สารประกอบแซนโทนจากเปลือกต้นมะดะหลวง

XANTHONES FROM THE STEM BARK OF *GARCINIA XANTHOCHYMUS*

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บทคัดย่อ

มะดะหลวง (*Garcinia xanthochymus* Hook. f.) เป็นพืชสมุนไพรที่พบได้ทางแถบประเทศจีน ซึ่งนิยมนำมาใช้เป็นยาถ่ายพยาธิ และใช้กำจัดสารพิษออกจากร่างกาย พืชในสกุล *Garcinia* จัดอยู่ในวงศ์ Clusiaceae มีองค์ประกอบทางเคมีส่วนใหญ่เป็นสารจำพวก Oxygenated xanthone จากการศึกษาองค์ประกอบทางเคมีจากเปลือกต้นมะดะหลวงที่เก็บมาจากทางภาคเหนือของประเทศไทย สามารถแยกสารประกอบแซนโทนได้ 5 ชนิด คือ 2,5-dihydroxy-1-methoxyxanthone (**1**), 2,6-dihydroxy-1,5-dimethoxyxanthone (**2**), 6-deoxyisojacareubin (**3**), 1,6-dihydroxy-4,5-dimethoxyxanthone (**4**) และ 12b-hydroxy-des-*D*-garcigerrin A (**5**) โดยที่สารประกอบ **1** และ **2** เป็นสารประกอบแซนโทนที่ยังไม่เคยมีผู้รายงานการพบในพืชชนิดนี้ การพิสูจน์โครงสร้างของสารบริสุทธิ์ใช้เทคนิคทางสเปกโทรสโคปี และใช้วิธีเปรียบเทียบข้อมูลทางสเปกโทรสโคปีของสารกับข้อมูลที่มีผู้รายงานไว้แล้ว

คำสำคัญ: มะดะหลวง Clusiaceae สารประกอบแซนโทน

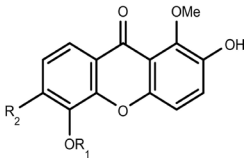
Abstract

Garcinia xanthochymus Hook. f. is a medicinal plant native to P. R. China and has been used as a traditional medicine for dispelling worms and removing food toxin. Phytochemicals investigation of the *G. xanthochymus* stem bark, collected from the northern part of Thailand, led to the isolation of five xanthones, 2,5-dihydroxy-1-methoxyxanthone (**1**), 2,6-dihydroxy-1,5-dimethoxyxanthone (**2**), 6-deoxyiso jacareubin (**3**), 1,6-dihydroxy-4,5-dimethoxyxanthone (**4**) and 12b-hydroxy-des-*D*-garcigerrin A (**5**). Their structures were elucidated by spectroscopic method, especially 1D and 2D NMR techniques, and by comparison of the data with the reported value. This is the first report of compounds **1** and **2** obtained from this plant species.

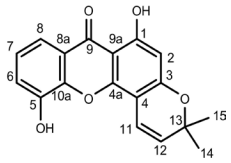
Keywords: *Garcinia xanthochymus*, Clusiaceae, Xanthone compound

Introduction

The genus *Garcinia* (Clusiaceae family) is well known to be a rich source of oxygenated xanthones. *Garcinia xanthochymus* Hook. f. (Clusiaceae) is a perennial medicinal plant native to the south and southwest of China [1–5] north of Thailand and Myanmar [6] which can grow up to 10–20 m. It is widely used as a traditional medicine for dispelling worms and removing food toxin [1–5]. Previous phytochemical studies of the bark [1–5, 7] twig bark [6], fruit [8], and heartwood of *G. xanthochymus* [9–10] have shown the presence of seven benzophenones [8], six biflavonoids [8], 46 xanthones [1–7, 9] and four triterpenes [11]. These compounds have shown a variety of bioactivities, including cytotoxic [6], antioxidant [3–5, 8], antiviral [12], antimalarial [13] and anti-HIV [14]. As part of our research on bioactive xanthones from *Garcinia* plants, a careful examination of the EtOAc extract obtained from stem bark of this plant species, collected from the northern part of Thailand, led to the isolation of three trioxygenated xanthones, including 2,5-dihydroxy-1-methoxyxanthone (**1**) [15], 6-deoxyisojacareubin (**3**) [7, 16] and 12b-hydroxy-des-*D*-garcigerrin A (**5**) [4, 17–18] and two tetraoxygenated xanthones: 2,6-dihydroxy-1,5-dimethoxyxanthone (**2**) [19] and 1,6-dihydroxy-4,5-dimethoxyxanthone (**4**) [1]. This is the first report on isolation of compounds **1** and **2** from *G. xanthochymus*.



1 : R_1 = H, R_2 = H
2 : R_1 = Me, R_2 = OH



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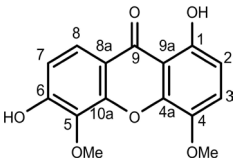

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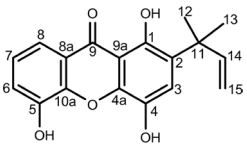

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Aims

To isolate, purifiy, and structure elucidations of xanthone from the stem bark of *G. xanthochymus*.

Materials and Methods

Plant materials

The stem bark of *G. xanthochymus* was collected from the northern part of Thailand in May 2003.

General experimental procedures

For the isolation and purification of the compounds, UV spectra were obtained on a Shimadzu UV-2450 PC spectrophotometer. Melting points were measured on a Griffin melting point apparatus in degree Celsius of temperature. Mass spectra ESIMS was measured on a Finnigan LC-Q. 1D and 2D NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR spectrometer, and chemical shifts were referenced to the residual solvent peaks

(δ_H 2.04 and δ_C 29.8 for acetone-d_6). Column chromatography was performed on silica gel (Silica gel 60, particle size finer than 0.063 mm and 0.063-0.0200 mm, Merck), and thin layer chromatography (TLC) was performed on pre-coated Silica gel 60 GF_{254} on aluminium plate (1.25 mm). Developing reagent: anisaldehyde-sulphuric reagent (0.5% anisaldehyde in methanolic solution containing 4.5% sulphuric acid and 10% glacial acetic acid) and heated at 100-110°C for 2-3 minutes, the spots of organic compounds gave specific colors with this reagent.

Extraction and purification

The dried stem bark of *G. xanthochymus* (1.15 kg) was extracted with EtOAc by exhaustive maceration (5 × 10 L) at room temperature for each 7 days and then with MeOH (5 × 10 L). Each extract was evaporated under reduced pressure at 40°C using a rotary vacuum evaporator to yield 90 g and 111 g of the EtOAc and MeOH extracts, respectively. Forty grams of EtOAc extract was fractionated by quick column chromatography (silica gel 60 GF_{254}, 150 g), eluted with n-hexane, n-hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-EtOAc, EtOAc, EtOAc-MeOH and MeOH with increasing amounts of the more polar solvent. Fractions were collected and checked by TLC on silica gel, fractions showing similar profiles were combined and 10 fractions (Fr.1-10) were obtained. Fraction 1 (12.2 g) was further purified by CC on silica gel and eluted with a gradient of n-hexane-acetone to give **3** (4.2 mg) as yellow solid. Two successive column chromatography eluted with n-hexane-acetone in a polarity-gradient manner of fractions 3-4 (1.5 g) gave **4** (3.4 mg) as yellow solid and **5** (416.3 mg) as orange solid. Fraction 10 (11.95 g) was applied to a silica gel column eluted with n-hexane-acetone in a polarity-gradient manner to give compounds **1** (3.2 mg) as yellow solid and **2** (10.4 mg) as pale yellow solid.

Results

The stem bark of *G. xanthochymus* was extracted at room temperature with EtOAc and MeOH. The EtOAc extract gave mainly a typical yellow and orange colorations with anisaldehyde-H_2SO_4 reagent which indicated for the presence of xanthones. Therefore the EtOAc extract was selected for further chromatographic isolation and purification and five xanthones **1-5** were obtained.

2,5-Dihydroxy-1-methoxyxanthone (**1**) (3.2 mg): yellow solid: mp 200-201°C, Lit: 214-218°C [15]; R_f 0.40 (40% acetone-hexane); ES-MS: *m/z* (rel. intensity): 257 $[M-H]^-$ (32), 273 (100); UV MeOH; λ_{max} (log ε) 241(3.6), 257(3.7, sh), 288(2.8), 309(2.6), 377(2.8); ^1H NMR and ^{13}C NMR (acetone-d_6) see Table 1.

2,6-Dihydroxy-1,5-dimethoxyxanthone (**2**) (10.4 mg): pale yellow solid: mp 240-241°C, Lit: 206-209°C [19]; R_f 0.39 (40% acetone-hexane); ES-MS: *m/z* (rel. intensity): 287 $[M-H]^-$ (79); UV MeOH; λ_{max} (log ε) 241(3.4), 249(3.4, sh), 282(2.8), 310(3.0), 360(2.6); ^1H NMR and ^{13}C NMR (acetone-d_6) see Table 1.

6-Deoxyisojacareubin (**3**) (4.2 mg): yellow solid: mp 233-234°C, Lit: 235-236°C [17]; R_f 0.45 (30% acetone-hexane); ES-MS: *m/z* (rel. intensity): 619 $[2M-H]^-$ (100); UV MeOH; λ_{max} (log ε) 222(3.1), 232(3.2), 250(3.5), 267(3.4), 308(2.9), 329(3.0), 378(2.4); ^{1}H NMR (acetone-d_6, 300 MHz): 13.0 (1H, *s*, 1-OH), 7.67 (1H, *d*, *J* = 7.8 Hz, H-8), 7.37 (1H, *dd*, *J* = 7.8, 1.7 Hz, H-6), 7.29 (1H, *d*, *J* = 7.8 Hz, H-7), 7.03 (1H, *d*, *J* = 10 Hz, H-11), 6.19 (1H, *s*, H-2), 5.74 (1H, *d*, *J* = 10 Hz, H-12), 1.48 (2x3H, *s*, H-14,15) and ^{13}C NMR (acetone-d_6, 75 MHz): 161.7 (C-1), 99.6 (C-2), 157.1 (C-3), 102.2 (C-4), 155.0 (C-4a), 146.9 (C-5), 122.0 (C-6), 125.1 (C-7), 115.7 (C-8), 120.5 (C-8a), 182.0 (C-9), 104.0 (C-9a), 145.2 (C-10a), 128.1 (C-11), 116.5 (C-12), 79.1 (C-13), 28.4 (C-14,15)

1,6-Dihydroxy-4,5-dimethoxyxanthone (**4**) (3.4 mg): yellow solid: mp 220-221°C; R_f 0.40 (30% acetone-hexane); ES-MS: *m/z* (rel. intensity): 287 $[M-H]^-$ (100); UV MeOH; λ_{max} (log ε) 242(3.5), 280(3.1), 298(3.0, sh), 314(3.0), 374(2.8); ^{1}H NMR (acetone-d_6, 300 MHz): 12.21 (1H, *s*, 1-OH), 7.87 (1H, *d*, *J* = 8.9 Hz, H-8), 7.43 (1H, *d*, *J* = 8.9 Hz, H-4), 7.04 (1H, *d*, *J* = 8.9 Hz, H-7), 6.69 (1H, *d*, *J* = 8.9 Hz, H-2), 4.05 (3H, *s*, 4-OMe), 3.97 (3H, *s*, 5-OMe) and ^{13}C NMR (acetone-d_6, 75 MHz): 155.6 (C-1), 109.5 (C-2), 122.0 (C-3), 141.2 (C-4), 145.2 (C-4a), 138.7 (C-5), 158.0 (C-6), 114.8 (C-7), 121.7 (C-8), 114.0 (C-8a), 179.7 (C-9), 108.3 (C-9a), 152.3 (C-10a), 57.9 (4-OMe), 61.7 (5-OMe)

12b-Hydroxy-des-*D*-garcigerrin A (**5**) (416.3 mg): orange solid: mp 229-230°C; R_f 0.32 (30% acetone-hexane); ES-MS: *m/z* (rel. intensity): 623 $[2M-H]^-$(100), 311 $[M-H]^-$ (24); UV MeOH; λ_{max} (log ε) 238(3.3), 249(3.3), 264(3.5), 317(2.8), 409(2.5); ^{1}H NMR (acetone-d_6, 300 MHz): 12.88 (1H,*s*, 1-OH), 7.69 (1H, *d*, *J* = 7.6, 1.8 Hz, H-8), 7.37 (1H, *dd*, *J* = 7.8, 1.8 Hz, H-6), 7.35 (1H, *s*, H-3), 7.28 (1H, *d*, *J* = 7.8 Hz, H-7), 6.28 (1H, *dd*, *J* = 10.7, 17.5 Hz, H-14), 5.02 (1H, *dd*, *J* = 17.5 Hz, H-15E), 4.98 (1H, *dd*, *J* = 10.7 Hz, H-15Z), 1.52 (2x3H, *s*, H-12,13) and ^{13}C NMR (acetone-d_6, 75 MHz): 153.2 (C-1), 129.5 (C-2), 123.2 (C-3), 136.8 (C-4), 142.0 (C-4a), 147.0 (C-5), 121.8 (C-6), 125.1 (C-7), 116.5 (C-8), 123.2 (C-8a), 183.8 (C-9), 109.3 (C-9a), 145.6 (C-10a), 40.9 (C-11), 26.8 (C-12, 13), 147.8 (C-14), 111.0 (C-15)

Discussion and Conclusions

From the EtOAc extract of the *G. xanthochymus* stem bark, collected from the northern part of Thailand, led to the isolation of three trioxygenated xanthones (**1**, **3** and **5**) and two tetraoxygenated xanthones (**2** and **4**). Their structures were identified by spectroscopic method, especially 1D and 2D NMR techniques, and by comparison of the data with the reported values.

Compound **1** was obtained as yellow solid and its molecular formula was determined to be $C_{14}H_{10}O_5$ by ESMS at *m/z* 257 $[M-H]^-$. The UV absorptions at 241, 257, 288, 309, 377 nm indicated that **1** to be a simple oxygenated xanthone structure. In its ^{13}C NMR and DEPT spectra, 14 carbon signals were observed including one methoxyl carbon, five

methines, seven quaternary carbons and a carbonyl carbon (Table 1).

The ^{1}H NMR spectrum of **1** (Table 1) showed a pair of *ortho*-coupled aromatic proton signals at δ 7.27 (*d*, *J* = 9.0 Hz) and 7.38 (*d*, *J* = 9.0 Hz), a singlet methoxyl signal at δ 3.91 as well as the presence of an ABM system signals at δ 7.20 (*t*, *J* = 7.8 Hz), 7.27 (*dd*, *J* = 7.8, 1.7 Hz) and 7.66 (*dd*, *J* = 7.8, 1.7 Hz) attributable to a 1,2,3-trisubstituted benzene ring. HMBC spectrum of **1** displayed a long range correlations from the methoxyl group at δ 3.91 with C-1 (δ 176.2) confirming the placement of the methoxyl group at C-1 (see Figure 1). The *ortho*-coupled aromatic proton at δ 7.38 (H-3) showed correlations with C-2 (δ 146.9) and C-4 (δ 114.6), the aromatic proton signal at δ 7.66 (H-8) showed correlations with C-7 (δ 124.1), C-8 (δ 116.8) and C-9 (δ 176.2) and correlations between δ 7.27 (H-6) with C-5 (δ 147.4) and C-7 (δ 124.1) were also observed. These observations together with the comparisons of the ^{1}H and ^{13}C NMR data of **1** with 2,5-dihydroxy-1--methoxyxanthone, led to the identification of **1** to be 2,5-dihydroxy-1-methoxyxanthone [15]. This xanthone has been isolated from the wood of *Garcinia subelliptica* [15].

Table 1 ^{1}H and ^{13}C NMR data of compounds **1-2** in acetone-d_6.

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		146.1		146.2
2	8.33 *s* (OH)	146.9	8.24 *s* (OH)	147.5
3	7.38 (*d*, *J* = 9.0 Hz)	123.9	7.33 (*d*, *J* = 9.1 Hz)	123.2
4	7.30 (*d*, *J* = 9.0 Hz)	114.6	7.28 (*d*, *J* = 9.1 Hz)	114.4
4a		151.1		150.9
5	9.21 *s* (OH)	147.4		135.1
6	7.27 (*dd*, *J* = 7.8, 1.7 Hz)	120.1	9.22 *s* (OH)	156.0
7	7.20 (*t*, *J* = 7.8 Hz)	124.1	6.95 (*d*, *J* = 8.9 Hz)	113.8
8	7.66 (*dd*, *J* = 7.8, 1.7 Hz)	116.8	7.83 (*d*, *J* = 8.9 Hz)	122.6
8a		123.8		117.1
9		176.2		175.3
9a		117.1		117.1
10a		145.5		151.2
1-OMe	3.91 *s*	62.1	3.90 *s*	61.6
5-OMe			3.99 *s*	62.1

Compound **2** was obtained as pale yellow solid. Its molecular formula $C_{15}H_{12}O_6$ was determined by the molecular ion peak at *m/z* 287 $[M-H]^-$ in the ESMS. The UV spectrum of **2** had characteristic xanthone skeleton. The ^{13}C, DEPT, HMQC and COSY provided 15 carbon signals. The ^{1}H NMR spectrum of **2** (Table 1) showed two sets of *ortho*-coupled

protons at δ 6.95 and 7.83 (each *d*, *J* = 8.9 Hz), and δ 7.28 and 7.33 (each *d*, *J* = 9.1 Hz). The presence of only one low field aromatic proton (δ 7.83), therefore, indicated that either C-1 or C-8 of the xanthone moiety is free. This was confirmed by the absence of a chelated hydroxyl group in the xanthone as shown by its ^{1}H NMR spectrum. Two hydroxyl groups at δ 8.24 and 9.22 and two methoxyl groups at δ 3.90 and 3.99 suggesting the xanthone to be tetraoxygenated. The presence of two pairs of *ortho*-coupled protons in two different aromatic rings is evident from the above ^{1}H NMR data. Comparisons of their ^{1}H and ^{13}C NMR data with the reported values, led to the identification of this compound could be 2,6-dihydroxy-1,5-dimethoxyxanthone [19]. In HMBC spectrum (see Figure 1), two methoxyl group signals at δ 3.90 and 3.99 showed correlations with C-1 (δ 146.2) and C-5 (δ 135.1), respectively. The *ortho*-coupled aromatic proton at δ 7.83 showed correlations with C-7 (δ 113.8), C-8 (δ 122.6) and C-9 (δ 176.3). The aforementioned spectral effect showed that there were two methoxyl and two hydroxyl groups at C-1, C-5 and C-2, C-6 on the xanthone ring, respectively. The structure of **2** was deduced as 2,6-dihydroxy-1,5-dimethoxyxanthone which has been isolated from the wood of *Garcinia subelliptica* [19].

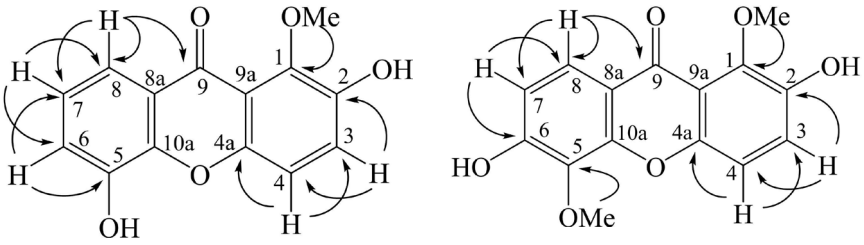

Figure 1 Selected HMBC correlations of compounds **1-2**.

Compounds **3**, **4** and **5** were identified as 6-deoxyisojacareubin [5, 15, 19], 1,6-dihydroxy-4,5-dimethoxyxanthone [12] and 12b-hydroxy-des-*D*-garcigerrin A [9, 16-17], respectively, by comparison of their spectral data with the literature values.

Acknowledgements

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