ケロウジ(Sarcodon scabrosus)培養菌糸体に含まれる新規カサン骨格ジテルペノイド

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The Cultured Mycelium of Fungus *Sarcodon* Produced A New Cassane Diterpenoid*

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Summary. We had isolated the cyathane diterpenoid named sarcodonins from the fruiting body of fungus *Sarcodon scabrosus*. These compounds showed diversity of biological activities such as bitter taste, antibacterial activity and anti-inflammatory activity. With the objective of the mass production of these sarcodonins, we cultured the mycelium of this fungus. From the mycelium of this fungus, we isolated ergosterol (1) and ergosterol peroxide (3) along with a new cassane diterpenoid named sarcolide A (cassa-14-(17),15-diene-1,5--dihydroxy-3,4-carbolactone), while we did not found any cyathane diterpenoids. The mycelium of *S. scabrosus* did not produce any sarcodonins in the culture condition.

Key words: *Sarcodon scabrosus* ; mycelium ; fungus metabolite ; cassane diterpenoid

Introduction

In the investigation of the constituents of the fruiting body of fungi, we had found many biologically active substances[1-5]. We had isolated the cyathane diterpenoids named sarcodonin A ~M from the fruiting body of fungus Sarcodon scabrosus. These compounds showed diversity of biological activities such as bitter taste[1], antibacterial activity[4] and anti-inflammatory activity[5].

Sarcodon scabrosus is not edible mushroom, because it contains many bitter principles[1]. It occurs in coniferous forests in mountain regions in Japan. The mycelium of this fungus makes the mycorrhiza with pine trees. Many reports on the constituents of the mycelium of the wood rotting fungi had reported, while there are few reports on the mycorhizal fungi. It is interesting to get these bioactive fungal metabolites from the cultured mycelium of the fungus, *S. scabrosus*. In this paper, we wish to report the fungal metabolites from the cultured mycelium of this fungus.

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*Studies on Components of Mushroom, Part X, For Part IX see ref. 4.

The mycelium of *S. scabrosus* was induced from the fruiting body by setting a piece of the tissue of this fungus on a potato-sucrose medium. This mycelium was cultured on the peptone agar medium. The mycelial mat was separated from the culture broth, air dried (490g), and extracted with EtOAc afforded a crude extract, from which the neutral fraction (5.7g) was given. The neutral fraction was chromatographed repeatedly and afforded the compound 1 (245mg), 2 (100mg) and 3 (102mg).

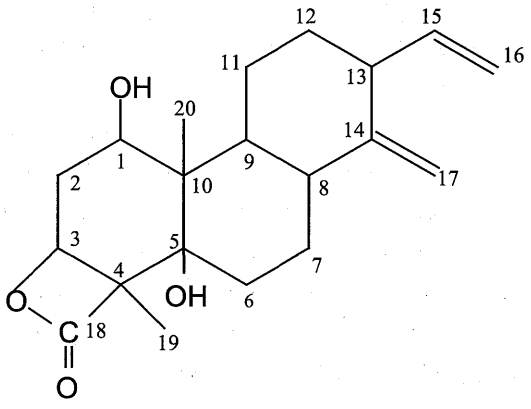
Compounds 1 (colorless needles, mp. 150-153°C) and 3 (colorless plates, mp. 166-168°C) were identified to be ergosterol and ergosterol peroxide, respectively, by directly comparing their spectral data with those of the authentic samples.

The molecular formula of 2, $C_{20}H_{26}O_4$ was assigned by HR-MS m/z 332.1981 (M^+, calcd. as 332.1985). Its IR spectrum showed the presence of the β-lactone (1820 cm^{-1}), and the hydroxyl groups (3300, 3175cm^{-1}). The ^{13}C-NMR spectrum of 2 (Table 1) showed 20 carbon signals which were assignable to two methyl, seven methylene, six methine and five quaternary carbons respectively. In the ^{13}C-NMR spectrum, five sp^2 signals (a lactone cabonyl : 174.6 ppm, a vinyl group : 139.1 and 117.0 ppm, and an exomethylene group : 150.6 and 106.9 ppm) were identified. The HR-MS and

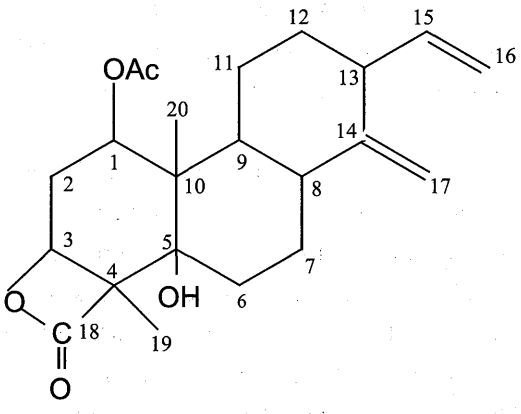
Table 1 NMR Data for Compound **2** and **2a** (250MHz in $CDCl_3$)

position	**2**			**2a**		
	^{13}C		^{1}H	^{13}C		^{1}H
1	71.5	CH	4.10 br.d (5.0)*	73.1	CH	5.19 br. d (5.9)
2	34.5	CH_2	2.54 m	31.5	CH_2	2.67 ddd (18.0, 6.5, 2.2)
						2.40 m
3	76.0	CH	4.50 dd (8.3, 3.0)	75.0	CH	4.43 dd (8.8, 2.0)
4	60.3	C		60.5	C	
5	75.0	C		73.8	C	
6	35.7	CH_2	2.33 m	35.7	CH_2	2.40 m
			1.76 m			1.95 m
7	26.2	CH_2	1.71 m	26.2	CH_2	1.75 m
8	40.1	CH	2.17 m	40.3	CH	2.00 m
9	39.8	CH	1.34 m	40.2	CH	1.34 m
10	43.1	C		43.3	C	
11	27.6	CH_2	1.76 m	27.2	C	
12	26.5	CH_2	1.65 m	26.5	CH_2	1.49 m
			1.37 m			1.23 m
13	54.7	CH	2.47 m	54.6	CH	2.45 m
14	150.6	C		150.2	C	
15	139.1	CH	5.64 ddd (17.1, 9.1, 6.0)	139.0	C	5.63 ddd (17.1, 10.0, 5.9)
16	117.0	CH_2	5.15 dd (9.9, 2.0)	116.9	CH_2	5.15 dd (10.0, 2.0)
			5.00 dd (17.1, 2.0)			
17	106.9	CH_2	4.70 d (1.7)	107.2	CH_2	4.68 d (1.7)
			4.60 d (1.3)			4.59 d (1.3)
18	174.6	C		174.2	C	
19	19.0	CH_3	1.57 s	19.0	CH_3	1.59 s
20	15.1	CH_3	1.15 s	15.2	CH_3	1.06 s
hydroxyl			2.90 s			3.40 s
			3.90 d (2.0)			
acetoxyl				21.2	CH_3	2.14 s
				168.5	C	

* Values in parentheses are coupling constants in Hz



SarcolideA (2)



2a

Fig 1 Structures of Compound **2** and Its Acetate **2a**

the ^{13}C-NMR spectral data indicated that **2** could have a tetracyclic ring system including one lactone moiety.

The presence of one secondary hydroxyl group in the compound **2** was revealed by formation of monoacetate derivative. In the ^{1}H-and ^{13}C-NMR spectra, the signals assignable to acetoxy group were shown at δ5.19 (1H, dd) and δ2,14 (3H, s) and at δ174.2 and 21.2 in the ^{1}H and ^{13}C-NMR spectra, respectively.

From the foregoing spectral data and chemical reactivity enable the structure of sarcolide A to be the tricyclic sesquiterpene, having a β-lactone, a secondary hydroxyl, a tertiary hydroxyl, a vinyl

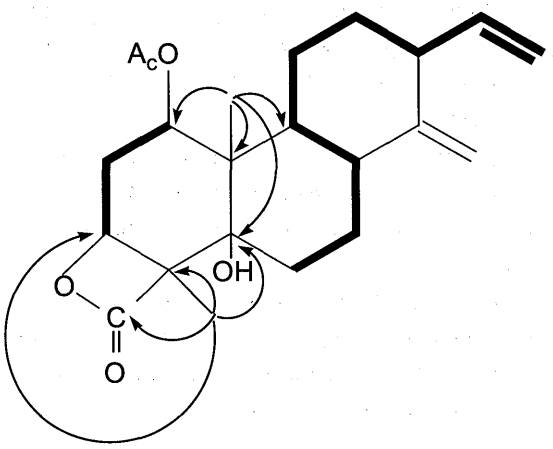


Fig. 2. H-H and H-C Correlations of **2a**
Arrows show the significant H-C correlation found by the COLOC spectrum of **2a**. Bold lines shows the H-H correlation found by the COSY spectrum of **2a**.

group, an exomethylene, and two tertiary methyl groups. Furthermore, from the COSY and COLOC spectra, the plane structure of sarcolide A was decided to be cassa-14(17),15-dien-1,5-di-hydroxy-3,4-carbolactone (2).

The cultured mycelium of *S. scabrosus* fungus produced ergosterol and ergosterol peroxide as the fruiting body of this fungus, but did not produce any diterpenoid, sarcodonins which were the characteristic bioactive cyathane diterpenoids of its fruiting body. It is very interesting to compare the constituents of the natural mycelium, mycorrhiza of *S. scabrosus* fungus with those of the cultured mycelium.

Experimental

Instrument : Optical rotations were measured in MeOH or $CHCl_3$ on Oyodenki MP1T polarimeter. Mass spectra were measured on a Hitachi GC/MS M-80B spectrometer. ^1H and ^{13}C-NMR, DEPT, COSY and COLOC spectra were recorded on a Bruker AC250 spectrometer operating at 250 MHz for proton and 62,5 MHz for carbon, with TMS as an internal standard. IR spectra were taken with a Hitachi EPI-G2 spectrometer.

Culture of mycelium of S. scabrosus : A piece of the fruiting body of *S. scabrosus* was set on the potato agar medium containing 20% of sucrose, at 27°C for 30 days. The induced mycelium of *S. scabrosus* was cultured on the peptone agar medium containing 0.6% of peptone and 3% of glucose, at 23°C for 15 days.

Isolation of constituents of mycelium of S. scabrosus : The mycelial mat was separated from the culture broth, air dried(490g) and extracted with EtOAc afforded a crude extract, from which the neutral fraction(5.7g) was given. The neutral fraction was chromatographed on aluminum oxide to give six fractions. The fraction eluted with MeOH(1.4g) was chromatographed on si-gel to afford five fractions. The $CHCl_3$/MeOH(20 : 1) eluate was recrystalyzed from hexane afforded compound **1** (245mg). The $CHCl_3$/MeOH (30 : 1) eluate (1.25g) was rechromatographed on si-gel, and the benzene/EtOAc (10 : 1) eluate to afford the compound **2** (100mg). The benzene/EtOAc (5 : 1) eluate was recrystaliyzed from hexane/benzene (1 : 1) to give a colorless plates (102mg).

Physicochemical data of compound **2** *(sarcolide A)* : mp 198-199°C ; $[\alpha]_D^{23}$-20° (c 0.1, acetone) ; IR ν_{max}(KBr)cm^{-1} : 3400, 3175, 3050, 2980, 1820, 1600, 1440, 1090, 1070, 1040, 990, 980, 820 ; ^1H and ^{13}C-NMR : in Table 1.

Acetylation of sarcolide A (Preparation of compound **2a**) : Sarcolide A (23mg) was treated with acetic anhydride (1ml) and pyridine (1ml) at room temperature overnight. Resultant reaction mixture was worked up with usual manner to afford an amorphous solid (**2a**, 20mg).

Physicochemical data of compound **2a** : $[\alpha]_D^{23}$-106° (c 0.05, MeOH) ; IR ν_{max}(KBr) cm^{-1} : 3550, 3050, 2950-2850, 1820, 1740, 1640, 1440, 1380, 1240, 1000, 940, 900, 820 ; ^1H and ^{13}C-NMR : in Table 1.

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ケロウジ（*Sarcodon scabrosus*）培養菌糸体に含まれる新規カサン骨格ジテルペノイド

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要　約

私達は担子菌ケロウジ子実体の成分として種々の生物活性（苦味，抗菌活性等）を有するシアタン骨格のジテルペノイド sarcodonin A～M を単離し、その構造を決定した。これらの生物活性物質を大量生産する目的で本菌の培養を試みた。子実体から誘導，純粋培養して得られた菌糸体を常法により抽出，単離操作を行い，カサン骨格を持つジテルペノイドを得た。その構造を機器分析および化学反応によって cassa-14(17),15-diene-1,5-dihydroxy-3,4-carbolactone と決定した。培養菌糸体からはカサン骨格のジテルペノイドが得られたが，シアタン骨格を持つ sarcodonin 類は全く得られなかった。以上の事から培養菌糸体の成分と天然の菌糸体であるケロウジ菌根の成分との比較に興味が持たれる。

キーワード：ケロウジ，培養菌糸体，キノコ代謝産物，カサンジテルペノイド，シアタンジテルペノイド