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THE SEPARATION AND IDENTIFICATION OF PHENOLS IN THE FORM OF *p*-(N,N-DIMETHYLAMINO)BENZENE *p'*-AZOBENZOATES BY MEANS OF PAPER AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates of differently substituted phenols have been prepared. Some of the derivatives are well crystallized substances that can be characterized by their melting-points. The others, however, are very difficult to purify. Nevertheless, their chromatographic identification can be achieved without difficulty. The esters are coloured, and consequently their detection is not necessary. Less than 0.5 μg of ester can be detected in the spot.

We did not succeed in separating and identifying phenols that have bulky groups in the *ortho* position to the hydroxyl group.

INTRODUCTION

The reactions used in the identification of phenols are mostly based on the reactivity of the hydroxyl group, and sometimes on the substitution of the aromatic nucleus. If the hydroxyl group is influenced by the neighbouring negative phenyl group, it gains a weakly acidic character. For the identification of phenols, as for alcohols, the reactions of the hydroxyl group with the chlorides[1] and anhydrides[2] of acids are used to form the corresponding esters. Monocyclic phenols can be easily identified by converting them into the esters of 3,5-dinitrobenzoic acid; for polycyclic phenols acetylation is more suitable. The least reactive phenols can be acetylated by acetyl chloride in the presence of powdered magnesium. In alkaline media, phenols react with monochloroacetic acid to form aryloxyacetic acids[3], which can be isolated and, if necessary, their neutralization equivalents can be determined and the unknown phenol can thus be identified. Esterification of phenols with the isocyanates or azides of acids produces urethanes[4,5]. Of other suitable derivatives, 2,4-dinitrophenyl ethers of phenols may be mentioned[6].

Being non-volatile and relatively easy to detect, almost all phenols can be subjected to paper chromatography (PC) or thin-layer chromatography (TLC) in their original state[7–26].

EXPERIMENTAL

The preparation of *p*-(N,N-dimethylamino)benzene-*p'*-azobenzoyl chloride, which is used as a reagent, is comparatively simple and has been described previously[27]. One of the most frequently used methods to prepare esters is the pyridine method, in which a mixture of benzene and pyridine serves as the reagent. For less reactive hydroxyl groups, the use of pyridine alone is preferable. The main reason for the presence of pyridine in the reagent mixture is its ability to bond with the hydrogen chloride formed. The dimethyl amino group in the molecule of the reagent has similar properties in that pyridine alone can be used. The esterification can be carried out either by heating the reagent mixture under reflux or in sealed ampoules that dip directly into boiling water. Many of the esters crystallize very well and have characteristic melting-points. For chromatographic purposes it is not necessary to purify the esters by crystallization, and the reagent solutions can be applied directly onto the chromatographic paper or the thin layer.

TABLE I

DERIVATIVES OBTAINED BY THE PYRIDINE REACTION

p-(N,N-dimethylamino)benzene p'-azobenzoates of	*Molecular weight*	*Melting point (°C) (corrected)*
Phenol	345	225–229
o-Cresol	359	196–199
p-Methoxyphenol	375	200–204
o-Nitrophenol	390	193–197
m-Nitrophenol	390	196–198
p-Nitrophenol	390	261–267
2,4-Dinitrophenol	435	277–278
2,6-Dinitrophenol	435	279–280
1-Naphthol	395	209–222

The pyridine method is carried out as follows (preparative scale). 0.5 g of phenol and 1.5 g of *p*-(N,N-dimethylamino)benzene-*p'*-azobenzoyl chloride are heated with 20 ml of pyridine under reflux for 1 h. After cooling, 20 ml of a 5% solution of sodium hydrogen carbonate are added to the mixture, then the solution is filtered and the separated derivative is crystallized from dimethylformamide. The esters obtained are red-orange crystalline substances with characteristic melting points (see Table I).

The other chromatographed derivatives of phenols were prepared by heating the corresponding phenol with the reagent in benzene under reflux for 3 h.

The derivatives for chromatographic purposes only are prepared on the microscale. The amount of the reagent and the sample is reduced about ten times and the mixture is heated in a sealed ampoule. After cooling, the mixture can immediately be put on the start of the chromatogram.

Paper chromatography

The work was carried out on impregnated Whatman No. 1 chromatographic papers by the descending technique. Purification and isolation of the esters in the solid state is not necessary for the stationary phase, and hexane or cyclohexane and benzene appeared to be the most convenient as the mobile phase.

The inverse systems with stationary paraffin oil and with dimethylformamide, methanol and water in the mobile phase also proved to be useful. The applied systems of solvents S_1–S_6 for PC are combinations of both of these basic systems, and they established the possibilities for the identification of different phenols in various combinations (R_F values are given in Table II). The systems of solvents used were:

S_1 45 % impregnated solution of dimethylformamide/cyclohexane–benzene (5:1).

S_2 30 % impregnated solution of dimethylformamide/hexane.

S_3 40 % impregnated solution of dimethylformamide/hexane–xylene (10:1).

S_4 10 % impregnated solution of paraffin oil/dimethylformamide–methanol–water (8:3:2).

S_5 10 % impregnated solution of paraffin oil/dimethylformamide–methanol–water (8:4:1).

S_6 10 % impregnated solution of paraffin oil/dimethylformamide–butanol–water (80:5:15).

TABLE II

R_F VALUES OF *p*-(N,N-DIMETHYLAMINOBENZENE) *p'*-AZOBENZOATES OF THE CORRESPONDING PHENOLS OBTAINED BY PC

Original phenol	*Solvent system*					
	S_1	S_2	S_3	S_4	S_5	S_6
Phenol	0.41	0.48	0.38	—	—	—
o-Cresol	0.49	0.52	0.51	—	—	—
m-Cresol	0.53	0.58	0.54	—	—	
o-Xylenol	0.56	0.58	0.70	—	—	—
o-Nitrophenol	0.19	0.11	—	—	—	—
m-Nitrophenol	0.23	—	0.16	—	—	—
p-Methoxyphenol	0.25	0.22	0.18	—	—	—
o-Chlorophenol	0.40	—	0.30	—	—	—
1-Naphthol	0.45	0.47	—	—	—	—
Octylphenol	—	—	—	0.20	0.60	0.51
Nonylphenol	—	—	—	0.13	0.52	0.39
Dodecylphenol	—	—	—	0.08	0.25	0.20
Cetylphenol	—	0.63	—	—	0.08	0.03
2-Methyl-6-octylphenol	—	—	0.82	—	—	—
p-*tert*.-Octylphenol	—	—	—	0.19	0.55	—
o-*sec*.-Butylphenol	0.76	—	—	0.50	—	—
o-Octyl-*o*-cresol	0.62	—	—	0.17	0.48	—
o-*tert*.-Butyl-*p*-cresol	—	—	—	0.46	—	—
p.-*tert*.-Butyl-*o*-cresol	0.73	0.66	0.85	0.49	—	0.68
4,6-Di-*tert*.-butyl-*o*-cresol	—	—	0.61	—	—	—

Thin-layer chromatography

Both impregnated and unimpregnated layers were used for the separation of *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates. The layers were prepared by pouring Silica Gel CH (Lachema) or Kieselgel G (Macherey, Nagel and Co.) on to glass plates

(18 × 24 cm). They were activated for 1 h at 110°. The following systems proved to be the best:

S_7 cyclohexane–methyl ethyl ketone (7:3) (Silica Gel CH).
S_8 cyclohexane–ethyl acetate (4:1) (Silica Gel CH).
S_9 hexane–ethyl acetate (4:1) (Kieselgel G).
S_{10} benzene–chloroform (1:1) (Kieselgel G).
S_{11} chloroform (Kieselgel G).

R_F values obtained are given in Table III.

TABLE III

R_F VALUES OF *p*-(N,N-DIMETHYLAMINO)BENZENE *p'*-AZOBENZOATES OF THE CORRESPONDING PHENOLS OBTAINED BY TLC

Original phenol	*Solvent system*				
	S_7	S_8	S_9	S_{10}	S_{11}
Phenol	0.66	0.53	0.33	0.42	0.55
o-Cresol	0.67	0.50	0.31	0.38	—
m-Cresol	0.69	—	0.45	0.50	0.75
p-Cresol	—	0.49	—	—	—
p-Methoxyphenol	0.64	0.39	0.15	0.25	0.46
o-Chlorophenol	0.75	—	0.25	0.50	0.80
o-Nitrophenol	0.54	0.32	—	0.32	—
m-Nitrophenol	0.63	0.40	0.20	—	0.49
p-Nitrophenol	—	0.47	—	—	—
o-Xylenol	0.55	0.51	—	—	—
Octylphenol	0.79	—	—	—	—
Nonylphenol	0.76	0.56	0.50	0.59	—
Dodecylphenol	0.80	—	—	0.69	0.80
Cetylphenol	0.80	—	—	0.70	0.83
o-*sec.*-Butylphenol	0.69	0.51	0.40	—	—
o-Octyl-*o*-cresol	0.75	0.55	0.45	—	—
o-tert.-Butyl-*o*-cresol	0.70	0.56	0.47	—	—
p-*tert.*-Butyl-*o*-cresol	0.73	0.56	—	—	—
p-*tert.*-Octylphenol	0.70	0.57	0.48	—	—
o-*tert.*-Butyl-*p*-cresol	—	0.52	—	—	—
4,6-Di-*tert.*-butyl-*o*-cresol	—	0.48	—	—	—
1-Naphthol	—	—	0.23	0.38	0.59

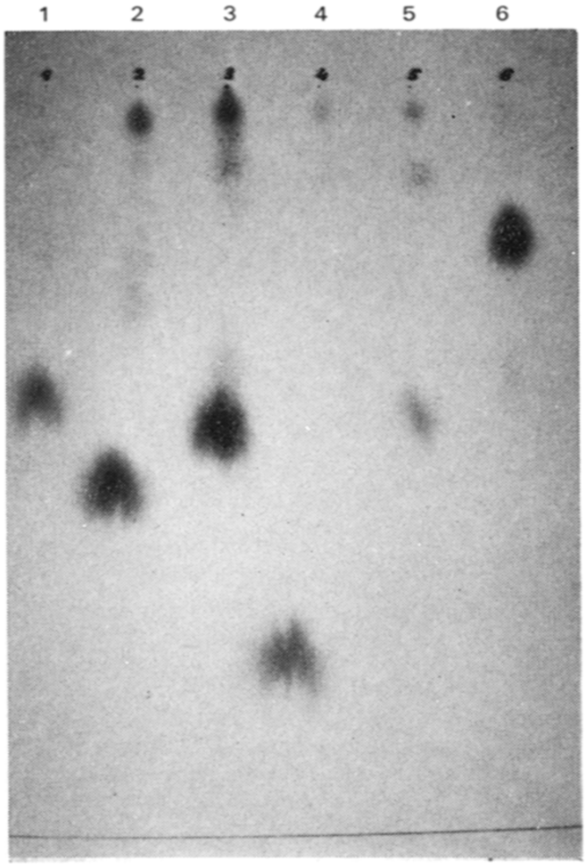
RESULTS AND DISCUSSION

p-(N,N-Dimethylaminobenzene)-*p'*-azobenzoyl chloride proved to be a suitable reagent for the chromatographic separation and identification of many of the phenols. The advantages of this reagent are that the corresponding esters are coloured, so that no detection reagent is needed. The preparations of both the reagent and the corresponding esters are simple. Some monovalent phenols have given esters after heating for 1 h. After cooling the reaction mixture and adding sodium hydrogen carbonate, esters have been isolated. Derivatives with constant melting points have been obtained by recrystallization. For the preparation of esters, dioxane, benzene, toluene, xylene, pyridine, dimethylaniline or their mixtures can be used as solvents.

We failed only to prepare the derivatives of phenols with bulky groups in the *ortho* position to the hydroxyl group. The difficulty in the reaction of these substances with the reagent is apparently caused by steric hindrance.

Of the chromatographic systems examined for PC, the systems with dimethylformamide (S_1–S_3) as the stationary phase proved to be the most convenient (Figs. 1 and 2). For the simple alkylphenols, the inverse system, with paraffin oil as the stationary phase, works well, for example S_5 where the R_M values are linearly dependent on the number of the carbon atoms in the alkyl group. In TLC a developing system must be chosen according to the esters present in the mixture (Figs. 3 and 4).

The method used until now for the separation of phenols in their free form and their subsequent detection by means of iron (III) hexacyanoferrate is reliable, but it does not allow the movement of spots during the development to be followed.



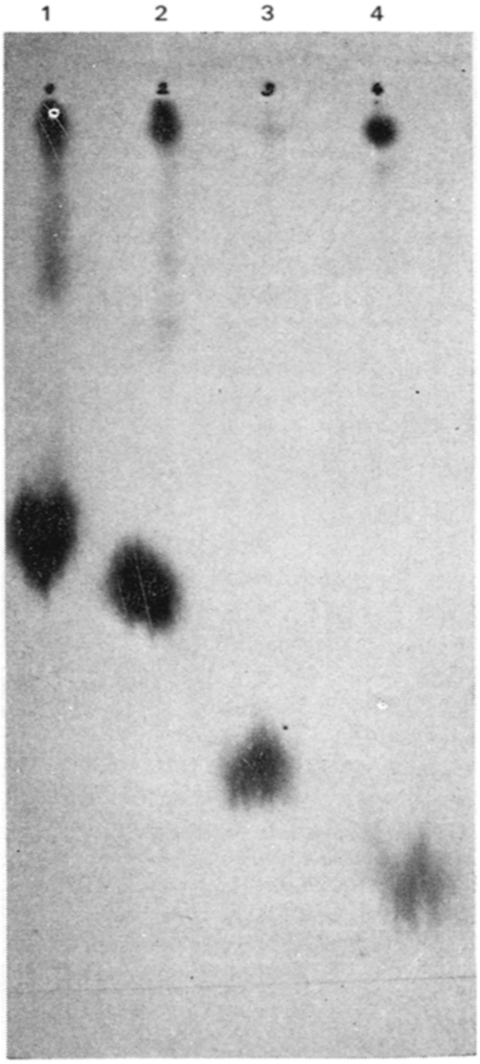
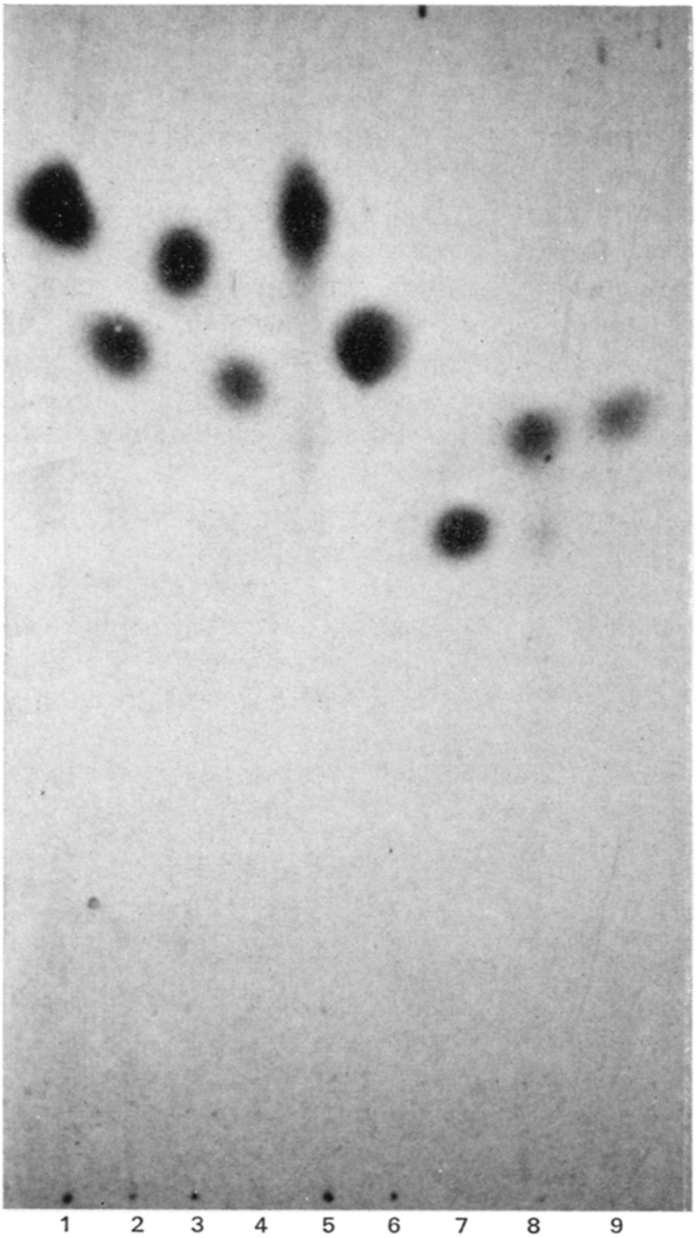


Fig. 1. Separation of *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates of phenols. System: 45% dimethylformamide/cyclohexane–benzene (25:1); Whatman No. 1. paper. 1 = Standard (phenol); 2 = *m*-cresol; 3 = α-naphthol; 4 = *o-sec.*-butylphenol; 5 = technical phenol; 6 = *o*-nitrophenol.

Fig. 2. Separation of *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates of phenols. System: 45% dimethylformamide/cyclohexane–benzene (25:1); Whatman No. 1 paper. 1 = *o*-Cresol; 2 = *m*-cresol; 3 = *o-sec.*-butylphenol; 4 = *o*-octyl-*o*-cresol.



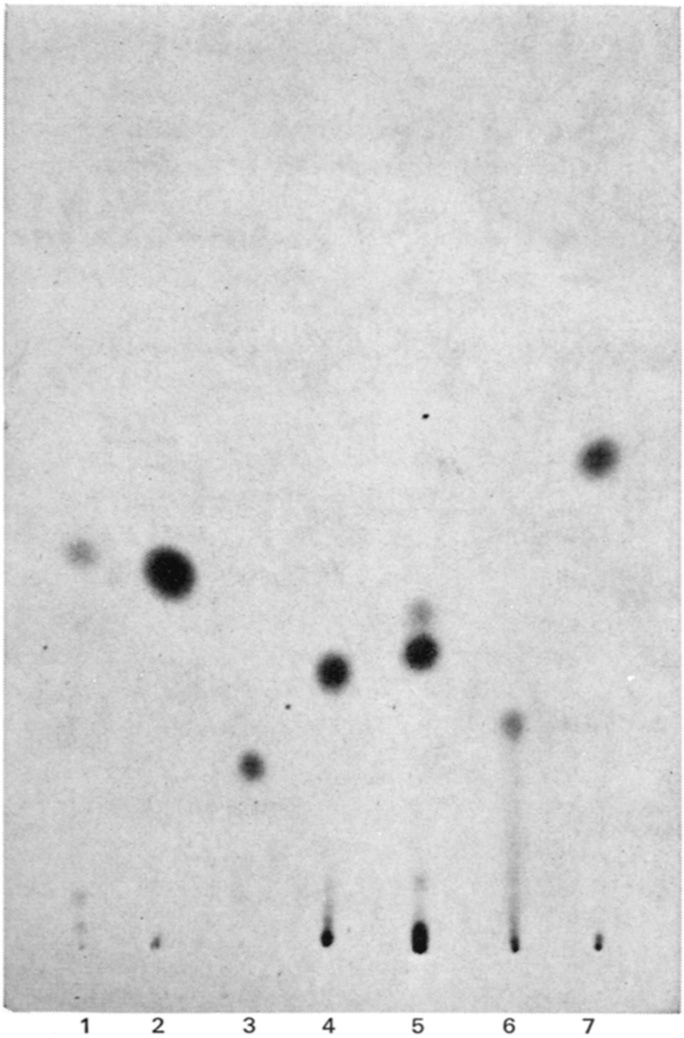


Fig. 3. Separation of *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates of phenols. System: thin layer of Silica Gel CH; cyclohexane–methyl ethyl ketone (7:3). 1 = *o*-Decylphenol; 2 = *m*-cresol; 3 = nonylphenol; 4 = *o*-cresol; 5 = *p*-cetylphenol; 6 = *o*-*sec*.-butylphenol; 7 = *o*-nitrophenol; 8 = *m*-nitrophenol; 9 = *p*-methoxyphenol.

Fig. 4. Separation of *p*-(N,N-dimethylamino)benzene *p'*-azobenzoates of phenols. System: thin layer of Kieselgel G; hexane–ethyl acetate (4:1). 1 = Phenol; 2 = *o*-cresol; 3 = *p*-methoxyphenol; 4 = α-naphthol; 5 = *o*-chlorophenol; 6 = *m*-nitrophenol; 7 = *o*-*sec*.-butylphenol.

Coloured derivatives enable a substantial shortening of the process of separation to be achieved in those cases when a few phenols only should be separated. The sensitivity of the method is very high — less than 0.5 μg of esters can be detected in a spot.

A great advantage of this method of separating coloured esters of phenols is the possibility of their quantitative analysis. With the low concentrations being dealt with in chromatography, the Lambert–Beer law is effective, and consequently the determination can be carried out photometrically either *in situ* or after elution from the chromatogram.

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