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Anti-plasmodial sesquiterpenoids from the African *Reneilmia cincinnata*

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Abstract

A new isodaucane sesquiterpenoid, 6,7,10-trihydoxyisodaucane, was isolated from the fruits of *Reneilmia cincinnata*, together with the known sesquiterpenoids oplodiol, oplopanone, 5*E*,10(14)-germacradien-1β,4β-diol, 1(10)*E*,5*E*-germacradien-4α-ol and eudesman-1,4,7-triol. A large amount of 5-hydroxy-3,7,4′-trimethoxyflavone was also isolated. Their structures were established by NMR techniques using 1D and 2D experiments. Three of the known sesquirernenoids exhibited noteworthy anti-plasmodial activity against *Plasmodium falciparum* strains. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Reneilmia cincinnata*; Zingiberaceae; Isodaucane; Germacrene; Eudesmane; Flavonoid; Antiplasmodial activity

1. Introduction

We have embarked on the discovery of potential therapeutic agents from plants of the humid rain forests of West and Central Africa within the framework of the NIH International Co-operative Bio-diversity Group (ICBG) Program (Suffness et al., 1995). Plant selection relies mostly on gathering ethnobotanical and ethnomedical information through interactions between taxonomists and traditional healers. Our interest has focused on the treatment for parasitic diseases caused by Protozoa which include malaria, leishmaniasis and trypanosomiasis. In a continuation of our collaborative phytochemical investigation (Tchuendem, Ayafor, Connolly & Sterner, 1998) and anti-parasitic drug discovery programs (Okunji, Iwu, Jackson & Tally, 1996), the plant species *Reneilmia cincinnata* (K. Schum.) Bak. (Zingiberaceae) (Koechlin, 1965) was identified as a potential anti-malarial treatment. In Cameroon, the powdered fruits of this plant are a major constituent of the ingredients of a steam-bath used traditionally to treat fevers. *R. cincinnata* is also a reputed spice. When tested in the Walter Reed Army Institute of Research (WRAIR) in vitro anti-malarial assay (Desjardins, Canfield, Haynes & Chulay, 1979; Mihous, Weatherly, Bowdre & Desjardins, 1985) the methylene chloride extract of *R. cincinnata* exhibited significant inhibitory activity. Bioassay-guided fractionation studies were therefore initiated. No previous work has been reported on this plant although labdane diterpenes have been reported from the sister species *R. alpina* (Zou et al., 1997) and *R. guianensis* (Ramiandrasoa, Chilon, Moretti & Kunesch, 1986).

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2. Results and discussion

A sample of the air-dried powdered fruits of *R. cincinnata* was macerated in methylene chloride and the extract tested in vitro for anti-malarial activity (Desjardins et al., 1979; Mihous et al., 1985). Activities in this bioassay are recorded as (IC_{50}) values for W-2 and D-6, which are the concentrations in (μg/ml) required to cause 50% inhibition of the two *Plasmodium falciparum* malaria parasite clones, Indochina W-2 and Sierra Leone D-6, respectively. The W-2 clone is resistant to chloroquine, pyrimethamine, sulfadoxine, and quinine, while the D-6 clone is resistant to mefloquine. The methylene chloride extract gave on IC_{50} of 4.136 μg/ml for D-6 clone and 25.517 μg/ml for the W-2 clone. This level of activity was considered to be significant.

Bioassay-guided fractionation of the extract following the protocol described in Section 3 led to the isolation of six sesquiterpenoids and a flavonoid. One of the sesquiterpenoids to which we have assigned the trivial name reneilmol (**1**), is new. The other five are known and have been identified as oplodiol (**2**) (Minato & Ishikawa, 1967), (−)-oplopanone (**3**) (Takeda, Minato & Ishikawa, 1966; Wratten & Faulkner, 1977), 5*E*,10(14)-germacradien-1β,4β-diol (**4**) (Kitagawa, Cui, Kobayashi & Kyogoku, 1987) 1(10)*E*,5*E*-germacradien-4α-ol (**5**) (Izac, Bandurraga, Wasylyk, Dunn & Fenical, 1982), eudesman-1,4,7-triol (**6**) (Sung, Steffan, Steglich, Klebe & Adam, 1992) (the structures of compounds **1**–**6** are shown in Fig. 1), and

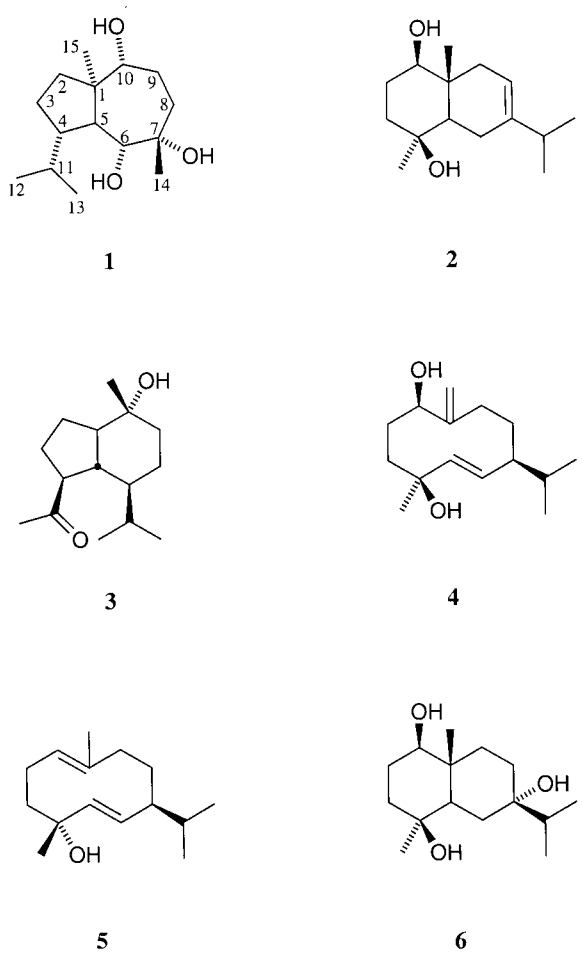

Fig. 1. Structures of the isolated compounds.

Table 1
^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data (δ, multiplicity, *J*) for **1** in $CDCl_3$–CD_3OD 4 : 1, and HMBC correlations[a]

Atom no.	^{13}C	^{1}H	HMBC correlations (H to C)
1	48.4, *s*	–	–
2	39.2, *t*	1.47, *m*	1, 3, 10
		1.37, *dt*, 6.4, 12.7	1, 3, 10
3	24.3, *t*	1.53, *m*	2, 4, 11
		1.28, *ddd*, 5.5, 11.2, 17.4	2, 4, 11
4	50.3, *d*	1.90, *m*	3, 5, 6, 11, 13, 14
5	51.6, *d*	1.56, *d*, 9.1	1, 2, 4, 6, 7, 11
6	76.7, *d*	3.26, *d*, 9.5	1, 5, 10
7	73.8, *s*	–	–
8	33.4, *t*	1.84, *dt*, 4.7, 13.3	7, 9, 10
		1.49, *m*	7, 9, 10
9	27.9, *t*	1.53/1.75, *m*	1, 8, 10
10	74.9, *d*	3.49, *dd*, 5.1, 9.5	1, 2, 5, 8, 9
11	31.9, *d*	1.63, *m*	3, 4, 12, 13
12	22.4, *q*	0.85, *d*, 6.9	4, 11, 12
13	17.6, *q*	0.78, *d*, 6.7	4, 11, 13
14	25.7, *q*	1.17, *s*	6, 7, 8
15	25.6, *q*	0.90, *s*	2, 3, 5, 10

[a] The $CHCl_3$/$CDCl_3$ signals (δ 7.26 and 77.0) were used as reference (the coupling constants *J* are given in Hz).

the flavonoid 5-hydroxy-3,7,4′-trimethoxyflavone (Wollenweber, Iinuma, Tanaka & Mizuno, 1990), by comparison of their physical data (mp, $[\alpha]_D$) and spectral data (IR, ^1H, ^3C, and EIMS) with those reported in the literature.

Reneilmol (**1**) was obtained as colourless crystals from a mixture of *n*-hexane-methylene chloride (7 : 3). Its IR spectrum showed absorptions for hydroxy groups at υ_{max} 3350 and 3290 cm^{-1}. The EIMS with peaks at *m*/*z* 238 $[M–H_2O]^+$ and 220 $[M–H_2O]^+$ and the presence of signals due to fifteen carbon atoms in the ^{13}C-NMR spectrum enabled us to deduce the molecular formula $C_{15}H_{28}O_3$. The ^1H-NMR spectrum (Table 1) showed signals for two tertiary methyl groups with singlets of three protons each at δ 1.17 and 0.90 ppm, two secondary methyl groups at δ 0.85 (*d*, J = 6.9 Hz) and 0.78 (*d*, J = 6.7 Hz), and two methine protons attached to oxygen-bearing carbons at δ 3.49 (*dd*, J = 5.1 and 9.5 Hz) and 3.26 (*d*, J = 9.5 Hz). The ^{13}C-NMR spectrum (Table 1) indicated the presence of three oxygenated carbon atoms at δ 76.7, 74.9 and 73.8 ppm. No olefinic carbon could be detected. The direct heteronuclear $^{13}C–^1H$ correlation spectrum (HMQC) led to the conclusion that the compound had two quaternary, five tertiary, four secondary and four primary carbon atoms. Finally, the use of long-range $^{13}C–^1H$ correlations (HMBC) associated with $^1H–^1H$–COSY permitted the complete assignment of structure **1**, 6,7,10-trihydroxyisodaucane, to reneilmol which is a new isodaucane sesquiterpene. The various HMBC correlations are shown in Table 1.

The relative stereochemistry of **1** has been determined using the NOESY spectrum (summarised in Fig. 2), while the absolute configuration remains to be clarified. Important correlations were observed between H-5 (δ 1.56), H-3a (δ 1.28), and H-15 (δ 0.90) suggesting that they were on the same side of the molecule. Further correlations were also observed between H-6 (δ 3.26), H-4 (δ 1.90), H-14 (δ 1.17) as well as between H-10 (δ 3.49), H-6, and H-14. Reneilmol (**1**) is thus 6,7,10-trihydroxyisodaucane and can be regarded as an oxidative product of 6,10-epoxy-7(14)-isodaucene isolated from *Salvia sclarea* (Maurer & Hauser, 1983). It belongs to the rare group of isodaucanes or salvialanes.

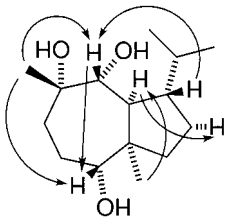

Fig. 2. Pertinent NOESY correlations observed with **1**.

The results of the anti-plasmodial testing of four of the sesquiterpenoids isolated from *R. cincinnata* are summarised in Table 2. The hydroxylated germacradienes (**4**) and (**5**) clearly have the strongest activity. Oplodiol, an eudesmenediol (**2**), showed moderately strong activity, while oplopanone (**3**) was inactive. The isolation of anti-plasmodial sesquiterpenoids from the fruits of *R. cincinnata* lends some support to its use in traditional anti-fever preparations.

3. Experimental

3.1. Plant material

The fruits of *Renealmia cincinnata* (K. Schum.) Bak. were collected in Bafut, Northwest province of Cameroon, in October 1997. The authentication was done by Paul Mezili, a botanist at the Cameroon National Herbarium where a voucher specimen is deposited.

3.2. Extraction and isolation

The air-dried and powdered fruits (200 g) of *R. cincinnata* were macerated in CH_2Cl_2 (2 litres) for four days. Removal of the solvent from the filtrate under

Table 2
Anti-plasmodial activities of *R. cincinnata* sesquiterpenoids

	Plasmodium falciparum clones (IC_{50}, μg/ml)	
	D-6	W-2
Reneilmol (**1**)	NT[a]	NT[a]
Oplodiol (**2**)	4.17	25.50
Oplopanone (**3**)	> 50.00	> 50.00
5*E*,10(14)-Germacradien-1β,4β-diol (**4**)	1.63	31.90
1(10)*E*,5*E*-Germacradien-4β-ol (**5**)	1.54	1.90
Eudesman-1,4,7-triol (**6**)	NT[a]	NT[a]
Cloroquine (as standard)	0.0028	0.075

[a] NT: not tested.

vacuum in a rotatory evaporator provided an organic extract (43 g) which was subjected to vacuum liquid chromatography on SiO_2 (200–400 Mesh) using hexane-EtOAc mixtures as eluent. 250 ml fractions were collected and grouped on the basis of their TLC profiles. The combined fractions eluted with hexane-EtOAc 9 : 1 (6.5 g) which showed the strongest antimalarial activity (IC_{50}: D-6 3.80 μg/ml; W-2 4.80 μg/ml) were further purified by repeated column chromatography on SiO_2 (70–230 Mesh) and on Sephadex LH-20 (*n*-hexane-CH_2Cl_2 9 : 1) to yield 1(10)*E*,5*E*-germacradien-4α-ol (**5**) (52 mg), 5*E*,10(14)-germacradien-1β,4β-diol (**4**) (20 mg), and 5-hydroxy-3,7,4′-trimethoxyflavone (150 mg). The fractions eluted with hexane-EtOAc (3 : 2) also showed activity in the antimalarial screen (IC_{50}: D-6 9.50 μg/ml; W-2 15.00 μg/ml). Further purification of this fraction by MPLC using the Baeckström Separo AB column (i.d. 15 mm) with a continuous gradient of EtOAc in *n*-hexane afforded oplopanone (**3**) (470 mg), oplodiol (**2**) (20 mg) and a mixture of reneilmol (**1**) and 1,4,7-trihydoxyeudesmane (**6**) (150 mg). Final purification of the later mixture (140 mg) by LH-20 gel permeation chromatography with CH_2Cl_2–MeOH (1 : 1) as eluent gave pure reneilmol (**1**) (5 mg) and pure 1,4,7-trihydroxyeudesmane (**6**) (60 mg). TLC experiments were performed on silica gel GF_{254} pre-coated plates and detection was accomplished by spraying with 50% H_2SO_4 followed by heating at 110° or by visualising with an UV lamp at 254 and 366 nm.

3.3. Spectroscopy

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125.8 MHz) were recorded at room temperature in $CDCl_3$ or in $CDCl_3$–CD_3OD using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in ppm with the solvent signals, δ_H 7.26 and δ_C 77.0 as reference, while the coupling constants (J) are given in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra evaluated with the standard Bruker UXNMR software (ref. 941001). The IR spectra were recorded with a Perkin–Elmer 298 spectrometer, and the mass spectra recorded with a Jeol SX102 spectrometer at 70 eV. The melting points (uncorrected) were determined with a Reichert microscope while the optical rotations were measured with a Perkin–Elmer 141 polarimeter at 22°C.

3.3.1. 6,7,10-Trihydroxyisodaucane (*1*)

This was obtained as colourless crystals from *n*-hexane- CH_2Cl_2 (7 : 3); mp 143–145°. $[\alpha]_D^{22}$-5.6° (MeOH; c {0.55}). Analysis: found C, 70.27; H, 11.01. $C_{15}H_{28}O_3$ requires: C, 70.26; H, 11.01%. IR υ_{max}^{KBr} cm^{-1}: 3350, 3290, 2970, 2930, 2890, 1455, 1445, 1360, 1250, 1090, 1055, 1020, 980. See Table 1 for ^{1}H- and ^{13}C-NMR data. FABMS *m*/*z* 257 ($[M+1]^+$. EIMS (probe) 70 eV *m*/*z* (relative intensity): 238 $[M–H_2O]^+$ (5), 220 $[M–2H_2O]^+$ (14), 195 (38), 177 (45), 167 (100), 159 (32), 151 (61), 136 (43), 123 (73), 93 (67), 81 (95), 71 (35), 55 (33), 43 (91) and 41 (31).

3.3.2. 7-Eudesmene-1β,4β-diol (oplodiol) (*2*)

Crystallised from a mixture of *n*-hexane-EtOAc as colourless plates, mp 107–109° (see Ref. Minato & Ishikawa, 1967, 106–107°). $[\alpha]_D^{28}$-53° ($CHCl_3$; c{0.68}) (see Ref. Minato & Ishikawa, 1967); $[\alpha]_D^{24}$-51.9°; dioxan, c {0.5}). IR, NMR and EIMS were in good agreement with published data.

3.3.3. Oplopanone (*3*)

3 was obtained as colourless prisms from *n*-hexane-EtOAc, mp 95–97° (see Refs. Takeda et al., 1966; Wratten & Faulkner, 1977), 96–97°). $[\alpha]_D^{26}$-16° ($CHCl_3$; c{0.57}) (see Refs. Takeda et al., 1966; Wratten & Faulkner, 1977); $[\alpha]_D^{25}$-20°; dioxane, c {0.6}). (−)-Oplopanone exhibited spectral data (IR, NMR and EIMS) comparable to published values (Wratten & Faulkner, 1977).

3.3.4. 5E,10(14)-Germacradien-1β,4β-diol (*4*)

4 was obtained as colourless prisms from *n*-hexane-EtOAc, mp 120–122° (see Ref. Kitagawa et al., 1987, 119°). The structure was assigned by comparison of IR, NMR and EIMS data with those found in the literature (Kitagawa et al., 1987).

3.3.5. 1(10)E,5E-Germacradien-4α-ol (*5*)

5 was obtained as brown oil $[\alpha]_D^{26} + 118°$ ($CHCl_3$; c{0.80}) (see Ref. Izac et al., 1982); $[\alpha]_D^{26} + 112°$). IR, NMR and EIMS were in good agreement with published data (Izac et al., 1982).

3.4. Anti-malarial assay

The in vitro anti-malarial assays were performed by using a modification of semi-automated microdilution technique described earlier (Desjardins et al., 1979; Mihous et al., 1985). Two *Plasmodium falciparum* malaria parasite clones designated Indochina (W-2) and Sierra Leone (D-6) were utilised in susceptibility tests. The W-2 clone is resistant to chloroquine, pyrimethamine, sulfadoxine, and quinine, while the D-6 clone is resistant to mefloquine. The test sesquiterpenoids, oplodiol (**2**), oplopanone (**3**), 5*E*,10(14)*E*-germacra-

dien-1β,4β-diol (**4**), 1(10)*E*,5*E*-germacradien-4α-ol (**5**) were each dissolved in DMSO and serially diluted using malarial growth medium. Drug-induced reduction in uptake of tritiated hypoxanthine was used as index of inhibition of parasite growth.

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