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THE DETERMINATION OF ORGANIC PEROXY COMPOUNDS

V. THE MECHANISM OF THE CHROMATOGRAPHIC SEPARATION OF SYMMETRICALLY SUBSTITUTED DIBENZOYL PEROXIDES ON VARIOUS SORBENTS*

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SUMMARY

The R_F values of seventeen isomeric substituted dibenzoyl peroxides on aluminium oxide, treated and untreated cellulose and silica gel are given. The best separations were obtained on silica gel and dimethylformamide impregnated paper. The so-called "ortho effect" and the effect of substituents on the migration behaviour of compounds under investigation are discussed.

INTRODUCTION

Although it has been found that *p,p'*-dichlorodibenzoyl peroxide and dibenzoyl peroxide can be separated by chromatography¹⁻⁴, no attempt has been made, to our knowledge, to investigate systematically the effect of substituents on the migration behaviour of these compounds. In this paper we present the results of the chromatographic study of seventeen symmetrically substituted dibenzoyl peroxides on various sorbents, and give some possible interpretations of the interactions between the compounds under investigation and the stationary phase, which could give rise to the observed separations.

EXPERIMENTAL

The substituted dibenzoyl peroxides were prepared from the corresponding benzoyl chlorides by known procedures⁵. The products were purified by recryst-

* Part III. R. KAVČIČ, B. PLESNIČAR AND A. PERDIH, *J. Chromatogr.*, 38 (1968) 515; Part IV: D. HADŽI, R. KAVČIČ AND B. PLESNIČAR, *Spectrochim. Acta*, 27A (1971) 179.

tallization and the purity and identity were checked by the usual physical and chemical methods. All the products were found to be more than 99% pure by iodometry.

The adsorbents used were Silica Gel G (Merck), pH 6.5, Aluminium Oxide G (Merck), pH 7.5, and Aluminium Oxide-Acid (Woelm), pH 4.3. Commercial cellulose-coated plates (CEL 300 and 300 AC-10) were obtained from Macherey-Nagel, Düren, G.F.R.

Thin-layer plates (50 × 20 and 20 × 20 cm) were prepared as follows:

(A) A slurry of 30 g of Silica Gel G in 60 ml of water was spread with the Stahl apparatus to a coating thickness of about 250 μm . After being coated, the plates were air-dried for 18 h at room temperature and a relative humidity of about 40%.

(B) 30 g of aluminium oxide were homogenized in 35 ml of water and applied as was silica gel. The plates were activated for 1 h at 120°, cooled and equilibrated with ambient air for 20 min. An activity grade of approximately II according to Brockmann and Schodder is expected under these conditions⁶.

The chromatographic paper used was Whatman No. 2. Different impregnations were performed by immersing the paper or thin layer in (a) 25% (v/v) of formamide in acetone; (b) 30% of N-methylformamide in acetone; (c) 30% of N,N-dimethylformamide in acetone; (d) 2% of paraffin oil in light petroleum (boiling range 40–70°); and (e) 2% of dinonyl phthalate in diethyl ether. The impregnated paper was dried for 15 min in a vertical position in the case of amides and for 24 h in other cases.

The compounds were applied as dilute solutions (10 mg/ml) in benzene in volumes of 0.25 μl (*o,o'*-dinitrodibenzoyl peroxide was dissolved in methylene chloride-methanol (1:1) and applied in a volume of 2.0 μl) 2.0 cm from the edge of the plate with a distance of 1.0 cm between them.

The spots were located with a 0.1% solution of diphenylamine in ethanol or a 0.1% solution of potassium iodide in 96% acetic acid. The latter detecting agent was the only one that was suitable for the detection of *o,o'*-dinitrodibenzoyl peroxide and for the detection of all dibenzoyl peroxides on aluminium oxide in the absence of formic acid.

Freshly opened commercial solvents *pro analysi* were used without further purification.

The plates and paper were developed in well sealed glass tanks saturated with the solvent vapour of the mobile phase used. The length of each run was 10 cm. The temperature around the chromatographic systems was $19 \pm 1^\circ$ and the relative humidity was 40%.

RESULTS AND DISCUSSION

The chromatographic data for dibenzoyl peroxides on various sorbents developed with the mobile phases which gave best separations are summarized in Table I. Some characteristic chromatographic patterns are given in Figs. 1–6.

Chromatography on cellulose

Direct phase systems. When untreated cellulose (thin layers as well as paper) was used as the stationary phase, it was observed that all dibenzoyl peroxides, except the *o,o'*-difluoro derivative, all dinitro and "tailing" dibenzoyl peroxides

TABLE I

 $R_F \times 100$ VALUES OF SYMMETRICALLY-SUBSTITUTED DIBENZOYL PEROXIDES ON VARIOUS SORBENTS

Substituents	Stationary ^a and mobile ^b phases														
	1,A	1,B	1,C	1,D	1,E	1,F	1,G	1,H	2,G	2,H	3,I	4,J	5,J	6,K	7,L
I	33	32	27	44	40	46	20	58	74	89	73	70	34	52	84
<i>o,o'</i> -Difluoro-	28	22	24	36	25	33	16	47	62	87	61	51	15	58	92
<i>m,m'</i> -Difluoro-	48	41	34	52	40	47	25	67	79	90	79	77	49	38	76
<i>p,p'</i> -Difluoro-	45	38	28	50	48	50	22	63	78	90	76	73	43	40	78
<i>o,o'</i> -Dichloro-	43	37	34	47	28	37	22	59	83	90	68	67	24	41	75
<i>m,m'</i> -Dichloro-	54	48	41	59	41	52	33	66	88	92	84	86	64	19	49
<i>p,p'</i> -Dichloro-	53	48	37	59	52	56	30	66	87	92	72 ^c	t	59	15	t ^d
<i>o,o'</i> -Dibromo-	41	39	35	47	28	34	20	60	85	90	63 ^t	62 ^t	25	36	68
<i>m,m'</i> -Dibromo-	51	45	41	57	41	51	27	67	88	93	78 ^t	82 ^t	62	11	t
<i>p,p'</i> -Dibromo-	54	49	37	58	47 ^t	52 ^t	21 ^t	67	80 ^t	93	65 ^t	t	53	11	t
<i>o,o'</i> -Dinitro-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>m,m'</i> -Dinitro-	13	16	8	13	0	20	0	22	7	71	0	0 ^t	4	34	0
<i>p,p'</i> -Dinitro-	18	21	9	16	0	25 ^t	0	23 ^t	9	71	0	0 ^t	3	30	0
<i>o,o'</i> -Dimethyl-	41	44	34	51	51	48	28	67	83	92	85	92	63	32	63
<i>m,m'</i> -Dimethyl-	35	38	30	45	47	49	23	62	79	92	82	91	60	30	63
<i>p,p'</i> -Dimethyl-	28	33	26	36	42 ^t	51	17	62	73 ^t	90	65 ^t	t	49	28	t
<i>o,o',p,p'</i> -Tetrachloro-	60	54	45	62	55	54	36	66	92	92	83	90	69	13	t

^a Stationary phases: 1, Silica Gel G; 2, Aluminium Oxide G; 3, Acetylated cellulose (pre-coated plates, 300 AC-10); 4, Whatman No. 2 paper, N-methylformamide; 5, Whatman No. 2 paper, dimethylformamide; 6, Whatman No. 2 paper, dinonyl phthalate; 7, Whatman No. 2 paper, paraffin oil.

^b Mobile phases: A, xylene (mixture of isomers, boiling range 137–140°); B, toluene; C, chlorobenzene; D, carbon tetrachloride–chloroform (3:1); E, light petroleum (boiling range 40–70°)–diethyl ether (4:1); F, light petroleum–acetone(4:1); G, light petroleum–formic acid (the paper in the tank was wetted with formic acid); H, carbon tetrachloride saturated with formic acid; I, *n*-hexane; J, light petroleum; K, acetone–water (3:2); L, methanol–benzene–water (8:1:2).

^c Tailing.

^d Tailing without the possibility of evaluation of the R_F values.

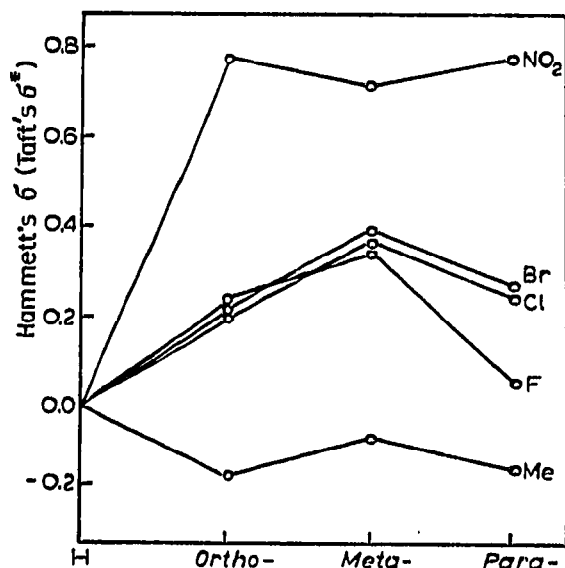


Fig. 1. Plot of Hammett's σ constants (Taft's σ^* constants for *o*-isomers) versus the position of the substituents.

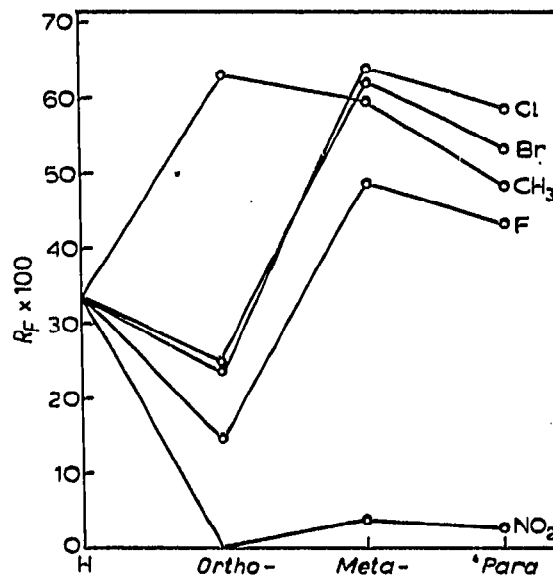


Fig. 2. Plot of R_F values of substituted dibenzoyl peroxides on paper impregnated with N,N-dimethylformamide (mobile phase, light petroleum) versus the position of the substituents.

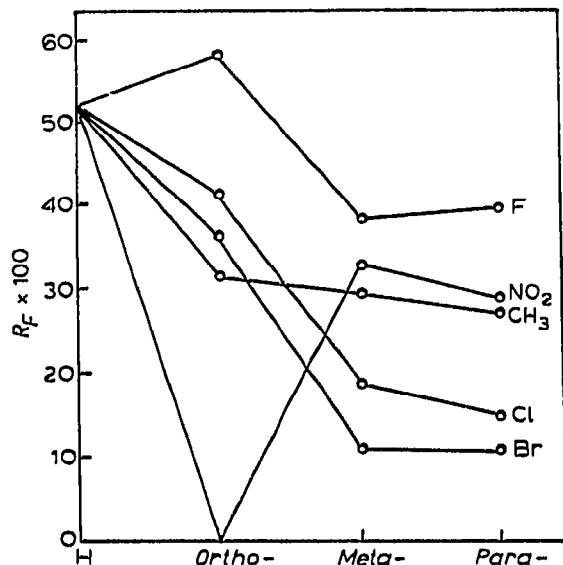


Fig. 3. Plot of R_F values of substituted dibenzoyl peroxides on paper impregnated with dinonyl phthalate (mobile phase, acetone-water (3:2)) versus the position of the substituents.

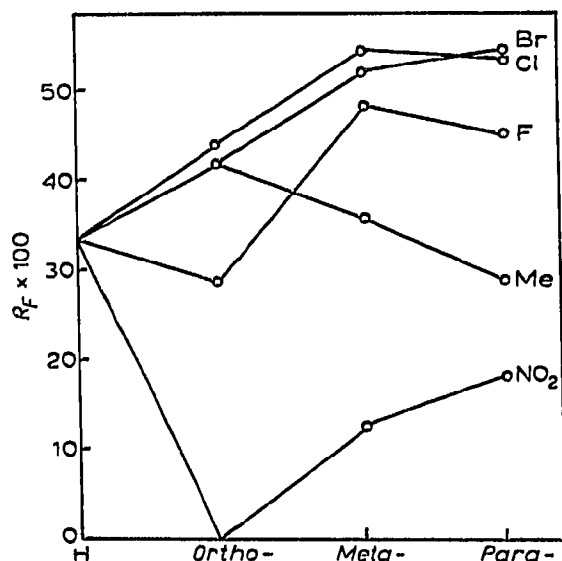


Fig. 4. Plot of R_F values of substituted dibenzoyl peroxides on Silica Gel G (mobile phase, xylene) versus the position of the substituents.

migrate with the front even when hexane is used as the mobile phase. On the other hand, separation occurred on acetylated cellulose and the pattern of separation was similar to that obtained on silica gel. Tailing was observed with bromo derivatives and also with *p,p'*-dichloro- and *p,p'*-dimethyldibenzoyl peroxides. All the spots were located in the upper part of the chromatogram.

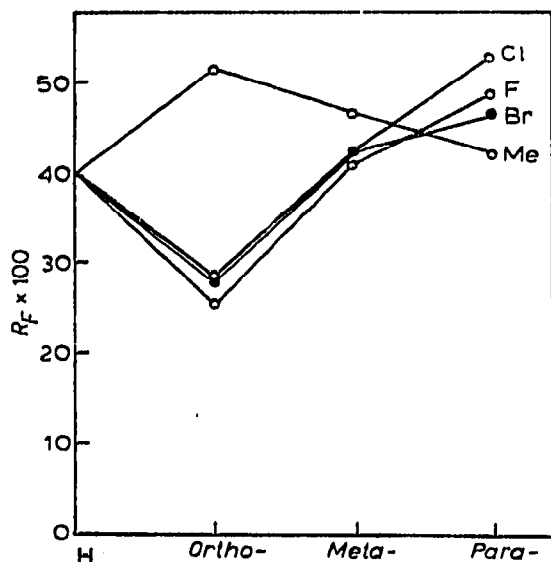


Fig. 5. Plot of R_F values of substituted dibenzoyl peroxides on Silica Gel G (mobile phase, light petroleum-diethyl ether (4:1)) versus the position of the substituents.

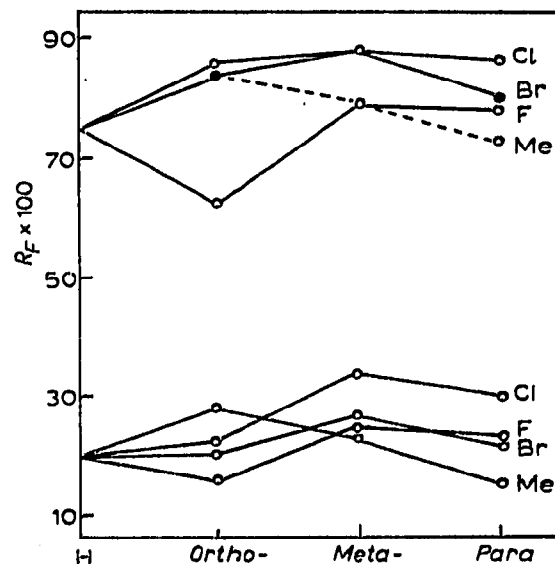


Fig. 6. Plot of R_F values of substituted dibenzoyl peroxides on (a) Silica Gel G and (b) Aluminium Oxide G (mobile phase, light petroleum-formic acid) versus the position of the substituents.

Impregnation of the paper with formamide did not give better separations in comparison with those obtained on untreated paper when the same mobile phases were used. On the other hand, fairly good separation was obtained when the paper was impregnated with N-methylformamide and excellent separation when N,N-dimethylformamide was used as the impregnating agent. Light petroleum ether (boiling range 40–70°) was found to be the best mobile phase in these cases (see Table I and Fig. 2).

Reversed phase systems. Heavy tailing was observed on acetylated cellulose thin-layer plates when water-containing mobile phases were used (e.g., methanol-water (8:2), isopropanol-water (1:1); and acetone-water (3:2)). Non-tailing compounds (all fluoro-substituted compounds, *o*-chloro-, *o*-bromo, *o*-methyl- and *m*-methyl-substituted compounds) did not separate well.

The results obtained on the paper impregnated with paraffin oil were similar to those obtained on acetylated cellulose. The composition of the mobile phase was the same although the proportions of the two components of the mobile phase were different. The addition of benzene or chloroform to the mobile phase used on acetylated cellulose caused a decrease in the tailing and separation was somewhat improved (see Table I).

By using paper impregnated with dinonyl phthalate and the mobile phases already used on acetylated cellulose, fairly good separations were observed. Tailing appeared with *p*-chloro-, *o,p*-dichloro-, *m*-bromo-, *p*-bromo- and *p*-methyl-substituted compounds. When alcohol-containing mobile phases were used, all nitro-substituted dibenzoyl peroxides remained at the start. By using acetone-water (3:2), the pattern of the chromatographic separation was similar to that obtained with alcohol-containing mobile phases, although *m*- and *p*-nitro-substituted compounds moved in this case (see Table I and Fig. 3). The tailing observed with all the above compounds is probably the result of their low solubility in the mobile phases used (hydrophobic bonds).

Thin-layer chromatography on aluminium oxide

In the thin-layer chromatography of substituted dibenzoyl peroxides on Aluminium Oxide G, tailing was observed when solvents of the eluotropic series, from light petroleum to diethyl ether, were used as mobile phases in all cases, except for nitro-substituted compounds, which remained at the start. On aluminium oxide-acid, similar results were obtained although *o,o'*-dimethyldibenzoyl peroxide moved without tailing and only slight tailing was observed with *m,m'*-dimethyldibenzoyl peroxide. The height of the run of the corresponding tails increased with the polarity of the mobile phases used, and in all cases the major part of the applied samples remained at the start. With diethyl ether as the mobile phase, dibenzoyl peroxides moved with the front, except for *o,o',p,p'*-tetrachlorodibenzoyl peroxide, the spot of which was spread all over the chromatogram.

Electrostatic interactions between strong positive electrostatic fields directed outward from the surface of the aluminium oxide and dibenzoyl peroxide probably cause the irreversible (or slowly soluble) formation of complexes on this sorbent⁶. Dibenzoyl peroxides can be freed only by using more basic mobile phases that are capable of preventing interactions with aluminium oxide, thus causing the migration of dibenzoyl peroxides with the front.

By developing with light petroleum or carbon tetrachloride in an atmosphere saturated with formic acid, fair separations were obtained that were very similar to those obtained on silica gel under the same conditions (see Table I and Fig. 4). In this case, the formation of the above complexes is prevented by the layer of formic acid adsorbed on the surface of the aluminium oxide.

Thin-layer chromatography on Silica Gel G

Contrary to the results with aluminium oxide and cellulose, well shaped spots located in the middle part of the chromatogram were obtained on Silica Gel G when solvents of the eluotropic series, from carbon tetrachloride to benzene, and also mixtures of more and less polar solvents, were used (Figs. 4-6).

The results seem to indicate that the prevailing factor that governs the mechanism of chromatographic separation of dibenzoyl peroxides on this sorbent is the formation of intermolecular hydrogen bonds. Acceptors for hydrogen bonds are carbonyl oxygen atoms and to a lesser extent the oxygen atoms of the peroxide bond. The substituents which increase the electronic density of the carbonyl oxygen or of the peroxide bond cause an increase in the strength of the hydrogen bond, and *vice versa*. The relatively weak intermolecular hydrogen bonds could also be formed between the hydrogen-donating stationary phase and the halogens or the aromatic system, and relatively strong hydrogen bonds between the hydrogen-donating stationary phase and the nitro group. In all these cases the possibility of the occurrence of simultaneous hydrogen bonds is plausible.

For the purpose of discussion, the compounds under investigation can be divided into three groups, the *ortho*-effect being dealt with separately.

Ortho-effect

Comparing the chromatographic behaviour of phenols^{7,8}, one of the most thoroughly studied aromatic systems, the positive *ortho*-effect was observed in direct phase systems only with dibenzoyl peroxides that have a methyl substituent. Chloro- and bromo-substituted compounds do not show such an effect, or it is negative. A negative *ortho*-effect was observed with fluoro-substituted dibenzoyl peroxides. These results strongly suggest that the *ortho*-effect is due to the sum of at least three components: steric hindrance, Hammett's sigma influence and simultaneous hydrogen bondings.

The methyl group and halogens appear to cause lesser shielding of the carboxyl group than the smaller phenol group.

Phenols show an increase in the steric effect and Hammett's sigma influence in the sequence of R_F values: $H < Me < F < Cl < Br$ ^{7,8}. On the other hand, the sequence of R_F values with dibenzoyl peroxides is $F < H < Br \approx Cl < Me$. The only exception was observed by using basic oxygen-containing mobile phases where the following order occurred: $F \leq Cl \approx Br < H < Me$. It can be concluded that the size of the halogen atom is not the deciding factor, most probably as a result of the rotation of the carboxyl group out of the plane of the aromatic ring. The fluorine atom is small in comparison with the sizes of the other substituents, and it can therefore produce a smaller declination of the carboxyl group, so it should remain almost coplanar with the aromatic ring.

If the Hammett sigma constants determined the mobility of dibenzoyl per-

oxides, one could expect the sequence of R_F values $\text{Me} < \text{H} < \text{Cl} < \text{Br} < \text{F}$. On the other hand, the predominating influence of simultaneous hydrogen bonds would favour the following order of R_F values: $\text{F} > \text{Cl} \geq \text{Br} > \text{H} > \text{Me}$.

There was good qualitative agreement between the sum of these effects and the actual migration when aromatic mobile phases and phases with chlorinated aliphatics were used. When mobile phases with basic oxygen were used on Silica Gel G, and systems with amide-impregnated paper, such an agreement was not observed. A logical explanation for this discrepancy could be the preferential adsorption of diethyl ether (and even more so of acetone) on the stationary phase, which causes a shortage of it in the mobile phase (light petroleum). In this way, most of the active hydrogen atoms on silica gel are blocked. Thus the dipole-dipole interaction between the stationary phase, which contains mainly molecules with basic oxygen with almost no active hydrogen atoms available, and dibenzoyl peroxide is relatively increased. As a result, *o*-halogenated dibenzoyl peroxides should migrate more slowly than *o,o'*-dimethyldibenzoyl peroxide. That this is not the only possible explanation is demonstrated by the migration of *o,o',p,p'*-tetrachlorodibenzoyl peroxide which shows lower R_F values than *p,p'*-dichlorodibenzoyl peroxide only when the mobile phase light petroleum-acetone was used, but not with the mobile phase light petroleum-diethyl ether or in the light petroleum-N,N-dimethylformamide system.

Contrary to the results with phenols^{7,8}, a positive *ortho*-effect was observed with *o,o'*-difluorodibenzoyl peroxide in reversed phase systems. The difference in the sequence of R_F values ($\text{H} > \text{F} > \text{Me} \geq \text{Cl} > \text{Br}$ for phenols and $\text{F} > \text{H} > \text{Cl} > \text{Br} > \text{Me}$ for dibenzoyl peroxides) can also be seen.

From the pattern in Fig. 3, it could be concluded that simultaneous hydrogen bonding plays a role in the chromatographic separation. This effect decreases in the previously mentioned sequence and it seems that simultaneous hydrogen bonding is more important than the size of the substituents.

Methyl-substituted dibenzoyl peroxides

The *m*- and *p*-substituted dibenzoyl peroxides show, in direct phase systems, R_F values that qualitatively correlate well with those expected on the basis of Hammett's sigma constants. The only exception was observed when the mobile phase light petroleum-acetone was used. In this case, the R_F values of *p,p'*-dimethyldibenzoyl peroxide were greater than those of the *m*-isomer. This supports our view that the high basicity of the oxygen atom in acetone causes the masking effect of *m*- and *p*-substituents as well as the dipole-dipole and pi-dipole interactions between dibenzoyl peroxide and the stationary phase.

The heavy tailing of the *p*-isomer prevents any conclusion for reversed phase systems.

Nitro-substituted dibenzoyl peroxides

The R_F values increase in the order $o < m < p$ for all the mobile phases used. *o,o'*-Dinitrodibenzoyl peroxide is only sparingly soluble in the mobile phases used, so no migration was observed. The Hammett sigma constants decrease in the order $p > o > m$. The pattern of the R_F values for *m*- and *p*-isomers is in accordance with the sigma values. The basicity of the carbonyl oxygen is lower for compounds with the nitro group in the *p*-position than for *m*-substituents, which means that *p,p'*-

dinitrodibenzoyl peroxide forms intermolecular hydrogen bonds having lower strengths with the more polar phases compared with *m,m'*-dinitrodibenzoyl peroxide. As a result, *p,p'*-dinitrodibenzoyl peroxide migrates faster in direct phase systems and more slowly in reversed phase systems.

In general, the R_F values of nitro-substituted dibenzoyl peroxides are lower in comparison with those of other dibenzoyl peroxides examined, except with acetone-water as the mobile phase on dinonyl phthalate-impregnated paper.

Halogen-substituted dibenzoyl peroxides

With respect to the Hammett sigma constants, it would be expected that the R_F values of *m*-isomers would be highest in direct phase systems. It can be seen from Table I that the pattern of the behaviour of these compounds is not that which would be expected on the basis of the above assumption. The lowering of the R_F values with fluoro-isomers in comparison with those of chloro- and bromo-isomers could probably be explained by following the order of the strength of the hydrogen bonds between the stationary phase and fluorine on the one hand and chlorine and bromine on the other ($F > Cl \geq Br$). By using mobile phases containing a basic oxygen atom on Silica Gel G, these differences diminish, but not in light petroleum-amide systems. In general, the sequence of R_F values is $F < Br \approx Cl$. With fluorine-substituted dibenzoyl peroxides, the trend is $o < p < m$. By using trichloroethylene and mobile phases having a basic oxygen atom, this trend was reversed on Silica Gel G, probably because of the dipole-dipole interactions between the mobile and the stationary phases.

With bromine as a substituent, the sequence $o < m < p$ was observed in aromatic mobile phases and $o < p < m$ in mobile phases containing haloalkanes on Silica Gel G and in the light petroleum-N,N-dimethylformamide system.

The behaviour of chloro-substituted dibenzoyl peroxides is in between those of bromo and fluoro derivatives. By using aromatic mobile phases, *m,m'*-dichloro- and *p,p'*-dichlorodibenzoyl peroxides are not resolved as well as the corresponding bromo and fluoro derivatives.

From these facts, it can be concluded that the chromatographic behaviour of halogenated dibenzoyl peroxides is subject to the influence of a number of interactions of similar strengths, but no precise evaluation of their relative contributions can be given.

In reversed phase systems, there are no significant differences between the R_F values of *m*- and *p*-isomers. The same was also observed in the case of phenols.

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