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ORIGINAL ARTICLE

Liquid–liquid extraction and transport through membrane of amino acid methylesters by calix[*n*]arene derivatives

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Abstract The extractability together with the transport through liquid membrane of some amino acid methylesters by using *p*-tert-butylcalix[4]arene as extractant or carrier was studied. In this context, *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$) were found to act as useful carriers or extractant reagents for L-tryptophan methylester and L-tyrosine methylester. The calix[*n*]arene derivatives used in experiments extracted amino acids methylesters from the aqueous phase into chloroformic phase in the presence of tropaeolin 00 ([4(4′-anilinophenylazo)benzenesulphonic acid]) as counterion at pH ≅ 5.0. The extraction and the transport depend on the structure of calixarenes, the structure of amino acids, the pH, and the nature of anion used as ion pair for cation-receptor complexes. The properties of solvent involved in liquid membrane play an important role in membrane stability and also in selecting membrane systems. The results demonstrated that the inclusion properties of the investigated hosts are correlated with their structural properties and also they suggest further possibilities for optimal separation of amino acids derivatives.

Keywords Amino acid methylesters · Derivative calixarenes · Liquid–liquid extraction · Transport · Liquid membrane

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Introduction

The calix[*n*]arenes with their unique three-dimensional surface and conformational rigid structures are involved as receptors in recognition of amino acids, that is, solvent extraction and transport through liquid membrane in order to perform selective separation of amino compounds from mixtures [1–10]. Intensive studies have been performed concerning their use in host-guest complexation and separation of chemical or biochemical compounds due to their easy chemical transformations and complexing abilities towards cations, anions, and neutral compounds [11–16]. Calixarenes have been used for the design of various receptors and sophisticated derivatives such as calixcrowns [17, 18], calixspherands, and dendrimers [19].

Particular interest in functionalized calixarenes stems from their ability to act as extractant in solvent extraction or carrier through liquid membrane for separation of various compounds. Thus, Chang et al. [20] reported the transport of *N*-benzoyl amino acids through a chloroform liquid membrane by employing a calix[6]arene derivative as selective carrier. Zolotov et al. [21] employed *p*-1-adamantylcalix[8]arene ethyl ester as extractant for amino acid esters**.** The selective extraction and transport of some amino acid ethyl and methyl esters and *Z*-amino acid carboxylates by using a new calix[4]arene having chiral pendant groups were performed by Okada et al. [22]. Hu et al. [23] synthetized (R)-cysteine-containing calix[4]arenas, which may serve as good chiral macrocyclic ligands in the studies of chiral recognition and chiral catalysis. Moreover, Shinkai et al. [24] spectroscopically proved that pseudo-C_2-symmetrical homooxacalix[3]arene exibits enantiomeric recognition properties towards alanine ethyl ester and phenylalanine ethyl ester. The separation of zwitterionic form of aromatic amino acids by calix[4]arene

based α-aminophosphonates through a supported liquid membrane composed of a porous polymeric support was reported by Antipin et al. [25]. The ability of calix[6]arene carboxylic acid derivatives to be extractants and/or carriers for transporting aromatic amino acids, nucleotide bases such as adenine, and catecholamine through a liquid membrane was reported by Oshima et al. [26–28]. The transport of aromatic amino acids methylesters through a chloroform liquid membrane containing *p*-tert-butylcalix[6]arene, and *p*-tert-butylcalix[8]arene as carriers in the presence of picrate was reported [29].

As part of an ongoing study, we have previously reported [30, 31] aspects of extractability and transport through liquid membrane of some amino acid methylesters by *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$), which revealed a calixarenes ability to act as extractant or carrier for amino acids. 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. Following our research in the recognition and separation of biological compounds by macrocyclic receptors we report here the study of the solvent extraction and transport through liquid membrane of some amino acid methylesters by *p*-tert-butylcalix[4]arene in chloroform compared with *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$). Moreover, we extend the studies of extraction abilities and the transport through chloroform liquid membrane of *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$) upon aromatic amino acids, L-tryptophane methylester and L-tyrosine methylester.

Experimental

All following amino acid derivatives: L-tryptophane methylester hydrochloride (L-TrpOMe), L-phenylalanine methylester hydrochloride (L-PheOMe), L-tyrosine methylester hydrochloride (L-TyrOMe), L-leucine methylester hydrochloride (L-LeuOMe), 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) were purchased from Fluka at the highest purity commercially available and employed without further purification (Chart 1).

The *p*-tert-butylcalix[*n*]arenes ($n = 4, 6, 8$) were obtained from Merck and used without further purification (Chart 1). Reagent grade tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) from Fluka (Chart 1), distilled (Millipore), and deionised water were used throughout the experiments. The organic solvent chloroform (dielectric constant $\varepsilon_r = 4.81$ [32]), was distilled before use.

Liquid–liquid extraction

The extraction measurements of amino acid methylesters from aqueous phase into chloroform were performed according to Pedersen's procedure [33]. Equal volumes (5 mL) of aqueous solution of amino acid methylester (3.0×10^{-4} M, except L-CysOMe = 3×10^{-3} M) and 6.0×10^{-4} M of tropaeolin 00 as counterion at pH ≅ 5.0 and chloroform solution (5 mL) of calixarene (5.0×10^{-3} M) were mixed and shaken for 30 min at $T = 25 \pm 1$ °C For extraction of L-CysOMe, the concentration of calixarene was $c_{calixarene} = 1.0 \times 10^{-2}$M. Chloroform and water were saturated with each other to prevent volume change during extraction. It was investigated the extraction of amino acid methyesters and the counter anion tropaeolin 00 in solvent in the absence of calixarene. No detectable amounts of any amino acids were extracted onto the organic phase under the experimental conditions.

The pH of the aqueous solutions was adjusted by the hydrochloric acid. The extractability was calculated as $E\ [\%] = \frac{(A_0 - A)}{A_0} \times 100$, where A_0 and A are the absorbencies of the aqueous phases before and after the extraction with calixarenes, respectively. The absorbency was determined by spectrophotometric measurements carried out by means of an UV–Vis Spectrometer JASCO V-530. Each experiment was repeated five times and reproducibility was ± 10%.

Liquid membrane transport

The experimental procedure was the same as the one described in the previous paper [30]. The transport experiments were carried out using a device [34] consisting of two concentric tubes: the inner one (acting as a stirer too) contained the source phase, 10 mL, of aqueous solution of amino acid (3×10^{-3} M) and 1×10^{-3} M of tropaeolin 00 as counterion at pH ≅ 5.0 whereas the receiving phase, 10 mL, of LiOH 0.01 N (pH = 13.0) together with the membrane phase, 35 mL, of calixarene derivative (5×10^{-3} M) in chloroform were introduced in the outer tube. Each experiment was repeated three times at least. Reproducibility was ±15%. Similar transport experiments were performed for reference in the absence of the amino acid methylester. The pH was measured by a digital MV-870 Pracitronic pH-meter with glass electrode and saturated calomel electrode.

Results and discussion

The extraction experiments of cationic species of amino acid methylesters from aqueous acidic phase (pH ≅ 5.0) into chloroform phase were carried out with *p*-tert-butylcalix[4]arene in the presence of tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) as counterion. The

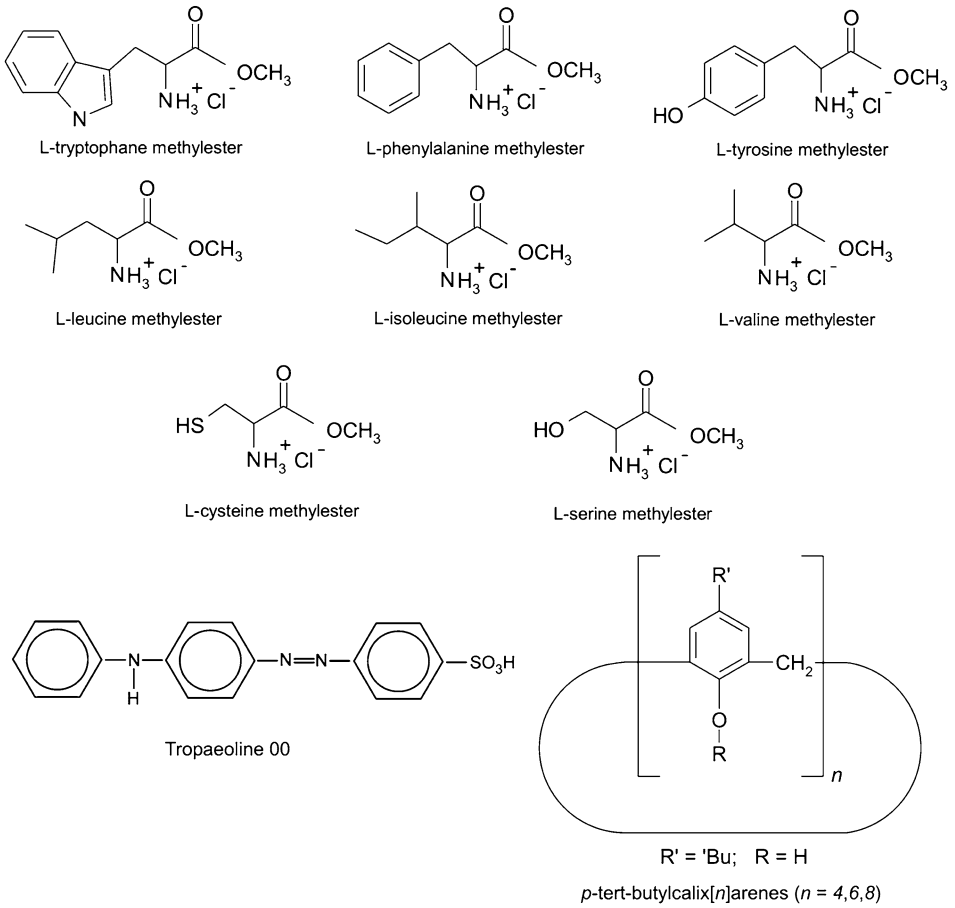

Chart 1 The chemical structure of compounds used throughout the experiments

values of extractability of amino acid methylesters obtained by using by *p*-tert-butylcalix[4]arene are presented in Fig. 1 together with the values of extractability of amino acid methylesters obtained by using *p*-tert-butylcalix[*n*]arenes (n = 6, 8) under the same experimental conditions [30].

Extraction experiments performed by using *p*-tert-butylcalix[4]arene as extractant show the following sequence of extractability: L-TrpOMe (–1.16) > L-CysOMe (–2.55) > L-PheOMe (–1.45) ≅ L-SerOMe (–3.00) > L-LeuOMe (–1.72) ≅ L-IleOMe (–1.80) > L-TyrOMe (–2.11) > L-ValOMe (–2.29) [35]. As one can see, there is no relationship between the extractability of amino acid methylesters carried out under experimental conditions and their hydrophobicity represented by log *P* (listed in parentheses) [35]. The extractability of amino acids by using the *p*-tert-butylcalix[6]arene as extractant decreases in the following sequence: L-TrpOMe > L-PheOMe > L-CysOMe > L-IleOMe > L-LeuOMe > L-TyrOMe > L-SerOMe > L-ValOMe [30], and in the case of the *p*-tert-butylcalix[8]arene it turns to: L-IleOMe > L-TrpOMe > L-PheOMe > L-ValOMe ≅ L-SerOM-

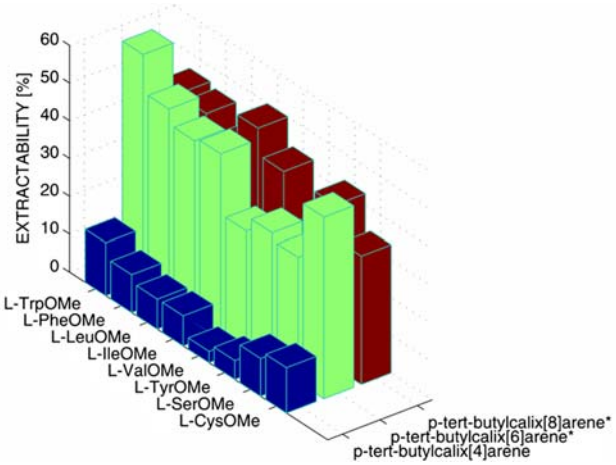

Fig. 1 Extractability of some amino acid methylesters from the aqueous phase into chloroform phase by *p*-tert-butylcalix[*n*]arenes (n = 4, 6, 8) in the presence of tropaeolin 00 at pH ≅ 5.0. T = 25 ± 1 °C. *From ref. [30] except values of L-TrpOMe and L-TyrOMe

e > L-LeuOMe > L-CysOMe > L-TyrOMe [30]. These results suggest that the macrocyclic structure of calixarenes influence their extraction ability upon amino acid methylesters. Thus, the *p*-tert-butylcalix[6]arene emphasized better extractability than that of both *p*-tert-butylcalix[8]arene, and *p*-tert-butylcalix[4]arene. The order of the extractabilities of amino acid methylesters with derivative calix[*n*]arenes is as follows: *p*-tert-butylcalix[6]arene > *p*-tert-butylcalix[8]arene > *p*-tert-butylcalix[4]arene. Both calixarenes, *p*-tert-butylcalix[4]arene and *p*-tert-butylcalix[6]arene provided the highest affinity towards L-TrpOMe. Calixarene *p*-tert-butylcalix[8]arene exhibits also ability to extract tryptophane but less than other calixarenes involved in our study. This host showed the highest extractability towards L-isoleucine methylester.

The experiments continued with the transport of amino acid methylesters through chloroform liquid membrane using *p*-tert-butylcalix[*n*]arenes ($n = 4, 6, 8$) as carriers. Thus, the previous study [30] concerning the ability of *p*-tert-butylcalix[*n*]arenes ($n = 4, 6$) to act as carriers is extended to L-tryptophane methylester and L-tyrosine methylester. In Fig. 2, the transport yields of amino acid methylesters through chloroform liquid membrane with *p*-tert-butylcalix[*n*]arene ($n = 4, 6, 8$) is displayed.

As in extraction experiments, *p*-tert-butylcalix[6]arene exhibited a high transport ability towards L-tryptophane in comparison with *p*-tert-butylcalix[*n*]arenes ($n = 4, 8$). The transport of amino acid methylesters through liquid membrane by the *p*-tert-butylcalix[4]arene as carrier is smaller than that of *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$) as carriers.

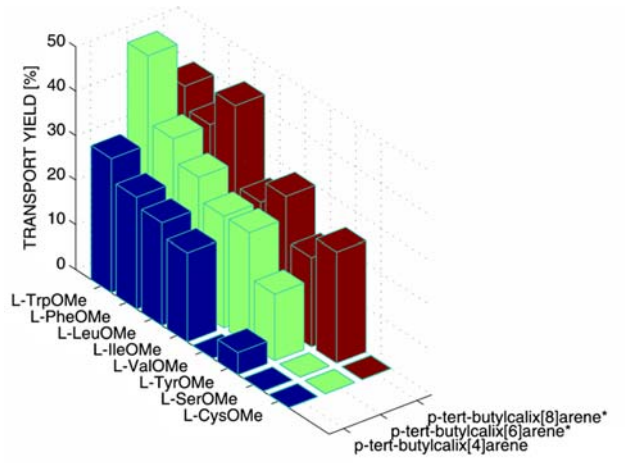

Fig. 2 The transport yield of some amino acid methylester hydrochlorides through chloroform liquid membrane by *p*-tert-butylcalix[*n*]arenes ($n = 4, 6, 8$) as carriers in the presence of tropaeolin 00 after 24 h of stirring 200 rpm. $T = 25 \pm 1$ °C. *Source phase*: [amino acid methylester] = 3×10^{-3} M; pH = 5.0; [tropaeolin 00] = 1×10^{-3} M; 10 mL *Membrane*: Chloroform, [calixarene] = 5×10^{-3} M, 35 mL. *Receiving phase*: LiOH 0.01 N (pH = 13.0), 10 mL

The sequence of decreasing transport yields of amino acids using *p*-tert-butylcalix[4]arene as carrier was the following: L-TrpOMe > L-PheOMe > L-LeuOMe > L-TyrOMe. In the membrane system, the *p*-tert-butylcalix[8]arene exhibited better transport ability than both *p*-tert-butylcalix[*n*]arenes ($n = 4, 6$) for the amino acids methylesters through chloroform liquid membrane, except L-tryptophane with *p*-tert-butylcalix[6]arene.

The sequence of the transport yields of amino acids using *p*-tert-butylcalix[6]arene as carrier was the following: L-TrpOMe > L-PheOMe > L-LeuOMe > L-IleOMe ≅ L-ValOMe > L-TyrOMe and with the *p*-tert-butylcalix[8]arene as carrier the sequence of amino acid yields is the following: L-LeuOMe > L-TrpOMe > L-PheOMe > L-ValOMe > L-IleOMe ≅ L-SerOMe > L-TyrOMe. The results pointed out that the structure of calixarene is one of the most important parameter for recognition of amino acids. As in the case of *p*-tert-butylcalix[*n*]arenes ($n = 6, 8$), *p*-tert-butylcalix[4]arene doesn't show any transport ability towards L-CysOMe through chloroform liquid membrane. The structure of L-cysteine could be responsible of this behavior. The same situation subsists for L-Ser and L-Val. The hydrophobicity of the amino acid is an important parameter, which has to be considered in both the extraction and transport experiments.

Conclusions

The extraction abilities and the transport through chloroform liquid membrane of *p*-tert-butylcalix[*n*]arenes ($n = 4, 6, 8$) upon some amino acid methylesters (L-tryptophane, L-phenylalanine, L-tyrosine, L-leucine, L-valine, L-cysteine, L-isoleucine, and L-serine) were investigated. The experimental results suggested that amino acid methylesters are extracted from aqueous phase (pH ≅ 5.0) into organic phase and transported through chloroform liquid membrane by *p*-tert-butylcalix[*n*]arenes ($n = 4, 6, 8$) in the presence of tropaeolin 00 ([4-(4′-anilinophenylazo)benzenesulphonic acid]) as counterion.

The extractability and the transport were proved to be essentially controlled by the structure of calix[*n*]arene and the nature of the amino acid. Some parameters, such as the pH and the nature of the anion used as counterion [29] influenced both the extractability and the transport through liquid membrane of amino acid methylesters. Moreover, the influence of the composition and structure of the compounds under study upon the partition processes occurring in biphasic and triphasic systems was also observed. The results suggested further possibilities for optimal separation of amino acids derivatives and other biological species by means of derivative calixarenes.

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