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Scabronine A, a Novel Diterpenoid Having Potent Inductive Activity of the Nerve Growth Factor Synthesis, Isolated From the Mushroom, *Sarcodon scabrosus*

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Abstract: A novel diterpenoid, scabronine A, having strong inductive activity of the nerve growth factor synthesis was isolated from the fruit body of the mushroom, *Sarcodon scabrosus* (Fr.) Karst. The stereostructure of scabronine A (**1**) was elucidated on the basis of the spectroscopic analysis of natural scabronine A and its derivatives.

Keywords: carboxylic acids and derivatives; fungi; stereochemistry; terpenes and terpenoids

Thelophoraceous brown fungus *Sarcodon scabrosus* (Fr.) Karst. which grows in coniferous and deciduous woods of Europe and Japan has been recognized as an inedible mushroom because of its strongly bitter taste which may derive from bitter terpenoids[1]. During our studies on biologically active constituents of Japanese mushrooms (Basidiomycetes)[2], we found a new diterpenoid, scabronine A (**1**) which showed strong inductive activity of the nerve growth factor (NGF) synthesis in 1321N1 human astrocytoma cells, from *S. scabrosus*. We wish to report here the isolation and structural elucidation of **1**.

The fruit body (1.4 kg) of *S. scabrosus* was extracted with MeOH and then the extract was partitioned between *n*-hexane-EtOAc and water. The *n*-hexane-EtOAc extract was subjected repeatedly to silica gel chromatography using *n*-hexane-EtOAc or chloroform-MeOH mixed solvent system of increasing polarity. The fraction obtained with chloroform-MeOH (30:1) elution was purified twice by reversed-phase HPLC on ODS. Elution with MeOH-H_2O (7:3) gave scabronine A (**1**, 213 mg, 1.5 x 10^{-2}%) as a colorless amorphous solid, mp 93.5-94.5 °C, $[\alpha]_D^{23}$ −15.0° (*c* 0.92, MeOH). The molecular formula, $C_{22}H_{34}O_6$ was determined by HREI-MS (*m/z* 394.2357, M^+, Δ mmu +0.2). Scabronine A (**1**) showed characteristic IR absorptions at 3410, 1700, 1100, 1080 cm^{-1} implying hydroxyl and carboxyl functions. The yellow coloration with BCG reagent on TLC and the methyl ester (**2**) given with diazomethane indicated the carboxylic acid substituent in **1**.

The ^1H and ^{13}C NMR spectra showed several sets of signals with the ratio of 2:1 due to equilibrium at the hemiacetal position (δ_H/δ_C 5.31/104.1 for major and 5.91/99.4 for minor isomers). The ratio was changed to 94:6[3] by the methylation of **1** with diazomethane. Both

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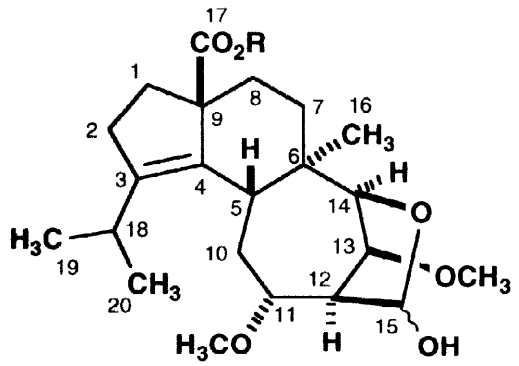
Table 1. ^{1}H and ^{13}C NMR spectral data for scabronine A (**1**) and the methyl ester (**2a**) in $CDCl_3$.[a)]

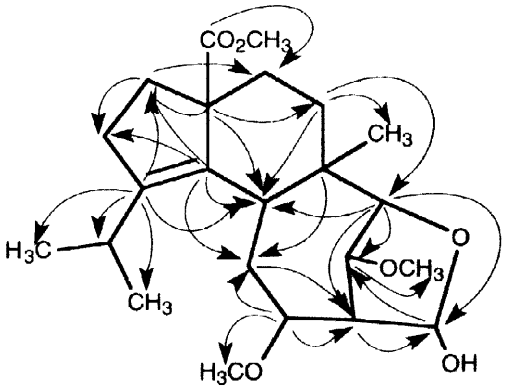
	Scabronine A (**1**) [b)]		Methyl ester (**2a**)	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	33.6 (t)	1.70-1.78 (1H, m)	33.8 (t)	1.68 (1H, ddd, *J*=6.8, 8.1, 13.2)
		1.90-2.03 (1H, m)		1.91 (1H, ddd, *J*=7.8, 9.3, 13.2)
2	29.7 (t)	2.43 (2H, m)	29.6 (t)	2.39 (2H, m)
3	143.7 (s)		143.1 (s)	
4	134.2 (s)		133.9 (s)	
5	37.7 (d)	2.21 (1H, *J*=12.4)	37.8 (d)	2.14 (1H, d, *J*=12.3)
6	41.9 (s)		41.6 (s)	
7	34.5 (t)	1.14 (1H, ddd, *J*=2.0, 2.0, 13.6)	34.4 (t)	1.10 (1H, ddd, *J*=2.6, 4.1, 14.0)
		1.85-2.03 (1H, m)		1.96 (1H, ddd, *J*=4.2, 13.6, 14.0)
8	31.4 (t)	1.48 (1H, ddd, *J*=4.8, 14.0, 14.0)	31.8 (t)	1.46 (1H, ddd, *J*=4.1, 13.6, 13.6)
		2.15 (1H, ddd, *J*=2.0, 2.0, 14.0)		2.17 (1H, ddd, *J*=2.6, 4.2, 13.6)
9	61.9 (s)		61.7 (s)	
10	32.8 (t)	1.71 (1H, ddd, *J*=10.5, 12.4, 14.0)	32.7 (t)	1.67 (1H, ddd, *J*=10.7, 12.3, 13.7)
		2.31 (1H, dd, *J*=6.0, 14.0)		2.25 (1H, dd, *J*=5.4, 13.7)
11	80.1 (d)	3.29 (1H, dd, *J*=6.0, 10.5)	80.5 (d)	3.17 (1H, dd, *J*=5.4, 10.7)
12	53.4 (d)	2.60 (1H, s)	54.1 (d)	2.54 (1H, s)
13	80.7 (d)	3.89 (1H, s)	81.0 (d)	3.93 (1H, s)
14	93.4 (d)	4.02 (1H, s)	92.1 (d)	3.96 (1H, s)
15	104.1 (d)	5.29 (1H, s)	104.1 (d)	5.10 (1H, s)
16	15.1 (q)	0.85 (3H, s)	15.2 (q)	0.83 (3H, s)
17	182.7 (s)		177.4 (s)	
18	27.0 (d)	2.94 (1H, sept, *J*=6.8)	27.0 (d)	2.93 (1H, sept, *J*=6.8)
19	21.8 (q)	1.05 (3H, d, *J*=6.8)	21.8 (d)	1.03 (3H, d, *J*=6.8)
20	21.4 (q)	0.97 (3H, d, *J*=6.8)	21.4 (d)	0.96 (3H, d, *J*=6.8)
11-OMe	56.6 (q)	3.40 (3H, s)	56.5 (q)	3.39 (3H, s)
13-OMe	56.8 (q)	3.36 (3H, s)	56.7 (q)	3.36 (3H, s)
17-OMe			51.8 (q)	3.69 (3H, s)

a) ^{1}H and ^{13}C NMR were recorded at 500 MHz and 125 MHz, respectively. Multiplicities in parentheses for ^{13}C signals were determined by DEPT experiments. *J*s were expressed in Hz.

b) Major isomer.



1: R=H
2a: R=Me, 15*S*-isomer
2b: R=Me, 15*R*-isomer

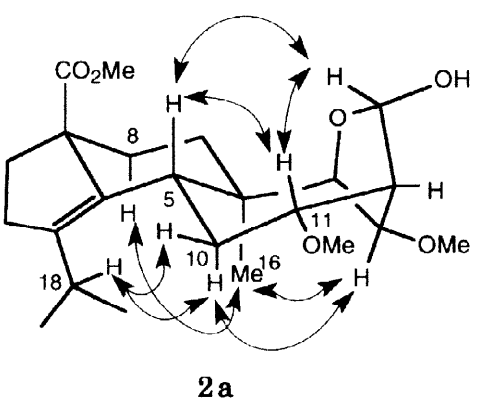


A

↶ : HMBC; COLOC correlation

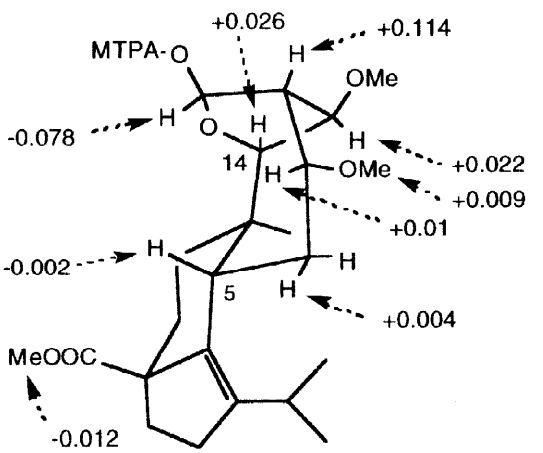
Figure 1

epimeric isomers could be isolated by silica gel chromatography with *n*-hexane-Et_2O (1:2) elution to give the methyl esters (**2a** and **2b**) in the ratio of 93.3:6.7. The major methyl ester (**2a**), $[\alpha]_D^{24}$ –29.6° (*c* 1.1, MeOH), *m/z* 482.507 (M^+, $C_{23}H_{36}O_6$), showed NMR signals and IR absorptions due to an acetal (δ_H/δ_C 5.10/104.1, ν 3511 cm^{-1}), three methoxyls (δ_H/δ_C 3.36/56.7, 3.39/56.5 and 3.69/51.8) one of which belonged to a methoxycarbonyl (δ_C 51.8, 177.4, ν 1721 and 1198 cm^{-1}), three carbinyl methines (δ_H/δ_C 3.17/80.5, 3.93/81.0 and 3.96/92.1), three methines (δ_H/δ_C 2.14/37.8, 2.54/54.1 and 2.93/27.0), five methylenes (δ_H/δ_C 1.68;1.91/33.8, 2.39;2.40/29.6, 1.10;1.96/34.4, 1.46;2.17/31.8, 1.67;2.25/32.7), three methyls (δ_H/δ_C 0.83/15.2, 0.96/21.4 and 1.03/21.8), two quaternary carbons (δ_C 41.6 and 61.7) and a fully substituted olefin (δ_C 133.9; 143.1). Six degrees of unsaturation derived from the molecular formula suggested that **2a** was a tetracyclic compound having a carbonyl and a double bond. 2D NMR analysis such as H-H and C-H COSY, COLOC and HMBC together with 1D H-H homo-decoupling experiment indicated a hemiacetal ring adjacent to a cycloheptane ring as illustrated in the gross structure **A** (Figure 1).



2a

↷ : NOE correlation



Δδ values for MTPA–**2a**

Figure 2

The relative stereochemistry was elucidated by the 1D differential NOE experiment as shown in Figure 2. Among those observed, the NOE between H-18 and H_2-10, H-8 and H_3-16, H-5 and H-11 suggested a chair conformation of the cyclohexane ring. This indicated that the methoxycarbonyl substituent at C-9 has an axial configuration, *S**.

The absolute stereochemistry was analyzed by the extended Mosher (^{1}H) method[4]. *S*- and *R*-MTPA esters of 17-*O*-methylscabronine A (**2a**) gave a reasonable distribution pattern of $\Delta\delta$ values as shown in Figure 2. Although the sign of $\Delta\delta$ value for H-14 did not follow the extended Mosher method, it went into the proper group when the O-C=O plane of the MTPA ester was twisted counterclockwise as illustrated in Figure 2. Consequently, the stereostructure **1** was established for scabronine A. Scabronine A (**1**) could be a highly oxidized analogue of allocyathin B_2 isolated from *S. scabrosus*[1].

Scabronine A (**1**) showed potent inductive activity of NGF synthesis in 1321N1 human astrocytoma cells[5]. Namely, **1** released 746 pg/mL of NGF at 100 μM from 1321N1 cells under basal release of 147 pg/mL of NGF during two days. Furthermore, reverse transcription polymerase chain reaction (RT-PCR) revealed that **1** increased NGF mRNA by 1.99-fold in 1321 N1 cells. Some NGF inducers such as catecholamines[6], benzoquinones[7], hericenones[8], fellutamides[9], kansuinin A[10], pyrroloquinoline quinones[11] and erinacines[12] have been hitherto reported. A strong NGF inducer with low molecular size such as scabronine A (**1**) will be a useful drug for serious neuronal diseases such as Alzheimer's disease. Scabronine A (**1**) will also be helpful to clarify the mechanism of NGF synthesis and secretion.

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