Sterols, aromatic compounds, and cerebrosides from the *Hericium erinaceus* fruiting body

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# Abstract

Chemical investigation of the methanolic extract from the fruiting bodies of *Hericium erinaceus*, led to the isolation of thirty-one compounds including thirty-five ergostane-type sterols (1–35), fourteen aromatic compounds (36–49), and two cerebrosides (50 and 51). Their structures were identified based on spectroscopic analyses and by comparison of their spectral data with those reported in literature. This is the first comprehensive low-polarity chemical investigation of *H. erinaceus*. Thirty-one of the compounds (6–8, 11–35, 39, 41, and 49) were isolated for the first time from the genus *Hericium* and the family Hericiaceae. The chemotaxonomic relationship between *H. erinaceus* and other *Hericium* species was also discussed.

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# 1. Subject and source

*Hericium erinaceus* (Bull.) Pers., which belongs to the family Hericiaceae, is an edible medicinal fungus found in China, India, Korea, and Japan. The mushroom is called “houtou”, which means “monkey head mushroom”, in China because of its shape. It is popular because of its culinary value and its application as an herbal medicine. (Mizuno et al., 1992). The reported health-promoting properties of the mushroom fruiting bodies, mycelia, and the pure bioactive compounds include anti-hypertensive, anti-hyperlipidemic, anti-senescence, antibiotic, anti-carcinogenic, anti-diabetic, anti-fatigue, cardioprotective, hepatoprotective, nephroprotective, and neuroprotective properties and improvement of anxiety, cognitive function, and depression (Friedman, 2015). Dried fruiting bodies of *H. erinaceus* were purchased from the herbal market in Kumsan, Chungnam Province, Korea in August 2013 (identified by Prof. Young Ho Kim). A voucher specimen (CNU 13110) was deposited at the Herbarium of the College of Pharmacy at Chungnam National University.

# 2. Previous work

Previous phytochemical investigations on *H. erinaceus* have revealed the presence of aromatic compounds such as hericerins, erinacerins, and erinaceolactones (Yaoita et al., 2005; Ueda et al., 2008); diterpenoids such as erinacines and their
glycosides (Kenmoku et al., 2001; Lee et al., 2000; Kawagishi et al., 2006); sterols such as ergosterol and ergosterol peroxide (Zan et al., 2015); alkaloids (Li et al., 2014a; Lu et al., 2014); and polysaccharides (Mizuno et al., 1992). Our recent study on *H. erinaceus* revealed the presence of three new aromatic compounds (Li et al., 2014b, 2015a) and ten new sterols (Li et al., 2014c, 2015b), indicating that this species could be a lucrative resource for producing novel constituents and thus, its chemotaxonomic profile needs to be updated.

3. Present study

Dried fruiting bodies (2.5 kg) of *H. erinaceus* were extracted with MeOH (5 L × 3) under reflux conditions. The MeOH extract (320.0 g) of *H. erinaceus* was suspended in water and partitioned with CHCl₃, yielding CHCl₃ (90.0 g) and water (220.0 g) fractions (Fig. 1). The CHCl₃ fraction (90.0 g) was separated through silica gel column (5.0 × 30 cm) 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; 4 L for each step) to yield eight fractions (Fr. 1A–1H).

Fraction 1A (8.2 g) was separated using silica gel (3.0 × 80 cm) column chromatography with a gradient of n-hexane-EtOAc (20:1 to 10:1, 10 L) to yield 11 sub-fractions (Fr. 1A-1–1A-11). Fraction 1A-7 (950.0 mg) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (10:10:1, 15:15:1; 2.0 L for each step) elution solvent to give compound 44 (102.0 mg). Fraction 1A-8 (1.8 g) was subjected to a C₁₈ (2.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (8:8:1, 10:10:1; 3.0 L for each step) elution solvent to yield compounds 3 (65.0 mg) and 4 (102.0 mg). Fraction 1A-9 (360.0 mg) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (8:8:1, 10:10:1, 15:15:1; 1.5 L for each step) elution solvent to give compounds 43 (36.0 mg) and 45 (9.0 mg).

Fraction 1C (12.5 g) was separated using silica gel (3.5 × 80 cm) column chromatography with a gradient of n-hexane-EtOAc (20:1 to 10:1, 10 L) to yield 11 sub-fractions (Fr. 1C-1–1C-11). Fraction 1C-3 (1.4 g) was subjected to a C₁₈ (1.5 × 80 cm) column chromatography with a MeOH-acetone-H₂O (3:3:1, 5:5:1, 8:8:1, 10:10:1; 800 mL for each step) elution solvent to give compounds 41 (210.0 mg) and 46 (8.0 mg). Fraction 1C-5 (520.0 mg) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (5:5:1, 8:8:1, 10:10:1; 1.0 L for each step) elution solvent to give compounds 47 (13.0 mg) and 48 (21.0 mg). Fraction 1C-6 (1.0 g) was subjected to a C₁₈ (1.5 × 80 cm) column chromatography with a MeOH-acetone-H₂O (3:3:1, 5:5:1, 8:8:1; 1.2 L for each step) elution solvent to give compounds 13 (7.0 mg), 17 (36.0 mg) and 18 (53.0 mg). Fraction 1C-7 (875.0 mg) was subjected to C₁₈ (2.0 × 80 cm) column chromatography with a CHCl₃-acetone (7:1, 9:1, 12:1; 1 L for each step) elution solvent to yield compounds 12 (11.0 mg), 29 (11.0 mg), 30 (8.0 mg), and 31 (3.0 mg). Fraction 1C-8 (950.0 mg) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (3:3:1, 4:4:1, 6:6:1, 9:9:1; 2.0 L for each step) elution solvent to yield compounds 6 (21.0 mg), 8 (7.0 mg), 22 (22.0 mg), 33 (7.0 mg), and 34 (16.0 mg). Fraction 1C-9 (1.0 g) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (3:3:1, 4:4:1, 6:6:1; 1.2 L for each step) elution solvent to give compounds 37 (86.2 mg) and 40 (43.6 mg).

Fraction 1C-11 (1.3 g) was subjected to a C₁₈ (1.5 × 80 cm) column chromatography with a MeOH-acetone-H₂O (3:3:1, 5:5:1, 7:7:1; 1.5 L for each step) elution solvent to yield compounds 24 (29.0 mg), 26 (9.6 mg), and 35 (6.0 mg).

Fraction 1D (11.6 g) was separated using silica gel (3.0 × 80 cm) column chromatography with a gradient of n-hexane-EtOAc (8:1 to 1:5:1, 20 L) to yield 14 sub-fractions (Fr. 1D-1–1D-14). Fraction 1D-4 (127.0 mg) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-H₂O (3.8:1; 1.0 L) elution solvent to give compound 39 (31.2 mg). Fraction 1D-6 (210.5 mg) was subjected to a C₁₈ (1.5 × 80 cm) column chromatography with a MeOH-acetone-H₂O (1.5:1:1, 3:1:5:1, 6:3:5:1, 9:5:1; 1.0 L for each step) elution solvent to give compound 42 (21.4 mg). Fraction 1D-7 (2.0 g) was subjected to silica gel (1.5 × 80 cm) column chromatography with a CHCl₃-acetone (20:1; 1 L) elution solvent to yield compounds 5 (450.0 mg), 16 (17.0 mg), and 28 (5.6 mg). Fraction 1D-8 (632.0 mg) was subjected to a C₁₈ (2.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (1:1:1; 2:2:1; 3:3:1, 5:5:1, 8:8:1, 10:10:1; 3 L for each step) elution solvent to yield eight sub-fractions (Fr. 1D-9-A–1D-9-H). Fraction 1D-9-D (112.0 mg) was subjected to silica gel (1.0 × 80 cm) column chromatography with a CHCl₃-acetone (7:1, 8:1; 1 L for each step) elution solvent to yield compounds 19 (90.0 mg) and 20 (110.0 mg). Fraction 1D-9-E (350.0 mg) was subjected to silica gel (1.5 × 80 cm) column chromatography with a CHCl₃-acetone (7:1, 9:1, 12:1; 1 L for each step) elution solvent to yield compounds 10 (12.0 mg), 11 (7.0 mg), and 32 (22.0 mg). Fraction 1D-9-F (4.0 g) was subjected to silica gel (2.0 × 80 cm) column chromatography with a CHCl₃-acetone (12:1; 1 L) elution solvent to yield compounds 1 (2.8 g), 25 (20.0 mg), and 27 (4.0 mg). Fraction 1D-9-G (102.0 mg) was subjected to silica gel (1.0 × 80 cm) column chromatography with a n-hexane-EtOAc-acetone (3:1:0.15; 750 mL) to give compound 36 (13.4 mg). Fraction 1D-10 (2.2 g) was subjected to a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (0.6:1:2:1; 2:2:1; 4:2:1; 3:1:5:1; 5:10:1; 1 L for each step) elution solvent to give 7 sub-fractions (Fr. 1D-11-A–1D-11-G). The fraction 1D-11-D (45.0 mg) was subjected to silica gel (1.0 × 80 cm) column chromatography with a CHCl₃-acetone (10:1, 5:1; 3:1; 500 mL for each step) elution solvent to give compound 14 (12.0 mg).

Fraction 1E (1.1 g) was separated using a C₁₈ (1.0 × 80 cm) column chromatography with a MeOH-acetone-H₂O (1:1, 3:1, 5:1, 7:5:1, 9:1, 15:1; 300 mL for each step) elution solvent to give compounds 38 (11.8 mg), 49 (7.3 mg), 50 (325.7 mg), and 51 (126.8 mg).

The structures of the isolated metabolites (compounds 1–51, Fig. 2) were identified on the basis of spectroscopic and spectrometric data and by comparison of these data with values in the literature. The fatty acid residues of compounds 3, 4, 12,
**Fig. 1.** Isolation scheme of compounds 1-51.

- **H. erinaceus** (2.5 kg)
  - MeOH (5 L × 3)
  - MeOH extract (320.0 g)
  - H₂O added (2.5 L)
  - H₂O solution
    - partitioned with CHCl₃ (2.5 L × 3 for each step)
  - CHCl₃ ext. (960.0 g)
  - NP CC IE:E:M (25:1:0-0.5:1:0)

**Fractions (A-H)**

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<th>1D</th>
<th>1E</th>
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<td>NP CC</td>
<td>NP CC</td>
<td>NP CC</td>
<td>RP CC</td>
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**Sub-fractions**

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<th>A8</th>
<th>A9</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C11</th>
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<tbody>
<tr>
<td>45</td>
<td>47 (13.0 mg)</td>
<td>12 (11.0 mg)</td>
<td>29 (11.0 mg)</td>
<td>40 (41.6 mg)</td>
<td>4 (65.0 mg)</td>
<td>31 (31.0 mg)</td>
<td>28 (5.6 mg)</td>
<td>16 (17.0 mg)</td>
<td>14 (12.0 mg)</td>
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</tr>
<tr>
<td>13</td>
<td>3 (7.0 mg)</td>
<td>6 (21.0 mg)</td>
<td>24 (29.0 mg)</td>
<td>33 (7.0 mg)</td>
<td>19 (9.0 mg)</td>
<td>1 (2.8 g)</td>
<td>2 (487.0 mg)</td>
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<tr>
<td>16</td>
<td>11 (7.0 mg)</td>
<td>20 (11.0 mg)</td>
<td>22 (22.0 mg)</td>
<td>34 (16.0 mg)</td>
<td>49 (7.3 mg)</td>
<td>9 (19.0 mg)</td>
<td>7 (42.0 mg)</td>
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*CC: Column Chromatography; NP: Normal Phase (Silica gel); RP: Reversed Phase (C₄); A: Acetone; C: CHCl₃; D: CH₂Cl₂; EA: EtOAc; H: n-hexane; M: MeOH; W: H₂O*
29–31, 33–35, 41, and 43–48 were confirmed using GC/MS analysis after enzymatic hydrolysis. The compounds were identified as ergosterol (1; Notaro et al., 1992a), ergosterol-3-O-β-D-glucopyranoside (2; Notaro et al., 1992a), ergosterol palmitate (3; Panthama et al., 2011), ergosterol stearate (4; Panthama et al., 2011), ergosterol peroxide (5; Notaro et al., 1992a), (22E)-ergosta-4,6,8,22-tetraen-3-one (6; Notaro et al., 1992a), 22E,24R)-ergosta-7,22-diene-3β,5α,6β,9α-tetraol (7; Ohnuma et al., 2000), fomentarol A (8; Zang et al., 2013), cerevisterol (9; Luo et al., 2006), blazein (10; Luo et al., 2006), 3β,5α-dihydroxy-6β-acetoxy-ergosta-7,22-diene (11; Yaotia et al., 2002), erinarol C (12; Li et al., 2014c), (3β,5α,6β,22E) ergosta-6,8(14),22-triene-3,5-diol (13; Li et al., 2015b), erinarol H (14; Li et al., 2015b), erinarol I (15; Li et al., 2015b), erinarol A (17; Li et al., 2014c), erinarol B (18; Li et al., 2014c), topsentisterol E1 (19; Luo et al., 2006), citreone-17α-thrasteroid (20; Nakada and Yamamura, 2000), (3β,7α,22E) ergosta-8(14),22-diene-3,7-diol (21; Notaro et al., 1992b), 7-ketobrassicasterol (22; Notaro et al., 1992a), (3β,22E) ergosta-5,8(14),22-triene-7-one (23; Lin et al., 1999), 5α,6α-epoxy-3β-hydroxy-ergosta-22-ene-7-one (24; Lee et al., 2008), 5α,6α-epoxy-(22E,24R)-ergosta-8(14),22-diene-3β,7β-diol (25; Ishizuka et al., 1997), 5α,6α-epoxy-(22E,24R)-ergosta-7,22-diene-3β,7β-diol (26; Bok et al., 1999), 5α,6α,8α-diepoxo-(22E,24R)-ergosta-22-ene-3β,7α-diol (27; Yaotia et al., 1999), 5α,6α,8α,9α-diepoxo-(22E,24R)-ergosta-22-ene-3β,7β-diol (28; Yaita et al., 1999), (3β,5α,6α,22E) ergosta-7,22-diene-3,5,6-triol 6-palmitate (29; Gao et al., 2007), (3β,5α,6α,22E) ergosta-7,22-diene-3,5,6-triol 6-oleate (30; Gao et al., 2007), (3β,5α,6α,22E)-ergosta-7,22-diene-3,5,6-triol 6-linoleate (31; Gao et al., 2007), (22E)-ergosta-7,22-diene-3β,5α,6β-triol (32; Ohnuma et al., 2000), erinarol D (33; Li et al., 2014c), erinarol E (34; Li et al., 2014c), erinarol F (35; Li et al., 2014c), isohericerin (36; Miyazawa et al., 2012), hericene A (37; Kobayashi et al., 2012), N-De phenylethyl isohericin (38; Miyazawa et al., 2012), isohericene A (39; Li et al., 2015a), hericene J (40; Ueda et al., 2008), ericinacene D (41; Li et al., 2014c), 4-[3',7'-dimethyl-2',6'-octadienyl]-2-formyl-3-hydroxy-5-methoxybenzylalcohol (42; Miyazawa et al., 2012), hericene A (43; Arnone et al., 1994), hericene B (44; Arnone et al., 1994), hericene C (45; Arnone et al., 1994), hericene C (46; Kawagishi et al., 1991), hericene D (47; Kawagishi et al., 1991), hericene E (48; Kawagishi et al., 1991), hericene A (49; Li et al., 2015a), cerebroside B (50; Lee et al., 2015), and cerebroside D (51; Lee et al., 2015).

4. Chemotaxonomic significance

The present study reports the isolation and structural elucidation of fifty-one compounds from the fruiting bodies of H. erinaceus, which can be divided into thirty-five ergostane-type sterols (1–35), fourteen aromatic compounds (36–49), and two cerebrosides (50 and 51). To our knowledge, this is the first comprehensive low-polarity chemical investigation of H. erinaceus.

Sterols are common components of most mushrooms and fungi, especially compounds 1–5, which are the primary constituents of H. erinaceus. Previous studies on H. erinaceus have focused on diterpenoids and aromatic compounds, but sterols have not been identified. Among the thirty-five isolated sterols, twenty-eight compounds (6–8, and 11–35) were isolated for the first time from the genus Hericium and the family Hericaceae. In addition, the compounds 19, 20, 23, 24, and 29–31 were isolated from mushrooms for the first time; they have been previously isolated from Aspergillus awamori (Gao et al., 2007) and the marine sponge Topsisia sp. (Luo et al., 2006). Compounds 9 and 10 have been reported previously from this mushroom (Lu et al., 2014), as well as from other species of the genus Hericium, for example H. coralloides (Zhang et al., 2012). These two compounds can be considered as characteristic sterols of the genus Hericium. Interestingly, compounds 6 and 25, have only been reported from Pleuratus ostreatus (Chobó et al., 1997) and Pleuratus eryngii (Kikuchi et al., 2016), which belong to the family Pleuraceae. Compounds 7, 21, and 22, have only been reported from Agaricus subrubescens (Kawagishi et al., 1988) and Chlorophyllum molybdates (Yoshikawa et al., 2001), which belong to the family Agaricaceae. Compounds 8 and 32 were found for the first time in this mushroom, but were previously reported from Fomes fomentarius (Zang et al., 2013), which belongs to the family Polyporaceae. The occurrence of these special ergostane-type sterols in species from these families implies they share similar biochemical pathways and thus, there might be a close relationship among the families Hericiaceae, Pleuraceae, Agaricaceae, and Polyporaceae. Sterols can be found in many fungal species, but, the isolation of compounds 1–35 from H. erinaceus might be of systematic importance.

Previous chemical investigations of H. erinaceus have revealed a series of sphingolipids, such as cerebroside B, cerebroside D, and cerebroside E (Lee et al., 2015). This study reports two of sphingolipids, cerebroside B (50) and cerebroside D (51), which are only found in H. erinaceus in the family Hericaceae (Lee et al., 2015). Thus, the presence of these two cerebrosides might serve as a chemotaxonomic marker of H. erinaceus.

Aromatic compounds such as isoindoline alkaloids, isobenzofuranones and related derivatives are the characteristic metabolites from genus Hericium. In this paper, four isoindoline alkaloids (36–38 and 49) were isolated from H. erinaceus. Isohericerin (36) is a primary component previously isolated only from this mushroom, along with hericerin A (49; Li et al., 2015a). Hericene (37) and N-De phenylethyl isohericin (38) were previously isolated from H. erinaceus and H. coralloides only (Zhang et al., 2012; Wittstein et al., 2016); thus, it can be confirmed that H. erinaceus is intimately related to H. coralloides. Among isobenzofuranones and its related derivatives (39–48), isohericinone J (39) and ericinacene D (41) were obtained from the family Hericaceae for the first time in this study (Li et al., 2014b, 2015a), whereas 4-[3',7'-dimethyl-2',6'-octadienyl]-2-formyl-3-hydroxy-5-methoxybenzylalcohol (42), hericene A (43), hericene C (46), hericene D (47), and hericene E (48) have not been reported in any species of the genus Hericium. These compounds could be valuable markers at the infrageneric level of the genus Hericium, as well as the family Hericaceae. Hericene J (40), hericene B (44), and hericene C (45) were also previously reported from cerebrosides alone (Zhang et al., 2012), further indicating a close relationship.
Fig. 2. The chemical structures of compounds 1–51 from *H. erinaceus*. 
between these two species. These evidences suggest that further studies on *H. erinaceus* will certainly prove very useful to clarify the phylogenetic relationships in the genus *Hericium*, as well as in the family Hericaceae.

This is the first comprehensive study of the low-polarity chemical constituents of *H. erinaceus*, which indicates that *H. erinaceus* shares some compounds with other species of the genus *Hericium*, such as *H. coralloides*, but the composition of most compounds in this species differs from others. From a chemotaxonomic perspective, some isoidolindle alkaloids and isobenzofuranones as well as a number of sterols, may be recognized as markers for the genus *Hericium*. This study enriched our knowledge of an edible mushroom of the family Hericaceae.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.12.011.

References