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IDENTIFICATION OF AROMATIC SULPHONIC ACIDS BY REACTION ELECTROPHORESIS ON PAPER

JAROSLAV FRANC and DAO VAN HOC

Research Institute of Organic Syntheses, Pardubice-Rybitví (Czechoslovakia)

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SUMMARY

A method was elaborated to establish the number of sulphonic groups bound to the aromatic ring of aromatic acids by means of a paper electrophoretic separation of the original compound from the derivative. The difference between the mobilities is directly proportional to the number of substituted sulphonic groups.

INTRODUCTION

This paper is another contribution in the series of papers concerned with establishing the number of different functional groups of molecules with an aromatic nucleus^{1–5}. Basically, we are concerned with comparing the mobility in paper chromatography or in electrophoresis on paper of the substance that we wish to identify, with the mobility existing after the reaction has been carried out. During the reaction, a change occurs in the functional group that we are investigating. The reaction that is being carried out must be on the one hand of such a type as to provide a molecule that has sufficiently changed characteristics and, on the other, of such a type as to enable us to chromatograph (electrophoretically separate) the original product as well as the reaction product within one system.

In this work, we were concerned with establishing the number of sulphonic groups, and in particular of naphthalenesulphonic and anthraquinonesulphonic acids. These acids are important intermediate dyestuff products and, in addition to the sulphonic group, the molecule also contains other functional groups, which affect the properties of the molecule and consequently also the mobility in paper chromatography or electrophoresis on paper.

The reaction selected in the present instance was the substitution of a hydroxyl group for the sulphonic group, by fusion with sodium hydroxide, according to the well known reaction



For the separation of the original sulphonic acid and the corresponding hydroxy derivative, the most suitable method proved to be electrophoresis on paper in 1 *N* acetic acid. In this system, the sulphonic group largely affects the mobility of the molecule, whereas the contribution by other functional groups is slight.

EXPERIMENTAL

Fusion with sodium hydroxide

The cleavage of the sulphonic group and its replacement by a hydroxyl group is carried out by a simple method. A few milligrams of the sample and 1–2 pieces of solid sodium hydroxide are heated carefully in a test-tube over a flame until a liquid is formed. This liquid is then heated for a further 2–3 min, then cooled and dissolved in ethanol. The original sample is also dissolved in ethanol, and the two solutions are then spotted next to each other on the start of the electropherogram.

TABLE I
MOBILITIES OF SULPHONIC ACIDS BEFORE AND AFTER REACTION
Potential gradient 7 V cm⁻¹.

<i>Acid</i>	<i>Mobility before fusion ($\mu \cdot 10^5$)</i>	<i>Mobility after fusion ($\mu \cdot 10^5$)</i>	$\Delta\mu$
1-Naphthalenesulphonic	7.9	0.0	7.9
2-Naphthalenesulphonic	7.9	0.0	7.9
2,6-Naphthalenedisulphonic	16.6	0.0	16.6
1,3,5,7-Naphthalenetetrasulphonic	23.5	0.0	23.5
1-Naphthol-4-sulphonic	7.6	0.0	7.6
1-Naphthol-5-sulphonic	8.3	0.0	8.3
1-Naphthol-8-sulphonic	7.8	0.0	7.8
2-Naphthol-3,6-disulphonic	12.9	0.0	12.9
2-Naphthol-6,8-disulphonic	13.0	0.0	13.0
1,8-Dihydroxy-6,8-disulphonic	14.5	0.0	14.5
1-Naphthol-3,6,8-trisulphonic	17.6	0.0	17.6
1-Nitro-4,8-naphthalenedisulphonic	19.1	0.0	19.1
2-Nitro-4,7-naphthalenedisulphonic	17.6	0.0	17.6
2-Nitro-4,8-naphthalenedisulphonic	17.6	0.0	17.6
Anthraquinone-1-sulphonic	7.4	0.0	7.4
Anthraquinone-2-sulphonic	7.4	0.0	7.4
Anthraquinone-2,7-disulphonic	15.5	0.0	15.5
Anthraquinone-1,5-disulphonic	15.8	0.0	15.8
1-Naphthylamine-4-sulphonic	1.3	-7.2 *	8.5
1-Naphthylamine-5-sulphonic	0.9	-7.1 *	8.0
1-Naphthylamine-6-sulphonic	0.5	-7.0 *	7.5
1-Naphthylamine-8-sulphonic	4.0	-7.4 *	11.4
2-Naphthylamine-5-sulphonic	0.0	-7.6 *	7.6
2-Naphthylamine-7-sulphonic	0.0	-7.5 *	7.5
1-Naphthylamine-3,6-disulphonic	13.1	0.0	13.1
1-Naphthylamine-3,8-disulphonic	11.4	0.0	11.4
2-Naphthylamine-5,7-disulphonic	11.9	0.0	11.9
2-Naphthylamine-4,8-disulphonic	11.9	0.0	11.9
1-Naphthylamine-3,6,8-trisulphonic	17.2	0.0	17.2
2-Naphthylamine-6-hydroxy-8-sulphonic	0.5	-8.5	9.0
1,8-Aminonaphthol-3,6-disulphonic	11.6	Standard	

* Another intensive spot on the start.

Electrophoresis

The separation by electrophoresis on paper was carried out using equipment that has been described previously⁶, *viz.*, in a damp chamber. Whatman No. 2 paper of dimensions 13 × 45 cm was used, with 1 *N* acetic acid as electrolyte. A potential of 260 V was applied to the electrodes and the duration of the electrophoresis was 2 h. In order to make possible the monitoring of the course of the electrophoresis and to permit the appropriate corrections to be carried out, a standard was spotted on the start of the electropherogram, *viz.*, the so-called H-acid (1,8-aminonaphthol-3,6-disulphonic acid), which has a mobility, under the conditions used, of $11.6 \cdot 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$. The established mobilities and their mutual differences are listed in Table I.

Detection

When dried, the electropherogram is observed under UV light, and then sprayed either with a saturated aqueous solution of pinacryptol yellow or diazotized sulphanilic acid, according to the reactions of the individual substances.

DISCUSSION

If we plot the differences in the mobilities between the original sulphonic acid and the corresponding hydroxy derivative (Δu) depending on the number of sulphonic groups present, we obtain the graph illustrated in Fig. 1. It is clear that, with only one exception, the Δu values for individual numbers of SO_3H groups vary within bounds that do not overlap. The only exception involves the following acids: 1-nitro-4,8-naphthalenedisulphonic and 2-nitro-4,7- and -4,8-naphthalenedisulphonic acids, for which a strong induction effect evidently occurs owing to the presence of the nitro group and consequently an abnormal mobility.

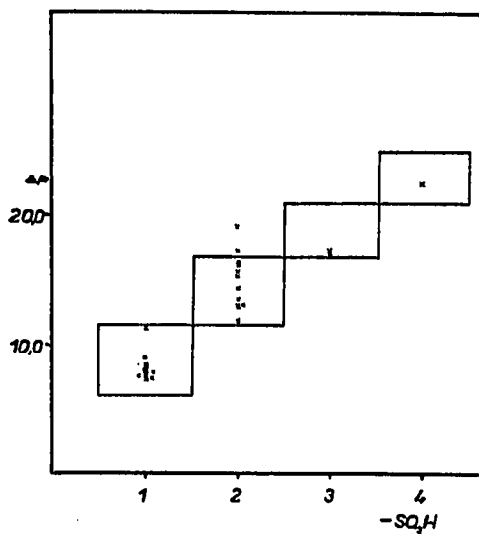


Fig. 1. Differences in mobilities (Δu) versus the number of SO_3H groups present.

The hydroxy group and the nitro group as such do not contribute towards increasing the mobility in the acetic acid medium. On the other hand, however, the amino group contributes towards a mobility opposite to that of the SO_3H group. This is the reason why aminomonosulphonic acids have virtually zero mobility, and the mobilities of aminodisulphonic acids are less than those of other disulphonic acids.

There is also another anomaly with the aminonaphthalenesulphonic acids. As a result of fusion with sodium hydroxide, a much larger portion of the substance is converted into a substance that has zero mobility (an oligomer may be formed) than into the corresponding aminonaphthol. This point must be borne in mind when investigating an unknown sulphonic acid.

The procedure can naturally be applied not only to establish the number of SO_3H groups present in the molecule, but can also serve as basic proof of its presence as such.

The Δu values, established for a certain number of SO_3H groups differ from the values previously found⁶, because, at the present stage of investigation, we had available a much larger number of experimental values.

Different detection reagents were used because sulphonic acids with different functional groups react differently with different reagents. For the identification, it is therefore expedient to spray with both reagents both the substance and the fused liquid before carrying out the electrophoresis, by spotting them on a separate strip of chromatographic paper.

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