Short Communication

Isolation and Structures of Four
New Metabolites from
Aspergillus wentii

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It is well known that a mold of *Aspergillus wentii* produces toxic metabolites such as aflatoxins¹⁾ and emodin.²⁾ Some papers have also reported that secondary metabolites such as kojic acid,³⁾ β -nitropropionic acid,⁴⁾ 1-amino-2-nitrocyclopentane-1-carboxylic acid,⁵⁾ wentilactone A and B,⁶⁾ and hexadecylcitraconic acid⁷⁾ can be isolated from the same fungus. In the course of our screening search for toxic compounds among fungal metabolites, we found four new compounds in the extracts of mycelial mats of *Aspergillus wentii* IFO 4108 apart from the toxic ones. We wish to report on the structural elucidation of these compound.

Aspergillus wentii IFO 4108 was grown by surface culture on a malt extract medium (100 liters) at 24°C for 21 days. The dried mycelial mats were extracted repeatedly with acetone and the combined extracts concentrated under reduced pressure to give dark brown solids, which were then extracted with hot acetone. After evaporation of the solvent, the residue was subjected to silicAR CC-4 adsorption column chromatography. The eluate with benzene gave a mixture of compounds **1**, **2** and **3**, which was rechromatographed with the same column to separate into individual compounds. The eluate with benzene containing 5% of acetone gave compound **4**.

Compound **2** was recrystallized from acetone to afford pale yellow plates (4009 mg), mp 160~161°C, and its molecular formula was found to be C₁₈H₁₈O₇ [m/z ; 346 (M⁺)] by

elemental analysis. Spectroscopic data of **2** are as follows. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹; 3360, 2950, 1720, 1630, 1600, 1470, 1440, 1240, 1220, 1150, 1065, 950, 910, 830. UV $\lambda_{\max}^{\text{EtOH}}$ nm (ϵ); 282 (13,400), 323 (sh. 5800). UV absorption maxima⁸⁾ together with IR absorption bands at 1630 and 1660 cm⁻¹ indicated the presence of a benzo-phenone skeleton. In the PMR spectrum [(CD₃)₂CO, TMS] of **2**, a 3H singlet due to an aromatic methyl was observed at δ 2.18 and two 3H singlets at δ 3.67 and 3.72 were assigned to two methoxyls. As the presence of the ester function was indicated by an IR absorption band at 1720 and 1240 cm⁻¹, a 3H singlet at δ 3.87 must be assigned to -COOCH₃. A 2H singlet at δ 6.18 was assigned to aromatic protons adjacent to a methyl group observed at δ 2.18, since irradiation at the methyl protons changed the 2H singlet to a sharp singlet. Moreover, two aromatic protons appeared as a meta-relationship at δ 6.80 (1H, d, $J=2$ Hz) and 7.06 (1H, d, $J=2$ Hz). A broad signal at δ 10.0~11.5, equivalent to two protons, disappeared on treatment with D₂O indicating the presence of two phenolic hydroxyls.

On treatment with acetic anhydride-pyridine, **2** afforded the diacetyl derivative **7**, mp 154~155°C, C₂₂H₂₂O₉ [m/z ; 430 (M⁺)]. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹; 2950, 1770, 1730, 1650, 1620, 1600, 1580, 1445, 1370, 1340, 1280, 1220, 1190, 1145, 1060, 1040, 930. UV $\lambda_{\max}^{\text{EtOH}}$ nm (ϵ); 251 (14,100), 316 (5900). PMR $\delta_{\text{TMS}}^{(\text{CD}_3)_2\text{CO}}$; 1.94 (6H, s), 2.79 (3H, s), 3.62 (3H, s), 3.76 (3H, s), 3.92 (3H, s), 6.76 (1H, d, $J=2$ Hz), 6.88 (3H, broad s). A signal at δ 6.88 suggested that a 2H singlet at δ 6.18 in **2** shifted to a lower magnetic field in **7**. This spectroscopic and chemical evidence indicated that two hydroxyl and a methyl group were present in the relationship such as 2,6-dihydroxy-4-methylphenyl in one aromatic ring of a benzophenone skeleton.

The monomethyl ether of **2** was obtained by treatment of **2** with diazomethane in ether. The physicochemical data of monomethyl ether **6** are as follows. Mp 155~156°C. UV $\lambda_{\max}^{\text{EtOH}}$ nm (ϵ); 283 (13,300), 323 (sh. 6700). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹; 2950, 1720, 1630, 1600, 1460, 1350, 1290, 1260,

1230, 1065, 830. PMR $\delta_{\text{TMS}}^{(\text{CD}_3)_2\text{CO}}$; 2.28 (3H, s), 3.34 (3H, s), 3.62 (3H, s), 3.69 (3H, s), 3.86 (3H, s), 6.20 (1H, s), 6.38 (1H, s), 6.82 (1H, d, $J=2$ Hz), 7.08 (1H, d, $J=2$ Hz). These spectroscopic data were found to be identical with those of dimethylsulochrin⁹⁾ which was obtained by methylation of sulochrin **5**⁹⁾ with diazomethane.

On the basis of these results, compound **2** was determined as methyl-2-(2,6-dihydroxy-4-methylbenzoyl)-3,5-dimethoxybenzoate.

Compound **1** was recrystallized from methanol to afford pale yellow needles (438 mg), mp 188~189°C and its molecular formula was determined as $\text{C}_{17}\text{H}_{14}\text{O}_6$ [m/z ; 314 (M^+)] by elemental analysis. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} ; 2950, 1720, 1660, 1620, 1580, 1440, 1430, 1340, 1330, 1290, 1260, 1220, 1160, 860, 845. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ); 235 (48,900), 243 (32,600), 267 (sh, 26,300), 303 (27,900), 351 (10,000). PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$; 2.35 (3H, s), 3.92 (3H, s), 4.03 (3H, s), 6.59 (2H, d, $J=2$ Hz), 6.82 (2H, s), 12.20 (1H, s). UV absorption maxima¹⁰⁾ together with IR absorption bands at 1660, 1620 and 1580 cm^{-1} indicated the presence of a xanthone skeleton

in **1**. These spectroscopic data were found to be identical with those of a xanthone derivative obtained by treatment of **2** with KOH solution. In the case of sulochrin,⁹⁾ a xanthone derivative was also obtained by methanol elimination between the hydroxyl and methoxyl groups of a benzophenone skeleton. From these results, compound **1** was determined as methyl-(1-hydroxy-6-methoxy-3-methylxanthone)-8-carboxylate.

Compound **3** was recrystallized from methanol to afford pale yellow needles (296 mg), mp 261~263°C. The molecular formula was determined as $\text{C}_{16}\text{H}_{12}\text{O}_6$ [m/z ; 310 (M^+)] by elemental analysis. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} ; 3350, 2950, 1700, 1660, 1620, 1510, 1445, 1385, 1340, 1280, 1220, 1180, 1160, 1030, 860. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ); 235 (49,100), 251 (sh, 28,700), 289 (12,900), 308 (11,400), 361 (26,000). PMR $\delta_{\text{TMS}}^{\text{Pyridine-}d_5}$; 2.22 (3H, s), 4.00 (3H, s), 6.60 (2H, s), 7.03 (1H, d, $J=2$ Hz), 7.20 (1H, d, $J=2$ Hz), 9.00~10.50 (2H, broad). On treatment with AlCl_3 in monochlorobenzene, **2** afforded a xanthone derivative which was extracted with ethyl acetate from a sodium bicarbonate so-

lution. The spectroscopic data of **3** were identical with those of the xanthone derivative derived from **2**. Thus the structure of **3** was determined as methyl-(1,6-dihydroxy-3-methylxanthone)-8-carboxylate.

Compound **4** was recrystallized from benzene to afford pale yellow plates (1443 mg), mp 185~186°C, and the molecular formula was found to be $C_{17}H_{16}O_7$ [m/z ; 332 (M^+)] by elemental analysis. The spectroscopic data of **4** are as follows. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3350, 2950, 1690, 1630, 1610, 1590, 1450, 1430, 1360, 1320, 1250, 1200, 1150, 1070, 1010, 910. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 282 (18,200), 325 (sh, 8300). PMR $\delta_{\text{TMS}}^{\text{Pyridine-}d_5}$: 2.07 (3H, s), 3.64 (3H, s), 3.67 (3H, s), 6.46 (2H, s), 6.98 (1H, d, $J=3$ Hz), 7.33 (1H, d, $J=3$ Hz), 10.00~11.50 (3H, broad). The UV spectrum was almost the same as that of **2** indicating the presence of a benzophenone skeleton. On treatment with diazomethane, **4** gave the dimethyl sulochrin mentioned above. Treatment of **4** with KOH solution afforded **1**. From these results, the structure of **4** was determined as methyl-2-(2,6-dihydroxyl-4-methylbenzoyl)-3-hydroxy-5-methoxybenzoate.

The mutagenicities of these compounds **1**, **2**, **3**, and **4**, together with emodin, physcion and 6,8-*O*-dimethyllemodin isolated from this fungus, were tested on *Salmonella typhimurium* tester strains TA 100 and TA 98 according to the method of Ames *et al.*⁽¹¹⁾ with some modifications.⁽¹²⁾

Though emodin, physcion and 6,8-*O*-dimethylemodin were mutagenic to TA 100 with S-9 mix, the four new compounds men-

tioned above were non-mutagenic to TA 100 and TA 98 with or without S-9 mix. These results might indicate that the mutagenicities of the metabolites isolated from *Aspergillus wentii* are responsible for the anthraquinone skeleton.

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