CHROM. 6092

Identification of aromatic nitro compounds by reaction paper electrophoresis

When identifying organic compounds and when determining numbers of functional groups, it is necessary in certain cases to determine the number of nitro groups coupled to the aromatic ring. Sometimes, this number can be evaluated in a different manner, for example, from the content of nitrogen. However, in this case, it is necessary to have certain data on the compound to be identified and, naturally, it cannot contain other nitrogen-containing groups. Typically, one knows nothing about the compound, so that a method is required that is general, is as far as possible independent of other functional groups, and is simple to perform. Usually, small amounts obtained after separation by paper or thin-layer chromatography are of interest.

Recently, the authors used reaction paper chromatography for solving an analogous problem—the determination of the number of carboxylic groups bound to the benzene ring, where the original compound and that obtained after a suitable reaction (electroreduction) were applied on to the chromatographic paper. The number of carboxylic groups was determined from the difference between the R_F values[1].

The authors tried to use this principle in determining nitro groups on the basis of applying an unreduced sample in the vicinity of that obtained after the reduction. However, the use of paper chromatography was unsuccessful, as the conversion of a nitro group into an amino group resulted in a large change in the dipole moment of the molecule and in unequal shifts of the R_F values.

Paper electrophoresis was shown to be much more advantageous. The authors formerly developed a procedure[2], which can easily be used for distinguishing amino groups from other functional groups in an acetic acid medium. This method was also used in the present case, *i.e.*, the original compound containing the nitro group was applied in the vicinity of the compound obtained after reduction with zinc. The number of nitro groups was determined from differences in mobilities.

Experimental

Electrophoresis. The separation by paper electrophoresis was carried out on the same device as described recently[2], *i.e.*, a moist chamber system. Whatman No. 1 filter-paper was used and 1 N acetic acid served as the electrolyte. A potential of 300 V was maintained for 2.5 h. The distance between electrolyte levels was 46 cm. For controlling the course of the electrolytic process, *p*-phenylenediamine prepared by reduction of *p*-nitroaniline was applied on to each electropherogram.

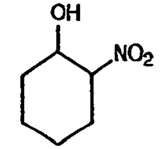
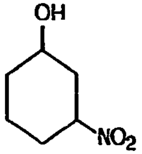
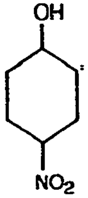
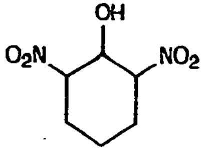
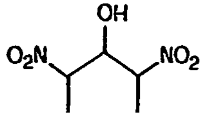
Reduction. The reduction of nitro compounds was carried out with pulverized zinc in the acetic acid or hydrochloric acid medium, which was more suitable for dissolving the sample. About 0.05–0.1 g of the compound was dissolved in 1–2 ml of 90% acetic acid (or hydrochloric acid diluted 1:2) and the mixture was boiled under a reflux condenser for 15 min. The amount of zinc added ranged from 0.5 to 1 g. After the reduction was complete, the mixture was applied directly on to the starting point of the electropherogram in the vicinity of the solution taken from the original sample.

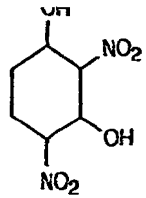
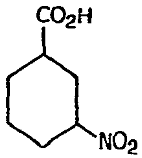
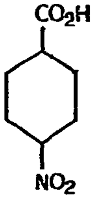
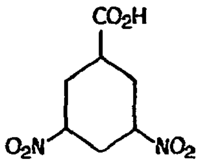
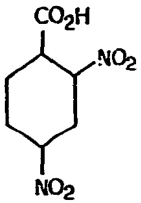
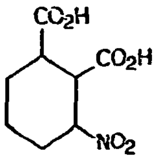
Detection. After drying, the detection on the electropherogram was carried out by spraying it with 5% tin(II) chloride solution in 2 N hydrochloric acid. The electropherogram was allowed to dry and was then sprayed with Ehrlich reagent. (During

TABLE I

VALUES OF MOBILITY, $u \cdot 10^5$ $cm^2 \cdot V^{-1} \cdot sec^{-1}$, OF NITRO COMPOUNDS BEFORE AND AFTER THE REDUCTION

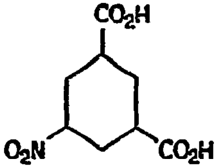
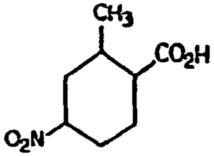
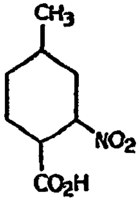
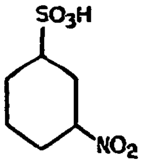
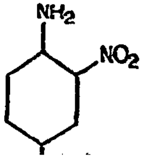
Values in parentheses are weak spots.

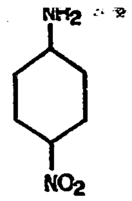
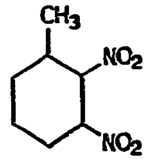
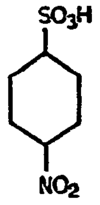
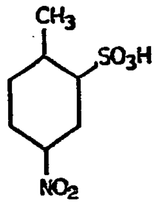
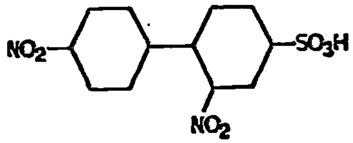
Compound	*Mobility*[b] *of the original compound, u* (NO_2)	*Mobility*[b] *of the compound after reduction, u* (NH_2)			*Δ u*			*Detection*[a]					
								D_1 (NH_2)			D_2 (NH_2)		
		Spot I	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*
	0.0	13.5			13.5			y			y		
	0.0	13.5			13.5			y			y		
	0.0	12.1			12.1			y			y		
	0.0	11.4	14.5		11.4	14.5		y	or		y	or	
	−5.3	−1.3	0.7	14.7	4.0	6.0	20.0	w br	w br	w br	w br	w br	

	−6.0	1.8	12·4	7.8	18.4	w br	w br r-br	w br	w br
	0.0	5·4		5·4		y		y or	
	0.0	6.1		6.1		y		y	
	0.0	8.2	11.5	8.2	11.5	y	or	y	or
	−5·3	(1·7)	13·0	(7.0)	18.3	(w br)y		-	y
	−2.6	1.1		3·7		or		y–or	

(*Continued on p. 160*)

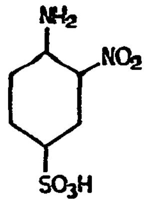
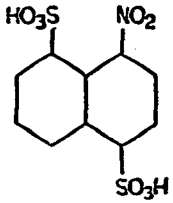
TABLE I (*continued*)

Compound	*Mobility of the original compound, $u(NO_2)$*	*Mobility of the compound after reduction, $u(NH_2)$*			*Δu*			*Detection*[a]					
								$D_1(NH_2)$			*$D_2(NH_2)$*		
		Spot I	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*
	0.0	4.0			4.0			y			or		
	0.0	7·7			7·7			y			y		
	0.0	7·7			7·7			y			y		
	−4.8	1.3			6.1			y			y-gr		
	1.1	14.8			13.6			y			y		

	1.7	18.8^c	17.1	dr	or (luminous)
	0.0	15.1	15.1	y	v
	−8.3	− 1.2	7.1	y	y
	−4.7	1.3	6.0	y	y
	−7.6	2.8	10.4	y	y

(*Continued on p. 162*)

TABLE I (*continued*)

Compound	*Mobility of the original compound, u*(NO_2)	*Mobility of the compound after reduction, u* (NH_2)			*Δ u*			*Detection*[a]					
								Dc(NH_2)			$D_2(NH_2)$		
		Spot I	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*	*Spot I*	*Spot II*	*Spot III*
	−4.1	1.6			5.7			or			y		
	−7.6	0.9			8.5			y			y-gr		

[a] Detection: D_1 = tin(II)chloride + Ehrlich reagent; D_2 + UV; y = yellow; or = orange; w = weak; br = brown; r-br = red-brown; dr = dark red; v = violet; ter = terracotta.

[b] The —sign indicates the motion to the anode.

[c] Used as a reference compound.

the evaluation, the colourations of the original and reduced compounds were also compared.)

The mobility values found are given in Table I.

Discussion

When differences between the mobilities, u, of the original and reduced compounds are plotted as a graph (Fig. 1), it can obviously be seen that, with a few exceptions, the number of nitro groups is directly proportional to the magnitude of the difference between the mobilities. The exceptions are certain nitrophenols with one nitro group and *p*-nitroaniline, which overlap into the group of compounds with two nitro groups. In many compounds, several spots were revealed, due to different intermediate degrees of reduction where first one and then the other nitro group is reduced. Various isomers can also be formed which can also be observed on the electropherogram.

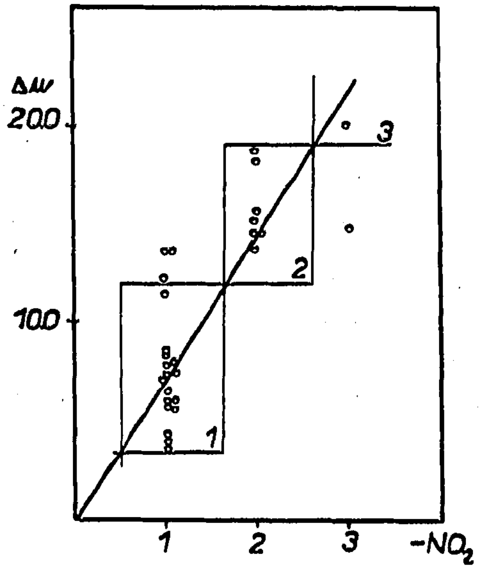


Fig. 1. Dependence of the number of nitro groups bound to the aromatic ring of the compound to be identified on the difference between the mobilities (Δu) of the original and reduced compound.

The procedure can also be used to demonstrate the existence of any nitro group. In the identification of nitrated hydrocarbons, the reduction is more difficult and the reduction products usually form bands on the electropherogram.

It is also obvious from Fig. 1 that the mobility of one amino group bound to the aromatic ring equals $7.2 \cdot 10^5$ cm^2 V^{-1}sec^{-1} in 1 N acetic acid. This represents a good agreement with the recently found[2] value of $7.1 \cdot 10^5$ cm^2 V^{-1} sec^{-1}.

The work presented here does not offer a large range of results, but it represents one part of the programme in which we are interested and which is aimed at identifying the greatest variety of functional groups with the aid of reaction chromatography.

Research Institute of Organic Syntheses, JAROSLAV FRANC
Pardubice-Rybitví (Czechoslovakia) KARLA POSPÍŠILOVÁ

1 J. FRANC AND K. POSPÍŠILOVÁ, *J. Chromatogr.*, 66 (1972) 329.
2 J. FRANC AND M. WURST, *Collect. Czech. Chem. Commun.*, 24 (1959) 857.

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