

Two New Aromatic Compounds from *Hericium erinaceum* (BULL.: FR.) PERS.¹⁾

Yasunori YAOITA, Kuniko DANBARA, and Masao KIKUCHI*

Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai, Miyagi 981–8558, Japan.

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Two new aromatic compounds, erinacerins A (1) and B (2), were isolated from the fruiting bodies of *Hericium erinaceum* (BULL.: FR.) PERS. (Hericiaceae) together with a known compound, hericenone A (3). The structures of the new compounds were elucidated on the basis of their spectral data. It was found that 1 occurred as a racemate.

Key words *Hericium erinaceum*; Hericiaceae; aromatic compound; mushroom

The fruiting bodies of *Hericium erinaceum* (BULL.: FR.) PERS. (Yamabushitake in Japanese, Hericiaceae) are known as an edible mushroom. The constituents of *H. erinaceum* have been previously investigated and shown to contain aromatic compounds,^{2–5} fatty acids,⁶ sterols⁷ and polysaccharides.⁸ Some aromatic compounds, including hericenone A (3), were found to have significant cytotoxicity against HeLa cells² and stimulating activity of the synthesis of the nerve growth factor.³ In a continuation of our investigation of chemical constituents from mushrooms, we describe here the isolation and structural elucidation of two new aromatic compounds, erinacerins A (1) and B (2), together with a known compound (3) from the fruiting bodies of *H. erinaceum*. Compound 3 was identified as hericenone A by comparison of its spectroscopic data with those previously described in the literature.^{2,4)}

Compound 1, called erinacerin A, was obtained as a colorless oil. The molecular formula was determined to be C₂₇H₃₁NO₄ by high-resolution (HR)-electron ionization (EI)-MS. The IR spectrum showed the presence of a γ -lactam (1705 cm⁻¹), an α,β -unsaturated ketone (1680 cm⁻¹) and phenyl groups (1614 cm⁻¹). The ¹H-NMR spectrum (*vide* Experimental) showed signals due to a tertiary methyl group [δ_{H} 1.41 (3H, CH₃-9')], two olefinic methyl groups [δ_{H} 1.85 (3H, CH₃-8'), 2.14 (3H, CH₃-10')], six methylenes [δ_{H} 1.90 (1H, H-2'a), 2.02 (1H, H-2'b), 2.64 (1H, H-4'a), 2.68 (1H, H-1'a), 2.69 (1H, H-1'b), 2.76 (1H, H-4'b), 2.98 (2H, H₂-2''), 3.83 (1H, H-1''a), 3.84 (1H, H-1''b), 4.11 (1H, H-3a), 4.17 (1H, H-3b)], a methoxyl group [δ_{H} 3.87 (3H)], a trisubsti-

tuted double bond [δ_{H} 6.05 (1H, H-6')] and six aromatic protons [δ_{H} 6.90 (1H, H-7), 7.20–7.32 (5H, H-4''–H-8'')]. The ¹³C-NMR spectrum (*vide* Experimental), obtained with the aid of a distortionless enhancement by polarization transfer (DEPT) spectrum, showed characteristic signals appearing to be due to a carbonyl [δ_{C} 198.1 (C-5')], a carbonyl of γ -lactam [δ_{C} 168.9 (C-1)],⁹ two methylene carbons linked to γ -lactam nitrogen [δ_{C} 44.4 (C-1''), 48.0 (C-3'')⁹] and an oxygenated quaternary carbon [δ_{C} 75.9 (C-3')]. There were thirteen degrees of unsaturation in the molecule according to the molecular formula. Two benzene rings, a γ -lactam and an α,β -unsaturated ketone accounted for twelve of those. One degree of unsaturation remained, implying that there was one more ring in this structure. The ¹H–¹H shift correlation spectroscopy (¹H–¹H COSY) spectrum of 1 implied connectivities for H₂-1'–H₂-2' and H₂-1''–H₂-2''. Interpretation of the ¹H-detected heteronuclear multiple-bond coherence (HMBC) spectrum revealed correlations from H₂-3 to C-1, C-3a and C-7a; H-7 to C-1 and C-6; H₂-1' to C-4, C-5 and C-6; H-4' and H-6' to C-5'; CH₃-8' and CH₃-10' to C-6'; CH₃-9' to C-2', C-3' and C-4'; H₂-1'' to C-3; H₂-2'' to C-4'' and C-8''; and CH₃O-6 to C-6. As a result, C-3' carbon is linked to an oxygen atom at C-4 to establish a dihydropyran ring. Therefore, the structure of 1 was deduced to be as shown in Fig. 1. Despite the presence of an asymmetric carbon at C-3', the specific rotation of 1 was almost zero, suggesting its racemic nature. This was proved by chiral reverse-phase HPLC, in which 1 was separated into two peaks in a ratio of 1 : 1 [peak A, (–)-1: [α_{D}^{25} –16.8° (MeOH); peak B, (+)-1: [α_{D}^{26} +16.1° (MeOH)], both of which gave ¹H-NMR spectra identical to those of the starting material. The circular dichroism (CD) spectra of (+)-1 and (–)-1 exhibited mirror images [(+)-1: 261.5 nm ($\Delta\epsilon$ +4.6), 224.0 nm ($\Delta\epsilon$ –5.2), (–)-1: 260.5 nm ($\Delta\epsilon$ –5.4), 223.1 nm ($\Delta\epsilon$ +5.2)], confirming their enantiomeric relationship or, in other words, the racemic na-

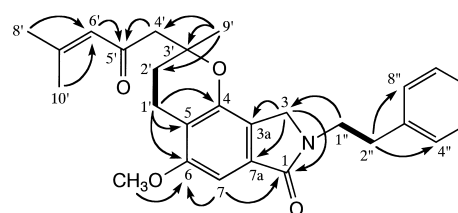
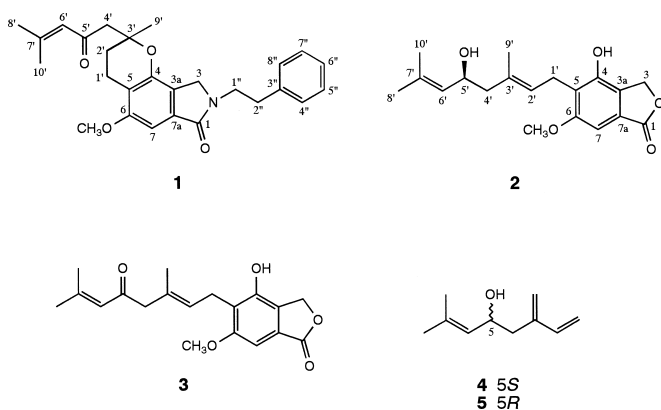


Fig. 1. ¹H–¹H COSY (Bold Line) and HMBC (Full-Line Arrows) Correlations for 1

* To whom correspondence should be addressed. e-mail: mkikuchi@tohoku-pharm.ac.jp

ture of our initial preparation.

Compound **2**, called erinacerin B, was obtained as an amorphous powder, $[\alpha]_D^{25} +12.7^\circ$. The molecular formula was determined to be $C_{19}H_{24}O_5$ by HR-EI-MS [m/z 314 ($M^+ - H_2O$)] and ^{13}C -NMR data. The IR spectrum showed the presence of hydroxyl groups (3383 cm^{-1}), a phthalide (1763 cm^{-1}) and a benzene ring (1603 cm^{-1}). The 1H - and ^{13}C -NMR spectra of **2** were similar to those of **3**, except that the C-5' carbonyl group in **3** was replaced by a hydroxyl group [δ_H 4.54 (H-5'), δ_C 66.0 (C-5')] in **2**. The position of this hydroxyl group was confirmed by the 1H - 1H COSY spectrum, in which H-5' showed connectivity to H₂-4' and H-6'. The absolute configuration of the hydroxyl group at C-5' was determined as *S* by comparing the specific rotation values of **2** [$[\alpha]_D^{25} +12.7^\circ$ (MeOH)], (*S*)-(+)-ipsdienol [**4**, $[\alpha]_D^{25} +15.7^\circ$ (MeOH)]¹¹ and (*R*)-(-)-ipsdienol [**5**, $[\alpha]_D^{25} -15.3^\circ$ (MeOH)].¹¹ On the basis of the above data, the structure of **2** was represented as shown in the formula.

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. IR spectra were recorded with a Perkin-Elmer Spectrum One FT-IR spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. 1H - and ^{13}C -NMR spectra were recorded on JEOL JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as internal standard. HR-EI-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on a Kieselgel 60 (230–400 mesh, Merck). HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, RI-8010).

Fungal Material The fresh fruiting bodies of *Hericium erinaceum* (from Sendai, Miyagi Prefecture, Japan) were purchased in a food market.

Extraction and Isolation The fresh fruiting bodies of *H. erinaceum* (1.3 kg) were extracted three times with Et₂O at room temperature for 2 weeks. The Et₂O extract (6.8 g) was chromatographed on a silica gel column using *n*-hexane–EtOAc (7:3–1:7), EtOAc, and MeOH to afford 32 fractions. Fraction 14 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40 °C; mobile phase, MeOH–H₂O (7:1); flow rate, 1.0 ml/min] to give **1** (3.6 mg) and **3** (5.0 mg). The separation of **1** into its enantiomers [(–)-**1** (1.2 mg, t_R 14.4 min), (+)-**1** (1.2 mg, t_R 19.2 min)] was achieved by HPLC using a chiral column chromatograph [column, Chiralpak AD-RH (4.6 mm i.d.×15 cm, Daicel Chemical Industries, Ltd.); mobile phase, CH₃CN–H₂O (7:3); flow rate, 0.5 ml/min]. Fraction 16 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d.×30 cm, Tosoh), column temperature, 40 °C; mobile phase, MeOH–H₂O (7:1); flow rate, 1.0 ml/min] to give **2** (1.6 mg).

Erinacerin A (**1**): Colorless oil. $[\alpha]_D^{21} \pm 0^\circ$ ($c=0.4$, MeOH). IR ν_{\max} (CHCl₃) cm^{-1} : 1705, 1680, 1614. UV λ_{\max} (MeOH) nm (log ϵ): 215 (4.6), 244 (4.2), 292 (3.4). HR-EI-MS m/z : 433.2237 (M^+ , Calcd for $C_{27}H_{31}NO_4$: 433.2253). 1H -NMR (400 MHz, CDCl₃) δ : 1.41 (3H, s, CH₃-9'), 1.85 (3H, d, $J=1.2$ Hz, CH₃-8'), 1.90 (1H, ddd, $J=13.9, 7.1, 6.8$ Hz, H-2'a), 2.02 (1H,

ddd, $J=13.9, 6.6, 6.6$ Hz, H-2'b), 2.14 (3H, d, $J=1.2$ Hz, CH₃-10'), 2.64 (1H, d, $J=14.1$ Hz, H-4'a), 2.68 (1H, dd, $J=7.1, 6.6$ Hz, H-1'a), 2.69 (1H, dd, $J=6.8, 6.6$ Hz, H-1'b), 2.76 (1H, d, $J=14.1$ Hz, H-4'b), 2.98 (2H, t, $J=7.6$ Hz, H₂-2''), 3.83 (1H, t, $J=7.6$ Hz, H-1'a), 3.84 (1H, t, $J=7.6$ Hz, H-1'b), 3.87 (3H, s, CH₃O-6), 4.11 (1H, d, $J=16.8$ Hz, H-3a), 4.17 (1H, d, $J=16.8$ Hz, H-3b), 6.05 (1H, br s, H-6'), 6.90 (1H, s, H-7), 7.20–7.32 (5H, m, H-4'–H-8''). ^{13}C -NMR (100 MHz, CDCl₃) δ : 17.4 (C-1'), 20.8 (C-10'), 24.8 (C-9'), 27.8 (C-8'), 30.6 (C-2'), 34.9 (C-2''), 44.4 (C-1''), 48.0 (C-3), 52.3 (C-4'), 55.9 (CH₃O-6), 75.9 (C-3'), 96.2 (C-7), 113.5 (C-5), 121.9 (C-3a), 125.0 (C-6'), 126.5 (C-6''), 128.6, 128.7 (C-4'', C-5'', C-7'', C-8''), 132.2 (C-7a), 138.8 (C-3''), 148.6 (C-4), 155.6 (C-7'), 158.5 (C-6), 168.9 (C-1), 198.1 (C-5').

(+)-**1**: Colorless oil. $[\alpha]_D^{26} +16.1^\circ$ ($c=0.1$, MeOH). CD λ_{ext} ($c=2.15 \times 10^{-5}$ M, MeOH) (nm) ($\Delta\epsilon$): 261.5 (+4.6), 224.0 (–5.2).

(–)-**1**: Colorless oil. $[\alpha]_D^{25} -16.8^\circ$ ($c=0.1$, MeOH). CD λ_{ext} ($c=2.06 \times 10^{-5}$ M, MeOH) (nm) ($\Delta\epsilon$): 260.5 (–5.4), 223.1 (+5.2).

Erinacerin B (**2**): Amorphous powder. $[\alpha]_D^{24} +12.7^\circ$ ($c=0.2$, MeOH). IR ν_{\max} (CHCl₃) cm^{-1} : 3383, 1763, 1603. UV λ_{\max} (MeOH) nm (log ϵ): 212 (4.5), 259 (4.0), 296 (3.5). HR-EI-MS m/z : 314.1526 ($M^+ - H_2O$, Calcd for $C_{19}H_{22}O_4$: 314.1518). 1H -NMR (600 MHz, CDCl₃) δ : 1.71 (3H, d, $J=1.1$ Hz, CH₃-10'), 1.72 (3H, d, $J=1.5$ Hz, CH₃-8'), 1.87 (3H, s, CH₃-9'), 2.21 (1H, dd, $J=13.6, 4.8$ Hz, H-4'a), 2.26 (1H, dd, $J=13.6, 8.8$ Hz, H-4'b), 3.51 (1H, dd, $J=16.5, 7.7$ Hz, H-1'a), 3.58 (1H, dd, $J=16.5, 6.2$ Hz, H-1'b), 3.87 (3H, s, CH₃O-6), 4.54 (1H, dt, $J=4.8, 8.8$ Hz, H-5'), 5.16 (1H, br d, $J=8.8$ Hz, H-6'), 5.20 (2H, s, H₂-3), 5.37 (1H, m, H-2'), 6.97 (1H, s, H-7). ^{13}C -NMR (150 MHz, CDCl₃) δ : 16.4 (C-9'), 18.2 (C-10'), 23.3 (C-1'), 25.8 (C-8'), 47.7 (C-4'), 56.2 (CH₃O-6), 66.0 (C-5'), 68.0 (C-3), 98.5 (C-7), 121.1 (C-5), 123.6 (C-2'), 125.1 (C-3a), 127.4 (C-6'), 127.8 (C-7a), 136.1 (C-7'), 137.1 (C-3'), 150.4 (C-4), 159.2 (C-6), 171.7 (C-1).

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References and Notes

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