

THE PAPER CHROMATOGRAPHY OF SOME ISOMERIC MONOSUBSTITUTED PHENOLS. I.

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(Received September 28th, 1964)

INTRODUCTION

In recent years the paper chromatography of phenols has been given extensive study. Many techniques have been developed for mobile solvent systems on plain or buffered papers and for methods of detecting the spots^{1, 2}.

The mobile solvents most frequently used consist of two or more solvents often containing an aqueous phase. EVANS *et al.*³ used butanol-pyridine-water saturated with NaCl (1:1:2) as the mobile solvent. BATE-SMITH⁴ used butanol-acetic acid-water (4:1:5) and *m*-cresol-acetic acid-water (50:2:48). RILEY⁵ used *n*-butanol-benzene-water (1:9:10) and *n*-butanol-benzene-water (1:19:20) as well as *n*-amyl alcohol saturated with water. SUNDT⁶ has used cyclohexane-ethyl acetate (5:1) saturated with a mixture of dimethylformamide-water (5:2). CLARK⁷ has obtained good results using cyclohexane-ethyl acetate-acetic acid (5:1:1) and butanol-water-acetic acid (6:2:1) on ion-exchange paper.

The use of mixed volatile solvents presents a disadvantage in that the solvent ratios may change on standing because of the different volatilities of the components. This problem can be minimized by mixing the solvents just before using. This procedure, however, is time consuming and requires the storage and handling of larger amounts of solvents.

There are many different techniques for locating phenols on paper chromatograms. The most frequently used methods require either specialized equipment or the use of unstable reagents. Examination of the papers under short and long wave ultraviolet light is often used either before or after chemical treatment⁸.

Chemical methods of spot location frequently involve spraying the paper with reagents such as diazotized sulfanilic acid, diazotized *p*-nitroaniline and diazotized benzidine^{3, 8}. Spraying the paper with silver nitrate followed by heating has been used⁴. Other reagents used as sprays for locating phenols are 2 % phosphomolybdic acid⁵ and equal volumes of 1 % ferric chloride and 1 % potassium ferricyanide⁹.

The work described in this paper was carried out to develop some simple solvent systems for the separation of isomeric monosubstituted phenols, to investigate the effect of the orientation of substituent groups on the R_F' value using the new solvent systems and to develop the use of ceric ammonium nitrate as a stable spot-locating agent for a number of phenols. Data are presented for some common phenols and for a number of phenols for which no references have been found in the paper chromatographic literature.

EXPERIMENTAL

Reagents

Toluene saturated by shaking with distilled water. Mineral spirits (Kauri-Butanol value 37-38, aniline point 56-59°) saturated with distilled water. Ceric ammonium nitrate reagent prepared by dissolving 200 g of ceric ammonium nitrate in 500 ml of 2 N nitric acid with heating. The solution was allowed to stand overnight and the clear supernatant liquid was decanted.

Procedure

The toluene and mineral spirits were kept saturated with water by the presence of a lower water layer in the tanks. The tanks were lined with paper sheets extending into the water layer. All chromatograms were run at 25° with Whatman No. 1 paper, using the ascending development method. The papers were allowed to equilibrate 20 min in the tank before immersion. The solvents were allowed to travel 10 cm requiring 30-45 min.

The paper sheets were sprayed with the reagent as soon as they were removed from the tanks and then washed thoroughly with water to remove the excess reagent. The papers were air dried. The R_F' values reported are the distance traveled by the spot front divided by the distance traveled by the solvent front³.

RESULTS AND DISCUSSION

Water and toluene saturated with water have been found to be satisfactory mobile solvents for use in the paper chromatography of phenols. Mineral spirits saturated with water also has been found to be quite satisfactory. Mineral spirits is a mixture of aliphatic and aromatic hydrocarbons, but because of its low volatility (usual b.p. range 150-200°) no difficulty has been observed due to evaporation. All three solvent systems have been used for 30 days with no variation in the R_F' values observed.

A solution of ceric ammonium nitrate in 2 N nitric acid as a spot-locating reagent for phenylphenols has been reported¹⁰. This reagent has been found to be a good spot-locating material for most of the phenols tested. The reagent solution is quite stable and has been stored under ordinary laboratory shelf conditions for a year or more without loss of effectiveness. The reagent also gives easily discernible colored spots with most phenols. These spots are permanent and will not wash out with water so that the paper chromatogram may be retained as a permanent record.

A comparison of the effect of the three solvent systems on isomeric groups of phenols shows that in general the highest R_F' values are obtained with toluene. The exceptions are the hydroxyphenols, the aminophenols and the hydroxybenzoic acids. These phenols have the greatest R_F' values with water.

Phenols having substituent groups that are not readily ionized or associated have greater R_F' values with mineral spirits than with water. These include the phenylphenols, the iodophenols, the *tert.*-butylphenols and the naphthols. Groups such as the cresols, hydroxyphenols, hydroxybenzoic acids, aminophenols and diphenols have higher R_F' values with water than with mineral spirits.

The "ortho effect" is quite obvious among isomeric groups where any significant

difference is found in the R_F' values of the three isomers. The *ortho* isomer has the largest R_F' value and is frequently separate from the *meta* and *para* isomers. The R_F' values of the *meta* and *para* isomers are usually close together, with the *meta* isomer having a slightly greater R_F' value than the *para* isomer. The only exceptions found are resorcinol, which has a greater R_F' value in toluene than either catechol or hydroquinone, and *m*- and *p*-aminophenol, which have slightly greater R_F' values in water than the *ortho* isomer. Table I shows the R_F' values found in the three solvents and the spot colors observed.

The differences in R_F' values of various isomeric groups in a single solvent show much variation depending on the nature of the substituent group and the solvent. For example, in both water and toluene the halophenols, the nitrophenols and the cresols show only a small spread in R_F' values between the three isomers. However, in mineral spirits a large difference is apparent. With the diphenols, large R_F' differences between the isomers are present with all three solvents. The phenylphenols show little difference in R_F' values in toluene but large differences in water and mineral spirits. The aminophenols show the greatest differences in toluene.

Fig. 1 shows the effect of the orientation of substituent groups on the R_F' value of phenols with water as the mobile solvent. The use of water as the mobile solvent points to adsorption as the mechanism involved. When toluene and mineral spirits are used as the mobile solvents, water is the more polar stationary phase indicating that the mechanism is partition.

Observation of the data in Fig. 1 shows that for *meta* and *para* substituted phenols the substituents are very nearly in the same order as the R_F' values decrease. For *ortho* substituted phenols the order is altered mainly by the effect of the carboxyl, chloro and hydroxyphenyl groups.

Figs. 2 and 3 show the effect of the orientation of the substituent groups on the R_F' values of phenols with both mineral spirits and toluene as the mobile phases.

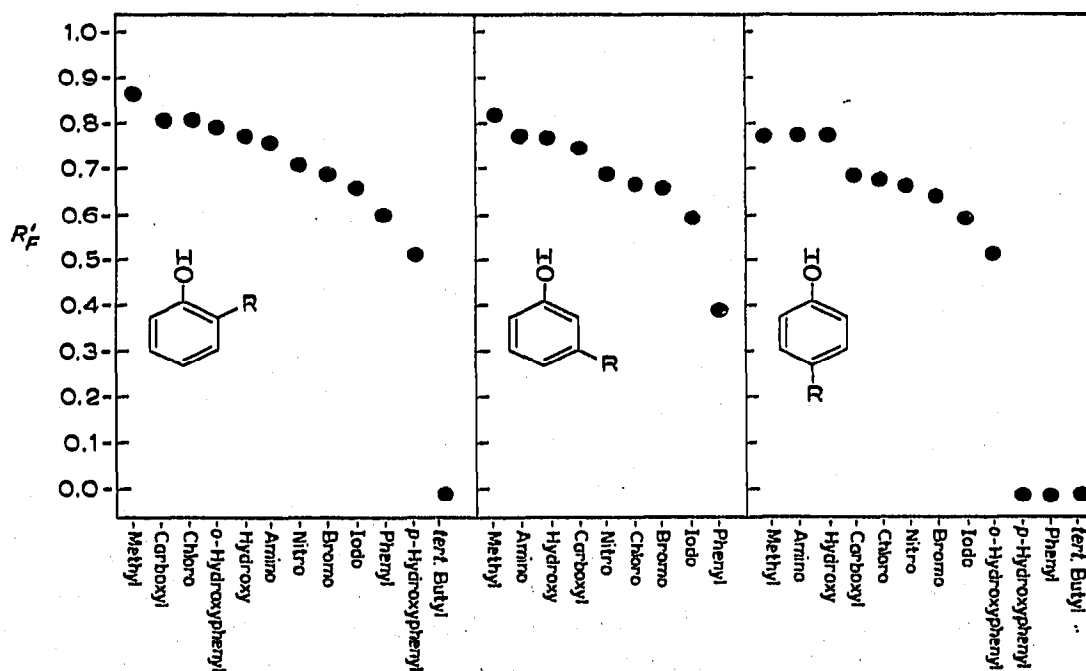


Fig. 1. Effect of the orientation of substituent groups on the R_F' values of phenols with water.

Within each table with *meta* and *para* substituted phenols, the substituents are in nearly the same order as the R_F' values decrease. The exceptions are phenyl substitution in Fig. 2 and iodo substitution in Fig. 3.

The data from both Figs. 2 and 3 show that in *ortho* substituted phenols the chloro, nitro and carboxyl groups have all advanced their relative positions on the list.

TABLE I

 R_F' VALUES OF ISOMERIC MONOSUBSTITUTED PHENOLS

Phenol	R_F' value, 25°			Color of spot
	Water	Mineral spirits	Toluene	
Phenol	0.81	0.36	0.88	Brown
1-Naphthol	0.51 ^a	0.71	0.99	Light purple
2-Naphthol	0.43 ^a	0.53	0.97	Light yellow
<i>o</i> - <i>tert.</i> -Butylphenol	0.00	0.97	1.00	Light brown
<i>p</i> - <i>tert.</i> -Butylphenol	0.00	0.89	1.00	Light tan
<i>o,o'</i> -Diphenol	0.80	0.37	0.83	Brown
<i>o,p'</i> -Diphenol	0.52	0.08	0.53	Light tan
<i>p,p'</i> -Diphenol	0.09 ^a	0.00	0.00	Light yellow
<i>o</i> -Phenylphenol	0.61	0.96	0.99	Rust red
<i>m</i> -Phenylphenol	0.40	0.77	0.97	Light tan
<i>p</i> -Phenylphenol	0.00	0.54 ^a	0.95	Light gray-green
<i>o</i> -Cresol	0.87	0.68	0.99	Very light yellow
<i>m</i> -Cresol	0.83	0.60	0.99	Light yellow
<i>p</i> -Cresol	0.78	0.54	0.99	Very light yellow
Salicylic acid	0.82 ^b	0.23 ^a	0.76	Light tan
<i>m</i> -Hydroxybenzoic acid	0.76 ^b	0.00	0.00	Tan
<i>p</i> -Hydroxybenzoic acid	0.69 ^b	0.00	0.00	Light brown to brown
<i>o</i> -Aminophenol	0.77	0.00	0.21 ^a	Tan to brown
<i>m</i> -Aminophenol	0.78	0.00	0.12 ^a	Brown
<i>p</i> -Aminophenol	0.78 ^c	0.00	0.07	Light tan
Catechol	0.78	0.00	0.00	Black
Resorcinol	0.78	0.00	0.11 ^a	Brown
Hydroquinone	0.78 ^d	0.00	0.00	Purple; fades rapidly
<i>o</i> -Bromophenol	0.70 ^e	0.94	0.98	Light yellow
<i>m</i> -Bromophenol	0.67	0.62	0.97	Light tan
<i>p</i> -Bromophenol	0.65	0.60	0.97	Light brown
<i>o</i> -Iodophenol	0.67	0.86	1.00	Pink to tan
<i>m</i> -Iodophenol	0.61	0.68	1.00	Light tan
<i>p</i> -Iodophenol	0.60	0.65	0.98	Brown
<i>o</i> -Chlorophenol	0.82	0.91	1.00	Light yellow
<i>m</i> -Chlorophenol	0.68	0.58	0.98	Light tan
<i>p</i> -Chlorophenol	0.68	0.53	0.97	Gray
<i>o</i> -Nitrophenol	0.72 ^{e,f}	0.94	1.00	Light tan
<i>m</i> -Nitrophenol	0.70 ^g	0.12 ^a	0.89	Tan
<i>p</i> -Nitrophenol	0.67 ^g	0.08 ^a	0.75	Yellow
<i>p</i> -Phenolsulfonic acid	0.95	0.00	0.00	Light yellow
<i>p,p'</i> -Sulfonyldiphenol	0.84	0.17 ^a	0.07 ^a	Light tan

^a Spot streaks.

^b The hydroxybenzoic acids gave two spots in water because of ionization. A 1% acetic acid solution was used to repress ionization.

^c Tan before developing; fades to white.

^d Bleaches spot white when developer is applied.

^e Spot spreads out rapidly.

^f Yellow before developing; fades to white.

^g Yellow before developing.

Two of these three groups showing increased activity in the *ortho* position were also observed to show the same effect in the adsorption chromatograms with water. The increased activity of these phenols is thought to be caused by intramolecular hydrogen bonding in the carboxyl, nitro and hydroxyphenyl groups and by a change in polarity in the chloro group.

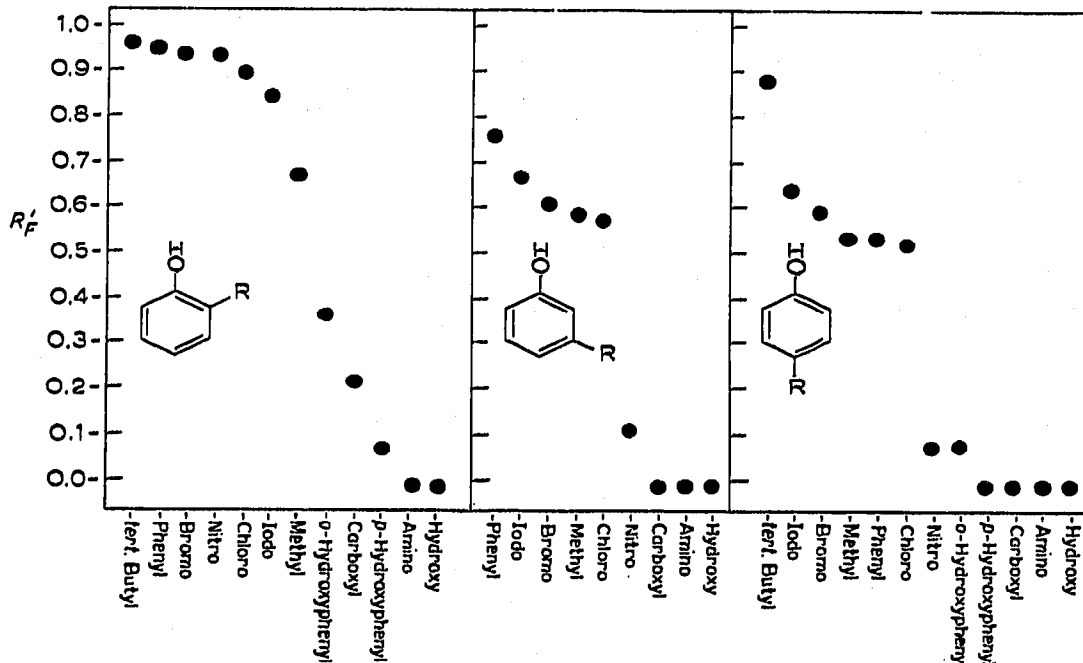


Fig. 2. Effect of the orientation of substituent groups on the $R_{F'}$ values of phenols with mineral spirits.

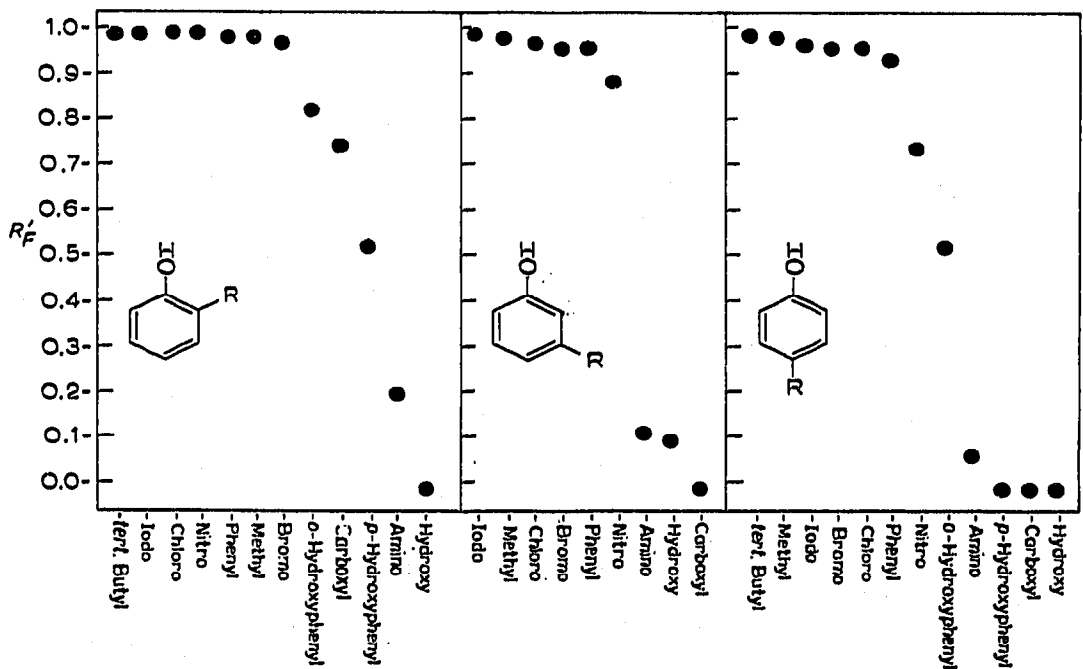


Fig. 3. Effect of the orientation of substituent groups on the $R_{F'}$ values of phenols with toluene.

The great difference between the adsorption and partition systems is pointed out by the data in Figs. 1-3. The data in the partition systems show an order of decreasing activity of the substituents that approximates the reverse of the order of decreasing activity in the adsorption system. This is more nearly true with *meta* and *para* substituted phenols since other factors may influence the *ortho* substituted phenols.

Ceric ammonium nitrate has been found to be quite useful as a spot-locating reagent for phenols. This reagent has been used in the detection of organic acids on paper chromatograms¹¹. However, no references to its use for detecting phenols have been found other than for the phenylphenols previously mentioned¹⁰. In the work reported here, this reagent has been quite useful with only a few exceptions. In using this reagent with the cresols, the spot color is very light yellow and if a small sample is used, the spot may be difficult to see because of the light color developed. Some of the phenols decolorize the reagent leaving a white spot on a yellow background before the paper is washed. This happens only when water is used as the mobile solvent. The phenols exhibiting this behavior are hydroquinone, *p*-aminophenol and *o*-nitrophenol.

From observation of the data in Table I, it is noted that only *p,p*-diphenol has no significant R_F' value in any of the three solvents. Work on this compound has shown that anhydrous methanol is a good mobile solvent giving an R_F' value of 0.85.

For purposes of comparison and information several phenols have been included in the data that are not presented as members of an isomeric series.

ACKNOWLEDGEMENTS

The author wishes to thank DONALD B. GRIFFIN, PATRICK J. RYAN and FRANK A. UNIETIS, all of Reichhold Chemicals, Inc., for their interest, support and helpful comments, and DOROTHY M. GUMPRECHT for invaluable help with the manuscript.

SUMMARY

The R_F' values of a number of isomeric monosubstituted phenols have been determined for three solvent systems. The solvent systems are water, toluene saturated with water and mineral spirits saturated with water. Most of the phenols show a significant variation in R_F' values between the three solvent systems. The R_F' values of isomeric phenols vary considerably in individual solvents depending on the substituent group and its orientation. The use of ceric ammonium nitrate as a spot-locating reagent is described. This reagent has been found to be more convenient to use and store than many other spot-locating agents commonly used for phenols.

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