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SEPARATION AND IDENTIFICATION OF POLYNUCLEAR AROMATIC COMPOUNDS IN COAL TAR BY USING GLASS CAPILLARY CHROMATOGRAPHY INCLUDING COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Glass capillary chromatography combined with mass spectrometry has been used for the qualitative analysis of high-temperature coal tar. A simple method for making thermally stable wall-coated open tubular (WCOT) columns coated with the stationary phase SE-54 is described. More than 140 components with boiling points up to 870 °K could be separated and identified or characterized. A typical pattern for polynuclear aromatics (PNAs) was found on columns having polarities ranging from the polar polyphenyl ether sulphone Poly S 179 to the less polar silicone liquids OV-61, OV-7 and SE-54. Comparison of the retention times of more than 40 standard compounds contained in a single test mixture enabled most of the major constituents to be assigned. A number of compounds that had not hitherto been reported to be constituents of coal tar were characterized by their mass spectra. These included two unknown compounds in the benzpyrene fraction.

Coal tar may now serve as an inexpensive but very complete mixture for the gas chromatographic analysis of PNAs, because the retentions and/or orders of elution of a large variety of PNAs obtained on WCOT columns coated with various stationary liquids of different polarity have now been established.

INTRODUCTION

In 1933, Cook *et al.*¹ isolated the polynuclear aromatics (PNAs) benzo[*a*]pyrene, benzo[*e*]pyrene and benzo[*a*]anthracene from coal tar and demonstrated that the first compound is highly carcinogenic when applied to the skin of mice. Since then a large number of tests have been developed to study the deleterious effects of chemical substances². Major efforts have also been made in analytical chemistry and especially in chromatography to improve the determination of PNAs at various concentrations and in various matrices. Considerable improvements in gas chromatography were achieved by Lijinski *et al.*³ in 1963. This group succeeded in eluting coal tar constituents and synthetic PNA mixtures with a wide range of volatilities (up to benzo[*a*]pyrene, boiling point 760 °K) on packed columns containing small amounts

of stationary liquids and exhibiting high permeabilities. They used as the support glass beads coated with SE-30 as the stationary phase.

In recent years great efforts have been made to improve the performance of gas-liquid chromatographic separations by (1) increasing the separation efficiencies in terms of theoretical plate numbers, n (it must be realized, however, that the resolution of two closely eluted compounds is governed by the square root of n in both the isothermal⁴ and the temperature-programmed mode⁵) and (2) developing more selective, thermally stable stationary phases.

(1) The greater permeabilities of wall-coated open tubular (WCOT) columns compared with packed columns enable longer columns with very small amounts of stationary liquid to be used and, as a result, the greatest possible overall plate numbers are obtained. In recent years glass WCOT columns have proved successful in the separation of isomers of low volatility. Various methods for preparing glass capillary columns have been published with a view to meeting the following requirements: high separation efficiency, minimal tailing behaviour, in particular for polar solutes, and the utilization of stationary phases with a wide range of polarities.

WCOT glass columns usually contain very small amounts of stationary liquid and exhibit high phase ratios. Therefore, the result is relatively low capacity ratios (k') for the eluted components at moderate retention temperatures at the expense, however, of very small sample capacities. Thus, in practical analysis wide ranges of PNAs can in general be eluted within 1-2 h. Columns packed with glass beads exhibit permeabilities and phase ratios between those of WCOT columns and columns packed with conventional supports.

As early as 1964-65 Liberti and co-workers^{6,7} introduced glass capillary columns (up to 40,000 theoretical plates) coated with the silicones SE-30, SE-52 and XE-60 for the determinations of PNAs in atmospheric dusts. They concluded that SE-52 is the most effective of these stationary liquids with respect to selectivity and temperature stability. In recent years, Lee *et al.* demonstrated the possibility of using glass capillary columns in gas chromatography-mass spectrometry (GC-MS) in PNA analysis and examined air particulates⁸ and tobacco and marijuana smoke⁹. There have been few attempts¹⁰⁻¹², however, to use glass capillary columns of high efficiencies in the determination of PNAs in coal tar, particularly in combination with mass spectrometry.

In parallel with the improvement of glass WCOT columns, Grimmer and co-workers^{13,14} developed packed columns of maximal length 20 m, yielding 50,000 theoretical plates. These were able to achieve about the same resolution as glass capillary columns of the same length but had the disadvantages of very long retention times, higher column temperatures and large pressure drops. For example, the retention time of the benzpyrene fraction was about 90 min at 553 °K, compared with 15 min at 523 °K for a 30-m WCOT glass column. Owing to the large amounts of stationary phase used, these packed columns have much greater sample capacities and show considerable bleeding rates in temperature programming at the necessarily higher temperatures than do capillary columns. Therefore, they can be used for the isolation of microgram amounts of unknown PNAs for UV analysis. Owing to the high bleeding rates at high temperatures only stationary liquids without any chromophore (*e.g.*, phenyl groups) can be used to avoid errors in the UV spectra of the isolated compounds.

Blomberg and Wännman¹² recently applied differently pre-treated WCOT glass columns in the GC of coal tar. They used SP 2100 and thin layers of non-extractable Carbowax as the stationary phase, according to the procedures of Aue and Younker¹⁵ and Cronin¹⁶. They observed different bleeding rates of the same stationary liquid depending on the kind of pre-treatment and type of glass drawn into capillaries. Similar experiences were reported by us recently^{17,18}.

(2) Many workers have tried to improve the separations of PNA isomers by using more selective stationary phases in packed columns. Optimal selectivities permit much lower separation efficiencies in terms of theoretical plate numbers. Stationary liquids with special selectivities must have sufficient thermal stability, which is not the case for most polar stationary phases.

In 1972, Sauerland and Zander¹⁹ tested poly-*m*-phenoxyene (synthesized by Beeson and Pescar²⁰) in coal tar analysis. Two years later, Mathews *et al.*²¹ developed two polyphenylether sulphones as the stationary liquid. One compound (Poly S 179) proved to be temperature stable up to 673 °K. They chromatographed a mixture of PNAs up to phenanthrene on a 10-ft. packed column that had been coated with this stationary liquid by programming from 473 to 623 °K at 10 °K/min. These retention temperatures indicate a strong retardation of the PNAs on this stationary liquid. Sauerland *et al.*²² succeeded in eluting the PNAs in coal tar up to benzo[*a*]coronene on even longer packed columns, containing small amounts of Poly S 179. By using very low heating rates in temperature programming, retention times of several hours had to be taken into account. In 1975, Lao *et al.*²³ examined coal tar on 12-ft. Dexsil 300 and OV-1 packed columns by GC-MS and identified or characterized about 50 components in a single run. Janini *et al.*²⁴ developed several nematic stationary liquids of increasing thermal stability. In this series the application²⁵ of N,N'-bis-(*p*-phenylbenzylidene)-*o*,*o'*-bi-*p*-toluidine was demonstrated for coal tar analysis. This compound, however, is limited in its use to the narrow temperature range between 528 and 563 °K, which severely restricts temperature programming.

In view of these results, our main objectives in this investigation were as follows:

- (i) to elute coal tar constituents on long capillaries with high separation efficiencies coated with selective stationary liquids;
- (ii) to check the use of Poly S 179 as the most temperature-stable polar stationary liquid in WCOT glass column GC-MS at temperatures above 573 °K;
- (iii) to identify and characterize as many compounds as possible in coal tar using the summary of coal tar constituents by Lang and Eigen²⁶;
- (iv) to investigate the applicability of coal tar as a test mixture containing most of the important carcinogenic PNAs in a non-artificial matrix;
- (v) to compare the separation of compounds of low volatility in glass capillary GC and in high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Manufacture and performance of glass capillary columns for high-temperature work

In general, the same method was used to prepare the columns as described previously⁹. Glass capillaries were drawn from soda-glass (Schott AR) and borosilicate glass (Schott Duran 50) tubing (O.D. 8 mm, I.D. 3 mm in both instances).

TABLE I
COLUMNS MADE FOR PNA ANALYSIS

Stationary liquid	Chemical composition	Temperature range (°K)	Quantity	Length (m)	Type of glass	Surface treatment	Time (min)	Coating	Film thickness (mm)
		Commercial	This work						
Poly S 179*	Polyphenyl sulphonyl ether	473-673	463-663	1	92	Alkali	HCl	120	Dynamic, mercury plug
OV-61**	Methyl- + phenylpolysiloxane (2:1)	323-623	-543	1	30	Alkali	HCl HF	120 40	Dynamic, mercury plug
OV-7***	Methyl- + phenylpolysiloxane (4:1)	293-623	-553	1	30	Alkali	HCl HF	120 40	Dynamic, mercury plug
SE-54†	Methyl- + phenyl- + vinyl- polysiloxane	373-573	353-563	3	45-48	Borosilicate	None		Dynamic, mercury plug 54-6†
SE-54				1	45		HCl HF	120 40	Dynamic, mercury plug 65
SE-54				2	45 and 35	Alkali	HCl H ₂ O (25 ml) HF	240 40	Static 110

* Stationary phase used by Mathews *et al.*²¹ and Sauerland *et al.*²².

** Stationary phase recommended by Grob *et al.*²³ for high-temperature work.

*** Stationary phase used by Lane *et al.*²⁰.

† Stationary phase used by Onuska *et al.*³¹ and Winkler *et al.*³².

resulting in capillaries of 0.27 mm I.D. and up to 150 m in length. The alkali-glass capillaries were etched with a continuous flow of hydrogen chloride for 2–4 h at 730 °K, followed by a stream of hydrogen fluoride diluted with nitrogen for 40 min at the same temperature. The borosilicate-glass capillaries were not etched for the applications in this work.

In continuation of our recent experiments¹⁷ on the influence of different glass surfaces on the temperature stability of methylsilicones, we removed the sodium chloride crystals that had been formed on the inner surface of the alkali-glass capillaries by the hydrogen-chloride treatment by forcing water (5 × 5 ml) through the etched capillaries followed by subsequent rinsings with acetone and diethyl ether. These capillaries were then dried overnight in a flow of nitrogen at 670 °K, followed by the same hydrogen fluoride treatment as described above. Three of these columns were then statically coated²⁸ with OV-101 and SE-54 as stationary phases to yield coating efficiencies of 67–90% (2800–3500 theoretical plates/m at $k' = 15$ –18). Without any prior deactivation with an organic material, the polar test compounds *n*-butanol and cyclopentanone showed no tailing on these columns.

The columns made for the PNA analysis described here are listed in Table I.

These columns were coated without any prior deactivation. Most of the substances used for this purpose tend to decompose at temperatures above 550 °K, whereas in the GC of PNAs higher column temperatures are usually required. For the dynamic coating procedure the concentrations of the stationary liquids in dichloromethane were 20% (w/w) for Poly S 179 and OV-61, 15% (w/w) for OV-7 and 5% (w/w) for SE-54. All columns (except for the Poly S 179 column, which can only be used above 463 °K) were tested by the above-mentioned polarity mixture. Tailing was observed with *n*-butanol and cyclopentanone on each of these columns, but not for dibutyl ether and hydrocarbons. With alkali-glass treated columns we explain this behaviour as the result of a deactivating effect caused by additional treatment with hydrogen fluoride³³. Very recent experiments have revealed the possibility of deactivating borosilicate-glass (Duran 50) to a certain degree with gaseous mixtures of hydrogen fluoride and nitrogen in the dynamic mode³⁴.

Thermal treatment of dynamically coated SE-54 columns

When testing the first of these SE-54 columns all peaks showed severe tailing. The performances were considerably improved by flushing the capillary columns with nitrogen, sealing their ends in a micro-flame and keeping them at 600 °K for 1 h. In contrast to the procedures described by Aue and Younker¹⁵ for the deactivation of WCOT glass columns, no rinsings with solvents were attempted for removing any of the stationary liquid. Three columns were made without any prior etching by simply coating the bare surface of borosilicate capillaries followed by the thermal treatment. The improved column behavior observed can be explained by better spreading of the gum phase on the glass surface at the applied temperature and/or by cross-linking of the vinyl groups incorporated in the silicone polymer. The Kováts retention indices of the test compounds measured on the SE-54 columns are given in Table II.

The retention indices of methyl butyrate show that alkali-glass columns treated with hydrogen fluoride are considerably less active than untreated borosilicate columns.

This method of preparing thin-film glass capillary columns of high thermal stability for the elution of compounds of low volatility and polarity is simple and relatively rapid. However, the highest coating efficiencies for silicone-gum phases can

TABLE II

KOVÁTS RETENTION INDICES OF TEST COMPOUNDS ON SE-54 COLUMNS, MEASURED AT 353 °K

The column numbers serve for the distinction of the SE-54 columns from each other and do not correspond to the numbers in Table I.

<i>Test compound</i>	<i>Column 1:</i> <i>Alkali-glass, HCl, HF etched, dynamic coating</i>	<i>Column 2</i> <i>Alkali-glass, HCl, H₂O, HF treated, static coating</i>	<i>Column 3</i>	<i>Column 4</i> <i>Borosilicate-glass, mercury plug, thermal treatment at 600 °K</i>	<i>Column 5</i>	<i>Column 6</i>
Benzene + <i>n</i> -butanol	673.89	662.92	664.32	676.43	674.86	675.28
Methyl butyrate	726.89	722.88	724.03	753.89	753.76	740.51
Toluene	777.79	775.51	777.37	777.13	776.59	775.55
Octene-1	792.03	790.99	791.73	791.86	791.65	791.99
Dibutyl ether	884.05	883.61	883.22	884.56	883.57	884.06

be obtained by the static coating procedure²⁸. No tailing was observed for the PNAs, except for quinoline and isoquinoline, both of which have approximately the same basicity as unsubstituted aniline.

Separation efficiencies in terms of theoretical plate numbers determined for components of the benzpyrene fraction for coal tar chromatograms are given in Table III.

TABLE III

SEPARATION EFFICIENCIES OF THIN-FILM GLASS WCOT COLUMNS COATED WITH DIFFERENT STATIONARY PHASES

<i>Column</i>	<i>Type of glass</i>	<i>Etching</i>	<i>Coating</i>	<i>Theoretical plates/m</i>	<i>k</i>	<i>Compound</i>	<i>Column temperature (°C)</i>
45-m SE-54	Alkali	HCl-HF	Dynamic thermal treatment	1530	10.5	Perylene	483
45-m SE-54	Borosilicate	None	Dynamic thermal treatment	1860	11.0	Perylene	483
48-m SE-54	Borosilicate	None	Dynamic thermal treatment	2150	12.0	Benzo[a]pyrene	483
45-m SE-54	Borosilicate	None	Dynamic thermal treatment	1750	8.2	Perylene	473
45-m SE-54	Alkali	HCl-H ₂ O-HF	Static	2800	16.8	Benzo[e]pyrene	483
35-m SE-54	Alkali	HCl-H ₂ O-HF	Static	2850	17.3	Benzo[e]pyrene	483
30-m OV-7	Alkali	HCl-HF	Dynamic	2860	10.2	Perylene	523
30-m OV-61	Alkali	HCl-HF	Dynamic	2000	16.1	Benzo[a]pyrene	523

Column operation

All columns coated with silicone phases were used under both isothermal and temperature-programmed conditions. The Poly S 179 column was used in temperature programming only. Hydrogen was used as the carrier gas, because it exhibits the least dependence of HETP on carrier gas velocities, u , (flat HETP *versus* u graphs) owing to the relatively large diffusion coefficients, D_g (ref. 35), of organic compounds in hydrogen. In addition, this gas has the lowest viscosity, which again exhibits the

weakest dependence on temperature of all carrier gases³⁶. Allowing for these facts, the carrier gas inlet pressure was adjusted in such a way that at the final temperature the gas velocity reached the HETP minimum for isothermal conditions (*ca.* 0.35 m/sec for hydrogen and 0.24 m/sec for helium used in GC-MS). Maximal efficiencies in terms of plate numbers are thus obtained when compounds of high molecular weight are eluted, which enables more possible isomers to be separated than for low-molecular-weight compounds.

Peaks were assigned by comparing their retention times with more than 40 test compounds in a single artificial mixture on OV-7 and Poly S 179 columns. The GC-MS measurements were carried out using the latter column. The non-polar SE-54 column was chosen to cover a wider range of PNAs, whereas the OV-61 column was selected mainly for an improved separation of the benzofluoranthenes. These C₂₀H₁₂ isomers also exhibit different carcinogenic activities, but are much more difficult to separate than benzo[*a*]pyrene from benzo[*e*]pyrene and perylene (see Fig. 4).

Temperature-programmed and isothermal runs on the Poly S 179 column and part of a computer-reconstructed total ion chromatogram are shown in Fig. 1. The peaks are arbitrarily assigned as in all other chromatograms according to Table IV. Alternative structures for isomers that cannot be distinguished from each other on the basis of their mass spectra have been suggested in those instances where no comparison of retention data with test compounds was possible.

It can be seen from Fig. 1a-c that the range of application for PNA analysis begins with acenaphthene for the Poly S 179 column, which has a very thin film of stationary liquid.

Therefore, we chromatographed compounds of greater volatility than acenaphthene on capillaries coated with silicone phases (see Fig. 3a). In this range, the separation of naphthalene and benzo[*b*]thiophene is of practical importance. It also proved to be possible to separate azulene (the first non-benzenoid aromatic compound reported in coal tar) from both methylnaphthalenes on the SE-54 and other apolar columns (see Fig. 3a). Inspection of the retention behaviour of PNAs and heterocycles with the same carbon skeleton shows that the order of elution is generally as follows: (1) O-PNA, (2) S-PNA, (3) PNA, (4) N-PNA. This sequence seems to be independent of the polarity of the stationary phase. A previously unidentified dinaphthofuran (compound 105b; characterized by MS), which is eluted shortly before the benzofluoranthenes, obeys this rule. The only exception is benzo[*b*]thiophene, which is eluted shortly after naphthalene (see Fig. 3a).

On Poly S 179 some anomalous orders of elution were observed in comparison with the less polar silicone phases SE-54, OV-7 and OV-61 (see Table V).

In comparison with the non-polar silicone stationary liquids the much higher thermal stability of the polar Poly S 179 phase is essential, because on the latter phase the retention temperatures are about 90 °K higher than on the former. In general, polar phases are thermally less stable than non-polar phases. The separations of the three benzofluorenes (peaks 67-69) and of benzo[*a*]anthracene, chrysene and triphenylene (peaks 86, 88, 89; Fig. 1a and b), and the strong retardation of the acidic carbazole and its homologues are remarkable features associated with Poly S 179. The latter observation may be explained by the formation of hydrogen bonds between the solute and the oxygen atoms of the stationary phase. Plotting the boiling points

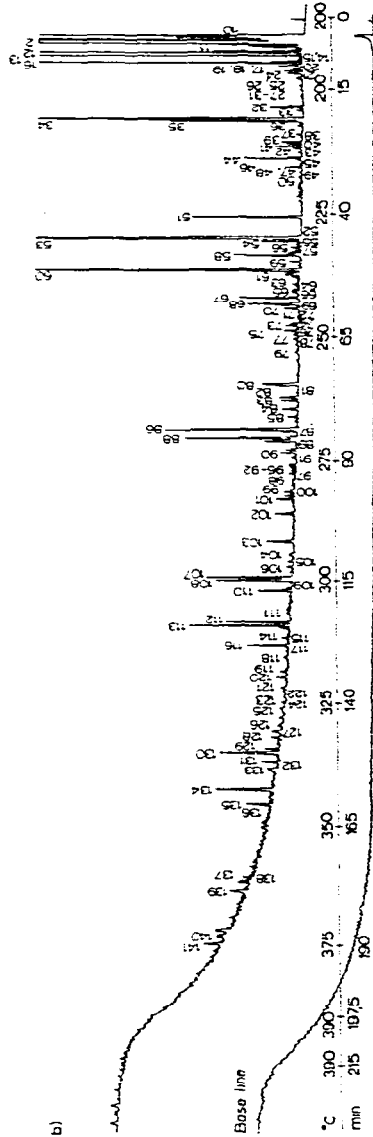
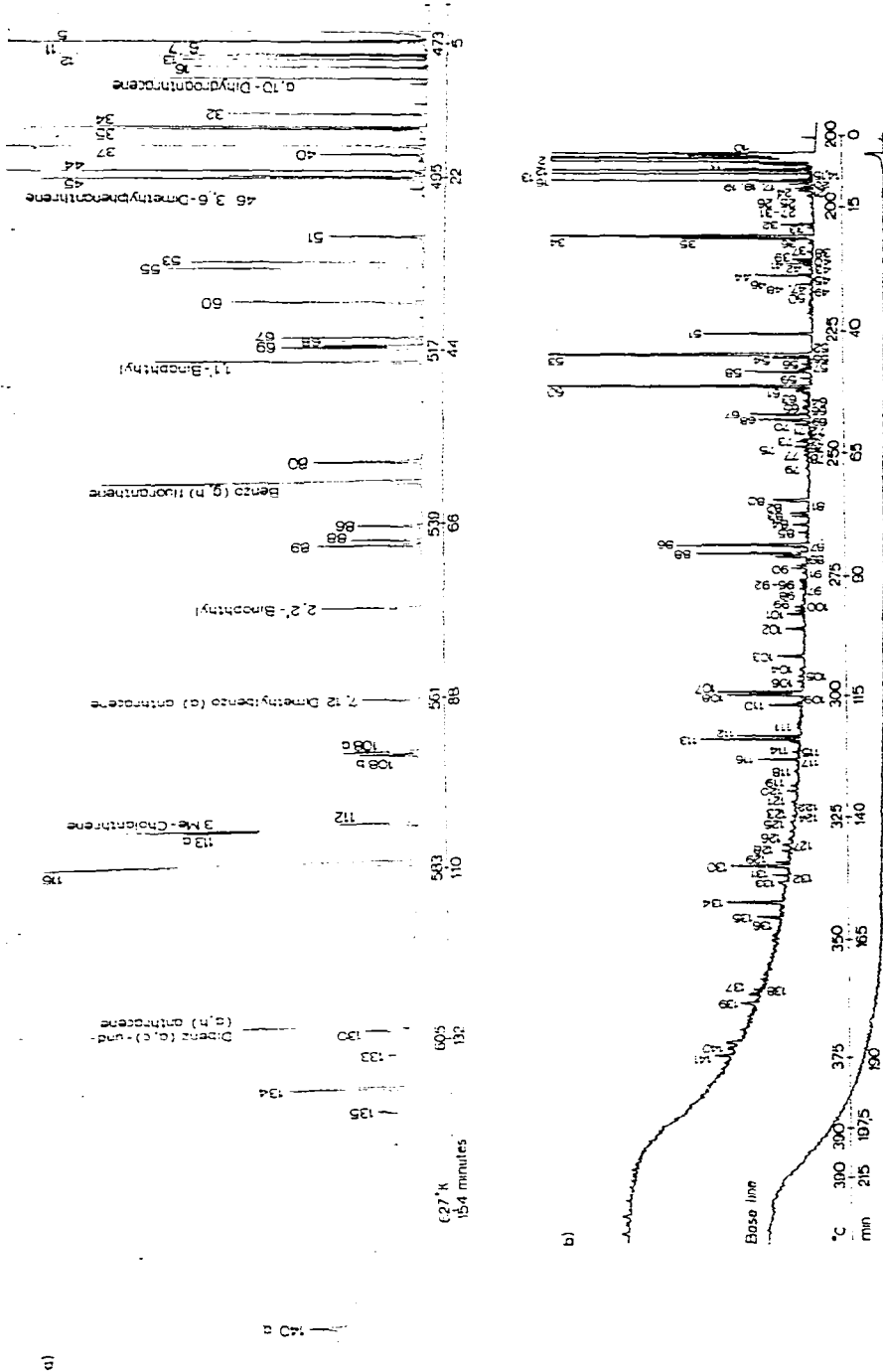


Fig. 1.

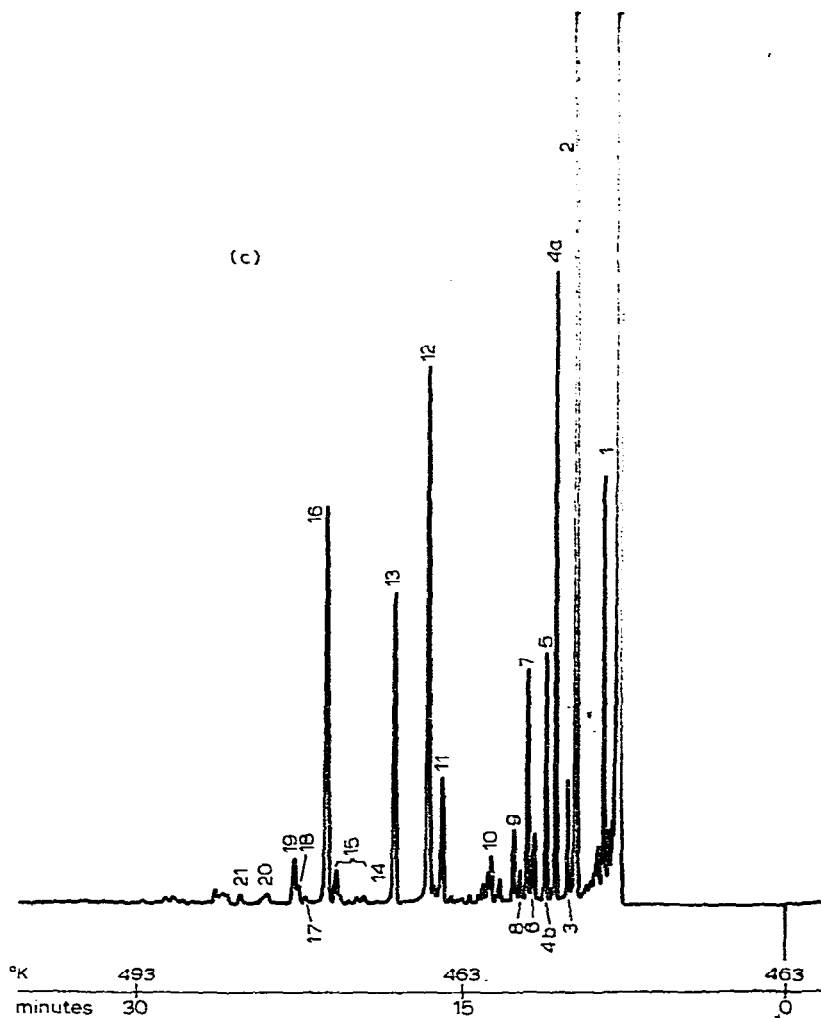


Fig. 1.

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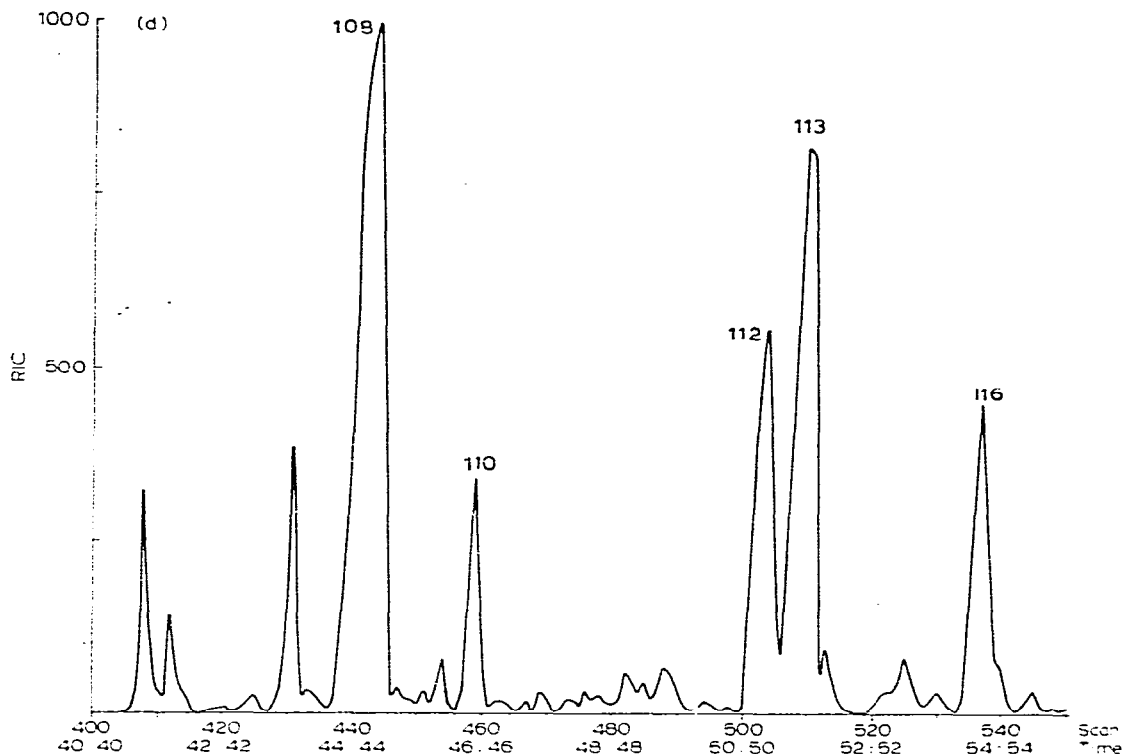


Fig. 1. Gas chromatography of PNAs and coal tar on Poly S 179. Peak numbers as in Table IV. (a) PNA test mixture. Column, 92-m Poly S 179, I.D. 0.27 mm alkali-glass, HCl-etched. Column temperature, programmed from 473 to 663 °K at 1 °K/min. Carrier gas, hydrogen, 0.35 m/sec at 663 °K. 34 = Phenanthrene; 35 = anthracene; 53 = fluoranthene; 60 = pyrene; 86 = benzo[*a*]anthracene; 88 = chrysene; 112 = benzo[*e*]pyrene; 113a = benzo[*a*]pyrene; 116 = perylene; 130 = indeno[1,2,3-*cd*]pyrene; 134 = benzo[*ghi*]perylene; 135 = anthanthrene; 140a = coronene. (b) Coal tar. Column, 92-m Poly S 179, I.D. 0.27 mm. Column temperature, programmed from 473 to 663 °K at 1 °K/min. Carrier gas, hydrogen, 0.35 m/sec at 663 °K. Peaks listed as in (a). (c) Coal tar (low-boiling fraction). Column, 92-m Poly S 179, I.D. 0.27 mm. Column temperature, programmed from 463 to 495 °K at 1 °K/min. Carrier gas, helium, 0.20 m/sec at 463 °K. 1 = Indene; 2 = naphthalene; 3 = benzo[*b*]thiophene; 4a = 2-methylnaphthalene; 4b = azulene; 5 = 1-methylnaphthalene; 7 = biphenyl; 11 = acenaphthene; 12 = acenaphthylene; 13 = dibenzofuran; 16 = fluorene. (d) Coal tar. Instrument, Finnigan 4000. Column, 92-m Poly S 179, I.D. 0.27 mm. Column temperature, 600–610 °K. Interface, Pt capillary, I.D. 0.1 mm. Interface temperature, 540 °K. Ion source temperature, 573 °K. Benzopyrene fraction of the computer-reconstructed total ion chromatogram. 107 = Benzo[*b*]fluoranthene; 108a = benzo[*j*]fluoranthene; 108b = benzo[*k*]fluoranthene; 112 = benzo[*e*]pyrene; 113a = benzo[*a*]pyrene; 116 = perylene.

(according to Lang and Eigen²⁶) of a number of PNAs against their retention temperatures leads to graphs in which all compounds containing an NH group or a single nitrogen atom are located on lines parallel to that for the unsubstituted PNAs. On the basis of this plot (Fig. 2), peak 77 was assigned as an azabenzofluorene and not as a benzocarbazole, and compound 82 as pyrrolo[*def*]phenanthrene, whereas compounds 103, 104a, 105a and 106 are characterized as benzocarbazoles and naphthoindole, respectively. In each instance the presence of these isomers was confirmed by mass spectrometry.

TABLE IV

ASSIGNMENT OF PEAKS BASED ON GC-MS AND COMPARISON OF RETENTION TIMES ON POLY S 179 AND OV-7

Peak No.	Molecular weight	Assignment	Remarks
1	116	Indene	
2	128	Naphthalene	
3	134	Benzo[b]thiophene	
4a	142	2-Methylnaphthalene	
4b	128	Azulene	
5	142	1-Methylnaphthalene	
6	129	Quinoline	
7	154	Biphenyl	
8	129	Isoquinoline	
9	156	Dimethylnaphthalene	
10	168	Methylbiphenyl	
11	154	Acenaphthene	
12	152	Acenaphthylene	
13	168	Dibenzofuran	
14	131	Methylindole	Or methylindolizine
15a	168	Methylbiphenyl	
15b	180	?	No diphenylethene
16	166	Fluorene	
17	153	Azaacenaphthylene	Or naphthonitrile
18	182	Methyldibenzofuran	
19	182	Methyldibenzofuran	
20a	166	Methylacenaphthylene	Or naphthocyclopentadiene
20b	183	?	
21	166	Methylacenaphthylene	Or naphthocyclopentadiene
22	153	Azaacenaphthylene	Or naphthonitrile
23	180	1,1'-Diphenylethene?	
24a	180	Methylfluorene	
24b	196	?	
25a	180	Methylfluorene	
25b	196	?	
26	180	9(?) -Methylfluorene	
27	196	?	
28	167	Azafluorene	Or methylcyanonaphthalene
29	180	Methylfluorene	
30	167	Azafluorene	Or methylcyanonaphthalene
31	144	1(?) -Naphthol	
32	184	Dibenzothiophene	
33	184	Naphthothiophene	
34	178	Phenanthrene	
35	178	Anthracene	
36	198	Methyldibenzothiophene	
37	179	Benzo[h]quinoline	
38	184	Naphthothiophene	
39	192	Methylphenanthrene, -anthracene	
40	179	Acridine	
41	192	Methylphenanthrene, -anthracene	
42	192	Methylphenanthrene, -anthracene	
43	192	Methylphenanthrene, -anthracene	

(Continued on p. 110)

TABLE IV (continued)

Peak No.	Molecular weight	Assignment	Remarks
44	190	4 <i>H</i> -Cyclopenta[<i>def</i>]phenanthrene	
45	179	Benzo[<i>f</i>]quinoline	
46	204	2-Phenylnaphthalene	
47	179	Azaphenanthrene, -anthracene	
48	206	Dimethylphenanthrene, -anthracene	
49	193	Phenylindole	Or methylbenzoquinoline
50	206	Dimethylphenanthrene, -anthracene	
51	167	Carbazole	
52	181	Methylcarbazole	
53	202	Fluoranthene	
54	218	Benzonaphthofuran	
55	206	9,10-Dimethylanthracene	
56	204	Dihydropyrene?	No 1-phenylnaphthalene
57	208	Thiopheno[<i>def</i>]phenanthrene	Lee and Hites ³⁷
58	202	Acephenanthrylene-, anthrylene	
59a	218	Benzonaphthofuran	
59b	167	Naphthopyrrole	
60	202	Pyrene	
61	216	Methylfluoranthene, -pyrene	
62	216	Methylfluoranthene, -pyrene	
63	218	Benzonaphthofuran	
64	204	4 <i>H</i> -Cyclopenta[<i>def</i>]phenanthren-4-one	According to Gold ³⁸
65	216	Methylfluoranthene, -pyrene	
66	216	Methylfluoranthene, -pyrene	
67	216	Benzo[<i>a</i>]fluorene	
68	216	Benzo[<i>b</i>]fluorene	
69	216	Benzo[<i>c</i>]fluorene	
70	216	Methylfluoranthene, -pyrene	
71	232	Tetrahydro derivative of benzo[<i>c</i>]phenanthrene, benzo[<i>a</i>]anthracene, chrysene, triphenylene	
72	216	Methylfluoranthene, -pyrene	
73	203	Azafluoranthene, -pyrene	Or phenanthrene or anthracene nitrile
74	230	Dimethylfluoranthene, -pyrene	Dihydro derivatives of benzo[<i>c</i>]phenanthrene, benzo[<i>a</i>]anthracene, chrysene, triphenylene possible
75	203	Azafluoranthene, -pyrene	
76	230	As peak 74	
77	217	Azabenzofluorene	No benzocarbazole
78	230	As peak 74	
79	203	Azafluoranthene, -pyrene	Or phenanthrene or anthracene nitrile
80	228	Benzo[<i>c</i>]phenanthrene	
81	254	1,2'-Binaphthyl	Or phenylphenanthrene, -anthracene
82	191	Pyrrolo[<i>def</i>]phenanthrene	
83a	226	Benzo[<i>mno</i>]fluoranthene	Or cyclopenta[<i>cd</i>]pyrene
83b	234	Benzonaphthothiophene	
84	229	Aza derivative of benzo[<i>c</i>]phenanthrene (presumably)	

TABLE IV (continued)

Peak No.	Molecular weight	Assignment	Remarks
85	234	Benzonaphthothiophene	
86	228	Benzo[<i>a</i>]anthracene	
87	228	?	
88	228	Chrysene	
89	228	Triphenylene	
90	229	Aza derivative of benzo[<i>c</i>]phenanthrene, benzo[<i>a</i>]anthracene, chrysene, triphenylene	
91	242	Methyl derivative of benzo[<i>c</i>]anthracene or isomers	
92	242	Methyl derivative of benzo[<i>c</i>]anthracene or isomers	
93	242	Methyl derivative of benzo[<i>c</i>]anthracene or isomers	
94	242	Methyl derivative of benzo[<i>c</i>]anthracene or isomers	
95	234	Phenylphenanthrene, -anthracene, no binaphthyl	
96	242	Methyl derivative of benzo[<i>a</i>]anthracene or isomers	
97	242	Methyl derivative of benzo[<i>a</i>]anthracene or isomers	
98a	242	Methyl derivative of benzo[<i>a</i>]anthracene or isomers	
98b	254	2,2'-Binaphthyl	
99	240	4 <i>H</i> -Cyclopenta[<i>def</i>] chrysene or 4 <i>H</i> -cyclopenta[<i>def</i>]triphenylene	
100	240	4 <i>H</i> -Benzo[<i>fg</i>]pyrene or dibenzo[<i>def,i</i>]fluorene	
101	240	As peak 100	
102	230	Benzanthrone	
103	217	Benzocarbazole	
104	217	Benzocarbazole	
105a	217	Benzocarbazole	
105b	268	Dinaphthofuran	
106	217	Naphthoindole	
107	252	Benzo[<i>b</i>]fluoranthene	
108a	252	Benzo[<i>k</i>]fluoranthene	
108b	252	Benzo[<i>j</i>]fluoranthene	
109	253	Azabenzofluoranthene or benzo[<i>c</i>]phenanthrene, benzo[<i>a</i>]anthracene, chrysene, triphenylene nitrile	
110	252	Isomer of benzofluoranthene, -pyrene	
111	266	Methylbenzofluoranthene	
112	252	Benzo[<i>e</i>]pyrene	
113a	252	Benzo[<i>a</i>]pyrene	
113b	252	?	
114	266	Methylbenzofluoranthene, -pyrene	
115	266	Methylbenzofluoranthene, -pyrene	
116	252	Perylene	
117a	266	Methylbenzofluoranthene, -pyrene, methylperylene	
117b	278	Dibenzophenanthrene, -anthracene	

(Continued on p. 112)

TABLE IV (continued)

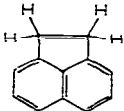
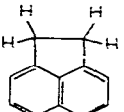
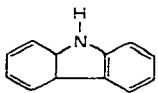
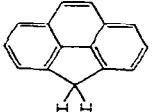
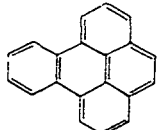
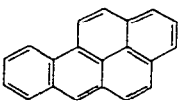
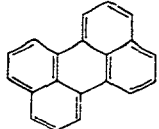
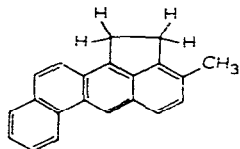
Peak No	Molecular weight	Assignment	Remarks
118	266	Methylbenzofluoranthene, -pyrene, methylperylene	
119a	264	Methylenebenzofluoranthene, -pyrene, methyleneperylene	
119b	253	Aza derivative of C ₂₀ H ₁₂ PNA or nitrile of C ₁₈ H ₁₂ PNA	
120	254	6 <i>H</i> -Benzo[<i>cd</i>]pyrene-6-one or isomer	Gold ³⁸
121a	284	Dinaphthothiophene	
121b	264	Methylene derivative of benzofluoranthenes, -benzpyrenes, perylene	
122	279	Dibenzoacridine or isomer	
123	278	Dibenzophenanthrene, -anthracene	
124	284	Dinaphthothiophene	
125a	278	Dibenzophenanthrene, -anthracene	
125b	284	Dinaphthothiophene	
126	279	Dibenzoacridine or isomer	
127	278	Dibenzophenanthrene, -anthracene	
128	276	Indenofluoranthene	
129	278	Dibenzo[<i>a,h</i>]- and/or dibenzo[<i>a,c</i>]anthracene	
130	276	Indeno[1,2,3- <i>cd</i>]pyrene	
131	278	Benzo[<i>b</i>]chrysene	
132	267	Dibenzocarbazole	
133	278	Picene	
134	276	Benzo[<i>ghi</i>]perylene	
135	276	Anthanthrene	
136	290	Methyl derivative of indenofluoranthene, -pyrene, benzo[<i>ghi</i>]perylene, anthanthrene	Methylene derivative of dibenzophenanthrenes or -anthracenes possible
137	302	Dibenzofluoranthene, -pyrene	
138	302	Dibenzofluoranthene, -pyrene	
139	302	Dibenzofluoranthene, -pyrene	
140a	300	Coronene	
140b	300	?	
141	302	Dibenzofluoranthene, -pyrene	

The PNAs are, in general, more strongly retarded than partially hydrogenated compounds, more or less independent of the polarity of the stationary phase. With the polar Poly S 179 this behaviour is so marked that benzo[*a*]pyrene (molecular weight 252) exhibits almost the same retention time as 3-methylcholanthrene (molecular weight 268; Fig. 1a). Allowance should be made for this effect if both carcinogenic compounds need to be separated from each other.

In temperature-programmed runs the major groups of PNAs (phenanthrene; anthracene; fluoranthene/pyrene; benzo[*a*]anthracene/chrysene; benzofluoranthene; benzpyrenes; indeno[1,2,3-*cd*]pyrene/benzo[*ghi*]perylene/anthanthrene; coronene and dibenzopyrenes are eluted at approximately equal time intervals, representing an increase of 24 (-C=C-) and 26 (-CH=CH-) daltons for each group. By extrapolation, we assume molecular weights of 324-328 daltons for the group of compounds eluted beyond coronene (Fig. 3a). This group has not, however, been examined by GC-MS up to now.

TABLE V

ORDER OF ELUTION OF SOME PNAs ON SLIGHTLY POLAR METHYL-/PHENYL-SILOXANES AND POLY S 179

Compound	Structure	Order of elution	
		SE-54, OV-61, OV-7	Poly S 179
Acenaphthylene		1	2
Acenaphthene		2	1
Carbazole		1	2
4H-Cyclopenta[def]phenanthrene		2	1
Benzo[e]pyrene		1	1
Benzo[a]pyrene		2	3
Perylene		3	4
3-Methylcholanthrene		4	2

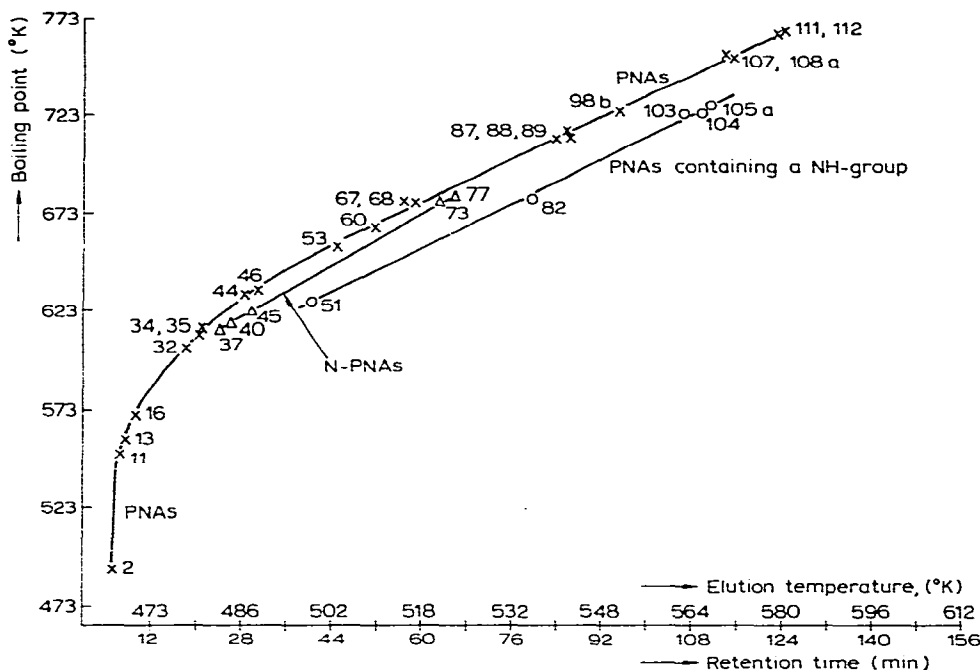


Fig. 2. Plot of boiling points of PNAs, N-PNAs and carbazole homologues (containing an NH group) versus elution temperature (retention time). Numbers indicate peaks according to Table IV. Column, 92-m Poly S 179, I.D. 0.27 mm. Column temperature, programmed from 473 to 663 °K at 1 °K/min. Carrier gas, hydrogen, 0.35 m/sec at 663 °K.

In PNA analysis, separations of isomers with different carcinogenic activities are of special interest and therefore we attempted to optimize the separation of the PNAs containing four, five and six rings. Owing to the very small film thickness of the SE-54 capillaries, we succeeded in eluting the components of coal tar up to anthanthrene at 473 °K, *i.e.*, *ca.* 320 °K below its boiling point (see Fig. 3b). Two compounds hitherto unreported in coal tar (peaks 110 and 113b) could be separated within the benzpyrene fraction. Peak 110 was confirmed as a further $C_{20}H_{12}$ isomer, in agreement with two isomers found in tobacco and marijuana smoke condensates by Lee *et al.*⁹ Another previously unreported component (113b) was separated from benzo[*a*]pyrene. Owing to the importance of quantitative benzo[*a*]pyrene determinations from various sources we eluted the benzpyrene fraction at different temperatures (Fig. 3b-f) to demonstrate the difficulty in separating this compound from both benzopyrenes and to obtain correct quantitative data. Benzo[*b*]-, -[*j*]- and -[*k*]-fluoranthene are only slightly separated on the SE-54 column but better on the OV-61 column (Fig. 4).

Having shown by GC-MS that dibenzo-, benzonaphtho- and dinaphthothio-phenes are present in coal tar, it seems doubtful that they could be used as indicators for oil spill contaminations as proposed by Warner³⁹ and Overton *et al.*⁴⁰. It should be noted that a number of these S-PNAs were identified many years ago in coal tar by Kruber⁴¹⁻⁴⁴.

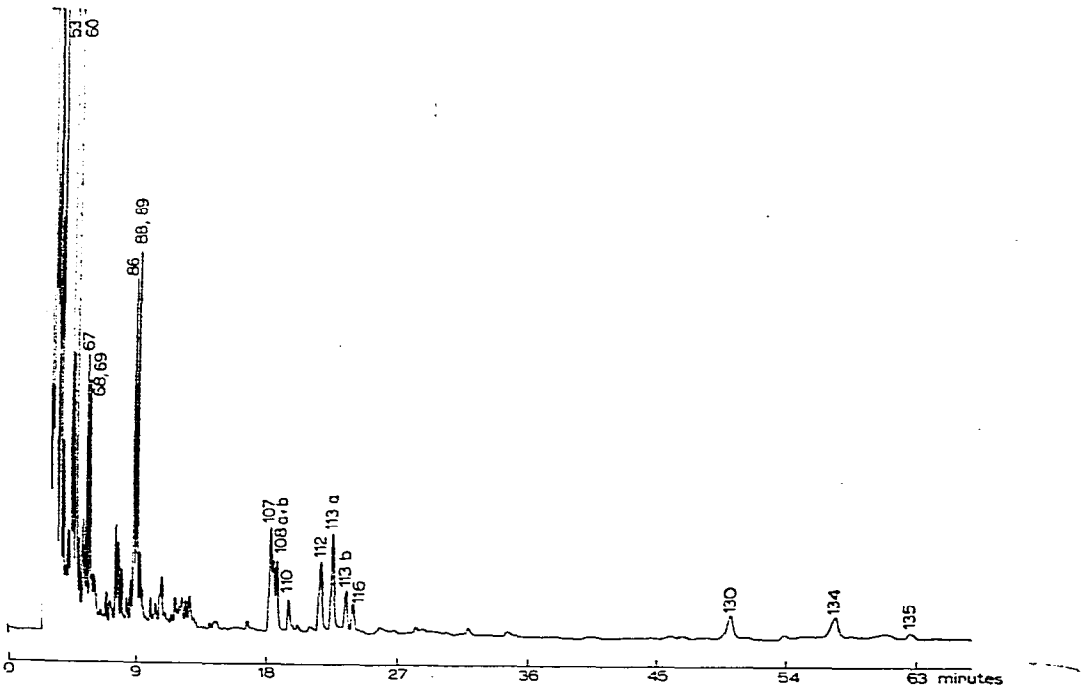
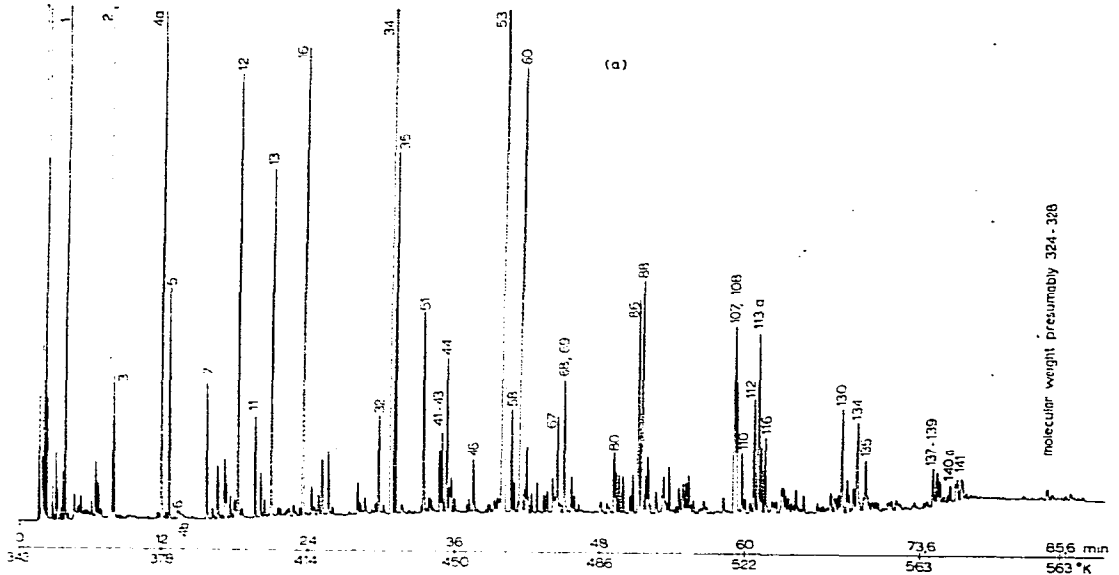


Fig. 3.

(Continued on p. 116)

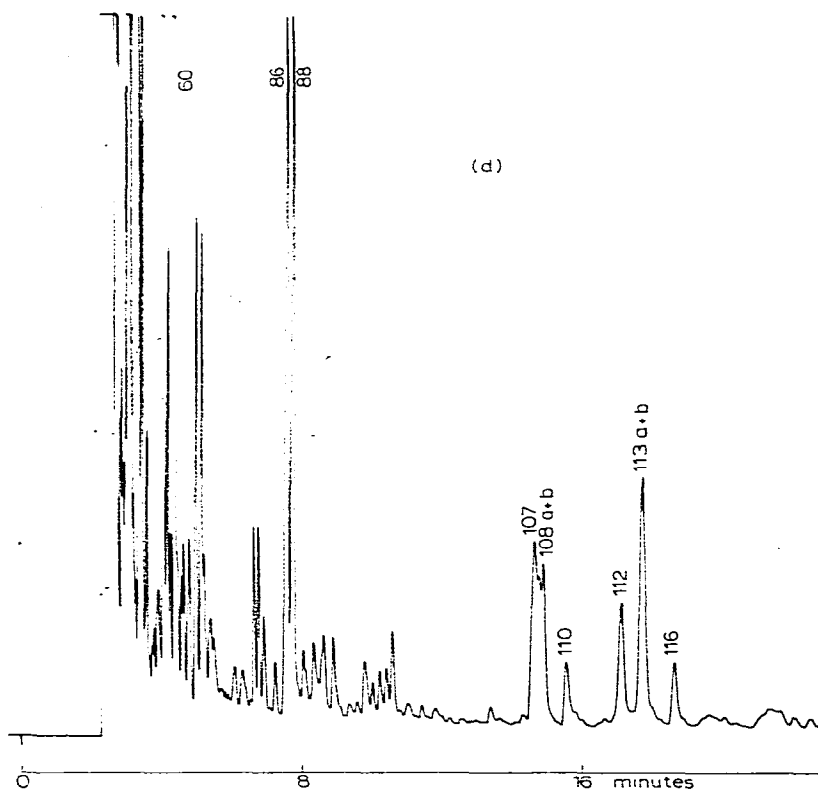
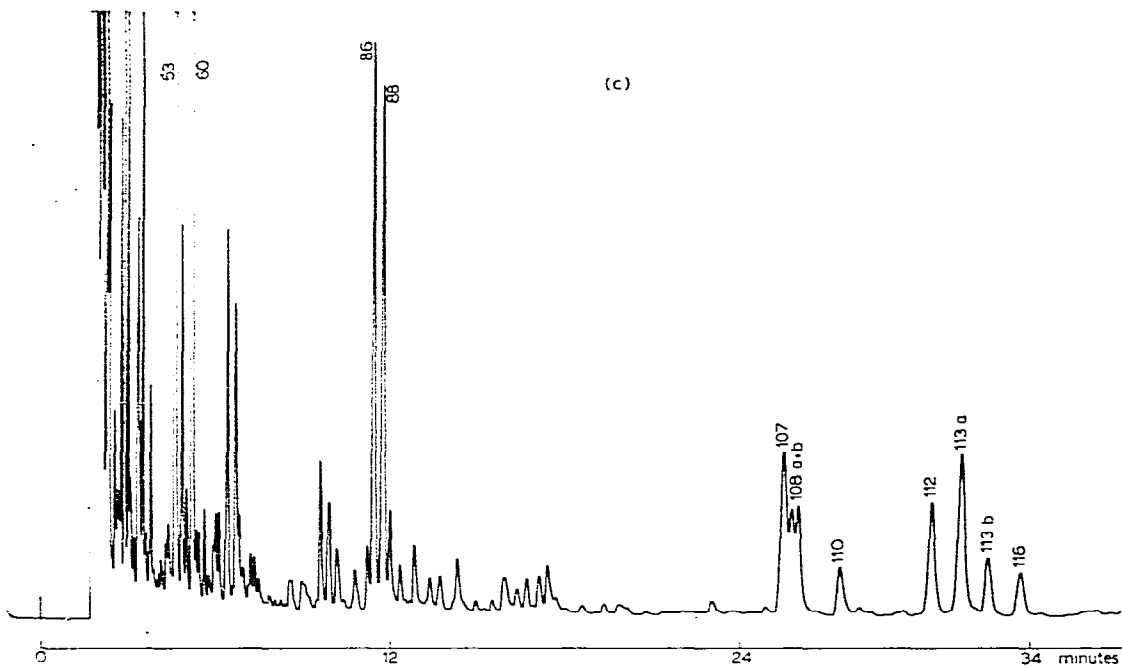


Fig. 3.

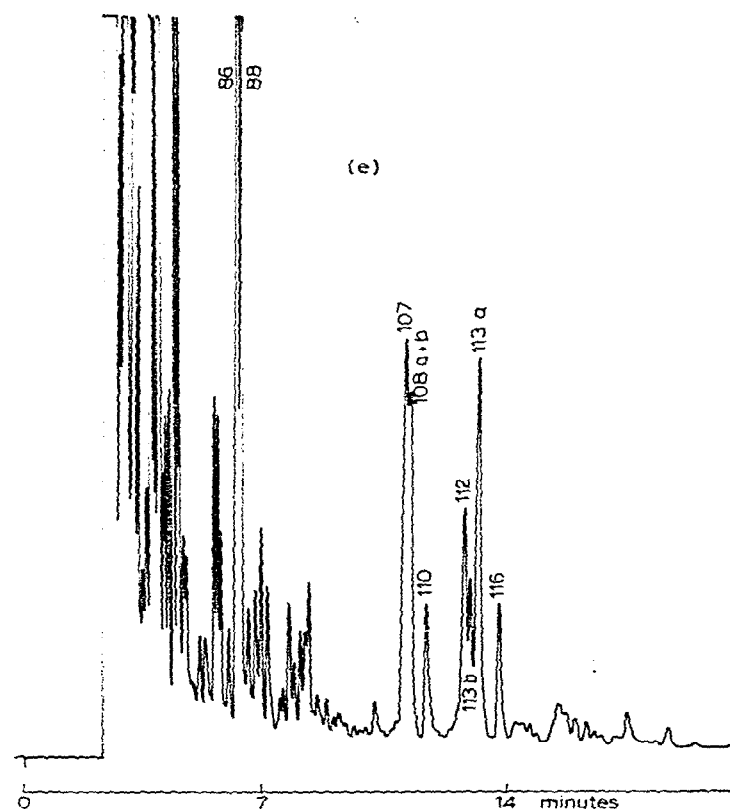


Fig. 3. Gas chromatography of coal tar on SE-54. Peak numbers according to Table IV. Figures in parentheses after names of compounds are wet retention times. Column, 48-m SE-54, I.D. 0.27 mm, borosilicate-glass. Column temperature, programmed from 342 to 563 °K at 3 °K/min. Carrier gas, hydrogen, 0.35 m/sec at 563 °K; 1 = indene; 2 = naphthalene; 3 = benzo[*b*]thiophene; 4a = 2-methylnaphthalene; 4b = azulene; 5 = 1-methylnaphthalene; 7 = biphenyl; 11 = acenaphthene; 12 = acenaphthylene; 13 = dibenzofuran; 16 = fluorene; 34 = phenanthrene; 35 = anthracene; 53 = fluoranthene; 60 = pyrene; 86 = benzo[*a*]anthracene; 88 = chrysene; 112 = benzo[*e*]pyrene; 113a = benzo[*a*]pyrene; 116 = perylene; 130 = indeno[1,2,3-*cd*]pyrene; 134 = benzo[*ghi*]perylene; 135 = anthanthrene; 140a = coronene. (b) Coal tar (fraction of 4-6-ring PNAs). Column, 45-m SE-54, I.D. 0.27 mm, borosilicate-glass. Column temperature, 473 °K. Carrier gas, hydrogen, 0.35 m/sec. 86 = Benzo[*a*]anthracene; 88 = chrysene; 107 = benzo[*b*]fluoranthene (0.733); 108a = benzo[*j*]fluoranthene (0.749); 108b = benzo[*k*]fluoranthene (0.758); 110 = undetermined isomer of benzo[*j*]fluoranthenes, -pyrenes (0.795); 112 = benzo[*e*]pyrene (0.898); 113a = benzo[*a*]pyrene (0.935); 113b = uncharacterized compound (0.981); 116 = perylene (1.000); 130 = indeno[1,2,3-*c,d*]pyrene; 134 = benzo[*g,h,i*]perylene; 135 = anthanthrene. (c) Coal tar (fraction of 4- and 5-ring PNAs). Column, 45-m SE-54, I.D. 0.27 mm, borosilicate-glass. Column temperature, 483 °K. Carrier gas, hydrogen, 0.35 m/sec. 53 = fluoranthene; 60 = pyrene; 86 = benzo[*a*]anthracene; 107 = benzo[*b*]fluoranthene (0.755); 108a = benzo[*j*]fluoranthene (0.764); 108b = benzo[*k*]fluoranthene (0.773); 110 = undetermined isomer of benzo[*j*]fluoranthenes, -pyrenes (0.816); 112 = benzo[*e*]pyrene (0.908); 113a = benzo[*a*]pyrene (0.939); 113b = unidentified (0.967); 116 = perylene (1.000). (d) Coal tar (fraction of 4- and 5-ring PNAs). Column, 45-m SE-54, I.D. 0.27 mm, alkali-glass. Column temperature 493 °K. Carrier gas, hydrogen, 0.35 m/sec. 60 = pyrene; 86 = benzo[*a*]anthracene; 88 = chrysene; 107 = benzo[*b*]fluoranthene (0.753); 108a = benzo[*j*]fluoranthene (0.762); 108b = benzo[*k*]fluoranthene (0.771); 110 = unknown isomer of benzo[*j*]fluoranthenes, -pyrenes (0.811); 112 = benzo[*e*]pyrene (0.909); 113a + b = benzo[*a*]pyrene and unknown compound (0.945); 116 = perylene. (e) Coal tar (4- and 5-ring PNAs). Column, 45-m SE-54, I.D. 0.27 mm, alkali-glass. Column temper-

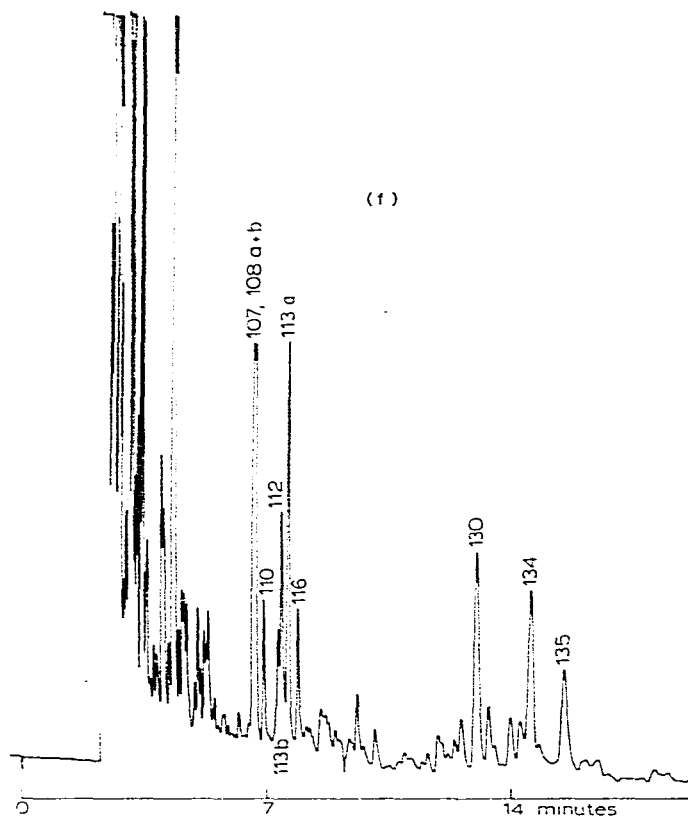


Fig. 3.

ature, 503 °K. Carrier gas, hydrogen, 0.35 m/sec. 86 = Benzo[*a*]anthracene; 88 = chrysene; 107 = benzo[*b*]fluoranthene (0.757); 108a = benzo[*j*]fluoranthene; 108b = benzo[*k*]fluoranthene; 110 = unknown isomer of benzofluoranthenes, -pyrenes (0.817); 112 = benzo[*c*]pyrene (0.904); 113b = unknown (0.922); 113a = benzo[*a*]pyrene (0.943); 116 = perylene (1.000). (f) Coal tar (5- and 6-ring PNAs). Column, 45-m SE-54, I.D. 0.27 mm, alkali-glass. Column temperature, 523 °K. Carrier gas, hydrogen, 0.35 m/sec. 107 = benzo[*b*]fluoranthene; 108a = benzo[*j*]fluoranthene; 108b = benzo[*k*]fluoranthene; 110 = unknown isomer of benzofluoranthenes, -pyrenes (0.875); 113b = unknown compound (0.925); 112 = benzo[*c*]pyrene (0.938); 113a = benzo[*a*]pyrene (0.938); 116 = perylene (1.000); 130 = indeno[1,2,3-*cd*]pyrene; 134 = benzo[*ghi*]perylene; 135 = anthanthrene.

GC-MS

A Finnigan 4000 instrument coupled to an Incos data system was used. The 92-m Poly S 179 capillary column was connected to the mass spectrometer by a platinum capillary (I.D. 0.1 mm), thermostated at 538 °K. The ion source was kept at 573 °K. Scans were taken from *m/e* 34 to 420, starting with 2 sec per scan and changing to 4 and 6 sec per scan for slower peaks. After an isothermal period of 15 min, the temperature was increased at 1 °K/min. A splitter with a septum purge was used, the splitting ratio being 180:1, with a velocity of helium of 0.49 m/sec at 363 °K. The background of the stationary phase measured at 519 °K is shown in Fig. 5, suggesting an upper temperature limit of approximately 620 °K for this phase in GC-MS. However, this limit will be lower if ions of low intensity have to be used for the interpretation of mass spectra.

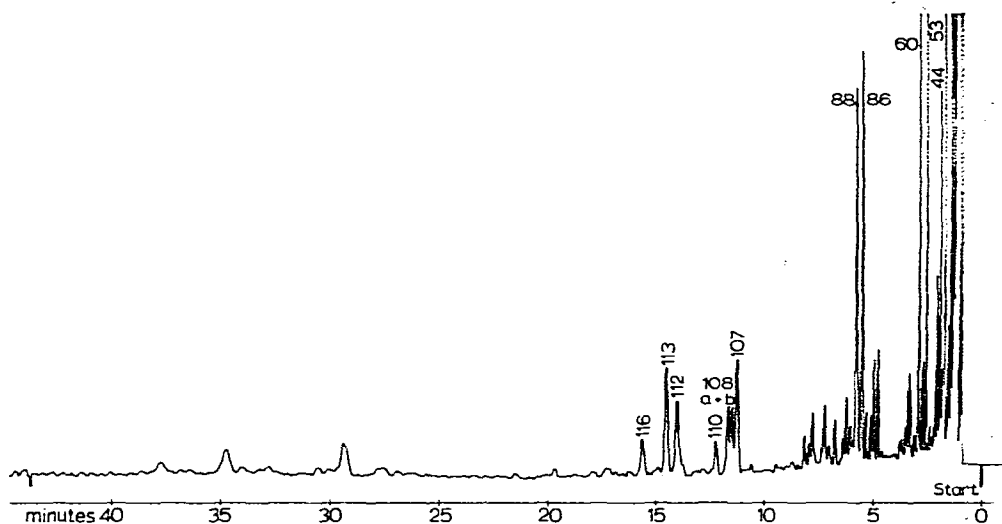


Fig. 4. Gas chromatography of coal tar (4- and 5-ring PNAs) on OV-61. Column, 30-m OV-61, alkali-glass. Column temperature, 523 °K. Carrier gas, hydrogen, 0.35 m/sec. Peak numbers according to Table IV. 53 = Fluoranthene; 60 = pyrene; 107 = benzo[*b*]fluoranthene; 108a = benzo[*j*]fluoranthene; 108b = benzo[*k*]fluoranthene; 110 = unknown isomer of benzofluoranthenes, -pyrenes; 113a = benzo[*a*]pyrene; 113b = unknown; 116 = perylene.

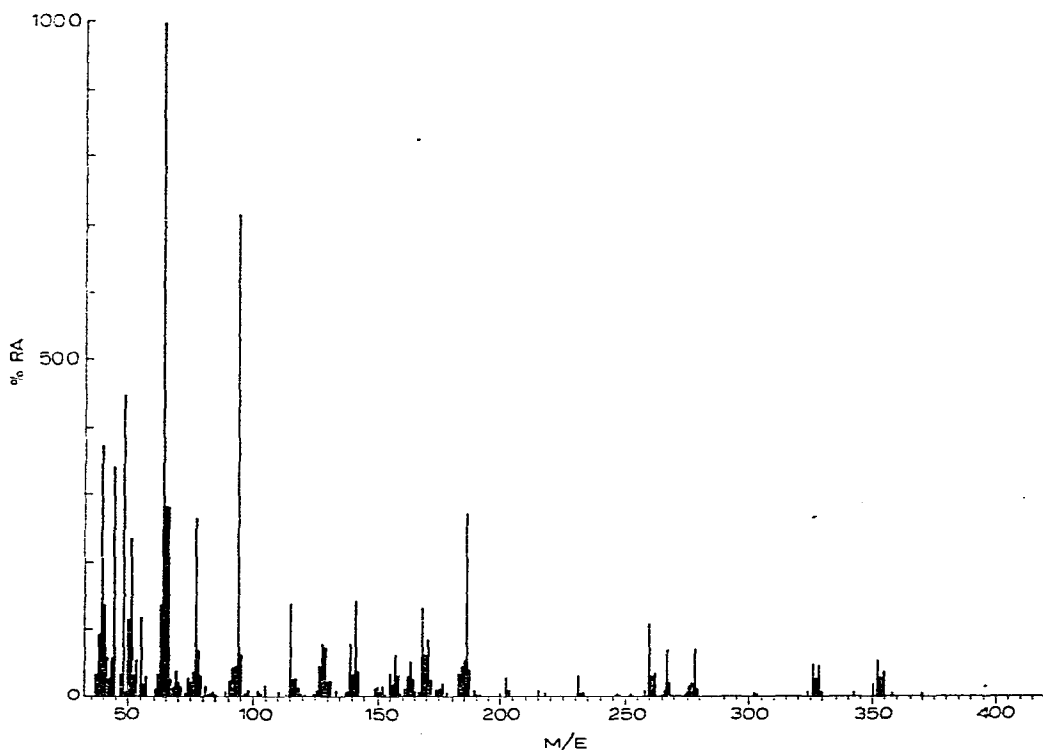


Fig. 5. Background pattern of stationary liquid Poly S 179 at 625 °K. Column length 92 m, I.D. 0.27 mm. Carrier gas, helium, 0.24 m/sec.

Interpretation of mass spectra of PNAs

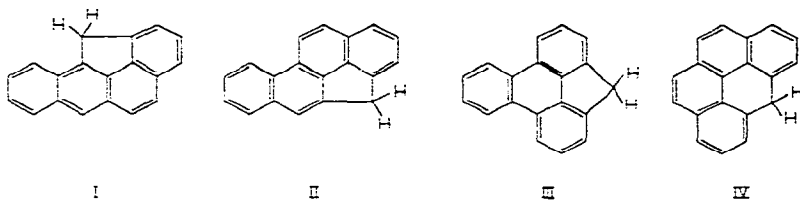
Unsubstituted PNAs, their monomethyl derivatives and hetero-atom substituted PNAs can be easily detected by their very intense molecular ions, which in general are the base peaks. A distinction of isomers is not possible in general.

The unsubstituted PNAs show hydrogen cleavage of the molecular ion leading to $M-1 < M-2$ in intensity. In addition, $M-26$ and the doubly charged ions $M/2e$ and $(M-26)/2e$ are observed. A detailed discussion of PNAs containing four rings and their derivatives was given by Grimmer and Glaser⁴⁵.

Monomethylated PNAs generally exhibit the molecular ions as the base peak, followed by $M-1$, which is of greater intensity than $M-2$ and $M-3$. The intensity of the important $M-1$ ion often increases if the methyl group is located at such a position that cleavage of hydrogen from a methyl group enables ring closure to a five- or six-membered ring to occur. This enables the positive charge to be stabilized by the surrounding π -electron system.

In addition to unsubstituted PNAs, the ions $M-14$, $M-15$ and $M-16$, with weak intensities, are often observed. Because of their molecular masses and these characteristics, monomethyl derivatives of five- and six-ring PNAs could be detected that have not yet been reported in coal tar. According to the "8 Index of Mass Spectra"⁴⁶, only with monomethyl derivatives of the strained acenaphthylene is the $M-1$ ion the base peak instead of the molecular ion.

PNAs carrying CH_2 bridges, e.g., fluorene, also exhibit the molecular ion as base peaks, followed by $M-1$, $M-2$, $M-3$ and $M-4$ due to hydrogen cleavage. $M-26$ and the doubly charged ions $M/2e$ and $(M-26)/2e$ are also observed. In many instances the partially hydrogenated PNAs can be distinguished from monomethylated PNAs by their molecular mass. However, with increasing molecular weights, isomers derived from both groups are possible. The intensity of the $M-1$ ion of PNAs carrying methylene bridges is often greater than that of monomethyl derivatives. Thus, peaks 99–101 were assigned to three of the four possible $\text{C}_{17}\text{H}_{12}$ isomers dibenzo[*def,i*]fluorene (I), 4*H*-cyclopenta[*def*]chrysene (II), 4*H*-cyclopenta[*def*]triphenylene (III) and 4*H*-benzo[*fg*]pyrene (IV).



The possibility of the isomeric methyl derivatives of $\text{C}_{16}\text{H}_{12}$ PNAs (benzo[*ghi*]fluoranthene, benzo[*mno*]fluoranthene, cyclopenta[*cd*]pyrene) was excluded, because of the intensity of the $M-1$ ion (70% of the base peak) and the absence of CH_3 cleavage. Moreover, methyl derivatives in coal tar always occur in lower concentrations than their parent PNAs, whereas these three compounds, which have not yet been reported in coal tar, appear in higher concentration than the only observed $\text{C}_{16}\text{H}_{12}$ PNA (peak 83a).

Unsubstituted PNAs containing hetero-atoms have the molecular ion as the base peak. Characteristic fragmentations are $M - 1$, $M - 2$, $M - 3$, $M - CHX$ ($X = O, S, N$), $M/2e$ and $(M - CHX)/2e$. Here again, distinction between the various isomers by interpretation of the mass spectra is in general not possible. Nevertheless, in combination with retention data, peak 105b could be assigned as a dinaphthofuran, peak 132 as a dibenzocarbazole and peaks 122 and 126 as dibenzoacridines. These compounds have not been previously reported to be constituents of coal tar.

With sulphur-containing PNAs, the ions $M - 32$, $M - 33$ and $M + 2$ (due to ^{34}S , relative abundance 4%) are additionally observed. In addition to the dibenzo-, naphtho- and benzonaphthothiophenes first described by Kruber³¹⁻³⁴, we detected three dinaphthothiophenes (peaks 121a, 124 and 125b) and the pericondensed thio-pheno[*def*]phenanthrene¹¹. These compounds are also reported for the first time in coal tar.

Nitrogen-containing PNAs of molecular weight 253 (peaks 109 and 119b) may be, in addition to azabenzofluoranthenes, -pyrenes or -perylene, nitriles of the $C_{18}H_{12}$ PNAs. The same difficulty arises for the assignment of peaks 17, 22, 28 and 30 as azaacenaphthylenes and azafluorenes cannot be distinguished from the isomeric nitriles of naphthalene and methylnaphthalene. Isonitriles can probably be excluded as coal tar constituents owing to their thermal sensitivity.

Carbonyl derivatives of PNAs

Peaks 102 and 120 were characterized as benzanthrone and 6*H*-benzo[*cd*]-pyrene-6-one by comparison of the mass spectra with those in the "Registry of Mass Spectral Data"⁴⁷ and Gold's results³⁸. Characteristic fragmentations from the molecular ion (base peak) are $M - 1$, $M - 28$, $M - 29$ and their doubly charged ions. $(M - 28)/2e$ and $(M - 29)/2e$ are of greater intensity than $M/2e$. These carbonyl compounds can be distinguished from the above-mentioned benzo- and naphthoanelated furans by their masses and missing $M - 2$ ions. Here again, isomers cannot be excluded.

CONCLUSIONS

By using the static coating procedure²⁸ preferably for silicone-gum phases we obtained up to 30% higher theoretical plate numbers than with the dynamic mercury plug method¹⁰. The use of glass capillary chromatography in combination with mass spectrometry proved to be valuable for the characterization of previously unknown PNAs and PNA derivatives in coal tar. The distinction of isomers by interpretation of mass spectra alone is in general not possible. We suggest that a collection of the GC data for available test compounds be compiled in order to ensure the GC determination of known compounds from different matrices. These should be obtained from WCOT glass columns of high performance measured isothermally within close interpolation limits (if possible, in between consecutive *n*-alkanes) under carefully controlled conditions, as started by Cantuti and co-workers^{7,48} a long time ago. In view of the complexity of PNA mixtures, stationary liquids of different polarity should be used for reliable determinations. Owing to the wide volatility range of the PNAs and necessarily larger sample capacities in GC-MS than in GC, temperature programming and the use of linear indices⁴⁹ seem to be appropriate to support the interpretation of mass spectra.

Most of the carcinogenic four- to six-ringed compounds (fluoranthene-anthanthrene) to be determined routinely can be handled in a single isothermal run. In these instances less volatile compounds (*e.g.*, coronene) should be removed from the chromatographic column by back-flushing. Further, the selectivity of the stationary liquid for the separation of certain isomers is more effective in the isothermal than in the temperature-programmed mode. The isothermally measured Kováts retention indices⁵⁰ calculated by interpolation between consecutive *n*-alkanes at carefully controlled temperatures are much more accurate than any other chromatographic data and should therefore be used for the identification of compounds.

An important part of this work was the temperature-programmed survey runs, separating various groups of isomers in the volatility range from indene beyond coronene. Recognizing the great complexity of natural PNA mixtures, which often contain more than 100 PNAs and their derivatives, we consider that gradient elution HPLC is inferior to temperature-programming in GC (polydimensional techniques are not discussed here), if thin-film capillary columns of high efficiencies are used. The main reasons are as follows:

(1) The relatively poor separation efficiency in terms of theoretical plate numbers in HPLC compared with capillary-column GC. HPLC systems normally do not exhibit more than 9000 theoretical plates in isocratic runs, compared with our average capillary columns of 90,000 theoretical plates (measured at k' values ≥ 10). These columns can be used at temperatures up to 300 °C, which is necessary in PNA analysis. Realizing that resolution increases only with the square root of the theoretical plate numbers, one achieves a more than three-fold increase in resolution by using capillary columns.

(2) The range of available chromatographic selectivities is far greater in HPLC than in GC. This advantage cannot be fully used in PNA analysis, however, owing to the poor solubilities of condensed PNAs. Further, the choice of mobile phases is restricted with regard to optimal detection. On the other hand, it must be pointed out that in GC progress has been made in applying more PNA-selective stationary liquids by some workers^{14,24,25,27} recently and by ourselves in this work. The separation of triphenylene from chrysene can nowadays be accomplished by GC, for example.

In view of these results and our own experiences with reversed-phase HPLC, we think that HPLC should be advantageously used for the following tasks in PNA analysis:

- (1) Separation of PNAs of lower volatility than coronene.
- (2) Isolation of unknown PNAs on a micro-preparative scale for identification by UV or fluorescence spectroscopy.
- (3) Quantitative routine analysis of a known and limited number of PNAs such as the benzpyrene fraction at optimized selectivities of both the chromatographic and the detection system. In this instance non-UV-absorbing or non-fluorescing components do not have to be removed in a previous separation step.
- (4) Fast group separations.

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