THE PAPER CHROMATOGRAPHY OF SOME ISOMERIC MONOSUBSTITUTED PHENOLS. III.

DONALD L. GUMPRECHT

Technical Service Department, Reichhold Chemicals, Inc., Tuscaloosa, Ala. 35401 (U.S.A.)

(Received June 21st, 1968)

SUMMARY

In a continuation of previous work, the R_F' values have been determined for a number of isomeric phenols in three polar and three non-polar solvent systems. The use of ceric ammonium nitrate as a spot-locating agent for phenols has been continued and found to be of much value. Some correlation has been found between the R_F' value of some alkoxyphenols and the molecular weight of the substituent, especially with the *meta* and *para* isomers. With the *ortho* isomers internal bonding effects upset this relationship. Mineral spirits has been found to be a good indicator of the degree of polarity of a molecule.

INTRODUCTION

The studies in the first two papers in this series were carried out on isomeric monosubstituted phenols.[1,2] The solvent systems used were water, toluene saturated with water and mineral spirits saturated with water. A solution of ceric ammonium nitrate was found to be a satisfactory spot-locating agent in most cases. In some cases, a correlation was found between R_F' values and the molecular weight of the substituent groups.

The effects of the orientation of substituents in the various solvents were studied. The findings confirm the work of many others in showing an *"ortho* effect" in which the *ortho* isomer has the highest R_F value in an isomeric series, followed by the *meta* and finally the *para* isomer. This *"ortho* effect" has also been shown with disubstituted phenols in which the 2,6-isomer has the highest R_F value in an isomeric series.[3-5]

The work presented in this paper is an extension of the previous data. Three additional solvent systems have been developed in order to obtain additional information. Isomeric monosubstituted phenols are still the main groups studied but some additional groups or single phenols have been included to provide additional information.

EXPERIMENTAL

Reagents

The mobile solvents used were divided into polar and non-polar solvents. The polar solvents were water, water–dioxane–acetic acid (75:24:1, v/v/v), and water–methanol–acetic acid (75:24:1, v/v/v). The non-polar solvents were benzene saturated with water, toluene saturated with water and mineral spirits saturated with water. The mineral spirits had a Kauri-butanol value of 37–38 and an aniline point of 56–59°. The spot-locating agent was a ceric ammonium nitrate solution which has been described earlier.[1]

Procedure

The non-polar solvents were kept saturated with water by the presence of a lower water phase in the chromatographic chamber. The chambers were all lined with paper extending into the liquid to maintain a saturated atmosphere at 25°. The ascending method of development was used with Whatman No. 1 paper. The papers were allowed to equilibrate in the chambers for 20 min before being immersed in the mobile solvents. This was omitted, however, when examining volatile materials such as the isopropylphenols. Solvent travel was 10 cm, requiring 30–60 min depending on the solvent used.

The paper sheets were removed from the chamber and sprayed immediately with the ceric ammonium nitrate reagent. When methanol was present in the solvent, the papers were dried before spraying. After spraying, any visible spots were marked, including a few in which the material bleached the reagent white. The papers were washed with warm tap water to remove excess reagent and then air-dried and the remaining spots marked. Nearly all the spots fade slightly on aging but are permanent enough to provide a good visual record. The R_F' values reported are the ratio of the spot front to the solvent front.[6]

RESULTS AND DISCUSSION

Previous work in this series has shown that water, toluene saturated with water and mineral spirits saturated with water are useful mobile solvents for substituted phenols. This has been found to be true in the present data. The three additional solvent systems examined have also been found to be of value. Care must be used with the two solvent systems B and C (Table I) since on prolonged usage the composition changes due to loss of volatile components. These solutions are renewed once a week or oftener if used frequently. Changing composition is not a problem with the other four solvents since three of them are pure liquids and the fourth is quite non-volatile. These solutions have been used over several months duration with no change observed in R_F' values obtained.

The data obtained for a number of phenols in the six solvent systems are shown in Table I. As in the previous work, a ceric ammonium nitrate solution was found to be a useful spot-locating agent. In a few cases, the spots were light in color and required larger samples than normally used but the majority of phenols gave easily discernable spots. In one case (*m*-hydroxybenzophenone) we were unable to locate any spot when solvent system B was used. Both *p*-nitrobenzenediazonium fluoborate

TABLE I

R_F' VALUES OF SOME ISOMERIC MONOSUBSTITUTED PHENOLS

Solvent systems: (A) water; (B) water–dioxane–acetic acid (75:24:1, v/v/v); (C) water–methanol–acetic acid (75:24:1, v/v/v); (D) benzene saturated with water; (E) toluene saturated with water; (F) mineral spirits saturated with water.

Phenol	R_F' Value at 25°					
	A	B	C	D	E	F
o-Cyanophenol	0.75	0.92	0.83	0.72	0.64	0.07[a]
m-Cyanophenol	0.73	0.90	0.81	0.88	0.79	0.10
p-Cyanophenol	0.71	0.88	0.79	0.73	0.61	0.00
o-Hydroxycinnamic acid	0.53	0.85	0.68	0.06	0.06	0.00
m-Hydroxycinnamic acid	0.50	0.84	0.65	0.05	0.04	0.00
p-Hydroxycinnamic acid	0.46	0.80	0.57	0.04	0.00	0.00
o-Hydroxyphenylacetic acid	0.84	0.91	0.86	1.00	1.00	0.58[a]
m-Hydroxyphenylacetic acid	0.86	0.93	0.86	1.00	0.97	0.15[a]
p-Hydroxyphenylacetic acid	0.80	0.89	0.84	1.00	0.96	0.11[a]
o-Isopropylphenol	0.88[b]	0.97[b]	0.85[b]	1.00	1.00	0.98
m-Isopropylphenol	0.75	0.95	0.80	1.00	1.00	0.94
p-Isopropylphenol	0.68	0.90	0.76	1.00	1.00	0.92
o-Hydroxybenzophenone	0.00	0.51[a]	0.62	1.00	1.00	1.00
m-Hydroxybenzophenone	0.00	—	0.63	1.00	1.00	0.20
p-Hydroxybenzophenone	0.00	0.86[a]	0.67	1.00	1.00	0.00
o,o'-Dihydroxybenzophenone	0.51[a]	0.88	0.70[a]	1.00	1.00	0.99
p,p'-Dihydroxybenzophenone	0.41[a]	0.90	0.56	0.11	0.06[a]	0.00
o-Hydroxydiphenylmethane	0.60[a]	0.83[a]	0.76[a]	1.00	1.00	0.95
p-Hydroxydiphenylmethane	0.44[a]	0.80[a]	0.66[a]	1.00	1.00	0.85
o,o'-Dihydroxydiphenylmethane	0.64[a]	0.90	0.75	1.00	0.98	0.38
p,p'-Dihydroxydiphenylmethane	0.60	0.90	0.75	0.97	0.56	0.00
o-tert.-Amylphenol	0.00	0.49[a]	0.00	1.00	1.00	1.00
p-tert.-Amylphenol	0.00	0.00	0.00	1.00	1.00	0.89
o,o'-Dihydroxyazobenzene	0.00	0.67[a]	0.00	1.00	1.00	0.99
p,p'-Dihydroxyazobenzene	0.00	0.64[a]	0.00	0.00	0.00	0.00
p,p'-Dihydroxyazoxybenzene	0.12[a]	0.65	0.20[a]	0.00	0.08[a]	0.00
p-Hydroxyazobenzene	0.24[a]	0.72[a]	0.36[a]	1.00	1.00	0.47[a]
m-Hydroxymandelic acid	0.91	0.94	0.93	0.33	0.18	0.00
p-Hydroxymandelic acid	0.90	0.92	0.90	0.27	0.15	0.00
m-Hydroxydiphenylamine	0.50[a]	0.84	0.61[a]	1.00	1.00	0.31[a]
p-Hydroxydiphenylamine	0.54[a]	0.86	0.63[a]	1.00	1.00	0.30[a]
m-Butoxyphenol	0.66[a]	0.83	0.72	1.00	1.00	0.91
p-Butoxyphenol	0.00	0.82	0.00	1.00	1.00	0.00
p-Hydroxyphenylsulfone	0.75	0.94	0.78	0.93[a]	0.54	0.00
p,p'-Dihydroxydiphenyl ether	0.00	0.82	0.67[a]	0.00	0.00	0.00

TABLE I (continued)

Phenol	R_F' Value at 25°					
	A	B	C	D	E	F
Diphenolic acid	0.70	0.91	0.83	0.08	0.12[a]	0.00
1-Hydroxyfluorene	0.19[a]	0.65	0.34[a]	1.00	1.00	0.67
2-Hydroxyfluorene	0.14[a]	0.66	0.30[a]	1.00	1.00	0.46[a]
3-Hydroxyfluorene	0.17[a]	0.73	0.34[a]	1.00	1.00	0.56
9-Hydroxyfluorene	0.22	0.77	0.45[a]	1.00	1.00	0.69
1-Hydroxyfluorenone	0.00	0.74[a]	0.28[a]	1.00	1.00	0.92
2-Hydroxyfluorenone	0.00	0.69[a]	0.27[a]	0.83	0.83	0.00
4-Hydroxyfluorenone	0.00	0.72[a]	0.27[a]	0.93	0.93	0.00
2-Hydroxypyridine	0.88	0.88	0.86	0.10[a]	0.06[a]	0.00
3-Hydroxypyridine	0.86	0.85	0.87	0.12[a]	0.08[a]	0.00
4-Hydroxypyridine	0.81	0.81	0.84	0.00	0.00	0.00
4-Hydroxyhydrindene	0.68	0.82	0.72	1.00	1.00	0.84
5-Hydroxyhydrindene	0.56	0.73	0.64	1.00	1.00	0.80
2-Hydroxyquinoline	0.52[a]	0.79	0.65	0.74	0.64	0.00
4-Hydroxyquinoline	0.71	0.83	0.78	0.00	0.00	0.00
5-Hydroxyquinoline	0.77	0.83	0.78	0.27[a]	0.20[a]	0.00
6-Hydroxyquinoline	0.77	0.80	0.74	0.00	0.00	0.00
7-Hydroxyquinoline	0.77	0.81	0.80	0.00	0.00	0.00
8-Hydroxyquinoline	0.81	0.84	0.78	1.00	1.00	0.94
2-Hydroxy-1,4-Naphthoquinone	0.00	0.83[a]	0.61[a]	0.87	0.91[a]	0.21[a]
8-Hydroxy-1,4-Naphthoquinone	0.00	0.81[a]	0.59[a]	1.00	1.00	0.93
p-Nitrosophenol	0.75	0.89	0.82	0.32[a]	0.23[a]	0.00
p-tert.-Octylphenol	0.00	0.00	0.00	1.00	1.00	0.90
p-Dodecylphenol	0.00	0.00	0.00	1.00	1.00	0.95

[a] Spot streaks.
[b] Vaporizes rapidly.

and alkaline potassium permanganate were ineffective in this case. The reasons for our failure to locate this spot in this solvent are not known at the present time.

It has been observed previously that less readily ionized phenols have greater R_F' values with mineral spirits and toluene than with water.[2] A number of additional cases have been observed in the current data including all of the isopropylphenols, both hydroxydiphenylmethanes, the tert.-amyl phenols and a number of others as shown in Table I. A number of ortho isomers are also in this group. The use of the additional solvent systems gives supporting evidence in most cases. The phenols that have higher R_F' values with mineral spirits and toluene also have higher R_F' values with benzene. Many of these values are greater than the R_F' values found not only with water but also the other two polar solvents. This is especially true with benzene and toluene since they are both extremely strong solvents with non-polar phenols. The presence of ortho substituted phenols in this group shows the reduced polarity caused by intramolecular bonding effects. The fact that the hydroxyfluorenes, hydroxy-

fluorenones and hydroxyhydrindenes are in this group shows that these compounds are not readily ionized and thus are non-polar in nature.

The use of benzene as a non-polar solvent has shown that it gives the highest R_F' values of any of this group. This result is not unexpected since benzene is slightly higher in various eluotropic series than is toluene.[7,8] In the three polar solvent systems, the solvent water–dioxane–acetic acid gives much higher R_F' values than the other two. This is somewhat unexpected since both water and methanol are high in the eluotropic series, both having greater dielectric constants than dioxane. In this case the dioxane should act as a non-polar diluent but apparently it acts as a strong organic solvent.

The solvent system water–methanol–acetic acid results in R_F' values that are higher than those obtained with water alone. Apparently the methanol (as does the dioxane) acts as an organic solvent at least partially independent of the polarity of the phenol or the dielectric constant of the solvent.

The effect of the substituent group orientation on R_F' values for four of the solvent systems is shown in Figs. 1–4. The data for the solvent systems water–methanol–acetic acid and benzene were not included since they closely resemble other solvent systems. In Figs. 1 and 2, the order of the substituents as the R_F' values decrease is quite similar in most cases. The use of dioxane–acetic acid along with water results in an overall increase in R_F' values as can be seen from comparing Fig. 1 with Fig. 2. As observed previously the mechanism involved in the case of the polar solvents is primarily adsorption.
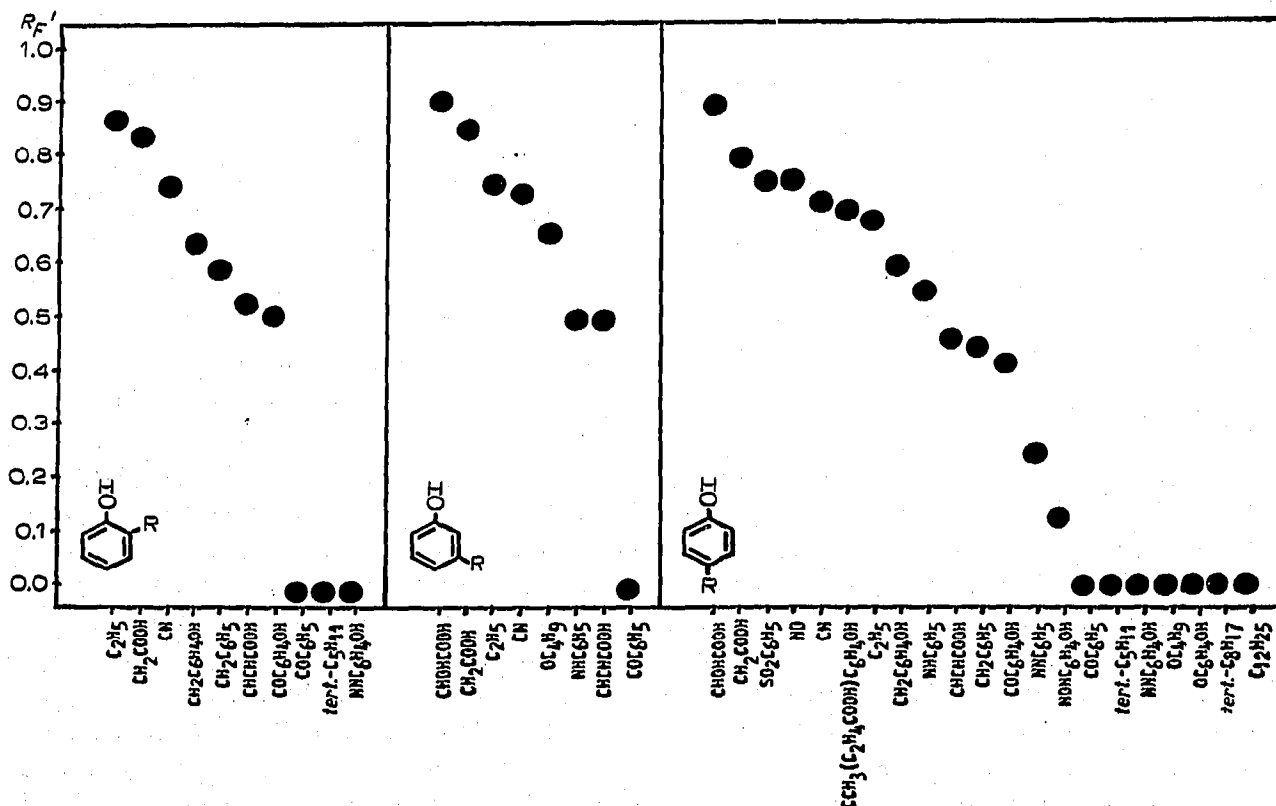

Fig. 1. Effect of orientation of substituents on the R_F' value with water as solvent.
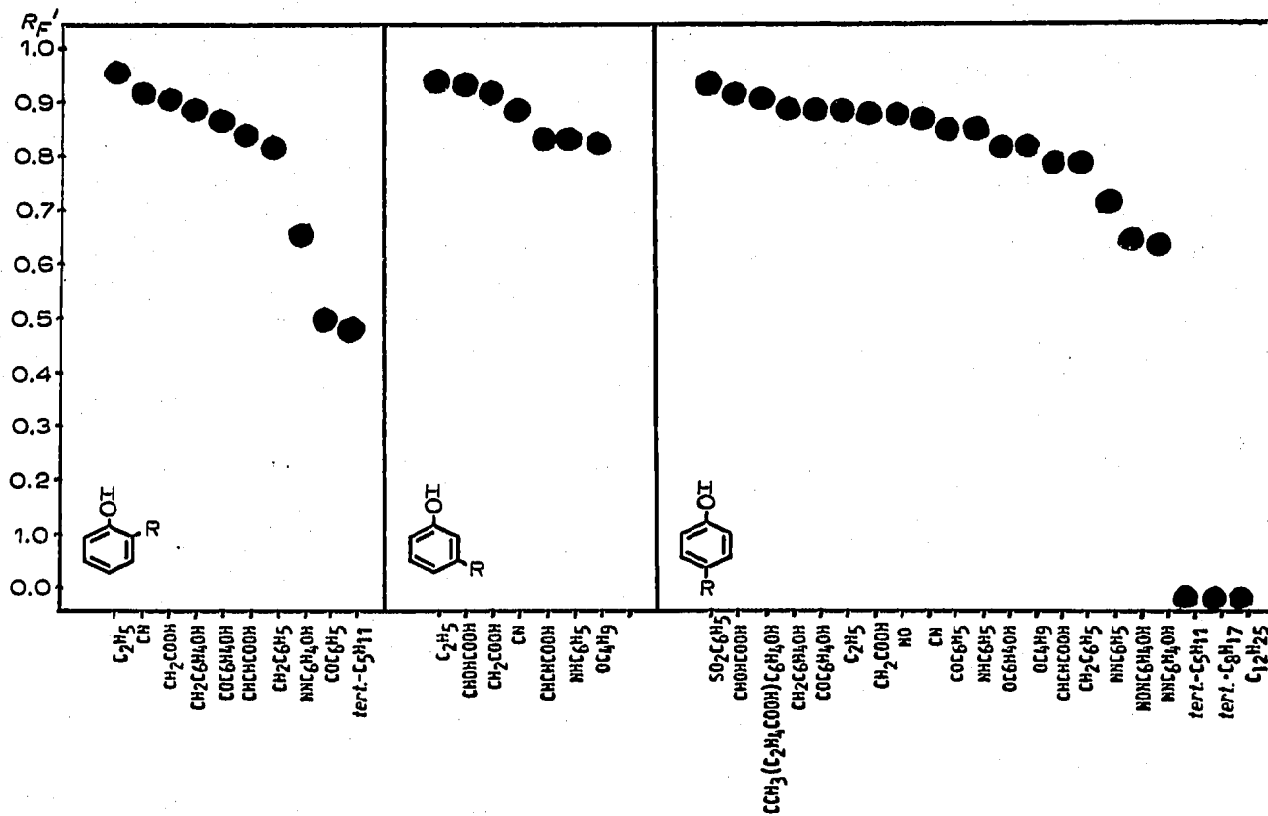
Fig. 2. Effect of orientation of substituents on the R_F' value with water–dioxane–acetic acid as solvent.
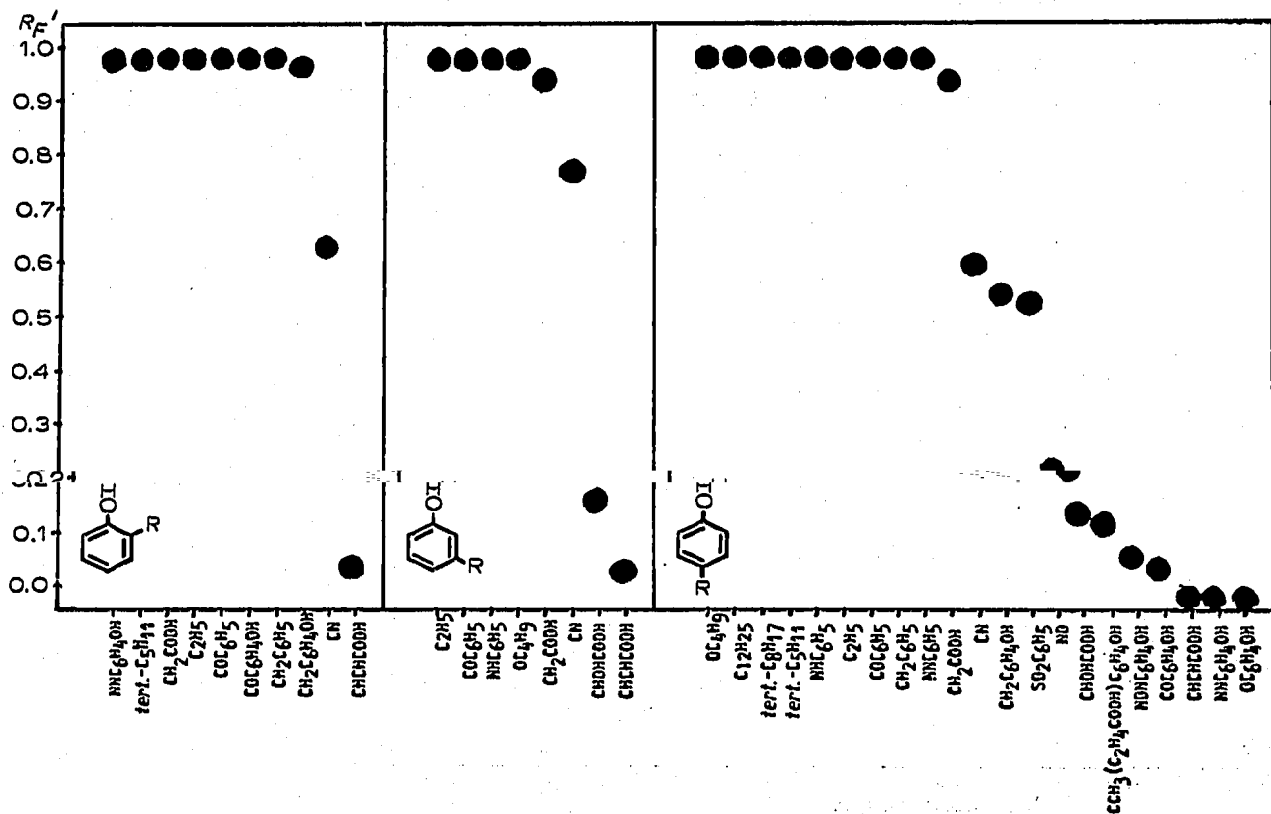

Fig. 3. Effect of orientation of substituents on the R_F' value with toluene as solvent.

Figs. 3 and 4 show the order of the substituents as the R_F' values decrease to be in nearly the same order with the non-polar solvents. In this case, the mechanism involved is partition and again the great differences between adsorption and partition can be seen by comparing the order of the substituents in the polar solvent systems with the order in the non-polar systems.

Since data are now available for the *meta* and *para* isomers of three alkoxy-phenols, a comparison was made of the R_F' values and the molecular weights of the substituents in water and mineral spirits. The data for the two lower members of the series were taken from Part II of this paper. The results of this comparison are shown in Fig. 5. Although not included in Fig. 5, the *ortho* isomers of the two lower members
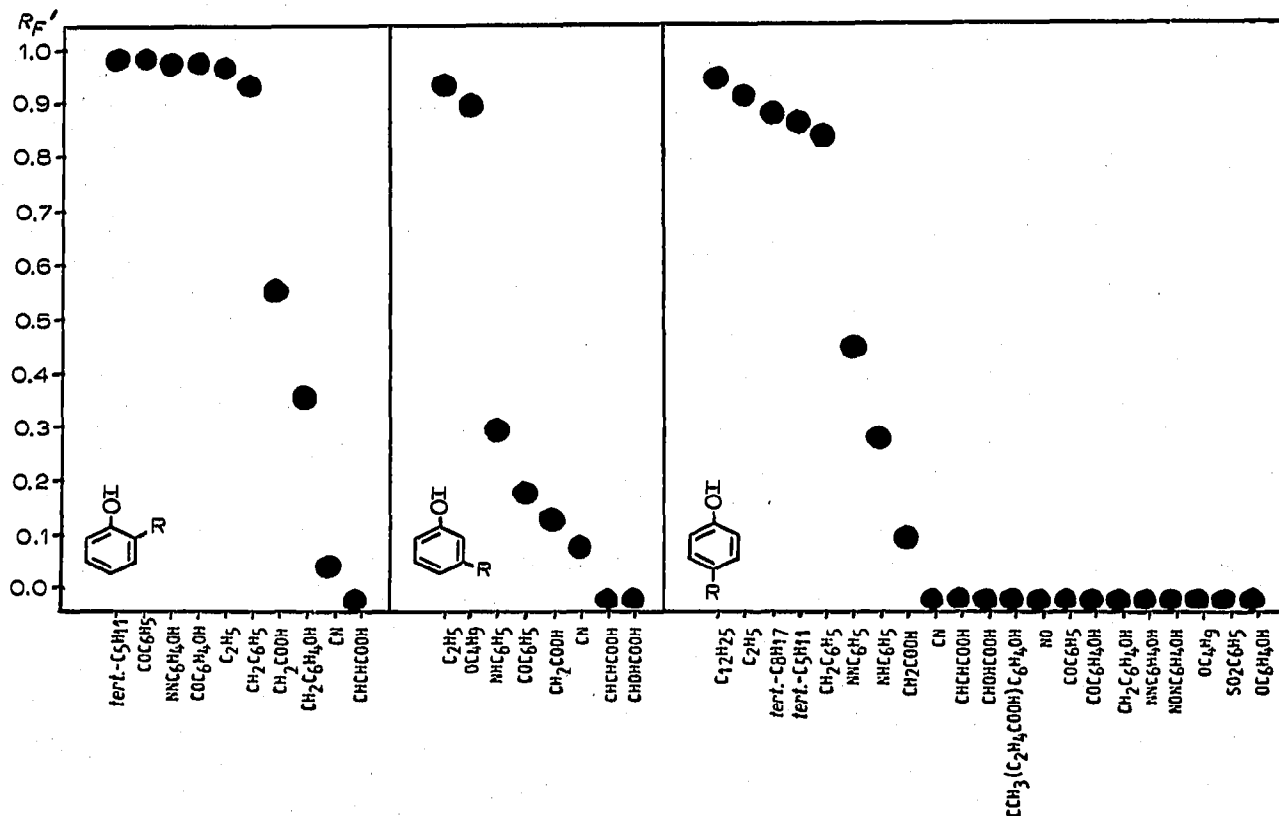
Fig. 4. Effect of orientation of substituents on the R_F' value with mineral spirits as solvent.
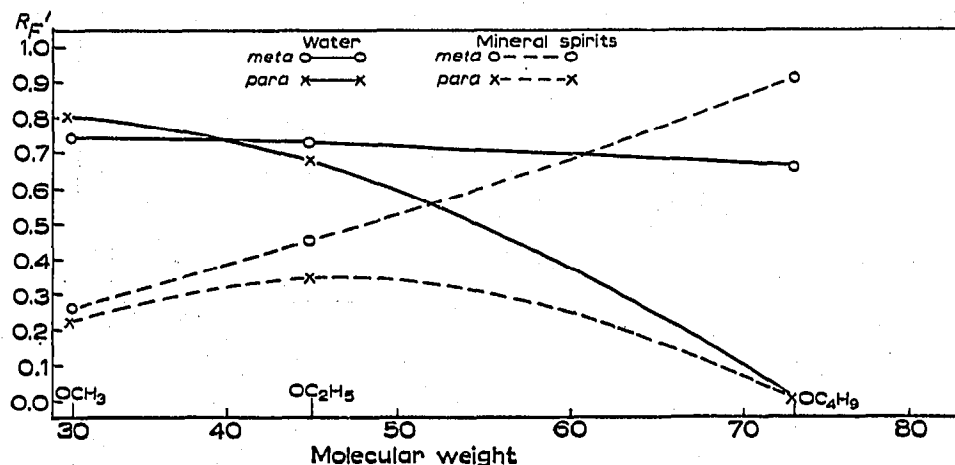

Fig. 5. R_F' values in water and mineral spirits *vs.* molecular weight of alkoxy side chain.

of the series show considerable deviation from the patterns shown by the *meta* and *para* isomers. The *ortho* isomers were not included since only two points would be available. Based on these values, it would not be possible to predict even the approximate R_F' value for *o*-butoxyphenol. Apparently the intramolecular bonding in the *ortho* isomers upsets the molecular weight–R_F' relationship.

The data for the isopropylphenols observed in the current work have been compared with those for the *n*-propylphenols in Part II. With both water and mineral spirits, the isopropylphenols had higher R_F' values than did the *n*-propylphenols. This was especially pronounced with water as the solvent.

Comparisons were made between the R_F' values for *p*-hydroxyazobenzene and *p,p'*-dihydroxyazobenzene to determine the effect of the extra hydroxyl. As might be expected, the extra hydroxyl (especially in the *para* position) lowers the R_F' values rather drastically.

A similar comparison was made between *o*- and *p*-hydroxybenzophenone and *o,o'*- and *p,p'*-dihydroxybenzophenone. In this case, the results were quite different. The extra hydroxyl group in the *ortho* position increased the R_F' values in the polar solvents but had little effect with the non-polar solvents. The extra *para* hydroxyl group increased the R_F' values in the polar solvents (except when methanol was present) but drastically reduced the R_F' values with the non-polar solvents. The *ortho* substituted benzophenones can readily form intramolecular hydrogen bonds and thus reduce the R_F' values in polar solvents. This is particularly true for the benzophenone containing only one hydroxyl group in the *ortho* position. The addition of a second *ortho* hydroxyl allows the molecule to become more polar and thus more susceptible to the effects of polar solvents.

A comparison of *o*- and *p*-hydroxydiphenylmethane with *o,o'*- and *p,p'*-dihydroxydiphenylmethane appears to give results analogous to the benzophenones. However, the variations in R_F' values are not as severe possibly because of weaker internal bonding.

Some comparisons have been made for the R_F' values of four sets of isomeric phenols containing an acidic substituent. These include the hydroxyphenylacetic acids, the hydroxybenzoic acids (data in Part I), the hydroxycinnamic acids and the hydroxymandelic acids. The results as shown in Fig. 6 indicate that the R_F' values are
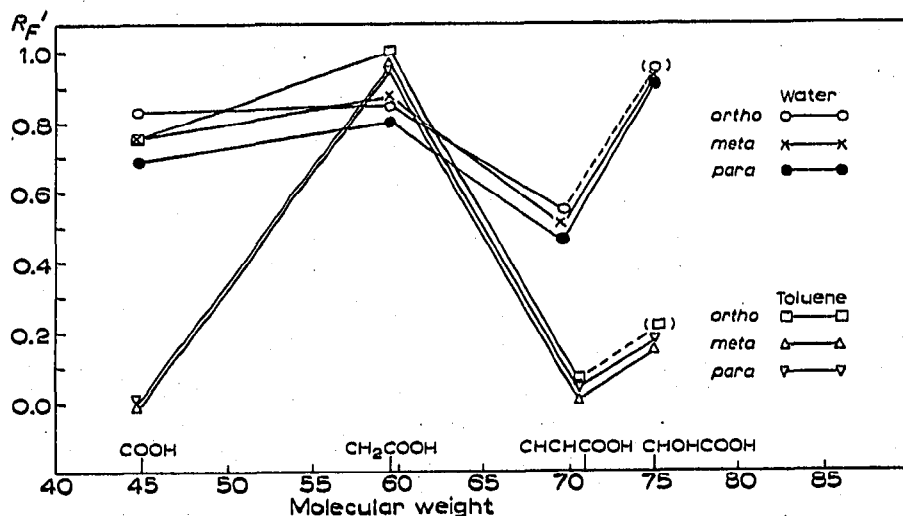

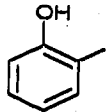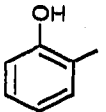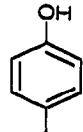Fig. 6. R_F' value of phenols *vs.* molecular weight of acidic substituent.
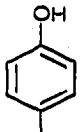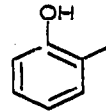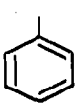
not a direct function of the molecular weights. With water as the solvent, the unsaturation present in the cinnamic acid substituent has a pronounced lowering effect on the R_F' values. The three saturated substituents hav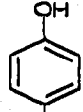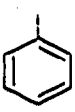e R_F' values that appear to be nearly proportional to the molecular weights. This does not hold true in toluene, however. The data do enable us to make a prediction for the R_F' values of o-hydroxymandelic acid, which is not available. Thus, based on Fig. 6, we can estimate the R_F' value with water to be between 0.91 and 0.95 and with toluene to be between 0.18 and 0.25. The estimated values are shown in parentheses in Fig. 6 and are connected with dotted lines.

The effect of inserting a methylene group between the ring and the carboxyl group can be seen by comparing the data for the hydroxybenzoic acids and the hydroxyphenylacetic acids. In the three solvents common to both sets of isomers it is seen that insertion of a methylene group between the ring and the carboxyl group causes an increase in R_F' values. This increase is quite drastic with the non-polar solvents, indicating that the polarity of the molecule has been reduced because of the longer chain length.

The data for a number of compounds containing two phenyl groups connected by some intermediate group have been examined. In a case where the two phenyl groups are identical, any variations in R_F' values must come from the connecting group. These data are shown in Table II. In the four separate groupings in this table,

TABLE II

R_F' VALUES OF SOME PHENOLS HAVING TWO PHENYL GROUPS WITH VARIABLE CONNECTING GROUPS

Phenyl	Phenyl	Connecting group	Solvent					
			A	B	C	D	E	F
(o-hydroxyphenyl)	(o-hydroxyphenyl)	$-CH_2-$	0.64[a]	0.90	0.75	1.00	0.98	0.38
		$-C(=O)-$	0.51[a]	0.88	0.70[a]	1.00	1.00	0.99
		$-N=N-$	0.00	0.67[a]	0.00	1.00	1.00	0.99
(p-hydroxyphenyl)	(p-hydroxyphenyl)	$H_3C-C-CH_2CH_2COOH$	0.70	0.91	0.83	0.08	0.12[a]	0.00
		$-CH_2-$	0.60	0.90	0.75	0.97	0.56	0.00
		$-C(=O)-$	0.41[a]	0.90	0.56	0.11	0.06[a]	0.00
		$-NON-$	0.12[a]	0.65	0.20	0.00	0.08[a]	0.00
		$-O-$	0.00	0.82	0.67[a]	0.00	0.00	0.00
		$-N=N-$	0.00	0.64[a]	0.00	0.00	0.00	0.00
(o-hydroxyphenyl)	(phenyl)	$-CH_2-$	0.60[a]	0.83[a]	0.76[a]	1.00	1.00	0.95
		$-C(=O)-$	0.00	0.51[a]	0.62	1.00	1.00	1.00
(p-hydroxyphenyl)	(phenyl)	$-SO_2-$	0.75	0.94	0.78	0.93	0.54	0.00
		$-NH-$	0.54[a]	0.86	0.63[a]	1.00	1.00	0.30[a]
		$-CH_2-$	0.44[a]	0.80[a]	0.66[a]	1.00	1.00	0.85
		$-N=N-$	0.24[a]	0.72[a]	0.36[a]	1.00	1.00	0.47[a]
		$-C(=O)-$	0.00	0.86[a]	0.67	1.00	1.00	1.00

[a] Spot streaks.

it is seen that when the connecting group is $-CH_2-$ the highest R_F' values are usually found. This appears to be independent of the fact that either one or both phenyl groups contain a hydroxyl substituent. When mineral spirits is the solvent, the $-C(O)-$ group gives higher R_F' values than the $-CH_2-$ because of reduced polarity from intramolecular bonding. The relationship of polarity to R_F' value with mineral spirits is discussed later.

In the event that both phenyl groups contain a hydroxyl substituent, the $-C(O)-$ connecting group usually has the second highest R_F' values. However, when only one phenyl group contains a hydroxyl substituent, the R_F' value for the connecting group $-C(O)-$ becomes 0.00 with water and in most other solvents is equal to or greater than the R_F' value for the $-CH_2-$ connecting group. This is because of the reduced polarity of the molecule caused by the loss of one of the hydroxyl groups.

The three non-polar solvents show generally high R_F' values independently of the connecting group except where both phenyl groups have *para* hydroxyl substituents. In this case the molecules are more polar since the opportunity for internal bonding is reduced.

When the connecting group is the azo-nitrogen configuration, some interesting comparisons can be made. With the three polar solvents, the R_F' value is higher when only one of the phenyl groups has a hydroxyl substituent. When both phenyl groups contain a hydroxyl substituent, the polarity is reduced possibly as a result of a resonance structure facilitated by the azo connecting group. This lowers the R_F' values. When only one hydroxyl is present, a resonance structure would be less likely to form resulting in higher polarity and higher R_F' values with polar solvents.

With the three non-polar solvents and an azo connecting group, when both phenyl groups have *para* hydroxyls, the R_F' values are 0.00. However, in both other cases with non-polar solvents the R_F' values are very high. Apparently the R_F' values with the non-polar solvents are not dependent on the solubility of the molecule but rather the polarity. When both phenyl groups have *para* hydroxyls, the polarity is high, resulting in low R_F' values with non-polar solvents. When both phenyl groups have *ortho* hydroxyls, internal bonding reduces the polarity, resulting in higher R_F' values with non-polar solvents.

The effect of adding oxygen to the azo connecting group can be seen by comparing the data for the azo and azoxy diphenols. There is little or no effect in the non-polar solvents but with polar solvents the R_F' values are higher. The addition of the oxygen would reduce the resonance effect and thus increase the polarity of the molecule, resulting in a higher R_F' value.

For purposes of obtaining additional information, a number of phenols have been examined that are not strictly classified as monosubstituted phenols. These include a number of isomeric groups such as hydroxynaphthoquinones, hydroxyfluorenones, hydroxyfluorenes, hydroxyhydrindenes, hydroxyquinolines and hydroxypyridines.

With only a few exceptions this group has relatively high R_F' values with the non-polar solvents, especially the highly aromatic solvents. This is because of the relatively large size of the molecules involved which tends to overshadow polar effects and thus results in compounds that are largely non-polar in composition. The major exceptions to this are the hydroxypyridines, which are relatively small in size and thus more likely to be affected by polarity. This is supported by the fact that all of the hydroxypyridines have high R_F' values with polar solvents.

Polar effects cannot be completely ignored, however, since the hydroxyquinolines show otherwise. The four isomeric hydroxyquinolines that have the least opportunity for intramolecular bonding are also seen to have low R_F' values with all of the non-polar solvents. These are the 4,5,6- and 7-hydroxyquinolines. If the 3-hydroxyquinoline were available, it too would presumably fall in this category. Conversely, the 2- and 8-hydroxyquinolines, which can easily form bonds because of their steric positions, have high R_F' values with non-polar solvents. The 8-hydroxyquinoline appears to bond more readily than the 2-isomer since its R_F' value is greater, especially with the mixed aromatic–aliphatic solvent mineral spirits.

A similar effect is seen with both the naphthoquinones and the fluorenones. In these examples the 1-hydroxyfluorenone and the 8-hydroxy-1,4-naphthoquinone are most tightly bonded internally as shown by the high R_F' value with non-polar solvents and especially with mineral spirits. With polar solvents 8-hydroxy-1,4-naphtho-quinone, as expected, has a slightly lower R_F' value than does the 2-isomer. This is not the case with 1-hydroxyfluorenone, however.

From a survey of the data in Table I, as well as that in Parts I and II of this paper, it appears that in most cases mineral spirits may be used as a good indicator of the degree of intramolecular bonding. When the bonding is strong the R_F' value with mineral spirits is high since the molecule tends to be non-polar. For this reason most *para* isomers have low R_F' values with mineral spirits except when the substituent is either a non-hydroxyl containing aromatic or a saturated aliphatic group. In these cases, the R_F' values are high because of the similarity in structure between the solvent and the substituent.

It is believed that bonding effects are weak with the hydroxyfluorenes and the hydroxyhydrindenes. Since 9-hydroxyfluorene has the highest R_F' value, it may bond with either of the aromatic rings to reduce its polarity. However, this reduction in polarity seems insufficient to cause low R_F' values with the polar solvents. This same fact appear to be true with 4-hydroxyhydrindene. With the fluorenes the compound in which the second greatest amount of bonding could occur would be that with the hydroxyl in the 1-position. In this position the hydroxyl could bond with a hydrogen from the 9-position. As before, however, this bond is so weak that the R_F' value with non-polar solvents is not affected.

ACKNOWLEDGEMENTS

REFERENCES

1 D. L. GUMPRECHT, *J. Chromatog.*, 18 (1965) 336.
2 D. L. GUMPRECHT AND F. SCHWARTZENBURG, JR., *J. Chromatog.*, 23 (1966) 134.
3 D. L. GUMPRECHT, *J. Chromatog.*, 30 (1967) 528.
4 L. S. BARK AND R. J. T. GRAHAM, *J. Chromatog.*, 23 (1966) 120.
5 L. REIO, *J. Chromatog.*, 1 (1958) 338.
6 R. A. EVANS, W. H. PARR AND W. C. EVANS, *Nature*, 164 (1949) 674.
7 I. M. HAIS AND K. MACEK, *Paper Chromatography*, 3rd Ed., Academic Press, New York, 1963.
8 E. LEDERER AND M. LEDERER, *Chromatography*, 2nd Ed., Elsevier, Amsterdam, 1957.