New Tricyclic Sesquiterpenes from the Fermentation Broth of *Stereum hirsutum*

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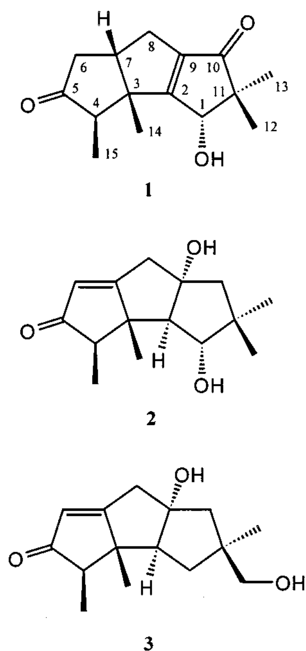
In search for novel metabolites from the culture broth of the mushroom *Stereum hirsutum*, three new tricyclic sesquiterpenes named hirsutenols A (**1**), B (**2**), and C (**3**) have been isolated. Their structures were assigned on the basis of various spectroscopic studies.

Mushrooms produce various classes of primary and secondary metabolites, many that exhibit significant antimicrobial, antitumor, and antiviral activity. Hence, some species of mushrooms have been used in the Orient as traditional medicines for treatment of immune diseases, diabetes, and cancer. We have investigated biologically active principles from basidiomycetes.[1,2] As part of our ongoing screening efforts, three new sesquiterpenes, hirsutenols A (**1**), B (**2**), and C (**3**), have been isolated from the culture broth of *Stereum hirsutum* (Willd.: Fr.) S. F. Gray (Stereaceae). *Stereum* species are known to produce sesquiterpenes with hirsutane,[3] sterepolide,[4] and sterpurene[5] skeletons, benzaldehydes,[6] and benzofurans.[7] In this paper, we describe the isolation and structure determination of **1**–**3**.



The producing strain obtained from tissue culture of *S. hirsutum* was fermented as outlined in the Experimental Section, and the fermentation broth was separated consecutively by Diaion HP-20 and silica gel column chromatographies, solvent partition between EtOAc and H_2O, and preparative HPLC to afford **1**–**3**.

The molecular formula of **1** was determined to be $C_{15}H_{20}O_3$ by HREIMS. Its IR spectrum suggested the presence of hydroxyl and carbonyl groups. The ^{1}H NMR spectrum of **1** in CD_3OD exhibited signals due to an oxygenated methine at δ 4.45, two methylenes and two methines between δ 2.0 to δ 3.0, and four singlet methyls (Table 1). The methylene proton signals due to H-6 at δ 2.57 and 2.40 were gradually quenched by deuterium exchange in CD_3OD. This suggested that this methylene was adjacent to a carbonyl carbon. The COSY spectrum that was measured after reexchange by addition of CD_3OH revealed a partial structure $-CH_2-CH-CH_2-$, and direct $^1H-^{13}C$ connectivity was established by HMQC experiment. The structure of **1** was assigned by interpretation of the HMBC spectrum. HMBC showed long-range correlations from H-12 and H-13 to C-1, C-10, and C-11, from H-1 to C-2 and C-9, from H-14 to C-2, C-3, C-4, and C-7, from H-15 to C-3, C-4, and C-5, from H-6 to C-5 and C-8, and from H-8 to C-2, C-3, C-6, C-7, and C-9 (Figure 1, Supporting Information). Finally, C-9 should be connected to the carbonyl carbon of C-10 by the process of elimination. The relative stereostructure was determined by the NOESY spectrum, which showed NOEs between H-1, H-7, H-12, and H-14.

The molecular formula of **2** was determined to be $C_{15}H_{22}O_3$ by HREIMS, and IR absorptions at 3400 and 1695 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^{1}H NMR spectrum of **2** showed signals attributable to an olefinic proton, an oxygenated methine doublet, two methylenes, two methines, and four methyl groups. In the ^{13}C NMR spectrum, a ketone carbonyl carbon (δ 214.7), two sp^2 carbons (δ 194.2 and 123.3), two hydrogenated carbons (δ 87.0 and 81.2), and 10 sp^3 carbons between δ 67.4 and 9.4 were evident. These signals were assigned as four methyl, two methylene, four methine, and five quaternary carbons using the DEPT spectrum. Direct $^1H-^{13}C$ connectivity ($^1J_{C,H}$) was also established with the aid of an HMQC spectrum. Partial structures $CH_3-CH<$ and $>CH-CH<$ were readily determined by interpretation of the proton coupling patterns in the ^{1}H NMR spectrum. The structure of **2** was finally assigned using the HMBC spectrum, which showed the critical long-range correlations from H-12 and H-13 to carbons C-1, C-10, and C-11, from H-1 to C-2 and C-3, from H-2 to C-3, C-4, and C-9, from H-14 to C-2, C-3, C-4, and C-7, from H-15 to C-3, C-4, and C-5, from H-6 to C-3, C-4, C-5, and C-7, from H-8 to C-2, C-3, C-6, C-7, and C-9, and from H-10 to C-1, C-2, and C-9, as shown in Figure 1 in the Supporting Information. The

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Table 1. ^{1}H NMR Spectral Data (δ) of **1**, **2**, and **3** in CD_3OD

no.	**1**	**2**	**3**
1	4.45 (1H, t, J = 2.3)[a]	3.66 (1H, d, J = 10.0)	1.73 (1H, dd, J = 13.0, 10.0) 1.54 (1H, ddd, J = 13.0, 9.0, 1.7)
2		2.25 (1H, d, J = 10.0)	2.41 (1H, t, J = 9.6)
4	2.38 (1H, br q)	2.41 (1H, q, J = 7.3)	2.38 (1H, q, J = 7.4)
6	2.57 (1H, dd, J = 19.3, 9.7) 2.40 (1H, dd, J = 19.3, 4.0)	5.79 (1H, s)	5.79 (1H, d, J = 1.8)
7	2.95 (1H, m)		
8	2.75 (1H, ddd, J = 16.4, 9.1, 2.5) 2.22 (1H, ddd, J = 16.4, 7.3, 2.0)	2.72 (2H, s)	2.84 (1H, dd, J = 15.8, 1.8) 2.76 (1H, d, J = 15.8)
10		1.87 (1H, d, J = 13.8) 1.71 (1H, d, J = 13.8)	1.87 (1H, d, J = 13.9) 1.72 (1H, br d, J = 13.9)
12	1.12 (3H, br s)	1.11 (3H, s)	3.35 (2H, s)
13	1.04 (3H, s)	1.04 (3H, s)	1.17 (3H, s)
14	1.18 (3H, s)	1.07 (3H, s)	0.95 (3H, s)
15	1.12 (3H, br s)	1.10 (3H, d, J = 7.3)	1.05 (3H, d, J = 7.4)

[a] Proton resonance integrals, multiplicities, and coupling constants (J = Hz) are in parentheses.

stereochemistry was determined by the NOESY spectrum in comparison with known hirsutane-skeletal compounds. The NOEs observed between H-1, H-12, H-14, and H-15 were well matched with the stereochemistry of hirsutanes previously reported.[3,8]

Compound **3** had the same molecular formula as **2**, and its UV and IR spectra were very similar to those of **2**. The ^{1}H and ^{13}C NMR spectra of **3** also resemble those of **2** except for the presence of two additional methylenes (C-1 and C-12) instead of a hydroxyl methine (C-1) and a methyl (C-12) in **2**. The structure of **3** was assigned by the HMBC experiment in combination with the DQF-COSY and HMQC spectra. HMBC showed the critical correlations from H-1 at δ 1.73 and 1.54 to C-3, C-9, C-10, C-12, and C-13 and from H-12 at δ 3.35 to C-1, C-10, C-11, and C-13, establishing the structure of **3** as shown. The relative stereochemistry was determined by a NOESY spectrum, which exhibited correlations between H-14 and H-1 at δ 1.73, H-1 at δ 1.73 and H-12, and H-12 and H-10 at δ 1.87 and between H-2 and H-1 at δ 1.54, H-1 at δ 1.54 and H-13, and H-13 and H-10 at δ 1.72.

Hirsutenols **1**−**3** possessed the hirsutane skeleton, and their relative stereochemistries were established on the basis of the NOESY experiments as *cis*, *trans*, *cis* configuration as consistently found in the hirsutane class. Namely, the hydrogen at C-2 was *cis* to the C-9 hydroxyl group and *trans* to the C-14 methyl group, which in turn was *cis* to H-7. In a literature survey on the absolute configurations of the reported hirsutane series, we noted that all C-1 hydroxyl groups were α-oriented, and C-14 and C-15 methyls were β-oriented. Therefore, the absolute configurations for **1**−**3** were assigned as shown. To the best of our knowledge, this is the first report of compounds **1**−**3**.

The hirsutane sesquiterpenes have been reported to exhibit weak antifungal activity. In an assessment of antimicrobial activity by the agar diffusion method (50 μg compounds/disk), however, hirsutenols **1**−**3** showed only moderate antibacterial activity against *Escherichia coli* and no activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Candida albicans*, *Aspergillus* spp., and *Chlorella regularis*.

Experimental Section

General Experimental Procedures. Specific rotation was determined using a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on a Shimadzu UV-260 and a FT-IR Equinox 55 spectrophotometer, respectively. NMR spectra were obtained using Varian UNITY 300 (300 MHz) and Bruker DMX600 (600 MHz) NMR spectrometers in CD_3OD with TMS as an internal standard. Chemical shifts are given in ppm (δ) values. EIMS and HREIMS spectra were taken on a JEOL JMS-SX 102A mass spectrometer, operating at 70 eV. Analytical SiO_2 TLC was performed with Kiesel gel $60F_{254}$ (Merck) without activation.

Fungal Material. *Stereum hirsutum* was collected at Dukyu-National Park, Korea, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the taxonomic key of Imazeki and Hongo.[9] A voucher specimen (MVS39) is deposited in the Antibiotics Research Laboratory, Korea Research Institute of Bioscience and Biotechnology. The hirsutenols-producing strain was obtained from the tissue culture of the fruiting body of *S. hirsutum*.

Fermentation. *S. hirsutum* grown on potato sucrose agar medium was used to inoculate two 500 mL Erlenmeyer flasks containing 100 mL of the seed medium consisting of yeast extract 0.4%, malt extract 1.0%, and glucose 0.4% (pH 6.0 before sterilization). The flasks were shaken on a rotary shaker for 3 days at 28 °C. The seed culture was transferred into a 5 L jar fermenter containing 3 L of the same medium as above for production of **1**−**3**, and cultivation was carried out at 28 °C for 14 days with aeration of 2 L/min and agitation of 250 rpm. Compounds **1**−**3** were evident at 5 days of cultivation and reached maximum concentration at 10 days.

Extraction and Isolation. The fermentation broth was separated into supernatant and mycelium by centrifugation. The supernatant was applied to a column of Diaion HP-20, and the column was washed with 30% aqueous MeOH and then eluted with 70% aqueous MeOH. After concentration of the eluate in vacuo, the resultant residue was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was concentrated and then subjected to a silica gel column eluting with $CHCl_3$−MeOH (50:1−1:1, stepwise) to give two fractions. An active fraction was purified by reversed-phase preparative HPLC (column: Metasil 5u ODS, 21.2 × 250 mm) developed with 50% aqueous MeOH at a flow rate of 5.5 mL/min to give **1** (3 mg) with a retention time of 34 min. Another fraction was further purified by preparative HPLC using the same conditions as above to afford **2** (2 mg) and **3** (4 mg) with retention times of 19 and 20 min, respectively.

Hirsutenol A (1): colorless oil; UV (MeOH) λ_{max} (log ε) 242 (4.07) nm; IR (KBr) ν_{max} 3430, 2960, 1735, 1690, 1635, 1560, 1460, 1380 cm^{-1}; $[\alpha]_D$ −160° (c 0.05, MeOH); for ^{1}H NMR, see Table 1; ^{13}C NMR (CD_3OD) δ 221.9 (C-5), 209.7 (C-10), 189.0 (C-2), 145.6 (C-9), 75.5 (C-1), 55.5 (C-11), 54.7 (C-3), 51.6 (C-4), 51.1 (C-7), 42.2 (C-6), 32.2 (C-8), 24.0 (C-12), 20.9 (C-13), 18.7 (C-14), 9.6 (C-15); HREIMS m/z 248.1414 M$^+$ ($C_{15}H_{20}O_3$ requires 248.1412).

Hirsutenol B (2): colorless oil; UV (MeOH) λ_{max} (log ε) 231 (3.97) nm; IR (KBr) ν_{max} 3400, 2930, 1695, 1635, 1575, 1445, 1245, 1040 cm^{-1}; $[\alpha]_D$ +112° (c 0.16, MeOH); for ^{1}H NMR, see Table 1; ^{13}C NMR (CD_3OD) δ 214.7 (C-5), 194.2 (C-7), 123.3 (C-6), 87.0 (C-9), 81.2 (C-1), 67.4 (C-2), 59.1 (C-4), 53.6 (C-3), 53.3 (C-10), 45.2 (C-11), 44.9 (C-8), 27.9 (C-13), 21.1 (C-12),

21.0 (C-14), 9.4 (C-15); HREIMS *m/z* 250.1575 M^+ ($C_{15}H_{22}O_3$ requires 250.1569).

Hirsutenol C (3): colorless oil; UV (MeOH) λ_{max} (log ϵ) 232 (3.97) nm; IR (KBr) ν_{max} 3390, 2965, 1695, 1635, 1460, 1380, 1245, 1080, 1035 cm^{-1}; $[\alpha]_D$ +160° (*c* 0.16, MeOH); for ^1H NMR, see Table 1; ^{13}C NMR (CD_3OD) δ 214.7 (C-5), 195.4 (C-7), 123.1 (C-6), 93.0 (C-9), 71.4 (C-12), 62.8 (C-2), 58.7 (C-4), 55.1 (C-3), 51.0 (C-10), 50.0 (C-1), 44.6 (C-8), 37.0 (C-11), 24.0 (C-13), 21.2 (C-14), 9.7 (C-15); HREIMS *m/z* 250.1576 M^+ ($C_{15}H_{22}O_3$ requires 250.1569).

Antimicrobial Activity. Antimicrobial activity of hirsutenols **1**−**3** was estimated by the agar diffusion method using paper disks of 8 mm diameter. Each paper disk with 50 μg of compound was placed on agar media suspended with test microorganisms. Inhibition zones were observed after incubation at 27 °C for 24 and 48 h for yeast and fungi, respectively, and at 37 °C for 24 h for bacteria.

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Supporting Information Available: Figure 1, showing HMBC spectral data for **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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