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Note

Thin-layer chromatographic separation of potential antineoplastic agents: 1-ethoxycarbonyl-2-arylazo-2-nitroethanes

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There is increasing interest in the synthesis, thin-layer chromatographic (TLC) separation and biological evaluation of compounds containing the N*–N*–S* or O*–N*–S* tridentate ligand system[1–5] or arylazo grouping[6,7]. This interest stems mainly from certain interesting biological activities[8,9], carcinostatic activities of heterocyclic carboxylaldehyde thiosemicarbazones and the interfering action of 5-arylazopyrimidines with nucleic acid synthesis.

As part of a general study directed towards the development of antineoplastic agents, the above-mentioned rationale led to the examination of the synthesis and biological properties of 1-ethoxycarbonyl-2-arylazo-2-nitroethanes. However, little or no information was available on the separation, identification and determination of these compounds. Hence the present study was undertaken in order to establish a sensitive and reproducible chromatographic procedure for the separation and identification of various 1-ethoxycarbonyl-2-arylazo-2-nitroethanes.

MATERIALS AND METHODS

TLC plates were prepared from a slurry of 40 g of silica gel G (Merck, Darmstadt, G.F.R.) in 80 ml of distilled water. The slurry was spread on 20 × 20 cm glass plates to a thickness of 0.20 mm with a Stahl applicator. The plates were air dried, activated at 110°C for 4 h and stored in a desiccator.

A 1% solution of each compound in acetone was prepared and 1 μl of the solution (corresponding to 10 μg of each compound) was spotted 2.0 cm from the edge of the TLC plate with a moiropipette. The chromatogram was developed with benzene–chloroform–*n*-hexane (5:1:5) until the solvent front had travelled 16 cm. About 70 min were usually required for the development of the plate.

The chromatogram was then dried with a hot-air blower, sprayed with 10% (w/v) methanolic potassium hydroxide solution and then heated at 50°C.

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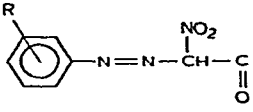
RESULTS AND DISCUSSION

The results are given in Table I. Each $R_F \times 100$ value represents the mean of five identical runs; each series of five determinations showed only slight variations, within the limits of experimental error.

No appropriate colour change occurred when the chromatograms were viewed under UV light.

TABLE I

TLC SEPARATION OF 1-ETHOXYCARBONYL-2-ARYLAZO-2-NITROETHANES

General formula of compounds:  Plate: silica gel G, 20 × 20 cm, 0.20 mm layer. Developing solvent: benzene–chloroform–*n*-hexane (5:1:5).

R	$R_F \times 100$*	*Colour of spots*
H	46	Yellow
2-NO_2	18	Orange
3-NO_2	22	Deep yellow
4-NO_2	12	Orange red
2,3-$(CH_3)_2$	34	Deep yellow
2,4-$(CH_3)_2$	40	Yellow
2-Cl	25	Yellow
3-Cl	30	Yellow
4-Cl	37	Deep yellow
2-Br	43	Yellow
2,3-(Cl_2)	51	Deep yellow
2,4-(Cl_2)	47	Deep yellow
4-Cl-2,5-$(OCH_3)_2$	15	Deep yellow

* Averages of five identical runs.

The detection limit was *ca.* 1 μg for each compound. The only adsorbent used was silica gel G. Activation of the TLC plates at 75–150°C was also examined. Heating the plate hardly affected the chromatograms, but the best separation was achieved with a plate heated at 110°C for 30 min.

Several other developing solvents, *e.g.*, benzene–chloroform (4:1), benzene–*n*-hexane (4:1) and benzene–chloroform–*n*-hexane (4:1:1) were also examined, but the chromatograms obtained had the disadvantage of incomplete separation of some of these compounds, which would be a serious handicap in analytical studies. Sharp spots free from tailing were obtained only with benzene–chloroform–*n*-hexane (5:1:5). Increasing the proportion of chloroform gave higher R_F values, but did not improve the separation.

The R_F values obtained with this system were adequate for the separation and identification of the compounds of interest.

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