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The Mass Spectra of the α-Amino Acids[1]

BY GREGOR JUNK AND HARRY SVEC

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The mass spectra of the α-amino acids have been obtained using a mass spectrometer equipped with a heated crucible ion source. Correlations of these data to molecular structure have been made and unimolecular decomposition mechanisms are proposed. Comparisons of these data have been made with the mass spectra of the ethyl esters of the amino acids and with other carboxylic acids and their esters.

Introduction

Anderson[2] and Biemann, *et al.*,[3] have reported the positive ion mass spectra of some of the methyl and ethyl esters of the α-amino acids. Both investigators found it necessary to convert the acids to volatile derivatives because of the tendency of the untreated acids to decompose and form some diketopiperazine at the temperature required to achieve adequate reservoir pressures in their mass spectrometers.

Appreciable structure changes for the amino acids were also observed here with an externally heated inlet system. However, the mass spectra of the amino acids, which do not include the spectra of decomposition products, and diketopiperazines have been obtained by resorting to a crucible ion source. When the solid acid is placed directly into the ionization chamber, the temperature required to produce adequate sample vapor in the ionization region is lower than with the conventional externally heated inlet systems used previously.[2,3] This lower temperature results in thermal stability and the amino acids yield reproducible and unique mass spectral patterns.

These mass spectra and the interpretations given here should provide further understanding of the processes which occur when complex organic molecules are subjected to electron impact.

Experimental

A General Electric analytical mass spectrometer whose ion source was modified to accommodate a small crucible was used to obtain the results reported. The collimating slits of the ion source were not disturbed. The filament, trap, repeller plate, ion chamber and first shield slit were removed. A new ion chamber similar to the chamber described by Cameron[4] was designed and mounted on the shield plate whose ion exit slit had been enlarged to 75 mils. The filament and trap were positioned on either side of slots in the ion chamber which allowed free passage of the ionizing beam of electrons from the filament through the chamber and onto the trap electrode. A 500° maximum temperature furnace was designed to fit over the ion chamber. The furnace is used as a source of heat when necessary during an assay or for baking out the source region between sample runs. Access to the source region is through a 3.5 in. diameter circular port at the top of the mass tube. A Teflon gasket is used for the vacuum seal at this port. A venting valve (Vactronic Laboratory Equipment, Inc.) was put into the system at the source end of the analyzer and a magnetically operated isolation valve of our own design was placed between the analyzer section and the cold trap of the vacuum system. These valves allowed venting the source chamber to change the sample without warming the trap or cooling the diffusion pump.

The acid whose spectrum was to be determined was introduced into the ion chamber *via* a small borosilicate glass crucible. After the system pumped down the filament was turned on. The same filament which provided the ionizing electrons ordinarily radiated enough energy to heat the ion chamber and vaporize the amino acid. One-half to one hour was allowed for temperature equilibrium to be established and the spectrum to stabilize. Several magnetic scans in the mass range of the acid were then made. After completing the scans the electronic components were turned off, the isolation valve was closed, the system was vented with dry nitrogen, the sample was removed and the system was re-evacuated. Ordinarily the small residue of the previous sample could be removed by increasing the filament emission up to 10-fold. The bake-out time at this higher emission was continued for about 3 hours. Then the analyzer section was vented again and the next sample to be assayed was placed in the ionization chamber.

Periodically the furnace was put in place over the ion chamber and the source region baked out at 400–500°. Using this method, background ion currents were not observed except for small currents corresponding to less than 10^{-13} ampere at masses 18, 28 and 44.

Appearance potentials, A.P., were determined by linear extrapolation of the ionization efficiency curves near the threshold. Argon was introduced simultaneously with the amino acid and was used as the electron voltage calibrating gas.

The carboxyl and amino hydrogens of eight acids were exchanged in an excess of 99+% D_2O. Approximately 2×10^{-4} mole of each acid was dissolved in 7×10^{-2} mole of D_2O. Open vessel and sealed tube deuterations at temperatures of 20 to 80° and for times of up to 40 hours were employed. As expected, the deuteration conditions employed did not affect the extent of the exchange as determined from the mass spectral patterns.

Results and Discussions

Molecular Ionizations.—It is proposed that the ionization process occurring with the aliphatic amino acids is the removal of one of the electrons from the unbonded pair on the nitrogen atom. This is very similar to the ionization of the amines as described by Collin.[5] To support this proposal, the A.P. of the parent ions of glycine and isoleucine were measured and calculated[6] and then compared with the average measured and

(1) Contribution No. 1149. Work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission.

(2) C. O. Anderson, *Acta Chem. Scand.*, **12**, 1353 (1958).

(3) K. Biemann, J. Seibl and F. Gapp, *Biochem. Biophys. Res. Commun.*, 1, 307 (1959).

(4) A. E. Cameron, *Rev. Sci. Instr.*, **25**, 1154 (1954).

(5) J. Collin, *Bull. soc. chim. Belges.*, **63**, 500 (1954).

(6) J. L. Franklin, *J. Chem. Phys.*, **22**, 1304 (1954).

TABLE I

APPEARANCE POTENTIAL VALUES

Sample	Calcd.[a]	(Parent)$^+$ meas.[b]	(P − COOH)$^+$ meas.[b]	(75)$^+$ meas.[b]
Glycine	9.18	9.5	10.1	—
Isoleucine	8.96	9.5	9.9	9.9
Methionine	8.87	9.5	10.2	—
Carboxylic acids	10.50[d]	10.7[c]	—	—
Satd. hydrocarbons	10.64[d]	10.6[c]	—	—
Amines	9.18[d]	9.4[c]	—	

[a] These values were calculated using the united atom approach of Franklin.[6] [b] The measured values are reproducible to ±0.2 e.v. The estimated accuracy is −0.2 to −0.4 e.v. [c] Average measured values taken from Field and Franklin.[7] [d] Average calculated values taken from Franklin.[6]

calculated A.P. of hydrocarbons, carboxylic acids and amines. These values are recorded in Table I.

The close agreement between the average measured A.P. of 9.5 e.v. for glycine and isoleucine and the average A.P. of 9.4 e.v. for thirteen aliphatic amines[7] suggests that the same ionization process occurs in amines and aliphatic amino acids. Additional evidence that this is true is given by the higher energy required to remove an electron from the hydrocarbon or carboxyl groups as indicated in Table I where the average values for thirteen saturated hydrocarbons and four aliphatic acids are listed.

This "amine type" ionization results in the charge being localized on the nitrogen and adjacent α-carbon atom. The mass spectra of the amino acids can be explained best by assuming this localization, which seems reasonable in view of the experimental evidence. It must be admitted that other electrons, possibly delocalized, can be removed at the high electron energy (75 volts) used to secure the mass spectra reported in Table II, but the dominant process is the most energetically favorable process which for the aliphatic amino acids is the removal of one of the nitrogen electrons. This hypothesis has been used by Sharkey, *et al.*,[8] Beynon, *et al.*,[9] and Chupka and Berkowitz[10] to explain the mass spectra of similar molecules containing a functional constituent.

The A.P. of the parent ion of methionine was determined to observe the effect of a sulfur atom in the R group. The spectroscopic ionization potential of CH_3SH[7] is 9.4 e.v. If R–S–R type compounds behave similarly, one would expect the A.P. due to the removal of one of the electrons on the sulfur atom of methionine to be approximately 9.4 e.v., the same energy necessary to remove one of the nitrogen electrons. The measured A.P. of methionine parent ion was 9.5 e.v. which leaves no preference in this case for charge localization on either the sulfur or nitrogen.

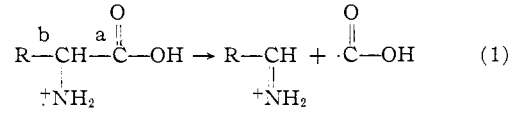
Instrumental difficulties prevented the observation of the A.P. of other substituted amino acids, but an inspection of their mass spectra indicates that the ionization process is dependent upon the substituent present in the R linkage. It is difficult to identify a single dominant ionization process which is consistent with the observed mass spectra.

Fragmentation when R is Alkyl

Table II shows the condensed fragmentation patterns[11] of the amino acids and the deuterated amino acids whose spectra have been established. Isotopic corrections have not been applied to the data presented in Table II but have been applied to all other tables in the text in a way that accounts for all isotopic forms of each fragment. The parent ions and those mass numbers where at least one acid has an ion intensity greater than 1% of the total ion current are included in the table. A representation based on the total ion yield is used because the interpretations presented here are more meaningful on this basis than they would be if the conventional method of recording mass spectra relative to the base peak had been used. The less complicated spectra of the aliphatic amino acids will be considered first.

Several ionic species can be positively identified by comparing the normal acid spectra to the deuterated acid spectra (see Table II) even though only semi-quantitative comparisons are possible due to the differences in the fragmentation patterns of molecules in which deuterium is substituted for hydrogen.[12] In some instances, the process by which the fragment ion is formed is supported by the observed proper metastable ion currents in both the normal and deuterated acid spectra. Discussions of the fragment ion currents from the aliphatic amino acids which are collected in Table II are given below.

(P − COOH) Fragments.—Table III gives the comparative amounts of the dominant amine fragments (P − COOH) as well as all the other fragments produced by rupture of bond a and/or b

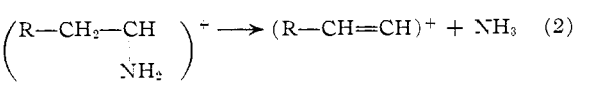


(1)

The listed values illustrate the preferred rupture of bond a with the charge remaining on the fragment containing the nitrogen as shown in eq. 1. The average A.P. of 10.0 e.v. for the (P − COOH) fragment produced by this process from glycine and isoleucine and 10.2 e.v. for the similar process from methionine is offered as supporting evidence for the above interpretation. The energy required to rupture bond a is estimated to be at least 3.0 e.v. Simple breaking of this bond should have resulted in an A.P. for the (P − COOH) fragment of >12.5 e.v. The difference of only 0.5 e.v. between the A.P. of the (P) ion and that of the (P − COOH) fragment thus requires that a bond-forming process occurs during this fragmentation.

The systematic decrease in the intensity of the amine ion fragment and all other fragments produced by the rupture of bond a and/or b as the hydrocarbon end of the molecule becomes larger can be attributed to the greater number of low activation energy reaction paths available to both the parent ion and the (P − COOH) fragment.

(P − COOH − NH_3) Fragments.—Table IV gives the relative intensities of the charged olefinic hydrocarbon fragment ions formed by the rupture of bond a and the elimination of NH_3 from the amine fragment. The general reaction for the formation of these hydrocarbon ion currents is



(2)

The reaction indicates that no mass shift should occur in the deuterated acid spectrum. This interpretation was confirmed in all the acids studied. Additional

(7) F. H. Field and J. L. Franklin, "Electron Impact Phenomena," Academic Press, Inc., New York, N. Y., 1957.

(8) A. G. Sharkey, Jr., J. L. Shultz and R. A. Friedel, *Anal. Chem.*, **31**, 87 (1959).

(9) J. H. Beynon, G. R. Lester and A. E. Williams, *J. Phys. Chem.*, **63**, 1861 (1959).

(10) W. Chupka and J. Berkowitz, *J. Chem. Phys.*, **32**, 1546 (1960).

(11) The complete mass spectra will be presented in the program of Uncertified Mass Spectra sponsored by Committee E-14 on Mass Spectrometry of ASTM.

(12) F. E. Condon, *J. Am. Chem. Soc.*, **73**, 4675 (1951).

TABLE IV

TENDENCY TO ELIMINATE THE ELEMENTS OF AMMONIA

	$(P - COOH)^+$	$(P - COOH - NH_3)^+$	$(P - COOH - NH_2)^+$
Glycine	68.9	0.2	0.5
Alanine	50.5	3.0	2.5
n-Butyric	33.8	9.6	0.6
Isobutyric	39.3	7.3	16.3
Valine	29.2	8.8	0.6
Norvaline	29.0	2.2	1.2
Leucine	24.6	0.5	0.8
Norleucine	28.4	1.7	.2
Isoleucine	22.1	2.5	.1

it is not possible to draw universally consistent correlations between structure and the extent of hydrocarbon ion formation by the process in eq. 2. Biemann, *et al.*,[13] state that the intensity of these ion currents is generally weak unless the β-carbon atom has a branch or contains an aryl substituent as is the case in valine, isoleucine and phenylalanine. Their ion currents at masses 55, 69 and 103 were 22, 11 and 11% of the amine ion currents. Our results for the same three acids are 30, 11 and 12% of the amine ion current which is excellent agreement. However, the values for α-amino-*n*-butyric acid and α-aminoisobutyric acid are 28 and 19% and neither of these acids has a substituent on the β-carbon atom.

(P − COOH − NH_2) Fragments.—Table IV also lists the occurrence of these fragments. Where possible the deuterated spectra have been compared to the normal spectra to get an estimate of the amount of this fragment. The amount is low due to formation of primary carbonium ions in all cases except α-aminoisobutyric acid. Here the greater number (16.3%) is due to the increased stability of a secondary carbonium ion. For example, the fragment ion is $(CH_3\text{–}\overset{+}{C}\text{–}CH_3)$ from α-aminoisobutyric acid whereas α-amino-*n*-butyric acid yields the fragment $(CH_3\text{–}CH_2\text{–}\overset{+}{C}H)$.

(P − R − COOH) Fragment.—This fragment appears at mass 29 from all the amino acids given in Table III except isobutyric. The values listed are estimates obtained by comparing the deuterated acid spectra to the normal acid spectra. Because of the large uncertainties in the listed values no interpretations of the comparative number of these fragments are offered other than to say that their general low amount is due to the necessary rupture of two bonds to produce the $(CH\text{–}NH_2)$ fragments.

(P − R) Fragment.—Most of the aliphatic amino acids have ion currents of moderate intensity due to the rupture of bond b with the charge going to the (P − R) fragment. The low value for glycine and alanine reflects the low probability for loss of hydrogen and methyl, but the loss of methyl from alanine is much greater than the loss of H from glycine.

The (P − R) fragment from α-aminoisobutyric acid appears at mass 88. The absence of mass 74 ions and the appearance of a fragment 14 mass units higher serves as an indication that the α-carbon atom has a methyl substituent. This conclusion is not positive, however, since a methyl substituent on the nitrogen atom could cause the same mass shift. Biemann, *et al.*,[13] discuss extensively the structural information obtained from a consideration of the mass spectra of several amino butyric acid isomers.

(R) Fragment.—The amount of fragmentation at bond b to produce (R) ions is low in all cases and this observation is offered as supporting evidence that the charge is preferably localized on the nitrogen and adjacent carbon atom when R is an alkyl group. Under this condition the probability of forming (R) ions is small even if R is the stable ethyl, propyl or butyl radicals.

(COOH) Fragments.—The amount of this fragment is small for all the amino acids studied. This is probably due to the instability of this ion compared to the other possible ions which can be formed. The observed small amount also lends some support for localizing the charge on the nitrogen and α-carbon atom.

Proline and Cycloleucine Mass Spectra.—The mass spectra of proline and cycloleucine show a stronger tendency for the (P − COOH) fragment to remain intact when the charged fragment is part of a ring structure. For example, the amounts of this fragment with valine and norvaline are 29.2 and 29.0%, respectively, while the number with proline is 45.6% of the total ion yield. The amounts from isoleucine, norleucine and leucine are 22.1, 28.4 and 24.6% while the number from cycloleucine is 39.9%.

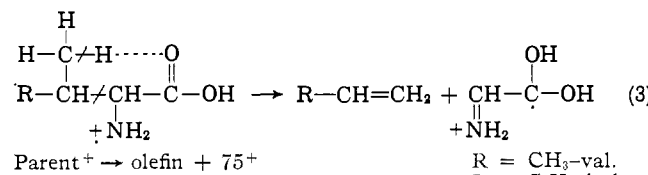
Major Rearrangement Ion Currents.—The amino acid molecule-ions exhibit strong tendencies to rearrange one hydrogen atom upon fragmentation. Table V lists the relative intensities of ion currents at masses 30, 44, 57 and 75 from a number of aliphatic amino acids. The 30, 44 and 75 ion fragments cannot be formed by simple bond ruptures and must be the result of hydrogen migration. The 57 fragment can be formed by simple bond rupture, but it is included in the discussion here because its occurrence reflects the tendency of the aliphatic acids to form mass 75 ions. Alanine is included in the table to show the limited tendency of this acid to undergo rearrangement processes of the type discussed here.

TABLE V

MAJOR REARRANGEMENT FRAGMENTS

Sample	75^+	57^+	44^+	30^+	Total
Alanine	—	0.1	—	0.5	0.6
Butyric	0.1	0.8	1.3	6.6	8.8
Valine	4.2	8.2	0.6	3.4	16.4
Norvaline	0.4	1.2	3.3	21.8	26.7
Leucine	1.1	1.7	16.3	14.1	33.2
Norleucine	0.5	1.3	4.9	18.0	24.7
Isoleucine	9.6	9.3	2.7	8.6	30.2

The processes leading to the formation of these rearranged fragments are given in eq. 3 through 7.



(3)

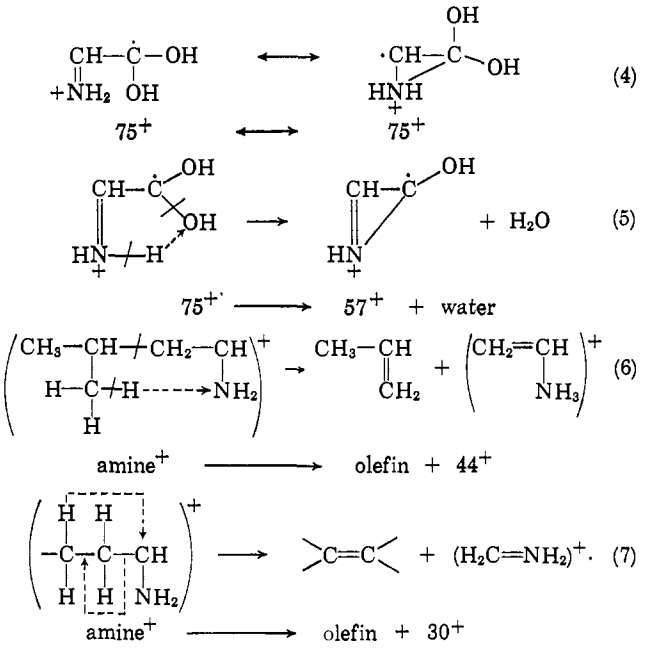
Parent$^+$ → olefin + 75^+

R = CH_3–val.
R = C_2H_5–isoleu.

The identities of the ions and the above processes have been established in some cases by the observation of metastable ion currents in both the deuterated and normal acid spectra and in all cases by the proper mass shifts when the normal acid spectra are compared to the deuterated acid spectra.

A few additional comments about the proposed reactions and the data in Table V seem desirable for clarity: 1. Only valine and isoleucine, which have methyl substituents on the β-carbon atom, have intense mass 75 ion currents. 2. A definite similarity exists between reactions 3 and 6. In both processes the methyl substituent is on a carbon atom which is twice removed from the carbon atom which has the substituent that accepts the migrating hydrogen and an olefin is eliminated. 3. Valine and isoleucine, which have intense 75 ion currents also have intense 57 currents. A metastable ion current at 43.3 is ob-

(13) K. Biemann, J. Seibl and F. Gapp, *J. Am. Chem. Soc.*, **83**, 3795 (1961).



served to support the process $75^+ \rightarrow 57^+ + 18$. Similar ion currents from isoleucine-d_3 at 43.1 and 44.6 confirm the processes $78^+ \rightarrow 58^+ + 20$ and $78^+ \rightarrow 59^+ + 19$. The process which occurs depends upon the elimination of OH or OD from the terminal carbon atom of the mass 78 fragment. With the structures given in 3 and 4 an equal probability exists for the two processes. The shift in the intensity of the 57 ion current in the undeuterated spectra of valine and isoleucine to 58 and 59 in the deuterated spectra also confirms the process in eq. 5. 4. The parent ion of mass 75 from glycine is structurally different from the 75 ion produced by fragmentation as given in 3. This explains why glycine produces no mass 57 ions even though the 75 ion intensity is 4.0% which is comparable to the 75 intensity from valine. 5. The extent of formation of the mass 30 ion cannot be strictly associated with the number of γ-hydrogens available or to their lability. Note the strong mass 30 ion current for norleucine (2 γ-H's) and norvaline (2 γ-H's) and the weak 30 for α-amino-*n*-butyric acid (3 γ-H's). Also compare norvaline and norleucine (*sec*-H) to leucine (*tert*-H).

When the sum of all the major rearrangement ion currents of the aliphatic amino acids are considered there is little correlation between structure and the extent of the rearrangements. However, the comparative extent of a particular rearrangement within an isomeric series such as the valines and the leucines does indicate structural features. Thus: (1) β-methyl substituents lead to intense 75 ion currents, (2) γ-methyl substituents encourage 44 ion formation, and (3) 30 ion formation predominates in straight chain isomers.

Ion fragments formed by selective rearrangements of the leucine and valine isomers of the amino acids and the ester derivatives are compared in Table VI. This table illustrates the greater tendency for rearrangements which the amino acids have in comparison to the esters. The same is true of the aliphatic acids compared to their esters since Happ and Stewart[14] observed selective rearrangement ion currents from the aliphatic acids which were of a higher relative intensity than has been indicated by Sharkey, *et al.*,[8] Beynon, *et al.*,[15]

(14) G. P. Happ and D. W. Stewart, *J. Am. Chem. Soc.*, **74**, 4404 (1952).

(15) J. H. Beynon, R. A. Saunders and A. E. Williams, *Anal. Chem.*, **33**, 221 (1961).

TABLE VI

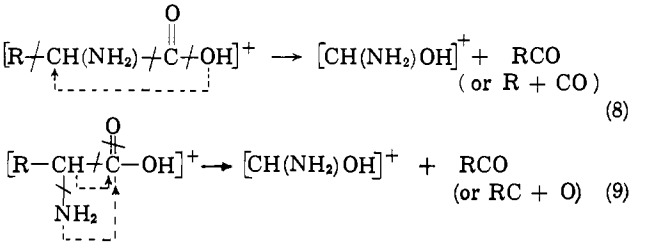
COMPARATIVE REARRANGEMENTS OF THE AMINO ACIDS AND ESTERS

	Per cent of the amine fragment					
Sample	Acid 75^+	Ester 103^{+a}	Acid 44^+	Ester 44^{+a}	Acid 30^+	Ester 30^{+a}
Leucine	6	1[b]	66	50	58	37
Norleucine	3	0[b]	18	10	64	45
Isoleucine	46	2[b]	12	10	39	31
Valine	16	2[b]	2	2[b]	12	10
Norvaline	2	0[b]	11	4[b]	77	54

[a] Values taken from Biemann, *et al.*[13] The 103 ion from the esters is analogous to the 75 ion from the acids. [b] Values not listed in text and roughly estimated from fragmentation graphs.

and Asselineau, *et al.*,[16] in the investigations of the aliphatic esters.

Mass 46.—All the 46 fragments of the normal acids are undoubtedly due to rearrangements. The structure of the 46 ion is not the expected formic acid structure since the 46 shifts to mass 49 instead of 47 or 48 in the deuterated acid spectra. Two possible rearrangements which could account for the observed mass shift are shown in eq. 8 and 9. Process 8 is preferred because it involves fewer bond ruptures.



This rearrangement ion is the only one observed which involves the migration of a functional group. In all the other cases only hydrogen is involved in the rearrangements.

Masses 18 through 22.—Fragments of masses 18 and 19 in the normal acid spectra and 19 through 22 in the deuterated spectra can be attributed to rearrangement processes. This observation was expected since H_2O rearrangement ions are observed from the aliphatic acids[14] and ammonion ions, formed by rearrangement, occur with the amines.[17] The resolution of the mass spectrometer was not sufficient to distinguish between like nominal masses containing the oxygen and nitrogen atoms. One process by which H_2O could be formed would be the same as that in eq. 5. The only change involves the charge going with the water fragment. Very probably some of these ion currents are due to residual water adsorbed on the solid acids. No positive means of distinguishing between rearranged H_2O and residual H_2O is available.

Fragmentation when R Contains Hetero Atoms

The introduction of an aryl, hydroxy or carboxyl substituent or a sulfur atom in the R group greatly alters the fragmentation processes of the amino acids. The reason for this can be summarized by stating that the electron removed upon electron impact is no longer primarily one of the electrons from the unbonded pair from the nitrogen atom with the resultant localization of the charge on this atom and the adjacent α-carbon atom.

Aryl Substituents.—The three aryl substituted acids studied are phenylalanine, tyrosine and tryptophan. The mass designations of the fragments from

(16) J. Asselineau, R. Ryhage and E. Stenhagen, *Acta Chem. Scand.*, **11**, 196 (1957).

(17) J. Collin, *Bull. soc. roy. sci., Liege*, **21**, 446 (1952).

tyrosine were checked repeatedly, but the expected major ion currents due to simple bond ruptures were invariably displaced one mass unit higher. This indicates that hydrogen migration is extensive. Thus tyrosine has a major ion current of 108 instead of 107. It also has an ion current at 137 comparable in intensity to the one observed at 136 (P − COOH). The reason for this unique behavior of tyrosine is not readily apparent.

A significant observation is the high 74 ion current (27%) from phenylalanine while tyrosine and tryptophan have low intensity 74 ion currents. The reason for this is the greater tendency of the (R–CH_2) fragments from tyrosine and tryptophan to accommodate the positive charge. Biemann, *et al.*,[13] give a table of the relative intensity of the amine, ester and (R–CH_2) fragments from the amino acid esters which pointedly demonstrates the increasing π-electron density in the ring through the series phenylalanine, tyrosine and tryptophan. The enhanced electron density increases the ability to accommodate the charge. The ester results and those for the acids are compared in Table VII.

TABLE VII

RELATIVE ABUNDANCE OF SELECTED PEAKS IN THE SPECTRA OF AROMATIC ACIDS AND ESTERS

	Acids			Esters[a]		
	Phe	Tyr	Try	Phe	Tyr	Try
Amine type peak	74	3.5	3.4	100	51	8.5
Ester type peak[b]	100	6.3	2.0	83	52	4
$(ArCH_2)^+$[c]	55	100	100	23	100	100

[a] Taken from Biemann, *et al.*[13] [b] Mass 74 for the acids and 102 for the esters. [c] $(ArCH_3)^+$ for tyrosine and tryptophan.

Hydroxy Substituents.—Two hydroxy substituted acids, serine and threonine, were studied. The relative amounts of the fragments discussed here are given in Table VIII. Ruptures at bond b are favored if the molecule-ion is formed by the removal of either an electron from the amino nitrogen or an electron from the hydroxyl oxygen. The presence of the hydroxyl group also enhances hydrogen rearrangements as reflected by the intense 75 ion currents and the formation of H_2O ions.

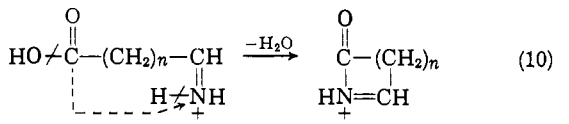
TABLE VIII

SELECTED IONS IN THE MASS SPECTRA OF SERINE AND THREONINE

Fragment	Serine	Threonine
$(P - COOH)^+$	12.8	5.7
$(P - H_2O)^+$	—	0.3
$(P - COOH - H_2O)^+$	5.4	3.7
$(75)^+$	5.1	18.2
$(H_2O)^+$	25.5	6.5

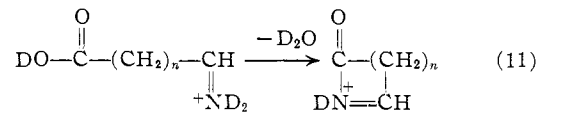
The relative amount of the amine fragment from serine at mass 60, where no interfering ion fragment can be formed readily, is 12.8% while the sum of the amine and the interfering $[CH(NH_2)COOH]$ fragments from threonine at mass 74 is only 5.7%. The elimination of H_2O from both amine fragments occurs to form significant amounts of mass 42 and 56 fragments, respectively. Also, H_2O is eliminated from the 75 fragments from both acids by the same process as that described earlier in eq. 5. Threonine exhibits a *meta* ion current at 43.3 to support the $75^+ \rightarrow 57^+ + 18$ process. The ion resulting from the elimination of the elements of water from the parent ion is small from both acids which indicates that either the ionic structure of $(P - H_2O)$ is unstable and rapidly decomposes to other products or it is formed to only a limited extent.

Carboxylic Acid Substituents.—The unimolecular decomposition processes which cause the large $(P - COOH - H_2O)$ ion currents with the di-acids were studied. It has been suggested[13] that the ion formed from aspartic ester is a four-membered ring structure and the ion from glutamic ester is a five-membered ring structure. These cyclic structures can be formed readily from the acids if the elements of water are eliminated from the (P − COOH) fragment with the OH radical coming from the acid group and the H radical coming from the amino group. The general equation for the reaction is



(10)

Energetic considerations for this mechanism are favorable due to the formation of the carbon–nitrogen bond during the decomposition. It was possible to check the mechanism by replacing the carboxyl and amino hydrogens with deuterium and then comparing the mass spectra of the normal acids with that of the tagged acids. The mass 70 fragment from aspartic acid shifted to mass 71 in aspartic acid-d_4 and the mass 84 fragment shifted to mass 85 in glutamic acid-d_4. The general reaction given in 11 shows that the observed mass shifts correspond to the elimination of the elements of water as outlined earlier. These results are offered as experimental evidence for the existence of the $(P - COOH - H_2O)$ ions in the cyclic state.



(11)

$m/e = 105$; $n = 2$ $m/e = 85$; $n = 2$

$m/e = 91$; $n = 1$ $m/e = 71$; $n = 1$

Table IX gives the relative amount of the (P − COOH), $(P - H_2O)$, $(P - COOH - H_2O)$ and (H_2O) fragments from the two di-acids. The values listed reflect the greater tendency of glutamic acid to eliminate water. This is reasonable in view of the stability of a 5-atom cyclic structure from glutamic acid compared to a 4-atom cyclic structure from aspartic acid.

TABLE IX

SELECTED VALUES FROM DI-ACID SPECTRA

Fragment	Aspartic	Glutamic
$(P - COOH)^+$	9.5	0.2
$(P - H_2O)^+$	0.2	1.9
$(P - COOH - H_2O)^+$	6.5	26.8
$(H_2O)^+$	13.3	18.9

Acetic acid ions at mass 60 contribute less than 1% of the total ion intensity from both dicarboxylic amino acids. This observation is somewhat surprising in view of the strong tendency to form this ion from the aliphatic acids.[14]

Sulfur Substituents.—Methionine and cystine were studied. The mass spectrum of cystine is not unique and reproducible. Observations indicate that cystine decomposes thermally and that the ion currents observed are those of the decomposition products. Methionine, however, gives a unique and reproducible mass spectrum. Ions of significant intensity result from the rupture of the bonds α and β to the sulfur atom as well as β to the nitrogen atom. The comparatively strong parent ion intensity (4.2%) reflects the ability of the sulfur atom to accommodate the positive charge. The $(P - H_2O)$ ion current at mass 131 is of moderate intensity. It fragments further to produce an ion at mass 83 and the CH_3SH neutral fragment. The appearance of the most intense ion current at mass 61, $(CH_3–S–CH_2)$, shows that most of the parent ions are

probably produced by removal of one of the sulfur electrons. The observed 74 and 75 ion currents are due to (CH_3–S–CH_2–CH) and (CH_3–S–CH_2–CH_2) fragments rather than (CH–(NH_2)COOH) and (CH–(NH_2)C($OH)_2$) fragments. Ion currents at masses 76 and 77 in the ratio of the sulfur isotopes were used to identify these fragments. The appearance of an ion current at mass 104 indicates that some molecule-ions are formed by removal of one of the nitrogen electrons. The preferred fragmentation of these molecule-ions results in the (P − COOH) fragments at mass 104. Very probably, the 56 ion current is due to the elimination of CH_3SH from the amine fragment as proposed by Biemann, *et al.*[13] The mass 57 fragment (CH_2—CH_2—CH=NH_2) is formed by a simple bond rupture of the amine fragment at one of the bonds α to the sulfur atom. The 55 ions are formed by the loss of two hydrogens from 57 or CH_3SH and H from the amine fragment.

The only ion of significant intensity formed in the sulfur atom range is at mass 35 (1.3%). The observed 35/37 ratio is in the $^{32}S/^{34}S$ ratio indicating that the 35 is H_3S^+. This was surprising since the formation of this fragment requires the rupture of two bonds and the migration of three hydrogen atoms. The absence of an ion current corresponding to H_2S ruled out the possibility that the H_3S was the result of a collision process.

The above consideration of the fragments from methionine shows that the presence of a single sulfur atom in the R group can readily be detected by the tendency for bond ruptures α and β to the sulfur atom as well as β to the nitrogen atom. Ion currents due to the sulfur isotopes help to indicate the presence of this atom.

Parent Ions.—These ions serve to establish the molecular weight of the amino acids and are of low intensity in all cases. Methionine, phenylalanine and tryptophan, with parent ion intensities of 4.2, 2.0 and 1.6%, respectively, are the only parent ions whose intensities are greater than 1% of the total ion yield. No (P + 1) ion currents[15-18] are observed from the amino acids at the low source operating pressures used for this work.

Conclusions

The conclusions concerning the mass spectra of the amino acids are in essential agreement with those summarized by Biemann, *et al.*,[13] who worked with the ester derivatives. Reference is made to their publication for listing of uses of the mass spectrometer in amino acid studies.

A satisfying similarity exists between the amount of the mass fragments and the interpretation of the mass spectra of the acids and the ester derivatives. Many of the notable differences such as: (1) the absence of the (P + 1) ion currents from the acids; (2) the more extensive rearrangement ion currents from the acids; and (3) minor differences in the amount of the fragments resulting from the rupture of the amine fragment, may be attributed to the different operating conditions of the mass spectrometers used. The most notable difference in the spectra of amino acids and their esters is the intensities of the (R), (P − R) and (P − R + H) ion currents. Evidently the destabilization of the charge on the α-carbon atom and the amino nitrogen referred to by Biemann, *et al.*,[13] is not as effective in the amino acids as in their esters. This is reflected in the general greater amount of the 74 and 75 fragments from the acids compared to the analogous 102 and 103 fragments from the esters.

To a large extent, the mass spectra of the amino acids can be best explained by assuming localization of the charge on one of the functional substituents.

The low vapor pressure of the amino acids has classically been attributed to their zwitterion character. Close examination of the (P − COO) ion currents in the mass spectra of the amino acids and deuterated amino acids reveals that the acids do not exist as an inner salt in the vapor state. As the tables indicate, this ion current is non-existent in most of the cases studied and of such low intensity in the cases where it is observed that its significance is doubtful. Loss of the inner salt structure when the vapor molecule is bombarded with the ionizing electron must be admitted as a possible explanation of the absence of appreciable (P − COO) ions.

Close comparisons of the normal acid spectra to the deuterated acid spectra also reveal that the extent of randomization of the hydrogen and deuterium atoms upon ionization and subsequent fragmentation with or without rearrangement is negligible.[19,20] In fact the limited degree of apparent randomization, indicated by comparing the spectra, can be attributed to incomplete deuteration of the amino group rather than randomization.

A subsequent publication will deal with the quantitative analysis of two- to five-component mixtures of the simple aliphatic amino acids. Intimate solid mixtures of these acids behave ideally and it is possible to use the large (P − COOH) ion currents to obtain quantitative results.

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