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A sensitive method for the detection of adenine compounds separated by paper or thin-layer chromatography

Purine compounds separated by paper chromatography (PC) or thin-layer chromatography (TLC) are most often detected by means of their fluorescence in UV light[1]. Purines can also be detected by PC by directing UV light at chromatograms pinned over a sheet of photographic paper; upon development of the photographic paper, the UV absorbing purines appear as white spots against a dark background[2]. Location reagents are less sensitive than the above techniques for locating purines[1]. One location reagent is the silver nitrate–Bromphenol Blue reagent of WOOD[3]. This reagent forms a blue purine-silver-dye complex which, under optimum conditions, can detect 0.05 μmole of purine derivatives.

In the present communication paper chromatograms are first dipped in an acidic vanillin reagent; they are then dried and dipped in a modified silver nitrate–Bromphenol Blue reagent. Adenyl compounds appear as pink spots on a gold background. The method is considerably more sensitive than any of the methods described above and specifically detects adenyl purines only. The same method can be applied to TLC by spraying instead of dipping. The specificity and sensitivity of the method are discussed.

Materials and methods

All the materials and reagents used were commercially available. The chromatographic paper used was Whatman No. 1. Microcrystalline cellulose, vanillin and silver nitrate were obtained from Merck AG, Darmstadt, G.F.R. Bromphenol Blue was obtained from May & Baker Ltd., Dagenham, Great Britain.

Reagent 1 consisted of 3.0 g of vanillin in 100 ml ethanol plus 0.5 ml sulphuric acid (*d* 1.84). Reagent 2 consisted of aqueous silver nitrate 2.5% mixed with an equal quantity of Bromphenol Blue 0.5% in acetone.

Paper chromatograms containing purines were first dipped in reagent 1, then immediately placed on filter paper in an oven at 90° for 5 min. They were then dipped in reagent 2, allowed to dry in air for 10 min, then placed in an oven at 90° for approximately 1½ min.

The presence of adenyl compounds on paper chromatograms was indicated by a characteristic pink spot on a gold background. The colour was stable for at least several months.

Results and discussion

The location method was most sensitive for ATP and adenine, which could be detected on chromatograms spotted with as little as 0.0005 and 0.0001 μmole respectively (Table I). Other adenine compounds could be located in spots containing a minimum of 0.01 μmole. Higher concentrations of inosine were needed for detection. Deamination of the purine nucleus as occurs in the conversion of adenine to hypoxanthine (Fig. 1) failed to decrease the sensitivity of the compound to colour development since spots containing 0.01 μmole hypoxanthine could still be detected (Table I). However, sensitivity to colour development is lost in the conversion of hypoxanthine to xanthine since the latter compound could not be detected in spots containing as much as 10 μmole (Table I). The conversion of hypoxanthine to xanthine involves substitution on carbon 2 of the purine nucleus (Fig. 1). It should be noted that guanine and its nucleotides also have a substituent on carbon 2 of the purine nucleus and also failed to be detected by this location method in relatively higher concentrations (Table I, Fig. 1). The pyrimidines cytosine, thymine and uracil were not detected in concentrations as high as 0.1 μmole. The nature of the coloured

TABLE I

MINIMUM QUANTITIES OF PURINES DETECTED BY LOCATION REAGENTS

Compound	*μmole*
ATP[a]	0.0005
ADP[a]	0.01
AMP[c]	0.01
Cyclic 3′,5′-AMP[a]	0.01
Adenosine[c]	0.01
Inosine[b]	0.05
Adenine[c]	0.0001
Guanine[c]	5.0 (not detected)
GMP[c]	2.0 (not detected)
Hypoxanthine[d]	0.01
Xanthine[d]	10.0 (not detected)
Uric acid[d]	10.0 (not detected)

[a] Calbiochem, Los Angeles, U.S.A.
[b] Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.
[c] The British Drug Houses Ltd., Poole, Great Britain.
[d] Koch-Light Laboratories, Ltd., Colnbrook, Bucks., Great Britain.
[e] Fluka AG, Buchs SG, Switzerland.

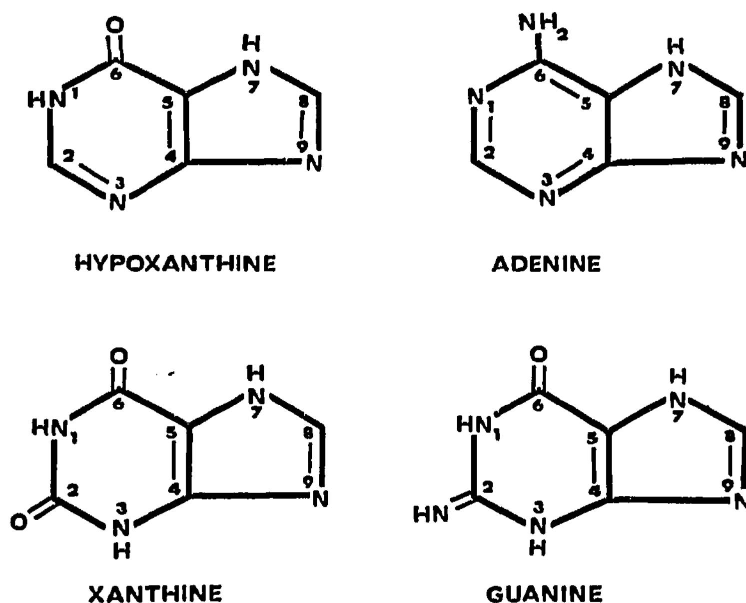

Fig. 1. The structures of hypoxanthine, adenine, xanthine and guanine.

TABLE II

COLOUR REACTIONS BY NON-PURINE COMPOUNDS

Spots contained 10 μmole of compound unless otherwise specified.

Compound	*Colour reaction*
Uracil[c,d]	—
UMP[c,f]	—
Thymine[c,h]	—
Cytosine[c,d]	—
DL-Phenylalanine[d]	brown
DL-Alanine[d]	—
Glycine[e]	—
Taurine[d]	—
DL-Leucine[d]	—
L(+)-Lysine[d]	—
Sucrose[e]	green
KCl[e]	—
Urea[b]	—
Noradrenaline[g]	green
Histamine[e]	—
5-Hydroxytryptamine[a,f]	—
Creatine[e]	—
Orcinol[e]	purple
Pyrogallol[e]	brown-purple
Cholesterol[e]	green

[a] Purple spot visible only after first dip.
[b] Yellow spot visible only after first dip.
[c] Spot contained 0.1 μmole.
[d] Koch-Light Laboratories Ltd., Colnbrook, Bucks., Great Britain.
[e] The British Drug Houses Ltd., Poole, Great Britain.
[f] Calbiochem, Los Angeles, U.S.A.
[g] Sterling Pharmaceuticals Ltd., Sydney, Australia.
[h] Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

complex is at present under investigation and will be the subject of a further communication.

A limited range of organic compounds was spotted on to the chromatogram and dipped in the location reagents in order to determine the specificity of the method. None of the compounds tested gave a pink spot, although in certain cases spots of other colours were observed (see Table II).

Non-phosphorylated adenyl compounds could be detected with sensitivity equal to that described above by omitting the step of heating the chromatogram in an oven at 90° for 5 min after dipping in Reagent I. This step was initially introduced because the phosphorylated adenyl compounds present on the chromatogram dipped in the acid Reagent I were hydrolysed upon heating and could be detected in smaller amounts.

The method has been applied to PC and microcrystalline cellulose coated TLC developed in a variety of solvent systems. However, it should be noted that solvents which are either strongly acidic or strongly alkaline may interfere with the location method.

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