

Production of Cyclopiazonic Acid by *Aspergillus flavus* Link

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Received for publication 17 August 1976

Production of cyclopiazonic acid by *Aspergillus flavus* is reported for the first time. A procedure for its production by agitated solid substrate fermentation on red wheat is described along with the isolation procedure and physical and chemical properties of this indole derivative. The compound has been found to exert antibacterial activity.

Cyclopiazonic acid is a toxic indole derivative, which until 1973 had been isolated only from liquid cultures of *Penicillium cyclopium* Westling (2, 3, 5). In 1973, Ohmomo et al. reported its production by *Aspergillus versicolor* (4). In the present communication, we described the isolation of this compound from *A. flavus* Link (the identity of the fungus was determined by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) grown on agitated red wheat.

The agitated solid substrate fermentation technique has been successfully used for the production of several mycotoxins (1). In the present study it was possible to increase the production of the crude toxin of *A. flavus* by almost 10-fold as compared with the more conventional static rice technique. The new procedure yielded an average of 1.6 g of crude toxin per 1 kg of red wheat having a mean lethal dose of about 25 mg/kg; details of the agitated solid substrate technique are described elsewhere (1). Moist sterile grains of red wheat were inoculated with spores of the fungus and incubated at 30°C for 10 days on a rotary shaker. After the incubation, the grains were extracted with dichloromethane, the filtrate was evaporated, and the residue was precipitated in petroleum ether. The precipitate (crude toxin) was collected, dissolved in chloroform, and extracted with 1 N potassium bicarbonate. The acidic extract was chromatographed on silica gel plates (impregnated with 6.3% tartaric acid and containing fluorescent indicator; about 40 mg of acidic extract per plate [20 by 20 by 0.1 cm]), using 20% 2-pentanone in chloroform as the solvent. The dark, nonfluorescent band with an R_f of about 0.6 was collected and eluted with chloroform-acetone to give a fraction which, after washing with water to remove tartaric acid, contained cyclopiazonic acid and minor traces of other acidic impurities. This mixture was then applied to a Dowex 1X8 (200 to 400

mesh, formate form, 150 mg of mixture per 20 g of resin) anion-exchange column and eluted with a gradient of 0 to 3.5 M formic acid in 1:1 aqueous methanol. Fractions containing cyclopiazonic acid were combined and crystallized from methanol to give pure cyclopiazonic acid (11% of the weight of the crude toxin): needles, melting point, 236 to 238°C; mass spectrum m/e (percent intensity) 336.14552 (81, M^+ , calculated for $C_{20}H_{20}N_2O_3$: 336.14739), 196 (50), 182 (100), 181 (58), 156 (39), 155 (44), 70 (48); ultraviolet (EtOH) 223 (ϵ 39,400), 277 (sh 18,400), 281 (19,300), 290 (sh 15,600) nm; on basification: 222 (40,300), 252 (16,300), 277 (sh 18,400), 281 (19,300), 291 (sh 15,600); CD (MeOH) 225 ($\Delta\epsilon$ -39), 235 (-22), 279 (-9), 289 (0), 320 nm (+4); infrared ($CHCl_3$) 3,520, 3,000, 2,945, 1,710, 1,640, 1,615, 1,450, 1,430, 1,390, 1,375, 1,360, 1,340, 1,290, 1,270, 1,260, 1,160, 1,110, 1,085, 1,045, 980 cm^{-1} ; nuclear magnetic resonance ($CDCl_3$) δ 1.65 (3H, s), 1.69 (3H, s), 2.34 (3H, s), 2.67 (1H, m), 3.02 and 3.11 (2H, 2 br s), 3.54 (1 H, dd, J - 5.5 and 10 Hz), 4.04 (1H, d, J - 10 Hz), 6.70-6.85 (1H, m), 6.98-7.10 (3H, m), 8.07 (1H, br, exchanges with D_2O), 12.1 (1H, br, exchanges with D_2O). Attempts to methylate or acetylate cyclopiazonic acid gave only very complex reaction mixtures, from which no pure product was isolated. Cyclopiazonic acid was not reduced by catalytic hydrogenation over 10% Pd on charcoal.

When its antibacterial activity was tested with the disk agar diffusion assay (1), the cyclopiazonic acid was found to be active against *Bacillus megaterium*.

This work was supported by Public Health Service contract 1CP33217 from the National Cancer Institute. High-resolution mass spectra were measured in the National Institutes of Health supported facility at Massachusetts Institute of Technology (Public Health Service Grant FR00317 from the Division of Research Facilities and Resources) under the direction of K. Biemann.

We are indebted to G. N. Wogan and P. Donehue, Department of Nutrition and Food Science, Massachusetts

Institute of Technology, for bioassays throughout the isolation work. The microbiological studies were under the direction of A. L. Demain.

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