SULPHENAMIDES AND SULPHINAMIDES

I. THE CHROMATOGRAPHY OF AROMATIC DERIVATIVES

R. F. BAYFIELD
Veterinary Research Station, Department of Agriculture, Glenfield, N.S.W. (Australia)
AND
V. CLARKE AND E. R. COLE
Department of Applied Organic Chemistry, University of New South Wales, Kensington, N.S.W. (Australia)
(Received June 24th, 1966)

INTRODUCTION

Studies on the stability, interchange and displacement reactions of alkyl and aryl sulphenamides (I) and sulphinamides (II), groups of organosulphur compounds whose basic structure differs by one oxygen atom, require that a suitable method be available for the detection and estimation of products, often in rather complex mixtures from reactions carried out on a small scale. Methods for the analysis of these compounds have not been extensively studied and the present report emphasizes the correlation in behaviour of the two series with the structural difference.

$$\underset{\text{(I)}}{R\cdot S\cdot NH\cdot R'} \qquad \underset{\text{(II)}}{R\cdot SO\cdot NH\cdot R'}$$

Previous work on organosulphur compounds[1–3] had shown the usefulness of paper chromatography in direct and reverse phase methods and as a matter of continuity it seemed desirable if possible to apply the same systems to these groups of compounds, together with extensions to thin-layer chromatography.

PAPER CHROMATOGRAPHY

Systems previously used involved impregnation of the paper with either liquid paraffin[1] or phenoxyethanol[3] and immediate differences in behaviour appeared when these systems were used for the present materials. Best separations of sulphenamides were achieved on the paraffined paper and these contrasted with the poorer separations on phenoxyethanol paper. Quite opposite results were obtained with the sulphinamides where poor separation was associated with the use of paraffined paper but good separations followed use of the phenoxyethanol paper. The suggestion thus arises that these different results are associated with the bonding between the sulphoxide group and the hydroxylic phase, and it has been interesting therefore, to observe similar results with sulphoxides themselves (*e.g.* diphenyl sulphoxide) and sulphides (diphenyl sulphide).

With the sulphenamides, use of a liquid paraffin system was profitably extended to zinc carbonate–fluorescein impregnated papers[4]. These combined better separation with a method of locating spots, by viewing under ultraviolet light, which allowed easy removal for spectroscopic identification and estimation after leaching with solvent from cut-out areas. Spots appeared as mauve ultraviolet absorbing areas on a light yellow fluorescent background.

THIN-LAYER CHROMATOGRAPHY

On silica gel, resolution of sulphenamides and sulphinamides was less satisfactory by thin-layer chromatography with a variety of solvents. Indeed pure materials which gave only one spot on paper chromatograms gave several spots on silica gel plates. It is possible that some of these, particularly with the sulphenamides, were due to decomposition on the plate, but it is conceivable that duplication of spots at least may be the result of different rates of migration of different forms of the compound. Sulphenamides have $pK_a \sim 3$[5] and it is possible that the molecule on silica gel may move in both ionized and unionized forms. It is not unusual to find that pure materials without decomposition give rise to more that one spot during chromatography[6].

EXPERIMENTAL

Materials

Sulphenamides and sulphinamides were prepared by standard methods to satisfactory analyses and constants.

Reagents

Liquid paraffin solution: 3 % v/v solution in light petroleum b.p. 40–60°. Phenoxyethanol solution: 10 % v/v solution in acetone. *p*-Dimethylaminobenzaldehyde solution: 0.1 % w/v in ethyl alcohol.

Preparation of papers and plates

Whatman No. 1 paper was impregnated by drawing it through the required solution then allowing excess solution to drain and the solvent to evaporate, from the vertically suspended paper. With the phenoxyethanol papers it was found that the distance of travel by a compound was dependent on the time of drying after treatment. About 16 h or overnight standing was taken as a practical standard treatment.

Zinc carbonate–fluorescein papers were prepared according to the directions of the Vitamin E Panel of the Analytical Methods Committee[4].

Kieselguhr G plates (thickness 250 μ) were allowed to stand for 30 min after spreading, then dried at 100° for 2 h.

Cellulose plates (thickness 250 μ) were prepared using a 20 % w/v mixture with water, treated in a Waring blendor for 5 min before spreading. Plates were then allowed to stand for 30 min before drying at 100° for 2 h.

Silica gel plates (thickness 250 μ) were prepared in the usual manner.

Plates treated with phenoxyethanol were prepared with the same precautions as used for the papers.

Developing solvents

Various strengths of methyl alcohol were used to develop chromatograms on paraffined papers and plates. Little difference in results appeared with stronger solutions (80–90 %) but gradual improvement was noted with lower strengths and results reported, unless otherwise stated, are those using 65 % methyl alcohol.

n-Heptane saturated with phenoxyethanol was used for papers and plates treated with this material.

Paper chromatograms were developed by the descending technique.

Method of loading chromatograms

Materials were loaded on to chromatograms from chloroform solution.

RESULTS*

(a) *Paper chromatography*

Sulphenamides. A comparison of results for a series of sulphenamides with the reverse phase paraffin system on paper and on zinc carbonate treated paper is presented in Table I.

TABLE I

PAPER CHROMATOGRAPHY OF SULPHENAMIDES

Compound		*System*											
R·S·NH·R′		*Reverse phase with paraffined paper*						*Reverse phase with paraffined zinc carbonate–fluorescein paper*					
R	*R′*	*cm**	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ
Ph	Ph	17.6	1.0	16.2	1.0	16.1	1.0	15.1	1.0	15.4	1.0	15.5	1.0
CH_3Ph	Ph	11.9	0.68	10.9	0.67	10.6	0.66	9.8	0.65	10.0	0.65	9.6	0.62
Ph	$PhCH_3$	11.1	0.63	10.9	0.67	10.6	0.66	10.1	0.67	10.0	0.65	9.6	0.62
CH_3Ph	$PhCH_3$	7.1	0.40	6.4	0.40	6.1	0.38	6.5	0.43	5.8	0.38	5.8	0.37
		Single spots		Mixtures well separated				Single spots		Mixtures well separated			
Ph	Ph	16.0	1.0	15.8	1.0	15.3	1.0	18.1	1.0	17.9	1.0	17.6	1.0
ClPh	Ph	11.6	0.72	10.9	0.69	10.7	0.70	13.0	0.72	12.4	0.69	12.2	0.69
Ph	PhCl	11.8	0.74	10.9	0.69	10.7	0.70	14.5	0.80	12.4	0.69	12.2	0.69
ClPh	PhCl	8.9	0.56	8.0	0.51	7.5	0.49	9.3	0.51	8.3	0.46	8.6	0.49
		Single spots		Mixtures well separated				Single spots		Mixtures well separated			

* Refers to movement by compound (30 μg).

For further comparison the behaviour of sulphenamides on phenoxyethanol-treated paper is presented in Table II.

Sulphinamides. Results for sulphinamides on phenoxyethanol paper are given in Table III, which also indicates the poorer separations on paraffined paper.

* Results are presented as R_ϕ values *i.e.* distance travelled compared to distance travelled by the parent member of each series where $R = R' = C_6H_5$.

TABLE II

PAPER CHROMATOGRAPHY OF SULPHENAMIDES

Compound $R \cdot S \cdot NH \cdot R'$		*System phenoxyethanol-treated paper*					
R	*R'*	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ
Ph	Ph	17.6	1.0	14.9	1.0	17.9	1.0
CH_3Ph	Ph	21.1	1.20	16.8	1.13	19.4	1.08
Ph	$PhCH_3$	21.0	1.19	17.5	1.18	20.9	1.17
CH_3Ph	$PhCH_3$	22.6	1.29	19.5	1.31	22.6	1.26
ClPh	Ph	17.2	0.98	14.4	0.97	17.9	1.0
Ph	PhCl	17.1	0.97	13.8	0.93	17.3	0.97
ClPh	PhCl	16.7	0.95	12.9	0.87	16.8	0.94
	Front	27.2		28.1		27.2	

TABLE III

PAPER CHROMATOGRAPHY OF SULPHINAMIDES

Compound $R \cdot SO \cdot NH \cdot R'$		*System*					
		Reverse phase with paraffined paper		*Phenoxyethanol-treated paper*			
R	*R'*	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ
Ph	Ph	No resolution achieved		3.3	1.0	3.2	1.0
CH_3Ph	Ph			4.0	1.2	4.1	1.3
Ph	$PhCH_3$	R_F values all between 0.9 and 0.97		4.2	1.3	4.2	1.3
CH_3Ph	$PhCH_3$			6.0	1.8	6.0	1.8
ClPh	Ph	No resolution achieved		3.7	1.1	3.8	1.2
Ph	PhCl			3.4	1.03	3.8	1.2
ClPh	PhCl	R_F values all between 0.9 and 0.97		4.6	1.4	4.3	1.35
Ph	$PhOCH_3$			1.1	0.3	1.0	0.3
$PhSON<^{CH_3}_{Ph}$				0.1	0.03	0.1	0.03

TABLE IV

THIN-LAYER CHROMATOGRAPHY OF SULPHENAMIDES ON KIESELGUHR G PLATES

Compound $R \cdot S \cdot NH \cdot R'$			*System*					
			Reverse phase paraffined plates		*Phenoxyethanol-treated plates*			
	R	*R'*	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ
1	Ph	Ph	13.0	1.0	3.9	1.0	3.4	1.0
2	CH_3Ph	Ph	11.2	0.87	4.9	1.26	4.1	1.21
3	Ph	$PhCH_3$	11.0	0.85	5.1	1.31	4.4	1.30
4	CH_3Ph	$PhCH_3$	8.7	0.68	6.2	1.59	5.3	1.56
5	ClPh	Ph	12.0	0.93	3.5	0.9	3.0	0.88
6	Ph	PhCl	12.5	0.97	2.9	0.74	2.5	0.73
7	ClPh	PhCl	11.3	0.88	2.7	0.70	2.2	0.65
Mixture of 1, 2, 3 and 4, on same plate			—	—	Spots at 3.9, 5.0 and 6.2 cm			
Mixture of 1, 5, 6 and 7 on same plate			—	—	Spots at 3.8, 3.0 and 2.6 cm			

TABLE V

THIN-LAYER CHROMATOGRAPHY OF SULPHENAMIDES

	Compound R·S·NH·R'		*System Kieselguhr G plates, treated with phenoxyethanol*									
			Two solvent ascents						*Three solvent ascents*			
	R	*R'*	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ	*cm*	R_ϕ
1	Ph	Ph	6.6	1.0	6.5	1.0	7.2	1.0	8.7	1.0	9.3	1.0
2	CH_3Ph	Ph	8.0	1.21	7.7	1.18	8.3	1.17	10.6	1.22		
3	Ph	$PhCH_3$	8.3	1.25	8.0	1.23	8.7	1.23	11.0	1.26		
4	CH_3Ph	$PhCH_3$	9.5	1.44	9.3	1.43	9.8	1.38	12.2	1.40		
5	ClPh	Ph	6.0	0.91	5.9	0.93	6.3	0.89			8.8	0.94
6	Ph	PhCl	5.2	0.79	4.9	0.75	5.4	0.76			7.8	0.84
7	ClPh	PhCl	4.5	0.68	4.3	0.66	4.7	0.66			7.0	0.75
Mixture of 1, 2, 3 and 4 on same plate			Spots at 6.9, 8.3, 8.8 and 9.8 cm						Spots at 9.2, 10.6, 11.0 and 12.1 cm			
Mixture of 1, 5, 6 and 7 on same plate			Spots at 6.9, 6.5, 5.5 and 4.8 cm						Spots at 9.5, 8.8, 7.8 and 6.9 cm			

TABLE VI

THIN-LAYER CHROMATOGRAPHY OF SULPHINAMIDES ON KIESELGUHR G PLATES

Compound R·SO·NH·R'		*System*				
		Reverse phase paraffined plates	*Phenoxyethanol-treated plates*			
R	*R'*		*cm*	R_ϕ	*cm*	R_ϕ
Ph	Ph		1.1	1.0	1.1	1.0
CH_3Ph	Ph	No resolution	1.3	1.2	1.3	1.2
Ph	$PhCH_3$	R_F values all between 0.9 and 0.97	1.4	1.3	1.4	1.3
CH_3Ph	$PhCH_3$		1.7	1.5	1.7	1.5
ClPh	Ph	No resolution	1.2	1.1	1.1	1.0
Ph	PhCl	R_F values all between 0.9 and 0.97	1.0	0.9	0.9	0.8
ClPh	PhCl		1.3	1.2	1.3	1.2
Ph	$PhOCH_3$		0.5	0.4	0.4	0.4
$PhSON<^{Ph}_{CH_3}$			0.1	0.1	0.1	0.1

TABLE VII

THIN-LAYER CHROMATOGRAPHY OF SULPHINAMIDES ON CELLULOSE PLATES

Compound R·SO·NH·R'		*System phenoxyethanol-treated plates*			
R	*R'*	*cm*	R_ϕ	*cm*	R_ϕ
Ph	Ph	2.0	1.0	1.8	1.0
CH_3Ph	Ph	2.3	1.15	2.8	1.55
Ph	$PhCH_3$	2.6	1.3	3.1	1.8
CH_3Ph	$PhCH_3$	3.0	1.5	3.6	2.0

(*b*) *Thin-layer chromatography*

Sulphenamides. Results for sulphenamides using kieselguhr impregnated with paraffin and with phenoxyethanol are shown in Table IV.

The effect of repeated solvent ascents on the behaviour of sulphenamides is shown in Table V.

Sulphinamides. The thin-layer chromatography of sulphinamides on kieselguhr plates variously treated is shown in Table VI.

The use of the cellulose–phenoxyethanol system is illustrated in Table VII.

Table VIII illustrates the use of *p*-dimethylaminobenzaldehyde as a reagent for detecting sulphenamides, sulphinamides and amines on the same chromatogram.

TABLE VIII

USE OF *p*-DIMETHYLAMINOBENZALDEHYDE AS DETECTION REAGENT

Compound		*System phenoxyethanol-treated Kieselguhr G plates*		
		Sulphenamides $R \cdot S \cdot NH \cdot R'$	*Sulphinamides* $R \cdot SO \cdot NH \cdot R'$	*Amines* $R' \cdot NH_2$
R	*R'*	R_ϕ	R_ϕ	R_ϕ
Ph	Ph	1.0	1.0	1.0
CH_3Ph	Ph	1.26	1.20	1.0
Ph	$PhCH_3$	1.31	1.30	1.18
CH_3Ph	$PhCH_3$	1.59	1.50	1.18
ClPh	Ph	0.90	1.10	1.0
Ph	PhCl	0.74	0.90	0.7
ClPh	PhCl	0.70	1.20	0.7
Distance moved in one ascent				
Ph	Ph	3.9 cm	0.3 cm	2.5 cm ($R' = C_6H_5$)

DISCUSSION

Location of spots

Sulphenamides were located on the paraffin-treated papers by exposure to bromine vapour when generally a blue to mauve spot formed. This colour deepened when sprayed with starch–iodide solution after removal of excess halogen in a current of warm air. Thus sensitivity was improved. The bromine/starch–iodide treatment applied to the zinc carbonate–fluorescein papers produced bright orange spots visible in ordinary light.

As indicated previously[2] bromine is not a good reagent for the detection of sulphinamides on chromatograms; apart from one unsymmetrical methoxyl derivative positive tests required very heavy and impractical loading. This applied irrespective of the chromatographic system used.

Departing from treatment with halogen, sulphenamides could be detected by treating with trichloracetic acid either in chloroform or aqueous solution. A series of colours was obtained similar to those obtained in the bromine test.

Fission reactions of sulphenamides with halogen acids to produce sulphenyl halides by the reversal of the condensation method of preparation are well recognized[7]:

$$R \cdot S \cdot Cl + H_2N \cdot R' \rightleftarrows R \cdot S \cdot NH \cdot R' + HCl$$

The ability to regenerate the amine by such a reverse reaction forms the basis of use of the *o*-nitrobenzene sulphenyl group as a protective agent in peptide synthesis[8]. Trichloracetic acid is a more than sufficiently strong acid to cause a scission reaction to produce an equivalent sulphenyl derivative. It was noted previously[2] that addition of excess sulphenyl chloride during the preparation of sulphenamides leads to colour formation and it is not improbable that a corresponding derivative from trichloracetic acid would do so even more readily. As evidence of the fission reaction aniline trichloracetate has been isolated and diphenyl disulphide detected on paper chromatograms.

No colour was obtained when sulphinamides were treated in this manner. However, when chromatograms of sulphinamides were exposed to hydrochloric acid fumes or treated with trichloracetic acid, in each case followed by starch–iodide spray, well defined blue-mauve spots appeared on a very clear background. Trichloracetic acid thus appeared as a selective reagent in producing immediate colours with sulphenamides but requiring starch–iodide with sulphinamides. This result was undoubtedly associated with the oxidizing power of the sulphoxide group for which a composite reagent of acid, iodide and starch has been described[9]. The acid treatment of sulphinamides also led to decomposition.

Based on this ease of breakdown of sulphenamides and sulphinamides in each case to give an amine, *p*-dimethylaminobenzaldehyde has been used successfully as an indirect means of detection on chromatograms. There is some selectivity in this test as sulphonamides without an additional amino group do not respond to the reagent. Similar decomposition with acid preceding location of the amine moiety in ultraviolet light has been applied to the indirect detection of sulphenamide accelerators themselves and as components of vulcanizates[10].

The colour obtained in the reaction of bromine with aryl sulphenamides was controlled by the amine component. Derivatives prepared from aniline gave a blue-mauve spot irrespective of substitution in the sulphenyl portion of the molecule. By contrast compounds prepared from *p*-toluidine gave a pink-brown colour even when prepared from thiophenol as the sulphur component. Slighter differences in colour appeared with derivatives of *p*-chloroaniline where a more purplish colour was formed. It may be remarked again that sensitivity was improved if exposure to bromine was followed by spraying with starch–iodide after suitable treatment for removal of excess halogen. The direct test was sensitive to 2–5 $\mu g/cm^2$ which improved to 1–2 $\mu g/cm^2$ with the starch–iodide treatment.

Sensitivity of detection of sulphinamides in the acid/starch–iodide test was 2–5 μg. The bromine/starch–iodide test and the *p*-dimethylaminobenzaldehyde test were also about this sensitivity.

Similar sensitivities were obtained with both groups of compounds on the thin-layer plates.

Separation by thin-layer chromatography had the advantage of much shorter development times—generally 2–3 h against 16 or so for paper chromatograms. This

advantage was maintained despite the use at times of repeated solvent ascent[11] to assist the resolution of mixtures (Table V). It must be noted however that paper chromatograms as on zinc carbonate–fluorescein paper, were considered more convenient for quantitative work.

The influence of the amine component extends to chromatographic behaviour in the series. As indicated through the tables the unsubstituted phenyl derivatives, N-phenyl benzenesulphenamide and N-phenyl benzenesulphinamide ($R=R'=C_6H_5$) were taken as reference compounds equivalent to diphenyl disulphide of earlier work[1].

The disulphides, ditolyl and dichlorodiphenyl* moved more slowly on paraffin treated paper ($R_\phi < 1$) an effect maintained with mono-substitution. Under similar conditions on paraffined paper and on paraffined zinc carbonate–fluorescein paper substituted sulphenamides also had $R_\phi < 1$ (Table I). By contrast, sulphinamides had $R_\phi \sim 1$ (Table III) and were not resolved by this system.

On phenoxyethanol-treated papers and plates more discrimination appeared. On these papers the tolylsulphenamides had $R_\phi > 1$ but the chlorophenyl derivatives had $R_\phi < 1$ (Table II). This effect did not appear with the sulphinamides which, while resolved, all had $R_\phi \geqslant 1$ (Table III).

The separation of sulphenamides in this manner was carried over to phenoxyethanol-treated kieselguhr plates (Table IV), a result emphasized in the multiple ascent series (Table V). Indeed best resolution of the sulphenamides, even of unsymmetrically substituted derivatives was achieved on these plates. Recognition of the separation was assisted by the differences in colour obtained according to the amine component as discussed above.

The phenoxyethanol-treated kieselguhr plates also gave the best separation of sulphenamides from sulphinamides. Comparisons may be based on the R_F values of the parent compounds, Tables II and III, for paper and, Tables IV and VI, for thin-layer chromatograms.

Separations achieved on phenoxyethanol-treated cellulose plates were about the same as those obtained on kieselguhr plates. Some irregularities may appear and this appears to be related not only to the drying time after treatment of the cellulose, but goes back further to the cellulose itself. Different batches appear to have different "wetting" capacity so that while relative performance of materials is maintained, nevertheless R_ϕ values may vary. However, comparison of the two sets of results in Table VII shows that each compound has proportionally about the same greater R_ϕ value, *i.e.* about 30 % increase in the value for the second set. Emphasis is therefore placed on the necessity for reference compounds other than the parent material. Despite this possibility of variation in results, a preference may be expressed for the cellulose plates as being less likely to be disturbed by the dipping and spraying steps.

Table VIII illustrates the correlation between the rate of movement of the amine component and the derived sulphenamides.

ACKNOWLEDGEMENTS

The interest in this work of the Director (Mr. J. C. KEAST) and Mr. R. H. FALK of the Veterinary Research Station, Glenfield, is gratefully appreciated.

* Refers to *para* substitution in this and present work.

SUMMARY

An examination has been made of the paper and thin-layer chromatography of a series of aromatic sulphenamides and sulphinamides. Attention is drawn to the correlation of structure with chromatographic behaviour.

REFERENCES

1 E. R. COLE, *Nature*, 198 (1963) 1083.
2 R. F. BAYFIELD, V. CLARKE AND E. R. COLE, *J. Chromatog.*, 19 (1965) 370.
3 D. BARNARD AND E. R. COLE, *Anal. Chim. Acta*, 20 (1959) 540.
4 *Report of the Analytical Methods Committee, Vitamin E Panel, Analyst*, 84 (1959) 356.
5 R. F. BAYFIELD AND E. R. COLE, to be published.
6 A. H. BECKETT AND N. CHOULIS, *J. Pharm. Pharmacol.*, 15 (1963) 242T.
7 J. H. BILLMAN AND E. O'MAHONY, *J. Am. Chem. Soc.*, 61 (1939) 2340.
8 L. ZERVAS, D. BOROVAS AND E. JAZIS, *J. Am. Chem. Soc.*, 85 (1963) 3660.
9 J. F. THOMPSON, W. N. ARNOLD AND C. J. MORRIS, *Nature*, 197 (1963) 380.
10 J. HERR, *Plaste u Kautschuk*, 10 (1963) 737; from *Anal. Abstr.*, 12 (March 1965) No. 1277.
11 G. URBACH, *J. Chromatog.*, 12 (1963) 196.