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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 fifty-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.

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-hyperlipodemic, 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

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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 $CHCl_3$, yielding $CHCl_3$ (90.0 g) and water (220.0 g) fractions (Fig. 1). The $CHCl_3$ 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).

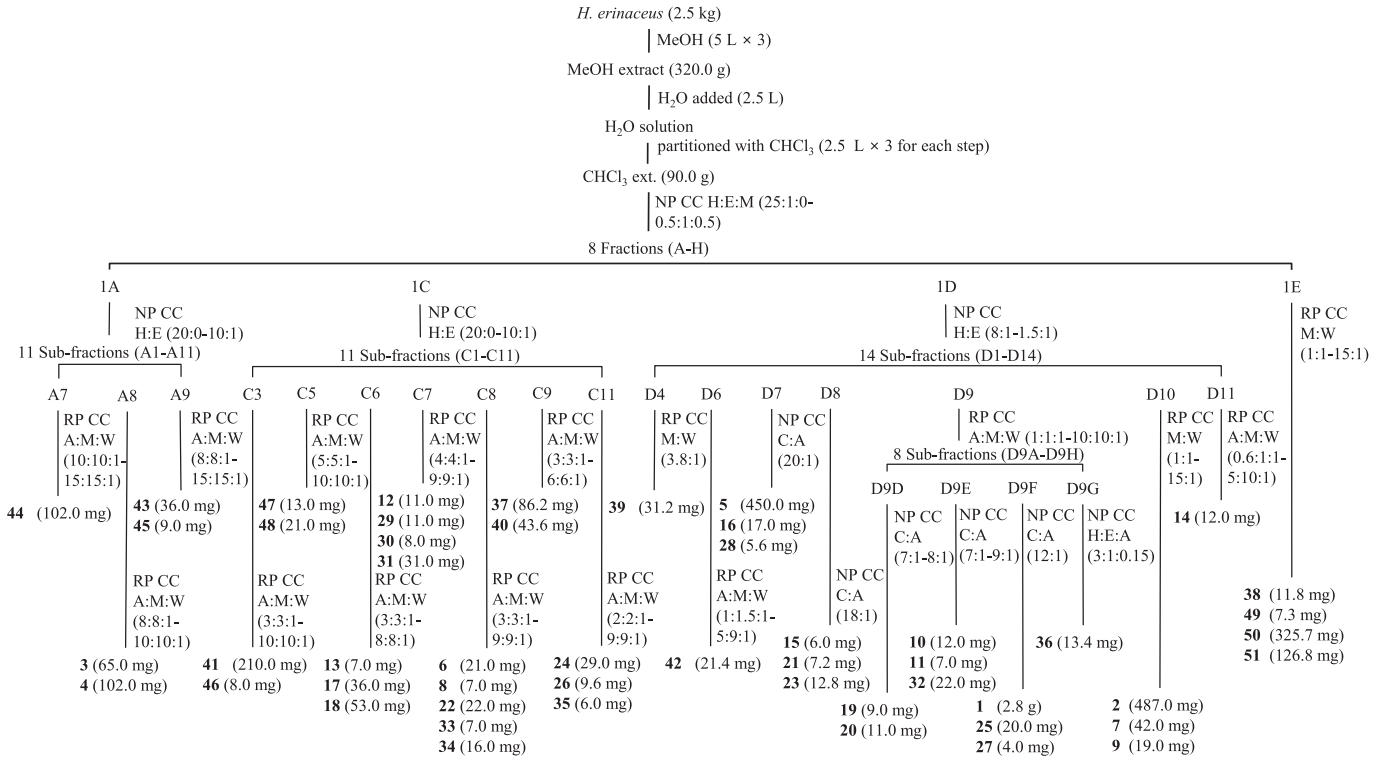
Fraction 1A (8.2 g) was separated using silica gel (3.0 × 80 cm) column chromatography with a gradient of *n*-hexane-EtOAc (20:1 to 10:1, 10 L) to yield 11 sub-fractions (Fr. 1A-1–1A-11). Fraction 1A-7 (950.0 mg) was subjected to a C_{18} (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (10:10:1, 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 C_{18} (2.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (1.5 × 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 **41** (210.0 mg) and **46** (8.0 mg). Fraction 1C-5 (520.0 mg) was subjected to a C_{18} (1.0 × 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 **47** (13.0 mg) and **48** (21.0 mg). Fraction 1C-6 (1.0 g) was subjected to a C_{18} (1.5 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (2.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (4:4:1, 6:6:1, 9:9:1; 2.0 L for each step) elution solvent to yield compounds **12** (11.0 mg), **29** (11.0 mg), **30** (8.0 mg), and **31** (31.0 mg). Fraction 1C-8 (952.0 mg) was subjected to C_{18} (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (1.5 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 C_{18} (1.0 × 80 cm) column chromatography with a MeOH -H_2O (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 C_{18} (1.5 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 $CHCl_3$-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 silica gel (1.0 × 80 cm) column chromatography with a $CHCl_3$-acetone (18:1; 1 L) elution solvent to yield compounds **15** (6.0 mg), **21** (7.2 mg), and **23** (12.8 mg). Fraction 1D-9 (5.6 g) was subjected to C_{18} (2.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (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 $CHCl_3$-acetone (7:1, 8:1; 1 L for each step) elution solvent to yield compounds **19** (9.0 mg) and **20** (11.0 mg). Fraction 1D-9-E (350.0 mg) was subjected to silica gel (1.5 × 80 cm) column chromatography with a $CHCl_3$-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 $CHCl_3$-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 C_{18} (1.5 × 80 cm) column chromatography with a MeOH-H_2O (1:1, 2:1, 3:1, 5:1, 7:1, 9:1, 11:1, 13:1, 15:1; 2.5 L for each step) elution solvent to yield compounds **2** (487.0 mg), **7** (42.0 mg), and **9** (19.0 mg). Fraction 1D-11 (240.0 mg) was subjected to a C_{18} (1.0 × 80 cm) column chromatography with a MeOH-acetone-H_2O (0.6:1.2:1, 1:2:1, 2:4:1, 3:6:1, 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 a silica gel (1.0 × 80 cm) column chromatography with a $CHCl_3$-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 C_{18} (1.0 × 80 cm) column chromatography with a MeOH-H_2O (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**,



CC: Column Chromatography; NP : Normal Phase (Silica gel); RP : Reversed Phase (C_{18}); A: Acetone; C: $CHCl_3$; D: CH_2Cl_2; EA: EtOAc; H: *n*-hexane; M: MeOH; W: H_2O

Fig. 1. Isolation scheme of compounds **1**-**51**.

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), ergosteryl-3-*O*-*β*-D-glucopyranoside (**2**; Notaro et al., 1992a), ergosteryl palmitate (**3**; Panthama et al., 2011), ergosteryl stearate (**4**; Panthama et al., 2011), ergosterol peroxide (**5**; Notaro et al., 1992a), (22*E*)-ergosta-4,6,8,22-tetraen-3-one (**6**; Notaro et al., 1992a), 22*E*,24*R*)-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**; Yaoita et al., 2002), erinarol C (**12**; Li et al., 2014c), (3*β*,5*α*,22*E*) 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 G (**16**; 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), citreoanthrasteroid (**20**; Nakada and Yamamura, 2000), (3*β*,7*α*,22*E*) ergosta-8(14),22-diene-3,7-diol (**21**; Notaro et al., 1992b), 7-ketobrassicasterol (**22**; Notaro et al., 1992a), (3*β*,22*E*) ergosta-5,8(14),22-triene-7-one (**23**; Lin et al., 1999), 5*α*,6*α*-epoxy-3*β*-hydroxy ergosta-22-ene-7-one (**24**; Lee et al., 2008), 5*α*,6*α*-epoxy-(22*E*,24*R*)-ergosta-8(14),22-diene-3*β*,7*β*-diol (**25**; Ishizuka et al., 1997), 5*α*,6*α*-epoxy-(22*E*,24*R*)-ergosta-7,22-diene-3*β*-ol (**26**; Bok et al., 1999), 5*α*,6*α*;8*α*,9*α*-diepoxy-(22*E*,24*R*)-ergosta-22-ene-3*β*-7*α*-diol (**27**; Yaoita et al., 1999), 5*α*,6*α*;8*α*,9*α*-diepoxy-(22*E*,24*R*)-ergosta-22-ene-3*β*-7*β*-diol (**28**; Yaoita et al., 1999), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-palmitate (**29**; Gao et al., 2007), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-oleate (**30**; Gao et al., 2007), (3*β*,5*α*,6*α*,22*E*)-ergosta-7,22-diene-3,5,6-triol 6-linoleate (**31**; Gao et al., 2007), (ergosta-7,22-diene-3β,5α,6α-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), hericerin (**37**, Kobayashi et al., 2012), *N*-De phenylethyl isohericerin (**38**, Miyazawa et al., 2012), isohericenone J (**39**; Li et al., 2015a), hericeone J (**40**, Ueda et al., 2008), erinacene D (**41**; Li et al., 2014c), 4-[3′,7'-dimethyl-2′,6'-octadienyl]-2-formyl-3-hydroxy-5-methyoxybenzylalcohol (**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), hericenone C (**46**; Kawagishi et al., 1991), hericenone D (**47**; Kawagishi et al., 1991), hericenone E (**48**; Kawagishi et al., 1991), hericerin 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 Hericiaceae. 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 *Topsentia* 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 haracteristic streols of the genus *Hericium*. Interestingly, compounds **6** and **25**, have only been reported from *Pleurotus ostreatus* (Chobot et al., 1997) and *Pleurotus eryngii* (Kikuchi et al., 2016), which belong to the family Pleurotaceae. Compounds **7**, **21**, and **22**, have only been reported from *Agaricus subrufescens* (Kawagishi et al., 1988) and *Chlorophyllum molybdites* (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, Pleurotaceae, 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 of two sphingolipids, cerebroside B (**50**) and cerebroside D (**51**), which are only found in *H. erinaceus* in the family Hericiaceae (Lee et al., 2015). Thus, the presence of these two cerebrosides might serve as a chemotaxonomic marker of *H. erinaceus*.

Aromatic compounds such as isoindolinone alkaloids, isobenzofuranones and related derivatives are the characteristic metabolites from genus *Hericium*. In this paper, four isoindolinone 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). Hericerin (**37**) and *N*-De phenylethyl isohericerin (**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**), isohericenone J (**39**) and erinacene D (**41**) were obtained from the family Hericiaceae 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-methyoxybenzylalcohol (**42**), hericene A (**43**), hericenone C (**46**), hericenone D (**47**), and hericenone 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 Hericiaceae. Hericeone J (**40**), hericene B (**44**), and hericene C (**45**) were also previously reported from *H. coralloides* alone (Zhang et al., 2012), further indicating a close relationship

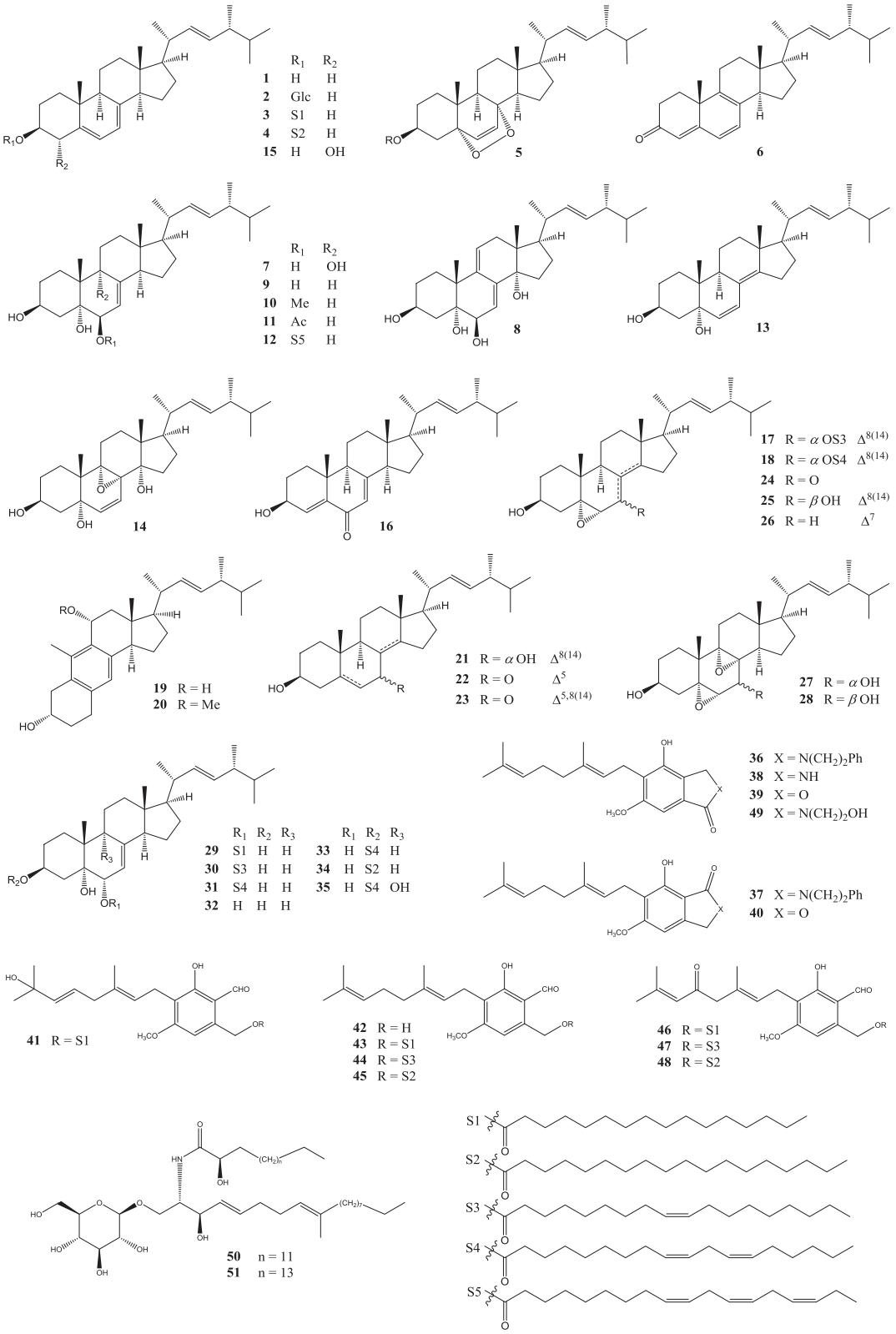


Fig. 2. The chemical structures of compounds **1**–**51** from *H. erinaceus*.

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 Hericiaceae.

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 perpective, some isoindolinone 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 Hericiaceae.

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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.

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