Paper Chromatography of 2,4-Dinitrophenylsulfide Derivatives of Mercaptans and Mercapto-Acids*

E. A. DAY[†] and S. PATTON, Department of Dairy Science, The Pennsylvania State University, University Park, Pennsylvania

INTRODUCTION

The 2,4-dinitrophenylsulfides (DNPS) exhibit characteristics which make them suitable as derivatives for identification of mercaptans and mercapto-acids. In view of this point, the lack of information on methods for separation of mixtures of the derivatives, their light absorption characteristics, and other properties is somewhat surprising. Bost et al.¹ first reported on the mercaptan derivatives in 1932, and Carson and Wong² have recently described a column chromatography procedure for resolving such mixtures. However, the procedure presents difficulties in locating bands on the column because of the faint yellow color of the compounds. This problem is further magnified when working with microquantities of material.

The work reported herein describes suitable paper chromatography methods for separating mixtures of DNPS of the C₁ to C₇ normal aliphatic mercaptans and of mixtures of mercapto-acids. As little as 0.03 μ g, of individual components can be detected. In addition, light absorption characteristics, melting points, and elemental analysis were determined on the authentic derivatives.

^{*}Authorized for publication as Paper No. 2206 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[†] Present address: Department of Food and Dairy Technology, Oregon State College, Corvallis, Oregon.

EXPERIMENTAL

Preparation of Authentic Derivatives

One milliliter of pyridine was added to 9 ml. of 0.1 M 1-fluoro-2,4dinitrobenzene in 95% ethanol followed by a slight excess of the mer-The mixture was warmed on a steam bath for 10 minutes, captan. and warm distilled water was added until the first appearance of The product formed bright vellow crystals when the cloudiness. mixture was cooled. The crystals were collected by filtration and were washed and recrystallized from a suitable mixture of ethanol and water until a constant melting point was obtained. Derivatives of the normal C_1 to C_7 mercaptans were prepared. Melting points were taken on a Fisher-Johns melting point apparatus and later verified on the Kofler micromelting point stage. The molecular extinction coefficients (ϵ) were determined by analyzing three separate solutions of each derivative at five concentrations ranging from 2.5 to 15.0 mg./ l. in 95% ethanol. Absorbance was measured at the ultraviolet absorption maxima with a Beckman Model DU Spectrometer. Elemental analyses were carried out and R_f values were determined by paper chromatography.

Preparations of Mercapto-Acid Derivatives

The DNPS derivatives of 2-mercapto-acetic, 3-mercapto-propionic, and 3-mercapto-butyric acids were prepared by reacting 1.0 g. of the acid dissolved in a few milliliters of pyridine with 1.5 g. of 1-chloro-2,4-dinitrobenzene dissolved in 10 ml. of pyridine. The mixture was heated on a steam bath for 30 minutes and then acidified with 20%H₂SO₄ until the odor of pyridine was no longer evident. The crude product, usually an oil, was recrystallized to constant melting point from a suitable mixture of benzene and petroleum ether (b.p. 40-55°C.). Melting points, light absorption characteristics, and elemental analyses were obtained in the manner described for the mercaptan derivatives.

Paper Chromatography of the DNPS Derivatives of Mercaptans

Ten μg . quantities of saturated ethanol solutions of the DNPS were spotted one inch from the edge of the shortest dimension of Whatman No. 1 paper (10 \times 15 in.). The sheet was fastened into the form of a MICROCHEMICAL JOURNAL, VOL. III, ISSUE 2 cylinder and placed in a 9-cm. Petri dish cover in the bottom of a 6×18 in. chromatography jar. It was equilibrated for 12 hours with methanol saturated with *n*-heptane and then developed with *n*-heptane saturated with methanol by the ascending method described by Huelin³ for separation of 2,4-dinitrophenylhydrazones. The spots were easily distinguishable on the paper when examined over an ultraviolet lamp at 360 m μ . Although the lower limits for detection of the DNPS on paper chromatograms were not determined, spots containing 0.03 μ g. of derivatives were visible with the aid of the ultraviolet lamp.

Paper Chromatography of DNPS Derivatives of Mercapto-Acids

Sheets of Whatman No. 1 paper (9 \times 10.5 in.) were spotted one inch from the edge of the shorter dimension with 10 μ g. of the DNPS derivative in acetone. The sheets were fastened into the shape of a cylinder and developed by ascending methods. Two procedures were found suitable for resolving the derivatives.

Procedure A. The lower (aqueous) phase of the equilibrated solvent system of *n*-butanol:ethanol:water (20:5:25) was placed in a 6×12 in. cylindrical chromatography jar, and two halves of a Petri dish, back to back, were placed in the bottom of the jar. The paper cylinder was then placed in the upper Petri dish half and the jar was sealed. After two hours of equilibration, the upper phase of the solvent system was added to the Petri dish containing the paper cylinder and the chromatogram was allowed to develop for 12 to 15 hours. The positions of the spots were readily noted by observation over the ultraviolet lamp.

Procedure B. This procedure was the same as Procedure A except that a solvent system of benzene and 1% aqueous acetic acid (25:25) was employed.

RESULTS

Observations on the DNPS derivatives of mercaptans are presented in Table I. A laboratory accident prevented elemental analyses of nitrogen for the C_2 to C_7 derivatives. The R_f values are averages of five chromatograms. As evidenced by the data, good separation of the compounds was obtained.

	Average	R_f	17 0.16	17 0.28	0 0.42	9 0.54	15 0.64	3 0.72	4 0.80		R_f Values	oc. A Proc. B	.59 0.20	0.67 0.71	0.75 0.81
rophenylsulfide Derivatives of Normal Mercaptans	Theory, % Found, %	Ø	14.9	14.0	13.3	12.4	12.0	11.4	10.7		Ì	Pr	21 0	0 62	8
		N	13.35									S	12.2	11.7	11.(
		Н	2.91	3.53	4.07	4.63	5.29	5.52	5.81	o-Acids	nd, %	Z	11.01	10.42	9.98
		C	9.08	2.32	4.23	7.17	8.81	0.66	2.46	ercapto	Four	Н	3.20	3.13	3.70
			97 3	05 4	23 4	51 4	86 4	27 5	74 5	ome M	ome M	С	37.26	40.35	42.69
		32	3 14.	3 14.	. 13.	12.	11.	11.	10.	s of S			41	.74	20
		z	13.08	12.28	11.57	10.93	10.36	9.85	9.39	vative		S	12	Ξ	11
		Н	2.83	3.54	4.17	4.73	5.23	5.68	6.09	sLE II e Deri	ory, <i>%</i>	N	10.85	10.25	6.76
		C	9.25	2.10	4.62	5.85	8.87	0.68	3.32	TAE ylsulfid	Thec	Н	2.34	2.96	3.52
	I	10-4	23 3	25 4	28 4	23 4	28 4	28 5	26 5	ophen		C	37.21	39.70	41-95
Dinit		× *	1.5	1	1		_	-	Ξ	Dinitr	×	4-	16	<u></u>	32
Properties of 2,4-1	λmax	πm	333	333	334	334	334	334	334	[2,4-]	ų	10			
	Ч.р., °С	128	115	81	66	80	74	82	rties of	λ	μm	332	333	335	
	C.b.	127	115	82	67	80	74	82	Prope	M.p.,	(obs.)	170	153	166	
	N N S4DNPS	Methanethiol	Ethanethiol	n-Propanethiol	n-Butanethiol	n-Pentanethiol	n-Hexanethiol	n-Heptanethiol		3 4-DNPS	derivative	2-Mercapto-acetic	3-Mercapto-propionic	3-Mercapto-butyric	

TABLE I Dinitrophenylsulfide Derivatives of N

MICROCHEMICAL JOURNAL, VOL. III, ISSUE 2

E. A. DAY AND S. PATTON

Table II contains data for the DNPS derivatives of mercaptoacids with average R_f values from three representative chromatograms. Although not at point in this study, separation of longer chain acid derivatives might be desirable in some instances. It was observed that R_f values for the three derivatives could be proportionately reduced by using a solvent system of heptane: benzene: 1%aqueous acetic acid (25:25:25). For this purpose, the methanolheptane system described above might also prove suitable.

Summary

Ascending paper chromatographic methods for the separation of 2,4-dinitrophenylsulfide derivatives of mercaptans and mercaptoacids are presented. A solvent system composed of methanol as the stationary phase and heptane as the mobile phase gave satisfactory separations of the normal C_1 to C_7 mercaptan derivatives on Whatman No. 1 paper. Two procedures were devised to separate the mercapto-acids derivatives. One method employed a solvent system of *n*-butanol:ethanol:water (20:5:25) with the water-rich layer as the stationary phase and the butanol-rich layer as the mobile phase. The solvent system for the second procedure was composed of equal parts of benzene and 1% acetic acid with the benzene-rich layer as the mobile phase in this case.

We are indebted to A. Steyermark, Micro-Chemical Division of Hoffmann-LaRoche, Inc., for the elemental analyses reported herein.

References

1. R. W. Bost, J. O. Turner, and R. D. Norton, J. Am. Chem. Soc., 54, 1985 (1932).

2. J. F. Carson and F. F. Wong, J. Org. Chem., 22, 1725 (1957).

3. F. E. Huelin, Australian J. Sci. Research, Ser. B, 5, 328 (1952).

Received November 16, 1958