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Abietane Diterpenoids from the Bark of *Cryptomeria fortunei*

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Four new abietane diterpenoids, fortunins A−D (**1**−**4**), and six new 18-hydroxymethylene abietane diterpenoids, fortunins E−J (**5**−**10**), along with 12 known diterpenoids, were isolated from the bark of *Cryptomeria fortunei*. The structures of new compounds were established on the basis of 1D and 2D NMR and other spectroscopic analyses.

The genus *Cryptomeria* D. Don (Taxodiaceae) includes important timber trees in China and Japan. Previous investigations on the constituents of the Japanese cedar *C. japonica* (L. f) D. Don resulted in the isolation of biflavones,[1] sesquiterpenes,[2] abietane-type diterpenes,[3–5] and some unique triterpenes.[6–8] Recently, abietane-type diterpenes with novel skeletons, such as diterpenes attached to sesquiterpenes[9,10] and dimeric diterpenes,[11] were reported from the bark and heartwood of *C. japonica*. However, no diterpenoids were reported from the Chinese cedar, *Cryptomeria fortunei* Hooibrenk ex Otto et Dietr.[12]

This paper describes a detailed investigation on the chemical constituents of *C. fortunei*. The plant is widely distributed south of the Yangtze River in China. Its root bark has been used in folk medicine as detoxification and insecticidal agents.[13] Bark of the title plant has now yielded four abietane diterpenoids, fortunins A−D (**1**−**4**), and six 18-hydroxymethylene abietane diterpenoids, fortunins E−J (**5**−**10**). Their structures were elucidated on the basis of extensive spectroscopic analyses. Also identified were 12 known diterpenes: salviviridinol[19] (**11**), sugikurojin D[9] (**12**), 6β-hydroxyferruginol[14] (**13**), iguestol[15] (**14**), callicarpone[21] (**15**), (+)-sugiol[4] (**16**), 11-hydroxysugiol[16] (**17**), hypargenin B[20] (**18**), 5,6-dehydrosugiol[17] (**19**), 6-hydroxy-5,6-dehydrosugiol[18] (**20**), 14-deoxycoleon U[16] (**21**), and 18-hydroxyferruginol[22] (**22**).

Results and Discussion

Fortunin A (**1**), an amorphous solid, had the molecular formula $C_{22}H_{32}O_3$ based on HREIMS (*m*/*z* 344.2342). The IR spectrum showed absorption bands at 3425 (OH), 1756 (C=O) and 1610 and 1494 cm^{-1} (aromatic). UV maxima at 267 and 275 nm supported the presence of an aromatic ring. The ^{13}C NMR and DEPT spectra indicated 22 resonances including signals of an acetyl group at δ_C 169.8 (C=O) and 20.9 (CH_3) (Table 1). The ^{1}H NMR spectrum of **1** (Table 2) showed signals of three singlet methyl groups at δ_H 0.93, 0.96, and 1.25, an isopropyl group at δ 1.20, 1.22 (each 3H, d, J = 6.9) and 2.89 (1H, sept, J = 6.9), two singlet *para* aromatic protons (δ 6.82 and 7.48), and an oxygenated methine at δ 4.80 (1H, dd, J = 8.6, 7.9). These data indicated an abietane diterpene skeleton similar to that of 7β-hydroxyferruginol[23] (**1a**) except for an extra acetoxyl group. The acetoxyl group was suggested to be at C-12 by the upfield shift of H-15 (δ 2.89 in **1** vs δ 3.20 in **1a**), and this was confirmed by the NOE difference correlation between the methyl signal at δ_H 2.32 and H-15. The ROESY correlation of H-7/H-5α suggested that H-7 was α-oriented. Thus, **1** was established as 7β-hydroxy-12-acetoxyabieta-8,11,13-triene.

Fortunin B (**2**) had the molecular formula $C_{24}H_{34}O_5$ as determined by HREIMS (*m*/*z* 402.2403). UV absorptions were present at 267 and 275 nm. The IR spectrum showed the presence of OH (3453 cm^{-1}), ketone (1739 cm^{-1}), and aromatic groups (1611 and 1498 cm^{-1}). The ^{1}H NMR spectrum (Table 2) displayed signals for a typical isopropyl moiety attached to a phenyl group, two *para* aromatic protons, two acetyl groups, three singlet methyls, and an ABX system at δ_H 1.75 (1H, d, J = 11.7), 5.63 (1H, dd, J = 11.7, 5.3), and 4.69 (1H, br d, J = 5.3). These data were similar to those of 6α,7β-dihydroxyferruginol[24] (**2a**) except for two extra acetyl groups, suggesting that **2** was a derivative of **2a**. The location of an acetoxyl group at C-6 was deduced from the HMBC correlation between H-6 and the acetyl carboxyl at δ 172.5, and H-6 was indicated to be β-oriented by the ROESY cross-peak of H-6/CH_3-20β. The second acetoxyl group was deduced to be at C-12 on the basis of the correlation between H-15 and the acetyl methyl at δ 2.18 in the ROESY spectrum. The β-OH at C-7 was confirmed by the ROESY cross-peak of H-7/H-5α. Thus, the structure of **2** was determined as 6α,12-diacetoxy-7β-hydroxyabieta-8,11,13-triene.

Fortunin C (**3**) was determined to have the molecular formula $C_{22}H_{34}O_4$ by HREIMS. UV absorption was present at 282 nm, and the IR spectrum showed the presence of OH (3363 cm^{-1}) and phenyl (1612 and 1488 cm^{-1}) groups. The MS and NMR data (Tables 1 and 2) revealed that **3** had a structural similarity to salviviridinol[19] (**11**) with an extra 15 mass units. This was consistent with an OCH_3 (δ_H 3.76) in **3** instead of an OH as in **11**. Attachment of the OCH_3 group to C-7 was elucidated from the HMBC correlation between H-7 and the methyl resonance at δ 53.3. ROESY cross-peaks of H-7/H-5α and H-6/CH_3-20β suggested an α-orientation of H-7 and a β-orientation of H-6 (Figure 1). Thus, **3** was designated as 6α,11-dihydroxy-7β,12-dimethoxyabieta-8,11,13-triene.

Fortunin D (**4**) was assigned the same molecular formula, $C_{22}H_{34}O_4$, as that of **3**. The ^{1}H and ^{13}C NMR data of **4** (Tables 1 and 2) closely resembled those of **3**, and further spectroscopic analysis revealed that **4** was a stereoisomer of **3**. The major ^{1}H and ^{13}C NMR differences were observed at C-7. Comparing the coupling constants of H-7 in these two compounds ($J_{6,7}$ = 8.8 Hz in **3** vs $J_{6,7}$ = 3.8 Hz in **4**), the relative configuration of H-7 in **4** was suggested to be β-oriented. Thus, **4** was identified as 6α,11-dihydroxy-7α,12-dimethoxyabieta-8,11,13-triene.

The HREIMS of fortunin E (**5**) (*m*/*z* 332.1989 [M]$^+$) suggested its molecular formula to be $C_{20}H_{28}O_4$. An aromatic system was indicated by the UV maxima at 230 and 284 nm, as well as IR absorptions at 1605 and 1506 cm^{-1}. The IR spectrum also showed absorptions indicating conjugated ketone (1663 cm^{-1}) and OH groups (3359 cm^{-1}). The ^{13}C NMR and DEPT experiments (Table 1) showed 22 signals ascribed to four methyls, four methylenes, five methines, and seven quaternary carbon atoms. The ^{1}H NMR spectrum (Table 3) displayed signals of two singlet methyl groups at δ 1.16 and 1.32 and an isopropyl group at δ 1.23, 1.25 (each 3H, d, J = 7.5) and 3.19 (1H, sept, J = 7.5), which was indicative of an abietane skeleton.[25] Comparing the ^{1}H and ^{13}C NMR data of compounds **1**−**4**, the absence of the fifth methyl and the presence

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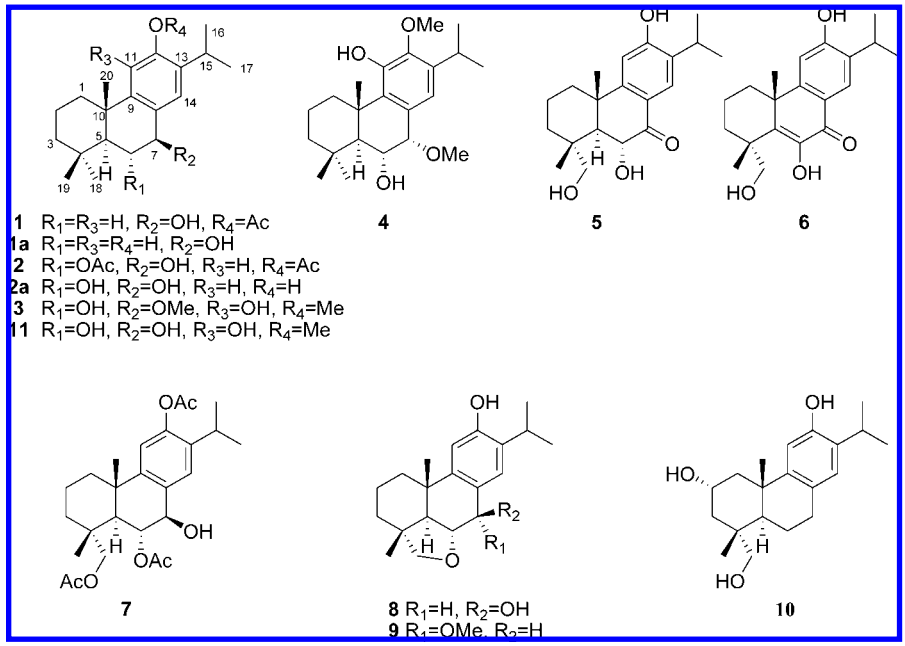
Chart 1



of a methylene [δ_C 75.0; δ_H 3.23 (1H, d, $J = 11.6$) and 3.56 (1H, d, $J = 11.6$)] suggested the presence of a hydroxymethylene instead of a methyl in **5**. This hydroxymethylene group was assigned to C-4 by the HMBC correlations of H-18/C-4, H-18/C-19, and H-19/C-18. Two aromatic proton signals at δ 6.74 (1H, s) and 7.93 (1H, s) were assigned to H-11 and H-14. The AX signals at δ 2.04 (1H, d, $J = 12.7$) and 4.50 (1H, d, $J = 12.7$) were attributed to H-5 and H-6, respectively. Thus, OH groups were assigned to C-6 and C-12. The carboxyl was confirmed at C-7 by a long-range correlation between H-14 (δ 7.93) and the ketone (δ 198.5) in the HMBC spectrum. ROESY cross-peaks of H-6/CH_3-20β, CH_3-19/CH_3-20β, and CH_3-6/CH_3-19β indicated the α-orientation of OH-6. Therefore, **5** was established as 6α,12,18-trihydroxyabieta-8,11,13-trien-7-one.

The HREIMS (*m*/*z* 330.1823 $[M]^+$) determined that fortunin F (**6**) had the molecular formula $C_{20}H_{26}O_4$, 2 mass units less than that of **5**. The UV maxima at 280 and 333 nm indicated an aromatic system. The IR spectrum confirmed the presence of the aromatic system (1596, 1467 cm^{-1}), conjugated ketone (1680 cm^{-1}), and OH (3369 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1) indicated 20 resonances including a conjugated ketone signal at δ_C 179.5 (C═O) and eight low-field signals at δ 137.1 (C), 142.5 (C), 120.7 (C), 155.1 (C), 111.1 (CH), 158.6 (C), 134.4 (C), and 125.9 (CH). These data suggested that **6** contained a ketone, a double bond, and a phenyl group. The ^1H NMR data of **6** (Table 2) were similar to those of **5**, except for the absence of the H-5 and H-6 AX signals in **5**. Thus, compound **6** was deduced to be a 5,6-dehydrogenated derivative of **5**. The double bond was assigned to C-5 and C-6 by HMBC correlations of CH_3-20/C-5 (δ 137.1), CH_3-19/C-5 (δ 137.1), and CH_2-18/C-5 (δ 137.1). Thus, **6** was established as 6,12-dihydroxyabieta-5,8,11,13-tetraene-7-one.

The molecular formula of fortunin G (**7**) was established as $C_{26}H_{36}O_7$ by its HRESIMS (*m*/*z* 483.2356 $[M + Na]^+$). The IR spectrum showed the OH (3442 cm^{-1}), aromatic (1605 and 1463 cm^{-1}), and acetyl (1735 and 1240 cm^{-1}) absorptions. The UV spectrum showed a maximum at 210 nm. The ^{13}C NMR and DEPT spectra (Table 1) showed 26 signals including three acetyl groups.

Table 1. ^{13}C NMR Data of Compounds **1**−**10** (in $CDCl_3$, 100 MHz)[a]

C	1	2	3	4	5	6	7	8[b]	9	10[c]
1	38.8 CH_2	38.8 CH_2	36.5 CH_2	37.1 CH_2	38.5 CH_2	35.6 CH_2	38.3 CH_2	38.9 CH_2	38.1 CH_2	48.9 CH_2
2	19.0 CH_2	18.6 CH_2	19.0 CH_2	19.2 CH_2	18.6 CH_2	17.9 CH_2	17.9 CH_2	20.3 CH_2	20.0 CH_2	66.6 CH
3	41.2 CH_2	43.3 CH_2	42.8 CH_2	42.4 CH_2	37.8 CH_2	35.5 CH_2	37.2 CH_2	35.2 CH_2	34.8 CH_2	45.8 CH_2
4	33.1 qC	33.4 qC	33.8 qC	33.6 qC	39.1 qC	41.9 qC	36.5 qC	40.0 qC	39.5 qC	40.5 qC
5	49.1 CH	52.3 CH	53.3 CH	50.7 CH	52.8 CH	137.1 qC	45.4 CH	55.0 CH	49.8 CH	44.7 CH
6	30.3 CH_2	78.9 CH	68.7 CH	69.7 CH	72.5 CH	142.5 qC	78.4 CH	79.3 CH	75.5 CH	20.4 CH_2
7	71.1 CH	76.7 CH	85.9 CH	81.9 CH	198.5 qC	179.5 qC	76.3 CH	77.0 CH	80.1 CH	30.8 CH_2
8	136.0 qC	133.1 qC	130.0 qC	130.4 qC	120.7 qC	120.7 qC	133.1 qC	130.1 qC	126.5 qC	127.1 qC
9	148.8 qC	148.0 qC	133.4 qC	132.0 qC	156.7 qC	155.1 qC	147.5 qC	147.4 qC	147.3 qC	148.9 qC
10	38.3 qC	39.4 qC	41.8 qC	41.5 qC	39.0 qC	40.8 qC	39.3 qC	37.9 qC	37.6 qC	40.6 qC
11	118.0 CH	117.1 CH	146.1 qC	146.6 qC	110.4 CH	111.1 CH	117.2 CH	111.2 CH	111.5 CH	111.8 CH
12	147.6 qC	148.0 qC	143.9 qC	144.7 qC	161.0 qC	158.6 qC	148.0 qC	155.1 qC	153.7 qC	153.8 qC
13	137.5 qC	138.2 qC	138.7 qC	138.0 qC	134.6 qC	134.4 qC	138.4 qC	133.9 qC	132.7 qC	134.0 qC
14	125.7 CH	127.3 CH	115.8 CH	118.1 CH	127.5 CH	125.9 CH	127.3 CH	127.0 CH	130.0 CH	127.8 CH
15	27.3 CH	27.4 CH	26.4 CH	26.4 CH	27.0 CH	26.9 CH	27.4 CH	27.7 CH	26.9 CH	28.2 CH
16	22.9 CH_3	22.8 CH_3	23.6 CH_3	23.5 CH_3	22.5 CH_3	22.2 CH_3	22.7 CH_3	23.1 CH_3	22.4 CH_3	23.7 CH_3
17	23.0 CH_3	22.9 CH_3	23.8 CH_3	23.8 CH_3	22.6 CH_3	22.4 CH_3	23.0 CH_3	23.1 CH_3	22.6 CH_3	23.7 CH_3
18	21.5 CH_3	22.1 CH_3	21.3 CH_3	21.1 CH_3	17.7 CH_3	22.6 CH_3	18.0 CH_3	19.0 CH_3	18.8 CH_3	19.3 CH_3
19	33.1 CH_3	35.7 CH_3	36.0 CH_3	35.5 CH_3	75.0 CH_2	71.8 CH_2	73.8 CH_2	83.5 CH_2	84.4 CH_2	72.1 CH_2
20	25.2 CH_3	25.8 CH_3	23.0 CH_3	22.8 CH_3	25.3 CH_3	22.6 CH_3	26.1 CH_3	24.0 CH_3	23.3 CH_3	27.1 CH_3

[a] $CH_3\underline{C}O$-12 (169.8, C), $\underline{C}H_3CO$-12 (20.9, CH_3) in **1**, $CH_3\underline{C}O$-6 (172.5, C), $\underline{C}H_3CO$-6 (20.9, CH_3), $CH_3\underline{C}O$-12 (169.8, C), $\underline{C}H_3CO$-12 (21.7, CH_3) in **2**, CH_3O-7 (53.3, CH_3), CH_3O-12 (61.8, CH3) in **3**, CH_3O-7 (58.1, CH_3), CH_3O-12 (61.7, CH_3) in **4**, $CH_3\underline{C}O$-6 (172.6, C), $\underline{C}H_3CO$-6 (20.9, CH_3), $CH_3\underline{C}O$-12 (169.7, C), $\underline{C}H_3CO$-12 (21.0, CH_3), $CH_3\underline{C}O$-18 (171.1, C), $\underline{C}H_3CO$-18 (21.6, CH_3) in **7**, CH_3O-7 (59.7, CH_3) in **9**. [b] Measured in Py-d_5. [c] Measured in CD_3OD; the others measured in $CDCl_3$.

Table 2. ^{1}H NMR Data of Compounds **1**−**4** (in $CDCl_3$, δ in ppm and J in Hz)

H	**1** (300 MHz)	**2** (300 MHz)	**3** (600 MHz)	**4** (600 MHz)
1α	1.39 dd, 1.4, 12.5	1.46 m	1.34 dd, 4.1, 13.9	1.30 ddd, 4.6, 13.6, 13.6
1β	2.15 dd, 2.4, 12.5	2.14 dd, 5.5, 11.7	3.07 ddd, 4.1, 4.6, 13.9	3.01 ddd, 4.6, 4.6, 13.6
2α	1.46 m	1.53 m	1.51, dd, 4.6, 13.6	1.49, ddd, 4.6, 4.6, 13.6
2β	1.51 m	1.53 m	1.68 ddd, 3.0, 12.0, 13.6	1.56 ddd, 3.3, 3.3, 13.6
3α	1.65 m	1.62 m	1.28 ddd, 3.0, 12.0, 14.7	1.54 dd, 3.3, 11.3
3β	1.76 m	1.68 m	1.48 br d, 14.7	1.72 dd, 3.3, 11.3
5	1.39 dd, 1.3, 12.5	1.75 d, 11.7	1.53 d, 11.1	1.86 d, 10.3
6α	2.27 dd, 7.9, 12.5			
6β	1.70 ddd, 1.3, 8.6, 12.5	5.63 dd, 5.3, 11.7	4.25 dd, 8.8, 11.1	4.19 br d, 10.3
7α	4.80 dd, 8.6, 7.9	4.69 br d, 5.3	4.47 d, 8.8	
7β				4.15 d, 3.8
11	6.82 s	6.84 s		
14	7.48 s	7.36 s	6.85 s	6.71 s
15	2.89 sept, 6.9	2.95 sept, 6.8	3.20 sept, 6.8	3.22 sept, 6.8
CH_3-16	1.20 d, 6.9	1.19 d, 6.8	1.24 d, 6.8	1.25 d, 6.8
CH_3-17	1.22 d, 6.9	1.21 d, 6.8	1.24 d, 6.8	1.25 d, 6.8
CH_3-18	0.96 s	1.08 s	1.23 s	1.20 s
CH_3-19	0.93 s	1.02 s	1.21 s	1.18 s
CH_3-20	1.25 s	1.26 s	1.43 s	1.35 s
OMe-7			3.27 s	3.61 s
OMe-12			3.76 s	3.76 s
CH_3CO-6		2.18 s		
CH_3CO-12	2.32 s	2.32 s		

The ^{1}H NMR spectrum (Table 3) exhibited typical signals of a 18-hydroxymethylene abietane: two tertiary methyls at δ_H 1.00 and 1.37, an isopropyl at δ 1.20, 1.21 (each 3H, d, J = 6.4) and 2.94 (1H, sept, J = 6.4), a hydroxymethylene at δ 3.67 (1H, d, J = 10.7) and 4.13 (1H, d, J = 10.7), and two singlet aromatic protons at δ 6.84 and 7.38. The ABX type signals at δ 2.13 (1H, d, J = 11.8), 5.39 (1H, dd, J = 11.8, 5.2), and 4.64 (1H, d, J = 5.2) were assigned to H-5, H-6, and H-7, respectively. The HMBC spectrum of **7** showed long-range correlations of H-19 (δ 3.67)/C═O (δ 171.1) and H-6 (δ 5.39)/C═O (δ 172.6), indicating that two acetoxyl groups were located at C-6 and C-18, respectively. The third acetoxyl group was assigned to C-12 by the ROESY correlation between the methyl at δ 2.32 and H-15. ROESY cross-peaks of H-6/CH_3-20β, CH_3-6/CH_3-19β, and H-7/H-5α indicated that the acetoxyl group at C-6 was α-oriented and the OH at C-7 was β-oriented. Thus, **7** was determined to be 6α,12,18-triacetoxy-7β-hydroxyabieta-8,11,13-triene.

The molecular formula of fortunin H (**8**) ($C_{20}H_{38}O_3$) was inferred from its HREIMS (m/z 316.2036 $[M]^+$), indicating that **8** had seven degrees of unsaturation, one more than those of **7**. The IR spectrum showed absorptions at 3407 (OH) and 1600 and 1504 cm^{-1} (aromatic). The latter was also supported by the UV data (λ_{max} 280 nm). The ^{1}H NMR spectrum of **8** (Table 3) was similar to that of **7** except for three additional acetoxyl groups. Considering one extra unsaturated degree of **8**, an epoxy group was suggested to be present in its structure. This conclusion was supported by the long-range HMBC correlation between H-6 and C-18. The ROESY spectrum of **8** (Figure 1) indicated the same relative configuration as that of **7**; H-6 and OH-7 were β-oriented, and H-7, OH-6, and H-18 were α-oriented. Accordingly, **8** was determined to be 7β,12-dihydroxy-6α,18-epoxyabieta-8,11,13-triene.

Fortunin I (**9**) had the molecular formula $C_{21}H_{30}O_3$, as determined by its HREIMS (m/z 330.2185 $[M]^+$), 14 mass units more than that of **8**. The ^{1}H and ^{13}C NMR data of **9** (Tables 1 and 3) were similar to those of **8**, except for an additional OCH_3 at δ_H 3.63 (3H, s). This OCH_3 group was assigned to C-7 by the HMBC correlation between the OCH_3 signal at δ 3.63 and C-7 (δ 80.1). The ROESY cross-peaks of H-6/CH_3-19β and H-6/CH_3-20β indicated a β-orientation for H-6. The NOE enhancement of MeO-7 was observed by irradiation of H-5α, indicating that H-7 was also β-oriented. Thus, **9** was established as 12-hydroxy-7α-methoxy-6α,18-epoxyabieta-8,11,13-triene.

Fortunin J (**10**) had the molecular formula $C_{20}H_{30}O_3$ with six degrees of unsaturation. The IR spectrum showed an OH absorption at 3411 cm^{-1} and an aromatic group at 1618 and 1508 cm^{-1}. The latter was supported by the UV data (λ_{max} 282 nm). The ^{1}H NMR spectrum (Table 3) showed signals of two singlet methyls at δ_H 0.89 and 1.22, an isopropyl at δ 1.17, 1.18 (each 3H, d, J = 6.4) and 3.18 (1H, sept, J = 6.4), and a hydroxymethylene at δ 3.13 (1H, d, J = 10.8) and 3.44 (1H, d, J = 10.8), indicative of a 18-hydroxymethylene abietane diterpene. The signal at δ 4.04 (1H, dddd, J = 4.3, 4.5, 11.3, 11.3) indicated the presence of a proton geminal to an OH group at C-2, similar to that of pomiferin D.[25] The OH group was determined to be α-oriented by the ROESY correlations of H-2/CH_3-20β and H-2/CH_3-19β. Thus, **10** was established as 2α,12,18-trihydroxyabieta-8,11,13-triene.

In conclusion, 22 diterpenoids were obtained for the first time from the bark of *C. fortunei*.[15] Seven 18-hydroxymethylene abietane diterpenes (**5**−**10**, **22**) were reported for the first time from the genus *Cryptomeria*. Differing from 6α,18-lactone abietane diterpenoids in previous reports,[26,27] fortunin H (**8**) was the first example of an abietane-type diterpene containing a 6α,18-epoxy functionality in the plant kingdom.

Abietane diterpenes were reported to exhibit antitumor activity.[28,29] The isolated compounds were tested on HL-60 and A549 cells by SRB and MTT methods. The result (see Supporting Information) showed that compounds **3**, **20**, and **21** have weak inhibitory activity on HL-60 cells, and **21** shows weak inhibitory activity on A549 cells.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer. NMR spectra were recorded on Bruker AM-300, Bruker AM-400, and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (J) are in Hz. EIMS and HREIMS spectra were recorded on a Finnigan MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Micromass LC-MS-MS mass spectrometer. Silica gel was used for flash chromatography and was produced by Qingdao Marine Chemical Industrials. TLC and preparative TLC were carried out on precoated silica gel GF254 plates (Yantai Chemical Industrials), and the TLC spots were viewed at 254 nm. Analytical HPLC was performed on a Waters 2690 instrument with a 996 PAD (photodiode array detector) and an Alltech ELSD 2000 detector. Preparative HPLC was carried out on a Varian Pro-star solvent delivery module with a Varian Pro-star UV−vis detector.

Plant Material. The bark of *C. fortunei* was collected in Chou County, Anhui Province, China, in September 2006 and identified by

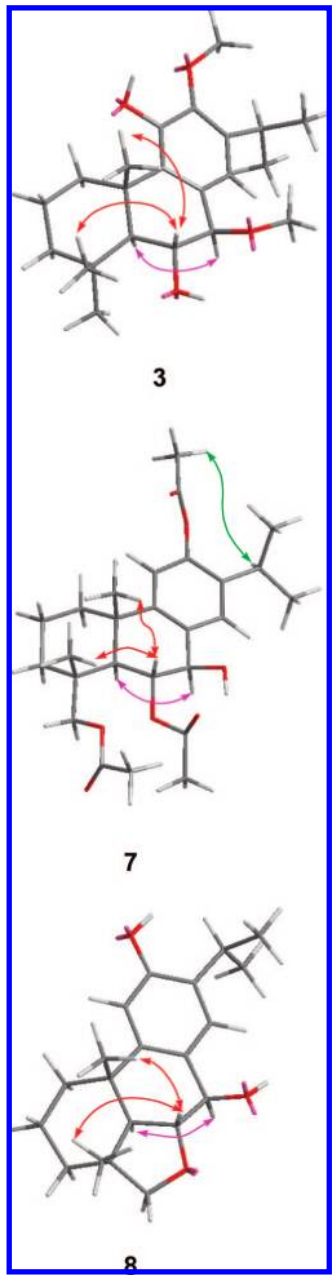


Figure 1. Key ROESY correlations of compounds **3**, **7**, and **8**.

Jingui Shen, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (20060928) has been deposited in the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. The air-dried bark of *C. fortunei* (9.5 kg) was ground into a powder and extracted with acetone. The extract was concentrated under reduced pressure to give a black syrup. The black syrup was suspended in water and then partitioned with petroleum ether (PE), CH_2Cl_2, and EtOAc successively to give fractions PE (500 g), CH_2Cl_2 (95 g), and EtOAc (160 g), respectively. The CH_2Cl_2 extract was subjected to column chromatography (CC) over silica gel and eluted with a gradient of PE/acetone to yield fractions 1−9. Fraction 2 was filtered to afford **16** (2.0 g), and the residue was subjected to CC over silica gel with PE−acetone (from 20:1 to 20:3) to give **14** (10 mg). Fraction 3 was separated over an MCI gel column with MeOH−H_2O (60%−100%) and then a silica gel column (PE−EtOAc, 100:6). Compounds **1** (35 mg), **3** (23 mg), **16** (1.4 g), **19** (15 mg), and **20** (46 mg) were obtained, respectively, after purification over a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1). Fraction 4 was separated on an MCI gel column with MeOH−H_2O (60%−100%) to give subfractions 4a−4d. From the subfractions 4a, 4c, and 4d, **2** (24 mg), **21** (49 mg), and **12** (38 mg) were obtained after purification over a Sephadex LH-20 column (MeOH). Subfraction 4b was separated by Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) and then purified with preparative HPLC (CH_3CN−H_2O from 45% to 70% in 0−192 min, 15 mL/min, 210 nm), affording **4** (14 mg). Fraction 5 was subjected to an MCI gel column eluted with MeOH−H_2O from 60% to 100% and then separated over a silica gel column eluted with CH_2Cl_2 to yield **17** (160 mg) and **18** (62 mg). Fraction 6 was subjected to an MCI gel column (MeOH−H_2O: 60%−100%) to give subfractions 6a−6d. Subfraction 6b was separated on a silica gel column eluted with CH_2Cl_2 to yield **11** (30 mg) and **22** (10 mg). Fraction 7 was separated into four subfractions, 7a−7d, by an MCI gel column (MeOH−H_2O: 60%−100%). Subfraction 7b was subjected to a silica gel column eluted with CH_2Cl_2 to CH_2Cl_2−acetone (100:6) and further purified by TLC (CH_2Cl_2−acetone, 100:6) to yield **15** (28 mg). Subfraction 7c was separated on Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) repeatedly to afford **7** (37 mg). Fraction 8 was first subjected to an MCI gel column and then separated over silica gel (CH_2Cl_2−acetone from 100:3 to 100:6) and Sephadex LH-20 (MeOH) to yield **5** (19 mg), **6** (29 mg), **8** (19 mg), and **9** (29 mg). Fraction 9 was separated over MCI gel and Sephadex LH-20 to give compound **10** (13 mg).

Fortunin A (1): white, amorphous powder; $[\alpha]^{20}_D$ +71 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max} 267 (5.09) and 275 (5.04) nm; IR ν_{max} (KBr) 3425, 1756, 1610, 1494, 1461, 1209, 1029, 912 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* 344 $[M]^+$, 302, 285, 269, 259, 227, 199, 178, 163, 149, 123, 91, 83, 69; HREIMS *m*/*z* 344.2342 (calcd for $C_{22}H_{32}O_3$, 344.2352).

Fortunin B (2): yellow, amorphous powder; $[\alpha]^{20}_D$ +72 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} 267 (5.15) and 275 (5.10) nm; IR ν_{max} (KBr) 3453, 1739, 1611, 1498, 1369, 1209, 1047 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m*/*z* 402 $[M]^+$, 384, 360, 342, 327, 300,

Table 3. ^1H NMR Data of Compounds **5**−**10** (in $CDCl_3$, δ in ppm and *J* in Hz)[a]

	5	6[b]	7	8[c]	9	10[d]
1	1.36 dd, 12.8	1.54 m	1.52 dd, 13.8	1.29 dd, 12.7	1.44 dd, 4.1, 13.0	1.26 br d, 10.2
	2.17 br d, 12.8	2.24 ddd, 4.2, 9.6,12.8	1.74 dd, 13.8	1.98 br d, 12.7	2.03 br d, 13.0	2.51 br d, 10.2
2	1.81 m	1.75 m	1.68 m	1.51 m	1.75 m	4.04 dddd, 4.5, 4.3, 11.3, 11.3
3	1.47 dd, 11.6, 13.9	1.86 m	1.42 dd, 11.0	1.56 br d, 12.4	1.30 dd, 4.1 10.4	1.47 dd, 12.0
	1.67 d, 13.9	1.96 m	1.58 dd, 11.0	1.63 dd, 13.3	1.82 dd, 4.1 10.4	1.68 dd, 3.3, 12.0
5	2.04 d, 12.7		2.13 d, 11.8	1.68 d, 12.7	2.14 d, 12.2	1.65 dd, 4.7,11.0
6	4.50 d, 12.7		5.39 dd, 5.2,11.8	4.38 dd, 7.0, 12.7	4.17 dd, 3.8, 12.2	1.61 dd, 8.2, 10.4
						1.80 dd, 4.7, 10.4
7			4.64 d, 5.2	5.10 d, 7.0	4.46 d, 3.8	2.51 dd, 4.7, 8.2
11	6.74 s	6.88 s	6.84 s	7.03 s	6.57 s	6.67 s
14	7.93 s	8.02 s	7.38 s	8.08 s	7.05 s	6.76 s
15	3.19 sept, 7.5	3.16 sept, 7.0	2.94 sept, 6.4	3.69 sept, 7.3	3.15 sept, 6.3	3.18 sept, 6.4
16	1.23 d, 7.5	1.28 d, 7.0	1.20 d, 6.4	1.36 d, 7.3	1.25 d, 6.3	1.17 d, 6.4
17	1.25 d, 7.5	1.30 d, 7.0	1.21 d, 6.4	1.37 d, 7.3	1.26 d, 6.3	1.18 d, 6.4
18	1.16 s	1.28 s	1.00 s	1.07 s	1.17 s	0.89 s
19	3.23 d, 11.6	3.35 d, 11.5	3.67 d, 10.7	3.47 d, 7.4	3.51 d, 6.7	3.13 d, 10.8
	3.56 d, 11.6	4.25 d, 11.5	4.13 d, 10.7	3.74 d, 7.4	3.77 d, 6.7	3.44 d, 10.8
20	1.32 s	1.50 s	1.37 s	1.11 s	1.11 s	1.22 s

[a] CH_3CO-6 (2.17, s), CH_3CO-12 (2.32, s), CH_3CO-18 (2.05, s) in **7**, OMe-7 (3.63, s) in **9**. [b] Observed in 300 MHz; the others observed in 600 MHz. [c] Measured in Py-d_5. [d] Measured in CD_3OD.

285, 260, 218, 187, 159, 115, 91, 69, 55; HREIMS *m/z* 402.2403 (calcd for $C_{24}H_{34}O_5$, 402.2406).

Fortunin C (3): yellow, amorphous powder; $[\alpha]^{20}_D$ +77 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} 282 (5.36) nm; IR ν_{max} (KBr) 3363, 1612, 1488, 1311, 1112, 1014, 860 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m/z* 362 $[M]^+$, 332, 315, 297, 287, 273, 259, 245, 217, 149, 109, 91, 59, 55; HREIMS *m/z* 362.2447 (calcd for $C_{22}H_{34}O_4$, 362.2457).

Fortunin D (4): white, amorphous powder; $[\alpha]^{20}_D$ +54 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} 281 (5.31) nm; IR ν_{max} (KBr) 3425, 1616, 1417, 1124, 615 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; EIMS *m/z* 362 $[M]^+$, 330, 315, 245, 209, 149, 1096, 91, 83, 69, 57; HREIMS *m/z* 362.2447 (calcd for $C_{22}H_{34}O_4$, 362.2457).

Fortunin E (5): yellow, amorphous powder; $[\alpha]^{20}_D$ +17 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} 230 (6.25) and 284 (6.14) nm; IR ν_{max} (film) 3359, 1663, 1605, 1506, 1465, 1277, 1259, 1110, 1035, 758 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; EIMS *m/z* 332 $[M]^+$, 284, 269, 255, 241, 229, 193, 161, 147, 109, 83, 69, 55; HREIMS *m/z* 332.1989 (calcd for $C_{20}H_{28}O_4$, 332.1988).

Fortunin F (6): yellow, amorphous powder; $[\alpha]^{20}_D$ −3 (*c* 0.22, $CHCl_3$); UV (MeOH) λ_{max} 280 (5.84) and 333 (5.79) nm; IR ν_{max} (film) 3369, 1680, 1596, 1467, 1313, 1178, 1047, 756 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; EIMS *m/z* 330 $[M]^+$, 299, 281, 245, 203, 165, 115, 97, 83, 69, 57; HREIMS *m/z* 330.1823 (calcd for $C_{20}H_{26}O_4$, 330.1831).

Fortunin G (7): yellow, amorphous powder; $[\alpha]^{20}_D$ +38 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} 221 nm; IR ν_{max} (film) 3442, 1735, 1605, 1463, 1371, 1240, 1041 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; ESIMS *m/z* 483.2 $[M + Na]^+$; HRESIMS *m/z* 483.2356 (calcd for $C_{26}H_{36}O_7Na$, 483.2359).

Fortunin H (8): yellow, amorphous powder; $[\alpha]^{20}_D$ +108 (*c* 0.29, MeOH); UV (MeOH) λ_{max} 280 (5.44) nm; IR ν_{max} (film) 3407, 1600, 1504, 1417, 1267, 999, 754 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; EIMS *m/z* 316 $[M]^+$, 302, 283, 255, 216, 203, 171, 145, 123, 115, 95, 81, 67, 55; HREIMS *m/z* 316.2036 (calcd for $C_{20}H_{28}O_3$, 316.2039).

Fortunin I (9): yellow, amorphous powder; $[\alpha]^{20}_D$ +62 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} 224 (5.85) and 277 (sh) (5.34) nm; IR ν_{max} (film) 3236, 1616, 1502, 1421, 1269, 1099, 987, 846 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; EIMS *m/z* 330 $[M]^+$, 299, 283, 255, 213, 189, 171, 159, 123, 95, 81, 69, 55; HREIMS *m/z* 330.2185 (calcd for $C_{21}H_{30}O_3$, 330.2195).

Fortunin I (10): yellow, amorphous powder; $[\alpha]^{20}_D$ +12 (*c* 0.22, MeOH); UV (MeOH) λ_{max} 282 (4.47) nm; IR ν_{max} (film) 3411, 1618, 1508, 1213, 1094, 1045, 752 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 3; EIMS *m/z* 318 $[M]^+$, 303, 285, 267, 255, 225, 189, 147, 133, 105, 91, 69, 55; HREIMS *m/z* 318.2199 (calcd for $C_{20}H_{30}O_3$, 318.2195).

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Supporting Information Available: The structures of known compounds (**11**−**22**), ^{13}C and ^1H NMR spectra for compounds **1**−**10**, ROESY spectra for compounds **3**, **7**, and **8**, and the result of antitumor activity tests are available free of charge via the Internet at http://pubs.acs.org.

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