THE PAPER CHROMATOGRAPHY OF SOME ISOMERIC DISUBSTITUTED PHENOLS

I. PHENOL CONTAINING ONE VARIABLE AND ONE HYDROXYL SUBSTITUENT

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(First received November 28th, 1966; modified March 21st, 1967)

INTRODUCTION

A great many investigations have been carried out in recent years on the paper chromatography of phenols, and there are many references to these studies in the literature[1-6]. In our previous work on isomeric monosubstituted phenols, the use of a ceric ammonium nitrate solution as a visualization agent has been studied. The mobile solvents used were water, toluene saturated with water, and mineral spirits saturated with water[7-9]. These studies have led to the development of a practical method for the qualitative and quantitative analyses of some phenolic by-products from the alkaline fusion of benzenesulfonic acid[10]. The effects of the orientation of substituents on the R_F' values were studied for a number of isomeric monosubstituted phenols.

The information presented in this paper is an extension of the previous work to include a number of isomeric disubstituted phenols. All phenols used in this work have at least two hydroxyl substituents in addition to a third variable substituent. A few phenols are presented for information purposes that are not strictly classified as disubstituted phenols. The use of ceric ammonium nitrate as a visualization agent has been continued since it was found to be quite satisfactory for the dihydric phenols. Some additional solvent systems have been developed in order to cover a wider area of information. The solvent systems consist of three polar solvents, two non-polar aromatic solvents and one non-polar aliphatic–aromatic solvent.

EXPERIMENTAL

Reagents

Toluene and benzene saturated by shaking with distilled water. Mineral spirits (Kauri-butanol value 37–38, aniline point 56–59°) saturated by shaking with distilled water. Methanol, acetic acid, heptane and 1,4-dioxane. Ceric ammonium nitrate solution prepared by dissolving 200 g of ceric ammonium nitrate in 500 ml of $2N$ nitric acid with heating. The solution was allowed to stand overnight and the clear supernatant liquid was decanted for use.

Procedure

The benzene, toluene and mineral spirits were kept saturated with water by the presence of a lower water layer in the chromatographic chamber. The chambers were

lined with paper to maintain a saturated atmosphere and were maintained at 25°. Whatman No. 1 paper was used with the method of ascending development. The papers were allowed to equilibrate in the chambers for 20 min before being immersed in the mobile solvents. The solvent travel was 10–11 cm and required about 30–45 min, depending on the solvent.

The paper sheets were removed from the chambers, sprayed immediately and then washed thoroughly with tap water before drying. A few of the phenols bleached the spray reagent and these were marked before the washing step. Papers in which the mobile solvent contained methanol were dried before spraying since methanol reacts with the ceric ammonium nitrate reagent. The R_F' values reported are the distance traveled by the spot fronts divided by the distance traveled by the solvent fronts[11].

RESULTS AND DISCUSSION

The data obtained in the course of this work confirm our earlier findings that toluene, mineral spirits, and water are suitable mobile solvents for many substituted phenols. Of the thirty-seven phenols studied in this work only three have R_F' values of zero in all three of these solvents. These are 2,6-dihydroxyanthraquinone, 1,4-dihydroxynaphthalene and 2,5-dihydroxyacetophenone. None of the phenols has an R_F' value of zero in all six solvent systems. Table I shows the R_F' values found for the various phenols with the exception of the anthraquinones. The anthraquinones are discussed separately and the data for them are shown in Table II.

The use of a ceric ammonium nitrate solution as a visualization reagent was continued. The reagent proved to be satisfactory for all the dihydric phenols studied. A visualization reagent was not required for any of the dihydroxyanthraquinones since they are all colored materials. A few of the phenols were located by the bleaching of spots when the reagent was sprayed. However, most left a definite colored spot.

Within an isomeric group the highest R_F' values are normally found with the dioxane containing solvent system, the methanol containing system and thirdly with water alone. These are all polar solvent systems involving adsorption as a means of separation. The three non-polar solvent systems usually give lower R_F' values although there are some exceptions, particularly with the two aromatic solvents. Thus 1,4-dihydroxynaphthalene, 2,4-dihydroxyacetophenone, 2,6-dihydroxyacetophenone and 2,4-dihydroxypropiophenone have higher R_F' values with benzene than with the polar solvents. This appears to be caused by intramolecular hydrogen bonding with the ketones and by reduced polarity resulting from the opposing hydroxyl groups on the naphthalene.

With the three non-polar solvents, benzene is most active, as would be expected. Toluene is slightly less active and mineral spirits is a poor third. In our earlier data on isomeric monosubstitued phenols it was shown that none of the phenols had higher R_F' values with mineral spirits than with toluene although some were equal[8,9]. The data developed here show the same to be true for the substituted dihydric phenols. This is not unexpected since it is felt that mineral spirits because of its predominantly aliphatic nonpolar composition is the least effective of all the solvents. In general we find that polar solvents are the best for more ionic or polar compounds such as hydroxybenzoic acids while aromatic solvents are best for less polar types such as the internally bonded phenols.

In a number of isomeric groups it is observed that the 2,6-isomer has the highest R_F' value. This effect is not consistent for all groups of isomers or all solvents but it does occur very frequently. All of the isomeric groups in which a 2,6-isomer is present exhibit this effect in at least two solvent systems. It can be seen from Table I that 2,6-dihydroxybenzamide has the highest R_F' value in four of the five solvent systems in which an R_F' value is shown for any of the three isomers. In a like manner 2,6-dihydroxytoluene has the highest R_F' of any of its group in three of six solvent systems, 2,6-dihydroxybenzoic acid has the highest R_F' in five of five solvents, 1,2,3-trihydroxybenzene in two of three solvents, and 2,6-dihydroxyacetophenone in all six solvents.

The effect of 2,6-substitution on the R_F' value is analogous to the "*ortho* effect" observed in monosubstituted phenols in which the *ortho* isomers nearly always have a

TABLE I

R_F' VALUES OF SOME ISOMERIC DISUBSTITUTED PHENOLS

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

Phenol	Solvent system					
	A	B	C	D	E	F
2,4-Dihydroxybenzaldehyde	0.62	0.86	0.70	0.51	0.44	0.07[a]
2,5-Dihydroxybenzaldehyde	0.64	0.88	0.73	0.47	0.41	0.05[a]
2,4-Dihydroxybenzamide	0.48	0.82	0.60	0.00	0.00	0.00
2,6-Dihydroxybenzamide	0.47	0.85	0.65	0.08[a]	0.07[a]	0.00
3,5-Dihydroxybenzamide	0.53	0.83	0.64	0.00	0.00	0.00
2,3-Dihydroxytoluene	0.67	0.81	0.76	0.71	0.59	0.08
2,5-Dihydroxytoluene (Toluhydroquinone)	0.68	0.84	0.79	0.13	0.11[a]	0.00
2,6-Dihydroxytoluene	0.73	0.87	0.81	0.23	0.20[a]	0.00
3,4-Dihydroxytoluene (4-Homopyrocatechol)	0.67	0.85	0.75	0.39	0.34	0.06
3,5-Dihydroxytoluene (Orcinol)	0.68	0.86	0.76	0.10[a]	0.08[a]	0.00
2,3-Dihydroxybenzoic acid (o-Pyrocatechuic acid)	0.57[b]	0.86	0.67	0.11[a]	0.07[a]	0.00
2,4-Dihydroxybenzoic acid (β-Resorcylic acid)	0.57[b]	0.85	0.69	0.00	0.00	0.00
2,5-Dihydroxybenzoic acid (Gentisic acid)	0.64[b]	0.89	0.70	0.00	0.00	0.00
2,6-Dihydroxybenzoic acid (γ-Resorcylic acid)	0.77[b]	0.90	0.81	0.14[a]	0.13[a]	0.00
3,4-Dihydroxybenzoic acid (Protocatechuic acid)	0.56[b]	0.83	0.67	0.00	0.06[a]	0.00
3,5-Dihydroxybenzoic acid (α-Resorcylic acid)	0.63[b]	0.85	0.69	0.00	0.00	0.00
1,2,3-Trihydroxybenzene (Pyrogallol)	0.67	0.81	0.75	0.00	0.00	0.00
1,2,4-Trihydroxybenzene (Hydroquinol)	0.63	0.88	0.73	0.00	0.00	0.00
1,3,5-Trihydroxybenzene (Phloroglucinol)	0.61	0.84	0.72	0.00	0.00	0.00
1,3-Dihydroxynaphthalene	0.35	0.78	0.53	0.27	0.17[a]	0.00
1,4-Dihydroxynaphthalene	0.00	0.00	0.00	1.00	1.00	0.00
1,5-Dihydroxynaphthalene	0.33[a]	0.81	0.51	0.29[a]	0.15[a]	0.00
1,6-Dihydroxynaphthalene	0.37	0.80	0.55	0.31	0.18[a]	0.00
1,7-Dihydroxynaphthalene	0.38	0.81	0.57	0.28	0.17[a]	0.00
2,3-Dihydroxynaphthalene	0.36	0.79	0.54	0.55	0.39	0.06[a]
2,6-Dihydroxynaphthalene	0.33	0.80	0.51	0.12[a]	0.10[a]	0.00
2,7-Dihydroxynaphthalene	0.30	0.81	0.50	0.14[a]	0.10[a]	0.00
2,4-Dihydroxyacetophenone	0.56[a]	0.81	0.69	0.86	0.59	0.06[a]
2,5-Dihydroxyacetophenone	0.00	0.77	0.65[a]	0.06	0.00	0.00
2,6-Dihydroxyacetophenone	0.57[a]	0.86	0.70	1.00	0.80	0.16[a]
2,4-Dihydroxypropiophenone	0.54[a]	0.83	0.65	1.00	0.94	0.15[a]
3,4-Dihydroxypropiophenone	0.55	0.87	0.70	0.18[a]	0.00	0.00

[a] Spot streaks.
[b] 1 % acetic acid required to prevent ionization.

higher R_F' value than the *meta* and *para* isomers. However in this case the net result is a "double *ortho*" effect. Fig. 1 shows the R_F' value *versus* the hydroxyl orientation for the dihydroxytoluenes and the dihydroxybenzoic acids with a polar solvent (water) and a non-polar aromatic solvent (toluene). The "double *ortho*" effect is shown in three of the four curves. Since 2,4-dihydroxytoluene was not available the R_F' values for this material were estimated and are enclosed in parentheses. The estimate was made by comparison of the other members of the two series of isomers with both solvents.
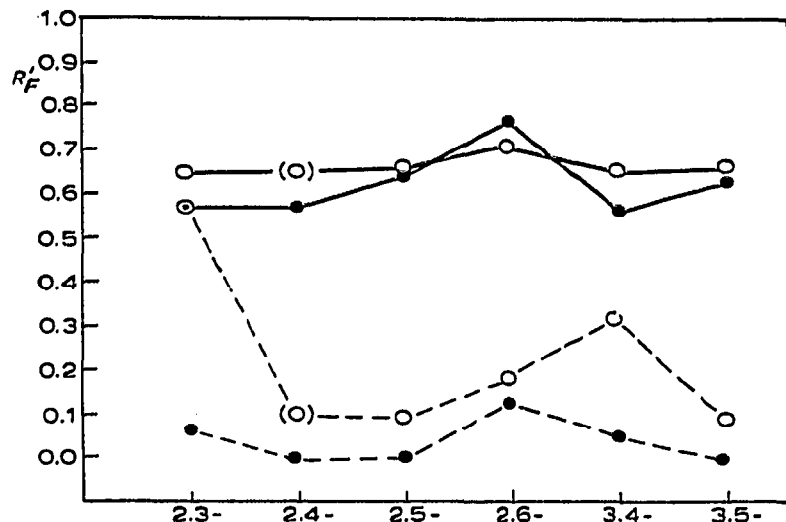
Fig. 1. Effect of hydroxyl orientation on R_F' values in water and toluene for methyl and carboxyl substituents. (O) Methyl-; (●) carboxyl-; (———) water; (– – –) toluene.

In two recently published papers, BARK AND GRAHAM present R_F data for a number of di-, tri- and tetra-methylated phenols. Their data show that for all methylated phenols the R_F value of the 2,6-isomer was higher than the others in the series[12]. When their system was a reversed phase, the R_F values of the 2,6-isomers were lowest in the series as would be expected[13]. The data presented here verify the results shown by these authors even though the relative positions of the substituents are reversed, *i.e.*, they had a hydroxyl with methyl groups in both *ortho* portions, while we have a substituent with hydroxyl groups in both *ortho* portions. This same effect has also been shown by REIO[14].

Another generalization that may be drawn from the data is that frequently phenols having adjacent hydroxyl groups have higher R_F' values than those in which the hydroxyls are separated. For example, with mineral spirits, benzene and toluene, 2,3-dihydroxytoluene and 3,4-dihydroxytoluene have higher R_F' values than the other members of the series. This is also true with 2,3- and 3,4-dihydroxybenzoic acids with benzene and toluene. However, in this case the 2,6- or "double *ortho* effect" shows up giving this isomer a higher R_F' value than either of the phenols containing adjacent hydroxyls. This "adjacent hydroxyl effect" is also found in 2,3-dihydroxynaphthalene with mineral spirits, benzene and toluene. These observations show that the "adjacent hydroxyl" effect is found primarily with the non-polar solvents. The only isomeric group in which polar solvents show this effect is the trihydroxybenzenes. This effect is caused by intramolecular bonding which reduces the polarity of the molecule.

Another interesting observation is the fact that in a series of isomers in which two members have adjacent hydroxyls, the member having the hydroxyls closest to the third substituent will have the higher R_F' value of the two. This is thought to be caused by reduced polarity resulting from a combination of bonding and steric effects. This can be seen by comparing 2,3-dihydroxytoluene and 3,4-dihydroxytoluene with mineral spirits, benzene and toluene as solvents. This same effect is shown with 2,3- and 3,4-dihydroxybenzoic acid with both polar and non-polar solvents and with 1,2,3- and 1,2,4-trihydroxybenzene with water and water–methanol–acetic acid.

A comparison was made of the various isomers in which the hydroxyl groups occupy the same position relative to the nonhydroxyl substituent. Fig. 2 shows the R_F' values of 2,3-dihydroxybenzoic acid, 2,3-dihydroxytoluene and 1,2,3-trihydroxybenzene with a polar solvent (water). This comparison shows the effect of the substituent group on the R_F' value since the hydroxyl groups are the same. Similar comparisons can be made in Figs. 3–7, in which are shown the R_F' values of the 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dihydroxy compounds, respectively.

In our earlier work on monosubstituted phenols, it was noted that a general pattern exists for the order of the non-hydroxyl substituent as the R_F' values decrease[8,9]. From observation of Figs. 2–7, it can be seen that this same general type of pattern is observed in the present work on substituted dihydric phenols. The phenol containing the methyl substituent nearly always has the highest R_F' value.
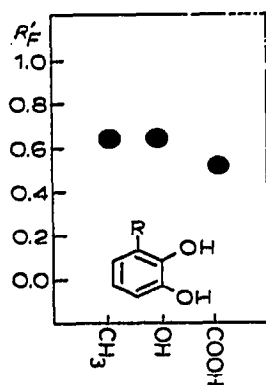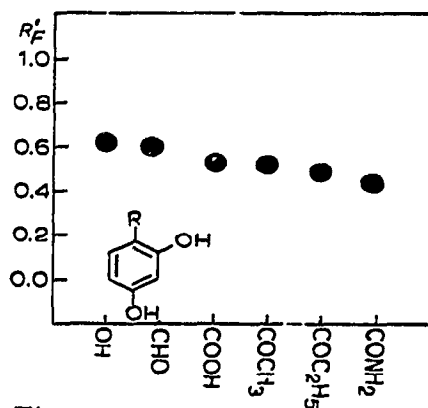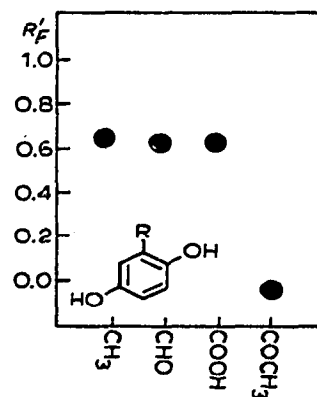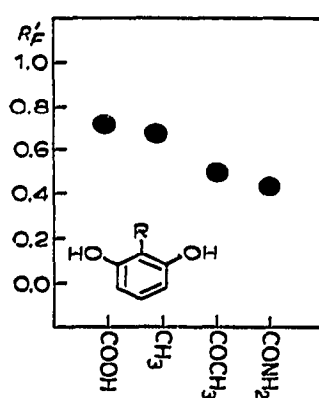

Fig. 2

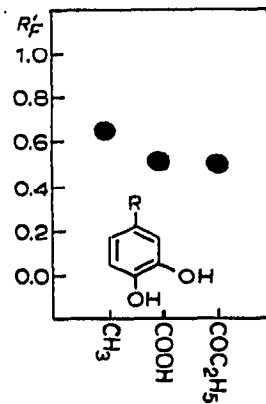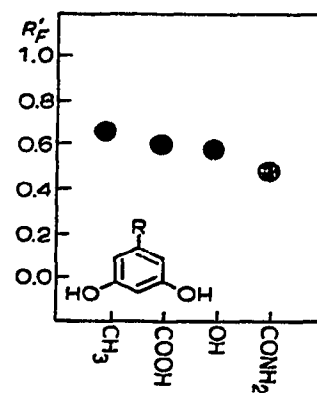
Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Figs. 2–7. Effect of substituents on R_F' value in water for various hydroxyl orientations.

The exception to this is 2,6-dihydroxytoluene, which ranks second in the 2,6-disubstituted phenols. The second ranking group in order of decreasing R_F' values is the hydroxyl substituent, followed by the aldehyde substituent, carboxyl, acetate, propionate and amide. There are some exceptions to this order, particularly at the higher R_F' values.
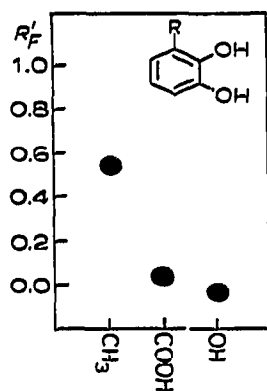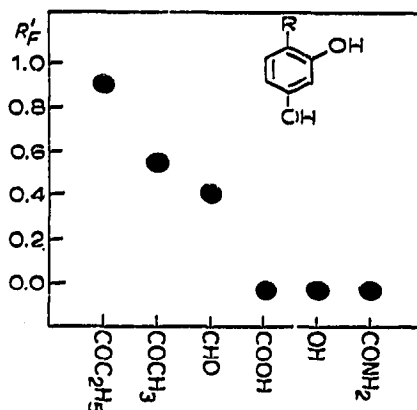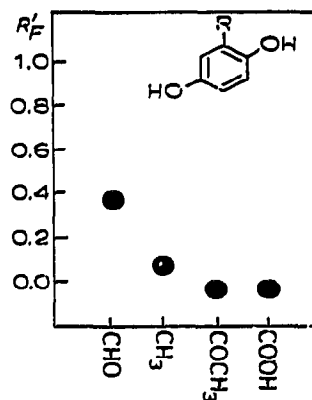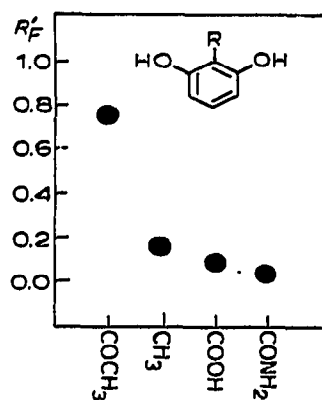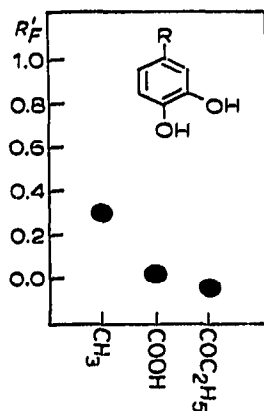

Fig. 8   Fig. 9   Fig. 10
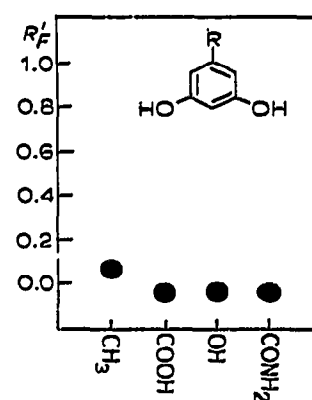

Fig. 11   Fig. 12   Fig. 13

Figs. 8–13. Effect of substituents on R_F' value in toluene for various hydroxyl orientations.

Figs. 8–13 show the R_F' values of the various phenols in relation to the non-hydroxyl substituents with a non-polar mobile solvent (toluene). A comparison of the results shown in Figs. 2–7 with Figs. 8–13 reveals differences in the magnitude of the R_F' values as well as the order of the substituents. This once again emphasizes the great difference between the partition and adsorption mechanisms in separations with paper chromatography. With a non-polar mobile solvent (partition), many of the substituents are nearly in the reverse order from that found with a polar mobile solvent (adsorption). The primary exception to this is the methyl substituent. This substituent is usually found at the top of the order with either solvent. This same fact was observed for methyl-substituted isomeric monosubstitued phenols with water and toluene but not with mineral spirits[8].

It can be seen from observation of the position of the substituents as the R_F' values decrease that with polar solvents the order is approximately the same as the

increasing molecular weights of the substituents. In our previous work, direct corre-
lation of R_F' and molecular weight has not been successful except for halogen substi-
tuents[9]. However, in this case a correlation is found as shown in Fig. 14. Only the
benzamide shows any large deviation from this curve. The values for this curve were
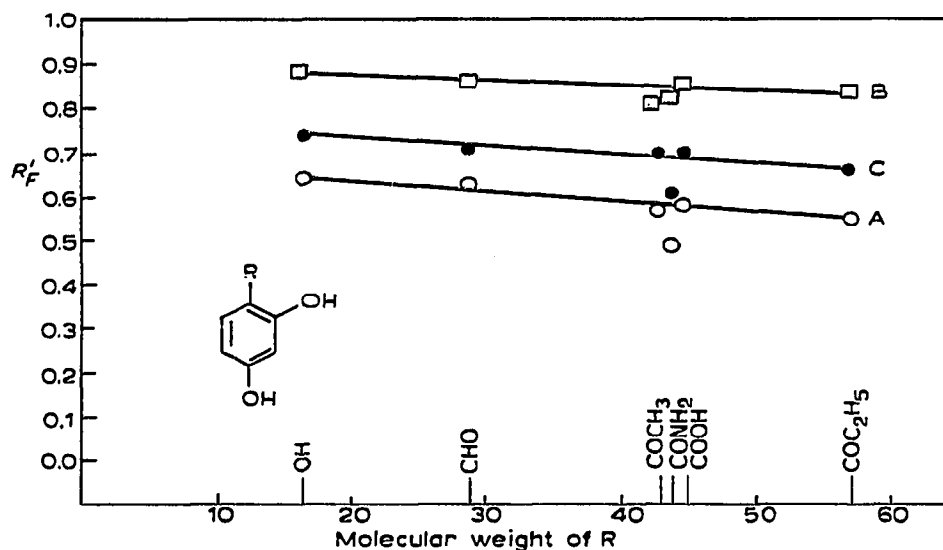taken from the 2,4-dihydroxy compounds since more of these were available.

Fig. 14. Molecular weight of non-hydroxyl substituent vs. R_F' value with polar solvents. A, B, and
C refer to the solvents mentioned in Tables I and II.

Since we are dealing with dihydric phenols, the effect of a third substituent is
not as pronounced as in our previous work. This minimizes steric and bonding effects
to the point that the R_F' value is more nearly proportional to the molecular weight.
The reduced effect of a single substituent can be observed in Figs. 2–7 where the
variations in R_F' value in a series are much less than have been previously observed
for monosubstituted phenols. With only one exception all the R_F' values in Figs. 2–7
range between 0.45 and 0.75. With monosubstituted phenols the R_F' range is 0.00
to 0.90.

With the aromatic mobile solvents, no correlation is found between R_F' and the
molecular weight of the substituent. However, the effect of a single substituent is
greater than when polar solvents are used as evidenced by the range of R_F' values
found in Table I. This shows again the difference between partition and adsorption
particularly with strongly aromatic solvents such as benzene or toluene.

The behavior of 2,5-dihydroxyacetophenone is an unusual factor. When
compared with other 2,4-, 2,5-, and 2,6-isomeric groups, it appears that the 2,5-
isomer should have an R_F' value nearly equal to the 2,4-isomer and less than the 2,6-
isomer. However, 2,5-dihydroxyacetophenone has an R_F' value lower than either the
2,4- or the 2,6-isomer in all six solvent systems. The difference is very large with water
and the non-polar solvents. It appears that in addition to bonding, steric hindrance
may affect the mobility of the 2,5-isomer quite markedly.

The studies on the dihydroxynaphthalenes have resulted in some interesting
observations. The "adjacent hydroxyl" effect described earlier is also found here.

With the three non-polar solvents 2,3-dihydroxynaphthalene has the highest R_F' value of any of the series. This effect is not seen with the polar solvents.

Another interesting observation is the behavior of 1,4-dihydroxynaphthalene. This compound has an R_F' of 0.00 in all solvents except the two aromatic systems and in these systems the R_F' value is 1.00. In this respect 1,4-dihydroxynaphthalene is acting like a completely non-polar molecule due to reduced polarity occasioned by the opposing location of the hydroxyls on the ring. The 2,6- and 2,7-isomers appear to be slightly more polar than the others since with the aromatic non-polar solvents they are more strongly adsorbed.

The dihydroxyanthraquinones are being discussed separately because of the difficulty in obtaining good separations. For this reason additional solvents have been studied. The separations obtained within any one solvent system still leave much to be desired but do show improvement over previously published data. For example using petroleum ether saturated with methanol, TAKIDO found only a difference of 0.01 R_F units between the 1,4-, 1,5- and 1,8-isomers[15]. The data of TAKIDO are included in Table II for purposes of comparison. His results compare quite closely to those obtained with water saturated mineral spirits. Solvent B shows a large difference in R_F' units between the extremes of the 1,4-, 1,5- and 1,8-isomers while solvents C, G, H, I and J show at least 0.05 R_F' units between the high and low values.

TABLE II

R_F' VALUES OF SOME DIHYDROXYANTHRAQUINONES

Solvent systems: (A) water; (B) water–dioxane–acetic acid (75:24:1, v/v); (C) water–methanol–acetic acid (75:24:1, v/v); (D) benzene saturated with water; (E) toluene saturated with water; (F) mineral spirits saturated with water; (G) methanol; (H) methanol–water–acetic acid (70:29:1, v/v); (I) methanol–water–toluene (70:13:17, v/v); (J) methanol saturated with heptane; (K) petroleum ether saturated with methanol.

Anthraquinone	Solvent system										
	A	B	C	D	E	F	G	H	I	J	K[b]
1,2-Dihydroxy (Alizarin)	0.00	0.53[a]	0.12[a]	1.00	1.00	0.32[a]	0.79	0.65	0.91	0.79	0.17
1,4-Dihydroxy (Quinizarin)	0.00	0.38[a]	0.07[a]	1.00	1.00	0.99	0.79	0.58	0.90	0.83	0.98
1,5-Dihydroxy (Anthrarufin)	0.00	0.56[a]	0.11[a]	1.00	1.00	0.96	0.80[a]	0.66	0.89[a]	0.85[a]	0.97
1,8-Dihydroxy (Chrysazin)	0.00	0.59[a]	0.14[a]	1.00	1.00	0.98	0.84	0.71	0.94	0.89	0.98
2,6-Dihydroxy (Anthraflavin)	0.00	0.58[a]	0.00	0.00	0.00	0.00	0.85	0.72	0.95	0.88	0.00

[a] Spot streaks.
[b] Data of TAKIDO[15].

The work of HOYER has shown that intramolecular hydrogen bonding has a pronounced effect on the adsorption of hydroxyanthraquinones and methyl hydroxyanthraquinones on silica gel[16,17]. This is found to be true in the present data in which only the 2,6-isomer does not have the opportunity to bond and therefore is the most polar of the isomers. This isomer is strongly adsorbed and has an R_F' value of 0.00 with all non-polar and even some polar aqueous solvents. The high polarity of this

isomer is also shown by the fact that in strongly polar solvents it is less adsorbed than the other isomers.

The 1,4-, 1,5- and 1,8-isomers appear to be less polar than both the 1,2- and the 2,6-isomers since they are not strongly adsorbed with the non-polar aliphatic solvents. The 1,2-isomer is of intermediate polarity since it is somewhat adsorbed by these same solvents. Among the 1,4-, 1,5-, and 1,8-isomers the 1,8-isomer appears to be the most polar followed by the 1,5-isomer although the differences in most cases are slight.

ACKNOWLEDGEMENTS

SUMMARY

The R_F' values have been determined in six solvent systems for a number of substituted dihydric phenols. Ceric ammonium nitrate has been found to be a good visualization reagent for these phenols. Three polar and three non-polar solvent systems were studied. The effect of orientation on the R_F' value has been studied. A "double ortho" and an "adjacent hydroxyl" effect were observed with a number of isomers.

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