Anthraquinones with Antiplasmodial Activity from the Roots of *Rennellia elliptica* Korth. (Rubiaceae)

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Received: 24 August 2010; in revised form: 6 October 2010 / Accepted: 11 October 2010 / Published: 20 October 2010

**Abstract:** Dichloromethane root extract of *Rennellia elliptica* Korth. showed strong inhibition of *Plasmodium falciparum* growth *in vitro* with an IC$_{50}$ value of 4.04 µg/mL. A phytochemical study of the dichloromethane root extract has led to the isolation and characterization of a new anthraquinone, 1,2-dimethoxy-6-methyl-9,10-anthraquinone (1), and ten known anthraquinones: 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (2), nordamnacanthal (3), 2-formyl-3-hydroxy-9,10-anthraquinone (4), dammacanthal (5), lucidin-ω-methyl ether (6), 3-hydroxy-2-methyl-9,10-anthraquinone (7), rubiadin (8), 3-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (9), rubiadin-1-methyl ether (10) and 3-hydroxy-2-hydroxymethyl-9,10-anthraquinone (11). Structural elucidation of all compounds was accomplished by modern spectroscopic methods, notably 1D and 2D NMR, IR, UV and HREIMS. The new anthraquinone 1, 2-formyl-3-hydroxy-9,10-anthraquinone (4) and 3-hydroxy-2-methyl-9,10-anthraquinone (7) possess strong antiplasmodial activity, with IC$_{50}$ values of 1.10, 0.63 and 0.34 µM, respectively.

**Keywords:** anthraquinone; *Rennellia elliptica*; antiplasmodial; Rubiaceae
1. Introduction

Rennellia elliptica Korth. (Rubiaceae) is a tropical shrub locally known in Malaysia as Segemuk or Mengkudu Rimba. This plant is widely distributed, along riverbanks or lowland forest, in Peninsular Malaysia, Sumatra and Borneo [1]. Decoctions of the root are taken by the locals for various purposes, including as an aphrodisiac, for body aches and as a post natal tonic [2]. Not much has been reported on the chemical constituents or biological properties of this plant. A preliminary study by Yusoff et al. on the roots of the plant reported one anthraquinone compound [3]. Our screening of some Malaysian plant extracts for antiplasmodial activity showed that the dichloromethane roots extract of *R. elliptica* is a potential source of antiplasmodial compounds (IC$_{50}$ = 4.04 µg/mL). This paper reports the isolation, structure elucidation and antiplasmodial activity of a series of anthraquinone compounds, including a new one, from the root of *R. elliptica*.

Figure 1. Anthraquinones 1-11, isolated from roots of *R. elliptica* Korth.

| Position | R$_1$      | R$_2$      | R$_3$ | R$_4$ |
|----------|------------|------------|-------|-------|
| 1        | OCH$_3$    | OCH$_3$    | H     | CH$_3$|
| 2        | OH         | OCH$_3$    | H     | CH$_3$|
| 3        | OH         | CHO        | OH    | H     |
| 4        | H          | CHO        | OH    | H     |
| 5        | OCH$_3$    | CHO        | OH    | H     |
| 6        | OH         | CH$_2$OCH$_3$ | OH | H     |
| 7        | H          | CH$_3$    | OH    | H     |
| 8        | OH         | CH$_3$    | OH    | H     |
| 9        | H          | OCH$_3$   | OH    | CH$_3$|
| 10       | OCH$_3$    | CH$_3$    | OH    | H     |
| 11       | H          | CH$_2$OH  | OH    | H     |

2. Results and Discussion

Eleven anthraquinones (Figure 1) were isolated from roots of *R. elliptica* after extensive chromatographic separation and were characterized by analysis of spectroscopic data and by comparison with literature values [4-13]. The structures of compounds 4 and 6 were also confirmed using x-ray crystallography [14,15]. The compound 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (2) was described by Mittie and Biswas in 1928 [16]. However, since then there have been no further reports on the natural occurrence of this compound and there is no spectroscopic data
available in the literature for comparison. Hence, we elucidated the structure by careful analysis of MS, IR, UV and NMR data, followed by confirmation through x-ray crystallography [17]. In this paper we include the full spectroscopic data for this compound.

The new compound, 1,2-dimethoxy-6-methyl-9,10-anthraquinone (1) was obtained as bright yellow amorphous solid. The HREIMS displayed a [M + H]^+ peak at 283.0968 [calc 283.3067] suggesting a molecular formula of C_{17}H_{14}O_{4}. The absorption maxima in the UV spectrum were observed at 373, 341 and 257 nm, indicative of an anthraquinone moiety [18]. The IR spectrum did not show presence of chelated carbonyl and hydroxyl groups. The sp^2 C-H stretch for the aromatic ring was observed at 3,081 cm^{-1}. With the exception of the sharp singlet in the downfield region for the hydrogen-bonded hydroxyl group, the ^1H-NMR spectrum resembles that of compound 2, suggesting a similar substitution pattern. Splitting pattern of the five aromatic proton signals suggested substitutions on both rings. Two overlapping doublets centered at δ_H 8.17 are due to H-8 (d, J = 7.8 Hz) and H-4 (d, J = 8.7 Hz), the peri-hydrogens. A doublet at δ_H 7.28 (J = 8.7 Hz) is due to H-3, meanwhile H-7 gave another doublet of doublet at δ_H 7.58 (J_o = 7.8 Hz, J_m = 1.7 Hz). These assignments were confirmed by their respective correlations in the COSY spectrum. H-5 resonated as a singlet at 8.06 ppm. In addition, two sharp singlets at δ_H 2.53 (3H, s) and 4.02 (6H, s) due to a methyl and two methoxy groups, respectively, were also observed.

The location of the methoxy groups were established at C-1 and C-2 of ring C based on its NOE correlation with H-3. Thus, the only possible location for the methyl substituent is at C-6. This assignment was confirmed through NOE correlations of the methyl group with H-5 and H-7. These NOE correlations are illustrated in Figure 2. The placement of methyl group at C-6 was further confirmed by HMBC experiment which showed a ^3J correlation with H-7. The methine carbons (C-3, C-4, C-5, C-7 and C-8) were assigned through HMQC correlations while the quaternary carbons (C-1, C-2, C-6, C-11, C-12, C-13 and C-14) were assigned based on careful analysis of HMBC spectrum. Both carbonyl carbons in this compound resonated very closely to each other with only 0.01 ppm difference at δ_C 182.70 and 182.71, which further confirmed the unchelated nature of the carbonyls.

Close inspection of all spectroscopic data confirmed that compound 1 is 1,2-dimethoxy-6-methyl-9,10-anthraquinone, the 1-methyl ether of compound 2. Complete ^1H and ^13C data of compounds 1 and 2 are presented in Table 1.

**Figure 2. NOESY Correlations of 1.**
Table 1. ¹H- (300 MHz) and ¹³C- (75.5 MHz) NMR Data of Compounds 1 and 2 in CDCl₃.

| Position | Compound 1 | | | Compound 2 | | |
|---|---|---|---|---|---|---|
| | δ_H | δ_C | HMBC | | δ_H | δ_C | HMBC |
| 1 | - | 159.1 | - | | 154.0 | - | |
| 2 | - | 149.6 | - | | 152.7 | - | |
| 3 | 7.28, 1H, _d_, J = 8.7 Hz | 159.9 | C-2, C14 | 7.19, 1H, _d_, J = 8.4 Hz | 154.0 | C-2, C-14 |
| 4 | 149.6 | C-3, C-10,C-13, C-14 | 7.89, 1H, _d_, J = 8.4 Hz | 152.7 | C-3, C-10,C-13, C-14 |
| 5 | 8.06, 1H, s | 126.9 | C-7, C-10, C-11 | 8.12, 1H, s | 127.8 | C-6-C9, C-10-C-11 |
| 6 | - | 144.6 | - | | 146.2 | - | |
| 7 | 7.58, 1H, _dd_, J_o = 7.8 Hz, J_m = 1.7 Hz | 134.7 | 6-CH₃, C-8, C-12 | 7.61, 1H, _d_, J = 8.1 Hz | 134.6 | 6-CH₃, C-6,C-8 |
| 8 | 8.17, 1H, d, J = 7.8 Hz | 127.0 | C-6, C-9 | 8.23, 1H, _d_, J = 8.1 Hz | 127.1 | C-7,C-9, C-12 |
| 9 | - | 182.7 | - | | - | 189.1 | - |
| 10 | - | 182.7 | - | | - | 181.8 | - |
| 11 | - | 132.9 | - | | - | 134.0 | - |
| 12 | - | 132.9 | - | | - | 131.1 | - |
| 13 | - | 127.4 | - | | - | 116.1 | - |
| 14 | - | 127.5 | - | | - | 125.5 | - |
| 1-OH | - | - | - | | 13.20, 1H, s | - | - |
| 1-OCH₃ | 4.02, 3H, s | 61.3 | C-1 | - | - | - |
| 2-OCH₃ | 4.02, 3H, s | 56.3 | C-1,C-2,C-3 | 4.04, 3H, s | 56.4 | C-1,C-3 |
| 6-CH₃ | 2.53, 3H, s | 21.8 | C-5,C-6,C-7 | 2.56, 3H, s | 22.0 | C-5,C-6,C-7 |

Anthraquinones isolated from the root of _R. elliptica_ exhibited the typical substitution pattern of _Rubia_ type anthraquinones, with most of them substituted only on ring C [19]. The anthraquinones from _Morinda_ and _Prismatomeris_ spp. in the same family also exhibited a similar substitution pattern [4-9,12,13,20-22]. Genus _Rennellia_ is classified in the same tribe as _Morinda_ and _Prismatomeris_ [23], so the anthraquinones may be produced through similar biosynthetic pathways, which explains the similarity in the substitution patterns [19]. However, anthraquinones 1, 2 and 9 which are substituted on both rings have a methyl substitution at C-6, differing from the anthraquinones of _Prismatomeris_ and _Morinda_ which are typically hydroxyl or methoxy substituted at C-6.

We screened the anthraquinones isolated from the roots of _R. elliptica_ for antiplasmodial activity based on the promising screening results of dichloromethane crude extract (IC₅₀ = 4.04 µg/mL). The _in vitro_ antiplasmodial activity of anthraquinones isolated from _R. elliptica_ against a chloroquine sensitive strain of _P. falciparum_ (3D7) is shown in Table 2. Compound 7 displayed the strongest inhibition activity, with an IC₅₀ value of 0.34 µM, followed by compound 4 with an IC₅₀ value of 0.63 µM. Sittie _et al._ established that an aldehyde group at C-2 and a phenolic hydroxy group at C-3 on the anthraquinone skeleton enhance the activity of anthraquinones against the growth of _P. falciparum_ [24]. Our results show that methyl group at C-2 together with phenolic hydroxy group at C-3 in compound 7 also gave significant activity. It should also be noted that both compounds 4 and 7 do not possess hydroxyl substituents at the peri positions. The new anthraquinone 1 also exhibited strong inhibition, with an IC₅₀ value of 1.1 µM. Interestingly, anthraquinone 2, which structurally differs only at C-1 (hydroxyl substituent instead of methoxy substituent) did not show any significant activity. The position of substituents on anthraquinone skeleton clearly influences the antiplasmodial activity, which warrants further investigation. We are currently synthesizing anthraquinone derivatives with various substitution patterns for the purpose of establishing structure-activity relationships.
Table 2. Antimalarial Activities of Anthraquinones from R. elliptica Korth.

| Sample | IC₅₀ (µM) |
|--------|-----------|
| 1      | 1.10      |
| 2      | na†       |
| 3      | 72.46     |
| 4      | 0.63      |
| 5      | 51.28     |
| 6      | 2.10      |
| 7      | 0.34      |
| 8      | na†       |
| 9      | nt‡       |
| 10     | na†       |
| 11     | nt‡       |

Chloroquine diphosphate 6.30*  

Each sample was tested in duplicate; The IC₅₀ values were obtained from average values of percent inhibition within a series of concentration; Notes: na†–no activity; nt‡–not tested; * unit in nM.

3. Experimental

3.1. Instrumentation

¹H- and ¹³C-NMR spectra were run on a Bruker 300 Ultrashield NMR (Switzerland) at 300 and 75.5 MHz, respectively, using CDCl₃ or acetone-d₆ (Merck) as solvent. Chemical shifts are reported in ppm and δ scale with the coupling constants given in Hz. Melting points were determined using a Hitotek X-4 (China) melting point apparatus equipped with a microscope and are uncorrected. HREIMS spectra were obtained on a Thermo Finnigan Automass Multi (Shimadzu, Japan). IR spectra were obtained using Perkin-Elmer 1600 series FTIR spectrometer (USA) using KBr pellets. UV spectra were recorded on Shimadzu UV-160A spectrometer (Japan) in absolute ethanol (Scharlau) and alkaline ethanol.

3.2. Chemicals and Reagents

Column chromatography was carried out using silica gel (silica gel 60, 230-400 mesh, Merck, Germany). n-Hexane, dichloromethane and methanol for column chromatography were freshly distilled from industrial grade solvents. The fractions collected were monitored using analytical TLC (Merck, Germany), pre-coated with silica gel 60 F₂₅₄ of 0.25 mm thickness and visualized under UV light at 245 nm and 356 nm. PTLC was carried out using pre-coated plate with PSC-Fertigplatten Kieselgel 60 F₂₅₄ (1.0 mm thickness, 20 × 20 cm) purchased from Merck. Plasmodium falciparum (3D7) strain was used for in vitro antiplasmodial tests. All chemicals used for determination of antiplasmodial activity were purchased from Sigma-Aldrich, unless otherwise stated.

3.3. Plant Material

The roots of R. elliptica were collected from Kuala Keniam, National Park, Pahang, Malaysia at altitude 165 m above sea level and were identified by Dr Shamsul Khamis, Universiti Putra Malaysia.
The voucher specimens (SK1512/08) were deposited at Herbarium of Institute of Bioscience, Universiti Putra Malaysia and Universiti Teknologi MARA.

3.4. Extraction and Isolation of Anthraquinones

The powdered air-dried roots of *R. elliptica* (1 kg) were successively extracted with hexane, dichloromethane and methanol (5 L each) at room temperature for 72 hours. The filtrate was concentrated *in vacuo* to give 27 g of a brown coloured residue. The dichloromethane crude extract (9 g) was pre-mixed with silica (1:1) and introduced onto a packed column (5 cm × 60 cm) of acid-washed silica gel (previously shaken with 2% oxalic acid for 2 hours, filtered and dried at 90 °C). The column was eluted gradiently using compositions of solvents of increasing polarity (*n*-Hex-CH$_2$Cl$_2$, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 100 CH$_2$Cl$_2$ v/v; CH$_2$Cl$_2$-MeOH, 99:1, 95:5, 9:1 v/v). Fractions of 200 mL each were collected and combined based on TLC pattern into eight major fractions (A to H) for further separation procedures.

Compound 3 (92 mg) was obtained upon recrystallization of fraction A from CH$_2$Cl$_2$. The golden yellow precipitate from fraction C was recrystallized from CH$_2$Cl$_2$ to yield crystals of 4 (42 mg). The remaining liquid from fraction C was dried and subjected to column chromatography (15 mm × 330 mm) eluted isocratically with CH$_2$Cl$_2$ to give 2 (7 mg) and 5 (20 mg). Fraction D was purified using PTLC (100% CH$_2$Cl$_2$) to furnish 6 (23 mg). Compound 7 (20 mg) was purified from fraction E after recrystallization of the dark yellow precipitate from CH$_2$Cl$_2$. The remaining portion of fraction E was rechromatographed using a small column (15 mm × 330 mm) eluted with a gradient of CH$_2$Cl$_2$-MeOH (100, 99:1, 98:2, v/v) to give 50 fractions. Repeated PTLC (CH$_2$Cl$_2$-MeOH, 99:1, v/v) of these fractions afforded compounds 1 (5 mg), 8 (32 mg) and 9 (7 mg). The pale yellow precipitate from fraction F was redissolved in CH$_2$Cl$_2$ and MeOH. Purification of the MeOH soluble portion using repeated PTLC (CH$_2$Cl$_2$-MeOH, 97:3, v/v) yielded 10 (23 mg). Compound 11 (5 mg) was obtained upon recrystallization of light yellow precipitate from CH$_2$Cl$_2$ of fraction F. Spectral data for compounds 3-9 are in good agreement with published data [4-13].

1,2-Dimethoxy-6-methyl-9,10-anthraquinone (1). Bright yellow amorphous solid (CH$_2$Cl$_2$); m.p. 193-196 °C. UV $\lambda_{max}$ (EtOH): 373, 341, 257 nm; IR $\nu_{max}$ (KBr): 3,081, 2,945, 1,666, 1,601 cm$^{-1}$; HREIMS 283.0968 [M + H]$^+$ (calc for C$_{17}$H$_{14}$O$_4$ 282.3067); For $^1$H-NMR (CDCl$_3$) and $^{13}$C-NMR (CDCl$_3$) data, see Table 1.

1-Hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (2). Red needles (CH$_2$Cl$_2$); m.p. 220-221 °C. UV $\lambda_{max}$ (EtOH): 421, 278, 262 nm; IR $\nu_{max}$ (KBr): 3,467, 1,653, 1,637 cm$^{-1}$; HREIMS 269.0867 [M + H]$^+$ (calc for C$_{16}$H$_{12}$O$_4$ 268.2796); For $^1$H-NMR (CDCl$_3$) and $^{13}$C-NMR (CDCl$_3$) data, see Table 1.

3.5. Determination of Antiplasmodial Activity

The antiplasmodial activity of dichloromethane extract and the isolated compounds were determined by methods as previously described [25]. The samples were dissolved in DMSO and kept at -20 °C until used. The malarial parasite *P. falciparum* (3D7) clone was propagated in a 24-well culture plate in the presence of 10, 1, 0.1, 0.01 and 0.001 µg/mL range of concentrations of each
compound. Chloroquine diphosphate was used as positive control. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa (Merck). The antiplasmodial activity of each compound was expressed as an IC$_{50}$ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

4. Conclusions

A phytochemical study on dried roots of R. elliptica afforded a new anthraquinone, 1,2-dimethoxy-6-methyl-9,10-anthraquinone (1), together with ten known anthraquinones 2-11. Several anthraquinones strongly inhibited in vitro growth of a chloroquine sensitive strain of Plasmodium falciparum (3D7), with the strongest inhibition shown by compound 7. The antiplasmodial data suggested that the roots extract of R. elliptica is a potential source of new antiplasmodial agents.

Acknowledgements

The authors would like to thank Ministry of Higher Education and Universiti Teknologi MARA for financial support (FRGS grant 600-IRDC/ST/FRGS/TN.5/3/2) and Shamsul Khamis from Universiti Putra Malaysia for plant identification. Special thanks to Aty Widyawaruyanti from Department of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, University of Airlangga, Indonesia for determination of antiplasmodial activities. Che Puteh Osman thanks Universiti Teknologi MARA for scholarship awarded to her.

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*Sample Availability:* Samples of the compounds are available from the authors.

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