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# Antimicrobial and Antiparasitic Abietane Diterpenoids from the Roots of *Clerodendrum eriophyllum*

**Francis Machumi**[a], **Volodymyr Samoylenko**[b], **Abiy Yenesew**[a], **Solomon Derese**[a], **Jacob O. Midiwo**[a], **Frank T. Wiggers**[b], **Melissa R. Jacob**[b], **Babu L. Tekwani**[b,c], **Shabana I. Khan**[b], **Larry A. Walker**[b,c], and **Ilias Muhammad**[b]

Ilias Muhammad: milias@olemiss.edu

[a]Department of Chemistry, University of Nairobi, P.O. Box 30197 (00100), Nairobi, Kenya

[b]National Center for Natural Products Research, University of Mississippi, Mississippi 38677, USA

[c]Department of Pharmacology, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Mississippi 38677, USA

## Abstract

Chromatographic separation of the roots of a Kenyan medicinal plant, *Clerodendrum eriophyllum*, led to the isolation of ten abietane diterpenoids (**1**-**10**), one of which (**1**) was isolated for the first time from a natural source. Using spectroscopic data, the structure of **1** was determined to be 12-hydroxy-8,12-abietadiene-3,11,14-trione. Circular dichroism (CD) spectra showed that the stereochemistry of compounds **1, 3**, and **6**-**8** belongs to the normal series of abietane diterpenes, which confirmed the absolute stereochemistry of the isolated compounds. Compounds **2**-**10** were evaluated for their *in vitro* antiplasmodial, antileishmanial, antifungal and antibacterial activities. Compounds **3** and **7** exhibited potent antifungal activity ($IC_{50}$/MIC 0.58/1.25 and 0.96/2.5 μg/mL, respectively) against *C. neoformans*, whereas **3, 6** and **7** showed strong antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* with $IC_{50}$/MIC values between 1.33–1.75/2.5–5 and 0.96–1.56/2.5 μg/mL, respectively. In addition, compounds **3** and **9** exhibited potent antileishmanial activity ($IC_{50}$ 0.08 and 0.20 μg/mL, respectively) against *L. donovani*, while **3** and **7** displayed weak antimalarial activity against *Plasmodium falciparum*, but **9** was inactive.

### Keywords

*Clerodendrum eriophyllum*; Verbenaceae; abietane diterpenoids; antimicrobial; antileishmanial; antimalarial

*Clerodendrum eriophyllum* Gürke (Verbenaceae), a small tree 0.5 – 2 m high, is scattered in the dry bushlands of Eastern Kenya where the plant is used by local communities for the treatment of malaria [1]. The plant has no record of previous phytochemical analysis. However, the methanol extract of *C. eriophyllum* root bark previously showed weak *in vitro* activity against *Plasmodium falciparum* D6 and W2 clones ($IC_{50}$ 9.51–10.56 μg/mL); while its methanol and aqueous extracts exhibited significant *in vivo* chemosuppression (i.e., 90.1% and 61.5%, respectively) against *P. berghei* infected mice treated intraperitoneally at a dose of 100 mg/kg body weight [2]. The genus *Clerodendrum* is known to contain iridoids [3, 4], abietane diterpenoids [5–7] and steroids [8]. In the quest for antiplasmodial

Correspondence to: Ilias Muhammad, milias@olemiss.edu.

compounds from Kenyan plants, we have investigated the roots of *C. eriophyllum* collected from Eastern Kenya. In this paper we report the isolation and structure elucidation of a new abietane diterpenoid, 12-hydroxy-8,12-abietadiene-3,11,14-trione (**1**), obtained alongside nine other known abietane diterpenoids (**2**-**10**).

We also report the antiplasmodial, antileishmanial, antibacterial, and antifungal activities of the isolated compounds.

The 1:1 MeOH/$CH_2Cl_2$ extract of roots of *C. eriophyllum* showed moderate antiplasmodial activity with $IC_{50}$ values of 8.8 μg/mL against chloroquine-sensitive (D6) and -resistant (W2) strains of *P. falciparum*. Repeated chromatographic purification of this extract gave 12-hydroxy-8,12-abietadiene-3,11,14-trione (**1**), as well as nine known abietane diterpenoids, namely royleanone (**2**) [9], taxodione (**3**) [10], 11- hydroxy-7,9(11),13-abietatrien-12-one (**4**) [11], sugiol (**5**) [12], ferruginol (**6**) [13], 6-hydroxysalvinolone (**7**) [14], 6,11,12,16-tetrahydroxy-5,8,11,13-abietatetra-en-7-one (**8**) [15], uncinatone (**9**) [16], and 11-hydroxy-8,11,13-abietatriene-12-*O*-*β*-xylopyranoside (**10**) [15].

The molecular formula of compound **1** was established as $C_{20}H_{26}O_4$ (*m/z* 331.1910 [M+$H^+$; calculated for *m/z* 331.1909) by HRESIMS. The UV absorption maxima at $\lambda_{max}$ 273 and 378 nm closely matched those of the *p*-quinone chromophore of royleanone **2** [9]. The IR spectrum indicated the presence of hydroxyl group(s) ($\nu_{max}$ 3080–3430 $cm^{-1}$), unconjugated carbonyl ($\nu_{max}$ 1704 $cm^{-1}$), together with olefinic and conjugated carbonyl absorptions of the *p*-quinone moiety ($\nu_{max}$ 1609, 1633 and 1650 $cm^{-1}$). The $^{13}C$ NMR spectrum showed 20 signals, with the $sp^2$ region displaying four olefinic quaternary carbons ($\delta_C$ 124.3, 144.1, 145. 8, 150.6) and two conjugated carbonyls ($\delta_C$ 183.2, 186.9) assignable to the *p*-quinone moiety. The $^1H$ NMR spectrum did not show signals in either the olefinic or aromatic regions, but did show signals for three methyl singlets at $\delta_H$ 1.09, 1.13 and 1.24, assignable to C-18, C-19 and C-20, respectively, of an abietane skeleton, and an isopropyl group, with two methyl doublets at $\delta_H$ 1.19 and 1.20 (each 3H, $J$ = 7.0 Hz) and a methine septet at $\delta_{H\text{-}15}$ 3.15. The position of the isopropyl group was deduced from HMBC correlations (Table 1) between $\delta_{H\text{-}15}$ 3.15 and $\delta_{C\text{-}12}$ 150.6, $\delta_{C\text{-}13}$ 124.3, and $\delta_{C\text{-}14}$ 186.9. The unconjugated carbonyl at $\delta_C$ 216.7 was established to be at C-3 from HMBC correlations between $\delta_{H\text{-}18}$ 1.13, $\delta_{H\text{-}19}$ 1.09 and the carbonyl carbon signal. Furthermore, a comparison of the $^{13}C$ NMR spectral data of **1** with those of the known compound royleanone **2** showed close similarities of the carbon signals, except for the differences associated with C-2 – C-5 due to the presence of a carbonyl group at the C-3 position ($\delta_C$ 33.8, 216.7, 46.9 and 50.8 *vs*. 18.5, 41.2, 33.8 and 44.4 for **2**, respectively). A complete set of 2D NMR experiments [$^1H$-$^1H$ COSY, $^1H$-$^{13}C$ HMQC, $^1H$-$^{13}C$ HMBC (Table 1), $^1H$-$^1H$ NOESY] allowed the unambiguous establishment of the structure of **1** as 12-hydroxy-8,12-abietadiene-3,11,14- trione.

Circular dichroism (CD) spectra showed that the stereochemistry of compounds **1, 3**, and **6**-**8** belong to the normal series (A/B *trans*) of diterpenes. The positive Cotton effect at 275 and 284 nm for compound **1** supports the β-orientation of the methyl group at C-10, i.e. (10*S*)-Me configuration, according to the rule for **π**-**π*** transition of an α,β-unsaturated ketone [17] (Figure 2). The CD spectra of compounds **3** and **6** are in agreement with those of the known taxodione analog [18] and ferruginol [19], respectively, confirming their absolute stereochemistry. CD spectra of **7** and **8**, not previously reported, are similar to the related abietane diterpene cyrtophyllone A, whose absolute stereochemistry was determined by X-ray crystallography [17]. Only recently, the absolute configuration of 6-hydroxysalvinolone (**7**) was determined as (10*R*)-Me by enhanced X-ray crystallography [20], thus, supporting its stereochemistry deduced from CD spectra.

The antiplasmodial, antileishmanial, antifungal, antibacterial and cytotoxic activities are summarized in Tables 2-4. Compounds **3** and **9** demonstrated potent antileishmanial activities with $IC_{50}$ values of 0.08 and 0.20 μg/mL, respectively, against *L. donovani*, compared with those observed for the standard drug amphotericin B ($IC_{50}$ 0.13 μg/mL).

On the other hand, the antiplasmodial activities of compounds **3, 7** and **8** were found to be very weak, with $IC_{50}$ values of 1.2 – 4.8 μg/mL, when compared with the standard artemisinin ($IC_{50}$ <0.026 μg/mL). Strong antifungal activities were also displayed by **3** and **7**, showing $IC_{50}$ values of 0.58 and 0.96 μg/mL, respectively, against *C. neoformans*, as compared with 0.44 μg/mL of the standard amphotericin B. Compounds **3, 6** and **7** showed strong antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* with $IC_{50}$/MIC values between 1.33–1.75/2.5–5 and 0.96–1.56/2.5 μg/mL, respectively. With regard to cytotoxicity, only 6-hydroxysalvinolone (**7**) showed moderate cytotoxic activity with an $IC_{50}$ value of 4.5 μg/mL against monkey kidney fibroblasts (VERO). Finally, due to paucity of material, compound **1** could not be evaluated for its *in vitro* antiparasitic, antimicrobial and cytotoxic activities.

## Experimental

### General

Optical rotations were measured in $CHCl_3$ or MeOH using an AUTOPOL IV® instrument at ambient temperature. Circular dichroism (CD) spectra were recorded in MeCN using an Olis DCM 20 CD spectrometer at ambient temperature. IR spectra were taken as films on a Bruker Tensor 27 FTIR instrument. UV spectra were obtained in MeCN using a Hewlett-Packard 8453 spectrophotometer; 1D and 2D NMR data were acquired on a Bruker BioSpin instrument at 600 MHz ($^1H$), 150 ($^{13}C$) in $CDCl_3$ using the residual solvent as int. standard. HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with electrospray ionization (ESI). For column chromatography (CC), Merck silica gel 60 (0.063–0.200 mm) and Fluka Sephadex LH-20 were used as stationary phases; For PTLC, Merck silica gel 60 $PF_{254+366}$, coated on glass plates to make 1.0 mm layers was used; Analytical TLC was carried out using factory prepared aluminum plates (0.25 mm) coated with silica gel (60 $F_{254}$, Merck); The isolated compounds were visualized by observing under UV light at 254 or 365 nm, followed by spraying with 1% vanillin-$H_2SO_4$ spray reagent.

### Plant material

The roots of *Clerodendrum eriophyllum* were collected from Machakos, Eastern Kenya in November 2007 and identified at the Department of Botany, University of Nairobi, Kenya, where a voucher specimen No. JMFM/2007/11 has been deposited.

### Extraction and isolation

The roots of *C. eriophyllum* were air dried and pulverized to give 1.8 kg of material. This was extracted by cold percolation at room temperature using 1:1 MeOH/ $CH_2Cl_2$ (3×4 L, 24 h each), followed by 100% methanol (1×4 L, 24 h) to give 65 g of brown gummy extract, of which 35 g was adsorbed onto 40 g of silica gel and subjected to CC on a silica gel column (300 g, 5×35 cm), eluted with *n*-hexane/$CH_2Cl_2$ (95:5, 3.5 L; 9:1, 1.25 L; 3:1, 2 L; 1:1, 3 L; 1:3, 1 L; 100% $CH_2Cl_2$1.5 L) followed by $CH_2Cl_2$/MeOH (99:1, 1.25 L; 98:2, 1 L; 95:5, 1 L). Sixty-two fractions of eluents, collected in 250 mL aliquots, were concentrated using a rotary evaporator and similar fractions were combined on the basis of TLC analysis. Combination of fraction 5–9 crystallized in *n*-hexane/$CH_2Cl_2$ (95:5) gave **2** (260 mg). Fractions 11–19 (640 mg) were rechromatographed over silica gel (50 g, 2.0×30 cm) and eluted with *n*-hexane/$CH_2Cl_2$(95:5) to give **4** (8.7 mg) after 0.6 L of elution, and **6** (65 mg) after 1.4 L of elution. The latter was further purified by PTLC developed with *n*-hexane/

$CH_2Cl_2$(8:2). Fraction 22–28 (160 mg) was purified on a Sephadex LH 20 column (100 g, 2.5×30 cm), eluted with MeOH/ $CH_2Cl_2$ 1:1 (0.3 L), followed by PTLC developed with *n*-hexane/$CH_2Cl_2$ 7:3 to give **3** (28 mg).

Fraction 34–45 (600 mg) was subjected to silica gel CC (50 g, 2.0×30 cm), eluted with $CH_2Cl_2$/ *n*-hexane (8:2) to give **1** (4.0 mg) after 0.28 L of elution, **9** (6.7 mg) after 0.35 L of elution, and **5** (10.3 g) after 0.8 L of elution. Fractions 52–57 (580 mg) were rechromatographed over silica gel (50 g, 2.0×30 cm) and eluted with $CH_2Cl_2$ to yield **7** (6.5 mg) and **8** (64.2 mg), both of which crystallized from $CH_2Cl_2$, after 0.52 L and 1.3 L of elution, respectively. Compound **10** (15 mg) was obtained after purifying fractions 58–60 on a Sephadex LH 20 column (100 g, 2.5×30 cm) eluted with MeOH/ $CH_2Cl_2$ 1:1 (0.3 L).

Royleanone (**2**) [9], taxodione (**3**) [10], 11-hydroxy-7,9(11),13-abietatrien-12-one (**4**) [11], sugiol (**5**) [12], ferruginol (**6**) [13], 6-hydroxysalvinolone (**7**) [14], 6,11,12,16-tetrahydroxy-5,8,11,13-abietatetra-en-7-one (**8**) [15], uncinatone (**9**) [16] and 11-hydroxy-8,11,13-abietatriene-12-*O*-*β*-xylopyranoside (**10**) [15] were identified by comparison of their full physical (mp and optical rotation) and spectral data (UV, IR, $^1H$ and $^{13}C$ NMR, and MS) with those reported in the literatures.

## 12-Hydroxy-8,12-abietadiene-3,11,14-trione (1)

Yellow solid $R_f$ 0.5 (*n*-hexane/$CH_2Cl_2$/MeOH 60:39:1) [$\alpha_D^{25}$ +184 (c 0.16, $CHCl_3$) UV (MeCN) $\lambda_{max}$ (lg ε), nm: 201 (3.94), 205 (3.86), 273 (3.98), 378 (2.78) CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 208 (+6.4·10$^3$), 275 (+18.9·10$^3$), 284 (+20.2·10$^3$), 330 (−2.3·10$^3$) IR (film) $\nu_{max}$cm$^{-1}$: 3430–3080 (OH), 2965 (C-H), 2934 (C-H), 2873 (C-H), 1704 (C=O), 1650 (C=O), 1633 (C=O), 1609 (C=C), 1462 (C-H), 1271 (C-O) $^1H$ and $^{13}C$ NMR: (see Table 1) HRESIMS: *m*/*z* 331.1910 [M+H$^+$ (calcd. for $C_{20}H_{27}O_4$, 331.1909); 329.1758 [M-H$^-$ (calcd. for $C_{20}H_{25}O_4$, 329.1753).

## Taxodione (3)

UV (MeCN) $\lambda_{max}$ (lg ε), nm: 211 (3.81), 315 (4.13), 325 (4.13), 334 (4.12), 337 (4.11), 394 (3.38); CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 205 (−20.2·10$^3$), 261 (−12.7·10$^3$), 321 (−18.7·10$^3$), 337 (19.2·10$^3$), 445 (+13.6·10$^3$).

## Ferruginol (6)

UV (MeCN) $\lambda_{max}$ (lg ε), nm: 211 (3.90), 219 (3.87), 281 (3.58); CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 206 (−2.8·10$^3$), 211 (+12.9·10$^3$), 227 (+9.5·10$^3$), 265 (−0.9·10$^3$), 301 (−2.3·10$^3$).

## 6-Hydroxysalvinolone (7)

UV (MeCN) $\lambda_{max}$ (lg ε), nm: 219 (3.97), 250 (3.98), 284 (3.94), 335 (4.00); CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 213 (+36.3·10$^3$), 281 (+30.4·10$^3$), 338 (−19.7·10$^3$).

## 6,11,12,16-Tetrahydroxy-5,8,11,13-abietatetra-en-7-one (8)

UV (MeCN) $\lambda_{max}$ (lg ε), nm: 218 (3.98), 251 (4.01), 285 (3.89), 339 (3.97), 407 (2.90); CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 212 (+48.9·10$^3$), 284 (+29.7·10$^3$), 339 (−19.7·10$^3$).

## Uncinatone (9)

UV (MeCN) $\lambda_{max}$ (lg ε), nm: 220 (4.06), 228 (4.08), 232 (4.09), 283 (4.17), 298 (4.17), 334 (4.01), 376 (3.95). CD (MeCN) $\lambda_{max}$ ([θ], deg·cm$^2$/dmol), nm: 205 (−18.8·10$^3$), 213

(−18.9·10$^3$), 234 (+24.5·10$^3$), 241 (+23.9·10$^3$), 271 (sh) (−4.3·10$^3$), 297 (−17.1·10$^3$), 323 (+12.7·10$^3$), 350 (−2.7·10$^3$), 381 (−5.6·10$^3$).

### Antimicrobial assay

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and included the fungi *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113 and *Aspergillus fumigatus* ATCC 90906; the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods [21, 22], as described by Samoylenko *et al* 23]. Drug controls, ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi, were included in each assay.

### Antimalarial/parasite LDH assay

The *in vitro* antimalarial activity was measured by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity [23, 24]. The assay was performed in a 96-well microplate and included two *P. falciparum* strains [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The $IC_{50}$ values were computed from the dose response curves generated by plotting percent growth against test concentrations. DMSO, artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively.

### Antileishmanial assay

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes. In a 96 well microplate assay, compounds with appropriate dilution were added to the *Leishmania* promastigotes culture (2×106 cells/mL). The plates were incubated at 26°C for 72 h and growth of *Leishmania* promastigotes was determined by Alamar blue assay [25]. Pentamidine and amphotericin B were used as standard antileishmanial agents. $IC_{50}$ values for each compound were computed from the growth inhibition curve.

### Cytotoxicity assay

The *in vitro* cytotoxic activity was determined against monkey kidney fibroblasts (VERO) following the method described by Samoylenko *et al* 23]. Doxorubicin was used as the positive and DMSO as the negative (vehicle) control.

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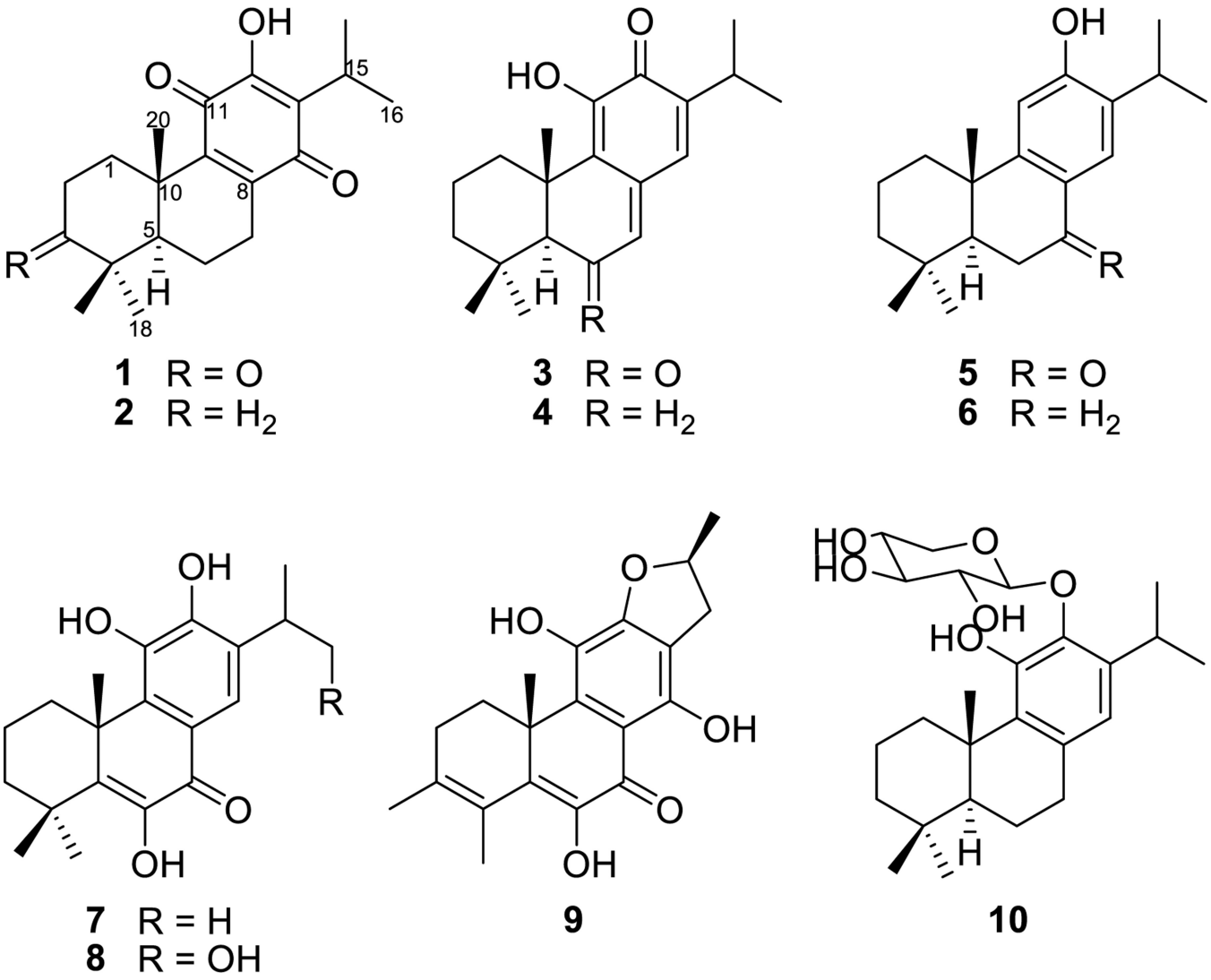


**Figure 1.**
Chemical structures of compounds **1**-**10** isolated from *C. eriophyllum*.

**Table 1**

$^{1}H$ and $^{13}C$ NMR spectroscopic data (*J* values in Hz, in parenthesis), and $^{1}H$-$^{13}C$ HMBC correlations of compound **1**.

| H/C | $\delta_H$ | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | 1.76, m; 2.81, m | 34.5, t | C-2, C-3, C-5, C-10, C-20 |
| 2 | 2.59, ddd (15.6, 9.2, 5.4); 2.51, dt (15.6, 7.2) | 33.8, t | C-1, C-3, C-4, C-10 |
| 3 | - | 216.7, s | |
| 4 | - | 46.9, s | |
| 5 | 1.76, m | 50.8, d | C-1, C-3, C-4, C-6, C-7, C-10, C-18, C-19, C-20 |
| 6 | 1.46, ddd (21.8, 12.0, 4.5); 1.76,m | 18.6, t | C-4, C-5, C-7, C-8, C-10 |
| 7 | 2.31, ddd (19.6, 12.0, 6.0); 2.84, br dd (19.6, 5.4) | 26.0, t | C-5, C-6, C-8, C-9. C-14 |
| 8 | - | 145.8, s | |
| 9 | - | 144.1, s | |
| 10 | - | 37.3 ,s | |
| 11 | - | 183.2 ,s | |
| 12 | - | 150.6 ,s | |
| 13 | - | 124.3, s | |
| 14 | - | 186.9, s | |
| 15 | 3.15, sept (7.0) | 24.1, d | C-12, C-13, C-14, C-16, C-17 |
| 16 | 1.20, d (7.0) | 19.9, q | C-13, C-15, C-17 |
| 17 | 1.19, d (7.0) | 19.8, q | C-13, C-15, C-16 |
| 18 | 1.13, s | 27.7, q | C-3, C-4, C-5, C-19 |
| 19 | 1.09, s | 20.0, q | C-3, C-4, C-5, C-18 |
| 20 | 1.24, s | 20.6, q | C-1, C-5, C-9, C-10 |
| 12-OH | 7.21, s | - | C-11, C-12, C-13 |

**Table 2**

Antiplasmodial, antileishmanial and cytotoxic activity of compounds **2**-**10**.

| Compound/extract | *P. falciparum* | | VERO | *L. donovani* | |
|---|---|---|---|---|---|
| | D6[a] | W2[b] | | | |
| | $IC_{50}$, µg/mL | | $TC_{50}$ µg/mL | $IC_{50}$ µg/mL | $IC_{90}$ µg/mL |
| *C.eriophyllum* extract | 8.8 | 8.8 | NC | NT | NT |
| **2** | - | - | NC | NT | NT |
| **3** | 1.2 | 1.2 | NC | 0.08 | 0.21 |
| **6** | - | - | NC | 4 | 13 |
| **7** | 1.8 | 2.5 | 4.5 | 3.2 | 6.5 |
| **8** | 3.0 | 4.8 | NC | 12 | 22 |
| **9** | - | - | NC | 0.2 | 0.9 |
| **10** | - | - | NC | NT | NT |
| Chloroquine | <0.026 | 0.14 | NC | NT | NT |
| Artemisinin | <0.026 | <0.026 | NC | NT | NT |
| Pentamidine | NT | NT | NT | 1.4 | 6 |
| Amphotericin B | NT | NT | NT | 0.13 | 0.3 |

[a]Chloroquine-sensetive clone;

[b]Chloroquine-resistant clone;

-=Not Active; NT = Not Tested; NC = Not cytotoxic (up to the maximum dose tested; 4.76 µg/mL for pure compounds and 47.6 *m*g/ml for crude extracts). $IC_{50}$ is the concentration that affords 50% inhibition of growth.

**Table 3**

Antifungal activities of compounds **2**-**10**.

| | $IC_{50}$/MIC, μg/mL | | | | |
|---|---|---|---|---|---|
| **Compound** | ***C. glabrata*** | ***C. krusei*** | ***C. neoformans*** | ***A. fumigatus*** | ***C. albicans*** |
| **2** | –/– | –/– | –/– | –/– | –/– |
| **3** | 5.2/10 | 12.0/– | 0.58/1.25 | 8.9/– | 12.5/– |
| **6** | –/– | –/– | –/– | –/– | –/– |
| **7** | –/– | –/– | 0.96/2.5 | 11.2/– | –/– |
| **8** | 14.9/20 | 14.5/20 | 5.9/20 | –/– | –/– |
| **9** | –/– | –/– | –/– | –/– | –/– |
| **10** | –/– | –/– | –/– | –/– | –/– |
| Amphotericin B | 0.31/0.65 | 0.95/1.25 | 0.44/1.25 | 1.29/2.50 | 0.43/1.25 |

-=Not Active; NT = Not Tested; $IC_{50}$ is the concentration that affords 50% inhibition of growth; MIC is the lowest test concentration that allows no detectable growth.

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**Table 4**

Antibacterial activities of compounds **2**-**10**.

| | $IC_{50}$/MIC, μg/mL | | | | |
|---|---|---|---|---|---|
| **Compound** | ***S. aureus*** | ***MRS*** | ***E. coli*** | ***P. aureginosa*** | ***M. intracellulare*** |
| **2** | −/− | −/− | −/− | −/− | −/− |
| **3** | 1.35/5 | 1.47/2.5 | −/− | −/− | 11.9/− |
| **6** | 1.33/2.5 | 0.96/2.5 | −/− | −/− | 14.5/− |
| **7** | 1.75/5 | 1.56/2.5 | −/− | −/− | −/− |
| **8** | 6.8/20 | 8.44/20 | −/− | −/− | −/− |
| **9** | −/− | −/− | −/− | −/− | −/− |
| **10** | −/− | −/− | −/− | −/− | −/− |
| Ciprofloxacin | 0.1/0.25 | 0.08/0.25 | 0.004/0.008 | 0.06/0.25 | 0.30/1.00 |

=Not Active; NT = Not Tested; $IC_{50}$ is the concentration that affords 50% inhibition of growth; MIC is the lowest test concentration that allows no detectable growth.