MHz, CDCl_3) of **3** (6-acetyldihydroavicine).

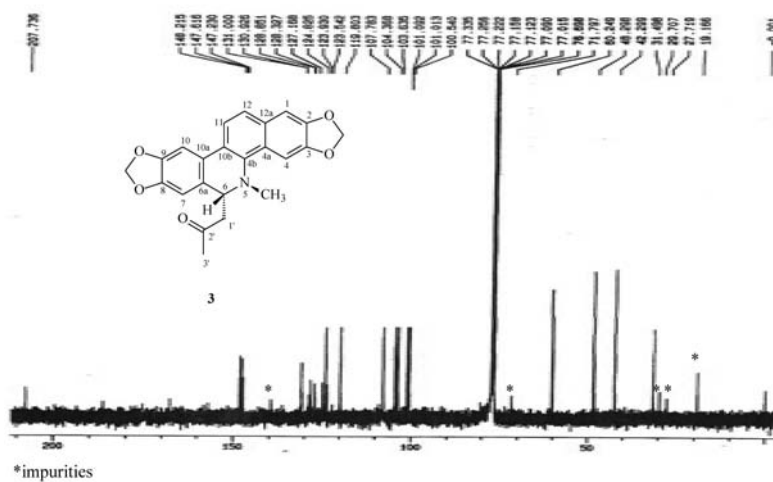


Figure S12. ^{13}C NMR spectrum (100 MHz, CDCl_3) of **3** (6-acetyldihydroavicine).

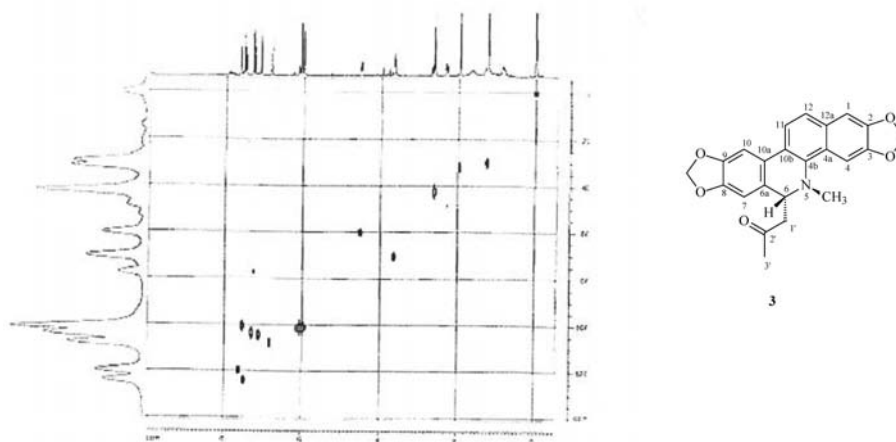


Figure S13. gHSQC spectrum (100 MHz, CDCl_3) of **3** (6-acetyldihydroavicine).

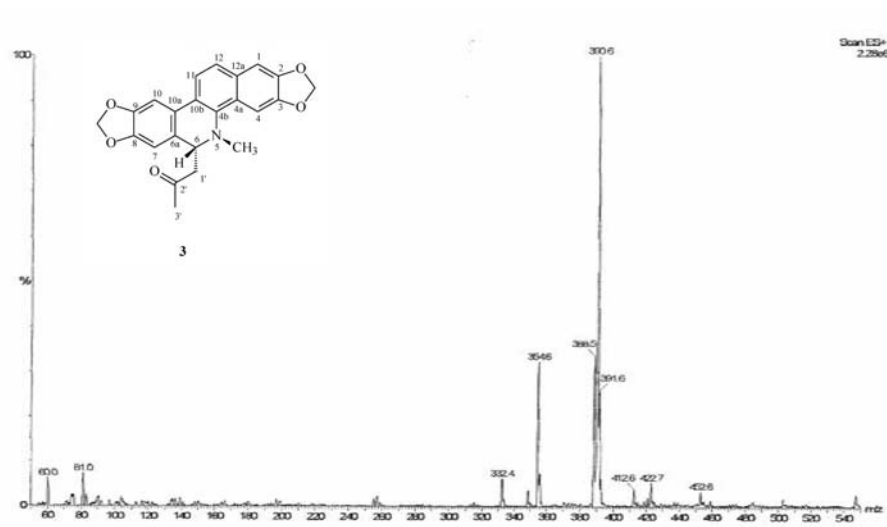


Figure S14. EIMS (+) of **3** (6-acetyldihydroavicine).