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Note

Thin-layer chromatographic separation of sterigmatocystin, 5-methoxysterigmatocystin and O-methylsterigmatocystin

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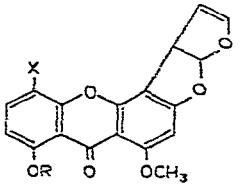
Sterigmatocystin (I), a mycotoxin known to be a potent hepatocarcinogen, has been isolated from the mycelium of *Aspergillus versicolor*, *Aspergillus nidulans*, and *Bipolaris* sp.[1]. A mutant strain of *A. versicolor* has been found to produce 5-methoxysterigmatocystin (II) in culture[2] and O-methylsterigmatocystin (III) was shown to be a metabolite of *Aspergillus flavus*[3]. Other closely related metabolites, such as the dihydro derivatives, have been isolated from *A. flavus*[4] and *A. versicolor*[5].



	R	X
I	H	H
II	H	OCH_3
III	CH_3	H

The presence of mold strains capable of producing these compounds has been detected during routine mycotoxin inspection of cereal grains and other agricultural commodities. Considerable attention has therefore been focused by the scientific community on this potential health hazard.

Previous investigators[3,6–8] have reported thin-layer chromatographic (TLC) procedures for the detection of sterigmatocystin employing silica gel, oxalic acid impregnated silica gel and Kieselgel D-5. A TLC method was also reported for the separation of dihydro-O-methylsterigmatocystin and O-methylsterigmatocystin[4]. These workers used sulfuric acid, alcoholic ferric chloride and ultraviolet light as detecting agents. In our investigation we found that iodine vapor, a well known and non-specific agent, was extremely useful in the detection of these xanthone derivatives.

From this study it is anticipated that future investigators could select the desired migration distance for sterigmatocystin and at the same time, have the capability of separating it from other closely related metabolites.

EXPERIMENTAL

Materials employed were as follows. Thin-layer plates: precoated silica gel G TLC plates (Uniplate, 5.08 × 20.32 cm; Analtech, Newark, Del., U.S.A.) with a

layer thickness of 250 μm; the plates were not activated prior to use. Solvents: All solvents utilized were analytical grade. Developing chamber: glass cylindrical chambers, 6.35 × 22.86 cm, were used as developing tanks. Detection: visualization was accomplished by exposing the developed TLC plate to iodine vapors.

The TLC plate was spotted with approximately 0.5 μg of each of the three compounds and developed for a distance of 10 cm in the appropriate solvent system. The developed plate was allowed to air-dry, exposed to iodine vapors, and the results were recorded.

RESULTS

Our TLC of sterigmatocystin, 5-methoxysterigmatocystin, and O-methylsterigmatocystin resulted in the perfection of seven different solvent systems which gave reproducible and discrete separation of these three compounds. None of these systems showed any evidence of compound tailing. The solvent systems and migration patterns of the three xanthone compounds are shown in Table I.

Previous investigators have reported the detection of sterigmatocystin and O-methylsterigmatocystin on TLC plates with the use of long-wavelength ultraviolet light. Both sterigmatocystin and the O-methyl derivative exhibit characteristic fluorescence, fluorescing red and pale blue, respectively. The lower limit of detection of these two compounds with ultraviolet light was determined to be 0.6 μg. Since 5-methoxysterigmatocystin does not exhibit fluorescence with ultraviolet light, it was necessary to develop another method for detection. Exposure to iodine vapors was found to be the most sensitive method. The lower limit of detection with iodine vapors was 0.0325 μg.

TABLE I

TLC OF STERIGMATOCYSTIN, 5-METHOXYSTERIGMATOCYSTIN AND O-METHYLSTERIGMATOCYSTIN

Solvent system	*Ratio*	$R_F \times 100$			*Developing time (min)*
		Sterigmatocystin	*5-Methoxy-sterigmatocystin*	*O-Methyl-sterigmatocystin*	
Benzene–acetone	10:0.2	26	18	1	25
Benzene–methanol	10:0.2	57	48	12	25
Carbontetrachloride–acetone	10:2.0	67	60	17	32
Chloroform–acetone	10:0.5	68	58	24	23
Carbontetrachloride–methanol	10:2.0	78	71	44	38
Dichloromethane–methanol	10:0.5	90	85	52	19
Chloroform–methanol	10:0.5	95	92	65	23

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