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THE STUDY OF THE COMPLEXATION OF CALIX[4]ARENE AND CALIX[4]RESORCINARENE WITH RESIN ACIDS BY THE RP HPLC METHOD. BINDING CONSTANTS DETERMINATION

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Key words: calix[4]arenes; resin acids; reversed-phase high performance liquid chromatography; inclusion complexes; binding constants; molecular modelling

The Host-Guest complexation of octakis-(diphenoxyphosphoryloxy)tetramethylcalix[4]resorcinarene (CR) and 5,17-bis-(N-tolyliminomethyl)-25,27-dipropoxycalix[4]arene (CA) with 6 diterpenoid (resin) acids has been studied by the reversed phase high-performance liquid chromatography (RP HPLC). The chromatographic characteristics (retention time t_R and retention factor k') of resin acids have been determined. The lipophilicity values $\log P$ of the acids, binding constants K_A (395-682 M^{-1} for CR and 844-1268 M^{-1} for CA), as well as Gibbs free energies ΔG (-14.79 – -16.14 kJ/mol for CR and -16.70 – -17.67 kJ/mol for CA) of the complexes with resin acids have been calculated. Molecular modelling of CA complexes has revealed the presence of hydrogen bonds between carboxylic groups of acids and nitrogen atoms of imino groups at the upper rim or oxygen atoms of the hydroxyl groups at the lower rim of the CA macrocycle. Molecular modelling of CR complexes has shown the presence of hydrogen bonds between carboxylic groups of acids and oxygen atoms of diphenoxyphosphoryloxy groups at the upper rim of the CR macrocycle. The effect of $\log P$ values on K_A values of the CR/CA 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. The relationship between supramolecular (K_A) and physicochemical ($\log P$, pK_a) characteristics of acids has been determined.

ДОСЛІДЖЕННЯ КОМПЛЕКСОУТВОРЕННЯ КАЛІКС[4]АРЕНУ ТА КАЛІКС[4]РЕЗОРЦИНАРЕНУ ІЗ СМОЛЯНИМИ КИСЛОТАМИ МЕТОДОМ ОФ ВЕРХ. ВИЗНАЧЕННЯ КОНСТАНТ ЗВ'ЯЗУВАННЯ

О.І.Кальченко, С.О.Черенок, А.В.Соловійов, В.В.Горбачук, С.Ю.Суйков, В.І.Кальченко

Ключові слова: калікс[4]арени; смоляні кислоти; обернено-фазна високоефективна рідинна хроматографія; комплекси включення; константи зв'язування; молекулярне моделювання

Комплексоутворення типу Гість-Господар октакіс-(дифеноксифосфорилокси)-тетраметилкалікс[4]резорцинарену (CR) та 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]арену (CA) з 6 дитерпеноїдними (смоляними) кислотами було досліджено методом обернено-фазної високоефективної рідинної хроматографії (ОФ ВЕРХ). Визначені хроматографічні характеристики (час утримання t_R та фактор утримання k') смоляних кислот. Розраховано значення ліпофільності $\log P$ смоляних кислот та констант зв'язування K_A комплексів (395-682 M^{-1} для CR та 844-1268 M^{-1} для CA), а також значення вільних енергій Гіббса ΔG (-14.79 – -16.14 кДж/моль для CR та -16.70 – -17.67 кДж/моль для CA) із смоляними кислотами. Молекулярне моделювання комплексів CA вказало на присутність водневих зв'язків між карбоксильними групами кислот та атомами азоту іміно-груп верхнього вінця CA макроциклу або атомами кисню ОН груп його нижнього вінця. Молекулярне моделювання комплексів CR вказало на присутність водневих зв'язків між карбоксильними групами кислот та атомами кисню дифеноксифосфорилокси-груп верхнього вінця макроциклу CR. Здійснено оцінку впливу $\log P$ на константи зв'язування K_A комплексів CR/CA. Лінійна залежність K_A від $\log P$ кислот вказує на роль сольофобних взаємодій на комплексоутворення. Встановлено взаємозв'язок між супрамолекулярними (K_A) та фізико-хімічними ($\log P$, pK_a) характеристиками кислот.

ИССЛЕДОВАНИЕ КОМПЛЕКСООБРАЗОВАНИЯ КАЛИКС[4]АРЕНА И КАЛИКС[4]РЕЗОРЦИНАРЕНА СО СМОЛЯНЫМИ КИСЛОТАМИ МЕТОДОМ ОФ ВЭЖХ. ОПРЕДЕЛЕНИЕ КОНСТАНТ СВЯЗЫВАНИЯ

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Ключевые слова: калікс[4]арени; смоляные кислоты; обращенно-фазная высокоэффективная жидкостная хроматография; комплексы включения; константы связывания; молекулярное моделирование

Комплексообразование типа Гость-Хозяин октакіс-(дифеноксифосфорилокси)-тетраэтилкалікс[4]резорцинарена (CR) и 5,17-біс-(N-толілімінометил)-25,27-дипропоксикалікс[4]арена (CA) с 6 дитерпеноидными (смоляными) кислотами было исследовано методом обращенно-фазной высокоэффективной жидкостной хроматографии (ОФ ВЭЖХ). Определены хроматографические характеристики (время удерживания t_R и фактор удерживания k') смоляных кислот. Рассчитаны значения липофильности $\log P$ смоляных кислот и констант связывания K_A их комплексов (395-682 M^{-1} для CR и 844-1268 M^{-1} для CA), а также значения свободных энергий Гиббса ΔG (-14.79 – -16.14 кДж/моль для CR и -16.70 – -17.67 кДж/моль для CA) со смоляными кислотами. Молекулярное моделирование комплексов CA показало наличие водородных связей между карбоксильными группами кислот и атомами азота имино-групп верхнего обода

макроцикла *CA* или атомами кислорода гидроксильных групп его нижнего обода. Молекулярное моделирование комплексов *CR* показало наличие водородных связей между карбоксильными группами кислот и атомами кислорода дифеноксифосфорилокси-групп верхнего обода макроцикла *CR*. Оценено влияние $\log P$ на константы связывания K_A комплексов *CR/CA*. Линейная зависимость K_A от $\log P$ кислот свидетельствует о влиянии сольвофобных взаимодействий на процесс комплексообразования. Установлена взаимосвязь между супрамолекулярными (K_A) и физико-химическими ($\log P$, pK_a) характеристиками кислот.

Naturally occurring di- and triterpenoid acids are isolated from different plant sources [1], have a wide variety of biological activities [2-9], and generate considerable interest in the pharmacological community. These compounds play an important role in searching new drugs for the treatment of different diseases. It is well known that abietic acid and abietane diterpenoids have the anti-inflammatory [10], phytoalexin-like [11], and anticonvulsant activities [12]. In the work [13] the antiviral activity against HHV-1 and HHV-2 for abietic and dehydroabietic acid was evaluated *in vitro*. Methyl abietate, abietinal, abietadienoic acid, methyl abietadienoate, abietadienol and dehydroabietinol acetate showed a significant anti-herpetic activity. Maleopimaric acid and its imide revealed the bacterial, fungicidal and nematocidal properties [14]. In the work [15] the antiulcer activity of quinopimaric acid was described.

Pentacyclic triterpenes – betulin, betulinic, oleanolic and ursolic acids possess the anticancer, anti-inflammatory and antiviral activity. Unfortunately, the biological activity of these compounds is reduced by their poor solubility or bioavailability. To improve these properties the supramolecular Host-Guest complexes of betulinic acid, ursolic acid (K_A 140 M^{-1}) or oleanolic acid (K_A 145 M^{-1}) with cyclodextrins were prepared and studied [16-18].

Along with cyclodextrins, calixarenes [19] are one of the most important categories of the supramole-

cular Hosts for application in pharmacology [20-22]. Compared to cyclodextrins, calixarenes exhibit a high degree of chemical functionalization, which leads to obtaining compounds with interesting physicochemical and binding properties. There are many conformational isomers of calixarenes, and a large number of cavities of different sizes and shapes, which can be involved in molecular recognition and binding processes.

Formerly it was shown that octakis(diphenoxyphosphoryloxy)calix[4]resorcinarene (**CR**) and bisiminocalix[4]arene (**CA**) appeared to be effective complexing agents for aromatic hydrocarbons [23], benzene carboxylic acids [24, 25], pyridine carboxylic acids [26] and 2,4-dichlorophenoxyacetic acid [27].

In this work for the first time the complexation **CR** and **CA** with 6 diterpenoid resin acids – pimaric **1**, maleopimaric **2**, palustric **3**, dehydroabietic **4**, abietic **5** and neoabietic **6** (Guest molecules) (Fig. 1) in water-containing solutions was studied, and stability constants of their supramolecular Host-Guest complexes were determined by the RP HPLC method. To the best of our knowledge, no complexation of any calixarenes with di- and triterpenoid acids was described in literature.

We believe that the study of the complexation of **CA** and **CR** with resin acids may be useful for understanding of the process of recognition and binding of diterpenoids in the aqueous medium. The results obtained can be used for developing drug delivery systems for these biologically active acids.

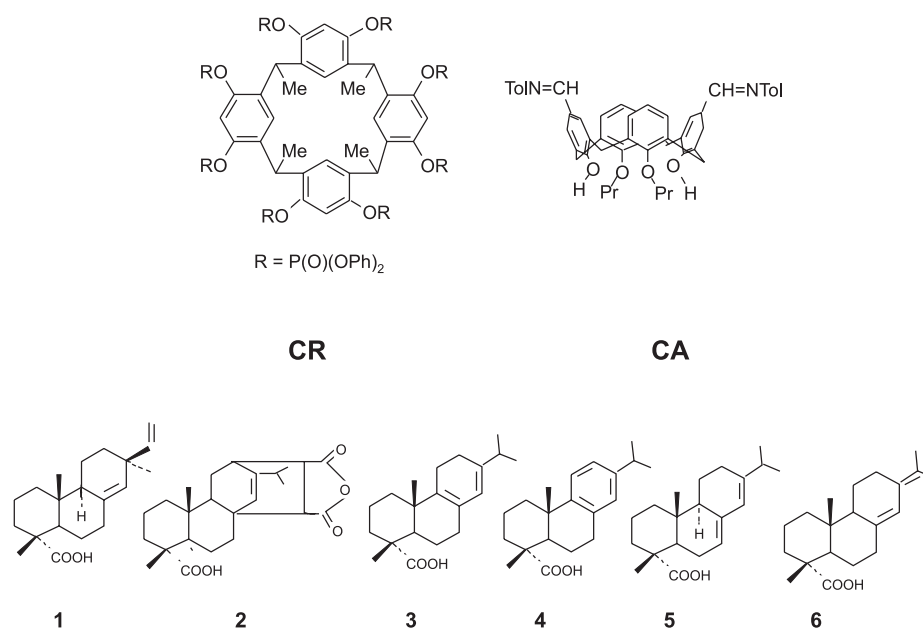


Fig. 1. Structural formulas of calix[4]resorcinarene **CR**, calix[4]arene **CA** (Hosts) and pimaric **1**, maleopimaric **2**, palustric **3**, dehydroabietic **4**, abietic **5**, neoabietic **6** acids (Guest).

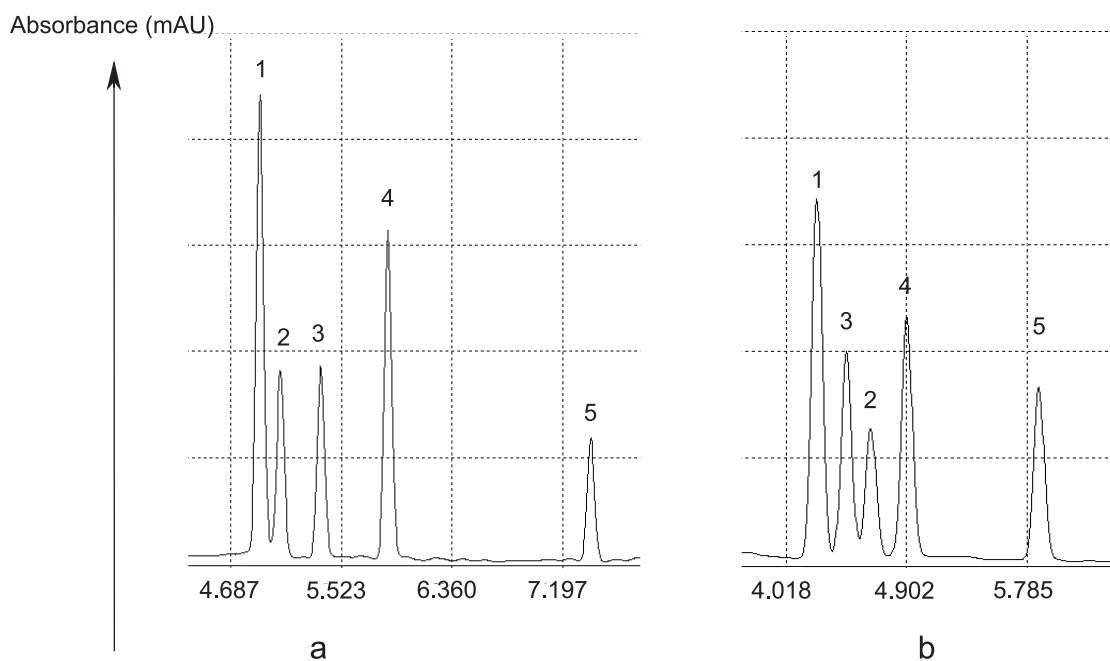


Fig. 2. Chromatograms of resin acids before (a) and after (b) **CR** addition to the mobile phase. Resin acids: **1** – pimaric; **2** – maleopimaric; **3** – palustric; **4** – dehydroabietic; **5** – abietic.

Results and Discussion

The **CR/CA** were registered on the chromatograms by sharp peaks with retention factors k' 8.65 (**CR**) and 0.89 (**CA**). Chromatograms of the resin acids obtained before and after **CR** and **CA** addition in the mobile phase are presented in Fig. 2, 3.

A comparative estimation of the chromatograms presented in Fig. 2, 3 shows that **CR** and **CA** addition to the mobile phase decreases the retention times and changes the elution order of acids. It should be noted **CA** addition allows separating abietic **5** and neoabietic **6** acids (Fig. 3). Formation of the Host-Guest inclusion complexes weakens the interaction of these

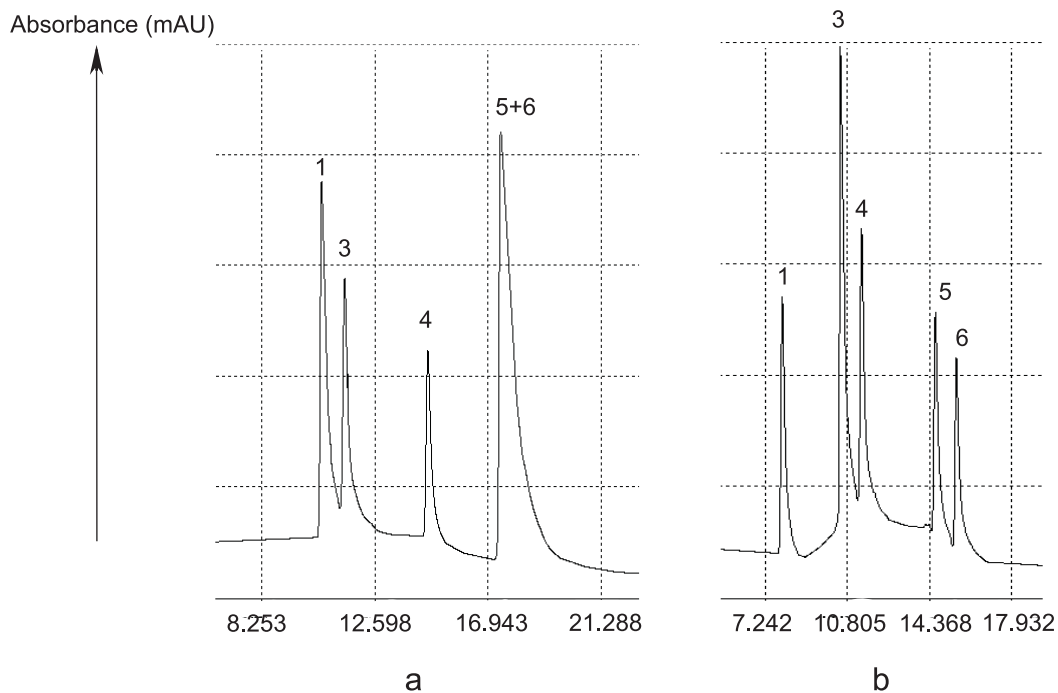


Fig. 3. Chromatograms of resin acids before (a) and after (b) **CA** addition to the mobile phase. Resin acids: **1** – pimaric; **3** – palustric; **4** – dehydroabietic; **5** – abietic; **6** – neoabietic.

Table 1Retention factors k' of resin acids **1-6** determined before and after **CR/CA** addition

Resin acid	Retention factor, k' (RSD = 3-5%)			
	Before CA addition	After CA addition	Before CR addition	After CR addition
Pimaric	10.17	7.38	3.80	3.17
Maleopimaric	12.21	8.91	4.66	4.0
Palustric	12.29	8.67	4.71	3.76
Dehydroabietic	13.38	9.79	5.17	3.41
Abietic	17.22	12.67	6.83	5.26
Neobietic	19.18	14.40	7.66	5.63

Table 2The values of K_A (M^{-1}) and ΔG (kJ/mol) of the calixarene complexes with resin acids **1-6**

Resin acid	CR		CA	
	K_A ($\bar{A}\pm s$)	ΔG	K_A ($\bar{A}\pm s$)	ΔG
Pimaric	395±71	-14.79	1268±228	-17.67
Maleopimaric	548±80	-15.60	1102±176	-17.33
Palustric	464±66	-15.19	1121±157	-17.37
Dehydroabietic	640±95	-15.98	1158±174	-17.45
Abietic	557±82	-15.64	862±121	-16.72
Neobietic	682±102	-16.14	844±135	-16.70

acids with the stationary phase in the RP HPLC conditions. The linear character of $1/k'$ vs plots on the calixarene concentration ($r = 0.99$) indicates formation of the Host-Guest supramolecular complexes with 1:1 stoichiometry. The retention factors k' of acids **1-6** obtained before and after addition of **CR** and **CA** to the mobile phase are presented in Tab. 1.

The binding constants K_A and free Gibbs energies ΔG ($\Delta G = -RT \ln K_A$) of the Host-Guest calixarene complexes with the acid molecules were calculated by the method described in and are presented in Tab. 2.

As shown in Tab. 2, the binding constants K_A of resin acids **1-6** are in the range of 395–682 M^{-1} for **CR** complexes and 844–1268 M^{-1} for **CA** complexes. The complexes of resin acids can be stabilized by different supramolecular interactions (hydrogen bonds, van der Waals, solvophobic interaction, etc.). Therefore, the

role of hydrophobic interactions for the complexes is confirmed by the binding constants correlation with $\log P$ of resin acids (Fig. 4, 5).

Increase of the $\log P$ values of acids increases the K_A values of their complexes with **CR** (Fig. 4), but decreases K_A values for the complexes with **CA** (Fig. 5).

To clarify the nature of supramolecular interactions the molecular modelling of **CA** and **CR** complexes with resin acids were carried out. The Host-Guest complexation with **CA** is presented in Fig. 6.

As shown in Fig. 6A-6F, all resin acids are included into the macrocyclic cavity of **CA**. The inclusion of Host-Guest complexes is stabilized by different supramolecular interactions, first of all, hydrogen bonds. In the complexes shown in Fig. 6A, 6C and 6D the hydroxyl group of pimaric, abietic and neobietic acids, respectively, form hydrogen bonds with a basic nitrogen atom

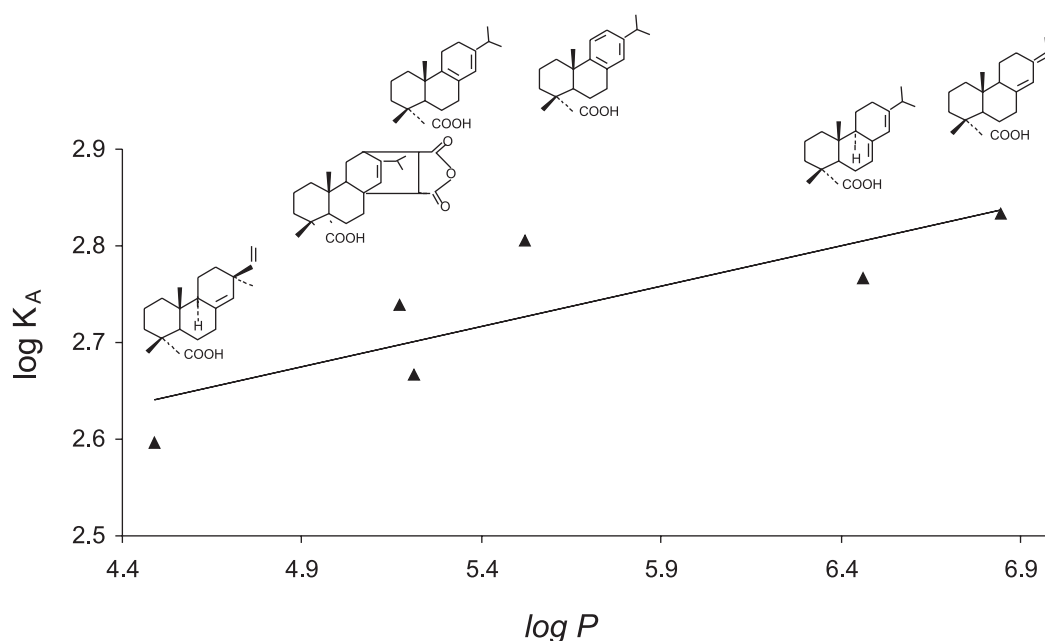


Fig. 4. The plot of $\log K_A$ vs $\log P$ for **CR** complexes with pimaric **1**, maleopimaric **2**, palustric **3**, dehydroabietic **4**, abietic **5**, neobietic **6** acids (the relationship described this correlation is: $y = 0.0835x + 2.2658$; $r = 0.83$).

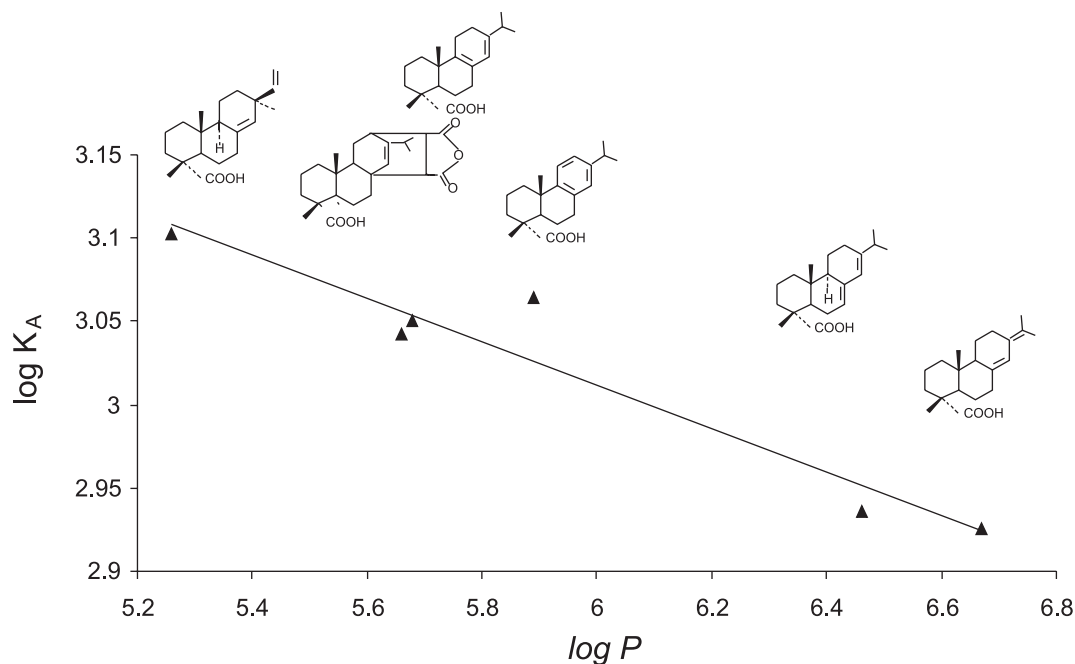


Fig. 5. The plot of $\log K_A$ vs $\log P$ for **CA** complexes with pimarinic **1**, maleopimaric **2**, palustric **3**, dehydroabietic **4**, abietic **5**, neoabietic **6** acids (the relationship described this correlation is: $y = -0.1307x + 3.7964$; $r = 0.96$).

of the upper rim imino group. The carboxylic groups of dehydroabietic, palustric and maleopimaric acids form hydrogen bonds with oxygen atoms on the **CA** lower rim (Fig. 6B, 6E, 6F).

Experimental Part

The methanol and acetonitrile were obtained from Acros Organics (Thermo Fisher Scientific, New Jersey – USA), and resin acids were obtained from Sig-

ma-Aldrich (Sigma-Aldrich Corporation, Sigma-Aldrich Box 14508, St. Louis Missouri, 63178, USA). **CR** was synthesized by the method [28] and **CA** – by the method [29].

RP HPLC analysis

The chromatographic experiment was performed on a Hitachi liquid chromatographic system (Hitachi, Ltd., Tokyo, Japan) with an UV detector ($\lambda = 254$ nm). The column (250×4.6 mm i.d.) was LiChrosorb RP 18

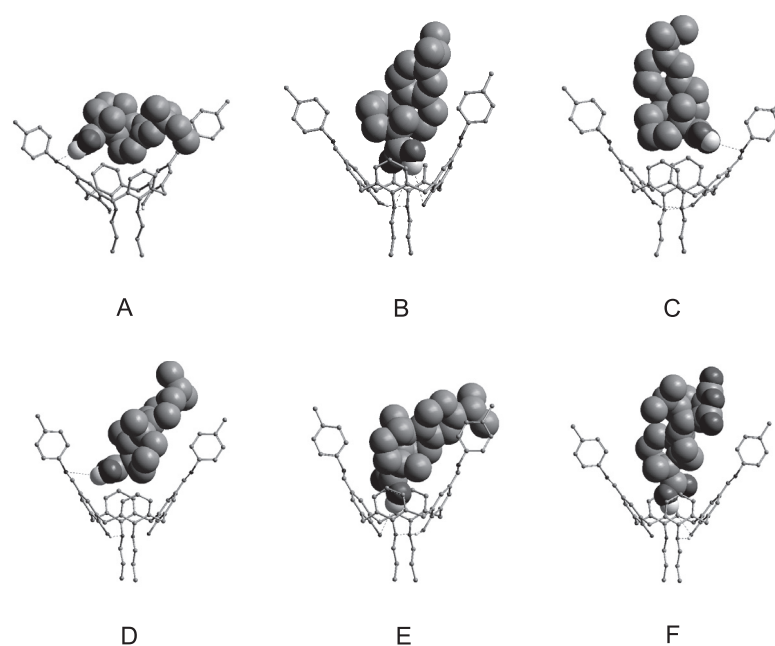


Fig. 6. Energy minimized structures of **CA** complexes with pimarinic (**A**), dehydroabietic (**B**), abietic (**C**), neoabietic (**D**), palustric (**E**) and maleopimaric (**F**) acids.

(Merck, Germany). The mobile phases based on **CR** and **CA** ($C = 0.01$ mM) were prepared by dissolving calixarenes in MeOH/H₂O/formic acid (75/25/0.01, v/v) (phase A for **CR**) and MeCN/H₂O/formic acid (86/14/0.01, v/v) (phase B for **CA**), respectively. The sample injected was 20 μ L. All chromatograms were obtained at 22°C. All measurements were performed in triplicate. The phases A and B were used as blank ones for **CR** and **CA** analysis, respectively.

The binding constants of **CR** and **CA** complexes with acids **1-6** were calculated by the RP HPLC method described in [30] by changing of the retention factor k' values for acids during complexation.

Determination of the $\log P$ values of resin acids and molecular modelling

The values of $\log P$ of acids **1-6** were calculated from the equation: $\log P = 7.746 \cdot (\log k')$ where coefficient 7.746 was the ratio of the experimental value of $\log P$ of abietic acid 6.46 [31] to $\log k'$ value of abietic acid 0.834 determined by the RP HPLC method in this work.

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