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

O.I.Kalchenko, A.V.Solovyov*, V.I.Kalchenko

Institute of Organic Chemistry, National Academy of Sciences of Ukraine
5, Murmanska str., Kyiv-94, 02660. E-mail: vik@ioch.kiev.ua
* Present address: Department of Chemical & Biomolecular Engineering, University of California, Berkeley, USA, CA 94720-1460

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 λ = 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 \(\Delta G\) (-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.
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,
Berkeley, USA) and an ultraviolet-visible detector. The column (250×4.6 mm i.d.) was packed with LiChrosorb 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 μL. All chromatograms were recorded at 254 nm. The dead time \( t_0 \) was measured with NaNO2.

**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 \( \log P = K x (\log k') \).

| Substrate | Retention time, \( t_{r, \text{min}} \), min | Capacity factor, \( k' \) | \( K_a \), M\(^{-1} \) | \( \Delta G^\circ \), 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]

In accordance with the data obtained (Table) the highest \( K_a \) was observed for cinnamic acid (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 \( t_{r, \text{min}} \), capacity factor \( k' \)), their binding constants \( K_a \) and free Gibbs energies \( \Delta G \) 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 \( 1/k'\nu \) 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’\ν vs the DPCA concentration (r = 0.98-0.99).](image-url)
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…OH formed by carboxylic groups of the Guest molecule with the oxygen atoms of the hydroxyl groups of the Host molecule.

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

Fig. 3. Plots of $K_A$ vs log P for diphenylacetic, caffeic, gallic, cinnamic and p-coumaric acids ($r = 0.83$).
The proportional dependence of the binding constants of the complexes on the long hydrogen bonds H–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–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.

References

1. Ludwig R. Microchimica Acta, 2005, Vol. 152, pp.1-19.
2. Menon K., Sewani M. Reviews in Analytical Chemistry, 2006. Vol. 25, pp.49-82.
3. Mutihac L., Buschmann H. J. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2005, Vol. 51(1), pp.53-57.
4. De Fátima A., Fernandes S. A., Sabino A. A. Current Drug Discovery Technologies, 2009, Vol. 6, pp.151-170.
5. Rodik R. V., Boyko V. I., Kalchenko V. I. Current Medicinal Chemistry, 2009. Vol. 16, pp.1630-1655.
6. Cherenok S., Kalchenko V. Topic Heterocycl. Chemistry, 2009, Vol. 20, pp.229-273.
7. Sansone F., Baldini L., Cassati A., Ungaro R. New Journal of Chemistry, 2010, Vol. 34, pp.2715-2728.
8. Gutsche C. D. Calixarenes: Introduction, Monographs in Supramolecular Chemistry, Cambridge, The Royal Society of Chemistry, 2008.
9. Asfari Z., Boehmer V., Harowfield J., Vicens J. (Eds.). Calixarenes 2001, Dordrecht, Kluwer Academic, 2001.
Acknowledgements

This work was supported by the State Fund for Fundamental Research of Ukraine.