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THE MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS OF CALIX[4] ARENEHYDROXYMETHYLPHOSPHONIC ACIDS

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The basic chromatographic characteristics (retention time, t_R, capacity factors, k', and coefficients of asymmetry peaks, K_S) of the number of biologically significant calix[4]arenehydroxymethylphosphonic acids and their sodium salts have been obtained in a reversed-phase high-performance liquid chromatography (RP HPLC) on Zorbax CN. The structure of calix[4] arenehydroxymethylphosphonic acids have been studied by the molecular modeling method (TURBOMOLE program). The relationship between the structure and chromatographic characteristics of the calix[4]arenes, as well as their sorption mechanism on the column surface have been discussed.

МОЛЕКУЛЯРНА БУДОВА ТА ХРОМАТОГРАФІЧНІ ХАРАКТЕРИСТИКИ КАЛІКС[4]АРЕНГІДРОКСИМЕТИЛФОСФОНОВИХ КИСЛОТ
О.І.Кальченко, С.О.Черенок, О.В.Роженко, О.А.Ющенко, В.І.Кальченко
Основні хроматографічні характеристики (часи утримання t_R, коефіцієнти ємкості k', коефіцієнти асиметрії піків K_S) низки біологічно значимих калікс[4]аренгідроксиметилфосфонових кислот та їх натрієвих солей були визначені методом обернено-фазної високоефективної рідинної хроматографії (ОФ ВЕРХ) при застосуванні хроматографічної насадки Zorbax CN. Будову калікс[4]аренгідроксиметилфосфонових кислот було досліджено методом молекулярного моделювання (програмний пакет TURBOMOLE). Обговорено взаємозв'язок між будовою, хроматографічною поведінкою та механізмом сорбції каліксаренів поверхнею хроматографічної насадки.

МОЛЕКУЛЯРНОЕ СТРОЕНИЕ И ХРОМАТОГРАФИЧЕСКИЕ ХАРАКТЕРИСТИКИ КАЛИКС[4]АРЕНГИДРОКСИМЕТИЛ-ФОСФОНОВЫХ КИСЛОТ
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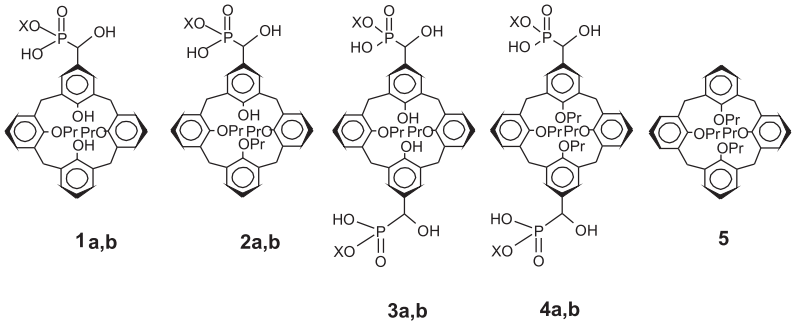
Основные хроматографические характеристики (времена удерживания t_R, коэффициенты емкости k', коэффициенты асимметрии пиков K_S) серии биологически значимых каликс[4]аренгидроксиметилфосфоновых кислот и их натриевых солей определены методом обращенно-фазной высокоэффективной жидкостной хроматографии (ОФ ВЭЖХ) при использовании хроматографической насадки Zorbax CN. Строение каликс[4]аренгидроксиметилфосфоновых кислот исследовано методом молекулярного моделирования (программный пакет TURBOMOLE). Обсуждена взаимосвязь между строением и хроматографическим поведением и механизмом сорбции каликсаренов поверхностью хроматографической насадки.

Calixarenes [1] are macrocyclic compounds with cone shaped structure which can be synthesized by the cyclocondensation of para-substituted phenols with formaldehyde. These compounds due to the ability to the selective formation of host-guest supramolecular complexes with substrates (cations, anions, organic molecules, biopolymers) are widely used in chemistry, physics, and biology [1-5].

Due to the ability to simulate the substrate-receptor interactions with biomolecules, calixarenes are objects of biomedical research [6, 7]. Calixarenes contained preorganized bio-affine groups are able to recognize and bind in supramolecular complexes different biologically active molecules such as amino acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, riboflavin, vitamin B_{12}, nucleotides, nucleosides and short DNA fragments [8-20]. The calixarenes substrate-receptor interactions in water solutions can be investigated by HPLC method [21].

The aim of this work is determination of the HPLC chromatographic characteristics of calix[4]arenehydroxymethylphosphonic acids 1a-4a, their sodium salts 1b-4b as well as modeling tetrapropoxycalixarene 5 (Scheme) and investigation of relationship of these characteristics with molecular structure of the calixarenes.

The calix[4]arenehydroxymethylphosphonic acids form host-guest inclusion complexes with amino acids



X = H (a), Na (b)

Scheme

and dipeptides [22, 23]. They are also selective modulators of calcium metabolism in cells [24] and inhibitors of proteintyrozynphosphataze [25]. Thus, the chromatographic characteristics of the acids may be useful for determination of the binding constants of their supramolecular complexes with the biomolecules in water solutions [26] as well as for their pharmacological investigations by the HPLC method.

Experimental

HPLC analysis. The solvents were obtained from Acros Organics. Calix[4]arenehydroxymethylphosphonic acids 1a-4a (Scheme 1) were synthesized by the reaction of the corresponding formylcalixarenes with sodium salts of dialkylphosphites and the next dealkylation of the esters formed by the consecutive treatment with trimethylbromosilan and methanol according to [25]. Sodium salts of calix[4]arenehydroxymethylphosphonic acids 1b-4b were obtained by addition of an equivalent quantity of sodium methylate to methanol solution of the acids. Unsubstituted at the upper rim tetrapropoxycalixarene 5 was synthesized by the method [26].

HPLC analysis was performed using the high pressure liquid chromatographic system Hitachi. The column (250 × 4,6 mm i.d.) was packed with Zorbax CN. The mobile phase THF/H_2O (90/10 v/v) was utilized for calix[4]arenehydroxymethylphosphonic acids 1a-4a and tetrapropoxycalixarene 5. The mobile phase H_2O/MeCN (99/1 v/v) was used for sodium salts 1b-4b. Flow rate of the both mobile phases was 0.8 ml/min. Chromatographic samples were prepared in the solvents identical to the mobile phases. Concentration of the calixarenes in samples for the analysis was 10^{-3}-10^{-4} M, the samples volume was 20 mkl. Each of the samples were analyzed three times. Wave length of UV detector was 254 nm. All chromatograms were obtained at temperature 26°C.

Molecular modelling. All the structures were first optimized using TURBOMOLE (version 6.02) program packet [27, 28] in order to utilize advantages of the Resolution Identity (RI) [29] algorithm implemented in the TURBOMOLE program packet. B97-D functional [32, 33] and standard triple-zeta basis sets (TZVP) [34] were used. The basis sets were contracted as (14s9p)/[5s4p] → {73211/6111} for Si, (11s6p)/[5s3p] → {62111/411} for C, N, O and (5s)/[3s] → {311} for H. One set of (five) d-functions was added for every heavy atom and one set of p-functions was used for hydrogen atoms. In order to find conformers with the lowest total energies optimized structures were modified. Maximal number of hydrogen bonds under condition of minimal steric interactions between the guest and the host has been chosen as criteria for modification. After modification the structures were fully optimized again and the resulted total energy was compared with that before modification. The procedure was repeated until no total energy lowering was observed. For the structure with the lowest total energy vibrational analysis was performed computing analytically first and second order derivatives. No imaginary frequencies were found for 3a, complex 6 and complex 7. Solvent effects (H_2O) were modeled using COSMO [35] procedure. Corrected and uncorrected total energy values and relative energy magnitudes are listed in Table 1. The DE values were calculated as a difference between the total energy magnitudes for the adduct and free host and guest structures corrected on zero point energy correction (ZPE). *DG* values were derived from the total energy magnitudes corrected on chemical potential (*ch. pot.*), computed for standard conditions (T= 298.15K, p= 0.1 MPa). VMD program packet [36] was used for graphical representation of the structures.

Chromatographic characteristics determination. The most suitable eluents for chromatographic analysis of acids 1a-4a and calixarene 5 was THF/H_2O mixture (90/10 v/v), and for sodium salts 1b-4b – H_2O/MeCN mixture (99/1 v/v). The main chromatographic parameters of calixarenes 1-5 – retention times t_R, retention volumes V_R, capacity factors k', and peak asymmetry coefficients K_S are given in Table 1.

Results and Discussion. The retention times of calixarene 5 and calix[4]arene-hydroxymethylphosphonic acids 1a-4a are within 3.34-6.55 min. The changing of calix[4]arenehydroxymethylphosphonic

Table 1

Retention times t_R, retention volumes V_R, capacity factors k', and peak asymmetry coefficients KS of calixarenes 1-5

Calixarene, №	Retention time, t_R, min	Retention volume, V_R, ml	Capacity factor, k'	Peak asymmetry coefficient, K_S
1a	3.34	2.67	1.30	1.00
1b	3.14	2.51	1.17	0.71
2a	4.16	3.33	1.87	1.50
2b	4.74	3.79	2.27	0.93
3a	5.35	4.28	2.69	1.30
3b	5.42	4.34	2.74	0.81
4a	6.55	5.24	3.52	2.00
4b	12.37	9.90	7.53	1.89
5	3.12	2.50	1.15	1.00

acids 1a-4a to their salts 1b-4b insignificantly changes their retention time (3.14-12.37 min), with the exception of calixarene 4 (6.55 min for the acid and 12.37 min for the salt). The retention volume V_R and capacity factor k' are increased as well. Calixarene peaks are characterized by reasonable values of the asymmetry coefficients within 0.71-2.0. Thus, for the acids and their salts the increasing of the number of propyl groups at the lower rim and phosphoryl groups at the upper rim of the macrocycle leads to the increasing of the retention parameters – t_R, V_R and k'. For the acids 1a-4a the inverse dependence of capacity factors k' from the molecule lipophilicity parameter log P calculated by the software package Hyper Chem, 8 is observed (Fig. 1).

Calixarenes 1-4 adsorb on the Zorbax CN surface, covered by cyanoalkyl groups, mainly by the upper rim of the macrocycle. This is confirmed by the greater retention time of tetrapropoxycalixarene-bis-phosphonic acid 4a (6.55 min) and its salt 4b (12.37 min) comparatively with unsubstituted at the upper rim model tetrapropoxycalixarene **5** (3.12 min) (Table 1).

To determine a nature of the adsorption the molecular structure of the calixarenes was studied. Conformation of macrocyclic skeleton of the calixarenes is confirmed by the ^{1}H NMR and quantum-chemical calculations methods. In the NMR spectra of dipropoxycalixarenes 1, 3 signals of $ArCH_2Ar$ methylene groups are observed as two doublets of axial and equatorial protons of the spin system AB ($^2J_{HH}$ = 13 Hz) with the differences between their chemical shifts Δδ 0.85-0.90 ppm. This indicates that calixarenes 1, 3 exist in the *flattened cone* conformation with C_{2v} symmetry of the macrocyclic skeleton. The *flattened cone* conformation is stereochemically rigid due to the intramolecular hydrogen bonds OH···OPr at the lower rim of the macrocycle. At the same time, the

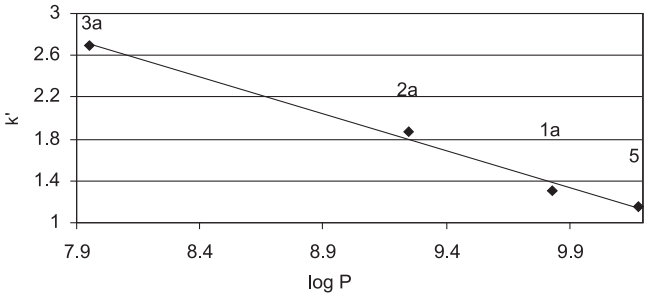
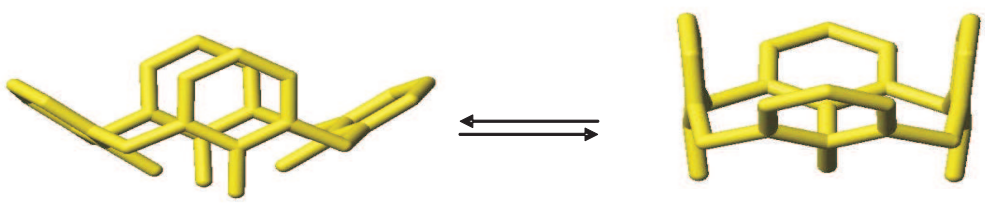
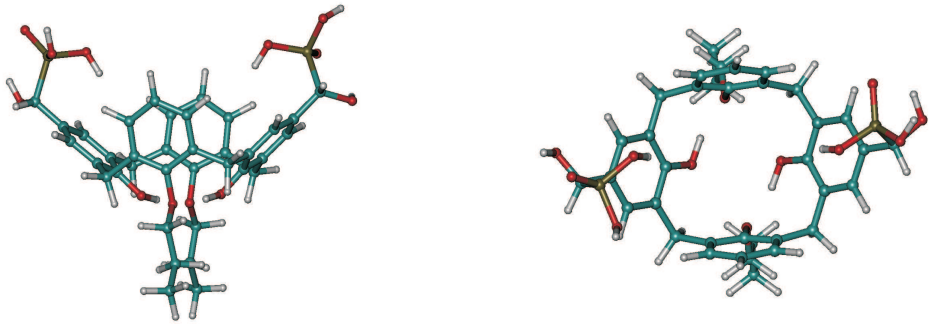


Fig.1. Correlation of capacity factors k' with lipophilicity log P of calixarenes 1a-3a, 5.



Flattened cone *Flattened cone*

Fig. 2. Conformational transitions *flattened cone* - *flattened cone* in tetrapropoxycalixarenes 4, 5.



a b

Fig. 3. The most favorable conformation calixarene 3a (a – side view, b – top view).

difference between chemical shifts of the axial and equatorial protons Δδ for tetrapropoxycalixarenes 4 and 5 consists 1.1-1.2 ppm which corresponds to the stereochemically mobile *regular cone* conformation with C_{4v} symmetry of the skeleton [2]. Rapid in the NMR scale *flattened cone – flattened cone* transitions take place for the tetrapropoxycalyxarenes (Fig. 2) [2]. Tripropoxycalixarene 2 occupies an intermediate position between these two conformations (Δd 1.01 ppm).

Quantum-chemical calculations for calixarene 3a structure were carried out at the DFT approximation. For geometry optimization, the modern DFT-functional Grimme B97-D was used [37]. This takes into account effects of electronic dispersion (for example, van der Waals interactions), which is of importance for molecular complexes. The most stable conformation for 3a (Fig. 3) is *flattened cone* where the aromatic fragments substituted with phosphoryl groups lay closer to the main plane of the molecule formed by methylene groups of their macrocyclic skeleton. Unsubstituted aromatic fragments are approximately perpendicular to this plane. Hydroxyl group of CH-O-H moiety and one of the hydroxyl groups of P-OH fragment form hydrogen bonds with oxygen of P=O groups. Hydroxyl group of remaining P-OH fragment interacts with the aromatic π-system.

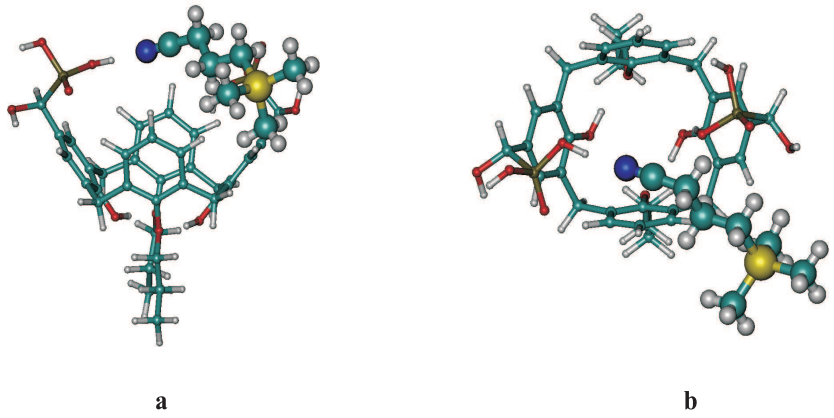
In order to elucidate influence of the calixarene nature on sorption processes we have calculated the structure of the host-guest complex for compound 3a with 4-(trimethylsilyl)butyronitrile $Me_3SiCH_2CH_2CH_2CN$ (complex 6), and 4-(trisiloxysilil)butyronitrile $(H_3SiO)_3SiCH_2CH_2CH_2CN$ (complex 7). These compounds model binding sites of calixarenes on the of

Table 2

The calculated values of total energies (E), total energies, taking into account amendments to the fluctuations at 0K (E + ZPE), chemical potential (E + h.p.) and relative energy of adduct formation (ΔE and ΔG)

Structure	E, Hartree	ZPE (*x.n.*)[a], Hartree	Hartree	ν, см$^{-1}$	ΔE *(ΔG)*[a], kcal / mol
3a	-2981.954273 -2982.004634[6]	0.718895 *0.623782* -	-2981.235378 *-2981.330491* -	9.9	0.00 *0.00* 0.00
6	-3601.910628 -3601.960179[6]	0.919571 *0.807206* -	-3600.991058 *-3601.103422* -	6.6	-16.11 *0.02* -9.63
7	-4581.938440 -4581.993561[6]	0.904090 *0.786235*	-4581.034349 *-4581.152204* -	14.5	-21.33 *0.14* -19.07
$Me_3Si(CH_2)_3CN$	-619.928202 -619.940194[6]	0.198196 *0.155239* -	-619.730006 *-619.772963* -	23.3	- - -
$(H_3SiO)_3Si(CH_2)_3CN$	-1599.944531 -1599.958538[6]	0.179548 *0.122601* -	-1599.764983 *-1599.821929* -	17.9	- - -

[a] Value *ch. pot.*, *E+ ch. pot.* and Δ*G* are shown in italics. [b] Value of total and relative energies, calculated with the COSMO procedure for water as solvent (ε = 78.39), uncorrected.



a b

Fig. 4. VMD plots of the most favorable conformation of complex **6,** (a) side view, (b) top view.

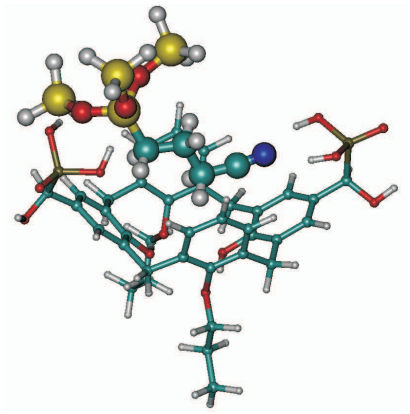


Fig. 5. VMD plot of adduct 7 structure (conformation with the lowest total energy).

Zorbax CN surface. In **6** (Fig. 4) hydrogen bonds P-O H···NC and P=O···H_2CCN with two calixarenes phosphonic residues are formed without including the guest molecule into the host cavity.

The reaction of the complex formation is exothermic (ΔE = -16.1 kcal/mol). However, the transition relative free Gibbs energy values (ΔG) decreases exothermicity reaction to zero (Table 2), due to decreasing entropy by formation of guest-host adduct. Consideration of the solvent influence (water) using of the empirical COSMO procedure reduces the ΔE value to -9.6 kcal/mol.

So, in polar solvent the binding efficiency of **3a** calixarene towards 4-(trimethylsilyl)butyronitrile is low. 4-(Trisiloxysilyl)butyronitrile better mimics the Zorbax CN silica gel surface. The theoretically calculated complex 3a with this guest molecule (7, Fig. 5) is additionally stabilized by two hydrogen bonds PO-H···OSi, so values of the calculated exothermic DE reaction energy increases to -21.3 kcal/mol (-19.1 kcal/mol in water). However the entropy effect reduces the exothermic Gibbs free energy of the reaction to zero (+0.1 kcal/mol). This corresponds to a dynamic equilibrium of the complex 7 with molecules 3a and 4-(trisiloxysilyl)butyronitrile.

However, it is noteworthy that in the Zorbax CN silica gel the butyronitrile moiety is covalently bound to the polysilicate matrix, and has a much lower number of degrees of freedom compared with model compound 7. Therefore, from point of view of entropy the guest-host interaction will be more favorable and the reaction can become exothermic. In addition, in the real chromatographic processes interactions of the calixarene host with residual silanol groups Si-OH of Zorbax CN should not be neglected as well.

Conclusion

Analyzing the data obtained one can conclude that the cone shaped calix[4]arenehydroxymethylphosphonic acids and their sodium salts in conditions of the high-performance liquid chromatography adsorb on Zorbax CN support by the upper rim of the macrocycle forming the hydrogen bonds with cyanoalkyl groups at the support surface. The calixarenes are registered as sharp peaks with the retention time within 3.14-12.37 min and can be analyzed by HPLC method in aqueous solutions, including biological fluids during bio-medical investigations.

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