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GANODERIC ACIDS T, S AND R, NEW TRITERPENOIDS FROM THE CULTURED MYCELIA OF *GANODERMA LUCIDUM*[1)]

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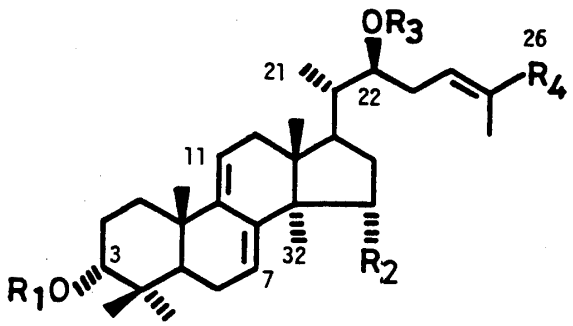
New lanostane-type triterpenoids, ganoderic acids T, S and R were isolated from the cultured mycelia of *Ganoderma lucidum*. The structure of ganoderic acid T was determined by the spectral data and X-ray analysis, and the structures of the other compounds were elucidated on the basis of their spectral data.

KEYWORDS —— *Ganoderma lucidum*; Polyporaceae; cultured mycelia; triterpenoid; lanostane; ganoderic acid T; ganoderic acid S; ganoderic acid R; antihepatotoxic agent

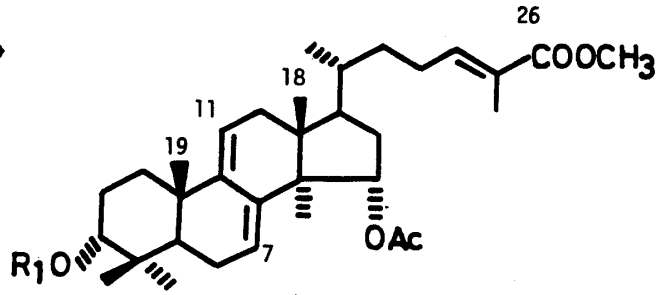
Highly oxygenated lanostane type triterpenoids, ganoderic acid and lucidenic acid derivatives were isolated from the fungus *Ganoderma lucidum* (called Reishi in Japan).[1-6] Toth et al.[7,8] isolated ganoderic acids Z, Y, X, W, V and U, which were cytotoxic to hepatoma cells in vitro, from the cultured mycelia of *G. lucidum*. These studies encouraged us to search for the triterpenoid components of the cultured mycelia of *G. lucidum*.

Mycelia of *G. lucidum* were cultured statically on soytone medium, containing glucose, soytone, yeast ext. and several inorganic salts, for 6 weeks in the dark at 25°C. The $CHCl_3$ extract of the mycelia afforded three new ganoderic acid derivatives, named ganoderic acids T, S and R, when repeatedly purified by Si-gel column chromatography and high performance liquid chromatography.

Ganoderic acid T (**1**), Anal. Calcd for $C_{36}H_{52}O_8$: C, 70.56; H,8.55, Found C, 70.40; H, 8.54, colorless needles, showed mp 200-202°C, $[\alpha]_D$+23°($CHCl_3$, c=0.13), UV λ nm (log ε) : 216(4.0), 225(4.0), 234(4.0), 242(4.0), IR ν : 3420(OH), 2940(CH), 1720(COO), 1240(C-O) cm^{-1}. The ^{1}H-NMR spectrum of **1** showed the presence of five tertiary methyl(δ 0.66, 0.88, 0.98, 0.99 and 1.03), one allyl methyl(δ 1.86) and one secondary methyl(δ 0.97, d, J=6.7) signals. Also observed were signals due to three secondary acetoxyl groups[δ 2.05(3H,s), 2.07(3H,s), 2.08(3H,s), 4.68(1H,dd J=3.0,3.0 H-3), 5.08(H, dd J=10.0,5.1 H-15), 5.03(H, ddd J=7.1,7.0,1.4 H-22)] and three olefinic proton signals [δ 5.32(1H, d, J=6.4 H-11), 5.48(1H, bs, H-7), 6.78(1H, dd, J=7.5,7.5 H-24)]. These spectral data suggest that **1** is a lanostane



1) R_1=R_3=Ac, R_2=OAc, R_4=COOH
2) R_1=R_2=H, R_3=Ac, R_4=COOH
3) R_1=R_3=Ac, R_2=H, R_4=COOH
4) R_1=R_3=H, R_2=OH, R_4=CH_2OH



5) R_1=H
6) R_1=Ac

type triterpenoid having heteroannular diene moiety and three acetoxyl groups. The ^{13}C-NMR spectral data of **1** supported this suggestion and revealed the presence of one carboxyl group (Table I). Comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data of **1** with those of methyl ganoderate X (**5**) and 3-*O*-acetyl methyl ganoderate X (**6**)[8] led to the conclusion that **1** is a triacetoxy-ganoderic acid of 7,9(11),24-triene type. Concerning the positions of three acetoxyl groups, two proved to be at C-3α and C-15α on the basis of the chemical shift values and the coupling patterns of the methine proton signals corresponding to those due to 3β-H(δ 4.67,t,J=2.5Hz) and 15β-H (δ 5.08,dd,J=9,5Hz) of **6**; the third one was located at C-22 by the ^{1}H-^{1}H shift correlation NMR spectrum, in which a correlation between 23-H (δ 2.34 and 2.57) and the third acetoxyl methine proton signal at δ 5.03 was observed clearly. The relative stereostructure of **1** was determined by X-ray analysis.[9] The structure in Fig. 1 was drawn using the *PLUTO* program.[10] The absolute stereostructure was determined by the CD spectrum of compound **4**[11] prepared from **1** by reduction, which showed a positive Cotton effect($[\theta]_{239}$ +12689, c=0.012 EtOH) like that of (26S)-26-*O*-methylperenniporiol($[\theta]_{240}$ +29824, c=0.012 EtOH).[12] Thus, the structure of **1** was determined to be (22*S*,24*E*)-3α,15α,22-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid.

Ganoderic acid R(**3**), Anal. Calcd for $C_{34}H_{50}O_6$: C,73.61;H,9.09, Found C,73.77; H,9.13, colorless needles, showed mp 201-202°C, $[\alpha]_D$ +8.7°(c=0.092; $CHCl_3$), UV λ nm (logε): 224(4.0), 234(4.0), 242(4.0), 250(3.8) and IR ν : 3400(OH), 2870(CH), 1720(COO), 1675(COO). The ^{1}H-NMR spectrum of **3** was very similar to that of **1** except for the loss of the methine proton signal at δ 5.03(15α-H) and of one acetoxyl methyl proton signal. The ^{13}C-NMR spectrum of **3** was also closely similar to that of **1** except for the appearance of a methylene carbon signal (δ 31.3) in **3** instead of the methine carbon signal (δ 77.4) in **1**. Investigation of these spectral data indicated that the structure of **3** is (22*S*,24*E*)-3α,22-diacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid.

Ganoderic acid S(**2**), Anal. Calcd for $C_{32}H_{48}O_5$: C,74.96; H,9.44, Found C,74.93; H,9.55, colorless needles, showed mp 194-196°C, $[\alpha]_D$ +19.8°(c=0.085;$CHCl_3$), UV λ nm (log ε) : 231(4.2), 240(4.2), 248(4.0) and IR ν : 3410(OH), 1720(COO), 1675(COO) cm^{-1}. The ^{13}C-NMR spectrum of **2** was very similar to that of **3**, except for the

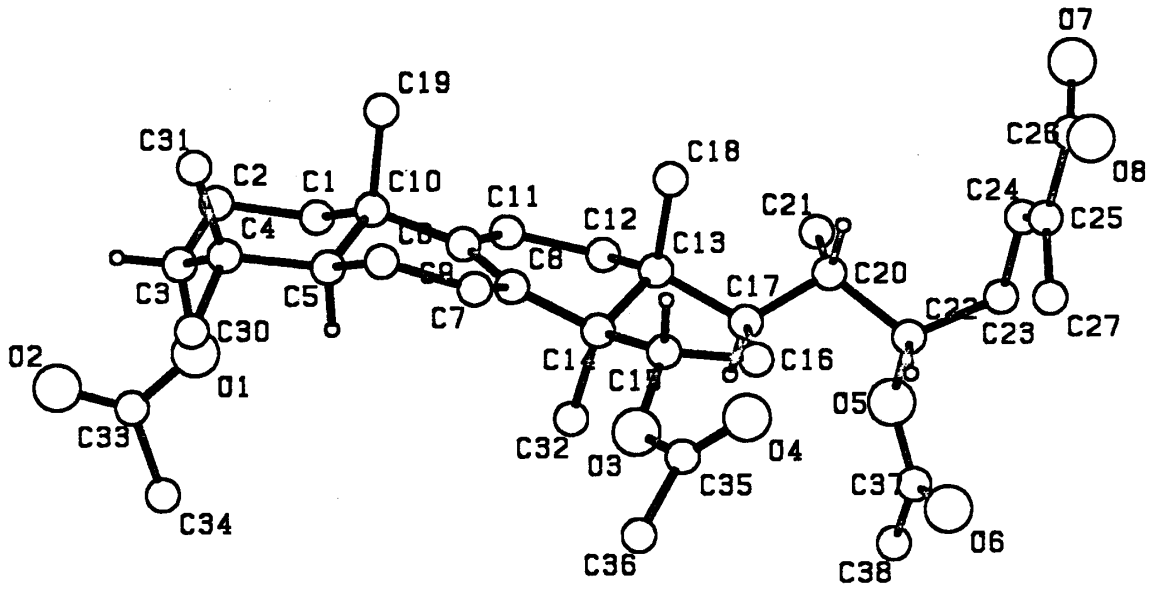


Fig. 1. A Computer-Generated Perspective Drawing of Ganoderic Acid T(1)

upfield shift of the C-3 carbon signal (δ 76.1) relative to that of 3(δ 78.1)and the loss of one acetoxyl group. In the ^{1}H-NMR spectrum of 2, a methine proton signal at δ 5.11(ddd,J= 6.8,6.0,1.7) due to 22-H was observed and a proton signal, which was absent in 1 and 3, appeared at δ 3.45(dd,J=1.4,1.4,3β-H). Based on this spectral evidence, 2 was deduced to be (22*S*,24*E*)-22-acetoxy-3α-hydroxy-5α-lanosta-7,9(11),24-trien-26-oic acid.

Table I. ^{13}C NMR Spectral Data of Compounds 1-3(75.2 MHz, $CDCl_3$)[a)]

Carbon	Compounds 1	2	3	5[b)]	Carbon	Compounds 1	2	3	5[b)]
1	30.8	29.8	30.5	30.0	19	23.0[c)]	22.7[d)]	22.4[c)]	22.8
2	23.3	25.5	23.0	25.7	20	39.8	39.3	39.3	35.9
3	78.2	76.0	78.0	76.0	21	12.8	12.6	12.6	19.0
4	36.8	37.1[c)]	36.4	37.1	22	74.6	74.6	74.6	34.8
5	44.1	43.1	44.0	43.0	23	32.1	31.7	31.8	25.7
6	22.7	27.5	27.5	23.0	24	139.2	139.5	139.6	142.7
7	121.6	120.3	120.2	121.3	25	129.4	129.0	129.0	127.4
8	140.2	142.2	142.2	140.3	26	172.0	172.2	172.3	168.7
9	146.2	145.9	145.8	146.1	27	12.4	12.2	12.2	12.4
10	37.5	37.3[c)]	37.1	37.4	30	27.9	28.1	27.7	28.2
11	115.5	115.5	115.5	115.6	31	22.6[c)]	22.5[d)]	22.5[c)]	22.7
12	38.1	37.6	37.6	38.0	32	18.6	25.7	25.6	18.2
13	44.1	43.6	43.6	44.2	AcMe	21.2	21.0	21.0	21.5
14	51.6	50.3	50.3	51.5	AcMe	21.4		21.3	21.5
15	77.4	31.2	31.3	77.5	AcMe	21.5			
16	36.7	22.9	22.8	Absent	C=O	170.7	170.6	170.6	171.2
17	45.6	47.3	47.3	48.9	C=O	170.9		170.6	171.2
18	15.9	15.4	15.4	16.0	C=O	170.2			

a) The number of protons directly attached to each individual carbon was verified experimental with the DEPT pulse sequence.

b) The data of compound 5 are cited from ref. 8).

c), d), Assignments may be interchanged in each compound.

Ganoderic acid derivatives isolated as main triterpenoid components from the cultured mycelia of *G. lucidum* have 3α substituents. In contrast, ganoderic acid derivatives from the fruit bodies of *G. lucidum* are 3β substituted or 3 keto compounds. These difference on C-3 substituent groups between ganoderic acid derivatives isolated from the cultured mycelia and those from the fruit bodies of *G. lucidum*, greatly interested us in the biosynthetic pathway of the triterpenoids of *G. lucidum*.

We recently observed, using galactosamine-induced cytotoxity in primary-cultured rat hepatocytes, that ganoderic acids R and S are strongly antihepatotoxic. The detail reports are now in preparation.

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9) Crystal data: m.w.=612.8, orthorhombic, space group $P2_12_12_1$, a=16.740(1), b=29.115(3), c=7.213(1) Å, V=3515.6(6) Å^3, z=4, d(calcd.)=1.157 gcm^{-3}, d(obsd.)=1.13 gcm^{-3}(flotation). The structure was solved by *MULTAN* 84[13] and refined by a block-diagonal least-squares technique[14] to R=0.057 for 2130 reflections[$|Fc|>\sigma(Fo)$ and $|\Delta F|<3\sigma(Fo)$] of 2994 unique ones obtained in the range of $\theta \leq 60°$ for Cu$K\alpha$ radiation.
10) W. D. S. Motherwell and W. Clegg, *PLUTO*, Program for plotting molecular and crystal structures. Univ. of Cambridge, England (1978).
11) Compound (**4**) was prepared by the reduction of methylated **1** with $LiAlH_4$. **4**, $C_{30}H_{48}O_4$(MS, M^+ at m/z 472.3534), colorless needles mp 201-202°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.63(3H,s), 0.89(3H,d,J=6.5Hz), 0.98(6H,s), 1.00(3H,s), 1.40(1H,m), 1.51(1H,dd,J=10.5, 5.0Hz), 1.69(3H,s), 3.45(1H,bs), 3.66(1H,bdd,J=9.0, 5.0Hz), 4.04(2H,s), 4.28(1H,bt,J=7Hz), 5.34(1H,bd, J=6.2Hz), 5.46(1H,bt,J=7Hz), 5.85(1H,bd,J=6Hz). ^{13}C-NMR (100.6MHz,$CDCl_3$): δ 11.8(3), 14.0(3), 15.9(3), 17.4(3), 22.7(3), 22.8(3), 22.9(2), 25.6(2), 28.2(3), 29.9(2), 33.9(2), 37.3(0), 37.4(0), 38.6(2), 39.5(2), 40.5(1), 43.1(1), 44.3(0), 45.3(1), 52.1(0), 68.7(2), 73.3(1), 74.8(1), 76.1(1), 115.7(1), 121.4(1), 122.1(1), 137.6(0), 140.8(0), 146.2(0).
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