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New Anthraquinones from the Roots of *Hedyotis dichotoma*

A. S. Hamzah,* H. Jasmani, R. Ahmad, and A. R. Baba

School of Applied Sciences, Institut Teknologi Mara, 40450 Shah Alam, Selangor, Malaysia

N. H. Lajis

Department of Chemistry, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia

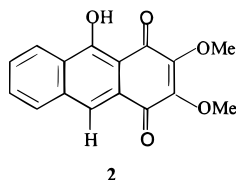
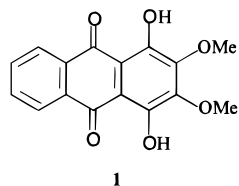
N. Aimi, M. Kitajima, and H. Takayama

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku Chiba 263, Japan

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Two new anthraquinones, 1,4-dihydroxy-2,3-dimethoxyanthraquinone (**1**) and 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone (**2**), were isolated from the roots of *Hedyotis dichotoma* along with two known plant metabolites, isovitexin and ursolic acid. The structures of **1** and **2** were elucidated using spectroscopic techniques.

Malaysian species of the genus *Hedyotis* (Rubiaceae) are distributed abundantly throughout peninsular Malaysia. Among 35 Malaysian species the most common representatives include *H. capitellata*, *H. dichotoma*, *H. diffusa*, *H. herbacea*, and *H. verticillata*.¹ *Hedyotis dichotoma* is a small herb (0.1–0.2 m tall) commonly found in open places, especially in sandy areas. Like the other species of the genus, it is used by the locals in traditional medicine. The whole dried plant is sold locally and used as a health tea. Phytochemical studies previously performed on the genus *Hedyotis* have led to the isolation of alkaloids, anthraquinones, flavonoids, iridoids, and triterpenes,^{2–11} but no study has yet appeared on *H. dichotoma*. After investigation of the constituents of roots of this plant, we have isolated two new anthraquinones: 1,4-dihydroxy-2,3-dimethoxyanthraquinone (**1**) and 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone (**2**), along with two known metabolites, isovitexin and ursolic acid.²



Compound **1** exhibited a melting point of 262–264 °C. The HRFABMS showed a pseudomolecular ion peak at m/z 301.0721, indicating the molecular formula to be $C_{16}H_{12}O_6$. The UV spectrum showed absorption maxima at 477 and 257 nm, and the former was shifted to 572 nm upon addition of base. This observation suggested that the compound is a 1,4-dihydroxy-type anthraquinone.¹² The IR spectrum showed an absorption band at 1624 cm^{-1} , suggesting the presence of a chelated carbonyl group.

The $^1\text{H-NMR}$ spectrum of **1** showed the presence of A_2B_2 aromatic protons that resonated at δ 8.34 (2H) and δ 7.82 (2H). A low field signal at δ 13.5 that integrated for two protons was attributed to two identical hydroxyl

protons that were strongly chelated to a carbonyl group. A singlet at δ 4.13, integrated for six protons, suggested the presence of two methoxyl groups. The symmetry of the structure of **1** was indicated by the appearance of only four kinds of proton signals. In a difference NOE study, irradiation of the methoxyl signal caused enhancement of the peak intensity of the OH signal, while no effect was observed on the aromatic protons. This observation suggested that the methoxyl group is not adjacent to any of the aromatic protons. The $^{13}\text{C-NMR}$ spectrum showed the presence of eight carbon signals for compound **1** corresponding to 16 carbons. This observation was attributed to the existence of C_2 symmetry in the molecular structure. On the basis of the analysis of all of its spectral data, the structure of **1** was assigned as 1,4-dihydroxy-2,3-dimethoxyanthraquinone (**1**).

Another new anthraquinone, compound **2**, mp 163–165 °C, showed a molecular ion peak at m/z 284 in the HRFABMS, which indicated the molecular formula of the compound to be $C_{16}H_{12}O_5$. The UV spectrum showed strong absorption maxima at 456, 286, and 233 nm. In alkaline EtOH, the absorption band with the longest wavelength was shifted to 537 nm. The IR spectrum showed a strong absorption at 1624 cm^{-1} , which once again suggested the presence of a chelated carbonyl group. The $^1\text{H-NMR}$ data showed a series of four adjacent aromatic protons at δ 8.45, 7.93, 7.72, and 7.68 and an isolated aromatic proton at δ 8.10 and suggested several possible structures, of which **2** was the most likely. Other possible structures were excluded by the absence of any NOE observations between the isolated aromatic proton (δ 8.10) and any of the aromatic methoxyl groups. On the other hand, a distinct NOE was observed between the isolated aromatic proton H-10 (11%) and the aromatic proton H-5 at δ 7.93. The *peri* position of the hydroxyl group was ascertained by a clean NOE between the 9-OH and H-8 (2%). In the HMBC spectrum of compound **2**, both of the methoxyl singlets at δ 4.15 and 4.16 exhibited a long-range carbon–proton coupling with that of C-2 (149.4 ppm) and C-3 (148.3 ppm), which is part of an α,β -unsaturated ketone. These spectral data strongly suggested that 2,3-dimethoxy-9-hydroxy-1,4-anthraqui-

* To whom correspondence should be addressed. Phone: 603-556-4840. FAX: 603-559-7681. E-mail: sazali@itm.edu.my.

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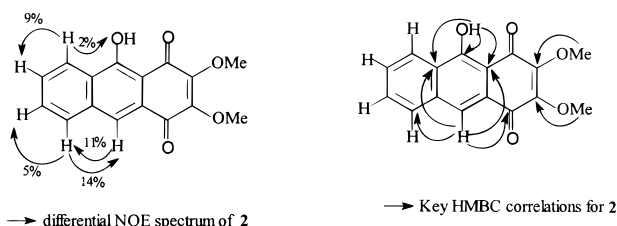


Figure 1.

none was the structure of **2**. Other NOE and HMBC correlations as indicated in Figure 1 further supported this conclusion. It should be noted that in the shikimate-derived anthraquinones, 1,4-quinones are quite rare; as far as we know, only one representative has been reported in the literature.¹³

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. UV and IR spectra were recorded on a Shimadzu UV-vis 160 spectrometer and a Perkin-Elmer 1600 FTIR spectrometer, respectively. ¹H- and ¹³C-NMR spectra were recorded on a JEOL JNM-A 500 spectrometer at 500 (¹H) and 125 (¹³C) MHz, respectively. MS were recorded on AEI-MS 12 spectrometer (Finnigan). Ionization was induced by electron impact at 70 eV. For column chromatography and analytical TLC, Merck 7734 and Merck DC-Plastikfollen 60 F₂₅₄, respectively, were used.

Plant Materials. *H. dichotoma* was collected at Gebeng, Kuantan, Malaysia, in June 1995. The plant was identified by Prof. Ruth Kiew of Universiti Pertanian Malaysia, and voucher specimens were deposited at the Herbarium of Biology Department, Universiti Pertanian Malaysia.

Extraction and Isolation. The ground roots of *H. dichotoma* (300 g) were soaked in CH₂Cl₂ for 48 h. The solvent was removed by filtration, and fresh CH₂Cl₂ was then added to the roots for another 48 h. The CH₂Cl₂ extracts were combined and evaporated under reduced pressure to give a reddish viscous mass (1.6 g). The crude extract was subjected to column chromatography using CHCl₃ with increasing amounts of MeOH. In all, 25 fractions were collected, of which fractions 12–14 contained two major compounds. Preparative TLC of fractions 12–14 using EtOAc–CH₂Cl₂ (8:2) afforded the two anthraquinones, **1** [orange crystals; 40 mg; *R*_f 0.61–0.63; Si gel, CH₂Cl₂–petroleum ether (7:3)] and **2** [orange crystals; 30 mg; *R*_f 0.38–0.40; Si gel, CH₂Cl₂–petroleum ether (7:3)].

1,4-Dihydroxy-2,3-dimethoxyanthraquinone (1): orange crystals (CH₂Cl₂–petroleum ether); mp 262–264 °C; UV (MeOH) λ max (log ε) 233 (0.71), 257 (1.67), 477 (0.36) nm; IR ν max (KBr disk) 3448, 2924, 2854, 1744, 1623, 1584, 1462, 1413, 1378, 1269, 1114,

1064 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 13.50 (1H, s, OH × 2), 8.34 (1H, d, *J* = 6.1 Hz, H-5), 8.34 (1H, d, *J* = 6.1 Hz, H-8), 7.82 (1H, d, *J* = 5.8 Hz, H-6), 7.82 (1H, d, *J* = 5.8 Hz, H-7), 4.13 (1H, s, O-Me × 2); ¹³C NMR (125 MHz, CDCl₃) δ 185.9 (C-9, C-10), 154.5 (C-1, C-4), 148.3 (C-2, C-3), 134.2 (C-6, C-7), 133.5 (C-11, C-12), 126.9 (C-5, C-8), 108.8 (C-13, C-14), 61.5 (O-Me × 2); FABMS *m/z* 300 (76), 285 (19), 270 (8), 254 (9), 158 (62), 129 (22), 102 (100); HRFABMS *m/z* found [MH]⁺ 301.0721 (C₁₆H₁₃O₆ requires 301.0711).

2,3-Dimethoxy-9-hydroxy-1,4-anthraquinone (2): orange crystals (CH₂Cl₂–petroleum ether); mp 163–165 °C; UV (MeOH) λ max (log ε) 233 (1.36), 286 (0.62), 456 (0.18), nm; IR ν max (KBr disk) 3450, 3018, 2958, 1704, 1656, 1624, 1506, 1230, 1076 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 13.7 (1H, s, OH), 8.45 (1H, br dd, H-8), 8.10 (1H, s, H-10), 7.93 (1H, br dd, H-5), 7.72 (1H, ddd, H-6), 7.68 (1H, ddd, H-7), 4.15 (1H, s, O-Me), 4.16 (1H, s, O-Me); ¹³C NMR (125 MHz, CDCl₃) δ 186.2 (C-1), 180.9 (C-4), 162.0 (C-9), 149.4 (C-2), 148.3 (C-3), 135.7 (C-11), 131.0 (C-6), 130.3 (C-5), 129.0 (C-7), 127.8 (C-12), 126.6 (C-14), 124.7 (C-8), 121.8 (C-10), 107.0 (C-13), 61.6 (O-Me), 61.4 (O-Me); FABMS *m/z* 284 (76), 269 (94), 255 (15), 239 (13), 171 (60), 142 (100); HRFABMS *m/z* found [MH]⁺ 285.0725 (C₁₆H₁₃O₅ requires 285.0763).

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