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NOTE

Erinacene D, a new aromatic compound from *Hericium erinaceum*

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Fruiting bodies of *Hericium erinaceum* (Hericiaceae) are known as a traditional edible mushroom. The main constituents of *H. erinaceum*, which include polysaccharides, aromatic compounds and fatty acids, have been investigated previously, and their bioactive effects are well known.[1–4] For example, hericenone A, an aromatic compound, causes significant cytotoxicity in HeLa cells.[5] Isohericerin and isohericenone significantly reduce the viability of A549, SK-OV-3, SK-MEL-2 and HCT-15 cell lines.[6] In addition, hericenones C–E stimulated the synthesis of nerve growth factor (NGF) *in vitro*.[2] However, few reports have described their nuclear factor kappa B (NF-κB) inhibition.

During the search for a NF-κB inhibitor from natural products, we determined that the $CHCl_3$ fraction of *H. erinaceum* showed significant effects on tumor necrosis factor alpha (TNF-α)–induced NF-κB inhibitory activity in human keratinocyte (HaCaT) cells. Bioassay-guided isolation of the $CHCl_3$ fraction resulted in a new aromatic compound named erinacene D (**1**), together with six known compounds (Figure 1). On the basis of spectral data and chemical evidence, which were consistent with previous reports, the structures of the known compounds were identified as hericene A (**2**),[7] hericene B (**3**),[7] hericene C (**4**),[7] hericenone C (**5**),[8] hericenone D (**6**)[8] and hericenone E (**7**).[8] In this study, we report the structural elucidation of erinacene D (**1**) and NF-κB inhibitory activity of compounds **1**–**7**.

NF-κB is the most extensively studied transcription factor in the immune system. It plays an important role in the transcriptional regulation of numerous cytokines and adhesion molecules. Known inducers of NF-κB activity are highly variable and include reactive oxygen species, TNF-α, interleukin (IL)-1β, bacterial lipopolysaccharide, isoproterenol, cocaine and ionizing radiation. The NF-κB activation causes transcription at the κB site, which is involved in many diseases, including inflammatory disorders and cancer. Hence, the inhibition of NF-κB signaling has become a therapeutic target for the treatment of inflammatory diseases and cancer.[9–11]

Dried fruiting bodies of *H. erinaceum* were purchased from an herbal market, kumsan, Chungnam Province, Korea, in August 2013. Its scientific name was determined by one of authors (Professor Young Ho Kim). A voucher specimen (CNU 13110) was deposited

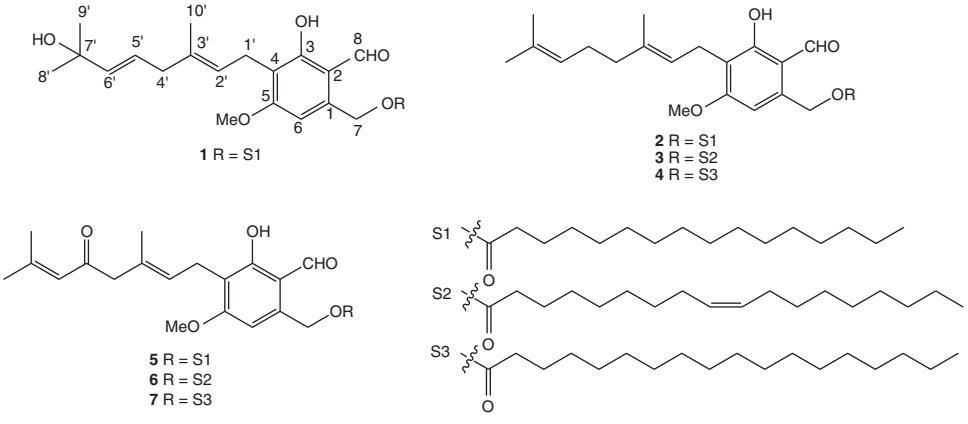

Figure 1 Structures of compounds **1**–**7** from *H. erinaceum*.

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at the Herbarium College of Pharmacy, Chungnam National University. Dried fruiting bodies (2.5 kg) of *H. erinaceum* were extracted thrice with MeOH (5 liters) under reflux. The MeOH extract (320.0 g) of *H. erinaceum* was suspended in water and partitioned with $CHCl_3$, yielding $CHCl_3$ (90.0 g) and water (220.0 g) fractions. These fractions were evaluated for NF-κB inhibitory activity using an NF-κB-luciferase assay.[12] Among them, the $CHCl_3$ fraction showed the strongest activity. Thus, we selected the $CHCl_3$ fraction for isolation. The $CHCl_3$ fraction (90.0 g) was subjected to silica gel (5.0 × 30 cm) column chromatography with a gradient of *n*-hexane-EtOAc-MeOH (25:1:0, 9:1:0, 5:1:0, 2.5:1:0, 1:1:0.1, 1:1:0.3, 0.5:1:0.5; 0:0:1; 4 l for each step) to give 8 fractions (Fr. 1A–1H). The fraction 1C was separated using a silica gel (2.0 × 80 cm) column chromatography with a gradient of *n*-hexane-EtOAc (20:1–10:1, 10 liters) to give 11 subfractions (Fr. 1C-1–1C-11). The fraction 1C–3 was subjected to an YMC (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (3:3:1, 5:5:1, 8:8:1, 10:10:1; 800 ml for each step) elution solvent to give compounds **1** (210.0 mg; 0.0084%) and **5** (8.0 mg; 0.0004%). The fraction 1C-5 was subjected to an YMC (2.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (5:5:1, 8:8:1, 10:10:1; 1.0 l for each step) elution solvent to give compounds **6** (13.0 mg; 0.0005%) and **7** (21.0 mg; 0.0008%). The fraction 1A was separated using a silica gel (3.0 × 80 cm) column chromatography with a gradient of *n*-hexane-EtOAc (1:0 to 6:1, 10 l) to give 9 subfractions (Fr. 1A-1–1A-9). The fraction 1A-7 was subjected to an YMC (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (10:10:1, 15:15:1; 2.0 liters for each step) elution solvent to give compound **2** (102.0 mg; 0.0041%). The fraction 1A-9 was subjected to an YMC (2.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (8:8:1, 10:10:1, 15:15:1; 1.5 liters for each step) elution solvent to give compounds **3** (36.0 mg; 0.0014%) and **4** (9.0 mg; 0.0004%).

Table 1 The ^{1}H- and ^{13}C-NMR data of compound 1

Position	δ_C	δ_H
1	138.5	
2	112.9	
3	162.9	
4	117.8	
5	163.5	
6	105.6	6.45 (s)
7	63.0	5.25 (s)
8	193.1	10.03 (s)
1′	21.5	3.27 (d, 7.0)
2′	122.3	5.13 (t, 7.0)
3′	134.4	
4′	42.5	2.58 (d, 6.4)
5′	125.4	4.15 (m)
6′	139.3	4.09 (d, 15.9)
7′	70.8	
8′	29.7	1.23 (s)
9′	29.7	1.23 (s)
10′	16.1	1.67 (s)
1″	173.3	
2″	34.3	2.26 (t, 7.5)
3″	24.9	1.53 (m)
4″–13″	29.2–29.8	1.1–1.27 (m)
14″	32.0	1.55 (m)
15″	22.7	1.1–1.27 (m)
16″	14.2	0.80 (t, 7.5)
5-OMe	56.0	3.84 (s)

Abbrevations: d, doublet; m, multiplet; s, singlet; t, triplet.
NMR data were obtained in 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$, and values in *n* parentheses are coupling constants in Hz.

Compound **1** was isolated as a colorless gum. The molecular formula was established as $C_{35}H_{56}O_6$ by HR-ESI-MS (*m/z* 595.3969 ($[M+Na]^+$); calcd 595.3975), corresponding to six degrees of unsaturation. The IR absorption bands at 3421 and 1730 cm^{-1} implied the presence of hydroxy and ester groups, respectively. The ^{1}H-NMR spectrum of compound **1** (Table 1) revealed a singlet at δ_H 6.45 (H-6), belonging to an aromatic ring, three olefinic proton signals at δ_H 4.09–5.13 (H-2′, 5′ and 6′), two methylene group signals at δ_H 2.58 (d, J = 6.4 Hz, H-4′) and 3.27 (d, J = 7.0 Hz, H-1′), and three methyl group signals at δ_H 1.23 (s, H-8′, 9′) and 1.67 (s, H-10′), belonging to a side chain of an aromatic ring. Other signals such as an aldehyde proton at δ_H 10.03 (s, H-8), a methoxy group at δ_H 3.84 (s) and a palmitoyl group at δ_H 0.80–2.26 (H-2″–16″) were also observed. The ^{13}C-NMR spectrum (Table 1) showed two characteristic carbonyl groups at δ_C 173.3 (C-1″) and 193.1 (C-8). Aromatic ring signals at δ_C 105.6 (C-6), 112.9 (C-2), 117.8 (C-4), 138.5 (C-1), 162.9 (C-3) and 163.5 (C-5); side chain signals including three

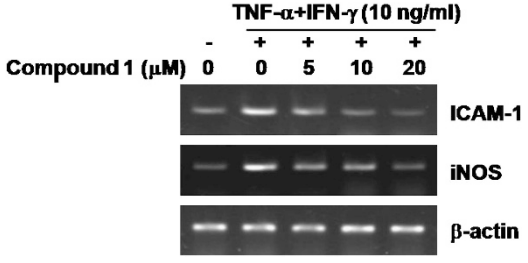

Figure 3 Effects of compound **1** on iNOS and ICAM-1 mRNA expression in HaCaT cells. −0: cells were treated without 10 ng ml^{-1} TNF-α + IFN-γ and compound **1**; + 0: cells were treated with 10 ng ml^{-1} TNFα + IFN-γ only; + 5, 10, 20: cells were treated with 10 ng ml^{-1} TNF-α and compound **1**. IFN-γ, interferon gamma; TNFα, tumor necrosis factor alpha.

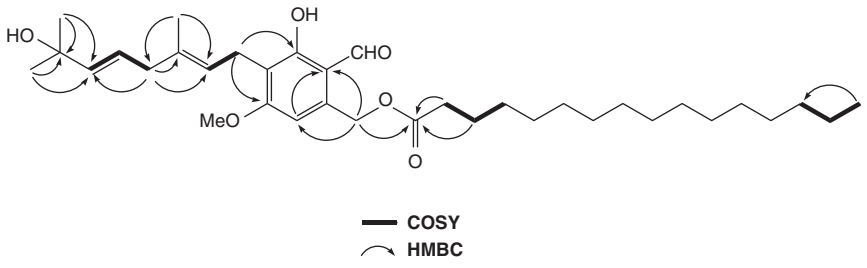

Figure 2 ^{1}H–^{1}H COSY and key HMBCs of compound **1**.

olefinic carbons at δ_C 122.3 (C-2′), 125.4 (C-5′), 134.4(C-3′) and 139.3 (C-6′), three methyl groups at δ_C 16.1 (C-10′) and 29.7 (C-8′, 9′), two methylene groups at δ_C 21.5 (C-1′) and 42.5 (C-4′), and a quaternary carbon at δ_C 70.8 (C-7′); and palmitoyl group signals at δ_C 14.2–34.3 (C-2″–16″) were also observed. Both ^{1}H- and ^{13}C-NMR signals were similar to hericene A (**2**), except for the positions of double bonds and a quaternary carbon. The HMBC spectrum showed correlations between H-9′, 10′ (δ_H 1.23)/C-6′ (δ_C 139.3) and 7′ (δ_C 70.8), indicated that the hydroxy group was located at C-7′. Correlations between H-4′ (δ_H 2.58)/C-2′ (δ_C 122.3), 3′ (δ_C 134.4), 5′ (δ_C 125.4) and 6′ (δ_C 139.3), as well as results from the ^{1}H–^{1}H COSY spectrum, suggest that the double bonds were located at C-2′, 3′ and C-5′, 6′ (Figure 2). Thus, the structure of compound **1** was assigned (Table 1) and named erinacene D.

The NF-κB inhibitory activity of compounds **1–7** was evaluated through the inhibition of TNF-α–induced NF-κB luciferase reporter assay.[12] Cell viability was measured using a cell-counting kit (*CCK*)-*8*. The results showed that compounds **1–4** did not exhibit significant cytotoxicity in HaCaT cells at the concentrations tested, whereas compounds **5–7** showed moderate cytotoxicity (see Supplementary Material).

HaCaT cells were treated with 10 ng ml^{-1} TNF-α and showed increased transcriptional activity compared to untreated cells. The transfected HepG2 cells were pretreated with compounds at various concentrations (0.1, 1, and 10 μM), followed by stimulation with TNF-α. Pyrrolidine dithiocarbamate was used as a positive control. Results showed that compound **1** significantly inhibited TNF-α–induced NF-κB transcriptional activity in a dose-dependent manner, with an IC_{50} value of 9.7 μM, whereas compounds **2–4** showed moderate activity, with IC_{50} values ranging from 17.2 to 38.6 μM. TNF-α plays important roles in inflammatory skin diseases such as psoriasis.[13] Inflammatory responses to TNF-α can be mediated through stimulation of the IL-1 expression and via distal proinflammatory cytokines, such as IL-2, IL-6, IL-10 and interferon gamma (IFN-γ).[14] Expression of these inflammatory cytokines is mainly mediated by the NF-κB or MAPK pathway.[15] Compound **1** was investigated for TNF-α– and IFN-γ–induced transcriptional inhibitory effects on iNOS and ICAM-1 gene expression (Figure 3). The results showed that compound **1** significantly inhibited the induction of both iNOS and ICAM-1 mRNA in a concentration-dependent manner, indicating that this compound reduced the transcription of these genes. The housekeeping protein β-actin was unchanged by the presence of compound **1** at the same concentration. These data show that compounds **1–4** isolated from *H. erinaceum* suppress TNFα–induced NF-κB transcriptional activity. This study shows that these compounds can be considered as contributors to NF-κB inhibitory activity of *H. erinaceum*.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)