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# SEPARATION OF AROMATIC COMPOUNDS BY LIQUID CHROMATOGRAPHY ON DIOL-BONDED PHASE COLUMNS

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## SUMMARY

Retention data of aromatic, polyaromatic, aza-aromatic and phenolic compounds have been measured on diol-bonded silica adsorbents from either laboratory or commercial preparation. Agreement between the data obtained with the two types of phases illustrates the ease of the synthesis of diol-bonded silica. This type of packing material provides a distinct separation of polynuclear aromatic hydrocarbons based on the number of condensed rings. Retention data are compared to similar data measured on amino-bonded silicas. The behavior of the two adsorbents towards polynuclear aromatic hydrocarbons is very similar but the diol phase is less retentive. The importance of substituents effect is determined by varying the degree of substitution on the basic ring structure. Retention of aza-aromatics is independent of the basicity but depends much on the environment of the nitrogen atom. Correlation between retention and the Hammett constants in the case of phenols is compatible with a donor–acceptor retention mechanism.

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## INTRODUCTION

Reversed-phase liquid chromatography (RPLC) using alkyl-bonded porous silica particles as stationary phase has become the most popular mode of chromatography during the last few years. The major advantages of this method are well known: the use of water–organic solvent mixtures as mobile phases with easily adjustable polarity, unique selectivity and weak energies of interaction between the solute and the non-polar stationary phase. Conversely, normal- (or straight) phase liquid chromatography (NPLC) offers some interesting features which often tend to be forgotten, such as the ability to afford class separations, the use of organic solvents which are less corrosive, do not dissolve silica and require markedly lower inlet pressures to achieve the proper mobile phase velocity. Moreover, the retention mechanism is much better understood in NPLC than in RPLC[1].

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For many years separations in NPLC were performed using metal oxide adsorbents (silica, alumina). These solids are very sensitive to the water content of the mobile phase and their retention properties can be affected strongly and sometimes permanently by minor changes in the concentration of the water sorbed on the solid surface. NPLC can be carried out, however, using moderately polar packing materials obtained by chemical bonding on the surface of the silica of functional alkyl silanes. The preparation of various types of such adsorbents, using different groups, *e.g.* $NH_2$, $R(NH_2)_2$, CN, ROR, diol, 2,4-dinitroanilinopropyl, 3-N-pyrrolidonepropyl, perfluoroalkyl, has been described[2-9]. They offer a wide range of selectivity and may be used advantageously. The amino phase is the most popular stationary phase of this group.

The amino-bonded phase is mainly used in two areas: the analysis of sugars[10], with a water–organic solvent mobile phase and the class separation of polyaromatic hydrocarbons, with the so-called "energy analysis column"[11]. The first system belongs more likely to RPLC and the second one to NPLC. Hammer *et al.* state that the $NH_2$ phase behaves as a partially silanized silica[12]. Snyder and Schunk have shown that the displacement model accounts well for the retention mechanism[13] and Snyder has extended this model to a wide range of adsorbents[14].

While there are many retention data on silica and on amino-bonded silica, they are rather scarce on diol-bonded silica. This packing is predominantly used in size-exclusion chromatography with particular reference to protein separations[15-17]. There have been few reports on its use as a stationary phase for NPLC, in the separation of steroids[18-22], emulsifiers[23], flavones[24], phenolic esters[25], herbicides[26] and hydrocarbon metabolites[27].

As for most chemically bonded silica materials, two types of diol-bonded silicas are available[16], the polymeric and the monomeric phases. The latter type is available from manufacturers and is said to be weakly polar, the general opinion being that the polarity increases in the order: diol < cyano < amino < diamino.

The purpose of this paper is to investigate the potentiality of diol packings for the separation of aromatic compounds and to compare their performance and retention patterns with those of other NPLC stationary phases.

## EXPERIMENTAL

### *Equipment*

The measurements have been performed with a Merck LMC 31 liquid chromatograph (Merck, Nogent sur Marne, France), equipped with a three-head piston pump, a Rheodyne 7125 injection port and a fixed-wavelength UV detector having a 8-$\mu$l cell volume. A programming keyboard permits the selection of the desired analytical procedure.

### *Materials*

Diol-bonded silica of the monomeric type is prepared as follows. An amount of 10 g of silica is dried for 48 h in an oven and put in a reaction flask with 100 ml dry toluene, 3 ml $\gamma$-glycidoxypropyl trimethoxy silane (Silar Lab., Scotia, NY, U.S.A.) and 0.1 ml pyridine. The reaction mixture is stirred and refluxed for 8 h under anhydrous conditions. The epoxy silica thus obtained is washed with toluene.

tetrahydrofuran and acetone. Hydrolysis is then carried out with 200 ml of aqueous nitric acid (pH 2.0). After thorough rinsing and mixing with water, the diol-bonded silica is dried overnight at 105°C.

### *Columns*

Two types of columns were used. Stainless-steel Hibar columns (250 × 4 mm) were packed with diol-bonded 5-$\mu$m LiChrosorb particles (irregular shaped), prepared according to the procedure described above, or with diol-bonded 10-$\mu$m LiChrospher particles (spherical shaped) obtained from Merck (Darmstadt, F.R.G.). Glass cartridges (150 × 3 mm) were packed with diol-bonded 5-$\mu$m LiChrosorb or with amino-bonded 5-$\mu$m LiChrosorb, both available from Merck. According to information given by the manufacturer, the average surface coverage of the bonded material is 2 $\mu$mol/m$^2$.

### *Chemicals*

HPLC-grade solvents of the LiChrosorb type (Merck) were used. Standard polyaromatic hydrocarbons were a gift from the Laboratoire de Chimie Macromoleculaire (Marseille Saint-Jerome, France). Authentic samples of aza-arenes were kindly given by J. M. Schmitter (Ecole Polytechnique, Palaiseau, France).

The determination of $t_0$ was carried out using *n*-pentane as an inert solute.

## RESULTS AND DISCUSSION

### *Column performance*

The performance characteristics of the two columns are described in Table I, where are reported the minimum value of the reduced plate height, the corresponding reduced velocity and the coefficients of the Knox equation. The LiChrospher column was found to be exceptionally well packed.

In contradiction to popular belief, we observed that the specific permeability of the column packed with spherical particles was markedly smaller than that of the column packed with irregular shaped particles. Data from the literature on this com-

TABLE I

CHARACTERISTICS AND PERFORMANCE OF DIOL COLUMNS

Solute: benzene; solvent: isooctane. $d_p$ = particle size; $h_{min}$ = minimum value of the reduced plate height; $\nu_{opt}$ = optimum reduced mobile phase velocity; $K$ = specific permeability of the column; $A$ and $C$ are coefficients in the Knox equation $\left(h = \frac{B}{\nu} + A\nu^{1/3} + C\nu\right)$; $R^2$ (Knox) and $R^2$ (Van Deemter) indicate the fit of the plate height *vs.* mobile phase velocity data to the Knox equation and Van Deemter equation, respectively. 1: column packed in the laboratory with home-made diol bonded silica. 2: column obtained from Merck.

| *Column* | *Characteristics* | | | | | *Performance* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Packing* | $d_p$ *(μm)* | $h_{min}$ | $\nu_{opt.}$ | $K \times 1000$ | $A$ | $C$ | $R^2$ *(Knox)* | $R^2$ *(Van Deemter)* |
| 1 | LiChrosorb | 5 | 5.35 | 4.8 | 1.34 | 2.76 | 0.77 | 0.968 | 0.971 |
| 2 | LiChrospher | 10 | 2.33 | 2.47 | 0.66 | 0.55 | 0.11 | 0.984 | 0.989 |

parison are inconsistent. It has been reported that the permeabilities of good columns, well packed with spherical and with irregular shape particles are either very different[28] or not very different[29]. This uncertainty is probably a consequence of the difficulty in defining and measuring correctly a particle size for irregular shaped particles.

These results could be explained easily if the average particle size of the LiChrospher was smaller than the value stated by the manufacturer for the particular batch we used. We did not measured the average particle size ourselves.

Finally, the optimum flow-rate was rather small, and the required inlet pressure was moderate.

### *Retention of benzene derivatives*

Table II lists the capacity factors ($k'$) of some benzene derivatives, together with those of a few alkylnaphthalenes.

With the classical polar adsorbents (silica, alumina), the addition of alkyl groups to an aromatic ring increases the retention[2] and Popl *et al.* have published retention indices illustrating this trend[30]. On the other hand, Martin *et al.*[31] and Chumakov and Lopatenko[32] observed that toluene is less retained than benzene. Also when the molecules are planar, branched alkyl substituents result in a lesser adsorption energy than linear ones and bulky groups (more than *ca.* 10 carbon atoms) also decrease the retention. The number and position of the alkyl substituents have

TABLE II

RETENTION OF BENZENE DERIVATIVES AND ALKYLNAPHTHALENES

Column, LiChrosorb Diol, solvent, isooctane.

| *Compound* | $k'$ |
|---|---|
| Benzene | 0.20 |
| Toluene | 0.20 |
| *m*-Xylene | 0.20 |
| *p*-Xylene | 0.20 |
| Durene | 0.20 |
| Butylbenzene | 0.16 |
| Octadecylbenzene | 0.08 |
| Styrene | 0.24 |
| Acetophenone | 1.40 |
| Chlorobenzene | 0.24 |
| Nitrobenzene | 0.94 |
| *p*-Dibromobenzene | 0.40 |
| *p*-Dicyanobenzene | 0.24 |
| *p*-Dimethoxybenzene | 0.70 |
| Naphthalene | 0.46 |
| 1-Methylnaphthalene | 0.42 |
| 2-Methylnaphthalene | 0.42 |
| 2-Ethylnaphthalene | 0.39 |
| 2,6-Dimethylnaphthalene | 0.39 |
| 1,5-Dimethylnaphthalene | 0.39 |
| 2,4-Dibutylnaphthalene | 0.20 |

a larger effect on the retention on all these NPLC sorbents than the size of these groups.

On the diol phases, benzene, toluene, xylenes and durene were eluted together. The retention of monoalkyl benzenes decreased slowly with increasing length of the alkyl chain. This effect may be ascribed in part to the electron donor effect of the alkyl chain on the aromatic ring. The substitution of an electron-attracting atom or group, such as chlorine or the nitro group, has the opposite effect of increasing the retention (*cf.* Table II).

This relationship between the electron density on the aromatic ring and the retention can be expressed in terms of Hammett constants, as will be discussed later.

The significant decrease in retention of aromatic and polyaromatic molecules with increasing size of the alkyl substituent(s) has important consequences for the ability of the corresponding chromatographic system to perform class separation. For example, dibutylnaphthalene elutes very closely to benzene and interfers with it. It is most probable that diphenylnaphthalene, along with many other polyalkylnaphthalenes with more than 10 alkyl carbon atoms will be eluted before benzene and thus will interfere with monoaromatic hydrocarbons. More complex alkylaromatics were not available to us, however, for further exploration of this problem.

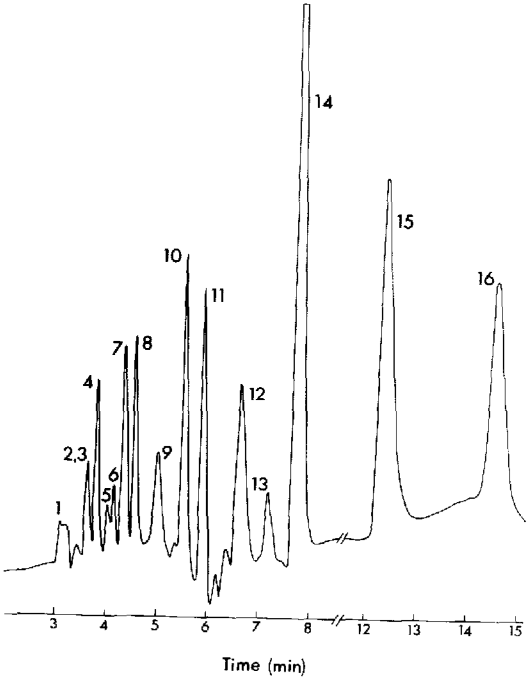

Fig. 1. Analysis of a mixture of authentic compounds on the diol column prepared in the laboratory (see Table I). Mobile phase, isooctane, 1 ml/min. Compounds: 1 = octadecyl benzene; 2 = toluene; 3 = benzene; 4 = chlorobenzene; 5 = 1-methylnaphthalene; 6 = naphthalene; 7 = *ortho*-chloro (chloromethyl) benzene; 8 = *para*-chloro (chloromethyl) benzene; 9 = anthracene; 10 = pyrene; 11 = nitrobenzene; 12 = acetophenone; 13 = *para*-methylacetophenone; 14 = benzopyrene; 15 = dibenzopyrene; 16 = *para*-methoxyacetophenone.

It has been observed that dimethylnaphthalenes are more retained than the methylnaphthalenes on bare silica[33]. This does not occur with the diol phases. Methyl substitution of naphthalene decreases the retention (*cf.* Table II) and the two dimethylnaphthalenes tested were not resolved from ethylnaphthalene.

The chromatogram in Fig. 1 shows the separation of a mixture of authentic compounds on the LiChrosorb column bonded and packed by us.

### *Retention of polynuclear aromatic hydrocarbons (PAHs)*

In Table III are given the retention data of model aromatic hydrocarbons, using either *n*-heptane or isooctane as eluent.

We observed first a marked difference between the two series of data, retention with *n*-heptane being systematically smaller than with isooctane. Both solvents were checked for purity and water content (Karl Fisher titration). The water content was respectively 50 ppm in *n*-heptane and 70 ppm in isooctane, which is not much below saturation.

It is difficult to explain the difference in retention observed with these two solvents of nearly identical elution strength. The larger retention obtained with isooctane results, however, in a better resolution between the early eluting compounds, so isooctane has been used in most cases.

Within 10% the retention data measured on the two LiChrosorb samples,

TABLE III

RETENTION OF POLYNUCLEAR AROMATIC HYDROCARBONS

(1) LiChrospher 100 Diol from Merck; (2) LiChrosorb 100 Diol from laboratory; (3) Diol cartridge, LiChrosorb 100 from Merck; (4) Diol cartridge, LiChrosorb 100 from Merck. Eluent: 1, 2, 3, isooctane; 4, *n*-heptane.

| *Compound* | *k'* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| 1 Benzene | 0.20 | 0.15 | 0.14 | 0.09 |
| 2 Naphthalene | 0.46 | 0.35 | 0.36 | 0.24 |
| 3 Fluorene | 0.68 | 0.49 | 0.51 | 0.33 |
| 4 Anthracene | 0.99 | | | |
| 5 Phenanthrene | 1.00 | 0.67 | 0.74 | 0.47 |
| 6 Pyrene | 1.44 | 0.91 | 1.01 | 0.64 |
| 7 Chrysene | 2.20 | 1.24 | | |
| 8 Perylene | 2.81 | 1.56 | | |
| 9 Fluoranthene | 1.52 | 0.95 | 1.02 | 0.67 |
| 10 Benzo[*a*]pyrene | 3.72 | 1.90 | 2.14 | 1.22 |
| 11 Dibenzopyrene | 5.49 | 2.84 | 3.18 | |
| 12 Coronene | 5.52 | | | |
| 13 Benz[*a*]anthracene | 2.08 | 1.16 | 1.28 | |
| 14 Benzo[*k*]fluoranthene | 2.62 | | 1.58 | |
| 15 Benzoperylene | 4.90 | 2.50 | | |
| 16 Pentacene | 4.56 | | 2.42 | 1.36 |
| 17 Triphenylmethane | 2.95 | | | |
| 18 Biphenyle | 0.46 | | 0.36 | 0.24 |
| 19 *p*-Terphenyle | 1.09 | | 0.94 | |

prepared either by Merck or by us, are identical. This shows that the method of preparation is not very critical. On the other hand, the retention on LiChrospher is 1.5–2 times larger.

Snyder and Poppe[1] have shown that, in agreement with the rule of additivity of free energy contributions, the adsorption energy and therefore the logarithm of the column capacity factor of catacondensed PAHs increase linearly with increasing number of aromatic carbon atoms in the molecule. A plot of log $k'$ *vs.* the carbon atom number is linear. Popl *et al.*[30] have suggested to define retention indices in a way similar to those used in gas chromatography. He assigned reference indices of 10 to benzene, 100 to naphthalene, 1000 to phenanthrene, 10000 to benz[*a*]-anthracene and 100000 to benzo[*b*]chrysene, so log $I$ rather than $I$ increases linearly with the ring number. The advantage of this reference system for retention data is supposed to be a better insight into the effects of substitution and pericondensation of phenyl rings. As pointed out by Karlesky *et al.*[34], substitution of phenanthrene by anthracene and of benzo[*b*]chrysene by 1,2,3,4-dibenzanthracene does not change significantly the results of the comparison.

In the determination of the retention index of a compound, only interpolation of data between the reference hydrocarbons eluted just before and just after it is allowed since a marked deviation from linear behavior may occur. However, the plot of log $k'$ *versus* the number of carbon atoms of the reference compounds has been found to be linear with many sorbents, such as alumina[30], silica[33], chemically bonded silica with non-polar or polar groups, *e.g.* $NH_2$, CN, $NO_2$ groups[2,6]. This linearity can be explained by the linear variation of the molecular (Van der Waals) volumes: benzene (48.36), naphthalene (73.96), anthracene and phenanthrene (99.56), chrysene (125.16). A similar result was obtained with the diol phase as exemplified in Fig. 2 with the results for LiChrospher 100 Diol (see Table III).

We observed that the diol phase exhibited the same behavior and a correlation

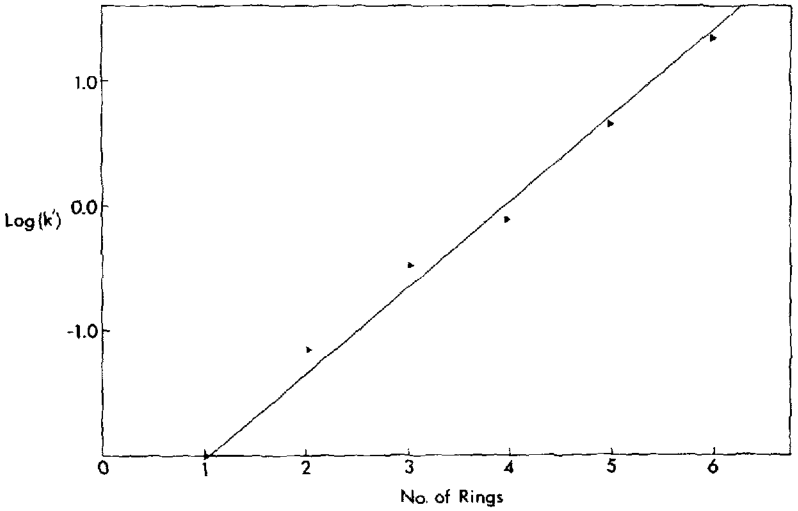

Fig. 2. Plot of the logarithm of the column capacity factor *versus* the number of aromatic rings for PAHs Column, LiChrospher 100 Diol (Merck, see Table I); mobile phase, isooctane.

coefficient of 0.964 testifies to an excellent fit. As pointed out by Karlesky *et al.*[34], substitution of benz[*a*]anthracene by chrysene does not change the regression equation: $\log I = 2.94 \log k' - 3.03$.

Naphthalene, biphenyl and acenaphthalene were eluted together, well before fluorene, which is usually considered as a three-ring aromatic hydrocarbon. With the diol phase, fluorene and fluoranthrene eluted with indices of 400 and 4000, respectively, *i.e.* with the same retention times as hypothetical PAHs having 2.6 and 3.6 rings, respectively. Anthracene and phenanthrene were poorly resolved and the pericondensed pyrene was the first four-ring PAH to be eluted, long after the last three-ring PAH. Although scatter increased with increasing ring number, there was no overlap and class separation remained possible, at least as long as there was no alkyl substitution. Preparative separations have been carried out at the 0.5-g scale, using a Lobar type column packed with LiChrosorb Diol, 10 μm.

Donor–acceptor interactions take place between the diol moieties and the aromatic molecules. A good correlation was observed between $\log k'$ and the number of double bonds in these molecules, but structural factors play a role which, however minor, must be taken into account. Thus, raw correlation of the *n*-conjugation *versus* $\log k'$ with all the data points yielded a correlation coefficient of 0.887, whereas within each of the peri-, cata- and non-condensed families the correlation coefficients were 0.984, 0.989 and 0.982, respectively. The planarity of the molecule seems to have a lesser influence as the diol moiety is flexible and adsorption is delocalized (admitedly the interaction between a PAH molecule and the diol group belongs as much to partition as to adsorption). A further attempt at checking correlations between the retention volumes of PAH and their electronic properties was performed and we found that the best one is obtained with:

$$E^{R}_{HMO} = E_{HMO} - \beta_z \qquad (1)$$

$E^{R}_{HMO}$ is the resonance energy and $E_{HMO}$ is the $\pi$ electron energy calculated by means as the Hückel approximation, and where $\beta_z$ is the resonance energy integral[9].

Since the concentration of groups chemically bonded to the surface is estimated to be around 2 μmole/m$^2$, the contribution of residual silanols to the retention cannot be completely neglected, however.

The chromatogram in Fig. 1 shows the separation of a number of polynuclear hydrocarbons with 2 to 6 rings.

### *Comparison with amino-bonded silica*

A large number of silica-based packing materials have been used for the analysis of PAH mixtures. A survey of the literature shows that similar adsorbents exhibit different retention volumes for the same compounds[2,6,11,12]. The origin of the silica, the bonding procedure used and the different ways to determine $t_0$ are responsible for these differences. For the sake of comparison, we have used first cartridges with $NH_2$ and diol packings obtained from the same manufacturer and packed in the same manner. To achieve a better efficiency, two such columns were coupled in series and four combinations were used: $NH_2$–$NH_2$, $NH_2$–diol, diol–$NH_2$ and diol–diol. Pentane was used as the inert solute for the determination of $V_0$.

The data in Table IV clearly demonstrate that the diol column retained PAHs

TABLE IV

COMPARISON BETWEEN THE RETENTION OF AROMATIC HYDROCARBONS ON THE AMINO AND THE DIOL COLUMNS

(1) Series of two amino silica cartridges, isooctane; (2) series of one amino silica and one diol cartridge, isooctane; (3) series of two diol silica cartridges, isooctane; (4) column of amino-bonded silica, *n*-heptane. All columns were packed with LiChrosorb 5 μm.

| *Compound* | *k'* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| Octadecylbenzene | 0.16 | 0.11 | 0.09 | 0.06 |
| Butylbenzene | 0.19 | 0.15 | 0.13 | 0.09 |
| Durene | 0.21 | 0.18 | 0.14 | 0.09 |
| Xylene | 0.21 | 0.18 | 0.14 | 0.10 |
| Toluene | 0.21 | 0.18 | 0.14 | 0.11 |
| Benzene | 0.21 | 0.18 | 0.14 | 0.11 |
| 2,4-Dibutylbenzene | 0.28 | 0.20 | 0.16 | – |
| 2-Ethylnaphthalene | 0.45 | 0.38 | 0.30 | 0.26 |
| 2,6-Dimethylnaphthalene | 0.45 | 0.38 | 0.30 | 0.26 |
| 1,5-Dimethylnaphthalene | 0.45 | 0.38 | 0.30 | 0.26 |
| Acenaphthalene | 0.51 | 0.43 | 0.36 | 0.30 |
| Naphthalene | 0.51 | 0.43 | 0.36 | 0.30 |
| Biphenyle | 0.51 | 0.43 | 0.36 | 0.33 |
| Fluorene | 0.67 | 0.66 | 0.51 | 0.47 |
| Phenanthrene | 1.09 | 0.89 | 0.74 | 0.66 |
| 2-Methylnaphthalene | 1.09 | 0.89 | 0.74 | 0.64 |
| *p*-Terphenyle | 1.17 | – | 0.94 | 0.84 |
| Pyrene | 1.47 | 1.25 | 1.01 | 0.91 |
| Fluoranthene | 1.56 | 1.34 | 1.02 | 0.85 |
| Benzo[*a*]pyrene | 3.16 | 2.70 | 2.14 | 1.77 |
| Pentacene | 3.51 | 3.02 | 2.42 | 2.00 |

less than unbonded silica[31] and than amino-bonded silica, but behaved quite similarly to the $NH_2$ column for the class separation of PAHs.

If we consider the difference in retention indices between the substituted *i*-ring compound which had the lowest retention and the *i*-ring compound itself, we observed, however, that the diol phase is less attractive for ring separation than the $NH_2$ phase, which itself is less attractive than the fluoro phase described by Berendsen *et al.*[8] and used by Ecknig *et al.*[9].

Two series of measurements were made with the combination $NH_2$–diol, reversing the order of the two columns. As the data were identical within the limits of experimental errors, only one set of data is reported. It can be seen in Table IV that the *k'* values measured on the $NH_2$–diol combination are approximately the average between the values obtained for the $NH_2$–$NH_2$ and the diol–diol column series.

The data in Table IV show again a systematic difference in retentions observed on the column (eluted with *n*-heptane) and on the cartridge (eluted with isooctane) packed with the amino-bonded silica, the retention on the column being about twice less. This is the same phenomenon as reported above (see top of previous section).

### *Retention of aza-arenes*

The results discussed in the previous section show that the retention of PAHs and of benzene derivatives on the chemically bonded diol phase depends on the electronic structure of the molecule and on its ability to form hydrogen bonds. With aza-arenes, steric hindrance and specific properties of the nitrogen atom are also involved in the retention mechanism.

Column capacity factors measured with isooctane–ethanol (97.5:2.5, v/v) as mobile phase are given in Table V. Ethanol is used as a polar modifier.

Although the retention tended to decrease with increasing number of rings, at difference of what is observed with PAHs, it mainly depended again on the donor–acceptor interactions and on the ability to form hydrogen bonding. The retention of aza-arenes was independent of the basicity of the compound, *i.e.* of its $pK_a$. This is illustrated by the comparison between the retention order and the $pK_a$ of two series of compounds. The basicity increases in the order: indole ≈ carbazole < 2-chloroaniline < pyridine and 4-methyl-pyridine < 3-methyl pyridine < pyridine. The re-

TABLE V

CAPACITY FACTORS OF AZA-ARENES ON LICHROSORB DIOL

Eluent: isooctane–ethanol (97.5:2.5, v/v).

| *Compound* | $k'$ | $pK_a$ |
|---|---|---|
| 7,5-Dimethylbenz[*a*]acridine | 0.90 | |
| 8,10-Dimethylbenz[*a*]acridine | 0.90 | |
| 5-Methylbenz[*a*]acridine | 1.10 | |
| 8-Methylbenz[*a*]acridine | 1.10 | |
| 9-Methylbenz[*c*]acridine | 1.10 | |
| 7-Methylbenz[*c*]acridine | 1.10 | |
| 2,2-Biquinoline | 1.30 | |
| Benzo[*h*]quinoline | 1.70 | 4.25 |
| 7,9-Dimethylbenz[*a*]acridine | 2.10 | |
| 10,12-Dimethylbenz[*a*]acridine | 2.10 | |
| 12 Methylbenz[*a*]acridine | 2.70 | |
| 9 Methylbenz[*a*]acridine | 2.70 | |
| 2,4,6-Trimethylpyridine | 3.30 | 7.43 |
| 1-Azafluoranthene | 3.40 | |
| Acridine | 3.70 | 5.58 |
| Phenanthridine | 3.70 | 5.58 |
| Quinoline | 3.90 | 4.94 |
| 2-Ethyl-4-methylpyridine | 3.90 | 6.99 |
| 3-Picoline | 4.10 | 5.68 |
| 7-Azafluoranthene | 4.50 | |
| Benzo[*f*]quinoline | 5.00 | 5.15 |
| *m*-Chloroaniline | 5.00 | |
| Isoquinoline | 6.70 | 5.42 |
| Pyridine | 6.70 | 5.25 |
| 4-Picoline | 6.70 | 6.02 |
| 3-Chloropyridine | 3.90 | 5.68 |
| 4-Chloropyridine | 6.70 | 6.02 |
| Carbazole | 7.90 | |
| Indole | 9.30 | |

tention increases in the order: 2-chloro-aniline < pyridine < carbazole < indole and 3-methyl-pyridine < pyridine ≈ 4-methyl-pyridine.

The same observation was made with multi ring systems (*cf.* Table V). We observed also that biquinoline is markedly less retained than quinoline. It seems that, whereas retention increases regularly with increasing number of aromatic rings for PAHs, it does decrease with increasing ring number for aza-arenes. A similar result was observed on bare silica[35].

### *Retention of phenols*

The column capacity factors for a number of phenols are listed in Table VI. With alkylphenols the elution order is: di-*ortho*, *mono*-ortho, *non*-ortho, but the retention depended also on the size of the substituent, and the *ortho-sec.*-butyl compound was eluted among the di-*ortho* derivatives. Thus, class separation of these compounds was impossible.

The mobile phase, isooctane–chloroform (50:50, v/v), permitted the elution of

TABLE VI

RETENTION OF PHENOLS

Solvent: isooctane–chloroform (50:50, v/v); column: diol-bonded silica.

| *Compounds* | *k'* |
|---|---|
| 1 2,4,6-Tributylphenol | 0.36 |
| 2 3,5-Dimethyl-2-isopropylphenol | 0.51 |
| 3 3,5-Di-*tert.*-butylphenol | 0.73 |
| 4 2,3,4,5-Tetramethylphenol | 0.73 |
| 5 2,4,5-Trimethylphenol | 0.80 |
| 6 2,3,5-Trimethylphenol | 0.84 |
| 7 2-*sec.*-Butylphenol | 0.88 |
| 8 2,3-Dimethylphenol | 1.13 |
| 9 3,5-Dimethyl-4-isopropylphenol | 1.21 |
| 10 3-*tert.*-Butylphenol | 1.28 |
| 11 3-Methyl-4-ethylphenol | 1.32 |
| 12 4-Amylphenol | 1.43 |
| 13 3,5-Dimethylphenol | 1.50 |
| 14 3,4-Dimethylphenol | 1.61 |
| 15 4-Ethylphenol | 1.76 |
| 16 4-Methylphenol | 1.88 |
| 17 Phenol | 2.31 |
| 18 3-Methyl-4-chlorophenol | 2.35 |
| 19 4-Phenylphenol | 2.42 |
| 20 4-Methoxyphenol | 2.53 |
| 21 4-Fluorophenol | 2.79 |
| 22 Naphthol-2 | 2.71 |
| 23 3-Chlorophenol | 2.97 |
| 24 3-Fluorophenol | 2.97 |
| 25 3,5-Dichlorophenol | 3.01 |
| 26 4-Chlorophenol | 3.01 |
| 27 4-Bromophenol | 3.01 |
| 28 3-Nitrophenol | 4.20 |
| 29 4-Nitrophenol | 5.50 |

TABLE VII

RETENTION OF PHENOLS

Solvents: (1) methylene chloride; (2) *n*-hexane–ethyl acetate. Column: diol-bonded silica.

| *Compound* | *k'* | |
|---|---|---|
| | *1* | *2* |
| 2,6-Dimethylphenol | 2.6 | 4.9 |
| 2,5-Dimethylphenol | 5.8 | 7.8 |
| 3,5-Dimethylphenol | 11.6 | 13.0 |

all the compounds listed in Table V. This is not possible on the $NH_2$ phase, as pointed out by Hammers *et al.*[12], which is consistent with the strong acid–base interactions between the amino group and the phenols.

In normal-phase systems the retention of phenols depends mainly on their ability to give hydrogen bonding, a mechanism in which the phenols behave as proton donors while the stationary phase acts as proton acceptor. The extent of the hydrogen bonding depends on the electronic environment of the phenolic oxygen. An increase

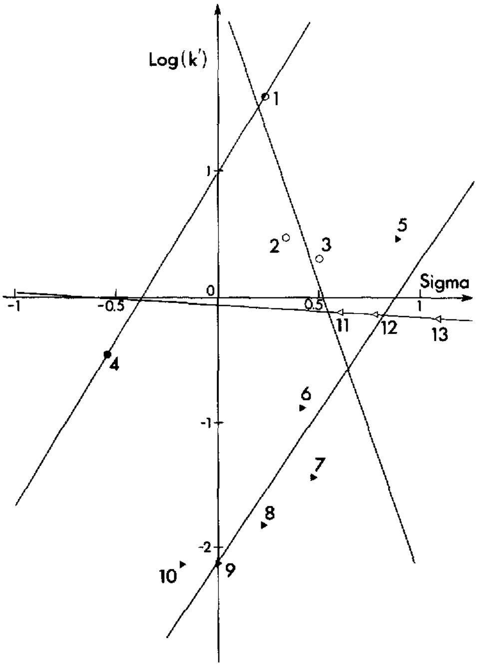

Fig. 3. Correlation between the logarithm of the column capacity factor of benzene derivatives and the Hammett constant (Sigma). Column LiChrospher 100 Diol (Merck, see Table I). Mobile phase, isooctane. Compounds: 1 = *para*-methoxyacetophenone; 2 = *para*-dimethoxybenzene; 3 = *para*-methylacetophenone; 4 = acetophenone; 5 = *para*-bromocyanobenzene; 6 = *para*-chloro (chloromethyl) benzene; 7 = *para*-dibromobenzene; 8 = chlorobenzene; 9 = benzene; 10 = toluene; 11 = *para*-chloronitrobenzene; 12 = nitrobenzene; 13 = *para*-nitrotoluene.

of the adsorption energy is observed with electron-donating substituents while electron-withdrawing groups will decrease the retention.

A comparison between the retention data on the diol phase and on silica[31] is rather difficult as different mobile phases were used. We performed some measurements using pure methylene chloride and *n*-hexane–ethyl acetate (90:10, v/v) (*cf.* Table VII). The elution strengths of methylene chloride, of *n*-hexane–ethyl acetate and of isooctane–chloroform are 0.32, 0.25 and 0.20, respectively, according to data from Snyder[36]. We can conclude that the retention of phenols on the diol phase is much weaker than on silica. The selectivity, however, is good, *e.g.* the relative retention of 3,4-DMP and 3,5 DMP is 1.07; their separation requires 15 500 plates, which is easily achieved with these columns.

The regression of the logarithm of the relative retention of the substituted phenols (reference phenol) *versus* the Hammett constant gives only fair correlation coefficients: for the *para* derivatives the regression coefficient is 0.839; for the *meta* derivatives it is 0.981; for the whole set of data it is 0.849, which means a fair amount of scatter (*cf.* Fig. 3). When a similar regression is performed on the data reported by Hammers *et al.*[12], the regression coefficients are 0.92 and 0.80 for the *para* derivatives and for the whole set of data, respectively. No particular significance seems to be associated to the larger scatter with the *meta* derivatives.

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