**Note**

Hydroxysulochrin, a Tea Pollen Growth Inhibitor from the Fungus *Aureobasidium* sp.

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A new plant growth regulator, hydroxysulochrin (1), together with sulochrin (2) was isolated from the culture filtrate of *Aureobasidium* sp. grown on a malt extract medium. The structures of 1 and 2 were established by spectroscopic methods. 1 and 2 inhibited tea pollen tube growth by 41% and 36% of the control value at a concentration of 100 mg/l, respectively. However, 1 and 2 showed no inhibitory effect on the growth of lettuce seedlings from 0.1 mg/l to 100 mg/l.

Key words: hydroxysulochrin; *Aureobasidium*; tea pollen

We have previously investigated fungal metabolites such as deoxycyclopaldic acid,10 chloroisosulochrin, chloroisosulochrin dehydrate, and pestheic acid2) for their potential as plant growth regulators. In the course of our screening search for new plant growth regulators suitable for developing new herbicides and for new lead compounds, we found the presence of pollen growth inhibitors in the cultural metabolite of *Aureobasidium* sp. which belongs to the *Dematiaceae* and is one of the wood-infesting fungi found in the litter layer.3) Bioassay-guided fractionation with tea pollen led to the isolation of a new compound, hydroxysulochrin (1), together with sulochrin (2). In this report, we describe the isolation, structural elucidation, and some biological activities of 1 and 2.

The fungus was cultured stationarily in a malt extract medium (50 L) at 24°C for 35 days. The culture filtrate was adjusted to pH 2.0, before being extracted with EtOAc. The EtOAc extract was fractionated by silica gel column chromatography and further separated by preparative TLC, and final purification by recrystallization afforded hydroxysulochrin (1) and sulochrin (2).

Compound 2 was identified as sulochrin (Fig. 1) by comparing its physicochemical properties with those reported.4–8) Compound 1 was obtained as a yellow amorphous compound. HREIMS of 1 gave [M]+ at 348.0868, consistent with a molecular formula C₁₇H₁₆O₈. The UV, IR, and ¹H- and ¹³C-NMR spectra of 1 revealed a close relationship to 2.

![Fig. 1. Structures of Hydroxysulochrin (1) and Sulochrin (2).](image-url)

The UV absorption maxima at 273 and 328 nm and the IR absorption band at 1640 cm⁻¹ indicate that 1 possessed a benzophenone skeleton.9) The IR band at 1696 cm⁻¹ and two signals at δ 52.0 and 165.7 in the ¹³C NMR spectrum indicate the presence of a methoxycarbonyl group. The ¹H-NMR spectrum of 1 showed two new resonances at δ 4.36 and 5.25, indicating that 1 differed from 2 only by the presence of a hydroxymethyl group on C-4 instead of a methyl group. The resonance at δ 62.7 assignable to a hydroxymethyl carbon in the ¹³C-NMR spectrum of 1 also support this structural assignment. From these...
results, 1 was identified as methyl-5-hydroxy-2-(2,6-dihydroxy-4-hydroxymethylbenzoyl)-3-methoxybenzoate, and the compound was named hydroxysulochrin (Fig. 1).

Sulochrin (2) has been isolated from Oospora sulphurea-ochracea, Aspergillus wentii and Aspergillus terreus var. aureus. Compound 2 has very weak antimicrobial activity and inhibits ecosinophil activation without cytotoxicity, but is not known as a plant growth regulator.4,7,8,10) The biological activities of sulochrin (Fig. 1) and sulochrin (2). Aureobasidium sp. was cultured stationarily in a malt extract medium (50 L) at 24°C for 35 days. The culture filtrate was adjusted to pH 2.0 with 2 N HCl, before being extracted twice with EtOAc. The combined extracts were concentrated in vacuo, and the resulting residue (7.5 g) was fractionated by column chromatography on silica gel (hexane-EtOAc). Two active fractions were obtained from the 40% and 60% EtOAc eluates. The fraction eluted with 40% EtOAc (630 mg) was further fractionated by column chromatography on silica gel (hexane-acetone). The fraction eluted with 30% acetone was purified by preparative TLC, developing with hexane-EtOAc (1:1, v/v), and the solid was recrystallized from acetone-hexane to afford 20 mg of 2 as a yellow amorphous solid. The fraction eluted with 60% EtOAc (327 mg) was further fractionated by column chromatography on silica gel (benzene-acetone). The fraction eluted with 20% acetone was purified by preparative TLC, developing with benzene-acetone (6:4, v/v), and the solid was recrystallized from EtOAc-hexane to afford 14 mg of 1 as a yellow amorphous solid.

**Experimental**

**Instruments.** Melting point (mp) data were determined with Yanagimoto micromelting point apparatus. UV and IR spectra were recorded by Shimadzu UV-2200 and Jasco FT/IR-7000 spectrometer, respectively. 1H- and 13C-NMR spectra were recorded with a Jeol JNM-GX 270 NMR spectrometer at 270 and 68 MHz, respectively. Chemical shifts are expressed in δ values against solvents as internal standards. MS spectra were recorded with a Jeol JMS-AX500 apparatus.

**Isolation and purification of hydroxysulochrin (1) and sulochrin (2).** Aureobasidium sp. was cultured stationarily in a malt extract medium (50 L) at 24°C for 35 days. The culture filtrate was adjusted to pH 2.0 with 2 N HCl, before being extracted twice with EtOAc. The combined extracts were concentrated in vacuo, and the resulting residue (7.5 g) was fractionated by column chromatography on silica gel (hexane-EtOAc). Two active fractions were obtained from the 40% and 60% EtOAc eluates. The fraction eluted with 40% EtOAc (630 mg) was further fractionated by column chromatography on silica gel (hexane-acetone). The fraction eluted with 30% acetone was purified by preparative TLC, developing with hexane-EtOAc (1:1, v/v), and the solid was recrystallized from acetone-hexane to afford 20 mg of 2 as a yellow amorphous solid. The fraction eluted with 60% EtOAc (327 mg) was further fractionated by column chromatography on silica gel (benzene-acetone). The fraction eluted with 20% acetone was purified by preparative TLC, developing with benzene-acetone (6:4, v/v), and the solid was recrystallized from EtOAc-hexane to afford 14 mg of 1 as a yellow amorphous solid.

**Hydroxysulochrin (1).** Mp 210–213°C. UV λmax (EtOH) nm (ε): 217 (20,100), 266 (7,700), 273 (9,500), 328 (4,600), IR νmax (KBr) cm⁻¹: 3378 (OH), 1696 (O=C = O), 1640 (C=O). 1H-NMR (270 MHz, DMSO-d6) δ: 3.63 (s, 3H, Ar-O Me), 3.64 (s, 3H, Ar-COO Me), 4.36 (d, J = 5.3 Hz, 2H, Ar–CH2OH), 5.25 (t, J = 5.3 Hz, 1H, Ar–CH2OH), 6.23 (s, 2H, 3'-H, 5'-H), 6.68 (d, J = 2.0 Hz, 1H, 4'-H), 6.90 (d, J = 2.0 Hz, 1H, 6'-H). 13C-NMR (68 MHz, DMSO-d6) δ: 52.0 (q), 56.0 (q), 62.7 (t), 103.5 (dX2), 104.2 (d), 107.3 (d), 109.9 (s), 126.3 (s), 128.1 (s), 152.1 (s), 156.9 (s), 158.2 (s), 161.7 (sX2), 165.7 (s), 199.8 (s). HRMS m/z (M⁺) calcd: for C17H16O8, 348.0844; found: 348.0868.

**Sulochrin (2).** Mp 250–252°C. UV λmax (EtOH) nm (ε): 217 (32,900), 265 (12,100), 283 (14,500), 324 (6,100). IR νmax (KBr) cm⁻¹: 3350 (OH), 1698 (O–C O), 1640 (C=O). 1H-NMR (270 MHz, DMSO-d6) δ: 2.11 (s, 3H, Ar–Me), 3.59 (s, 3H, Ar–OMe), 3.60 (s, 3H, Ar–COOMe), 6.05 (s, 2H, 3'-H, 5'-H), 6.64 (d, J = 2.0 Hz, 1H, 4'-H), 6.86 (d, J = 2.0 Hz, 1H, 6'-H). 13C-NMR (68 MHz, DMSO-d6) δ: 21.5 (q), 52.0 (q), 55.9 (q), 103.4 (dX2), 107.2 (d), 107.5 (d), 109.2 (s), 126.2 (s), 127.9 (s), 147.3 (s), 156.8 (s), 158.1 (s), 1640 (O–C O), 1640 (C=O).
Bioassay for tea pollen tube growth. Pollen grains of *Camellia sinensis* O. Kuntze were collected from an open flower, dried in a desiccator over silica gel and stored in a refrigerator. The grains were sown with the edge of a cover glass on a 1.5% agar medium containing 10% sucrose, 10 ppm boric acid and the compound to be tested at various concentrations on a microscopic slide, and then incubated in a moist chamber at 24°C in the dark. After cultivating for 12 h, the length of the pollen tube was measured and compared with that of an untreated control. Triplet experiments were conducted.

Bioassay for the growth of lettuce seedlings. Lettuce seeds (*Lactuca sativa* L.) were grown under continuous light (about 2000 lux) at 24°C in a Petri dish (150 × 25 mm) lined with a filter paper moistened with deionized water. After 1 day, 12 seedlings were selected for uniform size (2 mm-long radicles) and transferred to a mini-Petri dish (35 × 15 mm) lined with a filter paper moistened with deionized water (1 mL) and a measured amount of the test compound. The Petri dishes were kept at 24°C for 4 days under continuous light (about 2000 lux). The lengths of the hypocotyls and roots treated with the compounds were measured, and the mean values of the lengths were compared with those of an untreated control. Triplet experiments were conducted.

References