Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 1163–1167
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

An unusual quinolinone alkaloid from *Waltheria douradinha*

Solange C.S.M. Hoelzel, Eleno R. Vieira, Sandro R. Giacomelli, Ionara I. Dalcol, Nilo Zanatta, Ademir F. Morel *

Departamento de Química (NPPN), Universidade Federal de Santa Maria, CEP: 97105-900 Santa Maria, RS, Brazil

Received 9 November 2004; received in revised form 1 February 2005
Available online 3 May 2005

Abstract

The chemical investigation of the methanolic extract of the root bark of *Waltheria douradinha* (Sterculiaceae) afforded an unusual quinolinone alkaloid named waltherione-A (**1**). Its structure was determined mainly by NMR spectroscopic methods. The antibacterial activity of waltherione-A (**1**) and the corresponding *O*-methylated derivative (**2**) was tested against three Gram-negative and three Gram-positive bacteria, with only (**2**) having moderate activity.

Keywords: *Waltheria douradinha*; Sterculiaceae; Quinolinone-alkaloid; Structure determination; Antibacterial activity

1. Introduction

Waltheria douradinha St Hil. (Sterculiaceae), locally called ''douradinha-do-campo'', is a plant native to South America, mainly South Brazil, Uruguay, Paraguay, and Argentine (Schultz, 1980). It is used in folk medicine for the treatment of various diseases such as bronchitis, laryngitis, and as a wound cleansing and healing agent (Simões, 1986). In our previous paper on the chemical constituents of *W. douradinha*, our interest was directed toward the isolation and structural elucidation of their cyclopeptide alkaloids (Morel et al., 1999a,b). In that study, a unknown compound, which gave positive response in Dragendorff's reagent test was also isolated, but it did not show a peptide bond in the IR spectrum. However, due to the small quantity of the isolated compound, its structure was not elucidated. During a re-examination of a second collection of this plant in 2002, a larger amount of this alkaloid was isolated, which allowed the elucidation of its structure and to perform biological activity tests. Thus, this compound was ultimately deduced to be a new quinolinone alkaloid (**1**), named waltherione-A on the basis of its ^{1}H and ^{13}C NMR spectroscopic data, including 2D NMR experiments such as ^{1}H–^{1}H COSY, NOESY, and ^{1}H–^{13}C-COSY–$^{n}J_{HC}$ ($n = 1, 2$, and 3, HMQC, HMBC). This class of alkaloid is unusual in plants of the Sterculiaceae family. For example, in the literature search, we found only the alkaloids melochinone (Kapadia et al., 1975), and its open chain analog, melovinone (Kapadia et al., 1978), both isolated from *Melochia tomentosa* L. (Sterculiaceae). More recently, sterculinines I and II, which are 2-quinolinones structures instead of 4-quinolinones described by Kapadia et al. or our new isolated compound, were isolated from *Sterculia lychnophora* Hance (Sterculiaceae) (Wang et al., 2003). In a preliminary antibacterial screening, while waltherione-A (**1**) was inactive against three Gram-positive and three Gram-negative bacterial strains, its corresponding *O*-methylated derivative (**2**) was active against these bacteria.

* Corresponding author. Tel.: +55 55220 8869; fax: +55 55220 8031.
E-mail address: afmorel@base.ufsm.br (A.F. Morel).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.03.019

2. Results and discussion

Waltherione-A (**1**) was obtained as a white solid. Its molecular formula was determined to be $C_{23}H_{23}NO_5$ by means of HRMS, which gave a molecular ion at *m/z* 393.15777 (calcd. for $C_{23}H_{23}NO_5$ 393.15762), and from ^{13}C NMR spectroscopic data, which indicated the presence of 23 carbons. The ^1H NMR spectrum of **1** recorded in $CDCl_3$ indicated the presence of six aromatic hydrogens between δ 6.29 and 7.48 ppm, three methyl singlets [two at δ 3.97 and 3.77 (3C–OMe and 15C–OMe), and one at δ 2.41 (2C–Me)], two diastereotopic methylene at δ 1.90/2.13 and 1.82/1.92, and two methine hydrogens at δ 4.61 and 6.87. The ^1H NMR spectrum also allowed the assignment of one hydroxyl group at δ 5.01 and an amidic hydrogen at δ 11.27. The homonuclear ^1H–^1H-COSY spectrum revealed the presence of three spin systems in the molecule of **1**. The first spin system showed a connectivity between the C-7 aromatic hydrogen at δ 7.48 (d, J = 8.2 Hz) and the C-8 aromatic hydrogen at 7.41 (d, J = 8.2 Hz). In the second spin system, we observed vicinal coupling between the C-10 methine hydrogen at δ 4.61 (dd, J = 8.0; 1.7 Hz) and the C-11 methylene hydrogens at δ 1.90/2.13 (m), which showed coupling with the methylene C-12 hydrogens at δ 1.82/1.92 ppm. In turn, the latter exhibited vicinal coupling with the methine C-13 hydrogen at δ 6.87 (dd, J = 8.0; 1.5 Hz). In the third spin system, we observed vicinal coupling between C-3′, C-4′, C-5′, and the C-6′ aromatic hydrogens at δ 6.94, 7.20, 6.70, and 6.29 ppm, respectively. The NOESY spectra of **1** showed a cross peak between H-10 and H-13, indicating that these two hydrogens are in *syn* position. The relative configuration of C-9, C-10, and C-13 was proposed as 9R*, 10R*, 13S* from spectroscopic data, this being supported by AM1 calculation (Dewar et al., 1985). The ^{13}C NMR spectrum (100.6 MHz, $CDCl_3$) of **1** provided strong support for the proposed structure. The ^{13}C NMR chemical shifts of **1** were assigned from the analysis of the proton noise-decoupled ^{13}C, DEPT 135° and 90° spectra, 2D heteronuclear correlated spectra (HMQC and HMBC), and chemical shift comparison with known quinolinone alkaloids (Kapadia et al., 1975, 1978, 1980). The ^1H and ^{13}C NMR of **1**, along with the hydrogen coupling constants and the most important connectivities observed in the HMBC spectrum are presented in Table 1. Fig. 1 shows the most important connectivities observed in the HMBC spectrum of **1** that were supported by intra- and inter-residue heteronuclear correlations.

A ^1H NMR spectrum was registered in DMSO-d_6 in order to provide evidence of a possible tautomerism on the quinolinone moiety of **1**, as previously observed by Werner (Werner, 1969). In this spectrum, the additional signal observed at 8.2 ppm was assigned to the phenolic form (enol) of **1**. The increase in the size of the phenol peak with increasing temperature, observed in a ^1H NMR study of this sample with varying temperature from 25 to 80 °C is an indication of its tautomerism. To further confirm the tautomeric forms, compound **1** was methylated with diazomethane overnight to furnish the *O*-methylated product **2** (40%). This result also confirmed that **1** does exist in a tautomeric equilibrium between the keto (**1**)-phenol (**1′**) forms. Therefore, compound **1** is closely related to melochinone (Kapadia et al., 1975), except that the 7-membered ring fused to the quinolinone system of melochinone has been replaced by a bicyclo moiety with an epoxide bridge between carbons C-10 and C-13 and also bearing a hydroxyl group on C-9. In this manner, compound **1** was assigned as 9-hydroxy-3-methoxy-2-methyl-9-(2-methoxyphenyl)-14-oxa-biciclo[3.2.1]-octa-[*f*]quinolinone, and designated as waltherione A. Compound **2** (see Scheme 1) was obtained as a white solid. The EI-mass spectrum showed the molecular ion [M^+] at *m/z* 405.

The antibacterial activity of alkaloid **1** and the *O*-methylated derivative **2** was evaluated by means of direct bioautography in a TLC bioassay (Saxena et al., 1995; Rahalison et al., 1991). The amounts of waltherione-A (**1**) assayed were not active against the bacteria tested (*Staphylococcus aureus*, *Streptococcus epidermidis*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella setubal* and *Escherichia coli*), while its *O*-methyl derivative (**2**) was active against all tested strains. The detection limits were 25.0, 3.5, 6.5, 12.5, 12.5, and 6.5 μg, respectively. The largest sample amount tested was 50 μg.

3. Experimental

3.1. General methods

Melting points were determined in a MQAPF-301 melting point apparatus and are uncorrected. Optical rotations were taken on a Perkin–Elmer 341 digital Polarimeter. IR spectra were recorded on a Bruker IFS 28 spectrometer. NMR spectra were acquired on a Brüker DPX-400 operating at 400 and 100 MHz, for ^1H and ^{13}C, respectively. Chemical shifts are given in δ (ppm) using tetramethylsilane (TMS) as an internal standard. HREIMS were recorded on a VG Autospec mass spectrometer operating in the mode at 70 eV. Thin layer chromatography was performed on pre-coated TLC plates (Merk, silica 60 F-254). Spots were visualized by 10% H_2SO_4 in ethanol solution followed by heating or with Dragendorff's reagent.

3.2. Plant material

The root bark of *Waltheria douradinha* was collected in São Pedro, Rio Grande do Sul state, Brazil in 2002,

Table 1
^{1}H and ^{13}C NMR spectroscopic data for **1** (400/100.6 MHz, $CDCl_3$, δ-values)

Position	δ^{1} H[a] (ppm)	*J* (Hz)	δ^{13} C[b] (ppm)	HMBC (H to C)
1	11.27	–	–	–
2	–	–	141.6	–
3	–	–	139.3	–
4	–	–	174.4	–
4a	–	–	119.8	–
5	–	–	141.7	–
6	–	–	130.5	–
7	7.48*d*	8.2	131.8	C-5, C-8a, C9
8	7.41*d*	8.2	117.4	C-4a, C-6
8a	–	–	141.8	–
9	–	–	77.2	–
10	4.61*dd*	8.0; 1.7	80.16	C-6, C12
11	1.90/2.13*m*	–	21.6	C-9, C13
12	1.82/1.92*m*	–	33.7	C-5, C-10
13	6.87*dd*	8.0; 1.5	75.6	C-4a,C-6, C-11
1′	–	–	134.7	–
2′	–	–	156.4	–
3′	6.94*d*	8.0; 1.5	110.8	C-1′, C-2′, C-5′
4′	7.20*ddd*	8.0; 7.3; 1.5	128.5	C-2′, C-6′
5′	6.70*ddd*	7.6; 7.3; 1.5	120.6	C-1′, C-3′
6′	6.29*dd*	7.6; 1.5	131.1	C-9, C-2′
2C–CH_3	2.41*s*	–	14.3	C-3
3C–OMe	3.97*s*	–	55.4	C-3
2′C–OMe	3.77*s*	–	59.2	C-2′

[a] Assignments confirmed by ^{1}H–^{1}H COSY and NOESY.
[b] Assignments confirmed by HMQC, HMBC and DEPT.

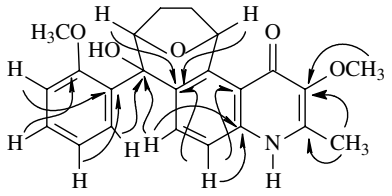
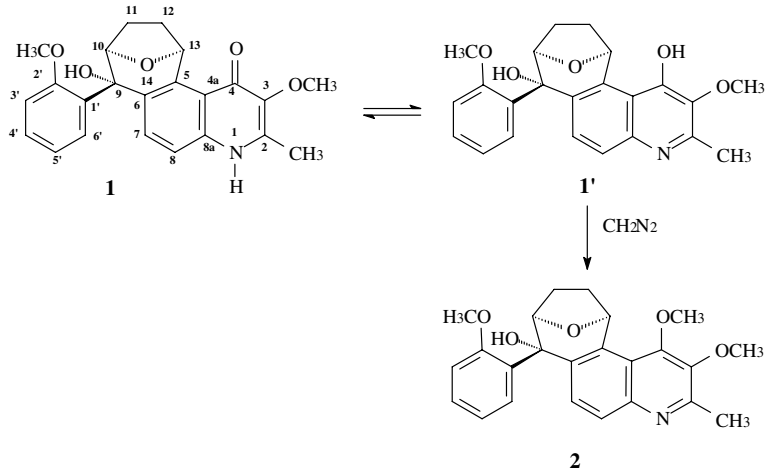


Fig. 1. Significant correlations in HMBC spectrum of **1**.

and was identified by Prof. Renato Záchia. The voucher specimen (SMDB 8073) is deposited in the Herbarium of the Federal University of Santa Maria.

3.3. Extraction and isolation

Dried ground root bark (5 kg) of *Waltheria douradinha* collected in São Pedro, Rio Grande do Sul state, Brazil in 2002 was exhaustively extracted with MeOH (5×5 L) at room temperature for 48 h. The resulting methanolic extract was filtered and concentrated in vacuo to obtain crude material (800 g). This extract was acidified to pH 2–3 and exhaustively extracted with Et_2O to yield the acidic ether extract (450 g). The pH of the aqueous fraction was adjusted (pH 8–9) and it was successively partitioned with *n*-hexane, Et_2O,



Scheme 1. Structures of waltherione-A (**1**), the phenolic form of **1**(**1′**) and of the corresponding *O*-methylated derivative (**2**).

EtOAc, and *n*-butanol. The Et_2O fraction (5 g) was disregarded in the present study, on the basis of a preliminary TLC analysis, which showed that all compounds were known cyclopeptide alkaloids. The known alkaloids were easily identified as adouetine Y′, scutianine B, franganine and the waltherines A, B and C, on the basis of their spectral properties and comparison with authentic samples. The EtOAc fraction (1.5 g) was subjected to a silica gel (230–400 mesh) CC eluted with a gradient of $CHCl_3$:MeOH (100:0 → 97:3 → 95:5 → 90:10 → 80:20 → 70:30 → 50:50 → 0:100, each volume 200 mL) to afford ten fractions (FA1–FA10). Fraction FA3 (150 mg), which contained a Draggendorf-positive spot in TLC, was further applied to silica gel (230–400 mesh) CC using $CHCl_3$:MeOH (98:2, v/v) as eluent to yield **1** (40 mg).

3.4. Waltherione-A (*1*)

White solid from $CHCl_3$–MeOH, m.p. 206.0–207.5 °C; $[\alpha]_D^{25}$: −25.5 (*c* 0.04, $CHCl_3$); IR ν_{max} cm^{-1}: 3400–3200 (NH), 1685–1635 (C=O); HREIMS: *m/z* = 393.15777 (Calc. for $C_{23}H_{23}NO_5$ 393.15762). For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

3.5. Methylation of *1*

Compound **1** (20 mg) was dissolved in an excess of ethereal diazomethane (20 mL) and the mixture was kept at room temperature overnight, following which the crude product (16 mg) was purified by preparative TLC ($CHCl_3$–MeOH, 10:1) to give the *O*-methylated derivative (**2**) as a white solid (10.5 mg); m.p. 188.0–189.0 °C; $[\alpha]_D^{25}$ − 21.5° (*c* 0.016, $CHCl_3$); EIMS: *m/z* = 405. ^1H NMR ($CDCl_3$, 400 MHz): δ = 2.60 (3H, *s*, C2–CH_3), 3.80 (3H, *s*, C3–OCH_3), 3.90 (3H, *s*, C2′–OCH_3), 4.1 (3H, *s*, C4–OCH_3), 4.75 (1H, *dd*, *J* = 8.0, 1.8 Hz, H-10), 6.18 (1H, *dd*, *J* = 8.0, 1.61 Hz, H-13), 6.30 (1H, *dd*, *J* = 7.5, 1.5 Hz, H-6), 6.85 (1H, *ddd*, *J* = 7.5, 7.2, 1.5 Hz, H-5′) 7.05 (1H, *ddd*, *J* = 8.0, 7.2, 1.5 Hz, H-4′), 7.20 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-3′), 7.60 (1H, *d*, *J* = 8.25 Hz, H-8), 7.85 (1H, *d*, *J* = 8.25 Hz, H-7), ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 158.0 (C-2), 143.0 (C-3), 155.0 (C-4), 120.5 (C-4a), 138.0 (C-5), 134.5 (C-6), 133.7 (C-7), 128.3 (C-8), 148.5 (C-8a), 77.5 (C-9), 80.6 (C-10), 22.5 (C-11), 34.2 (C-12), 75.6 (C-13), 134.5 (C-1′), 157.2 (C-2′), 110.5 (C-3′), 128.6 (C-4′), 120.5 (C-5′), 132.0 (C-6′), 19.5 (C2–CH_3), 55.6 (C3-OCH_3), 60.40 (C4–OCH_3), 59.4 (C2′–OCH_3).

3.6. AM1 calculation

The calculations were carried out by the Austin Model 1 (AM1) semiempirical method, implemented in the CS MOPAC 97 package. Geometries were completely optimized without fixing any parameter, thus bringing all geometric variables to their equilibrium values. The energy minimization protocol employs the Eigenvector Following routine, a conjugated gradient method. Convergence to a local minimum is achieved when the energy gradient is $\leqslant$0.01 kcal mol^{-1}. The calculations were performed on a PC Pentium III-550 MHz. AM1 calculation data are available under request.

3.7. Antibacterial assays

The antibacterial activity of **1** and **2** was tested against three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538p), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 9341), and three Gram-negative bacteria: *Klebsiela pneumoniae* (ATCC 10031), *Salmonella setubal* (ATCC 19196) and *Escherichia coli* (ATCC 11103), using the bioautography technique (Saxena et al., 1995; Rahalison et al., 1991). The microorganisms used in the antibacterial assay have been maintained at the Chemistry Departament of Universidade Federal de Santa Maria, RS, Brazil. For the antimicrobial assay, 25.0, 12.5, 6.25 and 3.12 μg of **1** and **2** was applied to pre-coated TLC plates. TLC plates were developed with $CHCl_3$:MeOH (95:5) and dried for complete removal of solvents. The inoculum was prepared by culturing each organism in tryptone soya agar (TSA, Oxoid) at 37 °C to a turbidity equivalent to McFarland 0.5 standard (1.5×10^8 CFU/mL). One microliter of each diluted inoculum (10^4–10^6 CFU) was applied onto Mueller Agar (MHA-DIFCO) plates. Amoxillin was used as positive control. Each assay was conduced in triplicate.

Acknowledgments

The authors thank FAPERGS (Fundação de Amparo à Pesquisa do Rio Grande do Sul) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support. We thank Prof. Paulo M Imamura (IQ-UNICAMP, SP, Brazil) for the HRMS measurements.

References

Dewar, M.J.S., Zoebisch, E.G., Healy, E.F., Stewart, J.J.P., 1985. AM1 a new general purpose quantum mechanical molecular model. Journal of the American Chemical Society 107, 3902–3909.

Kapadia, G.J., Shukla, Y.N., Basac, S.P., 1978. Melovinone, an open chain analogue of melochinone from *Melochia tomentosa*. Phytochemistry 17, 1444–1445.

Kapadia, J.G., Paul, B.D., Silverton, J.V., Fales, H.M., Sokoloski, E.A., 1975. Melochinone, a novel quinolinone from *Melochia tomentosa*. Journal of the American Chemical Society 12, 6814–6819.

Kapadia, G.J., Shukla, Y.N., Basac, S.P., Sololosky, E.A., Fales, H.M., 1980. The melosatins – a novel class of alkaloids from *Melochia tomentosa*. Tetrahedron 36, 2441–2447.

Morel, A.F., Gehrke, I.T.S., Mostardeiro, M.A., Ethur, E.M., Dessoy, E.C., 1999a. Cyclopeptide from the bark of *Waltheria douradinha*. Phytochemistry 51, 473–477.

Morel, A.F., Flach, A., Zanatta, N., Ethur, E.M., Mostardeiro, M.A., Gehrke, T.S., 1999b. A new cyclopeptide alkaloid from the bark of *Waltheria douradinha*. Tetrahedron Letters 40, 9205–9209.

Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M., Frenk, E., 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochemical Analysis 2, 199–203.

Saxena, G., Farmer, S., Towers, G.H.N., Hancock, R.E.W., 1995. Use of specific dyes in the detection of antimicrobial compounds from crude plant extracts using a thin layer chromatography agar overlay technique. Phytochemical Analysis 6, 125–129.

Schultz, A.R.H., 1980. Introdução à Botânica Sistemática, 5ª ed., vol. 2, Editora UFRGS, Porto Alegre, pp. 199–202.

Simões, C.M.O., 1986. Plantas Da Medicina Popular Do Rio Grande Do Sul. Editora UFRGS, Porto Alegre, pp. 56–57.

Wang, R.F., Yang, X.W., Ma, C.M., Shang, M.Y., Liang, J.Y., Wang, X., Cai, S.Q., Shoyama, Y., 2003. Alkaloids from the seeds of *Sterculia lychonophora* (Pangdahai). Phytochemistry 63, 475–478.

Werner, W., 1969. Methylierung und hydrierung von 2-alkyl-chinolon-4. Tetrahedron 25, 255–261.