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Short Report

6-Acetonyl-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-acetonildiidroqueleritrina 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-acetonyl-*N*-methyl-dihydrodecarine was isolated from *Zanthoxylum riedelianum* roots together with lupeol, 6-acetonyldihydrochelerythrine and 6-acetonyldihydroavicine. 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] cumarins,[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-HIV[13] and antiinflammatory,[8,10] as well as anti-helmintic.[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 oil[14] 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-acetonyl-*N*-methyl-dihydrodecarine (**1**), together with two known alkaloids 6-acetonyldihydrochelerythrine (**2**) and 6-acetonyldihydroavicine (**3**) from the roots of *Z. riedelianum*.

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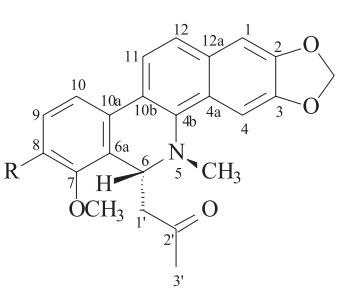
Experimental

General procedures

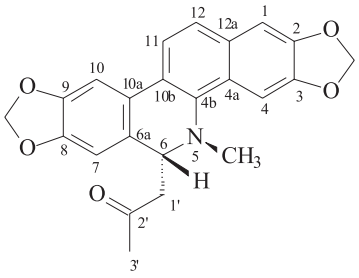
Melting points were uncorrected. IR spectra were recorded on FTIR-Bomem-MB/100 model spectrophotometer using NaCl film. NMR spectra in $CDCl_3$, were recorded on Bruker ARX-400 (400 MHz for ^1H and 100 MHz for ^{13}C); Bruker AC-200 (200 MHz for ^1H and 50 MHZ for ^{13}C) and Varian-Mercury 300 (300 MHz for ^1H and 75 MHz for ^{13}C) 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 PF_{254} (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



1: R = OH
2: R = OCH_3



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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-acetonyldihydrochelerythrine (**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-acetonyl-*N*-methyl-dihydrodecarine (**1**, 60.0 mg, mp 186-188 °C) and 6-acetonyldihydroavicine (**3**, 57.0 mg, mp 184-185 °C).

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

Brown amorphous solid. $[\alpha]_D^{21.5°}$ -5.625 ($CHCl_3$; conc. 0.014 g mL^{-1}). IR (NaCl film) ν_{max}/cm^{-1}: 3396, 1708, 1615, 1610, 1516, 1425, 1296, 1239. ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (Table 1). ESIMS/MS: *m/z* (rel. int.) 391 $[M+H]^+$.

Table 1. ^1H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data of **1** and **3**, in $CDCl_3$

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' (-$\underline{C}H_2CO$)	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' (-$\underline{C}OCH_3$)	207.6		207.7	
3'(-CO$\underline{C}H_3$)	31.5	2.02 (s)	31.4	1.97 (s)
OCH_2O	101.1	6.05 (s)	101.1 and 101.9	6.10 (d, 2.5) and 6.0 (d, 2.5)
7-O$\underline{C}H_3$	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-acetonyldihydrochelerythrine (**2**)[17] and 6-acetonyl-dihydroavicine (**3**)[18] were identified mainly by ^{1}H and ^{13}C 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 ($\nu_{C=O}$), 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 $C_{23}H_{21}NO_5$. The ^{1}H NMR spectrum of **1**, exhibited signals corresponding to six aromatic hydrogen, characteristic of a benzophenanthridine system.[18] Accordingly, the aromatic region from compound (**1**) ^{1}H 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 ^{1}H NMR spectrum also displayed signals of methylenedioxide 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 acetonyl 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 acetonyl methylene hydrogens in the ^{1}H NMR and ^{1}H, ^{1}H-COSY spectra. The ^{13}C NMR spectrum also confirmed the acetonyl moiety with signals at 207.6 (C=O), 31.5 ($CO\underline{C}H_3$), 46.5 ($-\underline{C}H_2CO$). Through the chemical shifts observed in the ^{13}C, ^{1}H-*g*HSQC 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 acetonyl moiety with C-6. Further correlations were observed between OCH_3 (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*-CH_3 (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 Δ_C = 54.9-60.2 = -5.3 ppm as γ effect. Therefore, the structure of **1** was established as 6-acetonyl-*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 aducts of two other benzophenantridine 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 aducts as compared to the parent alkaloids avicine and nitidine.

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Supplementary Information

Supplementary data of alkaloids structures as 1 and 2D ^{1}H and ^{13}C NMR spectra are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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