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Angiotensin Converting Enzyme-Inhibitory Triterpenes from *Ganoderma lucidum*

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The 70% MeOH extract of *Ganoderma lucidum* had an inhibitory effect on angiotensin converting enzyme activity, and from this extract, five new triterpenes, named ganoderal A, ganoderols A and B, and ganoderic acids K and S, were isolated. Their structures were determined on the basis of spectral evidence.

Keywords——*Ganoderma lucidum*; Polyporaceae; lanostane; triterpene; ganoderal A; ganoderol A; ganoderol B; ganoderic acid K; ganoderic acid S; angiotensin converting enzyme inhibitor

The fungus *Ganoderma lucidum* (Polyporaceae) has many pharmaceutical effects and has long been used as a home remedy. A number of triterpene constituents have been isolated and characterized from this fungus in relation to the biological activities.[1] In the course of our research on anti-hypertensive substances we found that the 70% MeOH extract of *G. lucidum* exhibited an inhibitory activity on angiotensin converting enzyme (ACE). From this extract we have now isolated and characterized five new triterpenes,[2] named ganoderal A (**1**), ganoderols A (**2**) and B (**3**),[3] and ganoderic acids K (**8**) and S (**4**), along with five known triterpenes. In this paper we describe the structure determination of the new compounds and the ACE-inhibitory activity of those triterpenes.

The dried fruiting body of *G. lucidum* KARST. produced in Nagano was extracted with 70% MeOH. The extract was adjusted to pH 2—3 with 1 N HCl and partitioned with ethyl acetate. The residue obtained by evaporation of the organic layer was separated on a column of silica gel into 14 fractions (Fr). Bioassays showed that Fr I, II, IV, V, VII, and XIII were ACE-inhibitory. Each bioactive fraction was repeatedly purified by silica gel column and/or preparative layer chromatography. From Fr I and II, compounds **1**, **2**, **3**, **4** and **5** were isolated. From Fr IV and V, compound **6**, from Fr VII, compounds **7**, **8** and **9**, and from Fr XIII, compound **10** were obtained. Compounds **5**, **6**, **7**, **9** and **10** were characterized as ganoderic acids Y,[4] F,[5] H,[6] B,[7] and D (C)[5,8] (in that order) based on the published physical and spectral data. The structures of the new triterpenes were determined as follows.

Compound **2**, named ganoderol A, showed characteristic peaks (236, 243 and 252 nm) of a 7,9(11)-diene tetracyclic triterpene in the ultraviolet (UV) spectrum, and gave the molecular (M^+) ion peak at *m/z* 438 ($C_{30}H_{46}O_2$) together with fragment peaks at 423 ($M-CH_3$), 337(c−2[9]), 309(b−2), 269(a−1). Further, the M^+ ion peak at *m/z* 510 of the trimethylsilyl derivative indicated the presence of a hydroxyl group. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I) showed signals attributable to one *sec*- and six *tert*-methyl groups, three olefinic protons and an allylic hydroxymethylene group. From the ^{1}H-NMR data the partial structural features of 3-oxo-7,9(11)-diene nucleus and 24-en-26-ol side chain

were easily assigned. The chemical shifts of 24-H (δ 5.40), 27-H_3 (δ 1.67) and 26-H_2 (δ 4.01) were in good agreement with the data published for a (24*E*)-isomer.[4] In view of these data, ganoderol A was determined to be (24*E*)-3-oxo-5α-lanosta-7,9(11), 24-trien-26-ol.

Compound **3**, named ganoderol B, showed the same UV spectrum as ganoderol A and gave the M^+ ion peak at *m/z* 440 ($C_{30}H_{48}O_2$), along with a characteristic fragment peak at 311 (b−2) in the mass spectrum (MS). The ^{1}H-NMR spectrum was closely similar to that of ganoderol A except for the presence of a new signal (δ 3.24) assignable to the 3α-proton of a 3β-alcohol derivative and the slight upfield shift of the 30- and 31-methyl signals. In view of these data, ganoderol B was established to be (24*E*)-5α-lanosta-7,9(11), 24-triene-3,26-diol.

Compound **4**, named ganoderic acid S, showed a peak at 226 nm, indicating a conjugated carboxylic group, in addition to the peaks due to 7,9(11)-diene in the UV spectrum and the M^+ ion peak at *m/z* 452 ($C_{30}H_{44}O_3$), together with fragment ion peaks at *m/z* 437 ($M-CH_3$), 419 ($M-CH_3-H_2O$), 353 (d), 309 (b−2), 269 (a−1) in the MS. Comparison of the ^{1}H-NMR spectrum with that of ganoderol A indicated the presence of a 24-en-26-oic acid unit instead of the 24-en-26-ol of ganoderol A. The stereochemistry of the 24-ene was deduced to be *E*, since the chemical shifts of 24-H (δ 6.91) and 27-H_3 (δ 1.84) of **4** resembled those of a (24*E*)-isomer such as ganoderic acid Y (3-oxo derivative of compound **4**) rather than a (24*Z*)-isomer such as tyromisic acid.[4,10] Therefore, the structure was determined to be (24*E*)-3-oxo-5α-lanosta-7,9(11), 24-trien-26-oic acid.

Compound **1**, named ganoderal A, showed the M^+ ion peak at *m/z* 436 ($C_{30}H_{44}O_2$), together with fragment peaks at *m/z* 337 (c−2), 309 (b−2), 281 and 269 (a−1). The ^{1}H-NMR spectrum was similar to that of ganoderol A, except for the presence of the aldehyde proton (δ 9.40) and a downfield shift of the 24-proton (δ 6.50) and the 27-methyl (δ 1.76) signals, indicating the presence of an unsaturated aldehyde in the side chain. The (24*E*)-stereochemistry was deduced from the published ^{1}H-NMR data for a triterpene having a (24*E*)-26-al moiety.[4] In view of these data, ganoderal A was deduced to be (24*E*)-3-oxo-5α-lanosta-7,9(11),24-trien-26-al.

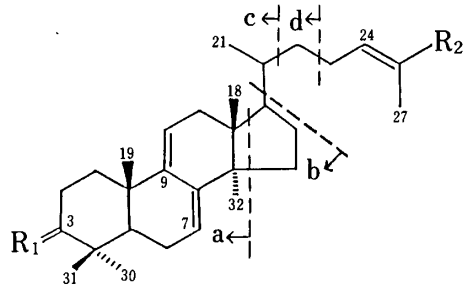
Compound **8**, named ganoderic acid K, showed a peak (252 nm) indicative of a

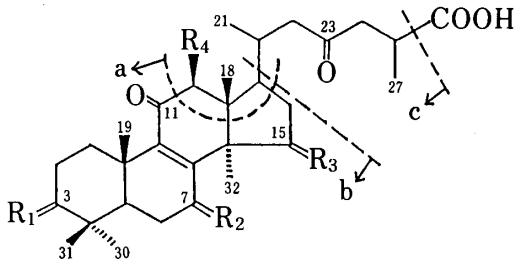
TABLE I. ^{1}H-NMR (200 MHz) Spectral Data for Ganoderal A (**1**), Ganoderols A (**2**) and B (**3**), and Ganoderic Acids S (**4**) and K (**8**) (in $CDCl_3$)

Assignment/ multiplicity (*J* in Hz)	Compounds				
	1	**2**	**3**	**4**	**8**
18-H_3 s	0.60	0.59	0.57	0.59	0.96
19-H_3 s	1.13	1.13	0.88	1.13	1.26
21-H_3 d (6)	0.96	0.93	0.92	0.94	0.96
27-H_3 s	1.76	1.67	1.67	1.84	1.22[a]
30-H_3 s	1.09	1.09	1.01	1.09	1.03
31-H_3 s	1.21	1.20	0.98	1.20	0.85
32-H_3 s	0.89	0.88	0.88	0.88	1.49
OAc s	—	—	—	—	2.26
2β-H dt (14.5, 6)	2.79	2.78	—	2.78	—
3α-H dd (10.5, 5)	—	—	3.24	—	3.20
7-H d (6)	5.52	5.51	5.47	5.51	4.80[b]
11-H d (6)	5.40	5.38	5.32	5.39	—
12α-H s	—	—	—	—	5.62
24-H t (7)	6.50	5.40	5.40	6.91	—
CHO s	9.40	—	—	—	—
CH_2OH s	—	4.01	3.99	—	—

a) (d, *J*=7Hz). *b*) (t, *J*=9Hz).

conjugated carbonyl group, presumably 8-en-11-one, in the UV spectrum and fragment ion peaks at *m/z* 556 ($M-H_2O$, $C_{32}H_{46}O_9$ for the molecular formula), 538 ($M-2H_2O$), 528 (c−1), 514 (M−AcOH), 468 (c−1−AcOH), 417 (b), 345, 306 (a+1). The ^{1}H-NMR spectrum had the combined features of the spectra of ganoderic acids B[7] and H,[6] showing characteristic signals assignable to two hydroxyl groups (δ 3.20, 3α-H; 4.80, 7β-H), an acetoxy methyl (δ 2.26) and a methine (δ 5.62, 12α-H). The chemical shifts of the *tert*-methyl signals were rationalized by assuming the nucleus to contain a 15-oxo group. The 27-methyl signal at δ 1.22 indicated the 23-oxo-26-acid side chain. On the basis of these data the structure of ganoderic acid K was assigned as 3β,7α-dihydroxy-12β-acetoxy-11,15,23-trioxo-5α-lanost-8-en-26-oic acid.





	R_1	R_2	IC_{50} (M)
1	=O	–CHO	$10^{-5}<$
2	=O	$-CH_2OH$	$10^{-4}<$
3	α-H,β-OH	$-CH_2OH$	2.2×10^{-4}
4	=O	–COOH	9.5×10^{-5}
5	α-H,β-OH	–COOH	4.3×10^{-5}

	R_1	R_2	R_3	R_4	IC_{50} (M)
6	=O	=O	=O	–OAc	4.7×10^{-6}
7	α-H,β-OH	=O	=O	–OAc	2.0×10^{-5}
8	α-H,β-OH	α-H,β-OH	=O	–OAc	2.6×10^{-5}
9	α-H,β-OH	α-H,β-OH	=O	–H	2.9×10^{-5}
10	α-H,β-OH	α-H,β-OH	α-OH,β-H	–H	3.0×10^{-5}

Chart 1

The inhibitory effects of compounds **1**—**10** on ACE activity were determined by a modification of the method described by Friedland and Silverstein,[11] and expressed in terms of IC_{50} (the amount of samples needed to inhibit 50% of ACE activity). Eight compounds isolated in the present study were inhibitory (data included in Chart 1). Ganoderic acid F had the highest inhibitory effect, $IC_{50}=4.7\times10^{-6}$ M, whereas the IC_{50} values of the other compounds were of the order of 10^{-5} M.

In summary, we have isolated and characterized from *G. lucidum* ten lanostane triterpenes which have ACE-inhibitory activity. Our results are in contrast to the conclusion by Arichi *et al.* that the compounds responsible for anti-hypertensive activity have molecular weights of more than 100000 daltons, based on their *in vivo* data in SHR rats.[12]

Experimental

Apparatus and Isolation——Melting points were measured on a Yazawa hot stage microscope and are uncorrected. UV spectra were recorded on a Shimadzu UV 200 spectrometer as a EtOH solution. The ^{1}H-NMR spectra were obtained on a JEOL FX-200 spectrometer at 200 MHz, with a tetramethylsilane as an internal standard. Mass spectra (70 eV) were measured on a Shimadzu GC-MS 9020 DF spectrometer using a direct inlet system. The *Rf* values reported were obtained on E. Merck Kiesel gel 60 F_{254} pre-coated plates (0.25 mm thickness). Preparative layer chromatography was done on E. Merck Kiesel gel 60 F_{254} pre-coated plates (0.5 mm thickness). Solvent systems for the silica gel (E. Merck Silica gel 60, 70—230 mesh) column chromatography of the ethyl acetate extract were $CHCl_3$ (Fr I and II), $CHCl_3$–methanol, 50:1 (Fr IV, V, and VII) and $CHCl_3$–methanol, 9:1 (XIII). Approximate yields (starting from 10 g of 70% methanol extract) of the compounds were **1** (7 mg), **2** (19 mg), **3** (12 mg), **4** (7 mg), **5** (34 mg), **6** (24 mg), **7** (20 mg), **8** (43 mg), **9** (18 mg), **10** (17 mg).

Compound 1 (Ganoderal A)——Compound **1** was obtained as colorless leaflets, mp 127—128 °C (from MeOH). $[\alpha]_D^{25}$ +27° (c=0.21, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 235 (4.17). *Rf* value in TLC: 0.50 (hexane–ethyl ace-

tate, 4:1)

Compound 2 (Ganoderol A)——Compound **2** was obtained as colorless leaflets, mp 99—101 °C (from MeOH). $[\alpha]_D^{25}$ +33° (c=0.31, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 236 (4.13), 243 (4.18), 252 (4.00). *Rf* value in TLC: 0.30 (hexane–ethyl acetate, 4:1).

Compound 3 (Ganoderol B)——Compound **3** was obtained as colorless needles, mp 171—173 °C (from MeOH). $[\alpha]_D^{25}$ +61° (c=0.18, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 237 (4.10), 244 (4.17), 252 (3.98). *Rf* value in TLC: 0.20 (hexane–ethyl acetate, 4:1).

Compound 4 (Ganoderic Acid S)——Compound **4** was obtained as colorless sticks, mp 168—169 °C (from MeOH). UV λ_{max}^{EtOH} nm (log ε): 226 (4.14), 235 (4.19), 243 (4.15), 252 (3.98). *Rf* value in TLC: 0.25 (hexane–ethyl acetate, 4:1).

Compound 8 (Ganoderic Acid K)——Compound **8** was obtained as an amorphous powder. $[\alpha]_D^{25}$ +48° (c=0.29, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 251.5 (4.14). *Rf* value in TLC: 0.47 ($CHCl_3$–MeOH, 9:1).

Bioassay——Purified ACE (9.2 units/mg protein) prepared from hog kidney was provided by Dr. T. Suzuki. Samples were dissolved either in water or in ethanol (ethanol concentration in the assay mixture was held constant at 10%). Inhibitory effects on ACE activity were determined by a modification of the published method, at least in duplicate.[11]

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