

COLUMN CHROMATOGRAPHIC SEPARATION OF BASIC POLYNUCLEAR AROMATIC COMPOUNDS FROM COMPLEX MIXTURES

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Airborne particulates and other complicated mixtures have been separated through alumina column chromatography, and many polynuclear aromatic hydrocarbons have been determined in the separated fractions through ultraviolet-visible absorption spectrophotometry. Pioneers in this development have been GOULDEN AND TIPLER¹, WEDGWOOD AND COOPER², FALK³ and KOE AND ZECHMEISTER⁴. This method has been applied extensively to the analysis of urban airborne particulates^{5,6}. Pentane with increasing amounts of ether has been used as the developing solvent. In this way organic air borne and air pollution source particulates are readily fractionated by one run through an alumina column and then analyzed spectrophotometrically for 9 to 12 polycyclic aromatic hydrocarbons⁵.

Another column adsorbent that has been shown to be of value in the separation of polycyclic aromatic hydrocarbons is cellulose acetate⁷. The method has been used extensively in pyrolysis studies⁸.

The possible use of π -complexing agents on a column for new types of separations is indicated by the isolation of the benzopyrene fraction minus benzo(*k*)fluoranthene from an airborne particulate sample⁹. Another system that is effective in separating mixtures of polycyclic aromatic hydrocarbons is a liquid-liquid partition chromatographic Fluorisil column consisting of a stationary liquid phase of *sym.*-trinitrobenzene in Carbowax-400 developed by iso-octane¹⁰.

Although the use of these various adsorbents for the separation of the more polar aromatic compounds has not been studied, these adsorbent systems should be of value in the analysis of polluted air and other complicated mixtures. In the work described in this paper the same type of alumina column so successful in hydrocarbon analysis⁵ has been used with a more polar solvent system for the separation of the basic fraction of complex mixtures into analyzable fractions. Of great value in the characterization and determination of the aza heterocyclic hydrocarbons are the absorption and fluorescence spectra of these compounds in pentane and pentane-1 % trifluoroacetic acid¹¹.

EXPERIMENTAL

Chemicals and equipment

All solvents were distilled before use. The pure aza compounds were obtained

from commercial sources and were purified by crystallization where evidence of impurity was found.

A Cary recording quartz spectrophotometer Model 11 with 1-cm cells was used for absorption wavelength determinations. Thermo-Vac ovens (Schaar and Company, Chicago, Illinois 60634) were used for careful evaporation of the solutions.

Chromatography

For the chromatographic work Merck acid-washed aluminum oxide was washed with ether, dried, and heated in an oven at 130° for 4 h. The alumina then contained approximately 12 % water (as determined by weighing a sample, heating to a standard red heat for 10 min, cooling in a desiccator, and reweighing). Enough water was then added to the oven-dried alumina to give a final concentration of 13.0 %. (Less water was added for greater resolution; more water for faster separation.) The mixture was shaken and allowed to stand for 12 h in a stoppered container before being used.

One gram of the treated alumina was added to a small volume of the chloroform solution containing 1 to 50 mg of the basic fraction. This fraction was obtained by extracting an organic airborne particulate or air pollution source sample or any other complicated mixture with 10 % aqueous sulfuric acid, neutralizing the acid with sodium carbonate, and extracting this mixture with chloroform. The chloroform on the alumina was evaporated so that the organic material was homogeneously dispersed. This alumina was then added to an 0.5- by 15-in. column, which contained a lower layer of 9 g of the previously prepared alumina and an upper layer of 0.5 g of silica gel, neither containing eluting solvent.

The column was eluted with successive 100 ml volumes of pentane solutions containing the following percentages: of ether—8, 16, 24, 32, 40, 48 and 56, of acetone—5, 10, 15, 20, 25, 30, 35, 40, and then with 100-ml volumes of ether followed by methanol, respectively. Although the column elution required approximately 9 h, most of the known aza compounds were collected before the acetone in pentane solutions were reached; therefore if the operation were stopped at this stage the procedure would involve less than 1 day of operation. The column was protected from light during this period. Fractions of approximately 15 ml were collected and then allowed to stand overnight in the hood.

Spectral analysis

After standing overnight most fractions had evaporated to dryness. The residue in each of these tubes was dissolved in a small volume of pentane and transferred quantitatively by repeated washings to a 3-ml spectral cell of 1-cm light path. The final volume was 3.0 ± 0.1 ml. The ultraviolet-visible absorption spectrum of each tube was then determined. While these spectra were being obtained, the tubes that still contained eluting solvent were placed in a vacuum oven and carefully evaporated at room temperature in the dark. Then the adsorption spectra of these fractions were also obtained. This procedure can be used with the baseline method for quantitative analysis of the separated basic organic compounds.

For further characterization a drop (about 0.03 ml) of trifluoroacetic acid can be added to the pentane solution in the cell. The absorption spectrum of the salt will then be obtained.

RESULTS AND DISCUSSION

An example of the separation is shown in Fig. 1. Note that the largest molecule comes off the column first. This is because the aza nitrogen is most sterically hindered in this molecule. The alumina adsorbent has a strong attraction for the aza nitrogen. In the figure a dividing line is shown between sterically hindered and non-sterically hindered molecules; this line comes at approximately the thirtieth tube.

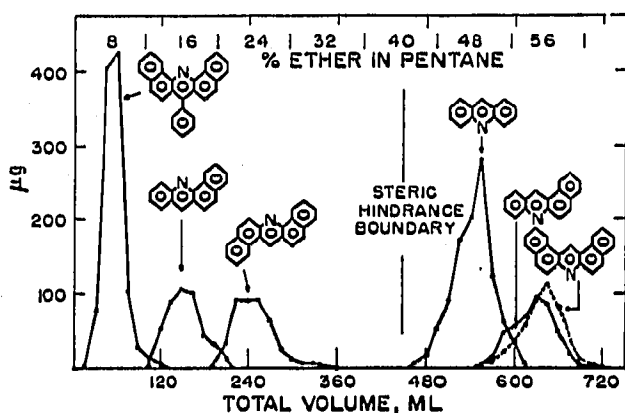


Fig. 1. Elution of 7-phenyldibenz(*c,h*)acridine, benz(*c*)acridine, dibenz(*a,h*)acridine, acridine, benz(*a*)acridine and dibenz(*a,j*)acridine on an alumina column. The three- and six-ring compounds in 1-mg amounts; all others in 0.5-mg amounts.

The dividing line can be moved back further while the elution volume for each compound is increased either by decreasing the percentage of ether in the pentane, by decreasing the amount of water on the alumina, or by lengthening the column. The composition of the mixture chromatographed also affects the position of the dividing line and the elution volumes.

With the described procedure, sterically hindered aza heterocyclic hydrocarbons up to about six or seven rings can be readily separated from the non-sterically hindered aza compounds. Among aza heterocyclic hydrocarbons with the same amount of steric hindrance at an aza nitrogen atom, those with fewer rings are eluted more quickly. Examples of this phenomenon are the pairs: benz(*c*)acridine and dibenz(*a,h*)acridine, acridine and benz(*a*)acridine (Fig. 1). This type of separation is dissimilar from that of the polycyclic aromatic hydrocarbons on alumina, in which the compounds are eluted mainly according to the number of rings in the molecule.

The elution properties of the first compound eluted in Fig. 1 resemble those of the polycyclic hydrocarbons mainly because of the large amount of steric hindrance around the aza nitrogen atom. This resemblance is shown by the fact that polycyclic hydrocarbons containing up to seven fused rings, *e.g.* benzene to coronene, would be eluted in the first six tubes, if they were present.

APPLICATION

The procedure was applied to a complicated mixture, the basic fraction of a sample of coal tar pitch. Coal tar pitch pollution is notorious in that very large concentrations of polynuclear hydrocarbons can be found in extremely high concentra-

tions in the air¹². The separation on the column worked very nicely. The absorption spectrum in pentane and pentane-1 % trifluoroacetic acid was obtained for each tube. Sharp spectral bands were found in all tubes. It was estimated that sharp bands were obtained for at least 100 basic aromatic compounds. On the basis of position on the column and the absorption spectra in pentane and pentane-1 % trifluoroacetic acid, the following compounds were characterized: an alkylbenzo(*h*)quinoline, benzo(*h*)quinoline, benzo(*f*)quinoline, acridine, benz(*a*)acridine, and benz(*c*)acridine. Some of the absorption spectra indicated that alkyl derivatives of the benzacridines may be present. The presence of these types of compounds should be more thoroughly investigated, since many alkylated benzacridines are carcinogenic¹³. Examples of the evidence are shown in Figs. 2 and 3. The spectrum of the sixth fraction obtained in this run is very closely similar in pentane (and acidic pentane) to that of benzo(*h*)quinoline (Fig. 2). The unknown compound in pentane solution absorbs 2 m μ further in the visible spectrum, *e.g.*, its long wave-length band is at 348 m μ while the comparable band for benzo(*h*)quinoline is at 346 m μ . From this evidence it is deduced that the

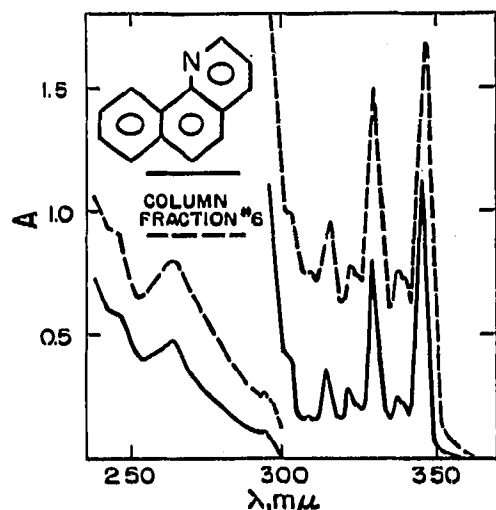


Fig. 2. Ultraviolet absorption spectra in pentane of benzo(*h*)quinoline (—) and subfraction 6 of a basic fraction from coal tar pitch (---).

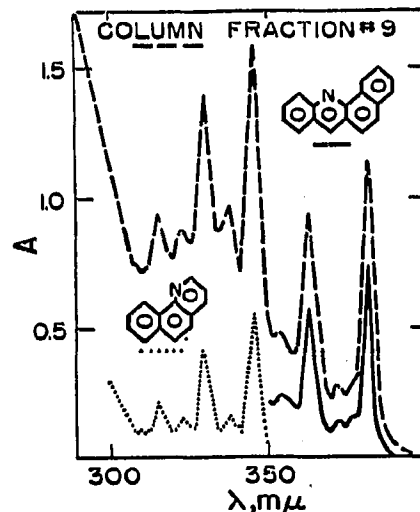


Fig. 3. Ultraviolet absorption spectra in pentane of benzo(*h*)quinoline (· · · ·), benz(*c*)acridine (—) and subfraction 9 of a basic fraction from coal tar pitch(---).

compound is an alkyl benzo(*h*)quinoline. A mixture of benzo(*h*)quinoline and benz(*c*)acridine is found in fraction 9. Some of the evidence is shown in Fig. 3. The bands for benzo(*h*)quinoline and benz(*c*)acridine coincide exactly with the spectral bands found for the fraction. In acidic pentane the fraction gave bands at 294, 332, 410 and 432 m μ ; these coincide with the bands obtained for benz(*c*)acridine in the same solvent. In addition the acidic solution of the fraction had bands at 277 (shoulder), 282, 310, 328, 356 and 372 m μ ; these coincide with the bands obtained for benzo(*h*)quinoline in the same solvent.

The same type of evidence was used for characterizing the other compounds. The absorption spectrum in pentane solution following column chromatography is the best simple method for characterizing these compounds. The absorption spectrum in acidic solution gives additional evidence, but the spectral fine structure is lost.

CONCLUSIONS

The column chromatographic system introduced in this paper can be used for the quantitative determination of aza heterocyclic hydrocarbons separated from the basic fraction of complex mixtures. Although only six aza heterocyclic hydrocarbons have been characterized in the basic fraction of coal tar pitch, the presence of approximately 100 unknown compounds was deduced from the presence of the many sharp bands in all subfractions. The absorption spectral picture obtained with these fractions is much more complicated than that obtained with the polynuclear aromatic hydrocarbon fractions separated from coal tar pitch.

Many pure aza heterocyclic hydrocarbons must be accumulated before these unknowns can be characterized. Additional means of characterization involving gas, paper, and thin-layer chromatographic methods combined with absorption, fluorescence and phosphorescence spectral methods must be applied before optimum results can be obtained for the routine quantitative determination of the basic polynuclear compounds. In addition, the method should be investigated qualitatively with the basic fractions of various types of urban atmospheric and pollution source particulates before it is applied quantitatively and routinely in air pollution analyses and studies.

SUMMARY

A new method for the column chromatographic separation of basic polycyclic fractions of extremely complicated mixtures is described. Heterocyclic hydrocarbons sterically hindered at the aza nitrogen atom are readily separated from non-sterically hindered aza compounds. Aza heterocyclic hydrocarbons with the same amount of steric hindrance are eluted in the order of their increasing number of fused rings per molecule. The method has been applied to the separation of the basic fraction of coal tar pitch. An alkylbenzo(*h*)quinoline, benzo(*h*)quinoline, benzo(*f*)quinoline, acridine, benz(*a*)acridine and benz(*c*)acridine have been identified. In addition, approximately 100 unknown compounds were believed to be present in the fractions. The evidence for this conclusion was derived from the absorption spectra.

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