On the Proton Affinity of Some α-Amino Acids and the Theory of the Kinetic Method[1]

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A rationalization of the kinetic method for determination of proton affinities (E_{pa}) has been formulated. When a proton-bound amine dimer with the general structure amine$_1$–amine$_2$–H$^+$ decomposes to either amine$_1$–H$^+$ or amine$_2$–H$^+$ the critical energies for the competing fragmentations can be calculated from a simplified version of the Marcus equation, which is supported by published values of molecular pair proton affinities. Consequently reaction rates of the metastable ions can be calculated from the expression $k(E) = \nu[(E - E_0)/E]^{s-1}$ and ion abundances from the expression $\int_E P(E)F(E)dE$, where $P(E)$ is the probability of reaction and $F(E)$ is the energy distribution function of the metastable ions. It is argued that for metastable ions generated by ionization methods such as CI or FAB, the energy distribution functions will be smooth and that consequently the relative ion abundances from two competing decompositions will not depend on $F(E)$. Model calculations of fragment ion abundances from metastable decomposition of ions with the general structure pentylamine–amine$_x$–H$^+$ show a linear relationship between the logarithm to the ratio: I(amine$_x$–H$^+$)/(pentylamine–H$^+$) and the E_{pa} of amine$_x$. This provides a rationalization of the kinetic method that avoids any introduction of a thermodynamic temperature. Determination of the fragment ion abundances from decomposition of metastable protonated clusters with the general structure α-amino acid–amine$_x$–H for 17 different α-amino acids gave the following E_{pa}/kcal mol^{-1} values: Ser, 217.2; Val, 218.1; Asp, 218.1; Leu, 218.7; Ile, 219.2; Thr, 219.2; Phe, 219.9; Tyr, 220.7; Met, 221.0; Asn, 222.1; Glu, 222.3; Pro, 222.4; Trp, 223.5; Gln, 226.9; Lys, 228.7; His, 230.5; Arg, >242.8.

Base strength, which is the object of this work, has been studied in the condensed phase since the last century, and studies of the basic properties of nitrogen and oxygen bases in the gas phase have played a seminal role in the development of methods and theories for the study of bimolecular reactions in the gas phase.[2]

In a proton exchange equilibrium such as eqn. (1), there are,

$$A^1H^+ + A^2 \rightleftharpoons A^1 + A^2H^+ \quad (1)$$

as for any equilibrium, two principally different methods available for determination of the equilibrium constant. One is to determine the concentrations of the products and reactants and the other is to determine the rates of the forward and backward reactions. When the equilibrium is established in the gas phase both methods require that both bases A^1 and A^2 can be vapourized. This generally means that direct determinations of equilibrium constants involving thermally labile molecules in the gas phase is impossible according to either of these principles. The kinetic method appears to provide a solution to this problem. It was first developed by Cooks and co-workers[3–6] for the determination of proton affinities (E_{pa}), and depends on a mathematically simple relationship between fragment ion abundances from unimolecular decompositions and the enthalpy changes associated with the fragmentations. However, the theory behind this method has well-recognized weaknesses, and the objectives of this paper are (1) to give a better foundation to the understanding of the kinetic method and (2) to apply the method to the determination of the E_{pa} of α-amino acids as examples of polar compounds, that are not readily studied with equilibrium methods.

Experimental

Proton bound cluster ions of amines and amino acids were generated by fast atom bombardment (FAB) using a Kratos MS 50RF mass spectrometer with the electric sector (E) before the magnet (B). The FAB gun was operating at 9 kV and the bombarding gas was Xe (Messer-Griesheim 99.99%). The resolving power of the mass spectrometer was set to 1200 (10% valley definition) and the accelerating voltage 8 kV. The post accelerating detector voltage was 15 kV. A linked scan (B/E) was used to observe fragmentations of metastable ions in the first field free region between the source and the electrostatic sector.

The α-amino acids were racemic mixtures from Sigma except for L-cysteine and L-glutamine. The matrix was a 75% solution of trichloroacetic acid in 87% aqueous glycerol. The cluster ions were generated from saturated solutions of the amino acids in the matrix with *ca.* 1 μl amine added to a volume of 150 μl. Formation of an insoluble salt was occasionally observed when the amine was added to the amino acid solution. This was removed by centrifugation, as its presence on the target gave rise to unstable ion-currents. The amines were analytical or synthetic grade from various suppliers.

A normal mass spectrum was recorded to check for the presence of the required cluster ion as well as to ensure the absence of interfering ions. The generally poor resolution of the parent ion under linked scan conditions means that fragment ions from parents in a window of approximately 3 Da are observed. The fragment ion spectra were recorded by a linked scan (B/E) at 10 s decade^{-1}. Typically 14–16 scans were added as non-centroided data and mass assigned according to a calibration on a mixture of LiI and CsI. The areas of the peaks were calculated after smoothing.

The necessary proton affinity values for the reference amines (except tetramethylguanidine) were taken from the tables published by Aue and Bowers.[7] The amines, with E_{pa} in parentheses, were the following: allylamine (216.5), ethylamine (217.1), propylamine (218.5), butylamine (219.0), pentylamine (219.6), hexylamine (220.1), *sec*-butylamine (220.5),

cyclohexylamine (221.2), *tert*-butylamine (221.3), norbornylamine (221.7), *tert*-pentylamine (222.3), diethylamine (225.1), dipropylamine (227.4), dibutylamine (228.4), diisopropylamine (228.9), di-*sec*-butylamine (230.9), triethylamine (231.2), tributylamine (234.8) and tetramethylguanidine (242.8).[8] Model calculations of relative ion abundance were done on a Macintosh computer with a curve-fitting and plotting program (Passage F).

Theory

The kinetic method is based on the measurement of relative abundances of fragment ions produced by metastable or collision induced dissociation of ion-bound clusters. Taking proton affinities as the example, the method is based on the observation that for closely related bases A^1 and A^2 the relationship (2) can be obtained: I_1 and I_2 are the abundances of

$$\ln (I^1/I^2) = [E_{pa}(A^1) - E_{pa}(A^2)]C \qquad (2)$$

A^1H^+ and A^2H^+ in a fragment ion spectrum of $A^1A^2H^+$, E_{pa} refers to the proton affinities and C is a constant. If one of the amines is kept constant and the other is varied eqn. (2) will represent a straight line.

The reactions which are observed are given in eqn. (3).

$$A^1{-}H{-}A^2H^+ \xrightarrow{k_1} A^1{-}H^+ + A^2 \qquad (3a)$$

$$A^1{-}H{-}A^2H^+ \xrightarrow{k_2} A^1 + A^2{-}H^+ \qquad (3b)$$

The rationalization of the observed simple relationship between proton affinity and ion abundances given by eqn. (2) has usually been based on four assumptions.[5,9] (1) The reverse activation energies for the reverse reactions in eqn. (3) are negligible. (2) The entropy effects can be ignored. (3) The rate constants can be described by the well-known equation from transition state theory, eqn. (4). And (4) the ratio of the rates in

$$k(T) = \frac{RT}{h}\frac{Q^*}{Q}\exp\left(\frac{-E_0}{RT}\right) \qquad (4)$$

eqn. (2) is equal to the ratio of the measured fragment ion abundances, eqn. (5).

$$\ln\frac{I_2}{I_1} = \ln\frac{k_2(E)}{k_1(E)} = \ln\frac{k_2(T)}{k_1(T)} \qquad (5)$$

The weaknesses of this rationalization are recognized. Firstly, eqn. (4) implies that the reacting ions are in thermal equilibrium. This is clearly not the case for isolated ions undergoing metastable or collision induced fragmentations. Secondly, an identification of ion abundance ratios with rate constant ratios [eqn. (5)] ignores the fundamental difference between micro- and macro-scopic rate constants, and the complicated interplay of ion energetics and instrumental parameters that determine relative ion abundances. This second weakness is also a problem in alternative rationalizations of eqn. (2).[10]

In the following it will be shown that the simple relationship expressed by eqn. (2) can be expected without assuming the precursor ions to be in thermal equilibrium, but is to be expected when the rates are calculated from reasonable estimates of ion energetics and instrumental parameters which determine the ion abundances.

It is a prerequisite for application of the kinetic method that only two different reaction pathways are available to the precursor ion; *e.g.* (3a) and (3b). The abundances of the fragment ions can be calculated from expressions (6a) and (6b).

$$I_1 = \int_E F(E)P_1(E)\,dE \qquad (6a)$$

$$I_2 = \int_E F(E)P_2(E)\,dE \qquad (6b)$$

$F(E)$ is a function describing the potential energy distribution of the precursor ion population, and $P_1(E)$ and $P_2(E)$ are functions describing the probabilities of fragmentation *via* (3a) and (3b). For metastable precursor ions $P_1(E)$ and $P_2(E)$ can be calculated from expressions (7a) and (7b).[11,12] $k_1(E)$ And $k_2(E)$

$$P_1(E) = \frac{k_1(E)}{k_1(E) + k_2(E)}\{\exp\{-t_1[k_1(E) + k_2(E)]\} - \exp\{-t_2[k_1(E) + k_2(E)]\}) \qquad (7a)$$

$$P_2(E) = \frac{k_2(E)}{k_1(E) + k_2(E)}\{\exp\{-t_1[k_1(E) + k_2(E)]\} - \exp\{-t_2[k_1(E) + k_2(E)]\}) \qquad (7b)$$

are the rate constants for reactions (3a) and (3b). t_1 and t_2 are the times after ionization for entrance and exit from the field free region where the reaction is observed. For reactions occurring in the first field free region t_1 is equal to the source residence time plus the time to pass the accelerating region. The source residence time is not known for the FAB source of a Kratos MS50 instrument. We have used a constant value of 2 μs. Calculations of source residence times in an AEI MS9 gives values of this order, and the similarity of the sources makes it reasonable to apply a similar value for the MS50 FAB source.[13] A source residence time of 2 μs is much longer than the time for passing the accelerating region and hence t_1 can be equated with the source residence time. t_2 can be calculated from t_1 and the flight-time through the first field free region.

According to the quasi equilibrium theory (QET) the rate constants can be calculated from the expression[14,15] (8), where ν is a frequency factor, which for simple bond cleavages can be

$$k(E) = \nu\left(\frac{E - E_0}{E}\right)^{(s-1)} \qquad (8)$$

equated with the vibrational frequency of the bond that is broken in the reaction. E is the potential energy of the precursor ions. E_0 is the critical energy for the fragmentation reaction, and s is the number of oscillators in the precursor ion. Such a simple expression is inferior to a proper RRKM treatment for calculations of absolute rates.[16,17] However, its very simplicity makes it well suited for qualitative arguments, and successful calculations of a number of mass spectra, including metastable ion abundances, have been based on this expression.[12,18–21]

The assumption that the activation energies of the reverse reaction in eqn. (3) are zero is the same as setting the activation energies equal to the difference in the heat of formation of the products and the reactants, *i.e.* the reaction enthalpy. Hence the critical energies E_1 and E_2 for reactions (3a) and (3b) can be calculated from molecular pair proton affinities (E_{mppa}) and E_{pa} as follows.

The proton affinities $E_{pa}(A^1)$ and $E_{pa}(A^2)$ for the bases A^1 and A^2, are equal to the ΔH for the reactions (9a) and (9b). The

$$A^1H^+ \longrightarrow A^1 + H^+ \qquad (9a)$$

$$A^2H^+ \longrightarrow A^2 + H^+ \qquad (9b)$$

molecular pair proton affinity [$E_{mppa}(A^1A^2)$] for the bases A^1 and A^2 is equal to ΔH for reaction (10). Subtraction of eqn.

$$A^1A^2H^+ \longrightarrow A^1 + A^2 + H^+ \qquad (10)$$

(9a) and eqn. (9b) from eqn. (10) gives eqn. (3a) and eqn. (3b), and hence the critical energies E_1 and E_2 can be calculated from expressions (11a) and (11b). E_{mppa} have been measured for a

$$E_1 = E_{mppa}(A^1A^2) - E_{pa}(A^1) \qquad (11a)$$

$$E_2 = E_{mppa}(A^1A^2) - E_{pa}(A^2) \qquad (11n)$$

number of amines, and it is found that they can be calculated from eqn. (12), where E_s is a constant generally close to 20.9 kcal

$$E_{mppa}(A^1A^2) = E_s + \frac{E_{pa}(A^1) + E_{pa}(A^2)}{2} \qquad (12)$$

mol^{-1}.[7] E_s can be called the intrinsic well-depth and is equivalent to the intrinsic barrier for exchange reactions solution.[22] Insertion of (12) into (11a) and (11b) gives eqns. (13a) and (13b).

$$E_1 = E_s - \frac{E_{pa}(A^1) - E_{pa}(A^2)}{2} \qquad (13a)$$

$$E_2 = E_s + \frac{E_{pa}(A^1) - E_{pa}(A^2)}{2} \qquad (13b)$$

The only missing parameters are now the frequency factor ν and the number of oscillators: $s - 1$ in the metastable precursor ion. The QET expression [eqn. (8)] gives the best agreement with experiments when these parameters are treated as variables.[12] For a simple bond fission a frequency factor of approximately 10^{13} s^{-1} can be assumed, and for metastable fragmentations, which occur close the threshold, the number of oscillators is often reduced by a factor of 3–5, so that the exponent $s - 1$ in eqn. (8) is replaced by $(s - 1)/5$.

The thermodynamic cycles used above in the calculations of activation energies for fragmentation of a protonated dimer, are illustrated in Fig. 1. Since E_{pa} values are known for a number of amines the $P(E)$ functions for decomposition of a given protonated cluster can now be calculated from the eqns. (7), (8) and (13). For the calculations of relative ion abundances [eqn. (6)] the potential energy distributions, $F(E)$ functions, must now be considered. However, at this stage it is useful to examine the $P(E)$ functions. In Figs. 2 and 3 are shown the $P(E)$ functions for fragmentation of a protonated dimer of pentylamine and isopropylamine into either protonated pentylamine or protonated isopropylamine. In the Fig. 3 the number of oscillators has been reduced by a factor of 5. It is noticeable that the $P(E)$ functions for the two competing reactions are very similar, and that their maxima occur at nearly the same potential energy. In Fig. 2 the maxima occur at 118.57 kcal mol^{-1} (formation of pentylamine H^+) and 118.64 (formation of isopropylamine–H^+). In Fig. 3 they occur at 33.66 and 33.69 kcal mol^{-1}. This is important when the significance of the $F(E)$ is considered.

It is a general problem in the study of unimolecular chemistry of ions in the gas phase with a sector instrument, that it is difficult to get hold of the energy distributions of the isolated ions. In the present context it is important that ion-bound dimers usually are generated by chemical ionization or fast atom bombardment and not by electron ionization (EI) of isolated molecules. It is often assumed that ions produced by EI

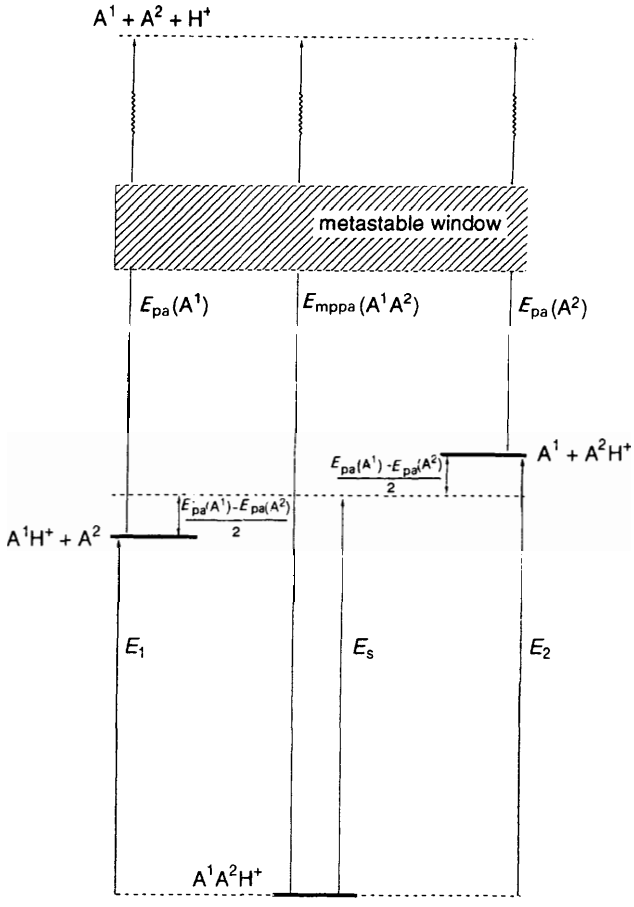


Fig. 1 Illustration of the thermodynamic cycles used to calculate the reaction enthalpies for fragmentation of a protonated dimer. The metastable window illustrates the internal energy range of the fragmenting metastable ions (see Fig. 2). E_s is the average well-depth for proton bound amine dimers. See the text for details.

have energy distribution functions, $F(E)$, that are qualitatively similar to photoionization spectra.[15,23] This means, that they can change very sharply within a narrow energy range. Since source reactions will deplete the population of ions with high potential energies, the effective energy distributions of the metastable ions will be different from that of the ions formed in the source.[24] However, any abrupt changes or discontinuities in the energy distributions will be preserved, and consequently a small shift of fractions of a kcal mol^{-1} in the position of the $P(E)$ function may give a large difference to the number of ions available for reaction, and hence in the relative fragment ion abundances. With CI or FAB the initial potential energy distributions of the ions, and hence also the effective energy distribution of the metastable ions, will be very different from photoionization spectra. For both methods the ions arise from regions of high pressure where frequent collisions and ion–molecule reactions can take place. For CI this region is the source, and for FAB it is the site of the initial impact and the volume above. The energy distributions of metastable ions coming from a CI or FAB ion-source will not be thermal, but it is reasonable to assume that they will be smooth and well-behaved, and without the sharp changes that could be expected if the ions were produced by EI. With this assumption, a change of a fraction of a kcal mol^{-1} in the position of the $P(E)$ function for the precursor ion population will not significantly change the number of ions that can react. Hence the ratio of relative ion abundances calculated from eqn. (6), will not significantly depend on the $F(E)$ functions. In the following calculations on model systems we have chosen to ignore it, and $F(E)$ is set to 1 independently of the energy of the ion.

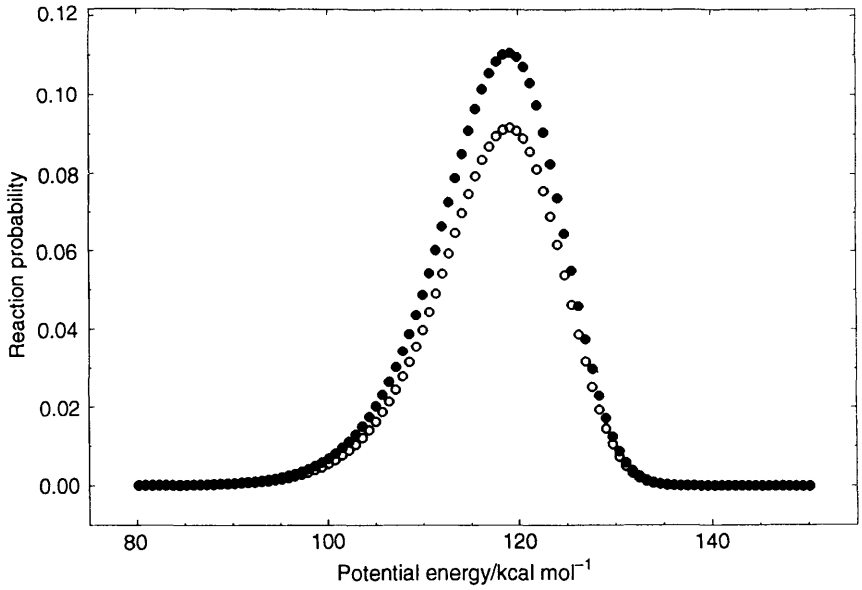


Fig. 2 Reaction probabilities for pentylamine–isopropylamine–H^+: ●, formation of pentylamine–H^+; ○, formation of isopropylamine–H^+. The E_{pa} of pentylamine and isopropylamine are 216.9 and 219.4 kcal mol^{-1}. From eqn. (13) the activation energies for the formation of pentylamine–H^+ and isopropylamine–H^+ can be calculated as 20.8 and 21.0 kcal mol^{-1}. With a total of 33 atoms s^{-1} becomes equal to 92. The frequency factors for both reactions were set to 10^{13} s^{-1} (333.7 cm^{-1}).

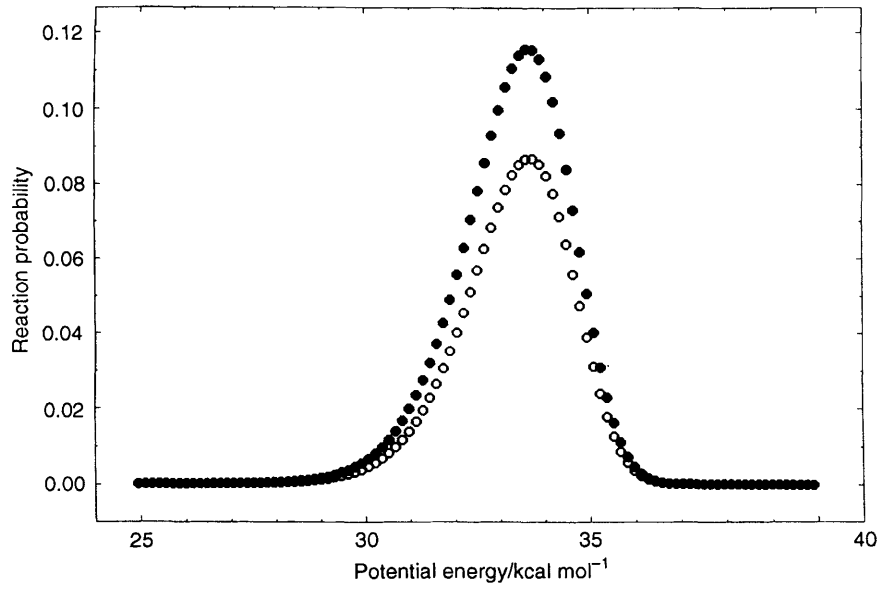


Fig. 3 Reaction probabilities for pentylamine–isopropylamine–H^+. The number of oscillators has been reduced by a factor of five. All other parameters as for Fig. 2.

The reduction in the number of oscillators by a factor of five is arbitrarily chosen to see the effect on the calculated ratio of fragment ion abundances. As can be seen from Fig. 3 this reduction in the number of active oscillators narrows the metastable window and moves it closer to the fragmentation thresholds.

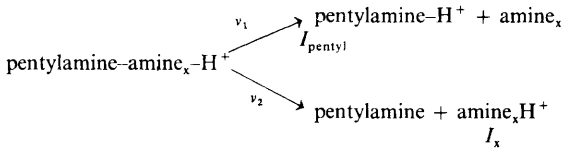
With E_{pa} values from the equilibrium experiments the relative fragment ion abundances from competitive unimolecular fragmentation of metastable ions with the general structure pentylamine–amine$_x$–H^+ have been calculated, and the results are given in Table 1 The results in the first column were calculated with the full number of oscillators, with an intrinsic well-depth of 20.9 kcal mol^{-1} and with the same frequency factor for the two competitive reactions. For the results in the second column the number of oscillators was reduced. The numbers in the third column were obtained with a reduced frequency factor for formation of pentylamine–H^+ and for the fourth column the intrinsic well-depth (E_s) was set to 22.9 kcal mol^{-1}. The data in Table 1 are shown in Fig. 4 with linear least-square fits.

That the calculated points fit so well to a linear regression, shows the validity of eqn. (2). For the kinetic method to be valid the regression lines should cross the x-axis at the E_{pa} value for pentylamine. The fitted lines (*a*), (*b*) and (*d*) in Fig. 4 give values within 0.05 kcal mol^{-1} of the 219.6 kcal mol^{-1}, which is the E_{pa} value of pentylamine from equilibrium measurements that was used in the calculations.[7] For line (*c*) the frequency factor for formation of pentylamine–H^+ has been reduced from 10^{13} to 10^{12} s^{-1}. This results in a lower and incorrect apparent E_{pa} for pentylamine (218.0 kcal mol^{-1}).

A lower frequency factor is the same as a higher activation entropy for the reaction. Thus fragmentations associated with opening of rings or rearrangements, where breaking or formation of several bonds is involved, have lower frequency factors than simple bond fissions.[15]

In principle the rationalization of the kinetic method given above only applies when the ion-bound dimers decompose as metastable ions. It can potentially be extended to collisionally activated ions if the collision region and the fragmenting region

Table 1 Calculated logarithms to fragment ion abundance ratios from decompositions according to



Amine$_x$	E_{pa}(x)	$\ln \frac{I_x}{I_{pentyl}}$ [a]	$\ln \frac{I_x}{I_{pentyl}}$ [b]	$\ln \frac{I_x}{I_{pentyl}}$ [c]	$\ln \frac{I_x}{I_{pentyl}}$ [d]
c-Hexyl	221.3	1.633	2.365		
tert-Butyl	221.3	1.645	2.523	4.787	2.275
Decyl	220.7	1.025	1.325		
sec-Butyl	220.5	0.863	1.291	3.568	1.176
Hexyl	220.1	0.472	0.659		
Isobutyl	219.5	−0.0956	−0.142	2.168	−0.129
Isopropyl	219.4	−0.193	−0.296		
Butyl	219.0	−0.574	−0.855	1.512	−0.789

[a] Full number of oscillators calculated from $s = 3N - 6$, where N is the number of atoms in the metastable precursor ion: pentylamine–amine$_x$–H$^+$. $\nu_1 = \nu_2 = 10^{13}$ s^{-1}. [b] Number of oscillators reduced by a factor of five, $\nu_1 = \nu_2 = 10^{13}$ s^{-1}. [c] As (*b*) but with $\nu_1 = 10^{12}$ s^{-1} and $\nu_2 = 10^{13}$ s^{-1}. [d] As (*b*) but with $E_s = 22.9$ kcal mol^{-1}.

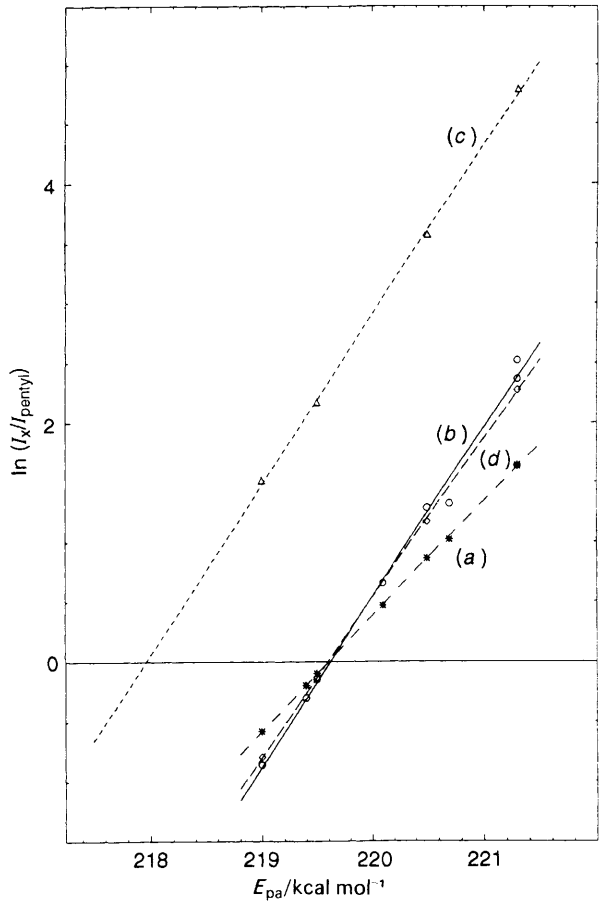


Fig. 4 Graphical representation of the data in Table 1. The lines are least-square fits.

are separated. If the fragmenting region is situated immediately after the collision cell, the observed fragmentations can be much faster and this may give broader $P(E)$ functions. The changes in the energy distribution functions, $F(E)$, upon collision activation may also have to be considered.

Other features of this approach to the kinetic method will be explored in the discussion section.

Results

The relationship between ion abundances and proton affinities expressed by eqn. (2) can be used in E_{pa} determinations in two different ways. One possibility is to construct a calibration curve based on comparisons of a reference base with a number of other bases of known E_{pa} and subsequently compare the reference base with the unknown. This method suffers from the disadvantage that the determination of the unknown E_{pa} depends on the behaviour of a single cluster ion. For this reason we have chosen to compare each amino acid with several amines.

How well eqn. (2) is followed for protonated dimers generated by FAB is shown in Fig. 5. In this figure the experimental data for unimolecular fragmentations of pentylamine–amine$_x$–H$^+$ dimers are shown. It would be possible to fit the calculated lines (Fig. 4) to the experimental results by changing the frequency factor or the extent of reduction in the number of active oscillators, but since also the source residence time is at best an estimate, the fitting of so many parameters is unlikely to yield much useful information.

The measurements give a value of 220.1 kcal mol^{-1} for the E_{pa} of pentylamine. Comparison with the value 219.6 kcal mol^{-1} from equilibrium studies illustrates the degree of accuracy that can be expected from application of the kinetic method when the ion-bound dimers are generated by FAB.

The data from analyses of protonated clusters with the general structure α-amino acid–amine$_x$–H$^+$ are shown in Fig. 6. Each amino acid has been compared to at least three different amines, and three or four measurements have been done on each protonated cluster. The E_{pa} of the 17 α-amino acids, which were obtained from the linear regressions, are listed in Table 2. It was not possible to determine E_{pa} for arginine but comparisons with tributylamine and tetramethylguanidine, indicate a value that is higher than for either of these compounds.

Discussion

Model Calculations.—The rationalization of the kinetic method provided by the model calculations summarized in Table 1 and Fig. 4 depends on the validity of the assumptions behind the calculations. The significance of some, but not all of them, can be assessed by comparing the different calculations.

An important question is to what extent the simplified QET formula [eqn. (8)] is valid. This is the same as asking to what extent the QET expression can reproduce the energy dependence of a rate constant that is calculated on a higher level of

Table 2 Comparison of E_{pa} values for 17 amino acids[a]

Amino acid	This work	Gorman *et al.*[a]	Li and Harrison[b]	Meot-Ner *et al.*[c]
Ser	217.2	214.1–214.8		
Val	218.1	214.8–217.1	215.0	216.6
Asp	218.1	214.1–214.8		
Leu	218.7	217.1–219.4	216.1	217.2
Ile	219.2	217.1–219.4	216.8	
Thr	219.2	220.4–221.3	216.6	
Phe	219.9	219.4–220.4	217.4	217.8
Tyr	220.7	219.4–220.4	217.9/217.7	
Met	221.0	220.4–221.3	218.0/218.4	
Asn	222.1	219.4–220.4	217.8/219.6	
Glu	222.3	224.3–225.1		
Pro	222.4	221.3–224.3	218.5/220.5	221.1
Trp	223.5	221.3–224.3	220.8	
Gln	226.9	220.4–221.3	221.4	
Lys	228.7	225.1–227.4	222.9	
His	230.5	220.4–221.3		
Arg	> 242.8	> 235.0		

[a] From ref. 29, the data have been adjusted to the E_{pa} values from ref. 7. [b] From ref. 32, the data have not been adjusted. [c] From ref. 31, the data have been adjusted to the E_{pa} values from ref. 7.

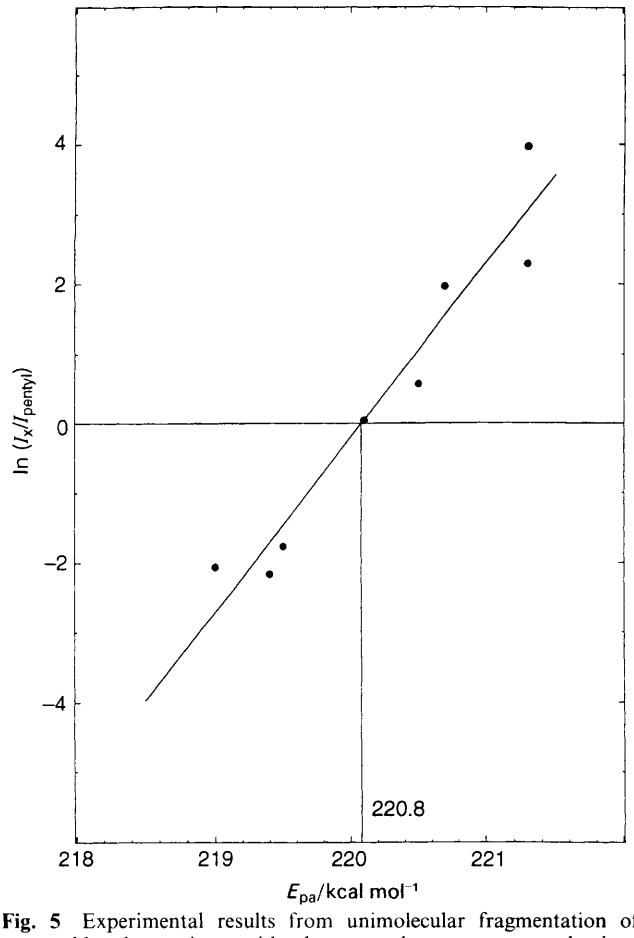


Fig. 5 Experimental results from unimolecular fragmentation of metastable cluster ions with the general structure pentylamine–isopropylamine–H^+

theory *e.g.* RRKM theory, in which the rate constants are calculated through a sum over states.[16,17] The only way to answer this question is to perform RRKM calculations on ion-bound dimers. For the present, the best argument for the simplified QET expression is the success with which it has been used in the past, in particular by Williams and his collaborators, to calculate mass spectra, including their energy dependence, abundance of metastable ions *etc.*[12,19,21]

The model calculations indicate that the linearity of the calibration curves will depend on the extent to which the number of oscillators is reduced. The linearity of the measured calibration curves (Fig. 6) suggests that for a given α-amino acid the number of active oscillators in the metastable precursor ions does not depend on the amine or in other words that the excess energy for the various metastable amino acid–amine$_x$–H^+ ions is the same for a given α-amino acid. However, it is not clear how a change in the number of active oscillators will affect a real reaction, but the question is clearly not very important for the validity of method.

What about the use of the simple additivity scheme to calculate the reaction enthalpies? Firstly it must be noted, that the rationalization of the kinetic method given here is based on the assumption that the critical energies for the decompositions are equal to the reaction enthalpies. The simple additivity scheme is an approximation of Marcus' theory,[25] and comparison with more complicated schemes, including the Marcus equation, suggests that the simple additivity scheme will give valid results, as long as the difference in E_{pa} of the amines in the protonated dimer is small.[22] The small differences obtained when the intrinsic well-depth is changed from 20.9 to 22.9 kcal mol^{-1} also support this approximation. That well-depths for proton-bound dimers can be calculated in such a relatively simple way is supported by calculations carried out on proton-bound alkoxide ions by Brauman and his collaborators,[26] who have shown that there is a reasonably good agreement between the well-depths calculated from Marcus' theory and RRKM theory.

The crucial question to be answered, when the validity of the kinetic method is considered, is clearly to what extent the frequency factors for the two competing reactions are the same. Consider decomposition of ions with the general structure amino acid–amine$_x$–H^+. If formation of the protonated amino acid proceeds with a lower frequency factor (the reaction leading to A^1H^+ and A^2 in Fig. 7), the measured E_{pa} will be too low [line (*c*) in Fig. 4], whereas it will be too high if it is the formation of the protonated amine that is associated with the low frequency factor. The present study cannot exclude that this will be the case for some of the investigated cluster ions. However, since it would be difficult to fit a line to points from line (*c*) and either line (*a*) or (*b*) in Fig. 4, the good linear fits of the data summarized in Fig. 6 indicate that, for a given amino acid, the frequency factors for the competitive reactions of amino acid–amine$_x$–H^+ are independent of the amine.

Experimental Results.—The proton affinities obtained here agree well with the order of those determined in earlier studies in

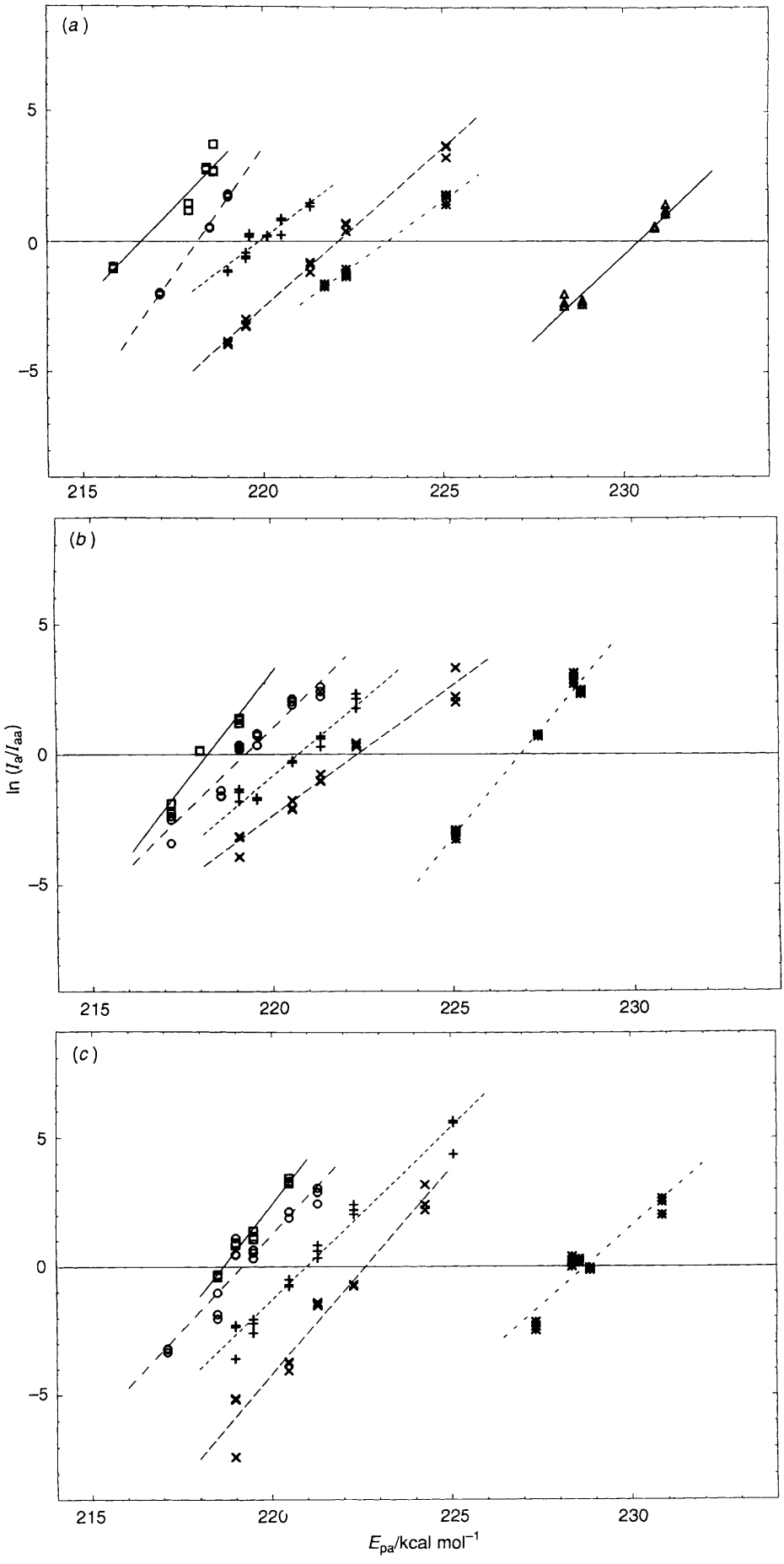


Fig. 6 Experimental results from unimolecular fragmentaton of metastable cluster ions with the general structure α-amino acid–amine$_x$–H$^+$. I_{aa} and I_a refer to relative abundances of protonated amino acid and amine, respectively.

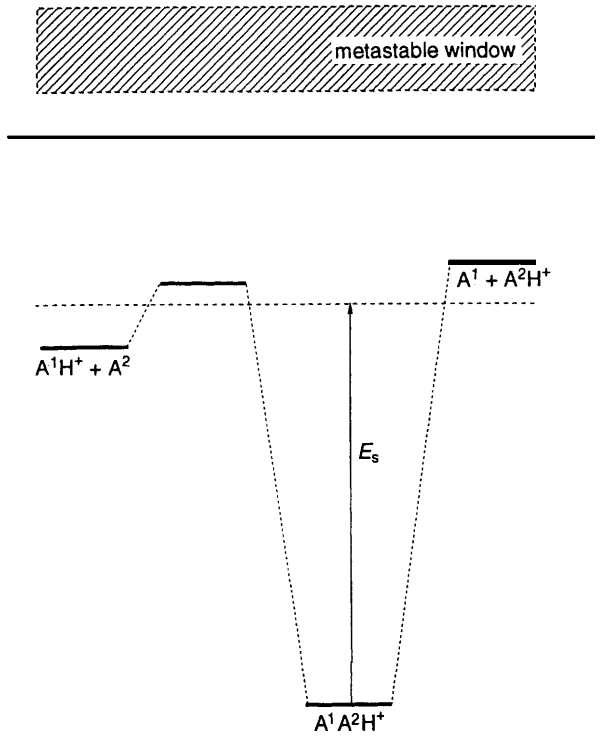


Fig. 7 Potential energy profile with a large reverse activation energy for one of the decomposition reactions. Notice that the overall reaction enthalpies may still follow eqn. (13).

which the α-amino acids were compared pairwise.* The order of Asp and Leu has changed compared with the earlier work. But the earlier order depended on a measurement on the fragmentation of a single protonated dimer (Asp-Leu–H^+) and even when it has been confirmed by others in a similar experiment,[27] the result of the present work must be more reliable. There is also a difference concerning proline. However, the earlier result was obtained by MIKE analysis without post acceleration detection, and it was assumed that the yield of secondary electrons was proportional to the energy, or mass, of the fragment ion.[28,29] We now believe that the mass discrimination in the detection system for instrumental reasons was stronger than that. Owing to the low molecular weight of proline compared to the other amino acids with high proton affinities, the proline–H^+ ions will give a relatively lower yield of secondary electrons. The present work was obtained with a post acceleration potential at 15 kV of fragment ion with kinetic energies in the order of 4 keV (half the kinetic energy of the precursor ions) and this should eliminate any significant mass discrimination arising from the mass-dependent yields of secondary electrons in the multiplier.

Gorman *et al.*[30] have published a study on the E_{pa} of α-amino acids wherein they employ a bracketing technique with laser desorption of the α-amino acids in an FTICR cell. FTICR results are also available for Gly and Ala.[31] Earlier equilibrium measurements have been done by Meot-Ner *et al.*[32] with a pulsed high-pressure mass spectrometer. The kinetic method has also been used by Li and Harrison[33] They have studied the fragmentations of cluster ions of some of the α-amino acids and ethylamine or dimethylamine at different collision energies in a collision quadrupole. Their results are in good agreement with the data presented here, as are the order of E_{pa} obtained by Wu and Fenselau.[27] The data are all summarized in Table 2. The most significant disagreements are between the result presented here and those obtained by Gorman *et al.* The order of E_{pa} is generally the same, but discrepancies are noticeable for the α-amino acids Thr, Asp, Asn, Glu, Gln and His. Gorman *et al.* find that Thr has a E_{pa} at least 5.6 kcal mol^{-1} above that of Ser. In the present work the difference obtained is 2 kcal mol^{-1}. The difference measured by Gorman *et al.* seems very large in view of the small structural difference between Ser and Thr, which consists of an extension of the carbon chain from three to four carbon atoms. In comparison the difference in E_{pa} of propyl- and butyl-amine is 0.5 kcal mol^{-1}. This is a difference of the same magnitude as measured between Val and Ile which also differ in structure by one CH_2-group. For these two α-amino acids the present work indicates a difference in the E_{pa} of 1.1 kcal mol^{-1} and the results by Gorman *et al.* a difference smaller than 2.3 kcal mol^{-1} and greater than the uncertainty of the measurements.

In view of the results from the model calculations, the most attractive way to account for the discrepancies between the kinetic and equilibrium results is to invoke an energy profile such as shown in Fig. 7, where one of the fragmentation reactions occurs with a significant reverse activation energy. Thus the high E_{pa} of histidine measured by the kinetic method could be explained by formation of $HisH^+$ from His–$amine_x$–H^+ by a reaction that has a much smaller reverse activation energy than the formation of $amine_x$–H^+ from the same precursor ion. If the formation of $HisH^+$ requires the opening or the formation of a ring, the reaction could possibly follow an energy profile of this kind. However, this is not consistent with other comparisons between the two sets of measurements. Thus the kinetic measurements and the FTICR results, both indicate that Asn has a higher proton affinity than Asp. However, for the homologous pair Glu and Gln, the FTICR measurements have the order reversed and assign the highest affinity to Glu. It is suggested by the authors that protonated Glu has a cyclic structure, whereas the protonated forms of Asp, Asn and Gln are linear, and that this may account for the discrepancy between the FTICR and the kinetic results. Comparison with the well-characterized structures of protonated α,ω-diaminoalkanes, indicate that Lys also may form a ring upon protonation.[34,35] However, the kinetic and FTICR measurements are in fair agreement on the proton affinity of Lys (228.7 *vs.* 225.1–227.4 kcal mol^{-1}), which indicate that the fragmentations of the Lys–$amine_x$–H^+ cluster ion into either Lys–H^+ or $amine_x$–H^+ is in agreement with the assumption in the kinetic method, *i.e.* the entropy effects and reverse activation energies can be neglected. Consequently it seems to be difficult to account for the discrepancies between the kinetic results and the bracketing results with regard to His, Glu and Gln, solely by reference to the structures of the protonated α-amino acids.

Conclusions

Based on a simplified version of the quasi-equilibrium theory (QET), a rationalization of the kinetic method based on analysis of metastable ions has been provided. It is based on calculation of well-depths for proton-bound dimers of amines from a simple additivity scheme. This is an approximation of Marcus' theory, which has been confirmed in experimental studies and it is likely that similar schemes are valid for other ionized dimers although fewer have been investigated in detail. Calculations of activation energies from Marcus theory lead to linear free energy relationships (LFERs) for molecules in thermal equilibrium, where the macroscopic rate-constants can be calculated from activated complex theory. It is noticeable that Marcus' theory can lead to a similar kind of expression for unimolecular reactions of isolated ions in the gas-phase, when the microscopic

* The increasing order of E_{pa} was found to be Gly, Ala, Cys, Ser, Val, Asp, Leu, Thr, Ile, Phe, Met, Tyr, Asn, Pro, Glu, Trp, [Gln, Lys], His, Arg.[37]

rate constants are calculated from the very different simplified QET expression.

The principal advantage of a rationalization of the kinetic method based on quasi-equilibrium theory, compared to one based on activated complex theory, is that any reference to a thermodynamic temperature is avoided. Thus it reinforces the validity of the kinetic method.

The calculations on model systems show that the crucial factor for the validity of results obtained with the kinetic method by analysis of metastable ions is the frequency factor or activation entropy for the competing unimolecular reactions. When the proton affinities for the 17 α-amino acids obtained by the kinetic method are compared with results from bracketing experiments on FTICR instruments, the agreement between the two sets of data is generally good when the accuracies of the methods are taken into account. The ion–molecule reactions in the FTICR cell passes across the same potential-energy well, from which the protonated cluster examined in the kinetic experiment originates. The work by Brauman[26,36] has shown, that even such relatively simple processes as proton exchange reactions between alkoxide ions are difficult to explain in detail. A more detailed characterization of protonated dimers and clusters of bidentate amines and the dynamics of their decomposition is clearly necessary, before the relative merit of results obtained by the kinetic method and by the FTICR bracketing experiments can be determined.

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