

## Paper Chromatography of Thioureas

ANDERS KJÆR and KURT RUBINSTEIN

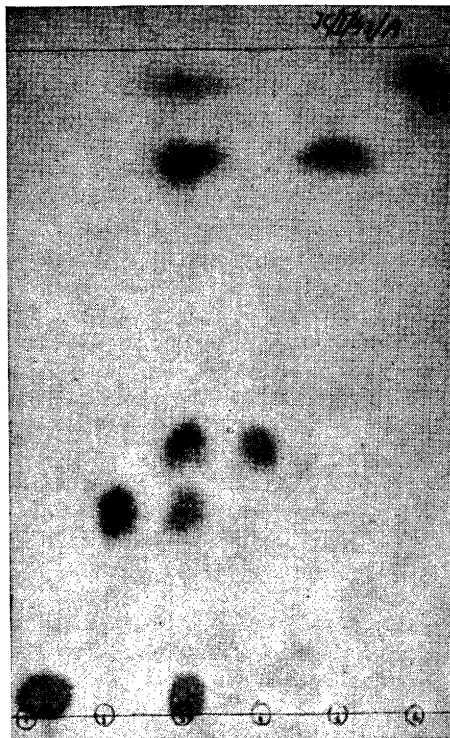
*Chemical Laboratory, University of Copenhagen, Copenhagen, Denmark*

In connection with a current investigation of some natural products it became necessary to separate and identify minute amounts of N-substituted thioureas. The success which has attended the use of paper chromatography for the identification of a great variety of widely differing substances suggested the application of this technique to the present problem. No previous examples of paper chromatography of thioureas seem to be on record. A satisfactory method for separating a large number of thioureas has been worked out and a brief account of the method given in a preliminary paper<sup>1</sup>. The present communication deals with a more detailed description of the technique and results.

Because, for other reasons, we were mainly interested in N-substituted thioureas, most of the substances studied were of this type. In addition, however, we investigated some N,N'- and N,N-disubstituted, N-trisubstituted and N-tetrasubstituted thioureas while isothioureas have not been considered. The scope of the method was further explored by studying some thiosemicarbazides, a few thio-amides and -hydrazides and a series of heterocyclic thio-urea-derivatives (*e. g.* thiobarbituric acids). Various substituents were considered, including alkyls, alkenyls, aralkyls and aryls as well as combinations of these. From the outset of the present investigation the highest accuracy attainable was aimed at, because highly reproducible results were a prerequisite for the application of the method to the currently studied problems.

### EXPERIMENTAL

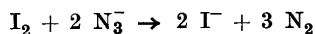
*Spraying Reagents.* For location of the spots on the paper two different and about equally satisfactory reagents were found to be applicable. Grote<sup>2</sup> described a colour reaction for thioureas and similar compounds with a specially prepared reagent, produced from sodium nitroprusside, bromine and hydroxylamine. We found the intensive blue or turquoise colours produced to be sufficiently stable<sup>3</sup> for application in paper chromato-



*Fig. 1. Paper-chromatogram on No. 1 Whatman paper of N-methylated thioureas. Solvent system chloroform-water. Temperature 23.5°. Sprayed with Grote's reagent. 1. Mono-methyl-; 2. N,N'-Dimethyl-; 3. Mixture of mono-methyl-, N,N'-dimethyl-, N,N-dimethyl-, trimethyl- and tetramethyl-; 4. N,N-Dimethyl-; 5. Trimethyl-; 6. Tetramethyl-.*

graphy. Dilution tests demonstrated that a solution containing 0.1 mg of thiosinamine per ml still gave an easily detectable blue spot on filter paper.

Likewise, good results were obtained on consecutive spraying of the paper with a starch-solution and the iodine-azide reagent of Feigl<sup>3</sup>. Owing to the ability of thioureas to catalyse the reaction



white spots on a uniformly blue background appeared where the individual thioureas were located. This catalytic action of sulphur compounds on the reaction above is of a rather general nature as far as thio-compounds containing divalent sulphur are concerned (*cf.* Ref.<sup>3</sup>). Therefore, we believe that the iodine-azide-reagent will prove useful for the detection of a great many sulphur compounds in paper chromatography. When this work was about complete, we incidentally noticed one previous application of the reagent to paper chromatography. Chargaff, Levine and Green<sup>4</sup> utilised the Feigl reagent for the

detection of cysteine, cystine and methionine, although without the preceding treatment with a starch-solution, which latter certainly increases the sensitivity considerably.

Because of its convenience, we generally preferred the Grote reagent in our paper-chromatographic work. Heating at 100° for about one minute accelerated the colour formation, whereas prolonged heating or the application of higher temperatures should be avoided due to the thermolabile character of the colours produced.

*Solvents.* After many experiments with various solvents and solvent mixtures, chloroform-water was eventually found to be the most suitable system for the separation of mixtures of mono-substituted thioureas. Water-saturated chloroform was used throughout as the mobile solvent with water as the stationary phase. The application of chloroform, apparently a little used solvent in paper chromatography, at first presented certain difficulties in this investigation. Due to the high density of chloroform it proved difficult to secure the necessary equilibrium in the chromatographic chamber until special precautions were introduced as described below. Also, mixtures of butanol-dibutyl ether proved to be of value in many cases but suffered from the inherent disadvantage of rapidly developing peroxides, deleterious to the thioureas under investigation.

*Paper.* Throughout this work, No. 1 Whatman paper was used, the strips always being cut parallel to the long side of the commercial sheets. Several experiments, however, were conducted on No. 2 Whatman paper with about equally satisfactory results. The very slow No. 50 Whatman paper proved useful in some separations, especially for substituted thioureas with  $R_{Fh}$ -values (see below) over 1.20. The texture of this paper, however, makes it less suitable for general application.

*Temperature.* In order to obtain highly reproducible results it appeared necessary to keep the temperature of the chromatographic system rather constant. Therefore, all determinations reported in the present paper were performed in a room kept at  $23.5^\circ \pm 0.2^\circ$ .

*Working procedure.* For several reasons the ascending technique was preferred during the present investigation. It combines speed and high reproducibility with simplicity in apparatus and performance. Especially when chloroform is used as the mobile solvent, the descending technique results in an inconveniently high flow-rate, mainly due to the high density of the solvent.

The chromatograms were run in a rectangular all-glass case (23 × 11 cm, 32 cm high), provided with a plane, ground upper edge and covered with a glass-plate, tightened by means of silicone grease. On the bottom of the chamber was placed a rectangular glass dish (20 × 10 cm, 5 cm high), containing water-saturated chloroform and surrounded by a thin layer of chloroform-saturated water. The back-wall of the chamber was completely lined with filter paper dipping into the chloroform in the dish. The two narrow end-walls were similarly lined with paper, soaked in the aqueous phase on the bottom of the chamber. Only with this arrangement was it possible to create the necessary liquid-vapour equilibrium throughout the entire system. A rack of pyrex glass rods, standing on the bottom and extending along the end-walls right to the top of the chamber, was here provided with two bendings at slightly different levels. These supported a horizontal glass rod to which the chromatogram was attached by means of small stainless steel springs.

The Whatman paper was cut into broad strips, 28 cm long and 17.5 cm wide. Two cm from the lower edge of the paper the starting line was marked, and another horizontal pencil-line drawn in a distance of 23 cm from the starting line, indicating the end-position of the rising liquid-front. The strip was fixed to the glass rod along its upper edge, and at

the opposite end it was burdened with another rod in order to keep the strip in a vertical position. The solutions to be investigated, usually containing 10 mg of solute per ml in ethanol or chloroform, were applied with micropipettes as circular spots of 6–8 mm diameter along the starting line at previously marked points. This required *ca.* 1–2  $\mu$ l of solution, *i. e.* each spot contained 10–20  $\mu$ g of the individual compound. It may be noticed, however, that mixtures containing as little as 1 mg/ml of each compound still gave detectable spots after development and spraying. Six individual samples were run simultaneously on the same strip. Spots No. 1 and 6 were applied at a distance of 2 cm from the vertical edges of the paper while the remaining samples were arranged equidistantly in between, all along the starting line.

The air-dried paper was now placed at the upper support of the rack in the chromatographic chamber with its lower edge a few mm above the chloroform-surface in the dish on the bottom. It was kept here for 16–18 hours to acquire complete equilibrium. This stage proved very important for the obtainment of reproducible results. Next day the glass cover was removed, the strip lowered to the second bending in the rack and the lid rapidly replaced. The chloroform was now allowed to rise to the upper line (23 cm). By proper operation the chloroform-front rose as an even, horizontal line, easily observed in transmitted light. The development should require 110–120 minutes. Periods outside this range were indicative of imperfect equilibrium and led to less satisfactory results. It should be pointed out, however, that no difficulties in this respect were encountered when the directions given above were carefully followed. The strip was now removed from the chamber, air-dried and sprayed with Grote's reagent. On brief heating at 100°, sharp, deep-blue spots revealed the location of the individual thioureas. The boundaries of the patches were marked with a pencil because the colour gradually faded.

Even in experiments carefully conducted as described, some variations in the  $R_F$ -values were noticed from one run to another, probably caused by factors outside the control of the experimenter (unevenness in paper texture, traces of impurities *etc.*). These fluctuations were circumvented, however, by relating the distances travelled by the individual compounds not as usually to the solvent front, but rather to some arbitrary reference compound. For this purpose we chose phenylthiourea which was placed as a standard sample on each chromatogram. For convenience, we introduced the term  $R_{Ph}$ , defined as the ratio between the distances travelled by the individual compounds and phenylthiourea.

*Quantitative studies.* No extensive study of the application of the present technique to quantitative determinations of thioureas was made. Exploratory experiments, however, indicated an accuracy of *ca.*  $\pm 5$  %. In these experiments the solutions to be investigated were applied to two identical paper sheets as 6 mm broad streaks along the starting lines. After chromatography in the usual way, one of the sheets was sprayed with Grote's reagent and the other cut in horizontal strips according to the locations determined on spraying. The strips were separately extracted in micro-Soxhlet extractors with methanol and the concentrations determined spectrophotometrically.

#### Model substances

Only a few of the substances examined and listed in the accompanying tables were commercially available. Most of them were synthesised according to descriptions in the literature and found to possess physical data in agreement with those previously reported. In some cases, however, we found physical properties which differed considerably from

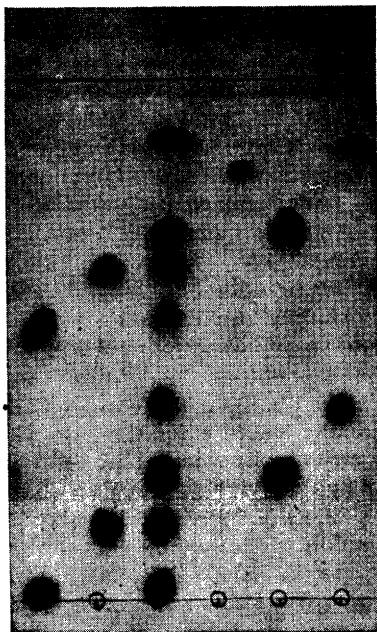


Fig. 2. Paper-chromatogram on No. 1 Whatman paper of different mono-substituted thioureas. Solvent system chloroform-water. Temperature 23.5°. Sprayed with Grote's reagent. 1. Methyl- and  $\beta$ -methallyl-; 2. Ethyl- and  $\alpha$ -methallyl-; 3. Mixture of methyl-, ethyl-, allyl-, iso-propyl-,  $\beta$ -methallyl-,  $\alpha$ -methallyl-, n-butyl- and  $\beta$ -phenylethyl-; 4. Phenyl-; 5. Allyl- and n-butyl-; 6. iso-Propyl- and  $\beta$ -phenylethyl-.

the literature values. The revised data together with compounds not previously reported will be briefly discussed below.

*N-Ethylthiourea*. Two widely differing melting points are found in the literature. Hofmann<sup>5</sup> reports the m.p. 113°, while Dyson and Hunter<sup>6</sup> list the value 144°\*. Our preparation, carefully recrystallised from ethyl acetate, melted at 110–111°\*\*.

$C_3H_8N_2S$ (104.2)	Calc.	N	26.89
	Found	»	26.93

*N-iso-Propylthiourea*. Jahn<sup>7</sup> reports the m.p. 157° while a non-analysed specimen is claimed by Mathes *et al.*<sup>8</sup> to melt at 168–169°. Our product, recrystallised from water as flat prisms, melted at 169–170°.

$C_4H_{10}N_2S$ (118.2)	Calc.	N	23.70
	Found	»	23.79

*N-iso-Butylthiourea*. This compound was previously reported by Hofmann<sup>9</sup> with m.p. 93.5°. Our preparation, synthesised from *iso*-butylamine and thiocarbonyl chloride, appeared as colourless, nacreous plates after recrystallisation from water. M. p. 100–101°.

$C_5H_{12}N_2S$ (132.2)	Calc.	C	45.42	H	9.15	N	21.20
	Found	»	45.63	»	8.96	»	21.13

*Compounds No. 14–17 in Table 1.* These substances have been reported in a previous paper<sup>10</sup>.

\* misprint for 114°?

\*\* all our melting points are uncorrected.

*N-n-Butyl-N'-methylthiourea*. This was prepared from *n*-butyl isothiocyanate and methylamine in the usual way. The thiourea was recrystallised from ethyl acetate-petroleum ether. M. p. 40–41°.

$C_8H_{14}N_2S$ (146.3)	Calc.	C	49.26	H	9.65	S	21.92
	Found	»	49.35	»	9.42	»	21.93

*4-Morpholinethiocarboxamide* (Table 2, No. 11). A compound with this alleged structure was recently described by Henry and Dehn<sup>11</sup>, m.p. 111.5–112.5°. It was prepared by evaporating an aqueous solution of morpholine hydrochloride and potassium thiocyanate on the water-bath. The generally experienced difficulties in rearranging thiocyanates of secondary amines in conjunction with the agreement in m. p. with the long known morpholinium thiocyanate, rendered it probable that the compound in question was in fact the salt. On repeating the experiment we found this to be the case.

An authentic sample of 4-morpholinethiocarboxamide was synthesised by the Wallach-procedure<sup>12</sup>. An ethereal solution of 7.3 g of cyanogen bromide was added dropwise to an ice-cooled solution of 12.0 g of morpholine in ether. The morpholine hydrobromide was removed by filtration, the ether evaporated at ordinary pressure and the residue distilled twice *in vacuo* to give 5.5 g of pure *4-cyanomorpholine* as a colourless liquid. B. p. 74.5° at 0.9 mm.

$C_5H_8N_2O$ (112.1)	Calc.	C	53.58	H	7.19	N	25.00
	Found	»	53.39	»	7.15	»	24.95

When a solution of 4-cyanomorpholine in ethanol was saturated with ammonia and hydrogen sulphide the separation of crystals started spontaneously. After standing overnight, the reaction mixture was concentrated on the water-bath and filtered hot. On dilution with ether crystalline 4-morpholinethiocarboxamide separated. It was recrystallised from ethanol-ether for analysis. M. p. 171°.

$C_5H_{10}N_2OS$ (146.2)	Calc.	C	41.07	H	6.90	N	19.17
	Found	»	41.14	»	6.82	»	19.16

*N-Methyl-4-morpholinethiocarboxamide*. This compound was prepared from methyl isothiocyanate and morpholine in the usual way. The m. p. was 102.5–103.5° after recrystallisation from ethyl acetate-petroleum ether.

$C_6H_{12}N_2OS$ (160.2)	Calc.	C	44.98	H	7.55	N	17.49	S	20.02
	Found	»	44.75	»	7.59	»	17.56	»	20.03

*N-Ethyl-4-morpholinethiocarboxamide*. The reaction of ethyl isothiocyanate and morpholine in ethanol yielded the substituted thiourea in high yield. It was recrystallised twice from ethanol-ether. M. p. 81.5–82.5°.

$C_7H_{14}N_2OS$ (174.3)	Calc.	C	48.24	H	8.10	N	16.08	S	18.40
	Found	»	48.32	»	8.19	»	00.00	»	18.51

*N-Allyl-4-morpholinethiocarboxamide*. Henry and Dehn<sup>11</sup> recently described a compound, m. p. 56–57°, with this alleged structure. On reaction of allyl isothiocyanate with morpholine we obtained, however, a compound which melted at 64.5° after recrystallisation from aqueous ethanol. This we consider to be authentic *N*-allyl-4-morpholine-thiocarboxamide.

$C_8H_{14}N_2OS$ (186.3)	Calc.	C	51.58	H	7.58	N	15.04	S	17.21
	Found	»	51.90	»	7.38	»	14.98	»	17.18

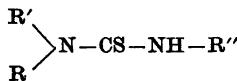
Table 1.  $R_{Ph}$ -values of *N*-mono-substituted thioureas on No. 1 Whatman filter paper with chloroform-water as the solvent system. Temperature 23.5°.

No.	Substituent	$R_{Ph}$	No.	Substituent	$R_{Ph}$
1.	Hydrogen	0.00	12.	$\beta$ -Phenylethyl	1.10
2.	Methyl	0.04	13.	Allyl	0.26
3.	Ethyl	0.15	14.	$\alpha$ -Methallyl	0.74
4.	<i>n</i> -Propyl	0.42	15.	$\beta$ -Methallyl	0.62
5.	<i>iso</i> -Propyl	0.41	16.	<i>trans</i> -Crotyl	0.78
6.	<i>n</i> -Butyl	0.84	17.	3-Butenyl	0.61
7.	<i>iso</i> -Butyl	0.76	18.	<i>p</i> -Bromophenyl	1.00
8.	DL- <i>sec.</i> -Butyl	0.74	19.	<i>p</i> -Iodophenyl	1.06
9.	<i>tert.</i> -Butyl	1.00	20.	<i>o</i> -Tolyl	1.19
10.	<i>iso</i> -Pentyl	1.07	21.	<i>m</i> -Tolyl	1.25
11.	Benzyl	0.90	22.	<i>p</i> -Tolyl	1.22

## RESULTS

The  $R_{Ph}$ -values for the compounds investigated were determined at least three times and found to be reproducible within  $\pm 0.01$ . No change in the values was observed even in mixtures containing eight or nine individual compounds. Only a few of the thioureas investigated had  $R_{Ph}$ -values which precluded their separation by the present technique. A general tendency to

Table 2.  $R_{Ph}$ -values of symmetrically and unsymmetrically *N*-disubstituted thioureas on No. 1 Whatman filter paper with chloroform-water as the solvent system. Temperature 23.5°.



No.	R	R'	R''	$R_{Ph}$
1.	Methyl	Hydrogen	Methyl	0.42
2.	Methyl	Hydrogen	Ethyl	0.89
3.	Ethyl	Hydrogen	Ethyl	1.24
4.	Methyl	Hydrogen	<i>n</i> -Butyl	1.35
5.	<i>n</i> -Butyl	Hydrogen	<i>n</i> -Butyl	1.41
6.	Methyl	Hydrogen	Phenyl	1.40
7.	Ethyl	Hydrogen	Phenyl	1.41
8.		Ethylthiourea		0.26
9.	Methyl	Methyl	Hydrogen	0.60
10.		Pentamethylene	Hydrogen	1.18
11.		3-Oxapentamethylene	Hydrogen	0.55

Table 3.  $R_{Fh}$ -values of *N*-tri- and *N*-tetrasubstituted thioureas on No. 1 Whatman paper with chloroform-water as the solvent system. Temperature 23.5°.

No.	Substance	$R_{Fh}$
1.	Trimethylthiourea	1.21
2.	<i>N</i> -Methyl-4-morpholinethiocarboxamide	1.17
3.	<i>N</i> -Ethyl-4-morpholinethiocarboxamide	1.27
4.	<i>N</i> -Allyl-4-morpholinethiocarboxamide	1.31
5.	<i>N</i> -Methyl-1-piperidinethiocarboxamide	1.37
6.	<i>N</i> -Methyl-1-pyrrolidinethiocarboxamide	1.35
7.	<i>N</i> -Ethyl-1-pyrrolidinethiocarboxamide	1.37
8.	Tetramethylthiourea	1.43

form green-blue spots of lower intensity was noticed for the aromatically substituted thioureas. Thiourea itself did not move on the paper, while gradual lengthening of the carbon chain of the substituents resulted in increasing  $R_{Fh}$ -values as would be expected. The aromatically substituted, as well as most di-, tri- and tetrasubstituted derivatives, possessed  $R_{Fh}$ -values above 1.00. For mixtures containing compounds with  $R_{Fh}$ -values far above 1.25 the present solvent system was not very suitable. No efforts were made, however, to extend the technique to such compounds because our main interest centred on the aliphatic mono-substituted derivatives. In Fig. 1 and Fig. 2 two typical chromatograms are shown.

Table 4.  $R_{Fh}$ -values of various thio-compounds on No. 1 Whatman filter paper with chloroform-water as the solvent system. Temperature 23.5°.

No.	Substance	$R_{Fh}$	Colour
1.	Thioacetamide	0.25	weakly violet
2.	Thiobenzamide	0.79	brownish
3.	Thiosemicarbazide	0.00	violet
4.	4-Methylthiosemicarbazide	0.20	violet
5.	1,1,4-Trimethylthiosemicarbazide	1.32	faintly blue
6.	1-Phenylthiosemicarbazide	0.55	turquoise
7.	Thiobarbituric acid	0.00	blue-green
8.	5-Ethylthiobarbituric acid	0.00	yellow
9.	5,5-Diethylthiobarbituric acid	1.30	yellow
10.	5-Methyl-5-allylthiobarbituric acid	1.19	yellow
11.	5,5-Diallylthiobarbituric acid	1.37	yellow



The compounds investigated are listed in the Tables 1, 2 and 3 along with their  $R_{PA}$ -values, determined as described in the experimental section.

A few thioamides and thiohydrazides were investigated. With these, Grote's reagent gave yellow or brownish spots of much lower intensity. Various substituted thiosemicarbazides were also studied but with less satisfactory results than the thioureas. Due to the formation of only weak and transient colours, varying in an unpredictable manner from blue or violet to red with change in kind and position of substituents, the present method is not considered suitable for this class of compounds. A series of thiobarbituric acids was investigated, all producing yellow spots on spraying with Grote's reagent. The results obtained are listed in Table 4.

#### SUMMARY

A paper-chromatographic method for the separation and identification of thioureas has been developed. Experimental data for 41 substituted thioureas are presented.

Some related compounds, *viz.* thioamides, thiohydrazides, thiosemicarbazides and thiobarbituric acids have been briefly studied by the same technique.

Some new thioureas are described and revised data for a few previously reported compounds given.

The valuable technical assistance of Mr. Josef Conti in part of this work is greatly acknowledged. The microanalyses were performed in this Laboratory by Mr. A. Grossmann. The photographic assistance of Dr. N. Berg is highly appreciated. Thanks are due also to Dr. A. Friediger and Dr. J. Hald for their valuable gifts of some of the compounds investigated.

#### REFERENCES

1. Kjær, A., and Rubinstein, K. *Nature*. *In press*.
2. Grote, I. W. *J. Biol. Chem.* **93** (1931) 25.
3. Feigl, F. *Qualitative Analysis by Spot Tests*. 3rd edit. Elsevier Publ. Co., Inc. New York—Amsterdam, 1946.
4. Chargaff, E., Levine, C., and Green, C. *J. Biol. Chem.* **175** (1948) 67.
5. Hofmann, A. W. *Ber.* **18** (1885) 2788.
6. Dyson, G. M., and Hunter, R. F. *Rec. trav. chim.* **45** (1926) 421.
7. Jahn, H. *Monatsh.* **3** (1882) 168.
8. Mathes, R. A., Stewart, F. D., and Swedish Jr., F. *J. Am. Chem. Soc.* **70** (1948) 1452.
9. Hofmann, A. W. *Ber.* **7** (1874) 511.
10. Kjær, A., Rubinstein, K., and Jensen, K. A. *Acta Chem. Scand.* **7** (1953) 518.
11. Henry, R. A., and Dehn, W. M. *J. Am. Chem. Soc.* **72** (1950) 2806.
12. Wallach, O. *Ber.* **32** (1899) 1874.

Received February 3, 1953.

*Acta Chem. Scand.* **7** (1953) No. 3