A New Cyathane-xyloside from the Mycelia of *Hericium erinaceum*

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A new cyathane-xyloside derivative, named erinacine R, was isolated from the mycelia of basidiomycete *Hericium erinaceum*. The structure of this compound was elucidated by spectral methods including 2D NMR experiments.

Key words: Hericium erinaceum, Basidiomycete, Erinacine R, Diterpenoid

Introduction

Erinacines and their xylosides are currently attracting much attention because of their unique biological activities. Erinacines are known to have a potent stimulating activity for nerve growth factor-synthesis and have been proposed as medicines for degenerative neuronal disorder such as Alzheimer's disease and peripheral nerve regeneration [1, 2]. *Hericium erinaceum* is a mushroom belonging to the family Hericiaceae and has been known as a Chinese medicine or food in China and Japan. Erinacines A, B, C, D, E, F, G, H, I, P, and Q were isolated from the mycelia of *Hericium erinaceum* [3 – 8]. All of these diterpenoids possess a cyathane skeleton consisting of angularly condensed five-, six-, and seven-membered rings.

In continuation of our studies on basidiomycete-derived bioactive secondary metabolites, we investigated the chemical constituents of the mycelia of *Hericium erinaceum*, the erinacine-producing basidiomycete. This report describes the structure elucidation of a new compound named erinacine R.

Results and Discussion

Table 1. ^{1}H and ^{13}C NMR (in $CDCl_3$) data of erinacine R.

Position	δC	δH (J in Hz)
1 (CH_2)	38.7	1.54 (m); 1.62 (m)
2 (CH_2)	28.5	2.23 (m)
3	140.6	
4	136.5	
5 (CH)	40.5	2.12 (m)
6	44.2	
7 (CH_2)	29.7	1.36 (d, 13.2); 1.48 (d, 13.2)
8	212.2	
9	47.8	
10 (CH_2)	29.7	1.88 (m)
11 (CH)	68.6	5.91 (t, 8.2)
12	138.6	
13 (CH)	155.7	6.88 (d, 5.6)
14 (CH)	85.5	4.36 (d, 5.6)
15 (CH)	191.7	9.44 (s)
16 (CH_3)	16.7	0.95 (3H, s)
17 (CH_3)	24.6	0.90 (3H, s)
18 (CH)	27.2	2.82 (d, 6.8)
19/20 (CH_3)	21.5, 21.8	0.96, 0.98 (each 3H, d, 6.8)
1′ (CH)	105.8	4.87 (d, 6.8)
2′ (CH)	73.3	3.56 (dd, 8.0, 6.8)
3′ (CH)	75.5	3.62 (dd, 8.0, 7.6)
4′ (CH)	70.0	3.79 (m)
5′ (CH_2)	65.3	3.48 (m); 4.43 (m)
*C*OCH$_3$	170.5	
CO*C*H$_3$	20.6	2.10 (3H, s)

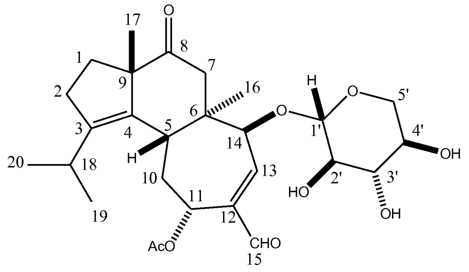

Fig. 1. The molecular formula of erinacine R.

The ethyl acetate-soluble fraction of the MeOH extract from the mycelia of *Hericium erinaceum* was successively subjected to silica gel and Sephadex LH-20 column chromatography to give the new compound, erinacine R. Its molecular formula was determined as $C_{27}H_{38}O_9$ from the $[M+Na]^+$ ion peak (m/z = 529.59512, calcd. 529.5920 for $C_{27}H_{38}O_9Na$; HRMS ((+)-ESI)), requiring nine degrees of unsaturation. Compared with the ^{13}CNMR spectrum of erinacine P [7], the molecular formula and the NMR data listed in Table 1 determine erinacine R to be a monoacetate of a cyathane-xyloside.

Compared with the ^{13}CNMR spectrum of erinacine P, erinacine R has one more carbonyl carbon (δ = 212.2) and one methylene less on ring B. The difference between erinacine R and erinacine P is the C-8 chemical shift (212.4 in erinacine R *vs*. 49.4 in erinacine P). An HMBC correlation between H-17 (δ = 0.90) and C-8 was observed. The acetoxy group is also located at C-11. The proton at this position appears at δ = 5.91 and is coupled to the C-10 methylene protons as evidenced in the ^{1}H-^{1}H COSY spectrum. It shows a correlation peak with the carbonyl carbon (δ = 170.5) of the acetyl group in the HMBC measurement. The double bond in ring C is located at the C-12/C-13 position. The olefinic proton appears at δ = 6.88 as a sharp doublet, with coupling to the C-14 oxylmethine proton at δ = 4.36. The correlation in the HMBC experiment between H-1$'$ and C-14 indicates that the xylose part is connected with C-14.

The relative stereochemistry of erinacine R was established by the ROESY experiment. ROESY correlations H-5/H-11 and H-14/H-16 indicated that these protons were situated at the same side. Thus, the structure of erinacine R was elucidated as represented in Fig. 1.

Experimental Section

General

Optical rotation was taken on a Horiba Sepa-300 polarimeter (Horiba, Tokyo, Japan). ^{1}H, ^{13}C and two-dimensional NMR spectra were recorded on a Bruker DRX-500 instrument (Karlsruhe, Germany). Chemical shifts are given as δ in ppm relative to TMS as internal standard and coupling constants in Hz. Mass spectra were measured with a VG Autospec 3000 spectrometer (VG, England). Infrared (IR) spectra were obtained on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Richmond, CA, USA) in KBr pellets.

Materials

Column chromatography was carried out on silica gel (200 – 300 mesh), and TLC was carried out on plates precoated with silical gel F_{254} (Qingdao Marine Chemical Ltd., Qingdao, P. R. China) and Sephadex LH-20 (Amersham Bioscinces, Uppsala, Sweden).

Culture conditions, extraction and isolation

The pure mycelia of *Hericium erinaceum* were subcultured for four weeks at 25 °C and grown in a 500 mL flask in 250 mL of a medium containing 20 g dextrose, 150 g husked potato, 3 g yeast, 1.5 g $MgSO_4$, 3 g KH_2PO_4, and 10 mg of VB_1 in 1 L of distilled H_2O. After four weeks culture (40 flasks), the mycelia were harvested with a nylon cloth and dried in an oven at 55 °C to dryness. The dry mycelia (60.5 g) of *Hericium erinaceum* was comminuted and extracted with MeOH (three times in a total of 1.2 L). The filtrates were combined, and the organic solvent was removed under reduced pressure. The MeOH extract (9.6 g) was subjected to chromatography over silica gel and then eluted with petroleum ether to give fraction A, and with ethyl acetate to give fraction B. Fraction B (1.8 g) was subjected to further silica gel column chromatography and eluted with $CHCl_3$/EtOH (95 : 5) to afford fraction B_1 (60 mg). Fraction B_1 was passed through a Sephadex LH-20 column with $CHCl_3$/MeOH (1 : 1, v/v) as eluent to yield erinacine R (22 mg).

Erinacine R: Pale-yellow amorphous powder. – $[\alpha]_D^{20}$ = -82.7 (c = 0.1, MeOH). – UV (MeOH): λ_{max} ($\log\varepsilon$) = 241 (0.45), 205 nm (0.24). – IR (KBr): ν = 3410 (OH), 2975, 2878, 1775, 1745, 1682 cm^{-1}. – HRMS ((+)-ESI): *m/z* = 529.59512 (calcd. 529.5920 for $C_{27}H_{38}O_9Na$, $[M+Na]^+$). – ^{1}H and ^{13}C NMR ($CDCl_3$): see Table 1.

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