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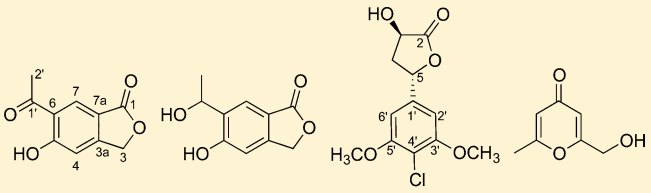
Erinaceolactones A to C, from the Culture Broth of *Hericium erinaceus*

Jing Wu,[†] Taiki Tokunaga,[‡] Mitsuru Kondo,[†,§] Kota Ishigami,[‡] Shinji Tokuyama,[‡] Tomohiro Suzuki,[†] Jae-Hoon Choi,[‡] Hirofumi Hirai,[†,‡] and Hirokazu Kawagishi*[,†,‡,⊥]

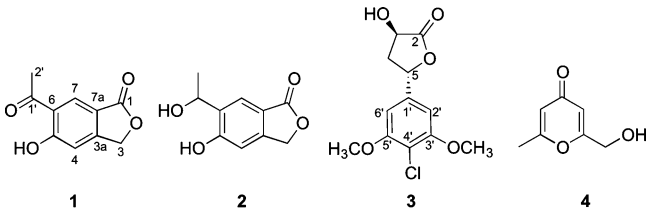
[†]Research Institute of Green Science and Technology, [‡]Graduate School of Agriculture, [§]Graduate School of Science, and [⊥]Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

Supporting Information

ABSTRACT: Three novel compounds, erinaceolactones A to C (**1**−**3**), and a known compound (**4**) were isolated from the culture broth of *Hericium erinaceus*. The planar structures of **1**−**3** were determined by the interpretation of spectroscopic data. The absolute configuration of **3** was determined by X-ray crystallography. Although compound **4** had been synthesized, it was isolated from a natural source for the first time. In the bioassay examining plant-growth regulatory activity of these compounds (**1**−**4**) and other components of the fungus (**5**−**8**), compounds **1**, **2**, and **4**−**8** suppressed the growth of lettuce.



Hericium erinaceus is an edible and medicinal mushroom belonging to the family Hericiaceae. It is called Yamabushitake in Japanese, Houtougu (monkey head) in Chinese, and Lion's Mane in English after its shape. In the past 25 years, we have reported phenols (hericenones A and B), a series of benzyl alcohol derivatives (hericenones C to H, 3-hydroxyhericenone F), and other hericenone analogues (hericenones I and J) from the fruiting bodies.[1−5] Chlorinated orcinol derivatives[6] (**8**) and a series of diterpenoid derivatives (erinacines A to K) have been isolated from the mycelia of the fungus.[7−12] Hericenones C to H and erinacines A to I significantly induced the synthesis of nerve growth factor (NGF) *in vitro* and/or *in vivo*. Erinacine K showed anti-MRSA activity. 3-Hydroxyhericenone F showed protective activity against endoplasmic reticulum (ER) stress-dependent cell death.[13,14] Several ER stress-suppressive compounds were also isolated from scrap cultivation beds of this mushroom.[15] From the culture broth of the fungus, erinapyrones A and B (**5**, **6**) and compound **7** have been isolated.[16,17] Erinapyrones A and B showed cytotoxicity toward HeLa cells. In our continuing search for bioactive compounds from the culture broth, we found three novel compounds (**1**−**3**) along with a known compound (**4**). Here we describe the isolation, structure determination, and biological activity of the compounds.



The culture broth of *H. erinaceus* was extracted with *n*-hexane and EtOAc, successively. The EtOAc-soluble part was fractionated by repeated chromatography. As a result, three novel compounds (**1**−**3**) and a known compound (**4**) were isolated.

Compound **1** was obtained as white crystals. Its molecular formula was determined as $C_{10}H_8O_4$ by HRESIMS (*m*/*z* 191.0321 $[M - H]^-$; calcd for $C_{10}H_7O_4$, 191.0344), indicating the presence of seven degrees of unsaturation in the molecule. The structure of **1** was elucidated by interpretation of NMR data, including DEPT, HMBC, and HMQC data (Figure 1).

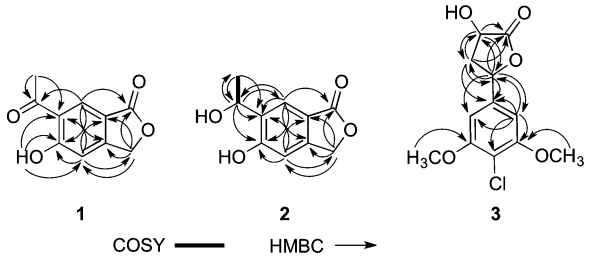


Figure 1. COSY and HMBC correlations of erinaceolactones A to C (**1** to **3**).

The DEPT experiment indicated the presence of a methyl, a methylene, two methines, and six quaternary carbons. The complete assignment of all the protons and carbons was accomplished as shown in Table 1. The benzolactone moiety was constructed by the HMBC correlations (H-3/C-1, 3a, 4, 7a; H-4/C-3, 5, 6, 7a; H-7/C-1, 3a, 5) (Figure 1). The position of the acetyl group at the benzolactone was elucidated by the HMBC correlations from the acetyl protons (H-2′) to C-6 and the phenyl proton (H-7) to the carbonyl (C-1′). The chemical shift of the hydrogen-bonded phenol (δ_H 12.89) and the HMBC correlations from the hydroxy to C-4, 5, and 6

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Table 1. NMR Spectroscopic Data (500 MHz) for Erinaceolactones A to C (1−3)

	erinaceolactone A		erinaceolactone B		erinaceolactone C		
	1 (in $CDCl_3$)		**2** (in CD_3OD)		**3** (in $CDCl_3$)		
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	position	δ_C, type	δ_H (J in Hz)
1	169.9, C		173.9, C		1		
2					2	176.8, C	
3	69.1, CH_2	5.28, s	70.7, CH_2	5.24, s	3	67.3, CH	4.52, dd (7.0, 7.0)
3a	153.4, C		149.9, C		4	38.5, CH_2	2.52, m, 2.68, m
4	111.6, CH	7.02, s	108.7, CH	6.89, s	5	78.4, CH	5.66, dd (7.0, 5.2)
5	167.1, C		161.8, C		1′	138.5, C	
6	120.4, C		136.6, C		2′, 6′	101.2, CH	6.49, s
7	129.6, CH	8.35, s	123.9, CH	7.85, s	3′, 5′	156.5, C	
7a	116.6, C		117.2, C		4′	110.8, C	
1′	204.4, C		66.1, CH	5.14, q (6.4)	OCH_3	56.5, CH_3	3.88, s
2′	26.8, CH_3	2.71, s	24.0, CH_3	1.42, d (6.4)			
5-OH		12.89, s					

suggested the presence of a hydroxy group at C-5. All the data allowed us to conclude that **1** was 6-acetyl-5-hydroxyisobenzofuran-1(3*H*)-one, and it was named erinaceolactone A.

Erinaceolactone A (**2**) was obtained as yellow crystals. Its molecular formula was determined as $C_{10}H_{10}O_4$ by HRESIMS (m/z 193.0480 $[M - H]^-$; calcd for $C_{10}H_9O_4$, 193.0501), indicating the presence of six degrees of unsaturation in the molecule. The NMR data of **2** were similar to those of **1** (Table 1). Comparison of the molecular formula of **2** with that of **1** and HMBC correlaions (H-1′/C-2′, 5, 6, 7; H-2′/C-1′, 6) indicated the presence of a hydroxy group in **2** instead of the ketone of the acetyl group in **1**. As a result, **2** was determined to be 5-hydroxy-6-(1-hydroxyethyl)isobenzofuran-1(3*H*)-one. The absolute configuration of **2** remains undetermined.

Erinaceolactone C (**3**) was obtained as white crystals. Its molecular formula was determined as $C_{12}H_{13}ClO_5$ by HRESIMS (m/z 295.0359 $[M + Na]^+$; calcd for $C_{12}H_{13}ClNaO_5$, 295.0349), suggesting the presence of six degrees of unsaturation in the molecule. The structure of **3** was elucidated by interpretation of NMR data, including DEPT, COSY, HMBC, and HMQC data (Figure 1). The DEPT experiment and the molecular formula indicated the presence of two methyls, a methylene, four methines, and five quaternary carbons. The complete assignment of all the protons and carbons was accomplished as shown in Table 1. The four-substituted symmetric phenyl moiety was elucidated by its NMR chemical shift [δ_C 138.5, 101.2, 156.5, 110.8; δ_H 6.49 (2H, s)] and HMBC correlations (H-2′/C-1′, 3′, 4′, 6′) (Figure 1 and Table 1). The position of the methoxy group was determined by the HMBC correlation from the protons to C-3′ and C-5′. The α-hydroxy-γ-lactone moiety was determined by the COSY (H-4/H-3, 5) and HMBC correlations (H-3/C-2, 4, 5; H-4/C-2, 3, 5; H-5/C-2, 3, 4) and the chemical shift of the H-3 methine [δ_C 67.3, δ_H 4.52 (2H, dd, J = 7.0, 7.0 Hz)]. The HMBC correlations (H-4/C-1′; H-5/C-1′, 2′, 6′; H-2′/C-5) indicated that the phenyl was attached at C-5 of the γ-lactone. The molecular formula and NMR chemical shift of C-4′ (δ_C 110.8) suggested that a chlorine was bound to C-4′. Confirmation of the planar structure and determination of its absolute configuration were performed by X-ray crystallography analysis (Figure 2). As a result, **3** was determined as (3*R*,5*S*)-5-(4-chloro-3,5-dimethoxyphenyl)-3-hydroxydihydrofuran-2(3*H*)-one.

Compounds **4** was identified as 2-(hydroxymethyl)-6-methyl-4*H*-pyran-4-one that had been synthesized.[18−20] However, this is the first time it has been reported as a natural product (Figure 3).

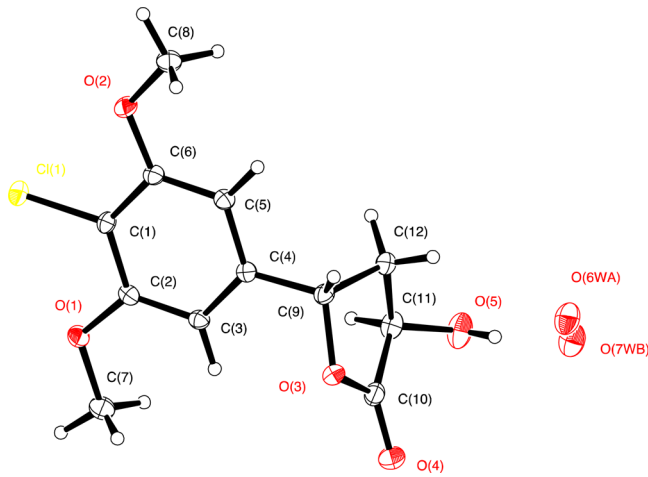

Figure 2. ORTEP drawing of erinaceolactone C (**3**) with ellipsoids at the 50% probability level. Hydrogen atoms are shown as small spheres of arbitrary radii.

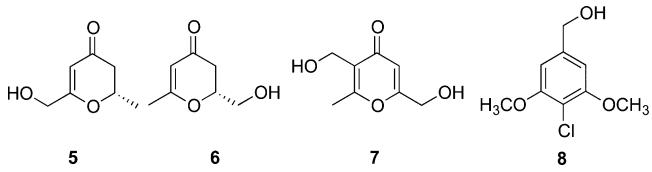

Figure 3. Structures of compounds **5**−**8**.

The effect of compounds **1** to **8** (compounds **5** to **8** had been already isolated from the same fungus[6,16,17]) on lettuce growth was examined (Figure 4). 2,4-Dichlorophenoxyacetic acid was used as a positive control, which inhibited the hypocotyl and root growth of lettuce dose-dependently. Compounds **1**, **7**, and **8** weakly inhibited the root growth of lettuce at 1 μmol/paper, while compounds **2**, **5**, and **6** showed an inhibition as low as 100 nmol/paper. As for the hypocotyl growth of lettuce, compounds **5** and **6** showed inhibition at 1 μmol/paper and 100 nmol/paper, respectively. Compound **3** exhibited no activity. Interestingly, compound **4** inhibited the growth of root and hypocotyl at lower doses (1 and 10 nmol/paper), but

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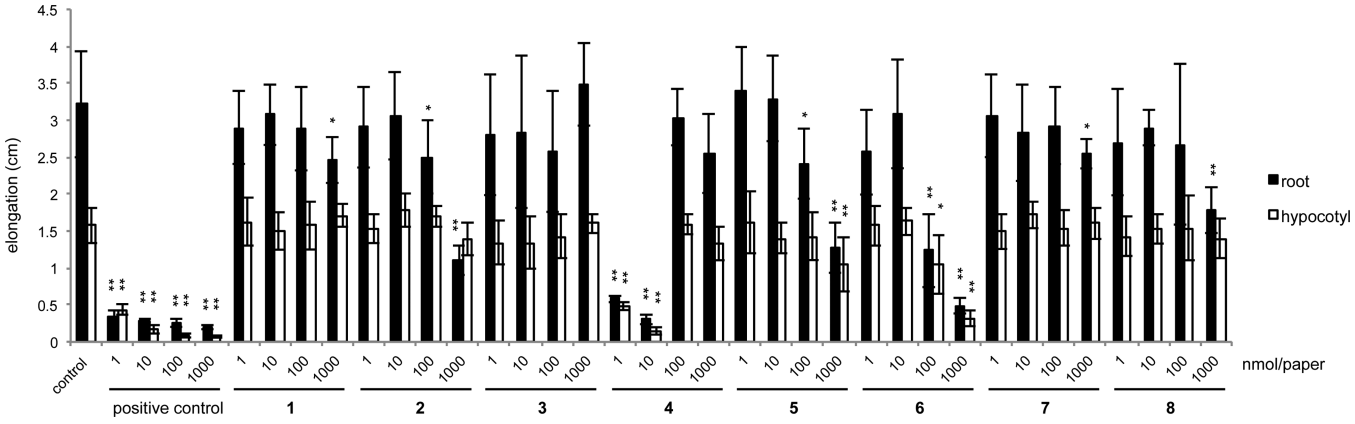

Figure 4. Effect of **1** to **8** on the growth of lettuce. Lettuce seedlings were treated with **1** to **8**. Respective length of growth compared with the control ± standard deviation (*p < 0.05, **p < 0.01 vs control, n = 7).

showed no activity at higher doses (100 nmol and 1 μmol/paper).

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H NMR spectra (one- and two-dimensional) were recorded on a Jeol lambda-500 spectrometer at 500 MHz, and ^{13}C NMR spectra were recorded on the same instrument at 125 MHz (Jeol, Tokyo, Japan). HRESIMS spectra were measured on a JMS-T100LC mass spectrometer (Jeol, Tokyo, Japan). A Jasco grating infrared spectrophotometer was used to record the IR spectra, and the specific rotation values were measured by a Jasco DIP-1000 polarimeter. HPLC separations were performed with a Jasco Gulliver system using reversed-phase HPLC columns (XBridge Phenyl, Waters, Milford, MA, USA; Phenyl, InertSustain, Tokyo, Japan). Silica gel plates (Merck F_{254}), ODS gel plates (Merck F_{254}), and silica gel 60 N (Kanto Chemical, Tokyo, Japan) were used for analytical TLC and for flash column chromatography, respectively.

Fungal Material. The strain of *Hericium erinaceus* was isolated from the fruiting body collected in Nagano Prefecture. Voucher material has been deposited in the research laboratory of Kubo Industry, Nagano Prefecture, Japan. Lettuce seeds (*Lactuca sativa* L. cv. Cisko; Takii Co., Ltd., Tokyo, Japan) were used in this study.

Incubation. The components of the culture medium were as follows: glucose 4%, peptone 0.3%, yeast extract 0.3%, KH_2PO_4 0.05%, and Na_2HPO_4 0.05% in distilled water. The culture medium was adjusted to pH 5.5. The aerated liquid culture was carried out in a 10 L jar fermenter containing 6 L of the medium and incubated at 25 °C for 14 days.

Extraction and Isolation. The culture broth of *H. erinaceus* (5 L) was filtered and then concentrated under reduced pressure. The concentrate was successively partitioned between *n*-hexane and water, and then EtOAc and water (2 L each, three times). The EtOAc-soluble part (3.52 g) was fractionated by silica gel flash column chromatography (EtOAc; 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% EtOAc/MeOH; and MeOH, 1 L each) to obtain 15 fractions (fractions 1−15), and fraction 3 (41.0 mg) was further separated by reversed-phase HPLC (XBridge Waters Phenyl, 40% MeOH) to afford **1** (0.7 mg). Fraction 4 (90.0 mg) was further separated by reversed-phase HPLC (Phenyl, 40% MeOH) to afford **2** (5.4 mg) and **3** (3.3 mg). Fraction 8 (116 mg) was further separated by reverse-phase HPLC (XBridge Waters Phenyl, 20% MeOH) to afford **4** (6.2 mg).

Erinaceolactone ***1:*** white crystals; IR (neat) 2921, 1789, 1651 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS m/z 191 $[M - H]^-$; HRESIMS m/z 191.0321 $[M - H]^-$ (calcd for $C_{10}H_7O_4$, 191.0344).

Erinaceolactone ***2:*** yellow crystals; $[\alpha]^{22}_D$ −26 (c 0.54, MeOH); IR (neat) 3383, 1731 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS m/z 193 $[M - H]^-$; HRESIMS m/z 193.0480 $[M - H]^-$ (calcd for $C_{10}H_9O_4$, 193.0501).

Erinaceolactone ***3:*** white crystals; $[\alpha]^{22}_D$ +6.4 (c 0.51, MeOH); IR (neat) 3444, 1783 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS m/z 295 $[M + Na]^+$; HRESIMS m/z 295.0359 $[M + Na]^+$ (calcd for $C_{12}H_{13}ClNaO_5$, 295.0349).

X-ray Crystallography Analysis. **3** was crystallized in MeOH. The single crystal was mounted on a loop for a measurement at 173 K. X-ray diffraction data were collected by a Rigaku VariMax Saturn (Mo Kα radiation, 1.2 kW rotating anode). For the measurement, 18 preliminary data frames were measured at 0.5° increments of ω, to assess the crystal quality and preliminary unit cell parameters. The intensity images were also measured at 0.5° intervals of ω. The intensity images were integrated using the CrystalClear program package, and the empirical absorption correction was applied for the data. The structures were solved by direct methods, the SIR92 program, and refined using the SHELXL-97 program. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1011415. The data can be obtained free of charge via www.ccdc.cam.ac.uk/products/csd/request.

Crystal data of **3**: $C_{12}H_{15}ClO_6$ (fw = 290.70); monoclinic, space group $P2_1$ (No. 4); a = 4.6451(3) Å, b = 11.3065(8) Å, c = 12.3885(7) Å, β = 94.458(2)°; V = 648.67(8) Å^3, T = 173(2) K, Z = 2, D_c = 1.488 g/cm^3, $F(000)$ = 304.00, reflections collected/unique reflections/parameters refined: 10 284/2931/181, final R_1 = 0.0555 ($I > 2\sigma(I)$), wR_2 = 0.0764 (all data), S = 1.022. Flack parameter = −0.08(7).

Bioassay. Lettuce seeds were put on filter paper (Advantec No. 2, ⌀ 55 mm; Toyo Roshi Kaisha, Japan), soaked in distilled water in a Petri dish (⌀ 60 × 20 mm), and incubated in a growth chamber in the dark at 25 °C for 1 day. Each sample was dissolved in 1 mL of methanol (1, 10^{-1}, 10^{-2}, and 10^{-3} μmol/mL) and then poured on filter paper (⌀ 55 mm) in a Petri dish (⌀ 60 × 20 mm). After the sample-loaded paper had been air-dried, 1 mL of distilled water was poured on the sample-loaded paper or intact filter paper (control). The preincubated lettuce seedlings (n = 9 in each Petri dish) were transferred onto the sample-loaded filter paper or control filter paper and incubated in a growth chamber in the dark at 25 °C for 3 days. The lengths of the hypocotyl and the root were measured using a ruler.

ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, and 2D NMR spectra of erinaceolactones A to C (**1** to **3**) and X-ray crystallographic data for **3** are given. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +81-54-238-4885. E-mail: achkawa@ipc.shizuoka.ac.jp.

Notes

The authors declare no competing financial interest.

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