CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **85** (4) (2012) 469–477.
http://dx.doi.org/10.5562/cca2125

Original Scientific Article

ESI MS/MS Study of Calix[4]arene Derivatives and their Metal Complexes†

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RECEIVED MAY 16, 2012; REVISED OCTOBER 9, 2012; ACCEPTED OCTOBER 12, 2012

Abstract. The peptidocalixarenes **1**–**3** bearing tryptophan, phenylglycine and leucil units at the lower rim and their complexes with alkali-metal (Li^+, Na^+, K^+, Rb^+, Cs^+) and selected lanthanide cations (La^{3+}, Ce^{3+}, Eu^{3+}, Yb^{3+}) were analyzed by ESI MS. The influences of the solvent (acetonitrile, methanol, addition of formic acid or sodium acetate) and the calixarene:cation molar ratio on signal intensities were investigated. Comprehensive MS/MS analyses were performed of all singly and doubly charged ions of **1**–**3** and their complexes, and fragmentation pathways were proposed. An inductive cleavage was observed during dissociation of protonated ions, while the presence of alkali-metal or lanthanide cations caused homolytic cleavage and formation of radical cations. The results of MS analysis were in accordance with those obtained by other techniques (spectrophotometric, potentiometric, and conductometric titrations). The MS/MS experiments could be used as fast and sensitive method for prediction of relative stabilities of calixarene complexes with metal ions. (doi: 10.5562/cca2125)

Keywords: calixarenes, alkali-metal cations, lanthanides, electrospray, mass spectrometry

INTRODUCTION

Calixarenes, a class of macrocyclic compounds prepared by the condensation of formaldehyde with phenol, as well as their derivatives, have been extensively studied as a host molecules in supramolecular chemistry.[1–3] Depending on a macrocyclic cavity size and substituents at upper or lower rim, calixarenes are suitable receptors for cations,[4,5] anions,[6] neutral[7] and chiral[8] molecules. Binding properties of these compounds were extensively studied by NMR, UV-Vis, vibrational and luminescence spectroscopy.[9]

Supramolecular interactions, including inclusion complexes of calixarene derivatives, can be analyzed by mass spectrometry using soft ionization techniques, like electrospray (ESI) and MALDI.[10,11] However, in most studies MS was used only for the determination of complex stoichiometry or for the confirmation of molecular mass of newly synthesized calixarene derivatives.[12–14] More detailed studies on underivatized calix[4]arenes and those whose phenolic hydroxyl groups were partially or completely substituted with ester or ether groups were performed by MALDI MS.[15] The signal of sodiated molecular ion was dominant in all spectra. The loss of hydroxyl groups was observed for underivatized calixarenes, whereas the most abundant fragment ion in MS/MS spectrum of substituted calixarenes was assigned to the loss of side chains. Breaking up of the calixarene macrocycle was not found. Goolsby *et al.* used MALDI and ESI-MS to determine Na^+/K^+ binding selectivities of calix[4]arene ether derivatives directly from the intensities of the corresponding complexes in mass spectra.[16] The strong binding of alkali-metal cations was confirmed by fragmentation patterns of the complexes where, instead of simple disassembly of the complex, the elimination of the side chains at the calixarene rims occured. The complex formation of *p*-*tert*-butilcalix[6]arene with ammonium hydroxide, ammonium and sodium ions, as well as fragmentation of these complexes, were studied by ESI-MS using triple-stage quadrupole mass spectrometer.[17] Monomer, dimer and trimer complexes with NH_4^+ ions were identified. In mass spectrum of acetonitrile solution of calix[6]arene (M[6]) and ammonium hydroxide, in addition to the monomer complex $([M[6]+NH_4])^+$, the ion corresponding to the loss of two phenolic units $([M[6]+NH_4]–M[2])^+$

† This article belongs to the Special Issue devoted to the 85th anniversary of *Croatica Chemica Acta*.
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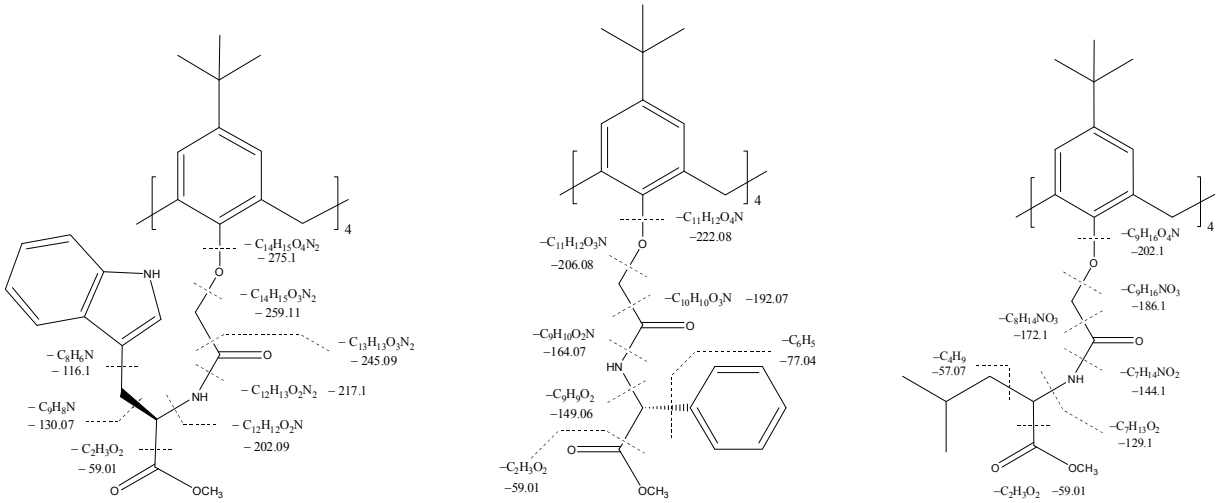


1 ($C_{100}H_{112}N_8O_{16}$, 1680.82) **2** ($C_{88}H_{100}N_4O_{16}$, 1468.71) **3** ($C_{80}H_{1116}N_4O_{16}$, 1388.84)

Scheme 1. Structures of compounds **1–3** and the exact masses of the parts of molecules which would occur by the cleavage of the bonds.

and the signal of complementary radical cation $M[2]^{+\bullet}$ were observed. Fragmentation of the monomer complex was characterized by the six successive losses of 56 Da, a C_4H_8 unit, formed by rearrangement of *tert*-butyl groups. Recently, a noncovalent complexation of monoamine neurotransmitters and related ammonium ions by glucosylcalix[4]arene was studied by ESI-FTICR MS.[18] The results obtained by H/D exchange indicated that the singly charged 1:1 complexes were mainly stabilized by intermolecular hydrogen bonds. Upon dissociation of the complexes induced by collision, the proton remained on the calixarene which than fragmented further. However, more information on the fragmentation pattern was not given.

Calixarene with carbamoyl phosphine oxide (CMPO) groups were shown to be good extracting agents for nuclear reprocessing since they can separate actinides from lanthanides. Complexation and extraction of La^{3+}, Eu^{3+} and Yb^{3+} by calix[4]arenes with CMPO groups attached to the lower or upper calixarene rim were monitored by ESI-MS. The stoichiometries of the corresponding complexes and selectivity of macrocycles towards lanthanides were determined.[19] Investigations carried out by Arnaud-Neu and coworkers[20] included determination of stability constants of La^{3+}, Eu^{3+} and Yb^{3+} with CMPO and related calix[4]arene derivatives by UV absorption spectrometry, and monitoring the reaction of La^{3+} with these ligands by ESI-MS, which provided the structural information for the complexes (*e. g.* coordination of anion or solvent molecules). Distribution curves of the complexes obtained by two different techniques were in good agreement.

Previously we have reported the results of spectrometric and electrochemical studies on binding properties of some peptidocalixarenes towards alkali metal cations.[21–24] In this paper we present mass spectrometric investigations of the following calix[4]arene derivatives: 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis(*O*-methyl-L-tryptophanylcarbonylmethoxy)calix[4]arene (**1**), 5,11,17,23-tetra-*tert*-butil-26,28,25,27-tetrakis-(*O*-methyl-D-α-phenylglycinecarbonylmethoxy)calix[4]arene (**2**) and 5,11,17,23-tetra-*tert*-butyl-26,28,25,27-(*O*-methyl-L-leucil-carbonylmethoxy)calix[4]arene (**3**) (Scheme 1), and their complexes with alkali-metal (Li^+, Na^+, K^+, Rb^+, Cs^+) and selected lanthanide (La^{3+}, Ce^{3+}, Eu^{3+}, Yb^{3+}) cations.

EXPERIMENTAL

Materials

The ligands **1-3** were prepared according to the procedures described elsewhere.[21,24] The solvents, acetonitrile and methanol, both HPLC grade, were purchased from J. T. Baker and used without further purification. The salts were nitrates ($La(NO_3)_3$, $Eu(NO_3)_3$, $RbNO_3$, $CsNO_3$, Merck; $Ce(NO_3)_3$, Hopkin & Williams), perchlorates ($LiClO_4$, $KClO_4$, Fluka; $NaClO_4$, Aldrich) and chlorides ($YbCl_3$, Aldrich). Some MS experiments were carried out using aqueous solutions of sodium acetate (c = 0.1 mol dm^{-3}, Kemika) and formic acid (w = 0.1 %, Kemika).

Electrospray Mass Spectrometry

Ligand solutions ($c = 5 \times 10^{-5}$ mol dm^{-3} to 10^{-3} mol dm^{-3}) were prepared in acetonitrile and methanol. Solutions with increasing metal:ligand molar ratios from 0.5 to 5 were prepared in acetonitrile. The ESI mass spectra of the solutions were obtained on Agilent 6410 Triple Quadrupole Mass Spectrometer. Mass spectra were

recorded in the range of m/z = 100 to m/z = 2000 in positive ion mode. Samples were introduced in mass spectrometer directly via Agilent 1260 HPLC (Agilent Technologies, Palo Alto, CA, SAD) by infusion. Capilary potential in mass spectrometer was 4 kV and fragmentor voltage was 135 V. Gas temperature was 350 °C and gas flow rate was 12 dm^3 min^{-1}. Tandem mass spectrometry was performed with collision energies of 10 eV up to 60 eV. Nitrogen was used as a collision gas.

Spectrophotometry

UV titrations of **1** in acetonitrile (V = 2.0 cm^3, c = 5.4 × 10^{-5} mol dm^{-3}, ϑ = (25.0 ± 0.1) °C) were carried out by the stepwise addition of lanthanide(III) salts directly into the measuring quartz cell (l = 1 cm). The spectrometric data were processed using SPECFIT program.[25]

RESULTS AND DISCUSSION

Full Scan Mass Spectra of Compounds 1–3

The MS spectra of peptidocalixarene acetonitrile solutions in the mass range from m/z 100 to m/z 2000 were acquired at three different concentrations: 5 × 10^{-5}, 1 × 10^{-4}, and 5 × 10^{-4} mol dm^{-3}. Since all compounds have nitrogen atoms which are easily protonated, the spectra were recorded in positive ion mode. The most abundant $[M+H]^+$ and $[M+2H]^{2+}$ ions were observed at 1 × 10^{-4} mol dm^{-3}, so all other measurements were performed at that concentration. In addition to signals of the ions mentioned above, the ones assigned to $[M+Na]^+$, $[M+H+Na]^{2+}$, and $[M+H+K]^{2+}$ ions were observed in MS spectra of **1**–**3** as well. The peaks corresponding to aducts with impurities already present in the system (Na^+, K^+, NH_4^+) are usually observed in MS spectra of calixarene derivatives.[12–14] As an example, the MS spectrum of **3** is shown in Figure 1.

The influence of solvent on signal intensities of **1** was studied by using methanol, and by adding the formic acid and sodium acetate in acetonitrile solution of **1**. The signal of singly protonated molecule $[M+H]^+$, which was not influenced by the solvent, was detected in all ESI MS spectra with very low intensity (0.25 to 1.2 %), while the signal of doubly charged calixarene $[M+2H]^{2+}$ increased from ≈2.5 % in acetonitrile to ≈8 % in methanol, and in acetonitrile solution containing formic acid. The most prominent change was observed in signal assigned to doubly charged molecule $[M+H+K]^{2+}$, going from ≈20 % in acetonitrile to ≈90 % in methanol. Also, the very intensive signal (≈94 %) in methanol was the one at 202.1 assigned to fragment ion. In acetonitrile, the intensity of this signal was ≈30 %. The base signal in acetonitrile solution with an excess of sodium ions was observed at m/z 863.8 and assigned to $[M+2Na]^{2+}$ ion.

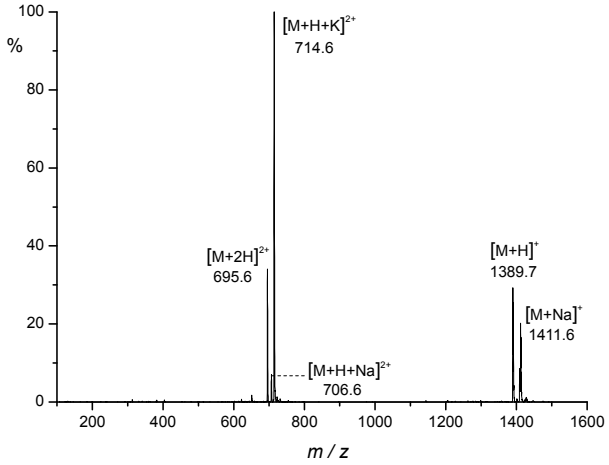


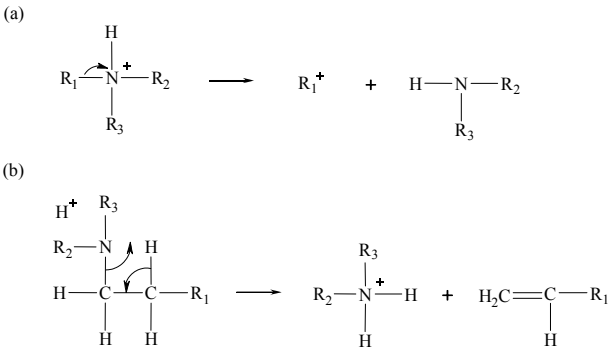
Figure 1. MS spectrum of **3** ($C_{80}H_{116}O_{16}N_4$, exact mass = 1388.84) in acetonitrile. c(**3**) = 1 × 10^{-4} mol dm^{-3}; fragmentor voltage = 135 V.

MS/MS Spectra of Compounds 1–3

The electrospray ionization usually generates even-electron ions (EE^+) which fragment according to the even-electron rule.[26] Two most important fragmentation reactions of EE^+ ions are shown in Scheme 2, and involve heterolytic or inductive cleavage (a) or a proton rearrangement (b). These reactions were observed for amines, amides, ethers and esters. However, in the study of pesticides, the formation of fragment ions with odd electrons ($OE^{+\bullet}$) was noticed in MS/MS spectra as well, but in extent less than 10 %.[27] The formation of unusual odd-electron fragments from EE^+ precursor ions were also recorded in MS/MS analysis of protonated natural occurring antibiotics.[28]

In the present paper, the comprehensive MS/MS analyses of all singly and doubly charged molecules observed in full scan mass spectra of peptidocalixarenes **1**–**3** were performed.

The fragmentation pathways of singly protonated molecules of compounds **1**–**3** were quite similar. The cleavage of C–O bond involving the phenolic oxygen atom and loss of the substituent at the lower rim were observed in all MS/MS spectra. In addition, a neutral



Scheme 2. Fragmentation of even-electron ions in MS/MS.

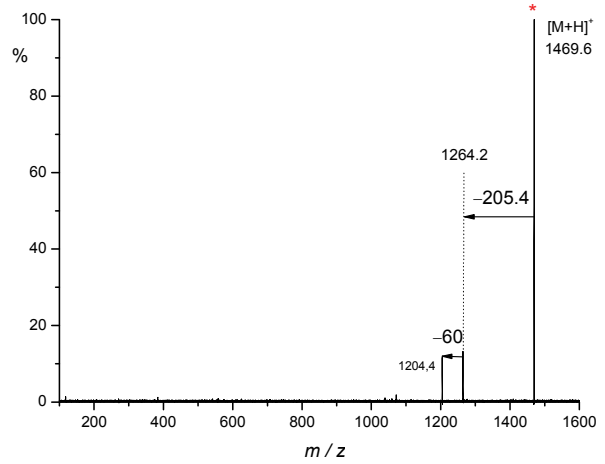


Figure 2. MS/MS spectrum of [**2**+H]$^+$ (*m/z* 1469.9) at 30 eV.

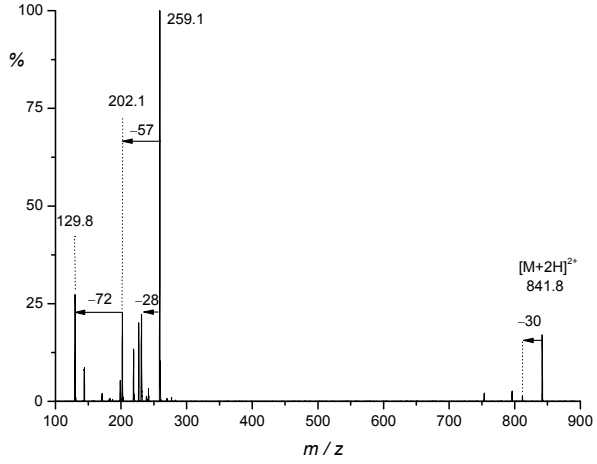
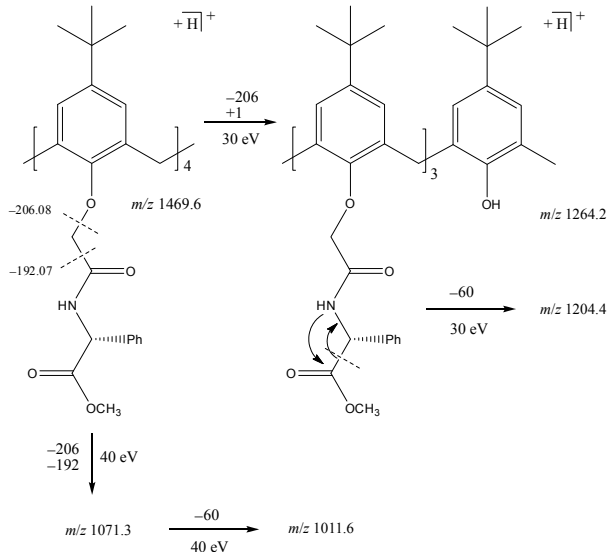
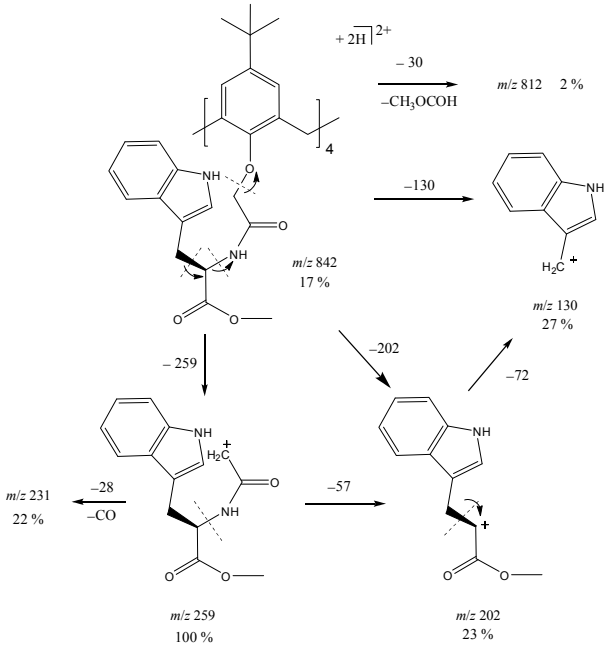


Figure 3. MS/MS spectrum of [**1**+2H]$^{2+}$ (*m/z* 841.8) at 20 eV.



Scheme 3. Proposed fragmentation pathway of protonated molecule [**2**+H]$^+$ (*m/z* 1469.6) at 30 eV and 40 eV.

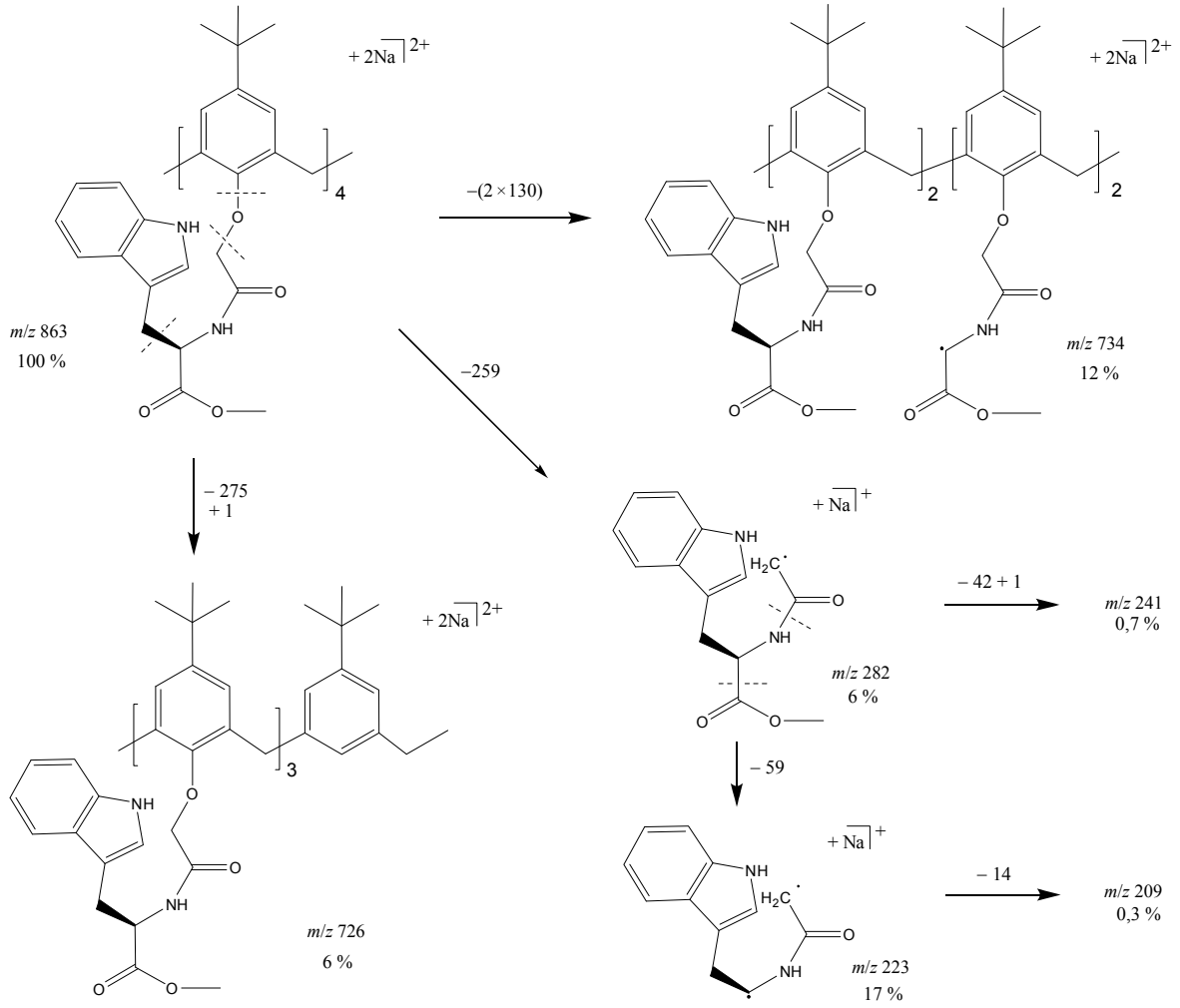


Scheme 4. Proposed fragmentation pathway of doubly protonated molecule [**1**+2H]$^{2+}$ (*m/z* 841.8) at 20 eV.

loss of 60 Da, assigned to CH_3OCOH group, was also recorded. As an example, the MS/MS spectrum of **2** at 30 eV is shown in Figure 2, and the corresponding fragmentation is proposed in Scheme 3. The loss of 60 Da could be a consequence of losing methyl methanoate (Scheme 3) or methanol and carbon monoxide. The cleavage of CH_2–CO bond in **2** was observed at higher potential (40 eV). The same bond was also cleaved in **3**, but a carbocation was formed ($C_{63}H_{87}N_2O_{10}$, exact mass = 1031.64), and the corresponding signal was noticed at *m/z* 1031.8. The collision potential applied amounted to 50 eV.

For fragmentations of doubly protonated [M+2H]$^{2+}$ peptidocalixarenes, less energy were needed (15-20 eV), as expected. However, beside the neutral loss of 60 Da (CH_3OCOH group), the formations of carbocations by heterolytic cleavage were observed for compounds **1-3**. An example corresponding to the calixarene derivative **1** is shown in Figure 3 and Scheme 4. The signals of analogous ions in MS/MS spectrum of **2** were at *m/z* 149 (100 %), 164 and 206, whereas in the spectrum of **3** the signal of carbocation was found at *m/z* 186.

The loss of small, neutral molecules like methanol and CO was also observed in MS/MS spectrum of [**1**+2H]$^{2+}$. The loss of methanol from protonated *N*-acetyl *O*-methoxy proline, with a same group as in **1** (–$COOCH_3$) was reported by I. Komáromi *et al.*[29] The possible explanation for the loss of 28 Da from ion with *m/z* 259 can be the migration of alkyl group followed by the loss of CO, as was observed in the metastable ion dissociation of acetylphenyl ions, $CH_3COC_5H_4^+$ (Ref. 30).



Scheme 5. Proposed fragmentation pathway of doubly sodiated molecule [**1**+2Na]$^{2+}$ (*m/z* 863.4) at 35 eV.

It is known that molecules may undergo the different fragmentation routes under the influence of the alkali-metal cations.[31] For that reason a MS/MS experiment on [**1**+2Na]$^{2+}$ ion has been also performed. As we were not able to isolate this ion form acetonitrile solution, we used the acetonitrile solution with an excess of sodium cation (the base signal in MS spectrum corresponded to [**1**+2Na]$^{2+}$), since once isolated, the ion gave the same MS/MS spectrum regardless from which solvent a parent ion was originated. The proposed fragmentation pathway of doubly sodiated molecule is shown in Scheme 5. As can be seen, the completely different mechanism compared to [**1**+2H]$^{2+}$, involving homolytic cleavage of bonds and formation of radicals, was observed. Probably that is a consequence of different charge distribution in the protonated and sodiated molecules. As generally observed, more energy was needed for this fragmentation, and still the most intensive signal was the one assigned to [**1**+2Na]$^{2+}$ ion.

The intensive signal in the MS spectra of peptidocalixarenes studied was the one assigned to [M+H+K]$^{2+}$ ion, so the MS/MS experiments on this ion were performed. The fragmentation was very similar for the compounds **1**–**3**, but different than those previously described. The loss of whole subunit including phenolic oxygen was dominant process (100 %), and then the radical loss, –130 Da for **1** (11 %), –206 Da for **2** (56 %), and –186 Da for **3** (11 %). The formations of carbocations were noticed for **1** and **2**, but the corresponding signals had intensities less than 1 %.

MS/MS Spectra of Complexes of 1 and 2 with Alkali-metal Cations

We have previously informed on coordination abilities of **1** and **2** towards alkali-metal cations.[21–24] The stability constants of the corresponding 1:1 complexes in acetonitrile were determined by spectrophotometric, conductometric and potentiometric titrations. Strong complexation was observed for lithium and sodium (lg $K_{\mathrm{Li}\mathbf{1}} > 6$, lg $K_{\mathrm{Li}\mathbf{2}} > 6$, lg $K_{\mathrm{Na}\mathbf{1}} = 8.25$, lg $K_{\mathrm{Na}\mathbf{2}} = 7.66$), moderate for potassium (lg $K_{\mathrm{K}\mathbf{1}} = 5.09$, lg $K_{\mathrm{K}\mathbf{2}} = 4.62$), whereas larger rubidium and cesium cations did not fit into the peptidocalixarenes ion binding sites. Herein, we

used ESI-MS to determine the stoichiometry of the complexes, and MS/MS experiments to easily and quickly predict the stability of the complexes formed.

The influence of metal to ligand molar ratio on the MS spectrum of **2** was studied by acquiring full scan mass spectra of solutions with following Na:**2** ratios: 0.5, 1.0, 2.0 and 5.0. In all spectra signals assigned to $[M+2H]^{2+}$, $[M+H+Na]^{2+}$, $[M+H+K]^{2+}$, $[M+2Na]^{2+}$, $[M+H]^{+}$, and $[M+Na]^{+}$ were observed. The signal of sodium complex $[M+Na]^{+}$ was the base signal, except for the last solution, where the signals at *m/z* lower than 350 were much more intensive than the others. Since the most abundant $[M+Na]^{+}$ ions were obtained at molar ratio 2.0, all other experiments were performed at this experimental condition.

The MS spectra of complexes with lithium and potassium cations showed the same pattern of signals as spectrum of sodium complex, and in addition the most intensive ones assigned to singly charged complexes of **2** with Li^{+} or K^{+}. Although the 2:1 complexes of calixarene derivatives with alkali metal cations were not observed by other techniques, in the MS spectrum signals corresponding to $[M+2Li]^{2+}$ and $[M+2K]^{2+}$ ions were present. These ions are probably formed during the electrospray process. The same stands for the $[M+Rb]^{+}$ and $[M+Cs]^{+}$ ions. In the case of the complexes with **1**, similar MS spectra were obtained.

The MS/MS experiments were performed on both singly and doubly charged ions of peptidocalixarenes complexes with alkali metal cations. The $[\mathbf{1}+Li]^{+}$ and $[\mathbf{1}+Na]^{+}$ ions were so stable that they did not fragment at the highest collision potential applied (60 eV). These results are in accordance with those obtained by "classical" methods.[21–24] The $[\mathbf{1}+K]^{+}$ ion was cleaved at potential of 60 eV, but still the signal of this ion remained the most intensive one. It should be noted that no dissociation of the complex was observed. Instead, the loss of radical (–130 Da) was a dominant process indicating a rather stable complex of the macrocycle with potassium ion. These results are in accordance with values of stability constants previously determined.[24] Although signals of $[\mathbf{1}+Rb]^{+}$ and $[\mathbf{1}+Cs]^{+}$ ions were present in the MS spectra, we could not isolate these ions due to their very low abundance.

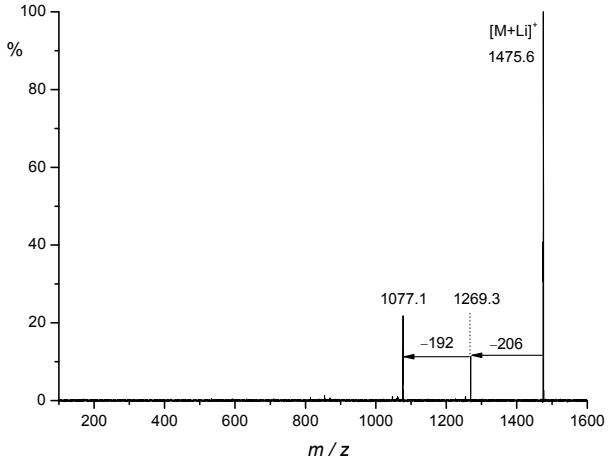


Figure 4. MS/MS spectrum of $[\mathbf{2}+Li]^{+}$ (*m/z* 1475.6) at 60 eV.

The tandem mass spectrometry investigations on complexes of alkali-metal cations with **2** were also carried out. The fragmentation of the singly charged complexes was observed at high collision potential (Figure 4), and loss of radical was recorded for Li^{+}, Na^{+} and K^{+} complexes. During fragmentation of sodium complex a loss of 2 calixarene subunits was observed, whereas in MS/MS experiments on $[\mathbf{2}+Rb]^{+}$ and $[\mathbf{2}+Cs]^{+}$ ions dissociation of adducts occurred. The results were in accordance with those obtained spectrophotometrically.

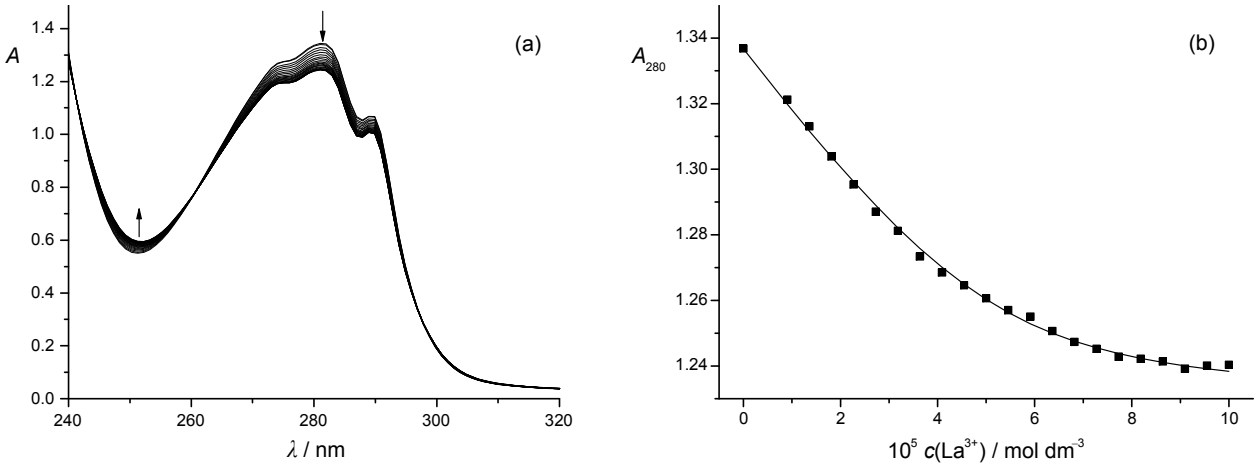


Figure 5. Spectrophotometric titration of **1** ($c = 5.39 \times 10^{-5}$ mol dm^{-3}) with $La(NO_3)_3$ in acetonitrile. $l = 1$ cm; $\vartheta = (25.0 \pm 0.1)$ °C; $c(La^{3+}) = 0$ (top curve) – 9.54×10^{-5} mol dm^{-3} (bottom curve); the spectra are corrected for dilution (a). Dependence of absorbance at 280 nm on La^{3+} concentration: experimental (■); calculated (—) (b).

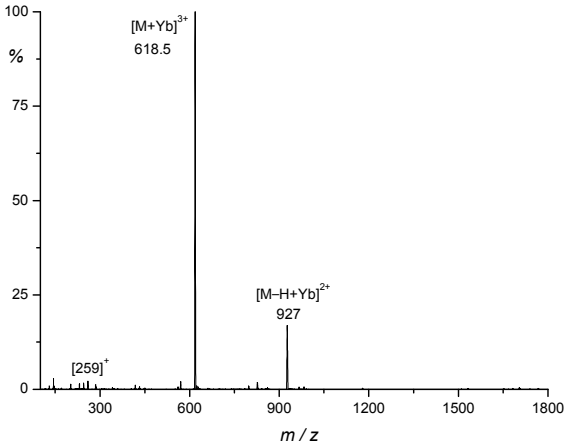


Figure 6. MS spectrum of acetonitrile $YbCl_3$ + **1** solution ($n(Yb^{3+}):n(\mathbf{1})$ = 1:1). $c(\mathbf{1}) = 1 \times 10^{-4}$ mol dm^{-3}; fragmentor voltage = 135 V.

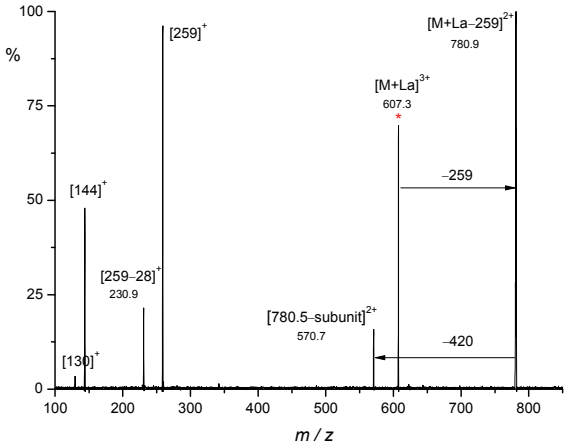
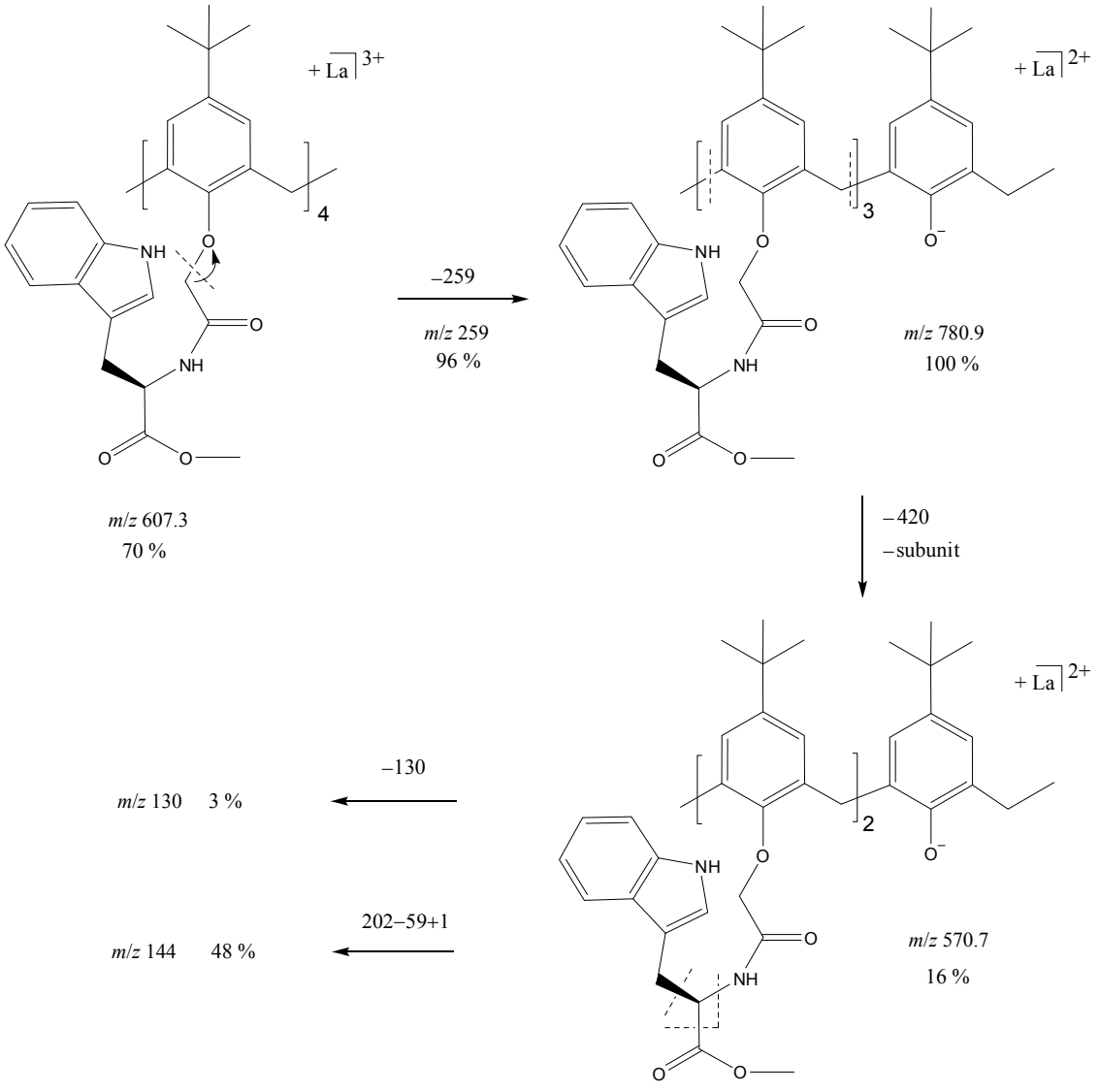


Figure 7. MS/MS spectrum of [**1**+La]$^{3+}$ (*m/z* 607.3) at 10 eV.



Scheme 6. Proposed fragmentation pathway of [**1**+La]$^{3+}$ (*m/z* 607.3) at 10 eV.

The fragmentation pathway of $[\mathbf{1}+2Li]^{2+}$ was similar to that of $[\mathbf{1}+2Na]^{2+}$ described previously. The radical loss (–192, –206 and –222 Da) was observed for $[\mathbf{2}+2Li]^{2+}$ and $[\mathbf{2}+2Na]^{2+}$ ions, while in the case of $[\mathbf{2}+2K]^{2+}$ ion dissociation leading to $[\mathbf{2}+K]^{+}$ ion occurred.

Complexes of 1 with Lanthanides

The study of coordination abilities of **1** was extended to lanthanide cations, *i.e.* La^{3+}, Ce^{3+}, Eu^{3+} and Yb^{3+}. The stability constants of 1:1 complexes in acetonitrile were determined by spectrophotometric titrations (an example is shown in Figure 5). It should be noted that the ion pairing, which is likely to occur to a significant extent in acetonitrile solutions, was not taken into account. Based on at least three titrations, the following results were obtained: lg $K_{\mathrm{La}\mathbf{1}}$ = 5.27 and lg $K_{\mathrm{Ce}\mathbf{1}}$ = 5.26. Stability constants for $Eu\mathbf{1}^{3+}$ and $Yb\mathbf{1}^{3+}$ complexes could be only estimated, lg $K \geq 6$, because of the limited sensitivity of spectrophotometry. From electrostatic point of view, the observed increase of stability constants along the series was expected.[20]

In the MS spectra of acetonitrile solutions of $La\mathbf{1}^{3+}$ complexes ($n(La^{3+})$: $n(\mathbf{1})$ = 0.5, 1 and 5) the base signal at *m/z* 154.9 was assigned to fragment ion $[La+171]^{+}$ containing tryptophan subunit. The $[\mathbf{1}+La]^{3+}$ and $[\mathbf{1}+La+NO_3]^{2+}$ ions were observed but with very low intensities. All other signals ($[\mathbf{1}+H]^{+}$, $[\mathbf{1}+Na]^{+}$, *etc.*) recorded in MS spectrum of **1** were also present. By lowering fragmenting potential from 135 to 40 V, only a small increase in signal intensity of $[\mathbf{1}+La]^{3+}$ was obtained, so all other measurements were performed at fragmentor potential of 135 V. The MS spectrum of acetonitrile Ce^{3+} + **1** solution ($n(Ce^{3+})$: $n(\mathbf{1})$ = 1) was very similar. However, the mass spectra of solutions of $Eu\mathbf{1}^{3+}$ and $Yb\mathbf{1}^{3+}$ complexes were considerably different. Only the signals assigned to $[M+Ln]^{3+}$ and $[M-H+Ln]^{2+}$ ions (Ln stands for lanthanide) were present (Figure 6), as well as the signal corresponding to carbocation at *m/z* 259 formed by the cleavage of the ether bond.

The results of MS/MS experiments performed on $[M+Ln]^{3+}$ ions can be related to the relative stability of the complexes studied. At the same collision potential applied (10 eV), more fragmentation was observed for La^{3+} and Ce^{3+} complexes. The tandem mass spectra of $[La\mathbf{1}]^{3+}$ ion, and the proposed fragmentation pathway are shown in Figure 7 and Scheme 6, respectively. The loss of 259 Da, as well as the loss of calix[4]arene subunit, was also recorded in fragmentation of $[Ce\mathbf{1}]^{3+}$, but in a smaller extent. During the fragmentation of the other two complexes, namely $[Eu\mathbf{1}]^{3+}$, and $[Yb\mathbf{1}]^{3+}$, only the cleavage of ether bond (–259 Da) and formation of carbocation was observed.

CONCLUSION

In this paper it has been shown that mass spectrometry enables fast and sensitive characterization of various peptidocalixarenes and their complexes with alkali-metal and lanthanide cations. Good correlations with the results obtained previously by other methods were achieved. Furthermore, the tandem mass spectrometry (a fast method which requires only small sample quantities) can rather simply and quickly provide information on the relative stabilities of the complexes.

Acknowledgements. This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (projects 119-1191342-2960).

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