CHEMICAL CONSTITUENTS FROM THE MYCELIUM OF ISARIA JAPONICA YASUDA

Nguyen Ngoc Tuan, Nguyen Tan Thanh, Dao Thi Thanh Xuan, Tran Dinh Thang *

Department of Chemistry, Vinh University, 182 Le Duan, Vinh City, Nghe An province, Vietnam

*Email: thangtd@vinhuni.edu.vn

Received: 15 June 2016; Accepted for publication: 23 October 2016

ABSTRACT

A chemical investigation of the mycelium of Isaria japonica Yasuda resulted in the identification of eight compounds, including two steroids (ergosterol, ergosterol peroxide), one flavonoid (tricine), one phenolic (2-hydroxy-3-phenylpropanoic acid), two nitrogenous compounds (adenosine, uracil), one saccharide (D-mannitol), one amino acid (3-amino butanoic acid). The chemical structures of nine compounds were determined on the basis of 1D and 2D NMR, UV, IR and MS analytical results.

Keywords: Isaria japonica, triterpenoid, steroid, flavonoid, phenolic.

1. INTRODUCTION

Natural remedies are becoming increasingly popular and important in the public and scientific communities. Historically, natural remedies have been shown to present interesting biological and pharmacological activity and are used as chemotherapeutic agents. For centuries Cordyceps is a genus of more than 400 species in the family Clavicipitaceae. All Cordyceps species are endoparasitoids, parasitic mainly on insects and other arthropods, a few are parasitic on other fungi. Until recently, the best known species of the genus was Cordyceps sinensis [1, 2, 3]. Isaria japonica Yasuda has traditionally been used as health foods for various diseases in Japan, Korea and China [2, 3, 5]. Myriocin, a sphingosine analog isolated from the culture filtrate of Isaria sinclairii (P. cicadae), showed inhibitory effect on T cell-dependent immune responses [5]. Recently, they reported about the success in cultivating I. japonica (P. tenuipes) in a liquid medium and demonstrated that this liquid medium augmented anti-sheep red blood cell IgM plaque-forming cells response upon oral administration in mice [5, 6].

In the present study, we have succeeded in cultivating Isaria japonica in a liquid medium and chemical investigation of the mycelium of I. japonica resulted in the identification of eight compounds including two steroids (ergosterol, ergosterol peroxide), one flavonoid (tricine), one phenolic (2-hydroxy-3-phenylpropanoic acid), two nitrogenous compounds (adenosine, uracil), one saccharide (D-mannitol), one amino acid (3-amino butanoic acid). The chemical structures of the eight compounds were determined on the basis of 1D and 2D NMR, UV, IR and MS analytical results.
2. EXPERIMENTS

2.1. General

Melting points were determined using Yanagimoto MP-S3 apparatus. Optical rotations were measured using a JASCO DIP-370 polarimeter. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer. $^1$H- and $^{13}$C-NMR, COSY, NOESY, HMQC, and HMBC spectra were obtained on the Bruker AV-500 NMR spectrometer, with tetramethylsilane (TMS) as the internal standard and chemical shifts were reported in $\delta$ values (ppm). The electrospray ionization (ESI) mass spectra were determined using an Agilent 1200 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, E. Merck). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 F 254 plates (Merck) and the compounds were visualized by spraying with 10 % (v/v) H$_2$SO$_4$ followed by heating at 110°C for 10 min.

2.2. Fungal material

The entomogenous fungi *I. japonica* Yasuda was collected at the Pumat National Park of Nghean Province, Vietnam, in November 2013 and identified by Assoc. Prof. Dr. Tran Ngoc Lan, Institute for Regional Research and Development, Ministry of Science and Technology, Vietnam. A voucher specimen (VU130805) was deposited at the herbarium of the Department of Chemistry, Vinh University. *I. japonica* was cultured in a Potato Dextrose Agar (PDA) (5 L), at 25 – 26 °C for 14 days.

2.3. Extraction and isolation

The cultures were filtered through cheese cloth to separate broth and mycelium. The mycelium of *I. japonica* was extracted with methanol (1 L × 3) at ambient temperature, and the combined extracts were concentrated under reduced pressure to give a deep brown syrup (25 g). The crude extract was suspended in water and partitioned with ethyl acetate to afford ethyl acetate (15 g) and water soluble (10 g) fractions, respectively. The ethyl acetate soluble extracts were applied to silica gel column chromatography with a mixture of hexane and acetone step gradient system (100:0, 25:1, 15:1, 10:1, 7:1, 5:1) and then eluted with a chloroform:methanol step gradient solvent mixture (10:0, 6:1, 3:1, 2:1, 1:1) to afford minor fractions. These fractions were monitored by TLC to combine into six fractions. Fraction 1 was subjected to the silica gel column chromatography (200 g, 60 × 3 cm) eluting with a hexan:axeton (15:1) to afford compound 1 (123 mg) and compound 3 (15 mg). Fraction 4 was subjected to the silica gel column chromatography (200 g, 60×3 cm) eluting with a hexan:axeton (9:1) to afford compound 2 (31 mg). Fraction 3 (1.2 g) was subjected to the silica gel column chromatography (200 g, 60 × 3 cm) eluting with a chloroform/methanol step gradient system (15:1, 9:1, each 200 mL) to afford compound 4 (13 mg) and compound 5 (25 mg). Fraction 3 was subjected to the silica gel column chromatography (200 g, 60×3 cm) eluting with a chloroform:methanol (30:1) to afford compound 5 (38 mg). Fraction 5 afforded compound 6 (30 mg), compound 7 (16.5 mg), and compound 8 (14.5 mg).

*Compound 1*: white powder, m.p. 165 – 167 °C; UV (MeOH) $\lambda_{max}$ nm: 211, 285. IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3433, 2959, 1726, 1090; EI-MS $m/z$ 396 [M]$^+$; $^1$H-NMR (500 MHz, CDCl$_3$) ($\delta$ ppm): 5.49 (1H, m), 5.35 (1H, m), 5.28 (1H, dd, $J = 15.5, 7.5$ Hz), 5.25 (1H, dd, $J = 15.5, 7.0$ Hz), 3.48
Compound 2: Colorless needles, m.p. 296 – 298 °C; ESI-MS m/z 428 [M]+; 1H-NMR (500 MHz, CDCl₃) (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); 13C-NMR (125 MHz, CDCl₃) (δ ppm): 135.6 (C-22), 135.2 (C-6), 131.5 (C-2), 127.6 (C-4'), 126.1 (C-2'), 129.1 (C-3'), 129.0 (C-5'), 127.9 (C-7), 127.3 (C-8), 124.2 (C-19), 123.2 (C-28), 121.9 (C-20), 114.9 (C-18), 114.2 (C-17), 109.1 (C-9), 104.0 (C-13), 42.0 (C-24), 40.1 (C-20), 38.7 (C-4), 36.9 (C-10), 36.5 (C-12), 34.5 (C-2'), 32.4 (C-25), 29.9 (C-1), 28.2 (C-16), 22.8 (C-15), 21.7 (C-11), 20.2 (C-21), 19.7 (C-27), 19.4 (C-26), 17.9 (C-19), 17.2 (C-28), 12.5 (C-18).

Compound 3: Pale yellow powder, m.p. 296 – 298 °C; ESI-MS m/z: 331 [M+H]+; 1H-NMR (500 MHz, DMSO-d₆) (δ ppm): 7.32 (2H, s, H-2 and H-6), 6.97 (1H, s, H-3), 6.55 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, dd, J = 2.0 Hz, H-6), 3.88 (6H, s, 3'-OCH₃ and 5'-OCH₃); 13C-NMR (125 MHz, DMSO-d₆) (δ ppm): 181.8 (C-4), 164.1 (C-7), 163.6 (C-2), 161.4 (C-9), 157.3 (C-5), 148.2 (C-3', and S'), 139.9 (C-4'), 120.4 (C-1'), 104.4 (C-2' and 6'), 103.7 (C-10), 103.6 (C-3), 98.8 (C-6), 94.2 (C-8), 56.4 (2C, 3'-OCH₃ and 5'-OCH₃).

Compound 4: White powder, [α]D 25° = −20.8(c 2, H₂O); m.p. 122 – 124 °C; 1H-NMR (CDCl₃&MeOD, 500 MHz) (δ ppm): 7.35 (2H, m, H-3',5'), 7.28 (1H, t, J = 7.5 Hz, H-4'), 7.11 (2H, brd, J = 7.0 Hz, H-2',6'), 4.10 (1H, dd, J = 6.5, 4.0 Hz, H-2), 2.94 (1H, dd, J = 7.0, 3.5 Hz, H-3), 2.17 (1H, dd, J = 8.0, 7.5 Hz, H-3b); 13C-NMR (CDCl₃&MeOD, 125 MHz) (δ ppm): 167.3 (C-1), 135.1 (C-1'), 130.2 (C-2',6'), 129.1 (C-3',5'), 127.6 (C-4'), 56.5 (C-2), 40.3 (C-3).

Compound 5: Colorless powder; [α]D 25° = -31.7 (c 0.04, MeOH); m.p. 235 - 237 °C; EI-MS (rel. int.): m/z 267 ([M]+, 3), 178(30), 164(78), 135(100); UV (MeOH) λmax: 261 nm; IR (neat) νmax: 3334, 1654 cm⁻¹; 1H-NMR (CDCl₃&CD₂OD, 500 MHz) (δ ppm): 3.77 (1H, dd, J = 12.4, 2.4 Hz, H-5'), 3.97 (1H, dd, J = 12.4, 2.4 Hz, H-5'), 4.27 (1H, q, J = 2.4 Hz, H-4'), 4.35 (1H, dd, J = 4.8, 2.4 Hz, H-3'), 4.79 (1H, dd, J = 7.2, 4.8 Hz, H-2'), 5.86 (1H, d, J = 7.2 Hz, H-1'), 8.05 (1H, s, H-8), 8.23 (1H, s, H-2); 13C-NMR (CDCl₃ & CD₂OD, 125 MHz) (δ ppm): 156.2 (C-6), 152.5 (C-2), 148.6 (C-4), 141.0 (C-8), 126.0 (C-5), 91.3 (C-1'), 87.6 (C-4'), 74.2 (C-2'), 71.9 (C-3'), 61.6 (C-5').

Compound 6: Light brown powder; m.p. 310 – 311 °C; EI-MS (rel. int.): m/z 112 ([M]+, 100), 69(52); UV (MeOH) λmax: 258 nm; IR (neat) νmax: 3116, 2926, 2856, 1711, 1671 cm⁻¹; 1H-NMR (DMSO-d₆, 500 MHz) (δ ppm): 5.44 (1H, d, J = 7.5 Hz, H-5), 7.37 (1H, d, J = 7.5 Hz, H-6), 10.81 (1H, br s), 10.98 (1H, br s).

Compound 7: Colorless powder; m.p. 166-168 °C, EI-MS (rel. int.): m/z 182 ([M]+, 3), 103(60), 73(100); IR (neat) νmax: 3207, 2918, 1420, 1380 cm⁻¹; 1H-NMR (DMSO-d₆, 500 MHz) (δ ppm): 4.40 (1H, d, J=5.0Hz, 2-OH), 4.31 (1H, t, J = 5.5 Hz, 1-OH), 4.12 (1H, d, J = 7.0 Hz, 3-OH), 3.35-3.63 (4H, m, H-1,2,3); 13C-NMR (DMSO-d₆, 125 MHz) (δ ppm): 64.1 (C-1), 69.9 (C-3), 71.5 (C-2).
Compound 8: Colorless powder; m.p. 189 – 190 °C; \(^1\)H-NMR (CDCl\(_3\), 500 MHz) (δ ppm): 5.25 (1H, dt, \(J = 6.5, 13.0\) Hz, H-3), 2.60 (1H, dd, \(J = 7.0, 7.0\) Hz, H-2), 1.27 (3H, \(d, J = 6.5\) Hz, H-4); \(^1^3\)C-NMR (DMSO-d\(_6\), 125 MHz) (δ ppm): 169.2 (C-1), 67.6 (C-3), 40.8 (C-2), 19.8 (C-4).

3. RESULTS AND DISCUSSION

Compound 1 was obtained as an optically active white powder. The EI-MS of 1 showed a pseudomolecular ion peak at \(m/z\) 396 \([M]^+\), corresponding to a molecular formula of \(C_{28}H_{44}O\). The UV spectrum of 1 exhibited absorption maxima at 211 nm and 285 nm. The IR absorption bands at 3433, 2959, 1726, 1090 cm\(^{-1}\) suggested the presence of hydroxy group, carbon–carbon double bond functionalities, respectively. The \(^1\)H-NMR spectrum of 1 displayed six methyl groups [δ\(_H\) 0.61 (3H, s), 0.82 (3H, \(d, J = 6.5\) Hz), 0.85 (3H, \(d, J = 6.5\) Hz), 0.93 (3H, \(d, J = 7.0\) Hz), 0.96 (3H, s), and 1.05 (3H, \(d, J = 7.0\) Hz)], respectively. In addition, the presence of one proton attached to an oxygen bearing carbon [δ\(_H\) 3.48 (1H, m)] and four protons attached to double bonds at [δ\(_H\) 5.28 (1H, \(dd, J = 15.5, 7.5\) Hz), 5.25 (1H, \(dd, J = 15.5, 7.0\) Hz), 5.35 (1H, m), and 5.49 (1H, m)]. Its \(^1^3\)C-NMR spectrum displayed totally 28 carbon signals, which confirmed the presences of six olefinic carbons [δ\(_C\) 116.3; 119.6; 130.0; 135.6; 140.7 and 142.0] and one oxygenated carbon [δ\(_C\) 70.5 ppm]. These facts indicated that compound 1 is ergosterol, confirmed by direct comparison with the literature data [7].

Compound 2 was obtained as an optically active colorless needles with optical rotation .... The EI-MS of 2 showed a pseudomolecular ion peak at \(m/z\) 428 \([M]^+\), corresponding to a molecular formula of \(C_{28}H_{44}O_3\). The \(^1\)H-NMR spectrum of 2 displayed six methyl at [δ\(_H\) 0.87 (s, H-18), 0.95 (s, H-19), 0.87 (d, \(J = 6.5\) Hz, H-26), 0.89 (d, \(J = 6.5\) Hz, H-27), 0.96 (d, \(J = 7.0\) Hz, H-28), 1.05 (d, \(J = 6.5\) Hz, H-21)], four protons attached to double bonds at [δ\(_H\) 5.28 (1H, \(dd, J = 15.5, 7.5\) Hz), 5.25 (1H, \(dd, J = 15.5, 7.0\) Hz), 5.35 (1H, m), and 5.49 (1H, m)], and one proton attached to an oxygen bearing carbon at [δ\(_H\) 3.58 (1H, m)], which suggested the presence of the sterol. Its \(^1^3\)C-NMR and DEPT spectrum displayed totally 28 carbon signals, which confirmed the presences of six methyl carbons, seven methylen, eleven methin and four quaternary carbons. In addition, the \(^1^3\)C-NMR spectrum of compound 2 exhibited signals consistent with the presence of three oxygenated carbons [81.4 (C-5); 78.4 (C-8); 64.6 (C-3)] and two C=C double bonds in the downfield region [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 ergosterol peroxide (5\(\alpha\), 8-epidioxyergosta-6,22-dien-3-ol) [7] and compound 2 was therefore characterized as deduced.

Compound 3 was obtained as an optically active pale yellow powder. The ESI-MS showed a pseudomolecular ion peak at \(m/z\) 331 [M+H]^+, corresponding to a molecular formula of \(C_{17}H_{14}O_7\). The \(^1\)H-NMR spectrum showed signals due to five sp\(^2\)-methine protons [δ\(_H\) 7.32 (2H, s, H-2 and H-6), 6.97 (1H, s, H-3), 6.55 (1H, \(d, J = 2.0\) Hz, H-8), 6.20 (1H, \(d, J = 2.0\) Hz, H-6)], and two methoxy protons [δ\(_H\) 3.88 (6H, s, 3‘-OCH\(_3\) and 5‘-OCH\(_3\)]. The \(^1^3\)C-NMR showed seventeen carbon signals at 181.8 (C-4), 164.1 (C-7),163.6 (C-2), 161.4 (C-9), 157.3 (C-5), 148.2 (2C, C-3’ and 5’), 139.9 (C-4’), 120.4 (C-1’), 104.4 (2C, C-2’ and 6’), 103.7 (C-10), 103.6 (C-3), 98.8 (C-6), 94.2 (C-8), 56.4 (2C, 3’-OCH\(_3\) and 5’-OCH\(_3\)). Compounds 3 was identified as
tricin by comparison of its physical and spectroscopic properties with those reported in the literature [8, 9].

Compound 4 was obtained as an optically active white powder, m.p. 122 – 124 °C. The 1H-NMR spectrum of 4 displayed the signals of the ABX system of a mono-substituted benzene ring [δH: 7.35 (2H, m, H-3,5), 7.28 (1H, J = 7.5 Hz, H-4'), 7.11 (2H, br, d, J = 7.0 Hz, H-2',6')], one oxygenated methine proton [δH: 4.10 (1H, dd, J = 6.5, 4.0 Hz, H-2)], two methylene protons [δH: 2.94 (1H, dd, J = 7.0, 3.5 Hz, H-3a), 2.17 (1H, dd, J = 8.0, 7.5 Hz, H-3b)]. The 13C-NMR spectrum of 4 showed one carbonyl carbon [δC 171.1 (C-7)], six aromatic carbons [δC 152.5 (C-4), 148.6 (C-5), 141.0 (C-6), 135.6 (C-1'), 130.2 (C-2',6'), 129.1 (C-3',5'), 127.6 (C-4')], one oxygenated carbon [δC 134.9 (C-1)], one methylene carbon [δC 67.9 (C-3)]. Compounds 1 and 5 showed absorption maxima at 3116, 2926, 2856, 1711, 1671 cm⁻¹. The UV spectrum of 1 showed one proton attached to an amino bearing carbon at 5.25 (1H, J = 5.0 Hz, H-3), methylene protons at 2.60 (1H, d, J = 7.0 Hz, H-2) and 2.47 (1H, d, J = 7.0 Hz, H-2), methyl proton at 1.27 (3H, d, J = 6.5 Hz, H-4). In addition, the 13C-NMR and DEPT spectrums

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Compound 5 was obtained as an optically active colorless powder. The IR absorption bands at 3334, 1654 cm⁻¹. The UV spectrum of 5 showed a pseudomolecular ion peak at m/z 267, corresponding to a molecular formula of C₄H₄N₂O₂. The 1H-NMR spectrum of 5 displayed the signals of a ribose sugar moiety at 3.77 (1H, d, J = 12.4, 2.4 Hz, H-5'), 3.97 (1H, dd, J = 12.4, 2.4 Hz, H-5'), 4.27 (1H, ddd, J = 2.4, 2.4, 2.4 Hz, H-4'), 4.35 (1H, dd, J = 4.8, 2.4 Hz, H-3'), 4.79 (1H, dd, J = 7.2, 4.8 Hz, H-2'), 5.68 (1H, d, J = 7.2 Hz, H-1'), and adenine moiety at 8.05 (1H, s, H-8), 8.23 (1H, s, H-2). The 13C-NMR spectrum of 5 also displayed the signals of a ribose sugar moiety at 91.3 (C-1'), 87.6 (C-4'), 74.2 (C-2'), 71.9 (C-3'), 61.6 (C-5'), and adenine moiety at 156.2 (C-6), 152.5 (C-2'), 148.6 (C-4'), 141.0 (C-8), 120.6 (C-5). The HMBC spectrum of 5 suggested adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β-N9-glycosidic bond. Compounds 5 was identified as adenosine by comparison of its physical and spectroscopic properties with those reported in the literature [11].

Compound 6 was obtained as an optically active light brown powder, m.p. 310 – 311 °C. The IR absorption bands at 3116, 2926, 2856, 1711, 1671 cm⁻¹. The UV spectrum of 6 exhibited absorption maxima at 258 nm. The El-MS of 6 showed a pseudomolecular ion peak at m/z 112, corresponding to a molecular formula of C₆H₇NO₃O. The 1H-NMR spectrum of 6 showed signals due to two sp²-methine protons [δH: 5.44 (1H, d, J = 7.5 Hz, H-5), 7.37 (1H, d, J = 7.5 Hz, H-6)], two proton NH at 10.81 (1H, br s), 10.98 (1H, br s). Compounds 6 was identified as uracil by comparison of its physical and spectroscopic properties with those reported in the literature [12].

Compound 7 was obtained as an optically active colorless powder, m.p. 166 - 168 °C. The IR absorption bands at 3207 (OH), 2918, 1420, 1380 cm⁻¹. The El-MS of 7 showed a pseudomolecular ion peak at m/z 182 corresponding to a molecular formula of C₆H₁₂O₅. The 1H-NMR spectrum of 7 displayed the signals of four hydroxyl groups [δH: 4.40 (1H, d, J = 5.0Hz, 2-OH), 4.31 (1H, t, J = 5.5 Hz, 1-OH), 4.12 (1H, d, J = 7.0 Hz, 3-OH)], and four methine protons at 3.35-3.63 (4H, m, H-1,2,3). The 13C-NMR spectrum of 7 showed three oxygenated carbons 64.1 (C-1), 69.9 (C-3), 71.5 (C-2). Compounds 7 was identified as D-mannitol by comparison of its physical and spectroscopic properties with those reported in the literature [13].

Compound 8 was obtained as an optically active colorless powder, m.p. 189 – 190 °C. The 1H-NMR spectrum of 8 displayed totally seven protons signals, which confirmed the presences of one proton attached to an amino bearing carbon at 5.25 (1H, dt, J = 6.5 Hz, H-3), methylene protons at 2.60 (1H, dd, J = 7.0, 7.0 Hz, H-2) and 2.47 (1H, dd, J = 7.0, 7.0 Hz, H-2), methyl proton at 1.27 (3H, d, J = 6.5 Hz, H-4). In addition, the 13C-NMR and DEPT spectrums
displayed totally four carbons signals, including one carbonyl carbon at 69.2 (C-1), one nitrogenated carbon 67.6 (C-3), one methylen carbon at 40.8 (C-2) and one methyl carbon 19.8 (C-4). Compounds 8 was identified as 3-amino butanoic acid by comparison of its physical and spectroscopic properties with those reported in the literature [14].

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\begin{align*}
&\text{Ergosterol (1)} & \text{Ergosterol peroxide (2)} & \text{Tricine (3)} & \text{Uracil (6)} \\
&\text{2-hydroxyl-3-phenylpropanoic acid (4)} & \text{Adenosine (5)} & \text{D-mannitol (7)} & \text{3-amino butanoic acid (8)}
\end{align*}
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4. CONCLUSION

In the present study, we have succeeded in cultivating *I. japonica* in a liquid medium and studying chemical composition of the mycelium of *I. japonica* Yasuda in Pumat, Nghean province resulted in the identification of eight compounds, including two steroids (ergosterol, ergosterol peroxide), one flavonoid (tricine), one phenolic (2-hydroxy-3-phenylpropanoic acid), two nitrogenous compounds (adenosine, uracil), one saccharide (D-mannitol), one amino acid (3-amino butanoic acid). The chemical structures of the eight compounds were determined on the basis of 1D and 2D NMR, UV, IR and MS analytical results.

REFERENCES


**Tóm tắt**

**Thành phần hóa học của loại Năm Kí sinh côn trùng (*Isaria japonica*) ở Việt Nam**

Nguyễn Ngọc Tuan, Nguyễn Tân Thành, Đào Thị Thanh Xuân, Trần Đình Thắng*

*Khoa Hóa, Trường Đại học Vinh, 182 Lê Duẩn, thành phố Vinh, tỉnh Nghệ An, Việt Nam*

*Email: thangtdl@vinhuni.edu.vn*

Nghiên cứu thành phần hóa học của năm kí sinh côn trùng (*Isaria japonica*) bằng các phương pháp sắc khí đã phân lập được 8 hợp chất bao gồm 2 steroid (ergosterol, ergosterol peroxit), 1 flavonoit (tricin), 1 phenolic (axit 2-hydroxyl-3-phenyl-propanoic), 2 hợp chất chiều nito (adenosin, uracil), 1 saccharit (D-mannitol), 1 axit amino (axit 3-amino butanoic). Các hợp chất này đã được xác định cấu trúc bằng các phương pháp phổ từ ngoại (UV), phổ hồng ngoại (IR), phổ khối lượng phân giải cao (HR-ESI-MS), phổ công hưởng từ (1H-, 13C-NMR, DEPT, HMBC HSQC và COSY).

Từ khóa: *Isaria japonica*, triterpenoit, steroid, flavonoit, phenolic.