New Biphenyl Constituents from *Garcinia oblongifolia*

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Four new biphenyl derivatives, oblongifoliagarcinines A–D (**1**–**4**), were isolated on the chemical investigation of the stems and leaves of *Garcinia oblongifolia*. The structures of **1**–**4** were established on the basis of 1D- and 2D-NMR and other spectroscopic analyses.

Introduction. – Plants of the genus *Garcinia*, widely distributed in tropical Africa, Asia, New Caledonia, and Polynesia, have yielded an abundance of biologically active and structurally intriguing natural products [1][2]. *Garcinia oblongifolia* is a medium-sized shrub found in the south of China and north of Vietnam. It was used to treat burns [3] and showed anti-inflammatory activity [4]. In a previous study, four new polyprenylated benzoylphloroglucinol derivatives, which were only very weak inhibitors of tubulin assembly, have been isolated from this plant [5]. To the best of our knowledge, only a few prenylated biphenyl natural compounds have been found in the plant kingdom [6–17], showing bioactivities such as DNA-strand scission [6], cytotoxicity [6–8], antibacterial activity [9–11], and neurite outgrowth-promoting activity [12]. Our careful chemical investigation of this plant collected from Guangxi Province of China led to the isolation of four new prenylated biphenyl compounds, named oblongifoliagarcinines A–D (**1**–**4**; *Fig. 1*); their structures were determined on the basis of 1D- and 2D-NMR analyses and other spectroscopic methods.

Results and Discussion. – Oblongifoliagarcinine A (**1**) was obtained as a pale brown powder and had the molecular formula $C_{17}H_{16}O_3$, as inferred from the HR-EI-MS showing the molecular-ion peak at *m/z* 268.1116 (M^+), indicating ten degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 3385 cm^{-1} for free OH groups, 2972 cm^{-1} for aromatic C–H moieties, 1215 cm^{-1} for C–O groups, and 835 cm^{-1} for a 1,2,3,5-tetrasubstituted benzene moiety [18]. The ^{1}H- and ^{13}C-NMR (*Table 1*), EI-MS, and HMBC data (*Table 1* and *Fig. 2*) allowed to determine the structure of oblongifoliagarnine A (**1**) as 6-(4-hydroxyphenyl)-2,2-dimethyl-2*H*-1-benzopyran-8-ol, which is a new biphenyl derivative.

The ^{1}H-NMR spectrum of **1**[1]) showed characteristics of a chromene ring, *i.e.*, of the olefinic H-atoms H–C(1′) (δ(H) 6.37 (*d*, $J=9.8$)) and H–C(2′) (δ(H) 5.65 (*d*, $J=9.8$)), and of a pair of magnetically

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[1]) Arbitrary atom numbering.

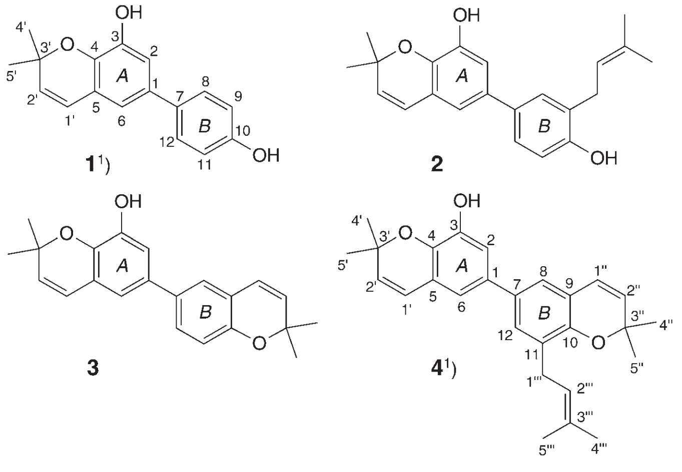

Fig. 1. *Compounds* **1**–**4**¹), *isolated from* G. oblongifolia

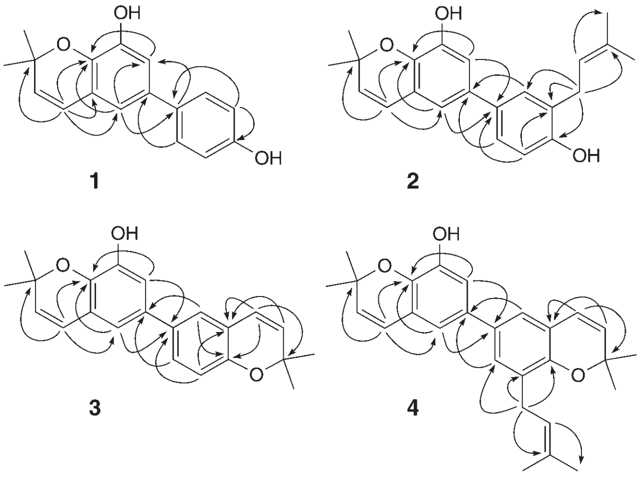

Fig. 2. *Selected HMBC correlation*s *of* **1**–**4**

equivalent Me groups (δ(H) 1.48 (*s*, Me(4′,5′))). The base peak at *m/z* 253 ($[M - \mathrm{Me}]^+$) in the EI-MS and a set of signals at δ(C) 28.0 (Me(4′,5′)), 77.3 (C(3′)), 130.9 (C(2′)), and 122.3 (C(1′)) in the ^{13}C-NMR spectrum provided further support for the presence of a geminally dimethyl-substituted chromene system. The remaining unsaturation degrees suggested the presence of a typical biphenyl unit which was supported by the remaining 12 aromatic C-atom signals in the ^{13}C-NMR spectrum. The existence of two phenolic OH groups was confirmed by the signals of two exchangeable H-atoms in the ^{1}H-NMR (δ(H) 5.48 and 5.05) and two oxygenated aromatic C-atoms in the ^{13}C-NMR spectrum (δ(C) 144.5 (C(3)) and 154.7 (C(10))). The ^{1}H-NMR spectrum showed a set of *d*s of *ortho*-coupled H-atoms at δ(H) 7.40 (*d*, *J* = 8.6, H–C(8,12)) and 6.86 (*d*, *J* = 8.6, H–C(9,11)), typical for a *para*-substituted aryl moiety. Therefore, one OH group was assigned to C(10). A set of *d*s of *meta*-coupled H-atoms at δ(H) 7.00 (*d*, *J* = 2.0, H–C(2)) and 6.75 (*d*, *J* = 2.0, H–C(6)) belonged to a 1,2,3,5-tetrasubstituted benzene moiety (ring *A*). A HMBC experiment allowed to position the substituents at ring *A*. The H–C(1′) signal at δ(H) 6.37

Table 1. *^{1}H- and ^{13}C-NMR and HMBC Data of Compound* **1**1) *in $CDCl_3$*

	δ(H)a)	δ(C)	HMBC
C(1)		133.9	
H−C(2)	7.00 (*d*, $J = 2.0$)	113.3	C(3), C(4), C(6), C(7)
C(3)		144.5	
C(4)		138.3	
C(5)		121.3	
H−C(6)	6.75 (*d*, $J = 2.0$)	116.1	C(2), C(4), C(5), C(7)
C(7)		133.6	
H−C(8,12)	7.40 (*d*, $J = 8.6$)	127.9	C(1), C(10), C(12,8), C(7)
H−C(9,11)	6.86 (*d*, $J = 8.6$)	115.5	C(7), C(11,9)
C(10)		154.7	
H−C(1′)	6.37 (*d*, $J = 9.8$)	122.3	C(3′), C(4), C(5), C(6)
H−C(2′)	5.65 (*d*, $J = 9.8$)	130.9	C(3′), C(5)
C(3′)		77.3	
Me(4′,5′)	1.48 (*s*)	28.0	
OH−C(3)	5.48 (br. *s*)		
OH−C(10)	5.05 (br. *s*)		

a) Measured at 300 MHz.

showed a two-bond connectivity with C(5) (δ(C) 121.3), and a three-bond connectivity with C(4) (δ(C) 138.3) and C(6) (δ(C) 116.1) in the HMBC plot (*Fig. 2*). H−C(2) at δ(H) 7.00 showed a two-bond connectivity with C(3) (δ(C) 144.5), and a three-bond connectivity with C(4) (δ(C) 138.3), C(6) (δ(C) 116.1), and C(7) (δ(C) 133.6), which suggested that the second OH group was attached to C(3). Further support for the determination of the structure was provided by the signal of H−C(6) at δ(H) 6.75 showing a two-bond connectivity with C(5) (δ(C) 121.3), and a three-bond connectivity with C(2) (δ(C) 113.3), C(4) (δ(C) 138.3), and C(7) (δ(C) 133.6) in the HMBC plot.

Oblongifoliagarcinine B (**2**) was obtained as a yellow oil. The HR-EI-MS suggested the molecular formula $C_{22}H_{24}O_3$ (M^+ at *m/z* 336.1725). The ^{1}H- and ^{13}C-NMR (*Table 2*) and HMBC data (*Fig. 2*) established the structure of oblongifoliagarcinine B (**2**) as 6-[4-hydroxy-3-(3-methylbut-2-en-1-yl)phenyl]-2,2-dimethyl-2*H*-1-benzopyran-8-ol.

In the ^{1}H- and ^{13}C-NMR of **2**1), signals due to a prenyl group attached to an aromatic C-atom were found, *i.e.*, CH_2(1″) (δ(H) 3.40 (*d*, $J = 7.2$); δ(C) 29.9), H−C(2″) (δ(H) 5.36 (*t*, $J = 7.2$); δ(C) 121.7), C(3″) (δ(C) 134.8), Me(4″) (δ(H) 1.79 (*s*); δ(C) 25.8), and Me(5″) (δ(H) 1.80 (*s*); δ(C) 17.9). Comparison with the ^{1}H- and ^{13}C-NMR spectra of **1** suggested that **2** was also a biphenyl compound but with an additional prenyl group. The ^{1}H- and ^{13}C-NMR signals of **2** for a chromene ring with H−C(1′) (δ(H) 6.38 (*d*, $J = 9.8$); δ(C) 122.3), H−C(2′) (δ(H) 5.65 (*d*, $J = 9.8$); δ(C) 130.9), C(3′) (δ(C) 77.3), Me(4′,5′)) (δ(H) 1.48 (*s*); δ(C) 28.0), H−C(2) δ(H) 7.01 (*d*, $J = 2.2$), and H−C(6) (δ(H) 6.76 (*d*, $J = 2.2$)), which were almost identical with those of **1**, indicated the presence of a similarly substituted ring *A*. In the aromatic region, the appearance of an *ABX* spin system at δ(H) 7.29 (*d*, $J = 2.4$, H−C(8)), 7.27 (*dd*, $J = 8.8$, 2.4, H−C(12)), and 6.83 (*d*, $J = 8.8$, H−C(11)) suggested that the prenyl group is attached to ring *B*. The prenyl group was located at C(9) on the basis of key HMBC data (*Fig. 2*), *i.e.*, by the correlations CH_2(1″) (δ(H) 340)/C(9) (δ(C) 127.0), C(8) (δ(C) 128.3), and C(10) (δ(C) 153.5).

Oblongifoliagarcinine C (**3**) gave a molecular-ion peak at *m/z* 334.1566 in its HR-EI-MS, corresponding to the molecular formula $C_{22}H_{22}O_3$. The ^{1}H- and ^{13}C-NMR

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **2**–**4**[1]) *in* $CDCl_3$

	2		**3**		**4**	
	δ(H)[a])	δ(C)	δ(H)[a])	δ(C)	δ(H)[a])	δ(C)
C(1)		134.1		134.0		134.4
H−C(2)	7.01 (*d*, *J* = 2.2)	113.4	7.00 (*d*, *J* = 2.1)	113.3	7.00 (*s*)	113.4
C(3)		144.5		144.6		144.5
C(4)		138.2		138.3		138.2
C(5)		121.2		121.2		121.2
H−C(6)	6.76 (*d*, *J* = 2.2)	116.1	6.75 (*d*, *J* = 2.1)	116.0	6.75 (*d*, *J* = 2.0)	116.0
C(7)		133.5		133.5		132.9
H−C(8)	7.29 (*d*, *J* = 2.4)	128.3	7.29 (*d*, *J* = 2.2)	127.3	7.00 (*d*, *J* = 2.0)	122.4
C(9)		127.0		121.3		121.0
C(10)		153.5		152.2		149.8
H−C(11) or C(11)	6.83 (*d*, *J* = 8.8)	115.9	6.80 (*d*, *J* = 8.3)	116.4		129.3
H−C(12)	7.27 (*dd*, *J* = 8.8, 2.4)	125.7	7.27 (*d*, *J* = 8.3)	124.5	7.15 (*d*, *J* = 2.0)	127.7
H−C(1′)	6.38 (*d*, *J* = 9.8)	122.3	6.37 (*d*, *J* = 10.0)	122.3	6.38 (*d*, *J* = 9.8)	122.4
H−C(2′)	5.65 (*d*, *J* = 9.8)	130.9	5.65 (*d*, *J* = 10.0)	130.9	5.64 (*d*, *J* = 9.8)	130.8
C(3′)		77.3		77.3		77.3
Me(4′,5′)	1.48 (*s*)	28.0	1.48 (*s*)	28.1	1.48 (*s*)	28.0
CH_2(1″) or H−C(1″)	3.40 (*d*, *J* = 7.2)	29.9	6.36 (*d*, *J* = 9.8)	122.4	6.35 (*d*, *J* = 9.8)	122.7
H−C(2″)	5.36 (*t*, *J* = 7.2)	121.7	5.64 (*t*, *J* = 9.8)	131.6	5.63 (*d*, *J* = 9.8)	130.7
C(3″)		134.8		76.3		76.1
Me(4″)	1.79 (*s*)	25.8	1.45 (*s*)	28.1	1.45 (*s*)	28.0
Me(5″)	1.80 (*s*)	17.9	1.45 (*s*)	28.1	1.45 (*s*)	28.0
CH_2(1‴)					3.31 (*d*, *J* = 7.5)	28.4
H−C(2‴)					5.32 (*t*, *J* = 7.5)	122.8
C(3‴)						131.9
Me(4‴)					1.73 (*s*)	25.8
Me(5‴)					1.75 (*s*)	17.9
OH−C(3)	5.49 (br. *s*)		5.47 (br. *s*)		5.45 (br. *s*)	
OH−C(10)	5.22 (br. *s*)					

[a]) Measured at 300 MHz.

spectra of **3**[1]) (*Table 2*) were similar to those of compound **2**, except for the prenyl functionality at C(9) in **2**. Signals for a further geminally dimethyl-substituted chromene system were present in the ^{1}H- and ^{13}C-NMR spectra of **3** (H−C(1″) (δ(H) 6.36 (*d*, *J* = 9.8); δ(C) 122.4), H−C(2″) (δ(H) 5.64 (*d*, *J* = 9.8); δ(C) 131.6), C(3″) (δ(C) 76.3), and Me(4″,5″)) (δ(H) 1.45; δ(C) 28.1)). These results suggested that compound **3** was formed by cyclization of the prenyl group in **2** with an adjacent OH group. Accordingly, oblongifoliagarcinine C (**3**) was assigned to be 2,2,2′,2′-tetramethyl[6,6′-bi-2*H*-1-benzopyran]-8-ol.

Oblongifoliagarcinine D (**4**) was shown to have the molecular formula $C_{27}H_{30}O_3$ from its HR-EI-MS (M^+ at *m*/*z* 402.2188). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** (*Table 2*) with those of **3** suggested that **4** was similar to **3** but with one more prenyl group. This was confirmed by the HMBC data (*Fig. 2*), and the structure of oblongifoliagarcinine D (**4**) was established as 2,2,2′,2′-tetramethyl-8′-(3-methylbut-2-en-1-yl)[6,6′-bi-2*H*-1-benzopyran]-8-ol.

The prenyl group of **4** consisting of $CH_2(1''')$ (δ(H) 3.31 (d, $J = 7.5$); δ(C) 28.4), H–C(2''') (δ(H) 5.32 (t, $J = 7.5$); δ(C) 122.8), C(3''') (δ(C) 131.9), Me(4''') (δ(H) 1.73 (s); δ(C) 25.8), and Me(5''') (δ(H) 1.75 (s); δ(C) 17.9) was attached to C(11) of ring *B*, as suggested by the set of *d*s of *meta*-coupled H-atoms at δ(H) 7.15 (d, $J = 2.0$, H–C(12)) and 7.00 (d, $J = 2.0$, H–C(8)) instead of an *ABX* spin system in **3**. This result was further supported by the key HMBC data (*Fig. 2*), *i.e.*, by the correlations $CH_2(1''')$ (δ(H) 3.31)/C(11) (δ(C) 129.3), C(12) (δ(C) 127.7), and C(10) (δ(C) 149.8).

The cytotoxic activities of the new compounds **1**–**4** against the growth of tumor cell lines A549 (human lung adenocarcinoma) and HL-60 (human leukemia) were evaluated. Unfortunately, all tested compounds were inactive *in vitro* against these cancer cells.

Experimental Part

General. Column chromatography (CC): commercial silica gel (*Qingdao Marine Chemical Industrials*; 200–300 and 300–400 mesh) and MCI gel *CHP20P*, 75–150 µm (*Mitsubishi Chemical Industries Ltd.*). TLC: precoated silica gel *GF254* plates (*Yantai Chemical Industrials*). UV Spectra: *Hewlett-Packard 8452A* diode array spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna-FT-IR-750* spectrophotometer; in cm^{-1}. NMR Spectra (^1H, ^{13}C, HSQC, and HMBC): *Bruker DRX-300* or *AM-400* spectrometer; chemical shifts δ in ppm, with $SiMe_4$ as internal standard, and coupling constants J in Hz. EI-MS and HR-EI-MS: *Finnigan MAT-95* mass spectrometer; in m/z (rel. %).

Plant Material. The stems and leaves of *Garcinia oblongifolia* (3.1 kg) were collected in Guangxi Province, P. R. China, in August 2005, and identified by Prof. *Jin-Gui Shen* of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM). A voucher specimen is deposited at the herbarium of the SIMM.

Extraction and Isolation. The air-dried stems and leaves of *G. oblongifolia* (3.5 kg) were ground into powder and extracted with 95% EtOH (3 ×). The conc. EtOH extract was suspended in H_2O and partitioned successively with AcOEt and BuOH. The AcOEt extract (130 g) was subjected to CC (SiO_2), petroleum ether/AcOEt 95:5 → 50:50): *Fr. A–L. Fr. D* (1.430 g) was subsequently subjected to CC (SiO_2 (small column), petroleum ether/AcOEt 95:5 → 75:25): *Fr. D.1–D.3. Fr. D.2* was separated by CC (MCI gel, Me_2CO/H_2O 60:40 → 80:20; then *Sephadex LH-20*, MeOH: **4** (13 mg). Similarly, **3** (5 mg) was purified from *Fr. E* (1.100 g), **2** (13 mg) from *Fr. H* (4.878 g), and **1** (14 mg) from *Fr. I* (1.523 g) by CC (SiO_2, petroleum ether/AcOEt 95:5 → 75:25; MCI gel, Me_2CO/H_2O 60:40 → 80:20; *Sephadex LH-20*, MeOH).

Oblongifoliagarcinine A (=*6-(4-Hydroxyphenyl)-2,2-dimethyl-2*H*-1-benzopyran-8-ol*; **1**): Pale brown power. UV (MeOH): 258 (3.1). IR (KBr): 3385, 2972, 1610, 1585, 1481, 1379, 1254, 1215, 1128, 835. ^1H- and ^{13}C-NMR: *Table 1*. EI-MS: 268 (46, M^+), 253 (100), 235 (6), 207 (4), 127 (15), 119 (6). HR-EI-MS: 268.1116 ($C_{17}H_{16}O_3^+$; calc. 268.1094).

Oblongifoliagarcinine B (=*6-[4-Hydroxy-3-(3-methylbut-2-en-1-yl)phenyl]-2,2-dimethyl-2*H*-1-benzopyran-8-ol*; **2**): Yellow oil. UV (MeOH): 250 (3.2). IR (film): 3423, 2974, 2926, 1587, 1483, 1379, 1254, 1198, 1126, 1065, 760. ^1H- and ^{13}C-NMR: *Table 2*. EI-MS: 336 (59, M^+), 321 (100), 319 (15), 303 (5), 265 (11), 189 (4), 153 (6), 133 (6), 119 (10), 69 (5). HR-EI-MS: 336.1725 ($C_{22}H_{24}O_3^+$; calc. 336.1720).

Oblongifoliagarcinine C (=*2,2,2',2'-Tetramethyl[6,6'-bi-2*H*-1-benzopyran]-8-ol*; **3**): Yellow oil. UV (MeOH): 259 (3.0). IR (KBr): 3431, 2974, 1637, 1589, 1479, 1361, 1257, 1205, 1126, 952, 719. ^1H- and ^{13}C-NMR: *Table 2*. EI-MS: 334 (36, M^+), 319 (100), 289 (5), 152 (26), 83 (7), 57 (7). HR-EI-MS: 334.1566 ($C_{22}H_{22}O_3^+$; calc. 334.1563).

Oblongifoliagarcinine D (=*2,2,2',2'-Tetramethyl-8'-(3-methylbut-2-en-1-yl)[6,6'-bi-2*H*-1-benzopyran]-8-ol*; **4**): Yellow oil. UV (MeOH): 254(3.1). IR (film): 3552, 2974, 2924, 1718, 1587, 1460, 1375, 1257, 1205, 1126, 950, 838, 725. ^1H- and ^{13}C-NMR: *Table 2*. EI-MS: 402 (48, M^+), 387 (100), 357 (3), 333 (3), 279 (5), 186 (24), 167 (11), 149 (49), 125 (7), 111 (10), 97 (14), 83 (14), 71 (21), 57 (30). HR-EI-MS: 402.2188 ($C_{27}H_{30}O_3^+$; calc. 402.2189).

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