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Cryptofolione derivatives from *Cryptocarya alba* fruits

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Abstract

Cryptofolione (**1**) and the new cryptofolione derivative 6-(4,6-dimethoxy-8-phenyl-octa-1,7-dienyl)-4-hydroxy-tetrahydro-pyran-2-one (**2**) were isolated from the fruits of *Cryptocarya alba*. The structures were elucidated by spectroscopic methods. Cryptofolione showed activity towards *Trypanosoma cruzi* trypomastigotes, reducing their number by 77% at 250 μg mL^{-1}. Cryptofolione showed moderate cytotoxicity in both macrophages and *T. cruzi* amastigotes. It also displayed a mild inhibitory effect on the promastigote form of *Leishmania* spp. As both cytotoxic and trypanocidal effects are similar, the compound presented little selectivity in our assay models.

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Introduction

Cryptocarya alba Mol. (Lauraceae) is a tree common in central Chile where it is known as peumo. The ripe fruits were eaten by the Mapuche either boiled in water or raw (Muñoz et al 1981; de Mösbach 1991) and are still consumed by the rural population. In a study of food plants gathered by Chilean amerindians, a methanolic extract of *C. alba* fruits showed a free radical scavenging activity and DNA binding effect (Schmeda-Hirschmann et al 1999). Following our studies on bioactive compounds from Chilean plants, we now report the isolation of cryptofolione derivatives from the basic extract of ripe *C. alba* fruits. As the anti-parasitic effect of the isolated compounds was not previously determined, the activity of cryptofolione (**1**) and its derivative, 6-(4,6-dimethoxy-8-phenyl-octa-1,7-dienyl)-4-hydroxy-tetrahydro-pyran-2-one (**2**) was assessed towards *Trypanosoma cruzi* and *Leishmania* spp.

Materials and Methods

Plant material

Cryptocarya alba (Mol.) Looser (Lauraceae) was collected at the Plaza de Armas, Talca city (35° 26′ S; 71° 39′ W, 90 m over sea level) on April 30, 1996. A voucher herbarium specimen (JIL 185) has been kept at the Herbario de la Universidad de Talca.

Table 1 ^{1}H NMR data of compounds **1** and **2** ($CDCl_3$, δ values)

H	Compound		
	1	**1a**	**2**
3	5.88 d br (9.8, 1.5)	6.02 ddd (10, 2, 1.5)	2.64 dd (17.5, 4.5) 2.43 dd (17.5, 7.5)
4	6.75 ddd (9.8, 8.3, 4,1)	6.84 ddd (10, 8.5, 4)	3.80 m
5	2.29 m(br dd) (5.9, 4.5)	2.39 m	1.78 m
6	4.75 ddbr (15, 6.6, 5)	4.86 dd br (15, 6.5)	4.28 m
1′	5.54 dd (15.5, 6.5)	5.64 dd (15.5, 3.5)	5.58 dd (15.5, 6.0)
2′	5.76 ddd (15.5, 7, 6.5)	5.76 ddd (15.5, 7.5, 7.5)	5.71 ddd (15.5, 7.5, 7.5)
3′	2.25 br dd (6.5, 6.5)	2.30 m	2.22 m
4′	3.93 m	5.03 m	4.00 m
5′	1.63 m (7.6, 6.7)	1.90 m	1.65 m
6′	4.50 dd br (10.4, 5.8)	5.44 m	4.60 m
7′	6.16 dd (15.9, 5.8)	6.08 dd (16, 7.5)	6.24 dd (16, 6)
8′	6.50 d (15.9)	6.59 d (16)	6.60 d (16)
10′, 14′	7.24 br d (7.4, 1.4)	7.34 d br (7.5, 1.5)	7.34 d br (7.5)
11′, 13′	7.18 ddbr (7.1,7.1,1.4)	7.28 dddbr (7.5,7.5,2)	7.28 ddbr (7.5, 7.5, 1.4)
12′	7.12 ddbr (7.1,7.1,1.4)	7.22 dddbr (7.5,7)	7.21 ddbr (7.5,7.5,1.4)
OH 4′	3.62 s		
OH 6′	3.63 s		
OAc		2.03 s	
CH_3		1.98 s	3.65 s 3.32 s

Isolation

The ripe fruits (1.8 kg) were oven-dried at 40°C, ground and extracted with methanol (2 × 10 L) at room temperature. The extract was filtered, concentrated and retaken in 2 L water. The suspension was made acidic by addition of 0.1 M HCl (pH 2) and filtered. The aqueous phase was basified with NaOH to pH 10 and extracted three times with $CHCl_3$, yielding 1.3 g of soluble materials. The extract was chromatographed over silica gel, eluted with a PE–PE : EtOAc–EtOAc gradient yielding eight fractions. Fraction 2–3 (200 mg) consisted of nearly pure cryptofolione. Some 50 mg of **1** were purified from fraction 4–5. Preparative TLC of fraction 6 (PE–EtOAc 4 : 1; silica gel) afforded 15 mg **1** and 20 mg **2**. The ^{1}H- and ^{13}C-NMR data of **1**, its acetate **1a** and **2** are presented in Tables 1 and 2.

Trypanocidal activity in-vitro

Activity in Trypanosoma cruzi *trypomastigotes*

Balb/c mice infected with Cl *T. cruzi* strain fourteen days after infection were used. Blood was obtained by cardiac puncture using 3.8% sodium citrate as anticoagulant in a 7 : 3 ratio. The compound was dissolved

Table 2 ^{13}C NMR data of compounds **1** and **2** ($CDCl_3$, δ values).

C	Compound		
	1 (75 MHz)	**1a** (125 MHz)	**2** (125 MHz)
2	164.84 s	163.68 s	172.00 s
3	121.33 d	121.64 d	38.72 t
4	145.81 d	144.53 d	77.39 d
5	29.84 t	29.64 t	42.36 t[a]
6	78.37 d	77.64 d	70.92 d
1′	129.76 d	130.49 d	135.76 d
2′	131.77 d	129.33 d	127.17 d
3′	40.56 t	37.60 t	40.34 t
4′	68.11 d	68.74 d	68.20 d
5′	42.88 t	38.50 t	41.39 t[a]
6′	69.90 d	70.74 d	70.08 d
7′	132.47 d	126.95 d	132.02 d
8′	129.65 d	132.87 d	129.69 d
9′	137.00 s	136.06 s	137.00 s
10′, 14′	126.68 d	126.62 d	126.39 d
11′, 13′	128.81 d	128.61 d	128.50 d
12′	127.78 d	128.12 d	127.50 d
Acetate			
C=O		170.51 s 170.23 s	
CH_3		21.19 q 21.08 q	56.68 q 51.74 q

[a]Assignations are interchangeable.

in cold DMSO to a final concentration of 250 μg mL^{-1}. Samples (10 μL) of the pure compounds at different concentrations were mixed (40, 100 and 250 μg mL^{-1}) in microtitre plates with 100 μL infected blood (10^5 parasites mL^{-1}). Infected blood and infected blood containing 250 μg mL^{-1} gentian violet were used as controls. Plates were kept at 4°C for 24 h. Each solution was microscopically observed at 400 × magnification, placing 5 μL per sample on a slide and covering it with a 22 × 22 mm cover-glass for parasite counting (Schempler 1988; Rojas de Arias et al 1994).

Activity in T. cruzi *amastigotes*

Resident macrophages were harvested from the peritoneal cavities of normal BALB/c mice in ice-cold PBS. The cells were plated at 2×10^6 mL^{-1} (0.1 mL per well) in Lab-Tek 24-chamber slides (Nunc, Naperville, IL) and incubated at 37°C under an atmosphere of 4% CO_2 for 1 h. *T. cruzi* amastigotes were isolated from the peritoneal cavity of infected mice and were added at a 5:1 parasite–macrophage ratio. Cultures were incubated for 4 h. Then, 0.5 mL of compound in RPMI medium was added at different concentrations in triplicate and the slides were further incubated for 48 h. Cultures were stained with trypan blue and examined under light microscopy. The number of dead intracellular amastigotes was determined by counting at least 100 macrophages. Results are shown as percent of dead amastigotes in relation to infected controls without the compound (Torres-Santos et al 1999). A similar procedure was carried out to test cytotoxicity. Macrophages free of infection were stained with trypan Blue and results were expressed as percent of dead macrophages in relation to controls without the compound. All assays were performed in triplicate. Results are presented as mean values ± s.d.

Leishmanicidal activity in-vitro

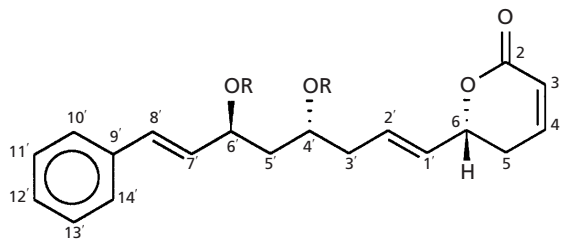
Promastigote inhibition studies were performed on *Leishmania amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903) and *L. donovani* (MHOM/BR/74/PP75) grown at 22°C in Schneider's *Drosophila* medium containing 20% fetal bovine serum. Promastigote cultures in the logarithmic phase were transferred at a concentration of 10^6 cells per mL. Compounds (1 mg) were dissolved in 40 μL DMSO and added to 1 mL of the medium from which samples were drawn. From this stock solution 200 μL were dissolved in 800 μL of medium. A100-μL sample of this second solution was mixed with 100 μL of parasite culture, reaching a compound concentration of 100 μg mL^{-1} and 0.4% of DMSO. The solution was placed in microtitre plates. All assays were carried out in triplicate. The activity of compounds was evaluated after 72 h by optical observation of a drop of each culture with a microscope and compared with control cells. Assays to assess the drug concentration required to inhibit parasite growth were performed in triplicate (Del Rey et al 1999).

Statistical analysis

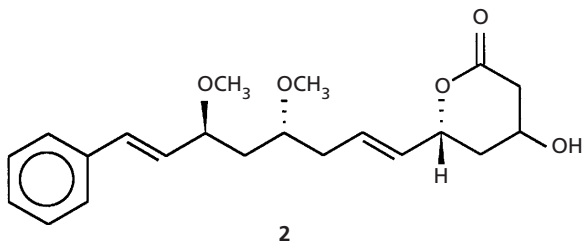
Results are presented as mean ± s.d. Statistical significance was determined by one-way analysis of variance followed by Scheffe's test, with the level of significance set at $P < 0.05$.

Results

From the basic extract of *C. alba* fruits, two main compounds were isolated. The NMR data of the compounds is presented in Tables 1 and 2 and the structures are shown in Figure 1. Compound **1** was identified as the α-pyrone derivative cryptofolione, previously isolated from *C. latifolia* and *C. myrtifolia* by Sehlapelo et al (1994). The ^{1}H NMR spectrum of **2** was close to that of cryptofolione. Instead of the signals at δ 6.75 and δ 5.88, characteristic of an α-pyrone nucleus, a multiplet at δ 3.80 and a pair of dd at δ 2.43 and δ 2.64 pointed to



R
1 H Cryptofolione
1a A Cryptofolione acetate



2

Figure 1 Structure of compounds **1** and **2**.

Table 3 Cytotoxicity in macrophages, trypanocidal effect in *T. cruzi* amastigotes and in-vitro activity of cryptofolione towards three strains of *Leishmania* spp promastigotes.

Parasite	Cryptofolione concn (μg mL^{-1})	Cytotoxicity	Trypanocidal effect	Leishmanicidal effect
T. cruzi	100	22 ± 3	25 ± 3	
T. cruzi	50	37 ± 4*	44 ± 3**	
T. cruzi	25	42 ± 4*	79 ± 4**	
Leishmania spp				
L. amazonensis PH8	100			+
L. brasiliensis 2903	100			+
L. donovani PP75	100			+

Data are presented as percentage of cell survival mean ± s.d. +: > 40% of cell survival. *$P < 0.01$ compared with 100 μg mL^{-1}; **$P < 0.001$ compared with 100 μg mL^{-1}.

a 3,4 saturated pyrone hydroxylated at C-4. The presence of two signals at δ 3.32 and δ 3.65 indicates methoxylation at 4′ and 6′. The stereochemistry at C-6, C-4′ and C-6′ is given by analogy with compounds **1** and **1a**, while the configuration at C-4 remains to be established. The ^{13}C NMR data of **2** is in complete agreement with the proposed structure.

The ^{1}H and ^{13}C NMR data of cryptofolione, **1**, completely agree with the literature values. $[\alpha]_{589}^{20}$: +22.0; c = 0.40; $CHCl_3$. MS: m/z 314.1503: calc. for $C_{19}H_{22}O_4$ $[M]^+$: 314.1518.

Cryptofolione acetate, **1a**, was obtained by treating **1** (15 mg) with acetic anhydride/pyridine overnight. After the usual work-up, 10 mg **1a** was obtained. MS: m/z 398.173: calc. for $C_{23}H_{26}O_6$ $[M]^+$: 398.173.

6-(4,6-Dimethoxy-8-phenyl-octa-1,7-dienyl)-4-hydroxy-tetrahydro-pyran-2-one, **2**: colourless gum, $[\alpha]_{589}^{20}$: −10.52; c = 0.475; $CHCl_3$. MS: m/z 360.194: calc. for $C_{21}H_{28}O_5$ $[M]^+$: 360.194.

Discussion

From the basic extract of ripe *C. alba* fruits, cryptofolione (**1**) was isolated as the main compound (0.015%) while the derivative **2** was obtained in 0.0001% yield. Cryptofolione was first reported as a constituent of *C. latifolia* and *C. myrtifolia* (Sehlapelo et al 1994).

In the trypanocidal assay, cryptofolione at 250 μg mL^{-1} reduced the number of trypomastigotes by 77% while **2** proved to be inactive (data not shown). Under the same conditions, gentian violet lysed 100% of the parasites. The leishmanicidal assays showed a moderate effect of this compound on promastigotes (< 70% of lysis) (Table 3).

Cytotoxicity in macrophages was also performed with cryptofolione (Table 3). Fifty percent of dead macrophages were observed at the lowest concentration tested (25 μg mL^{-1}), which was moderately active against *T. cruzi* amastigotes infecting mice peritoneal macrophages.

The results obtained confirm the in-vitro trypanocidal and leishmanicidal properties of cryptofolione. This compound shows activity against *T. cruzi* and *Leishmania* spp in-vitro when compared with gentian violet and their own controls. However, cryptofolione showed a moderate effect on *T. cruzi* amastigotes infecting mice peritoneal macrophages at the cytotoxic IC50. As the trypanocidal effect and cytotoxicity values are similar, the compound presented little selectivity.

Bioactive compounds reported from *Cryptocarya* species include (−)-caryachine (Chen et al 1996) and crychine (Ko et al 1993) from *C. chinensis*. The lignan (−)-grandisin was reported as the brine shrimp toxic compound from *C. crassinervia* (Saad et al 1991). The presence of cryptofolione and a derivative in *C. alba* is in agreement with the phytochemical trend observed in other *Cryptocarya* species (Sehlapelo et al 1994; Drewes et al 1995a, b, 1996).

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