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THE STUDY OF COMPLEXATION OF 5,17-BIS-(N-TOLYLIMINO-METHYL)-25,27-DIPROPOXYCALIX[4]ARENE WITH BENZOIC ACIDS BY RP HPLC AND MOLECULAR MODELING METHODS

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Key words: Calixarenes; benzoic acids; reversed-phase high performance liquid chromatography; inclusion complexes; binding constants

The Host-Guest complexation of 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene with benzoic acids has been studied by reversed-phase high-performance liquid chromatography (RP HPLC) method (the mobile phase – MeCN/H_2O, 86/14 v/v, the column support – LiChrosorb RP 18, UV detector, λ = 254 nm). The study of the chromatographic behaviour of 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene and benzoic acids, as well as determination of their main chromatographic characteristics – the retention times t_R and capacity factors k' have been performed. On the basis of the data obtained the lipophilicity log P, as well as the binding constants and Gibbs free energies of the complexes of 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene with benzoic acids have been calculated. The binding constants and Gibbs free energies of the complexes of 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene with benzoic acids are in the range of 335-910 M^{-1} or -14.38 – -16.85 kJ/mol, respectively. The influence of the benzoic acids lipophilicity log P and pKa values on the binding constants K_A of the complexes has been examined. It has been found that decrease of the log P and pKa values increases the binding constants K_A of the complexes. Molecular modeling of the complexes revealed the presence of hydrogen bonds between carboxylic groups of the acids and nitrogen atoms of imino-groups at the upper rim or oxygen atoms of the hydroxyl groups at the lower rim of the calixarene macrocycle. A linear dependence of the binding constants from the acid lipophilicity log P indicates a significant role of solvatophobic interactions during the complexation process.

ДОСЛІДЖЕННЯ КОМПЛЕКСОУТВОРЕННЯ 5,17-БІС-(N-ТОЛІЛІМІНОМЕТИЛ)-25,27-ДИПРОПОКСИКАЛІКС[4]АРЕНУ З БЕНЗОЙНИМИ КИСЛОТАМИ МЕТОДАМИ ОФ ВЕРХ ТА МОЛЕКУЛЯРНОГО МОДЕЛЮВАННЯ

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Ключові слова: *каліксарени; бензойні кислоти; метод обернено-фазної високоефективної рідинної хроматографії; комплекси включення; константи зв'язування*

Комплексоутворення типу Гість-Господар 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]арену з бензойними кислотами досліджено методом обернено-фазної високоефективної рідинної хроматографії (ОФ ВЕРХ) (рухома фаза - MeCN/H_2O, 86/14 за об'ємом, колоночна насадка марки LiChrosorb RP 18, УФ-детектор, λ = 254 нм). Проведено дослідження хроматографічної поведінки та визначені основні хроматографічні характеристики 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]арену та бензойних кислот-субстратів – час утримання t_R та коефіцієнти ємкості k'. На підставі отриманих даних розраховано значення ліпофільності log P бензойних кислот, а також констант зв'язування та вільних енергій Гіббса комплексів каліксарену з бензойними кислотами. Розраховані значення констант зв'язування та вільних енергій Гіббса комплексів 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]арену з бензойними кислотами знаходяться в межах 335-910 M^{-1} та -14.38 – -16.85 кДж/моль, відповідно. Досліджено вплив ліпофільності log P та pKa заміщених бензойних кислот на константи зв'язування K_A їх комплексів з каліксареном. Встановлено, що константи зв'язування K_A збільшуються по мірі зниження значень ліпофільності log P та pKa бензойних кислот. Проведено молекулярне моделювання комплексів включення, яке вказує на наявність водневих зв'язків між карбоксильними групами бензойних кислот та атомами водню іміногруп верхнього вінця або атомами кисню гідроксильних груп нижнього вінця каліксаренового макроциклу. Лінійна залежність констант зв'язування від ліпофільності log P кислот вказує на помітну роль сольватофобних взаємодій при їх комплексоутворенні з 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]ареном.

ИССЛЕДОВАНИЕ КОМПЛЕКСООБРАЗОВАНИЯ 5,17-БИС-(N-ТОЛИЛИМИНОМЕТИЛ)-25,27-ДИПРОПОКСИКАЛИКС[4]АРЕНА С БЕНЗОЙНЫМИ КИСЛОТАМИ МЕТОДАМИ ОФ ВЭЖХ И МОЛЕКУЛЯРНОГО МОДЕЛИРОВАНИЯ

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Ключевые слова: *каликсарены; бензойные кислоты; метод обращеннофазной высокоэффективной жидкостной хроматографии; комплексы включения; константы связывания*

Комплексообразование типа Гость-Хозяин 5,17-бис-(N-толилиминометил)-25,27-дипропоксикаликс[4]арена с бензойными кислотами исследовано методом обращеннофазной высокоэффективной жидкостной хроматографии (ОФ ВЭЖХ) (подвижная фаза – MeCN/H_2O, 86/14, колоночная насадка марки LiChrosorb RP 18, УФ-детектор, λ = 254 нм). Проведено исследование хроматографического поведения и опре-

делены основные хроматографические характеристики 5,17-бис-(N-толилиминометил)-25,27-дипропоксикаликс[4]арена-рецептора и бензойных кислот-субстратов – времена удерживания t_R и коэффициенты емкости k'. На основе полученных данных рассчитаны значения липофильности log P бензойных кислот, а также констант связывания и свободных энергий Гиббса комплексов 5,17-бис-(N-толилиминометил)-25,27-дипропоксикаликс[4]арена с бензойными кислотами. Рассчитанные значения констант связывания и свободных энергий Гиббса находятся в пределах 335-910 M^{-1} и -14.38 – -16.85 кДж/моль, соответственно. Исследовано влияние липофильности log P и pKa замещенных бензойных кислот на константы связывания K_A их комплексов с каликсареном. Установлено, что константы связывания K_A возрастают по мере снижения значений липофильности log P и pKa бензойных кислот. Проведенное молекулярное моделирование комплексов показало наличие водородных связей между карбоксильными группами бензойных кислот и атомами водорода иминогрупп верхнего обода или атомами кислорода гидроксильных групп нижнего обода каликсаренового макроцикла. Линейная зависимость констант связывания от липофильности log P кислот указывает на заметную роль сольватофобных взаимодействий при комплексообразовании с 5,17-бис-(N-толилиминометил)-25,27-дипропоксикаликс[4]ареном.

Molecular recognition, separation, membrane transport and analytical sensing of biorelevant molecules by artificial receptors constitute an important problem in chemistry and biology [1-7]. Calixarenes – «macrocyclic vases», which are easily available through the cyclocondensation of *para*-substituted phenols with formaldehyde, are widely used as molecular platforms for construction of specific receptors capable of highly selective recognition between fairly similar substrates [8, 9, 10]. Apparently, the outstanding receptor properties of functionalized calixarenes toward the biorelevant molecules make them highly promising materials for sensor technologies [11], as well as Host molecules for drug delivery systems in pharmaceutical science [5, 6, 8, 12-17].

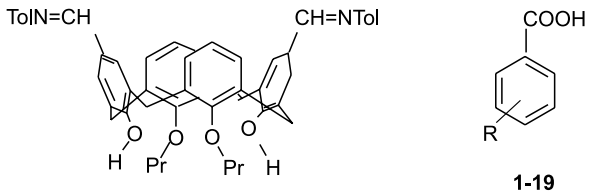
In this paper we report about the Host-Guest complexation study of 5,17-bis-(N-tolyliminomethyl)-25, 27-dipropoxycalix[4]arene Host (**CA**) with a series of *o-, м-, p*-substituded benzoic acids as the Guests (Chart).

Benzoic acids are widely used as drugs, and their salts as carriers of specific anions. In medical practice benzoic acid is used as an antibacterial and antifungal medicine for skin diseases and mycosis. Benzoic acid is a constituent of Whitfield's ointment, which is used for treating fungal skin diseases such as tinea, ringworm, and athlete's foot [18]. Its esters (from methyl to amyl) are used in the perfume industry. Hydrochloride of β-diethylaminoethyl ester of *p*-aminobenzoic acid is known as Novocain drug with a moderate anesthetic activity and a wide spectrum of the therapeutic action. *p*-Aminobenzoic acid (PABA) is the growth factor of microorganisms and its derivatives are used as effective antibacterial agents. PABA has been referred to as Vitamin B_{10}. Some bacteria in the human intestinal tract such as *E. coli* generate PABA from chorismate [19].

Information on the supramolecular Host-Guest interaction of **CA** with benzoic acids will be useful in design of calixarene based sensors or drug delivery systems for the biorelevant acids.

Experimental Part

The RP HPLC study was performed on a Hitachi chromatograph (Hitachi, Ltd., Tokyo, Japan) consisting of a high-pressure pump connected to a Rheodyne sample 7120 injector with a 20 μL loop (Rheodyne, Berkeley) and an ultraviolet-visible detector. The column (250×4.6 mm i.d.) was packed with LiChrosorb RP-18 (Merck, Germany). Acetonitrile was obtained from Acros Organics. Carboxylic acids were purchased from Sigma-Aldrich. **CA** was synthesized by the method [20]. The acetonitrile-water (86:14, v/v) mixture was used as a blank mobile phase. The calixarene based mobile phases were prepared by dissolving **CA** in the acetonitrile-water (86:14, v/v) mixture to obtain the **CA** concentration of 0.05-0.6 mM. The analytes for injections were dissolved in the same acetonitrile-water (86:14, v/v) mixture (C = 0.01 mM). The amount of the sample injected was 20 μL. All chromatograms were recorded at 22°C. The UV detector was operated at 254 nm. The mobile phase contain-



CA Host

1-19

Guests

R = H (1), *o*-Cl (2), *m*-Cl (3), *p*-Cl (4), *o*-CH_3 (5), *m*-CH_3 (6), *p*-CH_3 (7), *o*-NH_2 (8), *m*-NH_2 (9), *p*-NH_2 (10), *o*-NO_2 (11), *m*-NO_2 (12), *p*-NO_2 (13), *o*-OH (14), *m*-OH (15), *p*-OH (16), *o*-COOH (17), *m*-COOH (18), *p*-COOH (19)

Chart

Table

Retention times t_R, capacity factors k′, log P of benzoic acids **1-19**, K_A and DG values of their complexes with **CA**

Acid	Retention time, t_R, min	Capacity factor, k'	log P	K_A, M^{-1}	ΔG, kJ/mol
1	4.50	3.50	1.87	650±72	-16.02
2	3.72	2.72	1. 05	832±110	-16.63
3	3.74	2.74	1.51	798±89	-16.53
4	3.83	2.83	1.55	560±73	-15.65
5	5.50	4.50	2.25	401±42	-14.83
6	4.67	3.67	1.94	448±42	-15.10
7	3.83	2.83	1.55	439±53	-15.05
8	4.50	3.50	1.87	417±52	-14.92
9	3.90	2.90	1.59	510±61	-15.42
10	3.75	2.75	1.51	783±99	-16.48
11	3.65	2.65	1.46	623±93	-15.92
12	3.50	2.50	1.37	910±127	-16.85
13	3.91	2.91	1.60	608±63	-15.86
14	4.23	3.23	1.75	403±38	-14.84
15	3.33	2.33	1.26	335±35	-14.38
16	3.12	2.12	1.12	353±42	-14.51
17	4.27	3.27	1.77	749±75	-16.37
18	4.00	3.00	1.64	534±58	-15.53
19	3.80	2.80	1.54	450±38	-15.11

ing the **CA** additive was equilibrated for 3 h before the analysis. Under these conditions the column was saturated with the **CA** additive. The dead time t_0 was measured with $NaNO_2$.

Lipophilicity log P of acids **1-19** (Table) was calculated from equation log P = 3.438 (log *k'*). The coefficient 3.438 is the ratio of log P value of benzoic acid **1** (1.87) [21] to its log *k'* (0.544) determined by RP HPLC in this work (Table). Free Gibbs energy DG is determined by the equation DG = -RT ln K_A.

Molecular modelling of the **CA** complexes with acids **1-19** were carried out using Hyper Chem, 8.0 program [http://www.hyper.com/Download/AllDownloads/tabid/470/Default.aspx]. The structures were optimized by the semi-empirical PM3 method. The RMS gradient was equal to 0.01 kcal/A mol.

Results and Discussion

Calixarene **CA** and benzoic acids **1-19** in the given analysis conditions were registered on the chromatograms as sharp peaks. Calixarene **CA** retention time t_R and capacity factor *k'* were 5.67 min and 0.89, respectively. A linear isotherm character (Fig. 1) reflects reversible adsorption of calixarene **CA** on the LiChrosorb-RP18 support.

The chromatographic characteristics of acids **1-19** – retention times t_R, retention volumes V_R, capacity factors *k'*, their log P, as well as binding constants K_A

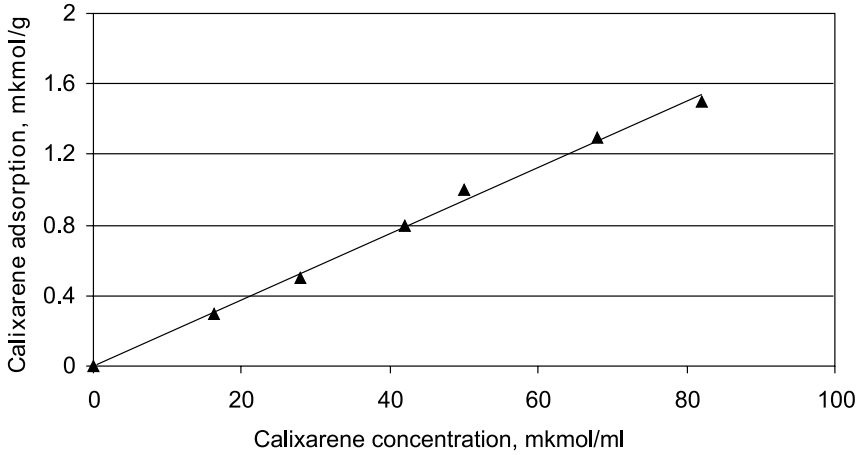


Fig. 1. The adsorption isotherm of calixarene (R^2=0.99).

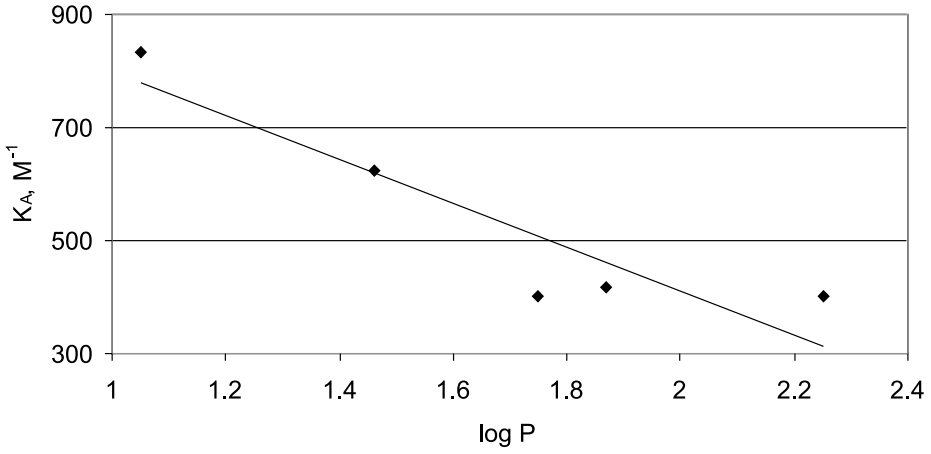


Fig. 2. Plots K_A *vs* log P for *o*-chlorobenzoic, *o*-nitrobenzoic, o-hydroxybenzoic, *o*-aminobenzoic, *o*-toluic acids (R^2=0.84).

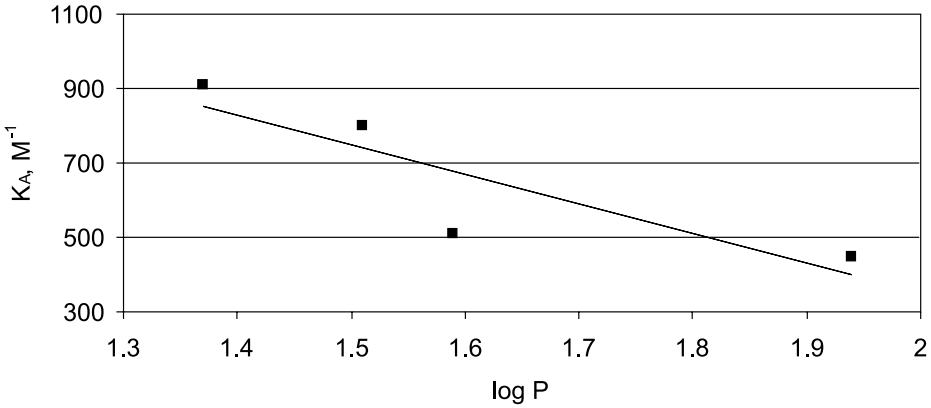


Fig. 3. Plots K_A *vs* log P for *m*-nitrobenzoic, *m*-chlorobenzoic, *m*-aminobenzoic, *m*-toluic acids (R^2=0.80).

and free Gibbs energies DG of their complexes with **CA** are presented in Table.

Binding constants of the supramolecular Host-Guest complexes of calixarene with benzoic acids **1-19** were determined by RP HPLC method in accordance with [20]. The method consists of the choice of analysis conditions of the Host and Guest, determination of the retention time t_R and the capacity factor k' of the Host, determination of the retention time t_R and the capacity factor k' of the Guest before and after Host addition to the mobile phase. Addition of calixarene to the mobile phase decreases the capacity factor k' values of benzoic acids. The linear character plots of k'*vs* the calixarene concentration (R^2 = 0.95-0.99) testifies formation of Host-Guest supramolecular complexes with 1:1 stoichiometry [20].

The binding constant K_A of the calixarene complex with the Guest molecule (the ratio of 1:1) can be calculated by equation (1):

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

where k_0' i k' – are capacity factors of the Guest molecule determined in the absence and the presence of **CA** in the mobile phase.

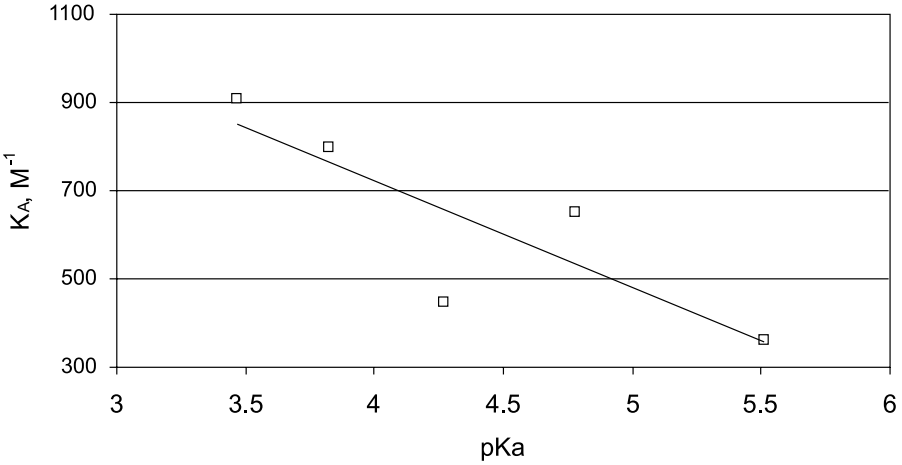


Fig. 4. Plots K_A *vs* pKa for *m*-nitrobenzoic, *m*-chlorobenzoic, *m*-aminobenzoic, *m*-phthalic acids (R^2=0.96).

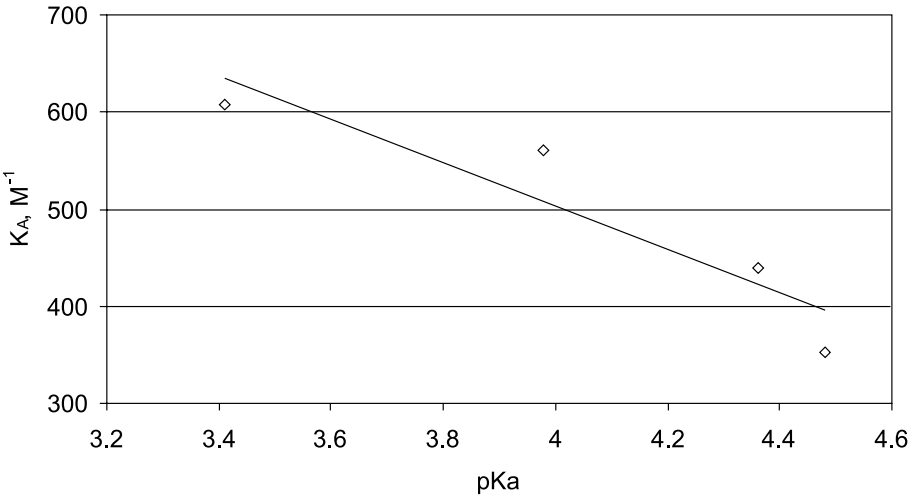


Fig. 5. Plots K_A *vs* pKa for *p*-nitrobenzoic, *p*-chlorobenzoic, *p*-toluic, *p*-hydroxybenzoic acids (R^2=0.94).

As shown in Table 1, the binding constants K_A of the complexes are in the range of 335-910 M^{-1}. The lowest K_A values were observed for hydroxybenzoic acids (335-403 M^{-1}) and toluic acids (401-448 M^{-1}). The highest K_A values were observed for phthalic (534-749 M^{-1}) and nitrobenzoic (608-910 M^{-1}) acids. It should be noted that the binding constants of **CA** with benzoic acid **1** is higher compared to its complex with *β*-cyclodextrin modified by three MeO-groups [22] (K_A = 650 M^{-1} and 550 M^{-1}, respectively).

Calixarene Host-Guest complexes are stabilized by various non-valence interactions – hydrogen bonds, Van der Waals, π-π, C-H...π, solvatophobic interactions [8]. It has been found that there are the linear dependence of the binding constants K_A from the lipophilicity log P of *o*-chlorobenzoic, *o*-nitrobenzoic, o-hydroxybenzoic, *o*-aminobenzoic, *o*-toluic acids (Fig. 2), as well as *m*-nitrobenzoic, *m*-chlorobenzoic, *m*-aminobenzoic, *m*-toluic acids (Fig. 3). It is interesting to note that increase of log P of the acids decreases K_A values of the complexes.

The binding constants K_A are strongly depended on pKa values of benzoic acids. These dependences for the Host-Guest complexes of *meta* and *para* substituted benzoic acids are demonstrated by Fig. 4 and Fig. 5, respectively. Unfortunately, for *p*-substituted acids such correlation has not been observed.

As it is shown in Fig.4, 5, the increase of pKa values of the acids decreases K_A of their complexes with **CA**. Unfortunately, for *o*-substituted acids such correlation was not observed.

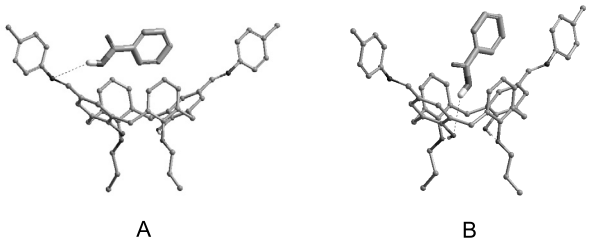


Fig. 6. Energy minimized structures of **CA** complexes with benzoic acid.

To clarify the nature of the supramolecular Host-Guest interactions the molecular modeling of **CA** complex with benzoic acid and *o*-, *m*-, *p*-phthalic acids was carried out. The inclusion complex of benzoic acid **1** shows two approximately energy equal structures **A** (ΔE=-29.38 kcal/mol) and **B** (ΔE=-31.15 kcal/mol) with one hydrogen bond between the Host and Guest molecules (Fig. 6).

In the structure **A** the hydrogen bond of the OH group of benzoic acid **1** with basic nitrogen of the imino group of **CA** is observed. In the structure **B** the OH group of the acid forms a hydrogen bond with the oxygen atom at the lower rim of the macrocycle.

Energy minimized structures of **CA** complexes with *o*-phthalic (**C**), *m* phthalic – (D) and *p*-phthalic (**E**) acids are presented in Fig. 7.

Calixarene complexes with *o*-phthalic **C** and *m*-phthalic **D** acids are stabilized by two hydrogen bonds. Thus, one carboxyl group forms a hydrogen bond with the imino nitrogen atom, and another with the oxygen atom of the hydroxyl groups at the lower rim of the macrocycle. Complex **E** with *p*-phthalic acid having the most distant carboxyl groups is stabilized by two hydrogen bonds with the distal imino nitrogen atoms.

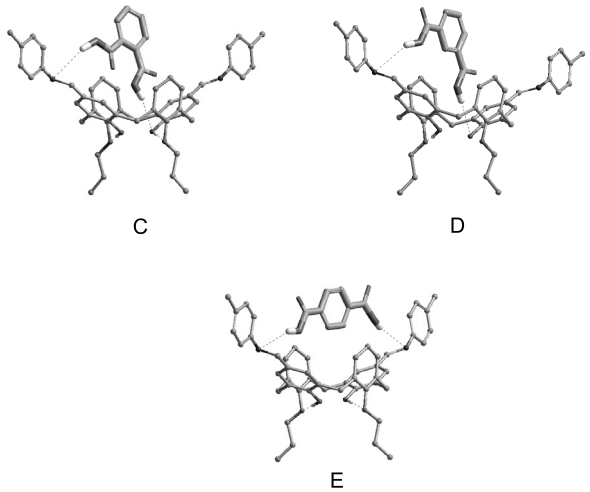


Fig. 7. Energy minimized structures of **CA** complexes with *o*-phthalic **C**, *m*-phthalic **D** and *p*-phthalic acid **E**.

It should be noted that the Host-Guest complexation does not change the *flattened-cone* conformation of calixarene confirming complementarity of benzoic acids to the molecular architecture of the calixarene macrocycle.

Conclusions

5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene containing two imino groups on the upper rim of the macrocycle forms the Host-Guest inclusion complexes with benzoic acids, which are characterized by binding constants 335-910 M^{-1} in acetonitrile-water solution. The data obtained provide the basis for creating a highly sensitive sensor for recognition and binding of biologically active benzoic acids in the biological media. Calixarene is a promising compound in design of the sensor devices or drug delivery systems for biorelevant acids.

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