The detection and chromatography on paper of boric acid, sodium tetraborate and benzene boronic acid and the use of chlorogenic and caffeic acids to detect the ions of B, W, Mo and Ge

Investigation of the function of boron in higher plants (for which it is an essential element) led to the following study of the paper chromatography of boric acid, sodium tetraborate and benzene boronic acid, all of which stimulate the growth of flax when they are added to a boron-free nutrient culture solution in the concentration range $5 \cdot 10^{-8}$ to $1 \cdot 10^{-6}$ *M*. At, and above, $1 \cdot 10^{-4}$ *M*, benzene boronic acid is toxic to growth, causing the initiation of adventitious buds on the hypocotyl and the suppression of lateral root growth.

Detection on paper

These three B compounds all have one or more pairs of hydroxyl groups which cause them to complex or esterify with other stereochemically complementary organic polyols, such as carbohydrates[1,2] polyphenols[3,4] and coumarins[5]. Where complexes are formed with compounds, such as 3,4-dihydroxycinnamic acids which already fluoresce in or absorb U.V. light, the formation of the borate–hydroxyphenol complex causes an increase in the wavelength of maximum absorption ($\lambda_{U.V.}$ max.)[4]. There is also a change in the intensity and quality of the U.V.-induced fluorescence, compared to that of the phenol alone. This latter property has been used here to detect these hydroxy-boron compounds on paper.

The compounds were spotted on to filter paper which had been wetted with 0.1 *M* NaOH and subsequently dried. The paper was then sprayed with a chlorogenic [3-(3,4-dihydroxycinnamoyl)-quinic] acid solution (0.1% w/v, in acetone) and dried. It was then placed under a U.V. lamp (Engelhard Hanovia, with Chance glass OX_1 filter, max. emission 3660 Å). Where the paper was impregnated with one of the boron compounds, the background fluorescence was greatly increased and altered in colour. By this method it is possible to detect 1 μg (in a spot 5 mm in diameter) of the three compounds used. The pre-existing U.V. fluorescence of benzene boronic acid was enhanced by a factor of approximately 10, and altered in colour from a dull blue to a bright light blue. Boric acid and sodium tetraborate themselves have no U.V. fluorescence until sprayed with chlorogenic acid. It seems possible that B could be quantitatively assayed by this method, using U.V. fluorescence spectrophotometry.

This method has been extended to detect, on paper, the metallic ions of Ge, W and Mo, the hydroxides of which complex in solution with polyols in a manner similar to the ions derived from H_3BO_3[6]. Table I lists the daylight and U.V. fluorescence colours of these metallic compounds on filter paper, when sprayed with either chlorogenic or caffeic (3,4-dihydroxycinnamic) acid solutions.

These results indicate that certain polyphenols (such as caffeic and chlorogenic acids) can be detected and identified on paper, and possibly quantitatively estimated in solution, by the choice of suitable metallic reagents. The converse is also true: certain hydrated metallic ions give distinctive shifts in U.V. fluorescence when complexed with a range of already fluorescent polyphenols, used as "spot" reagents.

The induction of U.V. fluorescence, or of changes in inherent U.V. fluorescence, by boric acid when mixed in solution with fluorescein or cochineal has been described

TABLE I

THE DAYLIGHT AND U.V. FLUORESCENCE COLOURS OF B, W, Mo AND Ge COMPOUNDS WHEN SPOTTED ONTO FILTER PAPER, AND SPRAYED WITH EITHER CHLOROGENIC OR CAFFEIC ACID SOLUTIONS

Solution spotted onto filter paper		*Spray used*			
Compound used		*Caffeic acid*	*Chlorogenic acid*	*Caffeic acid*	*Chlorogenic acid*
Solute	*Solvent*	*Colour of spot*			
		In daylight		*Under U.V. light*	
—	—	—	very pale yellow	bright blue	bright blue-green
H_3BO_3	0.1 *M* NaOH	—	—	bright blue	bright blue
Na_2WO_4	0.1 *M* NaOH	pale yellow	very pale yellow	brown	bright yellow
H_2MoO_4	0.1 *M* NaOH	intense brown	—	intense black	black
GeO_2	0.1 *M* NaOH	—	very pale yellow	light grey	bright light yellow

(from earlier papers) by RADLEY AND GRANT[11] in 1939. They also mention the effects of the salts of Be, Al and Mo upon the fluorescence colours of certain compounds[11]. Since the advent of paper chromatography, the use of inherent U.V. fluorescence is one of the many standard methods for the detection of substances on chromatograms. U.V. fluorescence has been *induced* by forming complexes with 8-hydroxyquinoline[7], and immobile fluorescent compounds have been introduced into the matrix of chromatographic columns[8]. KIRCHNER *et al.*[9] used the quenching of U.V. fluorescence to detect terpenes on chromatographic columns. BAKER AND COLLIS[10] appreciated the possibility of using a *specific* fluorescent reagent (a derivative of 7-hydroxycoumarin), which they synthesised, to detect hydroxy and amino compounds on chromatograms, but they do not appear either to have exploited this, or to have specifically used the shift in U.V. fluorescence for the purposes of detection and identification. The results of the experiments described in this paper indicate that this property can be exploited further by the *systematic* choice of fluorescent reagents or U.V. fluorescence-modifying metallic ions.

The chromatography of boric acid, benzene boronic acid and sodium tetraborate

These three boron compounds were run, by upwards displacement, on No. 1 Whatman paper in six solvents. The R_F values of each compound in each solvent are given in Table II. The compounds were detected by spraying successively with 0.1 *M* NaOH and chlorogenic acid.

TABLE II

THE R_F VALUES OF BORIC ACID, BENZENE BORONIC ACID AND SODIUM TETRABORATE IN SIX SOLVENTS

No.	*Solvent*	R_F		
		H_3BO_3	$Na_2B_4O_7$	$PhB(OH)_2$
1	H_2O	0.87	0.87	0.72
2	Ethanol	0.0	0.0	1.0
3	Ethanol–water (80:20)	0.65	0.16	0.80
4	Butan-1-ol–acetic acid–water (4:1:5, by vol.) (top layer)	0.47	0.25 0.50	0.90
5	Acetic acid (1 % solution)	0.71	(not detected)	0.75
6	Benzene	0.0	0.0	(streaked)

It is apparent that, of the solvents used, No. 4 was perhaps the most satisfactory, although with sodium tetraborate it induced a double spot. This may be attributed to dissociation from the tetraborate to give some monoborate ion.

It is thus evident that the biologically active boron compounds here used may be successfully separated by paper chromatography, and also detected in microgram quantities on paper. In addition, the method of detection appears to have a more general application to other metallic hydroxides.

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Chromatography on starch columns

MOORE AND STEIN[1] showed that phenylalanine, tyrosine and tryptophan could be separated on a column of starch using 0.1 *N* hydrochloric acid as the developing solvent, and suggested that the retardation was due to adsorption. Other workers have employed starch columns developed with immiscible solvents for the chromatography of the iodotyrosines[2] and purine–pyrimidine mixtures[3]. Using a starch (Morning Star Nicol, Inc., N.Y.) column and 0.1 *N* hydrochloric acid phenylalanine, tyrosine, monoiodotyrosine, tryptophan and diiodotyrosine have been easily and quantitatively resolved (Fig. 1).