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# ANTIPROTOZOAL 6-SUBSTITUTED-5,6-DIHYDRO-α-PYRONES FROM *RAIMONDIA CF. MONOICA*

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Dichloromethane extracts of both the roots and the leaves of *Raimondia cf. monoica* showed *in vitro* antiplasmodial and leishmanicidal activities against *Plasmodium falciparum* and *Leishmania panamensis*, respectively. Three 6-substituted 5,6-dihydro-*2H*-pyran-2-ones were isolated. (**1**) and (**2**) were identified as (6*S*)-(5′-oxohepten-1′*E*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**1**) and (6*R*)-(5′-oxohepten-1′*Z*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**2**), respectively. (–)-Argentilactone (**3**) was also isolated. The structure of the new compound (**1**) was determined by spectroscopic methods; additional spectroscopic data for (**2**) are reported for the first time.

*Keywords:* Annonaceae; α-pyrones; Leishmania; Malaria

## INTRODUCTION

Bioactive natural compounds possessing α,β-unsaturated lactones have been found mainly in *Lamiaceae* [1], *Lauraceae* [2,3] and *Annonaceae* families [4–7]. 6-Substituted derivatives of 5,6-dihydro-α-pyrones have been reported as insect antifeedants, antifungal, antitumor and plant growth inhibiting agents [8,9]. In our continuing search for biologically active constituents of medicinal plants of Colombia, we report here the isolation and structural elucidation of one new compound, (6*S*)-(5′-oxohepten-1′*E*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**1**), together with two known α-pyrones, (6*R*)-(5′-oxohepten-1′*Z*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**2**), and (–)-argentilactone (**3**), from the roots and the leaves of *Raimondia cf. monoica* (Fig. 1). The chemistry of this plant has not been previously studied, and there have been no reports of its use in folk medicine. The antimalarial and leishmanicidal activities of the isolated compounds against *Plasmodium falciparum* and *Leishmania panamensis* are also described.

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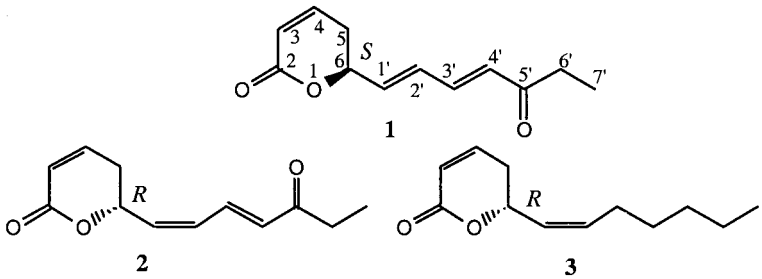

FIGURE 1 Structures of the isolated $\alpha$-pyrones (**1–3**).

## RESULTS AND DISCUSSION

Compound (**1**) was obtained as a pale yellow oil. It showed a molecular ion at $m/z$ 206 in the EIMS. The absorptions at 1720, 1695 and 1635 $cm^{-1}$ in the IR spectrum and a positive Kedde's reaction suggested the presence of both an $\alpha,\beta$-unsaturated lactone and ketone functionalities [10]. A carbon resonance at 163.2 ppm, and two olefinic resonances at 6.08 and 6.90 ppm, correlated with two signals at 121.7 and 144.2 ppm in the $^1$H-NMR and $^{13}$C-NMR spectra, respectively, were in agreement with an $\alpha,\beta$-unsaturated lactone subunit. The $^{13}$C-NMR spectrum (DEPT data) of (**1**) (Table I) exhibited 12 carbon signals consisting of one methyl carbon (8.1), two methylene carbons (29.4 and 34.2), one oxymethine carbon (76.5), two carbonyl carbons (163.2 and 200.8) and six methine carbons (121.7, 130.4, 131.0, 137.4, 139.9 and 144.2 ppm). The DQF-COSY and spin decoupling experiments of (**1**) clearly revealed two partial structural units (a,b) shown in Fig. 2, and connections of these two units and remaining two carbonyl carbons (C-2 and C-5′) were suggested by the HMBC correlations (Table I).

The configurations of the dienone group were deduced as 1′*E* and 3′*E* by considering the coupling constants: $J_{1',2'} = 15.1$ Hz, $J_{3',4'} = 15.5$ Hz, *versus* 9.8 Hz for the *Z* isomer (**2**), in the $^1$H-NMR spectrum. The proposed structure, (5′-oxohepten-1′*E*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**1**) was confirmed by DQF-COSY, DEPT, HMQC and HMBC spectral analysis (Table I). The absolute configuration at C-6 was assumed to be *S* due to the sign of specific rotation of (**1**) ($[\alpha]_D = +60.8$) compared to known pyranones (e.g. (–)-argentilactone (**3**), which possesses the *R* absolute configuration [6,12]). Furthermore, a positive cotton effect was observed ($\lambda_{max}$ 260 nm, $\Delta\varepsilon$ +5.24) in the CD spectrum, which is in agreement with the *S* absolute configuration, on the basis of Snatzke's rules [12].

Compound (**2**) showed by EIMS a $[M]^+$ ion at $m/z$ 206 corresponding to the molecular formula $C_{12}H_{14}O_3$, the MS, IR, CD and $^1$H-NMR data were in close agreement with those reported for (6*R*)-(5′-oxohepten-1′*Z*,3′*E*-dienyl)-5,6-dihydro-*2H*-pyran-2-one (**2**) isolated from *Chorisia crispiflora* [11]. The $^{13}$C-NMR, HMQC and HMBC spectra, previously unreported, fully supported structure (**2**) (Table II). Compound (**3**) was also a pale yellow oil. The spectroscopic data are similar to the reported data of (–)-argentilactone earlier isolated from *Aristolochia argentine*, *Chorisia crispiflora* and *Annona haematantha* [6,11,13,14].

Biological studies showed that (**1**) and (**2**) had interesting activities against *L. panamensis* ($ED_{50}$ (μg/mL) = 1.9 and 0.42, respectively), whereas (**3**) showed an $ED_{50}$ (μg/mL) = 0.1 against *P. falciparum*.

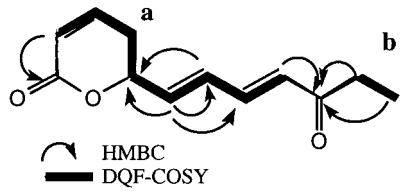


FIGURE 2 Selected 2D NMR correlations for (**1**).

TABLE I Correlated $^{13}$C-NMR, $^{1}$H-NMR, HMBC and COSY for compound (1)

| *Atom* | $\delta_C$ ppm | $\delta_H$ ppm | *HMBC* | $^{1}H$–$^{1}H$ *COSY* |
|---|---|---|---|---|
| 1 | – | – | – | — |
| 2 | 163.2 | – | – | – |
| 3 | 121.7 | 6.08 | 2.44 | 6.90 |
| 4 | 144.2 | 6.90 | 2.56 | 6.08, 2.56 |
| 5 | 29.4 | 2.56, 2.44 | 6.08 | 6.90, 5.06 |
| 6 | 76.5 | 5.06 | 6.51, 6.15 | 6.15 |
| 1′ | 137.4 | 6.15 | – | 6.51 |
| 2′ | 130.4 | 6.51 | 6.15 | 7.14 |
| 3′ | 139.9 | 7.14 | 6.15 | 6.51, 6.24 |
| 4′ | 131.0 | 6.24 | 6.24 | 7.14 |
| 5′ | 200.8 | – | 6.24, 2.59, 1.1 | – |
| 6′ | 34.2 | 2.59 | 1.10 | 1.10 |
| 7′ | 8.1 | 1.10 | 2.59 | 2.59 |

TABLE II Correlated $^{13}$C-NMR, $^{1}$H-NMR, HMBC and COSY for compound (**2**)

| *Atom* | $\delta_C$ ppm | $\delta_H$ ppm | *HMBC* | $^{1}H$–$^{1}H$ *COSY* |
|---|---|---|---|---|
| 1 | – | – | – | – |
| 2 | 163.1 | – | 6.08, 6.90 | – |
| 3 | 121.6 | 6.08 | 2.46 | 6.90 |
| 4 | 144.4 | 6.90 | 2.46, 5.47 | 6.08, 2.46 |
| 5 | 29.7 | 2.44, 2.47 | 6.90, 6.08 | 6.90, 5.47 |
| 6 | 73.6 | 5.47 | 2.46 | 2.46, 5.95 |
| 1′ | 135.1 | 5.95 | 5.47, 6.28 | 5.47, 6.28 |
| 2′ | 132.1 | 6.28 | 5.95 | 5.95, 7.37 |
| 3′ | 134.2 | 7.37 | 6.28, 5.95 | 6.28 |
| 4′ | 130.0 | 6.30 | 7.37 | 7.37 |
| 5′ | 200.5 | – | 2.58, 6.30, 1.09, 7.37 | – |
| 6′ | 34.7 | 2.58 | 1.09 | 1.09 |
| 7′ | 7.9 | 1.09 | 2.58 | 2.58 |

Compounds (**1**), (**2**) and (**3**) showed antileishmanial and antimalarial activity, but also showed cytotoxicity on U-937 cells (Table III).

## EXPERIMENTAL

### General

$^{1}$H and $^{13}$C-NMR spectra were recorded as $CDCl_3$ solutions with a Bruker AC-200 (200 MHz) and Bruker AM-400 (400 MHz). $^{1}$H–$^{1}$H (DQF–COSY), and $^{1}$H–$^{13}$C

TABLE III *In vitro* activity of pyrones (**1–3**)

| *Compound* | *L. panamensis* | | *P. falciparum* | *U-937 cells* |
|---|---|---|---|---|
| | $ED_{50}$ (μg/mL) | *SI*[a] | $ED_{50}$ (μg/mL) | $LD_{50}$ (μg/mL) |
| 1 | 1.9 | 1.1 | – | 2.1 |
| 2 | 0.42 | 2.3 | – | 1.0 |
| 3 | 10[b] | – | 0.1 | – |
| Glucantime | 6.7 | 59.6 | – | 416.4 |
| Chloroquine | – | – | 0.005 | – |

[a]Selectivity Index; [b]Literature data [6]; –: No data available.

(HMQC and HMBC) correlation spectra were recorded at 400 MHz. Chemical shifts ($\delta$) were expressed in ppm with the protonated solvant as reference, coupling constants ($J$) were given in hertz (Hz). Mass spectra were recorded on a Nermag–Sidar R10–10C with $NH_3$ as vector gaz (for CIMS), or at 70 eV (for EIMS). Optical rotations were obtained (Na–*D* line) using a cell (1.5 mL) with 1 dm path length, on a Polartronic E (Schimdt–Haensch) polarimeter. UV spectra were recorded on a JASCO J-720 instrument. Infra red spectra (IR) were recorded on a Perkin-Elmer RXI (FT-IR) apparatus. TLC were carried out on silica gel 60 F254 aluminium sheets (Merck), followed by detection at 254 nm and with Kedde reagent. Solvent system for TLC: hexane–EtOAc 6 : 4. Column chromatography was performed using silica gel 60 (70–200 μm) (Merck).

## Plant Material

Roots and leaves of *R. cf. monoica* were collected in the forest of Cordoba (Colombia) in January 2000. A voucher specimen has been deposited in the herbarium Joaquin Antonio Uribe of Medellin (N°0474 JAUM).

## Extraction and Isolation

The dried and powdered roots (1.4 kg) were extracted at room temperature with petroleum ether and $CH_2Cl_2$. The $CH_2Cl_2$ extract (20 g) was fractionated by column chromatography on silica gel with a stepwise gradient (petroleum ether–EtOAc 1 : 0 to 0 : 1) into 34 fractions (I–XXXIV). Fractions II–VII (4.6 g) were purified by column chromatography on silica gel eluted with hexane–EtOAc (10 : 1), yielding compound (**3**) (1.9 g).

Fractions VIII–XXXIV (15 g) were further purified by repeated column chromatography on silica gel eluted with hexane–EtOAc (6 : 14) yielding compounds (**2**) (100 mg) and (**1**) (90 mg).

The $CH_2Cl_2$ extracts of the leaves (30 g) were fractioned in the same way as above to give 60 fractions I–LX. Purification of fractions XXX–XL by column chromatography (hexane–EtOAc 6 : 4) afforded compound (**1**) (200 mg), a mixture of compounds (**1**) and (**2**) (40 mg) and (**2**) (50 mg).

*(6S)-(5′-oxohepten-1′E,3′E-dienyl)-5,6-dihydro-2H-pyran-2-one* (**1**): Pale yellow oil, $[\alpha]_D = +60.8$ ($c = 9.2$, EtOH); CD (MeOH: $c = 0.04$) $\lambda_{max}$ ($\Delta\varepsilon$): 260 (+5.24); IR (film) $\nu$ ($cm^{-1}$): 1720, 1695, 1635; CIMS $m/z$: 225 $[M + H + NH_4]^+$, 207 $[M + H]^+$; EIMS $m/z$ (rel. int): 206 (29), 177 (19), 149 (40), 138 (25), 109 (27), 81 (100); $^{13}$C-NMR, $^1$H–$^1$H COSY, HMBC: see Table I; $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ (ppm): 1.10

(3H, t, $J = 7.3$ Hz), 2.44 (1H, dddd, $J = 1.2$, 2.7, 9.1, 18.4 Hz), 2.56 (1H, ddd, $J = 1.2$, 5.1, 18.4 Hz), 2.59 (2H, q, $J = 7.3$ Hz), 5.06 (1H, dquint, $J = 4.3$, 9.7 Hz), 6.08 (1H, ddd, $J = 1.2$, 2.4, 10.0 Hz), 6.15 (1H, dd, $J = 5.4$, 15.1 Hz), 6.24 (1H, d, $J = 15.5$ Hz), 6.51 (1H, ddd, $J = 0.78$, 10.9, 15.3 Hz), 6.90 (1H, ddd, $J = 2.7$, 5.4, 9.8 Hz), 7.14 (1H, dd, $J = 10.9$, 15.5 Hz).

*(6R)-(5′-oxohepten-1′Z,3′E-dienyl)-5,6-dihydro-2H-pyran-2-one* **(2)**: Pale yellow oil, $[\alpha]_D = -48$ ($c = 0.125$, EtOH); UV (MeOH)$\lambda_{max}$ (nm) (log $\varepsilon$): 205 (4.0), 265 (5.0); CD (MeOH: $c = 0.04$) $\lambda_{max}$ ($\Delta\varepsilon$): 264 (−5.6); IR (film) $\nu$ ($cm^{-1}$): 1730, 1695, 1635; CIMS $m/z$: 225 $[M + H + NH_4]^+$, 207 $[M + H]^+$; EIMS $m/z$ (rel. int): 206 (8), 188 (7), 177 (48), 149 (41), 138 (17), 109 (79), 81 (100); $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ (ppm): 1.09 (3H, t, $J = 7.3$ Hz), 2.44 (ddd, 1H, $J = 2.7$, 5.9, 10.5 Hz), 2.47 (ddd, 1H, $J = 2.7$, 5.9, 10.5 Hz), 2.58 (2H, q, $J = 7.3$ Hz), 5.47 (1H, ddq, $J = 1.1$, 4.7, 7.3 Hz), 5.95 (1H, dd, $J = 5.4$, 15.1 Hz), 6.08 (1H, ddd, $J = 1.2$, 2.4, 9.8 Hz), 6.28 (1H, d, $J = 15.5$ Hz), 6.30 (1H, dd, $J = 0.7$, 15.1 Hz), 6.90 (1H, ddd, $J = 3.1$, 5.1, 9.8 Hz), 7.37 (1H, ddd, $J = 1.1$, 11.7, 15.5 Hz); $^{13}$C-NMR, $^1$H–$^1$H COSY, HMBC: see Table II.

(–)-*Argentilactone* **(3):** Pale yellow oil, $[\alpha]_D = -20$ ($c = 1$, EtOH); UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 216 (4.0); IR (film) $\nu$ ($cm^{-1}$): 2928, 1724, 1465, 1380; CIMS $m/z$: 225 $[M + H + NH_4]^+$, 195 $[(M + H]^+$; EIMS $m/z$ (rel. int): 194 (9), 152 (15), 123 (16), 110 (20), 97 (38), 68 (100); $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$ (ppm): 0.88 (3H, t, $J = 7.3$ Hz), 1.3 (6H, m, H 4′, 5′, 6′), 2.09 (2H, m, H-8), 2.38 (2H, m, H-4), 5.22 (1H, ddd, $J = 5.7$, 7.8, 9.5, Hz, H-6), 5.56 (1H, m, H-1′), 5.64 (1H, m, H-2′), 6.04 (1H, dt, $J = 1.2$, 9.7 Hz), 6.90 (1H, dddd, $J = 1.2$, 4.7, 5.4, 9.7 Hz); $^{13}$C-NMR ($CDCl_3$, 50 MHz) $\delta$ (ppm): 163.8 (C-2), 120.9 (C-3), 144.8 (C-4), 29.2 (C-5), 73.5 (C-6), 126.0 (C-1′), 135.1 (C-2′), 27.3 (C-3′), 28.6 (C-4′), 30.9 (C-5′), 22.0 (C-6′), 13.6 (C-7′).

### *In vitro* Antiparasitic Testing

*Antimalarial assays*. The *in vitro* antimalarial evaluation of compounds (**1–3**) was carried out according to Rieckmann *et al* [15], and Cruz-Mancipe and Fuenmayor-Pelaez [16]. Briefly, *P. falciparum* (strain FCB-2) was maintained in continuous culture as described by Trager and Jensen [17,18]. In each experiment, three microplates (96 wells/microplate) were used to evaluate the compounds (with chloroquine as reference). The percentage of parasitaemia inhibition by each extract/compound was estimated by the difference between % parasitaemia of the negative control and % parasitaemia of the experimental wells.

### Leishmanicidal Assays

*Leishmania panamensis* promastigotes, strain M/HOM/87/UA140, were grown at 28°C in Schneider's Drosophila medium (Sigma) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS). After 48 h of growth, U-937 cells [19,20] were washed twice with Dubelcco's phosphate buffer saline (DPBS) (Gibco BRL). 100.000 cells/mL were exposed to stationary phase growth promastigotes at a ratio of 25 parasites/cell. The range of concentration varied between 0.1 and 10 μg/mL, aseptically solubilized in DMSO. For each compound, three independent experiments were performed in triplicate. Percentage of infection was calculated by dividing the number of infected cells obtained in the presence of each compound by the number of infected

cells obtained in the absence of treatment. Glucantime was used as the positive control. Results were expressed as $ED_{50,}$ which was calculated by Probit analysis. The $ED_{50}$ of glucantime, used as the standard drug in these assays, was 6.7 μg/mL (SbV; MW not known).

## Cytotoxicity

Cytotoxicity was evaluated by enzymatic micromethod on human promonocytic U-937 cells. Briefly, U-937 cells were introduced into 96-well flat-bottomed microtiter plates at a density of 5000 cells/100 μL. The cultures were incubated for 96 h in the presence of various compound concentrations (3–100 μg/mL). The optical density at 570 nm was measured using an ELISA plate reader (Bio Rad). Cells cultivated in the absence of treatment but maintained under the same conditions were used as control. Three independent experiments were performed in triplicate for the determination of cytotoxicity of each compound. Results were expressed as $LD_{50}$ and calculated by Probit analysis.

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