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# IDENTIFICATION OF AROMATIC NITRILES BY REACTION PAPER CHROMATOGRAPHY

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## SUMMARY

A procedure involving reaction paper chromatography for the determination of the number of nitrile groups in molecules from the shift in the $R_M$ values of the substance before and after reaction has been developed. A technique for the detection of substances with a nitrile group, which can be used with advantage even for other substances, has been devised.

## INTRODUCTION

This paper is the latest in the series of studies[1–4] in which we set out to develop a procedure involving reaction paper chromatography and electrophoresis for determining the number of various functional groups in molecules, chiefly in aromatic compounds. As in previous work, this paper is concerned with determining the number of nitrile groups bound to the aromatic ring by using the differences in the $R_M$ values obtained in the separation of the unreacted and reacted substances in the presence of each other by paper chromatography. In this instance, the well-known reaction of the nitrile group with alkaline hydrogen peroxide was utilized:

$$RCN + 2\ H_2O_2 \rightarrow RCONH_2 + H_2O + O_2$$

The only difficulty in using this procedure was the non-existence of a reliable and sufficiently sensitive method for the detection of substances containing nitrile groups. This problem was successfully solved by spraying the chromatogram with hydroxylamine and copper(II) acetate. Intensely fluorescent spots are formed, not only with substances that contain a nitrile group but also with those that contain an amide group and with many others.

## EXPERIMENTAL

In order to convert the nitrile group into an amide group, the reaction with hydrogen peroxide described by Radziszewski[5] and modified for our purposes was used.

To 1 ml of 15–20% hydrogen peroxide, 1–2 drops of a 15% aqueous NaOH solution and several milligrams of a sample of a substance containing a nitrile group were added. The mixture was shaken and filtered after 2–3 min, and the

TABLE I

$R_F$ VALUES FOR SUBSTANCES BEFORE AND AFTER THEIR REACTION WITH HYDROGEN PEROXIDE, $\Delta R_M$ VALUES AND THE COLORATION OF TRACES ON WHATMAN No. 3 PAPER USING *n*-PROPANOL–AMMONIA (2:1) AS THE SOLVENT

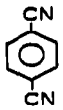
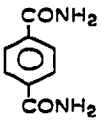
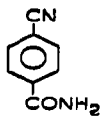
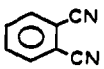
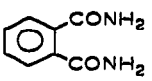
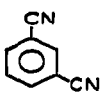
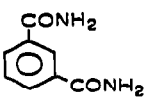
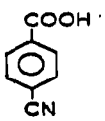
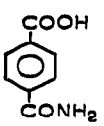
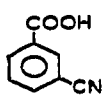
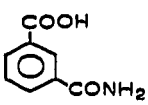
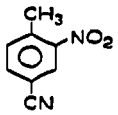
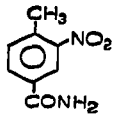
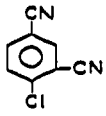
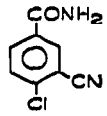
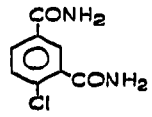
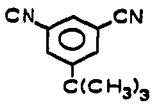
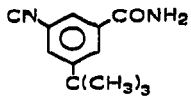
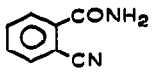
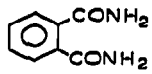
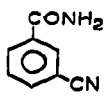
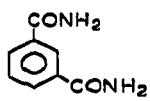
| *Substance* | *Substance after reaction* | $R_F$ | $\Delta R_M$ (*one CN group*) | $\Delta R_M$ (*two CN groups*) | *Detection** | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Immediately* | | *After drying* | |
| | | | | | *Visible* | *UV* | *UV* | *Visible* |
|  | | 0.95 | | | or | d r | d | or |
| |  | 0.64 | | +1.03 | – | y or | y or | – |
|  | | 0.85 | +0.50 | | – | y | y | – |
|  | | 0.95 | | | y | d | d | y |
| |  | 0.66 | | +0.99 | – | bl | bl | – |
|  | | 0.95** | | | sl y | bl | bl | – |
| |  | 0.64 | | +1.03 | – | y g | y g | – |
|  | | 0.69 | | | g | y g | sl | r b |
| |  | 0.46 | +0.42 | | – | y | y | – |
|  | | 0.69 | | | g | bl | y g | – |
| |  | 0.46 | +0.42 | | – | bl | y g | – |

TABLE I (*continued*)

| *Substance* | *Substance after reaction* | $R_F$ | $\Delta R_M$ (*one CN group*) | $\Delta R_M$ (*two CN groups*) | *Detection* * | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Immediately* | | *After drying* | |
| | | | | | *Visible* | *UV* | *UV* | *Visible* |
|  | | 0.94 | | | – | d | d | – |
| |  | 0.86 | +0.41 | | – | y g | y g | – |
|  | | 0.94 | | | – | sl bl | sl bl | – |
| |  | 0.85 | +0.44 | | – | y | y g | – |
| |  | 0.64 | | +0.95 | – | y g | y g | – |
|  | | 0.94 | | | – | sl bl | sl bl | – |
| |  | 0.84 | +0.48 | | – | y g | y g | – |
|  | | 0.85 | | | y | d | d | y |
| |  | 0.66 | +0.47 | | – | bl | bl | – |
|  | | 0.85 | | | – | y | y | – |
| |  | 0.64 | +0.51 | | – | y g | y g | – |

* Coloration: or= orange, d= dark, r= red, y= yellow, g= green, b= brown, bl= blue, sl= slightly.

** The spot is perceptible only after application on a previously washed chromatographic paper.

residue on the filter was dissolved in either dimethyl sulphoxide or ethanol. The solution obtained was applied directly on to the chromatographic paper as a 5% solution.

*Paper chromatography*

The substances were chromatographed on Whatman No. 3 paper using *n*-propanol–ammonia (2:1) as the solvent.

*Detection*

The detection was carried out by spraying with two reagents. The first reagent was prepared by mixing 5 g of hydroxylamine hydrochloride with an equivalent amount of anhydrous sodium carbonate and adding 200 ml of 96% ethanol. After stirring, the solution was filtered and the filtrate was used for spraying. The second reagent was a saturated aqueous solution of copper(II) acetate.

The chromatogram was sprayed with the first reagent and, after drying either freely in the air or in an oven at 80°, it was sprayed with the second reagent. The coloration of the spots was observed immediately under UV light. Some substances fluoresced brightly in various colours, and some were visible even under normal light. The $R_F$ values found and the corresponding colorations are shown in Table I. The hydroxylamine solution was followed for 14 days and it was found that its efficiency did not change substantially during this time.

## RESULTS AND DISCUSSION

As already mentioned, the Radziszewski reaction[5] was used for the reaction of the nitrile group with hydrogen peroxide. Radziszewski states that the reaction proceeds best at 40°, quantitatively and without the formation of side products. In our work, the laboratory temperature was chosen for the sake of simplicity and because a quantitative reaction is unimportant.

The *n*-propanol–ammonia solvent was used for the chromatographic separation for two reasons. Firstly, the separation of all of the given substances, *i.e.*, in the form of nitriles and the corresponding amides, is excellent, and secondly, it is simple, as we used this system in previous work on reaction paper chromatography.

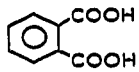
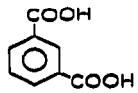
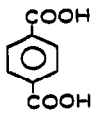
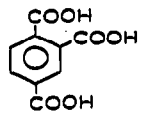
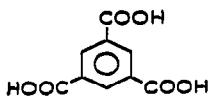
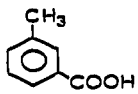
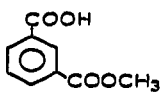
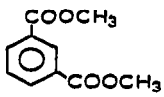
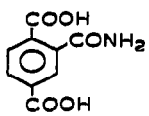
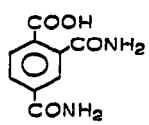
The greatest problem was a suitable method of detection. For the detection of amides, spraying with mercury(II) acetate and diphenylcarbazone was used. However, substances that contain a nitrile group cannot be detected in this way and there was no other suitable detection technique. However, Tiemann[6] found that when a nitrile is treated with hydroxylamine at 60–80°, the corresponding amide oxime is formed, and Pearse and Pflaum[7] further discovered that coloured complexes are formed by reaction of this amide oxime with various metal ions.

By combining these two reactions in spraying the chromatograms, spots that were coloured in visible light or that fluoresced strongly under UV light were formed. Furthermore, it was found that not only nitriles and amides but also other aromatic substances undergo this reaction and therefore a few of them were tested; their behaviour is shown in Table II. It is difficult to say to what degree amide oximes ($-C\lessdot^{NH_2}_{NOH} \leftrightarrow -C\lessdot^{NH}_{NHOH}$) are formed during the detection with

TABLE II

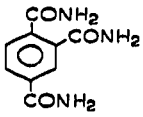
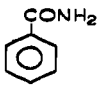
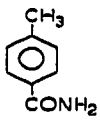
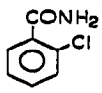
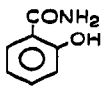
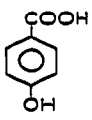
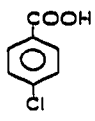
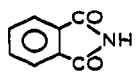
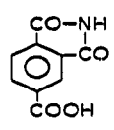
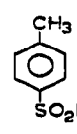
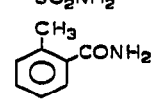
DETECTION OF VARIOUS AROMATIC SUBSTANCES WITH HYDROXYLAMINE AND COPPER(II) ACETATE

Chromatography on Whatman No. 3 paper using *n*-propanol–ammonia (2:1) as the solvent. Colours are represented as in Table I.

| *Substance* | $R_F$ | *Detection* | | | |
|---|---|---|---|---|---|
| | | *Immediately* | | *After drying* | |
| | | *Visible* | *UV* | *UV* | *Visible* |
|  | 0.65 | g | g bl | d bl | g b |
|  | 0.26 | g | g bl | bl | g b |
|  | 0.24 | g | bl | y g | g b |
|  | 0.24 | g | y or | y or | — |
|  | 0.04 | g | y | y | sl g |
|  | 0.04 | g | bl | bl | sl g |
|  | 0.69 | g | d | — | sl g |
|  | 0.70 | g | g bl | y g | — |
|  | 0.95 | — | sl bl | — | — |
|  | 0.10 | g | y | y | — |
|  | 0.23 | sl g | y | y | — |

(*Continued on p. 164*)

TABLE II (*continued*)

| *Substance* | *Rf* | *Detection* | | | |
|---|---|---|---|---|---|
| | | *Immediately* | | *After drying* | |
| | | *Visible* | *UV* | *UV* | *Visible* |
|  | 0.39 | – | y | y | – |
|  | 0.89 | r b | g bl | d | r b |
|  | 0.89 | r b | g bl | g bl | r b |
|  | 0.87 | – | g bl | d | r b |
|  | 0.64 | – | bl | bl | b |
|  | 0.35 | – | d | d | b |
|  | 0.65 | g | g bl | – | b |
|  | 0.66 | – | sl bl | d | r b |
|  | 0.23 | g | y g | d | r b |
|  | 0.92 | – | – | d | b |
|  | 0.87 | – | – | d | r b |

hydroxylamine, as the reaction also proceeds with amides, sulphonamides, acids, triazine derivatives, etc. Using this method of detection, it is also possible to

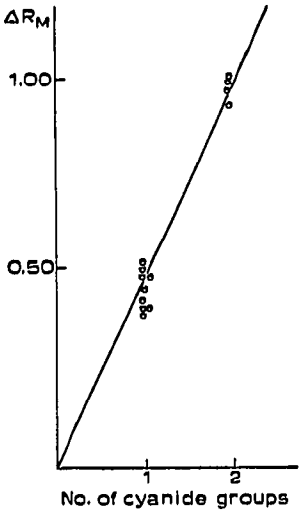



Fig. 1. Variation in $\Delta R_M$ values for initial and reacted substances containing one or two nitrile groups.

distinguish by their coloration individual isomers of, for example, dinitriles, diamides, monoamides of carboxylic acids, cyanobenzoic acid and dicarboxylic and tricarboxylic acids.

The sensitivity of detection was tested for phthalodinitrile on Whatman No. 3 paper. The lowest amount that could be detected was 5 $\mu$g.

No difficulties were encountered during the chromatography of the substances listed in Table I, except for phthalaminic acid, which rearranges to phthalic acid[8].

When differences in the $R_M$ values of the initial and the reacted substances were followed, values of $\Delta R_M$ of 0.47 for one nitrile group and 1.00 for two nitrile groups were obtained. The distribution for various substances is apparent from Fig. 1.

The results obtained in this work show therefore that the number of nitrile groups in an aromatic substance can be determined very easily by this method.

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