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CHEMICAL CONSTITUENTS OF THE DICHLOROMETHANE EXTRACT OF *GANODERMA COCHLEAR*

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Summary

Chemical Constituents of the Dichloromethane Extract of *Ganoderma cochlear*

From the dichloromethane extract of *Ganoderma cochlear*, five compounds (**1** - **5**) were isolated by column chromatography. Their chemical structures were identified as fornicatin F (**1**), 1-tetracosanoylglycerol (**2**), (22*E*)-ergosta-7,9(11),22-trien-3*β*-ol (**3**), polycarpol (**4**), and lucidenic acid N (**5**) based on spectral data and literature references. Compounds **2** - **5** were isolated from *G. cochlear* for the first time.

Keywords: *Ganoderma cochlear*, *1-tetracosanoylglycerol, (22E)-ergosta-7,9(11),22-trien-3β-ol, Lucidenic acid N.*

1. Introduction

Ganoderma cochlear (Nees) Merr., belongs to the Ganodermataceae family. The species is popular in Tay Nguyen and is often used as a substitute for *G. lucidum* to enhance immunity and hepatoprotective effects [1]. Up-to-date phytochemical studies have shown over 90 compounds, including mainly triterpenoids, sterols, phenols, and especially alkaloids [2],[3],[4],[5],[6],[7],[8],[9],[10],[11],[12]. Scientific studies *in vitro* have shown the species has valuable effects such as anti-inflammatory [4], hepatoprotective [2], renoprotective [3],[6],[8], neuroprotective [7], anti-oxidant [11], and cytotoxic activity [5],[13],[14]. These activities are mainly attributed to terpenoids and phenolic compounds. However, in Vietnam, there is only one study on the chemical constituents of this mushroom [15]. In this study, we isolated and identified five compounds (**1** - **5**) from the dichloromethane extract of *Ganoderma cochlear*: fornicatin F (**1**), 1-tetracosanoylglycerol (**2**), (22*E*)-ergosta-7,9(11),22-trien-3*β*-ol (**3**), polycarpol (**4**), and lucidenic acid N (Fig.1). Among them, compounds **2** - **5** are reported for the first time from *G. cochlear*.

2. Materials and methods

2.1. Plant materials

The samples were collected in Kon Ka Kinh Park, Gialai, in December 2023. The mushroom was identified as *Ganoderma cochlear* (Nees) Merr., which belongs to the Ganodermataceae family, by Assoc. Prof. Nguyen Phuong Dai Nguyen. A voucher specimen (GC122023) was deposited at the National Institute of Medicinal Materials.

2.2. General experiment procedures

The NMR measurements were conducted using CD_3OD and $CDCl_3$ as solvents on a Bruker NMR spectrometer with a frequency of 500 or 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 YMC (ODS-A 12 nm, S-75 µm, Japan) as the stationary phase. Thin Layer Chromatography (TLC) was carried out on *silica gel* 60 F_{254} 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 *G. cochlear* (10.0 kg) was extracted with 240 L 80% ethanol (v/v) for 3 hours at 70 °C, and concentrated under reduced pressure to yield total extract (TGC, 510 g, 5.1%). The total extract was suspended in water and successively extracted with dichloromethane (DCM), ethyl acetate (EtOAc), and *n*-butanol. The solvents were evaporated *in vacuo* to obtain corresponding dichloromethane (DGC, 37.3 g), ethyl acetate (EGC, 102 g), *n*-butanol (BGC, 85.6 g), and water (WGC, 274.5 g) extracts, respectively.

The DGC extract (35 g) was chromatographed on a *silica gel* column, eluting with gradient solvents of *n*-hexane/EtOAc/methanol (50/1/1 - 5/1/1 - 100% MeOH, v/v/v) to give 6 fractions (A-F). Fraction A (3.9 g) was continuously separated on a *silica gel* column, eluting with gradient solvents of *n*-hexane/acetone (15/1 - 1/1, v/v) to afford 3 fractions (A1 - A3). Fraction A1 (985 mg) was crystallized and washed five times with *n*-hexane/methanol (3/1, v/v) to yield compound **1** (21 mg) and a washing solution. Compound **2** (7 mg) was yielded from the concentrated residue of the washing solution (255 mg) by a *silica gel* column with an eluent of *n*-hexane/acetone (5/1, v/v). Fraction A3 (927 mg) was separated on a *silica gel* column, eluting with *n*-hexane/acetone (3/1, v/v) to afford compounds **3** (12 mg) and **4** (14 mg). Fraction C (1.1 g) was further separated on a *silica gel* column, eluting with DCM/MeOH (15/1, v/v) to give 3 fractions (C1 - C3). Compound **5** (12 mg) was obtained from fraction C1 (72 mg) by a YMC column with an eluent of MeOH/water (5/1, v/v).

Compound **1** (fornicatin F): colorless needles. UV (MeOH) λ_{max} 256 và 201 nm. $[\alpha]_D$ +87.2° (*c* 0.1, MeOH). ESI-MS *m/z*: 489.5 $[M+H]^+$, $C_{29}H_{44}O_6$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 5.07 & 4.84 (2H, s, H-28), 4.27 (1H, br s, H-7), 3.67 (3H, s, 24-OAc), 3.63 (3H, s, 3-OAc), 1.84 (3H, s, H-29), 1.27 (3H, s, H-19), 1.12 (3H, s, H-30), 0.95 (3H, s, H-18), 0.87 (3H, d, *J* = 6.6 Hz, H-21). ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 200.0 (C-11), 174.5 (C-24), 174.2 (C-3), 161.3 (C-8), 147.9 (C-4), 137.4 (C-9), 115.0 (C-28), 66.7 (C-7), 52.1 (C-14), 51.6 (3-OAc), 51.5 (24-OAc), 51.4 (C-12), 50.2 (C-17), 45.5 (C-13), 45.1 (C-5), 39.1 (C-10), 35.9 (C-20), 33.4 (C-1), 32.5 (C-6), 31.2 (C-22), 31.0 (C-23), 30.6 (C-15), 29.5 (C-2), 27.3 (C-16), 26.7 (C-30), 24.4 (C-29), 22.6 (C-19), 18.1 (C-21), 17.9 (C-18).

Compound **2** (1-tetracosanoylglycerol): white powder. ESI-MS *m/z*: 443.6 $[M+H]^+$, $C_{27}H_{54}O_4$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 4.20 (1H, dd, *J* = 3.6, 14.4 Hz, H-1′ *α*), 4.16 (1H, dd, *J* = 4.8, 11.4 Hz, H-1′ *β*), 3.90 (1H, br s, H-2′), 3.60 - 3.70 (2H, m, H-3′), 2.35 (2H, t, *J* = 6.6 Hz, H-2), 1.26 - 1.33 (40H, m, H-3→H-22), 1.26 - 1.33 (2H, m, H-23), 0.88 (3H, t, *J* = 6.6 Hz, H-24). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 174.2 (C-1), 70.3 (C-2′), 65.2 (C-1′), 63.4 (C-3′), 34.2 (C-2), 24.9 - 31.9 (C-3 - C-22), 22.7 (C-23), 14.1 (C-24).

Compound **3** ((22*E*)-ergosta-7,9(11),22-trien-3*β*-ol): white powder. ESI-MS *m/z*: 395.40 $[M-H]^-$, $C_{28}H_{44}O$. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 5.57 (1H, dd, *J* = 3.0, 5.5 Hz, H-11), 5.39 (1H, dd, *J* = 3.0, 5.5 Hz, H-7), 5.23 (1H, dd, *J* = 7.0, 15.0 Hz, H-22), 5.18 (1H, dd, *J* = 7.5, 15.0 Hz, H-23), 3.62 (1H, dd, *J* = 4.5, 10.5 Hz, H-3), 1.04 (3H, d, *J* = 6.5 Hz, H-21), 0.95 (3H, s, H-19), 0.92 (3H, d, *J* = 7.0 Hz, H-28), 0.84 (3H, d, *J* = 7.0 Hz, H-27), 0.83 (3H, d, *J* = 7.0 Hz, H-26), 0.63 (3H, s, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 141.8 (C-9), 139.8 (C-8), 135.6 (C-23), 132.0 (C-22), 119.6 (C-11), 116.3 (C-7), 70.5 (C-3), 55.8 (C-17), 54.6 (C-14), 46.3 (C-5), 42.9 (C-13), 42.9 (C-24), 40.8 (C-12), 40.4 (C-20), 39.1 (C-4), 38.4 (C-1), 37.1 (C-10), 33.1 (C-25), 32.0 (C-2), 28.3 (C-6), 23.2 (C-16), 21.1 (C-21), 21.1 (C-15), 20.0 (C-27), 19.7 (C-26), 17.6 (C-28), 16.3 (C-19), 12.1 (C-18).

Compound **4** (polycarpol): white amorphous solid. $[\alpha]_D$ +87° (*c* 1.25, $CHCl_3$). ESI-MS *m/z*: 439.35 $[M-H]^-$, $C_{30}H_{48}O_2$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 5.48 (1H, d, *J* = 6.0 Hz, H-7), 5.32 (1H, d, *J* = 6.0 Hz, H-11), 5.15 (1H, t, *J* = 7.2 Hz, H-24), 3.68 (1H, dd, *J* = 6.0, 9.6 Hz, H-15), 3.25 (1H, d, *J* = 4.2, 12.0 Hz, H-3), 1.73 (3H, s, H-

27), 1.64 (3H, s, H-26), 1.10 (3H, s, H-29), 0.99 (3H, s, H-19), 0.92 (3H, s, H-30), 0.91 (3H, d, J = 6.6 Hz, H-21), 0.89 (3H, s, H-28), 0.58 (3H, s, H-18). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 145.9 (C-9), 142.7 (C-8), 134.5 (C-25), 121.0 (C-24), 120.2 (C-7), 116.3 (C-11), 79.0 (C-3), 73.4 (C-15), 50.3 (C-14), 49.1 (C-17), 47.2 (C-5), 43.7 (C-13), 40.5 (C-20), 38.7 (C-4), 37.9 (C-16), 37.4 (C-10), 35.7 (C-12), 34.4 (C-22), 31.5 (C-1), 28.2 (C-28), 27.8 (C-2), 27.3 (C-23), 25.9 (C-27), 25.7 (C-19), 23.0 (C-6), 22.8 (C-21), 18.2 (C-26), 15.8 (C-30), 15.6 (C-18), 11.7 (C-29).

Compound **5** (lucidenic acid N): white power. ESI-MS m/z: 485.3 $[M-H]^-$, $C_{27}H_{40}O_6$. ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 4.85 (1H, dd, J = 9.5, 8.0 Hz, H-7), 3.17 (1H, dd, J = 5.0, 12.0 Hz, H-7), 1.39 (3H, s, H-30), 1.23 (3H, s, H-19), 1.04 (3H, s, H-29), 1.00 (3H, d, J = 7.0 Hz, H-21), 0.99 (3H, s, H-18), 0.86 (3H, s, H-28). ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 218.8 (C-15), 200.5 (C-11), 177.6 (C-24), 158.8 (C-8), 144.1 (C-9), 79.0 (C-3), 68.0 (C-7), 60.5 (C-14), 51.5 (C-12), 50.3 (C-5), 47.0 (C-17), 46.7 (C-13), 41.9 (C-16), 39.9 (C-10), 39.7 (C-4), 36.4 (C-20), 36.0 (C-1), 31.9 (C-22), 31.7 (C-23), 28.7 (C-29), 28.3 (C-2), 28.0 (C-6), 24.9 (C-30), 18.9 (C-19), 18.5 (C-21), 17.8 (C-18), 16.2 (C-28).

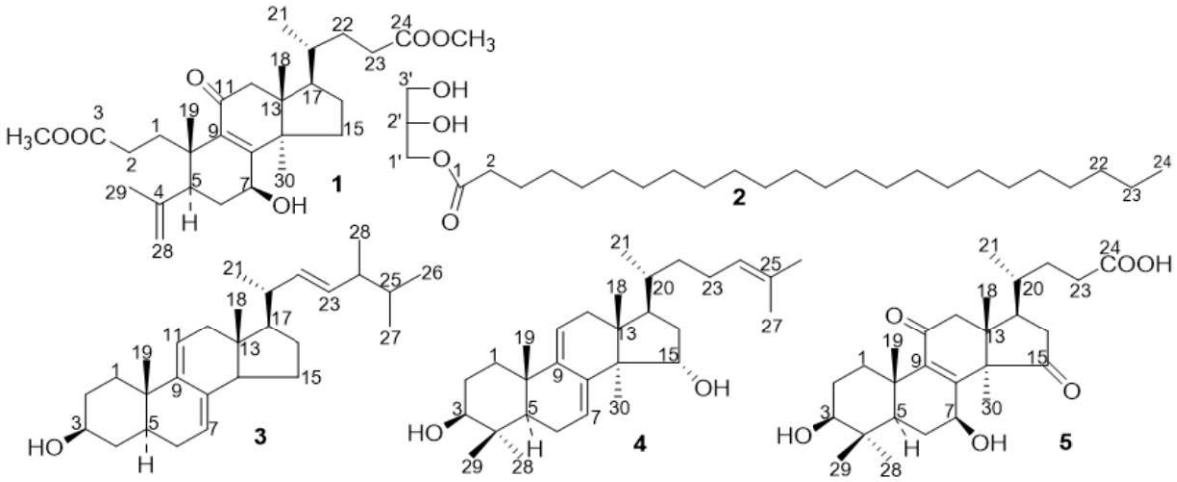

Fig. 1. Chemical structures of compounds (**1-5**)

3. Results and discussion

Compound **1** was isolated as colorless needles. It had a molecular formula of $C_{29}H_{44}O_6$ based on the ESI-MS spectrum with a peak at m/z 489.5 $[M+H]^+$. The 1D-NMR spectra of **1** showed the characteristic signals of a 3,4-*seco*-trinorlanostane-triterpenoid. The ^{1}H-NMR spectrum of **1** showed five methyl groups at δ_H 1.84 (3H, s, H-29), 1.27 (3H, s, H-19), 1.12 (3H, s, H-30), 0.95 (3H, s, H-18), and 0.87 (3H, d, J = 6.6 Hz, H-21); two methoxy groups at δ_H 3.67 (3H, s, 24-OAc) and 3.63 (3H, s, 3-OAc); one hydroxymethine group at δ_H 4.27 (1H, br s, H-7); and one methylene group at δ_H 4.84 and 5.07 (2H, s, H-28). The ^{13}C-NMR spectrum exhibited 29 carbons: five methyl signals at δ_C 26.7 (C-30), 22.6 (C-19), 24.4 (C-29), 17.9 (C-18), and 18.1 (C-21); one hydroxymethine signal at δ_C 66.7 (C-7); four olefinic signals at δ_C 115.0 (C-28), 147.9 (C-4), 161.3 (C-8), and 137.4 (C-9); and one ketone signal at δ_C 200.0 (C-11). Moreover, the ^{13}C-NMR spectrum showed 2 methyl signals at δ_C 51.6 and 51.5 and 2 carbonyl signals at δ_C 174.5 and 174.2, which indicated the presence of 2 ester groups. The HMBC correlations of H-22 (δ_H 2.33 - 2.34 and 2.22 - 2.27), H-23 (δ_H 2.34 - 2.36 and 1.26 - 1.36), and methyl (δ_H 3.67) with C-24 (δ_C 177.5) indicated the ester group at C-24; and the correlations of H-2 (δ_H 1.99 - 2.08 and 2.22 - 2.27), H-1 (δ_H 2.09 - 2.13), and methyl (δ_H 3.63) with C-3 (δ_C 174.2) indicated that the remaining ester group was positioned at C-3 (see Fig. 2). Furthermore, the long-range correlations of H-28 (δ_H 5.07 and 4.84) with C-5 (δ_C 45.1) and C-29 (δ_C 24.4) and the correlations of H-5 (δ_H 2.13) with C-4 (δ_C 147.9), C-6 (δ_C 32.5) and C-7 (δ_C 66.7) confirmed a 3,4-*seco*-lanostane compound, that is the characteristic structure from *G. cochlear*. The 7β-OH-configuration was assigned based on the coincidence of NMR spectroscopic

data and $[\alpha]_D$ value. Compound **1** was identified as methyl 7*β*-hydroxy-11-oxo-3,4-*seco*-25,26,27-trinorlanosta-4(28),8-diene-3,24-diester, and named fornicatin F [2].

Compound **2** was isolated as a white powder. Its molecular formula was determined to be $C_{27}H_{54}O_4$ based on the ESI-MS spectrum with a peak at *m/z* 443.6 $[M+H]^+$. The ^{1}H-NMR spectrum of **2** showed a triplet signal for a methyl group at δ_H 0.88 (3H, t, *J* = 6.6 Hz, H-24); signals of 22 methylene groups at δ_H from 1.26 to 2.35; and five signals at δ_H 4.20 (1H, dd, *J* = 3.6, 14.4 Hz, H-1′α), 4.16 (1H, dd, *J* = 4.8, 11.4 Hz, H-1′*β*), 3.90 (1H, br s, H-2′) and 3.60 - 3.70 (2H, m, H-3′) corresponding to two oxymethylene and one oxymethine groups. The ^{13}C-NMR spectrum displayed 27 carbon signals, including one carbonyl group at δ_C 174.2 (C-1); one oxymethine and two oxymethylene groups at δ_C 70.3 (C-2′), 65.2 (C-1′) and 63.4 (C-3′); 22 methylene groups at δ_C from 24.9 to 34.2; and one methyl group at δ_C 14.1 (C-24). The HMBC correlations of H-2 (δ_H 2.34) with C-1 (δ_C 174.2) and C-3 (δ_C 24.9); and H-1′ (δ_H 4.20 and 4.16) with C-1 (δ_C 174.2) indicated the ester group at C-1. Compound **2** was identified as 1-tetracosanoylglycerol, based on the above spectroscopic evidence and by comparison to the published literature [16]. Compound **2** is isolated from *G. cochlear* for the first time.

Compound **3** was obtained as a white 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 six methyl groups at δ_H 1.04 (3H, d, *J* = 7.0 Hz, H-21), 0.95 (3H, s, H-19), 0.92 (3H, d, *J* = 7.0 Hz, H-28), 0.84 (3H, d, *J* = 7.0 Hz, H-27), 0.83 (3H, d, *J* = 7.0 Hz, H-26), and 0.63 (3H, s, H-18); one hydroxymethine group at δ_H 3.62 (1H, dd, *J* = 4.5, 10.5 Hz, H-3); four olefinic protons at δ_H 5.57 (1H, dd, *J* = 3.0, 5.5 Hz, H-11), 5.39 (1H, dd, *J* = 3.0, 5.5 Hz, H-7), 5.23 (1H, dd, *J* = 7.0, 15.0 Hz, H-22) and 5.18 (1H, dd, *J* = 7.5, 15.0 Hz, H-23). The *E*-configuration of the C-22/C-23 double bond was deduced from the coupling constants *J* = 15.0 Hz of H-22 and H-23 [17]. The ^{13}C-NMR spectrum displayed 28 carbon signals, including six methyl 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 signal at δ_C 70.5; six olefinic 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 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 the 2 double bonds were located at C-7/C-8 and C-9/C-11. The HMBC correlations of H-22 (δ_H 5.23) with C-20 (δ_C 40.4) and C-23 (δ_C 135.6); and H-23 (δ_H 5.18) with C-22 (δ_C 132.0) and C-24 (δ_C 42.9) confirmed the remaining double bond at C-22/C-23. Based on the above evidence and by comparison to the published literature [17], compound **3** was identified as (22*E*)-ergosta-7,9(11),22-trien-3*β*-ol. This compound is reported for the first time from *G. cochlear*.

Compound **4** was obtained as a white amorphous solid. 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* 439.4 $[M-H]^-$. The 1D-NMR spectra of **4** showed the characteristic signals of a lanostane-triterpenoid. The ^{1}H-NMR spectrum of **4** showed 8 methyl groups, including 7 *singlet* signals at δ_H 1.73 (3H, s, H-27), 1.64 (3H, s, H-26), 1.10 (3H, s, H-29), 0.99 (3H, s, H-19), 0.92 (3H, s, H-30), 0.89 (3H, s, H-28), and 0.58 (3H, s, H-18) and 1 *doublet* signal at δ_H 0.91 (3H, d, *J* = 6.6 Hz, H-21); 2 hydroxymethine signals at δ_H 3.68 (1H, dd, *J* = 6.0, 9.6 Hz, H-15) and 3.25 (1H, dd, *J* = 4.2, 12.0 Hz, H-3); 3 olefinic signals at δ_H 5.48 (1H, d, *J* = 6.0 Hz, H-7), 5.32 (1H, d, *J* = 6.0 Hz, H-11) and 5.15 (1H, t, *J* = 7.2 Hz, H-24). The ^{13}C-NMR and DEPT showed thirty carbon signals, including 8 methyl groups at δ_C 28.2 (C-28), 25.9 (C-27), 25.7 (C-19), 22.8 (C-21), 18.2 (C-26), 15.8 (C-30), 15.6 (C-18) và 11.7 (C-29); 2 hydroxymethine groups at δ_C 79.0 (C-3) and 73.4 (C-15); 6 olefinic carbons at δ_C 145.9 (C-9), 116.3 (C-11), 120.2 (C-7), 142.7 (C-8), 121.0 (C-24) and 134.5 (C-25). Based on the comparison to the published literature [18], compound **4** was identified as polycarpol. This compound is isolated from *G. cochlear* for the first time.

Compound **5** was isolated as a white powder. The ESI-MS spectrum with a peak at *m/z* 459.5 $[M-H]^-$ suggested the molecular formula of **5** as $C_{27}H_{40}O_6$. The 1D-NMR spectra of **5** showed characteristic signals of a trinorlanostane-triterpenoid. The ^{1}H-NMR spectrum of **5** showed 6 methyl signals, including 5 singlet signals at δ_H 1.39 (3H, s, H-30), 1.23 (3H, s, H-19), 1.04 (3H, s, H-29), 0.99 (3H, s, H-18) and 0.86 (3H, s, H-28) and

1 *doublet* signal at δ_H 1.00 (3H, d, J = 7.0 Hz, H-21); two hydroxymethine signals at δ_H 4.85 (1H, dd, J = 9.5, 8.0 Hz, H-7) and 3.17 (1H, dd, J = 5.0, 12.0 Hz, H-3). The β-configuration of hydroxyl groups (3-OH and 7-OH) was deduced from the coupling constants of H-3 (J = 5.0, 12.0 Hz) and H-7 (J = 9.5, 8.0 Hz) and was compared with the published data [19]. The ^{13}C-NMR and DEPT showed twenty-seven carbon signals, including 6 methyl groups at δ_C 28.7 (C-29), 24.9 (C-30), 18.9 (C-19), 18.5 (C-21), 17.8 (C-18) and 16.2 (C-28); 2 hydroxymethine groups at δ_C 79.0 (C-3) and 68.0 (C-7); one olefinic group at δ_C 158.8 (C-8) and 144.1 (C-9); 3 carbonyl groups at δ_C 218.8 (C-15), 200.5 (C-11) and 177.6 (C-24). The carbon signals were assigned by comparison to the published literature [19]. Thus, compound **5** was identified as lucidenic acid N [19]. This compound is reported for the first time from *G. cochlear*.

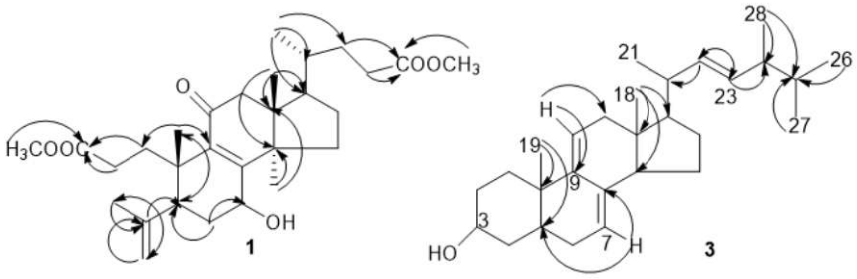

Fig. 2. The HMBC correlations of compounds **1** and **3**

4. Conclusion

Five compounds were obtained from the dichloromethane extract of *G. cochlear,* including fornicatin F (**1**), 1-tetracosanoylglycerol (**2**), (22*E*)-ergosta-7,9(11),22-trien-3β-ol (**3**), polycarpol (**4**) and lucidenic acid N (**5**). The structures of these compounds were established by spectral data and literature references. Among them, compounds **2** - **5** were isolated from *G. cochlear* for the first time.

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STEROID AND TRITERPENOID COMPOUNDS ISOLATED FROM *AMANITA PANTHERINA* AND THEIR ANTI-INFLAMMATORY ACTIVITY

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Summary

Steroid and Triterpenoid Compounds Isolated from *Amanita pantherina* and their Anti-Inflammatory Activity

Phytochemical research of the ethanolic extract of *Amanita pantherina* sensu Gonnermann & Rabenhorst led to the isolation of six steroids (**1–6**) and a triterpenoid (**7**), using various chromatographic separations. Their structures were elucidated to be β-sitosterol (**1**), stigmasterol (**2**), cycloeucalenol (**3**), spinasterol (**4**), daucosterol (**5**), stigmasterol 3-*O*-β-D-glucoside (**6**), and betulin (**7**) by detailed analysis of NMR spectroscopic records and comparison with those reported. Compound **7** showed the most inhibitory activity (IC_{50} = 33.4 µM) against the LPS-induced NO production in macrophage RAW 264.7 cells, followed by compound **3** with an IC_{50} value of 48.2 µM. Compounds **1**, **2**, and **4** exhibited weak inhibitory activity with IC_{50} values of 92.8, 64.9, and 62.4 µM, respectively, while compounds **5** and **6** were inactive (IC_{50} > 100 µM). This is the first time compounds **3** and **7** have been evaluated for their inhibitory effects on NO production. In addition, compounds **2**, **3**, and **7** showed weak inhibitory activity against tumor necrosis factor-alpha (TNF-α) with IC_{50} values of 83.9, 72.8, and 58.1 µM, respectively.

Keywords: *Amanita pantherina* sensu Gonnermann & Rabenhorst, *Sterol, Inflammatory activity, NO production, Cytotoxic, RAW264.7 cells.*

1. Introduction

Amanita pantherina sensu Gonnermann & Rabenhorst has fascinated both traditional cultures and modern researchers due to its striking appearance and complex biochemical properties. Historically, it has been used in rituals and conventional medicine. Today, its bioactivity, particularly muscimol and ibotenic acid, has drawn attention to potential therapeutic applications despite its toxicity [1],[2]. In contemporary research, the bioactivity of *A. pantherina* has garnered attention for its potential therapeutic applications. Studies have revealed that this mushroom contains compounds such as alkaloids (muscimol and ibotenic acid) and sterols [3],[4]. Research has highlighted various health benefits of *A. pantherina*, including its potential neuroactive properties [3]. While primarily recognized for its psychoactive properties, the anti-inflammatory effects of *A. pantherina*, specifically through nitric oxide inhibition, remain underexplored. Given the risks associated with its toxicity, this study focuses on *in vitro* research to evaluate the safety and efficacy of its compounds. Nitric oxide is produced from L-arginine by nitric oxide synthases (NOS), with the inducible form (iNOS) being upregulated by inflammatory stimuli. Elevated NO levels contribute to tissue damage and inflammatory diseases, making iNOS inhibitors of significant interest [5],[6]. Therefore, compounds inhibiting iNOS and reducing NO production greatly interest anti-inflammatory research. This