

**ANTIOXIDANT BENZOPHENONES AND XANTHONES FROM THE ROOT BARK  
OF *GARCINIA SMEATHMANNII***

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**ABSTRACT.** A new geranylated xanthone (**1**) was isolated from the root bark extract of *Garcinia smeathmannii* Oliver along with known guttiferone I, isoxanthochymol, smeathxanthones A and B, and triacontanyl caffeate. The structures of these compounds were elucidated by spectral analysis and by comparison with the reported data. These compounds showed significant antioxidant DPPH radical scavenging activities.

**KEY WORDS:** *Garcinia smeathmannii*, Xanthone, Antioxidant

## INTRODUCTION

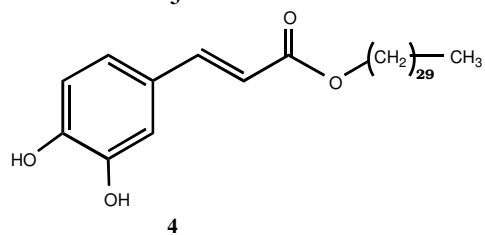
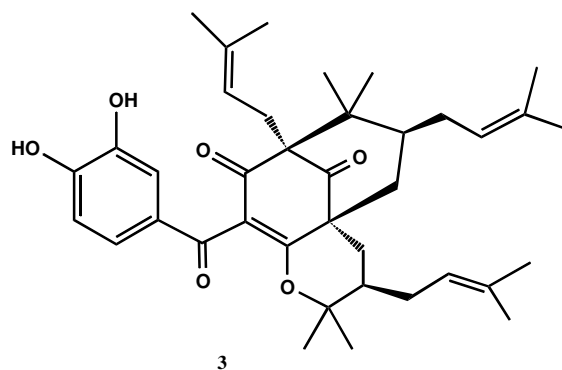
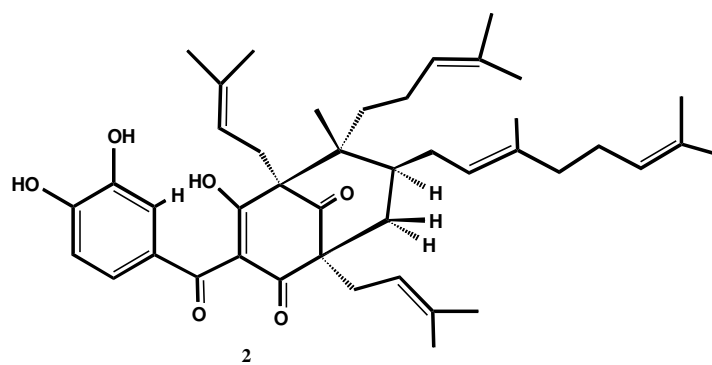
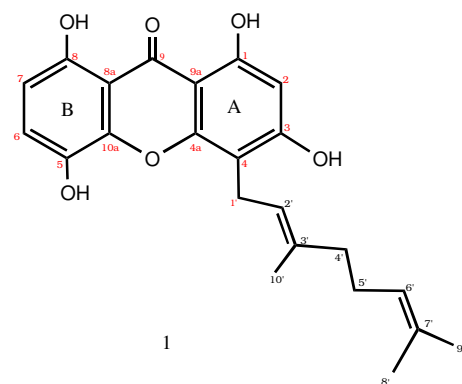
The genus *Garcinia* (Guttiferae) is known to yield a variety of oxygenated and prenylated xanthones, benzophenones and flavonoids, some of which have exhibited a wide range of antioxidant and other activities, such as HIV inhibitory, cytotoxic, anti-inflammatory, antibacterial and antifungal activities [1-5]. *Garcinia smeathmannii* is used as antidote for many poisons, and for the treatment of ophtalmia [6]. In continuation of our search for bioactive plant metabolites from medicinal plants, we have investigated the root bark extracts of *Garcinia smeathmannii* Oliver. (syn *Garcinia barteri*), a tree distributed in the lowland tropical rainforests of west and central Africa [7, 8].

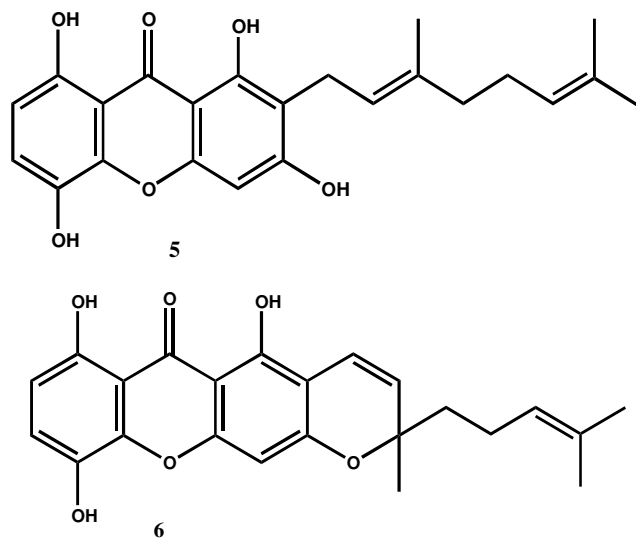
## RESULTS AND DISCUSSION

Extensive column chromatography of a chloroform extract of the root bark of *G. smeathmannii* led to the isolation of a new xanthone, cheffouxanthone (**1**), as well as four known compounds, guttiferone I (**2**) [9], isoxanthochymol (**3**) [1], triacontanyl caffeate (**4**) [10] and smeathxanthones A (**5**) and B (**6**) [5].

Cheffouxanthone (**1**) was obtained as yellow needles, m.p. 168-169 °C. The compound gave a dark green color with methanolic ferric chloride indicating that it was phenolic. The HREI MS supported a molecular formula at C<sub>23</sub>H<sub>24</sub>O<sub>6</sub> (*m/z* 396.1523). The UV spectrum showed absorption maxima at λ<sub>max</sub> 351, 307, 279, 284, 255, 241, 200 nm and IR exhibited strong bands at 3443 (OH), 1650 (chelated CO) and 1580 (aromatic ring) cm<sup>-1</sup>, suggesting a xanthone skeleton with a chelated phenolic hydroxyl group [11].

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 Table 1.  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of cheffouxanthone (**1**) in acetone- $d_6$ .

Position	<b>1</b>	
	$^{13}\text{C}$	$^1\text{H}$ [m, J (Hz)]
1	161.4	-
2	98.4	6.38 (s)
3	164.4	-
4	115.7	-
4a	144.6	-
10a	155.2	-
5	137.7	-
6	124.2	7.32 (d, 8.8)
7	109.5	6.63 (d, 8.8)
8	153.7	-
8a	107.7	-
9a	102.2	-
9	185.4	-
1'	21.5	3.59 (d, 7.2)
2'	122.6	5.38 (t, 7.2)
3'	135.2	-
4'	40.0	1.98 (t, 7.2)
5'	26.8	2.02 (m)
6'	124.5	5.15 (m)
7'	131.1	-
8'	25.2	1.54 (s)
9'	17.2	1.56 (s)
10'	15.9	1.86 (s)
1- OH		12.01 (s)
3- OH		10.20 (s)
5- OH		8.90 (s)
8- OH		11.30 (s)

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) of **1** showed resonances for two chelated phenolic hydroxyl groups at  $\delta$  12.01 (s, exchangeable with  $\text{D}_2\text{O}$ , 1-OH) and 11.30 (s, exchangeable with  $\text{D}_2\text{O}$ , 8-OH) and the corresponding chelated carbonyl carbon at  $\delta$  185.4 (C-9), two free hydroxyl groups at  $\delta$  10.20 (br s, exchangeable with  $\text{D}_2\text{O}$ , 3-OH), and 8.90 (br s, exchangeable with  $\text{D}_2\text{O}$ , 5-OH). Two coupled aromatic protons resonated at  $\delta$  7.32 (d,  $J = 8.8$  Hz, H-6) and 6.63 (d,  $J = 8.8$  Hz, H-7) with the corresponding carbons at  $\delta$  124.2 (C-6) and, 109.5 (C-7), respectively. One isolated aromatic proton resonated at  $\delta$  6.38 (s, H-2) with the corresponding aromatic carbon at  $\delta$  98.4 (C-2), while two trisubstituted double bond protons resonated at  $\delta$  5.38 (t,  $J = 7.2$ , H-2') and 5.15 (m, H-6') with corresponding carbons at  $\delta$  122.6 (C-2'), 124.5 (C-6'), respectively. One benzylic methylene group appeared at  $\delta$  3.59 (d, 7.2 Hz,  $\text{H}_2\text{-1}'$ ) and its corresponding carbon at  $\delta$  21.5 (C-1'), three vinyl methyls at  $\delta$  1.86 (s, H-10'), 1.56 (s, H-9') and 1.54 (s, H-8'), with corresponding carbons at  $\delta$  15.9 (C-10'), 17.2 (C-9') and 25.2 (C-8'), respectively. Two methylene protons at  $\delta$  1.98 (m) and 2.02 (m) with corresponding carbons at  $\delta$  40.0 (C-4') and 26.8 (C-5'), as well as nine substituted aromatic carbons at  $\delta$  164.4 (C-3), 161.4 (C-1), 115.7 (C-4), 144.6 (C-4a), 155.2 (C-10a), 133.7 (C-5), 153.7 (C-8), 107.7 (C-8a) and 102.2 (C-9a), six of which were inferred to be oxygenated were also observed. These data suggested a tetrahydroxyxanthone with a geranyl substituent.

HMQC, HMBC (Figure 1) and COSY  $45^\circ$  experiments were performed to determine the final structure of **1**. The chelated hydroxyl proton at C-1 ( $\delta$  12.01) showed the HMBC correlations with the oxygenated carbon at  $\delta$  161.4 (C-1), a protonated aromatic carbon at  $\delta$  98.4 (C-2), and a substituted aromatic carbon at  $\delta$  102.2 (C-9a). The aromatic proton (H-2) exhibited long-range correlations with the C-1, C-9a, C-3 ( $\delta$  164.4) and, C-4 ( $\delta$  115.7). The more deshielded benzylic methylene protons (H-1') showed cross-peaks with two oxygenated aromatic carbons ( $\delta$  164.4 and 144.6) indicating that C-3 was oxygenated and the geranyl group was present at C-4. The xanthone ring B therefore contain a chelated hydroxyl proton at C-8 ( $\delta$  11.30) correlated with an oxygenated carbon at  $\delta$  153.7 (C-8), a protonated aromatic carbon at  $\delta$  109.5 (C-7) and a substituted aromatic carbon at  $\delta$  107.7 (C-8a). The C-7 aromatic proton showed correlations with C-8, C-8a, C-6 ( $\delta$  124.2) and C-5 ( $\delta$  137.7) whereas the C-6 aromatic proton showed a cross-peak with the three oxygenated carbons at  $\delta$  137.7 (C-5), 153.7 (C-8) and 155.2 (C-10a).

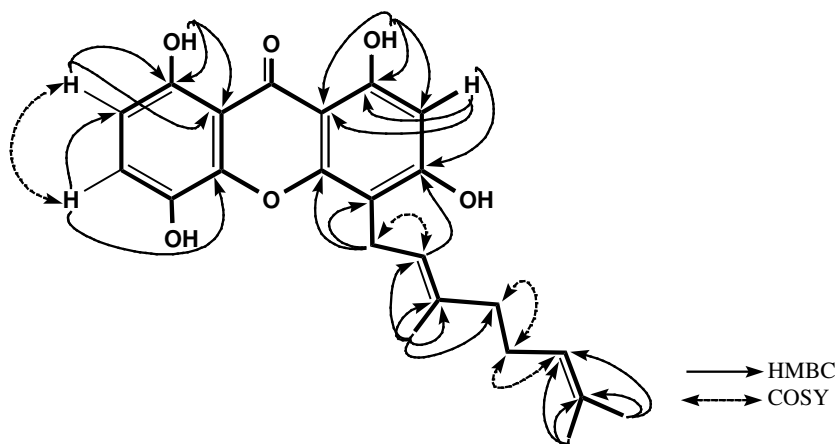


Figure 1. Key HMBC and COSY correlations in compounds **1**.

The presence of a geranyl unit was inferred from the major ions at  $m/z$  [M-69]<sup>+</sup> and [M-123]<sup>+</sup> in the mass spectrum, while its location near the hydroxyl group was inferred from an ion at  $m/z$  [M-111]<sup>+</sup> [12]. On the basis of above cited spectral data, cheffouxanthone (**1**) was characterized as 4-(3,7-dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone and has not previously been described in the literature.

#### Antioxydant activities

Compounds **1-6** were screened for DPPH radical scavenging activity. All the compounds tested showed interesting antioxidant activity compared to the standard, 3-*t*-butyl-4-hydroxyanisole (Table 2).

Table 2. Antioxidant activity of compounds **1-6**.

Name of compounds /code	% Scavenging activity (1000 $\mu$ M)	IC <sub>50</sub> ( $\mu$ M)
Isoxanthochymol ( <b>3</b> )	91.0	5.8
Guttiferone I ( <b>2</b> )	92.3	26.8
Triacontanil caffeate ( <b>4</b> )	89.1	42.0
Cheffouxanthone ( <b>1</b> )	92.0	47.8
Smeathxanthone A ( <b>5</b> )	92.0	47.8
Smeathxanthone B ( <b>6</b> )	93.0	87.0
3- <i>t</i> -butyl-4-hydroxyanisole (BHA) <sup>a</sup>	92.6	44.2

<sup>a</sup> Standard antioxidant.

## EXPERIMENTAL

**General.** The melting points were recorded on a micro melting point apparatus and are uncorrected. Optical rotations were measured on a digital polarimeter in acetone. Ultraviolet spectra were recorded in methanol on U3200 Hitachi spectrophotometer. Infrared spectra were recorded on an 8900 Shimadzu IR spectrometer. The mass spectra were recorded on a JMS-HX-110 double focusing mass spectrometer. Accurate mass measurements were performed with FAB source using glycerol as matrix on JMS-DA500, and HREI MS were recorded with the same instrument. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded respectively at 400 and 100 MHz, on Bruker spectrometers. Methyl, methylene and methine carbons were distinguished by DEPT NMR experiments. Homonuclear <sup>1</sup>H connectivities were determined using COSY experiments. One bond <sup>1</sup>H-<sup>13</sup>C connectivities were determined with the <sup>1</sup>H-detected HMQC spectrum. Two and three bond <sup>1</sup>H-<sup>13</sup>C connectivities were determined by HMBC experiment. Chemical shifts were reported in  $\delta$  (ppm) and coupling constants (*J*) were measured in Hz. Precoated TLC plates (silica gel) were used to check the purity of compounds, and ceric sulphate spraying reagent was used for the staining of compounds on TLC. All reagents used were of analytical grades.

**Plant material.** The root bark of *G. smeathmannii* was collected from Cheffou-Baham, western province, Cameroon in August 2003 and was identified by Dr. Tchiengue Bathelemy of the Cameroon National Herbarium (CNH), Yaoundé, where a voucher specimen (35169/HNC) has been deposited.

**Extraction and isolation.** Air dried root bark of *G. smeathmannii* (2 kg) were ground and extracted at room temperature successively with hexane and ethyl acetate, and respective fractions were concentrated under reduced pressure to obtain 18 g and 60 g of extracts,

respectively. Hexane extract (15 g) was chromatography over silica gel (5 x 80 cm), eluted with a mixture of petroleum ether/ethyl acetate to yield four fractions (10:1 fraction 1; 10:2 fraction 2; 10:4 fraction 3 and 10:5 fraction 4), and finally with MeOH. After evaporation of fraction 1, compounds **1** (30 mg), **2** (40 mg), **5** (50 mg) and **6** (100 mg) were obtained by purification with silica gel column as solid phase and petroleum ether-ethyl acetate as solvents. Compounds **3** (15 mg) and **4** (17 mg) were obtained from fraction 3 in the same conditions as fraction 1.

*Cheffouxanthone (I)*. Yellow needle like crystals; m.p. 168-169 °C; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 351 (4.459), 307 (4.018), 279 (4.2570), 284 (4.518), 255 (4.793), 241 (4.550), 200 (5.358) nm; IR (KBr)  $\nu_{\max}$ : 3443, 1650, 1580  $\text{cm}^{-1}$ ; EI MS:  $m/z = 396$  [M]<sup>+</sup> (41), 327 (10), 311 (11), 273 (100). HREI MS:  $m/z$  396.1523 (calcd 396.1572 for C<sub>23</sub>H<sub>24</sub>O<sub>6</sub>); <sup>1</sup>H NMR (400 MHz, C<sub>3</sub>D<sub>6</sub>O) and <sup>13</sup>C NMR (100 MHz) see Table 1.

*Determination of the radical scavenging activity*. The reaction mixture, containing 5  $\mu\text{L}$  of test sample (1 mM in DMSO) and 95  $\mu\text{L}$  of DPPH (Sigma, 300  $\mu\text{M}$  in EtOH), was taken in a 96-well micro titer plate and incubated at 37° C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity was determined by comparison with a DMSO containing control (Table 2). IC<sub>50</sub> values represent the concentration of a compound requires to scavenge 50% of DPPH radicals. BHA (3-*t*-butyl-4-hydroxyanisole) was used as a positive control.

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