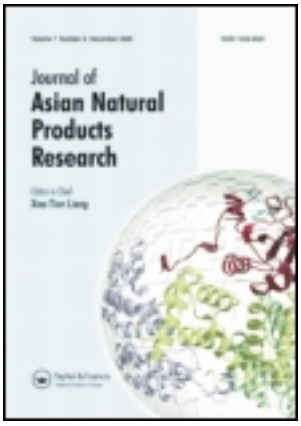


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

A new prenylated xanthone from the branches of *Calophyllum inophyllum*

Dai-Jing Wei [a b], Wen-Li Mei [a], Hui-Ming Zhong [b], Yan-Bo Zeng [a], Xu-Dong Wu [a] & Hao-Fu Dai [a]

[a] Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, China

[b] College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao, 266042, China

Available online: 15 Mar 2011

To cite this article: Dai-Jing Wei, Wen-Li Mei, Hui-Ming Zhong, Yan-Bo Zeng, Xu-Dong Wu & Hao-Fu Dai (2011): A new prenylated xanthone from the branches of *Calophyllum inophyllum* , Journal of Asian Natural Products Research, 13:03, 265-269

To link to this article: http://dx.doi.org/10.1080/10286020.2010.550884

Journal of Asian Natural Products Research
Vol. 13, No. 3, March 2011, 265–269

Taylor & Francis
Taylor & Francis Group

A new prenylated xanthone from the branches of *Calophyllum inophyllum*

Dai-Jing Wei[ab], Wen-Li Mei[a], Hui-Ming Zhong[b], Yan-Bo Zeng[a], Xu-Dong Wu[a] and Hao-Fu Dai[a]*

[a]*Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;* [b]*College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China*

(*Received 25 October 2010; final version received 22 December 2010*)

The investigation of chemical constituents from the branches of *Calophyllum inophyllum* Linn led to the isolation of a new prenylated xanthone, named caloxanthone Q (**1**), together with three known compounds, 2-deprenylrheediaxanthone B (**2**), jacareubin (**3**), and 6-deoxyjacareubin (**4**). Their structures were completely elucidated on the basis of spectroscopic methods (UV, IR, HR-ESI-MS, 1D NMR, and 2D NMR).

Keywords: Clusiaceae; *Calophyllum inophyllum* Linn; prenylated xanthone; caloxanthone Q

1. Introduction

Calophyllum inophyllum Linn (Clusiaceae) is an evergreen shrub mainly distributed in Yunnan, Guangxi, Hainan, and Taiwan Provinces of China. As a traditional medicinal plant, *C. inophyllum* is used for the treatment of eye diseases, wounds, rheumatisms, and inflammations in China [1,2]. Since inophyllums B and P isolated by Patil *et al.* [3] showed strong activity against HIV-1, much attention has been paid to research on the chemical components of *C. inophyllum*, and xanthones [4–6], pyranocoumarins [4–9], terpenoids [9,10], and flavonoids [11] were found to be the main components. In our previous study, three new prenylated xanthones [12,13] were isolated from the twigs of *C. inophyllum* collected in Hainan Province of China. Our further phytochemical work on this species has led to the isolation of another new prenylated xanthone (Figure 1), which contained an acid group and named as caloxanthone Q (**1**), together with three known compounds elucidated as 2-deprenylrheediaxanthone B (**2**), jacareubin (**3**), and 6-deoxyjacareubin (**4**), respectively. We report here about the isolation and structural elucidation of these compounds.

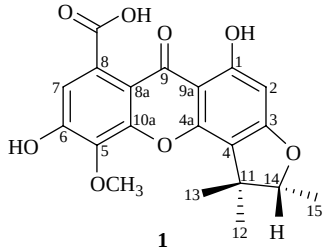
2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. The molecular formula of **1** was determined as $C_{20}H_{18}O_8$ by its HR-ESI-MS analysis, which was supported by ^{13}C NMR and DEPT spectral data (Table 1). The IR spectrum of **1** exhibited strong absorptions due to free hydroxyl (3473 cm^{-1}), chelated hydroxyl (3160 cm^{-1}), conjugated carbonyl group (1637 cm^{-1}), and aromatic groups (1584

*Corresponding author. Email: hfdai2001@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.550884
http://www.informaworld.com



1

Figure 1. Structure of compound **1**.

and 1390 cm^{-1}). These data, together with those obtained from the UV spectrum [λ_{max} (MeOH) 200, 243, 282, 325, 380 nm], were consistent with the presence of a xanthone skeleton [4,5]. The ^{13}C NMR spectrum of **1** showed 20 carbon signals, which were attributed by DEPT and HMQC techniques as 4 methyls, 3 methines, and 13 quaternary carbons including one carbonyl (δ_C 179.1) and one carboxylic acid (δ_C 170.1), six oxygenated sp^2 carbons, four sp^2 carbons, and one sp^3 carbon. The ^1H NMR spectrum (Table 1) revealed the presence of a chelated hydroxyl group at δ_H 12.99 (1H, s) and two aromatic signals [δ_H 6.26 (1H, s, H-2) and 6.84 (1H, s, H-7)]. Furthermore, it showed the presence of an α,α,β-trimethyldihydrofuran ring [δ_H 4.59 (1H, q, $J = 6.5$ Hz), 1.55, 1.25 (each 3H, s), and 1.36 (3H, d, $J = 6.5$ Hz)] and a methoxyl group (δ_H 3.86, 3H, s).

In the HMBC spectrum (Figure 2), the chelated hydroxyl group caused three cross peaks with three aromatic carbons C-9a (δ_C 102.9), C-2 (δ_C 94.1), and C-1 (δ_C 163.8), which suggested that the chelated hydroxyl group was located at C-1. The long-range correlations of H-12 (δ_H 1.25) and H-13 (δ_H 1.55) with C-4 (δ_C 113.5) and H-14 (δ_H 4.59) with C-3 (δ_C 166.2) in the HMBC spectrum indicated that the α,α,β-trimethyldihydrofuran ring was fused at C-4 through an oxygen at C-3 position [14]. By comparing the ^1H and ^{13}C NMR signals of **1** with those of caloxanthone N [12], the difference involved was only that the γ,γ-dimethyl-allyl chain at C-8 of caloxanthone N was replaced by the free carbonyl group (δ_C 170.1) in **1**. Strong interactions observed between the methoxyl protons and H-12 (δ_H 1.25) and H-13 (δ_H 1.55) in the ROESY spectrum (Figure 2) supported the placement of the methoxyl protons at C-5 in **1**. The presence of a hydroxyl group at C-6 was deduced from the downfield shift of C-6 (δ_C 157.2) and the chemical shift of the methoxyl group (δ_C 60.9) which proved to be di-*ortho* oxygen substituted, because it was observed at δ_C 60.9 in the ^{13}C NMR spectrum [15,16]. The location of the carboxylic acid (δ_C 170.1) was deduced at C-8 through the correlation of H-7 (δ_H 6.84) with the carboxylic acid

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **1** in DMSO-d_6.

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
1	163.8 (s)		9a	102.9 (s)	
2	94.1 (d)	6.26 (1H, s)	10a	152.4 (s)	
3	166.2 (s)		11	43.8 (s)	
4	113.5 (s)		12	21.9 (q)	1.25 (3H, s)
5	135.3 (s)		13	25.7 (q)	1.55 (3H, s)
6	157.2 (s)		14	91.2 (d)	4.59 (1H, q, $J = 6.5$ Hz)
7	112.8 (d)	6.84 (1H, s)	15	14.7 (q)	1.36 (3H, d, $J = 6.5$ Hz)
8	131.9 (s)		OMe-5	61.6 (q)	3.86 (3H, s)
9	179.1 (s)		COOH-8	170.1 (s)	
4a	150.8 (s)		OH-1		12.99 (1H, s)
8a	109.7 (s)				

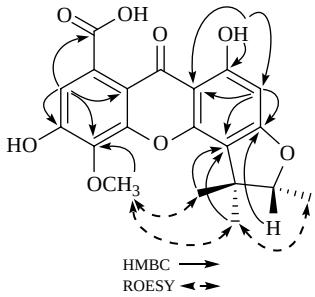


Figure 2. Key HMBC and ROESY correlations of compound **1**.

(δ_C 170.1) in the HMBC spectrum. The ROESY experiment showed that H-14 (δ_H 4.59) correlated with H-13 (δ_H 1.55), while not with H-12 (δ_H 1.25). This result indicated that when H-14 and CH_3-13 were assigned β-orientation, CH_3-12 and CH_3-15 were in α-orientation. Consequently, the structure of **1** was elucidated as shown in Figure 1, named caloxanthone Q. And this is the first report of prenylated xanthone derivative with a carboxylic acid group obtained from plants of the *Calophyllum* genus.

Three known compounds **2**–**4** were identified as 2-deprenylrheediaxanthone B, jacareubin, 6-deoxyjacareubin, respectively, by comparing their spectral data with those reported in literature [17,18].

Compounds **1**–**4** were evaluated for their cytotoxic activities against chronic myelogenous leukemia (K562), human gastric carcinoma (SGC-7901), and human hepatoma (SMMC-7721) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, but none of them were active ($IC_{50} > 20$ μg/ml).

3. Experimental

3.1 General experimental procedures

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

3.2 Plant material

The twigs of *C. inophyllum* were collected from the conservation area of a mangrove forest at Wenchang County of Hainan Province, China, in May 2006, and authenticated by Associate Professor Zheng-Fu Dai (Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China). A voucher specimen (No. 20060508) has been deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

3.3 Extraction and isolation

The dried and crushed branches of *C. inophyllum* (19.9 kg) were exhaustively extracted with 95% EtOH three times at room temperature and filtered. After evaporation, the EtOH residue was diluted in H_2O and successively extracted with petroleum ether to afford petroleum ether extract (290.0 g). The aqueous part was applied to a macroporous resin D-101 column eluted with H_2O, a gradient of 50, 95, and 100% MeOH, to afford 50% MeOH fraction (65.0 g), 95% MeOH fraction (245.0 g), and 100% MeOH fraction (70.0 g). The 50% MeOH fraction (65.0 g) was concentrated *in vacuo* to give a residue (65.0 g), which

was chromatographed on a silica gel column (200–300 mesh) with gradient $CHCl_3$–MeOH as eluents to give seven fractions. Fraction 1 (3.5 g) was subjected to silica gel column chromatography (100–200 mesh), eluting with a gradient of Me_2CO in petroleum ether (1:10 → 1:1) to yield 11 subfractions. Subfraction 6 (287.5 mg) was separated by Sephadex LH-20 column chromatography eluted with 95% EtOH to give **2** (7.5 mg) and **3** (12.1 mg). Fraction 2 (0.8 g) was subjected to silica gel column chromatography (100–200 mesh), eluted with petroleum ether–ethyl acetate (2:1) and recrystallized to afford compound **4** (9.0 mg). Fraction 3 (0.8 g) was subjected to a silica gel column (100–200 mesh) with gradient elution utilizing $CHCl_3$–MeOH as solvent system to give five fractions. Subfraction 1 (120.5 mg) was chromatographed over Sephadex LH-20 eluted with 95% EtOH, further purified by silica gel column chromatography eluted with petroleum ether–acetone (5:1), and recrystallized to afford compound **1** (11.0 mg).

*3.3.1 Caloxanthone Q (**1**)*

Yellow powder, $C_{20}H_{18}O_8$, mp 140–142°C, $[\alpha]_D^{36}$ +38.0 ($c = 0.5$, MeOH); UV (MeOH) λ_{max} 200, 243, 282, 325, 380 nm; IR (KBr) ν_{max} (cm^{-1}): 3473, 3160, 2925, 2851, 2374, 2336, 1637, 1584, 1390, 1332, 1266, 1189, 1117, 1058, 997, 860. ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS *m/z*: 409 $[M + Na]^+$; HR-ESI-MS *m/z*: 409.0897 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_8Na$, 409.0899).

3.4 Bioassay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the previously reported method [19]. The inhibition rates were calculated using optical density (OD) mean values from inhibition rate (%) = $(OD_{control} - OD_{sample})/OD_{control}$. 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.

Acknowledgements

This research was financially supported by National Nonprofit Institute Research Grant of CATAS-ITBB (ITBBZD0742) and National Basic Research Program of China (2009CB125904).

References

[1] H.Y. Chen, *Flora of Hainanica* (Science Press, Beijing, 1965), Vol. 2, p. 56.
[2] H.F. Dai and W.L. Mei, *Modern Research on Medicinal Plants in Hainan* (China Science and Technology Press, Beijing, 2007), p. 58.
[3] A.D. Patil, A.J. Freyer, D.S. Eggleston, R.C. Haltiwanger, M.F. Bean, P.B. Taylor, M.J. Caranfa, A.L. Breen, H.R. Bartus, and R.K. Johnson, *J. Med. Chem.* **36**, 4131 (1993).
[4] M. Iinuma, H. Tosa, T. Tanaka, and S. Yonemori, *Phytochemistry* **35**, 527 (1994).
[5] M. Iinuma, H. Tosa, T. Tanaka, and S. Yonemori, *Phytochemistry* **38**, 725 (1995).
[6] Y. Wu, P.C. Zhang, R.Y. Chen, D.Q. Yu, and X.T. Liang, *Acta Chim. Sin.* **61**, 1047 (2003).
[7] M. Itoigawa, C. Ito, H.T.-W. Tan, M. Kuchide, H. Tokuda, H. Nishino, and H. Furukawa, *Cancer Lett.* **169**, 15 (2001).
[8] C. Spino, M. Dodier, and S. Sotheeswaran, *Bioorg. Med. Chem. Lett.* **8**, 3475 (1998).
[9] M.C. Yimdjo, A.G. Azebaze, A.E. Nkengfack, A.M. Meyre, B. Bodo, and Z.T. Fomum, *Phytochemistry* **65**, 2789 (2004).
[10] T.R. Govindachari, N. Viswanathan, B.R. Pai, U.R. Rao, and M. Srinivasan, *Tetrahedron* **23**, 1901 (1967).
[11] Y.Z. Li, Z.L. Li, H.M. Hua, Z.G. Li, and M.S. Lui, *J. Chin. Med. Mater.* **32**, 692 (2007).

[12] Q. Xiao, Y.B. Zeng, W.L. Mei, Y.X. Zhao, Y.Y. Deng, and H.F. Dai, *J. Asian Nat. Prod. Res.* **10**, 993 (2008).
[13] H.F. Dai, Y.B. Zeng, Q. Xiao, Z. Han, Y.X. Zhao, and W.L. Mei, *Molecules* **15**, 606 (2010).
[14] Y.S. Zou, A.J. Hou, G.F. Zhu, Y.F. Chen, H.D. Sun, and Q.S. Zhao, *Bioorg. Med. Chem.* **12**, 1947 (2004).
[15] A. Makriyannis and S. Fesik, *J. Am. Chem. Soc.* **104**, 6462 (1982).
[16] I. Miura, K. Hostettmann, and K. Nakanishi, *Nouv. J. Chim.* **2**, 653 (1978).
[17] G. Rath, O. Potterat, S. Mavi, and K. Hostettmann, *Phytochemistry* **43**, 513 (1996).
[18] P.W. Westcrman, S.P. Gunasekera, M. Uvais, S. Sultanbawa, and R. Kazlauskas, *Org. Magn. Reson.* **9**, 631 (1977).
[19] T. Mosmann, *J. Immunol. Methods* **65**, 55 (1983).