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Extraction and DFT study on interaction of the cesium cation with enniatin B

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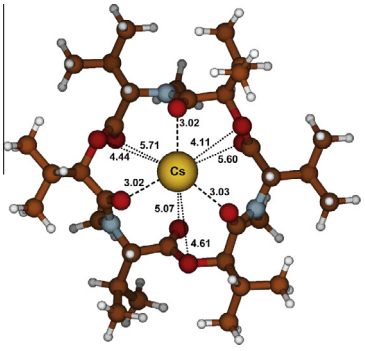
HIGHLIGHTS

- Stability of the enniatin B–Cs^+ complex was determined.
- Quantum mechanical DFT calculations were carried out.
- Structure of the resulting cationic complex was predicted.

GRAPHICAL ABSTRACT



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ABSTRACT

By using extraction experiments and γ-activity measurements, the extraction constant corresponding to the equilibrium $Cs^+(aq) + A^-(aq) + \mathbf{1}(nb) \leftrightarrows \mathbf{1}{\cdot}Cs^+(nb) + A^-(nb)$ taking place in the two-phase water–nitrobenzene system (A^- = picrate, **1** = enniatin B; aq = aqueous phase, nb = nitrobenzene phase) was evaluated as $\log K_{ex}(\mathbf{1}{\cdot}Cs^+, A^-) = 2.3 \pm 0.1$. Further, the stability constant of the $\mathbf{1}{\cdot}Cs^+$ complex in nitrobenzene saturated with water was calculated for a temperature of 25 °C: $\log \beta_{nb}(\mathbf{1}{\cdot}Cs^+) = 4.2 \pm 0.1$. Finally, applying quantum mechanical DFT calculations, the most probable structure of the cationic complex species $\mathbf{1}{\cdot}Cs^+$ was derived. In the resulting $\mathbf{1}{\cdot}Cs^+$ complex, which is most energetically favored, the "central" cation Cs^+ is bound by nine bonding interactions to the corresponding nine oxygen atoms of the parent enniatin B ligand. The interaction energy of the considered complex $\mathbf{1}{\cdot}Cs^+$ was found to be −228.3 kJ/mol, confirming the formation of this investigated complex as well.

Introduction

Enniatins are a group of cyclohexadepsipeptide mycotoxins produced by *Gnomonia errabuda* and several *Fusaria* species, with phytotoxic, antibiotic, and insecticidal activities [1–4]. They appear in nature as mixtures of cyclic depsipeptides – the main variants are enniatins A, A1, B, and B1, together with minor amounts of enniatins C, D, E, and F. Enniatins function as ionophores by their incorporation into the cellular membrane to form dimeric structures. They transport monovalent ions across the membrane, especially the mitochondrial membranes, affecting oxidative phosphorylation uncoupling [5–8]. It has been demonstrated that enniatins have a cytotoxic effect on human cancer cells [9]. Furthermore, incubation of H4IIE hepatoma cells with enniatins strongly diminished phosphorylation of ERK (p44/p42) [10]. Enniatins B and B1 were found to inhibit the multi-drug resistance transporter Pdr5p from *Saccharomyces cerevisiae* [11], indicating

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their beneficial potential in the cases of drug resistant patients. It was found that enniatins A1 and B1, and – to a lesser extent – enniatin B may possess anticarcinogenic properties by induction of apoptosis and disruption of ERK signaling pathway [11]. Enniatins have been also mentioned as potential anti-AIDS drugs.

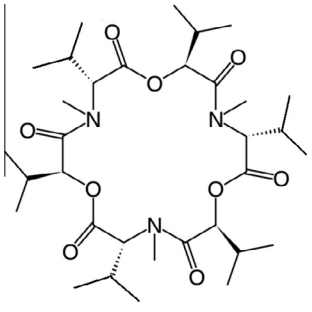
The X-ray crystal structure of free enniatin B ligand has been described by Kratky and Dobler [12]. The sandwich structure of the 2:1 complex of enniatin B with KI has been established by using X-ray diffraction [13]. In addition, the crystal structure of the 1:1 complex of enniatin B with KNCS has been determined by the same method [14]. However, up to now, interaction of the cesium cation with enniatin B has not been investigated. Therefore, in the current work, the solvent extraction of cesium picrate (abbrev. CsA) into nitrobenzene by means of the mentioned enniatin B ligand (abbrev. **1**; see Scheme 1) was studied. At this point it should be noted that the Cs^+ ion is a typical representative of the univalent cations with a large radius; besides, the radionuclide $^{137}Cs^+$ used (see Section 'Experimental') enables relatively fast and very accurate physical–chemical measurements. Thus, this cation was chosen for the present study. Moreover, the stability constant of the proved **1**·Cs^+ complex species in the organic phase of the water–nitrobenzene extraction system was determined. Finally, applying quantum mechanical DFT calculations, the most probable structure of this cationic complex species was predicted.

Experimental

Compound **1** (puriss., ⩾99%; see Scheme 1) was purchased from Aldrich and it was employed as received. The other chemicals used (Lachema, Brno, Czech Republic) were of reagent grade purity. A solution of cesium picrate, CsA, in water was prepared by dissolving stoichiometric amount of picric acid in an aqueous solution of CsOH. The radionuclide $^{137}Cs^+$ was supplied by Techsnaveksport, Russia; its radionuclidic purity was 99.9%.

The extraction experiments were carried out in 10 mL polypropylene test-tubes with polypropylene stoppers: 2 mL of an aqueous solution of CsA of the concentration in the range from 2×10^{-4} to 1×10^{-3} M and 10 kBq of $^{137}Cs^+$ were added to 2 mL of a nitrobenzene solution of **1**, the initial concentration of which varied from 1×10^{-3} to 5×10^{-3} M (in all experiments, the initial concentration of **1** in nitrobenzene, $C_{\mathbf{1}}^{\mathrm{in,nb}}$, was always higher than the initial concentration of CsA in water, $C_{\mathrm{CsA}}^{\mathrm{in,aq}}$). The test-tubes filled with the solutions were shaken for 3 h at 25 ± 1 °C, using a laboratory shaker. Then the phases were separated by centrifugation. Finally, 1 mL samples were taken from each phase and their γ-activities were measured by means of a well-type NaI(Tl) scintillation detector connected to a γ-analyzer Triathler (Hidex, Turku, Finland).

The equilibrium distribution ratios of cesium, D_{Cs}, were determined as the ratios of the measured radioactivities of $^{137}Cs^+$ in the nitrobenzene and aqueous samples.



1

Scheme 1. Structural formula of enniatin B (abbrev. **1**).

Results and discussion

Extraction experiments

Regarding the results of previous papers [15–17], the two-phase water–CsA (A^- = picrate)–nitrobenzene extraction system can be described by the following equilibrium

$$Cs^+(aq) + A^-(aq) \leftrightarrows Cs^+(nb) + A^-(nb); \quad K_{ex}(Cs^+, A^-) \tag{1}$$

with the corresponding extraction constant K_{ex} (Cs^+, A^-); aq and nb denote the presence of the species in the aqueous and nitrobenzene phases, respectively. For the constant K_{ex} (Cs^+, A^-) one can write [15]

$$\log K_{ex}(Cs^+, A^-) = \log K^{i}_{Cs^+} + \log K^{i}_{A^-} \tag{2}$$

where $K^{i}_{Cs^+}$ and $K^{i}_{A^-}$ are the individual extraction constants for Cs^+ and A^-, respectively, in the water–nitrobenzene system [15]. Knowing the values $\log K^{i}_{Cs^+} = -2.7$ [15] and $\log K^{i}_{A^-} = 0.8$ (A^- = picrate) [15], the extraction constant K_{ex} (Cs^+, A^-) was simply calculated from Eq. (2) as $\log K_{ex}$ (Cs^+, A^-) = −1.9.

From previous results [18,19] it follows that the two-phase water–CsA (A^- = picrate)–nitrobenzene–**1** (enniatin B) extraction system (see Section 'Experimental'), chosen for determination of the stability constant of the **1**·Cs^+ complex in nitrobenzene saturated with water, can be characterized by the main chemical equilibrium

$$Cs^+(aq) + A^-(aq) + \mathbf{1}(nb) \leftrightarrows \mathbf{1}\cdot Cs^+(nb) + A^-(nb); \quad K_{ex}(\mathbf{1}\cdot Cs^+, A^-) \tag{3}$$

to which the equilibrium extraction constant

$$K_{ex}(\mathbf{1}\cdot Cs^+, A^-) = \frac{[\mathbf{1}\cdot Cs^+]_{nb}[A^-]_{nb}}{[Cs^+]_{aq}[A^-]_{aq}[\mathbf{1}]_{nb}} \tag{4}$$

corresponds. It is necessary to emphasize that **1** is a considerably lipophilic ligand, practically present in the nitrobenzene phase only, where this ligand forms – with the Cs^+ cation – the relatively stable complex **1**·Cs^+, as given below.

Employing the conditions of electroneutrality in the organic and aqueous phases, the mass balances of **1** and CsA at equal volumes of the nitrobenzene and aqueous phases, as well as the measured equilibrium distribution ratio of cesium, $D_{\mathrm{Cs}} = [\mathbf{1}\cdot Cs^+]_{nb}/[Cs^+]_{aq}$, combined with Eq. (4), we gain the final expression for the extraction constant K_{ex} (**1**·Cs^+, A^-):

$$K_{ex}(\mathbf{1}\cdot Cs^+, A^-) = D_{Cs}^2 \Big/ \left\{ C_{\mathbf{1}}^{\mathrm{in,nb}} - \frac{D_{Cs}}{1 + D_{Cs}} C_{\mathrm{CsA}}^{\mathrm{in,aq}} \right\} \tag{5}$$

where $C_{\mathrm{CsA}}^{\mathrm{in,aq}}$ is the initial concentration of CsA in the aqueous phase and $C_{\mathbf{1}}^{\mathrm{in,nb}}$ denotes the initial concentration of **1** in the organic phase of the system under consideration.

In this work, from the extraction experiments and γ-activity measurements (see Section 'Experimental') by means of Eq. (5), the following value of the constant K_{ex} (1·Cs^+, A^-) was determined: $\log K_{ex}$ (1·Cs^+, A^-) = 2.3 ± 0.1 (see Table 1). This constant experimentally proves the justifying of the extraction mechanism and the presentation of the corresponding species, expressed by the two-phase chemical equilibrium (3).

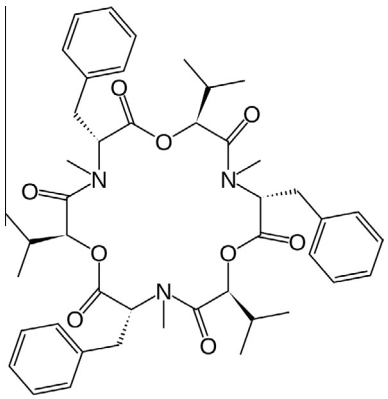
Furthermore, with respect to previous results [18,19], for the extraction constants K_{ex} (Cs^+, A^-) and K_{ex} (**1**·Cs^+, A^-) defined above, as well as for the stability constant of the complex **1**·Cs^+ in nitrobenzene saturated with water, denoted by β_{nb} (**1**·Cs^+), one gets

$$\log \beta_{nb}(\mathbf{1}\cdot Cs^+) = \log K_{ex}(\mathbf{1}\cdot Cs^+, A^-) - \log K_{ex}(Cs^+, A^-) \tag{6}$$

Using the constants $\log K_{ex}$ (Cs^+, A^-) and $\log K_{ex}$ (**1**·Cs^+, A^-) given above, and applying Eq. (6), we obtain the stability constant of

Table 1
Experimental data concerning determination of $\log K_{ex}$ ($\mathbf{1}\cdot Cs^+$, A^-) on the basis of Eq. (5).

$C_{CsA}^{in,aq}$ (M)	$C_{\mathbf{1}}^{in,nb}$ (M)	D_{Cs}	$\log K_{ex}$ ($\mathbf{1}\cdot Cs^+$, A^-)
2×10^{-4}	1×10^{-3}	0.47	2.4
4×10^{-4}	2×10^{-3}	0.60	2.3
6×10^{-4}	3×10^{-3}	0.79	2.4
8×10^{-4}	4×10^{-3}	0.88	2.3
1×10^{-3}	5×10^{-3}	0.89	2.2



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Scheme 2. Structural formula of beauvericin (abbrev. **2**).

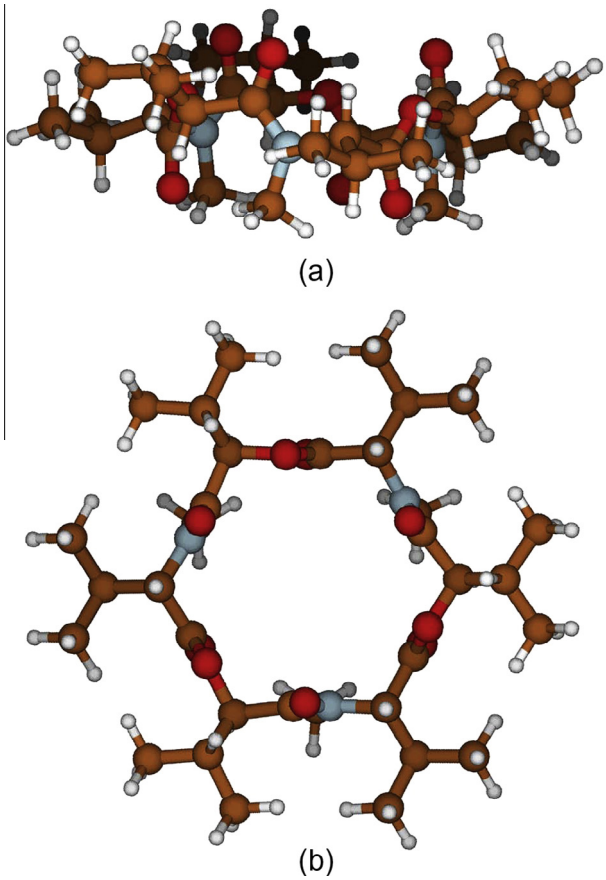

Fig. 1. Two projections of the DFT-optimized structure of free ligand **1** [B3LYP/6-31G(d,p)]: (a) side view and (b) top view.

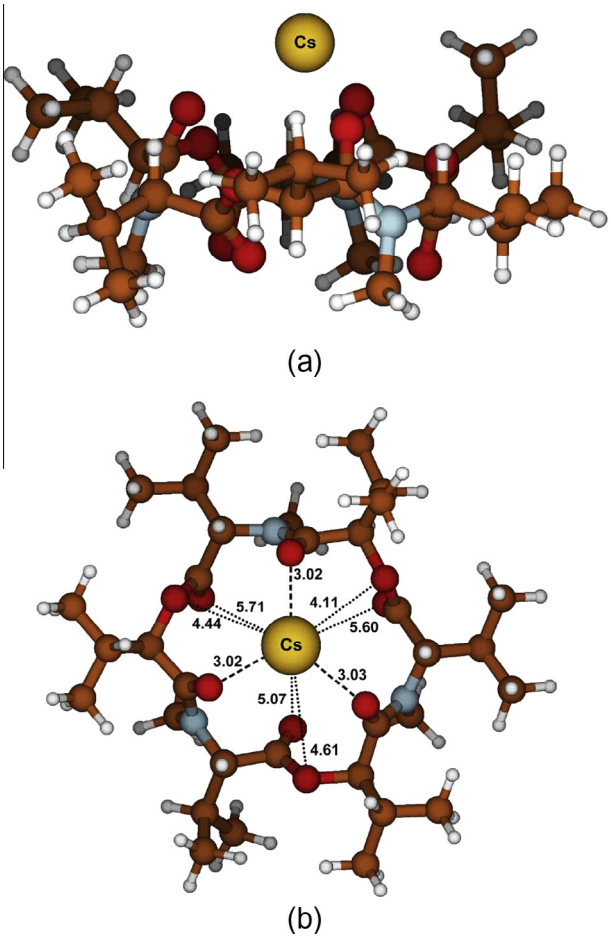

Fig. 2. Two projections of the DFT-optimized structure of the $\mathbf{1}\cdot Cs^+$ complex (B3LYP/LanL2DZ): (a) side view and (b) top view; distances/Å.

the $\mathbf{1}\cdot Cs^+$ complex in water-saturated nitrobenzene at 25 °C as $\log \beta_{nb}$ ($\mathbf{1}\cdot Cs^+$) = 4.2 ± 0.1. In this context it should be noted that the stability constant of the complex species $\mathbf{2}\cdot Cs^+$, where **2** denotes beauvericin (see Scheme 2), in nitrobenzene saturated with water is $\log \beta_{nb}$ ($\mathbf{2}\cdot Cs^+$) = 3.9 ± 0.1 [20]. This means that in the mentioned nitrobenzene medium, the stabilities of the considered cationic complexes $\mathbf{1}\cdot Cs^+$ and $\mathbf{2}\cdot Cs^+$ are nearly comparable.

Quantum mechanical calculations

The theoretical calculations were performed at the density functional level of theory (DFT, B3LYP functional) [21,22], employing the Gaussian 09 suite of programs [23]. The 6-31G(d,p) and LanL2DZ basis sets were used and the optimizations were unconstrained. In order to increase the numerical accuracy and to reduce oscillations during the molecular geometry optimization, two-electron integrals and their derivatives were calculated by using the pruned (99,590) integration grid, having 99 radial shells and 590 angular points per shell, which was requested by means of the Gaussian 09 keyword "Int = UltraFine". The most probable structure of the $\mathbf{1}\cdot Cs^+$ cationic complex was predicted on the basis of the thorough conformational analysis (i.e., different initial mutual positions of the ligand **1** and the Cs^+ cation were considered during the geometry optimization) and the respective vibrational frequency calculations.

In the model calculations, we optimized the molecular geometries of the parent enniatin B ligand (**1**) and its complex with Cs^+,

analogously as in our previous papers [24–27]. The optimized structure of the free ligand **1** is illustrated in Fig. 1.

In Fig. 2, the structure obtained by the full DFT-optimization of the **1**·Cs^+ complex is depicted, together with the lengths of the corresponding bonds (in Å). As follows from this figure, the complexation with the Cs^+ cation changes the overall shape of the parent ligand **1** only slightly. In the resulting **1**·Cs^+ cationic complex species, which is most energetically favored, the "central" cation Cs^+ is bound by nine bonding interactions to the respective nine oxygen atoms (3.02, 4.44, 5.71, 3.02. 4.11, 5.60, 3.03, 4.61, and 5.07 Å) of the parent ligand **1** (see Fig. 2). On the other hand, in the 2:1 sandwich complex of enniatin B with KI [13], in which the coordination environment around the K^+ cation is formed by the oxygen atoms of the amide groups, the average $K^+\cdots O$ distance is only 2.69 Å [13]. Cartesian coordinates (in Å) for the free ligand **1** and the **1**·Cs^+ complex are presented in Supplementary material.

Finally, the interaction energy, E(int), of the complex **1**·Cs^+ under study [calculated as the respective difference between the pure electronic energies of **1**·Cs^+ and isolated **1** and Cs^+ species: $E(\text{int}) = E(\mathbf{1}\cdot Cs^+) - E(\mathbf{1}) - E(Cs^+)$] was found to be −228.3 kJ/mol, which also confirms the formation of this cationic complex species **1**·Cs^+.

Conclusions

In this work, we have shown that the combination of theoretical DFT calculations with an experimental extraction method in the two-phase water–nitrobenzene system can provide relevant data on the noncovalent bonding interactions of the cesium cation (Cs^+) with the enniatin B ligand (**1**). By using this extraction method, the stability constant of the cationic complex **1**·Cs^+ in nitrobenzene saturated with water was determined as $\log \beta_{nb}(\mathbf{1}\cdot Cs^+) = 4.2 \pm 0.1$ (t = 25 °C). On the other hand, applying DFT calculations, the most probable structure of this **1**·Cs^+ cationic species was predicted. In the resulting complex, the "central" cation Cs^+ is bound by nine bonding interactions to the corresponding nine oxygens of the parent ligand **1**. It is obvious that the present work may be an important contribution predominantly to both theoretical and experimental study of the enniatin B ligand, as well as to supramolecular chemistry in general.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2014.07.072.

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