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Charge-remote Fragmentation in Dialkylaminostyryl Dyes

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Dialkylaminostyryl probes with two long alkyl chains were used to investigate the energetics of charge-remote fragmentation (CRF). These compounds provide high-quality liquid secondary ion (LSI) and electrospray ionization (ESI) mass spectra in which the intact cation predominates. The intact cation undergoes CRF in LSI but not in ESI, in which the cation is of lower internal energy. CRF is initiated in collision-induced dissociation (CID) and surface-induced dissociation (SID) for intact cations generated by either LSI or ESI. Single bond cleavage to lose one entire alkyl chain followed by CRF within the remaining alkyl chain seems to be the lowest energy CID and SID process. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: charge-remote fragmentation; liquid secondary ion mass spectrometry; electrospray ionization; tandem mass spectrometry; surface-induced dissociation

INTRODUCTION

Charge-remote fragmentation (CRF)[1] processes have been used to identify the branching points or double bond locations in long alkyl chains in compounds such as fatty acids, alkyl ammonium salts and bile acids.[1–7] Typically, CRF is observed following collision-induced dissociation (CID). The amount of energy required to initiate CRF appears to be both compound dependent and related to the inherent stability of the charge site.[4] The intact cations of carbocyanine dyes,[8] molecules with two long alkyl chains similar in structure to the compounds currently under study, undergo CRF[9] when formed by liquid secondary ion mass spectrometry (LSIMS). CRF also occurs in CID of the cations of these compounds created by both electrospray ionization (ESI) and LSIMS, and in surface-induced dissociation (SID) following LSIMS.

In this study, we investigated CRF in four substituted dialkylaminostyryl compounds. These lipophilic probes insert in membranes with their two alkyl tails and their fluorophore oriented parallel to the phospholipid acyl chains.[10] These compounds provide high-quality positive ion LSI and ESI mass spectra in which the intact cation is the dominant ion. CRF along each of the two chains is monitored as the collision energy in CID and SID is varied, allowing insight into the energetics of the CRF process, and possible confirmation of results from a previous carbocyanine dye study.[9] For the carbocyanine dyes, which also contain two long alkyl chains in the cation molecule, loss of one complete alkyl chain followed by second-chain CRF was shown to be the lowest energy process. In this work, ESI and LSI were used as ionization methods, and CRF dissociations of two exemplar probe molecules were studied specifically to reveal changes in CRF dissociation processes with varying internal energy deposition.

EXPERIMENTAL

Positive ion LSI spectra were recorded on a VG 70-SEQ hybrid mass spectrometer of EBqQ geometry (VG Analytical, Manchester, UK). SID tandem mass spectra were measured on an HX110/HX110 mass spectrometer of EBEB geometry (Jeol, Peabody, MA, USA). High-energy linked-scan experiments were completed on a VG 70-SE mass spectrometer of EB geometry (VG Analytical). Both the VG 70-SEQ and JEOL HX110/HX110 instruments used a cesium ion beam to sputter ions from sample solutions prepared in *m*-nitrobenzyl alcohol (*m*-NBA). The VG 70-SE instrument used a xenon atom beam to sputter ions from sample solutions prepared in *m*-NBA. Air was used as collision gas, and the acceleration voltage was 8000 V for the VG 70-SE instrument. The parent ion beam signal was attenuated by ~70% in the high-energy CID experiment and 50% in the low-energy CID experiment. SID was investigated on a perfluoropolyether (Krytox 1625, DuPont, Wilmington,

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DE, USA)-coated brass surface placed in the collision cell region of the JEOL mass spectrometer. A VAX-based computer system and OPUS 3.0 software were used to acquire and process data on the VG 70-SEQ instrument. JEOL Complement software was used to process and acquire the data on the HX110 instrument. Positive ion ESI and low-energy (<100 eV) CID spectra were recorded on a VG Quattro II triple-quadrupole mass spectrometer. Sample concentrations of methanol solutions used in ESI experiments were approximately 20 μM. The needle voltage was 3500–3700 V and the cone voltage was 70–80 V. The source temperature was $\sim 80\,°C$. In CID for ESI, the collision gas was 99.999% argon. The collision energies ranged from 5 to 100 eV. The collision cell pressure was $\sim 1.5 \times 10^{-3}$ Torr (1 Torr = 133.3 Pa), corresponding to a parent ion beam attenuation of 50%. The data system for the VG Quattro II triple quadrupole mass spectrometer was MassLynx version 2.22.

For LSIMS and fast atom bombardment (FAB) data, ion signals were recorded for 3–5 min or 50–90 scans and the spectra were averaged. In ESI experiments, samples were injected using a Kd Scientific Model 200 syringe pump at a flow rate of 10 μl min^{-1}. Mass spectra were obtained using the multi-channel averaging (MCA) mode and represent the average of 15–20 scans. A mass range of 100–1500 Da was scanned at a rate of 6 s per scan.

Compounds were purchased from Molecular Probes (Eugene, OR, USA) and used as received. The abbreviations used in the discussion are the catalog names.

RESULTS AND DISCUSSION

The dye compounds used in this study contain either two long alkyl (three examples) or two alkenyl (one example) chains (structures are given in Fig. 1). In the following discussion, 'first' alkyl (or alkenyl) chain processes refer to CRF fragmentations that produce ions between the *m/z* of the intact cation (or precursor ion) and the *m/z* corresponding to complete loss of one of the alkyl or alkenyl chains. 'Second' chain CRF processes generate fragment (or product) ions resulting from losses originating in the remaining alkyl chain, after complete loss of the first chain has occurred. For example, the positive ion LSI mass spectrum of D-291 (Fig. 2) contains an intense signal corresponding to the intact cation at *m/z* 491. Loss of one complete alkyl chain in this compound produces a fragment ion at *m/z* 350; this represents loss of the first alkyl chain. Loss of all of the remaining second alkyl chain produces a fragment ion at *m/z* 209.

D-291 and D-3897 are chosen as representative examples of these dialkylaminostyryl compounds. Figure 2 shows the positive ion LSI mass spectrum of D-291, and includes ions that correspond to CRF of both the first and the second alkyl chains. The intact cation of the probe at *m/z* 491 fragments to form the ion at *m/z* 350 by complete loss of the first chain. In the CRF series, the ion at *m/z* 475 corresponds to loss of CH_4 from the intact cation, and this first fragment ion is followed by a series of ions separated by 14 Da, terminating in the ion at *m/z* 350. Associated with each CRF fragment ion is a group of adjacent, lower abundance ions. As an example, the ion at *m/z* 447 is accompanied by lower abundance ions at *m/z* 448, 446 and 445. The reproducible pattern around each CRF ion suggests that a series of hydrogen transfers (losses of hydrogen but also hydrogen addition) accompanies each CRF loss. The reproducibility of this pattern is seen in the cluster of ions around the CRF fragment ions at *m/z* 461, 447, 433 and 419. This satellite ion pattern was observed in the mass spectra of the previously studied carbocyanine dyes[9] and in other CRF studies. These CRF losses correspond to a C_nH_{2n+2}[7] and/or a C_nH_{2n+1}[4] ion series.

The LSI mass spectrum of D-3897 (Fig. 3), a compound with two double bonds in each of the alkyl

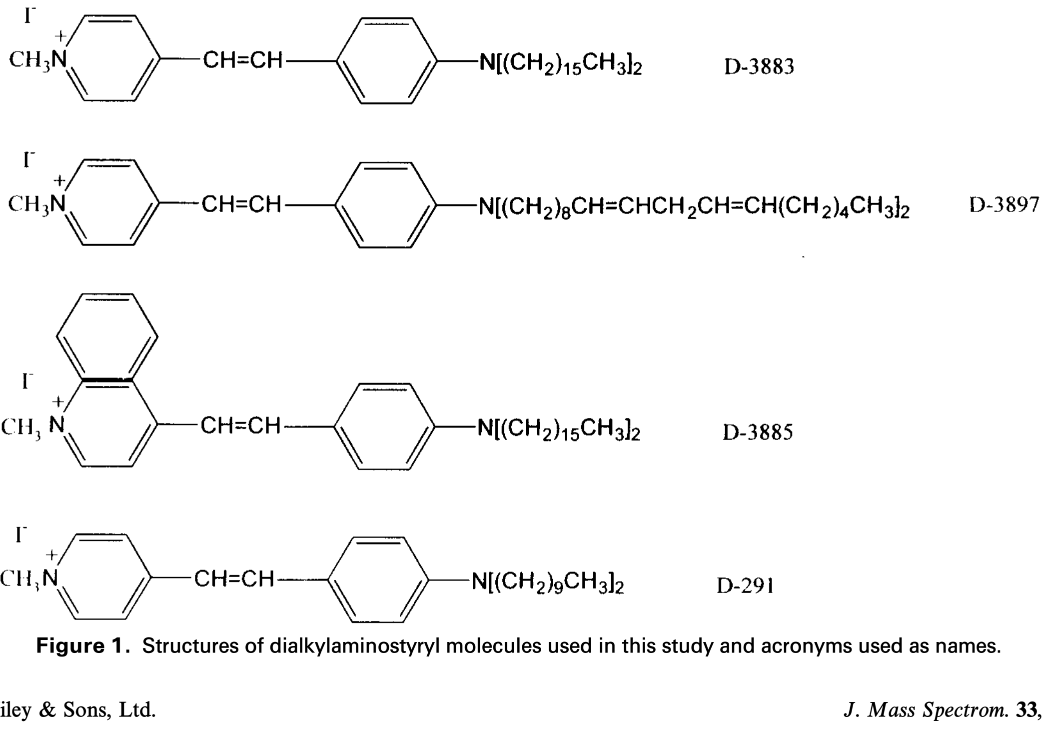

Figure 1. Structures of dialkylaminostyryl molecules used in this study and acronyms used as names.

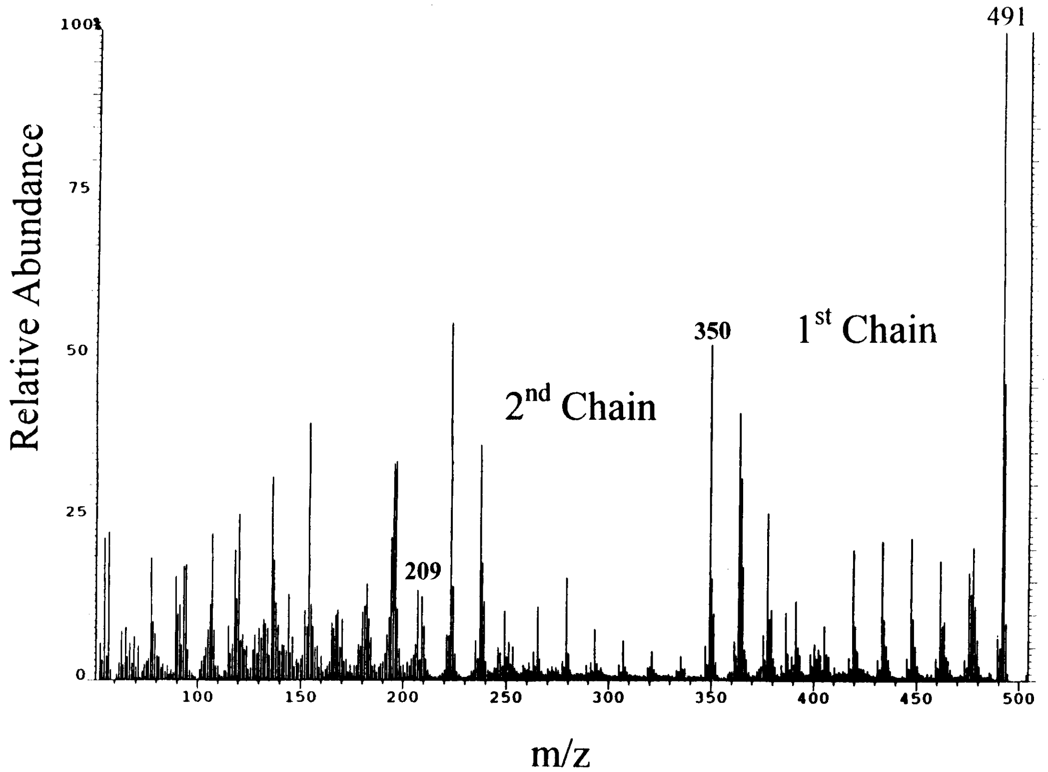

Figure 2. Positive-ion LSIMS of D-291. Areas of first- and second-chain CRF are noted. The intact cation is at *m*/*z* 491.

chains, is characterized by a neutral loss of CH_4 from the intact cation at *m*/*z* 708, followed by a series of four CRF ions separated by 14 Da, until the position of the double bond is reached. At the point of the double bond (indicated by an asterisk), the abundance of the CRF fragment ion is diminished, and the fragment ion separation becomes 12 Da. After this point, the normal CRF fragmentation (loss of 14 Da) pattern continues until the next double bond in the chain is reached, indicated again by a separation of 12 Da, and again a lower intensity signal. The position of the double bond is clearly indicated by the changes in abundance of the

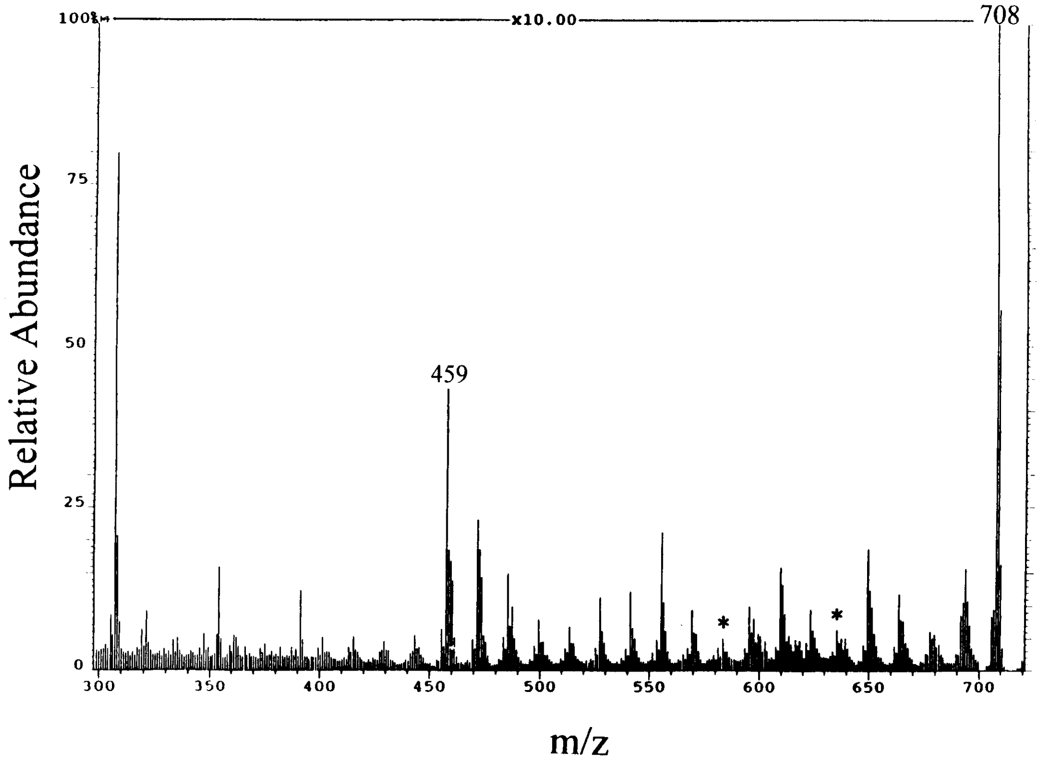

Figure 3. Positive-ion LSIMS of D-3897. The intact cation is at *m*/*z* 708. Asterisks indicate the position of the double bond in the alkenyl chain.

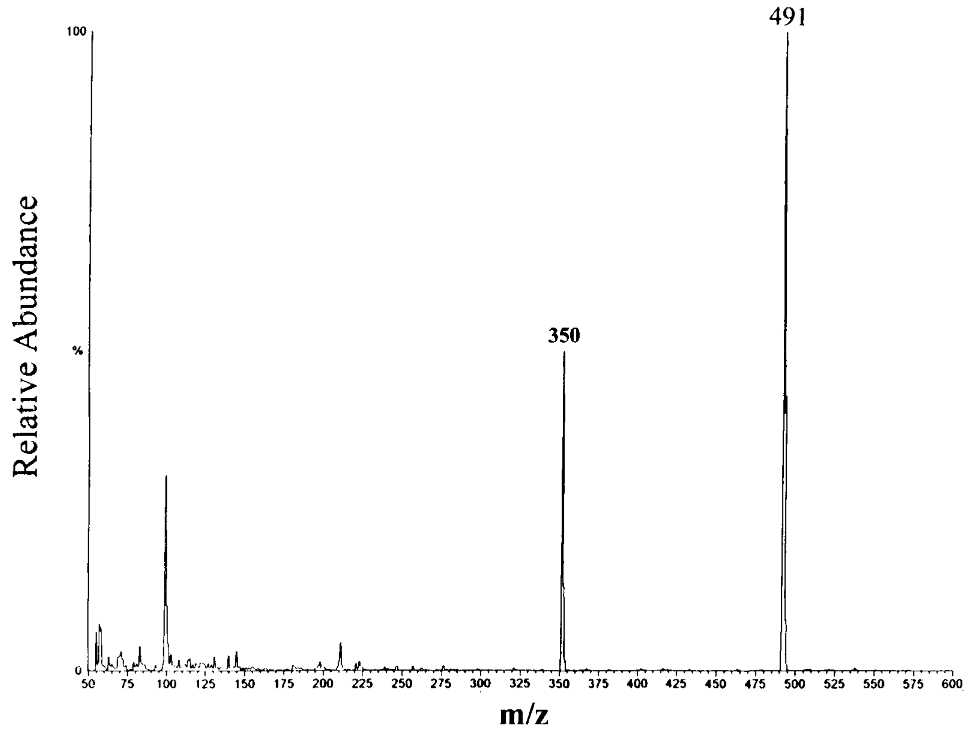

Figure 4. Positive-ion ESI mass spectrum of D-291. Intact cation at *m/z* 491.

CRF ions and changes in the mass separation between CRF ions. This pattern is similar to that seen in the carbocyanine dyes[9] and in compounds with double bonds in the alkyl chain.[6,7]

In an ESI source, cations are produced with a very low internal energy and source CRF is not expected. This initial expectation was borne out in our study of the carbocyanine dyes[8] and is confirmed here with the dialkylaminostyryl probe molecules.

Positive ion ESI mass spectra of D-291 and D-3897 are again discussed. Figure 4 shows a portion of the positive ion ESI mass spectrum of D-291. As expected, the amount of fragmentation is minimal and the intact cation dominates the mass spectrum. Careful examination of the mass region between *m/z* 350 and 491 shows that there is no CRF. None of the dialkylaminostyryl probes examined by ESI show CRF fragmentation in the mass spectrum. Figure 5 shows the positive ion ESI mass spectrum of D-3897. The intact cation appears at *m/z* 708 and complete loss of the first alkyl chain results in the ion at *m/z* 459, but there is no evidence of CRF for either chain in this positive ion

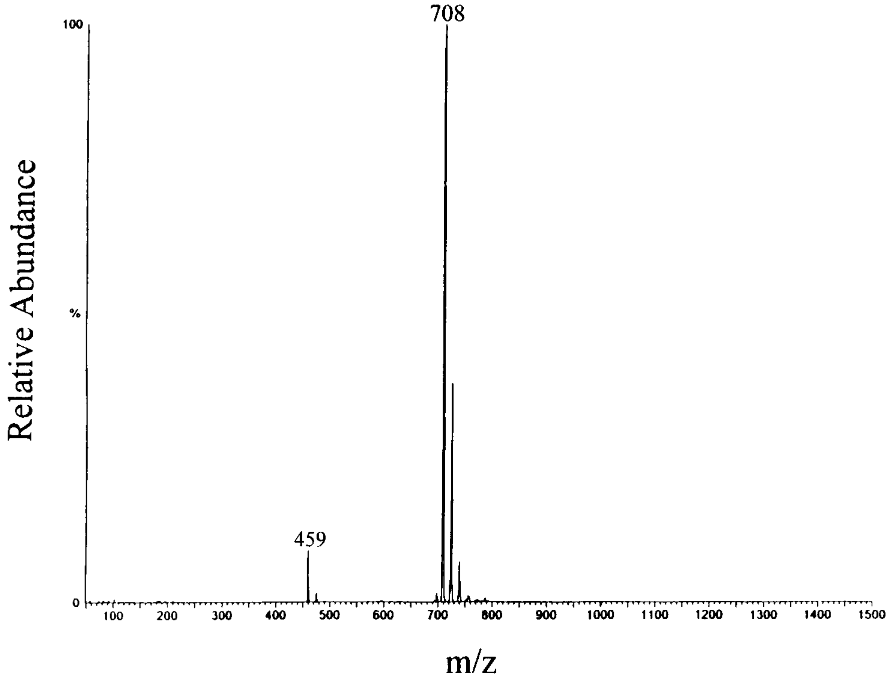

Figure 5. Positive-ion ESI mass spectrum of D-3897, with the intact cation at *m/z* 708.

mass spectrum. The ions at *m*/*z* 724 and 740 represent the adduct ions [$C^+ + O$] and [$C^+ + MeOH$], respectively. The oxygen adduct ion formation is due to the air-sensitive nature of the dialkylaminostyryl probe analyzed. The abundant cations obtained in ESI are ideal candidates for MS/MS.

Figure 6 shows the SID tandem mass spectra recorded for the cation of D-291 (LSIMS ionization) at a collision energy of 40 eV. At this collision energy, second-chain CRF product ions predominate, with only a few of the product ions corresponding to first-chain CRF.

The SID study included changing the energy of collision of the parent ion with the surface on the distribution of CRF product ions. When the collision energy was increased to 100 eV, the CRF dissociations of the second alkyl chain decreased and the mass spectrum became dominated by peaks associated with CRF dissociations of the first alkyl chain.

Figure 7 shows the ESI CID tandem mass spectrum of the intact cation (*m*/*z* 491) of D-291 recorded at a laboratory collision energy of 40 eV. First-chain CRF ions here are lower in relative abundance than ions from second-chain CRF, but both are observed. Figure 8 shows the ESI CID tandem mass spectrum of D-3897 recorded at a slightly higher laboratory collision energy of 50 eV. The precursor ion is the intact cation at *m*/*z* 708. In this tandem mass spectrum, CRF ions corresponding to losses from within the first alkyl chain (*m*/*z* 650, 636, 624, 610, 596, 555, 541 and 527) are present. CRF ions corresponding to losses from within the second alkyl chain are also present (*m*/*z* 443, 429, 415, 401, 322, 308, 294 and 280). CRF ions corresponding to losses from within the first alkyl chain are not present at lower collision energies (i.e. a collision energy of 40 eV) for this cation. At lower collision energies, only second-chain CRF occurs, and as the collision energy is increased, fragment ions corresponding to first-chain CRF appear. This suggests that the lower energy process is CRF within the second alkyl chain, after cleavage of the intact first alkyl chain. This pattern is reproduced in the tandem mass spectra of each of the four dialkylaminostyryl molecules studied.

The high-energy CID (8000 V) spectrum recorded for the intact cation of D-291 is shown in Fig. 9, and is representative of the tandem mass spectra obtained for each of the compounds in this study. As in the LSI mass spectral data, the tandem mass spectrum includes first-chain CRF with initial loss of CH_4 from the cation, followed by a series of product ions separated by 14 Da, with CRF of the first chain terminating in the ion at *m*/*z* 350. In sequence, the next CRF ion corresponds to loss of CH_4 from the fragment ion at *m*/*z* 350, and then CRF proceeds through a series of product ions separated by 14 Da corresponding to second chain CRF. The amount of internal energy deposited into the ion in high energy CID is sufficient for both first- and second-chain CRF.

Establishing a relative energy scale for CRF

We assume that ESI produces molecular cations with a small amount of internal energy, specifically, lower than the cations generated by LSI. This supposition is supported by the fact that there is very little in-source dissociation of the cation observed in the ESI mass spectrum. Low-energy CID tandem mass spectra were recorded in the collision energy range 5–70 eV for ESI-generated cations. At lower collision energies, fragment

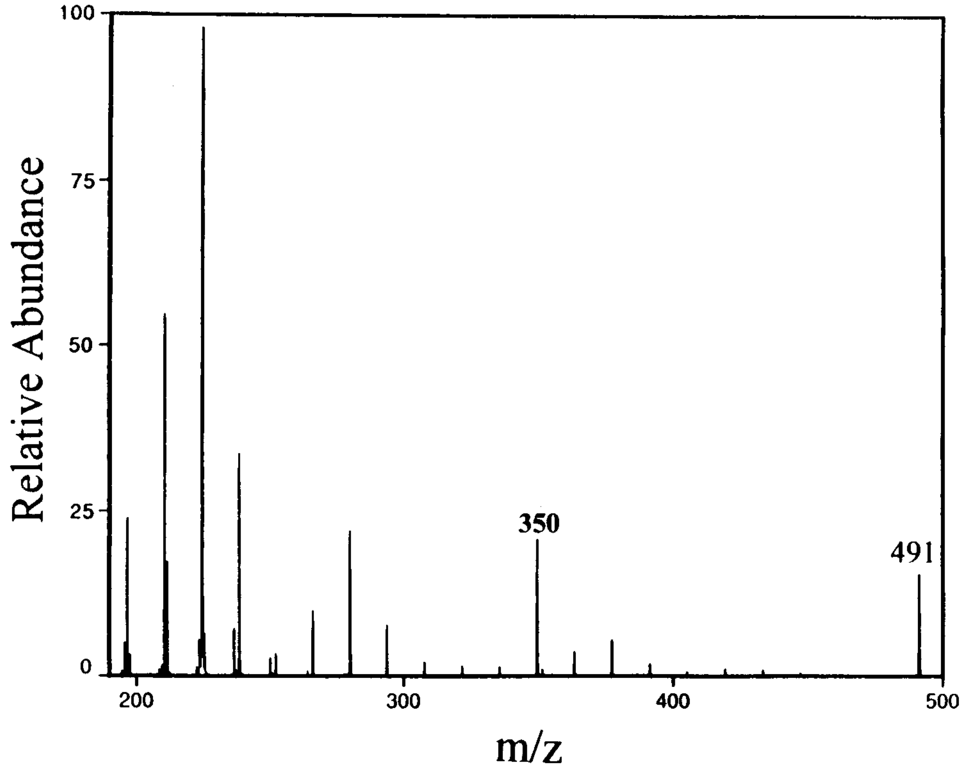

Figure 6. SID tandem mass spectrum of D-291 at a collision energy of 40 eV.

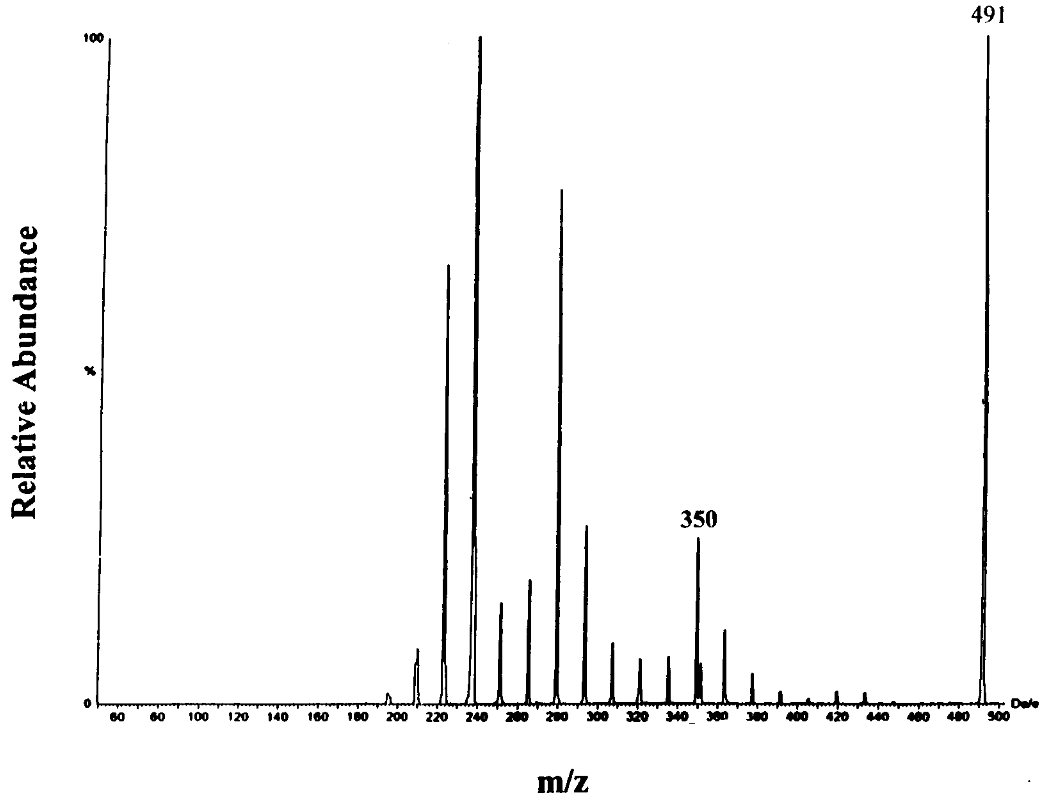

Figure 7. ESI CID tandem mass spectrum of D-291 at a collision energy of 40 eV.

ions corresponding to second-chain CRF appear first, and then increase in abundance as the collision energy is increased. Figure 10 shows a plot of the summed intensities of first- and second-chain CRF from the intact cation of D-291 at several collision energies. As the collision energy increases (and therefore as the amount of internal energy in the parent cation increases), the ratio of the abundances of second-chain

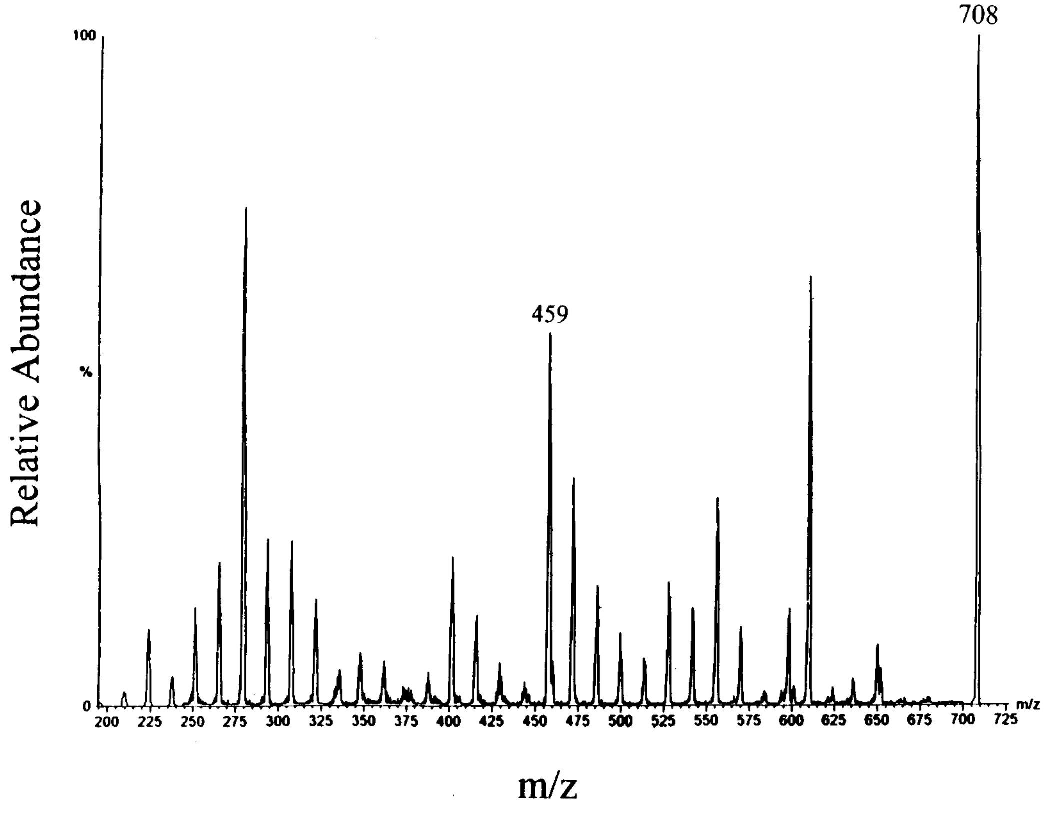

Figure 8. ESI CID tandem mass spectrum of D-3897 at a collision energy of 50 eV.

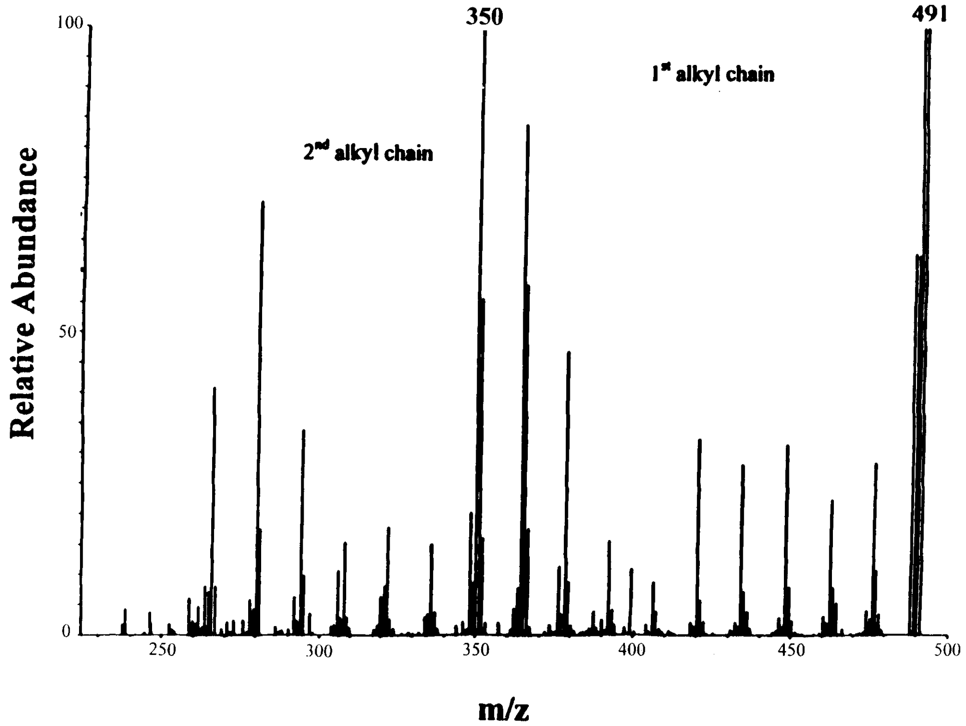

Figure 9. High-energy CID linked-scan mass spectrum of D-291 at a collision energy of 8.0 keV.

to first-chain CRF fragments increases. These results suggest that the lowest energy process available is cleavage of the entire first alkyl (or alkenyl chain), followed by CRF within the second chain. At higher collision energies, direct first-chain CRF processes can be observed.

With high energy CID, it was expected from our previous study[9] that a greater amount of internal energy is deposited into the cation than with ESI, ESI low-energy CID MS/MS or low-energy SID MS/MS. In these experiments, second-chain CRF products remain but first-chain CRF product ions predominate. Most of the cations now have the higher amount of energy necessary to initiate first-chain CRF.

CONCLUSIONS

These probes were chosen for study because the two distinct processes of first- and/or second-chain CRF can be monitored as the amount of internal energy in the cation is varied. The approach in the current study is similar to that for the carbocyanine dyes.[9] Both studies

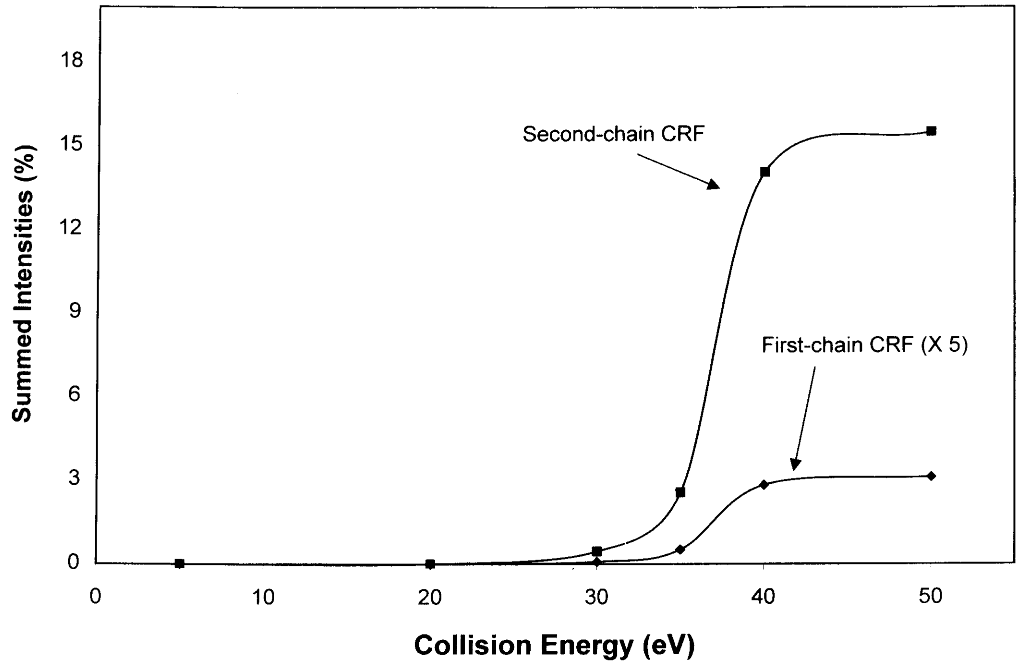

Figure 10. Summed abundances of first- and second-chain CRF for D-291 in ESI low-energy CID MS/MS.

indicate that second-chain CRF (after cleavage of the entire first chain) is the lower energy process. First-chain CRF from the intact cation requires a higher amount of internal energy. The relative extent of each of these processes therefore reflects the amount of ion internal energy. As reviewers have noted, it is unusual that the *lowest* energy process seems to be that in which a cleavage occurs first, followed by CRF as opposed to simple CRF from the intact cation. We note that the initial cleavage of the first alkyl chain leads to a radical ion, from which second chain CRF occurs. Claeys and Derrick[11] noted that initial radical ion formation is a rate-determining step in the CRF of $[M + Li]^+$ ions for monounsaturated compounds. Although the parallel in mechanism is not complete, the initially formed radical ion may be predisposed to undergo CRF, whereas direct CRF from the intact cation may proceed through a different mechanism.

Several 'thermometer' molecules have been used to determine the energetics of dissociations in mass spectrometry. Tungsten hexacarbonyl, via the abundances of successive losses of CO, has been used to establish an energy scale in CID and SID.[12] The dissociation of *n*-butylbenzene, particularly to ions at m/z 91 and 92, has been used to similar effect.[13] A higher mass molecule for which dissociations could reflect energy deposition across the wider range of energies encountered with various activation methods would be valuable. Multi-chain molecules such as the carbocyanine dyes or the dialkylaminostyryl molecules studied here may serve as a next generation of thermometer molecules if CRF is found to vary predictably within the energy range encompassed by low-energy CID, high-energy CID and SID. Additional work across a broader energy range or with compounds with additional numbers of alkyl chains, or different alkyl chains, is justified. Evidence so far suggests that such compounds offer a distinct, reproducible and predictable dissociation behavior with increasing amounts of cation internal energy.

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