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Erinacines J and K from the mycelia of *Hericium erinaceum*

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Abstract—Two novel compounds, erinacines J (**1**) and K (**3**), were isolated from the cultured mycelia of *Hericium erinaceum*. Their structures were determined by spectral analyses. Erinacine K showed anti-MRSA activity.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has developed resistance to most antibiotics and is one of the most prevalent pathogen in nosocomial infections. Therefore, anti-MRSA substances are urgently required. We screened extracts of various mushrooms and their mycelia for anti-MRSA activity. Since the extract from the cultured mycelia of *Hericium erinaceum* showed potent activity, we tried to isolate the active principles from the fungus. We wish to report here the isolation, the structure determination of two novel compounds named erinacine J (**1**) and K (**2**), and the inhibitory activity of the two compounds and the other erinacines.

2. Results and discussion

The extract of the lyophilized mycelia of *H. erinaceum* was successively extracted with $CHCl_3$, EtOAc, acetone, and MeOH. Since the $CHCl_3$-soluble fraction showed significant activity, this fraction was repeatedly chromatographed on the basis of the result of the bioassay. As a consequence, two new compounds were purified and named as erinacine J (**1**) and K (**3**).

Erinacine J (**1**) was isolated as colorless crystals.[1] FABMS of **1** showed ion peaks at *m/z* 467 ($[M+H]^+$) and *m/z* 489 ($[M+Na]^+$). Its molecular formula was determined as $C_{25}H_{38}O_8$ by HRESIMS, indicating the presence of seven degrees of unsaturation in the molecule. The ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of **1** along with DEPT and HMQC showed two methyls attached at quaternary carbons, an isopropyl attached at an sp^2 carbon [δ_H 1.07 (6H, d, J=7.0), δ_C 18.7; 2.68 (1H, heptet, J=7.0), 41.8], seven sp^3 methylenes, eight sp^3 methines, three sp^3 quaternary carbons (δ_C 43.6, 47.4, 81.0), two carbonyls (δ_C 216.7, 217.4) (Tables 1 and 2). These data suggested that this compound is an analog of erinacines.[2–6] The HMBC correlations (H19,20/C3, H18/C3, H1/C3, H2/C3) indicated a partial structure, $(CH_3)_2CHCOCH_2CH_2$–, (Fig. 1). Cross peaks (H1/C9, H1/C4, H17/C1, H8/C1) between the terminal methylene (C1) in the sequence and the other protons or carbons suggested the further linkage, C1–$C(CH_3)$(CO–)CH_2–. The other part of the structure except for the sugar part was determined by the following HMBC correlations; H17/C8, H17/C9, H17/C4, H8/C17, H8/C4, H8/C7, H10/C4, H5/C4, H5/C7, H5/C6, H5/C10, H5/C11, H7/C5, H10/C5, H11/C5, H16/C5, H16/C7, H16/C6, H16/C14, H7/C16, H14/C16, H14/C7, H14/C6, H14/C13, H14/C12, H10/C12, H11/C12, H11/C13, H13/C11, H13/C6, and H15/C11. The structure of the highly modified sugar part and the bonds between the sugar and the aglycon was also confirmed by the HMBC correlations; cross peaks between H1′/C2′, H3′/C2′, H3′/C4′, H5′/C1′, H5′/C3′, H5′/C4′, H1′/C14, H1′/C13, H3′/C13, H3′/C15, H13/C2′, H14/C1′, H14/C2′, and H15/C3′ were observed in the spectrum. The COSY data of **1** also supported this structure (data not shown). The chemical shifts of the ^1H and ^{13}C NMR signals of the compound were similar to those of **2** obtained in this study, and especially those of the sugar part were almost same as each other. Compound **2**, CJ-14,258, which has been reported as a κ opioid receptor agonist, and its stereochemistry except for C-2′ has been determined.[2] Formation of **1** in the mycelia can be envisioned as an oxidative cleavage of C3–C4 bond of **2**.

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Table 1. ^{1}H NMR data [δ_H (the number of protons, multiplicity, *J* in Hertz)] for erinacine J (**1**), CJ-14,258 (**2**), and erinacine K (**3**) (in CD_3OD)[a]

Position	**1**	**2**	**3**
1	1.65 (2H, dd, 7.9, 7.9)	1.56 (1H, m); 1.61 (1H, m)	1.50 (1H, m); 1.58 (1H, m)
2	2.52 (1H, m); 2.58 (1H, m)	2.29 (2H, m)	2.30 (2H, m)
5	2.72 (1H, m)	2.30 (1H, m)	2.81 (1H, m)
7	1.72 (1H, m); 1.94 (1H, m)	1.36 (1H, m); 1.51 (1H, m)	1.12 (1H, m); 1.99 (1H, m)
8	1.72 (1H, m); 1.84 (1H, ddd, 13.3, 13.1, 5.4)	1.38 (2H, m)	1.40 (1H, m); 1.51 (1H, m)
10	1.21 (1H, m); 2.05 (1H, m)	1.70 (1H, m); 1.82 (1H, m)	2.10 (2H, m)
11	0.93 (1H, m); 2.17 (1H, m)	1.01 (1H, m); 2.21 (1H, m)	4.66 (1H, m)
12	1.68 (1H, m)	1.68 (1H, m)	—
13	1.98 (1H, dd, 12.2, 8.9)	1.97 (1H, dd, 12.2, 8.5)	5.78 (1H, d, 5.8)
14	3.89 (1H, d, 8.9)	3.99 (1H, d, 8.5)	3.81 (1H, m)
15	4.82 (1H, d, 8.5)	4.82 (1H, d, 8.5)	4.60 (1H, d, 13.0); 4.67 (1H, d, 13.0)
16	0.88 (3H, s)	0.96 (3H, s)	0.83 (3H, s)
17	1.20 (3H, s)	1.08 (3H, s)	1.07 (3H, s)
18	2.68 (1H, heptet, 7.0)	2.79 (1H, heptet, 7.0)	3.02 (1H, heptet, 6.7)
19,20	1.07 (6H, d, 7.0)	0.96 (3H, d, 7.0); 0.97 (3H, d, 7.0)	0.98 (6H, d, 6.7)
1′	4.95 (1H, s)	4.91 (1H, s)	4.23 (1H, d, 7.2)
2′	—	—	3.22 (1H, dd, 7.2, 8.9)
3′	3.82 (1H, d, 8.9)	3.81 (1H, d, 8.5)	3.30 (1H, dd, 8.9, 8.5)
4′	4.14 (1H, m)	4.16 (1H, m)	3.45 (1H, ddd, 8.5, 9.9, 5.2)
5′	3.13 (1H, dd, 11.0, 11.3); 3.79 (1H, dd, 11.0, 5.2)	3.12 (1H, dd, 11.3, 11.0); 3.79 (1H, m)	3.13 (1H, dd, 11.6, 9.9); 3.79 (1H, dd, 11.6, 5.2)
CH_3CO–			2.05 (3H, s)

[a] These assignments were established by decoupling, COSY, DEPT, HMQC, and HMBC experiments.

Erinacine K (**3**) was purified as pale yellow oil, and its molecular formula, $C_{27}H_{42}O_8$, was determined by HRESIMS. The NMR data (Tables 1 and 2) and HMBC experiment (Fig. 1) indicated that this compound was also an erinacine analog having a simple sugar.[3–7] The plane structure of the compound was determined by HMBC correlations (Fig. 1); H1/C2, H1/C3, H1/C4, H1/C9, H1/C17, H1/C8, H2/C3, H2/C4, H2/C9, H18/C2, H18/C3, H18/C4, H19,20/C3, H19/C20, H20/C19, H5/C3, H5/C4, H5/C10, H17/C1, H17/C9, H17/C4, H17/C8, H8/C9, H8/C4, H8/C6, H7/C6, H16/C6, H16/C7, H16/C5, H16/C14, H11/C5, H11/C10, H13/C11, H13/C12, H13/C6, H13/C14, H13/C15, H15/C11, and H15/C13. The structure of the compound was also supported by its COSY experiments (data not shown). This compound had an acetoxy group at C15; cross peaks between H15/CH_3CO and CH_3CO/CH_3CO were detected in its HMBC spectrum. The sugar was identified as D-xylose by hydrolysis of **3** with β-glucosidase and ^{1}H NMR data of the product. The position of the glycosidic linkage between the aglycon and the sugar was determined by H14/C1′ and C1′/H14 cross peaks in the HMBC spectrum (Fig. 1). The relative stereochemistry of **3** was deduced by NOESY and ROESY experiments; cross peaks between H5/H17 and H14/H16 were observed. The stereochemistry at C11 remains undetermined since its NOESY and ROESY experiments did not give any valuable information.

Compounds **1**, **3**, and the other erinacine analogs obtained in this study [**2**, erinacine A (**4**), C (**5**), and E (**6**)] were evaluated in anti-MRSA assays.[6,7] MIC of **2**, **3**, **4**, and **5** were 62.5, 500, 500, and 62.5 μM, respectively. Although **6** gave halos by the direct drop method, this compound was inert even at 1 mM by the micro-plate method. Compound **1** did not show any activity by the both method. This result indicated that the three-ring skeleton of the aglycon in the active compounds was indispensable to the anti-MRSA activity.

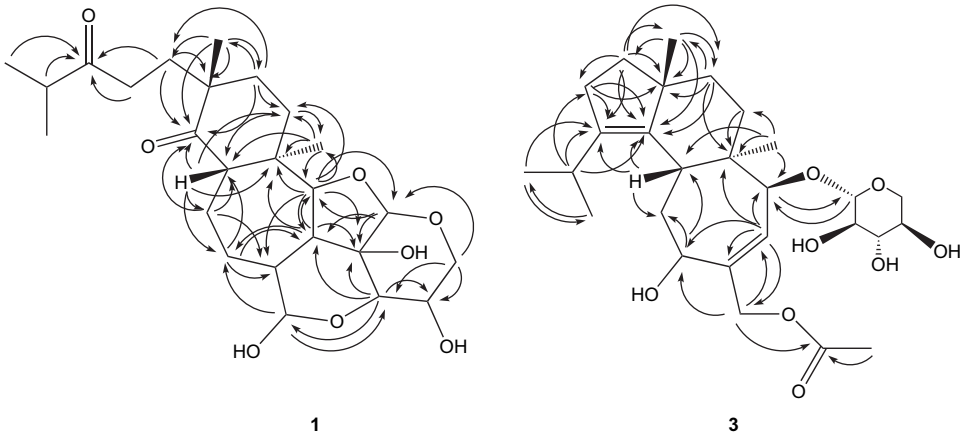

Figure 1. HMBC correlations in **1** and **3**.

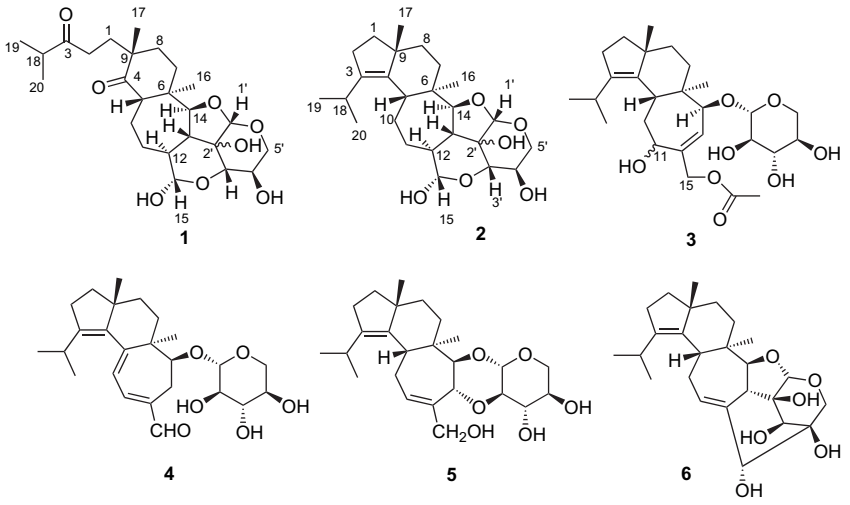

Table 2. ^{13}C NMR data (δ_C) for erinacine J (**1**), CJ-14,258 (**2**), and erinacine K (**3**) (in CD_3OD)[a]

Position	1	2	3
1	33.3	39.3	39.2
2	36.3	28.9	29.4
3	217.4	139.3	140.4
4	216.7	140.2	140.2
5	56.2	48.3	41.3
6	43.6	41.6	44.1
7	27.6	39.2	33.0
8	35.2	37.7	38.1
9	47.4	50.6	50.5
10	19.8	26.4	37.2
11	34.8	36.9	71.6
12	45.8	45.6	132.4
13	47.9	47.1	129.9
14	93.3	94.6	86.4
15	97.6	97.9	66.7
16	19.7	19.6	17.0
17	25.2	25.5	24.9
18	41.8	28.2	28.1
19, 20	18.7	21.7	21.0
		22.3	22.0
1′	108.6	108.4	107.2
2′	81.0	81.3	75.2
3′	85.8	86.0	77.8
4′	69.4	69.4	71.2
5′	65.5	65.5	66.7
CH$_3$CO–			21.0
CH$_3$*CO*			172.7

[a] These assignments were established by DEPT, HMQC, and HMBC experiments.

3. Experimental

3.1. General

^{1}H NMR spectra (one- and two-dimensional) were recorded on a JEOL lambda-500 spectrometer at 500 MHz, while ^{13}C NMR spectra were recorded on the same instrument at 125 MHz. The FABMS spectra were recorded on a JEOL DX-303HF and the HRESIMS spectra were measured on a JMS-T100LC mass spectrometer. A JASCO grating infrared spectrophotometer was used to record the IR spectra. The $[\alpha]_D$ spectra were measured by using a JASCO DIP-1000 spectropolarimeter. HPLC separations were performed with a JASCO Gulliver system using an ODS column (Grandpack ODS-A S-5 YC, 20×300 mm, Masis, Japan). Silica gel plate (Merck F_{254}) and silica gel 60 N (Merck 100–200 mesh) were used for analytical TLC and for flash column chromatography, respectively.

3.2. Fungus materials and cultivation

The strain, HE-01003, of *H. erinaceum* was collected in Fukushima Prefecture, Japan, in October, 2001. The isolate is deposited in the culture collection of IBI Corporation. The components of the mycelia culture were as follows; glucose 4%, polypeptone 0.3%, yeast extract 0.3%, KH_2PO_4 0.05%, and Na_2HPO_4 0.05% in the distilled water. The culture medium was adjusted to pH 5.5. The aerated liquid culture was carried out in a 1.5 ton tank containing 1000 L of the medium, and incubated at 25 °C for 30 days.

3.3. Extraction and isolation

After the cultivation, the wet mycelia of *H. erinaceum* were obtained by centrifugation (7000×g, 30 min) and lyophilized. The dried mycelia (about 200 g) were successively extracted with $CHCl_3$ (4 L, twice), EtOAc (4 L, twice), acetone (6 L, three times), and then MeOH (8 L). The residue (4.97 g) obtained after removing $CHCl_3$ was fractionated by silica gel flash column chromatography (100%, 80%, 60% $CHCl_3$/acetone, 90%, 70% $CHCl_3$/MeOH, MeOH, each 1 L) to obtain 13 fractions. Fraction 12 (464.8 mg) was further separated by silica gel flash column chromatography (90% $CHCl_3$/MeOH) and six fractions were obtained. Fraction 12–3 (122.0 mg) was separated by reversed-phase HPLC (80% MeOH) to afford compound **1** (12.7 mg). On the other hand, fraction 13 (142.9 mg) was subjected to reversed-phase HPLC (80% MeOH) to provide compounds **2** (0.9 mg) and **3** (3.1 mg).

3.3.1. Erinacine J (1). Colorless crystals, mp 125–128 °C. $[\alpha]_D$ −16.1 (*c* 1.00, MeOH). IR ν_{max} (KBr) cm^{-1}: 3418, 2934, 1706. HRESIMS *m/z* 489.2461 [M+Na]$^+$ (calcd for $C_{25}H_{38}NaO_8$, 489.2464).

3.3.2. Erinacine K (3). Pale yellow oil. $[\alpha]_D$ −18.4 (*c* 0.290, MeOH). IR ν_{max} (neat) cm^{-1}: 3398, 2926, 1725. HRESIMS *m/z* 517.2782 [M+Na]$^+$ (calcd for $C_{27}H_{42}NaO_8$, 517.2777).

3.4. Bioassay

3.4.1. Direct drop method. Pre-incubated MRSA suspension (100 μl) is spread on the surface of agar medium in Petri dishes. Solution of each sample (2 μl) was dropped on the agar. After incubation at 37 °C overnight, the activity (formation of halo) was observed.

3.4.2. Micro-plate method. Each sample at various concentrations was added to each well in a 96-well micro-plate. Pre-incubated MRSA suspension is added to the wells. After incubation at 37 °C for 16 h, inhibition was evaluated on the basis of turbidity of the culture media in the wells. Minimum inhibitory concentration (MIC) of each sample was defined as the minimum concentration that gave no turbidity of the culture media.

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References and notes

1. Kawagishi, H.; Nakamura, T.; Akiyama, Y.; Tokuyama, S. Japanese Patent 2,005,239,669, 2005.
2. Shimbo, M.; Kawagishi, H.; Yokogoshi, H. *Nutr. Res.* **2005**, *25*, 617–623.
3. Lee, E. W.; Shizuki, K.; Hosokawa, S.; Suzuki, M.; Suganuma, H.; Inakuma, T.; Li, J.; Ohnishi-Kameyama, M.; Nagata, T.; Furukawa, S.; Kawagishi, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2402–2405.
4. Kawagishi, H.; Simada, A.; Shizuki, K.; Mori, H.; Okamoto, K.; Sakamoto, H.; Furukawa, S. *Heterocycl. Commun.* **1996**, *2*, 51–54.
5. Kawagishi, H.; Shimada, A.; Shirai, R.; Okamoto, K.; Ojima, F.; Sakamoto, H.; Ishiguro, Y.; Furukawa, S. *Tetrahedron Lett.* **1994**, *35*, 1569–1572.
6. Kawagishi, H.; Shimada, A.; Hosokawa, S.; Mori, H.; Sakamoto, H.; Ishiguro, Y.; Sakemi, S.; Bordner, J.; Kojima, N.; Furukawa, S. *Tetrahedron Lett.* **1996**, *37*, 7399–7402.
7. Saito, T.; Aoki, F.; Hirai, H.; Inagaki, T.; Matsunaga, Y.; Sakakibara, T.; Sakemi, S.; Suzuki, Y.; Watanabe, S.; Suga, O.; Sujaku, T.; Smogowicz, A. A.; Truesdell, S. J.; Wong, J. W.; Nagahisa, A.; Kojima, Y.; Kojima, N. *J. Antibiot.* **1998**, *51*, 983–990.