Metabolites of *Aspergillus cervinus* Massee (Moniliaceae)

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Abstract □ An investigation of the metabolites produced by *Aspergillus cervinus* Massee (Moniliaceae), when grown in submerged culture on a malt extract medium, resulted in the isolation and identification of the quinol terremutin and 3,6-dihydroxy-2,5-toluquinone.

Keyphrases □ *Aspergillus cervinus* Massee (Moniliaceae)—isolation and identification of terremutin and 3,6-dihydroxy-2,5-toluquinone □ Terremutin—isolation and identification from *A. cervinus* □ 3,6-Dihydroxy-2,5-toluquinone—isolation and identification from *A. cervinus*

The genus *Aspergillus* (Moniliaceae) may be divided into 18 groups based on cultural and morphological considerations (1). In accordance with this division, *Aspergillus cervinus* Massee belongs to the *A. cervinus* group, a group consisting of four species and having an ill-defined place within the genus. Similarities between members of this group and members of other groups within the genus makes exact placement difficult. *A. cervinus* bears some similarities to *A. terreus* and was at one time assigned to its group (2). Since *A. cervinus* had not been studied phytochemically and since this knowledge could be of help to its taxonomy, the metabolites of this organism were studied.

DISCUSSION

For this study, *A. cervinus* was grown in a malt extract medium by submerged fermentation. At harvest the mycelium was removed by filtration, and the clear broth was acidified and extracted with ethyl acetate. Concentration of the ethyl acetate extract afforded terremutin (I) as a crystalline mass. Terremutin has previously been isolated only from cultures of a mutant strain of *A. terreus* (3).

Chromatography of the mother liquors from the crystallization of terremutin over silicic acid afforded 3,6-dihydroxy-2,5-toluquinone (II). This compound has been obtained as a degradation product of actinomycin C (4), has been isolated from cultures of *A. fumigatus* (5), and has been prepared synthetically (6).

Minute quantities of several other metabolites have been detected in cultures of *A. cervinus* by TLC, and work is underway to isolate and identify them.

The isolation of terremutin from *A. cervinus* tends to indicate a relationship to *A. terreus*, while the isolation of 3,6-dihydroxy-2,5-toluquinone could indicate a relationship to *A. fumigatus*. Thus, the taxonomical position of *A. cervinus* within the genus *Aspergillus* must remain as it is until further work establishes it firmly.

EXPERIMENTAL[1]

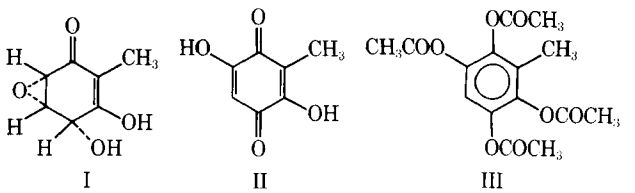
Organism—*A. cervinus*[2] (ATCC 16915) was maintained on slopes of Mycophil[3] agar.

Cultural Conditions—The organism was grown in a medium consisting of malt extract[4] (20 g), glucose[5] (20 g), Phytone[3] (1 g), and distilled water (1 liter). Five liters of this medium was distributed in 125-ml quantities into 40, 250-ml, wide-mouth erlenmeyer flasks; the flasks were stoppered with foam plugs[6] and autoclaved at 15 psi for 20 min. Each flask was inoculated with 1 ml of a spore suspension prepared by adding 10 ml of sterile 1:10,000 sodium lauryl sulfate solution to a heavily sporulated agar slope of *A. cervinus*. The flasks were incubated in the dark on a rotary shaker at 150 rpm for 14 days at 25–30°.

Isolation of Terremutin (I)—The contents of the flasks were pooled and filtered, and the filtrate was acidified to pH 2–3 with concentrated hydrochloric acid and extracted with 3 × 5-liter portions of ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate and concentrated *in vacuo* at 40° to about 25 ml. A crystalline mass (1.7 g) was deposited overnight. Recrystallization of this material first from methanol and then from ethyl acetate afforded large prisms of I (1.56 g), mp 146–147°; $[\alpha]_D^{25.5}$ −264.2° (c 1.00, methanol); λ_{max} (CH_3OH): 272 (log ε 4.03) and 315 (sh)(3.77) nm, with a bathochromic shift of the 272-nm peak to 310 nm in 0.01 *N* methanolic potassium hydroxide; ν_{max} (KBr): 3320, 3123, 2953, 1630, 1400, and 1015 cm^{-1}; NMR (at 60 MHz in D_2O): δ 1.68 (s, 3H, CCH_3), 3.65 (d, 1H, J = 4 Hz), 3.85 (d, 1H, J = 4 Hz, epoxide H), and 4.78 (s, 1H, OCH). The mass spectrum gave significant peaks at *m/e* 156 (1.5%) (M^+), 73 (30), 71 (43), and 56 (100).

The isolated material was identical by melting point, mixed melting point, IR, UV, NMR, and mass spectroscopic analyses to an authentic sample of terremutin.

Isolation of 3,6-Dihydroxy-2,5-toluquinone (II)—The ethyl acetate mother liquors from the initial crystallization of terremutin were evaporated to give a residue (6.40 g). This residue was chromatographed over a column of silicic acid[7] (220 g) and eluted with benzene (2-liter fractions). The third and fourth fractions were combined and evaporated to yield an orange residue (190 mg). Crystallization of this material from benzene–methanol afforded orange scales of II (126 mg), mp 160–165° [lit. (4) mp 157–163° and (6) mp 177°]; λ_{max} (CH_3OH): 287 (log ε 3.56) and 420 (2.38) nm [lit. (5) λ_{max} 282 and 420 nm]; ν_{max} (KBr): 3320, 2940, 2860, 1610, 1370, 1310, 1170, 1040, 855, 765, and 700 cm^{-1}; NMR (at 60 MHz in trifluoroacetic acid): δ 2.05 (s, 3H, CCH_3) and 6.29 (s, 1H, quinone H). The mass spectrum gave significant peaks at *m/e*



I II III

[1] Melting points were taken on a Thomas-Hoover Uni-Melt capillary apparatus and are corrected. IR spectra were determined on a Beckman model IR-8 spectrometer in KBr pellets. UV spectra were obtained on a Perkin-Elmer model 202 recording spectrometer. NMR spectra were obtained on a Perkin-Elmer model R-24 spectrometer in D_2O or trifluoroacetic acid solutions. Optical rotations were measured in a Rudolph polarimeter. Mass spectra were obtained with an LKB-9000 mass spectrometer. Fermentations were carried out on a New Brunswick scientific model G-10 gyrotory shaker.

[2] Obtained from the American Type Culture Collection.

[3] Baltimore Biological Laboratory, Baltimore, Md.

[4] Fisher Scientific Co., Pittsburgh, Pa.

[5] Dextrose, laboratory guide, Fisher Scientific Co.

[6] DiSP., Scientific Products, Edison, N.J.

[7] Mallinckrodt Chemical Works, St. Louis, Mo.

154 (100%) (M^+), 126 (13), 98 (16), and 80 (27), compatible with II (7). Attempts to obtain an authentic sample of II for direct comparison were unsuccessful.

Reductive Acetylation of 3,6-Dihydroxy-2,5-toluquinone—The identification of 3,6-dihydroxy-2,5-toluquinone was further substantiated by its conversion to 2,3,5,6-tetraacetoxytoluene (III) by reductive acetylation (4, 6). Twenty milligrams of II was refluxed for 2 hr with 200 mg of powdered zinc and 5 ml of acetic anhydride. The mixture was cooled and filtered, and the filter was washed with 2 × 5 ml of hot acetic acid. The combined filtrate and washings were diluted to 30 ml with distilled water and extracted with 4 × 30 ml of chloroform. Evaporation of the pooled, dried chloroform extracts gave a white, crystalline residue (17 mg). Recrystallization of this residue from chloroform gave white needles of III, mp 197–198°, whose physical characteristics (melting point, mixed melting point, IR, NMR, and mass spectroscopy) were identical to those of an authentic sample.

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Synthesis of 5,5-Diethyl-2-ethoxytetrahydro-4,6-pyrimidinedione

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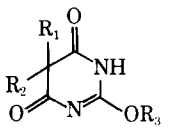
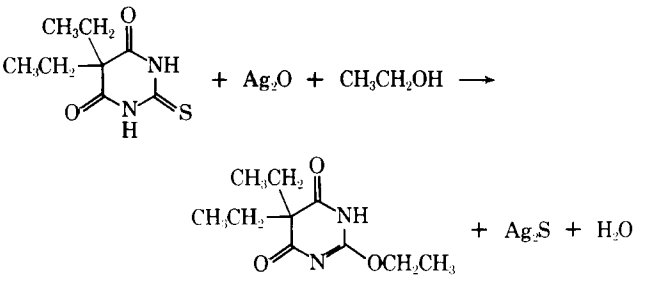
Abstract □ The original synthesis of 5,5-diethyl-2-ethoxytetrahydro-4,6-pyrimidinedione, a new pyrimidine derivative, is described. Evidence of its structure is given.

Keyphrases □ 5,5-Diethyl-2-ethoxytetrahydro-4,6-pyrimidinedione—synthesis, structure determination □ Pyrimidine derivatives—synthesis of 5,5-diethyl-2-ethoxytetrahydro-4,6-pyrimidinedione, structure determination

Alkoxy tetrahydro-4,6-pyrimidinediones (I), related to the family of barbituric acid derivatives, were prepared by a number of workers, starting from isourea alkyl ethers and condensing with substituted malonic esters or nitriles (1–3). They have also been obtained as by-products of the methylation of barbituric acid derivatives (4, 5). These synthesis methods reported require several steps. In the present paper, a new synthesis is reported for a one-step preparation in which silver oxide acts upon 5,5-diethyl-2-thiobarbituric acid in ethanol to give a new pyrimidine derivative, 5,5-diethyl-2-ethoxytetrahydro-4,6-pyrimidinedione



I



II

Scheme I

(II) (Scheme I). This reaction is similar to the general reaction of amides with silver oxide to form a salt (6) which is readily transformed into imidocarboxylic

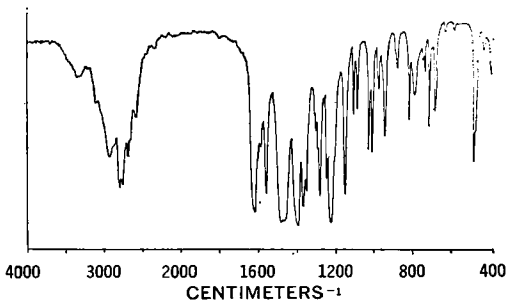


Figure 1—*IR spectrum of II.*