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CAPILLARY GAS CHROMATOGRAPHY OF AZAARENES

II. APPLICATION TO PETROLEUM NITROGEN BASES

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SUMMARY

The retention behaviour of azaarenes on several stationary phases was investigated. Retention indices were measured with a reference system constituted by quinoline benzologues. Steric hindrance in the vicinity of the nitrogen atom was found to influence drastically the retention of azaarenes. Further important parameters are the selectivity of the stationary phase towards the location of the heteroatom and the degree of alkyl substitution of the solutes. The most useful and complementary selection of stationary phases was OV-73, OV-61 and SP-2340. Examples of separations of complex azaarene mixtures extracted from crude oils are discussed.

INTRODUCTION

Nitrogen bases (nitrogenated polynuclear aromatic hydrocarbons, NPAHs) found in crude petroleum are complex mixtures of alkyl-substituted azaarenes containing two to seven fused rings, which mostly contain a single nitrogen atom[1–5]. Four determinations are required for the total identification of individual structures: the location of the nitrogen atom, the arrangement of fused rings (linear, angular, clustered) and the location and type of substituents. As pointed out by Lee and Wright[6], great complexity may arise from the first two aspects; for instance, eight possible isomers of unsubstituted triaromatic compounds (benzoquinolines) can be found, whereas there are 29 isomers of tetraaromatic structures. Adding alkyl substituents results in a drastic increase in the number of isomers: for instance, there are 68 methylbenzoquinolines and 319 monomethylated tetraaromatic azaarenes.

As a consequence of the complexity of petroleum NPAHs, total identifications of individual structures are usually obtained by means of co-injections on three different stationary phases. Thus, the selection of suitable stationary phases is of considerable importance. In a previous paper[7], we described three methods for the reproducible preparation of thermally stable and inert columns in order to be able to use the overall range of available stationary phases up to their stability limits. The result of the analysis of a test mixture consisting of homologues and isomeric azaarenes is

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indicative of the choice of the most useful stationary phases in each of the three classes apolar, medium-polar and polar.

Combined capillary gas chromatography–mass spectrometry (GC–MS) is the method of choice for the investigation of petroleum azaarenes, but usually provides only a restricted amount of information, because mass spectra do not contain structurally indicative fragments for any type of determination cited above[4,8]. Thus, the full potential of GC–MS can be achieved only with columns that are selective enough by using the information derived from retention data. A series of azaarenes with two to five fused aromatic rings were chosen as internal references for the determination of retention indices using linear temperature programming conditions, in an analogous manner to Novotny and co-workers[9,10]. This base index was used as an aid for identifications during sample screening and also for the tentative prediction of retention data.

Micropreparative fractionations of total basic fractions were effected by means of reversed-phase liquid chromatography (RPLC)[4] in order to facilitate GC separations and to be able to use stationary phases having less extended working ranges than apolar ones. The most useful stationary phases for petroleum azaarene analysis were selected by considering the type of separation achieved for different crude oil samples.

EXPERIMENTAL

The preparation of capillary columns has been described in a previous paper[7]. The method of selective extraction of petroleum bases was published in part in ref. 3 and will be completed elsewhere.

Micropreparative RPLC fractionations were performed on a C_{18} (LiChrosorb RP-18, 5 μm, from Merck, Darmstadt, G.F.R.) column (15 cm × 4 mm I.D.) using methanol–water (90:10, v/v).

RESULTS AND DISCUSSION

Stationary phase selection

Polar stationary phases are more difficult to use than apolar ones, but their application to the separation of azaarenes is to be justified owing to the lack of selectivity of the latter[7]. Changing the polarity of the stationary phase has only minor effects on the selectivity for neutral PAHs, as emphasized by several workers[11,12]. Bartle *et al.*[13] have shown recently that the retention indices of planar PAHs on methylphenylsilicones are strictly related to their boiling points. On the other hand, the behaviour of NPAHs can be completely different, in particular if polar stationary phases are used. Steric hindrance effects due to substituents located near the nitrogen atom and particular positions of this atom can dramatically affect the GC retention, in a similar manner to the behaviour of azaarenes in normal-phase liquid chromatography[14].

A series of isomeric and homologues di- and tricyclic aromatic NPAHs were used in a capillary column test for the evaluation of the degree of selectivity provided by a given stationary phase. Other azaarenes (from tetra- to heptacyclic aromatic compounds) were also analysed in order to determine the useful working range, *i.e.*, the maximum size of solutes that can be routinely analysed[7]. The elution sequence observed for the test mixture on eight stationary phases is shown in Fig. 1.

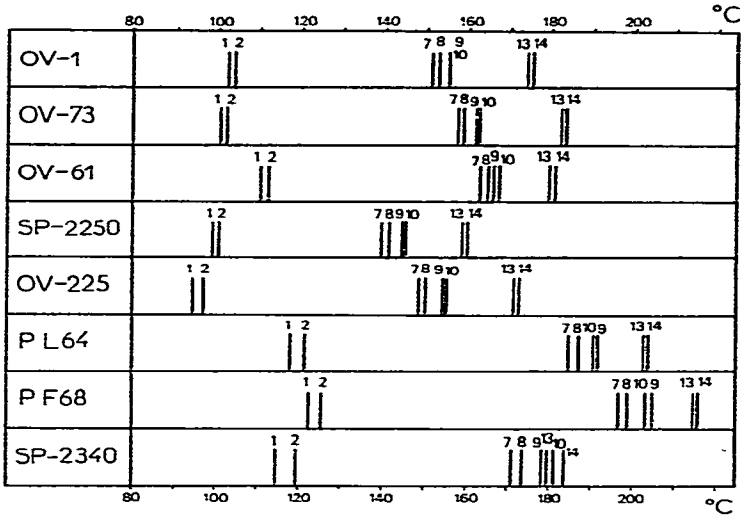

Fig. 1. Peak sequence of a base test mixture[7] observed on various stationary phases under standard temperature programming conditions. 1, Quinoline; 2, isoquinoline; 7, benzo[*h*]quinoline; 8, acridine; 9, phenanthridine; 10, benzo[*f*]quinoline; 13, 2,4-dimethylbenzo[*h*]quinoline; 14, 2,3-dimethylbenzo[*h*]-quinoline. PL64 and PF68 are Pluronic L64 and F68 stationary phases, respectively.

As expected, only minor selectivity changes are observed for the separation of NPAHs with methylphenyl-silicones when the number of phenyl groups of the phase is increased. This effect can be evaluated from the resolution achieved between the pair of isomers benzo[f]quinoline and phenanthridine: no separation on apolar OV-1 (100% methyl), partial separation on a 50-m OV-73 column (5.5% phenyl, analogous to SE-52) and baseline separation on a 25-m OV-61 column (33% phenyl). The conclusion that can be drawn from the evaluation of these stationary phases is that the elution sequence of azaarenes on silicone stationary phases follows the order of increasing number of carbon atoms, the best separations between closely related isomers (location of nitrogen atom) being obtained with medium-polar stationary phases. However, the higher thermal stability of columns coated with an apolar phase such as OV-73 (preferred to SE-52 because of a slightly higher stability) must also be considered when choosing the stationary phase for petroleum azaarene separations, particularly if high-molecular-weight compounds are present.

Polyglycols are also interesting as stationary phases. The elution sequence observed for the base test mixture with Pluronics (polyethylene–polypropylene block copolymers) (*cf.*, Fig. 1) is similar to that obtained with OV-61 or SP-2250 (50% phenyl-, 50% methyl-silicone), but the size of solutes that can be analysed is limited to four aromatic rings. However, the ease of preparation of chemically inert glass capillary columns coated with a polyglycol stationary phase is still an attractive feature. In agreement with other workers[15], we prefer Pluronic F68 to other polyglycols (Pluronic L64 and Carbowaxes) because of its high thermal stability and narrow range of molecular weight distribution.

Two silicone polymers incorporating cyano groups in their structures have been tested, namely OV-225 (25% phenyl-, 25% cyanopropyl-methyl-silicone) and SP-2340 (75% cyanopropyl-, 25% methyl-silicone). The first seems of less interest

than OV-61 or Pluronic F68, but SP-2340 leads to completely different selectivities. As shown previously[7], the separation is no longer closely related to the number of carbon atoms in NPAH molecules, but is strongly influenced by steric hindrance around the nitrogen atom.

Thus, taking in account the thermal stability of stationary phases, the coating efficiencies achieved with our methods of column preparation[7] and the retention patterns observed for reference azaarenes, the following stationary phases may be selected: OV-73, OV-61, Pluronic F68 and SP-2340.

Measurement of retention data

The comparison of GC retention data measured in different laboratories depends on numerous factors, all of which become critical when polar compounds are analysed with glass capillary columns. Although Kováts retention indices (*I*) have found wide acceptance, the most reproducible results are usually obtained with reference compounds that have chemical functions or structures as similar as possible to those of the solutes being studied. Thus, by analogy with the work of Novotny and co-workers[9,10], we chose a reference system consisting of azaarene benzologues ranging from two to five fused rings. The base retention indices (*BI*) 200, 300, 400 and 500 have been attributed to quinoline, acridine, benz[a]acridine and dibenz[*a*, *j*]acridine, respectively. The first two compounds have already been used in the same way[10]; although the other two are less commonly available, they were chosen because a plot of the elution temperature of the four compounds against their retention indices is nearly a straight line on most of the stationary phases that we have used.

BI values were calculated according to the following equation, which applies to linear temperature programming conditions[16,17]

$$BI = 100 \cdot \frac{T_R - T_x}{T_{x+1} - T_x} + 100x$$

where T_R = retention time of a compound, T_x = retention time of NPAH internal standard eluting prior to the compound, T_{x+1} = retention time of NPAH internal standard eluting after the compound and x = number of rings of NPAH internal standard eluting prior to the compound.

BI values of 30 reference NPAHs were calculated and are listed in Table I; structures and numbering systems are shown in Fig. 2. Our observations are in agreement with the conclusions of Lee *et al.*[9]. No significant difference between *BI* values measured on two columns, both coated with SE-52 stationary phase but with film thicknesses of 0.15 and 0.25 μm were observed. The average confidence limit is 0.2 index unit. Furthermore, *BI* values are more reproducible than *I* values. Nevertheless, a correspondence between *I* and *BI* has been established, permitting the use of *I* values for NPAHs measured by other workers[18,20]. As shown in Fig. 3, the three straight-line sections are nearly coincident.

A quasi-linear relationship was found between the boiling points of azaarenes and their *BI* values measured on SE-52 (*cf.*, Fig. 4 and Table I). This information can be valuable as a preliminary determination of the type of nitrogen compounds that can be expected in a distillate fraction.

The prediction of the retention sequence of azaarenes, and in particular of

TABLE I

VALUES OF BASE INDICES OF REFERENCE AZAARENES, MEASURED ON SE-52 STATIONARY PHASE

The temperature was programmed from 50 to 280°C at 2°C/min.

No.	*Compound*	*BI*	*Standard deviation*	*No. of determinations*	*Boiling point* (°C)*
1	Quinoline	200.00	–	–	237.1
2	Isoquinoline	203.47	0.37	5	243.2
3	2-Methylquinoline	212.56	0.15	6	246.5
4	2,6-Dimethylquinoline	233.00	0.13	3	266
5	2-Benzylpyridine	243.13	0.19	4	276 742 mmHg
6	5H-Indeno[1,2-*b*]pyridine	272.81	0.39	3	314
7	Benzo[*h*]quinoline	298.50	0.11	4	338 719 mmHg
8	Acridine	300.00	–	–	345
9	Phenanthridine	303.55	0.30	4	360
10	Benzo[*f*]quinoline	304.40	0.17	4	350 721 mmHg
11	2-Methylbenzo[*h*]quinoline	312.08	0.27	5	324
12	9-Methylacridine	332.33	0.05	2	
13	2,4-Dimethylbenzo[*h*]quinoline	334.30	0.14	6	
14	2,3-Dimethylbenzo[*h*]quinoline	335.48	0.17	8	
15	1,3-Dimethylbenzo[*f*]quinoline	341.00	0.35	5	
16	Indeno[1,2,3-*ij*]isoquinoline	345.82	0.15	4	394
17	Acenaphtho[1,2-*b*]pyridine	348.70	0.03	2	396
18	2,4,6-Trimethylbenzo[*h*]quinoline	356.10	0.11	5	
19	7,6-Ethylenebenzo[*h*]quinoline	356.94	0.28	7	
20	Benzo[*l*,*m*,*n*]phenanthridine	357.40	0.44	3	407
21	2-Tolyl-3-methylquinoline	362.10	0.21	3	
22	2,3,4-Trimethylbenzo[*h*]quinoline	363.34	0.32	5	
23	11H-Indeno[1,2-*b*]quinoline	372.19	0.11	4	410
24	Benz[*c*]acridine	393.75	0.09	4	434
25	Benz[*a*]acridine	400.00	–	–	438
26	Naphtho[2,1-*f*]quinoline	409.50	0.20	5	
27	8-Methylbenz[*a*]acridine	411.54	0.08	9	
28	10-Methylbenz[*a*]acridine	420.94	0.04	2	
29	8-Ethylbenz[*a*]acridine	423.09	0.11	3	
30	12-Methylbenz[*a*]acridine	427.39	0.36	2	
31	8,9,10-Trimethylbenz[*a*]acridine	431.14	0.08	4	
32	Indeno[1,2-*de*]benzo[*h*]quinoline	448.19	0.16	3	
33	7-Methylindeno[7,1-*bc*]acridine	481.81	0.37	7	
34	Dibenz[*a*,*j*]acridine	500.00	–	–	

* Values from refs. 18 and 19.

isomeric compounds, is still very difficult, even on apolar stationary phases. The prediction of the location of methyl substituents in petroleum azaarene molecules, once the ring system and the location of the nitrogen atom are known, would be a considerable help for the elucidation of these structures. For instance, alkylated benzo[*h*]quinolines have been identified as major triaromatic NPAHs in crude oils[4,5], but the total determinations of individual structures could be achieved only after having synthesized authentic compounds. Because of the considerable number of theoretically possible isomers of alkylated azaarenes, some indications about the location of substituents are very helpful as an orientation for the preparation of

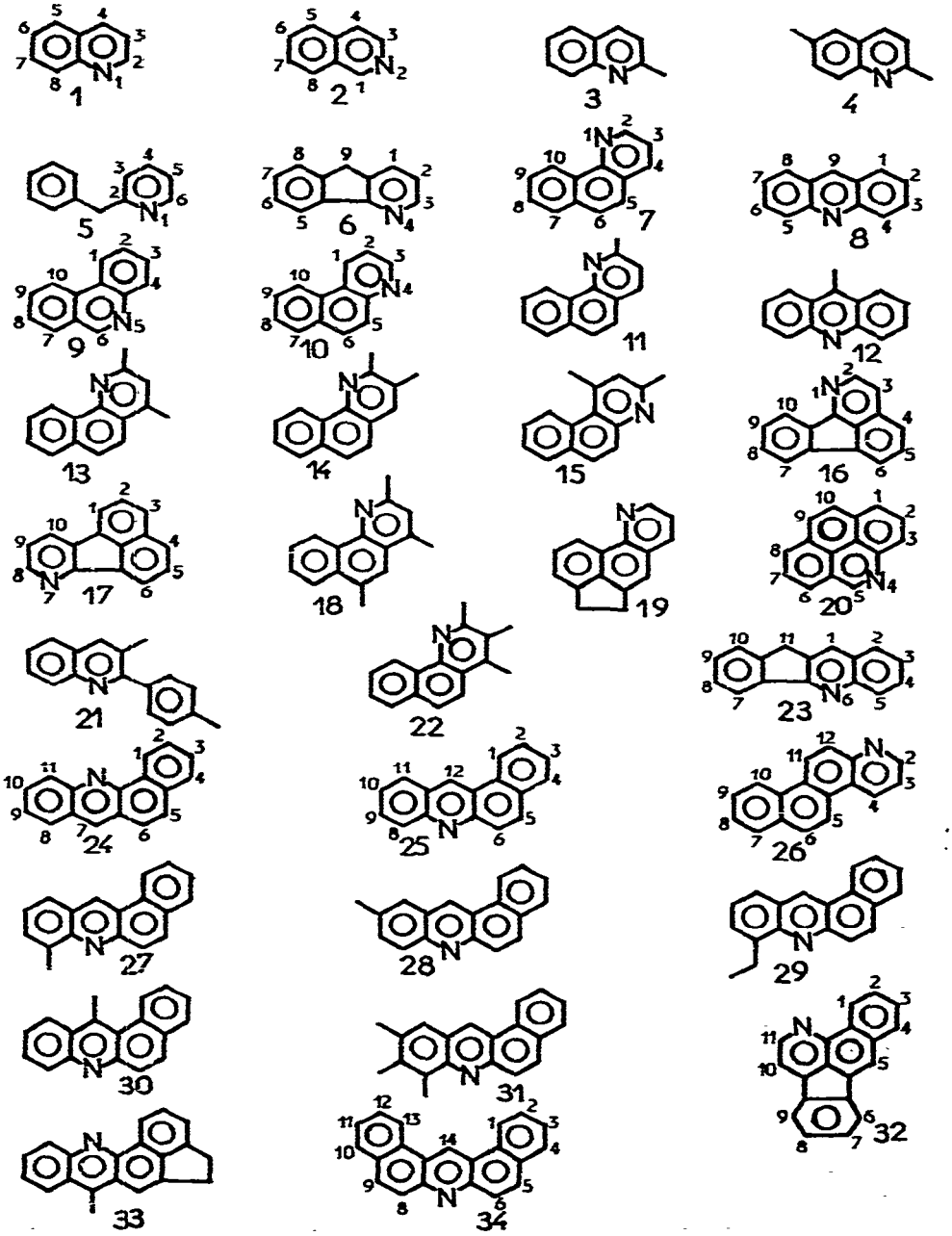

Fig. 2. Structures and numbering systems of reference azaarenes (*cf.*, Table I).

reference molecules. Elution sequences observed for neutral PAHs, a well documented series for which numerous standards are available[9,21,22], may provide such indications.

Independently of the hydrocarbon skeleton of azaarenes, the introduction of a

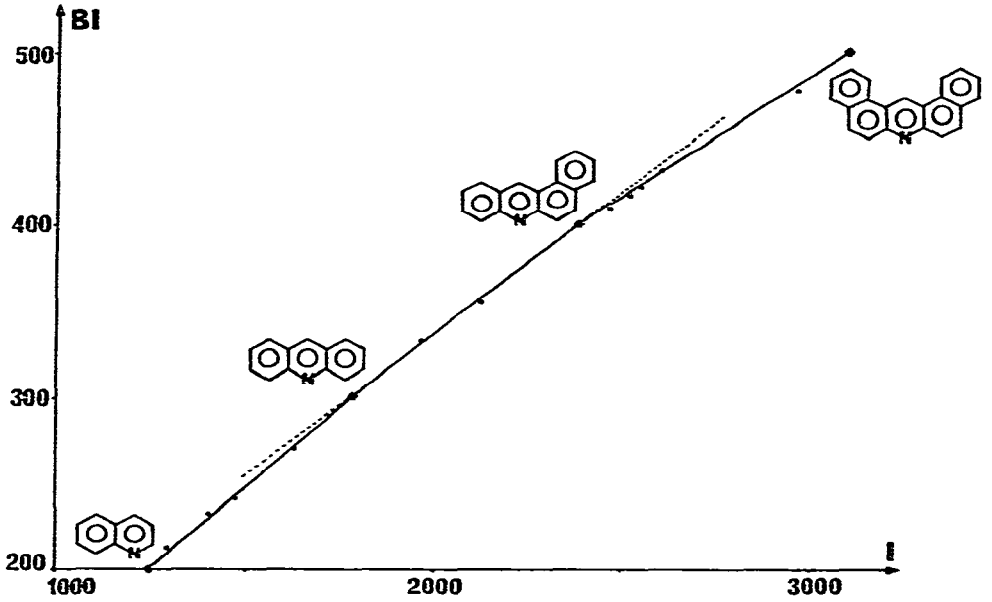

Fig. 3. Correlation between Kováts retention indices (*I*) and base indices (*BI*) of reference NPAHs measured on SE-52 under temperature programming conditions.

methyl substituent in the α-position to the nitrogen atom produces a *BI* increment of about 13 units on SE-52 (about 80 *I* units), which is smaller than the increment observed for a methyl group in any other location (about 22 *BI* units or 120 *I* units, a value comparable to the CH_3 increments observed with neutral PAHs on the same stationary phase[9]). This effect, which is related to the steric hindrance of the nitrogen atom, plays an important role not only on polar but also on apolar stationary phases, as can be seen for various series of comparisons between PAH and NPAH elution sequences on SE-52 (Fig. 5).

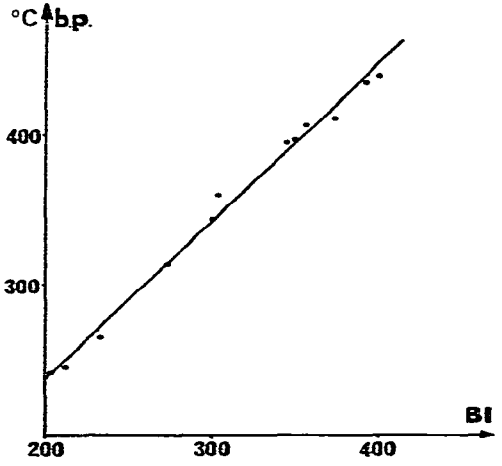

Fig. 4. Correlation between *BI* measured on SE-52 and boiling points (b.p.) of reference azaarenes (*cf.*, Table I).

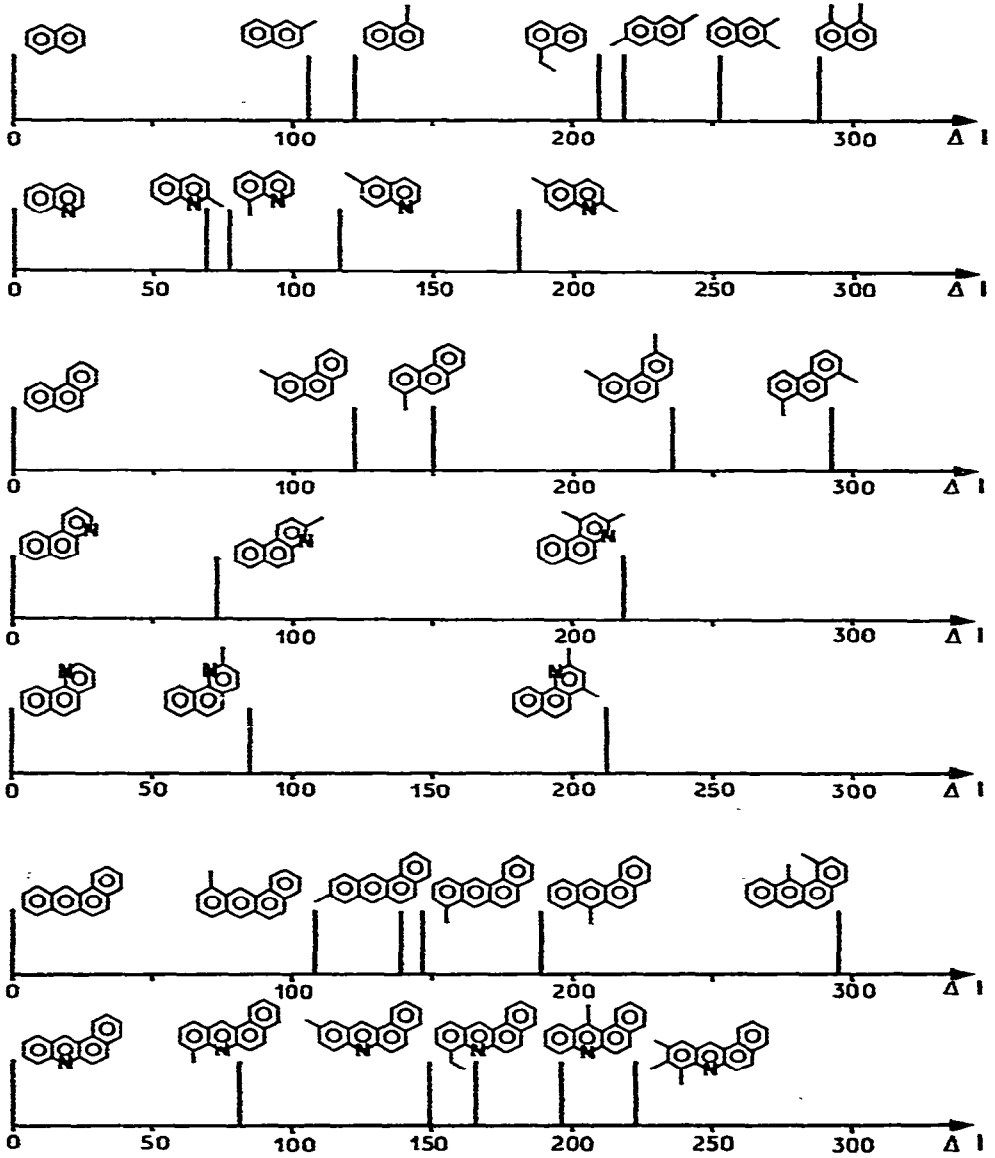

Fig. 5. Comparison of the retention behaviours of alkyl-substituted PAHs and NPAHs on SE-52. *ΔI* are increments in the Kovàts retention indices observed relative to the unsubstituted compound of each series.

Apart from the special case of α-substituted NPAHs, comparison between the *BI* values of PAHs and NPAHs does not lead to valuable predictions for most available reference azaarenes. NPAH retention indices estimated on the basis of the corresponding values for PAHs are sometimes found to be in close agreement with experimental values (*e.g.*, less than 5 *BI* units), but even this is clearly insufficient if one considers the complexity of petroleum NPAH mixtures. Sophisticated approaches involving topological descriptors[23,24], which might be more helpful, are plagued

by the major difficulty of the lack of availability of authentic alkyl-substituted azaarene samples.

A representation on a triangular diagram of the retention indices measured for eleven solutes was used for a more complete evaluation of the preliminarily selected stationary phases. Base retention index values on three stationary phases, x, y and z, are converted into retention index fractions, F_I, according to the following definitions[25]:

$$F_{I_x} = \frac{I_x}{I_x + I_y + I_z}$$

and

$$F_{I_x} + F_{I_y} + F_{I_z} = 1$$

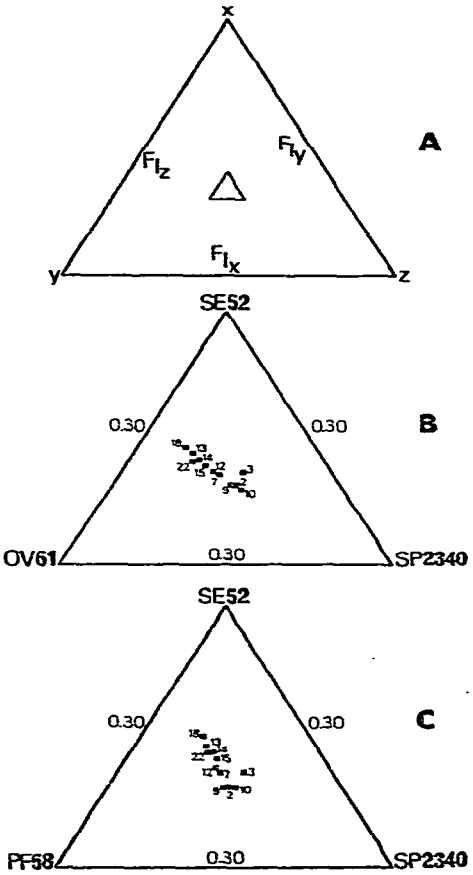

Fig. 6. Representation of base indices of reference NPAHs on a triangular diagram. The retention fractions corresponding to the apex of triangles B and C (small triangle shown in A) are all 0.4. For structures, see Table I and Fig. 2.

Thus, three F_I values define a point on a triangular diagram, F_I being equal to zero for each side and equal to 1 for each apex of the triangle (*cf.*, Fig. 6A).

The two plots of F_I values, which are triangles extracted from the central part of the diagram, obtained with the combinations SE-52–OV-61–SP-2340 (Fig. 6B) and SE-52–Pluronic F68–SP-2340 (Fig. 6C) are very similar. All values of F_I are between 0.318 and 0.350, which is very close to the centre of the triangle, confirming that minor selectivity changes only can be achieved with these stationary phases. Therefore, the final selection of stationary phases will be strongly influenced by practical considerations such as sample complexity and range of molecular weight of azaarenes occurring in crude oils.

Separation of azaarenes from crude oils

After a prior RPLC clean-up procedure[4], total basic fractions extracted from crude oils can be analysed on apolar columns (*cf.*, Fig. 7). The analysis is more easily performed, however, on narrower cuts delineated by the number of carbon atoms in azaarene molecules, that is, with fractions obtained after a micropreparative fractionation by RPLC, using a water-methanol mixture as the eluent[4,14]. Most of the stationary phases that have been evaluated with reference compounds in this study have also been applied to the separation of such fractions (*cf.*, Fig. 8).

Because practically the only types of di- and tricyclic aromatic NPAHs occurring in crude oils are alkyl-substituted quinolines and benzo[*h*]quinolines, respectively[4,5], the greatest difficulty in the capillary GC of petroleum azaarenes with sizes not larger than tricyclic aromatics resides in the separation of isomers resulting from various locations and types of alkyl substituents. The chromatograms shown in Fig. 8, obtained with apolar and moderately polar stationary phases, all of which provide a separation controlled by the number of carbon atoms in the solute molecules,

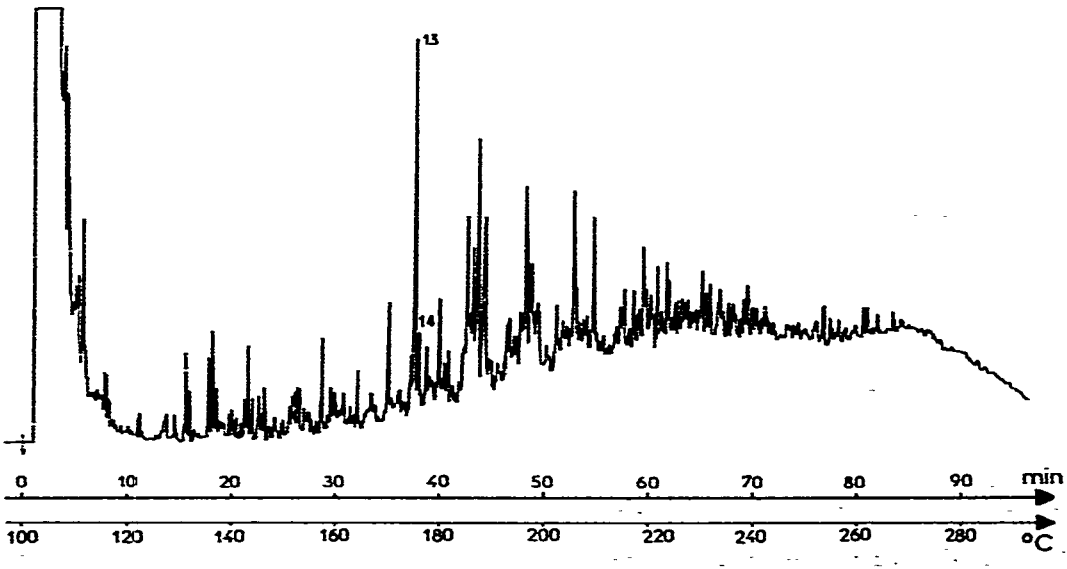

Fig. 7. Gas chromatogram of a total base fraction extracted from an Iranian crude oil. Capillary column 35 m × 0.3 mm I.D., coated with OV-73, film thickness 0.15 μm. Peak numbering as in Fig. 1 and Table I.

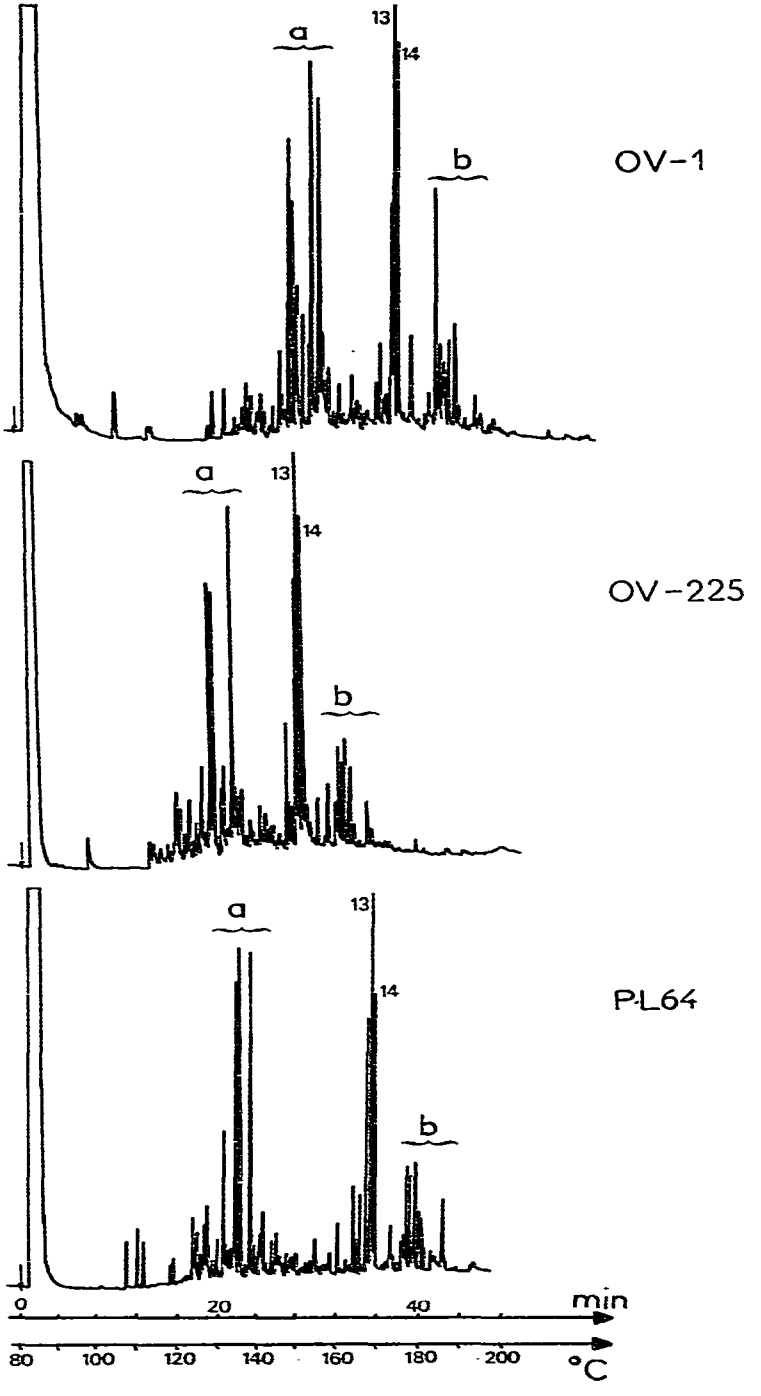

Fig. 8. Comparison of retention patterns of petroleum azaarenes on three stationary phases. The analysed fraction, extracted from a crude oil from the Congo, was obtained by micropreparative RPLC and contained mainly C_4- and C_5-alkylquinolines (group a), dimethylbenzo[*h*]quinolines (peaks 13 and 14) and C_3-alkylbenzo[*h*]quinolines (group b). All columns were 25 m × 0.3 mm I.D. and the film thickness was between 0.15 and 0.20 μm.

illustrate this situation. Only small changes in the overall separation pattern are observed, whereas some differences are found within groups of isomers such as a and b. Therefore, the most suitable column for the analysis of such samples should be the most easily prepared one, which in our opinion is a column coated with OV-73 or a similar silicone. Other stationary phases are then mostly useful for identification purposes by means of co-injections with reference compounds.

Use of the very polar cyano-silicone SP-2340 results in many interferences

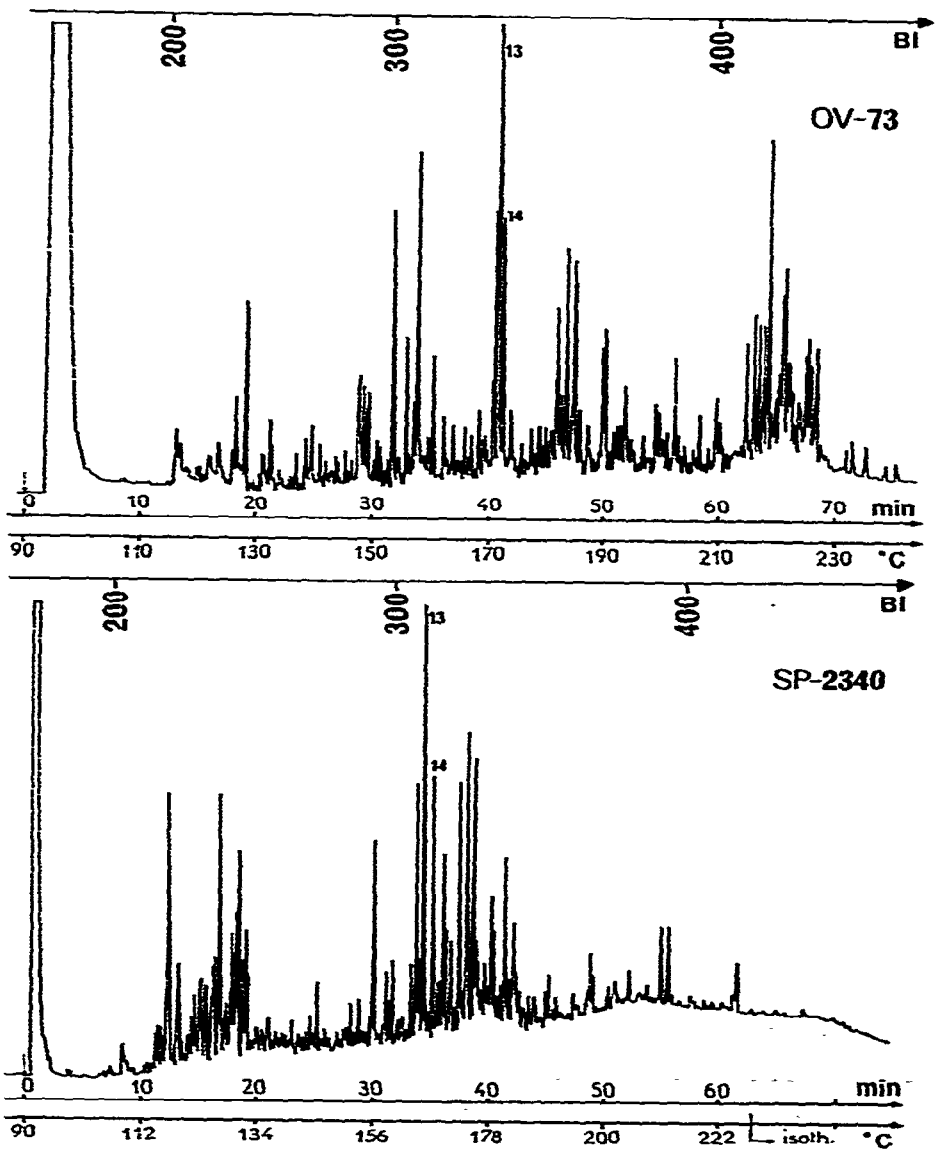

Fig. 9. Effect of alkyl substitution on the separation of petroleum azaarenes (crude oil from the Congo) on apolar OV-73 (column: 55 m × 0.3 mm I.D., film thickness 0.18 μm) and very polar SP-2340 (40 m × 0.3 mm I.D., film thickness 0.15 μm).

between azaarenes having different numbers of rings, an effect of particular importance with petroleum NPAHs. A comparison of the separations achieved with a broad fraction of di- and tricyclic aromatic bases on OV-73 and SP-2340 illustrates this feature (Fig. 9). Thus, SP-2340, which we found to be most selective in terms of location of the nitrogen atom, is not very helpful for the investigation of crude oil samples from different origins. On the other hand, it is completely satisfactory for the analysis of NPAHs in environmental samples, where numerous types of azaarenes are found with a low degree of alkyl substitution[26,27].

An ideal stationary phase for azaarene separation should provide the best selectivity for isomers differing in the location of the nitrogen atom and at the same time produce minimum interferences between alkylated benzologues. These two requirements are well fullfilled by OV-61, as illustrated by the separation of the base fraction extracted from a coking gas oil (Fig. 10). This sample, the detailed investigation of which will be reported in a forthcoming paper, shows the occurrence of a wide range of alkylquinolines together with several ring isomers of benzoquinolines and with alkylated aromatic amines. Interferences between the various series of compounds are minimized with OV-61, which leads us to prefer it to Pluronic F68, a choice which is also influenced by the higher thermal stability of OV-61.

A detailed investigation of the structures of tetracyclic aromatic NPAHs will

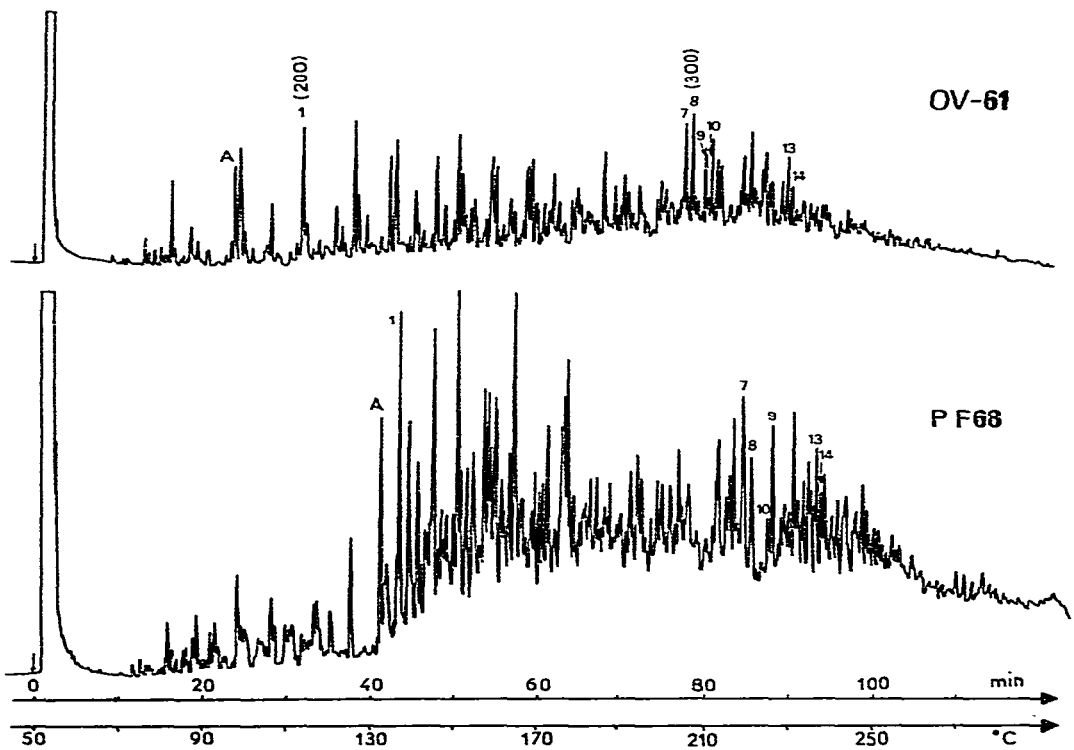

Fig. 10. Separation of a total nitrogen base fraction extracted from a coking gas oil. OV-61 column: 41 m × 0.3 mm I.D., film thickness 0.21 μm. Pluronic F68 column: 40 m × 0.3 mm I.D., film thickness 0.15 μm. Peak numbering as in Fig. 1 and Table I. A: 2,6-Dimethylaniline.

be conducted in the near future, using a combination of OV-73, OV-61 and SP-2340 for the identification of individual compounds.

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