

CHROM. 3790

CHROMATOGRAPHY OF ISOMERIC PEROXYBENZOIC ACIDS

II. THE THIN-LAYER CHROMATOGRAPHIC SEPARATION MECHANISM OF ISOMERIC MONO- AND DISUBSTITUTED PEROXYBENZOIC ACIDS ON VARIOUS POLYAMIDE AND CELLULOSE ADSORBENTS

RAJKO KAVČIČ AND BOŽO PLESNIČAR

*Organic Chemistry Laboratory, Department of Chemistry,
University of Ljubljana, Ljubljana (Yugoslavia)*

AND

ANTON PERDIH

Public Health Institute, Kranj (Yugoslavia)

(Received September 4th, 1968)

SUMMARY

The migration behaviour of several mono- and disubstituted peroxybenzoic acids has been studied on seven thin-layer chromatographic adsorbents. Some effects of the molecular structure of the peroxy acids on their chromatographic behaviour and the interactions between the stationary or the mobile phase and peroxy acids are discussed.

INTRODUCTION

In connection with our research on organic peroxy acids and related materials, we have recently shown that positional isomerism of substituted peroxybenzoic acids could have an appreciable effect on the mobilities of such isomers on paper chromatography¹. In an attempt to gain further insight into the factors involved in these variations between positional isomers, we have investigated the migration behaviour as well as the nature of hydrogen bonding in the TLC of a series of isomeric mono- and disubstituted peroxybenzoic acids on a variety of thin-layer adsorbents.

EXPERIMENTAL

The mono- and disubstituted peroxybenzoic acids were synthesized by oxidizing small portions of parent aromatic acids with 95 % hydrogen peroxide in methanesulphonic acid according to the method of SILBERT *et al.*². The products were purified by recrystallization from a mixture of hexane and diethyl ether, and in a few cases, pure compounds were obtained by sublimation *in vacuo*. Purity and identity of the individual isomers was checked by the usual physical and chemical methods.

MATERIALS AND METHODS

The following commercial products (obtained from Macherey-Nagel & Co., Düren, Germany) were used as adsorbents: MN-Cellulose Powder 300 HR, MN-Cellulose Powder 300 Ac/ca. 10%, MN-Cellulose Powder 300 Ac/ca. 20%, MN-Cellulose Powder 300 Ac/ca. 30%, MN-Cellulose Powder 300 Ac/ca. 40%, MN-Polyamide TLC 6/6, MN-Polyamide TLC 6 and MN-Polyamide TLC 11.

Thin-layer plates (5 × 20 cm and 20 × 20 cm) were prepared by one of the procedures outlined below:

(A) Acetylated cellulose adsorbents were applied as a slurry prepared as follows: 15 g of acetylated cellulose (for five plates) was mixed with a few ml of 95% ethanol in a mortar, and 60 ml of ethanol were added with stirring in an electric mixer for approx. 1 min. The slurry obtained was applied to the plates in the usual way, using Desaga equipment. The plates were dried in air for 24 h before use.

(B) 15 g of Polyamide MN-6 and Polyamide MN-6/6 were homogenized with about 65 ml of water for 1 min in an electric mixer. Polyamide MN-11 was homogenized with 55 ml of methanol, and the slurry was applied to the plates, which were dried in air for 24 h before use.

The samples were applied as dilute (about 4–5%) solutions in ethyl acetate, in quantities of 20 μg, by means of a micropipette. After applying samples, the plates were allowed to stand in air for half an hour before chromatography. The solvents were commercial products "for chromatography." The plates were developed in well-sealed glass tanks saturated with solvent vapours of a composition suitable for the TLC of the compounds being examined. When the solvent front had reached the 10-cm line on the layer—the time of development was between 20 and 30 min—the plates were removed and the spots located with an aqueous acetic acid solution of potassium iodide or with an aqueous acetic acid solution of *p*-aminodimethylaniline hydrochloride¹.

The temperature around the chromatographic systems was $21^{\circ} \pm 1^{\circ}$, and the relative air humidity 50–60%.

RESULTS AND DISCUSSION

The TLC data for various isomeric mono- and disubstituted peroxybenzoic acids on seven different polyamide and cellulose adsorbents are summarized in Table I.

Thin-layer chromatography on Silica Gel G

Attempts have been made to obtain some thin-layer chromatographic data concerning the peroxy acids examined on silica layers (Silica Gel G, according to STAHL, Merck). Unfortunately, partial or complete decomposition was observed during the migration, and this was even more pronounced when developing solvents did not contain acetic acid. On the other hand, we noticed previously¹ that the same peroxy acids are stable during chromatography on acetylated and non-acetylated paper. On activated silica layers, the time for decomposition was about 10 min after application, on air-dried silica layers 20 min. The impregnation of silica gel with dimethylformamide enhances the stability for 30 min. Better results were obtained by lowering the temperature around the chromatographic system to 6°. The peroxy

TABLE I

 R_F VALUES $\times 100$ OF MONO- AND DISUBSTITUTED PEROXYBENZOIC ACIDS ON VARIOUS CELLULOSE AND POLYAMIDE ADSORBENTS

Substituted peroxybenzoic acid	Stationary ^a and mobile phases ^b														
	1		2		3		4		5		6		7		8
	A	A	A	B	A	B	A	B	A	A	B	A	B	A	
2-Nitro-4-chloro-		26	27		17		13		9	34	12	40		10	
2-Nitro-5-chloro-		25	29	62	16	44	11	7	31	10	38		8		
2-Chloro-5-nitro-		28	22	58	17		12	11	37	14	43		11		
4-Chloro-3-nitro-	76	41	37		24	49	18	14	43	16	48		14		
2-Nitro-5-methyl-	69	22	21	60	14	45	10	4	36	6	42		8		
4-Nitro-3-methyl-	85	50	43	70	33	56	28	21	52	24	56		21		
2,4-Dichloro-	92	75	67	72	53	57	42	33	51	36	55		28		
2,5-Dichloro-	91	74	65	64	45	58	37	22	48	31	51		21		
3,4-Dichloro-	96	81	76	73	59	59	54	35	57	43	59		30		
<i>o</i> -Chloro-	91	69	61	62	40	51	36	25	46	29	49		25		
<i>m</i> -Chloro-	93	77	68	73	53	63	48	39	52	41	58		30		
<i>p</i> -Chloro-	97	81	73	80	54	65	47		56	39	57		33		
<i>o</i> -Nitro-	68	20	18	55	9	44	5	5	26	6	31		5		
<i>m</i> -Nitro-	84	40	37	68	20	51	16	12	39	16	46		12		
<i>p</i> -Nitro-	71	32	32	63	18	48	16	10	35	15	44		11		
<i>m</i> -Fluoro-		75	71	78	46	61	43	34	51	41	55		30		
<i>p</i> -Fluoro-		74	68	73	45	60	45	40	54	45	58		31		
<i>m</i> -Bromo-		80	77		53			37	56	42	57		29		

^a Stationary phases: (1) MN-Cellulose Powder 300 HR; (2) MN-Cellulose Powder 300 Ac/ca. 10%; (3) MN-Cellulose Powder 300 Ac/ca. 20%; (4) MN-Cellulose Powder 300 Ac/ca. 30%; (5) MN-Cellulose Powder 300 Ac/ca. 40%; (6) MN-Polyamide TLC 6; (7) MN-Polyamide TLC 6/6; and (8) MN-Polyamide TLC 11 (Macherey-Nagel).

^b Mobile phases: (A) carbon tetrachloride-acetic acid (10:1); (B) chlorobenzene-acetic acid (10:1).

acids are partly or completely decomposed on silica gel, presumably by the action of the inorganic binders (CaSO_4). This explanation could be supported by our results (obtained on Eastman-Kodak K 301R2 plates with polyvinyl alcohol as a binder), which showed that no decomposition or very slow decomposition was observed during the chromatographic separation on these plates of the peroxy acids examined.

Another possible explanation of the observed phenomena would be similar to that apparent in the reaction of unsaturated fatty acids on Silica Gel G, where the energy-rich activated sites (non-solvatized silanol groups which are very weakly hydrogen bonded if at all) catalyse the decomposition⁹. If polyvinyl alcohol is used as a binder, these sites are occupied by the binder, which enables the formation of hydrogen bonds. The same experimental observations were made in our preliminary study of the decomposition kinetics of the peroxy acids examined using ethyl acetate as a solvent, in the presence of small amounts of both kinds of adsorbent. A more detailed investigation of this phenomenon is under way.

Thin-layer chromatography on acetylated celluloses

In general it is possible to use two different systems of developing solvents relative to the stationary phase on acetylated cellulose: (1) on a less polar mobile phase than acetylated cellulose or (2) on a more polar mobile phase. In order to fine

mobile phases which are less polar than acetylated cellulose, mixtures of different solvents (carbon tetrachloride, petroleum ether, aromatic hydrocarbons plus different quantities of acetic acid) were tested. On acetylated celluloses, the best results were obtained by using the combinations of carbon tetrachloride-acetic acid (10:1) and chlorobenzene-acetic acid (10:1). On non-acetylated cellulose the best separation was achieved by using the mobile phase light petroleum (b.p. 40–70°)-acetic acid (10:1). The results obtained by using mobile phases which are more polar than acetylated cellulose were not satisfactory. Small differences in polarity of isomeric peroxybenzoic acids are more pronounced in non-polar media.

The comparison of R_F values on various acetylated celluloses shows the progressive decrease when we go from the non-acetylated cellulose to cellulose thin layers of an increasing degree of acetylation (Figs. 1 and 2).

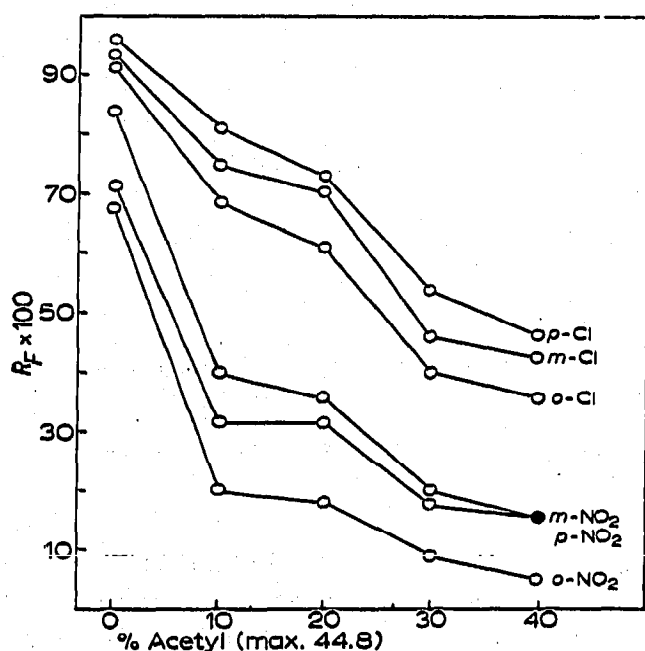


Fig. 1. Plot of R_F values of some monosubstituted peroxybenzoic acids on variously acetylated cellulose layers against the degree of acetylation.

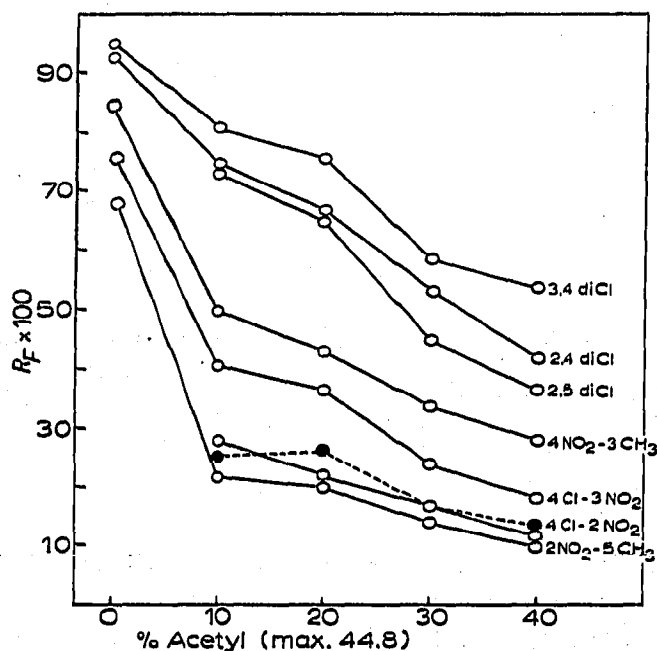
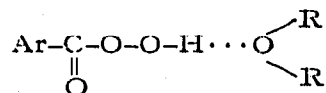


Fig. 2. Plot of R_F values of some disubstituted peroxybenzoic acids on variously acetylated cellulose layers against the degree of acetylation.

The differences in mobilities of isomeric mono- and disubstituted peroxybenzoic acids on various acetylated celluloses are most probably the result of the following two factors: (1) the distribution between the water phase (up to 11% in the case of non-acetylated cellulose and somewhat lower in acetylated celluloses) and the mobile phase; and (2) the formation of intermolecular hydrogen bonds between the molecules of peroxy acids and relatively basic oxygen atoms of the hydroxy, ether and ester groups of the cellulose.

Our I.R. investigation^{4, 5} indicates that aromatic peroxy acids appear in solvents which are not capable of forming hydrogen bonds as intramolecularly hydrogen-bonded monomers. Solvents and systems with basic oxygen, on the other hand, are

capable of forming intermolecularly hydrogen-bonded adducts by combining a molecule of the peroxy acids with a molecule of the Lewis base, according to the following scheme:



It is reasonable to assume that the influence of the first factor could be important, especially on non-acetylated cellulose and on celluloses of a low degree of acetylation. On the other hand, the second factor is more apparent with highly acetylated celluloses. The increasing migration of substituted peroxybenzoic acids on non-acetylated cellulose layers in comparison with that on acetylated ones, could be explained by the low solubility of these peroxy acids in water as compared to that in the mobile phase. The distribution coefficient between the mobile phase and the stationary phase is large. By increasing the degree of acetylation, the hydrophobicity is increased and therefore, the distribution coefficient becomes smaller. Although the shapes of the curves obtained by plotting the R_F values against the degree of acetylation are roughly hyperbolic (Figs. 1 and 2), there are small deviations from the ideal hyperbolic shape, especially between the 10 % and 20 % acetylated cellulose layers. These deviations are possibly due to a slight difference between the declared and actual degrees of acetylation of commercial adsorbents. In fact, however, this is probably not the most important factor, since the deviations due to the type of substituent are considerably greater. An additional possible explanation of this phenomenon could be the occurrence of simultaneous mechanisms of distribution and/or binding of peroxy acids on these layers, which are not dependent in the same manner on the degree of acetylation. The suggestion of hydrogen bonding in which the peroxy acid group or a nitro group acts as the proton acceptor, while the adsorbent acts as donor, could be plausible.

In Fig. 3 are plotted the moisture contents of variously acetylated celluloses, as well as the R_F values of one peroxy acid (*m*-nitroperoxybenzoic acid) against the degree of acetylation. The differences in the shapes of the two curves allow us to conclude that the moisture content in acetylated celluloses is not the predominant cause of decreasing mobility.

Thin-layer chromatography on various polyamides

Graphic presentation of the TLC migration patterns of mono- and disubstituted peroxybenzoic acids on different polyamides is given in Fig. 4.

The question of a correlation of R_F values obtained cannot be answered directly. There are somewhat greater differences in the pattern on the polyamide adsorbents than in that on the cellulose layers. Thus, the R_F values are greatest on Polyamide 6/6 and lowest on Polyamide 11, although the dielectric constant of Polyamide 6 is highest, and that of Polyamide 11 lowest. The apparent discrepancy between the R_F values on polyamide adsorbents and the values of dielectric constants does not provide a rational explanation for the above-mentioned phenomena. Still, we would like to mention the intensity of tailing on various polyamide layers examined. The observed tailing was directly proportional to the dielectric constant of the polyamide adsorbent. It appears that the formation of hydrogen bonds between the peroxy acids and the polyamide plays an important role in the TLC separation of peroxy

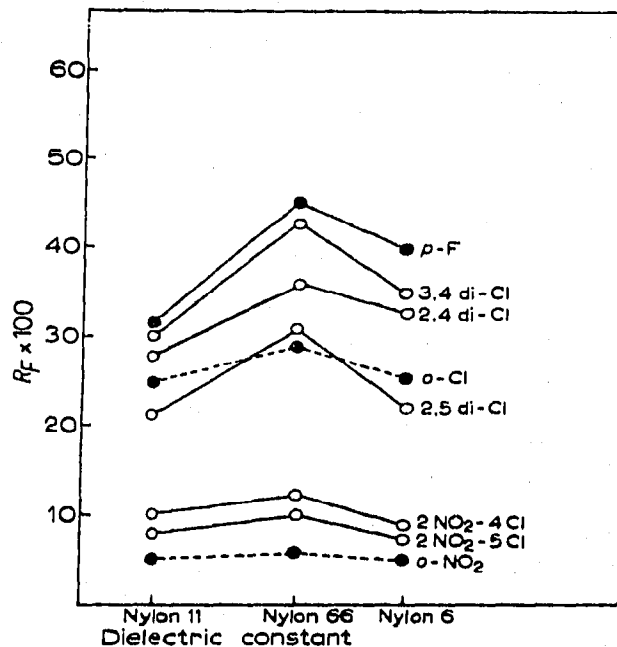
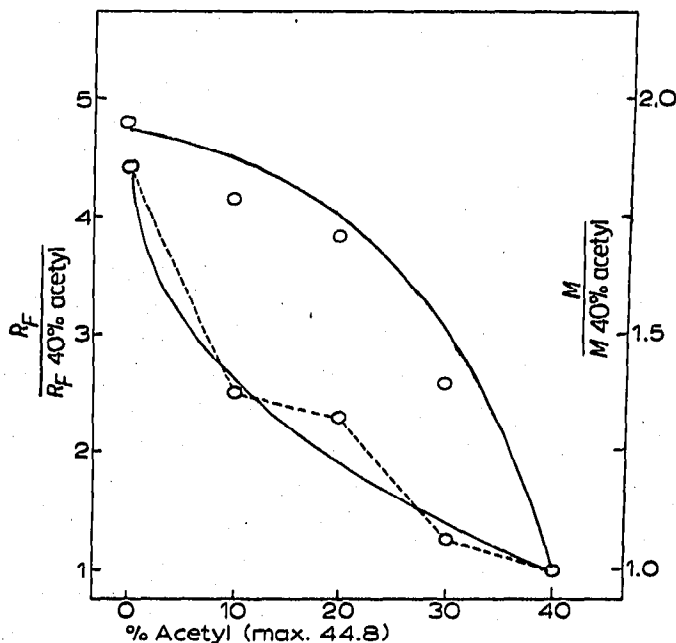
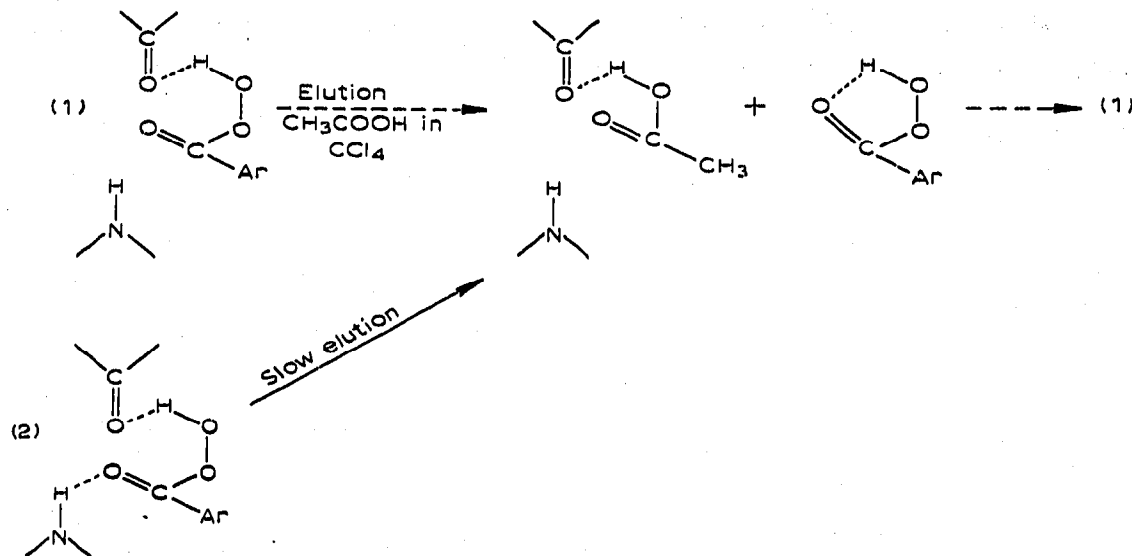


Fig. 3. Plot of R_F values of *m*-nitroperoxybenzoic acid on variously acetylated cellulose layers and moisture content of acetylated cellulose adsorbents against the degree of acetylation.

Fig. 4. Plot of R_F values of some mono- and disubstituted peroxybenzoic acids on various polyamide layers against the approximate trend of increasing dielectric constant of the same adsorbents.

acids on these adsorbents. Apparent irreversibility of such intermolecular hydrogen bond formation is quite in keeping with the tailing observed which was greatest on Polyamide 6 and smallest on Polyamide 11. We believe that the chromatographic separation of the peroxy acids examined on polyamide adsorbents is not connected with the formation of new types of products which would be formed by a reaction between peroxy acids and polyamides. The main factor in this separation is the formation of partially reversible intermolecular hydrogen bonds. The fact that developing systems such as acetic acid-, dimethylformamide- and alcohol-containing systems do produce well defined spots without tailing, whereas there is heavy tailing with the acetone-acetic acid-water (1:1:3) system, provides additional support for the above-mentioned conclusion. In addition, the structure of the polyamide can also influence the formation of hydrogen bonding between the peroxy acid and the adsorbent. In ideal crystalline polyamide, all carbonyl groups are blocked with hydrogen bonds to parallel NH groups. In a commercially available polyamide, in powder form, less than 50% is crystalline⁶. Besides, in Polyamide 6, nearly 40% of the NH groups are free⁷, which is also in accordance with the intensity of tailing observed. In Polyamide 11 and Polyamide 6/6, the number of free NH groups is much smaller. The results of the TLC of substituted peroxybenzoic acids on polyamide layers described above led us to the conclusion that during the separation, we are observing partially reversible formation of hydrogen bonds between the molecule of peroxy acid and polyamide. In the case of phenols⁸ there exists the possibility of formation of only one hydrogen bond between the carboxyl oxygen atom of the polyamide and

the hydrogen atom of the phenolic group. With peroxy acids, the formation of one as well as two hydrogen bonds could be expected, according to the following scheme:



The formation of both types of hydrogen bond is conditioned by a rather favourable steric configuration of a peroxy-carboxyl group which is not planar but somewhat skewed^{9,10}. It is our assumption that appearance of the tailing is a result of the formation of double hydrogen bonds. The mobile phase (acetic acid in carbon tetrachloride or chlorobenzene) is not capable of simultaneously taking over both hydrogen bonds.

ACKNOWLEDGEMENT

The authors wish to thank the Boris Kidrič Fund for financial support of this research.

REFERENCES

- 1 R. KAVČIČ, B. PLESNIČAR AND B. HRISTOVSKI, *J. Chromatog.*, **22** (1966) 381.
- 2 L. S. SILBERT, E. SIEGEL AND D. SWERN, *J. Org. Chem.*, **27** (1962) 1336.
- 3 E. STAHL (Editor), *Dünnschichtchromatographie*, Springer-Verlag, Berlin, 1962.
- 4 R. KAVČIČ, B. PLESNIČAR AND D. HADŽI, *Spectrochim. Acta*, **23A** (1967) 2483.
- 5 D. HADŽI, R. KAVČIČ AND B. PLESNIČAR, in preparation.
- 6 P. GUSTAVSON, *Kem. Ind. (Zagreb)*, **3** (1968) 97.
- 7 N. G. McCURM, B. E. READ AND G. WILLIAMS, *Anelastic and Dielectric Effects in Polymeric Solids*, John Wiley, New York, 1967, p. 478.
- 8 L. HÖRHAMMER AND H. WAGNER, *Pharm. Ztg.*, **104** (1959) 783.
- 9 J. R. RITTENHOUSE, W. LOBUNEZ, D. SWERN AND J. G. MILLER, *J. Am. Chem. Soc.*, **80** (1958) 4850.
- 10 D. BELITSKUS AND G. A. JEFFREY, *Acta Cryst.*, **18** (1965) 3.