

## An Uncommon Naphthaquinoid and a New Lignan Derivative from the Roots of *Cordia leucocephala* Moric

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Two uncommon compounds, a meroterpene naphthoquinoid (cordiaquinone P) and a new lignan derivative, were isolated from the hexane extract of roots of *Cordia leucocephala* Moric and their structures, including the relative stereochemistry, were determined by nuclear magnetic resonance (NMR) (<sup>1</sup>H and <sup>13</sup>C NMR, HSQC, HMBC and NOESY) and high resolution electrospray ionization mass spectrometry (HRESIMS) techniques.

**Keywords:** *Cordia leucocephala*, Boraginaceae, naphthoquinone

### Introduction

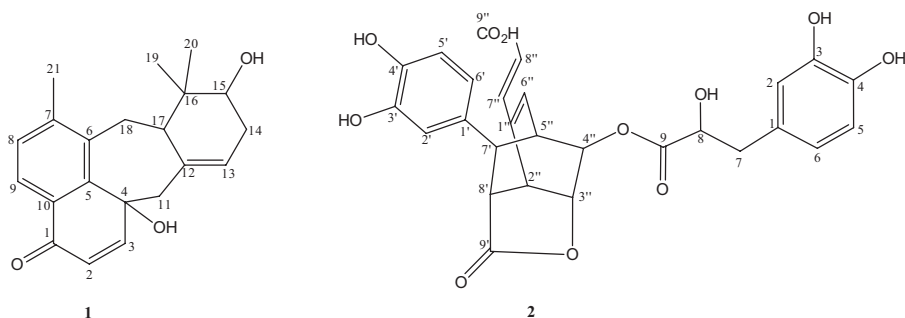
*Cordia* comprises one of the major and most important genus of the Boraginaceae family. For this genus are recognized ca. 300 species, many of which are used in traditional medicine.<sup>1</sup> It is known as a prolific source of terpenoids, phenolic compounds and saponins,<sup>2-6</sup> however, it is famous for its ability to biosynthesize meroterpenoid benzoquinone and naphthoquinones, including their reduced forms.<sup>7,8</sup> These type of compounds, many of which are named as cordiaquinones or cordiachromes, an allusion to the genus, seems to be of chemotaxonomic significance.<sup>8-11</sup> Interestingly, the occurrence of such secondary metabolites seems to be restrict to the roots. Previous work with the roots of *C. polycephala* resulted in the isolation of the new cordiaquinones P and Q, besides the known cordiaquinone J, whose cytotoxic properties were evaluated.<sup>12</sup> Our efforts to find anticancer compounds led us to continue the chemical investigation of the EtOH extract from roots of *C. polycephala*. In the present work, it is described the isolation and characterization of two further new compounds, a naphthoquinoid and a lignan derivative (Figure 1).

### Results and Discussion

The new compounds **1** and **2** were isolated from the root EtOH extract of *C. leucocephala* and their structures were unequivocally elucidated by spectrometric methods, including 1D and 2D nuclear magnetic resonance (NMR) experiments and high resolution electrospray ionization mass spectrometry (HRESIMS).

Compound **1**, a yellow resin, possesses molecular formula C<sub>21</sub>H<sub>24</sub>O<sub>3</sub> (ten degrees of unsaturation), which was determined by HRESIMS through the sodium adduct ion at *m/z* 347.1633 [M + Na]<sup>+</sup> (calcd. 347.1623). The infrared (IR) spectrum exhibited absorption bands for chelated and nonchelated hydroxyl (3512 and 3346 cm<sup>-1</sup>), C–H stretching of sp<sup>2</sup> and sp<sup>3</sup> carbon (3065–2886 cm<sup>-1</sup>), conjugated carbonyl (1661 cm<sup>-1</sup>), carbon-carbon double bond (1634 cm<sup>-1</sup>), skeletal bands of benzene ring (1588 and 1470 cm<sup>-1</sup>), C–O stretching (1087 and 1039 cm<sup>-1</sup>) and the =C–H out-of-plane bending (816 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) displayed signals for two *ortho* coupled protons at δ 7.87 (d, *J* 8.0 Hz, H-8) and 7.23 (d, *J* 8.0 Hz, H-7), olefinic proton signals at δ 6.80 (d, H-3) and 6.29 (d, H-2) compatible with a *cis* (*J* 10.0 Hz) double bond, and an isolated signal at δ 5.53 (br s, H-13) indicating a trisubstituted double bond. A signal for an oxymethine proton at δ 3.51 (t like, *J* 5.3 Hz, H-15), and two singlets

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**Figure 1.** Structures of compounds **1** and **2** isolated from *C. leucocephala*.

for methyl groups attached to a quaternary carbon at  $\delta$  1.09 (3H-19) and 1.08 (3H-20), and a singlet for a methyl attached to a benzene ring at  $\delta$  2.46 (3H-21). Additionally, a set of signals in the range of  $\delta$  3.45-1.75 indicated protons attached to nonfunctionalized  $sp^3$  carbons. The  $^1H$ - $^1H$  COSY contour map showed cross-peaks evidencing the presence of two isolated spin systems through the signals at  $\delta$  5.53 (H-13) with  $\delta$  2.34 and 2.20 (2H-14), and the later with  $\delta$  3.51 (H-15) for AMM'X, as well as for an AXX' at  $\delta$  1.75 (H-17) with  $\delta$  3.45 and 3.15 (2H-18).

The  $^{13}C$  NMR spectrum revealed 21 carbon signals (Table 1), to which, based on the DEPT and HSQC experiments, were assigned an oximethine ( $\delta$  74.2), a methine ( $\delta$  49.4), five monohydrogenated  $sp^2$  carbons ( $\delta$  154.4, 130.1, 126.4, 124.9 and 124.4), three methylenes ( $\delta$  49.2, 32.2 and 29.7), three methyls (26.1, 22.0 and 18.6), and eight nonhydrogenated carbons, five of which  $sp^2$  ( $\delta$  144.2, 142.4, 140.6, 137.4 and 128.2), one oxygenated ( $\delta$  69.7), one saturated ( $\delta$  38.2), and one carbonyl group ( $\delta$  185.0). A 4-hydroxy-7-methyl-naphthoquinone moiety (seven degrees of unsaturation) was supported by  $^1H$  and  $^{13}C$  NMR data and was confirmed by the HMBC contour map through the correlations of H-9 ( $\delta$  7.87) with C-1 ( $\delta$  185.0) and C-7 ( $\delta$  142.4); H-8 ( $\delta$  7.23) with C-6 ( $\delta$  140.6) and C-21 ( $\delta$  22.0); H-2 ( $\delta$  6.29) with C-10 ( $\delta$  128.2) and C-4 ( $\delta$  69.8), as well as the Me-21 ( $\delta$  2.46) with the carbons 6-8 ( $\delta$  140.6, 142.4 and 130.1, respectively). The three remaining unsaturation degrees were attributed to an extra carbon-carbon double bond and two cycles, which are part of a monoterpene ( $C_{10}$ ) chain positioned at C-4 and C-6, through the HMBC correlations of the methylene protons 2H-11 ( $\delta$  2.69 and 2.38) with C-3 ( $\delta$  154.4) and C-4 ( $\delta$  69.8), as well as the methylene protons 2H-18 ( $\delta$  3.45 and 3.15) with C-5 ( $\delta$  144.2), C-6 ( $\delta$  140.6) and C-7 ( $\delta$  142.4). Finally, the correct localization of the geminal methyls at C-16 ( $\delta$  38.2), the hydroxyl group at C-15 ( $\delta$  74.2) and the double bond between C-12 ( $\delta$  137.4) and C-13 ( $\delta$  124.4), were also confirmed by the HMBC contour map as depicted in Figure 2. The relative configuration of the stereocenters of **1**, was determined by NOESY analysis (Figure 3). The

hydroxyl group at C-15 was  $\alpha$ -positioned, particularly due to the dipolar interactions of the methine proton at  $\delta$  1.75 (d,  $J$  11.6 Hz, H-17) with the proton signals at  $\delta$  3.51 (t,  $J$  5.3 Hz, H-15) and the methyl signal at  $\delta$  1.09 (s, 3H-19). Both H-15 and 3H-19 also showed dipolar coupling, confirming the relative stereochemistry. The dipolar coupling of H-3 ( $\delta$  6.8 d,  $J$  10.0 Hz) with the H $\beta$ -11 ( $\delta$  2.69 d,  $J$  14.3 Hz), and of the later with H-13 ( $\delta$  5.53 br s) suggested that the hydroxyl at C-4 is also  $\alpha$ . Thus, the structure of **1** was established as the terpene 4 $\alpha$ ,15 $\alpha$ -dihydroxy-naphthoquinone derivative named as cordiaquinone P.

Compound **2**, was isolated as a brown resin, its HRESIMS exhibited a deprotonated molecule at  $m/z$  523.126 [ $M - H$ ] $^-$ , indicating as the  $C_{27}H_{24}O_{11}$  molecular formula, which requires 16 degrees of unsaturation. The  $^1H$  NMR spectrum (500 MHz,  $CD_3OD$ ) showed signals for two AMX systems:  $\delta$  6.74 (d,  $J$  1.0 Hz, H-2), 6.69 (d,  $J$  8.0 Hz, H-5) and 6.61 (br d,  $J$  8.0 Hz, H-6), and  $\delta$  6.64 (d,  $J$  8.1 Hz, H-5'), 6.42 (d,  $J$  1.6 Hz, H-2') and 6.33 (d,  $J$  8.1 Hz, H-6'), indicating two trisubstituted aromatic rings. Olefinic protons signals at  $\delta$  7.42 (d,  $J$  15.9 Hz, H-7''), 6.52 (d,  $J$  6.4 Hz, H-6'') and 6.26 (d,  $J$  15.9 Hz, H-8'') for a  $\alpha,\beta,\gamma,\delta$ -conjugated system, and signals for three oxymethine protons at  $\delta$  5.20 (dd,  $J$  8.0 and 3.7 Hz, H-8), 4.24 (d,  $J$  4.8 Hz, H-3'') and 3.99 (d,  $J$  3.2 Hz, H-4''), were also observed. In addition, the  $^1H$  NMR spectrum exhibited signals for diastereotopic methylene protons at  $\delta$  3.11 (dd,  $J$  14.3, 8.0 Hz, H-7a) and 3.00 (dd,  $J$  14.3, 3.7 Hz, H-7b), besides signals for three methine protons at  $\delta$  4.1 (br s, H-2''), 3.31 (br s, H-5'') and 2.67 (br s, H-8') (Table 1).

The combination of  $^{13}C$  NMR, DEPT and HSQC spectra were consistent with the presence of one methylene, sixteen methines and ten nonhydrogenated carbons (Table 1). A detailed NMR data analysis allowed to identify two aromatic rings, three carboxyl carbons [ $\delta$  181.0 (C-9), 173.7 (C-9') and 168.2 (C-9'')], two double bonds [ $\delta$  144.3 (C-7''), 141.9 (C-6''), 135.3 (C-1'') and 117.4 (C-8'')], and three oxymethine carbons [ $\delta$  84.2 (C-3'') and 75.1 (C-4'' and C-8)]. Based on the carbon hydrogenation pattern, chemical shift values and HMBC correlations, two caffeic acid moieties

were easily identified, as well as an  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -conjugated carboxylic acid system, Table 1 and Figure 2. Comparison of the NMR data of **2** with those reported for the bicyclic [2.2.2] octane lignin (rufescenolide), an adduct of two caffeic acid molecules, previously isolated of *C. rufescens*,<sup>13</sup> revealed a close similarity among them, differing only by the substitution of the hydroxyl proton at C-4'' of the bicyclic [2.2.2] octane lignan for another caffeic acid moiety, as well as by the presence of a carboxylic acid function instead the methyl ester. The COSY and HMBC contour map supported the structure of the new lignan derivative after the esterification with 2-hydroxy-dihydrocaffeic acid (Figure 2). The relative stereochemistry suggested for **2** was established by the NOESY analysis (Figure 3), which showed correlations between H $\alpha$ -7' ( $\delta$  3.14) and H $\alpha$ -4'' ( $\delta$  3.99, d,  $J$  3.2 Hz) allowing to set the aromatic ring at C-7' and the 2-hydroxy-dihydrocaffeic acid moiety at C-4'', both  $\beta$ -positioned. This proposition also was supported by the NOE correlations observed for H $\beta$ -8' ( $\delta$  2.67) with H-2' ( $\delta$  6.42, d,  $J$  1.6 Hz) and H-6' ( $\delta$  6.33, dd,  $J$  8.1, 1.6 Hz).

A speculative biogenetic route for the formation of **2**, involving three caffeic acid units and common enzymatic reactions, such as reduction, oxidation, hydrogenation and cyclization, is suggested (Figure 4).

## Conclusions

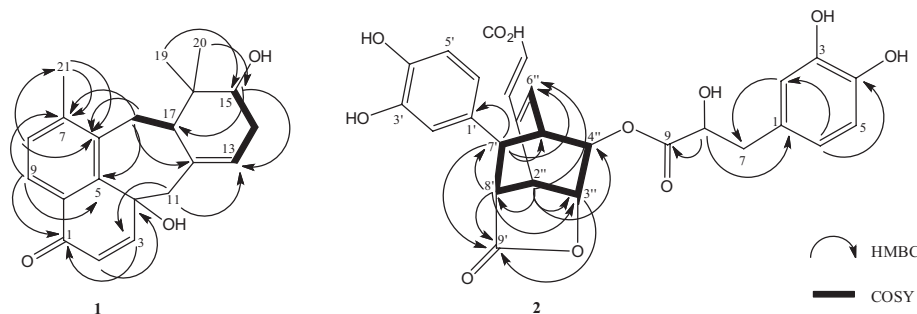
From *C. leucocephala* were isolated two new compounds, a meroterpene naphthoquinoid which was

designated as cordiaquinone P, and a lignan trimmer-like of caffeic acid, the acid form of rufescenolide esterified with a 2-hydroxy-dihydrocaffeic acid moiety at C-4''. Based on the current results, rufescenolide, previously isolated from *C. rufescens*, could be speculated as being an artifact, since the solvent used during the extraction process was methanol.<sup>13</sup> The cytotoxicity of both compounds **1** and **2** have being assayed against a panel of tumor cells, unfortunately, neither one showed any cytotoxic activity.

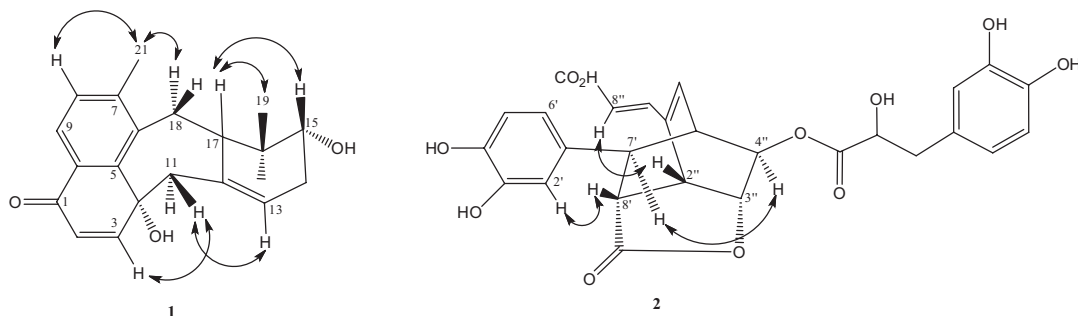
## Experimental

### General methods

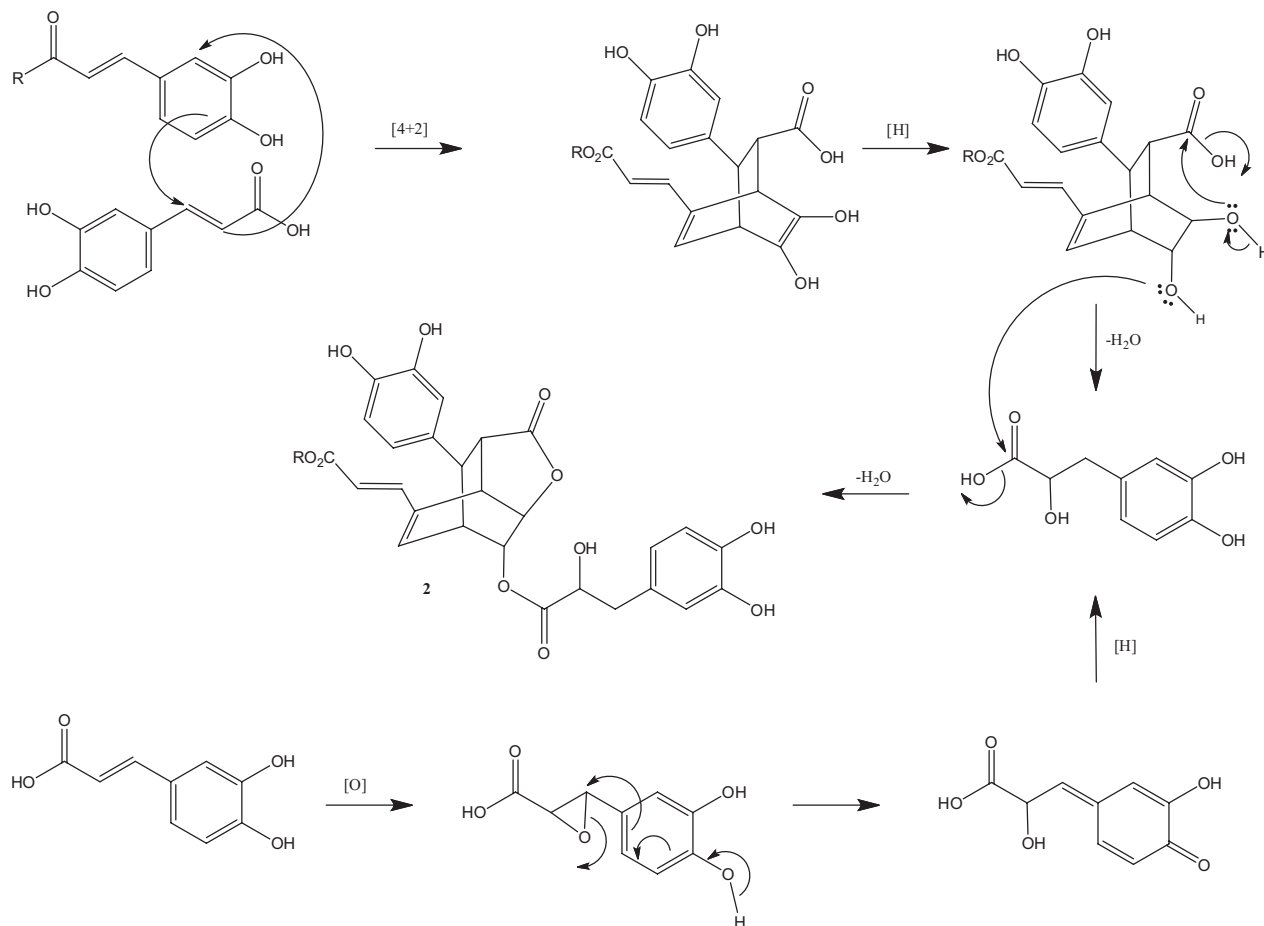
The optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. The HRESIMS were acquired using a LCMS-IT-TOF (SHIMADZU) spectrometer. IR spectra (KBr pellets) were recorded using a Perkin-Elmer FT-IR 1000 spectrometer. All NMR experiments were recorded on a Bruker DRX-500 spectrometer operating at 500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively. The high performance liquid chromatography (HPLC) analysis was carried out using a Class LC-10 (SHIMADZU) system equipped with a SPD-M10Avp diode array UV-Vis detector, two LC10ATvp pumps and LC-Si column (250 mm  $\times$  10 mm i.d.  $\times$  5  $\mu$ m) from Supelco. Chromatography columns were carried out on silica gel 60 (Merck) and Sephadex LH-20 (Pharmacia), while thin layer chromatography (TLC) was performed on



**Figure 2.** <sup>1</sup>H-<sup>1</sup>H COSY (bold) and HMBC (arrows) key correlations observed for compounds **1** and **2**.



**Figure 3.** Key NOESY correlations observed for compounds **1** and **2**.



**Figure 4.** Hypothetical biogenetic route suggested for the formation of **2**.

precoated silica gel polyester sheets (kieselgel 60 F254, 0.20 mm, Merck). The fractions and pure compounds were monitored by spraying with vanillin/perchloric acid/EtOH solution followed by heating at 105 °C.

#### Plant material

*C. leucocephala*, collected in May 2006, was harvested at Mossoró County, Rio Grande do Norte State, Brazil, and identified by the botanist Odaci Fernandes de Oliveira. A voucher specimen (MOSS 8827) has been deposited at the Herbarium Dárdano de Andrade Lima of the Universidade Federal Rural do Semi-Árido (UFERSA), Rio Grande do Norte, Brazil.

#### Extraction and isolation

Dried and powdered roots of *C. leucocephala* (650.0 g) were extracted, at room temperature, with hexane followed by a 70% aqueous ethanol solution to yield 9.3 and 57.0 g, respectively, of crude extracts. The crude hydroalcoholic extract was dissolved in H<sub>2</sub>O (700 mL) and partitioned

with CHCl<sub>3</sub>, EtOAc and *n*-BuOH (3 × 200 mL, each), to yield, after the solvent evaporation, 28.5, 2.71 and 7.26 g, respectively. The CHCl<sub>3</sub> fraction (28.5 g) was subjected to chromatography column (CC) over silica gel, by elution with binary mixtures of CHCl<sub>3</sub>/MeOH of increasing degree of polarities to yield 57 fractions (20 mL each), which after TLC analysis were pooled together to six fractions (F1A to F6A). The F3A (170.2 mg) was subjected to a Sephadex LH-20 column, using MeOH as eluent, to give 19 subfractions of 8 mL, that after TLC analysis were grouped to 5 subfractions (F1B to F5B). The F3B (80.0 mg), was purified by semipreparative HPLC analysis using H<sub>2</sub>O (0.1% TFA)/ACN (42:58; v/v) at a flow rate of 1.9 mL min<sup>-1</sup> to yield compound **1** (67.0 mg, *t<sub>R</sub>* = 16.2 min). The EtOAc fraction (2.71 g) was fractionated by silica gel CC, using as eluent a ternary mixture of AcOEt/MeOH/H<sub>2</sub>O (70:22:08 v/v), to give 29 fractions (8 mL). TLC analysis showed a major spot for fractions 11-14 (687.3 mg) that were pooled together. The final fraction was subjected to a Sephadex LH-20 column, followed by semipreparative HPLC using H<sub>2</sub>O (0.1% formic acid)/ACN (75:25; v/v) at flow rate of 2.9 mL min<sup>-1</sup> to yield compound **2** (70.2 mg, *t<sub>R</sub>* = 22.1 min).

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR data for compounds **1** (CDCl<sub>3</sub>) and **2** (MeOD)<sup>a</sup>

Position	<b>1</b>		Position	<b>2</b>	
	$\delta_c$	$\delta_H$		$\delta_c$	$\delta_H$
1	185.0	–	1	129.5	–
2	126.4	6.29 (d, <i>J</i> 10.0 Hz)	2	117.7	6.74 (d, <i>J</i> 1.0 Hz)
3	154.4	6.80 (d, <i>J</i> 10.0 Hz)	3	146.2	–
4	69.8	–	4	145.4	–
5	144.2	–	5	116.5	6.69 (d, <i>J</i> 8.0 Hz)
6	140.6	–	6	121.9	6.61 (br d, <i>J</i> 8.0 Hz)
7	142.4	–	7	38.1	3.11 (dd, <i>J</i> 14.3 and 3.7 Hz) 3.00 (dd, <i>J</i> 14.3 and 8.0 Hz)
8	130.1	7.23 (d, <i>J</i> 8.0 Hz)	8	75.1	5.20 (dd, <i>J</i> 8.0 and 3.7 Hz)
9	124.9	7.87 (d, <i>J</i> 8.0 Hz)	9	173.7	–
10	128.2	–	1'	134.7	–
11	49.2	2.69 (d, <i>J</i> 14.3 Hz) 2.39 (d, <i>J</i> 14.3 Hz)	2'	116.1	6.42 (d, <i>J</i> 1.6 Hz)
12	137.4	–	3'	146.2	–
13	124.4	5.53 (br s)	4'	145.4	–
14	32.2	2.34 (br d) 2.20 (br d)	5'	116.3	6.64 (d, <i>J</i> 8.1 Hz)
15	74.2	3.51 (t, <i>J</i> 5.3 Hz)	6'	120.4	6.33 (dd, <i>J</i> 8.1 and 1.6 Hz)
16	38.2	–	7'	45.8	3.14
17	48.4	1.75 (d, <i>J</i> 11.6 Hz)	8'	45.7	2.67 (br s)
18	29.7	3.45 (dd <i>J</i> 14.2 and 11.6 Hz) 3.15 (br d <i>J</i> 14.2 Hz)	9'	181.0	–
19	26.1	1.09 (s)	1''	135.5	–
20	18.6	1.08 (s)	2''	40.4	4.1 (br s)
21	22.0	2.46 (s)	3''	84.2	4.24 (d, <i>J</i> 4.8 Hz)
			4''	75.1	3.99 (d, <i>J</i> 3.2 Hz)
			5''	48.7	3.31 (br s)
			6''	141.9	6.52 (d, <i>J</i> 6.4 Hz)
			7''	144.3	7.42 (d, <i>J</i> 15.9 Hz)
			8''	117.4	6.26 (d, <i>J</i> 15.9 Hz)
			9''	168.2	–

<sup>a</sup> <sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz).  $\delta$  in ppm and *J* in Hz. Assignments were based on HSQC, COSY, HMBC and NOESY experiments. Overlapped proton NMR signals are reported without specify the multiplicity.

Compound **1**: yellow resin;  $[\alpha]_D^{25} -70.0$  (*c* 0.45, CHCl<sub>3</sub>); for <sup>1</sup>H-(500 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) data, see Table 1; positive HRESIMS: *m/z* 347.1633 [M + Na]<sup>+</sup> (calcd. 347.1623 for C<sub>21</sub>H<sub>24</sub>NaO<sub>3</sub>).

Compound **2**: brown resin;  $[\alpha]_D^{25} +310.4$  (*c* 0.45, MeOH); for <sup>1</sup>H (500 MHz, MeOD) and <sup>13</sup>C NMR (125 MHz, MeOD) data, see Table 1; negative HRESIMS: *m/z* 523.1264 [M - H]<sup>-</sup> (calcd. 523.1240 for C<sub>27</sub>H<sub>23</sub>O<sub>11</sub>).

## Supplementary Information

Supplementary information (Figures S1-S16) is available free of charge at <http://jbc.s.org.br> as a PDF file.

## Acknowledgments

The authors thank the Governmental Brazilian Agencies CNPq, FUNCAP, CAPES, PRONEX and INCT-SisBio for financial support.

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Submitted on: July 8, 2014

Published online: November 28, 2014