PAPER CHROMATOGRAPHY OF PHENOLS

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The separation and identification of free phenols by paper chromatography have been studied extensively during the last 15 years, and a very great number of solvents have been proposed. The literature has been reviewed up to 1956 mainly by BLOCK AND ZWEIG[1], DIETRICH[2], LEDERER AND LEDERER[3] and HAIS AND MACEK[4]. Of the more recent work in this field the publications should be mentioned of REIO[5], COULSON AND EVANS[6] and PRIDHAM[7], who proposed a great many solvent systems, using plain or buffer-treated paper. KUNG-TSUNG WANG[8] uses a polyamide-impregnated paper and solvents such as *n*-butanol, ethyl acetate, toluene, benzene, cyclohexane, cyclohexene or *n*-hexane. MACÁK AND KUBEŠ[9] recently described a method using cyclohexane as the mobile phase on paper impregnated with formamide.

In connection with extensive analytical work on phenols in natural products, we needed a paper chromatographic method that would give a precise and good separation. In our hands many of the solvent systems described in the literature failed to give the results desired. Stimulated by our previous good results with two-phase solvent systems[10, 11] and by the comprehensive work of GASPARIČ AND VEČEŘA[12], we devised a simple method using dimethylformamide as the stationary phase and a mixture of cyclohexane and ethyl acetate as the mobile phase. A variety of free monophenolic compounds have been separated satisfactorily.

GENERAL PROCEDURE

Sheets of Schleicher & Schüll paper No. 2043b Mgl (or Macherey, Nagel & Co., Düren, No. 2214), 19 × 54 cm, were impregnated by drawing them through a mixture of dimethylformamide–acetone (75:25, v/v); then they were lightly pressed between sheets of filter paper and hung in the air for a short time to evaporate the acetone. The phenols to be chromatographed were dissolved in chloroform (1 mg per ml) and 3–5 μl of the solution was placed, in the usual way by means of a micropipette, on the starting line, 12 cm from one end of a sheet prepared as described above (6 substances on each sheet).

After 15 minutes equilibration of the sheet in the chromatography chamber,

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the mobile phase, consisting of a mixture of cyclohexane–ethyl acetate (5:1, v/v), which had been previously saturated by agitating with a mixture of dimethylform-amide–water (5:2, v/v), was poured into the trough. It is possible to increase the R_F values by increasing the quantity of ethyl acetate in the mobile phase. The proportion cyclohexane–ethyl acetate should not exceed 5:2 v/v, other experimental conditions remaining unchanged. The chromatograms were run by the descending method at 25°, the solvent front travelling about 35 cm from the starting line in 3 1/4–3 1/2 hours. The paper sheets are kept and prepared in a laboratory with a constant relative humidity of 45 %.

As already observed during our earlier work with similar two-phase solvent systems[10,11], correct saturation of the atmosphere in the chromatography chamber is essential for good separation and round spots (no "tailing"). The two side walls (20 × 50 cm) of a rectangular glass jar (20 × 30 cm and 50 cm high) were lined with filter papers dipping into crystallizing dishes containing dimethylformamide–water (5:2, v/v). The lining papers were completely moistened every time a new chromatogram was placed in the jar. On the bottom of the chromatography chamber two small crystallizing dishes containing the mobile phase were placed.

The finished chromatogram was dried for 10 min at 40° and in most instances sprayed with diazotized sulphanilic acid and sodium carbonate as described in the literature[13,1]. Diazotized p-nitroaniline and ferric chloride solution as described[1], were also used as spraying agents in some cases. Examination of the chromatogram in ultraviolet light may sometimes help in the identification (fluorescence).

RESULTS AND DISCUSSION

In spite of very constant experimental conditions, the R_F values in a two-phase solvent system as used here, may show small variations (up to 0.05 units) from one chromatogram to another, the principal cause being probably due to differences in the impregnation grade.

The R_F values in Table I are relative values obtained by running a special reference mixture of five substances (R_F values 0.12–0.70) on every chromatogram. The R_F values of the reference mixture itself were determined as the average value of more than 30 chromatograms. The R_F of each substance in the list is an average value obtained from 5 to 20 test chromatograms, each test chromatogram being in correct relationship to the values of the reference mixture.

As shown in the photographs (Fig. 1), the method gives sharp round spots without "tailing" and a good separation of a great many mono-phenols.

A difference of 0.05 in the R_F values is necessary to obtain separation of two compounds. In general, substances chromatographed separately and in mixtures show the same R_F values. The sensitivity of the method is satisfactory. With diazotized sulphanilic acid, 0.1 μg of substance could still be detected.

The method is based on a partition between a strongly polar stationary phase (dimethylformamide) and a less polar mobile phase (cyclohexane–ethyl acetate).

TABLE I

R_F VALUES AND COLOUR REACTIONS OF VARIOUS MONOPHENOLIC COMPOUNDS

Descending paper chromatography at 25°. Paper: Schleicher & Schüll, No. 2043 b Mgl, impregnated with dimethylformamide–acetone (75:25, v/v); mobile phase: cyclohexane–ethyl acetate (5:1, v/v), saturated with dimethylformamide–water (5:2, v/v). Spraying agent: diazotized sulphanilic acid, then 20 % Na_2CO_3[13].

| Compound	R_F value	Colour
p-Aminophenol	0	grey-blue
m-Aminophenol	0	yellow
o-Aminophenol	0.05	light brown
o-Hydroxybenzyl alcohol	0.06	yellow
Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	0.10	light brown
4-(p-Hydroxyphenyl)-butan-2-one	0.12	rose
4-(m-Hydroxyphenyl)-butan-2-one	0.14	yellow-orange
p-Nitrophenol	0.18	lemon-yellow
4-(p-Hydroxyphenyl)-butan-3-one	0.18	rose
2-Hydroxy-1,3-dimethoxybenzene (Pyrogallol 1,3-dimethyl ether)	0.19	red
Hydroquinone monomethyl ether (1-Hydroxy-4-methoxybenzene)	0.19	violet
Ethylvanillin (4-Hydroxy-3-ethoxybenzaldehyde)	0.21	pale brown
5-(p-Hydroxyphenyl)-pentan-2-one	0.25	rose
Phenol	0.34	yellow
Guaiacol (1-Hydroxy-2-methoxybenzene)	0.35	orange
4-(o-Hydroxyphenyl)-butan-2-one	0.35	orange
o-Vanillin (2-Hydroxy-3-methoxybenzaldehyde)	0.39	yellow
p-Phenylphenol (4-Hydroxydiphenyl)	0.40	dark violet
Vinylguaiacol (1-Hydroxy-2-methoxy-4-vinylbenzene)	0.41	rose-violet
m-Cresol (m-Methylphenol)	0.41	yellow
p-Cresol (p-Methylphenol)	0.42	rose
3,4-Dimethylphenol (4-Hydroxy-1,2-dimethylbenzene)	0.45	rose
Creosol (2-Methoxy-4-methylphenol)	0.46	rose-light violet
p-Benzylphenol (4-Hydroxydiphenylmethane)	0.48	pink, pale
o-Cresol (o-Methylphenol)	0.49	yellow-orange
3,5-Dimethylphenol (5-Hydroxy-1,3-dimethylbenzene)	0.50	yellow
o-Phenylphenol (2-Hydroxydiphenyl)	0.51	orange
Isoeugenol (4-Hydroxy-3-methoxy-1-propenylbenzene)	0.52	violet
p-Ethylphenol (4-Hydroxy-1-ethylbenzene)	0.54	rose-violet
2,3-Dimethylphenol (3-Hydroxy-1,2-dimethylbenzene)	0.56	orange
2,5-Dimethylphenol (2-Hydroxy-1,4-dimethylbenzene)	0.57	orange
2,4-Dimethylphenol (4-Hydroxy-1,3-dimethylbenzene)	0.59	violet
Eugenol (4-Hydroxy-3-methoxy-1-allylbenzene)	0.60	violet
o-Nitrophenol	0.60	pale yellow
4-Hydroxy-3-methoxy-1-ethylbenzene (p-Ethylguaiacol)	0.61	violet
2,6-Dimethylphenol (2-Hydroxy-1,3-dimethylbenzene)	0.65	orange
2-Ethoxy-4-methylphenol	0.66	violet
2-Hydroxy-1-ethoxy-4-propenylbenzene	0.68	orange
p-tert.-Butylphenol (4-Hydroxy-1-tert.-butylbenzene)	0.70	pink, pale
Butylhydroxyanisole (2- and 3-tert.-Butyl-4-methoxyphenol)	0.74	pink, pale
Carvacrol (2-Hydroxy-1-methyl-4-isopropylbenzene)	0.76	orange
Thymol (3-Hydroxy-1-methyl-4-isopropylbenzene)	0.79	orange
o-tert.-Butylphenol (2-Hydroxy-1-tert.-butylbenzene)	0.81	orange
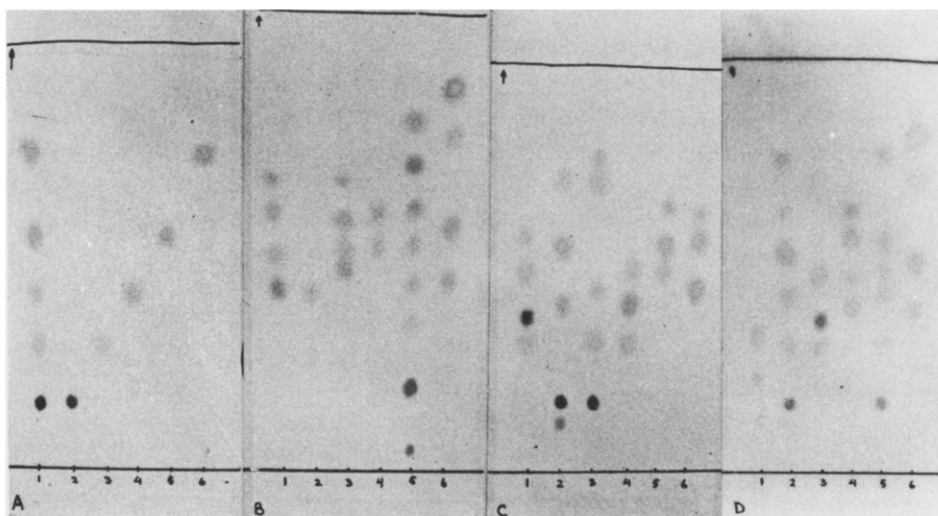
Fig. 1. Photographs of mono-phenolic compounds (sprayed with diazotized sulphanilic acid and sodium carbonate).

A. (1) 2-Hydroxy-1,3-dimethoxybenzene, phenol, creosol, eugenol, thymol; (2) 2-Hydroxy-1,3-dimethoxybenzene; (3) Phenol; (4) Creosol; (5) Eugenol; (6) Thymol.

B. (1) *p*- and *o*-Cresol, 2,4- and 2,6-dimethylphenol; (2) *m*-Cresol; (3) 3,4-; 3,5-; 2,3- and 2,6-Dimethylphenol; (4) Isoeugenol, eugenol; (5) *o*-Aminophenol, hydroquinone monomethyl ether, phenol, *p*-phenylphenol, *p*-benzylphenol, eugenol, 2-hydroxy-1-ethoxy-4-propenylbenzene, carvacrol; (6) *p*- and *o*-Phenylphenol, *p*- and *o-tert.*-butylphenol.

C. (1) Phenol, vinylguaiacol, *o*-cresol, eugenol; (2) 4-(*p*-Hydroxyphenyl)-butan-2-one, 2-hydroxy-1,3-dimethoxybenzene, *p*-phenylphenol, *p*-ethylphenol, *p-tert.*-butylphenol; (3) 2-Hydroxy-1,3-dimethoxybenzene, phenol, creosol, *p-tert.*-butylphenol, carvacrol; (4) Phenol, *p*- and *o*-cresol; (5) 3,5-; 2,3- and 2,6-Dimethylphenol; (6) 3,4-; 2,4- and 2,6-Dimethylphenol.

D. (1) 4-(*m*-Hydroxyphenyl)-butan-2-one, 5-(*p*-hydroxyphenyl)-pentan-2-one, 4-(*o*-hydroxyphenyl)-butan-2-one; (2) 2-Hydroxy-1,3-dimethoxybenzene, phenol, creosol, *p*-ethylphenol, 2-ethoxy-4-methylphenol, carvacrol; (3) Guaiacol, vinylguaiacol, *p*-benzylphenol, 4-hydroxy-3-methoxy-1-ethylbenzene, butylhydroxyanisole; (4) *p*- and *o*-Cresol, 2,4- and 2,6-dimethylphenol; (5) 2-Hydroxy-1,3-dimethoxybenzene, phenol, creosol, isoeugenol, eugenol, thymol; (6) *p*- and *o*-Phenylphenol, *p*- and *o-tert.*-butylphenol.

A sufficiently great difference in polarity (acidity) between two compounds is the condition for their separation on the paper chromatogram.

These experimental results show an interesting relationship between R_F value and acidity (pK_a). To a certain extent, this relationship permits one to predict the structure from the R_F value. The influence of the substituents of the phenols is due partly to their *nature*, and partly to their *position*. The chief effects are: (a) inductive effect, (b) steric hindrance, (c) hyperconjugation, (d) mesomeric effect. For general information on these effects, we refer the reader to the literature[14].

Some of these relationships are illustrated in Table II.

In addition to the relationships shown in Table II, some interesting facts are briefly discussed below.

Cresols

The replacement of one hydrogen in the phenol nucleus by the electron-*donating* methyl group ($+ I$ substituent[14]) decreases the acidity of the phenolic hydroxyl

TABLE II

RELATIONSHIPS BETWEEN ACID STRENGTH (pK_a), R_F VALUE,
ELECTRONIC AND STERIC EFFECTS, OF SOME SUBSTITUTED
MONOPHENOLIC COMPOUNDS

Substances	pK_a*	R_F**	Principal effects (relative strength)		
			Inductive	Steric	Hyperconjugation
Phenol	9.98[15]	0.34			
m-Cresol	10.08[15]	0.41	—	—	—
p-Cresol	10.10[16]	0.42	+	—	+
o-Cresol	10.19[16]	0.49	+	++	+
3,5-Dimethylphenol	10.23[17]	0.50	—	—	—
3,4-Dimethylphenol	10.43[17]	0.45	+	—	+
2,5-Dimethylphenol	10.46[17]	0.57	+	+	+
2,3-Dimethylphenol	10.57[17]	0.56	+	+	+
2,4-Dimethylphenol	10.63[17]	0.59	++	+	+
2,6-Dimethylphenol	10.66[17]	0.65	++	+++	+

* Literature values. Reference given for each one.
** Experimental results from this work (taken from Table I).

group, as expected. The additional steric effect in *ortho*-cresol explains the relatively great decrease of the acid strength, and a corresponding increase of the R_F value, sufficient to obtain a good separation from the *meta*- and *para*-isomers. However, the two latter compounds are not separated from each other. (A mixture is detectable by spraying with diazotized sulphanilic acid, which gives an orange spot (*meta*-) surrounded by a rose border (*para*-).)

Dimethylphenols (xylenols) (Table II)

Of special interest in this series is the fact that from the pK_a values alone, one would expect that the difference between 2,5- and 2,3-dimethylphenol would be large enough for separation to be possible. However, the R_F values are too close and no separation is obtained. This suggests that the acidity is not the only influence on the migration on the dimethylformamide-impregnated paper.

According to electronic reasoning, it would be predicted that a *para*-methyl group has an increased inductive effect (+ *I*). This is confirmed by the pK_a values of 3,4- and 2,4-dimethylphenols, compared with those of the 3,5- and 2,5-; 2,3-isomers respectively. 2,4-Dimethylphenol has also a slightly higher R_F value than the isomers with the second methyl group in *meta*-position. In the case of 3,4- and 3,5-dimethylphenol, the R_F values are in inverse proportion to the pK_a values, but different enough to give separation.

Further examples (not shown in Table II) which demonstrate clearly the influence of the steric effect of an *ortho* substituent are given by *para*- and *ortho-tert.*-butylphenol (R_F 0.70 and 0.81 respectively). These are easily separated from each other, as are *para*- and *ortho*-phenylphenol (R_F 0.40 and 0.51 respectively).

It is remarkable and interesting to note the good separation of eugenol (I) and isoeugenol (II).

R_F 0.60 R_F 0.52

(I)   (II)
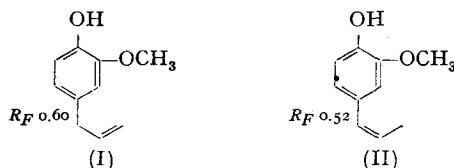
The double bond in *conjugation* with the aromatic nucleus in isoeugenol increases the polarity of the molecule, which may be predicted from the greater stability of the anion (delocalization of the charge, mesomeric effect, $+ M$). The same explanation is valid for p-phenylphenol (R_F 0.40) and p-benzylphenol (R_F 0.48).

This brief discussion of some of the relationships between R_F value, acid strength and structure, based on experimental results, should aid in the interpretation of the electronic and steric effects of substituted phenols in relation to paper-chromatographic results. This may be of some help in the identification of unknown compounds.

SUMMARY

A convenient two-phase solvent system for descending paper chromatography of free, mono-phenolic compounds is described. Some relationships between R_F value, acidity (pK_a) and structure are discussed.

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