ent-Kaurane Diterpenoids from the Liverwort *Jungermannia atrobrunnea*

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Three new rearranged *ent*-kaurane-type diterpenoids (**1**−**3**) and seven new *ent*-kaurane-type diterpenoids (**4**−**10**) have been isolated from the liverwort *Jungermannia atrobrunnea*. Their structures were determined by extensive spectroscopic techniques and X-ray crystallographic analysis. The absolute configurations of these compounds were clarified by CD spectroscopic studies. Compound **1** is the first example of a rearranged *ent*-kaurane diterpenoid possessing a peroxide bridge.

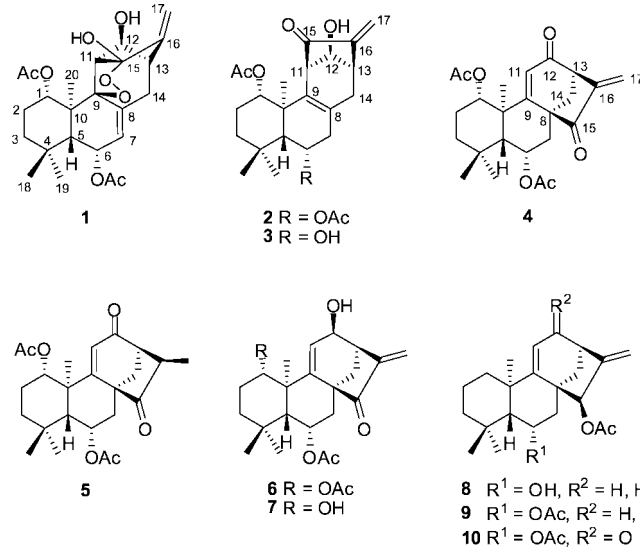
Liverworts (Hepaticae), widely distributed spore-forming terrestrial green plants with thallose or leafy character, have been used as medicinal plants in North America, China, and Europe for a long time.[1] It is known that liverworts are rich sources of sesquiterpenoids, diterpenoids, and bis(bibenzyls), which show diverse biological activities, including cytotoxic, antimicrobial, antifungal, and antioxidative properties.[1−4] The *Jungermannia* species generally contain a variety of diterpenoids such as the *ent*-kaurane, clerodane, trachylobane, pimarane, and labdane types,[1] and some species also contain sesquiterpenoids of the chilocyphane, cuparane, and gymnomitrane types.[5−7] Nearly no bis(bibenzyl) compounds have been isolated from *Jungermannia* species, besides perrottetin E from *J. comata*.[8]

In our ongoing search for bioactive substances from Chinese liverworts,[9−16] an aquatic habitated liverwort *Jungermannia atrobrunnea*, collected in the mountainous (1610 m) area of Zhejiang Province, was investigated phytochemically. Three new rearranged *ent*-kaurane- (**1**−**3**) and seven new *ent*-kaurane-type diterpenoids (**4**−**10**) were obtained. Their structures were elucidated by means of MS, NMR, and CD spectroscopic analysis, as well as single-crystal X-ray diffraction analysis performed on compound **1**. All the *ent*-kaurane diterpenoids were evaluated for their antifungal activity against *Candida albicans*. We herein report the isolation, identification, and biological evaluation of these new compounds.

Results and Discussion

The air-dried material of *J. atrobrunnea* (80 g) was extracted exhaustively with Et_2O at room tempreture. The ether extract (2.03 g) was fractionated by repeated column chromatography on Si gel and Sephadex LH-20, followed by semipreparative high-performance liquid chromatography (HPLC) and preparative TLC, resulting in the isolation of three new rearranged *ent*-kaurane-type diterpenoids (**1**−**3**) and seven new *ent*-kaurane-type diterpenoids (**4**−**10**).

Compound **1** was isolated as colorless needles (MeOH). The IR spectrum of **1** showed absorptions for hydroxy (3434 cm^{-1}) and olefinic (1629 cm^{-1}) groups. The molecular formula $C_{24}H_{32}O_8$ was determined from the $[M + NH_4]^+$ ion peak at *m/z* 466.2430 in the HRESIMS (calcd, 466.2441), which required nine degrees of unsaturation. The ^1H NMR spectrum (Table 1) of **1** showed the presence of an *exo*-methylene at δ_H 5.09 (s) and 5.31 (s), an olefinic proton at δ_H 5.66 (dd, J = 5.2, 2.4 Hz), three oxygenated methine protons at δ_H 3.82 (m), 5.28 (dd, J = 11.8, 4.5 Hz), and 5.41 (m), three tertiary methyls at δ_H 0.93 (s, 3H), 1.14 (s, 3H), and 1.45 (s, 3H), and two acetyl methyls at δ_H 2.01 (s, 3H) and 2.03 (s, 3H). The ^{13}C NMR (Table 3) and HMQC spectra confirmed the presence of a trisubstituted double bond, an *exo*-methylene, three methyls, three methylenes, six methines (including three oxygenated methines), four quartenary carbons, and two acetoxy groups.



Three partial linkage moieties, $-CHCH_2CH_2-$ (C-1 to C-3), $-CHCHCH=$ (C-5 to C-7), and $-CHCHCHCH_2-$ (C-11 to C-14) (shown as bold lines in Figure 1), were established on the basis of the $^1H-^1H$ COSY and HMQC spectra of **1**. Connection of these partial structures and the quarternary carbons to constitute a rearranged kauranoid[17,18] was based on the cross-peaks of H_3-20/C-1, C-5, C-9, and C-10; H-5/C-1, C-4, C-9, and C-10; and H-11/C-8, C-9, C-10, C-15, and C-16 in the HMBC spectrum (Figure 1). In addition, HMBC correlations of the hydroxy proton at δ_H 3.99 (d, J = 3.1 Hz) to C-11, C-12 (δ_C 73.5), and C-13 and of the hydroxy proton at δ_H 5.78 (s) to C-11, C-15 (δ_C 113.2), and C-16 confirmed the presence of one hydroxy group at C-12 and the other at the hemiketalic C-15. The two acetoxy groups were placed at C-1 and C-6 due to the cross-peaks of H-1 and H-6 with the *O*-acetyl carbonyls at δ_C 170.4 and 170.1, respectively, in the HMBC spectrum. Furthermore, the two oxygenated quaternary carbons C-9 (δ_C 94.3) and C-15 (δ_C 113.2) together with the

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Table 1. ^{1}H NMR Data of Compounds **1**−**5** (600 MHz)[a]

position	**1**[b]	**2**[c]	**3**[b]	**4**[d]	**5**[d]
1	5.28 dd (11.8, 4.5)	4.39 dd (11.0, 4.1)	4.23 dd (11.0, 4.1)	4.49 dd (11.3, 3.6)	4.47 dd (10.8, 5.3)
2α	1.70 qd (11.5, 4.5)	1.64 qd (11.3, 4.1)	1.67 qd (11.3, 4.1)	1.75 qd (11.3, 3.6)	1.74 m
2*β*	1.80 dq (11.5, 4.5)	2.10 dq (11.3, 4.1)	2.06 m	1.80 dq (11.3, 3.6)	1.79 m
3	α 1.32 dt (13.4, 3.2)	1.34−1.37 m	1.30−1.35 m	1.40 m	1.43 m
	β 1.38 td (13.4, 3.2)				
5	1.62 d (4.3)	1.24 s	1.07 s	1.68 s	1.73 s
6	5.41 m	5.48 d (5.2)	4.42 d (4.4)	5.80 m	5.72 m
7	5.66 dd (5.2, 2.4)	α 1.99 d (18.8)	α 1.96 d (17.9)	α 2.63 dd (15.6, 8.0)	α 2.54 dd (15.1, 7.2)
		β 2.23 dd (18.8, 5.2)	*β* 2.22 dd (17.9, 4.4)	*β* 1.68 dd (15.6, 4.5)	*β* 1.68 dd (15.1, 3.7)
11	3.70 d (2.0)	3.80 s	3.81 s	6.08 s	6.10 s
12	3.82 m	4.08 s	3.99 s		
13	2.75 m	2.99 br s	2.97 br s	3.57 d (2.7)	3.03 dd (7.0, 4.2)
14α	2.77 m	2.53 dd (17.3, 4.9)	2.57 dd (17.2, 4.8)	2.41 d (11.8)	2.41 d (12.2)
14*β*	2.56 m	2.09 d (17.3)	2.03 m	2.07 dd (11.8, 2.7)	2.08 dd (12.2, 4.2)
16					1.04 m
17	5.09 s	5.52 s	5.43 s	5.73 s	1.04 d (7.8)
	5.31 s	6.01 s	5.84 s	6.17 s	
18	0.93 s	0.95 s	0.96 s	1.08 s	1.08 s
19	1.14 s	1.01 s	1.24 s	1.13 s	1.12 s
20	1.45 s	1.54 s	1.59 s	1.59 s	1.59 s
OAc-1	2.01 s	2.14 s	2.03 s	2.08 s	2.10 s
OAc-6	2.03 s	2.07 s		2.11 s	2.10 s

[a] Assignments are based on 1D and 2D NMR experiments. Figures in parentheses are coupling constants (*J*) in Hz. [b] Measured in acetone-d_6. [c] Measured in $CDCl_3$. [d] Measured in $CDCl_3$−CD_3OD (1:1).

Table 2. ^{1}H NMR Data of Compounds **6**−**10** (600 MHz)[a]

position	**6**[b]	**7**[c]	**8**[b]	**9**[d]	**10**[b]
1	4.47 m[e]	3.40 dd (11.2, 5.2)	α 1.93 br d (13.5)	α 1.94 br d (13.2)	α 2.04 br d (13.2)
			β 1.37 m	*β* 1.40 td (13.2, 3.9)	*β* 1.47 m
2α	1.60 m	1.60 m	1.72 qt (13.5, 3.5)	1.70 qt (13.2, 3.9)	1.61 m
2*β*	2.07 m	2.07 m	1.49 dm (13.5)	1.49 dm (13.2)	1.53 m
3	1.31−1.36 m	α 1.28 dt (13.5. 3.6)	α 1.34 m	α 1.37 dm (13.2)	α 1.46 m
		β 1.21 td (13.5, 3.5)	*β* 1.13 td (13.3, 3.5)	*β* 1.13 td (13.2, 3.9)	*β* 1.17 td (13.2, 3.2)
5	1.66 d (1.8)	1.63 d (4.9)	1.45 d (6.4)	1.67 d (6.0)	1.68 d (5.7)
6	5.64 m[f]	5.78 ddd (9.2, 6.1, 4.9)	4.31 m	5.34 ddd (9.6, 7.2, 6.0)	5.47 ddd (9.4, 6.9, 5.7)
7α	2.47 dd (15.2, 7.6)	2.35 dd (14.4, 9.2)	1.95 dd (14.3, 8.8)	2.42 dd (14.7, 9.6)	2.65 dd (15.0, 9.4)
7*β*	1.51 dd (15.2, 2.7)	1.59 dd (14.4, 6.1)	1.26 m	1.75 dd (14.7, 7.2)	1.89 dd (15.0, 6.9)
11	5.64 m[f]	6.58 d (1.6)	5.46 m	5.46 t (3.3)	6.03 s
12	4.47 m[e]	4.56 m	α 2.14 br d (17.3)	α 2.13 br d (17.3)	
			β 2.53 ddd (17.3, 4.2, 3.3)	*β* 2.51 ddd (17.3, 4.2, 3.3)	
13	3.07 t (4.8)	3.04 t (4.4)	2.77 br s	2.75 br s	3.40 br s
14	α 1.88 s	α 1.90 d (11.4)	α 1.46 d (11.1)	α 1.50 d (11.2)	2.02 m
	β 1.87 d (4.8)	*β* 1.87 dd (11.4, 4.4)	*β* 1.79 dd (11.1, 5.1)	*β* 1.78 dd (11.2, 5.1)	
15			5.45 s	5.42 t (2.4)	5.70 t (2.2)
17	5.54 s	5.45 s	4.83 s	4.86 br s	5.12 br s
	6.18 s	5.94 s	5.05 s	5.03 dd (2.4, 0.8)	5.39 br s
18	1.02 s	0.96 s	1.00 s	0.90 s	0.99 s
19	1.02 s	1.12 s	1.26 s	1.13 s	1.23 s
20	1.46 s	1.39 s	1.31 s	1.31 s	1.49 s
OAc-1	2.05 s				
OAc-6	2.04 s	2.04 s		2.15 s	2.13 s
OAc-15			2.16 s	2.04 s	2.19 s

[a] Assignments are based on 1D and 2D NMR experiments. Figures in parentheses are coupling constants (*J*) in Hz. [b] Measured in $CDCl_3$. [c] Measured in acetone-d_6. [d] Measured in $CDCl_3$−CD_3OD (1:1). [e] Signals were overlapped. [f] Signals were overlapped.

remaining two unassigned oxygen atoms in the structure of **1** indicated a peroxide bridge between C-9 and C-15, which satisfied the nine degrees of unsaturation and the low chemical shifts of C-9 and C-15.[19]

The NOE correlations of H-1 to H-5, H-6 to H_3-18, and H-12 to H_3-20 and H-14α in the NOESY spectrum of **1** (Figure 2) revealed the *O*-acetyl groups at C-1 and C-6 to both be α-oriented, while the hydroxy group at C-12 was *β*-oriented. The relative configuration was further supported by the results of a single-crystal X-ray crystallographic analysis for compound **1** (Figure 3). Compound **1** is the first example of a rearranged *ent*-kaurane diterpenoid with a peroxide bridge and was named jungermatrobrunin A.

Compound **2**, colorless needles, had the molecular formula $C_{24}H_{32}O_6$ by HRESIMS (*m*/*z* 434.2525 $[M + NH_4]^+$; calcd 434.2537). Analysis of the ^{1}H and ^{13}C NMR data (Tables 1 and 3) of **2** also revealed a rearranged *ent*-kaurane diterpenoid that has a similar structural skeleton to jungermannenone C, a compound previously isolated from the same genus,[17,18] except for the additional resonances of two acetoxy groups and two oxymethines in **2**. The two acetoxy groups were assigned at C-1 and C-6, respectively, on the basis of the HMBC correlations of H-1 to one acetoxy carbonyl (δ_C 170.6) and from H-6 to the other acetoxy carbonyl (δ_C 170.4). The NOESY correlations of H-1 to H-5 and of H-6 to H_3-18 established the acetoxy groups at C-1 and C-6 to both be α-oriented. Accordingly, compound **2** was determined to be 1α,6α-diacetoxyjungermannenone C.[17] Compound **3** was a deacetyl derivative of **2**. The HRESIMS gave a molecular formula of $C_{22}H_{30}O_5$ ($[M + NH_4]^+$ at *m*/*z* 392.2424, calcd 392.2432), which indicated the absence of one acetyl group in **3**. The upfield shift of the H-6*β* resonance from δ_H 5.48 in **2** to δ_H 4.42 in **3** revealed that the hydroxy group was at the C-6α position in **3**. The downfield shift of C-7 (δ_C 43.6) in **3** due to a *β*-downfield effect of the

Table 3. ^{13}C NMR Data of Compounds **1**−**10** (150 MHz)[a]

position	**1**[b]	**2**[c]	**3**[b]	**4**[d]	**5**[d]	**6**[c]	**7**[b]	**8**[c]	**9**[d]	**10**[c]
1	75.8	79.3	80.4	78.9	78.8	79.3	78.6	41.0	41.5	40.3
2	24.9	24.6	25.4	25.0	25.2	25.8	30.0	19.0	19.4	19.6
3	42.1	40.1	40.9	40.2	44.0	40.1	42.8	45.1	45.3	44.9
4	34.5	33.7	34.4	33.8	33.7	33.6	34.5	34.1	34.2	34.4
5	47.9	51.2	53.8	47.5	47.8	48.5	47.7	49.1	48.1	46.9
6	67.4	67.4	65.1	68.1	68.1	68.5	67.9	65.8	69.2	68.2
7	125.4	37.6	43.6	34.5	35.3	35.2	34.4	40.9	38.0	37.4
8	136.3	126.1	127.5	52.9	52.6	49.7	50.9	42.9	42.6	45.7
9	94.3	133.3	134.0	170.2	169.7	147.5	149.8	153.2	153.1	178.2
10	44.2	42.9	42.4	44.1	43.9	42.8	44.8	38.5	37.8	39.4
11	66.8	52.7	52.6	125.2	126.1	128.1	127.9	116.7	117.6	122.6
12	73.5	74.6	74.6	196.8	200.4	69.8	71.2	37.8	38.9	198.4
13	47.9	44.9	45.8	52.3	49.6	42.9	44.8	37.8	38.0	55.1
14	39.6	40.9	42.4	45.2	45.7	42.1	44.8	41.4	41.8	46.2
15	113.2	200.1	201.5	200.5	212.8	202.7	204.5	86.0	86.7	82.4
16	156.5	148.1	151.6	138.2	41.9	142.5	145.6	155.5	155.6	145.4
17	108.5	118.7	117.2	122.0	14.1	118.7	120.5	108.0	108.8	114.3
18	32.4	33.0	33.5	32.1	32.2	32.7	32.3	32.2	32.6	32.6
19	25.0	22.8	23.6	23.9	23.6	22.8	24.6	24.0	24.3	24.3
20	14.4	16.6	16.8	19.4	19.3	19.9	19.9	27.0	24.3	26.1
OAc-1	170.4	170.6	169.9	170.6	170.7	171.1				
	21.8	22.4	22.3	21.4	21.3	21.8				
OAc-6	170.1	170.4		170.4	170.5	170.4	170.4		170.4	170.1
	21.6	21.7		21.2	21.1	21.7	21.7		21.7	21.7
OAc-15								171.1	170.3	170.0
								21.3	21.8	21.5

[a] Assignments are based on 1D and 2D NMR experiments. [b] Measured in acetone-d_6. [c] Measured in $CDCl_3$. [d] Measured in $CDCl_3$−CD_3OD (1:1).

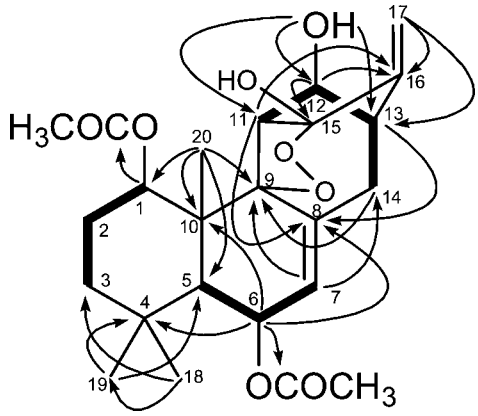

Figure 1. Key ^{1}H−^{1}H COSY (—) and HMBC (H→C) correlations of **1**.

6-hydroxy group[20,21] confirmed this arrangement. Accordingly, **3** was determined to be 1α-acetoxy-6α-hydroxyjungermannenone C.[17]

The absolute configurations of compounds **1**−**3** were proposed from their CD spectra. The CD spectra of **2** and **3** both showed a negative Cotton effect at 345−355 nm corresponding to an enone system[17−19,22] and a positive Cotton effect at 260−270 nm, which coincided with rearranged *ent*-kaurane-type diterpenoids jungermannenones A−E.[17] The CD spectrum of **1** indicated an positive Cotton effect at 260 nm identical with that of **2** and **3**, although there was a positive Cotton effect at 320 nm instead of a negative Cotton effect due to the absence of the enone system. Thus, compound **1** was also elucidated as a rearranged *ent*-kaurane diterpenoid of the same absolute configuration.

Compound **4** was isolated as a white, amorphous powder. The molecular formula was established as $C_{24}H_{30}O_6$ from its positive HRESIMS (*m*/*z* 415.2099 $[M + H]^+$; calcd 415.2115). The NMR data (Tables 1 and 3) suggested that **4** was an *ent*-9(11),16-kauradien-12,15-dione derivative.[18] The resonances of the two acetoxy groups appeared in the NMR spectra at δ_H 2.08 (s, 3H) and 2.11 (s, 3H); δ_C 170.6, 170.4, 21.4, and 21.2, and were linked to C-1 and C-6, respectively, by the HMBC correlations of H-1

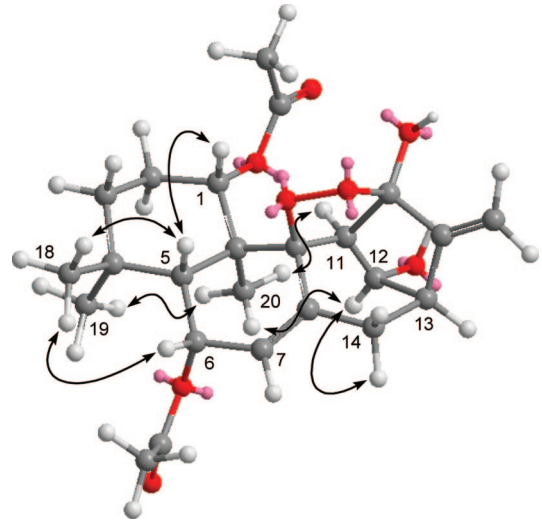

Figure 2. Key NOESY correlations (H↔H) of **1**.

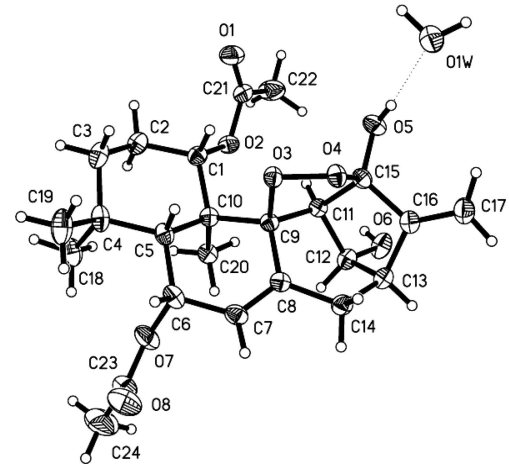

Figure 3. ORTEP drawing of **1**.

(δ_H 4.49, dd, J = 11.3, 3.6 Hz) to one carbonyl at δ_C 170.6 and H-6 (δ_H 5.80, m) to the other carbonyl at δ_C 170.4. In the NOESY spectrum, NOEs of H-1 to H-5 and of H-6 to H_3-18 clarified that OAc-1 and OAc-6 were both α-oriented. Thus, the identity of compound **4** was established as 1α,6α-diacetoxy-*ent*-kaura-9(11),16-dien-12,15-dione. The ^{1}H and ^{13}C NMR data (Tables 1 and 3) of **5** were similar to those of **4**, which revealed that **5** was also an *ent*-9(11),16-kauradien-12,15-dione derivative.[18] HRESIMS of **5** gave a quasi-molecular ion peak $[M + H]^+$ at *m*/*z* 417.2262, corresponding to a molecular formula of $C_{24}H_{32}O_6$ (calcd 417.2272), which suggested one less degree of unsaturation than in **4**. According to the NMR data, the double bond between C-16 and C-17 disappeared in **5**. Instead, a methyl group at C-16 was evident on the basis of a doublet at δ_H 1.04 (3H, d, J = 7.8 Hz) in the ^{1}H NMR spectrum. Me-17 could be assigned as β-oriented on the basis of the NOE correlations of H-16α with H-13α and H-14β in the NOESY spectrum. Thus the structure of **5** was determined as 16*R*-1α,6β-diacetoxy-*ent*-9(11)-kauren-12,15-dione.

Compound **6**, isolated as colorless needles, was assigned the molecular formula $C_{24}H_{32}O_6$ from its positive HRESIMS (*m*/*z* 434.2526 $[M + NH_4]^+$; calcd 434.2537). The ^{1}H and ^{13}C NMR data (Tables 2 and 3) of **6** resembled those of **4** except for the resonance of an oxymethine group at C-12 (δ_H 4.47, m and δ_C 69.8) instead of a ketone carbonyl in the NMR spectra of **6**, which indicated a hydroxy group at C-12. This assignment was confirmed by the HMBC correlations of H-12 (δ_H 4.47, m) to C-9 (δ_C 147.5), C-14 (δ_C 42.1), and C-16 (δ_C 142.5). The β-orientation of OH-12

was suggested from the NOESY correlation of H-12 to H_α-14 (δ_H 1.8, s). Therefore, the structure of **6** was determined as 12β-hydroxy-1α,6α-diacetoxy-*ent*-kaura-9(11),16-dien-15-one.

Compound **7** possessed the molecular formula $C_{22}H_{30}O_5$, confirmed by the HRESIMS (*m/z* 392.2425 $[M + NH_4]^+$; calcd 392.2432). It differed from **6** in not having an acetyl group. The upfield shift of H-1 (δ_H 3.40, dd, J = 11.2, 5.2 Hz) and the downfield shift of C-2 (δ_C 30.0) in **7** due to a β-downfield effect of the 1-hydroxy group[20] indicated that the hydroxy group at the C-1 position in **7** had replaced the acetoxy group in **6**. In the NOESY spectrum of **7**, the correlations of H-1 to H-5, of H-6 to H_3-18, and of H-12 to H-14α confirmed that the 1-OH, 6-OAc, and 12-OH in **7** were α-, α-, and β-oriented, respectively. Therefore, compound **7** was established as 1α,12β-dihydroxy-6α-acetoxy-*ent*-kaura-9(11),16-dien-15-one.

The ^{1}H and ^{13}C NMR (Tables 2 and 3) data of **8** and **9** revealed that these two compounds were both *O*-acetyl derivatives of exsertifolin H.[23] The molecular formula $C_{22}H_{32}O_3$ of **8**, which was deduced from the pseudo molecular ion peak $[M + NH_4]^+$ at *m/z* 362.2687 (calcd 362.2695) by HRESIMS, confirmed the presence of an ester group. The HMBC correlation of H-15 (δ_H 5.45, s) to the acetyl carbonyl at δ_C 171.1 indicated that the hydroxy group at C-15 in exsertifolin H has been replaced by an acetoxy group in **8**. The molecular formula of **9** was established as $C_{24}H_{34}O_4$ from its HRESIMS (*m/z* 404.2786 $[M + NH_4]^+$; calcd 404.2795), which showed one more acetyl group in the structure compared with **8**. The HMBC correlation of H-6 (δ_H 5.34, ddd, J = 9.6, 7.2, 6.0 Hz) to the ester carbonyls at δ_C 170.4 suggested that the hydroxy group at C-6 in exsertifolin H has also been acetylated in **9**. Thus, the identity of compound **8** was established as 6α-hydroxy-15β-acetoxy-*ent*-kaura-9(11),16-diene, and **9** was elucidated as 6α,15β-diacetoxy-*ent*-kaura-9(11),16-diene.

Compound **10** had a molecular formula of $C_{24}H_{32}O_5$ deduced from HRESIMS (*m/z* 401.2297 $[M + H]^+$; calcd 401.2328). NMR data of **10** indicated that it was an oxygenated derivative of **9**, as a methylene group at C-12 in **9** was replaced by a ketone function in **10**. In the HMBC spectrum of **10**, the correlation of H_2-14 (δ_H 2.02, m) with C-12 (δ_C 198.4) also confirmed the above deduction. Compound **10** was thus deduced as 6α,15β-diacetoxy-*ent*-kaura-9(11),16-dien-12-one.

The CD data indicated the absolute configurations of compounds **4**−**10**. They all showed a positive Cotton effect at 312−350 nm and a negative Cotton effect at 235−253 nm in their CD spectra, which supported them as *ent*-kaurane-type diterpenoids.[17]

All of the isolates **1**−**10** were evaluated for their antifungal activity against *C. albicans*. Their minimal inhibitory concentration (MIC) values were all 128 μg/mL, which clarified their weak antifungal activity.

Experimental Section

General Experimental Procedures. Melting points were measured on an X-6 micromelting point apparatus without correction. Optical rotations were determined on a GYROMAT-HP polarimeter. UV spectra were measured on a Shimadzu UV-2450 spectrophotometer. CD spectra were obtained on a Chirascan spectropolarimeter. IR spectra were recorded on a Thermo-Nicolet 670 spectrophotometer using KBr disks. NMR spectra were measured on a Bruker Avance DRX-600 spectrometer operating at 600 (^{1}H) or 150 (^{13}C) MHz with TMS as internal standard. HRESIMS were carried out on a VG ZAB-2F mass spectrometer, and ESIMS was carried out on an API 4000 triple-stage quadrupole instrument. All solvents used were analytical grade (Laiyang Chemical Reagent Co., Ltd., Shandong, People's Republic of China). Column chromatography was performed on either Si gel (200−300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China) or Sephadex LH-20 (25−100 μm; Pharmacia). Preparative TLC was performed on HP-TLC plates precoated with Si gel GF_{254} (Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a ZORBAX Eclipse XDB-C18, 4.6 mm × 150 cm column. Fractions were monitored by TLC, and spots were visualized under UV (254 nm) light and by heating Si gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The liverwort *J. atrobrunnea* Amakawa was collected in Fengyangshan, Zhejiang Province, People's Republic of China, in July 2006. The sample was identified by one of the authors (R.-L.Z.), and a voucher specimen (no. 20060729-4) was deposited in the School of Pharmaceutical Sciences, Shandong University.

Extraction and Isolation. Air-dried and powdered liverwort *J. atrobrunnea* (80 g) was extracted with Et_2O (0.5 L × 3, each 2 days) at room temperature and filtered. The filtrate was evaporated under reduced pressure at 40 °C to afford a residue. The Et_2O extract (2.03 g) was applied to Si gel (200−300 mesh) column chromotography eluting with a petroleum ether−acetone gradient system (100% petroleum ether, 100:1, 50:1, 20:1, 10:1, 5:1) to give fractions A−F. Fraction C (100 mg) was subjected to Si gel column chromotography using petroleum ether−acetone (90:1) as the eluent to yield mixtures C1−C3. Fraction C1 (50 mg) was purified by Sephadex LH-20 column chromatography, eluted with $CHCl_3$−MeOH (1:1), to give **8** (4 mg). Compounds **10** (3 mg) and **9** (3 mg) were obtained from fractions C2 (30 mg) and C3 (20 mg), respectively, by preparative TLC (petroleum ether−acetone, 10:1). Fraction D (200 mg) was chromatographed on Sephadex LH-20 using $CHCl_3$−MeOH (1:1) as eluant and then purified by semipreparative HPLC (56% MeOH−H_2O) to yield **6** (4 mg), **4** (4 mg), and **5** (6 mg). Fraction E (400 mg) was subjected to Si gel column chromatography and eluted in a step gradient manner with petroleum ether−acetone (from 20:1 to 5:1) to afford E1 (100 mg) and E2 (50 mg). Compound **2** (15 mg) was obtained from E1 by Sephadex LH-20 column chromatography ($CHCl_3$−MeOH, 1:1). E2 (50 mg) was purified by semipreparative HPLC (50% MeOH−H_2O) to give **1** (5 mg) and **7** (3 mg). Fraction F (45 mg) was subjected to preparative TLC (petroleum ether−acetone, 4:1) to obtain compound **3** (3 mg).

Jungermatrobrunin A (1): colorless needles (MeOH); mp 157−159 °C; $[\alpha]^{20}_D$ +35.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 230 (1.67) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 320 (+0.5), 260 (+0.4) nm; IR (KBr) ν_{max} 3434, 2929, 1732, 1629, 1429, 1369, 1241 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 466 $[M + NH_4]^+$; positive HRESIMS *m/z* 466.2430 $[M + NH_4]^+$ (calcd for $C_{24}H_{36}NO_8$, 466.2441).

1α,6α-Diacetoxyjungermannenone C (2): colorless needles (MeOH); mp 196−197 °C; $[\alpha]^{20}_D$ −36.2 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 235 (2.06) nm, 265 (1.97) nm, 348 (1.86) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 347 (−2.6), 265 (+2.1) nm; IR (KBr) ν_{max} 3450, 2931, 1736, 1647, 1433, 1373, 1228 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 417 $[M + H]^+$; HRESIMS *m/z* 434.2525 $[M + NH_4]^+$ (calcd for $C_{24}H_{36}NO_6$, 434.2537).

1α-Acetoxy-6α-hydroxyjungermannenone C (3): white, amorphous powder; $[\alpha]^{20}_D$ −34.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 232 (2.23) nm, 272 (2.11) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 350 (−2.1), 268 (+2.4) nm; IR (KBr) ν_{max} 3428, 2921, 1729, 1644, 1462, 1384, 1256 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS 392 $[M + NH_4]^+$; positive HRESIMS *m/z* 392.2424 $[M + NH_4]^+$ (calcd for $C_{22}H_{34}NO_5$, 392.2432).

1α,6α-Diacetoxy-*ent*-kaura-9(11),16-dien-12,15-dione (4): white, amorphous powder; $[\alpha]^{20}_D$ +42.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 230 (2.02) nm, 268 (1.94) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 312 (+5.3), 235 (−9.2) nm; IR (KBr) ν_{max} 2931, 1738, 1681, 1582, 1375, 1237 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 415 $[M + H]^+$; positive HRESIMS *m/z* 415.2099 $[M + H]^+$ (calcd for $C_{24}H_{31}O_6$, 415.2115).

16*R*-1α,6β-Diacetoxy-*ent*-9(11)-kauren-12,15-dione (5): white, amorphous powder; $[\alpha]^{20}_D$ +61.8 (*c* 0.70, MeOH); UV (MeOH) λ_{max} (log ε) 228 (2.34) nm, 260 (2.22) nm, 310 (2.13) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 314 (+5.4), 238 (−3.3) nm; IR (KBr) ν_{max} 2934, 1732, 1678, 1576, 1429, 1369, 1234 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS 434 $[M + NH_4]^+$; positive HRESIMS *m/z* 417.2262 $[M + H]^+$ (calcd for $C_{24}H_{33}O_6$, 417.2272).

12β-Hydroxy-1α,6α-diacetoxy-*ent*-kaura-9(11),16-dien-15-one (6): colorless needles (MeOH); mp 194−197 °C; $[\alpha]^{20}_D$ +51.2 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 238 (2.38) nm, 350 (2.01) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 346 (+4.0), 240 (−7.5) nm; IR (KBr) ν_{max} 3435, 2950, 1734, 1645, 1449, 1378, 1243 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS 434 $[M + NH_4]^+$; positive HRESIMS *m/z* 434.2526 $[M + NH_4]^+$ (calcd for $C_{24}H_{36}NO_6$, 434.2537).

1α,12β-Dihydroxy-6α-acetoxy-*ent*-kaura-9(11),16-dien-15-one (7): white, amorphous powder; $[\alpha]^{20}_D$ +45.2 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 237 (2.40) nm, 344 (2.00) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 350 (+4.4), 239 (−7.0) nm; IR (KBr) ν_{max} 3436, 2932, 1733, 1645, 1456, 1382, 1365, 1248 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS 392 $[M + NH_4]^+$; positive HRESIMS *m/z* 392.2425 $[M + NH_4]^+$ (calcd for $C_{22}H_{34}NO_5$, 392.2432).

6α-Hydroxy-15β-acetoxy-*ent*-kaura-9(11),16-diene (8): white, amorphous powder; $[\alpha]^{20}_D$ −55.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 236 (1.90) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 331 (+0.2), 253 (−0.7) nm; IR (KBr) ν_{max} 2917, 2849, 1738, 1586, 1471, 1398, 1235 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z*, 362 $[M + NH_4]^+$; positive HRESIMS *m/z* 362.2687 $[M + NH_4]^+$ (calcd for $C_{22}H_{36}NO_3$, 362.2695).

6α,15β-Diacetoxy-*ent*-kaura-9(11),16-diene (9): white, amorphous powder; $[\alpha]^{20}_D$ −46.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 246 (1.94) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 330 (+0.7), 253 (−0.5) nm; IR (KBr) ν_{max} 2916, 1739, 1462, 1260 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z*, 404 $[M + NH_4]^+$; positive HRESIMS *m/z* 404.2786 $[M + NH_4]^+$ (calcd for $C_{24}H_{38}NO_4$, 404.2795).

6α,15β-Diacetoxy-*ent*-kaura-9(11),16-dien-12-one (10): white, amorphous powder; $[\alpha]^{20}_D$ −24.7 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 247 (2.05) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 332 (+4.0), 247 (−17.0) nm; IR (KBr) ν_{max} 2928, 1742, 1677, 1459, 1367, 1244 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 418 $[M + NH_4]^+$; positive HRESIMS *m/z* 401.2297 $[M + H]^+$ (calcd for $C_{24}H_{33}O_5$, 401.2328).

Crystal Structure of Jungermatrobrunin A (1). Single crystals suitable for X-ray analysis were obtained by recrystallization from methanol. A colorless platelet crystal having approximate dimensions of 0.21 × 0.15 × 0.11 mm^3 was used for analysis. All measurements were made on a Bruker APEX2 CCD area-detector diffractometer employing graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 293 K and operating in the $\phi-\omega$ scan mode. Crystal data: $C_{24}H_{32}O_8 \cdot H_2O$, M = 466.51, monoclinic, space group $P2_1$, a = 6.2573(2) Å, b = 12.0494(5) Å, c = 16.0026(6) Å, V = 1188.45(8) Å^3, Z = 2, D_{calcd} = 1.304 g/cm^3, $F(000)$ = 500, and μ(Mo Kα) = 0.099 mm^{-1}. Cell refinement and data reduction: APEX2 Software Suite.[24] Program used to refine structure: SHELXL-97;[25] refinement on F^2, full-matrix least-squares calculations. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in geometrically calculated positions and refined as riding atoms with the relative isotropic parameters. One lattice water molecule was contained in the structure. A total of 9199 reflections (2850 unique, R_{int} = 0.0290) were collected from 1.29° to 27.50° in θ and index ranges $5 \geq h \geq -8$, $15 \geq k \geq -14$, $20 \geq l \geq -17$. The final stage converged to R_1 = 0.0488 (wR_2 = 0.1057) for 2850 observed reflections [with $I > 2\sigma(I)$] and 302 variable parameters, R_1 = 0.0386 (wR_2 = 0.0992) for all unique reflections and GoF = 1.031.

Details of crystallographic data (excluding structure factors) for the structure analysis have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 683122. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: C44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Antifungal Assays. MIC values were determined by the broth microdilution method.[15] All tests were performed in RPMI-1640 broth supplemented with DMSO at a final concentration of 0.5% (v/v). *C. albicans* ATCC10231 was incubated for 48 h before MIC determination. Serial double dilutions of the diterpenoids were prepared in a 96-well microtiter plate and ranged from 0.5 to 254 mg/mL. The final inocula were adjusted to 2.5 × 10^4 cfu/mL. The inoculated plates were incubated aerobically at 35 °C for 24 h. Growth of the microorganism was indicated by the presence of turbidity and a pellet on the well bottom. The MIC value was recorded as the lowest concentrations at which no microorganism growth was observed. Fluconazole was used as a positive control drug.

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Supporting Information Available: 1D and 2D NMR spectra as well as IR spectra of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Asakawa, Y. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer: Vienna, 1995; Vol. 65, pp 1−618.
(2) Scher, J. M.; Burgess, E. J.; Lorimerb, S. D.; Perry, N. B. *Tetrahedron* **2002**, *58*, 7875–7882.
(3) Niu, C.; Qu, J. B.; Lou, H. X. *Chem. Biodiversity* **2006**, *3*, 34–40.
(4) Schwartner, C.; Michel, C.; Stettmaier, K.; Wagner, H.; Bors, W. *Free Radical Biol. Med.* **1996**, *20*, 237–244.
(5) Matsuo, A.; Terada, I.; Nakayama, M.; Hayashi, S. *Tetrahedron Lett.* **1977**, *43*, 3821–3824.
(6) Carman, R. M.; Craig, W. J.; Shaw, I. M. *Aust. J. Chem.* **1973**, *26*, 215–214.
(7) Nagashima, F.; Kondoh, M.; Uematsu, T.; Nishiyama, A.; Saito, S.; Sato, M.; Asakawa, Y. *Chem. Pharm. Bull.* **2002**, *50*, 808–813.
(8) Nagashima, F.; Toyota, M.; Asakawa, Y. *Phytochemistry* **1990**, *29*, 2169–2174.
(9) Li, Z. J.; Lou, H. X.; Yu, W. T.; Fan, P. H.; Ren, D. M.; Ma, B.; Ji, M. *Helv. Chim. Acta* **2005**, *88*, 2637–2640.
(10) Niu, C.; Qu, J. B.; Lou, H. X. *Chem. Biodiversity* **2006**, *3*, 34–40.
(11) Lu, Z. Q.; Fan, P. H.; Ji, M.; Lou, H. X. *J. Asian Nat. Prod. Res.* **2006**, *8*, 187–192.
(12) Ma, B.; Lu, Z. Q.; Guo, H. F.; Lou, H. X. *Helv. Chim. Acta* **2007**, *90*, 52–57.
(13) Ma, B.; Guo, H. F.; Lou, H. X. *Helv. Chim. Acta* **2007**, *90*, 58–62.
(14) Xie, C. F.; Qu, J. B.; Sun, B.; Guo, H. F.; Lou, H. X. *Biochem. Syst. Ecol.* **2007**, *35*, 162–165.
(15) Qu, J. B.; Xie, C. F.; Guo, H. F.; Yu, W. T.; Lou, H. X. *Phytochemistry* **2007**, *68*, 1767–1774.
(16) Qu, J. B.; Xie, C. F.; Ji, M.; Shi, Y. Q.; Lou, H. X. *Helv. Chim. Acta* **2007**, *90*, 2109–2115.
(17) Nagashima, F.; Kondoh, M.; Fujii, M.; Takaoka, S.; Watanabe, Y.; Asakawa, Y. *Tetrahedron* **2005**, *61*, 4531–4544.
(18) Nagashima, F.; Kasai, W.; Kondoh, M.; Fujii, M.; Watanabe, Y.; Braggins, J. E.; Asakawa, Y. *Chem. Pharm. Bull.* **2003**, *51*, 1189–1192.
(19) Kubo, M.; Minami, H.; Hayashi, E.; Kodama, M.; Kawazu, K.; Fukuyama, Y. *Tetrahedron Lett.* **1999**, *40*, 6261–6265.
(20) Qin, C. Q.; Liu, C. J.; Li, J. C.; Sun, H. D.; Lin, Z. W. *Acta Bot. Yunnannica* **1984**, *6*, 333–335.
(21) Jiang, B.; Lu, Z. Q.; Hou, A. J.; Zhao, Q. S.; Sun, H. D. *J. Nat. Prod.* **1999**, *62*, 941–945.
(22) Hong, S. S.; Lee, S. A.; Han, X. H.; Jin, H. Z.; Lee, J. H.; Lee, D.; Lee, J. J.; Hong, J. T.; Kim, Y.; Ro, J. S.; Hwang, B. Y. *J. Nat. Prod.* **2007**, *70*, 632–636.
(23) Nagashima, F.; Tanaka, H.; Tataoka, S.; Asakawa, Y. *Phytochemistry* **1996**, *41*, 1129–1141.
(24) *APEX II Users Manual*; Bruker AXS Inc., 2005.
(25) Sheldrick, G. M. *SHELXTL, v. 5, Reference Manual*; Siemens Energy and Automation, Inc., 1997.

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