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Note

Thin-layer chromatography of some new angular heterocyclics

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Benzophenazine derivatives have played an important role in pharmacology and in the chemistry of dyes, being widely used as antibacterial agents, antibiotics, fungicides, anticonvulsants, sensitizers for film formers, antioxidants and vat dyes. The necessity for their rapid separation and identification thus merits further investigation.

Although there are a number of references[1–4] to the thin-layer chromatography (TLC) of phenazines, there is no report in the literature on the TLC of benzophenazines. We have successfully applied this technique to the separation and identification of submicrogram quantities of these angular heterocyclic compounds on silica gel G in various non-aqueous solvent systems.

EXPERIMENTAL

Apparatus and reagents

Standard TLC equipment (plates, atomizers, fixed-thickness applicator and developing tanks) were obtained from Adair Dutt & Co, New Delhi, India. Silica gel G was obtained from E. Merck (Darmstadt, G.F.R.). Acetic acid, *o*-dichlorobenzene and carbon tetrachloride were analytical-grade reagents. Benzene and toluene were dried over sodium wire before use.

6-Chloro-5-hydroxybenzo[a]phenazines. A suspension of 2.27 g (0.01 mole) of 2,3-dichloro-1,4-naphthoquinone and the appropriately substituted *o*-phenylenediamine (0.01 mole) in 100 ml of dry ethanol was heated under reflux for 4 h. The resulting mixture was chilled, collected and washed with hot water, slurried in hot glacial acetic acid, filtered and crystallized from ethanol to give the corresponding substituted 6-chloro-5-hydroxybenzo[*a*]phenazines.

6-Pyridiniobenzo[a]phenazine-5-ones. A mixture of 9.04 g (0.04 mole) of 2,3-dichloro-1,4-naphthoquinone and the corresponding *o*-phenylenediamine (0.04 mole) in 100 ml of dry pyridine was heated on a steam bath for 1 h. The resulting orange solid was collected on a suction pump and re-crystallized from pyridine to give analytically pure samples of substituted 6-pyridiniobenzo[*a*]phenazine-5-ones.

5,6-Benzo[a]phenazinequinones. A suspension of 5.0 g of the substituted 6-chloro-5-hydroxybenzo[*a*]phenazine in 60 ml of glacial acetic acid containing 5 ml of nitric acid (sp.gr. = 1.42) and 5 ml of water was heated on a steam bath for 1.5 h.

The resulting bright yellow precipitate was filtered off and re-crystallized from benzene to give the substituted 5,6-benzo[*a*]phenazinequinone.

Chromatographic procedure

The thin-layer plates (20 × 20 cm; thickness, 0.02 cm) were prepared by the usual manner[5] from silica gel G. The activated plates were stored over calcium chloride until required. Columns (1.0 cm wide) parallel to the direction of coating were drawn on each plate using a scriber. The outside columns of each plate were not used. Spots containing 2–4 μl of each sample (6–8 μg of the compound in methanol) were made in each column 3.5 cm from the base of the plate. The plates were then placed in developing tanks to which 200 ml of solvent had been added (at least 2 h prior to use). The end walls of the tanks were lined with strips of filter paper which had been 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 the starting point. The plates were removed from the tank and the positions of the solvent front and spots were immediately marked. The colours of the spots were noted in visible light and after spraying with 50% sulphuric acid.

RESULTS AND DISCUSSION

Many solvent systems were tried, the best for these angular heterocycles being (A) acetic acid–toluene (3:2), (B) acetic acid–*o*-dichlorobenzene (3:2) and (C) acetic acid–carbon tetrachloride–benzene (5:3:2). The higher the content of acetic acid in each system the higher were the R_F values. Combinations of other solvents such as methanol, hexane, tetrahydrofuran, acetone, chloroform, 1,4-dioxane with or without

TABLE I

MELTING POINTS, R_F VALUES AND SPOT COLOURS OF SUBSTITUTED BENZO[*a*]-PHENAZINES

Compound	*M.p. (°C)*	*Solvent*			*Spot colour*	
		A	*B*	*C*	*In visible light*	*With sulphuric acid*
6-Chloro-5-hydroxybenzo[*a*]phenazine	268	0.59	0.61	0.64	pink	light green
6,10-Dichloro-5-hydroxybenzo[*a*]-phenazine	289	0.65	0.66	0.73	pink	light green
6-Chloro-5-hydroxy-10-nitrobenzo-[*a*]phenazine	310–313d	0.63	0.65	0.72	pink	orange
6-Pyridiniobenzo[*a*]phenazine-5-one	323	0.08	0.12	0.09	pale yellow	violet
10-Chloro-6-pyridiniobenzo[*a*]phena-zine-5-one	335	0.17	0.30	0.21	yellow	violet
10-Nitro-6-pyridiniobenzo[*a*]phena-zine-5-one	>360	0.14	0.26	0.14	yellow	violet
5,6-Benzo[*a*]phenazinequinone	265	0.52	0.58	0.60	yellow	orange
10-Chloro-5,6-benzo[*a*]phenazine-quinone	271	0.55	0.64	0.65	yellow	orange
10-Nitro-5,6-benzo[*a*]phenazine-quinone	232	0.51	0.63	0.64	light yellow	orange

acetic acid were also used for the development of the chromatograms but did not give a good separation. This was due to tailing of the spots, to lower or higher R_F values, and substituted 6-pyridiniobenzo[*a*]phenazine-5-ones remained at the starting point resulting in incomplete separation. Table I shows that the 10-chloro-substituted phenazine derivatives had higher R_F values than the other substituted or unsubstituted angular heterocyclics. It is also interesting that the hydroxyphenazine derivatives had higher R_F values than corresponding benzo[*a*]phenazinones and benzo[*a*]phenazinequinones.

It was noticeable that application of a series of spots of one sample, allowing the solvent to evaporate between each application, affected the shape of the spots and the R_F values of the sample. This may be due to radial chromatography. In order to obtain reproducible R_F values, each sample was applied in one application. The ranges of R_F values obtained from the five plates used in Table I were compared with those from five plates developed in tanks in which the filter paper had not been saturated with the solvent. Plates developed in the latter tanks did not give reproducible results, whereas those developed in tanks with saturated filter paper gave values within the accepted tolerance limit of $\pm$ 0.02 unit. The plates were developed at room temperature (25–30°). The R_F values in Table I are the reproducible values from at least five experiments on different plates.

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REFERENCES

1 L. Birkofer and G. Leithaeuser-Weitecki, *Naturwissenschaften*, 46 (1959) 669.
2 L. R. Snyder, *J. Chromatogr.*, 17 (1965) 73.
3 C. R. Engel and E. Sawicki, *J. Chromatogr.*, 31 (1967) 109.
4 S. Caroli and M. Lederer, *J. Chromatogr.*, 37 (1968) 333.
5 J. G. Kirchner, *Thin-layer Chromatography*, in A. Weissberger (Editor), *Technique of Organic Chemistry*, Interscience, New York, 1967.