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′-acetylrubrenolide (1), 2′,3′-diacetylrubrenolide (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. Compounds 3 showed significant cytotoxicity against UACC-62, MCF-7, HT-29, and PC-3 human cancer cell lines, with 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

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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 γ-lactones 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.) Taubert ex Mez, a tree popularly known as “cumbruninha” that has not been previously chemically investigated, showed activity in the brine shrimp (Artemia salina) lethality test (LD50 = 734.8 µg/mL). After partitioning of this bioactive extract, the activity was found to rest only on the dichloromethane soluble (LD50 = 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 (Table 1). 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.

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Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>Active against UACC-62, MCF-7, HT-29, and PC-3</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>Active against UACC-62, MCF-7, HT-29, and PC-3</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>Active against UACC-62, MCF-7, HT-29, and PC-3</td>
</tr>
</tbody>
</table>

The IR, 1H, and 13C NMR spectra of 2 (Table 1) showed striking resemblance with those described for 3, except for 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 (δ1 3.59 and δ2 3.44, assigned to 2-H'), as a result of the presence of an acetate group at C-3'. Furthermore, long-range correlations discernible in the HMBC spectrum of 3 between the oxy-methylene carbon at δ79.7 and the methylene hydrogens at δ1 63.6–1.77 and δ2 1.42–1.61, the connectivities of which to the carbon resonance at δ35.3 were observed in the HSQC spectrum, as well as further information provided by the correlation spectroscopy (COSY) spectrum of 3, indicated that a five-membered lactone moiety bearing side chains at C-2 (δ1 38.8) and C-4 (δ2 79.7) was present in the structure of 3. Detailed analysis of the NMR spectra of 3 revealed that 3 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 acetoxyl 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 (δ1 3.59 and δ2 3.44, assigned to 2-H', as a result of the presence of an acetate group at C-3'. Furthermore, long-range correlations discernible in the HMBC spectrum of 3 between the oxy-methylene hydrogens at δ35.3 and the acetoxyl carbon at δ171.1 confirmed the location of this group at C-3' as well as the coupling of these hydrogens to the oxy-methylene 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]20 +20.86 (c 0.12, acetone), suggested that its absolute configuration is the same as that of 3, namely (2S,4R,2'R). Therefore, the structure of compound 1, which is being described for the first time, was unambiguously shown to be 3'-acetylrubrenolide (Fig. 1).
lowed compound 2 to be determined as 2′,3′-diacetylrubrenolide, which is being reported for the first time as a natural product, yet it was formerly obtained by the acetylation of rubrenolide [6]. The 1H and 13C NMR spectral data of 2, not previously reported in the literature, are now presented in \( \textit{Table 1} \).

The molecular formula of 3 was established as \( \text{C}_{13}\text{H}_{20}\text{O}_{4} \) on the basis of its HRESIMS (m/z 321.2035 [M + Na]+). Its 1H and 13C NMR spectral data (\( \textit{Table 1} \)) and specific rotation value agreed with those reported for rubrenolide, a butyrolactone that has been previously obtained from only one Lauraceae species, \( \textit{Nectandra rubra} \) (Mez) C.K. Allen (synonym \( \textit{Sextonia rubra} \) (Mez) van der Werff) [6,7], and also by stereoselective syntheses [8,9] (\( \textit{Fig. 1} \)). The initially assigned (2S,4R,2R) configuration of rubrenolide was later shown to be (2S,4R,2R) instead through its total synthesis [8]. Although the 13C 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 (\( \textit{Fig. 2} \)), 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 \textit{in vitro} antiproliferative effects against seven cancer cell lines using the SRB assay. As depicted in \( \textit{Table 2} \), rubrenolide (3) was shown to be the most active butanolide, on the basis of its significant \( \text{GI}_{50} \) values in the range of 3.3 and 9.9 \( \mu \text{g/mL} \) 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 \( \textit{M. crassiramea} \) were collected in Campo Grande, MS, Brazil, in July 2011. A voucher specimen (No. 33014) 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 \( \textit{M. crassiramea} \) were extracted with EtOH (20 L) at room temperature. Partitioning of the EtOH extract gave the hexane, \( \text{CH}_2\text{Cl}_2 \), and EtOAc phases with the brine shrimp toxicity residing in the \( \text{CH}_2\text{Cl}_2 \) phase. Repeated CC procedures on silica gel and Sephadex LH-20 of the bioactive \( \text{CH}_2\text{Cl}_2 \) 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 (\( \textit{A. salina} \)) lethality test was performed with extracts and phases according to Meyer et al. [10]. Cytotoxicity of compounds 1–3 was measured \textit{in vitro} by growth inhibition of six human...
cancer cell lines – namely, 786–0 (kidney carcinoma), MC-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 $^1$H and $^{13}$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.

References
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Table 2 Antiproliferative effects of compounds 1–3 on human cancer cell lines (GI50, µg/mL).

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

Supporting information available as Supporting Information.