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Anthraquinones from Cell Suspension Culture of *Morinda elliptica*

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Abstract – The chemical investigation on the cell suspension culture of *Morinda elliptica* L. yielded eight anthraquinones, two of which, anthragallol-1,2-dimethyl ether (**3**) and purpurin-1-methyl ether (**4**), have not been isolated from the original plant. Other compounds isolated include nordamnacanthal (**1**), alizarin-1-methyl ether (**2**), rubiadin (**5**), soranjidiol (**6**), lucidin-ω-methyl ether (**7**), and morindone (**8**). The structures of anthraquinones were established based on spectral studies.
Key words – Anthraquinones, Cell Suspension Culture, *Morinda elliptica,* Rubiaceae.

Introduction

Morinda is a genus of the family Rubiaceae which grows as shrub or small tree. Various species are widely distributed in tropical Asia through out Malaysia, Indonesia and India, and they have been known to contain anthraquinone compounds (Thomson, 1971). *Morinda elliptica* L. is used traditionally for the treatment of a number of health problems and ailments including loss of appetite, headaches, cholera, diarrhoea, fever and haemorrhoids (Burkill and Haniff, 1930). Previous study on the roots of *M. elliptica* had resulted in the isolation of a new anthraquinone along with ten other known ones (Ismail *et al.*, 1997). Several of these anthraquinones have shown strong antimicrobial and cytotoxic activities.

Experimental

Cultivation of cell suspension cultures – Cell suspension culture of *M. elliptica* was established from callus cells which were initially induced from young leaves (Aziz *et al.*, 1997). Callus cultures were grown on MS (Murashige and Skoog, 1962) solid medium, added with 30 g/L sucrose, 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/L 6-furfurylaminopurine (kinetin) and 2.5 g/L phytogel (Sigma Chemicals Co.), under illumination of 2000 lux of white fluorescent lamp for 16 hrs photoperiod. Subculture of callus cells was made at a regular period of one month.

The cell suspension culture was developed by inoculating 3-5 g callus cells into the liquid medium. The cell suspension culture was subsequently subcultured every 6-8 days. A volume of 10 ml of cell suspension was inoculated into 90 ml of liquid medium in a 300 ml Erlenmeyer flask. Before subculture, all flasks containing medium were closed with aluminium foil and autoclaved at 121°C, for 15 minutes. The optimised medium formulation involved manipulation of culture age, inoculum age and inoculum preparation strategies, and utilising MS medium enriched with 80 g/L sucrose, 0.5 mg/L α-naphthaleneacetic acid (NAA) and 0.5 mg/L kinetin (Abdullah *et al.*, 1998). The cell suspension cultures were incubated at 27±3°C, on an orbital shaker at 135 rpm. After 18 days of incubation period, cells were harvested by suction filtration, dried in a draft oven at 70°C, for 24 hours, to obtain dry cell weight.

Extraction and isolation – The dried cells (250 g) were successively extracted with CH_2Cl_2 (3×500 ml) at room temperature, followed with MeOH using soxhlet apparatus for 12 hours. The CH_2Cl_2 and MeOH extracts were concentrated *in vacuo* to give residues of 2.8 and 16 g, respectively. The CH_2Cl_2 extract (2.5 g) was fractionated by column chromatography (40 cm×2.5 cm i.d.) on acid washed silica gel (previously shaken with 4% oxalic acid for 30 minutes, filtered and dried at 90°C). The column

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was eluted successively with petroleum ether followed by CH_2Cl_2, and then with CH_2Cl_2 enriched with MeOH in an increasing polarity manner. Fractions of 20 ml each were collected. The combined fraction B (8-24) was crystallized from $CHCl_3$ to give compound **1**. The combined fraction D (41-60) was rechromatographed and eluted with CH_2Cl_2 followed by CH_2Cl_2:MeOH mixture (in increasing polarity manner, collecting fractions of 20 ml each). The combined sub-fraction D2 (7-9) from this chromatography yielded compound **8** while the combined sub-fraction D3 (10-23) was subjected to preparative reversed phase HPLC on Waters Prep-Pak μBondapak C18 (10 μm) column using methanol:water as the solvent system to give compounds **2**, **3**, **4**, **5**, and **6**.

The MeOH extract (10 g) was partitioned with EtOAc:H_2O from which the EtOAc fraction (3.2 g) was further subjected to column chromatography (45 cm×3.5 cm i.d.) and successively eluted with petroleum ether, $CHCl_3$ and $CHCl_3$:MeOH (in increasing polarity) mixture. The fraction A (1-4) was rechromatographed on acid washed silica gel column (40 cm×2.0 cm i.d.) with $CHCl_3$:MeOH as the eluent, and further purified by preparative TLC to give compounds **7** and **8**.

Spectral analyses – UV spectra were recorded on Shidmadzu UV-VIS 160 and IR spectra on Perkin Elmer 1650 FTIR spectrometers. Mass spectra were recorded on Finnigan Mat SSQ 710 spectrometer with ionization induced by electron impact at 70 eV. ^1H and ^{13}C-NMR were recorded on JEOL GX-500 spectrometer.

Compound 1 – bright orange crystals; m.p. 230-232°; UV λ_{max} (MeOH): 205, 246, 271, 344 and 472 nm; UV λ_{max} (MeOH/$^-$OH): 206, 245, 272, 346 and 472 nm; IR ν_{max} (KBr): 3431, 1679, 1651, 1635 and 1592 cm^{-1}; EI-MS m/z (rel. int.): 268 (M^+, 70), 240 (100) and 212 (33); ^1H-NMR ($CDCl_3$, 500 MHz) δ ppm: 14.06 (1H, s, 1-OH), 12.68 (1H, s, 3-OH), 10.51 (1H, s, 2-CHO), 8.33 (1H, dd, J_m = 2.1 Hz, J_o = 7.0 Hz, H-8), 8.29 (1H, dd, J_m = 2.0 Hz, J_o = 6.9 Hz, H-5), 7.84 (2H, m, H-6, H-7), 7.34 (1H, s, H-4).

Compound 2 – yellow-orange crystals; m.p. 166-168°; UV λ_{max} (MeOH): 206, 247, 269 and 382 nm; λ_{max} (MeOH/$^-$OH): 206, 249, 313 and 477 nm; IR ν_{max} (KBr): 3442, 2930, 1668 and 1591 cm^{-1}; EI-MS m/z (rel. int.): 254 (M^+, 62), 236 (22), 208 (100); ^1H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.27 (2H, m, H-5, H-8), 8.14 (1H, d, J_o = 8.5 Hz, H-4), 7.78 (2H, m, H-6, H-7), 7.36 (1H, d, J_o = 8.5 Hz, H-3), 6.73 (1H, s, 2-OH), 4.04 (3H, s,1-OCH_3).

Compound 3 – yellow crystals; m.p. 236-238°; UV λ_{max} (MeOH): 206, 238, 279 and 312 nm; λ_{max} (MeOH/$^-$OH): 206, 243, 311 and 470 nm; IR ν_{max} (KBr): 3310, 2933, 1670 and 1590 cm^{-1}; EI-MS m/z (rel. int.): 284 (M^+, 100), 269 (92), 267 (15) and 241 (38); ^1H-NMR (CD_3OD, 500 MHz) δ ppm: 8.21 (1H, dd, J_m = 1.5 Hz, J_o = 7.7 Hz, H-8), 8.17 (1H, dd, J_m = 1.5 Hz, J_o = 7.6 Hz, H-5), 7.81 (1H, ddd, J_m = 1.5 Hz, $J_{7,8}$ = 7.7 Hz, $J_{7,6}$ = 7.3 Hz, H-7), 7.76 (1H, ddd, J_m = 1.5 Hz, $J_{6,5}$ = 7.6 Hz, $J_{6,7}$ = 7.3 Hz, H-6), 7.55 (1H, *s*, H-4), 3.97 (3H, *s*, 2-OCH_3), 3.95 (3H, *s*, 1-OCH_3)

Compound 4 – yellow crystals; UV λ_{max} (MeOH): 208, 244, 281 and 402 nm; λ_{max} (MeOH/$^-$OH): 206, 242, 312 and 470 nm; IR ν_{max} (KBr): 3412, 2923, 1670 and 1635 cm^{-1}; EI-MS m/z (rel. int.): 270 (M^+, 100), 252 (54) and 227(53); ^1H-NMR (CD_3OD, 500 MHz) δ ppm: 8.28 (1H, dd, J_m = 2.2 Hz, J_o = 6.6 Hz, H-8), 8.21 (1H, dd, J_m = 2.2 Hz, J_o = 6.5 Hz, H-5), 7.83 (2H, m, H-6, H-7), 7.29 (1H, s, H-4), 4.54 (1H, s, 2-OH), 3.97 (3H, s, 1-OCH_3).

Compound 5 – yellow crystals; m.p. 224° (dec.); UV λ_{max} (MeOH): 205, 244, 278 and 402 nm; λ_{max} (MeOH/$^-$OH): 206, 311 and 474 nm; IR ν_{max} (KBr): 3402, 2925, 1663, 1626 and 1592 cm^{-1}; EI-MS m/z (rel. int.): 254 (M^+, 100), 226 (11), 197 (13); ^1H-NMR (DMSO-d_6, 500 MHz) δ ppm: 13.17 (1H, s, 1-OH), 8.21 (1H, dd, J_m = 1.5 Hz, J_o = 7.6 Hz, H-8), 8.13 (1H, dd, J_m = 1.8 Hz, J_o = 7.3 Hz, H-5), 7.89 (2H, m, H-6, H-7), 7.23 (1H, s, H-4), 2.07 (3H, s, 2-CH_3).

Compound 6 – yellow needles; m.p. 270-272°; UV λ_{max} (MeOH): 216, 269 and 404 nm; λ_{max} (MeOH/$^-$OH): 205, 247, 307 and 470 nm; EI-MS m/z (rel. int.): 254 (M^+, 100), 226 (9), 197 (22); IR ν_{max} (KBr): 3426, 2928, 1664, 1636 and 1596 cm^{-1}; ^1H-NMR (DMSO-d_6, 500 MHz) δ ppm: 13.10 (1H, s, 1-OH), 11.21 (1H, br s, 6-OH), 8.10 (1H, d, J_o = 8.5 Hz, H-8), 7.63 (1H, d, J_o = 7.6 Hz, H-3), 7.55 (1H, d, J_o = 7.6 Hz, H-4), 7.45 (1H, d, J_m = 2.7 Hz, H-5), 7.25 (1H, dd, J_o = 8.5 Hz, J_m = 2.7 Hz, H-7), 2.07 (3H, *s*, 2-CH_3).

Compound 7 – yellow needles; m.p. 180-182°; UV λ_{max} (MeOH): 207, 245, 281 and 413 nm; λ_{max} (MeOH/$^-$OH): 207, 250, 313 and 482 nm; IR ν_{max} (KBr): 3440, 2923, 1675 and 1621 cm^{-1}; EI-MS m/z (rel. int.): 284 (M^+, 55), 253 (99), 196 (87) and 139 (100); ^1H-NMR ($CDCl_3$, 500 MHz) δ ppm: 13.30

(1H, s, 1-OH), 9.30 (1H, s, 3-OH), 8.27 (2H, m, H-5, H-8), 7.78 (2H, m, H-6, H-8), 4.94 (2H, s, 2-CH_2O-), 3.58 (3H, s, -OCH_3).

Compound 8 – orange crystals; m.p. 272-274°; UV λ_{max} (MeOH): 230, 258 and 448 nm; λ_{max} (MeOH/$^-$OH): 208, 259, 333 and 542 nm; IR ν_{max} (KBr): 3456, 2924 and 1628 cm^{-1}; EI-MS m/z (rel. int.): 270 (M^+, 100), 242 (12) and 139 (13); ^{1}H-NMR (CD_3OD, 500 MHz) δ ppm: 7.80 (1H, d, J_o= 8.2 Hz, H-8), 7.75 (1H, d, J_o=7.6 Hz, H-4), 7.59 (1H, d, J_o = 7.6 Hz, H-3), 7.16 (1H, d, J_o = 8.2 Hz, H-7), 2.35 (3H, s, CH_3).

Results and Discussion

The identity of all compounds (Table 1), except for **3** and **4** was confirmed based on comparison of their physical and spectroscopic data with those of the authentic samples (Ismail *et al.*, 1997). The spectral data of compound **4** were identical with those in the literature (Wijnsma *et al.*, 1986). The structure of compound **3** was assigned based on comparison of UV, IR, ^{1}H NMR data with those in the literature and was further confirmed by the assignment of ^{13}C NMR data which was not previously reported, utilizing FGHMBC and FGHMQC experiments (Fig. 1). Similarly, the assignment of ^{13}C NMR data of compound **6** (not available from literature) was also achieved using the correlation experiments (Fig. 2).

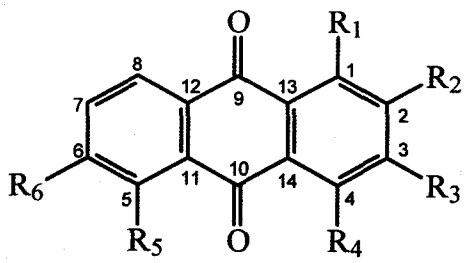

Table 1. Compounds isolated from cell suspension culture

Name of compounds	R_1	R_2	R_3	R_4	R_5	R_6
Nordamnacanthal (**1**)	OH	CHO	OH	H	H	H
Alizarin-1-methyl ether (**2**)	OCH_3	OH	H	H	H	H
Anthragallol-1,2-dimethyl ether (**3**)	OCH_3	OCH_3	OH	H	H	H
Purpurin-1-methyl ether (**4**)	OCH_3	OH	H	OH	H	H
Rubiadin (**5**)	OH	CH_3	OH	H	H	H
Soranjidiol (**6**)	OH	CH_3	H	H	H	OH
Lucidin-ω-methyl ether (**7**)	OH	CH_2OCH_3	OH	H	H	H
Morindone (**8**)	OH	CH_3	H	H	OH	OH

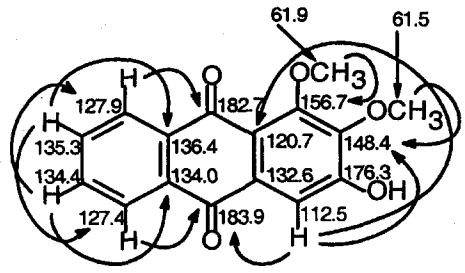

Fig. 1. FGHMBC correlation of compound **3**.

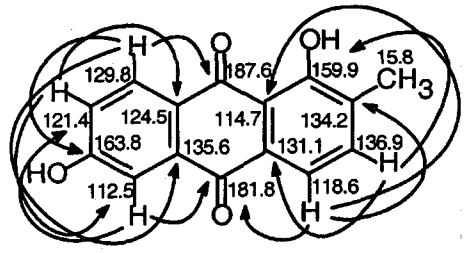

Fig. 2. FGHMBC correlation of compound **6**.

Comparison of the constituents isolated from both the plant and cell culture showed that only nordamnacanthal appeared to be present in both sources while other components varied in concentrations. Previous studies on the constituents of the cell suspension culture of *M. citrifolia* have shown the presence of eight anthraquinone derivatives, as well as some of their respective glycosides (Inoue *et al.*, 1981 and Zenk *et al.*, 1975). Compounds **2**, **3**, **4**, **6** and **7** isolated in the current study were absent in the previous study on the root of the original plant.

Acknowledgements

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