ERINACINE D, A STIMULATOR OF NGF-SYNTHESIS, FROM THE MYCELIA OF *HERICIUM ERINACEUM*

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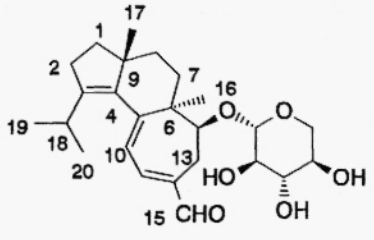
Abstract: A novel diterpenoid, erinacine D, was isolated from the cultured mycelia of *Hericium erinaceum*. The structure of the compound was determined by interpretation of the spectral data and chemical reaction. This compound showed stimulating activity of nerve growth factor (NGF)-synthesis.

Introduction

Stimulators of NGF-synthesis have been expected to be used as drugs for degenerative neuronal disorders such as Alzheimer's disease and for peripheral nerve regeneration, and some natural products exhibiting such activity have been reported (1-5). In the previous papers, we reported the isolation of the stimulators, hericenones C to H from the fruiting bodies of *Hericium erinaceum*, which were first active compounds isolated from other than animals (1, 2). Furthermore, new cytotoxic γ-pyrones, antimicrobial orcinol-derivatives and strong stimulators of NGF-synthesis, erinacines A (1) to C (3) have been isolated from the mycelia of this fungus (3, 6, 7). In this paper, we describe further isolation of a new stimulator of NGF-synthesis, erinacine D (4) from the mycelia.



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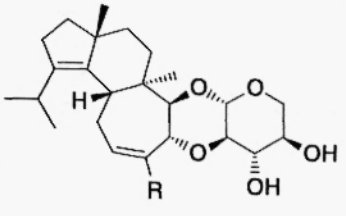
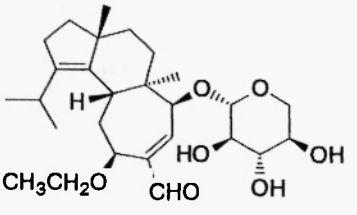


2 R = CHO
3 CH_2OH



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Results and Discussion

The fungus was cultivated by shaking at 30°C for 4 weeks. The culture was centrifuged, and the precipitate (mycelia, wet weight; 1.38 kg) were extracted with ethanol and the extract after concentrating the solvent was fractionated by solvent partition between ethyl acetate and water. Repeated silica gel column chromatography and preparative TLC ($CHCl_3$:MeOH = 95:5) of the ethyl acetate-extract gave 4 (4.9 mg, mp 121-123°C) as white crystals.

The molecular formula $C_{27}H_{42}O_7$ of erinacine D (4) (8) was determined by high resolution

Table ^{13}C and ^{1}H NMR data for compounds 1 and 4 (in $CDCl_3$)[a]

position	ppm(multiplicity, J in Hz)			
	1		4	
1	38.2	1.67(m) 1.57(m)	36.9	1.52(m)
2	28.8	2.34(m)	28.4	2.26(m)
3	145.4		140.3	
4	141.6		139.0	
5	154.0		34.0	
6	47.9		43.4	
7	33.2	2.36(m) 1.30(br.d,13.21)	34.2	
8	36.3	1.61(m)	35.8	1.44(m) 1.51(m)
9	49.1		49.5	
10	119.8	5.81(d,8.07)	30.8	1.93(dd, 13.12, 12.21) 2.26(m)
11	145.4	6.72(d,8.07)	68.7	4.61(d, 4.88)
12	138.5		145.3	
13	27.5	3.24(dd,17.60,5.87) 2.48(d,17.60)	154.4	6.93(d, 7.93)
14	84.0	3.60(d,5.87)	85.0	3.72(d, 7.93)
15	194.2	9.31(s)	193.9	9.42(s)
16	26.3	0.93(s)	17.2	0.77(s)
17	23.8	0.93(s)	23.9	1.08(s)
18	26.8	2.77(heptet,6.60)	27.1	2.93(heptet, 6.72)
19,20	21.4	0.98(d,6.60)	21.6	0.97(d, 6.72)
	21.4	0.91(d,6.60)	22.1	0.95(d, 6.72)
1'	104.8	4.48(d,5.14)	104.0	4.77(br.s)
2'	71.1	3.38(dd,5.14, 6.59)	68.9	3.73(m)
3'	73.2	3.46(dd, 6.59, 6.96)	69.5	3.73(m)
4'	69.3	3.50(m)	69.2	3.73(m)
5'	63.5	3.74(dd,11.74,2.93) 3.20(dd, 11.74, 6.96)	61.1	4.04(d, 11.90) 3.46(dd, 11.90,11.36)
CH_2			65.6	3.53(q, 7.02)
CH_3			15.4	1.15(t, 7.02)

[a] These assignments were established by HMBC, HMQC, HSQC, DEPT, NOESY, and/or NOE-difference experiments.

FABMS of the MH^+ ion (479.3034, Δ+2.5 mmu). The ^{13}C and ^1H NMR data were similar to those of erinacine A (1) (Table); both are diterpenoids having "cyathane" skeleton and xylose (9). The sugar part of 4 was confirmed by the results that acetylation of the compound with acetic anhydride in pyridine afforded a triacetate [^1H NMR; δ 2.04(s, Ac), 2.05(s, Ac), 2.05(s, Ac), 4.56(d, *J* =6.59 Hz, H1'), 4.99(dd, *J* =6.59, 8.79 Hz, H2'), 5.16(dd, *J* =8.79, 8.43 Hz, H3'), 4.92(ddd, *J* =8.43, 8.13, 5.13 Hz, H4'), 4.13(dd, *J* =5.13, 12.09 Hz, H5'a), 3.30(dd, *J* =8.13, 12.09 Hz, H5'b)]. Compound 4 has two double bonds, whereas compound 1 possesses three ones. Furthermore, this compound has two additional carbons as an ethoxy group compared with compounds 1 to 3. The complete plane structure was determined by interpretation of HMBC correlation (Figure). The relative stereochemistry was deduced by NOESY experiments; the NOE appeared between H5 and H17, H5 and CH_2 in the ethoxy group, H5 and H10b (d, 2.26), H10a (d, 1.93) and H16, H10a and H14, and H14 and H16. Since this compound was also obtained by no use of EtOH through the isolation, it is not an artifact. Determination of the absolute configuration of the compound is now in progress.

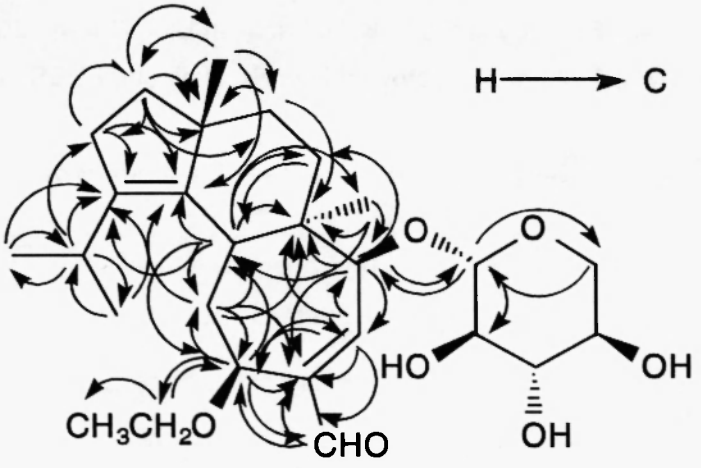

Figure. HMBC correlation in compound 4

Compound 4 showed stimulating activity to NGF-synthesis by rat astroglial cells (1-3, 10-12); the amount of NGF secreted into culture medium in the presence of 4 (1.67 mM) was 141.5±18.2 pg/ml. This activity was stronger than that (69.2±17.2 pg/ml at 1.0 mM) of a positive control compound, epinephrine.

References and Notes

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(8) FABMS (positive; matrix, 3-nitrobenzyl alcohol) of **4**: *m/z* 501 $(M+Na)^+$, 479 $(MH)^+$. IR ν_{max} cm^{-1}: 3406, 1691. UV λ_{max} nm(ε) (EtOH): 229 (5900), 204 (7300). CD: nm(Δε) (EtOH) 250(-2.25)

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