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Anthraquinones from *Morinda citrifolia* Roots

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Following our finding of 2-ethoxy-1-hydroxyanthraquinone (**1**) from *Morinda citrifolia* root, we further investigated the same part of this plant and it gave us 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (**2**). Along with these new compounds, five other known anthraquinones, 1-hydroxy-2-methylanthraquinone (**3**), damnacanthal (**4**), nordamnacanthal (**5**), 2-formyl-1-hydroxyanthraquinone (**6**) and morindone-6-methyl-ether (**7**) were also isolated from the same plant. The structures of these compounds were elucidated based on spectroscopic analysis such as NMR, MS and IR.

Key Words: *Morinda citrifolia*, 2-Ethoxy-1-hydroxyanthraquinone, 2-(Ethoxy-ethyl)-1-hydroxyanthraquinone, Anthraquinones.

INTRODUCTION

Morinda citrifolia L. (noni) belongs to the Rubiaceae family, a which is well known for its abundance in anthraquinones[1]. This plant is native in South East Asia and Australia and is used by the polynesians for more than 2000 years as food and medicine[2,3], herbal remedies to treat diabetes, as well as for swollen spleen, liver diseases and cough[4] and to cure several diseases. Nowadays, it is even largely sold as food supplement[5]. Various anthraquinones[6,7,8] and some terpenes[9] have been reported from the roots of this plant. Some of these anthraquinones have been found to exhibit anticancer properties[10] against human breast cancer and lung cancer cell lines[11]. In our previous paper, we reported the isolation and elucidation of 2-ethoxy-1-hydroxyanthraquinone (**1**)[12]. In this paper, we report the isolation and identification of another new anthraquinone derivative with an ethoxy moiety, 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (**2**) and the comparison data of **1** and **2**.

EXPERIMENTAL

The roots of *M. citrifolia* were collected from Wakaf Bharu, Kelantan, Malaysia, in December 2006 and the plant was identified by Dr. Rusea Go of the Biology Department, UPM, Serdang, Selangor, Malaysia. A voucher specimen (voucher specimen number: RG2103) was deposited in the Herbarium at Biology Department, UPM.

Melting points were determined using the digital precision melting point apparatus. Infrared spectra were measured in NaCl/KBr pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shidmazu GCMS-QP5050A spectrometer. ^{1}H (400 MHz), ^{13}C (100 MHz) and ^{2}D NMR were obtained using Jeol 400 MHz FT-NMR spectrometer with TMS (tetramethylsilane) as internal standard. Ultraviolet spectra were recorded in MeOH and $CHCl_3$ on a Shidmazu UV-160A, UV-vis spectrophotometer.

Extraction and isolation: Dried roots of *M. citrifolia* (5 kg) were finely ground into powder form. The sample was extracted using solvents with increasing polarity to give *n*-hexane (22.9 g), petroleum ether (1.7 g), chloroform (28.0 g), acetone (10.1 g) and methanol (70.4 g) extracts. The *n*-hexane, petroleum ether, chloroform (9.25 g) and acetone extracts were chromatographed on a silica gel column chromatography (Kieselgel 60 PF_{254}) with a gradient of hexane-chloroform, chloroform-ethyl acetate, ethyl acetate-methanol and methanol. Fractions from the column chromatography of the chloroform extract were subjected repeatedly to further purifications using hexane-chloroform or petroleum ether-chloroform to furnish 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (**2**) (4.8 mg), 2-ethoxy-1-hydroxyanthraquinone (**1**) (6.5 mg), 1-hydroxy-2-methylanthraquinone (**3**) (30.9 mg), damnacanthal (**4**) (19.32 mg), nordamnacanthal (**5**) (140.7 mg) and 2-formyl-1-hydroxyanthraquinone (**6**) (10.4 mg). Meanwhile, the hexane extract was further purified and eluted with petroleum ether/chloroform to yield damnacanthal (**4**) (58.1 mg). The acetone extract gave morindone-6-methyl-ether or 1,5-dihydroxy-6-methoxy-2-methylanthraquinone (**7**) (10.3 mg) after further purification using 100 % petroleum ether in a mini column. The petroleum ether extract did not furnish any significant

compounds. The methanol extract is still being investigated for its chemical constituents.

2-(Ethoxy-ethyl)-1-hydroxyanthraquinone (2): Yellow solid, m.p. 109-110 °C. UV (MeOH) λ_{max} nm (log ε): 210 (2.97), 227 (3.44), 250 (3.91), 335 (0.42), 402 (0.83). IR (KBr, ν_{max}, cm^{-1}): 2924 (asym. sp^3 C-H stretch), 2854 (sym. sp^3 C-H stretch), 1671 (C=O unchelated), 1632 (C=O chelated), 1590 and 1475 (aromatic C=C), 1255 and 1030 (C-O). EI-MS m/z (rel. int.): 296 (M^+, 82 %), 268 (2), 251 (96), 224 (100), 194 (20), 167 (14), 139 (65), 111 (7), 98 (8), 83 (10), 69 (13). ^1H and ^{13}C NMR (Table-1).

TABLE-1
^1H (400 MHz, $CDCl_3$) AND ^{13}C NMR (100 MHz, $CDCl_3$) SPECTRAL DATA OF **1** [Ref. 12] AND **2**

Position	Compd. **2**		Compd. **1**	
	δ_H	δ_C	δ_H	δ_C
1-OH	13.54 (1H, s)	162.4	13.54 (1H, s)	162.4
2	–	125.1	–	164.7
3	8.24 (1H, d, *J* = 7.8 Hz)	138.6	8.24 (1H, d, *J* = 8.3 Hz)	138.6
4	7.84 (1H, d, *J* = 7.8 Hz)	118.1	7.85 (1H, d, *J* = 9.2 Hz)	118.1
4a	–	136.0	–	136.0
5	8.36 (1H, t, *J* = 3.7 Hz)	127.4	8.35 (1H, d, *J* = 9.2 Hz)	127.5
5a	–	133.1	–	133.2
6	7.85 (1H, dd, *J* = 8.2, 3.7 Hz)	134.9	7.84 (1H, t, *J* = 3.6 Hz)	134.9
7	7.85 (1H, dd, *J* = 8.2, 3.7 Hz)	134.8	7.84 (1H, t, *J* = 3.6 Hz)	134.6
8	8.36 (1H, d, *J* = 3.7 Hz)	127.2	8.31 (1H, d, *J* = 9.2 Hz)	127.2
8a	–	133.2	–	133.1
9	–	188.4	–	188.4
9a	–	117.2	–	117.1
10	–	182.0	–	182.0
11	1.44 (2H, t, *J* = 7.3 Hz,)	29.7	4.47 (2H, q, *J* = 7.4 Hz)	61.7
12	4.50 (2H, t, *J* = 7.3 Hz,)	64.0	1.44 (3H, t, *J* = 7.4 Hz)	14.2
13	3.88 (2H, br, t)	69.2	–	–
14	1.23 (2H, br, t)	14.3	–	–

2-Ethoxy-1-hydroxyanthraquinone (1): Yellow solid, m.p. 123-124 °C[12]. UV (MeOH) λ_{max} nm (log ε): 210 (2.97), 227 (3.44), 250 (3.91), 335 (0.42), 402 (0.83). IR (KBr, ν_{max}, cm^{-1}): 3447 (OH stretch), 2924 (asym. sp^3 C-H stretch), 2854 (sym. sp^3 C-H stretch), 1671 (C=O unchelated), 1632 (C=O chelated), 1590 and 1475 (aromatic C=C), 1255 and 1030 (C-O). EI-MS m/z (rel. int.): 268 (M^+, 2 %), 251 (96), 224 (100), 194 (20), 167 (14), 139 (65), 111 (7), 98 (8), 83 (10), 69 (13). ^1H and ^{13}C NMR (Table-1).

1-Hydroxy-2-methylanthraquinone (3): Yellow solid, m.p. 180-181 ºC (Lit. 183-184 °C)[13]. UV (MeOH) λ_{max} nm (log ε): 409 (0.27), 324 (0.15), 253 (1.49), 225 (0.95), 204 (1.19). IR (NaCl, ν_{max}, cm^{-1}): 3438 (br, H), 2926 (asym. sp^3 C-H stretch), 2856 (sym. sp^3 C-H stretch), 1670 (unchelated C=O), 1634 (chelated C=O), 1590 and 1456 (C=C), 1290 (C-O). EI-MS m/z (rel. int.): 238 (M^+, 100 %), 209 (9), 181 (25), 152 (21), 76 (29). ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are in agreement with published data[14].

Damnacanthal (4): Pale yellow needles, m.p. 217-218 ºC (Lit. 210-211 ºC)[15]. UV ($CHCl_3$) λ_{max} nm (log ε): 205 (0.67), 253 (4), 261 (4), 271 (4), 329 (4), 389 (1.83). IR (KBr, ν_{max}, cm^{-1}): 3448 (br, OH), 2940 (asym. sp^3 C-H stretch), 2862 (sym. sp^3 C-H stretch), 1652 (br, C=O), 1564 and 1446 (C=C). EI-MS m/z (rel. int.): 282 (M^+, 39 %), 254 (100), 225 (37), 208 (19), 168 (15), 152 (10), 139 (30), 113 (9), 76 (11), 69 (13), 50 (7). ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are in agreement with published data[15].

Nordamnacanthal (5): Orange-yellow solid, m.p. 224-225 ºC (Lit. 220-221 ºC)[16]. UV (MeOH) λ_{max} nm (log ε): 214 (2.89), 246 (3.76), 253 (3.91), 262 (3.76), 290 (2.99), 420 (0.97). IR (KBr, ν_{max}, cm^{-1}): 3440 (OH), 2926 (asym. sp^3 C-H stretch), 2856 (sym. sp^3 C-H stretch), 1736 (C=O), 1644 (aldehyde C=O), 1592 and 1464 (aromatic C=C), 1268 (C-O). EI-MS m/z (rel. int.): 268 (M^+, 85 %), 240 (100), 212 (26), 184 (22), 155 (6), 138 (18), 77 (15), 69 (136). ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are in agreement with published data[16].

2-Formyl-1-hydroxyanthraquinone (6): Orange solid, m.p. 181-182 ºC (Lit. 183-185 ºC)[17]. UV (MeOH) λ_{max} nm (log ε): 207 (2.44), 249 (2.83), 309 (0.76), 385 (0.45). IR (KBr, ν_{max}, cm^{-1}): 3440 (OH), 2926 (asym. sp^3 C-H stretch), 2856 (sym. sp^3 C-H stretch), 1732 (C=O), 1674 (C=O unchelated), (C=O chelated), 1590, 1426 (C=C), 1212 (C-O). EI-MS m/z (rel. int.): 252 (M^+, 30 %), 224 (100), 196 (15), 168 (31), 150 (20), 139 (57), 119 (8), 69 (21). ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data are in agreement with published data[17].

Morindone-6-methyl-ether (7): Red solid, m.p. 250-251 ºC (Lit. 252-253 ºC)[17]. UV (MeOH) λ_{max} nm (log ε): 299 (0.03), 303 (0.22), 405 (0.42), 420 (0.60), 435 (0.75), 448 (0.79), 462 (0.70), 480 (0.44). IR (KBr, ν_{max}, cm^{-1}): 3438 (br, OH), 2924 (asym. sp^3 C-H stretch), 2855 (sym. sp^3 C-H stretch), 1620 (C=O), 1461 (aromatic C=C), 1254 and 1055 (C-O). EI-MS m/z (rel. int.): 284 (M^+, 100 %), 269 (35), 255 (95), 241 (34), 185 (24), 169 (3), 157 (8), 139 (11), 115 (11), 99 (9), 92 (12), 77 (29), 63 (16), 51 (18). ^1H NMR (400 MHz, $CDCl_3$) data are in agreement with published data[18].

RESULTS AND DISCUSSION

Compound **2** is a yellow solid, with m.p. 123-124 ºC. Based on the molecular ion peak at m/z 296, in the EI-MS spectrum, the molecular formula of this compound was deduced to be $C_{18}H_{16}O_4$. The alpha cleavage of the molecule at C-13 to yield a fragment ion that bears a positive charge on the oxygen was shown by the weak fragment ion peak at m/z 282. Further fragmentation that occurs on this fragmented ions, ($C_{17}H_{13}O_4^+$) as a rearrangement reaction forms another intense peak at m/z 251. The UV spectrum of **1** revealed characteristic absorption bands of an anthraquinone skeleton at 210, 227, 250 and 335 nm. The presence of the hydroxyl group at position **1** in ring A was confirmed by the peak at 402 nm. Meanwhile, the presence of chelated and unchelated quinone carbonyls was exhibited clearly by two intense peaks in the FTIR spectrum at 1632 and 1671 cm^{-1}. These fragmentation information, NMR and IR data, indicated the presence of the ethoxy-ethyl moiety in the structure of the compound.

Besides the singlet for the hydroxyl proton at δ 13.54, the ^{1}H NMR of **2** also showed two-proton triplets at δ 4.50 (*J* = 7.3 Hz, H-12) and δ 1.44 (*J* = 7.3 Hz, H-11) and broad triplets at δ 3.88 (H-13) and δ 1.23 (H-14). These signals indicated the presence of oxygenated -CH_2CH_2- and ethyl groups. In addition, the ^{13}C NMR and HMQC spectral analysis supported this suggestion by showing carbon peaks at δ: 29.7 (1.44), 64.0 (4.50), 69.2 (3.88) and 14.3 (1.23) for C-11, C-12, C-13 and C-14, respectively. Furthermore, the couplings of H-12 (δ 4.50) to H-11 (δ 1.44) and H-13 (δ 3.88) as displayed by the COSY spectrum affirmed the presence of the ethoxy-ethyl moiety. Meanwhile, the doublets at δ 8.24 (*J* = 7.8 Hz, H-3) and 7.84 (*J* = 7.8 Hz, H-4) were assigned to the remaining protons in the 1,2-disubstituted ring A of this anthraquinone. This assignment can be explained not only by the splitting pattern of these two protons, but also the confirmation by the HMBC and COSY spectra. From the HMBC experiment (Table-2), long-range connectivities ^3J were observed between H-3 (δ 8.24) and C-1 (δ 162.4) and between H-4 (δ 7.84) and C-2 (δ 125.1). H-3 (δ 8.24) and H-4 (δ 7.84) were also observed to have cross peaks with each other in the COSY spectra.

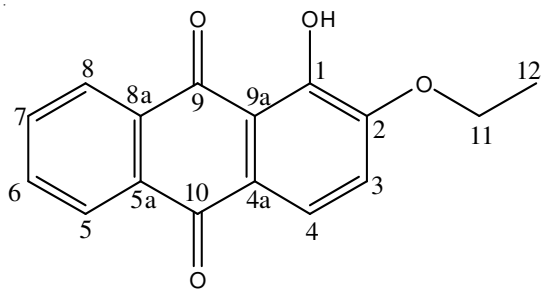
TABLE-2
HMBC AND COSY CORRELATIONS OF COMPOUND **2**

Position	HMBC	COSY
1-OH	C-1 (162.4) (^2J), C-2 (125.1) (^3J)	
3	C-1 (162.4) (^{3}J), C-4a (^{3}J)	H-4 (7.84)
4	C-2 (136.0) (^{3}J), C-9a (117.2) (^{3}J), C-10 (182.0) (^{3}J)	H-3 (8.24)
5	C-7 (134.8) (^{3}J)	H-6 (7.85)
6		H-5 (8.36)
7		H-8 (8.36)
8	C-7 (134.8) (^{2}J)	H-7 (7.85)
11		H-12 (4.50)
12		H-11 (1.44), H-13 (3.88)
13		H-12 (4.50)

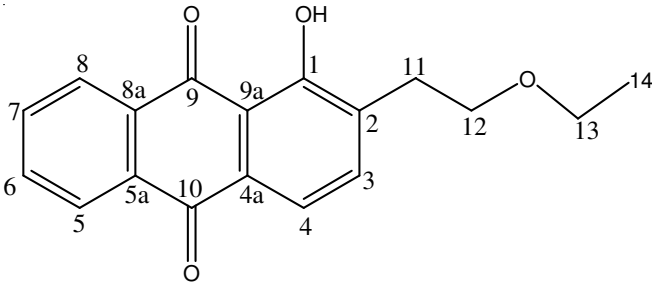
On the other hand, signals at δ 8.36 (H-5, H-8) and δ 7.85 (H-6, H-7) in the ^{1}H NMR spectrum were clearly shown to consist of two protons in each signal. Thus, they were assigned to the protons of the unsubstituted ring C as follows: δ 8.36 as H-5 and H-8 and δ 7.85 as H-6 and H-7. The HMBC spectrum displayed the ^2J and ^3J couplings of both H-5 (δ 8.36) and H-8 (δ 8.36) to C-7 (δ 134.8), respectively. Meanwhile, from the COSY spectrum, cross peaks were observed between H-5 (δ 8.36) and H-6 (δ 7.85) and between H-7 (δ 7.85) to H-8 (δ 8.36).

Hence, the structure of compound **2** is elucidated as 2-(ethoxy-ethyl)-1-hydroxyanthraquinone and the spectral data are summarized in Table-1.

Compound **1**, bright yellow soild, $C_{16}H_{12}O_4$, m.p. 123-124 ºC, displayed a quartet at δ 4.47 (H-11) and a three-proton triplet at δ 1.44 (H-12), which coupled to each other. These signals indicated the presence of the CH_3CH_2- group in the structure. Meanwhile, the presence of the ethoxy moiety (O-CH_2CH_3) was suggested when the quartet was observed to be correlated to the carbon peak at δ 164.7 in the HMBC spectrum, implying the presence of an oxygenated carbon. The suggestion was further confirmed by the HMQC spectral analysis where these protons were seen to be attached to the carbons at δ 61.7 (C-11) and δ 14.2 (C-12), respectively. The hydroxyl proton was found as a singlet proton at δ 13.54 and was assigned to position **1**.

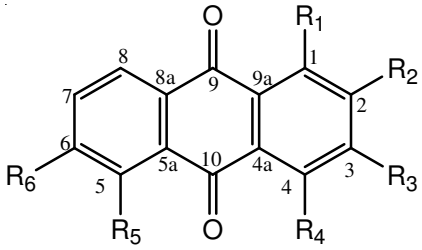


(**1**)



(**2**)

The other compounds which are 1-hydroxy-2-methyl-anthraquinone (**3**), damnacanthal (**4**), nordamnacanthal (**5**) and 2-formyl-1-hydroxyanthraquinone (**6**) and morindone-6-metylether (**7**) were identified by their spectral data and by comparison with literature data[13-17], respectively.



Anthraquinones	R1	R2	R3	R4	R5	R6
1-Hydroxy-2-methyl anthraquinone (**3**)	OH	CH_3	H	H	H	H
Damnacanthal (**4**)	OMe	CHO	OH	H	H	H
Nordamnacanthal (**5**)	OH	CHO	OH	H	H	H
2-Formyl-1-hydroxyanthraquinone (**6**)	OH	CHO	H	H	H	H
Morindone-6-methylether (**7**)	OH	CH_3	H	H	OH	OMe

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