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CYTOTOXIC ANTILEUKEMIC ANTHRAQUINONES FROM *MORINDA PARVIFOLIA**

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Key Word Index—*Morinda parvifolia*; Rubiaceae; anthraquinones; antitumour activity.

Abstract—Bioassay-directed fractionation of an antileukemic extract of *Morinda parvifolia* (Hong-Zhu-Teng) has led to the isolation and characterization of cytotoxic anthraquinones which include the new morindaparvin-B and the known lucidin-ω-ethyl ether, lucidin-ω-methyl ether, digiferruginol, 1-hydroxy-6 or 7-hydroxymethylanthraquinone and 2-hydroxymethylanthraquinone in addition to the previously reported antileukemic morindaparvin-A and alizarin-1-methyl ether. 2-Hydroxymethylanthraquinone demonstrated significant activity (T/C = 150%) in the P-388 lymphocytic leukemia screen at 10 mg/kg/day. The structures of these compounds were determined by physico-chemical data and spectral evidence.

INTRODUCTION

We reported recently on the isolation of morindaparvin-A (**1**), a new anti-leukemic anthraquinone, and alizarin-1-methyl ether (**2**) from *Morinda parvifolia* (Hong-Zhu-Teng or Bai-Yen-Teng), and the antileukemic activity of the related derivatives [1]. Further investigation of the antileukemic fractions after the isolation of **1** and **2** has now led to the isolation of the new morindaparvin-B (**3**) and five known anthraquinones lucidin-ω-ethyl ether (**4**), lucidin-ω-methyl ether (**5**), digiferruginol (**6**), 1-hydroxy-6- or 7-hydroxymethylanthraquinone (**7**) and 2-hydroxy-methylanthraquinone (**8**) (Table 1). Compounds **3**, **5**, **6**, **7** and **8** showed significant cytotoxicity with ED_{50} = 4.0, 0.62, 0.09, 2.6 and 2.6 μg/ml, respectively, against the *in vitro* growth of KB tissue culture cells. Compound **8** also demonstrated a T/C = 150% against the *in vivo* growth of P-388 lymphocytic leukemia in mice at 10 mg/kg/day. The structures of **3**–**8** were determined based upon physico-chemical data and spectral evidence.

RESULTS AND DISCUSSION

Compounds **3**–**8** were isolated by silica gel column and repeated preparative thin-layer chromatography from the active fractions after the isolation of **1** and **2**. Morindaparvin-B (**3**), $C_{15}H_{10}O_5$, exhibited a molecular ion peak at *m/z* 270 as base peak in the mass spectrum. Compound **3** is an anthraquinone bearing two hydrogen-bonded hydroxy groups as revealed by its comparable UV-VIS spectrum with a 1,5-dihydroxyanthraquinone system.‡ This is confirmed by the presence of two hydrogen-bonded carbonyl groups (1635 and 1610 cm^{-1}) in the IR spectrum and two hydrogen-bonded hydroxyl groups (δ12.68 and 13.07, exchangeable with D_2O) in a 250 MHz NMR ($CDCl_3$) spectrum.

The presence of a hydroxymethyl group in **3** was indicated by a one-proton triplet at δ5.31 (*J* = 5.3 Hz, $CH_2O\underline{H}$) [$CDCl_3$: DMSO-d_6 (2:1)], which disappeared upon addition of D_2O, and a two-proton doublet at δ4.73 (*J* = 5.3 Hz, $C\underline{H}_2OH$) in the NMR spectrum, and substantiated by an intense mass peak at *m/z* 241 ($[M-29]^+$, 52%) [4].

The assignment of the remaining five aromatic protons, which appeared at δ7.34–7.87, in the NMR spectrum of **3** was achieved by double resonance experiments. Thus, irradiation of H-6 (δ7.34, *dd*, *J* = 8.8 and 1.3 Hz) converted a pair of double doublets at δ7.69 (*J* = 8.8 and 7.5 Hz, H-7) and 7.85 (*J* = 7.5 and 1.3 Hz, H-8) into two doublets (*J* = 7.5 Hz). Irradiation of H-7 collapsed the signals of H-6 and H-8 to a pair of doublets (*J* = 1.3 Hz). The remaining AB quartet (δ7.80 and 7.92, *J* = 7.90 Hz), which was intact during the irradiation process, was assigned to two adjacent protons with δ7.80 for H-3 and δ7.92 for H-4 as the latter appeared at low field comparable to H-8, which was adjacent to the quinone carbonyl.

The above evidence established the structure of **3** as 1,5-dihydroxy-2-hydroxymethylanthraquinone.§ Added confirmation was based upon the evidence that the mass spectral fragmentation pattern of **3** was identical with that of 1-hydroxy-2-hydroxymethylanthraquinone (**6**) [5] except that the former showed 16 mass units more than the latter.

*Part 67 in the series "Antitumor Agents". For Part 66 see I. H. Hall, Y. F. Liou, C. B. Oswald and K. H. Lee, *J. Pharm. Sci.* (submitted).

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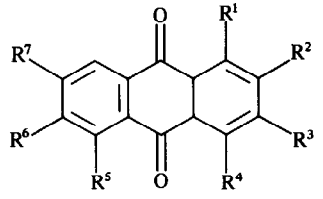
‡For example, 1,5-dihydroxyanthraquinone showed λ_{max}^{EtOH} nm (log ε): 254 (4.33), 275 (sh, 4.05), 285 (4.03), 418 (4.00) and 432 (4.00). 1,5-Dihydroxy-3-hydroxymethylanthraquinone showed λ_{max}^{EtOH} nm (log ε): 254 (4.37), 279 (sh, 4.03), 289 (4.04), 418 (4.06) and 428 (4.06) [2, 3].

§An alternate proof of the structure of **3** by a total synthesis has recently been achieved in this laboratory [P. Chang and K. H. Lee, unpublished data].

Table 1. Cytotoxic antileukemic anthraquinones isolated from *M. Parvifolia*



Compound	Formula	MW	Quantity isolated (mg)	Yield (% dry wt)	R^1	R^2	R^3	R^4	R^5	R^6	R^7	ED_{50}* P-388 (μg/ml)	ED_{50} KB (μg/ml)	T/C (%)† P-388 (mg/kg)
1	$C_{15}H_8O_4$	252	37	0.00081	$-OCH_2O-$		H	H	H	H	H	1.85	10	112 (20), 129 (10)
2	$C_{15}H_{10}O_4$	254	95	0.00208	OMe	OH	H	H	H	H	H	10	inactive	136 (10)
3	$C_{15}H_{10}O_5$	270	7	0.00015	OH	CH_2OH	H	H	OH	H	H	10.5	4.0	
4	$C_{17}H_{14}O_5$	298	3	0.00007	OH	CH_2OEt	OH	H	H	H	H			
5	$C_{16}H_{12}O_5$	284	5	0.00011	OH	CH_2OMe	OH	H	H	H	H	inactive	0.62	
6	$C_{15}H_{10}O_4$	254	5	0.00011	OH	CH_2OH	H	H	H	H	H	5.9	0.09	94 (10)
7	$C_{15}H_{10}O_4$	254	12	0.00026	OH	H	H	H	H	CH_2OH	H	inactive	2.6	
8	$C_{15}H_{10}O_3$	238	20	0.00044	H	CH_2OH	H	H	H	H	H	inactive	2.6	150 (10)

*ED_{50}: The drug concentration in μg/ml which inhibits the growth by 50% as compared with controls. KB (human epidermoid carcinoma of the nasopharynx) response of $\leqslant 4$ μg/ml is considered as indicative of cytotoxicity [6].

†T/C: The ratio of mean survival rate of treat animls to the mean survival rate of control animals. According to NCI protocols, T/C $\geqslant 125\%$ is required for significant activity in the P-388 lymphocytic leukemia screen [6].

EXPERIMENTAL

General procedures. Mps are uncorr. ^{1}H NMR spectra were determined at 60 or 250 MHz, chemical shifts were given in δ-values with TMS as the int. standard. MS were recorded using a direct inlet system at 70 eV. CC was performed using silica gel; fractions (250 ml each) were monitored by TLC and assayed by an *in vitro* P-388 lymphocytic leukemia tissue culture method [6]. Prep. TLC was carried out on silica gel G (1000 microns) and solvents as indicated below; compounds were visualized by UV lights and recovered by $CHCl_3$–Me_2CO (1:1).

Plant material. The rhizome and root of *M. parvifolia* used were collected in December 1979 in Mt. Kuan-Ying, Kaohsiung Shen, Taiwan. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction. The ground air-dried rhizome and root (4.55 kg) were exhaustively extracted with MeOH; after concn a syrup-like material remained. Guided by the *in vivo* P-388 assay [6], this active extract was dissolved in MeOH–H_2O (3:1) and extracted with hexane (1 l., × 6). The aq. layer was concd and then extracted with $CHCl_3$ (1 l., × 5). The active $CHCl_3$ layer, when combined, dried over Na_2SO_4, and then evaporated *in vacuo* gave 43.2 g of a residue.

Isolation of anthraquinones. The foregoing residue (43.2 g) was chromatographed on silica gel eluted with $CHCl_3$ followed by conventional adsorption CC techniques. Fractions 1–4 which showed significant cytotoxicity, were combined and purified by repeated prep. TLC ($CHCl_3$). The main component which exhibited yellow fluorescence under UV light was recovered to afford 37 mg of morindaparvin-A (**1**) as fluffy crystals after recrystallization from $CHCl_3$.

Fractions 5–7 were combined and subjected to prep. TLC ($CHCl_3$–Me_2CO, 15:1). Two orange–yellow bands gave, on treatment in the usual way, 3 mg of lucidin-ω-ethyl ether (**4**) and 5 mg of lucidin-ω-methyl ether (**5**). The main components of fractions 12–15 were rechromatographed on a silica gel column followed by prep. TLC ($CHCl_3$–Me_2CO, 15:1). A major band was purified by prep. TLC (use the same solvents) and recrystallized from MeOH to give 95 mg of alizarin-1-methyl ether (**2**). Another band was separated and purified by repeated prep TLC using the same solvents to give, after crystallization from $CHCl_3$, 7 mg of morindaparvin-B (**3**) and 5 mg of digiferruginol (**6**).

The combined fractions 16–27 were separated by CC and purified by prep. TLC ($CHCl_3$), followed by crystallization from $CHCl_3$ to afford 12 mg of 1-hydroxy-6- or 7-hydroxymethylanthraquinone (**7**) and 20 mg of 2-hydroxymethylanthraquinone (**8**).

Morindaparvin-B (**3**). Orange–yellow needles, mp 208.5–209.5°. UV λ_{max}^{EtOH} nm (log ε): 227 (4.32), 255 (4.13), 277 (sh) (3.64), 287.5 (3.65), 420 (3.64) and 430 (3.65), bathochromic shifts upon addition of KOH, and color changed to orange–red. MS *m/z* (rel. int.): 270 $[M]^+$ (100), 252 (9), 241 (51), 224 (13), 213 (4), 196 (5), 168 (6), 139 (9) and 121 (8). [Found: M^+, 270.0524; $C_{15}H_{10}O_5$ requires: 270.0527.]

Lucidin-ω-ethyl ether (**4**)*. Orange–yellow powder, mp 151° (dec.) (lit. [7] 168–170°, lit [8] 182–183°). UV λ_{max}^{EtOH} nm (log ε): 245 (4.21), 277 (4.11), 313 (sh) (3.71) and 410 (3.33), bathochromic shifts in KOH soln and color changed to red; IR ν_{max}^{neat} cm^{-1}: 3220 (OH), 1673 (C=O), 1625 (C=O, chelated) and 1585; ^{1}H NMR (250 MHz, $CDCl_3$): δ1.35 (3H, *t*, *J* = 7.8 Hz, –OCH_2CH_3), 3.74 (2H, *q*, *J* = 7.8 Hz, –OCH_2CH_3), 4.98 (2H, *s*, benzyl-CH_2), 7.32 (1H, *s*, H-4), 7.76 (2H, *m*, H-6 and H-7), 8.21 (2H, *m*, H-5 and H-8), 9.65 (1H, *br*, 3-OH, exchangeable with D_2O) and 13.30 (1H, *s*, 1-OH, exchangeable with D_2O); MS *m/z* (rel. int.): 298 $[M]^+$ (25), 269 (11), 254 (34), 253 (57), 252 (100), 224 (8), 196 (15), 168 (7) and 139 (10). [Found: M^+, 298.0844; Calc for $C_{17}H_{14}O_5$: 298.0840.] This compound was identical with an authentic sample (IR, NMR, TLC) [7].

Lucidin-ω-methyl ether (**5**)*. Orange–yellow powder, mp 170° (dec.) (lit. [7] 163–166°). UV λ_{max}^{EtOH} nm (log ε): 246 (4.51), 282 (4.40), 315 (sh) (4.07) and 417 (3.72) bathochromic shifts in KOH soln and color changed to red; IR ν_{max}^{neat} cm^{-1}: 3180 (OH), 1673 (C=O) 1625 (C=O, chelated) and 1592; ^{1}H NMR (60 MHz $CDCl_3$): δ3.57 (3H, *s*, OMe), 4.92 (2H, *s*, benzyl-CH_2), 7.30 (1H, *s*, H-4), 7.73 (2H, *m*, H-6 and H-7), 8.24 (2H, *m*, H-5 and H-8) and 13.27 (1H, *s*, 1-OH, exchangeable with D_2O); ^{1}H NMR (250 MHz, Me_2CO-d_6): δ3.42 (3H, *s*, OMe), 4.71 (2H, *s*, benzyl-CH_2), 7.32 (1H, *s*, H-4), 7.92 (2H, *m*, H-6 and H-7), 8.26 (2H, *m*, H-5 and H-8) and 13.35 (1H, *s*, 1-OH), H-4 had no NOE upon irradiation of δ3.42 or 4.71; MS *m/z* (rel. int.): 284 $[M]^+$ (30), 269 (10), 254 (10), 253 (32), 252 (100), 224 (10), 196 (31), 168 (9) and 139 (11). [Found: M^+, 284.0687; Calc for $C_{16}H_{12}O_5$: 284.0684.] The IR, UV and MS spectral data of **5** were identical with those of lucidin-ω-methyl ether reported in the literature [7] except for the NMR data which were not reported.

Digiferruginol (**6**). Orange–yellow needles, mp 209° (lit. [9] 210°]; UV λ_{max}^{MeOH} nm (log ε): 223 (4.24), 253 (4.47), 330 (3.35) and 405 (2.61), bathochromic shifts in KOH soln and color changed to red; IR ν_{max}^{neat} cm^{-1}: 3200 (OH), 1671 (C=O), 1635 (C=O, chelated) and 1594; ^{1}H NMR [60 MHz, $CDCl_3$: DMSO-d_6 (2:1)]: δ4.75 (2H, *d*, *J* = 5.5 Hz, $C\underline{H}_2OH$), 5.22 (1H, *t*, *J* = 5.5 Hz, $CH_2O\underline{H}$, exchangeable with D_2O), 7.75 (1H, *d*, *J* = 8 Hz, H-3), 7.85 (2H, *m*, H-6 and H-7), 7.89 (1H, *d*, *J* = 8 Hz, H-4), 8.25 (2H, *m*, H-5 and H-8) and 12.82 (1H, *s*, 1-OH, exchangeable with D_2O); ^{1}H NMR (60 MHz, $CDCl_3$): δ2.34 (1H, *t*, *J* = 6.1 Hz, $CH_2O\underline{H}$), 4.87 (2H, *d*, *J* = 6.1 Hz, $C\underline{H}_2OH$), 7.83 (4H, *m*, H-3, H-4, H-6 and H-7), 8.30 (2H, *m*, H-5 and H-8) and 13.04 (1H, *s*, 1-OH); MS *m/z* (rel. int.): 254 $[M]^+$ (100), 236 (13), 225 (62), 208 (11), 197 (5), 180 (6) and 152 (13), the MS spectral fragmentation has been reported in the literature [5]. [Found: M^+, 254.0577; Calc for $C_{15}H_{10}O_4$: 254.0577.] This compound was also identified by direct comparisons of its IR and NMR with those of 1-hydroxy-2-hydroxymethylanthraquinone [9].

1-Hydroxy-6- or 7-hydroxymethylanthraquinone (**7**). Orange–yellow crystals, mp 172° (lit. [2] 208–210°). UV λ_{max}^{EtOH} nm (log ε): 210 (4.67), 256 (4.78), 280 (sh) (4.36), 335 (4.32) and 398 (4.58), color changed to red in KOH soln; IR ν_{max}^{neat} cm^{-1}: 3535 (OH), 1662 (C=O), 1631 (C=O, chelated) and 1595; ^{1}H NMR (60 MHz, $CDCl_3$): δ1.98 (1H, *t*, *J* = 6.0 Hz, $CH_2O\underline{H}$, exchangeable with D_2O), 4.93 (2H, *d*, *J* = 6.0 Hz, $C\underline{H}_2OH$), 7.30–8.50 (6H, *m*, aromatic Hs) and 12.64 (1H, *s*, 1-OH, exchangeable with D_2O), ^{1}H NMR (60 MHz, DMSO-d_6): δ5.62 (1H, *t*, *J* = 5.6 Hz, $CH_2O\underline{H}$), 4.93 (2H, *d*, *J* = 5.6 Hz, $C\underline{H}_2OH$), 7.25–8.48, (6H, *m*, aromatic Hs) and 12.47 (1H, *s*, 1-OH); MS *m/z* (rel. int.): 254 $[M]^+$ (100), 226 (13), 225 (50), 208 (7), 197 (9), 180 (6), 169 (5), 168 (4), 152 (8) and 139 (9). [Found: M^+, 254.0577; Calc for $C_{15}H_{10}O_4$: 254.0578]. The IR and NMR spectra of this compound were identical with those of 1-hydroxy-2-hydroxymethylanthraquinone [2].

2-Hydroxymethylanthraquinone (**8**). Pale yellow needles, mp 194° (lit. [10] 192–193°); no color change in KOH soln. IR ν_{max}^{neat} cm^{-1}: 3520 (OH), 1670 (C=O) and 1586; ^{1}H NMR (60 MHz, $CDCl_3$): δ1.99 (1H, *t*, *J* = 6.0 Hz, $CH_2O\underline{H}$, exchangeable with D_2O), 4.91 (2H, *d*, *J* = 6.0 Hz, $C\underline{H}_2OH$), 7.82 (3H, *m*, H-3, H-6 and H-7) and 8.31 (4H, *m*, H-1, H-4, H-5 and H-8); MS *m/z*

*The lucidin ω-ethers could be artefacts derived from lucidin as methanol and chloroform were used as solvents for extraction, although there was no direct proof for this possibility.

(rel. int.): 238 $[M]^+$ (97), 210 (27), 209 (100), 192 (11), 181 (20), 164 (9) and 152 (21). [Found: M^+, 238.0633; Calc for $C_{15}H_{10}O_3$: 238.0629]. This compound was identical with an authentic sample which is commercially available from Aldrich.

Biological activity. Test results (Table 1) were obtained from Professor I. H. Hall of the UNC School of Pharmacy and Professor Y. C. Cheng and Mr. Michael Fisher of the UNC Cancer Research Center. The anthraquinones were tested for both *in vitro* and *in vivo* cytotoxic antileukemic activities against KB and P-388 tissue culture cells and P-388 lymphocytic leukemia growth in BDF_1 male mice, respectively, according to a literature method [6].

Most of the eight anthraquinones isolated showed potent cytotoxic activity in KB cells (Table 1). For example, compound **1** exhibited both cytotoxicity (ED_{50} = 1.85 μg/ml) and antileukemic activity (T/C = 129% at 10 mg/kg) against P-388 lymphocytic leukemia. Compounds **2** and **8** showed significant antileukemic activity with T/C values of 136 and 150%, respectively, at 10 mg/kg. Compound **8** was also reported before as a toxic agent to hypoxic EMT 6 tumor cells in culture [10]. Compound **6** exhibited potent cytotoxicity in KB cells (ED_{50} = 0.09 μg/ml), but failed to produce *in vivo* antileukemic activity at a dose of 10 mg/kg. Because of the scarcity of the samples, the other isolated anthraquinones have not been screened *in vivo*. Thus, compound **4** was not screened *in vitro* due to its scarcity and instability. Compound **4** was reported to have potent mutagenic activity [7].

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