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Quinolone alkaloids from *Waltheria douradinha*

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Abstract

A phytochemical investigation of the stems of *Waltheria douradinha* resulted in isolation of two 4-quinolone alkaloids, waltherione B and vanessine, along with three known alkaloids, waltherione A, antidesmone and *O*-methyltembamide. Their structures were elucidated on the basis of their 2D NMR spectroscopic analyses, and from X-ray crystallographic analysis of waltherione A and the *O*-methyl derivative of waltherione B. Additionally, waltherione B and vanessine, and the *O*- and *N*-methyl derivatives of waltherione A and waltherione B, were evaluated for their antimicrobial activities; only vanessine displayed any (weak) antimicrobial activity.

Keywords: *Waltheria douradinha*; Sterculiaceae; Quinolone alkaloids; Stereochemistry; Waltherione B; Vanessine; Antimicrobial activity

1. Introduction

The Sterculiaceae family has approximately 70 genera and 1500 species (Mabberley, 1997). In Brazil, the species *Waltheria douradinha* Saint-Hilaire, a shrub that grows in sandy and stony soils, is used (as the whole plant) in traditional medicine for the treatment of respiratory disorders, as a stimulant, emetic, and diuretic, to treat urinary diseases and, externally, as a wound cleansing and healing agent (Corrêa, 1931; Simões, 1986; Lorenzi and Matos, 2002). In a previous paper (Morel et al., 1999a, 1999b, 2005) on the chemical constituents from the roots of *W. douradinha*, the isolation and structure elucidation of some peptide alkaloids and an unusual 4-quinolone alkaloid was reported. Preliminary studies showed that the stems of this species contain similar alkaloids. Because the stem is easy to collect, it was used for the current investigation. In the course of the studies of the stem bark, two new 4-quinolone alkaloids, named waltherione B (**1**) and vanessine (**2**), together with the known alkaloids waltherione A (**3**), antidesmone (**4**) and *O*-methyltembamide (**5**) were isolated. Their structures were determined on the basis of ^{1}H and ^{13}C NMR spectroscopic data and on the X-ray crystallographic analysis of either the alkaloids or their *N*- or *O*-methyl derivatives. In addition, a revised relative stereochemistry for waltherione A (**3**) is presented. Alkaloids **1** and **3** were methylated with methyl bromide to furnish the *O*-and *N*-derivatives **6**–**9**.

2. Results and discussion

Waltherione B (**1**) was obtained as a white solid after isolation and purification. HRESIME showed $[M+Na]^{+}$ at $m/z = 416.14694$, in agreement with the molecular formula $C_{23}H_{23}NO_5$. This was further supported by the ^{13}C NMR spectroscopic data, which contained 23 distinct signals. The ^{13}C NMR, DEPT, and HMQC spectra also contained resonances that were attributed to a carbonyl at δ_C 174.2, two oxygenated methine carbons at δ_C 75.2 and 82.5, an oxygenated quaternary carbon at δ_C 78.0, two methylene carbons at δ_C 23.3 and 32.0, two methoxyl groups at δ_C 55.5 and 59.7, as well as fourteen aromatic carbons between δ_C 111.8 and 158.0.

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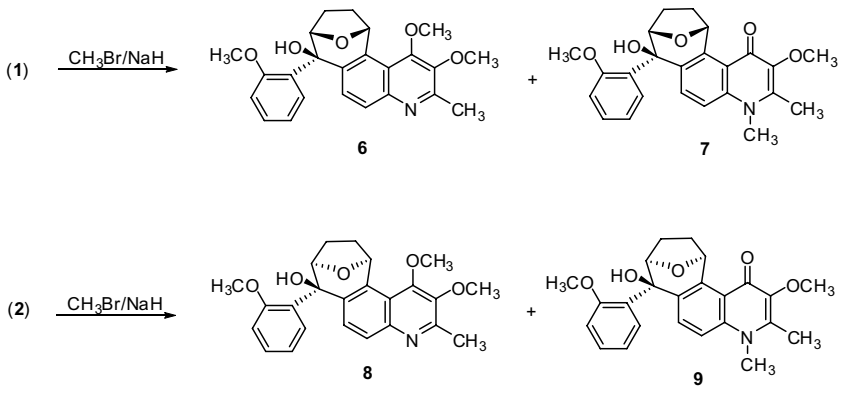


Scheme 1.

The ^{1}H NMR spectrum of **1** determined in $CDCl_3$ indicated the presence of six aromatic hydrogens between δ_H 6.70 and 7.40 ppm, three methyl singlets, two superimposed signals at δ_H 3.98 (3C-OMe and 15C-OMe), and one methyl group at δ_H 2.35 (2C-Me), two diastereotopic methylene groups at δ_H 1.37/1.67 and 1.95/2.23, two methine hydrogens at δ_H 4.75 and 6.68, and an amide proton at δ_H 11.10. The isomeric relationship between **1** and **3** was evident from the mass spectroscopic data, which indicated an identical molecular formula $C_{23}H_{23}NO_5$. The complete assignments of the ^{1}H and ^{13}C signals were readily accomplished by the analysis of the 1D and 2D NMR spectroscopic data, and by comparison to those of **3** (Morel et al., 2005). The spectroscopic similarities between alkaloids **1** and **3** indicated that they possess the same connectivity, and suggested that they were diastereoisomeric at C-10 and C-13, and/or C-9. The relative stereochemistry of the chiral centers of alkaloids **1** and **3** was determined by X-ray crystallographic analysis. Treatment of alkaloids **1** and **3** with methyl bromide at room temperature resulted in the formation of the *O*- and *N*- methyl derivatives **6**–**9** (Scheme 1), confirming the tautomerism of the quinolone moiety of alkaloids **1** and **3** (Werner, 1969). X-ray quality single crystals of **6**, the *O*-methylated product of waltherione B (**1**), of watherione A (**3**), and of its *N*-methylated product (**9**) were obtained. Compound **6** was used to determine the relative stereochemistry of alkaloid **1**. Figs. 1 and 2 show the results of the X-ray analysis of **3** and **6**, establishing that the new alkaloid **1** possesses a relative stereochemistry 9*R**, 10*R**, 13*S**. Consequently, the previously reported stereochemistry for alkaloid **3**, proposed by AM1 calculation (Morel et al., 2005), must be changed to 9*R**, 10*S**, 13R*. On the basis of the results above, alkaloid **1** was identified as (9*R**, 10*R**, 13*S**)-9-hydroxy-3-methoxy-2-methyl-9-(2-methoxyphenyl)-14-oxa-biciclo[3.2.1]-octa [*f*] quinolone, and designated as waltherione B.

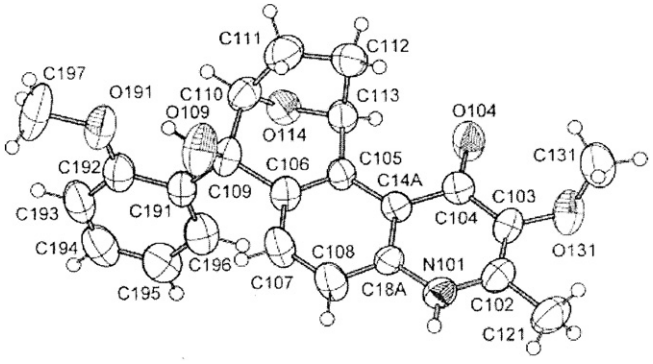


Fig. 1. Perspective view of the X-ray structure of one molecule of waltherione-A (**3**).

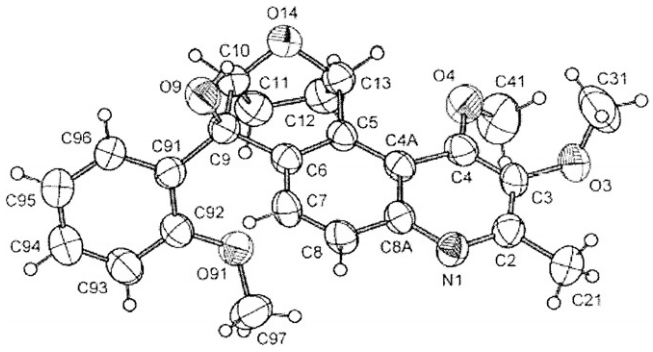
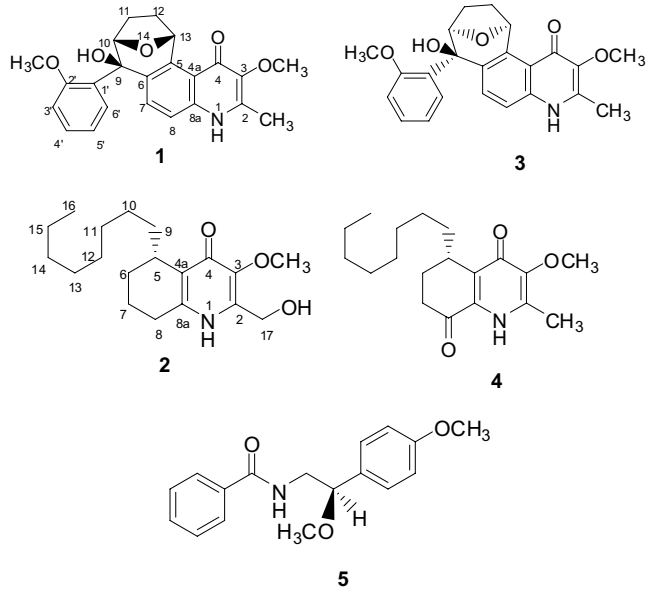


Fig. 2. Perspective view of the X-ray structure of the compound **6**.



Vanessine (2) was obtained as a yellowish viscous oil, and showed an accurate [M+H]+ ion at m/z 322.23772 in the HRESIMS, two mass units more than that of antidesmone (4) (Buske et al., 1999, 2002), and corresponding to the empirical molecular formula C19H31NO3. The formula was also deduced on the basis of the 13C NMR spectrum, combined with DEPT data. The 1H NMR spectrum of 2 recorded in CDCl3 indicated the presence of ten methylene groups between δH 1.23 and 1.46, and one bearing oxygen at δH 4.76, a methine hydrogen at δH 3.0, two methyl groups, a singlet at δH 3.75 (3C-OMe), and a triplet at δH 0.85 (16C-Me). The methine hydrogen, attached at position 5, was shifted downfield (δH 3.0) due to the presence of the adjacent carbonyl. In the 1H–1H–COSY spectrum, H-5 has a correlation with the signals at δH 1.21/1.71 (2H, m), which corresponds to H2-6, and with the resonances at δH 1.49/1.88 (2H, m), which correspond to H2-9. The placement of the octanyl side chain at C-5, was based on the HMBC spectrum, which showed connectivities between H-5 and C-8′ (δC 128.9) and C-4 (δC 172.3). In the same spectrum, the methylene proton signal at δH 4.76 (H-17) showed a cross-peak with an O-linked carbon resonance at δC 143.5 (C-3) that was consistent with placement of the hydroxymethyl group at C-2. The terminal methyl group of the octanyl side-chain appeared at δH 0.85 (3H, t, J = 8.2 Hz), the C10–C15 methylenes between δH 1.23 and 1.29 (12H, m), and the C9 methylene at δH 1.49/1.88 (2H, m). The signals at δH 1.21/1.71 (2H, m), 1.28 (2H, m) and 2.60 (2, m), were attributed to the internal methylenes C6, C7, and C8, respectively, on the basis of analysis of the COSY spectrum, combined with DEPT and HMQC data. The 13C NMR spectrum (100.6 MHz, CDCl3) of 2 provided strong support for the proposed structure. 13C NMR chemical shifts of 2 were assigned from the analysis of the proton noise-decoupled 13C, DEPT 135° and 90° spectra, 2D heteronuclear correlated spectra (HMQC and HMBC), and chemical shift comparison with 4 and other known quinolinone alkaloids (Kapadia et al., 1975, 1978, 1980, 1993; Morel et al., 2005). Detailed 1H and 13C NMR comparisons established that 2 differed structurally from 4 at the C-2 position, which has a methylene carbon bearing oxygen at δ 4.76/56.1 in place of the methyl group, and at C-8, which has a methylene group in place of the carbonyl group. On the basis of the results above, alkaloid 2, named vanessine, was identified as 2-(hydroxymethyl)-3-methoxy-5-octanyl-5,6,7,8-tetrahydroquinolin-4 (1H)-one. The relative stereochemistry of 2 {$[\alpha]_D^{25}$ + 18.5 (c 0.12, CHCl3)} was not directly determined, but was assumed to be 5S, as in alkaloid 4 {$[\alpha]_D^{25}$+ 24 (c 0.2, CHCl3)} (Buske et al., 1999), on the base of the same clockwise sense of the plane-polarized light.

Preliminary antimicrobial activity of alkaloids **1** and **2**, and of the derivatives **6**–**9**, was determined by means of bioautography (Saxena et al., 1995). These results show that only alkaloid **2** demonstrated antibacterial activity against *Escherichia coli*, *Salmonela setubal* and *Klebsiella pneumoniae*. The minimal inhibitory concentrations (MIC) obtained for the active alkaloid, used as recommended by NCCLS (2000), showed that alkaloid **2** has very weak antimicrobial activity against *E. coli* (25.0 μg/mL), *S. setubal* (50.0 μg/mL) and *K. pneumoniae* (25.0 μg/mL), compared to chloramphenicol (3.12, 3.12, and 1.56 μg/mL, respectively).

3. Experimental

3.1. General experimental procedures

Melting points were determined with an "MQAPF-301" (Micro-Química, Florianópolis, Brazil) apparatus and are uncorrected. Optical rotations were taken on a Perkin–Elmer 341 digital polarimeter. IR spectra were acquired on a Bruker IFS 28 spectrometer. High-resolution ESI mass spectra were recorded on a Bruker Bio Apex 70 eV FT-ICR (Bruker Daltonic, USA) instrument. Low resolution MS were recorded on a Varian-3800 Saturn system operating at an ionization potential of 70 eV. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 spectrometer at 400.1/100.6 MHz using $CDCl_3$ as a solvent and TMS as an internal standard. All crystallographic measurements were made on a Bruker X8 Kappa Apex II area detector with graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$). Thin layer chromatography was performed on a pre-coated TLC plates (Merck, silica 60 F-254), sprayed with Dragendorff's reagent, and 10% H_2SO_4 in EtOH followed by heating.

3.2. Plant material

W. douradinha stems were collected in April 2004 in São Pedro do Sul, Rio Grande do Sul, Brazil. Prof. Thais S.C. Dorow (Department of Botany, Federal University of Santa Maria, Brazil) identified the plant and a voucher specimen (SMDB 8073) is deposited in the herbarium of the Federal University of Santa Maria.

3.3. Extractions and Isolation

Air-dried stems of *W. douradinha* (5.1 kg) were pulverized and extracted exhaustively with MeOH (5 L ×5, 3 days each time), at room temperature. Concentration of the combined MeOH extracts yielded a dark crude extract (1.47 kg). This extract was suspended in H_2O (1.0 L) and successively partitioned with *n*-hexane (HF, yield 10.5 g) to remove lipid material. The remaining aqueous extract was acidified with 2 N HCl to pH 2–3. After exhaustive extraction with Et_2O (0.5 L ×5), the acidic solution was made basic with NH_4OH to pH 9 and extracted with Et_2O (0.5 L ×5) to yield the basic ether extract (5.8 g). The hexane fraction (EF) containing, in minor amounts, two Dragendorff-positive spots in TLC, was subjected to silica gel CC (800 g), eluted with *n*-hexane–EtOAc mixtures (95:5, 90:10, 80:20, 60:40, 50:50, and 40:60, v/v) to give 68

fractions (100 mL each). Fractions 10–14 (*n*-hexane–EtOAc, 90:10) containing the same Dragendorff-positive spots by TLC were combined (25 mg) and subjected to preparative TLC (*n*-hexane–EtOAc 8:2, twice) to give **4** (11 mg). Fraction 21 (120 mg), containing another Dragendorff-positive spot in TLC, was subjected to preparative TLC (silica gel, *n*-hexane–EtOAc, 80:20, twice) to give **5** (85 mg). The basic ethyl ether fraction, which gave a positive response on TLC with Dragendorff's reagent for three alkaloids, was further fractionated by silica CC (400 g) eluted with CH_2Cl_2 containing increasing amounts of MeOH (up to 50% v/v) to give 55 fractions (200 mL each). Fractions with similar TLC profiles were combined to produce 20 sub-fractions. Sub-fraction 8 that eluted with CH_2Cl_2–MeOH (97:3) was subjected to preparative TLC (silica gel, CH_2Cl_2–MeOH, 98:2) to give **2** (12 mg). Sub-fraction 14 (CH_2Cl_2–MeOH, 90:10) was submitted to preparative TLC (silica gel, diisopropyl ether–MeOH, 95:5) to yield **3** (60 mg). Sub-fraction 15 (CH_2Cl_2–MeOH, 90:10) was submitted to preparative TLC (silica gel, hexane–acetone, 60:40). The positive Dragendorff's spot was extracted (80 mg) and resubmitted to further preparative TLC (silica gel, CH_2Cl_2–MeOH, 95:5) to give **1** (40.5 mg).

3.4. Waltherione B (*1*)

White solid from $CHCl_3$–MeOH, m.p. 170.5–171.0 °C; $[\alpha]_D^{25}$ +2.1 (*c* 0.01, $CHCl_3$); IR ν_{max} cm^{-1}: 3400–3200 (NH), 1684–1636 (C=O); HRESIMS $[M+Na]^+$: *m/z* = 416,14694 (Calc. for $C_{23}H_{23}NO_5$ + Na 416,1468439). ^1H NMR ($CDCl_3$, 400 MHz): δ_H =1.37/1.67 (2H, *m*, H-11), 1.95/2.23 (2H, *m*, H-12), 2.35 (3H, *s*, 2C-Me), 3.97 (3H, *s*, 3C-OCH_3), 3.98 (3H, *s*, 2′C –OCH_3), 4.75 (1H, *br s*, H-10), 5.14 (1H, *s*, 9-OH), 6.68 (1H, *dd*, 7.5;1.5 Hz, H-6′), 6.72 (1H, *ddd*, 7.5, 7.2,1.5 Hz, H-5′), 6.90 (1H, *d*, 8.1 Hz, H-7), 6.95 (1H, *d*, 8 Hz, H-3′), 7.18 (1H, *d*, 8.1 Hz, H-8), 7.22 (1H, *ddd*, 8.0; 7.2;1.5 Hz, H-4′). ^{13}C NMR (CD_3, 100 MHz): δ_C = 14.4 (2C-*C*H_3), 23.3 (C-11), 32.0 (C-12), 55.5 (3C-O*C*H_3), 59.7 (2′C-O*C*H_3), 72.2 (C-13), 78.0 (C-9), 82.5 (C-10), 111.8 (C-3′), 117.1 (C-8), 120.4 (C-4a), 120.7 (C-5′), 128.9 (C-4′), 129.0 (C-7), 130.4 (C-6), 131.4 (C-6′), 133.4 (C-1′), 139.4 (C-3), 141.7 (C-2), 141.8 (C-5), 141.9 (C-8a), 157.9 (C-2′), 174.2 (C-4).

3.5. Vanessine (*2*)

Yellowish viscous oil. $[\alpha]_D^{25}$ +18.5 (*c* 0.12, $CHCl_3$); ν_{max} cm^{-1}: 3580–3400 (OH), 3390–3210 (NH), 1680–1635 (C=O); HRESIMS ($[M+H]^+$ at 322.23772 ($C_{19}H_{31}NO_3$ + H (Calc. 322.237670). EIMS: *m/z* = 321 $[M]^+$, 209 $\{[M]^+-C_8H_{16}\}$.^1H NMR ($CDCl_3$, 400 MHz): δ_H = 0.85 (3H, *t*, H-16), 1.23–1.29 (12H, *m*, H10–H15), 1.21/1.71 (2H, *m*, H-6), 1.28 (2H, *m*, H-7), 1.49/1.88 (2H, *m*, H-9), 2.60 (2H, *m*, H-8), 3.0 (1H, *m*, H-5), 3.75 (3H, *s*, 3C-O*C*H_3), 4.76 (2H, *s*, H-17). ^{13}C NMR (CD_3, 100 MHz): δ_C = 14.0 (C-16), 16.9 (C-6), 22.6–33.5 (C10–C15), 24.6 (C-9), 27.0 (C-8), 27.9 (C-7), 31.21 (C-5), 56.0 (C-17), 60.4 (C-18), 128.9 (C-8′), 141.8 (C-2), 142.5 (C-4′), 143.5 (C-3), 172.3 (C-4).

3.6. Alkaloids *3*–*5*

Identified by direct comparison with authentic samples, with spectroscopic data corresponding to those reported in the literature for waltherione A (Morel et al., 2005), antidesmone (Buske et al., 1999, 2002), and *O*-methyltembamide (Moura et al., 2002), respectively.

3.7. Methylation of *1* and *3*

Alkaloid **1** (20 mg) was dissolved in acetone (10 mL) and treated with an excess of $CH_3Br_{(g)}$ and catalytic amounts of NaH. The reaction mixture was kept at room temperature overnight, then concentrated to dryness (22 mg), dissolved in H_2O, and extracted with EtOAc (5 mL, 3×). The crude EtOAc soluble product (19 mg) was purified by preparative TLC (CH_2Cl_2–MeOH 95:5) to give the *O*-methylated derivative **6** (7.0 mg,) and the *N*-methylated derivative **7** (8.5 mg**)**. The same method was applied to alkaloid **3** (20 mg), to give the *O*-methylated derivative **8** (5.5 mg) and the *N*-methylated derivative **9** (9.0 mg). Compound **6,** (9*R**, 10*R**, 13*S**)-9-hydroxy-3,4-dimethoxy-9-(2′-methoxyphenyl)-14-oxa-bicyclo[3.2.1]-octa-[*f*] 2-methylquinoline colorless crystal from CH_2Cl_2–MeOH (8:2); m.p. 150 °C (decomposition); $[M]^+$ 405; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 1.35–2.45 (4H, *m*, H-11 and H-12), 2.80 (3H, *s*, H-20), 3.80 (3H, *s*, 4-OMe), 3.90 (3H, *s*, 3-OMe), 4.02 (3H, *s*, 15-OMe), 4.70 (1H, *s*l, H-10), 5.40 (1H, *s*, 9-OH), 6.20 (1H, *d*, 7 Hz, H-13), 6.90–7.4 (six aromatic hydrogens). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 20.0 (C-20), 24.0 (C-11), 34.5 (C-12), 56.5 (C-21), 60.2 (C-22), 60.5 (C-23), 76.0 (C-13), 79.5 (C-9), 80.5 (C-10), 112.0 (C-3′), 119.8 (C-4a), 120.6 (C-5′), 127.3 (C-8), 129.3 (C-7), 129.8 (C-4′), 131.3 (C-6′), 131.6 (C-6), 134.0 (C-1′), 141.2 (C-5), 144.2 (C-2), 145.2 (C-8a), 157.2 (C-2′), 158.1 (C-4), 159.0 (C-3). Compound **7,** (9*R**, 10*R**, 13*S**)-9-hydroxy-3-methoxy-1,2-dimethyl-9-(2′-methoxyphenyl)-14-oxa-bicyclo[3.2.1]-octa-[*f*] quinolone: yellowish crystal, m.p. 111.6–112.0 °C; EIMS: *m/z* = $[M]^+$ 405; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 1.37-2.47 (4H, *m*, H-11 and H-12), 2.60 (3H, *s*, H-20), 3.90 (6H, *s*, 3-OMe and 15-OMe), 3.60 (3H, *s*, NMe), 4.70 (1H, *brs*, H-10), 6.25 (1H, *d*, 7 Hz, H-13), 6.60–7.60 (six aromatic hydrogens). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 20.0 (C-20), 24.0 (C-11), 33.0 (N–Me), 34.5 (C-12), 56.5 (C-21), 60.2 (C-22), 61.5 (C-23), 76.3 (C-13), 79.5 (C-9), 80.5 (C-10), 110.8 (C-3′), 118.2 (C-8), 119.8 (C-4a), 121.3 (C-5′), 129.2 (C-4′), 129.4 (C-7), 129.8 (C-6), 132.1 (C-6′), 133.8 (C-1′), 142.0 (C-2), 142.2 (C-5), 142.5 (C-8a), 157.5 (C-2′), 160.0 (C-3), 176.5 (C-4). Compound **8,** (9*R**, 10*S**, 13*R**)-9-hydroxy-3,4-dimethoxy-9-(2′-methoxyphenyl)-14-oxa-bicyclo[3.2.1]-octa-[*f*]2-methylquinoline: colorless crystal from CH_2Cl_2:MeOH (8:2); m.p. 187–188 °C; EIMS: *m/z* = $[M]^+$ 405; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 1.50–2.50 (4H, *m*, H-11 and H-12), 2.75 (3H, *s*, H-20),

3.95 (3H, *s*, 4-OMe), 3.92 (3H, *s*, 3-OMe), 4.05 (3H, *s*, 15-OMe), 4.74 (1H, *brs*, H-10), 5.35 (1H, *s*, 9-OH), 6.40 (^{1}H, *brs*, H-13), 6.20–7.80 (six aromatic hydrogens). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 20.2 (C-20), 24.5 (C-11), 35.5 (C-12), 56.0 (C-21), 60.2 (C-22), 60.7 (C-23), 76.3 (C-13), 79.8 (C-9), 81.5 (C-10), 110.2 (C-3′), 120.4 (C-4a), 120.5 (C-5′), 128.0 (C-8), 128.3 (C-7), 130.8 (C-4′), 131.3 (C-6), 132.0 (C-6′), 134.5 (C-1′), 144.0 (C-2), 145.4 (C-8a), 157.0 (C-2′), 158.2 (C-4), 159.5 (C-3). Compound **9,** (9*R**, 10*S**, 13*R**)-9-hydroxy-3-methoxy-1,2-dimethyl-9-(2′-methoxyphenyl)-14-oxa-bicyclo[3.2.1]-octa-[*f*]quinolone: colorless crystal from CH_2Cl_2:MeOH (8:2); mp 165 °C (decomposition); ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.35–2.50 (4H, *m*, H-11 and H-12), 2.63 (3H, *s*, H-20), 3.92 (3H, *s*, 3-OMe), 3.98 (3H, *s*, 15-OMe), 3.68 (3H, *s*, NMe), 4.74 (1H, *brs*, H-10), 6.35 (1H, *brs*, H-13), 6.70–7.80 (six aromatic hydrogens). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 20.7 (C-20), 24.7 (C-11), 32.8. (N–Me), 34.5 (C-12), 57.2 (C-21), 61.2 (C-22), 61.8 (C-23), 76.5 (C-13), 79.2 (C-9), 81.2 (C-10), 111.2 (C-3′), 117.7 (C-8), 120.2 (C-4a), 122.3 (C-5′), 129.0 (C-4′), 130.8 (C-7), 131.0 (C-6), 131.6 (C-6′), 135.2 (C-1′), 141.9 (C-2), 142.3 (C-5), 142.2 (C-8a), 157.6 (C-2′), 159.5 (C-3), 176.8 (C-4).

3.8. X-ray crystallographic analysis

Alkaloids **1** and **3**, and their derivatives **6**–**9** were submitted to crystallization, but only **3**, **6**, and **9**, afforded crystals suitable for X-ray diffraction determination.

Alkaloid **3** crystallized from an *n*-hexane:MeOH 1:1 solution at room temperature with MeOH and H_2O solvate, positionally disordered over two sites. The asymmetric unit contains four molecules of **3**, joined by hydrogen bonding to the solvate (disordered water/methanol). The only difference between the molecules is the dihedral angles of the methoxy groups. A perspective view of the final X-ray model of one molecule of **3** is shown in Fig. 1. Crystal data of **3**: $(C_{23}H_{24}NO_5)_4 \cdot CH_3OH \cdot H_2O$, tetragonal, space group $P4_3$, $a = b = 15.5109(3)$ Å, $c = 33.6208(11)$ Å, $V = 8088.8(3)$ Å^3, $Z = 4$, $D_c = 1.333$ Mg m^{-3}, $F(000) = 3440$, absorption coefficient = 0.095 mm^{-1}, $T = 293(2)$ K, crystal size = 0.20 × 0.10 × 0.07 mm^3. CCDC No. 626424.

Compounds **6** and **9** were crystallized from CH_2Cl_2–MeOH (4:1, v/v) solutions at room temperature. A view of the X-ray model of **6** is shown in Fig. 2. Crystal data of **6**: $C_{24}H_{25}NO_5$, orthorhombic, space group $P2_12_12_1$, $a = 7.6938(4)$ Å $b = 12.2862(7)$ Å, $c = 20.8109(12)$ Å, $V = 1967.20(19)$ Å^3, $Z = 4$, $D_c = 1.376$ Mg m^{-3}, $F(000) = 864$, absorption coefficient = 0.096 mm^{-1}, $T = 296(2)$ K, crystal size = 0.30 × 0.25 × 0.15 mm^3. Crystal data of **9**: $C_{24}H_{25}NO_5$, orthorhombic, space group $P2_12_12_1$, $a = 9.2078(5)$ Å, $b = 9.4827(5)$ Å, $c = 23.65473(13)$ Å, $V = 2056.03(19)$ Å^3, $Z = 4$, $D_c = 1.316$ Mg m^{-3}, $F(000) = 864$, absorption coefficient = 0.092 mm^{-1}, $T = 296(2)$ K, crystal size = 0.35 × 0.20 × 0.04 mm^3. CCDC Nos. 626425 and 626426, respectively. Crystallographic data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk) by quoting the CCDC Nos. listed above.

3.9. Antimicrobial assay

Preliminary antimicrobial activities of alkaloids **1** and **2**, and of compounds **6**–**9**, were obtained using a bioautography technique (Saxena et al., 1995). The Minimal Inhibitory Concentration (MIC) was determined in 96-well culture plates by a micro-dilution method using a microorganism suspension with a density of 10^5 CFU/mL in Casein Soy Broth (CSB) incubated for 24 h at 37 °C for bacteria, and Sabouraud Broth (SB) incubated for 72 h at 30 °C for yeasts, as recommended by NCCLS, for determination of the MIC (NCCLS, 2000). Proper blanks were assayed simultaneously with samples tested in triplicate. A collection of ten microorganisms was used, including three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538p), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), four Gram-negative bacteria: *Klebsiella pneumoniae* (ATCC 10031), *E. coli* (ATCC 11103) *S. setubal* (ATCC 19196) and *Pseudomona aeruginosa* (ATCC 9341), and three yeasts: *Saccharomyces cerevise* (ATCC 2601), *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 289). Standard strains of microorganisms were maintained at the Chemistry Department of the University of Santa Maria, RS, Brazil. Chloramphenicol and mystatin were used in order to control sensitivity of the test microbials. Samples were tested in triplicate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.10.018.

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