

ANTHRAQUINONES FROM *MORINDA ELLIPTICA*

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Key Word Index *Morinda elliptica*; Rubiaceae; anthraquinone; 2-formyl-1-hydroxy-anthraquinone.

Abstract Phytochemical studies on roots of *Morinda elliptica* have resulted in the isolation of a new anthraquinone, 2-formyl-1-hydroxyanthraquinone, and 10 known anthraquinones. The structures of the anthraquinones were established based on spectral studies. © 1997 Elsevier Science Ltd. All rights reserved

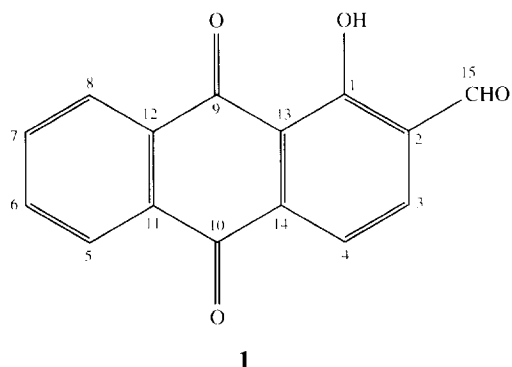
INTRODUCTION

Morinda elliptica L. is a shrub or small tree, growing wild in newly developed areas or in bushes throughout the Malay Peninsula. Traditionally, different parts of the plant are used in various ways for a number of health problems and ailments including loss of appetite, headaches, cholera, diarrhoea, fever and haemorrhoids [1].

RESULTS AND DISCUSSION

A new anthraquinone (**1**) along with 10 known ones, 1-hydroxy-2-methylantraquinone, nordanacanthal, damnacanthal, lucidin-*o*-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-1-methyl ether, alizarin-1-methyl ether and morindone-5-methyl ether, were isolated from roots of *M. elliptica*.

The new anthraquinone (**1**) ($R_f = 0.66$, silica gel, CHCl_3) gave a molecular ion peak at m/z 252.0414 (HR-MS) corresponding to the molecular formula $\text{C}_{15}\text{H}_8\text{O}_4$ (calculated for $\text{C}_{15}\text{H}_8\text{O}_4$ 252.0422). The UV-vis spectrum showed absorption maxima at 229, 278, 331 and 407 nm. These were suggestive of a 9,10-anthraquinone structure. A bathochromic shift (407 to 531 nm) on adding NaOH suggested the presence of OH at C-1. This was further confirmed by the IR spectrum, which showed bands for a chelated carbonyl at 1638 cm^{-1} , besides the absorption for free carbonyl (1676 cm^{-1}) groups. In addition, the ^1H NMR spectrum showed the characteristic downfield signal for the chelated hydroxyl group at δ 13.26 (1H,



s). The ^1H NMR spectrum also revealed the presence of an aldehyde proton at δ 10.63 (1H, s) and six aromatic protons. The ^{13}C NMR showed 15 carbon atoms which include three carbonyl carbons (downfield signals at δ 181.8, 188.0, and 188.9).

The exact location of the substituents was established after careful examination of the NMR pattern of the aromatic protons using correlation experiments, FGHMQC and FGHMBC. There were five signals in the aromatic region and the pattern suggested that ring A of the anthraquinone moiety was unsubstituted. Two sets of multiplets centred at δ 8.35 and 8.32, integrating for one proton each, were attributed to H-8 and H-5, respectively. Another set of multiplets at δ 7.88, integrating for two protons, was assigned to H-6 and H-7. This left two aromatic protons, a hydroxyl and a formyl group, to be accounted for as substituents on ring C. A set of *ortho* coupled doublets at δ 8.23 (1H, $J = 8.0\text{ Hz}$) and 7.89 (1H, $J = 8.0\text{ Hz}$) indicated that the two aromatic protons were next to each other. This ruled out the possi-

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bility of placing the formyl group at C-3, since the position of hydroxyl group had already been established at C-1.

A FGHMQC experiment through carbon-proton coupling, identified the formyl carbon (C-15 (δ 188.0) as well as C-5 (δ 127.7), C-8 (δ 127.2), C-6 (δ 134.7) and C-7 (δ 135.3). The proton giving the signal at δ 8.23 was attached to the carbon giving the ^{13}C NMR signal at δ 135.4 and similarly the proton giving the signal at δ 7.89 was attached to the carbon giving the ^{13}C NMR signal at 118.7 ppm.

The ^{13}C NMR assignments for C-1, C-2 and C-13 were accomplished through an FGHMBC experiment where the hydroxyl proton was seen to correlate with peaks at δ 164.5 (C-1), 128.4 (C-2) and 117.4 (C-13). A carbon-proton coupling between the formyl proton and C-1 (164.5) was observed, confirming that the formyl group was located next to the hydroxyl group. The signal for H-3 was assigned at δ 8.23 based on its correlation in the FGHMBC spectra with C-15 (δ 188.0), C-1 (δ 164.5) and C-14 (δ 137.2), all of which are three bonds away from H-3. This gave the final assignment for H-4 at δ 7.89 which agreed well with its correlation with C-2 (δ 128.4), C-14 (δ 117.4) and C-10 (δ 181.8). This allowed us to support the assignment of the formyl group at C-2. Compound **1** was, therefore, 2-formyl-1-hydroxyanthraquinone, a new naturally occurring anthraquinone derivative. The structure of other known compounds were confirmed by comparison with literature values.

EXPERIMENTAL

General. Mps: uncorr; MS: Finnigan Mat SSQ 710 spectrometer, 70 eV; HR-MS: Jeol JMS HX-110A; ^1H and ^{13}C NMR: 500 and 125.65 MHz, respectively, in CDCl_3 or $\text{DMSO}-d_6$ as solvent. CC: acid washed silica gel (Merck 7734) and silica gel (Merck 9385), eluates monitored by analytical TLC; Prep and analytical TLC: pre-coated PLC plates silica gel 60 F_{254} 2.0 mm thickness and Merck DC-Plastikfollen 60 F_{254} , respectively. The compounds were visualized by UV at 254 and 366 nm. Prep HPLC: Waters PrepPak cartridge 25×100 mm, with $\mu\text{Bondapak C18}$ 10 μm 125 Å packing.

Plant material. Roots of *Morinda elliptica* were collected from Port Dickson, Negeri Sembilan, and identified by Mr Anthonysamy Sivarimuthu of the Department of Biology. A voucher specimen was deposited in the herbarium of the Department of Biology, Universiti Pertanian Malaysia.

Extraction. The ground air-dried roots of *M. elliptica* (12 kg) were soaked at room temp. in CH_2Cl_2 for 36 hr. Solvent removal left a brown coloured residue (142 g). A portion of this crude extract (26 g) was introduced into a packed column (10 cm $\times$ 50 cm) of acid-washed silica gel (previously shaken with 4% oxalic acid for 30 min, filtered and dried at 90 °C). The column was eluted with petrol, CH_2Cl_2 , and CH_2Cl_2 enriched with increasing percentages (1, 2, 5 and 10%)

of MeOH. Frs of 150 ml each were collected and combined (based on TLC pattern) into six major quinone-containing frs for further sepn procedures.

Isolation of anthraquinones. Fr. A (1–6) was rechromatographed using a smaller column (3.5 cm $\times$ 30 cm) eluted with a mixture of CH_2Cl_2 –petrol 2:3. 1-Hydroxy-2-methylanthraquinone (95.5 mg) was isolated after purification by prep TLC developed in the same solvent. The latter frs of the same column were sepd by prep TLC developed in CHCl_3 . Recovery of the yellow-orange band followed by recrystallization from CHCl_3 gave 80.2 mg of the new anthraquinone (**1**) as orange crystals, mp 183–185 °C. Nordamnacanthal, mp 214–218 °C (1.02 g), was the major component of fr. B (7–12) and was isolated after recovery of the orange band from CC on acid-washed silica gel eluted with CHCl_3 . Chromatotron sepn of the yellow band gave more of the new anthraquinone (**1**) and 196.4 mg of damnacanthal. The last orange band was purified on a smaller column followed by prep TLC developed in CHCl_3 and Me_2CO (9:1) to yield 56.6 mg of lucidin- ω -methyl ether. Fr. C (13–27) consisted of mainly morindone, and nordamnacanthal.

The components of fr. D (28–42) were sepd by prep RP-HPLC on a C-18 column using MeOH– H_2O as the solvent system. Recovery of the two collected peaks gave 86 mg of alizarin-1-methyl ether and a mixt. of rubiadin (143 mg) and soranjidiol (165 mg). This mixt. was sepd by repetitive prep TLC. Fr. E (42–92) was column chromatographed as before (eluted with CHCl_3 containing increasing amounts of MeOH). Fr. 15–19 of this column were recrystallized from hot Me_2CO giving 806 mg of rubiadin-1-methyl ether. Frs 6–9 were subjected to HPLC sepn to give more rubiadin-1-methyl ether, rubiadin, soranjidiol, and another anthraquinone, morindone-5-methyl ether (65 mg).

2-Formyl-1-hydroxyanthraquinone (1). Orange crystals, mp 183–185 °C (CHCl_3); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 229, 278, 331, 407; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 229, 280, 308, 531; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3448, 1696, 1676, 1638, 1592; EIMS (probe) 70 eV m/z (rel. int.): 252 [$\text{M}]^+$ (57), 224 (100), 196 (11), 168 (30); ^1H NMR (CDCl_3 , 500 MHz) δ : 13.26 (1H, s, 1-OH), 10.63 (1H, s, CHO), 8.35 (1H, m, H-8), 8.32 (1H, m, H-5), 8.23 (1H, d, J = 8.0 Hz, H-3), 7.89 (1H, d, J = 8.0 Hz, H-4), 7.88 (2H, m, H-6 and H-7); ^{13}C NMR (CDCl_3 , 125.65 MHz) δ : 164.5 (C-1), 128.4 (C-2), 135.4 (C-3), 118.7 (C-4), 127.7 (C-5), 134.7 & 135.3 (C-6 & C-7), 127.1 (C-8), 188.9 (C-9), 181.8 (C-10), 133.3 (C-11), 134.8 (C-12), 117.4 (C-13), 137.2 (C-14), 188.0 (C-15).

Known anthraquinones were identified by comparison of their spectroscopic data with literature values [2–5].

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