Cytotoxic Constituents Isolated from the Fruit Bodies of Hypsizigus marmoreus

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The bioactivity-guided fractionation of chloroform extracts of the fruit bodies of Hypsizigus marmoreus led to our isolation of (22E,24R)-ergosta-7,22-diene-3(5,6)-diol (1), ergosterol-3-O-[D-glucopyranoside (2), 5x,8x-epidioxyergosta-6,22-diene-3]-ol (3), hypsizigrenol A4 (4), hypsizigrenol A4 (5), hypsizigrenol A4 (6) and hypsizigrenol B4 (7). Among these seven isolates, compound 2 was identified for the first time from this plant. All compounds (1-7) exhibited moderate cytotoxicity towards cultured human colon carcinoma (HT-29), human breast carcinoma (MCF-7) and human hepatoblastoma (HepG-2) cell lines.

Key words: Hypsizigus marmoreus, cytotoxicity

INTRODUCTION

Mushrooms have long been valued by humans as both edible and medical resources. A number of bioactive molecules, some with antitumor properties, have been identified in several different mushroom species. Mushrooms, such as the Trametes versicolor, Lentinus edodes, and Gentinella frondosa are known to be effective against advanced liver, stomach, lung and brain cancers of patients and in animals (Kodama et al., 2002; Kabir et al., 1989; Wasser and Weis, 1999; Mizuno, 1999). Of particular interest, Hypsizigus marmoreus has recently become a popular edible mushroom in Asia where its many biological properties (anti-tumor and anti-oxidant) are well known (Matsuzawa et al., 1996).

As a preliminary search to identify new cytotoxic compounds from natural products, we have screened solvent extracts of more than 100 Korean folk medicines and edible mushrooms for their cytotoxic activity. In this study, we report on the isolation of three sterols and four hypsizigrenols from CHCl3 extracts of H. marmoreus. The structure of these compounds was identified as follows: (22E,24R)-ergosta-7,22-diene-3(5,6)-diol (1), ergosterol-3-O-[D-glucopyranoside (2), 5x,8x-epidioxyergosta-6,22-diene-3]-ol (3), hypsizigrenol A4 (4), hypsizigrenol A4 (5), hypsizigrenol A4 (6) and hypsizigrenol B4 (7) on the basis of their spectral properties. All seven compounds were tested for their cytotoxic effects against various cancer cell lines, such as human colon carcinoma (HT-29), human breast carcinoma (MCF-7) and human hepatoblastoma (HepG-2). The inhibitory effects of these compounds against DNA topoisomerase (I and II) were also evaluated.

MATERIALS AND METHODS

General experimental procedures

The optical rotations of the compounds were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. The NMR spectra were recorded on a Bruker 250 MHz (DMX 250, UK) spectrometer using Bruker’s standard pulse program. Samples were dissolved in either CD3OD or CDCl3 and pyridine-d5, and the chemical shifts reported in δ (ppm) downfield from TMS. The FAB-MS

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spectra were measured by a VG TRIO 2A (UK) mass spectrometer. Silica gel 60 (70-230 and 230-400 mesh, Merck), Lichroprep RP-18 gel (40-63 μm, Merck) and TLC plates (Si-gel 60 F254 and RP18 F254) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation and by spraying with 10% H2SO4 in EtOH followed by heating. An LC-10AD pump (Shimadzu, Japan), SPD-10A detector (Shimadzu, Japan) and Shim-Pack Prep-ODS (20 × 25 mm) column were used for preparative HPLC. All other chemicals and solvents were analytical grade and used without further purification.

Plant materials
Dried fruit bodies of cultivated H. marmoreus were provided from the Yonam College of Agriculture, Cheonan, Republic of Korea. The voucher specimen (YN0505) was deposited with the Department of Food and Nutrition, Yeungnam University, Republic of Korea.

Extraction and isolation
Dried fruit bodies of cultivated H. marmoreus (4 kg) were extracted three times with MeOH (5 L) at 60°C for 8 h. The combined MeOH extracts were concentrated under reduced pressure to yield the MeOH extract (860 g). The extract was suspended in H2O (1 L) and then fractionated successively with n-hexane, chloroform, ethyl acetate and water-saturated n-butanol (3 times with each solvent). Removal of the solvents yielded 140.0 g, 36.0 g, 4.2 g, 125.0 g and 541.0 g of the n-hexane, chloroform, ethyl acetate, n-butanol and water fractions, respectively (Table 1).

The CHCl3 extract (35.0 g) was loaded on a silica-gel column (60 × 9 cm, Silica-gel 70-230 mesh) and the column eluted with a CHCl3-MeOH gradient (gradient: CHCl3, MeOH = 0%, 1%, 2%, 4%, 8%, 15%, 30%, 50%, 100%). The eluates were combined on the basis of the TLC results, so that 26 fractions (HMC1-26) were produced. Fraction HMC9 (550 mg) was rechromatographed on a silica-gel column (60 × 3 cm, Silica-gel 70-230 mesh) with n-hexane-EtOAc (gradient from n-hexane 50% to EtOAc 100%), affording 1 (12 mg) and 2 (15 mg), respectively. Fraction HMC10 (600 mg) was rechromatographed on a LiChroprep RP-18 column (60 × 4 cm) with MeOH-H2O (gradient from 10:90 to 100% MeOH), which afforded 19 subfractions (HMC10-1-19). Subfraction HMC10-15 was further purified to afford 3 (11 mg) using HPLC with MeOH-H2O gradients. The fraction HMC16 (1.3 g) was chromatographed on a LiChroprep RP-18 column (60 × 4 cm) with MeOH-H2O (gradient from 50% to 100% MeOH), yielding 12 subfractions (HMC16-1-12), and subfraction HMC16-8 was further purified giving compound 4 (43 mg) using HPLC with MeOH-H2O gradients. Fraction HMC18 (1.1 g) was chromatographed on a reverse-phase column (80 × 3.0 cm, LiChroprep RP-18) with MeOH-H2O (gradient from 2:8 to 100% MeOH) to afford 22 fractions (HMC18-1-18-22). The subfractions HMC18-15 and HMC18-20 were purified to afford 6 (25 mg) and 5 (10 mg), respectively, using HPLC with MeOH-H2O gradients. Fraction HMC20 (750 mg) was chromatographed on a reverse-phase column (60 × 2 cm, LiChroprep RP-18) with MeOH-H2O (gradient from 2:9 to 100% MeOH) to afford 7 (HMC20-3, 18 mg).

The physico-chemical properties of the seven isolated compounds were as follows:

Table 1. Inhibitory effects of solvent fraction of Hypsizigus marmoreus on the growth of Hep-G2 and HT-29 cells when applied at a concentration of 50 μg/mL.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Inhibition rate (%)</th>
<th>Hep-G2</th>
<th>HT-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>42</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>54</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>CHCl3 fraction</td>
<td>91</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
</tr>
<tr>
<td>BuOH fraction</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
</tr>
<tr>
<td>Water fraction</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
<td>&lt; 0</td>
</tr>
</tbody>
</table>

Fig. 1. Proposed structures of compounds 1-7.

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