CHROM. 5389

THE SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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(Received March 30th, 1971)

SUMMARY

A mixture of polycyclic aromatic hydrocarbons was separated in a column, packed with Al_2O_3 containing 2% by weight of water, by eluting with a gradient of cyclopentane–ether. By means of a flow-through-cell ultraviolet spectrophotometer, the extinctions of the eluted components were recorded at the wavelengths of 260, 275 and 296 nm at 30-sec intervals. Sensitivity and selectivity of the determination was thus considerably increased.

INTRODUCTION

As has been shown earlier[1], adsorption chromatography using a gradient of two solvents having different elution powers affords a number of advantages in group analyses of mixtures containing aromatic hydrocarbons with one to four rings. The time required for analysis is shortened and the eluates form sharp peaks which permit the detection of substances even though present in low concentrations. The earlier investigation[1] dealt with hydrocarbons containing four rings among which fluoranthene and pyrene were recorded separately and the others as a sum. There has been an ever growing interest in the analysis of polycondensed aromatic hydrocarbons, especially in connection with the carcinogenic properties that some of them have been found to posses. The recent stricter sanitary regulations and atmospheric purity control measures require new effective and sensitive analytical methods for the determination of these substances.

Most studies dealing with the determination of polycyclic aromatic hydrocarbons are based on preliminary separation by adsorption chromatography, using columns with Al_2O_3 adsorbent or silica gel, or by thin-layer chromatographic methods. After separation, the substance in question is determined in the fractions obtained, using UV spectrophotometry or low-temperature fluorescence. Separation on alumina-packed columns was used by CLEARY[2], GRIMMER AND HILDEBRANDT[3], SFORZOLINI *et al.*[4], who all subsequently measured the UV spectra of the individual fractions. Thin-layer chromatography was used by JANÁK AND KUBECOVÁ[5], HOOD AND WINEFORDNER[6], BIERNOTH[7], SCHAAD *et al*[8]. and MALÝ[9] to separate polycyclic aromatics.

The present report shows the application of gradient elution chromatography for analysing aromatic hydrocarbons comprising four and more nuclei. Separation is carried out in a column packed with alumina wetted with 2% w/w of water. The

solvent gradient formerly employed, pentane–ether, was replaced by cyclopentane–ether. Cyclopentane is a more polar solvent than pentane, has a low viscosity which is advantageous for chromatographic separation, and a low boiling point, which allows the easy concentration of the collected fractions. A steeper gradient was also used at the beginning of analysis since the method was being used for the determination of four and more cyclic aromatic hydrocarbons without regard to the more detailed separation of the lower components, *i.e.* aromatics with one to three nuclei. The UV spectrophotometric detection method which had been developed for this purpose allowed the selective determination of the components in question. Application of the method is demonstrated on a mixture of standards as well as on a black coal pitch extract.

EXPERIMENTAL

Materials

The details are given in our earlier work[1].

Alumina Woelm Eschwege Neutral with 2% H_2O was used as adsorbent. Before use the alumina was heated for 8 h at 400° and then deactivated by addition of 2% water.

A column of 4 mm inner diameter and 1 m in length, and a gradient elution pump (Dialagrad Model 190, ISCO) were used. The UV spectrophotometer was an SP 800 B (Pye Unicam). The spectrophotometer was connected to a three-channel point recorder allowing three-color records with 30-sec intervals between the points. The wavelength was changed at the beginning of each interval, and at its end the respective extinction value was recorded. Three records differing in color were thus obtained, each color corresponding to an extinction at one wavelength. For the purpose of increasing sensitivity, use was made of a flow-through quartz cell of 10 mm pathlength and 0.2 ml dead volume capacity.

Procedure

The method was verified with a mixture of standards containing fluoranthene, triphenylene, 1,2-benzanthracene, chrysene, 3,4-benzopyrene and coronene. 100 mg of a mixture of standards were dissolved in 1 ml 1-methylnaphthalene. The column was first wetted with pure cyclopentane and 1.5 μl of the standard mixture was then introduced. After injecting, the 2-h program of the gradient cyclopentane–ether elution was started. The flow rate of the eluents through the column was 60 ml/h. The detection of the eluted components at the wavelengths of 260, 275 and 296 nm is shown in Fig. 1 together with the gradient course.

Fig. 2 shows an example of analysis of a cyclohexane coal tar pitch extract. 5 g of black coal pitch was extracted in a Soxhlet with 200 ml cyclohexane for 10 h. The cyclohexane solution was evaporated *in vacuo* and 0.5 g of extract was dissolved in 1 ml benzene. 1.0 μl of benzene solution was dosed into the column. In the analysis of the black coal pitch the flow rate through the column and the gradient course were the same as in the case of separating the standard mixture. The fractions collected during analysis were subjected to UV spectral measurements. The individual eluates were identified by comparing the spectra with those of the standards. Fig. 3 shows the spectrum of a fraction containing 3,4-benzopyrene in comparison with a standard spectrum.

RESULTS AND DISCUSSION

The method reported earlier[1] in which the eluates were detected at 260 nm is not suitable for separating polycondensed aromatic hydrocarbons since at this wavelength some of these hydrocarbons do not provide a satisfactory response, and thus require considerably larger amounts of sample to be introduced which reduces the separating efficiency of the column. Simultaneous detection at several wavelengths was therefore chosen in order to ensure that most of the components are recorded in this manner. The earlier procedure, *i.e.* detection at a single wavelength, would require as many analyses as would correspond to the number of wavelengths selected for detection. Such a procedure would be very time consuming and any inaccuracy in the reproducibility of the experiments might cause serious errors. The present procedure makes use of the possibilities of flow-through cell UV spectrophotometry and the UV extinctions were recorded at three wavelengths at 30-sec intervals. Recording at the wavelengths of 260, 275 and 296 nm was proved suitable.

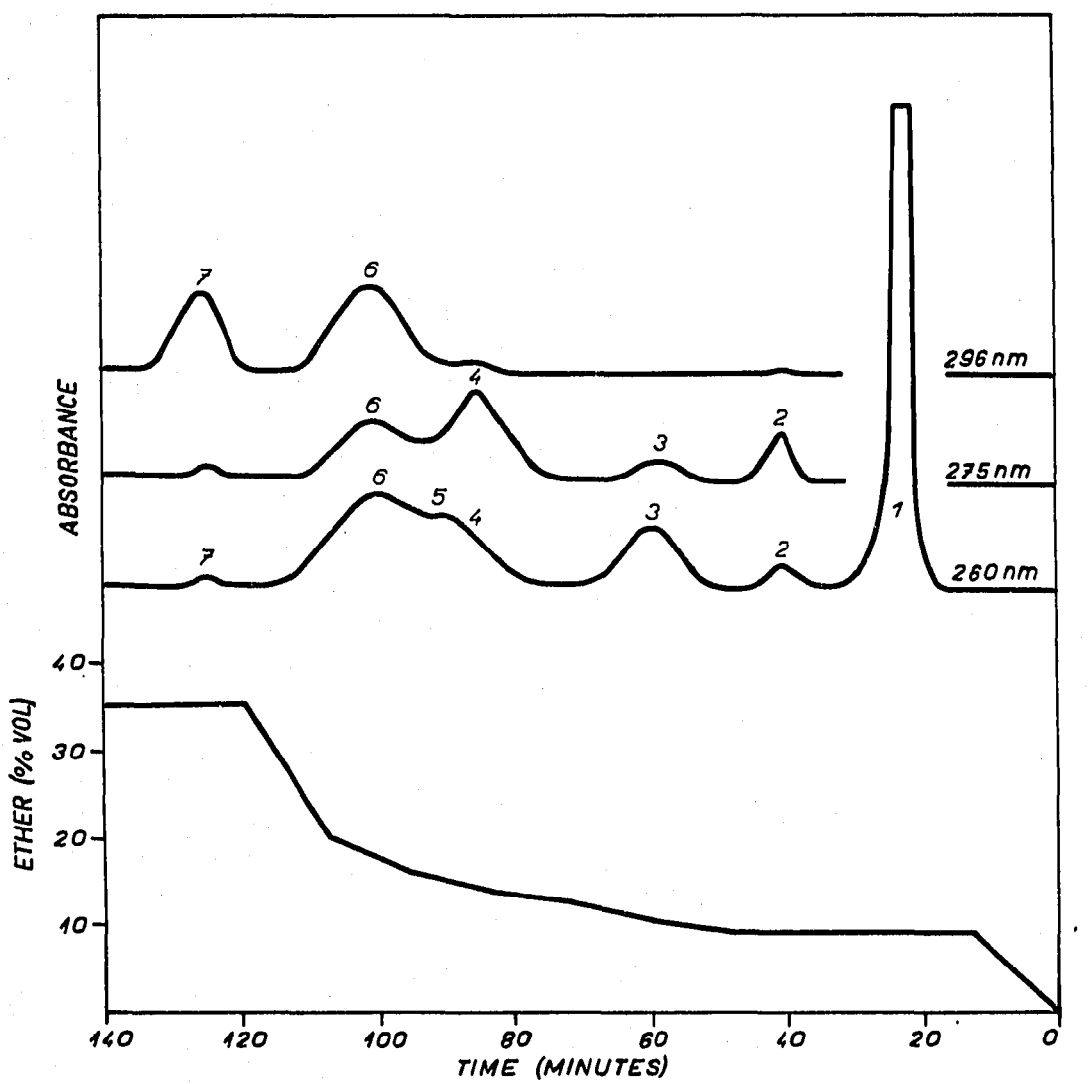

Fig. 1. Separation of the mixture of standards with recordings of the eluted compounds at three wavelengths. 1 = 1-methylnaphthalene (solvent); 2 = fluoranthene; 3 = triphenylene; 4 = 1,2-benzanthracene; 5 = chrysene; 6 = 3,4-benzopyrene; 7 = coronene.

Fig. 1, showing the separation of a mixture of standards, indicates an obviously satisfactory separation of 1-methyl naphthalene, fluoranthene, triphenylene, and coronene; the example of the threesome, 1,2-benzanthracene, chrysene and 3,4-benzopyrene also shows the advantage of simultaneous extinction recording at three different wavelengths. At 260 nm all the components remain virtually within a single extended peak. At 275 nm it is possible to distinguish clearly the peaks of 1,2-benzanthracene and 3,4-benzopyrene, while the extinction of chrysene is low and practically non-interfering at this wavelength. At 296 nm the extinction of 1,2-benzanthracene and chrysene is suppressed to a minimum and the record shows a sharp peak of 3,4-benzopyrene.

The method is sensitive, allowing the detection of 0.5 μg of 3,4-benzopyrene in the sample without amplification. When the quantity injected is known and the respective calibrations have been performed it is thus possible to determine quantitatively the content of 3,4-benzopyrene in a mixture.

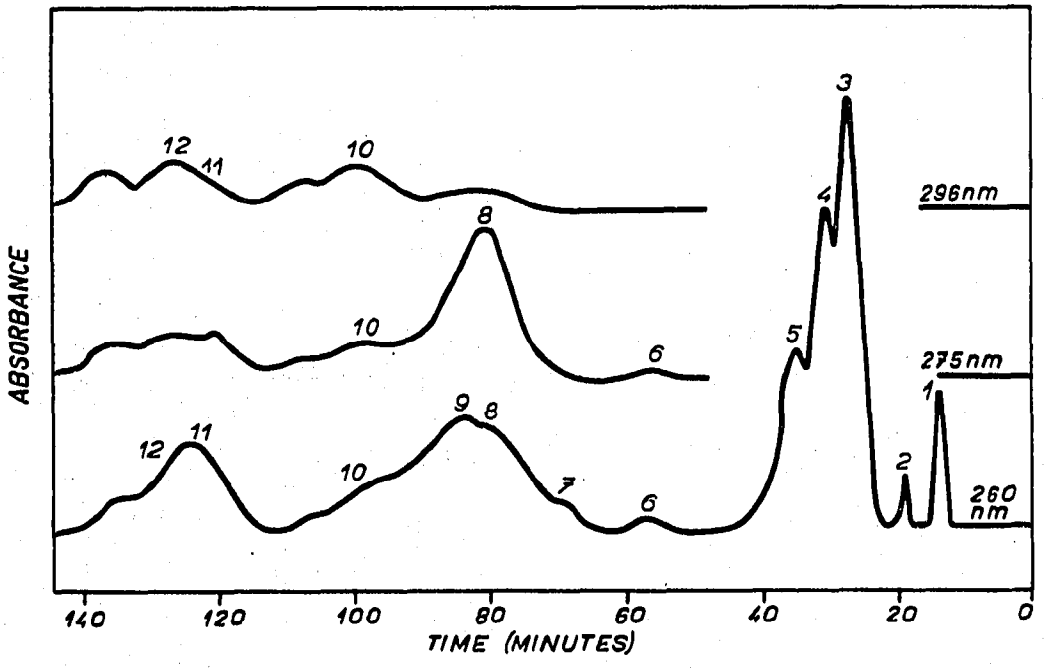

Fig. 2. Separation of coal tar pitch extract. 1 = benzene (solvent); 2 = two-nuclei hydrocarbons; 3 = phenanthrene, anthracene; 4 = pyrene; 5 = fluoranthene; 6 = triphenylene; 7 = benzofluorenes; 8 = 1,2-benzanthracene; 9 = chrysene; 10 = 3,4-benzopyrene; 11 = 1,12-benzoperyene; 12 = coronene.

As indicated by Fig. 2, which shows the analysis of a cyclohexane extract from black coal pitch recorded at 260 nm, the sample also contains aromatic hydrocarbons with two nuclei. Peak No. 1 pertains to benzene which was employed as solvent, peak No. 2 to a mixture of hydrocarbons with two rings. The further three partially separated components represent phenanthrene and anthracene (peak No. 3), pyrene and fluoranthene. Triphenylene is completely separated. Peak No. 7, pertaining to 1,2- and 2,3-benzofluorene, is quite small. Then further components follow in the order: 1,2-benzanthracene; chrysene and 3,4-benzopyrene, which at 260 nm constitute a single elongated peak. As shown by the UV spectrum of the 3,4-benzopyrene

fraction in Fig. 3, separation of this component from the other substances is very satisfactory and allows quantitative evaluation. The double peak following next was found to belong to 1,12-benzoperylene and coronene. At the 275 nm wavelength a well-defined peak of 1,2-benzanthracene and small peaks of triphenylene and 3,4-benzopyrene in addition to other less distinguishable components are detected. At 296 nm well discernible peaks of 3,4-benzopyrene and coronene appear as well as two further components which have not been identified. It should be noted that the substance analyzed as an example in Fig. 2 was neither treated nor purified in advance.

The reported method of gradient elution adsorption analysis of polycondensed aromatics, including simultaneous recording of the extinction at several wavelengths, has a number of advantages. In addition to the already mentioned possibility of detecting most of the eluted components, suitable selection of wavelength permits records of some eluates to be obtained either selectively with maximum suppression of interference from other substances or to achieve maximum sensitivity by choosing the wavelength so that the substance in question exhibits maximum extinction. Another advantage is afforded by the possibility of comparing the extinction records at three different wavelengths which may show whether a peak pertains to one or

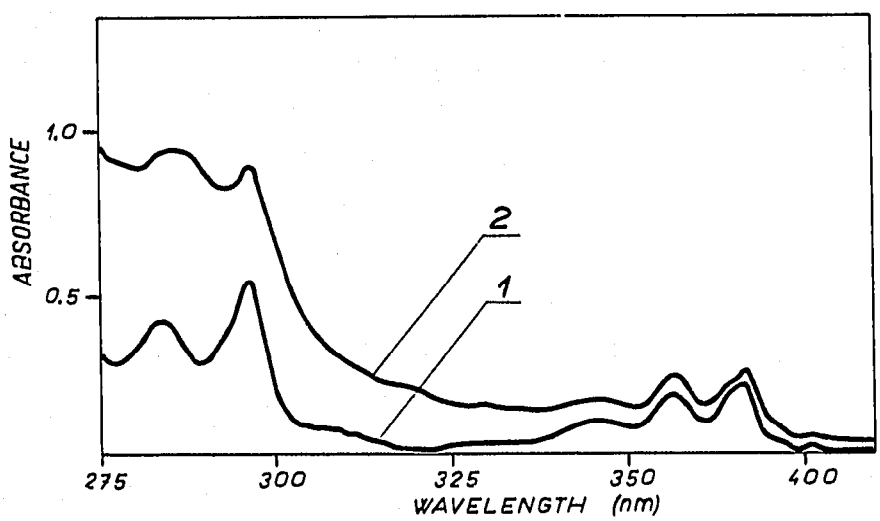

Fig. 3. UV spectrum of 3,4-benzopyrene. Curve 1 = standard; curve 2 = eluted fraction.

more components; in the case of known substances it may serve as evidence of their identity; it may also help in the identification of unknown substances. As shown in the example of 3,4-benzopyrene and 1,2-benzanthracene, this method allows the determination over the entire course of elution for a certain component which may then be collected and determined separately by spectrophotometry. In spite of the fact that the undesirable interference cannot be eliminated completely at any wavelength in such rich mixtures of substances, the method can nevertheless even serve for quantitative orientation determinations of certain substances.

For example, it is possible to prepare a calibration curve for 3,4-benzopyrene by plotting the values of the areas corresponding to the extinction of 3,4-benzopyrene at 296 nm *vs.* the quantity of 3,4-benzopyrene in the feed. When analysing an unknown mixture of polycondensed aromatic hydrocarbons the maximum amount of

3,4-benzopyrene in the feed is determined from the area corresponding to this substance. The course of extinction at two other wavelengths permits one to assess the possible interference of the other components. It is obviously necessary to maintain identical conditions of analysis as compared to those of the standards, especially as regards the flow rate of the eluent through the column.

The main advantage of the method is its speed, as the entire analysis takes only 2.5 h. The amount of sample required, which ranges from 0.1 to 0.5 mg, is likewise advantageous for determining polycyclic aromatic hydrocarbons in foodstuffs, in the atmosphere, etc. In addition to this the small quantity of sample required allows one to work within the linear adsorption isotherm region, this being significant from the standpoint of the reproducibility of results. No special treatment of the sample is necessary since saturated hydrocarbons and olefins are eluted at the beginning of the analysis and polar substances have much larger retention volumes than the aromatic hydrocarbons in question. It should be noted that hydrocarbons having a linear molecule, *e.g.* tetracene or pentacene, likewise have much higher elution volumes and are not eluted during the analysis described.

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