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Xanthones from *Garcinia smeathmannii* (Oliver) and their antimicrobial activity

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Abstract

Two new xanthones, smeathxanthone A (**1**) (2-(3,7-dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone) and smeathxanthone B (**2**) (5,7,10-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)[2*H*, 6*H*]pyrano[3,2-*b*]xanthen-6-one), have been isolated from the stem bark of *Garcinia smeathmannii,* and their structures elucidated on the basis of 1D and 2D NMR experiments. 1,3,5-Trihydroxyxanthone and 1,5-dihydroxyxanthone were also obtained. The compounds showed only modest activity against a range of bacteria and yeasts.

Keywords: *Garcinia smeathmannii*; Guttiferae; Stem bark; Xanthone; Smeathxanthones A and B; Antimicrobial activity

1. Introduction

The genus *Garcinia* (Guttiferae) is rich in a variety of oxygenated and prenylated xanthones. Some of these compounds exhibit a wide range of microbial and other pharmacological activity, e.g., cytotoxic, anti-inflammatory, antimicrobial, antifungal, xanthine oxidase and monoamine oxidase inhibitory activity (Hiroyuki et al., 1996; Nkengfack et al., 2002). In our search for biologically active substances, we extracted the stem bark of *Garcinia smeathmannii* Oliver (syn. *Garcinia barteri*) which has not been previously investigated. *G. smeathmannii* is widely distributed in the lowland tropical rainforests of West and Central Africa (Ampofo and Waterman, 1986; Berhaut, 1975; Bouquet, 1969). We now report the isolation and characterisation of two new xanthone derivatives, smeathxanthone A (**1**), smeathxanthone B (**2**), and their antimicrobial activity.

2. Results and discussion

Air-dried and ground stem bark of *G. smeathmannii* was extracted successively with hexane and ethyl acetate at room temperature. Chromatography of the hexane (18 g) and ethyl acetate (60 g) extracts afforded the new compounds smeathxanthone A (**1**) and smeathxanthone B (**2**), as well as the known 1,3,5-trihydroxyxanthone and 1,5-dihydroxyxanthone (Zhang et al., 2002).

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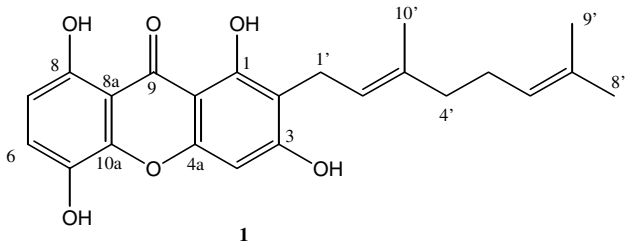
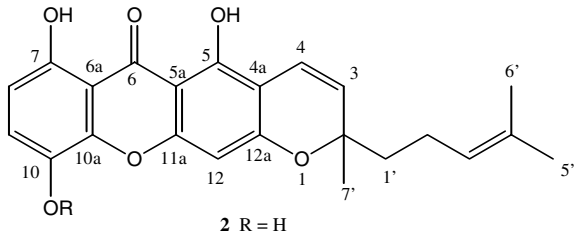


1



2 R = H
3 R = Me

Compound (**1**), yellow crystals from hexane–ethyl acetate, gave a positive $FeCl_3$ test indicating the presence of phenolic groups. CIMS (*m/z* 396.91 $[M + H]^+$) indicated a molecular formula $C_{23}H_{24}O_6$, with 12 double bond equivalents. The IR spectrum exhibited strong absorption bands characteristic of hydroxyl, conjugated carbonyl and aromatic ring moieties. The UV spectrum showed absorptions at λ_{max} nm (log ϵ): 205 (5.00), 223 (1.27), 297 (2.63), 337 (0.78) characteristic of a 1,3,5,8-tetraoxygenated xanthone (Hiroyuki et al., 1996; Nkengfack et al., 2002). The ^{13}C NMR spectrum revealed the presence of 23 carbon signals, including three methyl, five methine, three methylene and 12 quaternary carbons, one of which was a carbonyl function (δ_C 185.6). The ^1H NMR of **1** (Table 1) revealed the presence of two chelated hydroxyl groups at δ_H 12.31 (1H, *s*) and 11.30 (1H, *s*) one of which must be placed at C-1 (δ_H 12.31) and the second at C-8 (δ_H 12.31). The absence of aromatic signals around δ_H 7.40–7.60 in the ^1H NMR spectrum was consistent with the lack of protons *peri* to the carbonyl group (Nkengfack et al., 2002). Three aromatic protons at δ_H 6.58 (1H, *s*); 6.63 (1H, *d*, $J = 8.8$ Hz) and 7.29 (1H, *d*, $J = 8.8$ Hz) were attributed to H-4, H-7 and H-6, respectively, on the basis of the HMBC spectrum. The ^1H NMR of **1** also exhibited characteristic signals for a geranyl moiety at δ_H 3.38 (2H, *d*, $J = 7.2$ Hz, H-1′); 5.30 (1H, *t*, $J = 7.2$ Hz, H-2′); 1.98 (2H, *t*, $J = 7.0$ Hz, H-4′); 2.09 (2H, *m*, H-5′); 5.08 (1H, *t*, $J = 7.0$ Hz, H-6′); 1.61 (3H, *s*, H-8′); 1.56 (3H, *s*, H-9′) and 1.81 (3H, *s*, H-10′) (Merza et al., 2004). The presence of a geranyl moiety was further confirmed by a main fragment at *m/z* 273 corresponding to $[M - 123]^+$ in the mass spectrum and the set of signals at δ_C 16.3, 17.7, 21.9, 25.8, 27.4, 40.5, 122.9, 125.1, 131.6 and 135.6 in the ^{13}C NMR spectrum. In the HMBC spectrum (Table 1), the chelated phenolic protons observed at δ_H 12.31 and 11.30 showed correlations with aromatic carbons at δ_C 102.5 (C-2), 112.1 (C-9a), 160.9 (C-1) and δ_C 108.4 (C-8a), 110.2 (C-7), respectively, confirming their location at C-1 and C-8 of the xanthone nucleus. The proton at δ_H 7.29 (*d*, $J = 8.8$ Hz, H-6) correlated with carbons at δ_C 138.0 (C-5), 144.5 (C-10a) and 154.1 (C-8). The signal due to the benzylic protons (2-H-1′) of the geranyl moiety showed cross peaks with carbon signals at δ_C160.9 (C-1), 165.0 (C-3), 135.6 (C-3′) and 122.9 (C-2′), demonstrating that the geranyl group was located at C-2 of ring A. Hence, smeathxanthone A is 2-(3,7-dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone (**1**), which, to our knowledge, has not yet been described in the literature.

Smeathxanthone B (**2**), m.p. 187–189 °C, $[\alpha]_D^{22} + 30.3$ was obtained as granules from hexane–ethyl acetate

Table 1
^1H NMR data of smeathxanthone A (**1**) and B (**2**)

1			**2**		
Position	$^1H^A$ [*m*, *J* (Hz)]	HMBC	Position	$^1H^A$ [*m*, *J* (Hz)]	HMBC
4	6.58 (*s*)	9a, 2, 4a, 3	3	5.79 (*d*, 10.2)	2, 4a
6	7.29 (*d*, 8.8)	5, 10a, 8	4	6.78 (*d*, 10.2)	2, 4a
7	6.63 (*d*, 8.8)	8, 5, 8a	8	6.65 (*d*, 8.8)	10, 6a
1′	3.38 (*d*, 7.2)	2′, 3′, 1, 3	9	7.33(*d*, 8.8)	7, 8, 10a
2′	5.30 (*d*, 7.2)	2, 1	12	6.41 (*s*)	5a, 4a, 12a, 5
3′			1′	2.06 and 1.62 (*dt*, 13.1, 2.2)	2, 7′, 2′
4′	1.98 (*t*, 7.0)		2′	2.16 (*m*)	1′, 3′, 2
5′	2.09 (*m*)	6′, 7′, 3′	3′	5.16 (*t*, 7.8)	
6′	5.08 (*t*, 7.0)		5′	1.66 (*s*)	4′, 3′, 6′
8′	1.61 (*s*)	7′, 9′, 6′	6′	1.58 (*s*)	
9′	1.56 (*s*)	7′, 8′, 6′	7′	1.49(*s*)	1′, 2′, 2, 3
10′	1.81 (*s*)	2′, 3′, 4′			
1-OH		1, 2, 9a	5-OH	12.31 (*s*)	
8-OH		8a, 8	7-OH	11.30 (*s*)	
3-OH					
5-OH	9.67 (*brs*)		10-OH	9.67 (*brs*)	10a

[A] Recorded in acetone-d_6 at 400 MHz.

and also reacted positively with $FeCl_3$, suggesting the presence of phenolic groups. The molecular formula $C_{23}H_{22}O_6$ was obtained on the basis of its CIMS, *m/z* 394.99 $[M + H]^+$. Its IR and UV spectra were similar to those of **1**, indicating the presence of a xanthone nucleus (Yoshio et al., 1990; Hostettmann and Hostettmann, 1989). The ^{13}C NMR spectrum (Table 2), displayed 12 quaternary, six methine, two methylene and three methyl carbons. The ^1H NMR spectrum of **2** showed many similarities with that of **1**, particularly the signals of three phenolic hydroxyl groups, two chelated (δ_H 12.31 and 11.30) and one free (δ_H 9.67). This was confirmed by methylation of compound **2** (CH_3I) to give a monomethyl derivative **3**, exhibiting an *O*-methyl group at δ_H 3.97 in its ^1H NMR spectrum. The HMBC experiment (Table 1) indicated that ring B of xanthone (**2**) was identical to that of smeathxanthone A (**1**) and thus characterized by hydroxyls at C-7 and C-10, the former being chelated with the C-6 carbonyl. However, in contrast to **1**, compound **2** contained a substituted pyran ring [δ_H 5.79 (1H, *d*, *J* = 10.2 Hz, H-3) and δ_H 6.78 (1H, *d*, *J* = 10.2, H-4)]. These signals, in association with a methyl group at δ_H 1.49 (3H, *s*), indicated the presence of a 2-methylpyran ring in compound **2**. In the HMBC spectrum, H-4 (δ_H 6.78) correlated with C-4a (δ_C 105.4) and C-12a (δ_C 157.8) while H-3 (δ_H 5.79) correlated with C-4a. This information enabled us to locate the chromene on C-12a/C-4a. A 4-methylpent-3-enyl moiety was also observed, which was located at C-2 of the 2-methyl-3-chromene ring on the basis of HMBC correlations of the C-2 methyl group with C-1′ (δ_C 42.3) and C-3 (δ_C 128.3) and of the methylene protons 2H-1′ with C-2 (δ_C 82.1). Thus, smeathxanthone B was characterized as 5,7,10-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)[2*H*,6*H*]pyrano[3,2-*b*]-xanthen-6-one (**2**). It presumably arises from an oxidative cyclisation of smeathxanthone A (**1**) and has not previously been described in the literature.

Table 2
^{13}C NMR assignments (ppm) of compounds **1** and **2**

Carbon	1[A]	Carbon	2[A]
1	160.9	2	82.1
2	102.5	3	128.1
3	165.0	4	115.9
4	94.6	4a	105.4
4a	156.7	5	162.9
10a	144.5	5a	103.1
5	138.0	6	185.7
6	124.2	6a	108.4
7	110.2	7	154.1
8	154.1	8	110.6
8a	108.4	9	124.8
9	185.6	10	138.1
9a	102.5	10a	144.3
1′	21.9	11a	158.1
2′	122.9	12	95.8
3′	135.6	12a	157.8
4′	40.5	1′	42.3
5′	27.4	2′	23.4
6′	125.1	3′	124.7
7′	131.6	4′	132.3
8′	25.8	5′	25.8
9′	17.7	6′	18.1
10′	16.3	7′	27.4

[A] Recorded in acetone-d_6 at 100 MHz.

Table 3
In vitro antimicrobial activity of compounds **1**–**2**

Tested micro-organisms	MIC[A] (μg/ml) of the compounds		
	1	**2**	GM/N[B]
Bacteria			
E. coli	156.25	625	10
K. pneumoniae	312.5	625	10
P. vulgaris	312.5	312.5	05
S. typhi	156.25	625	05
S. aureus	312.5	312.5	10
S. faecalis	156.25	625	10
Yeasts			
C. albicans	312.5	312.5	30
C. krusei	312.5	312.5	30

[A] Result of the MIC recorded as of triplicated experiments.
[B] GM: gentamicin; N: nystatin.

Compounds **1** and **2** showed antibacterial and antifungal activity (Table 3) but the MIC values (Table 3) were much higher than those of the reference antibiotics.

3. Experimental

All melting points were determined on a Buchi apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter; IR spectra were determined as KBr pellets; UV spectra were determined in 80% EtOH as solvent. MS were recorded on FINNIGAN MAT SSQ-7000. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using acetone-d_6. Chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as internal standard. Thin layer chromatography was performed on precoated Kielselgel F_{254} (Merck) and spots were visualized under UV (254 nm) and revealed with H_2O–H_2SO_4 (1:1); flash chromatography and CC separations were carried out using silica gel 60 particle size 0.063–0.200 mm.

3.1. Plant material

The stem bark of *G. smeathmannii* Oliv. was collected in August 2003 at Cheffou-Baham, West-Province, Cameroon and was identified by Dr. Barthélemy Tchiengue of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (35169/HNC) is deposited.

3.2. Isolation

Ground stem bark (5 kg) was extracted successively at room temperature with hexane and ethyl acetate and the solvent removed under reduced pressure to yield 18 and 60 g of respective extracts. The hexane extract was first subjected to silica gel flash chromatography, eluting with hexane–ethyl acetate mixtures of increasing polarity. The fractions were purified by CC to yield compound (**2**) (120 mg). The ethyl acetate extract was also subjected to flash chromatography using the same solvent and the fractions purified by CC to yield compound (**1**) (65 mg), 1,5-dihydroxyxanthone (35 mg) and 1,3,5-trihydroxyxanthone (20 mg).

3.2.1. 2-(3,7-Dimethyl-2,6-octadienyl)-1,3,5,8-tetrahydroxyxanthone (smeathxanthone A) (*1*)

Yellow crystals, m.p. 216–218 °C, CI-MS *m/z* 396.91 $[M + H]^+$ (100), 273.18 $[M - 123]^+$ (50), 274.23 $[M + H - 123]^+$ (5); IR (KBr) ν_{max} cm^{-1} 3315, 2891, 2350, 2200, 1962, 1869, 1579, 1440, 1290, 1193, 1084, 936, 822, 784; UV λ_{max} EtOH ($\log \varepsilon$) 408 (0.32), 338 (0.21), 337 (0.78), 297 (2.63), 223 (1.27), 205 (5.00). For ^1H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) see Tables 1 and 2.

3.2.2. 5,7,10-Trihydroxy-2-methyl-2-(4-methylpent-3-enyl)[2H,6H] pyrano[3,2-b]xanthen-6-one (smeathxanthone B) (*2*)

Yellow powder; m.p. 187–189 °C; $[\alpha]_D^{22} + 30.3$ (*c*, 0.02 MeOH); CIMS *m/z* 394.99 $[M + H]^+$(100) 379.04 (19); 273.16 (20); UV λ_{max} EtOH ($\log \varepsilon$) 408 (0.32), 338 (0.21), 337 (0.78), 297 (2.63), 223 (1.27), 205 (5.00); IR (KBr) ν_{max} cm^{-1} 3727, 3414, 2965, 2359, 2262, 2062, 1987, 1636, 1586, 1346, 1052, 1000, 935, 862, 774. For ^1H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) see Tables 1 and 2. The methylated compound (**3**), prepared by treatment (5 mg) of **2** with CH_3I (K_2CO_3/acetone), was obtained as yellow oil (3 mg); $[\alpha]_D^{22} + 30.3$ (*c*, 0.02 MeOH); CIMS *m/z* 408.99 $[M + H]^+$ (100); 394.02 (50); 379.04 (19); 273.16 (20); UV λ_{max} EtOH ($\log \varepsilon$) 408 (0.32), 338 (0.21), 337 (0.78), 297 (2.63), 223 (1.27), 205 (5.00); IR (KBr) ν_{max} cm^{-1} 3736; 3394; 2919; 1629; 1582; 1205; 1169; 1042; 1002; 822; 752. ^1H NMR (acetone-d_6, 400 MHz) *O*-methyl group at δ_H 3.97.

3.3. Antimicrobial assay

The antimicrobial activity of compounds **1** and **2** was studied using a micro dilution assay on a total of eight microbial cultures belonging to six aerobic bacterial species (*Escherichia coli* LMP0101U, *Staphylococcus aureus* LMP 0206U, *Proteus vulgaris* LMP0103, *Klebsiella pneumoniae* LMP0210U, *Streptococcus faecalis* LMP0207U, *Salmonella typhi* LMP0209U) and 2 *Candida* species (*Candida albicans* LMP0204 and *Candida kruse* i LMP0311U). These strains were clinically isolated from the urogenital discharges of patients in the "*Centre Pasteur du Cameroun*" health institute and monitored in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) of the University of Yaoundé I. The strains were activated at 37 °C for 24 h on nutrient agar (NA) (aerobic bacteria) or Sabouraud glucose agar (SGA) (fungi).

The antimicrobial activity was evaluated according to the Minimal Inhibition Concentration (MIC). The inoculates of the microorganisms were prepared from 12 h broth cultures and the suspensions were adjusted to 0.5 McFarland turbidity. The test compounds were first dissolved in dimethylsulfoxide (DMSO), 10% to the highest dilution (625 μg/ml), and serial twofold dilutions were made over a concentration range of 2.44–625 μg/ml in the 96 wells micro plate containing nutrient broth. The MIC values of the test compounds against the above pathogens were determined on the basis of the micro dilution method (Zgoda and Porter, 2001). Gentamicin (bacteria) and nystatin (yeasts), diluted in water, were used as reference antibiotics. The results are presented in Table 3.

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