SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY THIN-LAYER CHROMATOGRAPHY ON IMPREGNATED LAYERS

ARNE BERG AND JØRGEN LAM

*Department of Organic Chemistry, Chemical Institute,
University of Aarhus (Denmark)*

(Received February 21st, 1964)

INTRODUCTION

The separation of polycyclic aromatic hydrocarbons from complex mixtures as encountered, for example, in tobacco smoke, air pollution studies[1,2], and in pyrolysis experiments[3] has been achieved mainly by chromatographic means, usually column or paper methods.

A small number of studies have been carried out on known mixtures of hydrocarbons using paper chromatography[4-7]. Quite good separations have been accomplished, but the method suffers, among other things, from non-reliability of R_F values unless the conditions for running the chromatograms are controlled very strictly. The chief difficulty is due to the necessity of using specially impregnated (acetylated) paper, the reproducibility of the grade being rather poor.

The possibility of finding a more easily accessible (and much less expensive) chromatographic adsorbent prompted the use of thin-layer chromatography (TLC). Although the reproducibility of R_F values by this method is not better than in paper chromatography, other advantages (shorter running time, sharper spots and a broader spectrum of spray reagents, for instance) connected with TLC, made this method promising for the present purpose.

Since this work was started two papers have appeared dealing with the problem. WIELAND, LÜBEN AND DETERMANN[8] succeeded in separating a mixture of hydrocarbons* on a plate covered with *acetylated* cellulose powder. The chromatogram was developed with a methanol–ether–water mixture. Only very few details are given.

In a more detailed study KUCHARCZYK, FOHL AND VYMĚTAL[9] used silica gel and alumina as adsorbents. The R_F values (with *n*-hexane and with carbon tetrachloride as solvents) of a number of hydrocarbons (and some heterocyclic compounds) are reported, as well as their colours in ultraviolet light and when sprayed with tetracyanoethylene and with formaldehyde sulphuric acid reagent. It is emphasized, however, that the R_F values depend very much on experimental conditions. As to the condensed polycyclic hydrocarbons examined the separation, judged by the reported R_F values, is poor.

In the present study we have examined a number of polycyclic aromatic hydro-

---

* Anthracene, phenanthrene, fluoranthene, pyrene, 1,2-benzanthrene, chrysene, perylene and 3,4-benzopyrene. Phenanthrene and pyrene were not separated.

carbons on plates covered with alumina or silica gel, and a variety of developing solvents have been tried.

Following the idea that a complexing reagent, when mixed (in relatively small amounts) with the adsorbent, might cause a further separation due to different complexing power toward different hydrocarbons, we have studied the effect of various admixtures.

EXPERIMENTAL

Hydrocarbons

The hydrocarbons used are summarized in Table I. Except for numbers 12, 14 and 19, supplied by Aldrich Chemical Co., U.S.A., No. 2 from Heyl and Co., Germany, and No. 5 and 10, which were synthesized in this laboratory, the rest of the compounds was supplied by L. Light & Co., Great Britain. The purity of most of these substances, purified if necessary, was indicated by non-resolvability in a chromatographic test. Anthracene was scintillator grade. Coronene, chrysene and 3,4-benzofluoranthene were rather impure. They were all purified by column chromatography.

Test solutions were prepared in benzene, the concentration of polycyclic hydrocarbon being 0.5 %. When this concentration could not be obtained saturated solutions were used (see Table I). The solutions were kept in darkness.

TABLE I

	Hydrocarbon[a]	Fluorescent spots[b]				Coloured spots[b]	
		Al	Sil	Caf Sil	DMF Al	TNF Sil	TNF Al
1	Anthracene	v	v	v	v	v	r-br
2	Pyrene	g	g	v	g	br	br
3	Chrysene[c]	bl-v	v	v	v	y-br	y-br
4	3,4-Benzofluoranthene	bl	bl	bl	bl	y	y-br
5	3,4-Benzopyrene[d]	y	y	bl(v)	y	y-gr	gr-g
6	Perylene[c,e]	bl(br)	bl(br)	bl-g	bl(y)	y	y-g
7	1,12-Benzoperylene	y	y-g	v	y-g	bl-gr	br
8	Coronene[c]	v	v	v	v	br	r-br
9	Fluoranthene	bl	l.bl	bl			
10	1,2-Benzanthracene	l.bl	l.bl	v			
11	1,2-5,6-Dibenzanthracene[c]	bl-g	bl	v			
12	1,2-3,4-Dibenzanthracene	bl	bl	v			
13	1,2-3,4-Dibenzopyrene[d]	bl	y	y			
14	1,2-4,5-Dibenzopyrene[c,d]	g	bl	v			
15	3,4-9,10-Dibenzopyrene[c,d]	br	y-br	bl-v			
16	Fluorene	—	—	—		y	
17	Acenaphthylene	—	—	—		y	
18	Phenanthrene	—	—	—		y	
19	Triphenylene	—	—	—		y	
20	1,2-Benzopyrene[c,d]	bl	bl	v		br	
21	2,3-Benzanthracene	—	g	g		v	

[a] Hydrocarbon mixtures used were as follows: I = 1–3; II = 1–8; III = 9–15; IV = 16–21.
[b] Abbreviations: Al = alumina; Sil = silica gel; Caf = caffeine; DMF = dimethylformamide; TNF = 2,4,7-trinitrofluorenone; bl = blue; br = brown; g = green; gr = grey; l = light; r = red; v = violet; y = yellow. A dash indicates that the spot is non-fluorescent.
[c] A saturated solution (in benzene) was used for spotting the plates.
[d] Concerning the numbering of positions in pyrene see ref. 11.
[e] In most cases a perylene spot in U.V. light was bright blue with a distinct brown or yellow-brown centre region.

Unless otherwise indicated 1 μl of sample solution was used for spotting the plate. The spotting was done by means of a micropipette of the constriction type (total volume 1 μl).

Solutions containing more than one hydrocarbon (test mixtures) were prepared by mixing equal volumes of the solutions of the pure substances, and as many micro-liters were applied to the plate as the number of hydrocarbons in the mixture. A 2 μl pipette was used. The test mixtures used are indicated in Table I.

For quantitative work (extraction and estimation by U.V.-spectroscopy) greater amounts of hydrocarbons are needed*.

Plates

Glass plates, 20 × 20 cm, were used. The adsorbent layers were prepared by means of a Mutter-Hofstetter device** and the thickness of the layer was in all but a few instances 0.25–0.30 mm. Prescribed procedures (see *e.g.*, ref. 10) were followed. After air-drying for half an hour the plates were activated at 150° for 3 h (alumina) or at 120° for 2 h (silica gel). The plates were stored in a desiccator over silica gel. Plates that were not heat-activated, *i.e.* simply dried in air for 24 h, were also used.

Adsorbents

The adsorbents used were the following: Alumina (Aluminiumoxyd-G, Merck) and silica gel (Kieselgel-G, Merck, nach Stahl).

Impregnated layers

The following substances were used for impregnating the layers: picric acid, styphnic acid, 2,4,7-trinitrofluorenone, caffeine, urea, dimethylformamide, and silver nitrate.

The active layers were prepared as follows: for the three first-named compounds the one in question was dissolved in 1 or 2 ml of acetone or alcohol, and the solution added to the amount of water to be used for preparing the layer in the usual way. In this way the complexing reagent was evenly distributed on the adsorbent.

The amounts of these three nitro compounds used per unit area of plate were about equivalent to (or twice that of) the amounts of hydrocarbons per unit area of the separated spots. This means that, according to the amounts of hydrocarbons used (see above), each plate was loaded with 7.5 mg (or 15 mg) of the reagent.

Caffeine (0.5 g per plate), urea (0.5 g per plate) or silver nitrate (2.5 g per plate) were dissolved in the amount of water necessary for preparing the plates. It was necessary to dissolve caffeine at 50–60° taking advantage of the largely enhanced so-lubility of caffeine at increased temperature. In order to avoid recrystallization of the caffeine when suspending the adsorbent in the solution, the adsorbent too was preheated to the same temperature. The silver nitrate plates were dried and stored in the dark. The amount of caffeine corresponded to a molar ratio of caffeine to hydrocarbon of roughly 100 to 1.

For the preparation of dimethylformamide-impregnated plates, heat activated alumina-covered plates were dipped for 5 min in an ethereal solution of the reagent (0.5, 5 or 10 %), and then, after air-drying for half an hour, they were dried for a further five minutes at 70°. Silica gel layers loosened from the glass when treated in this way.

---

* Work on this line is in progress and will be published soon.
** Camag & Co., Muttenz, Switzerland.

Solvents

Pure solvents as well as solvent mixtures were tried. Except for benzene, the solvents used were not analytical grade or specified for chromatographic use, and were in most cases chemically pure laboratory reagents, which, if necessary, were further purified. Ethyl ether was freed from peroxides. Light petroleum, the bulk solvent used, was a fraction with b.p. 40–50°. All the solvents used were non-fluorescent in ultraviolet light. Per cents indicated for mixed solvents are by volume.

Development

The distance travelled by the solvent front was 13 cm in all cases, ascending technique being used. The development tank (9 × 22 × 23 cm) was lined with filter paper on one side. The running time varied between 30 and 50 min. All experiments were run at room temperature, 22° ± 1°. Very often, repeated developments were performed on a plate. The plate was dried for a short time in a stream of air (room temperature) before the next run.

Detection and identification

The spots on the developed chromatograms were located by inspection in ultraviolet light (3660 Å). It is interesting to note that the fluorescence colours on caffeine-impregnated silica gel plates are somewhat different from (see Table I) and much more brilliant than the colours on ordinary plates. Equally interesting is the fact, that the spots, when developed on such plates, are distinctly sharper than usual.

When nitro compounds are added to the active layer, the fluorescence in ultra-violet light disappears. With 2,4,7-trinitrofluorenone the spots of the hydrocarbon–trinitrofluorenone complexes become visible. The fluorescence is not quenched on plates impregnated with dimethylformamide or with silver nitrate.

Spraying with conc. sulphuric acid or nitric acid followed by heating in an oven to 180–200°, as well as contact with iodine vapour, has been used for detection.

RESULTS AND DISCUSSION

Table I records the fluorescence colours in ultraviolet light and the colours in daylight of the hydrocarbons on different types of layers. It should be emphasized that the fluorescence colours depend somewhat on such factors as concentration of hydrocarbon in the spot, possible impurities, and whether the plate is wetted by the solvent or not. The colours indicated apply to a plate dried in air.

In Figs. 1–3 a series of representative chromatograms are recorded. For each the following details are given: hydrocarbon mixture (II, III or IV); type of layer; mode of impregnation and activation of layer; developer; number of repeated runs on the plate using the same developer; mode of detection (ultraviolet light (U.V.), and colour of complex (CC)).

Developers, size of samples

Some indication for the selection of suitable developers was obtained from a preliminary study involving the three hydrocarbons anthracene (1), pyrene (2), and 3,4-benzopyrene (5). Only ordinary alumina or silica gel plates, activated as described, were used for this purpose.
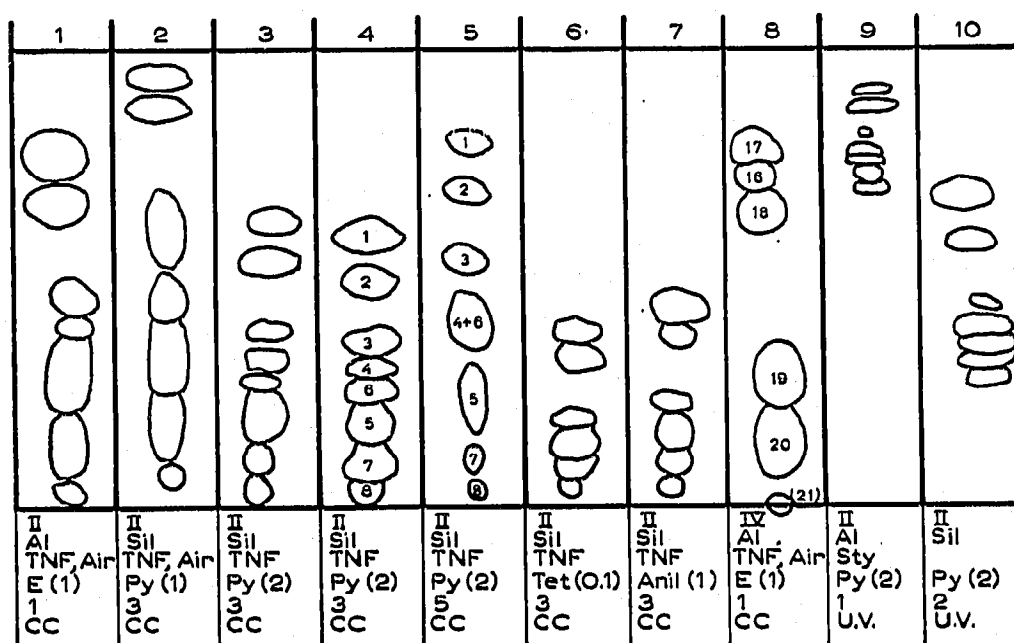
Fig. 1. Effect of 2,4,7-trinitrofluorenone (TNF). Symbols: II and IV = hydrocarbon mixtures II and IV; Sil = silica gel G; Al = alumina G; Sty = styphnic acid; Air = plate air-dried only; E = ether; Py = pyridine; Tet = tetralin; Anil = aniline. Bracketed figures indicate per cent (by volume) of the named component in a mixture with light petroleum. Figures in the fifth row indicate number of runs on a plate. Order of appearance of hydrocarbons II given in chromatograms 4 and 5 apply to the other chromatograms, too. CC = detection by colour of complex.
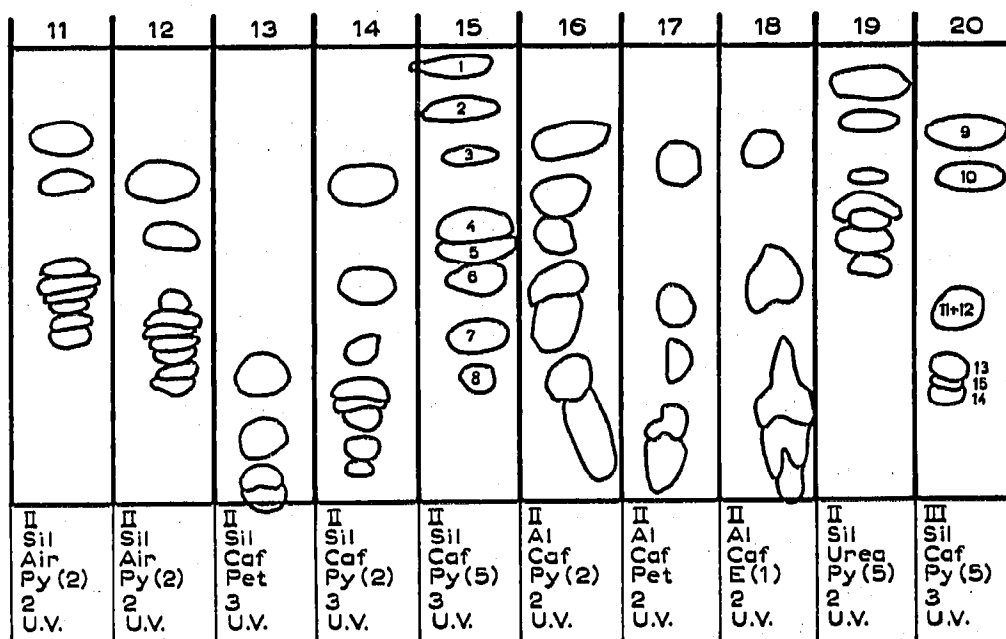

Fig. 2. Effect of caffeine (Caf). Symbols: II and III = hydrocarbon mixtures II and III; Pet = light petroleum; U.V. = detection by fluorescence in ultraviolet light. For the other abbreviations see legend to Fig. 1. Order of appearance of hydrocarbons II given in Expt. 15 apply to the other chromatograms, too.
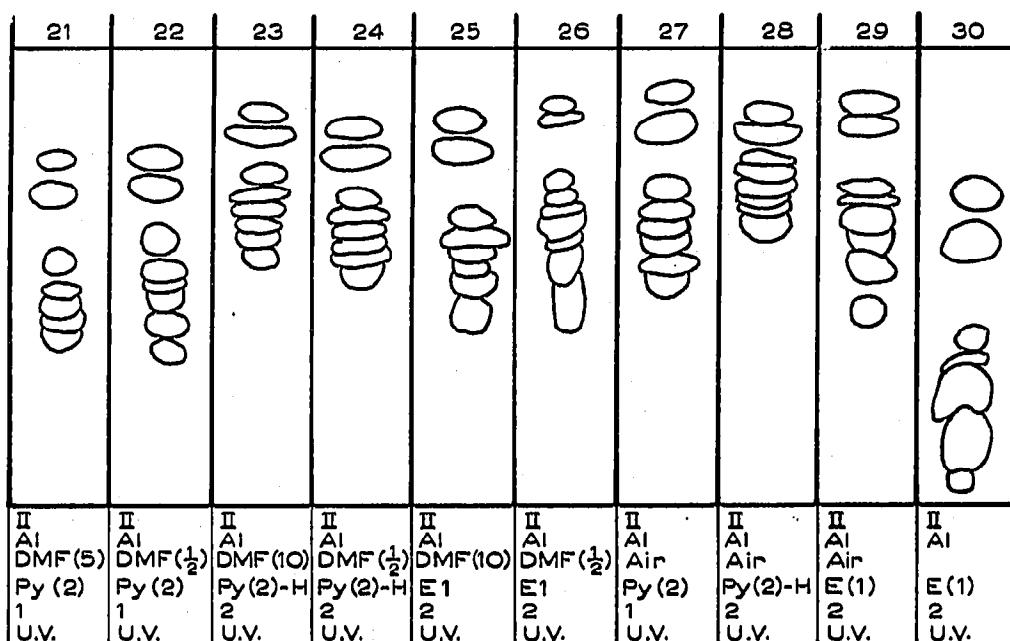
Fig. 3. Effect of N,N-dimethylformamide (DMF, bracketed figures indicate concentration of DMF in the ethereal solution used for impregnation) and of air-drying (Air). Symbols: H means that n-heptane was used instead of light petroleum. For the other abbreviations see legends to Figs. 1 and 2. Order of appearance of hydrocarbons II in all chromatograms is as indicated in Expt. 15 (Fig. 2).

The eluting power in a series of pure solvents was found to follow the order usually called the eluotropic series[10]. Only aliphatic and alicyclic hydrocarbons showed any promising separating effects whereas other solvents, including chlorinated aliphatic hydrocarbons, were found to be quite unsatisfactory. This is in contrast to the findings of KUCHARCZYK, FOHL AND VYMĚTAL[9] who used chloromethanes. The reported R_F values of the polycyclic aromatics are, however, not very different from each other.

Light petroleum was found to be the most suitable pure solvent, especially when used on alumina plates. But with more complex hydrocarbon mixtures it failed to separate the slower running hydrocarbons.

Much better results were obtained with mixed developers with light petroleum as the bulk solvent. Polar solvents, in small amounts, modified the developer to give fairly good separations. Pyridine (2–5 %), ether (1 %) and acetic acid (0.04 %) were found most useful. Ether and acetic acid were especially so on alumina plates whereas pyridine worked better on silica gel. A variety of other polar and some non-polar solvents were also possible additives, e.g. aniline and tetralin (Expt. 6 and 7, Fig. 1).

Adsorbents and impregnating agents

The obvious idea that a complexing agent, when admixed with the adsorbent, may influence the mobility of the substances to be separated, has been utilized in very few instances[10] and then only silver nitrate and boric acid have been used as complexing agents. Separation of some *cis–trans* isomers, for instance, has been thus achieved.

Nitro compounds. With polycyclic aromatics, the agents of choice would seem to be such well-known substances as picric acid and styphnic acid, 2,4,7-trinitro-

fluorenone, *sym*-trinitrobenzene, and tetracyanoethylene. Whereas the two last-mentioned compounds still have to be tried, the three others are included in this study. Picric acid is valueless, but styphnic acid showed some effect (Expt. 9, Fig. 1), and trinitrofluorenone proved to be excellent (Fig. 1). Since the complexes formed are coloured, the possibility of direct detection of the spots is offered by this method. This of course is of special value for non-fluorescent hydrocarbons. Detection, undoubtedly, can be difficult in some instances due to faintly coloured complexes. Identification of the different hydrocarbons cannot be done by colours alone, but must be supplemented by R_F values or, preferentially, by running pure test substances on the same plate. Use of the last-mentioned technique is appropriate for a number of reasons. First, R_F values in thin-layer chromatography are often not very well-defined due to difficulties in reproducing experimental conditions exactly, this being the case when complexing agents are admixed with the adsorbent. Furthermore it was noticed repeatedly, though not always, that hydrocarbons with only slightly different R_F values, when separating from a mixture, move faster than when running alone. This is probably due to overloading of the starting spot containing the mixture. Secondly, R_F values refer to a single run on a plate, whereas in this study it has been found advantageous to repeat a run on a plate two, three or even more times. Yet, the reproducibility of experiments is satisfactory, as illustrated by experiments 3 and 4 in Fig. 1. These considerations apply to other types of plates too (Expt. 11 and 12, Fig. 2). The effect of repeated runs is illustrated by Expt. 4 and 5 (Fig. 1).

Caffeine. It is well known that association colloids which, in aqueous solution, form micelles at a critical concentration exert a solubilizing effect on polycyclic aromatic hydrocarbons[12]. The same is true for lactic and butyric acid[13], though with these substances it is not necessarily due to micelle formation. The solubilizing effect exerted on the aromatics by purines and related substances in aqueous solution is evidently caused by complex formation. The ability of caffeine in this respect is outstanding. This effect was first noticed by BROCK, DRUCKREY AND HAMPERL[14] and later studied thoroughly by WEIL-MALHERBE[15] and by BOYLAND AND GREEN[16].

It is now found that caffeine, used in admixture with thin-layer adsorbents (silica gel), has a pronounced influence on the separation of mixtures of polycyclic aromatic hydrocarbons. Probably complex formation is the cause in this case too. As far as the polar character of the aqueous solvent is of importance for the complex formation, this possibility has its counterpart in chromatography in the polarity of the adsorbent. As can be seen from experiments 10, 11, 14, 15 and 16, 17, 18 (Figs. 1 and 2), the effect is most evident on silica gel plates.

The brilliancy of the fluorescence colours on caffeine-impregnated plates can possibly be explained as a result of diminished transparency of the silica gel layer, light-scattering in the layer causing increased secondary excitation of the hydrocarbons. The observed shifts in colours are quite understandable on account of complex formation.

BOYLAND AND GREEN[16] found that urea was without effect on the solubilization of polycyclic aromatic hydrocarbons, in agreement with our finding that urea was of no value for the separation of these substances (compare Expt. 10 (Fig. 1), 11 and 19 (Fig. 2)).

Attempts to utilize the solubilization effect by developing the chromatograms with aqueous solutions of caffeine, with or without added pyridine, failed. On alumina

layers the hydrocarbon spots move rapidly, but unfortunately with severe tailing and very poor separation. On silica gel, on the contrary, the spots move very slowly being accelerated with increasing concentration of caffeine and still more so by addition of pyridine. In the last case tailing becomes pronounced and at any rate no separation is obtained.

Silver nitrate. The ability of the silver ion to bind to π-electron structures has been demonstrated by fractional extraction of benzene homologues with aqueous solutions of silver nitrate. The principle, as stated above[10], has proved valuable in some instances in thin-layer chromatography on silica gel plates, and the possibility of applying it to the problem at hand was examined. Separations, however, were not satisfactory.

Dimethylformamide. The effect of impregnating alumina layers with dimethylformamide is seen from Fig. 3, experiments 25 and 30. The spots move faster than on unimpregnated plates, but separation is almost the same. Furthermore, there is no significant difference between the developers used (experiments 21 to 26), and the concentration of the solution of dimethylformamide used to prepare the plates seems to be of no importance. An interesting observation can be made by comparing experiment 28 with numbers 23 and 24, and experiment 29 with numbers 25 and 26. These chromatograms show that drying of the alumina layers in air, without subsequent activation by heat, has almost the same effect as impregnating activated layers with dimethylformamide. In experiment 27 the spots have moved a little further than in numbers 21 and 22, but otherwise these chromatograms are very much alike.

Often chromatography on adsorbents impregnated with dimethylformamide or similar substances is regarded as purely underlying the partition principle, although it is argued, too, that adsorption may play a rôle. This evidently must be the case with polycyclic aromatic hydrocarbons in view of the observed similar effects of water and dimethylformamide and since partition between two components, one of which is water, is not very likely without participation of the alumina to which the water is adsorbed.

R_F values

Tabling of reliable R_F values and use of such figures demands a precisely standardized technique which must be strictly followed. As this is not necessary in order to obtain good separations, as discussed above, no list of R_F values will be given here. We believe it safe to say that too many R_F values recorded in the literature are of limited value for identification purposes beyond being merely suggestive. Qualitatively, a general order of appearance of the different hydrocarbons in a chromatogram can be stated. From top to bottom, the order for the most frequently used mixture (II) almost invariably is as follows: anthracene (1), pyrene (2), chrysene (3), 3,4-benzofluoranthene (4), 3,4-benzopyrene (5), perylene (6), 1,12-benzoperylene (7), and coronene (8). This order is maintained even when other adsorbents or developers are used except for (5) and (6), which are interchanged on layers impregnated with TNF (see Expt. 4, 5 (Fig. 1) and 15 (Fig. 2).

For the two other mixtures the order, as far as they have been examined, is (only numbers are given) for III: 9, 10, 11, 12 (or 12, 11), 13, 14 (or 14, 13), and 15; and for IV: 16, 17 (or 17, 16), 19, 20, and 21. The alternative orders are connected with the lack of degree of separation of such closely related hydrocarbons as 11 and 12

and similarly 13, 14 and 15. Whereas the three last-mentioned ones can be distinguished from each other, by their fluorescence, in the chromatogram (Expt. 20, Fig. 2), this is not the case with 11 and 12. With mixture IV the best results were obtained on TNF-impregnated plates (Expt. 8, Fig. 1). As our experimental evidence, concerning the hydrocarbons of III and IV, is rather scarce only the two chromatograms mentioned are shown in this paper.

In some of the chromatograms, although separation has occurred, there are more spots lying very close together. Certainly such spots do not represent pure hydrocarbons, but separation is sufficient to secure, in many instances, the identity of the main component in a spot, when relative position is considered and some colour test is applied and, preferentially, comparison with test substances is made.

Two-dimensional development

This technique has been used in order to increase the number of consecutive runs on a plate using the same developer. No change in development conditions is introduced by this procedure, but well developed chromatograms are obtained. A single experiment is recorded showing the successful separation of ten spots from a mixture of eleven hydrocarbons (Fig. 4).
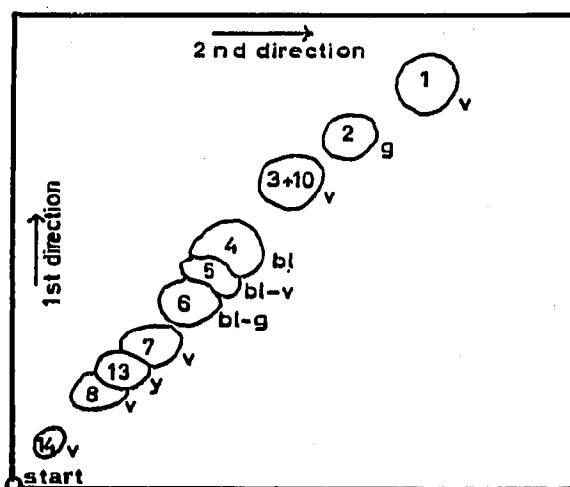

Fig. 4. Development in two directions in a separation of eleven hydrocarbons including test mixture II and hydrocarbons 10, 13, and 14. Adsorbent, caffeine on silicagel. Developer, pyridine (5%) in light petroleum. Two runs in each direction. Numbers in spots indicate hydrocarbons identified by fluorescence colours and distances travelled. Abbreviations: bl = blue; g = green; v = violet; y = yellow.

SUMMARY

Thin-layer chromatographic separation on alumina and on silica gel of mixtures of polycyclic aromatic hydrocarbons is promoted by modifying the adsorbent with

small amounts of complex forming substances. 2,4,7-Trinitrofluorenone on alumina and caffeine on silica gel are very efficient. Mixtures of light petroleum with small amounts of polar solvents serve well as developers.

NOTE ADDED IN PROOF

The use of some nitro compounds as complexing agents in TLC is reported in a note by FRANCK-NEUMANN AND JÖSSANG[17].

REFERENCES

[1] J. BONNET AND S. NEUKOMM, Oncologia, 10 (1957) 124.
[2] B. L. VAN DUUREN, J. Natl. Cancer Inst., 21 (1958) 1.
[3] J. LAM, Acta Pathol. Microbiol. Scand., 39 (1956) 198; ibid. 45 (1959) 237.
[4] D. S. TARBELL, E. G. BROOKER, A. VANTERPOOL, W. CONWAY, C. J. CLAUS AND T. J. HALL, J. Am. Chem. Soc., 77 (1955) 767.
[5] T. WIELAND AND W. KRACHT, Angew. Chem., 69 (1957) 172.
[6] F. MICHEL AND W. SCHMINKE, Angew. Chem., 69 (1957) 334.
[7] T. M. SPOTSWOOD, J. Chromatog., 2 (1959) 90; ibid. 3 (1960) 101.
[8] TH. WIELAND, G. LÜBEN AND H. DETERMANN, Experientia, 18 (1962) 430.
[9] N. KUCHARCZYK, J. FOHL AND J. VYMĚTAL, J. Chromatog., 11 (1963) 55.
[10] E. V. TRUTER, Thin Film Chromatography, Cleaver Hume Press, London, 1963.
[11] H. VOLLMANN, H. BECKER, M. CORELL AND H. STREECK, Ann., 531 (1937) 1.
[12] P. EKWALL AND K. SETÄLÄ, Acta Chem. Scand., 2 (1948) 733.
[13] P. EKWALL AND L. SJÖBLOM, Acta Chem. Scand., 6 (1952) 96.
[14] N. BROCK, H. DRUCKREY AND H. HAMPERL, Arch. Exptl. Pathol. Pharmakol., 189 (1938) 709.
[15] H. WEIL-MALHERBE, Biochem. J., 40 (1946) 351.
[16] E. BOYLAND AND B. GREEN, Brit. J. Cancer, 16 (1962) 347.
[17] M. FRANCK-NEUMANN AND P. JÖSSANG, J. Chromatog., 14 (1964) 280.