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Note

Thin-layer chromatography of biologically active agents

I. Thiosemicarbazones

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The thiosemicarbazones are a group of substances of clinical interest because of their wide spectrum of biological activity. French and Blanz[1,2] demonstrated that several α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, from pyridine, pyrazine, pyridazine, isoquinoline, quinazoline, phthalazine and purine, possess significant antineoplastic activity when the carbonyl group of the side chain is located at an α-position to the ring nitrogen atom. Prescott and Li[3] showed that thiosemicarbazones exhibit low toxicity. Several thiosemicarbazones have been demonstrated to possess antivirus effects[4].

Franc[5] separated thiosemicarbazones of aliphatic ketones by paper chromatography; only one other paper refers to the thin-layer chromatography (TLC) of these substances[6]. In this paper we describe a convenient TLC method for separation and identification of thiosemicarbazones.

MATERIALS AND METHODS

The thiosemicarbazones (Table I) were synthesized according to the method of Bernstein *et al.*[7].

Thin-layer chromatographic plates were prepared from silica gel G (SMI; 20 × 20 cm, thickness 0.25 mm) and MN-Polyamide-DC6 (Macherey, Nagel & Co.; 20 × 20 cm, thickness 0.25 mm) according to the manufacturer's instructions. The silica gel G plates were air-dried, activated at 110°C for 3 h and stored in a desiccator. The solvent system comprised benzene–chloroform–methanol (9:3:2 or 9:3:3).

A 1% methanolic solution of each compound was prepared and 1 μl (corresponding to 10 μl of each compound) was spotted 2.0 cm from the edge of the plate. The solvent was allowed to migrate 15 cm from the starting line. The chromatograms were developed at room temperature (20 ± 3°C) in a normal chromatographic chamber presaturated with the solvents for at least 30 min. Two different spray reagents were used. One was ferric chloride in butanolic (1%, w/v); in this case, heating at 110°C was required. The other was a new and sensitive spray reagent, 2,4-dinitrophenylhydrazine dissolved in ethanol (3%, w/v); it is, furthermore, suitable for testing semicarbazones.

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RESULTS AND DISCUSSION

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

The detection limit was found to be *ca.* 1 μg for each compound. The new spray, 2,4-dinitrophenylhydrazine, located the thiosemicarbazone spots unequivocally and did not require heating. It is also useful in semicarbazones detection.

Several developing solvents, *e.g.*, benzene–ethanol (8:2), benzene–methanol–hexane (7:1:2), benzene–ethyl acetate (1:1), chloroform–methyl ethyl ketone–formic acid (9:2:1) and toluene–chloroform–acetone (8:5:7), were examined, but incomplete separations were obtained. Sharp spots free from tailing were found only with the solvent system benzene–chloroform–methanol (9:3:2 or 9:3:3).

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

TABLE I

COLOUR REACTIONS AND ULTRAVIOLET SPECTRA OF THIOSEMICARBAZONES

Tsc = Thiosemicarbazone; Bz = benzaldehyde; Thph = thiophenecarboxaldehyde; Fur = furaldehyde; AcNaph acetylnaphthalene. Solvent: A = benzene–chloroform–methanol (9:3:2); B = benzene–chloroform–methanol (9:3:3 S = Silica gel G; P = polyamide; D_1 = 2,4-dinitrophenylhydrazine; D_2 = ferric chloride. Colours: Y = yellow; Br brown; R = red; Or = orange; M = magenta; L = light; D = dark; G = grey; P = pink. Each $R_F \times 100$ val represents the mean of five determinations.

Thiosemicarbazone	$R_F \times 100$ *in solvent*				*Colour reaction*		*Ultraviolet spectra*	
	A		*B*		D_1	D_2	λ_{max}	*log ε*
	S	*P*	*S*	*P*				
Tsc Bz	48	76	70	80	Y	LBr	312	4.39
Tsc *o*-OH-Bz	45	41	73	56	Y	DBr	328	4.18
Tsc *m*-OH-Bz	21	29	63	44	Y	LBr	309	4.24
Tsc *p*-OH-Bz	11	23	49	39	Br-R	LBr	319	4.42
Tsc 2,3-diOH-Bz	7	12	41	22	Or	G	335/296	4.40/4.15
Tsc 3,4-diOH-Bz	10	10	32	14	Or	DG	330	4.35
Tsc 2,4-diOH-Bz	12	21	40	35	Y	G	312	4.39
Tsc 3,4-diOH-5-OCH_3-Bz	22	17	29	31	Or-G	G	328/302	4.19/4.06
Tsc Fur	44	65	64	71	Or-R	Br-Y	316	4.61
Tsc 2-Thph	45	64	58	71	Or-R	Br	328	4.38
Tsc Acetophenone	56	86	69	94	Or-R	Br-Y	302	4.36
Tsc 5-NO_2-2-Fur	25	50	51	73	Or	Y	283	3.98
Tsc Cyclohexanone	58	92	90	94	Y	Br	268	4.33
Tsc 9-Anthraldehyde	79	87	88	95	Y	Y	362/344	3.85/3.72
Tsc AcNaph	78	94	89	96	Or	Or-R	320/278	4.59/4.37
Tsc Acetone	62	89	50	92	Y-R	P	252	4.04
Tsc 2-Pentanone	62	97	68	97	Or	P	266	4.27
Tsc Benzophenone	–	–	79	95	–	Y	312	4.33
Tsc Acetaldehyde	34	47	46	76	–	M	261	4.10
Tsc 2-Butanone	45	68	72	89	–	Br	293	4.83
Tsc CH_3–CO–iso-C_3H_7	37	36	25	21	–	P	260	4.01

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