Chemical Constituents from the Mangrove Plant, *Aegiceras corniculatum*

Minjuan Xu,[†] Zhiwei Deng,[‡] Min Li,[†] Jun Li,[†] Hongzheng Fu,[†] Peter Proksch,[§] and Wenhan Lin*,[†]

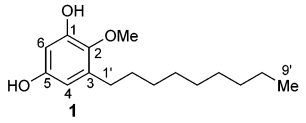
State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100083, People's Republic of China, Analytical and Testing Center, Beijing Normal University, Beijing 100073, People's Republic of China, and Institute of Pharmaceutical Biology, Heinrich-Heine University, Universitaetsstrasse 1, D-40225 Duesseldorf, Germany

Received October 30, 2003

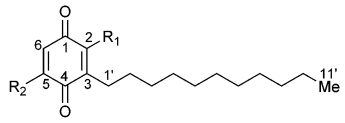
From the stems and twigs of the mangrove plant, *Aegiceras corniculatum*, seven new compounds, namely, 2-methoxy-3-nonylresorcinol (**1**), 5-*O*-ethylembelin (**2**), 2-*O*-acetyl-5-*O*-methylembelin (**3**), 3,7-dihydroxy-2,5-diundecylnaphthoquinone (**4**), 2,7-dihydroxy-8-methoxy-3,6-diundecyldibenzofuran-1,4-dione (**5**), 2,8-dihydroxy-7-methoxy-3,9-diundecyldibenzofuran-1,4-dione (**6**), and 10-hydroxy-4-*O*-methyl-2,11-diundecylgomphilactone (**7**), were isolated together with three known compounds, 5-*O*-methylembelin (**8**), 3-undecylresorcinol, and 2-dehydroxy-5-*O*-methylembelin. The structures of **1**−**7** were determined by spectroscopic methods. Compound **2** and 5-*O*-methylembelin showed in vitro cytotoxicity against the HL-60, Bel_{7402}, U937, and Hela cell lines.

Mangrove forests occur in most tropical and subtropical regions of the world and are important both ecologically and economically, as they protect coastlines from erosion and provide resources for utilization in forestry, fisheries, and food production.[1−3] *Aegiceras corniculatum* (L.) Blanco (Aegiceratataceae) is a shrub or small tree that grows in mangrove swamps of Asia and Australia.[3] In mainland China, *A. corniculatum* is one of two species of the genus and is widely distributed along the coastline in tropical and subtropical areas.[4] The bark and seeds of *A. corniculatum* possess ichthyotoxicity,[4] but no other pharmacological activities of the plant have been reported. A number of saponins and triterpenes[5−8] as well as hydroquinones[5,9] were previously isolated from this species, and it was demonstrated that the hydroquinone, 5-*O*-methylembelin, shows fish toxicity.[9]

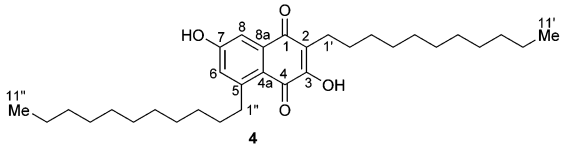
In our ongoing research program to investigate bioactive natural products from mangrove plants, seven new compounds (**1**−**7**) as well as three known compounds were isolated from this plant. In this report, we describe the structural elucidation of these new compounds, whose structures were determined on the basis of spectroscopic analysis.



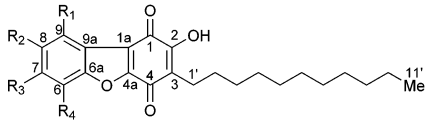
1



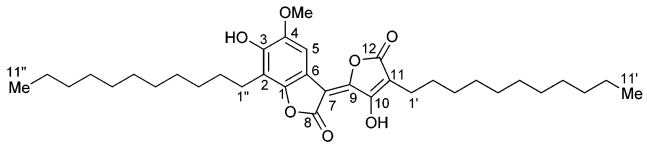
2 R_1 = OH, R_2 = OCH_2CH_3
3 R_1 = $OCOCH_3$, R_2 = OCH_3
8 R_1 = OH, R_2 = OCH_3



4



5 R_1 = H, R_2 = OCH_3, R_3 = OH, R_4 = *n*-$C_{11}H_{23}$
6 R_1 = *n*-$C_{11}H_{23}$, R_2 = OH, R_3 = OCH_3, R_4 = H



7

Results and Discussion

The petroleum ether fraction of the EtOH extract from *A. corniculatum* was subjected to repeated column chromatography to afford 10 aromatic constituents, of which seven (**1**−**7**) were determined as new natural products. 3-Undecylresorcinol was originally obtained from the wood of *Persoonia elliptica*,[10,11] 5-*O*-methylembelin (**8**)[9] was previously isolated from the same source collected from a Philippines mangrove forest, and 2-dehydroxy-5-*O*-methylembelin was previously reported as a synthesized product.[12]

Compound **1** was isolated as a white solid, and its molecular formula, $C_{16}H_{26}O_3$, was afforded from the HREIMS (*m*/*z* 266.1889 $[M]^+$, calcd 266.1882). The IR absorptions at 3383, 1601, 1493, and 1466 cm^{-1} suggested the presence of hydroxyl and aromatic functions. The ^1H NMR spectrum showed two aromatic protons at δ 6.19 (1H, d, $J_{4,6}$ = 1.5 Hz, H-4) and 6.31 (1H, d, $J_{6,4}$ = 1.5 Hz, H-6), a methoxy group signal at δ 3.73 (3H, s), and a saturated alkyl chain at δ 2.56 (2H, t, $J_{1',2'}$ = 7.0 Hz, H-1′), 1.27−1.63 (14H, m), and 0.88 (3H, t, $J_{8',9'}$ = 7.0 Hz, H-9′). The ^{13}C NMR spectrum displayed six aromatic carbons at δ 149.5 (s, C-1), 139.2 (s, C-2), 136.5 (s, C-3), 107.5 (d, C-4), 152.3 (s, C-5), and 100.5 (d, C-6), indicating the presence of a tetrasubstituted aromatic ring. The remaining carbons contained eight methylenes at δ 22.7−32.5 (t, C×8), a methoxy carbon at δ 61.6 (q), and a methyl group at δ 14.1 (q), which was consistent with a nonyl group as a side chain. The HMQC spectrum enabled the signals of all protonated carbons and their associated protons to be

* To whom correspondence should be addressed. Tel: (86) 10-62062210. Fax: (86) 10-62092724. E-mail: whlin@bjmu.edu.cn.
[†] Peking University.
[‡] Beijing Normal University.
[§] Heinrich-Heine University.

10.1021/np030477z CCC: $27.50
Published on Web 04/07/2004

assigned. The HMBC spectrum exhibited correlations for the H-6 proton with C-1, C-2, C-4, and C-5, and H-4 with C-2, C-5, C-6, and a methylene carbon C-1′ (δ 32.5, t), as well as the correlation of the methoxyl protons at δ 3.73 (3H, s) with C-2, permitting the location of two hydroxyl groups at C-1 and C-5 and the methoxyl group at C-2, respectively. The alkyl moiety was positioned at C-3 due to the correlation between H-4 and C-1′ in the HMBC spectrum as well as the small coupling between H-4 and H-1′ observed in the ^{1}H−^{1}H COSY spectrum. Therefore, the structure of **1** was determined as 2-methoxy-3-nonylresorcinol.

Compound **2** was isolated as orange crystals, and the molecular formula, $C_{19}H_{30}O_4$, was established from the HREIMS (*m/z* 322.2136 $[M]^+$, calcd 322.2144). The IR spectrum exhibited hydroxyl group (3347 cm^{-1}), alkene (1634 cm^{-1}), and quinoid carbonyl (1606 cm^{-1}) absorption bands. The ^{1}H and ^{13}C NMR data of **2** were closely comparable with those of 5-*O*-methylembelin (**8**),[9] with the exception of signals for an additional ethoxyl group [δ 4.06 (2H, q, $J_{1'',2''}$ = 7.0 Hz, H-1″), 1.54 (3H, t, $J_{2'',1''}$ = 7.0 Hz, H-2″), 65.8 (t, C-1″), 13.8 (q, C-2″)], which was shown to replace the methoxyl group signal of 5-*O*-methylembelin (**8**) at C-5, on the basis of a correlation of signals H-1″ and δ 5.83 (1H, s, H-6) with C-5 (δ 160.3, s) in the HMBC spectrum. The saturated alkyl chain was consistent with an undecyl moiety since the molecular weight of **2** was 14 amu higher than that of 5-*O*-methylembelin (**8**). The structure of **2** was thus identified as 5-*O*-ethylembelin.

Compound **3** was isolated as a light yellow amorphous powder. The ^{1}H and ^{13}C NMR data of **3** were similar to those of **2** and 5-*O*-methylembelin. However, **3** differed from 5-*O*-methylembelin (**8**) in the group at C-2, with a hydroxyl group of the latter compound replaced by an acetoxyl group in **3** [δ 2.30 (3H, s); 168.0 (s), 20.2 (q)]. Accordingly, the structure of **3** was assigned as 2-*O*-acetyl-5-*O*-methylembelin.

Compound **4** was isolated as a yellow powder, and its molecular formula was obtained by HREIMS (*m/z* 498.3692 $[M]^+$, calcd for $C_{32}H_{50}O_4$, 498.3709). The IR spectrum showed absorption bands indicating hydroxyl groups (3307 cm^{-1}), quinoid carbonyls (1642 cm^{-1}), and an aromatic ring (1591, 1564, 1467 cm^{-1}). The ^{1}H NMR spectrum displayed two *meta*-coupled aromatic protons at δ 6.93 (1H, d, $J_{6,8}$ = 1.5 Hz, H-6) and 7.72 (1H, d, $J_{8,6}$ = 1.5 Hz, H-8), two exchangeable protons at δ 7.84 (1H, s, OH-3) and 7.10 (1H, s, OH-7), and two saturated alkyl chains resonating at δ 2.55 (2H, t, $J_{1',2'}$ = 7.5 Hz, H-1′), 3.11 (2H, t, $J_{1'',2''}$ = 7.0 Hz, H-1″), 1.22−1.67 (36H, m), and 0.90 (6H, t, J = 7.0 Hz, H-11′ and H-11″). The ^{13}C NMR spectrum exhibited 10 downfield carbon signals, of which six [δ 151.1 (s, C-5), 122.2 (d, C-6), 161.4 (s, C-7), 113.6 (d, C-8), 138.0 (s, C-8a), 120.2 (s, C-4a)] were attributed to the aromatic ring, whereas four [δ 122.2 (s, C-2), 154.7 (s, C-3), 185.8 (s, C-1), and 180.9 (s, C-4)] were characterized as being part of a benzoquinone moiety of a naphthoquinone molecule. The DEPT spectrum was used to classify the remaining carbons as methylenes δ 23.1−35.7 (t, C×20) and methyls δ 14.5 (q, CH_3 × 2), which belonged to two alkyl side chains. The HMQC spectrum exhibited various cross-peaks, which were used to assign the protons and their connected carbons. In the HMBC spectrum, an exchangeable proton at δ 7.84 (s, OH-3) correlated with signals at δ 180.9 (s, C-4), 154.7 (s, C-3), and 122.2 (s, C-2), and the side chain methylene protons at H-1′ correlated with the resonance at δ 185.8 (s, C-1) and those for C-2 and C-3, resulting in the assignment of a hydroxyl group at C-3 and one of the alkyl chains at C-2 of the benzoquinone ring. Furthermore, the H-8 aromatic proton showed long-range correlations with C-1, C-7 (δ 161.4, s), C-6 (δ 122.2, d), and C-4a (δ 120.2, s), and the remaining H-6 aromatic proton correlated with C-7, C-4a, C-8 (δ 113.6, d), and C-1″ (δ 35.7, t). An exchangeable proton at δ 7.10 (1H, s, OH-7) correlated with C-6, C-7, and C-8, indicating the attachment of a second hydroxyl to C-7 of the aromatic ring. The second alkyl chain was located at C-5 due to the correlations of the methylene protons H-1″ with C-5 (δ 151.1, s), C-6, and C-4a and from the correlation of H-6 with the methylene carbon C-1″ in the HMBC spectrum. The length of each alkyl chain could be determined by interpretation of the fragmentation pattern of **4** in the EIMS spectrum (Figure S1).[13,14] Hence, the alkyl chains were composed of undecyl moieties. On the basis of the spectral analysis discussed above, the structure of **4** was determined as 3,7-dihydroxy-2,5-diundecylnaphthaquinone.

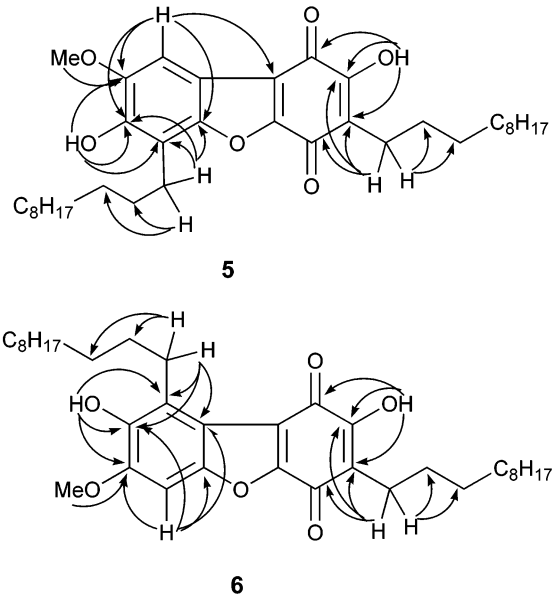


Figure 1. Main HMBC correlations of **5** and **6** (H→C).

Compound **5** was isolated as a dark red solid, and its molecular formula, $C_{35}H_{52}O_6$, was obtained from its HREIMS (*m/z* 568.3740 $[M]^+$, calcd 568.3764). The IR absorption bands at 3542, 3314, 1639, and 1567 cm^{-1} suggested the presence of hydroxyl and carbonyl groups as well as an aromatic ring. The gross structure of **5** was established by means of 2D NMR spectroscopic studies as being characterized by a dibenzofuran-1,4-dione basic nucleus substituted with two saturated alkyl side chains. In the HMBC spectrum, an exchangeable proton at δ 7.06 (1H, s, OH-2) displayed correlations with carbon signals at δ 179.9 (s, C-1), 152.0 (s, C-2), and 120.3 (s, C-3). In turn, the methylene protons at δ 2.52 (2H, t, $J_{1',2'}$ = 7.0 Hz, H-1′), belonging to one of the alkyl side chains, exhibited correlations with C-2, C-3, and C-4 (δ 177.9, s), indicating that a hydroxyl group was present at C-2 and the alkyl side chain was connected to C-3 of the quinone part of the molecule. The HMBC spectrum also exhibited correlations of an aromatic proton signal at δ 7.26 (1H, s, H-9) with C-1a (δ 113.4, s), C-6a (δ 151.7, s), C-8 (δ 147.2, s), and C-7 (δ 145.9, s). Other HMBC correlations were observed for a methoxyl group signal at δ 4.01 (3H, s) with C-8, an exchangeable proton at δ 6.11 (1H, s, OH-7) with C-6 (δ 114.4, s), C-7, and C-8, and the methylene protons at δ 2.94 (2H, t, J = 7.0 Hz, H-1″) with C-6, C-6a, and C-7, respectively (Figure 1). These observations permitted the

assignment of the methoxyl group at C-8, a hydroxyl group at C-7, and the second alkyl side chain at C-6, respectively. These proposed substitution patterns were also supported by NOE cross-peaks between MeO-8 and H-9 and between H-1″ and OH-7 in the NOESY spectrum. The lengths of two saturated side chains were established as undecyl residues from the EIMS. The substitution pattern of **5** was further supported by the comparison of its NMR data with those of related structures that were prepared by an unambiguous synthetic route.[15] Therefore, the structure of **5** was determined as 2,7-dihydroxy-8-methoxy-3,6-diundecyldibenzofuran-1,4-dione.

Compound **6** was isolated as a dark red solid and had the same molecular formula as that of **5** based on its HREIMS. Its IR and ^{1}H NMR spectra were very similar to those of compound **5**, but differed in the ^{13}C NMR spectrum with regard to the signals of the dibenzofuran-1,4-dione residue, indicating that **6** has a different substitution pattern in this substructure as compared with **5**. The HMBC correlations of an exchangeable proton at δ 7.38 (1H, s, OH-2) and the alkyl methylene protons at δ 2.54 (2H, t, $J_{1',2'} = 7.5$ Hz, H-1′) with carbons in the quinone ring were comparable to those described for **5** and hence consistent with the same substitution pattern. However, the additional alkyl methylene protons resonating at δ 3.25 (2H, t, $J_{1'',2''} = 7.5$ Hz, H-1″) correlated with carbons resonating at δ 115.8 (s, C-9a), 122.3 (s, C-9), and 142.3 (s, C-8), supporting the assignment of the second alkyl side chain at C-9 rather than at C-6 as in **5**. Moreover, an exchangeable proton at δ 5.79 (1H, s, OH-8) correlated with C-7 (δ 148.0, s), C-8, and C-9, and the methoxyl protons at δ 4.01 (3H, s) correlated with C-7, thereby proving the substitution pattern of the aromatic ring system (Figure 1). Since the EIMS fragmentation pattern of **6** was identical to that of **5**, the two alkyl side chains of **6** were also assigned as undecyl moieties. Consequently, the structure of **6** was identified as 2,8-dihydroxy-7-methoxy-3,9-diundecyldibenzofuran-1,4-dione.

Compound **7** was isolated as an orange solid, and its molecular formula, $C_{35}H_{52}O_7$, was obtained by HREIMS (m/z 584.3688 $[M]^+$, calcd 584.3713). The IR spectrum displayed absorption bands at 3430, 1798, 1720, 1646, and 1616 cm^{-1}, suggesting the presence of hydroxyl, unsaturated γ-lactone, and aromatic groups. The ^{1}H NMR spectrum showed signals for one aromatic proton at δ 7.21 (1H, s, H-5), two exchangeable protons at δ 11.83 (1H, s, OH-10) and 6.24 (1H, s, OH-3), a methoxy group at δ 3.98 (3H, s), 20 methylenes at δ 2.74 (2H, t, $J_{1'',2''} = 7.0$ Hz, H-1″), 2.40 (2H, t, $J_{1',2'} = 7.0$ Hz, H-1′), and 1.20−1.60 (36H, m, H-2′-10′ and H-2″-10″), and two methyl groups at δ 0.91 (6H, t, $J = 7.0$ Hz, H-11′ and H-11″). The ^{13}C NMR spectrum displayed resonances for one methine carbon at δ 104.7 (C-5), 11 quaternary carbons [δ 173.9 (C-8), 168.3 (C-12), 162.4 (C-10), 150.6 (C-9), 148.6 (C-1), 147.6 (C-3), 144.9 (C-4), 114.3 (C-2), 112.3 (C-6), 108.7 (C-11), and 107.0 (C-7)], three methyl groups at δ 57.3 (q, OCH_3), 14.52 (q, CH_3-11′ or CH_3-11″), and 14.49 (q, CH_3-11″ or CH_3-11′), and 20 methylene signals between δ 22.3−32.3 (t, $CH_2 \times 20$). The skeletal structure of **7** was established to be that of gomphilactone[16] on the basis of extensive 2D NMR spectral analysis. In the HMBC spectrum, an exchangeable proton at δ 11.83 (1H, s, OH-10) correlated with carbons at δ 150.6 (s, C-9), 162.4 (s, C-10), and 108.7 (s, C-11), and the alkyl methylene protons H-1′ correlated with C-10, C-11, and C-12, suggesting that a hydroxyl group substituent was present at C-10 and an alkyl side chain was located at C-11 of an unsaturated γ-lactone ring. With respect to the aromatic ring A, HMBC correlations were observed between the methylene protons H-1″ and δ 148.6 (s, C-1), 114.3 (s, C-2), and 147.6 (s, C-3) and between an exchangeable proton at δ 6.24 (1H, s, OH-3) and C-2, C-3, and C-4 (δ 144.9, s). Further correlations included those between the aromatic proton at δ 7.21 (s, H-5) and C-3, C-6, C-1, C-4, and C-7 (δ 107.0, s), thereby revealing the positions of the alkyl side chain at C-2 and the hydroxyl and methoxyl groups at C-3 and C-4, respectively. The length of the two alkyl side chains could be determined by the EIMS fragment pattern of compound **7**, which supported the presence of two undecyl side chain units. The stereochemistry of compound **7** with regard to the orientation of the lactone rings was based on the downfield signal of the exchangeable proton (δ 11.83, s) at C-10, which suggested the formation of a hydrogen bond with the carbonyl oxygen group at ring B. Accordingly, the structure of **7** was established as 10-hydroxy-4-*O*-methyl-2,11-diundecylgomphilactone.

Table 1. ^{13}C NMR Data of Compounds **4**−**7**[a]

position	**4**	**5**	**6**	**7**
1	185.8	179.9	177.8	148.6
1a		113.4	118.8	
2	122.2	152.0	151.9	114.3
3	154.7	120.3	118.3	147.6
4	180.9	177.9	177.4	144.9
4a	120.2	153.3	153.3	
5	151.1			104.7
6	122.2	114.4	92.0	112.3
6a		151.7	150.4	
7	161.4	145.9	148.0	107.0
8	113.6	147.2	142.3	173.9
8a	138.0			
9		99.4	122.3	150.6
9a		119.5	115.8	
10				162.4
11				108.7
12				168.3
1′	23.7	23.1	22.1	24.0
2′	30.3	30.0	30.4	30.1
3′	28.6	28.8	27.9	27.8
4′−8′	29.5−30.2	29.8−30.1	29.0−30.5	27.9−32.3
9′	32.3	32.3	31.5	32.3
10′	23.1	23.1	22.3	23.1
11′ or 11″	14.5	14.5	13.7	14.52
1″	35.7	24.0	22.3	22.3
2″	30.9	30.1	30.5	30.6
3″−8″	28.6−30.9	29.75−30.1	29.0−30.5	27.9−32.3
9″	32.3	32.3	31.5	32.3
10″	23.1	23.1	22.3	23.1
11″ or 11′	14.5	14.5	13.7	14.49
OMe		57.0	56.1	57.3

[a] Recorded in $CDCl_3$. Chemical shifts (δ) in ppm.

To investigate whether the 1,4-dibenzofurandione derivatives **5** and **6** and the furanylidene benzofuranone derivative **7** originated from dimerization of embelin,[17,18] the plant material was percolated in acetone, and the acetone extract was examined by negative-ion ESIMS/MS, in which molecular ions at m/z 567 $[M - 1]^+$, 583 $[M - 1]^+$, 497 $[M - 1]^+$, and 307 $[M - 1]^+$, corresponding with those of compounds **5**, **6**, **7**, **4**, and **8**, were present. This evidence demonstrated that compounds **5** and **7** were not obtained in this investigation as artifacts of extraction.

Compounds **1**−**10** were submitted for bioassay against the HL-60, Bel_{7402}, U937, and Hela cultured human tumor cell lines. Of these **2** and 5-*O*-methylembelin (**8**) showed cytotoxicity toward the four cell lines, as shown in Table 2. The remaining compounds showed negative activity ($IC_{50} > 100$ μg/mL).

Table 2. Cytotoxicity Data of 5-*O*-Ethylembelin (**2**) and 5-*O*-Methylembelin (**8**)[a]

compound	cell line			
	HL-60	Bel_{7402}	Hela	U937
5-*O*-ethylembelin (**2**)	2.5	2.7	3.9	1.3
5-*O*-methylembelin (**8**)	3.0	3.6	9.0	1.5
colchicine	1.6	0.4	0.1	0.1

[a] Results are expressed as IC_{50} values in μg/mL.

Experimental Section

General Experimental Procedures. Melting points were measured on a XT-4A micromelting point apparatus without correction. The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-500 FT NMR spectrometer using TMS as an internal standard. EIMS were performed with a Bruker APEX II mass spectrometer, and ESIMS were recorded on a PE Q-STAR ESI-TOF-MS/MS spectrometer. Column chromatography was carried with silica gel (200−300 mesh), and HF254 silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, People's Republic of China. Sephadex LH-20 (18−110 μm) was obtained from Pharmacia.

Plant Material. The specimen of *Aegiceras corniculatum* was collected at the coastline close to Xiamen, Fujian Province, People's Republic of China, in July 2002. The species was identified by Prof. Lin Peng of Xia Men University. A voucher specimen (HN-032) was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

Extraction and Isolation. The stems and twigs of *A. corniculatum* (6.0 kg) were air-dried and then ground. The powdered sample was percolated with 95% EtOH twice at room temperature and then extracted with hot 95% EtOH at 60 °C. Both extracts were combined and concentrated in a vacuum to afford a black residue. The residue was dissolved in water and partitioned against petroleum ether, EtOAc, and *n*-BuOH, successively. The petroleum ether extract (37 g) was subjected to passage over a silica gel column and eluted with cyclohexane−acetone gradients (10% and 20% cyclohexane). From portions 6−7 and 15−16 of the 10% cyclohexane eluents, **2** (30 mg) and **8** (100 mg) were collected, respectively. The remaining 10% cyclohexane fractions (1.5 g) showed mixtures of spots by TLC and were subjected to Sephadex LH-20 column chromatography with 90% MeOH as eluent. Each portion was collected in 50 mL flasks. Fractions 7−8 yielded **5** (3.0 mg), fractions 13−15 afforded **6** (3.5 mg), and fractions 18−20 afforded 2-dehydroxy-5-*O*-methylembelin (3.0 mg). The 20% cyclohexane fractions (2.6 g) were separated further by silica gel chromatography eluting with petroleum ether and chloroform gradients to afford **4** (5:1, 1.5 mg) and **7** (3:1, 2.0 mg). The eluted fractions (1:1, 0.9 g) were further separated by reversed-phase semipreparative HPLC in 95% MeOH to afford **1** (4.0 mg), **3** (11.6 mg), and 3-undecylresorcinol (4.5 mg).

The stems and twigs of *A. corniculatum* (50 g) were ground and percolated in acetone for one week. The acetone extract was concentrated in vacuum and detected by LC-(−)-ESIMS/MS, in which the peaks for molecular ions at *m*/*z* 567 [M − 1]$^+$, 583 [M − 1]$^+$, 497 [M − 1]$^+$, and 307 [M − 1]$^+$, corresponding with those of compounds **5**, **6**, **7**, **4**, and **8**, were observed.

2-Methoxy-3-nonylresorcinol (1): white powder; mp > 300 °C; UV (MeOH) λ_{max} (log ϵ) 216 (3.92), 283 (3.71) nm; IR (KBr) ν_{max} 3383, 1601, 1493, 1466 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.19 (1H, d, J = 1.5 Hz, H-4), 6.31 (1H, d, J = 1.5 Hz, H-6), 3.73 (3H, s, OCH_3), 2.56 (2H, t, J = 7.0 Hz, H-1′), 1.27−1.63 (14H, m, H-2′-8′), 0.88 (3H, t, J = 7.0 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 152.3 (s, C-5), 149.5 (s, C-1), 139.2 (s, C-2), 136.5(s, C-3), 107.5 (d, C-4), 100.5 (d, C-6), 61.5 (q, OCH_3), 32.5 (t, C-1′), 29.3−31.9 (t, C-2′-7′), 22.7 (t, C-8′), 14.1 (q, C-9′); EIMS *m*/*z* 266[M]$^+$ (38), 251 (8), 236 (7), 154 (44), 139 (100); HREIMS *m*/*z* 266.1889 [M]$^+$ (calcd for $C_{16}H_{26}O_3$, 266.1882).

5-*O*-Ethylembelin (2): orange crystals; mp 59−60 °C; UV (MeOH) λ_{max} (log ϵ) 220 (3.62), 289 (3.68) nm; IR (KBr) ν_{max} 3347, 1666, 1634, 1606 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23 (1H, s, OH-2), 5.83 (1H, s, H-6), 4.06 (2H, q, J = 7.0 Hz, H-1″), 2.46 (2H, t, J = 7.5 Hz, H-1′), 1.54 (3H, t, J = 7.0 Hz, H-2″), 1.27−1.46 (18H, m, H-2′-10′), 0.90 (3H, t, J = 6.8 Hz, H-11′); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 183.0 (s, C-1), 181.8 (s, C-4), 160.3 (s, C-5), 151.4 (s, C-2), 119.2 (s, C-3), 102.4 (d, C-6), 65.8 (t, OCH_2, C-1″), 28.0−31.9 (t, C-2′-9′), 22.7 (t, C-10′), 22.6 (t, C-1′), 14.1 (q, C-11′), 13.8 (q, CH_3, C-2″); EIMS *m*/*z* 322 [M]$^+$ (58), 182 (100), 170 (12), 153 (71), 139 (10), 124 (21); HREIMS *m*/*z* 322.2136 [M]$^+$ (calcd for $C_{19}H_{30}O_4$, 322.2144).

2-*O*-Acetyl-5-*O*-methylembelin (3): yellow amorphous powder; UV (MeOH) λ_{max} (log ϵ) 233 (3.93), 251 (3.72), 280 (3.52) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.73 (1H, s, H-6), 3.85 (3H, s, OCH_3), 2.32 (2H, t, J = 7.5 Hz, H-1′), 2.30 (3H, s, $COCH_3$), 1.22−1.42 (18H, m, H-2′-10′), 0.85 (3H, t, J = 6.6, 6.8 Hz, H-11′); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 179.4 (s, C-4), 178.1 (s, C-1), 168.0 (s, CO), 164.1 (s, C-5), 151.1 (s, C-2), 133.2 (s, C-3), 101.8 (d, C-6), 57.1 (q, OCH_3), 28.2−31.9 (t, C-2′-9′), 23.9 (t, C-1′), 22.7 (t, C-10′), 20.2 ($COCH_3$), 14.1 (q, C-11′); EIMS *m*/*z* 308 [M]$^+$ (23), 280 (31), 168 (39), 152 (11), 139 (13), 109 (18), 91 (27), 69 (67); EIMS *m*/*z* 350 [M$^+$], 308, 280, 166.

3,7-Dihydroxy-2,5-diundecylnaphthoquinone (4): yellow powder; mp 75−77 °C; UV (MeOH) λ_{max} (log ϵ) 222 (4.02), 272 (3.87), 302 (3.65), 346 (3.53) nm; IR (KBr) ν_{max} 3307, 1701, 1642, 1591, 1564, 1467 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (1H, s, OH-3), 7.72 (1H, d, J = 1.5 Hz, H-8), 7.10 (1H, s, OH-7), 6.93 (1H, d, J = 1.5 Hz, H-6), 3.11 (2H, t, J = 7.0 Hz, H-1″), 2.55 (2H, t, J = 7.5 Hz, H-1′), 1.22−1.65 (18H, m, H-2′−10′), 1.22−1.67 (18H, m, H-2″−10″), 0.90 (6H, t, J = 7.0 Hz, H-11′ and H-11″); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 498 [M]$^+$ (100), 470 (5), 358 (29), 231 (9), 218 (23), 189 (11), 129 (10), 43 (62); HREIMS *m*/*z* 498.3692 (calcd for $C_{32}H_{50}O_4$, 498.3709).

2,7-Dihydroxy-8-methoxy-3,6-diundecyldibenzofuran-1,4-dione (5): dark red solid ($CHCl_3$); mp 65−66 °C; UV (MeOH) λ_{max} (log ϵ) 220 (3.94), 258 (3.72), 289 (3.55) nm; IR (KBr) ν_{max} 3542, 3314, 1739, 1674, 1639, 1567, 1467, 1278 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (1H, s, H-9), 7.06 (1H, s, OH-2), 6.11 (1H, s, OH-7), 4.01 (3H, s, OCH_3), 2.94 (2H, t, J = 7.0 Hz, H-1″), 2.52 (2H, t, J = 7.0 Hz, H-1′), 1.25−1.68 (36H, m, H-2′-10′ and H-2″-10″), 0.87 (6H, t, J = 7.0 Hz, H-11′ and H-11″); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 568 [M]$^+$ (43), 428 (30), 288 (35), 287 (11), 259 (10), 97 (28); HREIMS *m*/*z* 568.3740 (calcd for $C_{35}H_{52}O_6$, 568.3764).

2,8-Dihydroxy-7-methoxy-3,9-diundecyldibenzofuran-1,4-dione (6): dark red solid ($CHCl_3$); mp 88−89 °C; UV (MeOH) λ_{max} (log ϵ) 222 (4.21), 258 (3.80), 295 (3.65) nm; IR (KBr) ν_{max} 3537, 3393, 1650, 1622, 1554, 1469, 1282 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 (1H, s, OH-2), 7.00 (1H, s, H-6), 5.79 (1H, s, OH-8), 4.01 (3H, s, OCH_3), 3.25 (2H, t, J = 7.5, Hz, H-1″), 2.54 (2H, t, J = 7.5, Hz, H-1′), 1.24−1.67 (36H, m, H-2′−10′ and H-2″−10″), 0.91 (3H, t, J = 7.0 Hz, H-11′ or H-11″), 0.87 (3H, J = 7.0 Hz, H-11″ or H-11′); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 568 [M]$^+$ (100), 453 (10), 429 (30), 428 (35), 289 (20), 287 (18), 273 (10), 259 (9); HREIMS *m*/*z* 568.3740 (calcd for $C_{35}H_{52}O_6$, 568.3764).

10-Hydroxy-4-*O*-methyl-2,11-diundecylgomphilactone (7): orange solid ($CHCl_3$); mp 103−105 °C; UV (MeOH) λ_{max} (log ϵ) 220 (3.81), 273 (3.67), 453 (3.58) nm; IR (KBr) ν_{max} 3430, 1798, 1720, 1646, 1616, 1465, 1339, 1266 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.83 (1H, s, OH-10), 7.21 (1H, s, H-5), 6.24 (1H, s, OH-3), 3.98 (3H, s, OCH_3), 2.74 (2H, t, J = 7.0, Hz, H-1″), 2.40 (2H, t, J = 7.0, Hz, H-1′), 1.20−1.60 (36H, m, H-2′-10′ and H-2″−10″), 0.91 (6H, t, J = 7.0 Hz, H-11′ and H-11″); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 584 [M]$^+$ (100), 443 (9), 360 (50), 334 (18), 303 (20), 219 (11); HREIMS *m*/*z* 584.3688 (calcd for $C_{35}H_{52}O_7$, 584.3713).

Cytotoxicity Assays. The cytotoxic activity of the isolated compounds was investigated on a small panel of human cancer cell lines, comprising HL-60 (human acute promyelocytic leukemia), Bel_{7402} (human hepatocellular carcinoma), U937 (human monocytic leukemia), and Hela (cervical carcinoma). The cell lines were maintained in RPMI-1640 (Hyclone)

medium supplemented with 10% (v/v) fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin, at 37 °C in a humidified atmosphere containing 5% CO_2. Each tested compound was dissolved in DMSO to a concentrations of 50 μg/mL and diluted to the required concentrations with the medium when used. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed to evaluate the in vitro cytotoxic activity of tested compounds. Cancer cells (180 μL) were seeded at 2000/mL onto the 96-well plates. After 24 h of incubation at 37 °C, 20 μL of compound in serial dilutions (from 100 to 1 μg/mL) were added, and 0.1% DMSO was used as the control. Following 48 h of incubation with each compound, 20 μL of 5 mg/mL MTT (Sigma) was added to each well. After an additional 4 h of incubation, the medium was discarded and dried in the air, then 200 μL of acid-isopropyl alcohol was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm by a microplate reader. The experiments were run in triplicate. Colchicine was used as the positive control.

Acknowledgment. This work was supported by grants from National High Technology Development Project (863 project) (No. 2001AA620403 and 2002AA217081), NSFC (No. 40176038, 30171106), and the International Cooperation Projects of BMBF-CNCBD. We would like to thank Prof. P. Lin of Xia Men University (People's Republic of China) for identification of the plant material.

Supporting Information Available: Scheme showing proposed mass spectral fragmentation pattern for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Miles, D. H.; Kokpol, U.; Chittawong, V.; Santi, T. P.; Tunsuwan, K.; Chi, N. *Pure Appl. Chem.* **1998**, *70*, 2113−2122.
(2) Dodd, R. S.; Fromard, F.; Rafii, Z. A.; Blasco, F. *Biochem. System. Ecol.* **1995**, *23*, 859−868.
(3) Li, M. S.; Lee, S. Y. *Forest Ecol. Manage.* **1997**, *96*, 241−259.
(4) Lin, P.; Fu, Q. *Environmental Ecology and Economic Utilization of Mangroves in China*; Higher Education Press: Beijing, 1995; pp 1−95.
(5) Hensens, O. D.; Lewis, K. G. *Aust. J. Chem.* **1966**, *19*, 169−174.
(6) Rao, K. V. *Tetrahedron* **1964**, *20*, 973−977.
(7) Rao, K. V.; Bose, P. K. *Tetrahedron* **1962**, *18*, 461−464.
(8) Rao, K. V.; Bose, P. K. *J. Indian Chem. Soc.* **1959**, *36*, 358−360.
(9) Gomez, E.; Cruz-Giron, O. D. L. *J. Nat. Prod.* **1989**, *52*, 649−651.
(10) Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 1393−1405.
(11) Cannon, J. R.; Metcalf, B. W. *Aust. J. Chem.* **1971**, *24*, 1925−1931.
(12) Koenig, W. A.; Faasch, H.; Hortsch, H.; Colberg, C.; Hausen, B. M. *Z. Naturforsch.* **1993**, *48b*, 387−393.
(13) Steglich, W.; Esser, F.; Pils I. *Z. Naturforsch.* **1971**, *26b*, 336−338.
(14) Chong, P. Z. *The Application of Mass Spectroscopy on Natural Organic Chemistry*; Academic Press: Beijing, 1987; 845 pp.
(15) Carney, J. R.; Scheuer, P. J. *Tetrahedron Lett.* **1993**, *34*, 3727−3730.
(16) Jaegers, E.; Steffan, B.; Ardenne, R.; Steglich, W. *Z. Naturforsch.* **1981**, *36C*, 488−489.
(17) Kiuchi, F.; Suzuki, N.; Fukumoto, Y.; Goto, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1225−1228.
(18) Ravikanth, V.; Ramesh, P.; Diwan, P. V.; Venkateswarlu, Y. *Heterocycl. Commun.* **2000**, *6*, 315−317.

NP030477Z