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Study of Calixarene Complexation with Biologically Active Carboxylic Acids by RP HPLC Method

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Host-Guest complexation of octakis(diphenoxyphosphoryloxy)tetramethylcalix[4]resorcinarene **CRA** and 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene **CA** with bio relevant aromatic, pyridine and diterpenoid carboxylic acids in water-organic solution had been studied by the RP HPLC and molecular modelling methods. The stability constants K_A (387-1914 M^{-1}) of the supramolecular complexes had been determined. It was shown the Host-Guest interactions are depended on structure of the Host molecules and *log P* values of the Guests. The complexation is determined by the hydrogen bonds of the COOH group of the carboxylic acids with P=O oxygen atom of diphenoxyphosphoryl group of the calixresorcinarene **CRA**, and oxygen or nitrogen atoms located on the lower or the upper rim of the calixarene **CA**.

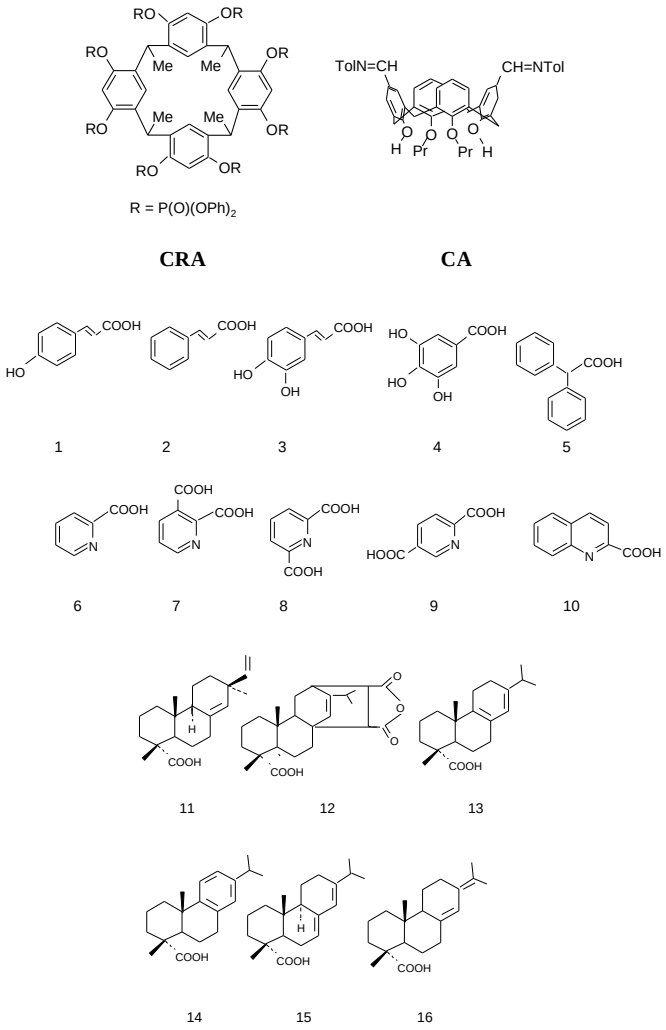
Introduction

Molecular recognition, membrane transport and analytical sensing of bio relevant carboxylic acids [1,2,3,4] by artificial receptors constitute an important problem in chemistry and biology [5,6]. The receptor properties of functionalized calixarenes toward the bio relevant molecules [7] make them highly promising materials for sensor technologies [8], as well as Host molecules for drug delivery systems in pharmaceutical science [9,10].

Calixarenes [11] contained preorganized bio-affine groups are able to recognize different bio relevant molecules such as aromatic acids, dipeptides, proteins, choline and acetylcholine, carbohydrates, nucleotides, nucleosides and other organic substances [12,13,14,15]. Calixarene derivatives can also be fastened on the surface of proteins [16,17]. Calixarenes also simulate substrate-receptor interactions in bio-chemical processes [18,19].

Formerly it was shown that calix[4]arenes functionalized by appopriate

functional groups are effective receptors for aromatic compounds [20,21,22]. The receptor activity is essentially depended from the number of the functional groups, geometrical parameters and stereochemical configuration of the macrocyclic platform as well.



Scheme. The **CRA** and **CA** (Hosts) and *p*-coumaric **1**, cinnamic **2**, caffeic **3**, gallic **4**, diphenylacetic **5**, picolinic **6**, quinolinic **7**, dipicolinic **8**, izocinchomeronic **9,** quinaldinic **10,** pimaric **11**, maleopimaric **12 ,** palustric **13**, dehydroabietic **14,** abietic **15** and neoabietic **16** acids (Guests).

The aim of this work was to study the Host-Guest complexation of the octakis(diphenoxyphosphoryloxy)tetramethyl-calix[4]resorcinarene **CRA** and 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]-arene **CA** with the aromatic, pyridine and diterpenoid carboxylic acids **1-16** (**Scheme**).

Reversible-phase high-performance liquid chromatography method (RP HPLC) was used for determination of the stability constants of the supramolecular complexes formed.

Results and discussion

Usually, for the determination of the stability constants of the calixarene Host-Guest complexes with organic molecules the NMR, calorimetry, UV, fluorescent spectroscopy methods are widely used [9,23,24,25,26]. However in some cases due to poor solubility of the calixarenes or the Guest molecules or the absence of a corresponding instrumental response on the complexation process the above menthioned methods are inefficient. To solve this problem we have developed RP HPLC method for the determination stability constants of the calixarene complexes with organic compounds in water or water-organic solutions [27,28,29,30]. The method includes the determination of retention factor k' of the Guest molecule before and after calixarene addition to the mobile phase. The stability constant K_A of the calixarene complex with the Guest molecule (for 1:1 stoichiometry) can be calculated by equation (1) [20]:

$1/k' = 1/k_0' + K_A \times [CA]/k_0'$ (1),

where k_0' and k' are retention factors of the Guest molecule determined in the absence and in the presence of the calixarene in the mobile phase.

The calixarenes and the acids were registered on the chromatograms by the sharp peaks. Linear adsorption isotherm (r=0.99) indicated on its reversible sorption on the solid support surface (LiChrosorb RP 18).

An addition of the **CA** or **CRA** to the mobile phase decreases the retention factor k' of the acids. The linear character plots of k' *vs* the **CA** and **CRA** concentration testifies the formation of the Host-Guest inclusion complexes with 1:1 stoichiometry and allows the correct calculation K_A values by the equation (1).

The stability constants K_A and free Gibbs energy ΔG ($\Delta G = -RT\ lnK_A$) for **CA** and **CRA** complexes with the acids are presented in **Table 1.**

Table 1. Stability constants K_A (M^{-1}) and free Gibbs energy ΔG (kJ/mol) of the complexes

№	Acid	**CRA**		**CA**	
		K_A	ΔG	K_A	ΔG
Aromatic acids					
1	*p*-Coumaric	692	-16,18	-	-
2	Cinnamic	941	-16.94	-	-
3	Caffeic	520	-15.47	-	-
4	Gallic	625	-15.92	-	-
5	Diphenyl acetic	387	-14.74	-	-
Pyridine acids					
6	Picolinic	770	-16.44	-	-
7	Quinolinic	1914	-18.69	-	-
8	Dipicolinic	1572	-18.20	-	-
9	Isocinchomeronic	1895	-18.67	-	-
10	Quinaldinic	584	-15.76	-	-
Diterpenoid acids					
11	Pimaric	395	-14.79	1268	-17.67
12	Maleopimaric	548	-15.60	1102	-17.33
13	Palustric	464	-15.19	1121	-17.37
14	Dehydroabietic	640	-15.98	1158	-17.45
15	Abietic	557	-15.64	862	-16.72
16	Neoabietic	682	-16.14	844	-16.70

*relative standard deviation (*RSD*) is 5-24 %

The stability constants are depended on structure of Host and Guest molecules and vary from 387 M^{-1} (complex **CA** with diphenylacetic acid **5**) to 1914 M^{-1} (complex **CA** with quinolinic acid **7**) (**Table 1)**.

Hydrophobic interactions between the Host and Guest molecules influence the complex stability in the water-organic solutions. This is clear to see from the dependence of K_A values of the **CRA** and **CA** complexes from *log P* of the diterpenoid acids **11-16** [31] **(Figures 1,2**).

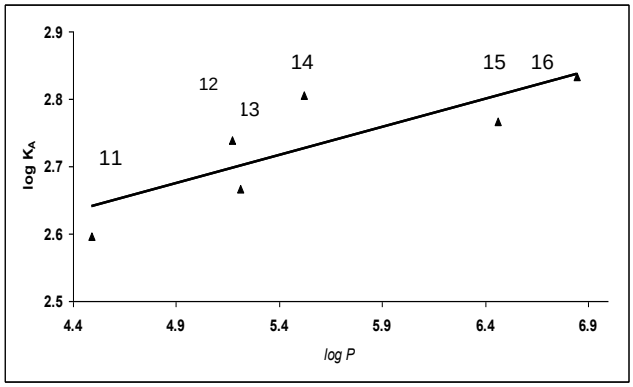


Figure 1. Plots of *log P of* diterpenoid acids **11-16** on their K_A complexes with **CRA**.

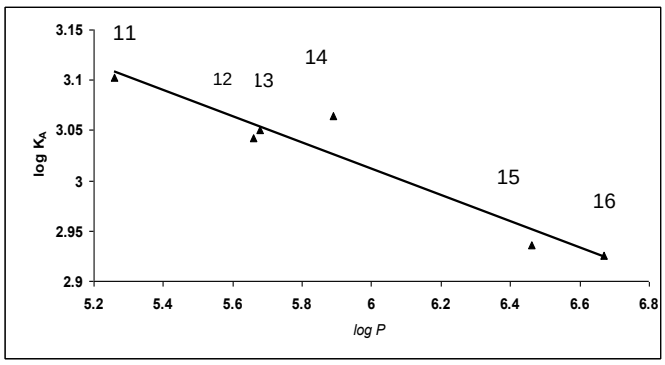


Figure 2. Plots of *log P* of diterpenoid acids **11-16** on their K_A complexes with **CA**.

As is shown in **Figure 1** K_A values for **CRA** complexes increase with increasing of the *log P* diterpenoid acids. At the same time increasing of the *log P* decreases K_A values for the **CA** complexes (**Figure 2**).

To clarify the nature of the Host-Guest interaction the molecular modelling of the **CRA** and **CA** Hosts **(A, B**) as well as their complexes with pimariic acid (**C**), abietic acid (**D**), and dehydroabietic acid (**E**) was performed (**Figure 3**).

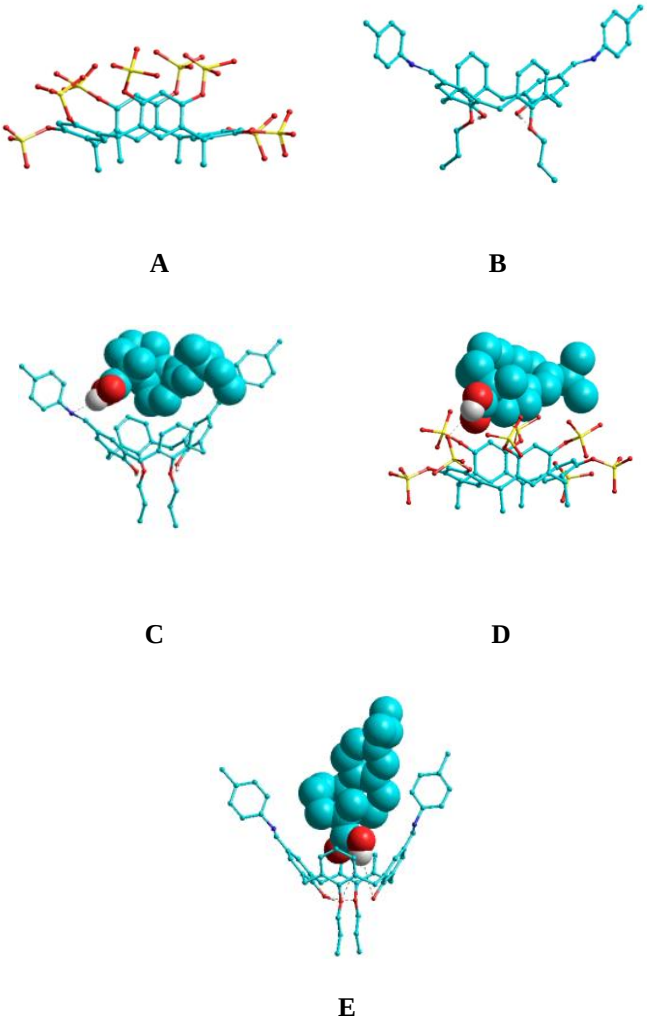


Figure 3. Energy minimized structures of **CRA (A**), **CA** (**B**), and complexes **CA**-pimaric acid (**C**), **CRA**-abietic acid (**D**), and **CA**-dehydroabietic acid (**E**). **(**Hyrogen bonds are presented by dotted lines. PhO substituents in **CRA** molecule are omitted for clarity).

In the complexes **C,D,E** the acid Guests **11,14,15** are included into three-dimentional molecular cavities of the Hosts **CA** and **CRA** decorated with imino or phosphoryl groups respectively. The complexes are stabilized by the hydrogen bonds of C(O)OH groups of the Guests with nitrogen or oxygen atoms of the calixarene Hosts. For example, pimaric acid **11** forms hydrogen bonds with nitrogen atom of C=N group at the upper rim of calixarene **CA** (N···O distance 3.11-3.17 Å). At the same time dehydroabietic acid **14** forms hydrogen bond with oxygen atom of OH group at the **CA** lower rim. Carboxylic group of abietic acid **15** in complex **D** interacts with oxygen atom of P=O group at the upper rim of **CRA** (O···O distance 3.45-3.65Å).

Conclusions

CA and **CRA** decorated with protonoaccepting imino or phosphoryl groups at the upper rim of macrocycle form stable supramolecular Host-Guest complexes with bio-active aromatic, pyridine and diterpenoid carboxylic acids in water-organic solution. The stability constants determined by RP HPLC method are depended on structure of the Host and Guest molecules and vary from 387 M^{-1} to 1914 M^{-1}. Molecular modelling show that the inclusion complexes formed are stabilized by the intermolecular hydrogen bonds of C(O)OH groups of the Guests with protonoaccepting nitrogen or oxygen atoms at the upper or the lower rim of the calixarene Hosts.

Acknowledgements

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Experimental part

Reagents

CRA was synthesized by the method [32] and **CA** by the method [33].

Methods and equipment

RP HPLC analysis

RP HPLC analysis was performed on the high pressure liquid chromatograph Hitachi (Hitachi, Ltd., Tokyo, Japan) in isocratic conditions using the column LiChrosorb RP 18 (Merck, Darmstadt, Germany) as a stationary phase. The calixarene based mobile phases were prepared by dissolving of the **CRA** in mixture MeOH/H_2O/formic acid (75/25/0.01, v/v) or **CA** in mixture MeCN/H_2O/formic acid (86/14/0.01, v/v). The calixarene concentration in the mobile phase vary from 0.1 x 10^{-4} M to 1.20 x 10^{-4} M. A volume of the sample injected was 20 μL. All chromatograms were obtained at 26 °C. UV detector was operated at 254 nm and the flow rate was 0.8 ml/min.

Determination of log P of the resin acids

Values of *log P* of the resin acids **11-16** were calculated from the equation *log P* = 7.746 · (log *k'*). The coefficient 7.746 is the relation of *log P* value of abietic acid (6.46) [34] to its log *k'* (0.834), determined by RP HPLC in this work.

Abietic acid was chosen as a standard compound in the calculation of the lipophilicity other log P resin acids **11-16.** It should be noted log P values of the acids determined by RP HPLC method are slightly different for mobile phases MeOH/H_2O/formic acid (75/25/0.01, v/v) (**CRA**) and MeCN/H_2O/formic acid (86/14/0.01, v/v) (**CA**).

Molecular modelling

The molecular modelling of **CA** and **CRA** and their complexes with the acids was carried out by molecular mechanics MM+ method, force field PM3 (software package Hyper Chem, version 8) [35]. RMS gradient was 0.01 kcal/mol**.**

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