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Paper Chromatography of Amines

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(Received 16 February 1951)

Although the method of paper chromatography has been employed successfully for the separation of amino-acids, sugars, purines, pyrimidines, phenolic compounds and other naturally occurring substances, relatively few attempts have so far been made to apply it to the separation and identification of amines. The behaviour of various primary aromatic amines on paper chromatograms has received some attention (Ekman, 1948; Tabone, Robert & Troestler, 1948; Tabone & Robert, 1948; Kelemen, Tanos & Halmagyi, 1950), but of the non-aromatic amines only the following groups of biologically important amines have been successfully separated: creatine, creatinine and their demethylated analogues (Maw, 1948; Ames & Risley, 1948); histamine and acetylhistamine (Urbach, 1949); adrenaline, noradrenaline and methyladrenaline (James, 1948); histamine, glucosamine, chondrosamine, choline, and ethanolamine (Dent, 1949; Partridge, 1948; Chargaff, Levine & Green, 1948; Aminoff, Morgan & Watkins, 1950).

The need for a rapid method of identifying small amounts of amines in biological materials became evident during the course of investigations on the metabolism of amino-acids in soil and in studies on plant amine oxidase. The results presented in this paper suggest that this problem can be solved by application of paper chromatography. The \( R_f \) values in various solvents of a large number of amines, including those of two homologous series (n-alkyl monoamines and polymethylene diamines), have been determined.

The results obtained with the two homologous series studied provided an opportunity to test the deduction (Martin, 1949) that the addition of a given group to a molecule will change its partition coefficient in a ratio depending only on the group and the phase pair concerned. From this it follows that the same ratio will relate the partition coefficients of all neighbouring pairs of a homologous series. Bate-Smith & Westall (1950) have recently shown that this deduction holds well enough for hydroxyl and glucose groups in a large number of phenolic substances. They tested the validity of the deduction by plotting \( R_f \) against the number of groups of the chromatogram, against the number of groups and obtained series of approximately parallel straight lines for several homologous series. The same method was used in the present work.

METHODS

Apparatus. The apparatus used for determining the \( R_f \) values of the substances was similar to that described by Dent (1948). The only modification was that the lid of the cabinet had a small hole fitted with a rubber bung which could be removed to permit introduction of solvent into the trough.

Procedure. The amines were normally used in the form of 0.1 M aqueous solutions of their hydrochlorides. Where the hydrochlorides were not available aqueous solutions of the free bases were neutralized with HCl to pH 7.0 and diluted to 0.1 M. Agmatine was used in the form of an 0.1 M aqueous solution of its sulphate.

1–3 \( \mu \)l of the amine solutions, containing 0–40 \( \mu \)g of each amine, were applied at intervals of 3 cm to a horizontal line ruled 6 cm. from the top of a large sheet of Whatman no. 4 filter paper. The papers were allowed to dry and then equilibrated overnight in the cabinet with the vapour of the aqueous phase of the solvent-water mixture to be used for irrigation. After this preliminary equilibration the solvent was added through the hole in the lid, and irrigation was continued until the leading edge of the solvent had travelled a distance of 35–45 cm. from the line of application. Depending on the solvent used the time required was 7–24 hr. The sheets were then removed from the cabinet, dried at room temperature or in an oven at 105\(^\circ\)C, sprayed with a 0.1 % (w/v) solution of ninhydrin in CH\(_2\)Cl\(_2\) and placed in an oven at 105\(^\circ\)C for 5 min. The positions of the solvent fronts were marked immediately after removal of the sheets from the cabinet. \( R_f \) values reported were determined at 16\(^\circ\) ± 2\(^\circ\).

Solvents. (All percentages are v/v.) The solvents used were phenol, 'collidine', n-butanol, n-butanol-acetic acid and m-cresol-acetic acid. The 'collidine' mixture used was that described by Dent (1948), consisting of equal volumes of 2:4-lutidine and 2:4:6-collidine saturated with water. The n-butanol (40 %)-acetic acid (10 %)-water (50 %) mixture was that employed by Partridge (1948) for the separation of sugars. Before use this mixture was shaken thoroughly and allowed to stand for 3 days (Bate-Smith & Westall, 1950). The m-cresol (50 %)-acetic acid (2 %)-water (48 %) mixture used was that described by Bate-Smith & Westall (1950). Phenol runs were carried out both in the presence of NH\(_3\) and HCN, and in an atmosphere saturated with 50 % aqueous acetic acid; 'collidine' runs were conducted in the presence of diethylamine (Dent, 1948).
RESULTS

$R_p$ values of various amines in the solvents tested are recorded in Table 1. They were obtained with one batch of filter paper and represent the means of several determinations. In agreement with Bate-Smith (1949) the values were found to vary from batch to batch of filter paper, but to be reasonably constant within individual batches. Although the $R_p$ values obtained with other batches of Whatman no. 4 paper were somewhat different, the degree of separation obtained was similar.

Small well defined spots were obtained using butanol or butanol-acetic acid as solvent; somewhat larger, elongated spots were obtained when phenol or m-cresol-acetic acid was used as solvent.

![Chromatogram](image)

**Fig. 1.** Chromatogram on Whatman no. 4 filter paper of primary alkylamines run in butanol-acetic acid and sprayed with ninhydrin. Before photographing, the visible areas of the spots were outlined with pencil.

The tint and intensity of the colours obtained on spraying the chromatograms with ninhydrin were found to depend, to some extent, on the solvent used for irrigation. The colours were strongest when m-cresol-acetic acid was used as solvent and weakest when 'collidine' was employed. No attempt was made to determine the lower limit of sensitivity for the detection of each amine. However, the ninhydrin colour reaction seemed to occur as readily with the primary amines tested as with $\alpha$-amino-acids, 10 $\mu$g. of most of the compounds being easily detectable. With the exception of ephedrine, the secondary amines tested gave much weaker colours with ninhydrin than did the primary amines. Trimethylamine, hordenine, choline and urea could not be detected with ninhydrin. Weak yellow spots of identical $R_p$ value were obtained with ninhydrin when hydroxyamine and hydrazine were run in butanol ($R_p$ 0.02) and butanol-acetic acid ($R_p$ 0.12).

Butanol-acetic acid proved to be the most effective solvent for the separation of the primary $n$-alkylamines (see Fig. 1). The latter did not appear on chromatograms run in 'collidine', presumably owing to their loss by volatilization under the basic conditions employed.

Phenol alone and m-cresol-acetic acid were found to be the most satisfactory solvents for separating the polymethylene diamines.

It is obvious from Table 1 that useful separations of the other amines tested can be achieved by the choice of suitable solvents.

**Fig. 2.** $R_M = \log_{10} \left( \frac{1}{R_p} - 1 \right)$ values in various solvents of primary $n$-alkyl monoamines (-----) and primary polymethylene diamines(------). O, n-butanol; x, n-butanol-acetic acid; □, m-cresol-acetic acid; △, phenol. For details of solvent mixtures and experimental conditions see text.

The $R_M = \log_{10} \left( \frac{1}{R_p} - 1 \right)$ values for members of the two homologous series of amines studied in those solvents in which there were appreciable differences in $R_p$ within the series are plotted against the number of methylene groups in Fig. 2. The results obtained for the diamines with phenol in an atmosphere of acetic acid are not included since, under the experimental conditions employed, the solvent-water mixture and the atmosphere may not have been in equilibrium. The curve in this case showed a sharp break.

DISCUSSION

The results presented above show that a large number of amines can be separated and identified by the paper-chromatographic technique. The use of ninhydrin for the detection of the amines studied again raises the question of the specificity of the
Table 1. *R*<sub>f</sub> values of amines in various solvents on Whatman no. 4 paper at room temperature

<table>
<thead>
<tr>
<th>Solvent (% v/v)</th>
<th>n-Butanol</th>
<th>n-Butanol (40%), acetic acid (10%), water (50%)</th>
<th>‘Collidine’</th>
<th>m-Cresol (50%), acetic acid (2%), water (48%)</th>
<th>Phenol</th>
<th>Phenol</th>
<th>Phenol</th>
<th>Colour with ninhydrin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylamine</td>
<td>0.10</td>
<td>0.37</td>
<td>—</td>
<td>0.52</td>
<td>0.96</td>
<td>0.91</td>
<td>0.72</td>
<td>P</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>0.18</td>
<td>0.45</td>
<td>—</td>
<td>0.67</td>
<td>0.95</td>
<td>0.94</td>
<td>0.80</td>
<td>P</td>
</tr>
<tr>
<td>n-Propylamine</td>
<td>0.28</td>
<td>0.58</td>
<td>—</td>
<td>0.76</td>
<td>0.97</td>
<td>0.98</td>
<td>0.86</td>
<td>P</td>
</tr>
<tr>
<td>n-Butylamine</td>
<td>0.31</td>
<td>0.70</td>
<td>—</td>
<td>0.86</td>
<td>0.96</td>
<td>0.98</td>
<td>0.91</td>
<td>P</td>
</tr>
<tr>
<td>n-Amyleamine</td>
<td>0.53</td>
<td>0.77</td>
<td>—</td>
<td>0.89</td>
<td>0.96</td>
<td>0.98</td>
<td>0.92</td>
<td>P</td>
</tr>
<tr>
<td>n-Heptylamine</td>
<td>0.58</td>
<td>0.85</td>
<td>—</td>
<td>0.93</td>
<td>0.95</td>
<td>0.98</td>
<td>0.94</td>
<td>P</td>
</tr>
<tr>
<td>iso-Propylamine</td>
<td>0.27</td>
<td>0.57</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>P</td>
</tr>
<tr>
<td>iso-Amyleamine</td>
<td>0.52</td>
<td>0.77</td>
<td>—</td>
<td>0.97</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>P</td>
</tr>
<tr>
<td>1:2-Diaminoethane</td>
<td>0.02</td>
<td>0.14</td>
<td>0.20</td>
<td>0.02</td>
<td>0.93</td>
<td>0.23</td>
<td>0.18</td>
<td>P</td>
</tr>
<tr>
<td>1:3-Diaminopropene</td>
<td>0.02</td>
<td>0.15</td>
<td>0.30</td>
<td>0.03</td>
<td>0.93</td>
<td>0.35</td>
<td>0.25</td>
<td>P</td>
</tr>
<tr>
<td>1:4-Diaminobutane (putrescine)</td>
<td>0.02</td>
<td>0.16</td>
<td>0.20</td>
<td>0.06 (0.38)‡</td>
<td>0.94</td>
<td>0.89</td>
<td>0.45</td>
<td>P</td>
</tr>
<tr>
<td>1:5-Diaminopentane (cadaverine)</td>
<td>0.02</td>
<td>0.17</td>
<td>0.22</td>
<td>0.09 (0.50)‡</td>
<td>0.94</td>
<td>0.94</td>
<td>0.59</td>
<td>P</td>
</tr>
<tr>
<td>1:6-Diaminohexane</td>
<td>0.03</td>
<td>0.20</td>
<td>0.24</td>
<td>0.14 (0.64)‡</td>
<td>0.94</td>
<td>0.97</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>Benzyamine</td>
<td>0.38</td>
<td>0.68</td>
<td>0.79</td>
<td>0.86</td>
<td>0.95</td>
<td>0.98</td>
<td>0.91</td>
<td>RP§</td>
</tr>
<tr>
<td>β-Phenylethylamine</td>
<td>0.51</td>
<td>0.72</td>
<td>0.80</td>
<td>0.88</td>
<td>0.95</td>
<td>0.97</td>
<td>0.91</td>
<td>B</td>
</tr>
<tr>
<td>ββ-Diphenylethylamine</td>
<td>0.50</td>
<td>0.72</td>
<td>0.80</td>
<td>0.86</td>
<td>0.94</td>
<td>0.97</td>
<td>0.90</td>
<td>B</td>
</tr>
<tr>
<td>β-Phenyl-β-hydroxyethylamine</td>
<td>0.35</td>
<td>0.65</td>
<td>0.79</td>
<td>0.84</td>
<td>0.94</td>
<td>0.96</td>
<td>0.86</td>
<td>P</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>0.14</td>
<td>0.45</td>
<td>Streak</td>
<td>0.58</td>
<td>—</td>
<td>0.91</td>
<td>0.74</td>
<td>P</td>
</tr>
<tr>
<td>Agmatine</td>
<td>0.00</td>
<td>0.05</td>
<td>0.16</td>
<td>0.06 (0.64)‡</td>
<td>0.94</td>
<td>0.60 (0.93)‡</td>
<td>0.42 (0.75)‡</td>
<td>P</td>
</tr>
<tr>
<td>Allyamine</td>
<td>0.03</td>
<td>0.50</td>
<td>—</td>
<td>0.14</td>
<td>0.95</td>
<td>0.95</td>
<td>0.86</td>
<td>P</td>
</tr>
<tr>
<td>Ethanamine</td>
<td>0.09</td>
<td>0.33</td>
<td>0.44</td>
<td>0.47</td>
<td>0.90</td>
<td>0.85</td>
<td>0.65</td>
<td>P</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>0.12</td>
<td>0.43</td>
<td>—</td>
<td>—</td>
<td>0.97</td>
<td>0.95</td>
<td>0.95</td>
<td>P</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>0.33</td>
<td>0.75</td>
<td>0.86</td>
<td>0.95</td>
<td>0.94</td>
<td>0.96</td>
<td>0.94</td>
<td>P</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>0.04</td>
<td>0.24</td>
<td>0.50</td>
<td>0.03</td>
<td>0.67</td>
<td>0.42</td>
<td>0.30</td>
<td>P</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.03</td>
<td>0.19</td>
<td>0.44</td>
<td>0.09</td>
<td>0.96</td>
<td>0.94</td>
<td>0.68</td>
<td>BG</td>
</tr>
<tr>
<td>Spermine</td>
<td>0.00</td>
<td>0.07</td>
<td>0.10</td>
<td>0.02</td>
<td>0.93</td>
<td>0.33</td>
<td>0.24</td>
<td>P</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>0.35</td>
<td>0.67</td>
<td>0.84</td>
<td>0.86</td>
<td>0.95</td>
<td>0.96</td>
<td>0.91</td>
<td>GBr</td>
</tr>
<tr>
<td>Tyramine</td>
<td>0.30</td>
<td>0.62</td>
<td>0.84</td>
<td>0.70</td>
<td>0.95</td>
<td>0.96</td>
<td>0.85</td>
<td>GP</td>
</tr>
</tbody>
</table>

* Final colours on chromatograms run in n-butanol-acetic acid: P, purple; R, red; B, blue; G, grey; Br, brown.
† These amines were not revealed by ninhydrin in this solvent.
‡ Values in brackets refer to weaker spot detected.
§ Colour initially yellow.
|| Weak coloration with ninhydrin.
ninhydrin colour reaction. As Dent (1948) pointed out, there is still much confusion on this subject. He found that, contrary to the statement of Calverly (1944), $\beta$- and $\gamma$-amino-acids gave the reaction. He further showed that substances containing a primary aliphatic group, namely ethanolamine, glucosamine and histamine, gave colours with ninhydrin similar to those obtained with $\alpha$-amino-acids. The results reported here indicate that this reaction is typical of compounds containing an aliphatic primary amino group. In addition, it appears that some compounds containing a secondary aliphatic amino group give the ninhydrin colour reaction. Tertiary amines do not appear to give the reaction.

Dent (1948) is of the opinion that only a limited number of ninhydrin-reacting substances other than $\alpha$-amino-acids are likely to be present in mixtures obtained from natural sources. This is not borne out, however, by the recent work of Steward & Thompson (1950), who detected in plant materials some twenty ninhydrin-reacting substances which could not be identified with any of the known naturally occurring amino-acids. Several of these substances were found to be stable to acid hydrolysis and were, therefore, probably not peptides. The possibility that some of these acid-stable compounds were amines is suggested by the fact that several amines have recently been found in plant materials.

The chances of confusing amino-acids with amines when chromatographing mixtures of unknown composition would appear to depend largely upon the solvents employed for chromatography. Several of the solvents employed in this work have also found favour in paper-chromatographic analysis of amino-acid mixtures. The results obtained in this work show that in some of these solvents, e.g. butanol-acetic acid, many amines run at speeds similar to some of the naturally occurring amino-acids. However, under the conditions commonly employed for two-dimensional chromatographic analysis of amino-acid mixtures, i.e. using phenol-ammonia as first solvent and 'collidine' or butanol-acetic acid as second solvent, it would appear that only those amino-acids which have high $R_F$ values in phenol-ammonia are likely to be confused with amines.

In this respect it may be pointed out that the chromatographic behaviour and properties of Dent's (1948) 'fast arginine'—believed to be a new basic amino-acid—are consistent with those of putrescine.

Although ninhydrin has been used for the detection of the amines employed in this work, it is obvious that there is much scope for the development of more specific methods of detection. For example, histamine, tyramine and tryptamine could be detected by the Pauly reagent, adrenaline by potassium ferricyanide (James, 1948) and agmatine by the Sakaguchi reaction.

Dent (1948) found that when phenol was used as solvent the $R_F$ value of glucosamine in an atmosphere saturated with 1:1 (v/v) aqueous acetic acid was markedly smaller than the value obtained in an atmosphere of ammonia and hydrocyanic acid. We have confirmed the effect of acidity with this substance. However, it is clear from Table 1 that the majority of the amines tested were unaffected by this change of atmosphere, and that this test cannot be used to reveal the presence of amines in the mixtures under investigation.

It can be seen from Fig. 2 that when the $R_M$ values in various solvents of the two homologous series of amines studied are plotted against the number of methylene (—$\text{CH}_2$—) groups a series of approximately parallel straight lines is obtained. It would appear, therefore, that Martin's (1949) deduction concerning the relationship between constitution and partition coefficient holds well enough for —$\text{CH}_2$— groups in primary $n$-alkyl monoamines and polymethylene diamines.

Finally, it may be pointed out that the findings in this work with regard to the effect of molecular structure on mobility in phenol are in agreement with those of other workers. For example, mobility is increased by decarboxylation (cf. $R_F$ values of corresponding amino-acids) or substitution by a phenyl group of a hydrogen atom and decreased by replacement of a hydrogen atom by a hydroxyl group.

**SUMMARY**

1. The $R_F$ values on filter-paper chromatograms of a large number of amines in various solvents have been determined.

2. Ninhydrin was found to be an effective reagent for the detection of primary aliphatic amines on paper chromatograms.

3. The results obtained suggest that amines likely to be present in biological materials could be separated and identified by paper chromatography.

4. Relationships between the molecular structures of the amines studied and their $R_F$ values are discussed.
β-(3:4-Dihydroxyphenyl)ethylamine (hydroxytyramine) in Normal Human Urine

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(Received 16 March 1951)

Following the discovery by Holtz, Heise & Lüdtke (1938) of L-dopa decarboxylase in animal tissues, Holtz, Credner & Koepp (1942) demonstrated that after administration of 3:4-dihydroxyphenylalanine (DOPA) to man, guinea pigs, rabbits and cats, the urine contained considerable quantities of hydroxytyramine present in free and conjugated form. They also obtained some evidence for the presence normally of hydroxytyramine in urine. Holtz, Credner & Kroneberg (1947) later showed that normal urine contained what appeared to be a mixture of hydroxytyramine, adrenaline and noradrenaline (urosympathin). In a recent paper, however, Kroneberg & Schimann (1950) express the opinion that in man and certain animals noradrenaline forms most if not all of the catechol amines in urine.

The presence in normal urine of adrenaline and noradrenaline could be demonstrated biologically after adsorption on aluminium hydroxide (von Euler & Hellner, 1951 a, b). When purified urine extracts were submitted to paper chromatography, a mixture of n-butanol and N-hydrochloric acid being used as a solvent, not only noradrenaline and adrenaline were found, but also considerable quantities of another catechol amine occupying the site of hydroxytyramine on the chromatogram. The present paper deals with the separation and identification of hydroxytyramine from extracts of human urine by use of partition chromatography on a starch column.

EXPERIMENTAL

Preparation of extracts. Urine was obtained from medical students. The catechol amines were separated by adsorption in alumina in portions of 0-5-2 l. according to the technique described previously (von Euler & Hellner, 1951 a). In order to facilitate filtration of the alumina precipitate Hyflo Superces (Johns Manville and Co.) was added when necessary in amounts of 30-50 g./l.

In two preparations smaller portions of whole urine were boiled for 20 min. at pH 2 in order to split conjugated catechol amines (von Euler & Hellner, 1951 a). This procedure generally increased the yield of biologically active catechol amines by 30-100%, presumably by splitting ethereal sulphates (Richter, 1940). The glucuronides (Clark, Akawie, Poglund & Geissman, 1950) would presumably not be split by this mild hydrolysis, which, however, was chosen in order to minimize the inactivation of the free catechol amines (by racemization). No qualitative differences were observed between untreated and hydrolysed urine.

Partition chromatography on starch column. The catechol amines of the urine extract were taken up in n-butanol by repeated evaporation of the aqueous solution with n-butanol at low temperature in vacuo. The butanol extract thus obtained was suitably concentrated and used for partition chromatography on a starch column with n-butanol/ N-HCl as solvent according to the technique used by Hamberg & von Euler (1950). Each 2 hr. sample was evaporated to dryness in vacuo and dissolved in 2 ml. of water. These solutions were used for colorimetric determination, paper chromatography and biological assay. A control run was made with a mixture of adrenaline, noradrenaline and hydroxytyramine, together with urine and the R values determined.

REFERENCES
