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Studies on Fungal Products. XVI.¹⁾ New Metabolites Related to 3-Methylorsellinate from *Aspergillus silvaticus*

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Aspergillus silvaticus grown on Raulin-Thom medium produced a series of new fungal metabolites: ethyl 3-methylorsellinate (**1**), 6-hydroxy-4-methoxy-5-methylphthalimidine (**2**), and 3,6-dimethyl-4-hydroxy-2-methoxybenzaldehyde (**3**), along with quadrilineatin (**4**). These metabolites may be precursors in the biogenesis of silvaticol (**6**), which has been isolated previously as a metabolite of the same fungus.

Keywords—*Aspergillus silvaticus*; 3-methylorsellinate; phthalimidine; quadrilineatin; versicol; silvaticol

Aspergillus silvaticus FENNEL *et* RAPER, strain IFO 8173, produces a nitrogen-containing “secoanthraquinone,” silvaticamide,²⁾ and phthalides, silvaticol (**6**) and nidulol (**7**).³⁾ Recently we reported the isolation of dioxopiperazine derivatives, dithiosilvatin and silvathione,⁴⁾ and arugosins,¹⁾ from the same fungus. Compound **2** was also isolated from the culture filtrate as a minor component. In all cases, Czapek-Dox or modified Czapek-Dox medium was employed as a substrate for the production of these metabolites.

When cultivated in Raulin-Thom medium, which contains ammonium ion instead of the nitrate ion used in Czapek-Dox medium as a nitrogen source, however, this fungus produces a further series of secondary metabolites **1**, **3**, **4**, and **5** as main components of the culture filtrate, as well as compound **2**. Compounds **4** and **5** were identical with quadrilineatin originally isolated from *Emericella quadrilineata* (THOM *et* RAPER) C. R. BENJAMIN (anam. *Aspergillus tetrazonus* SAMSON *et* GAMS)⁵⁾ and versicol isolated from *Aspergillus versicolor* (VUILL.) TIRABOCHI⁶⁾ and *Sporormia affinis* SACC., BOMM. *et* ROUSS (later transferred to the genus *Sporormiella*).⁷⁾

The spectra of compound **1**, mp 124—126 °C, C₁₁H₁₄O₄, are almost superimposable on those of methyl 3-methylorsellinate (**8**), with the exceptions that the parent peak shows a mass number 14 units higher than that of **8** in the mass spectra (MS) and the appearance of proton nuclear magnetic resonance (¹H-NMR) signals at δ 1.41 (3H, t) and 4.39 (2H, q), which were assigned to the ethyl group of the carboxylate (Table I). Compound **1** was identified as ethyl

Chart 1

TABLE I. ^1H -NMR Chemical Shifts of 3-Methylorsellinate and Related Compounds in CDCl_3

Proton ^{a)}	1 ^{b)}	2 ^{c)}	3	4	6	7	8 ^{d)}
1-CH ₂		4.47			5.39		
1-CHO			10.35	10.37			
2-OH	12.11						12.04
2-OMe		3.89	3.83	3.93	3.91	4.08	
3-Me	2.10	2.16	2.18	2.23	2.23	2.19	2.10
4-OH	5.12	— ^{e)}	6.97	—	—	—	5.23
5-H	6.20	6.91	6.52	7.14	7.04	6.59	6.20
6-Me	2.48		2.54				2.45
6-CH ₂						5.15	
6-CHO				10.37			

a) Numberings of the above compounds correspond to that of 1. b) The signals of the ethyl group of the carboxylate were observed at δ 1.41 and 4.39. c) This compound was measured in CD_3OD . d) The signal of the methyl group of the carboxylate was observed at δ 3.92. e) Lines indicate that the signals were not observed.

2,4-dihydroxy-3,6-dimethylbenzoate (ethyl 3-methylorsellinate), which had been prepared as a synthetic intermediate by Elix and Norfolk.⁸⁾ Recently Aldridge and Moore isolated this compound along with quadrilineatin (**4**) from the same fungus but they could not determine the exact structure.⁹⁾ Though the methyl ester (**8**) has already been reported from *Aspergillus terreus* THOM,¹⁰⁾ etc., this is the first time that the ethyl ester (**1**) of 3-methylorsellinic acid has been isolated as a natural product.

The molecular formula of **2**, mp 217 °C (subl.), was determined from the high-resolution MS as $\text{C}_{10}\text{H}_{11}\text{NO}_3$, which corresponds to the replacement of the lactonic oxygen atom of silvaticol (**6**) or nidulol (**7**) with a nitrogen atom. The ^1H -NMR chemical shifts of **2** are correspond well to those of **6** rather than **7** (Table I). The upfield shift of the signal at δ 5.39 in **6** to the signal at δ 4.47 in **2** indicates that the lactone moiety is replaced with a lactam moiety. From the above results, the structure of **2** was assumed to be 6-hydroxy-4-methoxy-5-methylphthalimidine. It is very interesting that **2** was isolated from *A. silvaticus*, considering that a nitrogen-containing compound, silvaticamide,²⁾ has been isolated from the same fungus.

Compound **3**, mp 151–153 °C, $\text{C}_{10}\text{H}_{12}\text{O}_3$, was considered to be a benzaldehyde derivative, because the ^1H -NMR signal at δ 10.35 can be assigned to the aldehyde conjugated with the benzene ring. The signals of two aromatic methyl groups were observed at δ 2.18 and 2.54. The latter signal was assigned to the methyl group at the *ortho* position of the aldehyde because of its downfield shift compared to the normal aromatic methyl group. The other functional groups of **3** were one methoxyl, one hydroxyl, and one aromatic proton (Table I). In order to determine the exact structure, heteronuclear long-range selective decoupling experiments on **3** were carried out. When the methyl protons adjacent to the aldehyde (δ 2.54, 6-Me) were irradiated, the carbon-13 nuclear magnetic resonance (^{13}C -NMR) signals at δ 114.57 (Dq, C-5), 121.01 (Sdm, C-1), and 141.39 (Sm, C-6) were changed to D, Sdd, and Sd, respectively. On the other hand, the ^{13}C -NMR signals at δ 159.93 (Sm, C-4) and 165.30 (Sm, C-2) were changed into Sd and Sqd, respectively, by selective irradiation of the other methyl protons (δ 2.18, 3-Me). From the above results and the chemical shifts and multiplicity of the ^{13}C -NMR signals, compound **3** was confirmed to be 3,6-dimethyl-4-hydroxy-2-methoxybenzaldehyde.

Prior to this study, epidithiodioxopiperazine, dithiosilvatin,⁴⁾ “seco-anthraquinone,” arugosins,¹⁾ and phthalides, silvaticol (**6**) and nidulol (**7**)³⁾ had been found as secondary

metabolites of *A. silvaticus* in Czapek-Dox medium. Although factors affecting silvaticol (**6**) production, especially in Czapek-Dox medium, have not yet been studied, its biogenesis is presumably as follows. 3-Methylorsellinate (**1** or a corresponding compound), formed *via* the acetate-malonate pathway followed by cyclization and introduction of a C₁ unit at the C-3 position, would be transformed into the aldehyde (**3**) by *O*-methylation at C-2 followed by reduction of the carboxylate. Then the oxidation of the methyl group at C-6 in **3** gives quadrilineatin (**4**), which is the key intermediate to **6** and **7**.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Infrared (IR) and ultraviolet (UV) spectra were recorded on a Hitachi 215 spectrophotometer and a Hitachi 124 spectrophotometer, respectively. MS were obtained on a JEOL JMS-D 300 spectrometer. ¹H- and ¹³C-NMR spectra were measured with a JEOL JNM-FX 100 at 99.60 MHz and a JEOL JNM-GX 400 at 100.43 MHz, respectively, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet=S or s, doublet=D or d, triplet=t, quartet=Q or q, multiplet=m, and broad=br. Capital letters refer to the pattern resulting from directly bonded coupling (¹J_{C,H}).

Isolation of Metabolites from *Aspergillus silvaticus*—*A. silvaticus*, strain IFO 8173, was incubated at 27 °C for 14 d in Raoult-Thom medium [(NH₄)₂HPO₄ 0.6 g, (NH₄)₂SO₄ 0.25 g, K₂CO₃ 0.6 g, MgCO₃ 0.4 g, FeSO₄·7H₂O 0.07 g, ZnSO₄·7H₂O 0.07 g, tartaric acid 4.0 g, ammonium tartrate 4.0 g, glucose 75 g, water 1500 ml]. The culture filtrate (22.5 l) was extracted with dichloromethane at pH 2. The evaporated residue (5.3 g) was chromatographed on silica gel. Elution with chloroform afforded **1** (50 mg), 3,6-dimethyl-4-hydroxy-2-methoxybenzaldehyde (**3**) (40 mg), and versiol (**5**) (100 mg), elution with chloroform-methanol (50:1, v/v) gave quadrilineatin (**4**) (20 mg), and elution with chloroform-methanol (20:1, v/v) provided **2** (5 mg). Compounds **4** and **5** were identified by comparison of the spectral data, including optical rotation, with those of authentic samples.

Ethyl 3-Methylorsellinate (**1**): Colorless needles, mp 124–126 °C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3450 (OH), 1620 (COO). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 217 (4.40), 268 (4.31), 295 (3.79). MS m/z : 210 (31%, M⁺), 164 (97), 136 (100). ¹H-NMR (CDCl₃) δ : 1.41 (3H, t, $J=7.1$ Hz, -CH₂CH₃), 2.10 (3H, s, Me), 2.48 (3H, s, Me), 4.39 (2H, q, $J=7.1$ Hz, -OCH₂CH₃), 5.12 (1H, s, OH), 6.20 (1H, s), 12.11 (1H, s, OH). Compound **1** was identified by comparison of the IR, ¹H-NMR, and MS with those of an authentic sample.

6-Hydroxy-4-methoxy-5-methylphthalimidine (**2**): Colorless crystalline powder, mp 217 °C (subl.). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3190 (OH), 1710, 1660. UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 212 (3.95), 252 (3.29), 295 (2.98). MS m/z : 193 (100%, M⁺), 178 (56), 162 (46), 149 (30). High-resolution MS m/z : 193.0682 (Calcd for C₁₀H₁₁NO₃: 193.0737). ¹H-NMR (C₅D₅N) δ : 2.50 (3H, s, Me), 3.84 (3H, s, OMe), 4.55 (2H, s), 7.61 (1H, s), 9.27 (1H, s, NH or OH); (CD₃OD) δ : 2.16 (3H, s, Me), 3.89 (3H, s, OMe), 4.47 (2H, s), 6.91 (1H, s).

3,6-Dimethyl-4-hydroxy-2-methoxybenzaldehyde (**3**): Colorless crystalline powder, mp 151–153 °C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3100 (OH), 1660 (COO). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 232 (4.11), 284 (4.15). MS m/z : 180 (100%, M⁺), 165 (35), 163 (85), 149 (16), 135 (35), 120 (37), 107 (14), 91 (31), 77 (26). High-resolution MS m/z : 180.0788 (Calcd for C₁₀H₁₂O₃: 180.0787). ¹H-NMR (CDCl₃) δ : 2.18 (3H, s, Me), 2.54 (3H, s, Me), 3.83 (3H, s, OMe), 6.52 (1H, s), 6.97 (1H, br s, OH), 10.35 (1H, s, CHO). ¹³C-NMR (CDCl₃) δ : 8.12 (Q, 3-Me), 21.43 (Qd, 6-Me), 63.10 (Q, 2-OMe), 114.57 (Dq, C-5), 115.41 (Sm, C-3), 121.01 (Sdm, C-1), 141.39 (Sm, C-6), 159.93 (Sm, C-4), 165.30 (Sm, C-2), 191.48 (D, 1-CHO).

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