sorbed by many solvents, etc. We therefore prepared plates containing a material (about 1 % w/w) which fluoresces at 365 mμ, such as Riedel de Haen Leuchtstoffe Grun N, Vanino's pigment and salts of 3,5-dihydroxypyrene-8,10-disulphonic acid[15]. In the few cases where the spots to be visualized did not absorb 365 mμ UV-light, we used 254 mμ sensitized plates, but in these cases the choice of eluents is restricted (must be non-absorbers for the actual wavelength) and special arrangements must be made to allow the light to enter the chamber.

No difficulty should be experienced in collecting fractions provided that the outlet capillaries are of suitable length and diameter. To avoid the eluent creeping between the capillaries and the stainless steel plate, the capillaries were inserted in a melting point tube about 10 mm shorter than the tiny capillaries in such a way that the melting point tube did not touch the eluent-carrying coated layer.

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The separation and detection of several mycotoxins by thin-layer chromatography

The study of mycotoxicoses[1] especially aflatoxicosis[2,3] emphasized the existence of fungal metabolites harmful to higher organisms. This evidence and the ability of various ubiquitous fungi, *e.g. Aspergillus flavus*[3] and *Penicillium islandicum*[4] to elaborate potent carcinogens prompted theories on a possible relationship between the consumption of mycotoxins and diseases of unknown etiology, *e.g.* the high incidence of hepatocarcinogenicity in Africa[5]. It is therefore essential that rapid and sensitive analytical methods be developed for the detection of these hazardous compounds in

agricultural commodities and consumer products. These methods can be used to study the factors which would influence the growth of the toxigenic fungi in nature and their production of mycotoxins. It is of importance to note that the presence only of toxigenic fungi on a specific product does not necessarily indicate the presence of any mycotoxin.

Several excellent methods have been reported for the screening and quantitative estimation of the aflatoxins (see *e.g.* Ref. 6). Recentley EPPLEY[7] introduced a method for the screening for zearalenone, aflatoxin and ochratoxin. This paper describes a new developing system for the simultaneous separation and detection of eleven different mycotoxins. The water soluble and lipid material can usually be removed from an extract of mouldy material by liquid–liquid partition. For mixtures containing acidic mycotoxins, *e.g.* cyclopiazonic acid, secalonic acid D, and ochratoxins A and B extensive purification is achieved by removal of the neutral material by standard procedure.

Experimental

Silica Gel G (Merck) was slurried with 0.4 *N* aqueous oxalic acid in a (1:2) ratio. The separation was achieved on 20 × 20 cm plates using an 0.25 mm layer of the above-mentioned slurry. The plates were air dried, activated at 100° for 40 min and kept at room temperature. The solvent combination used was chloroform–methylisobutylketone (4:1). The plate was spotted with each of the mycotoxins in a solution of chloroform–methanol (1:1) and allowed to develop *ca.* 14 cm from the spotting line in a tank saturated with the solvent vapour. The plate was removed from the chamber and dried at room temperature. The spots were detected by exposure to long wavelength (366 mμ) UV illumination and spraying with colour reagents.

The spray reagents used were: (a) Concentrated sulphuric acid. After spraying the plate was heated at *ca.* 110° for 10 min. (b) One per cent ethanolic ferric chloride.

TABLE I

THE SEPARATION OF MYCOTOXINS ON 0.25 mm LAYER OF SILICA GEL G IMPREGNATED WITH OXALIC ACID

Mycotoxin	*Reference*	*Fungus*	R_F *value* (× 100)	*Fluorescence*	*Colour reagents*	
					H_2SO_4	$FeCl_3$
Aspertoxin	8	*A. flavus*	12	light yellow	green-yellow	—
Ochratoxin B	9	*A. ochraceus*	20	blue	—	red-brown
Secalonic acid D	10	*P. oxalicum*	23	dark	light brown	light brown
8α-(3-methylbutyryloxy)-4β,15-diacetoxyscirp--9-en-3α-ol	11	*F. tricinctum*	28	—	lead grey	—
Aflatoxin G_1	12	*A. flavus*	30	green	green-grey	—
Aflatoxin B_1	12	*A. flavus*	40	blue	green-grey	—
6β-Hydroxyrosenonolactone	13	*T. roseum*	44	—	orange-red	—
Ochratoxin A	9	*A. ochraceus*	48	green	—	red-brown
Cyclopiazonic acid	14	*P. cyclopium*	65	dark	red-brown	red-brown
Zearalenone	15	*F. graminearum*	72	faint blue	light yellow	red-brown
Sterigmatocystin	16	*A. nidulans* *A. versicolor* *Bipolaris sp.*	85	orange	green-grey	green

Results and discussion

The colours of the various mycotoxins under UV light and after spraying with the colour reagents are recorded in Table I. Also included in Table I are the fungal sources and the R_F ($\times$ 100) values. The reported R_F value for each mycotoxin is the average of ten independant determinations. It is apparent from the R_F values that the mycotoxins are well resolved in this system.

A suitable chromogenic reagent for these mycotoxins is a solution of one per cent ceric sulphate in 6 *N* sulphuric acid. Some compounds give a characteristic colour with a specific reagent, *e.g.* cyclopiazonic acid gives a violet colour on spraying with Ehrlich reagent. Cyclopiazonic acid also turns violet-red on prolonged standing on the silica gel plates impregnated with oxalic acid.

If oxalic acid is omitted from the silica gel slurry, the mobility of the neutral metabolites are virtually unaffected, whereas the acidic compounds *e.g.* cyclopiazonic acid, secalonic acid D, and ochratoxins A and B do not move. This can be employed as a confirmation. Absolute confirmatory tests, *e.g.* by direct comparison with a standard reference sample, by physico-chemical methods or bio-assay[17] are essential for the final proof for the presence of a suspected mycotoxin in foodstuffs.

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