62. Structures of Ganoderic Acid A and B, Two New Lanostane Type Bitter Triterpenes from *Ganoderma lucidum* (FR.) KARST.[1])

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Dedicated to Prof. Dr. *Conrad Hans Eugster* on the occasion of his 60th birthday

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Summary

The structures of two new bitter triterpenes, ganoderic acid A and B, isolated from a mushroom *Ganoderma lucidum* (FR.) KARST. *(Polyporaceae)* were determined as **1** and **2** on the basis of spectral data. Ganoderic acid A is a novel highly oxidized triterpene bearing a boat-shaped A-ring of lanostane.

1. Introduction. – In connection with our work on the search for bitter principles in natural products, we investigated the bitter constituents of *Ganoderma lucidum* (FR.) KARST. This mushroom is distributed in northern Asia, being called 'holy mushroom' in China and Japan, and has long been used as a home remedy. It secretes bitter substances over the entire surface of the basidiocarp. Recently, we isolated two kinds of new bitter principles from the chloroform extract of its dried epidermis and named them ganoderic acid A and B. Both structures were elucidated by analysis of 100-MHz-^{13}C-NMR., 400-MHz-^{1}H-NMR. and mass spectra.

2. Structure of ganoderic acid A (1). – The molecular formula of **1**, $C_{30}H_{44}O_7$, was deduced by high-resolution mass spectroscopy (M^+ 516.3013, Calc. 516.3087). The 100-MHz-^{13}C-NMR. spectrum of **1**, analyzed by off-resonance and selective decoupling techniques, showed the presence of 30 C-atoms, which were assigned to seven methyl, seven methylene and six methine groups, including two methine groups bearing O-atoms, as well as to four fully substituted C-atoms, one tetra-substituted double bond, three carbonyl groups and one carboxyl group (see *Table 1*).

[1]) Key words: *Ganoderma lucidum* (FR.) KARST., Ganoderic acid A and B, Bitter triterpene, 7β,15α-Dihydroxy-3,11,23-trioxo-5α-lanost-8-en-26-oic acid, 3β,7β-Dihydroxy-11,15,23-trioxo-5α-lanost-8-en-26-oic acid.

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Table 1. *^{13}C-NMR. chemical shifts of ganoderic acid A* (**1**), *its derivatives* **3–5** *and methyl ganoderate B* (**6**)[a])

C-Atom	1	3	4	5	6
C(1)	35.7	35.6	35.3	37.4	34.8
C(2)	34.4	34.4	34.1	34.7	28.3
C(3)	208.4	208.7	207.9	208.4	78.5
C(4)	47.0	46.7	46.4	44.1	39.0
C(5)	49.2	48.8	48.4	44.8	45.7
C(6)	29.2	29.1	25.9	33.8	27.8
C(7)	69.1	68.9	70.6	200.0	67.0
C(8)	159.6	159.5	154.9	150.4	155.0
C(9)	140.6	140.1	144.4	147.7	142.9
C(10)	46.8	46.8	46.7	47.1	45.4
C(11)	200.0	199.7	198.3	200.2	200.0
C(12)	51.9	51.8	51.4	51.3	51.0
C(13)	38.2	38.0	37.5	39.6	38.8
C(14)	54.2	54.1	52.5	57.4	58.5
C(15)	72.6	72.4	74.8	207.5	207.5
C(16)	36.2	36.3	35.6	39.9	41.0
C(17)	48.3	48.2	48.3	49.1	49.2
C(18)	17.4	17.3	17.2	17.0	17.2
C(19)	19.6	19.4	18.1	18.5	17.6
C(20)	32.8	32.8	32.8	32.1	32.1
C(21)	19.4	19.7	19.2	19.8	19.8
C(22)	49.8	49.7	49.2	49.3	49.3
C(23)	217.3	217.4	216.1	215.9	215.9
C(24)	46.7	46.8	46.5	46.9	46.9
C(25)	34.8	34.8	34.5	34.8	34.9
C(26)	27.4	27.5	26.7	27.6	26.8
C(27)	180.1	176.3	176.2	176.8	176.2
$COOCH_3$	–	52.0	52.0	52.0	51.7
C(30)	17.0	17.2	20.8	16.5	15.5
C(31)	20.8	20.8	17.0	21.0	24.5
C(32)	19.8	19.7	22.0	20.4	18.7

[a]) The spectra were measured at 100.61 MHz in $CDCl_3$; δ-values are given in ppm relative to TMS (= 0 ppm); assignments were made by off-resonance and selective proton-decoupling techniques.

The presence of a carboxyl group in **1** was revealed by the ^{1}H-NMR. signal at 12.05 ppm in addition to the ^{13}C-NMR. signal at 180.1 ppm, and by the formation of its methyl ester **3**, m.p. 196–197°, $C_{31}H_{46}O_7$ (M^+ 530.3263, Calc. 530.3244). One of the three carbonyl groups of **3** was shown to be an α,β-unsaturated one by the signal of a carbonyl C-atom at 199.7 ppm and by the signals of a double bond at 140.1 and 159.5 ppm. The presence of two secondary hydroxyl groups in **1** was indicated by the ^{1}H-NMR. signals at 5.32 and 4.50 ppm (D_6-DMSO) and two methine signals at 4.79 and 4.62 ppm, and also by two methine C-atom signals at 69.1 and 72.6 ppm. This was supported by the formation of the di-*O*-acetyl methyl ester **4**, m.p. 196–197°, $C_{35}H_{50}O_9$ (M^+ 614.3451, Calc. 614.3455) on treatment of **3** with acetic anhydride/pyridine. The ^{1}H-NMR. spectrum of **4** showed signals at 5.32 and 5.54 ppm due to the two methine groups bearing the acetoxy group, in addition to the signals at 1.99 (*s*, 3 H) and 2.00 (*s*, 3 H) of the two acetoxy groups. The ^{13}C-NMR. spectrum of **4** showed signals at 21.2 (*qa*), 21.0 (*qa*), 171.3 (*s*)

and 170.4 (*s*) due to the acetoxy groups. Oxidation of **3** with *Collin*'s reagent gave a pentaketo methyl ester **5**, m.p. 182.5–183.5°, $C_{31}H_{42}O_7$ (M^+ 526.2913, Calc. 526.2931), the ^{13}C-NMR. spectrum of which showed signals at 208.4, 207.5, 200.0, 200.2 and 215.9 ppm due to the carbonyl groups.

The 400-MHz-^{1}H-NMR. spectra of **4** and **5** were extensively studied employing double-resonance experiments in several solvents, partially relaxed *Fourier* transform spectra *(Fig. 1)* and a combination of both techniques in order to determine the positions of functional groups and assign 18 protons appearing as complex signals in the region from 0.5 to 3 ppm. From these experiments the partial structures **I**–**VI** of **4** were obtained.

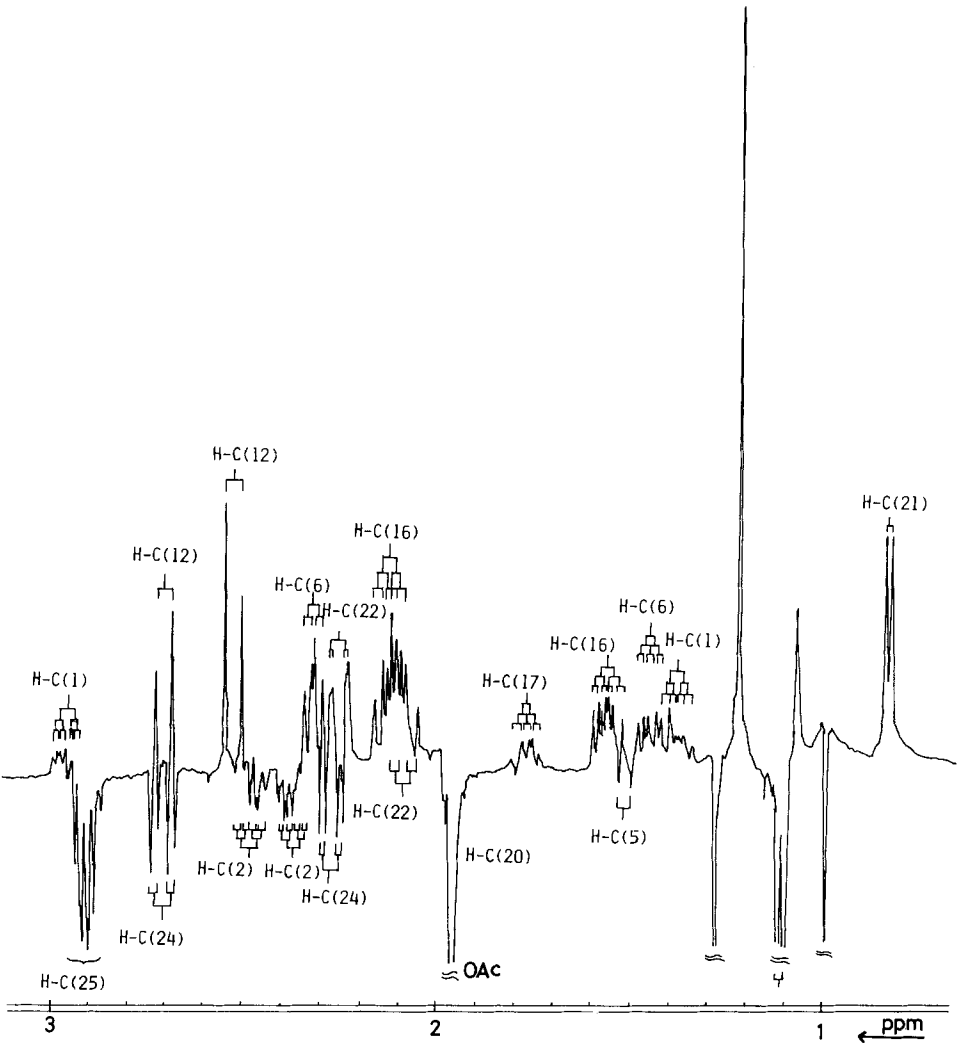


Fig. 1. *Partially relaxed FT-^{1}H-NMR. spectrum of* **4**

I. $H_2C(1)-H_2C(2)-OC(3)-(H_3C)_2C(4)$;
II. $HC(5)-H_2C(6)-(AcO)HC(7)$;
III. $C(8)=C(9)-OC(11)-H_2C(12)$;
IV. $(AcO)HC(15)-H_2C(16)-HC(17)-(H_3C)HC(20)-H_2C(22)-OC(23)$;
V. $H_2C(24)-(COOCH_3)(CH_3)HC(25)$;
VI. 3 CH_3.

From these partial structures together with ^{13}C-NMR. spectral data mentioned above, ganoderic A was suggested to be a triterpenoid of a highly oxidized lanostane type. This suggestion was supported by the fact that all the members of C_{30} triterpene carboxylic acids from *Polyporaceae* known to date had the oxigenated lanostane skeletons [1] [2], *e.g.* trametenolic acid B (=3β-hydroxy-8,24-lanostadien-21-oic acid) [3–5] [8] [9] [11], 15α-hydroxy-trametenolic acid [5] [9] [10], 3α-hydroxy-8,24-lanostadien-21-oic acid [3], pinicolic acid (=3-oxo-8,24-lanostadien-21-oic acid) [3] [8] [11], tyromycic acid (=3-oxo-7,9(11),24-lanostatrien-26-oic acid) [6], 3β-hydroxy-7,9(11),24-lanostatrien-21-oic acid [7], 3β,15α-dihydroxy-8,24-lanostadien-26-oic acid [10], and senexdielic acid (=3β,22-dihydroxy-8,24-lanostadien-29-oic acid) [11].

The structure of side chain was deduced from the fragmentation pattern in the mass spectra of **4** and **5**, as shown in *Table 3*. Both spectra showed the common fragments *m/z* 171, 144, 129 and 59, corresponding to $C_9H_{15}O_3^+$, $C_7H_{12}O_3^+$, $C_6H_9O_3^+$ and $C_2H_3O_2^+$, from which the structure of the side chain was deduced as $(CH_3)HC(20)-H_2C(22)-OC(23)-H_2C(24)-(CH_3)(COOCH_3)HC(25)$. This was supported by the geminal ^{1}H-NMR. coupling constants of the 2 H–C(24) (J=18 Hz) and the 2 H–C(22) (J=17 Hz), and by the chemical shifts of the 2 H–C(24) at 2.77 and 2.39 ppm and of the 2 H–C(22) at 2.36 and 2.19 ppm in **4**.

In the ^{13}C-NMR. spectrum of pentaketo ester **5** the appearance of the new signal at 200.0 ppm due to a newly formed α,β-unsaturated carbonyl group and the disappearance of the signal at 68.9 ppm due to a methine group in **3** were observed. This result demonstrated the formation of a tetrasubstituted 2-en-1,4-dione system in **5** which was produced by the oxidation of a tetrasubstituted 4-hydroxy-2-en-1-one system in **3**. The ^{13}C-NMR. spectrum confirmed this, the signal of C(8) at 159.5 ppm in **3** being shifted upfield to 150.4 ppm in **5** and of C(9) at 140.1 downfield to 147.7 ppm. The only place, the tetrasubstituted 4-hydroxy-2-en-1-one moiety of **3** can be located in the ring part of the lanostane skeleton, is at C(7)–C(8)–C(9)–C(11). Accordingly, C(7) of the partial structure **II** is connected to C(8) of **III**, and the position of the allylic secondary hydroxyl is at C(7) of lanostane. Then, the *ABX*-type protons H–C(5) and the 2 H–C(6) of pentaketo ester **5** could be assigned to C(5) and C(6) of ring B of lanostane, and the typical *AB*-type protons the 2 H–C(12) observed in both **4** and **5** to C(12) of ring C.

The position and configuration of the acetoxy groups and the side chain were determined by a study of nuclear *Overhauser* effects (NOE) and of long-range spin coupling in the ^{1}H-NMR. spectrum of **4**.

Observation of a 10% NOE enhancement of the H–C(15) signal **4** at 5.32 ppm upon irradiation at 1.02 ppm ($H_3C(18)$), which showed a long-range coupling

Table 2. *^{1}H-NMR. chemical shifts of ganoderic acid A* (**1**), *its derivatives* **4** *and* **5** *and methyl ganoderate B* (**6**)[a])

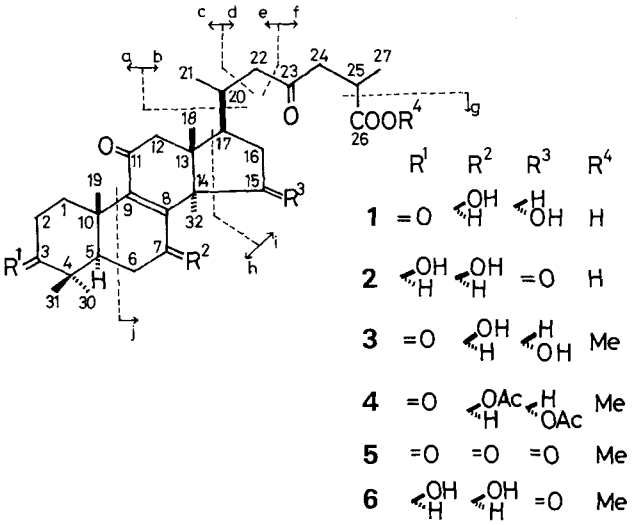
	1	**4**	**5**	**6**
H_β–C(1)	*ca.* 2.85	2.92 (*d*×*d*×*d*, 14, 7.7, 5.0)	2.82 (*d*×*d*×*d*, 14, 8.7, 6.1)	2.66 (*d*×*d*×*d*, 13.6, 3.6, 3.6)
H_α–C(1)	1.42 (*d*×*d*×*d*, 14, 8, 8)	1.42 (*d*×*d*×*d*, 14, 9.6, 7.7)	1.65 (*d*×*d*×*d*, 14, 9.7, 6)	1.17 (*m*)
H_β–C(2)		2.51 (*d*×*d*×*d*, 16, 9.6, 7.7)	2.55 (*d*×*d*×*d*, 15.7, 9.7, 6)	1.65 (*m*)
H_α–C(2)		2.40 (*d*×*d*×*d*, 16, 7.7, 5)	2.40 (*d*×*d*×*d*, 15.7, 8.7, 6)	1.65 (*m*)
H_α–C(3)	–	–	–	3.19 (*d*×*d*, 11, 5.4)
H_α–C(5)	1.67 (*d*×*d*, 12.5, 1.5)	1.55 (*d*×*d*, 14, 1.5)	2.40 (*d*×*d*, 13, 2.7)	0.88 (*d*×*d*, 11, 5.4)
H_α–C(6)	1.70 (*d*×*d*×*d*, 12.5, 12.5, 9.7)	1.44 (*d*×*d*×*d*, 14, 14, 8.5)	2.63 (*d*×*d*×*d*, 14.8, 13, 1.3)	1.60 (*d*×*d*×*d*, 13.3, 13.3, 8.2)
H_β–C(6)	2.06 (*d*×*d*×*d*, 12.5, 7.6, 1.5)	2.29 (*d*×*d*×*d*, 13.8, 8, 1.5)	2.25 (*d*×*d*, 14.8, 2.7)	2.17 (*d*×*d*×*d*, 13.3, 8.2, 1.3)
H_α–C(7)	4.62 (*d*×*d*, 9.7, 7.6)	5.54 (*d*×*d*, 8.5, 8)	–	4.78 (*d*×*d*×*d*, 8.2, 8.2, 3.6)
H_α–C(12)		2.54 (*d*×*qa*, 17.0, 0.8)	2.81 (*d*, 16.2, 0.9)	2.68 (*d*, 16.7)
H_β–C(12)		2.77 (*d*, 17.0)	2.69 (*d*, 16.2)	2.76 (*d*, 16.7)
H_β–C(15)	4.79 (*d*×*d*, 9.2, 7.8)	5.32 (*d*×*d*, 6, 6)	–	–
H_β–C(16)	1.81	1.61 (*d*×*d*×*d*, 14, 10, 6)	1.79 (*d*×*d*, 18.1, 8.2)	2.05 (*d*×*d*, 17.4, 5.6)
H_α–C(16)		2.16 (*d*×*d*×*d*, 14, 10, 6)	2.67 (*d*×*d*, 18.1, 9.4)	2.65 (*d*×*d*, 17.4, 5.6)
H_α–C(17)		1.84 (*d*×*d*×*d*, 10, 10, 8)	2.16 (*d*×*d*×*d*, 11, 9.4, 8.2)	2.10 (*m*)
3 H–C(18)	0.98 (*s*)	1.02 (*s*)	0.82 (*s*)	0.99 (*s*)
3 H–C(19)	1.26 (*s*)	1.27 (*s*)	1.21 (*s*)	1.20 (*s*)
H–C(20)		2.00 (*qa*×*d*×*d*×*d*, 6, 10, 9, 2)	2.03 (*qa*×*d*×*d*×*d*, 6.6, 8, 5, 11)	2.10 (*m*)
3 H–C(21)	0.90 (*d*, 6.2)	0.86 (*d*, 6)	0.90 (*d*, 6.6)	0.97 (*d*, 7)
H–C(22)	2.41 (*d*×*d*, 17.6, 3.2)	2.36 (*d*×*d*, 17, 2)	2.30 (*d*×*d*, 17, 5)	2.35 (br. *d*, 5)
H–C(22)	2.30 (*d*×*d*, 16.7, 8.6)	2.19 (*d*×*d*, 17, 9)	2.29 (*d*×*d*, 17, 8)	2.35 (br. *d*, 5)
H–C(24)	2.85 (*d*×*d*, 18, 8.6)	2.77 (*d*×*d*, 18, 8)	2.78 (*d*×*d*, 17.6, 8.5)	2.83 (*d*×*d*, 17.4, 8.7)
H–C(24)	2.50 (*d*×*d*, 18, 4.9)	2.39 (*d*×*d*, 18, 5)	2.37 (*d*×*d*, 17.6, 5.0)	2.39 (*d*×*d*, 17.4, 5.6)
H–C(25)	2.97 (*qa*×*d*×*d*, 7.3, 8.6, 4.9)	2.90 (*qa*×*d*×*d*, 7, 8, 5)	2.88 (*qa*×*d*×*d*, 7.2, 8.5, 5.0)	2.97 (*qa*×*d*×*d*, 7.2, 8.7, 5.6)
3 H–C(26)	1.23 (*d*, 7.3)	1.14 (*d*, 7)	1.12 (*d*, 7.2)	1.17 (*d*, 7.2)
3 H–C(30)	1.10 (*s*)	1.03 (*s*)	1.05 (*s*)	1.84 (*s*)
3 H–C(31)	1.12 (*s*)	1.09 (*s*)	1.07 (*s*)	1.02 (*s*)
3 H–C(32)	1.29 (*s*)	1.28 (*s*)	1.57 (*s*)	1.33 (*s*)
COOH(DMSO)	12.05 (br. *s*)	–	–	–
$COOCH_3$	–	3.64 (*s*)	3.61 (*s*)	3.67 (*s*)
OH(DMSO)	5.32 (*s*) and 4.50 (*s*)	–	–	–
OAc	–	2.00 (*s*) and 1.99 (*s*)	–	–

[a]) The spectra were measured at 400 MHz in $CDCl_3$; δ-values are given in ppm relative to TMS (=0 ppm); multiplicity and *J*-values (in Hz) are in parenthesis.

Table 3. *Mass fragmentations of ganoderic acid A* (**1**), *its derivatives* **4** *and* **5** *and methyl ganoderate B* (**6**)[a])

	M^+	a	b	b – R^4OH	c	d + H	f	g	h + H	i – H	j
1	516			139			115				
	(50)			(52)			(57)				
4	614		171	139	471	144	129	59			
	(24)		(22)	(29)	(7)	(47)	(43)	(54)			
5	526	355	171	139	383	144	129	59	301	225	
	(39)	(6)	(11)	(19)	(13)	(11)	(83)	(100)	(21)	(6)	
6	530		171	139		144	129	59			390
	(45)		(16)	(28)		(5)	(58)	(100)			(21)

[a]) Mass numbers *m/z* and relative intensity in parenthesis.



($J \approx 0.8$ Hz) with H_α–C(12) at 2.54 ppm, revealed that an acetoxy group was situated at C(15) and its configuration was α. This was supported by the long-range coupling ($J \approx 0.1$ Hz) between H_β–C(15) (5.32 ppm) and $H_3C(32)$ (1.28 ppm), and by a vicinal H, C-coupling between H_β–C(15) and C(32) (22.0 ppm) in the ^{1}H-undecoupled ^{13}C-NMR. spectrum. When the signal of $H_3C(32)$ was irradiated, 11 and 10% NOE enhancement was observed for the H–C(17) and H–C(7) signals, respectively. The former indicated that the side chain was at C(17) and in β-position which was in agreement with the observation of the long-range coupling ($J \approx 0.1$ Hz) between H–C(17) (1.84 ppm) and $H_3C(18)$ (1.02 ppm); the latter indicated β-configuration for the allylic acetoxy group at C(7), which was supported by a vicinal coupling between H–C(7) and the 2 H–C(6) (8.5 and 8 Hz, respectively).

The assignment of $H_2C(1)$ in **4** (partial structure **I**) to H_α–C(1) and H_β–C(1) of ring A of lanostane was based on the long-range coupling ($J \approx 0.1$ Hz) between $H_3C(19)$ (1.27 ppm) and H_α–C(1) (1.42 ppm), and on the downfield shift of

H_β–C(1) (2.92 ppm) due to the anisotropic effect of the carbonyl group at C(11). Then, the position of a carbonyl group in partial structure **I** was situated at C(3). Also observed were the long-range coupling ($J \approx 0.1$ Hz) between H–C(5) (1.55 ppm) and $H_3C(31)$ (1.09 ppm) and a 10% NOE enhancement of the H–C(5) signal at 1.55 ppm upon irradiation of the $H_3C(30)$ signal at 1.03 ppm. This suggested that H–C(5) in the partial structure **II** was assigned to C(5) of lanostane. The configuration of H–C(5) was α (axial) since it showed the typical vicinal coupling constants of an axial proton [J(H–C(5), H_α–C(6)) = 14 Hz, J(H–C(5), H_β–C(6)) = 1.5 Hz)].

The above observation thus established the structure of ganoderic acid A as *7β,15α-dihydroxy-3,11,23-trioxo-5α-lanost-8-en-26-oic acid* (**1**).

3. Structure of ganoderic acid B (2). Ganoderic acid B (**2**) has the same molecular formula as ganoderic acid A, $C_{30}H_{44}O_7$, as deduced by high resolution mass spectroscopy of its methyl ester **6**, $C_{31}H_{46}O_7$ (m.p. 202.5–205°; M^+ 530.3263, Calc. 530.3244). The IR. spectrum of **6** is very similar to that of methyl ganoderate A (**3**). The presence of two secondary hydroxyl groups and three carbonyl groups is shown by the ^{13}C- and ^1H-NMR. data of **6** (*Table 1* and *2*). Oxidation of **6** with *Collin*'s reagent yielded the pentaketo ester **5**, which was identical with the product obtained from **3** by the same treatment. This suggested that **1** and **2** differ from one another in the position and/or configuration of the secondary hydroxyl groups. In fact, the ^{13}C- and ^1H-NMR. data of **6** were similar to those of **3** except for the signals of the C- and H-atoms of rings A and D. The ^1H-NMR. spectrum of **6** lacked the signal at 4.79 ppm (H–C(15)) of **1**, but showed a new signal 3.19 ppm (H–C(3)) which was shifted to 4.67 ppm by trifluoroacetylation. The signals of 2 H–C(16) of **6** at 2.05 and 2.65 ppm were shifted downfield compared with **4** (1.61 and 2.16 ppm, respectively), and those of the 2 H–C(2) at 1.65 and 1.65 ppm upfield (**4**: 2.51 and 2.40 ppm, respectively). These observations indicated the presence of a secondary hydroxyl group at C(3) and a carbonyl group at C(15)

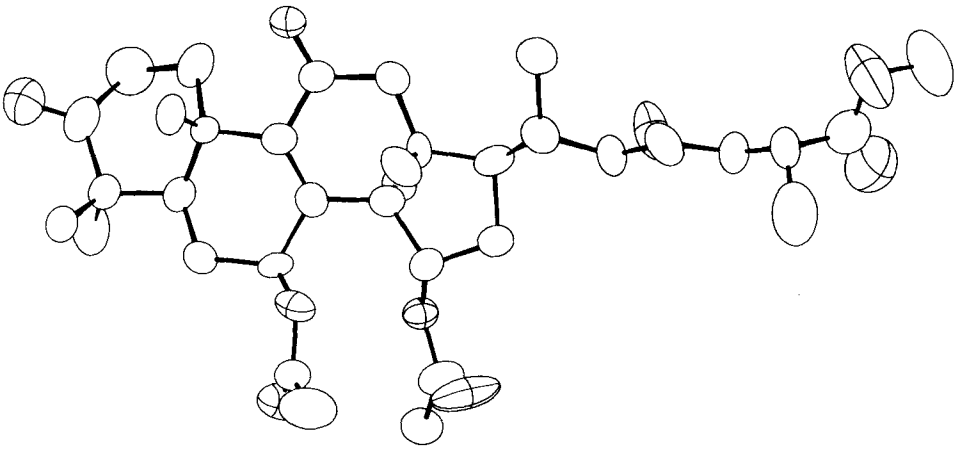


Fig. 2. *Stereoview of the molecule of methyl di-O-acetylganoderate A* (**4**) *obtained by X-ray analysis*

in **6**. The configuration of HO–C(3) was assigned as β (equatorial) on the basis of two coupling constants J(H–C(3), H_2–C(2)) (11 and 5.4 Hz, respectively). Thus, the structure of ganoderic acid B was established as *3β,7β-dihydroxy-11,15,23-trioxo-5α-lanost-8-en-26-oic acid* (**2**).

Interestingly, the C(3)-carbonyl compounds of this series, such as **1** and **3**–**5**, have a boat-shaped ring A, while the C(3)-hydroxyl compound **6** has a chair-shaped one. This was deduced from ^{1}H-NMR. spectrum of **4**, the vicinal coupling constant J(H_α–C(1), H–C(2)) (9.6 and 7.7 Hz, resp.) and J(H_β–C(1), HC(2)) (7.7 and 5.0 Hz, resp.) showed that dihedral angles between H_α–C(1) and H_α–C(2), and between H_β–C(1) and H_β–C(2) are approximately zero. Finally, the structure of **1** suggested above, including the boat-shaped ring A, was confirmed by X-ray analysis of methyl di-*O*-acetylganoderate A (**4**) *(Fig. 2)*. The details of the X-ray analysis will be reported in *Chem. Pharm. Bull.* (Tokyo).

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Experimental Part

General remarks. Melting points are uncorrected. IR. spectra (cm^{-1}) were measured on a *Hitachi 215* IR. spectrophotometer. UV. spectra (λ_{max}(ε)) were recorded on a *Shimazu UV 200* spectrophotometer. ^{13}C-NMR. (100.61 MHz, δ in ppm) were recorded on a *Bruker WH 100*, solvent $CDCl_3$; sweep width 24,000 Hz; data point 16 K. ^{1}H-NMR. (400.10 MHz, δ in ppm) were measured on a *Bruker WH 400*, solvent $CDCl_3$; sweep width 4400 Hz; data point 16 K. In the partially relaxed FT. (180°-τ-90°)n T pulse method was used, τ = 0.5 s, T = 8 s. Mass spectra (*m/z*) were recorded on a *Varian MAT 312* double focusing mass spectrometer with a *Varian MAT 188* data system, operating at 70 eV (high resolution). Samples were introduced *via* a direct insertion probe. Accurate mass measurements were made by means of computer-assisted acquisition or peak-matching technique using perfluorokerosene as a reference standard at a resolving power of 8000.

Isolation of Ganoderic acid A (**1**) *and B* (**2**). The dried chipped epidermis of *Ganoderma lucidum* (FR.) KARST. (100 g) were extracted with $CHCl_3$ at room temp. for 1 week (2 times). The combined extracts were evaporated at 40–45° under reduced pressure to about 1/10 of the original volume, and the resulting solution was extracted with sat. aq. $NaHCO_3$-solution. The combined extracts were acidified with 6N HCl (pH 3–4) at 0°. The precipitate on the water was extracted with $CHCl_3$, the extract dried (Na_2SO_4) and the solvent removed under reduced pressure, leaving crude material (5.5 g). A part of crude material (1 g) was chromatographed on silica gel. Elution with $CHCl_3$/MeOH 98:2 gave a bitter substance as an amorphous powder (263 mg **1**). Further elution with $CHCl_3$/MeOH 95:5 gave a slight bitter component as an amorphous powder (135 mg **2**).

Data of ganoderic acid A (= 7β,15α-dihydroxy-3,11,23-trioxo-5α-lanost-8-en-26-oic acid; **1**). $[\alpha]_D^{27}$ = +153.8° (c = 0.156, $CHCl_3$), single spot on TLC. (*Merck* silica gel GF-254). – IR. ($CHCl_3$): 3300, 2600–2400, 1700, 1650, 1250, 1100 and 1000. – MS.: 516.3013 (50, M^+; Calc. for $C_{30}H_{44}O_7$, 516.3087), 498 (21), 139 (52), 115 (57).

Data of ganoderic acid B (= 3β,7β-dihydroxy-11,15,23-trioxo-5α-lanost-8-en-26-oic acid; **2**). Single spot on TLC. – IR. ($CHCl_3$): 3400, 2600–2400, 1720, 1700, 1640 and 1270.

Synthesis of methyl ganoderate A (methyl 7β,15α-dihydroxy-3,11,23-trioxo-5α-lanost-8-en-26-oate; **3**). An amorphous powder of **1** (65 mg) was methylated with ethereal diazomethane to give crystalline **3** (25 mg), m.p. 196–197° (from EtOH). – IR. ($CHCl_3$): 3300, 1700, 1650, 1260, 1060 and 990.

Synthesis of methyl di-O-acetylganoderate A (= methyl 7β,15α-diacetoxy-3,11,23-trioxo-5α-lanost-8-en-26-oate; **4**). Compound **3** (68 mg) was acetylated as usual to give **4** (45 mg), m.p. 196–197° (from EtOH), $[\alpha]_D^{20}$ = +205.9° (c = 0.24, MeOH). – UV. (C_2H_5OH): 245 (9670) and 345 (39). – MS.: 614.3451 (24, M^+; Calc. for $C_{35}H_{50}O_9$, 614.3455), 572 (79), 554 (25), 512 (90), 171 (22), 144 (47), 139 (29), 129 (43), 59 (54).

Synthesis of methyl ganoderate B (= methyl 3β,7β-dihydroxy-11,15,23-trioxo-5α-lanost-8-en-26-oate; **6**). An amorphous powder of **2** (40 mg) was methylated with ethereal diazomethane to give **6** as needles (11.8 mg), m.p. 202.5–203° (from EtOAc). – MS.: 530.3263 (45, M^+; Calc. for $C_{31}H_{46}O_7$, 530.3244), 512 (8), 502 (23), 390 (21), 358 (36), 331 (23), 171 (16), 139 (28), 129 (58), 59 (100).

Synthesis of methyl 3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oate; **5**. – *(i) From* **3**. A solution of **3** (7 mg) in CH_2Cl_2 (1 ml) was treated with slight excess of *Collin's* reagent at 0°. After 30 min, the mixture was diluted with 10 ml of CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The product was purified by prep. TLC. with $CHCl_3$/MeOH 95:5 to give **5** as crystals (1.6 mg), m.p. 182.5–183.5° (from EtOAc). – MS.: 526.2913 (39, M^+, Calc. for $C_{31}H_{42}O_7$, 526.2931), 495 (8), 383 (13), 301 (21), 139 (19), 129 (83), 59 (100).

(ii) From **6**. Compound **6** (6 mg) was oxidized and purified as indicated above giving 1.0 mg of **5**. – ^{1}H-NMR.: superimposable to that obtained in *(i)*.

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