Lanostanoids Isolated from *Ganoderma lucidum* Mycelium Cultured by Submerged Fermentation

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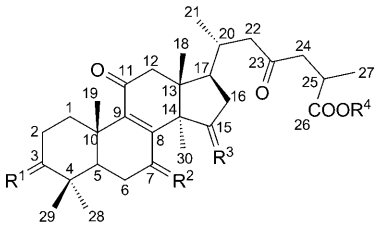
Three new lanostane triterpene acids, 3-*O*-acetylganoderic acid B (**1**), 8*β*,9*α*-dihydroganoderic acid C (**3**), and 3-*O*-acetylganoderic acid K (**4**), as well as two new lanostane triterpene acid ethyl esters, ethyl 3-*O*-acetylganoderate B (**2**) and ethyl ganoderate J (**5**), were isolated and characterized from *Ganoderma lucidum* mycelia which was cultured by submerged fermentation method. Their structures were elucidated on the basis of spectroscopic methods. In addition, the identification of two known lanostane triterpene acid methyl esters, methyl *O*-acetyl ganoderate C and methyl 3,7,11,15,23-pentaoxo-lanost-8-en-26-oate were identified by comparison of the NMR data with those reported in the literature.

Introduction. – *Ganoderma lucidum* (Ling Zhi), a well-known Chinese drug, was clinically used in Asian countries for thousands of years. Lanostane triterpenoids, known to represent the main bioactive compounds in *G. lucidum*, were claimed to possess antimicrobial [1][2], anti-HIV [3], antitumor [4][5], and anti-oxidation [6] activities. Submerged fermentation is a promising method to produce active lanostane triterpenoids from the cultured mycelium of *G. lucidum* on a large scale. The production of lanostane triterpenoids was increased to 754.6 mg/l through a pH-change, DOT-shift integrated fed-batch fermentation process, which was reported by our group [7]. To elucidate the structure of lanostane triterpenoids in detail, the cultured mycelium was phytochemically investigated. We examined the AcOEt extract of the dried, crushed powder of mycelium. Solvent partitioning and repeated column chromatography resulted in the isolation of seven highly oxidized lanostane triterpenoids. These were 3-*O*-acetylganoderic acid B (**1**), ethyl 3-*O*-acetylganoderate B (**2**), 8*β*,9*α*-dihydroganoderic acid C (**3**), 3-*O*-acetylganoderic acid K (**4**), ethyl ganoderate J (**5**), and two known triterpenes, namely methyl *O*-acetylganoderate C [8] and methyl 3,7,11,15,23-pentaoxolanost-8-en-26-oate [8]. The structures of the known compounds were identified by comparison of the NMR data with those reported in the literature. The elucidation of the structure of the new compounds **1**–**5** (*Fig. 1*) is presented in this article.

Results and Discussion. – *Isolation and Characterization of the Compounds.* An AcOEt extract (24 g) of the cultured *Ganoderma lucidum* mycelium was chromatographed on silica gel and separated into fractions *A*–*F* (see *Exper. Part*). *Fr. B*, *C*, and *D* were rechromatographed on *Sephadex LH-20* gel and then reversed-phase HPLC



	R^1	R^2	R^3	R^4
1	α-H, β-OAc	α-H, β-OH	O	H
2	α-H, β-OAc	α-H, β-OH	O	Et
4	α-H, β-OAc	O	α-OH, β-H	H
5	O	O	α-OH, β-H	Et

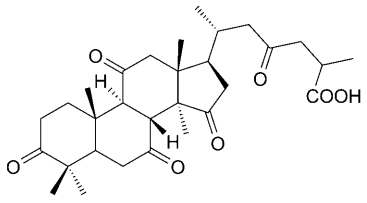


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Fig. 1. *Structures of compounds isolated from* Ganoderma lucidum *mycelium cultured by submerged fermentation*

separately, to afford the new compounds **1** (5 mg), **2** (10 mg), **3** (3 mg), **4** (5 mg), and **5** (3 mg), together with two known compounds.

The molecular formula of **1** was determined as $C_{32}H_{46}O_8$ by the HR-ESI-MS (*m*/*z* 557.3189 ($[M-H]^-$, calc. for $C_{32}H_{45}O_8^-$, 557.3114)). The IR spectrum showed absorption bands corresponding to OH groups (3520 cm^{-1}) and α,β-unsaturated CO groups (1660 cm^{-1}). The ^{1}H-NMR spectrum (*Table 1*) of compound **1** showed the presence of six tertiary Me groups with signals at δ(H) 0.92 (*s*), 1.23 (*s*), 0.92 (*s*), 0.89 (*s*), 1.33 (*s*), and 2.03 (*s*); two secondary Me groups at δ(H) 0.98 (*d*) and 1.17 (*d*). The ^{13}C-NMR spectrum and DEPT measurement revealed that the molecule contained 32 C-atoms, including eight Me, seven CH_2, and six CH groups, as well as five CO groups, two quaternary olefinic, and four quaternary C-atoms (*Table 2*). Among the five CO group signals, one was a COOH group at δ(C) 179.9, one was an ester CO group at δ(C) 171.1, and the other three were ketone CO groups at δ(C) 197.9, 217.6, 207.8. The presence of two O-bearing CH groups was supported by the signals at δ(H) 4.78 (*dd*, J = 8.9, 8, 1 H), 4.46 (*dd*, J = 11.1, 5.3, 1 H) and δ(C) 66.9, 80.2. The presence of an AcO group was confirmed by the C-atom signals at δ(C) 171.1 and 21.5, coupling with the down-field shifted Me group at δ(H) 2.03 (*s*). The NMR data of compound **1** are similar to those of ganoderic acid B [9]. However, the presence of an AcO group, the down-field shifts of C(3) and Me(29) to 80.2 and 16.7, and the up-field shift of C(2) and C(4) to 24.2 and 37.8, suggested that C(3) of compound **1** was substituted by AcO instead of OH. The HMBC spectrum, which showed long-range C-atom to H-atom connectivities from the CO of the AcO group to H–C(3) (*Fig. 2*), further confirmed this assumption. The 3β-acetoxy configuration was supported by the characteristic chemical shift of the H_α–C(3) at 4.46 (*dd*, J = 11.1, 5.3), which was also observed for methyl *O*-acetylganoderate C [8]. Based on the above conclusions, compound **1** was identified as 3-*O*-acetylganoderic acid B (3β-acetoxy-7β-hydroxy-11,15,23-trioxo-lanost-8-en-26-oic acid). Other assignments for H- and C-atoms were defined on the basis of the correlations observed in the HSQC and HMBC spectra, which are shown in *Fig. 2*.

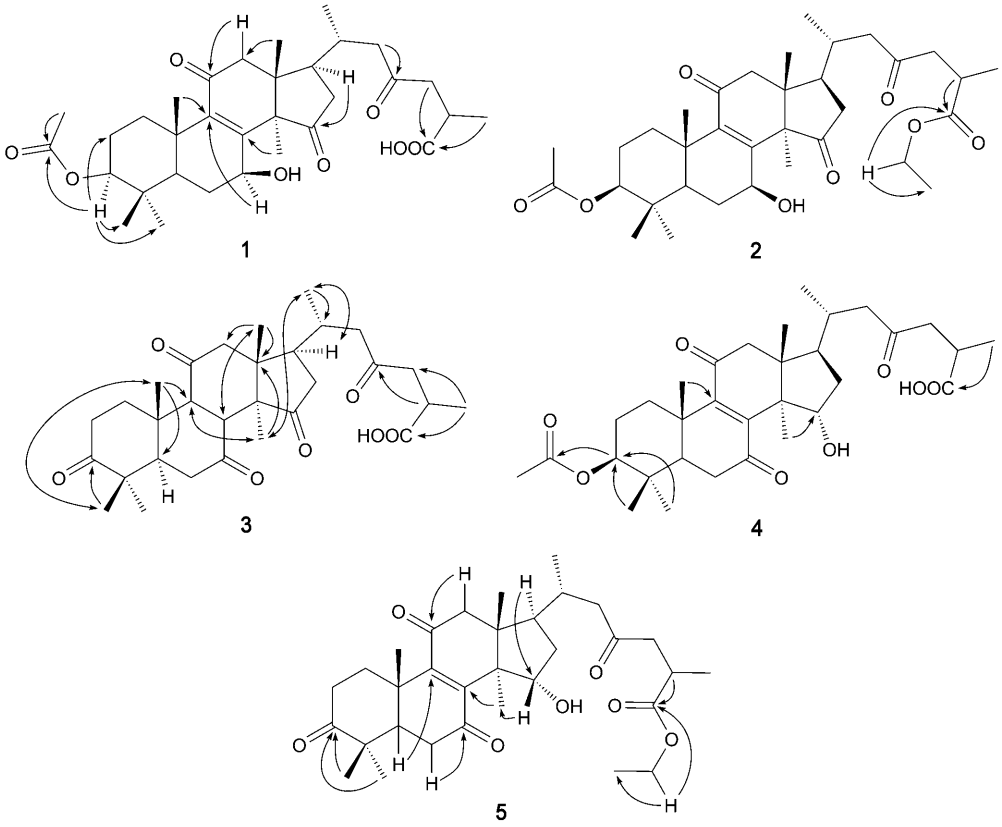

Fig. 2. *Long-range and NOE correlations observed in the HMBC and NOESY spectra of* **1**–**5** (HMBC →, NOE ↔)

HR-ESI-MS and ^{13}C-NMR data provided the molecular formula $C_{34}H_{50}O_8$ (*m*/*z* 585.3432 ([*M* – H]$^-$; calc. 585.3427)) for compound **2**. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were very similar to those of **1**. Analysis of the 2D-NMR data including HSQC and HMBC spectra showed compound **2** to be the ethyl ester of compound **1**. This was evident from the presence of a CH_2 group with signals at δ(H) 4.12 and δ(C) 60.9, a Me group with signals at δ(H) 1.23 and δ(C) 14.4, and a CO group with a signal at δ(C) 175.8 (C(26)). The CH_2 group was found to have a cross-peak with C(26) and a Me group in the HMBC spectrum, establishing compound **2** to be ethyl 3-*O*-acetylganoderate B. Compound **2** might be an artefact of isolation, arising through *trans*-esterification with AcOEt used for the extraction.

Compound **3** had the molecular formula $C_{30}H_{42}O_7$ (*m*/*z* 513.2865 ([*M* – H]$^-$; calc. 513.2842)) as determined by HR-ESI-MS and ^{13}C-NMR. The ^{1}H-NMR spectrum (*Table 1*) showed the presence of five quaternary Me groups at δ(H) 0.75 (*s*), 1.48 (*s*), 1.02 (*s*), 1.06 (*s*), and 1.62 (*s*), and two secondary Me groups at δ(H) 1.15 (*d*) and 0.92 (*d*). The ^{13}C-NMR spectrum (*Table 2*) and DEPT experiment revealed the presence of

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **1**–**5**. δ in ppm[a]).

	1	**2**	**3**	**4**	**5**
H_β–C(1)	2.89 (*dt*, *J* = 13.6, 3.5)	2.87 (*dt*, *J* = 13.6, 3.5)	2.98 (*ddd*, *J* = 13.5, 13.5, 5.4)	2.89 (*dt*, *J* = 13.5, 3.5)	2.97 (*ddd*, *J* = 14.2, 9.4, 6.6)
H_α–C(1)	1.21–1.25 (*m*)	1.21–1.26 (*m*)	1.51–1.56 (*m*)	1.26–1.30 (*m*)	1.78–1.83 (*m*)
CH_2(2)	1.68–1.71 (*m*)	1.68–1.71 (*m*)	2.33–2.36 (*m*)	1.68–1.77 (*m*)	2.51–2.56 (*m*)
H_α–C(3)	4.46 (*dd*, *J* = 11.1, 5.3)	4.48 (*dd*, *J* = 11.0, 5.3)		4.57 (*dd*, *J* = 11.1, 5.0)	
H_α–C(5)	1.03 (*dd*, *J* = 12.8, 1.3)	1.02 (*dd*, *J* = 13.3, 2.0)	1.82–1.84 (*m*)	1.54 (*dd*, *J* = 12.5, 4.5)	2.26 (*dd*, *J* = 14.9, 2.7)
H_α–C(6)	2.17–2.19 (*m*)	2.17–2.19 (*m*)		2.52–2.54 (*m*)	2.46–2.49 (*m*)
H_β–C(6)	1.59–1.62 (*m*)	1.59–1.62 (*m*)	2.72 (*dd*, *J* = 15.7, 14.6)	2.62–2.63 (*m*)	2.61–2.63 (*m*)
H_α–C(7)	4.78 (*dd*, *J* = 8.9, 8.0)	4.79 (*dd*, *J* = 8.9, 8.0)			
H–C(8)			3.12 (*d*, *J* = 13.2)		
H–C(9)			2.51 (*d*, *J* = 13.2)		
H_α–C(12)	2.78 (*d*, *J* = 17.2)	2.77 (*d*, *J* = 17.2)	2.92–2.96 (*m*)	2.76–2.82 (*m*)	2.82 (*d*, *J* = 17.7)
H_β–C(12)	2.68 (*d*, *J* = 17.2)	2.68 (*d*, *J* = 17.2)	2.41–2.43 (*m*)		2.57 (*d*, *J* = 17.7)
H–C(15)				4.38 (*dd*, *J* = 7.5, 1.8)	4.31 (*td*, *J* = 7.2)
H_β–C(16)	2.03 (*dd*, *J* = 14.7, 9.0)	2.03 (*dd*, *J* = 14.7, 9.0)	1.89–1.91 (*m*)	1.83–1.86 (*m*)	1.86–1.87 (*m*)
H_α–C(16)	2.67 (*dd*, *J* = 14.7, 6.4)	2.67 (*dd*, *J* = 14.7, 6.4)			
H–C(17)	2.11–2.13 (*m*)	2.11–2.13 (*m*)	1.90–1.92 (*m*)	1.83–1.86 (*m*)	1.81–1.84 (*m*)
Me(18)	0.92 (*s*)	0.92 (*s*)	0.75 (*s*)	0.89 (*s*)	0.91 (*s*)
Me(19)	1.23 (*s*)	1.23 (*s*)	1.48 (*s*)	1.27 (*s*)	1.26 (*s*)
H–C(20)	2.17–2.18 (*m*)	2.17–2.18 (*m*)	1.95–1.98 (*m*)	2.00–2.02 (*m*)	2.03–2.06 (*m*)
Me(21)	0.98 (*d*, *J* = 6.3)	0.98 (*d*, *J* = 6.3)	0.92 (*d*, *J* = 6.0)	0.87 (*d*, *J* = 6.3)	0.89 (*d*, *J* = 6.3)
CH_2(22)	2.36 (*d*, *J* = 2.7)	2.36 (*d*, *J* = 2.7)	2.31–2.35 (*m*)	2.34–2.37 (*m*)	2.41 (*d*, *J* = 2.7)
CH_2(24)	2.44 (*dd*, *J* = 10.8, 2.1), 2.82–2.84 (*m*)	2.44 (*dd*, *J* = 10.8, 2.1), 2.82–2.84 (*m*)	2.46–2.51 (*m*), 2.81–2.83 (*m*)	2.42 (*dd*, *J* = 17.5, 4.9), 2.83 (*dd*, *J* = 17.5, 8.5)	2.39–2.44 (*m*), 2.82–2.84 (*m*)
H–C(25)	2.96–2.97 (*m*)	2.92–2.93 (*m*)	2.88–2.90 (*m*)	2.92–2.96 (*m*)	2.96–2.97 (*m*)
Me(27)	1.17 (*d*, *J* = 7.1)	1.17 (*d*, *J* = 7.1)	1.15 (*d*, *J* = 7.1)	1.18 (*d*, *J* = 7.1)	1.17 (*d*, *J* = 7.1)
Me(28)	0.92 (*s*)	0.93 (*s*)	1.02 (*s*)	0.90 (*s*)	1.14 (*s*)
Me(29)	0.89 (*s*)	0.89 (*s*)	1.06 (*s*)	1.13 (*s*)	0.93 (*s*)
Me(30)	1.33 (*s*)	1.33 (*s*)	1.62 (*s*)	1.01 (*s*)	1.20 (*s*)
MeCH_2O		4.12 (*q*, *J* = 7.5)			4.15 (*q*, *J* = 7.2)
MeCH_2O		1.23 (*t*, *J* = 6.6)			1.28 (*t*, *J* = 7.2)
AcO–C(3)	2.03 (*s*)	2.03 (*s*)		2.11 (*s*)	

[a]) Values in parentheses are coupling constants in Hz.

Table 2. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *of Compounds* **1**–**5**. δ in ppm.

	1	**2**	**3**	**4**	**5**
CH_2(1)	34.7	34.7	35.9	34.8	34.7
CH_2(2)	24.2	24.2	33.8	24.1	24.2
H–C(3) or C(3)	80.2	80.2	215.3	79.3	80.2
C(4)	37.8	37.7	47.7	37.9	37.8
H–C(5)	49.2	49.5	54.4	49.9	49.2
CH_2(6)	26.7	26.7	40.3	37.0	26.7
H–C(7) or C(7)	66.9	66.9	204.8	202.8	66.9
C(8)	157.0	157.0	46.2	154.7	157.0
C(9)	142.8	142.8	59.1	149.9	142.8
C(10)	38.9	38.9	37.5	40.1	38.9
C(11)	197.9	197.9	207.7	199.5	197.5
CH_2(12)	50.4	50.4	50.6	52.3	50.4
C(13)	45.6	45.6	46.2	48.1	45.6
C(14)	59.6	59.6	54.7	53.0	59.6
C(15) or H–C(15)	217.6	217.6	210.4	72.3	217.6
CH_2(16)	41.1	41.1	40.2	36.5	41.1
H–C(17)	45.7	45.8	44.5	48.3	45.7
Me(18)	17.6	17.6	15.8	17.5	17.6
Me(19)	18.7	18.7	13.0	18.0	18.7
H–C(20)	32.2	32.2	32.1	32.6	32.2
Me(21)	19.9	19.9	19.4	19.6	19.9
CH_2(22)	49.5	49.5	49.1	49.5	49.5
C(23)	207.8	207.8	207.7	208.5	207.8
CH_2(24)	46.8	46.9	47.1	46.8	46.8
H–C(25)	34.6	35.0	34.5	34.8	34.6
C(26)	179.9	175.8	176.2	179.3	179.9
Me(27)	17.1	17.4	16.7	17.1	17.1
Me(28)	28.3	28.3	25.4	17.3	28.3
Me(29)	16.7	16.7	20.7	20.3	16.7
Me(30)	24.5	24.6	12.7	28.3	24.5
$COOCH_2$Me		60.9			60.8
$COOCH_2$*Me*		14.4			14.4
CO of AcO–C(3)	171.1	171.1		170.9	
Me of AcO–C(3)	21.5	21.5		21.4	

seven Me, seven CH_2, six CH, and five ketone CO groups, as well as one CO group and four quaternary C-atoms. These NMR characteristics are very similar to those of methyl ganoderate C [8]. However, no olefinic H- or C-atom was found in the NMR spectra, and no chromophore was found in the UV spectrum. This was substantiated by the appearance of a pair of *doublet*s at δ(H) 3.12 (d, J = 13.2, H–C(8)) and δ(H) 2.51 (d, J = 13.2, H–C(9)) in ^{1}H-NMR spectrum, and two CH groups with signals at δ(C) 46.2 (C(8)) and 59.1 (C(9)) in the ^{13}C-NMR spectrum. Further more, the distinctive MeO group present in methyl ganoderate C was not found in compound **3**, which indicated C(26) to be a COOH group instead of an ester. The HMBC spectrum showed long-range C- to H-atom connectivities from C(3) to Me(28) and Me(29); from C(7) to CH_2(6) and H–C(9); from C(11) to H–C(12), H–C(8), H–C(9); from C(15) to Me(30), H–C(8) and H–C(17); from C(23) to H–C(20) and H–C(25); and from

C(26) to Me(27), establishing the position of the C-atoms containing functional groups, including one COOH (C(26)), five ketone CO (C(3), C(7), C(11), C(15), and C(23)). The relative configuration of H–C(8) and H–C(9) was defined by NOESY NMR correlations. Cross peaks were observed from H–C(8) to Me(18) and Me(19); and from H–C(9) to Me(30). Thus, H–C(8) was assigned β-configuration and H–C(9) α-configuration, which is identical to 8β,9α-dihydroganoderic acid J [10]. On the basis of the above evidence, compound **3** was established as 8β,9α-dihydroganoderic acid C (3,7,11,15,23-pentaoxolanostan-26-oic acid).

Compound **4** had the molecular formula $C_{32}H_{46}O_8$ (*m/z* 559.3208 ($[M+H]^+$; calc. 559.3272)), as determined by HR-ESI-MS. The NMR spectra suggested **4** to be a further ganoderic acid derivative, and similar to the known compound methyl ganoderate K. However, the distinctive MeO group of methyl ganoderate K was not found in compound **4**, and the chemical shift of C(26) shifted down-field to 179.3, which demonstrated C(26) to be a COOH group instead of an ester group. Furthermore, 32 C-atom signals were observed in compound **4** according to the ^{13}C-NMR data, besides the similar chemical shift as methyl ganoderate K [11], the additional two C-atom signals at δ(C) 170.9 and 21.4 indicated the presence of an AcO group, and the Me *singlet* at δ(H) 2.11 (*s*) supported this conclusion. The HMBC spectrum which showed long-range C- to H-atom connectivities from the CO group of AcO to H–C(3), further confirmed this assumption. Additionally, the 3β-configuration for the AcO group was indicated by the characteristic chemical shift of the H_α–C(3) at 4.57 (*dd*, $J = 11.1, 5.0$) which was also observed in compound **1**. Thus, compound **4** was established as 3-*O*-acetylganoderic acid K (3β-acetyloxy-15α-hydroxy-7,11,23-trioxolanost-8-en-26-oic acid).

Compound **5** had the molecular formula $C_{32}H_{46}O_7$ (found *m/z* 541.3070, calc. 541.3165 for $[M-H]^-$), as determined by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were closely related to those of methyl ganoderate J [11]. The only differences were the presence of an additional CH_2 signal at δ(H) 4.15, δ(C) 60.8 and a Me group with signals at δ(H) 1.28, δ(C) 14.4, which indicated the appearance of an ethyl ester in the side chain of compound **2**. Analysis of the HMBC spectra showed the connectivities from a CH_2 group (δ(H) 4.15, *q*, $J = 7.2$) to a Me group (δ(C) 14.4) and C(26) (δ(C) 176.0) separately, establishing compound **5** to be ethyl ganoderate J. As in the case of compound **2**, compound **5** could possibly be an artifact of isolation.

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Experimental Part

General. All solvents of analytical grade were purchased from *BoDi Chemical Factory* (Tianjin, P. R. China). Column chromatography (CC): *Sephadex LH-20* gel (*Amersham Pharmacia Biotech China Ltd.*, Shanghai). Flash column chromatography (FC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). TLC: silica gel *G* (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Precoated Silica gel *G* plates used for TLC: *Merck Inc.* (Darmstadt, Germany). TLC spots were viewed at 254 nm after spraying with H_2SO_4/MeOH (10:1, *v*/*v*) and heating at 110°. Preparative HPLC: *Waters 600* instrument coupled with a *2487* multiple wave detector, *ODS* column (10 × 25 mm, 5 μm; *Agela Technologies Inc.*; Beijing, P. R. China), system of H_2O, MeCN, and MeOH at a flow rate of 4 ml/min. Optical rotations: *Perkin-Elmer 314* polarimeter. IR Spectra: *Nicolet NEXUS 670* FT-IR spectrometer. NMR Spectra: *Varian Mercury-400BB* NMR instrument; chemical shifts (δ) are given in ppm, with TMS as the internal standard; coupling constants (J) are given in Hz. EI-MS: *Shimadzu GC/MS-QP 2010 plus* mass spectrometer. HR-ESI-MS: *Bruker Bio TOF-Q plus* mass spectrometer.

Mycelium Material. The strain of *G. lucidum* (CGMCC 5.616) provided by Chinese General Microbiological Fermentation Collection Center (Beijing, P. R. China) was maintained on potato-dextrose agar slants. A novel pH-shift, DOT-shift integrated fed-batch fermentation process was applied to obtain mycelium [7].

Extraction and Isolation. G. lucidum mycelium was harvested from a liquid culture after 18 d. After filtration through four layers of cheesecloth and washing with H_2O, the biomass (*ca.* 3 kg) was powdered and extracted with MeOH (3 ×). After evaporation of the collected percolate, the concentrated extracts were partitioned between petroleum ether (PE) and H_2O, and the aq. layer was re-extracted with AcOEt. The pooled AcOEt fraction was subjected to CC on a SiO_2 column (60 × 4 cm) and eluted with a gradient of $CHCl_3$/acetone (50:1, 30:1, 20:1, 10:1, and finally EtOH). On the basis of the differences in composition indicated by TLC, six crude fractions (*A* – *F*) were collected. *Fr. B*, *C*, and *D* were subjected to a *Sephadex LH-20* gel column eluted with MeOH resp., to afford a series of sub-fractions (*B-1* to *B-3*; *C-1* to *C-5*; *D-1* to *D-3*). Subfractions *B-3*, *C-2*, *D-3* were further purified by prep. HPLC separately. Compounds **1** (10.3 mg) and **5** (2 mg) were well separated by eluting with MeCN/H_2O/MeOH (56:36:8, adjusted pH to 3.0 with formic acid); compound **2** (2.1 mg) was obtained by eluting with MeCN/H_2O/MeOH 56:36:8 (adjusted pH to 3.0 with HCOOH); and compounds **3** (5.0 mg), **4** (3.0 mg) was isolated with MeCN/H_2O/MeOH 40:56:4 (adjusted pH to 3.0 with HCOOH) as the eluent.

3-O-Acetylganoderic Acid B (*= (3β,7β)-3-(Acetyloxy)-7-hydroxy-11,15,23-trioxolanost-8-en-26-oic Acid*; **1**). White amorphous powder. $[\alpha]_D^{25} = +142.3$ ($c = 0.2$; MeOH). UV (MeOH): 254 (3.8). IR (KBr): 3520, 2950, 2920, 2860, 1735, 1705, 1660. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 558 (12, M^+), 541 (25), 481 (100), 463 (23), 445 (8), 387 (10), 351 (21), 307 (3), 239 (7). HR-ESI-MS: 557.3189 ($[M-H]^-$, $C_{32}H_{45}O_8^-$; calc. 557.3114).

Ethyl 3-O-Acetylganoderate B (*= Ethyl (3β,7β)-3-(Acetyloxy)-7-hydroxy-11,15,23-trioxolanost-8-en-26-oate*; **2**). Pale yellow amorphous solid. $[\alpha]_D^{25} = +162.1$ ($c = 0.2$; MeOH). UV (MeOH): 258 (3.8). IR (KBr): 3512, 2950, 2920, 2860, 1735, 1701, 1663. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 586 (8, M^+), 569 (12), 509 (21), 481 (100), 415 (6), 379 (23), 333 (52), 239 (2). HR-ESI-MS: 585.3432 ($[M-H]^-$, $C_{34}H_{49}O_8^-$; calc. 585.3427).

8β,9α-Dihydroganoderic Acid C (= 3,7,11,15,23-Pentaoxolanostan-26-oic Acid; **3**). White amorphous solid. $[\alpha]_D^{25} = +61.9$ ($c = 0.2$; MeOH). IR (KBr): 3481, 2950, 2921, 2851, 1735, 1701. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 515 (25, $[M+1]^+$), 495 (12), 483 (100), 451 (24), 339 (21), 325 (10). HR-ESI-MS: 513.2865 ($[M-H]^-$, $C_{30}H_{41}O_7^-$; calc. 513.2842).

3-O-Acetylganoderic Acid K (*= (3β,15α)-3-(Acetyloxy)-15-hydroxy-7,11,23-trioxolanost-8-en-26-oic Acid*; **4**). Pale yellow amorphous solid. $[\alpha]_D^{25} = +182.1$ ($c = 0.2$; MeOH). UV (MeOH): 278 (3.9). IR (KBr): 3452, 2948, 2920, 2860, 1725, 1700, 1680. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 559 (30, $[M+1]^+$), 541 (12), 523 (20), 505 (25), 481 (100), 463 (28), 445 (10), 351 (6), 333 (12). HR-ESI-MS: 559.3208 ($[M+H]^+$, $C_{32}H_{47}O_8^+$; calc. 559.3272).

Ethyl Ganoderate J (*= Ethyl (15α)-15-Hydroxy-3,7,11,23-tetraoxolanost-8-en-26-oate*; **5**). Yellow amorphous solid. $[\alpha]_D^{25} = +172.6$ ($c = 0.2$; MeOH). UV (MeOH): 251 (3.8). IR (KBr): 3458, 2945, 2920, 1720, 1710, 1680. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS (70 eV): 543 (24, $[M+1]^+$), 525 (30), 507

(22), 497 (100), 479 (32), 461 (7), 405 (11), 367 (5). HR-ESI-MS: 541.3070 ($[M-H]^-$, $C_{32}H_{45}O_7^-$; calc. 541.3165).

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