Lucidenic Acids P and Q, Methyl Lucidenate P, and Other Triterpenoids from the Fungus *Ganoderma lucidum* and Their Inhibitory Effects on Epstein−Barr Virus Activation

Kenji Iwatsuki,[†] Toshihiro Akihisa,*[,‡] Harukuni Tokuda,[§] Motohiko Ukiya,[‡] Manabu Oshikubo,[‡] Yumiko Kimura,[⊥] Takeshi Asano,[∥] Atsushi Nomura,[†] and Hoyoku Nishino[§]

K-Laboratories for Intelligent Medical Remote Services, 2266-22 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-0003, Japan, College of Science and Technology, Nihon University, 1-8 Kanda Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan, Department of Biochemistry and Department of Orthopaedics, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-0841, Japan, and College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

Received May 16, 2003

A new triterpene acid, lucidenic acid P (**1a**), and two new triterpene acid methyl esters, methyl lucidenates P (**1b**) and Q (**2b**), were isolated and characterized from the fruiting body of the fungus *Ganoderma lucidum*. Their structures were elucidated on the basis of spectroscopic methods. In addition, eight known triterpene acids, lucidenic acids A (**3a**), C (**4a**), D_2 (**5a**), E_2 (**6a**), and F (**7a**) and ganoderic acids E (**9a**), F (**10a**), and T-Q (**11a**), and six known triterpene acid methyl esters, methyl lucidenates A (**3b**), D_2 (**5b**), E_2 (**6b**), F (**7b**), and L (**8b**) and methyl ganoderate F (**10b**), were isolated and identified from the fungus. All of the triterpenoids, with the exception of **7a**, were evaluated with respect to their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, which is known to be a primary screening test for antitumor promoters. All of the compounds tested showed potent inhibitory effects on EBV-EA induction (96−100% inhibition at 1×10^3 mol ratio/TPA).

The fruiting bodies of *Ganoderma lucidum* KARST (Polyporaceae), commonly known as the Reishi mushroom, are 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 100 oxygenated triterpenoids have been isolated from this mushroom,[2−8] and these compounds displayed cytotoxic[8,9] and anticomplement activities[10] and inhibitory activities on human immunodeficiency virus (HIV)-1 protease,[7] histamine release,[11] angiotensin converting enzyme,[12] cholesterol synthesis,[13,14] and eukaryotic DNA polymerases.[15] In the course of our search for potential antitumor promoters (chemopreventive agents) from natural sources, we have found various types of triterpenoids exhibiting potent inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation, a primary screening test for antitumor promoters.[16−18] In this paper, we report the isolation and characterization of three new oxygenated lanostane-type triterpenoids, lucidenic acid P (**1a**) and methyl lucidenates P (**1b**) and Q (**2b**), along with 14 known triterpenoids from the fruiting body of the fungus *G. lucidum* and their inhibitory effects on EBV-EA activation induced by TPA.

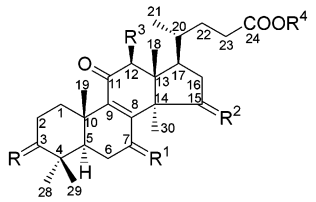
Results and Discussion

The fruiting bodies of *G. lucidum* were extracted with methanol, and the extract was chromatographed on silica gel followed by preparative reversed-phase HPLC, yielding a new triterpene acid, **1a**, and two new triterpene acid methyl esters, **1b** and **2b**, along with eight known triterpene acids, **3a**, **4a**, **5a**, **6a**, **7a**, **9a**, **10a**, and **11a**, and six known triterpene acid methyl esters, **3b**, **5b**, **6b**, **7b**, **8b**, and **10b**.

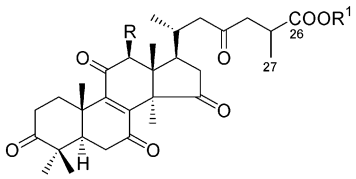
The molecular formula of **1a** was determined as $C_{29}H_{42}O_8$ from its HREIMS ($[M]^+$ *m*/*z* 518.2827) as well as from the ^{13}C NMR. The UV absorbance at 255 nm indicated the presence of an α,β-unsaturated ketone system. Its IR absorption bands suggested the presence of hydroxyl (3446 cm^{-1}), carbonyl (1729 cm^{-1}), and carboxyl (1681 cm^{-1}) groups. The ^1H NMR spectrum showed signals for five tertiary methyl [δ_H 0.85, 0.99, 1.03, 1.27, and 1.49 (each s)], a secondary methyl [δ_H 1.00 (d, $J = 6.4$ Hz)], an *O*-acetyl [δ_H 2.22 (s)], and three oxymethine [δ_H 3.18 (dd, $J = 6.8$, 9.3 Hz), 4.80 (dd, $J = 8.9$, 8.9 Hz), 5.62 (s)] groups (Table 1). The ^{13}C NMR, combined with DEPT and HMQC, showed that **1a** had seven methyls (including an acetyl methyl), six methylenes, six methines (including three oxymethines), four quaternary carbons, two sp^2 carbons, and four carbonyls (including two ketones) (Table 1). The MS of **1a** showed diagnostic fragment ions at *m*/*z* 355 $[C_{22}H_{27}O_4]^+$, corresponding to the loss of a side chain ($C_5H_9O_2$) and acetic acid with one H transfer, and 306 (base peak) $[C_{18}H_{26}O_4]^+$. The latter ion is formed by the loss of a side chain and part of rings C and D via cleavage at the C-11−C-12, C-13−C-14, and C-16−C-17 bonds, which is characteristic for the lanostane-type triterpenoid possessing a 12-hydroxy (or acetoxy)-11,15-dioxo-Δ^8-skeletal structure.[2,4,8] On the basis of these data, in combination with the ^{13}C and ^1H NMR spectral comparison with methyl lucidenate C (methyl 3β,7β,12β-trihydroxy-25,26,27-trinor-11,15-dioxolanost-8-en-24-oate)[2] and lucidenic acid N (3β,7β-dihydroxy-25,26,27-trinor-11,15-dioxolanost-8-en-24-oic acid),[8] the structure of compound **1a** was assigned as 3β,7β-dihydroxy-12β-acetoxy-25,26,27-trinor-11,15-dioxolanost-8-en-24-oic acid (12-*O*-acetyllucidenic acid C), which

* To whom correspondence should be addressed. Tel: +81-3-3259-0806. Fax: +81-3-3293-7572. E-mail: akihisa@chem.cst.nihon-u.ac.jp.
[†] K-Laboratories for Intelligent Medical Remote Services.
[‡] College of Science and Technology, Nihon University.
[§] Department of Biochemistry, Kyoto Prefectural University of Medicine.
[⊥] College of Pharmacy, Nihon University.
[∥] Department of Orthopaedics, Kyoto Prefectural University of Medicine.

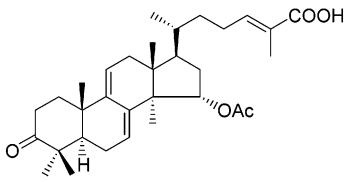
10.1021/np0302293 CCC: $25.00
Published on Web 12/03/2003



	R	R^1	R^2	R^3	R^4
1a	α-H, β-OH	α-H, β-OH	O	OAc	H
1b	α-H, β-OH	α-H, β-OH	O	OAc	Me
2b	O	α-H, β-OH	α-OH, β-H	H	Me
3a	O	α-H, β-OH	O	H	H
3b	O	α-H, β-OH	O	H	Me
4a	α-H, β-OH	α-H, β-OH	O	OH	H
5a	O	O	O	OAc	H
5b	O	O	O	OAc	Me
6a	α-H, β-OH	O	O	OAc	H
6b	α-H, β-OH	O	O	OAc	Me
7a	O	O	O	H	H
7b	O	O	O	H	Me
8b	α-H, β-OH	O	O	OH	Me



	R	R^1
9a	H	H
10a	OAc	H
10b	OAc	Me



11a

we named lucidenic acid P.[19] Analysis of $^1H-^1H$ COSY, HMQC, HMBC, and phase-sensitive NOESY spectra supported the proposed structure of **1a**.

Compound **1b**, which showed $[M]^+$ at *m/z* 532.3036 ($C_{40}H_{44}O_8$) in the HREIMS, had two secondary hydroxyl groups [ν_{max} 3459 cm^{-1}; δ_H 3.20 (1H, dd, J = 5.6, 10.7 Hz) and 4.80 (1H, dd, J = 8.5, 8.7 Hz)], a secondary acetoxyl group [ν_{max} 1680 cm^{-1}; δ_H 2.22 (3H, s) and 5.61 (1H, s)], an α,β-unsaturated ketone (λ_{max} 252 nm; ν_{max} 1733 cm^{-1}), four tertiary methyls [δ_H 0.85, 1.03, 1.27, and 1.49 (each 3H and s)], a secondary methyl [δ_H 0.99 (3H, d, J = 6.3 Hz)], and an *O*-methyl group [δ_H 3.68 (3H, s)]. In addition, compound **1b** exhibited a diagnostic ion at *m/z* 306 (base peak) in the EIMS. These data are in good agreement with those of **1a**, except for the molecular ion in the MS and the methoxyl ^1H signal, and, hence, **1b** was the methyl ester of **1a**, i.e., methyl lucidenate P. This was confirmed by the preparation of methyl ester (**1b**) from **1a** by treatment with diazomethane.

Compound **2b** showed an $[M]^+$ at *m/z* 474.2979 ($C_{28}H_{42}O_6$) in the HREIMS. The compound had two secondary hydroxyl groups [ν_{max} 3445 cm^{-1}; δ_H 4.63 (1H, dd, J = 6.9, 10.8 Hz) and 4.80 (1H, dd, J = 7.1, 9.5 Hz)], two carbonyl moieties [ν_{max} 1736, 1707 cm^{-1}; δ_C 199.6 and 216.9 (each s)] of which one is an α,β-unsaturated system [λ_{max} 252 nm; δ_C 140.3, 159.2 (each s)], a carboxyl group [ν_{max} 1661 cm^{-1}; δ_C 174.3 (s)], a methoxyl group [δ_C 51.6 (q); δ_H 3.67 (3H, s)], five tertiary methyls [δ_H 0.96, 1.10, 1.12, 1.26, 1.28 (each 3H and s)], and a secondary methyl [δ_H 0.88 (3H, d, J = 6.3 Hz)]. In the EIMS, **2b** exhibited a diagnostic fragment ion at *m/z* 336 $[C_{19}H_{26}O_5]^+$ due to the loss of ring A by cleavage of the C-5−C-6 and C-9−C-10 bonds.[3] Comparison of these data with those of ganoderic acid A (7β,15α-dihydroxy-3,11,23-trioxolanost-8-en-26-oic acid),[11] and its methyl ester,[3] and **1a** and **1b** (Table 1) enabled the assignment of the structure of **2b** as methyl 7β,15α-dihydroxy-25,26,27-trinor-3,11-dioxolanost-8-en-24-oate, which we named methyl lucidenate Q. Analysis of $^1H-^1H$ COSY, HMQC, HMBC, and phase-sensitive NOESY spectra supported the proposed structure of **2b**.

Fourteen known compounds were identified by spectral comparison with the corresponding and/or relevant compounds as lucidenic acid A (**3a**),[2] methyl lucidenate A (**3b**),[3] lucidenic acid C (**4a**),[2,4] lucidenic acid D_2 (**5a**),[4] methyl lucidenate D_2 (**5b**),[4] ludicenic acid E_2 (**6a**),[4] methyl lucidenate E_2 (**6b**),[4] lucidenic acid F (**7a**),[3] methyl lucidenate F (**7b**),[3] methyl lucidenate L (**8b**),[5] ganoderic acid E (**9a**),[5,20] methyl ganoderate E (**9b**),[5,20] ganoderic acid F (**10a**),[4] methyl ganoderate F (**10b**),[4] and ganoderic acid T-Q (**11a**).[21]

The inhibitory effects on the induction of EBV-EA induced by TPA were examined as a preliminary evaluation of the potent antitumor-promoting activities for 16 *G. lucidum* triterpenoid constituents, viz., eight carboxylic acids (**1a**, **3a**, **4a**, **5a**, **6a**, **9a**, **10a**, and **11a**) and eight methyl esters (**1b**, **2b**, **3b**, **5b**, **6b**, **7b**, **8b**, and **10b**). The inhibitory effects (Table 2) were compared with those of β-carotene, a vitamin A precursor that has been studied extensively in cancer chemoprevention using animal models.[22] All the compounds tested exhibited potent inhibitory effects (96−100% at 1 × 10^3 mol ratio/TPA) on EBV-EA induction by TPA, which were more inhibitory than β-carotene, with preservation of the high viability (70%) of the Raji cells. With the exception of compound **10b**, all the compounds tested showed 1−7% inhibition even at a lower concentration (1 × 10 mol ratio/TPA). The inhibitory effects against EBV-EA induction have been demonstrated to be closely parallel with those against tumor promotion in vivo,[23] and the oxygenated triterpenoids of *G. lucidum* are, therefore, suggested to be potent cancer chemopreventive agents as antitumor promoters.

Experimental Section

General Experimental Procedures. Crystallizations were performed in acetone−methanol (MeOH), and melting points measured on a Yanagimoto micro melting point apparatus are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter in $CHCl_3$ at 25 °C. UV spectra on a Shimadzu UV-2200 spectrometer and IR spectra on a JASCO IR-300 IR spectrometer were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded with a JEOL LA-400 spectrometer at 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ with tetramethylsilane (TMS; ^1H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Electron-impact mass spectra (EIMS) and high-resolution EIMS (HREIMS) were recorded on a JEOL JMS-GC mate spectrometer (70 eV) using a direct inlet system. 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 (silica gel 60, 220−400 mesh, Merck) was used for column chromatography. Reversed-phase preparative HPLC was carried out on a 25 cm × 10 mm i.d. Pegasil ODS II (Senshu Scientific Co., Ltd., Tokyo, Japan) C_{18} silica column, at 25 °C with MeOH−H_2O−AcOH [80:20:1, v/v/v; HPLC(I)] and MeOH−H_2O−AcOH [60:40:1, v/v/v; HPLC(II)] as mobile phase at 2 mL/min. A refractive index detector was used for reversed-phase HPLC.

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

	1a				**2b**			
C no.	δ_C		δ_H[a]	HMBC (H to C)	δ_C		δ_H[a]	HMBC (H to C)
1	34.4	t	0.92 (α), 2.61 (*β*; dt, 13.7, 3.6)	2, 10, 19	35.6	t	1.46 (α), 2.85 (*β*; ddd, 6.3, 7.1, 13.9)	2, 3, 5, 9, 10, 19
2	27.2	t	1.65 (2H)	3	34.3	t	2.49 (2H, dd, 7.1, 7.7)	1, 3, 4, 10
3	78.0	d	3.18 (dd, 6.8, 9.3)	28, 29	216.9	s		
4	38.5	s			46.8	s		
5	49.1	d	0.88	4, 6, 7, 9, 10, 28, 29	48.8	d	1.68 (dd, 9.5, 13.2)	4, 6, 7, 10, 28, 29
6	26.6	t	2.20 (α), 1.65 (*β*)	4, 5, 7, 8, 10	29.0	t	2.04 (α), 1.66 (*β*)	4, 5, 7, 8, 10
7	66.1	d	4.80 (dd, 8.9, 8.9)	6, 9	68.8	d	4.63 (dd, 6.9, 10.8)	6, 8, 9
8	155.9	s			159.2	s		
9	142.9	s			140.3	s		
10	38.5	s			38.0	s		
11	192.3	s			199.6	s		
12	79.8	d	5.62 (s)	11, 13, 17, 18, *C*OMe	51.8	t	2.75 (α; d, 15.8), 2.52 (*β*; d, 15.8)	9, 11, 13, 14, 18
13	50.4	s			46.6	s		
14	60.6	s			53.9	s		
15	216.7	s			72.6	d	4.80 (dd, 7.1, 9.5)	8, 14, 30
16	37.4	t	2.71 (α; dd, 8.1, 19.0), 2.31 (*β*)	13, 15, 17	36.6	t	1.84 (α), 1.96 (*β*)	14, 15, 17, 20
17	46.0	d	2.40	13, 16, 18	48.5	d	1.78	13, 16, 18
18	13.1	q	0.99 (s)	12, 13, 14, 17	17.3	q	0.96 (s)	12, 13, 14, 17
19	18.6	q	1.27 (s)	1, 4, 5, 9, 10	19.4	q	1.28 (s)	1, 5, 9, 10
20	31.8	d	1.62	21, 22	35.7	d	1.43	21, 22
21	20.4	q	1.00 (d, 6.4)	20, 22	18.1	q	0.88 (d, 6.3)	17, 20, 22
22	29.5	t	1.25, 1.86	20, 23, 24	30.0	t	1.32, 1.81	20, 23, 24
23	30.0	t	2.30, 2.40	22, 24	31.0	t	2.25, 2.38	20, 22, 24, COO*Me*
24	178.2	s			174.3	s		
28	28.0	q	1.03 (s)	3, 4, 5, 10, 29	27.4	q	1.12 (s)	3, 4, 5, 29
29	15.3	q	0.85 (s)	3, 4, 5, 10, 28	20.7	q	1.10 (s)	3, 4, 5, 28
30	24.0	q	1.49 (s)	8, 13, 14, 15	19.4	q	1.26 (s)	8, 13, 14, 15
*C*OMe	170.5	s						
CO*Me*	20.7	q	2.22 (s)	*C*OMe				
COO*Me*					51.6	q	3.67 (s)	23, 24

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

Table 2. Percentage of Epstein−Barr Virus Early Antigen Induction in the Presence of Triterpene Acids from *Ganoderma lucidum* with Respect to a Positive Control (100%)[a]

		concentration (mol ratio/32 pmol TPA)				IC_{50}[b]
	compound	1000	500	100	10	(mol ratio/ 32 pmol TPA)
1	lucidenic acid P	0 (70)	27.5	74.2	96.6	286
1a	methyl lucidenate P	2.3 (70)	28.9	77.4	97.6	293
2a	methyl lucidenate Q	0 (70)	24.6	73.7	95.4	283
3	lucidenic acid A	0 (70)	22.7	73.0	96.1	280
3a	methyl lucidenate A	0 (70)	24.9	75.1	95.0	287
4	lucidenic acid C	0 (70)	24.0	72.6	93.1	284
5	lucidenic acid D_2	2.0 (70)	26.1	73.8	96.1	287
5a	methyl lucidenate D_2	2.2 (70)	29.3	76.9	98.0	290
6	lucidenic acid E_2	0 (70)	23.5	73.1	94.0	280
6a	methyl lucidenate E_2	1.2 (70)	27.9	76.0	95.4	288
7a	methyl lucidenate F	2.1 (70)	26.8	75.4	98.3	285
8a	methyl lucidenate L	0 (70)	21.7	71.9	92.6	275
9	ganoderic acid E	0 (70)	28.3	79.0	97.4	281
10	ganoderic acid F	3.6 (70)	28.5	77.1	98.7	293
10a	methyl ganoderate F	2.3 (70)	26.4	75.3	100	289
11	ganoderic acid T-Q	0 (70)	26.4	74.6	95.3	281
	β-carotene[c]	8.6 (70)	34.2	82.1	100	400

[a] Values represent percentages relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cell. [b] IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA. [c] Reference compound.

Chemicals and Materials. Fruiting bodies of *Ganoderma lucidum* Karst (Polyporaceae), which were cultivated in Gunma prefecture (Japan) in 2001, were obtained from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo, Japan), and their identification was done by description (1). A voucher specimen has been deposited in the College of Science and Technology, Nihon University. TPA was purchased from ChemSyn Laboratories (Lenexa, KS). The cell culture reagent, *n*-butyric acid, and other reagents were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation. Dried and chipped fruiting bodies of *G. lucidum* (373 g) were extracted with MeOH (3 L) for 2 weeks each at room temperature three times. The combined solutions were concentrated in vacuo to give an extract (30.0 g), which was subjected to chromatography on silica gel (1 kg). The column 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] as eluant with monitoring by TLC, and the eluates were arranged into six fractions. 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. Upon HPLC (I), a portion (427 mg) of fraction A yielded **11a** (20.2 mg; retention time (t_R) 39.6 min). A portion (250 mg) of fraction B, on HPLC (II), afforded nine compounds, **1b** (11.0 mg; t_R 41.1 min), **2b** (4.9 mg; t_R 26.4 min), **3b** (14.2 mg; t_R 39.4 min), **5b** (1.6 mg; t_R 35.4 min), **6b** (6.8 mg; t_R 29.9 min), **7b** (5.1 mg; t_R 36.7 min), **8b** (0.8 mg; t_R 21.2 min), **9a** (4.9 mg; t_R 22.8 min), and **10b** (1.4 mg; t_R 30.7 min). HPLC (II) of a portion of fraction C (556 mg) gave seven compounds, **2a** (11.0 mg; t_R 16.3 min), **3a** (4.7 mg; t_R 27.2 min), **4a** (9.0 mg; t_R 15.8 min), **5a** (44.1 mg; t_R 23.3 min), **6a** (27.7 mg; t_R 20.6 min), **7a** (2.7 mg; t_R 24.8 min), and **10a** (8.0 mg; t_R 25.2 min). Some physical characteristics and the spectral data of three new compounds, **1a**, **1b**, and **2b**, are shown below. The ^{1}H NMR data of five known triterpene acids, **5a**, **6a**, **7a**, **9a**, and **10a**, are also described below since these were not previously reported.

Lucidenic Acid P (1a): colorless needles from acetone-MeOH, mp 135−137 °C; $[\alpha]^{25}_D$ +14.7°(*c* 0.38, $CHCl_3$); UV

(MeOH) λ_{max} 255 nm; IR ν_{max} 3446, 1755, 1729, 1681 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; EIMS *m/z* 518 $[M]^+$ (9), 503 (7), 490 (25), 472 (3), 458 (8), 440 (4), 430 (4), 355 (5), 329 (6), 306 (100), 277 (7), 255 (3), 199 (3), 171 (3), 153 (10), 135 (3); HREIMS *m/z* 518.2827 (calcd for $C_{29}H_{42}O_8$, 518.2880). Treatment of **1a** with ethereal CH_2N_2 afforded **1b**.

Methyl lucidenate P (1b): colorless needles from acetone−MeOH, mp 83−85 °C; $[\alpha]^{25}_D$ +77.6° (*c* 0.41, $CHCl_3$); UV (MeOH) λ_{max} 252 nm; IR ν_{max} 3459, 1733, 1680 cm^{-1}; ^1H NMR δ 0.85 (3H, s, H-29), 0.99 (3H, s, H-18), 0.99 (3H, d, J = 6.3 Hz, H-21), 1.03 (3H, s, H-28), 1.27 (3H, s, H-19), 1.49 (3H, s, H-30), 2.22 (3H, s, 12β-OAc), 3.20 (1H, dd, J = 5.6, 10.7 Hz, H-3α), 3.68 (3H, s, COO*Me*), 4.80 (1H, dd, J = 8.5, 8.7 Hz, H-7β), 5.61 (1H, s, H-12α); EIMS *m/z* 532 $[M]^+$ (12), 517 (4), 504 (23), 472 (13), 454 (6), 444 (14), 417 (2), 332 (7), 329 (7), 306 (100), 288 (4), 277 (10), 255 (5), 241 (4), 227 (7); HREIMS *m/z* 532.3036 (calcd for $C_{30}H_{44}O_8$, 532.3036).

Methyl lucidenate Q (2b): colorless needles from acetone−MeOH, mp 130−131 °C; $[\alpha]^{25}_D$ +58.5° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} 252 nm; IR ν_{max} 3445, 1736, 1707, 1661 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; EIMS *m/z* 474 $[M]^+$ (100), 456 (42), 441 (16), 425 (17), 413 (12), 336 (92), 330 (17), 318 (46), 313 (22), 299 (14), 287 (14), 276 (19), 259 (28), 245 (13), 203 (28), 161 (29), 137 (24); HREIMS *m/z* 474.2979 (calcd for $C_{29}H_{42}O_8$, 474.2981).

Lucidenic acid D_2 (5a): ^1H NMR δ 0.86 (3H, s, H-18), 1.02 (3H, d, J = 6.6 Hz, H-21), 1.12 (3H, s, H-29), 1.14 (3H, s, H-28), 1.33 (3H, s, H-19), 1.81 (3H, s, H-30), 2.22 (3H, s, 12β-OAc), 5.68 (1H, s, H-12α); EIMS *m/z* 514 $[M]^+$ ($C_{29}H_{38}O_8$).

Lucidenic acid E_2 (6a): ^1H NMR δ 0.82 (3H, s, H-18), 0.88 (3H, s, H-29), 0.98 (3H, d, J = 6.6 Hz, H-21), 1.02 (3H, s, H-28), 1.33 (3H, s, H-19), 1.73 (3H, s, H-30), 2.21 (3H, s, 12β-OAc), 3.23 (1H, dd, J = 4.8, 10.8 Hz, H-3α), 5.62 (1H, s, H-12α); EIMS *m/z* 516 $[M]^+$ ($C_{29}H_{40}O_8$).

Lucidenic acid F (7a): ^1H NMR δ 0.86 (3H, s, H-18), 0.96 (3H, d, J = 6.6 Hz, H-21), 1.12 (3H, s, H-29), 1.14 (3H, s, H-28), 1.28 (3H, s, H-19), 1.65 (3H, s, H-30); EIMS *m/z* 456 $[M]^+$ ($C_{27}H_{36}O_6$).

Ganoderic acid E (9a): ^1H NMR δ 0.88 (3H, s, H-18), 0.98 (3H, d, J = 6.4 Hz, H-21), 1.12 (3H, s, H-29), 1.14 (3H, s, H-28), 1.23 (3H, d, J = 7.0 Hz, H-27), 1.28 (3H, s, H-19), 1.64 (3H, s, H-30); EIMS *m/z* 512 $[M]^+$ ($C_{30}H_{40}O_7$).

Ganoderic acid F (10a): ^1H NMR δ 0.85 (3H, s, H-18), 0.99 (3H, d, J = 6.4 Hz, H-21), 1.12 (3H, s, H-29), 1.14 (3H, s, H-28), 1.22 (3H, d, J = 7.2 Hz, H-27), 1.34 (3H, s, H-19), 1.80 (3H, s, H-30), 2.25 (3H, s, 12β-OAc), 5.68 (1H, s, H-12α); EIMS *m/z* 570 $[M]^+$ ($C_{32}H_{42}O_9$).

In Vitro EBV-EA Activation Experiment. The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells; nonproducer type), cultivated in 10% fetal bovine serum (FBS) RPMI-1640 medium (Sigma, St. Louis, MO). The indicator cells (Raji cells; 1×10^6 cells/mL) were incubated in 1 mL of the medium containing 4 mM *n*-butyric acid as an inducer, 32 pM of TPA [20 ng/mL in dimethyl sulfoxide (DMSO)], and a known amount (32, 16, 3.2, 0.32 nmol) of the test compound at 37 °C in a CO_2 incubator. After 48 h, the cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was removed. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted and the experiments were repeated three times. The average extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with *n*-butyric acid plus TPA, where the extent of EA induction was ordinarily more than 40%. The viability of treated Raji cells was assayed by the Trypan Blue staining method.[24] In this experiment, all candidate and cell activated reagents were dissolved in a small volume of DMSO and added into the basic screening culture solution. The small volume of basic solvent did not induce specific potency in these cells at all.

Acknowledgment. This study was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan, and by a grant from the National Cancer Institute (CA 177625).

References and Notes

(1) Namba, T. *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures*, revised ed.; Hoikusya: Osaka, 1994; Vol. II, pp 244−250.
(2) Nishitoba, T.; Sato, J.; Kasai, T.; Kawagishi, H.; Sakamura, S. *Agric. Biol. Chem.* **1985**, *49*, 1793−1798.
(3) Kikuchi, T.; Kanomi, S.; Kadota, S.; Murai, Y.; Tsubono, K.; Ogita, Z. *Chem. Pharm. Bull.* **1986**, *34*, 3695−3712.
(4) Kikuchi, T.; Kanomi, S.; Murai, Y.; Kadota, S.; Tsubono, K.; Ogita, Z. *Chem. Pharm. Bull.* **1986**, *34*, 4018−4029.
(5) Nishitoba, T.; Sato, H.; Sakamura, S. *Phytochemistry* **1987**, *26*, 1777−1784.
(6) Shiao, M.-S.; Lin, L.-J.; Yeh, S.-F. *Phytochemistry* **1988**, *27*, 2911−2914.
(7) Min, B.-S.; Nakamura, N.; Miyashiro, H.; Bae, K.-W.; Hattori, M. *Chem. Pharm. Bull.* **1998**, *46*, 1607−1612, and references therein.
(8) Wu, T. S.; Shi, L. S.; Kuo, S. C. *J. Nat. Prod.* **2001**, *64*, 1121−1122.
(9) Lin, C. N.; Tome, W. P.; Won, S. J. *J. Nat. Prod.* **1991**, *54*, 998−1002.
(10) Min, B. S.; Gao, J. J.; Hattori, M.; Lee, H. K.; Kim, Y. H. *Planta Med.* **2001**, *67*, 811−814.
(11) Kohda, H.; Tokumoto, W.; Sakamoto, K.; Fujii, M.; Hirai, Y.; Yamasaki, K.; Komoda, Y.; Nakamura, H.; Ishihara, S.; Uchida, M. *Chem. Pharm. Bull.* **1985**, *33*, 1367−1374.
(12) Morigiwa, A.; Kitabatake, K.; Fujimoto, Y.; Ikekawa, N. *Chem. Pharm. Bull.* **1986**, *34*, 3025−3028.
(13) Sonoda, Y.; Sekigawa, Y.; Sato, Y. *Chem. Pharm. Bull.* **1988**, *36*, 966−973.
(14) Komoda, Y.; Shimizu, M.; Sonoda, Y.; Sato, Y. *Chem. Pharm. Bull.* **1989**, *37*, 531−533.
(15) Mizushina, Y.; Takahashi, N.; Hanashima, L.; Koshino, H.; Esumi, Y.; Uzawa, J.; Sugawara, F.; Sakaguchi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2047−2052.
(16) Akihisa, T.; Tokuda, H.; Ichiishi, E.; Mukainaka, T.; Toriumi, M.; Ukiya, M.; Yasukawa, K.; Nishino, H. *Cancer Lett.* **2001**, *173*, 9−14.
(17) Ukiya, M.; Akihisa, T.; Tokuda, H.; Suzuki, H.; Mukainaka, T.; Ichiishi, E.; Yasukawa, K.; Kasahara, Y.; Nishino, H. *Cancer Lett.* **2002**, *177*, 7−12.
(18) Ukiya, M.; Akihisa, T.; Tokuda, M.; Hirano, M.; Oshikubo, M.; Nobukuni, Y.; Kimura, Y.; Tai, T.; Kondo, S.; Nishino, H. *J. Nat. Prod.* **2002**, *65*, 462−465.
(19) Three oxygenated 25,26,27-trinorlanostane-type triterpenoids recently isolated from *G. lucidum* have been named lucidenic acids N,[8] O,[15] and SP1.[10]
(20) Hirotani, M.; Furuya, T. *Phytochemistry* **1986**, *25*, 1189−1193.
(21) Lin, L.-J.; Shiao, M.-S.; Yeh, S.-F. *Phytochemistry* **1988**, *27*, 2269−2271.
(22) Murakami, A.; Ohigashi, H.; Koshimizu, K. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1−8.
(23) Akihisa, T.; Yasukawa, K. In *Studies in Natural Products Chemistry, Vol. 25, Bioactive Natural Products (Part F)*; Atta-ur-Rahman, Ed.; Elsevier Science B.V.: Amsterdam, 2001; pp 43−87.
(24) Takaishi, Y.; Ujita, K.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. *Cancer Lett.* **1992**, *65*, 19−26.

NP0302293