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BENZOPHENANTHRIDINE ALKALOIDS FROM *ZANTHOXYLUM RHOIFOLIUM*

NEUSA F. DE MOURA, HERALDO B. RIBEIRO, EMÍLIA C. S. MACHADO, EDUARDO M. ETHUR, NILO ZANATTA and ADEMIR F. MOREL*

Departamento de Química, Universidade Federal de Santa Maria, 97119-900, Santa Maria, RS, Brazil

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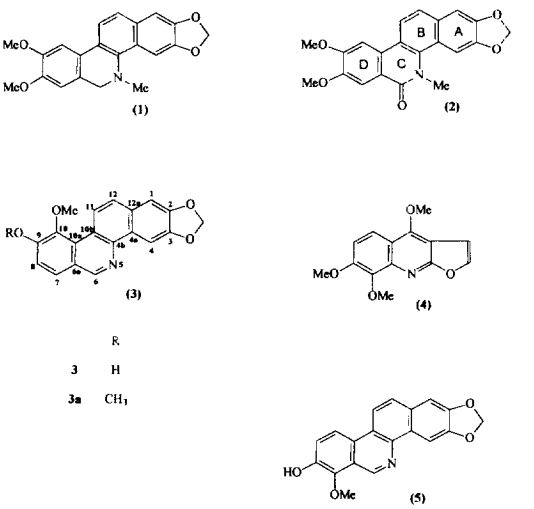
Key Word Index—*Zanthoxylum rhoifolium*; Rutaceae; bark; benzophenanthridines; alkaloids; zanthoxyline.

Abstract—A novel benzophenanthridine alkaloid, named zanthoxyline, was isolated from the bark of *Zanthoxylum rhoifolium*, together with the known compounds, dihydronitidine, 6-oxynitidine and skimmianine. The structure of the new compound was elucidated on the basis of spectroscopic investigations and elemental analysis. © 1997 Elsevier Science Ltd

INTRODUCTION

Plants of the Rutaceae are common in the south of Brazil. They are currently used in popular medicine as teas or infusions against a variety of inflammatory diseases. The main objective of the present work was the isolation and structural determination of benzophenanthridinic alkaloids present in *Zanthoxylum rhoifolium*. Our interest in this type of alkaloid is based on their pharmacological properties, such as, anti-leukaemic [1], antitumour [2], anti-inflammatory [3, 4] and antimicrobial effects [5–7].

From methanolic extracts of the bark of *Z. rhoifolium*, three benzophenanthridine alkaloids have been isolated and their structures determined by spectroscopic methods. Two of these compounds were the dihydrobenzophenanthridine alkaloids dihydronitidine (**1**) and oxynitidine (**2**), previously isolated from *Zanthoxylum nitidium* [8]; the other one is a novel tertiary benzophenanthridine alkaloid, which we named zanthoxyline (**3**). Skimmianine (**4**), a very common furoquinoline alkaloid, was also isolated [9].



Structure 1.

RESULTS AND DISCUSSION

The structures of alkaloids **1**–**4** were determined by IR, mass, HR-mass, ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR data of dihydronitidine (**1**) and oxynitidine (**2**) were compared with those reported by Ishi *et al.* [11] and by Wall *et al.* [13]. A close agreement between our data and those given in the literature was observed, although the assignments of the chemical shifts of H-1 and H-4 in **1**, H-4 and H-10 in **2** should be revised (see Table 1). These chemical shifts were corrected based on the data obtained from NOESY and COLOC experiments.

Compound **3** has a structure closely related to decarine (**5**), another benzophenanthridine alkaloid previously isolated from *Zanthoxylum decaryi* [10]. In contrast to **5**, the hydroxyl group is attached to C-9 and the methoxyl group to C-10. The ^{1}H-NMR spectrum of **3** shows seven aromatic protons, a phenolic -OH (δ 10.07, *s*), a methoxyl group (δ 4.03, *s*) and an -OCH_2O- group (δ 6.02, *s*). This compound was named zanthoxyline. Unambiguous assignments

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR assignments of alkaloids **1**–**3** and **3a**

Proton	**1**[a]	**1**[b]	**2**[a]	**2**[d]	**3**[c]	**3a**[a]
1	7.54	7.11	7.18 *s*	7.13 *s*	7.46	7.12 *s*
4	7.11	7.67	7.64 *s*	7.54 *s*	8.57	8.65 *s*
6	3.13, 4.03 *dd*	4.2 *s*	—	—	9.61	9.67 *s*
7	6.79 *s*	6.80 *s*	7.93 *s*	7.92 *s*	8.42 *d*	8.24 *d*
8	—	—	—	—	7.61 *d*	7.27 *d*
10	7.25 *s*	7.31 *s*	7.58 *s*	7.61 *s*	—	—
11	7.64 *d*	7.71 *d*	7.98 *d*	7.95 *d*	8.46 *d*	8.24 *d*
12	7.33 *d*	7.49 *d*	7.55 *d*	7.45 *d*	7.92 *d*	7.59 *d*
N-CH_3	2.73 *s*	2.51 *s*	3.98 *s*	3.95 *s*	—	—
-O-CH_2-O	6.04 *s*	6.04 *s*	6.10 *s*	6.09 *s*	6.09 *s*	6.05 *s*
8-OCH_3	3.94 *s*	3.94*s*	4.06 *s*	4.04 *s*	—	—
9-OCH_3	3.99 *s*	3.98 *s*	4.10 *s*	4.04 *s*	—	4.02 *s*
10-OH	—	—	4.03 *s*	—	—	—
10-OCH_3	—	—	—	—	4.09 *s*	4.06 *s*

[a] Recorded in $CDCl_3$;
[b] Data taken from ref [12];
[c] Recorded in DMSO-d_6;
[d] Data taken from ref. [13].

of all protons of **3** were made by a series of 1D- and 2D-NMR experiments and are reported in Table 1.

From the contour plot of the COSY ^{1}H-^{1}H experiment of **3**, the existence of four spin systems can be observed: (i) protons H-6, H-7 and H-8, (ii) protons H-1, H-4, H-11 and H-12, (iii) aliphatic protons of the methylenodioxy group (-OCH_2O-) and (iv) the methoxyl protons.

Proton H-6 (δ 9.61) of the first spin-system, because of its easy way of identification, was the starting point for the assignment of all other protons in the molecule. Due to the proximity of the nitrogen, this proton resonates at the lowest field in the ^{1}H NMR spectrum. H-6 shows a cross-peak with H-7 at δ 8.42 in the NOESY spectrum, suggesting a through-space interaction among these two protons. The proton H-7, in turn, exhibits a cross-peak with a signal at δ 7.61, which then was assigned as H-8. This demonstrates the absence of substituents on C-6, C-7 and C-8, and the presence of substituents on C-9 (OH).

The second spin-system formed by the protons H-1, H-4, H-11 and H-12, was securely assigned starting from the strong coupling between H-11 and H-12, which implies an *ortho*-relationship (J = 9.0 Hz), with chemical shifts at δ 7.92 and 8.46, respectively. The unambiguous assignment of H-11 and H-12 was done by the observation of two long-range cross-peaks in the COSY spectrum of the resonance at δ 7.92 (H-12) with the signals at δ 7.46 (H-1) and δ 8.57 (H-4). Only H-12, because of its unique position in the molecule, can have cross-peaks with both H-1 and H-4. Proton H-4 (δ 8.57) exhibits a cross-peak with H-1 in the *para*-position. The correct assignment of H-1 and H-4 was possible by comparison with the NOESY spectrum. In this spectrum, the cross-peak between H-12 and H-4, which are spatially far apart, disappears, whereas the cross-peak between H-1 and H-12, closer in space, remains. The protons of the -OMe group resonate at δ 4.09 and those of the methylenedioxyl group at a typical chemical shift of δ 6.09.

We propose the structure of zanthoxyline based not only on the spectroscopic data of zanthoxyline itself, but also on similar NMR studies with two other zanthoxyline-related compounds, **1** and **2**. For compounds **1** and **2**, we observed cross-peaks between H-10 and H-11 in the NOESY H-H spectrum, which were not observed in the corresponding spectrum of zanthoxyline.

The ^{13}C NMR chemical shifts of **3** were assigned from analysis of the proton noise-decoupled ^{13}C spectrum, DEPT 135° and DEPT 90° spectra, two dimensional heteronuclear correlated spectroscopy (HETCOR), and carbon-coupled spectra. Unambiguous assignment of the protonated carbon resonances of **3** are given in Table 2 and is in accordance with the related ^{1}H NMR data.

Tentative assignment of the non-protonated carbon resonances of **3** was done by analysing the long-range carbon-proton coupling constants on the coupled ^{13}C NMR spectrum, taking long-range C-H coupling values of related compounds as reference [11].

The mass spectral data of **3** not only reveal the correct M_r but show the expected peaks of CH_3-, C≡O- and the total side-chain fragmentation (m/z 304, 276 and 246, respectively) followed by two further CO eliminations to give $C_{15}H_8NO$ and $C_{14}H_8N$ with m/z 218 and 190, respectively.

To confirm the position of hydroxyl group in **3**, this group was methylated with dimethylsulphate to afford the dimethoxy derivative (**3a**). The ^{1}H NMR spectrum of **3a** exhibits a singlet at δ 4.02, in addition to a singlet at δ 4.09 of **3**. To assign the correct position of the new singlet a NOESY experiment (Fig. 1) was performed, which showed a cross-peak between the singlet at δ 4.02 with the H-8 at δ 7.27, indicating that the alkylated hydroxyl was attached to C-9. This unam-

Table 2. ^{13}C NMR assignments of alkaloids **1**–**3** and **3a**

C	1[a]	2[a]	3[b]	3a[a]
1	99.8	104.8	105.1	104.3
2	149.2	147.5	148.7	148.4
3	149.2	147.0	148.9	148.5
4	104.6	102.6	102.4	102.3
4a	127.0	121.0	—	129.3
4b	124.4	135.9	—	140.2
6	69.9	164.3	145.5	146.5
6a	123.5	119.2	127.4	128.3
7	110.8	108.7	118.9	118.2
8	148.5	149.7	125.6	119.2
9	147.9	153.5	148.1	149.4
10	106.6	102.8	143.2	145.6
10a	124.4	128.9	121.8	121.9
10b	137.9	116.7	—	120.0
11	119.6	118.3	119.0	118.2
12	124.3	123.2	128.1	127.0
12a	129.1	131.8	129.9	129.8
N-CH_3	42.7	41.2	—	—
8-OCH_3	56.1	56.2	—	—
9-OCH_3	65.4	56.1	—	57.0
10-OCH_3	—	—	61.9	61.8
-O-CH_2-O-	101.1	102.6	102.0	101.3

[a] Recorded in $CDCl_3$.
[b] Recorded in DMSO-d_6.
Chemical shift values reported as δ values from TMS at 100.6 MHz.

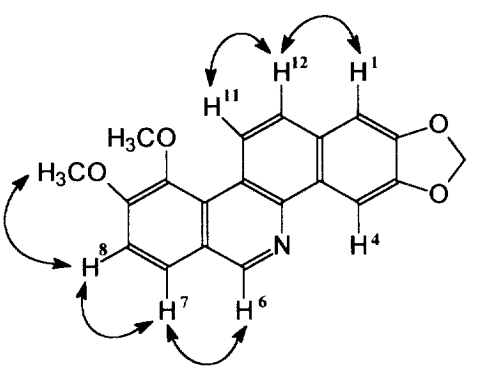


Fig. 1. NOESY cross-peaks observed for compound **3a**.

biguously defined that **3** has a methoxyl group attached to C-10 and that the hydroxyl group is bound to C-9.

In the COLOC spectrum of **3a** (Fig. 2), the proton at δ 8.24 (H-7) shows a cross-peak with C-6a (δ 128.31), which, in turn, exhibits a cross-peak with the proton at δ 9.67 in the HETCOR spectrum. This is a unique chemical shift which arises from the neighbouring position to the nitrogen atom on the pyridine ring. Moreover, H-7 shows cross-peaks (^3J) with C-9 at δ 149.39 and with C-10a at δ 121.93. The aromatic proton (H-8) attached to C-8 (cross-peak in the HETCOR) shows cross-peaks of the $^3J_{CH}$ type with the carbon at δ 145.58 (C-10) and $^3J_{CH}$ with the carbon at δ 128.31 (C-6a). In the COLOC spectrum of **3**, the carbon at δ 143.18 (C-10) exhibits a cross-peak with a methoxylic

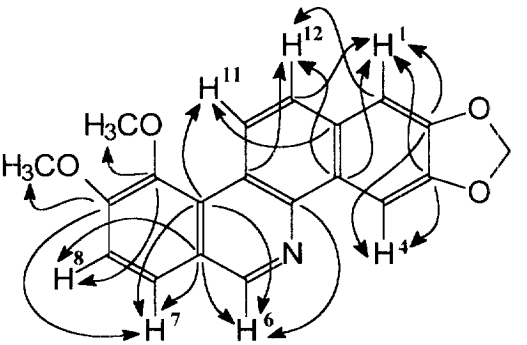


Fig. 2. COLOC correlations observed for compound **3a**.

proton at δ 4.09. The cross-peaks confirm the presence of a hydroxyl group on C-9 and a methoxyl group on C-10, characterizing the A and B rings. The cross-peaks at ^2J and ^3J, observed for the protons and the carbons of the C and D rings, confirm the structure of compound **3**, which was characterized as zanthoxyline.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr discs. Low resolution MS were obtained by GC/MS, high resolution MS at 70 eV. ^1H and ^{13}C NMR were recorded on a Bruker AC80 operating at 80.13 and 20.15 MHz, respectively, and on a Varian VXR at 400 and 100.6 MHz, respectively. Chemical shifts are given in δ using TMS as int. standard.

Plant material. Bark of *Z. rhoifolium* Eng. was collected in the vicinity of Santana do Livramento, State of Rio Grande do Sul, Brazil, in 1993. A voucher specimen is deposited at the Herbarium of the University of Santa Maria.

Isolation of alkaloids. Bark was oven-dried at 50°, ground in a Wiley mill and extracted with MeOH in a Soxhlet extractor. Solvent was evapd under red. pres. to obtain 1.5 kg of a dark viscous residue (crude extract). To this extract, H_2O–Et_2O (1:1) (2 l) was added and acidified with 2N HCl to pH 1.5. The organic phase was sepd and the H_2O layer extracted $\times 2$ with Et_2O. The Et_2O phases were combined and not analysed. The aq. acidic soln was basified with NH_4OH to pH 9.0 and extracted with Et_2O until the Dragendorff's test was negative. The organic phases were combined, washed with H_2O (2×100 ml), dried (Na_2SO_4) and the solvent evapd: 10 g (0.8%) of a dark residue was obtained. The basic extract (5 g) was sepd by CC on silica gel H (500 g) using $CHCl_3$–MeOH mixts of decreasing vol. ratios as mobile phase. Frs were monitored by silica gel TLC run with various solvent systems using Dragendorff's reagent for visualisation.

Isolation of dihydronitidine (**1**). The crystalline residue (90 mg) of fr. 1, recrystallized from $CHCl_3$–Et_2O gave **1** (mp 189–190°, lit. 210 [12]), homogeneous on TLC. IR ν cm^{-1}: 3490, 2800, 2930, 1660, 1530, 1500. GC-MS (70 eV) *m/z* (rel. int.): 349 (15, $[M]^+$), 348 (100), 304 (10), 247 (8), 174 (13), (1), 88 (4), 75 (3). NMR: Tables 1 and 2.

Oxynitidine (**2**). Recrystallization of the resultant yellow solid (20 mg) of fr. II, from diisopropylether gave **2** (mp 276–278°, lit. 284–285° [8]), homogeneous on TLC. GC-MS (70 eV) *m/z* (rel. int.): 364 $[M]^+$, 348, 320, 307, 157 (100). NMR: Tables 1 and 2.

Zanthoxyline (**3**). Recrystallization of the resultant solid (240 mg) of fr. II from $CHCl_3$–Et_2O gave **3** (mp 220–222°) IR ν cm^{-1}: 2920, 1540, 1470, 1320, 1250. GC-MS (70 eV) *m/z* (rel. int.): 319 (100, $[M]^+$), 304 (78) 276 (96), 246 (7), 190 (36), 163 (29), 138 (37), 87 (13), 82 (8). HR-MS (70 eV) *m/z*: $C_{19}H_{13}NO_4$ $[M]^+$ calcd 319.0844, found 319.0852; $C_{18}H_{10}NO_4$ calcd 304.0607, found 304.0609; $C_{17}H_{10}NO_4$ calcd 276.2709, found 276.0762. NMR: Tables 1 and 2.

Skimmianine (**4**). Fr. III recrystallized from $CHCl_3$ gave **4** (mp 140°). GC-MS (70 eV) *m/z* (rel. int.): 259 (100, $[M]^+$), 258 (50), 244 (65), 230 (40), 216 (30), 201 (25), 184 (3), 173 (20), 156 (10), 130 (20), 87 (10). ^1H NMR (80 MHz, $CDCl_3$): δ 7.97 (1H, *d*, $J = 12$ Hz), 7.54 (1H, *d*, $J = 4$ Hz), 7.19 (1H, *d*, $J = 12$ Hz), 6.99 (1H, *d*, $J = 4$ Hz), 4 (3H, *s*), 4.11 (3H, *s*), 4.01 (3H, *s*). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ 164.4, 157.2, 149.2, 143.0, 142.1, 141.5, 118.5, 114.9, 112.2, 61.7, 59.0, 56.9. These data comply with those given in ref. [9].

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