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Anthraquinones from *Hedyotis herbacea*

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A new anthraquinone, 2-hydroxymethyl-10-hydroxy-1,4-anthraquinone (**1**), was isolated from *Hedyotis herbacea* along with three other known derivatives: 1,4-dihydroxy-2-hydroxymethylanthraquinone (**2**); 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone; and 1,4-dihydroxy-2,3-dimethoxyanthraquinone. The structure of **1** was determined based on analysis of its spectroscopic data.

Many species of *Hedyotis* are used in Malay and Chinese traditional medicine for various purposes such as tonics and for treatment of appendicitis, boils, dysentery, hepatitis, snake bites, and tonsillitis.[1,2] They are classified as herbaceous plants that usually grow as weeds in lowland areas. *Hedyotis herbacea* Linn. (Rubiaceae) is a small herb, commonly found in sandy open places. It usually stands upright, reaching a height of 0.1−0.3 m. The fresh leaves are sold locally and used as a poultice to improve blood circulation. Previously we reported the isolation of anthraquinones from *H. dichotoma*.[3] We also reported on the isolation of flavonoid glycosides from this species, including kaempferol 3-*O*-arabinopyranoside and kaempferol 3-*O*-rutinoside, as well as ursolic acid from the fresh whole plant extract.[4] In view of the potential of *H. herbacea* to be developed as the new source for arbutin,[5] a cultivation of this plant was conducted. The plant matured in three to four months, after which time some of the leaves began to turn black and slowly wilted. Upon harvesting, air-drying, and storage, the whole plant sample turned dark. We now report the isolation of anthraquinones from this darkened harvest of cultivated *H. herbacea*.

Extraction of the ground whole plant with MeOH followed by conventional purification procedures resulted in the isolation of a new anthraquinone (**1**), as red needles, mp 190−192 °C. The compound gave a quasi-molecular ion peak at *m*/*z* 255.066 (calcd *m*/*z* 255.0658) by HRFABMS, corresponding to the molecular formula $C_{15}H_{11}O_4$. The 1,4-anthraquinone chromophore was evident from its UV absorption curve, showing the absorption maxima at 302 and 464 nm, with the latter being shifted to 514 nm upon addition of base, suggesting the presence of a hydroxyl group.[6] This was further confirmed by the IR spectrum, which showed a band for a chelated carbonyl at 1639 cm^{-1} in addition to an absorption for a free carbonyl at 1658 cm^{-1}. The ^1H NMR spectrum also supported the chelated nature of the hydroxyl group to the carbonyl by the characteristic downfield signal at δ 13.87 (1H, s). The ^{13}C NMR spectrum showed 15 carbon atoms, which included two carbonyl downfield signals at δ 189.1 and 184.4, indicative of the presence of the chelated and nonchelated carbonyl, respectively.

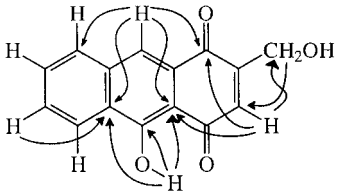

Figure 1. Selected HMBC correlations of compound **1**.

The assignment of the structure of **1** was carried out by interpretation of the FGHMQC and FGHMBC correlation NMR spectra.[7,8] There were six aromatic protons as indicated by the ^1H NMR spectrum, and its pattern suggested that ring A of the anthraquinone moiety was nonsubstituted. Two sets of double doublets at δ 8.51 and 7.98, each integrating for one proton, were attributed to H-5 and H-8, respectively. The deshielded signal observed for H-5 can be attributed to the occurrence of an oxygen atom at its proximity (C-10).[9] A similar deshielding effect was also observed for H-8 in 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone. A multiplet centered at δ 7.73, integrating for two protons, was assigned to H-6 and H-7. Two other aromatic signals that appeared as a singlet at δ 8.13 and broad triplet at δ 7.10 (J = 1.8 Hz) were allocated to H-9 and H-3, respectively. The benzylic hydroxymethyl protons (H-15) were represented by a singlet peak integrating for two protons at δ 4.73.

In the FGHMBC NMR spectrum of **1** an aromatic proton at δ 8.13 (s, H-9) showed C−H long-range correlations with the carbon signals at δ 184.3 (C-1), 130.6 (C-8), 127.9 (C-11), and 108.7 (C-14), indicating that this singlet aromatic proton occurred at C-9 in ring B. Further, the chelated hydroxyl proton at δ 13.87 correlated with C-14 (δ 108.7) and C-11 (δ 127.9), indicating that the phenolic OH was located at C-10 in ring B. Therefore, **1** possesses a 10-hydroxy-1,4-anthraquinone skeleton; this finding clearly rules out the possibility that this anthraquinone exists as the alternative 1,10-anthraquinone tautomeric form **1′**. Another aromatic proton at δ 7.10 (br t, H-3) also correlated with carbon signals at δ 184.3 (C-1) and 108.7 (C-14). These results, as well as other correlations observed between protons in ring A and the respective carbons, strongly supported the proposed structure of **1** as the new 2-hydroxymethyl-10-hydroxy-1,4-anthraquinone (Figure 1).

The identity of the known compound 1,4-dihydroxy-2-hydroxymethylanthraquinone (**2**) was confirmed based on its spectral data as well as FGHMBC and FGHMQC NMR experiments, showing cross-peaks between δ 8.35 (H-5,

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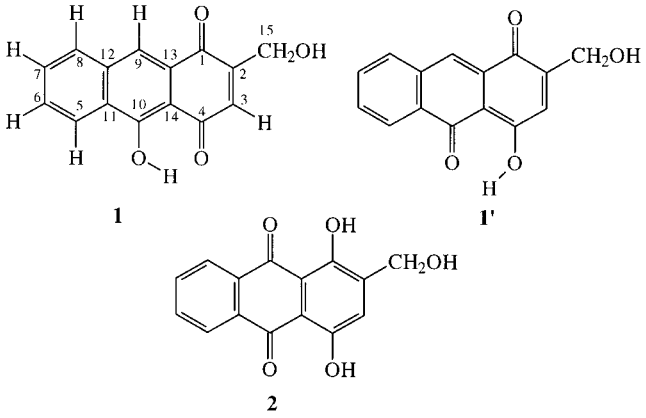
H-8, m) and the corresponding carbonyl carbons of C-9 and C-10 (δ 186.1 and 186.9, respectively). Thus, the possibility of the compound being a 1,4-anthraquinone could be eliminated. The ^{13}C NMR data of this compound have not been reported in the literature before. The other known compounds, 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone and 1,4-dihydroxy-2,3-dimethoxyanthraquinone, were determined based on spectroscopic data and comparison with the data in the literature.[3,10]

Only a few 1,4-anthraquinones have been isolated from plants,[11−13] although bianthraquinones having a 1,4-anthraquinone moiety were reported from plants the genera *Senna* (Leguminosae)[12−14] and *Galium* (Rubiaceae).[15]



1 1' 2

Experimental Section

General Experimental Procedures. Melting points were determined on Kofler hot-stage apparatus and are uncorrected. UV (in absolute EtOH) and IR (in mini KBr form) spectra were recorded on a Shimadzu UV−vis 160 and Perkin−Elmer 1650 FTIR spectrometers, respectively. ^1H and ^{13}C NMR spectra ($CDCl_3$ or DMSO-d_6) were determined on a JEOL JNM-A 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). The spectra were interpreted by the aid of the FGCOSY, FGHMBC, and FGHMQC techniques. MS were recorded using Finnigan MAT SSQ 710 spectrometer, with ionization being induced by electron impact at 70 eV. HRFABMS were obtained using a JEOL JMS HX-110A mass spectrometer. Column chromatography and analytical TLC utilized Merck 9385 and Merck DC−Plastikfollen 60 F_{254}, respectively.

Plant Material. *Hedyotis herbacea* was obtained from the cultivation experimental plot at the farm unit of Universiti Putra Malaysia. The plant was harvested three and a half months after germination from seeds collected from Tanjung Tualang district in Perak, Malaysia. It was air-dried and ground before extraction. The voucher specimen (no. 06365) has been deposited at the herbarium of the Biology Department, Universiti Putra Malaysia.

Extraction and Isolation. The ground dried sample of *H. herbacea* (100 g) was extracted three times overnight with MeOH, (3 L). The combined extracts were evaporated under reduced pressure to give a brown gum (20 g). The gum was shaken with H_2O−MeOH (2:1) (750 mL) and extracted with $CHCl_3$ (3 × 250 mL). Removal of the solvent under reduced pressure gave a brownish gum (5.2 g). The extract (5.0 g) was then subjected to Si gel column chromatography and successively eluted with $CHCl_3$, followed by $CHCl_3$−EtOAc (1:1) mixture, and finally with EtOAc to give 35 (15-mL) fractions. Fractions 3−7 were combined to give 2.4 g of crude product after removal of solvent. These combined fractions were rechromatographed on a Si gel column and eluted with $CHCl_3$ to give 20 (15-mL) fractions from which the combined fractions 3−5 and combined fractions 8−11 yielded 700 mg and 1.2 g of residue, respectively, upon evaporation. Combined fractions 8−11 were again chromatographed on a Si gel column and eluted with $CHCl_3$ to give 30 fractions (15 mL) of which fractions 11 and 12 were combined to yield 200 mg of residue designated as A, while fractions 15−30 were also combined and evaporated to afford 400 mg of residue designated as B. Further Si gel column chromatography on product A using $CHCl_3$ as eluent gave compound **2** as red needles (8 mg), mp 183−184 °C, crystallized from MeOH. Product B was subjected to silica gel column chromatography to provide 15 mg of compound **1**, as red needles, mp 190−192 °C after recrystallization from MeOH.

The combined fractions 3−5 (650 mg) from the second column chromatographic step were subjected to further column chromatography on Si gel with $CHCl_3$−hexane (1:1) mixture as eluent to give 30 (15-mL) fractions. Fractions 5−10 were combined, evaporated, and rechromatographed on Si gel column to finally afford 8 mg of 2,3-dimethoxy-9-hydroxy-1,4-anthraquinone as orange needles, mp 159−160 °C. Fractions 12−21 were combined and subjected to further Si gel column chromatography to yield 5 mg of 1,4-dihydroxy-2,3-dimethoxyanthraquinone as orange needles after recrystallization from MeOH, mp 175−176 °C.

2-Hydroxymethyl-10-hydroxy-1,4-anthraquinone (1): obtained as red needles; mp 190−192 °C (MeOH); UV (MeOH) λ_{max} (log ϵ 302 (3.72), 464 (3.94) nm; UV (MeOH/NaOH) λ_{max} 302, 514 nm; IR (KBr) ν_{max} 3395 (OH), 2921 (C−H), 1658 (C═O), 1639 (C═O, chelated), 1591, 1458, 1353, 1253, 1091, 1030, 888, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 13.87 (1H, s, −OH), 8.51 (1H, dd, J_{ortho} = 7.0 Hz, J_{meta} = 1.5 Hz, H-5), 8.13 (1H, s, H-9), 7.98 (1H, dd, J_{ortho} = 7.0 Hz, J_{meta} = 1.2 Hz, H-8), 7.73 (2H, m, H-6 and H-7), 7.10 (1H, br t, J = 1.8 Hz, H-3), 4.73 (2H, s, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184.3 (C-1), 151.3 (C-2), 134.8 (C-3), 189.1 (C-4), 124.9 (C-5), 129.3 (C-6), 131.2 (C-7), 130.6 (C-8), 122.0 (C-9), 162.5 (C-10), 127.9 (C-11), 135.9 (C-12), 127.6 (C-13), 108.7 (C-14), 60.4 (CH2); EIMS m/z 254 (M^+, 100), 225 (81), 197 (49), 171 (18), 152 (37), 139 (44), 115 (70), 88 (18), 76 (15); HRFABMS m/z found $[MH]^+$ 255.0670 ($C_{15}H_{11}O_4$ requires 255.0658).

1,4-Dihydroxy-2-hydroxymethylanthraquinone: obtained as red needles; mp 183−184 0C (lit. 200−202 °C);[7] UV, IR, ^1H NMR, EIMS, consistent with literature values;[7] ^{13}C NMR (DMSO-d_6, 125 MHz) δ 154.2 (C-1), 144.6 (C-2), 124.5 (C-3), 156.9 (C-4), 126.5 (C-5), 134.9 (C-6), 135.0 (C-7), 126.6 (C-8), 186.1 (C-9), 186.9 (C-10), 132.8 (C-11), 132.9 (C-12), 111.7 (C-13), 110.9 (C-14), 57.5 (C-15).

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