Studies on the Triterpenoid Constituents of the Spores from *Ganoderma lucidum* Karst

Ruo-Yun Chen and De-Quan Yu

Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050

Received June 8,1992; Accepted May 29,1993

Abstract Five compounds were isolated from the ether-soluble fraction of the spores of *Ganoderma lucidum*. On the basis of their chemical properties and spectral data (MS, UV, IR, ^1H and $^{13}CNMR$), they were identified as 3, 7, 11, 12, 15, 23-hexaoxo-5α-lanosta-8-en-26-oic acid (I), ganoderic acid B (II), C (III), D (IV) and ganodermanontriol (V). Compound I is a new natural product, named ganosporeric acid A. Compounds II, III, IV and V are known compounds and were obtained for the first time from the spores of *Ganoderma lucidum*. Pharmacological experiments showed that ganosporeric acid A has an activity for lowering the levels GPT in mice with liver injury by CCl_4 and GaNI and exhibits heptoprotective effects.

Key words Spores of *Ganoderma lucidum* ; Ganosporeric acid A; Ganoderic acid B, C, E; Ganodermanontriol

Ganoderma lucidum Karst. is a famous tonic in Chinese traditional medicine. The extract of its spores was shown to be efficacious for hepatic protection and used clinically for the treatment of atrophic muscle rigidity[1].

In the previous papers we isolated and identified several steroids and triterpene lactons from the title spores [2,3]. In continuation of our study on the effective principles we have examined the minor constituents and isolated five triterpenoids, ganosporeric acid A (I), ganoderic acids B (II), C(III), E(IV) and ganodermanontriol (V) from the acidic fraction. Ganosporeric acid A (I) is a new compound. In this paper we report the structural elucidation of ganosporeric acid A (I) as well as the compounds II, III, IV and V.

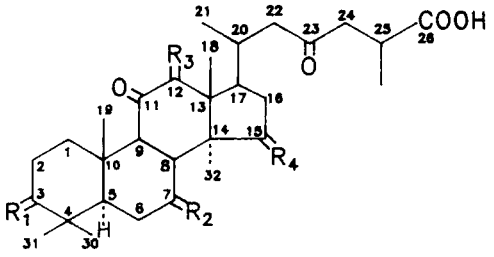
Ganosporeric acid A is a yellow crystal, mp 115~116 °C, $[\alpha]_D^{28}$+48. The FD-MS gave M^+ at m/z 526 corresponding to the molecular formula as $C_{30}H_{38}O_8$, which was established by elementary analysis. IR data showed the major absorption bands at 2200~2000, 1740, 1725, 1700, 1690, 1275, 1215 and 1050 cm^{-1}, displaying the characteristic absorption of polycarbonyl groups, which is similar to known compound ganoderic acid E (IV)[4]. UV spectra showed the λ_{max}^{MeOH} nm (log ε) at 205 (3.77), 224 (sh) and 265 (3.75). The $^{13}CNMR$ and DEPT spectra indicated that

This project was supported by National Natural Science Foundation of China

ganosporeric acid A possesses 7 CH_3, 6 C=O, 1 COOH, 1 >C=C<, 6 CH_2, 4 CH and 4 sp^3 quaternary carbons. Comparison of the ^{13}CNMR spectra of ganosporeric acid A to ganoderic acid E (IV) displayed that both are almost superposable. In fact the only difference between the ^{13}CNMR spectra is the lacking of a C-12 signal at δ 48.6 ppm for a CH_2 group and the addition of a signal at δ 192.6 ppm for a C=O group in the spectrum of ganosporeric acid A, showing the methylene group of C-12 might be converted into a carbonyl group. Correspondingly, the signal of C-13 was changed to the low field region from δ 43.9 to δ 59 ppm due to the deshielding effect of C-12 carbonyl and 6 ppm upfield shifts of C-17 and C-18 were observed arising from the γ-effect of C-12. In addition, the signal of C-21 was also changed to the low field region from δ 18.6 ppm to δ 23.3 ppm since C-21 is close to C-12 carbonyl in space.

The ^{1}HNMR spectra of ganosporeric acid A and ganoderic acid E are also compatible with the proposed structure. The coupling relationship of 1-H and 2-H showed a typical ddd spliting pattern excluding the possibility of C-1 or C-2 carbonyl group. The coupling constants of H-17 with H-20 and H-16 eliminated the C-16 carbonyl and there were absent of the H-12 signal.

The MS spectra of ganosporeric acid A showed it to be accommodated to ganoderic acid derivatives[11]. The fragment ion at m/z 369 of ganosporeric acid A was derived from the breaking of C-17 and C-20 bond which is also showing the absence of carbonyl group on the side chain. Thus the structure of ganosporeric acid A was elucidated as 3, 7, 11, 15, 23-hexaoxo-5α-lanosta-8-en-26-oic acid. The ^{1}H and ^{13}CNMR data are given in Tables 1 and 2. The structures of compound I to V are shown as follows.

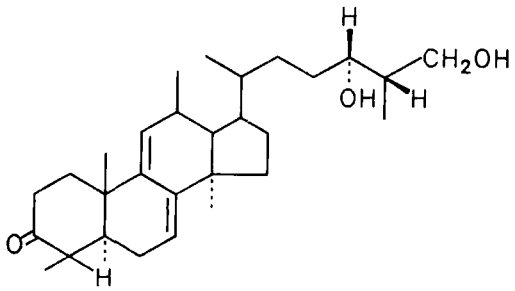


I: $R_1=R_2=R_3=R_4=O$

II: $R_1=R_2=\beta$-OH , H; $R_3=2H$; $R_4=O$

III: $R_1=R_4=O$, $R_2=\beta$-OH, H; $R_3=2H$

IV: $R_1=R_2=R_4=O$; $R_3=2H$



V

Experimental

Apparatus

Melting points were measured on Boetius melting point apparatus and uncorrected. UV spectra were taken on Shimadzu UV 240 instrument. IR spectra were recorded with a Perkin-Elmer 399 instrument. MS spectra were determined with MAT 711 and ZAB-2F equipment. Optical rotations were determined with a Perkin-Elmer 241 instrument and ^{1}H and ^{13}CNMR spectra were taken on a Bruker AM-500 and JNM-GX 400 spectrometer respectively, in $CDCl_3$ solution with

TMS as an internal standard.

Isolation

The ether-soluble fraction of spores of *Ganoderma lucidum* (8 kg) was dissolved in 12 L of 4% KOH-water and extracted with ether, the ether solution was combined and washed to neutrality by water, then evaporated *in vacuo* to yield 2900 g of a neutral fraction. The aqueous phase was acidified to pH 2 with HCl and extracted with ether. The ethereal solution was evaporated to give 2950

Table 1. ^{1}HNMR spectral data of compounds I~V

Proton	I	II	III	IV
1	1.76 ddd (14.3, 9.8, 6.3)	0.93 m	1.46 ddd (13.9, 8.5, 4.8)	1.74 ddd (14.2, 8.3, 5.7)
1	2.8 ddd (14.3, 8.1, 5.9)	2.82 ddd (17.2, 10.5)	2.96 ddd 13.9, 9.8, 7.7)	2.89 ddd (14.2, 5.5, 6)
2	2.44 ddd (14.5, 8.1, 6.3)	1.64 ddd (14.9, 7.5, 5)	2.51 ddd (15.7, 7.3, 4.8)	2.40 ddd (15.5, 7. 5, 5)
2	2.63 ddd (14.5, 9.8, 5. 9)	1.66 ddd (14.9, 10, 8)	2.55 ddd (15.7 8.5 8.3)	2.61 ddd (15, 9.3, 6)
3	-	3.20 dd (11, 5.3)	-	-
5	2.50 dd (13.2, 2.6)	0.87 dd (13.1, 1.8)	1.58 d (13.5)	2.30 dd (14.9, 2.6)
6	2.54 dd (13.5 2.6)	2.20 ddd (13 8.1 1.8)	2.10 m	2.52 m
6	2.75 d (13.5)	1.59 ddd (13, 9.5, 5.4)	1.68 ddd (13, 9.5, 4)	2.63 m -
7	-	4.79 dd (9.5, 8.1)	4.86 dd (7.7, 9.5)	-
OH	-	3.9	4.10	-
12	-	2.72 d (25)	2.79 d (17.6)	2.88 d (16.1)
12	-	2.64 d (25)	2.74 d (17.6)	2.74 d (16.1)
16	2.85 dd (13.3, 5)	2.65 dd (19, 7.8)	2.65 m	2.72 d (18.2, 5.3)
16	2.01 m	2.03 dd '19, 9.5)	2.05 m	1.85 dd (18.2, 8.3)
17	1.93 ddd (13.3, 12.7, 5)	2.15 m	2.15 m	2.26 ddd (11.4, 10, 8.3)
18-CH_3	1.19 s	1.00 s	1.04 s	0.88 s
19-CH_3	1.39 s	1.21 s	1.27 s	1.27 s
20	2.01 m	2.16 m	2.15m	2.10 m
21-CH_3	0.89 d (6.6)	0.98 d (6)	1.01 d (5.5)	0.98 d (6.5)
22	2.39 dd (5.0, 2.4)	2.36 d (4)	2.39 d (5.2)	2.37 d (6.8)
22	2.36 dd (5.0, 2.6)	2.36 d (4)	2.39 d (5.2)	2.36 d (6.8)
24	2.92 dd (11.1, 8.4)	2.83 dd (17.9, 8.6)	2.86 dd (17.9, 8.8)	2.82 dd (17.8, 8.6)
24	2.47 dd (11.1, 6.5)	2.46 dd (17.9, 5.0)	2.47 dd (17.9, 4.7)	2.48 dd (17.8, 6)
25	2.96 qdd (7.2, 8.4, 6.5)	2.97 dqd (7.2, 8.6, 5.0)	2.96 qdd (7.0, 8.8, 4.7)	2.95 qdd (7.3, 8.6, 6)
27-CH_3	1.23 d (7.2)	1.22 d (7.2)	1.25 d (7.0)	1.23 d (7.3)
30-CH_3	1.15 s	1.03 s	1.14 s	1.13 s
31-CH_3	1.14 s	0.85 s	1.35 s	1.11 s
32-CH_3	1.55 s	1.33 s	1.12 s	1.64 s

Compound II was taken in 400 MHz, $CDCl_3$

g of acidic fraction, which was chromatographed on a silica gel column and eluted with petroleum ether, Et_2O, Me_2CO and MeOH successively. The ethereal fraction (500 g) was further chromatographed on a silica gel column using $CHCl_3$-MeOH in different ratios as eluants. The $CHCl_3$ fraction (212 g) was chromatographed repeatedly on a silica gel column by low pressure and reduced pressure chromatography to give compounds I 120 mg, II 42 mg, III 716 mg, IV 220 mg and V 37 mg respectively.

Identification

Compound I, yellow needles, mp 115~118 °C, $[\alpha]_D^{28}$+48 (c 0.1, $CHCl_3$), FD-MS, m/z 526. The molecular formula was established by MS and the elementary analysis. Anal. calcd. for $C_{30}H_{38}O$. 1/2 H_2O, C 67.2, H 7.2; found C 67.5; H 7.2. IR (KBr) cm^{-1}: 3460, 2970, 2200~2000, 1740, 1725, 1700, 1690, 1460, 1380, 1275, 1215, 1170, 1050. UV λ_{max}^{MeOH} nm ($\log \varepsilon$): 205 (3.77), 224 (sh), 26 (3.75). EI-MS m/z 526 (M^+,2), 508 (M^+-H_2O, 4), 480 (M^+-H_2O-CO, 4) 369 (4), 353 (4), 302 (17), 285 (5), 207 (59), 193 (14), 179 (100), 149 (10), 115 (57), 83 (49), 69 (21). ^1H and ^{13}CNMR data are shown in Tables 1 and 2.

Compound II, colourless needles, mp 205~208°C, $[\alpha]_D^{28}$+49.1, (c 0.083, $CHCl_3$). Anal. calcd. for $C_{30}H_{44}O_7$, 1/2H_2O: C 68.5, H 8.5; found C 68.2, H 8.3. IR (KBr) cm^{-1}: 3400,

Table 2. ^{13}CNMR spectral data of compounds I~V

Carbon	I	II	III	IV	V
1	37.2	34.6	35.5	37.2	36.6
2	34.5	26.0	34.2	34.6	34.8
3	214.5	78.3	216.7	215.2	216.7
4	47.4	38.8	46.7	47.0	47.4
5	50.9	49.1	48.7	50.7	50.7
6	33.4	27.6	27.5	33.8	23.6
7	198.2	66.6	66.2	199.3	119.9
8	150.0	156.6	157.7	149.7	142.8
9	149.5	142.7	141.1	146.6	144.5
10	39.2	38.6	38.1	39.0	37.2
11	197.0	197.8	197.6	199.3	117.2
12	192.6	50.2	50.0	48.6	37.8
13	59.0	45.3	44.9	43.9	43.7
14	61.0	59.3	59.3	57.1	50.3
15	203.8	217.4	217.6	206.6	27.6
16	38.9	40.8	40.9	39.6	28.8
17	38.3	45.5	45.5	44.1	51.0
18	12.4	17.4	17.9	16.0	15.7
19	18.5	18.4	18.1	19.7	22.4
20	32.2	31.9	31.9	32.0	36.5
21	23.3	19.6	19.5	18.6	18.6
22	48.6	49.0	48.8	48.9	31.4
23	207.3	207.6	207.0	207.5	33.5
24	46.5	46.5	46.5	46.5	79.2
25	33.6	34.3	34.3	34.3	73.9
26	180.3	179.4	180.6	179.8	67.6
27	16.8	16.9	16.8	16.9	22.0
30	27.4	28.1	26.9	27.5	25.3
31	20.3	15.3	20.7	20.3	20.9
32	19.3	24.4	24.6	20.9	25.4

Compound II was taken in 100 MHz, $CDCl_3$

Compounds I, III~V were taken in 125 MHz, $CDCl_3$

2980, 2930, 2880, 2200~2000, 1730, 1710, 1650, 1460, 1420, 1370, 1220, 1170, 1140, 1060, 1020, 1000. UV λ_{max}^{MeOH}: 253 nm (log ε 3.9), EI-MS m/z: 516 (M^+, 39), 498 (M^+-H_2O, 13), 488 (M^+-CO, 30), 470 (7), 376 (31), 358 (50), 331 (43), 313 (8), 275 (11), 246 (24), 175 (20), 139 (34), 121 (27), 95(24), 65 (55), 43 (100), ^{1}H and ^{13}HNMR data are shown in Tables 1 and 2. The spectral data of compound II were found to be identical with those of ganoderic acid B[5~7], thus compound II was identified as 3β, 7β-dihydroxy-11, 15, 23-trioxo-5α-lanosta-8-en-26-oic acid (II).

Compound III, colourless needles, mp 125~127°C, $[\alpha]_D^{14}$+18.3(c 0.153, $CHCl_3$), molecular formula was determined as $C_{30}H_{42}O_7$ by HR-MS m/z: 514.2931 (calcd, 514.2930), 496 (M^+-H_2O, 48), 486 (M^+-CO, 23), 478 (26), 468 (14), 411 (33), 408 (18), 386 (19), 376 (39), 366 (16), 356 (79), 329 (56), 303 (10), 273 (16), 246 (20), 167 (22), 149 (14), 139 (89), 115 (30), 87 (26), 55 (34), 43 (97), 31 (100). IR (KBr) cm^{-1}: 3400, 2980, 1760, 1720, 1700, 1680, 1640, 1450, 1415, 1380, 1370, 1270, 1170, 1130, 1060, 1045. UV λ_{max}^{MeOH}: 252 nm (log ε 3.88). ^{1}H and ^{13}CNMR data are shown in Tables 1 and 2. The spectral data of compound III were found to be identical with those of the known compound ganoderic acid C[6,7]. Hence, compound III was identified as 7β-hydroxy-3, 11, 15, 23-tetraoxo-5α-lanosta-8-en-26-oic acid (III).

Compound IV, yellowish needles, mp 105~106°C, $[\alpha]_D^{13}$ +165.3 (c 0.0635, $CHCl_3$). HR-MS m/z 512.2777 (calcd. 512.2773), showed the molecular formula as $C_{30}H_{40}O_7$. EI-MS m/z: 512 (M^+, 67), 494 (M^+-H_2O, 14), 382 (7), 302 (31), 301 (98), 247 (10), 215 (14), 193 (24), 189 (16), 179 (16), 175 (14), 165 (29), 149 (46), 139 (100), 115 (91), 105 (21), 69 (94), IR (KBr) cm^{-1}: 3460, 2950, 1720, 1710, 1680, 1660, 1440, 1400, 1360, 1260, 1200, 1160, 1090, 1030, 920. UV λ_{max}^{MeOH} nm (log ε): 210 (3.4), 252 (3.87). ^{1}H and ^{13}CNMR data are shown in Tables 1 and 2. The spectral data of compound IV were found to be identical with those of the known compound ganoderic acid E[4,8], thus, the compound IV was identified to be 3, 7, 11, 15, 23-pentaoxo-5α-lanosta-8-en-26-oic acid (IV).

Compound V, colourless needles, mp 128~130°C, $[\alpha]_D^{28}$+34.8 (c 0.064, $CHCl_3$). Molecular formula was determined by the elementary analysis as $C_{30}H_{48}O_4$ (anal. cal. for $C_{30}H_{48}O_4 \cdot 1/2\ H_2O$, C 72.1, H 10.2, found C 72.3, H 9.7). IR (KBr) cm^{-1}: 3400, 2965, 2880, 1700, 1460, 1435, 1380, 1115, 1040, 1010, 815. UV λ_{max}^{MeOH} nm (log ε): 235 (4.1), 242 (4.18), 251 (4.0). EI-MS m/z: 473 (M^++1, 76), 454 (M^+-H_2O, 15), 439 (M^+-H_2O-CH_3, 8), 396 (24), 311 (28), 309 (50), 269 (55), 107 (24), 95 (36), 75 (100), 69 (52), 55 (76), 43 (95). ^{1}HNMR ($CDCl_3$) δ 5.39 (1H, d, J=6.4 Hz, H-7), 5.51 (1H, d, J=6.4 Hz, H-11), 3.83 (1H, d, J=11.2 Hz, H-26), 3.48 (1H, d, J=11.2 Hz, H-26), 1.20 (3H, s, CH_3-27), 1.12 (3H, d, CH_3-31), 1.11 (3H, s, CH_3-30), 1.08 (3H, s, CH_3-19), 0.92 (3H, d, J=6. 4 Hz, CH_3-21), 0.88 (3H, s, CH_3-32), 0.59 (3H, s, CH_3-18), ^{13}CNMR data are shown in Table 2. The spectral data of compound V were found to be identical with those of known compound ganodermanontriol[9,10], therefore compound V is 24, 25, 26-trihydroxy-5α-lanosta-7, 9(11)-dien-3-one (V).

Acknowledgments We are thankful to the Pharmaceutical Factory of the Institute of Materia Medica, Chinese Academy of Medical Sciences, for supplying the extract of spores of *Ganoderma lucidum* and the Department of Instrumental Analyses for the measurements of spectral data.

References

1 Fu HD. *Traditional Med*, 1981, **22**: 22
2 Chen RY, Wang YH, Yu DQ. *Acta Botan*, 1991, **33**: 65
3 Chen RY, Yu DQ. *Acta Pharm Sin*, 1991, **26**: 430
4 Kikuchi T, Satsuda S, Kadota S, Murai Y, Ogita Z. *Chem Pharm Bull*, 1985, **33**: 2624
5 Kubota T, Asaka Y, Miura I, Mori H. *Helv Chim Acta*, 1982, **65**: 611
6 Nishitoba T, Sato H, Kasai T, Kawagishi H, Sakanura S. *Agr Biol Chem*, 1985, **49**: 1793
7 Kohda H, Tokumota W, Sakamoto K, Fujii M, Hirai Y, Yamasaki K, Komoda Y, Nakamura H, Ishihara S, Uchida M. *Chem Pharm Bull*, 1985, **33**: 1367
8 Hirotani M, Furaya T. *Phytochemistry*, 1986, **25**: 1189
9 Fujita A, Arisawa M, Saga M, Hayashi T, Morita N. *J Nat Prod*, 1986, **49**: 1122
10 Sato H, Nishitoba T, Shhirasu S, Oda K, Sakamura S. *Agr Biol Chem*, 1986, **50**: 2887
11 Kikuchi T, Kanomi S, Kadota S, Murai Y, Tsubono K, Ogita Z. *Chem Pharm Bull*, 1986, **34**: 3695

赤芝孢子粉三萜化学成分研究

陈若芸 于德泉

中国医学科学院药物研究所，北京 100050

提要 从赤芝孢子粉(*Ganoderma lucidum* Karst)酯溶部分分离得到五种三萜类化合物经理化常数和光谱(UV, IR, MS, ^{1}H 和 $^{13}CNMR$)分析分别鉴定为赤芝孢子酸A (ganosporeric acid A, I), ganoderic acid B (II), ganderic acid C (III), ganoderic acid E (IV), ganodermanontriol (V)。其中赤芝孢子酸A是新化合物，其余均为首次从赤芝孢子粉中得到。经药理实验表明赤芝孢子酸A对CCl_4和GaNI引起的小鼠转氨酶升高有降低作用，对丙酸杆菌引起的小鼠免疫性肝损伤有保护作用。

关键词 赤芝孢子粉；赤芝孢子酸A；Ganoderic acid B, C, E；Ganodermanontriol