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Note

High-performance liquid chromatography of the mycotoxin sterigmatocystin and its application to the analysis of mouldy rice for sterigmatocystin

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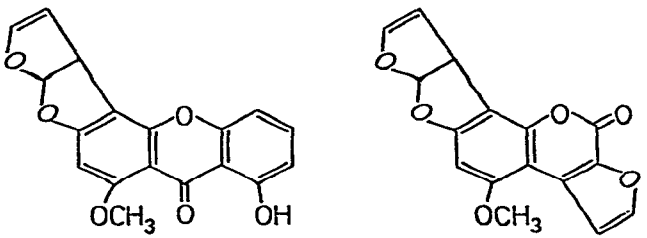
Sterigmatocystin (Fig. 1) was first isolated in 1954 by Hatsuda *et al.*[1] and 8 years later its structure was established by Bullock *et al.*[2].

In spite of the close relationship between the well known aflatoxin B_1 (Fig. 1), which is also a very potent carcinogenic mycotoxin, and sterigmatocystin an intensive survey of foods for sterigmatocystin has not yet been carried out. Sterigmatocystin is known to be hepatotoxic. It also causes severe necrosis of the kidneys and is a dermatotoxic agent[3]. A detailed report on its carcinogenic effects and the regular dietary daily dosage (10–100 ppm) required to induce hepatoma in mice was published by Chu[4]. In contrast to aflatoxin B_1, no teratogenic effect could be observed[5].



STERIGMATOCYSTIN AFLATOXIN B_1

Fig. 1. Structures of sterigmatocystin and aflatoxin B_1.

Sterigmatocystin can be produced by *Aspergillus versicolor*, *A. nidulans*, *A. rugulosum*, a Bipolaris species and a Penicillium species[6]. The main producer, *A. versicolor*, is ubiquitous. It has been found to grow on corn, foods, bread, dried fruits, cheese, rice, soymeal, berries, confitures and meat products[7].

We have previously described a procedure for the determination of sterigmatocystin in extracts from vegetable foods by thin-layer chromatography (TLC)[8]. A method for the analysis of pre-purified samples of sterigmatocystin by high-performance liquid chromatography (HPLC) was published earlier by Stack *et al.*[9]. Kingston *et al.*[10] determined sterigmatocystin in a synthetic mixture after separation by HPLC. In this paper we describe a simple extraction procedure and a separation system that allows the determination of sterigmatocystin in mouldy rice.

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EXPERIMENTAL

Production of sterigmatocystin on rice by A. versicolor

Flasks containing 60 g of rice and 32 ml of water were stoppered with cotton-wool plugs and, after 2 h, were autoclaved for 20 min at 121°C. The rice was inoculated with spores of *A. versicolor* St. 599 (Prof. Dr. Leistner, Bundesanstalt für Fleischforschung, 8650 Kulmbach, G.F.R.) and incubated at room temperature.

Sample preparation

The extraction of mouldy material has been described previously[8]. Extraction with acetonitrile–water (containing 4% of potassium chloride) was followed by partitioning against *n*-hexane and chloroform. After evaporation of the solvent, the residue was dissolved in an appropriate amount of methanol and 200 μl of the solution obtained were pipetted on to a Sep-Pak C_{18} Cartridge (Waters Assoc., Milford, MA, U.S.A.) and eluted with 3 ml of 50% methanol. Sterigmatocystin was completely retained while other compounds passed through the bed. The sterigmatocystin was then eluted with 10 ml of methanol–water (60:40) and the extract was transferred into a 10-ml Luer-Lok syringe containing a Swinney filter holder and a 0.5-μm Millipore filter through which the sample was filtered into a test-tube ready for injection into the HPLC system.

High-performance liquid chromatography

HPLC separations were conducted with a Model ALC/GPC 201 instrument (Waters Assoc.), equipped with a μBondapak C_{18} (particle size 10 μm) column (30 cm × 4 mm I.D.), M6000A and M45 pumps, a U6K septumless injector, an M660 solvent gradient programmer, a variable-wavelength UV absorbance detector (Pye Unicam, Cambridge, Great Britain) and an M730 Data Module dual-pen recorder with an electronic integrator. According to the maximal absorbance[7] all measurements were carried out at a wavelength of 246 nm.

RESULTS

Table I gives the retention times of sterigmatocystin using different methanol–water mixtures, and shows a decrease in retention time with increasing methanol concentration (60 to 70%).

TABLE I

RETENTION TIMES (min) OF STERIGMATOCYSTIN IN DIFFERENT METHANOL–WATER SOLVENT SYSTEMS ON A μBONDAPAK C_{18} COLUMN

Solvent	*Flow-rate (ml/min)*	
	1.0	*1.5*
Methanol–water (6:4)	24.7	17.1
Methanol–water (6.5:3.5)	15.6	11.2
Methanol–water (7:3)	10.1	7.2
Methanol–water (8:2)	5.6	3.8
Methanol–water (9:1)	4.0	2.6
Methanol	3.1	2.2
Gradient: methanol–water (6:4) to methanol–water (7:3) within 10 min	16.8	12.8

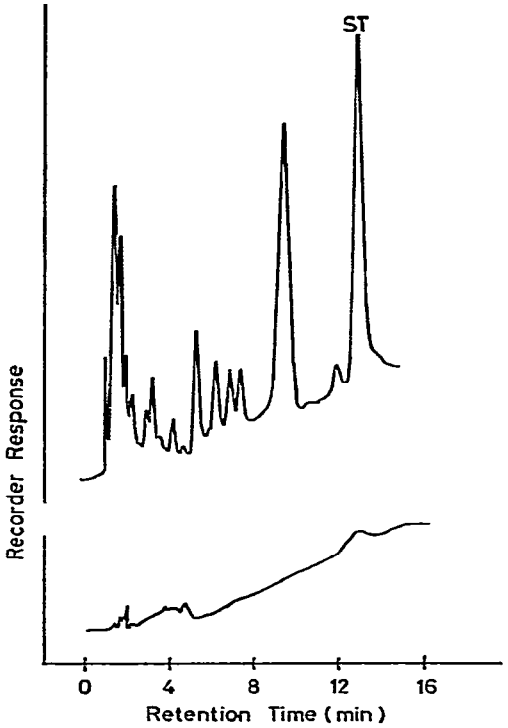

Fig. 2. Top: chromatogram of an extract from mouldy rice; ST = sterigmatocystin. Bottom: representative chromatogram of an extract from non-mouldy rice.

As the sterigmatocystin peak interfered with that of another compound in the extract from mouldy rice, a linear solvent gradient was applied. The chromatogram (Fig. 2, top) illustrates the resolution attained using a linear gradient of methanol–water from 60:40 to 70:30 within 10 min at a flow-rate of 1.5 ml/min. The elution pattern for extracts from non-mouldy rice shows no characteristic peaks (Fig. 2, bottom).

The retention times for this gradient were highly reproducible. Thirty-seven injections of pure sterigmatocystin (Sigma, St. Louis, MO, U.S.A.) over 2 weeks gave a mean retention time of 12.8 min with a coefficient of variation of 1.22% (Table II).

TABLE II

REPRODUCIBILITY OF RETENTION TIME FOR STERIGMATOCYSTIN BY APPLYING A LINEAR SOLVENT GRADIENT IN HPLC

Parameter	*Value*
No. of injections (*N*)	37
Retention time (sec):	
Range	750–789
Mean	769
Mean retention time (min)	12.8
Standard deviation (sec)	9.38
Coefficient of variation (%)	1.22

The reproducibility of the determination was tested by injecting ten 10-μl aliquots of a standard containing 10 μg/ml of sterigmatocystin in methanol. The injections were made using a microlitre syringe (Hamilton, Reno, NV, U.S.A.). The reproducibility of peak measurements was 4.39% (coefficient of variation) (Table III).

TABLE III

PEAK AREA REPRODUCIBILITY IN STERIGMATOCYSTIN SEPARATION BY GRADIENT HPLC

Parameter	*Value*
No. of injections (N)*	10
Peak area:	
Range	1114.7–1305.8
Mean	1188.3
Standard deviation	52.2
Coefficient of variation (%)	4.39

* 10-μl injections of 0.1-μg sterigmatocystin standard, 0.04 a.u.f.s. using a linear HPLC gradient (60 to 70% methanol, within 10 min, flow-rate 1.5 ml/min).

The relationship between peak area and amount of mycotoxin injected was linear over the range 25–1250 ng, with a correlation coefficient of 0.9994.

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REFERENCES

1 Y. Hatsuda, S. Kuyama and N. Terashima, *J. Agr. Chem. Soc. Jap.*, 28 (1954) 992.
2 E. Bullock, J. C. Roberts and J. G. Underwood, *J. Chem. Soc.*, (1962) 4179.
3 J. J. van der Watt, in I. F. H. Purchase (Editor), *Mycotoxins*, Elsevier, Amsterdam, 1974, p. 369.
4 F. S. Chu, *Advan. Appl. Microbiol.*, 22 (1977) 83.
5 A. W. Hayes, *Toxicon* (1978) Suppl. 1, 739.
6 G. N. Wogan, *Amer. Rev. Pharmacol.*, 15 (1975) 437.
7 R. Orth, *Inaugural Dissertation*, Naturwissenschaftliche Gesamtfakultät der Universität Heidelberg, Heidelberg, 1973.
8 R. Schmidt, K. Neunhoeffer and K. Dose, *Z. Anal. Chem.*, 299 (1979) 382.
9 M. E. Stack, S. Nesheim, N. L. Brown and A. E. Pohland, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 966.
10 D. G. I. Kingston, P. N. Chen and J. R. Vercellotti, *J. Chromatogr.*, 118 (1976) 414.