

the present photoinduced electron transport shown in Figure 5 strongly suggests that both electron transports are mainly controlled by the same principle, the proposed flux conjugation.

Experimental Section

Instruments. Electronic absorption spectra were measured with either a Union SM-401 high-sensitivity spectrometer or a Hitachi 340S spectrophotometer. Fluorescence spectra were measured with a Union FS-301 high-sensitivity fluorescence spectrometer. Sonication was performed with an ultrasonic disruptor, Model UR-200P (Tomy Seiko). Centrifugation was carried out with a refrigerated centrifuge, Tomy Seiko Model RS-20III. Steady-state irradiation was carried out using a WACOM 500-W xenon lamp in conjunction with a grating monochromator (1200 grooves/nm) (Shimadzu, Bausch & Lomb). Fluorescence lifetimes were measured with a Horiba NAES-1100 equipped with a 540-nm cut-off filter (SC-54, Fuji). Flash photolysis studies were carried out using a Model DL-2100 C dye laser (Phase-R) and a RA-1200 (Union) detector. Rapid reactions in the dark were recorded with a stopped-flow spectrophotometer, Union RA 401, at 25 °C under Ar atmosphere.

Materials. Commercially available reagents were used without further purification except otherwise stated. Egg yolk lecithin was carefully purified according to the literature method.⁷ Zn-TSO₃NaPP and alkylviologen dications were prepared as described previously.⁸ All solutions used in the present study were deaerated using a sequence of evacuation and argon back-flushing in an ice bath (the procedure was repeated 20 times per sample).

Preparation of Artificial Single-Wall Bilayer Liposomes. Artificial lecithin liposomes containing FMN and C₄V²⁺ in its interior aqueous solution were prepared according to a slight modification of our previously reported procedure.²

A solution of 100 mg of carefully purified egg yolk lecithin² and 10 mg of C₄V²⁺ in 5 mL of CH₂Cl₂ was gently evaporated under reduced pressure under Ar. Into the resulting lecithin thin layer film a solution of 200 mg of FMN in 5 mL of 50 mM phosphate buffer (pH 6.8) was added, and the lecithin film was suspended in the solution. The suspension was sonicated in an ice bath in an Ar box for 3 min followed by further sonication for 2 min. The procedure was repeated five times. The resulting solution was centrifuged (1.8 × 10⁴ rpm) for 30 min at 4

°C. The supernatant was applied to Sepharose 4B column (1.6 cmφ × 35 cm) and eluted with 50 mM phosphate buffer (pH 6.8) at 6 °C. Single-wall bilayer liposomes were obtained after an elution volume of 42 to 54 mL had passed.

Artificial liposomes containing Zn-TSO₃NaPP, Na₂S₂O₃, and C₄V²⁺ in the interior aqueous phase were prepared by a procedure similar to that described above for FMN and C₄V²⁺ containing liposomes.

Determination of the Quantum Yield for the Formation of FMNH. The quantum yield for the formation of FMNH in the artificial liposome, under conditions of steady-state photolysis, was determined using ferrioxalate actinometry.⁹ Both incident and transmitted light were measured at 22 °C using 0.1 mol of ferrioxalate solution (2 cm depth). An unmodified liposome solution was used as a reference.

Steady-State Photolysis. The deaerated liposome solutions in 10-mm quartz cells were irradiated with a 500-W xenon lamp equipped with a grating monochromator (1200 grooves/nm) (425 ± 5 nm). Reduction of FMN was followed spectrophotometrically (by monitoring change in absorbance of between 300 and 500 nm).

Typical Procedure of the Na₂S₂O₄ Reduction of FMN Present in the Interior Aqueous Solution of the Functionalized Liposome (FMN, C₄V²⁺⁽ⁱ⁾|Lip-C₄V²⁺). A freshly prepared liposome solution (1.5 mL) (FMN, C₄V²⁺⁽ⁱ⁾|Lip-C₄V²⁺), kept at pH 6.8 with a 5 mM phosphate buffer containing 45 mM sodium bromide, was placed in a 10-mm quartz cell equipped with a three-way stopcock. Into this solution 0.5 mL of a 0.1 mM solution of C₄V²⁺ was added. The solution was deaerated as described earlier, and into the deaerated liposome solution, at 25 °C, 0.2 mL of a freshly prepared Na₂S₂O₄ solution (7.1 mM) (by titration with K₃FeCN₆(aq)) was added using a specially designed syringe.² Reduction of the internal FMN and formation/consumption of C₄V^{•+} were followed by monitoring the changes in absorbance at 450 and 605 nm, respectively.

Registry No. CH₃V²⁺, 4685-14-7; C₃H₇V²⁺, 46903-41-7; C₄H₉V²⁺, 47082-19-9; C₅H₁₁V²⁺, 47230-70-6; C₆H₁₃V²⁺, 47369-13-1; C₁₀H₂₁V²⁺, 47709-85-3; FMN, 146-17-8; FMNH, 5666-16-0; Zn-TSO₃NaPP, 80004-36-0; H⁺, 12408-02-5; Na₂S₂O₄, 7775-14-6.

(9) $\phi_{425} = (2[\text{FMNH}]_{\text{formed}}/[\text{h}\nu]_{\text{absd}}) = 2[\text{FMNH}]_{\text{formed}}/([\text{modified Lip}]_{\text{absd}} - [\text{unmodified Lip}]_{\text{absd}})$; ^aBased on UV determination (FMN: $\epsilon_{316} = 2.7 \times 10^3$, $\epsilon_{370} = 1.2 \times 10^4$, $\epsilon_{470} = 1.05 \times 10^4$, FMNH: $\epsilon_{316} = 7.4 \times 10^3$, $\epsilon_{370} = 3.9 \times 10^3$, $\epsilon_{470} = 7 \times 10^2$). ^bChemical Actinometry using ferrioxalate. ^cLight diffraction.

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Gas-Phase Ion Decompositions Occurring Remote to a Charge Site

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Abstract: Several classes of closed-shell, gas-phase ions have been found to decompose upon collisional activation in a manner which does not rely on charge initiation. The reactions provide specific and highly informative structural information. This type of fragmentation, which was observed first in a study of carboxylate anions of unsaturated fatty acids, is now identified as parallel losses of the elements of C_nH_{2n+2} initiated from the alkyl terminus. A homologous series of saturated fatty acid anions ranging in chain length from 5 to 18 carbons has been used as a model system to characterize the phenomenon. The fragmentation occurs also for collisionally activated sulfate and sulfonate anions and long-chained amine and phosphonium cations. Deuterium labeling was used to determine that the C_nH_{2n+2} losses are from the alkyl terminus and that a probable mechanism is a process which involves a 1,4 hydrogen elimination with subsequent formation of terminally unsaturated carboxylate anions and neutral olefins.

The mechanisms of decomposition of gas-phase ions such as those which are produced in a mass spectrometer have been explained in terms of charge-site or radical-site initiation.^{1,2} The

role of charge site is particularly well accepted for fragmentations of radical cations produced by electron or photon impact. The McLafferty rearrangement is illustrative. Hydrogen transfer takes

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(2) Williams, D. H.; Howe, I. "Principles of Organic Mass Spectrometry"; McGraw Hill: London, 1972; pp 94-121.

place via a six-membered ring transition state to an electron-deficient heteroatom such as that in a carbonyl group. An identical rearrangement called the Norrish type II cleavage takes place in the neutral molecule after absorption of light by a $n-\pi^*$ transition. It is either electron removal (McLafferty rearrangement) or electron promotion (Norrish type II) that "triggers" the chemical process.

The question of whether the charge can be pictured as localized at certain sites has been the subject of some debate;³⁻⁶ however, the issue does *not* involve the role of charge in triggering fragmentation. In fact, the ability of the charge site to migrate in olefins or other double bond-containing compounds probably causes double bond isomerization and limits the specificity of the information obtained from EI mass spectra of these compounds. Moreover, the key role of the charge site is apparent in that many compounds are more amenable to EI analysis if derivatized to accommodate better the positive charge.

The discovery of a set of highly specific decomposition reactions of gas-phase carboxylate anions of unsaturated fatty acids may point to a new class of fragmentation reactions which do not rely on charge initiation.⁷ These decompositions are manifested as a series of regularly spaced peaks separated by 14 amu in the collisionally activated decomposition (CAD) spectra.^{8,9} The intensities of the peaks follow a distinctive pattern with a smooth "envelope" appearance in which the most intense peaks are those corresponding to higher mass ions and the number of peaks in the series may be correlated to the length of the carbon chain. This type of decomposition is not limited to fatty acids as it has also been found to occur for sulfate and sulfonate anions which possess long alkyl chains.¹⁰ Unlike the decomposition of radical cations, these fragmentations have proven to be highly specific and very useful for determining the location of the double bond of monounsaturated fatty acids⁷ and the structure of anionic surfactants.¹⁰ However, the reactions are not readily explained in terms of charge site-initiated fragmentation.

The goals of the research reported here are (1) to determine the precise identity of fragments observed earlier by us⁷ in a study of the collisionally induced dissociation of carboxylate anions of unsaturated fatty acids, (2) to provide evidence that the fragmentation is not restricted to this group of compounds, and (3) to establish the mechanism by studying the CAD spectra of isotopically labeled compounds.

Results and Discussion

Identity of Fragments. In previous work in this laboratory, Tomer et al.⁷ have shown that the double bond position in monounsaturated fatty acids can be located by collisionally activating the $(M-H)^-$ anion. This gives rise to a series of regularly spaced peaks in the CAD spectrum. A pattern of two intense peaks corresponding to cleavage allylic to the double bond with three very minor intervening peaks can be used to locate unequivocally the position of the double bond. However, neither the precise identity of the fragments nor the mechanism was presented in that communication.

Careful examination of the spectra and spectra of other related compounds shows that these fragmentations involve the loss of the elements of C_nH_{2n+2} , i.e., CH_4 , C_2H_6 , etc. (we cannot easily distinguish between the loss of C_nH_{2n+2} as an alkane or as an

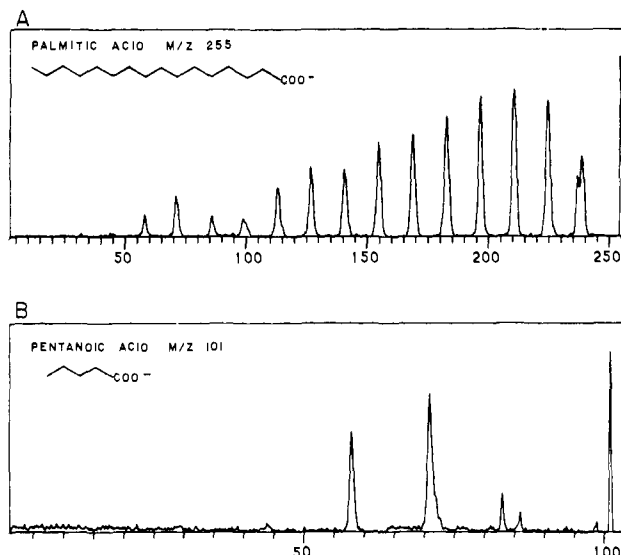
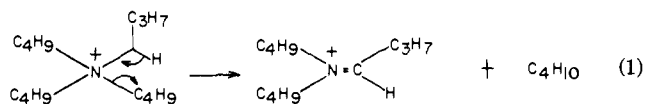


Figure 1. Spectra of the daughter ions produced by collisionally activating the $(M-H)^-$ ions of palmitic acid (A) and pentanoic acid (B). Only the m/z 71 ion is formed by the loss of a C_nH_{2n+2} fragment from the pentanoic anion. Ions m/z 71, 99, 113, 127, 155, 169, 183, 197, 211, 225, and 239 are formed by the loss of C_nH_{2n+2} fragments from the $(M-H)^-$ ion of palmitic acid.

alkene and H_2). The loss of the elements of neutral alkane fragments is not common in mass spectral decompositions, but it has been reported by several groups for a range of compounds which include amines, secondary alcohols, ketones, and quaternary ammonium ions.¹¹⁻¹⁷ These losses characteristically occur where there is branching at a charge site as in the quaternary ammonium ion shown in eq 1. Veith¹¹ accounted for this loss by proposing



a mechanism in which hydrogen is transferred from one substituent group to a second and the second group is lost as is shown in eq 1. However, the parallel losses of C_nH_{2n+2} from the uncharged end of a carboxylate anion seem to occur by a distinctly different process than that described by Veith and require a hydrogen transfer to occur from the portion of the carbon chain which retains the charge. Hence, the distinctive interruption in the C_nH_{2n+2} loss pattern in the CAD spectra of the monounsaturated carboxylate anions may be attributed to the difficulty of transferring vinylic hydrogens and cleaving double bonds.

Based on the CAD spectra of *unsaturated* fatty acids, one might expect the pattern for C_nH_{2n+2} elimination to be smooth and relatively regular for *saturated* carboxylate anions. Thus, we began the study of the scope, requirements, and mechanism of these decomposition reactions by investigating a simple homologous series of saturated carboxylic acids.

The mass spectra of fatty acid anions desorbed by using fast atom bombardment and analyzed in the negative ion mode *without* collisional activation consist of the $(M-H)^-$ anion and no apparent fragments.¹⁸ If the $(M-H)^-$ ion is selected by using MS-I of the tandem instrument and its metastable or spontaneous de-

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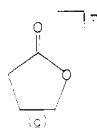
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(16) Holmes, J. L.; Burgers, P. C.; Mollah, Y. A. *Org. Mass Spectrom.* **1982**, *17*, 127-130.

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(18) See ref 7 for an example of the fatty acid spectra.

compositions followed by scanning the ESA of MS-II, the anion shows very little fragmentation except for a low abundance loss of H₂O for the longer chained acids. However, upon collisional activation, decompositions characterized as the parallel losses of C_nH_{2n+2} fragments from the alkyl terminus become the significant feature of the decomposition spectra of (M - H)⁻ ions (Table I and Figure 1A) (see ref 19). While shorter chained acids such as pentanoic (Figure 1B) decompose to ions that may be attributed to C_nH_{2n+2} loss (in this case the ion of *m/z* 71), the "envelope" of C_nH_{2n+2} loss ions as seen in Figure 1A becomes more defined and increasingly prominent as the chain length increases. Also, the abundances of fragment ions formed increase substantially for the longer-chain acids. It should be noted that the only other decompositions observed, in addition to the C_nH_{2n+2} losses, are those which lead to ions *m/z* 58, *m/z* 86, and ions resulting from the loss of H₂O from the (M - H)⁻ ions. The *m/z* 58 ion (CH₂COO)⁻ results from cleavage β to the carboxyl group. The ion *m/z* 86 is not part of the alkane loss series since an *m/z* 85 ion would be expected. It could possibly arise from the formation of a cyclic daughter ion which incorporates the charge site (see structure a) or it may exist as a ring-opened structure with the



negative charge on the oxygen and the radical site located on the carbonyl carbon (structure b).

The charge site, which would be expected to reside with the carboxylate group, appears to be involved in only the fragmentations just discussed, i.e., the loss of water and those leading to



formation of ions *m/z* 58 and 86, with the remainder of the decompositions initiated remote from the charge site. It is possible that coiling of the carbon chain of the isolated ionic species, as described by Meyerson,²⁰ could disperse the charge partially into the alkyl chain by polarization or completely by H⁺ transfer to the carboxylate. However, for these fatty acids, it appears that a distinction can be made between charge-effected fragmentations and ones not readily associated with the charge site. This is especially apparent when these spectra are compared to those in Stenhagen and co-workers's classic studies of carboxylate ester radical cations in which the preferred fragmentations are those which form low mass ions.²¹⁻²³

To determine if remote site decompositions involve the losses of species other than hydrocarbons, the CAD spectrum of the fragment ions from the (M - H)⁻ ion of 11-aminoundecanoic acid was obtained (Figure 2). This spectrum shows that prominent losses of the elements of neutral amines, e.g., NH₃, CH₃NH₂, C₂H₅NH₂, etc., occur. In the presence of both the amine and carboxyl functional groups, the negative charge would be expected to reside with the carboxyl group. Hence, decomposition by losses of various C_nH_{2n+1}NH₂ is another example of fragmentation remote from the charge site.

(19) The processes discussed here are those observed under negative ion conditions. This does not eliminate the potential for other processes such as charge reversal. In fact charge reversal has been observed for the (M - H)⁻ ions of oleic acid and eicosanoic acid. It is reasonable to assume that this is a significant process since for palmitic-7,7,8,8-*d*₄ acid, the positive ion decompositions of the (M + H)⁺ ion which produce mostly low mass ions represent 5% of the main beam while for the (M - H)⁻ ion of the compound decompositions represent 1% of the main beam.

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(21) Odham, G.; Stenhagen, E. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Ed.; Wiley-Interscience: New York, 1972; pp 211-227.

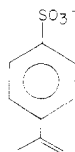
(22) Dihn-Nguyen, N.; Ryhage, R.; Stallberg-Stenhagen, S.; Stenhagen, E. *Ark. Kemi* **1961**, 393-399.

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Table I. Relative Abundances of Ions Formed in the Collisionally Activated Decompositions of Anions

carboxylate anion	loss of																		
	<i>m/z</i> 58	<i>m/z</i> 86	H ₂ O	CH ₄	C ₂ H ₆	C ₃ H ₈	C ₄ H ₁₀	C ₅ H ₁₂	C ₆ H ₁₄	C ₇ H ₁₆	C ₈ H ₁₈	C ₉ H ₂₀	C ₁₀ H ₂₂	C ₁₁ H ₂₄	C ₁₂ H ₂₆	C ₁₃ H ₂₈	C ₁₄ H ₃₀	C ₁₅ H ₃₂	
pentanoic (C ₄ H ₉ CO ₂ ⁻)	76	17	35		100														
hexanoic (C ₅ H ₁₁ CO ₂ ⁻)	44	33	25	12	100														
heptanoic (C ₆ H ₁₃ CO ₂ ⁻)	49	36	41	29	30	100													
octanoic (C ₇ H ₁₅ CO ₂ ⁻)	77	45	68	71	72	39	100												
nonanoic (C ₈ H ₁₇ CO ₂ ⁻)	64	40	90	70	98	86	51	100											
decanoic (C ₉ H ₁₉ CO ₂ ⁻)	40	37	60	61	83	100	59	28	75										
lauric (C ₁₁ H ₂₃ CO ₂ ⁻)	31	18	37	55	76	100	83	65	42	20									
palmitic (C ₁₅ H ₃₁ CO ₂ ⁻)	25	25	41	53	78	100	81	62	51	37	23	19	12	5				8	
stearic (C ₁₇ H ₃₅ CO ₂ ⁻)			55	55	75	100	88	76	67	64	53	43	30	29	21			11	9

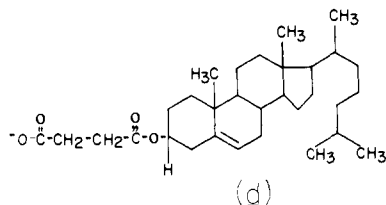
Fragmentation of Other Classes of Compounds. Decompositions occurring remote from a charge site have been observed for several other classes of compounds studied in this laboratory. The collisionally activated decompositions of surfactant anions reported by Lyon et al.¹⁰ are one example. The CAD spectrum of the $(M - H)^-$ ion of pure tetradecyl sulfate shows prominent SO_3^- and SO_4^- ions and an "envelope" of ions which we now know arise by C_nH_{2n+2} losses analogous to those decompositions of long-chained fatty acid anions. Shorter chain alkyl sulfate compounds such as octyl sulfate, like their carboxylate counterparts, show C_nH_{2n+2} losses, but the loss pattern is not well developed in the CAD spectrum. The CA decompositions of the $(M - H)^-$ ion of dodecyl benzenesulfonate, a species with a branched alkyl chain, involve C_nH_{2n+2} losses terminating with an ion which probably has the structure c.



Ethoxy alkyl sulfates likewise give the C_nH_{2n+2} losses as well as oxygen-containing fragment losses which may be an extension of phenomenon. It should be noted that for branched sulfonate and ethoxy alkyl sulfates, all ions resulting from losses of C_nH_{2n+2} are observed, but the relative abundances of the ions in the "envelope" or loss pattern are perturbed with respect to the abundance observed in the unbranched alkyl sulfates and carboxylates.

The decomposition of dodecyl benzenesulfonate is particularly noteworthy because the presence of the ring limits the potential for coiling of the molecule. Analysis of molecular models shows that coiling could occur to allow interactions starting at the C-C bond between the sixth and seventh carbon atoms of the alkyl chain. Cleavage of C-C bonds located closer to the phenyl ring could not occur with interaction with the charge site. Yet, these bonds are cleaved. Hence the decomposition of this compound by parallel C_nH_{2n+2} losses provides evidence that coiling²⁰ does not play an important role in the phenomenon.

The cholesteryl hemisuccinate $(M - H)$ anion (structure d) is another example in which remote site C_nH_{2n+2} losses are observed in the decomposition of a rigid ion. In this case, losses of C_nH_{2n+2}



occur from the alkyl chain remote from the charge-bearing carboxylate. As for the sulfonate, study of a model of this ion indicates that a minimum length of seven carbons in the alkyl chain is necessary for any interaction with the charge site.

The phenomenon of decompositions remote from a charge site has also been observed for collisionally activated positive ions; however, the product ions are of reduced relative abundances. One example of this is produced by collisionally activating long-chained alkyl ammonium ions. The spectrum of the fragments from the $(M + H)^+$ ion of octadecylamine (Figure 3) is a typical example. The ions below m/z 90 can be accounted for by expected fragmentations proximal to the NH_3^+ functional group. However, the fragment ions above m/z 90 are due to parallel losses of C_nH_{2n+2} . The spectrum of octadecylamine-*N,N,N*-*d*₃ shows no mass shifts corresponding to loss of deuterium as part of C_nH_{2n+2} , and this indicates that the losses of C_nH_{2n+2} do not result from the transfer of deuterium from the ammonium functionality.

A second example of a class of positive ions which undergo remote charge site fragmentations are the triphenylalkylphosphonium salts.²⁴ The CAD spectrum of the fragments of

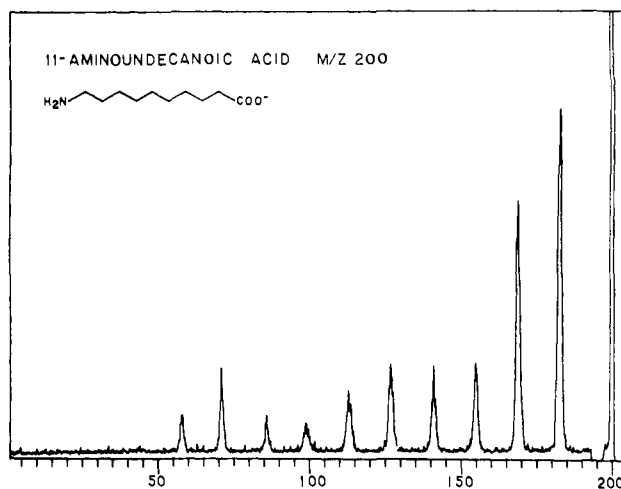


Figure 2. Spectra of the daughter ions produced by collisionally activating the $(M - H)^-$ ion of 11-aminoundecanoic acid. Fragment ions are formed by losses of NH_3 , CH_3NH_2 , $C_2H_5NH_2$, etc.

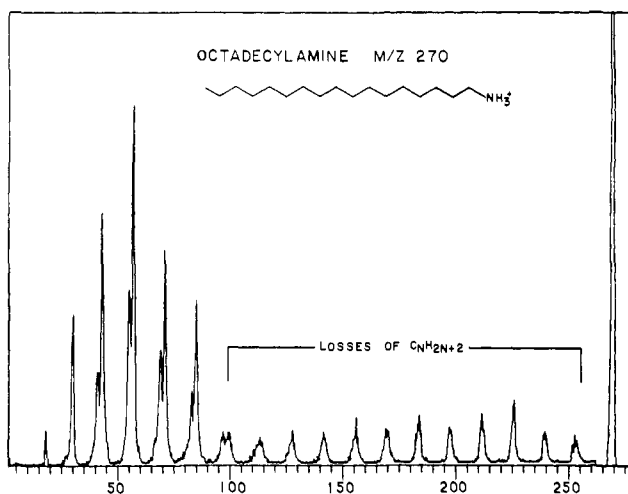


Figure 3. Spectra of the daughter ions produced by collisionally activating the $(M + H)^+$ ion of octadecylamine. Ions m/z 254-100 are formed by remote site C_nH_{2n+2} losses. Ions below m/z 100 are formed by alkyl losses initiated at the charge site.

n-decyltriphenylphosphonium ion desorbed directly by using FAB shows that a prominent ion, m/z 262, is formed which may be attributed to the $(C_6H_5)_3P^+$ ion. However, major fragment ions in the range m/z 275-387 are formed by losses of C_nH_{2n+2} .²⁵ The pattern of peaks caused by these losses from phosphonium ions is somewhat different than those seen for the other classes of compounds having straight chain alkyl groups. Normally the abundance of the charged fragments diminishes as the fragmentation approaches the charge site; i.e., the lowest abundance daughter ions are those which would represent the fragmentation nearest the charge site. Because this trend does not apply for the phosphonium salts, two types of decompositions are suggested: those initiated remote from the charge as seen before and those proximate to the charge site which produce presumably $Ph_3P=CH=CH_2^+$ (m/z 289) and $Ph_3P=CH^+$ (m/z 275).

Mechanism. The general features of the reactions are their relatively high energy requirements and their location remote from a charge site. The processes can be viewed as analogous to thermal reactions rather than the typical charge or radical site-initiated reaction so common in mass spectrometry. To explore more deeply

(24) McCrery, D. A.; Peake, D. A. Paper presented at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 1984, FPA 13.

(25) Ions m/z 275, 289, 303, 317, 331, 345, 359, 373, and 387 with relative abundances to the base peak m/z 262 of 47, 55, 5, 8, 11, 17, 19, 18 and 10 respectively.

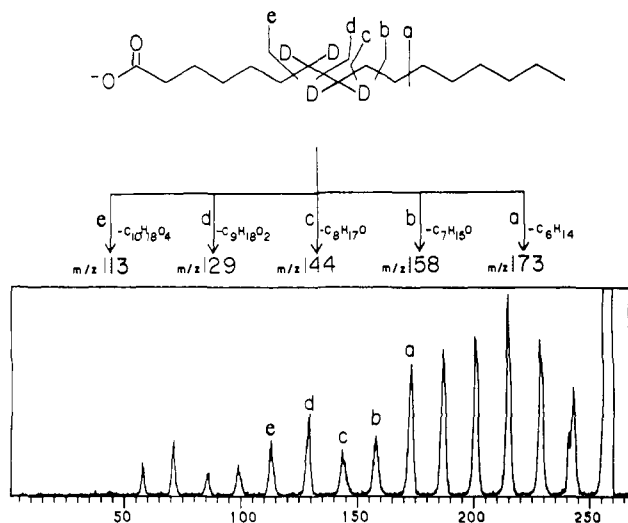


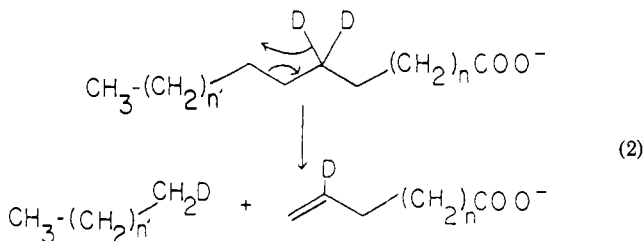
Figure 4. Spectra of the daughter ions produced by collisionally activating the $(M - H)^-$ ion of palmitic-7,7,8,8- d_4 acid.

the mechanism, the decompositions of various deuterium-labeled fatty acid carboxylates were investigated.

Deuterium-labeled acids including palmitic-16,16,16- d_3 acid, palmitic-7,7,8,8- d_4 acid, stearic-9,10- d_2 acid, myristic-9,10- d_2 acid, and octanoic-2,3- d_2 acid were used to probe the mechanism of the reaction. The CAD spectrum of the palmitic-16,16,16- d_3 acid differs from the spectrum of the unlabeled acid only in the mass of the $(M - H)^-$ ion. All other product ions have the same masses as revealed by comparing their respective spectra. Thus, the loss of CH_4 occurs only from the alkyl end of the molecule, and it proceeds *without* H/D interchange.

The fragmentations of carboxylate anions labeled at internal chain positions are more informative. The CAD spectrum of palmitic-7,7,8,8- d_4 acid (Figure 4) shows mass shifts above m/z 113 as compared to the unlabeled acid whereas ions below that m/z have the same mass for both compounds. As indicated in the figure, the ions m/z 129, 144, 158, and 173 of the labeled acid correspond to the ions m/z 127, 141, 155, and 169 of the unlabeled acid. The reduced abundance of the ions m/z 144 and 158 of the labeled compared to the corresponding ions m/z 141 and 155 of the unlabeled acid is taken to indicate a kinetic isotope effect. The decompositions of stearic-9,10- d_2 acid, myristic-9,10- d_2 acid, and octanoic-2,3- d_2 acid²⁶ supply similar evidence.

These results are most easily rationalized in terms of a mechanism for the loss of C_nH_{2n+2} which involves a 1,2-elimination of an alkane (see eq 2). This type of process would certainly be

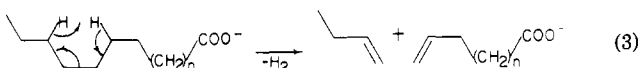


a high-energy process and is symmetry forbidden. However, Williams et al.²⁷ have reported that 1,2-eliminations do occur for MS fragmentations and that such reactions give substantial kinetic energy release.

(26) Myristic-9,10- d_2 acid CAD spectrum: m/z (rel abundance), 58 (13), 71 (23), 86 (11), 99 (14), 113 (29), 127 (44), 141 (53), 156 (73), 170/171 (63), 184/185 (89), 199 (100), 211 (56), 213 (66). Octanoic-2,3- d_2 acid CAD spectrum: m/z (rel abundance), 58/59 (85), 72/73 (62), 88 (50), 101 (66), 115 (100), 126 (60), 129 (77). Stearic-9,10- d_2 acid CAD spectrum: m/z (rel abundance), 58 (7), 71 (11), 86 (7), 99 (9), 113 (17), 127 (22), 141 (29), 156 (32), 170-171 (43), 184/185 (45), 199 (70), 213 (79), 227 (79), 241 (100), 255 (88), 267 (39), 269 (57)9

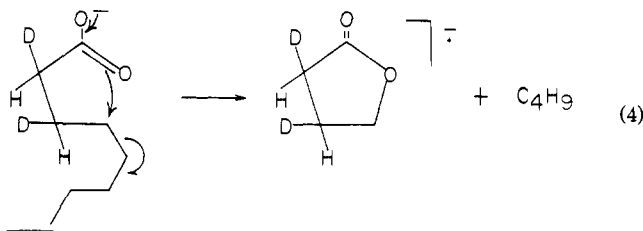
(27) Williams, D. H. *Acc. Chem. Res.* **1977**, *10*, 280-286.

Another mechanism can be proposed which is also consistent with the isotopic-labeling results. This mechanism involves a 1,4-loss of H_2 to give a terminally unsaturated fatty acid carboxylate and a 1-alkene (see eq 3). Although the neutral products



are different (and not detected in the mass spectrometry experiments conducted here), the ionic products are the same for both mechanisms. The 1,4-elimination mechanism is preferred on the basis of the following considerations. (1) The transition state is six-electron- or aromatic-like, and the reaction should be thermally allowed. (2) *Exclusive* production of an unsaturated fatty acid carboxylate and an alkene is predicted according to the 1,4-elimination. On the basis of a 1,2-elimination, an alkene and a saturated fatty acid should be as likely to be formed as an alkane and an unsaturated fatty acid. Yet only the unsaturated fatty acid carboxylate is observed. (3) The products of a 1,4-elimination are consistent with those found in the pyrolysis of fatty acid esters.²⁸ Pyrolysis at 650 °C leads to production of a series of alkenes and unsaturated fatty acid esters. These results substantiate the thermal analogy mentioned above.

The CAD spectrum of octanoic-2,3- d_2 acid also provides information regarding the mechanism for water loss and for production of the ion m/z 86, both of which are seen for all saturated fatty acid fragmentations. Water loss from the unlabeled acid anion results in the ion m/z 125. However, for the labeled acid, the corresponding ion is m/z 126. This is interpreted to indicate that the hydrogens lost as H_2O were originally attached to the 2- or 3-carbon atom. The m/z 126 ion was examined more closely with a narrow scan over the mass range of m/z 120-135 which allowed for greater sensitivity. The narrow scan spectrum of the labeled acid shows only a single peak at m/z 126 which corresponds to the loss of HDO and *not* a multiplet which implies that both the hydrogen and deuterium lost as HDO in the labeled octanoate ion are attached to the same carbon atom initially. This somewhat unexpected observation certainly merits further investigation with other deuterium-labeled acids. The ion m/z 86 which is produced in CA decompositions of all unlabeled fatty acids and which is assigned to possess a cyclic structure (see structure a) is m/z 88 in the octanoic-2,3- d_2 acid. This supports



the theory that this ion is formed by a simple or assisted cleavage reaction (eq 4), a process quite different than the decompositions which produce the main series of ions.

A final point to be noted regarding the deuterium-labeled compounds studied was the lack of evidence for hydrogen/deuterium exchange within the limits of resolution available in the experiments. This is quite evident if one examines superimposed narrow scans of labeled and unlabeled palmitic acid in the range of m/z 108-135. Both spectra have peaks at m/z 113 which are indistinguishable, and the respective peaks at m/z 129 and 127 differ in position but not peak width.

Although MS-II of the instrument used in the laboratory has an energy-resolving power of 8000, the mass-resolving power is much less due to unavoidable energy release in the decomposition. That energy release serves to broaden the peaks in the CAD spectra. The masses of ions are subsequently assigned from the centroids of these broadened peaks. The validity of this approach has been substantiated in at least three ways. First, the results

(28) Sun, K. K.; Hayes, H. W.; Holman, R. T. *Org. Mass Spectrom.* **1970**, *3*, 1035-1042.

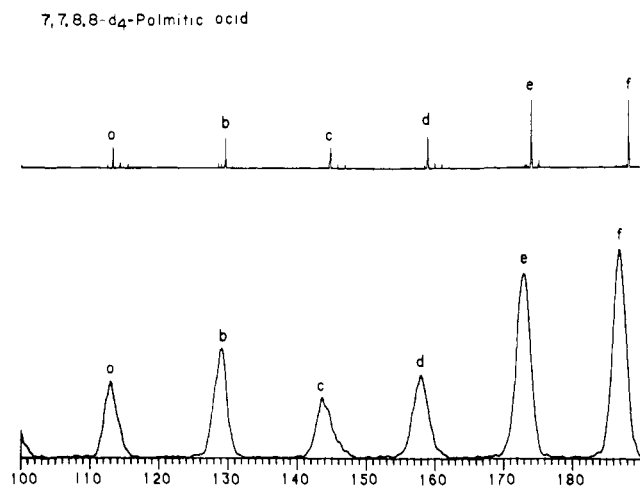


Figure 5. Spectra of the daughter ions in the range m/z 100–290 produced by collisionally activating the $(M - H)^-$ ion of palmitic-7,7,8,8- d_4 acid. The upper spectrum which was obtained by using a four-sector instrument is compared to a spectrum obtained with the three-sector instrument used in this study.

are reproducible to within a few tenths of an atomic mass unit. Second, expanded portions of the spectra as well as spectra obtained from narrow scans, both of which allow for more careful scrutiny of the peaks and assignment of the mass, show agreement with mass assignments based on full CAD spectra. Finally, the broadening of the peaks decreases with decreasing mass, resulting in centroids that are more accurately assigned. Mass assignments obtained for larger mass ions correspond to values expected based on the more precise values obtained for their smaller homologues.

Nevertheless, it would be advantageous to obtain a CAD spectrum at unit mass resolution. Therefore, a four-sector instrument at the National Institutes of Environmental Health was employed to obtain a CAD spectrum of palmitic-7,7,8,8- d_4 acid.²⁹ This is a tandem instrument with double-focusing MS-I (BE) and double-focusing MS-II (EB) which has an ultimate resolving power of 10 000 for MS-II. The data for palmitic-7,7,8,8- d_4 acid with unit resolution for the collisionally dissociated daughter ions indicate that at least 90% of the ions have m/z values consistent with those expected for remote site fragmentation. A comparison of a CAD spectrum with unit resolution for the daughter ions with a spectrum of palmitic-7,7,8,8- d_4 acid obtained in this laboratory in the range of m/z 113–187 is shown in Figure 5.

In summary, fragmentation initiated remote from the charge site is observed for several classes of collisionally activated ions and can provide specific and highly informative structural information. Examination of the conditions for which the observation is made suggests reasons why this chemistry has previously avoided detection. Prior to the advent of negative ion chemical

ionization and FAB, it has been difficult to produce easily $(M - H)^-$ anions of carboxylates, sulfates, etc., or closed shell positive ions such as alkyl triphenylphosphonium ions. These anions can now be produced in high abundance. However, carboxylate anions desorbed by fast atom bombardment show virtually no fragmentation. If these $(M - H)^-$ anions are collisionally activated, the remote site fragmentation occurs. These processes resemble high-energy thermal reactions and appear to be significant only under collisional activation conditions.

Experimental Section

Mass Spectrometry. Mass spectra were obtained with a Kratos MS-50 triple analyzer tandem mass spectrometer which has been described previously.³⁰ This instrument consists of a high-resolution MS-I of Nier-Johnson geometry followed by an electrostatic analyzer used as MS-II. Fast atom bombardment (FAB)³¹ was used to ionize desorptively the preformed conjugate bases from the basic matrix triethanolamine. CAD spectra of the fragments from negative $(M - H)^-$ ions of the fatty acids were taken by activating the ions in the third-field free region by using helium gas (sufficient helium was added to suppress the ion beam by 50%) and by scanning MS-II; 15–30 scans were signal-averaged for each spectrum.

Saturated fatty acids ranging in length from 5-carbon pentanoic acid to 18-carbon stearic acid were obtained from Sigma Chemical Co. In each case a negative ion mass spectrum and unimolecular decomposition spectrum were obtained in addition to the CAD spectrum.

Deuterium-Labeled Compounds. Stearic-9,10- d_2 acid, myristic-9,10- d_2 acid, and octanoic-2,3- d_2 acid were prepared from their respective monounsaturated acids by reacting diimide (N_2D_2) with the unsaturated fatty acids to add deuterium to the double bond.^{32,33} In each case 15 mg of the unsaturated acid in 1 mL of H_2O was neutralized by the addition of 0.1 F LiOH. The solvent was evaporated, and the residue was washed with acetone and then dried. This residue was then heated at reflux for 3 to 4 days with 0.4 mL of D_2O and 0.4 mL of hydrazine- d_4 . The appearance of a white foam followed by its gradual disappearance was evidence of reaction. At the end of the reflux period the solvent was evaporated and the remaining residue acidified with 1 F HCl. The product was then extracted into ether and recovered by evaporating the solvent. The identity of the product was consistent with the negative ion FAB mass spectrum. Palmitic-16,16,16- d_3 acid and palmitic-7,7,8,8- d_4 acid were obtained from MSD Isotopes. Octadecylamine- N,N,N - d_3 was prepared by placing the unlabeled amine in a glycerol- d_8 matrix.

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