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IDENTIFICATION OF ORGANIC COMPOUNDS

LXXXIX*. PAPER AND THIN-LAYER CHROMATOGRAPHY OF MONOHYDRIC PHENOLS AS ANTIPYRYLQUINONEIMINE DYES

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SUMMARY

The conversion of phenols into coloured derivatives using the reaction with 4-aminoantipyrine and an oxidizing agent has been described. *p*-Unsubstituted phenols or phenols substituted with halogeno, carboxy or sulpho groups in the *p*-position react to form red antipyrylquinoneimines. Phenols with *p*-alkoxy groups react only partially. *p*-Alkylphenols give yellow products under the same conditions. The red dyes are most effectively separated using the partition system formamide/*n*-hexane–benzene (1:1) on paper or thin layers of cellulose, whereas the solvent system dimethylformamide/*n*-hexane is suitable for the yellow products. The separation of isomeric *m*- and *p*-alkylphenols is carried out on Silufol sheets using benzene–acetone (3:1) as the mobile phase. The separation of *m*- and *p*-halogenophenols is achieved by the elimination of the halogen in *p*-position. The yellow products are relatively less stable both in solution and on chromatograms, and therefore the coloured derivatives should be freshly prepared.

INTRODUCTION

Monohydric phenols represent a group of compounds that can easily be subjected to paper or thin-layer chromatography (TLC). Many solvent systems and methods of detection have been reported[1–3]. Some problems, however, have been encountered, caused by poor separation of *m*- and *p*-isomers, *e.g.* *m*- and *p*-cresols, especially when partition systems were used. Therefore, various methods have been recommended for the conversion of phenols into derivatives with greater structural differences. Azo dyes prepared by reaction with arenediazonium salts, and antipyrylquinoneimine dyes, prepared by reaction with 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-5-pyrazolone) and an oxidizing agent are examples of such derivatives. These reagents attack different positions of the benzene nucleus depending on the type and position of the substituents present.

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The conversion of phenols into antipyrylquinoneimine dyes and the chromatographic separation of these dyes has been recommended by several authors, especially for the separation of isomeric cresols[4–6], xylenols[7] and naphthols[8]. Several other papers have dealt with the problem of the separation of phenols in water, etc.[9,10].

Phenols react with the Emerson reagent to form intensely red antipyrine dyes (antipyrylquinoneimines). The *m*- and *p*-isomers can be separated because the dyes are formed only from phenols with the position *para* to the hydroxy group either unsubstituted or substituted by such groups (*e.g.* the halogens) as are eliminated during the oxidative coupling reaction. In the case of *p*-alkyl-substituted phenols the products are yellow and their chromatographic behaviour is significantly different from that of the red dyes.

We have extensively studied the Emerson reaction of monohydric phenols from the point of view of the photometric determination of variously substituted phenols[11–14]. The structure of the resulting antipyrylquinoneimines has been confirmed, and their chemical and spectral properties, the optimum reaction conditions and the effect of different substituents on the course of the reaction have been determined. Chromatographic methods were used to evaluate the reaction products throughout the investigation. A special study dealt with the chromatographic behaviour of the yellow products derived from *p*-alkyl-substituted phenols[15]. In this paper, we use the above results to evaluate the application of these derivatives to the chromatographic separation of phenols, and summarize the scope and limitation of the procedure.

EXPERIMENTAL

Materials

The phenols used for the reaction were standard chemicals from our collection, the identity and purity of which were checked by melting-point determination and paper chromatography[16].

All reagents were reagent-grade chemicals.

General procedure for the preparation of the dyes

A 2.5-ml volume of a 0.01 *M* solution of a particular phenol in ethanol was treated with 1 ml of 4-aminoantipyrine solution (0.5% solution in the buffer solution), 25 ml of buffer solution (Britton-Robinson buffer, pH 7.5–10.0) and 1 ml of a 2% potassium hexacyanoferrate(III) solution in the buffer solution. (The hexacyanoferrate solution was stable when stored in darkness.) The reaction mixture was extracted with 1 ml of chloroform, and a 5-μl aliquot of the separated chloroform layer was spotted on the starting line of the chromatogram.

Paper chromatography

Whatman No. 3 paper was used throughout the experiments. The impregnation was carried out with a 20% solution of formamide in ethanol or 50% solution of dimethylformamide in benzene. The impregnated paper was dried for 10–15 min at room temperature, and the chloroform solutions were then applied. Development was carried out with heptane–benzene (1:1) in the former case and heptane in the latter.

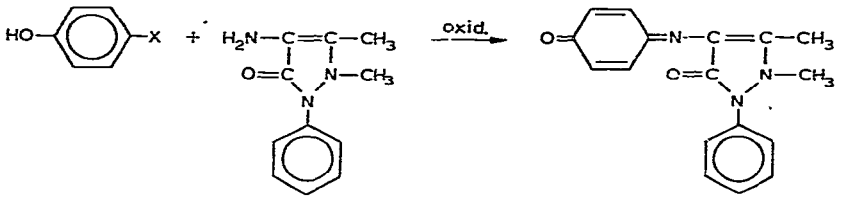
Thin-layer chromatography

Silufol pre-coated sheets (Sklárny Kavalier, Votice, Czechoslovakia) were used for adsorption chromatography. Benzene–acetone (3:1) was used as mobile phase.

Lucefol pre-coated sheets (Kavalier) impregnated with formamide (a 10%-alcoholic solution of formamide) were used for partition chromatography. *n*-Heptane–benzene (1:1) was used as the mobile phase.

RESULTS AND DISCUSSION

The reaction of phenols with 4-aminoantipyrine and the oxidizing agent in which the red quinoneimines are formed takes place at the position *para* to the hydroxy group. All other original substituents are retained. Quantitative elimination of the *p*-substituent occurs if it is hydrogen, a halogen, a carboxy group or a sulpho group:

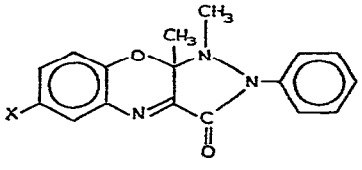


X = H, halogens, COOH or SO_3H

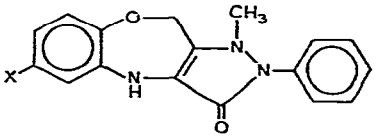
In the case of *p*-alkoxyphenols only partial elimination of the *p*-substituent was observed. Thus, these phenols also show spots of the corresponding *p*-unsubstituted phenols on the chromatograms. This can result in errors when the reaction is used for the identification of unknown mixtures of phenols, *e.g.* in water.

p-Alkyl-substituted phenols react to form yellow precipitates. We have previously reported[15] that this reaction takes place in one position *ortho* to the hydroxy group only, even if both *o*-positions are free (e.g. in *p*-cresol). Furthermore, quantitative elimination of the *o*-halogen atom was observed in the case of 6-bromo-2,4-dimethylphenol, whereas two products were found in the reaction with 6-chloro-3,4-dimethylphenol. 3,4-Dialkylphenols also give two spots due to the formation of two possible isomeric products. The yellow products characteristic of the *p*-alkylphenols are less stable than the red dyes, both in solution and on the chromatograms. Therefore, freshly prepared products should be used for chromatography when *p*-alkylphenols are expected in the reaction mixture.

Canadian authors[17,18] have reported that the yellow products have structures I and are converted easily into white compounds II.



I ; X = alkyl



II ; X = alkyl

Preparation of derivatives

Our previous investigation of the Emerson reaction of phenols[11] established the optimum reaction conditions. The optimum conditions for the photometric procedure were found, and discrepancies between the results of previous authors could be explained.

Chromatography of the red antipyrylquinoneimines

The most efficient separation of the red dyes was achieved using the partition system formamide/*n*-hexane–benzene (1:1), in both paper and cellulose TLC. Adsorption chromatography on silica gel layers was studied for comparison. R_F values obtained using both separation mechanisms are summarized in Table I. The relationship between the structures of the dyes and their chromatographic behaviour was studied from four points of view.

(1) Homologous series. The R_F values of 2- and 3-alkylsubstituted phenol derivatives increase in all cases with the length of the alkyl chain. The ΔR_M values for the methylene group are of the same order in paper and cellulose TLC as expected, whereas they are considerably lower in adsorption chromatography (see Table II).

(2) Influence of the halogen atoms. On substitution by halogen atoms the R_F values are increased in the series F < Cl < Br; in the case of iodine, however, some

TABLE I

R_F VALUE OF PHENOLS CHROMATOGRAPHED IN THE FORM OF THE RED ANTIPYRYLQUINONEIMINE DYES

Method I: paper chromatography, formamide/*n*-heptane–benzene (1:1). Method II: thin-layer chromatography, Lucefol sheets impregnated with formamide/*n*-heptane–benzene (1:1). Method III: thin-layer chromatography, Silufol sheets/benzene–acetone (3:1).

Phenol	R_F			*Phenol*	R_F		
	I	*II*	*III*		*I*	*II*	*III*
Phenol	0.05	0.07	0.20	3-Fluorophenol	0.09	0.11	0.26
2-Methylphenol	0.16	0.21	0.26	2-Chlorophenol	0.12	0.13	—
3-Methylphenol	0.11	0.13	0.22	3-Chlorophenol	0.20	0.22	0.28
2-Ethylphenol	0.35	0.45	0.29	2-Bromophenol	0.13	0.15	0.25
3-Ethylphenol	0.23	0.29	0.24	3-Bromophenol	0.22	0.26	0.27
2-*n*-Propylphenol	0.53	0.62	0.33	2-Iodophenol	0.22	—	0.30
2-Isopropylphenol	0.55	0.66	0.34	3-Iodophenol	0.19	0.21	0.30
2-*sec.*-Butylphenol	0.69	0.82	0.36	2,6-Dichlorophenol	0.24	0.23	0.35
2-*tert.*-Butylphenol	0.72	0.83	0.44	2,6-Dibromophenol	0.25	0.25	0.37
3-*tert.*-Butylphenol	0.45	0.56	—	2-Bromo-6-chlorophenol	0.23	0.25	0.36
2-Phenylphenol	0.39	0.46	0.30	2,6-Di-iodophenol	0.32	0.33	0.47
3-Phenylphenol	0.30	0.33	0.26	2,3,6-Trichlorophenol	0.33	0.47	0.47
2,5-Dimethylphenol	0.34	0.39	0.27	2,3,5,6-Tetrachlorophenol	0.65	0.52	—
2,6-Dimethylphenol	0.39	0.48	0.28	6-Bromo-2-methylphenol	0.67	0.48	0.47
3,5-Dimethylphenol	0.43	0.49	0.26	6-Bromo-2,5-dimethylphenol	0.32	0.40	0.45
3-Methyl-5-isopropylphenol	—	0.53	0.27	2-Chloro-3,5-dimethylphenol	0.23	0.26	—
2-Isopropyl-5-methylphenol	0.72	—	0.45	2,6-Dibromo-3,5-dimethylphenol	0.34	0.43	0.47
2-Methyl-6-*tert.*-butylphenol	0.82	0.92	0.52	2,6-Di-iodo-3,5-dimethylphenol	0.39	0.45	0.57
2,6-Diethylphenol	0.76	0.84	0.47	Dichlorophene	—	—	0.02
2-Ethyl-6-*tert.*-butylphenol	—	—	0.77	Hexachlorophene	—	—	0.47
2-Fluorophenol	0.07	0.08	0.26				

TABLE II

ΔR_M VALUES FOR METHYLENE GROUPS IN POSITIONS 2 AND 3

Methods I, II and III as in Table I.

Substituent	*I*		*II*		*III*	
	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M
H	1.279	—	1.123	—	0.602	—
2-CH_3	0.720	−0.559	0.575	−0.548	0.454	−0.148
2-C_2H_5	0.269	−0.451	0.087	−0.488	0.389	−0.065
2-*n*-C_3H_7	−0.052	−0.321	−0.213	−0.300	0.307	−0.082
3-CH_3	0.908	−0.371	0.825	−0.298	0.545	−0.057
3-C_2H_5	0.525	−0.383	0.389	−0.436	0.501	−0.044

irregularities were observed. The difference between the influence of chlorine and bromine is small. The relationship can be seen from the data in Table I. The influence of the halogen atoms in 2,6-dihalogenophenols can be seen from Table III. Again the ΔR_M values for the 2,6-dihalogeno groups are higher in the partition systems, but the differencies are practically the same.

TABLE III

ΔR_M VALUES FOR 2,6-DIHALOGENO SUBSTITUTION

Methods I, II and III as in Table I.

Substituents	*I*		*II*		*III*	
	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M
H,H	1.279	—	1.123	—	0.602	—
2,6-Cl_2	0.501	−0.778	0.525	−0.598	0.269	−0.333
2,6-Br_2	0.477	−0.802	0.477	−0.646	0.231	−0.371
2,6-I_2	0.327	−0.952	0.308	−0.815	0.052	−0.550

(3) Influence of the number of halogen atoms. The presence of each further chlorine atom results in an increase of the R_F values in the case of phenol, so that the R_F values increase in the series mono < di < tri < tetra. The influence of the halogen atom in the case of 3,5-dimethylphenol is very interesting. Whilst the R_F values are increased when phenol or 2-methylphenol are substituted by halogens, in the case of 3,5-dimethylphenol the R_F values are decreased when the partition systems are used (see Table I).

(4) Separation of 2- and 3-isomers. Eight such isomeric pairs of phenols were investigated (see Table IV). 2-Alkylphenols always have higher R_F values. The difference in their chromatographic behaviour when compared with 3-isomers and expressed as the ΔR_M values is of the same order in both partition systems, but considerably lower in the adsorption system.

Chromatography of the yellow dyes

The yellow dyes derived from *p*-alkylphenols behave chromatographically in a considerably less polar manner than the red dyes. Therefore, they have high R_F

TABLE IV

SEPARATION OF 2- AND 3-ISOMERS

Methods I, II and III as in Table I.

Substituent	*I*		*II*		*III*	
	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M
2-CH_3	0.720		0.575		0.454	
		0.188		0.250		0.091
3-CH_3	0.908		0.825		0.545	
2-C_2H_5	0.269		0.087		0.389	
		0.256		0.302		0.112
3-C_2H_5	0.525		0.389		0.501	
2-*tert.*-C_4H_9	−0.410		−0.689		—	
		0.323		0.584		—
3-*tert.*-C_4H_9	−0.087		−0.105		—	
2-Phenyl	0.194		0.070		0.368	
		0.174		0.238		0.086
3-Phenyl	0.368		0.308		0.454	
2-F	1.123		1.061		0.454	
		−0.118		−0.153		0.0
3-F	1.005		0.908		0.454	
2-Cl	0.865		0.825		—	
		−0.263		−0.280		—
3-Cl	0.602		0.545		—	
2-Br	0.825		0.753		0.477	
		−0.280		−0.299		−0.045
3-Br	0.545		0.454		0.432	
2-I	0.545		—		0.368	
		0.085		—		0.0
3-I	0.630		—		0.368	

values in all systems used for the separation of red dyes. The partition system dimethylformamide/*n*-hexane was found to be the most efficient for the separation of their homologous series. (For R_F values see ref. 15.)

Separation of m- and p-isomers

It has been found that the yellow products derived from *p*-alkylphenols are well separated from the red dyes in both partition and adsorption systems. Silica gel layers (Silufol), with the mobile phase benzene–acetone (3:1), can be used for rapid separation of a particular pair of isomers, *e.g. m*- and *p*-cresols, whereas the partition system must be used when unknown mixtures of phenols are to be separated.

Individual pairs of 3- and 4-halogenophenols can be separated, because red dyes identical with those from *p*-unsubstituted phenols are formed from 4-halogenophenols, whereas the *m*-isomer does not lose its halogen atom under the conditions.

CONCLUSIONS

The chromatography of phenols after conversion into antipyrine dyes represents a sensitive and suitable method for the identification of phenols in mixtures. The advantage lies in the simple preparation of the coloured derivatives and

their isolation. A further advantage is that when small or trace concentrations of phenols are involved, the concentration and isolation of the coloured derivatives is more convenient in comparison with that of the free phenols.

A suitable solvent system must be chosen according to the separation desired. The partition solvent system formamide/*n*-hexane–benzene (1:1) was found to be the most efficient for the separation of the red dyes, whereas the less polar yellow derivatives of *p*-alkylphenols were separated using the system dimethylformamide/*n*-hexane. The separation of 3- and 4-alkylphenols (*e.g. m*- and *p*-cresol) was achieved on silica gel thin-layers (Silufol), using the mobile phase benzene–acetone (3:1). The 3- and 4-halogenophenols were also separated, owing to the elimination of the *p*-halogen atom.

If this method is used for the identification of phenols in unknown mixtures, such factors should be borne in mind as the elimination of some substituents in *p*- or *o*-positions, respectively, the formation of identical derivatives from different phenols, the formation of two yellow products from one phenol, and the decreased stability of the yellow products. However, some of these factors can be used to advantage in the separation of particular pairs of compounds.

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