

CHROM. 493I

**Argentation thin-layer chromatography with silver oxide\*****II. Amines, unsaturated and aromatic carboxylic acids**

Recently a method of argentation thin-layer chromatography (TLC), with silver oxide on silica gel was shown to separate pyridine and some of its homologs<sup>1</sup>. For isomeric compounds, the order of the  $R_F$  values was shown to be the same as that of the  $pK_a$  values, the dipole moments, or the stability constants of known silver ion complexes of the corresponding pyridine homologs.

As argentation TLC using silver nitrate has been used for the separation of fatty acids, lipids, steroids, etc.<sup>2</sup>, we tried to develop the method with  $Ag_2O$  for several other classes of compounds. As it has the advantage of allowing the use of a more polar solvent than chromatography with  $AgNO_3$ , it was thought that compounds such as amines and acids might be separated, and probably many others, as well as the compounds known to be separated in other forms of argentation chromatography.

*Preparation of the plates*

The  $Ag_2O$  plates were prepared as previously described<sup>1</sup>.  $Ag_2O$ /Silica Gel GF<sub>254</sub> (Merck) plates were also prepared in the same way as some compounds can easily be identified by UV absorption on a fluorescent background, even with  $Ag_2O$  plates. In this work, only activated plates were used. The activation time was reduced to 45 min, giving better plates.

TABLE I

$R_F \times 100$  VALUES OF SUBSTITUTED BENZOIC ACIDS, AND PHTHALIC, MALEIC AND FUMARIC ACIDS  
Solvent I: ethanol-ethyl acetate-conc.  $NH_4OH$  (9:3:2); solvent II ethanol-water-conc.  $NH_4OH$  (10:1.2:1.6). The solvents were selected by preliminary scanning. The detection of the spots was by UV.

| Substituent    | Solvent I                                 |                                 | Solvent II                                |                                 |
|----------------|---|---------------------------------|---|---------------------------------|
|                | Silica Gel<br>GF <sub>254</sub> + $Ag_2O$ | Silica Gel<br>GF <sub>254</sub> | Silica Gel<br>GF <sub>254</sub> + $Ag_2O$ | Silica Gel<br>GF <sub>254</sub> |
| 2,6-di-OH      | 90  | 92                              | 92  | 94                              |
| 3,5-di- $NO_2$ | 88  | 91                              | 91  | 92                              |
| 2-OH           | 82  | 88                              | 86  | 90                              |
| H              | 70  | 77                              | 73  | 81                              |
| 2,4-di-OH      | 65  | 83                              | 69  | 85                              |
| 3-OH           | 59  | 78                              | 68  | 82                              |
| 4-OH           | 50  | 80                              | 64  | 84                              |
| Fumaric acid   | 26  | 41                              | 38  | 61                              |
| Phthalic acid  | 20  | 30                              | 29  | 46                              |
| Maleic acid    | 14  | 22                              | 19  | 33                              |

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*Carboxylic acids*

We investigated some hydroxybenzoic acids, and phthalic, maleic and fumaric acids to test the method. The  $R_F$  values, solvents, and methods of detection are given in Table I.

The order of  $R_F$  values on the  $Ag_2O$  plates is 2,6-di-OH > 3,5-di-NO<sub>2</sub> > 2-OH > benzoic > 2,4-di-OH > 3-OH > 4-OH in both solvents (the numbers indicating substituents in the benzoic acid) and the separations are good. This is the same order as the acidity of the compounds and the inverse order of the  $pK_a$  values<sup>5-7</sup> and this order is maintained even if we include the dicarboxylic acids, if the  $pK_a$  value for the second carboxyl group<sup>6</sup> is used, giving: 4-OH > fumaric > phthalic > maleic, for the  $R_F$  values, and the inverse for the  $pK_a$  values.

It seems that the more dissociated the carboxylic acid is in our solvents (the carboxylate ion is stabilized by resonance or hydrogen bonding or both<sup>3</sup>) the further it is carried on the  $Ag_2O$  plate.

The order of dipole moments<sup>4</sup> (4-hydroxy > 2-hydroxy > 3-hydroxy; fumaric acid > 3-hydroxy > maleic acid > phthalic acid) is different from the order of the  $R_F$  values, but would explain the "anomalous"  $R_F$  of 2,4-dihydroxybenzoic acid. Considering the  $pK_a$  values of 2-hydroxy-, 4-hydroxybenzoic and benzoic acids, the difference in  $pK_a$  values, in  $pK_a$  units, is: 2-OH - H = -1.17 4-OH - H = +0.36.

TABLE II

$pK_a$  VALUES OF SUBSTITUTED BENZOIC ACIDS, AND PHTHALIC, MALEIC, AND FUMARIC ACIDS

| <i>Substituent</i>       | <i>pK<sub>a</sub></i> |
|--------------------------|-----------------------|
| 2,6-di-OH                | 1.30 <sup>a</sup>     |
| 3,5-di-NO <sub>2</sub>   | 2.7-2.9 <sup>b</sup>  |
| 2-OH                     | 3.00 <sup>a</sup>     |
| H                        | 4.17 <sup>d</sup>     |
| 3-OH                     | 4.08 <sup>c</sup>     |
| 4-OH                     | 4.53 <sup>c</sup>     |
| Fumaric acid ( $pK_2$ )  | 4.38 <sup>d</sup>     |
| Phthalic acid ( $pK_2$ ) | 5.408 <sup>d</sup>    |
| Maleic acid ( $pK_2$ )   | 6.23 <sup>d</sup>     |

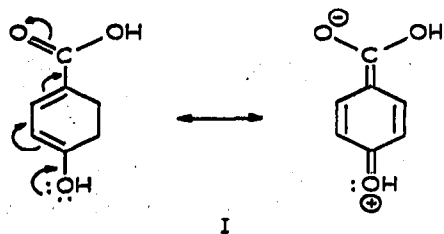
<sup>a</sup> Ref. 3. 2,4-Dihydroxybenzoic acid should have a  $pK_a$  value between 2-hydroxybenzoic and benzoic acids.

<sup>b</sup> Calculated from the known effect of NO<sub>2</sub> as substituent.

<sup>c</sup> Ref. 6.

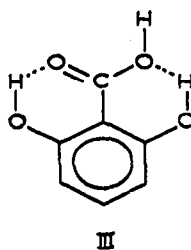
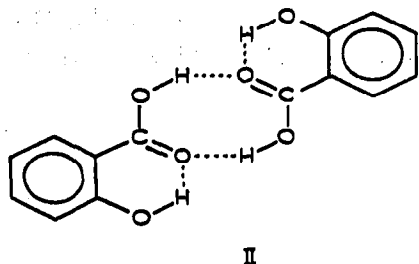
<sup>d</sup> Ref. 7.

If there is additivity of the  $pK_a$  values, which is a reasonable hypothesis, the  $pK_a$  of 2,4-dihydroxybenzoic acid, having the contributions of the 2- and 4-hydroxy, should be approximately  $4.17 - 1.17 + 0.36 = 3.36$ , *i.e.*, between 2-hydroxybenzoic acid and benzoic acid itself. That the  $R_F$  is between that of benzoic acid and 3-hydroxybenzoic acid can be understood if we consider the polarity of the compounds. The 4-hydroxy group, in the benzoic acid group, is an electron donor as the  $pK_a$  of 4-hydroxybenzoic acid clearly shows, and could be expected from resonance form (I) that opposes dissociation of the benzoic acid. The direction of electron movement



determines an increase in the dipole moment of the compound, and the increase in polarity has a lowering effect on the  $R_F$  value.

It should be noted that the dipole moments of the mono-hydroxybenzoic acids are in the order expected from the direction of the dipole moments (electronic effects) of the substituent groups. The order of the  $pK_a$  values is different because of hydrogen bonding, polar effects of substituents, and association<sup>3</sup> giving structures like II and III for 2-hydroxybenzoic acid and 2,6-dihydroxybenzoic acid and increasing the acidity of both to a value higher than the non-hydrogen-bonding forms would have.

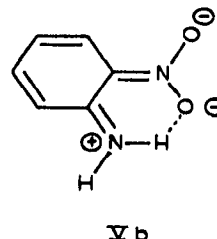
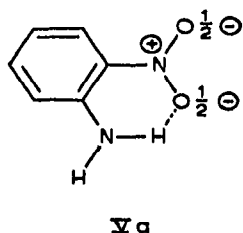
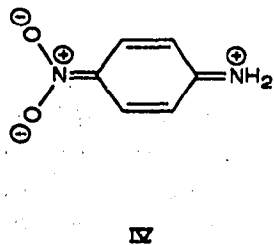


The inverse order of  $pK_a$  values shows that complexing has a major role in the mechanism of the chromatography of the investigated compounds.

### Amines

We studied the chromatography of toluidines, chloroanilines and nitroanilines, comparing the *o*-, *m*-, and *p*-isomers in each case. This gives us a good understanding of substituent effect, as compared with aniline. In Table III the  $R_F$  values, solvents and method of identification are given.

The analysis of the  $R_F$  values on  $Ag_2O$  on Silica Gel GF<sub>254</sub> plates shows clearly that, as in the case of the pyridines<sup>1</sup> and carboxylic acids, complexation ( $R_F$  values



in the inverse order of  $pK_a$  values) is always present but, as the effect of substituents on polarity is quite different for Me (+I effect), Cl (-I effect) and  $NO_2$  (-I, -M) and the steric effect of the substituents in the *o*-position is also important, there is always

TABLE III

 $R_F \times 100$  OF SUBSTITUTED ANILINES

Solvent I: petroleum ether-ethyl acetate (1:1); solvent II: petroleum ether-ethyl acetate-benzene (6:3:1). Detection was done under a UV lamp or with ninhydrin reagent. If the chromatogram on  $Ag_2O$  was dried after development and left on the table, without further treatment, dark spots developed slowly for the toluidines and chloroanilines. The nitroanilines, if in a high concentration, could be seen as yellow spots.

| Substi-<br>tuent          | Solvent I                        |                          | Solvent II                       |                          |
|---------------------------|----------------------------------|--------------------------|----------------------------------|--------------------------|
|                           | Silica Gel<br>$GF_{254} + Ag_2O$ | Silica Gel<br>$GF_{254}$ | Silica Gel<br>$GF_{254} + Ag_2O$ | Silica Gel<br>$GF_{254}$ |
| <i>o</i> -Cl              | 93                               | 90                       | 79                               | 82                       |
| <i>o</i> -NO <sub>2</sub> | 85                               | 81                       | 68                               | 65                       |
| <i>o</i> -CH <sub>3</sub> | 73                               | 79                       | 52                               | 65                       |
| <i>m</i> -Cl              | 79                               | 83                       | 65                               | 68                       |
| <i>m</i> -NO <sub>2</sub> | 69                               | 65                       | 50                               | 48                       |
| <i>m</i> -CH <sub>3</sub> | 62                               | 76                       | 41                               | 59                       |
| <i>p</i> -Cl              | 60                               | 68                       | 35                               | 50                       |
| <i>p</i> -NO <sub>2</sub> | 56                               | 59                       | 32                               | 37                       |
| <i>p</i> -CH <sub>3</sub> | 52                               | 71                       | 30                               | 52                       |
| H                         | 61                               | 75                       | 38                               | 58                       |

competition between the  $pK_a$  value and the dipole moment  $\mu$ , the more polar compounds tending to have a lower  $R_F$  value.

Taking both these effects into account the order of the  $R_F$  values can easily be rationalized, and used as a measure of the extent of substituent effects.

The order of  $R_F$  values is: *o*-Cl > *o*-NO<sub>2</sub> > *m*-Cl > *o*-CH<sub>3</sub> > *m*-NO<sub>2</sub> > *m*-CH<sub>3</sub> > H > *p*-Cl > *p*-NO<sub>2</sub> > *p*-CH<sub>3</sub>. It is interesting that for all 3 series, we had the  $R_F$  values in the order *o* > *m* > *p*. For the toluidines and chloroanilines, this is the inverse order of the  $pK_a$  values, but is not so for the nitroanilines, for which the  $pK_a$  values are in the order *m* > *p* > *o*<sup>6,7</sup>, because of the resonance and steric effects in the *p* and *o*

TABLE IV

 $pK_a^*$  VALUES AND DIPOLE MOMENTS ( $\mu$ ) OF SUBSTITUTED ANILINES

The  $pK_a$  values were taken from ref. 6, pp. 593-594 except for aniline, which was taken from ref. 7. The dipole moments are from ref. 6, p. 506; aniline and nitroanilines were taken from ref. 4; to have comparable results for each series, data was taken from the same author, wherever possible. References to the original articles are given in the above books.

| Substi-<br>tuent          | $pK_a$ | $\mu$ |
|---------------------------|--------|-------|
| <i>o</i> -CH <sub>3</sub> | 4.42   | 1.58  |
| <i>m</i> -CH <sub>3</sub> | 4.73   | 1.44  |
| <i>p</i> -CH <sub>3</sub> | 5.08   | 1.31  |
| <i>o</i> -Cl              | 2.62   | 1.77  |
| <i>m</i> -Cl              | 3.32   | 2.66  |
| <i>p</i> -Cl              | 3.81   | 2.97  |
| <i>o</i> -NO <sub>2</sub> | -0.28  | 4.45  |
| <i>m</i> -NO <sub>2</sub> | 2.45   | 4.72  |
| <i>p</i> -NO <sub>2</sub> | 0.98   | 7.10  |
| H                         | 4.58   | 1.52  |

positions, respectively, giving rise to the structures IV<sup>8</sup> and V. For the chloroanilines and nitroanilines, the  $R_F$  values are in the inverse order of the dipole moments<sup>4</sup> which gives a reasonable explanation, the more polar isomer having a lower  $R_F$ . Thus the order for the toluidines can be explained by the inverse  $pK_a$  order, *i.e.*, the isomer having more complexing power having a lower  $R_F$ .

The order of dipole moments for chloro- and nitroanilines is  $p\text{-NO}_2 > m\text{-NO}_2 > o\text{-NO}_2 > p\text{-Cl} > m\text{-Cl} > o\text{-Cl}$ ; if we take the  $R_F$  values of each pair of anilines substituted by Cl and NO<sub>2</sub> in the same position, we have, as expected from the polarities:  $o\text{-Cl} > o\text{-NO}_2$ ,  $m\text{-Cl} > m\text{-NO}_2$ ,  $p\text{-Cl} > p\text{-NO}_2$ .

From the discussion above it is also easy to deduce that for each position, *o*, *m*, and *p*, we will always have  $-\text{Cl} > -\text{NO}_2 > -\text{CH}_3$  for the  $R_F$  values. The fact that *m*-chloroaniline has a higher  $R_F$  than *o*-toluidine is only a consequence of the  $R_F$  values of the chloroanilines obeying the inverse order of polarities, while the  $R_F$  values of the toluidines obey the inverse order of the  $pK_a$  values. Aniline itself can be included in the toluidine series, with a  $pK_a$  higher than *o*-toluidine (steric effect) and lower than *m*- and *p*-toluidine (inductive effect).

What is more difficult to understand is that in the series of Cl and NO<sub>2</sub> substituted compounds, which seem to obey an inverse polarity law, when the substituent is at the same position, the  $R_F$  values of the *o*- and *m*-nitroanilines are higher than that of *m*- and *p*-chloroanilines, respectively. For *o*-nitroaniline it could be explained that the  $pK_a$ , which is much lower than all the others, is the reason for the compound being in the second place according to  $R_F$  value, because of its high polarity. For *m*-nitroaniline, where the resonance effect is supposed to affect the  $pK$  and polarity only to a small extent and the ratio of  $pK_a/\mu$  is not so small, the explanation is not so good.

There are some small differences, especially in the position of the toluidines, on the pure silica gel plates, but in each series the relative positions are maintained, showing that, apart from the complexing, some of the mechanisms are the same in both cases.

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