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THE TRITERPENOID AND STEROID FROM THE FRUITING BODY OF *Ganoderma applanatum* (Pers.) Pat. IN VIET NAM

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Abstract. Five compounds, ergosterol (**1**), 5α,8α-epidioxy-22E-ergosta-6,22-dien-3*β*-ol (**2**); ergosta-7,22-dien-3*β*-ol (**3**); lanosta-7,9(11),24-triene-3,26-diol (**4**) and 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (**5**) were isolated from fruiting body of *Ganoderma applanatum* (Pers.) Pat. (Ganodermataceae). The structures of the isolated compounds were established by spectroscopic methods (MS, ^{1}H-NMR, ^{13}C-NMR, DEPT).

Keywords: Ganoderma applanatum, Ganodermataceae, triterpenoid, steroid, lanosta-7,9(11),24-triene-3,26-diol.

Classification numbers: 1.1.1; 1.1.6.

1. INTRODUCTION

"Linh chi" - the Vietnamese name for *Ganoderma* can be used in the prevention and treatment of various types of disease. Various compounds have been isolated from the fruiting bodies, spores, gills, and mycelia of many *Ganoderma* mushrooms. *Ganoderma* contains many bioactive natural components, including triterpenes, steroid, polysaccharides, proteins, and unsaturated fatty acids [1-5]. It has been identified as new potent lead structures for the development of novel pharmaceuticals against infectious diseases, cancer, and other diseases. The majority of biological activities are its immunomodulatory and antitumor activities [1–8]. The majority of triterpenes and steroid of *Ganoderma* exhibit a wide range of biological activities, including antitumor, immunomodulatory, anti-HIV-1, antioxidant, antimicrobial antihypertensive, antiangiogenic, antiandrogenic, and antihepatitis B activities [9-12].

Ganoderma applanatum (Ganodermataceae) that has been widely used as a folk medicine for the treatment and prevention of various diseases [13]. Herein, we report the isolation of five compounds (ergosterol (**1**), 5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol (**2**); ergosta-7,22-dien-3*β*-ol (**3**); lanosta-7,9(11),24-triene-3,26-diol (**4**) and 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-

oic acid (**5**)) from fruiting body of *Ganoderma applanatum* (Pers.) Pat. (Ganodermataceae). The structures of these compounds were elucidated using a combination of 1D and 2D NMR techniques (^{1}H-, ^{13}C-NMR, HSQC and HMBC).

2. EXPERIMENTAL

2.1. General

Melting points were recorded on MP55 Melting Point System; Optical rotations were measured using an AUTOPOL VI Automatic Polarimeter; 1D-NMR and spectra were obtained on the Bruker AV-III 500 NMR spectrometer; The electrospray ionization mass spectrometry (ESI-MS); Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, E. Merck); Thin layer chromatography was conducted on precoated Kieselgel 60 F 254 plates (Merck); the compounds were visualized by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 °C for 10 min.

2.2. Fungal material

In August 2015, the basidiomycetes of *Ganoderma applanatum* (Ganodermataceae) were collected at the Puhuong of Nghe An Province, Vietnam. It was identified by Prof. Dr. Ngo Anh, Department of Biology, Hue University. A voucher specimen (Vinh-TSWu 20150815) was deposited at the herbarium of the School of Chemistry, Biology and Environment, Vinh University.

2.3. Extraction and isolation

The dried basidiomycetes (3.0 kg) of *Ganoderma applanatum* were extracted with MeOH to give a deep brown crude (128 g) after concentration in *vacuo*. The crude extract was suspended in water and subjected to a liquid/liquid partition using ethyl acetate and butanol to afford ethyl acetate (32 g), butanol (33 g) and water soluble (55 g) fractions. The crude ethyl acetate were applied to silica gel column chromatography using gradient of hexane and acetone increasing polarity (100:0 to 2:1) to afford minor fraction s, and were monitored by TLC to combine into ten major fractions (Frs. G1-G10). Purification of fraction G1 (1.2 g) by the silica gel column chromatography eluting with a mixture of hexane and acetone (15:1) afforded compound **1** (123 mg). G3 (2.6 g) was subjected to the silica gel column chromatography eluting with a hexane and ethyl acetate solvent mixture (15:1) to yield compound **4** (38 mg). G4 (2.5 g) was subjected to the silica gel column chromatography eluting with a mixture of hexane and acetone (9:1) to produce compound **2** (10 mg) and **3** (31 mg). G6 (2.9 g) was subjected to the silica gel column chromatography eluting with chloroform and methanol solvent mixture (10:1) and further recrystallization of the minor fraction to afford compound **5** (30 mg).

Compound **1**, ergosterol**:** white powder, m.p. 166-167 ^{o}C; EI-MS *m/z* 396 $[M]^{+}$; ^{1}H-NMR (500MHz, $CDCl_3$, δ ppm): 5.49 (1H, *m*, H-7), 5.35 (1H, *m*, H-6), 5.28 (1H, *dd*, *J* = 15.5, 7.5 Hz, H-22), 5.25 (1H, *dd*, *J* = 15.5, 7.0 Hz, H-23), 3.48 (1H, *m*, H-3), 1.05 (3H, *d*, *J* = 7.0 Hz, H-28), 0.96 (3H, *s*, H-19), 0.93 (3H, *d*, *J* = 7.0 Hz, H-27), 0.85 (3H, *d*, *J* = 6.5 Hz, H-26), 0.82 (3H, *d*, *J* = 6.5 Hz, H-21), 0.61 (3H, s, H-18); ^{13}C-NMR (125MHz, $CDCl_3$, δ ppm): 11.5 (C-18), 15.8 (C-19), 17.0 (C-28), 19.1 (C-21), 19.4 (C-26), 20.4 (C-27), 20.6 (C-11), 22.2 (C-15), 27.4 (C-16), 31.5 (C-2), 32.2 (C-25), 37.7 (C-10), 38.2 (C-12), 39.0 (C-1), 40.0 (C-20), 40.4 (C-4), 41.8 (C-24), 42.2 (C-13), 45.5 (C-9), 53.6 (C-14), 55.0 (C-17), 68.3 (C-3), 115.8 (C-7), 118.2 (C-6), 131.2 (C-22), 135.0 (C-23), 139.8 (C-8), 140.3 (C-5).

Compound **2**, 5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol: white powder, m.p.: 177-178 ^{o}C; EI-MS *m/z* 428 $[M]^+$; ^{1}H-NMR (500MHz, $CDCl_3$, δ ppm): 6.44 (1H, *d*, *J* = 8.5 Hz, H-7), 6.23 (1H, *d*, *J* = 8.5 Hz, H-6), 5.28 (1H, *m*, H-22), 5.18 (1H, *m*, H-23), 3.58 (1H, *m*, H-3), 1.05 (3H, *d*, *J* = 6.5 Hz, H-21), 0.96 (3H, *d*, *J* = 7.0 Hz, H-28), 0.95 (3H, *s*, H-19), 0.89 (3H, *d*, *J* = 6.5 Hz, H-27), 0.87 (3H, *s*, H-18), 0.87 (3H, *d*, *J* = 6.5 Hz, H-26); ^{13}C-NMR (125MHz, $CDCl_3$, δ ppm): 12.5 (C-18), 17.2 (C-28), 17.9 (C-19), 19.4 (C-26), 19.7 (C-27), 20.2 (C-21), 21.7 (C-11), 22.8 (C-15), 28.2 (C-16), 29.9 (C-2), 32.4 (C-25), 34.5 (C-1), 36.5 (C-10), 36.9 (C-4), 38.7 (C-12), 40.1 (C-20), 42.0 (C-24), 44.0 (C-13), 50.9 (C-9), 51.2 (C-14), 55.4 (C-17), 64.6 (C-3), 78.4 (C-8), 81.4 (C-5), 130.1 (C-7), 131.5 (C-23), 135.2 (C-6), 135.6 (C-22).

Compound **3**, ergosta-7,22-dien-3*β*-ol: colorless needles; $[\alpha]_D^{20}$ -5 (*c* = 0.85, $CHCl_3$); m.p. 185.5-187 °C ; EI-MS (*rel. int.*): *m/z* 398($[M]^+$, 4), 217(8), 255(9), 107(15), 69(24), 43(100); IR (KBr) ν_{max}: 3341, 2951, 2928, 2870, 1663, 1458, 1377, 1044, 970 cm^{-1} ; ^{1}H-NMR ($CDCl_3$, 500 MHz) (δ ppm): 5.22 (1H, *dd*, *J* =15.0, 7.5 Hz, H-22), 5.16 (1H, *m*, H-7), 5.19 (1H, *dd*, *J* =15.0, 8.0 Hz, H-23), 3.59 (1H, *m*, H-3), 1.01 (3H, *d*, *J* = 7.0 Hz, H-21), 0.91 (3H, *d*, *J* = 7.0 Hz, H-28), 0.83 (3H, *d*, *J* = 7.5 Hz, H-27), 0.80 (3H, *d*, *J* = 8.0 Hz, H-26), 0.79 (3H, *s*, H-19), 0.55 (3H, *s*, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) (δ ppm):139.6 (C-8), 135.7 (C-22), 131.9 (C-23), 117.5 (C-7), 71.1 (C-3), 56.0 (C-17), 55.1 (C-14), 49.5 (C-9), 43.3 (C-13), 42.8 (C-24), 40.5 (C-20), 40.3 (C-5), 39.5 (C-12), 38.0 (C-4), 37.2 (C-1), 34.2 (C-10), 33.1 (C-25), 31.5 (C-2), 29.7 (C-6), 28.1 (C-16), 22.9 (C-15), 21.6 (C-11), 21.1 (C-21), 20.0 (C-27), 19.7 (C-26), 17.6 (C-28), 13.0 (C-19), 12.1 (C-18).

Compound **4**, lanosta-7,9(11),24-triene-3,26-diol: white powder, m.p. 171-172 ^{0}C. EI-MS *m/z* 440 $[M]^+$; EI-MS *m/z* 412 $[M\text{-}H_2O]^+$ (13), 397(6), 394(19), 383(10), 379(21), 376(15), 269(15), 251(57), 69(100); ^{1}H-NMR (500 MHz, $CDCl_3$) (δ ppm): 5.46 (1H, *s*, H-7); 5.32 (1H, *d*, *J* = 13.5 Hz, H-11); 5.32 (1H, *d*, *J* = 13.5 Hz, H-24); 3.76(2H, *d*, *J* = 4.0 Hz, H-26); 3.00 (1H, *d*, *J* = 3.5Hz, H-3); 2.15(1H, *s*); 2.08 (1H, *s*, H-12); 2.08 (3H, *s*, H-19); 2.08(3H, *s*, H-29); 1.54 (1H, *s*, H-17); 1.54 (3H, *s*, H-27); 1.54 (3H, *s*, H-28);1.36 (2H, *m*, H-15); 1.36 (2H, *m*, H-1); 1.27 (2H, *m*, H-2); 1.27 (2H, *m*, H-20); 1.05 (2H, *d*, *J* = 8.5Hz, H-22); 0.98 (1H, *d*, *J* = 8.5Hz, H-5); 0.91 (2H, *s*, H-6); 0.91 (2H, *s*, H-16); 0.91 (3H, *s*, H-21); 0.84 (2H, *s*, H-23); 0.77 (3H, *s*, H-30); 0.53 (3H, *s*, H-18); ^{13}C-NMR (125 MHz, $CDCl_3$) (δ ppm): 142.7(C-8); 146.0 (C-9); 134.4(C-25); 127.0(C-24); 120.2(C-7); 116.3(C-11); 79.0(C-3); 69.1(C-26); 50.9(C-17); 50.3(C-14); 49.2(C-5); 43.8(C-13); 37.9(C-12); 38.7(C-4); 35.9(C-22); 35.7(C-1); 37.4(C-10); 36.1(C-20); 31.5(C-15) ;27.9(C-16); 27.8(C-2); 25.6(C-28); 22.8(C-19); 24.6(C-23); 28.2(C-29); 23.0(C-6); 15.8(C-30); 15.7(C-18); 18.4(C-21); 13.7(C-27).

Compound **5,** 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid**:** colorless needles; m.p. 243-244 ^{o}C; ESI-MS *m/z* 453 $[M\text{-}H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 6.75 (1H, *t*, *J* = 7.0 Hz, H-24); 5.42 (1H, *s*, H-7); 5.26 (1H, *s*, H-11); 3.18 (1H, *dd*, *J* = 4.7, 11.2 Hz, H-3); 1.76 (3H, *s*, H-27); 0.93 (3H, *s*, H-19); 0.92 (3H, *s*, H-29); 0.87 (3H, *d*, *J* = 5.6 Hz, H-21); 0.81 (6H, *s*, H-18, H-28); 0.50 (3H, *s*, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$) (δ ppm): 170.9 (C-26); 146.0 (C-9); 144.0 (C-24); 142.6 (C-8); 127.2 (C-25); 120.3 (C-7); 116.2 (C-11); 78.8 (C-3); 50.9 (C-17); 50.3 (C-14); 48.7 (C-5); 43.8 (C-13); 38.7 (C-4); 37.8 (C-12); 37.4 (C-10); 36.2 (C-20); 35.8 (C-1); 34.9 (C-22); 31.5 (C-16); 28.1 (C-29); 27.9 (C-2); 27.5 (C-15); 25.8 (C-23); 25.5 (C-28); 23.0 (C-6); 22.7 (C-19); 18.3 (C-21); 15.7 (C-18); 15.8 (C-30); 12.1 (C-27).

3. RESULTS AND DISCUSSION

The dried fruiting bodies of *Ganoderma applanatum* were powdered and refluxed with methanol, and the resulted extracts were partitioned with ethyl acetate and butanol to afford

ethyl acetate and butanol fractions successively. The ethyl acetate layer was subjected into purification by a repeated column chromatography to result in five compounds ergosterol (**1**), 5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol (**2**), ergosta-7,22-dien-3*β*-ol (**3**), lanosta-7,9(11),24-triene-3,26-diol (**4**), 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (**5**) (Figure 1), respectively.

Compound **1** was obtained as white powder. The molecular formula of **1** was established through the EI-MS analysis. The EI-MS showed a pseudomolecular ion peak at *m/z* 396 $[M]^+$ (suggesting to a molecular formula of $C_{28}H_{44}O$), indicating seven indices of hydrogen deficiency. The ^{1}H-NMR exhibited the signals of one oxygenated methine proton [δ_H 3.48 (H-3)], six methyl groups [δ_H 1.05 (H-28), 0.96 (H-19), 0.93 (H-27), 0.85 (H-26), 0.82 (H-21), and 0.61 (H-18)], and four olefinic protons [δ_H 5.28 (1H, *dd*, *J* = 15.5, 7.5 Hz, H-22), 5.25 (1H, *dd*, *J* = 15.5, 7.0 Hz, H-23), 5.35 (1H, *m*, H-6), and 5.49 (1H, *m*, H-7)]. Moreover, the ^{13}C-NMR spectrum displayed the signals of 28 carbons, including six olefinic carbons [δ_C 115.8 (C-7); 118.2(C-6); 131.2(C-22); 135.0 (C-23); 139.8(C-8) and 140.3(C-5)] and one oxygenated carbon [δ_C 68.3 (C-3)]. According to the UV, IR, EI-MS, ^{1}H-, ^{13}C-NMR, DEPT spectral analysis and comparison of the spectral data between **1** and ergosterol, the structure of **1** was identified as ergosterol [14,15].

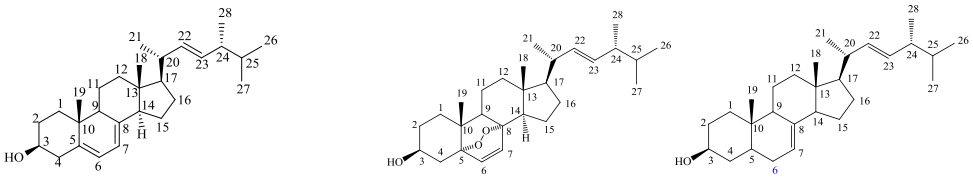
Compound **2** was obtained as white powder. The ^{1}H-NMR displayed the presence of one oxygenated methine proton [δ_H 3.58 (H-3)], six methyl groups [δ_H 1.05 (H-21), 0.96 (H-28), 0.95 (H-19), 0.89 (H-27), 0.87 (H-18), 0.87 (H-26)], and four olefinic protons [δ_H 6.23 (H-6), 6.44 (H-7), 5.28 (H-22) and 5.18 (H-23)]. The ^{13}C-NMR and DEPT spectrum of **2** displayed signals for six methyl carbons, seven methylens, eleven methines, and four quaternary carbons. In addition, the ^{13}C-NMR spectrum of compound **2** showed signals consistent with the presence of three oxygenated carbons [δ_C 81.4 (C-5); 78.4 (C-8); 64.6 (C-3)] and four olefnic carbons [δ_C 135.6 (C-22) and 131.5 (C-23); 135.2 (C-6) and 130.1 (C-7)], which were similar to those observed for compound **1**, except for the absence of one C=C double bond. Notably, these spectroscopic data were consistent with those reported in the literature for a known compound 5α,8*α*-epidioxyergosta-6,22-dien-3*β*-ol [16].

Compound **3** was obtained as optically active white powder. The ^{1}H-NMR spectrum of **3** showed clear signals for six methyl groups [δ_H 0.55 (3H, *s*), 0.79 (3H, s), 0.80 (3H, *d*, *J* = 8,0 Hz), 0.83 (3H, *d*, *J* = 7,5), 0.91 (3H, *d*, *J* = 7.0 Hz) và 1.01 (3H, *d*, *J* = 7.0 Hz)], a carbinol proton [δ_H 3.59 (1H, m)] and three olefinic protons [δ_H 5.22 (1H, *dd*, *J* = 15.0, 7.5 Hz), 5.19 (1H, *dd*, *J* = 15.0, 8.0 Hz) and 5.16 (1H, *m*)]. Additionally, the ^{13}C-NMR displayed signals of 28 carbons, including 4 olefinic carbons (δ_C 117.5, 139.6, 131.9 and 135.7) and one oxygenated carbon (δ_C 71.1). Based on the above evidence, the chemical structure of **3** was established as ergosta-7,22-dien-3-ol [17,18]. It was reported from *Coriolus sanguineus, Fomes sp., Polyporus sp...*

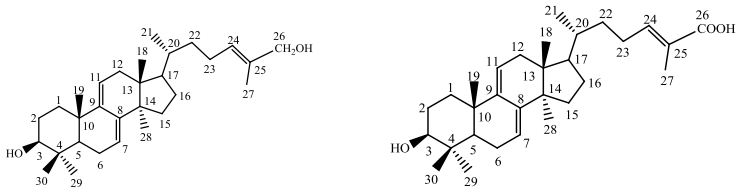
Compound **4** was obtained as white powder. The molecular formula of **4** was established through the EI-MS analysis. The EI-MS showed a pseudomolecular ion peak at *m/z* 440 $[M]^+$, combination with ^{1}H- and ^{13}C-NMR spectral data allowed to propose the molecular formula of $C_{30}H_{48}O_2$. The ^{1}H-NMR spectrum showed signals due to three olefinic protons [δ_H 5.46 (1H, *s*, H-7); 5.32 (1H, *d*, *J* = 13.5Hz, H-11); 5.32(1H, *d*, *J* = 13.5 Hz, H-24)], and one oxygenated methine proton [δ_H 3.00 (1H, *d*, *J* = 3.5Hz, H-3)]. Additionally, its ^{1}H-NMR showed signals due to protons for six methyl groups [δ_H 2.08 (3H, *s*, H-29); 2.08 (3H, *s*, H-19); 1.54 (3H, *s*, H-27); 0.91 (3H, *s*, H-21); 0.77 (3H, *s*, H-30); 0.53 (3H, *s*, H-18)]. The ^{13}C-NMR and DEPT spectra of **4** exhibited 30 carbon signals, corresponding to a triterpenoid (including 7 methyl, 6 methine,

10 methylene and 7 nonprotonated carbons). Its ^{13}C-NMR showed the typical signals of olefinic carbons [δ_C 142.7(C-8); 146.0 (C-9); 134.4(C-25); 127.0(C-24); 120.2(C-7); 116.3(C-11)]. Compounds **4** was identified as lanosta-7,9(11),24-triene-3,26-diol by comparison of its physical and spectroscopic properties with those reported in the literature [8]. This compound has been isolated from *Ganoderma lucidum* [9].

Compound **5** was obtained as white powder. The EI-MS of **5** showed a pseudomolecular ion peak at *m/z* 454 $[M]^+$, combination with ^{1}H- and ^{13}C-NMR spectral data allowed to propose the molecular formula of $C_{30}H_{46}O_3$. In its ^{1}H-NMR spectrum, there were proton signals characteristic of three olefinic protons [δ_H 5.47 (1H, *d*, H-7); 6.91 (1H, *t*, H-24) and 5.32 (1H, *d*, H-11)]. In the downfield region, one oxygenated proton at δ_H 3.26 (1H, *d*, H-3) suggested the C-3 hydroxylation. Moreover, the ^{1}H-NMR spectrum displayed signals of seven methyl groups [δ_H 1.00 (H-29); 0.98 (H-19); 0.88 (H-28); 0.88 (H-30); 0.57 (H-18), 1.84 (H-27), 0.92 (H-21)]. There were proton signals characteristic of the triterpenoid basic skeleton (lanostane). The ^{13}C-NMR and DEPT spectrum of **5** displayed characteristic signals for 30 carbons, including: seven methyl, eight methylens, seven methines and seven quaternary carbons. In its ^{13}C-NMR spectrum, there were signals characteristic of olefinic carbons [δ_C 146.0 (C-9); 143.0 (C-8); 146.0 (C-25); 127.0 (C-26); 120.3 (C-7); 116.2 (C-11)], seven carbon methyl groups [δ_C 16.0 (C-30); 28.1 (C-29); 26.0 (C-28); 22.7 (C-19); 18.3 (C-21); 16.0 (C-18); 12.0 (C-27)]. Notably, these spectroscopic data were consistent with those reported in the literature for a known compound 3*β* -hydroxy- 5α-lanosta 7,9,24 (*E*)-trien-26-oic acid. The compound **5** defined as 3*β*-hydroxy-5α-lanosta 7,9,24 (*E*)-trien-26-oic acid. This compound has been isolated from *Ganoderma lucidum* [19].



(**1**) Ergosterol (**2**) 5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol (**3**) Ergosta-7,22-dien-3*β*-ol



(**4**) lanosta-7,9(11),24-triene-3,26-diol (**5**) 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid

4. CONCLUSION

Finally, we have succeeded in studying chemical composition of the *Ganoderma applanatum* (Pers.) Pat. and resulted in the identification of five compounds, including ergosterol (**1**), 5α,8α-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol (**2**); ergosta-7,22-dien-3*β*-ol (**3**); lanosta-7,9(11),24-triene-3,26-diol (**4**) and 3*β*-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (**5**). The structure elucidation of the five compounds were determined on the basis of MS and NMR spectrometric methods.

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