# Anthraquinone-Benzisochromanquinone Dimers from the Roots of *Berchemia floribunda*

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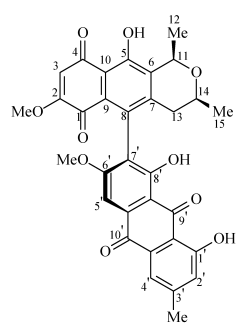
**Four novel anthraquinone-benzisochromanquinone dimers, named floribundiquinones A, B, C, and D (1—4), along with six known anthraquinones, 10-(chrysophanol-7′-yl)-10-hydroxychrysophanol-9-anthrane (5), physcion (6), chrysophanol (7), 1,5,8-trihydroxy-3-methyl-anthraquinone (8), aloe-emodin (9), and xanthorin (10), were isolated from the roots of *Berchemia floribunda*. Their structures including the absolute axial stereochemistry were elucidated on the basis of spectroscopic methods. Floribundiquinones represent a novel carbon skeleton with an anthraquinone-benzisochromanquinone unit. Hepatoprotective activities were evaluated against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells.**

**Key words** *Berchemia floribunda*; anthraquinone; floribundiquinone A; floribundiquinone B; floribundiquinone C; floribundiquinone D

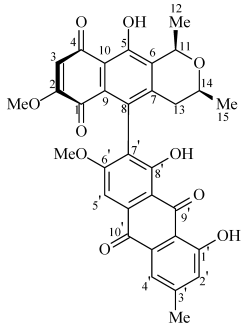
The genus *Berchemia* (Rhamnaceae) with more than 31 species occurred in Asia. About eighteen species are distributed in south part of China. Their roots have been used as a remedy for gall stones, stomach-ache, rheumatism and lumbago in Chinese folk medicine.[1] Hitherto, *B. racemosa*, *B. pakistanica*, *B. zeyheri*, *B. formosana*, *B. polyphylla*, and *B. floribunda* were chemically investigated and some lignans,[2] flavones,[3—9] aromatic glycosides[10,11] and quinines[12—14] were isolated. In the course of our search for active components from the genus *Berchemia*, ethanol extracts of the roots of *B. floribunda* showed obviously hepatoprotective effects against D-galactosamine-induced toxicity. Furthermore, from bioactivity-directed fractionation four novel anthraquinone-benzisochromanquinone dimers, named floribundiquinones A—D (**1**—**4**) were isolated, along with six known anthraquinones, 10-(chrysophanol-7′-yl)-10-hydroxy-chrysophanol-9-anthrane (**5**),[15] physcion (**6**),[16] chrysophanol (**7**), 1,5,8-trihydroxy-3-methyl-anthraquinone (**8**),[17] aloe-emodin (**9**),[18] and xanthorin (**10**).[17] Their structures including the absolute axial stereochemistry were elucidated on the basis of spectroscopic methods. Hepatoprotective activities of compounds **1**—**4**, **6**, and **7** were evaluated against D-galactosamine-induced toxicity in WB-F344.

Floribundiquinone A (**1**) was obtained as a brown amorphous powder. The IR spectrum indicated the presence of hydroxyl, hydrogen bonded carbonyl and aromatic groups. The high resolution (HR)-EI-MS of **1** showed a molecular ion peak at *m*/*z* 570.1513 corresponding to molecular formula $C_{32}H_{26}O_{10}$. The UV spectrum of **1** showed absorption maxima at 203, 222, 254, 283, and 434 nm in MeOH. These data suggested that **1** has quinone groups.

The $^1$H-NMR spectrum of **1** (Table 1) showed the presence of three phenolic hydroxyl groups at $\delta_H$ 13.41 (1H, s), 12.27 (1H, s), and 12.03 (1H, s), two methoxy groups at $\delta_H$ 3.90 (3H, s) and 3.82 (3H, s), one aromatic methyl group at $\delta_H$ 2.47 (3H, s), two doublet methyl groups at $\delta_H$ 1.23 (3H, d, $J$=6.5 Hz) and 1.70 (3H, d, $J$=6.5 Hz), two methine protons at $\delta_H$ 5.15 (1H, q, $J$=6.5 Hz) and 3.65 (1H, m), and a broad singlet at $\delta_H$ 2.24 (2H, br s). Additionally, the $^1$H-NMR spectrum revealed the presence of two *meta* coupled protons at $\delta_H$ 7.09 (1H, d, $J$=2.0 Hz) and 7.67 (1H, d, $J$=2.0 Hz) and two sharp singlets at $\delta_H$ 6.05 and 7.50. In the $^1$H–$^1$H correlated spectroscopy (COSY), the proton at $\delta_H$ 5.15 (1H, q, $J$=6.5 Hz) correlated with protons at $\delta_H$ 1.70 (3H, d, $J$=6.5 Hz), while the proton at $\delta_H$ 3.65 (1H, m) correlated with protons at $\delta_H$ 2.24 (2H, br s) and methyl protons at $\delta_H$ 1.23 (3H, d, $J$=6.5 Hz), suggesting the existence of the moieties –CH(CH$_3$)O– and –CH$_2$CH(CH$_3$)O–. Furthermore, in view of the correlations between C-6 at $\delta_C$ 136.8 and H-11 at $\delta_H$ 5.15 (1H, q, $J$=6.5 Hz), H-12 at $\delta_H$ 1.70 (3H, d, $J$=6.5 Hz), and H-13 at $\delta_H$ 2.24 (2H, br s), C-7 at $\delta_C$ 143.9 and H-11 at $\delta_H$ 5.15 (1H, q, $J$=6.5 Hz), H-13 at $\delta_H$ 2.24 (2H,



**1**



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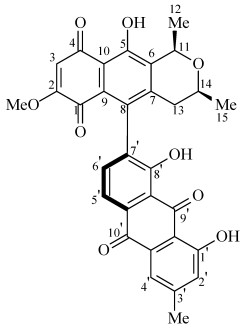

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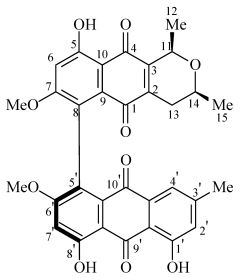

**4**

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Table 1. $^1$H-NMR Data for Compounds **1**, **2**, **3** and **4** in $CDCl_3$ (*J* Values in Parentheses)

| Proton | **1** $\delta_H$ (mult. Hz) | **2** $\delta_H$ (mult. Hz) | **3** $\delta_H$ (mult. Hz) | **4** $\delta_H$ (mult. Hz) |
|---|---|---|---|---|
| 3-H | 6.05 (1H, s) | 6.06 (1H, s) | 6.07 (1H, s) | |
| 5-H | 13.41 (1H, s) | 13.43 (1H, s) | 13.39 (1H, s) | 13.14 (1H, s) |
| 6-H | | | | 6.76 (1H, s) |
| 11-H | 5.15 (1H, q, 6.5) | 5.16 (1H, q, 6.5) | 5.16 (1H, q, 6.5) | 4.80 (1H, q, 7.0) |
| 12-H | 1.70 (3H, d, 6.5) | 1.70 (3H, d, 6.5) | 1.68 (3H, d, 6.5) | 1.60 (3H, d, 7.0) |
| 13-H | 2.24 (2H, br s) | 2.16 (1H, dt, 16.5, 2.0) | 2.14 (1H, dt, 16.5, 3.0) | 2.04 (1H, ddd, 18.0, 10.0, 4.0) |
| | | 2.40 (1H, ddd, 16.5, 11.0, 4.0) | 2.32 (1H, dd, 16.5, 13.0) | 2.46 (1H, dt, 18.0, 2.5) |
| 14-H | 3.65 (1H, m) | 3.63 (1H, m) | 3.62 (1H, m) | 3.47 (1H, m) |
| 15-H | 1.23 (3H, d, 6.5) | 1.23 (3H, d, 6.0) | 1.23 (3H, d, 6.5) | 1.25 (3H, d, 6.5) |
| 2-OMe | 3.82 (3H, s) | 3.83 (3H, s) | 3.83 (3H, s) | 3.70 (3H, s) |
| 1′-H | 12.03 (1H, s) | 12.01 (1H, s) | 11.97 (1H, s) | 12.15 (1H, s) |
| 2′-H | 7.09 (1H, d, 2.0) | 7.11 (1H, d, 2.0) | 7.11 (1H, d, 2.0) | 7.03 (1H, d, 2.0) |
| 4′-H | 7.67 (1H, d, 2.0) | 7.67 (1H, d, 2.0) | 7.69 (1H, d, 2.0) | 7.36 (1H, d, 2.0) |
| 5′-H | 7.50 (1H, s) | 7.51 (1H, s) | 7.94 (1H, d, 7.5) | |
| 6′-H | | | 7.41 (1H, d, 7.5) | |
| 7′-H | | | | 6.71 (1H, s) |
| 8′-H | 12.27 (1H, s) | 12.29 (1H, s) | 12.36 (1H, s) | 13.08 (1H, s) |
| 3′Me | 2.47 (3H, s) | 2.48 (3H, s) | 2.49 (3H, s) | 2.34 (3H, s) |
| 6′-OMe | 3.90 (3H, s) | 3.86 (3H, s) | | 3.75 (3H, s) |

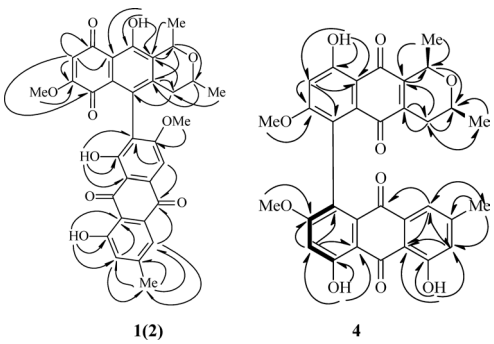


Fig. 1. Key HMBC of Compounds **1**(**2**) and **4**

br s) and H-14 at $\delta_H$ 3.65 (1H, m), and C-11 at $\delta_C$ 71.1 and H-14 at $\delta_H$ 3.65 (1H, m) in the heteronuclear multiple-bond correlation (HMBC) (Fig. 1), it was confirmed that a 5,6-dihydro-2,6-dimethyl-2*H*-pyran system was fused at the 3,4-position. The signal at $\delta_H$ 6.05 was assigned to a quinonoid proton adjacent to the methoxyl group. Careful analysis of the HMBC data revealed that **1** has a 3,4-dihydro-1,3-dimethyl-1*H*-naphtha[2,3-*c*]pyran-6,9-dione unit (benzisochromanquinone unit).[19,20] The remaining NMR data were similar to those of physcion[16,21,22] except for the absence of an aromatic proton at $\delta_H$ 6.67. It was evident from the HMBC correlations as summarized diagrammatically in Fig. 1. On the basis of this observation, it is proposed that **1** is a combination of benzisochromanquinone and anthraquinone. The biaryl connectivity was determined at the C-8 and C-7′ positions on the basis of the facts that C-7′ and C-8 were quaternary carbons and their chemical shifts were downfield. On the other hand, in HMBC spectrum, the C-8 terminus of biaryl bond at $\delta_C$ 126.3 correlated with H-13 at $\delta_H$ 2.24, while C-7′ at $\delta_C$ 121.4 correlated with H-5′ at $\delta_H$ 7.50.[23—25] The relative *cis*-configurations at C-11 and C-14 in **1** could be deduced from the nuclear Overhauser effect (NOE) experiment in which on irradiation of H-12 signal at $\delta_H$ 1.70 (3H, d, $J$=6.5 Hz) an enhancement of H-15 signal at $\delta_H$ 1.23 (3H, d, $J$=6.5 Hz) was observed, while on irradiation of H-11 signal at $\delta_H$ 5.15 (1H, q, $J$=6.5 Hz) an enhancement of H-14 signal at $\delta_H$ 3.65 (1H, m) was observed. Furthermore, the chirality at the asymmetric centers must be 11*R*, 14*S* considering the fact that presumably all of the related benzisochromanquinone isolated to date from the Rhamnaceae genus.[19,20] On the other hand, the absolute configuration at the chiral axis was also determined from the circular dichroism (CD) spectrum. The spectrum exhibited a strong negative Cotton effect at longer wavelength and a strong positive Cotton effect at shorter wavelength close to 280 nm. Compound **1** was designated to be *S* according to the Prelog–Helmchen.[26—28] Therefore, compound **1** was elucidated as depicted and named floribundiquinone A.

Floribundiquinone B (**2**) was obtained as red needles. The IR and UV spectra and optical rotation were very similar to **1**. The HR-EI-MS of **2** showed a molecular ion peak at *m*/*z* 570.1535 corresponding to the same molecular formula $C_{32}H_{26}O_{10}$ as **1**. Compared the NMR data of **2** with the corresponding signals of **1** (Tables 1, 2), the only difference was that two methylene protons of benzisochromanquinone unit were well separated and split to regular multiplets at $\delta_H$ 2.16 (1H, dt, $J$=16.5, 2.0 Hz) and 2.40 (1H, ddd, $J$=16.5, 11.0, 4.0 Hz), respectively, in **2**, instead of that they were overlapped and appeared as an irregular broad singlet at $\delta_H$ 2.24 (2H, br s) in **1**.[19] All of above proposed that the two compounds were a pair of diasteroisomers.[29] Furthermore, the same relative configurations as **1** at C-11 and C-14 were determined based on the NOE experiments. But the CD spectrum of **2** showed a strong positive Cotton effect at longer wavelength and a strong negative Cotton effect at shorter wavelength close to 280 nm, which was just opposite to **1**. So, it is obvious that **1** and **2** are atropisomers. Therefore, the absolute stereochemistry of the biaryl axis in **2** must be *R*.

Floribundiquinone C (**3**) was obtained as a brown powder. Its UV and IR spectra were very similar to **2**. The HR-EI-MS of **3** showed a molecular ion peak at *m*/*z* 540.1432 corresponding to molecular formula $C_{31}H_{24}O_9$. The $^1$H-NMR spectrum of **3** (Table 1) indicated the presence of three phenolic hydroxyl groups at $\delta_H$ 13.39 (1H, s), 12.36 (1H, s), and 11.97

Table 2. $^{13}$C-NMR Data for Compounds **1**, **2**, **3** and **4** in $CDCl_3$

| Carbon | 1 | 2 | 3 | 4 | Carbon | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 179.5 | 179.4 | 179.4 | 183.6 | 1′ | 162.5 | 162.5 | 162.7 | 162.1 |
| 2 | 160.5 | 160.7 | 160.8 | 143.7 | 2′ | 124.5 | 124.6 | 124.3 | 123.8 |
| 3 | 108.5 | 108.5 | 108.5 | 145.9 | 3′ | 148.6 | 148.6 | 149.4 | 148.1 |
| 4 | 191.2 | 191.3 | 191.3 | 187.5 | 4′ | 121.4 | 121.4 | 121.4 | 121.0 |
| 5 | 159.0 | 159.0 | 159.0 | 164.9 | 4′a | 133.1 | 133.1 | 132.9 | 133.7 |
| 6 | 136.8 | 136.8 | 137.3 | 104.4 | 5′ | 103.7 | 103.4 | 120.4 | 123.4 |
| 7 | 143.9 | 144.0 | 143.1 | 165.8 | 6′ | 163.3 | 162.8 | 136.5 | 164.8 |
| 8 | 126.3 | 126.2 | 128.9 | 123.4 | 7′ | 121.4 | 121.3 | 135.7 | 103.9 |
| 9 | 125.4 | 125.5 | 126.2 | 128.9 | 8′ | 160.7 | 161.0 | 159.7 | 164.0 |
| 10 | 112.6 | 112.6 | 112.3 | 110.3 | 8′a | 111.2 | 111.2 | 115.7 | 109.7 |
| 11 | 71.1 | 71.0 | 71.1 | 69.7 | 9′ | 191.2 | 191.2 | 192.8 | 190.8 |
| 12 | 20.9 | 21.0 | 20.9 | 21.1 | 9′a | 113.6 | 113.6 | 113.8 | 113.7 |
| 13 | 34.9 | 34.9 | 36.6 | 30.7 | 10′ | 182.0 | 182.1 | 181.9 | 182.7 |
| 14 | 69.0 | 69.0 | 69.0 | 68.6 | 10′a | 134.6 | 134.7 | 133.4 | 128.9 |
| 15 | 21.3 | 21.4 | 21.3 | 21.1 | 3′-Me | 22.2 | 22.2 | 22.3 | 22.1 |
| 2-OMe | 56.5 | 56.5 | 56.6 | 56.5 | 6′-OMe | 56.5 | 56.6 | | 56.4 |

(1H, s), one methoxy at $\delta_H$ 3.83 (3H, s), one aromatic methyl group at $\delta_H$ 2.49 (3H, s), two *meta*-coupled protons at $\delta_H$ 7.11 (1H, d, $J$=2.0 Hz) and 7.69 (1H, d, $J$=2.0 Hz), and two *ortho*-coupled protons at $\delta_H$ 7.94 (1H, d, $J$=7.5 Hz) and 7.41 (1H, d, $J$=7.5 Hz). Additionally, the resonances of two doublet methyl groups at $\delta_H$ 1.23 (3H, d, $J$=6.5 Hz) and 1.68 (3H, d, $J$=6.5 Hz), two methine protons at $\delta_H$ 5.16 (1H, q, $J$=6.5 Hz) and 3.62 (1H, m), and two regular multiplets at $\delta_H$ 2.14 (1H, dt, $J$=16.5, 3.0 Hz) and 2.32 (1H, dd, $J$=16.5, 13.0 Hz) suggested the existence of a 3,4-fused 5,6-dihydro-2,6-dimethyl-2*H*-pyran system. While two methylene protons of benzisochromanquinone unit were also split to two multiplets as **2**. Further compared the NMR data of **3** with those of the corresponding signals in **2**, the significant difference was absence of a methoxy signal at C-6′ in **3**. The detail assignments were accomplished by means of the NMR spectroscopic analysis including the heteronuclear multiple-quantum coherence (HMQC) and HMBC (Tables 1, 2). The same relative configurations at C-11 and C-14 were determined as **1** based on the NOE experiments. The chirality of axis was determined as *S* from the CD spectrum. Thus, all these results indicated structure of **3** as shown.

Floribundiquinone D (**4**) was a red powder. The $^1$H-NMR spectrum (Table 1) of **4** exhibited the presence of three phenolic hydroxyl groups at $\delta_H$ 13.14 (1H, s), 13.08 (1H, s) and 12.15 (1H, s), two methoxy groups at $\delta_H$ 3.75 (3H, s) and 3.70 (3H, s), one aromatic methyl group at $\delta_H$ 2.34 (3H, s), two doublet methyl groups at $\delta_H$ 1.25 (3H, d, $J$=6.5 Hz) and 1.60 (3H, d, $J$=7.0 Hz), two methine protons at $\delta_H$ 4.80 (1H, q, $J$=7.0 Hz) and 3.47 (1H, m), and methylene protons at $\delta_H$ 2.04 (1H, ddd, $J$=18.0, 10.0, 4.0 Hz) and 2.46 (1H, dt, $J$=18.0, 2.5 Hz). The spectrum also revealed the presence of two *meta* coupled protons at $\delta_H$ 7.36 (1H, d, $J$=2.0 Hz) and 7.03 (1H, d, $J$=2.0 Hz) and two sharp singlets at $\delta_H$ 6.76 (1H, s) and 6.71 (1H, s). The $^{13}$C-NMR spectrum of **4** (Table 2) contained 32 carbon signals with four carbonyl carbons. These observations suggested that **4** was still a combination of physcion and naphthoquinone with a 5,6-dihydro-2,6-dimethyl-2*H*-pyran ring. But careful analysis revealed that two protons at $\delta_H$ 6.76 (1H, s) and 6.71 (1H, s) appeared in **4**, instead of the quinonoid proton at $\delta_H$ 6.05 adjacent to the methoxyl group and the proton at $\delta_H$ 7.50 (1H, s, 5′-H) in **1**,[21,22] suggesting that the pattern of a 5,6-dihydro-2,6-dimethyl-2*H*-pyran ring fused to naphthoquinone unit and the biaryl connectivity in **4** were not identical with those in **1**. These were further confirmed by HMBC spectrum (Fig. 1). In HMBC spectrum the correlations of H-6 at $\delta_H$ 6.76 (1H, s) with C-7 at $\delta_C$ 165.8, C-8 at $\delta_C$ 123.4 and C-10 at $\delta_C$ 110.3 and H-7′ at $\delta_H$ 6.71 (1H, s) with C-5′ at $\delta_C$ 123.4, C-6′ at $\delta_C$ 164.8 and C-8′a at $\delta_C$ 109.7 were observed. So, the 5,6-dihydro-2,6-dimethyl-2*H*-pyran system was fused at the 3,4-position[19,28] and the biaryl connectivity was determined at the C-8 and C-5′ positions associated with the shift principle.[25,29] The same relative configurations at C-11 and C-14 as **1** were determined based on the NOE experiments. The chirality of axis was determined as *R* from the CD spectrum. Thus, compound **4** was elucidated as depicted and named floribundiquinone D.

Table 3. Hepatoprotective Effects of Compounds **1**—**4**, **6**, and **7** against D-Galactosamine-Induced Toxicity in WB-F344 Cells[a]

| Compounds | Cell survival rate (% of normal) | Inhibition (% of control) |
|---|---|---|
| Normal | 100.0±4.1 | |
| Control | 47.5±13.1 | |
| Bicyclol[b] | 55.5±4.6 | 15.2 |
| **1** | 68.9±6.5 | 40.9 |
| **2** | 55.8±6.9 | 15.8 |
| **3** | 81.1±15.5* | 64.0 |
| **4** | 76.0±6.0* | 54.3 |
| **6** | 36.1±3.8 | −21.8 |
| **7** | 63.1±10.5 | 29.8 |

*a*) Results are expressed as means S.D. ($n$=3; for normal and for control, $n$=6). *b*) Positive control substance. * $p<0.01$, significantly different from control by Students's $t$-test.

Hepatoprotective activities of compounds **1**—**4**, **6** and **7** from EtOAc fraction of *B. floribunda* were examined against D-galactosamine-induced toxicity in WB-F344 cells (Table 3) using bicyclol, a drug showing hepatoprotective activity[30] as a positive control. Compounds **3** and **4** showed a potent inhibitory activity at $10^{-4}$ M *in vitro*.

## Experimental

**General Experimental Procedures** All melting points were determined on a Reichert Nr-229 micromelting point apparatus and are uncorrected. The

optical rotations were measured on a Perkin-Elmer 34/LC polarimeter. UV spectra were recorded on HP 8453 UV–Visible spectrophotometer. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. $^1$H-NMR (500 MHz), $^{13}$C-NMR (125 MHz), NOE, HMQC and HMBC spectra were run on an INOVA-500 spectrometer with tetramethylsilane (TMS) as internal standard and values were given in ppm ($\delta$). Electrospray ionization (ESI) was performed on Agilent 1100 series LC/MSD Trap mass spectrometer (SL). EI-MS and HR-EI-MS were performed on AutoSpec Ultima-TOF mass spectrometer. HR-ESI-MS was performed on Finnigan LTQ FTMS. Silica gel (100—200, 200—300 mesh) (Qingdao) and silica gel GF-254 (Qingdao) for TLC.

**Plant Material** The roots of *B. floribunda* (WALL.) BRONGN. were collected from Jianfengling in Hainan province of People's Republic of China in July 2005. The plant material was identified by Professor Shi-Man Huang. A voucher specimen has been deposited in the Herbarium of the Department of Medicinal plants, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P. R. China.

**Extraction and Isolation** The dried roots of *B. floribunda* (WALL.) BRONGN. (8.5 kg) were exhaustively extracted four times with 95% EtOH under reflux. The EtOH extract was then concentrated under reduced pressure to give a residue (817.0 g), which was suspended in $H_2O$, and the suspension was then extracted with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (50.8 g) was chromatographed over silica gel column eluting with petroleum ether–acetone (in gradient), followed by MeOH to yield 8 fractions (Fr. 1—8). Compound **6** [(80.0 mg) petroleum ether/acetone (50 : 1)] was crystallized in acetone as yellow needles. Compound **7** [(46.0 mg) petroleum ether/acetone (30 : 1)] was crystallized in acetone as yellow needles from Fr. 1. Compound **8** [(14.0 mg) petroleum ether/acetone (30 : 1)] was crystallized in acetone as yellow needles from Fr. 2. Fr. 3 was chromatographed over a silica gel column and eluted with petroleum ether–acetone (in gradient) to give 4 subfractions and then Fr. 3-2 was chromatographed over a silica gel column and eluted with petroleum ether–acetone (in gradient) again to give 2 subfractions, compound **4** [(33.0 mg) petroleum ether/acetone (10 : 1)] was crystallized in acetone as a red powder from Fr. 3-2-1 and compound **5** [(18.0 mg) petroleum ether/acetone (10 : 1)] was crystallized in acetone as a yellow amorphous powder from Fr. 3-2-2. Fr. 4 was chromatographed over a silica gel column and eluted with petroleum ether–acetone (in gradient) to give 4 subfractions and then Fr. 4-2 was crystallized in acetone to give compound **3** [petroleum ether/acetone (8 : 1) 28.0 mg]. Fr. 5 was chromatographed over a silica gel column and eluted with petroleum ether–acetone (in gradient) to give 4 subfractions and then Fr. 5-1 was crystallized in acetone to give compound **1** [(36.0 mg) petroleum ether/acetone (4 : 1)], Fr. 5-2 was crystallized in acetone to give compound **2** [(43.0 mg) petroleum ether/acetone (4 : 1)]. Compound **9** (26.0 mg) was crystallized in acetone as a yellow amorphous powder from Fr. 6 [petroleum ether/acetone (3 : 1)]. Compound **10** (22.0 mg) was crystallized in acetone as a red amorphous powder from Fr. 8 [petroleum ether/acetone (1 : 1)].

Floribundiquinone A (**1**): Brown powders; mp 195—197 °C, $[\alpha]_D^{20}$ +167°($c$=0.06, $CHCl_3$), UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$): 203 (4.55), 222 (4.63), 254 (4.34), 283 (4.45), 434 (4.11) nm; CD (MeOH, $c=3.3\times10^{-4}$) nm ([$\theta$], deg·cm$^2$·dmol$^{-1}$): 235 (+47500), 262 (+1450), 267 (+9360), 292 (−102940), 311 (+9060), 400 (+1650), 461 (+25730); IR (KBr) $\nu_{max}$ 2974, 2850, 1682, 1626, 1603, 1481, 1381, 1277, 1205, 1095 cm$^{-1}$; $^1$H-NMR (500 MHz, $CDCl_3$): see Table 1; $^{13}$C-NMR data (125 MHz, $CDCl_3$): see Table 2; ESI-MS(+) *m/z* 593 [M+Na]$^+$, ESI-MS(−) *m/z* 569 [M−1]$^-$, EI-MS *m/z* (rel. int.) 570 (39), 555 (73), 439 (64), 331 (100), 171 (42), 115 (15), HR-EI-MS *m/z* 570.1513 (Calcd for 570.1526).

Floribundiquinone B (**2**): Red needles; mp 195—197 °C, $[\alpha]_D^{20}$ +161° ($c$=0.08, $CHCl_3$), UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$): 202 (4.48), 222 (4.54), 254 (4.24), 283 (4.42), 434 (3.99) nm; CD (MeOH, $c=4.2\times10^{-4}$) nm ([$\theta$], deg·cm$^2$·dmol$^{-1}$): 245 (−4300), 257 (+3690), 274 (−20080), 291 (+32300), 308 (−11950), 400 (+7300), 461 (−3950); IR (KBr) $\nu_{max}$ 2980, 2850, 1680, 1630, 1600, 1480, 1380, 1280, 1210, 1100 cm$^{-1}$; $^1$H-NMR (500 MHz, $CDCl_3$): see Table 1; $^{13}$C-NMR data (125 MHz, $CDCl_3$): see Table 2; HR-EI-MS *m/z* 570.1535 (Calcd for 570.1526).

Floribundiquinone C (**3**): Brown powders; mp 124—126 °C, $[\alpha]_D^{20}$ +461°($c$=0.12, $CHCl_3$), UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$): 202 (4.50), 228 (4.55), 261 (4.38), 294 (4.22), 434 (4.14) nm; CD (MeOH, $c=2.8\times10^{-4}$) nm ($\theta$, deg·cm$^2$·dmol$^{-1}$): 232 (+51870), 281 (−36450), 301 (+5090) 311 (−5090), 347 (+4430), 408 (+60), 463 (+14540); IR (KBr) $\nu_{max}$ 2960, 2924, 2850, 1734, 1684, 1628, 1603, 1479, 1423, 1383, 1265, 1201, 1113, 1099, 1076, 1024, 989 cm$^{-1}$; $^1$H-NMR (500 MHz, $CDCl_3$): see Table 1; $^{13}$C-NMR data (125 MHz, $CDCl_3$): see Table 2; HR-EI-MS *m/z* 540.1432 (Calcd for 540.1420).

Floribundiquinone D (**4**): Red powders; mp 165—168 °C, $[\alpha]_D^{20}$ −48° ($c$=0.05, $CHCl_3$), UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$): 202 (4.26), 225 (4.30), 251 (4.16), 257 (4.16), 305 (3.88) (sh), 452 (3.83) nm; CD (dioxane, $c=1.4\times10^{-4}$) nm ($\theta$, deg·cm$^2$·dmol$^{-1}$): 229 (−61070), 241 (−20360), 256 (−119290), 276 (+71250), 309 (+27030), 351 (−730), 431 (+22030), 488 (−20440); IR (KBr) $\nu_{max}$ 2922, 2850, 1628, 1604, 1464, 1388, 1261, 1246, 1207, 1103, 1030, 995 cm$^{-1}$; $^1$H-NMR data (500 MHz, $CDCl_3$): see Table 1; $^{13}$C-NMR data (125 MHz, $CDCl_3$): see Table 2; ESI-MS(+) *m/z* 571, [M+1]$^+$ HR-ESI-MS [M+H]$^+$ *m/z* 571.1617 (Calcd for 571.1604).

**Hepatoprotective Activity Assay. Protective Effect on Cytotoxicity Induced by D-Galactosamine in WB-F344 Cells** The hepatoprotective effects of compounds **1**—**4**, **6** and **7** were determined by a 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[31,32] in WB-F344 Cells, with some modification. Each cell suspension of $1\times10^4$ cells in 200 $\mu$l of Dulbecco's modified Eagle's medium containing fetal calf serum (3%), penicillin (100 units/ml), and streptomycin (100 $\mu$g/ml) was planted in a 96-well microplate and precultured for 24 h at 37 °C under a 5% $CO_2$ atmosphere. Fresh medium (200 $\mu$l) containing bicyclol and test samples was added, and the cells were cultured for 1 h. Then, the cultured cells were exposed to 40 mM D-galactosamine for 24 h. Cytotoxic effects of test samples were measured simultaneously in the absence of D-galactosamine. The medium was changed into a fresh one containing 0.5 mg/ml MTT. After 3.5 h incubation, the medium was removed and 150 $\mu$l of dimethyl sulfoxide was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm. Inhibition (%) was obstained by the following formula:

$$\text{inhibition (\%)}=[(OD_{(sample)}-OD_{(control)})]/[(OD_{(normal)}-OD_{(control)})]\times100$$

**Statistical Analysis** All values were expressed as ±S.D. The Student's *t*-test for unpaired observations between normal or control and tested samples was carried out to identify statistical differences; $p$ values less than 0.01 were considered as significantly different.

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