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Note

Thin-layer chromatographic separation of the potential antituberculous agents N-aryl-N'-*p*-methylbenzenesulphonyl thiosemicarbazides

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Considerable interest has been focused on the synthesis, thin-layer chromatographic (TLC) separation and biological evaluation of substituted thiosemicarbazides that have been shown to possess antituberculous activity[1–3]. The substituted thiosemicarbazides are of interest to the pharmacologist because of their wide spectrum of biological activity, and their unique physical and chemical properties make the compounds potentially useful in drug receptor studies. Prescott and Li[4] showed that the substituted thiosemicarbazides possess low toxicity.

A series of N-aryl-N'-*p*-methylbenzenesulphonyl thiosemicarbazides were found to possess antituberculous activity. However, little or no information was available on the separation, identification and determination of these compounds, and this study was therefore performed in order to establish a sensitive and reproducible chromatographic procedure for their separation and identification.

MATERIALS AND METHODS

The TLC plates were prepared from a slurry of 40 g of silica gel G (E. 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° for 4 h and stored in a desiccator.

A 1% methanolic solution of each compound 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 activated TLC plate with the help of a micropipette. The spots were allowed to air dry and then developed by elution at constant temperature ($30 \pm 1°$) with benzene–carbon tetrachloride–chloroform–propanol-2 (4:7:2:1). About 70 min were usually required for the development. The chromatogram was then dried with a hot-air blower, sprayed with a 2% (w/v) methanolic solution of copper(II) sulphate and heated at 50°.

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 variation, which was regarded as being within the limits of experimental error.

TABLE I

TLC SEPARATION OF N-ARYL-N'-*p*-METHYLBENZENESULPHONYL THIOSEMICARBAZIDES

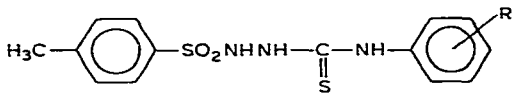

Plate: silica gel G, 20 × 20 cm, 0.20 mm layer. Developing solvent: benzene–carbon tetrachloride–chloroform–propanol-2 (4:7:2:1). Detection reagents (2%, w/v): D_1 = copper(II) sulphate; D_2 = cobalt(II) sulphate; D_3 = basic lead acetate. Colours: B = black; Br = brown; D = dark; G = green; V = violet; Y = yellow.

R	$R_F \times 100$*	*Detection*		
		D_1	D_2	D_3
H	68	BV	G	DBr
2-CH_3	61	YV	V	Br
3-CH_3	50	BV	G	DBr
4-CH_3	47	BV	G	DBr
2-OCH_3	73	Y	G	Br
4-OCH_3	44	V	BV	Br
2-Cl	65	V	GV	Br
3-Cl	54	BV	GV	Br
4-Cl	40	BV	GBr	DBr
4-OC_2H_5	57	V	GV	Br

* These $R_F \times 100$ values are averages of five identical runs.

The detection limit was found to be *ca.* 1 μg for each compound. The only absorbent used was silica gel G, but heating of the TLC plate at various temperatures (75–150°) was also examined. Heating the plate hardly affected the chromatograms, but the best separation was achieved with heating at 110° for ½ h.

Several developing solvents, *e.g.*, benzene, benzene–chloroform (4:1), benzene–propanol-2 (3:1), benzene–carbon tetrachloride–chloroform (4:2:1) and benzene–chloroform–propanol-2 (4:2:1), were also examined, but incomplete separations were obtained. Sharp spots free from tailing were found only with the solvent system benzene–carbon tetrachloride–chloroform–propanol-2 (4:7:2:1). Increasing the proportion of propanol-2 gave higher R_F values, but did not improve the separation.

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

REFERENCES

1 R. Behnisch, F. Mietzsch and H. Schmidt, *Amer. Rev. Tuberc.*, 61 (1950) 1.
2 K. A. Brownlee and D. Hamre, *J. Bacteriol.*, 61 (1951) 127.
3 R. L. Thompson, M. L. Price and S. A. Minton, Jr., *Proc. Soc. Exp. Biol. Med.*, 78 (1951) 11.
4 B. Prescott and C. Li, *J. Med. Chem.*, 7 (1964) 383.