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Note

Separation of the mycotoxins α- and β-cyclopiazonic acids using DEAE-cellulose

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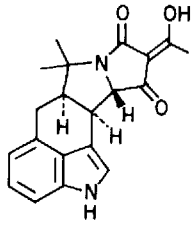
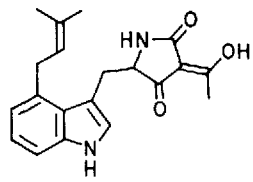
α-Cyclopiazonic acid[1] (α-CPA) (I) is an important mycotoxin produced by the ubiquitous fungi *Aspergillus flavus*, *A. oryzae* and a variety of *Penicillium* species, including *P. griseofulvum*, *P. viridicatum*, *P. crustosum*, *P. puberulum* and *P. patulum*[2,3]. The immediate biogenetic precursor of α-CPA, β-cyclopiazonic acid[4] (β-CPA) (II), frequently accompanies α-CPA in extracts of the above fungi. Mixtures of these metabolites have proved difficult to separate and purify; several stationary phases and eluents have been used[1,3–5] and recent biosynthetic investigations[5,6] have relied on manipulation of fungal growth conditions to circumvent this problem[7]. We report here an efficient and simple method to purify α-CPA and β-CPA in the presence of other co-metabolites using DEAE-cellulose. This method is of particular applicability in preparative separation of α-CPA and β-CPA.



I



II

EXPERIMENTAL

Activated Whatman DE-52 DEAE-cellulose[8] was washed with ammonium hydrogen carbonate solution (0.02 *M*) until the washings had essentially the same pH as the initial solution (7.95). A slurry of the cellulose in this initial buffer was then packed into a glass column (48 × 1.9 cm I.D.) and allowed to settle under gravity. The column was connected to a Gilson Minipuls 2 peristaltic pump, flow-rate 30 ml h^{-1}, and the ultraviolet absorption at 280 nm of the eluent was compared before and after passing through the column using an ISCO UA-2 ultraviolet analyzer. A crude extract (500 mg) of *P. griseofulvum* Dierckx. CSIR 1082, a known producer of α-CPA

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and β-CPA[5], in ammonium hydrogen carbonate solution (0.02 *M*, 2 ml) was injected onto the column and fractions (7.5 ml each) were collected. Solvent and ammonium hydrogen carbonate were removed by freeze-drying for 24 h. Initially ammonium hydrogen carbonate solution (0.02 *M*) was pumped through the column for 2 h and then a linear gradient from 0.02 *M* to 0.2 *M* was started, the final molarity being reached after a further 26 h. Elution with this final buffer was then maintained until both α-CPA and β-CPA had eluted from the column (the identity of the metabolites was confirmed by thin-layer chromatography and comparison of physical data with authentic standards).

RESULTS AND DISCUSSION

Despite the presence of numerous co-metabolites both α-CPA and β-CPA were obtained in a pure state using the above system. β-CPA (5 mg) was eluted first and was contained in fractions 170–192; α-CPA (11 mg) was present in fractions 230–260.

These results are important for future biosynthetic experiments on the metabolites, obviating the separation problems encountered previously. In addition this method may be used as an analytical probe for the presence of α-CPA, and coupled with thin-layer chromatography[3] should provide unambiguous evidence for contamination by this toxin. Finally the method is ideal for preparative separation and accumulation of substantial amounts of the two metabolites.

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