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Meroterpenoid Pigments from the Basidiomycete *Albatrellus ovinus*

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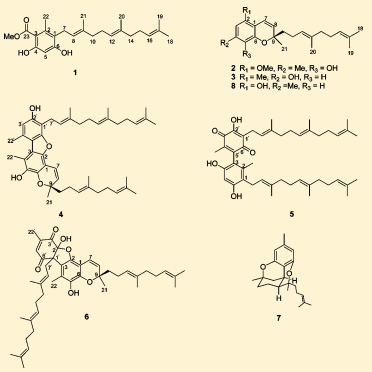
Supporting Information

ABSTRACT: Eight grifolin derivatives, involving three new monomers, albatrelins A−C (**1**−**3**), three novel dimers (meroterpenoid pigments), albatrelins D−F (**4**−**6**), and two known ones, 6a,7,8,9,10,10a-hexahydro-3,6,9-trimethyl-6-(4-methyl-3-penten-1-yl)-1,9-epoxy-6*H*-dibenzo[*b*,*d*]pyran (**7**) and confluentin (**8**), were isolated from *Albatrellus ovinus*. Their structures were established by extensive spectroscopic analysis. The absolute configurations of compounds **2**−**4** were determined as 9*R* by comparing their optical rotations with data reported in the literature. Albatrelin F (**6**) was isolated as a pair of C-2′ tautomers with a ratio of 1.3:1. Confluentin (**8**) showed weak cytotoxicity against four human tumor cell lines, HL-60, SMMC-7712, A-549, and MCF-7, *in vitro*.



Pigments of fungi have aroused great interest from mycological chemists because of their intriguing colors, complex structures, and diverse bioactivities. Presently, more than 1000 fungal pigments have been reported including compounds from the shikimate−chorismate pathway, acetate−malonate pathway, and mevalonate pathway and those containing nitrogen.[1−6] Dimeric grifolin meroterpenoid pigments originate from the shikimate−chorismate pathway.[1] Only four grifolin dimers, namely, grifolinones B[7] and C,[8] albatrellin[9] and 16-hydroxyalbatrellin,[9] have been isolated from natural sources. These four reported dimers could be divided into two types depending on the connection of the two monomeric parts: (A) head−tail type, the aromatic ring (head) of one monomer connected with the side-chain (tail) of the other; (B) head−head type, two aromatic rings (heads) of monomers directly connected. Structurally, grifolinone B, albatrellin, and 16-hydroxyalbatrellin belong to head−tail-type dimers, while grifolinone C is the only example of a head−head type of grifolin dimer from a natural source.

RESULTS AND DISCUSSION

Albatrellus ovinus is a nontoxic but inedible mushroom, mainly distributed through Asia, North America, and Europe.[10] Previous investigations on *A. ovinus* revealed the presence of various grifolin monomers but no dimers.[11,12] With the consideration that the congeneric fungi usually have similar constituents, an extensive investigation on the fruiting bodies of *A. ovinus* was performed and resulted in three novel grifolin dimers, albatrelins D−F (**4**−**6**), together with three new grifolin monomers, albatrelins A−C (**1**−**3**), and two known ones, 6a,7,8,9,10,10a-hexahydro-3,6,9-trimethyl-6-(4-methyl-3-penten-1-yl)-1,9-epoxy-6*H*-dibenzo[*b*,*d*]pyran (**7**)[13] and confluentin (**8**),[14] being isolated. Their structures were established by extensive spectroscopic analysis. The absolute configurations of compounds **2**−**4** were determined as 9*R* based on their optical rotations. Albatrelin F (**6**) was isolated as a pair of C-2′ tautomers with a ratio of 1.3:1. Compound **8** showed weak cytotoxic activity against four human tumor cell lines, HL-60, SMMC-7712, A-549, and MCF-7, with IC_{50} values of 15.05, 17.08, 18.48, and 23.01 μM, respectively, based on a cytotoxicity assay *in vitro*.

Compound **1**, a colorless oil, displayed a $[M + Na]^+$ ion at m/z 409.2347 in the positive HRESIMS, corresponding to the molecular formula $C_{24}H_{34}O_4$. The ^{1}H NMR spectrum indicated one aromatic proton at δ_H 6.29 (1H, s, H-5), three olefinic protons at δ_H 5.05 (1H, t, H-8) and 5.07 (2H, m, H-12 and 16), and one singlet methyl at δ_H 2.45 (3H, s, H-22). In the ^{13}C NMR (DEPT) spectrum, 24 carbons were recognized as one carbonyl group, one phenyl ring, three trisubstituted double bonds, five methylenes, and six methyls (including one methoxyl). Its NMR spectroscopic data were close to those of 1-formylneogrifolin, except that a methoxycarbonyl group was observed in the downfield region instead of the formyl group in 1-formylneogrifolin.[11] In the HMBC spectrum, the correlations from H-5, H-22, and OH-4 to C-3 and from OMe to C-23 suggested that the methoxycarbonyl group was located at the C-3 position. Therefore, the structure of compound **1** was determined and named albatrelin A.

Compound **2** possessed a molecular formula of $C_{23}H_{32}O_3$ according to the HRESIMS, which showed the quasi-molecular ion peak at m/z 379.2244 ($[M + Na]^+$), requiring eight degrees

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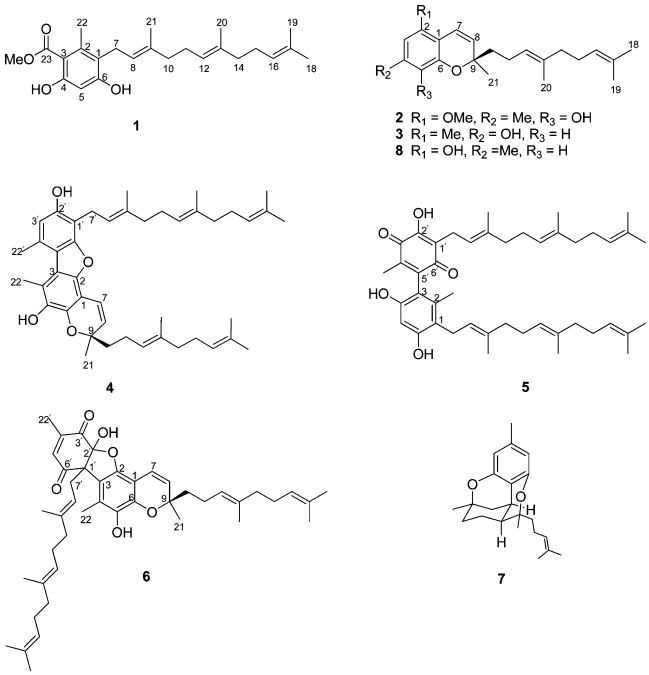

of unsaturation. The ^{13}C NMR (DEPT) spectra indicated six methyls (including one methoxyl), four methylenes, five sp^2 methines, and eight quaternary carbons. The characteristic 2*H*-chromene moiety was revealed from the aromatic proton at δ_H 6.15 (1H, s, H-3) and two *cis*-coupled olefinic protons at δ_H 6.63 (1H, d, J = 10.3 Hz, H-7) and 5.46 (1H, d, J = 10.3 Hz, H-8) in the ^1H NMR spectrum, as well as eight sp^2 carbons (from δ_C 104.7 to 148.0) and one quaternary carbon at δ_C 79.5 (s, C-9) in the ^{13}C NMR spectrum. Its NMR spectroscopic data were comparable to those of confluentin with the exception of one additional methoxyl group. Therefore, the key to determine the structure of compound **2** was the location of the hydroxyl and methoxyl group on the phenyl ring. In the ROESY spectrum, the correlation of H-3 with Me-22 and OMe, as well as the HMBC cross-peak of OMe/C-2, suggested that OMe was substituted at C-2. Similarly, the residual hydroxyl group was proposed at C-5 by HMBC correlation of OH with C-4 and C-6. The optical rotation ($[\alpha]^{16}_D$ −18.5, $CHCl_3$) was measured, and the only chiral center (C-9) in compound **2** was deduced as *R* by comparing with the rotation values of daurichromenes C ($[\alpha]^{26}_D$ −32.0, MeOH) and D ($[\alpha]^{26}_D$ −26.0, MeOH) and daurichromenic acid ($[\alpha]^{20}_D$ +30.0, $CHCl_3$)[15,16] (Figure 1).

Compound **3** was determined to have a molecular formula of $C_{22}H_{30}O_2$ from HREIMS at m/z 326.2251. Its ^1H and ^{13}C NMR spectra were similar to those of compound **2** except for the absence of one methoxyl group and the singlet aromatic proton in compound **2** being replaced by two *m*-coupling aromatic protons [δ_H 6.20 (d, J = 1.8 Hz, H-3) and 6.13 (d, J = 1.8 Hz, H-5)]. On the basis of the above analysis and its structural features, the hydroxyl and methyl groups on the phenyl ring should adopt a *meta*-substitution. In the HMBC spectrum, the observed cross-peaks of H-22 with C-1, C-2, and C-3 and of H-7 with C-1 and C-2 supported the location of methyl at C-2. The hydroxyl group was deduced at C-4 from HMBC correlations of OH (δ_H 8.27) with C-3, C-4, and C-5. Its absolute stereochemistry was reasonably characterized as 9*R* based on the optical rotation ($[\alpha]^{16}_D$ = −17.2, $CHCl_3$).

Compound **4** was obtained as a yellow oil, with the molecular formula of $C_{44}H_{58}O_4$ according to the HREIMS at m/z 650.4329, corresponding to 16 degrees of unsaturation. The ^{13}C NMR spectrum showed 36 signals; in addition four signals [δ_C 17.6 (q), 25.7 (q), 26.6 (t), and 39.6 (t)], each representing two overlapped ones, of which 10 methyls, 9 methylenes, 8 methines, and 17 quaternary carbons were recognized, indicated a dimeric grifolin skeleton. Extensive 1D and 2D NMR analysis allowed the establishment of partial structures **4a** and **4b**. The characteristic carbon signals of three trisubstituted double bonds [δ_C 121.4 (d, C-8′), 123.6 (d, C-12′), 124.4 (d, C-16′), 138.6 (s, C-9′), 135.5 (s, C-13′), 131.4 (s, C-17′)] and four singlet methyls (δ_H 1.58, 1.59, 1.67, 1.89) suggested a farnesyl moiety. The methylene at δ_H 3.68 (d, J = 7.2 Hz, H-7′) showed HMBC correlations to carbons at δ_C 152.8 (s, C-2′) and 155.7 (s, C-6′), suggesting the connection of C-1′/C-7′. Similarly, Me-22′ (δ_H 2.80) was proposed to be located at C-4′ by a ROESY correlation of H-3′/Me-22′ and HMBC cross-peaks from H-3′ to C-1′ (δ_C 107.7), C-2′ (δ_C 152.8), and C-5′ (δ_C 117.8). Thus, the partial structure **4b** was determined as a grifolin-like moiety with its C-5′ position being substituted (Figure 3).[14] The remaining NMR data belonging to the partial structure **4a** were very similar to those of compound **2**, except for the absence of an OMe and an aromatic proton (H-3). The nucleus of 2*H*-chromene was proved by the characteristic signals of two *cis*-coupled protons at δ_H 6.90 (1H, d, J = 9.9 Hz, H-7) and 5.64 (1H, d, J = 9.9 Hz, H-8) and a quaternary carbon at δ_C 80.2 (C-9), as well as the HMBC correlations from H-7 to C-2 and C-6 and from H-8 to C-1. In addition, the HMBC correlations from δ_H 2.75 (Me-22) to δ_C 138.7 (C-5, s), 117.6 (C-3, s), and 116.4 (C-4, s) and from δ_H 5.44 (OH) to δ_C 138.7 (C-5), 145.7 (C-6), and 116.4 (C-4) suggested the substitution of Me-22 and OH-5 on the phenyl ring. HMBC cross-peaks and a ROESY correlation between Me-22 and Me-22′ revealed that partial structures **4a** and **4b** connected through position 3 to 5′. Up to now, 15 degrees of unsaturation was addressed from the above analysis, and one additional ring was needed to fulfill its unsaturation. Therefore, an oxygen bridge was proposed between C-2 and C-6′. The stereochemistry of compound **4** was determined to be 9*R* by comparing its optical rotation ($[\alpha]^{24}_D$ −21.7, $CHCl_3$) with those of **2** and **3**.

Compound **5** was a deep yellow powder, with a molecular formula of $C_{44}H_{60}O_5$ based on HREIMS at m/z 668.4429 ($[M]^+$), requiring 15 degrees of unsaturation. Inspection of the ^{13}C NMR spectrum revealed 30 signals with an additional seven signals each representing two carbon atoms due to the presence of two identical farnesyl groups. Partial structure **5a** was elucidated based on extensive MS, IR, and NMR spectroscopic

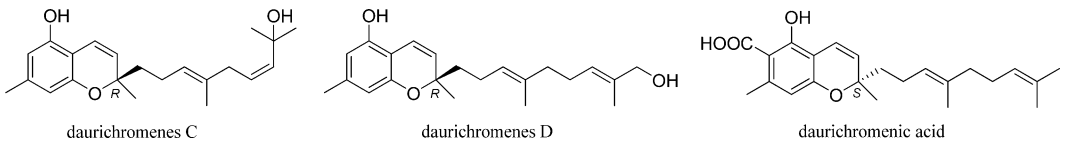

Figure 1. Structures and absolute configurations of daurichromens C and D and daurichromenic acid.

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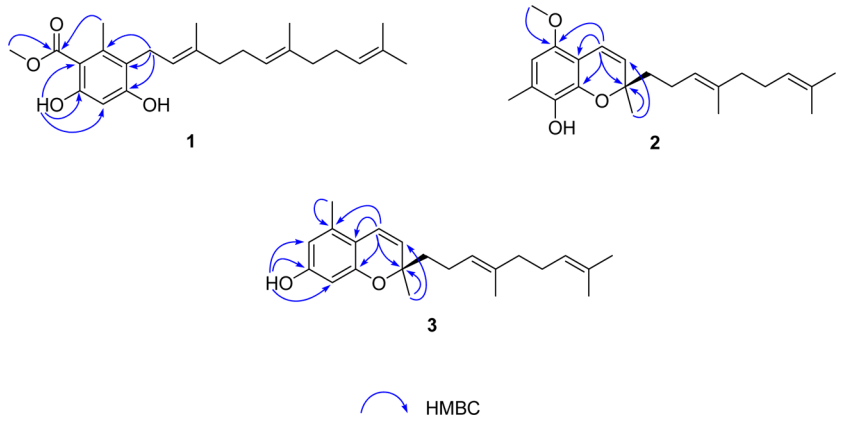

Figure 2. Key HMBC correlations for compounds **1**−**3**.

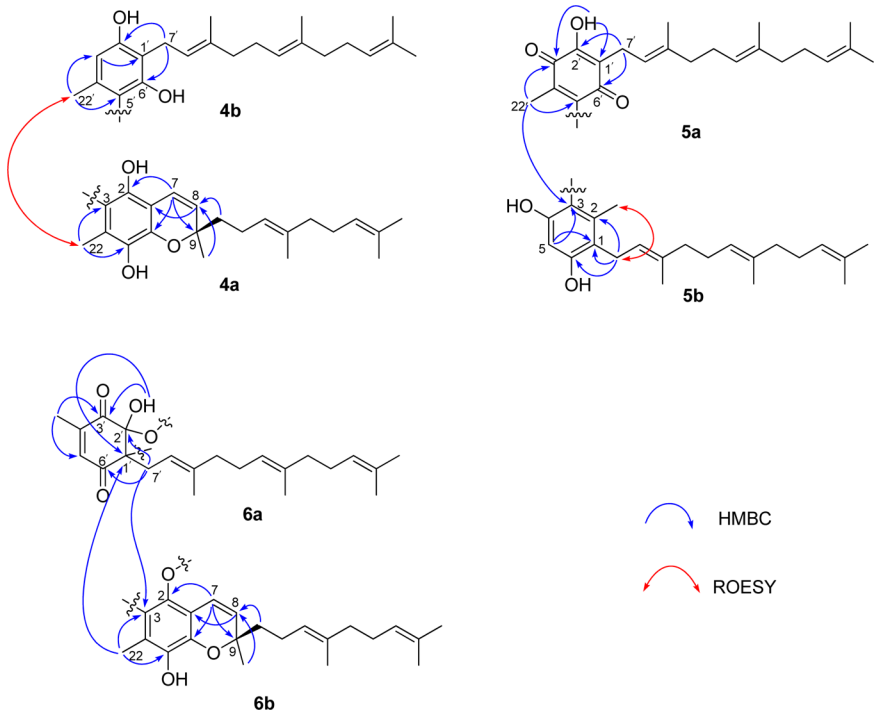

Figure 3. Key HMBC and ROESY correlations for partial structures **4a**, **4b**, **5a**, **5b**, **6a**, and **6b**.

analysis. An intensive $[M + H]^+$ ion peak in the EIMS spectrum, a strong IR absorption at 1647 cm^{-1}, and two downfield carbon signals at δ_C 184.1 and 186.9 were characteristic for a quinone moiety.[9] Further, The HMBC cross-peaks from H-7′ (δ_H 3.17) to C-2′, C-6′, and C-1′ and from OH-2′ to C-1′, C-3′, and C-2′ revealed that **5a** was a *p*-benzoquinone with a farnesyl group at position 1′. Me-22′ was located at position 4′ based on the HMBC cross-peaks from Me-22′ to C-3′, C-4′, and C-5′ (Figure 3). The quaternary carbon C-5′ was proposed to connect with partial structure **5b**. The remaining ^1H and ^{13}C NMR data were indicative of a 3-substituted neogrifolin moiety (**5b**),[14] which was supported by the HMBC correlations from H-7 to C-1, C-2, and C-6, from Me-22 to C-1, C-2, and C-3, and from H-5 to C-1 and C-3, combined with ROESY correlation of Me-22/H-7. HMBC correlations from δ_H 1.84 (Me-22′) to δ_C 113.4 (C-3) and 151.1 (C-4) suggested that fragments **5a** and **5b** were connected by C-3 and C-5′. Thus, compound **5** was elucidated and named albatrelin E.

Compound **6**, a deep yellow powder, exhibited a molecular ion peak at *m*/*z* 682.4229 ($[M]^+$), corresponding to a molecular formula of $C_{44}H_{58}O_6$ with 16 degrees of unsaturation. Extensive analysis of 1D and 2D NMR spectra of **6** revealed a fragment structurally the same as **4a**. The remaining 1D NMR signals included two carbonyls, one trisubstituted double bond, two quaternary carbons, one singlet methyl, and a farnesyl group. A hemiketal with a free hydroxyl at C-2′ was proved by the observed carbon signal at δ_C 104.0 (s) and HMBC cross-peak of OH-2′/C-2′. HMBC correlations from H-7′ to C-1′, C-2′, and C-6′ revealed the connection of C-1′ and the farnesyl group. The ROESY correlation of H-5′/Me-22′ and HMBC correlations from Me-22′ to C-5′ and C-3′ supported the location of Me-22′ at position 4′. The observed HMBC cross-peaks from H-7′ to C-3 and from H-22 to C-1′ revealed that C-1′ was connected to C-3. As a consequence, there was one unsaturation remaining, which meant that another ring needed to be formed to complete the structure of compound **6**. The last possible ring could be formed only

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 1−3

	1[a]		2[b]		3[c]	
pos.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	119.6, s		108.5, s		112.9, s	
2	140.9, s		148.0, s		135.6, s	
3	107.0, s		104.7, d	6.15, s	110.2, d	6.20, d (1.8)
4	162.6, s		123.8, s		158.7, s	
5	101.5, d	6.29, s	137.0, s		102.0, d	6.13, d (1.8)
6	159.2, s		139.6, s		155.6, s	
7	25.1, t	3.34, d (6.7)	117.8, d	6.63, d (10.3)	120.4, d	6.51, d (10.1)
8	121.9, d	5.05, t (6.7)	127.0, d	5.46, d (10.3)	126.7, d	5.52, d (10.1)
9	137.1, s		79.5, s		78.3, s	
10	39.7, t	1.93−2.22, m	41.1, t	1.75, ddd (14.0, 11.0, 5.5) 1.67, ddd (14.0, 11.4, 5.5)	41.6, t	1.67, overlapped
11	26.4, t	1.93−1.22, m	22.9, t	2.04−2.14, m	23.3, t	2.08−2.14, m
12	123.7, d	5.07, overlapped	124.0, d	5.08, t (7.1)	125.1, d	5.14, t (7.1)
13	135.3, s		135.7, s		135.7, s	
14	39.6, t	1.93−2.11, m	39.9, t	2.02, overlapped 1.93, overlapped	40.4, t	1.94−1.97, m
15	26.7, t	1.93−2.11, m	26.8, t	2.02, overlapped 1.93, overlapped	27.3, t	2.03−2.06, m
16	124.3, d	5.07, overlapped	124.5, d	5.05, t (7.0)	125.1, d	5.09, t (6.9)
17	131.3, s		131.6, s		131.6, s	
18	25.1, q	1.67, s	25.9, q	1.65, s	25.8, q	1.64, s
19	17.7, q	1.58, s	17.9, q	1.57, s	17.7, q	1.57, s
20	16.0, q	1.57, s	16.2, q	1.54, s	16.0, q	1.57, s
21	16.2, q	1.78, s	26.5, q	1.37, s	26.4, q	1.33, s
22	18.8, q	2.45, s	16.2, q	2.21, s	18.5, q	2.19, s
23	172.3, s					
-OCH_3	51.9, q	3.92. s	56.2, q	3.73, s		
4-OH		11.2, s				8.27, s

[a]Spectra were measured in $CDCl_3$ at 400 MHz. [b]Spectra were measured in $CDCl_3$ at 600 MHz. [c]Spectra were measured in acetone-d_6 at 400 MHz.

Table 2. ^{1}H NMR Spectroscopic Data for Compounds 4−6

	δ_H (J in Hz)				δ_H (J in Hz)		
pos.	4[a]	5[a]	6[b]	pos.	4[a]	5[a]	6[b]
5		6.07, s		3′			6.82, s
7	6.90, d (9.9)	3.28, d (6.3)	6.36, d (9.9)	5′	6.59, s		
8	5.64, d (9.9)	5.13, t (6.3)	5.63, d (9.9)	7′	3.68, d (7.2)	3.17, d (7.4)	3.50, dd (13.0, 8.6) 2.78, dd (13.0, 6.3)
10	1.77−1.85, m	2.02−2.06, m	1.68−1.80, m	8′	5.39, t (7.2)	5.16, t (7.4)	5.07, m
11	2,14−2.16, m	2.06−2.10, m	2.09−2.15, m	10′	2.08−2.12, m	1.93−1.97, m	1.85−1.87, m
12	5.12, t (6.7)	5.06−5.08, m	5.13, m	11′	2.10−1.16, m	1.93−1.97, m	1.85−1.87, m
14	2.08−2.12, m	2.02−2.06, m	1.94−1.98, m	12′	5.06−5.10, m	5.06−5.08, m	5.04−5.07, m
15	2.02−2.06, m	2.02−2.06, m	1.94−1.98, m	14′	1.95−1.98, m	2.02−2.06, m	2.03−2.07, m
16	5.06−5.10, m	5.06−5.08, m	5.04−5.07, m	15′	2.02−2.06, m	2.02−2.06, m	2.03−2.07, m
18	1.67, s	1.67, s	1.65, s	16′	5.06−5.10, m	5.06−5.08, m	5.04−5.07, m
19	1.59, s	1.59, s	1.58, s	18′	1.67, s	1.67, s	1.65, s
20	1.58, s	1.58. s	1.54; 1.56 s	19′	1.59, s	1.59, s	1.58, s
21	1.48, s	1.77, s	1.34; 1.38, s	20′	1.58, s	1.57, s	1.58, s
22	2.75, s	1.93, s	2.50, s	21′	1.89, s	1.72, s	1.60, s
5-O*H*	5.44, s		7.14, s	22′	2.80, s	1.84, s	2.11, s
2′-O*H*	5.31, s	7.08, s	6.77, s				

[a]Spectra were measured in CDCl3 at 400 MHz. [b]Spectrum was measured in acetone-d_6 at 400 MHz.

between C-2 and C-2′ by an oxygen bridge. The instability of the C-2′ hemiketal structure resulted in the splitting of several ^{1}H and ^{13}C NMR signals (see Tables 2 and 3). Since all compounds from this species showed a 9*R* configuration, we deduced that the stereochemistry at C-9 of compound **6** was also *R*. The absolute configuration of C-1′ and C-2′ could not be identified because this compound was isolated as a pair of C-2′ tautomers, which could not be separated. The ratio of the tautomers was approximately 1.3:1 because the area ratio of the splitting signals of CH_3-21 was about 1.3:1 in the ^{1}H NMR spectrum. The major tautomer was deduced to be the one with 2′-OH and the farnesyl moiety in the same orientation,

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Table 3. ^{13}C NMR Spectroscopic Data for Compounds 4−6

pos.	δ_C, type 4[a]	5[a]	6[b]	pos.	δ_C, type 4[a]	5[a]	6[b]
1	103.4, s	118.9, s	104.4, s	1′	107.7, s	120.4, s	67.6, s
2	137.6, s	136.4, s	140.7, s	2′	152.8, s	150.8, s	104.0, s
3	117.6, s	113.4, s	118.1, s	3′	114.2, d	184.1, s	192.5, s
4	116.4, s	151.1, s	124.0, s	4′	129.5, s	139.8, s	149.6, s
5	138.7, s	101.4, d	139.8, s	5′	117.8, s	143.6, s	141.5, d
6	145.7, s	155.3, s	146.2, s	6′	155.7, s	186.9, s	196.4; 196.3, s
7	117.0, d	25.3, t	117.3, d	7′	22.9, t	22.3, t	31.2, t
8	128.2, d	122.1, d	129.2; 129.1, d	8′	121.4, d	119.6, d	120.0, d
9	80.2, s	137.1, s	80.0, s	9′	138.6, s	137.2, s	140.0, s
10	40.9, t	39.73, t	41.6; 41.4, t	10′	39.6, t	39.73, t	40.5, t
11	22.7, t	26.5, t	23.4; 23.2, t	11′	26.3, t	26.6, t	27.4, t
12	123.6, d	123.8, d	124.8; 124.7, d	12′	123.6, d	124.1, d	124.7, d
13	135.6, s	135.3, s	135.8; 135.7, s	13′	135.5, s	135.0, s	135.6, s
14	39.7, t	39.68, t	40.33; 40.28, t	14′	39.6, t	39.68, t	40.4, t
15	26.6, t	26.70, t	27.28, 27.25, t	15′	26.6, t	26.73, t	27.3, t
16	124.3, d	124.3, d	125.1, d	16′	124.4, d	124.4, d	125.1, d
17	131.2, s	131.3, s	131.6, s	17′	131.4, s	131.3, s	131.6, s
18	25.7, q	25.7, q	25.8, q	18′	25.7, q	25.7, q	25.8, q
19	17.6, q	17.1, q	17.7, q	19′	17.6, q	17.1, q	17.7, q
20	16.0, q	16.0, q	16.03; 15.98, q	20′	16.0, q	16.0, q	16.0, q
21	26.3, q	16.2, q	26.5; 26.4, q	21′	16.3, q	16.15, q	16.7, q
22	15.9, q	17.1, q	11.8, q	22′	25.1, q	12.9, q	16.2, q

[a]Spectra were measured in $CDCl_3$ at 100 MHz. [b]Spectrum was measured in acetone-d_6 at 100 MHz.

supported by the weak ROESY correlation between δ_H 6.77 (2′-OH) and 2.78 (H-7′).

All the isolates were assayed for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7712, A-549, MCF-7, and SW480) by the MTT method *in vitro*, with DDP and paclitaxel as positive controls. The result showed that compound **8** exhibited weak cytotoxic activities against four cancer cell lines, HL-60, SMMC-7712, A-549, and MCF-7, with IC_{50} values of 15.05, 17.08, 18.48, and 23.01 μM, respectively, and compounds **1**−**6** exhibited IC_{50} values ≥ 40 μM to the cell lines mentioned above and can be regarded as inactive.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco model 1020 polarimeter (Jasco International Co. Ltd., Tokyo, Japan). UV spectra were recorded on a Shimadzu double-beam 2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Ettlingen, Germany) using KBr pellets. 1D and 2D NMR spectra were acquired on Bruker AV-600, DRX-500, and AM-400 instruments at room temperature with TMS as internal standard (Bruker, Rheinstetten, Germany). Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra (MS) were recorded on an API QSTAR time-of-flight spectrometer (MDS Sciex, Ontario, Canada) or a VG Autospec-3000 spectrometer (VG, Manchester, England). Silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Sweden), and RP-18 gel (40−75 μm, Fuji Silysia Chemical Ltd. Japan) were used for column chromatography. Preparative HPLC (Prep-HPLC) was performed on an Agilent 1100 liquid chromatography system equipped with a Zorbax SB-C_{18} column (9.4 mm × 150 mm). Precoated silica gel GF254 plates (Qingdao Marine Chemical Inc., Qingdao, China) were used for TLC. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Fungal Material. The fungus *A. ovinus* was collected in Anhui Province, China, in October 2011. The mushroom was identified by Prof. Zhu-Liang Yang (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The fruiting bodies of *A. ovinus* (about 500 g) were extracted twice with chloroform/methanol (1:1). Evaporation of the solvent under reduced pressure gave the extract (40 g), which was subjected to silica gel column chromatography (CC) using a petroleum ether/acetone gradient (1:0 → 0:1) to afford fractions A−G. Fraction A was separated with petroleum ether to give **7** (6.0 mg). Fraction C was subjected to CC over silica gel with a petroleum ether/acetone system (20:1 → 10:1) to yield two subfractions, C_1 and C_2. Fraction C_1 was purified by preparative HPLC (CH_3CN/H_2O, 6:4 → 9:1) to give **2** (21.2 mg) and **8** (15.0 mg), while fraction C_2 was chromatographed by CC over RP-18 gel to provide **1** (11.0 mg) and **3** (3.0 mg). Fractions D−G appeared dark red, showing the presence of pigments. Fraction D was purified using Sephadex LH-20 column chromatography ($CHCl_3$/MeOH, 1:1) and then repeated CC over silica gel and preparative HPLC to afford compound **4** (8.6 mg). Fraction E was subjected to Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and then further purified by prep-HPLC (CH_3CN/H_2O, 9:1) to yield compound **5** (20.0 mg). Fraction F was applied on a RP-18 gel column (95% methanol) to obtain compound **6** (16.0 mg).

*Albatrelin A (**1**):* colorless oil; UV (MeOH) λ_{max} (log ε) 308 (2.97), 266 (3.32), 230 (3.57) nm; IR (KBr) ν_{max} 3303, 2965, 2922, 2851, 1657, 1605, 1321, 1259 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m*/*z* 409 (100) $[M + Na]^+$; HRESIMS (positive) *m*/*z* 409.2347 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_4Na$, 409.2354).

*Albatrelin B (**2**):* colorless oil; $[\alpha]^{16}_D$ −18.5 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 320 (2.84), 286 (3.02), 223 (3.43) nm; IR (KBr) ν_{max} 3303, 2965, 2922, 2851, 1657, 1605, 1321, 1259 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m*/*z* 379 (30) $[M + Na]^+$; HRESIMS (positive) *m*/*z* 379.2244 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_3Na$, 379.2249).

*Albatrelin C (**3**):* colorless oil; $[\alpha]^{16}_D$ −17.2 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 320 (2.62), 286 (2.92), 220 (3.47) nm; IR (KBr) ν_{max} 3441, 2966, 2924, 2853, 1613, 1460, 1144 cm^{-1}; ^1H and

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^{13}C NMR data, see Table 1; EIMS) *m/z* 326 (50) $[M]^+$, 175 (100); HREIMS *m/z* 326.2251 $[M]^+$ (calcd for $C_{22}H_{30}O_2$, 326.2246).

*Albatrelin D (**4**):* yellow oil; $[\alpha]^{24}_D$ −21.7 (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 342 (3.01), 286 (3.42), 255 (3.62) nm; IR (KBr) ν_{max} 3441, 2967, 2923, 2854, 1620, 1444, 1081 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; ESIMS *m/z* 673 (100) $[M + Na]^+$, 1323 (10) $[2\ M + Na]^+$; HREIMS (positive) *m/z* 650.4329 $[M]^+$ (calcd for $C_{44}H_{58}O_4$, 650.4335).

*Albatrelin E (**5**):* deep yellow powder; UV (MeOH) λ_{max} (log ε) 424 (2.77), 330 (3.13), 282 (3.48) nm; IR (KBr) ν_{max} 3417, 2967, 2922, 2854, 1647, 1615, 1443, 1369, 1312, 1259, 1087 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; ESIMS (positive) *m/z* 691 (100) $[M + Na]^+$; EIMS (positive) *m/z* 668 (50) $[M]^+$, 669 (40), 670 (50), 517 (50), 69 (100); HREIMS *m/z* 668.4429 $[M]^+$ (calcd for $C_{44}H_{60}O_5$, 668.4441).

*Albatrelin F (**6**):* deep yellow powder; $[\alpha]^{24}_D$ −2.3 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 417 (2.52), 330 (2.88), 282 (3.31), 225 (3.83) nm; IR (KBr) ν_{max} 3441, 2966, 2922, 2853, 1667, 1635, 1437, 1378, 1069 cm^{-1}; ^{1}H NMR data, see Table 2;^{13}C NMR data, see Table 3; ESIMS (positive) *m/z* 705 (100) $[M + Na]^+$, 1387 (30) $[2\ M + Na]^+$; EIMS *m/z* 682 (30) $[M]^+$, 683 (20), 684 (10), 531 (40); HREIMS *m/z* 682.4229 $[M]^+$ (calcd for $C_{44}H_{58}O_6$, 682.4233).

Cytotoxicity Assays. The human tumor cell lines HL-60 (promyelocytic leukemia), SMMC-7712 (hepatocarcinoma), A-549 (lung carcinoma), MCF-7 (breast), and SW 480 (colon adenocarcinoma) were used. All the cells were cultured in RMPI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compounds at various concentrations in triplicate for 48 h, with DDP and toxal as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells lysed with 200 μL of SDS after removal of 100 μL of medium. The optical density of lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[17]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra for compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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