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Hepatoprotective Effects of Triterpenoids from *Ganoderma cochlear*

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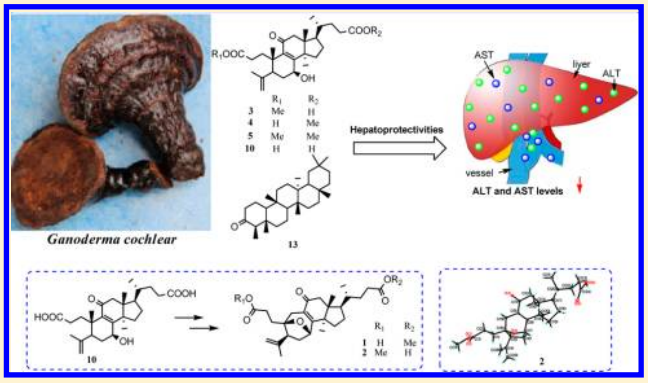
Supporting Information

ABSTRACT: Two novel trinorlanostanes, cochlates A and B (**1** and **2**), with a 3,4-*seco*-9,10-*seco*-9,19-cyclo skeleton, as well as six new triterpenoids, fornicatins D−F (**3**−**5**) and ganodercochlearins A−C (**6**−**8**), together with five known triterpenoids (**9**−**13**), were obtained from the fruiting bodies of *Ganoderma cochlear*. The structural elucidation was achieved by interpretation of spectroscopic data, and compounds **2** and **7a** were further characterized by X-ray crystallographic analysis. Fornicatins A, D, and F (**10**, **3**, and **5**) and fredelin (**13**) lowered the ALT and AST levels in HepG2 cells treated with H_2O_2, suggesting that they could display in vivo hepatoprotective activities.



The fungal family Ganodermataceae contains more than 200 species, which mostly occur in subtropical and tropical regions.[1] *Ganoderma* has been used as a folk medicine to treat and prevent various diseases. The identified chemical constituents of *Ganoderma* include polysaccharides, a triterpene, sterols, lectins, and so on, of which Ganoderma triterpenoids (GTs) is one of the main components,[2] that show diverse biological activities, including cytotoxicity against HepG2 and P-388 tumor cells (lucidenic acids A and N and ganoderic acid E)[3] and HIV-1 protease inhibitory (schisanlactones A and G),[4] antiplasmodial (ganoderic acid TR1, ganoderic aldehyde TR, and 23-hydrocyganoderic acid S),[5] and antiviral activities (ganoderone A and lucialdehyde B).[6]

Ganoderma sinense, a traditional Chinese medicine, is registered in the Chinese Pharmacopoeia together with *Ganoderma lucidum* and has been used widely in Asian countries. Our previous research on the chemical constituents of this fungus has led to the isolation of ganoderma acids,[7] ganoderma acohols,[7] novel skeleton lanostane-triterpenoids (methyl ganosinensate A, ganosinensic acids A and B),[8] and alkaloids (sinensines B−E and sinensine).[9]

Ganoderma cochlear has the same morphological characteristics as *G. sinense*, but the fungus stipe of *G. cochlear* lies in the back of the pileus. Our initial phytochemical investigation on *G. cochlear* has resulted in the identification of two new 3,4-*seco*-trinorlanostane triterpenoids,[10] suggesting that different types of GTs exist in *G. cochlear*. To discover additional biologically functional triterpenoids from *G. cochlear*, we systematically isolated and characterized the constituents of *G. cochlear*.

Two novel trinorlanostanes, cochlates A and B (**1** and **2**), with a 3,4-*seco*-9,10-*seco*-9,19-cyclo skeleton, and six new triterpenoids, named fornicatins D−F (**3**−**5**) and ganodercochlearins A−C (**6**−**8**), together with five known compounds, were isolated from the fruiting bodies of *G. cochlear*. New compounds were identified by spectroscopic technology and X-ray crystallographic analysis. An in vitro H_2O_2-induced HepG2 cell injury model was used to evaluate inhibitory activities of compounds **1**, **3**, **4**, **5**, **9**, **10**, and **13** against the increase of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels in the range of their maximum nontoxic concentration.

RESULTS AND DISCUSSION

Cochlate A (**1**) was assigned a molecular formula of $C_{28}H_{40}O_6$ by HRESIMS and 1D NMR spectroscopic data (Tables 1 and 2). The hydroxyl (3433 cm^{-1}), carbonyl (1708 and 1742 cm^{-1}), and conjugated carbonyl groups (1656 cm^{-1}) were identified from the IR spectrum. The UV spectrum showed an absorption band at 258 nm due to the α,β-unsaturated carbonyl group. The ^1H NMR spectrum showed four methyl signals at δ 1.77 (s), 1.19 (s), 0.90 (s), and 0.89 (d, J = 6.0 Hz) and a methoxyl signal at δ 3.67 (s). The ^{13}C NMR DEPT spectra displayed 28 carbon resonances for five methyls (one methoxyl), 10 methylenes (one sp^2 carbon), four methines (one oxymethine), six quaternary carbons (three sp^2 carbons and one oxygenated quaternary carbon), and three carbonyls (one ketone, one carboxyl, and one ester). These NMR data of **1** were characterized for a 3,4-*seco*-trinorlanostane triterpenoid, which was similar to fornicatin B.[11]

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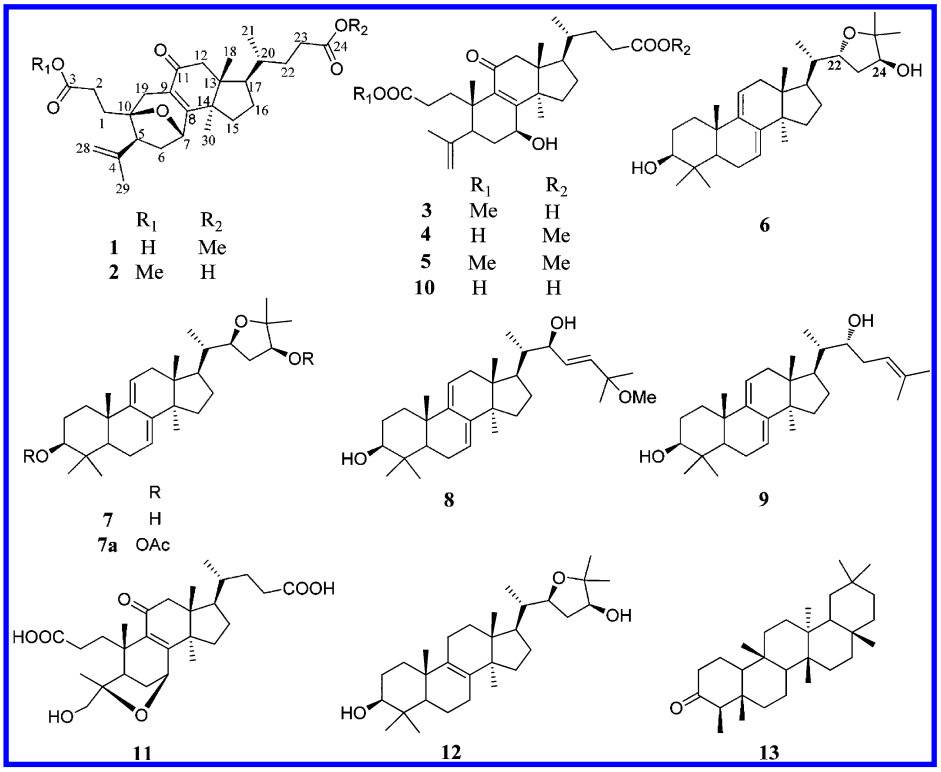
Chart 1



However, comparison of the 1D NMR data of **1** with those of fornicatin B showed that **1** had four methyl groups, one quaternary methyl less than fornicatin B. In addition, the presence of an additional methylene (δ 35.0) and the double-bond between C-8 and C-9 determined that the gross structure of **1** had the same seven-membered B ring (9,19-cyclo-9,10-*seco*) as colossolactone D.[12] Long-range HMBC correlations (Figure 1) from H-5 (δ 2.69) to C-1 (δ 30.4), C-4 (δ 144.0), C-6 (δ 41.4), C-7 (δ 73.4), C-10 (δ 82.4), and C-19 (δ 35.2) and from H-19 (δ 2.11, 2.39) to C-1 (δ 30.4), C-5 (δ 53.5), C-8 (δ 166.0), C-9 (δ 125.5), and C-7 (δ 73.4), together with the ^{1}H−^{1}H COSY correlations of H-5/H$_2$-6/H-7, further confirmed a seven-membered ring B in **1**. Meantime, the ether bond between C-10 and C-7 was identified by the HMBC correlation from H-7 (δ 4.56) to C-10 (δ 82.4).

In the ^{13}C NMR spectrum of **1**, a methoxyl (δ 51.5) and a low-field quaternary carbon (δ 174.6) indicated the presence of a carboxyl ester. Furthermore, the HMBC correlations of H-22 (δ 1.32), H$_2$-23 (δ 2.27, 2.39), and OMe (δ 3.67) with the ester group (δ 174.6) speculated that the ester was located at C-24.

Compound **2**, obtained from the same polarity fraction as **1**, was isolated as colorless crystals. The molecular formula of **2** was the same as that of **1**, assigned as $C_{28}H_{40}O_6$ ([M + Na]$^+$, *m*/*z* 495.2731; calcd 495.2722) by HRESIMS. The IR and UV absorption were similar to **1** and showed the presence of hydroxyl, ketone carbonyl, and α,β-unsaturated carbonyl groups. The ^{1}H and ^{13}C NMR spectroscopic data of **2** were also analogous to those of **1** and are shown in Tables 1 and 2. The only distinction between **1** and **2** could be the position of the ester. The HMBC correlations of H$_2$-1 (δ 2.08, 2.30) with C-2 (δ 28.9), C-3 (δ 174.1), C-10 (δ 82.3), and C-19 (δ 35.0) and of H$_2$-2 (δ 2.46) and OMe (δ 3.64) with C-3 (δ 174.1) indicated that the carboxyl ester was attached to C-3 in **2**. A single X-ray crystallographic analysis of **2** clarified the stereochemistry (Figure 2). The absolute configurations of C-7 and C-10 were deduced as *S** and *S**, respectively.

Therefore, the structures of **1** and **2** were established as (7*S*,10*S*)-7,10-epoxy-3,4-*seco*-9,10-*seco*-9,19-cyclo-25,26,27-trinorlanosta-4(28),8-dien-3-oic-24-ester and (7*S*,10*S*)-7,10-epoxy-3,4-*seco*-9,10-*seco*-9,19-cyclo-25,26,27-trinorlanosta-4-(28),8-dien- 24-oic-3-ester, named cochlates A (**1**) and B (**2**), respectively.

Fornicatin D (**3**) and fornicatin E (**4**) gave the same molecular formula, $C_{28}H_{42}O_6$ (HRESIMS). The IR spectrum of **3** showed absorption bands at 3452, 1730, 1695, and 1643 cm^{-1}, indicating the presence of hydroxyl, carbonyl, and α,β-unsaturated carbonyl groups. The 1D NMR data of **3** suggested that **3** is similar to fornicatin B,[11] except for the presence of an additional methoxyl in **3**. The HMBC correlations of H$_2$-1 (δ 3.09), H$_2$-2 (δ 2.48), and OMe (δ 3.61) with C-3 (δ 174.2) indicated that the carboxyl group at C-3 in fornicatin B was converted into the ester group in **3**.

Comparison of spectroscopic data of **4** and **3** allowed us to deduce that **4** is an isomer of **3**. In the HMBC spectrum of **4**, H$_2$-22 (δ 2.48, 2.30), H$_2$-23 (δ 1.92, 1.38), and OMe (δ 3.63) showed correlations with C-24 (δ 174.3), suggesting that the ester group was positioned at C-24 in **4**. A correlation of H-5/H-7, which was observed in the ROESY spectra of both **3** and **4**, suggested 7-OH as β-oriented. Accordingly, the structures of **3** and **4** were methyl 7β-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-4(28),8-dien-24-oic-3-ester and methyl 7β-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-4(28),8-dien-3-oic-24-ester, named fornicatins D and E (**3**, **4**).

Fornicatin F (**5**) possessed a molecular formula of $C_{29}H_{44}O_6$ as determined by HRESIMS (*m*/*z* 511.3039, [M + Na]$^+$; calcd 511.3035). The 1D NMR data (Tables 1 and 2) of **5** were similar to those of **3**, and the major difference was that an additional methoxyl and the high-field shift of C-24 (2 ppm)

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−8 [δ in ppm, J in Hz]

position	1[a]	2[b]	3[b]	4[b]	5[a]	6[b]	7[b]	8[b]
1	1.87, m	2.08, m	3.09, m	3.25, m	2.08, m	1.49, m	2.04, m	1.54, m
	2.03, m	2.30, m				2.04, m		2.07, m
2	2.46, m	2.46, m	2.48, m	2.65, m	2.23, m	1.30, m	1.97, m	1.99, m
			2.09, m		2.00, m			
3						3.49, t (7.2)	3.49, t (7.8)	3.50, m
5	2.69, m	2.69, m	2.41, dd (5.0, 15.0)	2.48, m	2.12, m	1.27, m	1.28, m	1.31, m
6	2.26, m		2.23, m	2.26, m	2.12, m	2.19, m	1.31, m	2.19, m
					2.04, m			
7	4.56, d (6.1)	4.54, d (6.3)	4.69, m	4.70, m	4.25, m	5.57, d (5.1)	5.58, d (6.0)	5.61, d (5.7)
11						5.38, d (6.0)	5.38, d (6.1)	5.44, d (6.1)
12	2.52, d (20.0)	2.50, d (20.0)	2.64, d (20.0)	2.68, d (16.0)	2.53, d (17.8)	2.17, d (17.4)	2.16, d (17.4)	2.22, m
	2.64, d (20.0)	2.64, d (20.0)	2.84, d (20.0)	2.81, d (16.0)	2.64, d (17.8)	2.28, d (17.4)	2.28, d (17.4)	2.37, m
15	1.45, m	1.43, m	3.00, m	2.99, m	2.46, m	2.71, m	2.17, m	1.53, m
				1.65, m	1.41, m			1.82, m
16	1.54, m	1.54, m	1.51, m	1.45, m	2.02, m	1.44, m	1.47, m	1.62, m
	2.11, m	2.13, m		1.92, m	1.42, m	1.71, m	1.75, m	2.34, m
17	1.80, m	1.78, m	1.66, m	1.59, m	1.66, m	2.21, m	2.22, m	2.50, m
18	0.90, s	0.88, s	1.03, s	1.02, s	0.94, m	0.70, s	0.70, s	0.82, s
19	2.11, m	2.11, m	1.42, s	1.48, s	1.26, m	1.11, s	1.12, s	1.14, s
	2.39, m	2.39, m						
20	1.47, m	1.48, m	1.48, m	1.36, m	1.44, m	1.48, m	1.55, m	1.74, m
21	0.89, d (6.0)	0.88, d (6.0)	0.89, d (6.5)	0.81, d (6.0)	0.88, d (6.4)	1.05, d (6.6)	1.06, d (6.6)	1.30, d (6.7)
22	1.32, m	1.40, m	2.00, m	2.48, m	2.37, m	4.54, m	4.21, m	4.71, m
			1.47, m	2.30, m	2.24, m			
23	2.27, m	2.28, m	2.61, m	1.92, m,	1.44, m	2.19, m	2.21, m	5.95, dd (4.1, 15.9)
	2.39, m	2.40, m		1.38, m		1.44, m	1.40, m	
24						4.27, m	4.36, m	6.00, dd (1.7, 15.9)
26						1.39, s	1.40, s	1.37, s
27						1.54, s	1.54, s	1.37, s
28	4.81, s	4.79, s	4.89, s	4.97, s	4.83, s	1.17, s	1.16, s	1.25, s
	4.95, s	4.92, s	5.02, s	5.03, s	5.07, s			
29	1.77, s	1.76, s	1.78, s	1.82, s	1.83, s	1.24, s	1.24, s	1.17, s
30	1.19, s	1.18, s	1.23, s	1.23, s	1.10, s	0.91, s	0.91, s	1.02, s
OMe (3)		3.64, s	3.61, s		3.63, s			
OMe (24)	3.67, s			3.63, s	3.66, s			
OMe (25)								3.19, s

[a]Measured in $CDCl_3$. [b]Measured in C_5D_5N. ^{1}H NMR spectra (δ) were measured at 400 MHz for **1** and **3**−**5** and at 600 MHz for **2** and **6**−**8**. The assignments were based on ROESY, HSQC, and HMBC experiments.

were newly observed in the ^{13}C NMR spectrum of **5**. The interference was confirmed by the HMBC correlations of H_2-22 (δ 2.37, 2.24), H_2-23 (δ 1.44), and OMe (δ 3.66) with C-24 (δ 174.4). Consequently, the structure of **5** was established as methyl 7β-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-4-(28),8-diene-3,24-diester and named fornicatin F (**5**).

The molecular formula of **6** was assigned as $C_{30}H_{48}O_3$ by HRESIMS ([M + Na]$^+$, m/z 479.3514; calcd 479.3501). The IR spectrum showed the presence of a hydroxyl group (ν_{max} 3505, 3359 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **6** exhibited the following signals: seven singlet methyls (δ 0.70, 0.91, 1.11, 1.17, 1.24, 1.39, 1.54); a doublet methyl (δ 1.05, d, J = 6.6 Hz); seven methylenes; seven methines, including three oxymethines [δ 3.49 (t, J = 7.2 Hz); δ 4.27, (m); δ 4.54, (m)] and two aromatic methines [δ 5.57, (d, J = 5.1 Hz); δ 5.38, (d, J = 6.0 Hz)]; seven quaternary carbons including one oxyquaternary carbon δ 82.6 (s) and two tetrasubstituted double bonds (δ 134.4 and 146.9).

These data showed that **6** was similar to inonotsuoxide A,[13] possessing a tetrahydrofuran ring in the side chain, except for the presence of two additional aromatic methine signals. The signals of two methine proton doublets each at δ 5.57 (d, J = 5.1 Hz) and 5.38 (d, J = 6.0 Hz) in the ^{1}H NMR spectrum and the UV absorption band at 244 nm indicated the presence of a conjugated double bond. Furthermore, the conjugated double bonds were located at $\Delta^{7,8}$ and $\Delta^{9,11}$ by the HMBC correlations (Figure 3) of H-5 (δ 1.27) and H_2-6 (δ 2.19) with C-7 (δ 121.4); of H-7 (δ 5.57, d, J = 5.1 Hz) with C-8 (δ 134.4) and C-9 (δ 146.9); of Me-19 (δ 1.11) and H_2-1 (δ 1.49, 2.04) with C-9 (δ 146.9); and of H-11 (δ 5.38, d, J = 6.0 Hz) with C-9 (δ 146.9). Therefore, the planar structure of **6** was established as 22,25-epoxylanost-7,9-diene-3,24-diol.

Compound **7** had the same molecular formula, $C_{30}H_{48}O_3$ ([M + Na]$^+$; m/z 479.3495), as **6** by its HRESIMS and 1D NMR data (Tables 1 and 2). Acetylation of **7** results in ganodecochlearin B diacetate (**7a**), and a single X-ray crystallographic analysis (Cu Kα radiation) of **7a** assigned C-22 and C-24 as *S* and *S*, respectively. Meanwhile, the observed ROESY correlations of H-24 with H-22, H-23α, and Me-27 indicated that H-22 was coplanar with H-24 in **7**.

However, comparison of their ROESY spectra showed the correlations of H-23α/H-24 and of H-24/Me-27, as well as no

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Table 2. ^{13}C NMR Spectroscopic Data for Compounds 1−8

position	1[a]	2[b]	3[b]	4[b]	5[a]	6[b]	7[b]	8[a]	8[b]
1	30.4, CH_2	30.4, CH_2	32.4, CH_2	32.6, CH_2	33.4, CH_2	36.7, CH_2	36.7, CH_2	35.8, CH_2	36.8, CH_2
2	28.8, CH_2	28.9, CH_2	30.1, CH_2	30.8, CH_2	29.3, CH_2	30.4, CH_2	29.1, CH_2	27.9, CH_2	29.3, CH_2
3	176.1, C	174.1, C	174.2, C	176.4, C	174.1, C	78.4, CH	78.5, CH	79.1, CH	78.6, CH
4	144.0, C	143.9, C	146.3, C	146.4, C	147.8, C	39.8, C	39.8, C	38.8, C	39.9, C
5	53.5, CH	53.4, CH	45.0, CH	44.8, CH	44.9, CH	50.9, CH	50.1, CH	49.2, CH	50.2, CH
6	41.4, CH_2	41.4, CH_2	35.4, CH_2	35.5, CH_2	32.3, CH_2	38.7, CH_2	38.5, CH_2	23.1, CH_2	24.0, CH_2
7	73.4, CH	73.4, CH	67.8, CH	67.8, CH	66.5, CH	121.4, CH	121.4, CH	120.5, CH	121.5, CH
8	166.0, C	165.7, C	165.8, C	165.3, C	161.2, C	134.4, C	134.4, C	142.7, C	143.4, C
9	125.5, C	125.5, C	137.0, C	137.3, C	137.2, C	146.9, C	146.9, C	146.0, C	147.0, C
10	82.4, C	82.3, C	41.1, C	41.2, C	38.8, C	38.2, C	38.2, C	37.5, C	38.6, C
11	199.8, C	199.6, C	200.4, C	200.3, C	199.9, C	117.0, CH	117.0, CH	116.3, CH	117.1, CH
12	48.9, CH_2	48.9, CH_2	52.0, CH_2	52.0, CH_2	51.5, CH_2	38.4, CH_2	38.2, CH_2	37.9, CH_2	38.2, CH2
13	47.8, C	47.8, C	47.2, C	47.2, C	45.4, C	44.5, C	44.6, C	43.8, C	44.5, C
14	49.5, C	49.5, C	53.3, C	53.3, C	51.9, C	53.0, C	50.9, C	50.5, C	51.2, C
15	29.8, CH_2	29.8, CH_2	32.2, CH_2	32.2, CH_2	30.4, CH_2	32.3, CH_2	32.4, CH_2	31.7, CH_2	32.5, CH_2
16	27.3, CH_2	27.3, CH_2	27.7, CH_2	27.6, CH_2	27.2, CH_2	23.8, CH_2	23.9, CH_2	27.5, CH_2	28.3, CH_2
17	49.4, CH	49.4, CH	50.1, CH	49.9, CH	50.1, CH	48.5, CH	48.8, CH	47.3, CH	48.2, CH
18	17.4, CH_3	17.5, CH_3	17.7, CH_3	17.6, CH_3	17.8, CH_3	16.2, CH_3	16.3, CH_3	15.8, CH_3	16.6, CH_3
19	35.2, CH_2	35.0, CH_2	22.3, CH_3	22.3, CH_3	22.5, CH_3	23.5, CH_3	23.5, CH_3	22.9, CH_3	23.6, CH_3
20	35.7, CH	35.7, CH	36.2, CH	36.0, CH	35.7, CH	40.8, CH	40.2, CH	41.9, CH	43.2, CH
21	17.9, CH_3	17.9, CH_3	18.4, CH_3	18.2, CH_3	18.0, CH_3	13.2, CH_3	13.5, CH_3	12.0, CH_3	13.3, CH_3
22	30.9, CH_2	30.8, CH_2	31.8, CH_2	31.1, CH_2	31.1, CH_2	77.9, CH	77.3, CH	73.8, CH	73.2, CH
23	31.0, CH_2	31.0, CH_2	31.8, CH_2	31.4, CH_2	30.9, CH_2	28.2, CH_2	28.2, CH_2	134.9, CH	135.0, CH
24	174.6, C	176.1, C	176.4, C	174.3, C	174.4, C	78.3, CH	78.4, CH	132.8, CH	135.2, CH
25						82.6, C	82.2, C	74.9, C	75.3, C
26						22.2, CH_3	24.4, CH_3	25.9, CH_3	26.7, CH_3
27						28.3, CH_3	27.3, CH_3	25.9, CH_3	26.9, CH_3
28	114.2, CH_2	114.3, CH_2	115.6, CH_2	115.5, CH_2	114.9, CH_2	17.1, CH_3	17.1, CH_3	28.3, CH_3	29.3, CH_3
29	22.3, CH_3	22.5, CH_3	23.2, CH_3	23.3, CH_3	24.3, CH_3	29.3, CH_3	29.3, CH_3	16.0, CH_3	17.2, CH_3
30	26.1, CH_3	26.2, CH_3	27.6, CH_3	27.5, CH_3	26.6, CH_3	26.3, CH_3	26.4, CH_3	26.2, CH_3	26.4, CH_3
OMe (3)		51.6, CH_3	51.4, CH_3		51.6, CH_3				
OMe (24)	51.5, CH_3			51.3, CH_3	51.6, CH_3				
OMe (25)								50.7, CH_3	50.6, CH_3

[a]Measured in $CDCl_3$. [b]Measured in C_5D_5N. ^{13}C NMR spectra (δ) were measured at 100 MHz for **1** and **3**−**5** and at 150 MHz for **2** and **6**−**8**. The assignments were based on ROESY, HSQC, and HMBC experiments.

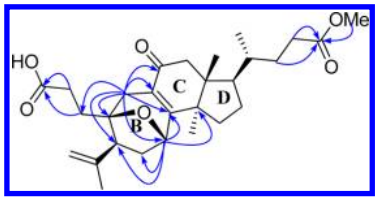

Figure 1. Key HMBC (H→C) correlations of **1**.

correlation of H-22/H-24 in **7**, which suggested 24*S* and 22*R*. Accordingly, the structures of **6** and **7** were determined to be 22*R*,25-epoxylanost-7,9-diene-3β,24*S*-diol and 22*S*,25-epoxylanost-7,9-diene-3β,24*S*-diol, named ganodecochlearins A and B.

The molecular formula of compound **8** was established as $C_{31}H_{50}O_3$ by HRESIMS. The IR, UV absorption, and 1D NMR data of **8** were similar to those of lanosta-7,9(11),23*E*-triene-3β,22*R*,25-triol.[14] However, an additional methoxyl signal at δ 50.7 and the HMBC correlations of H_2-23, H_2-24, and OMe with C-25 confirmed that the methoxyl group was connected to C-25 in **8**.

On the basis of detailed analysis of coupling constants at δ 5.95 (dd, J = 4.1, 15.9 Hz, H-23) and 6.00 (dd, J = 1.7, 15.9 Hz, H-24), the geometry of the double bond was established to be *E*. The absolute configuration of C-22 was assigned as *R* based

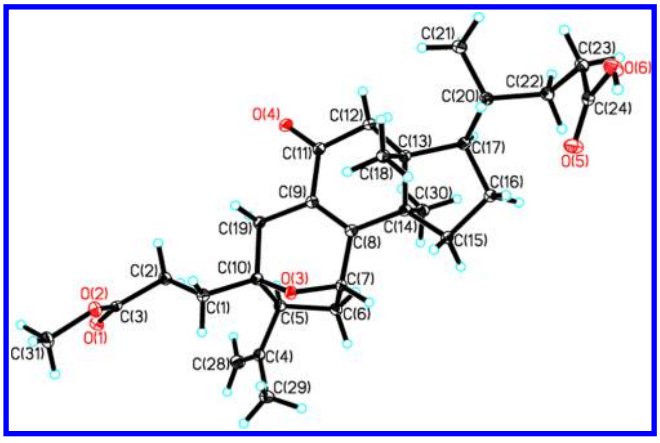

Figure 2. X-ray crystal structure of **2**.

on the coupling constant $J_{20/22}$ = 2.8 Hz, in accordance with the corrected Karplus curve.[15] 3β-OH was determined by the ROESY correlation of H-3/H-5. Therefore, the structure of **8** was assigned as lanosta-7,9(11),23*E*-triene-25-methoxyl-3β,22*R*-diol, named ganodercochlearin C (**8**).

The known isolates were identified by comparing their physical and spectroscopic data from the literature. They are

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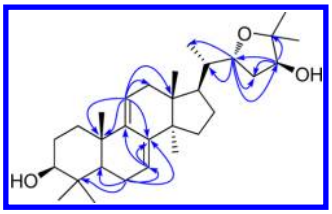

Figure 3. Key HMBC (H→C) correlations of compound **6**.

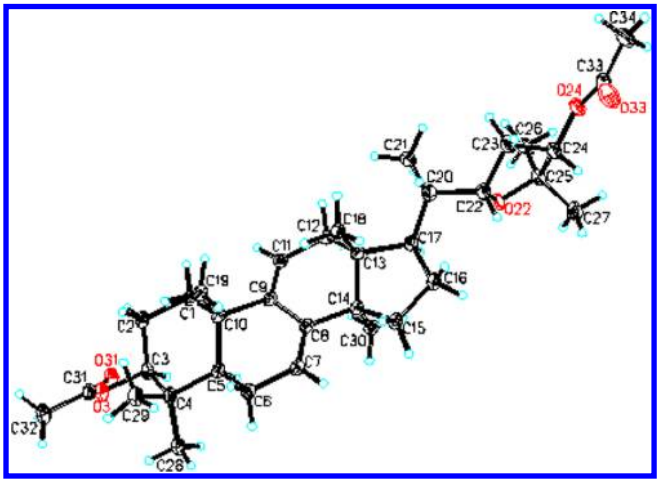

Figure 4. X-ray crystal structure of **7a**.

3*β*,22*S*-dihydroxylanosta-7,9(11),24-triene (**9**),[16] fornicatin A (**10**),[11] fornicatin B (**11**),[11] inonotsuoxide B (**12**),[13] and fredelin (**13**).[17]

Considering the wide distribution of fornicatin B (**10**) in *G. cochlear*, cochlates A and B (**1**, **2**) could be derived from modification of fornicatin B (**10**). Therefore, we proposed a possible biosynthetic route of cochlates A and B (**1**, **2**) as shown in Scheme 1 (see Supporting Information).

In the present study, the inhibitory effects of compounds **1**, **3**, **4**, **5**, **9**, **10**, and **13** against the increase of ALT and AST levels in H_2O_2-induced HepG2 cells (Table 3) were studied. The maximum nontoxic concentrations of seven tested compounds on HepG2 cells were 74.60, 18.40, 15.50, 59.90, 150.20, 65.70, and 15.00 μM, respectively.

A set of cells in culture medium treated with H_2O_2 was used as the model group, and in comparison to the model group, fornicatin D (**3**), fornicatin F (**5**), fornicatin A (**10**), and fredelin (**13**) notably lowered ALT (16.06−21.01 U L^{-1}) and AST levels (19.09−30.38 U L^{-1}) in a concentration-dependent manner, while fornicatin E (**4**) inhibited the increase of AST levels (19.56−22.93 U L^{-1}) in the range of the maximum nontoxic concentration (see Supporting Information).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zurich, Switzerland) using TMS as internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm with reference to the TMS resonance. ESIMS and HRTOF-ESIMS data were recorded on an API QSTAR Pulsar spectrometer. Infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets. An agilent 1100 series instrument equipped with an Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 250 mm) was used for high-performance liquid chromatography (HPLC) analysis.

Table 3. Effects of Different Concentrations of Compounds 3, 5, 10, and 13 on ALT and AST Levels in HepG2 Cells Induced by H_2O_2[a]

groups	concentration (μM)	ALT (U·L^{-1})	AST (U·L^{-1})
control		7.22 ± 1.16	10.53 ± 0.61
model		23.22 ± 1.22[b]	32.36 ± 0.79[b]
3	3.88	21.01 ± 1.00	28.23 ± 2.07[c]
	7.75	19.32 ± 0.27[c]	26.54 ± 0.63[d]
	15.5	19.73 ± 3.19	26.19 ± 4.67
5	14.98	19.32 ± 1.59[c]	28.28 ± 1.40[c]
	29.95	18.51 ± 0.70[d]	26.77 ± 2.07[c]
	59.90	17.81 ± 0.70[d]	22.11 ± 0.79[d]
10	4.60	19.61 ± 1.58	30.38 ± 1.72
	9.20	20.20 ± 1.29[c]	23.80 ± 2.88[d]
	18.40	20.84 ± 2.11	20.66 ± 1.16[d]
13	16.43	18.92 ± 0.66[c]	29.45 ± 0.72[c]
	32.85	18.27 ± 2.67[c]	25.43 ± 3.53[c]
	65.70	16.06 ± 3.33[c]	19.09 ± 1.94[d]

[a] n = 3, mean ± SD. Control: a set of cells maintained in culture medium with DMSO. Model: a set of cells maintained in culture medium with DMSO and treated only with H_2O_2. [b] $p < 0.01$, compared to control group. [c] $p < 0.05$, compared to model group. [d] $p < 0.01$, compared to model group.

Fungal Material. The fruiting bodies of *G. cochlear* were purchased in April 2010 from Juhuacun Traditional Chinese Medicine Market in Kunming. The mushroom was identified by Prof. Liu Peigui, Kunming Institute of Botany, Chinese Academy of Science. A specimen (No. 10042501) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. *G. cochlear* (38 kg) mushrooms were chipped and extracted with 95% EtOH under reflux three times. The combined ethanol extracts were evaporated under reduced pressure. The residue was suspended in H_2O and extracted with $CHCl_3$. The volume of the combined $CHCl_3$ extracts was reduced to one-third under vacuum. The residue was fractionated by CC (silica gel; $CHCl_3$−MeOH step gradients): fractions I−IV (100:1, 50:1, 20:1, 5:1). Fraction II was treated by CC (silica gel; $CHCl_3$−MeOH step gradients) to give **13** (24 mg). Fraction III was also subjected to silica gel column chromatography and gave four subfractions (III-1−III-4). Compounds **6** (35 mg), **7** (40 mg), **8** (45 mg), **9** (20 mg), and **12** (64 mg) were obtained from fraction III-2 by CC (silica gel; $CHCl_3$−MeOH) and recrystallization. Fraction IV (434 g) was absorbed to macroreticular resin and eluted with 50% MeOH−H_2O and then MeOH. Fraction IV-3 (130 g) was fractionated by reversed-phase silica gel chromatography to obtain three fractions (50% MeOH−H_2O → 100% MeOH). Fraction IV-3-3 (56 g) was further separated by reversed-phase HPLC (CH_3CN−H_2O, 60%) to afford compounds **1** (40 mg) and **2** (50 mg). By repeated column chromatography, HPLC separation, and recrystallization, from fraction IV-4 compounds **3** (50 mg), **4** (50 mg), **5** (25 mg), and **11** (45 mg) were isolated. The spectroscopic data of new compounds (**1**−**8**) are listed herein.

Cochlate A (1): white powder; $[\alpha]_D^{12}$ +82.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.91), 203 (4.24) nm; IR (KBr) ν_{max} 3433, 2954, 1742, 1708, 1656, 1378, 1269, 1173, 1055 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; positive ESI *m*/*z* 473 $[M + H]^+$; HRESIMS *m*/*z* 473.2906 $[M + H]^+$ (calcd for $C_{28}H_{41}O_6$, 473.2903).

Cochlate B (2): colorless needles (MeOH−H_2O); mp 195−196 °C; $[\alpha]_D^{12}$ +8.8 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 259 (3.91), 202 (3.79) nm; IR (KBr) ν_{max} 3432, 2967, 1739, 1704, 1648, 1630, 1380, 1267, 1176, 1052 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; positive ESI *m*/*z* 495 $[M + Na]^+$; HRESIMS *m*/*z* 495.2731 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_6$, 495.2722).

X-ray Crystallography of 2. The structure was solved by direct methods using the program SHELXS-97 (Sheldrich, G. M., University of Gottingen, Gottingen, Germany, 1997), then refined by SHELXS,

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with refinement on F^2 against all reflections. All esd's are estimated using the full covariance matrix. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located by geometry and riding on the related atoms during refinements with a temperature factor of 1.2 or 1.5 times the latter.

Crystal data of **2**:[18] $C_{28}H_{40}O_6$, M = 472.60, colorless needles, space group $P2_12_12$, a = 11.28200(10) Å, b = 13.67650(10) Å, c = 16.2336(2) Å, $\alpha = \beta = \gamma = 90°$, V = 2504.82(4) Å^3, Z = 4, d = 1.253 g/cm^3, crystal dimensions 0.13 × 0.15 × 0.88 mm were used for measurements on a Bruker APEX DUO diffractometer with a graphite monochromator (Φ/ω scans, $2\theta_{max}$ = 69.62°), Cu Kα radiation. The total number of independent reflections measured was 14 593, of which 4319 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_1 = 0.0272, wR_2 = 0.0701 ($w = 1/\sigma|F|^2$), S = 1.044.

*Fornicatin D (**3**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{17}$ +133.9 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.69), 200 (3.62) nm; IR (KBr) ν_{max} 3452, 2932, 2871, 1730, 1643, 1453 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 475.3061 $[M + H]^+$ (calcd for $C_{28}H_{43}O_6$, 475.3059).

*Fornicatin E (**4**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{22}$ +117.7 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 257 (4.25), 201 (3.91) nm; IR (KBr) ν_{max} 3525, 2953, 1740, 1710, 1645, 1453 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 475.3063 $[M + H]^+$ (calcd for $C_{28}H_{43}O_6$, 475.3059).

*Fornicatin F (**5**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{21}$ +87.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.82), 201 (3.69) nm; IR (KBr) ν_{max} 3432, 2961, 1741, 1641, 1453 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 511.3039 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_6Na$, 511.3035).

*Ganodercochlearin A (**6**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{25}$ +24.2 (c 0.1, $CHCl_3$); UV (MeOH; CH_2Cl_2) λ_{max} (log ε) 244 (4.08) nm; IR (KBr) ν_{max} 3383, 2965, 2930, 2851, 1464 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 479.3514 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501).

*Ganodercochlearin B (**7**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{25}$ +26.2 (c 0.1, $CHCl_3$); UV (MeOH; CH_2Cl_2); λ_{max} (log ε) 244 (4.28) nm; IR (KBr) ν_{max} 3385, 2972, 2961, 2931, 2849, 1460 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 479.3495 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501).

*Ganodercochlearin B Diacetate (**7a**).* A mixture of compound **7** (10 mg) and Ac_2O (1 mL) in pyridine (1 mL) was kept at room temperature overnight. Usual workup gave a residue (10.5 mg), which was recrystallized from MeOH to a corresponding ganodercochlearin B diacetate (9.4 mg).

Crystal data of **7a**:[18] $C_{34}H_{52}O_5$, M = 540.76, monoclinic, space group $P21$, a = 6.9978(3) Å, b = 11.1764(5) Å, c = 20.4376(9) Å, α = 90.00°, β = 98.054(2)°, γ = 90.00°, V = 1582.66(12) Å^3, T = 100(2) K, Z = 2, crystal dimensions 0.29 × 0.27 × 0.26 mm was used for measurements on a Bruker APEX DUO diffractometer with a graphite monochromator (Φ/ω scans, $2\theta_{max}$ = 69.17°), Cu Kα radiation; 13 325 reflections measured, 5162 independent reflections (R_{int} = 0.0428). The final R_1 values were 0.0410 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1177 ($I > 2\sigma(I)$). The final R_1 values were 0.0416 (all data). The final $wR(F^2)$ values were 0.1186 (all data). The goodness of fit on F^2 was 1.096. Flack parameter = −0.05(18).

*Ganodercochlearin C (**8**):* colorless needle (MeOH–H_2O); $[\alpha]_D^{25}$ +27.8 (c 0.1, $CHCl_3$); UV (MeOH; CH_2Cl_2) λ_{max} (log ε) 237 (4.28) nm; IR (KBr) ν_{max} 3340, 2977, 2952, 2912, 2833, 1468 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS m/z 493.3661 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_3Na$, 490.3657).

Assessment of Hepatoprotective Activity. HepG2 cells (hepatocellular carcinoma cells) in logarithmic growth phase were seeded in 96-well plates (1 × 10^4 cells/well, 200 μL/well) in minimum essential media (MEM) with 10% FBS for 24 h at 37 °C. The maximum nontoxic concentration of DMSO in HepG2 cells was determined prior to the cytotoxicity analysis. The maximum nontoxic concentration of DMSO was 1% (v/v). Thus, subsequently all compounds were prepared in DMSO at concentrations lower than the DMSO toxic dose. The concentrated stocks of compounds were mixed with MEM of 2% FBS and divided into different concentrations (6.25, 12.5, 25, 50, 100, 200, and 400 μM) before being added to a preformed monolayer of HepG2 cells in 96-well plates. The maximum nontoxic concentration of test compounds was analyzed with a colorimetric assay using the tetrazolium salt MTT after 96 h postincubation. The percentage of growth inhibition in HepG2 cell was calculated using the following formula:

$$\%\text{cell inhibition} = (OD_{570\text{control}} - OD_{570\text{test compounds}})/OD_{570\text{control}} \times 100\%$$

The H_2O_2 concentration used for cell culture treatment was previously determined according to a modified method of Gu et al.[19] HepG2 cells in logarithmic growth phase were made into a single-cell suspension and seeded in 96-well plates (1 × 10^4 cells/well, 200 μL/well) for 24 h at 37 °C. Then 3.2 mM H_2O_2 was added to the cell medium, and the mixture was further incubated at 37 °C for 2 h. The medium with different concentrations of test compounds (200 μL) was dissolved in DMSO, mixed in cell medium, and incubated for 24 h. The obtained reacted supernatant was directly used to detect ALT and AST levels. The control group was a set of cells maintained in culture medium with DMSO, while the model group was a set of cells maintained in culture medium with DMSO and treated only with H_2O_2. All data are expressed as the mean ± SD of at least three independent experiments as indicated. The test for the paired samples was used to determine statistical difference between parameters. These differences were considered significant for $p < 0.05$, 0.01, or 0.001 and nonsignificant for $p > 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra for compounds **1**–**8**, proposed biogenetic pathway for cochlates A (**1**) and B (**2**) (Scheme 1), and effects of different concentrations of test compounds **1**, **4**, and **9** on ALT and AST levels in H_2O_2-induced HepG2 cells (Table 1). This information 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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