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Erinacines E, F, and G, Stimulators of Nerve Growth Factor (NGF)-Synthesis, from the Mycelia of *Hericium erinaceum*

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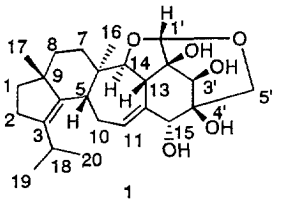
Abstract: The structures of erinacines E, F and G from mycelia of *Hericium erinaceum* were determined by spectroscopic and/or X-ray analysis. Erinacines E and F exhibited potent stimulating activity against NGF synthesis by astroglial cells.

Stimulators of NGF-synthesis have been expected as medicines for degenerative neuronal disorders such as Alzheimer's disease and peripheral nerve regeneration, and some natural products exhibiting such activity have been reported.[1-7] In the previous papers, we reported the isolation of the stimulators, erinacine A to D, from the mycelia of *Hericium erinaceum* whose fruiting bodies have been known as Chinese medicine or food in Japan and China.[3,4] Those compounds were diterpene-xyloside possessing "cyathan" skeltons.[3,4,7-14] Our further search for the stimulators in the mycelia of this fungus has resulted in the isolation of novel stimulators having unique structures and stimulating activity. We report here the isolation, the structure, and the activity of the compounds, erinacines E to G.



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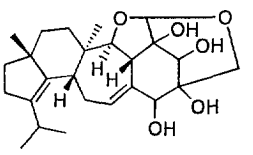

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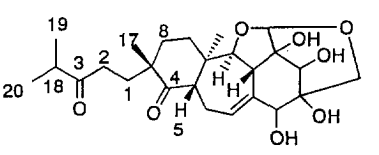

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The fungus was cultivated by shaking at 30°C for 4 weeks. The culture (15 l) was centrifuged, and the resulting residue (mycelia, wet weight 1.38 kg) was extracted with 85% ethanol and the extract after evaporating the solvent was partitioned between ethyl acetate and water. Repetitive silica gel chromatography and HPLC of the ethyl acetate-extract (33.1 g) gave 1 (4.1 mg, mp 161-163°C), 2 (19.7 mg, mp 228-230°C), and 3 (4.9 mg, mp 135-137°C) as white crystals.

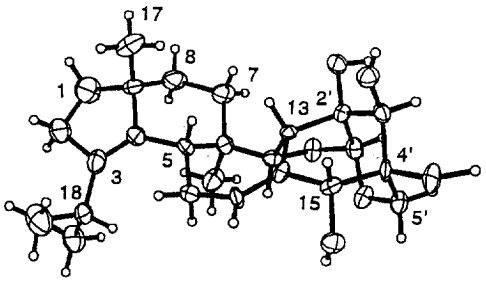

Fig. 1. A stereo ORTEP plot of erinacine E (1).

The molecular formula of 1 was determined to be $C_{25}H_{36}O_6$ ([M+H]$^+$ *m/z* 433.2602 Δ +1.2 mmu) by HRFABMS. IR (KBr) 3394, 1078, 1043 cm^{-1}; UV (MeOH) λ_{max} 207 (ε 7100); $[\alpha]^{25}_D$ = -144° (*c* 0.50, MeOH). The analyses of ^{1}H-NMR, ^{13}C-NMR, COSY, DEPT, HSQC, and HMBC data showed that this compound has a diterpene having "cyathan" skeleton like erinacines A to D.[3,4,15] However, the sugar part of 1 was highly modified. C-C bonds between C13 and C2', and C15 and C4', and a C-O-C bond between C14 and C1' were suggested by HMBC correlations; cross peaks between H1'/C13, H3'/C13, H5'/C15, and H1'/C14 appeared in the spectrum. Finally, the structure including the relative stereochemistry was determined from a single crystal X-ray diffraction analysis.[16] A stereo ORTEP plot of a single molecule of 1 is shown in Figure 1.

Erinacine F (2) showed the same molecular formula ($C_{25}H_{36}O_6$, [M-H]$^-$ *m/z* 431.2429 Δ -0.5 mmu by HRFABMS) as 1. IR (KBr) 3429, 1051, 1016 cm^{-1}; UV (MeOH) λ_{max} 203 (ε 8000); $[\alpha]^{25}_D$ = -31° (*c* 0.20, MeOH). The ^{1}H-NMR, ^{13}C-NMR and COSY data, and HMBC and/or COLOC correlations were quite similar to those of 1.[17] In addition, the coupling constant between H13 and H14 (*J* = 10.62) in its ^{1}H-NMR spectrum indicated that their relative configuration was *trans* and NOESY experiment showed that the relative stereochemistry of the aglycone part in 2 was the same as that in 1; NOESY correlations between H5/H13, H5/H17, and H14/H16 were observed. All the data allowed us to conclude that 2 was a diastereomer of 1 in the sugar part. However, the stereochemistry of the sugar part in 2 remains undetermined since its NOESY experiments did not give any valuable information.

Erinacine G (3), IR (KBr) 3394, 1708, 1076, 1053 cm^{-1}; UV (MeOH) λ_{max} 204 (ε 13000); $[\alpha]^{25}_D$ = -13° (*c* 0.25, MeOH); Molecular formula, $C_{25}H_{36}O_8$ (HRFABMS [M+H]$^+$ *m/z* 431.2429 Δ -0.8 mmu). The interpretation of ^{1}H-NMR, ^{13}C-NMR,[18] COSY, DEPT, NOESY, HSQC, and HMBC spectra of erinacine G (3) gave the deduced structure; cross peaks between H17/C1, H17/C4, H1/C3, H19/C3, H20/C3, and H5/C4 were observed in its HMBC spectrum. The coupling constant between H13 and H14 (*J* = 9.81) in ^{1}H-

NMR spectrum of 3 and NOESY experiment suggested that the relative stereochemistry of the aglycone part in 3 was also the same as that in 1.[18] The relative stereochemistry of the sugar part in 3 has not been determined yet, but the part has probably the same stereochemistry as that of 2 since the chemical shifts in ^{1}H-NMR of the part of 3 in CD_3OD [δ 5.07 (s, H1'), 3.75 (br.s, H3'), 3.63 (br.s., H5')] were very similar to those of 2 in CD_3OD.[17] Formation of 3 in the mycelia can be envisioned as oxidative cleavage of C3-C4 bond of 2.

Erinacines E (1) and F (2) exhibited potent stimulating activity against NGF synthesis by astroglial cells (erinacine G has not been tested yet).[1-4, 19,20] In the bioassay using rat astroglial cells, the amounts of NGF secreted into the medium in the presence of 1 and 2 at 5.0 mM were 105 ± 5.2 and 175 ± 52 pg/ml, respectively. These activities were stronger than that (70.2 ± 5.4 pg/ml at 1.0 mM) of a known potent stimulator, epinephrine used as a positive control in this assay.

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15 ^{1}H-NMR of 1 (400 MHz, CD_3OD); δ 0.96(3H, s, H16), 0.99(3H, d, J=6.96, H19 or 20), 1.02(3H, d, J=6.96, H20 or 19), 1.10(3H, s, H17), 1.36(1H, m, H7a), 1.56(3H, m, H1a and H8), 1.69(1H, m, H1b), 1.76(1H, m, H7b), 2.33(2H, m, H2), 2.61(1H, m, H10a), 2.69(1H, m, H10b), 2.88(1H, br.d, J=12.46, H5), 2.96(1H, qq, J=6.60, 6.96, H18), 3.12(1H, m, H13), 3.23(1H, d, J=12.28, H5'a), 3.91(1H, s, H3'), 3.92(1H, d, J=12.28, H5'b), 4.22(1H, d, J= 6.23, H14), 4.70(1H, br.s, H15), 4.94(1H, s, H1'), 5.62(1H, m, H11). ^{13}C-NMR of 1 (100 MHz, CD_3OD); δ 17.5(C16), 22.2(C19 and 20), 25.2(C17), 28.3(C18), 28.7(C7), 29.3(C2), 32.1(C10), 38.3(C8), 40.2(C1), 42.4(C6), 44.1(C13), 44.4(C5), 51.0(C9), 66.7(C5'), 72.3(C15), 75.0(C4'), 76.0(C3'), 80.3(C2'), 97.6(C14), 106.4(C1'), 124.2(C11), 138.8(C4), 140.3(C3), 142.7(C12).

16 Crystallographic data: $C_{25}H_{36}O_6+H_2O$, FW=450.6, crystallized from methanol and water; crystal size, 0.08 x 0.54 x 0.78 nm; cell dimensions, a = 46.274(7)Å, b = 7.928(2) Å, c = 13.297(1) Å, α = 90.0°, β= 95.20(1)°, γ = 90.0°, V = 4858(2) Å^3; space group, C2; molecules/unit cell, 8; density calcd.g/cm^3, 1.232; linear absorption factor, 0.725 nm^{-1}, number of reflections, 2730; nonzero reflections ($I>3.0\sigma$), 2519; R-index, 7.27%; GOF, 1.45; secondary extinction factor, χ 25(4) x 10^{-4}. A representative crystal was surveyed and a 1 Å data set (maximum sin Θ/λ= 0.5) was collected on a Siemens R3RA/v diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography . All crystallographic calculations were facilitated by the SHELXTL system. A trial structure was obtained by direct methods. This trial structure was refined routinely. Hydrogen positions were calculated wherever possible . The methyl hydrogens and the hydrogens on oxygen were located by difference Fourier techniques . The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations . The final R-index was 7.27%. A final difference Fourier revealed no missing or misplaced electron density. The refined structure was plotted using the SHELXTL plotting package. The absolute configuration was not determined in this analysis because no 'heavy atoms' were present in the structure.

17 ^{1}H-NMR of 2 (400 MHz, CD_3OD); δ 0.97(3H, s, H16), 0.97(3H, d, J=6.60, H19 or 20), 1.00(3H, d, J=6.60, H20 or 19), 1.08(3H, s, H17), 1.42(1H, m, H7a), 1.50(1H, m, H8a), 1.58(1H, m, H8b), 1.60(2H, m, H1), 1.80(1H, ddd, J=13.21,13.55,4.40, H7b), 2.31(2H, m, H2), 2.41(1H, m, H10a), 2.57(1H, m, H10b), 2.70(1H, br.d, J=11.36, H5), 2.89(1H, heptet, J=6.60, H18), 3.15(1H, br.d, J=10.62, H13), 3.68(2H, br.s, H5'), 3.76(1H, br.s, H3'), 4.26(1H, d, J=10.62, H14), 4.72(1H, br.s, H15), 5.03(1H, s, H1'), 5.51(1H, m, H11). ^{13}C-NMR of 2 (100 MHz, CD_3OD); δ 18.6(C16), 22.6(C20 or 19), 23.1(C19 or 20), 25.9(C17), 29.1(C18), 29.3(C7), 29.9(C2), 31.4(C10), 38.1(C8), 40.1(C1), 42.7(C6), 45.7(C5), 51.8(C9), 54.0(C13), 65.1(C5'), 71.9(C3'), 80.4(C4'), 83.6(C2'), 86.8(C15), 92.4(C14), 109.3(C1'), 122.7(C11), 140.2(C4), 141.0(C3), 141.1(C12). HMBC correlations are as follows; H1/C2, H1/C8, H1/C17, H2/C4, H5/C4, H5/C6, H5/C16, H7/C16, H8/C9, H8/C17, H10/C6, H13/C2', H14/C16, H15/C12, H15/C4', H16/C5, H16/C6, H17/C1, H17/C4, H17/C8, H17/C9, H18/C2, H18/C19, H18/C20, H19/C18, H19/C20, H20/C18, H20/C19, H1'/C14, H3'/C2', H3'/C4', H5'/C1', H5'/C4'.

18 ^{1}H-NMR of 3 (400 MHz, $CDCl_3$); δ 0.94(3H, s, H16), 1.09(6H, d, J=6.96, H19 and 20), 1.20(3H, s, H17), 1.70(3H, m, H1 and H8a), 1.78(1H, m, H7a), 1.86(1H, m, H8b), 2.03(1H, m, H7b), 2.09(1H, m, H10a), 2.51(2H, m, H2), 2.58(1H, m, H10b), 2.62(1H, heptet, J=6.96, H18), 3.00(1H, d, J=10.62, H5), 3.20(1H, br.d, J=9.81, H13), 3.74(1H, d, J=12.82, H5'a), 3.79(1H, d, J=12.82, H5'b), 3.83(1H, br.s, H3'), 3.96(1H, br.d, J=9.81, H14), 4.73(1H, br.s, H15), 5.17(1H, s, H1'), 5.57(1H, m, H11). ^{13}C-NMR of 3 (100 MHz, $CDCl_3$); δ 18.0(C16), 18.4(C19 and 20), 23.6(C10), 24.8(C17), 25.8(C7), 32.3(C1), 34.0(C8), 35.4(C 2), 40.9(C18), 43.1(C6), 46.9(C9), 51.2(C5), 53.1(C13), 63.6(C5'), 70.1(C3'), 78.4(C4'), 81.4(C2'), 85.9(C15), 90.0(C14), 107.9(C1'), 120.8(C11), 138.8(C12), 214.2(C4), 214.9(C3). NOESY correlations are as follows; H5/H13, H5/H17, H14/H16.

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