Article

Caloxanthones O and P: Two New Prenylated Xanthones from *Calophyllum inophyllum*

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Abstract: Chemical investigation of the EtOH extract of the twigs of *Calophyllum inophyllum* collected in Hainan Province of China resulted in the isolation of two new prenylated xanthones, caloxanthone O (**1**) and caloxanthone P (**2**). Their structures were elucidated by a study of their physical and spectral data. Compound **1** exhibited cytotoxicity against human gastric cancer cell line (SGC-7901), with an IC_{50} value of 22.4 μg mL^{-1}.

Introduction

Calophyllum inophyllum Linn, which belongs to the family Clusiaceae, is an evergreen shrub widely distributed in tropical areas. It is used in traditional Chinese folk medicine for the treatment of eye diseases, wounds, rheumatism and inflammations [1,2]. The chemical literature reflects the existence of wide variety of natural products in this plant, such as pyranocoumarins [3–9], xanthones [4,5,8], triterpenes [9,10], and flavonoids [11], which possessed various bioactivities such as anti-HIV-1 [3], anti-microbial [9], and cytotoxic activities [9,12]. Previously, we isolated a new cytotoxic prenylated xanthone from the twigs of *C. inophyllum* collected in Hainan Province of China [12]. In the continuous search for bioactive constituents, two new prenylated xanthones, named caloxanthones O (**1**) and P (**2**) were obtained. Compound **1** exhibited cytotoxicity against human gastric cancer cell line. The present paper discusses their structural elucidation and cytotoxicity.

Results and Discussion

Compound **1**, was obtained as a yellow powder that reacted positively to the Gibbs and $FeCl_3$ reagent, indicating the presence of phenolic groups. Its HR-ESI-MS spectrum showed the quasi-molecular $[M+Na]^+$ ion peak at *m/z* 467.1681 (calc. 467.1676), corresponding to the molecular formula $C_{24}H_{28}O_8$. This formula could also be validated through its ^{1}H-NMR, ^{13}C-NMR and DEPT data. The IR spectrum displayed free hydroxyl (3,560 cm^{-1}), chelated hydroxyl (3,160 cm^{-1}), conjugated carbonyl (1684 cm^{-1}), and aromatic ring (1,589, 1,540 cm^{-1}) absorptions. These data, together with those obtained from the UV spectrum [λ_{max} (MeOH) 217, 222, 252, 282, 321 nm] were consistent with the presence of a xanthone skeleton [4, 5]. In the ^{1}H-NMR spectrum, a chelated hydroxyl group δ_H 13.73 (s, 1H), two isolated aromatic protons δ_H 6.19 (s, 1H), 6.78 (s, 1H) and one methoxyl (δ_H 3.84) were observed. The ^{1}H-NMR spectrum of **1** also showed two methyl singlets (δ_H 1.25 and δ_H 1.55), a methyl doublet (δ_H 1.36, *J* = 6.5 Hz) and one-proton quartet (δ_H 4.56, *J* = 6.2 Hz), which suggested the presence of an α,α,β-trimethyldihydrofuran ring [4]. Furthermore, two methyl singlets (δ_H 1.15, δ_H 1.17), an oxymethine proton (δ_H 3.41, 1H, dd, *J* = 6.3, 9.6 Hz)，and two methylene protons (δ_H 2.62 and 3.78, each 1H) were observed in the ^{1}H-NMR spectrum, which indicated the presence of a C_5 unit characterized as a 2,3-dihydroxy-3-methylbutyl chain in **1** [13]. Assignment of the ^{13}C-NMR spectral data are shown in Table 1. A combination of the ^{1}H-^{1}H COSY and HMQC experiments permitted the assignment of all the protonated carbons. It remained to establish the positions of the substituents on the xanthone skeleton. In the HMBC spectrum (Figure 2), the proton of chelated hydroxyl group 1-OH (δ_H 13.73) was correlated to three carbons C-9a (δ_C 102.7), C-2 (δ_C 92.9), and C-1 (δ_C 163.5), which suggested that the chelated hydroxyl group was located at C-1. Aromatic carbons with an oxygen function were observed at C-1 (δ_C 163.5), C-3 (δ_C 164.9) and C-4a (δ_C 151.3) in the ^{13}C-NMR spectrum, which indicated that this aromatic ring was a phloroglucinol ring [14]. Therefore, the α,α,β-trimethyldihydrofuran ring was clearly fused at C-4 through an oxygen at C-3 position. This result was further confirmed by the long-range correlations of H-12 (δ_H 1.25) and H-13 (δ_H 1.55) with C-4 (δ_C 112.0) and H-14 (δ_H 4.56) with C-3 (δ_C 164.9) in the HMBC spectrum. The positions of the 2,3-dihydroxy-3-methylbutyl chain and the remaining phenolic hydroxyl group were established as follows. In the HMBC spectrum (Figure 2), the methylene protons H_2-16 (δ_H 3.78 and δ_H 2.62) were correlated to three aromatic carbons C-8a (δ_C 110.8), C-7 (δ_C 117.7), and C-8 (δ_C 139.4). The latter

resonance at C-8 also gave cross-peak with the oxymethine proton H-17 (δ_H 3.41). These results demonstrated clearly that the 2,3-dihydroxy-3-methylbutyl moiety was located at C-8. The cross-peaks from the aromatic proton (δ_H 6.78) to C-16 (δ_C 36.7), C-8a (δ_C 110.8), and C-5 (δ_C 132.8) in the HMBC spectrum indicated that the aromatic proton (δ_H 6.78) was assigned at C-7 position. The methoxyl group was deduced to be located at C-5 position by ROESY experiment revealing the cross-peak from the 5-OMe (δ_H 3.84) to H-13 (δ_H 1.55) (Figure 2). The downfield shifts of C-6 (δ_C 155.3) and C-18 (δ_C 71.9) revealed that each carbon should be connected to a hydroxyl group. The configuration at C-17 has not been determined in this study because the amount of compound **1** is too low. On the basis of the above results, the structure of compound **1** was thus elucidated and named caloxanthone O.

Figure 1. Structures of compounds **1** and **2**.
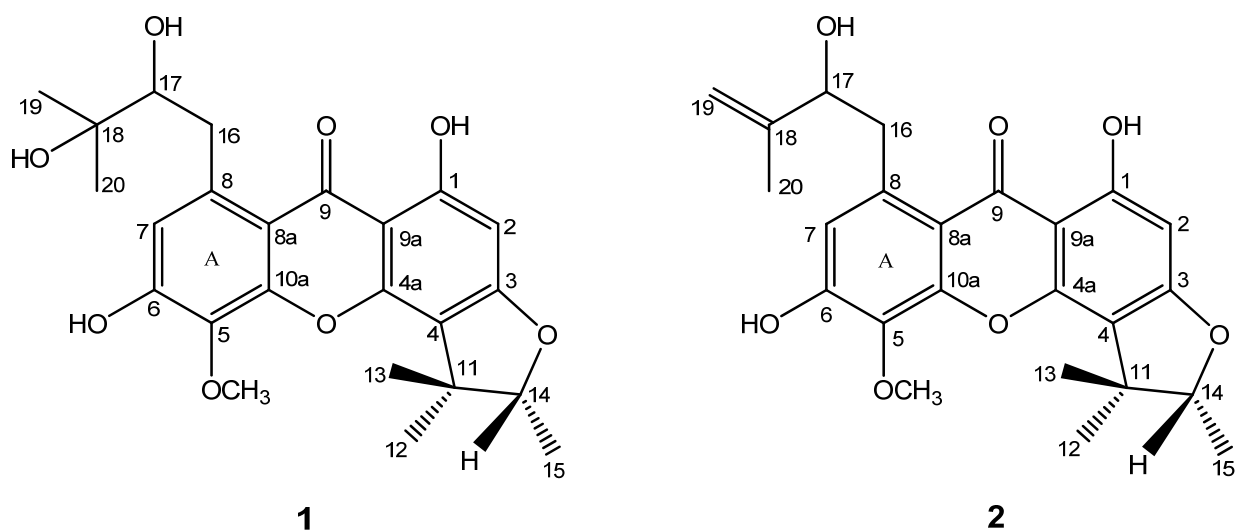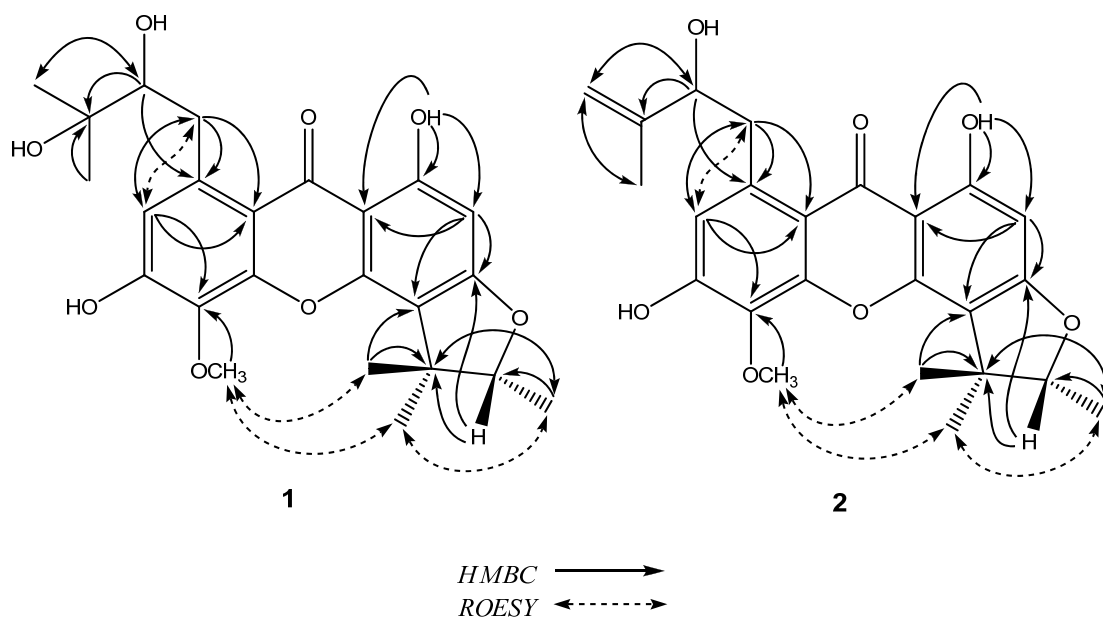
Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

HMBC ——→
ROESY ◄------

Compound **2**, was obtained as yellow powder and reacted positively to the Gibbs and FeCl$_3$ reagent. The [M+Na]$^+$ at *m/z* 449.1576 in the HR-ESI-MS spectrum corresponds to the molecular formula C$_{24}$H$_{26}$O$_7$ (calc. 449.1571). The IR and UV spectra were suggestive of a xanthone derivative. The ^{1}H- and ^{13}C-NMR were closely related to those of **1**, except for the substitution of C$_5$ unit side chain in ring A. The ^{1}H-NMR spectrum of **2** showed a methyl singlet (δ_H 1.86), an olefinic methylene (δ_H 4.77 and 4.96, each 1H), an oxymethine (δ_H 4.35, dd, *J* = 8.8, 3.6 Hz) and a methylene (δ_H 3.02, dd, *J* = 12.6, 3.6 Hz and δ_H 3.76, dd, *J* = 12.6, 3.6 Hz), which established a 2-hydroxy-3-methylbut-3-enyl group. The positions of the substitutions in **2** were established by HMBC and ROESY spectra (Figure 2). The configuration at C-17 has not been determined in this study because the amount of compound **2** is too low. From the above evidence, the structure of the compound **2** was thus confirmed, and given the trivial name caloxanthone P.

Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **2**. (^{1}H at 400 and ^{13}C at 100 MHz; *J* in Hz).

No.	**1**[a]		**2**[b]	
	δ_C	δ_H	δ_C	δ_H
1	163.5 (*s*)		166.5 (*s*)	
2	92.9 (*d*)	6.19 (1H, *s*)	95.1 (*d*)	6.13 (1H, *s*)
3	164.9 (*s*)		167.7 (*s*)	
4	112.0 (*s*)		114.1 (*s*)	
5	132.8 (*s*)		135.4 (*s*)	
6	155.3 (*s*)		153.8 (*s*)	
7	117.7 (*d*)	6.78 (1H, *s*)	119.5 (*d*)	6.83 (1H, *s*)
8	139.4 (*s*)		140.6 (*s*)	
9	181.5 (*s*)		184.2 (*s*)	
4a	151.3 (*s*)		153.9 (*s*)	
8a	110.8 (*s*)		113.8 (*s*)	
9a	102.7 (*s*)		105.3 (*s*)	
10a	151.3 (*s*)		156.8 (*s*)	
11	43.0 (s)		45.5 (s)	
12	21.1 (*q*)	1.25 (3H, *s*)	22.7 (*q*)	1.33 (3H, *s*)
13	25.0 (*q*)	1.55 (3H, *s*)	26.9 (*q*)	1.63 (3H, *s*)
14	90.3 (*d*)	4.56 (1H, *q*, 6.2 Hz)	92.6 (*d*)	4.57 (1H, *q*, 6.5 Hz)
15	13.9 (*q*)	1.36 (3H, *d*, 6.5 Hz)	15.5 (*q*)	1.41 (3H, *d*, 6.6 Hz)
16	36.7 (*t*)	3.78 (1H, *d*, 12.8 Hz, H-16a)	43.5 (*t*)	3.76 (1H, *dd*, 12.6, 3.6 Hz, H-16a)
		2.62 (1H, *t*, 11.3 Hz, H-16b)		3.02 (1H, *dd*, 12.6, 3.6 Hz, H-16b)
17	77.2 (*d*)	3.41 (1H, *dd*, 9.6, 6.3 Hz)	77.0 (*d*)	4.35(1H, *dd*, 3.6, 8.8 Hz)
18	71.9 (*s*)		150.6 (*s*)	
19	25.5 (*q*)	1.15 (3H, *s*)	111.0 (*t*)	4.96 (1H, *s,* H-19a)
				4.77 (1H, *s,* H-19b)
20	25.7 (*q*)	1.17 (3H, *s*)	19.6 (*q*)	1.86 (3H, *s*)
OMe−5	60.7 (*q*)	3.84 (3H, *s*)	62.8 (*q*)	3.96 (3H, *s*)
OH−1		13.73 (1H, *s*)		13.68 (1H, *s*)

^{a} In DMSO-*d$_6$*. ^{b} In acetone-*d$_6$*.

Compounds **1** and **2** were evaluated for their cytotoxic activity against human gastric cancer cell line (SGC-7901) using the MTT method. Compound **1** showed cytotoxic activity against the SGC-7901 cell line with the IC_{50} value of 22.4 μg mL^{-1}, while compound **2** was inactive ($IC_{50} > 100$ μg mL^{-1}).

Experimental

General

Melting points were obtained on a Beijing Taike X-5 stage apparatus and are uncorrected. Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rudolph Research Analytical, NJ, USA). The UV spectra were measured on a Shimadzu UV-2550 spectrometer. The IR spectra were obtained on a Nicolet 380 FT-IR instrument, as KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HRESIMS spectra were measured with an API QSTAR Pulsar mass spectrometer. Column chromatography was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck). TLC was preformed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China).

Plant Material

The twigs of *Calophyllum inophyllum* L. were collected in Wenchang county, Hainan Province, China in May 2006, the plant was identified by Associate Professor Zheng-Fu Dai of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 20060508) of *C. inophyllum* was deposited.

Extraction and Isolation

The dried and crushed twigs of *C. inophyllum* (19.9 kg) were extracted with 95% EtOH (70 L) three times at room temperature. After removal of EtOH by evaporation, the EtOH extract was suspended in water (6.0 L) and successively partitioned with petroleum ether (4 L) three times to give a Petro-soluble extract (290.0 g) and an aqueous residue. The Petro-soluble extract (290.0 g) was applied to a silica gel (200-300 mesh) column packed in CHCl$_3$. The column was then eluted in gradient elution with CHCl$_3$-acetone (1:0, 20:1, 10:1, 5:1, 2:1, 0:1) to afford 16 fractions. The active fraction (fraction 16, 44.0 g) was then subjected to repeated column chromatography over silica gel using Pet-EtOAc as eluent and further separated by column chromatography over Sephadex LH-20 using 95% EtOH as eluent to afford **1** (5.6 mg). The 95% MeOH fraction (245.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel, eluting with gradient elution CHCl$_3$-MeOH to afford 10 fractions. The active fraction (fraction 3, 5.7 g) was then subjected to repeated column chromatography over silica gel using Pet-acetone as eluent and further separated by column chromatography over Sephadex LH-20 using 95% EtOH as eluent to afford **2** (6.2 mg).

Caloxanthone O: Yellow powder, M.p. 228– 230 ºC. $[\alpha]_D^{25} = + 32.0$ (*c* = 0.50, MeOH). UV (MeOH): λ_{max} (log ε_{max}): 217 (1.25), 222 (sh), 252 (0.51), 282 (0.32), 321 (0.28) nm. IR (KBr): ν = 3560, 3160, 2314, 1684, 1589, 1540, 1419, 1384, 843 cm^{-1}. HRMS ((+)-ESI): *m/z* = 467.1681 (calcd. 467.1676 for $C_{24}H_{28}O_8Na$, [M + Na]$^+$). ^{1}H and ^{13}C-NMR: see Table 1.

Caloxanthone P: Yellow powder, M.p. 203 − 204 ºC. $[\alpha]_D^{25}$ = + 56.0 (*c* = 0.50, MeOH). UV (MeOH): λ_{max} (log ε_{max}): 219 (1.95), 258 (2.17), 281 (1.36), 325 (1.47) nm. IR (KBr): ν = 3556, 3473, 3414, 2414, 2230, 1725, 1619, 1585, 1343, 1276, 1048, 907, 861, 830 cm^{-1}. HRMS ((+)-ESI): *m/z* = 449.1576 (calcd. 449.1571 for $C_{24}H_{26}O_7Na$, $[M + Na]^+$). ^{1}H and ^{13}C-NMR: see Table 1.

Bioassay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the previously reported method [15]. The inhibition rates (IR%) were calculated using OD mean values from IR% = $(OD_{control} − OD_{sample})/OD_{control}$. The IC_{50} value, which was defined as the concentration of sample needed to reduce 50% of absorbance relative to the vehicle-treated control, was determined using the Bliss method. The same experiment was repeated independently three times to obtain a mean IC_{50} and its standard deviation.

4. Conclusions

It was reported that a wide variety of bioactive natural products exist in the plant *Calophyllum inophyllum*, such as pyranocoumarins, triterpenes, and flavonoids, together with xanthones, especially prenylated xanthones. In our continuous search for bioactive constituents, two new prenylated xanthones, named caloxanthones O (1) and P (2) were obtained. Compound 1 showed cytotoxic activity against the SGC-7901 cell line with the IC_{50} value of 22.4 μg mL^{-1}, while compound 2 was inactive (IC_{50} > 100 μg mL^{-1}).

Acknowledgements

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Sample Availability: Samples of the compound **1** and **2** are available from the authors.