Sesquiterpenoids from *Bombax malabaricum*

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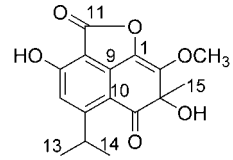
Phytochemical investigation of the chemical constituents of the roots of *Bombax malabaricum* afforded nine cadinane sesquiterpenoids, including five new compounds (bombamalones A–D, **1**–**4**; bombamaloside, **5**), and four known compounds (isohemigossypol-1-methyl ester, **6**; 2-*O*-methylisohemigossylic acid lactone, **7**; bombaxquinone B, **8**; and lacinilene C, **9**). The structures of **1**–**5** were identified by spectroscopic methods and comparison with literature values. Compounds **1**–**9** were evaluated against the HGC-27 human gastrointestinal cancer cell line, but all were inactive (IC_{50} >10 μM).

Bombax malabaricum DC (syn. *Salmalia malabaricum* DC) (Bombacaceae),[1] a medium-sized deciduous tree, is distributed widely in tropical areas of mainland China and India, and it is used as a folk medicine because of its demulcent, diuretic, restorative, aphrodisiac, and emetic properties.[2–4] Cadinane sesquiterpenoids are the most representative constituents of this family, and their antifungal activity was reported previously.[2,4–6] Recent investigations on other families have reported cadinane sesquiterpenoids possessing cytotoxicity[7] (Sterculiaceae) and anti-HIV activity[8] (Aristolochiaceae), which stimulated the present study on the cadinane sesquiterpenoids from *B. malabaricum*.

The air-dried roots of *B. malabaricum* were extracted with H_2O/acetone (3:7). Separation and purification of this extract by repetitive chromatography led to the isolation and characterization of five new sesquiterpenoids, named bombamalones A–D (**1**–**4**) and bombamaloside (**5**), along with four known compounds, isohemigossypol-1-methyl ester (**6**),[5] 2-*O*-methylisohemigossylic acid lactone (**7**),[6] bombaxquinone B (**8**),[2] and lacinilene C (**9**).[9,10]

Compound **1** was obtained as a yellow amorphous powder and was shown to possess a molecular formula of $C_{16}H_{16}O_6$ by HRESIMS. The IR spectrum of **1** indicated the presence of hydroxyl (3431 cm^{-1}), carbonyl (1751 cm^{-1}), lactone (1699 cm^{-1}), and olefinic (1612 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) showed an aromatic methyl group at δ_H 1.51 (3H, s), a singlet aromatic proton at δ_H 7.00 (1H, s), a methoxy at δ_H 4.20 (3H, s), and an isopropyl side chain [two methyl signals at δ_H 1.23 (d, J = 6.1 Hz), 1.25 (d, J = 6.1 Hz) and a septet methine at δ_H 4.23 (J = 6.1 Hz)], which supported a cadinane sesquiterpenoid structure.[2,5,6,9,10] In the ^{13}C NMR spectrum, 16 carbon resonances (Table 2) were differentiated into 3 methyls, 2 methines, 1 methoxy, and 10 quaternary carbons by a DEPT experiment. When these data were compared with those of 2-*O*-methylisohemigossylic acid lactone (**7**),[6] an additional carbonyl (δ_C 196.8) and an sp^3 oxygenated quaternary carbon (δ_C 77.9) were observed in **1**, whereas conjugation of the naphthol rings in **1** was absent. The placement of a hydroxyl at C-3 (δ_C 77.9) and a ketone at C-4 (δ_C 196.8) was inferred from the HMBC correlations of these two carbons with the C-15 methyl resonance. Other NMR assignments (Tables 1 and 2) were facilitated by comparison with the data of 2-*O*-methylisohemigossylic acid lactone (**7**)[5] and a HMBC experiment (Figure 1). Accordingly, compound **1** was identified as 3,7-dihydroxy-5-isopropyl-8-methoxy-7-methyl-7*H*-naphtho[1,8-*bc*]furan-2,6-dione and was named bombamalone A.



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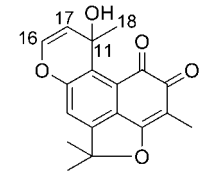

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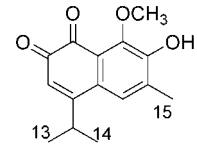

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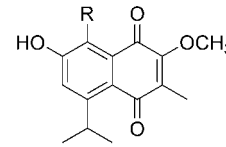
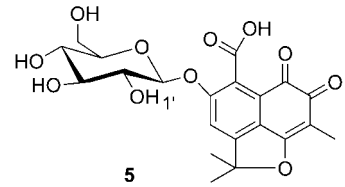


4 R = COOH
8 R = CHO



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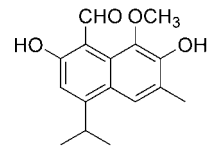

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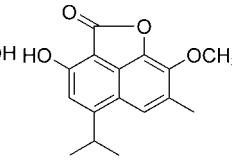

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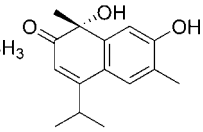

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A molecular formula of $C_{18}H_{16}O_5$ and 11 degrees of unsaturation were determined for compound **2** on the basis of the HRESIMS. The red color, IR bands at 1691 and 1610 cm^{-1}, and ^1H NMR resonances of the *gem*-dimethyl singlet signal at δ_H 1.70 (6H, s) and of an aromatic methyl group at δ_H 1.86 (3H, s), respectively, along with a singlet aromatic proton at δ_H 7.21 (1H, s), suggested a cadinane skeleton possessing an *o*-naphthoquinone chromophore,[11] analogous to hibiscoquinone D.[12] Inspection of the ^1H, ^{13}C, and DEPT NMR spectra (Tables 1 and 2) of **2** revealed the presence of a *cis*-olefin [δ_H 7.78 (d, J = 10.0 Hz), δ_C 121.8 and δ_H 6.21 (d, J = 9.7 Hz), δ_C 132.9], an aliphatic quaternary carbon (δ_C 97.6), and an additional methyl group ($\delta_{H/C}$ 1.66/28.7). Analysis of the HMBC spectrum (Figure 1) indicated that this methyl and olefinic carbon (δ_C 132.9) are connected to the hydroxylated quaternary carbon (δ_C 97.6), whereas the other olefinic carbon (δ_C 121.8) is cyclized with C-7 (δ_C 158.2) via an oxygen. All the ^1H

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Table 1. ^{1}H NMR Data of Compounds **1**–**5** (400 Hz)[a]

position	**1**[b]	**2**[c]	**3**[c]	**4**[d]	**5**[d]
4			7.35 (s)		
6	7.00 (s)	7.21 (s)	6.21 (s)	7.22 (s)	7.48 (s)
12	4.23 (sept, 6.1)		3.30 (sept, 6.7)	4.01 (sept, 6.8)	
13	1.23 (d, 6.1)	1.70 (s)	1.27 (d, 6.7)	1.15 (d, 6.8)	1.829 (s)
14	1.25 (d, 6.1)	1.70 (s)	1.27 (d, 6.7)	1.15 (d, 6.8)	1.834 (s)
15	1.51 (s)	1.86 (s)	2.30 (s)	1.97 (s)	1.91 (s)
16		7.78 (d, 10.0)			
17		6.21 (d, 9.7)			
18		1.66 (s)			
OMe	4.20 (s)		3.81 (s)	3.91 (s)	
1′					5.32 (d, 7.4)
2′					3.70–3.81 (m)
3′					3.75–3.81 (m)
4′					3.53 (t, 8.7, 8.4)
5′					3.65–3.75 (m)
6′α					3.80–3.85 (m)
6′β					4.03 (dd 14.3, 5.4)

[a] TMS was used as internal standard; δ_H in ppm; *J* values (Hz) are in parentheses. Assignments are based on HMQC and HMBC spectra. [b] Recorded in acetone-d_6. [c] Recorded in CD_3OD. [d] Recorded in D_2O.

Table 2. ^{13}C NMR Data of Compounds **1**–**5** (100 Hz, δ values)[a]

position	**1**[b]	**2**[c]	**3**[c]	**4**[d]	**5**[d]
1	149.7 (s)	181.6 (s)	153.1 (s)	185.0 (s)	181.6 (s)
2	142.2 (s)	181.4 (s)	153.5 (s)	158.6 (s)	183.8 (s)
3	77.9 (s)	107.8 (s)	134.6 (s)	137.5 (s)	110.3 (s)
4	196.8 (s)	171.3 (s)	126.7 (d)	191.3 (s)	174.2 (s)
5	163.2 (s)	149.2 (s)	168.1 (s)	156.9 (s)	150.2 (s)
6	113.4 (d)	116.4 (d)	121.3 (d)	121.2 (d)	116.3 (d)
7	162.8 (s)	158.2 (s)	183.0 (s)	159.1 (s)	158.6 (s)
8	103.6 (s)	123.8 (s)	181.1 (s)	129.3 (s)	134.6 (s)
9	123.5 (s)	122.2 (s)	123.8 (s)	132.1 (s)	124.2 (s)
10	111.9 (s)	127.6 (s)	128.1 (s)	124.3 (s)	128.9 (s)
11	164.6 (s)	97.6 (s)		177.6 (s)	175.1 (s)
12	29.7 (d)	98.6 (s)	30.8 (d)	31.5 (d)	102.2 (s)
13	22.9 (q)	27.4 (q)	22.9 (q)	25.3 (q)	28.4 (q)
14	23.0 (q)	27.4 (q)	22.9 (q)	25.3 (q)	28.4 (q)
15	26.5 (q)	26.9 (q)	17.1 (q)	11.8 (q)	9.5 (q)
16		121.8 (d)			
17		132.9 (d)			
18		28.7 (q)			
OMe	60.5 (q)		62.1 (q)	63.5 (q)	
1′					102.7 (d)
2′					75.4 (d)
3′					78.9 (d)
4′					72.3 (d)
5′					78.2 (d)
6′					63.4 (t)

[a] TMS was used as internal standard. Assignments are based on HMQC and HMBC spectra. [b] Recorded in acetone-d_6. [c] Recorded in CD_3OD. [d] Recorded in D_2O.

and ^{13}C NMR assignments of **2** were confirmed by HMBC experiment. Therefore, the structure of this novel sesquiterpenoid, bombamalone B, was elucidated as 10-hydroxy-3,5,5,10-tetramethylbenzofuro[4,3-*fg*]chromene-1,2(5*H*,10*H*)-dione. It was not possible to ascertain the configuration of the C-11 hydroxyl group.

The molecular formula of compound **3** was determined by HRESIMS as $C_{15}H_{16}O_4$. An *O*-naphthoquinone chromophore was suggested by its red color and IR bands at 1653 and 1587 cm^{-1}.[11] The ^{1}H and ^{13}C NMR data (Tables 1 and 2) implied that **3** is a norsesquiterpenoid based on a 14-carbon cadinane architecture, bearing a methoxy group ($\delta_{H/C}$ 3.81/62.1), closely related to a previous proposed structure for hibiscoquinone B.[12] HMBC correlations (Figure 1) allowed the assignment of the four oxygenated carbons in the naphthoquinone scaffold. The two carbonyl groups were placed at C-7 (δ_C 183.0) and C-8 (δ_C 181.1). Accordingly, the structure of **3** was characterized as 7-hydroxy-4-isopropyl-8-methoxy-6-methylnaphthalene-1,2-dione (bombamalone C).

Compound **4** was assigned the elemental composition $C_{16}H_{16}O_6$ from analysis by HRESIMS. When comparing the NMR data

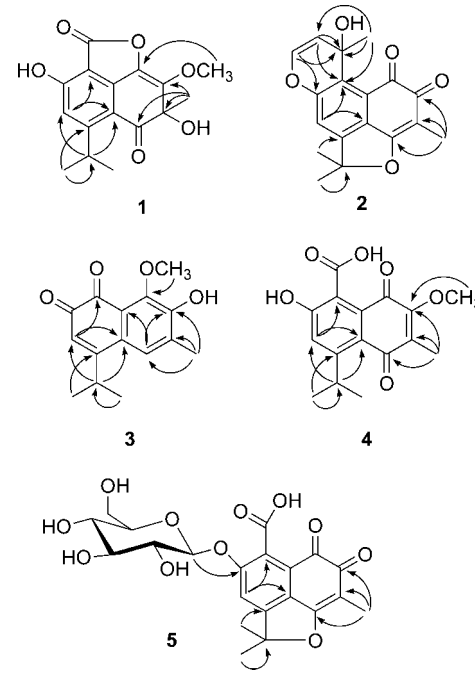

Figure 1. Key HMBC (H → C) correlations of compounds **1**–**5**.

(Tables 1 and 2) of **4** with those of bombaxquinone (**8**),[2] it was evident that the aldehyde function of **8** was replaced by a carboxyl group at C-11 (δ_C 177.6). Other connectivities were confirmed by HMBC experiment (Figure 1). Therefore, compound **4** was identified as 5,8-dihydro-2-hydroxy-4-isopropyl-7-methoxy-6-methyl-5,8-dioxonaphthalene-1-carboxylic acid (bombamalone D).

The presence of an *o*-naphthoquinone system in compound **5** was also deduced from its red color and IR bands at 1701 and 1593 cm^{-1} (broad with shoulders at 1580 and 1650 cm^{-1}).[11] In addition, the ^{1}H NMR spectrum (Table 1) displayed characteristic resonances of a sugar moiety, besides the typical signals for the analogous compound, hibiscoquinone D.[12] The ^{13}C NMR spectrum (Table 2) and molecular formula of $C_{21}H_{22}O_{11}$ (established by HRESIMS) confirmed **5** to be a monoglycosylated cadinane sesquiterpenoid. The C-11 aldehyde function of hibiscoquinone D[12] was replaced by a carboxyl group (δ_C 175.1), and other connectivities of the aglycon were confirmed by a HMBC experiment (Figure 1). The location of the sugar unit was determined to be C-7 from the HMBC correlation between H-1′ at δ_H 5.32 and C-7 at δ_C 158.6. Acid hydrolysis of **5** afforded glucose, identified by co-TLC with an authentic sample. The glucose unit was determined to have the

β-glycosidic form on the basis of the ^{1}H–^{1}H coupling constant (J = 7.4 Hz) of the anomeric proton. On the basis of these data, compound **5** was assigned as 4-*O*-β-glucopyronosyl-6,7-dihydro-2,2,8-trimethyl-6,7-dioxo-2*H*-naphtho[1,8-*bc*]furan-5-carboxylic acid and given the trivial name bombamaloside.

Compounds **1**–**9** were screened against the HGC-27 human gastrointestinal cancer cell line, using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-terazolium bromide (MTT) assay.[13] However, all of the compounds were inactive (IC_{50} >10 μM) against this cell line. Compound **2** was also inactive against the A549 lung carcinoma, MCF-7 breast carcinoma, and HeLa cervical human cancer cell lines.

Experimental Section

General Experimental Procedures. The optical rotations were obtained on a Perkin-Elmer 341 polarimeter, and the UV and IR spectra were recorded on a Shimadzu UV-2450 and a Perkin-Elmer 577 spectrometer, respectively. The NMR spectra were taken in D_2O, CD_3OD, and acetone-d_6 on a Varian Mercury NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ESI mass spectra were recorded on a Bruker Esquire-3000 mass spectrometer. Column chromatography was carried out on Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd.), TSK gel Toyopearl HW-40F (30–60 μm; Toso Co., Ltd.), MCI gel CHP-20P (75–150 μm; Mitsubishi Chemical Industries Co., Ltd.), and Cosmosil 75 C_{18}-OPN (40–105 μm; Nacalai Tesque Inc.) columns. TLC was performed on $HSGF_{254}$ silica gel plates (Yantai).

Plant Material. The roots of *Bombax malabaricum* were collected at Guangxi, China, in August 2005, and identified by Prof. Heming Yang. A voucher specimen (No. BM001) has been deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The air-dried and powdered roots (8 kg) were extracted with H_2O/acetone (3:7) at room temperature (15 L × 3). After concentration under a vacuum to remove the organic solvent, the suspended residue was removed by centrifugation. The aqueous solution was submitted to Diaion HP-20 gel column chromatography and eluted with MeOH–H_2O (0%, 25%, 50%, 100%). The aqueous sugar-containing fraction was discarded, and the three other fractions were subjected repeatedly to column chromatography on MCI gel CHP-20P, Cosmosil 75 C_{18}-OPN, and Toyopearl HW-40F columns. Fraction B (25% MeOH eluate) yielded compounds **1** (12.2 mg), **2** (2.8 mg), **4** (5.0 mg), and **5** (3.0 mg). Fraction C (50% MeOH eluate) afforded compounds **9** (4.2 mg) and **5** (4.8 mg). Fraction D (100% MeOH eluate) afforded compounds **6** (120.2 mg), **7** (24.8 mg), and **8** (5.7 mg).

Bombamalone A (1): yellow amorphous powder; $[\alpha]^{20}_D$ +15 (*c* 0.25; MeOH); UV (MeOH) λ_{max} (log ε) 291 (4.85), 361 (4.33), 4.60 (3.48) nm; IR (KBr) λ_{max} 3431, 3232, 2966, 1751, 1699, 1612 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 327 [M + Na]$^+$; ESIMS (negative-ion mode) *m*/*z* 303 [M − H]$^-$; HRESIMS *m*/*z* 327.0818 [M + Na]$^+$ (calcd for $C_{16}H_{16}O_6Na$, 327.0845).

Bombamalone B (2): red amorphous powder; $[\alpha]^{20}_D$ −25 (*c* 0.35; MeOH); UV (MeOH) λ_{max} (log ε) 279 (4.63), 472 (3.62) nm; IR (KBr) λ_{max} 3425, 2925, 1691, 1610 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 313 [M + H]$^+$; ESIMS (negative-ion mode) *m*/*z* 311 [M − H]$^-$; HRESIMS *m*/*z* 335.0920 [M + Na]$^+$ (calcd for $C_{18}H_{16}O_5Na$, 335.0895).

Bombamalone C (3): red amorphous powder; UV (MeOH) λ_{max} (log ε) 274 (4.60), 371 (3.62) nm; IR (KBr) ν_{max} 3417, 2968, 1653, 1587 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 283 [M + Na]$^+$; ESIMS (negative-ion mode) *m*/*z* 259 [M − H]$^-$; HRESIMS *m*/*z* 283.0949 [M + Na]$^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946).

Bombamalone D (4): red amorphous powder; UV (H_2O) λ_{max} (log ε) 273 (4.30), 358 (3.48) nm; IR (KBr) λ_{max} 3423, 3218, 2972, 1645, 1593 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 327 [M + Na]$^+$; ESIMS (negative-ion mode) *m*/*z* 303 [M − H]$^-$; HRESIMS *m*/*z* 327.0830 [M + Na]$^+$ (calcd for $C_{16}H_{16}O_6Na$, 327.0845).

Bombamaloside (5): red amorphous powder; UV (H_2O) λ_{max} (log ε) 275 (4.83), 326 (3.87), 411 (3.52), 472 (3.82) nm; IR (KBr) λ_{max} 3403, 2927, 1701, 1593 (broad with shoulders at 1580 and 1650) cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 473 [M + Na]$^+$; ESIMS (negative-ion mode) *m*/*z* 449 [M − H]$^-$; HRESIMS *m*/*z* 473.1061 [M + Na]$^+$ (calcd for $C_{21}H_{22}O_{11}Na$, 473.1060).

Acidic Hydrolysis of Compound 5. Compound **5** (1.5 mg) was dissolved in 5% HCl aqueous solution and then heated in a boiling water bath for 5 h. After cooling, the reaction mixture was neutralized with 10% Na_2CO_3, and glucose was identified by co-TLC with an authentic glucose sample (EtOAc–MeOH–H_2O–HOAc, 13:3:3:4; R_f 0.46).

Determination of Cell Proliferation Using a MTT Assay. The cells were seeded in a 96-well plate (5 × 10^3 cells/well) and cultured overnight. The appropriate drug was then added at various concentrations (1, 10, 50, 100, 250, 500, 750, and 1000 nM), and the wells were incubated for an additional 48 h. Cell proliferation was determined by the MTT assay.[13] The UV/vis absorbance at 570 nm was measured with a microplate reader. Cytotoxicity is expressed in terms of IC_{50} values as means of three determinations. Paclitaxel was used as positive control (IC_{50} values: HGC-27, 777.8 ± 102.4 nM; A549, 12.6 ± 5.2 nM; MCF-7, 12.4 ± 3.2 nM; HeLa, 5.6 ± 1.8 nM).

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