ANTITUMOR AGENTS 50.[1] MORINDAPARVIN-A, A NEW ANTILEUKEMIC ANTHRAQUINONE, AND ALIZARIN-1-METHYL ETHER FROM *MORINDA PARVIFOLIA*, AND THE ANTILEUKEMIC ACTIVITY OF THE RELATED DERIVATIVES

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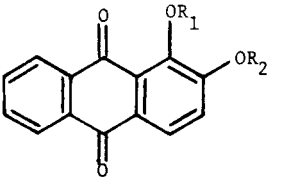
ABSTRACT.—Bioassay directed isolation of an antileukemic extract of *Morinda parvifolia* (Hong-Zhu-Teng) (Rubiaceae) has led to the characterization of morindaparvin-A (**1**), a new cytotoxic and antileukemic anthraquinone, and the known alizarin-1-methyl ether (**2**). The structure of **1** was identified as 1,2-methylenedioxyanthraquinone by synthesis from alizarin (**3**) and dibromomethane. The mono- and di-acetates (**4** and **5**), cinnamates (**6** and **7**) and senecioates (**8** and **9**) of alizarin were prepared and tested for their *in vivo* antileukemic activity against P-388 lymphocytic leukemia growth in BDF_1 mice at 10 mg/kg/day and were found to be inactive, whereas **3** and its related derivatives **2**, **10** (alizarin-2-methyl ether) and **11** (1,2-ethylenedioxyanthraquinone) demonstrated marginal activity (T/C~126–136%) in the P-388 lymphocytic leukemia screen at 10 mg/kg/day.

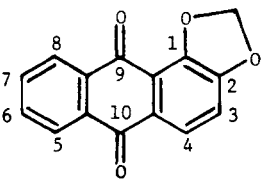
The rhizome and root of *Morinda parvifolia* Bartl. (Rubiaceae) are known as "Hong-Zhu-Teng" (1) or "Bai-Yen-Teng" (2) in Chinese folklore as herbal remedies for the treatment of human bronchitis and whooping cough (2). As a result of a continued search among Chinese medicinal plants for new naturally occurring potential antitumor agents,[1] the methanolic extract of the rhizome and root of *M. parvifolia* was found to show significant inhibitory activity *in vivo* against the P-388 lymphocytic leukemia growth in mice (T/C = 152%) at 50 mg/kg/day, I.P. Bioassay-directed fractionation of the active extract has led to the isolation and characterization of a new cytotoxic and antileukemic compound,[2] morindaparvin-A (**1**) and the known alizarin-1-methyl ether (**2**). The latter lacks cytotoxic activity.[2] Morindaparvin-A showed significant cytotoxicity with an ED_{50} = 1.85 μg/ml against the *in vitro* growth of P-388 lymphocytic leukemia tissue culture cells. Morindaparvin-A (**1**), $C_{15}H_8O_4$, *m/z* 252.0425 (M^+), mp 257° (dec.), was isolated in 0.00081% yield as fluffy crystals. The ir spectrum of **1** showed strong absorption bands at 1675, 1587 and 1455 cm^{-1}, while its uv spectrum indicated absorption maxima at 248 and 273 mμ. These spectral data are characteristic of a 1,2-dioxygenated anthraquinone system (3,4). The absence of absorption bands for hydroxyl groups in the ir spectrum and the presence of a low field two-proton singlet at δ 6.32 in the nmr (100 MHz, $CDCl_3$, TMS) spectrum suggested the presence of a methylenedioxy group in **1**. This methylenedioxy group was assigned to the 1,2-position as H-3 and H-4 appeared as a pair of doublets at δ 7.15 (J = 9 Hz, H-3) and 7.98 (J = 9 Hz, H-4), respectively. The protons of the unsubstituted aromatic ring in **1** were observed as multiplets at δ 7.80 (2H, H-6 and H-7) and 8.31 (2H, H-5 and H-8). On the basis of these data, **1** was regarded as a 1,2-methylenedioxyanthraquinone. The confirmation of the structure of **1** was determined by a direct comparison of the physical data with a product synthesized from alizarin (**3**) and dibromomethane.

[1]For part 49 see K. H. Lee, K. Tagahara, H. Suzuki, R. Y. Wu, M. Haruna, I. H. Hall, H. C. Huang, K. Ito, T. Iida and J. S. Lai, *J. Nat. Prod.*, **44**, 530 (1981).

[2]Compounds **1** and **2** were all inactive in the KB test system (ED_{50} > 10 μg/ml). Compound **2** was also inactive in the *in vitro* P-388 lymphocytic leukemia test system with an ED_{50} = 27 μg/ml.

Compound **2**, $C_{15}H_{10}O_4$, *m/z* 254.0575 (M^+), mp 179°,[3] was isolated as orange-yellow needles in 0.0021% yield. Compound **2** showed the presence of a hydroxyl group (ir band at 3580 cm^{-1} and nmr one-proton broad singlet at δ 6.82, which disappeared upon addition of D_2O) and an aromatic methoxy group (nmr three-proton singlet at δ 4.03). The lack of methylenedioxy methylene moiety as seen in **1**, coupled with the similarity of the remaining nmr (60 MHz, $CDCl_3$, TMS) aromatic resonances [δ 7.34 (d, 1H, $J=9.0$ Hz, H-3), 8.10 (d, 1H, $J=9.0$ Hz, H-4), 7.73 (m, 2H, H-6 and H-7) and 8.24 (m, 2H, H-5 and H-8)] of **2** compared to **1**, led to the assignment of the structure of 1-methoxy-2-hydroxyanthraquinone, i.e., alizarin-1-methyl ether, for **2**. Alizarin-1-methyl ether was recently isolated from *Morinda lucida* (3-5).





2, R_1 = Me, R_2 = H

1

3, $R_1 = R_2$ = H

4, R_1 = H, R_2 = COMe

5, $R_1 = R_2$ = COMe

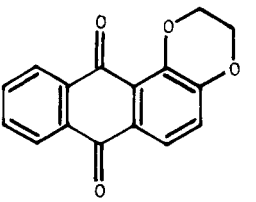
6, R_1 = H, R_2 = CO-CH=CH-(1' 2' 3')

7, $R_1 = R_2$ = CO-CH=CH-

8, R_1 = H, R_2 = CO (2' 3' 4' 5' 1')

9, $R_1 = R_2$ = CO

10, R_1 = H, R_2 = Me



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Due to the fact that structures **1** and **2** are actually derivatives of alizarin (**3**) which is also commercially available,[4] alizarin was selected as a model compound for a structure-antileukemic activity relationships study. Alizarin was first assayed for its *in vivo* antileukemic activity against P-388 lymphocytic leukemia growth in BDF_1 mice according to the standard National Cancer Institute procedures (6). Since alizarin (**1**) gave a significant antileukemic activity[5] with a T/C = 134% at 10 mg/kg in this test system,[6] esters of **1**, such as the acetates (**4** and **5**), the cinnamates (**6** and **7**) and the senecioates (**8** and **9**), were prepared by general methods for possible enhancement of the *in vivo* antileukemic activity. The importance of an ester group in conferring cytotoxicity and antileukemic activity on compounds in certain series has been described (8,9). The assignment of the hydroxyl group at C-1 instead of at C-2 for compounds **4**, **6**, and **8** was based upon the extremely low field one-proton hydroxy doublets at δ 12.76, 12.83 and 12.80, respectively, resulting from the hydrogen bonding between the C-1 hydroxyl group and the C-9 carbonyl. The lack of *in vivo* antileukemic activity

[3]Lit. 3 reported mp 182-184° for alizarin-1-methyl ether.

[4]Product of Aldrich Chemical Co., Milwaukee, Wisc.

[5]According to NCI protocol (6,10) T/C ≥ 120% is required for significant activity in the P-388 screen.

[6]Alizarin was previously reported by NCI (7) to be active in the Adenocarcinoma 755 assay, causing 60% reduction of tumor weight increase at a dosage of 450 mg/kg in mice.

of these esters (**4–9**) and the marginal antileukemic activity demonstrated by compounds **1** (T/C=129%) and **2** (T/C=136%) in the P–388 systems at 10 mg/kg/day led to the further synthesis of analogous compounds alizarin-2-methyl ether (**10**) and 1,2-ethylenedioxyanthraquinone (**11**). Compounds **10** and **11** were prepared by methylation of **3** with diazomethane and by treatment of **3** with 1,2-dibromoethane, respectively. Compounds **10** and **11** exhibited the same marginal antileukemic activity in the P–388 screen with T/C=126% and 130%, respectively, at 10 mg/kg/day level.

EXPERIMENTAL[7]

PLANT MATERIAL.—The rhizome and root of *Morinda parvifolia* (Rubiaceae) used was from a collection made in December 1979 in Mt. Kuan-Ying, Kaohsiung Shen. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

EXTRACTION OF *M. parvifolia*.—The ground air-dried rhizome and root (4.55 kg) were exhaustively extracted with methanol; after removal of the solvent, a syrup remained. Guided by the *in vivo* P–388 assay (6), this active syrup was dissolved in methanol-water (3:1) and extracted several times with hexane. The aqueous layer was concentrated and then extracted several times with chloroform. The active chloroform layers, when combined, dried over anhydrous sodium sulfate, and then evaporated *in vacuo* gave 43.2 g of a residue.

ISOLATION OF MORINDAPARVIN-A (**1**) AND ALIZARIN-1-METHYL ETHER (**2**).—The foregoing residue (43.2 g) was chromatographed on silica gel (2.1 kg), eluted with chloroform (4.5 liters), chloroform-acetone [3:1 (4 liters), 7:3 (1.5 liters), 3:2 (0.5 liter), 1:1 (0.5 liter), 1:2 (0.5 liter) and 1:4 (0.5 liter)], acetone (0.75 liter), acetone-methanol [10:1 (0.25 liter), 5:1 (0.25 liter), 3:1 (1.5 liters), 2:1 (2 liters), 1:1 (1 liter), 1.5:3.5 (1 liter) and 1:5 (1 liter)] and methanol (4.5 liters). Fractions of 250 ml each were collected, examined by tlc, and assayed by an *in vitro* P–388 lymphocytic leukemia tissue culture method. Fractions 1–9, which showed significant cytotoxicity, were combined and purified by repeated preparative thin-layer chromatography on silica gel. The main component which exhibited yellow fluorescence under short and long wave uv light was extracted with chloroform-acetone (1:1) to afford 37 mg of **1** as fluffy crystals after recrystallization with chloroform. Compound **1**, mp 257° (dec.), showed ir bands (neat) at 1675, 1587, 1455, 1295, 1260, 1075, 1040, 994 and 934 cm^{-1}. The mass spectrum revealed M^+ at m/z 252.0425 ($C_{15}H_8O_4$ requires 252.0422) and other pertinent mass peaks at m/z 223 (M^+–CO) and 196 (M^+–CO–CO).

Combination of fractions 12–15 followed by rechromatography on silica gel column (200 g) in chloroform (250 ml) yielded compound **2**. Further purification by preparative tlc and recrystallization from methanol gave 95 mg of **2** as orange-yellow needles: mp 179°; ir (neat) 3580 (OH), 1670, 1632, 1560, 1380, 1330, 1310, 1250, 1054 and 970 cm^{-1}; and mass spectrum: m/z 254.0575 (M^+, $C_{15}H_{10}O_4$ requires 254.0578), 236 (M^+–H_2O), 226 (M^+–CO) and 208 (M^+–H_2O–CO).

SYNTHESIS OF MORINDAPARVIN-A (**1**) FROM ALIZARIN (**3**).—To a stirred suspension of alizarie (**3**, 4.8 g) in *N,N*-dimethylformamide (44.5 ml), potassium carbonate (0.6 g), cuprous oxidn (4.8 g) and then dibromomethane (10.44 g) were added slowly. The mixture was heated at 105° with reflux for 9 hours and filtered. The filtrate was evaporated under reduced pressure to dryness. The residue was washed several times with 1N sodium hydroxide and extracted with chloroform (1.5 liters). The chloroform extract was evaporated *in vacuo* ,dried and crystallized from methanol to furnish 2 g of yellow crystals which was identical by mixed mp, tlc, ir and nmr spectra to that of morindaparvin-A(**1**) described above.

ALIZARIN-2-ACETATE (**4**) AND ALIZARIN-1,2-DIACETATE (**5**).—Acetylation of alizarin (**3**, 5 g) with acetic anhydride (4 g) and pyridine (3 ml) at room temperature for 5 hours under stirring followed by the usual workup yielded products **4** and **5**. These two compounds were further purified by column chromatography on silica gel (320 g) in chloroform.

Compound **4** (0.678 g, orange needles) gave the following data: mp 199–201° [chloroform-ethanol (1:4)]; ir (KBr) 3500, 1775, 1675, 1640, 1592, 1440, 1368, 1290, 1270, 1200, 1000 and 890 cm^{-1}; and nmr (60 MHz, $CDCl_3$) δ 2.41 (3H, s, OCO*Me*), 7.45 (1H, d, J=9 Hz, H–3), 7.80 (1H, d, J=9 Hz, H–4), 7.81 (2H, m, H–6 and H–7), 8.29 (2H, m, H–5 and H–8) and 12.76 (1H, d, J=1 Hz, 1-*OH*) Calc. for $C_{16}H_{10}O_6$: C, 68.08; H, 3.57. Found: C, 67.53, H, 3.14.

Compound **5** (2.9 g, yellow needles) gave the following data: mp 182–184° [chloroform-ethanol (1:5)]; ir (KBr) 1775, 1675, 1589, 1422, 1367, 1320, 1280, 1200, 1030, 1000, 900 and 860

[7]Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-nuclear magnetic resonance (^{1}H-nmr) spectra were recorded on a Varian XL 100 and Jeol JNM-FX 60 spectrometers and are given in parts per million (δ) downfield from an internal tetramethylsilane (TMS) standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV with a direct inlet system. The abbreviation M^+ refers to the molecular ion. Silica gel for column chromatography refers to Mallinckrodt CC-7. Silica gel for preparative thin layer chromatography (ptlc) refers to Analtech silica gel G (1000 microns). Compounds were visualized by ultraviolet light.

cm^{-1}; nmr (60 MHz, $CDCl_3$) δ 2.37 (3H, s, 2-OCO*Me*), 2.50 (3H, s, 1-OCO*Me*), 7.60 (1H, d, J=9 Hz, H-3), 8.30 (1H, d, J=9 Hz, H-4), 7.75 (2H, m, H-6 and H-7), and 8.20 (2H, m, H-5 and H-8); Calc. for $C_{18}H_{12}O_6$: C, 66.67; H, 3.73. Found: C, 66.41, H, 3.54.

ALIZARIN-2-CINNAMATE (**6**) AND ALIZARIN-1,2-DICINNAMATE (**7**).—To a mixture of **3** (5 g) in dry benzene (30 ml) and pyridine (3.5 ml) was added cinnamoyl chloride (7 g). The mixture was refluxed for 3 hours and worked up as usual to yield a residue which, upon column chromatography on silica gel (450 g) in chloroform, afforded compounds **6** and **7**.

Compound **6** (1.85 g, orange crystals) exhibited the following properties: mp 209–210° [chloroform-ethanol (1:4)]; ir (KBr) 3420, 1736, 1720 (shoulder), 1668 1640, 1593, 1428, 1355, 1280, 1259, 1235 and 1120 cm^{-1}; nmr (60 MHz, $CDCl_3$); δ 7.55 (1H, d, J=9 Hz, H-3), 7.93 (1H, d, J=9 Hz, H-4), 8.30 (2H, m, H-5 and H-8), 7.81 (2H, m, H-6 and H-7), 7.95 (1H, d, J=16.5 Hz, H-3'), 6.70 (1H, d, J=16.5 Hz, H-2'), 7.54 (5H, m, cinnamoyl aromatic protons) and 12.83 (1H, d, J=1 Hz, 1-*OH*); Calc. for $C_{23}H_{14}O_5$: C, 74.59; H, 3.81. Found: C, 74.05; H, 3.58.

Compound **7** (5.3 g, yellow needles) gave the following data: mp 205.5–206.5° [chloroform-methanol (1:4)]; ir (KBr) 1740, 1672, 1620, 1589, 1330, 1275 and 1115 cm^{-1}; nmr (60 MHz, $CDCl_3$) δ 7.41 (1H, d, J=9 Hz, H-3), 7.75 (1H, d, J=9 Hz, H-4), 8.13 (2H, m, H-5 and H-8), 7.64 (2H, m, H-6 and H-7), 8.13 (1H, d, J=16.5 Hz) and 7.96 (1H, d, J=16.5 Hz) (two H-3'), 6.78 (1H, d, J=16.5 Hz) and 6.60 (1H, d, J=16.5 Hz) (two H-2') and 7.40 (10H, m, two cinnamoyl aromatic protons); Calc. for $C_{32}H_{20}O_6$: C, 76.79; H, 4.03. Found: C, 76.44; H, 3.81.

ALIZARIN-2-SENECIOATE (**8**) AND ALIZARIN-1,2-DISENECIOATE (**9**).—To a mixture of **3** (2.5 g) in dry benzene (20 ml) and pyridine (2 ml) was added senecioyl chloride (2.47 g). The mixture was refluxed for 1 hour and worked up as described above for the preparation of **6** and **7** to give **8** and **9**.

Compound **8** (1.6 g, orange crystals) exhibited the following properties: mp 175–177.5° [chloroform-ethanol (1:3)]; ir (KBr) 3480, 1745, 1665, 1646, 1591, 1435, 1350, 1280, 1260, 1240, 1213, 1118 and 1065 cm^{-1}; nmr (60 MHz, $CDCl_3$) δ 2.04 (3H, d, J=1.5 Hz, Me-5'), 2.28 (3H,d, J=1.5 Hz, Me-4'), 6.02 (1H, m, H-2'), 7.50 (1H, d, J=9.0 Hz, H-3), 7.85 (1H, d, J=9.0 Hz, H-4), 8.25 (2H, m, H-5 and H-8), 7.78 (2H, m, H-6 and H-7) and 12.80 (1H, d, J=1 Hz, 1-*OH*); Calc. for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.36; H, 4.19.

Compound **9** (2.4 g, yellow crystals) gave the following data: mp 154–156° [chloroform-ethanol (1:4)]; ir (KBr) 1742, 1675, 1645, 1585, 1325, 1318, 1278, 1215, 1123, 1060 and 930 cm^{-1}; nmr (60 MHz $CDCl_3$) δ 2.01 (3H, d, J=1.5 Hz) and 2.03 (3H, d, J=1.5 Hz) (two Me-5'), 2.23 (6H, s, two Me-4'), 5.92 (1H, m) and 6.10 (1H, m) (two H-2'), 7.64 (1H, d, J=9.0 Hz, H-3), 8.30 (1H, d, J=9.0 Hz, H-4), 8.21 (2H, m, H-5 and H-8), 7.75 (2H, m, H-6 and H-7); Calc. for $C_{24}H_{20}O_6$: C, 71.28; H, 4.99. Found: C, 71.49; H, 5.15.

ALIZARIN-2-METHYL ETHER (**10**).—To a mixture of **3** (2.5 g) in chloroform (25 ml) was added dropwise an excess amount of diazomethane in ether with stirring for 5 hours followed by the usual workup. The product was purified by column chromatography on silica gel (200 g) eluted with chloroform (200 ml) and chloroform-ether [4:1 (100 ml); 20:7 (270 ml)] to give 0.65 g of **10** as orange needles: mp 231° (chloroform); ir (neat) 1664, 1645, 1590, 1455, 1370, 1300, 1269, 977, 798, 785, and 714 cm^{-1}; nmr (60 MHz, $CDCl_3$) δ 4.03 (3H, s, OCH_3), 7.17 (1H, d, J= 8.3 Hz, H-3), 7.79 (2H, m, H-6 and H-7), 7.84 (1H, d, J=8.3 Hz, H-4), 8.80 (2H, m, H-5 and H-8), 13.03 (1H, d, J=1 Hz, 1-*OH*) Calc. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.97. Found: C, 70.62, H, 4.64.

1,2-ETHYLENEDIOXYANTHRAQUINONE (**11**).—Alizanin (**3**, 1.6 g) was treated with 1,2-dibromoethane (23 ml) in dimethylformamide (11 ml) and chloroform (11 ml) in the presence of anhydrous potassium carbonate (0.3 g) with stirring and reflux for 14 hours. The reaction mixture was filtered and evaporated *in vacuo* to dryness and then chromatographed on a silica gel column (500 g) with chloroform as solvent. Evaporation of the chloroform eluate gave a residue which, when further purified by recrystallization with chloroform-ether (3:1), yielded 75 mg of **11** as yellow needles: mp 233.5~235°; ir (neat) 1670, 1570, 1474, 1428, 1335, 1288, 1275, 1082, 896 and 710 cm^{-1}; nmr (60 MHz, $CDCl_3$) 4.48 (4H, s, -O-CH_2CH_2-O-), 7.23 (1H, d, J= 8.4 Hz, H-3), 7.75 (2H, m, H-6 and H-7), 7.93 (1H, d, J=8.4 Hz, H-4), 8.22 (2H, m, H-5 and H-8). Calc for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79. Found: C, 72.16; H, 3.70.

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