Two New Xanthones from the Stems of *Garcinia cowa*

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Two new xanthones, 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone (1) and 1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl)xanthone (2), have been isolated together with six known xanthones: 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone (3), dulxanthone A (4), 1,5,6-trihydroxy-3,7-dimethoxyxanthone (5), 1,7-dihydroxyxanthone (6), 1,3,5-trihydroxy-6-methoxyxanthone (7), 1,3,6,7-tetrahydroxyxanthone (8), from the stems of *Garcinia cowa* (Guttiferae).

Key words *Garcinia cowa*; Guttiferae; xanthone

The plant *Garcinia cowa* ROXB. is a medium size tree found in Southeast China and Southeast Asia. The fruits are edible and abundant in (−)-hydroxycitric acid,[1] and the latex and the bark have some prenylated xanthones which show mainly a 1, 3, 6, 7 oxygenation pattern.[2—6] The constituents of the young stems of the species from Southeast China were investigated for bioactive xanthones. This paper reports the isolation and characterization of two new xanthones, 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone (**1**) and 1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl)xanthone (**2**), along with six known xanthones: 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone (**3**),[7] dulxanthone A (**4**),[8] 1,5,6-trihydroxy-3,7-dimethoxyxanthone (**5**),[9] 1,7-dihydroxyxanthone (**6**),[10] 1,3,5-trihydroxy-6-methoxyxanthone (**7**),[11] 1,3,6,7-tetrahydroxyxanthone (**8**),[12] from the stems of this plant. Compounds **1**—**4**, **7** have a 1, 3, 5, 6 oxygenation pattern of the xanthone nucleus. Examination of their bioactivities is now in progress.

Compound **1**, obtained as a pale yellow amorphous powder, had the molecular formula $C_{19}H_{20}O_7$ based on *m/z* 360.1216 (M^+) by high-resolution electron impact mass spectroscopy (HR-EI-MS). Its UV (234, 252, 285, 326 nm) and IR (3469, 1641 cm^{-1}) showed absorptions characteristic of 1,3,5,6-tetraoxygenated xanthone.[8,9,13] In the ^{1}H-NMR spectrum, four hydroxyl signals [δ_H 4.32, 9.06, 10.51 (1H, each, br s), and 13.11 (1H, s, chelated)] were observed; two one-proton doublets [δ_H 6.87, 7.46, each J=9 Hz] were further exhibited in addition to a methoxyl singlet [δ_H 3.85 (3H, s)]. The spectrum also showed the presence of two methyl groups in a singlet [δ_H 1.12 (6H, s)] and two methylene groups as two triplets [δ_H 1.52, 2.77, each J=8.5 Hz], implying the presence of a 3-hydroxyl-3-methylbutyl side chain.

The ^{13}C-NMR data of **1** (see Table 1) had a strong resemblance to **4** (characterized as dulxanthone A) indicating a xanthone skeleton with a similar substitution pattern.[8] Furthermore, the ^{1}H- and ^{13}C-NMR spectroscopic assignments for **1** were determined by the HSQC and HMBC spectrum (see Fig. 2). In the HMBC spectrum, cross-peaks the chelated hydroxyl proton δ_H 13.11 (1-OH)/δ_C 94.1 (C-2) and 101.9 (C-9a), δ_H 6.42 [H-2]/δ_C 160.9 (C-1), 101.9 (C-9a) and 163.4 (C-3), δ_H 3.85 (3-OCH_3)/δ_C 163.4 (C-3) were observed; correlations δ_H 2.77 [H-1′]/δ_C 163.4 (C-3), 153.5 (C-4a) and 108.5 (C-4), δ_H 1.52 [H-2′]/δ_C 108.5 (C-4) were present, which confirmed the A-ring feature of **1**. Furthermore, one hydroxyl group δ_H 9.06 [5-OH] showed correlations to δ_C 152.2 (C-6), 132.5 (C-5) and 146.5 (C-10a), another hydroxyl group δ_H 10.51 [6-OH] was correlated to δ_C 152.2 (C-6), 132.5 (C-5) and 112.9 (C-7); correlation between δ_H 7.46 [H-8] and δ_C 180.8 (C-9) was observed, which indicated the position of two hydroxyl groups in the B-ring. Thus, compound **1** was determined to be 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone.

Compound **2** was isolated as yellow needles, mp 217—218 °C, $C_{24}H_{24}O_6$ (*m/z* 408.1552), had UV and IR spectral data suggestive of a xanthone derivative. What is more, **2** showed analogous UV features to nigrolineaxanthone B (1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-hydroxyl-3-methylbutyl)xanthone) also suggesting the presence of a 1,3,5,6-tetraoxygenated xanthone chromophore.[13] In the ^{1}H-NMR spectrum, two hydroxyl groups [δ_H 9.37 (1H, br s), and 13.14 (1H, s, chelated)] were observed; in addition to a methoxyl singlet [δ_H 3.90], a set of signals due to a prenyl group [δ_H 3.46 (2H, d, J=7.5 Hz), 5.20 (1H, t, J=7.5 Hz), 1.79 (3H, s), 1.61 (3H, s)] was present; there were two aromatic proton signals at δ_H 7.38 and 6.49 (each 1H, s), the signal in lower field [δ_H 7.38] was assigned to H-8 which was strongly deshielded by the carbonyl, and the other signal at δ_H 6.49 (1H, s) was indicative of H-2 or H-4. Two one-proton doublets [δ_H 6.57, 5.88, each J=10 Hz] along with two methyl groups [δ_H 1.45, 6H, s] suggested the presence of a 2,2-dimethylpyran ring.

Thus, the structure of **2** was characterized by the HSQC and HMBC spectrum (see Fig. 2). In the HMBC spectrum (as shown), cross peaks δ_H 13.14 (1-OH)/δ_C 94.3 (C-2) and 102.0 (C-9a), δ_H 3.90 (3-OCH_3)/δ_C 163.6 (C-3) were observed; δ_H 3.46 [H-1″] was correlated to δ_C 163.6 (C-3), 153.1 (C-4a) and 107.5 (C-4), which confirmed a prenyl group was attached to C-4. Furthermore, the A-ring feature of **2** was confirmed by comparison of the ^{13}C-NMR data with the isolated compounds **1** and **4** (see Table 1) including literature values. The presence of a dimethylpyran ring fused with the B ring of the 1,3,5,6-tetraoxygenated xanthone nucleus at C-6 and C-7 was determined by three C–H three-bond correlations δ_H 5.88 [H-5′]/δ_C 118.2 (C-7), δ_H 6.57 [H-4′]/δ_C 146.3 (C-6) and 112.1 (C-8). Since there was no other proton signal the substituent at C-5 must be a hydroxyl group, which was established by HMBC correlations δ_H 9.37 [5-OH]/δ_C 133.2 (C-5), 146.3 (C-6) and 146.3 (C-10a). Accordingly, compound **2** was elucidated as 1,5-dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl)xanthone.

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Table 1. ^{13}C-NMR Data of Compounds **1**—**5** (Values in (δ) ppm)

	1	**2**	**3**	**4**	**5**
C-1	160.9	161.2	162.8	161.2	162.3
C-2	94.1	94.3	97.9	94.2	96.7
C-3	163.4	163.6	165.2	163.5	165.6
3-OCH_3	56.3	56.4		56.3	55.9
C-4	108.5	107.5	93.9	107.1	92.3
C-4a	153.5	153.1	157.2	153.2	157.1
C-5	132.5	133.2	133.0	132.6	133.3
C-6	152.2	146.3	146.0	152.3	142.2
C-7	112.9	118.2	118.2	112.9	146.0
C-8	116.0	112.1	112.0	116.0	95.3
C-8a	112.8	113.5	113.7	112.8	111.2
C-9	180.8	180.1	179.4	180.2	179.4
C-9a	101.9	102.0	101.6	101.8	102.4
C-10a	146.5	146.3	146.0	146.5	142.2
Others	17.2 (C-1′)	121.1 (C-4′)	121.1 (C-4′)	21.1 (C-1′)	55.9 (7-OCH_3)
	42.7 (C-2′)	131.7 (C-5′)	131.6 (C-5′)	122.2 (C-2′)	
	69.3 (C-3′)	77.8 (C-6′)	77.6 (C-6′)	130.9 (C-3′)	
	29.2 (C-4′)	27.8 (6′-CH_3)	27.9 (6′-CH_3)	25.5 (C-4′)	
	29.2 (C-5′)	27.8 (6′-CH_3)	27.9 (6′-CH_3)	17.7 (C-5′)	
		21.1 (C-1″)			
		122.1 (C-2″)			
		131.0 (C-3″)			
		25.5 (C-4″)			
		17.7 (C-5″)			

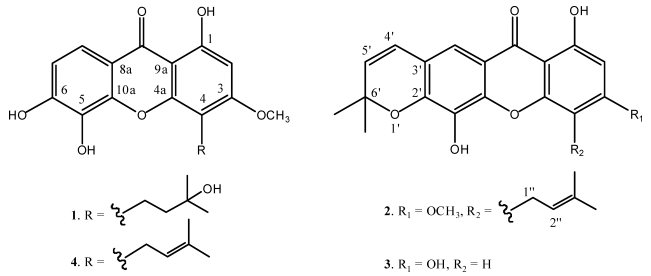

Fig. 1. Chemical Structures of **1**—**4**

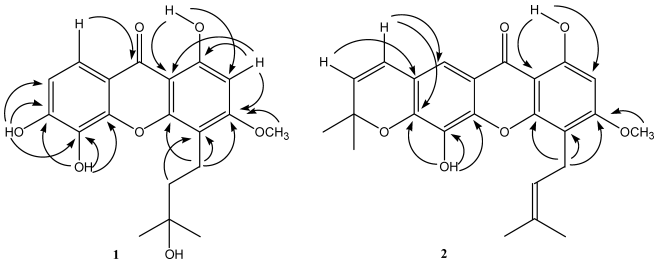

Fig. 2. Selected HMBC Correlations of **1** and **2**

Compounds **3** and **5** were determined as 1,3,5-trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone and 1,5,6-trihydroxy-3,7-dimethoxyxanthone on the basis of the HSQC and HMBC spectrum involving comparison of their MS and ^{1}H-NMR data. The ^{13}C-NMR spectra of 1,5,6-trihydroxy-3,7-dimethoxyxanthone (**5**) are reported here for the first time.

Experimental

General Experimental Procedures Melting points were determined on an X4 micro-melting point apparatus and are uncorrected. UV spectra were measured with a Hitachi UV-2201 spectrophotometer and IR spectra with an Impact 400 FTIR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ with an INOVA spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), using visual DMSO-d_6 resonances for internal reference. The spectra were interpreted with aid of the HMBC and HSQC techniques. Mass spectra were recorded on an AutoSpec Ultima-TOF spectrometer. Silica gel (200—300 mesh) and silica gel GF_{254} sheets (0.20—0.25 mm) (from Qingdao Haiyang Chemical Co., Qingdao, P. R. China) were used for column chromatography and TLC, respectively. Sephadex LH-20 (25—100 μm, Sigma-Aldrich) was also used for column chromatography.

Plant Material The stems of *Garcinia cowa* were collected from Yunnan Province P. R. China and identified by Prof. ShaoRong Guo. A voucher specimen (YA-04-0418) is deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, P. R. China.

Extraction and Isolation The dried stems of *Garcinia cowa* (20 kg) were extracted three times with 95% EtOH for 2 h under reflux, and then extracted two times with 70% EtOH for 2 h under reflux. After combination and removal of solvent, the residue (2.1 kg) was suspended in water (5000 ml) and partitioned successively with petroleum ether (5000 ml), EtOAc (5000 ml), and *n*-BuOH (5000 ml). The EtOAc-soluble fraction (272 g) was chromatographed over silica gel and eluted with $CHCl_3$–MeOH (10 : 0—1 : 1) to give 16 fractions. Fraction 3 (1.5 g) was separated by a silica gel (200—300 mesh) column eluting with ether–EtOAc (7 : 3) to afford **2** (35 mg). Fraction 5 (61.5 g) was separated by a silica gel (200—300 mesh) column eluting with $CHCl_3$–MeOH (9 : 1) to afford **1** (200 mg), and on Sephadex LH-20, eluting with MeOH, to afford **3** (80 mg). Fraction 6 (11.3 g) was purified by Sephadex LH-20, and elution with MeOH–H_2O (95 : 5) gave **4** (18 mg) and **5**. Fractions 8—10 were also separated by Sephadex LH-20 with MeOH : H_2O (95 : 5) to afford **6** (110 mg), **7** (23 mg), and **8** (300 mg).

1,5,6-Trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone (**1**): Pale yellow amorphous powder. ^{1}H-NMR δ: 13.11 (H, br s, 1-OH), 10.51 (H, br s, 6-OH), 9.06 (H, br s, 5-OH), 7.46 (H, d, J=9 Hz, H-8), 6.87 (H, d, J=9 Hz, H-7), 6.42 (H, s, H-2), 4.32 (H, br s, 3′-OH), 3.85 (3H, s, 3-OCH_3), 2.77 (2H, t, J=8.5 Hz, H-1′), 1.52 (2H, t, J=8.5 Hz, H-2′), 1.12 (6H, s, H_3-4′, H_3-5′). ^{13}C-NMR data (see Table 1). IR (KBr) ν_{max} cm^{-1}: 3469, 3312, 2960, 2820, 1641, 1585, 1466, 1432, 1365, 1262. UV (CH_3OH) λ_{max} (log ε): 234 (4.35), 252 (4.57), 285 (3.89), 326 (4.21). HR-EI-MS *m*/*z* 360.1216 $[M]^+$ (Calcd for $C_{19}H_{20}O_7$: 360.1209). EI-MS *m*/*z* 360 ($[M]^+$, 13), 342 (21), 327 (8), 301 (7), 287 (73), 286 (100), 257 (14).

1,5-Dihydroxy-3-methoxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)-4-(3-methylbut-2-enyl)-xanthone (**2**): Yellow needles, mp 217—218 °C ($CHCl_3$). ^{1}H-NMR δ: 13.14 (H, br s, 1-OH), 9.37 (H, br s, 5-OH), 7.38 (H, s, H-8), 6.57 (H, d, J=10 Hz, H-4′), 6.49 (H, s, H-2), 5.88 (H, d, J=10 Hz, H-5′), 5.20 (H, t, J=7.5 Hz, H-2″), 3.90 (3H, s, 3-OCH_3), 3.46 (2H, d, J=7.5 Hz,

H-1″), 1.79 (3H, s, H-4″), 1.61 (3H, s, H-5″), 1.45 (6H, s, 6′,6′-CH_3). ^{13}C-NMR data (see Table 1). IR (KBr) ν_{max} cm^{-1}: 3334, 2970, 2854, 1637, 1581, 1498, 1377, 1261 cm^{-1}. UV (CH_3OH) λ_{max} (log ε): 240 (4.31), 274 (4.74), 323 (4.25), 377 (3.95). HR-EI-MS *m*/*z* 408.1552 $[M]^+$ (Calcd for $C_{24}H_{24}O_6$: 408.1573). EI-MS *m*/*z* 408 ($[M]^+$, 64) 393 (100), 353 (29), 337 (14), 325 (17).

1,3,5-Trihydroxy-6′,6′-dimethyl-2*H*-pyrano(2′,3′:6,7)xanthone (**3**): Yellow needles, mp, UV, EI-MS and ^{1}H-NMR spectral data comparable with literature values. ^{13}C-NMR data (see Table 1).

Dulxanthone A (**4**): Yellow powder, UV, IR, EI-MS, ^{1}H- and ^{13}C-NMR spectral data comparable with literature values. ^{13}C-NMR data (see Table 1).

1,5,6-Trihydroxy-3,7-dimethoxyxanthone (**5**): Pale brown powder, UV, EI-MS and ^{1}H-NMR spectral data comparable with literature values. ^{13}C-NMR data (see Table 1).

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