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Article

Meroterpenoid Hydroquinones from *Cordia globosa*

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Two new meroterpenoid hydroquinones, *rel*-(4b*E*,6*Z*,8*E*,9a*S*,10*S*)-1,4-dihydroxy-9a,10-dihydro-10,12-epoxy-5-methylbenzo[*a*]azulen-12-one and *rel*-(4b*Z*,6*Z*,8*E*,9a*S*,10*S*)-1-hydroxy-9a,10-dihydro-4,11:10,12-diepoxy-benzo[*a*]azulen-11,12-dione, along with the known peptide derivative (*S*)-*N*-benzoylphenylalanine-(*S*)-2-benzamide-3-phenylpropyl ester, were isolated from the roots of *Cordia globosa*. Their structures were determined by 1D and 2D nuclear magnetic resonance (NMR) spectrometry, Fourier transform infrared (FTIR) spectroscopy and high resolution atmospheric pressure chemical ionization mass spectrometry (HRAPCIMS) data analysis. The new compounds were tested against three human cancer cell lines (colon adenocarcinoma, ovarian carcinoma and glioblastoma), but none of them exhibited any activity.

Keywords: *Cordia globosa*, Boraginaceae, hydroquinones

Introduction

The *Cordia* genus (Boraginaceae) comprises approximately 300 species widespread worldwide,[1] many of which used in traditional medicine for different purposes as cicatrizing, anti-inflammatory, anthelmintic, antimalarial, diuretic and to treat urinary infections.[2-4] *Cordia* has proved to be a prolific source of meroterpenoid quinones, chromenes, hydroquinones and hydrochromenes.[5-9]

Previously, we have investigated some plants belonging to the genus *Cordia*, including *C. globosa* and evaluated the antiproliferative properties of the isolated terpenoid quinones.[10-12] In the present work the EtOH extract from roots of *C. globosa*, an annual and aromatic shrub native to the northeast of Brazil, was investigated, which led to the isolation and characterization of two new terpenoid hydroquinones (**1** and **2**), and a known peptide derivative (**3**) (Figure 1).

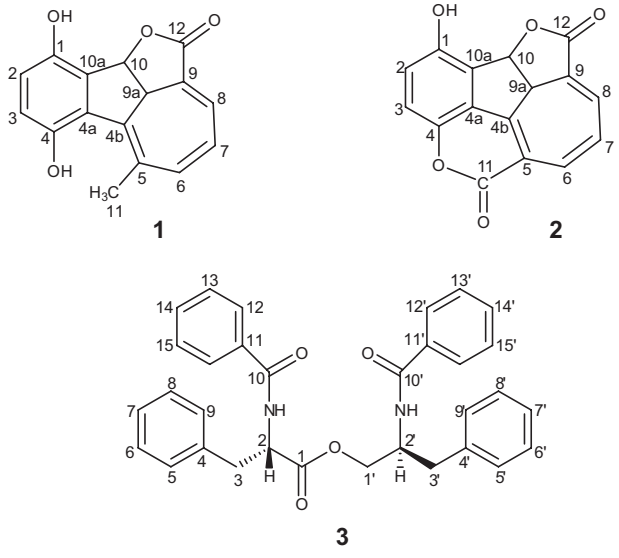

Figure 1. Structures of compounds **1**-**3** isolated from *C. globosa*.

Experimental

General experimental procedures

The Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer spectrum 100 equipped with a universal attenuated total reflectance (UATR) accessory. Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. One-dimensional [^{1}H, ^{13}C, distortionless enhancement by polarization transfer (DEPT)] and two-dimensional nuclear magnetic resonance (NMR) experiments [correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC),

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heteronuclear multiple-bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY)] were recorded on a Bruker DRX-500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard pulse sequences supplied by the manufacturer. The high resolution mass spectrometry (HRMS) using atmospheric pressure chemical ionization (HRAPCIMS) was performed on a liquid chromatography-mass spectrometry ion trap and time-of-flight (LCMS-IT-TOF, Shimadzu) spectrometer. The positive ion mass spectra were recorded in the *m/z* 200-700 range, using a potential of 4.0 kV on the capillary and He as the collision gas. The high performance liquid chromatography (HPLC) analysis was carried out using an ultra-fast liquid chromatography (UFLC, Shimadzu) system equipped with a SPD-M20A diode array UV-Vis detector and a Phenomenex C-18 column, 5 μm (4.6 × 250 mm^2). The mobile phase consisted of H_2O (trifluoroacetic acid (TFA) 0.2% v/v) and MeCN with a 4.5 mL min^{-1} flow rate, oven temperature of 40 °C and the chromatograms were monitored at 210-350 nm. Low performance liquid chromatography was carried out in glass columns packed with silica gel 60 (70-230 mesh, Vetec or 230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on silica gel precoated aluminum sheets (kieselgel 60 F_{254}, 0.20 mm, Merck). Fractions and pure compounds were monitored by TLC, and the spots visualized by heating (at ca. 100 °C) the plates sprayed with a vanillin/perchloric acid/EtOH solution.

Plant material

Roots of *C. globosa* were collected at Pico Alto, located at an approximate altitude of 1000 m, in Guaramiranga County, Ceará State, northeast of Brazil. The plant material was identified by PhD Maria Iracema B. Loiola, botanist of the Departamento de Biologia, Universidade Federal do Ceará (UFC). A voucher specimen (No. 39.851) has been deposited at the Herbário Prisco Bezerra, UFC.

Extraction and isolation

The air-dried and powdered roots (7.5 kg) of *C. globosa* were extracted with hexane (3 × 10 L) followed by EtOH (3 × 10 L), at room temperature for 24 h, and the resulting solutions were concentrated under reduced pressure to yield 13.9 g (0.001%) and 67.8 g (0.001%) of the hexane and EtOH extracts, respectively. The EtOH extract (67.0 g) was fractionated over silica gel by elution with CH_2Cl_2, followed by EtOAc, to yield two main fractions weighting 24.0 and 9.0 g, respectively. The CH_2Cl_2 fraction (24.0 g) was subjected to a silica gel column chromatography using hexane-EtOAc (2:8, 4:6, 6:4 and 8:2, v/v) and EtOAc as eluents, providing 28 fractions, which were monitored by TLC and then pooled to 8 subfractions. Subfraction 23-27 (hexane-EtOAc 8:2, 1.9 g) was subjected to repeated fractionation over silica gel eluted with hexane-EtOAc, to yield a main fraction of 129.0 mg [hexane-EtOAc (4:6, v/v)]. This material was subjected to semi-preparative HPLC using H_2O (TFA 0.2% v/v)-MeCN 6.5:3.5 to yield the pure compounds **1** (6.0 mg, t_R 8.7 min) and **2** (8.0 mg, t_R 15.4 min). The EtOAc fraction (3.4 g) was subjected to flash chromatography using an isocratic solution of CH_2Cl_2-EtOAc 8:2 (v/v) to yield 95 subfractions of 8 mL. Sub-fraction 56-72 (80.0 mg) was further purified by HPLC using the mobile phase H_2O (TFA 0.2% v/v)-MeCN 6:4 to afford compound **3** (5.5 mg, t_R 12.8 min).

rel-(4b*E*,6*Z*,8*E*,9a*S*,10*S*)-1,4-Dihydroxy-9a,10-dihydro-10,12-epoxy-5-methylbenzo[*a*]azulen-12-one (**1**)

Yellow powder; m.p. 197-203 °C; $[\alpha]_D^{20}$ –195.3° (*c* 0.01, MeOH); IR (ATR) ν_{max} / cm^{-1} 3317, 1718, 1684, 1639, 1215, 1260; HRAPCIMS calcd. for $C_{16}H_{13}O_4$ $[M + H]^+$: 269.0808; found: 269.0808; ^{1}H and ^{13}C NMR spectral data, see Table 1.

rel-(4b*Z*,6*Z*,8*E*,9a*S*,10*S*)-1-Hydroxy-9a,10-dihydro-4,11:10,12-diepoxy-benzo[*a*]azulen-11,12-dione (**2**)

Yellow powder; m.p. 200-205 °C; $[\alpha]_D^{20}$ –129.3° (*c* 0.9, EtOAc); IR (ATR) ν_{max} / cm^{-1} 3466, 1750, 1628, 1465, 1188, 1209; HRAPCIMS calcd. for $C_{16}H_9O_5$ $[M + H]^+$: 281.0444; found: 281.0469; ^{1}H and ^{13}C NMR spectral data, see Table 1.

Cytotoxicity evaluation

Cytotoxicity was evaluated against three human cancer cell lines provided by the National Cancer Institute (Bethesda, MD, USA): colon adenocarcinoma (HCT-116), ovarian carcinoma (OVCAR-8) and glioblastoma (SF-295). Cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mmol L^{-1} glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin at 37 °C under a 5% CO_2 atmosphere. For all experiments, cells were plated in 96-well plates (10^5 cells *per* well for adherent cells or 0.3 × 10^5 cells *per* well for suspended cells in 100 μL of medium). After 24 h, all the compounds (0.048-5.0 μg mL^{-1}) dissolved in 1% dimethylsulfoxide (DMSO) were added to each well using a high throughput screening system (Biomek 3000, Beckman Coulter, Inc.), and the cultures were incubated for 72 h. Doxorubicin (Zodiac) was used as a positive control. Control groups received the same amount

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2**

Position	**1**[a]		**2**[b]	
	δ_H / ppm	δ_C / ppm	δ_H / ppm	δ_C / ppm
1	–	149.4	–	153.9
2	6.85 (d, *J* 8.5 Hz)	120.1	7.23 (d, *J* 8.5 Hz)	118.4
3	6.82 (d, *J* 8.5 Hz)	118.8	7.33 (d, *J* 8.5 Hz)	123.2
4	–	147.6	–	146.8
4a	–	133.3	–	124.1
4b	–	129.4	–	143.5
5	–	128.5	–	122.6
6	7.02 (d, *J* 11.4 Hz)	144.2	7.62 (d, *J* 11.8 Hz)	131.2
7	6.77 (dd, *J* 11.4, 5.8 Hz)	128.8	6.82 (dd, *J* 11.8, 6.1 Hz)	130.2
8	7.03 (dd, *J* 5.8, 1.6 Hz)	127.6	7.26 (bd, *J* 6.1 Hz)	130.3
9	–	125.9	–	116.7
9a	2.96 (d, *J* 8.0 Hz)	47.7	4.08 (d, *J* 6.9 Hz)	45.8
10	6.17 (d, *J* 8.0 Hz)	79.8	6.56 (d, *J* 6.9 Hz)	82.1
10a	–	127.8	–	123.4
11	2.48 (s)	23.6	–	161.6
12	–	167.3	–	167.4

[a]500/125 MHz, acetone-d_6; [b]500/125 MHz, pyridine-d_5. δ: Chemical shift.

of DMSO. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazoliumbromide (MTT) to a purple formazan product as previously described.[13] At the end of the incubation, the plates were centrifuged and the medium was replaced with fresh medium (150 µL) containing MTT (0.5 mg mL^{-1}). Three hours later, the plates were centrifuged, the MTT formazan product was dissolved in 150 µL DMSO, and the absorbance was measured using a multiplate reader (Spectra Count, Packard). The drug effect was quantified as the percentage of the control absorbance of the reduced dye at 550 nm. The concentration values that inhibit growth in 50% (IC_{50}) were calculated, along with the respective 95% of confidence interval (CI), by non-linear regression using the software GraphPad Prism 5.0.

Results and Discussion

Compound **1**, a yellow powder, showed IR absorption bands for hydroxyl groups (3317 cm^{-1}), conjugated carboxyl of γ-lactone moieties (1718 cm^{-1}), carbon-carbon double bonds (1684 and 1639 cm^{-1}) and carbon-oxygen bonds (1215-1260 cm^{-1}). The molecular formula of $C_{16}H_{12}O_4$ (11 degrees of unsaturation) was determined by HRAPCIMS through the molecule protonated peak $[M + H]^+$ at *m/z* 269.0808 (calcd. *m/z* 269.0808). The ^{1}H NMR spectrum (Table 1) exhibited signals for an aromatic ring at δ_H 6.85 (d, *J* 8.5 Hz, H-2) and 6.82 (d, *J* 8.5 Hz, H-3), indicating an AB system similar to those of a 1,4-hydroquinone moiety. In addition, signals at δ_H 7.03 (dd, *J* 5.8 and 1.6 Hz, H-8), 7.02 (d, *J* 11.4 Hz, H-6) and 6.77 (dd, *J* 11.4 and 5.8 Hz, H-7) were related to a coupling system of olefinic protons, while the signals at δ_H 6.17 (d, *J* 8.0 Hz, H-10) and 2.96 (d, *J* 8.0 Hz, H-9a) were associated with methines, one of which corresponding to an oxymethine proton. Finally, a singlet at δ_H 2.48 (s, Me-11), was compatible with a vinyl methyl. Besides the vicinal correlations for the protons H-2/H-3, H-6/H-7, and H-9a/H-10, the COSY spectrum exhibited the allylic coupling for H-9a and H-8, as well as the homoallylic coupling of H-9a and the Me-11. The ^{13}C NMR spectrum (Table 1) displayed signals for 16 carbon atoms, 13 of which corresponding to sp^2 hybridized carbons. The DEPT spectrum revealed a methyl group at δ_C 23.6 (C-11), an oxymethine at δ_C 79.8 (C-10) and another methine at δ_C 47.7 (C-9a), in addition to five monohydrogenated sp^2 carbon atoms at δ_C 118.8-144.2. Comparison of DEPT with ^{13}C NMR spectra revealed eight non-hydrogenated carbon atoms, one characteristic of a γ-lactone carboxyl at δ_C 167.3 (C-12), as well as the signals at δ_C 149.4 (C-1) and 147.6 (C-4) related to the oxygenated carbons of the 1,4-hydroquinone moiety. In addition, signals for three olefinic double bonds were observed, which, after COSY and HMBC analyses (Figure 2), were shown to make part of an extensive conjugated system involving the lactone

carboxyl and the phenyl moiety. The long range correlations displayed by the oxymethine proton at δ_H 6.17 (d, *J* 8.0 Hz, H-10) with the carbon atoms at δ_C 149.4 (C-1), 127.8 (C-10a) and 129.4 (C-4b), were fundamental to assign the structure of compound **1** as a 1,4-hydroquinone bearing a monoterpene side chain constituted of a γ-lactone fused to a seven members ring. Unfortunately, the NOESY spectrum (Supplementary Information Figure S8) was not decisive to help defining the relative stereochemistry of **1**. The only undoubtful NOE observed was that of CH_3-11 with H-6, whose cross peaks have not shown-up on the COSY spectrum. All the other observed cross peaks can either be assigned to COSY breakthrough or chemical exchange. However, the C-9a and C-10 stereocenters were proposed to be *trans*, in agreement with the corresponding protons coupling constant of 8.0 Hz. Based on the above mentioned data, the structure of **1** was established as *rel*-(4b*E*,6*Z*,8*E*,9a*S*,10*S*)-1,4-dihydroxy-9a,10-dihydro-10,12-epoxy-5-methylbenzo[*a*]azulen-12-one.

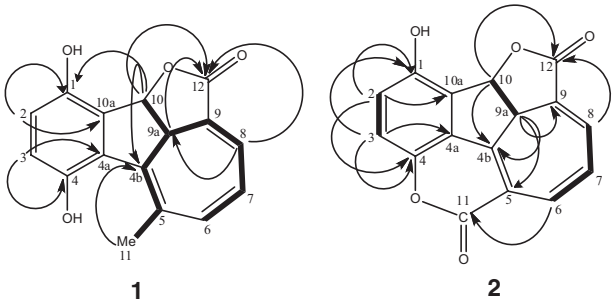

Figure 2. Selected COSY (H▬H) and HMBC (H→C) correlations observed for compounds **1** and **2**.

Compound **2** was also isolated as a yellow powder. Its FTIR spectrum showed absorption bands for hydroxyl groups (3466 cm^{-1}), conjugated carboxyl groups (1750 cm^{-1}), carbon-carbon double bonds (1628 and 1465 cm^{-1}) and carbon-oxygen bonds (1188 and 1209 cm^{-1}). The molecular formula of $C_{16}H_8O_5$ (13 degrees of unsaturation) was determined by HRAPCIMS analysis through the molecule protonated peak $[M + H]^+$ at *m/z* 281.0469 (calcd. *m/z* 281.0444). Despite the ^1H NMR spectrum of **2** (Figure 1) being run in a different solvent (C_5D_5N) than that used for **1** ($(CD_3)_2CO$), it showed the same number of protons and splitting pattern, except for the disappearance of methyl group, revealing the same backbone structure of **1**.

The ^{13}C and DEPT NMR spectra of **2** were also similar to those of **1** (Table 1). The main difference was the appearance of an additional δ-lactone carboxyl group at δ_C 161.6 (C-11) in compound **2**, in substitution of the Me-11 of **1**. The HMBC correlation of the proton signal at δ_H 7.62 (H-6) with the carboxyl at δ_C 161.6 supported the lactonization between C-4 and C-5 (Figure 2). Additional long range correlations, depicted in Figure 2, corroborated the structure of **2**. The structures of compounds **1** and **2** show a high degree of similarity, and one could then speculate on the biogenetic formation of **2** simply by the oxidation of Me-11 of **1** to the correspondent carboxyl acid followed by an intramolecular nucleophilic substitution reaction yielding the δ-lactone moiety. Thus, the structure of **2** was established as *rel*-(4b*Z*,6*Z*,8*E*,9a*S*,10*S*)-1-hydroxy-9a,10-dihydro-4,11:10,12-diepoxy-benzo[*a*]azulen-11,12-dione.

Additionally to the new compounds, the peptide (*S*)-*N*-benzoylphenylalanine-(*S*)-2-benzamide-3-phenylpropyl ester (**3**) was also isolated, currently designated as asperphenamate,[14] anabellamide[15] or auranamide[16] (Figure 1). This is the first report on the occurrence of this compound in *Cordia* spp.

Compounds **1**-**3** were tested *in vitro* for their antiproliferative effects against cancer cell lines HCT-116, OVCAR-8 and SF-295, however they didn't show cytotoxic activity (IC_{50} > 5.0 μg mL^{-1}).

Conclusions

In this work two new meroterpenoid hydroquinones (**1** and **2**) were isolated from the EtOH extract of roots of *Cordia globosa*, in addition to a peptide derivative (**3**) not yet reported for this genus. Compounds **1** and **2** are 1,4-hydroquinones fused to a monoterpene moiety, a structural feature that is frequently found in *Cordia* species, particularly in roots.

Supplementary Information

Supplementary information (^1H and ^{13}C NMR, COSY, HSQC, HMBC, NOESY, HRMS and FTIR spectra) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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