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BIOACTIVE COMPOUNDS FROM THE ROOT OF *MYRSINE AFRICANA*

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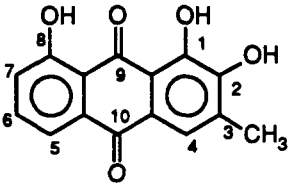
ABSTRACT.—Bioactivity-directed fractionation of the EtOH extract of the roots of *Myrsine africana*, using lethality to brine shrimp, led to the isolation and identification of emodin and 2-hydroxychrysophanol as cytotoxic components, the latter being a new natural product. Nepodin and 5-methoxy-7-hydroxyphthalide were also isolated but were not significantly cytotoxic.

Myrsine saponin (an uncharacterized glycoside of primulagenin A) was isolated by Kupchan *et al.* (1) from the twigs and leaves of *Myrsine africana* L. (Myrsinaceae). This compound showed significant inhibitory activity against the Walker intramuscular carcinosarcoma 256 in rats and seemed to explain the Walker activity of the EtOH extracts. During the screening of some of the Kupchan plant materials for bioactivity, the root of this species showed significant lethality to brine shrimp (BS) (2) in the EtOH extract (F001, LC_{50} 114 ppm). Myrsine saponin was expected to concentrate in the H_2O-soluble fraction (F002), which was inactive; thus, the BS bioassay suggested that some additional bioactive components must be present.

Concentration of the aqueous MeOH residue (F005) produced a large quantity of a weakly bioactive compound that was identified as the naphthalene derivative, nepodin (3). Cc of the mother liquor was performed over Si gel with gradient elutions (see Experimental). Yellow crystals of emodin (4), orange crystals of an unknown labile compound **1**, and colorless crystals of 5-methoxy-7-hydroxyphthalide (5) were obtained. Tlc was used to facilitate the pooling of similar fractions. The BS activity was concentrated in the pools that yielded emodin and compound **1**.



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The brilliant orange compound **1** gave ms patterns ($[MH]^+$ 271.056 for $C_{15}H_{10}O_5$) that were almost identical to those of emodin; thus, **1** appeared to be a positional isomer of emodin with a different arrangement of substitutions around the 9,10-anthraquinone ring. Preparation of the di- and triacetates of **1** produced a clearer picture of the ^{1}H-nmr shifts for the four aromatic protons, one of which (H-4) was isolated and three of which (H-5–H-7) were adjacent as evidenced by their coupling patterns (see Experimental). The lability of **1** indicated catechol hydroxyls, and the usual biogenetic placement of the methyl at C-3 suggested that **1** might be 2-hydroxychrysophanol.

Weak three-bond coupling between H-4 and the methyl proton at C-3 supported the placement of the methyl group at C-3. The downfield signal of the free hydroxyl in the diacetate was indicative of hydrogen bonding to the carbonyl oxygen at C-9, placing the free hydroxyl at C-1 and the acetates at either C-2 and C-5 or C-8. The ir spectrum of compound **1** had free and chelated carbonyl absorptions at 1663 and 1617 cm^{-1} ($\Delta = 46\ cm^{-1}$), which showed that

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two of the hydroxyl groups are in the 1,8 positions (6). Large downfield shifts of the protons at C-4 and C-5 when the triacetate was formed supported the positioning of the hydroxyls at C-1 and C-8, respectively, para to these positions. 2-Hydroxychrysophanol [**1**] is a new natural compound, but it has previously been obtained by demethylation of obtusifolin and is also known as norobtusifolin (7).

The three other compounds were identified by their ms, ^{1}H-nmr, ir, and/or uv spectra and by comparisons of mp values with those in the literature. An authentic sample of emodin was purchased (Aldrich) for direct comparison. All three are previously known natural products. Emodin inhibited the growth of crown gall tumors on potato discs (8), and it has been previously reported to have murine antileukemic (P-388 127% T/C) and Walker sarcoma antitumor activities (9). Both emodin and 2-hydroxychrysophanol exhibited moderate cytotoxicities in our panel of three human tumor cell lines. The bioactivities of the isolated compounds are summarized in Table 1.

These results demonstrate that a bioassay-directed re-examination of previously investigated antitumor plant species, using improved bioassays, can quickly yield additional, chemically diverse, active compounds that may have been overlooked in previous investigations. The members of the Myrsinaceae are known to contain labile hydroxybenzoquinones of unknown bioactivities (10); such compounds likely explain the darkening of extracts and a decrease of bioactivities that we observed during the fractionation work.

EXPERIMENTAL

PLANT MATERIAL.—The roots of *M. africana* were collected in July 1973, in Kenya under the direction of Robert E. Perdue Jr., U.S. Department of Agriculture, Beltsville, Maryland, where voucher specimens are maintained (B-653559 D062, PR-36428).

EXTRACTION AND ISOLATION.—The dried, ground, root material (8.0 kg) was extracted by percolation with 95% EtOH. The EtOH residue (F001, 455 g, BS LC_{50} 146 ppm) was partitioned between $CHCl_3$ and H_2O. The H_2O residue (F002, 200 g, BS LC_{50} >1000 ppm) was inactive, while the interface (F004, 55 g, BS LC_{50} 113) and the $CHCl_3$ residue (F003, 130 g, BS LC_{50} 31 ppm) were active. During condensation of the $CHCl_3$ layers, 40 g of nepodin crystallized. The remaining $CHCl_3$ residue (F003, 90 g) was partitioned between hexane and 10% H_2O in MeOH; however, there was some loss in bioactivity, as neither the hexane residue (F006, 14 g, BS LC_{50} 255 ppm) nor the aqueous MeOH residue (F005, 41 g, BS LC_{50} 114 ppm) was as active as F003. The solutions and extracts quickly darkened, indicating phenolic/quinone decomposition (10).

Cc of F005 (40 g) was performed over Si gel (1 kg) eluted with hexane, hexane-$CHCl_3$-EtOAc (2:2:1), $CHCl_3$-MeOH (10:1, 5:1, 2:1), and MeOH. At the beginning the progress of the separation was monitored by the collection of several colored bands and tlc. After tlc four pools were made of the 20 fractions collected. The best activity (BS LC_{50} 58 ppm) was in the second pool (fractions 4–8). The residue of this pool (5.457 g) was

TABLE 1. Bioactivities of Compounds Isolated from *Myrsine africana*.

Compound	Brine Shrimp (LC_{50} in ppm[a])	Potato Disc (% tumor inhibition)	Human Tumor Cytotoxicities (ED_{50} in mcg/ml)		
			A-549 (lung)	KBMRI (nasal)	HT-29 (colon)
Nepodin	105(66–156)	—[b]	64.3	36.7	31.0
Emodin	35(21–54)	40	4.2	6.0	>10
2-Hydroxychrysophanol	—	—	3.1	5.7	2.8
5-Methoxy-7-hydroxyphthalide	>1000	—	>100	>100	28.0

[a]95% confidence intervals in parentheses.
[b]— indicates not tested.

chromatographed over Si gel (100 g) eluted with hexane, hexane-$CHCl_3$ (1:3), and $CHCl_3$. After tlc seven pools were made from the 23 fractions. The second and third pools were BS-inactive but yielded colorless crystals of 5-methoxy-7-hydroxyphthalide from $CHCl_3$/MeOH. Three pools were active (fractions 8–10, BS LC_{50} 103 ppm; fractions 15–17, BS LC_{50} 105 ppm; fractions 18–23, BS LC_{50} 50 ppm). The material in the first active pool was highly labile and decomposed. The crystalline residue of the second active pool was recrystallized (MeOH) to form yellow crystals of emodin (25 mg). The third active pool left a labile, orange, crystalline residue which was recrystallized (Me_2CO) and identified as 2-hydroxychrysophanol [**1**] (20 mg).

NEPODIN.—Yellow crystals; 0.5% yield; mp 159–161° ($CHCl_3$) [lit. (3) 162–163°]; eims *m/z* (%) $[M]^+$ 216 (69), 201 (100); ir and ^1H nmr as reported (3). Diacetate mp 185–187° [lit. (3) 186–187°].

EMODIN.—Yellow crystals; 0.0003% yield; mp 256° [lit. (4) 255–256°]; eims *m/z* $[M]^+$ 270 (100%); ir and ^1H nmr as reported (4); co-tlc with authentic emodin purchased from Aldrich Chemical Company.

2-HYDROXYCHRYSOPHANOL [**1**].—Orange crystals; 0.00025% yield; mp 265°; ir (KBr) ν max 3410, 1663, 1617 cm^{-1}; eims *m/z* (%) $[M]^+$ 270 (100), 253 (3.6), 242 (12), 213 (7.8), 168 (16.9), 139 (48.4), 128 (14.6), 115 (9.5); hr cims $[MH]^+$ 271.056 (calcd 271.061) for $C_{15}H_{11}O_5$; ^1H nmr (200 MHz, Me_2CO-d_6) ppm 2.32 (s, 3H, Me at C-3), 7.32 (m, 1H, H-7), 7.60 (s, 1H, H-4), 7.76 (m, 2H, H-6 and H-5). Triacetate: mp 234–235°; ^1H nmr (200 MHz, $CDCl_3$) 2.35 (s, 3H, Me at C-3), 2.28 (s, 3H, Ac), 2.43 (s, 3H, Ac), 2.44 (s, 3H, Ac), 7.39 (dd, 1H, J = 7.9, 1.0 Hz, H-7), 7.76 (t, 1H, J = 7.9 Hz, H-6), 8.21 (dd, 1H, J = 7.9, 1.0 Hz, H-5), 8.11 (s, 1H, H-4), Me at C-3 and H-4 showed long-range coupling. Diacetate: mp 188–189°, ^1H nmr (200 MHz, $CDCl_3$) 2.35 (s, 3H, Me at C-3), 2.43 (s, 3H, Ac), 2.47 (s, 3H, Ac), 7.43 (d, 1H, J = 7.9 Hz, H-7), 7.83 (t, 1H, J = 7.9 Hz, H-6), 8.28 (d, 1H, J = 7.9 Hz, H-5), 7.72 (s, 1H, H-4), and 12.73 (s, 1H, OH at C-1).

5-METHOXY-7-HYDROXYPHTHALIDE.—Colorless crystals; 0.0015% yield; mp 180° [lit. (5) 186–188°]; eims *m/z* (%) $[M]^+$ 180 (37), 151 (100); uv λ max (MeOH) nm (log ϵ) 219 (4.7), 256 (4.43), 291 (3.92), plus $AlCl_3$ 225 (4.37), 270 (4.42), 320 (3.79); ^1H nmr as reported (5). Acetate mp 140° [lit. (5) 140–142°].

BIOASSAYS.—Lethality to brine shrimp and the inhibition of crown gall tumors on potato discs were determined as previously described (2,6). Cytotoxicities to three human tumor cell lines (Table 1) were determined at the Cell Culture Laboratory, Purdue Cancer Research Center, using standard protocols of the National Cancer Institute. KB MRI is a human nasopharyngeal carcinoma (11); HT-29 is a human colon adenocarcinoma (12); and A-549 is a human lung carcinoma (13).

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LITERATURE CITED

1. S.M. Kupchan, P.S. Steyn, M.D. Grove, S.M. Horsfield, and S.W. Meitner, *J. Med. Chem.*, **12**, 167 (1969).
2. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **15**, 31 (1982).
3. J.L. Suri, K.L. Dhar, and C.K. Atal, *J. Indian Chem. Soc.*, **55**, 292 (1978).
4. A.W.K. Chan and W.D. Crow, *Aust. J. Chem.*, **19**, 1701 (1966).
5. S.B. Xalidhar and P. Sharma, *J. Indian Chem. Soc.*, **62**, 411 (1985).
6. Anonymous, "Chemistry of Chinese Traditional Herbs," Beijing Medical College and Beijing Traditional Medical College, Beijing, 1981, p. 233.
7. M. Takido, *Chem. Pharm. Bull.*, **6**, 397 (1958).
8. N.R. Ferrigni, J.E. Putnam, B. Anderson, L.B. Jacobsen, D.E. Nichols, D.S. Moore, and J.L. McLaughlin, *J. Nat. Prod.*, **45**, 679 (1982).
9. J.L. Hartwell, *Cancer Treat. Rep.*, **60**, 1031 (1976).
10. H. Ogawa and S. Natori, *Phytochemistry*, **7**, 773 (1968).
11. H. Eagle, *Proc. Soc. Exp. Biol. Med.*, **89**, 362 (1955).
12. J. Fogh (Ed.), "Human Tumor Cells, In Vitro," Plenum Press, New York, 1975, p. 115–159.
13. D.J. Giard, S.A. Aaronson, G. J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik, and W.P. Parks, *J. Natl. Cancer Inst.*, **51**, 1417 (1973).

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