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THE RESERVED-PHASE HPLC STUDY OF THE COMPLEXATION OF 5,17-BIS-(N-TOLYLIMINO-METHYL)-25,27-DIPROPOXYCALIX[4]ARENE WITH AROMATIC CARBOXYLIC ACIDS

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Key words: Calix[4]arene; reversed-phase high performance liquid chromatography; aromatic carboxylic acids; molecular modelling; Host-Guest complexation

The Host-Guest complexation of 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene with a number of aromatic carboxylic acids has been studied by reversed-phase high-performance liquid chromatography. The mobile phase was acetonitrile-water (80/20, v/v) with addition of 0.1% formic acid. The column was LiChrosorb RP 18, the UV detector operated at $\lambda = 254$ nm and at 26°C. The main chromatographic characteristics (retention time t_R and capacity factor k') of the aromatic carboxylic acids have been determined. The lipophilicity values of $\log P$ of carboxylic acids, as well as the binding constants K_A (387-941 M⁻¹) and Gibbs free energies ΔG (-14.74 – -16.94 kJ/mol) of the calixarene complexes with aromatic carboxylic acids have been calculated. The molecular modelling (Hyper Chem, version 8.0) of the calixarene complexes has 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 hydroxyl groups at the lower rim of the calixarene macrocycle. The influence of $\log P$ lipophilicity of acids on K_A values of the calixarene complexes has been assessed. The linear dependence of the binding constants on the acid lipophilicity indicates a significant role of solvophobic interactions on the complexation process. The relationship between supramolecular (K_A) and physicochemical (molecular weight, $\log P$, pKa) characteristics of acids has been found. The binding constants K_A of the complexes increase with increase of their molecular weight and $\log P$ values.

ОБЕРНЕНО-ФАЗНЕ ВЕРХ-ДОСЛІДЖЕННЯ КОМПЛЕКСООТВОРЕННЯ 5,17-БІС-(N-ТОЛІЛІМІНОМЕТИЛ)-25,27-ДИПРОПОКСИКАЛІКС[4]АРЕНУ З АРОМАТИЧНИМИ КАРБОНОВИМИ КИСЛОТАМИ

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Ключові слова: калікс[4]арен; обернено-фазна високоефективна рідинна хроматографія; ароматичні карбонові кислоти; молекулярне моделювання; комплексоутворення типу Гість-Господар
Комплексоутворення типу Гість-Господар 5,17-біс-(N-толлілімінометил)-25,27-дипропоксикалікс[4]арену з низкою ароматичних карбонових кислот досліджено методом обернено-фазної високоефективної рідинної хроматографії. Рухомою фазою – ацетонітрил-вода, (80/20, за об'ємом) з додаванням 0,1% мурашиної кислоти. Колонка – LiChrosorb RP 18, УФ детектор працював при довжині хвилі $\lambda = 254$ нм за температури 26°C. Визначені основні хроматографічні характеристики (час утримання t_R та коефіцієнт ємності k') карбонових кислот. Розраховані значення ліпофільності $\log P$ кислот, а також значення констант зв'язування K_A (387-941 M⁻¹) та вільних енергій Гібса ΔG (-14.74 – -16.94 kJ/mol) комплексів каліксарену з ароматичними карбоновими кислотами. Молекулярне моделювання (Hyper Chem, версія 8.0) каліксаренових комплексів вказало на присутність водневих зв'язків між карбоксильними групами кислот та атомами азоту іміно-груп на верхньому вінці або атомами кисню гідроксильних груп на нижньому вінці каліксаренового макроциклу. Оцінено вплив ліпофільності $\log P$ кислот на значення K_A каліксаренових комплексів. Лінійна залежність констант зв'язування K_A від ліпофільності кислот вказує на суттєву роль сольвофобних взаємодій у процесі комплексоутворення. Встановлено взаємозв'язок між супрамолекулярними (K_A) та фізико-хімічними характеристиками (молекулярна маса, $\log P$) кислот. Константи зв'язування K_A комплексів зростають зі збільшенням молекулярної маси кислот та їх ліпофільності $\log P$.

ОБРАЩЕННО-ФАЗНОЕ ВЭЖХ ИССЛЕДОВАНИЕ КОМПЛЕКСООБРАЗОВАНИЯ 5,17-БИС-(N-ТОЛИЛИМИНОМЕТИЛ)-25,27-ДИПРОПОКСИКАЛИКС[4]АРЕНА С АРОМАТИЧЕСКИМИ КАРБОНОВЫМИ КИСЛОТАМИ

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Ключевые слова: калікс[4]арен; обращенно-фазная высокоэффективная жидкостная хроматография; ароматические карбоновые кислоты; молекулярное моделирование; комплексообразование типа Гость-Хозяин
Комплексообразование типа Гость-Хозяин 5,17-біс-(N-толлілімінометил)-25,27-дипропоксикалікс[4]арена с некоторыми ароматическими карбоновыми кислотами исследовано методом обращенно-фазной высокоэффективной жидкостной хроматографии. Подвижная фаза – ацетонитрил-вода, (80/20, по объему) с добавкой 0,1% муравьиной кислоты. Колонка – LiChrosorb RP 18, УФ детектор работал при длине волны $\lambda = 254$ нм и температуре 26°C. Определены основные хроматографические характеристики (время удерживания t_R и коэффициент емкости k') ароматических карбоновых кислот. Рассчитаны

значения липофильности $\log P$ кислот, а также значения констант связывания K_A ($387\text{--}941\text{ M}^{-1}$) и свободных энергий Гиббса ΔG (-14.74 – -16.94 kJ/mol) комплексов каликсарена с ароматическими карбоновыми кислотами. Молекулярное моделирование (Nurag Chem, версия 8.0) каликсареновых комплексов показало присутствие водородных связей между карбоксильными группами кислот и атомами азота имино-групп на верхнем ободе или атомами кислорода гидроксильных групп на нижнем ободе каликсаренового макроцикла. Оценено влияние липофильности $\log P$ кислот на значения K_A каликсареновых комплексов. Линейная зависимость констант связывания K_A от липофильности кислот свидетельствует о существенной роли сольвофобных взаимодействий в процессе комплексообразования. Установлена взаимосвязь между супрамолекулярными (K_A) и физико-химическими характеристиками (молекулярная масса, $\log P$) кислот. Константы связывания K_A комплексов растут с увеличением молекулярной массы кислот и повышением значений их липофильности $\log P$.

An important problem in chemistry and biology is molecular recognition, separation, membrane transport and analytical sensing of biorelevant molecules by artificial receptors [1-7]. Calixarenes – “macro-cyclic vases”, which are easily available through the cyclocondensation of *para*-substituted phenols with formaldehyde, – are widely used as molecular platforms for constructing specific receptors capable of highly selective recognition between fairly similar substrates [8-10]. 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].

Aromatic carboxylic acids, such as benzoic, *p*-coumaric, cinnamic, gallic, diphenylacetic acid and their different derivatives are used in medical practice as antibacterial and antifungal agents for skin diseases and mycosis [18-23]. Many naturally occurring phenolic acids and analogues, namely caffeic and gallic

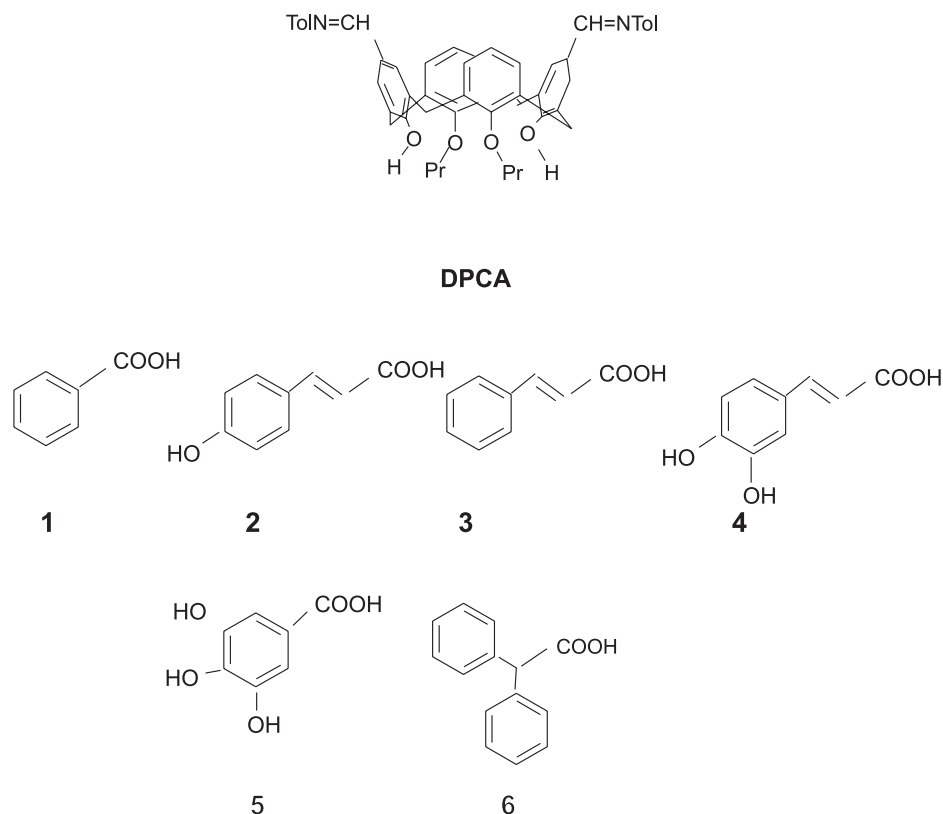
acids, are known to exhibit a wide variety of biological functions, in addition to their primary antioxidant activity, which are mainly related to modulation of carcinogenesis [24].

The information on the supramolecular Host-Guest interaction of calix[4]arenes with the aromatic carboxylic acids will be useful in the design of artificial receptors for such biorelevant compounds.

In this paper we reported the Host-Guests complexation study of 5,17-bis-(*N*-tolyliminomethyl)-25,27-dipropoxycalix[4]arene (**DPCA**) with benzoic **1**, *p*-coumaric **2**, cinnamic **3**, caffeic **4**, gallic **5**, diphenylacetic acid **6** (Scheme) by the reversed-phase high-performance liquid chromatography (RP HPLC) method in acetonitrile-water solution.

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,



Retention times t_{Rr} , capacity factors k' of carboxylic acids **1-6**, K_A and ΔG values of their complexes with **DPCA**

Substrate	Retention time, t_{Rr} min	Capacity factor, k'	K_A , M^{-1}	ΔG^a , kJ/mol
1 ^b	4.50	0.50	650±72	-16.02
2	3.68	0.23	692±111	-16.18
3	3.80	0.27	941±175	-16.94
4	3.90	0.30	520±70	-15.47
5	4.0	0.33	625±88	-15.92
6	4.48	0.49	387±48	-14.74

^a $\Delta G = -RT \ln K_A$

^b K_A was determined in [28]

Berkeley, USA) and an ultraviolet-visible detector. The column (250×4.6 mm i.d.) was packed with Li-Chrosorb RP 18 (Merck, Darmstadt, Germany). Acetonitrile was bought from the Acros Organics. Carboxylic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). **DPCA** was synthesized by the method [25]. The acetonitrile-water (80/20, v/v) mixture was used as a blank mobile phase. The calixarene based mobile phases were prepared by dissolving **DPCA** in acetonitrile-water (80/20, v/v), 0.1% formic acid mixture to obtain the **DPCA** concentration of 0.05-0.6 mM. The analytes for injections were dissolved in the mixture of acetonitrile-water (80/20, v/v) ($C = 0.01$ mM). The amount of the sample injected was 20 μ L. All chromatograms were recorded at 26°C. The UV detector operated at 254 nm. The dead time t_0 was measured with $NaNO_2$.

Determination of lipophilicity of $\log P$ of acids **1-6**

Lipophilicity of $\log P$ of acids **1-6** (Table) was calculated by the HPLC method from equation $\log P = K \times (\log k')$.

Table The coefficient K being the relationship of $\log P$ value of benzoic acid **1** (1.87) [26] to its $\log k'$ was determined by RP HPLC in this work.

Molecular modelling

Molecular modelling of **DPCA** complexes with acids **1-6** were carried out using a Hyper Chem, version 8.0 programme [27]. The structures were optimized by the semi-empirical PM3 method.

Results and Discussion

Calixarene **DPCA** and carboxylic acids **1-6** in the given conditions of analysis were registered on the chromatograms as sharp peaks. The chromatographic characteristics of carboxylic acids **1-6** (retention time t_{Rr} , capacity factor k'), their binding constants K_A and free Gibbs energies ΔG of their complexes with **DPCA** are presented in Table.

Binding constants of the inclusion Host-Guest complexes of **DPCA** with aromatic carboxylic acids **1-6** were determined by the RP HPLC method described in [29] and based on determination of retention factor k' of the Guest – carboxylic acids prior to and after the Host addition to the mobile phase. The **DPCA** addition to the mobile phase decreases retention factor k' of carboxylic acids **1-6**. The linear character plots of $1/k'$ vs the **DPCA** concentration (Fig. 1) indicate formation of the Host-Guest supramolecular complexes with 1:1 stoichiometry.

In accordance with the data obtained (Table) the highest K_A was observed for cinnamic acid ($941 M^{-1}$), and the lowest K_A was observed for the most bulky diphenylacetic acid ($387 M^{-1}$). The binding constants K_A strongly depended on the molecular weight (Fig. 2) and lipophilicity of $\log P$ (Fig. 3) of the acids.

There is the linear dependence of the binding constants K_A on lipophilicity of $\log P$ of cinnamic, *p*-coumaric, gallic, caffeic, benzoic and diphenylacetic acid (Fig. 3).

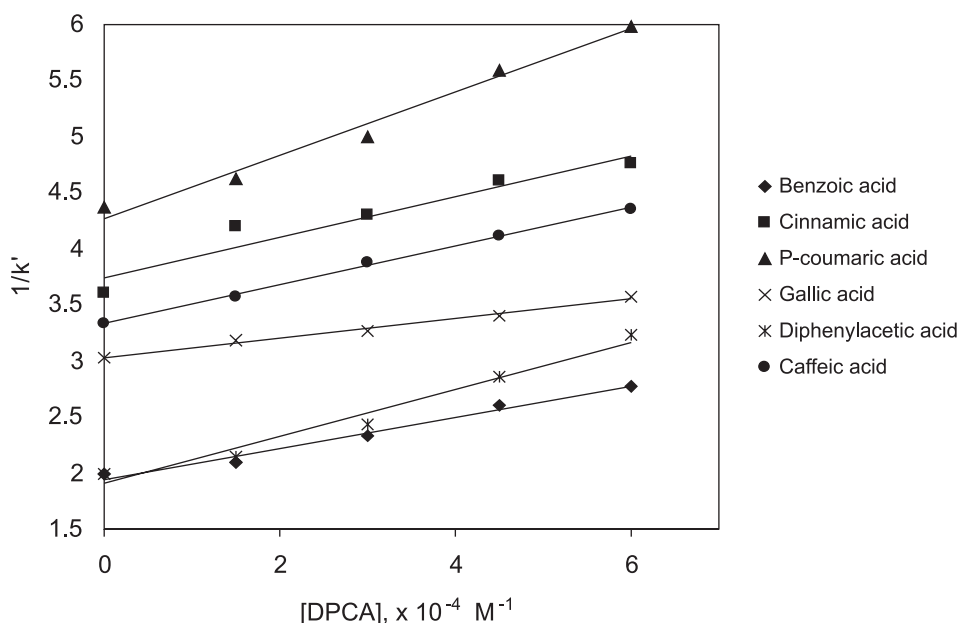


Fig. 1. Plots of $1/k'$ vs the **DPCA** concentration ($r = 0.98-0.99$).

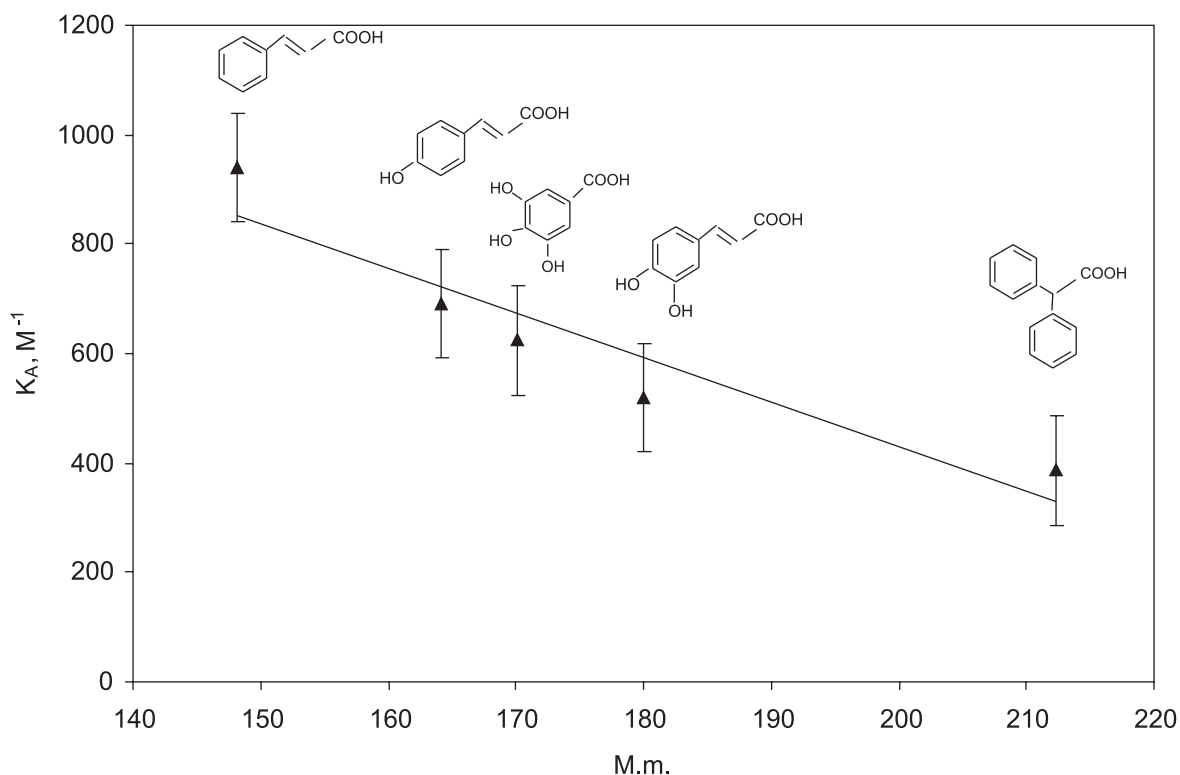


Fig. 2. The influence of the molecular weight of cinnamic, *p*-coumaric, gallic, caffeic and diphenylacetic acids on K_A of their complexes ($r = 0.98$).

The increase of $\log P$ values of the acids leads to increase of K_A values of their complexes with **DPCA**.

To clarify the nature of the supramolecular Host-Guest interactions the molecular modelling of **DPAA** complexes with cinnamic acid and diphenylacetic acid was carried out (Fig. 4).

Carboxylic acids deeply penetrate in the calixarene cavity (Fig. 4) with formation of the supramolecular Host-Guest complexes. The complexes are stabilized by the intermolecular hydrogen bonds $C(O)O-H \cdots OH$ formed by carboxylic groups of the Guest molecule with the oxygen atoms of the hydroxyl groups of the Host molecule.

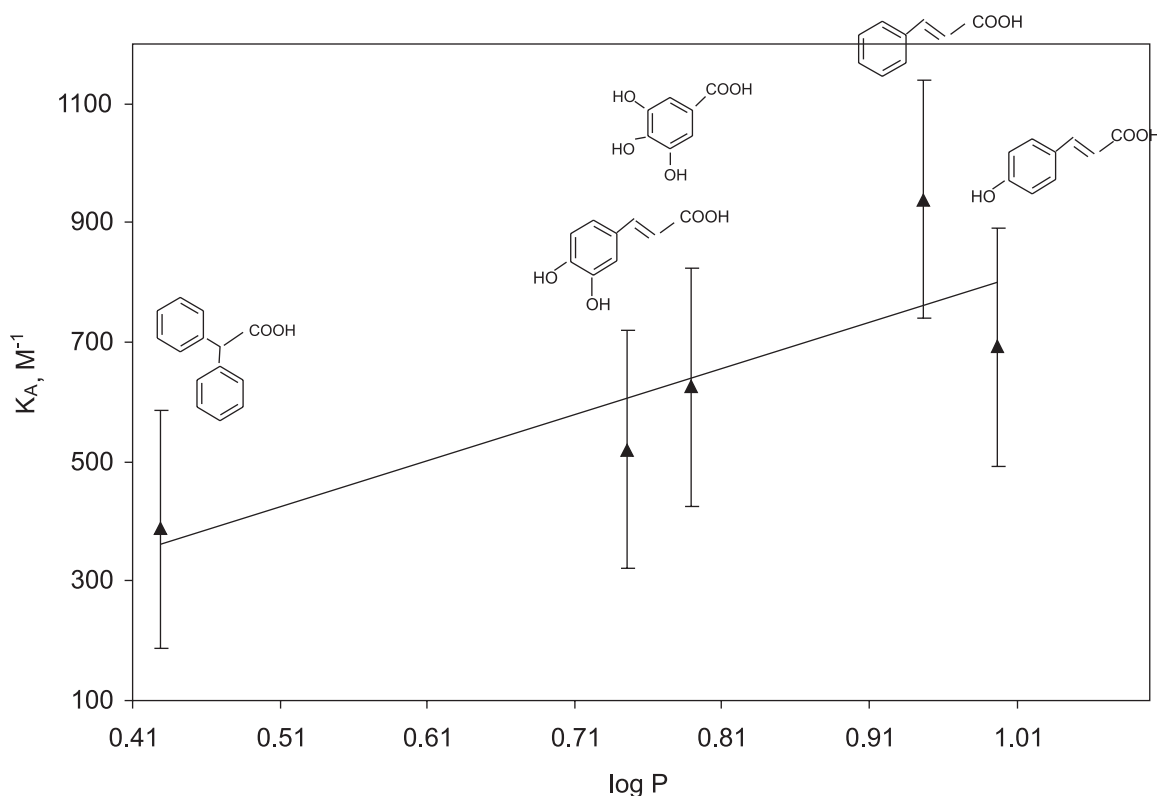


Fig. 3. Plots of K_A vs $\log P$ for diphenylacetic, caffeic, gallic, cinnamic and *p*-coumaric acids ($r = 0.83$).

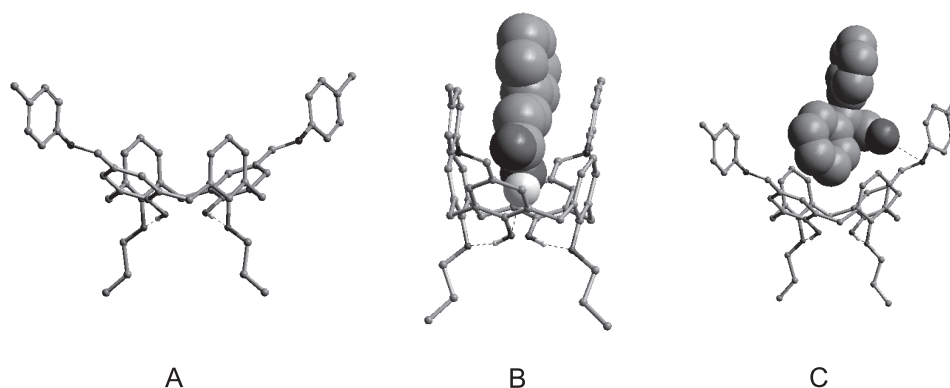


Fig. 4. The lowest energy structures of **DPCA (A)** and its complex with cinnamic (**B**) and diphenylacetic acid (**C**). Intermolecular and intramolecular hydrogen bonds are presented by dotted lines.

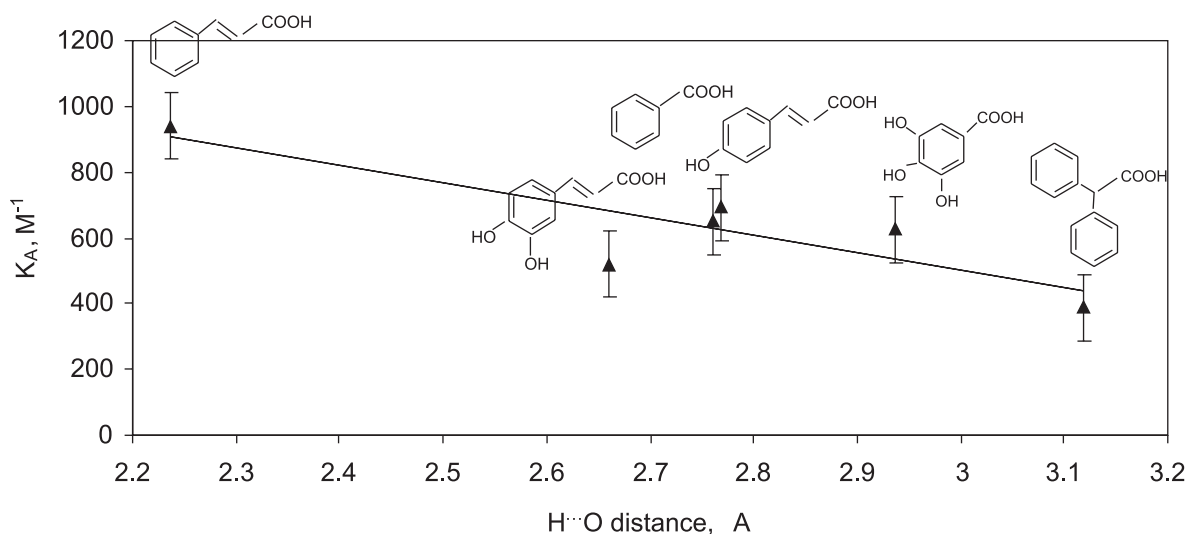


Fig. 5. Plots of K_A values vs $H\cdots O$ distances of the intermolecular hydrogen bonds between the Guest carboxylic groups and the Host hydroxyl groups at the lower rim of the macrocycle for cinnamic, caffeic, benzoic, *p*-coumaric, gallic and diphenylacetic acids ($r = 0.86$).

The proportional dependence of the binding constants of the complexes on the long hydrogen bonds $H\cdots O$ is observed (Fig. 5). Additionally, the complexes can be stabilized by the van der Waals stacking and interactions between the Host and Guest molecules (Fig. 4). In the case of diphenylacetic and benzoic acids the other hydrogen bonding is observed. Carboxylic groups form intermolecular bonds with the basic nitrogen atoms of imino groups (Fig. 4C). Phenyl groups of these acids are included into the molecular cavity as a result of π - π -stacking interactions. Plots of K_A values vs $H\cdots O$ distances of the intermolecular hydrogen bonds between the Guest carboxylic groups and the Host hydroxyl groups at the lower rim of the macrocycle is presented in Fig. 5.

Conclusions

DPCA containing two imino groups at the upper rim of the macrocycle forms the Host-Guest inclusion complexes with biorelevant aromatic carboxylic acids. Their binding constants (387 - $941 M^{-1}$) in acetonitrile-water solution depend on the molecular weight and $\log P$ of the acids. The complexes are stabilized by intermolecular hydrogen bonds between the Guest carboxylic groups and the Host hydroxyl groups, van der Waals stacking and solvophobic interactions. Calixarene is a promising compound in the design of sensor devices or drug delivery systems for such biorelevant compounds.

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