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XANTHONES FROM THE ROOTS OF *Calophyllum membranaceum*

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The genus *Calophyllum* of the Guttiferae family is a kind of evergreen plant, mostly growing in damp tropic jungles. Plants in this genus are a rich source of xanthones, flavonoids, coumarins and terpenoids [1].Various bioactivities such as antibacterial [2], antifungal [3], antiviral [4], antimalarial [5], antiplatelet aggregation [6], immunomodulatory [7], and cancer chemopreventive activities [8] have been reported. The stems and bark of *C. membranaceum* have been used in Chinese folk medicine for the treatment of rheumatism, arthritis, and lumbago in Hainan Island, P. R. China. In a previous paper, we reported the isolation and identification of triterpenoids, flavonoids, and xanthones from the leaves, stems, and roots of *C. membranaceum* [9, 10]. To explore the plant further and make use of Chinese herbal sources and to clarify the active components, in this paper, part of the chloroform fraction was subjected to a series of chromatographic techniques, such as silica gel column, Sephadex LH-20, and PTLC, yielding compounds **1**–**8**. The chemical constituents of *C. membranaceum* and their anticholinesterase (AChE) and antitumor activity were illuminated.

The root of *C. membranaceum* was collected in April 2007 from Hainan Province, P. R. China, and authenticated by Professor Qiong-xin Zhong (College of Life Science, Hainan Normal University). A voucher specimen of *C. membranaceum* was deposited in the Key Laboratory of Tropical Medicinal Plant Chemistry of Hainan Province, Hainan Normal University, Haikou, P. R. China.

Shade-dried plant material (16 kg) was milled and then extracted with 70% ethanol (3 × 20 L, each for 4 h) at 80°C. After evaporation of the solvents in vacuo, 3.2 kg residue was obtained. The extract was suspended in H_2O (2.0 L) and partitioned successively with petroleum ether (3 × 2 L), chloroform (3 × 2 L), ethyl acetate (3 × 2 L), and *n*-BuOH (3 × 2 L) to yield 102.5, 78.0, 146.5, and 200.0 g of extracts, respectively. The chloroform extract (392.0 g) was subjected to column chromatography (CC) on silica gel eluted with petroleum ether–ethyl acetate gradient (100:1→0:100) to obtain ten fractions, A–J. Fraction A (15.1 g) was separated by silica gel CC eluted with petroleum ether–ethyl acetate (20:1–3:1) to yield seven fractions, A1–A7. Recrystallization of fraction A3 (1.3 g) yielded the compound brasixanthone F (**1**, 7 mg); fraction A5 (3 g) was separated by silica gel CC eluted with petroleum ether–ethyl acetate (20:1, 10:1, 5:1, 1:1, 0:1) to give six fractions, A5-1–A5-6. Fraction A5-3 was further purified by Sephadex LH-20 with CH_3OH–$CHCl_3$ (6:4) to yield compound brasilixanthone B (**2**, 50 mg) and 2-methoxy-3-hydroxyxanthone (**8**, 20 mg). Fraction A6 (1.8 g) was subjected to silica gel CC eluted with petroleum ether–ethyl acetate (10:1→0:1) to yield the compound isoapetalic acid (**4**, 50 mg). Fraction B (23 g) was separated out by crystallization and recrystallized to give the compound gracilixanthone (**3**, 100 mg). Fraction B′ residue was subjected to silica gel CC eluted with petroleum ether–ethyl acetate (10:1→1:2) to give five fractions B1–B5. Fraction B2 was then purified by C-18 reverse-phase silica gel CC eluted with CH_3OH–H_2O (4:6) and Sephadex LH-20 with CH_3OH–$CHCl_3$ (6:4) to yield the compound rheediachromenoxanthone (**5**, 40 mg) and dehydrocycloguanandin (**6**, 15 mg). Fraction B4 was separated by silica gel CC eluted with petroleum ether–ethyl acetate (10:1, 5:1, 1:1) to yield the compound 2-hydroxy-1-methoxyxanthone (**7**, 6 mg).

The compounds were identified using UV, IR, mass, and NMR spectra, and all the data were in good agreement with the literature data [11–18]. All these compounds were isolated from *C. membranaceum* for the first time.

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The structures of the eight compounds were elucidated as follows:

Brasixanthone F (1).Yellow crystals. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.47 (6H, s, H-14, 15), 4.01 (3H, s, MeO-7), 5.59 (1H, d, J = 10, H-12), 6.33 (1H, s, H-4), 6.73 (1H, d, J = 10, H-11), 6.95 (1H, s, H-5), 7.58 (1H, s, H-8), 13.10 (1H, s, 1-OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 28.3 (C-14, 15), 56.5 (MeO-7), 78.0 (C-13), 94.9 (C-4), 102.6 (C-5), 103.2 (C-9a), 104.6 (C-2), 104.5 (C-8), 113.4 (C-8a), 115.5 (C-11), 127.4 (C-12), 144.3 (C-7), 152.6 (C-10a), 152.5 (C-6), 157.4 (C-1), 157.2 (C-4a), 160.1 (C-3), 179.9 (C-9) [11].

Brasilixanthone B (2). Yellow amorphous powder, mp 207°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.47 (6H, s, H-14, 15), 1.50 (6H, s, H-19, 20), 5.59 (1H, d, J = 10, H-12), 5.84 (1H, d, J = 10.4, H-17), 6.23 (1H, s, H-4), 6.37 (1H, d, J = 10.0, H-11), 8.04 (1H, d, J = 10.4, H-16), 13.54 (1H, s, 1-OH), [12].

Gracilixanthone (3).Yellow crystals, mp 204–205°C ($CHC1_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.48 (6H, s, H-14, 15), 1.52 (6H, s, H-24, 25), 1.69 (3H, s, H-20), 1.87 (3H, s, H-19), 3.50 (2H, d, J = 7.2, H-16), 5.28 (1H, m, J = 6, H-17), 5.59 (1H, d, J = 10, H-12), 5.70 (1H, d, J = 10.0, H-22), 6.41 (1H, d, J = 10.0, H-21), 6.73 (1H, d, J = 9.6, H-11), 7.44 (1H, s, H-8), 12.72 (1H, s, H-1). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 155.8 (C-1), 104.5 (C-2), 157.9 (C-3), 107.6 (C-4), 145.2 (C-4a), 132.3 (C-5), 144.6 (C-6), 117.7 (C-7), 113.3 (C-8), 114.5 (C-8a), 180.6 (C-9), 103 (C-9a), 154.0 (C-10a), 115.8 (C-11), 127.1 (C-12), 77.9 (C-13), 28.26 (C-14, C-15), 21.5 (C-16), 122.3 (C-17), 131.5 (C-18), 25.7 (C-19), 17.8 (C-20), 121.5 (C-21), 130.8 (C-22), 78.7 (C-23), 28.3 (C-24,C-25) [13].

Isoapetalic Acid (4). Yellow oily liquid. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz) : 0.83 (3H, t, J = 7.2, H-21), 1.11 (3H, d, J = 7.6, H-22), 1.13 (2H, m, H-19), 1.33 (6H, d, J = 6.4, H-18, H-20), 1.41 (3H, s, H-17), 1.50 (1H, m, H-15), 1.82 (1H, m, H-15), 2.50 (1H, dd, J = 3.2, 7.2, H-13), 2.62 (1H, dd, J = 6.4, 14.8, H-14), 2.83 (1H, dd, J = 8.8, 14.8, H-14), 3.68 (1H, m, H-16), 4.41 (1H, br.s, H-12), 5.42 (1H, d, J = 10, H-6), 6.56 (1H, d, J = 10, H-7). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 201.2 (C-1), 179.5 (C-2), 159.9 (C-3), 159.8 (C-4), 157.2 (C-5), 125.5 (C-6), 115.5 (C-7), 108.6 (C-8), 102.4 (C-9), 101.1 (C-10), 77.3 (C-11), 76.6 (C-12), 44.1 (C-13), 38.6 (C-14), 35.4 (C-15), 30.5 (C-16), 28.3 (C-17), 28.0 (C-18), 20.7 (C-19), 16.2 (C-20), 13.9 (C-21), 9.1 (C-22) [14].

Rheediachromenoxanthone (5). Yellow crystals, mp 223–224°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.57 (6H, s, H-14, 15), 5.74 (1H, dd, J = 0.4, 10, H-12), 6.44 (1H, d, J = 10, H-11), 6.80 (1H, dd, J = 1.2, 8.4, H-2), 7.02 (1H, dd, J = 1.2, 8.8, H-4), 7.53 (1H, s, H-8), 7.56 (1H, t, J = 8.4, H-3), 12.79 (1H, s, 1-OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 110.6 (C-2), 136.3 (C-3), 107.0 (C-4), 156.1 (C-4a), 132.1 (C-5), 145.4 (C-6), 117.9 (C-7), 113.7 (C-8), 114.8 (C-8a), 181.6 (C-9), 108.5 (C-9a), 145.2 (C-10a), 121.3 (C-11), 131.3 (C-12), 79.1 (C-13), 28.5 (C-14, 15) [15].

Dehydrocycloguanandin (6). Yellow crystals, mp 167–169°C. ESI-MS *m/z*: 295 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.57 (6H, s, H-14, 15), 5.85 (1H, d, J = 10.0, H-12), 6.43 (1H, d, J = 10.0, H-11), 6.79 (1H, dd, J = 0.8, 8.8, H-4), 7.02 (1H, d, J = 8.0, H-7), 7.05 (1H, dd, J = 0.8, 8.4, H-2), 7.60 (1H, t, J = 8.0, H-3), 7.75 (1H, d, J = 8.0, H-8), 12.71 (1H, s, 1-OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 161.9 (C-1), 107.4 (C-2), 136.5 (C-3), 110.3 (C-4), 156.3 (C-4a), 141.2 (C-5), 126.7 (C-6), 121.5 (C-7), 116.8 (C-8), 121.1 (C-8a), 182.1 (C-9), 145.8 (C-10a), 121.9 (C-11), 134.0 (C-12), 77.7 (C-13), 27.9 (C-14, 15), 108.9 (C-19a) [16].

2-Hydroxy-1-methoxyxanthone (7). Yellow crystals. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.05 (3H, s, MeO-1), 5.99 (1H, s, 2-OH), 7.25 (1H, d, J = 9.2, H-4), 7.37 (1H, t, J = 8.0, H-7), 7.41 (1H, d, J = 9.2, H-3), 7.45 (1H, d, J = 8.4, H-5), 7.72 (1H, m, H-6), 8.32 (1H, dd, J = 1.6, 8.0, H-8). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 62.6 (MeO-1), 145.3 (C-1), 144.4 (C-2), 122.0 (C-3), 114.2 (C-4), 151.0 (C-4a), 117.5 (C-5), 134.5 (C-6), 123.7 (C-7), 126.6 (C-8), 122.3 (C-8a), 176.3 (C-9), 116.0 (C-9a), 155.4 (C-10a) [17].

2-Methoxy-3-hydroxyxanthone (8). Yellow crystals, mp 226–228°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.03 (3H, s, MeO-2), 6.39 (1H, s, H-3), 7.02 (1H, s, H-4), 7.37 (1H, m, J = 0.8, 8.0, H-6), 7.49 (1H, dd, J = 0.8, 8.8, H-5), 7.69 (1H, m, J = 1.6, 8.4, H-7), 7.71 (1H, s, H-1), 8.35 (1H, dd, J = 1.2, 8.0, H-8) [18].

The anticholinesterase assay used in this study was described by [19]. Tacrine was used as reference compound for the anticholinesterase assays. The antitumor activity assay was based on the MTT colorimetric assay method [20]. The test compounds were dissolved in DMSO. Compared to tacrine, most of the xanthones showed weak inhibitory activity against AChE, except brasixanthone F (**1**), which inhibited AChE with an inhibition rate of 34.3% (brasilixanthone B (**2**), AchE 19.1%); the reference compound has an inhibition rate of 42.45%. The test compounds showed no activity on tumor; brasixanthone F showed good inhibitory activity against A549 (90), hepG2 (65), and MCF-7 (85).

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