A new pyranoxanthone from the stems of *Calophyllum membranaceum*

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Abstract

A new pyranoxanthone, membraxanthone A (**1**) has been isolated from the ethanol extract of the stem of *Calophyllum membranaceum*, together with three known pyranoxanthones, nigrolineaxanthone W (**2**), calophinone (**3**) and caloxanthone I (**4**), and three known triterpenoids, friedelin (**5**), canophyllol (**6**) and canophyllic acid (**7**). Their structures were elucidated on the basis of chemical evidence and intensive spectroscopic analysis including HRESI-MS, 1D- and 2D-NMR. All of the compounds were isolated from this plant for the first time. These xanthones showed no activities towards human cancer cell lines KB, BC-1 and NCI-4460.

Keywords: *Calophyllum membranaceum,* Guttiferae, pyranoxanthones, membraxanthone A, triterpenoids

Introduction

Since the research group of the National Cancer Institute reported that (+)-calanolide A and inophyllum B isolated from *Calophyllum lanigerum* Miq. and *C. inophyllum* L., respectively, which showed strong activity against human immunodeficiency virus type 1 (HIV-1),[1-2] a considerable number of studies have been performed on plants of the Calophyllum genus in the family of Guttiferae. Besides pyrancoumarins,[3-5] the genus is considered as a rich source of xanthone derivatives which possess antibacterial,[6] antifungal,[7] antiviral,[8] antimalarial,[9] antiplatelet aggregation,[10] immunomodulatory,[11] and cancerchemopreventive activities.[12]

Calophyllum membranaceum Gaertn. et Champ. is an evergreen tree which is only distributed in the Hainan island, P. R. China. In a previous paper, we reported the isolation and identification of triterpenoids and flavonoids from the leaves of *C. membranaceum*.[13] Further

investigation on the stems of this plant led to the isolation of a new pyranoxanthone, membraxanthone A (**1**), together with three known pyranoxanthones, nigrolineaxanthone W (**2**), calophinone (**3**) and caloxanthone I (**4**), three known triterpenoids, friedelin (**5**), canophyllol (**6**) and canophyllic acid (**7**) (Figure 1). Their structures were established using spectral methods, especially 1D- and 2D-NMR. The xanthones were screened for their cytotoxicities against the human cancer cell lines KB, BC-1 and NCI-4460. Unfortunately, the results showed no activities towards these human cancer cells.

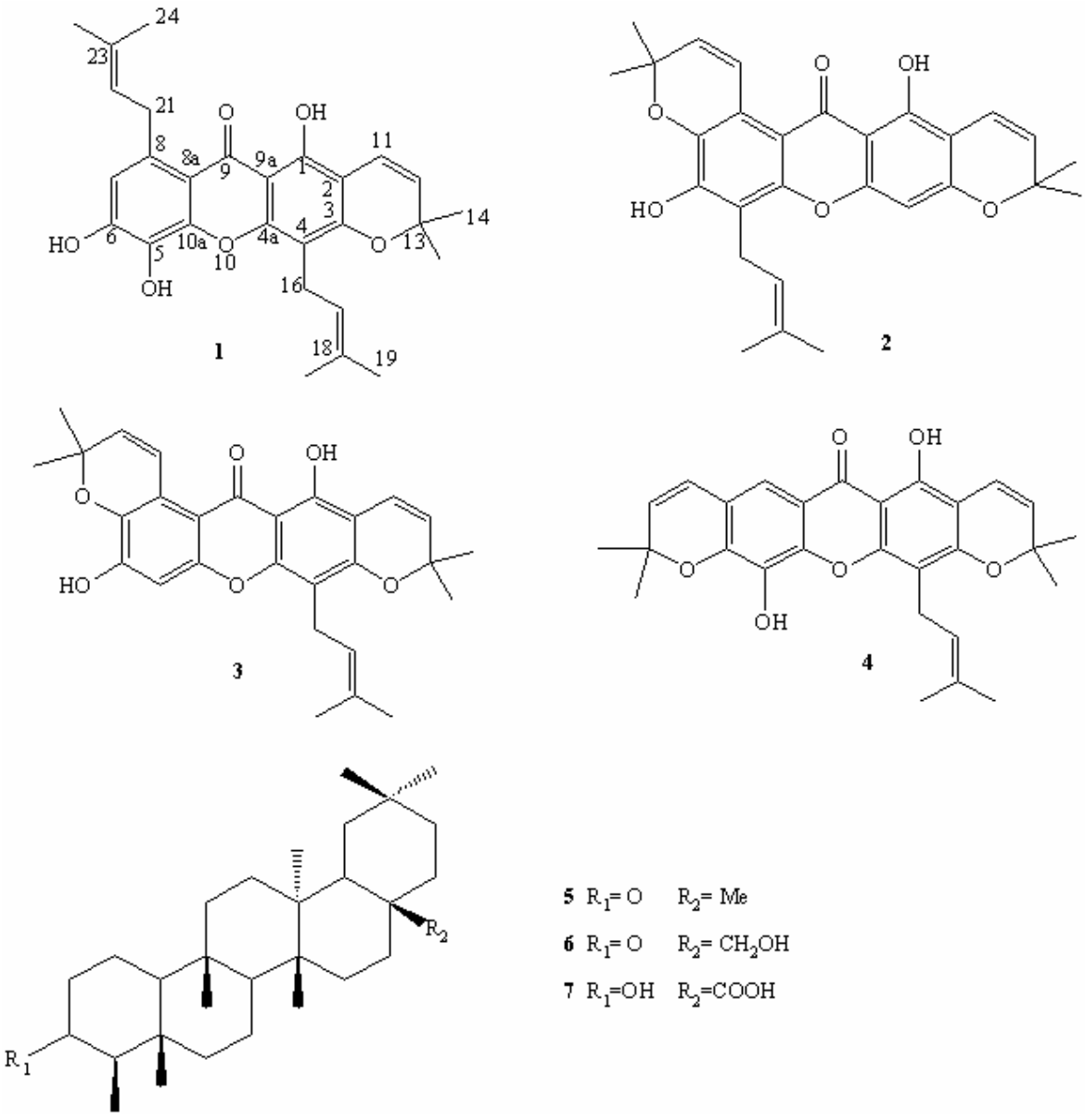

Figure 1. Chemical Structures of the compounds **1-7**.

Results and Discussion

General Procedures. Column chromatography of the ethanol extract from the stem of the plant gave eight main fractions. Further purification of fraction 4 gave compound **1** as a yellow amorphous powder, which gave a positive $FeCl_3$ test. Its molecular formula was determined as $C_{28}H_{30}O_6$ on the basis of negative HR-ESI-MS *m/z* 461.1957 $[M-H]^-$. The maximum UV absorption at λ_{max} 247, 258, 298 and 319 nm suggested the existence of a xanthone skeleton.[6] IR spectrum indicated the presence of hydroxyls (3452 cm^{-1}) and a conjugated carbonyl group (1654 cm^{-1}). The ^{1}H-NMR (Table 1) showed an aromatic singlet proton at δ 6.77 and a chelated hydroxyl proton at δ 13.5. The ^{13}C-NMR data (Table 1) demonstrated a carbonyl signal at δ 182.9 along with twelve aromatic carbon signals, six of them oxygenated at δ 157.2, 155.9, 153.6, 153.5, 150.7 and 139.6. The distribution of these aforementioned data was very similar to that of calophinone (**3**), except for the difference in ring A. The differences in ^{1}H-NMR spectrum of compound **1** from those of **3** included the absence of two olefinic hydrogen resonances at δ 8.03 (d, *J* = 10 Hz) and 5.83 (d, *J* = 10 Hz), and one aromatic singlet proton at δ 6.85 (s), and the appearance of other one olefinic proton at δ 5.21 (t, *J* = 6.8 Hz), two methylene protons at 3.42 (d, *J* = 6.0 Hz) and one aromatic singlet proton at δ 6.77 (s). The differences in ^{13}C-NMR spectrum of compound **1** from those of **3** are similar to those observed in ^{1}H-NMR spectrum. These different signals between the two compounds suggested that the dimethylpyrene ring attached to C-8/C-7 in calophinone (**3**) had been opened to be a 3-methylbut-2-enyl substituent in compound **1**. According to HMBC data, the methylene protons at δ 3.42 (H-21) correlated with carbons at δ 106.4 (C-7), δ 111.3 (C-8a) and δ 131.8 (C-23), while δ 5.21 (H-22) correlated with carbon at δ 127.2 (C-8), indicating that the isoprenyl group attached to C-8. The aromatic singlet proton at δ 6.77 caused cross peaks with carbons at δ 153.5 (C-6), δ 139.6 (C-5), δ 111.3 (C-8a) and δ 21.3 (C-21), which suggested the aromatic proton located on C-7. Therefore, membraxanthone A was assigned as 1, 5, 6-trihydroxy-4, 8-diisoprenyl-6′, 6′-dimethyl pyrano(2′, 3′: 2, 3)xanthone.

In addition to compound **1**, a number of other known compounds including three pyranoxanthones, nigrolineaxanthone W (**2**),[14] calophinone (**3**)[15] and caloxanthone I (**4**),[15] and three triterpenoids, friedelin (**5**),[13] canophyllol (**6**)[13] and canophyllic acid (**7**)[13] were also isolated from the plant. They were identified by comparison with spectral data of related compounds. Physical and spectroscopic details obtained for the known compounds **2-7** are available in the Supplementary Information.

Experimental Section

General Procedures. The IR spectra were recorded on a Nicolet 5DX-FTIR spectrophotometer. The UV spectra were measured on a Shimadzu UV-240 spectrophotometer. The NMR spectra were recorded on a Bruker Avance-400 M instrument. ESI-MS were obtained on a Finnigan

LCQ Advantage mass spectrometer and HRESI-MS on a API Qstar Pulsar-LC/TOF mass spectrometer. Silica gel (200-300 mesh, Qingdao marine Chemical, Qingdao, P.R.China), RP-18 silica gel (50 μm, Merck, Darmstadt, Germany) and Pharmadex LH-20 (Amersham Pharmacia Biotech., Hongkong, P.R.China) were used for column chromatography. Precoated silica gel GF254 plates and RP-18 F254 plates (0.25 mm, Merck, Darmstadt, Germany) were used for TLC.

Table 1. NMR data of **1** (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, $CDCl_3$)

Carbons	DEPT	δ_C (ppm)	δ_H (ppm)	HMBC*
1		155.9		
2		104.2		
3		157.2		
4		103.6		
4a		153.6		
5		139.6		
6		153.5		
7	CH	106.4	6.77 (1H, s)	5, 6, 8a
8		127.2		
8a		111.3		
9		182.9		
9a		101.7		
10a		150.7		
11	CH	116.1	6.72 (1H, d, *J* 10 Hz)	1, 3, 13
12	CH	126.9	5.76 (1H, d, *J* 10 Hz)	2, 13
13		77.7		
14	CH_3	28.2	1.47 (3H, s)	12, 14
15	CH_3	28.2	1.47 (3H, s)	12, 14
16	CH_2	26.0	4.34 (1H, d, *J* 7.2 Hz)	3, 4a, 18
17	CH	121.4	5.30 (2H, t, *J* 7.2 Hz)	4
18		135.8		
19	CH_3	18.1	1.88 (3H, s)	17, 18
20	CH_3	25.9	1.78 (3H, s)	17, 18
21	CH_2	21.3	3.42 (1H, d, *J* 6.8 Hz)	7, 8a, 23
22	CH	122.4	5.21 (2H, t, *J* 6.8 Hz)	8
23		131.8		
24	CH_3	17.9	1.88 (3H, s)	22
25	CH_3	25.8	1.67 (3H, s)	22
1-OH			13.50 (1H, s)	2, 9a

* long-range 1H-13C coupling, carbons to protons

Plant materials. The stem of *Calophyllum membranaceum* Gaertn. et Champ. was collected in April 2004 from Lingshui County, Hainan Province, P. R. China, and authenticated by Professor Qiong-xin Zhong (Department of Biology, Hainan Normal University, Hainan Province). A voucher specimen (20040419) was deposited in the Herbarium of the Department of Chemistry, Hainan Normal University.

Extraction and isolation. The air-dried and powdered stem of *C. membranaceum* (5 kg) was extracted with 70 % ethanol (3×30 L, each for 7 d) at room temperature. After evaporation of solvents in vacuo, 520 g 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 afford the corresponding fractions. The $CHCl_3$ extract (50 g) was subjected to column chromatography (CC) on silica gel eluted with petroleum ether-acetone gradient (1000:1 → 0:100) to obtained eight fractions, namely A1-A8. Fraction A2(2.5g) was separated by silica gel CC eluted with petroleum ether-ethyl acetate(20:1-5:1) to obtain compound friedelin (**5**, 50mg), canophyllol (**6**, 45mg). Fraction A4 (2 g) was separated by silica gel CC eluted with petroleum ether-acetone (20:1, 10:1, 5:1, 1:1) to give four fractions, namely B1-B4. Fraction B3 was further purified by C-18 reverse-phase silica gel CC eluted with CH_3OH-H_2O (8:2) to obtained compound membraxanthone A (**1,** 20 mg), nigrolineaxanthone W (**2**, 15 mg) and calophinone (**3**, 35 mg). Fraction A6 (1.6 g) was subjected to silica gel CC eluted with petroleum ether-acetone (10:1 → 1:2) to give five fractions C1-C5. Fraction C4 was then purified by C-18 reverse-phase silica gel CC eluted with CH_3OH-H_2O (4:6) and Sephadex LH-20 (CH_3OH) to yield compound caloxanthone I (**4**, 15 mg), canophyllic acid (**7**, 30mg).

Membraxanthone A 1. A pale-yellow needle. IR: 3455, 2960, 2926, 2858, 1662, 1600, 1458, 1293, 1127, 843 cm^{-1}; UV (MeOH) λ_{max} nm (logε): 216 (1.73), 291 (1.91), 303 (1.96), 334 (1.66); ESI-MS (positive mode): m/z 463.1 $[M+H]^+$; ESI-MS (negative mode): m/z 461.3 $[M-H]^-$; ESI-MS/MS (positive mode): m/z 463.1 $[M+H]^+$ to 407.1 $[M+H-C_4H_8]^+$; HR-ESI-MS (negative mode): m/z 461.1957 ($[M-H]^-$, calculated for $C_{28}H_{29}O_6$, 461.1964); ^1H and ^{13}C NMR see Table 1.

Cytotoxicity bioassays. The cytotoxicity of compounds **1-6** was determined employing the colorimetric method as described by Skehan et al.[16] The reference substance, ellipticine, exhibited cytotoxic activity against KB, BC-1 and NCI-4460 cells with IC_{50} values of 1.45, 1.60, and 0.56 mg/mL, respectively.

Physical and spectroscopic details obtained for the known compounds **2-7** are available in the Supplementary Information.

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