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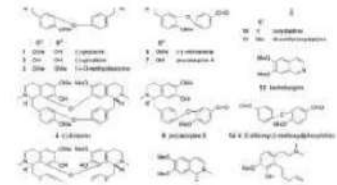
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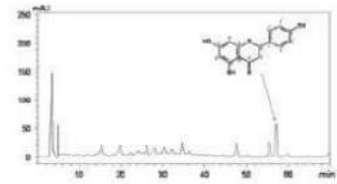
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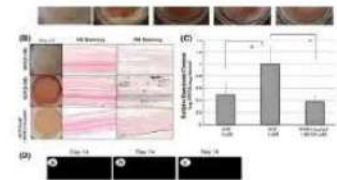
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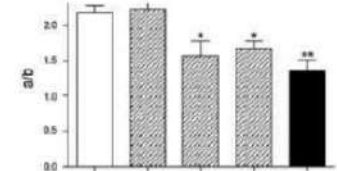
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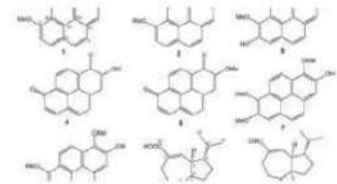
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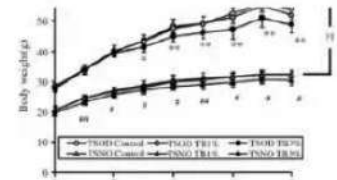
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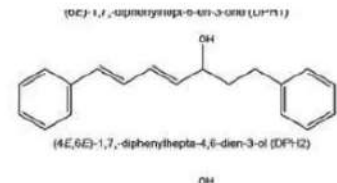
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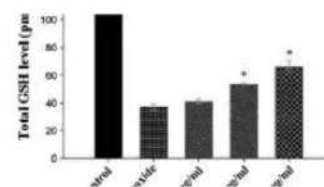
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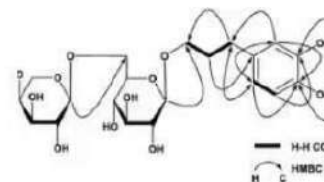
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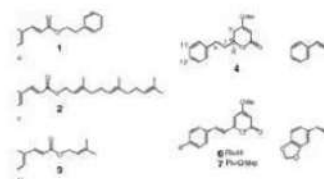
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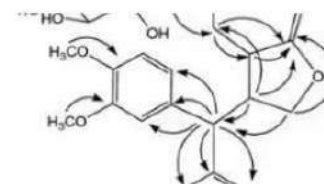
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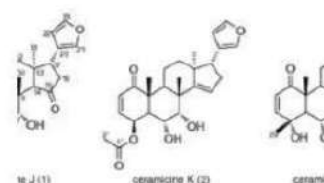
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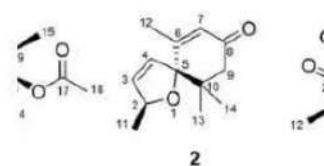
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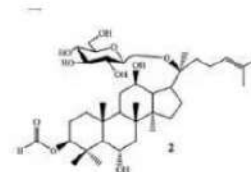
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Antiplasmodial decarboxyportentol acetate and 3,4-dehydrotheaspirone from *Laumoniera bruceadelpha*

Hiroshi Morita · Reika Mori · Jun Deguchi ·
Shiori Oshimi · Yusuke Hirasawa · Wiwied Ekasari ·
Aty Widyawaruyanti · A. Hamid A. Hadi

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Abstract A new spiro heterocycle, decarboxyportentol acetate (**1**) was isolated from the barks of *Laumoniera bruceadelpha* Nootboom (Simaroubaceae), together with 3,4-dehydrotheaspirone (**2**), and their structures were elucidated by 2D NMR analysis. 3,4-Dehydrotheaspirone (**2**) showed potent antiplasmodial activity against *Plasmodium falciparum* 3D7.

Keywords Decarboxyportentol acetate ·
3,4-Dehydrotheaspirone · *Laumoniera bruceadelpha* ·
Simaroubaceae · Antiplasmodial activity ·
Plasmodium falciparum 3D7

Introduction

Malaria is one of the crucial infectious diseases in the world and continues to cause morbidity and mortality on a large scale in tropical countries [1]. The antimalarial potential of compounds derived from plants has been proven by examples such as quinine from *Cinchona* species and artemisinin from *Artemisia annua* [2]. The plants belonging to Simaroubaceae are known to contain various

terpenoids with biological activities such as antimalarial, antifeedant, anti-inflammatory, antiulcer, antipyretic, and cytotoxic activities [3–6]. We have previously reported that two new quassinoids, delaumonones A and B, isolated from *Laumoniera bruceadelpha* Nootboom (Simaroubaceae) showed potent antiplasmodial activity [7].

In our search for bioactive constituents targeting malaria from medicinal plants, two spiro heterocycles, decarboxyportentol acetate (**1**) and 3,4-dehydrotheaspirone (**2**), were isolated from the barks of *L. bruceadelpha*, both of which showed potent antiplasmodial activity. This paper describes the isolation and structural elucidation of **1** and **2** with antiplasmodial activity against *Plasmodium falciparum* 3D7 (chloroquine-sensitive clone).

The barks of *L. bruceadelpha*, which were collected in Malaysia, were extracted with MeOH, and the extract was suspended in H₂O and then partitioned between CHCl₃ and *n*-BuOH, successively. The CHCl₃-soluble materials were subjected to a silica gel column, and then an ODS column followed by an ODS HPLC to afford decarboxyportentol acetate (**1**) and 3,4-dehydrotheaspirone (**2**).

Decarboxyportentol acetate (**1**), colorless amorphous solid, $[\alpha]_{\text{D}}^{20} +58$ (c 0.5, CHCl₃), showed molecular formula, C₁₈H₂₈O₄, which was determined by HRESIMS [*m/z* 331.1861 (M + Na)⁺, Δ -2.4 mmu]. IR absorptions implied the presence of carbonyl (1739 and 1653 cm⁻¹) and ether (1242 cm⁻¹) functionalities. ¹H and ¹³C NMR data are presented in Table 1. The ¹³C NMR spectrum revealed 18 carbon signals due to one *sp*³ quaternary carbon, two carbonyl carbons, one olefinic carbon, one *sp*² methine, six *sp*³ methines, and seven methyls. Among them, four carbons (δ_C 76.9, 80.7, 170.6, and 200.5) were ascribed to those bearing an oxygen atom.

The structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the

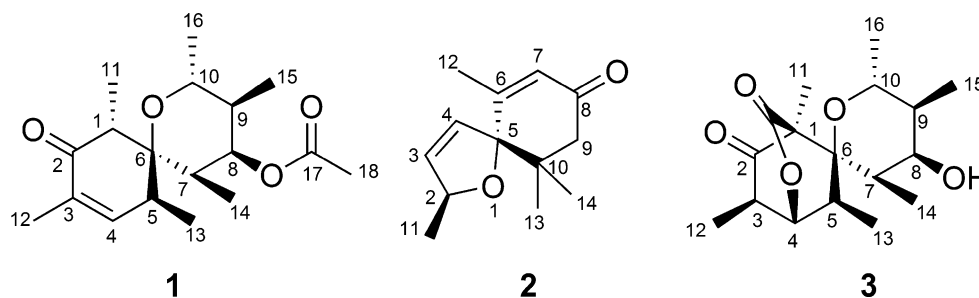
H. Morita (✉) · R. Mori · J. Deguchi · S. Oshimi · Y. Hirasawa
Faculty of Pharmaceutical Sciences, Hoshi University,
Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan
e-mail: moritah@hoshi.ac.jp

W. Ekasari · A. Widyawaruyanti
Faculty of Pharmacy, Airlangga University, Jalan
Dharmawangsa Dalam, Surabaya 60286, Indonesia

A. H. A. Hadi
Department of Chemistry, Faculty of Science,
University of Malaya, 50603 Kuala Lumpur, Malaysia

Table 1 ^1H and ^{13}C NMR data for decarboxyportentol acetate (**1**) and 3,4-dehydrotheaspirone (**2**) at 300 K

Position	δ_{H} [int., mult., J (Hz)]		δ_{C}	
	1 ^a	2 ^b	1 ^a	2 ^b
1	2.79 (1H, q, 6.8)		46.6	
2		4.32 (1H, dq, 4.2, 6.2)	200.5	68.7
3		5.75 (1H, m)	133.5	136.9
4	6.62 (1H, d, 6.6)	5.77 (1H, m)	147.4	130.1
5	3.37 (1H, dq, 6.6, 7.0)		33.9	79.9
6			80.7	168.0
7	2.09 (1H, dq, 2.9, 7.3)	5.87 (1H, m)	37.1	127.1
8	5.13 (1H, dd, 3.0, 2.9)		76.9	201.3
9a	1.56 (1H, m)	2.15 (1H, d, 17.4)	40.5	50.7
9b		2.52 (1H, d, 17.4)		
10	3.56 (1H, dq, 12.2, 6.3)		66.3	49.0
11	1.16 (3H, d, 6.8)	1.24 (3H, d, 6.2)	7.3	23.8
12	1.72 (3H, s)	1.91 (3H, s)	15.6	19.5
13	1.13 (3H, d, 7.0)	1.00 (3H, s)	14.7	24.4
14	0.62 (3H, d, 7.3)	2.52 (3H, s)	17.0	23.4
15	0.79 (3H, d, 6.9)		12.9	
16	1.14 (1H, d, 6.3)		19.0	
17			170.6	
18	2.10 (3H, s)		20.9	

 δ in ppm^a 600 MHz, CDCl_3 ^b 400 MHz, CD_3OD 

^1H - ^1H COSY, HSQC, and HMBC spectra in CDCl_3 (Fig. 1). The ^1H - ^1H COSY spectrum revealed connectivity of three partial structures **a** (C-1 and C-11), **b** (C-4 to C-5 and C-13), and **c** (C-7 to C-10, C-14, C-15, and C-16) as shown in Fig. 1. Connectivity of units **a** and **b** was implied by HMBC correlations for H_3 -11 to C-2 (δ_{C} 200.5) and H_3 -12 to C-2, C-3 (δ_{C} 133.5), and C-4 (δ_{C} 147.4). HMBC correlations were observed for H_3 -11, H-4, H-10 and H_3 -14 to C-6 (δ_{C} 80.7) suggesting that units **a**, **b**, and **c** connected through C-6. Judging from the ^{13}C chemical shift of C-6, **1** was deduced to have a spiro cyclic structure at C-6 incorporated in a tetrahydropyran ring. The presence of an acetate at C-8 (δ_{C} 76.9) was implied by the HMBC correlations for H-8 and H_3 -18 to C-17 (δ_{C} 170.6).

The relative configuration of **1** was elucidated by NOESY correlations and $^3J_{\text{H-H}}$ couplings as depicted in the computer-generated three-dimensional drawing (Fig. 2).

The NOESY correlation for H-7/H-9 and $^3J_{(\text{H-9}/\text{H-10})}$, $^3J_{(\text{H-7}/\text{H-8})}$, and $^3J_{(\text{H-8}/\text{H-9})}$ coupling values (12.2, 2.9, and 3.0 Hz, respectively) suggested that the tetrahydropyran ring took a chair form with axial orientations for H-7, H-9, and H-10, and equatorial orientation for H-8. The orientations of C-11 and C-13 and the relative configuration of C-6 were elucidated by NOESY correlations for H-1/ H_3 -13, H-7/ H_3 -11, and H-5/H-10, and the high field ^1H chemical shift (δ_{H} 0.62) of H_3 -14 by the anisotropic effect of C-3 to C-4 double bond. Thus, the relative configuration of **1** was assigned as in Fig. 2.

Portentol (**3**) was first isolated as the lichen constituent from *Rocella portentosa* [8]. Decarboxyportentol acetate (**1**) has been derived from portentol though decarboxyportentol followed by acetylation [8]. The absolute structure of **1** was also assigned to be the same as that derived from portentol [8]. Therefore, it is a first isolation from plants as a natural product.

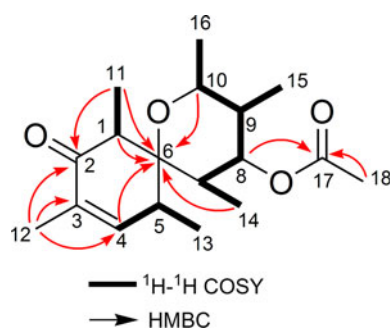


Fig. 1 Selected 2D NMR correlations for decarboxyportentol acetate (**1**)

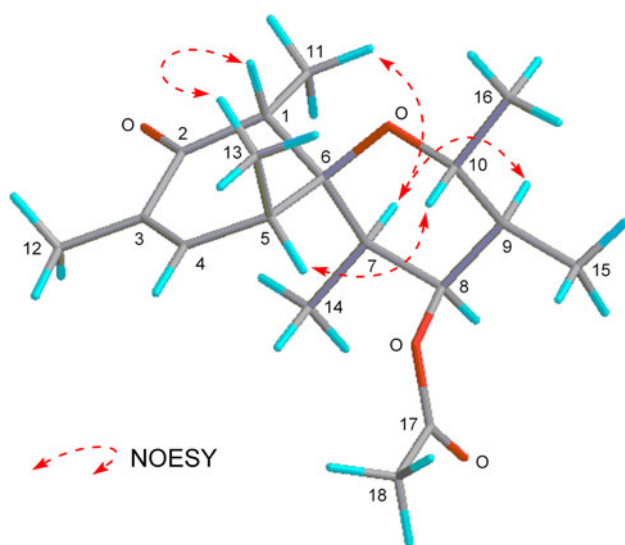


Fig. 2 Selected NOESY correlations and relative configuration for decarboxyportentol acetate (**1**)

3,4-Dehydrotheaspirone (**2**), colorless amorphous solid, $[\alpha]_D^{20} +155$ (c 1.0, MeOH), showed molecular formula, $C_{13}H_{18}O_2$, which was determined by HRESITOFMS [m/z 207.1376 ($M + H$) $^+$, $\Delta -0.9$ mmu]. IR absorptions implied the presence of carbonyl (1671 cm^{-1}) and ether (1249 cm^{-1}) functionalities. ^1H and ^{13}C NMR data are presented in Table 1. The ^1H NMR spectrum of **2** was similar to that of excoecariol B [9] except for an oxymethylene signal at the low field region (δ_{H} 4.08 and 4.23) in place of a methyl signal. By using spectroscopic analysis, **2** was assigned as 3,4-dehydrotheaspirone [10], which was recently isolated from the leaves of *Juniperus brevifolia* [11].

Decarboxyportentol acetate (**1**) and 3,4-dehydrothespirone (**2**) showed potent in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7 (IC_{50} **1**: 16 μM , IC_{50} **2**: 0.027 μM). In particular, 3,4-dehydrothespirone (**2**) did not show potent cytotoxicity against HL-60 cells (IC_{50} **1**: >100 μM , IC_{50} **2**: 2.7 μM). 3,4-Dehydrotheaspirone (**2**) had a high selectivity index (>100) and may have potential

as an antiplasmodial agent. Further studies on **2** including the mode of action of its antiplasmodial activity are under investigation.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO P-1030 polarimeter. Mass spectra were obtained with a Micromass LCT spectrometer. IR spectra were recorded on a JASCO FTIR-230 spectrometer and UV spectra on a Shimadzu UV-250 spectrophotometer. CD spectra were measured on a JASCO J-820 polarimeter. ^1H and 2D NMR spectra were recorded on a 600 and 400 spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Each NMR sample was prepared by dissolving in 100 μL of CDCl_3 and CD_3OD in 2.5 mm micro cells (Shigemi Co. Ltd.) and chemical shifts were reported using residual CHCl_3 (δ_{H} 7.26 and δ_{C} 77.0) and CH_3OH (δ_{H} 3.31 and δ_{C} 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. COSY and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1 K data points for each of 256 t_1 increments. NOESY spectra in the phase-sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HSQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. For HMBC spectra with Z axis PFG, a 50-ms delay time was used for long-range C–H coupling. Zero-filling to 1 K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. Merck silica gel 60 (40–60 μm) and Cosmosil 140C $_{18}$ -OPN were used for the column chromatography. Waters Sunfire ODS Pro C18 (5 μm , 10 \times 250 mm) column was used for HPLC analysis.

Plant material

The barks of *Laumoniera bruceadelpha* were collected at Mersing, Malaysia in 2001. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. Voucher specimens (KL4099) are deposited in the Herbarium of Chemistry Department, University of Malaya.

Extraction and isolation

The barks of *L. bruceadelpha* (1.4 kg), were extracted with MeOH, and a part (40 g) of the extract (126 g) was

suspended in H₂O and then partitioned between CHCl₃ and *n*-BuOH, successively. The CHCl₃-soluble materials were subjected to a silica gel column (CHCl₃/MeOH, 1:0 → 0:1), in which a fraction eluted by CHCl₃/MeOH (1:0) was further purified on an ODS HPLC with 40% MeOH to afford decarboxyportentolacetate (**1**, 2.2 mg). A fraction eluted by CHCl₃/MeOH (40:1) was further purified on an ODS column with MeOH/H₂O (3:7 → 1:0) followed by an ODS HPLC with MeOH/H₂O (2:3) to afford 3,4-dehydrotheaspirone (**2**, 2.3 mg).

Decarboxyportentol acetate (**1**): colorless amorphous solid, $[\alpha]_D^{20} +58$ (*c* 0.5, CHCl₃); IR (CHCl₃) ν_{\max} 1739, 1653, and 1242 cm⁻¹; UV (MeOH) λ_{\max} 240 (ϵ 1298) nm; CD (MeOH) λ_{\max} 238 ($\Delta\epsilon$ 0.59) nm; ¹H and ¹³C NMR (Table 1); ESIMS (pos.) *m/z* 331 (M + Na)⁺; HRESI-TOFMS (pos.) *m/z* 331.1861 (M + Na)⁺, calcd. for C₁₈H₂₈O₄Na, 331.1885.

3,4-Dehydrotheaspirone (**2**): colorless amorphous solid, $[\alpha]_D^{20} +155$ (*c* 1.0, MeOH); IR (CHCl₃) ν_{\max} 1671 and 1249 cm⁻¹; UV (MeOH) λ_{\max} 235 (ϵ 8870) nm; CD (MeOH) λ_{\max} 204 ($\Delta\epsilon$ 2.29) nm; ¹H and ¹³C NMR (Table 1); ESIMS (pos.) *m/z* 207 (M + H)⁺; HRESI-TOFMS (pos.) *m/z* 207.1376 (M + H)⁺, calcd. for C₁₃H₁₉O₂, 207.1385.

Antiplasmodial activity

Human malaria parasites were cultured according to the method of Trager and Jensen [12]. The antimalarial activity of the isolated compounds was determined by the procedure described by Budimulja et al. [13]. In brief, stock solutions of the samples were prepared in DMSO (final DMSO concentrations of <0.5%) and were diluted to the required concentration with complete medium (RPMI 1640 supplemented with 10% human plasma, 25 mM HEPES and 25 mM NaHCO₃) until the final concentrations of samples in culture plate wells were 10, 1, 0.1, 0.01, and 0.001 μg/ml. The malarial parasite *P. falciparum* 3D7 clone was propagated in 24-well culture plates. Growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain. The antimalarial activity of each compound was expressed as an IC₅₀ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control (*n* = 2).

The percentage of growth inhibition was expressed according to following equation:

$$\text{Growth inhibition \%} = 100 - \left[\frac{\text{(test parasitaemia / control parasitemia)} \times 100 \right]. \text{Chloroquine : IC}_{50} 0.011 \mu\text{M}.$$

Cytotoxic activity

HL-60 human promyelocytic leukemia cells were maintained in RPMI-1640 medium. The growth medium was supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The cells (5 × 10³ cells/well) were cultured in Nunc disposable 96-well plates containing 90 μl of growth medium per well and were incubated at 37°C in a humidified incubator of 5% CO₂. 10 μl of samples were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, 15 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml) were added to each of the wells. The cultures were incubated for another 3 h before the cell supernatants were removed. After the removal of the cell supernatants, 50 μl of dimethyl sulfoxide (DMSO) was added to each well. The formazan crystal formed was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad, USA) at 550 nm with reference wavelength at 700 nm. In all experiments, three replicates were used. Cisplatin was used as positive control (IC₅₀: 0.87 μM for HL-60).

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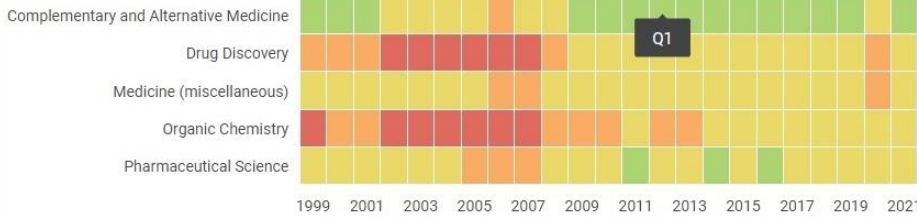
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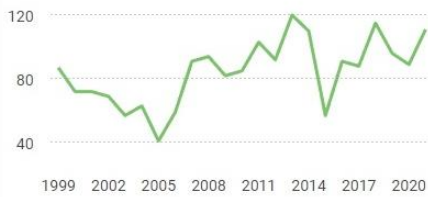
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