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Note

Thin-layer chromatographic separation and differentiation of some nuclear acetamides

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Thin-layer chromatographic (TLC) systems for the separation and differentiation of nuclear acetamides are of particular value as intermediates in the synthesis of dyestuffs and drugs.

There are only a few reports on the TLC of phenoxazines[1–4] and no reports on the TLC of acetamidobenzophenoxazines and benzophenazines. TLC methods involving the use of silica gel G plates have been developed for these nuclear-substituted angular heterocyclic compounds that permit their separation, differentiation and identification in submicrogram amounts on a single chromatoplate. R_F values for three solvent systems are reported.

EXPERIMENTAL

Apparatus and reagents

Standard TLC equipment (plates, atomizers, applicator with a fixed thickness and developing tanks) were supplied by Adair Dutt & Co. (New Delhi, India). Acetone, benzene, *n*-butanol, carbon tetrachloride, cyclohexane, chloroform, chlorobenzene, toluene and tetrahydrofuran were carefully purified and dried before use. Silica gel G was obtained from E. Merck (Darmstadt, G.F.R.). Concentrated sulphuric acid was used as the spray reagent.

Synthesis of substituted N-(5-oxo-5H-benzo[a]phenoxazin-6-yl)acetamides and N-(5-hydroxybenzo[a]phenazin-6-yl)acetamides

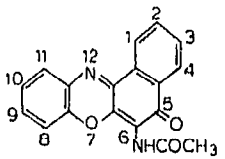
To a stirred, refluxing suspension of N-(3-chloro-1,4-dihydro-1,4-dioxo-2-naphthyl)acetamide (0.01 mole) in 35 ml of dry ethanol containing 1.56 g of anhydrous potassium acetate (0.02 mole) was added dropwise an alcoholic solution of the corresponding substituted *o*-aminophenol or *o*-phenylenediamine (0.01 mole). The stirring and refluxing were continued for 2–3 h and the reaction mixture was then cooled to room temperature. The solid that separated was collected by filtration, washed with hot water and recrystallized from benzene (Table I).

Preparation of chromatoplates

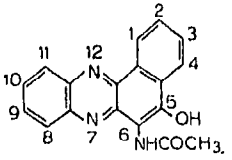
A number of 20 × 20-cm glass plates were coated (0.2 mm) with a silica gel G slurry, which was prepared by mixing thoroughly silica gel G with distilled water

TABLE I

R_F VALUES OF SOME SUBSTITUTED N-(5-OXO-5H-BENZO[*a*]PHENOXAZIN-6-YL)ACETAMIDES (A) AND N-(5-HYDROXYBENZO[*a*]-PHENAZIN-6-YL)ACETAMIDES (B)



(A)



(B)

Type	*Compound*	*R_F value**			*M.p. (°C)*	*Spot colour in visible light*	*Spot colour after H_2SO_4 spray*
		System A	*System B*	*System C*			
A	N-(5-Oxo-5H-benzo[*a*]phenoxazin-6-yl)acetamide	0.57	0.63	0.60	>300	Yellow	Brown
	N-(10-Chloro-5-oxo-5H-benzo[*a*]phenoxazin-6-yl)-acetamide	0.58	0.71	0.66	270	Yellow	Brown
	N-(9-Nitro-5-oxo-5H-benzo[*a*]phenoxazin-6-yl)-acetamide	0.65	0.81	0.70	>300	Yellow	Yellow
	N-(10-Nitro-5-oxo-5H-benzo[*a*]phenoxazin-6-yl)-acetamide	0.59	0.66	0.63	>300	Yellow	Yellow
	N-(5-Oxo-8,10-dinitro-5H-benzo[*a*]phenoxazin-6-yl)-acetamide	0.61	0.67	0.63	210	Light yellow	Reddish
	N-(11-Aza-5-oxo-5H-benzo[*a*]phenoxazin-6-yl)-acetamide	0.31	0.24	0.48	242	Yellow	Red
B	N-(5-Hydroxybenzo[*a*]phenazin-6-yl)acetamide	0.61	0.66	0.46	263	Brown	Violet
	N-(5-Hydroxy-10-nitrobenzo[*a*]phenazin-6-yl)-acetamide	0.46	0.63	0.30	>300	Brown	Violet
	N-(10-Chloro-5-hydroxybenzo[*a*]phenazin-6-yl)-acetamide	0.59	0.65	0.45	270	Brown	Violet

* Solvent systems: A, acetone–toluene–cyclohexane (3:1:1); B, *n*-butanol–benzene–carbon tetrachloride (2:2:1); C, tetrahydrofuran–chlorobenzene–chloroform (3:1:1).

(1:2, w/v). Spreading was carried out by the method of Davidek and Prochazka[5]. The chromatoplates were allowed to stand for 30 min at room temperature for superficial drying and were then activated by heating at 110–120° for 1 h in an electric oven. The activated chromatoplates were cooled and stored in a desiccator until required.

Chromatographic procedure

A Hamilton syringe was used to apply accurately 4-μl volumes of solutions of 4–5 μg of the compound[6] in acetone to the mid-points of the 10-mm lanes in a straight line at a distance 25 mm from the lower edge of the plates. The plates were placed in the tanks, to which had been added 200 ml of the solvent at least 1 h prior to use. The ends of the tanks were lined with strips of filter-paper freshly saturated with the solvent and the tank lids were sealed with vacuum grease. The solvent was allowed to ascend to a height of 14–16 cm from starting point, then the plates were removed from the tanks and the solvent front and spots were immediately marked. The colours of the spots were noted in visible light and after spraying with concentrated sulphuric acid.

RESULTS AND DISCUSSION

Combinations of many solvents were examined for developing the chromatograms, the most suitable being found to be (A) acetone–toluene–cyclohexane (3:1:1), (B) *n*-butanol–benzene–carbon tetrachloride (2:2:1) and (C) tetrahydrofuran–chlorobenzene–chloroform (3:1:1). Methanol, hexane, pyridine, 1,4-dioxan and acetic acid, with or without water, did not give good separations, owing to tailing of the spots or too low R_F values. It was observed that N-(9-nitro-5-oxo-5H-benzo[a]phenoxazin-6-yl)acetamide has higher R_F values than the other corresponding angular heterocyclic compounds (Table I). It was also interesting that N-(11-aza-5-oxo-5H-benzo[a]-phenoxazin-6-yl)acetamide has the lowest R_F value in comparison with other angular heterocyclic compounds (Table I).

All of the compounds were applied in a straight line, because the distance travelled by the spots has an effect on the R_F value as there is actually a separation of the components of the solvent on the layer and the components of higher R_F value which travel with the less polar solvent are not affected by the distance of the spot above the solvent level, but the lower R_F components which are moved by the solvent mixture are affected by the distance of spotting from the solvent level. The higher the sample is spotted, the longer it will take the solvent mixture to reach the spot and start to move it because of the separation effect of the layer on the solvent mixture. Naturally, the total distance that the solvent travels will affect the R_F value, because the greater the distance the solvent travels the greater will be the distance the spots are moved.

After development of three plates, the solvent system B (Table I) was changed as the R_F values were lower on subsequent plates. This change in R_F value is probably due to the selective adsorption of *n*-butanol on the thin layers. Great care was taken to obtain both a uniformly graded thickness and uniform activity of silica gel G in order to give reproducible[7] R_F values. The plates were developed at room temperature (25–30°) and all of the R_F values were obtained as described above. The R_F values

given in Table I are the reproducible R_F values from at least five experiments on different plates.

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