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Erinacines A, B and C, strong stimulators of nerve growth factor (NGF)-synthesis, from the mycelia of *Hericium erinaceum*.

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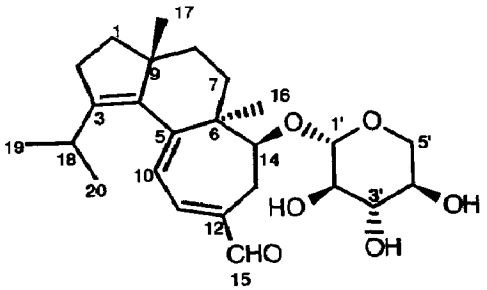
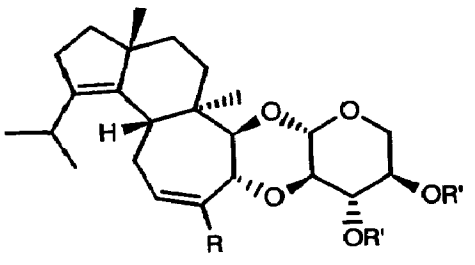
Abstract: The structures of novel diterpenoids, erinacines A, B, and C, isolated from the cultured mycelia of *Hericium erinaceum* were determined by interpretation of the spectral data, and chemical and enzymatic reactions. These compounds showed potent stimulating activity of nerve growth factor (NGF)-synthesis.

Stimulators of NGF-synthesis have been expected to become drugs for degenerative neuronal disorders such as Alzheimer's disease and peripheral nerve regeneration, and some natural products exhibiting such activity have been reported[1-4]. In the previous papers, we reported the isolation of the stimulators from the fruiting bodies of *Hericium erinaceum*[1,2]. Further search for the stimulators in the mycelia of this fungus has resulted in the isolation of novel diterpenoid-xylosides 1, 2 and 3 named erinacines A, B, and C, possessing potent stimulating activity to NGF-synthesis *in vitro*. In this paper, we describe the structures of these new compounds.



1



2 R = CHO R' = H
3 CH_2OH H
4 CHO Ac

Table ^{1}H-NMR data ($CDCl_3$, 400 MHz) for compounds 1 - 4*

position	ppm (multiplicity, J in Hz) 1	2	3	4
1	1.67(m)	1.58(m)	1.52(m)	1.60(m)
	1.57(m)	1.62(m)	1.63(m)	
2	2.34(m)	2.26(m)	2.27(m)	2.27(m)
5	-	2.30(m)	2.32(m)	2.37(m)
7	2.36(m)	1.58(m)	1.66(m)	1.50-1.72(m)
	1.30(br.d, 13.2)	1.69(m)		
8	1.61(m)	1.50(m)	1.50(m)	
10	5.81(d, 8.1)	2.72(m)	2.41(m)	2.69(m)
			2.48(m)	
11	6.72(d, 8.1)	6.98(m)	6.01(br.d, 7.3)	6.99(m)
13	3.24(dd, 17.6, 5.9)	5.14(br.d, 9.7)	4.81(br.d, 9.7)	5.15(d, 9.9)
	2.48(d, 17.6)			
14	3.60(d, 5.9)	3.82(d, 9.7)	3.89(d, 9.7)	3.82(d, 9.9)
15	9.31(s)	9.56(s)	4.31(d, 11.9)	9.63(s)
			4.00(d, 11.9)	
16	0.93(s)	0.99(s)	1.00(s)	1.00(s)
17	0.93(s)	1.02(s)	1.02(s)	1.02(s)
18	2.77(heptet, 6.6)	2.75(m)	2.75(heptet, 6.6)	2.78(qq, 6.2, 6.6)
19, 20	0.98(d, 6.6)	0.98(d, 6.2)	0.96(d, 6.6)	0.99(d, 6.2)
	0.91(d, 6.6)	0.97(d, 6.6)	0.97(d, 6.6)	0.97(d, 6.6)
1'	4.48(d, 5.1)	4.58(d, 8.4)	4.58(d, 8.6)	4.70(d, 8.8)
2'	3.38(dd, 5.1, 6.6)	3.20(dd, 8.4, 8.8)	3.41(dd, 8.6, 8.9)	3.43(dd, 8.8, 9.9)
3'	3.46(dd, 6.6, 7.0)	3.60(dd, 8.8, 8.8)	3.56(dd, 8.9, 8.6)	5.16(dd, 9.9, 9.2)
4'	3.50(m)	3.69(m)	3.63(m)	4.94(m)
5'	3.74(dd, 11.7, 2.9)	4.03(dd, 11.7, 5.9)	3.99(dd, 10.6, 5.4)	4.12(dd, 11.7, 5.9)
	3.20(dd, 11.7, 7.0)	3.35(dd, 11.7, 10.6)	3.31(dd, 10.6, 10.9)	3.36(dd, 11.7, 10.6)
Ac				2.02(s)
				2.09(s)

*Assignments were made by the decoupling, DEPT, HSQC, HMBC, and NOESY analyses.

The fungus was cultivated by shaking at 30°C for 4 weeks. The culture was centrifuged, and the resulting residue (mycelia, wet weight; 1.1 kg) was extracted with 85% ethanol and the extract after evaporating the solvent was partitioned between ethyl acetate and water. Repeated silica gel chromatography and HPLC of the ethyl acetate-extract gave 1 (398.7 mg, mp 74-76°C), 2 (24.5 mg, mp 125-127°C), and 3 (4.9 mg, mp 115-118°C) as white crystals.

Erinacine A (1)[5] had the molecular formula $C_{25}H_{36}O_6$ determined by HR-FAB-MS of the MH^+ ion (433.2566, Δ -2.4 mmu). Treatment of 1 with acetic anhydride and pyridine gave a triacetate (data not shown). The ^{1}H-NMR (Table) and ^{13}C-NMR[5] showed this compound was a glycoside of a diterpenoid. Aglycone of the compound could not be obtained by hydrolysis with dilute alkaline since many products formed, but treatment of the compound with β-glucosidase gave the aglycone and D-xylose without any by-product. All the data (^{1}H-NMR, IR, MS, $[\alpha]_D$) for the aglycon agreed with those of allocyathin B_2 whose absolute configuration was already determined[6]. Thus the structure of erinacine A including the absolute configuration was proposed as 1.

The molecular formula $C_{25}H_{36}O_6$ (MH^+ ion, *m/z* 433.2598, Δ +0.8 mmu from HR-FAB-MS) of erinacine B (2)[7] was the same as that of 1, and the NMR data (Table)[7] were similar to those of 1. But 2 has two double bonds and gave a diacetate 4 by acetylation. These results indicated that 2 contained a double-linked xylose moiety. The linkage positions were determined by HMBC experiment (Figure); the cross peaks between H1' and C14, and H13 and C2' were observed. The relative stereochemistry was shown by NOESY correlation; the NOE appeared between H5 and H13, H16 and H14, H14 and H2', H1' and H3', and H1' and H5'. The absolute configuration of 1 was deduced by comparison of CD data of 1 and 2: 1, Δε +47.1 at 344 nm, -12.4 at 273 nm (EtOH); 2, +3.46 at 353 nm, -3.46 at 278 nm (EtOH).

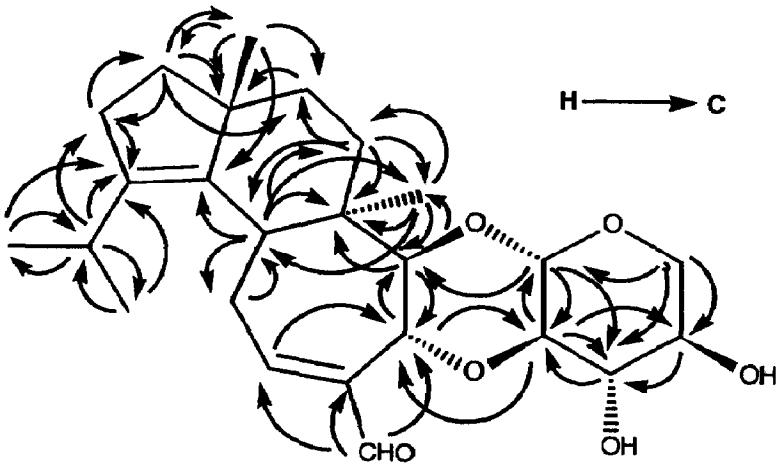


Figure HMBC correlation of erinacine B (2)

Erinacine C (3)[8] showed M+Na$^+$ ion at *m/z* 457 by FAB-MS. The ^{1}H-NMR (Table) and CD data [+5.66 at 345 nm, -2.97 at 250 nm (EtOH)] of 3 were similar to those of 2 except for the absence of a formyl group, suggesting the formyl group in 2 was just reduced to a hydroxy group in 3. This was confirmed by the direct conversion of 3 to 2 by oxidation with DDQ in dioxane.

In the bioassay using mouse astroglial cells[1,2, 9-11], the amounts of NGF secreted into the medium in the presence of 1, 2 or 3 at 1.0 mM were 250.1±36.2, 129.7±6.5, and 299.1±59.6 pg/ml, respectively. These activities were much stronger than that (69.2±17.2 pg/ml at 1.0 mM) of a known potent stimulator, epinephrine used as a positive control-compound in this assay.

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5) ^{13}C-NMR of 1 ($CDCl_3$, 100 MHz): 194.2 (1), 154.0 (5), 145.4 (3), 145.4 (11), 141.6 (4), 138.5 (12), 119.8 (10), 104.8 (1'), 84.0 (14), 73.2 (3'), 71.5 (2'), 69.3 (4'), 63.5 (5'), 49.1 (9), 47.9 (6), 38.2 (1), 36.3 (8), 33.2 (7), 28.8 (2), 27.5 (13), 26.8 (18), 26.3 (16), 23.8 (17), 21.4 (19,20). FAB-MS *m/z* (positive; matrix, 3-nitrobenzyl alcohol): 455, 433, 301, 283. IR ν_{max} cm^{-1}: 3423, 3012, 1675, 1654, 1577, 1560, 1457, 1430, 1361. UV λ_{max} nm(ε): 339 (11300), 201 (10500). $[\alpha]_D$ +216° (*c*=0.28, MeOH).
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7) ^{13}C-NMR of 2 ($CDCl_3$, 100 MHz): 193.4 (15), 155.4 (11), 142.1 (12), 140.2 (3), 135.9 (4), 98.5 (1'), 79.1 (14), 74.9 (3'), 71.1 (13), 70.9 (4'), 70.7 (2'), 66.9(5,), 49.4 (9), 41.9 (5), 41.6 (6), 38.1 (1), 36.4(8), 29.7 (10), 28.4 (2), 27.7 (7), 27.1 (18), 24.4 (17), 21.9, 21.4 (19, 20), 16.4 (16). FAB-MS *m/z* (positive; matrix, 3-nitrobenzyl alcohol): 433, 301. IR ν_{max} cm^{-1}: 3421, 1691, 1616, 1458, 1377. UV λ_{max} nm(ε): 230, (8400), 200(20800). $[\alpha]_D$ -34.9° (*c*=0.18, MeOH).
8) ^{13}C-NMR of 3 ($CDCl_3$, 100 MHz): 139.8 (3), 139.5 (12), 136.8 (4), 135.7 (11), 98.7 (1'), 79.8 (14), 74.7 (3'), 73.3 (13), 71.3 (4'), 69.9 (2'), 66.9 (5'), 66.0 (15), 49.4 (9), 42.6 (5), 41.5 (6), 38.1 (1), 36.5 (8), 28.4 (10), 28.3 (2), 27.8 (7), 26.9 (18), 24.5 (17), 21.9 (20), 21.4 (19), 16.5 (16). FAB-MS *m/z* (positive, matrix thioglycerol): 457, 433, 417, 399, 285. IR ν_{max} cm^{-1}: 3384, 1377, 1173, 1063, 1041, 1009. UV λ_{max} nm(ε): 203 (8100). $[\alpha]_D$ -72.5° (*c*=0.73, MeOH).
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