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Erinacol (Cyatha-3,12-dien-14β-ol) and 11-O-Acetylcyathin A_3, New Cyathane Metabolites from an Erinacine Q-Producing Hericium erinaceum

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Note

Erinacol (Cyatha-3,12-dien-14β-ol) and 11-*O*-Acetylcyathin A_3, New Cyathane Metabolites from an Erinacine Q-Producing *Hericium erinaceum*

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In our search for new cyathane metabolites related to the biosynthesis of erinacine Q in *Hericium erinaceum*, we isolated a novel cyatha-3,12-dien-14β-ol named erinacol together with known 11-*O*-acetylcyathatriol (the erinacine Q aglycon) and new metabolite 11-*O*-acetylcyathin A_3 from the mycelial extract. The structure of each compound was determined by spectral methods. Possible biosynthetic relationships of these metabolites are discussed from their structural features.

Key words: erinacol; cyathane; erinacine Q; 11-*O*-acetylcyathin A_3; *Hericium erinaceum*

Erinacines, diterpene-xylosides possessing an unusual 5/6/7-tricyclic cyathadiene skeleton, are currently attracting attention because of their unique biological activities toward mammalian cells.[1,2] In our investigation of erinacine biosynthesis in the basidiomycte, *Hericium erinaceum* YB4-6237, we isolated new parental erinacines P[3] and Q[4] (Fig. 1) from the mycelial extract and demonstrated that erinacine Q was metabolized to erinacine C through erinacine P in the basidiomycete.[4] Furthermore, (−)-cyatha-3,12-diene (Fig. 1) has been isolated as the possible tricyclic-hydrocarbon intermediate in erinacine biosynthesis from the basidiomycete.[5] Our continuing search for new cyathadiene metabolites biosynthetically related to the erinacine Q aglycon from the basidiomycete resulted in the isolation of a novel cyathadien-14β-ol named erinacol (**1**, Fig. 1) and new fungal metabolite 11-*O*-acetylcyathin A_3 (**2**) together with 11-*O*-acetylcyathatriol (**3**)[6] as the erinacine Q aglycon (Fig. 1). We report here the isolation of **1**, **2** and **3** from the basidiomycete and their structural elucidation. Possible biosynthetic relationships of these metabolites in the biosynthetic pathway of erinacine Q are discussed from a biosynthetic point of view.

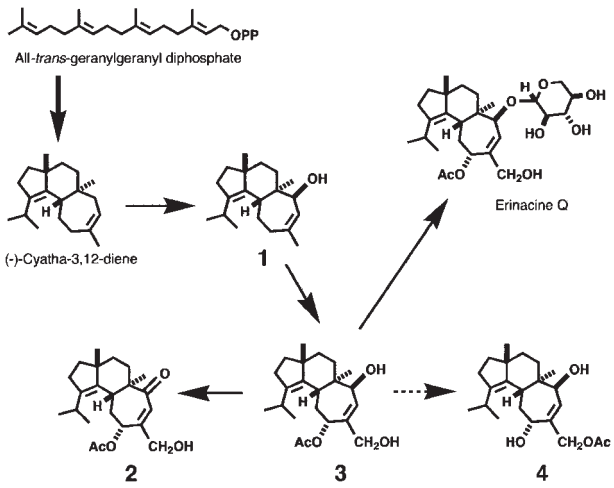

Fig. 1. Structures of Erinacine Q, (−)-Cyatha-3,12-diene and the Isolated Compounds, Erinacol (Cyatha-3,12-dien-14β-ol (**1**)), 11-*O*-Acetylcyathin A_3 (**2**), 11-*O*-Acetylcyathatriol (**3**) and 15-*O*-Acetylcyathatriol (**4**), and the Possible Biosynthetic Pathway to Erinacine Q from GGDP in *Hericium erinaceum* YB4-6237.

Hericium erinaceum YB4-6237 was reciprocally shake-cultured for 15 days at 25 °C in 500-ml Sakaguchi flasks, each containing a 5.0% glucose–0.5% peptone-1.0% Pharmamedia (Traders Protein Co.)–0.5% NaCl medium. Mycelia (117 g) obtained from 15 flasks by filtration were homogenized in acetone. From this aqueous acetone solution, an *n*-hexane extract (705 mg) containing the basic and neutral substances was prepared in the usual way. The extract incorporating **1**

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Table 1. NMR* Data for Erinacol (**1**), 11-*O*-Acetylcyathin A_3 (**2**), 11-*O*-Acetylcyathatriol (**3**), and 15-*O*-Acetylcyathatriol (**4**)

Position	**1**			**2**			**3**		**4**	
	δ_C		δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C		δ_C	
1	38.0	CH_2	1.41 (m)	38.1	CH_2	1.4∼1.5 (m)	38.0	CH_2	38.1	CH_2
2	28.5	CH_2	2.26 (td-like, 8, 2)	28.9	CH_2	2.30 (t-like, 8)	28.6	CH_2	28.6	CH_2
3	139.1	C		140.8	C		139.7	C	139.7	C
4	139.7	C		135.3	C		138.1	C	138.3	C
5	43.2	CH	2.92 (dm, 12)	38.9	CH	2.80 (dd, 11, 2)	39.9	CH	39.8	CH
6	42.7	C		54.0	C		41.9	C	41.8	C
7	33.8	CH_2	1.04 (ddd, 14, 4, 3) 2.11 (ddd, 4, 14, 5)	34.9	CH_2		33.1	CH_2	32.9	CH_2
8	36.9	CH_2	1.41 (ddd, 13, 5, 3) 1.51 (ddd, 4, 13, 4)	36.2	CH_2	1.4∼1.5 (m)	36.8	CH_2	36.9	CH_2
9	49.3	C		49.1	C		49.3	C	49.3	C
10	26.6	CH_2	1.81 (dddd, 13, 6, 3, 3) 1.92 (dddd, 13, 13, 12, 3)	31.5	CH_2	2.21 (ddd, 14, 11, 6) 2.47 (ddd, 14, 6, 2)	33.9	CH_2	36.8	CH_2
11	33.3	CH_2	1.98 (ddd, 15, 6, 3) 2.51 (dd, 15, 13)	71.8	CH	5.38 (dd, 6, 6)	73.6	CH	71.4	CH
12	143.0	C		147.5	C		143.0	C	141.0	C
13	125.8	CH	5.61 (br. d, 7)	125.9	CH	6.17 (br. s)	127.8	CH	129.9	CH
14	77.6	CH	3.68 (d, 7)	209.7	C		76.1	CH	76.3	CH
15	26.2	CH_3	1.77 (br. s)	64.2	CH_2	4.21 and 4.27 (ABqd, each 14, 5)	64.4	CH_2	66.0	CH_2
16	17.1	CH_3	0.77 (s)	14.6	CH_3	1.16 (s)	16.7	CH_3	16.8	CH_3
17	24.3	CH_3	1.09 (s)	24.0	CH_3	1.04 (s)	24.3	CH_3	24.4	CH_3
18	26.9	CH	3.01 (septet, 7)	27.2	CH	2.90 (septet, 7)	26.8	CH	26.8	CH
19/20	21.7	CH_3	0.95 (d, 7)	21.7	CH_3	1.00 (d, 7)	21.8	CH_3	21.7	CH_3
	22.0	CH_3	0.97 (d, 7)	21.8	CH_3	0.98 (d, 7)	21.9	CH_3	21.9	CH_3
CH_3CO	—	—		20.9	CH_3	2.02 (s)	21.2	CH_3	21.1	CH_3
CH_3CO	—	—		170.6	C		170.5	C	170.8	C

* Measured in $CDCl_3$ at 400 MHz for ^1H and at 100 MHz for ^{13}C.

and **2** was carefully separated by silica gel flash chromatography, using step-wise elution with mixtures of *n*-hexane/EtOAc as the eluent. A mixture of the 50:1 *n*-hexane/EtOAc fraction afforded **1** as a colorless solid (1.4 mg); this gave the characteristic purple color on a TLC plate after spraying the vanillin–H_2SO_4 reagent and then heating. **2** was eluted in a 5:1 mixture of the *n*-hexane/EtOAc fraction and further separated by a similar chromatographic procedure employing a mixture of 15:1 $CHCl_3$/acetone as the eluent to give **2** as a colorless solid (1.3 mg). Mycelia (222 g) similarly obtained from 20 flasks of the 11-day culture were homogenized in acetone. From this aqueous acetone solution, the *n*-hexane extract and then an additional extract with a mixture of 2:1 *n*-hexane/$CHCl_3$ were prepared in the usual way. The latter extract (461 mg) incorporating **3** and **4** was similarly separated by silica gel flash chromatography, using mixtures of $CHCl_3$/EtOH as the eluent. **3** was eluted with a 60:1 mixture of $CHCl_3$/EtOH and obtained as a colorless solid (2.8 mg). **4** was eluted with a 40:1 mixture of $CHCl_3$/EtOH and rechromatographed by using a 5:1 mixture of $CHCl_3$/acetone as the eluent to give a colorless solid (**4**, 0.8 mg).

1, $[\alpha]_D^{24}$ −22° (*c* 0.05, $CHCl_3$), had the molecular ion peak at *m/z* 288.2446 in the HR-EIMS data, indicating the molecular formula of $C_{20}H_{32}O$ (Calcd. molecular weight: 288.2453). Its spectral data were as follows. EIMS *m/z* (%): 288[M^+, 12], 270(100), 255(55), 227(58), 204(30), 189(67), 161(54), 119(48), 105(57); ^1H- and ^{13}C-NMR data are shown in Table 1. Its ^1H-NMR spectrum showed characteristic signals to those of (−)-cyatha-3,12-diene.[5] The presence of a characteristic signal of a hydroxymethine group (δ_H 3.68 and δ_C 77.6) in the ^1H- and ^{13}C-NMR spectra suggested that **1** was a monohydroxy derivative of (−)-cyatha-3,12-diene. In addition, the ^1H-NMR spectrum of **1** showed no signals for the H-14 methylene protons [δ 1.70 (dd, $J = 14$, 9 Hz) and 2.11 (dd, $J = 14$, 5 Hz)] that were observed for (−)-cyatha-3,12-diene, suggesting the presence of a hydroxy group of **1** at the C-14 position. Its ^1H- and ^{13}C-NMR signals (Table 1) were fully assigned by H–H and C–H COSY, and HMBC methods; the presence of the hydroxyl group at C-14 was well interpreted by corresponding changes in the ^{13}C-chemical shifts of its adjacent carbons of C-5, 6, 7 and 13 (Table 1). The structure of **1** was thus assigned as cyatha-3,12-dien-4-ol. The stereochemistry and conformation of **1** were elucidated as shown in Fig. 2 by ^1H-NMR spectrometry; clear NOEs were observed between H-14 and H-16, H-5 and H-7, H-5 and H-17, and H-5 and the axially oriented H-11 [δ 2.51 (dd, $J = 15$, 13 Hz)], and the appearance of the protons of H-5 (δ 2.92) and H-7 (δ 2.11) at a considerably lower field than those (δ 2.27 and 1.50–1.60, respectively) of (−)-cyatha-3,12-diene could be well interpreted by their 1,3-diaxial-like relationship to the hydroxy group at C-14. To determine the absolute stereochemistry of **1** by an allylic benzoate method,[7] the benzoate of **1** was prepared by treating with benzoyl

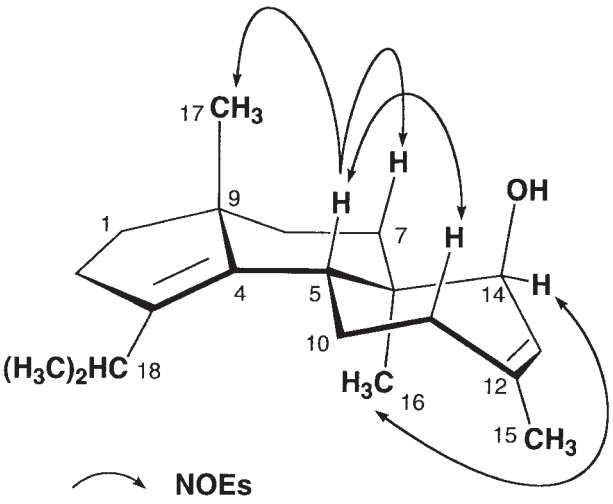

Fig. 2. Stereostructure and Conformation of Erinacol (**1**).

chloride in a mixture of 1:1 ethyl ether/pyridine. EIMS *m/z* (%): 392[M^+, 0.4], 377(2), 270(99), 105(100); UV λ_{max} (EtOH) nm (ε): 232 (16,800); δ_H (400 MHz, $CDCl_3$): 4.91 (1H, d, 7.6; H-14), 7.46 (2H, d, H-2′) 7.57 (1H, dd, 7.8, 7.8, H-3′), and 8.12 (2H, d, 7.8, H-1′). The benzoate showed a positive Cotton effect ($\Delta\varepsilon +$ 3.2) at 234 nm, indicating the configuration at the C-14 position to be *S*.[7] The absolute stereostructure of **1** was thus determined to be cyatha-3,12-dien-14β-ol (Fig. 2); its configuration at C-14 was the same as that of the aglycon of erinacine Q.

3, $[\alpha]_D^{25}$ -37° (*c* 0.26, $CHCl_3$), had the molecular ion peak at *m/z* 362.2457 in the HR-EIMS data, indicating the molecular formula of $C_{22}H_{34}O_4$ (Calcd. molecular weight: 362.2457). Its ^{1}H-NMR spectrum showed many signals closely resembling those of the aglycon moiety of erinacine Q.[4] Furthermore, the ^{13}C-NMR signals (Table 1) of **3** were in good agreement with those of the erinacine Q aglycon, 11-*O*-acetylcyathatriol, which has been isolated from basidiomycetous *Cyathus earlei* by Ayer *et al.*[6] Although its $[\alpha]_D$ value has not been reported in the literature, **3** and erinacine Q showed almost same CD spectra, having a negative Cotton effect at 207 nm. The structure of **3** was thus identified as 11-*O*-acetylcyathatriol. The ^{1}H and ^{13}C-NMR signals (Table 1) of **4** were basically similar to those of **3**. These data, together with the $[\alpha]_D$ value of **4** {$[\alpha]_D^{17}$ -9° (*c* 0.11, $CHCl_3$)} were in good agreement with those of 15-*O*-acetylcyathatriol, which has also been isolated from *C. earlei*[6] and was the positional isomer of the acetyl group of **3**. **3** in a methanolic Na_2CO_3 solution (4 mM) at room temperature gave **4** together with a small amount of cyathatriol, suggesting that **4** was an artifact formed during the preparation of the basic and neutral substances from the mycelial extract of *H. erinaceum* YB4-6237.

2, $[\alpha]_D^{16}$ -56° (*c* 0.12, EtOH), had the molecular ion peak at *m/z* 360.2297 in the HR-EIMS data, indicating the molecular formula of $C_{22}H_{32}O_4$ (Calcd. molecular weight: 360.2301). Its spectral data were as follows. EIMS *m/z* (%): 360[M^+, 55], 345(37), 317(19), 300(24), 285(51), 282(36), 272(42), 257(68), 231(48), 203(100), 189(97), 175(70); IR ν_{max} (film) cm^{-1}: 1743, 1672; the ^{1}H- and ^{13}C-NMR data are shown in Table 1. Its ^{1}H-NMR spectrum showed similar signals to those of **3**, except for the absence of the hydroxymethine signal of H-14. The ^{13}C-NMR signal (δ 209.7 in Table 1) and IR band ($\nu_{C=O}$: 1672 cm^{-1}) revealed **2** to have a conjugated carbonyl group in the molecule. These observations suggested that **2** was the 14-oxo-derivative of **3**. This was sufficiently supported by comparing the ^{13}C-NMR data between **2** and *O*,*O*-diacetylcyathin A_3, the latter has been chemically derived from 11,15-*O*,*O*-diacetylcyathatriol and cyathin A_3.[6,8–10] The ^{13}C-NMR signals of **2** were in good agreement with those of *O*,*O*-diacetylcyathin A_3, except for the acetylation-shifted carbons at 12, 13 and 14 (Δ4.1, 1.5 and 1.0 ppm, respectively). The structure of **2** was thus identified as 11-*O*-acetylcyathin A_3. Its ^{1}H- and ^{13}C-NMR signals (Table 1) were fully assigned by H–H and C–H COSY, and HMBC methods.

Cyatha-3,12-dien-14β-ol (**1**) and 11-*O*-acetylcyathin A_3 (**2**) are new fungal metabolites from erinacine Q-producing *H. erinaseum* YB4-6237. In particular, **1** is not only a novel monohydroxy cyatha-3,12-diene, but also a putative intermediate in the biosynthesis of the erinacine Q aglycon. This suggests that the 14β-hydroxyl was introduced first in three hydroxyls of the erinacine Q aglycon. 11-*O*-Acetylcyathatriol (**3**) as the erinacine Q aglycon was newly isolated from the mycelial extract of the basidiomycete and spectroscopically identified. Possible biosynthetic relationships of these metabolites to erinacine Q on the basis of the observations described here are shown in Fig. 1. **1** and **2** as important metabolites in the erinacine Q-biosynthetic pathway between (−)-cyatha-3,12-diene and parental erinacine Q in erinacines were revealed in this study. **2** being an oxidation metabolite of **3** of the erinacine Q aglycon. **4** as the positional isomer of the acetyl group in **3** is considered to have been an artifact derived from **3**.

We are now investigating the biosynthetic pathway of erinacine Q in the basidiomycete on the basis of incorporation experiments on labeled **1** and **2** to erinacine Q and its aglycon, and the isolation and structural determination of other hydroxycyathadiene metabolites.

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