

## A paper-chromatographic method for the identification of $\alpha$ - and $\beta$ -naphthol when present together

$\alpha$ -Naphthol and  $\beta$ -naphthol are constituents of high-boiling coal tar fractions and of the two the latter is especially important for it is the starting material for a wide range of synthetic dyes and their intermediates<sup>1</sup>. Several methods for the detection of  $\alpha$ - and  $\beta$ -naphthol when they are present together have been suggested. One such method is based on the fact that the phenyl-azo derivative of  $\alpha$ -naphthol is soluble in water, whereas that of  $\beta$ -naphthol is insoluble<sup>2</sup>. Methods based on the chromatography of naphthols or of their derivatives have also been proposed<sup>3</sup>. EVANS *et al.*<sup>4</sup> obtained paper chromatograms of free naphthols using a mixture of butanol, pyridine and sodium chloride solutions as developing agent and a solution of diazotised sulphanilic acid for the detection. Although the spots of the two phenols are somewhat different in colour their  $R_F$  values are identical, thus making it difficult to distinguish between them. RILEY<sup>5</sup> used *n*-amyl alcohol saturated with water or *n*-butanol-benzene-water for developing the spots and detected them by spraying with phosphomolybdic acid. With both solvents the  $R_F$  values of the two phenols were nearly equal. BARTON, EVANS AND GARDNER<sup>6</sup> used  $H_2CO_3$  solution for the development of the chromatograms and  $FeCl_3$  or  $K_3Fe(CN)_6$  as spraying reagents for the detection of the phenols.

In a series of papers, HOSSFELD *et al.* (see *e.g.*<sup>7</sup>) have attempted to identify phenols by coupling them with diazotised sulphanilic acid and then chromatographing the resulting dye. They used this technique for the identification of several phenols, including the naphthols. In the case of phenols whose derivatives have almost identical  $R_F$  values, the technique of two-dimensional chromatography was applied for affecting the separation.

We were interested in developing paper chromatographic methods<sup>8</sup> and during our investigations we observed that dyes lend themselves to easy separation if a suitable developing agent is used. Some of the phenols<sup>9</sup> can be identified by paper chromatographic separation of their diazo derivatives. We therefore thought that it would be possible to develop a method for the identification of phenols, particularly the naphthols, which would involve the preparation of diazo derivatives of the phenols followed by paper chromatographic development of these derivatives.

Amino-J acid (disodium salt of 2-naphthylamine-5,7-disulphonic acid) on diazotisation and coupling with the various phenols gave distinctly coloured spots on filter paper, which could be developed with a suitable eluent. Especially the results obtained with  $\alpha$ - and  $\beta$ -naphthol were encouraging because the  $R_F$  values obtained with certain developing agents differed appreciably for the two naphthols. The following method was, therefore, developed for the identification of  $\alpha$ - and  $\beta$ -naphthol when present together in a mixture.

*Experimental.* 0.1 % solutions of  $\alpha$ - and  $\beta$ -naphthol were prepared separately

TABLE I

 $R_F$  VALUES OF DYES OBTAINED BY COUPLING DIAZOTISED AMINO-J ACID WITH  $\alpha$ - AND  $\beta$ -NAPHTHOL

Eluent	$R_F$ value	
	Dye from $\alpha$ -naphthol	Dye from $\beta$ -naphthol
1. Isoamyl alcohol-ethyl alcohol-ammonia (40:40:25)	0.14	0.28
2. Isoamyl alcohol-ethyl alcohol-water (40:40:25) *	0.10	0.24
3. Methyl isobutyl ketone-ethyl alcohol (70:30) saturated with ammonia (10 %)	0.04	0.18

\* Paper impregnated with 3 % caustic soda solution before applying the spots.

in alcohol and one drop of each solution was placed about 2 inches apart on the base line of Whatman filter paper No. 1. The spots were coupled *in situ* with a cold alkaline solution of diazotised Amino-J acid (approx.  $M/50$ ). A dye was immediately formed. The paper was allowed to dry, rolled into the form of a cylinder and placed in a trough containing the desired eluent and then covered immediately with a bell jar. The usual precautions of saturating the atmosphere inside the bell jar and sealing the flange of the bell jar with silicone grease after assembly, were taken. The chromatogram was developed until the liquid front had moved up about 7-8 inches. This took nearly 4 hours. The spots of the dye formed from  $\alpha$ - or  $\beta$ -naphthol also moved up. The  $R_F$  values of these dyes with different developing agents are recorded in Table I.

The colour of the spots, when wet, were rose-red for  $\alpha$ -naphthol and reddish orange for  $\beta$ -naphthol in the case of all three eluents. On drying, the spots obtained

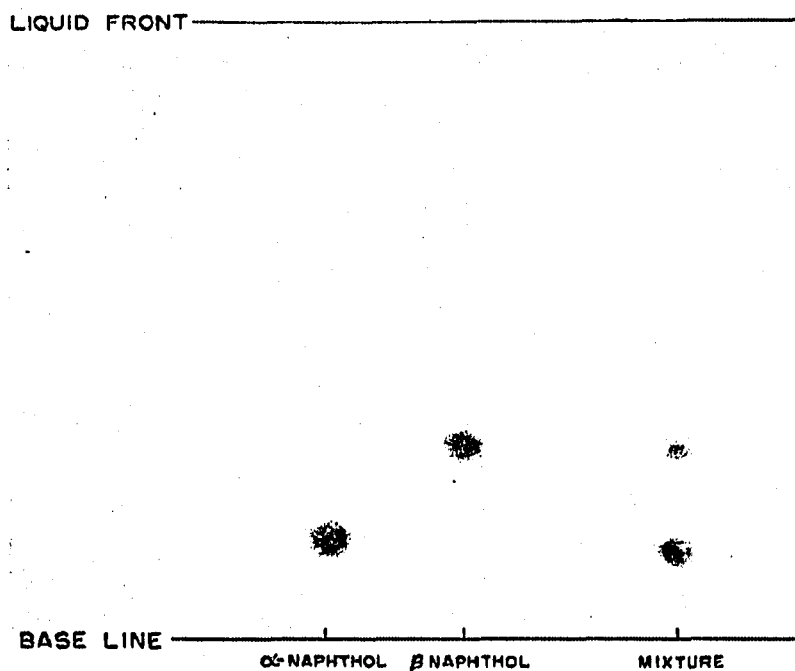


Fig. 1.

with eluents (1) and (3) acquired the same rose red colour; in the case of eluent (2) the dye spots retained their original shade.

It can be seen from Table I that the  $R_F$  values for the  $\alpha$ - and  $\beta$ -naphthol derivatives differ considerably in all three systems. In a separate experiment, one drop of a solution containing both  $\alpha$ - and  $\beta$ -naphthol was placed alongside the two drops of the  $\alpha$ -naphthol and  $\beta$ -naphthol solutions. After coupling all three spots separately with diazotised Amino-J acid, the paper was dried and developed with isoamyl alcohol-ethyl alcohol-ammonia solution. A typical chromatogram is shown in Fig. 1. It can be seen that on development the spot of the mixture of the two naphthols is separated into two spots, which move parallel to those of  $\alpha$ - and  $\beta$ -naphthol respectively.

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### Quantitative determination of proline by paper chromatography

Since the ninhydrin reagents are not very sensitive to proline, the isatin method for the detection of this amino acid has frequently been used, even for quantitative purposes. PASIEKA AND MORGAN<sup>1</sup> used the isatin reagent according to the method of ACHER *et al.*<sup>2</sup>. As these authors were not able to elute the coloured product obtained from proline with water or with other mineral or organic solvents from paper, they evaluated the spots—after washing out other spots and the background—by measuring the density of the proline areas in the cuvettes of a spectrophotometer. Since it is well known that densitometric methods *in situ* are subject to inaccuracies, it is to be expected that higher precision could be obtained if it were possible to elute the dye and measure its density in solution.

For the purpose of detecting proline on chromatograms the reagent according to ACHER *et al.*<sup>2</sup> and the reagent according to BARROLLIER *et al.*<sup>3</sup>, containing Zn acetate and acetic acid, were compared. The latter is more sensitive and therefore it was used in further work. The coloured reaction product of proline can be eluted from