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ANTIMALARIAL SESQUITERPENES FROM TUBERS OF *CYPERUS ROTUNDUS*: STRUCTURE OF 10,12-PEROXYCALAMENENE, A SESQUITERPENE ENDOPEROXIDE

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Key Word Index—*Cyperus rotundus*; antimalarial; endoperoxide sesquiterpene; sesquiterpenes; 10,12-peroxycalamenene; patchoulenone; caryophyllene α-oxide; 4,7-dimethyl-1-tetralone.

Abstract—Activity-guided investigation of *Cyperus rotundus* tubers led to the isolation of patchoulenone, caryophyllene α-oxide, 10,12-peroxycalamenene and 4,7-dimethyl-1-tetralone. The antimalarial activities of these compounds are in the range of EC_{50} 10^{-4}–10^{-6} M, with the novel endoperoxide sesquiterpene, 10,12-peroxycalamenene, exhibiting the strongest effect at EC_{50} 2.33×10^{-6} M.

INTRODUCTION

In a multidisciplinary research programme in a search for antimalarial natural products we came across the alleged antimalarial activity of *Cyperus rotundus* Linn. tubers (a common weed distributed throughout the country) reported in the guidelines of Thai medicinal plants used in primary health care [1]. *C. rotundus* Linn. has already been investigated by many groups and several mono- and sesquiterpenes have been isolated [2–9]. Some terpenes isolated from this plant have also been tested for antimalarial activity, with moderate *in vitro* activities being recorded [9]. In our screening through the usual procedure [10] it was noted that the crude hexane extract of the air-dried tubers of *C. rotundus* Linn. showed high potency in the *in vitro* test against *Plasmodium falciparum* (EC_{50} = 0.66 μg ml^{-1}); we therefore undertook a systematic study employing biological testing of the constituents of the crude hexane extract to track down the active principles.

RESULTS AND DISCUSSION

Antimalarial activity-guided quick column chromatography of the crude hexane extract of *C. rotundus* Linn. tubers using EtOAc–hexane (1:19) as eluent provided two active fractions. On further purification the first fraction yielded, after crystallization, patchoulenone (**1**), caryophyllene α-oxide (**2**) and 10, 12-peroxycalamenene (**3**) in 0.0030%, 0.0004% and 0.000045% yields from the dried tubers, respectively. Purification by HPLC (Nova Pak C18, pure acetonitrile as eluent) of the second fraction gave 4,7-dimethyl-1-tetralone (**4**) (0.0007%) as an oil.

Patchoulenone (**1**) and caryophyllene α-oxide (**2**) were obtained as needles from cold hexane, mp 52–53° and 60–61.5°, respectively, and exhibited physical data identical in all respects with those reported [2, 11]. Compound **1** is already known to be a constituent of *C. rotundus* Linn. [9], but there has been no report, until now, of the isolation of **2** from this plant. The *in vitro* activity against *Plasmodium falciparum* (EC_{50}) of pure **1** and **2** are in the order of 10^{-4}M (1.08×10^{-4} and 3.45×10^{-4}M respectively).

Isolation of the novel sesquiterpene endoperoxide, 10,12-peroxycalamenene (**3**) was achieved by activity-guided HPLC using 300 × 7.8 mm μ-Porasil and CH_2Cl_2–hexane (1:1) as eluent. Obtained as needles, mp. 67–68.5° (from cold hexane), $[\alpha]_D^{20} = -67.22°$ ($CHCl_3$; *c* 0.2765), the compound was shown by its mass spectral and elemental analysis to possess the molecular formula $C_{15}H_{20}O_2$. ^1H and ^{13}C NMR spectra of **3** (Table 1) showed the presence of a 1,2,4-trisubstituted aromatic nucleus, three aliphatic and one aromatic methyl groups (singlets at δ 1.01, 1.27, 1.59 and 2.34, respectively) and a three-carbon chain, $-CH-CH_2CH_2-$. The complete structure of **3** was finally confirmed by DEPT, ^1H–^1H COSY, ^{13}C–^1H COSY and NOE experiments (see Fig. 1). Although present in a very small amount in the

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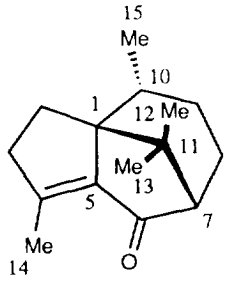

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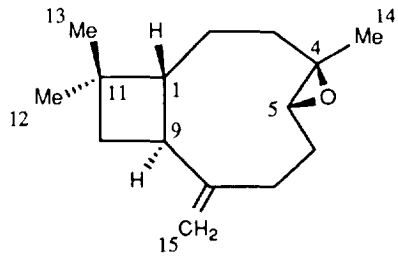

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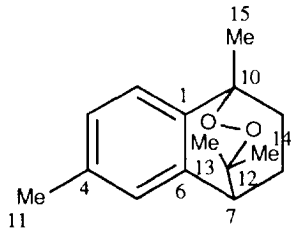

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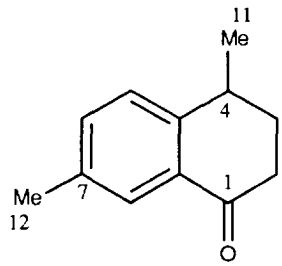

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Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectral data of compound **3** (in ppm, $CDCl_3$)

C	δ_C	H	δ_H
1	142.2 or 137.0	1	-
2	122.4	2	7.14 (1H) *d* (7.6)
3	126.8	3	7.02 (1H) *br d* (7.6)
4	142.2 or 137.0	4	
5	128.2	5	6.92 (1H) *br s*
6	142.2 or 137.0	6	
7	50.5	7	2.77 (1H) *m*
8	22.5	8	1.64–1.75 (H_a) *m*
			2.34 (H_b) *m*
9	32.9	9	1.64–1.75 (H_a) *m*
			2.58 (H_b) *br m*
10	83.1*	10	
11	21.1	11	2.34 (H) *s*
12	82.8*	12	
13	24.2†	13	1.01 (3H) *s**
14	25.2†	14	1.27 (3H) *s**
15	22.6†	15	1.59 (3H) *s**

*,† Signals may be interchangeable.

tubers of *C. rotundus* Linn. **3** exhibits pronounced *in vitro* antimalarial activity with an EC_{50} value of 2.33×10^{-6} M.

Identification of the fourth antimalarial component of *C. rotundus* Linn., the structurally simple and moderately active 4,7-dimethyl-1-tetralone **4** (EC_{50} 8.62×10^{-5} M), was straightforward. Compound **4** was previously reported to be a constituent of lavender oil [12] and its co-isolation with **3** from *C. rotundus* Linn. suggests that the two compounds share an intermediate.

The discovery of the highly promising antimalarial agents artemisinin [13–15] and Yingzhaozu [16] from the respective Chinese herbs *Artemisia annua* Linn. and *Artabotrys uncinatus* Linn., and the accumulation of evidence that the peroxide moiety plays an important role in the activity against malarial parasites, have spurred much effort towards the search for new members of naturally occurring peroxides as well as towards the studies of their syntheses [17–19] and mechanisms of action [20, 21]. The work described here demonstrates further progress in our own search for more such naturally occurring peroxides.

EXPERIMENTAL

General. CC was carried out on silica gel 60, 63–200 μ and silica gel 60, 40–63 μ (Merck). MPLC was carried out using a Büchi system and the packing material LiChroprep® Si 60, 15–25 μ (Merck). HPLC was performed on a Waters system (HPLC pump Waters 510 and detector Waters 484, $\lambda = 254$ nm), using μ-Porasil and Nova Pak C18. Prep. TLC was carried out on silica gel 60 PF_{254} (2 mm thick). ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-A500 instrument in $CDCl_3$ with TMS as int. standard. IR spectra were taken on a Jasco model A-302 spectrometer. Low-resolution mass spectra (70 eV) were run on Finnigan MAT INCOS 50 mass spectrometer. Elemental analyses were carried out on a Perkin Elmer Elemental Analyzer 2400 CHN. UV spectra were measured with a Jasco Uvidex-650 double beam spectrometer using EtOH as the solvent. Optical rotation was measured on a Perkin Elmer 241 spectrometer. Mps: uncorr.

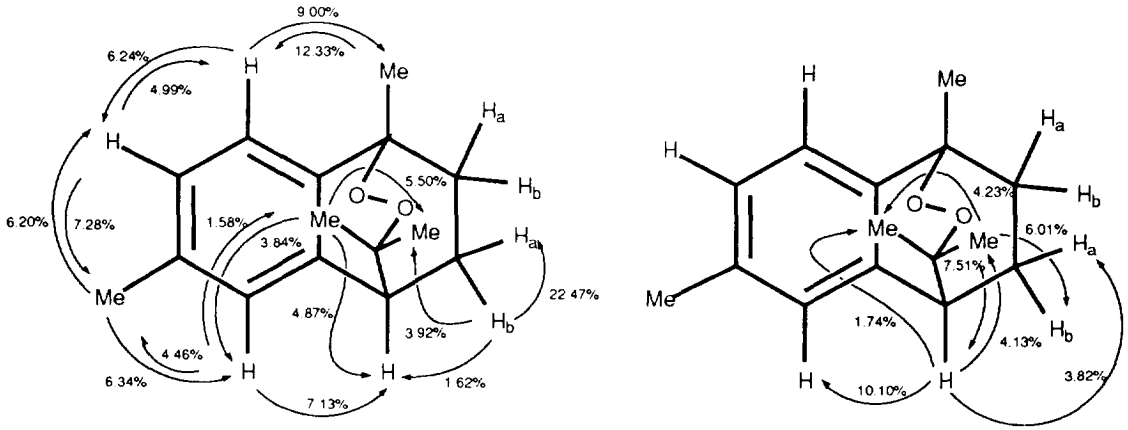

Fig. 1. NOEDF of 10,12-peroxycalamenene (**3**).

Plasmodium falciparum culture and in vitro testing. Continuous *in vitro* cultures of asexual erythrocytic stages of *P. falciparum* (K1, multidrug-resistant strain) were maintained following the method of Trager and Jensen [22]. Quantitative assessment of antimalarial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins [10]. Effective concentration (EC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]hypoxanthine by *P. falciparum*. EC_{50} value of 1.41×10^{-4} M was observed when a commercial sample of caryophyllene α-oxide (tech., 90%, Aldrich, cat. no. 36, 199-2) was subjected to the same test system.

Plant material. The air-dried tubers of *C. rotundus* Linn. were purchased from a Thai traditional dispensary, Bangkok, Thailand.

Extraction and isolation. The air-dried tubers of *C. rotundus* Linn. (10 kg) were ground and extracted by soaking in hexane at room temperature for 2 days, then filtered. The process was repeated three times. The filtrates were then combined and evapd under vacuum to dryness (140 g, 1.4% yield). The hexane crude extract was subjected to silica gel column chromatography using hexane and ethyl acetate as mobile phases with gradient elution, and separated into 5 frs (C1–C5). The antimalarial activities of these frs were determined from their EC_{50} values against *P. falciparum*, from which frs C2 and C3 were selected for further study. Fr. C2 (EC_{50} 0.56 μg ml^{-1}) was subjected to further chromatography by MPLC using EtOAc–hexane (1:19) as eluent to give five frs among which antimalarial activity could be detected in frs 3 and 4. Fr. 4 was further purified by MPLC using pure CH_2Cl_2 as eluent to afford, after crystallization from cold hexane, patchoulenone (**1**, 0.30 g), and caryophyllene α-oxide (**2**, 0.0400 g). MPLC of fr. 3 using EtOAc–hexane (1:99) as eluent followed by HPLC (μ-Porasil, EtOAc–hexane (1:400) and CH_2Cl_2–hexane (1:1) as eluents) provided 10,12-peroxycalamenene (**3**, 0.0045 g). The C3 fr. (EC_{50} = 0.70 μg ml^{-1}), on successive purification by MPLC (EtOAc–hexane, 3:97), prep. TLC (*iso*-PrOH–cyclohexane, 1:24, as developing solvent) and HPLC (Nova Pak C18, pure MeCN as eluent) yielded 4,7-dimethyl-1-tetralone (**4**, 0.0706 g).

Patchoulenone (**1**). Needles, mp 52–53° (cold hexane or MeOH), lit. [2] mp 52.2° (MeOH); $[\alpha]_D^{20}$: −113.33° ($CHCl_3$; *c* 0.4950), lit. [2] $[\alpha]_D^{20}$: −97.1° ($CHCl_3$; *c* 8.029). UV λ_{max}^{EtOH} nm (log ε): 256 (3.93), 335 (1.99). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1710 (C=O, *s*), 1658 (C=C, *s*), 1395 (*m*), 1375 (*m*). EIMS 70 eV, *m/z* (rel. int.): 218 ([M]$^+$, 100), 203 (24), 185 (5), 175 (84), 161 (24), 147 (38), 133 (17), 105 (24), 91 (30), 77 (20). Found: C, 82.36; H, 10.38. Calc. for $C_{15}H_{22}O$: C, 82.52; H, 10.16%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.64 (1H, *ddd*, *J* = 13.5, 9.5, 1.4 Hz, H-2a), 1.89 (1H, *dt*, *J* = 13.5, 9.5 Hz, H-2b), 2.44 (1H, *br dd*, *J* = 18.0, 9.5 Hz, H-3a), 2.78 (1H, *br dt*, *J* = 18.0, 9.5 Hz, H-3b), 2.05 (1H, *t*, *J* = 3.5 Hz, H-7), 1.73 (1H, *ddd*, *J* = 14.0, 7.0, 3.5 Hz, H-8a), 1.93 (1H, *ddt*, *J* = 6.5, 3.5, 14.0 Hz, H-8b), 1.15 (1H, *ddt*, *J* = 12.0, 7.0, 14.0 Hz, H-9a), 1.57 (1H, *dt*, *J* = 14.0, 6.5 Hz, H-9b), 2.16 (1H, *ddq*, *J* = 12.0, 6.5, 6.5 Hz, H-10), 1.01 (3H, *s*, H-12), 0.88 (3H, *s*, H-13), 2.07 (3H, *q like*, H-14), 0.84 (3H, *d*, *J* = 6.5 Hz, H-15). ^{13}C NMR (100 MHz, $CDCl_3$): δ 63.7 (C-1), 26.2 (C-2), 43.5 (C-3), 148.5 (C-4), 139.7 (C-5), 207.3 (C-6), 63.0 (C-7) 25.9 (C-8), 28.1 (C-9), 34.6 (C-10), 41.4 (C-11), 19.0 (C-12), 26.4 (C-13), 15.2 (C-14), 17.9 (C-15).

Caryophyllene α-oxide (**2**). Needles, mp 60–61.5° (cold hexane); lit. [23] mp 63.5–64°; $[\alpha]_D^{20}$: −70.12° ($CHCl_3$; *c* 0.6460), lit. [23] $[\alpha]_D^{20}$: −79.4° ($CHCl_3$; *c* 2.32). UV λ_{max}^{EtOH}: no maximum UV absorption above 220 nm. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3075 (*w*), 1630 (*m*), 900 (*m*). EIMS 70 eV, *m/z* (rel. int.): 220 ([M]$^+$, 1), 205 (5), 187 (5), 177 (11), 161 (11), 138 (24), 121 (31), 109 (66), 93 (99), 79 (100), 69 (68), 55 (48). Found: C, 81.53; H, 11.27. Calc. for $C_{15}H_{24}O$: C, 81.76; H, 10.98%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.76 (1H, *t*, *J* = 10 Hz, H-1), 1.32 (1H, *m*, H-2a), 1.55–1.72 (1H, *m*, H-2b), 0.95 (1H, *m*, H-3a), 2.10 (1H, *dt*, *J* = 3.5, 12.5 Hz, H-3b), 2.87 (1H, *dd*, *J* = 10.6, 4.1 Hz, H-5), 1.42 (1H, *m*, H-6a), 2.25 (1H, *ddd*, *J* = 16.4, 7.8, 4.1 Hz, H-6b), 2.11 (1H, *t*, *J* = 12.4 Hz, H-7a), 2.34 (1H, *ddd*, *J* = 12.4, 8.1, 4.4 Hz, H-7b), 2.61 (1H, *br q*, *J* = 10 Hz, H-9), 1.55–1.72 (2H, *m*, H-10), 0.98 (3H, *s*, H-12), 1.00 (3H, *s*,

H-13), 1.20 (3H, *s*, H-14), 4.97 (1H, *s*, H-15a), 4.85 (1H, *s*, H-15b). ^{13}C NMR (100 MHz, $CDCl_3$): δ50.8 (C-1), 27.2 (C-2), 39.1 (C-3), 59.8 (C-4), 63.7 (C-5), 30.2 (C-6), 29.8 (C-7), 151.8 (C-8), 48.7 (C-9), 39.8, (C-10), 34.0 (C-11), 29.9 (C-12), 21.6 (C-13), 17.0 (C-14), 112.7 (C-15).

10,12-*Peroxycalamenene* (**3**). Needles, mp 67–68.5° (cold hexane); $[\alpha]_D^{20}$: −67.22° ($CHCl_3$; *c* 0.2765). UV λ_{max}^{EtOH}: no maximum UV absorption above 220 nm. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1615 (*m*), 1501 (*m*) 1458 (*m*), 1372 (*m*), 1362 (*m*). EIMS 70 eV, *m/z* (rel. int.): 232 ([M]$^+$, 0.14), 215 (0.18), 198 (0.30), 185 (1.80), 157 (56), 146 (100), 128 (10), 115 (14), 103 (2), 91 (11), 77 (10). Found: C, 78.08; H, 8.74 $C_{15}H_{20}O_2$ requires: C, 77.55; H, 8.68%. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.14 (1H, *d*, *J* = 7.6 Hz, H-2), 7.02 (1H, *br d*, *J* = 7.6 Hz, H-3), 6.92 (1H, *br s*, H-5), 2.77 (1H, *dd like*, H-7), 1.64–1.75 (1H, *m*, H-8a), 2.34 (1H, *m*, H-8b), 1.64–1.75 (1H, *m*, H-9a), 2.58 (1H, *s*, H-9b), 2.34 (3H, *s*, H-11), 1.01 (3H, *s*, H-13), 1.27 (3H, *s*, H-14), 1.59 (3H, *s*, H-15). ^{13}C NMR (100 MHz, $CDCl_3$): δ142.2 or 137.0 (C-1), 122.4 (C-2), 126.8 (C-3), 142.2 or 137.0 (C-4), 128.2 (C-5), 142.2 or 137.0 (C-6), 50.5 (C-7), 22.5 (C-8), 32.9 (C-9), 83.1 (C-10 or C-12), 21.1 (C-11), 82.8 (C-12 or C-10), 24.2 (C-13, C-14 or C-15), 25.2 (C-14, C-13 or C-15), 22.6 (C-15, C-13 or C-15).

4,7-*Dimethyl*-1-*tetralone* (**4**). Oil; $[\alpha]_D^{20}$ + 5.60° ($CHCl_3$; *c* 0.3035). UV λ_{max}^{EtOH} nm (log ε): 252 (4.03), 305 (3.31). IR ν_{max}^{neat} cm^{-1}: 1685 (C=O, *s*), 1615 (*m*), 1570 (*m*), 1500 (*m*). EIMS 70 eV, *m/z* (rel. int.): 174 ([M]$^+$, 80), 159 (100), 146 (26), 132 (52), 118 (37), 91 (20), 77 (50). HRMS found: 174.1087 [M]$^+$, Calc. for $C_{12}H_{14}O$: 174.1045. ^{1}H NMR (400 MHz, $CDCl_3$): δ2.56 (1H, *ddd*, *J* = 17.3, 8.8, 4.6 Hz, H-2a), 2.77 (1H, *ddd*, *J* = 17.3, 8.5, 4.6 Hz, H-2b), 1.87 (1H, *dddd*, *J* = 13.3, 8.8, 7.4, 4.6 Hz, H-3a), 2.22 (1H, *ddt*, *J* = 13.3, 8.5, 4.6 Hz, H-3b), 3.04 (1H, *ddq*, *J* = 7.4, 4.6, 7.0 Hz, H-4), 7.22 (1H, *d*, *J* = 7.2 Hz, H-5), 7.32 (1H, *dd*, *J* = 7.2, 1.7 Hz, H-6), 7.83 (1H, *br s*, H-8), 1.37 (3H, *d*, *J* = 7.0 Hz, H-11), 2.35 (3H, *s*, H-12). ^{13}C NMR (100 MHz, $CDCl_3$): δ198.6 (C-1), 36.4 (C-2), 30.6 (C-3), 32.4 (C-4), 127.2 (C-5), 134.5 (C-6), 131.5 (C-7), 127.2 (C-8), 136.0 (C-9), 146.0 (C-10), 20.6 (C-11), 20.8 (C-12).

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