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THE CHROMATOGRAPHY OF POLYCYCLIC AROMATIC
HYDROCARBONS ON PARTIALLY ACETYLATED CELLULOSE

PART II

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In an earlier publication¹, the application of reversed-phase chromatography on partially acetylated paper to the separation and identification of polycyclic aromatic hydrocarbons was described. The techniques previously reported have now been supplemented by alternative methods of locating compounds on the developed chromatograms, by the isolation of the compounds in crystalline form, and by the introduction of an improved technique of acetylation which has been found to produce papers of increased resolving power and better mechanical properties.

The separations obtained on partially acetylated paper have been reproduced on a larger scale by the use of columns of partially acetylated cellulose powder, using similar solvent systems to those used with partially acetylated paper. The resolutions obtained are comparable to those obtained on partially acetylated paper and in the case of compounds of relatively low R_F values, are sometimes superior. The technique is of particular value in the separation of the higher polycyclic aromatic hydrocarbons, which are eluted almost simultaneously from an alumina column but have widely differing R_F values. For example, a mixture of 3:4-benzopyrene, perylene, 3:4-benzofluoranthene, 10:11-benzofluoranthene, 11:12-benzofluoranthene, 1:12-benzoperylene and 2:3:10:11-dibenzoperylene can be resolved into constituents by this method without appreciable overlap of fractions.

CHROMATOGRAPHY ON ACETYLATED PAPER

The technique of acetylation described previously¹ required the use of purified reagents in order to obtain acetylated papers of approximately reproducible properties. The amount of sulphuric acid used as catalyst was small compared with the total weight of the acetylating mixture, making the degree of acetylation obtained strongly dependent on relatively minor variations in the purity of the acetylating reagents. However, it has been found that chromatography paper can be successfully acetylated at room temperature, using appreciably larger concentrations of sulphuric acid as catalyst, so that minor variations in the purity of the acetylating reagents no longer produced marked variations in the properties of the papers produced.

The method is more fully described in the experimental section and gives papers

of a very even degree of acetylation, and of excellent mechanical strength, which retain most of the fibre structure of the original paper. The R_F values obtained with different batches of acetylated paper can be reproduced within close limits by exercising reasonable care in the acetylation process. The papers are sufficiently uniform to be developed by either the ascending or descending technique and show no variation in the rate of solvent travel across the width of the paper. The uniformity of the acetylation is of particular importance in the separation of compounds of similar, relatively low R_F values, where the solvent front is allowed to over-run the end of the paper.

It is possible to vary the resolving power of the acetylated paper within wide limits by varying the catalyst, concentration and the time of acetylation. The papers produced vary between 21% and 31% acetyl content, but the separations obtained depend not only on the acetyl content of the paper, but also on the degree to which the fibre structure of the paper is retained. The greatest separations are obtained with those papers in which the fibre structure is well retained. Such papers, however, have a lower capacity than those in which some fibre structure has been destroyed with consequent swelling of the paper.

A summary of the R_F values obtained for several polycyclic aromatic hydrocarbons on various types of acetylated paper is given in Tables I and II. The most satisfactory paper for the separation of two particular compounds depends both on the R_F values of the compounds concerned and on the technique of identification used. Where the compounds are to be identified by comparison of R_F values alone, and the distance of solvent travel is fixed, it is obvious that the papers of type I and II will give the best separation; but where the compounds are to be identified by extracting the spots and determining the ultra-violet absorption spectra of the extracts, the ratio of the R_F values becomes of greater importance than their absolute values. The solvent front can be allowed to over-run the end of the paper and the greatest separations are then governed by the ratio of the two R_F values and by the absolute mag-

TABLE I
 R_F VALUES FOR VARIOUS TYPES OF PAPER

Compound	R_F values for paper type			
	I	II	III	IV
3:4-Benzopyrene	0.240	0.186	0.115	0.055
Chrysene	0.460	0.410	0.272	0.164
Perylene	0.630	0.524	0.410	0.250
Pyrene	0.765	0.630	0.552	0.365
Phenanthrene	0.801	0.680	0.610	0.400

Solvent: methanol-ether-water, (4:4:1, v/v). 35 cm solvent ascent.

Catalyst concentration: based on 700 ml acetic anhydride and 1500 ml of thiophen-free benzene.

Paper I 1.6 g 92% sulphuric acid (21.2% acetyl content).

Paper II 2.0 g 92% sulphuric acid (23.7% acetyl content).

Paper III 2.5 g 92% sulphuric acid (25.1% acetyl content).

Paper IV 3.4 g 92% sulphuric acid (28.0% acetyl content).

TABLE II
COMPARISON OF R_F VALUES FOR PAPER IV WITH DIFFERENT SOLVENT SYSTEMS

Compound	R_F values	
	A	B
3:4-Benzopyrene	0.055	0.183
Chrysene	0.164	0.400
1:2-Benzanthracene	0.260	0.500
Perylene	0.250	0.530
3:4-Benzophenanthrene	0.352	0.570
Pyrene	0.400	0.640

Solvent A: methanol-ether-water (4:4:1, v/v).

Solvent B: ethanol-toluene-water (17:4:1, v/v).

nitude of the higher R_F . Papers of type IV will, then, often give better separations of particular compounds, although the absolute R_F values are considerably less than those obtained with papers of types I and II. For identification by this method, the papers are invariably run by the descending technique, and uniform acetylation of the papers is, therefore, of particular importance. When compounds of very low R_F value are being separated by this method, it is possible to decrease the amount of streaking obtained by attaching a strip of ordinary chromatography paper to the end of the acetylated paper, so that the solvent supply to the acetylated paper is metered by passing through the untreated paper before reaching the actual chromatogram. The rate of solvent travel is reduced, and the spots are more discrete.

The position of the compounds on the developed chromatograms may usually be determined by viewing the papers under ultra-violet light, and the position of non-fluorescent compounds determined by spraying with a solution of tetrachlorophthalic anhydride¹. Both high pressure and low pressure mercury lamps are normally used for exciting the fluorescence of the compounds as chrysene, phenanthrene, the phenylphenanthrenes and some other simple polycyclic compounds are not fluorescent under the usual high pressure mercury lamp with Woods glass filter, while coronene is difficult to locate under a low pressure lamp filtered to transmit mainly the 2537 Å mercury line. However, certain types of compounds, such as dibenzyl, stilbene, the isomeric phenylphenanthrenes, phenanthrene, and simple derivatives of naphthalene, are difficult to locate by these techniques when they are present in relatively low concentration. Similarly, a weakly fluorescent compound present in relatively large amounts may often be masked by the presence of a more strongly fluorescent compound of similar R_F value. Such compounds are often more easily located by a modification of the photo-print technique described by MARKHAM AND SMITH². The light source used is a 15-W low pressure mercury lamp without a filter, and the chromatogram held in firm contact with a strip of photographic paper sensitive to ultra-violet light by a quartz plate, is exposed and developed in the usual way. Similar techniques have been extensively used for several types of compounds chromatographed on cellulose, and partially acetylated paper appears to be almost as trans-

parent to ultra-violet light at 2537 Å as ordinary chromatography paper, so that special techniques of rendering the paper transparent are unnecessary. The light emitted from the low-pressure lamp consists essentially of the 2537 Å mercury line, and it has been found that no advantage is gained by filtering the small percentage of light of longer wavelength. Fluorescent compounds may also be located by this method, the photographic paper being insensitive to the emitted light; but the location of these compounds by their fluorescence on the chromatogram is appreciably more sensitive than the photographic technique.

The well-known technique of locating compounds on a developed chromatogram by the use of a spectrophotometer³ has also been applied successfully to partially acetylated paper. Most polycyclic aromatic hydrocarbons have intense absorption bands in the 2500–2800 Å region and are readily located by this method, although strongly fluorescent compounds sometimes appear as areas having a negative absorption relative to the background absorption. The technique has been fully described for the location of compounds on ordinary chromatography paper, but it should be pointed out that the success of the method depends on obtaining a constant reading for the absorption of the chromatogram itself, thus requiring an evenly acetylated paper. The papers produced by the method described below are particularly suitable for this type of location and show little variation in background absorption, whereas papers acetylated at 60 to 70° tend to give a variable reading which makes the location of small concentrations of compounds difficult. However, the photographic technique already described is equally as sensitive as the spectrophotometric location, which appears to offer no marked advantage.

While the techniques described have proved satisfactory for the identification of many compounds, it is often desirable to complete the identification of a compound from its melting point. Obviously it is possible to obtain milligram quantities of a compound by running a sufficiently large number of chromatograms, but it has been found that it is possible to isolate certain compounds from the extracts obtained from a limited number of chromatograms by using the technique of microsublimation. As the amounts of material concerned are small, the "sublimations" are carried out at atmospheric pressure using a very short path length, and the compounds are sublimed directly onto a microscope cover slip; the melting point is then determined in the usual way on a hot-stage microscope. Using an ordinary microscope slide with a depression into which the compound is introduced, and subliming onto a microscope cover slip covering the depression, it is possible to sublime hydrocarbons boiling as high as 500° at temperatures of 150–170° at atmospheric pressure. Where the compound has been extracted from a large area of paper it is necessary to rechromatograph the extract before sublimation in order to reduce the amount of resinous material and fibre in the extract. The technique is not invariably successful, particularly if the compound is relatively low-melting, but in many cases good melting points can be obtained. The technique may also be used to purify an extract before determining the ultra-violet absorption spectrum, thus reducing the amount of background absorption.

CHROMATOGRAPHY OF SUBSTITUTED POLYCYCLIC HYDROCARBONS
 ON ACETYLATED PAPER

The separation of aromatic hydrocarbons containing a single alkyl grouping is a problem of particular difficulty and is of importance in the study of the free radical reactions of hydrocarbons. Thus, although SZWARC⁴ has studied the relative rates of substitution of many aromatic hydrocarbons by methyl radicals, it has not been possible to establish the position of the methyl grouping in the substituted compounds. The R_F values obtained for methyl substituted 1:2-benzanthracenes and benzo-(c)-phenanthrenes are summarised in Table III, where the R_F values have been referred

 TABLE III
 R_F VALUES FOR ACETYLATED PAPER TYPE IV

Compound	R_F (relative to 3:4-benzopyrene)	
	A	B
Benzo-(c)-phenanthrene	2.96	5.86
1-Methyl-benzo-(c)-phenanthrene	2.64	5.20
2-Methyl-benzo-(c)-phenanthrene	3.38	6.95
3-Methyl-benzo-(c)-phenanthrene	2.90	5.76
4-Methyl-benzo-(c)-phenanthrene	2.40	3.47
5-Methyl-benzo-(c)-phenanthrene	3.25	5.84
6-Methyl-benzo-(c)-phenanthrene	3.66	7.44
1:2-Benzanthracene	2.65	4.44
3-Methyl-1:2-benzanthracene	3.30	5.90
4-Methyl-1:2-benzanthracene	2.82	5.00
5-Methyl-1:2-benzanthracene	2.74	3.80
6-Methyl-1:2-benzanthracene	3.00	4.52
7-Methyl-1:2-benzanthracene	3.45	5.95
9-Methyl-1:2-benzanthracene	1.93	2.94
10-Methyl-1:2-benzanthracene	2.80	3.64
1'-Methyl-1:2-benzanthracene	2.04	2.50
2'-Methyl-1:2-benzanthracene	2.98	5.25
3'-Methyl-1:2-benzanthracene	3.20	5.70
4'-Methyl-1:2-benzanthracene	2.50	3.34

Solvent A: ethanol-toluene-water (17:4:1, v/v).

Solvent B: methanol-ether-water (4:4:1, v/v).

to 3:4-benzopyrene as standard, since this method of comparison appears to give more reproducible results than do the absolute R_F values. The separations recorded are for a given distance of solvent travel, and may be greatly increased by allowing the solvent front to over-run the end of the paper. It is apparent that the position of the methyl grouping in the two molecules studied can often be assigned unequivocally, and can be limited to two positions in the most unfavourable cases. It is interesting to observe that although the sterically hindered isomers of 1:2-benzanthracene, the 1'- and 9-methyl, have the lowest R_F values of this group, the same is not true of the benzo-(c)-phenanthrenes, where the parent molecule already has a non-planar structure.

Similar separations can be expected on columns of partially acetylated cellulose, but unfortunately, insufficient material was available to test this hypothesis fully.

From the results obtained it is apparent that the separation of methyl substituted polycyclic aromatic hydrocarbons by chromatography on partially acetylated cellulose may be of considerable assistance in the study of free radical reactions between aromatic hydrocarbons and alkyl radicals.

ACETYLATION TECHNIQUE

Sixteen strips of Whatman's chromatography paper (No. 1), 24 in. by 6 in. were rolled together and immersed in an acetylating mixture comprising acetic anhydride (redistilled, 700 ml), thiophen-free benzene (1500 ml) and sulphuric acid (92%, 2.0 to 3.0 g) and allowed to stand for 24 h at 18° with frequent stirring and separation of the papers. After this time the acetylating mixture was poured off, the papers allowed to drain, and then allowed to stand for 24 h in ethanol, with occasional stirring. The papers were washed with a further quantity of ethanol and then in running water for 6 h and finally allowed to stand in distilled water for 2 h. After this time the papers were removed and air-dried.

The acetylating mixture can be used for another two batches of paper, with the addition of 0.5 g of fresh sulphuric acid for each successive batch, before the papers become unsuitable through destruction of the fibre structure; the properties of the successive batches are different but all give good resolutions. Where reproducible results are desired, fresh acetylating mixture should be used for each acetylation.

CHROMATOGRAPHY OF POLYCYCLIC AROMATIC HYDROCARBONS ON A COLUMN OF PARTIALLY ACETYLATED CELLULOSE

For many years the method of choice for the purification and separation of polycyclic aromatic hydrocarbons has been by liquid phase chromatography on alumina, both because of the high resolution obtained and the large capacity of the columns.

However, in the analysis of complex mixtures of hydrocarbons obtained in pyrolytic reactions, it has been found that it is extremely difficult to effect good resolutions of the higher polycyclic fractions, which often contain six or seven compounds, by this method. Chromatography of the fractions obtained from an alumina column by the conventional methods, on a column of partially acetylated cellulose has been found to give excellent resolutions of the compounds present in such fractions, and the number of compounds present in each fraction limited to two at the most and usually to a single major compound. The separations obtained are not usually as complete as those obtained by paper chromatography on partially acetylated paper, mainly because of the deficiencies of a dynamic method of resolution as opposed to a static method, but in conjunction with paper chromatography, the method provides a powerful analytical tool for the analysis of these fractions. While the separations obtained for compounds of relatively high R_F are not as efficient as those obtained by

paper chromatography, the resolution obtained for compounds of low R_F are often better because of the increased distance available for resolution. Thus, it is possible to obtain complete resolution of 3:4-benzopyrene, which has a particularly low R_F , from the compounds accompanying it in some pyrolysis fractions, such as perylene, 1:2-benzopyrene, chrysene and 3:4-benzofluoranthene.

The technique of acetylation and the method of packing the column are described in the experimental section. Good resolutions of polycyclic aromatic hydrocarbons have been obtained on columns of 24% and 32% acetyl content, the more highly acetylated cellulose giving more discrete bands and also retaining the compounds for a shorter time. The solvent systems used for the development of the columns, and also for the introduction of the compounds onto the column, were similar to those used for paper chromatography on partially acetylated paper, namely ethanol-benzene-water (17:4:1, v/v), ethanol-toluene-water (17:4:1, v/v) and methanol-toluene-water (10:1:1, v/v). The solvent systems containing ethanol have a greater eluting power than that containing methanol, but the latter is often useful in effecting increased resolution of the compounds of relatively high R_F value, particularly on the column of lower acetyl content. Some variation in R_F values was observed between the three solvent systems, but in the majority of cases the order of elution was the same.

The presence of water in the eluting solvent increases the rate at which the compounds move through the column and also serves to keep the limits of the individual bands sharply defined. While good resolutions have been obtained using mixtures of ethanol and toluene, and ethanol and benzene alone, the proportion of hydrocarbon in the eluting solvent being gradually increased during development, the absence of water causes extensive tailing of the bands, so reducing the effectiveness of the separations. However, compounds differing in R_F by 0.15 to 0.2 or more have been resolved with these solvent systems.

With the three solvent systems described above, it is possible to resolve compounds having an R_F difference of 0.08 with an absolute R_F value greater than 0.4, and compounds with an R_F difference of 0.05 with an absolute R_F value less than 0.4 on a column of partially acetylated cellulose (32% acetyl content) 70 cm long and 2.5 cm diameter. The capacity of the column is low, and it has been found that approximately 200 g of partially acetylated cellulose is required to give good resolution of 400 mg of a mixture of polycyclic aromatic hydrocarbons having a range of R_F values. The efficiencies of the separations obtained depend not only on the ratio of the R_F values of the two compounds concerned, but also on the extent to which the bands tail during development. Fluoranthene and 9,10-diphenylanthracene are particularly bad in this respect, and are often difficult to separate from the compounds of similar R_F .

The order of elution from a column of partially acetylated cellulose is given in Table IV, together with the R_F values obtained using the same solvent system on partially acetylated papers of slightly lower acetyl content (28.0%), and it will be seen that the order of elution follows the order of R_F values, as would be expected. It is

obvious that many compounds have similar R_F values and cannot be satisfactorily resolved by this means, but in most cases these compounds can be separated by first effecting a preliminary separation on an alumina column by the usual methods, or by fractional distillation. The technique is of particular value in the isolation of small

TABLE IV

ORDER THROUGH A COLUMN OF PARTIALLY ACETYLATED CELLULOSE

Solvent: ethanol-toluene-water (17:4:1, v/v).

Compound	R_F
9,10-Diphenylanthracene	0.675
Acenaphthylene	0.576
2:3-Benzofluorene	0.539
1,1'-Dinaphthyl	0.539
Phenanthrene	0.501
Anthracene	0.494
3:4-Benzophenanthrene	0.483
Pyrene	0.471
Triphenylene	0.446
Fluoranthene	0.441
Coronene	0.410
Fluorene	0.401
1:2-Benzanthracene	0.396
2,2'-Dinaphthyl	0.359
Perylene	0.351
1:2:5:6-Dibenzanthracene	0.246
3:4-Benzofluoranthene	0.209
3:4:9:10-Dibenzopyrene	0.209
Chrysene	0.206
Anthanthrene	0.163
3:4-Benzopyrene	0.093
2:3:10:11-Dibenzoperylene	0.042
Violanthrene	0.023

TABLE V

ORDER THROUGH ACTIVATED ALUMINA COLUMN

Solvent: *n*-hexane with gradually increasing benzene concentration.

Acenaphthene
Acenaphthylene
Fluorene
Phenanthrene
Anthracene
Pyrene
Fluoranthene
1:2-Benzanthracene
Chrysene
1:2:5:6-Dibenzanthracene
Perylene
3:4-Benzopyrene
3:4:9:10-Dibenzopyrene
Coronene
2:3:10:11-Dibenzoperylene
Violanthrene

amounts of the higher polycyclic aromatic hydrocarbons containing five or more rings, which are eluted almost simultaneously from an alumina column. The order of elution from a column of activated alumina with a solvent of continuously increasing strength, from hexane to benzene, is given in Table V. In practice it has been found that the complex mixtures of hydrocarbons obtained in pyrolytic reactions of hydrocarbons can be satisfactorily fractionated by distillation of the fractions boiling below 280° and by chromatography of the residue on activated alumina followed by chromatography of the fractions on partially acetylated cellulose. Where the fractions obtained from the column of partially acetylated cellulose contain two compounds, the fractionation may usually be completed by using the techniques of paper chromatography on partially acetylated paper already described, allowing the solvent front to over-run the end of the paper to obtain maximum resolution. The division of a fraction into groups of compounds of approximately the same R_F value is also of advantage in the analysis of the fraction by paper chromatography since the type of

acetylated paper can then be chosen so that maximum resolution is obtained. Thus it has been found that a paper of relatively high acetyl content (27–29%) is most suitable for the separation of compounds having a high R_F and for compounds of relatively low R_F , the best results are obtained with papers of lower acetyl content (20–24%).

Similar separations have been obtained from columns prepared by pulping partially acetylated papers, but the difficulties encountered in packing the fibrous mass obtained into an efficient column usually causes extensive over-lapping of the fractions.

Chromatography on partially acetylated cellulose columns is not proposed as an alternative method to the more usual technique of chromatography on activated alumina, but as an adjunct to this technique by which many difficult resolutions can be effected more easily. The R_F values recorded for methyl substituted 1:2-benzanthracenes and benzo-(c)-phenanthrenes (Table III) suggest that the technique may be of considerable assistance in the analysis of mixtures of these compounds. Complete resolutions of 4-, 7- and 5-methyl-benzo-(c)-phenanthrene and of 6-, 5-, 4- and 3-methyl-1:2-benzanthracene have been obtained on a column 70 cm long and 2.5 cm in diameter.

EXPERIMENTAL

Acetylation of cellulose powder

Whatman's cellulose chromatography powder (200 g) was acetylated in a mixture of thiophen-free benzene (1700 ml) and acetic anhydride (800 ml) containing 4.5 g of 92% sulphuric acid. The mixture was stirred for 24 h at 18°, filtered and the acetylated pulp stirred with 2,500 ml of ethanol for 4 h. The pulp was again filtered and stirred with a further 2,500 ml of ethanol for 5 h, filtered and dried at 70°.

The acetyl content of the powder was found to be 32%. An acetylation carried out under the same conditions using 3.0 g of 92% sulphuric acid as catalyst gave a pulp of 24% acetyl content.

Preparation of columns

The partially acetylated cellulose was slurried with the developing solvent in a Waring blender and the columns packed by pouring the slurry into a suitable column and allowing it to settle with intermittent vibration. The density of the partially acetylated pulp is considerably greater than that of cellulose powder, and a coherent column of good density can be obtained without compressing the powder. The columns can be used repeatedly without any obvious decrease in efficiency.

The use of a short column of smaller diameter to straighten the fronts of the eluates is an advantage in increasing the sharpness of the fractionation.

In establishing the order of elution from these columns the purity of the fractions obtained was determined by chromatography on partially acetylated paper.

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SUMMARY

The techniques previously described¹ for the separation of polycyclic aromatic hydrocarbons by chromatography on acetylated paper have been supplemented by the introduction of alternative methods of locating compounds on the developed chromatograms, by the isolation of the compounds in crystalline form from extracts from paper chromatograms, and by the use of an improved technique of acetylation which has been found to produce papers of increased resolving power and better mechanical properties.

The separations obtained on partially acetylated paper have been reproduced on the larger scale on columns of partially acetylated cellulose powder, using the same solvent systems as those used in the paper chromatography of these compounds.

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Note added in proof

The method of acetylation of cellulose powder described in this paper has proved unsatisfactory with some batches of Whatman's standard chromatography powder, which do not acetylate appreciably under these conditions. However, the following catalysts, based on the acetylating mixture already described, gave a satisfactory product.

I. 92 % sulphuric acid (4-5 g) with 72 % perchloric acid (1 g).

II. 92 % sulphuric acid (4 g) with 72 % perchloric acid (4 g).

III. 72 % perchloric acid (10 g), acetylated for 10 hours only.

These methods have been developed in collaboration with Dr. M. J. THOMPSON of this Department.