PHEBALOSIN FROM THE BARK OF *MICROMELUM MINUTUM*

V. TANTISHAIYAKUL, S. PUMMANGURA,* C. CHAICHANTIPYUTH,

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10500, Thailand

WEN-WEN MA, and J.L. MCLAUGHLIN

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

In Thailand, the shrub *Micromelum minutum* (Forst. f.) Seem (syn. *M. pubescens* Blume) (Rutaceae) (1) is named "Mui-chang" or "Hat-sa-khum," and the stems, fruits, flowers, leaves, and roots are employed medicinally for a variety of indications (2). Previous phytochemical studies have yielded several prenylated coumarins (3-8) and a pyranoquinoline alkaloid (7). Of the coumarins tested biologically, microminutin is weakly cytotoxic (7), and micromelin has antitumor activities (9). From the stem bark, we have now isolated phebalosin, a known prenylated coumarin (10-12) new to this species, along with a trace of micromelin (3,4). The ^{13}C-nmr spectrum of phebalosin, which has not been previously reported (7,9), along with ^{1}H nmr, high resolution ms, eims, uv, ir, mp, and co-tlc were useful in identifying the isolated compounds (10-14). The co-occurrence of the epoxide (phebalosin) with the related carbonyl compound (micropubescin) may be of biogenetic interest (15).

Phebalosin was significantly toxic to brine shrimp (16) (LC_{50} 47 ppm, 95% confidence interval 31-69 ppm) and significantly inhibitory to the development of crown gall tumors on potato discs (17) (−64% and −70% in two independent determinations). However, insignificant cytotoxic activity (18) was observed (9KB ED_{50}>20 μg/ml; 9PS ED_{50} 27 μg/ml), and no activity was observed in the 9ASK astrocytoma reversal assay (19). In the 3PS (P-388) in vivo murine leukemia system (18), phebalosin was inactive in doses up to 25 mg/kg, but insufficient material was available for testing at higher doses.

EXPERIMENTAL

PLANT MATERIAL.—The stem bark of *M. minutum* was collected from the Nakorn-sri-thamarat Province in the southern part of Thailand in May 1984. A voucher specimen was deposited at The Royal Forest Department, Ministry of Agriculture and Cooperative, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—The air-dried, powdered, stem-bark (500 g) was refluxed three times, for 5 h, with *n*-hexane. The combined filtrates were reduced to a syrupy residue (12 g). The entire residue was dissolved in Et_2O. Upon standing overnight, crude crystals of phebalosin (2 g) were deposited. Chromatography of the mother liquor over three columns of silica gel 60 yielded a trace of micromelin (0.025 g, 0.005% yield), mp 211-212° (uncorr.), lit. 216-218° (9), co-tlc, eims, uv, ir, and ^{1}H nmr (3).

Details of the procedures and identifications are available from the senior author.

IDENTIFICATION OF PHEBALOSIN.—Recrystallization from Me_2CO of the crude crystals yielded white, needle-like, crystals of phebalosin (0.6809 g, 0.14% yield); mp 127-128° (uncorr.), lit. 120.5-121.5° (9-11); hrms obs. 258.0862, calcd. 258.0892 for $C_{15}H_{14}O_4$; eims *m/z* (%, rel. int. 258 (M^+, 100), 229 (51), 199 (49), 189 (87), 131 (5); uv MeOH λ max 322 (log ε=4.13), 217 (log ε=4.53); ir (KBr) ν max 1705, 1715 (coumarin lactone), 1605 (C=C stretching), 1100 (C−O stretching), 890, 810 (assymmetrical stretching of epoxide ring) (20); 90 MHz ^{1}H nmr ($CDCl_3$) 1.87 (m, 3H), 14-CH_3, *J*-value is very small due to allylic coupling, 3.96 (s, 3H, OCH_3), 3.91-4.00 (2H, protons on C_{11} and C_{12}, 5.07 (m, 1H, C_{15}-H, proton which is *cis* to epoxide ring), 5.29 (m, 1H, C_{15}-H proton which is *trans* to epoxide ring), 6.15 (d, 1H, *J*=9 Hz, C_3-H), 8.01 (d, 1H, *J*=9 Hz, C_4-H), 6.86 (d, 1H, *J*=9 Hz, C_6-H), and 7.41 (d, 1H, *J*=9 Hz, C_5-H); ^{13}C nmr ($CDCl_3$) ppm 162.0, m, C_7; 160.3, dd, 3.7 Hz (H_3), 7.6 Hz (H_4), C_2; 152.7, m, C_9; 143.4, dd, 163.1 (H_4), 5.0 (H_5), C_4; 141.3, d, 5.6 Hz (H_{11}), C_{13}; 128.9, dd, 165.3 Hz (H_5), 3.9 Hz (H_4), C_5; 113.5, td, 157.4 (H_{15}), 3.3 Hz (H_{12} or H_{14}), C_{15}; 113.5, d, 173.0 Hz, C_3; 112.8, m, C_8; 112.8, m, C_{10}; 107.6, d, 161.2 (H_6), C_6; 60.7, dd, 181.3 (H_{11}), 3.4 (H_{12}), C_{11}; 56.3, q, 145.2 (H_{16}), C_{16}; 51.8, d, 180.4 (H_{12}), C_{12}; 17.4, q, 122.1 (H_{14}), 4.4 (H_{15}), C_{14}; these spectral data were indicative of phebalosin (10); co-tlc confirmed the identification.

BIOLOGICAL EVALUATIONS.—The brine shrimp lethalities and the crown gall antitumor assays were performed in our laboratory as previously described (16,17). 9KB and 9PS cytotoxicities and 9ASK results were determined in the Purdue Cell Culture Laboratory following protocols established by the National Cancer Institute (18,19). The 3PS (P-388) in vitro antileukemic assay (18) was performed at Illinois Institute of Technology, Life Science Division, Chicago, IL 60616, under NCI contract.

ACKNOWLEDGMENTS

This work was supported by grants from the Graduate School, Chulalongkorn University, and from the National Institutes of Health, National Cancer Institute, CA-30909. ^{13}C-nmr spectra were recorded at the Purdue University Biological Magnetic Resonance Laboratory which is supported, in part, by NIH grant no. PR01077 from the Division of Research Resources. The cooperation and help of Miss S. Suwan, Miss W. Jinsart, T.G. McCloud, and M.D. Antoun are gratefully acknowledged.

LITERATURE CITED

1. I.H. Burkill, "Dictionary of the Economic Products of the Malay Peninsula," vol. II (I-Z). Crown Agents for the Colonies, London, 1935, pp. 1468-1469.
2. S. Pongboonrod, "Maitet Muang Thai," Kasembunakij Press, Bangkok, 1958, pp. 624-625.
3. J.A. Lamberton, J.R. Price, and A.H. Redcliffe, *Aust. J. Chem.*, **20**, 973 (1967).
4. A. Chatterjee, C.P. Dutta, and S. Bhattacharyya, *Sci. Cult.*, **33**, 371 (1967).
5. A. Chatterjee, C.P. Dutta, and S. Chattacharyya, *Sci. Cult.*, **34**, 366 (1968).
6. P.P. Joshi, Y.N. Shuklan, D.S. Bhakuni, and M.M. Dhar, *Ind. J. Chem.*, **13**, 772 (1975).
7. P. Tantivatana, N. Ruangrungsi, V. Vaisiriroj, D.C. Lankin, N.S. Bhacca, R.P. Borris, G.A. Cordell, and L.F. Johnson, *J. Org. Chem.*, **48**, 268 (1983).
8. S. Das, R.H. Baruah, R.P. Sharma, J.N. Barua, P. Kulanthaivel, and W. Herz, *Phytochemistry*, **23**, 2317 (1984).
9. J.M. Cassady, N. Ojima, C.J. Chang, and J.L. McLaughlin, *J. Nat. Prod.*, **42**, 274 (1979).
10. P.W. Chow, A.M. Duffield, and P.R. Jefferies, *Aust. J. Chem.*, **19**, 483 (1966).
11. P.K. Sangal and P.K. Bose, *Sci. Cult.*, **35**, 332 (1969).
12. J.T. Pinhey and I.A. Southwell, *Aust. J. Chem.*, **26**, 409 (1973).
13. T.R. Seshadri and Vishwapaul, *J. Scient. Ind. Res.*, **32**, 227 (1973).
14. R.D.H. Murray, J. Mendez, and S.A. Brown, "The Natural Coumarins," John Wiley and Sons, New York, 1982, pp. 27-53.
15. A.I. Gray and P.G. Waterman, *Phytochemistry*, **17**, 845 (1978).
16. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
17. N.R. Ferrigni, J.E. Putnam, B. Anderson, L.B. Jacobsen, D.E. Nichols, D.S. Moore, J.L. McLaughlin, R.G. Powell, and C.R. Smith, Jr., *J. Nat. Prod.*, **45**, 679 (1982).
18. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep. (part 3)*, **3**, 1 (1972).
19. M. Suffness and J. Douros, *J. Nat. Prod.*, **45**, 1 (1982).
20. K. Nakanishi and P.H. Solomon, "Infrared Absorption Spectroscopy," Holden-Day Inc., San Francisco, 1977, p. 31.

Received 24 July 1985

FLAVONOLS AND FLAVONOL GLYCOSIDES FROM *EUPATORIUM AREOLARE* VAR. *LEIOCARPUM*

SANG GONG YU,[1] DOUGLAS A. GAGE,* NIANBAI FANG,[2] and TOM J. MABRY

Department of Botany, University of Texas at Austin, Austin, Texas 78713

In a continuation of our chemotaxonomic studies in the tribe Eupatorieae (Compositae) (1-8), we have investigated *Eupatorium areolare* var. *leiocarpum* B. L. Robins. Our work has focused upon finding chemical correlates with the generic limits proposed by King and Robinson in their taxonomic revision of the Eupatorieae (9, 10 and references therein). These authors recognize 180 genera (many of which they have created from the large genus *Eupatorium* L.) in a tribe where traditionally fewer than 50 genera have been accepted. The subject of this work, *E. areolare* var. *leiocarpum*, has not been directly treated by King and Robinson, but they would presumably include it within the small genus *Piptothrix* Gray (subtribe Oxylobinae) to which they have transferred the typical variety of this species, *E. areolare* DC. (11). In this

[1]Wuhan Institute of Medical Sciences, Wuhan, China.
[2]Hubei College of Chinese Traditional Medicine, Wuhan, China.