N-Nitrosoanonaine and *N*-Nitrosoxylopine, Aporphine Alkaloids from *Duguetia furfuracea*

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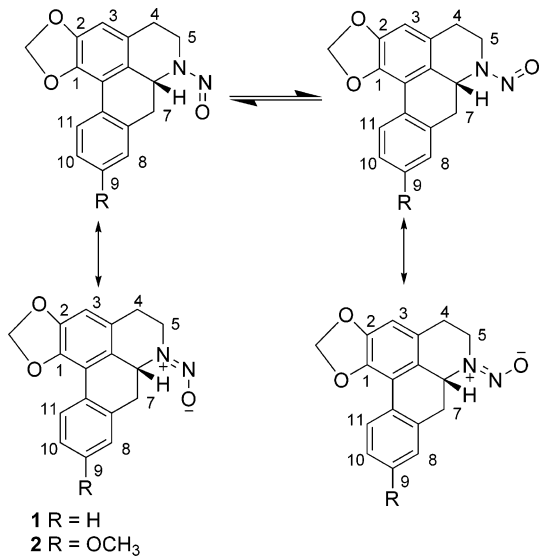
Two new aporphine alkaloids, *N*-nitrosoanonaine (**1**) and *N*-nitrosoxylopine (**2**), were isolated from the aerial parts of *Duguetia furfuracea*, a weed found in several Brazilian regions. Their structures were elucidated by NMR analysis, CHN analysis, CD, IR, and MS data. The single-crystal X-ray strucutural determination of the structure of **1** was also perfomed.

Duguetia, a genus of the family Annonaceae, has nearly 80 known species.[1] In the past two decades, chemical studies of the genus have grown in number, although pharmacological and biological evaluations have not been conducted so extensively. Several isoquinoline-derived alkaloids and sesquiterpene-type structures have been reported.[2−5] Of those constituents, the alkaloids have been most widely tested, exhibiting antimalarial, cytotoxic, and antimicrobial activities.[1,6]

Duguetia furfuracea is a weed found in several regions in Brazil. In the state of Mato Grosso do Sul, where it is called "araticum-seco",[7] the species is known as a pasture-invading plant,[8] and in folk medicine its powdered seeds are mixed with water for use against pediculosis,[7] whereas an infusion of the twigs and leaves is used to treat rheumatism,[9] and capsules containing "*D. furfuracea* material" are claimed to be useful for renal disorders.[10] From *D. furfuracea*, flavonoids[11] and sesquiterpenoids[12] have been isolated.

In the present study, we investigated the alkaloid extract obtained from the dried leaves of *D. furfuracea* and report the isolation and structure elucidation of two new alkaloids (**1** and **2**) containing a *N*-nitroso functionality. *N*-Acetyl and *N*-formyl aporphinoid alkaloid derivatives have been obtained from plants in the Annonaceae as both natural products and by synthetic procedures.[13,14] The determination of volatile *N*-nitroso compounds in foodstuffs and tobacco products has been documented and some volatile *N*-nitrosamines have been detected and quantified in medicinal plant preparations.[15] External and internal exposures to *N*-nitrosamine-containing compounds have provided circumstantial evidence of their carcinogenicity and mutagenicity.[16] On the other hand, compounds in this class can be regarded as potential NO/NO$^+$ donors, thus playing an important role in the regulation of many physiological functions.[17]

N-Nitrosoanonaine (**1**) was obtained as colorless crystals, at a yield of 0.012% from leaves. The compound exhibited $[\alpha]^{25}_D$ −298 (*c* 0.50 $CHCl_3$). Because of the close resemblance of the ^{1}H and ^{13}C NMR spectra of **1** to those of *N*-acetylanonaine,[13,14] it was possible to compare the chemical shifts of the protons and carbons in these two molecules. The comparison revealed that the main chemical shift differences in **1** were apparent in the neighborhood of the *N*-heterocycle,[13,14] suggesting the presence of a substituent at this nitrogen atom, though differing from that present in *N*-acetylanonaine. The (+) ESIMS-MS of **1** revealed a pseudomolecular ion at *m*/*z* 295 ([M + H]$^+$) and a fragment ion at *m*/*z* 265 ([M − 30 + H]$^+$), which were consistent with the loss of a NO group linked to the *N*-heterocycle.



The ^{1}H and ^{13}C NMR spectra (300 and 75 MHz, respectively) in $CDCl_3$ of **1** at room-temperature enabled the proposal of two rotational isomers in equilibrium (*E*- and *Z*-rotamer populations).[13,14,18] The diagnostic signals for *E*-**1** and *Z*-**1** were based on carbons C-4, C-5, C-6a, and C-7, showing that the effect of the shielding region of the *N*-nitroso group may be more or less pronounced, depending on the rotamer considered. In the case of the *E*-rotamer, the methine (C-6a) and methylene (C-7) carbons lie in the shielding region of the *N*-nitroso group; in the *Z*-rotamer, shielding occurs at both methylene carbons at C-4 and C-5.[17,18] Single-crystal X-ray crystallography of **1** was obtained to confirm its structure and relative configuration (Figure 1).

N-Nitrosoxylopine (**2**) was obtained as colorless amorphous solid, at a yield of 0.007% from leaves. The compound exhibited $[\alpha]^{25}_D$ −264 (*c* 0.50 $CHCl_3$). The (+) ESIMS-MS showed a pseudomolecular ion at *m*/*z* 325 ([M + H]$^+$) and a fragment ion at *m*/*z* 295 ([M − 30 + H]$^+$). By carrying out the same type of analysis performed for **1**, the ^{1}H and ^{13}C NMR spectra of **2** yielded almost similar data to those found for **1**, except for the additional signals in δ_H 3.83 (^{1}H NMR) and δ_C 55.2 (^{13}C NMR) and the mass fragment observed in the mass spectrum, as described above.

The assignment of the absolute configuration at the chiral center C-6a of the aporphine alkaloids **1** and **2** was determined from the sign of their circular dichroism (CD) spectra centered at 235 nm. Both compounds have *R*-(−)-configuration.[19]

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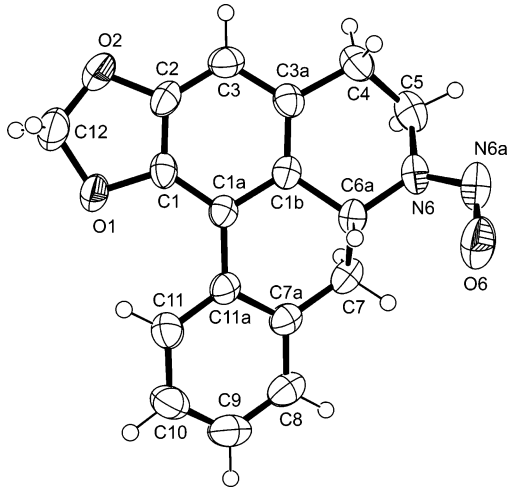

Figure 1. ORTEP diagram of **1** in the thermal ellipsoids drawn at the 50% probability level.

Experimental Section

General Experimental Procedures. Optical rotations were run in chloroform on a Perkin-Elmer 341 instrument. Detection of on-line CD spectra was perfomed on a Jasco-CD-1595 spectrometer with a flow-through cell (10 mm light path and 1 mm diameter, 1 mL/min). IR spectra were recorded on a Perkin-Elmer 1420. All NMR experiments were performed on a DPX-300 Bruker instrument (^{1}H: 300 MHz; ^{13}C: 75 MHz) using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are reported in δ units and coupling constants (J) in Hz. Mass spectrometric analyses were performed at low resolution on a Quattro-LC instrument Micromass (Manchester, UK) provided with an ESI ion source and a triple quadrupole mass analyzer. Solutions were infused into the ESI source at a flow-rate of 10 μL·min^{-1}, using a Harvard Apparatus model 1746 (Holliston, MA) syringe pump. Experiments were carried out using cone voltages from 10 to 20 V in the positive ion mode of analysis. CID fragmentation was performed using argon collision gas (7 psi) on an isolated parent ion.

Plant Material. The aerial parts (leaves and twigs) of *Duguetia furfuracea* (A. St.-Hil.) Benth. & Hook. f. (Annonaceae) were collected in May 2002, from the UFMS campus in Campo Grande, MS, Brazil, and identified by Prof. R. Mello Silva. A voucher specimen (number 023) has been deposited in the GC-MS Herbarium (Campo Grande, MS).

Extraction and Isolation. Air-dried, ground leaves of *D. furfuracea* (4.8 kg) were submitted to percolation with 95% ethanol for 72 h, yielding 970 g of ethanolic extract. The extract was shaken exhaustively with 3% HCl and the resulting acid fraction was adjusted to pH 9.0 with ammonium hydroxide and then extracted with methylene chloride. The resulting methylene chloride fraction (14 g) was solubilized in chloroform and extracted with 3% HCl and the resulting chloroform layer was concentrated under reduced pressure and precipitate fraction was obtained. This precipitate fraction was solubilized in methylene chloride with the help of a small amount of methanol and again exhaustively extracted with 3% HCl. A brown precipitate (4 g) and an acid layer were obtained. The precipitate was submitted to column chromatography (silica gel, 150 g, chloroform/methanol gradient), yielding *N*-nitrosoanonaine (**1**) (550 mg) and *N*-nitrosoxylopine (**2**) (320 mg).

***N*-Nitrosoanonaine (1)** ({5*H*-benzo[*g*]-1,3-benzodioxolo[5,6,4]-quinoline,6,7,7a,8-tetrahydro-7-nitroso-(7a*R*)}: colorless crystal ($CHCl_3$−MeOH, 1:1): mp 174−175 °C; $[\alpha]^{25}_D$ −298 (*c* 0.50 $CHCl_3$); CD $\Delta\epsilon$ (*c* 0.03 in 95% EtOH) (λ nm) −0.09 (235); IR (KBr) ν_{max} 2941, 2902, 1621, 1578, 1496, 1454, 1426, 1363, 1328, 1207, 1130, 1040, 924 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) *E*-**1**, δ 8.01 (1H, dd, J = 7.6, 2.0 Hz, H-11), 7.31−7.17 (3H, m, H-8, 9, 10), 6.52 (1H, s, H-3), 6.00, 5.90 (each 1H, d, J = 1.8 Hz, OCH_2O), 5.37 (1H, dd, J = 14.0, 4.3 Hz, H-6a), 5.08 (1H, ddd, J = 12.5, 4.5, 1.6 Hz, H-5α), 3.95 (1H, td, J = 12.5, 12.5, 3.3 Hz, H-5β), 3.12 (1H, dd, J = 14.0, 4.3 Hz, H-7β), 3.03 (1H, ddd, J = 15.4, 12.5, 4.5 Hz, H-4β), 2.87 (1H, ddd, J = 15.4, 3.3, 1.6 Hz, H-4α), 2.59 (1H, br t, J = 14.0 Hz, H-7α); ^{13}C NMR ($CDCl_3$, 75 MHz) *E*-**1**, δ 147.3 (C, C-2), 143.5 (C, C-1), 134.0 (C, C-7a), 130.1 (C, C-11a), 128.7 (CH, C-8), 128.0 (CH, C-9), 127.3 (CH, C-11), 127.1 (CH, C-10), 126.0 (C, C-3a), 123.3 (C, C-1b), 117.2 (C, C-1a), 107.5 (CH, C-3), 101.1 (CH_2, OCH_2O), 50.6 (CH, C-6a), 46.7 (CH_2, C-5), 31.6 (CH_2, C-7), 30.4 (CH_2, C-4); ^{1}H NMR ($CDCl_3$, 300 MHz) *Z*-**1**, δ 8.08 (1H, dd, J = 7.6, 2.0 Hz, H-11), 7.20−7.30 (3H, m, H-8, 9, 10), 6.55 (1H, s, H-3), 6.07, 5.95 (each 1H, br s, OCH_2O), 5.02 (1H, dd, J = 14.0, 4.3 Hz, H-6a), 4.43 (1H, dt, J = 13.3, 5.2, 5.2 Hz, H-5α), 3.45 (1H, dd, J = 14.0, 4.3 Hz, H-7β), 3.20 (1H, br t, J = 14.0 Hz, H-7α), 3.69 (1H, ddd, J = 13.3, 8.7, 5.2 Hz, 5β), 2.84 (1H, dt, J = 15.1, 5.2, 5.2 Hz, H-4α), 2.73 (1H, ddd, J = 15.1, 8.7, 5.2 Hz, H-4β); ^{13}C NMR ($CDCl_3$, 75 MHz) *Z*-**1**, δ 147.6 (C, C-2), 143.4 (C, C-1), 130.3 (C, C-11a), 133.9 (C, C-7a), 128.6 (CH, C-8), 128.1 (CH, C-9), 127.1 (CH, C-10), 126.3 (CH, C-11), 125.8 (C, C-3a), 123.8 (C, C-1b), 116.7 (C, C-1a), 107.5 (CH, C-3), 101.1 (CH_2, OCH_2O), 57.4 (CH, C-6a), 36.8 (CH_2, C-5), 35.9 (CH_2, C-7), 28.6 (CH_2, C-4); (+) ESIMS-MS *m*/*z* 295 $[M + H]^+$ (10), 265 (25), 249 (100), *anal.* C 69.11%, H 5.15%, N 9.30%, calcd for $C_{17}H_{14}N_2O_3$.

***N*-Nitrosoxylopine (2)** ({5*H*-benzo[*g*]-1,3-benzodioxolo[6,5,4-*de*]quinoline,6,7,7a,8-tetrahydro-7-nitroso-10-methoxy-(7a*R*)}: colorless, amorphous solid ($CHCl_3$-MeOH, 1:1): $[\alpha]^{25}_D$ −264 (*c* 0.50 $CHCl_3$); CD $\Delta\epsilon$ (*c* 0.03 in 95% EtOH) (λ nm) −0.05 (235); IR (KBr) ν_{max} 2925, 2843, 1609, 1506, 1462, 1429, 1320, 1210, 1110, 1042, 928 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) *E*-**2**, δ 7.99 (1H, d, J = 8.3 Hz, H-11), 6.83 (1H, dd, J = 8.3, 2.6 Hz, H-10), 6.77 (1H, br d, J = 2.6 Hz, H-8), 6.53 (1H, s, H-3), 6.05, 5.95 (each 1H, d, J = 1.2 Hz, OCH_2O), 5.33 (1H, dd, J = 14.0, 4.3 Hz, H-6a), 5.08 (1H, ddd, J = 12.3, 4.5, 1.3 Hz, H-5α), 3.96 (1H, dt, J = 12.3, 12.3, 2.2 Hz, 5β), 3.83 (3H, s, OCH_3), 3.09 (1H, dd, J = 14.0, 4.3 Hz, H-7β), 3.03 (1H, ddd, J = 15.4, 12.3, 4.5 Hz, H-4β), 2.87 (1H, ddd, J = 15.4, 2.2, 1.3 Hz, H-4α), 2.59 (1H, br t, J = 14.0 Hz, H-7α); ^{13}C NMR ($CDCl_3$, 75 MHz) *E*-**2**, δ 159.2 (CH, C-9), 147.3 (C, C-2), 142.8 (C, C-1), 135.9 (C, C-7a), 128.5 (CH, C-11), 126.0 (C, C-3a), 122.9 (C, C-11a), 122.6 (C, C-1b), 117.3 (C, C-1a), 113.8 (CH, C-8), 113.0 (CH, C-10), 106.6 (CH, C-3), 101.0 (CH_2, OCH_2O), 55.2 (OCH_3), 50.6 (CH, C-6a), 46.8 (CH_2, C-5), 32.0 (CH_2, C-7), 30.4 (CH_2, C-4); ^{1}H NMR ($CDCl_3$, 300 MHz) *Z*-**2**, δ 8.03 (1H, d, J = 8.3 Hz, H-11), 6.88 (1H, br d, J = 2.6, H-8), 6.82 (1H, dd, J = 8.3, 2.6 Hz, H-10), 6.60 (1H, s, H-3), 6.08, 5.95 (each 1H, d, J = 1.2 Hz, OCH_2O), 5.33 (1H, dd, J = 14.0, 4.3 Hz, H-6a), 4.45 (1H, dt, J = 13.2, 5.3, 5.3 Hz, H-5α), 3.66 (1H, ddd, J = 13.2, 8.7, 5.3 Hz, H-5β), 3.82 (3H, s, OCH_3), 3.43 (1H, dd, J = 14.0, 4.3 Hz, H-7β), 3.20 (1H, br t, J = 14.0 Hz, H-7α), 2.76 (1H, ddd, J = 15.1, 5.3, 4.5 Hz, H-4β), 2.73 (1H, ddd, J = 15.1, 8.7, 5.3 Hz, H-4α); ^{13}C NMR ($CDCl_3$, 75 MHz) *Z*-**2**, δ 159.3 (C, C-9), 147.5 (C, C-2), 142.7 (C, C-1), 135.7 (C, C-7a), 128.5 (CH, C-11), 126.0 (C, C-3a), 123.2 (C, C-1b), 123.1 (C, C-11a), 116.8 (C, C-1a), 113.9 (CH, C-8), 112.9 (CH, C-10), 106.6 (CH, C-3), 101.1 (CH_2, OCH_2O), 57.4 (CH, C-6a), 55.2 (OCH_3), 36.9 (CH_2, C-5), 36.4 (CH_2, C-7), 28.6 (CH_2, C-4); (+) ESIMS-MS *m*/*z* 325 $[M+ H]^+$ (25), 295 (20), 279 (100); *anal.* C 66.01%, H 5.29%, N 8.24%, calcd for $C_{18}H_{16}N_2O_4$.

Single-Crystal X-ray Crystallography of 1. Single crystals of **1** were slowly grown from a methanol−chloroform mixture (1:1). A suitable crystal (0.10 × 0.20 × 0.30 mm) was mounted on a Siemens P4 diffractometer and analyzed with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å, T = 298 K. Crystal data of **1**: $C_{17}H_{14}N_2O_3$, M_r = 294.30 g mol^{-1}, orthorhombic, $P2_12_12_1$. Unit-cell parameters determined from 96 reflections, 9.0° ≤ 2θ ≤ 15.0°, a = 7.011(1) Å, b = 11.185(1) Å, c = 17.190(1) Å, V = 1347.9(3) Å^3, Z = 4, F(000) = 616, D_x = 1.450 Mg·m^{-3}. The

number of measured reflections was 6252, $-1 \le h \le 6$, $-9 \le k \le 9$, $-20 \le l \le 20$, $2\theta_{max} = 60.0°$. $R_{int} = 0.0288$ for 2411 unique reflections, Friedel opposites not merged. The structure (Figure 1) was solved by direct methods[11] and refined by full-matrix least-squares on F^2 using the program SHELXS-97.[20,21] Isotropic extinction correction was performed.[20,21] For the non-hydrogen atoms, the anisotropic thermal parameters were refined. All hydrogen atoms were found from Fourier maps. Although they were fixed at geometrically idealized calculated positions; one isotropic thermal parameter was refined for all hydrogen atoms. In the final refinement, the R indexes were $R(F) = 0.0450$ for 1582 reflections with $F \ge 4\sigma(F)$, $wR(F^2) = 0.0821$ for all unique data and $S = 1.002$ for all unique reflections. Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 274744. The data can be obtained free of charge via www.ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 IEZ, UK, fax +44 1223 336033.

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Supporting Information Available: ^{1}H and ^{13}C NMR data including HMBC correlations for compounds **1** and **2** (Tables S1 and S2), as well a figure of the ^{13}C NMR spectrum of *N*-nitrosoanonaine (*E*-**1** and *Z*-**1** rotamers) in $CDCl_3$. These materials are available free of charge via the Internet at http://pubs.acs.org.

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