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Note

Thin-layer chromatography of some 2,6-dioxopyrimidines and -purines

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An investigation of the Traube synthesis as a suitable method for small scale preparation of isotopically labelled xanthine and its methyl derivatives, revealed that no satisfactory rapid method of analysis of reaction mixtures was available. The compounds of interest were all derivatives of uracil (1,2,3,6-tetrahydro-2,6-dioxopyrimidine) and xanthine (1,2,3,6-tetrahydro-2,6-dioxopurine).

PREVIOUS WORK

Low solubilities of 2,6-dioxopyrimidines in common solvents result in low R_F values in chromatography. Similar problems are encountered with xanthine and its monomethyl derivatives but the di- and trimethyl xanthines are more soluble and have been analysed by thin-layer chromatography (TLC)[1].

Water and aqueous acids or alkalis are the best solvents for these substituted pyrimidines and purines and have been used for paper chromatography (PC), often as mixtures with propanol or butanol[2,3]. Unfortunately R_F values for some compounds are low, resolution is poor and development slow. The advantages of acetonitrile as a solvent in PC of purines and pyrimidines have been discussed previously[4] but the solvent mixtures described proved unsuitable for many of the compounds listed in Tables I and II because of low R_F values.

PRESENT WORK

2,6-Dioxopyrimidines

Two solvent systems were found to give satisfactory results on either Kieselgel or Kieselguhr TLC plates (Table I). Solvent system A consisted of *n*-propanol–3.5% aqueous ammonium hydroxide (3:1) and system B consisted of acetonitrile–3.5% aqueous ammonium hydroxide (3:1). System B was found to give particularly good separations of the various pyrimidines on one or other of the two adsorbents, the only difficulties arising with the 4,5-diaminouracils.

4,5-Diaminouracils

4,5-Diaminouracil, together with the 3-methyl and 1,3-dimethyl derivatives

TABLE I

TLC OF 2,6-DIOXOPYRIMIDINES (URACIL DERIVATIVES)

Solvent system A: *n*-propanol–3.5% aqueous ammonium hydroxide (3:1); solvent system B, acetonitrile–3.5% aqueous ammonium hydroxide (3:1).

Derivative	*Observed R_F value*			
	Kieselgel		*Kieselguhr*	
	A	*B*	*A*	*B*
4-Amino-	0.36	0.32	0.86	0.77
4-Amino-5-nitroso-	0.40	0.39	0.85	0.79
4,5-Diamino-	0–0.4	0–0.5	0.73	0–0.8
4-Amino-5-formamido-	0.20	0.21	0.65	0.56
3-Methyl-4-amino-	0.38	0.42	0.89	0.84
3-Methyl-4-amino-5-nitroso-	0.44	0.38	0.85	0.79
3-Methyl-4,5-diamino-	0.2–0.5	0.3–0.5	0.8–0.95	0.7–0.9
3-Methyl-4-amino-5-formamido-	0.27	0.30	0.74	0.73
1,3-Dimethyl-4,5-diamino-	0.4–0.58	0.5–0.8	0.9–1.0	0.9–1.0
Run time (min)	240	45	90	10

gave a characteristic pattern of spots on TLC. This occurred when the original sample was either a free base or its acid salt and was probably due to oxidation of the 4,5-diamino system as reported for other 4,5-diaminopyrimidines[5]. Some observations on the fluorescence of a few oxidation products have been made[6] but there is no report of their number or identity. In the present work a considerable number of compounds could be distinguished —at least six for 4,5-diaminouracil, four for the 3-methyl derivative and seven for the 1,3-dimethyl derivative. In each case, R_F values in Table I are quoted as the range over which the pattern of spots was observed.

2,6-Dioxopurines

Solvent systems A and B were also satisfactory for the monomethyl xanthines

TABLE II

TLC OF 2,6-DIOXOPURINES (XANTHINE DERIVATIVES) ON KIESELGEL

See for solvent systems Table I.

Derivative	*Observed R_F value*	
	A	*B*
Xanthine	0.31	0.33
1-Methyl-	0.38	0.37
3-Methyl-	0.45	0.38
7-Methyl-	0.34	0.34
1,3-Dimethyl-	0.47	0.41
1,7-Dimethyl-	0.43	0.42
3,7-Dimethyl-	0.42	0.47
1,3,7-Trimethyl-	0.53	0.60
Run time (min)	240	45

on Kieselgel though R_F values were still rather low (Table II). On Kieselguhr streaking occurred and separation was poor.

EXPERIMENTAL

Materials

Pyrimidines were synthesised by the method of Traube[7] (see also ref. 8). Additional commercial samples were 4,5-diaminouracil (Koch-Light and Fluka) and 1,3-dimethyl 4,5-diaminouracil (Courtorch Chemicals). Xanthine and di- and trimethylxanthines (BDH) and monomethylxanthines (Fluka) were commercial samples used without further purification. Acetonitrile was reagent grade (98%) and other solvents were AnalaR grade. Kieselgel F_{254} and Kieselguhr F_{254} TLC plates (Merck pre-cast) were standard 20 × 20 cm with 0.25 mm layer.

Procedure

All compounds were applied as 5-μl samples of 1% solutions in 0.25 *M* aqueous sodium hydroxide except caffeine which was applied in chloroform. The plates were developed to a height of approx. 10 cm in tanks saturated with solvent vapour. Spots were located under UV light of wavelength 254 nm or 350 nm. All compounds quenched the background fluorescence of the plate at 254 nm except a few of the oxidation products of the 4,5-diaminouracils, which were fluorescent and best observed in light of wavelength 350 nm.

CONCLUSION

The TLC systems reported above facilitate analysis for some sparingly soluble 2,6-dioxopyrimidines and -purines. Good resolution with rapid development time suggests that these systems should have wide application to other classes of substituted pyrimidines and purines.

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