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Chemical Ionization of Amino Acids

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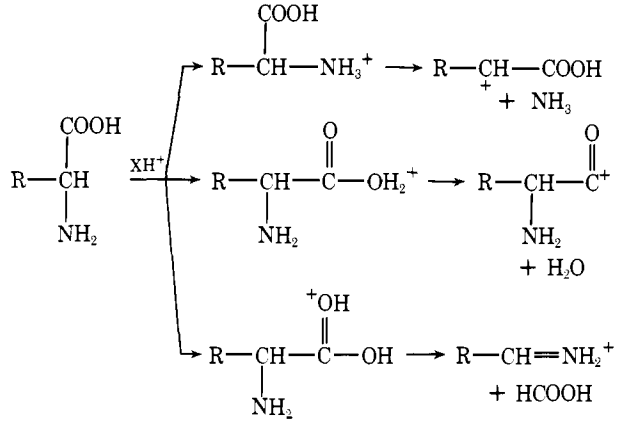
Abstract: The H_2 chemical ionization (CI) mass spectra of 14 α-amino acids have been determined and compared with the CH_4 CI mass spectra. The H_2 CI spectra show a much lower MH^+ abundance and increased abundances of fragment ions formed by sequential fragmentation of MH^+. The latter has potential advantages in determining the structure of R in $RCH(NH_2)CO_2H$. The CH_4 and H_2 CI mass spectra of β-alanine, 3-aminobutyric acid, 4-aminobutyric acid, and 6-aminohexanoic acid also have been determined. For the β-amino acids, the dominant fragmentation of MH^+ is sequential loss of H_2O and CH_2CO, while, for the terminal amino acids, MH^+ fragments by loss of both NH_3 and H_2O. By using CD_4 as reagent gas it has been found that the peak previously attributed to loss of H_2O from MH^+ in the CH_4 CI of α-amino acids arises instead by $C_2H_5^+$ addition followed by the loss of the elements of formic acid. When R in $RHC(NH_2)CO_2H$ contains a hydroxyl (including carboxyl) substituent loss of H_2O from MH^+ is observed in addition to the ethyl ion addition reaction. Using both CD_4 and D_2 as reagent gases, it has been found that there is substantial deuterium retention in the ions originating from fragmentation of MD^+. The results are not consistent with protonation at a specific site with the subsequent fragmentation being determined by the site of protonation, but rather indicate that there is extensive intramolecular proton transfer in MH^+ prior to fragmentation.

Some years ago, Milne et al.[1] reported the methane chemical ionization (CI) mass spectra of the biologically important α-amino acids. They interpreted the observed spectra in terms of protonation at a specific site followed by fragmentation reactions determined by the site of protonation, as outlined in Scheme I. The loss of NH_3 was observed

Scheme I



only when R contained a functional group capable of backside attack at the site of the amine function resulting in stabilization of the fragment ion formed. In general, they found that the CH_4 CI spectra gave abundant MH^+ ions,[2] confirming the molecular weight,[1] M, and abundant ($MH^+ - COOH_2$) ions (i.e., $RCH\text{-}NH_2^+$), providing the mass of R. However, they observed insufficient fragmentation to reveal details concerning the structure of R. Leclerq and Desiderio[3] also have examined the CH_4 CI of amino acids and derivatives, paying particular attention to the formation and subsequent fragmentation of cluster ions. From comparisons of the mode of fragmentation of the various cluster ions, they suggested that at least part of the ion signal attributed by Milne et al.[1] to H_2O loss from MH^+ arose instead by ethyl ion addition followed by loss of the elements of formic acid. Such a process would yield an ion isobaric with the ($MH^+ - H_2O$) ion, i.e., $(M - 17)^+$.

More recently, Meot-Ner and Field[4] have studied the temperature dependence of the isobutane CI of a number of α-amino acids. They observed mainly formation of the protonated molecule, MH^+, with only a slight amount of fragmentation. No peak corresponding to loss of H_2O from MH^+ was observed and this was rationalized in terms of initial protonation at the amine function with insufficient energy available for proton transfer to the hydroxyl group, a necessary prerequisite for water elimination. They did observe a fragment ion corresponding to loss of the elements of formic acid from MH^+. The temperature dependence of the relative ion intensities of ($MH^+ - COOH_2$) and MH^+ indicated a low Arrhenius A factor for the reaction leading to this fragment and this was interpreted in terms of a doubly cyclic intermediate, a, in the reaction leading to the loss of formic acid.

The present study of the chemical ionization of amino

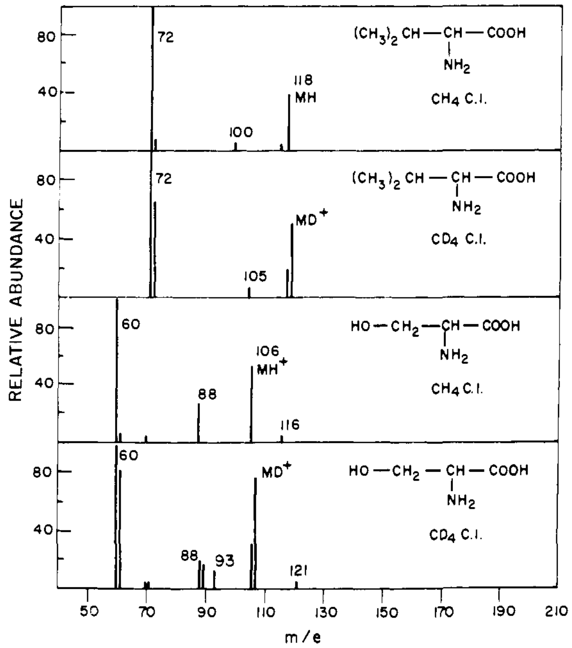
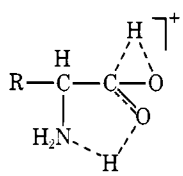


Figure 1. CH_4 and CD_4 CI mass spectra of valine and serine.



a

acids had three main aims. First, by using CD_4 as reagent gas, we have determined the relative importance of the ethyl ion addition reaction proposed by Leclerq and Desiderio[3] compared to loss of H_2O from MH^+. Second, we have determined the H_2 CI mass spectra of a representative selection of amino acids to determine whether the strongly exothermic protonation by H_3^+ would promote more fragmentation than is observed in the CH_4 CI and, thus, might provide additional information relevant to the structure of the R group in $RCH(NH_2)CO_2H$. Finally, the use of CD_4 and D_2 as reagent gases has allowed us to determine the importance of intramolecular proton shifts in MH^+ prior to fragmentation. As discussed above, previous workers have proposed protonation at specific sites without proton or hydrogen interchange in the MH^+ ion prior to fragmentation.

Experimental Section

The chemical ionization mass spectra were determined using a Dupont 21-490 mass spectrometer equipped with a high-pressure chemical ionization source. Further details concerning the operation of the mass spectrometer and the reagent gases used are given elsewhere.[5] The source temperature was varied between 150 and 250 °C, depending on the temperature necessary to volatilize the sample from the solid probe. Samples were introduced into the source from the solid probe system with the probe temperature used being between 125 and 225 °C; in each case the minimum temperature necessary to achieve adequate ion currents was used. For each amino acid the H_2, D_2, CH_4, and CD_4 CI spectra were obtained under identical conditions.

The amino acids were commercially available samples of high purity and were used as received.

Results and Discussion

Formation of ($MH^+ - 18$) in CH_4 CI Mass Spectra. As discussed above, Leclerq and Desiderio[3] have proposed that at least part of the ($MH^+ - 18$) ion signal in the CH_4 CI mass spectra of amino acids arises by ethyl ion addition followed by loss of the elements of formic acid (reaction 1) rather than by the loss of H_2O from MH^+ (reaction 2). To determine the relative importance of these two reactions we have examined the CD_4 CI mass spectra of a representative selection of α-amino acids, since, with CD_4 as reagent gas, the ion resulting from reaction 1 will shift by 5 mass units and no longer will be isobaric with the ion resulting from reaction 2.

Table I. ($MH^+ - H_2O$) and ($M{\cdot}C_2H_5^+ - COOH_2$) in the CH_4 CI of Hydroxy-Substituted Amino Acids

Amino acid	($MH^+ - H_2O$)[a]	($M{\cdot}C_2H_5^+ - COOH_2$)[a]
Serine	16.3	10.0
Threonine	61.8	10.7
Aspartic	42.5	2.4
Tyrosine	0.0	12.1

[a] Intensities as percent of base peak.

$$C_2H_5^+ + RCH(NH_2)COOH \longrightarrow RCH(^+NH_2C_2H_5)COOH \longrightarrow RCH{=}\overset{+}{N}HC_2H_5 + COOH_2 \quad (1)$$

$$RCH(NH_2)COOH \xrightarrow{XH^+} RCH(NH_2)COOH{\cdot}H^+ \longrightarrow RCH(NH_2){-}\overset{O}{\overset{\|}{C^+}} + H_2O \quad (2)$$

Figure 1 compares the CH_4 and CD_4 CI mass spectra of two α-amino acids, valine and serine. Both acids show significant ($MH^+ - 18$) peaks at *m/e* 100 and *m/e* 88, respectively, in their CH_4 CI spectra. For valine this peak shifts entirely to *m/e* 105 when CD_4 is used as reagent gas, indicating that the ion results entirely by reaction 1, ethyl ion addition, rather than by reaction 2. This was found to be the case for all α-amino acids not containing a second hydroxyl function. The amino acids investigated, with the intensity (% of base peak) of ($MH^+ - 18$) in parentheses were: glycine (14.4), alanine (8.4), α-aminobutyric (6.0), valine (4.7), leucine (3.1), isoleucine (3.5), methionine (4.0), phenylalanine (8.4), proline (2.0), and tryptophan (0.0). In these cases *no* peak was observed in the CD_4 CI spectra corresponding to loss of $(H,D)_2O$ from MD^+.

By contrast, in the chemical ionization of the hydroxy-substituted amino acid, serine, only part of the ($MH^+ - 18$) (*m/e* 88) peak shifts upwards by five mass units when CD_4 is used as reagent gas. The remainder of the ion signal is observed at *m/e* 88 and 89 corresponding to loss of HDO and H_2O from the MD^+ ion. This result indicates that both reactions 1 and 2 are occurring. This was found to be the case when there was a second hydroxyl group present in the molecule, even as part of a second carboxyl group, as in aspartic acid. The relative intensities for the ($MH^+ - H_2O$) and ($M{\cdot}C_2H_5^+ - COOH_2$) peaks are recorded in Table I. Although tyrosine contains a second hydroxyl function this is bound to the aromatic ring and loss of H_2O from MH^+ was not observed in the CH_4 CI, although it is observed in the H_2 CI (see below).

Meot-Ner and Field[4] did not observe a peak for the ($MH^+ - H_2O$) ion in the isobutane CI mass spectra of simple α-amino acids and, as discussed in detail below, we have not observed the peak in the H_2 CI mass spectra of simple α-amino acids. Thus, there is no evidence, in any CI studies, for formation of a stable ion by reaction 2 for cases where R does not contain a second hydroxyl function. We do not

Table II. Methane CI Mass Spectra of α-Amino Acids[a]

Amino acid (mol wt)	MH^+	$MH^+ - NH_3$	$MH^+ - H_2O$	$MH^+ - NH_3 - H_2O$	$MH^+ - 2H_2O$	$MH^+ - COOH_2$	$MH^+ - H_2O - COOH_2$	$MH^+ - NH_3 - COOH_2$	Other ions
Glycine (75)	44					100			
Alanine (89)	30					100			
α-Aminobutyric (103)	27					100			
Valine (117)	38					100			
Leucine (131)	67					100			*m/e* 74 = 8
Isoleucine (131)	67					100			*m/e* 74 = 4
Proline (115)	100					69			
Serine (105)	53	1	16		3	100			
Threonine (119)	100	2	62		12	100	27		
Aspartic (133)	94	2	43	1		100	2		*m/e* 74 = 60, *m/e* 102 = 10
Methionine (149)	85	49				100			*m/e* 102 = 31, *m/e* 56 = 9
Phenylalanine (165)	100	9		2		92			*m/e* 74 = 6
Tyrosine (181)	83	77		8		100		4	*m/e* 107 = 22
Tryptophan (204)	31	100		1		15		3	*m/e* 130 = 19

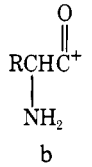
[a] Abundances relative to base peak assigned intensity of 100.

Table III. Hydrogen CI Mass Spectra of α-Amino Acids[a]

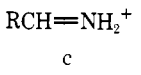
Amino acid (mol wt)	MH^+	$(M - H)^+$	$MH^+ - NH_3$	$MH^+ - H_2O$	$MH^+ - NH_3 - H_2O$	$MH^+ - 2H_2O$	$MH^+ - COOH_2$	$MH^+ - NH_3 - COOH_2$	$MH^+ - H_2O - COOH_2$	*m/e* 74	Other ions
Glycine (75)	1	1					100			*b*	
Alanine (89)	19	1					100			1	
α-Aminobutyric (103)	1	2					100	8		5	
Valine (117)	4	2					100	7		2	
Leucine (131)	4	2					100			36	*m/e* 44 = 12
Isoleucine (131)	2	3					100	13		18	
Proline (115)	8	4					100				
Serine (105)	32	2		5		12	100		24	2	
Threonine (119)	14	2		8		16	100	22	100	*c*	*m/e* 45 = 20
Aspartic (133)	26	1		6	2		48		36	100	
Methionine (149)	28	7	21				47	12		35	*m/e* 102 = 10, *m/e* 61 = 89, *m/e* 56 = 100
Phenylalanine (165)	6	2	2		10		100	4		10	*m/e* 91 = 4
Tyrosine (181)	40		17	14	16		100	18			*m/e* 107 = 56
Tryptophan (204)	20		100		7		59				*m/e* 130 = 89

[a] Abundances relative to base peak assigned intensity of 100. [b] Coincides with $(M - H)^+$. [c] *m/e* 74 is $MH^+ - COOH_2$.

consider that this is due to the failure to eliminate water from MH^+ but rather probably results from the instability of the resulting acyl ion b which rapidly loses CO to form the resonance stabilized ion c. The ion c forms the base



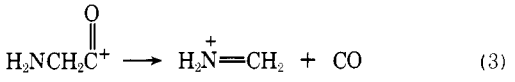
b



c

peak in the CH_4 and H_2 CI mass spectra of many α-amino acids (see below). Where a stable $(MH^+ - H_2O)$ ion is observed, Table I, it is probable that the second hydroxyl function present in the molecule is the one lost.

If one makes the reasonable assumption that the NH_2 group will not have any significant stabilizing effect on the ion b, one calculates, using available thermochemical data,[6] that reaction 3 is approximately 6 kcal mol^{-1} exothermic.



(3)

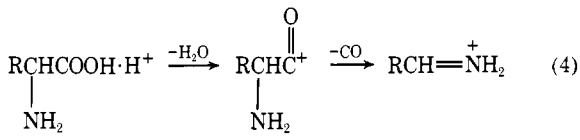
Since, in the chemical ionization of unsubstituted acids, abundant loss of H_2O to form RCO^+ is observed, one must conclude that the formal loss of formic acid from protonated α-amino acids is, to a large extent, the sequential loss of $H_2O + CO$.

Comparison of CH_4 and H_2 CI Mass Spectra. The CH_4 and H_2 CI mass spectra of the 14 α-amino acids studied are presented in tabular form in Tables II and III, respectively. The CH_4 CI data do not include the cluster ions $C_2H_5^+ \cdot M$ and $C_3H_5^+ \cdot M$ or ions derived from fragmentation of these clusters. In the latter category are the $(C_2H_5^+ \cdot M - COOH_2)$ ions discussed above; the $(MH^+ - H_2O)$ ion intensities, reported for water loss from the protonated molecule, have been corrected for this contribution using the data from the previous section. The CH_4 CI spectra are in reasonable agreement with the spectra reported by Milne et al.,[1] the difference in relative intensities undoubtedly arising from differences in reagent gas pressure, source temperature, and probe temperature.

A cursory examination of the data in Tables II and III shows that, in general, many of the same ions are observed in the H_2 CI spectra as are found in the CH_4 CI spectra. However, since protonation by H_3^+ is considerably more exothermic than protonation by CH_5^+ or $C_2H_5^+$, the H_2 CI spectra show a much lower intensity of MH^+ and increased intensities for fragment ions arising by decomposition of MH^+. Generally, in the CH_4 CI spectra only those ions originating directly by fragmentation of MH^+ are observed in any significant abundance, while in the H_2 CI further decomposition of these initial fragment ions is much more pronounced. This fact is of considerable assistance in deriving fragmentation schemes for MH^+, and the schemes proposed below are based to a large extent on comparisons of

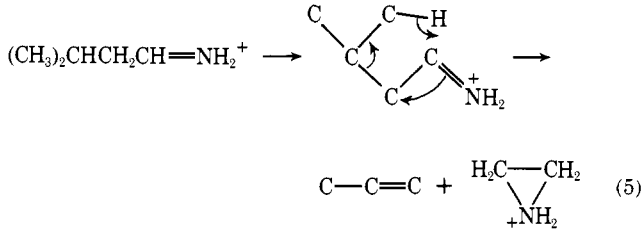
ion intensities in the H_2 and CH_4 CI spectra. These schemes should be considered as logical rationalizations of the observed spectra; confirmation of each step would require extensive deuterium labeling and detailed searching for metastable transitions, which are normally not seen with our instrument.

For the simple α-amino acids, glycine to proline, the major fragmentation reaction of MH^+ in both the CH_4 and H_2 CI systems involves the loss of the elements of formic acid, reaction 4. We have written reaction 4 as involving the



(4)

sequential loss of H_2O and CO rather than the loss of formic acid in line with the discussions of the previous section. For 2-aminobutyric acid, valine, isoleucine, and leucine, further fragmentation of $RCH{=}NH_2^+$ is observed in the H_2 CI spectra. For the first three cases, loss of NH_3 is observed, giving $(MH^+ - COOH_2 - NH_3)$ ion signals which are 8, 7, and 13%, respectively, of the base peak. For leucine no loss of NH_3 from $(MH^+ - COOH_2)$ is observed, but rather loss of C_3H_6 to give an ion at *m/e* 44 occurs. A possible reaction scheme is



(5)

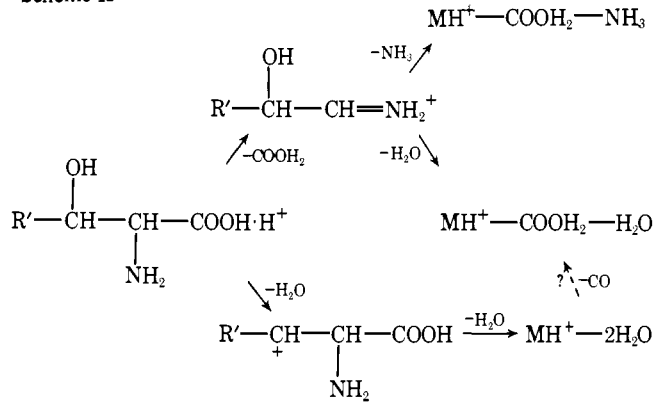
Leucine and isoleucine can thus be distinguished on the basis of their H_2 CI mass spectra, since only the latter shows a significant peak corresponding to $(MH^+ - COOH_2 - NH_3)$.

In the H_2 CI spectra low abundance $(M - H)^+$ ions are observed, as well as ions at *m/e* 74. The latter probably correspond to $H_2{}^+N{=}CHCOOH$, as observed in the electron impact mass spectra.[7] The much more intense *m/e* 74 peaks in the leucine and isoleucine H_2 CI spectra are difficult to rationalize; however, it should be noted that these are the only simple amino acids for which *m/e* 74 is observed in the CH_4 CI spectra.

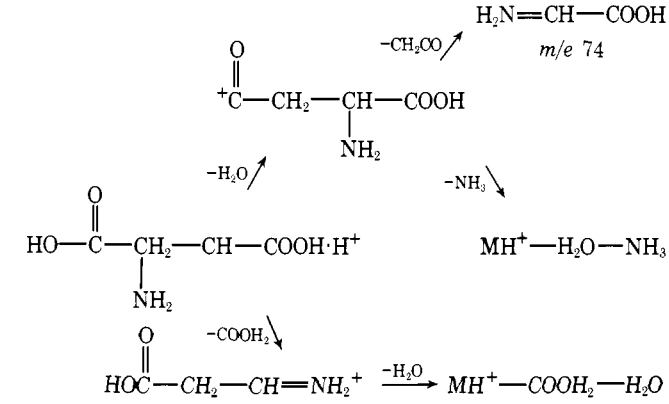
The H_2 and CH_4 CI mass spectra of the two hydroxy-substituted amino acids, serine (R′ = H) and threonine (R′ = CH_3), can be rationalized by the reaction sequence shown in Scheme II. The major fragmentation pathway for MH^+ remains loss of $COOH_2$, which, in the H_2 CI of both acids, is followed by loss of H_2O, giving for threonine the base peak $(MH^+ - COOH_2 - H_2O)$. The H_2 CI spectrum of threonine also shows a peak corresponding to $(MH^+ - COOH_2 - NH_3)$. In the CH_4 CI, the only further fragmentation of $(MH^+ - COOH_2)$ observed is the formation of $(MH^+ - COOH_2 - H_2O)$ in the fragmentation of threonine. In both the H_2 and CH_4 CI a peak is observed corresponding to $(MH^+ - H_2O)$, which fragments further by loss of another molecule of H_2O. In the H_2 CI this fragmentation may be followed by loss of CO to give the $(MH^+ - COOH_2 - H_2O)$ ion. We consider that the stable $(MH^+ - H_2O)$ ion arises from elimination of the hydroxyl group adjacent to the amine function rather than elimination of the carboxylic hydroxyl group.

The H_2 and CH_4 CI mass spectra of aspartic acid can be rationalized by Scheme III for fragmentation of MH^+. The

Scheme II

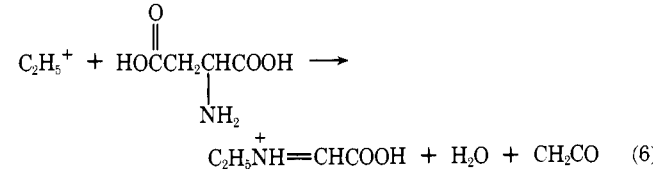


Scheme III



ion at *m/e* 74 forms the base peak in the H_2 CI spectrum and is 60% of the base peak in the CH_4 CI spectrum. The formation of this ion is most easily rationalized as ketene loss following the elimination of H_2O from MH^+, the H_2O loss involving the carboxyl group more remote from the amine function. A similar fragmentation is found in the CI mass of β-amino acids (see below).

An ion signal (10% of base peak) was observed at *m/e* 102 in the CH_4 CI spectrum of aspartic acid and was not reported by Milne et al. In the CD_4 spectrum this ion signal moved to *m/e* 107 indicating the incorporation of five deuterium atoms and the occurrence of the following reaction involving $C_2H_5^+$.

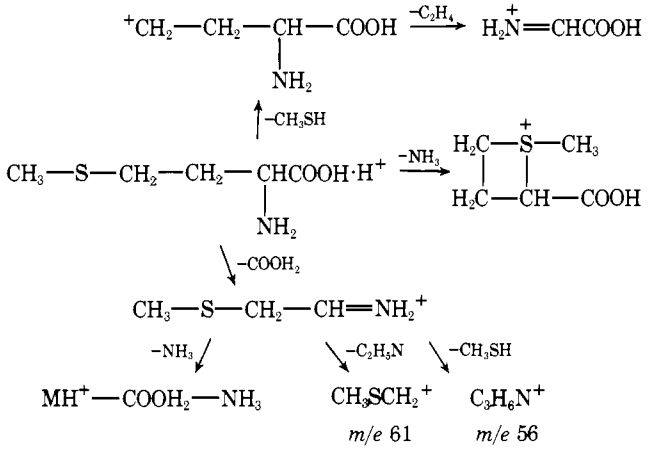


(6)

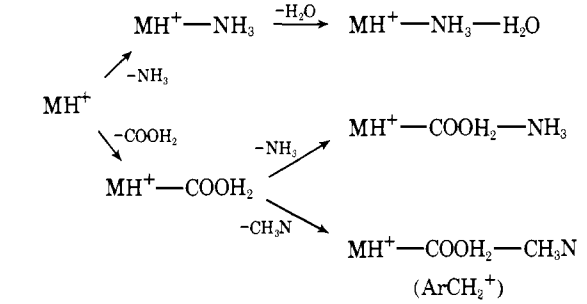
The H_2 CI spectrum of methionine is considerably more complex than the CH_4 CI spectrum, although the same fragmentation reactions appear to be involved. The *m/e* 56, 61, and 74 ions are of low intensity in the CH_4 CI spectrum but are much more intense in the H_2 CI spectrum with *m/e* 56 constituting the base peak. This is consistent with the sequential fragmentation scheme shown (Scheme IV) and the more exothermic protonation by H_3^+.

The remaining three α-amino acids, phenylalanine, tyrosine, and tryptophan, all contain aromatic substituents. The fragmentation of MH^+ following its formation by either H_2 CI or CH_4 CI is illustrated by the reactions shown in Scheme V. For phenylalanine fragmentation of MH^+ produces primarily $(MH^+ - COOH_2)$ with loss of NH_3 from MH^+ occurring to only a minor extent. The importance of NH_3 loss increases for tyrosine and becomes the most important fragmentation route for tryptophan. As expected,

Scheme IV



Scheme V



the ions arising from further fragmentation of the ($MH^+ - NH_3$) and ($MH^+ - COOH_2$) ions are more prominent in the H_2 CI spectra than they are in the CH_4 CI spectra. An unexpected fragmentation in the H_2 CI of tyrosine is loss of H_2O to form a stable ($MH^+ - H_2O$) ion. This undoubtedly involves loss of the *p*-hydroxy group from the phenyl ring. The H_2 CI of phenol shows a significant peak for loss of H_2O from MH^+.

In summary, the H_2 CI mass spectra of α-amino acids are similar to the CH_4 CI mass spectra; however, they show a much reduced MH^+ ion intensity and increased intensities for ions resulting from sequential fragmentation reactions originating from MH^+. Since in both H_2 CI and in EI much of the fragmentation goes through the intermediacy of the $RCH{=}N^+H_2$ ion, there are many similarities between the H_2 CI and the EI spectra. Thus H_2 CI has the potential advantage, shared with EI, of permitting distinction between isomeric R groups, as in leucine and isoleucine. On the other hand, a distinct disadvantage of the H_2 CI compared to CH_4 CI is the much lower abundance of the MH^+ ion, which could make it difficult to establish the molecular weight. However, it is likely that the advantage of increased fragmentation afforded by H_2 CI could be retained, and enhanced abundances of MH^+ obtained, by using a small amount of a weaker protonating agent, such as H_2O or NH_3, admixed with the reagent gas.

Effect of Position of NH_2 Group on the CI Mass Spectra. The effect of the position of the amino group, with respect to the carboxyl group, was investigated by determining the H_2 and CH_4 CI mass spectra of two β-amino acids, β-alanine and 3-aminobutyric acid, and two terminal amino acids, 4-aminobutyric acid and 6-aminohexanoic acid. The spectra obtained are shown, along with the electron impact mass spectra, in Figures 2 to 5.

The CH_4 CI mass spectrum of β-alanine shows an abundant ion signal at *m/e* 72, corresponding to ($MH^+ - H_2O$). This peak is almost absent in the H_2 CI spectrum. The base peak in both spectra is at *m/e* 30, corresponding to the

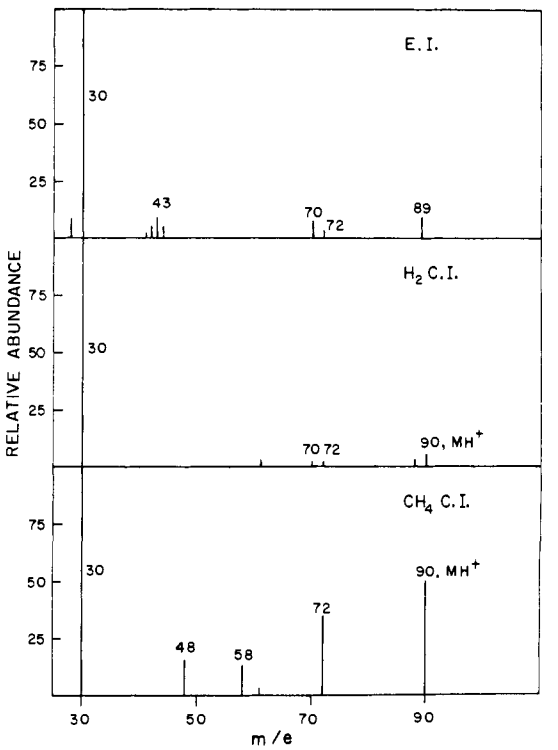


Figure 2. EI, H_2 CI, and CH_4 CI mass spectra of β-alanine.

$$RCH(NH_2)CH_2COOH\cdot H^+ \xrightarrow{-H_2O} H_2NCH(R)CH_2\overset{O}{\overset{\|}{C}}{}^+ \xrightarrow{-CH_2CO} H_2\overset{+}{N}{=}CHR \quad (7)$$

$CH_2{=}NH_2^+$ ion originating by the reaction sequence 7 (R = H). The same reaction sequence undoubtedly accounts for the base peak at *m/e* 44 (R = CH_3) in the H_2 and CH_4 CI mass spectra of 3-aminobutyric acid. The reaction is also the same as that proposed for formation of *m/e* 74 in the chemical ionization of aspartic acid (Scheme III), which can also function as a β-amino acid. The reaction sequence given by (7) is the major fragmentation route for MH^+ in β-amino acids.

The CH_4 CI of β-alanine shows a low intensity ion signal (10% of base peak) at *m/e* 58, while 3-aminobutyric acid shows a similar peak at *m/e* 72 (9% of base peak). Both these peaks shifted upwards by five mass units when CD_4 was used as reagent gas indicating the ethyl ion addition reaction 8. The analogous reaction, reaction 6, was observed in the CH_4 CI of aspartic acid.

$$C_2H_5^+ + RCH(NH_2)CH_2COOH \longrightarrow RCH(\overset{+}{N}H_2C_2H_5)CH_2COOH \longrightarrow RCH{=}\overset{+}{N}HC_2H_5 + (CH_2CO + H_2O) \quad (8)$$

The CH_4 CI of 4-aminobutyric acid and 6-aminohexanoic acid show that the major fragmentation routes of MH^+ are loss of H_2O and loss of NH_3. For both compounds there is a moderate intensity peak for loss of both H_2O and NH_3. In the H_2 CI of 4-aminobutyric acid the ($MH^+ - H_2O - NH_3$) ion results in the base peak in the spectrum, while for 6-aminohexanoic acid the further loss of CO from ($MH^+ - H_2O - NH_3$) leads to the base peak $C_5H_9^+$, at *m/e* 69. In summary, the fragmentation of terminal amino acids on chemical ionization consists largely of the loss of H_2O and/or NH_3 from MH^+.

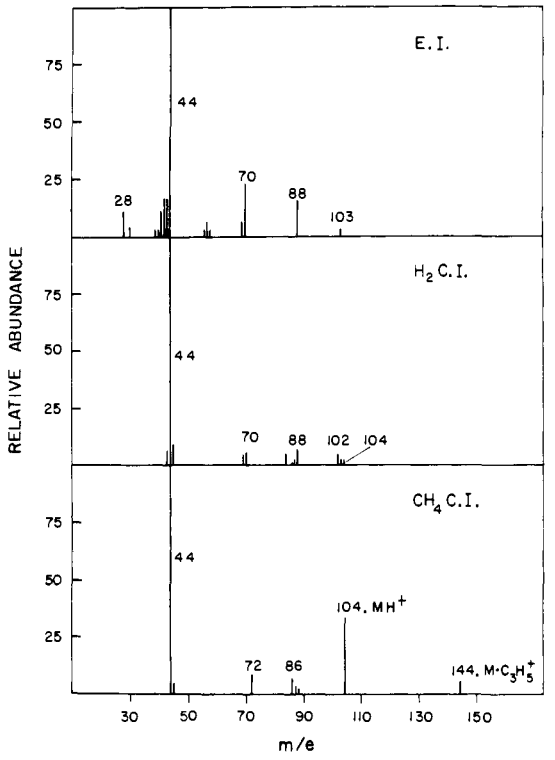


Figure 3. EI, H_2 CI, and CH_4 CI mass spectra of 3-aminobutyric acid.

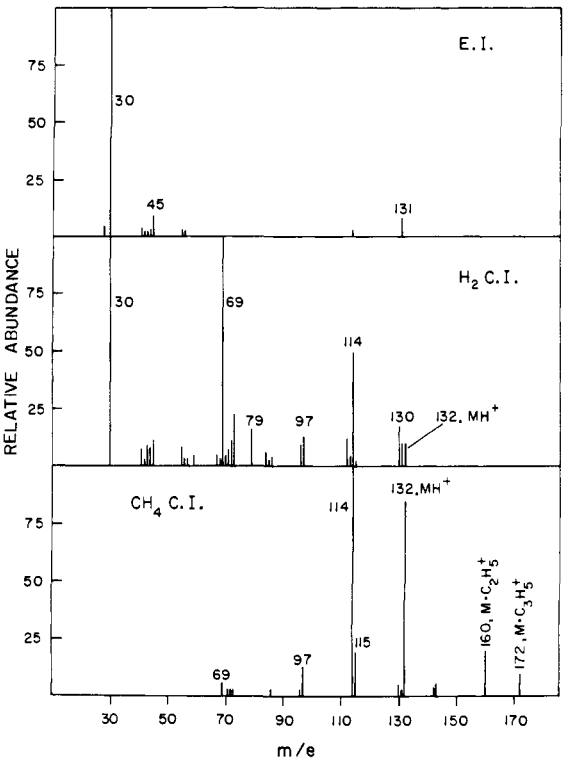


Figure 5. EI, H_2 CI, and CH_4 CI mass spectra of 6-aminohexanoic acid.

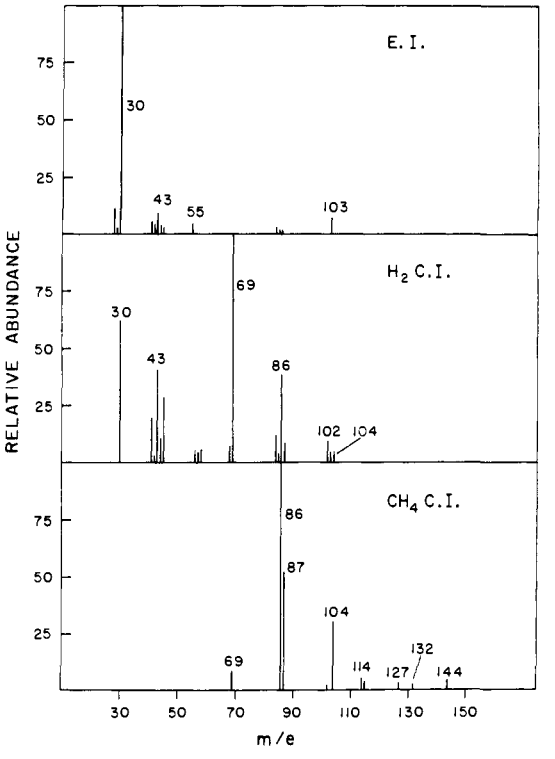


Figure 4. EI, H_2 CI, and CH_4 CI mass spectra of 4-aminobutyric acid.

Meot-Ner and Field[4] observed no MH^+ ions in the isobutane CI of 6-aminohexanoic acid and 11-aminoundecanoic acid, the base peak in both cases being $(M - 18)H^+$. They interpreted this as being due to lactam formation, probably prior to volatilization of the sample, and, therefore, suggested that the free amino acid cannot be obtained in the vapor phase when the amino and carboxyl groups are separated by more than one carbon atom. The CH_4 CI mass spectra in Figures 2 and 5 all show abundant ions corresponding to MH^+ indicating that we have been successful in obtaining the free amino acid in the gas phase by careful control of the heating of the solid sample.

Intramolecular Hydrogen Interchange in MH^+. Milne et al.[1] have postulated that the site of protonation determines the fragmentation mode of MH^+. They considered that protonation at the carboxyl group resulted in the loss of the elements of formic acid, while protonation at the amine led to loss of NH_3 under suitable conditions. By contrast, from their isobutane CI studies, Meot-Ner and Field[4] proposed that protonation occurred at the amine and that subsequent proton transfer to the carboxyl group resulted in the elimination of formic acid. If either one of these distinctive modes of protonation followed by fragmentation occurred, and there was no hydrogen interchange between sites within the molecule by intramolecular proton transfer, one would expect to see characteristic results when deuterated reagent gases are used, i.e., when MD^+ is formed and fragments. In the case of protonation at the carboxyl group followed by fragmentation, one should observe no deuterium retention in the appropriate fragment ion. On the other hand, addition of D^+ to the amino group followed by a single $(H/D)^+$ transfer to the carboxyl group should lead to 67% D retention in the fragment ion resulting from the loss of the elements of formic acid from the MD^+ ion. In this case the added D and the two H originally bonded to nitrogen become equivalent prior to transfer.

The percent retentions of deuterium in the $(MD^+ - COO(H,D)_2)$ ion in the CD_4 CI and D_2 CI of the amino acids studied are given in Table IV. It is obvious that the results fit neither of the above simple models. Before discussing the results in detail two experimental difficulties must be considered. First, there is the possibility that the deuterium incorporation observed in the various fragment ions (including those for which data are presented in Tables V to VII) may result from ion-molecule reactions of the nondeuterated ions with the reagent gas molecules, CD_4 or D_2, which are present in large excess. One would not antici-

Table IV. Percent Deuterium Retention in $MD^+ - COO(H,D)_2$

Amino acid	CD_4 CI	D_2 CI	Calcd[a]
Alanine	24	30	50
α-Aminobutyric	34	41	50
Valine	34	46	50
Leucine	38	43	50
Isoleucine	39	44	50
Proline	35	23	33
Serine	45	49	60
Threonine	64	58	60
Aspartic	38	29	60
Methionine	36	34	50
Phenylalanine	42	49	50
Tyrosine	41	47	60
Tryptophan	40	47	60

[a] Calculation assumes complete randomization of all H/D bonded to oxygen and nitrogen in MD^+.

Table V. Percent Deuterium Retention in $MD^+ - (H,D)_2O$

Amino acid	CD_4 CI	D_2 CI	Calcd[a]
Serine	48	41	60
Threonine	53	58	60
Aspartic	34	26	60
4-Aminobutyric	42	34	50
6-Aminohexanoic	44	34	50

[a] Calculation assumes complete randomization of all H/D bonded to oxygen and nitrogen in MD^+.

pate that such reactions would be equally facile for all fragment ions and with both reagent gases, yet similar results are obtained for all ions and in both the CD_4 and D_2 CI systems. Further, no significant variation of the percent incorporation was observed when the reagent gas pressure was varied by a factor of 2 to 3. If ion-molecule reactions were important such a pressure variation should be reflected in significant changes in the percent D incorporation. Thus, we conclude that ion-molecule reactions of fragment ions with the reagent gas play, at most, a minor role in deuterium incorporation.

It was noted that, when CD_4 and D_2 were used as reagent gases, significant MH^+ ion intensities were observed, often approaching the MD^+ intensities. The major part of this MH^+ ion signal undoubtedly arises from an isotopic exchange reaction of the MD^+ ion with the neutral amino acid. Other work in our laboratory[8] has shown that symmetrical proton transfer reactions of the type

$$RXHD^+ + RXH \longrightarrow RXH_2^+ + RXD \qquad (9)$$

are quite rapid in amines, alcohols, and mercaptans. In all of the amino acid CI spectra we observed measurable ion signals for the dimeric species M_2H^+, indicating that there had been a significant number of collisions of MH^+ with M. Only a small fraction of these collisions will lead to stable M_2H^+ ions; the majority will lead only to isotopic exchange by proton transfer (reaction 9). The extent of formation of MH^+ when deuterated reagent gases are used depends, in part, on the number of labile or exchangeable hydrogens; in the D_2 and CD_4 CI of methyl alkanoates we have not observed formation of MH^+.[9] This latter observation suggests that the MH^+ ions observed do not arise to a significant extent by reactions involving background H_2O. In the calculation of the percent deuterium retention in the various fragment ions we have assumed that the MH^+ ions formed by this exchange reaction do not fragment. Essentially we are assuming that the exchange reaction dissipates the excess energy originally present in MD^+ which is necessary for fragmentation. Some support for this assumption can be derived from the observation that the retention figures did not correlate with the MD^+/MH^+ ratio. To the extent that the assumption is not valid, the calculated retention figures will be low; however, the general conclusions drawn below will not be invalidated.

Table VI. Percent Deuterium Retention in $MD^+ - N(H,D)_3$

Amino acid	CD_4 CI	D_2 CI	Calcd[a]
Methionine	21	25	25
Tyrosine	45	36	40
Tryptophan	49	57	40
Phenylalanine	41	41	25
4-Aminobutyric	36		25
6-Aminohexanoic	38	54	25

[a] Calculation assumes complete randomization of all H/D bonded to oxygen and nitrogen in MD^+.

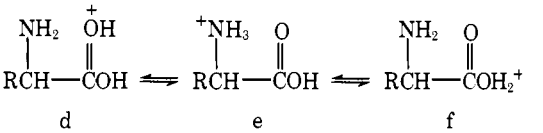
Table VII. Percent Deuterium Retention in $MD^+ - (H,D)_2O - N(H,D)_3$

Amino acid	CD_4 CI	D_2 CI
4-Aminobutyric	24	22
2-Aminohexanoic	27	21

The percent deuterium retention figures for the $(MD^+ - COO(H)_2)$ ion given in Table IV vary from 23 to 64% and obviously fit neither of the simple models discussed above. The results do indicate significant H/D interchange in the MD^+ ion prior to fragmentation. This interchange should occur primarily by H^+/D^+ transfer between electron-rich centers in the molecule and in the limit, one would anticipate complete equilibration of the H/D bonded to nitrogen and oxygen, with the result that the H/D lost upon elimination of water (or ammonia) should be selected at random from among all the labile H/D. The final column of Table IV records the percent deuterium retention expected for this limiting situation. The experimental results do not agree quantitatively with this model, being generally lower; however, there is little doubt that extensive interchange has occurred.

Table V records the percent deuterium retention in the fragment ion $(MD^+ - (H,D)_2O)$, where such a fragment ion was observed. Again, significant retention is observed, although less than that predicted for complete equillibration. Table VI records similar results for the $(MD^+ - N(H,D)_3)$ fragment ion. In this case the retention is much higher than the 0% predicted if D^+ addition occurs at the amino group and fragmentation follows without interchange and is even higher than the 25 to 40% predicted for complete equilibration of the labile hydrogens; there is a distinct preference for retention of the added proton in the fragment ion.

It is difficult to derive quantitative conclusions from the data in Tables IV to VI. However, one can state that the model of protonation at a specific site, with the subsequent fragmentation being determined by the site of protonation, is not valid. Nor can the results be accommodated by assuming initial protonation at one site only,[10] such as protonation at the amine function. Rather the results are best accommodated by assuming protonation at any one of several sites, to give species such as d, e, and f, with extensive intra-



d e f

molecular proton transfer between sites prior to fragmentation. Since complete equilibration of the labile hydrogens would require several transfer steps, and fragmentation

may occur at any stage, it is not surprising that complete equilibrium is not observed. It is probable that the various fragmentation reactions occur from specific canonical forms, i.e., loss of NH_3 from e and loss of H_2O from f (and possibly d). However, even this model of equilibration involving only the labile hydrogens may be too simple. One would anticipate that the loss of ammonia and water from the MH^+ ion of 4-aminobutyric acid and 6-aminohexanoic acid should remove all the labile hydrogens along with the added proton. However, as summarized in Table VII, we find 21-27% deuterium retention in the fragment ions resulting from loss of ammonia and water from the MD^+ ion in the D_2 and CD_4 CI of these amino acids. This result indicates that there may be significant interchange of the labile hydrogens with hydrogens bonded to carbon prior to fragmentation of MH^+.

Several other deuterium retention results are of interest. In line with the results in Tables IV to VI, the fragment ion resulting from elimination of methyl mercaptan from MD^+ in methionine showed 20% deuterium retention in the CD_4 CI and 52% retention in the D_2 CI. This compares with the 75% calculated on the basis of equilibration. Obviously, fragmentation by this mode does not result solely from protonation at this site although there is a preference for loss of the added proton.

The *m/e* 74 ion ($H_2N^+{=}CHCOOH$) is particularly abundant in the H_2 CI of leucine, isoleucine, aspartic acid, and methionine. For leucine and isoleucine there was practically no deuterium incorporation in this fragment when D_2 was used as reagent gas, indicating that the ion does not originate by fragmentation of the MD^+ ion. The most likely origin is either by charge transfer, possibly involving excited states of H_3^+, or by decomposition of the $(M - H)^+$ ion. By contrast, in the D_2 CI *m/e* 74 showed 57% deuterium retention for aspartic acid and 60% retention for methionine. The *m/e* 74 ion also is observed in the CH_4 CI of aspartic acid and using CD_4 the deuterium retention was found to be 50%. These retention figures are consistent with extensive intramolecular hydrogen interchange and fragmentation by the mechanisms outlined in Schemes III and IV. In the same vein, the *m/e* 44 ion in the CI of 3-aminobutyric acid showed approximately 40% deuterium retention in both the D_2 and CD_4 CI. Again this is consistent with the mechanism of formation outlined in reaction 7.

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(2) Throughout this paper MH^+ designates the protonated amino acid, while $(MH^+ - X)$ designates the ion resulting from the loss of X from the protonated molecule.
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(10) Initial protonation at a specific site, followed by incomplete equilibration through proton transfer, would result in a preference for loss of the added proton (deuteron) in only one fragmentation reaction. Since such a unique preference is not observed we conclude that the initial protonation must occur at any one of several sites.

The Dependence of Geminal H-H Spin-Spin Coupling Constants on ϕ and ψ Angles of Peptides in Solution

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Abstract: A theoretical study is presented of the conformational dependence of geminal H-H coupling constants in compounds which provide models for the peptide structure. Calculated results for Fermi contact coupling in *N*-methylacetamide, N^{α}-acetylglycinamide, *cyclo*-(-Gly-Gly-), and a three-peptide fragment having a γ turn are based on the finite perturbation theory (FPT) formulation in the semiempirical approximation of intermediate neglect of differential overlap (INDO). It is shown that the effect of the amide carbonyl is to produce a shift in the value of the geminal coupling constant to more negative values, depending on the value of the dihedral angle ψ. However, the effect of the amide nitrogen is to shift the geminal coupling constants toward more positive values depending on the dihedral angle ϕ. Under the combined effects of the two groups, as in N^{α}-acetylglycinamide, the total variation of the coupling is calculated to be 8 Hz. Agreement of calculated and experimental values is quite satisfactory in those cases in which x-ray structural data for the molecules are known. Although polarity of the solvent is known to have an effect on geminal H-H coupling, the calculated results for the three-peptide fragment having a γ turn suggests that intramolecular hydrogen bonding may not be an important factor. Based on these results, it is concluded that geminal H-H coupling constants can complement other NMR parameters as a probe of peptide structure in solution.

Studies of the conformations of peptides in solution[1] have made extensive use of spin-spin coupling constants from nuclear magnetic resonance spectra. Especially important, in this regard, are the vicinal H-N-C-H coupling constants, which provide a measure of the dihedral angle ϕ measured about the N-C_{α} bond in the peptide backbone **1**. It has also been suggested that the vicinal ^{15}N-C'C_{α}-H coupling constants would provide a measure of the dihedral angle ψ measured about the C_{α}-C' bond in the peptide backbone.[2-5] However, it appears that the difficulties of in-