Chemical Ionization Mass Spectrometry. XX. Energy Effects and Virtual Ion Temperature in the Decomposition Kinetics of Amino Acids and Amino Acid Derivatives

M. Meot-Ner and F. H. Field*

Contribution from the Rockefeller University, New York, New York 10021.
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Abstract: The i-C_4H_{10} CI mass spectra of several amino acids and peptides were determined. Temperature dependences of the mass spectra of proline, valine, and *N*-valerylleucine were determined. Activation energies and preexponential factors were obtained for the prominent fragmentation reactions, *i.e.*, the loss of HCOOH from the free amino acids and the loss of H_2O and HCOOH from the acylated compound. A fragmentation mechanism controlled by the energy requirements for internal proton transfer from amino to carboxyl groups is proposed. The kinetic data are found to exhibit a linear correlation between E_a and log A. The use of kinetic measurements for the determination of internal ion energies is demonstrated by the finding that the protonated *N*-valerylleucine ion formed in a methane plasma decomposes from an energetic state equivalent to that of the same ion formed in an isobutane plasma with a temperature 167 ± 10° higher than that of the methane plasma.

The temperature dependence of fragmentation reactions in gaseous ions by chemical ionization mass spectrometry is useful for the determination of the activation energies and preexponential factors of the reactions. Such studies were conducted previously on ester decompositions.[1-5] The spectra of amino acids and peptides in the strongly protonating methane plasma were investigated by Milne, *et al.*[6] Investigation of the CI mass spectrometry of these compounds under mild protonating conditions, and of the temperature dependence thereof, is important both for analytical purposes and for a deeper understanding of the physical chemistry of these biologically essential structures. The kinetic data can also be utilized to obtain information on the relative internal energies of the protonated ions in different protonating environments.

Experimental Section

Mass spectrometric studies were carried out on The Rockefeller University chemical physics mass spectrometer which was described previously.[7] Mass spectra were obtained at a source pressure of 1.0 ± 0.1 Torr of i-C_4H_{10} and 5 V/cm repeller field. An indirect solid probe *ca.* 40 cm upstream from the source was used to introduce solid samples, and it was heated to about 140° to effect volatilization.

Amino acids and peptides of commerical quality were used. CH_3CONH-Val-Val-OCH_3 was prepared by acylation of the dipeptide in acetic anhydride–methanol and esterification in methanol–HCl. The product was washed in alkaline and basic aqueous solution to remove residual free reactants. *N*-Valerylleucine was obtained by acylation of the amino acid with valeryl chloride, and it was washed with aqueous HCl to remove residual free leucine. Each kinetic study was replicated two to six times. Error estimates are based on the maximum deviation from the mean values obtained in these experiments.

Results and Discussion

A. CI Mass Spectra. The chemical ionization mass spectra obtained for several compounds at low (440°K) and high (630°K) source temperatures are shown in Tables I and II. Little fragmentation is observed in the low-temperature mass spectra of proline, valine, leucine, and the derivatized compounds *N*-valerylleucine and *N*-acetylvalylvaline methyl ester. The protonated molecular ion, MH^+, the protonated dimer, M_2H^+, and the addition complexes, $(M + 57)^+$ and $(M + 39)^+$, formed by the addition of reactant ions to the neutral additive are the most important species present. With increasing source temperature the relative intensities of the fragment ions increase, and in addition new fragment ions appear in the mass spectra of the derivatized compounds. In CH_3CO-Val-Val-$OCH_3 \cdot H^+$ the C-terminal ions of m/e 132 and the N-terminal acylium ion of m/e 142 are of analytical value, and their intensities increase sharply with increasing temperature. Similar behavior is to be observed for the m/e 85 acylium ion produced from CH_3CONH-$(C_4H_9)COOH \cdot H^+$. It may be inferred that temperature will be a useful variable in i-C_4H_{10} CI mass spectrometric analysis of many amino acids and peptides.

In contrast to the above compounds, the protonated molecular ion, MH^+, is absent even from the low-temperature (440°) spectra of underivatized alanylalanine and of 6-aminohexanoic and 11-aminoundecanoic acids (Table III). The presence of the dimers $(M - 18)_2H^+$ and of the addition complexes $(M - 18 + 39)^+$ and $(M - 18 + 57)^+$ indicates that the major gaseous neutral species in these cases is $(M - 18)$. In these compounds where more than one carbon atom separates the amino and carboxyl groups, lactam formation prior to ionization, probably prior to volatization, is a likely process. The observation of dimers and addition complexes is thus useful as a diagnostic technique to help distinguish between the thermal decomposition of neutrals and the fragmentations of ions.

B. Fragmentation Kinetics and Mechanisms. Rate constants, activation energies, and log A values for various ionic decomposition reactions were obtained from spectra measured at different temperatures utilizing procedures developed in earlier works in this laboratory.[1] Sample Arrhenius plots are shown in Figure 1. The results are summarized in Table IV.

The A factors found for the reactions involving loss

(1) F. H. Field, *J. Amer. Chem. Soc.*, **91**, 2827 (1969).
(2) F. H. Field, *ibid.*, **91**, 6334 (1969).
(3) W. A. Laurie and F. H. Field, *ibid.*, **94**, 2913 (1972).
(4) W. A. Laurie and F. H. Field, *ibid.*, **94**, 3359 (1972).
(5) W. A. Laurie and F. H. Field, *J. Phys. Chem.*, **76**, 3917 (1972).
(6) (a) G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Amer. Chem. Soc.*, **92**, 5170 (1970); (b) A. A. Kiryushkin, H. M. Fales, T. Axenrod, E. J. Gilbert, and G. W. A. Milne, *Org. Mass. Spectrom.*, **5**, 19 (1971).
(7) M. S. B. Munson and F. H. Field, *J. Amer. Chem. Soc.*, **88**, 2621 (1966).

Table I. Low-Temperature (440°K) Isobutane CI Mass Spectra of Some Amino Acids and Amino Acid Derivatives

Ion	Proline		Valine		Leucine		*N*-Valerylleucine		CH_3CO-Val-Val-OCH_3	
	m/e	*I*[a]	*m/e*	*I*	*m/e*	*I*	*m/e*	*I*	*m/e*	*I*
$(M_2H)^+$	231	2.8	235	4.2	263	2.8	431	4.2		
$(M + 57)^+$	172	1.8	174	3.0	188	3.5	272	1.4	329	2.3
$(M + 39)^+$	154	2.3	156	2.5	170	2.8	254	2.4	311	2.1
MH^+ (i)	117	5.2	119	5.0	133	5.5	217	11.4	274	13.7
MH^+	116	84.4	118	83.0	132	80.8	216	77.3	273	80.0
$(M - 1)^+$	114	1.6	116	1.0	130	1.0	214	1.8		
$MH^+ - 18$							198	0.6		
$MH^+ - 46$	70	1.4	72	1.3	86	1.6	170	0.9		
$H_3NCH(C_4H_9)COOCH_3^+$									132	5.9

[a] In Tables I–III, $I = 100(I_i/\Sigma I_i)$ where I_i is the intensity of the given ion and ΣI_i is the total ionization attributed to the additive compounds.

Table II. High-Temperature (630°K) Isobutane CI Mass Spectra of Some Amino Acids and Amino Acid Derivatives

Ion	Proline		Valine		Leucine		*N*-Valerylleucine		CH_3CO-Val-Val-OCH_3	
	m/e	*I*	*m/e*	*I*	*m/e*	*I*	*m/e*	*I*	*m/e*	*I*
MH^+ (i)	117	5.2	119	5.5	133	5.4	217	11.5	274	5.2
MH^+	116	82.0	118	84.9	132	81.0	216	66.1	273	26.8
$(M - 1)^+$	114	1.4	116	1.6	131	1.4	214	1.4	271	1.1
$NH^+ - 18$ (i)							119	1.4		
$MH^+ - 18$							198	9.5		
$MH^+ - 46$ (i)	71	1.6	73	0.5	87	1.8	171	0.5		
$MH^+ - 46$	70	9.8	72	7.6	86	10.4	170	3.4		
$H_3N^+CH(C_4H_9)COOH$							132	3.7		
$H_2N^+{=}CH(C_4H_9)$							86	2.1		
$C_4H_9CO^+$							85	0.4		
$CH_3CONHC(C_3H_7)CHO^+$									142	4.4
$H_3N^+CH(C_3H_7)COOCH_3$									132	53.7
$H_2N^+{=}CHC_3H_7$									72	5.0

Table III. Isobutane CI Mass Spectra of 6-Aminohexanoic Acid, 11-Aminoundecanoic Acid, and Alanylalanine[a]

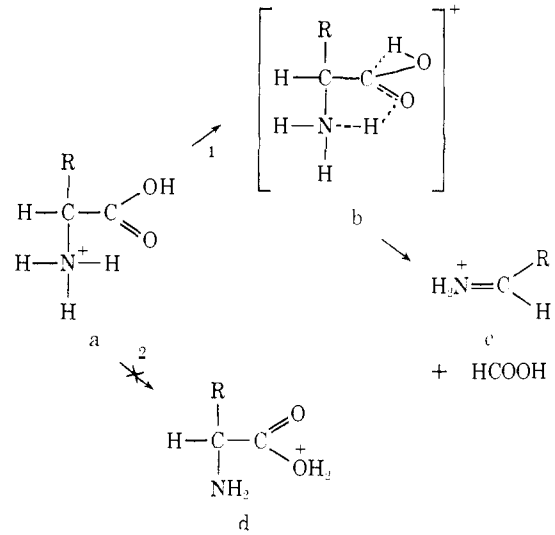
Ion	6-Aminohexanoic acid		11-Aminoundecanoic acid[b]		Alanylalanine	
	m/e	*I*	*m/e*	*I*	*m/e*	*I*
$(M - 18)_2H^+$	227	4.6			286	22.3
$(M - 18) + 57^+$	170	1.9				
$(M - 18) + 39^+$	152	2.0			181	1.3
$(M - 18)H^+$ (i)	115	6.8	185	11.4	144	6.7
$(M - 18)H^+$	114	82.7	184	88.6	143	69.8

[a] Source temperature = 440°K. [b] Low intensity of the signal in this compound prevented the detection of additional products.

of H_2O and loss of HCOOH from the two protonated amino acids and from *N*-valerylleucine are all quite low by absolute standards. Variations occur in the log *A* values obtained for the loss of HCOOH from all three compounds, but we are inclined to consider that the differences existing between the three values lie within the limits of experimental error. We think that a value of about 8.0 for log *A* may be taken as characteristic of the loss of HCOOH from all three compounds. Kinetic quantities for water loss were determined only for *N*-valerylleucine, and the value obtained for log *A* is 9.7. We very tentatively considered this value to be meaningfully larger than the log *A* value of approximately 8.0 associated with the HCOOH loss reactions.

The low absolute magnitudes of the *A* factors suggest that constrained, cyclic intermediates are involved in the processes leading to loss of H_2O and loss of HCOOH. A rationalization for the occurrence of such intermediates is that the proton affinity of an amino group is higher than that of a carboxyl group,[8] and thus the most stable form of a protonated amino acid is likely to be that of ion a (Scheme I). The reaction

Scheme I



involving loss of formic acid can be thought of as proceeding through a doubly cyclic intermediate such as ion b, and this reaction might be expected to have a particularly low *A* factor.

Pursuing this general point of view somewhat further, we suggest that water loss from protonated amino acids would occur with reaction 2 as a first step, and ion d

(8) J. Long and B. Munson, *J. Amer. Chem Soc.*, **95**, 2427 (1973).

Table IV. Kinetic Parameters of Decomposition Reactions in Protonated α-Amino Acids and an Acylated α-Amino Acid[a]

Ion reaction	Proline			Valine			*N*-Valerylleucine		
	E_a, kcal/mol	log A	log k_{300}	E_a, kcal/mol	log A	log k_{300}	E_a, kcal/mol	log A	log k_{300}
$MH^+ \longrightarrow (MH - 18)^+ + H_2O$							15.2	9.7	−1.2
$MH^+ \rightarrow (MH^+ - 46)^+ + HCOOH$	10.2	8.2	0.9	9.3	8.4	0.9	8.6	7.0	0.5
$C_4H_9CONHCH(C_4H_9)COOH \cdot H^+ \rightarrow H_3N^+CH(C_4H_9)COOH + (C_4H_8CO)$							22.1	12.1	−4.6

[a] Error estimates: E_a, ±1.5; log A, ±1.0; log k_{300}, ±1.5.

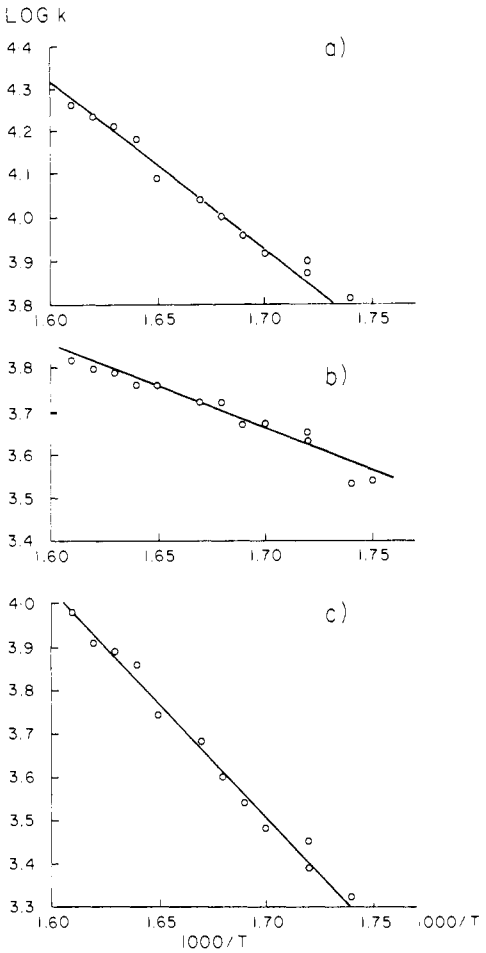
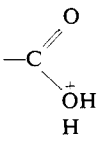
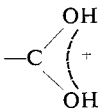


Figure 1. Arrhenius plots of three reactions in protonated *N*-valerylleucine: (a) $C_4H_9CONH(C_4H_9)COOH \cdot H^+ \rightarrow C_4H_9CONH(C_4H_9)C{\equiv}O^+ + H_2O$; (b) $C_4H_9CONH(C_4H_9)COOH \cdot H^+ \rightarrow C_4H_9CONH(C_4H_9)^+ + HCOOH$; (c) $C_4H_9CONH(C_4H_9)COOH \cdot H^+ \rightarrow H_3NCH(C_4H_9)COOH^+ + C_4H_8CO$.

would lose H_2O to complete the reaction. In fact, we do not observe water loss from the protonated amino acids, and the most obvious explanation for this is that because of difference in proton affinity, the proton attached to the amino group in ion a is held too firmly for transfer to occur to the –OH of the carboxyl group; *i.e.*, reaction 2 does not occur at an observable rate. This explanation is consistent with the results of theoretical studies on protonated carboxylic acids.[9] On the other hand, we find that loss of water does occur from protonated *N*-valerylleucine, and, indeed, it constitutes the most intense fragmentation process observed in this compound at high temperature (Table II). The value of log A for the reaction (Table IV) is larger than those found for the HCOOH reactions but still relatively small. We rationalize this behavior first by noting that the proton affinities of amides are generally accepted as being lower than those of amines but presumably higher than those of carbonyl groups or alcohols. This was confirmed recently for CH_3CONH_2.[10] We then suggest that under these circumstances the protonated *N*-valerylleucine will exist in a form similar to that of ion a (*i.e.*, with the proton attached to the amide group), but the acidity of the protonated amide group is sufficiently high that transfer of the proton to the adjacent –OH of the carboxyl group can occur, although the activation energy (E_a) for the process is relatively high (Table IV). The frequency factor is higher than those found for HCOOH loss, and the combination of the magnitudes of E_a and log A for *N*-valerylleucine are such as to make water loss the dominant fragmentation process in this compound at high temperature. We further point out that according to Scheme I, HCOOH loss involves an initial transfer of a proton to the carbonyl group, whereas H_2O loss would involve proton transfer to the hydroxyl group. It is of interest that the E_a value for loss of HCOOH is significantly lower (6.5 kcal/mole) than that for loss of H_2O. It is generally the case that the proton affinities of carbonyl groups are higher than those of hydroxyl groups.[8] Likewise,[9] the energy required for the formation of the structure



of a protonated carboxyl group is significantly higher than the energy required for the formation of



This may explain the observed differences in the E_a values of HCOOH loss and H_2O loss and provides some corroboration for the validity of Scheme I.

Finally, it has been found[3] that H_2O loss occurs readily from amino acids in methane chemical ionization, and this doubtless occurs because the much higher exothermicity of the protonation of the amino acid by CH_5^+ and $C_2H_5^+$ in methane provides energy for the occurrence of reaction 2. Thus, all the observations are consistent with H_2O and HCOOH fragmentation mechanisms controlled by an internal proton transfer rearrangement reaction.

(9) P. Ros, *J. Chem. Phys.*, **49**, 4902 (1968).

(10) R. Yamdagni and P. Kebarle, *J. Amer. Chem. Soc.*, **95**, 3504 (1973).

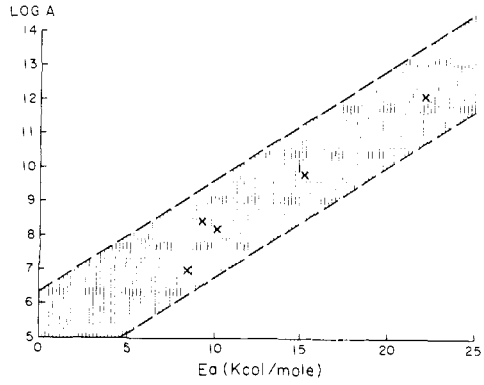


Figure 2. Isokinetic behavior in ionic fragmentation kinetics.

C. Enthalpy–Entropy Correlation. Our data exhibit isokinetic behavior; that is, a linear correlation exists between the activation energy, E_a, and the entropy related log A value. This correlation is shown in Figure 2. Such correlations in equilibrium[11,12] and kinetic[13,14] data have been the subject of past interest and theoretical analysis.[15,16]

Our kinetic measurements were made under conditions such that neither MH^+ nor fragment ion currents were less than 2% of the total sample ion current. At a characteristic residence time of 2×10^{-5} sec, these limits correspond to an accessible rate constant range of 2×10^3 sec$^{-1} \leqslant k \leqslant 4 \times 10^6$ sec^{-1}, *i.e.*, a range of 2000. Isokinetic equations relate the range of E_a and log A values which correspond to a fixed value of log k, namely

$$\log A = \log k + E_a/2.303RT \qquad \text{(I)}$$

Substitution of the maximum rate constant accessible in our experiments (4×10^6 sec^{-1}) into eq I yields the a straight line comprising the upper bound of the shaded region in Figure 2, and analogously the minimum accessible rate constant yields the lower bound in Figure 2. The shaded region comprises the range of E_a and log A values which are accessible in our experiments.

Our experimental values of E_a and log A from Table IV are plotted in Figure 2, and it is clear that they form straight line; *i.e.*, isokinetic behavior occurs. If the dynamic range of a set of kinetic measurements is relatively low, the accessible range of E_a–log A values (the shaded area in a plot such as Figure 2) may be so small that E_a and log A are forced into a linear relation, a phenomenon which may be referred to as observational selectivity. We are of the opinion that the dynamic range of our experimens is sufficiently large that the observed linearity of our experimental points is not the result of observational selectivity, but rather an intrinsic isokinetic behavior is exhibited. We present this solely as an experimental observation, for we can offer no explanation for the occurrence of the phenomenon. However, we wish to point out that mass spectrometric kinetic studies seem to provide an excellent technique for observing and investigating this phenomenon for the following reasons: (1) the dynamic range of the measurements is large, (2) the reactions occurring are simple and reasonably well characterized, (3) wall effects and heterogeneous processes are absent, and (4) solvent effects are absent.

D. Kinetic Effects of the Protonating Reagent. Studies of the decomposition kinetics in benzyl acetate[1] showed significant differences in the amounts of fragmentation produced by isobutane and methane as reactant gases, and similar effects of the acid strengths of the reactant gases are commonly found in CI mass spectrometry. Fales, Milne, and coworkers[6] have investigated the CI spectra of amino acids and peptides using methane as the reactant gas. As is to be expected, they observed more fragmentation with methane than we have with isobutane. As a matter of interest we have investigated the fragmentation of *N*-valerylleucine in methane at 0.7 Torr of source pressure at source temperatures between 390 and 450°K. We find that we are able to relate the fragmentation occurring in methane to that in isobutane in a semiquantitative way. We find that the fragmentation in methane may be looked upon as being equivalent to that which would be formed from isobutane at a significantly higher source temperature.

In the case of an ion undergoing several competitive unimolecular decomposition processes with rate constants k_i given by

$$k_i = A_i e^{-E_{a_i}/RT} \qquad \text{(II)}$$

The ratio of the concentration of the ith and jth product is given by

$$I_{P_i^+}/I_{P_j^+} = k_i/k_j = (A_i/A_j)e^{(E_{a_j}-E_{a_i})/RT} \qquad \text{(III)}$$

Equation III can be rearranged to give explicitly the temperature which would correspond to an observed pair of product ions and the E_a and ln A values for the reactions producing the product ions. Thus we have

$$T = (E_{a_j} - E_{a_i})/R[\ln (I_{P_i^+}/I_{P_j^+}) + \ln A_j - \ln A_i] \qquad \text{(IV)}$$

As may be seen from Table IV, we have kinetic parameters for three decomposition reactions of *N*-valerylleucine usiing sobutane as reactant, and we utilize these in eq IV in pairs along with corresponding pairs of product ion intensities obtained using methane as reactant. The decomposition processes considered are those producing the fragment ions with *m/e* 198 ($(MH - 18)^+$), *m/e* 170 ($(MH - 46)^+$), and *m/e* 136 ($H_3NCH(C_4H_9)COOH^+$). We give in Table V the intensities of these ions obtained in the methane CI spectra of *N*-valerylleucine at four different source tem-

Table V. Absolute Intensities[a] of Selected Ions from *N*-Valerylleucine[b]

Source temp, °K	I_{198}	I_{170}	I_{132}
390	1491	1058	567
410	835	561	613
447	1037	471	960
460	1421	648	1201

[a] Arbitrary units. [b] Methane chemical ionization. $P_{CH_4} = 0.70$ Torr.

(11) J. R. McCreary and R. J. Thorn, *J. Chem. Phys.*, **50**, 3725 (1969).
(12) J. R. McCreary and R. J. Thorn, *ibid.*, **53**, 3771 (1970), and references therein.
(13) J. Leffler, *J. Org. Chem.*, **20**, 1202 (1955).
(14) L. L. Schalenger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).
(15) R. J. Thorn, *J. Chem. Phys.*, **51**, 3582 (1969).
(16) E. R. Plante and R. C. Paule, *ibid.*, **53**, 3770 (1970).

peratures. These intensities were combined in pairs and used in eq IV along with the proper values of E_a and log A to obtain the virtual temperatures tabulated in Table VI.

Table VI. Virtual Temperatures in CH_4 Chemical Ionization of *N*-Valerylleucine[a]

Actual source temp, °K	Virtual temp ($T_{i,j}$), °K[b]				
	$T_{198,170}$	$T_{170,132}$	$T_{132,118}$	T_{av}	$T_{av} - T_{source}$
390	569	538	552	553	162
410	574	586	598	586	176
447	615	619	623	619	172
460	615	614	622	617	157
				Av = 167 ± 10	

[a] Source pressure of CH_4 = 0.70 Torr. [b] $T_{i,j}$ represents the temperature calculated from eq 4 from the pair of product ions with *m/e* values of *i* and *j*.

The virtual temperatures given in Table VI are all higher than the actual source temperatures, and the agreement between the values obtained using the three possible product ion pairs is surprisingly good. Furthermore, the extent to which the average virtual temperatures exceed the actual source temperatures shows an acceptably small variation from one actual source temperature to another. As a basis for comparison, variations of one unit in log A value for the fragmentation reactions causes variations of several hundred degrees in the virtual temperatures calculated from eq IV, the actual value depending upon the magnitudes of the E_a values involved. Conversely, variations of I kcal/mol in E_a when combind with our experimental log A values produce variations of about 100° in the virtual temperatures.

The overall average of the extent to which the virtual temperature exceeds the actual source temperature is 167 ± 10°K. The origin of this temperature difference is easily identified; namely, the acid strength of CH_5^+ is much greater than that of t-$C_4H_9^+$, and the exothermicity of the protonation of *N*-valerylleucine is much greater in methane chemical ionization. With the experimental conditions obtaining in these measurements, not all of the exothermicity is dissipated by the protonated *N*-valerylleucine before dissociation occurs, and our results show that the excited state involved in the dissociation is equivalent to the thermal equilibrium state which would be achieved were the gas 167° hotter. The fact that much the same results are obtained from the three different dissociation processes considered indicates that a relatively well-defined state of protonated *N*-valerylleucine is involved in the dissociation processes. Furthermore, the excess temperatures calculated in the methane chemical ionization depend upon kinetic parameters determined from isobutane chemical ionization measurements, and the consistent values of excess temperatures calculated from the three pairs of methane chemical ionization product ions constitute a consistency check on the isobutane kinetic quantities.

It is hoped that kinetic studies of competitive decomposition reactions may be useful for the evaluation of ion excitations in other suitable systems. The extent of applicability of such measurements requires further investigation.

Acknowledgment. This work was supported in part by a grant from the National Science Foundation.