Suillusin, a Unique Benzofuran from the Mushroom *Suillus granulatus*

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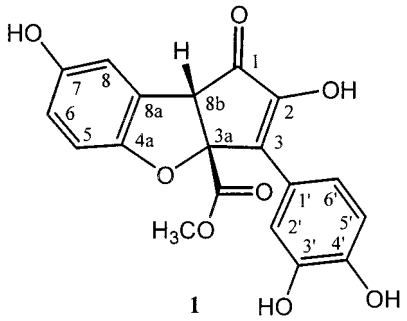
A unique benzofuran named suillusin was isolated from the methanolic extract of the fruiting body of the mushroom *Suillus granulatus*. Its structure was assigned on the basis of various spectroscopic analyses as a highly substituted novel 1*H*-cyclopenta[*b*]benzofuran (**1**). Suillusin is suggested to be biogenerated from polyporic acid.

Mushrooms have been used in Asia as traditional foods and medicines. Mushrooms produce various classes of primary and secondary metabolites, many that exhibit significant antimicrobial, antitumor, and antiviral activity. Despite their potential for drug development, few bioactive metabolites have been reported from mushrooms as compared with the higher plants and microbes. We have screened biologically active and chemically novel principles from the fruiting bodies of basidiomycetes[1,2] using an analytical HPLC system. As part of our ongoing screening efforts, a highly substituted 1*H*-cyclopenta[*b*]benzofuran, named suillusin (**1**), has been isolated from the mushroom *Suillus granulatus* (L.: Fr.) O. Kuntze (Boletaceae). *Suillus* spp. are known to produce various phenolic compounds, such as variegatic acid,[3] variegatorubin,[4] suillin,[5] grevillines,[6,7] trihydroxypulvinone,[8] and thelephoric acid.[8] In this paper, we describe the isolation, structural elucidation, and biological activity of **1**.



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Fresh fruiting bodies of *Suillus granulatus* were extracted with MeOH. The methanolic extract was partitioned between EtOAc and H_2O, and the EtOAc extract was purified consecutively on silica gel and Sephadex LH-20 flash columns and HPLC to afford compound **1**.

UV absorption maxima of **1** at 223 (sh), 261, 311 (sh), and 348 nm suggested that this compound had a unique chromophore. The molecular formula of **1** was determined to be $C_{19}H_{14}O_8$ by HREIMS. IR absorption at 1728 and 1703 cm^{-1} and ^{13}C NMR peaks at δ 173.5 and 197.8 implied the presence of ester carbonyl and α,β-unsaturated ketone carbonyl groups, respectively. The ^1H NMR spectrum in CD_3OD showed two sets of AMX spin systems due to 1,2,4-trisubstituted benzenes (δ 7.64, 7.42, 6.79 and 6.81, 6.68, 6.64), a methine proton at δ 4.15, and a methoxy proton at δ 3.72. Nineteen signals were observed by ^{13}C NMR. The DEPT spectrum revealed 11 quaternary, seven methine, and one methyl carbon signals. One bonded $^1H-^{13}C$ connectivity was established by PFG-HMQC spectra.[9]

Eleven of 13 degrees of unsaturation were satisfied with two benzene rings, two carbonyls, and one double bond. Further structural information was obtained by PFG-HMBC experiments[10,11] measured with delay times of 60, 100, and 140 ms, respectively. HMBC revealed the presence of the two 1,2,4-trisubstituted benzene systems and a carbomethoxyl group (Figure 1, Supporting Information).

Aromatic methine protons at δ 7.64 (H-2′) and 7.42 (H-6′) showed long-range correlations to a sp^2 quaternary carbon at δ 137.2 (C-3) that was conjugated with the sp^2 quaternary carbon at δ 151.0 (C-2), a remaining olefinic carbon. Long-range correlations from a methine proton at δ 4.15 (H-8b) to sp^2 carbons at δ 153.8 (C-4a), 123.9 (C-8a), and 112.2 (C-8) and from a methine proton at δ 6.68 (H-5) to quaternary carbons at δ 153.4 (C-7) and 123.9 (C-8a) revealed the presence of a 2,5-dioxyphenyl group.

From the molecular formula, four carbons among the six oxygenated carbons (δ 153.8, 153.4, 151.0, 148.3, 146.2, and 91.9) should have a hydroxyl group attached. Additionally the ^1H NMR spectrum in acetone-d_6 showed four exchangeable protons (δ 9.23, 8.44, 8.32, and 8.08) that were quenched by addition of D_2O. ^{13}C chemical shift values measured in CD_3OH were compared with those in CD_3OD ($\Delta\ \delta CD_3OH - \delta CD_3OD$) to assign the hydroxyl-attached carbons. As a result, four quaternary carbons at δ 153.4 (+0.10, C-7), 151.0 (+0.16, C-2), 148.3 (+0.18, C-4′), and 146.2 (+0.18, C-3′) were determined to be the hydroxyl-attached carbons, while two carbons at δ 153.8 (0, C-4a) and 91.9 (+0.01, C-3a) should be bridged by an ethereal oxygen to satisfy the molecular formula.

On the basis of the downfield shift (δ +0.11) of a carbonyl carbon in CD_3OH and chemical shift values of α and β carbons at δ 151.0 and 137.2, respectively, an α,β-unsaturated ketone carbonyl carbon at δ 197.8 was connected to C-2 at δ 151.0. If this partial structure was a 3-hydroxycyclopentenone system instead of the proposed 2-hydroxycyclopentenone, the chemical shifts of C-2 and C-3 would be shifted upfield and low-field, respectively. Two alternative structures were suggested for suillusin on the basis of the above results and additional HMBC data (Figure 1, Supporting Information).

The planar structure of **1** was finally determined by the NOE differential spectra. Important NOEs are summarized on the structure shown in Figure 1 in the Supporting

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Information, which showed NOEs between the methoxy protons at δ 3.72 and two aromatic methine protons at δ 7.64 (H-2′) and 7.42 (H-6′). The relative stereochemistry of two chiral centers was also established as *cis* by the NOEs observed between the methine proton at δ 4.15 (H-8b) and methoxy methyl protons at δ 3.72.

Suillusin (**1**) is a highly substituted 1*H*-cyclopenta[*b*]-benzofuran probably biosynthesized from polyporic acid.[12] A proposed biosynthesis of **1** is summarized in Scheme 1 in the Supporting Information. Details of the suillusin biosynthetic pathway remain to be investigated.

The antioxidative activity of suillusin was evaluated by the DPPH radical scavenging.[13] Activity was compared with well-known antioxidants, butylated hydroxyanisole (BHA) and vitamin E. Although suillusin was less effective than BHA (76%) and vitamin E (73%), the DPPH radical-scavenging effect was 30% at 100 μg/mL. Suillusin also showed specific cytotoxic effects against the UACC62 melanoma and SW620 colon cell lines, with IC_{50} values of 12 and 20 μg/mL, and exhibited mild toxicity against the HCT15 colon, A549 lung, SK-OV-3 ovary, and PC-3 prostate cell lines at a concentration of 30 μg/mL.

Experimental Section

General Experimental Procedures. Specific rotation was determined using a Polartronic 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 a JEOL JNM-A600 spectrometer in acetone-d_6, CD_3OD, or CD_3OH 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. Analytical HPLC was performed on a YMC ODS-H80 column (i.d. 4.6 × 250 mm) with a flow rate of 0.5 mL/min using a gradient of MeOH/H_2O as a mobile phase and by monitoring with a photodiode-array detector (190−650 nm).

Fungal Material. *Suillus granulatus* was collected near Yusong, Chungnam Province, Korea, in September to October, 1998, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the taxonomic key of Imazeki and Hongo.[14] A voucher specimen (MVS33) is deposited in the Antibiotic Research Laboratory, Korea Research Institute of Bioscience and Biotechnology.

Extraction and Isolation. *S. granulatus* (fresh weight 120 kg) was extracted twice with MeOH at room temperature for 3 days and filtered. The methanolic extract was concentrated under reduced pressure, and the aqueous residue was partitioned between hexane, $CHCl_3$, EtOAc, and BuOH. The EtOAc-soluble portion was concentrated in vacuo, and the concentrate was subjected to a column of silica gel eluted with stepwise additions of $CHCl_3$/MeOH. Two fractions [$CHCl_3$/MeOH (10:1, 5:1)] showing unique UV absorption were combined, concentrated in vacuo, and subjected to Sephadex LH-20 column chromatography eluted with MeOH to afford **1** (15 mg). The purity (>95%) of compound **1** was checked by HPLC using an ODS column with 30% aqueous MeOH as the elution solvent at a flow rate of 0.5 mL/min.

Suillusin (1): yellow powder; UV (MeOH) λ_{max} (log ϵ) 223 (sh) (3.56), 261 (3.21), 311 (sh) (3.30), 348 (3.42) nm; IR (KBr) ν_{max} 3412, 2924, 2852, 1728 (sh), 1703, 1611, 1523, 1490, 1439, 1386, 1284, 1218 cm^{-1}; $[\alpha]_D$ +4° (*c* 1.0, MeOH); ^1H NMR (CD_3OD, 600 MHz) δ 7.64 (1H, d, J = 2.0 Hz, H-2′), 7.42 (1H, dd, J = 8.8, 2.0 Hz, H-6′), 6.81 (1H, dd, J = 2.4, 1.0 Hz, H-8), 6.79 (1H, d, J = 8.8 Hz, H-5′), 6.68 (1H, d, J = 8.8 Hz, H-5), 6.64 (1H, ddd, J = 8.8, 2.4, 1.0 Hz, H-6), 4.15 (1H, br s, H-8b), 3.72 (3H, s, OC*H*$_3$); ^1H NMR (acetone-d_6, 600 MHz) δ 9.23 (1H, s, O*H*), 8.44 (1H, s, O*H*), 8.32 (1H, s, O*H*), 8.08 (1H, s, O*H*), 7.67 (1H, d, J = 2.2 Hz, H-2′), 7.52 (1H, dd, J = 8.4, 2.2 Hz, H-6′), 6.90 (1H, d, J = 8.4 Hz, H-5′), 6.84 (1H, m, H-8), 6.69 (1H, m, H-5), 6.69 (1H, m, H-6), 4.27 (1H, br s, H-8b), 3.71 (3H, s, OC*H*$_3$); ^{13}C NMR (CD_3OD, 150 MHz) δ 197.8 (C-1), 173.5 (*C*OOCH$_3$), 153.8 (C-4a), 153.4 (C-7), 151.0 (C-2), 148.3 (C-4′), 146.2 (C-3′), 137.2 (C-3), 125.3 (C-1′), 123.9 (C-8a), 122.8 (C-6′), 117.4 (C-6), 117.1 (C-2′), 116.2 (C-5′), 112.2 (C-8), 111.7 (C-5), 91.9 (C-3a), 56.9 (C-8b), 53.7 (COO*C*H$_3$); EIMS *m*/*z* 370 $[M]^+$; HREIMS *m*/*z* 370.0705 ($C_{19}H_{14}O_8$ requires 370.0689).

Measurement of DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Activity. A 100 μg sample of suillusin (**1**) dissolved in 20 μL of MeOH was added to 980 μL of 150 μM DPPH EtOH. After vortex mixing, the mixture was incubated for 20 min at room temperature and the absorbance at 517 nm was measured. Differences in absorbance between a test sample and a control (MeOH) were measured, and the activity was also compared with those of butylated hydroxyanisole and vitamin E, at 100 μg/mL.

Cell Culture and Assay for Cytotoxic Activity. Cytotoxicity of **1** was estimated using the human cancer cell lines HCT15 colon, SW620 colon, A549 lung, LOX-IMVI melanoma, UACC62 melanoma, SK-OV-3 ovary, and PC-3 prostate. After all of the adherent cell lines were detached from the culture flasks by addition of 2−3 mL of 0.05% trypsin-EDTA, they were washed and resuspended in the 10% calf serum-containing RPMI1640 medium. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. The microtiter plates containing the cells were preincubated for 24 h at 37 °C in 5% CO_2 to allow stabilization. Suillusin (**1**), dissolved in a small amount of MeOH, was added to give final concentrations of 0.3−30 μg/mL in the microtiter plates. Cells were incubated for 48 h in the presence of suillusin, and then cell growth was evaluated with an SRB (sulforhodamine B) assay. Adherent cell cultures were fixed by adding 100 μL of cold 50% trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and dried. A 100 μL sample of 0.4% SRB solution dissolved in 0.1% acetic acid was added to each well and incubated for 30 min. Unbound SRB was removed by washing with 0.1% acetic acid, and then the plates were dried. Bound stain was solubilized with 10 mM Tris base (pH 10.5) and then read on a microplate reader at 540 nm. A dose−response curve was plotted and a concentration giving 50% inhibition (IC_{50}) was calculated.

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Supporting Information Available: Figure 1, showing two alternative structures for **1** and HMBC and NOE differential spectral data, and Scheme 1, showing the proposed biosynthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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