

# CHARACTERIZATION OF POLYNUCLEAR AZA HETEROCYCLIC HYDROCARBONS SEPARATED BY COLUMN AND THIN-LAYER CHROMATOGRAPHY FROM AIR POLLUTION SOURCE PARTICULATES

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## INTRODUCTION

Two recently developed thin-layer chromatographic systems give entirely different separation patterns for the polynuclear aza heterocyclic hydrocarbons<sup>1</sup> and thus make possible the separation of aza compounds from complex mixtures. In addition, a column chromatographic system for the separation of basic compounds from complex mixtures makes possible the collection of large partially separated fractions<sup>2</sup>. Extensive characterization work can be done with these large fractions. With the recent development of a procedure for the direct spectrophotofluorometric examination of thin-layer chromatograms<sup>3</sup>, it is now possible to characterize the unknowns on the chromatogram without tedious extraction or elution. The analytical procedure has been standardized with coal tar pitch<sup>4</sup>.

In the work reported here the separation-characterization procedure is applied extensively to gaseous effluents trapped from various air pollution sources. Examples of the evidence on which the characterizations are based are given.

## EXPERIMENTAL

### *Reagents*

Most of the aza heterocyclic hydrocarbons were obtained from Aldrich Chemical Company of Milwaukee or K and K Laboratories, Plainview, Long Island. A few of these compounds were synthesized and purified by literature procedures. 11H-Indeno-(1,2-b)quinoline was obtained from Dr. J. SMOLICZ.

### *Equipment*

An Aminco-Bowman spectrophotofluorometer was used with the following settings: sensitivity 50, slit arrangement No. 2, and phototube RCA type 1P21. The instrument was equipped with an Aminco solid-sample accessory to obtain the fluorescence spectra of spots on thin-layer chromatograms. Details for the direct spectrophotometric analysis of aromatic compounds on thin-layer chromatograms were presented in a previous paper<sup>3</sup>.

### *Thin-layer chromatography*

Either glass plates less than 0.1 in. thick or plastic sheets ranging from 0.015 to 0.06 in. thick can be used. Ordinary window glass works nicely; a glass cutter must be used to cut out the spots for spectrofluorometric examination. Our best results were obtained with sheets of 0.015 in.-thick vinyl plastic VSA-3310 Clear 31 Matte 06, which are easily cleaned and coated with cellulose or alumina. Following development, spots are readily cut out with scissors. Window glass is less expensive than the glass usually used in thin-layer chromatography, and the plastic sheets are much less expensive. The main difficulty with the plastic material is its solubility in some organic solvents. Although the solvent systems used in this research did not affect the vinyl plastic, the possible effects of solvents should be explored for each plastic material. With the Teflon type of plastic, of course, all types of solvent systems can be used.

Aluminium oxide G and MN-cellulose powder 300 G (Brinkmann Instruments, Long Island) in 250- $\mu$  layers were used as the adsorbents.

### *Analytical procedures*

The collection of particulates from urban atmospheres polluted by coal-tar-pitch fumes<sup>5</sup> and from the effluent gases emanating from incinerators and the stacks of residential coal-burning furnaces<sup>6</sup> has been described. The particulate matter and other condensed material from about 5 to 20 m<sup>3</sup> of effluent gas or from a 2- to 24-h collection of coal-tar-pitch-polluted atmosphere was Soxhlet-extracted with benzene for about 6 to 8 h. The basic fraction was obtained from about 0.4 to 1 g of the benzene-soluble material from incinerators or stacks or from about 0.1 to 0.4 g of the coal-tar-pitch sample; it was then chromatographed on an alumina column with 100-ml volumes of pentane solutions containing 8, 16, 24, 32, 40, 48 and 56 % ether and 5, 10, 15, 20, 25, 30, 35 and 40 % acetone followed by 100-ml volumes of ether and then methanol<sup>2</sup>. The fractions, consisting of 15-ml volumes of eluent, were separated further on thin-layer plates coated with cellulose. Dimethylformamide-water (35:65) was used as the developer.

In some cases the basic fraction was separated with the thin-layer system of alumina plus pentane-ether (9:1, v:v). In a few instances these steps were followed by extraction of appropriate spots, then by further separation with a thin-layer system of cellulose dimethylformamide-water (35:65).

### DISCUSSION

Occasionally after the column chromatographic separation of a benzene-soluble fraction preliminary to analysis for polynuclear aromatic hydrocarbons, the presence of aza heterocyclic hydrocarbons can be ascertained in the absorption spectrum of the benzoperylene or coronene fraction. This phenomenon is shown in Fig. 1 for a column chromatographic subfraction of a benzene-soluble fraction of a coal-tar-pitch-polluted air sample. Bands at 330, 338 and 346 m $\mu$  are indicative of carbazole, coronene and benzo(*h*)quinoline, respectively. Bands at 363 and 382 m $\mu$  are indicative of benz(*c*)acridine. When this sample was chromatographed on a cellulose thin-layer plate with water-dimethylformamide as the developer, the four main components of this fraction were readily separated (Fig. 2). Direct spectrophoto-

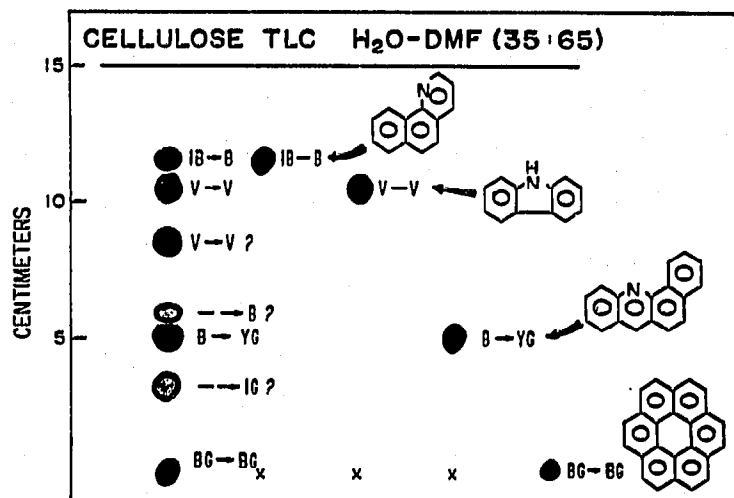
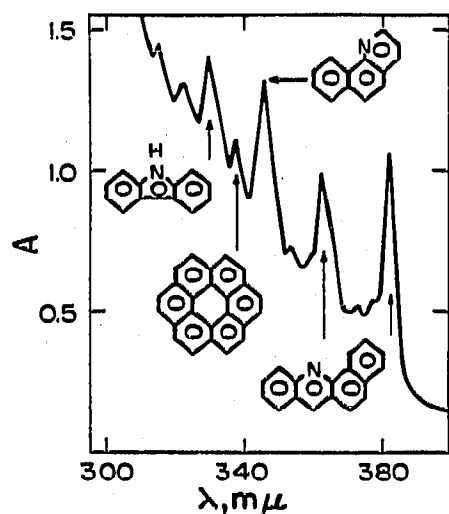


Fig. 1. Ultraviolet absorption spectrum of the carbazole fraction obtained through alumina column chromatographic separation<sup>8</sup> of an airborne particulate sample polluted with coal-tar-pitch fumes.

Fig. 2. Thin-layer chromatogram of the carbazole column chromatographic fraction, benzo(*h*)-quinoline, carbazole, benz(*c*)acridine, and coronene. Fluorescent colors of spots: B = blue; G = green; l = light; V = violet; Y = yellow.

fluorometric examination of the separated spots under neutral, alkaline and acid conditions characterized the four compounds unequivocally.

A few of the aza compounds could be characterized, following column chromatography of the basic fraction, by means of the similarity of the longer wavelength bands of the absorption spectrum of the appropriate fraction in neutral and acid solutions to that of the pure compound. Acridine can be characterized in this fashion.

In another method of characterization used to identify the unknowns unequivocally the basic fraction was thin-layer chromatographed twice, first with the alumina pentane-ether (9:1) system and then with the cellulose/dimethylformamide-water (35:65) system. The separation of the acridine spot is shown in Fig. 3. It must be emphasized that the final green-fluorescing acridine spot still contained other compounds. For these mixtures it can be stated as a general rule that after two separations each spot has a group of compounds with a common  $R_F$  value on alumina and another common  $R_F$  value on cellulose. Thus, the  $R_F$  values by themselves do not give proof of identity. With the high order of selectivity of fluorometric methods, it is a fairly simple matter to characterize the spots on the plate directly, if standards are available. Selectivity can be enhanced considerably through use of quenchofluorometric techniques<sup>7</sup>. The thin-layer chromatogram of the first column chromatographic fraction containing recognizable aza compounds is shown in Fig. 4. Alkylbenzo(*h*)quinoline, benz(*c*)acridine, and alkylbenz(*c*)acridine were characterized by  $R_F$  values, direct fluorometric analysis on the plate under neutral and acid conditions, and other fluorometric and phosphorimetric techniques, many of which have been described in a previous paper<sup>3</sup>. In some cases additional information was obtained by extracting the spot from the thin-layer chromatogram, evaporating the solvent, and then obtaining the spectra in appropriate solvent systems. Figs. 5 and 6 present examples of such supplementary characterization techniques. In Fig. 5 the extract of a spot with the same  $R_F$  value as acridine is characterized readily through its fluorescence spec-

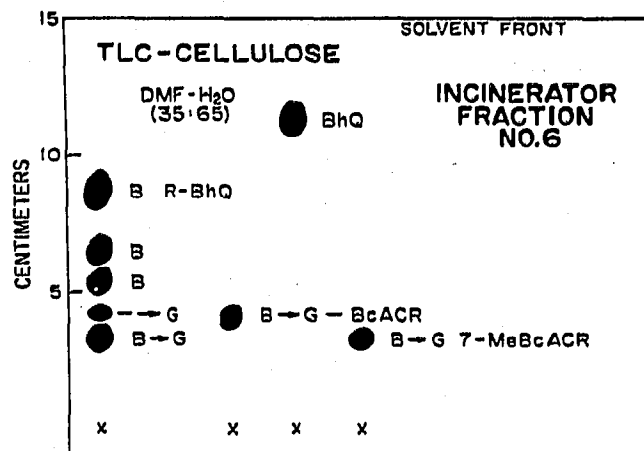
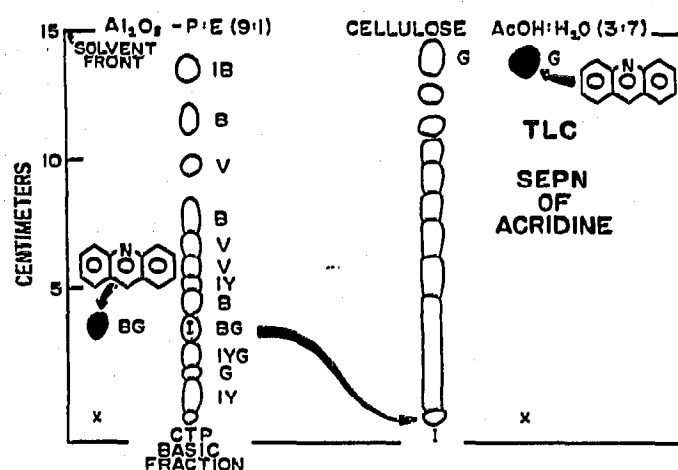


Fig. 3. Thin-layer chromatogram on alumina of the basic fraction of a coal-tar-pitch-polluted air sample followed by separation of the acridine fraction, I, on a cellulose thin-layer chromatogram. For explanation of letters see legend to Fig. 2.

Fig. 4. Thin-layer chromatogram of a column chromatographic fraction obtained from the basic fraction of the particulates collected from incinerator effluents, benzo(*h*)quinoline, benz(*c*)acridine, and 7-methylbenz(*c*)acridine. For explanation of letters see legend to Fig. 2.

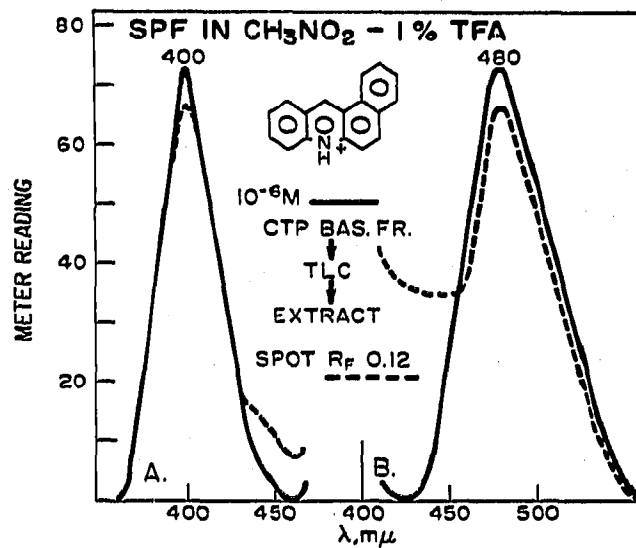
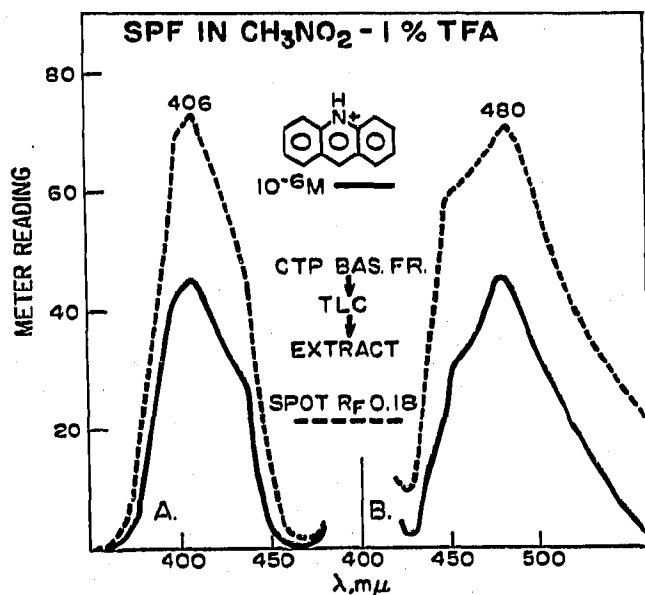


Fig. 5. Fluorescence excitation (A) and emission (B) spectra of acridine (—) and an extract of the spot,  $R_F$  0.18, obtained from an alumina thin-layer chromatogram (pentane-ether, 9:1, v:v) of the basic fraction of a coal-tar-pitch-polluted air sample (---). Meter multiplier reading (MM) 0.01.

Fig. 6. Fluorescence excitation (A) and emission (B) spectra of benz(*a*)acridine (—) at MM 0.03 and an extract of the spot,  $R_F$  0.12, obtained from an alumina thin-layer chromatogram (pentane-ether, 9:1, v:v) of the basic fraction of a coal-tar-pitch-polluted air sample (---) at MM 0.01.

trum in acidic nitromethane. In alkaline or acidic pentane the spectrum in solution or on the plate was not clear-cut because of the presence of bands from other compounds.

The acidic nitromethane solvent system quenches the fluorescence of practically all aromatic compounds except the aza heterocyclic and fluoranthenic hydrocarbons. The fluorescence spectra of the spot  $R_F$  0.12 in Fig. 6, dissolved in pentane or acidic pentane, show the presence of benzo(*f*)quinoline and unknown compounds in addition to benz(*a*)acridine. A solution of the extracted spot,  $R_F$  0.12, in alkaline nitromethane does not fluoresce. This is in line with the non-fluorescence of benz(*a*)acridine and benzo(*f*)quinoline in this solvent. In acidic nitromethane, however, the fluorescence of the extract of this spot is identical to that of the benz(*a*)acridine salt (Fig. 6).

For some compounds the fluorescence spectra on an adsorbent are different from those in solution. This difference can be used to strengthen the evidence for the characterization of a compound. For example, the emission spectrum of benz(*c*)acridine shows three sharp bands in solution, while the same spectrum on the plate shows a broad doublet at 400 and 410  $m\mu$ . This type of evidence, e.g. solution spectra, is shown in Figs. 7 and 8, in which the fluorescence spectrum of an extracted spot with the same  $R_F$  value as benz(*c*)acridine is compared to the spectra of that standard in pentane and acidic pentane solutions. This technique has been used to characterize many of the compounds separated by chromatography from various air pollution source effluents.

All compounds were characterized further by their positions in the effluent from an alumina column chromatogram, their  $R_F$  values on cellulose thin-layer chromatograms, and their fluorescence excitation and emission spectra (taken

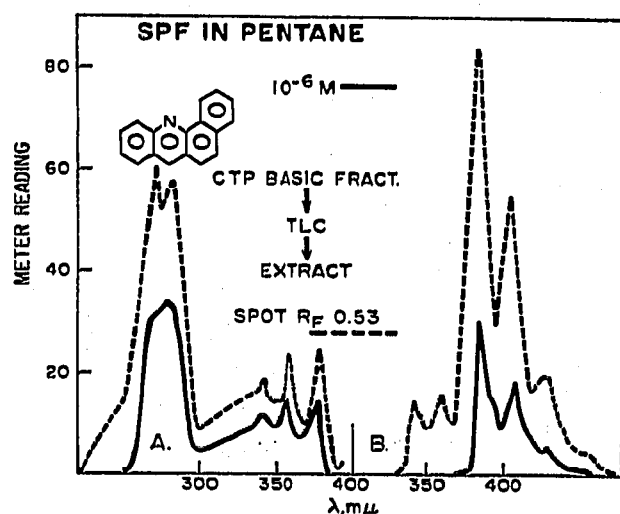


Fig. 7. Fluorescence spectra of benz(*c*)acridine at MM 0.03 (—). A. Excitation spectrum at emission wavelength 384  $m\mu$ . B. Emission spectrum at excitation wavelength 280  $m\mu$ . An extract of the spot,  $R_F$  0.53, obtained from an alumina thin-layer chromatogram (pentane-ether, 9:1, v:v) of the basic fraction of a coal-tar-pitch-polluted air sample at MM 0.01 (---). A. Excitation spectrum at emission wavelength 402  $m\mu$ . B. Emission spectrum at excitation wavelength 280  $m\mu$ .

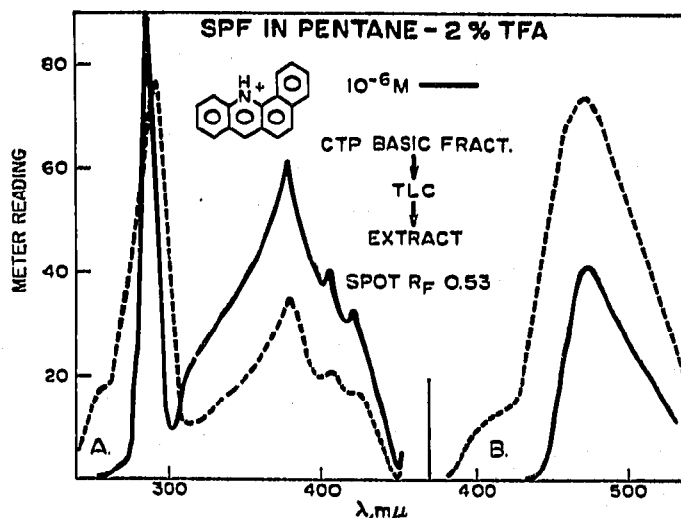


Fig. 8. Same fractions as in Fig. 7 but in acidic pentane at MM 0.01. Excitation spectra at emission wavelength 473  $m\mu$  and emission spectra at excitation wavelength at 287  $m\mu$ .

directly from the chromatogram) in the dry state, wet with ammonium hydroxide, and wet with aqueous trifluoroacetic acid. All these properties of an unknown had to be identical to those of a standard before the characterization was termed unequivocal.

## RESULTS

Table I shows the various aza heterocyclic hydrocarbons that have been characterized in coal tar pitch, coal-tar-pitch-polluted air, incinerator effluents from two different petroleum refineries, and the effluent from a domestic coal-burning furnace. Although the  $R_F$  evidence indicates that the alkyl group in many of the compounds is a methyl group, positive identification of the alkyl group and its position on the molecule could not be made because of a lack of standards.

The 25 aza heterocyclic hydrocarbons and the 8 polynuclear aromatic hydrocarbons that were extracted into the basic fraction are shown in Figs. 9a and 9b. The analyzed sample contained a large quantity of alkylated polycyclic hydrocarbons, as shown by the few that slipped into the basic fraction. These figures show the type

TABLE I

AZA HETEROCYCLIC HYDROCARBONS FOUND IN AIR POLLUTION SOURCE EFFLUENTS

No.	Compound*	Coal-tar-pitch	Coal-tar-pitch air sample	Pet. refinery I incinerator effluent	Pet. refinery II incinerator effluent	Coal combustion stack effluent
1	$R_h$ Benzo( <i>h</i> )quinoline	*	*	*	*	*
2	Benzo( <i>h</i> )quinoline	*	*	*	*	*
3	$R_b$ Benzo( <i>h</i> )quinoline	*	*	*	*	*
4	$R_c$ Benzo( <i>h</i> )quinoline	*	*	*	*	*
5	Benz( <i>c</i> )acridine	*	*	*	*	*
6	$R_h$ Benzo( <i>c</i> )acridine	*	*	*	*	*
7	$R_b$ Benzo( <i>c</i> )acridine	*	*	*	*	*
8	Dibenz( <i>ah</i> )acridine	*	*	*	*	*
9	5H-Indeno(1,2- <i>b</i> )pyridine ?				*	*
10	11H-Indeno(1,2- <i>b</i> )quinoline	*	*	*		*
11	Indenoquinoline I ?					*
12	Indenoquinoline II ?					*
13	Indenoquinoline III ?					*
14	Phenanthridine	*	*	*	*	*
15	R Phenanthridine					*
16	Acridine	*	*	*	*	*
17	Indeno(1,2,3- <i>ij</i> )isoquinoline	*	*	*	*	*
18	Benzo( <i>f</i> )quinoline	*	*	*	*	*
19	$R_h$ Benzo( <i>f</i> )quinoline	*	*			*
20	$R_b$ Benzo( <i>f</i> )quinoline	*	*			*
21	Benzo( <i>lmn</i> )phenanthridine	*	*			*
22	Benzo( <i>a</i> )acridine	*	*	*	*	*
23	$R_h$ Benzo( <i>a</i> )acridine	*	*			*
24	$R_b$ Benzo( <i>a</i> )acridine	*	*			*
25	Dibenz( <i>af</i> )acridine	*	*	*		*

\*  $R_h$ ,  $R_b$ , and  $R_c$  represent simple alkyl groups differing in position of substitution, size (methyl or ethyl) or number (1 or 2).

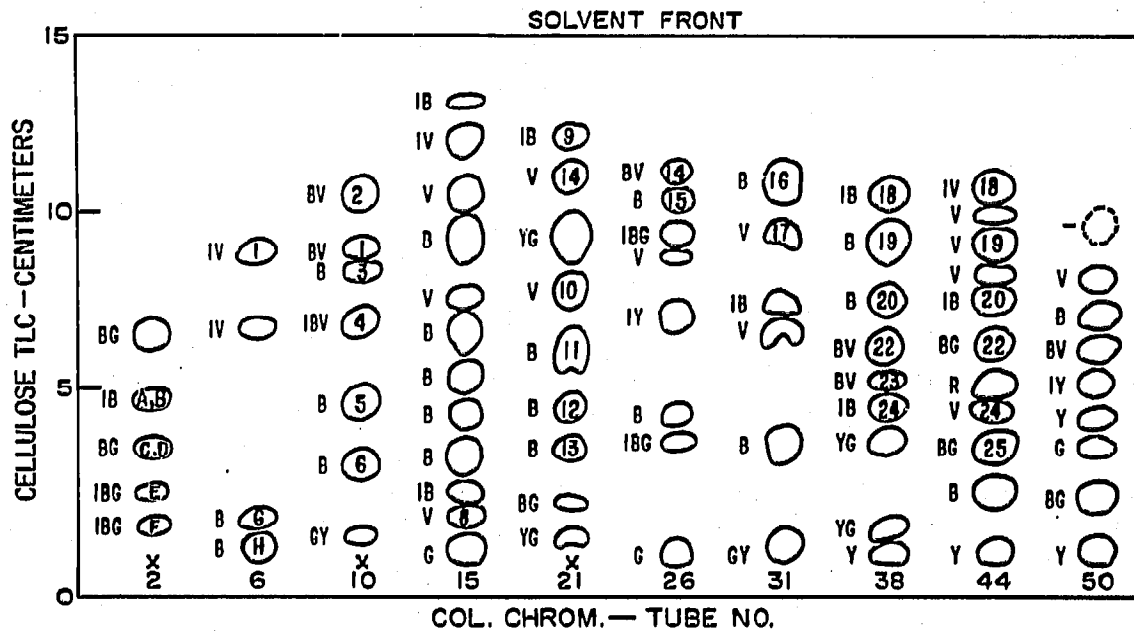


Fig. 9a. Thin-layer chromatograms (cellulose/dimethylformamide-water, 35:65) of the alumina column chromatographic fractions of the basic fraction of particulates collected from the chimney effluent of a domestic coal-burning furnace. The aza heterocyclic compounds, numbers 1 to 25, are identified in Table I. A = anthracene; B = phenanthrene; C = pyrene; D = fluoranthene; E = an alkylpyrene; F = another alkylpyrene; G = benzo(*a*)pyrene; and H = benzo(*b*)fluoranthene.

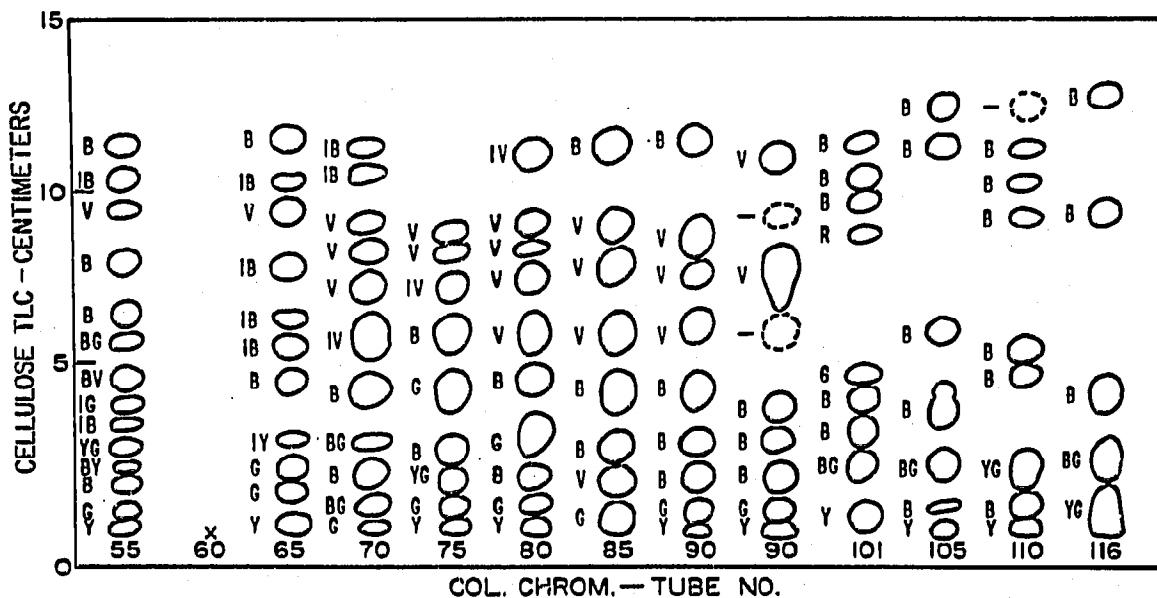


Fig. 9b. Continuation of Fig. 9a.

of separation possible with thin-layer chromatography of about every fifth column chromatographic tube. Each spot contained several compounds, most of which were not identified. Not one identified compound was found in the thin-layer chromatograms in tubes No. 50 through 116. These two figures are a reminder of the vast number of unknown compounds present in the basic fraction of these mixtures.

#### SUMMARY

By column and thin-layer chromatography approximately 200 spots have been obtained in the analysis of various air pollution sources. With the help of direct spectrophotofluorometric analysis of the spots on the thin-layer chromatograms 25 aza heterocyclic and 8 polynuclear aromatic hydrocarbons were characterized according to the parent ring structure. Eleven of the aza heterocyclic hydrocarbons were identified unequivocally.

The following carcinogens have been found in air pollution source effluents: dibenz(*a h*)acridine and dibenz(*a j*)acridine. In addition, alkyl derivatives of benz(*a*)-acridine and benz(*c*)acridine were obtained, some of which could be carcinogenic.

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