SEPARATION AND DETERMINATION OF AMINOANTHRAQUINONES BY MEANS OF THIN-LAYER CHROMATOGRAPHY

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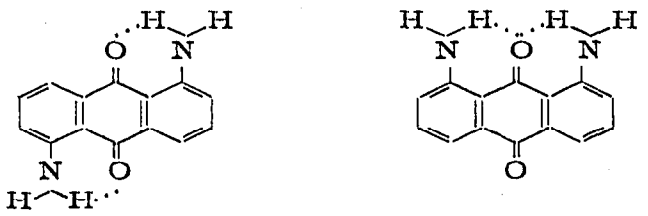
The 1,5- and 1,8-dinitroanthraquinones prepared by nitrating anthraquinone are important intermediates in the production of dyes. It is known that in this nitration process other isomers are also produced, namely 1,6-, 1,7-, 2,6- and 2,7-, together with 1- and 2-nitroanthraquinone. The qualitative separation of all these isomers has been reported earlier[1]. Petroleum-impregnated chromatographic paper was used for the separation, the R_F value of the 1,8-isomer was about 0.8, while the 1,5-isomer remained at the start.

In practice, however, this procedure is unsuitable owing to the fact that it is never known whether or not a number of other substances are remaining at the start together with the 1,5-isomer and thus escaping quantitative determination. Also, when the evaluation is done by colorimetric measurement directly on the paper, the determination of substances remaining at the start is subject to a large error.

Since the corresponding amino-derivatives are also used as intermediates in the preparation of dyes, a separation of various isomers in this form has been attempted.

The separation of the amino-derivatives, however, presents a certain difficulty. Whereas the dinitro-derivatives have widely differing R_F values due to large differences in their dipole moments, 1,5- and 1,8-diaminoanthraquinone are very difficult to separate because they have only a relatively low difference in their dipole moments which is practically totally removed by the formation of inner hydrogen bonds:



For this reason the separation of these isomers by means of paper chromatography was not successful. However, it was possible to achieve a separation which was good enough for quantitative evaluation by means of thin-layer chromatography. We have also succeeded in separating 1-aminoanthraquinone, which sometimes accompanies the diaminoanthraquinone derivatives mentioned in smaller or larger amounts, together with other isomers, such as the 1,7- and 1,6-isomers.

The quantitative evaluation of individual components is carried out by means of an instrument for the automatic evaluation of paper chromatograms in reflected light.

EXPERIMENTAL

The separation is carried out by means of chromatography on a thin layer of Al_2O_3, using glass plates of 4×15 cm size. The reason for selecting this size is that it is the maximum size for the automatic evaluation instrument used; the layer thickness is 1.5 mm.

Technical Al_2O_3 of grain size less than 100 DIN is used. The Al_2O_3 is first neutralised with HCl and after washing with water activated for 2 h at 350°.

Deactivation is carried out in a desiccator, which is first evacuated for one hour in order to remove dimethylformamide from the sample, and then water is placed in the desiccator and left there for $^1/_2$ h. The activity of aluminum oxide prepared in this way corresponds to activity III according to Brockmann. The aminoanthraquinone sample is dissolved in dimethylformamide to make an 0.2 % solution, which is then diluted with dimethylformamide to 0.04 %. 0.02 ml of this solution (*i.e.* a total of 8 μg) is placed on the start of the chromatogram. The developing solvent is a cyclohexane–ether mixture (1:1).

After separation, the solvent is allowed to evaporate and the intensity of the spots, with the chromatogram in the horizontal position, is measured by means of the Extinktions-Registriergerät with Integrator ERI-10 (Zeiss, Jena), which can work with transmitted as well as reflected light. It is possible to measure spots even in a freely poured layer of Al_2O_3. A suitable filter is used for the measurement (for aminoanthraquinones it is yellow-green, with an absorption maximum at about 510 mμ). Fig. 1 shows a typical result for a mixture of diaminoanthraquinones.

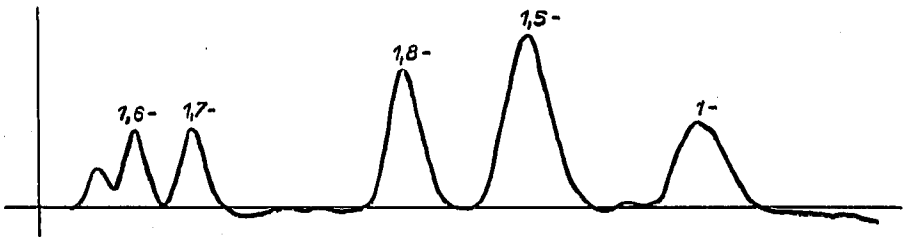


Fig. 1. Densitometer recording after chromatographic separation of various aminoanthraquinone isomers.

Since it has been found that at the concentrations used there is a linear relationship between the area of the spots and the concentration, it is sufficient to determine the ratio of the areas of individual pure components mixed in the same weight ratio.

The magnitude of the coefficient of variation, which has a mean value of $\pm$ 6 %, has been determined by statistical evaluation of results obtained for synthetic mixtures.

RESULTS AND DISCUSSION

In the course of our work several thousand determinations have been carried out and thus considerable experience with the method has been gained. It was found that it is very important to work with a suitable activity, or otherwise the spots will be smudged and overlap due to the relatively close R_F values of 1,5- and 1,8-diaminoanthraquinone.

Originally, the Al_2O_3 was deactivated by simply exposing the layer to atmospheric moisture, which was measured by means of a hygrometer. This method, however, was not suitable, since at 95% relative humidity deactivation took 4 h, and at 75% took $1^1/_2$days. The deactivation is therefore carried out in a moist chamber. This also protects aluminum oxide from contamination by other substances in the laboratory atmosphere, which might interfere with the separation process. It is also possible to evacuate the desiccator first and thus remove dimethylformamide, whose presence interferes in the separation. The aluminum oxide must not be basic.

Owing to the high volatility of one of the components of the developing mixture it is necessary to work as far as possible at temperatures around 20°.

Quantitative evaluation was possible with a Zeiss densitometer, which measures the chromatograms in a horizontal position and can work with transmitted as well as reflected light. It was designed originally for electropherograms, and we believe there has been no previous report of its application to thin-layer chromatograms.

The mixtures most frequently analysed contained the following components: 1,5-, 1,8-, 1,6- and 1,7-diaminoanthraquinone and 1-aminoanthraquinone. The relatively high coefficient of variation is caused by the lack of precision of the determination of 1-aminoanthraquinone and 1,7-diaminoanthraquinone. In the case of 1-aminoanthraquinone this is caused by the continuity of the aluminum oxide layer being slightly disturbed during the process of development, so that behind the 1-aminoanthraquinone the zero line is slightly distorted. 1,7-Diaminoanthraquinone is close to the starting line, where impurities, probably hydroxyanthraquinones or amino-hydroxyanthraquinones, are always present and interfere in the determination of the zero line of this isomer. 2-Aminoanthraquinone is usually also present, but mostly in small amounts, and is difficult to separate from 1,8-diaminoanthraquinone.

One advantage of this aminoanthraquinone separation is that owing to the fact that these substances are self-coloured, it is unnecessary to use any further means of detection of the spots. After removal of the plate from the development chamber the colour is relatively intense, but weakens after evaporation of the solvent; after that, however, it does not change for more than 24 h.

Although the precision of the determination is not very great, the error may be considerably decreased by repeated determinations. It is, furthermore, the only method for the determination of the 1,5- and 1,8-isomers in the presence of each other. It would be advantageous if the chromatographic separation could be performed on larger plates, but unfortunately we are limited by the dimensions of the densitometer. To increase precision, especially where the ratios of the components are unfavourable, it is recommended that a synthetic mixture, whose composition corresponds approximately to that of the sample, is analysed at the same time. Thus it is possible to determine which of the components causes the greatest error.

The time for the complete analysis is relatively short, the chromatographic separation taking about 10 min, and evaluation on the densitometer about 2.5 min. It is unnecessary to state that all operations must be performed with the utmost care, since this is an ultramicroanalysis.

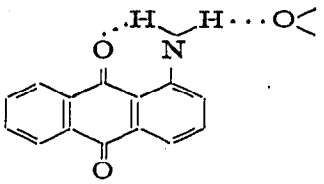
From Table I it would seem that the separation process is mainly controlled by the formation of hydrogen bonds. For example, 1-aminoanthraquinone has a high R_F value, since it has an internal hydrogen bond, and thus cannot form an intermolecular bond with the immobile phase; 2-aminoanthraquinone, on the other hand, has a far

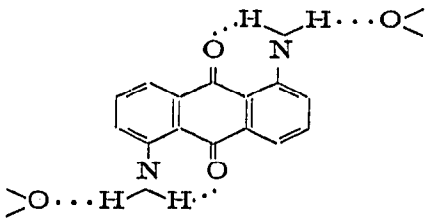
TABLE I

R_F VALUES OF AMINOANTHRAQUINONES

Anthraquinone	R_F	*Colour of spot*	*Number of hydrogen bonds (O···H–N)*
1-Amino-	0.62	orange	1
2-Amino-	0.23	yellow	0
1,2-Diamino-	0.065	violet	1
1,4-Diamino-	0.10	violet	2
1,5-Diamino-	0.46	orange-red	2
1,8-Diamino-	0.35	red-violet	2
2,6-Diamino-	0.00	orange-brown	0
1,6-Diamino	0.08	red	1
1,7-Diamino	0.14	orange	1

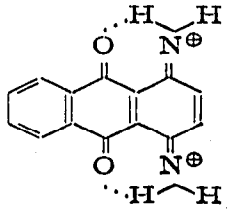
lower R_F value (R_F = 0.39), due to the absence of an internal hydrogen bond. The case of the diaminoanthraquinones is similar, the isomers which have two internal hydrogen bonds having higher R_F values than those which have only one, and these again have higher R_F values than isomers which have no internal hydrogen bond. 1-Aminoanthraquinone still, however, has a higher R_F value than the diaminoanthraquinones which have two internal hydrogen bonds, owing to the fact that only one hydrogen atom of the $-NH_2$ group forms a chelate bond:





Thus, the diaminoanthraquinones are more strongly bound to the stationary phase, in this case to water.

In the case of diaminoanthraquinones which have two internal hydrogen bonds, it is possible, up to a certain measure, to assess the energy of this bond from the R_F values. The 1,5-isomer has the highest R_F value, because the energy of both bonds is equal. In the 1,8-isomer, both protons form a chelate bond with the same oxygen, resulting in the fact that the second bond is weaker due to electrons being exhausted by the first bond, so that a part of this bond participates in the intermolecular bond with the immobile phase. It is interesting to compare the 1,4- and 1,5-isomers, whose R_F values ought to be equal. In fact, the R_F value of the 1,4-isomer is lower. This would seem to show that the second hydrogen bond is not formed, or if it is formed then the energy of the intermolecular hydrogen bond is far greater than would correspond to the structure:



The colour of the individual isomers is also related to the number of internal hydrogen bonds. It seems that the colour is darker with a larger number of internal hydrogen bonds. Comparison is also possible with respect to substitution in the 1- and 2-positions, since 1-substituted isomers are darker.

Another point of interest is the chromatographic equivalent of the hydrogen bond for this system, *viz.* aluminum oxide saturated with water and a cyclohexane–ether mobile phase. Considering that the energy of the hydrogen bond in 1-aminoanthraquinone is 4.8 kcal/mole (ref. 2), we obtain for the chromatographic equivalent of the hydrogen bond a value of $R_E = 0.65$ kcal/mole, the same value as was calculated for most separations (with an immobile aqueous phase; $R_E = 0.59 \pm 0.05$ kcal/mole) in paper chromatography.

SUMMARY

A procedure for the separation and determination of mono- and di-aminoanthraquinones has been developed. The separation is carried out on a thin layer of poured granular Al_2O_3, and the colorimetric evaluation of spots carried out with a Zeiss Type ERI-10 instrument. The precision of the method is $\pm$ 6 %.

REFERENCES

1 J. FRANC, *Chem. Listy*, 49 (1955) 872; *Collection Czech. Chem. Commun.*, 20 (1955) 1384.
2 J. FRANC, *Chem. Listy*, 52 (1958) 13; *Collection Czech. Chem. Commun.*, 24 (1959) 250.