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Cytotoxic caged-polyprenylated xanthonoids and a xanthone from *Garcinia cantleyana*

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Abstract

Phytochemical studies on the leaves and trunk bark of *Garcinia cantleyana* yielded five caged-xanthonoids including one tetra- and four tri-prenylated xanthones, cantleyanone A (**1**), 7-hydroxyforbesione (**2**) and cantleyanones B–D (**4**–**6**), as well as a simple xanthone, 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (**3**). Eight other known compounds, deoxygaudichaudione A, gaudichaudione H, friedelin, garbogiol, macranthol, glutin-5-en-3β-ol, and a mixture of sitosterol and stigmasterol were also isolated. Their structures were elucidated by means of spectroscopic data and comparison of their NMR data with literature values. Significant cytotoxicity against MDA-MB-231, CaOV-3, MCF-7 and HeLa cancer cell-lines was demonstrated by cantleyanones B–D, 7-hydroxyforbesione, deoxygaudichaudione A and macranthol, with IC_{50} values ranging from 0.22 to 17.17 μg/ml.

Keywords: *Garcinia cantleyana*; Caged-polyprenylated xanthonoids; Cantleyanones

1. Introduction

In the course of our ongoing search for bioactive compounds from natural sources, we have screened eleven *Garcinia* species found in the Malaysia Peninsula for cytotoxic activity. Among the species showing strong activity was the MeOH extract of *Garcinia cantleyana* Whitmore. The species is endemic to Malaysia, found mostly in the upper mountain forest (Whitmore, 1983). It is a small tree, occasionally reaching 15 m tall and 90 cm girth. The inner bark is thick, opaque, and produces sulphur-yellow exudates. The crude chloroform extract of leaves and trunk barks of *G. cantleyana* were both found to be cytotoxic against MDA-MB-231, MCF-7, CaOV-3, and HeLa cell-lines with IC_{50} values ranging from 2.33 to 8.33 μg/ml. The leaf extract afforded the new tetra-prenylated caged-xanthone cantleyanone A (**1**), 7-hydroxyforbesione (**2**), and 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (**3**), in addition to the known compounds deoxygaudichaudione A (Han et al., 2006), garbogiol (Iinuma et al., 1998), macranthol (Kouno et al., 1991), and glutin-5-en-3β-ol (Leong and Harrison, 1999). Meanwhile the three new tri-prenylated caged-xanthones, cantleyanones B–D (**4**–**6**) were isolated from the trunk bark extract together with two known compounds; gaudichaudione H (Cao et al., 1998) and friedelin. Macranthol which also exhibited strong cytotoxic activity was isolated for the first time from *Garcinia*. Herein, we report the structure elucidation of compounds **1**–**6**, and their cytotoxic properties against cancer cell-lines Fig. 1.

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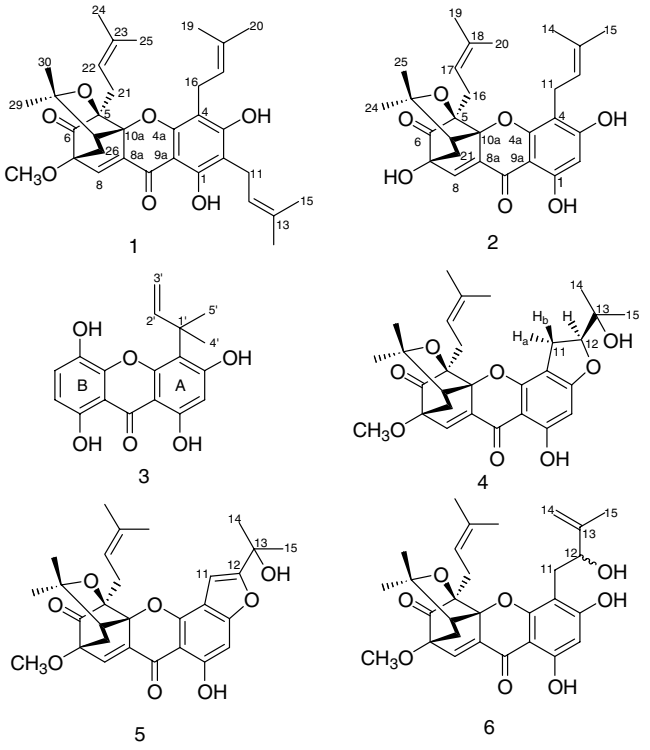

Fig. 1. Structures of cantleyanone A (**1**), 7-hydroxyforbesione (**2**), 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (**3**) cantleyanones B–D (**4**–**6**).

2. Results and discussions

Cantleyanone A (**1**) was isolated as yellow oil, and found to have the molecular formula $C_{34}H_{42}O_7$ by HRESIMS. Its IR spectrum exhibited absorption bands at 3449 (hydroxyl group), 1718 (unconjugated carbonyl group) and 1628 cm^{-1} (*ortho* hydroxyl chelated carbonyl group). These data and the UV absorption band at λ_{max} 359 nm for a conjugated carbonyl chromophore were similar to those of deoxygaudichaudione A (Han et al., 2006) suggesting that **1** has a similar caged-polyprenylated structure. The ^1H and ^{13}C NMR data were also comparable to those of deoxygaudichaudione A (Han et al., 2006), showing characteristic signals for the caged-prenylated and phloroglucinol halves of the structure. The only difference was the absence of the H-7 signal seen in the ^1H NMR spectrum of deoxygaudichaudone A, which has been replaced by a methoxyl singlet at δ_H 3.66. The attachment of the methoxy group to the bridge-head carbon was apparent from HMBC correlation observed between its protons to C-7 (δ_C 85.0). The olefinic proton H-8 (δ_H 7.51) also appeared as a singlet since it no longer has a coupling partner on C-7. The ^1H and ^{13}C NMR, as well as the HMBC data for cantleyanone A are summarized in Tables 1 and 2. A NOESY experiment supported the same relative stereochemistry for the tricyclic core of **1** as previously reported caged-xanthonoids (Cao et al., 1998; Rukachaisirikul et al., 2000; Xu et al., 2000). Cantleyanone A is thus assigned as structure **1**.

Table 1
^{13}C NMR data in $CDCl_3$ of compounds **1**–**6** (δ in ppm)

Position	1	2	3	4	5	6
1	160.5	163.4	161.6	166.3	161.0	164.2
2	106.5	97.3	101.0	92.1	93.3	99.0
3	163.2	164.6	163.4	169.4	162.0	167.0
4	107.7	106.4	111.5	104.6	110.3	105.1
4a	156.3	158.2	155.5	155.4	153.6	158.6
5	84.4	84.3	136.3	84.2	84.3	84.3
6	202.8	203.9	123.3	201.5	201.3	201.9
7	85.0	79.5	110.2	84.9	85.0	85.0
8	134.2	137.9	153.7	134.5	135.3	134.6
8a	132.4	131.4	107.2	132.2	132.2	132.2
9	179.3	179.3	185.1	178.3	180.0	179.0
9a	101.1	101.4	103.4	101.3	102.5	101.2
10a	89.2	89.7	143.0	89.5	89.8	89.6
11	22.9	22.3		26.9	97.8	28.9
12	121.6	121.5		92.1	162.7	78.9
13	135.6	135.3		71.8	69.4	146.0
14	26.0	26.0		26.4	28.9	113.8
15	18.3	18.2		24.5	28.8	17.1
16	21.4	29.1		29.2	29.3	29.0
17	121.9	117.4		117.8	118.2	117.4
18	134.7	135.9		135.5	135.8	135.8
19	26.1	25.7		25.8	25.7	25.7
20	18.2	17.0		17.1	17.1	16.9
21	29.1	30.8		30.1	29.9	30.41
22	117.8	50.1		49.8	49.8	50.0
23	135.8	84.2		84.0	84.1	83.6
24	25.8	29.3		29.2	30.7	30.3
25	17.0	30.4		30.5	29.2	29.1
26	30.5					
27	49.9					
28	83.7					
29	29.3					
30	30.4					
1′			152.2			
2′			41.6			
3′			109.6			
4′			28.3			
5′			28.3			
7-O$\underline{C}H_3$	54.2			54.2	54.3	54.2

7-Hydroxyforbesione (**2**) with the molecular formula $C_{28}H_{32}O_7$ determined from HRESIMS, showed UV and IR absorptions similar to those of **1** suggesting that **2** also possessed similar structural features as **1** and deoxygaudichaudione A. However, a closer examination of the ^1H and ^{13}C NMR spectra of **2** showed that, apart from the characteristic –$OC(Me)_2$–$CHCH_2$–C– unit for the caged-prenylated moiety and the phloroglucinol-type aromatic ring, **2** has only two other free prenyl groups in its molecular structure. One of these was the prenyl group attached to C-5 (δ_C 84.3), as in **1**, leaving the remaining prenyl group which must thus be attached on ring-A. The methoxy group at C-7 is also absent in this compound. However, the deshielded nature of C-7 (δ_C 79.5) indicated that this bridge-head carbon was hydroxylated.

The ^1H NMR spectrum of **2** exhibited an aromatic proton singlet at δ_H 6.07 (δ_C 97.3) which could be assigned as H-2 based on the ^2J and ^3J correlations observed from it to C-1 (δ_C 163.4, ^2J), C-3 (δ_C 164.6, ^2J), C4 (δ_C 106.4, ^3J) and

Table 2
^{1}H and HMBC NMR data ($CDCl_3$) for compounds **1**–**6**

Position	^{1}H **1**	HMBC **1**	^{1}H **2**	HMBC **2**	^{1}H **3**	HMBC **3**
2			6.07 (1H, *s*)	C-1, 3, 4, 9a	6.28 *s*	C-1, 3, 4, 9a, 9
6					7.25 *d* (9)	C-5, 8, 10[a]
7					6.71 *d* (9)	C-5, 8, 8a[a]
8	7.51 (1H, *s*)	C-6, 8[a], 9, 10[a]	7.29 (1H, *s*)	C-6, 8[a], 9, 10[a], 21		
11	3.42 (2H, *d*, 7.0)	C-2, 12, 13	3.46 (2H, *m*)	C-4, 4a, 12, 13		
12	5.25 (1H, *t*, 7.0)	C-2, 11, 14	5.25 (1H, *t*, 6.5)	C-4, 11, 14, 15		
14	1.84 (3H, *s*)	C-12, 13, 15	1.76 (3H, *s*)	C-12, 13, 15		
15	1.78 (3H, *s*)	C-12, 13, 14	1.81 (3H, *s*)	C-12, 13, 14		
16	3.42 (2H, *d*, 7.0)	C-3, 4a, 4, 17	2.67 (2H, *m*)	C-5, 6, 10a, 17, 18		
17	5.25 (1H, *t*, 7.0)	C-16, 19	4.42 (1H, *t*, 7.0)	C-16, 19, 20		
19	1.75 (3H, *s*)	C-17, 18, 20	1.39 (3H, *s*)	C-17, 18, 20		
20	1.80 (3H, *s*)	C-17, 18, 19	1.07 (3H, *s*)	C-17, 18, 19		
21	2.63 (1H, *m*)	C-5, 6, 10a, 22	2.18 (1H, *d*, 13.0) 1.85 (1H, *dd*, 13.0, 9.5)	C-6, 7, 8, 10a, 22, 23		
22	4.44 (1H, *m*)	C-24	2.57 (1H, *d*, 9.5)	C-5, 7, 10a, 21, 23, 25		
24	1.39 (3H, *s*)	C-22, 23, 25	1.28 (3H, *s*)	C-22, 23, 25		
25	1.04 (3H, *s*)	C-22, 23, 24	1.70 (3H, *s*)	C-22, 23, 24		
26	2.38 (1H, *d*,12.5) 1.64 (1H, *m*)	C-6, 7, 8, 10[a], 27, 28				
27	2.57 (1H, *d*, 10.5)	C-5, 10a, 26				5.29 *d* (10.5)
29	1.32 (3H, *s*)	C-27, 28, 30				
30	1.69 (3H, *s*)	C-27, 28				
2′					6.59 (1H, *dd*, 17.5, 10.5)	C-1′, 4, 3′, 4′/5′
3′					5.39 (1H, *d*, 17.5)	C-2′, 1′
					5.29 (1H, *d*, 10.5)	C-1′
4′					1.73 (3H, *s*)	C-4, 1′, 2′, 5′
5′					1.73 (3H, *s*)	C-4, 1′, 2′, 4′
1-OH	12.89 (1H, *s*)	C-1, 2, 9a	12.55 (1H, *s*)	C-1, 2, 9a	12.32 (1H, *s*)	C-1, 2, 9a
3-OH	6.53 (1H, *s*)	C-2, 3, 4				
8-OH					11.16 (1H, *s*)	C-7, 8, 8a
7-OCH_3	3.66 (3H, *s*)	C-7				

(*continued on next page*)

Table 2 (*continued*)

Position	^{1}H **4**	HMBC **4**	^{1}H **5**	HMBC **5**	^{1}H **6**	HMBC **6**
2	6.07 (1H, *s*)	C-1, 3, 4, 9a	6.64 (1H, *s*)	C-1, 3, 4, 9a	6.19 (1H, *s*)	C-1, 3, 4
6						
7						
8	7.50 (1H, *s*)	C-6, 7, 8a, 9, 10a, 21	7.57 (1H, *s*)	C-6, 9, 10a, 21	7.52 (1H, *s*)	C-6, 7, 8a, 9, 10a
11	3.17 (1H, *dd*, 15.0, 9.0)	C-3, 4a, 4, 13	6.57 (1H, *s*)	C-4, 4a, 11	3.19 (1H$_a$, *d*, 15.0)	C-3, 4, 4a, 12
	3.07 (1H, *dd*, 15.0, 9.0)				2.83 (1H$_b$, *dd*, 15.0, 10.0)	
12	4.76 (1H, *m*)	C-3, 4, 14, 15			4.38 (1H, *d*, 10.0)	C-4, 11, 14, 15
14	1.40 (3H, *s*)	C-12, 13, 15	1.69 (3H, *s*)	C-12, 13, 15	4.93 (1H, *br s*)	C-12, 13, 15
					4.95 (1H, *br s*)	
15	1.27 (3H, *s*)	C-12, 13, 14	1.69 (3H, *s*)	C-12, 13, 14	1.90 (3H, *s*)	C-12, 13, 14
16	2.60 (2H, *m*)	C-5, 6, 10a, 17, 18	2.64 (2H, *m*)	C-5, 6, 10a, 17, 18	2.50 (2H, *dd*, 14.0, 10.0)	C-5, 6, 10a, 17, 18
17	4.54 (1H, *t*, 7)	C-16, 19, 20	4.51 (1H, *m*)	C-16, 19, 20	4.45 (1H, *m*)	C-19, 20
19	1.42 (3H, *s*,)	C-17, 18, 20	1.35 (3H, *s*,)	C-17, 18, 20	1.39 (3H, *s*)	C-17, 18, 20
20	1.26 (3H, *s*)	C-17, 18,19	0.93 (3H, *s*)	C-17, 18, 19	1.04 (3H, *s*)	C-17, 18, 19
21	2.38 (1H, *d*, 12.5)	C-6, 7, 8, 10a, 22, 23	2.43 (1H, *d*, 13.0)	C-6, 7, 8, 10a, 22, 23	2.37 (1H$_a$, *d*, 13.5)	C-6, 7, 8, 10a, 22, 23
					1.66 (1H$_b$, *dd*, 13.5, 10.0)	
	1.64 (1H, *dd*, 12.5, 9.0)		1.64 (1H, *dd*, 10.0, 3.0)			
22	4.44 (1H, *m*)	C-5, 7, 10a, 21, 23, 25	2.60 (1H, *d*, 10.0)	C-5, 10a, 21, 23, 24, 25	2.56 (1H, *d*, 10.0)	C-5, 7, 10a, 21, 25
24	1.33 (3H, *s*)	C-22, 23, 25	1.78 (3H, *s*)	C-22, 23, 25	1.59 (3H, *s*)	C-22, 23, 25
25	1.67 (3H, *s*)	C-22, 23, 24	1.37 (3H, *s*)	C-22, 23, 24	1.30 (3H, *s*)	C-22, 23, 24
26						
27						
29						
30						
2′						
3′						
4′						
5′						
1-OH	12.72 (*s*)	C-1, 2, 9a	12.20 (*s*)	C-1, 2, 9a	12.59 (*s*)	C-1, 2, 9a
3-OH						
8-OH						
OCH_3	3.65 (*s*)	C-7	3.67 (*s*)	C-7	3.65 (*s*)	C-7

C-9a (δ_C 101.4, ^3J). The remaining prenyl group was thus placed at C-4. The HMBC correlations observed between H_2-11 (δ_H 3.46, m) with C-4 (^2J) and C-4a (δ_C 158.2, ^3J) further support this assignment. Based on the above considerations, compound **2** was assigned as 7-hydroxyforbesione. The ^1H and ^{13}C NMR, as well as the HMBC data for **2** are summarized in Table 1 and 2. It is worth noting that this finding represents the first report of a 7-hydroxylated caged-xanthonoid.

The molecular formula for the new compound (**3**) was established as $C_{18}H_{16}O_6$ by HRESIMS. Its UV (257 and 343 nm) and IR (3435 cm^{-1} and 1629 cm^{-1}) absorptions were typical of a xanthone derivative (Minami et al., 1996). Its ^1H NMR spectrum showed two sharp singlets at δ_H 12.32 and 11.16 which suggested the presence of two chelated hydroxyl groups on C-1 and C-8, respectively. A pair of *ortho*-coupled AB-type aromatic proton signals at δ_H 6.71 (d, $J = 9.0$ Hz, H-7), and 7.25 (d, $J = 9.0$ Hz, H-6) were also observed in addition to an aromatic one-proton singlet at δ_H 6.28 (H-2), suggesting disubstituted and trisubstituted rings B and A, respectively, of the xanthone molecule. In the HMBC spectrum, cross-peaks for one of the chelated hydroxyl proton {δ_H 11.16 (8-OH)/δ_C 153.7 (C-8), 107.3 (C-8a), 110.2 (C-7)}, and the *ortho*-coupled pair of aromatic protons {δ_H 6.71 (H-7)/δ_C 153.7 (C-8), 136.3 (C-5), 107.2 (C-8a) and 7.25 (H-6)/δ_C 153.7 (C-8), 136.3 (C-5), 143.0 (C-10a)} were observed, confirming a disubstituted ring-B feature for **3**. Similarly, HMBC cross-peaks observed for the other chelated hydroxyl proton {δ_H 12.32 (1-OH)/δ_C 101.0 (C-2), 161.6 (C-1), 103.4 (C-9a)}, and the singlet aromatic proton {δ_H 6.28 (H-2)/δ_C 111.5 (C-4), 163.4 (C-3), 161.6 (C-1), 103.4 (C-9a)} established a trisubstituted ring-A feature, with hydroxyl groups located at C-1 and C-3, and a third substituent at C-4. The third substituent represented by the set of signals at δ_H 1.73 (6H, *s*, H_3-4′ and H_3-5′), δ_H 5.39 (1H *d*, $J = 17.5$ Hz, H_a-3′), 5.29 (1H *d*, $J = 10.5$ Hz, H_b-3′) and δ_H 6.59 (1H *dd*, $J = 17.5$, 10.5 Hz, H-2′) in the ^1H NMR spectrum, was established to be a 1,1-dimethylprop-2-enyl group on the basis of 2D (^1H–^1H COSY, HSQC and HMBC) spectral data. Compound **3** was therefore assigned as 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone.

Cantleyanones B, C and D (**4**, **5** and **6**), obtained as yellow solids from the trunk bark extract, were all 7-methoxy caged-polyprenylated xanthones with a phloroglucinol-type aromatic ring-A. The three compounds showed UV and IR characteristics similar to **1** and **2** (UV absorption bands at λ_{max} 358–359 nm for a conjugated carbonyl chromophore, and IR absorption bands at 3423–3449 cm^{-1} for hydroxyl, 1715–1718 cm^{-1} for an unconjugated carbonyl, and 1636–1637 cm^{-1} for a chelated *ortho*-hydroxyl carbonyl groups). The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) also showed the common signals for a caged-prenylated and phloroglucinol halves of the molecule. The C-7 (δ_C 85.0) was methoxylated, and the phloroglucinol-type aromatic ring carries a single aromatic proton on C-2 based on the observed HMBC cross-peaks from H-2 to C-1 (^2J), C-3 (^2J), C4 (^3J) and C-9a (^3J). The structures of **4**, **5** and **6** differ from each other by the modified prenyl side-chain on C-4.

Canthelyanone B (**4**) has a molecular formula $C_{29}H_{34}O_8$ as determined by HRESIMS. Analysis of the ^1H, ^{13}C NMR, ^1H–^1H COSY, HSQC and HMBC data (Table 2) showed that the modified prenyl side-chain on C-4 for **4**, represented by the set of signals at δ_H 3.17 (*dd*, $J = 9.0$ and 15.0 Hz, H_a-11), 3.07 (*dd*, $J = 9.0$ and 15.0 Hz, H_b-11), 4.76 (*m*, H-12), 1.40 (*s*, H_3-14), and 1.27 (*s*, H_3-15), was in the form of a 2-(2-hydroxypropyl)dihydrofuran ring, similar to that found in mangostanin (Suksamrarn et al., 2006). The furan ring was further established to be fused to the C-3,4 position on ring-B based on the HMBC correlations H_2-11/δ_C 169.4 (C-3), 104.6 (C-4), 155.4 (C-4a), and H-12/169.4 (C-3), 104.6 (C-4). The relative stereochemistry at C-12 was deduced from a NOESY experiment. Whilst showing very strong NOEs with both Me-14 and Me-15, the oxymethine proton H-12 (δ_H 4.76) showed very strong NOE interactions to only one of the C-11 methylene protons (δ_H 3.17, H_a-11). On the other hand, the other C-11 methylene proton at δ_H 3.07 (H_b-11) was observed to show strong NOE interactions with both Me-14 and Me-15 as well as Me-25, which implied that they were on the same side of the molecule. This was further supported from the stronger NOE interactions observed between Me-25 and H_b-11 when compared to H_a-11. The structure of **4** was thus established and given the trivial name cantleyanone B.

The molecular formula of cantleyanone C (**5**) was determined to be $C_{29}H_{32}O_8$. The ^1H and ^{13}C NMR spectra of **5** were very similar to cantleyanone E (**4**), the only difference being the absence of the C-11 methylene group. In its place, the ^1H and ^{13}C NMR spectra of **5** exhibited an olefinic proton singlet at δ_H 6.57 (δ_C 97.8) and a very deshielded C-12 (δ_C 162.7), indicating the presence of a furan ring. Thus, **5** was assigned as the dehydrogenated product of cantleyanone E (**4**) and named cantleyanone C.

Cantleyanone D (**6**) has a molecular formula of $C_{29}H_{34}O_8$ as determined from its HRESIMS. The ^1H and ^{13}C NMR spectra of **6** showed a different type of prenyl side-chain on C-4 when compared to **4** and **5**. From the set of signals observed at δ_H 2.83 (*dd*, $J = 15.0$, 10.0 Hz, H_b-11), 3.19 (*d*, $J = 15.0$ Hz, H_a-11), 4.38 (*d*, $J = 10.0$ Hz, H-12), 4.93 and 4.95 (H_2-14), 1.90 (*s*, H_3-15), the prenyl side-chain was found to be in the form of a 2-hydroxy-3-methylbut-3-enyl group. The assignment was supported by ^1H–^1H COSY and the HMBC correlations observed between the protons at δ_H 2.83 and 3.19 ($H_{a/b}$-11) with C-4 (δ_C 105.1), C-4a (δ_C 158.6), C-3 (δ_C 167.0), C-12 (δ_C 78.9), C-13 (δ_C 146.0), and between H-12 (δ_H 4.38) with C-4, C-11 (δ_C 105.1), C-14 (δ_C 113.8) and C-15 (δ_C 17.1). The stereochemistry at C-12 was left unresolved. The above spectral considerations allowed the assignment of cantleyanone D as the structure **6**, which has not been reported before.

Table 3
Cytotoxic activities of the isolated natural products and $CHCl_3$ crude extract of *G. cantleyana*

Compounds	IC_{50} μg/ml[a]			
	MDA-MB-231	MCF-7	CaOV-3	HeLa
Cantleyanone A (**1**)	9.67 ± 1.53	24.33 ± 1.53	13.83 ± 1.26	20.67 ± 3.06
7-Hydroxyforbesione (**2**)	2.17 ± 0.31	0.42 ± 0.08	0.28 ± 0.07	0.22 ± 0.03
4-(1,1-Dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (**3**)	25.50 ± 1.32	17.67 ± 1.53	27.50 ± 0.87	13.33 ± 0.58
Cantleyanone B (**4**)	6.23 ± 0.93	0.83 ± 0.07	0.28 ± 0.04	0.43 ± 0.10
Cantleyanone C (**5**)	6.70 ± 0.36	1.48 ± 0.37	0.44 ± 0.06	0.48 ± 0.07
Cantleyanone D (**6**)	17.17 ± 0.76	4.40 ± 0.46	3.47 ± 1.23	2.8 ± 0.3
Deoxygaudichaudione A	0.44 ± 0.02	0.38 ± 0.06	0.44 ± 0.02	0.34 ± 0.03
Garbogiol	>30	>30	>30	>30
Macranthol	4.17 ± 0.35	3.70 ± 0.56	1.53 ± 0.57	2.53 ± 0.49
G. cantleyana (chloroform extract)	2.71 ± 0.35	4.17 ± 0.25	4.63 ± 0.15	4.37 ± 0.15

[a] Results are expressed as IC_{50} values (μg/ml) ±SD of three experiments performed in triplicate, CaOV-3, human ovarian cancer; HeLa, human cervical cancer; MDA-MB-231, human breast cancer (Re−); MCF-7, human breast cancer (Re+).

The isolated compounds were assayed for their cytotoxic activity on four cell-lines. The results are shown in Table 3. Compounds **2**, **4**–**6**, deoxygaudichaudione A and macranthol exhibited strong activity against the four cell-lines tested, with IC_{50} values ranging from 0.22 to 7 μg/ml, except for compound **6** which was weakly cytotoxic against MDA-MB-231 with an IC_{50} value of 17.7 μg/ml. Compounds **2**, **4** and **5** were also less active towards the MDA-MB-231 cell-line. Compounds **1** and **3** exhibited moderate to weak activity with IC_{50} values ranging from 9.67 to 27.50 μg/ml, respectively. Garbogiol showed weak activity against all tested cell-lines with IC_{50} values more than 30 μg/ml.

3. Conclusions

Similar to several other *Garcinia* species such as *G. morella*, *G. hanburyi*, *G. gaudichaudii*, *G. bracteata*, *G. forbesii* and *G. scortechinii*, *G. cantleyana* also elaborated caged-xanthones. The possible biosynthetic pathways of caged-xanthonoids have been discussed. From this investigation, the xanthonoids isolated from *G. cantleyana* seemed to be derived from mono-, tri- and tetra-prenylated xanthones. The prenyl moieties on the non-caged section of these xanthonoids retained their forms or are further oxidized and cyclized to form dihydrobenzofuran or benzofuran moieties. It is interesting to note the different attachment of the prenyl group in the mono-prenylated xanthone (**3**). The isolated xanthonoids are strongly cytotoxic except for cantleyanone A (**1**), 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (**3**) and garbogiol. Comparing the structures of the bioactive compounds **2**, **4**, **5** and **6** with that of **1** and **3**, it is clear that the caged nature of ring-B as well as the presence of the peri-hydroxyl group on ring-A are important structural features for bioactivity. These are in agreement to previous findings (Cao et al., 1998; Mackeen et al., 2000). The C8-8a double bond is also thought to be important for bioactivity (Zhang et al., 2004). However, as indicated by the weak cytotoxic activity exhibited by compound **1**, prenylation on C-2, appears to reduce the cytotoxic activity of this class of compounds quite significantly.

4. Experimental section

4.1. General

Melting points were determined on a Fisher-Johns hot stage apparatus and are uncorrected. UV and IR spectra were recorded on CARY 100 Conc UV–Vis (Varian) and Perkin–Elmer RXI FTIR spectrometers, respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. Mass spectra were recorded on Polaris Q Mass Spectrometers (ThermoFinnigan San Jose CA), with ionization being induced by electron impact at 70 eV. HRESIMS were measured using Finnigan MAT95XL-T spectrometers. ^1H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 500 Spectrometer using $CDCl_3$ as solvent unless otherwise stated. Adsorbents used for vacuum liquid chromatography (VLC) and column chromatography (CC) were silica gel Merck 7749 and Silica gel Merck 7734 or 9385, respectively. Sephadex LH-20 was used for gel permeation chromatography (GPC). For analytical and preparative TLC, Merck TLC plates, Silica gel 60 F_{254} and Merck PLC plates, Silica gel 60 F_{254} (2 mm) were, respectively, employed.

4.2. Plant material

The plant materials were collected in July 2001, from the Cameron Highlands in Pahang, Malaysia, and were botanically identified by Mr. Shamsul Khamis. A voucher specimen (SK 58/01) has been deposited at the Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia.

4.3. Extraction and isolation

The air-dried, milled leaves of *G. cantleyana* were extracted in two batches. In the first batch, 425 g of the material was macerated in MeOH for 72 h and the extraction process repeated three times. The extracts were combined and the solvent removed under reduced pressure to give 89 g of the crude methanolic extract. A portion of the extract (42 g) was then triturated with chloroform (300 ml) by continuous shaking in a chloroform suspension for 10 min followed by filtration. The procedure was repeated four times (4 × 300 ml), and the collected filtrates combined and concentrated under reduced pressure to give 17 g of the chloroform soluble fraction. The fraction was further fractionated by VLC, eluting with hexane, $CHCl_3$, EtOAc and MeOH to afford seven fractions, (A1–A7). Fraction A4 (400 mg) was further chromatographed over silica gel, eluting with 100% dichloromethane to yield deoxygaudichaudione A (9 mg). Meanwhile, fraction A6 (2.6 g) was separated by a silica gel CC, eluting with hexane–EtOAc, 95:5 to give five sub-fractions, B1–B5. Sub-fraction B2 (400 mg) was further purified on silica gel column, eluting with 10% ethyl acetate–hexane, followed by repeated preparative TLC to afford **1** (6.9 mg) and **2** (16 mg). Separation of sub-fraction B3 (155 mg) was performed by CC over silica gel, eluting with 100% dichloromethane to afford garbogiol (8 mg). Fractions A2 (500 mg) and A3 (700 mg) were each re-crystallized to yield glutin-5-en-3β-ol (16 mg) and a mixture of sitosterol and stigmasterol (29 mg), respectively.

In the second batch of extraction, 256 g of the raw material was extracted three times with chloroform, and the combined extracts were concentrated under reduced pressure to give 15 g of the crude chloroform extract. The extract was then fractionated on a Sephadex LH-20 column, eluting with 100% MeOH, yielding four fractions, C1–C4. Further purification of fraction C2 (300 mg) on Sephadex LH-20, eluting with MeOH, afforded **3** (11.6 mg). Finally, fraction C3 (5 g) was separated by CC, eluting with 1% MeOH in $CHCl_3$, to afford seven sub-fractions, D1–D7. Sub-fraction D2 (1.2 g) was further purified over Sephadex LH-20 column, eluting with MeOH to yield macranthol (7.9 mg).

The air-dried, milled trunk bark (1 kg) was macerated in chloroform for 72 h. The extraction process was repeated three times, the extracts combined and the solvent removed to yield 20 g of the crude chloroform extract. The extract was chromatographed over silica gel (250 g, 7734), eluting with 100% chloroform and finally flushed with methanol to afford seven major fractions (L–R). Further purification of fraction O (4 g) was performed by column chromatography over silica gel (110 g) with 4% MeOH in $CHCl_3$ as eluent. This afforded four combined sub-fractions which was further rechromatographed over silica gel (25 g, 9835) using 1% MeOH in $CHCl_3$ as eluent to afford **4** (8 mg), **5** (5.2 mg), and **6** (10 mg). Further separation of fraction Q (380 mg) by column chromatography over silica gel 9835 (22 g) was performed with 20% ethyl acetate in hexane as eluent to afford three combined sub-fractions Q1 (1–29), Q2 (30–45) and Q3 (46–72). Column chromatography of sub-fraction Q2 (60 mg) on silica gel using 2% ethyl acetate in dichloromethane as eluent, afforded three sub-fractions Q2.1 (1–13), Q2.2 (14–19) and Q2.3 (20–31). Sub-fraction Q2.2 (20 mg) was repeatedly chromatographed over Sephadex LH-20, eluting with MeOH to give gaudichaudione H (12 mg).

4.3.1. Cantleyanone A (*1*)

Yellow oil; $[\alpha]_D^{25} - 125^\circ$ (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 201 (3.40), 241 (3.47), 359 (3.65); IR (KBr) ν_{max} cm^{-1}: 3449, 1718, 1628, 1054; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESIMS *m/z*: 561.2839 $[M-H]^-$ (calcd. for $C_{34}H_{41}O_7$, 561.2931).

4.3.2. 7-Hydroxyforbesione (*2*)

Yellow amorphous solid, m.p. 188 °C; $[\alpha]_D^{25} - 135^\circ$ (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 350 (3.50), 347 (3.49), 241 (3.43); IR (KBr) ν_{max} cm^{-1}: 3431, 2929, 1730, 1636, 1426; For ^1H and ^{13}C NMR spectroscopic data see Table 2; HRESIMS: *m/z* 479.2118 $[M-H]^-$ (calcd. for $C_{28}H_{31}O_7$, 479.2149).

4.3.3. 4-(1,1-Dimethylprop-2-enyl)-1,3,5,8-tetrahydroxyxanthone (*3*)

Yellow crystal, m.p. 182–184 °C; UV ($CHCl_3$) λ_{max} nm (log ε): 343 (3.69), 300 (3.63), 279 (3.60), 257 (3.56); IR (KBr) ν_{max} cm^{-1}: 3435, 2964, 1629, 1497, 1401, 1284, 1205, 1158; HRESIMS *m/z*: 327.0903 $[M-H]^-$ (calcd. for $C_{18}H_{15}O_6$, 327.0947).

4.3.4. Cantleyanone B (*4*)

Yellow solid, m.p. 168 °C; $[\alpha]_D^{25} - 45^\circ$ (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 359 (3.60), 348 (3.58), 334 (3.56), 263 (3.46); IR (KBr) ν_{max} cm^{-1}: 3448, 1718, 1637, 1388, 1303 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 2; EIMS *m/z* (rel. int.): 510 $[M]^+$ (3), 482 (70), 467 (20), 413 (100), 385 (55), 353 (20), 251 (20), 245 (25), 203 (19); HRESIMS *m/z*: 509.2157 $[M-H]^-$ (calcd. for $C_{29}H_{33}O_8$: 509.2253).

4.3.5. Cantleyanone C (*5*)

Yellow solid, m.p. 153 °C; $[\alpha]_D^{25} + 40^\circ$ (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 318 (3.80), 261 (3.85), 241 (3.68); IR (KBr) ν_{max} cm^{-1}: 3433, 1716, 1637, 1364 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 2; ESIMS *m/z* (rel. int.): 507 $[M-H]^-$ (100), 462 (25), 411 (86), 397 (90), 351 (24), 245 (88), and 217 (15); HRESIMS *m/z*: 507.2004 $[M-H]^-$ (calc. for $C_{29}H_{31}O_8$: 507.2097).

4.3.6. Cantleyanone D (*6*)

Yellow amorphous solid, m.p. 175 °C; $[\alpha]_D^{25} + 122^\circ$ (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 358 (3.55), 333 (3.52); IR ν_{max}^{KBr} cm^{-1}: 3423 (–OH), 1715 (C=O), 1636 (C=O), 1333 cm^{-1}; ESIMS *m/z* (rel. int.): 509 $[M-H]^-$ (84), 464

(35), 411 (100), 396 (35), 351 (83), 245 (23), and 165 (24); HRESIMS $[M-H]^-$ m/z 509.2163 (calc. for $C_{29}H_{33}O_8$: 509.2253); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

4.3.7. Cytotoxicity

Cytotoxic activities of new compounds (**1**–**6**) together with known compounds was measured against a panel of four human cancer cell lines as described previously (Mackeen et al., 2000).

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References

Cao, S.G., Sng, V.H.L., Wu, X.H., Sim, K.Y., Tan, B.H.K., Pereira, J.T., Goh, S.H., 1998. Novel cytotoxic polyprenylated xanthonoids from *Garcinia gaudichaudii* (Guttiferae). Tetrahedron 54, 10915–10924.

Han, Q.B., Wang, Y.L., Yang, L., Tso, T.F., Qiao, C.F., Song, J.Z., Xu, L.J., Chen, S.L., Yang, D.J., Xu, H.X., 2006. Cytotoxic polyprenylated xanthones from the resin of *Garcinia hanburyi*. Chem. Pharm. Bull. 54, 265–267.

Iinuma, M., Ito, T., Miyake, R., Tosa, H., Tanaka, T., Chelladurai, V., 1998. A xanthone from *Garcinia cambogia*. Phytochemistry 47, 1169–1170.

Kouno, I., Morisaki, T., Hara, Y., Yang, C.S., 1991. Two new sesquineolignans from the bark of *Illicium dunnianum*. Chem. Pharm. Bull. 39, 2606–2608.

Leong, Y.W., Harrison, L.J., 1999. (20*R*,23*E*)-Eupha-8,23-diene-3β,25-diol from *Tripetalum cymosum*. Phytochemistry 50, 849–857.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y., Mohamed, S.M., 2000. Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. J. Ethnopharmacol. 72, 395–402.

Minami, H., Takahashi, E., Kodama, M., Fukuyama, Y., 1996. Three xanthones from *Garcinia subelliptica*. Phytochemistry 41, 629–633.

Rukachaisirikul, V., Adair, A., Dampawan, P., Taylor, W.C., Turner, P.C., 2000. Lanostanes and friedolanostanes from the pericarp of *Garcinia hombroniana*. Phytochemistry 55, 183–188.

Suksamrarn, S., Komutiban, O., Ratananukul, P., Chimnoi, N., Lartpornmatulee, N., Suksamrarn, A., 2006. Cytotoxic prenylated xanthones from the young fruit of *Garcinia mangostana*. Chem. Pharm. Bull. 54, 301–305.

Whitmore, T.C., 1983. Tree Flora of Malaya – A Manual for Foresters. Longman, Kuala Lumpur, Malaysia.

Xu, Y.J., Yip, S.C., Kosela, S., Fitri, E., Hana, M., Goh, S.H., Sim, K.Y., 2000. Novel cytotoxic, polyprenylated heptacyclic xanthonoids from Indonesian *Garcinia gaudichaudii* (Guttiferae). Org. Lett. 2, 3945–3948.

Zhang, H.-Z., Kasibhatla, S., Wang, S.Y., Herich, J., Guastella, J., Tseng, B., Drew, e.J., Cai, S.C., 2004. Discovery, characterization and SAR of gambogic acid as a potent apoptosis inducer by a HTS assay. Bioorg. Med. Chem. 12, 309–317.