Selective Cyclooxygenase-2 Inhibitors from *Calophyllum membranaceum*

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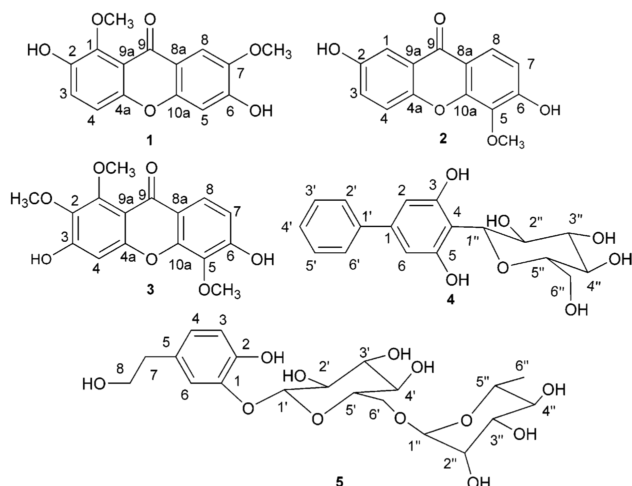
Received June 30, 2005

Chemical investigation of the anti-inflammatory Chinese folk medicine *Calophyllum membranaceum* has resulted in the isolation and characterization of three new xanthones (**1**−**3**), one new biphenyl *C*-glycoside (**4**), and one new phenylethanoid glycoside (**5**) along with 17 known compounds. Their structures were characterized on the basis of spectroscopic and chemical methods. Two xanthones, 2,6-dihydroxy-1,7-dimethoxyxanthone (**1**) and 3,4-dihydroxyxanthone, were found to exhibit selective inhibitory activity against cyclooxygenase-2 (IC_{50} = 2.99 and 1.80 μM) in vitro.

The stems and bark of *Calophyllum membranaceum* (Guttiferae) have been used in Chinese folk medicine for the treatment of rheumatism, arthritis, lumbago, and wounds.[1] Previous phytochemical studies have revealed the genus *Calophyllum* to be a rich source of secondary metabolites, such as xanthones,[2,3] coumarins,[4] chromenes,[5] and flavonoids.[6] The pyranocoumarin calanolide A showed significant inhibitory activity against HIV-1 reverse transcriptase and is now in phase II clinical trial for the treatment of AIDS.[7] To our best knowledge, no chemical investigation has been made on the species *C. membranaceum*. In an effort to find new bioactive natural products from Chinese herbal medicines and the genus *Calophyllum*, constituents of *C. membranaceum* were studied systematically. We herein report the isolation and structural characterization of three new xanthones (**1**−**3**), one new biphenyl *C*-glycoside (**4**), and one new phenylethanoid glycoside (**5**) along with 17 known compounds from *C. membranaceum*. In a preliminary pharmacological test, 2,6-dihydroxy-1,7-dimethoxyxanthone (**1**) and 3,4-dihydroxyxanthone were found to exhibit selective cyclooxygenase-2 inhibitory activity in vitro.



Results and Discussion

Powdered air-dried whole plants of *C. membranaceum* (15.5 kg) were percolated with 95% EtOH three times at room temperature. The filtrate was concentrated to dryness in vacuo and then suspended in 20% EtOH. After filtration of the precipitated chlorophyll and evaporation of EtOH from the filtrate, the aqueous residue was extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-butanol, successively. The latter three extracts were subjected to a series of column chromatography and PTLC steps to afford 22 compounds.

Compound **1** was obtained as a yellow amorphous powder with an elemental formula of $C_{15}H_{12}O_6$ determined by HREIMS and NMR analysis. The IR spectrum of **1** revealed the existence of hydroxyl (3462 cm^{-1}), conjugated ketone (1616 cm^{-1}), and aromatic groups (1589, 1475, 1429 cm^{-1}). Its UV absorption maxima at 364, 323, 238, and 202 nm suggested a xanthone skeleton in its structure.[8] The ^{13}C NMR spectrum of **1** showed 15 carbon signals including two methyls, four sp^2 methines, and nine sp^2 quaternary carbons. The ^1H NMR spectrum of **1** revealed the presence of two *ortho*-coupled protons at δ_H 7.40 (1H, d, J = 9.0 Hz) and 7.21 (1H, d, J = 9.0 Hz), two singlet protons at δ_H 7.70 (1H, s) and 6.96 (1H, s), and two methoxyls at δ_H 4.01 (3H, s) and 4.07 (3H, s). Thus, a xanthone skeleton substituted by two methoxyls and two hydroxyl groups was suggested in the structure of **1**. In its HMBC spectrum, $^{13}C-^{1}H$ long-range correlations were observed at C-1/H-3 and 1-OCH_3; C-2/H-4; C-4a/H-3 and H-4; C-7/H-5, H-8 and 7-OCH_3; C-8a/H-5 and H-8; C-9/H-8; C-9a/H-4; and C-10a/H-8. In its NOESY spectrum, NOE correlations were found between H-3 and H-4 and between 7-OCH_3 and H-8. Therefore, **1** was established to be 2,6-dihydroxy-1,7-dimethoxyxanthone. The structure of **1** was further supported by comparing its NMR signals with those of 7-hydroxy-1,2,8-trimethoxyxanthone.[9] To our best knowledge, compound **1** is a new compound, and it has been named calophymembranol A.

Compound **2** was isolated as a yellow amorphous powder with an elemental formula of $C_{14}H_{10}O_5$ determined by HREIMS. Its UV spectrum exhibited absorption maxima characteristic of xanthones at 357, 312, 249, and 202 nm.[8] The IR spectrum of **2** revealed the existence of hydroxyl (3267 cm^{-1}), conjugated ketone (1616 cm^{-1}), and aromatic groups (1583, 1473, 1448 cm^{-1}) in its structure. In the ^{13}C NMR spectrum of **2**, 14 carbon signals including one methyl, five sp^2 methines, and eight sp^2 quaternary carbons

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10.1021/np0502342 CCC: $30.25 © 2005 American Chemical Society and American Society of Pharmacognosy
Published on Web 09/27/2005

were observed. The ^{1}H NMR spectrum of **2** revealed the presence of one methoxyl at δ_H 3.98 (3H, s), two *ortho*-coupled aromatic proton signals at δ_H 7.01 (1H, d, $J = 8.8$ Hz) and 7.88 (1H, d, $J = 8.8$ Hz), and signals due to a 1,3,4-trisubstituted benzene ring at δ_H 7.61 (1H, d, $J = 3.1$ Hz), 7.52 (1H, d, $J = 9.2$ Hz), and 7.34 (1H, dd, $J = 3.1$ Hz, 9.2 Hz). In the HMBC spectrum of **2**, ^{13}C−^{1}H long-range correlation signals were observed at C-1/H-3; C-2/H-1, H-3, and H-4; C-3/H-1, H-4; C-4/H-3; C-4a/H-1, H-3, and H-4; C-5/H-7, H-8, 5-OCH_3; C-6/H-7, H-8; C-7/H-8; C-8a/H-7; C-9/H-1, H-8; C-9a/H-1, H-4; and C-10a/H-8. In the NOESY spectrum of **2**, NOE correlation signals were found between H-3 and H-4 and between H-7 and H-8. Therefore, the structure of **2** was deduced except for the position of the methoxyl group in ring B. A previous investigation indicated that the chemical shift of a methoxyl group on a benzene ring bearing two *ortho O*-substituted functions usually occurred at δ_C 60−61, while that for those bearing less than two *ortho O*-substituted functions occurred at δ_C 55−56.[10,11] Therefore, the chemical shift of the methoxyl group (δ_C 61.9) indicated its location at C-5 instead of C-6. Compound **2** was thus characterized as 2,6-dihydroxy-5-methoxyxanthone, a conclusion supported by comparing its NMR data with those of 6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2,3′:3,4)-xanthone.[12] Compound **2** is a new compound and has been given the trivial name calophymembranol B.

Compound **3** also possessed a xanthone skeleton as indicated by its characteristic UV absorption maxima at 370, 240, and 223 nm.[8] The IR spectrum of **3** revealed the existence of hydroxyl (3404 cm^{-1}), conjugated ketone (1605 cm^{-1}), and aromatic groups (1585, 1458 cm^{-1}). In the ^{13}C NMR spectrum of **3**, 16 carbon signals including three methyls, three sp^2 methines, and 10 sp^2 quaternary carbons were observed. The ^{1}H NMR spectrum of **3** showed the presence of two *ortho*-coupled aromatic proton signals at δ_H 7.82 (1H, d, $J = 7.8$ Hz) and 6.98 (1H, d, $J = 7.8$ Hz), one singlet proton signal at δ_H 6.82 (1H, s), and three methoxyls at δ_H 3.98 (3H, s), 3.95 (3H, s), and 4.00 (3H, s). In the HMBC spectrum of **3**, ^{13}C−^{1}H long-range correlation signals were observed at C-1/1-OCH_3; C-2/H-4 and 2-OCH_3; C-3/H-4; C-4a/H-4; C-5/H-7 and 5-OCH_3; C-6/H-7, H-8; C-8a/H-7; C-9/H-8; C-9a/H-4; and C-10a/H-8. In the NOESY spectrum of **3**, NOE correlation signals were found between 1-OCH_3 and 2-OCH_3 and between H-7 and H-8. Consequently, **3** was established to be 3,6-dihydroxy-1,2,5-trimethoxyxanthone. Its structure was confirmed by comparing its NMR data with those of 1,6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2′,3′:3,4)-xanthone[12] and 3,5-dihydroxy-1,2-dimethoxyxanthone.[13] Compound **3** has been assigned the trivial name calophymembranol C.

Compound **4** was obtained as a white amorphous powder with an elemental formula of $C_{18}H_{20}O_7$. UV absorptions at 259, 224, and 205 nm suggested a biphenyl skeleton.[14] The IR spectrum of **4** revealed the existence of hydroxyl (3404 cm^{-1}) and aromatic groups (1572, 1458, 1419 cm^{-1}) in its structure. In the ^{13}C NMR spectrum, 18 carbon signals including one methylene, 12 methines (seven for sp^2 methines), and five sp^2 quaternary carbons were observed. Its ^{1}H NMR spectrum suggested the existence of one monosubstituted benzene ring [δ_H 7.58 (2H, dd, $J = 8.1$, 1.5 Hz); 7.40 (2H, dt, $J = 8.1$, 1.5 Hz); 7.31 (1H, tt, $J = 8.1$, 1.5 Hz)], one symmetrical 1,2,3,5-tetrasubstituted benzene ring at δ_H 6.81 (2H, s), and one sugar unit. Analysis of 1D and 2D NMR spectra of **4** and also comparison of its NMR data with those of a known C-glucopyranoside revealed glucose as its sugar moiety, and the glucose moiety was connected to the aglycon in a C-glucopyranosidic linkage.[15] The anomeric proton signal at δ_H 4.95 (1H, d, $J = 10.0$ H_Z) indicated that the glucose unit was in a β glycosidic linkage. In the HMBC spectrum of **4**, ^{13}C−^{1}H long-range correlation signals were observed at C-1/H-2′, H-6′; C-3/H-1″, H-2; C-4/H-2, H-6; C-5/H-1″, H-6; C-1′/H-2, H-6, H-3′, and H-5′; C-2′/H-4′; C-4′/H-2′, H-6′; and C-6′/H-4′. Thus, the structure of **4** was established as 3,5-dihydroxybiphenyl-4-*C*-β-glucopyranoside. To our best knowledge, compound **4** is a new compound, and it has been assigned the trivial name calophymembranside A.

Compound **5** was obtained as a white amorphous powder with an elemental formula of $C_{20}H_{30}O_{12}$. Its UV absorptions at 277, 218, and 202 nm suggested an aromatic ring in its structure. The IR spectrum exhibited strong absorption bands due to hydroxyl (3400 cm^{-1}) and aromatic groups (1603, 1516, 1437 cm^{-1}). In its ^{13}C NMR spectrum, 20 carbon signals including one methyl, three methyenes, 13 methines (three for sp^2 methines), and three quaternary carbons (all for sp^2 carbons) were observed. The ^{1}H NMR spectrum of **5** in CD_3OD revealed three aromatic proton signals with two of them overlapped. Further analysis of the ^{1}H NMR spectrum of **5** in C_5D_5N indicated a 1,2,4-trisubstituted benzene ring [δ_H 7.64 (1H, d, $J = 1.9$ Hz); 7.19 (1H, d, $J = 8.1$ Hz); and 7.00 (1H, d, $J = 8.1$, 1.9 Hz)] in its structure. Acidic hydrolysis of **5** yielded glucose and rhamnose in addition to its aglycone. The planar structure of **5** was established on the basis of its HMBC spectrum, in which ^{13}C−^{1}H long-range correlation signals were observed at C-1/H-3, H-1′; C-2/H-4, H-6; C-4/H-6, H-7; C-5/H-3, H-6, H-7, and H-8; C-6/H-4, H-7; C-7/H-4, H-6, and H-8; C-8/H-7; C-1′/H-3′ and H-5′; C-6′/H-1″; and C-1″/H-6′. The structure of **5** was further confirmed by the NOE correlation signals between H-6 and H-1′; H-6 and H-7; H-7 and H-4; H-7 and H-8; and H-1″ and H-6′ in its ROESY spectrum. The anomeric proton signal at δ_H 4.70 (1H, d, $J = 7.4$ Hz) revealed that the glucose was present in a β glycosidic linkage, and the rhamnose residue was in the α configuration from the ^{13}C NMR chemical shifts of C-3″ and C-5″.[16] Thus, **5** was characterized as 2-hydroxy-5-(2-hydroxyethyl)phenol-1-*O*-α-rhamnopyranosyl-(1→6)-*O*-β-glucopyranoside. Compound **5** has been assigned the trivial name calophymembranside B.

In addition to the new compounds **1**−**5**, 17 known compounds were also isolated and characterized as calanolide E_2,[17] chapelieric acid,[18] apetalic acid,[19] isocalolongic acid,[20] blancoic acid,[21] 2-hydroxyxanthone,[22] 4-hydroxyxanthone,[23] 1,5-dihydroxy-3-methoxyxanthone,[24] 3-hydroxy-4-methoxyxanthone,[25] 3,4-dihydroxyxanthone,[26] 2-methoxyxanthone,[27] 6-(4-hydroxy-3-methylbut-2-enyl)-1,5-dihydroxyanthone,[28] 1,2,3-trihydroxy-5-methoxyxanthone,[29] 2,7-dihydroxy-1,8-dimethoxyxanthone,[30] 1,3-dimethoxy-5-hydroxyxanthone,[31] 1-methoxy-2-hydroxyxanthone,[27] and 1,8-dimethoxy-2-hydroxyxanthone[32] by comparison with data in the literature.

Cyclooxygenase-2 is normally unexpressed in most cells or tissues, but elevated levels are observed due to inflammatory events.[33] Another cyclooxygenase isoform, cyclooxygenase-1, is constitutively expressed in many tissues and predominates, for example, in gastric mucosa and in the kidney.[33] Inhibition of cyclooxygenase-2 is thought to mediate the therapeutic actions of nonselective nonsteroidal anti-inflammatory drugs (NSAIDs), while inhibition of cyclooxygenase-1 results in unwanted side effects, particularly in the gastrointestinal (GI) tract.[34] Thus, selective cyclooxygenase-2 inhibitors appear to possess improved

safety profiles compared with traditional nonsteroidal anti-inflammatory drugs.[35]

Stem and bark of *C. membranaceum* are used in several southern provinces of China for treatment of inflammatory diseases such as rheumatism, arthritis, and lumbago.[1] A natural xanthone, 1,5-dihydroxy-3,8-dimethoxyxanthone, was found to exhibit significant anti-inflammatory activity in vivo,[36] and another natural xanthone, γ-mangostin, was found to exhibit inhibitory activities against both cyclooxygenase-1 and cyclooxygenase-2 in vitro.[37] Cyclooxygenase-1 and cyclooxygenase-2 inhibitory activities of several xanthones isolated from *C. membranaceum* were selected for evaluation. As a result, 2,6-dihydroxy-1,7-dimethoxyxanthone (**1**) and 3,4-dihydroxyxanthone were found to exhibit significant cyclooxygenase-2 inhibitory activity ($IC_{50} = 2.99$ and 1.89 μM, respectively) and were inactive (both $IC_{50} >$ 100 μM) against cyclooxygenase-1 in vitro. Celecoxib and diclofenac were used as positive controls (celecoxib: IC_{50} >100 μM against cyclooxygenase-1, $IC_{50} = 0.08$ μM against cyclooxygenase-2; diclofenac: IC_{50} = 0.03 μM against cyclooxygenase-1, $IC_{50} = 0.07$ μM against cyclooxygenase-2). According to the above pharmacological results, and considing the existence of a series of xanthones in the plant, xanthones appear to contribute to the anti-inflammatory activity of *C. membranaceum*.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba Sepa-300 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. NMR spectra were run in DMSO-d_6, DMCO-d_6, CD_3OD, or $CDCl_3$ on Bruker AM-400 or Bruker AM-300 spectrometers with TMS as internal standard. LREIMS and HREIMS measurements were made with a Finnigan MAT 95 instrument. LRESIMS were measured using a Finnigan LCQ-DECA instrument, and HRESIMS data were obtained on a Mariner spectrometer. Preparative HPLC was carried out using a Varian SD-1 instrument, equipped with a Merck NW25 C_{18} column (10 μm, 20 mm × 250 mm), and ProStar 320 UV/vis detector. Column chromatography was carried out using silica gel H_{60} (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. PTLC was carried out using silica gel G_{60}. $HSGF_{254}$ silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) were used for analytical TLC.

Plant Material. Whole plants of *C. membranaceum* were collected in the suburb of Nanning, Guangxi Province, People's Republic of China, in May 2003, and identified by Mr. Huizhang Chen of Guangxi Medicinal Herb Company. A voucher specimen (No. SIMMZWM040315) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Powdered air-dried whole plants of *C. membranceum* (15.5 kg) were percolated at room temperature with 95% EtOH three times. The filtrate was concentrated to dryness in vacuo and suspended in 20% EtOH and filtered. After evaporation of EtOH from the filtrate, the aqueous residue (1.5 L) was extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-butanol (1.0 L × 3), successively, yielding a petroleum ether extract (24.3 g), a $CHCl_3$ extract (55.0 g), an EtOAc extract (25.6 g), and a *n*-butanol extract (243.0 g), respectively. The $CHCl_3$ extract (55.0 g) was subjected to a silica gel H column (1000 g) eluting with a petroleum ether−acetone gradient (20:1, 10:1, 8:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0:1) to give fractions A (1.25 g), B (2.20 g), C (3.50 g), D (4.60 g), E (3.10 g), F (3.20 g), G (4.50 g), H (6.0 g), and I (4.25 g). Fraction C (3.50 g) was separated using preparative HPLC (ODS), eluted with $MeOH-H_2O-$formic acid (3:1:0.02), to give calanolide E_2 (150.5 mg), chapelieric acid (20.4 mg), apetalic acid (75.6 mg), isocalolongic acid (120.8 mg), and blancoic acid (7.5 mg). Fraction D (4.60 g) was subjected to preparative HPLC, eluted with a $MeOH-H_2O$ gradient (3:1 → 9:1), to give 2-hydroxyxanthone (285.5 mg), 4-hydroxyxanthone (102.1 mg), 1,5-dihydroxy-3-methoxyxanthone (55.2 mg), and 3-hydroxy-4-methoxyxanthone (25.4 mg). Fraction F (3.20 g) was subjected to repeated column chromatography on silica gel eluted with a $CHCl_3$−acetone gradient (20:1 → 5:1) to give 3,4-dihydroxyxanthone (24.9 mg), 2-methoxyxanthone (15.5 mg), and 6-(4-hydroxy-3-methylbut-2-enyl)-1,5-dihydroxyanthone (18.2 mg). Fraction G (4.5 g) was separated by column chromatography on silica gel eluted with a $CHCl_3-MeOH$ gradient (60:1 → 10:1) to give fractions G1 (1.1 g), G2 (0.95 g), and G3 (1.85 g). Fraction G1 was passed through a Sephadex LH-20 column with 95% EtOH and then subjected to preparative TLC developed with $CHCl_3-MeOH$ (20:1) to give 1,2,3-trihydroxy-5-methoxyxanthone (7.8 mg) and **1** (9.0 mg). Fraction G2 was chromatographed on a silica gel column with toluene−MeOH (14:1) as eluent to give 2,7-dihydroxy-1,8-dimethoxyxanthone (15.5 mg), **2** (5.7 mg), and **3** (7.8 mg). Fraction G3 was separated by preparative TLC developed with $CHCl_3-MeOH$ (60:1) to give 1,3-dimethoxy-5-hydroxyxanthone (36.5 mg), 1-methoxy-2-hydroxyxanthone (10.6 mg), and 1,8-dimethoxy-2-hydroxyxanthone (7.9 mg). The EtOAc extract (24.3 g) was subjected to a silica gel H column, eluted with a $CHCl_3-MeOH$ gradient (12:1, 8:1, 5:1, 3:1, 1:1, 0:1), to give fractions 1 (0.69 g), 2 (2.15 g), 3 (10.54 g), 4 (1.69 g), and 5 (3.52 g). Fraction 2 was passed through a silica gel column with $CHCl_3-MeOH$ (10:1) to give **4** (10.2 mg). The *n*-butanol extract (100.0 g) was separated using a silica gel column, eluted with a $CHCl_3-MeOH$ gradient (4:1, 3:1, 2:1, 1:1,0:1), to give fraction 1 (10.5 g), fraction 2 (9.8 g), fraction 3 (25.5 g), and fraction 4 (36.6 g). Fraction 2 was subjected to a RP-18 column with $MeOH-H_2O$ (2:1) as eluent to give **5** (12.6 mg).

Calophymembranol A (1): yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 202 (4.68), 238 (4.71), 323 (4.31), 364 (4.08) nm; IR (KBr) ν_{max} 3462, 2939, 1616 (C=O), 1589, 1475, 1429 (aromatic ring), 1301, 1211, 1139, 1031, 788 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 288 [M^+] (75), 270 (100), 245 (30), 227 (17), 214 (30), 199 (13), 187 (6), 151 (19), 79 (8); HREIMS *m/z* 288.0623 (calcd for $C_{15}H_{12}O_6$, 288.0633).

Calophymembranol B (2): yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 202 (4.07), 249 (4.28), 312 (3.79), 357 (3.46) nm; IR (KBr) ν_{max} 3267, 2925, 2852, 1728, 1616 (C=O), 1583, 1473, 1448 (aromatic ring), 1333, 1242, 1072, 1020, 827, 789 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 258 [M^+] (100), 243 (94), 215 (36), 187 (21), 149 (9), 131 (7), 107 (3), 94 (5), 77 (4); HREIMS *m/z* 258.0534 (calcd for $C_{14}H_{10}O_5$, 258.0529).

Calophymembranol C (3): yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 223 (4.05), 240 (4.48), 370 (3.85) nm; IR (KBr) ν_{max} 3404 (OH), 3253, 1732, 1605 (C=O), 1585, 1458 (aromatic ring), 1306, 1262, 1095, 1134, 825 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 318 [M^+] (27), 303 (100), 288 (30), 260 (15), 167 (4), 149 (25); HREIMS *m/z* 318.0759 (calcd for $C_{16}H_{14}O_7$, 318.0740).

Calophymembranside A (4): white amorphous powder; $[\alpha]^{20}_D$ +13.0° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.38), 224 (4.20), 259 (4.09) nm; IR (KBr) ν_{max} 3404 (OH), 2924, 1701, 1631, 1572, 1458, 1419 (aromatic ring), 1363, 1198, 1074, 1020, 903, 764, 698 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 348 [M^+] (35), 258 (33), 240 (29), 228 (100), 215 (50), 200 (79), 171 (22), 152 (31), 128 (31), 115 (31), 77 (14); HREIMS *m/z* 348.1213 (calcd for $C_{18}H_{20}O_7$, 348.1209).

Calophymembranside B (5): white amorphous powder; $[\alpha]^{20}_D$ −40.9° (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.51), 218 (3.94), 277 (3.51) nm; IR (KBr) ν_{max} 3400, 2922, 1707, 1603, 1516, 1437, 1279, 1230, 1066, 912, 810 cm^{-1}; ^1H and ^{13}C NMR data (in CD_3OD), see Table 2; ^1H NMR data (in C_5D_5N, 300 Hz) δ_H 7.64 (1H, d, J = 1.9 Hz, H-6), 7.19 (1H, d, J = 8.1 Hz, H-3), 7.00 (1H, dd, J = 8.1, 1.9 Hz, H-4), 5.50, (1H, d, J = 0.9 Hz, H-1″), 5.31, (1H, d, J = 7.7 Hz, H-1′), 4.05 (2H, t, J = 5.9 Hz, H-8), 3.02 (2H, t, J = 5.9 Hz, H-7), 1.60 (3H, d, J = 6.0 Hz, H-6″); ESIMS *m/z* 485.2 [M + Na]$^+$; HRESIMS (positive-ion mode) *m/z* 463.1818 [M + H]$^+$ (calcd for $C_{20}H_{31}O_{12}$, 463.1816).

Table 1. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 MHz) Data of **1**–**3** (δ ppm)

	1[a]		**2**[b]		**3**[b]	
no.	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1		144.2, C	7.61, d (3.1)	110.2, CH		154.9, C
2		145.2, C		155.2, C		140.0, C
3	7.40, d (9.0)	121.6, CH	7.34, dd (3.1, 9.2)	124.7, CH		155.5, C
4	7.21, d (9.0)	114.0, CH	7.52, d (9.2)	120.3, CH	6.82, s	100.7, CH
4a		151.1, C		151.0, C		157.9, C
10a		152.3, C		152.3, C		151.0, C
5	6.96, s	102.2, CH		135.9, C		135.5, C
6		152.0, C		157.0, C		156.3, C
7		144.7, C	7.01, d (8.8)	114.6, CH	6.98, d (7.8)	114.2, CH
8	7.70, s	105.2, CH	7.88, d (8.8)	122.2, CH	7.82, d (7.8)	122.7, CH
8a		115.2, C		116.3, C		117.4, C
9a		115.6, C		123.2, C		110.6, C
9		175.3, C		176.8, C		175.0, C
OCH_3-1	4.07, s	62.6			3.98, s	62.4
OCH_3-2					3.95, s	61.9
OCH_3-5			4.00, s	61.9	4.00, s	61.9
OCH_3-7	4.01, s	56.5				

[a] In $CDCl_3$. [b] In DMCO-d_6.

Table 2. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 MHz) Data of **4** and **5** (δ ppm)

	4[a]		**5**[b]	
no.	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1		142.4, C		147.2, C
2	6.81, s	107.0, CH		147.1, C
3		157.4, C	6.78, m	117.5, CH
4		111.4, C	6.79, m	125.6, CH
5		157.4, C		132.3, C
6	6.81, s	107.0, CH	7.02, d (1.2)	119.7, CH
7			2.85, t (6.8)	40.3, CH_2
8			3.70, m	65.1, CH_2
1′		141.1, C	4.70, d (7.4)	104.8, CH
2′	7.58, dd (8.1, 1.5)	127.1, CH	3.49, dd (7.4, 9.4)	75.3, CH
3′	7.40, dt (8.1, 1.5)	129.2, CH	3.46, t (9.4)	78.1, CH
4′	7.31, tt (8.1, 1.5)	127.8, CH	3.32, t (9.4)	72.2, CH
5′	7.40, dt (8.1, 1.5)	129.2, CH	3.56, m	77.6, CH
6′	7.58, dd (8.1, 1.5)	127.1, CH	4.07, dd (12.0, 6.2) 3.56, dd (12.0, 2.0)	68.5, CH_2
1″	4.95, d (10)	76.3, CH	4.73, d (1.6)	102.6, CH
2″	3.75, t (9.9)	74.0, CH	3.87, dd (1.6, 3.4)	72.7, CH
3″	3.59, t (9.9)	78.9, CH	3.71, dd (3.4, 9.4)	72.9, CH
4″	3.63, t (9.9)	70.0, CH	3.37, t (9.4)	74.4, CH
5″	3.49, m	81.5, CH	3.64, m	70.4, CH
6″	3.87, dd (12.0, 3.8) 3.81, dd (11.9, 2.8)	61.1, CH_2	1.27, d (6.1)	18.5, CH_3

[a] In DMCO-d_6. [b] In CD_3OD.

Acidic Hydrolysis of 5. Compound **5** (3 mg) in 50% MeOH (3 mL) containing 5% HCl was refluxed in a boiling H_2O bath for 5 h. After cooling, the reaction mixture was poured into 10 mL of H_2O. The mixture was extracted with EtOAc, and the aqueous residue was then checked by co-TLC together with authentic sugar samples (EtOAc−MeOH−H_2O−HOAc, 13:3:3:4, glucose, $R_f = 0.45$; rhamnose, $R_f = 0.56$).

Assay for Inhibition of COX-1 and COX-2 Activity. The assay for inhibition of COX-1 and COX-2 activity is based on the model previously reported in our laboratory.[38] Briefly, after 24 h infection with human COX-1 or COX-2 recombinant baculovirus, sf9 cells expressed abundant COX-1 or COX-2 protein. Expressing cells, 1×10^6 COX-1 or 1×10^6 COX-2, were dispensed to 24-well plates. Various concentrations of calophymembranols A (**1**) and 3, 4-dihydroxyxanthone, or the positive controls diclofenac and celecoxib were added to the appropriate wells containing the cell suspension. Following a 15-min incubation, the cells were challenged with 10 μmol/L arachidonic acid (Sigma, final concentration) in EtOH (<1% v/v) and incubated for 10 min at 37 °C. Reactions were terminated by the addition of 100 μL of 1 M HCl, then neutralized with 100 μL of 1 M NaOH. The cells were pelleted for 10 min at 300*g*, and the production of PGE2 in the supernant was determined by a PGE2-specific RIA (Beijing East Asia Institute of Immunology). The production of PGE2 was then determined by interpolation from a standard curve, and the inhibitory rate was calculated by comparison of the PGE2 production by drug-treated groups with those of no drug, control groups.

Acknowledgment. The authors are grateful to Shanghai Committee of Science and Technology for the partial financial support (No. 03DZ19228).

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NP0502342