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## Thin-layer chromatographic studies of some new 5,10-dihydrophenazines

5,10-Dihydrophenazines, which are the reduced form of the corresponding phenazines, have been used in medicine and industry as antifilariatic agents and also as dyestuffs. Their rapid separation and identification thus merit further investigation.

Although a number of papers[1-8] have dealt with the chromatographic separation and identification of phenazines, there is no report in the literature on the thin-layer chromatography (TLC) of 5,10-dihydrophenazines. We successfully applied TLC to the separation and identification of these compounds in submicrogram quantities on a thin-layer of Silica Gel G using non-aqueous developing solvent systems.

*Experimental*

*Apparatus and reagents.* Standard TLC equipment (plates, atomizers, applicator with a fixed thickness and developing tanks) was obtained from Adair Dutt and Co. (New Delhi, India). Benzene, hexane, carbon tetrachloride and acetone were of analytical grade. Silica Gel G was of Merck quality.

*Preparation of 5,10-dihydrophenazines.* Halogenonitrobenzene (0.01 *M*), substituted *o*-phenylenediamine (0.012 *M*) and anhydrous sodium acetate (0.04 *M*) were dissolved in 50 ml of dry ethanol. The mixture was heated under reflux for 6–8 h and allowed to cool. The solid was removed by filtration and washed several times with water, dilute hydrochloric acid and ethanol. The residue was crystallised from a mixture of acetic acid and ethanol to give the desired product. The compounds were stored in specially designed flasks to avoid contact with air.

*Chromatographic procedure.* The glass plates (20 × 20 cm) were coated (0.3 mm) with a well-stirred mixture of 50 g of Silica Gel G and 100 ml of distilled water. The plates were first dried in air for 20–30 min and then activated by heating for 1 h at 110–120° in an oven. The plates were then cooled and stored in a desiccator before use.

The compounds were dissolved in acetone and spotted by means of a micropipette on the plates at 1-cm intervals on the starting line of the chromatogram in order to keep the diameters of the spots to less than 3 mm. The plates were then placed in developing tanks to which 200 ml of solvent had been added at least 1 h before use. The ends of the tanks were marked with strips of filter paper freshly saturated with the solvent, and the lids of the tanks were sealed with vacuum grease. Development was allowed to proceed until the solvent front had risen 10–15 cm beyond the original spots. The plates were removed from the tanks and the solvent front was immediately marked. The plates were allowed to dry in air. The spots were visible in daylight. Standard $R_F$ values for 5,10-dihydrophenazines were obtained using this method for the three solvent systems. 1,3-Dinitro-5,10-dihydrophenazine was included on every plate as a standard so that relative $R_F$ values could be calculated. The results were reproducible.

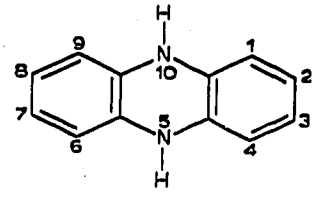
*Results and discussion*

Combinations of many solvents were examined for developing the chromatograms on Silica Gel G; the best of these were found to be (A) benzene–acetone (75:25); (B) hexane–acetone (60:40); and (C) carbon tetrachloride–acetone (70:30). It was observed that benzene, hexane, petroleum ether and various systems of methanol with or without water did not yield good separations. This was due to tailing of the spots or the lower $R_F$ values obtained that resulted in incomplete separation. It was observed that 5,10-dihydrophenazines prepared from 4-chloro-*o*-phenylenediamine had higher $R_F$ values than those prepared from the corresponding 4-nitro-*o*-phenylenediamine when compared with 5,10-dihydrophenazines prepared from *o*-phenylenediamine in all the solvent systems studied.

It is difficult to obtain reproducible $R_F$ values in TLC (ref. 9) when working with activated layers and non-aqueous solvent systems. For this reason, 1,3-dinitro-5,10-dihydrophenazine was selected as a standard and spotted with the other compounds on all the plates in a series. The $R_F$ values given in Table I are the reproducible $R_F$ values of at least five experiments on different plates.

TABLE I

$R_F$ AND RELATIVE $R_F$ VALUES OF SOME 5,10-DIHYDROPHENAZINES




System A: benzene–acetone (75:25); system B: hexane–acetone (60:40); system C: carbon tetrachloride–acetone (70:30).

| *5,10-Dihydrophenazine derivative* | *System A* | | *System B* | | *System C* | | *M.P. (°C)* | *Spot colour* |
|---|---|---|---|---|---|---|---|---|
| | $R_F$ | *Rel.* $R_F$ | $R_F$ | *Rel.* $R_F$ | $R_F$ | *Rel.* $R_F$ | | |
| 1,3-Dinitro- | 0.72 | 1.00 | 0.64 | 1.00 | 0.63 | 1.00 | 200[a] | Brown |
| 1-Nitro-3-chloro- | 0.76 | 1.05 | 0.73 | 1.14 | 0.69 | 1.09 | 145 | Red |
| 1,7-Dinitro-3-chloro- | 0.64 | 0.88 | 0.47 | 0.73 | 0.43 | 0.68 | 257 | Yellow |
| 3-Nitro-7-chloro | 0.73 | 1.01 | 0.64 | 1.00 | 0.52 | 0.82 | 197 | Yellow |
| 1,3,7-Trinitro- | 0.51 | 0.71 | 0.43 | 0.67 | 0.38 | 0.60 | 270 | Yellow |
| 1,3-Dinitro-7-chloro- | 0.71 | 0.98 | 0.53 | 0.82 | 0.49 | 0.77 | 217 | Violet |
| 1-Nitro-3,7-dichloro- | 0.74 | 1.02 | 0.57 | 0.89 | 0.61 | 0.96 | 175 | Yellow |
| 3,7-Dinitro | 0.55 | 0.76 | 0.46 | 0.71 | 0.42 | 0.66 | 245 | Yellow |

[a] Decomposition.

After development of three plates, the solvent was changed since $R_F$ values were lower on subsequent plates. This change in $R_F$ value is probably due to the selective evaporation of acetone.

The plates were developed at room temperature (22–28°) and all the $R_F$ values were obtained as described above. The range of $R_F$ values obtained from five plates used for the data in Table I were compared with those from five plates developed in unsaturated tanks. It was found that plates developed in unsaturated tanks did not

give reproducible results, whereas plates developed in saturated tanks gave values within the accepted tolerance normal for work on TLC ($\pm$0.02 $R_F$ units).

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