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Gas chromatographic study of the inclusion properties of calixarenes
II. Selective properties of cyclic tetra- to octamers derived from phenol, and some problems associated with the use of calixarenes in capillary gas chromatography

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Abstract

Fused-silica capillary columns have been prepared containing calixarene oligomers derived from *p-tert.*-butylphenol as the selectors. Using the quantitative structure–retention relationships (QSRR) method, general selectivity of the studied calixarenes toward aromatic analytes and highly pronounced selectivity of *p-tert.*-butylcalix[4]arene and *p-tert.*-butylcalix-[8]arene toward positional isomers of xylenes, ethyltoluenes and diethylbenzenes, and toward butylbenzene isomers, have been demonstrated. *p-tert.*-Butylcalix[8]arene exhibits selectivity toward chloromethanes. The efficiency of these columns was rather low because of the very poor solubility of the basic, unsubstituted calixarenes in common GC stationary phases.

Keywords: Structure–retention relationships; Inclusion complexes; Calixarenes; *p-tert.*-Butylcalixarenes

1. Introduction

Calixarenes, i.e., macrocyclic phenol–formaldehyde polycondensates, posses interesting basket-shaped intramolecular cavities [1] (Fig. 1). They are capable of selective interactions with many metal ions and organic molecules. X-ray structural analysis and ^{1}H NMR spectroscopy have demonstrated that *p-tert.*-butylcalix[4]arene (ptBC4A) forms inclusion complexes with toluene [2], benzene [3], *p*-xylene [3] and trichloromethane [4]; a ptBC4A derivative forms a complex with dichloromethane [5,6]. *p-tert.*-Butylcalix[6]arene (ptBC6A) forms complexes with trichloromethane and methanol [4] and benzene [7], while the octamethylether of *p-tert.*-butylcalix-[8]arene (ptBC8A) complexes with trichloromethane [4,8].

Calixarenes have been used for separations, first in gas chromatography [9–11] and then also in selective extraction [12–15], liquid chromatography [16–19] and electrophoresis [20].

The present paper is related to the previous study of the inclusion properties of *p-tert.*-butylcalix-[4]arene in gas–solid microcolumn chromatography

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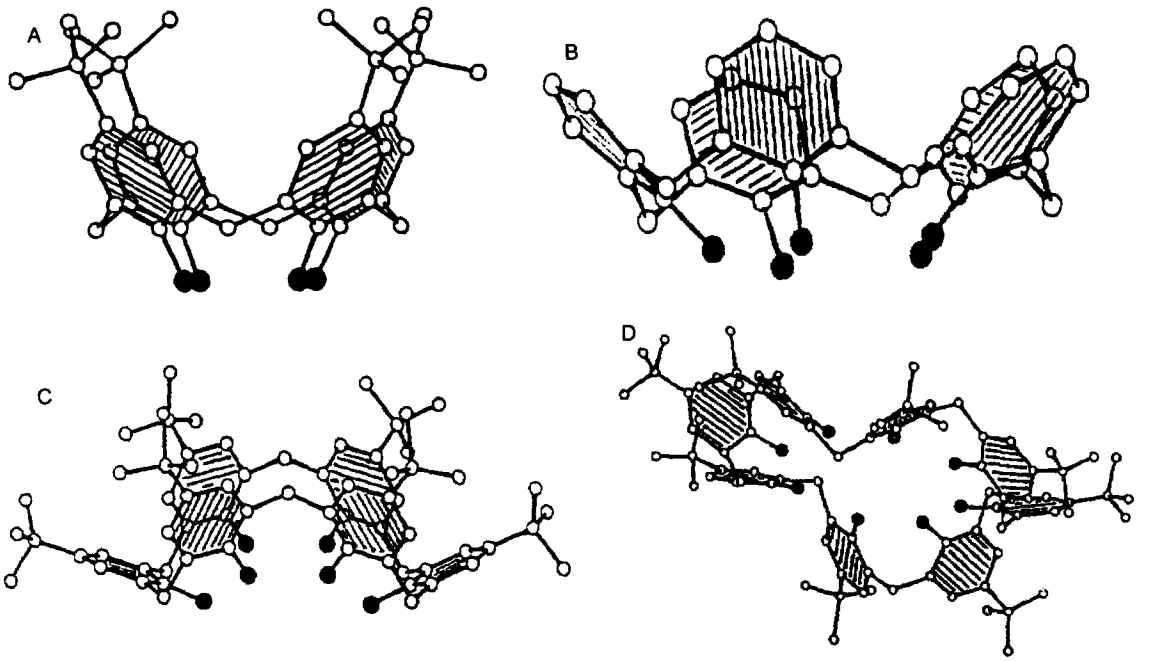

Fig. 1. Schematic conformational arrangement of some calixarenes in the crystalline state. (A) *p-tert.*-Butylcalix[4]arene, (B) calix[5]arene, (C) *p-tert.*-butylcalix[6]arene, (D) *p-tert.*-butylcalix[8]arene. ● represents phenolic oxygens.

[11], where ptBC4A selectivity toward analytes containing π-electron donor aromatic systems was found. Especially noteworthy is the capability of the cyclic tetramer to selectively interact with geometric isomers of dialkyl-substituted benzenes.

This paper aims at using calixarenes as selective components of stationary phases in capillary gas chromatography and studying the selectivity of the basic calixarene series toward alkyl-substituted benzenes, dimethylcyclohexane isomers and chloromethanes. The chosen potential selectors involved basic tetra- to octamer calixarenes derived from *tert.*-butylphenol, namely, *p-tert.*-butylcalix[4]arene (ptBC4A), tetrakis(trimethylsilylether) of *p-tert.*-butylcalix[4]arene (tms-ptBC4A), *p-tert.*-butylcalix-[5]arene (ptBC5A), *p-tert.*-butylcalix[6]arene (ptBC6A) and *p-tert.*-butylcalix[8]arene (ptBC8A). These oligomers, carrying free hydroxyl groups at the lower rim (except for tms-ptBC4A), are very poorly soluble in common organic solvents and nearly insoluble in common liquid–gas chromatographic stationary phases. The solubility can be considerably enhanced by judiciously substituting the basic calixarene skeleton, which, however, often leads to intramolecular inclusion of flexible substituents and thus to partial or complete occupation of the calixarene intramolecular cavity [21–27]. This intramolecular self-inclusion has not yet been demonstrated for the studied basic calixarene series containing the conformationally stable *tert.*-butyl-*p*-substituents. In spite of their poor solubility the *p-tert.*-butyl-substituted calixarenes represent conformationally stable basic selectors applicable to capillary gas chromatography. At present, we are continuing in this study by investigating the selectivity of stationary phases obtained by depositing a thin layer of a calixarene onto the surface of a pure fused-silica capillary column. A further work will be devoted to the selective properties of suitably substituted calixarenes with enhanced solubility.

2. Theoretical

Two principal problems are often encountered in studies of this kind: What reference substance is to

be employed for intercomparisons of the retention behaviour of various analytes and how to eliminate the effects of contingent fluctuations in the temperature and carrier gas flow-rate? How to quantify the degree of selectivity of the stationary phase toward analytes with widely differing physico-chemical properties?

Because of high complexation ability of calixarenes (which was studied in detail with ptBC4A [11]), no reference compound has been found with which the calixarene would not interact. Therefore, a whole group of compounds has been selected as a reference level, the reference homologous series (RHS) of lower monoalkylbenzenes (toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, *n*-hexylbenzene). All the RHS compounds interact with ptBC4A according to a certain common mechanism in all the systems that have so far been studied (RHS always yielded linear dependences of log k on the boiling points of the homologues – coefficients of correlation were from 0.9975 to 0.9999). Further, when using the method of quantitative structure–retention relationships (QSRR) [28], it is possible to evaluate the analyte–stationary phase interactions in a congeneric analyte group along homologous series by plotting retention data (e.g., log k) against analyte molecular descriptors [e.g., boiling point (b.p.), molar volume (MV), polarizability (α), dipole moment (μ), etc.]. These dependences are usually linear in congeneric analyte series, or are readily linearized. Relevant deviations from linearity indicate possible specific interactions. For quantitative evaluation of these deviations in a congeneric analyte series, the IRT_S^D quantity (increase/decrease in retention time) has been defined by the equation [11],

$$IRT_S^D = \frac{t'_{R,X} - t'_{R,H}}{t'_{R,H}} \cdot 100 \qquad (1)$$

where D relates to a defined molecular descriptor and S to a reference homologous series, $t'_{R,X}$ is the experimental adjusted retention time for analyte X and $t'_{R,H}$ is the calculated adjusted retention time obtained by regression analysis of the dependence log k=f(D) for a hypothetical analyte that is eluted within the homologous series S and whose physico-chemical characteristics D are identical with those of analyte X. A positive IRT_S^D value indicates an increase in retention time, while a negative value indicates a decrease in retention time in comparison with the defined homologous series. Fig. 2 depicts the separation of a hypothetical mixture eluted within 2.5 min, with a dead time of 1.0 min. For the peak X with the adjusted retention time on the stationary phase without selector, 1.0 min, the retardation caused by the presence of a selector and described by the value, $IRT_S^D = 50$, represents a retention time shift of 0.5 min, i.e., the compound is now eluted with an adjusted retention time of 1.5 min.

In this work the shifts in the retention times caused by the presence of the calixarenes as the selector were observed in the range of IRT_S^D values of −70 to +276.

3. Experimental

The calixarene oligomers were kindly provided by Professor S. Shinkai (Kyushu University, Kyushu, Japan) and Mr. Toru Sasaki (Shinkai Chemirecognics Project, Chikushino, Japan): ptBC4A, ptBC6A and ptBC8A; Professor M.A. McKervey (The Queen's University of Belfast, Belfast, UK): ptBC5A and Dr. G. Speier (University of Veszprém, Veszprém, Hungary): tms-ptBC4A.

3.1. Chromatographic column preparation

3.1.1. Stationary phases based on a thin film of calixarene deposited on an OV-1 surface

A precisely weighed amount of an oligomer was dissolved in a defined volume of dichloromethane and a fused-silica capillary column (11.55 m×0.20 mm I.D.), coated with the chemically bonded methylsilicone phase OV-1 (film thickness=0.25 μm, a commercial product from CS-Chromatographie Service), was filled with the solution. The solvent was evaporated in vacuum at 33°C (the static coating according to Bouche and Verzele [33]). The selector (calixarene) concentration was expressed in terms of weight fraction (%) of the calixarene per total volume of the pure methylsilicone phase in the

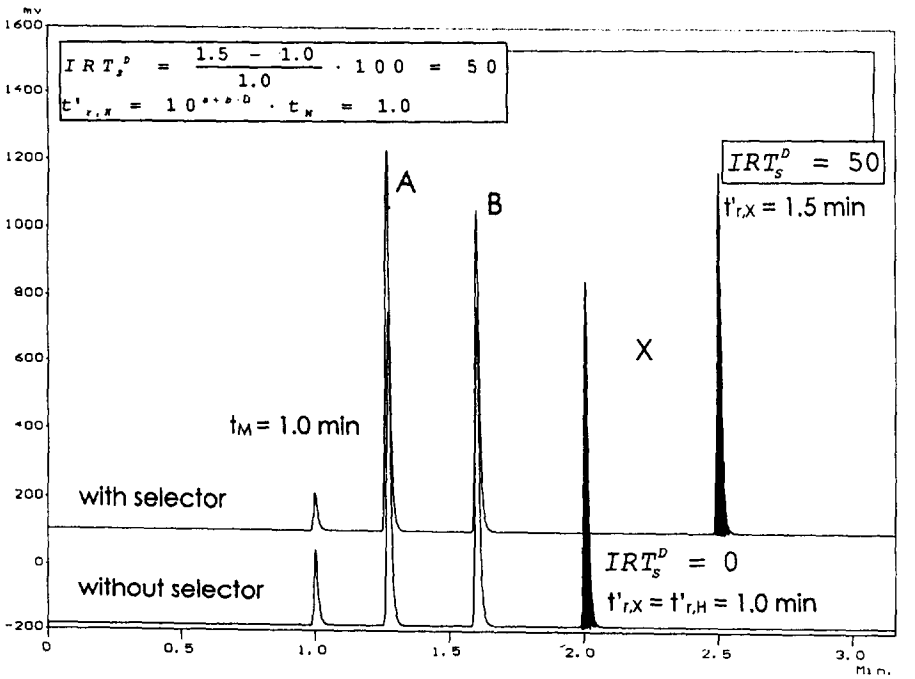

Fig. 2. Quantitative evaluation of the shifts in the retention times in the presence of the selector (the IRT_S^D quantity). Chromatogram of the hypothetical mixture. The peaks A and B represent the two members of a selected homologous series S, their retention behaviour is not affected by the selector. The analyte X elutes on the stationary phase without selector in $t'_R = 1.0$ min according to the homologous series S – its IRT_S^D quantity is therefore by the definition 0 (see text, Eq. 1). In the presence of the selector the adjusted retention time of the analyte X is shifted to $t'_{R,X} = 1.5$ min. This increase in the retention time can be quantified by the IRT_S^D value, $IRT_S^D = +50$. For details see Theoretical.

column. The prepared column was then conditioned in a hydrogen stream at a temperature of up to 120°C for minimal 3 h.

Two reference stationary phases were used, the pure, chemically bonded methylsilicone phase OV-1 (11.55 m×0.20 mm I.D., film thickness 0.25 μm, from CS-Chromatographie Service) and a glass micropacked column (0.62 m×1 mm I.D.) with pure silanized Porapak P, from Waters Assoc.

The chromatographic measurements were carried out on a Chrompack CP 9001 instrument with a flame ionisation detector (capillary columns). Hydrogen was used as the carrier gas at a flow-rate of 1.2 to 1.4 ml min^{-1}. The column temperature was constant, 100.0°C as a compromise for the elution of analytes with a wide range of boiling points and QSRR requirements, or 30.0°C when using squalane. Sample aliquots of 0.04 μl were injected at a split ratio of 1:150. The data were handled by MOSAIC from Chrompack. The glass micropacked columns were studied on a CHROM 61 instrument with a flame ionisation detector (Laboratorní přístroje, Prague, Czech Republic). Nitrogen was used as the carrier gas at a flow-rate of 20.0 ml min^{-1}. The column temperature was constant at 130.0°C. Aliquots of 50 μl of analytes were injected at 25°C using a head-space method.

4. Results and discussion

4.1. Solubility of calixarene oligomers

The calixarene solubility in some organic solvents (pentane, cyclohexane, cyclohexene, benzene, toluene, ethylbenzene, xylenes, diethyl ether, chloro-

methanes) primarily depends on the substitution of the basic skeleton. The maximum solubility of unsubstituted calixarenes, derived from *p-tert.*-butylphenol, ranges from 0.1 g/100 ml to (exceptionally) 1 g/100 ml (ptBC4A in *o*-xylene) or 2.6 g/100 ml (ptBC5A in diethyl ether). However, the solubility of tms-ptBC4A is an order of magnitude higher, from 4 to 8 g/100 ml.

The solubility of ptBC4A in some gas chromatographic stationary phases (squalane, SE-30, OV-61, OV-17, XE-60) was experimentally found to be very low. The calixarene is completely insoluble in nonpolar phases; the highest solubility, 0.08% (w/w) ($1.2 \cdot 10^{-3}$ mol kg^{-1}), was attained in the OV-17 phase. The solubility can be somewhat improved by silylation of the phenolic hydroxy group; silylated ptBC4A is permanently soluble in squalane at a concentration of 1% (w/w) ($1.1 \cdot 10^{-2}$ mol kg^{-1}) and the concentration can be increased for a short time to 5% (w/w) at 120°C. The solubilities of the silylated ptBC4A in the nonpolar SE-30 phase and the phenylsilicone phases OV-61 and OV-17 are ca. 0.1 and 0.5% (w/w), respectively.

The ptBC8A oligomer is completely insoluble in OV-101 and SE-54 phases. Very low solubility [0.01% (w/w)] was found in the squalane and OV-225 phases.

4.2. Retention behaviour of selected analytes on the stationary phase based on a thin film of calixarene deposited on the OV-1 surface

The maximum attainable calixarene concentrations, as selectors in stationary phases, are too low for practical purposes. Therefore, a thin layer of a crystalline calixarene selector was deposited on the surface of the methylsilicone phase OV-1, chemically bonded to the surface of a fused-silica capillary column. It can be assumed that the selector is sufficiently strongly immobilised in the medium of a relatively stable and chemically inert matrix. The calixarene concentration in this system was expressed in terms of the weight percentage of the calixarene, related to the total volume of the pure methylsilicone phase. In this way, stationary phases containing all the studied calixarenes were prepared and tested, at selector concentrations of 10, 25 and 50% (w/v). The reference columns were described in the Experimental section. The structure of a cross-linked divinylbenzene–styrene copolymer (Porapak P) forms a dense network of aromatic (π-electron donor) centres without defined cavities. The study of ptBC4A selectivity in microcolumn gas chromatography [11] has shown that the cyclic tetramer is generally selective toward π-electron donor aromatic analytes and specifically toward dialkylbenzene positional isomers. Therefore, to test the selective properties of the prepared stationary phases, homologous series of mono- and dialkyl-substituted benzenes were used, together with some other compounds (positional and geometric isomers of dimethylcyclohexane, chloromethanes). The selectivity toward the analytes was evaluated using the QSRR method. The mono-*n*-alkylbenzene homologous series (toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, *n*-hexylbenzene) always yielded linear dependences of log k on the boiling points of the homologues (coefficients of correlation were from 0.9975 to 0.9999). Hence, this series satisfied the conditions for the reference homologous series (RHS) and the relative retention of the other analytes was related to it. The selectivity of the studied calixarenes toward individual analytes was then quantified using the IRT_S^D value that represents the relative increase/decrease in the analyte retention with respect to the RHS (see Theoretical section).

4.2.1. Retention of dialkylbenzene positional isomers

Figs. 3–5 summarise the results obtained with the reference columns and with the columns containing the calixarene selectors. It can be seen from the first two columns in the figures that the methylsilicone reference stationary phase is quite nonselective toward the positional isomers of xylenes, ethyltoluenes and diethylbenzenes. Porapak P is quite nonselective toward xylene isomers, but with ethyltoluenes the *p*-isomer is eluted prior to a mixture of the *m*- and *o*-isomers.

The phase containing 53% (w/v) of the ptBC4A selector exhibits an increased retardation of *p*-dialkylbenzenes (*p*-xylene, *p*-ethyltoluene, *p*-diethylbenzene), in relation to the RHS; $IRT_{RHS}^{b.p.} = +13$ to +276 (Figs. 3–5 and Fig. 6). The retardation of

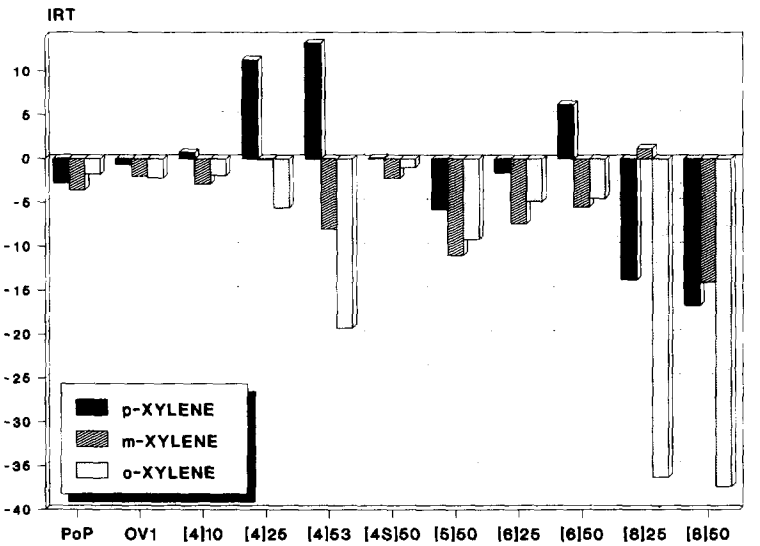

Fig. 3. Retention behaviour of xylene isomers on the stationary phase consisting of a thin layer of calixarene deposited on the OV-1 surface. Horizontal axis: PoP=Porapak P, OV1=chemically bonded methylsilicone phase OV-1, [4]10–[4]53=stationary phases containing 100, 250 and 530 mg of ptBC4A per ml OV-1, [4S]50=500 mg of tms-ptBC4A per ml OV-1, [5]50=500 mg of ptBC5A per ml OV-1, [6]25–[6]50=250 and 500 mg of ptBC6A per ml OV-1, [8]25–[8]50=250 and 500 mg ptBC8A per ml OV-1. Vertical axis: IRT=the relative retention with respect to the reference homologous series of *n*-monoalkylbenzenes (for details see Theoretical section). The isomers order follows the boiling points' [29] order: *p*-xylene, b.p.=138.3°C; *m*-xylene, b.p.=139.1°C; *o*-xylene, b.p.=144.4°C.

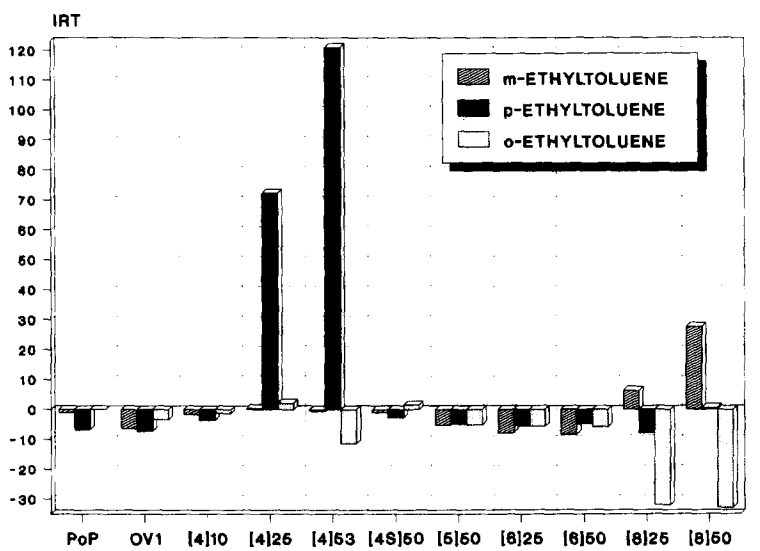

Fig. 4. Retention behaviour of ethyltoluene isomers on the stationary phase consisting of a thin layer of calixarene on the OV-1 surface. For labelling of the axes see Fig. 3. The isomers order follows the boiling points' order: *m*-ethyltoluene, b.p.=161.3°C; *p*-ethyltoluene, b.p.=162.5°C; *o*-ethyltoluene, b.p.=165.2°C.

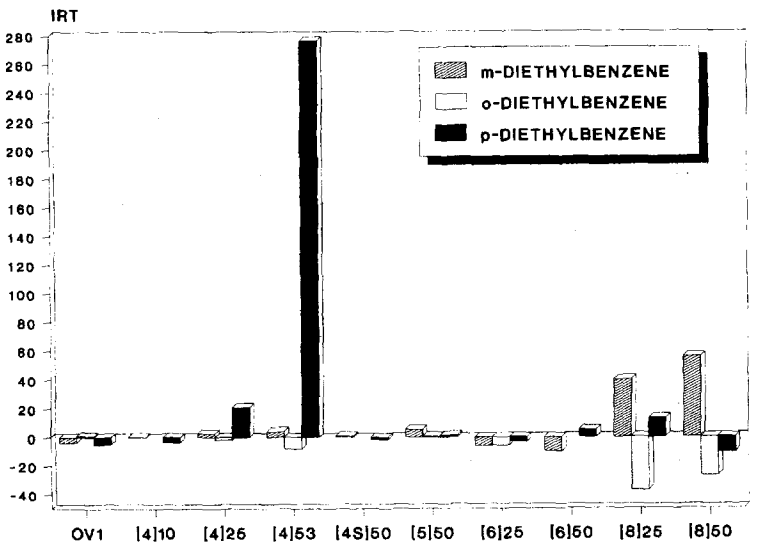

Fig. 5. Retention behaviour of diethylbenzene isomers on the stationary phase consisting of a thin layer of calixarene on the OV-1 surface. For labelling of the axes see Fig. 3. The isomers order follows the boiling points' order: *m*-diethylbenzene, b.p.=181.0°C; *o*-diethylbenzene, b.p.=183.4°C; *p*-diethylbenzene, b.p.=183.8°C.

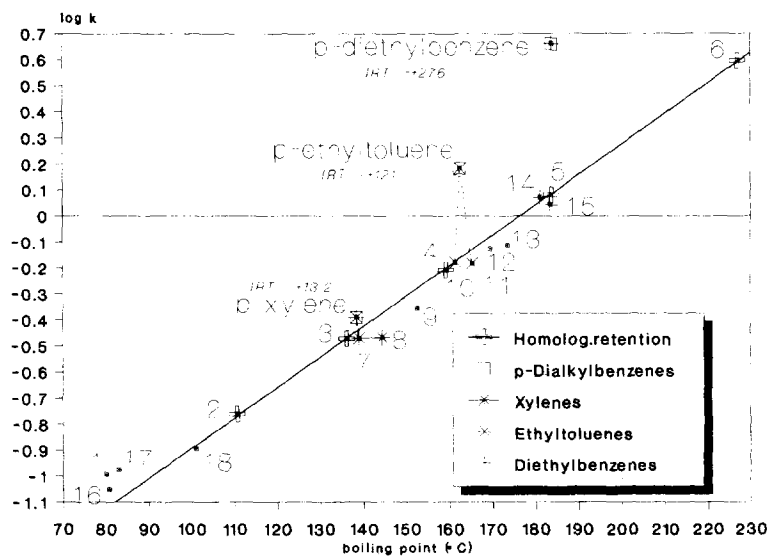

Fig. 6. Retention behaviour of aromatics on the stationary phase consisting of a thin layer of ptBC4A [53% (w/v)] on the OV-1 surface (column temperature, 100.0°C; hydrogen carrier gas, 1.4 ml min^{-1}, 0.04-μl liquid samples with a split ratio of 1:150. Dependence of log k on the analyte boiling points. Comparison of *p*-dialkylbenzenes with the following compounds: 1=benzene, 2=toluene, 3=ethylbenzene, 4=*n*-propylbenzene, 5=*n*-butylbenzene, 6=*n*-hexylbenzene, 7=*m*-xylene, 8=*o*-xylene, 9=isopropylbenzene, 10=*m*-ethyltoluene, 11=*o*-ethyltoluene, 12=*tert.*-butylbenzene, 13=*sec*-butylbenzene, 14=*m*-diethylbenzene, 15=*o*-diethylbenzene, 16=cyclohexane, 17=cyclohexene, 18=methylcyclohexane.

p-dialkylbenzenes rapidly increases with increasing ptBC4A content in the stationary phase, leading to peak broadening, probably due to a specific analyte–stationary phase interaction with slow analyte desorption. *o*-Dialkylbenzenes (*o*-xylene, *o*-ethyltoluene, *o*-diethylbenzene) are much less retarded with respect to the RHS ($IRT^{b.p.}_{RHS} = -19$ to -8), their retention decreases with increasing selector concentration in the stationary phase and the peak width is virtually unchanged. Whereas *m*-xylene is retarded somewhat less than corresponds to the RHS, *m*-ethyltoluene and *m*-diethylbenzene are eluted in agreement with the RHS. The column with pure OV-1 phase does not resolve the positional isomers of xylenes, ethyltoluenes and diethylbenzenes, but an addition of 53% (w/v) of ptBC4A leads to pronounced resolution and an elution order of *ortho*< *meta*≪*para*. These results indicate that there is a relationship between the shape of the analyte molecule and its retention behaviour. *p*-Dialkylbenzenes are strongly retarded in the presence of ptBC4A, while *o*-dialkylbenzenes are weakly retained, without respect to the isomer boiling points. An inclusion complex of ptBC4A with *p*-xylene has been described [3]. The mean diagonal distance of the geometric centres of the aromatic nuclei within the ptBC4A cavity is 0.681 nm [30]. However, the presence of π-electron orbitals above and below the aromatic ring plane somewhat decreases the real size of the calixarene conical cavity. The cavity diagonal dimension in the range of *tert.*-butyls is estimated at 0.6 nm from a computer model. The size of the benzene molecule (aromatic ring) is given as 0.55× 0.37 nm [31]. The Van der Waals radius of the methyl group is 0.20 nm [32]. On the basis of these facts and our previous results [11] it can be assumed that strong retardation of *p*-dialkylbenzenes in the presence of ptBC4A is probably caused by inclusion of these molecules into the ptBC4A cavity. The overall interaction mechanism probably involves the CH_3(guest)–π(host) and CH_3(host)–π(guest) interactions. The shape of the *o*-dialkylbenzene molecules is apparently unsuitable for inclusion into the ptBC4A cavity.

Xylene isomers are partially resolved only at high selector concentrations [50% (w/v)] for the ptBC6A selector, whereas the isomers are eluted together on the two reference stationary phases (Fig. 3). In the presence of ptBC6A, *p*-xylene is somewhat more retarded and the *o*- and *m*-xylene are somewhat less retarded than corresponds to the RHS.

The stationary phase with ptBC8A strongly differentiates the dialkylbenzene isomers. The positional isomers are eluted in the order, *ortho*<*para*< *meta*, independently from their boiling points order. Worth of attention is the strong retardation of *m*-ethyltoluene and *m*-diethylbenzene, which increases with increasing ptBC8A concentration in the stationary phase. The retention behaviour of *ortho* and *meta* isomers is not unambiguously dependent on the selector concentration. *o*-Dialkylbenzenes (*o*-xylene, *o*-ethyltoluene, *o*-diethylbenzene) are retained considerably less than corresponds to the RHS ($IRT^{b.p.}_{RHS} = -37$ to -27).

The accessible literature does not describe the cavity dimensions for ptBC6A and ptBC8A, neither gives data on their inclusion complexes with the test alkyl-substituted benzenes. Thus it can only be stated that the retention behaviour of dialkylbenzenes in the presence of ptBC8A is primarily determined by the shape of the isomer molecules and it can be assumed that inclusion of *m*-dialkylbenzenes into the ptBC8A cavity plays an important role in the analyte–stationary phase interaction mechanism.

Stationary phases containing the tms-ptBC4A or ptBC5A selectors do not significantly resolve the positional isomers of xylenes, ethyltoluenes and diethylbenzenes, similar to the pure methylsilicone phase.

4.2.2. Retention behaviour of butylbenzene isomers

The Porapak P and OV-1 reference stationary phases do not significantly resolve the butylbenzene positional isomers (Fig. 7).

On the methylsilicone phase with the ptBC4A selector, the retardation of *tert.*-butylbenzene and *sec*-butylbenzene strongly decreases with increasing selector concentration, compared with the RHS retention. The best resolution is attained with the maximum selector concentration in the stationary phase.

However, the greatest decrease in the retardation of *tert.*- and *sec.*-butylbenzene ($IRT^{b.p.}_{RHS} = -69$ and -38, respectively) was attained with the ptBC8A selector, again in dependence on the selector concentration. Whereas *sec.*-butylbenzene is eluted be-

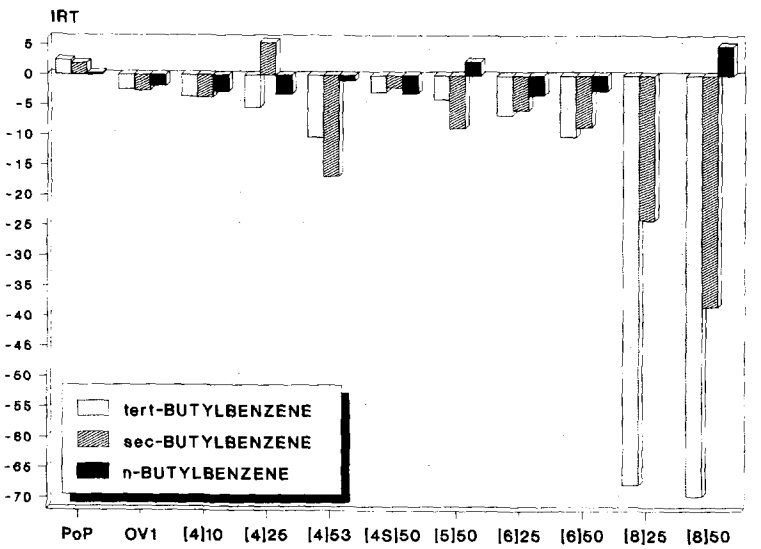

Fig. 7. Retention behaviour of butylbenzene isomers on the stationary phase consisting of a thin layer of calixarene on the OV-1 surface. For labelling of the axes see Fig. 3. The isomers' order follows the boiling points' order: *tert.*-butylbenzene, b.p.=169.5°C; *sec*-butylbenzene, b.p.=173.5°C; *n*-butylbenzene, b.p.=183.6°C.

fore *tert.*-butylbenzene in the presence of the cyclic tetramer and pentamer, the elution order is reversed for the cyclic hexamer and octamer. The retention behaviour of the butylbenzene isomers is primarily determined by the shape of the isomer molecule, without respect to their boiling points. It can be assumed that the inclusion plays an important role in the butylbenzene–calixarene interaction mechanism.

4.2.3. Retention behaviour of isopropylbenzene, dimethylcyclohexane isomers and chloromethanes

Isopropylbenzene is eluted in agreement with the RHS on the pure methylsilicone phase and on the phases containing 10 and 25% (w/v) ptBC4A; at the 53% (w/v) selector concentration it is retarded considerably less than corresponds to the RHS ($IRT^{b.p.}_{RHS}=-16$) (Fig. 6). A similar selectivity is also exhibited by ptBC8A, at a lower selector concentration ($IRT^{b.p.}_{RHS}=-17$). This discrimination of branched isopropylbenzene compared with linear *n*-propylbenzene (or the RHS) in the presence of ptBC4A is in agreement with the conclusions of our previous work [11] and may be related to differences in the shapes of the molecules and the ptBC4A cavity. The other oligomers studied exhibit no selectivity toward isopropylbenzene.

Dimethylcyclohexanes can be considered as nonaromatic analogues of xylenes. The isomers available (1,1-, 1,2- and 1,3-dimethylcyclohexane) are eluted in agreement with the RHS on the pure methylsilicone phase, as well as in the presence of the ptBC4A and tms-ptBC4A selectors. However, all the isomers are considerably less retarded compared with the RHS in the presence of ptBC8A ($IRT^{b.p.}_{RHS}=-62$ to -74). However, no selector leads to more pronounced resolution of the positional or geometric (*cis/trans*) isomers.

Di-, tri- and tetrachloromethane are eluted in the order of their boiling points, i.e. $CH_2Cl_2<CHCl_3<CCl_4$ from the pure methylsilicone phase. An addition of 25% (w/v) ptBC8A changes the retention order into $CH_2Cl_2=CCl_4<CHCl_3$. In the presence of 50% (w/v) of the selector, the CCl_4 retardation further decreases and the retention order is $CCl_4<CH_2Cl_2<CHCl_3$. This retention order corresponds to that obtained in GSC with a packed column [11].

4.3. Statistical evaluation of the results

Standard deviations (S.D.) and relative standard deviations (R.S.D.) of the selected quantities were

Table 1
The mean marginal values of relative standard deviations (R.S.D.) of selected quantities

Quantity	Marginal value	R.S.D. (%)
t_m^a	Minimal	0.4
	Maximal	2.5
t_R^b	Minimal	0.0
	Maximal	3.0
	Exceptionally[c]	5.0
t'_R	Minimal	0.4
	Maximal	3.5
	Exceptionally	7.2
	Fast analytes[d]	23.6
k	Minimal	0.6
	Maximal	4.3
	Exceptionally	7.6
	Fast analytes	23.7

[a] The dead time measured as retention time of methane, 15–30 replicates during experiment.
[b] 3–5 Replicates for each single analyte.
[c] For analytes with a broad peak shape – tailing due to strong specific interactions analyte–stationary phase (calixarene).
[d] The analytes eluting with short reduced retention times (b.p.< 110°C).

determined on the basis of the experimental results and the law of error propagation. The accuracy of the IRT_S^D quantity is presented as the range of values. Table 1 summarizes the mean marginal values of relative standard deviations of selected quantities. Table 2 presents as an example the results obtained on the stationary phase containing 50% (w/v) of ptBC6A.

5. Conclusion

It can be seen from the results that the studied calixarenes exhibit pronounced selectivity toward π-electron donor analytes (aromatics) and those carrying weakly electron-deficient C-H_X groups (alkyl-substituted aromatics, compounds analogous to di- and trichloromethane), when used in capillary gas chromatography. The extent of selectivity depends on the selector content in the stationary phase. The example of the tetrakis(trimethylsilyl ether)-substituted ptBC4A demonstrates that a substitution on the

Table 2
The measured values and statistical evaluation of the results obtained on the stationary phase consisting of a thin layer of ptBC6A [50% (w/v)] on the OV-1 surface

Analyte	b.p. (°C)	t_R (min)	S.D. (t_R) (min)	R.S.D. (t_R) (%)	log k	S.D. (log k)	IRT	IRT_{max}	IRT_{min}
Methane		0.270	0.002	0.7					
Cyclohexane	80.7	0.297	0.001	0.3	−1.005	0.036	33	38	29
Methylcyclohexane	100.9	0.311	0.002	0.6	−0.822	0.030	13	17	10
Cyclohexane	83.0	0.303	0.005	1.7	−0.917	0.072	52	57	47
Benzene	80.1	0.294	0.001	0.3	−1.057	0.041	20	22	19
Toluene	110.6	0.318	0.004	1.3	−0.753	0.041	1	1	0
Ethylbenzene	136.2	0.371	0.001	0.3	−0.429	0.010	2	9	−5
p-Xylene	138.3	0.382	0.001	0.3	−0.384	0.009	6	15	−1
m-Xylene	139.1	0.372	0.002	0.5	−0.425	0.012	−6	0	−10
o-Xylene	144.4	0.390	0.003	0.8	−0.354	0.013	−5	1	−9
n-Propylbenzene	159.2	0.459	0.001	0.2	−0.156	0.006	−2	6	−9
iso-Propylbenzene	152.4	0.429	0.001	0.2	−0.231	0.007	1	9	−6
m-Ethyltoluene	161.3	0.457	0.002	0.4	−0.161	0.007	−8	−2	−14
p-Ethyltoluene	162.5	0.471	0.003	0.6	−0.129	0.008	−5	2	−11
o-Ethyltoluene	165.2	0.485	0.002	0.4	−0.100	0.007	−6	1	−12
m-Diethylbenzene	181.0	0.593	0.002	0.3	0.077	0.005	−10	−3	−16
o-Diethylbenzene	183.4	0.329	0.003	0.9	−0.663	0.027	−85	−84	−85
p-Diethylbenzene	183.8	0.679	0.003	0.4	0.180	0.005	5	15	−3
tert.-Butylbenzene	169.5	0.502	0.000	0.0	−0.067	0.005	−10	−3	−16
sec.-Butylbenzene	173.5	0.535	0.002	0.4	−0.009	0.006	−9	−2	−15
n-Butylbenzene	183.6	0.648	0.003	0.5	0.145	0.005	−2	6	−9
n-Hexylbenzene	227.0	1.644	0.012	0.7	0.706	0.005	2	11	−6

Column temperature, 100.0°C; hydrogen carrier gas, 1.4 ml min^{-1}; 0.04-μl liquid samples with a split ratio of 1:150. S.D.=standard deviation; R.S.D.=relative standard deviation. IRT_{max}–IRT_{min}: the range of IRT_S^D values (similar to confidence interval).

basic calixarene skeleton may sometimes lead not only to an improvement in the calixarene solubility, but also to the loss of selectivity. The experimental data show a direct relationship between the steric arrangement of analyte molecules (*p*-dialkylbenzenes vs. *o*-dialkylbenzenes) and their retention behaviour and point to an important contribution of inclusion of these molecules into calixarene cavities to the overall interaction mechanism. In view of the very limited solubility of the studied calixarenes in common liquid stationary phases, the separation systems used were rather unusual, based on deposition of a thin layer of crystalline calixarene on the surface of a chemically bonded methylsilicone phase. These highly heterogeneous systems exhibit considerable selectivity, but their efficiency is relatively poor. The aim of our present study is to develop a system with an efficiency comparable to modern analytical columns, utilising inclusion phenomena and retaining the achieved high selectivity. The statements concerning inclusion of the analyte molecules into the calixarene cavities should be supported by other measurements, e.g., NMR spectroscopy and X-ray crystallography.

6. Unlisted Reference

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References

[1] C.D. Gutsche, in J.F. Stoddart (Editor), Calixarenes, Royal Society of Chemistry, Cambridge, 1989.
[2] G.D. Andreetti, R. Ungaro and A. Pochini, J. Chem. Soc. Chem. Commun., (1979) 1005.
[3] M. Coruzzi, G.D. Andreetti, V. Bocchi, A. Pochini and R. Ungaro, J. Chem. Soc. Perkin 2, (1982) 1133.
[4] C.D. Gutsche, B. Dhawan, K.H. No and R. Muthukrishnan, J. Am. Chem. Soc., 103 (1981) 3782.
[5] J.L. Atwood, S.G. Bott, C. Jones and C.L. Raston, J. Chem. Soc. Chem. Commun., (1992) 1349.
[6] V. Böhmer, G. Ferguson, J.F. Gallagher, A.J. Lough, M.A. McKervey, E. Madigan, M.B. Moran, J. Phillips and G. Williams, J. Chem. Soc. Perkin 1, (1993) 1521.
[7] H. Halit, D. Oehler, M. Perrin, A. Thozet, R. Perrin, J. Vicens and M. Bourakhouadar, J. Incl. Phenom., 6 (1988) 613.
[8] A.W. Coleman, S.G. Bott and J.L. Atwood, J. Incl. Phenom., 4 (1986) 247.
[9] E. Smolková-Keulemansová and L. Feltl, Abstracts of the 2nd International Symposium on Clathrate Compounds and Molecular Inclusion Phenomena, Parma, 1982, p.45.
[10] A. Mangia, A. Pochini, R. Ungaro and G.D. Andreetti, Anal. Lett., 16 (1983) 1027.
[11] P. Mŏuk and L. Feltl, J. Chromatogr. A, 696 (1995) 101.
[12] J. Vicens, A.E. Armah, S. Fujii and K.-I. Tomita, J. Incl. Phenom., 10 (1991) 159.
[13] Y. Okada, Y. Kasay and J. Nishimura, Tetrahedron Lett., 36 (1995) 555.
[14] F. Nakashio, M. Goto, K. Oowatari, T. Yamamoto, S. Shinkai, T. Nagasaki and H. Tsuboi, Jpn. Kokai Tokkyo Koho, JP 05320092 A2-931203, idem. JP 05271149 A2-931019
[15] J. Soedarsono, M. Burgard, Z. Asfari and J. Vicens, New J. Chem., 17 (1993) 601.
[16] J.D. Glennon, K. O'Connor, S. Srijaranai, K. Manley, S.J. Harris and M.A. McKervey, Anal. Lett., 26 (1993) 153.
[17] J.H. Park, Y.K. Lee, N.Y. Cheong and M.D. Jang, Chromatographia, 37 (1993) 221.
[18] K. Iwata, H. Kimizuka and H. Suzuki, Jpn. Kokai Tokkyo Koho, 7 pp.
[19] J.D. Glennon, E. Horne, K. O'Connor, G. Kearney, S.J. Harris and M.A. McKervey, Anal. Proc., 31 (1994) 33.
[20] D. Shohat and E. Grushka, Anal. Chem., 66 (1994) 747.
[21] G.D. Andreetti, A. Pochini and R. Ungaro, J. Chem. Soc., Perkin 2, (1983) 1773.
[22] F. Gharnati, M. Perrin, S. Rantsordas, H. Goldmann and V. Böhmer, J. Crystallogr. Spectroscop. Res., 21 (1991) 69.
[23] S. Rantsordas, M. Perrin, F. Gharnati, S. Lecocq, S. Vogt, T. Fey and V. Bohmer, J. Incl. Phenom., 9 (1990) 145.
[24] G.R. Newkome, Y.F. Hu, M.J. Saunders and F.R. Fronczek, Tetrahedron Lett., 32 (1991) 1133.
[25] G.D. Andreetti, G. Calestani, F. Ugozoli, A. Arduini, E. Ghidini, A. Pochini and R. Ungaro, J. Incl. Phenom., 5 (1987) 123.

[26] R. Ungaro, A. Pochini, G.D. Andreetti and P. Domiano, J. Incl. Phenom., 3 (1985) 35.
[27] A.W. Coleman, S.G. Bott and J.L. Atwood, J. Incl. Phenom., 4 (1986) 247.
[28] R. Kaliszan, Quantitative Structure–Chromatographic Retention Relationships, J. Wiley, New York, 1987.
[29] CRC Handbook of Chemistry and Physics, 74th ed., 1993–1994, CRC Press, Boca Raton, Florida.
[30] K.B. Lipkowitz and G.Pearl, J. Org. Chem., 58 (1993) 6729.
[31] D.V. Kiselev and J.I. Jashin, Adsorption Gas and Liquid Chromatography, SNTL, Prague, 1988.
[32] A. Bondi, J. Phys. Chem., 68 (1964) 441.
[33] J. Bouche and M. Verzele, J. Gas Chromatogr., 6 (1968) 501.