

PAPER PARTITION CHROMATOGRAPHY OF ALKYL-SUBSTITUTED
1-PHENYLAZO-2-NAPHTHOLS

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Although paper chromatography of water-soluble dyes has received a considerable amount of attention, very little has been published to date on the application of this technique to problems of separation and identification of oil-soluble dyes. The first use of paper chromatography for oil-soluble dye mixtures was reported by THALER AND SCHELER¹, who separated two simple dye mixtures on untreated paper. Separations of more complex dye mixtures by paper chromatography, again on untreated paper, have been reported by JAX AND AUST² and by FUJII³ using a variety of solvent mixtures for development. The experiments of THALER AND SCHELER¹ and those of JAX AND AUST², using untreated paper, were repeated by VERMA AND DASS⁴ and by MARK AND McKEOWN⁵ and in each case it was reported that poor resolution was obtained due to a considerable amount of tailing of the spots. The use of reversed-phase paper chromatography would be expected to lead to improved resolution of mixtures of oil-soluble dyes, and indeed, LINDBERG⁶, VERMA AND DASS⁴, and MARK AND McKEOWN⁵ all have reported excellent separations by using paper impregnated with a variety of lipophilic substances and development with a number of aqueous solutions of organic solvents.

This paper is concerned with the reversed-phase paper chromatographic behaviour of a series of alkyl-substituted 1-phenylazo-2-naphthols, all of which are oil-soluble and either isomers or homologues of one another. The specific compounds chosen for study were 1-phenylazo-2-naphthol, all three isomers of 1-(methylphenylazo)-2-naphthol, all six isomeric 1-(dimethylphenylazo)-2-naphthols, 1-(2-ethylphenylazo)-2-naphthol, 1-(2,4,5-trimethylphenylazo)-2-naphthol, and 1-(2,4,6-trimethylphenylazo)-2-naphthol. Data reported previously by other workers⁴⁻⁶ on the reversed-phase paper chromatography of two homologous series of dyes indicate that the R_F values of the dyes within each series differ sufficiently to allow at least the lower members of each series to be separated from one another. One of these groups of dyes consisted of commercial samples of Sudan I, Orange SS, and Oil Red XO which are mainly 1-phenylazo-2-naphthol, 1-(2-methylphenylazo)-2-naphthol, and a mixture of 1-(2,4-dimethylphenylazo)-2-naphthol and 1-(2,5-dimethylphenylazo)-2-naphthol respectively; these compounds have been included in the work reported here. The other group of homologous dyes was comprised of commercial

samples of Oil Yellow AB and Oil Yellow OB which are respectively 1-phenylazo-2-naphthylamine and 1-(2-methylphenylazo)-2-naphthylamine. Although Oil Red XO is known to be a mixture of two isomeric dyes, resolution of this mixture was not reported in the work referred to above. One might conclude from this that the difference in R_F values of isomeric dyes that differ only in the relative positions of methyl groups is too small to permit their separation; however, it has been observed in this laboratory⁷ that resolution of the two isomeric dyes comprising Oil Red XO could be accomplished under appropriate conditions of development. Thus the most interesting aspect of the work reported here is the chromatographic behaviour of the isomeric dyes within the group of compounds which were chosen for study.

EXPERIMENTAL

I. Amine intermediates

The following amines were purchased from Eastman Kodak Company, Rochester, N.Y., and were used without further purification.

2-Methylamiline. Reagent grade, Cat. No. 253; b.p. 83–85°/15 mm.

3-Methylamiline. Reagent grade, Cat. No. 862; b.p. 92–93°/15 mm.

4-Methylamiline. Reagent grade, Cat. No. 254; m.p. 43–45°.

2-Ethylamiline. Practical grade, Cat. No. P3066; b.p. 90–92°/8 mm.

2,3-Dimethylamiline. Practical grade, Cat. No. P5952. This amine was received in the form of the hydrochloride salt and was used as such without liberation of the free amine. Since this salt decomposes on heating, a purity check was done by liberating a small quantity of the amine, b.p. 220–221°/atm.

3,5-Dimethylamiline. Reagent grade, Cat. No. 2324; b.p. 104–105°/14 mm.

Pure samples of four other amines were obtained as follows:

Aniline. Reagent grade aniline (Fisher Scientific Company, New York, N.Y., Cat. No. A-740) was distilled from zinc dust in an atmosphere of nitrogen. The distillation, which was conducted at atmospheric pressure and without fractionation, gave a centre-cut distillate which boiled at 184°.

3,4-Dimethylamiline. This amine was synthesized from fenchone (technical grade, Cat. No. T4571, Eastman Kodak Company, Rochester, N.Y.), b.p. 190–197°/atm, according to the procedure described by ZAUGG⁸. The product was obtained in white crystalline plates, b.p. 97–98°/10 mm; b.p. 225–226°/atm; m.p. 49–50°. Reported for 3,4-dimethylaniline, b.p. 224°/atm; and m.p. 48°⁹; m.p. 50–51°⁸.

2,4,5-Trimethylamiline (Pseudocumidine). 100 g of commercial Ponceau 3R (F.D. & C. Red No. 1) (Calco Chemical Co., Inc., Boundbrook, N.J.) was dissolved in 2 l of water and made alkaline by the addition of 10 g of sodium hydroxide. The Ponceau 3R was reduced at 60° by the dropwise addition, with vigorous stirring, of a saturated aqueous solution (ca. 30%) of sodium hydrosulphite. Addition of the hydrosulphite was continued until the reaction mixture was no longer red in colour; at the end of the reduction the reaction mixture was murky and yellow-brown in colour. The substituted anilines liberated during the reduction were isolated from the reaction mixture by steam distillation and extraction of the distillate with ether. The ethereal

extract was washed with water, dried over potassium hydroxide pellets, and the ether removed by distillation. The oily liquid residue was then fractionally distilled under reduced pressure in an atmosphere of nitrogen. The fraction which boiled at 104–105°/10 mm was collected and most of this fraction crystallized when chilled in a refrigerator. The small quantity of uncrystallized material was separated from the solid by pressing the mixture between pads of filter paper. Recrystallization of the solid material from ethanol gave colourless needles, m.p. 65–66°. Reported for 2,4,5-trimethylaniline, m.p. 66°¹⁰.

2,4,6-Trimethylaniline (Mesidine). Reagent grade acetone was converted to mesitylene using the procedure described by ADAMS AND HUFFERD¹¹. Nitration of the mesitylene as described by POWELL AND JOHNSON¹², gave 2-nitromesitylene and this was then reduced with tin and hydrochloric acid using a procedure similar to that described by FIESER¹³ for the reduction of nitrobenzene. When the reduction had been completed, the reaction mixture was made strongly alkaline with sodium hydroxide and then steam-distilled to isolate the amine. The steam distillate was extracted with ether and the ether extract was dried over potassium hydroxide pellets. Distillation of the ether extract gave a colourless liquid, b.p. 232–233°/atm. Reported for 2,4,6-trimethylaniline, b.p. 232–233°/atm¹⁴.

2. Azo dyes

Each of the amines described above was diazotized and coupled with 2-naphthol using the following procedure:

A solution containing 0.050 mole of the amine and 8.4 ml (0.100 mole) of concentrated hydrochloric acid in 150 ml of water was prepared and cooled to 0–5° in an ice bath. To this solution was added, dropwise with vigorous stirring, a solution of 3.5 g (0.051 mole) of sodium nitrite in 50 ml of water. After addition of the nitrite had been completed, stirring was continued at 0–5° for 15 min and then 10% aqueous sulphamic acid solution was added dropwise until the excess nitrous acid had been destroyed (negative test with starch-iodide paper).

Concurrent with the diazotization of the amine, a solution containing 7.9 g (0.055 mole) of 2-naphthol (technical grade, m.p. 119–122°, Cat. No. T171, Eastman Kodak Company, Rochester, N.Y.), 4.0 g of sodium hydroxide, and 8.0 g of sodium acetate in 150 ml of water was prepared and cooled to 0–5° in an ice bath. The diazonium salt solution was then added dropwise, with vigorous stirring, over 30 min and was maintained at 0–5° by the addition of small quantities of ice. The ice bath was removed and stirring was continued for 1 h. The azo dye was collected by extraction of the reaction mixture with chloroform. The chloroform extract was washed with water and then evaporated to dryness on a steam bath. The residual, crude azo dye was recrystallized repeatedly from mixtures of ethanol and benzene until the product had a constant melting point.

In the case of the 2,3-dimethylaniline, which was used in the form of the hydrochloride salt, the above procedure was modified slightly to correct for the hydrogen chloride which was added via the salt. In this case, 0.050 mole of the salt and 4.2 ml

(0.050 mole) of concentrated hydrochloric acid were used in the diazotization step, the remainder of the procedure being unchanged.

The following pure azo dyes were prepared:

1-Phenylazo-2-naphthol. Dark red needles, m.p. 133.5–134.5°. Reported m.p. 132–133¹⁵.

1-(2-Methylphenylazo)-2-naphthol. Dark red needles, m.p. 131.9–132.4°. Reported m.p. 130.5–131.5¹⁶.

1-(3-Methylphenylazo)-2-naphthol. Dark red rods, m.p. 141.3–141.7°. Reported m.p. 139–140¹⁶.

1-(4-Methylphenylazo)-2-naphthol. Dark red rods, m.p. 135.3–135.8°. Reported m.p. 133–135°¹⁵; m.p. 135.5–137.0¹⁶.

1-(2-Ethylphenylazo)-2-naphthol. Dark red needles, m.p. 123.0–123.5°. No melting point has been reported previously for this compound although DOLINSKY AND JONES¹⁷ have reported its infrared spectrum.

1-(2,3-Dimethylphenylazo)-2-naphthol. Dark red needles, m.p. 187.5–188.0°. Reported m.p. 125–130¹⁸.

*1-(2,4-Dimethylphenylazo)-2-naphthol**

*1-(2,5-Dimethylphenylazo)-2-naphthol**

*1-(2,6-Dimethylphenylazo)-2-naphthol**

1-(3,4-Dimethylphenylazo)-2-naphthol. Dark red needles, m.p. 149.6–150.1°. Reported m.p. 146¹⁹.

1-(3,5-Dimethylphenylazo)-2-naphthol. Dark red needles, m.p. 196.4–196.9°. This is the first synthesis reported for this compound.

1-(2,4,5-Trimethylphenylazo)-2-naphthol. Dark orange needles, m.p. 164.4–164.9°. Reported m.p. 160–161¹⁹.

1-(2,4,6-Trimethylphenylazo)-2-naphthol. Dark red clusters of needles, m.p. 135.3–135.8°. Reported m.p. 134–135¹⁵.

3. Reversed-phase paper chromatography

A paper chromatographic apparatus supplied by Canadian Laboratory Supplies Limited, Montreal (Cat. Nos. 49-730, 18-310, and 18-316) was used for the development of the chromatograms. It consisted of a cylindrical glass tank (h. 24 in.; d. 12 in.) with a close fitting plate glass cover and was equipped with a stainless-steel rack that supported two 8 in. glass solvent troughs. The troughs were positioned for descending development of the chromatograms. Since the chromatograms being run were of the "Durchlauf" type, where the solvent front is allowed to run off the end of the paper and the running time is rather long, the tank was fitted with a constant-head apparatus. This consisted of a 2 l Erlenmeyer flask closed by a one-hole rubber stopper through which there extended a short piece of 8 mm glass tubing. A second short piece of 8 mm glass tubing was connected, by means of rubber tubing, to the one through the stopper such that the total length of tubing extending from the flask

* The samples of 2,4-, 2,5-, and 2,6-dimethyl-derivatives were obtained from Mr. W. PRZYBYLSKI of this Laboratory, to whom the authors wish to express their gratitude.

was about 2 in. From 1 l to 1.5 l of developing solvent was placed in the flask and the delivery tube was closed by a pinch-clamp applied to the rubber tubing. The flask was suspended in an inverted position above the tank with the delivery tube extending through a 0.5 cm hole in the glass cover and into one of the solvent troughs. The end of the delivery tube was positioned in the trough at the desired solvent level and, if necessary, the position of the rubber tubing was adjusted so that it closed off the small hole in the cover. The pinch-clamp was removed from the delivery tube so as to allow solvent to run into the trough and solvent was transferred to the second trough by a siphon made from glass tubing. When the level of liquid in the troughs fell below the tip of the delivery tube, air was admitted to the flask thus allowing fresh solvent to flow into the troughs until the original level was restored. Before developing chromatograms with a new solvent, the tank was equilibrated by placing 200 ml of the solvent in the bottom of the tank and allowing it to stand overnight. It was not necessary to equilibrate the tank again until the composition of the developing solvent was altered.

The paper used for the chromatograms was Whatman No. 3MM cut into strips 6 in. \times 22.5 in. and serrated at one end. Two pencil lines were drawn 2.25 in. and 2.75 in. respectively from the opposite end of the paper with the latter being marked every 19 mm. Impregnation of the paper with non-polar stationary phase was accomplished by passing it, at uniform speed, through a trough containing a solution of 5 g white mineral oil, U.S.P. XII (Fisher Scientific Co., New York, N.Y.; Cat. No. O-119) dissolved in 100 ml ether. The paper was then suspended in a vertical position and allowed to dry at room temperature.

Solutions of each of the oil-soluble dyes were prepared by dissolving 10 mg of the dye in acetone and diluting to 25 ml in a volumetric flask. These solutions were spotted along the second pencil line on the dried, impregnated paper, a single solution being applied to each mark on the starting line. The volume of each dye solution used was $5 \mu\text{l}$ (*i.e.* 2 μg of dye) delivered from a micro pipette and confined to a spot 0.5 cm in diameter. When the spots had dried, the paper was folded along the first pencil line and placed over a supporting rod in the developing tank with the end of the paper dipping into the solvent in the trough. One of the spots on each paper was 1-phenylazo-2-naphthol which was used as a reference compound for determining relative R_F values. The chromatograms were developed with mixtures of acetone and water using proportions of 40:60, 45:55, and 50:50 by volume. Development of the chromatograms was continued until the 1-phenylazo-2-naphthol spot had travelled about 30 cm down the paper from the starting line. Each of the oil-soluble dyes was chromatographed, with a given solvent, on four different strips of paper to obtain averaged values. After development of the chromatogram had been completed, the paper was removed from the tank, suspended in a vertical position, and dried at room temperature. R_S values relative to the 1-phenylazo-2-naphthol spot were then calculated and averaged for the four papers. The R_F value of 1-phenylazo-2-naphthol in each of the developing solvents was determined in quadruplicate and the averaged value was used to calculate the apparent R_F values of the other dyes.

DISCUSSION

The initial difficulty in this investigation was the procurement of suitable samples of the alkyl-substituted 1-phenylazo-2-naphthols. Since the dyes differed only slightly in R_F values, especially among isomeric dyes, it seemed desirable to obtain these dyes in as pure a state as possible. Only three dyes of this type, Sudan I, Orange SS, and Oil Red XO, are commercially available and although Sudan I and Orange SS are relatively free from isomeric contaminants, Oil Red XO is known to be a mixture of two isomeric dyes in approximately equal amounts. Rather than using any of these commercial products, all of the dyes employed in this investigation were synthesized in an identical manner by diazotization of the corresponding amines and coupling the resulting diazonium salts with 2-naphthol. Many of the amines needed for the syntheses of these dyes were available from chemical suppliers, usually in reagent grade purity, and were used without further purification. Those amines not commercially available were synthesized using methods which, for the most part, already appear in the literature. The crude product from each of the coupling reactions was purified by repeated recrystallization, to constant melting point, from mixtures of benzene and ethanol; these dyes are readily soluble in benzene but only sparingly soluble in ethanol.

Only one of the dyes reported here, 1-(3,5-dimethylphenylazo)-2-naphthol, was previously unknown. The 2-ethyl-derivative has been reported only once¹⁷ and although its infrared spectrum was given, the melting point for this compound was not reported. All of the remaining dyes appear in the literature one or more times and the melting points given here for these compounds generally agree quite well with the reported values, the exception being that of 1-(2,3-dimethylphenylazo)-2-naphthol which is considerably higher than the sole reported value by MAY AND HUNT¹⁸. At first it was thought that the material obtained as the 2,3-dimethyl-derivative was not the expected compound. The 2,3-dimethylaniline hydrochloride used in the preparation of this dye was only of practical grade and no information was available as to its identification or its purity since this material decomposes on heating and no melting point could be obtained. The amine was identified by distillation of the free amine after first liberating it from the salt. Almost all of the amine distilled within the range 220–221° which is correct for 2,3-dimethylaniline; unlike most of the isomeric dimethylanilines which boil at or about the same temperature, 2,3-dimethylaniline has a unique boiling point. Additional evidence as to the identity of this amine was obtained by conversion of the distilled amine to its acetyl derivative, m.p. 134–135°; reported m.p. 135.5°¹⁰. A centre-cut fraction of the distilled amine, when coupled with 2-naphthol in the usual manner, gave the same product as was obtained previously from the hydrochloride salt. Finally, the infrared spectrum of the dye was almost identical with that reported by DOLINSKY AND JONES¹⁷. Thus it seems to be evident that the dye which was obtained was indeed the expected 2,3-dimethyl-derivative. The validity of the melting point reported by MAY AND HUNT¹⁸ for this compound is somewhat in doubt since it spans a 5° range and also because their melting

points for other oil-soluble dyes of this type are consistently much lower than the values reported by other workers.

The method used for development of the chromatograms was a standard reversed-phase technique with descending flow of the developing solvent. Since the chromatograms were of the "Durchlauf" type and were developed for several days, the chromatography tank was fitted with a constant-head device for supplying fresh developing solvent so that the apparatus required little or no attention. Experimentation revealed that equilibration of the tank for several hours or preferably overnight was necessary in order to obtain reproducible R_F values but that it was not necessary to treat the paper in any manner, exclusive of impregnation, prior to development of the chromatogram. Whatman No. 3MM chromatographic paper was chosen as the support after running comparative chromatograms using this paper and Whatman papers numbers 1, 31, 31 extra thick, and 54. The 31 and 54 papers had the apparent advantage of being faster than the 1 and 3MM papers but it was found that the differences in R_F values of the dyes were smaller, under uniform chromatographic conditions, on these faster papers than on the slower ones. Whatman No. 1 paper was found to be roughly equivalent to 3MM paper in its ability to resolve mixtures of the dyes but it suffered from the disadvantage of having considerably less capacity than that of the thicker 3MM paper. These observations may not be directly attributable to the papers themselves but rather to the amounts of oil which were retained by the papers after impregnation. These papers vary greatly in their abilities to absorb liquids and since the oil was applied in ether solution to the paper, different amounts of this solution would be retained by the various papers and thus, on drying, these papers would contain different amounts of the stationary phase. It was found that varying the amount of oil on the paper, by altering the concentration of the impregnating solution, had a marked effect both on the R_F values and on the degree of separation which was achieved with dye mixtures. Only one stationary phase, light mineral oil, was used in this investigation because numerous chromatograms of oil-soluble dyes run in this laboratory have indicated that this material is at least as good as, and is sometimes superior to, the other commonly used stationary phases. It also has the added advantage of being chemically inert. For impregnation of the paper, it was found that a solution of 5 g of the oil in 100 ml of ether provided the best results both from the standpoint of the capacity of the paper and of the resolutions which could be achieved when using dye mixtures. Lower oil concentrations resulted in reduced capacities while higher oil concentrations gave inferior resolutions and required excessively long developing times.

The choice of acetone-water mixtures as developing solvents for the chromatograms was based on a number of considerations. The dyes were fairly soluble in acetone but insoluble in water and thus the R_F values of the dyes could be easily changed by altering the water content of the developing solvent. In addition, since acetone is completely miscible with water, it provided an infinite range of solvent mixtures of readily ascertained compositions, and also, it prevented separation of the solvent into two phases during development of the chromatograms due to fluctuations in

temperature. The mineral oil employed as the stationary phase in the chromatograms was relatively insoluble in acetone and even less so in acetone-water mixtures and thus this solvent system prevented excessive depletion of the stationary phase during development of the chromatograms. Some of the stationary phase was eluted from the paper during development of the chromatograms and presumably this could have been prevented by saturation of the developing solvent with mineral oil prior to the development, however, when this was tried it was found to have no observable effect on the chromatograms. Finally, acetone-water provided good resolution of mixtures of the dyes. It was thought that ethanol-water mixtures might also be used despite the fact that the dyes were considerably less soluble in ethanol than in acetone but this solvent system gave inferior resolutions to those obtained with acetone-water mixtures.

Acetone was selected as the solvent for the dyes in preparing stock solutions for application to the paper for some of the same reasons that it was chosen as the organic component of the developing solvents. It also had the desirable features of rapid evaporation so that the spots could be dried quickly and, being part of the developing solvent, small traces which remained after drying the spots would not influence the chromatograms. The amount of each dye which was applied to the papers was arrived at by trial and error and was such that the colour intensity of the spots made observations of their positions easy and yet it did not overload the paper. It was found that 2 μg of each of the dyes gave the best results, although several times this quantity could be applied to the paper without serious overloading taking place. The stock solutions were prepared such that 5 μl of solution contained 2 μg of dye, this being done purely for convenience since a 5 μl pipette was used for application of the dye solutions to the paper.

Table I summarizes the relevant data obtained from the chromatograms of individual 1-arylozo-2-naphthols in the three acetone-water developing solvents used in this investigation. Each figure in the table is an averaged value from four separate chromatograms each of which had a given dye spotted at different positions along the starting line so as to account for any localized differences in the paper or in the stationary phase. It was found that, for a given dye, values varied somewhat from one chromatogram to another depending on the position at which the dye was spotted, however, these deviations were never greater than 4% from the arithmetic mean. Each of the papers was spotted at the extreme left-hand mark on the starting line with 1-phenylazo-2-naphthol which served as a reference compound and thus allowed the calculation of R_S values (*i.e.* relative displacements) for each of the other dyes on the paper; these values appear in Table I under the heading R_S . Since the phenyl derivative was spotted at the same position on each of the papers, data obtained from different papers can be directly compared. This is important since it was not convenient to spot all thirteen of the dyes on a single paper. Under the heading R_F in Table I, the values for the phenyl derivative are averaged values which were obtained experimentally by retaining the solvent front on the paper. Values appearing under this heading for the other compounds were calculated by multiplication of R_S for the compound by R_F for the phenyl derivative.

TABLE I
 DATA OBTAINED FROM CHROMATOGRAMS OF 1-ARYLAZO-2-NAPHTHOLS DEVELOPED WITH ACETONE-WATER MIXTURES

Compound (Aryl group)	40:60 Acetone-water Running time 14 1/2 h			45:55 Acetone-water Running time 90 h			50:50 Acetone-water Running time 5 h			
	RS	R _F	RM Length of spot (cm)	RS	R _F	RM Length of spot (cm)	RS	R _F	RM Length of spot (cm)	
Phenyl	1.00	0.048	1.30	1.00	0.089	1.01	1.00	0.16	0.73	3.9
2-Methylphenyl	0.55	0.026	1.57	0.59	0.052	1.23	0.65	0.10	0.95	4.0
3-Methylphenyl	0.50	0.024	1.61	0.55	0.050	1.28	0.61	0.095	0.98	4.0
4-Methylphenyl	0.45	0.022	1.66	0.49	0.044	1.34	0.57	0.088	1.01	3.4
2-Ethylphenyl	0.30	0.015	1.85	0.35	0.031	1.49	0.41	0.064	1.16	3.1
2,3-Dimethylphenyl	0.29	0.014	1.85	0.33	0.029	1.52	0.39	0.060	1.19	4.6
2,4-Dimethylphenyl	0.25	0.012	1.91	0.29	0.026	1.57	0.37	0.056	1.23	3.2
2,5-Dimethylphenyl	0.29	0.014	1.85	0.34	0.031	1.49	0.41	0.064	1.17	3.2
2,6-Dimethylphenyl	0.37	0.018	1.74	0.42	0.037	1.41	0.46	0.072	1.11	3.2
3,4-Dimethylphenyl	0.27	0.013	1.88	0.33	0.029	1.52	0.39	0.061	1.19	2.7
3,5-Dimethylphenyl	0.24	0.012	1.91	0.28	0.025	1.59	0.35	0.055	1.24	3.1
2,4,5-Trimethylphenyl	0.16	0.0079	2.10	0.20	0.018	1.74	0.25	0.039	1.39	2.6
2,4,6-Trimethylphenyl	0.17	0.0081	2.09	0.21	0.019	1.72	0.26	0.040	1.38	2.6

Several papers have appeared in the literature, dealing with the relation between chemical structure and chromatographic behaviour, which are applicable to the work reported here. In their classical paper, MARTIN AND SYNGE²⁰ derived a general theoretical equation for the rate of movement of a solute during column partition chromatography. The same equation was later derived more simply by the use of the kinetic theory^{21,22}. CONSIDEN *et al.*²³ demonstrated that this equation could be applied to paper partition chromatography with some modification and introduced the concept of R_F values which now has achieved general acceptance. For ideal solutions, it was demonstrated mathematically by MARTIN²⁴ that the partition coefficient, to which the R_F value is related, is dependent on the free energy required to transport one mole of a compound from the stationary phase to the mobile phase. It was also shown by MARTIN that the addition of a given group to a chemical structure will alter the partition coefficient of the compound by a constant factor which is dependent only on the added group and the nature of the two partitioning phases and not on the rest of the molecule. BADE-SMITH AND WESTALL²⁵ suggested the use of R_M , which has a linear relation to the logarithm of the partition coefficient, in order to facilitate practical application of MARTIN'S rather involved equation. It follows directly from the equation derived by MARTIN that, for compounds comprising a homologous series, a linear relation exists between the R_M values and the number of like substituent groups present in the compounds. The equation also suggests that isomeric compounds should possess identical R_M values, although this conclusion is not strictly valid since, in deriving the equation, no consideration was given to interaction of the groups within a molecule. Indeed, FRANC *et al.*²⁶⁻²⁹ found that isomeric substituted benzenes had different R_M values and modified the linear relation for R_M values, derivable from MARTIN'S equation, by a term involving the dipole moments of the compounds. Accounting for polarity differences among isomers in this manner, these workers have obtained good agreement between calculated and observed R_M values.

The chromatographic data obtained in this investigation, which appear in Table I, agree fairly well with the relationships outlined above. Plotting the R_M values obtained with each solvent system against the number of methyl groups present in the dyes gave a straight line in agreement with MARTIN'S relationship although there was some scattering within the groups of isomeric compounds (Fig. 1). Using the method of least squares, a straight line was positioned on each plot and it was found that the slope gradually increased as the concentration of acetone in the solvent system was decreased. This increase in the slope corresponds to an increase in the spread of R_M values and hence to a greater separation of the spots on the chromatogram. The deviations of the R_M values from linearity did not exceed 6%, 7%, and 6% for the 40:60, 45:55, and 50:50 acetone-water solvent systems respectively.

The small but reproducible differences in the R_M (or R_F) values within the three isomeric groups indicate the effect of positional isomerism. Since the separations involve a process of continuous partition between a stationary non-polar phase and a mobile polar phase, any variation in the polarity of the isomers would lead to a corresponding variation in their rates of travel on a chromatogram. A more polar isomer

would favour the mobile phase and hence exhibit a higher R_F value than a less polar isomer. FRANC and co-workers (see above) have used dipole moments with considerable success as a measure of polarity differences between isomers. Unfortunately, this treatment is not directly applicable here because 1-arylazo-2-naphthol compounds

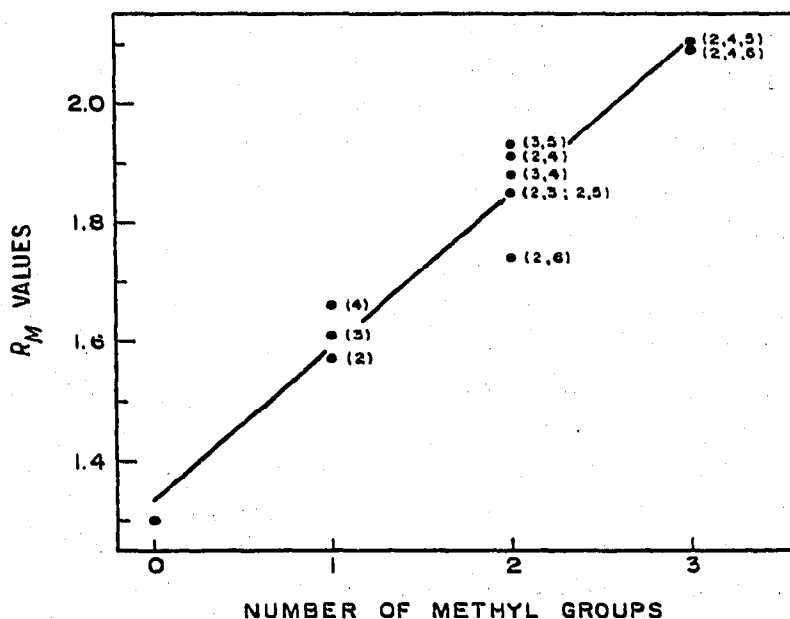


Fig. 1. R_M values of methyl-substituted 1-phenylazo-2-naphthols chromatographed with 40:60 acetone-water plotted against number of methyl groups. The figures in the brackets denote the positions of the methyl groups on the phenyl ring.

do not exist as single species amenable to precise dipole moment determinations but rather as equilibria of azo and hydrazone tautomers³⁰. The position of equilibrium in these tautomeric systems is dependent on the nature of the solvent and on the position and character of the substituents.

The relative polarities of the compounds in the three isomeric groups can be determined by considering the known effects of alkyl substituents attached to the phenyl ring on the position of the azo-hydrazone equilibrium. BURAWOY *et al.*³⁰ have shown that methyl groups favour the more polar hydrazone tautomer in the order 2-methyl > 3-methyl > 4-methyl. Thus, the 2-methylphenyl derivative should be more polar than the 3-methylphenyl derivative and this, in turn, should be more polar than the 4-methylphenyl derivative. Applying the same reasoning to the dimethylphenyl derivatives, the order of polarity should be 2,6-dimethyl > 2,5-dimethyl \equiv 2,3-dimethyl > 2,4-dimethyl > 3,5-dimethyl > 3,4-dimethyl and for the trimethylphenyl derivatives, 2,4,6-trimethyl more polar than 2,4,5-trimethyl. Inspection of Table I and Fig. 1 reveals that the isomers do follow generally the predicted order. A persistent exception is the 3,4-dimethyl isomer which, while it should show the lowest R_F value, shows intermediate behaviour. Bond deformation of the adjacent alkyl groups may be a factor in this instance. It is particularly gratifying to note that even in the highly substituted trimethyl derivatives, the effect of altering the posi-

tion of one methyl group is a small but measurable difference in R_F in the predicted direction.

Almost all the chromatograms run in connection with this work were performed on the individual dyes, however, the data which have been obtained could have practical application in the analysis of aromatic amine mixtures by first coupling the mixed amines with 2-naphthol. This technique was applied to the amine mixtures obtained by reduction of several commercial samples of Ponceau 3R. The azoic component of Ponceau 3R is a mixture of alkyl-substituted anilines including both methylated and ethylated compounds. Chromatography of the dye mixture, thus produced, using 40:60 acetone-water as the developing solvent readily separated the major components. Some of the dyes were not separable even with 40:60 acetone-water, which gave the greatest spread in R_F values. On the basis of the separations which were achieved in this solvent and the R_F values in Table I, two dyes can be separated if their R_F values differ by more than 0.001. Since increased resolution is obtained on decreasing the acetone concentration in the developing solvent, presumably improved separations could be achieved using a smaller concentration of acetone than was used in this work, however, the time required for the development would be rather lengthy. An ethyl derivative, 1-(2-ethylphenylazo)-2-naphthol, was included among the dyes studied here to see whether it would behave like a mono-methylated or like a di-methylated compound. Its R_F was similar to those of the di-methylated derivatives and thus the molecular weight of the compound appears to be the dominating factor in the R_F value of these compounds. The averaged length of the spot for each dye after development is included in Table I. There is no significant difference in these values from one solvent to another and it appears that the length of the spots is roughly proportional to the distance travelled down the paper, after an initial rapid lengthening of the spots. Lateral spreading was small except in the lower 25% of the paper. Longitudinal spreading could be minimized by the use of smaller amounts of the dyes. The size of the spot after development is important when considering the resolution which may be obtained with mixtures of the dyes.

SUMMARY

A number of alkyl-substituted 1-phenylazo-2-naphthols were prepared, of which 1-(3,5-dimethylphenylazo)-2-naphthol is reported for the first time, and these were subjected to reversed-phase paper chromatography using mineral oil-impregnated papers and acetone-water mixtures as developing solvents. R_F values are given for thirteen compounds. R_M values were calculated and these are discussed in the light of present knowledge of the relationship between chemical structure and chromatographic behaviour.

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