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Cytotoxic Caged Tetraprenylated Xanthonoids from *Garcinia gaudichaudii* (Guttiferae)

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Abstract: Leaves of the Malaysian medicinal plant *Garcinia gaudichaudii* yielded novel and cytotoxic gaudichaudiones A-D which are pentacyclic tetra-isoprenylated xanthonoids. These compounds which exhibited significant cytotoxicity to several cancer cell lines, had their structures determined by detailed spectral analysis.

Garcinia gaudichaudii Planch. et Triana is indigenous to Indo China, Malay Peninsula and Borneo and the juice from the plant's leaves is used by the natives to rub on cuts and minor wounds.[1] In continuation of our phytochemical studies, we have examined the chloroform extract of the leaves and succeeded, by bioassay directed fractionation, in isolating four novel constituents, gaudichaudiones A-D (**1** - **4**), which showed significant cytotoxic potency. The UV, IR, MS and NMR spectral data were indicative that they were tetraprenylated xanthonoids having a complex caged structural moeity.

Compound **1** was obtained as a yellow oil with the following spectral characteristics: $[\alpha]_D$–571.7° (c = 0.12, $CHCl_3$); IR (KBr) (ν_{max} 3350, 1733, 1687, 1650 cm^{-1}; UV (EtOH) (λ_{max} 356 nm); HREIMS [M^+], 546.2626, calcd. for $C_{33}H_{38}O_7$ 546.2618; ^1H and ^{13}C NMR, Tables 1 and 2. The NMR, ^1H-^1H COSY, NOESY and HMQC spectra of **1** revealed the presence of two phenolic hydroxy groups [δ_H 12.80 (s, C1-OH), 6.57 (s, C2-OH)], one aldehyde group [δ_H 9.23 (s, H-24)], and four proton-coupled systems involving isoprenyl groups. There were two 3-methylbut-2-en-1-yl groups [δ_H 3.33 (m, H_2-11), 5.22 (tm, 7.1 and 1.2, H-12), 1.82 (s, H_3-14), 1.77 (s, H_3-15), 3.33 (m, H_2-16), 5.12 (tm, 6.6 and 1.2, H-17), 1.77 (s, H_3-19), and 1.71 (s, H_3-20], one 3-formylbut-2-en-1-yl group [δ_H 2.68 (m, H_2-21), 6.38 (tq, 7.1 and 1.3, H-22), 9.23 (s, H-24), and 1.31 (br s, H_3-25)], and a fourth isoprenyl group which formed a CH-CH_2-CH-CH=C system [δ_H 2.55 (d, 9.5, H-27), 2.36 (dd, 4.6 and 13.4, H-26), 1.41 (dd, 9.5 and 13.4, H-26), 3.53 (dd, 4.6 and 7.1, H-7), and 7.57 (d, 7.1, H-8). In the HMBC spectrum of **1**, the correlations of the chelated (C1-OH) and unchelated (C3-OH) hydroxy protons, the protons at C-11 and C-16 with the coresponding carbons indicated that one part of the molecule was a phloroglucinol-type aromatic ring with two hydroxy and two *meta*-linked isoprenyl groups. The deshielded signal at δ_H 12.80 (s, C1-OH) suggested that a carbonyl group was *peri* to the hydroxy group. These observations provided the partial structure of **1** which resembles that of gartanin.[2] The correlations of the methine proton (δ_H 2.55, H-27) to C-5, C-7, C-8a and C-10a, and of the methylene protons (δ_H 1.41 and 2.36, H_2-26 to C-6, C-7, C-8, and C-10a confirmed that another part of the structure of **1** contained a rare tricyclo-4-oxa[4.3.1.$0^{3,7}$]decane system.[3] The methylene protons (δ_H 2.68, H_2-21) of the 3-formylbut-2-en-1-yl group correlated with C-10a, C-5 and C-6, which enabled its placement at C-5. The configuration at C-22 double bond could be determined as *E* from NOESY correlations. This was confirmed by the relatively shielded aldehyde group [δ_H 9.23 and δ_C 194.5 (values of δ_H 9.0, and δ_C 190 are

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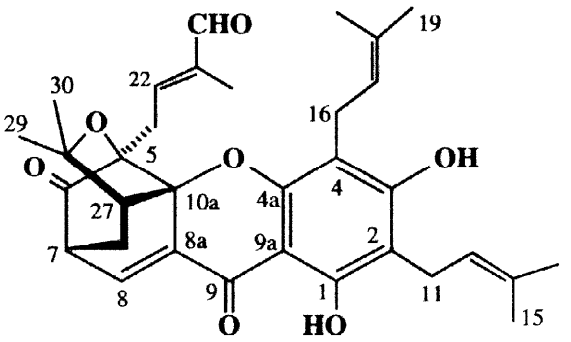
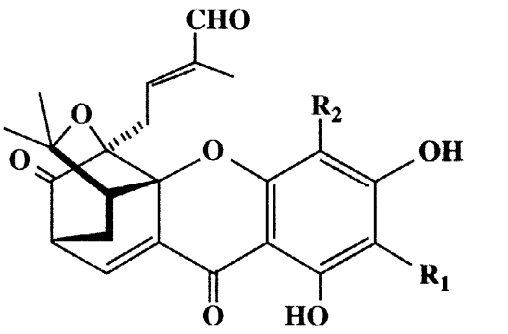
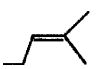
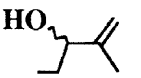
expected for a *Z* configuration)]. The chemical shifts of C-28 and C-5 which appeared at δ_C 83.9 and 83.3 respectively revealed that there was an ether linkage was between these two carbons, neither of which was linked with C-4a, otherwise they should appear at about 90 ppm, e.g. C-10a. Furthermore, if the *O*-linkage at C-5 and C-28 were hydroxy groups, their ^{13}C NMR chemical shifts (C-5 and C-28) should be close to 70 ppm.[4,5] The above spectral considerations allowed the assignment of structure **1** to gaudichaudione A.

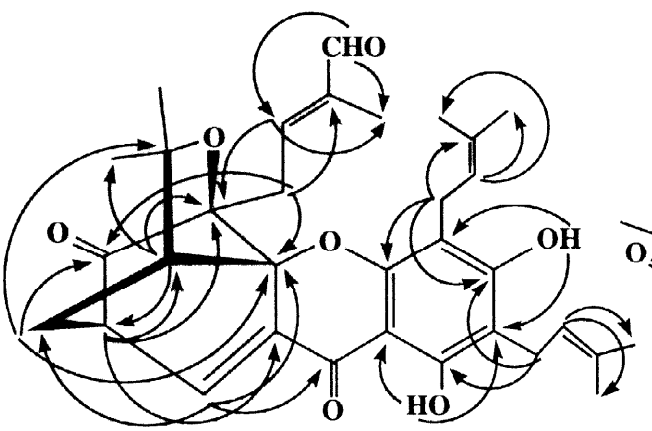


1

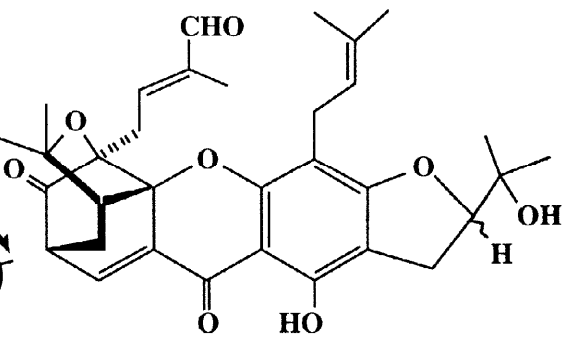


2 : R_1 =  ; R_2 = 

3 : R_1 =  ; R_2 = 



Diagnostic ^{3}J Correlations in the HMBC Spectrum of 1



4

The other isomeric compounds **2, 3** and **4** were related oxygenated derivatives of **1** because their HREIMS, [$M^{+\cdot}$] m/z 562.2558, 562.2566 and 562.2568, respectively, indicated they had the same molecular formula, $C_{33}H_{38}O_8$ (Table 3). A comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **3** with those of **1** revealed that the only differences were in the C-2 and C-4 substitutions respectively. In **2** the 2-hydroxy-3-methyl-3-butenyl group was located at C-2, whilst in **3** it was at C-4. Compound **4** differed from **1** only in the cyclised substituents at C-2 and C-3. In the HMBC spectrum of **4**, the correlations from the methylene protons (δ_H 3.13 and 3.02, H_2-11) to C-1, C-2 and C-3, and the methine protons (δ_H 4.96, H-12) to C-2 and C-3 indicated that a hydroxyisopropyldihydrofuran substituent[6] was fused linearly to the xanthonoid (C-2 and C-3). The structures of gaudichaudiones B, C and D were thus determined as **2, 3** and **4**.

Representative ED_{50}s (0.4 - 8 μg/mL) of compounds **1** and **2** to a number of cancer cell lines are given in Table 4 and are indicative of moderate to good cytotoxic activities.

Complex caged xanthanoids such as **1** - **4** as found from the present *Garcinia* plant may now be considered as a useful phytochemical characteristic of a selected group of plants from this genus, e.g. *Garcinia morella*, *G. hanburyi* and *G. forbesii* all provide various isoprenylated xanthonoids[7-14] resulting from a mixed shikimate and isoprenoid biosynthetic origin.[7,15]

Table 1. ^{13}C NMR Chemical Shifts for Compounds 1 - 4 (in $CDCl_3$)

C#	1	2	3	4	C#	1	2	3	4
1	160.5	160.9	161.5	157.8	15	25.8	18.5	25.8	25.6
2	107.9	106.9	110.9	106.1	16	22.1	22.3	29.1	22.3
3	163.7	165.4	165.1	168.4	17	121.6	122.2	78.4	121.5
4	106.3	108.4	104.3	103.7	18	134.4	132.5	146.1	132.6
4a	155.8	156.4	156.0	158.1	19	18.0	18.1	113.3	18.0
5	83.3	83.7	83.2	83.3	20	25.7	25.7	17.0	25.7
6	203.1	203.4	203.1	203.2	21	28.9	29.1	29.0	28.9
7	46.9	46.9	46.9	46.9	22	146.6	146.9	146.3	146.6
8	135.7	135.4	135.6	135.5	23	140.2	139.9	140.4	140.2
8a	133.4	133.8	133.4	133.6	24	194.5	194.8	194.4	194.5
9	180.0	178.8	178.8	179.3	25	8.6	8.7	8.7	8.7
9a	100.8	100.4	100.6	101.2	26	25.3	25.1	25.3	25.2
10a	90.6	90.5	90.8	90.9	27	48.9	49.1	49.1	49.1
11	21.2	28.4	21.5	26.7	28	83.9	84.0	83.8	84.0
12	121.2	77.5	122.1	91.9	29	29.1	28.8	28.7	29.0
13	135.7	146.6	132.5	71.9	30	29.9	29.9	30.8	30.2
14	17.9	110.5	17.9	23.9					

Table 2. ^{1}H NMR Data of Compounds 1 - 4 (in $CDCl_3$)

C#	1	2	3	4
7	3.53 (1H, dd, 4.6, 7.1)*	3.52 (1H, dd, 4.7, 6.8)	3.52 (1H, dd, 4.7, 6.9)	3.52 (1H, dd, 4.6, 6.8)
8	7.57 (1H, d, 7.1)	7.55 (1H, d, 6.8)	7.57 (1H, d, 6.9)	7.56 (1H, d, 6.8)
11	3.33 (2H, m)	2.66 (1H, dd, 9.2, 15.0)	3.32 (2H, m)	3.02 (1H, dd, 7.8, 15.4)
		3.11 (1H, dd, 1.9, 15.0)		3.13 (1H, dd, 9.5, 15.4)
12	5.22 (1H, tm, 7.1, 1.2)	4.28 (1H, m)	5.22 (1H, tm, 7.0)	4.96 (1H, dd, 7.8, 9.5)
14	1.82 (3H, s)	4.88 (1H, br s)		
		5.04 (1H, br s)	1.78 (3H, s)	1.19 (3H, s)
15	1.77 (3H, s)	1.85 (3H, s)	1.70 (3H, s)	1.32 (3H, s)
16	3.33 (2H, m)	3.24 (1H, dd, 5.4, 14.8)	2.77 (1H, dd, 10.3, 14.9)	3.24 (2H, d, 9.1)
		3.37 (1H, dd, 7.6, 14.8)	3.07 (1H, dd, 1.8, 6.9)	
17	5.12 (1H, tm, 6.6, 1.2)	5.10 (1H, m)	4.32 (1H, m)	5.15 (1H, m)
19	1.77 (3H, s)	1.74 (3H, s)	4.91 (1H, br s)	1.72 (3H, s)
			4.89 (1H, br s)	
20	1.71 (3H, s)	1.66 (3H, s)	1.86 (3H, s)	1.66 (3H, s)
21	2.68 (2H, m)	2.57 (1H, dd, 7.8, 16.2)	2.62 (2H, d, 7.0)	2.64 (1H, dd, 6.8, 16.8)
		2.76 (1H, dd, 6.7, 16.2)		2.73 (1H, dd, 7.6, 16.8)
22	6.38 (1H, tq, 7.1, 1.3)	6.45 (1H, m)	6.37 (1H, tm, 7.0)	6.39 (1H, m)
24	9.23 (1H, s)	9.15 (1H, s)	9.23 (1H, s)	9.22 (1H, s)
25	1.31 (3H, br s)	1.26 (3H, s)	1.32 (3H, s)	1.32 (3H, s)
26	1.41 (1H, dd, 9.5, 13.4)	1.43 (1H, dd, 9.4, 13.5)	1.40 (1H, dd, 9.5, 13.2)	1.41 (1H, dd, 9.5, 13.4)
	2.36 (1H, dd, 4.6, 13.4)	2.35 (1H, dd, 4.7, 13.5)	2.33 (1H, dd, 4.7, 13.2)	2.36 (1H, dd, 4.6, 13.4)
27	2.55 (1H, d, 9.5)	2.59 (1H, d, 9.4)	2.51 (1H, d, 9.5)	2.56 (1H, d, 9.4)
29	1.32 (3H, s)	1.31 (3H, s)	1.28 (3H, s)	1.32 (3H, s)
30	1.71 (3H, s)	1.74 (3H, s)	1.61 (3H, s)	1.72 (3H, s)
OH	C1-OH: 12.80	C1-OH: 12.83	C1-OH: 12.78	C1-OH: 12.56
	C3-OH: 6.57			

*Chemical shifts in ppm and in parenthesis (no. of protons, multiplicity, coupling constants in Hz)

Table 3. Summary of Selected Spectral Data of Compounds 1 - 4

	1	2	3	4
HREIMS	546.2626	562.2558	562.2566	562.2568
Mol. Formula (calcd)	$C_{33}H_{38}O_7$ (546.2618)	$C_{33}H_{38}O_8$ (562.2567)	$C_{33}H_{38}O_8$ (562.2567)	$C_{33}H_{38}O_8$ (562.2567)
UV in EtOH λ_{max} nm (log ε)	338 sh (4.23) 356 (4.28)	334 sh (4.16) 356 (4.25)	338 sh (4.11) 356 (4.17)	336 sh (4.04) 358 (4.12)
IR (KBr) ν_{max} cm^{-1}	3454, 1733 1687, 1650	3447, 1745 1691, 1640	3450,1745 1691, 1625	3474, 1741 1690, 1663
$[\alpha]_D$	-517.7 ° c = 0.120, $CHCl_3$	-446.2 ° c = 0.676, $CHCl_3$	-438.5 ° c = 0.203, $CHCl_3$	-335.8 ° c = 0.522, $CHCl_3$

Table 4. Cytotoxicity Data for compounds 1 and 2

Compound	P388	WEH164	MOLT4	HePG2	LL/2
1	0.42	2.85	1.30	8.0	0.50
2	0.51	0.42	0.38	1	3.5

P388 = mouse leukemia, WEHI1640 = mouse fibrosarcoma, THP-1 = human monocytic leukemia, MOLT4 = lymphoblastic leukemia, human, HePG2 = human hepatocellular carcinoma, LL/2 = mouse Lewis lung carcinoma

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