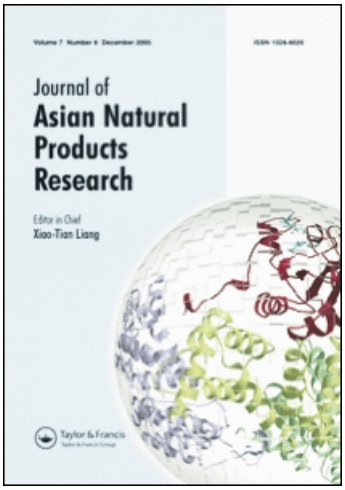

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Two new lanostanoids from *Ganoderma resinaceum*

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Two new lanostane triterpenoids, 3-epipachymic acid (3α-acetoxy-16α-hydroxy-24-methylene-5α-lanost-8-en-21-oic acid, **1**) and 3α-(3-hydroxy-5-methoxy-3-methyl-1,5-dioxopentyloxy)-24-methylene-5α-lanost-8-en-21-oic acid (**2**), together with a known compound, 3-oxo-5α-lanosta-8,24-dien-21-oic acid (**3**), were isolated from the fruiting body of *Ganoderma resinaceum*. The structure elucidation was accomplished by spectroscopic methods, especially NMR experiments. Compound **2** showed significant cytotoxic activity with IC_{50} value of 2.5 μg/ml in Hep-2 cell line.

Keywords: Lanostane triterpenoids; *Ganoderma resinaceum*; Lingzhi; Cytotoxicity

1. Introduction

Lingzhi (*Ganoderma* spp., Ganodermataceae) is a well-known Chinese medicine dating from ancient times. It is recorded in the most highly ranked herb category for its health-promoting and therapeutic properties without adverse effects [1]. In the literature, about 20 *Ganoderma* species, along with the well-known species of *G. lucidum*, have been reported to have medicinal values, and they are collectively known as Lingzhi [1–3]. So far, research work has been focused on *G. lucidum*, which is the most widely used species of Lingzhi, on its chemical and biologically active constituents [4–9]. Phytochemical studies on other Lingzhi species have been rarely reported [4,10]. As a member of *Genoderma*, *G. resinaceum* Boud. (Polyporaceae) has long been used for enhancement of internal balance and functions of the immune system [1,11]. A recent report described the antibacterial and antioxidant effects of this fungus [12]. Our study on *G. resinaceum* has now led to the isolation of two new lanostane triterpenoids (**1** and **2**; figure 1), together with a known compound, 3-oxo-5α-lanosta-8,24-dien-21-oic acid (**3**). Compounds **1**–**3** were evaluated for *in vitro* cytotoxicity in Hep-2 cell line.

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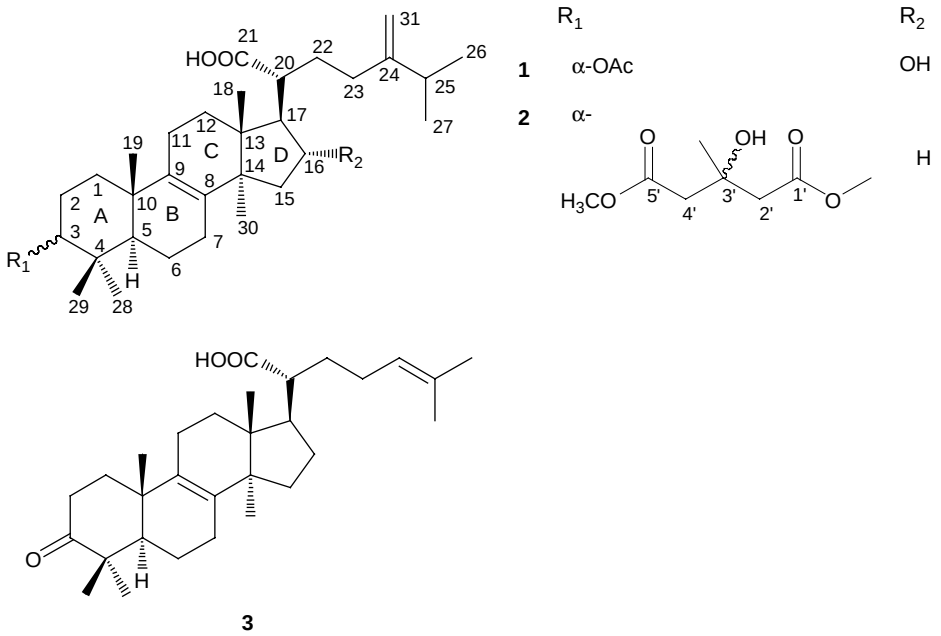

Figure 1. Structures of compounds **1**–**3**.

2. Results and discussion

Compound **1** ($C_{33}H_{52}O_5$) displayed a quasi-molecular ion peak at *m/z* 527 $[M - H]^-$ in negative FAB-MS. The molecular formula was confirmed by HRESI-MS at *m/z* 527.3712 $[M - H]^-$ and NMR spectral data (tables 1 and 2). The ^{1}H NMR, ^{13}C NMR and DEPT spectra of **1** exhibited signals for five tertiary and two secondary methyls, ten methylenes (including an exomethylene), six methines (including two oxymethines), seven quaternary carbons (including three olefinic carbons), a carboxylic group, and an acetoxy group. These NMR properties were characteristic of a 24-methylene-5α-lanostane triterpenoid [4]. Comparison of the ^{13}C NMR data of **1** with those of pachymic acid [13] indicated that the two compounds shared the same molecular formula and their structures were similar except for A-ring. An obvious difference observed in the ^{1}H NMR spectrum was that the doublet of doublet arising from H-3 in pachymic acid was replaced by a singlet in **1** at δ_H 4.86 (1H, s), suggesting that **1** was a 3-epimer of pachymic acid. In addition, the similarity between the NMR data of A-ring of **1** and those of tsugaric acid B [14] further supported that the stereochemistry of the acetoxy group attached to C-3 was in α-configuration. Direct evidence came from the NOE correlation between H-3 and CH_3-19β in the ROESY spectrum of **1**. Full assignment of **1** was finally achieved on the basis of 2D NMR analysis. Thus, compound **1** was characterised as 3-epipachymic acid, namely, 3α-acetoxy-16α-hydroxy-24-methylene-5α-lanost-8-en-21-oic acid.

The molecular formula of **2** ($C_{38}H_{60}O_7$) was determined by HRESI-MS at *m/z* 627.4254 $[M - H]^-$. The ^{1}H NMR and ^{13}C NMR spectra (tables 1 and 2) of **2** displayed signals for six tertiary and two secondary methyls, thirteen methylenes (including an exomethylene), five methines (including one oxymethines), eight quaternary carbons (including an oxygenated one and three olefinic ones), two ester carbonyl groups, one carboxylic group, and a methoxy group, which was indicative of a skeleton of 24-methylene-5α-lanost-8-en-21-oic acid as

Table 1. The ^{1}H NMR data of compounds **1** and **2** (400 MHz, δ in ppm, pyridine-d_5).

Position	***1***	***2***
1	1.37–1.43 (1H, overlap)	1.30–1.38 (1H, overlap)
	1.51–1.57 (1H, overlap)	1.53–1.60 (1H, overlap)
2	1.77–1.82 (1H, m)	1.68–1.83 (2H, overlap)
	1.68–1.72 (1H, overlap)	
3β	4.86 (1H, s)	4.97 (1H, s)
5α	1.66–1.70 (1H, overlap)	1.65–1.69 (1H, overlap)
6	1.60–1.64 (1H, overlap)	1.55–1.61 (1H, overlap)
	1.47–1.52 (1H, m)	1.42–1.47 (1H, overlap)
7	1.98–2.08 (2H, overlap)	1.97–2.10 (2H, overlap)
11	2.12–2.20 (2H, overlap)	1.90–2.10 (2H, overlap)
12	1.94–2.10 (2H, overlap)	1.57–1.75 (2H, overlap)
15	2.38–2.42 (1H, overlap)	1.97–2.05 (1H, overlap)
	1.66–1.70 (1H, overlap)	1.37–1.43 (1H, overlap)
16	4.53 (1H, m, β)	1.89–1.95 (2H, overlap)
17	2.81 (1H, dd, $J = 6.5, 5.8$ Hz)	2.41–2.37 (1H, overlap)
18	1.13 (3H, s)	1.06 (3H, s)
19	0.94 (3H, s)	0.94 (3H, s)
20	2.93–2.97 (1H, m)	2.61–2.65 (1H, m)
22	2.58–2.63 (1H, m)	1.85–1.93 (1H, overlap)
	2.42–2.46 (1H, overlap)	2.00–2.07 (1H, overlap)
23	2.48–2.53 (1H, m)	2.41–2.45 (1H, overlap)
	2.32–2.36 (1H, overlap)	2.30–2.34 (1H, m)
25	2.23–2.28 (1H, m)	2.23–2.28 (1H, m)
26	0.96 (3H, d, $J = 6.7$ Hz)	0.98 (3H, d, $J = 6.8$ Hz)
27	0.98 (3H, d, $J = 6.7$ Hz)	1.02 (3H, d, $J = 6.8$ Hz)
28	0.91 (3H, s)	0.84 (3H, s)
29	0.83 (3H, s)	1.01 (3H, s)
30	1.36 (3H, s)	0.91 (3H, s)
31	4.99 (1H, s)	4.96 (1H, s)
	4.85 (1H, s)	4.87 (1H, s)
OCH_3		3.59 (3H, s)
OAc	1.92 (3H, s)	
2′		2.97 (1H, d, $J = 14.7$ Hz)
		2.94 (1H, d, $J = 14.7$ Hz)
4′		3.05 (1H, d, $J = 14.6$ Hz)
		3.02 (1H, d, $J = 14.6$ Hz)
3′-CH_3		1.64 (3H, s)

occurred in **1**. Comparison of the NMR spectra of **2** with those of **1** revealed that, along with the disappearance of the hydroxyl group at C-16, the acetoxy group at C-3 in **1** was replaced by a seven-carbon side chain in **2**, consisting of two ester carbonyl groups [δ_C 171.2 (s) and 171.9 (s)], two methylenes [δ_C 46.4 (t), 46.1 (t); δ_H 2.97 and 2.94 (each 1H, d, $J = 14.7$ Hz), 3.05 and 3.02 (each 1H, d, $J = 14.6$ Hz)], one oxygenated quaternary carbon [δ_C 69.9 (s)], one methyl group [δ_C 28.4 (q), δ_H 1.64 (3H, s)], and one methoxy group [δ_C 51.2 (q), δ_H3.59 (3H, s)]. Careful analysis of this seven-carbon side chain based on HMQC and HMBC results established the identity of a 3-hydroxy-5-methoxy-3-methyl-1,5-dioxopentyloxy moiety. The ^{1}H–^{13}C long-range correlation between H-3 and the carbonyl carbon at δ_C171.2 (s) verified the attachment of the ester chain to C-3. The singlet of H-3 at δ_H4.97 (1H, s) implied that its coupling pattern between H-3 and H_2-2 was similar to that of **1**, thus suggesting an β-orientation of H-3. The stereochemistry of the hydroxyl group on the side chain remained undetermined. With all the available evidence, **2** was elucidated to be 3α-(3-hydroxy-5-methoxy-3-methyl-1,5-dioxopentyloxy)-24-methylene-5α-lanost-8-en-21-oic acid. It is the first example of a triterpenoid compound containing a 3-hydroxy-5-methoxy-3-methyl-1,5-dioxopentyloxy group being isolated from the *Ganoderma* species.

Table 2. The ^{13}C NMR data of compounds **1**–**2** (100 MHz, δ in ppm, pyridine-d_5).

No.	***1***	***2***	*No.*	***1***	***2***
1	31.1 t	31.3 t	21	178.8 s	178.5 s
2	23.6 t	23.6 t	22	31.6 t	31.9 t
3	77.8 d	78.3 d	23	33.2 t	32.8 t
4	37.0 s	37.0 s	24	156.1 s	156.0 s
5	45.8 d	46.0 d	25	34.1 d	34.3 d
6	18.3 t	18.4 t	26	21.9 q	22.0 q
7	26.4 t	26.3 t	27	21.9 q	21.9 q
8	134.7 s	134.4 s	28	27.8 q	28.0 q
9	134.9 s	135.1 s	29	22.0 q	21.9 q
10	37.2 s	37.3 s	30	25.7 q	24.5 q
11	21.0 t	21.2 t	31	107.0 t	107.1 t
12	29.6 t	30.8 t	$\underline{C}H_3CO$	21.1 q	
13	46.2 s	45.0 s	$\overline{C}H_3CO$	170.4 s	
14	48.8 s	49.9 s	OCH_3		51.2 q
15	43.6 t	27.5 t	1′		171.2 s
16	76.6 d	29.4 t	2′		46.4 t
17	57.3 d	47.7 d	3′		69.9 s
18	17.8 q	16.4 q	4′		46.1 t
19	19.0 q	19.1 q	5′		171.9 s
20	48.7 d	49.1 d	3′-CH_3		28.4 q

Compound **3** was obtained as white powder; $[\alpha]_D^{21} + 6.4$ (c 0.3, $CHCl_3$). The NMR spectral data were agreeable with those reported for 3-oxo-5α-lanosta-8,24-dien-21-oic acid [15].

Compounds **1**, **2** and **3** were evaluated for *in vitro* cytotoxicity against Hep-2 (human larynx carcinoma) cell line. Compound **2** exhibited significant inhibitory activity with an IC_{50} value of 2.5 μg/ml, similar to that of a positive control, cisplatin ($IC_{50} = 2.1$ μg/ml). No cytotoxic effect was observed for **1** and **3**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer, and IR spectra on a Bio-Rad FTS-135 infrared spectrophotometer. 1D-and 2D-NMR experiments were run on a Bruker AM-400 instrument. Chemical shifts (δ) were expressed in ppm with reference to TMS. MS spectra were recorded on a VG Auto Spec-3000 spectrometer or a Finnigan MAT 90 instrument. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), silica gel H (10–40 μm, Qingdao Marine Chemical), Lichroprep RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany), or MCI CHP-20P gel (70–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Fractions were monitored by TLC and spots were visualised by spraying with 10% H_2SO_4 in EtOH.

3.2 Plant material

The fruiting bodies of *G. resinaceum* were purchased from a Hong Kong market and authenticated according to the literature [16]. A sample (CUSCM-2004–0308) has been deposited in the School of Chinese Medicine, The Chinese University of Hong Kong.

3.3 Extraction and isolation

The fruiting bodies of *G. resinaceum* (1.5 kg) were extracted with 70% aqueous acetone (3 × 10 L). After evaporation of the acetone *in vacuo*, the concentrated extract was suspended in water and partitioned with EtOAc (3 × 2000 ml) to afford 80.0 g of EtOAc soluble residue, which was subjected to column chromatography over Diaion 101 macroporous resin (800 g) eluting with H_2O and MeOH (5000 ml each eluent) to provide two portions. The MeOH portion (35.3 g) was chromatographed over silica gel (200–300 mesh, 100 g) eluting with chloroform, gradually increasing the solvent strength with acetone to acquire 4 fractions: fractions A (3000 ml, chloroform), B (2000 ml, chloroform/acetone 9:1), C (1500 ml, chloroform/acetone 4:1) and D (1500 ml, acetone). Fraction A (10.2 g) was rechromatographed over silica gel (200–300 mesh, 200 g) developed with petroleum ether/acetone (9:1, 8:2, 6:4, 1:1, 0:1) in stepwise gradient mode to obtain 9 fractions: fractions A-1 to A-2 (each 1500 ml, petroleum ether/acetone 9:1), A-3 to A-7 (each 1500 ml, petroleum ether/acetone 8:2), A-8 (2000 ml, petroleum ether/acetone 6:4), A-9 (2000 ml, acetone). Compound **3** (60 mg) was obtained after repeated crystallisation from fraction A-5. The fraction A-3 was successively chromatographed on MCI-gel CHP-20P (100 g) eluting with aqueous MeOH (85%, 90%) and MeOH (1500 ml each eluent) to afford **1** (51 mg) from the eluate of 90% MeOH. The fraction A-7 (60 mg) was repeatedly rechromatographed over Lichroprep Rp-18 gel (80 g) developing with aqueous MeOH (80%, 85%, 90%) and MeOH (1500 ml each eluent) to yield compound **2** (15 mg).

3.3.1 3α-Acetoxy-16α-hydroxy-24-methylene-5α-lanost-8-en-21-oic acid (1). $C_{33}H_{52}O_5$, white powder; $[\alpha]_D^{21}$ − 11.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} log ε: 204.3 (4.0) nm; IR (KBr) ν_{max} 3432, 2957, 2877, 1736, 1707, 1639, 1457, 1376, 1250, 1181, 1061, 1035, 1017, 968, 934, 890, 592 cm^{-1}; ^1H NMR and ^{13}C NMR data, see tables 1 and 2; Negative FAB-MS *m/z* 527 $[M - H]^-$ (100), 281 (37), 255 (31); HRESI-MS *m/z* 527.3712 $[M - H]^-$ (calcd for $C_{33}H_{51}O_5$, 527.3736).

3.3.2 3α-(3-Hydroxy-5-methoxy-3-methyl-1,5-dioxopentyloxy)-24-methylene-5α-lanost-8-en-21-oic acid (2). $C_{38}H_{60}O_7$, white powder; $[\alpha]_D^{21}$ + 6.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} log ε: 204.4 (4.0) nm; IR (KBr) ν_{max} 3430, 3085, 2959, 2925, 2874, 2854, 1721, 1661, 1642, 1515, 1455, 1439, 1413, 1376, 1342, 1259, 1215, 1193, 1177, 1118, 1093, 1059, 1034, 993, 953, 931, 891 cm^{-1}; ^1H NMR and ^{13}C NMR data, see tables 1 and 2; Negative FAB-MS *m/z* 627 $[M - H]^-$ (100), 609 (30); HRESI-MS *m/z* 627.4254 $[M - H]^-$ (calcd for $C_{38}H_{59}O_7$, 627.4260).

3.4 Cytotoxicity assay

Cytotoxicity assays were performed using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] (MTT) reduction method [17,18]. In the assay, different concentrations (0, 1.57, 3.125, 6.25, 12.5, 25, 50 μg/ml) of samples (100 μl) were applied to the wells of 96-well plate containing confluent cell monolayer in triplication, while the dilution medium without the samples was used as negative control, and cisplatin as positive control. After 3 days of incubation, 12 μl of the MTT solution (5 mg/ml in phosphate

buffered saline) were added to each well. The trays were further incubated for 3 h to allow MTT formazan formation. After removing the medium, 100 µl of dimethyl sulfoxide (DMSO) were added to dissolve the formazan crystals. After 15 min, the contents in the wells were homogenised on a microplate shake. The optical densities (OD) were then read at a microplate spectrophotometer at double wavelengths of 540 and 690 nm. The median cytotoxic concentration (IC_{50}) was calculated as the concentration of the sample that decreased the number of viable cells to 50% of the cell control through the OD values of viable cells in comparison with non-viable cells.

References

[1] J.D. Zhao, X.Q. Zhang (Eds.). *Mycoflora of China – Ganodermataceae*, **Vol. 18**, Science Press, Beijing (2000).
[2] Traditional Chinese Medicine Bureau of China (Ed.), *Zhonghua Ben Cao*, **Vol. 1**, Shanghai Science and Technology Press, Shanghai (1999).
[3] H.M. Chang, P.P.H. But (Eds.). *Pharmacology and Application of Chinese Materia Medica*, **Vol. 1**, World Scientific, Singapore (1986).
[4] R.Y. Chen, D.Q. Yu. *Acta Pharm. Sin.*, **25**, 940 (1990).
[5] B.S. Min, J.J. Gao, M. Hattori, H.K. Lee, Y.H. Kim. *J. Nat. Prod.*, **67**, 811 (2001).
[6] T.S. Wu, L.S. Shi, S.C. Kuo. *J. Nat. Prod.*, **64**, 1121 (2001).
[7] T.B.T. Ha, C. Gerhauser, W.D. Zhang, N. Ho-Chong-Line, I. Fouraste. *Planta Med.*, **66**, 681 (2000).
[8] S. Lee, S. Park, J.W. Oh, C.H. Yang. *Planta Med.*, **64**, 303 (1998).
[9] J. Kenneth. *Reishi: Ancient Herb for Modern Times*, Sylvan Press, London (1992).
[10] X.M. Niu, M.H. Qiu, Z.R. Li, Y. Lu, P. Cao, Q.T. Zheng. *Tetrahedron Lett.*, **45**, 2989 (2004).
[11] S.N. Zhu. *Study on Lingzhi*, Shanghi Medical University Press, Shanghai (1994).
[12] M. Al-Fatimi, M. Wurster, H. Kreisel, U. Lindequist. *Pharmazie*, **60**, 776 (2005).
[13] T. Tai, T. Shingu, T. Kikuchi, Y. Tezuka, A. Akahori. *Phytochemisty*, **40**, 225 (1995).
[14] C.N. Lin, Y.F. Fann, M.I. Chung. *Phytochemistry*, **46**, 1143 (1997).
[15] H.J. Su, Y.F. Fann, M.I. Chung, S.J. Won, C.N. Lin. *J. Nat. Prod.*, **63**, 514 (2000).
[16] X.L. Wu, M. Zang, T.H. Xia. *Colored Illustrations of the Ganodermataceae and Other Fungi*, Guizhou Science and Technology Publishing House, Guizhou (1997).
[17] L.S.M. Ooi, H. Wang, C.W. Luk, V.E.C. Ooi. *J. Ethnopharmacol.*, **94**, 117 (2004).
[18] W.K. Liu, J.C.K. Ho, F.W.K. Cheung, B.P.L. Liu, W.C. Ye, C.T. Che. *Eur. J. Pharmacol.*, **498**, 71 (2004).