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A New Prenylated Xanthone from Latex of *Garcinia cowa* Roxb.

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Abstract: A new prenylated xanthone, 1,6-dihydroxy-3,7-dimethoxy-2-(3,7-dimethyloct-2,6-dienyl) xanthone (3-*O*-methylcowaxanthone) (**1**), together with four known xanthones, cowaxanthone (**2**), 7-*O*-methylgarcinone (**3**), α-mangostin (**4**) and γ-mangostin (**5**) were isolated from the latex of *Garcinia cowa*. The structure of compound **1** was elucidated on the basis of spectroscopic data interpretation, including 1D and 2D NMR and HREIMS. The cytotoxic activitiy of **1** against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7 and SW480, was evaluated, but it was inactive (IC_{50}>40μM).

Keywords: *Garcinia cowa* Roxb.; Guttiferae; prenylated xanthone; 3-*O*-methylcowaxanthone.

1. Introduction

Plants of the genus *Garcinia* (Guttiferae) have been extensively investigated from both phytochemical and biological points of view, and they are well known as rich natural sources of xanthones, benzophenones, and biflavonoids [1]. These phenolic constituents have been reported to possess a wide range of biological and pharmacological properties, such as antibacterial [2], antimalarial [3], antioxidant [4], anti-inflammatory [5], and cytotoxic [6-7] activities. *Garcinia cowa* Roxb. is a medium size tree with edible fruits and is distributed in the southern and western parts of Yunnan Province, People's Republic of China and Southeast Asia [8]. It has been used in the folk medicine as antipyretic and anti-inflammatory [9]. Previous phytochemical investigations of *G. cowa* resulted in the isolation of xanthones [10-15], benzophenone [16], and acylphloroglucinol derivative [17]. As part of our continuing studies on bioactive compounds from tropical medicinal plants, we further investigated the chemical constituents of this species. A new prenylated xanthone, 3-*O*-methylcowaxanthone (**1**), together with four known xanthones, cowaxanthone (**2**), 7-*O*-methylgarcinone (**3**), α-mangostin (**4**) and γ-mangostin (**5**) (Figure 1) was isolated from the latex of *G. cowa*. This paper mainly deals with the isolation, structural elucidation and cytotoxicity evaluation of the new xanthone.

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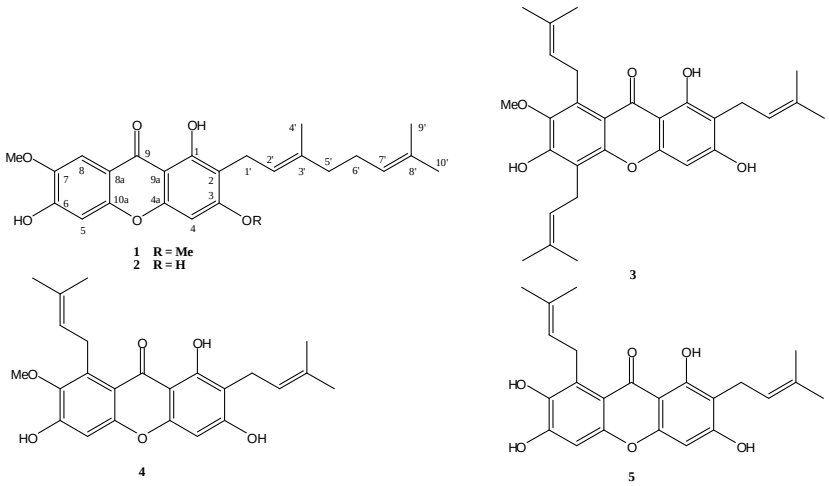

Figure 1. Structures of compounds **1-5**

2. Materials and Methods

2.1. General

TLC was preformed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China), and the spots were detected with a UV254 lamp and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. Column chromatography was performed using silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), reverse-phase C18 silica gel (40-63 μm, Merck, Darmstadt Germany) and Sephadex LH-20 (GE healthcare, Sweden), MCI-gel CHP 20P (75–150 μm; Mitsubishi Chemical Co., Japan). UV spectra were measured with a Shimadzu UV-2401 PC spectrophotometer. IR spectra were recorded on a Bruker Tensor-27 infrared spectrophotometer with KBr pellets. All NMR experiments were performed on a Bruker AM-400 and DRX-500 instruments with TMS as the internal standard. HREIMS spectra were recorded on a Waters AutoSpec Premier P776 instrument.

2.2. Plant Material

The latex of *Garcinia cowa* were collected from Xishuangbanna, Yunnan Province, P.R. China in August 2012, and authenticated by Prof. Hong Wang, herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No 20120801) was deposited in the ethnobotany research group of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

2.3. Extraction and Isolation

The yellow viscous latex (30 g) contaminated with little bark material was treated with warm EtOH and the mixture was filtered to remove the bark material. Evaporation of the solvent under reduced pressure gave a yellow-brown gum (28 g). The crude material was separated on a silica gel column chromatography (CC) using petroleum ether (PE)-EtOAc step-gradient elution (9:1– 4:6) to yield 5 fractions. Fr 2 was further chromatographed over silica gel (PE/EtOAc 4:1) to provide **3** (25 mg) and **5** (35 mg). Fr 3 was purified by RP-18 CC (eluted with 70–90% MeOH/H_2O) and Sephadex LH-20 (eluted with MeOH) to give **1** (16 mg) and **2** (21 mg). Fr 4 was subjected to RP-18 CC (60–80% MeOH/H_2O) to afford **4** (22mg).

3-O-methylcowaxanthone (**1**): Yellow amorphous powder, UV (MeOH) λ_{max} nm (log ε): 245 (4.44), 259 (4.37), 320 (4.19), 359 (3.90); IR (KBr) ν_{max}: 3386, 1658, 1609, 1577, 1521, 1483, 1287,

1157 cm^{-1}; EIMS *m/z* (rel. int.): 424 $[M]^+$ (29), 381(6), 367(5), 355(81), 356(18), 341(12), 325(11), 313(18), 302(28), 301(100), 288(15), 271(12), 69(23); HREIMS: *m/z* 424.1871 (calc. for $C_{25}H_{28}O_6$, 424.1886). ^{1}H- and ^{13}C-NMR see Table 1.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as yellow amorphous powder. The HREIMS exhibited a molecular ion at *m/z* 424.1871 (calcd. 424.1886), suggesting the molecular formula $C_{25}H_{28}O_6$. The IR spectrum showed the presence of hydroxyl group at 3386 cm^{-1} and a conjugated carbonyl at 1658 cm^{-1}. The UV spectrum had maxima absorptions at 245, 259, 320 and 359 nm. The ^{1}H NMR spectrum of 1 (Table 1) exhibited signals of a chelated phenolic hydroxy proton at δ_H 13.12 (s, 1-OH); a phenolic hydroxy proton at δ_H 6.39 (brs, 6-OH); two methoxy groups at δ_H 3.92 (s, 3-OCH_3) and 4.01 (s, 7-OCH_3) and three singlet aromatic protons at δ_H 6.42 (s, H-4), 6.93 (s, H-5) and 7.59 (s, H-8). The aromatic proton (δ_H 7.59) was assigned to be H-8 as it was deshielded by the C-9 carbonyl group. The ^{1}H NMR spectrum also displayed typical signals of one 3,7-dimethyloct-2,6-dienyl (geranyl) at δ_H 1.57, 1.64 and 1.80 (3H each, s, CH_3×3), 1.97, 2.05 (2H each, m), 3.38 (2H, d, J = 6.8 Hz), 5.06 and 5.23 (1H each, t, J = 6.8 Hz). In fact, the ^{1}H NMR spectral properties of **1** was very similar to those of cowaxanthone (**2**) except for the presence of an additional methoxy resonance at δ_H 3.92 (s, 3-OCH_3) in **1** instead of a hydroxyl group in **2**. To unequivocally corroborate the substitution pattern of **1**, the ^{1}H- and ^{13}C-NMR spectra of the known compound **2** were also listed in Table 1.The presence of the methoxyl group was confirmed by the oxymethyl carbon signal at δ_C 55.9 (3-OCH_3) in the ^{13}C NMR spectrum. From the mass difference (14 amu) between 1 and 2, it could be inferred that **1** was an O-methyl derivative of **2**. Careful comparison of the ^{13}C NMR data of **1** and **2**, particularly the shifts of C-2, C-3 and C-4, indicated that the additional methoxy substituent in **1** should be located at C-3, which confirmed by HMBC correlation between the methoxy proton (δ_H 3.92) and an oxyquaternary carbon (δ_C 163.8). NOESY correlations of methoxy proton between a lone H-4 also supported the above conclusion (Figure 2).

Furthermore, the extensive analysis of the correlation peaks in the HMBC and NOESY spectra established attachments of all remaining substituents identical to those of **2**. Another methoxy group was assigned to be at C-7 (δ_C 144.3) due to the methoxy proton (δ_H 4.01) showed correlation with C-7 in HMBC spectrum together with an observation of NOE correlations of methoxy proton between H-8. The geranyl group was confirmed and located at C-2 by the HMBC correlations of: H-1' with C-2, C-1, C-3, C-2', C-3'; H-2' with C-2, C-1', C-4'; H-5' with C-2', C-3', C-4', C-6' and C-7' ; H-6' with C-3', C-5', C-7' and C-8'; H-7' with C-9' and C-10'. Therefore, **1** was determined as1,6-dihydroxy-3,7-dimethoxy-2-(3,7-dimethyloct-2,6-dienyl) xanthone.

Additionally, compounds **2-5** were identified as cowaxanthone [18], 7-*O*-methylgarcinone [19], α-mangostin [20] and γ-mangostin [21], respectively, on the basis of ^{1}H NMR and ^{13}C NMR data and comparison with those reported in the literature.

3.2. Cytotoxicity

Cytotoxicity of compound **1** was evaluated against human leukemia HL-60, liver cancer SMMC-7721, lung cancer A549, breast adenocarcinoma MCF-7 and colon cancer SW480 cell lines by the MTT assay with *cis*-platinum (MW 300) and taxol as positive reference substance. However, **1** showed no cytotoxicity against five cancer cell lines with IC_{50} values over 40 μM.

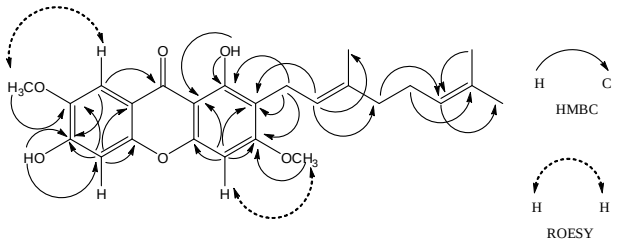

Figure 2. Key HMBC and ROESY correlations of compound **1**.

Table 1. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR data of **1** (in $CDCl_3$) and **2** in (DMSO-d_6).

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		159.2		159.5
2		111.7		109.8
3		163.8		162.5
4	6.42 (1H, *s*)	89.5	6.39 (1H, *s*)	93.0
4a		156.2		155.0
5	6.93 (1H, *s*)	102.4	6.87 (1H, *s*)	102.7
6		152.3		154.4
7		144.3		145.9
8	7.59 (1H, *s*)	104.5	7.40 (1H, *s*)	104.7
8a		113.5		111.5
9		179.8		178.8
9a		103.3		101.4
10a		152.4		151.8
1'	3.38 (2H, *d*, *J* = 6.8)	21.2	3.21 (2H, *d*, *J* = 6.9)	20.9
2'	5.23 (1H, *t*, *J* = 6.8)	122.0	5.17 (1H, *t*, *J* = 6.9)	122.2
3'		135.3		134.1
4'	1.80 (3H, *s*)	16.1	1.71 (3H, *s*)	15.9
5'	1.97 (2H, *m*)	39.8	1.89 (2H, *m*)	39.3
6'	2.05 (2H, *m*)	26.7	1.98 (2H, *m*)	26.2
7'	5.06 (1H, *t*, *J* = 6.8)	124.4	5.00 (1H, *t*, *J* = 6.9)	124.1
8'		131.2		130.7
9'	1.57 (3H, *s*)	17.6	1.49 (3H, *s*)	17.5
10'	1.64 (3H, *s*)	25.7	1.55 (3H, *s*)	25.5
1-OH	13.12 (1H, *s*)		13.35 (1H, *s*)	
3-OH			10.86 (1H, *s*)	
3-OMe	3.92 (3H, *s*)	55.9		
6-OH	6.39 (1H, *s*)		10.86 (1H, *s*)	
7-OMe	4.01 (3H, *s*)	56.5	3.86 (3H, *s*)	55.8

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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