Chromanones with Leishmanicidal Activity from *Calea uniflora*

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The dichloromethane extract of *Calea uniflora* afforded a mixture of two novel chromanones, uniflorol-A (**1**) and uniflorol-B (**2**), and one known chromanone, 2,2-dimethyl-6-(1-hydroxyethyl)-chroman-4-one (**3**). The structures of these compounds were determined by spectroscopic methods. Biological activity of the compounds against *Leishmania major* promastigotes was evaluated. Mixture of the novel chromanones **1** and **2** showed significant growth inhibition of the parasite in the micrograms per milliliter range.

Key words: Calea uniflora, Asteraceae, Chromanones, Leishmanicidal Activity

Introduction

The genus *Calea* L. (Asteraceae, tribe Heliantheae) includes 110 species of shrubs, small trees or perennial herbs, distributed in tropical and subtropical regions of the New World (Karis and Ryding, 1994; Pruski and Urbatsch, 1988). The literature reports some biological activities for the genus *Calea*, such as antifungal, anti-inflammatory, cytotoxic, larvicidal, antiplasmodial and antihypertensive (Flach *et al*., 2002; Vichnewski *et al*., 1982; Cerain *et al*., 1996; Bork *et al*., 1997; Köhler *et al*., 2002; Guerrero *et al*., 2002). Chemical studies carried out on *Calea* species have revealed the occurrence of a variety of compounds including sesquiterpene lactones (Ober *et al*., 1985), *p*-hydroxyacetophenone derivatives (Bohlmann *et al*., 1981a), thymol derivatives (Metwally and King, 1985), benzofurans (Bohlmann *et al*., 1982), chromenes (Steinbeck *et al*., 1997) and others. Recently, we described the isolation of four *p*-hydroxyacetophenone derivatives from the underground parts of *Calea uniflora* (Nascimento *et al*., 2004). In addition to these compounds here we report the isolation and the structure elucidation of a mixture of two novel (**1**, **2**) and one known chromanone (**3**) from this plant. A mixture of the novel (**1**, **2**) and the known chromanone (**3**) were also tested for their leishmanicidal activity against *Leishmania major* Friedling promastigotes.

Results and Discussion

Uniflorol-A (**1**) and uniflorol-B (**2**) (Fig. 1) were isolated as a mixture of amorphous white solids. The molecular formula $C_{18}H_{22}O_5$ was determined by a pseudomolecular ion at m/z 341.1271 $[M+Na]^+$ in the positive high resolution ESI mass spectrum. The IR spectrum indicated the presence of a hydroxy group (3450 cm^{-1}), an ester and cetone carbonyl function (1690, 1720 cm^{-1}) and an aromatic ring (1570, 1370 cm^{-1}). Two sets of data appeared in the ^1H, ^{13}C NMR, and DEPT spectra, indicating that this compound was a mixture of two isomers (diastereomeres). The ^1H NMR spectrum (Table I) indicated signals for six methyl groups at δ 1.46 (12H, s) for **1** and **2**, 1.58 (3H, d, J = 6.6 Hz), 2.08 (3H, d, J = 7.2 Hz) for **1** and 1.60 (3H, d, J = 6.6 Hz), 1.91 (3H, d, J = 7.3 Hz) for **2**, aromatic hydrogen atoms at δ 7.87–7.86 (2H, m), 7.51–7.47 (2H, m) and 6.93–6.90 (2H, m) for **1** and **2**, two olefinic hydrogen atoms at δ 6.38 (1H, q, J = 7.2 Hz) for **1** and 7.02 (1H, q, J = 7.3 Hz) for **2**, four methylene groups at δ 2.72 (4H, s) for **1** and **2**, 4.24 (2H, s) for **1** and 4.35 (2H, s) for **2**, and two methine groups at δ 5.98–5.92 (2H, m) for **1** and **2**.

The ^{13}C NMR spectrum (Table I) showed that chemical shifts of C-3′, C-4′, and C-5′ signals of **1** were downfield to δ 141.3 (+ 0.4), 15.8 (+ 1.5), 65.4 (+ 8.3) compared with corresponding signals in **2**, while those signals of C-8, C-1′ and C-2′ were upfield at δ 21.9 (– 0.1), 166.4 (– 0.3) and 131.7 (– 0.2).

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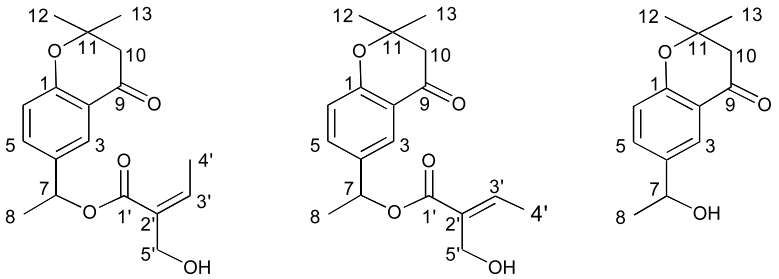

Fig. 1. Structures of uniflorol-A (**1**), uniflorol-B (**2**), and 2,2-dimethyl-6-(1-hydroxyethyl)-chroman-4-one (**3**).

Table I. NMR spectral data of compounds **1** and **2** (in $CDCl_3$).

Position	**1**			**2**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1		159.7 s	H-6,5,3		159.7 s	H-6,5,3
2		119.9 s	H-6,10		119.9 s	H-6,10
3	7.87–7.86 m	124.0 d	H-5	7.87–7.86 m	124.0 d	H-5
4		133.9 s	H-8,6		133.9 s	H-8,6
5	7.51–7.47 m	134.4 d		7.51–7.47 m	134.4 d	
6	6.93–6.90 m	118.7 d		6.93–6.90 m	118.7 d	
7	5.98–5.92 m	71.9 d		5.98–5.92 m	71.9 d	
8	1.58 d (6.6)	21.9 q		1.60 d (6.6)	22.0 q	
9		192.4 s	H-3,10,12,13		192.4 s	H-3,10,12,13
10	2.72 s	48.8 t		2.72 s	48.8 t	
11		79.4 s	H-10,12,13		79.4 s	H-10,12,13
12	1.46 s	26.6 q		1.46 s	26.6 q	
13	1.46 s	26.6 q		1.46 s	26.6 q	
1′		166.4 s	H-5′		166.7 s	H-5′
2′		131.7 s	H-4′,5′		131.9 s	H-4′,5′
3′	6.38 q (7.2)	141.3 d	H-4′,5′	7.02 q (7.3)	140.9 d	H-4′,5′
4′	2.08 d (7.2)	15.8 q		1.91 d (7.3)	14.3 q	
5′	4.24 s	65.4 t		4.35 s	57.1 t	

Compound	Leishmanicidal activity (% growth inhibition)		
	Dose [μg/ml]		
	100	50	25
1 and **2**	88.9	81.5	54.8
3	no activity	no activity	no activity

Table II. Percentages of *L. major* Friedlin promastigote growth inhibition by compounds **1**, **2** and **3**.

Those data suggested that **1** was the isomer of **2**. The structures of **1** and **2** were deduced from detailed analysis of the ^1H and ^{13}C NMR data aided by 2D NMR experiments (HMQC and HMBC).

A mixture of the new natural products **1** and **2** significantly inhibited *L. major* Friedling promastigotes growth by 88.9, 81.5 and 54.8% at concentrations of 100, 50 and 25 μg/ml, respectively (Table II). Compound **3** had no detectable inhibitory activity.

Experimental

General

The UV spectra were obtained by a Hitachi U-3501 spectrophotometer. IR spectra were re-

corded on a Nicolet Protégé 460 spectrophotometer. ESI-MS was performed on a Ultra TOF™-Q-Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometer. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker DRX 400 spectrometer in $CDCl_3$ using TMS as internal standard; chemical shift are in δ (ppm) and coupling constants (*J* values) in Hz. 2D NMR experiments (^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC) were performed using a Bruker DRX 500 spectrometer.

Plant material

The plant was collected in March 1997, at the Washington Luis highway, 1 km from Posto Castelo, Brazil, and was identified by Dr. Jose L. Panero, Department of Botany, University of Texas. A voucher specimen (SPFR 04003) is deposited in the Herbarium of Department of Biology, FFCLRP/USP, Ribeirão Preto, Brazil.

Extraction and isolation

Dried and powdered underground parts of *C. uniflora* (200 g) were exhaustively extracted with dichloromethane at room temperature. The solvent was evaporated under vacuum to afford 4.2 g of crude extract. The crude extract was chromatographed by vacuum liquid chromatography (VLC) on silica gel and eluted with *n*-hexane, *n*-hexane/ethyl acetate (gradient), ethyl acetate/methanol (gradient) and methanol to give 14 fractions (F1–F14). Fraction F6 eluted with *n*-hexane/ethyl acetate (7:3 v/v) was chromatographed over silica gel (7 × 30 cm, 70–230 mesh, *n*-hexane/ethyl acetate gradient 9:1 to 1:9 v/v, final 100% methanol) resulting in eleven subfractions (F601–F611). Fraction F608 was then subjected to preparative TLC using a solvent system (8:1:1 *n*-hexane/ethyl acetate/chloroform) to afford fraction F608-B (11 mg), which was submitted to HPLC [Shimpack PREP SIL (H) column, 20 × 250 mm, *n*-hexane/isopropyl alcohol 98:2, flow rate 9 ml min^{-1}] to give 2 mg of the mixture of compounds **1** and **2**. Fraction F14 which was obtained from methanol was purified by preparative TLC (*n*-hexane/ethyl acetate 7:3) to yield compound **3** (2 mg).

Leishmanicidal activity

The effect of the compounds on the viability of *L. major* promastigotes was performed using parasites cultured according to established protocols (Napolitano *et al.*, 2004). Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO) at 1.0 mg/ml. In all experiments the final content of DMSO was kept below 0.5% (v/v), a content that does not affect the parasite growth rate, mobility or morphology (Zhai *et al.*, 1999). Further dilutions of compounds were made directly in the *L. major* culture medium immediately before use. The parasite cultures were prepared with or without compounds at final concentrations of 100, 50, 25 μg/ml; a control culture contained only DMSO. Promastigote viability was assessed colorimetrically by the reduction of the salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the formazan dye (Berg *et al.*, 1994). Absorbances at 290 nm were expressed as percentages relative to untreated controls. The bioassays were performed in triplicate. Results are shown in Table II.

Uniflorol-A (**1**): Amorphous white solid. – IR (KBr): ν_{max} = 3450, 1720, 1690, 1570, 1370, 835, 795 cm^{-1}. – HR-ESI-MS: *m*/*z* = 341.1271 [M+Na]$^+$. – ^{1}H and ^{13}C NMR (400 and 100 MHz, $CDCl_3$): see Table I.

Uniflorol-B (**2**): Amorphous white solid. – IR (KBr): ν_{max} = 3450, 1720, 1690, 1570, 1370, 835, 795 cm^{-1}. – HR-ESI-MS: *m*/*z* = 341.1271 [M+Na]$^+$. – ^{1}H and ^{13}C NMR (400 and 100 MHz, $CDCl_3$): see Table I.

2,2-Dimethyl-6-(1-hydroxyethyl)-chroman-4-one (**3**): Yellow gum. – IR and ^{1}H NMR data were in agreement with the literature values (Bohlmann *et al.*, 1981b). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 193.0 (s, C-9), 159.8 (s, C-1), 138.5 (s, C-4), 134.1 (d, C-5), 123.6 (d, C-3), 120.1 (s, C-2), 119.1 (d, C-6), 79.7 (s, C-11), 70.0 (d, C-7), 49.2 (t, C-10), 27.0 (q, C-12/13), 25.4 (q, C-8). The ^{13}C NMR data of this compound has not been published previously.

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