

PAPER AND THIN-LAYER ELECTROPHORETIC SEPARATIONS OF POLYNUCLEAR AZA HETEROCYCLIC COMPOUNDS

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INTRODUCTION

Polynuclear aza heterocyclic compounds have been found in urban atmospheres¹, in effluents from air pollution sources such as those involving coal-burning operations, coal-tar-pitch fumes, some industrial operations², and automotive transportation³.

These aza compounds can be separated by thin-layer chromatography⁴. Cellulose and alumina thin-layer chromatography give two widely different types of separations. Three- to six-ring aza heterocyclic compounds⁵ and the smaller aza compounds have also been separated by paper chromatography⁶. Column chromatography has been found useful especially where test mixtures are available in amounts from 5 to 50 mg.

In this paper the electrophoretic separation of these types of compounds is discussed.

EXPERIMENTAL*

Equipment

An Aminco-Bowman spectrophotofluorimeter was used with the following settings: sensitivity, 50; slit arrangement No. 2; and phototube RCA type 1P21. An Aminco thin-film scanner was used in fluorimetric scanning of the paper and thin-layer pherograms. Electrophoretic separations were done with a Gelman chamber and a 0-500 V d.c. power supply.

Reagents

The aza heterocyclic compounds were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisc., and K & K Laboratories, Inc., Jamaica 33, New York.

Buffer. Formic acid (31.2 ml) and glacial acetic acid (59.2 ml) were made up to 1 l with distilled water. The pH of the solution was 2.0. When stored in a refrigerator, the solution is stable for one month.

Ascending column chromatography was accomplished with a silica gel Unibar (Analtech Inc., Wilmington, Del.).

* Mention of commercial products does not constitute endorsement by the Public Health Service.

Procedure

Paper electrophoresis. Whatman No. 1 paper was used in paper electrophoresis. It was spotted with the samples (equivalent to about 25 ng of the compound under investigation) 2 in. from the edge of the paper. The cathode end of the paper was placed in the buffer solution so that most of the paper was wet except for the spotted end. The buffer was allowed to diffuse toward the spots from both ends. When the entire paper was wet, the mixtures were separated at 500 V. The spots were allowed to migrate toward the cathode for about 75 min. The pherogram was then examined under ultraviolet light and scanned at the appropriate excitation and emission wavelengths. If desirable, the pherogram can be dried, fumed with trifluoroacetic acid fumes, scanned, and examined fluorimetrically.

Thin-layer electrophoresis. In thin-layer electrophoresis the 8 by 8 in. dry-cellulose plate was spotted with the samples. The cathode and anode edges of the plate were connected with the cold buffer solution by means of paper strips wet with buffer. Except for the spot area, the whole plate was "painted" with a piece of filter paper wet with buffer. When the entire plate was wet, the mixtures were separated at 500 V. Cooling was not necessary. The separation took about 75 min. The thin-layer pherogram was examined under ultraviolet light and scanned. If the pherogram is dried, it should be fumed with trifluoroacetic acid before fluorimetric examination and scanning.

RESULTS

Paper electrophoretic separations were rapid, much more rapid than with comparable paper chromatographic methods. Resolution was as good as with paper chromatography. Fluorimetric scans were readily performed; in addition, characterization of the spots by direct fluorimetric examination with the solid-state attachment⁷ was considerably simplified since the areas of interest could be readily cut out and examined at leisure. The various polynuclear aza heterocyclic compounds separated by this technique are listed in Table I. The smallest molecules move fastest. In 75 min acridine, benz(*c*)acridine, 8,10-dimethylbenz(*a*)acridine, pyrenoline, dibenz(*a,j*)acridine, and 7-phenyldibenz(*c,h*)acridine move distances of approximately 130, 90, 53, 25, 14, and 0 mm. All results are expressed in terms of mobility of test substance relative to mobility of benz(*c*)acridine, *e.g.*

$$\text{Relative migration rate} = 100 \cdot M_X / M_{\text{BcAcR}}$$

Separation of a mixture of standards by paper electrophoresis on Whatman No. 1 paper is shown in Fig. 1. Mixtures of seven standards, each present in amounts from 10 to 100 ng, were separated. Each row of nine spots was scanned with the appropriate excitation and emission wavelengths. The acridine spots were scanned at F 350/475. The type of scan obtained is shown in Fig. 1. The other six standards were also scanned at their appropriate fluorescence excitation, and emission wavelength maxima. The area of the scan of each spot was obtained by triangulation.

As shown for acridine, the area thus obtained gave a linear relation through the origin with the amount of acridine in the spot.

Cellulose thin-layer electrophoresis was as rapid a paper electrophoresis. Resolution and sensitivity were better than in paper chromatographic and electro-

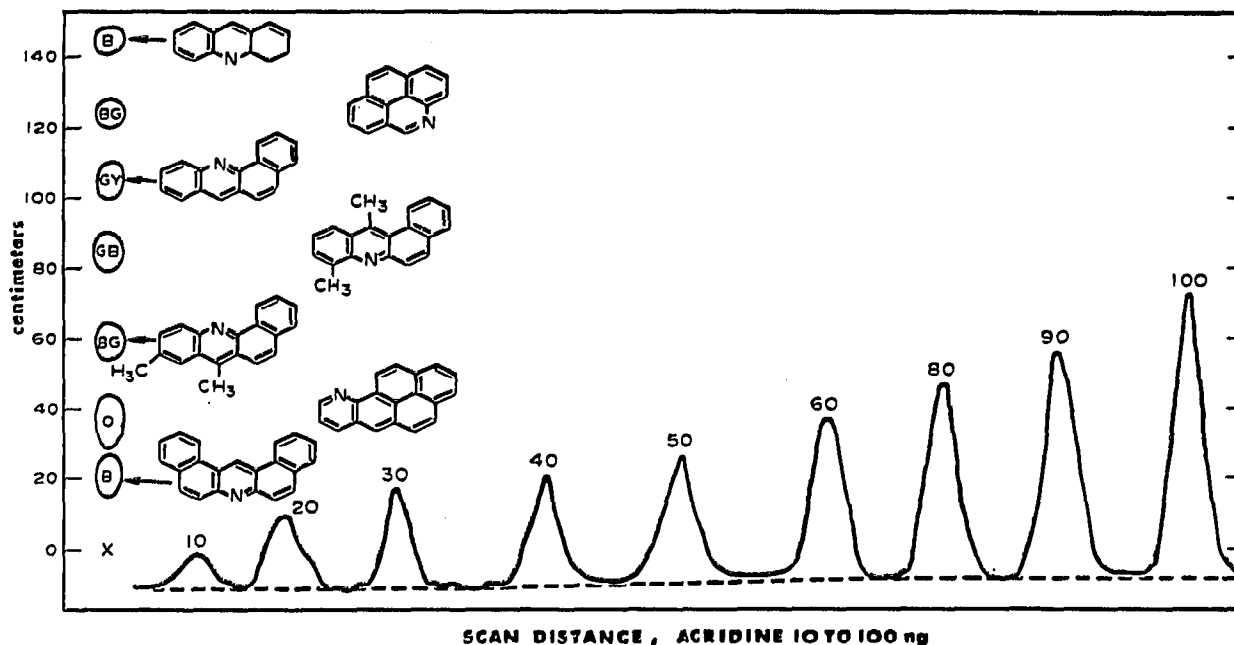


Fig. 1. Paper electrophoretic separation of seven standard compounds, 25 ng each, *viz.*, dibenz(*a,j*)acridine, pyrenoline, 7,9-dimethylbenz(*c*)acridine, 8,12-dimethylbenz(*a*)acridine, benz(*c*)acridine, benzo(*l,m,n*)phenanthridine, and acridine. Various concentrations were separated. Scan of the acridine spots at F 350/475. Pherograms were sprayed with trifluoroacetic acid fumes before scanning.

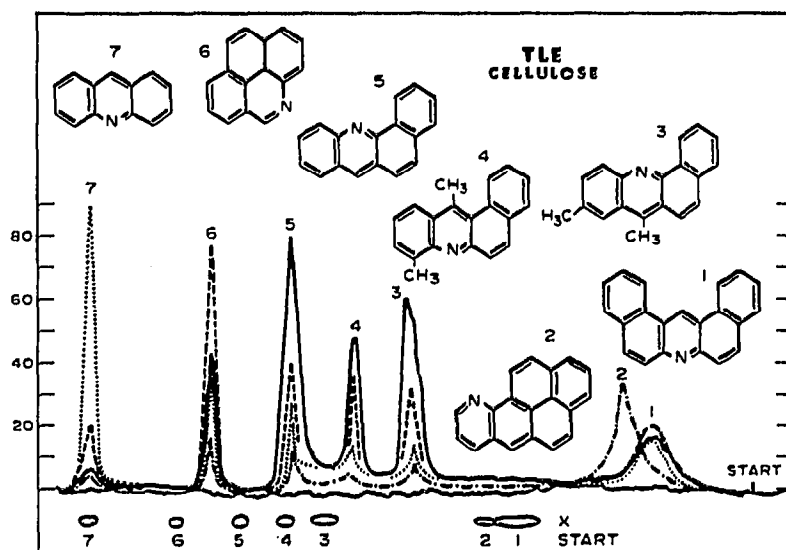


Fig. 2. Cellulose thin-layer electrophoretic separation of seven standard compounds. Fluorimetric scan of 25 ng each of benz(*c*)acridine (—) F 295/480 and meter multiplier (MM) 0.01; benzo(*l,m,n*)phenanthridine (---) F 394/470 and MM 0.1; acridine (···) F 350/475 and MM 0.03; and pyrenoline (·—·—·) F 320/548 and MM 0.01. Lowest tracing is baseline. Pherograms were sprayed with trifluoroacetic acid fumes before scanning.

phoretic separations, mainly because of the small size of the spots. Diameters of 3 to 4 mm clearly indicated the resolution possible with this type of separation. Resolution in the separation of a mixture of seven aza heterocyclic compounds can be seen in Fig. 2. Scanning at the appropriate excitation and emission wavelengths further

improves the selectivity of the method. As shown in Fig. 2, this method of scanning improves sensitivity and selectivity in the analysis of acridine, benzo(*l,m,n*) phenanthridine, benz(*c*)acridine, pyrenoline, and dibenz(*a,j*)acridine. With plastic less heat is produced during a run than with a glass plate, and the spots can be outlined on the plate. The outlined areas can be colored, the required information can be written on the plate, and the record of the separation can be saved for future use.

Results of the thin-layer electrophoretic separation of a large number of polynuclear aza heterocyclic compounds are shown in Table I.

TABLE I

ELECTROPHORETIC MOBILITIES OF SOME AZA HETEROCYCLIC COMPOUNDS RELATIVE TO BENZ(*c*)ACRIDINE

Compound ^a	PE Whatman No. 1	TLE cellulose on plastic ^b	Fluor. color ^c
Benzo(<i>h</i>)quinoline	145	155	P
3-Methylacridine	140	160	G
Phenanthridine	145	165	P
Benzo(<i>f</i>)quinoline	145	175	P
3-Methylbenzo(<i>f</i>)quinoline	140	165	P
9-H-Pyrido(2,3- <i>b</i>)indole	135	150	B P
Acridine	135	165	G
Benzo(<i>l,m,n</i>)phenanthridine	125	115	G
10-H-Indeno(1,2- <i>b</i>)quinoline	120	120	P
Indeno(1,2,3- <i>i,j</i>)isoquinoline	120	135	Y O
Acenaphtho(1,2- <i>b</i>)pyridine	120	145	P
Benz(<i>b</i>)acridine ^d	120	70	l O
Benz(<i>c</i>)acridine	100	100	
12-Methylbenz(<i>a</i>)acridine	95	85	P
Benz(<i>a</i>)acridine	90	90	B
7-Methylbenz(<i>c</i>)acridine	80	75	G
8,12-Dimethylbenz(<i>a</i>)acridine	80	70	G
9,12-Dimethylbenz(<i>a</i>)acridine	65	40	B
8,10-Dimethylbenz(<i>a</i>)acridine	55	60	G
7,9-Dimethylbenz(<i>c</i>)acridine	55	40	B
7,10-Dimethylbenz(<i>c</i>)acridine	55	90	G
Pyrenoline	25	10	O
Dibenz(<i>a,j</i>)acridine	15	10	B
1,4-Phenyldibenz(<i>a,j</i>)acridine	15	5	Y G
Dibenz(<i>a,h</i>)acridine	10	5	B
7-Phenyldibenz(<i>c,h</i>)acridine	0.0	0.0	P

^a All compounds were separated in 25-ng amounts except for indeno (1,2,3-*i,j*)isoquinoline and benz(*b*)acridine, which were separated in 100-ng amounts.

^b Similar results were obtained with cellulose glass plates.

^c Obtained under long-wavelength ultraviolet light. B = blue, G = green, l = light, O = orange, P = purple, Y = yellow.

^d Dip into ammonium hydroxide and read under U.V. immediately.

APPLICATION

Various samples of polluted urban atmospheres and industrial effluents were investigated. In most cases basic fractions were obtained by extraction; then the basic fraction was separated by column⁸ or thin-layer chromatography⁴ followed by

paper or thin-layer chromatography. Dependent on how much information is desired, any of the above basic fractions can be separated by the electrophoretic procedure.

An example of a characterization procedure is shown in Fig. 3. The basic subfraction of the benzene-soluble fraction of an urban air-borne particulate sample was separated on a silica gel Unibar column, 2.5 cm diameter and 12 cm long. About 30 min were required for separation by ascending chromatography. The column was examined under ultraviolet light, and the various bands were cut out and eluted with methanol. As shown in Fig. 3, the brilliant green fluorescent band contained benz(*c*)-acridine. A very small aliquot of this fraction was separated by paper electrophoresis. This fraction contained quite a few compounds, as shown by the pherogram. Fluorimetric examination of these spots and comparison of their mobilities with those of appropriate standards showed that two of these spots could be identified as benz(*c*)-acridine and an alkylbenz(*c*)acridine (Fig. 3).

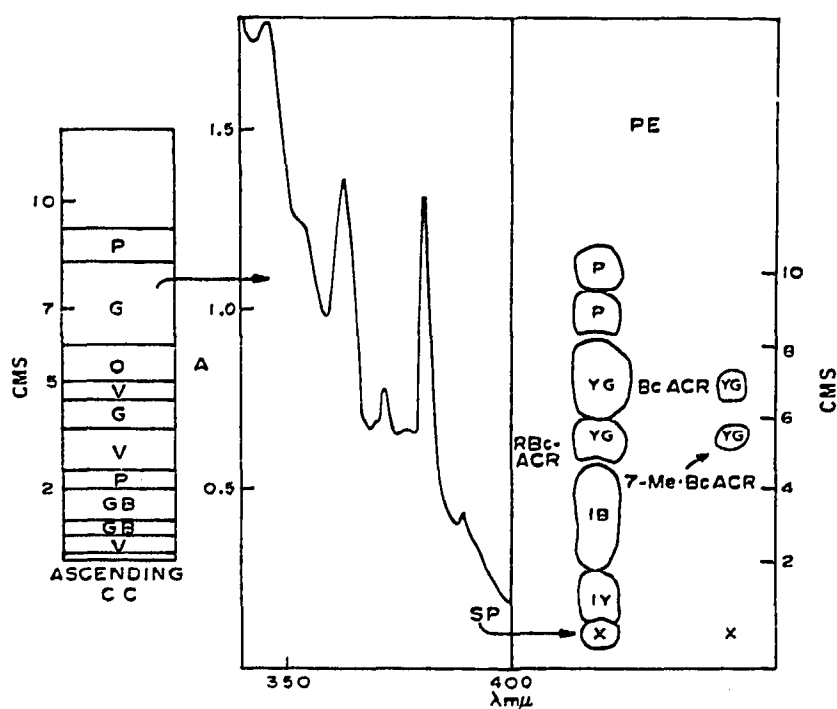


Fig. 3. Ascending silica gel column chromatogram of a basic subfraction from a benzene-soluble fraction of an urban air-borne particulate sample. Development was with pentane-ether (9:1). Absorption spectrum of the brilliant green fluorescent fraction in pentane. Paper electrophoretic separation of the brilliant green fluorescent fraction, benz(*c*)acridine and 7-methylbenz(*c*)acridine.

Our experience with electrophoresis indicates that this technique will be of definite value in the separation and analysis of various types of air pollution samples.

SUMMARY

A large number of polynuclear aza heterocyclic compounds have been separated by paper and thin-layer electrophoresis. The pherograms were scanned fluorimetrically. Many of the separated compounds were capable of being characterized and assayed by the scanning procedure. Analysis by fluorimetric scanning of the phero-

grams was much more selective when the appropriate excitation and emission wavelengths were used. Samples of urban airborne particulate can be separated and analyzed with the help of paper or thin-layer electrophoresis.

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