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Griffipavixanthone, a Novel Cytotoxic Bixanthone from *Garcinia griffithii* and *G. pavifolia*

Yuan-Jian Xu,[a] Shu-Geng Cao,[a] Xiao-Hua Wu,[ab] Yee-Hing Lai,[a] B. H. K. Tan,[b] J. T. Pereira,[c] S. H. Goh,[a]* Ganapathi Venkatraman,[a] Leslie J. Harrison,[a] and Keng-Yeow Sim[a]

Departments of Chemistry[a] and Pharmacology,[b] National University of Singapore
Kent Ridge Crescent, Singapore 119260
Forest Research Centre,[c] Sepilok, Sandakan, Sabah, Malaysia

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Abstract: A bioactivity-directed fractionation of the extracts of the Malaysian plant *Garcinia pavifolia* and a phytochemical study of *G. griffithii* led to the discovery of griffipavixanthone (**1**), a novel cytotoxic bixanthone with cyclized prenyl groups providing the xanthone-xanthone linkage. Spectroscopic data and preparation of methylated derivatives allowed for the complete structural elucidation of **1**.

It is well known that *Garcinia* species are rich in secondary metabolites especially xanthonoids,[1] biflavonoids,[2] and triterpenoids.[3] Our continuing phytochemical study of Southeast Asian plants as a source of bioactive compounds led to the isolation of a novel bixanthone, griffipavixanthone (**1**),[4] from the bark of *Garcinia griffithi* and *G. pavifolia*. **1** was an unique bixanthone with one xanthonoid linked to the other by putative tandem cyclization involving prenyl groups. The structure of **1** was elucidated by modern spectroscopic methods.

Griffipavixanthone (**1**) showed high *in vitro* cytotoxity against P388, LL/2 and Wehi164 cell lines with ED_{50} = 3.40, 6.80 and 4.60 μg/ml, respectively. The molecular formula $C_{36}H_{28}O_{12}$ was deduced from FABMS of **1** and HREIMS of the octamethylated derivative **2**. ^{13}C NMR and HMQC spectra showed resonances for 23 quaternary, nine CH, one CH_2 and three methyl carbons. HMBC spectral correlations established the connectivities for H-2–C(1, 3, 4, and 9a), H-4–C(2, 3, 4a, and 9a), H-2'–C(1', 3', 4', and 9a'), and H-4'–C(2', 3', 4a', and 9a'). The deshielded phenolic signals at δ_H 13.62 and δ_H 13.48 (^1H NMR spectrum, 300 MHz, acetone-d_6) suggested that two carbonyl groups were *peri* to these signals. Hence, the basic xanthonoid moieties of A/C and A'/C' can be drawn as shown in the figure.

In the HMBC spectrum of **1**, the methylene protons (δ_H 2.55 and 1.56/δ_C 41.3) correlated with C-12 (δ_C 62.7), C-13 (δ_C 34.0), C-15 (δ_C 132.5), and C-16 (δ_C 123.3) (see Figure). The olefinic proton (δ_H 5.45/δ_C 123.3) caused five crosspeaks with C-8 (δ_C 139.2), C-12 (δ_C 62.7), C-14 (δ_C 41.3), C-17 (δ_C 46.5), and C-20 (δ_C 24.4). Data from ^2J and ^3J correlations of the methine protons (δ_H 6.58/δ_C 43.2, C-11; δ_H 2.60/δ_C 62.7, C-12; δ_H 4.80/δ_C 46.5, C-17) with their neighbouring carbons (Figure) allowed for the construction of a cyclohexene ring (E) fused to a five-membered ring (D) adjoining the xanthonoid ring B. Further HMBC correlations of H-11 to C-8', C-8a' and C-7' extended the connectivity to ring

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B'. The aromatic proton of ring B' (δ_H 6.81/δ_C 114.2, C–7') correlated with C-11, C-8a', C–5' and C-6', verifying the linkage of rings D and B'. The ^{13}C NMR chemical shifts of the six carbons in ring B' matched those of caloxanthone B,[5] which confirmed our deductions. Furthermore, C-7 (δ_C 144.2), C-8 (δ_C 139.4) and C-8a (δ_C 110.1) together with the remaining three unassigned carbon chemical shifts at δ_C 132.0, 147.6, and 150.2 suggested the presence of fully substituted ring B (see Figure). The two quaternary carbons at the B/D ring junction appeared at relatively low field, resembling those of 1-(3'-hydroxy-4'-methylphenyl)-1,3,3,6-tetramethylindan-5-ol.[6] For the selective INEPT experiments[7] two methine protons H-11 and H-17 were selected. For the observation of the three bond and two bond C-H couplings for the aromatic carbon with the aliphatic methine protons, the optimum values for the delays Δ_1 and Δ_2 for $J = 4$ Hz and $J = 7$ Hz were determined. Thus for $J = 4$ Hz, irradiation of the methine proton at δ_H 4.80 (H-17) enhanced the carbon resonance at δ_C 110.1 (C-8a), whereas the irradiation of the methine proton at δ_H 6.58 (H-11) resulted in the enhancement of the signal at δ_C 139.4 (C-8). For $J = 7$ Hz, irradiation of the methine proton at δ_H 6.58 (H-11) led to the enhancement of the signal at δ_C 144.2 (C-7) and irradiation of the methine proton at δ_H 4.80 (H-17) resulted in the enhancement of the

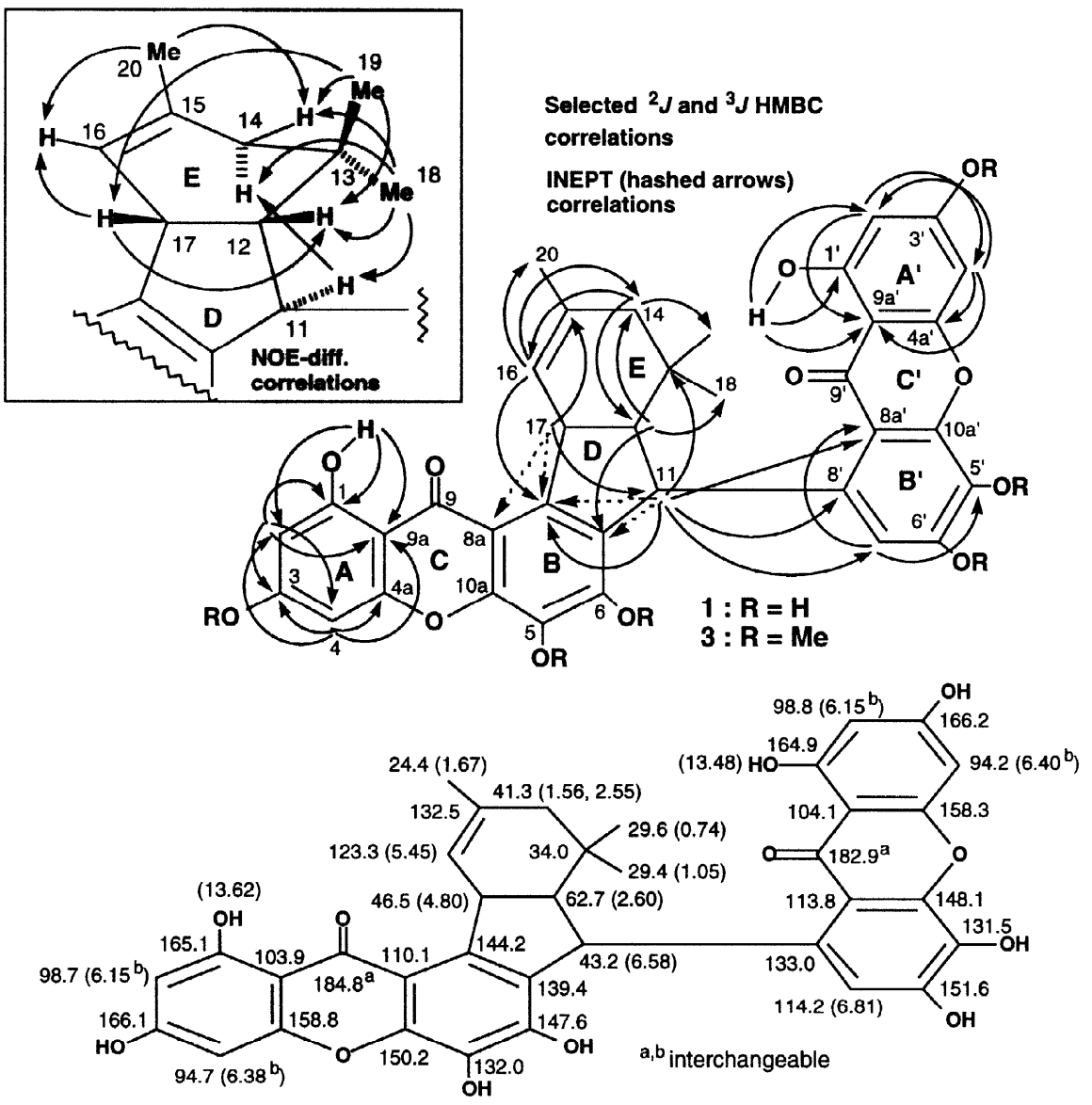


Figure. Selected HMBC, INEPT and NOE-diff. correlations and δ-assignments of **1**

signal at δ_C 139.4 (C-8). These results showed that ring D was fused to ring B at C-7/C-8 and the partial structure containing rings B', D and E could be extended to ring B.

The relative stereochemistry of optically active **1** was determined by NOE difference enhancements as shown. The *cis*-junction between rings D and E was determined from NOE-difference spectra. When H-17 was irradiated an enhancement of the signal of H-12 was observed. H-11 was *trans* to H-12 since the signal of H-12 was not enhanced when H-11 was saturated.

Compound **1** on methylation with methyl iodide gave an octamethyl ether **2** but with diazomethane gave a hexamethyl ether, **3**. The ^{1}H NMR spectrum of **3** showed signals for six methoxyl groups at δ_H 3.30, 3.81, 3.85, 3.89, 3.91, 3.98 (each 3H, s) and two chelated hydroxyl protons, indicating the presence of eight hydroxyl groups in **1**. The appearance of the *meta*-coupled protons in the ^{1}H NMR spectrum of **3** indicated that rings A and A' were phloroglucinol-type aromatic rings which were confirmed by HMBC spectra described above. This was also supported by the NOE enhancement observed between the methoxyl groups at C-3 with H-2 and H-4 and at C-3' with H-2' and H-4'.

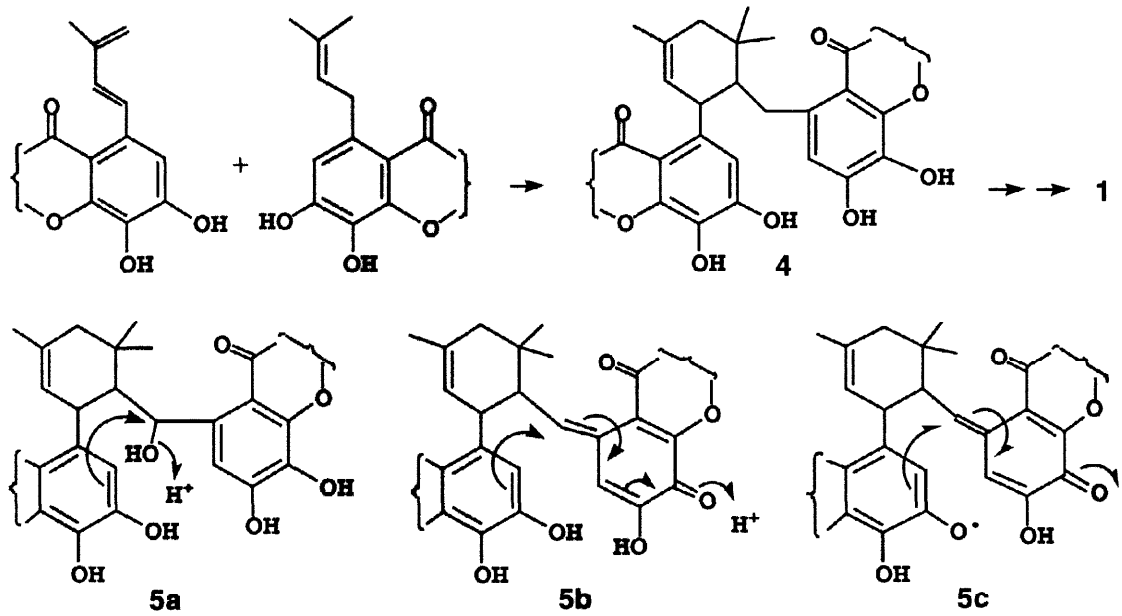
Table: ^{1}H, ^{13}C and HMBC data of compound **1** and ^{1}H NMR data of **3**

#	δ_H (**1**)[a]	δ_C (**1**)[a]	HMBC (**1**)[b]	δ_H (**3**)[c]
1-OH	13.62 (1H, s)	165.1		13.48 (1H, s)
2	6.15 (1H, m)	98.7	C1,C3,C4,C9a	6.35 (1H, m)
3		166.1		3.89 (3H, s, OMe)
4	6.38[d](1H, d, *J*=1.5)	94.7	C2,C3,C4a,C9a	6.46 (1H, d, *J*=2.3)
4a		158.8		
5		132.0		3.81 (3H, s, OMe)
6		147.6		3.99 (3H, s, OMe)
7		144.2		
8		139.4		
8a		110.1		
9		184.8[e]		
9a		103.9		
10a		158.8		
11	6.58 (1H, d, *J*=9.7)	43.2	C6,C7,C8,C12,C13,C17,C7',C8',C8a'	6.79 (1H, d, *J*=9.8)
12	2.60 (1H, m)	62.7	C7,C11,C13,C14,C16,C17,C18,C19	
13		34.0		
14	1.56 (1H, d, *J*=16.7)	41.3	C13,C15,C16,C18,C19,C20	
	2.55 (1H, d, *J*=16.7)			
15		132.5		
16	5.45 (1H, s)	123.3	C8,C12,C14,C17,C20	
17	4.80 (1H, br)	46.5	C8,C11,C15,C16	4.92 (1H, br)
18	0.74 (3H, s)	29.6	C12,C13,C14	0.69 (3H, s)
19	1.05 (3H, s)	29.4	C12,C13,C14	1.07 (3H, s)
20	1.67 (3H, s)	24.2	C14,C15,C16	1.69 (3H, s)
1'-OH	13.48 (1H, s)	164.9		13.20 (1H, s)
2'	6.15 (1H, m)	98.8	C1',C3',C4',C9a'	6.35 (1H, m)
3'		166.2		3.90 (3H, s , OMe)
4'	6.40[d](1H, d,*J*=1.5)	94.2	C2',C3',C4a',C9a'	6.50 (1H, d, *J*=2.3)
4'a		158.3		
5'		131.5		3.29 (3H, s, OMe)
6'		151.6		3.84 (3H, s, OMe)
7'	6.81	114.2	C11,C5',C6'C8a'	6.71 (1H, s)
8'		133.0		
8'a		113.8		
9'		182.9[e]		
9'a		104.1		
10'a		158.3		

[a] 300 MHz, acetone-d_6; [b,c] 500 MHz, acetone-d_6; [d,e] δ values with same superscripts are interchangeable.

Irradiation of the methoxyl protons at C-5 resulted in the enhancement of the methoxyl signal at C-6 and *vice versa* indicating that they were *ortho* to each other. Similarly, it was determined that MeO at C-6' was flanked by the methoxyl group at C-5' and the proton at C-7'. These results indicated that **1** was composed of two xanthone nuclei which were 1,3,5,6-tetraoxygenated. The structure of the bixanthone was assigned as shown and the NMR data are given in the Table.

Bixanthone (**1**) is the first example in which the two xanthone nuclei are linked *via* 5- and 6-membered rings from a double cyclization involving two prenyl groups. A less likely possibility involves a tandem radical cyclization to form a 5-membered ring followed by a 6-membered one. However, simple linkages *via* 6-membered rings formed by formal Diels-Alder of prenyl groups are known, e.g. garcilivins A-C.[8] The biosynthesis of **1** can be envisaged as arising from an initial Diels-Alder reaction of the prenyl groups of two xanthones to provide a cyclohexene derivative (**4**) and this is followed by another cyclization, ionic (**5a** or **5b**) or radical (**5c**), to form a fused 5-membered ring as shown below.



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4. Griffipavixanthone (**1**): yellow powder; $[\alpha]_D = +162.8$ (MeOH, *c* 0.13); m.p. >275 °C; UV λ_{max} nm (log ε) (MeOH) 226 (sh), 254 (4.80), 282 (sh), and 330 (4.58); IR (KBr) ν_{max} cm^{-1} 3393 (br), 3185, 2970, 2923, 1654, 1648, 1606, 1578, 1517, 1456, 1329, 1288, 1160, 1100, 1019, 972, and 831; FAB MS $[M+1]^+$ 653 ($C_{36}H_{28}O_{12}$); octamethyl derivative **2**, HREIMS 764.28293 (calcd. 764.28326); ^1H and ^{13}C NMR spectra of **1** and **3** are given in the Table.
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