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Isolation of erinacine P, a new parental metabolite of cyathane-xylosides, from *Hericium erinaceum* and its biomimetic conversion into erinacines A and B

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Abstract

A new cyathane-xyloside, erinacine P, was isolated from mycelia of basidiomycetous *Hericium erinaceum* YB4-6237 in a shaken culture. Since its aglycon closely resembles typical cyathane diterpenoids such as cyathins and cyathatriols in its substitution pattern, this glycoside seems to be an important metabolite in the biosynthesis of erinacines and striatins. In fact, according to our biogenesis studies, erinacine P can be successfully converted chemically into erinacine B and, further, to erinacine A under mild conditions.

Keywords: biomimetic reactions; biosynthesis; glycosides; terpenes and terpenoids.

Cyathane-xylosides of erinacines[1–3] and striatins[4] 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[1–3] and agonistic activity towards the κ opioid receptor.[5] Striatins have leishmanicidal activity.[6] In the course of our studies[7,8] to isolate cDNAs encoding novel fungal diterpene cyclases, we attempted the isolation of the cyathane hydrocarbon from *Hericium erinaceum* YB4-6237, the erinacine-producing basidiomycete. During the investigation,[†] we succeeded in isolating a new cyathane-xyloside named erinacine P (**1**) from the mycelial extract of this basidiomycete. Here, we report the structural elucidation of **1** and its in vitro biomimetic conversions into erinacine A (**2**) and erinacine B (**3**) (Fig. 1).

The brownish aqueous residue from a dipping extract of wet mycelia of *H. erinaceum* YB4-6237 with acetone obtained from 32 culture flasks, each containing 100 cm^3 of the culture medium {5.0% glucose, 0.5% peptone, 1.0% Pharmamedia® (Traders Protein Co.) and 0.5%

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† We have already isolated a cyathane hydrocarbon and the results will be reported in the near future.

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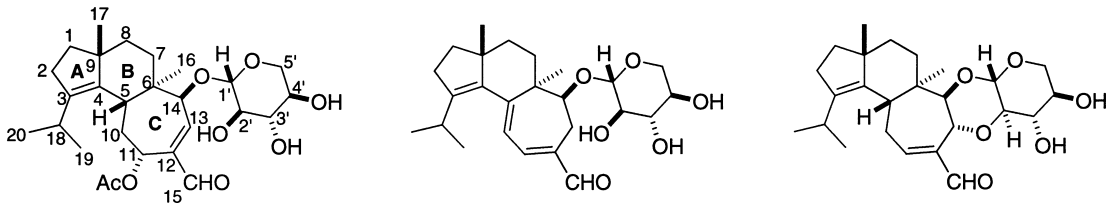


Figure 1.

NaCl in deionized water},‡ was extracted with EtOAc at pH 9. The residue of the organic extract was separated by silica gel flash chromatography using mixtures of $CHCl_3$ and EtOH (20:1–5:1) to afford a new metabolite **1** (155 mg) as the major diterpene-glycoside together with the known erinacines, A (**2**, 2.9 mg), B (**3**, 23 mg), and C (3.4 mg).[1]

The most polar metabolite, **1**, was obtained as a pale yellow amorphous solid {$[\alpha]_D^{25}$ –52.4 (*c* 0.23, MeOH); ν_{max} (film) 3384, 1743, and 1693 cm^{-1}; λ_{max} (MeOH) 206 (ε 7500) and 228 (ε 6900)}. Its molecular formula was determined as $C_{27}H_{40}O_8$ from the $(M+Na)^+$ ion peak (515.2629, Δ+0.8 mmu) in HR-FABMS. The molecular formula and the NMR data listed in Table 1 as well as its IR and UV data determine **1** to be a mono-acetate of a cyathane-xyloside. The difference between **1** and erinacine B (**3**) is the substitution pattern on the C-ring. The former has an additional acetoxy group at C11. The methine proton at this position appears at δ 5.88 and is coupled to the C10 methylene protons as evidenced in the H–H COSY spectrum. It has a correlation peak with the carbonyl carbon (δ 170.2) of the acetyl group in HMBC measurement. The double bond in the C-ring is located at the C12–C13 position. The olefinic proton at δ 6.89 appears as a sharp doublet, which is coupled to the C14 oxymethine proton at δ 4.43. The

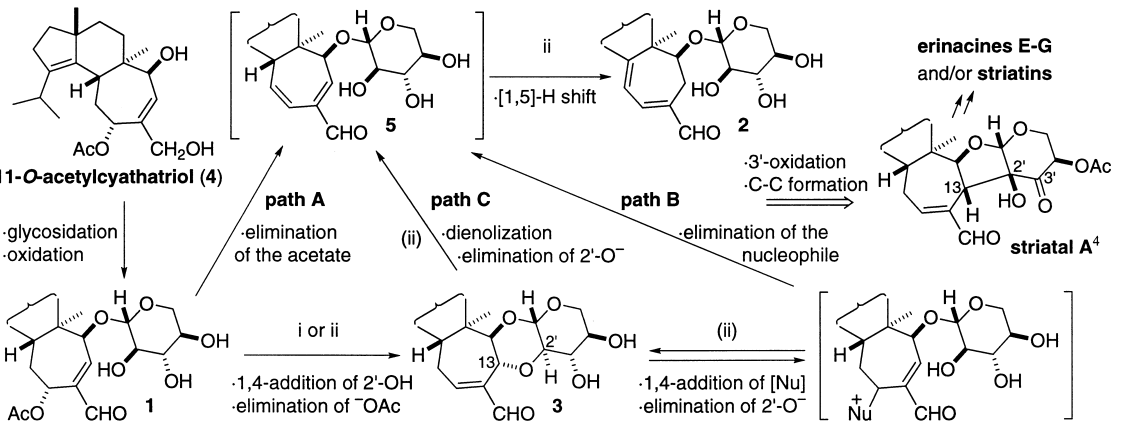
Table 1
NMR data for erinacine P (**1**) in $CDCl_3$ at +50°C;[a] 600 MHz for ^1H and 150 MHz for ^{13}C

Position[b]	^1H [ppm (multiplicity, *J* in Hz)	^{13}C [ppm (type)]	Position[b]	^1H [ppm (multiplicity, *J* in Hz)	^{13}C [ppm (type)]
1	1.50 (dt, 13.2, ~8)	38.5 (CH_2)	14	4.43 (br d, 5.5)	85.2 (br, CH)
	1.63 (dt, 13.2, ~7)		15	9.44 (s)	191.5 (CH)
2	2.28 (2H, m)	28.6 (CH_2)	16	0.98 (3H, s)	16.9 (CH_3)
3	—	140.4 (C)	17	0.96 (3H, s)	24.5 (CH_3)
4	—	136.5 (C)	18	2.77 (septet, 6.8)	27.1 (CH)
5	2.17 (br d, 11.2)	40.2 (CH)	19/20	0.97, 0.98 (each 3H, d, 6.8)	21.5, 21.8 (CH_3)
6	—	44.1 (C)	1'	4.40 (d, 6.6)	105.3 (CH)
7	1.47-1.56 (2H, m)	31.2 (br, CH_2)	2'	3.49 (dd, 8.2, 6.6)	73.4 (CH)
8	1.44-1.48 (2H, m)	37.0 (CH_2)	3'	3.56 (dd, 8.2, 8.0)	75.5 (CH)
9	—	49.4 (CH)	4'	3.74 (ddd, 8.8, 8.0, 4.8)	69.7 (CH)
10	1.86 (ddd, 13.9, 11.2, 8.2)	29.7 (br, CH_2)	5'	3.30 (dd, 11.9, 8.8)	65.0 (CH_2)
	2.57 (br dd, 13.9, 8.2)			4.03 (dd, 11.9, 4.8)	
11	5.88 (t, 8.2)	68.2 (br, CH)			
12	—	138.6 (br, C)	Ac/CO	—	170.2 (C)
13	6.89 (d, 5.5)	155.7 (br, CH)	Ac/Me	2.04 (3H, s)	21.0 (CH_3)

[a]Probably due to the contribution of conformers around the C-ring, several signals are heavily broadened especially in ^{13}C NMR spectrum at ambient temperature. [b]Assignments were confirmed by H-H COSY, HMQC and HMBC measurements.

‡ The basidiomycete in a shaken culture was grown at 25°C in this medium for 18 days. The wet weight of the obtained mycelia was ca. 157 g.

stereochemistry of C11 could be assigned unambiguously as depicted, since a clear NOE enhancement was observed on the C5 methine proton upon irradiation of the C11 methine.[§] Thus, the structure of **1** was elucidated to be an erinacine-like xyloside of which the aglycon has the typical substitution pattern of cyathane diterpenoids exemplified by 11-*O*-acetylcyathatriol (**4**)[9] shown in Scheme 1.



Scheme 1. A proposed biogenesis for the erinacine family. *Reagents and conditions for in vitro conversions*: (i) Et_3N (3 equiv.)–LiBr (0.5 equiv.), THF, rt (40 h) (**3**, 72%); (ii) DABCO (2 equiv.)–LiBr (0.5 equiv. then 2.5 equiv.), THF-d_8, rt (22 h) then 50°C (65 h) (**2**, 88%)

The characteristic structural features[¶] of the aglycon in **1** led us to propose a biogenesis that links cyathins to erinacines as shown in Scheme 1. First, the typically oxygenated cyathane diterpenoid such as **4** may form erinacine P (**1**). The 1,4-addition of the 2′-hydroxy group onto the β-position of the α,β-unsaturated aldehyde followed by the elimination of the acetate would form erinacine B (**3**). Erinacine A (**2**) may also be derived from **1** via an intermediary conjugated diene **5** (path A). Alternatively, **5** may be formed from **3** by either the 1,4-addition–elimination with participation of a nucleophile (path B) or a simple dienolate formation followed by the elimination of the 2′-hydroxy group (path C). For the final positional isomerization of the diene moiety from **5** to **2**, a [1,5]-sigmatropic hydrogen shift is more plausible than a simple protonation–deprotonation process[9] since the latter requires an unfavorable protonation at the conjugation terminus of the α,β-unsaturated aldehyde moiety. Similarly, when the 3′-position of the xylose is oxidized to a ketone prior to or during these transformations, a C–C bond between C13 and C2′ may be formed, leading to erinacines E–G[3] and/or striatins.[4] Thus, in this proposed biogenesis, **1** can be regarded as the parental metabolite of the cyathane-xyloside family. In fact, from a preliminary analysis on the time-dependent formation of the metabolites, it was clear that **1** is formed in advance of **2** and **3**.

To support the above-mentioned biogenesis, we investigated chemical conversions of **1** to **2** and **3**. In a treatment of a THF solution of **1** with Et_3N in the presence of LiBr,[‖] the formation of **3** was

[§] The NOEs were measured in a mixture of $CDCl_3$:CD_3OD (10:1) to avoid oligomeric contributions of the substrate.

[¶] Although erinacine D[2] has a similar substitution pattern, its stereochemistry at C-11 (β-OEt) is opposite to that of the known cyathins[9,10] and scabronines.[11,12]

[‖] Without LiBr, the reaction does not proceed smoothly. For use of lithium halide-tertiary amine, see Ref. 13 and Ref. 14.

actually realized even at ambient temperature. The absolute stereo-structure of **1** was unambiguously confirmed, since **3** obtained here as a main product in 72% yield is identical with the natural substance including optical rotation {$[\alpha]_D^{26}$ –123.0 (*c* 0.20, MeOH); natural, $[\alpha]_D^{26}$ –118.5 (*c* 0.20, MeOH)}.[#] Similarly, **1** was treated with 1,4-diazabicyclo[2.2.2]octane (DABCO, 2 equiv.) and LiBr (0.5 equiv.) in THF-d_8. Again, the predominant formation of **3** at an early stage of the reaction was observed by ^{1}H NMR analysis. After 22 h, another two sets of signals, which are ascribable to **5**[**] and **2**, were detected. These results indicate that the biogenesis in Scheme 1 is quite plausible and the reaction proceeds not through path A but through paths B or C. The mixture was warmed at 50°C for 65 h with additional LiBr (2 equiv.) in order to complete the reaction. During this period, the formation of **2** occurred without an increase in the amount of **5**, showing that the [1,5]-hydrogen shift proceeds smoothly taking advantage of the formation of the fully conjugated triene-system in **2**. Eventually, **2** was isolated as the sole product from the reaction mixture. Since it is well known that DABCO acts as a nucleophile and forms an intermediary adduct in the Baylis–Hillman reaction,[15] it is plausible that this chemical conversion takes place via path B.

Consequently, the accomplishment of the chemical conversions of **1** into **2** and **3** under mild conditions supports the proposed biogenesis shown in Scheme 1.[††]

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[#] The absolute value of optical rotation of **3** differs greatly from the reported one (–34.9).[1] The reason for this difference is uncertain at this moment.

[**] The diagnostic ^{1}H signals for **5** are: δ (THF-d_8) 6.21, 6.31(each dm, J = 12.2 Hz, 10/11-H), 6.82 (d, J = 8.1 Hz, 13-H), and 9.40 (s, 15-H).

[††] Added in proof: We noticed in a review that the planar structure of erinacine P (**1**) has been reported as herical isolated from *Hericium ramosum*; see, Lorenzen, K.; Anke, T. *Curr. Org. Chem*. **1998**, *2*, 329–364.

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