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. Compound **3** showed significant cytotoxicity against UACC-62, MCF-7, HT-29, and PC-3 human cancer cell lines, with GI_{50} 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 http://www.thieme-connect.de/products

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 "cumbuquinha" that has not been previously chemically investigated, showed activity in the brine shrimp (*Artemia salina*) lethality test (LD_{50} = 734.8 µg/mL). After partitioning of this bioactive extract, the activity was found to rest only on the dichloromethane solubles (LD_{50} = 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 $C_{19}H_{32}O_5$, as determined by the $[M + Na]^+$ ion at *m/z* 363.2141. The ^1H, ^{13}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 ^{13}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_H 2.05 and the carbon resonances at δ_C 20.8 and 171.1. The signal at δ_C 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 δ79.7 and the methylene hydrogens at δ1.63–1.77 and δ1.42–1.61, 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 (**Fig. 2**), indicated that a five-membered lactone moiety bearing side chains at C-2 (δ_C 38.8) and C-4 (δ_C 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_H 3.59 and δ_H 3.44, assigned to 2 H-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**, $[\alpha]_D^{20}$ + 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′-acetylrubrenolide (**Fig. 1**).

The IR, ^1H, and ^{13}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 ^1H 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 ^{13}C NMR spectrum, the signals assignable to the acetoxyl group at C-2′ were observed at δ 20.6 and δ 170.5. The ion at *m/z* 405.22455 $[M + Na]^+$ in the positive HRESIMS of **2**, consistent with the molecular formula $C_{21}H_{34}O_6$, 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_H 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-

Table 1 ^{1}H and ^{13}C NMR data for compounds **1–3** in $CDCl_3$[a].

Position	1[b]		2[b]		3[c]	
	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_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[d]	1.20–1.40 *br s*	29.2[e]	1.25–1.40 *br s*	29.3[f]
8	1.20–1.40 *br s*	28.0[d]	1.20–1.40 *br s*	29.0[e]	1.25–1.40 *br s*	29.0[f]
9	1.20–1.40 *br s*	29.3[d]	1.20–1.40 *br s*	29.3[e]	1.25–1.40 *br s*	29.3[f]
10	1.20–1.40 *br s*	28.8[d]	1.20–1.40 *br s*	28.8[e]	1.25–1.40 *m*	28.8[f]
11	1.20–1.40 *br s*	29.3[d]	1.20–1.40 *br s*	29.3[e]	1.25–1.40 *br s*	29.3[f]
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_1	–	–	2.02 *s*	20.6, 170.5	–	–
R_2	2.05 *s*	20.8, 171.1	2.05 *s*	20.9, 170.6	–	–

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

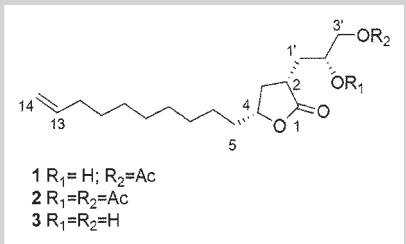

Fig. 1 Chemical structures of compounds **1–3**.

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 ^{1}H and ^{13}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 $C_{17}H_{30}O_4$ on the basis of its HRESIMS (*m/z* 321.20350 $[M + Na]^+$). Its ^{1}H and ^{13}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 ^{13}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 (δ 70.2) and C-2′ (δ 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 δ 79.9 and δ 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_{50} values in the range of 3.3 and 9.9 µg/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. 33014) was deposited at the CGMS Herbarium of the Univesidade 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_2Cl_2, and EtOAc phases with the brine shrimp toxicity residing in the CH_2Cl_2 solubles. Repeated CC procedures on silica gel and Sephadex LH-20 of the bioactive CH_2Cl_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 (*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}H-^{1}H COSY (–) correlations of **1**–**3**.

Table 2 Antiproliferative effects of compounds **1**–**3** on human cancer cell lines (GI_{50}, µg/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}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.

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