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Electron capture dissociation Fourier transform ion cyclotron resonance mass spectrometry of cyclodepsipeptides, branched peptides, and ε-peptides

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Abstract

Although electron capture dissociation (ECD) offers many advantages for structural elucidation, a fundamental understanding of all possible processes following electron capture is necessary if ECD is to succeed in the characterization of unknowns. Many biologically active compounds have non-standard structures, e.g, *N*-alkylation, branching, cyclization, and ester linkages. Here we report ECD of cyclodepsipeptides (valinomycin and beauvericin), including *N*-methylated structures (beauvericin), branched peptides ($AcA_3K(G_3)A_3$–NH_2 and $A_3K(G_3)A_3$–NH_2), and oligomers of ε-amino acids (ε-peptides) ($Ac(Ahx)_6K$ and $(Ahx)_6K$) to establish the behavior of such non-standard structures. ECD of cyclodepsipeptides yielded numerous backbone fragments but no charge-reduced species, consistent with a radical cascade mechanism. ECD of ε-peptides resulted in $a^\bullet$ and y fragments only, suggesting that the N–Cα $c/z^\bullet$ fragmentation channel is impeded in those structures. ECD of branched peptides resulted in complex fragmentation patterns, characterized by the presence of the immonium related m ion from the modified residue.

Keywords: ECD; FT-ICR; Cyclodepsipeptides; Branched peptides; ε-Peptides

1. Introduction

Tandem mass spectrometry (MS/MS) [1], in which an ionic precursor is characterized according to its fragments, is well established. Methods for inducing fragmentation in Fourier transform ion cyclotron resonance (FT-ICR) [2,3] mass spectrometry include infrared multiphoton dissociation (IRMPD) [4,5], sustained off-resonance irradiation collision-induced dissociation (SORI CID) [6], blackbody infrared radiative dissociation (BIRD) [7,8], and surface-induced dissociation (SID) [9]; and electron capture dissociation (ECD) [10–12]. Following its introduction in 1998 [10], electron capture dissociation demonstrated several unique features. As summarized in a recent review [13], ECD results in cleavage of peptide backbone N–Cα bonds to produce c and $z^\bullet$ (or $c^\bullet$ and z) fragment ions, whereas traditional techniques cleave peptide backbone amide bonds and form b and y ions [14,15]. Other unique features include preferential cleavage of disulfide bonds [16,17] and retention of post-translational modifications [18], e.g., phosphorylation [19,20] and glycosylation [21,22], for peptide backbone fragments. Sites of modification may thus be identified. That feature represents a significant advantage over traditional techniques, which typically result in the loss of labile modifications. Although the *presence* of a modification may be inferred by comparing the parent peptide ion mass to that of its MS/MS fragment ions, knowledge of the *site* of modification is lost. Clearly, ECD is a valuable tool for the structural elucidation of peptides and proteins and has high potential for the analysis of unknowns. In order for ECD to fulfill that potential, a fundamental understanding of all processes that occur following electron capture is required.

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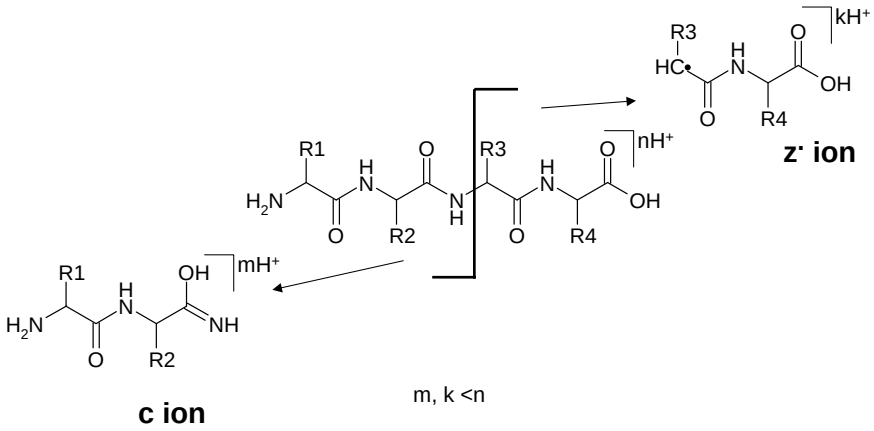
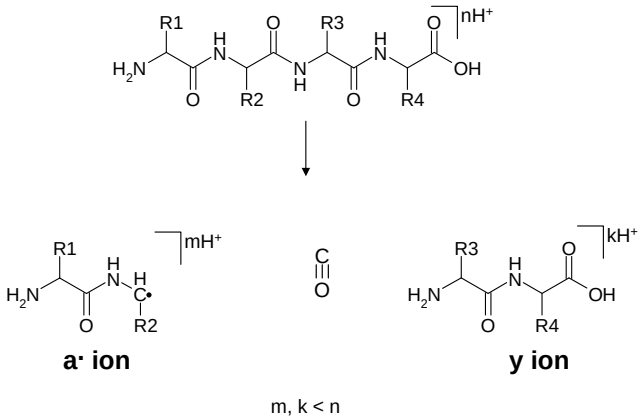


Scheme 1. N–Cα cleavage to produce c and z• fragment ions observed in ECD of standard peptides.

Typically, the major product of electron capture is the charge-reduced species, $[M+nH]^{(n-1)+\bullet}$, i.e., the precursor $[M+nH]^{n+}$ captures an electron but does not fragment. Hydrogen atom loss, i.e., production of $[M+(n-1)H]^{(n-1)+}$, is another common channel [23]. In terms of peptide backbone cleavage, the main process following electron capture is production of c and z• (or c• and z) as mentioned above, see Scheme 1. A second fragmentation pathway is production of a• and y ions [16], see Scheme 2. Although these pathways are (generally) dominant, several other processes may be initiated by electron capture. For example, cleavages within amino acid side-chains [10,24] can produce fragment ions of abundances comparable to those arising from backbone cleavage. Cooper et al. [24] demonstrated that fragment ions resulting from side-chain cleavage may even be the major products of ECD, with abundances greater than the charge-reduced species.

Kjeldsen et al. [25] introduced the technique of hot electron capture dissociation (HECD). Unlike standard ECD which employs electrons of low energy (>0.2 eV), HECD utilizes electrons of ∼10 eV. HECD results in extensive secondary fragmentation. In particular, dissociation of z•



Scheme 2. Generation of a• and y fragment ions observed in ECD of standard peptides.

ions results in the formation of characteristic w ions. Thus isoleucine and leucine may be distinguished. Cooper et al. [26] and Kjeldsen et al. [27] separately demonstrated that w ions may also result under standard ECD conditions. Additionally, secondary fragmentation involving the loss of entire amino acid side-chains can occur in standard ECD [26]. HECD also results in the formation of u ions [28], which are generated through secondary fragmentation of z• ions. Creation of a γ-lactam by bond formation between the N-terminal α- and β-carbon within the adjacent amino acid side chain results in side-chain cleavage in the second residue. Although u ions are less abundant than w ions, they are also useful in distinguishing Ile/Leu.

Formation of c and z• ions requires the cleavage of the N–Cα bond. For proline, N–Cα cleavage alone would not result in observable fragments because the precursor ion would remain intact by virtue of the cyclized side-chain. Recently, Cooper et al. [26] demonstrated that N–Cα cleavage within proline can be accompanied by secondary cleavage within the cyclized side-chain. Hence z-type fragments could be observed. Other examples in which capture of a single electron results in multiple bond cleavage include coincident cleavage of disulfide and backbone amine bonds in disulfide-cyclized peptides [16], and backbone fragmentation of doubly-charged cyclic peptides [29]. Leymarie et al. [29] proposed a mechanism in which the initial α-carbon radical species is propagated along the peptide chain to explain backbone cleavage in cyclic peptides.

In the present work, we investigate ECD of several non-standard peptides with the aim of furthering the knowledge of the processes that can occur following electron capture, and particularly the application of the technique to the structural elucidation of unknowns. First, we performed ECD of cyclodepsipeptides, or peptide lactones, which contain one or more ester linkages in the backbone. Cyclodepsipeptides occur widely in nature and many exhibit potentially exploitable biological activities such as anti-cancer, anti-bacterial, anti-viral, anti-fungal, anti-inflammatory, and anti-clotting properties [30]. Tandem mass spectra of cy-

clodepsipeptides are complex due to multiple ring-opening pathways [31], and sequence analysis is non-trivial. That problem has been addressed by chemical pre-treatment [32–34] and analysis of MS/MS of $[M + Na]^+$ precursor ions [35].

Second, we examined ECD of branched peptides. Branched peptides have applications in vaccine development [36,37], antibiotics [38], and protein models [39–41]. Typically, branching is achieved through a bivalent lysine [36,37,40–42]; however, alternative templates include α,α-disubstituted β-alanines [43] and pentaerythritol tetraacrylate [44]. In the present examples, branching occurs through bivalent lysine. The bivalent lysine structure emulates that of the ubiquitination modification of proteins. Ubiquitination is achieved by covalent addition of ubiquitin to the ϵ-amino group of the side chain of a lysine residue in the substrate protein. Ergo, a further reason for studying the ECD of branched peptides was to investigate the usefulness of ECD in ubiquitination analysis. Finally, we considered ECD of ε-peptides to investigate the effect of remote adjacent peptide bonds.

2. Experimental methods

2.1. Samples

Cyclodepsipeptides, valinomycin, and beauvericin were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification. The branched peptides ($AcA_3K(G_3)A_3$–NH_2 and $A_3K(G_3)A_3$–NH_2) and the ε-peptides ($Ac(Ahx)_6K$–OH and $(Ahx)_6K$–OH, Ahx is aminohexanoic acid) were synthesized by the BASS facility at Florida State University. Stock solutions of the depsipeptides in toluene (∼1 mM) were diluted to 1 μM in 1:1 water:acetonitrile (J.T. Baker, Philipsburg, NJ), 1% formic acid (Aldrich, Milwaukee, WI), and microelectrosprayed. The branched and ε-peptides were microelectrosprayed at 10 μM from solutions of 1:1 water:methanol (J.T. Baker), 2% formic acid (Aldrich).

2.2. Electron capture dissociation FT-ICR mass spectrometry

Peptide samples were analyzed with a homebuilt, passively-shielded, 9.4 Tesla FT-ICR mass spectrometer [45] equipped with an external microelectrospray ionization source [46]. The samples were infused at a flow rate of 300 nl/min through an electrospray emitter consisting of a 50 μm i.d. fused silica capillary which had been mechanically ground to a uniform thin-walled tip [47]. Two kilovolts was applied between the microspray emitter and the capillary entrance. The electrosprayed ions were delivered into the mass spectrometer through a Chait-style atmosphere-to-vacuum interface [48] and externally accumulated [46] for 8–20 s in an rf-only octapole. The ions were transferred through multipole ion guides and trapped in an open [49] cylindrical cell (Malmberg–Penning trap) [50]. A front-end resolving quadrupole [51] and/or stored-waveform inverse Fourier transform (SWIFT) [52,53] ejection served to isolate the peptide ion under investigation.

An indirectly heated dispenser cathode (Heat Wave, Watsonville, CA) mounted on the central axis of the system provided the electrons for ECD [54]. A potential of either −1.6 or −4.5 V was applied to the cathode during the irradiation event. A grid situated in front of the filament was kept at −200 V for most of the experiment and pulsed to either +6 or +200 V during the ECD event. The isolated parent ions were irradiated with electrons for 17–250 ms.

Ions were frequency-sweep ("chirp") [55] excited (72–640, or 72–1270 kHz, at 150 Hz/μs) and detected in direct mode (512 kword time-domain data). Between 50 and 200 time-domain data sets were co-added, Hanning apodized, zero-filled once and subjected to fast Fourier transform (FFT) followed by magnitude calculation. The experimental event sequence was controlled by a modular ICR data acquisition system (MIDAS) [56]. The FT-ICR mass spectra were internally frequency-to-*m*/*z* calibrated [57,58] with respect to the precursor ion, and the charge-reduced species or backbone fragments. The FT-ICR mass spectra were analyzed by use of the MIDAS analysis software package [59].

3. Results and discussion

3.1. ECD of cyclodepsispeptides

Figs. 1 and 2 show mass spectra obtained following ECD of valinomycin (cyclo-(Val-Hval-Val-Lac)$_3$, in which HVal is hydroxyisovaleric acid, Lac is lactic acid) $[M + H + K]^{2+}$ ions; and beauvericin (Cyclo-(HVal-NMePhe)$_3$) $[M + H + K]^{2+}$ ions. Both spectra were calibrated internally with respect to backbone fragments and the root mean square errors were 0.4 ppm, for a mass accuracy of ∼1.5 ppm. The fragments are detailed in Tables 1 and 2. Interestingly, all fragments retain the potassium cation. Numerous backbone fragments are observed for both species, i.e., capture of a single electron results in multiple bond cleavages. This result is supported by the ECD of cyclic peptides reported by O'Connor and co-workers [29], who proposed a radical migration mechanism to explain their findings. Furthermore, charge-reduced species were not observed for beauvericin and were observed in very low abundance (S/N ∼ 1:1) for valinomycin. Typically, the first charge-reduced species is the dominant product of electron capture. Generation of radical ions by electron-initiated backbone cleavage of these species is thus accompanied by immediate further radical chemistry in virtually all cases.

Electron capture initiates two major fragmentation pathways for valinomycin. First, N–Cα c/z-type cleavage within a valine residue, in combination with N–Cα c/z-type cleav-

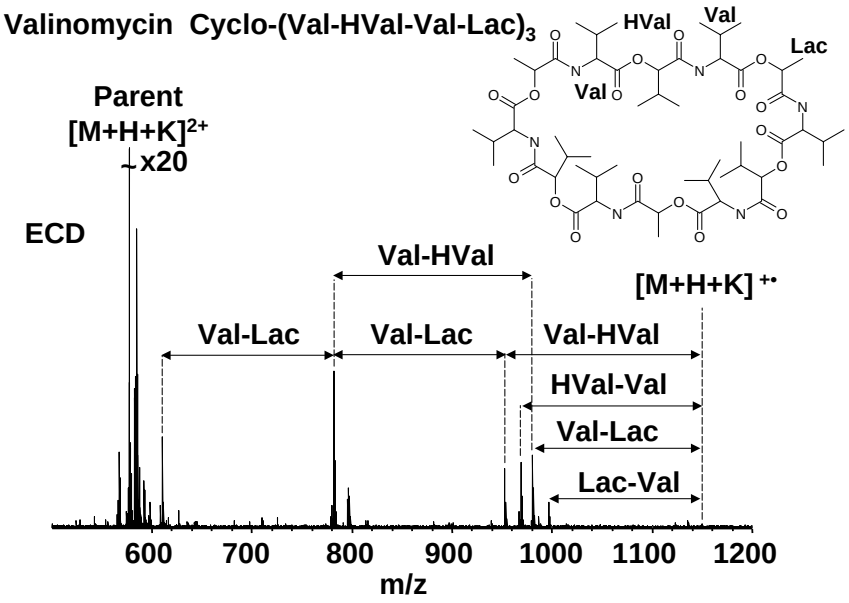


Fig. 1. ESI ECD FT-ICR mass spectrum of valinomycin $[M+H+K]^{2+}$ ions. *Inset*: Structure of valinomycin. Fragments are shown as amino acid losses from charge-reduced species (refer to Table 1 for cleavage types).

age in another valine residue was observed. Fragments observed at *m/z* 980.520, 952.490, 781.400, 610.310, and 411.187 correspond to N–Cα c/z• ring opening, of the type observed in the ECD of standard linear peptides (see Scheme 1), accompanied by N–Cα cleavage in which a z-type fragment is generated, i.e., a radical species is lost. The results may be explained by the radical cascade mechanism proposed by O'Connor and co-workers [29], see Scheme 3. In that mechanism, ring opening through cleavage of an N–Cα bond and generation of a radical species (as typically observed for ECD) is followed by further N–Cα bond cleavage by either direct radical rearrangement, as shown in Scheme 3, or following migration of the radical by hydrogen atom abstraction (not shown). The fragments observed at *m/z* 779.384 and 608.294 correspond to N–Cα c/z• ring opening with homolytic N–Cα cleavage and additional H• loss. Again, generation of those fragments may be explained by the radical migration mechanism.

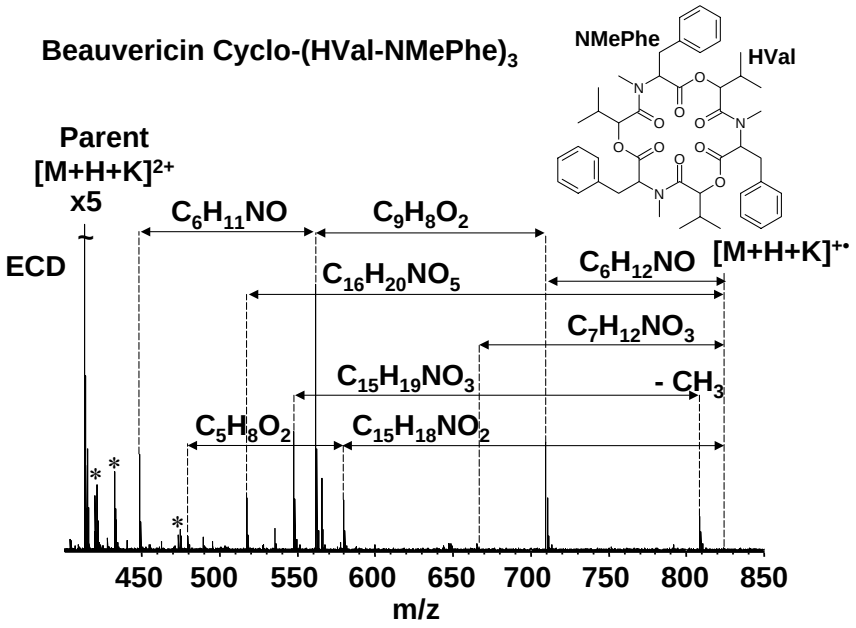


Fig. 2. ESI ECD FT-ICR mass spectrum of beauvericin $[M+H+K]^{2+}$ ions. *Inset*: Structure of beauvericin. Fragments are shown as losses from charge-reduced species (refer to Table 2 for cleavage types). Losses are not shown as amino acids due to complexity of cleavage channels. No charge-reduced species is observed. (∗): Doubly-charged species not removed during isolation.

Table 1
Fragments observed following electron capture dissociation of $[M + H + K]^{2+}$ ions of valinomycin

Measured *m/z*	Calculated *m/z*	Assignment
411.1871	411.1891	–($C_{36}H_{59}N_4O_{12}$) (Val-Lac-Val-HVal-Val-Lac-Val-HVal)[a]
608.2941	608.2943	–($C_{26}H_{44}N_3O_9$) (Val-Lac-Val-HVal-Val-Lac)[b]
610.3098	610.3100	–($C_{26}H_{42}N_3O_9$) (Val-Lac-Val-HVal-Val-Lac)[a]
626.3045	626.3049	–($C_{26}H_{42}N_3O_8$) (Lac-Val-Hval-Val-Lac-Val)[c]
779.3840	779.3839	–($C_{18}H_{31}N_2O_6$) (Val-HVal-Val-Lac) or (Val-Lac-Val-HVal)[b]
781.3999	781.3995	–($C_{18}H_{29}N_2O_6$) (Val-HVal-Val-Lac) or (Val-Lac-Val-HVal)[a]
795.3792	795.3788	–($C_{18}H_{31}N_2O_5$) (HVal-Val-Lac-Val) or (Lac-Val-HVal-Val)[d]
796.3872	796.3866	–($C_{18}H_{30}N_2O_5$) (HVal-Val-Lac-Val) or (Lac-Val-HVal-Val)[e]
952.4896	952.4891	–($C_{10}H_{16}NO_3$) (Val-HVal)[a]
966.4697	966.4683	–($C_{10}H_{18}NO_2$) (HVal-Val)[d]
968.4845	968.484	–($C_{10}H_{16}NO_2$) (HVal-Val)[c]
980.5201	980.5204	–($C_8H_{12}NO_3$) (Val-Lac)[a]
996.5156	996.5153	–($C_8H_{12}NO_2$) (Lac-Val)[c]

Assignments are expressed as losses from the charge-reduced precursors.
[a] N–Cα c/z• ring opening with N–Cα cleavage to produce z-type ions.
[b] N–Cα c/z• ring opening with homolytic N–Cα cleavage and H• loss.
[c] Ester (a•/y) cleavage with O–Cα cleavage to produce c-type ions.
[d] Ester (a•/y) cleavage with homolytic O–Cα cleavage and H• loss.
[e] Ester (a•/y) cleavage with homolytic O–Cα cleavage.

An interesting point to consider is that ECD of linear peptides generates radical species (typically z• ions). Given that radical cascade reactions are observed for depsipeptides, and even more pertinently cyclic peptides [29], one might expect a similar phenomenon for z• ions of linear peptides. Perhaps degradation of z• ions in this manner does occur, accounting for their low abundances relative to c ions. However, if such secondary fragmentation occurred, one would expect to see internal fragment ions, and they are not typically seen following ECD.

Second, ECD of valinomycin results in ester bond cleavage in combination with O–Cα c/z-type cleavage in the lactone moieties. Fragments observed at *m/z* 996.516, 968.485, and 626.305 correspond to ring opening by a•/y-type cleavage of an ester bond (similar to that shown in Scheme 2) accompanied by O–Cα cleavage, in which a c-type fragment is generated, i.e., a radical species is lost. The fragment observed at 796.387 corresponds to ester bond (a•/y) cleavage accompanied by homolytic O–Cα cleavage, and those at *m/z* 966.470 and 795.379 to ester bond (a•/y) cleavage with homolytic O–Cα cleavage and additional H• loss. Electron capture-initiated ester bond (a•/y) cleavage was also observed by Guan [60] in the ECD of acylated peptides. A proposed mechanism is shown in Scheme 4. Ring opening is achieved by cleavage of the ester bond, generating an α-carbon radical. As above, O–Cα bond cleavage occurs either by direct radical rearrangement (as shown in Scheme 4) or following migration of the radical by hydrogen abstraction (not shown).

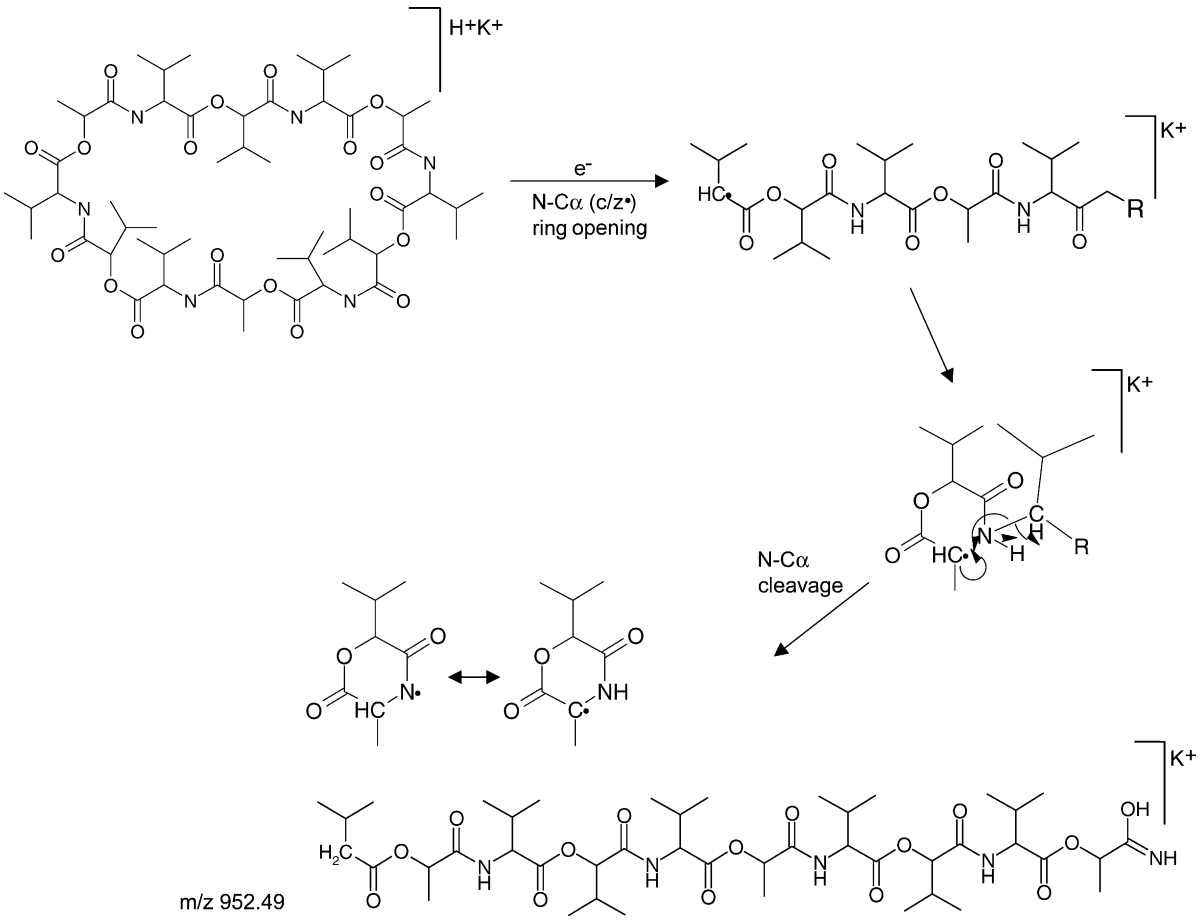
The ECD behavior of beauvericin is more complex. Beauvericin contains three *N*-methylated amino acid residues (NMePhe). A peak corresponding to loss of a methyl group was observed at *m/z* 808.357. That observation may be predicted and has been observed for other cyclic peptides containing *N*-alkylated amino acid residues (data not shown). ECD of standard peptide ions results in cleavage of the N–Cα bond. N-substituted residues present two possibilities for such cleavage.

In addition to the N–Cα and N–Cα-type fragmentation (see Scheme 3) observed for valinomycin, beauvericin ions undergo O–Cα fragmentation in combination with O–Cα

Table 2
Fragments observed following electron capture dissociation of $[M + H + K]^{2+}$ ions of beauvericin

Measured *m/z*	Calculated *m/z*	Assignment
404.1616	404.1621	–($C_{22}H_{31}N_2O_6$) (HVal-NMePhe-HVal)[a]
411.6938	411.6900	Parent $[M + H + K]^{2+}$
448.1519	448.1521	–($C_{21}H_{31}N_2O_4$) (Hval-NMePhe-HVal)[b]
479.1942	479.1943	–($C_{20}H_{26}NO_4$)(Hval-NMePhe-HVal)[c]
489.2150	–	
517.2465	517.2464	–($C_{16}H_{20}NO_5$) (Hval-NMePhe)[a]
535.1844	–	
547.2207	547.2205	–($C_{16}H_{22}NO_3$) ((NmePhe-HVal)+CH_3)[d]
561.2365	561.2361	–($C_{15}H_{20}NO_3$) (Hval-NMePhe) or (NMePhe-HVal)[b,e]
565.2581	–	
579.2470	579.2467	–($C_{15}H_{18}NO_2$) (Hval-NMePhe)[f]
665.2987	665.2988	–($C_7H_{12}NO_3$) (Hval)[a]
709.2887	709.2886	–($C_6H_{12}NO$) (Hval)[b]
808.3567	808.3570	–CH_3

Assignments are expressed as losses from the charge-reduced precursors. Amino acid residues originate at residue in which N–Cα or O–Cα, is cleaved.
[a] Ester (a•/y) cleavage with homolytic N–Cα or O–Cα cleavage.
[b] N–Cα (c/z•) ring opening with homolytic N–Cα or O–Cα backbone cleavage.
[c] Amide a•/y ring opening with O–Cα cleavage to produce a c-type ion.
[d] N–Cα (c/z•) with homolytic N–Cα backbone cleavage and methyl loss.
[e] O–Cα (c/z•) ring opening with homolytic O–Cα backbone cleavage.
[f] Ester a•/y ring opening with O–Cα cleavage to produce a c-type ion.



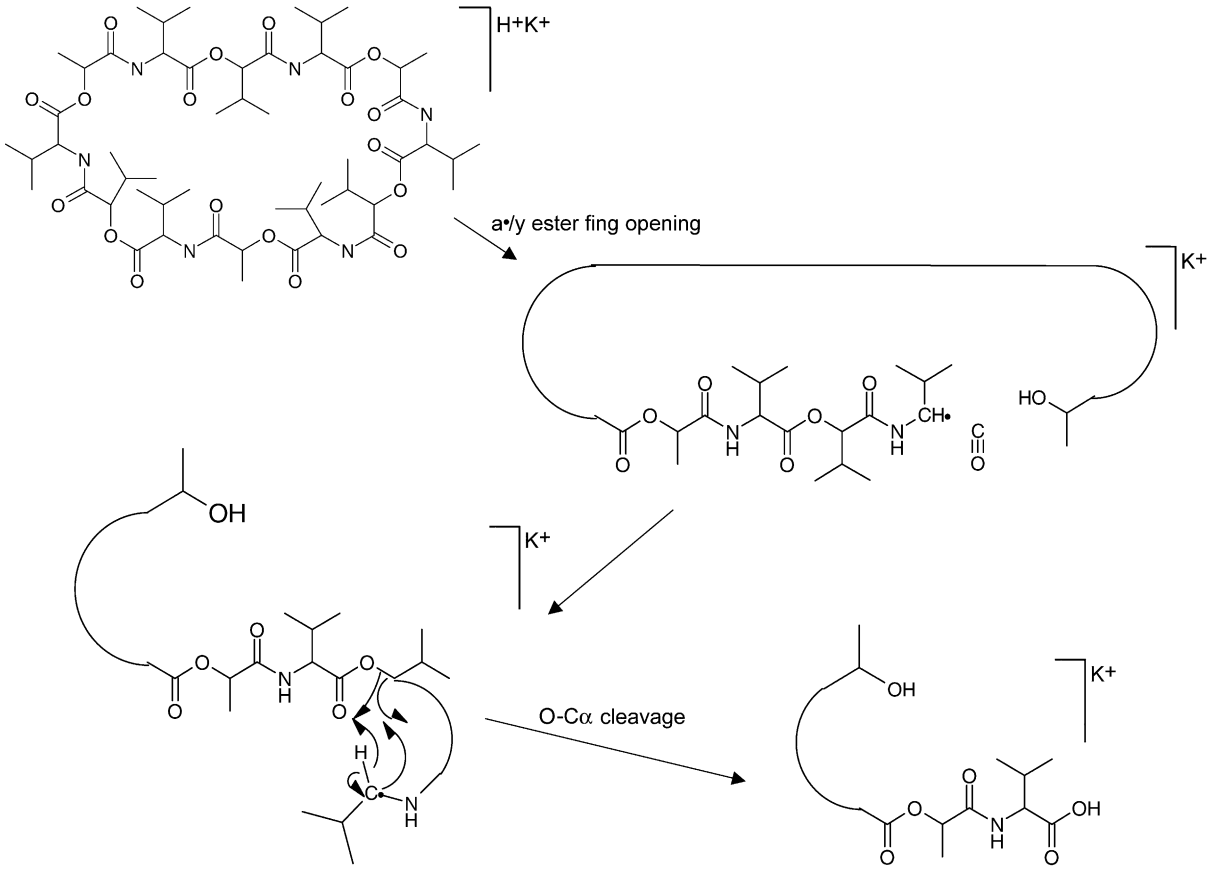
Scheme 3. N–Cα c/z-type cleavages observed for ECD of valinomycin.

and N–Cα fragmentation. For an example see Scheme 5. Again, the results may be explained by a radical cascade mechanism. Ions of *m/z* 561.237 may arise either by N–Cα (c/z•) ring opening accompanied by homolytic N–Cα cleavage, or by O–Cα (c/z•) ring opening with homolytic O–Cα cleavage in the lactone structures. The two possible fragmentation pathways may account for the greater abundance of those ions. The peaks observed at *m/z* 665.299, 517.247, and 404.162 correspond to ester (a•/y) cleavage with homolytic N–Cα or O–Cα cleavage; see e.g., Scheme 6. Ions of *m/z* 579.247 correspond to ester (a•/y) cleavage with O–Cα cleavage to produce c-type ions, see Scheme 7. Ions of *m/z* 479.194 correspond to a•/y cleavage of a peptide bond, i.e., within an NMePhe residue, with O–Cα cleavage to produce a c-type fragment; see Scheme 8.

Ions of *m/z* 547.221 correspond to loss of $C_{16}H_{22}NO_3$ from the parent ion. That loss can be attributed to N–Cα (c/z•) ring opening and homolytic N–Cα backbone cleavage (loss of $C_{15}H_{19}NO_3$), in conjunction with loss of a methyl group from an *N*-methylated residue. Although those two fragmentation pathways may be separate events, another possibility is that such a loss involves methyl migration as described by Turecek and Carpenter [61] in their study of glycine radicals in the gas-phase; see Scheme 9.

3.2. ECD of branched peptides

Fig. 3 shows the mass spectrum obtained following ECD of the branched peptide $AcA_3K(G_3)A_3–NH_2$ $[M+H+Na]^{2+}$ ions. The spectrum was internally calibrated with respect to the precursor and charged-reduced ions. The fragmentation sites are shown schematically in Fig. 4 and detailed in Table 3. Similar results were obtained for the branched peptide $A_3K(G_3)A_3–NH_2$ (data not shown). c and z• ions arising from cleavage within both the alanine and glycine branches (denoted (A) and (G), respectively) are observed, as are y ions deriving from the glycine branch. No cleavage of the N–Cε bond in the modified lysine side chain was found. That result agrees with those obtained for the ε-peptides (see below). Ions of *m/z* 577.271 correspond to $[b_4 + Na]^+$, i.e, cleavage of the amide bond C-terminal to the modified lysine residue. b ions are rarely observed in ECD spectra [26]. Of particular note are the ions of *m/z* 564.263, corresponding to $[m_4+Na]^{+\bullet}$; i.e., cleavage of the entire lysine side-chain glycine branch. After c_6 and c_5, the



Scheme 4. Ester ($a^{\bullet}$/y) cleavage with O–Cα cleavage observed for ECD of valinomycin.

Table 3
Fragments observed following electron capture dissociation of $[M + H + Na]^{2+}$ ions of the branched peptide, $AcA_3K(G_3)A_3–NH_2$

Measured *m/z*	Calculated *m/z*	Assignment
536.2683	536.2683	$[z_{4(A)}^{\bullet} + Na]$
564.2632	564.2633	$[m_4^{\bullet} + Na]$
572.3157	572.3156	c_4
577.2712	577.2711	$[[b_4 + Na]$
594.2975	594.2976	$[c_4 + Na]$
607.3054	607.3055	$[z_{5(A)}^{\bullet} + Na]$
614.3623	614.3626	$y_{4(G)}$
636.3446	636.3446	$[y_{4(G)} + Na]$
643.3525	643.3527	c_5
665.3347	665.3347	$[c_5 + Na]$
677.3473	677.3473	$[z_{5(G)}^{\bullet} + Na]$
678.3423	678.3426	$[z_{5(G)} + Na]/[z_{6(A)}^{\bullet} + Na]$
693.3661	693.3660	$[y_{5(G)} + Na]$
714.3894	714.3899	c_6
734.3684	734.3688	$[z_{6(G)}^{\bullet} + Na]$
736.3714	736.3719	$[c_6 + Na]$
749.3794	749.3791	Loss of acetyl group from $[M + Na]^+$
785.4268	785.4270	$[M + H]^+$
791.3893	791.3897	Loss of NH_3 from $[M + H + Na]^{\bullet +}$
807.4084	807.4084	$[M + Na]^+$

(G) and (A) refer to glycine and alanine branches, respectively.

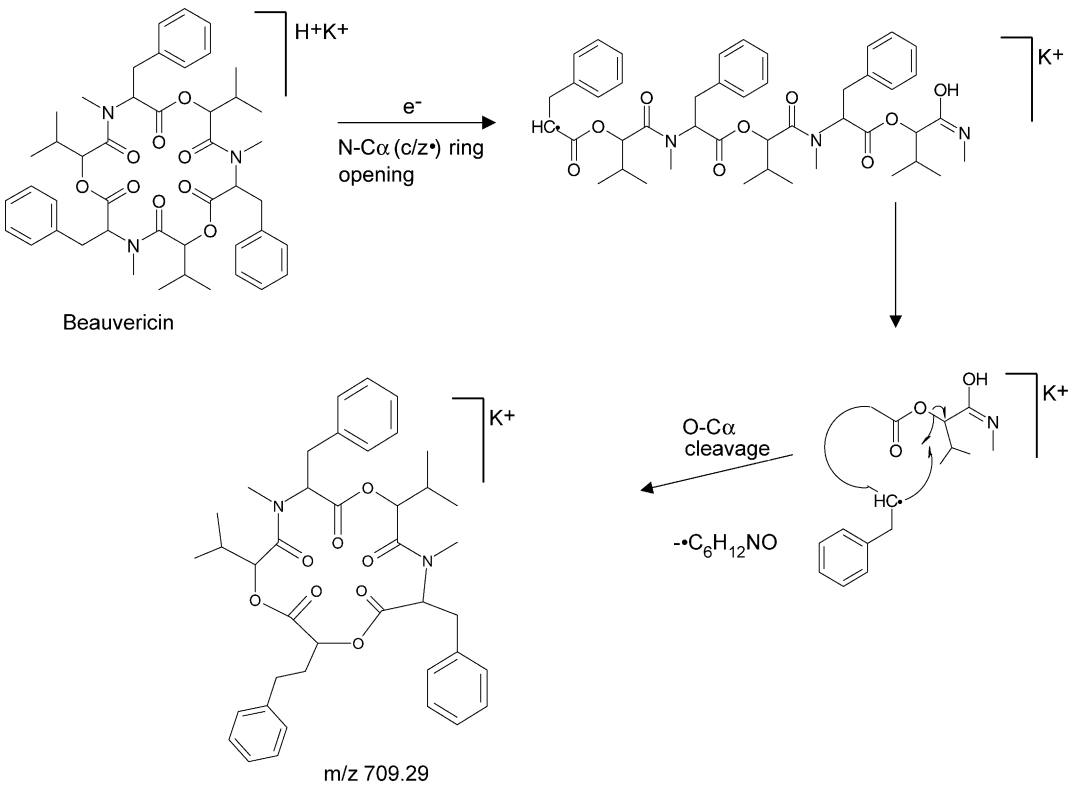
$m_4^{\bullet}$ ion is the most abundant product and is characteristic in the ECD of branched peptides.

3.3. ECD of ε-peptides

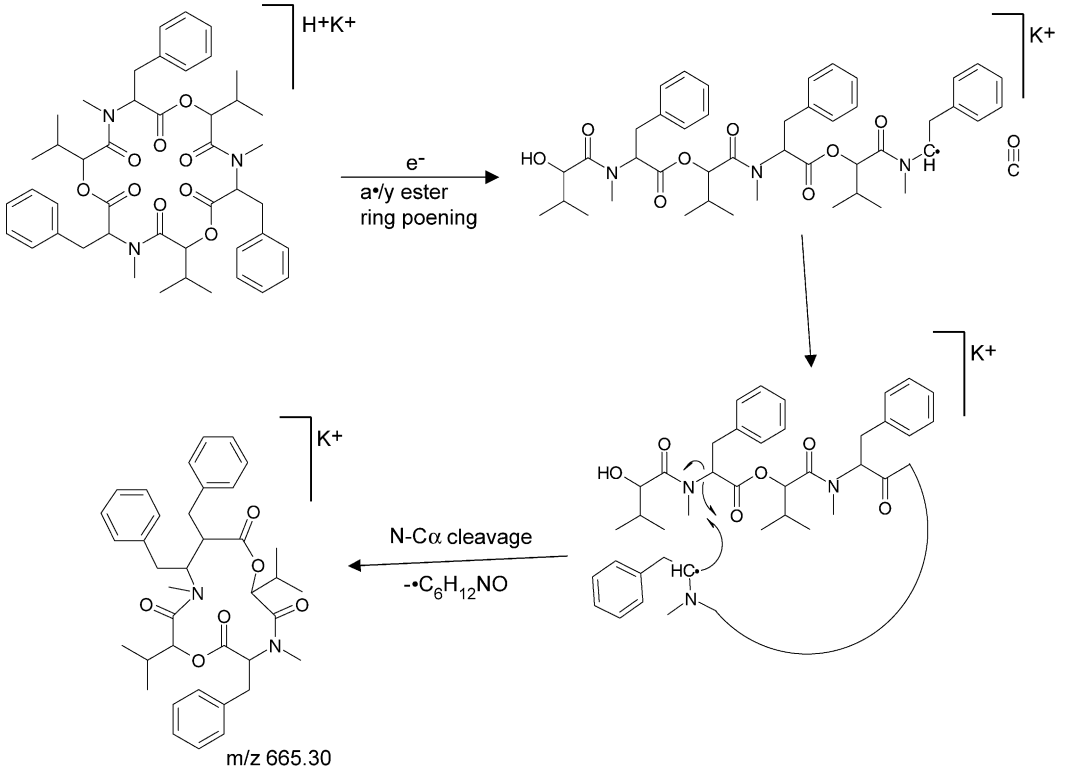
Fig. 5 shows the ECD FT-ICR mass spectrum of the $[M + 2H]^{2+}$ ions of the ε-peptide $(Ahx)_6K–OH$, in which Ahx is aminohexanoic acid. The spectrum was internally calibrated with respect to the precursor and charge-reduced ions. The fragment ions are listed in Table 4. Similar results were obtained for ECD of $Ac(Ahx)_6K–OH$ $[M+2H]^{2+}$ ions

Table 4
Fragments observed following electron capture dissociation of $(Ahx)_6$-Lys-OH $[M + 2H]^{2+}$ ions

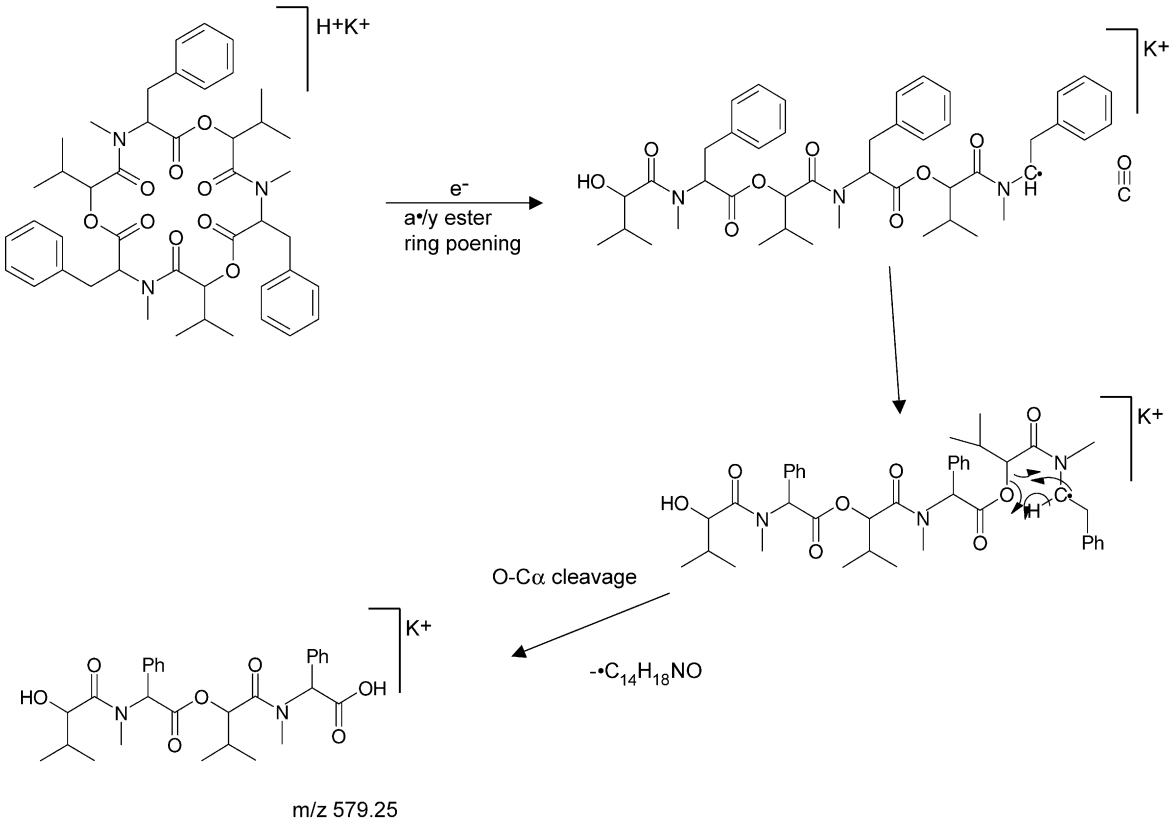
Measured *m/z*	Calculated *m/z*	Assignment
413.3123	413.3122	Parent $[M + 2H]^{2+}$
426.3566	426.3570	$a_4^{\bullet}$
486.3651	486.3655	y_4
539.4405	539.4410	$a_5^{\bullet}$
599.4487	599.4496	y_5
696.5375	696.5388	c_6
712.5328	712.5337	y_6
825.6172	825.6172	$[M + H]^+$



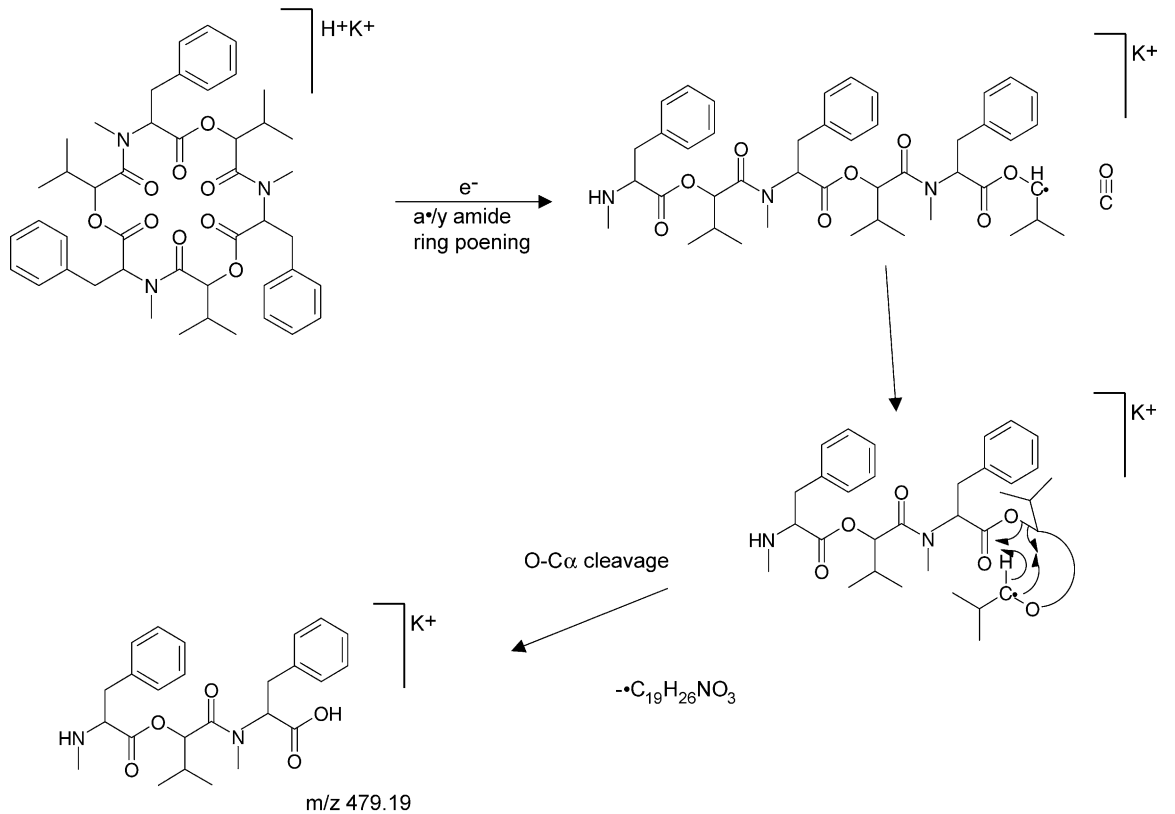
Scheme 5. Example of N–Cα (c/z•) ring opening with homolytic O–Cα cleavage observed for ECD of beauvericin.



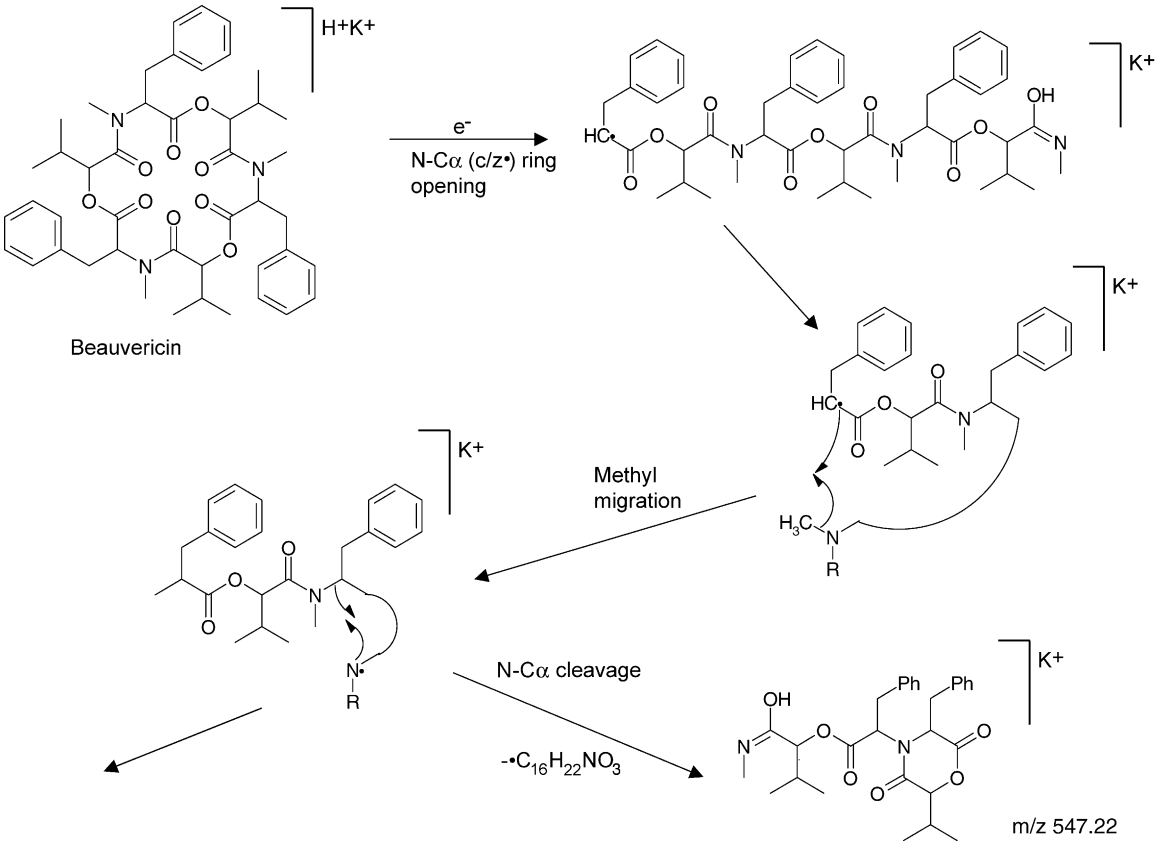
Scheme 6. Example of ester a•/y cleavage with homolytic N–Cα cleavage observed for ECD of beauvericin.



Scheme 7. Example of ester a•/y cleavage with O–Cα cleavage to produce c-type ions observed for ECD of beauvericin.



Scheme 8. Amide a•/y cleavage with O–Cα c/z• cleavage to produce c-type ions observed for ECD of beauvericin.



Scheme 9. Proposed mechanism for N–Cα (c/z•) ring opening, homolytic N–Cα backbone cleavage with methyl migration, observed for ECD of beauvericin.

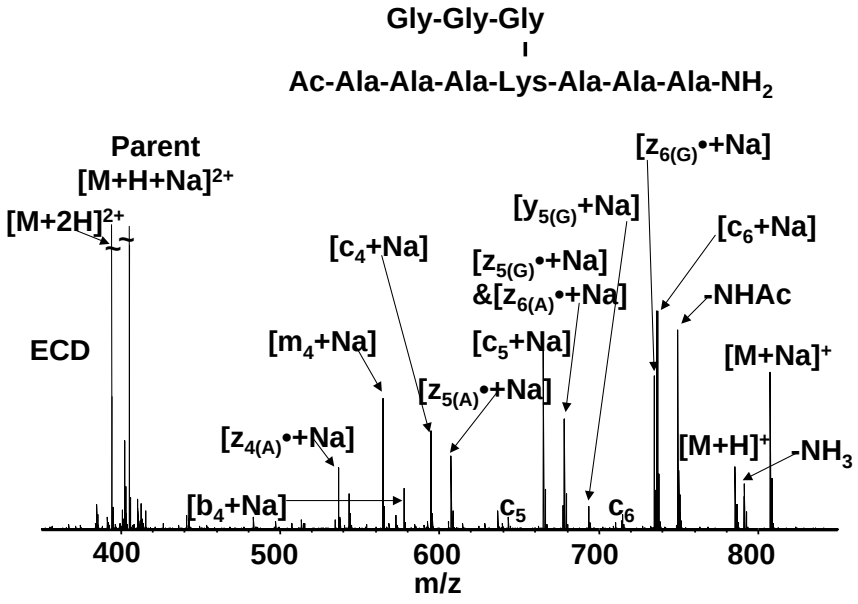


Fig. 3. ESI ECD FT-ICR mass spectrum of the branched peptide $AcA_3K(G_3)A_3–NH_2$ $[M + H + Na]^{2+}$ ions. Inset: structure of $AcA_3K(G_3)A_3–NH_2$. (A) and (G) refer to cleavages within the alanine and glycine branches, respectively.

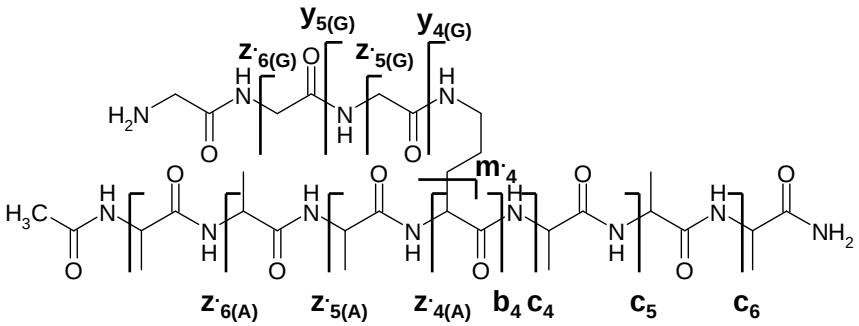


Fig. 4. Summary of fragments observed following ECD of the branched peptide $AcA_3K(G_3)A_3–NH_2$. (A) and (G) refer to cleavages within the alanine and glycine branches, respectively.

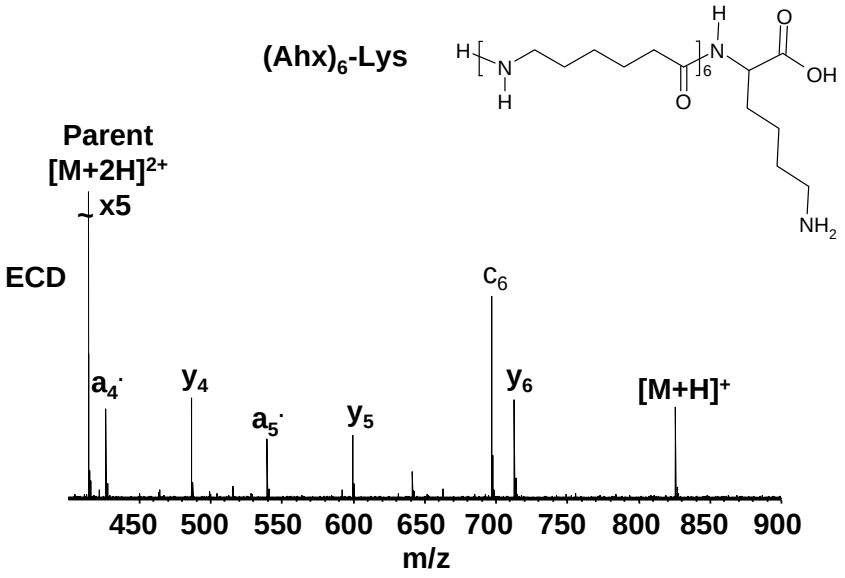


Fig. 5. ESI ECD FT-ICR mass spectrum of the ϵ-peptide, $(Ahx)_6K–OH$, Ahx is aminohexanoic acid. *Inset*: Structure of $(Ahx)_6K–OH$.

(data not shown). Ions of *m*/*z* 696.538 correspond to the c_6 fragment, i.e., derive from backbone amine cleavage within the lysine residue. Backbone N–Cε bond (c/z•) cleavage was not observed within the ε-amino acid residues. Backbone cleavage in the ε-amino acids resulted in a• and y ions only. Typically, a•/y backbone cleavage represents a minor fragmentation channel for ECD. Olsen et al. [62] investigated the ECD behavior of peptide nucleic acids (PNAs). The polyamide backbone of PNAs comprises *N*-(2-aminoethyl) glycine units and has some similarity, in terms of remote amide linkages, to the ε-peptides studied here. The dominant *backbone* fragmentation channel in PNAs is c/z• cleavage. The present results suggest that either the a•/y fragmentation channel is promoted, or the c/z• fragmentation channel is impeded in these ε-peptides. Thus, an isolated peptide unit may not fragment in typical c/z• fashion following ECD, and the influence of adjacent peptide units in α-peptides may be required to produce c/z• fragments at a given backbone site.

4. Conclusion

The present results establish the fragmentation behavior following electron capture for cyclodepsipeptides, branched peptides and ε-peptides. Electron capture by depsipeptides is accompanied by prompt secondary fragmentation in all cases; i.e., no or few charge-reduced species are observed. In addition to c/z• and a•/y cleavage of the N–Cα bonds, typically observed in ECD of peptides, both O–Cα c/z• and ester (a•/y) cleavages were observed in the lactone moieties. For beauvericin, the *N*-methylated depsipeptide, methyl group loss was observed. Methyl group loss was also observed in conjunction with backbone cleavage and that channel may involve methyl migration. The observation of backbone fragments in the ECD of these structures is consistent with a radical cascade mechanism proposed by O'Connor and coworkers [29].

The characteristic feature of mass spectra following ECD of ε-peptides is the dominant a•/y cleavage and absence of

c/z$^{\bullet}$ cleavage. Typically, a$^{\bullet}$/y cleavage represents a minor fragmentation channel in the ECD of peptides; ergo either a$^{\bullet}$/y cleavage is promoted or c/z$^{\bullet}$ cleavage is impeded in these structures. ECD of branched peptides produced complex spectra. N–Cα c/z$^{\bullet}$ cleavage was observed in both branches. No N–Cε bond cleavage was observed in the modified lysine side-chain, in agreement with the findings for ε-peptides. Peptide bond cleavage was observed at the site of the modified lysine residue. The characteristic feature of the ECD spectra of the branched peptides is observation of the immonium-related m ion for the modified amino acid.

The present results extend the range of application of ECD to the structural elucidation of unknowns. Additionally the results provide further insight into to the processes that follow electron capture.

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References

[1] F.W. McLafferty, Tandem Mass Spectrometry, Wiley, New York, 1983.
[2] A.G. Marshall, Int. J. Mass Spectrom. 200 (2000) 331.
[3] A.G. Marshall, C.L. Hendrickson, G.S. Jackson, Mass Spectrom. Rev. 17 (1998) 1.
[4] R.L. Woodlin, D.S. Bomse, J.L. Beauchamp, J. Am. Chem. Soc. 100 (1978) 3248.
[5] D.P. Little, J.P. Speir, M.W. Senko, P.B. O'Connor, F.W. McLafferty, Anal. Chem. 66 (1994) 2809.
[6] J.W. Gauthier, T.R. Trautman, D.B. Jacobson, Anal. Chim. Acta 246 (1991) 211.
[7] W.D. Price, P.D. Schnier, E.R. Williams, Anal. Chem. 68 (1996) 859.
[8] R.C. Dunbar, T.B. McMahon, Science 279 (1998) 194.
[9] R.A. Chorush, D.P. Little, S.C. Beu, T.D. Wood, F.W. McLafferty, Anal. Chem. 67 (1995) 1042.
[10] R.A. Zubarev, N.L. Kelleher, F.W. McLafferty, J. Am. Chem. Soc. 120 (1998) 3265.
[11] R.A. Zubarev, D.M. Horn, E.K. Fridriksson, N.L. Kelleher, N.A. Kruger, M.A. Lewis, B.K. Carpenter, F.W. McLafferty, Anal. Chem. 72 (2000) 563.
[12] F.W. McLafferty, D.M. Horn, K. Breuker, Y. Ge, M.A. Lewis, B. Cerda, R.A. Zubarev, B.K. Carpenter, J. Am. Soc. Mass Spectrom. 12 (2000) 245.
[13] R.A. Zubarev, Mass Spectrom. Rev. 22 (2003) 57.
[14] K. Biemann, Biomed. Environ. Mass 16 (1988) 99.
[15] P. Roepstorff, J. Fohlman, Biol. Mass Spectrom. 11 (1984) 601.
[16] R.A. Zubarev, N.A. Kruger, E.K. Fridriksson, M.A. Lewis, D.M. Horn, B.K. Carpenter, F.W. McLafferty, J. Am. Chem. Soc. 121 (1999) 2857.
[17] C.K. Fagerquist, R.R. Hudgins, M.R. Emmett, K. Hakansson, A.G. Marshall, J. Am. Soc. Mass Spectrom. 14 (2003) 302.
[18] N.L. Kelleher, R.A. Zubarev, K. Bush, B. Furie, B.C. Furie, F.W. McLafferty, C.T. Walsh, Anal. Chem. 71 (1999) 4250.
[19] A. Stensballe, O.N. Jensen, J.V. Olsen, K.F. Haselmann, R.A. Zubarev, Rapid Commun. Mass Spectrom. 14 (2000) 1793.
[20] S.D.-H. Shi, M.E. Hemling, S.A. Carr, D.M. Horn, I. Lindh, F.W. McLafferty, Anal. Chem. 73 (2001) 19.
[21] E. Mirgorodskaya, P. Roepstorff, R.A. Zubarev, Anal. Chem. 71 (1999) 4431.
[22] K. Håkansson, H.J. Cooper, M.R. Emmett, C.E. Costello, A.G. Marshall, C.L. Nilsson, Anal. Chem. 73 (2001) 4530.
[23] K. Breuker, H. Oh, B. Cerda, D.M. Horn, F.W. McLafferty, Eur. J. Mass Spectrom. 8 (2002) 177.
[24] H.J. Cooper, R.R. Hudgins, K. Håkansson, A.G. Marshall, J. Am. Soc. Mass Spectrom. 13 (2002) 241.
[25] F. Kjeldsen, K.F. Haselmann, B.A. Budnik, F. Jensen, R.A. Zubarev, Chem. Phys. Lett. 356 (2002) 201.
[26] H.J. Cooper, R.R. Hudgins, K. Håkansson, A.G. Marshall, Int. J. Mass Spectrom. 228 (2003) 723.
[27] F. Kjeldsen, K.F. Haselmann, E. Sorensen, R.A. Zubarev, Anal. Chem. 75 (2003) 1267.
[28] F. Kjeldsen, R.A. Zubarev, J. Am. Chem. Soc. 125 (2003) 6628.
[29] N. Leymarie, C.E. Costello, P.B. O'Connor, J. Am. Chem. Soc. 125 (2003) 8949.
[30] C.E. Ballard, H. Yu, B. Wang, Curr. Med. Chem. 9 (2002) 471.
[31] K. Eckart, Mass Spectrom. Rev. 13 (1994) 23.
[32] D. Zink, O.D. Hensens, Y.K.T. Lam, J.J. Reamer, J. Liesch, J. Antibiot. 45 (1992) 1717.
[33] K. Sugawara, K. Numata, M. Konishi, H. Kawaguchi, J. Antibiot. 37 (1984) 958.
[34] K.P. Bateman, P. Thibault, K.Q. Yang, R.L. White, L.C. Vining, J. Mass Spectrom. 32 (1997) 1057.
[35] L.C.M. Ngoka, M.L. Gross, P.L. Toogood, Int. J. Mass Spectrom. 182/183 (1999) 289.
[36] J.P. Tam, Proc. Nat. Acad. Sci. U.S.A. 85 (1988) 5409.
[37] J.P. Defoort, B. Nardelli, W. Huang, D.D. Ho, J.P. Tam, Proc. Nat. Acad. Sci. U.S.A. 89 (1992) 3879.
[38] M. Yoshida, J.P. Tam, R.B. Merrifield, Peptide chemistry, in: Proceedings of the Second Japanese Symposium on Peptide Chemistry, 1993.
[39] K. Hahn, W.A. Klis, J.M. Stewart, Science 248 (1990) 1544.
[40] M. Pellegrini, R.H. Ebright, J. Am. Chem. Soc. 118 (1996) 5831.
[41] J. Zeigler, R.T. Chang, D.W. Wright, J. Am. Chem. Soc. 121 (1999) 2395.
[42] M. Montal, M.S. Montal, J.M. Tomich, Proc. Nat. Acad. Sci. U.S.A. 87 (1990) 6929.
[43] P. Heinonen, J. Rosenberg, H. Lonnberg, Eur. J. Org. Chem. (2000) 3647.
[44] C.-F. Liu, J.P. Tam, Chem. Commun. (1997) 1619.
[45] M.W. Senko, C.L. Hendrickson, L. Pasa-Tolic, J.A. Marto, F.M. White, S. Guan, A.G. Marshall, Rapid Commun. Mass Spectrom. 10 (1996) 1824.
[46] M.W. Senko, C.L. Hendrickson, M.R. Emmett, S.D.-H. Shi, A.G. Marshall, J. Am. Soc. Mass Spectrom. 8 (1997) 970.
[47] J.P. Quinn, M.R. Emmett, A.G. Marshall, A device for fabrication of emitters for low-flow electrospray ionization, in: Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, FL, 1998.
[48] S.K. Chowdhury, V. Katta, B.T. Chait, Rapid Commun. Mass Spectrom. 4 (1990) 81.
[49] S.C. Beu, D.A. Laude Jr., Int. J. Mass Spectrom. Ion Proc. 112 (1992) 215.
[50] J.H. Malmberg, T.M. O'Neil, Phys. Rev. Lett. 39 (1977) 1333.
[51] C.L. Hendrickson, J.P. Quinn, M.R. Emmett, A.G. Marshall, Mass selective external ion accumulation for FTICR MS, in: Proceeding

of the 49th ASMS Conference on Mass Spectrometry and Allied Topics, Chicago, IL 2001.
[52] A.G. Marshall, T.-C.L. Wang, T.L. Ricca, J. Am. Chem. Soc. 107 (1985) 7893–7897.
[53] S. Guan, A.G. Marshall, Int. J. Mass Spectrom. Ion Proc. 157/158 (1996) 5.
[54] Y.O. Tsybin, P. Hakansson, B.A. Budnik, K.F. Haselmann, F. Kjeldsen, M. Gorshkov, R.A. Zubarev, Rapid Commun. Mass Spectrom. 15 (2001) 1849.
[55] A.G. Marshall, D.C. Roe, J. Chem. Phys. 73 (1980) 1581.
[56] M.W. Senko, J.D. Canterbury, S. Guan, A.G. Marshall, Rapid Commun. Mass Spectrom. 10 (1996) 1839.
[57] E.B. Ledford Jr., D.L. Rempel, M.L. Gross, Anal. Chem. 56 (1984) 2744.
[58] S.D.-H. Shi, J.J. Drader, M.A. Freitas, C.L. Hendrickson, A.G. Marshall, Int. J. Mass Spectrom. 195/196 (2000) 591.
[59] G.T. Blakney, G. van der Rest, J.R. Johnson, M.A. Freitas, J.J. Drader, S.D.-H. Shi, C.L. Hendrickson, N.L. Kelleher, A.G. Marshall, Further improvements to the MIDAS data station for FT-ICR mass spectrometry, in: Proceedings of the 49th American Society on Mass Spectrom, Conference on Mass Spectrometry & Allied Topics, Chicago, IL, 2001.
[60] Z. Guan, J. Am. Soc. Mass Spectrom. 13 (2002) 1443.
[61] F. Turecek, F.H. Carpenter, J. Chem. Soc., Perkin Trans. 2 (1999) 2315.
[62] J.V. Olsen, K.F. Haselmann, M.L. Nielsen, B.A. Budnik, P.E. Nielsen, R.A. Zubarev, Rapid Commun. Mass Spectrom. 15 (2001) 969.