An Assay of Sterigmatocystin and Related Metabolites of *Aspergillus versicolor* by High Speed Liquid Chromatography

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It is well-known that sterigmatocystin is a carcinogenic metabolite⁽¹⁾ of *Aspergillus versicolor* (Vuillemin) Tiraboschi,⁽²⁾ *Aspergillus nidulans* (Eidam) Winter,⁽³⁾ and *Aspergillus rugulosus* Thom et Raper.⁽⁴⁾ *Aspergillus versicolor* is widely distributed and frequently found in grains and stored foods.⁽⁵⁾ Together with sterigmatocystin this mold produces demethylsterigmatocystin, carcinogen,⁽⁶⁾ and many related metabolites. No standard, routine method has not yet been described for detection of demethylsterigmatocystin. Several methods have been reported for the assay of sterigmatocystin by Vorster *et al.*⁽⁷⁾ and Stack *et al.*⁽⁸⁾ These methods were based on measuring the fluorescence intensity of a spot of sterigmatocystin on TLC plate. Owing to the low sensitivity, these methods were not well enough to be useful in a routine screening procedure.

This paper describes an analytical method by high speed liquid chromatography of sterigmatocystin, demethylsterigmatocystin,⁽⁹⁾ 5-methoxysterigmatocystin,⁽¹⁰⁾ sterigmatin,⁽¹¹⁾ 6-deoxyversicolorin A,⁽⁹⁾ 6,8-O-dimethylversicolorin A,⁽¹²⁾ aversin (6,8-O-dimethylversicolorin B),⁽¹³⁾ and 6,8-O-dimethylaverufin,⁽¹⁴⁾ all of which are metabolites of *Aspergillus versicolor*.

These metabolites were isolated from the mycelial mat of this mold and purified by the column chromatography over silica gel and recrystallization. Shimadzu-DuPont Model LC-830 liquid chromatograph was used with Shimadzu UV-202 ultraviolet spectromonitor.

Eight strains of *Aspergillus versicolor* were grown on malt extract medium at 24°C. After 14 days each mycelial mat was harvested and dried. Two grams of the dried mycelial mat were placed in a 500 ml of Erlenmeyer flask fitted with a reflux condenser and 200 ml of acetone was added. The solution was refluxed for 24 hr and filtered. This extraction was repeated five times. The combined solutions were

evaporated *in vacuo* to produce dark brown extracts. For removing interfering metabolites, a certain amount of the dark brown extracts which contained less than ca. 0.5 mg of sterigmatocystin was subjected to chromatography on a column (350×22 mm *i.d.*) of 10 g of silica gel [silic AR CC-4 (200~325 mesh, Mallinckrodt)]. Elution with 530 ml of chloroform was performed at a rate of 5 ml per min. The first eluate (30 ml) was discarded and 500 ml of the next eluate was collected and concentrated. The residue was dissolved in 1 to 5 ml of chloroform. The chloroform solution was subjected to high speed liquid chromatography.

A Zorbax SIL column was employed for the separation of metabolites. Various solvent combinations were examined including chloroform-*n*-hexane-acetic

FIG. 1. Typical Elution Chromatogram of Sterigmatocystin and Related Metabolites.

Operating conditions

Instrument: Shimadzu-DuPont LC-830 liquid chromatograph

Column: Zorbax SIL (250×2.1 mm *i.d.*)

Column temperature: room temp. (20°C)

Mobile phase: 75% *n*-hexane, 20% methylene chloride (25% water saturated), 5% chloroform (25% water saturated), 0.1% acetic acid

Column pressure: 90 kg/cm²

Flow rate: 0.45 ml/min

Detector: UV detector at 320 nm (0.08 A.U.F.S.)

Peak identity

1. Sterigmatin 200 ng
2. Demethylsterigmatocystin 200 ng
3. 6-Deoxyversicolorin A 500 ng
4. 6,8-O-Dimethylaverufin 200 ng
5. 6,8-O-Dimethylversicolorin A 200 ng
6. Sterigmatocystin 166.7 ng
7. Aversin 200 ng
8. 5-Methoxysterigmatocystin 200 ng

TABLE I. ANALYTICAL RESULTS OF STERIGMATOCYSTIN IN THE DRIED MYCELIAL MATS OF *Aspergillus versicolor* BY HIGH SPEED LIQUID CHROMATOGRAPHY

Strains of <i>Aspergillus versicolor</i>	Sterigmatocystin content (ppm)
IFO 4105	22400
NI 5346	1480
NI 5361	111
IFO 6220	176
IFO 6282	20800
IFO 6576	1720
IFO 7082	7980
IFO 7781	1442

acid, methylene chloride-*n*-hexane-acetic acid, and chloroform-methylene chloride-*n*-hexane-acetic acid, in which the last one was selected as the best combination.

As shown in Fig. 1, the complete separation of sterigmatocystin and related metabolites was obtained with a solvent consisting of 75% *n*-hexane, 20% methylene chloride (25% water saturated), 5% chloroform (25% water saturated), and 0.1% acetic acid. Sterigmatocystin has UV absorption maxima at 325, 246, 233 and 205 nm in ethanol. UV absorption detection at 320 nm was most effective, because on this condition the peak area of sterigmatocystin was proportional to its quantity in the range of 6~180 ng and 3 ng of sterigmatocystin was detectable.

The mycelial mats of eight strains of *Aspergillus versicolor* were extracted with acetone. After removal of the interfering metabolites by column chromatography, sterigmatocystin was analyzed. Results obtained are shown in Table I.

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