Notes

A note on the paper chromatography of some indole derivatives on acetylated paper

A very considerable interest has developed in the paper chromatography of indole derivatives in the past decade and there have been numerous publications on the subject (for lists of references see JEPSON[1] and BLOCK *et al.*[2]).

In these laboratories we have been concerned with two problems involving the paper chromatography of indole derivatives, firstly, the examination of the urinary indoles of mental patients and secondly, investigations into the chemistry of the aminochromes involving the necessity of obtaining suitable systems for chromatographing 5,6-dihydroxyindoles and indoxyls[3-5]. The difficulties involved in the former study, which are not entirely chromatographic, are well known and in the latter case the relative instability of many of the substances involved under the conventional conditions for indole chromatography instigated the investigation of the use of unconventional chromatographic systems for these compounds. The 5,6-dihydroxyindoles and their diacetyl derivatives can be separated very easily on formamide-treated paper with non-polar running solvents[5], but this type of system is not very satisfactory for the aminochromes or 5,6-dihydroxyindoxyls owing to the very low solubility of this latter group of compounds in non-polar solvents. Satisfactory R_F values for indole derivatives can often be obtained on formamide-treated paper with a suitable mobile phase[6,7]; with dichloromethane or benzene–ethyl acetate (3:1) as running solvent it is possible to effect certain separations that have proved difficult with the classical methods (*i.e.* (a) 3-indolealdehyde from 3-indoleacetonitrile and (b) 3-indoleacetic acid from 3-indolelactic acid)[7].

The use of acetylated paper, particularly for the separation of non-polar substances (such as the sugar acetates, aromatic hydrocarbons, etc., (*cf.* refs. [8-12]) has been known for some time, but as far as the authors are aware the use of this type of modified paper for studying the paper chromatographic behaviour of indoles has not yet been reported, and it was considered that such reversed-phase systems might offer certain advantages particularly when dealing with some of the relatively non-polar indoles.

This note reports the use of Whatman No. 1 paper (approximately 22% acetylated[9]) and the upper phase of a chloroform–methanol–water mixture (10:10:6), *cf.* ref.[13], as running solvent. A very good separation of a number of 3-indoleacetic acid derivatives could be obtained (see Table I): for instance, 3-indoleacetonitrile (R_F = 0.02) was easily separated from 3-indolealdehyde (R_F = 0.47). The hydrophobic indoles tested, such as indole itself, skatole, 3-indoleacetonitrile and ethyl 3-indoleacetate had relatively low R_F values (0.02; 0.08; 0.02 and 0.22 respectively) under

these conditions, whilst the more polar substances tended to be relatively fast running.

This chromatographic system also appears to have certain potential uses when working with many of the substances encountered in studying aminochrome chemistry, *cf.* ref.[14] (see Table I), for instance adrenochrome (R_F = 0.82) was quite easily separated from two of its more common transformation products, adrenolutin (R_F = 0.72) and 5,6-dihydroxy-N-methylindole (R_F = 0.37); furthermore these substances seem relatively stable under these chromatographic conditions, in contrast to the ease with which compounds of this nature often decompose during chromatographic examination[3,4]. However, on drying the papers, after development with the solvent, the red adrenochrome spot slowly faded and was replaced by a yellow fluorescent spot, due to its isomerisation to adrenolutin (similar to the phenomenon observed on ordinary paper[3]).

The results obtained with fifteen different indole derivatives are given in Table I. The R_F values were found to be reasonably reproducible between different batches of paper. Although it might be considered desirable not to actually quote R_F values,

TABLE I

R_F VALUES OF SOME INDOLE DERIVATIVES ON ACETYLATED PAPER*

Compound	*Average R_F value* **	*Method of detection of spot***	*Source of compound*
5-Hydroxy-3-indoleacetic acid	71	A	Regis Chemical Company
3-Indoleacetic acid	57	A	Eastman Kodak Company
3-Indoleacetonitrile	02	A	Regis Chemical Company
3-Indolealdehyde	47	B	Regis Chemical Company
3-Indoleacetamide	67	A	Mann Research Laboratories Inc.
Ethyl 3-indoleacetate	22	A	Regis Chemical Company
Indole	02	A	Eastman Kodak Company
Skatole	08	A	Eastman Kodak Company
Tryptamine	04	A	Eastman Kodak Company
Lysergic acid diethylamide	04	A	Sandoz Pharmaceuticals (Canada) Ltd.
Adrenochrome	82	C	Prepared by the method of HEACOCK *et al.*[3]
Adrenolutin	72	C+	Prepared by the method of HEACOCK AND MAHON[4]
Adrenochrome monosemicarbazone	73	C	Labaz Company
5,6-Dihydroxy-N-methylindole	37	A	Prepared by the method of HEACOCK *et al.*[5]
5,6-Diacetoxy-N-methylindole	18	A	Prepared by the method of HEACOCK *et al.*[5]

* Using the upper phase of a chloroform–methanol–water (10:10:6) solvent system as running solvent.

** R_F values × 100.

*** A = Positive reaction (red to blue colours) with Ehrlich's reagent; B = Orange colour with 2,4-dinitropenylhydrazine; C = Self indicating (coloured substances); + = Exhibits a marked yellow-green fluorescence in ultraviolet light.

but to refer the migration of a given substance to a standard substance each time, the actual R_F values obtained have been reported here to demonstrate the general order (which is always the same) of the mobility of the substances investigated.

A few preliminary experiments were carried out with alternative running solvents including: (a) methanol; (b) various methanol–water mixtures and (c) various methanol–toluene–water (and related systems, *cf.* ref.[12]). However, with the current

group of compounds, the separations achieved with these systems were not as good as with the chloroform–methanol–water system.

Experimental

Indole derivatives. The sources of the indole derivatives used in this investigation are given in Table I.

Paper. Whatman No. 1 paper was acetylated according to the method of MICHEEL AND SCHWEPPE[9]. The paper was immersed in a solution of acetic anhydride in benzene (1:3), containing 0.1% of concentrated sulphuric acid by volume; the mixture was heated, under reflux, at 70° for six hours. The acetylation mixture was decanted; the paper washed with cold water and then allowed to stand in methanol overnight. Finally the paper was soaked in water for three hours and air dried. It was washed for 24 hours with the running solvent, and dried, immediately prior to use.

Solvent system. A chloroform*–methanol*–water (10:10:6) system was prepared and allowed to stand at room temperature for 1 hour. The upper phase was removed and used directly.

Procedure. The chromatography was carried out at room temperature, in the ascending direction. A total rise of *ca.* 30 cm, taking *ca.* 5 hours was employed.

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* Reagent grade solvents were used.