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Benzophenones and xanthones from *Garcinia cantleyana* var. *cantleyana* and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation

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ABSTRACT

Three benzophenones, 2,6,3′,5′-tetrahydroxybenzophenone (**1**), 3,4,5,3′,5′-pentahydroxybenzophenone (**3**) and 3,5,3′,5′-tetrahydroxy-4-methoxybenzophenone (**4**), as well as a xanthone, 1,3,6-trihydroxy-5-methoxy-7-(3′-methyl-2′-oxo-but-3′-enyl)xanthone (**9**), were isolated from the twigs of *Garcinia cantleyana* var. *cantleyana*. Eight known compounds, 3,4,5,3′-tetrahydroxy benzophenone (**2**), 1,3,5-trihydroxyxanthone (**5**), 1,3,8-trihydroxyxanthone (**6**), 2,4,7-trihydroxyxanthone (**7**), 1,3,5,7-tetrahydroxyxanthone (**8**), quercetin, glutin-5-en-3β-ol and friedelin were also isolated. The structures of the compounds were elucidated by spectroscopic methods. The compounds were investigated for their ability to inhibit low-density lipoprotein (LDL) oxidation and platelet aggregation in human whole blood *in vitro*. Most of the compounds showed strong antioxidant activity with compound **8** showing the highest inhibition with an IC_{50} value of 0.5 μM, comparable to that of probucol. Among the compounds tested, only compound **4** exhibited strong inhibitory activity against platelet aggregation induced by arachidonic acid (AA), adenosine diphosphate (ADP) and collagen. Compounds **3**, **5** and **8** showed selective inhibitory activity on platelet aggregation induced by ADP.

1. Introduction

Oxidative modification of low-density lipoprotein (LDL) involves lipid peroxidation and the modification of apolipoprotein B-100, followed by macrophage uptake and cell accumulation of cholesterol to generate foam cells, causing early atherosclerotic lesions (Heinecke, 2006). Many natural and synthetic antioxidants have been identified and developed to exhibit antiatherogenic activities by inhibiting foam cell formation in animal models (Bjorkhem et al., 1991). Antioxidant therapy may be one of the most important therapeutic approaches to prevent or retard the onset and progression of the atherogenic process. Although the antioxidant activity of many plants has been demonstrated, direct evidence of acute therapeutic benefits of plant extracts and their phenolic compounds in cardiovascular disorders remains sparse and data on LDL oxidation have been few (Okoko, 2009; Kolodziejczyk et al., 2009).

Platelets have been implicated in the pathogenesis of arterothrombotic conditions and play a key role in acute arterial thrombosis. Platelet aggregation is induced by the action of endogenous agonists such as arachidonic acid (AA), adenosine diphosphate (ADP), platelet activating factor (PAF), thrombin and collagen (Gibbins, 2004). In recent years, phenolic compounds (Nurtjahja-Tjendraputra et al., 2003), oxygenated xanthones (Chung et al., 2002; Jantan et al., 2009), coumarins (Tsai et al., 1998), isothiocyanates (Morimitsu et al., 2000), a diterpene (Shen et al., 2000), quinines (Liao et al., 2000), prenylflavonoids (Lin et al., 1993) and alkaloids of diverse chemical structures (Jantan et al., 2006), which have been isolated from various plants, showed potent antiplatelet activity. The chronic antioxidant, hypolipidemic, antithrombotic and antiplatelet activities of these compounds have important roles in prevention of lipoprotein oxidation and atherosclerotic lesion development (Wollin and Jones, 2001). However, direct evidence of acute therapeutic benefits of phenolic compounds in cardiovascular disorders remains sparse.

Recently, the methanol extract of the twigs of *Garcinia cantleyana* var. *cantleyana* Whitmore was reported to have significant antioxidative properties against copper-induced LDL oxidation and marked inhibitory effects on platelet aggregation caused by AA, ADP and collagen in human whole blood *in vitro* (Jantan et al., 2011). Phytochemical studies on the leaves and trunk bark of *G. cantleyana* have yielded five caged-xanthonoids, a xanthone and eight known compounds (Shadid et al., 2007). In this study, the isolation and structure elucidation as reported of three new benzophenones, 2,6,3′,5′-tetrahydroxybenzophenone (**1**), 3,4,5,3′,5′-pentahydroxybenzophenone (**3**) and 3,5,3′,5′-tetrahydroxy-4-methoxybenzophenone (**4**), one new xanthone, 1,3,6-trihydroxy-5-methoxy-7-(3′-methyl-2′-oxo-but-3′-enyl)xanthone (**9**) and eight known compounds (**2**, **5-8**,

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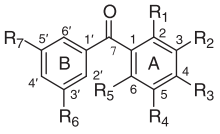
10–12) from the twigs of *G. cantleyana* var. *cantleyana*. The compounds were investigated for their ability to inhibit human LDL peroxidation and platelet aggregation *in vitro*.

2. Results and discussion

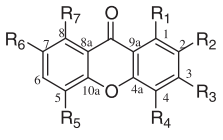
2.1. Structural identification of the isolated compounds

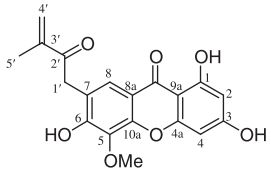
The air-dried twigs of *G. cantleyana* var. *cantleyana* (800 g) were successively extracted with *n*-hexane and MeOH. The hexane extract was subjected to silica gel column chromatography (CC) to give the known triterpenoids, glutin-5-en-3β-ol (**11**) and friedelin (**12**). Successive separations of the MeOH extract by CC using silica gel and Sephadex LH-20 afforded the new benzophenones, 2,6,3′,5′-tetrahydroxybenzophenone (**1**), 3,4,5,3′,5′-pentahydroxybenzophenone (**3**), 3,5,3′,5′-tetrahydroxy-4-methoxybenzophenone (**4**), and a new xanthone, 1,3,6-trihydroxy-5-methoxy-7-(3-methyl-2-oxo-but-3-enyl)xanthone (**9**), in addition to the known compounds, 3,4,5,3′-tetrahydroxy benzophenone (**2**), quercetin (**10**), 1,3,5-trihydroxyxanthone (**5**), 1,3,8-trihydroxyxanthone (**6**), 2,4,7-trihydroxyxanthone (**7**) and 1,3,5,7-tetrahydroxyxanthone (**8**) (Fig. 1). The structures of the known compounds were elucidated by a combination of ESIMS, ^{1}H and ^{13}C NMR techniques, and by comparison of their spectral data with literature values (Rao et al., 1974; Spino et al., 1995; Lin et al., 1996, 1998; Ungwitayatorn et al., 1997; Choudhary et al., 2005; Lannang et al., 2010).

Compound **1** was obtained as a yellow solid and found to have the molecular formula $C_{13}H_{10}O_5$ by HRESIMS (*m/z* 245.0447 [M-H]$^-$). The ^{1}H NMR spectrum of **1** exhibited signals for six aromatic protons at δ_H 6.41 (2H, *d*, *J* = 8.4 Hz, H-3 and H-5), δ_H 6.49 (1H, *t*, *J* = 1.8 Hz, H-4′), δ_H 6.78 (2H, *d*, *J* = 1.8 Hz, H-2′ and H-6′) and δ_H 7.13 (1H, *t*, *J* = 8.4 Hz, H-4). The ^{13}C NMR spectrum of **1** indicated the presence of 13 signals, i.e. six methine carbons (δ_C 106.4, 106.4, 106.9, 107.4, 107.4, 131.1) and seven quaternary carbons (δ_C 114.9, 140.4, 156.4, 156.4, 158.2, 158.2, 198.1). The resonance at δ_C 198.1 was typical for a carbonyl group and the four signals at δ_C 156.4, 156.4, 158.2, 158.2 were typical of hydroxylated aromatic carbons. This led us to deduce that the compound was a benzophenone. The complete ^{1}H and ^{13}C NMR assignments for **1** based on COSY-135, HMQC and HMBC experiments are shown in Table 1. The COSY spectrum exhibited a spin system for the resonances of the protons H-3, H-4 and H-5. Important observations from the HMBC experiment were as follows. (a) The protons H-2′ and H-6′ showed a ^2J correlation with C-1′ (δ_C 140.4) and ^3J correlations with C-4′ (δ_C 106.9) and C-7 (δ_C 198.1). (b) The proton H-5 showed a ^2J coupling with C-6 (δ_C 156.4) and a ^3J coupling with C-1 (δ_C 114.9). (c). The proton H-4 showed a ^2J coupling with C-3 (δ_C 106.4) and a ^3J coupling with C-2 (δ_C 156.4). Based on these data compound, **1** was elucidated as the new compound, 2,6,3′,5′-tetrahydroxybenzophenone.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
(**1**)	OH	H	H	H	OH	OH	OH
(**2**)	H	OH	OH	OH	H	OH	H
(**3**)	H	OH	OH	OH	H	OH	OH
(**4**)	H	OH	OMe	OH	H	OH	OH





(**9**)

	R_1	R_2	R_3	R_4	R_5	R_6	R_7
(**5**)	OH	H	OH	H	OH	H	H
(**6**)	OH	H	OH	H	H	H	OH
(**7**)	H	OH	H	OH	H	OH	H
(**8**)	OH	H	OH	H	OH	OH	H

Fig. 1. Structures of benzophenones and xanthones from *Garcinia cantleyana* var. *cantleyana*.

Analysis on the ^{1}H and ^{13}C NMR spectra of compounds **2**-**4** indicated that they were also benzophenones and structurally similar to **1**. The ^{1}H and ^{13}C NMR spectra of **2** showed the presence of six aromatic protons and 13 carbons, of which seven were quaternary carbons and one was attributed to a carbonyl group. Compound **2** was identified as a known compound, 3,4,5,3′-tetrahydroxybenzophenone as confirmed by the comparison of the spectroscopic data with that reported in the literature. Compound **2** has been isolated for the first time as a new natural product from *Garcinia mangostana* (Jiang et al., 2010).

Compound **3** was obtained as yellow crystals. The HRESIMS spectrum exhibited molecular ion peaks $[M + H]^+$ at *m/z* 263.0296 suggesting a molecular formula of $C_{13}H_{10}O_6$. The ^{1}H and ^{13}C NMR spectra of **3** indicated that it has the same substitution pattern of the aromatic system in ring A as compound **2**. The three protons in ring B showed *meta* coupling to each other, based on their COSY correlations and coupling constants; δ_H 6.40 (1H, *t*, *J* = 2.4 Hz, H-4′), δ_H 6.55 (2H, *d*, *J* = 2.4 Hz, H-2′ and H-6′) (Table 1). The chemical shifts of the quaternary aromatic protons indicated that five of the carbons were hydroxylated (δ_C 157.6, 157.7, 162.0, 162.0, 164.1). The connectivities between protons and carbons in the HMQC and HMBC spectrum indicated that the protons H-2 and H-6 were ^3J coupled with C-4 (δ_C 164.1) and C-7 (δ_C 199.2); the protons H-2′ and H-6′ showed ^2J coupling with C-1′ (δ_C 143.3) and ^3J correlation with C-4′ (δ_C 105.0) and C-7; the proton H-4′ showed ^2J coupling with C-3′ (δ_C 157.6) and C-5′ (δ_C 157.7) and ^3J correlation with C-2′ (δ_C 106.3) and C-6′ (δ_C 106.3). Conclusively, we identified **3** as a new compound, 3,4,5,3′,5′-pentahydroxybenzophenone.

Compound **4** was obtained as yellow crystals and has a molecular formula of $C_{14}H_{12}O_6$ as determined by HRESIMS (*m/z* 299.0336 $[M + Na]^+$, 575.0809 $[2 M + Na]^+$, 851.1273 $[3 M + Na]^+$). The ^{1}H and ^{13}C NMR spectra of **4** were very similar to **3**, except for the presence of a methoxylic carbon at δ_C 54.5 (3H, *s*) and methoxy signals at δ_H 3.57 (3H, *s*). There were HMBC correlations between the

Table 1
NMR spectroscopic data (600 MHz, MeOD) for benzophenones (δ in ppm).

Position	**1**		**3**		**4**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	114.9	–	104.9	–	106.4	–
2	156.4	–	94.4	5.86, *s*	91.1	6.01, *s*
3	106.4	6.41, *d* (8.4)	162.0	–	161.6	–
4	131.1	7.13, *t* (8.4)	164.1	–	163.5	–
4-OMe	–	–	-	–	54.5	3.57, *s*
5	106.4	6.41, *d* (8.4)	162.0	–	161.6	–
6	156.4	–	94.4	5.86, *s*	95.3	6.01, *s*
7	198.1	–	199.2	–	198.3	–
1′	140.4	–	143.3	–	142.8	–
2′	107.4	6.78, *d* (1.8)	106.3	6.55, *d* (2.4)	106.4	6.56, *d* (1.8)
3′	158.2	–	157.6	–	157.9	–
4′	106.9	6.49, *t* (1.8)	105.0	6.40, *t* (2.4)	105.5	6.43, *t* (1.8)
5′	158.2	–	157.7	–	157.9	–
6′	107.4	6.78, *d* (1.8)	106.3	6.55, *d* (2.4)	106.4	6.56, *d* (1.8)