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Some Aspects of Extractability and Transport of Amino Acid Esters by Calixarenes

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Abstract

The extraction abilities and the transport through chloroform liquid membrane of *p*-tertbutylcalix[n]arenes (n = 6, 8) upon some amino acid methylesters have been investigated. The experimental results suggested that amino acid methylesters are extracted into organic phase and transported by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) as counterion. The extractability and the transport have been proved to be essentially controlled by the structure of calixarene, the nature of amino acid and the nature of anion used as ion pair for cation-receptor complexes. Also, the results suggested that there is no relationship between the extraction efficiency and the hydrophobicity of amino acid esters. The results suggest further possibilities for optimal separation of amino acids derivatives and other biological species.

Introduction

Calixarene derivatives as important receptors of supramolecular hosts have the ability to form complexes with several charged or uncharged guest species. The calixarenes formed by condensation of phenols with formaldehydes have properties, which make them interesting receptors especially in enzyme mimics, catalysis, separation of organic compounds, field effect transistors, chemical sensors and binding biological substrates [1–12]. Much work has been dedicated to the synthesis of calixarenes modified on the upper [1, 2] and lower rims [13]. These derivatives present the ability to form complexes with different compounds. There are studies dedicated to the molecular inclusion of biological substrates, like biogenic amines, amino acids, and peptides by these receptors [14–17]. Comparing with cyclodextrins, the calixarene molecules exhibit large sterical flexibility. Chiral recognition of α-amino acids by calixarenes has been investigated [18, 19].

Of particular interest of some calixarene derivatives is their ability to form inclusion complexes in water [7, 14]. Thus many complexation studies have been reported with water-soluble calixarenes and neutral and charged guests by means of ^{1}H NMR, induced circular dichroism, spectrophotometric or calorimetric measurements [16, 17, 20–22]. The water-soluble tetrasulfonated calix[4]arene and hexasulfonated calix[6]arenes bind very strongly the quaternary ammonium cations, and also acetylcholine [23]. From X-ray crystallographic studies of the choline tetrasulfonated calix[4]arene complex established that the choline has its N-terminal inside the aromatic cavity of the receptor.

The interesting studies have been realized by using calixarene compounds for separation of positional isomers in capillary electrophoresis [24]. The mass spectrometry studies have evidenced that calix[6]arene derivatives present stronger binding of amino acids in the gas phase than calix[4]arenes [25]. This suggested that size is an important factor for increasing the strength of the interaction between the amino acid and the host. Also, similarly the underivatized calix[6]arene and calix[4]arene did not form complexes [25]. The complex of calix[4]arene with benzylammonium cation has been studied by gas phase ion molecule reactions [26, 27]. A series of calixarene carboxylic acid derivatives were used as host molecules for the quantitative extraction of amino acids in a liquid-liquid extraction system [28]. The interaction between the ammonium cation of the amino acid and the oxygen atoms of the host molecule was the main driving force for the complexation and the extraction mechanism was established by slope analysis and the Job method. Using calix[4]arene-based aminophosphonates as a carrier through membrane, the transport of zwitterionic amino acids have been realized [29]. The aminophosphonate groups were responsible for the transport efficiency and the order of the transport rate.

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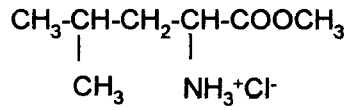
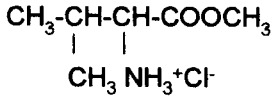


L-leucine methylester hydrochloride



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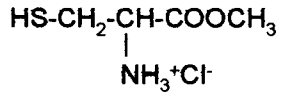
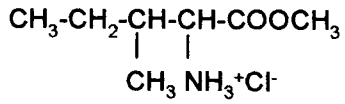
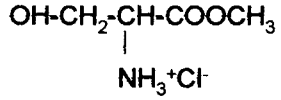


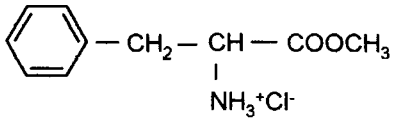
L-cysteine methylester hydrochloride



L-isoleucine methylester hydrochloride



L-serine methylester hydrochloride



L-phenylalanine methylester hydrochloride

Chart 1. The chemical structures of amino acid derivatives used in experiments.

The calix[4]arene with chiral pendant groups has been used for chiral recognition of some L-amino acid derivatives through liquid membrane [30].

In our previous study on the transport of the biogenic amines and aromatic amino acid methylesters in protonated form as ion pairs in the presence of picrate anion by using *p*-tert-butylcalix[n]arenes (n = 6, 8) as carries, we have shown that the above-mentioned calixarenes transport these amino acids through liquid membrane [31]. The results showed that the inclusion abilities of the investigated hosts were correlated with their conformational properties.

In order to observe the possibility of calixarenes to act as an extractant or carrier through membrane we have investigated some aspects of the liquid–liquid extraction and the transport through chloroform liquid membrane of amino acid methyl esters with *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00. The effects of physicochemical parameters such as the structure of calixarene and the nature of amino acid have been studied. Additionally, the influence of the composition and the structure of the compounds under study upon the partition processes occurring in biphasic and triphasic systems have been reported. The main goal of our experiments was performing the solubilization of some biomolecules in an organic medium and their optimal separation through liquid membrane.

Experimental

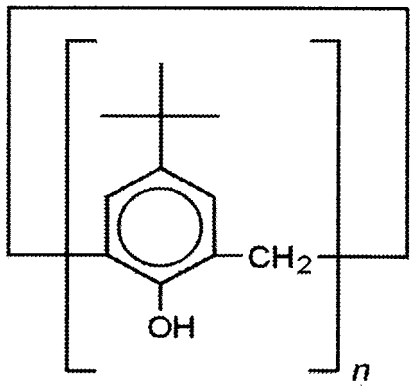
The following amino acid methylester hydrochlorides given in Chart 1 were obtained from Fluka at the highest purity commercially available: L-phenylalanine methylester hydrochloride (L-PheOMe), L-leucine methylester hydrochloride (L-LeuOMe)3 L-isoleucine methylester hydrochloride (L-IleOMe), L-valine methylester hydrochloride (L-ValOMe), L-cysteine methylester hydrochloride (L-CysOMe), and L-serine methylester hydrochloride (L-SerOMe).

Reagent grade-*p*-tert-butylcalix[6]arene and *p*-tert-butylcalix[8]arene were obtained from Merck and they were used throughout the experiments without further purification. The structures of calixarenes are shown in Figure 1. Chloroform (dielectric constant $\epsilon_r = 4.81$ [32]), the organic solvent employed, was distilled before use. Reagent grade tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) from Fluka (Figure 1), distilled, and deionised water were used throughout the experiments.

The transport experiments were carried out using a device previously described [33] consisting of two concentric tubes: the inner one, which also acted as a stirrer, contained the source phase (10 ml), whereas the receiving phase (10 mL) together with the membrane phase (35 mL) were introduced in the outer tube.

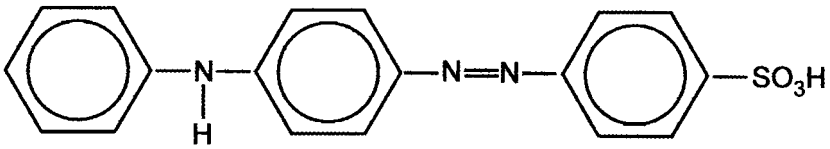
The phases were stirred at 200 rpm for 24 h. Each experiment was repeated at least three times. Reproducibility was ±10%. Similar transport experiments were performed in the absence of the carrier, for reference. The pH was measured by a digital MV-870 Pracitronic pH-meter with glass electrode and saturated calomel electrode.

Equal volumes of 3.0×10^{-4}–3.0×10^{-3} M of amino acid methylester and 6.0×10^{-4} M of tropacolin 00 as counterion at pH ~ 5.0 in aqueous phases were extracted with 5.0×10^{-3}–1.0×10^{-2} M calixarenes in the chloroform phase. In order to prevent volume change during extraction, the chloroform and water were saturated with each other. The phases were mixed and shaken for 30 minutes at 25 ± 1° C. The pH of the aqueous solutions was adjusted by the hydrochloric acid. The extractability was calculated as the ratio $E[\%] = \frac{(A_0 - A)}{A_0} \times 100$, where A_0 and A are the absorbencies of the aqueous phases before and after the extraction with calixarenes, respectively. The absorbency was



n=6,8

(a)



(b)

Figure 1. Structures of calixarenes (a) used throughout the experiments and tropaeolin 00: (([4-(4′-anilinophenylazo)benzenesulfonic acid]) (b).

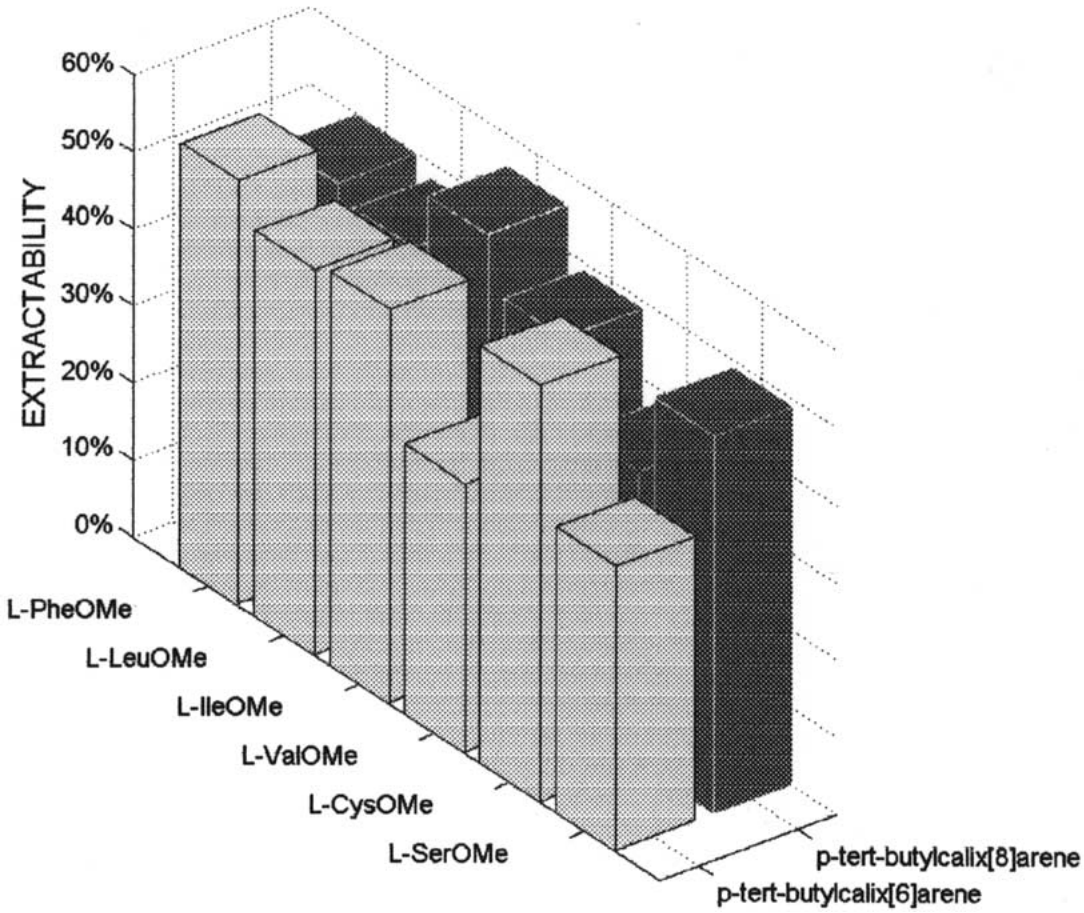


Figure 2. Experimental data of the extractability of some amino acid methylester hydrochlorides from the aqueous phase into chloroform phase by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaelon 00.

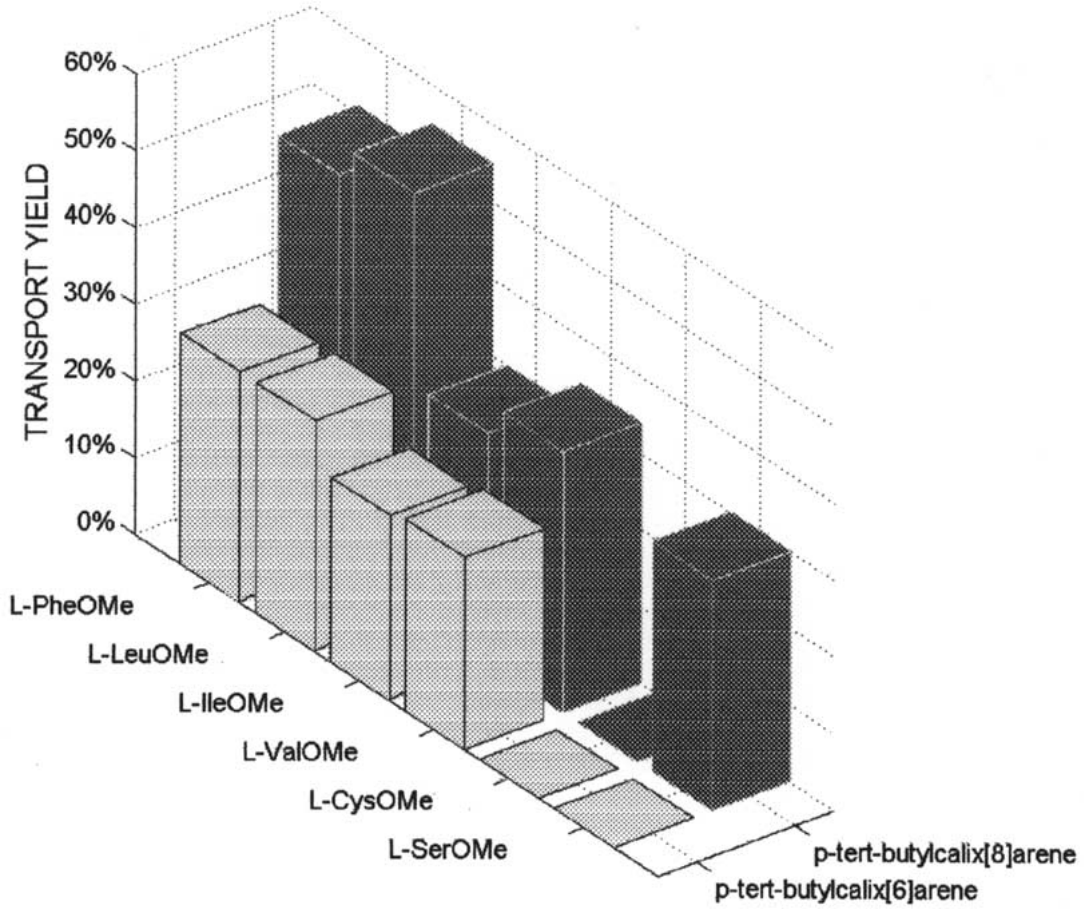


Figure 3. Experimental data of the transport of some amino acid methylester hydrochlorides through chloroform liquid membrane by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 alter 24 hours of stirring 200 rpm. *Source phase*: [amino acid methylester hydrochloride] $= 3 \times 10^{-3}$ M; pH = 5.0; [tropaeolin 00] $= 1 \times 10^{-3}$ M; 10 mL. *Membrane*: Chloroform, [calixarene] $= 5 \times 10^{-3}$ M, 35 mL. *Receiving phase*: LiOH 0.01 N (pH = 13.0), 10 mL.

determined by spectrophotometric measurements carried out by means of an UV-Vis Spectrometer JASCO V530. Each experiment was repeated five times.

Results and discussion

The values of extractability of amino acids methylesters from aqueous phase at pH = 5.0 into chloroform phase by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 are presented in Figure 2. The extraction ability of both calixarenes under study were studied under the same experimental conditions.

As one can see from Figure 2, both calixarenes are efficient extractants for the amino acid methylesters. Except of L-ValOMe (35% extractability) and L-SerOMe (37% extractability), the *p*-tert-butylcalix[6]arene provided better extractability than the *p*-tert-butylcalix[8]arene. The sequence of extractability using the *p*-tert-butylcalix[6]arene as extractant is the following: LPheOMe (−1.45) > L-CysOMe (−2.55) > L-IleOMe (−1.80) > L-LeuOMe (−1.72) > L-SerOMe (3.00) > L-ValOMe (−2.29) [34], and by using the *p*-tert-butylcalix[8]arene it turns to: L-IleOMe > L-PheOMe > L-ValOMe = L-SerOMe > L-LeuOMe > L-CysOMe. The results show that the structure of calixarenes is one of the most important factors for the recognition of amino acid. Extraction experiments in this case did not show any relationship between the extraction efficiency and the hydrophobicity of amino acid esters. It is known that the values of log *P* (listed in parentheses) is a quantitative indicator for the hydrophilic/lipophilic balance [34].

In contrast, in the triphasic system, the *p*-tert-butylcalix[8]arene exhibits better transport ability than *p*-tert-butylcalix[6]arene for the above amino acids methylesters through chloroform liquid membrane (Figure 3). The sequence of the transport yields of amino acids using *p*-tert-butylcalix[6]arene as carrier is the following; L-PheOMe > L-LeuOMe > L-IleOMe $\simeq$ ValOMe and with the *p*-tert-butylcalix[8]arene as carrier the sequence of amino acid yields is the following; L-LeuOMe > L-PheOMe > L-ValOMe > L-IleOMe $\simeq$ L-SerOMe.

Even though the L-CysOMe was extracted by both calixarenes with relatively good yields, it was not transported by all means. It was noted that the sulfhydryl group on the side chain of L-cysteine confers to this amino acid an increase in solubility. Similarly, the L-SerOMe has not been transported by *p*-tert-butylcalix[6]arene, whereas a 30% transport yield was obtained using *p*-tert-butylcalix[8]arene as carrier. One possible cause might be attributed to the structure of L-serine, which has a hydroxyl group in its molecule, hence

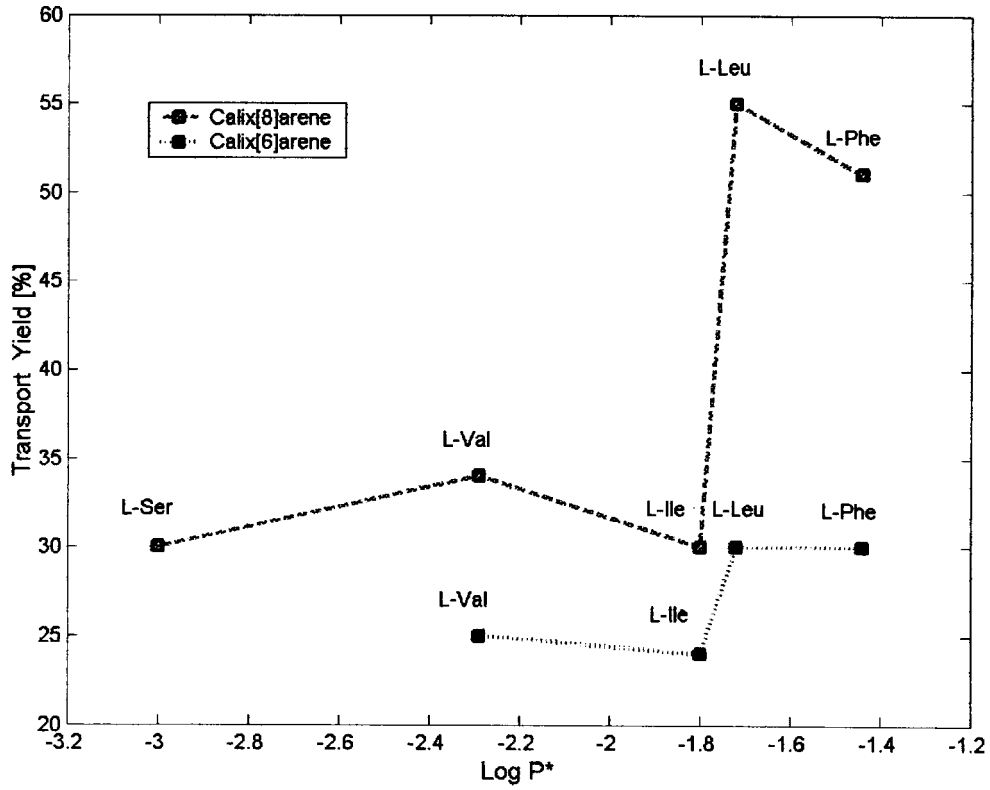


Figure 4. The relationship between the yields (%) of amino acid methylester hydrochlorides transport through liquid membrane by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 and their hydrophobicity (log *P*). * From Ref. [34].

the higher solubility of this amino acid in comparison with the others. At the same line of reasoning, the nature of the calixarene employed was evident in both the extraction and the transport of the amino acid methyl esters under study. It is known that the hydrophobicity of amino acids plays an important role on the rate of transport through liquid membrane. From the correlation between the yields of amino acids transport and their hydrophobicity one can see that the hydrophobic salts like L-phenylalanine and L-leucine have been transported with higher yields than the other amino acids under study (Figure 4). Except L-IleOMe there is a good correlation between the yields of amino acid esters transport and their hydrophobicity.

Further interpretations of the present results and deeper insight into the occurring phenomena require more experiments, which we are presently involved in.

Conclusions

Our experimental results have confirmed that the amino acid methylesters are effectively extracted and transported by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00. The *p*-tert-butylcalix[n]arene provided better extractability than the *p*-tert-butylcalix[8]arene to the most of amino acids methylesters studied (L-LeuOMe, L-PheOMe, L-IleOMe, L-CysOMe). In contrast, in the triphasic system, the *p*-tert-butylcalix[8]arene exhibits better transport ability than *p*-tert-butyicalix[6]arene for the amino acids through chloroform liquid membrane.

We have proved that the extractability and the transport are essentially controlled by the macrocyclic structure of calixarene, and the nature of the amino acid. Also the experimental results showed that there is no relationship between the extraction efficiency and the hydrophobicity of amino acid esters above mentioned; instead, in the transport through chloroform liquid membrane the hydrophobic salts like L-phenylalanine and L-leucine have been transported with higher yields than the other amino acids under study.

The results suggest new means to experimental design of optimal separation of some amino acid derivatives and other biological species.

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