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SEPARATION AND IDENTIFICATION OF ALCOHOLS AS N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOATES BY PAPER AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Crystalline esters of alcohols were prepared by means of N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride as reagent, and suitable conditions were found for the separation of these esters by means of paper and thin-layer chromatography. A detection reagent can be dispensed with as N,N-dimethyl-*p*-aminobenzeneazobenzoates are highly coloured substances. Less than 0.5 μg can be determined in a spot.

INTRODUCTION

For the identification and separation of low and medium molecular weight alcohols by paper (PC) and thin-layer (TLC) chromatography, esters of benzoic acid[1–8], nitrated or substituted in different ways, are used most frequently. For the identification of low molecular weight alcohols, 2,4-dinitrobenzyl bromide can be used as the agent for etherification[9]. N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride, because of its reactivity and detectability, has also proved to be very suitable for the preparation of esters. Its preparation is relatively simple, and the synthesis of the esters is rapid. The esters obtained are crystalline, bright red substances with sharp characteristic melting points which can be used for their identification by classical methods[10].

EXPERIMENTAL

Preparation of N,N-dimethyl-p-aminobenzeneazobenzoates

In contrast to the classical identification procedure where perfectly pure esters are required, the impure reaction mixture can be applied directly to the paper or thin layer for identification by chromatography. The preparation of esters is, therefore, greatly simplified.

1 mg of N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride, 2–5 μl of alcohol, 1 drop of pyridine and 0.3–0.5 ml of benzene are placed in a micro test tube. The mixture is heated to dissolve the esterification reagent, and then kept boiling for an appropriate time. In medium and high molecular weight alcohols, the reaction mix-

ture is heated for several minutes or the micro test tube is sealed and placed in a boiling water bath for 5–10 min. Immediately after cooling, the reaction mixture can be applied to the chromatogram without further purification. The high reactivity of the reagent, even without addition of pyridine, is very evident in the preparation of esters of low molecular weight alcohols when merely mixing the given components without heating produces a sufficient quantity of ester within seconds.

Paper chromatography

Chromatography was carried out on Whatman No. 2 paper and under various experimental conditions. Mobile phases were saturated with a stationary phase in all cases. The paper was impregnated by passing it through a solution of the stationary phase. The paper was allowed to hang for about 20 sec with the start end uppermost, the drops of surplus solution were wiped off with filter paper, and the paper was then hung with the start end downwards until the solvent had evaporated completely. In this way the stationary phase was distributed more uniformly throughout the length of the paper. The time for drying the paper after impregnation and for the application of the esters to the start is arranged so that the chromatogram can be placed in the chromatography chamber, previously saturated with vapours of the solvents used, in the 15th minute after beginning the impregnation. These working conditions must be strictly observed in order to achieve good reproducibility of the R_F values; this applies in other cases as well, but especially when working with dimethylformamide[11].

For chromatographic separation of esters a whole series of combinations of polar stationary phases was tested with non-polar solvents in mobile phases, and so were several reversed-phase combinations. The following systems were found most suitable:

S_1: a 60% solution of dimethylformamide in methanol/petroleum (boiling range 60–75°)

TABLE I

R_F AND R_M VALUES OBTAINED BY PC OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOATES OF ALIPHATIC C_1–C_{10} ALCOHOLS

Alcohol	*System*							
	S_1		S_2		S_3		S_4	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
Methyl	0.31	0.35	0.48	0.04	0.82	−0.66		
Ethyl.	0.45	0.09	0.61	−0.19	0.76	−0.50		
Propyl	0.57	−0.12	0.68	−0.33	0.65	−0.27		
Isopropyl	0.55				0.67			
Butyl	0.68	−0.33	0.75	−0.48	0.55	−0.09		
Isobutyl	0.65				0.58			
Amyl	0.78	−0.55	0.79	−0.58	0.43	0.12	0.67	−0.31
Isoamyl	0.75							
Hexyl			0.84	−0.72	0.32	0.33	0.57	−0.12
Heptyl					0.23[a]		0.47[a]	
Octyl			0.88	−0.87	0.16	0.72	0.38	0.21
Nonyl			0.89	−0.91	0.10	0.95	0.29	0.39
Decyl					0.07	1.13	0.22	0.55

[a] Calculated values.

S_2: a 20% solution of dimethylformamide in methanol/cyclohexane–benzene (10:1)

S_3: a 10% solution of paraffin oil in pentane/dimethylformamide–water (4:1)

S_4: a 10% solution of paraffin oil in pentane/dimethylformamide–methanol–water (8:2:1)

2–5 μl of a benzene or dimethylformamide solution of the ester was applied to the start; each sample contained 2–10 μg of ester.

The results are given in Table I and in Figs. 1–3.

The dependence of the R_M values on the number of C atoms in the alcohol molecule is demonstrated in Fig. 4.

Thin-layer chromatography

Silica Gel G thin layers were used for the separation of the esters; in some cases they were impregnated with a stationary phase. The plates (12 × 22 cm) were activated for 30 min at 120°.

The impregnation was made in a chromatography chamber by allowing a solution of the stationary phase to ascend the plate by capillary action. The volatile solvent was then allowed to evaporate into the air from the layer at laboratory temperature. In all cases, and especially when working with an impregnated layer, it is necessary to ensure that the chromatography chamber is completely saturated with developing solvent vapours. The esters were applied to the plate in the form of benzene solutions of the same concentration as indicated in PC. The following solvent systems were used:

S_5: a 40% solution of dimethylformamide in methane/cyclohexane–benzene (25:1)

S_6: a 10% solution of paraffin oil in pentane/dimethylformamide–water (4:1)

S_7: a 10% solution of paraffin oil in pentane/dimethylformamide–methanol–water (4:1:1)

S_8: a 10% solution of paraffin oil in pentane/dimethylformamide–methanol–water (8:2:1)

S_9: a 10% solution of paraffin oil in pentane/dimethylformamide–water (3:1)

S_{10}: cyclohexane–ethyl acetate (4:1)

S_{11}: hexane–ethyl acetate (4:1)

S_{12}: cyclohexane–methyl ethyl ketone (4:1)

The results are shown in Table II and Fig. 5.

Detection

As N,N-dimethyl-*p*-aminobenzeneazobenzoates are coloured substances, a special detection reagent is not necessary. However, when only low concentrations of sample have been applied to the paper or layer, the intensity of spots can be increased by spraying the chromatogram with 0.01 *N* H_2SO_4.

Sensitivity determination

The following amounts of the esters of C_2 and C_9 alcohols were gradually applied to the paper: 0.2; 0.4; 2; 4 μg and the limit of detection was determined. The development and the method of increasing the detection sensitivity were as described above.

TABLE II

R_F AND R_M VALUES OBTAINED BY TLC OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOATES

Alcohol	*System*															
	S_5		S_6		S_7		S_8		S_9		S_{10}		S_{11}		S_{12}	
	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
Methyl	0.32	+0.33			0.80	−0.60					0 42	+0.14	0.33	+0.31		
Ethyl	0 38	+0.21	0 82	−0 66	0.79	−0.58					0 47	+0.05	0.37	+0.23		
Propyl	0 44	+0.11	0 74	−0.45	0 74	−0.45					0.50	0.00	0.41	+0.16		
Butyl	0.51	−0.02	0 64	−0.25	0 68	−0.33					0.55	−0.09	0.44	+0.11		
Amyl	0.59	−0.16	0 53	−0.05	0.61	−0.19					0.58	−0.14	0.50	0 00		
Hexyl	0.67	−0.31	0 42	+0.14	0.54	−0.07					0.61	−0.19	0.60	−0.18		
Octyl							0 66	−0 29								
Nonyl							0.59	−0 16								
Decyl							0.53	−0.05								
Lauryl									0.45	+0 09					0 73	−0.43
Cetyl									0.41	+0 16					0.80	−0.60
Benzyl									0.64	−0.25					0 46	+0 07
Phenyl ethyl									0.63	−0.23					0 47	+0 05
Anisyl									0 70	−0.37					0.38	+0 21
Cinnamyl									0 61	−0.19					0 48	+0.04

RESULTS AND DISCUSSION

The chromatographic behaviour of N,N-dimethyl-*p*-aminobenzeneazobenzoates of aliphatic alcohols is similar to that of other esters of substituted benzoic acids. The whole series of esters of the C_1–C_{10} alcohols cannot be completely separated in a single solvent system; that ideal state is most nearly approached by the separation in system S_3 (10% solution of paraffin oil/dimethylformamide–water, 4:1) on Whatman No. 2 paper (Fig. 1), where all the esters are separated well except the lowest and highest homologues, *i.e.* the esters of the C_1 and C_2 alcohols and C_9 and C_{10} alcohols are not well separated. If a good separation of the methyl alcohol and ethyl alcohol

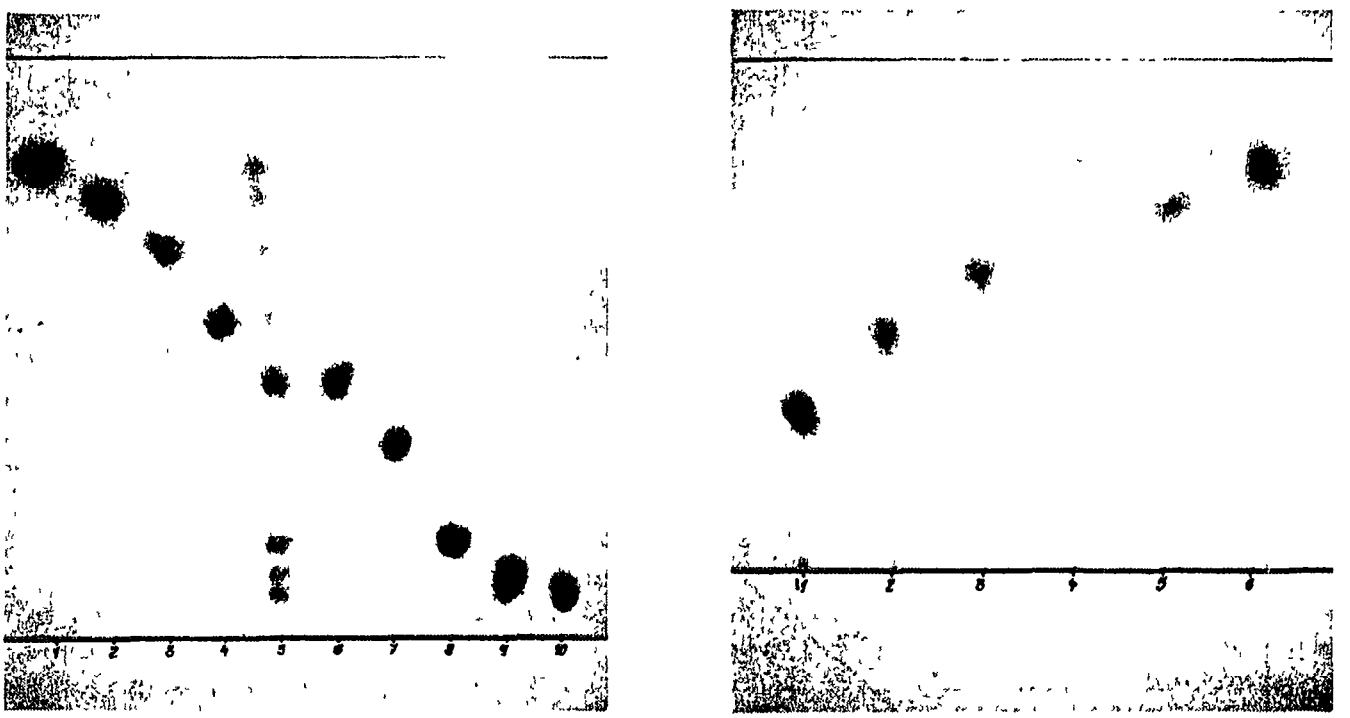

Fig 1 System, S_3; Whatman No. 2 paper. N,N-Dimethyl-*p*-aminobenzeneazobenzoates of alcohols 1 = methyl, 2 = ethyl, 3 = *n*-propyl, 4 = *n*-butyl, 5 = mixture C_1–C_{10}, 6 = *n*-amyl, 7 = *n*-hexyl, 8 = *n*-octyl; 9 = *n*-nonyl, 10 = *n*-decyl.

Fig 2. System, S_1, Whatman No 2 paper N,N-Dimethyl-*p*-aminobenzeneazobenzoates of alcohols 1 = methyl, 2 = ethyl, 3 = *n*-propyl, 4 = mixture C_1–C_5, 5 = *n*-butyl, 6 = *n*-amyl.

esters is of importance, the S_1 (Fig. 2) and S_2 systems are the most suitable for paper, and the S_5, S_{11}, or S_{10} systems for thin layers. If we want to separate the higher alcohols completely, the most suitable system for paper (Fig. 3) is the S_4 system: paraffin oil/dimethylformamide–methanol–water (8:2:1), or for thin layers, the S_9 system: paraffin oil/dimethylformamide–water (3:1), and S_{10} or S_{12} system. From the course of the separation on paper (Figs. 1 and 3) it is evident that an increase in the amount of dimethylformamide in the mobile phase would make a separation of esters of alcohols higher than C_{10} possible. If pure dimethylformamide is used as the mobile phase, the hexylalcohol ester moves practically with the front.

In all the systems mentioned a linear dependence was found between R_M values

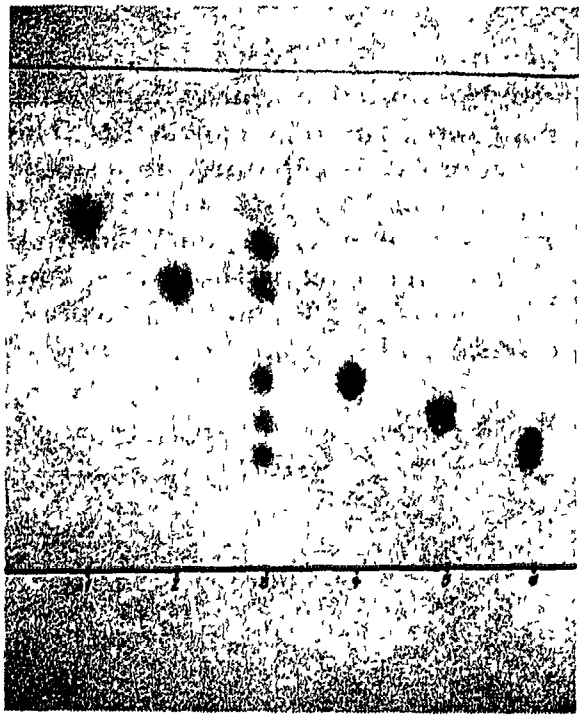

Fig. 3. System, S_4; Whatman No. 2 paper. N,N-Dimethyl-*p*-aminobenzeneazobenzoates of alcohols: 1 = *n*-amyl; 2 = *n*-hexyl; 3 = mixture C_5–C_{10}, 4 = *n*-octyl, 5 = *n*-nonyl; 6 = *n*-decyl.

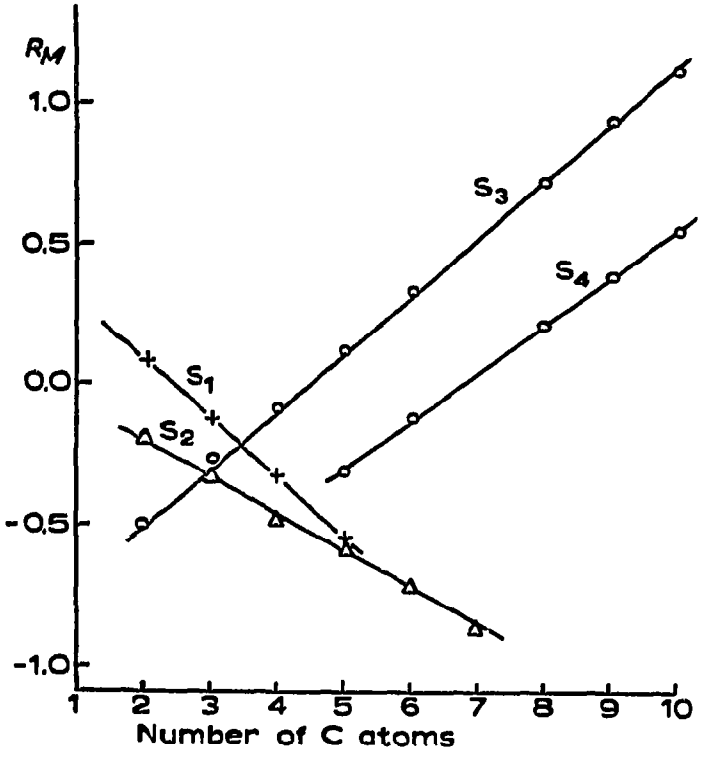

Fig. 4. Dependence between R_M values and the number of carbon atoms in the alcohol. R_M values obtained by PC of the esters

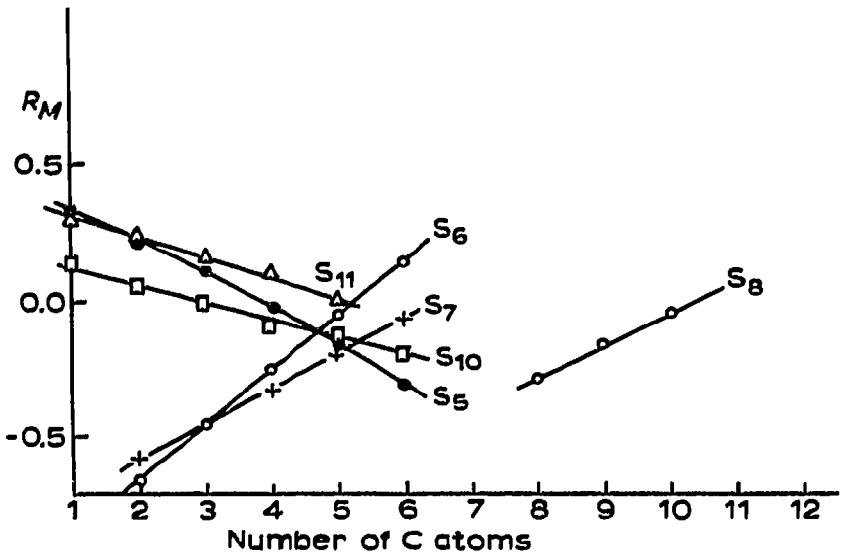

Fig. 5. Dependence between R_M values and the number of carbon atoms in the alcohol. R_M values obtained by TLC of the esters.

and the number of carbons in the alcohol molecule (Figs. 4 and 5). It is thus possible to identify the alcohols on this basis. The R_F values of heptyl N,N-dimethyl-*p*-aminobenzeneazobenzoate was calculated from this relationship, as the substance was not available (see Table I). In fair agreement with previous experience, isomeric alcohols or respectively their esters, were not separated, and were therefore not identifiable in this way.

The reagent, N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride, does not interfere with the identification in either its normal or hydrolysed form.

The sensitivity of the method described is high, and can be increased by spraying the chromatogram with 0.01 *N* sulphuric acid. From the series of known quantities of esters of ethyl and nonyl alcohol, in the range of 0.2–4 μg, it is evident that as little as 0.4 μg of ester in a spot can be easily detected. With the nonyl alcohol, resp. its ester, as little as 0.2 μg in a spot can be seen. Two alcohols differing by one carbon atom can be identified using this method, even in the ratio of 1:1000. However, the ester of the alcohol occurring as the insignificant quantity should have a higher R_F value than the alcohol in excess; this can easily be arranged with the solvent systems given.

EVALUATION OF THE METHOD

The method most used for the chromatographic identification of alcohols is their separation in the form of 3,5-dinitrobenzoates. This method, described below, was therefore chosen for comparison with the results obtained in the present work.

If we examine the various stages of both methods, the advantages and disadvantages pertaining to each operation can be evaluated.

The reagent and its preparation

The stability of 3,5-dinitrobenzoyl chloride as exposed to the air in a reagent

bottle with a ground glass stopper is rather low, and on storage under these conditions it rapidly loses its efficiency. Immediately after the preparation it should be distributed in small quantities into ampoules, and sealed. On the other hand, N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride is very stable. Even after long-term storage (several weeks) in an open vessel in the laboratory atmosphere no change in its reactivity was observed.

While the preparation of 3,5-dinitrobenzoyl chloride, and especially, its purification by means of distillation is very difficult, and even risky, the preparation of N,N-dimethyl-*p*-aminobenzoyl chloride is relatively simple[10].

Ester preparation

The use of a coloured reagent for the preparation of esters for the chromatographic microidentification of alcohols is advantageous. In the work described, long heating in sealed ampoules is unnecessary. In the lower and medium molecular weight alcohols the reaction rate is fairly high, and it is sufficient to heat the reaction mixture for the preparation of the respective esters for only a relatively short time. The presence of a small quantity of water in the reaction mixture is harmless, so that lower alcohols can also be identified in aqueous solutions.

Chromatography

When both the methods are compared from the viewpoint of the chromatographic separation both on paper and on thin layers, it was found that the behaviour of both the groups of esters is about the same. A great advantage of the new derivatives, however, is their colour, making a detection reagent unnecessary. The sensitivity of the new method is slightly higher. If we consider that 0.4 μg of ester can be easily seen, it means that the concentration of the alcohol concerned can be approximately five times lower. Coupled with the sensitivity is also the possibility of identifying very low quantities of one alcohol in admixture with a much higher concentration of a neighbouring homologue, even in a ratio as high as 1:1,000.

Time taken for the analysis

In the case of a micropreparation, the time for the identification of the alcohols according to the method described is considerably lower than that with the method using 3,5-dinitrobenzoyl chloride. The time consumption for coloured ester prepa rations is neglibgile in comparison with 3,5-dinitrobenzoates. The time for the identification of the alcohols is substantially reduced in the case of the coloured esters; with 3,5-dinitrobenzoates it takes a considerable time. Another advantage of the coloured esters is the possibility of a substantial reduction of chromatogram development time owing to the fact that the separation process is plainly visible.

Possibility of quantitative analysis

As the esters of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid are essentially azodyes, at low concentrations it should be possible to carry out quantitative chromatographic analysis, providing the colour–concentration relation obeys the Lambert–Beer law. This problem is now being investigated.

If we consider all the known methods of separation and identification of alcohols by means of paper and thin-layer chromatography, we come to the con-

clusion that the newly suggested reagent, N,N-dimethyl-*p*-aminobenzeneazobenzoyl chloride has a number of advantages over all the methods used hitherto. From preliminary tests, its high reactivity can also be used for the chromatographic separation and identification of other classes of substances, such as glycols, cellosolves, phenols, amines, etc., where it reacts with the hydroxy or amino groups.

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