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CHEMICAL CONSTITUENTS OF DICHLOROMETHANE EXTRACT FROM THE STEM BARK OF *GARCINIA FAGRAEOIDES*

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Abstract. The *Garcinia* genus belonging to the Clusiaceae family (Guttiferae) has been reported to be a rich source of polyisoprenylated benzophenones, xanthones and biflavonoids with various biological activities. *Garcinia fagraeoides* A.Chev is an evergreen hardwood tree found only in the north provinces of Viet Nam and Guangxi province of China. Its stem bark and leaves have been used in Vietnamese traditional medicine for treatment of malaria and dermatitis diseases. In our continuing studies on chemical constituents and biological activities from the genus *Garcinia*, the phytochemical of *Garcinia fagraeoides*'s stem bark collected from Thua Thien Hue province was investigated. Repeated chromatography steps over silica gel and Sephadex LH-20 of the dichloromethane partition from the methanol extract of *Garcinia fagraeoides*'s stem bark led to the isolation of five compounds including one polyisoprenylated benzophenone (isogarcinol (**1**)), two tetraoxygenated xanthones (rubraxanthone (**2**), α-mangostin (**3**)), ferulic acid (**4**), and pyromeconic acid (**5**). Their structures were elucidated mainly by analysis of their 1D, 2D NMR spectroscopy and ESI-MS data, and by comparison with reported data. The absolute configuration of isogarcinol **(1)** was determined by comparison of its experimental optical rotation and NMR data with the values of its stereoisomer. This is the first reported isolation of compounds **1-5** from the stem bark of *Garcinia fagraeoides*.

Keywords: Garcinia fagraeoides, polyisoprenylated benzophenone, xanthone.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

The genus *Garcinia* belongs to the family Clusiaceae (Guttiferae), with about 250 species worldwide and the majority of which are found in the tropical regions of Asia and Africa. *Garcinia* plants are known to produce a variety of polyisoprenylated benzophenones [1, 2] and xanthones [3] among other biologically active metabolites. *Garcinia fagraeoides* A.Chev is an evergreen hardwood tree found only in the north of Viet Nam and Guangxi province of China [4]. Its stem bark and leaves have been used in local traditional medicine for treatment of malaria and dermatitis diseases. There is no prior literature on the chemistry and bioactivity of *Garcinia fagraeoides*.

As part of our phytochemical studies of *Garcinia* species growing in Viet Nam, we describe herein the isolation and structural elucidation of chemical constituents from the dichloromethane extract of *Garcinia fagraeoides'*s stem bark.

2. MATERIALS AND METHODS

2.1. General

NMR spectra were recorded on a Bruker Advance 500 MHz and 600 MHz spectrometer (Germany) at the Institute of Chemistry, VAST. Chemical shifts are reported in δ (ppm) with tetramethylsilane (TMS) as an internal reference and coupling constants (*J*) are given in Hertz (Hz). ESI-MS spectra were performed on an LC-MS Agilent 1100 (USA). Melting points for isolated compounds were measured on Büchi B-545 apparatus. Specific rotation was obtained with a JASCO P-1010 polarimeter. Column chromatography (CC) was carried out on silica gel 60 (Merck, 5 - 40 μm), silica gel 100 (Merck, 63 - 200 μm), Sephadex LH-20 (GE Healthcare), and C_{18}-reversed-phase silica gel (RP-18, Merck, 15 - 25 μm). Thin-layer chromatography (TLC) was performed on silica gel 60 coated plates F254 (Merck). Visualization of TLC plates was performed under UV light (254 and 365 nm), staining with 5 % vanillin/H_2SO_4 or 10 % H_2SO_4 solution. Commercial solvents were distilled and dried, when necessary, by standard methods just prior to use.

2.2. Plant materials

The stem bark of *Garcinia fagraeoides* A. Chev was collected in Phong Dien, Thua Thien Hue province, in July 2020. The plant material was identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology. The voucher specimen No. GF202007 is deposited at the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The stem bark of *G. fagraeoides* (2.0 kg) was cut into small pieces and dried in an oven at 45 ^oC for three days. The dried stem bark (1.2 kg) was ground into powder and extracted with methanol (MeOH) (3 L × 3) at room temperature using conventional ultrasound-assisted technique. After removing the solvent, the residue (224 g) was dissolved in water and partitioned successively with dichloromethane (DCM), ethyl acetate (EtOAc), and *n*-butanol. DCM solution was evaporated under reduced pressure to yield DCM extract (67.9 g).

CC of the DCM extract over silica gel eluted with a gradient of *n*-hexane-EtOAc (100:0 to 0:100, v/v) yielded fifteen fractions (Frs. F1–F15). Fraction F4 was separated by silica gel CC (*n*-hexane-EtOAc, 100:0 to 50:50, v/v) to give seven subfractions F4.1-F4.7. Purification of subfraction F4.5 by repeated CC over silica gel (*n*-hexane-EtOAc, 5:1, v/v) provided compound **2** (11 mg) as yellow needles. Compounds **3** (8 mg) was obtained as yellow needles from subfraction F5.5 by chromatography over silica gel (*n*-hexane-EtOAc (4:1, v/v) followed by recrystallization in DCM. Fraction F10 was chromatographed over silica gel with *n*-hexane-acetone (30:1, v/v) to afford compound **4** (6 mg) as a colourless solid. Fraction F11 was recrystallized two times in *n*-hexane-acetone mixture (2:1, v/v) to yield compound **5** (124 mg) as colourless needles. Compound **1** (81 mg) was obtained as a dark yellow solid from fraction F12 by CC over silica gel column eluted with *n*-hexane-acetone (20:1, v/v) followed by CC on Sephadex LH-20 column using MeOH as eluent.

Isogarcinol (**1**): dark yellow solid. $[\alpha]_D$ -167(c 1.0, $CHCl_3$). ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6), see *Table 1*. ESI-MS *m/z* 603.36 $[M + H]^+$ (calcd. for $C_{38}H_{51}O_6$, 603.37).

Rubraxanthone (**2**): yellow needles, m.p. 207-208 ^oC.^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see *Table 2*. ESI-MS*m/z* 411.18 $[M + H]^+$ (calcd. for $C_{24}H_{27}O_6$, 411.18).

α-Mangostin (**3**): yellow needles, m.p. 180-181 ^oC.^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see *Table 2*. ESI-MS *m/z* 411.19 $[M + H]^+$ (calcd. for $C_{24}H_{27}O_6$, 411.18).

Ferulic acid (**4**): colourless solid, m.p. 169-170 ^oC.^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.59 (1H, d, *J* = 15.6 Hz, H-7), 7.23 (1H, d, *J* = 1.8 Hz, H-3), 7.13 (1H, dd, *J* = 7.8, 1.8 Hz, H-5), 6.84 (1H, d, *J* = 7.8 Hz, H-6), 6.65 (1H, d, *J* = 15.6 Hz, H-8), 3.93 (3H, s, H-10). ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 184.8 (C-9), 150.5 (C-1), 149.4 (C-2), 142.1 (C-7), 128.6 (C-4), 124.1 (C-5), 122.3 (C-8), 116.6 (C-6), 111.8 (C-3), 56.5 (C-10). ESI-MS*m/z* 195.06 $[M + H]^+$ (calcd. for $C_{10}H_{11}O_4$, 195.07).

Pyromeconic acid (**5**): colourless needles, m.p 117-118 ^oC. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.88 (1H, d, *J* = 0.5 Hz, H-3), 7.79 (1H, dd, *J* = 5.5, 1.0 Hz, H-4), 6.50 (1H, d, *J* = 5.5 Hz, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 173.7 (C-1), 155.5 (C-2), 146.8 (C-4), 138.8 (C-3), 113.7 (C-5). ESI-MS *m/z* 113.02 $[M + H]^+$ (calcd. for $C_5H_5O_3$, 113.02).

3. RESULTS AND DISCUSSION

The DCM extract was subjected to chromatographic steps over silica gel and Sephadex to yield five compounds (**1**-**5**, Figure 1). Their structures were elucidated by extensive spectroscopic methods including 1D- and 2D-NMR (COSY, HSQC, HMBC, NOESY), ESI-MS analysis as well as by comparison with reported data.

Compound **1** was isolated as a dark yellow solid, $[\alpha]_D$ -167 (c 1.0, $CHCl_3$). The ESI-MS data of **1** (*m/z* 603.36 $[M + H]^+$, calcd. for $C_{38}H_{51}O_6$, 603.37), suggested a molecular formula of $C_{38}H_{50}O_6$. The ^1H, ^{13}C NMR, COSY and HSQC spectra of **1** revealed the presence of three carbonyl groups [δ_C 207.4 (C-9), 194.3 (C-4), 192.2 (C-10)], one 1,2,4 trisubstitutedbenzene ring, three isoprenyl groups and four methyl groups. Analysis of COSY and HMBC spectra (Figure 2) allowed to determine a garcinol skeleton [5, 6] fused with a tetrahydropyrane ring by the correlations between H-12, H-16 (δ_H7.36, 7.11) and C-10 (δ_C 192.2); H-17 (δ_H 2.42, 2.63)

and C-4 (δ_C 194.3), C-5 (δ_C 68.9), C-6 (δ_C 46.6); H-24 (δ_H 2.13, 2.75) and C-6, C-7 (δ_C 47.0), C-8 (δ_C 39.6); H-29 (δ_H 1.08, 3.02) and C-1 (δ_C 52.0), C-2 (171.3), C-30 (δ_C 43.8), C-31 (δ_C 87.1). C-6 and C-31 were found bound to the two pairs of methyl groups by HMBC cross peaks between H-22 (δ_H 0.98), H-23 (δ_H 1.14) and C-6, H-32 (δ_H 1.27), H-33 (δ_H 0.93) and C-31. The HMBC correlations between H-34 (δ_H1.86, 2.05) and C-29 (δ_C 29.1), C-30, C-31 revealed that anisoprenyl group was attached to the pyrane ring at CH-30.

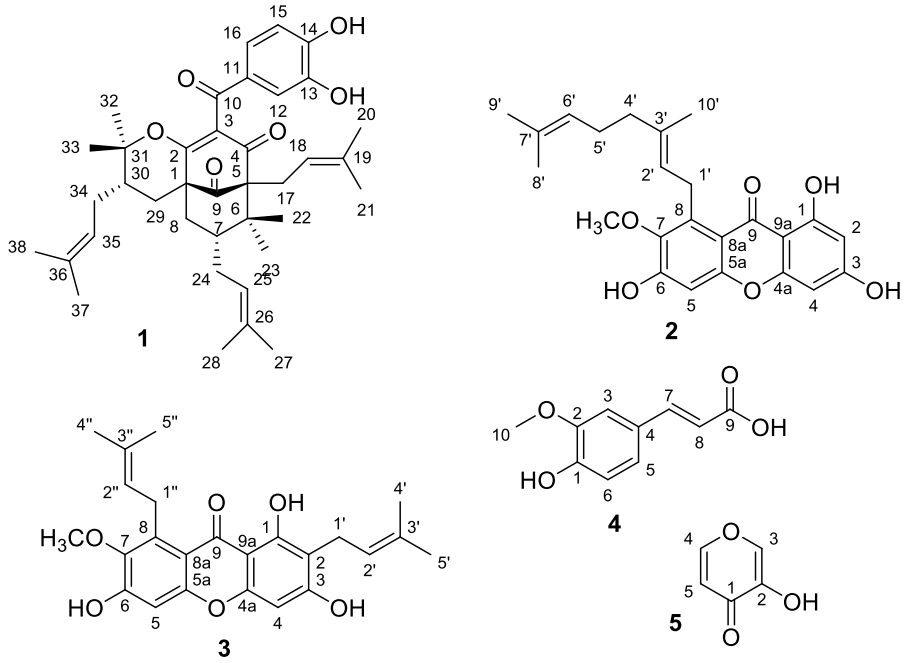

Figure 1. Chemical structures of compounds **1**-**5**.

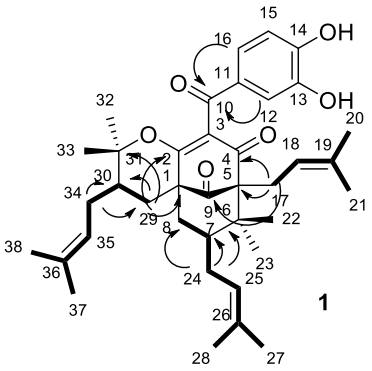

Figure 2. Key HMBC (arrows) and COSY (bold lines) correlations for compound **1**.

Examination of 1D and 2D NMR of **1** associated with reported data [7] (*Table 1*) confirmed that compound **1** was isogarcinol. The prenyl group at C-7 was suggested in α-orientation by slight differences of chemical shifts of C-7, C-8, C-22, C-23 and C-24 between **1** and 7-*epi*-isogarcinol which contains 7β-prenyl group [7] (see *Table 1*). Furthermore, NOESY correlations between H-24 and H-23 confirmed the configuration 7-α of **1**.

Table 1. NMR data for compound **1** and reported data of isogarcinol and 7-*epi*-isogarcinol [7].

Position	Compound **1**[a]		Isogarcinol[b]	7-*Epi* isogarcinol[b]
	δ_C	δ_H, *J* (Hz)	δ_C	δ_C
1	52.0		52.2	52.2
2	171.3		171.4	170.8
3	126.6		127.2	129.1
4	194.3		195.0	194.9
5	68.9		69.2	**71.4**
6	46.6		46.8	46.8
7	47.0	1.53 m	47.0	**42.2**
8	39.6	2.06 (overlapped) 2.29 d (14.5)	39.8	**43.1**
9	207.4		207.9	207.3
10	192.2		193.0	193.2
11	131.2		130.9	131.0
12	115.8	7.36 d (2.0)	116.6	117.1
13	145.8		147.8	147.9
14	151.2		153.7	153.7
15	115.5	6.83 d (8.0)	116.5	116.5
16	123.7	7.11 dd (8.0, 2.0)	124.3	124.4
17	26.3	2.42 dd (13.5, 5.0) 2.63 dd (13.5, 8.0)	26.7	25.9
18	121.5	4.96 (overlapped)	121.7	122.1
19	134.0		134.5	134.2
20	26.3	1.56 s	26.6	26.2
21	18.2	1.62 s	18.8	18.8
22	26.9	0.98 s	27.1	**16.6**
23	22.7	1.14 s	23.1	22.8
24	30.1	2.13 m 2.75 m	30.4	**28.5**
25	126.3	4.96 (overlapped)	126.4	**123.8**
26	133.1		132.9	132.9
27	25.9	1.76 s	26.5	26.2
28	18.5	1.69 s	19.0	18.4
29	29.1	1.08 t (13.5) 3.02 dd (14.0, 3.5)	29.1	28.5
30	43.8	1.45 m	43.8	43.8
31	87.1		87.2	87.6
32	21.6	1.27 s	21.7	21.7
33	28.9	0.93 s	29.4	29.2
34	30.1	1.86 (overlapped) 2.05 (overlapped)	30.4	30.4
35	122.9	5.20 m	122.8	122.9
36	133.8		133.7	133.7
37	26.1	1.68 s	26.2	26.2
38	18.0	1.55 s	18.3	18.3

[a]in acetone-d_6; [b]in pyridine-d_5. (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz).

Compounds **2** and **3** shared common spectral characteristics of a tetraoxygenated xanthone skeleton (Table 2): one carbonyl group (δ_C 181.9 - 182.0, C-9), twelve aromatic carbons including six oxygenated carbons (δ_C 142.6 - 163.9) and two or three methines. The ESI-MS data of **2** (*m/z* 411.18 $[M + H]^+$; calcd. for $C_{24}H_{27}O_6$, 411.18), suggested a molecular formula of $C_{24}H_{26}O_6$.

NMR spectra of **2** revealed the presence of a geranyl side chain with two olefinic CH groups [δ_H 5.26 (t, H-2'), δ_C 123.2 (C-2'); δ_H 5.03 (t, H-6'), δ_C 124.3 (C-6')], two quaternary sp^2 carbons [δ_C 135.7 (C-3'), 131.3 (C-7')], three CH_3 [δ_H 1.55, 1.60, 1.83 (s) (H-8', 9', 10')] and three CH_2 [δ_H 4.09, 2.02, 2.05 (m) (H-1', 4', 5')]. The HMBC correlations of H-1' with C-8 (137.2) and C-7 (142.8) allowed to locate this geranyl group at C-8 of the xanthone skeleton. On the other hand, resonance signal of CH_3 at [δ_H (3.71, s), δ_C 62.0] crossed with signal of C-7 in HMBC spectrum suggesting that OH-7 was methylated. Compound **2** was thus determined as rubraxanthone [8, 9].

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **2** and **3** (in $CDCl_3$).

Position	**2**		**3**		Position	**2**		**3**	
	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)	δ_C		δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)	δ_C
1		162.7		160.5	3'		135.7		135.2
2	6.22 d (2.0)	98.3		108.8	4'	2.02 m	39.7	1.84 s	17.9
3		163.9		161.5	5'	2.05 m	26.5	1.77 s	25.8
4	6.28 d (2.0)	93.4	6.29 s	93.3	6'	5.03 t	124.3		
4a		157.0		154.9	7'		131.3		
5	6.84 s	103.9	6.82 s	101.6	8'	1.55 s	17.6		
5a		155.8		154.6	9'	1.60 s	25.6		
6		154.7		155.7	10'	1.83 s	16.5		
7		142.8		142.6	1"			4.09 d (5.5)	26.5
8		137.2		137.1	2"			5.27 m	123.2
8a		112.3		112.1	3"				132.1
9		181.9		182.0	4"			1.84 s	18.2
9a		101.7		103.6	5"			1.69 s	25.8
1'	4.09 d (6.0)	26.5	3.45 d (6.0)	21.4	7-OMe	3.71 s	62.0	3.81 s	62.0
2'	5.26 t (5.5)	123.2	5.28 m	121.6	1-OH	13.45 s		13.78 s	

The ^{1}H and ^{13}C NMR of **3** confirmed the presence of two prenyl side chains (C(1')-C(5') and C(1")-C(5")) (*Table 2*). Moreover, there were only 2 singlet signals of two aromatic protons in the ^{1}H NMR spectrum of **3** instead of three proton signals compared to **2**. The observed HMBC correlations between H-1' (δ_H 3.42) and C-2 (δ_C 108.8), C-3 (δ_C 161.5), C-1 (δ_C 160.5); H-1" (δ_H 4.07) and C-7 (δ_C 142.6), C-8 (δ_C 137.1) suggested the locations of the two prenyl groups at C-2 and C-8, respectively. The OH-7 of **3** was also methylated as in the case of **2**,

confirmed by analysis of HMBC cross peaks of methyl protons with C-7. The NMR spectroscopic data of **3** were similar to those of α-mangostin [10]. The ESI-MS data of **3** (*m/z* 411.19 $[M + H]^+$; calcd. for $C_{24}H_{27}O_6$, 411.18)., 411.18), suggested a molecular formula of $C_{24}H_{26}O_6$. Compound **3** was thus determined as α-mangostin.

Compound **4** was isolated as a colorless solid, m.p. 169 - 170 ^oC. Its NMR spectra indicated the presence of a 1,2,4-trisubstituted benzene ring with an unsaturated C_3 side chain (*Figure 1*). The signals in ^1H NMR spectrum at δ_H 7.23 (1H, d, J = 1.5 Hz), 7.13 (1H, dd, J = 6.5, 1.5 Hz) and 6.84 (1H, d, J = 6.5 Hz) were assigned to H-3, H-5 and H-6 of the benzene ring, respectively. The observed correlation between methoxy group at δ_H 3.93 (3H, s, H-10) with C-2 (δ_C 149.4) confirmed the location on C-2 position of the methoxy group. The ethylene group [δ_H 7.59 (d, J = 15.6 Hz, H-7),δ_C 142.1 (C-7); δ_H 6.65 (d, J = 15.6 Hz, C-8), 122.3 (C-8)] was determined to be bound to the benzene ring and carbonyl group by the HMBC correlations between H-7 with C-5 (124.1), C-3 (111.8), C-4 (128.6); H-7 and H-8 with C-9 (184.9). The value of $J_{H\text{-}7,H\text{-}8}$ (15.6 Hz) suggested the *trans* configuration of the double bond. The ESI-MS showed a molecular ion peak at *m/z* 195.06 $[M+H]^+$ in agreement with the proposed structure of the known phenolic compound, ferulic acid ($C_{10}H_{10}O_4$). Compound **4** was thus identified as ferulic acid [11].

Compound **5** was isolated as colorless needles, m.p. 117 - 118 ^oC. The ^1H NMR spectrum showed only three olefinic protons at δ_H7.88 (d, J = 0.5 Hz, H-3), 7.79 (dd, J = 5.5, 1.0 Hz, H-4) and 6.50 (d, J = 5.5 Hz, H-5). The ^{13}C NMR and HSQC spectra displayed resonances for five carbons including one carbonyl group (δ_C 173.7, C-1), three olefinic CH [δ_C 146.8 (C-4), 138.8 (C-3), 113.5 (C-5)] and one oxygenated carbon (δ_C 155.5, C-2). The ESI-MS showed a molecular ion peak at *m/z*113.02 $[M+H]^+$ suggesting a molecular formula $C_5H_3O_3$. Examination of NMR, MS data and reported data [12] allowed to determine compound **5** as pyromeconic acid.

4. CONCLUSIONS

Phytochemical investigation of the stembark of *Garcinia fagraeoides* collected from Thua Thien Hue province led to the isolation of five compounds including one polyisoprenylated benzophenone (isogarcinol), two tetraoxygenated xanthones (rubraxanthone, α-mangostin), ferulic acid and pyromeconic acid. Their structures were substantially elucidated by one-dimensional (1D) and two-dimensional (2D) NMR spectroscopy and ESI-MS techniques, and by comparison with reported data. The absolute configuration of isogarcinol **(1**) was determined by comparison of its experimental optical rotation and NMR data with the values of its stereoisomer. This is the first reported isolation of compounds **1**-**5** from the stem bark of *Garcinia fagraeoides.*

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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