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

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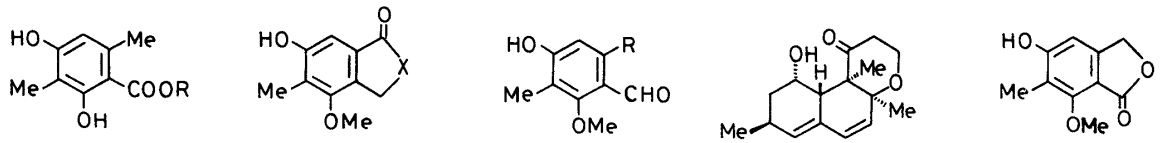
Aspergillus silvaticus grown on Raulin-Thom medium produced a series of new fungal metabolites: ethyl 3-methylorsellinate (**1**), 6-hydroxy-4-methoxy-5-methylphthalimidine (**2**), and 3,6-dimethyl-4-hydroxy-2-methoxybenzaldehyde (**3**), along with quadrilineatin (**4**). These metabolites may be precursors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

Keywords——*Aspergillus silvaticus*; 3-methylorsellinate; phthalimidine; quadrillineatin; versiol; silvaticol

Aspergillus silvaticus FENNELL *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 versiol isolated from *Aspergillus versicolor* (VUILL.) TIRABOCHI[6] and *Sporormia affinis* SACC., BOMM. *et* ROUSS (later transferred to the genus *Sporormiella*).[7]

The spectra of compound **1**, mp 124—126 °C, $C_{11}H_{14}O_4$, 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 (^{1}H-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



1 : R = Et
8 : R = Me

2 : X = NH
6 : X = O

3 : R = Me
4 : R = CHO

5

7

Chart 1

TABLE I. ^{1}H-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 $C_{10}H_{11}NO_3$, which corresponds to the replacement of the lactonic oxygen atom of silvaticol (**6**) or nidulol (**7**) with a nitrogen atom. The ^{1}H-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 δ4.47 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, $C_{10}H_{12}O_3$, was considered to be a benzaldehyde derivative, because the ^{1}H-NMR signal at δ10.35 can be assigned to the aldehyde conjugated with the benzene ring. The signals of two aromatic methyl groups were observed at δ2.18 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 (δ2.54, 6-Me) were irradiated, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals at δ114.57 (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 δ159.93 (Sm, C-4) and 165.30 (Sm, C-2) were changed into Sd and Sqd, respectively, by selective irradiation of the other methyl protons (δ2.18, 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 C_1 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. ^{1}H- and ^{13}C-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 Raulin-Thom medium [$(NH_4)_2HPO_4$ 0.6 g, $(NH_4)_2SO_4$ 0.25 g, K_2CO_3 0.6 g, $MgCO_3$ 0.4 g, $FeSO_4 \cdot 7H_2O$ 0.07 g, $ZnSO_4 \cdot 7H_2O$ 0.07 g, tartaric acid 4.0 g, ammonium tartarate 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 ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1620 (COO). UV λ_{max}^{MeOH} nm (log ε): 217 (4.40), 268 (4.31), 295 (3.79). MS *m/z*: 210 (31%, M^+), 164 (97), 136 (100). ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz, $-CH_2\underline{CH}_3$), 2.10 (3H, s, Me), 2.48 (3H, s, Me), 4.39 (2H, q, J = 7.1 Hz, $-O\underline{CH}_2CH_3$), 5.12 (1H, s, OH), 6.20 (1H, s), 12.11 (1H, s, OH). Compound **1** was identified by comparison of the IR, ^{1}H-NMR, and MS with those of an authentic sample.

6-Hydroxy-4-methoxy-5-methylphthalimidine (**2**): Colorless crystalline powder, mp 217 °C (subl.). IR ν_{max}^{KBr} cm^{-1}: 3190 (OH), 1710, 1660. UV λ_{max}^{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 $C_{10}H_{11}NO_3$: 193.0737). ^{1}H-NMR (C_5D_5N) 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); (CD_3OD) 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 ν_{max}^{KBr} cm^{-1}: 3100 (OH), 1660 (COO). UV λ_{max}^{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 $C_{10}H_{12}O_3$: 180.0787). ^{1}H-NMR ($CDCl_3$) 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). ^{13}C-NMR ($CDCl_3$) 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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