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# Characterization of Aza-Arenes in Basic Organic Portion of Suspended Particulate Matter

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■ Aza-arenes, formed as trace pollutants by incomplete combustion of *N*-containing organic matter, are found in the basic fraction of New York City's suspended particulate matter. We describe an isolating technique utilizing extraction, solvent partitioning, and a sequence of chromatographic separations. Particulate matter collected on filters is Soxhlet extracted with benzene/methanol, and the basic fraction derived from the extractable matter is prefractionated by HPLC. The subfractions are further separated by GC/MS and HPLC, followed by spectroscopic identification. This approach leads to the unambiguous identification of over 20 aza-arenes and other *N*-bases previously unidentified, and gives insight into the complexity of the basic fraction. Quantitative data also show an unanticipated abundance of quinolines, isoquinolines, and their alkyl derivatives in the fraction.

Analytical methods for polynuclear aza-heterocyclic hydrocarbons (aza-arenes) have received some attention in the past, since a number of these compounds, such as dibenz(a,j)acridine, dibenz(a,h)acridine, and some alkyl benzacridines, are known animal carcinogens (*1, 2*). Recently, quinoline has been found to induce hepatomas in rats (*3*). Although bioassays on aza-arenes have been few, it is suspected that many of these compounds might have tumorigenic activity (*2*). Aza-arenes are formed, as trace pollutants, during incomplete combustion of nitrogen-containing substances and are therefore found in urban suspended particulate matter (*4, 5*), tobacco smoke (*6, 7*), automobile exhausts (*8*), and many pollution source effluents (*9*).

Separation methods for aza-arenes have been well documented. They have been separated by thin-layer chromatography (TLC) (*10*), paper chromatography (*11*), electrophoresis (*12*), and conventional liquid chromatography using adsorption (*13*) and ion-exchange packings (*14*). Gas chromatography (GC) has also been successfully applied with a flame ionization detector (*15*) or with mass spectrometry as a specific ion detector (*4*). Various modes of high-pressure liquid chromatography, including reversed phase (*16*), adsorption (*16*), complexation (*17*), and liquid-liquid partitioning (*18*) have also been used for the separation of aza-arene standard compounds.

Aza-arenes, with the exception of neutral indole and carbazole homologs, are found in the basic organic fraction of suspended particulate matter. Although, in general, this fraction only constitutes a small percentage (0.5–3%) of the organic matter (*19*), bioassay data have shown the basic fraction to be carcinogenic to infant mice when administered subcutaneously (*20*). Sawicki and coworkers identified aza-

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arenes in the basic fraction after chromatographic enrichment by spectroscopic and spectrofluorimetric means (*5, 9*). Nineteen aza-arenes (10 parent ring compounds and 9 alkyl derivatives) were found in a Nashville sample (*5*). These procedures yield semiquantitative data and were most helpful in the past, although they may lead to composite quantitative results for several aza-arenes.

More recently, modern analytical instrumentation has been used to recheck earlier results. Alberini et al. (*15*) identified 11 aza-arenes in the basic fraction of suspended particulate matter by retention times on glass capillary columns. Using a different approach, Brocco et al. employed a TLC–GC method and identified seven aza-arenes, including quinoline which had not been previously identified (*21*). The results of the two studies were quite different from those reported by Sawicki et al., but since identifications were based solely on GC retention times, their data were questionable. Very recently, Cautreels and Van Cauwenberghe reported a detailed study of the basic fraction by mass fragmentography (*4*). Fifteen aza-arene compound types were identified in an Antwerp air particulate sample. Each type contains many isomers, undistinguishable by mass spectrometry.

Frei et al. (*22*) attempted to measure aza-arenes in the air particulate matter by injecting untreated particulate extracts into a silver-impregnated column in their HPLC system. However, no aza-arene peak was identifiable from the chromatogram.

In our approach, we prefractionate the basic fraction by HPLC into nine subfractions and characterize each subfraction independently by GC/MS and HPLC reversed phase followed by UV and fluorescence spectroscopy of the isolated peak. In view of the complexity of the basic fraction, we believe that this approach offers the most reliable qualitative and quantitative analysis of aza-arenes and other unknown components in the fraction. Since mass spectra of isomeric aza-arenes, e.g., phenanthridine and benzo(f)quinoline, are identical, the use of UV and fluorescence techniques supplements GC/MS and gives unambiguous identification and quantitation of these isomers.

## *Experimental*

The experimental procedure is outlined in Figure 1.

**Reagents.** Reference compounds were obtained from K&K, Aldrich Chemical Co., and Pfaltz & Bauer. 4-Azapyrene, 2-aza-fluoranthene, 13-azafluoranthene, 11H-indeno(1,2-b) quinoline, and four dimethylquinolines were kindly donated by Dr. Sauerland of the Rütgerswerke AG, Duisburg, West Germany. All solvents used were spectrograde from Fisher Scientific or from Burdick & Jackson.

**Sampling.** Particulate matter samples were provided by the New York City Department of Air Resources. They were collected at various sites in New York City's aerometric network on glass fiber filters by use of high-volume samplers. The average rate of sampling is 40–50 $ft^3$/min. The weight of a 24-h sample ranges from 90 to 200 mg depending on site, season, and weather conditions. Sample 1 is a 180-filter combined sample collected during February–April 1975, randomly selected from different stations. Sample 2 is a similar 100-filter sample collected during January–March 1975. The two samples were supplied separately from the Department of Air Resources.

**Extraction.** Extraction from soiled filters was carried out for 8 h in two large soxhlet extraction apparatus with preextracted Whatman cellulose thimbles (123 × 43 mm, Fisher Scientific). Each thimble accommodates five filters. Benzene/methanol (4:1) was used as the extracting solvent (*23*). (Since the azeotropic mixture is 60.5:39.5, the actual extracting solvent in the soxhlet apparatus may not be 4:1.) Other workers have used $CHCl_3$ (*5*), benzene (*4, 21*), or cyclohexane (*15*). We chose this solvent because it gives the highest amount of extractable organic matter and yields the most biologically active extract (*19*). We also believe that since aza-arenes are weak bases, and salt formation with inorganic and organic acids on the filter is a possibility, a polar solvent must be used.

**Partitioning.** The extract was evaporated to near dryness in a rotary evaporator at water bath temperatures under 45 °C, redissolved in 100 mL $CHCl_3$, and partitioned twice with equal volumes of water. The aqueous layer containing the water-soluble compounds was discarded. The $CHCl_3$ layer was extracted twice with 10% $H_2SO_4$ and once with 20% $H_2SO_4$ solution. The use of a strong acid is recommended since aza-arenes are weak bases. Emulsions were often encountered in these partitioning steps but could be minimized by sonicating in an ultrasonic bath. The acidic layers were combined, cooled in an ice bath, and neutralized by adding saturated NaOH solution until pH 12 was reached. This solution (ca. 300 mL) was then back-extracted three times into equal volumes of $CHCl_3$. The combined $CHCl_3$ solution was dried over anhydrous $NaSO_4$, filtered, and concentrated to 0.1 mL. The resulting dark brown solution, the basic fraction of particulate organic matter, contained aza-arenes and other *N*-bases.

**Prefractionation.** A prefractionation step is necessary since the basic fraction, especially when derived from a benzene/MeOH extraction, is too complex for any single separation technique. A high-pressure liquid chromatograph Model ALC/GPC 202 (Waters Associates) equipped with Model M-6000 pumps, Model 660 solvent programmer, and a 254-nm differential UV detector was used. Lichrosorb SI 60 silanized (30 $\mu$ diameter) (Brinkman Instruments, Inc.) was dry packed into a 2.1 mm i.d. × 60 cm stainless steel column for the prefractionation. Figure 2 shows a typical prefractionation chromatogram. With a solvent composed of 0.5% propanol-2 in *n*-hexane and a flow rate of 3.0 mL/min, most aza-arenes will elute between 4–25 min. Fifteen minutes after the injection of an 100-$\mu$L aliquot of the basic fraction, the solvent composition was linearly programmed to 20% propanol-2 in $CHCl_3$ in 20 min. Eight subfractions (10–50 mL) were collected, concentrated, and redissolved in 100 $\mu$L of MeOH.

**GC/MS Analysis.** Aliquots of these subfractions were injected into a Hewlett-Packard Model 5982 dual source combined GC/MS system interfaced with a Hewlett-Packard Model 5933A computerized data system. Mass spectra were obtained by electron impact at 70 eV. A 10-ft, ⅛-in. o.d. stainless steel column, packed with 6% Dexsil 300 GC on Chromosorb W(HP), 80/100 mesh, was used at 60 mL/min

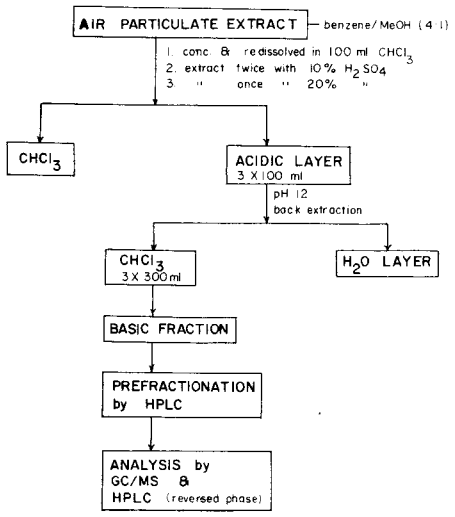



**Figure 1.** Analytical scheme for characterization of basic fraction of suspended particulate matter

flow rate. Temperature programming from 160 to 300 °C at 8 °C/min was initiated immediately after injection of each sample. Under these conditions the retention time of dibenz(a,h)acridine was 60 min. Other gas chromatograms were obtained on a Hewlett-Packard 5701A gas chromatograph with dual columns and flame ionization detectors.

**HPLC Reversed-Phase Analysis.** A 30 cm × 4 mm i.d. μ-Bondapak/$C_{18}$ reversed-phase column (Waters Assoc.) was used on the same HPLC described above. A solvent gradient from 20% $CH_3CN/H_2O$ to 80% $CH_3CN/H_2O$ at 4%/min and at a 3.0-mL/min flow rate was found to give satisfactory separation of most aza-arenes in 20 min. The chromatographic behavior of aza-arenes on reversed-phase columns is similar to that of the polynuclear aromatic hydrocarbons (*24, 25*) and is governed primarily by the number of aromatic rings.

Injections of 1–2% of the subfraction were sufficient for good HPLC chromatograms. However, use of over 30% of the subfraction was necessary when effluent fractions were to be analyzed by UV. Fractions (about 1 mL) sufficient to fill a UV microcell (4 × 10 mm) were taken. A Cary 14 UV–VIS spectrophotometer and a Perkin-Elmer MFP-2A spectrofluorometer were used to obtain spectra. Reference spectra were either obtained from aza-arene standards or from literature (*26*).

Extraction of 10 unexposed filters (blanks) and subsequent analysis by the same procedure yielded no aza-arenes.

*Results and Discussion*

**Difficulties in This Analysis.** The concentrations of aza-arenes in urban air ranged from 10 to 100 ng/g of the organic particulate extracts (10–100 ppb), roughly 100 times lower than the concentration of benzo(a)pyrene. The total amount of individual aza-arenes isolated rarely exceeded a few μg even after sampling large volumes of air. The analysis of these trace quantities in a complex environmental matrix presents several difficulties. In the past these problems were partially circumvented by relying heavily on fluorescence techniques which are very selective methods for identifying aza-arenes. However, we believe that our approach, using two separate high-resolution chromatographic methods followed by a battery of spectroscopic techniques, gives the most detailed and unequivocal characterization of the basic fraction.

Sample contamination is another problem in trace analyses. All glassware was washed in chromic acid, thoroughly rinsed with water, and triply rinsed with spectrograde solvent. Trace contaminants in TLC and conventional LC packings are avoided completely by using a HPLC adsorption prefractionation. This step gives quantitative recovery and is extremely reproducible as well as convenient. The entire prefractionation can be completed in 1 h, and cut-off points between fractions are easily determined by the UV monitor (Figure 2). Silanized silica, a weaker adsorbent than regular silica, was used to prevent any possible irreversible adsorption of the sample.

**Sample Size.** In our study, over 100 filters were used for detailed analyses. For routine analysis of major aza-arenes, a sample size of 10 filters should be quite sufficient.

**GC/MS of Subfractions.** The use of a computerized data system greatly enhances the power of a GC/MS system. It simplifies data handling and allows convenient mass spectral background subtraction. It enables the use of mass fragmentography or specific ion monitoring, which has become a powerful tool for routine analysis and sample screening. Also, the use of a mass spectral search system greatly facilitates mass spectral comparisons and, therefore, the identification of unknowns.

It should be indicated that many components of the basic fraction are only tentatively identified or unidentified even after rigorous efforts in searching reference mass spectra in the literature (*27*) and in our Hewlett-Packard disc reference library. The acute lack of reference mass spectral data, especially in the field of air pollution, is still a major hindrance to a more complete characterization. Fortunately, the aza-arenes as a class give very characteristic fragmentation patterns and are quite easily recognizable. Under electron impact, they yield strong molecular ion peaks and usually M-27 or M-28 peak corresponding to the loss of HCN or $H_2CN$ fragments (*28*). The mass spectrum of quinoline shown in Figure 3 is quite representative of other aza-arenes and almost identical to the mass spectrum of isoquinoline. The mass spectrum of quinoline, isolated from Sample 1, Fraction IV, is very similar to that of pure quinoline (Figure 3). This is important in quantitative analysis by GC since one peak often contains composite components. Figures 4–6 are gas chromatograms of Fractions III, IV, and IVA, respectively. Most labeled peaks were identified by both retention time and by mass spectral data.

**Specific Ion Monitoring.** Figure 7 demonstrates the use of mass fragmentography on Fraction IV and shows the simultaneous monitoring of quinolines, isoquinolines, and many

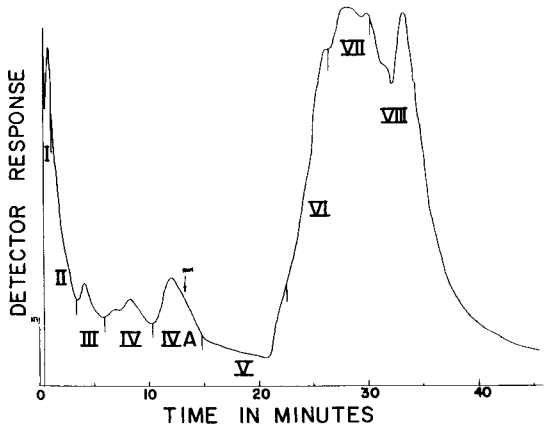



**Figure 2.** Typical HPLC prefractionation trace
Lichrosorb SI 60 column, 3.0 mL/min, 0.5 propanol-2 in hexane programmed after "RUN" to 20% propanol-2 in $CHCl_3$ in 20 min, sensitivity setting at nonlinear

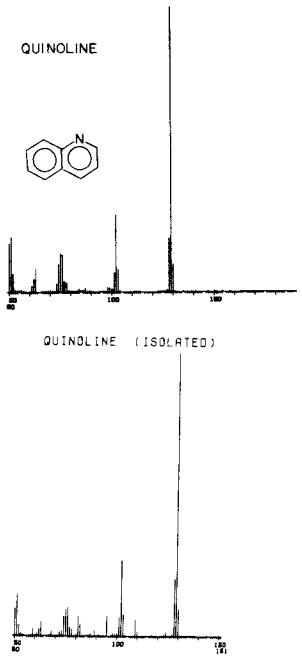



**Figure 3.** Mass spectra of pure quinoline and quinoline isolated in Sample 1

alkyl quinolines in the sample. The traces are normalized with respect to the quinoline peak. The relative concentration of each alkyl quinoline can be estimated from its total integrated area under each chromatographic peak. Figure 7 illustrates isoquinoline eluting after quinoline and shows the surprisingly high concentration of dimethylquinolines in New York City air (c.f., methylquinolines). The $C_3$-alkyl quinoline certainly would have escaped detection without the use of this technique.

**HPLC.** Figures 8–10 are HPLC chromatograms on Subfractions III, IV, and IVA, respectively. Most labeled peaks are identified by retention times, UV, and fluorescence spectra on the collected fractions. Compared to the GC traces (Figures 4–6), HPLC gives cleaner traces since the UV detector is quite sensitive to aza-arenes which have molar absorptivities of $10^4$–$10^5$ at 254 nm. The detection limit for most aza-arenes is $\simeq$1 ng (*16*) which compares favorably with that of GC, especially for the 5-ring, high boiling aza-arenes, e.g., dibenzacridines.

**UV and Fluorescence Spectra.** Figure 11 shows a UV spectrum of isoquinoline isolated from Fraction IVA of Sample 1. The dotted line is the spectrum from the pure compound at similar concentration taken from the literature (*26*). Figures 12 and 13 are similar fluorescence and excitation spectra of isolated benzo(f)quinoline. The spectra are not

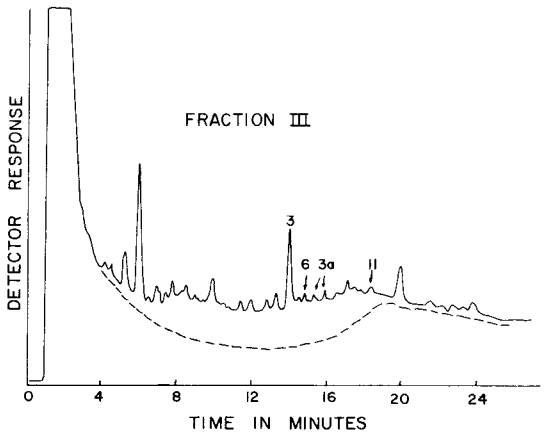



**Figure 4.** Gas chromatogram of Fraction III of Sample 1
Chromatographic conditions: 160 °C programmed to 300 °C at 8 °C/min, helium flow rate 30 mL/min, hydrogen pressure 20 psi, air pressure 30 psi. Column = 10-ft, ⅛-in., 6% Dexsil 300 GC on Chromosorb W(HP), 80/100 mesh; sensitivity = X80. Numbers correspond to compounds listed in Table I

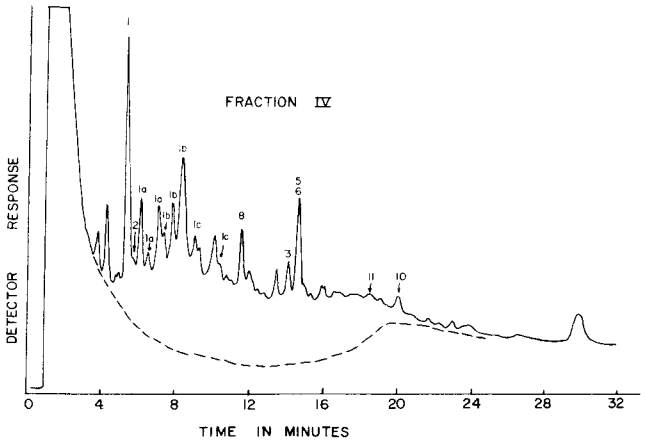



**Figure 5.** Gas chromatogram of Fraction IV of Sample 1
Chromatograph conditions same as in Figure 3

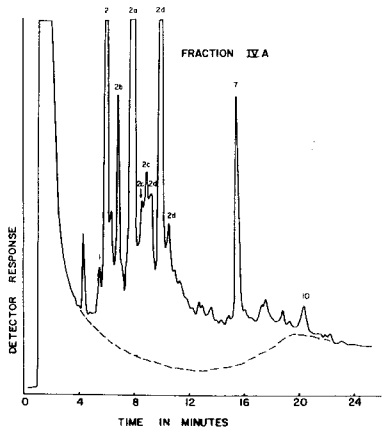



**Figure 6.** Gas chromatogram of Fraction IVA of Sample 1
Chromatographic conditions same as in Figure 3

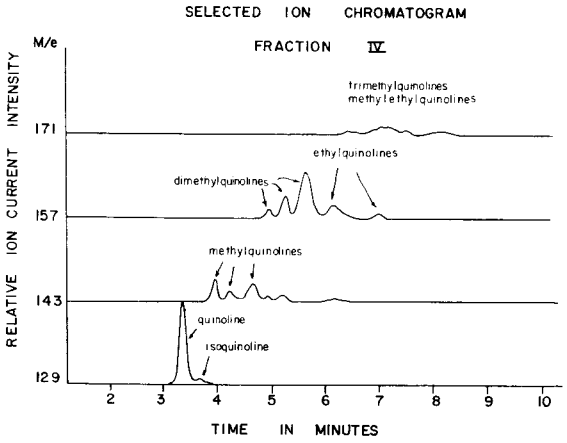



**Figure 7.** Selected ion chromatograms on Fraction IV, quinoline fraction
Chromatographic conditions same as in Figure 4

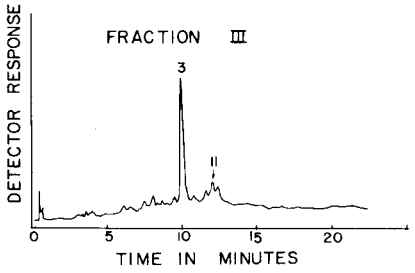



**Figure 8.** Liquid chromatogram of Fraction III of Sample 1
Chromatographic conditions: 20% $CH_3CN$ in $H_2O$ programmed to 80% $CH_3CN$ in $H_2O$ at 4%/min at 3.0 mL/min flow rate; column: μ-Bondapak/C18; sensitivity = 0.2 full-scale absorbance unit. Numbers correspond to compounds listed in Table I

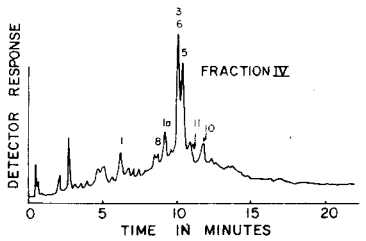



**Figure 9.** Liquid chromatogram of Fraction IV of Sample 1
Chromatographic conditions same as in Figure 8

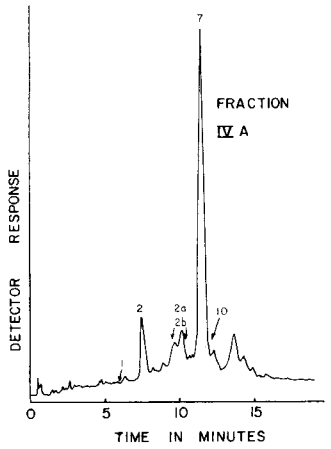



**Figure 10.** Liquid chromatogram of Fraction IVA of Sample 1
Chromatographic conditions same as in Figure 8

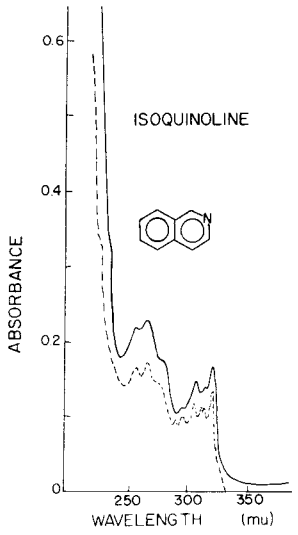



**Figure 11.** UV spectrum of isoquinoline isolated in Sample 1

corrected for source intensity and detector wavelength response variations.

**Quantitation.** Quantitative data on Samples 1 and 2 were obtained as follows: Two synthetic mixtures of aza-arene reference compounds were carefully prepared. An aliquot of these solutions was injected routinely to generate retention data and to check column reproducibility. The area under each peak was compared to that derived from an aliquot injection of a sample subfraction solution, usually after its identity and purity had been assured by mass spectrometry. Isomers like phenanthridine and benzo(f)quinoline were quantitated from UV spectra of collected fractions separated by HPLC.

**Concentrations of Aza-Arenes in New York City Suspended Particulate Matter.** The concentrations of aza-arenes and other *N*-bases from the basic fraction are summarized in Table I.

**2-Ring Aza-Arenes.** Quinolines escaped identification by Sawicki et al. since they possess low molar absorptivity ($3.8 \times 10^3$ at 308 nm) and do not fluoresce. We have confirmed Cautreels and Van Cauwenberghe's finding (*4*) by independently discovering quinoline in our New York City samples, and have extended their observations by demonstrating with quantitative data the complexity of the quinoline (IV) and isoquinoline (IVA) fractions. In our two New York City samples, the concentration of isoquinoline exceeds that of quinoline by 3–7 times. The highest concentration of aza-arenes (310 ng/1000 $m^3$ in Sample 1) is associated with a methyl derivative of isoquinoline. The exact structure has not yet been determined because of the lack of a reference compound; however, boiling point data (*29*) suggest its identity to be a 5- or 8-methylisoquinoline. It is possible that there are very specific precursors for this compound.

It should be emphasized that the reported levels of quinolines and isoquinolines represent the minimum concentrations in ambient air because quinoline (bp 237 °C) and isoquinoline (bp 242 °C) are probably not quantitatively trapped by the high-volume samplers. Their retention on glass fiber filters might be correlated to their hygroscopicity or possibly to their basicity.

**3-Rings.** Of the five isomers of 3-ring aza-arenes, benzo(f)-isoquinoline has the highest concentration (three times that of acridine in Sample 1) in our samples. This compound has

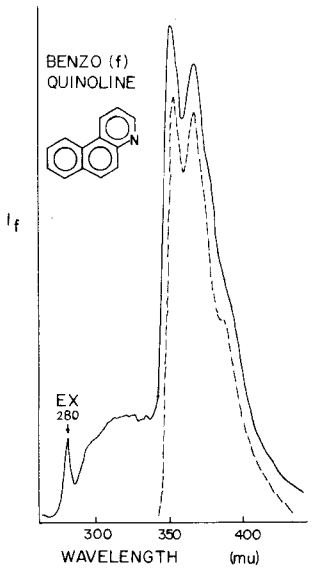



**Figure 12.** Fluorescence spectrum of benzo(f)quinoline isolated in Sample 2
Excitation slit width, 4 nm; emission slit width, 4 nm

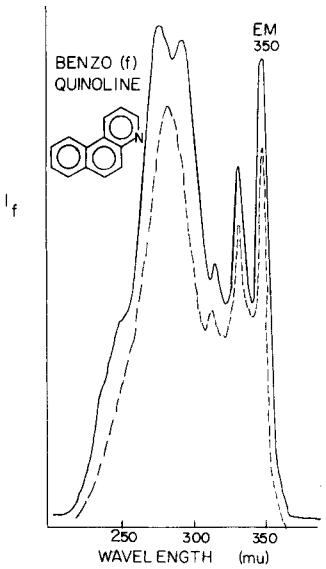


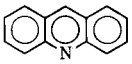
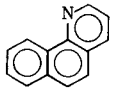
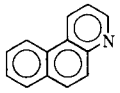
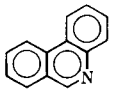
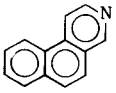
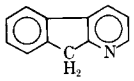
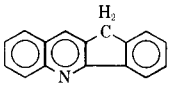
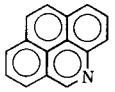
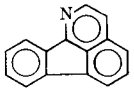
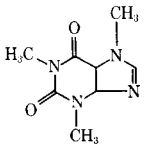

**Figure 13.** Excitation spectrum of benzo(f)quinoline isolated in Sample 1
Excitation slit width, 4 nm; emission slit width, 4 nm

not been previously isolated. It was identified by its chromatographic behavior on silica, mass spectrum, UV, and fluorescence spectra.

**4- and More Rings.** Azapyrenes, azafluoranthene, and traces of 11H-indeno(1,2-b)quinoline are the only 4-ring aza-arenes found in our samples. No benzacridine or dibenzacridine was found, even though our detection limit for these compounds in the HPLC reversed phase is $\simeq 1$ ng.

It is interesting to note that we found much higher concentrations of 2-ring aza-arenes and progressively smaller amounts of higher ring compounds in our samples. The reverse observation was reported for the Antwerp sample studied by

**Table I. Concentration of Aza-Arenes in New York City Suspended Particulate Matter**

| No. | Aza-arenes | Name | Mol wt | Concn in ng/1000 m$^3$ Sample 1 | Sample 2 |
|---|---|---|---|---|---|
| 1 |  | Quinoline | 129 | 69 | 22 |
| 1a | | Methylquinolines | 143 | 35 | 33 |
| 1b | | Dimethylquinolines | 157 | 48 | 44 |
| 1c | | Ethylquinolines | 157 | 14 | 22 |
| 1d | | 3C-quinolines | 171 | 10 | ND[a] |
| 2 |  | Isoquinoline | 129 | 180 | 140 |
| 2a | | 5 or 8 methylisoquinoline | 143 | 310 | 170 |
| 2b | | Other methylisoquinolines | 143 | 76 | 70 |
| 2c | | Dimethylisoquinolines | 157 | 62 | ND |
| 2d | | Ethylisoquinolines | 157 | 160 | 68 |
| 2e | | 3C-isoquinolines | 171 | 28 | ND |
| 3 |  | Acridine | 179 | 41 | 40 |
| 3a | | Methylacridines | 183 | 7 | ND |
| 4 |  | Benzo(h)quinoline | 179 | 10 | 13 |
| 5 |  | Benzo(f)quinoline | 179 | 11 | 10 |
| 6 |  | Phenanthridine | 179 | 22 | 18 |
| 7 |  | Benzo(f)isoquinoline | 179 | 110 | 34 |
| 8 |  | 4-Azafluorene (5-H-indeno-[1,2-b]-pyridine)[b] | 167 | 5 | 5 |
| 9 |  | 11H-indeno(1,2-b)quinoline | 217 | Trace | Trace |
| 10 |  | 4-Azapyrene (benzo (lmn)-phenanthridine) | 203 | 21[c] | 22[c] |
| 11 |  | 1-Azafluoranthene (indeno-[1,2,3-ij]isoquinoline) | 203 | 5[c] | 5[c] |
| 12 |  | Benzothiazole | 135 | 14 | 20 |
| 13 |  | Caffeine | 194 | 3400 | 7000 |

[a]ND: not determined. [b]Name according to Patterson's Ring Index. All structures are also drawn according to the ring index. [c]Includes other isomers.

Cautreels and Van Cauwenberghe (*4*). They found relatively higher concentrations of 4- and 5-ring aza-arenes compared to the 2-ring compounds. The fact that European cities generally burn more coal might explain this important difference.

**Others.** Benzothiazole, a common component in fuel oil, was found in Fraction 2. So far, no S-containing organic compound from particulate matter has ever been reported (*19*). The discovery of more S-containing compounds is expected to be found by using a sulfur selective flame photometric detector in GC.

Caffeine, in relatively high concentration, was found in Fractions 7 and 8. The ubiquity of coffee-roasting plants in New York City and in adjacent New Jersey might explain its presence. This finding is of biological interest since a recent assay has revealed that caffeine can inhibit the carcinogenic activity of polynuclear aromatic hydrocarbons (*30*).

This analytical study reveals valuable data on the composition of the basic fraction, both in terms of new compounds identified and their relative concentrations. The abundance and complexity of the quinoline and isoquinoline fractions, relative to other aza-arenes, should be noted. The recent findings on the carcinogenic properties of quinoline should be an additional reason for initiating more analytical work on the basic fraction. Nevertheless, since the observed quantities of aza-arenes are so minute and sufficient bioassays on these compounds are lacking, we hesitate to assign a biological significance to their presence in the basic fraction of urban pollutants.

Since the majority of the bicyclic compounds was probably lost during particulate matter collection by high-volume samplers, we are currently investigating trapping devices for quantitative sampling of semivolatile organics in the atmosphere. Improved sampling, coupled with a radioactive tracer for determining analytical recoveries, will definitely provide a more accurate and realistic assessment of the level of quinolines and other harmful volatiles in our cities.

Our approach should also be adaptable to other environmental sample types such as cigarette smoke, coal tar, and petroleum products where aza-arenes are present in relatively higher concentrations. Since aza-arenes, like polynuclear aromatic hydrocarbons, are pyrosynthesized during combustion, it is expected that they are widely distributed in the environment (*31*) and that they will receive more attention in future environmental research.

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*Literature Cited*

(1) Shubik, P., Hartwell, J. L., "Survey of Compounds Which Have Been Tested for Carcinogenic Activity", U.S. Public Health Service Publ. 149, 1951; Suppl. 1, 1957; Suppl. 2, 1969; 1968–1969 Vol, 1972; 1961–1967 Vol, Sect. I, Sect. II, 1973; 1970–1971 Vol, 1974.
(2) Arcos, J. C., Argus, M. P., "Chemical Induction of Cancer", Vol IIA, Academic Press, New York, N.Y., 1974.
(3) Hirao, K., Shinohara, Y., Tsuka, H., Fakushima, S., Takahashi, M., Ito, N., *Cancer Res.*, **36,** 329 (1976).
(4) Cautreels, W., Van Cauwenberghe, K., *Atmos. Environ.*, **10,** 447 (1976).
(5) Sawicki, E., McPherson, S. P., Stanley, T. W., Meeker, J., Elbert, W. C., *Int. J. Air Water Pollut.*, **9,** 515 (1965).
(6) Van Duuren, B. L., Bilbao, J. A., Joseph, C. A., *J. Nat. Cancer Inst.*, **25,** 53 (1960).
(7) Wynder, E. L., Hoffmann, D., "Tobacco and Tobacco Smoke", Academic Press, New York, N.Y., 1967.
(8) Sawicki, E., Meeker, J. E., Morgan, M. J., *Arch. Environ. Health,* **11,** 773 (1965).
(9) Sawicki, E., Meeker, J. E., Morgan, M. J., *Int. J. Air Water Pollut.*, **9,** 291 (1965).
(10) Engel, C. R., Sawicki, E., *J. Chromatogr.*, **31,** 109 (1967).
(11) Sawicki, E., Pfaff, J. P., *Anal. Chim. Acta,* **32,** 521 (1965).
(12) Sawicki, E., Guyer, M., Engel, C. R., *J. Chromatogr.*, **30,** 522 (1967).
(13) Sawicki, E., Stanley, T. W., Elbert, W. C., *ibid.*, **13,** 512 (1965).
(14) Snyder, L. R., *Anal. Chem.*, **41,** 314 (1969).
(15) Alberini, G., Cantuti, V., Cartoni, G. P., in "Gas Chromatography", A. B. Littlewood, Ed., p 258, inst. of Petrol., London, England, 1967.
(16) Dong, M., Locke, D. C., Hoffmann, D., *J. Chromatogr. Sci.*, **15,** 32 (1977).
(17) Vivilecchia, R., Thiebaud, M., Frei, R. W., *ibid.*, **10,** 441 (1972).
(18) Ray, S., Frei, R. W., *J. Chromatogr.*, **71,** 45 (1972).
(19) Hoffmann, D., Wynder, E. L., in "Air Pollution", 3rd ed., Vol 2, A. C. Stern, Ed., Academic Press, New York, N.Y., in press.
(20) Asahina, S., Andrea, J., Carmel, A., Arnold, E., Bishop, Y., Joshi, S., Coffin, D., Epstein, S. S., *Cancer Res.*, **32,** 2263 (1972).
(21) Brocco, D., Cimmino, A., Possanzini, M., *J. Chromatogr.*, **84,** 371 (1973).
(22) Frei, R. W., Beall, K., Cassidy, J., *Microchim. Acta,* **12,** 859 (1974).
(23) Hoffmann, D., Wynder, E. L., *Cancer,* **15,** 93 (1962).
(24) Dong, M., Locke, D. C., Ferrand, E., *Anal. Chem.*, **48,** 368 (1976).
(25) Locke, D. C., *J. Chromatogr. Sci.*, **12,** 433 (1974).
(26) "UV Atlas of Organic Compounds", 5 vols, Plenum Press, New York, N.Y., 1967–68.
(27) Stenhagen, E., Abrahamson, S., McLafferty, F. W., "Registry of Mass Spectral Data", Wiley, New York, N.Y., 1974.
(28) Porter, Q. N., Baldas, J., in "Mass Spectrometry of Heterocyclic Compounds", Wiley-Interscience, New York, N.Y., 1971.
(29) Lang, K. F., Eigen, I., *Fortschr. Chem. Forsch.*, **8,** 91 (1967).
(30) Rothwell, K., *Nature,* **252,** 69 (1974).
(31) Blumer, M., Youngblood, W. W., *Science,* **188,** 53 (1974).

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