

## Antiproliferative Butyrolactones from *Mezilaurus crassiramea*

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### Abstract

Bioassay-guided fractionation of the ethanol extract from the leaves of *Mezilaurus crassiramea*, which was toxic to *Artemia salina* larvae, afforded 3'-acetylubrenolide (**1**), 2',3'-diacetylubrenolide (**2**), and rubrenolide (**3**) from the active dichloromethane soluble fraction. Compound **1** is new, while **2** and **3** are first reported from a natural source and in the *Mezilaurus* genus, respectively. Compound **3** showed significant cytotoxicity against UACC-62, MCF-7, HT-29, and PC-3 human cancer cell lines, with GI<sub>50</sub> values ranging from 3.3 to 9.9 µg/mL, while **1** and **2** exhibited marginal activities against at least five of the six investigated cell lines. The structures of **1–3** were established on the basis of 1D- and 2D-nuclear magnetic resonance analyses, high-resolution electrospray ionization mass spectrometry data and specific optical rotation values.

### Key words

*Mezilaurus crassiramea* · Lauraceae · γ-lactones · rubrenolide · cytotoxicity

Supporting information available online at  
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*Mezilaurus* (Lauraceae) comprises about 20 species and is distributed from Costa Rica to Southern Brazil [1,2]. Although it belongs to a widely studied plant family, the chemical composition of members from this genus has been scarcely investigated. Previous works revealed the presence of neolignans, volatile terpenoids, alkaloids, and/or a γ-lactone in *Mezilaurus itauba* (Meisn.) Taubert ex Mez, *Mezilaurus duckei* H. van der Werff, and *Mezilaurus synandra* (Mez) Kosterm. [3–5]. As part of our continuing research on potential anticancer constituents from lauraceous species occurring in the “Cerrado” ecosystem of Midwest Brazil, we found that the ethanol extract of the leaves of *Mezilaurus crassiramea* (Meisn.) Taub. ex Mez, a tree popularly known as “cumbuquina” that has not been previously chemically investigated, showed activity in the brine shrimp (*Artemia salina*) lethality test (LD<sub>50</sub> = 734.8 µg/mL). After partitioning of this bioactive extract, the activity was found to rest only on the dichloromethane solubles (LD<sub>50</sub> = 29.8 µg/mL). Bioassay-directed column chromatography separations of this phase led to the isolation of the polyketide derived γ-lactones **1–3**, which were evaluated for their antiproliferative effects on six human cancer cell lines (786–0, MCF-7, PC-3, HT-29, UACC-62, and NCI/ADR-RES).

The high-resolution electrospray ionization mass spectrometry (HRESIMS; positive ion mode) of compound **1** suggested the molecular formula C<sub>19</sub>H<sub>32</sub>O<sub>5</sub>, as determined by the [M + Na]<sup>+</sup> ion at

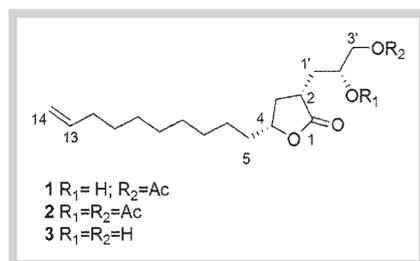
*m/z* 363.2141. The <sup>1</sup>H, <sup>13</sup>C, and distortionless enhancement by polarization transfer (DEPT) NMR spectra of **1** (Table 1) showed resonances attributable to a long linear alkyl chain (multiplets ranging from δ 1.20 to 1.61) bearing a terminal olefinic bond, as shown by a pair of broad doublets at δ 4.95 (*J* = 16.8 Hz) and δ 4.89 (*J* = 11.2 Hz), and a double double triplet at 5.76 (*J* = 16.8, 11.2, and 6.6 Hz), wherein the respective vinyl carbon signals were observed at δ 114.1 and δ 139.1 in the <sup>13</sup>C NMR spectrum (Fig. 1). Other characteristic features in the NMR spectra of **1** accounted for the presence of an acetoxy function, as deduced from the methyl singlet at δ<sub>H</sub> 2.05 and the carbon resonances at δ<sub>C</sub> 20.8 and 171.1. The signal at δ<sub>C</sub> 180.0 was indicative of the presence of an additional ester carbonyl carbon, which showed long-range correlations in the heteronuclear multiple-bond correlation (HMBC) spectrum to the methine hydrogen at δ 2.82–2.95 (m), and the methylene hydrogens at δ 1.42–1.61 (m) and δ 2.44–2.56 (m), which in turn showed connectivities in the heteronuclear single quantum coherence (HSQC) spectrum to the carbons at δ 38.8 and δ 35.7, respectively (Fig. 2). This data, along with a long-range heteronuclear correlation between the oxymethine carbon at δ 79.7 and the methylene hydrogens at δ 1.63–1.77 and δ 1.42–1.61, the connectivities of which to the carbon resonance at δ 35.3 were observed in the HSQC spectrum, as well as further information provided by the correlation spectroscopy (COSY) spectrum (Fig. 2), indicated that a five-membered lactone moiety bearing side chains at C-2 (δ<sub>C</sub> 38.8) and C-4 (δ<sub>C</sub> 79.7) was present in the structure of **1**. Detailed analysis of the remainder of the 1D- and 2D-NMR spectra revealed that **1** and rubrenolide (**3**) [6], a known polyketide-derived γ-lactone that was also isolated in this investigation, are closely related (Fig. 1). They were shown, however, to differ structurally only in the presence of an acetoxy functionality in **1** instead of a hydroxyl. This inference was in accordance with the downfield shifted signals of two diastereotopic oxymethylene hydrogens at δ 3.98 (dd, *J* = 11.2 and 6.3 Hz) and δ 4.08 (dd, *J* = 11.2 and 3.7 Hz) compared with those of **3** (δ<sub>H</sub> 3.59 and δ<sub>H</sub> 3.44, assigned to 2H-3'), as a result of the presence of an acetate group at C-3' in **1**. Furthermore, long-range correlations discernible in the HMBC spectrum of **1** between the oxymethylene hydrogens H-3' and the acetoxy carbonyl at δ 171.1 confirmed the location of this group at C-3' as well as the coupling of these hydrogens to the oxymethine H-2' at δ 3.80–3.91 (m) as revealed by the COSY spectrum. The positive value of the specific rotation of **1**, [α]<sub>D</sub><sup>20</sup> + 20.86 (c 0.12, acetone), suggested that its absolute configuration is the same as that of **3**, namely (2*S*,4*R*,2'*R*). Therefore, the structure of compound **1**, which is being described for the first time, was unambiguously shown to be 3'-acetylubrenolide (Fig. 1).

The IR, <sup>1</sup>H, and <sup>13</sup>C NMR spectra of **2** (Table 1) showed a striking resemblance with those described for **1**, except for the downfield shifted resonance of H-2', which was observed as a multiplet at δ 5.05–5.14 in the <sup>1</sup>H NMR spectrum of **2**, and the presence of an additional methyl singlet at δ 2.02 attributable to an acetate function at C-2', thus suggesting that **2** was the corresponding acetyl derivative of **1** (Fig. 1). Likewise, in the <sup>13</sup>C NMR spectrum, the signals assignable to the acetoxy group at C-2' were observed at δ 20.6 and δ 170.5. The ion at *m/z* 405.22455 [M + Na]<sup>+</sup> in the positive HRESIMS of **2**, consistent with the molecular formula C<sub>21</sub>H<sub>34</sub>O<sub>6</sub>, reinforced the foregoing proposal. In addition, long-range connectivities discernible in the HMBC spectrum of **2** from the hydrogen H-2' on the acetate-bearing carbon (δ<sub>H</sub> 5.05–5.14) to the acetoxy carbonyl at δ 170.5 provided further support for these assignments (Fig. 2). Therefore, the above data al-

Position	1 <sup>b</sup>		2 <sup>b</sup>		3 <sup>c</sup>	
	$\delta_{\text{H}}$ mult. (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult. (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult. (J in Hz)	$\delta_{\text{C}}$
1	–	180.0	–	178.0	–	180.0
2	2.82–2.95 m	38.8	2.45–2.64 m	37.7	2.83–2.94 m	38.7
3	2.44–2.56 m 1.42–1.61 m	35.7	2.45–2.64 m 1.40–1.57 m	35.5	2.53 ddd (12.3, 8.4, 5.4) 1.54–1.62 m	35.7
4	4.26–4.48 m	79.7	4.25–4.36 m	79.0	4.31–4.42 m	79.9
5	1.63–1.77 m 1.42–1.61 m	35.3	1.62–1.76 m 1.57–1.62 m	35.3	1.71–1.81 m 1.56–1.66 m	35.3
6	1.20–1.40 br s	25.1	1.31–1.49 m	25.1	1.42–1.52 m 1.32–1.42 m	25.2
7	1.20–1.40 br s	29.2 <sup>d</sup>	1.20–1.40 br s	29.2 <sup>e</sup>	1.25–1.40 br s	29.3 <sup>f</sup>
8	1.20–1.40 br s	28.0 <sup>d</sup>	1.20–1.40 br s	29.0 <sup>e</sup>	1.25–1.40 br s	29.0 <sup>f</sup>
9	1.20–1.40 br s	29.3 <sup>d</sup>	1.20–1.40 br s	29.3 <sup>e</sup>	1.25–1.40 br s	29.3 <sup>f</sup>
10	1.20–1.40 br s	28.8 <sup>d</sup>	1.20–1.40 br s	28.8 <sup>e</sup>	1.25–1.40 m	28.8 <sup>f</sup>
11	1.20–1.40 br s	29.3 <sup>d</sup>	1.20–1.40 br s	29.3 <sup>e</sup>	1.25–1.40 br s	29.3 <sup>f</sup>
12	1.90–2.00 m	33.7	1.95–2.00 m	33.7	2.00 dd (14.0, 6.6)	33.7
13	5.76 ddt (16.8, 11.2, 6.6)	139.1	5.76 ddt (16.8, 11.2, 6.6)	139.1	5.75 ddt (16.8, 11.2, 6.6)	139.1
14	4.95 br d (16.8) 4.89 br d (11.2)	114.1	4.95 br d (16.8) 4.89 br d (11.2)	114.1	4.95 br d (16.8) 4.89 br d (11.2)	114.1
1'	1.90–2.00 m 1.42–1.61 m	34.0	2.29 ddd (14.1, 10.8, 3.6) 1.53–1.62 m	32.0	1.90–2.00 m 1.55–1.61 m	33.7
2'	3.80–3.91 m	68.2	5.05–5.14 m	69.0	3.65–3.75 m	70.2
3'	4.08 dd (11.2, 3.7) 3.98 dd (11.2, 6.3)	68.4	4.22 dd (12.0, 3.6) 3.99 dd (12.0, 6.3)	64.9	3.59 br d (10.8) 3.44 dd (10.8, 6.3)	66.6
R <sub>1</sub>	–	–	2.02 s	20.6, 170.5	–	–
R <sub>2</sub>	2.05 s	20.8, 171.1	2.05 s	20.9, 170.6	–	–

**Table 1** <sup>1</sup>H and <sup>13</sup>C NMR data for compounds **1–3** in CDCl<sub>3</sub><sup>a</sup>.

<sup>a</sup> Assignments were confirmed by DEPT, <sup>1</sup>H-<sup>13</sup>COSY, HSQC, and HMBC experiments; <sup>b</sup> Recorded at 300.13 MHz (<sup>1</sup>H) and 75.47 MHz (<sup>13</sup>C); <sup>c</sup> Recorded at 500.13 MHz (<sup>1</sup>H) and 125.76 MHz (<sup>13</sup>C); <sup>d, e, f</sup> Interchangeable values within column



**Fig. 1** Chemical structures of compounds **1–3**.

lowed compound **2** to be determined as 2',3'-diacetyl-rubrenolide, which is being reported for the first time as a natural product, yet it was formerly obtained by the acetylation of rubrenolide [6]. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of **2**, not previously reported in the literature, are now presented in **Table 1**.

The molecular formula of **3** was established as C<sub>17</sub>H<sub>30</sub>O<sub>4</sub> on the basis of its HRESIMS (*m/z* 321.20350 [M + Na]<sup>+</sup>). Its <sup>1</sup>H and <sup>13</sup>C NMR spectral data (**Table 1**) and specific rotation value agreed with those reported for rubrenolide, a butyrolactone that has been previously obtained from only one Lauraceae species, *Nectandra rubra* (Mez) C.K. Allen (synonym *Sextonia rubra* (Mez) van der Werff) [6,7], and also by stereoselective syntheses [8,9] (**Fig. 1**). The initially assigned (2*S*,4*R*,2*S'*) configuration of rubrenolide was later shown to be (2*S*,4*R*,2'*R*) instead through its total synthesis [8]. Although the <sup>13</sup>C NMR data of **3** agreed with those published for rubrenolide [6], information provided by the HMBC spectrum of the former revealed that the previously reported resonance values due to C-4 ( $\delta$  70.2) and C-2' ( $\delta$  79.7) should be interchanged. This assumption was substantiated by

long-range correlations observed from H-3 and H-5 to C-4, and from H-1' and H-3' to C-2' in the HMBC spectrum of **3** (**Fig. 2**), thus allowing unambiguous assignments of C-4 and C-2' resonances as  $\delta$  79.9 and  $\delta$  70.2, respectively.

Following isolation, compounds **1–3** were further assessed for their *in vitro* antiproliferative effects against six cancer cell lines using the SRB assay. As depicted in **Table 2**, rubrenolide (**3**) was shown to be the most active butanolide, on the basis of its significant GI<sub>50</sub> values in the range of 3.3 and 9.9  $\mu$ g/mL against four of the six cell lines tested (UACC-62, MCF-7, HT-29, and PC-3), while **1** and **2** showed moderate antiproliferative effects.

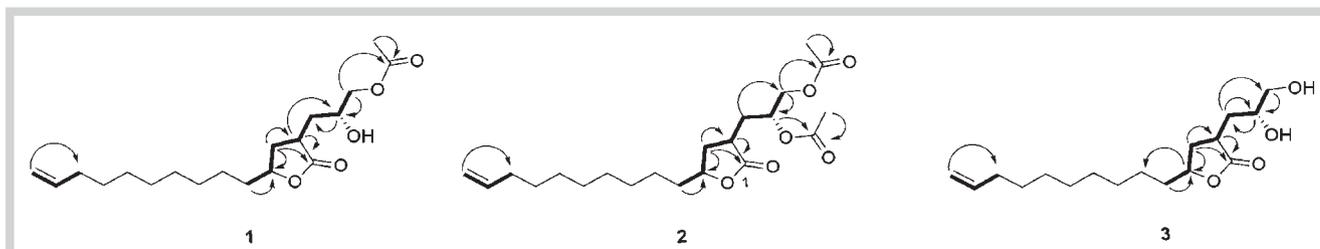
## Material and Methods



**Plant material:** The leaves of *M. crassiramea* were collected in Campo Grande, MS, Brazil, in July 2011. A voucher specimen (No. 33 014) was deposited at the CGMS Herbarium of the Universidade Federal de Mato Grosso do Sul.

**Extraction and isolation:** Air-dried and powdered leaves (1.96 kg) of *M. crassiramea* were extracted with EtOH (20 L) at room temperature. Partitioning of the EtOH extract gave the hexane, CH<sub>2</sub>Cl<sub>2</sub>, and EtOAc phases with the brine shrimp toxicity residing in the CH<sub>2</sub>Cl<sub>2</sub> solubles. Repeated CC procedures on silica gel and Sephadex LH-20 of the bioactive CH<sub>2</sub>Cl<sub>2</sub> phase afforded compounds **1–3**. The detailed extraction and isolation procedures of **1–3** are available as Supporting Information.

**Brine shrimp lethality and in vitro cytotoxic assays:** The brine shrimp (*A. salina*) lethality test was performed with extracts and phases according to Meyer et al. [10]. Cytotoxicity of compounds **1–3** was measured *in vitro* by growth inhibition of six human



**Fig. 2** Key HMBC (→) and  $^1\text{H}$ - $^1\text{H}$  COSY (–) correlations of 1–3.

**Table 2** Antiproliferative effects of compounds 1–3 on human cancer cell lines ( $\text{GI}_{50}$ ,  $\mu\text{g}/\text{mL}$ ).

Cell lines	1	2	3	Doxorubicin
PC-3	21.2 ± 1.2	19.1 ± 1.4	9.9 ± 2.0	0.2 ± 0.1
786-0	31.5 ± 0.2	16.5 ± 0.5	18.8 ± 1.0	0.7 ± 0.1
HT-29	29.5 ± 0.1	30.7 ± 0.3	5.1 ± 2.0	0.3 ± 0.1
MCF-7	26.4 ± 1.9	17.1 ± 2.2	3.7 ± 0.8	0.1 ± 0.1
UACC-62	46.5 ± 4.3	>250.0	3.3 ± 1.0	0.3 ± 0.1
NCI/ADR-RES	21.5 ± 2.5	23.5 ± 0.2	22.6 ± 2.1	3.0 ± 0.4

cancer cell lines – namely, 786-0 (kidney carcinoma), MCF-7 (breast adenocarcinoma), PC-3 (prostate carcinoma), HT-29 (colon adenocarcinoma), UACC-62 (melanoma), and NCI/ADR-RES (ovarian multidrug-resistant) using the SRB assay, as described elsewhere [11,12]. The detailed experimental procedures are available as Supporting Information.

### Supporting information

The detailed extraction and isolation data of compounds 1–3, as well as their  $^1\text{H}$  and  $^{13}\text{C}$  1D- and 2D-NMR, IR, and HR-ESIMS spectra, specific optical rotation values, and bioassays procedures are available as Supporting Information.

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### Conflict of Interest

The authors declare no conflicts of interest.

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