Oxygenated Lanostane-Type Triterpenoids from the Fungus *Ganoderma lucidum*

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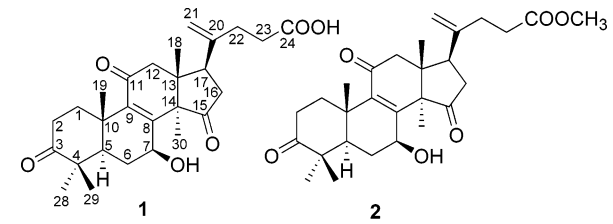
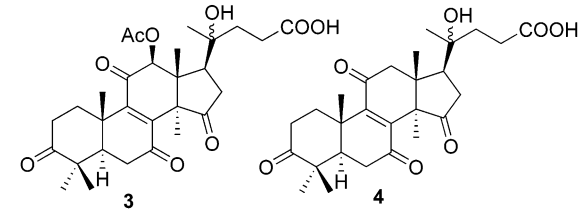
Two new triterpenoids, 20(21)-dehydrolucidenic acid A (**1**) and methyl 20(21)-dehydrolucidenate A (**2**), and five new 20-hydroxylucidenic acids, 20-hydroxylucidenic acid D_2 (**3**), 20-hydroxylucidenic acid F (**4**), 20-hydroxylucidenic acid E_2 (**5**), 20-hydroxylucidenic acid N (**6**), and 20-hydroxylucidenic acid P (**7**), were isolated from the fruiting body of the fungus *Ganoderma ludicum*, and their structures were established on the basis of spectroscopic methods.

The fruiting body of *Ganoderma lucidum* Karst (Polyporaceae), commonly known as the Reishi mushroom, is widely used in China, Japan, and Korea as a valuable crude drug, especially in the treatment of chronic hepatitis, nephritis, hepatopathy, neurasthenia, arthritis, bronchitis, asthma, gastric ulcer, and insomnia.[1] Over one hundred oxygenated triterpenoids have been isolated from this mushroom.[2−4] In the course of our search for potential antitumor-promoters (chemopreventive agents) from natural sources,[5,6] we have isolated and characterized three new and 14 known oxygenated lanostane-type triterpenoids from the fruiting body of the fungus *G. lucidum* and have reported their inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[4] Our continuing study on the constituents of *G. lucidum* fruiting body led to the isolation of seven further new triterpenoids (**1**−**7**), and we report their structure elucidation in this paper.



1 **2**



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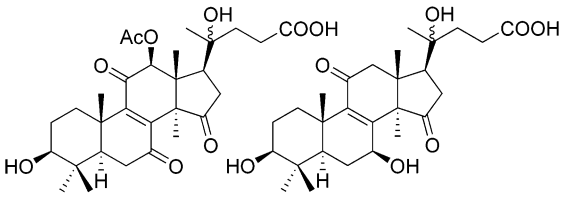

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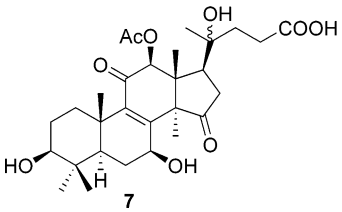

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Results and Discussion

The molecular formula of **1** was determined as $C_{27}H_{36}O_6$ from its HREIMS ($[M]^+$ *m/z* 456.2512) as well as from its ^{13}C NMR DEPT. The UV absorbance at 253 nm indicated the presence of an α,β-unsaturated ketone system. Its IR absorption bands suggested the presence of hydroxyl (3445 cm^{-1}), carbonyl (1735 cm^{-1}), carboxyl (1659 cm^{-1}), and terminal methylene (897 cm^{-1}) groups. The ^1H NMR spectrum showed signals for five tertiary methyl [δ_H 0.90, 1.11, 1.13, 1.26, and 1.39 (each s)], a terminal methylene [δ_H 4.91 and 5.08 (each 1H and s)], and an oxymethine [δ_H 4.87 (dd, J = 7.6, 9.6 Hz)] groups (Table 1). The ^{13}C NMR, combined with DEPT and HMQC, showed that **1** had five methyls, eight methylenes (including one sp^2 methylene carbon), three methines (including one oxymethine carbon), seven quaternary carbons (including three sp^2 carbons), and four carbonyls (including three ketones) (Table 1). The EIMS of **1** showed diagnostic fragment ions at *m/z* 355 $[C_{22}H_{27}O_4]^+$, corresponding to the loss of a side-chain ($C_5H_7O_2$) with concomitant 2H loss, 318 $[C_9H_{14}O_1]^+$, formed by the loss of ring A by the cleavage of C-5−C-6 and C-9−C-10, and 300 $[C_8H_{12}O_3]^+$, due to the loss of ring D plus 2H by the cleavage of C-13−C-17 and C-14−C-15. Comparison of these data with those of lucidenic acid A (7β-hydroxy-3,11,15-trioxo-25,26,27-trinorlanost-8-en-24-oic acid)[4,7,8] suggested that compound **1** possesses the same structure as that of lucidenic acid A with the exception of the side-chain. Compound **1** had an additional double bond in the side-chain as a terminal methylene group most probably located at C-20(21). The structure of compound **1** was, therefore, assigned as 7β-hydroxy-3,11,15-trioxo-25,26,27-trisnorlanosta-8,20(21)-dien-24-oic acid, which we

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Table 1. ^{13}C, ^{1}H, and HMBC NMR Spectral Data for Triterpenoids **1**–**3** ($CDCl_3$)

	1				**2**[a]				**3**			
C no.	δ_C		δ_H[b]	HMBC (H to C)	δ_C		δ_H[b]	HMBC (H to C)	δ_C		δ_H[b]	HMBC(H to C)
1	35.7	t	α: 1.48 ddd (8.2, 8.6, 13.8) β: 2.95 ddd (5.2, 7.6, 13.8)	2, 3, 9, 10, 19 2, 3, 5, 10, 19	36.0	t	α: 1.63 ddd (8.2, 8.2, 13.7) β: 3.18 ddd (6.5, 6.5, 13.7)		34.0	t	α: 1.73 ddd (6.9, 9.7, 14.3) β: 2.76 ddd (6.3, 8.3, 14.3)	2, 10, 19 2, 5, 10, 19
2	34.3	t	α: 2.45 ddd (5.2, 8.2, 16.5) β: 2.53 ddd (7.6, 8.6, 16.5)	1, 3, 10 1, 3, 10	34.5	t	2.57 (2H) dd like (8.3, 8.3)		33.6	t	α: 2.48 ddd (6.9, 8.3, 15.4) β: 2.60 ddd (6.3, 9.7, 15.4)	1, 3, 4, 10 1, 3, 10
3	216.5	s			215.9	s			214.9	s		
4	46.8	s			46.8	s			46.9	s		
5	49.0	d	1.58 dd (1.7, 13.7)	4, 7, 10, 19, 28, 29	48.9	d	1.76 dd (1.7, 13.7)		50.9	d	2.31 dd (2.3, 14.9)	1, 4, 6, 10, 28, 29
6	27.7	t	α: 2.12 ddd (1.7, 7.6, 13.1) β: 1.68 ddd (9.6, 13.1, 13.7)	7, 8, 10 5, 7	29.1	t	α: 2.22 ddd (1.7, 8.0, 13.1) β: 1.87 ddd (9.3, 13.1, 13.7)		37.4	t	α: 2.50 dd (2.3, 13.8) β: 2.74 dd (13.8, 14.9)	5, 7, 8, 10 5, 7, 10
7	66.3	d	4.87 dd (7.6, 9.6)	6, 9	65.7	d	5.19 dd (8.0, 9.3)		198.4	s		
8	157.8	s			159.8	s			145.6	s		
9	141.3	s			140.9	s			149.6	s		
10	38.3	s			38.5	s			39.3	s		
11	197.5	s			198.0	s			193.3	s		
12	49.1	t	α: 2.83 d (16.8) β: 2.65 d (16.8)	11, 13, 18 11, 14, 18	49.8	t	α: 3.06 d (16.8) β: 2.80 d (16.8)		78.6	d	5.70 s	11, 13, 14, COMe
13	45.3	s			45.5	s			47.9	s		
14	58.8	s			58.3	s			58.9	s		
15	217.7	s			215.5	s			203.8	s		
16	38.7	t	2.61 (2H) d like (9.0)	15	39.5	t	α: 2.62 dd (7.6, 18.9) β: 2.85 dd (11.0, 18.9)		35.4	t	α: 2.84 dd (10.0, 18.3) β: 2.27 dd (8.3, 18.3)	13, 15 14, 15, 17, 20
17	46.3	d	3.01 dd (9.0, 9.0)	13, 16, 17, 18, 20, 24	46.7	d	3.12 dd (7.6, 11.0)		48.8	d	2.95 dd (8.3, 10.0)	13, 16, 18, 20
18	18.8	q	0.90 s	12, 14, 17	19.3	q	1.10 s		13.0	q	0.96 s	13, 14
19	18.2	q	1.26 s	1, 5, 9, 10	18.6	q	1.35 s		18.7	q	1.34 s	1, 5, 9, 10
20	143.9	s			145.8	s			86.4	s		
21	112.3	t	4.91 s, 5.08 s	17, 22	111.6	t	4.93 s, 5.05 s		26.1	q	1.49 s	17
22	31.3	t	2.31 (1H) ddd (7.9, 7.9, 16.2) 2.48 (1H) ddd (5.2, 7.9, 13.4)	20, 21, 22, 23, 24 20, 21, 22, 23, 24	31.8	t	2.36 (1H) ddd (7.2, 15.5, 16.2) 2.48 (1H) ddd (7.6, 15.8, 16.2)		34.5	t	2.04 (1H) ddd (3.4, 10.3, 13.0) 2.10 (1H) ddd (2.7, 10.0, 13.0)	21, 23, 24 17, 21, 23
23	31.9	t	2.59 (2H) dd like (7.6, 7.6)	20, 24	32.6	t	2.61 (2H) d like (7.2)		28.0	t	2.56 (1H) m 2.69 (1H) m	22, 24 22, 24
24	175.1	s			173.2	s			175.6	s		
28	27.0	q	1.13 s	5, 29	27.0	q	1.15 s		27.6	q	1.14 s	3, 4, 5, 29
29	20.8	q	1.11 s	3, 4, 28	20.9	q	1.12 s		20.4	q	1.12 s	3, 4, 5, 28
30	24.6	q	1.39 s	8, 13, 14, 15	25.2	q	1.43 s		21.1	q	1.85 s	8, 13, 14, 15
*C*OMe									170.1	s		
CO*Me*									21.0	q	2.26 s	*C*OMe
COO*Me*					51.5	q	3.64 s					

[a] Determined in $CDCl_3$. [b] Figures in parentheses denote J values (hertz).

named 20(21)-dehydrolucidenic acid A. Analysis of $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra supported the proposed structure of **1**.

Compound **2**, $C_{28}H_{38}O_6$ (HREIMS *m/z* 470.2668 $[M]^+$), having 14 mass units (CH_2) higher than compound **1**, showed ^{13}C and ^1H NMR signal patterns very similar to those of **1** except for the presence of an additional methoxyl signal [δ_C 51.5 (q); δ_H 3.64 (3H, s)] for **2** (Table 1). The methoxyl group was unambiguously assigned at C-24 as a methyl ester group due to the presence of a significant cross-peak ($^3J_{C-H}$) between signals of δ_H 3.64 and δ_C 173.2 (C-24) in the HMBC spectrum. Hence, compound **2** was characterized as methyl 7β-hydroxy-3,11,15-trioxo-25,26,27-trisnorlanosta-8,20(21)-dien-24-oate [methyl 20(21)-dehydrolucidenate A]. Analysis of the UV, IR, and EIMS spectra (see Experimental Section) and the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of **2** supported the proposed structure.

Compound **3** was assigned the molecular formula $C_{29}H_{38}O_9$, as determined from its ^{13}C DEPT NMR, HREIMS (*m/z* 512.2410 $[M - H_2O]^+$), and FABMS (*m/z* 553 $[M + Na]^+$) data. The UV absorbance at 254 nm indicated the presence of an α,β-unsaturated ketone system. Its IR absorption bands suggested the presence of hydroxyl (3452 cm^{-1}), carbonyl (1752 cm^{-1}), and carboxyl (1698 cm^{-1}) groups. The ^{13}C and ^1H NMR data showed that compound **3** had six tertiary methyls, four ketones, a secondary acetoxyl, a tertiary hydroxyl, and a carboxyl group (Table 1). The ^1H NMR spectrum of **3** was very similar to that of lucidenic acid D_2 (12β-acetoxy-3,7,11,15-tetraoxo-25,26,27-trisnorlanost-8-en-24-oic acid).[4] The only exceptions were that **3** exhibited the C-21 methyl signal as a singlet (δ_H 1.49), instead of a doublet observed for lucidenic acid D_2, and the C-18 methyl singlet at somewhat lower field (δ_H 0.96) than lucidenic acid D_2, which suggested that **3** had the tertiary hydroxyl group at C-20. The presence of an EIMS fragment ion at *m/z* 413 $[M -$ side-chain $(C_5H_9O_3)]^+$ was consistent with this supposition. The above evidence coupled with analyses of the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra allowed the assignment of **3** as (20ξ)-12β-acetoxy-20-hydroxy-3,7,11,15-tetraoxo-25,26,27-trisnorlanost-8-en-24-oic acid (20-hydroxylucidenic acid D_2).

Compound **4** was assigned the molecular formula $C_{27}H_{36}O_7$ (HREIMS *m/z* 454.2355 $[M - H_2O]^+$; FABMS *m/z* 495 $[M + Na]^+$), corresponding to one acetoxyl group (58 mass units: $CH_3OCO - H$) less than that of **3**. In the ^1H NMR spectrum of **4** (Table 2), signals due to the ring system were in good agreement with those of lucidenic acid F (3,7,11,15-tetraoxo-25,26,27-trisnorlanost-8-en-24-oic acid),[4] while the ^{13}C and ^1H NMR signals arising from the side-chain moiety were superimposable with those of **3**. This suggested that **4** was a C-20-hydroxylated analogue of lucidenic acid F, viz., (20ξ)-20-hydroxy-3,7,11,15-tetraoxo-25,26,27-trisnorlanost-8-en-24-oic acid (20-hydroxylucidenic acid F). Analysis of the UV, IR, and EIMS spectra and the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of **4** supported this conclusion.

Compound **5** had the molecular formula $C_{29}H_{40}O_9$ (HREIMS *m/z* 514.2566 $[M - H_2O]^+$; FABMS *m/z* 555 $[M + Na]^+$) and exhibited ^1H NMR signals (Table 2) for the ring-system moiety very similar to those of lucidenic acid E_2 (12β-acetoxy-3β-hydroxy-7,11,15-trioxo-25,26,27-trisnorlanost-8-en-24-oic acid).[4] The ^{13}C and ^1H NMR signals for the side-chain moiety of **5** (Table 2) were, on the other hand, almost indistinguishable from those of **3**. The above evidence coupled with analyses of the UV, IR, and EIMS spectra, as well as the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra, indicated that **5** was (20ξ)-12β-acetoxy-3β,20-dihydroxy-7,11,15-trioxo-25,26,27-trisnorlanost-8-en-24-oic acid (20-hydroxylucidenic acid E_2).

Compound **6** was assigned the molecular formula $C_{27}H_{40}O_7$ (HREIMS *m/z* 458.2668 $[M - H_2O]^+$; FABMS *m/z* 499 $[M + Na]^+$). The ^{13}C and ^1H NMR signals (Table 2) of the ring system were very similar to those of lucidenic acid N (3β,7β-dihydroxy-11,15-dioxo-25,26,27-trisnorlanost-8-en-24-oic acid),[3] while the ^{13}C and ^1H NMR signals for the side-chain moiety were superimposable with those of **3**. This information suggested that **6** is a C-20-hydroxylated analogue of lucidenic acid N, viz., (20ξ)-3β,7β,20-trihydroxy-11,15-dioxo-25,26,27-trisnorlanost-8-en-24-oic acid (20-hydroxylucidenic acid N). Analyses of the UV, IR, and EIMS spectra and the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of **6** supported its proposed structure.

Compound **7** had the molecular formula $C_{29}H_{42}O_9$ (HREIMS *m/z* 516.2723 $[M - H_2O]^+$; FABMS *m/z* 557 $[M + Na]^+$) and showed ^{13}C and ^1H NMR signals (Table 2) arising from the ring-system moiety that were very similar to those of lucidenic acid P (12β-acetoxy-3β,7β-dihydroxy-11,15-dioxo-25,26,27-trisnorlanost-8-en-24-oic acid),[4] whereas the ^{13}C and ^1H NMR signals of the side-chain moiety were nearly indistinguishable from those of **3**. These findings indicated that **7** was a C-20-hydroxylated analogue of lucidenic acid P and that it has the structure (20ξ)-12β-acetoxy-3β,7β,20-trihydroxy-11,15-dioxo-25,26,27-trisnorlanost-8-en-24-oic acid (20-hydroxylucidenic acid P). Analyses of the UV, IR, and EIMS spectra and the ^{13}C DEPT, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra of **7** supported this structure.

This is the first report of the isolation of lanostane-type triterpenoids possessing a $\Delta^{20(21)}$-unsaturated side-chain, **1** and **2**, from a natural source, although several $\Delta^{20(21)}$-unsaturated dammarane-type triterpenoids have been reported in some higher plants.[9−11] In addition, although several C-20-hydroxylated ganoderic acids, highly oxygenated lanostane-type triterpenoids possessing a C_8-side-chain, have been reported as constituents of *G. lucidum*,[12−14] this is the first instance of the isolation of C-20-hydroxylated lucidenic acids, highly oxygenated lanostane-type triterpenoids with a C_5-side-chain, **3**−**7**, from a natural source. The absolute configuration at C-20 of compounds **3**−**7** remained undetermined in this study.

Experimental Section

General Experimental Procedures. Crystallizations were performed in acetone−MeOH, and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 polarimeter in acetone or in $CHCl_3$ at 25 °C. UV spectra, on a Shimadzu UV-2200 spectrometer, and IR spectra, on a JASCO FTIR-300E spectrometer, were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a JEOL ECX-500 (^1H: 500 MHz; ^{13}C: 125 MHz) or with a JEOL ECA-600 (^1H: 600 MHz, ^{13}C: 150 MHz) spectrometer in $CDCl_3$ or in C_5D_5N with tetramethylsilane as internal standard. Electron-ionization mass spectra (EIMS; 70 eV) and high-resolution EIMS (HREIMS) were recorded on a JEOL JMS-BU20 spectrometer using a direct inlet system. FABMS and HRFABMS were obtained with a JEOL JMS-BU20 spectrometer using glycerol as a matrix. Analytical TLC on silica gel (silica gel F_{254}, Merck; 10 × 10 cm) was developed using *n*-hexane−ethyl acetate (EtOAc)−acetic acid (AcOH) (50:50:0.5, v/v/v). Silica gel (Kieselgel 60, 230−400 mesh, Merck) was used for open column chromatography. Reversed-phase preparative HPLC was carried out on a 25 cm × 10 mm i.d.

Table 2. ^{13}C, ^{1}H, and HMBC NMR Spectral Data for Triterpenoids **4**–**7** ($CDCl_3$)

	4			**5**			**6**			**7**	
C no.	δ_C	δ_H[a]	HMBC(H to C)	δ_C	δ_H[a]	HMBC(H to C)	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]
1	34.6 t	α: 1.74 ddd (5.6, 9.8, 13.9)	2, 3, 9, 10, 19	33.2 t	α: 1.18 m	2, 19	34.8 t	α: 0.99 m	2, 3, 10	34.4 t	α: 0.96 ddd (4.3, 13.2, 14.3)
		β: 2.63 m	2, 3, 10, 19		β: 2.73 m	2, 3, 5		β: 2.85 ddd (3.4, 3.6, 13.7)	2, 3, 10		β: 2.63 ddd (3.7, 3.7, 13.2)
2	33.8 t	α: 2.52 ddd (5.6, 7.3, 15.1)	1, 3	27.3 t	1.71 (2H) m	4, 10	27.6 t	1.66 (2H) m	1, 3	27.4 t	1.65 (2H) m
		β: 2.62 m	1, 3								
3	215.1 s			77.4 d	3.26 dd (4.9, 11.2)	28, 29	78.2 d	3.21 dd (5.6, 10.7)	4, 28, 29	78.1 d	3.20 dd (5.1, 11.2)
4	47.0 s			40.5 s			38.6 s			38.6 s	
5	50.9 d	2.32 dd (2.7, 12.2)	4, 7, 10, 19, 28	51.4 d	1.56 dd (2.7, 14.4)	4, 6, 10, 19, 28, 29	49.1 d	0.88 brd (13.7)	4, 7, 19, 29	49.1 d	0.89 brd (14.9)
6	37.2 t	2.48 dd (2.7, 13.7)	5, 7, 8	36.6 t	α: 2.59 dd (2.7, 13.6)	5, 7	26.6 t	α: 2.19 br dd (8.5, 12.2)	5, 7, 8, 10	26.7 t	α: 2.24 brdd (9.2, 13.5)
		2.73 dd (13.7, 14.9)	5, 7		β: 2.68 dd (13.6, 14.4)	5, 7		β: 1.62 m	5, 7, 8, 10		β: 1.65 m
7	199.1 s			198.0 s			66.0 d	4.80 dd (8.5, 8.8)	6, 8, 9, 30	66.1 d	4.83 dd (6.0, 13.5)
8	146.2 s			151.4 s			156.5 s			155.9 s	
9	149.6 s			145.4 s			142.6 s			142.9 s	
10	34.6 s			39.1 s			38.9 s			38.6 s	
11	198.0 s			193.0 s			197.0 s			191.0 s	
12	48.8 t	α: 2.93 d (16.19	11, 13	78.9 d	5.65 s	11, 13, 14, 18, COMe	50.2 t	2.81 (2H) s	9, 11, 13, 14, 17, 18	79.1 d	5.64 s
		β: 2.86 d (16.4)	9, 11, 13								
13	44.3 s			48.2 s			45.4 s			49.8 s	
14	57.1 s			58.9 s			59.3 s			61.0 s	
15	204.9 s			203.9 s			215.7 s			214.5 s	
16	34.3 t	α: 2.64 m	15, 17	35.7 t	α: 2.81 dd (9.8, 18.1)	13, 14, 15, 17	35.7 t	α: 2.61 m	13, 15, 17, 20	37.1 t	α: 2.81 dd (8.6, 15.5)
		β: 2.28 dd (5.9, 14.9)	15, 17, 20		β: 2.28 dd (8.5, 18.1)	13, 14, 15, 17, 20		β: 2.49 m	13, 15, 17, 20		β: 2.52 dd (9.7, 16.9)
17	48.1 d	2.65 m	13, 15	49.2 d	2.93 dd (8.5, 9.8)	12, 13, 16, 18, 20, 21	49.5 d	2.52 m	13, 15, 16, 18, 20	50.2 d	2.84 dd (8.9, 8.9)
18	17.4 q	1.02 s	12, 13, 14	13.2 q	0.93 s	12, 13, 14	18.8 q	1.13 s	12, 13	14.2 q	1.08 s
19	18.6 q	1.28 s	1, 5, 9, 10	17.9 q	1.33 s	1, 4, 5, 8	18.3 q	1.22 s	1, 5, 9, 10	18.5 q	1.26 s
20	86.0 s			86.6 s			85.9 s			86.7 s	
21	26.3 q	1.50 s	17, 20, 22	26.1 q	1.48 s	17, 20	25.9 q	1.51 s	17, 20, 22	25.2 q	1.49 s
22	34.2 t	2.02 (1H),	20, 24	34.5 t	2.05 (2H) m	17, 20, 21, 23, 24	34.2 t	2.06 (2H) m	20, 21, 23, 24	34.6 t	2.08 (2H) m
		2.07 (1H) m	20, 23								
23	27.3 t	2.61 (1H) m	22, 24	28.1 t	2.52 (1H) m	22, 24	27.5 t	2.54 (1H) m	20, 22, 24	28.3 t	2.54 (1H) m
		2.67 (1H) m	22, 24		2.68 (1H) m	22, 24		2.64 (1H) m	20, 22, 24		2.70 (1H) m
24	175.8 s			175.6 s			175.9 s			175.5 s	
28	27.6 q	1.14 s	2, 4, 5, 29	27.9 q	1.03 s	3, 5, 10, 29	28.1 q	1.04 s	3, 4, 5, 29	28.1 q	1.04 s
29	20.3 q	1.12 s	3, 4, 28	15.5 q	0.89 s	3, 5, 10, 28	15.4 q	0.85 s	3, 4, 5, 28	15.4 q	0.86 s
30	21.3 q	1.68 s	8, 13, 14, 15	21.6 q	1.78 s	9, 13, 14, 15	24.7 q	1.38 s	8, 13, 15, 18	24.5 q	1.54 s
*C*OMe				170.1 s						170.3 s	
CO*Me*				21.0 q	2.26 s	*C*OMe				21.2 q	2.26 s

[a] Figures in parentheses denote J values (hertz).

C_{18} silica column (Pegasil ODS II column; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C eluting with MeOH−H_2O−AcOH (60:40:1, v/v/v) as mobile phase at 2 mL/min. A refractive index detector was used for reversed-phase HPLC.

Materials. Fruiting bodies of *Ganoderma lucidum* Karst (Polyporaceae) used in this study were described previously.[4]

Extraction and Isolation. Column chromatography on silica gel (1 kg) of the MeOH extract (30 g) of dried and chipped fruiting bodies of *G. lucidum* (373 g) which was eluted successively with *n*-hexanes−EtOAc [1:0 (2.5 L), 19:1 (6.5 L), 9:1 (2.5 L), 4:1 (3.0 L), 7:3 (10.0 L), 3:7 (9.0 L), 0:1 (7:0 L), v/v] gave six fractions.[4] A portion (5.0 g) of the most polar fraction (6.9 g) eluted by *n*-hexanes−EtOAc [7:3, 3:7, and 0:1] was further chromatographed on silica gel (200 g) with a stepwise gradient of *n*-hexanes−EtOAc [9:1 (4.5 L), 4:1 (5.8 L), 7:3 (3.0 L), 1:1 (3.4 L), 2:3 (0.6 L), 3:7 (5.2 L), 1:4 (0.8 L), 0:1 (1.0 L), v/v], which yielded fractions A (R_f ca. 0.7 on TLC; 707 mg), B (R_f ca. 0.5; 916 mg), and C (R_f ca. 0.2; 1.83 g) from the eluates of *n*-hexanes−EtOAc (7:3), (1:1 and 2:3), and (3:7, 1:4, and 0:1), respectively.[4] A portion (173 mg) of fraction B, separated by HPLC, afforded compound **2** (2.8 mg; t_R 27.6 min). HPLC of a portion (800 mg) of fraction C gave six compounds, **1** (2.0 mg; t_R 19.0 min), **3** (2.0 mg; t_R 13.0 min), **4** (4.0 mg; t_R 8.4 min), **5** (2.6 mg; t_R 15.0 min), **6** (14.2 mg; t_R 6.6 min), and **7** (2.6 mg; t_R 7.6 min).

20(21)-Dehydrolucidenic acid A (1): colorless needles, mp 135−137 °C; $[\alpha]^{25}_D$ +69.9° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} 253 nm (log ϵ 3.78); IR ν_{max} 3445, 1735, 1702, 1659, 897 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 456 $[M]^+$ (90), 438 $[M - H_2O]^+$ (75), 428 $[M - CO]^+$ (100), 397 (loss of C-23−C-24 by the cleavage of C-22−C-23 bond) (7), 395 (*m/z* 397−2H) (7), 369 (25), 355 [M − side-chain ($C_5H_7O_2$) − 2H]$^+$ (13), 331 (13), 318 (loss of ring A by the cleavage of C-5−C-6 and C-9−C-10 bonds) (85), 312 (98), 300 (loss of ring D plus 2H by the cleavage of C-13−C-17 and C-14−C-15 bonds) (20), 275 (55), 261 (25); HREIMS *m/z* 456.2512 (calcd for $C_{27}H_{36}O_6$ $[M]^+$, 456.2511).

Methyl 20(21)-dehydrolucidenate A (2): colorless needles, mp 123−125 °C; $[\alpha]^{25}_D$ +151.2° (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} 254 nm (log ϵ 3.88); IR ν_{max} 3458, 2928, 1733, 1706, 1660, 899 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 470 $[M]^+$ (91), 455 $[M - Me]^+$ (19), 452 $[M - H_2O]^+$ (11), 442 $[M - CO]^+$ (100), 397 (loss of C-23−C-24 by the cleavage of C-22−C-23 bond) (5), 369 (29), 355 [M − side-chain ($C_5H_7O_2$) − 2H]$^+$ (19), 345 (13), 332 (loss of ring A by the cleavage of C-5−C-6 and C9−C10) (87), 304 (29), 300 (loss of ring D plus 2H by the cleavage of C-13−C-17 and C14−C15 bonds) (87), 285 (20), 275 (35), 261 (19); HREIMS *m/z* 470.2668 (calcd for $C_{28}H_{38}O_6$ $[M]^+$, 470.2671).

20-Hydroxylucidenic acid D_2 (3): colorless needles, mp 123−125 °C; $[\alpha]^{25}_D$ +54.7°(*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} 254 nm (log ϵ 3.97); IR ν_{max} 3452, 1752, 1698 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 512 $[M - H_2O]^+$ (30), 470 $[M - HOAc]^+$ (100), 452 $[M - H_2O - HOAc]^+$ (10), 437 $[M - Me - H_2O - HOAc]^+$ (3), 427 (6), 413 [M − side-chain ($C_5H_9O_3$)]$^+$ (5), 397 (4), 371 (*m/z* 413 − Me − CO + H) (15), 354 (*m/z* 413 − HOAc + H) (18), 302 [M − $C_{11}H_{17}O_5$ (species formed by the cleavage of C-11−C-12, C-13−C-14, and C-16−C17 bonds) + H]$^+$ (60), 169 $[C_{11}H_{17}O_5 - HOAc]^+$ (60); HREIMS *m/z* 512.2410 (calcd for $C_{29}H_{36}O_8$ $[M - H_2O]^+$, 512.2411); FABMS *m/z* 553 $[M + Na]^+$.

20-Hydroxylucidenic acid F (4): colorless needles, mp 162−164 °C; $[\alpha]^{25}_D$ +128.6° (*c* 0.10, acetone); UV (MeOH) λ_{max} 255 nm (log ϵ 3.99); IR ν_{max} 3449, 1772, 1750, 1698, 1680 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; MS *m/z* 454 $[M - H_2O]^+$ (100), 439 $[M - H_2O - Me]^+$ (7), 426 $[M - COOH - H]^+$ (7), 411 (8), 399 (5), 383 (5), 355 [M − side-chain ($C_5H_9O_3$)]$^+$ (7), 327 (*m/z* 355 − CO) (30), 306 (25) [loss of ring A ($C_9H_{14}O$) plus CO by the cleavage of C-5−C-6 and C-9−C-10 bonds], 300 (loss of ring D by the cleavage of C-13−C-17 and C-14−C-15 bonds) (24), 285 (*m/z* 300 − Me) (7). HREIMS *m/z* 454.2355 (calcd for $C_{27}H_{34}O_6$ $[M - H_2O]^+$, 454.2355); FABMS *m/z* 495 $[M + Na]^+$.

20-Hydroxylucidenic acid E_2 (5): colorless needles, mp 147−149 °C; $[\alpha]^{25}_D$ +78.0° (*c* 0.16, acetone); UV (MeOH) λ_{max} 255 nm (log ϵ 3.85); IR ν_{max} 3466, 1753, 1697 cm^{-1}; EIMS *m/z* 514 $[M - H_2O]^+$ (10), 472 $[M - HOAc]^+$ (22), 454 $[M - HOAc - H_2O]^+$ (10), 439 (*m/z* 454 − Me) (3), 415 [M − side-chain ($C_5H_9O_3$)]$^+$ (4), 373 (*m/z* 415 − Me − CO + H) (22), 356 (*m/z* 415 − HOAc + H) (13), 304 [M − $C_{11}H_{17}O_5$ (species formed by the cleavage of C-11−C-12, C-13−C-14, and C-16−C17 bonds) + H]$^+$ (100), 169 $[C_{11}H_{17}O_5 - HOAc]^+$ (38). HREIMS *m/z* 514.2566 (calcd for $C_{29}H_{38}O_8$ $[M - H_2O]^+$, 514.2571); FABMS *m/z* 555 $[M + Na]^+$.

20-Hydroxylucidenic acid N (6): colorless needles, mp 268−270 °C; $[\alpha]^{25}_D$ +150.4° (*c* 0.23, acetone); UV (MeOH) λ_{max} 255 nm (log ϵ 3.85); IR ν_{max} 3434, 1771, 1721, 1661 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; MS *m/z* 458 $[M - H_2O]^+$ (48), 440 $[M - 2H_2O]^+$ (10), 430 $[M - COOH - H]^+$ (100), 407 (4), 371 (9), 357 [M − side-chain ($C_5H_9O_3$) − 2H]$^+$ (4), 331 (*m/z* 357 − CO) (13), 318 [loss of ring A ($C_9H_{14}O$) by the cleavage of C-5−C-6 and C-9−C-10 bonds] (45), 304 (loss of ring D by the cleavage of C-13−C-17 and C-14−C-15 bonds) (10); HREIMS *m/z* 458.2668 (calcd for $C_{27}H_{38}O_6$ $[M - H_2O]^+$, 458.2667); FABMS *m/z* 499 $[M + Na]^+$.

20-Hydroxylucidenic acid P (7): colorless needles, mp 125−127 °C; $[\alpha]^{25}_D$ +77.7° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} 254 nm (log ϵ 3.87); IR ν_{max} 3451, 1758, 1695 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; MS *m/z* 516 $[M - H_2O]^+$ (7), 501 $[M - H_2O - Me]^+$ (2), 488 $[M - COOH - H]^+$ (10), 474 $[M - HOAc]^+$ (4), 456 $[M - HOAc - H_2O]^+$ (4), 441 (*m/z* 454 − Me) (2), 417 [M − side-chain ($C_5H_9O_3$)]$^+$ (1), 375 (*m/z* 417 − Me − CO + H) (10), 356 (*m/z* 415 − HOAc + H) (13), 306 [M − $C_{11}H_{17}O_5$ (species formed by the cleavage of C-11−C-12, C-13−C-14, and C-16−C17 bonds) + H]$^+$ (100), 169 $[C_{11}H_{17}O_5 - HOAc]^+$ (10); HREIMS *m/z* 516.2723 (calcd for $C_{29}H_{40}O_8$ $[M - H_2O]^+$, 516.2722); FABMS *m/z* 557 $[M + Na]^+$.

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