Indole metabolites of *Penicillium cyclopium* NRRL 6093[1]

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Summary. The indoles penitrem A and B and roquefortine were isolated from fungal cultures of *Penicillium cyclopium* grown on Czapek-Dox medium at 25 °C for 2 weeks.

We report the isolation of the indoles penitrem A and B and roquefortine from the mycelium of *P. cyclopium* NRRL 6093 grown on Czapek Dox medium. *Penicillium cyclopium* strain NRRL 6093 isolated from dry sausage[2] was selected for our study because of its ability to produce tremorgenic compounds and other unknown secondary metabolites.

Penicillium strains have been shown by a number of investigators to produce a variety of toxic metabolites. *Penicillium cyclopium,* the principal organism found in feedstuffs that caused toxicoses in sheep and horses, also produced tremorgenic compounds in liquid culture[3]. 2 of these compounds, penitrem A ($C_{37}H_{44}NO_6Cl$) and penitrem B ($C_{37}H_{45}NO_6$), appeared to be structurally related and both caused tremors in mice[4]. In a neurobehavioral study of penitrem A in mice, it was found that the i.p. median tremorgenic dose was 0.19 mg/kg[5]. Birkinshaw et al.[6] found penicillic acid in culture extracts of *P. cyclopium.* Ciegler et al.[2] detected ochratoxin A in liquid cultures of *P. cyclopium,* a metabolite first discovered in fungal cultures of *Aspergillus ochraceus*[7].

Roquefortine is an indole alkaloid metabolite of *P. roqueforti*[8,9], the mold commonly found in fermenting silage and the usual mold found in cheeses of Roquefort type[10]. This toxin is reported to cause convulsive seizures in mice when injected i.p. at a dose of 10 mg/kg; its LD_{50} to male mice was 15–20 mg/kg i.p.[8].

P. cyclopium was maintained on malt extract agar slants. Fernbach flasks, each containing 500 ml of Czapek-Dox media supplemented with 2% corn steep liquor[11], were inoculated with 1 ml of a *P. cyclopium* spore suspension. The suspension was made by adding 5 ml sterile distilled water to a 2-week-old malt extract agar slant and dislodging the spores with a sterile loop. After inoculating, the flasks were incubated in static culture at 25 °C for 2 weeks. The mycelium was separated from the liquid medium by filtering through cheesecloth. The mycelium was extracted with chloroform-methanol (70:30) and filtered. The filtrate was dehydrated over anhydrous sodium sulfate, filtered, and then evaporated to dryness under vacuum. The remaining residue was placed on a Florisil column, (30 g, 60–100 mesh) which was eluted with chloroform. Eluates contained penitrems A and B along with other compounds, including viridicatin and substances that fluoresce yellow and blue-green under long UV light. Subsequent elution with chloroform-methanol (95:5) eluted a bright yellow fraction in yields of 148 mg/l. Purification by crystallization from methanol-water gave a white solid m.p. 193–196 °C (Fisher Johns uncorrected) and a formula composition $C_{22}H_{23}N_5O_2$ (m/e calculated 389.1851; found, 389.1850) as determined by high-resolution mass spectroscopy. UV-spectra showed $\lambda_{max}CH_3OH$ 325, 240 and 206 nm (log $\varepsilon = 4.42$, 4.20 and 4.49, respectively); IR-analysis γ_{max} ($CHCl_3$) 3420, 3290, 3200, 1680, 1660, 1600 cm^{-1}. These spectroscopic analyses, as well as ^{13}C and ^1H NMR-spectra, and the elemental analysis agreed with those of roquefortine reported by Scott et al.[8]. In addition, the mass spectrum[12] indistinguishable from that of roquefortine corresponded to $C_{22}H_{23}N_5O_2$.

The elaboration of the neurotoxin roquefortine by *P. cyclopium* concurrent with other tremorgenic compounds suggests the potential hazard of this mold, particularly since it is commonly found in stored grains and various cereal products[13]. Additional investigation of this alkaloid's role in mycotoxicoses of farm animals is indicated.

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