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<th>項目</th>
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<td>タイトル</td>
<td>新たな生体活性化合物のスパラシスキノコから</td>
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<td>発表者</td>
<td>川崎宏和; 林香子; 与山真季; 浩水南子; 木村貴正; 今村義昌</td>
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<tr>
<td>引用</td>
<td>生物学・生物技術・生化学. 71(7), p. 1804-1806</td>
</tr>
<tr>
<td>発行日</td>
<td>2007-07</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10297/2297">http://hdl.handle.net/10297/2297</a></td>
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<tr>
<td>版本</td>
<td>オリジナル</td>
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| 权利 | 日本農芸化学会 [本文データは学会協会の許諾に基づき CiNiiから複製したものである]
A novel compound (2) and a known one (1) were isolated from the mushroom, Sparassis crispa. Both compounds inhibited melanin synthesis and MRSA growth.

Key words: anti-melanin synthesis; anti-methicillin-resistant Staphylococcus aureus; mushroom; Sparassis crispa

The mushroom, Sparassis crispa (Hanabiratake in Japanese and cauliflower mushroom in English), grows on acicular trees and is widespread in northern temperate zones throughout the world. This mushroom has been used as a food and a home remedy in Asian countries. There are some reports on the isolation of β-glucan and antifungal benzoate derivatives.1–6 We examined the biological activity of an extract of the mushroom, and found inhibitory activity against melanin synthesis by mouse B16 melanoma cells and against the growth of methicillin-resistant Staphylococcus aureus (MRSA). We report here the isolation, structural determination of a novel compound (2) and a known one (1), and their biological activities.

General. 1H-NMR spectra (one- and two-dimensional) were recorded by a Jeol lambda-500 spectrometer at 500 MHz, while 13C-NMR spectra were recorded on the same instrument at 125 MHz. The FABMS data were recorded by a Jeol DX-303HF instrument and the HRESIMS spectra were measured by a JMS-T100LC mass spectrometer. A Jasco grating infrared spectrophotometer was used to record the IR spectra, and CD spectra were measured by a Jasco J-820 spectropolarimeter. MPLC was done with a Yamazen MPLC system and an UltraPak ODS-S50D column (50 × 300 mm, Yamazen, Japan). HPLC separation was performed with a Jasco Gulliver system, using an ODS column (Wakopak Wakosil ODS, 20 × 300 mm, Wako Pure Chemicals, Japan) or a C30 column (Develosil C30-UG-5, 20 × 250 mm, Nomura Chemicals, Japan). Silica gel plates (Merck F254) and silica gel 60N (Merck 100–200 mesh) were respectively used for analytical TLC and for flash column chromatography.

Extraction and isolation. Dried fruiting bodies of Sparassis crispa (10.0 kg) were successively extracted with hexane (4-liter, twice), EtOAc (4-liter, three times) and then MeOH (8-liter). The residue (75.1 g) obtained after removing EtOAc was fractionated by silica gel flash column chromatography (90%, 70%, 60%, 50% hexane/EtOAc, 90%, 50% EtOAc/EtOH, MeOH, each 2-liter) to obtain twelve fractions. Fraction 8 (6.501 g) was further separated by reversed-phase MPLC (90% MeOH), and ten fractions were obtained. Fraction 8–2 (212.1 mg) was separated by reversed-phase HPLC (70% MeOH) to afford compound 2 (4.0 mg). While fraction 8–3 (31.1 mg) was subjected to reversed-phase HPLC (70% MeOH) to provide compound 1 (8.2 mg).

Anti-MRSA. Methicillin-resistant Staphylococcus aureus 2932 (a clinical isolate of MRSA) was kindly supplied by Prof. Michio Ohta (Nagoya University Graduate School of Medicine, Nagoya, Japan). Each sample at various concentrations was added to each well in a 96-well micro-plate. The pre-incubated MRSA suspension was then added to the wells. After incubating at 37 °C for 16 h, inhibition was evaluated on the basis of the turbidity of the culture medium in the wells. The minimum inhibitory concentration (MIC) of each sample is defined as the minimum concentration that gave no turbidity of the culture medium.

Inhibition of melanin production in B16 melanoma cells. B16 mouse melanoma cells were purchased from American Type Culture Collection (ATCC) and grown in a humidified atmosphere with 5% CO2 at 37 °C. The cells were placed in flat-bottomed 6-well plates (Asahi Techno Glass, Tokyo, Japan) at a density of 1.0 × 104 cells/well in an RPMI 1640 medium (Wako Pure Chemicals, Japan) containing 10% (v/v) fetal bovine...
serum (FBS) and 0.5% (v/v) penicillin (10000 U/ml)-
streptomycin (10 mg/ml). After 24 h of cultivation, the
medium was replaced with a new RPMI 1640 medium
containing each sample (25 μg/ml final conc.). After
further cultivation for 48 h, the medium was again
replaced by a new quantity of the same medium con-
taining the test samples, and cultivation was continued
for 24 h. The adherent cells were washed with 10 mM
phosphate-buffered saline (pH 7.4) (PBS) and detached
from the wells by trypsinization. The cells were col-
lected in a test tube by centrifugation at 3000 rpm for
3 min, and the cell pellet was washed with PBS twice.
In order to extract melanin from the B16 mouse
melanoma cells, the cell pellet was suspended in 0.5
ml of 3 N NaOH and stored at 37 °C for 2 h. After
mixing well, the melanin content was determined by
measuring value of the absorbance at 450 nm minus that
at 690 nm by using a microplate reader (Viento XS,
Dainippon Pharma Co., Osaka, Japan. Arbutin was
used as the positive control.

The dried fruiting bodies of Sparassis crispa
were successively extracted with CHCl₃, EtOAc, and MeOH.
Since the EtOAc-soluble fraction showed significant
anti-melanin-synthesis and anti-MRSA activity, this
fraction was repeatedly chromatographed as guided by
the bioassay. As a result, two compounds (1 and 2) were
purified as active principles.

All the spectral data for 1 were identical with those of
a cytotoxic compound isolated from the mycelium of
Antrodia camphorata.⁷

Compound 2 was isolated as colorless oil. IR νmax (neat) cm⁻¹: 3502, 1792. FABMS of 2 showed the ion
peak at m/z 332 ([M + H]⁺). Its molecular formula was
determined as C₁₉H₂₅NO₄ by HRESIMS [m/z 330.1718
[M – H]⁻ (calcd. for C₁₉H₂₅NO₄, 330.1705)], indicating
the presence of eight degrees of unsaturation in the
molecule. The ¹H- and ¹³C-NMR spectra of 2 (Table 1)
共同 with DEPT, COSY and HMQC data suggested
the presence of an isobutyl attached at an sp³ carbon [δH
0.80 (3H, d, J = 6.7), δC: 22.5; 0.85 (3H, d, J = 6.7),
22.8; 1.93 (1H, m), 27.6; 2.31 (2H, m), 32.9], a methine
[5.73 (1H, s), 84.2], a p-substituted phenyl [6.93 (2H, d,
J = 8.9), 114.8; 7.40 (2H, d, J = 8.9), 130.2], −CH₃−CH=:
[4.52 (2H, d, J = 7.0), 148.9, 5.47 (1H, m), 119.2],
and two methyls attached at sp² carbon(s) [1.73 (3H, s),
18.2; 1.78 (3H, s), 25.8]. These NMR data were similar
to those of 1, and the molecular formulas of the two
compounds were the same. However, the structure of the
five-membered ring of 2 was different from that of 1.
The complete structure was determined by interpreting
the HMBC correlations (Fig. 1). Significant cross peaks
to connect each partial structure and to determine the
complete structure were as follows: H¹/C₂, H¹/C₃,
H¹/C₄, H₅/C₃, H₅/C₄, H²/H⁶'/C₄ and H₁⁰'/C⁴'.
This compound had an asymmetric carbon, but was
racemic since the CD spectrum of it showed a plane line.

Compounds 1 and 2 were evaluated in anti-MRSA
and melanin-production inhibition assays. MIC values of
1 and 2 in the anti-MRSA assay were 0.5 and 1.0 mM,
respectively. IC₅₀ values of 1 and 2 in the melanin

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**Table 1.** ¹H-NMR (δH (number of protons, multiplicity, J in Hz)) and ¹³C-NMR (δC) Data for 1 and 2 in CDCl₃.

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<tr>
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*These assignments were established by COSY, DEPT, HMQC and HMBC experiments.

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**Fig. 1.** HMBC Correlations in 2.
production inhibition assay were 33 μM and 12 μM, respectively. On the other hand, IC50 value of the positive control, arubutin, was 1.32 mM.

Acknowledgment

The authors thank Professor Masao Hattori (Toyama Medical and Pharmaceutical University, Japan) for providing the NMR data for compound 1 and Professor Michio Ohta (Nagoya University Graduate School of Medicine, Nagoya, Japan) for giving the MRSA sample. This work was partially supported by grant-in-aid for scientific research on priority areas ‘Creation of Biologically Functional Molecules’ (No. 17035037) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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