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Mass Spectrometric Analyses of *β*-Ketolactone Oligomers, Macrocyclic or Catenane Structures?

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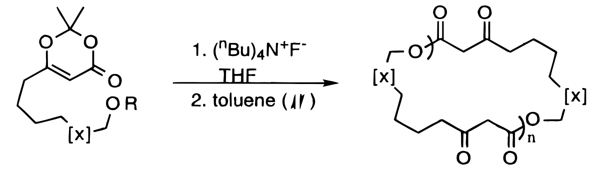
Mass spectrometry is used to develop an analytical method for a new class of organic oligomeric material, *β*-ketolactones. Three different series with varying oligomeric sizes are examined. The oligomers may form at least two different structures, a macrocyclic and a catenane ring. Fast atom bombardment coupled with Fourier transform mass spectrometry provides a rapid and convenient method that provides both molecular weight information and abundant fragment ions that are structurally relevant. All the compounds are found to work well with the mass spectrometric analysis. Electrospray ionization coupled with triple−quadrupole mass spectrometry was also evaluated and found to yield results that are similar to FAB. On the basis of the FAB and the low-energy collisionally activated dissociation spectra, we conclude that these compounds are macrocyclic rings and not catenanes.

β-Ketolactones comprise a new class of organic oligomeric material.[1−4] The recent interest in these types of macrolides is prompted by the discovery of their biological importance as antibiotics.[1−3] Several *β*-ketolactones have been synthesized using a general method of thermal cycloreversion of 2,2-dimethyl-6-(*ω*-hydroxyalkyl)-4*H*-1,3-dioxin-4-one (Scheme 1)[4]. Characterization and structural elucidation of the compounds remain a difficult task. Because of their apparent symmetry, the ^{1}H and ^{13}C NMR spectra are identical for the monomer and all higher homologues.[5] Elemental analyses also produce identical results. Furthermore, the cyclic oligomers have flexible structures that can assume various conformations, making it difficult to crystallize for X-ray crystallography. Only the dimer has so far been characterized by X-ray, showing a single macrocyclic ring structure. The structure need not be general for all homologues, however, as the possibility for a catenane structure increases with molecular size (Chart 1).

Mass spectrometry is based on the mass-to-charge ratio measurement and can overcome these limitations as each homologue can be distinguished by its mass. Moreover, the fragmentation pattern of the spectra can provide structural information that allows one to resolve whether some of the homologues have macrocyclic or catenane ring structures. In this study, we evaluate the mass spectrometry of *β*-ketolactones to develop a rapid and sensitive method for the analysis of these compounds. We find that the combination of fast atom bombardment (FAB) and Fourier transform mass spectrometry provides both structural and molecular weight information. Furthermore, electrospray ionization with a triple−quadrupole instrument provides the incorporation of minimal internal energy, producing few fragment ions and making the method suitable for the study of oligomeric mixtures.

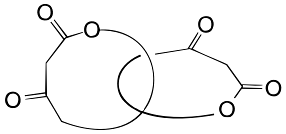
Scheme 1



series **1**: [x] = $-CH_2-$
series **2**: [x] = $-CH_2CH_2-$
series **3**: [x] = $-CH_2CH_2CH_2-$

a: n = 1 (dimer)
b: n = 2 (trimer)
c: n = 3 (tetramer)
d: n = 4 (pentamer)
e: n = 5 (hexamer)
f: n = 6 (heptamer)
g: n = 7 (octamer)

Chart 1



Fourier transform mass spectrometry has increasingly been used to analyze polymers.[6−13] FAB-Fourier transform mass spectrometry (FTMS)[14,15] features high mass measurement accuracy, ultrahigh resolution, and long ion storage times. It also offers high resolution even under MS/MS conditions.[16] The coupling of external sources such as FAB,[17,18] matrix-assisted laser

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desorption ionization (MALDI),[19,20] and electrospray with FTMS[21-25] has further expanded its capability. FAB ionization is found to produce a great deal of structurally important fragment ions.[18,26] Because of the long time scale of the FTMS technique, slow metastable decomposition occurs, producing large amounts of fragment ion and little matrix background during FAB ionization.[27-29] More importantly, MS/MS information is obtained without additional manipulation. It is therefore ideal for structural elucidation of a large number of homologous compounds and highly suitable for the characterization of these macrolides.

Electrospray ionization (ESI) with a triple−quadrupole instrument was also used to analyze some of the compounds. ESI is believed to be a significantly softer ionization method than FAB. In which case, the two ionization methods could be complementary; the latter can provide a rapid method for structural elucidation providing both intense quasimolecular and fragment ions, while ESI-MS can provide the capability for the direct analyses of reaction mixtures.

EXPERIMENTAL SECTION

FTMS Analyses. The experiments were performed in an external source quadrupole Fourier transform mass spectrometer (FTMS) which was built at the Chemistry Department of UC Davis. This instrument has been described in detail in earlier publications.[28,29] Samples were prepared by dissolving the β-ketolactones directly into *m*-nitrobenzyl alcohol (NBA) matrix. The oligomeric samples were either solids or oils, and we found this direct method to be the best. Other matrices such as glycerol and thioglycerol were also tried but did not produce results as good. The resulting solution was placed on a copper probe tip of a direct insertion probe. The probe was placed in the external ionization source. Secondary ions were produced in the source by bombarding the sample with a Cs^+ beam generated from an ion gun (Antek) operating at 7 keV. The ions were transported through a single-stage quadrupole ion guide into the ion cyclotron resonance (ICR) analyzer cell where the ions were detected. The entire sequence was controlled by the Omega data system (IonSpec Inc., Irvine, CA).

In the CID experiments, collision gas (N_2) was introduced into the ICR cell through a precision leak valve as background gas with pressures of $\sim 1 \times 10^{-7}$ Torr. The desired ion was isolated using a series of ejection pulses in a standard method. It was then translationally excited by applying an electric field with a frequency offset from its natural cyclotron frequency.[30] Collision with the background N_2 gas produced fragmentation.

Liquid Chromatography/Mass Spectrometry. The analyses of tetramer **2C**, hexamer **2E**, and octamer **2G** were performed in the VG Quattro triple−quadrupole mass spectrometer with electrospray ionization source and MassLynx data system. During the experiment, sample was dissolved in $HCCl_3$/MeOH (50:50) mix solvent to form a solution in the concentration of $<10^{-3}$ M. A 10 μL sample of this solution was injected through the injector valve and then carried by a mixture of acetonitrile and water (50:50) with 1% formic acid at a flow rate of 6 μL/min to the electrospray probe and ionized. The capillary voltage was maintained at 3.5 kV, cone voltage at $\sim$50 V, and a nebulizing gas (N_2) at flow rate of 20 L/h. The ions were then focused into the quadrupole mass analyzer and detected by an electron multiplier detector. For the CID experiment, the ion was selected by the first quadrupole mass filter, then collided with Ar (3×10^{-3} mbar) in the collision cell, and fragmented. The fragments were then analyzed by the second quadrupole analyzer.

Sample Preparation. The macrocyclic β-ketolactones were synthesized using thermal cycloreversion of 2,2-dimethyl-6-(ω-hydroxyalkyl)-4*H*-1,3-dioxin-4-one (Scheme 1). The polymeric mixtures were separated and purified by preparative TLC.[4,5] The purities of the compounds are between 95 and 99% as determined by HPLC and TLC.

RESULTS

FAB-FTMS of Poly(β-ketomacrolide) Series 1. Six polymers in this series, dimer **1a** through heptamer **1f**, were analyzed by FAB-FTMS. The three spectra corresponding to oligomers **1a**, **1b**, and **1e** are representative of the series (Figure 1). The FAB spectrum of the dimer **1a**, 1,11-dioxacycloeicosane-2,4,12,-14-tetraone (Figure 1a), shows primarily four dominant ions with *m/z* 341 [(the protonated molecule (MH^+)], 323 (loss of water from MH^+, $MH^+ - H_2O$), 171 (loss of 170 u, the monomer unit, from MH^+, $MH^+ - 170$), and 153 (loss of water from ion *m/z* 171, $171 - H_2O$). We believe that the 170 u neutral fragment lost is a cyclic ζ-lactone species (vide supra). Typically, large organic compounds with mainly oxygen-containing functional groups also show strong signals from metalated species, from background concentrations of alkali metal ions, but none were found. We have found that doping the samples with alkali metal chloride produces significantly stronger quasimolecular ions but a smaller number of structurally important fragment ions. Similarly, the trimer **1b**, 1,11,21-trioxacyclotriacontane-2,4,12,14,22,-24-hexaone, also produces the protonated molecular ion with *m/z* 511 along with sequential losses of the oligomeric unit (−170 u, *m/z* 341 and 153) and the corresponding loss of water from each oligomeric unit (*m/z* 493, 323, and 135) (Figure 1b). New types of fragments are observed in the trimer that are not present in the dimer. The signals at *m/z* 475, 305, and 135 appear to be the result of a loss of a second water molecule from the oligomeric ions. The ions with *m/z* 383 and 213 are derived from losses of 128 u from the protonated parent and the protonated fragment dimer. These two fragments (*m/z* 383 and 213) further lose water to yield ions with *m/z* 365 and 195, respectively. As the molecule becomes larger, the fragment yield increases further, thereby decreasing the abundance of the quasimolecular ion. The increase

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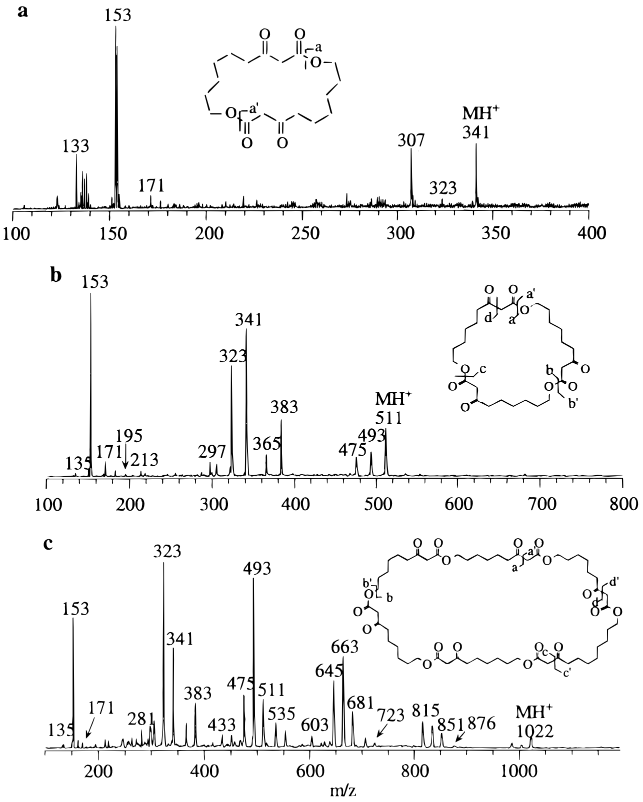

Figure 1. FAB spectra of (a) dimer **1a** (b) trimer **1b**, and (c) hexamer **1e** performed in an FTMS instrument. The samples were dissolved directly in *m*-nitrobenzyl alcohol. The ion with *m*/*z* 133 is Cs^+ and with *m*/*z* 307 is the protonated dimer of the matrix in the spectrum of **1a**. All other labeled peaks belong to the sample.

in fragment ions with increasing molecular size is a general trend. The hexamer **1e** shows a very weak protonated molecular species signal at *m*/*z* 1022 (7%) (Figure 1c). As in the trimer, abundant fragment series due to the losses of 18, 170, and 128 u are observed from the oligomeric ions.

Table 1 lists the major fragment ions, and some proposed neutrals lost, observed in the FAB spectra ranging in size from the dimer homologue **1a** to the heptamer **1f**. In all cases, the spectra have the same excellent quality with regard to signal to noise and the lack of matrix background, as the example spectra shown. There are general similarities in the fragments including losses of 170 u, an oligomeric unit, resulting in fragments with masses corresponding to smaller oligomers. Losses of 18 (one and two) and 128 u are from the protonated parent. The oligomeric fragments are also observed often with large abundances. Other less abundant fragments are also observed corresponding to losses of 60 ($C_2H_4O_2$) and 44 u (CO_2).

FAB-FTMS of Poly(*β*-keto macrolide) Series 2 and Series 3. The FAB-FTMS spectra of two oligomers in series **2**, dimer **2a** and trimer **2b**, are shown (Figure 2). As with the dimer **1a**, the spectrum of dimer **2a** shows the protonated molecular species, *m*/*z* 369, and the ions due to the losses of the oligomer unit, *m*/*z* 185 ($MH^+ - 184$ u), and water molecule(s), *m*/*z* 351 ($MH^+ - H_2O$) and 333 ($MH^+ - 2H_2O$), from the molecular ion (Figure 2a). Water is also lost from the monomeric fragments *m*/*z* 167 ($185 - H_2O$) and 149 ($167 - H_2O$). Likewise, the trimer **2b**, *m*/*z* 553 (MH^+), produces the same kinds of ions as trimer **1b** (Figure 2b). Losses of water are observed, *m*/*z* 535 ($MH^+ - H_2O$) and 517 ($MH^+ - 2H_2O$), as well as losses of monomeric units, *m*/*z* 369 ($MH^+ - 184$) and 185 ($MH^+ - 2 \times 184$). As with **1b**, losses of water from the oligomeric fragments are also observed with *m*/*z* 351 ($369 - H_2O$), 333 ($351 - H_2O$), 167 ($185 - H_2O$), and 149 ($167 - H_2O$). The loss of *ζ*-lactone is replaced by the loss of *η*-lactone molecule (142 u) from the quasimolecular ion to produce a fragment with *m*/*z* 411 ($MH^+ - \eta$-lactone).

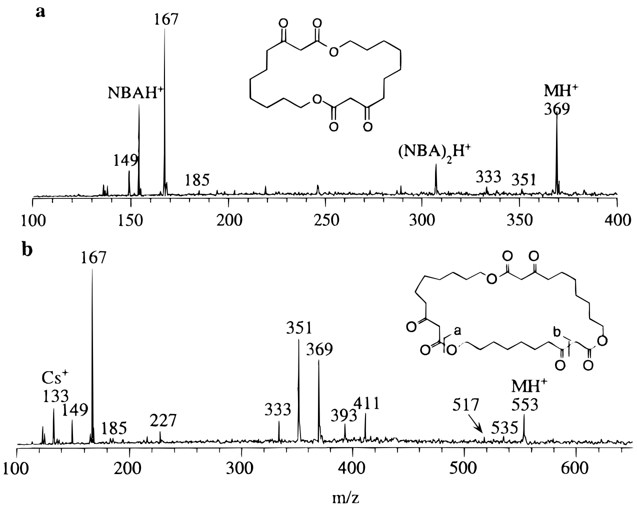

Figure 2. FAB-FTMS spectra of (a) dimer **2a** and (b) trimer **2b**.

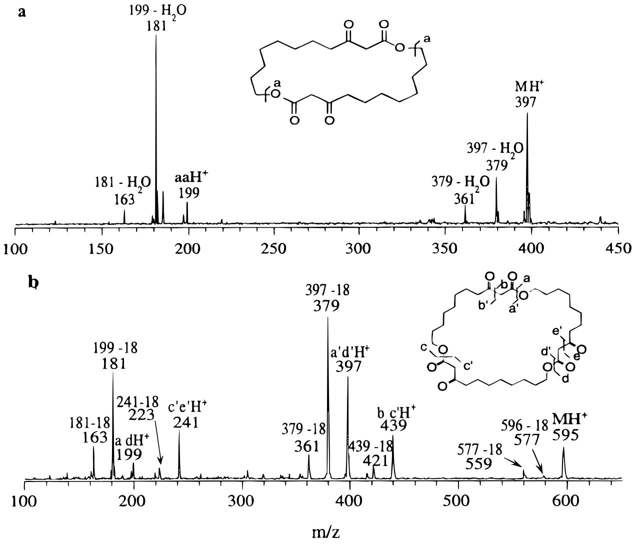

Figure 3. FAB spectra of (a) dimer **3a** and (b) trimer **3b**.

The FAB-FTMS spectra of two oligomers from series **3** compounds, dimer **3a** and trimer **3b**, are shown (Figure 3). As expected, the fragmentation patterns of the two oligomers are analogous to those found in series **1** and **2**. The two spectra show the protonated molecular ions (*m*/*z* 397 and 595, respectively) and abundant fragments resulting from the losses of repeating oligomeric unit(s) (198 u) and water molecule(s) from the quasimolecular ion and the oligomeric fragment ions. A similar trend with series **3** compounds is observed between the intensity of the quasimolecular ion and molecular size. The trimer **3b** also yields the fragments corresponding to the loss of *θ*-lactone molecule from the molecular ion and from the oligomeric fragments, analogous to losses of *ζ*-lactone or *η*-lactone molecules for the other two series.

CID of *β*-Keto Macrolides Formed by FAB-FTMS. Sustained off-resonance excitation was the method used for CID in the FTMS experiments.[30] In this method, ions are translationally

Table 1. Summary of Mass Spectral Intensities for the Oligomers of Compounds in Series **1**

		1a dimer	**1b** trimer	**1c** tetramer	**1d** pentamer	**1e** hexamer	**1f** heptamer
I	MH^+	341 (40%)	511 (25%)	681 (20%)	851 (22%)	1022 (7%)	1192 (8%)
I_1	$MH^+ - H_2O$	323 (6%)	493 (15%)	663 (3%)	833 (10%)	1003 (2%)	1174 (2%)
I_2	$I_1 - H_2O$		475 (10%)	645 (4%)	815 (18%)	985 (3%)	1156 (6%)
I_3	$MH^+ - 128$		383 (30%)	553 (6%)			
I_4	$I_3 - H_2O$		365 (10%)	535 (6%)	705 (6%)		
II	$MH^+ - 170$	171 (8%)	341 (80%)	511 (67%)	681 (14%)	851 (8%)	1022 (7%)
II_1	$II - H_2O$	153 (100%)	323 (60%)	493 (100%)	663 (31%)	833 (11%)	1003 (4%)
II_2	$II_1 - H_2O$	135 (6%)	305 (5%)	475 (74%)	645 (22%)	815 (14%)	985 (9%)
II_3	$II_1 - CO_2$		297 (6%)	467 (12%)			
II_4	$II - C_2H_4O_2$			451 (8%)			
II_5	$II_4 - H_2O$			433 (6%)			
II_6	$II - 128$		213 (2%)	383 (55%)	553 (8%)	723 (3%)	
II_7	$III_6 - H_2O$			365 (18%)	535 (14%)	705 (5%)	
III	$MH^+ - 2 \times 170$		171 (10%)	341 (53%)	511 (30%)	681 (17%)	851 (5%)
III_1	$III - H_2O$		153 (100%)	323 (83%)	493 (95%)	663 (46%)	833 (9%)
III_2	$III_1 - H_2O$		135 (2%)	305 (7%)	475 (33%)	645 (37%)	815 (10%)
III_3	$III - CO_2$			297 (4%)	467 (7%)	637 (4%)	
III_4	$III - C_2H_4O_2$			281 (3%)	451 (6%)	621 (4%)	
III_5	$III_4 - H_2O$			263 (4%)	433 (10%)	603 (7%)	
III_6	$III - 128$			213 (4%)	383 (48%)	553 (9%)	723 (2%)
III_7	$III_6 - H_2O$			195 (2%)	365 (21%)	535 (13%)	705 (4%)
IV	$MH^+ - 3 \times 170$			171 (2%)	341 (60%)	511 (26%)	681 (12%)
IV_1	$IV - H_2O$			153 (65%)	323 (100%)	493 (92%)	663 (27%)
IV_2	$IV_1 - H_2O$			135 (2%)	305 (14%)	475 (29%)	645 (23%)
IV_3	$IV - CO_2$				297 (10%)	467 (5%)	637 (3%)
IV_4	$IV - C_2H_4O_2$				281 (8%)	451 (7%)	
IV_5	$IV_4 - H_2O$					433 (7%)	603 (4%)
IV_6	$IV - 128$				213 (3%)	383 (25%)	553 (4%)
IV_7	$IV_6 - H_2O$					365 (13%)	535 (6%)
V	$MH^+ - 4 \times 170$				171 (8%)	341 (54%)	511 (15%)
V_1	$V - H_2O$				153 (76%)	323 (100%)	493 (60%)
V_2	$V1 - H_2O$				135 (4%)	305 (15%)	475 (15%)
V_3	$V - CO_2$					297 (12%)	467 (3%)
V_4	$V - C_2H_4O_2$					281 (10%)	451 (5%)
V_5	$V_4 - H_2O$					263 (6%)	433 (4%)
V_6	$V - 128$					213 (5%)	383 (16%)
V_7	$V_6 - H_2O$					195 (3%)	365 (13%)
VI	$MH^+ - 5 \times 170$					171 (3%)	341 (59%)
VI_1	$VI - H_2O$					153 (70%)	323 (100%)
VI_2	$VI1 - H_2O$					135 (2%)	305 (15%)
VI_3	$VI - CO_2$						297 (18%)
VI_4	$VI - C_2H_4O_2$						281 (9%)
VI_5	$VI_4 - H_2O$						263 (6%)
VI_6	$VI - 128$						213 (4%)
VI_7	$VI_6 - H_2O$						195 (2%)
VII	$MH^+ - 6 \times 170$						171 (3%)
VII	$VII - H_2O$						153 (50%)
VII	$VII_1 - H_2O$						135 (2%)

excited with energies that are less than the dissociation threshold. Through a series of collisions, the internal energy is increased until the threshold energy is reached. The fragment ions observed in the spectra are therefore the result of cleavage reactions near threshold energies allowing us to identify the lowest energy fragmentation pathways of the chosen ion. CID experiments were performed with the protonated trimer **1b** to determine whether a catenane structure exists for this compound. Under low-energy collision conditions and a background N_2 pressure of 1×10^{-7} Torr, several product ions were formed including those corresponding to the loss of water molecules (m/z 493 and 475), which are also the dominant fragment ions (Figure 4a). Losses of 170 u also occur to produce ions with m/z 341 and 153, but these are not dominant signals. For comparison, the low-energy CID of a m/z 511 fragment, isolated from the protonated pentamer (**1d**), was also performed (Figure 4b). This spectrum yields the same fragment as that of protonated **1b**, with some variation in relative abundances, suggesting that the two isobaric ions have similar structures. Similar comparisons were performed with the protonated tetramer (**1c**) and an isobaric ion from fragmentation of the hexamer (**1e**) to observe whether the trimer and its isobaric ion were somewhat unique. The comparison of the two spectra again yields similar behavior (Figure 5). The neutral losses that yield oligomeric ions from protonated **1c**, i.e., m/z 511, 341, and 171 (not observed), are even less abundant. Instead, losses of water from the oligomeric units (m/z 493, 323, and 153) appear as the dominant fragments. The isobaric fragment from the dissociated protonated hexamer **1e** yields the same product ions.

ESI-MS of *β*-Keto Macrolides. Because ESI-MS can be a useful method for the separation and analyses of mixtures of the oligomeric compounds, experiments were performed with electrospray ionization to assess the technique for the analyses of the compounds. The ESI-MS required a little more effort than the FAB-FTMS but yielded similar results. The spectrum for **2c** is shown (Figure 6) and is representative of the ESI-MS spectra of the macrolides. By varying the cone (or skimmer) voltage in the source, ionization conditions can be chosen to provide only quasimolecular ion or some degree of fragmentation. The

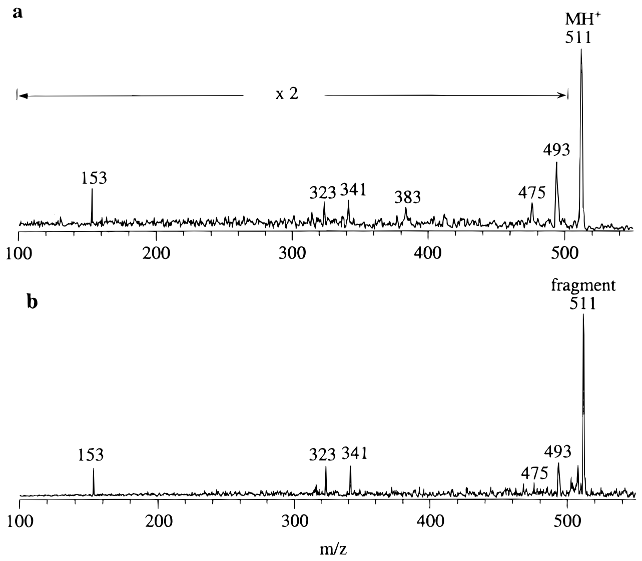

Figure 4. Low-energy CID spectrum of (a) the protonated trimer **1b**, m/z 511, and (b) an isobaric fragment isolated from the FAB-FTMS of the pentamer **1d**. The strong similarities of the two spectra indicate that the two ionic species have the same structure.

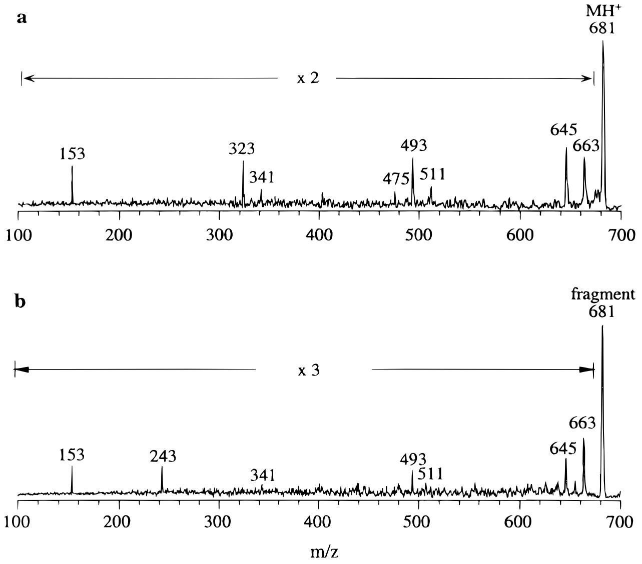

Figure 5. CID spectrum of (a) protonated tetramer **1c**, m/z 681, and (b) an isobaric fragment isolated from the FAB-FTMS of the hexamer **1e**. The signal at m/z 243 is an electrical noise.

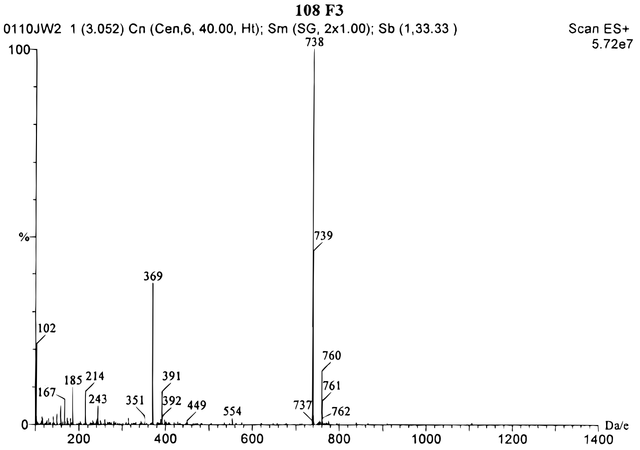

Figure 6. ESI-MS full mass spectrum of tetramer **2c** performed with an ESI sources mounted to a triple−quadrupole instrument. The quasimolecular ion has m/z 738.

spectrum of **2c** was obtained under conditions of maximum fragmentation (Figure 6). The spectrum shows a strong quasimolecular signal at m/z 738 (737.5) and fragments at m/z 553 ($MH^+ - 184$), 369 ($MH^+ - 2 \times 184$), 351 ($369 - H_2O$), 185 (MH^+ − trimer), and m/z 167 ($185 - H_2O$). The protonated molecular species is the base peak in all the ESI-MS spectra. The fragment ions observed are the same as those found in the FAB-FTMS spectra (for example, Figure 2). The intensity of the fragment ions can be further increased by performing CID (Figure 7). In the triple−quadrupole instrument, CID is performed by using the first set of quadrupole rods to mass select, the second as a collision cell, and the third to analyze the fragments. The CID spectrum of m/z 738 yields more abundant fragments and some new fragments not found in the ESI-MS spectrum, such as m/z 411, corresponding to the loss of an η-lactone from the m/z 553 oligomeric fragment. The loses of water molecules from the quasimolecular ion and the oligomeric fragments are also significantly increased in the CID spectrum. The CID spectrum has relative abundances that are more similar to the FAB-FTMS spectra than to the FTMS-CID spectra (for example, Figures 4 and 5). This behavior is consistent if we posit that off-resonance CID provides only threshold internal energies while the FAB process and CID in the triple−quadrupole provides significantly more.

DISCUSSION

The dissociation products of oligomers with macrocyclic ring structures should behave differently from those with catenane structures. A single ring fragments by first opening to produce a linear species that further dissociates to fragments. In catenane structures, two or more rings are intertwined. After opening of one of the rings, the molecule can unravel, producing smaller oligomeric species. With macrocyclic rings, therefore, at least two bonds are broken before fragments are observed, while with catenanes only a single bond is broken. Under the conditions of low-energy fragmentation, catenanes should produce only oligomeric ions. Macrocyclic rings may produce several other fragments if other cleavage pathways are as accessible as oligomeric bond cleavages. In the FAB spectra of the three different series of compounds, oligomeric fragment ions are not generally dominant. For example, in the series **1** compounds, the oligomeric fragment ions, separated by 170 u, have strong signals with the smaller oligomeric compounds but are weaker than signals from the losses of water molecules in the larger compounds. The FAB spectra are, however, not definitive as FAB ionization is capable of producing vibrationally excited ions with internal energies significantly greater than threshold. On the other hand, CID with FTMS can be used to introduce internal energies at or near the lowest dissociation threshold. Under the mild conditions of off-resonance CID, catenane structures should initially ring-open and dissociate leaving lower molecular weight oligomeric ions as the primary ionic products. The presence of other fragments (e.g., loss of H_2O) in the CID spectra strongly indicates that compounds have structures corresponding to single macrocyclic rings. Degradation of the quasimolecular ion apparently occurs by ring opening followed by cleavage of a second bond

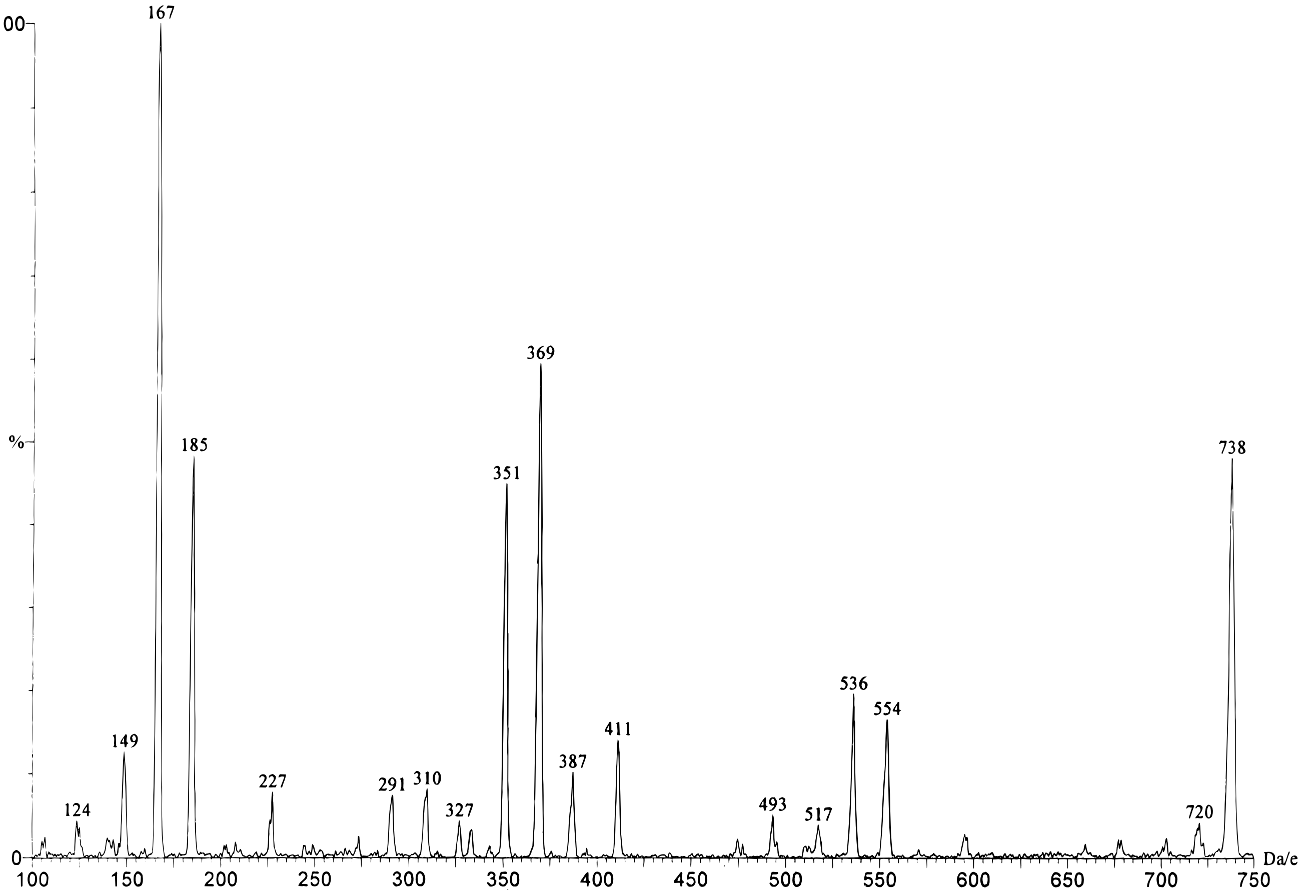

Figure 7. ESI-MS-CID spectrum of tetramer **2c**. The quasimolecular ion, *m/z* 738, was mass selected and fragmented by collision with argon gas.

that can be either an oligomeric bond or one that causes hydrogen migration resulting in the loss of water.

The macrocyclic ring structure is also supported by the comparison of the CID spectra between protonated oligomers and the isobaric fragments of larger compounds. For example, the CID spectra of protonated trimer **1b** (*m/z* 511) is consistent with a macrocyclic ring structure where the ion ring-opens to a linear form and fragments. The CID of the quasimolecular ion yields strong signals corresponding to losses of water molecules (*m/z* 493, 475, and 323). An isobaric ion, *m/z* 511 from the pentamer **1d**, was randomly chosen for CID. This species yields a CID spectrum similar to **1b**. The oligomeric fragment is also likely to be a linear species if it is a piece of a larger macrocyclic ring. The similarities in the CID spectra indicate that the two species have the same linear structure. The comparison of *m/z* 681 from protonated **1c** and the fragment of **1e** also yields similar spectra, indicating that the two ions also have similar structures. On the basis of these experiments, we conclude that the oligomeric compounds are generally macrocyclic rings rather than catenanes.

A readily apparent trend observed with increasing molecular size is decreasing quasimolecular ion intensity. On the basis of our experience with other oligomeric compounds such as oligosaccharides and oligopeptides, we believe that this is a general trend in FAB-FTMS. The intense fragment ion abundances observed with FAB-FTMS have been attributed to metastable dissociation.[27,31,32] It would appear therefore that the rates of metastable dissociation increases with molecular size. It is known that introducing a discrete amount of energy to a series of molecules with increasing molecular size produces relatively rapid fragmentation rates for smaller ions than larger ones.[33] It has also been suggested that larger molecular species fragment less when formed by some type of plasma desorption.[34,35] One possible explanation is that larger molecular ions need more extreme conditions to form than smaller mass ions (e.g., more energetic primary beam, higher extraction voltages, and higher rf amplitude for the quadrupole ion guide). This causes the larger ions to form with greater internal energy. Alternatively, the ions may be formed with internal energies that are scaled to size even under the same ionization conditions. That is, larger molecules with more degrees of freedom have more internal energy than smaller molecules. In any case, from the point of view of analyses, the intense fragmentation makes FAB-FTMS ideal for obtaining greater amounts of structural information on large molecules without collisional activation. However, it imposes a natural limit on the mass range obtainable by FAB. For this reason, larger molecules when analyzed by FTMS will require a relatively softer ionization technique such as matrix-assisted laser desorption and electrospray ionization.

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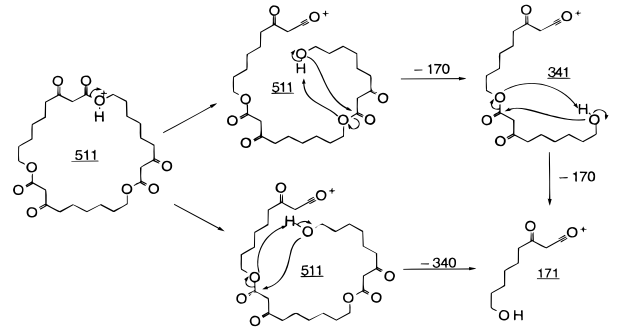
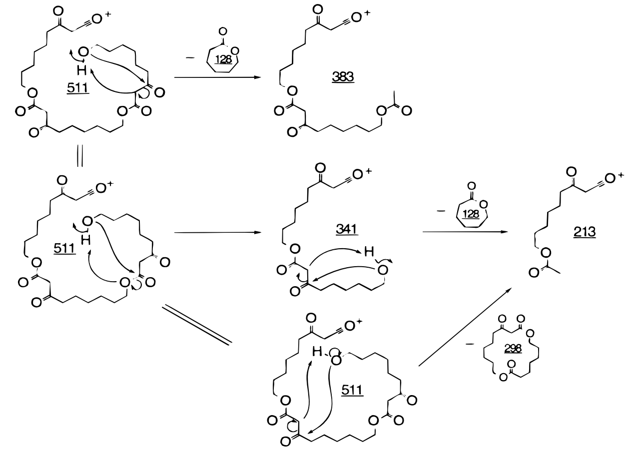
Scheme 2



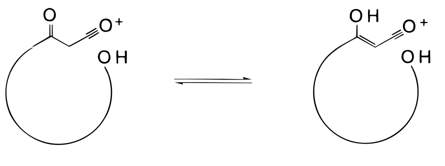
Scheme 3



A possible mechanism for the losses of 170 u is proposed and illustrated with protonated trimer **1c** (Scheme 2). Like the more commonly known cyclic lactones, the protonated β-ketolactone trimer likely undergoes ring opening by the charge-induced cleavage of an ester linkage[36] to form an acylium ion on one end and a hydroxyl group in the other. In the ring-opened form, the hydroxyl oxygen can attack the ester carbonyl group promoting charge-remote cleavage of the ester linkage to lose a 170 u neutral fragment. The loss of two 170 u fragments can happen either sequentially or as a loss of a dimeric ring by attack of an adjacent site. In either case, the neutral fragment lost is always cyclic while the charged fragment remains linear. An attack on the other carbonyl carbon of the β-ketolactone group yields the loss of 128 u (Scheme 3). For **1c**, this unimolecular reaction yields a neutral ζ-lactone. As outlined in Scheme 3, attack of the hydroxyl group at either carbonyl group can occur as the molecule fragments to smaller ions. For example, the molecule can ring-open and lose an oligomeric unit to produce m/z 341. This can be followed by an attack on the other carbonyl group to lose 128 u forming m/z 213. Alternatively, m/z 213 can be formed directly from m/z 511 by attack of the same carbonyl group in the second β-keto group to lose 298 u. In this way, losses of 128 can also occur for any of the oligomeric fragment.

The low-energy CID is informative for relating the energetics of the two fragmentation reactions (loss of 170 and 128 u). The final dissociations, as outlined in Schemes 2 and 3, can be described as charge-remote fragmentation. The presence of these reactions as major modes under relatively low energy conditions are unusual given the high activation barriers usually associated with these reactions.[37−40] However, the neutral products formed are highly stable and there appears to be no other modes of charge-induced fragmentation than the initial ring-opening reaction and the losses of water molecules. It is also apparent that loss of 170 u (and multiples) is a more facile reaction at threshold energies than the loss of 128 u. In the CID of the protonated trimer (Figure 4a), m/z 383 is relatively small but is significant in the FAB spectrum (Table 1). It is not observed in the CID of the m/z 511 fragment (Figure 4b) or in the CID of the protonated tetramer (Figure 5a). The relatively favorable loss of 170 u is consistent with the proposed mechanism. Cleavage of the ester bond (Scheme 2) is expected to be more facile than cleavage of the α-keto bond.

Scheme 4



The most abundant fragments in the majority of the spectra of the series **1** compounds are those resulting from the loss of one and two water molecules. The loss of one water molecule is common for γ-lactones.[36] It results from the dehydration of an open-chain molecular ion (presumably due to cleavage of the lactone linkage). The losses of two water molecules are, however, not observed for common lactones. We expect the loss of one water to be the result of a similar mechanism in the oligomers. The loss of the second water molecule is unusual given that no other hydroxyl group is apparent. In the oligomeric material, the second ketone group is β to the ketolactones, separated by a methylene group. The two keto groups make the methylene group acidic, allowing it to readily undergo tautomerization and form the enol. This enol hydroxyl group may be the source of the second water molecule. In solution, the enol form is observed in the ^{1}H NMR spectrum, which shows the vinylic proton peak at δ 5 ppm as a small triplet. In the gas phase, the open-chain molecular ion may similarly tautomerize to form a enolacylium ion that is stabilized by the conjugated double and triple bonds (Scheme 4).

CONCLUSION

On the basis of the FAB-FTMS, the low-energy CID, and the ESI-CID experiments, we conclude that the oligomers of β-ketolactone generally have a macrocyclic ring structure rather than a catenane ring. The similarities of the fragmentation patterns point to a general fragmentation behavior and similarities in structures of the three series. Primarily three types of products are observed corresponding to losses of oligomeric units, losses of water, and losses of cyclic lactones that are consistent with ζ-, η-, and θ-lactone molecules.

FAB-FTMS and ESI-MS are both useful for obtaining quasimolecular ions. FAB-FTMS provides the greatest amount of

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fragmentation. For routine analysis, FAB-FTMS would provide both molecular weight and structural information without the additional manipulation required for CID. ESI-MS provides a similarly convenient method for the analyses of the macrolides. The lack of fragment ions in the electrospray spectra compared to the FAB-FTMS spectra illustrates the relatively lower internal energy obtained with electrospray. As a caveat, it should be emphasized that a direct comparison between FAB-FTMS and ESI-triple-quadrupole MS cannot be easily made. The time between ion production and detection is significantly longer in the FTMS than in the triple−quadrupole instrument, allowing metastable ions to dissociate. This makes the difference in the internal energies of the ions formed by the two methods seem significantly more different.

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