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CHEMICAL CONSTITUENTS OF *GANODERMA MULTIPILEUM* DING HOU, GANODERMATACEAE

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Summary

Five compounds, lanosta-7,9(11),24-triene-3*β*,26-diol (**1**), 3*β*-hydroxy-lanosta-7,9(11),24-trien (**2**), (22*E*)-ergosta-7,9,22-trien-3*β*-ol (**3**), (22*E*)-ergosta-7,22-dien-3-one (**4**), and methyl 2-hydroxynonacosanoate (**5**) were isolated from *Ganoderma multipileum* Ding Hou, Ganodermataceae, collected in Taynguyen. Their structures were elucidated by the spectroscopic methods, such as the ESI-MS and NMR (1D and 2D) spectroscopy and comparison with published data. Five compounds (**1–5**) are the first report of the chemical composition from *G. multipileum* in Vietnam.

Keywords: *Ganoderma multipileum;* Ganodermataceae; *Lanostane; Ergostrol.*

1. Introduction

Ganoderma consists of almost 300 species of the family Ganodermataceae globally, but about 26 species in Tay Nguyen [1]. Among them, *G. lucidum* has the most comprehensive research, but the number of publications on the species continues to increase annually. Because the species has many valuable activities as immunomodulatory [2], antitumor [3], anti-inflammation [4], cardio-vascular [5], liver protection, detoxification, hepatitis, and gastric ulcer [6],[7]. *G. multipileum* is quite popular in Kon Ka Kinh Park, Gialai [8]. *G. multipileum* parasitizes living plants of the *Delonix* genus in subtropical to tropical forests [9]. Morphologically, *G. multipileum* is quite similar to *G. lucidum*, leading to easy confusion between the two species. Research on the chemical composition and biological activity of this fungus is still very limited in Vietnam and worldwide. Only one study by Mei-Juan Li *et al.* [10] isolated 16 lanostane-triterpenoids from *G. multipileum* and their anti-inflammatory activity. In this report, we isolated and determined the structure of 5 compounds (Fig. 1): lanosta-7,9(11),24-triene-3*β*,26-diol (**1**), 3*β*-hydroxy-lanosta-7,9(11),24-trien (**2**), (22*E*)-ergosta-7,9,22-trien-3*β*-ol (**3**), (22*E*)-ergosta-7,22-dien-3-one (**4**), and methyl 2-hydroxynonacosanoate (**5**). This is the first study on the chemical composition of this species in Vietnam.

2. Materials and methods

2.1. Plant material

The fruiting bodies of the samples were collected in Kon Ka Kinh Park, Gialai, in November 2024. The mushroom was identified as *Ganoderma multipileum* Ding Hou, belonging to the Ganodermataceae family by Assoc. Prof. Nguyen Phuong Dai Nguyen. A voucher specimen (GM112024) was deposited at the National Institute of Medicinal Materials.

2.2. Methods

The NMR measurements were conducted using $CDCl_3$ as a solvent on a Bruker NMR spectrometer with a frequency of 600 MHz. Tetramethylsilane was used as an internal standard, and chemical shifts were reported in δ (ppm). Electrospray Ionization Mass Spectrometry (ESI-MS) was performed using an Agilent 1100 series LC-MSD ion trap spectrometer. Column chromatography was performed using *silica gel* (70-230 or 230-400 mesh, Merck) and reversed-phase resin (YMC ODS-A 12 nm, S-75 μm, Japan) as a stationary phase. Thin Layer Chromatography (TLC) was carried out on *silica gel* 60 $F_{254}S$ plates (Merck). Spots were detected by UV radiation (245 and 365 nm) or by spraying with 10% H_2SO_4 followed by heating.

2.3. Extraction and isolation

The dried and pulverized fruiting body of *G. multipileum* (7.0 kg) was extracted with 80% ethanol (v/v) for 3 hours at 70 °C, and concentrated under reduced pressure to yield total extract (TGM, 245 g, 3.5%). The total extract was suspended in water and successively extracted with dichloromethane (DCM) and ethyl acetate (EtOAc). The solvent was evaporated *in vacuo* to obtain the corresponding dichloromethane extract (DGM, 28.1 g), ethyl acetate extract (EGM, 13.4 g), and water extract (WGC, 197.6 g), respectively. TLC chromatograms of the fractions showed that the

EtOAc and DCM extracts were quite similar, so they were combined for column chromatography (DEGM, 42.4 g).

The DEGM extract (40 g) was chromatographed on a *silica gel* column, eluting with gradient solvents of DCM–MeOH (50:1-5:1-100% MeOH, v/v) to give 8 fractions (A-H). The A fraction (2.9 g) was continuously separated on a *silica gel* column, eluting with solvents of *n*-hexane–acetone (10:1, v/v) to afford 3 fractions (A1 – A3). Compound **4** (9 mg) was yielded from crystallizing and washing A.1 precipitation (107 mg) with an eluent of *n*-hexane–acetone (2:1, v/v). Compound **5** (6 mg) was yielded from the A3 fraction (156 mg) by reversed-phase column with an eluent of acetone–water (10:1, v/v). The C fraction (7.8 g) was separated three times on a *silica gel* column, eluting with solvents of DCM–MeOH (30:1-10:1, v/v) to yield the C.1.1.1 fraction. Compound **2** (5 mg) was yielded from the C.1.1.1 fraction (125 mg) by reversed-phase column with an eluent of MeOH–water (7:1, v/v). Compound **3** (7 mg) was yielded from preparative thin-layer chromatography, using the prepared solvents of DCM–MeOH (15:1, v/v) and eluting with DCM. The D fraction (1.7 g) was separated three times on a *silica gel* column, eluting with solvents of DCM–MeOH (15:1-5:1, v/v) to yield 4 fractions (D1 - D4). Compound **1** (12 mg) was yielded from the D2 fraction (125 mg) by reversed-phase column with an eluent of MeOH–water (5:1, v/v).

Lanosta-7,9(11),24-triene-3β,26-diol (1): colorless needles; mp. 168-170°C; ESI-MS *m/z*: 441.5 $[M+H]^+$, $C_{30}H_{48}O_2$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$) see table 1.

3β-Hydroxy-lanosta-7,9(11),24-triene (2): white power; ESI-MS *m/z*: 425.50 $[M+H]^+$, $C_{30}H_{48}O$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$) see Table 1.

(22E)-Ergosta-7,9,22-trien-3β-ol (3): white powder; ESI-MS *m/z*: 395.4 $[M-H]^-$, $C_{28}H_{44}O$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$) see table 1.

(22E)-Ergosta-7,22-dien-3-one (4): white powder; mp. 178-180°C; ESI-MS *m/z*: 419.0 $[M+Na]^+$, $C_{28}H_{44}O$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm) and ^{13}C-NMR (150 MHz, $CDCl_3$) see Table 1.

Methyl 2-hydroxynonacosanoate (5): white powder; ESI-MS *m/z*: 467.5 $[M-H]^-$, $C_{30}H_{60}O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 4.19 (1H, dd, J = 4.0, 7.5 Hz, H-2), 3.79 (3H, s, OCH_3), 1.26 - 1.80 (52xH), 0.88 (3H, t, J = 14.0 Hz, H-29). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 175.9 (COO), 70.5 (C-2), 52.5 (OCH_3), 34.5 - 22.7 (26xCH_2), 14.1 (C-29).

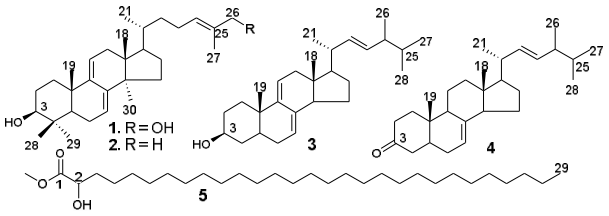

Fig. 1. Chemical structure of compounds **1** – **5**

3. Results and discussion

Compound **1** was obtained as colorless needles. Its molecular formula was determined to be $C_{30}H_{48}O_2$ based on the ESI-MS spectrum with a peak at *m/z* 441.5 $[M+H]^+$. The 1D-NMR spectra of **1** showed the characteristic signals of a lanostane-triterpenoid. The ^{1}H-NMR spectrum of **1** showed 7 methyl groups, including 6 *singlet* signals at δ_H 1.67 (3H, s, H-27), 1.09 (3H, s, H-29), 0.99 (3H, s, H-19), 0.88 (6H, s, H-28, H-30), 0.57 (3H, s, H-18) and 1 *doublet* signal at δ_H 0.92 (3H, d, J = 6.3 Hz, H-21); three oxygen-linked proton signals at δ_H 4.0 (2H, s, H-26) and 3.25 (1H, dd, J = 2.4, 9.0 Hz, H-3); 3 olefinic proton signals at δ_H 5.48 (1H, d, J = 6.0 Hz, H-11), 5.40 (1H, t, J = 7.2 Hz, H-24) and 5.32 (1H, d, J = 6.0 Hz, H-7). The ^{13}C-NMR spectrum exhibited 30 carbons: seven methyl carbon signals at δ_C 28.2 (C-29), 25.6 (C-30), 22.8 (C-19), 18.4 (C-21), 15.8 (C-28), 15.7 (C-18) and 13.6 (C-27); two oxygen-linked carbon signals at δ_C 79.0 (C-3) and 69.1 (C-26); and six olefinic carbon signals at δ_C 120.3 (C-7),143.0 (C-8), 146.0 (C-9),116.3 (C-11), 127.0 (C-24), and 134.0 (C-25). The HMBC correlations of H-28 (δ_H 0.88) and H-29 (δ_H 1.09) with C-3 (δ_C 79.0), C-4 (δ_C 38.7), and C-5 (δ_C 49.2) indicated that the hydroxy location was at C-3; the correlations of H-27 (δ_H 1.67) and H-24 (δ_H 4.0) with C-25 (δ_C 134.0) and C-26 (δ_C 69.1) indicated that the hydroxy location was at C-26, and one double bond is located at C-24/C-25. Furthermore, the other long-range correlations of H-7 (δ_H 5.32) with C-8 (δ_C 143.0) and C-6 (δ_C 23.0), and the correlations of H-11 (δ_H 5.48) with C-9 (δ_C 146.0) indicated that two double bonds are at C-7/C-8 and C-9/C-11. Based on these spectroscopic data and by comparison to published literature [11], compound **1** was identified as lanosta-7,9(11),24-triene-3β,26-diol and named ganodermadiol. This compound was isolated from *G. multipileum* Mei-Juan Li *et al.* [10]

Compound **2** was obtained as a white powder. Its molecular formula was determined to be $C_{30}H_{48}O$ based on the ESI-MS spectrum with a peak at *m/z* 425.50 $[M+H]^+$. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **2** were similar to those of **1**, a lanostane-triterpenoid, except for a methyl signal at δ_H 1.69 (H-26). And the proton and carbon signals were identified in Fig. 1 and

corresponded completely to published spectral data [12], compound **2** was identified as 3*β*-hydroxy-lanosta-7,9(11),24-triene. Compound **2** was isolated for the first time from *G. multipileum*.

Compound **3** was obtained as a yellow amorphous powder. The ESI-MS spectrum with a peak at *m/z* 395.4 $[M-H]^-$ suggested the molecular formula of **3** as $C_{28}H_{44}O$. The ^{1}H-NMR spectrum of **3** showed the presence of 6 methyl groups at δ_H 1.04 (3H, d, J = 6.4 Hz, H-21), 0.95 (3H, s, H-19), 0.92 (3H, d, J = 7.2 Hz, H-28), 0.84 (3H, d, J = 7.2 Hz, H-27), 0.83 (3H, d, J = 7.2 Hz, H-26), and 0.63 (3H, s, H-18); one hydroxymethine group at δ_H 3.62 (1H, dd, J = 4.4 & 10.6 Hz, H-3); 4 olefinic protons at δ_H 5.57 (1H, dd, J = 3.1, 5.4 Hz, H-11), 5.39 (1H, dd, J = 3.1, 5.6 Hz, H-7), 5.23 (1H, dd, J = 7.2 & 15.0 Hz, H-22) and 5.18 (1H, dd, J = 7.2, 15.0 Hz, H-23). The *E*-configuration of the double bond at C-22/C-23 was deduced from the coupling constants J = 15.0 Hz of H-22 and H-23. The ^{13}C-NMR spectrum displayed 28 carbon signals, including 6 methyl carbon signals at δ_C 21.1 (C-21), 20.0 (C-27), 19.7 (C-26), 17.6 (C-28), 16.3 (C-19) and 12.1 (C-18); one hydroxymethine carbon signal at δ_C 70.5; six olefinic carbon signals at δ_C 116.3 (C-7), 139.8 (C-8), 132.0 (C-22), 135.6 (C-23), 141.8 (C-9) and 119.6 (C-11). The HMBC correlations of H-7 (δ_H 5.39) with C-8 (δ_C 139.8) and C-5 (δ_C 46.3); and the correlations of H-11 (δ_H 5.57) with C-12 (δ_C 40.8), C-10 (δ_C 37.1), and C-9 (δ_C 141.8) indicated that two double bonds are at C-7/C-8 and C-9/C-11. Furthermore, the correlations of H-22 (δ_H 5.23) with C-20 (δ_C 40.4) and C-23 (δ_C 135.6); and the correlations of H-23 (δ_H 5.18) with C-22 (δ_C 132.0) and C-24 (δ_C 42.9) confirmed that one olefinic pair is C-22/C-23. Based on the above evidence and by comparison to published literature [13], compound **3** was identified as (22*E*)-ergosta-7,9,22-trien-3*β*-ol.

Compound **4** was isolated as a white amorphous powder with a melting point 178-180°C. Its molecular formula was determined as $C_{28}H_{44}O$ by the ESI-MS at *m/z* 419.0 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR spectra of compound **4** were similar to those of **3**, an ergostane-structure, except for a keton-carbon signal at δ_C 212.0 (C-3) and no olefinic signal at C-9/C-11. And the proton and carbon signals were identified in Table 1 and corresponded completely to published spectral data [14], compound **4** was identified as (22*E*)-ergosta-7,22-dien-3-one. Compound **4** was the first isolated from *G. multipileum*.

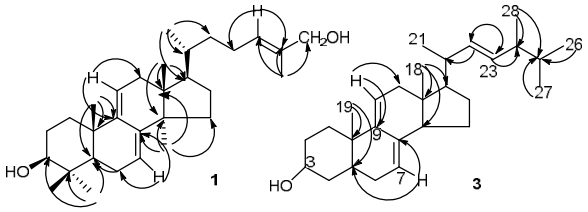

Fig. 2. The HMBC correlations of compounds **1** and **3**

Compound **5** was obtained as a white amorphous powder. The ESI-MS spectrum with a peak at *m/z* 467.5 $[M-H]^-$ suggested the molecular formula of **5** as $C_{30}H_{60}O_3$. The ^{1}H-NMR spectrum of **5** showed the presence of one *triplet* methyl group at δ_H 0.88 (3H, t, J =14.0 Hz, H-29); one *singlet* methoxy group at δ_H 3.79; 26 methylene groups at δ_H to 1.26 from 1.80; and one hydroxymethine group at δ_H 4.19 (1H, dd, J = 4.0 & 7.5 Hz, H-2). The carbon signals in the ^{13}C-NMR and DEPT spectra displayed 30 carbon signals and corresponded completely to the proton signals in the ^{1}H-NMR spectrum, including: one methyl signal at δ_C 14.1; one methoxy signal at δ_C 52.5; 26 methylene signals at δ_C 22.7 to 34.5; and one hydroxymethine signal at δ_C 70.5. Based on the 1D-NMR and MS spectral analysis, and comparison to published literature [15], compound **5** was identified as methyl 2-hydroxynonacosanoate.

The five compounds (**1**–**5**) were isolated for the first time from *G. multipileum* in Vietnam. Among them, four compounds (**1**–**4**) were isolated from *G. lucidum* [11-14], and two compounds (**2** and **5**) were published from *G. tropicum* [15]. Morphologically, the three species *G. lucidum*, *G. tropicum*, and *G. multipileum* are quite similar, so the isolated compounds can be used as chemical markers to distinguish these species.

Table 1. ^{1}H- (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$) data of compounds **1**–**4**

No.	1			2			3			4		
	δ_H (mult., J, Hz)	δ_C	δ_C[a] [11]	δ_H (mult., J, Hz)	δ_C	δ_C[b] [12]	δ_H (mult., J, Hz)	δ_C	δ_C[c] [13]	δ_H (mult., J, Hz)	δ_C	δ_C[a] [14]
1		35.8	35.8		35.8	35.7		38.4	34.6		38.8	38.8
2		28.9	28.2		27.9	27.8		32.0	31.6		38.1	38.1
3	3.25 (dd, 2.4, 10.9)	79.0	79.0	3.25 (dd, 4.6, 11.3)	79.0	79.0	3.62 (dd, 4.4, 10.6)	70.5	70.3		212.0	211.9
4		38.7	38.7		38.7	38.7		39.1	37.7		44.2	44.3
5		49.2	50.4		49.2	49.1		46.3	39.1		42.8	42.8
6		23.0	23.1		23.0	23.0		28.3	29.8		30.0	30.1
7	5.32 (d, 6.0)	120.3	120.3	5.47 (br d, 6.2)	120.2	120.1	5.39 (dd, 3.1, 5.6)	116.3	118.0	5.18 (br s)	117.0	117.0
8		143.0	142.7		142.8	142.7		139.8	135.7		139.5	139.5

No.	1			2			3			4		
	δ_H (mult., *J*, Hz)	δ_C	δ_C[a] [11]	δ_H (mult., *J*, Hz)	δ_C	δ_C[b] [12]	δ_H (mult., *J*, Hz)	δ_C	δ_C[c] [13]	δ_H (mult., *J*, Hz)	δ_C	δ_C[a] [14]
9		146.0	145.9		146.0	145.9		141.8	143.4		48.8	48.9
10		37.4	37.4		37.4	37.3		37.1	35.6		34.4	34.4
11	5.48 (m, 6.0)	116.3	116.3	5.32 (br d, 6.0)	116.4	116.3	5.57 (dd, 3.1, 5.4)	119.6	118.8		21.7	21.7
12		37.9	37.9		37.9	37.8		40.3	42.1		39.4	39.4
13		43.7	43.8		43.8	43.7		42.9	42.7		43.3	43.3
14		50.7	49.2		50.4	50.3		54.6	51.5		55.0	55.0
15		27.9	28.0		31.6	31.5		23.0	23.1		22.9	22.9
16		31.5	31.5		27.9	27.9		28.3	28.8		28.1	28.1
17		51.0	50.9		51.0	50.9		55.8	56.0		55.9	56.0
18	0.57 (s)	15.7	15.7	0.57 (s)	15.7	15.6	0.63 (s)	12.1	11.4	0.56 (s)	12.1	12.2
19	0.99 (s)	22.8	22.8	0.99 (s)	22.8	22.7	0.95 (s)	16.3	19.3	1.02 (s)	12.5	12.4
20		36.1	36.1		36.1	36.0		40.4	40.3		40.5	40.5
21	0.92 (d, 6.3)	18.4	18.4	0.91 (d, 6.4)	18.5	18.4	1.04 (d, 6.4)	21.1	19.6	1.03 (d, 6.4)	21.1	21.1
22		36.0	36.0		36.3	36.3	5.23 (dd, 7.2,15.0)	132.0	131.5	5.19 (dd, 7.8, 15.2)	135.6	135.6
23		24.6	24.6		25.0	24.9	5.18 (dd, 7.2,15.0)	135.6	135.0	5.22 (dd, 7.8, 15.2)	132.0	132.0
24	5.42 (t, 7.6)	127.0	127.0	5.11 (t, 6.4)	125.2	125.2		42.9	42.7		42.9	42.9
25		134.0	134.3		131.0	131.0		33.1	32.9		33.1	33.1
26	4.00 (s)	69.1	69.1	1.69 (s)	25.7	25.7	0.83 (d, 7.2)	19.7	19.8	0.92 (d, 7.8)	19.9	19.9
27	1.67 (s)	13.6	13.7	1.61 (s)	17.6	17.6	0.84 (d, 7.2)	20.0	20.6	0.83 (d, 7.2)	19.6	19.6
28	0.88 (s)	15.8	15.8	0.88 (s)	15.8	15.8	0.92 (d, 7.2)	17.6	17.6	0.84 (d, 7.2)	17.6	17.6
29	1.09 (s)	28.2	27.8	1.01 (s)	28.2	28.1	-	-	-	-	-	-
30	0.88 (s)	25.6	25.6	0.88 (s)	25.6	25.6	-	-	-	-	-	-

[a:] Measured in $CDCl_3$ and 125 MHz, [b:] Measured in $CDCl_3$ and 75.5 MHz, [c:] Measured in $CDCl_3$ and 25 MHz.

4. Conclusion

Five compounds were obtained from the DCM and EtOAc extracts of *G. multipileum*, including lanosta-7,9(11),24-triene-3β,26-diol (**1**), 3β-hydroxy-lanosta-7,9(11),24-trien (**2**), (22*E*)-ergosta-7,9,22-trien-3β-ol (**3**), (22*E*)-ergosta-7,22-dien-3-one (**4**), and methyl 2-hydroxynonacosanoate (**5**). The structures of these compounds were established by spectral data and literature references.

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