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A HOPANE TRITERPENOID FROM THE MYCELIUM OF *Isaria japonica* IN VIETNAM

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ABSTRACT

Compound 11β,22-dihydroxyhopane was isolated from the methanol extract of the mycelium of *Isaria japonica* in Vietnam. Its structure was elucidated by spectral data analysis, including MS and 2D NMR. This is the first complete NMR assignment for 11β,22-dihydroxyhopane.

Keywords: Isaria japonica, triterpenoid, 11β, 22-dihydroxyhopane, NMR technique, mass spectrometry.

1. INTRODUCTION

Cordyceps belongs to the family Clavicipitaceae of the order Hypocreales in the class Pyrenomycetes of ascomycetous fungi, and is known to be parasitized on insects [1, 2]. Various bioactive components were found in the genus *Cordyceps*. *Isaria japonica* is entomogenous fungi thought to be of the anamorph stage of *Cordyceps* [3]. This fungus with its host insects have been used in traditional medicine for various purposes in Japan, Korea and China [4, 5, 6]. *Isaria japonica* (*Paecilomyces tenuipes*) possesses anti-cancer activity *in vivo* [7] and significant cytotoxicity against cancer cell lines [8]. The culture liquid of *Isaria japonica* have been found to enhance the production of anti-sheep red blood cell IgM plaque-forming cells response upon oral administration in mice [6, 9].

In this article, we report the isolation and structural characterization of a hopane triterpenoid from the entomogenous fungi, *Isaria japonica* Yasuda.

2. EXPERIMENTAL

2.1 General

Melting point was measured on a Yanagimoto MP-S3 apparatus. NMR spectra were recorded on a Bruker AV-500 NMR spectrometer. ESI-MS and HR-ESI-MS spectra were

recorded on a micr OTOF-Q II 10187 spectrometer. Silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh) and TLC (precoated Kieselgel 60 F 254 plates) were purchased from Merck.

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 (VN112013) was deposited at the herbarium of the Department of Chemistry, Vinh University.

2.3. Extraction and isolation

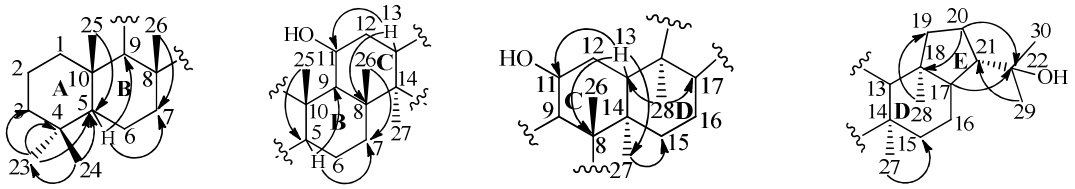
The dried mycelium of *I. japonica* (100 g) was extracted with methanol (1 L × 3) at ambient temperature, and concentrated under reduced pressure to give a deep brown syrup (25 g). The crude extract was suspended in water and partitioned with ethyl acetate. The EtOAc and aqueous solutions were concentrated to afford the residues of 15 g and 10 g, respectively. The ethyl acetate solubles were purified on silica gel column chromatography, eluted with a mixture of *n*-hexane/acetone (100/0 to 5/1) to afford six fractions. Fraction 5 (1.2 g) was subjected to silica gel column chromatography eluting with ethyl acetate/*n*-hexane (7/3) to yield compound **1** (13 mg).

Compound **1:** white powder, m.p. 216-217 ^{o}C; HR-ESI-MS *m*/*z*: 445.4038 $[M+H]^+$; NMR data see Table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as optically active yellow powder, m.p. 216 - 217 ^{o}C. The positive-mode HR-ESI-MS of **1** showed a pseudomolecular ion peak at *m/z* 445.4038 ($[M+H]^+$, calcd. for $C_{30}H_{53}O_2$, 445.4046) corresponding to a molecular formula of $C_{30}H_{52}O_2$. In its ^{1}H-NMR spectrum, signals of eight methyl groups, characteristic of a triterpene were observed at δ_H| 1.21 (H-30), 1.18 (H-29), 0.96 (H-27), 0.95 (H-25), 0.92 (H-24), 0.86 (H-26), 0.84 (H-23), and 0.76 (H-28). Additionally, a proton at δ_H 3.87 (1H, *m*, H-11) together with a set of complex signals in the aliphatic region were present.

In the ^{13}C-NMR and DEPT spectra of **1**, the resonances of eight methyls, ten methylens, six methines, and six quaternary carbons (Table 1) were noted. The chemical shifts of CH-11 and C-22 (Table 1) suggested their linkages to oxygens. Analysis of 2D NMR spectra, especially HMBC experiment revealed a hopane-type triterpene skeleton for **1**.



(a) A/B ring fusion in fragment 1 (c) C/D ring fusion in fragment 3
(b) B/C ring fusion in fragment 2 (d) D/E ring fusion in fragment 4

Figure 1. Key HMBC correlations of fragment 1-4.

HMBC correlations for the fusion of the A/B rings was determined by the correlations of H-23 to C-3/C-4/C-5; H-24 to C-5/C-23; H-25 to C-5; H-5 to C-9/C-7 and H-26 to C-7. Similarly, the B/C ring fusion was established by correlations of H-13 to C-8/C-11; H-26 to C-7. After the construction of the C/D ring fusion at positions C-13 and C-14, it was confirmed by correlations of H-13 to C-27; H-27 to C-15; and H-28 to C-13/C-17, respectively.

Table 1. ^{1}H-, ^{13}C-NMR, DEPT and HSQC, HMBC data for compound **1.**

Carbon	**DEPT**	**δ_C(ppm)**	**δ_H (ppm)**	**HMBC (H→ C)**
C-1	CH_2	24.1	1.45 (2H, *m*)	
C-2	CH_2	18.5	1.53 (2H, *m*)	
C-3	CH_2	51.2	1.08 (2H, *br t*, *J* = 12.0 Hz)	
C-4	C	39.2		
C-5	CH	55.6	0.72 (1H, *m*)	C-9
C-6	CH_2	21.1	1.53 (2H, *m*)	
C-7	CH_2	49.7	0.69 (1H, *m*) 2.06 (1H, *m*)	
C-8	C	35.0		
C-9	CH	50.4	1.25 (1H, *m*)	
C-10	C	50.8		
C-11	CH	65.4	3.87 (1H, *m*)	
C-12	CH_2	33.2	1.45 (2H, *m*)	
C-13	CH	49.9	0.69 (1H, *m*)	C-8, C-27, C-11
C-14	C	41.9		
C-15	CH_2	34.4	1.23 (2H, *m*)	
C-16	CH_2	22.0	1.94 (2H, *m*)	
C-17	CH	54.0	1.45 (2H, *m*)	C-22
C-18	C	44.1		
C-19	CH_2	41.3	1.59 (2H, *m*)	
C-20	CH_2	26.6	1.74 (2H, *m*)	C-18, C-22
C-21	CH	51.2	2.24 (1H, *m*)	
C-22	C	74.0		
C-23	CH_3	22.5	0.84 (3H, *s*)	C-4, C-3, C-5, C-24
C-24	CH_3	33.5	0.92 (3H, *s*)	C-23, C-5
C-25	CH_3	17.0	0.95 (3H, *s*)	C-5
C-26	CH_3	16.8	0.86 (3H, *s*)	C-7
C-27	CH_3	17.1	0.96 (3H, *s*)	
C-28	CH_3	16.1	0.76 (3H, *s*)	C-13, C-17
C-29	CH_3	30.9	1.18 (3H, *s*)	C-21
C-30	CH_3	28.8	1.21 (3H, *s*)	

δ_H (ppm) 500 MHz; *s*: singlet; *br s*: broad singlet; *d*: doublet; *m*: multiplet; δ_C (ppm) 125 MHz; ^{1}H-, ^{13}C-NMR recorded in $CDCl_3$.

Thus from the 2D NMR data analysis, the structure of compound **1** was established as 11β,22-dihydroxyhopane (Figure 2) which has been previously reported [10]. However, this is the first complete NMR assignment for this compound.

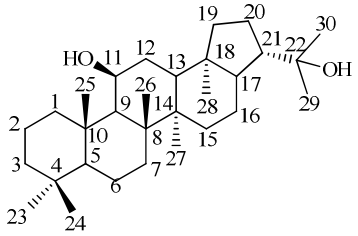


Figure 2. Structure of compound **1.**

4. CONCLUSION

In the present study, we have succeeded in studying chemical composition of the mycelium of *I. japnonica* Yasuda in Pumat, Nghean province and these experiments resulted in the identification of a hopane triterpene (11β,22-dihydroxyhopane). The chemical structures of the hopane triterpenoid compound were determined on the basis of spectroscopic and spectrometric analysis.

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