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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-3β-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).

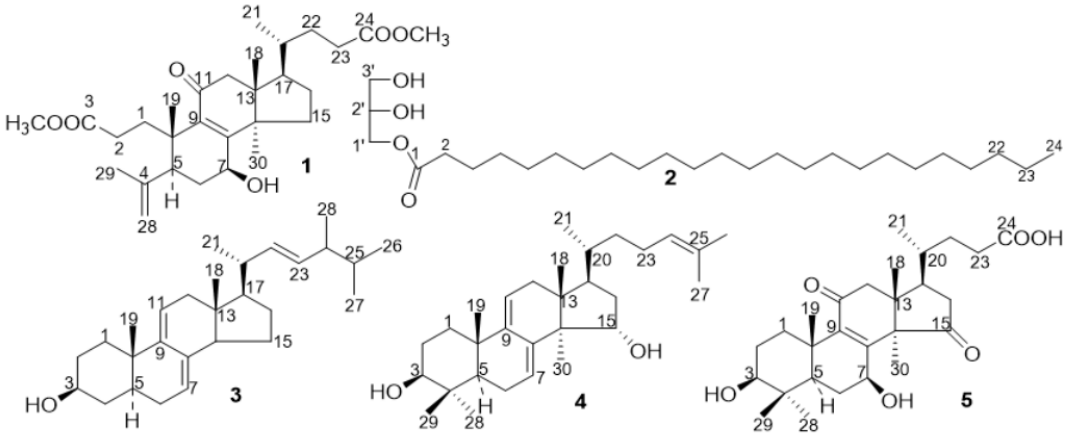

Fig. 1. Chemical structures of compounds (**1**-**5**)

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 7β-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-3β-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*.

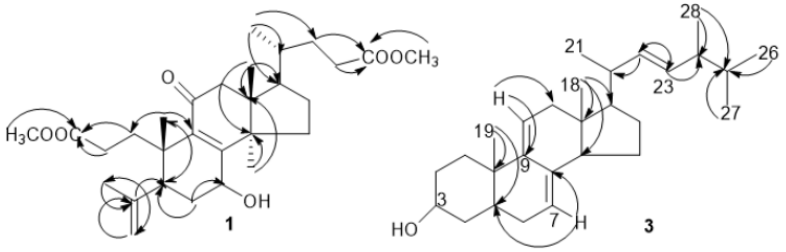

Fig. 2. The HMBC correlations of compounds **1** and **3**

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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