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Note

Identification of organic compounds

LXXXI*. A contribution to the chromatography of phenols as antipyryne dyes

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Antipyrylquinoneimine dyes, formed by the reaction of phenols with 4-aminoantipyryne and an oxidizing agent, have been recommended as suitable derivatives for use in the thin-layer chromatographic separation of phenols¹⁻⁷, especially of the isomeric cresols¹⁻⁴, xylenols⁵ and naphthols^{2,3}. Both Kamp¹ and Thielemann²⁻⁵ have found that cresols and xylenols with a free *para*-position to the hydroxyl group give red dyes, whereas *p*-cresol and xylenols with a methyl group in the *para*-position to the hydroxyl group give yellow products, the chromatographic migration of which is different from that of the corresponding red dyes. Hence it is possible to distinguish *m*- and *p*-cresols and the isomeric xylenols.

The colour reaction of phenols with 4-aminoantipyryne and an oxidizing agent has been extensively investigated in our laboratories and the results have been summarized in several papers⁸⁻¹¹ dealing with the formation and structure of the red antipyrylquinoneimine dyes. The yellow product formed in the reaction with *p*-cresol is under investigation at present. In this paper, work on the chromatographic behaviour of the yellow products resulting from the reaction with a series of *p*-alkyl-substituted phenols is described.

In the previous papers, it was pointed out that the reaction of phenols with 4-aminoantipyryne and potassium hexacyanoferrate(III) must be carried out under certain conditions involving the optimum pH range and buffer capacity, the ratio of the concentrations of the two reagents and their excess over the phenol being determined. The sequence of the addition of reagents was also found to be important. From this point of view, there are great differences in the experimental conditions used by the various workers, and these conditions are summarized in Table I.

Our preliminary experiments with *p*-alkylphenols and repetition of the procedures used by previous workers have shown that no uniform product is formed and that

* Part LXXX: *Mikrochim. Acta*, in press.

TABLE I

EXPERIMENTAL CONDITIONS USED FOR THE IDENTIFICATION OF PHENOLS AS PRODUCTS OF THE REACTION WITH 4-AMINOANTIPYRINE AND POTASSIUM HEXACYANOFERRATE(III)

Reference	Amount of reagent used			Chromatography
	Phenols	4-Aminoantipyrine	$K_3Fe(CN)_6$	
Kamp ¹	100 mg (<i>p</i> -cresol)	30 mg	200 mg	TLC*
Thielemann ²⁻⁵	not stated (cresols, xylenols)	6 mg	20 mg	TLC**
Svobodová and co-workers ⁸⁻¹¹	0.1 mg (phenol)	8.1 mg	26.3 mg	PC***
Chambon <i>et al.</i> ⁶	2.5 μ g (phenol)	60 mg	240 mg	TLC†
This work	2.7 mg (<i>p</i> -cresol)	15.4 mg	300 mg	PC††, TLC†††

* Silica gel G with chloroform-diethyl ether-*n*-butanol-diethylamine (50:30:10:0.5).

** Silica gel G impregnated with formamide-benzene or butyl acetate or their mixtures.

*** Formamide-benzene.

† Silica gel with chloroform-ethyl acetate (8:2).

†† Dimethylformamide-*n*-hexane.

††† Silufol-chloroform.

the number and amount of by-products formed are dependent on the reaction conditions used. The pH of the reaction mixture and the ratio of the reagents have been found to influence the results significantly. Spots of different products were found when the procedures of different workers were used. Our previous procedure⁸, which was suitable for the preparation of the red quinoneimine dyes, had to be modified because the sensitivity of the reaction is lower for *p*-alkylphenols.

EXPERIMENTAL

Materials

The phenols used for the reaction were standard chemicals from our collection, the identities and purity of which were checked by melting-point determinations and paper chromatography.

All reagents were reagent-grade chemicals.

General procedure for the preparation of the yellow products

A 2.5-ml volume of a 0.01 *M* solution of a particular *p*-alkylphenol in ethanol was treated with 3.5 ml of 4-aminoantipyrine solution (440 mg in 100 ml of the buffer solution), 25 ml of buffer solution (Britton-Robinson buffer, pH 9.9) and 5 ml of potassium hexacyanoferrate(III) solution (6.0 g in 100 ml of buffer solution). After about 60 min, the reaction mixture was extracted with 1 ml of chloroform and 5-10 μ l of the separated chloroform layer were 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 50% solution of dimethylformamide in benzene. After drying the impregnated sheet for 10–15 min at room temperature, the chloroform solutions were applied. Development was carried out with *n*-hexane. When detection was required, the chromatograms were sprayed with a 0.01–0.1% solution of diazotized 2,4-dinitroaniline in water¹². When iron(III) hexacyanoferrate(III) was used, the chromatograms were sprayed with a freshly prepared mixture of equal volumes of a 1% aqueous solution of iron(III) chloride and a 1% solution of potassium hexacyanoferrate(III) in water.

Thin-layer chromatography

Silufol pre-coated sheets (Kavalier, Czechoslovakia) were used. Chloroform and in some instances benzene–acetone (3:1) were used as the mobile phase. Detection was carried out in the same manner as for paper chromatography.

RESULTS AND DISCUSSION

The reaction conditions for the preparation of the yellow products described under Experimental were determined empirically and were applied to a series of *p*-alkylphenols. By using this procedure, we obtained the yellow products in relatively good yields and the formation of by-products was suppressed to a minimum.

TABLE II

R_F VALUES OF THE YELLOW PRODUCTS FROM *p*-ALKYLPHENOLS

Whatman No. 3 paper impregnated with 50% dimethylformamide solution; mobile phase, *n*-hexane.

Phenol	R_F
4-Methylphenol	0.34
4-Ethylphenol	0.45
4-Isopropylphenol	0.54
4- <i>sec.</i> -Butylphenol	0.64
4- <i>tert.</i> -Butylphenol	0.58
4-(1,1-Dimethylpropyl)phenol	0.69
4-Benzylphenol	0.25
4-Cyclohexylphenol	0.69
4-(1,1,3,3-Tetramethylbutyl)phenol	0.85
2,4-Dimethylphenol	0.43
3,4-Dimethylphenol	0.39
	0.49
2,3,4-Trimethylphenol	0.49
2- <i>tert.</i> -Butyl-4-methylphenol	0.76
2-Methyl-4- <i>tert.</i> -butylphenol	0.76
2-Ethyl-4- <i>tert.</i> -butylphenol	0.81
2,4-Di- <i>tert.</i> -butylphenol	0.95
2,4,6-Trimethylphenol	—
2,6-Di- <i>tert.</i> -butyl-4-methylphenol	—
2,6-Dimethyl-4- <i>tert.</i> -butylphenol	—
2-Bromo-4,6-dimethylphenol	0.43
2-Chloro-4,5-dimethylphenol	0.40
	0.60

For the paper chromatographic separation and characterization of the yellow products, papers impregnated with dimethylformamide and *n*-hexane as the mobile phase were found to be the most efficient. The R_F values are summarized in Table II. On thin layers of silica gel with chloroform as the mobile phase, the differences between the R_F values of the individual yellow products were much lower. When only a differentiation between isomers, *e.g.*, of the cresols or the xylenols is required, however, the thin-layer chromatographic separation was advantageous. Using Silufol sheets and benzene-acetone (3:1) as the mobile phase, the following R_F values were obtained: *o*-cresol, 0.39; *m*-cresol, 0.33; and *p*-cresol, 0.92.

The spots of the yellow products can be rendered visible by spraying the paper or thin-layer chromatograms with a solution of diazotized 2,4-dinitroaniline. Very intense red spots are formed within a few seconds.

We have also observed that the yellow products characteristic of *p*-alkylphenols are not stable. Exposure of the chloroform solution to heat or light or of the spotted chromatograms to air and light destroys the yellow products completely within a short time. The products of this destruction can be detected by applying 2,4-dinitrodiazobenzene reagent or iron(III) hexacyanoferrate(III) reagent. The instability of the yellow product from *p*-cresol can be illustrated by the chromatogram in Fig. 1.

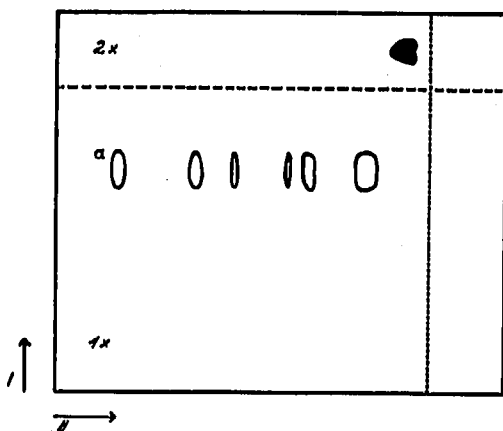


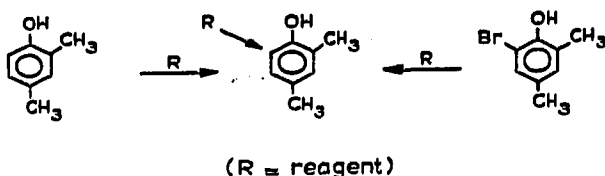
Fig. 1. Two-dimensional chromatogram of the yellow product from *p*-cresol (see text): a, yellow spot after development in the first direction; shaded area, yellow spot.

The solution of the yellow product from *p*-cresol was applied to point 1 and developed in the first direction until the dotted line was reached by the solvent front. Chloroform was used as the mobile phase in this direction. The chromatogram was then dried and exposed to light and air for 4 h and the same amount of the yellow solution was applied to point 2. The second development was carried out at right-angles to the first using benzene-acetone (3:1) as the mobile phase. No yellow product could be observed in the first sample applied to point 1 after the second development. After spraying with the reagents mentioned above, the decomposition products appeared.

The reaction is independent on the type of alkyl group in the *para*-position: the yellow products were formed in case of *p*-methyl-, *p*-ethyl-, *p*-isopropyl-, *p*-sec-

butyl-, *p*-*tert*-butyl-, *p*-(1,1-dimethylpropyl)-, *p*-benzyl-, *p*-cyclohexyl- and *p*-(1,1,3,3-tetramethylbutyl)phenol. The yellow products formed must contain the alkyl groups because their R_F values increase with increase in the length of the alkyl group.

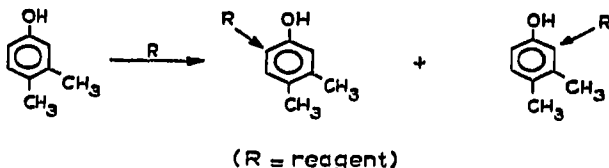
The yellow products were formed by the reaction of all tested *p*-alkylphenols that had at least one free *ortho*-position to the hydroxyl group. No yellow products were found in the reaction products from 2,4,6-trialkylphenols. Hence it can be deduced that the substitution takes place in this *ortho*-position, e.g.:



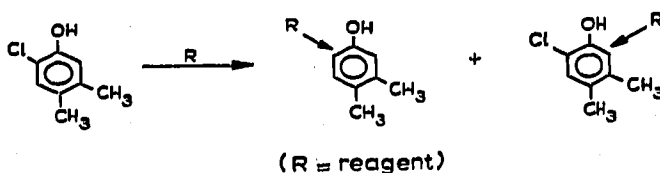
The yellow products from *p*-alkylphenols that have both *ortho*-positions unsubstituted must also represent structures in which one position has been substituted with a reagent molecule, because their R_F values are close to those of the corresponding 2,4-dialkylphenol derivatives.

When the last free *ortho*-position is substituted with a halogen atom, as for example in 2-bromo-4,6-dimethylphenol, replacement of the halogen atom takes place and the resulting yellow product has an R_F value the same as that derived from 2,4-dimethylphenol (see the above reaction scheme).

With unsymmetrically substituted phenols with two free *ortho*-positions, e.g. 3,4-dimethylphenol, the formation of two yellow spots is observed, probably due to the formation of two positional isomers:



The formation of two yellow products has also been observed with 2-chloro-4,5-dimethylphenol, one having the same R_F value as 3,4-dimethylphenol and the second having a considerably higher R_F value. The second product could be assumed to contain the chlorine atom in the molecule:



CONCLUSION

The results of this study have shown that when 4-aminoantipyrine is used as a reagent for the paper chromatographic separation and identification of phenols,

the following facts must be taken into consideration: the lower sensitivity of the reaction with *p*-alkylphenols in comparison with substances unsubstituted in the *para*-position, the instability of the yellow products formed with these phenols and the possibility of the formation of two yellow products in case of some phenols. The reaction is positive with all *p*-alkylphenols that have at least one free *ortho*-position to the hydroxyl group.

REFERENCES

- 1 W. Kamp, *Pharm. Weekbl.*, 101 (1966) 857.
- 2 H. Thielemann, *Z. Chem.*, 9 (1969) 350.
- 3 H. Thielemann, *Mikrochim. Acta*, (1970) 994.
- 4 H. Thielemann, *Pharmazie*, 25 (1970) 418.
- 5 H. Thielemann, *Pharmazie*, 24 (1969) 784.
- 6 P. Chambon, R. Chambon-Mougenot and J. Bronguier, *Rev. Inst. Pasteur Lyon*, 3 (1970) 395; *C.A.*, 76 (1972) 49603g.
- 7 O. M. Aly, *Water Res.*, 2 (1968) 587; *C.A.*, 69 (1968) 99175v.
- 8 D. Svobodová and J. Gasparič, *Collect. Czech. Chem. Commun.*, 33 (1968) 42.
- 9 D. Svobodová, J. Gasparič and L. Nováková, *Collect. Czech. Chem. Commun.*, 35 (1970) 31.
- 10 D. Svobodová and J. Gasparič, *Collect. Czech. Chem. Commun.*, 35 (1970) 1567.
- 11 D. Svobodová and J. Gasparič, *Mikrochim. Acta*, (1971) 384.