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XANTHONES FROM THE TWIGS OF *MAMMEA SIAMENSIS*

ONOOMAR POOBRASERT, HOWARD L. CONSTANT, CHRISTOPHER W. W. BEECHER, NORMAN R. FARNSWORTH, A. DOUGLAS KINGHORN, JOHN M. PEZZUTO, GEOFFREY A. CORDELL,* THAWATCHAI SANTISUK† and VICHAI REUTRAKUL‡

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Illinois 60612, U.S.A.; † The Forest Herbarium, Royal Forest Department, Bangkok 10900, Thailand; ‡ Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

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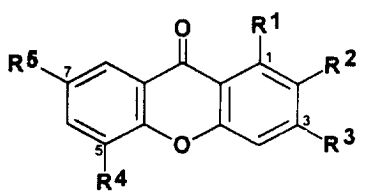
Key Word Index—*Mammea siamensis*; Guttiferae; 1,2-dimethoxy-5-hydroxyxanthone; xanthones.

Abstract—1,2-Dimethoxy-5-hydroxyxanthone, a new xanthone, was isolated from the twigs of *Mammea siamensis*, in addition to six known xanthones (5-hydroxy-1-methoxy-, 1,3-dimethoxy-5-hydroxy-, 2,5-dihydroxy-1-methoxy-, 1,7-dihydroxy-, 1,3,7-trihydroxy- and 3,5-dihydroxy-1-methoxyxanthone). Structures for these compounds were deduced from their spectral data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Mammea siamensis (Miq.) T. Anders. (*Ochrocarpus siamensis* Anders.), a small, evergreen tree up to 15 m tall and 10–30 cm in diameter, is native to Myanmar, Thailand, Laos, Cambodia and Vietnam [1]. The genus *Mammea* of the family Guttiferae is reported to contain various types of xanthones [2–10]. Previously, two phenylcoumarins, 6-butyryl-5-hydroxy-4-phenylseselin and 6-butyryl-5,7-dihydroxy-8-(3,3-dimethylallyl)-4-phenylcoumarin, were isolated from the flowers of *M. siamensis* [11], and ochrocarpus proanthocyanidin 1 has been isolated from its leaves and found to have piscicidal and molluscicidal activities [12]. Fractionation of the MeOH extract of the twigs of *M. siamensis* resulted in the isolation of a novel xanthone **3**, and the six known analogues, **1**, **2** and **4**–**7**.



	R_1	R_2	R_3	R_4	R_5
1	OCH_3	H	H	OH	H
2	OCH_3	H	OCH_3	OH	H
3	OCH_3	OCH_3	H	OH	H
4	OH	H	H	H	OH
5	OCH_3	OH	H	OH	H
6	OH	H	OH	H	OH
7	OCH_3	H	OH	OH	H

RESULTS AND DISCUSSION

The MeOH extract of the twigs of *M. siamensis* was partitioned against petroleum ether and $CHCl_3$, respectively. An LC-MS dereplication process of the $CHCl_3$–MeOH extract showed marginal cytotoxic activity in the BC1 (human breast cancer) (ED_{50} = 8.3 μg/ml) and ZR-75-1 (hormone-dependent human breast cancer) (ED_{50} = 8.6 μg/ml) cell lines, and chromatography of this extract by semi-preparative RP-HPLC or by silica gel columns resulted in the isolation of xanthones **1**–**7**.

Compounds **1**–**2**, and **4**–**7** were identified by spectroscopic data as 5-hydroxy-1-methoxyxanthone (**1**) [2, 3], 1,3-dimethoxy-5-hydroxyxanthone (**2**), 1,7-dihydroxyxanthone (euxanthone) (**4**) [2, 3, 13], 2,5-dihydroxy-1-methoxyxanthone (**5**) [14], 1,3,7-trihydroxyxanthone (**6**) [3], and 3,5-dihydroxy-1-methoxyxanthone (**7**) [15], respectively.

Compound **3**, 1,2-dimethoxy-5-hydroxyxanthone, is a yellow amorphous solid. The HREI-MS gave a $[M]^+$ at *m/z* 272.0675 ($C_{15}H_{12}O_5$). The ^1H NMR

* Author to whom correspondence should be addressed.

spectrum displayed two methoxyl groups (δ 3.77, *s*; 4.15, *s*), five aromatic protons (δ 7.11, *d*; 7.27, *t*; 7.34, *d*; 7.51, *dd*; 8.08, *dd*), and a hydroxyl group (δ 12.67, *br s*). The COSY experiment showed an *ortho* correlation (J = 7.9 Hz) between the *dd* at δ 8.08 and the *t* at δ 7.26. A weak *meta* correlation (J = 1.6 Hz) was also observed between the resonances at δ 8.08 and at δ 7.51. These correlations suggested that these three protons were on the same aromatic ring. Consequently, the *ortho* coupled (J = 9.2 Hz) doublets at δ 7.34 and at δ 7.11 in the COSY spectrum were placed on the other aromatic ring. The assignments of these five protons were in accordance with those of 2,5-dihydroxy-1-methoxyxanthone (**5**), which is the 2-hydroxy analogue of this xanthone. In the NOE experiment, irradiation of the methoxyl resonance at δ 3.77 enhanced the resonance at δ 7.34 (5.1%). On the other hand, irradiation of the methoxyl resonance at δ 4.15 or the proton at δ 12.67 did not show any NOE effect. These NOE results indicated that the methoxyl group (δ_H 3.77, δ_C 56.8) was adjacent to the proton at δ 7.34. Unambiguous carbon assignments for this xanthone were not possible because of sample size. However, following the establishment, using 2D NMR techniques, of the unambiguous assignments of 2,5-dihydroxy-1-methoxyxanthone (**5**), tentative assignments were made for **3** which established the structure as 1,2-dimethoxy-5-hydroxyxanthone.

Compounds **1**, **2**, and **5** were evaluated for their cytotoxic activity against various human cancer cell lines, i.e., Lu-1 (human lung cancer), KB (human oral epidermoid carcinoma), LNCaP (hormone-dependent human prostate cancer), and ZR-75-1, using the procedures described previously [16], and deemed to be inactive in these assay systems (ED_{50} values 20 μg/ml). However, compound **2** has been reported to inhibit type A and type B monoamine oxidases [17], and compound **6** was reported to possess tuberculostatic activity [18].

EXPERIMENTAL

General

Mps: Fisher-Johns melting apparatus (uncorr). UV: Beckman DU-7 spectrometer. IR: Midac Collegian FT-IR spectrometer. HREIMS and EIMS: Finnigan MAT 90 mass spectrometer. ^{1}H-, ^{13}C-NMR, NOE, and COSY: Varian XL-300 NMR spectrometer (299.9 MHz for ^{1}H and 75.4 MHz for ^{13}C), using TMS as an int. st. Selective INEPT spectra: Nicolet NMC-360 NMR spectrometer (360 MHz for ^{1}H and 90.8 MHz for ^{13}C). HMBC: General Electric GE-500 (500.12 MHz).

Plant material

Mammea siamensis (Miq.) T. Anders. was collected in Saraburi, Thailand in October 1991. A voucher specimen (AA768) is deposited at the Field Museum of Natural History, Chicago, Illinois, U.S.A.

Extraction and isolation

Dried and ground twigs of *M. siamensis* (3.63 kg) were extracted with 95% MeOH (13 l × 4). The combined extract was concentrated *in vacuo* (356 g), redissolved in 1 l water and extracted with petroleum ether (3 × 1 l). The aq. MeOH fr. was then extracted with $CHCl_3$ (1:1), and the $CHCl_3$–MeOH fr. was washed with 1% NaCl soln to remove tannins. This $CHCl_3$–MeOH fr. was dried *in vacuo* (12.9 g) and subjected to Si gel CC, eluting with a mixture of $CHCl_3$–MeOH. Fr. 6 (100 mg) from the $CHCl_3$–MeOH (100:0) eluate was further purified by semi-prep. HPLC (Kromasil® C_{18} 250 × 20 mm), using a gradient mobile phase [H_2O:CH_3CN (65:35) to H_2O:CH_3CN (0:100) in 50 min], and the detector was set at 280 nm to yield the xanthones **1** (6.8 mg), **2** (6.2 mg), **3** (2.9 mg), and **4** (7.2 mg) with retention times of 13.2, 14.3, 16.2, and 31.8 min, respectively. Frs 15–17 from the $CHCl_3$–MeOH (95:5) eluate were combined (600 mg) and chromatographed on Si gel CC, using $CHCl_3$–MeOH (99:1) as an eluent, to afford the xanthones **5** (36.0 mg) and **6** (9.5 mg). Fr.18 (600 mg) from the $CHCl_3$–MeOH (95:5) eluate was further purified on a Si gel CC, eluting with $CHCl_3$–MeOH (90:10), to yield the xanthone **7** (1.2 mg).

LC-MS dereplication procedure

The $CHCl_3$–MeOH extract of *M. siamensis* twigs, prepared as indicated above, was subjected for dereplication analysis, employing a previously published protocol, with the published chromatographic conditions being used, and the ZR-75-1 cytotoxicity assay used to monitor activity [19, 20].

1,2-Dimethoxy-5-hydroxyxanthone (**3**). Yellow amorphous residue. HREI-MS *m/z* 272.0675 for $C_{15}H_{12}O_5$ (calcd 272.0685). EI-MS *m/z* (rel. int.): 272 $[M]^+$ (100), 258 (11), 257 (76), 253 (16), 243 (33), 239 (15), 229 (42), 113 (38). UV λ_{max}^{MeOH} nm (log ε): 255 (2.66), 372 (1.49). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3459 (OH), 1640 (> C=O), 1584. ^{1}H NMR (299.9 MHz, pyridine-d_5): 3.77 (3H, *s*, OCH_3-2), 4.15 (3H, *s*, OCH_3-1), 7.11 (1H, *d*, J = 9.2 Hz, H-4), 7.27 (1H, *t*, J = 7.9 Hz, H-7), 7.34 (1H, *d*, J = 9.2 Hz, H-3), 7.51 (1H, *dd*, J = 1.6, 7.9 Hz, H-6), 8.08 (1H, *dd*, J = 1.6, 7.9 Hz, H-8), 12.67 (1H, *br s*, OH-5). ^{13}C NMR (75.4 MHz, pyridine-d_5) 56.8 (*s*, OCH_3-2), 61.6 (*s*, OCH_3-1), 113.4 (*d*, C-4), 116.2 (*d*, C-8), 117.5 (*s*, C-9a), 120.4 (*d*, C-6), 120.6 (*d*, C-3), 124.0 (*s*, C-8a), 124.2 (*d*, C-7), 135.6 (*s*, C-4a), 145.9 (*s*, C-10a), 147.8 (*s*, C-5), 149.0 (*s*, C-1), 151.4 (*s*, C-2), 176.5 (*s*, C-9).

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