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TETRAHEDRON

Scabronines B, C, D, E and F, Novel Diterpenoids Showing Stimulating Activity of Nerve Growth Factor-Synthesis, from the Mushroom *Sarcodon scabrosus*

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Abstract: Novel cyathane-type diterpenoids, scabronines B – F (**1** – **5**) were isolated from the mushroom *Sarcodon scabrosus*. Their structures were determined by interpretation of their spectral data and chemical transformation. The absolute stereochemistry of scabronine C (**2**) was confirmed by the exciton chirality method, and the chemical transformation of scabronines B, C and D (**1, 2** and **3**) clarified their absolute structures. The absolute stereochemistries of scabronines E and F (**4** and **5**) were determined by comparison of their CD spectra with that of erinacine A (**7**). Among these compounds, scabronines B, C and E (**1, 2** and **4**) exhibit stimulating activity of the synthesis of a nerve growth factor.

Keywords: fungi; diterpenoids; nerve growth factor synthesis

INTRODUCTION

Sarcodon scabrosus is a mushroom belonging to the family Thelephoraceae and has a strongly bitter taste. Diterpenoids, sarcodonins A – H, have previously been isolated from this mushroom as the bitter principles.[1] All these diterpenoids possess a cyathane skeleton consisting of angularly condensed five-, six- and seven-membered rings. In addition, erinacines A – F,[2-4] diterpenoid-xylosides with the same carbon skeleton, were isolated from the cultured mycelia of *Hericium erinaceum*, and they were reported to exhibit potent stimulating activity of nerve growth factor (NGF)-synthesis *in vitro*.[5] It was anticipated from the evidence that the cyathane-type diterpenoids contained in the mushroom *S. scabrosus* may possess the activity. Thus, we focused our study of the mushroom on isolation of cyathane-type diterpenoids having NGF-synthesis stimulating activity, and we have already isolated scabronine A (**6**) with the activity from the mushroom.[6] Further investigation on the mushroom led us to isolate five novel cyathane-type diterpenoids named scabronines B – F (**1** – **5**). In this paper, we describe the structures of the new diterpenoids and their stimulating activity of NGF-synthesis.

RESULTS AND DISCUSSION

Isolation of scabronines B – F (1 – 5) Dried fruit body of the mushroom (1.4 kg) was extracted twice with methanol. The extracts were concentrated *in vacuo* and partitioned between *n*-hexane-EtOAc (1:1) and water. Repeated chromatography of the *n*-hexane-EtOAc (1:1)-soluble fraction gave five new diterpenoids (**1** – **5**).

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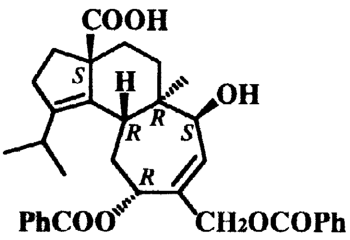
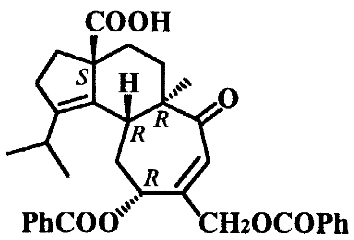
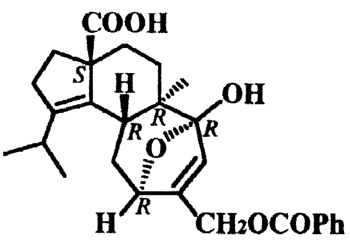


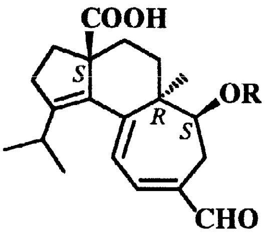
scabronine B (**1**)



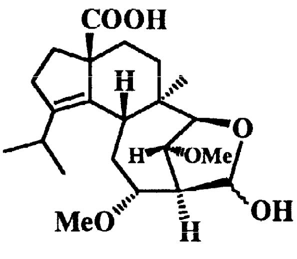
scabronine C (**2**)



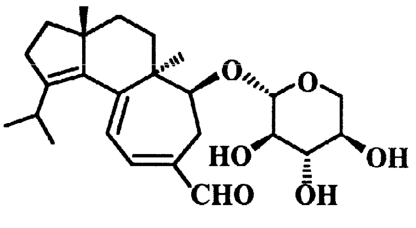
scabronine D (**3**)



scabronine E (**4**): R=Ac
scabronine F (**5**): R=H



scabronine A (**6**)



erinacine A (**7**)

Structure of scabronine B (1) Scabronine B (**1**), $[\alpha]^{25}_{D}$ +14.6° (*c* 0.39, MeOH), gave an ion peak at *m/z* 512.2571 (*m/z* 512.2563 calculated for $C_{33}H_{36}O_5$) in its HREI-MS. The presence of a carboxyl group was suggested by an IR absorption band at 1715 cm^{-1} and a ^{13}C-NMR signal at δ 182.6 (s), and this was substantiated by the formation of the monomethyl ester of scabronine B by treatment with diazomethane. ^1H-NMR spectrum of scabronine B (**1**) showed two secondary methyl and one tertiary methyl hydrogen signals at δ 0.97, 1.00 (each 3H, d, *J*=6.3 Hz) and 0.87 (3H, s), respectively. The former two methyl hydrogen signals were spin-coupled with a methine hydrogen signal at δ 2.98 (1H, septet, *J*=6.3 Hz), demonstrating the presence of an isopropyl group in the molecule. Aromatic ^1H-NMR signals (10H) at δ 7.35 – 7.97, twelve ^{13}C-NMR signals between δ 128 – 133 and two carbonyl carbon signals at δ 165.9 and 166.1 (each s) indicated two benzoate moieties. Moreover, ^{13}C-NMR of scabronine B (**1**) showed two oxymethine and one oxymethylene carbons (δ 75.4, 73.4 (each d) and 65.9 (t), respectively), one tetrasubstituted double bond (δ 144.2 and 134.0 (each s)) and one trisubstituted double bond (δ 139.0 (s) and 131.2 (d)). Based on the above partial structures, the construction of the molecular framework was deduced from H-H COSY, HMQC and HMBC spectra. HMBC correlations

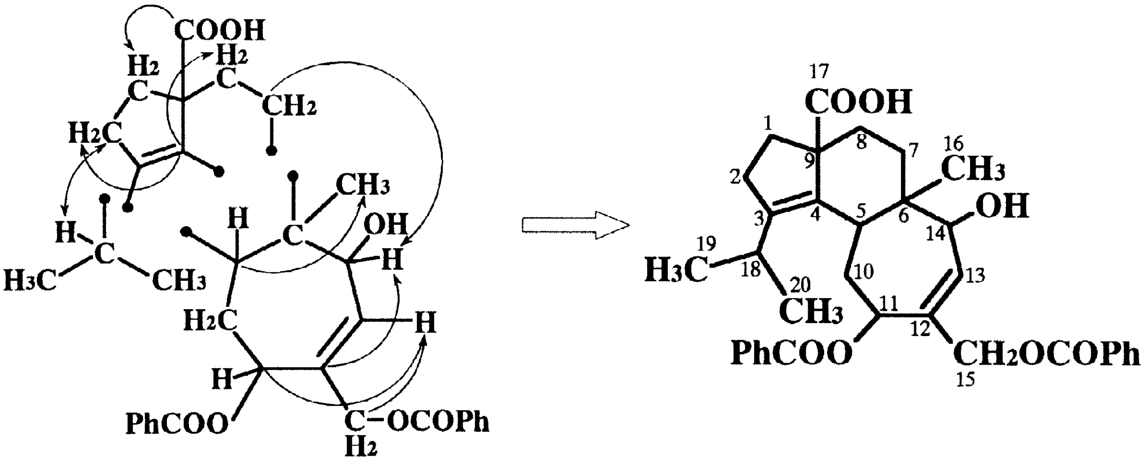


Fig. 1 HMBC correlations and planar structure of scabronine B (**1**).

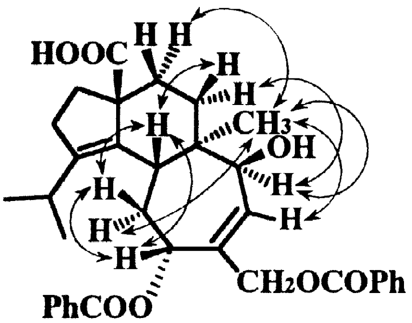


Fig. 2 NOE's of scabronine B (1).

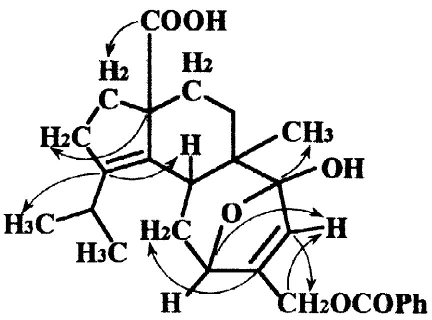


Fig. 3 HMBC correlations of scabronine D (4).

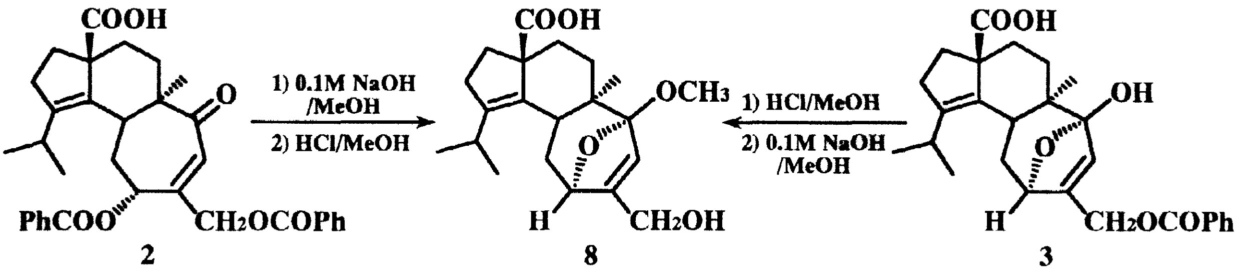
between C-2 (δ 30.5)/H-18 (δ 2.98), C-7 and C-12 (δ 34.7 and 139.0)/H-14 (δ 3.96), C-17 (δ 182.6)/H-1β and H-8α (δ 2.13 and 1.47), C-4 (δ 134.0)/H-2α and H-8β (δ 2.45 and 2.20), and C-15 (δ 65.9)/H-13 (δ 6.08) allowed the gross structure as shown in Fig. 1.

The relative stereochemistry of scabronine B (**1**) was established by NOE experiments. NOE's at H-5β/ H-7β and H-5β/H-11 indicated that these hydrogens were situated in the same side. In addition, NOE's at H-7α/H-14, H-8/H-16, H-10α/H-16 and H-13/H-16 confirmed the structure of scabronine B (**1**) represented as Fig. 2. The carboxyl group was determined to be oriented to the β side by a Dreiding model study which satisfied all the NOE's and coupling constants between the hydrogens.

Structure of scabronine C (2) Molecular formula of scabronine C (**2**), $[\alpha]_D^{25}$ -40.8° (*c* 0.31, MeOH), was analyzed to be $C_{34}H_{36}O_7$ by HREI-MS in conjunction with ^1H and ^{13}C-NMR data. The NMR spectra of scabronine C (**2**) was similar to those of scabronine B (**1**). The only difference is that a carbonyl carbon (δ 208.2) in the ^{13}C-NMR spectrum of scabronine C (**2**) was shown instead of the oxymethine carbon at δ 75.4 in that of scabronine B (**1**). Interpretation of the ^1H- and ^{13}C-NMR spectra of scabronine C (**2**) revealed that the compound **2** corresponded to an oxidized compound of scabronine B (**1**) at the hydroxyl group on C-14.

Structure of scabronine D (3) The molecular formula $C_{27}H_{32}O_6$ (EI-MS: *m/z* 452 $[M]^+$) of scabronine D (**3**), $[\alpha]_D^{25}$ -58.4° (*c* 1.30, MeOH), is different from that of scabronine C (**2**) by C_7H_4O. The ^1H-NMR spectrum showed signals for only one benzoyl moiety (δ 7.42 (2H, t, *J*=7.2 Hz), 7.55 (1H, t *J*=7.2 Hz) and 7.99 (2H, d, *J*=7.2 Hz)), and this suggested the compound **3** is a monobenzoyl derivative of the compound **2**. A comparative study of the ^1H-NMR spectra of the compounds **3** and **2** showed that the C-15 oxymethylene hydrogen signals appeared almost at the same positions (δ 4.92 (2H, s) and 4.97 (2H, d, *J*=1.1 Hz), respectively), while the C-11 oxymethine hydrogen signals were observed at different positions (δ 4.78 (1H, s) and 5.76 (1H, t, *J*=6.6 Hz), respectively). Furthermore, the ^{13}C-NMR spectrum of scabronine D (**3**) indicated the presence of an acetal carbon at δ 110.2. From the information regarding the structure mentioned above, a planar structure which forms an acetal linkage between the C-11 hydroxyl and C-14 carbonyl functions was suggested, and this was substantiated by 2D-NMR spectra (Fig. 3).

Absolute configuration of scabronines B (1), C (2) and D (3) The exciton chirality method could be applied for the determination of the absolute configuration, because the compound **2** possesses benzoate and conjugated enone groups at C-11. The CD spectrum of scabronine C (**2**) exhibited Cotton effects at 236.0 and 205.6 nm (Δε +14.3 and -16.6), indicating *R* configuration at C-11 and, consequently, the absolute configuration of scabronine



Scheme 1

C (2) was determined as 5*R*, 6*R*, 9*S* and 11*R*. The absolute configuration of scabronine B (1) was determined as 5*R*, 6*R*, 9*S*, 11*R* and 14*R* by the transformation of scabronine B (1) to scabronine C (2) with pyridinium dichromate. Conformational information of scabronine D (3) obtained from the ^{1}H-NMR spectrum was scanty, because of its complicatedly overlapped signals in the aliphatic hydrogen region. The absolute stereochemistry of scabronine D (3) was deduced by chemical transformation as shown in Scheme 1. Treatment of scabronine D (3) with acidic methanol followed by debenzoylation with 0.1 M sodium hydroxide afforded a compound **8**. The compound **8** was also derived from scabronine C (2) by debenzoylation followed by acidic methanol treatment. The absolute configuration of scabronine D (3) was thus confirmed as 5*R*, 6*R*, 9*S*, 11*R* and 14*R*.

Structure of scabronine E (4) A conjugated enal system in scabronine E (4), $C_{22}H_{28}O_5$ (EI-MS: *m/z* 372 [M]$^+$), $[\alpha]_D^{25}$ +872.7° (*c* 0.29, MeOH), was suggested by six olefinic ^{13}C-NMR signals at δ 151.8 (s), 151.2 (s), 144.8 (d), 138.4 (s), 135.5 (s) and 120.9 (d), and an aldehyde (δ 9.41) and two olefinic hydrogen signals (δ 5.98 and 6.76) in its ^{1}H NMR spectrum. Splitting patterns of the ^{1}H NMR signals and analysis of the H-H COSY showed that scabronine E (4) also possesses a cyathane skeleton. The planar structure for scabronine E (4) was obtained from the following cross peaks in the HMBC spectrum (Fig. 4): H-1α (δ 1.83)/C-17 (δ 181.8), H-8α (δ 1.64)/C-17 (δ 181.8), H-10 (δ 5.98)/C-4 (δ 135.5), H-18 (δ 2.91)/C-4 (δ 135.5), H-2 (δ 2.46 – 2.57)/C-4 (δ 135.5), H-14 (δ 4.97)/C-5 (δ 151.8), H-14 (δ 4.97)/C-12 (δ 138.4), H-13β (δ 3.16)/C-11 (δ 144.8), and H-13β (δ 3.16)/C-15 (δ 194.1). NOE's between H-14 (δ 4.97)/H-16 (δ 1.04), H-14 (δ 4.97)/H7α (δ 1.41), and H-16 (δ 1.04)/H-8α (δ 1.64) determined the relative stereochemistry of scabronine E (4), as shown in Fig. 5.

Structure of scabronine F (5) Scabronine F (5), $[\alpha]_D^{25}$ +782.6° (*c* 0.30, MeOH), was estimated to have a deacetylated structure of scabronine E (4), since scabronine F (5) showed no acetyl methyl signal in its ^{1}H-

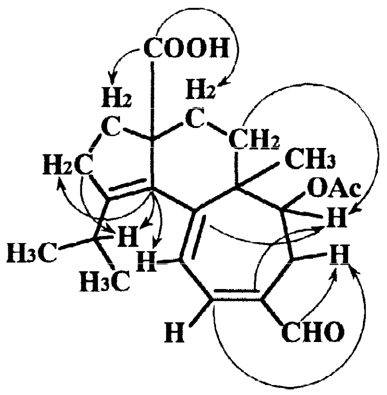


Fig.4 HMBC correlations of scabronine E (4).

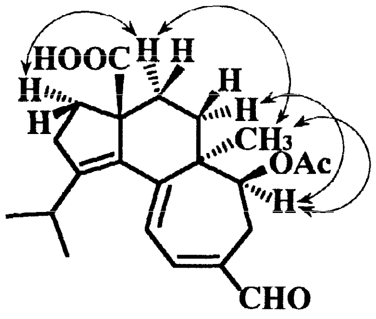


Fig. 5 NOE's of scabronine E (4).

Table 1. CD spectral data of scabronines E (4), F (5) and erinacine A (7).

Scabronine E (4) (MeOH)	Scabronine F (5) (MeOH)	Erinacine A (7) (EtOH)
337 nm (Δε +25.5)	337 nm (Δε +18.2)	344 nm (Δε +47.1)
268 nm (Δε -6.10)	270 nm (Δε -4.82)	273 nm (Δε -12.4)

NMR spectrum, and the ^{1}H-NMR signal for H-14 was observed at δ 3.71. Acetylation of scabronine F (5) with acetic anhydride in pyridine afforded scabronine E (4), substantiating the structure of scabronine F.

Absolute configuration of scabronines E (4) and F (5) The conjugated trienal system is common to scabronines E (4), F (5) and erinacine A (7). The CD spectra of these three compounds have Cotton effects with the same signs almost at the same wavelengths,[2] revealing the absolute configurations of scabronines E and F (4 and 5) to be 6*R*, 9*S* and 14*S* (Table 1).

Stimulating activity of NGF-synthesis of scabronines B, C, E and F (1, 2, 4 and 5) NGF-synthesis stimulating activity using rat astroglial cells was carried out in the manner described by Furukawa.[7] In this assay, epinephrine was used as a positive control. As shown in Fig. 6, the amount of NGF secreted into the medium was increased in the presence of scabronine B (1) (31.2±7.1 and 31.1 pg/ml at 33.3 and 100 μg/ml (60 and 179 μM), respectively) and scabronine E (4) (21.4±5.5 pg/ml at 33.3 μg/ml (90 μM)), whereas NGF was increased by epinephrine (52.3±7.0 pg/ml at 3.7 μg (20.2 μM)). Scabronines C (2) and F (5) (100 μg/ml) also slightly increased the amount of NGF (12.3±6.5 pg/ml and 4.75±0.48 pg/ml, respectively).

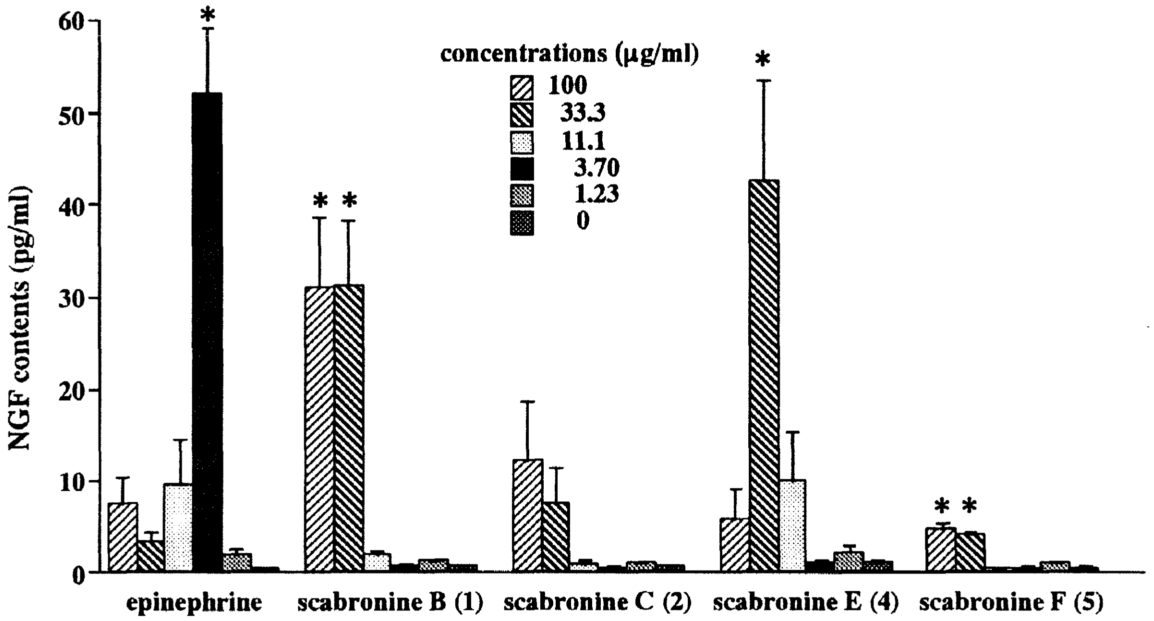


Fig. 6 Stimulating activity of the synthesis of NGF of scabronines.
* denotes $p<0.03$.

Table 2. ^{1}H-NMR spectral data of scabronines B - E (**1** - **4**) in $CDCl_3$.

Position No.	Chemical shifts (multiplicities and coupling constans in Hz)			
	Scabronine B (**1**)	Scabronine C (**2**)	Scabronine D (**3**)	Scabronine E (**4**)
1α	1.65 (ddd, 13.2, 8.8, 5.5)	1.67 (ddd, 13.6, 9.5, 6.6)	1.52-1.68 (m)	1.83 (dt, 13.7, 8.8)
1β	2.13 (ddd, 12.8, 8.8, 4.7)	2.18 (ddd, 13.6, 9.5, 4.4)	2.15 (m)	2.29 (ddd, 13.7, 8.4, 4.2)
2α	2.40-2.50 (m)	2.45 (ddd, 15.8, 9.5, 4.4)	2.27-2.38 (m)	2.46-2.57 (m)
2β	2.40-2.50 (m)	2.52 (br.ddd, 15.8, 9.5, 6.6)	2.40-2.50 (m)	2.46-2.57 (m)
5	3.18 (br.d, 11.7)	2.91 (dd, 11.4, 2.6)	2.40-2.50 (m)	—
7α	1.20 (m)	1.47 (ddd, 13.6, 4.0, 2.6)	1.35 (m)	1.41 (dt, 13.7, 3.9)
7β	2.18-2.35 (m)	1.74 (dt, 4.0, 13.6)	1.52-1.68 (m)	2.00 (dt, 3.9, 13.7)
8α	1.47 (br.t, 13.3)	1.53 (ddd, 13.6, 13.2, 4.0)	1.44 (m)	1.64 (ddd, 13.7, 13.5, 3.9)
8β	2.18-2.35 (m)	2.25 (ddd, 13.2, 4.0, 2.6)	2.27-2.38 (m)	2.30 (dt, 13.5, 3.9)
10α	2.18-2.35 (m)	2.32 (ddd, 14.3, 11.4, 6.6)	1.52-1.68 (m)	5.98 (d, 8.0)
10β	2.40-2.50 (m)	2.74 (ddd, 14.3, 6.6, 2.6)	2.27-2.38 (m)	
11	6.42 (br.d, 9.9)	5.76 (t, 6.6)	4.78 (s)	6.76 (dd, 8.0, 2.2)
13α	6.08 (d, 6.6)	6.27 (t, 1.1)	6.10 (s)	2.46-2.57 (m)
13β				3.16 (dd, 18.7, 6.0)
14	3.96 (d, 6.6)	—	—	4.97 (br.d, 6.0)
15α	4.86 (d, 12.8)	4.97 (2H, d, 1.1)	4.92 (2H, s)	9.41 (s)
15β	4.87 (d, 12.8)			
16	0.87 (3H, s)	1.24 (3H, S)	1.07 (3H, s)	1.04 (3H, s)
18	2.98 (septet, 6.3)	2.94 (septet, 7.0)	2.96 (septet, 6.6)	2.91 (septet, 6.9)
19	1.00 (3H, d, 6.3)	1.01 (3H, d, 7.0)	1.02 (3H, d, 6.6)	1.09 (3H, d, 6.9)
20	0.97 (3H, d, 6.3)	1.00 (3H, d, 7.0)	0.86 (3H, d, 6.6)	1.00 (3H, d, 6.9)
14-OAc	—	—	—	1.89 (3H, s)
(2')	7.97 (2H, br.d, 7.4)	8.01 (2H, br.d, 7.3)	7.99 (2H, J =7.2)	—
	7.93 (2H, br.d, 7.4)	7.96 (2H, br.d, 7.3)	—	—
(3')	7.36 (2H, br.t, 7.4)	7.42 (2H, br.t, 7.3)	7.42 (2H, t, 7.2)	—
	7.35 (2H, br.t, 7.4)	7.43 (2H, br.t, 7.3)	—	—
(4')	7.51 (br.t, 7.4)	7.55 (br.t, 7.3)	7.55 (t, 7.2)	—
	7.50 (br.t, 7.4)	7.56 (br.t, 7.3)	—	—

Table 3. ^{13}C-NMR spectral data of scabronines B - E (**1** - **4**) in CDCl$_3$.

	Chemical shifts			
Position No.	Scabronine B (**1**)	Scabronine C (**2**)	Scabronine D (**3**)	Scabronine E (**4**)
1	34.2 (t)	34.7 (t)	36.5 (t)	35.1 (t)
2	30.5 (t)	30.6 (t)	30.6 (t)	29.9 (t)
3	144.2 (s)	145.8 (s)	145.5 (s)	151.2 (s)
4	134.0 (s)	130.4 (s)	129.5 (s)	135.5 (s)
5	42.5 (d)	41.1 (d)	41.8 (d)	151.8 (s)
6	41.3 (s)	53.4 (s)	41.6 (s)	46.0 (s)
7	34.7 (t)	36.0 (t)	31.6 (t)	34.3 (t)
8	32.5 (t)	32.2 (t)	32.8 (t)	32.4 (t)
9	61.4 (s)	61.1 (s)	60.0 (s)	59.9 (s)
10	34.0 (t)	31.9 (t)	26.7 (t)	120.9 (d)
11	73.4 (d)	72.3 (d)	79.8 (d)	144.8 (d)
12	139.0 (s)	143.1 (s)	142.7 (s)	138.4 (s)
13	131.2 (d)	127.8 (d)	130.5 (d)	26.3 (t)
14	75.4 (d)	208.2 (s)	110.2 (s)	74.7 (d)
15	65.9 (t)	65.2 (t)	60.3 (t)	194.1 (d)
16	16.7 (q)	14.8 (q)	11.9 (q)	20.9 (q)
17	182.6 (s)	180.8 (s)	181.8 (s)	181.8 (s)
18	27.0 (d)	27.2 (d)	26.5 (d)	27.1 (d)
19	21.8 (q)	21.6 (q)	22.1 (q)	21.4 (q)
20	21.7 (q)	21.6 (q)	21.1 (q)	21.4 (q)
11-OCO	165.9 (s)	165.5 (s)	166.2 (s)	—
15-OCO	166.1 (s)	165.7 (s)	—	—
14-OAc	—	—	—	170.6 (s)
	—	—	—	25.9 (q)
Ph (1')	130.0 (s)	129.5 (s)	129.7 (s)	—
	130.0 (s)	129.4 (s)		
(2')	129.7 (d)	129.8 (d)	129.7 (d)	—
	129.5 (d)	129.7 (d)		
(3')	128.3 (d)	128.5 (d)	128.5 (d)	—
	128.2 (d)	128.5 (d)		
(4')	133.0 (d)	133.4 (d)	133.3 (d)	—
	132.8 (d)	133.3 (d)		

CDCl$_3$ as internal standard (δ 77.1). Multiplicity was based on the DEPT spectra, q: primary carbon, t: secondary carbon, d: tertiary carbon, s: quaternary carbon.

EXPERIMENTAL SECTION

UV and IR spectra were recorded on Hitachi U-3200 and JASCO FT/IR-5300 spectrometers, respectively. Optical rotations and CD spectra were measured with a HORIBA SEPA300 polarimeter and a JASCO J-720 circular dichroism spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL LAMBDA-600 (^{1}H: 600 MHz and ^{13}C: 150 MHz) and Varian Gemini 2000 (^{1}H: 300 MHz and ^{13}C: 75 MHz) spectrometers (TMS as internal standard). LR and HR EI-MS were obtained with JEOL JMS DX-303 and AX-500 mass spectrometers.

Isolation of scabronines Dried fruit body (1.4 kg) of *Sarcodon scabrosus*, collected in Sendai, Japan, were extracted twice with methanol at room temperature to give the extract (78 g). The methanol extract (78 g) was partitioned with *n*-hexane-EtOAc (1:1) and water to yield *n*-hexane-EtOAc (1:1) (18 g) and water solubles. *n*-Hexane-EtOAc (1:1) solubles were chromatographed over silica gel, and the column was eluted with *n*-hexane-EtOAc mixtures by increasing polarity. A repeated fractionation of the *n*-hexane-EtOAc (1:1)-eluting fraction (2.3 g) using silica gel (Kieselgel 60, Merck) and ODS (Cosmosil 75C18-OPN, Nacalai Tesque) columns afforded scabronine B (**1**) (100 mg), scabronine C (**2**) (18 mg), scabronine D (**3**) (27 mg), and scabronine E (**4**) (58 mg). Scabronine F (**5**) (190 mg) was obtained from EtOAc-eluting fraction (2.6 g) by fractionation with silica gel and ODS columns.

Scabronine B (1) Yellowish oil, $[\alpha]_D^{25}$ +14.6° (*c* 0.39, MeOH); UV (MeOH) nm (ε) 273.2 (1800), 229.0 (26000); IR ($CHCl_3$) ν 3495 (br), 2963, 2936, 1715, 1281, 1270 cm^{-1}. ^1H and ^{13}C-NMR (Table 2 and 3, respectively); HR EI-MS *m/z* 512.2571 $[M-COOH-H]^+$ (*m/z* 512.2562 calcd for $C_{33}H_{36}O_5$).

Scabronine C (2) Yellowish oil, $[\alpha]_D^{25}$ -40.8° (*c* 0.31, MeOH); UV (MeOH) nm (ε) 273.4 (2100), 230.4 (32000); IR ($CHCl_3$) ν 3513 (br), 2965, 2940, 1721, 1697, 1660, 1269, 1249 cm^{-1}. ^1H and ^{13}C-NMR (Table 2 and 3, respectively); HREI-MS *m/z* 556.2487 $[M]^+$ (*m/z* 556.2461 calcd for $C_{34}H_{36}O_7$).

Scabronine D (3) Yellowish oil, $[\alpha]_D^{25}$ -58.4° (*c* 1.3, MeOH); UV (MeOH) nm (ε) 273.2 (1000), 229.0 (16000); IR ($CHCl_3$) ν 3513 (br), 3375 (br), 3028, 2961, 1719, 1693, 1603, 1271, 1249 cm^{-1}. ^1H and ^{13}C-NMR (Table 2 and 3, respectively); HREI-MS *m/z* 452.2188 $[M]^+$ (*m/z* 452.2199 calcd for $C_{27}H_{32}O_6$).

Alkaline hydrolysis of scabronine C (2) Scabronine C (**2**, 24.6 mg) was hydrolyzed in 0.1 M NaOH/MeOH-H_2O (1:1) for 30 min at room temperature. The reaction mixture was neutralized with hydrochloric acid and extracted with ethyl acetate. The solvent was removed *in vacuo*, and chromatography of the residue over silica gel afforded debisbenzoate scabronine C (**9**, 12.9 mg) as a mixture of keto- and hemiacetal-form (1:1): ^1H-NMR ($CDCl_3$, 300 MHz) δ 6.10 (0.5H, br.s, H-13), 5.97 (0.5H, br.s, H-13), 4.82-4.95 (0.5H, m, H-11), 4.73 (0.5H, br.s, H-11), 4.37 (0.5H, br.d, *J* =16.4 Hz, H-15), 4.30 (0.5H, br.d, *J* =16.4 Hz, H-15), 3.88 (0.5H, d, *J* =12.6 Hz, H-15), 3.68 (0.5H, d, *J* =12.6 Hz, H-15), 3.09 (0.5H, septet, *J* =7.0 Hz, H-18), 3.05 (0.5H, septet, *J* =7.0 Hz, H-18), 2.62-2.74 (1H, m), 2.53 (1H, t, *J* =7.0 Hz, H-2), 2.43 (1H, t, *J* =7.4 Hz, H-2), 1.31-2.37 (9H, m), 0.98-1.20 (9H, m, H-16, H-19, H-20); HREI-MS *m/z* 348.1958 $[M]^+$ (*m/z* 362.2093 calcd for $C_{20}H_{28}O_5$).

Methyl acetalization of 9 **9** (12.9 mg) in 0.7 ml of 10 % methanolic hydrogen chloride was stirred in the presence of molecular sieves 3A for 9 h at room temperature. The reaction mixture was filtered and evaporated, and then column chromatography of the residue over silica gel gave a methyl acetal compound (**8**, 4.1 mg): $[\alpha]_D^{25}$ -74.9° (*c* 0.39, MeOH); ^1H-NMR ($CDCl_3$, 300 MHz) δ 6.04 (1H, t, *J* =1.9 Hz, H-13), 4.71 (1H, br.s, H-11), 4.36 (1H, dd, *J* =15.4, 1.6 Hz, H-15), 4.21 (1H, dd, *J* =15.4, 1.6 Hz, H-15), 3.31 (3H, s, OMe), 3.02 (1H, septet, *J* =6.9 Hz, H-18), 2.24-2.53 (5H, m), 2.14-2.22 (1H, m), 1.54-1.66 (3H, m), 1.47 (1H, dt, *J* =4.1, 13.5 Hz), 1.23-1.34 (1H, m), 1.05 (3H, d, *J* =6.9 Hz, H-19), 1.03 (3H, s, H-16), 0.97 (3H, d, *J* =6.9 Hz, H-20); ^{13}C-NMR ($CDCl_3$, 75 MHz) d 182.3 (s, C-17), 148.5 (s), 145.4 (s), 131.2 (s, C-4), 124.2 (d, C-13), 114.5 (s, C-14), 78.6 (d, C-11), 60.5 (s, C-9), 59.0 (t, C-15), 51.2 (q, OMe), 42.0 (s, C-6), 41.8 (d, C-5), 36.2 (t), 33.1 (t), 31.5 (t), 30.7 (t), 26.7 (t), 26.5 (d, C-18), 22.1 (q, C-19), 21.2 (q, C-20), 12.3 (q, C-16); HREI-MS *m/z* 362.2058 $[M]^+$ (*m/z* 362.2093 calcd for $C_{21}H_{30}O_5$).

Methyl debenzoyl scabronine D (8) Scabronine D (**3**, 12.2 mg) in 0.7 ml of 10 % methanolic hydrogen chloride was stirred in the presence of molecular sieves 3A for 9 h at room temperature. The reaction mixture was filtered. Evaporation of solvent followed by column chromatography of the residue over silica gel gave methyl scabronine D (**10**, 9.5 mg): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02 (2H, dd, J =7.9, 1.6 Hz), 7.57 (1H, tt, J =7.9, 1.6 Hz), 7.43 (2H, t, J =7.9 Hz), 6.10 (1H, t, J =1.0 Hz, H-13), 4.94 (2H, d, J =1.0 Hz, H-15), 4.79 (1H, br.s, H-11), 3.31 (3H, s, OMe), 2.97 (1H, septet, J =6.6 Hz, H-18), 2.20-2.46 (5H, m), 2.11 (1H, ddd, J =13.0, 8.0, 2.2 Hz), 1.64 (1H, ddd, J =12.4, 3.2, 1.6 Hz), 1.49-1.59 (2H, m), 1.41 (1H, dt, J =4.7, 13.7 Hz), 1.01 (3H, s, H-16), 1.01 (3H, d, J =6.6 Hz, H-19), 0.85 (3H, d, J =6.6 Hz, H-20); HR EI-MS *m/z* 466.2352 $[M]^+$ (*m/z* 466.2355 calcd for $C_{28}H_{34}O_6$).

Methyl scabronine D (**10**, 8.4 mg) was hydrolyzed in 0.1 M NaOH/MeOH-H_2O (1:1) for 30 min at room temperature. The reaction mixture was neutralized with hydrochloric acid and extracted with ethyl acetate. The solvent was removed *in vacuo*, and chromatography of the residue over silica gel afforded methyl debenzoyl scabronine D (**8**, 4.8 mg): $[\alpha]_D^{25}$ -72.6° (c 0.53, MeOH); ^{1}H, ^{13}C NMR spectra and EI-MS were identical with those of **8** derived from scabronine B (**1**).

Scabronine E (4) Yellowish oil, $[\alpha]_D^{25}$ +872.7° (*c* 0.29, MeOH); UV (MeOH) nm (ε) 331.8 (13000); IR ($CHCl_3$) ν 3318 (br), 2964, 2740, 1728, 1698, 1671, 1246 cm^{-1}. ^{1}H- and ^{13}C-NMR (Table 2 and 3, respectively); HREI-MS *m/z* 372.1939 $[M]^+$ (*m/z* 372.1937 calcd for $C_{22}H_{28}O_5$).

Scabronine F (5) Yellowish oil, $[\alpha]_D^{25}$ +782.6° (*c* 0.30, MeOH); UV (MeOH) nm (ε) 336.0 (14000); IR ($CHCl_3$) ν 3399 (br), 2934, 2740, 1698, 1672, 1169 cm^{-1}. ^{1}H-NMR (CD_3OD, 300 MHz) δ 9.41 (1H, s, H-15), 6.90 (1H, dd, J =8.2, 2.1 Hz, H-11), 6.07 (1H, d, J =8.2 Hz, H-10), 3.71 (1H, d, J =6.0 Hz, H-14), 3.01 (1H, dd, J =18.1, 6.0 Hz, H-13b), 2.98 (1H, septet, J =6.9 Hz, H-18), 2.51-2.61 (3H, m), 2.21-2.47 (3H, m), 1.84 (1H, dt, J =13.2, 8.8 Hz), 1.70 (1H, dt, J =13.2, 4.1 Hz), 1.45 (1H, dt, J =13.4, 4.1 Hz), 1.13 (3H, d, J =6.9 Hz, H-19), 1.05 (3H, d, J =6.9 Hz, H-20), 1.04 (3H, s, H-16); HREI-MS *m/z* 330.1831 $[M]^+$ (*m/z* 330.1831 calcd for $C_{20}H_{26}O_4$).

Acetylation of scabronine F (5) To a pyridine solution (0.4 ml) of scabronine F (**5**, 8.2 mg) was added 0.2 ml of acetic anhydride at room temperature. The reaction mixture was stirred at 20 h. The residue after evaporation of the mixture was submitted to silica gel column chromatography to afford acetylated scabronine F (6.1 mg): $[\alpha]_D$ +887.4° (*c* 0.21, MeOH); other spectral data were identical with those of scabronine E (**4**).

NGF synthesis stimulating activity Quiescent fetal rat astroglial cells, which were prepared in the modified manner described by Furukawa *et al.*,[7] were used for estimation of NGF synthesis stimulating activity. Test samples in Dulbecco's MEM medium containing 0.5 % bovine serum albumin were added to the culture. Media conditioned for 24 h by the cells were collected and provided for NGF measurement. NGF content was determined by a sensitive enzyme immunoassay as reported.[8] The increase of NGF content in the media was considered as the index of stimulating activity of NGF synthesis.

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