CHROM. 3469

IDENTIFICATION OF ORGANIC COMPOUNDS*

LXX. DETECTION AND DETERMINATION OF THE NUMBER OF HYDROXY GROUPS IN DISPERSE AZO DYES BY MEANS OF ACETYLATION AND ΔR_{Mr} VALUES

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SUMMARY

Acetylation of hydroxyazo dyes was shown to result in characteristic changes in the chromatographic behaviour of the compounds considered. These changes can be expressed quantitatively by means of ΔR_{Mr} values which are obtainable with satisfactory reproducibility, indicating not only the presence of a hydroxy group but also the number of them and their type. The whole procedure is quite simple and realisable in any laboratory. It can be used satisfactorily for structure elucidation of azo dyes.

During recent years the relationship between molecular structure and chromatographic behaviour has become an important tool in structure elucidation of organic compounds[1,2]. It is based on Martin's additivity principle which is valid only within a group of compounds of related structure and of the same inter- and intramolecular interactions[3]. ΔR_M values express quantitatively the changes in chromatographic behaviour of a compound caused by the introduction of a further functional group. When the changes in the molecules are caused by a chemical reaction and the chromatographic behaviour of the compound under investigation before and after the reaction is compared, use is made of ΔR_{Mr} values. These values, defined as:

$$R_{Mr} = R_{M''} - R_{M'}$$

where R_M' is the R_M value of the original compound, and R_M'' the R_M value of the reaction product, will be characteristic for each chemical change within an organic molecule. They will also be constant for other compounds of nearly similar chemical structure treated by the same reaction, provided that the same chemical changes occurred and the same inter- and intramolecular interactions are involved in the resulting compounds.

The acetylation of hydroxy compounds, followed by chromatographic inves-

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tigation of the original hydroxy compound and the resulting acetylation product has been shown to permit the determination of the number of hydroxy groups in steroids[4] or the simple phenolic compounds and sugars[5]. The changes in chromatographic behaviour caused by acetylation of the free hydroxy groups in steroid compounds are, however, in many cases so significant that the resulting compounds must be chromatographed in a different solvent system from the original compound, and a further correction (ΔR_{Ms}) is necessary to express the change caused by the use of another solvent system. In the case of simple phenols[5], no quantitative correlations have been made. ΔR_{Mr} values for conversions of certain functional groups in C_{19}-steroids to several types of derivatives have been reported recently[6,7].

The purpose of this paper is to describe the results obtained by attempting to use the acetylation reaction and ΔR_{Mr} values for the detection and determination of the number of hydroxy groups in azo dyes, especially those insoluble in water but soluble in organic solvents.

EXPERIMENTAL

Azo dyes

The azo dyes used in the course of our investigation were substances from our collection of model chromatographically pure dyes. For the most part they have been described in one of our previous communications[3].

Acetylation

Reagent. This is prepared by mixing 20 ml freshly distilled pyridine, 0.35 ml perchloric acid and 6.7 ml freshly distilled acetic anhydride. The reagent was prepared daily.

Procedure for partial acetylation. 50 mg of the dye under examination were mixed with 0.1 ml of the acetylation reagent and kept at room temperature overnight or at 40° for 30 min. Then the mixture was diluted with three volumes of water and the resulting oil or precipitate separated, washed twice with water and dissolved in 0.5 ml benzene. 5 μl of the benzene layer were applied to the chromatogram.

Procedure for complete acetylation. 50 mg of the azo dye and 0.2 ml of the reagent were treated as described above.

Procedure for arylazo-2-naphthol derivatives. 50 mg of the corresponding dye were mixed with 2 ml reagent and treated as described above.

Hydrolysis of the dye 4-nitro-2-chloroaniline —→ N-cyanoethyl-N-acetyloxyethylaniline. 0.5 g of the dye were suspended in a mixture of 5 ml conc. hydrochloric acid, 80 ml methanol and 20 ml water and boiled for 30 min. After neutralization the precipitate thus formed was dissolved in pyridine and applied to the chromatogram.

Paper chromatography

The method described previously[3,8] using Whatman paper No. 3MM impregnated with a 10% solution of 1-bromonaphthalene in chloroform was used with a pyridine–water (2:1) mixture saturated with 1-bromonaphthalene as mobile phase.

The R_F values were measured against an internal standard and are summarized in Table I. R_M values were calculated in accordance with BATE-SMITH AND WESTALL's[9] equation.

TABLE I

R_F AND R_M VALUES OF AZO DYES AND THEIR ACETYL DERIVATIVES

No.	*Azo compound*[a]	*Colour*[b]	R_F	R_M	ΔR_M	
I	2,6-Dichloro-4-nitroaniline ⟶ N-methyl-N-hydroxyethylaniline	A Br B BrOr	0.34 0.19	+ 0.288 + 0.630	+ 0.342	
II	4-Nitroaniline ⟶ N-ethyl-N-hydroxyethylaniline	A ROr B Or	0.45 0.26	+ 0.087 + 0.454	+ 0.367	
III	4-Nitroaniline ⟶ N-hydroxyethyl-N-cyanoethylaniline	A ROr B Or	0.58 0.40	— 0.140 + 0.176	+ 0.316	
IV	4-Nitroaniline ⟶ N-hydroxyethyl-N-cyanoethyl-*m*-toluidine	A R B ROr	0.53 0.36	— 0.052 + 0.250	+ 0.302	
V	4-Nitroaniline ⟶ N,N-bishydroxyethyl-*m*-chloroaniline	A brick R B brick Or C Or	0.61 0.42 0.26	— 0.194 + 0.140 + 0.454	+ 0.334 + 0.314	+ 0.64
VI	2-Methoxyaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A Go B GoY C Y	0.84 0.67 0.49	— 0.721 — 0.308 + 0.017	+ 0.413 + 0.325	+ 0.73
VII	4-Nitroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A dark R B brick R C brick R	0.65 0.45 0.25	— 0.269 + 0.087 + 0.477	+ 0.356 + 0.390	+ 0.74
VIII	4-Chloro-2-nitroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A OrR B OrR C Or	0.61 0.41 0.23	— 0.194 + 0.158 + 0.528	+ 0.352 + 0.370	+ 0.72
IX	2-Chloroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A Go B Go C Y	0.71 0.51 0.32	— 0.389 — 0.017 + 0.327	+ 0.372 + 0.344	+ 0.71
X	4-Chloroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A Go B Go C Y	0.66 0.48 0.32	— 0.288 + 0.035 + 0.327	+ 0.323 + 0.292	+ 0.61
XI	2-Methyl-5-nitroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A YOr B Go C YGo	0.65 0.46 0.31	— 0.269 + 0.070 + 0.347	+ 0.339 + 0.277	+ 0.616
XII	2-Methyl-5-chloroaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A Go B Y C Y	0.65 0.46 0.28	— 0.269 + 0.070 + 0.411	+ 0.339 + 0.341	+ 0.680
XIII	2-Nitro-4-methoxyaniline ⟶ N,N-bishydroxyethyl-*m*-toluidine	A Go B Y C Y	0.74 0.58 0.35	— 0.456 — 0.140 + 0.269	+ 0.316 + 0.409	+ 0.725
XIV	Aniline ⟶ phenol	A Y B Y	0.55 0.45	— 0.087 + 0.087	+ 0.174	
XV	*m*-Nitroaniline ⟶ phenol	A Y B OrY	0.42 0.31	+ 0.140 + 0.347	+ 0.207	
XVI	*p*-Toluidine ⟶ phenol	A Y B dull Y	0.47 0.40	+ 0.052 + 0.176	+ 0.124	
XVII	Aniline ⟶ 2-methoxy-5-methylaniline ⟶ phenol	A OrBr B OrBr	0.30 0.21	+ 0.368 + 0.575	+ 0.207	
XVIII	*p*-Chloroaniline ⟶ phenol	A Y B Or	0.40 0.29	+ 0.176 + 0.389	+ 0.213	

(*continued on p. 365*)

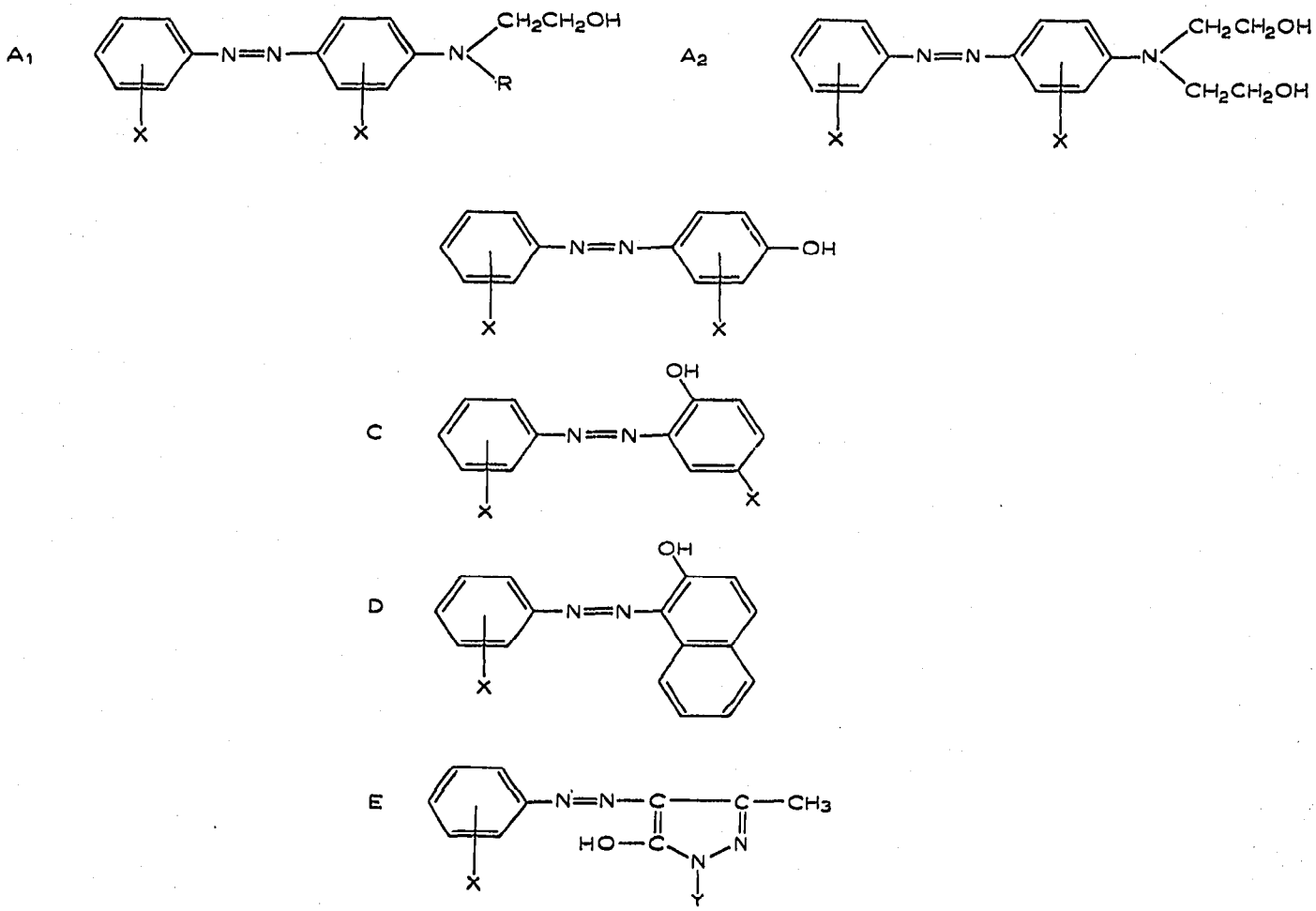
TABLE I (*continued*)

No.	*Azo compound*[a]	*Colour*[b]	R_F	R_M	ΔR_M
XIX	Aniline ⟶ *o*-cresol	A Y B Or	0.49 0.42	+ 0.017 + 0.140	+ 0.123
XX	1-Naphthylamine ⟶ *o*-cresol	A Y B Or	0.37 0.30	+ 0.231 + 0.368	+ 0.137
XXI	Aniline ⟶ *o*-chlorophenol	A Y B YOr	0.52 0.43	— 0.034 + 0.122	+ 0.156
XXII	*p*-Aminoazobenzene ⟶ *o*-cresol	A YGo B Or	0.21 0.14	+ 0.575 + 0.790	+ 0.215
XXIII	Aniline ⟶ *m*-cresol	A Y B Or	0.50 0.38	0 + 0.212	+ 0.212
XXIV	Aniline ⟶ *m*-chlorophenol	A Y B Or	0.51 0.43	— 0.017 + 0.122	+ 0.139
XXV	1-Naphthylamine ⟶ *m*-cresol	A Y B OrY	0.39 0.27	+ 0.194 + 0.432	+ 0.238
XXVI	4,4'-Dihydroxyazobenzene	A Y B Y	0.67 0.59	— 0.308 — 0.158	+ 0.150
XXVII	*p*-Aminoazobenzene ⟶ *p*-cresol	A YOr B Or	0.12 0.19	+ 0.870 + 0.630	— 0.240
XXVIII	Aniline ⟶ *p*-cresol	A Y B Or	0.31 0.43	+ 0.347 + 0.122	— 0.225
XXIX	Aniline ⟶ 3,4-xylenol	A Y B Or	0.25 0.39	+ 0.477 + 0.194	— 0.283
XXX	1-Naphthylamine ⟶ *p*-cresol	A Y B Or	0.22 0.30	+ 0.549 + 0.368	— 0.181
XXXI	Aniline ⟶ 2-naphthol	A Or B OrY	0.28 0.39	+ 0.411 + 0.194	— 0.217
XXXII	2-Methoxy-4-chloroaniline ⟶ 2-naphthol	A R B dull Or	0.26 0.40	+ 0.454 + 0.176	— 0.278
XXXIII	4-Chloroaniline ⟶ 2-naphthol	A ROr B dull Or	0.25 0.34	+ 0.477 + 0.288	— 0.189
XXXIV	4-Methoxyaniline ⟶ 2-naphthol	A R B dull Or	0.32 0.47	+ 0.327 + 0.052	— 0.275
XXXV	2-Methyl-5-chloroaniline ⟶ 2-naphthol	A ROr B dull Or	0.21 0.30	+ 0.575 + 0.368	— 0.207
XXXVI	2,5-Dichloroaniline ⟶ 1-phenyl-3-methylpyrazolone-5	A Y B —	0.15 —	—	—
XXXVII	2-Chloro-4-nitroaniline ⟶ 1-phenyl-3-methylpyrazolone-5	A Y B —	0.65 —	—	—
XXXVIII	Ethyl *p*-aminobenzoate ⟶ 1-phenyl-3-methylpyrazolone-5	A Y B —	0.21 —	—	—

[a] A = original dye; B = acetyl derivative; C = diacetyl derivative.
[b] Br = brown; Go = golden; Or = orange; R = red; Y = yellow.

RESULTS AND DISCUSSION

The azo compounds investigated in this study involved the following types:



where R = H, CH_3, C_2H_5, CH_2CH_2CN etc., X = various substituents except SO_3H and COOH, and Y = H, phenyl etc.

As far as these compounds are soluble in organic solvents, they are easily chromatographed when reversed-phase systems are used, as described in our previous communications[3,8]. Our studies concerning the relationship between the chromatographic behaviour and molecular structure of these azo dyes confirmed that the correlation is strictly valid, provided that only compounds of the same inter- and intramolecular interactions are considered[3]. We also observed that there is a significant difference in the R_F values of the azo dyes derived from N-cyanoethyl-N-hydroxyethyl- and N-cyanoethyl-N-acetoxyethylaniline (type A_1) and this leads us to the conclusion that this difference could be taken advantage of for the detection of hydroxy groups by means of acetylation. The fact that both the hydroxy compound and the acetyl derivative were coloured and migrated satisfactorily in the same solvent system seemed to be an evident advantage.

Acetylation

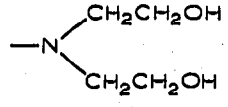
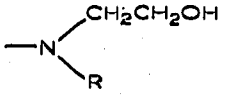
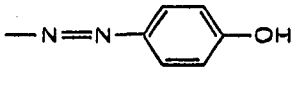
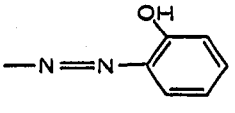
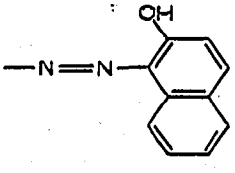
Acetylation in pyridine catalyzed by perchloric acid[10–12] proved to be the most convenient method. The reaction conditions could easily be modified so that complete or partial acetylation was obtained. In the latter case not only the completely acetylated compound but also the original hydroxy compound are present in the reaction mixture on the chromatogram. When more than one hydroxy group is present in the dye molecule under investigation, spots corresponding to all acetylation stages appear. Thus the R_F values of all reaction components can be measured under the same conditions. This is important since the presence of the reagent on the starting point of the chromatogram might have some influence on the R_F values. Furthermore acetylation also results in characteristic colour changes (see Table I). The acetylation rate was found to be influenced by the type of the azo dye in some cases, and also the solubility of some arylazo-2-naphthol dyes (type D) and pyrazolone derivatives (type E) sometimes made it necessary to use an excess of the reagent.

R_{Mr} values

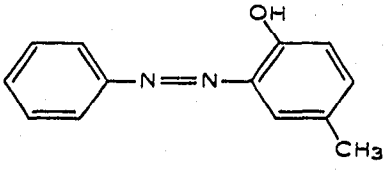
The results obtained are summarized in Table I. Thirteen dyes of type A, thirteen of type B, four of type C, five of type D and three of type E were examined. The average values for each type of the hydroxy groups can be found in Table II. It is evident that the ΔR_{Mr} values are considerably affected by the type of the hydroxy group, *i.e.* by the rest of the molecule, but that they are sufficiently reproduc-

TABLE II

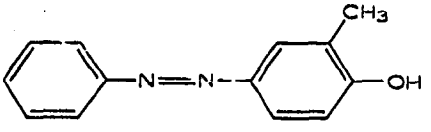
AVERAGE ΔR_{Mr} VALUES FOR DIFFERENT TYPES OF HYDROXY GROUPS

Type	ΔR_{Mr}	
	+ 0.349 + 0.340	+ 0.689
		+ 0.332
		+ 0.176
		− 0.232
		− 0.233

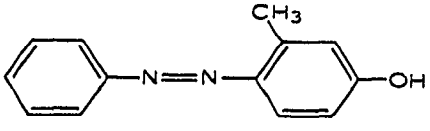
ible within each group of dyes. In the case of the aliphatic hydroxy groups in type A dyes and the phenolic *p*-hydroxy groups in type B dyes, acetylation diminishes the hydrophilic character of the hydroxy groups which results in the decrease of the R_F values. In type A_2 dyes, the contribution of both hydroxy groups seems to be equivalent. In *o*-hydroxyazo dyes either of the phenol (type C) or naphthol (type D) series, however, the R_F values increase after acetylation. The chromatographic behaviour of *o*-hydroxyazo dyes is influenced by the strong intramolecular bonding. These dyes therefore migrate more slowly than the corresponding *p*-hydroxyazo dyes on chromatograms with non-polar stationary phases. The acetyl derivatives of both dyes, however, do not differ in their intramolecular interactions and their chromatographic behaviour is much closer. This can be demonstrated using compounds XIX, XXIII and XXVIII as model substances.

(XXVIII) 

$R_{F_{OH}} = 0.31 \quad R_{F_{acetyl}} = 0.43$

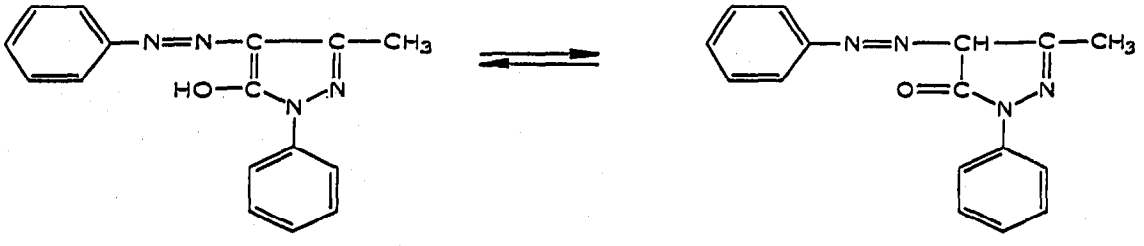
(XIX) 

$R_{F_{OH}} = 0.49 \quad R_{F_{acetyl}} = 0.42$

(XXIII) 

$R_{F_{OH}} = 0.50 \quad R_{F_{acetyl}} = 0.38$

No positive results have been obtained with pyrazolone dyes (Type E). This may be due to the fact that under the reaction conditions these dyes are present in their keto form.



The free amino and alkylamino groups might have been expected to cause some interference but none was observed in the case of the alkylamino groups. Free amino groups undergo the acetylation reaction and the ΔR_{Mr} values obtained are characteristic. This problem will be discussed in a further communication. In the same way acetoxyazo dyes can be hydrolyzed to the corresponding hydroxyazo

dyes and the process followed by chromatography and the ΔR_{Mr} values calculated. The ΔR_{Mr} values thus obtained correspond to the ΔR_{Mr} values for acetylation and can be used to confirm the presence of O-acetyl groups.

The procedure recommended in this communication has been used in structure elucidation of azo dyes. It is quite simple and realisable in any laboratory.

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