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Triterpene Lactones from Cultures of *Ganoderma* sp. KM01

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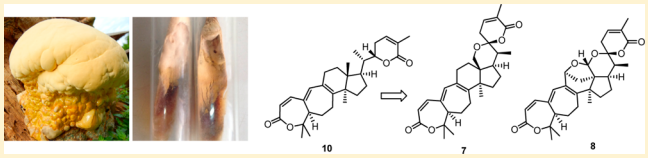
Supporting Information

ABSTRACT: A revised structure of colossolactone G (**1**), seven new triterpene lactones, ganodermalactones A–G (**2**–**8**), and five known triterpene lactones, colossolactone I (**9**), schisanlactone B (**10**), colossolactone B (**11**), colossolactone E (**12**), and colossolactone IV (**13**), and ergosterol have been isolated from cultured biomass of the macrofungi *Ganoderma* sp. KM01. Their structures were identified by spectroscopic methods. Structures and relative configurations of **3**, **7**, and **8** were confirmed by X-ray crystallographic analysis. Compounds **7**, **10**, and **12** exhibited antimalarial activity against *Plasmodium falciparum* in the range 6.0–10.0 μM (IC_{50}).



Ganoderma is a basidiomycete macrofungi that belongs to the family Ganodermataceae, order Polyporales. It is a large fruiting basidiocarp, club shaped with a rounded head, and mostly found in decayed wood and logs, serving as a saprophytic fungi.[1] The fruiting bodies of *Ganoderma* species have been widely used traditionally by the Chinese, Japanese, and Koreans to treat various diseases.[2] Previous investigations of secondary metabolites from both fruiting bodies and cell cultures of *Ganoderma* species have resulted in the isolation of compounds, most of which are lanostane terpenoids such as ganoderic acids,[3] lucidenic acids, and ganoderenic acids.[4,5] Moreover, structures and biological activities of triterpene lactones colossolactones A–G,[6] colossolactones I–IV,[7] and colossolactones V–VIII[8] have been reported from the fruiting body of *G. colossum*. These types of terpenoids have also been reported from plants including schisanlactones A and B (*Schisandra* sp.),[9,10] kadsulactone A (*Katsura heteroclita*),[11] and lancilactones A–C (*K. lancilimba*).[12] As part of our search for bioactive compounds from fungi and mushrooms, the EtOAc extract of the cultured biomass of *Ganoderma* sp. KM01 showed antimalarial activity with an IC_{50} value of 4.0 μg/mL. We herein present the isolation, structural elucidation, and bioactivity of compounds from the cultures of *Ganoderma* sp. KM01. It should be noted that this is the first report of triterpene lactones **7** and **8**, which contain a spiroketal-lactone and a bicyclo-spiroketal-lactone skeletons, from a natural source.

RESULTS AND DISCUSSION

The EtOAc extract of dried fungal biomass of *Ganoderma* sp. KM01 was fractionated by flash column chromatography on silica gel and preparative TLC to yield seven new triterpene lactones, ganodermalactones A–G (**2**–**8**), six known triterpene lactones (**1**, **9**–**13**), and ergosterol (Figure 1). The structures of these known compounds were identified by physical and spectroscopic data measurements (IR, ^{1}H and ^{13}C NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as colossolactone I (**9**),[7] schisanlactone B (**10**),[10] colossolactone B (**11**),[6] colossolactone E (**12**),[6] colossolactone IV (**13**),[7] and ergosterol.[13] Among these, the structure of known colossolactone G (**1**) has been revised. Since the previous structure elucidation of **1** was briefly reported, details of its revised structure have been described.

Compound **1** was obtained as colorless needles, and its molecular formula, $C_{32}H_{42}O_7$, was determined from the HRESITOFMS (observed m/z 539.3013 $[M + H]^+$), indicating 12 degrees of unsaturation. The IR spectrum of **1** showed characteristics of hydroxyl (3458 cm^{-1}) and carbonyl (1708 cm^{-1}) groups. The UV spectrum showed absorption maxima at 229, 274, and 332 nm indicative of a conjugated carbonyl group.[14] The ^{13}C NMR and DEPT spectra of **1** (Table 1) displayed 32 carbon signals attributable to three carbonyl (164.0, 166.2, and 170.0), four sp^2 quaternary (149.2, 132.8, 128.2, and 127.6), four sp^3 quaternary (92.7, 77.3, 55.1, and

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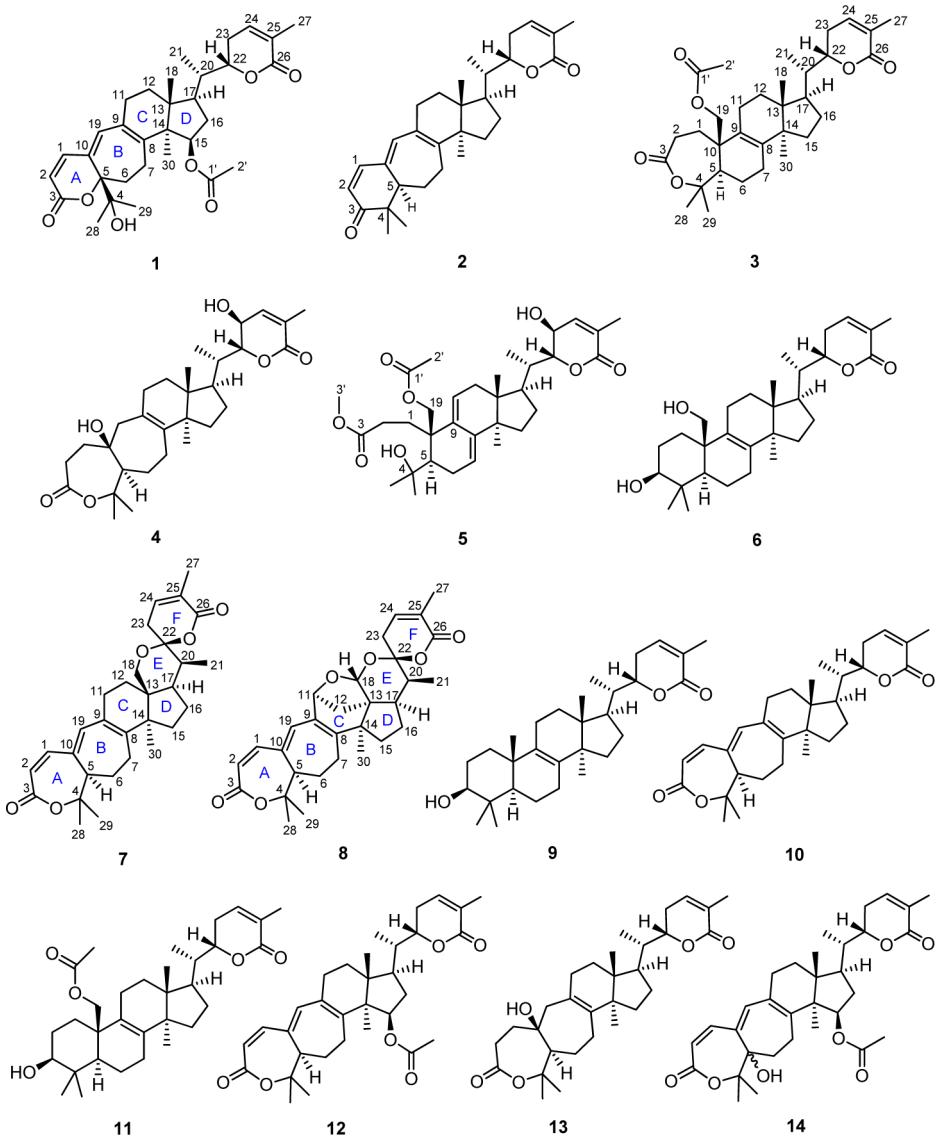

Figure 1. Structures of compounds **1**−**14**.

44.2), four sp^2 methine (147.9, 139.4, 139.4, and 116.5), four sp^3 methine (79.7, 78.3, 45.7, and 39.7), six sp^3 methlylene, and seven methyl carbons. The ^{1}H NMR spectrum of **1** (Table 1) showed signals that were assigned from intensive COSY, HSQC, HMBC, and NOESY experiments to seven methyl groups, characterized as four tertiary methyls as singlets at δ 0.94 (H-30), 1.02 (H-18), 1.12 (H-29), and 1.19 (H-28), an allylic methyl at δ 1.86 (s, H-27), an acetoxy methyl at δ 1.98 (H-2′), one secondary methyl at δ 1.03 (overlap, H-21), two oxymethines at δ 4.39 (dd, J = 2.4, 13.2 Hz, H-22) and 4.81 (brd, J = 7.2 Hz, H-15), and two methines at δ 2.13 (m, H-17) and 1.62 (m, H-20). The COSY and HMBC correlations (Figure 2) indicated that **1** has the ring B−D system and a side chain δ-lactone the same as isolated colossolactone E (**12**) and the reported colossolactone G (**14**).[6] The remaining part of the NMR spectra of **1** showed resonance signals with an excellent match to those of a seven-membered α,β-unsaturated lactone ring reported for **14** for the olefinic protons at δ_H 6.90 and 5.82 (both d, J = 9.6 Hz, H-1 and H-2), two methyl groups at δ_H 1.19 (s, H-28) and 1.12 (s, H-29), and the carbon signals at δ_C 147.9 (C-1), 116.5 (C-2), 164.0 (C-3), 77.3 (C-4), 92.7 (C-5), 24.3 (C-28), and 24.9 (C-29). The olefinic protons H-1 and H-2 showed a coupling constant of 9.6 Hz, which is the same J value as that of the isolated six-membered α,β-unsaturated ketone **2**. The coupling constants of H-1 and H-2 of the isolated seven-membered α,β-unsaturated lactones **7**, **8**, **10**, and **12** have J values of 12.0−12.2 Hz. These data lead to the conclusion that ring A of **1** is a six-membered ring with a 2-hydroxy propyl unit, rather than a seven-membered ring with a hydroxy at C-5 and a *gem*-dimethyl at C-4 as reported for **14**.[6] The 2-hydroxy propyl unit at C-5 of **1** was confirmed by the HMBC correlations of H-1 to C-5, C-10, and C-19, H-2 to C-3 and C-10, methyl groups of H_3-28 and H_3-29 to C-4, and H-7 to C-5. In our study, X-ray crystallographic analysis of **1** was performed. Unfortunately, the data quality of our X-ray measurement was incomplete due to poor diffraction of the crystal. However, its ORTEP plot could be used as a guide for assignment of a six-membered α,β-unsaturated lactone of ring A (see the Supporting Information). The NOESY spectrum of **1** showed correlations between H-15 and H_3-30, H_3-17 and H-30, H-12 (δ_H 1.89) and H-18, H-17 and H-21, H-18 and H-20, H-20 and H-22, H-21 and H-23$_\alpha$, and H-22 and H-23$_\beta$,

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Table 1. ^{13}C and ^{1}H NMR Spectral Data of Compounds 1, 2, and 3 ($CDCl_3$)

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	147.9	6.90, d (9.6)	149.0	6.96, d (9.6)	34.7	1.72, 1.82, m
2	116.6	5.82, d (9.6)	122.7	5.77, d (9.6)	32.6	2.60, 2.44, m
3	164.0		205.1		173.7	
4	77.3		45.7		85.7	
5	92.7		49.5	2.66, dd (4.8, 12.4)	48.9	2.05, m
6	44.1	2.26, 2.37, m	28.7	2.09, 1.69, m	24.1	1.47, m
7	26.7	1.95, 2.05, m	27.3	2.19 m	26.1	2.03, m
8	149.2		149.6		138.0	
9	128.2		128.2		127.9	
10	132.8		137.0		41.7	
11	27.9	2.02, 2.22, m	28.5	2.12, 1.87, m	21.3	1.95, m
12	31.0	1.72, 1.89, m	30.3	1.91, 1.73, m	30.6	1.76, 1.23, m
13	44.2		44.3		44.0	
14	55.1		52.3		50.5	
15	78.3	4.81, brd (7.2)	30.9	1.89, 1.37, m	31.0	1.22, 1.64, m
16	38.3	1.37,dd (9.0, 15.2)	31.6	1.77, 2.14, m	27.3	1.33, 2.00, m
		2.69, dt (7.6, 15.2)				
17	45.7	2.13, m	45.6	2.16, m	45.5	2.05, m
18	16.8	1.02, overlap	16.0	0.78, s	15.5	0.70, s
19	139.4	6.20, s	138.9	6.07, s	66.8	4.30, d (12.0)
						4.21, d (12.0)
20	39.7	1.62, m	40.4	1.57, m	40.3	1.54, m
21	13.3	1.03, overlap	13.4	1.05, d (6.4)	13.3	0.95 d (6.8)
22	79.7	4.39, dd (2.4, 13.2)	80.1	4.48, dd (3.2, 13.2)	80.1	4.41 dd (3.2, 13.2)
23β	27.6	2.52, m	27.8	2.56, m	27.7	2.51, m
23α		1.99, m		1.99, m		1.93, m
24	139.4	6.57, brd (6.0)	139.6	6.60, brd (6.4)	139.7	6.56, brd (6.4)
25	127.6		125.8		129.1	
26	166.2		166.5		166.5	
27	17.0	1.86, s	17.1	1.90, s	17.0	1.83, s
28	24.3	1.19, s	24.4	1.17, s	25.8	1.32, s
29	24.9	1.12, s	20.2	0.92, s	29.8	1.47, s
30	24.7	0.94, s	25.8	1.02, s	25.2	0.88, s
1′	170.0				170.1	
2′	21.3	1.98, s			20.9	1.93, s

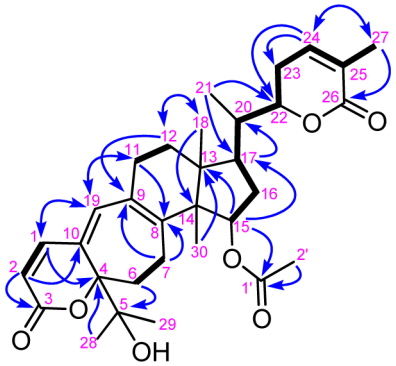

Figure 2. COSY (bold lines) and selected HMBC (arrows) correlations of 1.

indicating the relative configuration within the molecule. The assignment of the absolute configuration at C-22 was concluded to be *S* by the CD measurement. The CD spectrum of **1** showed a negative Cotton effect at 249 nm ($\Delta\varepsilon$ −36.9, $CHCl_3$), which is similar to that of analogues schinsanlactone B [240 nm ($\Delta\varepsilon$ −7.1)][10] and colossolactone VII [258 nm ($\Delta\varepsilon$ −4.1, $CHCl_3$)]. It was found that **1** and a known colossolactone G (**14**)[6] have identical NMR data. However, on the basis of the above evidence, the structure of **1** was different from that reported for **14** (Figure 1). Therefore, **1** was a new and revised structure for colossolactone G (**14**).

Compound **2** was obtained as a yellow solid, and its molecular formula, $C_{30}H_{40}O_3$, was determined from the HRESITOFMS (observed m/z 471.2863 $[M + Na]^+$), indicating 11 degrees of unsaturation. The IR spectrum of **1** showed the characteristics of conjugated carbonyl lactone (1713 cm^{-1}) and α,β-unsaturated ketone (1663 cm^{-1}) groups. The UV spectrum showed absorption maxima at 230, 252, and 350 nm, indicating a conjugated carbonyl group. The ^{1}H and ^{13}C NMR spectra of **2** are similar to those of isolated schisanlactone B (**10**),[10] except for the conjugated lactone in ring A, which appeared as a six-membered α,β-unsaturated ketone by showing $\delta_{H/C}$ 6.96 (d, J = 9.6 Hz H-1)/149.0, 5.77 (d, J = 9.6 Hz H-2)/122.7, 2.66 (dd, J = 4.8, 12.4 Hz, H-5)/49.5, 1.17 (H-28)/24.4, 20.2 (H-29)/20.2, δ_C 137.0 (C-10) and 205.1 (C-3). This was confirmed by the correlations of H-1 to C-3, C-5, C-9, and C-10; H-2 to C-4, and C-10; H-5 to C-3, C-4, and C-19; H-19 to C-1, C-5, C-8, C-10, and C-11; H-28 to C-3, C-4, C-5, and C-29; and H-29 to C-3, C-4, C-5, and C-28 in the HMBC spectrum. The ^{1}H and ^{13}C NMR data of **2**

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(Table 1) were established as a result of conclusive DEPT, COSY, HMBC, and NOESY experiments. The absolute configuration at C-22 was concluded to be *S* by CD measurement, which showed a negative Cotton effect at 257 nm ($\Delta\varepsilon$ −39.7, $CHCl_3$).[8,10] Thus, the structure of **2** was determined as a new triterpene lactone, which has been named ganodermalactone A.

Compound **3** was obtained as a white solid and was assigned the molecular formula $C_{32}H_{46}O_6$ as deduced from HRESI-TOFMS *m/z* 549.3138 $[M + Na]^+$, implying 10 degrees of unsaturation. The UV spectrum showed absorption maxima at 230 nm, indicating a conjugated carbonyl group. The IR spectrum of **3** showed the characteristics of carbonyl ester (1734 cm^{-1}), lactone, and α,β-unsaturated lactone (1712 cm^{-1}) groups. The ^{13}C NMR (Table 1) and DEPT spectra showed 32 carbon signals attributable to seven downfield signals corresponding to three carbonyl groups (δ 173.7, 170.1, and 166.5), four olefinic carbons (δ 139.7, 138.0, 129.1, and 127.9), three oxygenated carbons (δ 85.7, 80.1, and 66.8), and high-field signals that were assigned to seven methyl, 10 methylene, four methine, and four sp^3 quaternary carbons. The chemical shifts of protons and carbons of rings C and D and the side chain with a δ-lactone ring were similar to those of **2** (Table 1). The six-membered ring B, fused to ring C through C-8−C-9, was deduced from the COSY correlations between H-5 (δ 2.05, m)/H-6 (δ 1.47, m)/H-7 (δ 2.03, m), as well as the HMBC correlations of H-5 to C-9 and C-10, H-6 to C-8, and H-7 to C-5, C-9, and C-14. The assignment of ring A as a seven-membered lactone ring was indicated by the resonance signals at δ_C 173.7 (C-3) and 85.7 of a quaternary C-4 and δ_H 2.05 (m, H-5) as well as the correlations of H-1 (δ_H 1.72, 1.82, m) and H-2 (δ_H 2.60, 2.44, m) to C-3; H-5 to C-4, C-10, and C-19; and H-28 and H-29 to C-4 in the HMBC experiment. In addition, the side chain at C-10 was assigned as a $-CH_2-O-COCH_3$ unit from the NMR resonaces at δ 4.30 and 4.21 (both d, *J* = 12.0 Hz, H_2-19), δ_H 1.93 (s, H_3-2′), and δ_C 66.8 (C-19), 170.1 (C-1′), and 20.9 (C-2′). We found that our proposed structure of **3** has previously been reported as colossolactone C.[6] However, its ^1H and ^{13}C NMR spectral data did not agree well with **3**, especially around the positions 1−10. The differences of chemical shift value between **3** and colossolactone C were noted at $\delta_{H/C}$ 1.72, 1.82 (m)/34.7 and 1.66, 2.55 (m)/28.7 for H-1; 2.60, 2.44 (m)/32.6 and 2.21, 2.28 (m)/28.4 for H-2; 2.05 (m)/48.9 and 1.50 (m)/47.8 for H-5; δ_C 173.7 and 178.9 for C-3; and δ_C 85.7 and 75.2 for C-4, respectively. Finally, the structure of **3** was confirmed by X-ray crystallography (Figure 3). Thus, the reported structure of colossolactone C[6] should be revised. The absolute stereochemistry at C-22 of **3** was assigned as *S* from the CD spectrum at 260 nm ($\Delta\varepsilon$ −28.0, $CHCl_3$).[8,10]

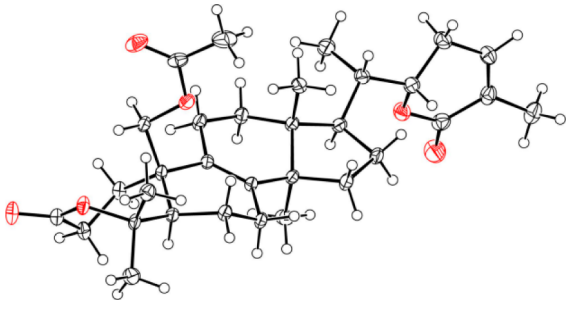

Figure 3. ORTEP plot of **3**.

On the basis of the above evidence, **3** was determined as a new triterpene lactone, which has been named ganodermalactone B.

Compound **4** was obtained as a white solid and was assigned the molecular formula $C_{30}H_{44}O_6$ as deduced from HRESI-TOFMS *m/z* 523.3026 $[M + Na]^+$, implying 9 degrees of unsaturation. The UV spectrum showed absorption maxima at 230 nm as in **3**. The IR spectrum of **4** showed the characteristics of hydroxy (3439 cm^{-1}), lactone carbonyl (1742 cm^{-1}), and conjugated carbonyl lactone (1696 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **4** (Table 2) were similar to those of colossolactone IV (**13**),[7] except for the appearance of a hydroxyl group at C-23 on the δ-lactone ring of the side chain by showing ^1H NMR signals at δ_H 4.20 (d, *J* = 10.4 Hz, H-22), 4.47 (brd, *J* = 10.4 Hz, H-23), 6.35 (s, H-24), and 1.93 (s, H-27) and ^{13}C NMR signals at δ_C 165.8 (C-26), 143.8 (C-24), 127.7 (C-25), 84.3 (C-22), and 63.6 (C-23). This was confirmed by correlations of an oxymethine proton H-23 to C-20, C-22, and C-25; H-24 to C-23; and H-20 to C-23 in the HMBC spectrum. The relative stereochemistry was determined by a combination of coupling constants and analysis of the NOESY spectrum, which was comparable to those of both **3** and colossolactone IV (**13**).[7] The large coupling constant (10.4 Hz) of H-23 to H-22, and no correlation between H-23 and H-22 in the NOESY spectrum, indicated an anti configuration of these two protons. The absolute stereochemistry of C-22 was assigned as *S* from the CD measurement, which showed a negative Cotton effect at 265 nm ($\Delta\varepsilon$ −13.5, $CHCl_3$ in the CD spectrum).[8,10] Thus, compound **4** was a new triterpene lactone, which has been named ganodermalactone C.

Compound **5** was obtained as a yellow, amorphous solid, and its molecular formula of $C_{33}H_{48}O_8$ was deduced from the HRESITOFMS (observed *m/z* 595.3238 $[M + Na]^+$), implying 10 degrees of unsaturation. The UV spectrum showed an absorption by the conjugated carbonyl group at 244 nm. The IR spectrum of **5** showed the characteristics of hydroxyl (3436 cm^{-1}), carbonyl ester (1723 cm^{-1}), and conjugated carbonyl lactone (1718 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **5** (Table 2) were similar to those of colossolactone VII,[8] except for the appearance of conjugated double bonds at C-7−C-8 and C-9−C-11 [$\delta_{H/C}$ 5.26 (m)/117.9 and 5.28 (m)/120.5], as in colossolactone VI,[8] and a hydroxyl group at C-23 in the δ-lactone ring ($\delta_{H/C}$ 4.47/63.4). The positions of these two functional groups were confirmed by HMBC correlations of H-6 to C-7 and C-8, H-7 to C-5, C-8, C-9, and C-14, H-11 to C-12, C-13, C-8, C-9, and C-10, and H-23 to C-22, C-20, C-26, and C-24. The relative stereochemistry of the lactone side chain was determined by a combination of coupling constants and analysis of the NOESY spectrum, which was similar to that of **4**. Since the CD spectrum showed a strong negative Cotton effect at 267 nm ($\Delta\varepsilon$ −21.3, $CHCl_3$), the absolute configuration at C-22 was assigned as *S*.[8,10] Thus, the structure of **5** was determined as a new triterpene lactone, which has been named ganodermalactone D.

Compound **6** was obtained as a white solid, and its molecular formula, $C_{30}H_{46}O_4$, was deduced from the HRESITOFMS (observed *m/z* 493.3289 $[M + Na]^+$), indicating 8 degrees of unsaturation. The UV spectrum showed an absorption maximum at 230 nm, indicating a conjugated carbonyl group. The IR spectrum of **6** showed the characteristics of hydroxyl (3446 cm^{-1}) and conjugated carbonyl lactone (1699 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **6** (Table 2) were similar to those of colossolactone B (**11**),[6] except for an acetoxy

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Table 2. ^{13}C and ^{1}H NMR Spectral Data of Compounds 4, 5 and 6 ($CDCl_3$)

	4		5		6	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	27.5	1.35, 1.98, m	27.0	1.72, 2.17, m	32.3	1.27, 2.02, m
2	28.3	1.32, 2.57, m	25.5	1.89, 2.45, m	27.7	1.50, 1.60, m
3	177.3		174.5		78.6	3.20, dd (11.6, 4.4)
4	74.7		75.1		38.9	
5	55.3	1.80, m	48.0	1.96, m	50.0	1.11, dd (2.8, 13.6)
6	33.9	2.15, 2.41, m	28.2	2.20, m	17.7	1.65, m
7	32.7	2.08, m	117.9	5.26, m	26.1	2.12, m
8	139.5		134.4		139.4	
9	122.0		141.2		130.5	
10	91.7		42.3		42.0	
11	31.1	2.03, 2.45, m	120.5	5.28, m	22.0	2.10, m
12	30.8	1.64, 1.84, m	38.7	2.15, 2.31, m	30.7	1.62, 1.95, m
13	44.5		43.8		44.3	
14	51.1		50.0		50.4	
15	30.1	1.21, 1.53, m	30.7	1.67, m	30.6	1.22, 1.57, m
16	25.3	1.61 m	31.5	1.38, 1.99, m	27.5	1.30, 1.89 m
17	45.3	2.10, m	46.0	1.59, m	45.8	2.05, m
18	15.7	0.81, s	16.6	0.62, s	16.3	0.73, s
19	41.5	1.62, d (14.4)	66.5	4.58, d (11.2)	65.7	3.53, d (11.2)
		3.17, d (14.4)		4.36, d (11.2)		3.92, d (11.2)
20	36.5	1.57, m	36.0	1.84, m	40.3	1.47, m
21	12.8	1.02, d (6.8)	12.5	1.00, d (6.8)	13.2	1.00, overlap
22	84.3	4.20, d (10.4)	84.3	4.18, d (10.8)	80.2	4.42, dd (13.2, 3.2)
23	63.6	4.47, brd (10.4)	63.4	4.47, brd (10.8)	27.8	2.53(β), m
						1.98(α), m
24	143.8	6.53, brs	144.3	6.52, brs	139.9	6.59, d (6.4)
25	127.7		127.4		128.0	
26	165.8		165.2		166.8	
27	16.8	1.93, s	16.8	1.91, s	17.0	1.87, s
28	27.0	1.33, s	33.7	1.28, s	28.2	0.98, overlap
29	32.1	1.29, s	25.9	1.19, s	15.4	0.87, s
30	24.5	0.94, s	24.1	0.87, s	24.6	0.93, s
1′			171.0			
2′			21.1	2.07, s		
3′			51.6	3.61, s		

group at C-19, which was displaced by a hydroxyl group [$\delta_{H/C}$ 3.53 and 3.92 (both d, J = 11.2 Hz)/65.7]. The absolute stereochemistry of C-22 was assigned as *S* from the CD spectrum at 261 nm ($\Delta\varepsilon$ −7.8, $CHCl_3$).[8] Thus, compound **6** was deduced as a new triterpene lactone, which has been named ganodermalactone E.

Compound **7** was obtained as colorless needles and was assigned the molecular formula $C_{30}H_{38}O_5$ as deduced from HRESITOFMS m/z 501.2619 $[M + Na]^+$, indicating 12 degrees of unsaturation. The UV spectrum showed absorption maxima at 230 and 327 nm, indicating a conjugated carbonyl group. The IR spectrum of **7** displayed the characteristics of two conjugated carbonyl lactones (1718 and 1672 cm^{-1}). The ^{13}C NMR and DEPT spectra of **7** (Table 3) showed 30 carbon signals attributable to two carbonyl (166.9 and 164.3), four sp^2 quaternary (149.3, 139.6, 129.8, and 126.8), four sp^2 methine (143.5, 142.8, 136.9, and 117.9), four sp^3 quaternary (104.6, 80.2, 48.9, and 44.5), three sp^3 methine (49.0, 42.9, and 35.3), eight sp^3 methylene, and five methyl carbons. The ^1H NMR spectrum of **7** (Table 3) showed signals that were assigned from COSY, HSQC, HMBC, and NOESY experiments to five methyl groups, characterized as three tertiary methyl singlets at δ 1.06 (H_3-30), 1.38 (H_3-29), and 1.50 (H_3-28), an allylic methyl at δ 1.87 (s, H_3-27) and a secondary methyl at δ 1.09 (d, J = 7.2 Hz, H_3-21), two oxymethylene protons at δ 3.17 and 3.61 (both d, J = 12.0 Hz, H_2-18), two methine protons at δ 2.01 (m, H-17), a pair of coupled olefinic protons at δ_H 6.63 (d, J = 12.0 Hz, H-1) and 5.78 (d, J = 12.0 Hz, H-2), and two olefinic protons at δ 6.13 (s, H-19) and 6.44 (brd, J = 5.2 Hz, H-24). The rest of the signals were assigned to seven methylene protons. The COSY and HSQC spectra indicated the presence of five substructures, i, ii, iii, iv, and v (bold lines in Figure 4). The assignment of ring A as a seven-membered α,β-lactone ring has been indicated by the signals at δ_C 166.9 (C-3) and 80.2 of an oxygenated carbon (C-4) and δ_H 6.63 (H-1), 5.78 (H-2), and 2.45 (m, H-5), which showed the correlations of H-1 to C-3, C-5, and C-19; H-2 to C-3 and C-10; H-5 to C-1, C-4, C-10, and C-19 (δ_C 142.8); and H_3-28 and H_3-29 to C-4 and C-5 in the HMBC experiment (Figure 5). That the seven-membered ring B containing two olefinic groups (C-10−C-19 and C-8−C-9) was fused to ring A at C-5−C-10 has been deduced on the basis of COSY correlations between H-5 ↔ H-6 ↔ H-7 and also the correlations of H-19 to C-1 and C-8 (δ_C 149.3); H-6 to C-8 and C-10; and H-7 to C-5, C-8, and C-9 in the HMBC spectrum. The methyl protons H_3-30 showed the HMBC correlations to C-8 and C-13 (δ_C 44.5), methylene protons H_2-

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Table 3. ^{13}C and ^{1}H NMR Spectral Data of Compounds 7 and 8 ($CDCl_3$)

	7		8	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	143.5	6.63, d (12.0)	143.2	6.67, d (12.2)
2	117.9	5.78, d (12.0)	118.3	5.81, d (12.2)
3	166.9		166.7	
4	80.2		80.0	
5	49.0	2.45, m	49.1	2.43, m
6	39.2	2.20,[a] 2.40, m	21.8[a]	1.86, 2.43, m
7	27.6	1.83, 2.50 m	28.1	1.92, 2.15, m
8	149.3		153.2	
9	129.8		134.9	
10	139.6		140.7	
11	25.8	2.10, 2.30, m	77.4	4.37, d (5.0)
12a	22.1	1.59, m	29.8	2.04, d (11.4)
12b		2.07, m		2.23, dd (11.4, 5.0)
13	44.5		54.6	
14	48.9		52.2	
15	31.0	1.50, 1.88, m	30.3	1.67, 1.82, m
16	22.4	1.87, 2.09, m	21.8[a]	1.90, 2.51, m
17	42.9	2.01, m	39.9	2.50, m
18β	61.0	3.17, d (12.0)	99.2	5.14, s
18α		3.61, d (12.0)		
19	142.8	6.13, s	140.6	6.35, s
20	35.3	1.81, m	35.0	1.96, m
21	14.8	1.09, d (7.2)	14.5	1.13, d (7.2)
22	104.6		105.4	
23α	32.9	2.20[a]	32.6	2.32, m
23β		2.83, brd (18.8)		2.82, dt (18.4, 2.4)
24	136.9	6.44, brd (5.2)	137.0	6.42, d (6.0)
25	126.8		126.4	
26	164.3		163.9	
27	17.0	1.87, s	16.8	1.85, s
28	26.2	1.50, s	26.2	1.50, s
29	29.0	1.38, s	29.1	1.38, s
30	26.8	1.06, s	25.8	1.20, s

[a]Overlapping of signals.

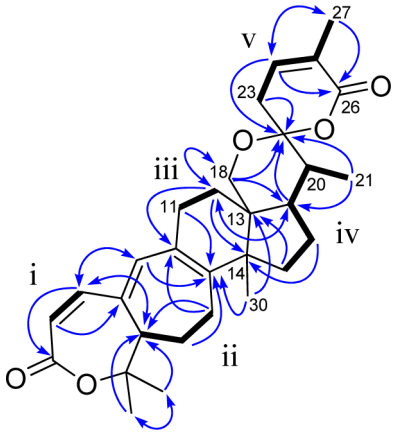

Figure 4. Selected HMBC correlations of 7.

11 to C-8, and H_2-12 to C-9 and C-14, indicating a six-membered ring C fused to ring B via C-8−C-9. These NMR data indicated a partial structure of 7, rings A−C as a 7/7/6 skeleton, which was similar to isolated schisanlactone B (10).[10] The COSY spectrum showed correlations between methylene groups at δ_H 1.50, 1.88 (H_2-15) and 1.87, 2.09 (H_2-16), H-16

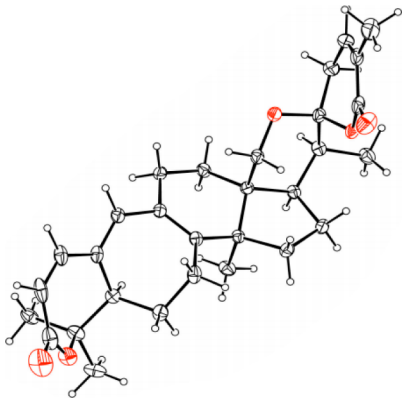

Figure 5. ORTEP plot of 7.

and H-17, H-17 and the methine proton H-20, and methyl protons H_3-21 (δ_H 1.09) and H-20, together with the HMBC correlations of H-12 to C-17 and C-18; H-16 to C-14; and oxymethylene protons H_2-18 to a ketal carbon at δ_C 104.6 (C-22), C-12, and C-17, supporting the connectivity of rings C, D, and E. The last ring, F, was identified as a six-membered α,β-unsaturated lactone from the COSY correlation of methylene protons H_2-23 to H-24, which has an allylic coupling to methyl protons H_3-27. In addition, the HMBC correlations of H-23 and H-24 to C-22, H-24 to carbonyl carbons C-26 (δ_C 164.3) and C-27, and H-27 to C-24, C-25, and C-26 confirmed the existence of ring F, which is connected to ring E at C-22 as a spiro-ketal lactone. The NOESY spectrum of 7 showed correlations between H-1 and H-2, H-1 and H-19, H-19 and H-11, H-18α and H-20, H-17 and H-20, H-17 and H-30, H-21 and H-23α,β and H-24, and H-24 and H-27, indicating the orientation of those protons in the molecule. Finally, the relative configuration of 7 was confirmed by single-crystal X-ray diffraction (Figure 5). On the basis of the above data, 7 was identified as a novel spirotriterpene lactone, which has been named ganodermalactone F.

Compound 8 was obtained as colorless needles and was assigned the molecular formula $C_{30}H_{36}O_6$, as deduced from HRESITOFMS m/z 493.2601 $[M + H]^+$, indicating 13 degrees of unsaturation. The UV and IR spectra of 8 showed the characteristic absorptions of conjugated carbonyl lactones as in 7. The ^1H and ^{13}C NMR spectroscopic data of 8 (Table 3) showed signals of rings A and B and a spiro-ketal lactone ring (C-22−C-27). However, the NMR spectra of 8 showed signals of an oxymethine group at $\delta_{H/C}$ 4.37/77.4 for H/C-11 instead of a methylene group ($\delta_{H/C}$ 2.10, 2.30/25.8), and an acetal unit at $\delta_{H/C}$ 5.14/99.2 for H/C-18 instead of the oxymethlene protons ($\delta_{H/C}$ 3.17, 3.61/61.0) as compared to 7. The COSY spectrum of 8 showed correlations between H-11 ↔ H-12 and H-15 ↔ H-16 ↔ H-17 ↔ H-20 ↔ H-21, indicating the partial structures of rings C, D, and E. The key HMBC spectrum of 8 demonstrated the correlations of H-11 to C-8, C-18, C-19, and C-13; H-18 to C-11, C-12, C-13, and C-14; H-17 to C-13, C-18, C-20, and C-22; H-12 to C-9, C-11, C-13, C-14, and C-18; H-21 to C-17, C-20, and C-22; and H-24 to C-22, C-23, and C-27, confirming the structure of 8 (Figure 6). The NOESY spectrum of 8 demonstrated the correlations between H-2 ↔ H-1 ↔ H-19 ↔ H-11 ↔ H-12β, H-12α ↔ H-17 ↔ H-20, H-15β (δ_H 1.67) ↔ H-18, and H-23α,β ↔ H-24 ↔ H-27, which indicate the relative configuration of 8. Finally, the configuration of 8 was confirmed by single-crystal X-ray diffraction (Figure 7). On the basis of the above deduction, the carbon

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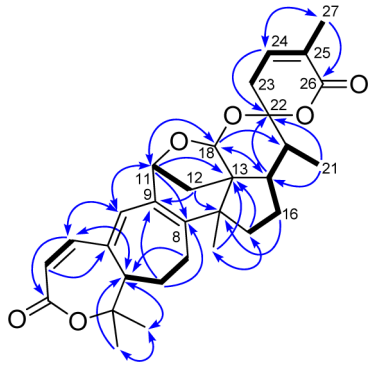

Figure 6. Selected HMBC correlations of **8**.

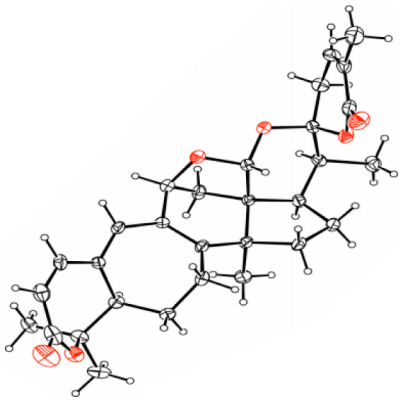

Figure 7. ORTEP plot of **8**.

backbone of **8** was unambiguously established as a novel bicyclo-spirotriterpene lactone, and it has been named ganodermalactone G.

Biogenetically, it is not unreasonable to propose that compounds **7** and **8** may derive from schisanlactone B (**10**) via a hydrolysis ring opening at C-22 to give a theoretical δ-hydroxycarboxylic acid, **A**.[15] Further oxidation at C-22 and hydroxylation at C-18 of **A** would provide **B**, which then would cyclize and lactonize to give the spiroketal **7**, while the oxidation at C-18 and hydroxylation at C-11 of **B** would provide **C**, which cyclizes to a bicyclo-spiroketal **8** (Figure 8).

Compounds **7**, **10**, and **12** showed antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 10.0, 6.0, and 10.0 μM, respectively (standard drug dihydroartemisinine, IC_{50} 0.004 μM). None of the compounds showed cytotoxicity against KB, NCI-H187, and MCF-7 cell lines at <10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter, and CD spectra were obtained using a JASCO J-810 apparatus. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were taken on a PerkinElmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as internal standard. HRESITOFMS were recorded on a Micromass Q-TOF-2 spectrometer. Column chromatography was carried out on Merck silica gel 60 (230−400 mesh). TLC was performed with precoated Merck silica gel 60 PF_{254} aluminum sheets; compounds were visualized under UV light (254 and 366 nm) and spraying with anisaldehyde and further by heating until charred.

Fungal Materials. The fruiting bodies were collected on decayed wood at Nakornprathom Province, Thailand, in November 2011 and identified by one of the authors (K.S.). Fruiting bodies are club-shaped, 30−50 cm wide and 60−70 cm long, yellowish-orange to brown on the upper surface and white when young, with many pores beneath the fruiting body. Pure culture was done using a tissue transplanting method, and the growth rate reached a 9 cm colony diameter in 7−10 days. The young colony was white, then become cream when mature. The mycelia were at the advanced margin of the colony hyaline. The texture of the colony was rinaceous in the early growth stage, then become farinaceous and much denser at a later stage. A voucher specimen (Gn-KM01) was deposited at the Department of Plant Production Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Pure culture was grown in conical flasks (1 L, 80 flasks) with potato dextrose broth (200 mL/flask) and incubated stationary at 28−30 °C for 30 days. The

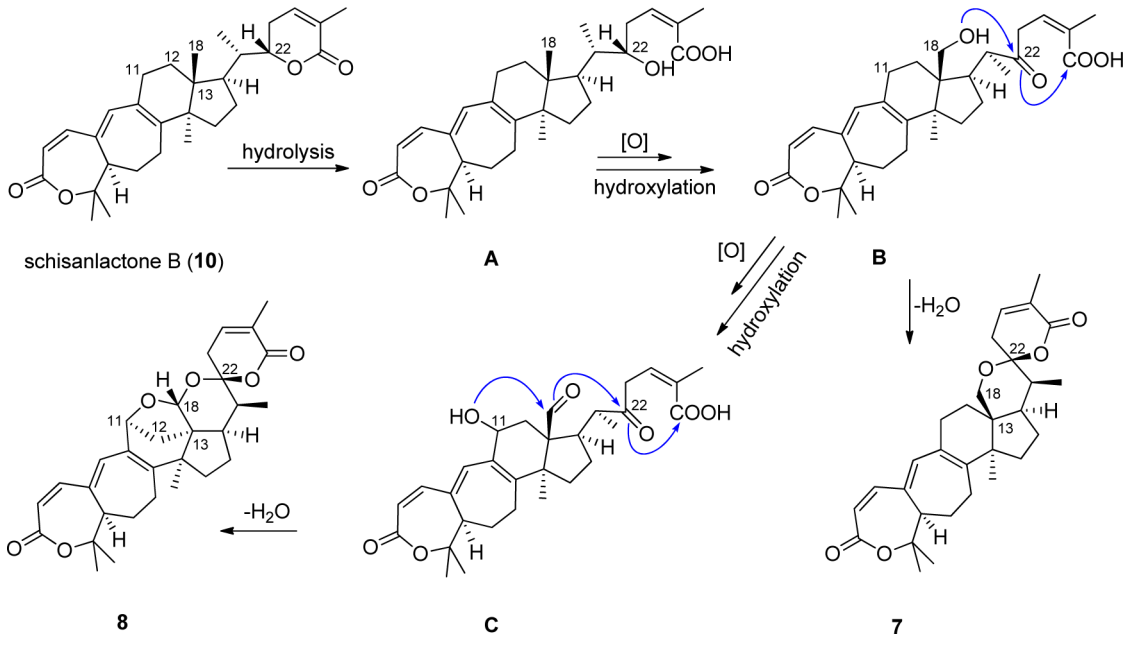

Figure 8. Plausible biogenetic pathway of **7** and **8**.

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culture broth was filtered to give a fungal biomass and then air-dried at room temperature.

Extraction and Isolation. The air-dried fungal biomass (155 g) was ground into a powder and extracted successively at room temperature with EtOAc (3 × 700 mL) and MeOH (3 × 700 mL). Removal of solvents was performed under reduced pressure to give crude EtOAc (15 g, 9.68%) and MeOH extracts (5 g, 3.23%), respectively. The solid material present in the EtOAc extract was filtered out to give **3** (1.5 g), while its filtrate was separated over silica gel flash column chromatography (FCC), eluted with a gradient system of hexane−EtOAc and EtOAc−MeOH, to give seven fractions, E_1−E_7. Fraction E_2 gave ergosterol (20.0 mg). Fraction E_4 yielded the yellow solid **2** (69.5 mg). The solid in fraction E_5 was filtered and purified to give the solid **8** (79.0 mg), and its filtrate was purified by FCC, eluted with 10% EtOAc−hexane, to yield 13 subfractions, $E_{5.1}$−$E_{5.13}$. Subfraction $E_{5.2}$ gave an additional amount of **2** (12.0 mg). The solid in subfractions $E_{5.4}$−$E_{5.7}$ was filtered out and was recrystallized from EtOAc−hexane to give colossolactone I (**9**) (282.7 mg). Subfraction $E_{5.8}$ yielded schisanlactone B (**10**) (791.9 mg). Subfraction $E_{5.9}$ gave **7** (69.7 mg). Subfraction $E_{5.10}$ was filtered to give an additional amount of **3** (207.5 mg), and its filtrate was purified by FCC, eluted with10% EtOAc−hexane, to give colossolactone B (**11**, 21.0 mg). Subfraction $E_{5.11}$ was purified by FCC, eluted with 30% EtOAc−hexane, to yield five subfractions, $E_{5.11.1}$−$E_{5.11.5}$. Subfraction $E_{5.11.1}$ gave an additional amount of **3** (4 mg). Subfraction $E_{5.11.2}$ was recrystallized from EtOAc to give colossolactone E (**12**, 497.2 mg). Subfraction $E_{5.12}$ was purified by FCC, eluted with 20% EtOAc−hexane, to yield seven subfractions, $E_{5.12.1}$−$E_{5.12.7}$. A solid in subfraction $E_{5.12.2}$ was filtered out to give colossolactone IV (**13**) (21.0 mg). Subfraction $E_{5.12.4}$ yielded **6** (9 mg). Subfraction $E_{5.12.5}$ was purified by preparative TLC and developed with 1% MeOH−CH_2Cl_2 (×3) to give an additional amount of **8** (3.3 mg) and **4** (5 mg). Subfraction $E_{5.12.6}$ was filtered out to give an additional amount of **8** (49.8 mg). Fraction E_6 was purified by FCC, eluted with 40% EtOAc−hexane, to give seven subfractions, $E_{6.1}$−$E_{6.7}$. Subfraction $E_{6.1}$ was purified to give an additional amount of colossolactone I (**9**) (41.7 mg). Subfraction $E_{6.3}$ yielded **8** (2.9 mg). Subfraction $E_{6.4}$ was recrystallized from EtOAc to give **1** (235.0 mg). The crude MeOH extract was separated over silica gel FCC, eluted with a gradient system of hexane−EtOAc and EtOAc−MeOH, to give six fractions, M_1−M_6. Fraction M_2 was filtered out to yield an additional amount of colossolactone I (**9**, 13.0 mg). Fraction M_3 gave an additional amount of **8** (7.5 mg). The filtrate of fraction M_3 was purified by FCC eluted with 40% EtOAc−hexane to give four subfractions, $M_{3.1}$−$M_{3.4}$. Subfraction $M_{3.2}$ was purified by preparative TLC and developed with 1% MeOH−CH_2Cl_2 (×3) to give **5** (6 mg). Fraction M_4 was filtered out to give colorless needles of ergosterol (21.6 mg). The filtrate of fraction M_4 was purified by FCC eluted with an isocratic system of hexane−EtOAc (60:40) to give four subfractions, $M_{4.1}$−$M_{4.4}$. Subfraction $M_{4.2}$ was filtered out to give an additional amount of **1** (263.4 mg).

*Colossolactone G (**1**):* colorless crystals; mp 149−151 °C; CD (*c* 0.000 37, $CHCl_3$) nm 249 (−36.9); $[\alpha]^{28}_D$ +2.40 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 229 (3.93), 274 (3.51), 332 (4.18); IR (KBr) ν_{max} cm^{-1} 3458, 2971, 1708, 1600, 1578, 1452, 1375, 1345, 1246, 1185, 1141, 1086, 1049, 1022; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 539.3013 $[M + H]^+$ (calcd for $C_{32}H_{42}O_7$ + H, 539.3009).

*Ganodermalactone A (**2**):* yellow solid; mp 185−187 °C; CD (*c* 0.000 32, $CHCl_3$) nm 257 (−39.7); $[\alpha]^{26}_D$ +82.6 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 230 (4.16), 252 (4.06), 350 (4.42); IR (film) ν_{max} cm^{-1} 2951, 2925, 2876, 1713, 1659, 1575, 1450, 1379, 1343, 1269, 1239, 1209, 1130, 1069, 1047, 1011; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 471.2863 $[M+ Na]^+$ (calcd for $C_{30}H_{40}O_3$ + Na, 471.2875).

*Ganodermalactone B (**3**):* white solid; mp 240−242 °C; CD (*c* 0.000 32, $CHCl_3$) nm 260 (−28.0); $[\alpha]^{22}_D$ +22.6 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.80); IR (KBr) ν_{max} cm^{-1} 2973, 2952, 2940, 2928, 1734, 1712, 1447, 1379, 1344, 1209, 1268, 1247, 1201, 1134, 1097, 1041, 1013; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 549.3138 $[M + Na]^+$ (calcd for $C_{32}H_{46}O_6$ + Na, 549.3192).

*Ganodermalactone C (**4**):* white solid; mp 258−260 °C; CD (*c* 0.000 32, $CHCl_3$) nm 265 (−13.5); $[\alpha]^{26}_D$ +36.8 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.29); IR (film) ν_{max} cm^{-1} 3439, 2921, 2852, 1742, 1696, 1463, 1376, 1260, 1152, 1032; ^1H and ^{13}C NMR spectral data, see Table 2; HRESITOFMS *m*/*z* 523.3026 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_6$ + Na, 523.3036).

*Ganodermalactone D (**5**):* yellow, amorphous solid; mp 153−155 °C; CD (*c* 0.000 28, $CHCl_3$) nm 267 (−21.3); $[\alpha]^{27}_D$ +122.3 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 244 (3.99); IR (KBr) ν_{max} cm^{-1} 3436, 2940, 1723, 1718, 1575, 1384, 1247, 1143, 1030; ^1H and ^{13}C NMR spectral data, see Table 2; HRESITOFMS *m*/*z* 595.3238 $[M + Na]^+$ (calcd for $C_{33}H_{48}O_8$ + Na, 595.3247).

*Ganodermalactone E (**6**):* white solid; mp 268−270 °C; CD (*c* 0.000 34, $CHCl_3$) nm 261 (−7.8); $[\alpha]^{27}_D$ +93.2 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 230 (4.10); IR (film) ν_{max} cm^{-1} 3446, 2945, 2878, 1699, 1452, 1371, 1343, 1249, 1143, 1130, 1091, 1027; ^1H and ^{13}C NMR spectral data, see Table 2; HRESITOFMS *m*/*z* 493.3289 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4$ + Na, 493.3294).

*Ganodermalactone F (**7**):* colorless needles; mp 250−252 °C; $[\alpha]^{27}_D$ +317.6 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 230 (3.81), 327 (3.99); IR (film) ν_{max} cm^{-1} 2960, 2928, 1718, 1672, 1598, 1566, 1459, 1370, 1295, 1174, 1126, 1059, 1006; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 479.2793 $[M + H]^+$ (calcd for $C_{30}H_{38}O_5$ + H, 479.2797).

*Ganodermalactone G (**8**):* colorless needles; mp 264−266 °C; $[\alpha]^{26}_D$ +291.8 (*c* 0.1, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 231 (4.14), 327 (4.35); IR (film) ν_{max} cm^{-1} 2956, 2938, 1716, 1668, 1592, 1562, 1449, 1391, 1356, 1291, 1177, 1130, 1056, 1033, 1019; ^1H and ^{13}C NMR spectral data, see Table 1; HRESITOFMS *m*/*z* 493.2601 $[M + H]^+$ (calcd for $C_{30}H_{36}O_6$ + H, 493.2590).

X-ray Crystallographic Analyses of 3, 7, and 8. X-ray diffraction data were measured on a Bruker-Nonius kappaCCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 298(2) K. The structures were solved by direct methods by SIR97[16] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[17] Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under the reference numbers CCDC 963331, 949503, and 949504. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

*X-ray data of **3**:* $C_{32}H_{46}O_6$, MW = 526.69, orthorhombic, dimensions 0.25 × 0.10 × 0.08 mm^3, D = 1.223 g/cm^3, space group $P2_12_12_1$, Z = 4, a = 9.2640(1) Å, b = 10.1610(3) Å, c = 30.3990(7) Å, V = 2861.5(1) $Å^3$, reflections collected/unique 27 750/7948, number of observations [>2σ(I)] 5837, final R indices [I > 2σ(I)] R_1 = 0.0538, wR_2 = 0.1334.

*X-ray data of **7**:* $C_{30}H_{38}O_5$, MW = 478.60, monoclinic, dimensions 0.25 × 0.15 × 0.10 mm^3, D = 1.257 g/cm^3, space group $P2_1$, Z = 2, a = 7.3040(3) Å, b = 15.5760(8) Å, c = 11.3010(6) Å, β = 100.378(3)°, V = 1264.7(1) $Å^3$, reflections collected/unique 5988/3271, number of observations [>2σ(I)] 3046, final R indices [I > 2σ(I)] R_1 = 0.0378, wR_2 = 0.0949.

*X-ray data of **8**:* $C_{30}H_{36}O_6$, MW = 492.59, orthorhombic, dimensions 0.20 × 0.10 × 0.07 mm^3, D = 1.283 g/cm^3, space group $P2_12_12_1$, Z = 4, a = 7.1300(3) Å, b = 15.5750(11) Å, c = 22.9690(15) Å, V = 2550.7(3) $Å^3$, reflections collected/unique 11 006/2010, number of observations [>2σ(I)] 1735, final R indices [I > 2σ(I)] R_1 = 0.0425, wR_2 = 0.1097.

Antimalarial Assay. Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen.[18] Quantitative assessment of activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[19] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The positive control was dihydroartemisinin.

Cytotoxicity Assay. Cytotoxic assays against human epidermoid carcinoma KB, NCI-H178, and MCF-7 cell lines were performed employing the colorimetric method as described by Skehan and co-workers.[20] The reference substance was ellipticine.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR and ^{13}C NMR spectra for compounds **1**−**8**, ORTEP plot for **1**, and X-ray crystallographic data (CIFs) for **3**, **7**, and **8**. 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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■ DEDICATION

Dedicated to Professor Dr. Yodhathai Thebtaranonth in honor of his 70th birthday.

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