ACTA CHEMICA SCANDINAVICA 6 (1952) 219–222

Detection, Separation, and Identification of Aliphatic Amines by Paper Chromatography

R. SCHWYZER

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

It has previously been difficult to detect, separate, and identify volatile aliphatic amines in quantities of a few gammas. The microdiffusion method of Conway, as applied by Richter [1] probably has been the best approach to this problem. For the purpose of studying the uptake and metabolism of lower aliphatic amines by plants, we have developed a paperchromatographic method, which allows the detection, separation, and identification of as little as 1γ of aliphatic amines in biological materials. The method is very simple and quick to carry out, the results being ready in about two hours. A minimum of preparatory operations are required, as very few naturally occurring substances interfere.

The chromatogram is run with butanol-acetic acid and the amine spots are made visible by spraying with a suitable indicator solution (bromphenol-blue). Blue spots appear immediately on a yellow background, varying in the intensity of colour according to the quantity of amine present. Basic amino acids, of course, give similar spots.

EXPERIMENTAL

Procedure

According to our experience the fast running paper Munktell OB (J. H. Munktells Pappersfabriksaktiebolag, *Grycksbo*, Sweden) is very well suited for our purpose. It is somewhat more acid than the Whatman No. 1, thereby offering a better and more evenly yellow background when sprayed with indicator solution. With Whatman No. 1 it is possible, though, to detect somewhat smaller amounts of amines (about 1 γ against 2 γ with Munktell).

a b

Fig. 1. a) Chromatogram of 5, 10, 15, and 20 γ (from left to right) of dimethylamine-HCl, ethylamine-HCl, n-*propylamine-HCl, and* i-*propylamine-HCl. b) Separation of ethylamine-HCl,* n-*propylamine-HCl,* i-*propylamine-HCl, and trimethylamine-HCl from ammonia (2, 5, 10 resp. 30 γ of amine).*

The ascending chromatogram method has been used, placing the strips or cylinders of paper in small receptacles and running the chromatogram for 1 hour with Munktell-paper (solvent ascends 13—14 cm) or 3 hours with Whatman, thus keeping to a minimum the losses of amines due to volatilization.

The amine solution is transferred to the paper in a current of warm air, evaporating the solvent during application. Care is taken to keep the diameter of the initial spots below 6 to 8 mm. Immediately after transfer and drying of the spots, the paper is placed into the chamber containing the solvent, and the chromatogram is started.

Care must be taken in preparing the amine solution, as strong acids (mineral acids, trichloroacetic acid) interfere with the chromatogram. Solutions containing such acids must, previous to application, be neutralized to a pH value of 3—6 by sodium acetate or by other means. It is also possible to neutralize acid solutions on the paper by ammonia vapour, the amine spots then appearing above a diffuse blue band of ammonium salts. The R_F values are in this case somewhat enhanced due to a replacing effect of the ammonium ions. Strong acids appear as bright yellow spots below the amines and the ammonia. The best solvent for preparing extracts of amines from biological material is absolute alcohol containing 0.1 to 2 per cent of glacial acetic acid.

The chromatograms are developed with *n*-butanol saturated with 25 % acetic acid. Before use, the solvent must be left at room temperature for about three days to allow esterification to proceed to equilibrium. Phenol-water may also be employed, although the high R_F values and long drying-time are objections to its use.

After running, the paper is dried at room temperature for one half to three quarters of an hour. A slow current of acid- and base-free air is very helpful. Spraying the chromatogram prepared in this manner immediately after drying with 0.2 % bromphenolblue in absolute alcohol develops the spots. As the

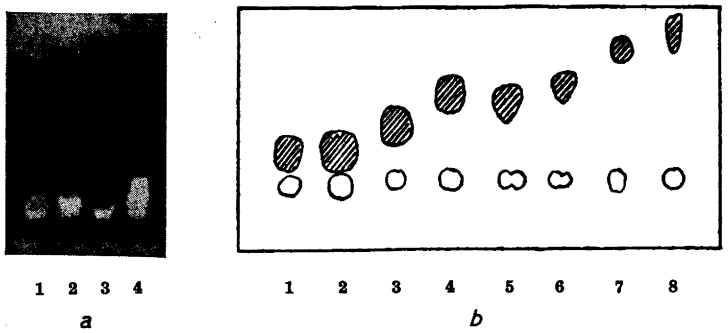

Fig. 2. a) Separation of mixtures: (1) dimethylamine-trimethylamine, (2) ethylamine-n-propylamine, (3) dimethylamine-ethylamine, (4) n-propylamine-i-propylamine. b) Chromatogram of some aliphatic and aromatic amines and piperidine.

1 = Dimethylamine-HCl	*5 γ*
2 = Trimethylamine-HCl	*10 γ*
3 = Ethylamine-HCl	*5 γ*
4 = n-Propylamine-HCl	*5 γ*
5 = i-Propylamine-HCl	*5 γ*
6 = Piperidine-HCl	*5 γ*
7 = Benzylamine-HCl	*5 γ*
8 = Phenylethylamine-HCl	*5 γ*

colour tends to fade after a few days, due to evaporation of amine (trimethylamine spots will fade especially rapidly), we have usually made photographic prints on document contact paper immediately after spraying.

Experiments

Amine	R_F	Minimum amount		Amine	R_F	Minimum amount	
Methylamine	0.28	3 γ	HCl-salt	Benzylamine	0.59	2 γ	HCl-salt
Dimethylamine	0.30	3 γ	»	Phenylethylamine	0.64	2 γ	»
Trimethylamine	0.31	4 γ	»	Cadaverine	0.12	2 γ	»
Ethylamine	0.35	3 γ	»	Piperidine	0.49	2 γ	»
n-Propylamine	0.46	2 γ	»	Arginine	0.13	1 γ	free base
i-Propylamine	0.45	2 γ	»				

Fig. 1a and 2b illustrate the chromatograms of these amines and of piperidine.

To test the efficacy of separation we have tried separation of the following pairs of substances (Figs. 1b and 2a):

NH_3 — all amines	neat separation.
Dimethylamine-trimethylamine	trimethylamine is somewhat ahead and evaporates completely in 12 hours.
Trimethylamine-ethylamine	trimethylamine is somewhat behind and evaporates, leaving ethylamine.
Dimethylamine-ethylamine	partial separation with constriction of area between the spots.
Ethylamine-*n*-propylamine	neat separation.
n-Propylamine — *i*-propylamine	partial separation with constriction.

Pairs of substances with greater differences in R_F values may, of course, easily be separated.

By applying different volumes of an unknown solution side by side with a known solution, it has been possible to determine the amount of amine present in the initial spot to about $\pm$ 1 γ. Best results are obtained by applying dilution series and by observing the minimum quantity giving a just visible spot after chromatography.

For identifying the more volatile amines we have also used the microdiffusion methods of Richter [1] and of Opfer-Schaum [2]. After spraying with indicator we have cut out the areas containing about 5 γ of amine, placed them in small crucibles of about 1 ml volume, and covered the containers with airtight lids which carried micro drops of picric acid solution (less than the required amount). After moistening the paper disc with saturated K_2CO_3 solution, crystals of the picrates appeared on standing. After draining the mother liquor, they were dried, and identified by their melting-point.

SUMMARY

A simple and rapid method of detecting, separating and identifying volatile aliphatic amines by paper chromatography is presented. By comparing the spots with those derived from solutions of known amine content, semiquantitative estimations of the amines may be made. Combination of the method with microdiffusion technique offers a possibility of separating amine mixtures and preparing derivatives of the components.

I wish to thank Prof. P. Karrer for a leave of absence from his institute, the *Erziehungsdirektion des Kantons Zürich* for a grant from the *Stiftung zur Förderung des akademischen Nachwuchses*, and Prof. A. I. Virtanen for suggesting this work and for his hospitality.

REFERENCES

1. Richter, D. *Biochem. J.* **31** (1937) 2022.
2. Opfer-Schaum, R. *Mikrochemie ver. Mikrochim. Acta* **31** (1944) 324; Opfer-Schaum, R., and Piristi, M. *Ibid.* **32** (1944) 148.

Received September 30, 1951.