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The Mass Spectra of the α -Amino Acids¹

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Abstract--The mass spectra of the α -amino acids have been obtained in a mass spectrometer equipped with a heated crucible ion source. Correlations of these data have been made with the molecular formulae of the ion fragments, and mechanisms leading to these fragments 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

Anderson² and Biemann et al.³ have reported the 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

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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 Cameron⁴ 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 furnace capable of heating the ion chamber to over 500°C 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 1/2" 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 analyser and a magnetically operated isolation valve of our own design was placed between the analyser 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 three hours. Then the analyser 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°C. Using this method, background ion currents were not observed except for small currents corresponding to less than 10^{-13} amperes 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 used as the voltage calibrating gas.

Deuterations of the carboxyl and amino hydrogens were carried out in an excess of 99+% D_2O . Approximately 2×10^{-4} moles of each acid were dissolved in 7×10^{-2} moles of D_2O . Open vessel and sealed tube deuterations at temperatures of 20 to 80°C and for times of up to 40 hours were employed. The deuteration conditions employed did not appear to affect the extent of the exchange as determined from the mass spectral patterns. In all probability the exchange of the two amino hydrogens and the acid hydrogen was completed almost instantaneously after dissolution.

Results and Discussions

Molecular Ionization

Sharkey et al.⁵ have summarized some of the evidence for appreciable localization of the charge on the oxygen atom of several oxygen-containing compounds. Beynon et al.⁶ discuss the increase in valence of several oxygen and nitrogen compounds. Collin⁷ has

proposed localization of the charge on the nitrogen atom when amines are ionized. Chupka and Berkowitz⁸ placed the charge on the nitrogen atom in their study of the unimolecular decomposition of alkyl amines. It seems reasonable that the same localization of the charge on either one of the oxygen atoms or the nitrogen atom occurs in the saturated amino acid molecule-ions which contain no hetero-atoms in the R group. Table I gives the corrected appearance potentials of the parent ions $(P)^+$ and the parent-minus-carboxyl ions $(P - COOH)^+$ of glycine, isoleucine and methionine.

The average measured A.P. of 9.5 electron volts (ev) agrees quite well with the average A.P. of 9.4 ev for thirteen aliphatic amines tabulated by Field and Franklin.⁹ This indicates that the amino acid parent-ions are probably produced by a process similar to that which occurs in the aliphatic amines. Table I also gives the average A.P. of four aliphatic acids and 13 hydrocarbons taken from the tables of Field and Franklin. These values are included in the table to serve as a posteriori evidence that the electron removed by electron impact of the amino acid molecule is one from the unbonded pair on the nitrogen atom. The measured appearance potentials for the amino acids are too low to allow for removal of one of the bonded electrons of the hydrocarbon end of the molecule or one from the unbonded pairs on the carboxyl oxygen atoms. It must be admitted that other electrons may be removed at the high electron energy (75 v) used to secure the mass spectra tabulated in Table II, but the dominant

TABLE I
APPEARANCE POTENTIAL VALUES

Sample	(Parent) ⁺		(P - COOH) ⁺		(75) ⁺
	Calc. ^a	Meas. ^b	Meas. ^b	Meas. ^b	
glycine	9.18	9.5	10.1	-	-
isoleucine	8.96	9.5	9.9	9.9	9.9
methionine	8.87	9.5	10.2	-	-
carboxylic acids	10.50 ^d	10.7 ^c	-	-	-
saturated 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.¹⁰

^b The measured values are reproducible to ± 0.2 ev. The estimated accuracy is -0.2 to -0.4 ev.

^c Average measured values taken from Field and Franklin.⁹

^d Average calculated values taken from Franklin.¹⁰

process with the saturated amino acids is the removal of one of the nitrogen electrons, that is, the most energetically favorable process.

A reasonable mechanism which accounts for the large (P - COOH)⁺ ion currents with the saturated aliphatic amino acids can readily be developed. The parent molecule absorbs sufficient energy from the impacting electron to remove one of the electrons of the unbonded pair on the nitrogen atom. Additional absorbed energy produces the parent ion, with the charge essentially localized on the nitrogen atom, in an excited state. This excitation energy is available as activation energy for the unimolecular decomposition of the parent ion to produce a

CONDENSED FRAGMENTATION PATTERNS IN PERCENT TOTAL ION YIELD $\times 10$

	gly	gly-d ₃	ala	ala-d ₃	i-but	but	but-d ₃	n-val	val	val-d ₃	cyclo-leu	n-leu	leu	leu-d ₃	i-leu	iso-leu-d ₃	pro-line	ser.	thereo.	asp. acid	glu. acid	trypt.	tyro.	ph. ala	ph. tyro.	ph. onine
14	5	5	5	3	4	2	1	1	1	2	1	1	-	-	-	1	2	2	4	1	5	2	1	1	1	
15	2	2	26	15	29	15	6	10	5	2	11	7	7	4	4	2	13	20	8	16	4	1	6	2	5	
17	5	4	7	6	9	4	3	3	2	2	2	3	2	1	1	1	2	43	13	30	38	31	16	1	11	
18	10	18	117	13	59	37	3	26	11	4	18	24	20	3	22	5	12	255	65	132	189	173	222	7	48	
19	4	14	3	28	1	6	5	3	8	1	1	2	2	5	2	5	-	4	8	2	1	-	-	1	1	
20	-	13	-	131	-	-	25	-	-	9	-	-	-	15	-	16	-	-	-	-	1	-	-	-	-	
21	-	4	-	8	-	-	11	-	-	5	-	-	-	6	-	6	-	-	-	-	-	-	-	-	-	
26	2	2	8	7	5	11	7	4	2	1	5	4	1	2	3	5	3	2	2	9	7	6	7	-	2	
27	16	5	29	26	14	33	21	35	26	19	22	36	26	24	25	20	20	7	16	21	20	18	19	2	18	
28	140	46	99	25	76	149	24	89	85	20	54	72	52	13	66	17	121	142	81	200	147	155	177	40	76	
29	9	112	18	69	10	41	119	37	57	71	15	34	22	49	73	87	5	44	115	16	15	18	15	4	16	
30	675	48	6	21	27	66	24	215	34	25	38	177	138	11	86	23	17	30	36	16	9	14	21	9	18	
31	14	72	1	9	2	2	23	4	1	31	1	5	2	28	2	30	1	19	4	2	1	11	2	-	-	
32	-	538	-	3	-	-	61	-	-	26	-	-	-	125	-	72	1	3	-	2	-	2	3	-	1	
33	-	32	-	2	-	-	2	-	-	1	-	-	-	3	-	2	-	-	-	-	-	-	-	-	1	
34	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	
39	-	-	4	2	24	14	13	16	21	16	26	17	17	15	17	13	26	2	4	7	8	10	14	8	16	
40	1	1	11	3	17	4	3	3	3	3	7	3	3	3	2	3	8	2	1	8	4	2	3	1	1	
41	2	3	18	8	71	94	90	18	23	19	53	47	37	30	54	44	87	11	5	24	76	24	19	5	16	
42	6	4	59	14	158	21	9	22	11	5	48	20	20	8	8	6	28	54	9	51	9	10	13	9	16	
43	2	1	21	38	24	26	12	23	19	16	9	35	58	52	8	7	102	21	18	85	5	14	14	7	10	
44	18	6	491	30	9	13	12	33	6	8	4	49	160	17	26	7	6	26	17	69	24	51	58	2	13	
45	22	1	33	51	17	11	23	11	8	3	11	10	8	25	6	5	9	7	48	20	10	11	5	1	27	
46	3	15	10	413	1	16	12	14	15	9	1	12	9	133	11	23	-	12	8	27	2	2	2	16	14	
47	1	-	1	77	-	1	8	1	1	-	-	-	-	11	-	1	-	1	1	-	-	-	-	-	27	
48	-	1	-	3	-	-	1	-	-	2	-	-	-	2	-	1	-	-	-	-	-	-	-	-	11	
49	-	1	-	7	-	-	15	-	-	12	-	-	-	9	-	8	-	-	-	-	-	-	-	-	7	
54	1	-	1	2	4	-	4	5	1	43	3	2	1	5	2	2	-	3	2	10	2	2	-	-	7	
55	1	-	4	3	1	2	3	20	84	76	35	4	3	3	5	5	2	2	3	7	15	9	8	-	35	
56	-	1	3	1	5	16	3	12	31	9	26	16	7	3	23	5	1	2	37	6	43	11	8	2	73	
57	-	1	1	2	10	8	9	12	80	15	13	13	17	5	90	18	-	33	181	4	5	16	10	1	20	
58	-	-	-	378	325	12	1	10	41	1	1	1	1	12	13	45	-	4	20	1	1	3	2	-	3	
59	-	-	-	17	13	19	-	1	46	1	-	-	2	9	1	48	-	2	5	-	-	1	-	-	7	
60	-	-	-	1	1	322	-	-	2	-	-	-	2	-	-	5	-	128	5	6	1	2	2	9	3	
61	-	-	-	-	-	13	-	-	6	-	-	-	-	-	-	5	-	2	-	-	-	-	-	-	140	
65	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	2	3	28	-	
67	-	-	-	-	-	-	-	-	-	-	47	2	1	1	2	1	3	-	-	1	1	2	2	-	-	
68	-	-	-	-	-	-	-	-	-	-	5	1	2	1	2	1	40	-	-	2	2	1	3	-	-	
69	-	-	-	-	1	1	1	1	1	-	3	17	5	4	25	25	22	1	1	3	1	2	2	-	1	
70	-	-	1	-	2	1	1	4	4	-	2	3	9	2	7	3	434	1	-	65	1	2	2	-	3	
71	-	-	-	-	1	1	1	1	1	-	2	2	2	4	2	2	20	1	-	3	-	2	1	-	1	
72	-	-	1	-	1	-	-	276	278	3	-	-	-	6	1	4	-	1	4	2	1	3	2	-	-	
73	-	-	1	-	-	1	-	14	14	26	-	2	2	1	-	-	1	-	1	1	4	2	1	-	2	
74	-	-	-	-	-	1	-	61	94	277	1	57	79	1	67	-	2	73	57	14	2	4	9	269	35	
75	40(P)	-	-	-	-	3	-	5	44	13	-	1	7	14	2	96	1	-	51	182	-	3	7	49	-	
76	2	-	-	2	-	-	4	-	2	14	-	-	-	11	4	9	-	1	-	-	-	1	1	2	2	
77	-	3	-	10	-	-	61	-	-	95	-	-	-	66	-	80	-	-	-	-	-	13	17	20	2	
78	-	17(P)	-	-	-	-	2	-	-	42	-	-	-	11	-	100	-	-	-	-	-	2	5	5	-	
82	-	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	1	1	-	-	
83	-	-	-	-	-	-	-	-	-	-	8	2	-	-	-	-	-	-	-	1	9	1	1	-	27	
84	-	-	-	-	-	-	-	-	2	-	377	2	-	-	2	-	-	-	1	4	268	3	1	-	2	
85	-	-	-	-	-	-	-	-	2	-	24	-	-	-	-	1	-	-	-	1	14	4	1	-	2	
86	-	-	-	-	-	-	-	1	-	-	1	271	232	1	208	1	1	1	-	1	3	1	-	-	3	
87	-	-	-	-	-	-	-	-	-	-	10	16	14	23	13	16	5	2	-	8	1	1	1	-	13	
88	-	-	-	-	-	-	-	-	-	-	1	1	2	214	-	215	-	-	-	95	-	-	-	-	14	
89	-	1(P)	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	4	-	2	1	-	1	
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	10	148	-	
92	-	-	1(P)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	49	-	
101	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-	2	-	-	-	-	-	24	
103	-	-	-	-	0.3(P)	0.3(P)	-	-	-	-	-	-	-	-	-	-	-	-	6	-	2	13	-	24	1	
104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	24	-	
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03(P)	-	-	-	-	1	-	1	
106	-	-	-	-	-	-	-	0.2(P)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
108	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	142	-	-	
109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-	
115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8(P)	-	2	-	3	-	-	-	
116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	4	17	
117	-	-	-	-	-	-	-	0.3(P)	0.1(P)	-	-	-	-	-	-	-	-	-	-	-	-	7	-	8	2	
119	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1(P)	-	-	-	-	1	8	-	
120	-	-	-	-	-	-	-	-	-	-	0.1(P)	-	-	-	-	-	-	-	-	-	-	-	2	199	-	
121	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	18	-	
129	-	-	-	-	-	-	-	-	-	-	0.01(P)	-	-	-	-	-	-	-	-	-	-	19	-	-	-	
131	-	-	-	-	-	-	-	-	-	-	-	2(P)	3(P)	-	3(P)	-	-	-	-	-	-	212	1	-	33	
132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	6	
133	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.001(P)	-	-	-	-	-	2	
134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
149	-	-	-	-	-	-	-	-																		

fragment ion and one or more neutral species. The particular decomposition which occurs is dependent on the amount of excitation energy absorbed, the energy of the products of the reaction as compared to the parent ion, and the activation energy necessary for the reaction to occur.^{9,11} Since the $(P - \text{COOH})^+$ ion was observed in the mass spectrometer, it can be assumed that the activation energy is relatively small resulting in a very rapid reaction. Thus a plausible explanation is needed which is in agreement with experimental observations, of why the products, $(P - \text{COOH})^+ \cdot (\text{COOH})$ are more energetically favorable than $(P)^+$. The parent ion structure and the reaction it undergoes is given in Eq. (1) where R designates an alkyl group.

Rupture of one of the bonds β to the NH_2 group (bond a) is the only reasonable process whereby a $(P - 45)^+$ ion current could be produced. This occurs to a considerable extent because the rupture of this bond with the concurrent formation of a π bond between the α carbon atom and the amino nitrogen effectively reduces the charge on the nitrogen by allowing a greater inductive shift of the bonding electrons towards the charged nitrogen atom. The result is a molecule-ion with the charge localized on the nitrogen and the adjacent α carbon atom. The average A.P. of 10.0 ev for the $(P - \text{COOH})^+$ fragment from glycine and isoleucine is offered as supporting evidence for the above interpretation. The energy required to rupture bond a is estimated

to be at least 3.0 ev. Simple breaking of this bond should have resulted in an A.P. for the $(P - COOH)^+$ fragment of > 12.5 ev. The difference of only 0.5 ev. between the A.P. of the $(P)^+$ ion and that of the $(P - COOH)^+$ fragment thus requires that a bond-forming process occurs during the fragmentation.

Other evidence that the charge is essentially localized is also available. Most of the fragment ion currents contain the nitrogen atom and the greatest proportion of the ions produced result from the rupture of bond a and/or bond b with the charge going either way but preferentially to the fragment which has the nitrogen atom.

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^9 is 9.4 ev. 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 ev, the same energy necessary to remove one of the nitrogen electrons. The measured A.P. of methionine parent ion was 9.5 ev which leaves no preference in this case for charge localization on either the sulfur or nitrogen.

The appearance of an ion current at $(P - COOH)^+$ suggests that a significant process which occurs is the removal of one of the nitrogen electrons. The resulting molecule-ion decomposes in much the same manner as that indicated for the simple aliphatic amino acids as illustrated by the glycine and isoleucine examples. However, a second significant process which occurs with methionine is the removal of one of the unbonded electrons on the sulfur atom. This latter type of

ionization can appreciably alter the fragmentation processes with respect to the aliphatic amino acids. If the charge is localized on the sulfur atom considerable bond breaking α and β to the sulfur atom would be expected. Close inspection of the fragmentation pattern of methionine (Table II) bears this out. (A detailed consideration of the fragments from methionine will be presented later.)

Unfortunately, instrumental difficulties prevented the observation of the A.P. of the aromatic amino acids and other substituted amino acids, but an inspection of their fragmentation patterns indicates that the mode of ionization is complicated by the unsaturated groups in tyrosine, phenylalanine and tryptophane, and the substituents on the alkyl group in threonine, serine, glutamic and aspartic acids.

Fragmentation When R is Alkyl

Table II shows the condensed fragmentation patterns¹² of the amino acids and the deuterated amino acids whose spectra have been established. Isotopic corrections have not been applied to the data tabulated in Table II but have been applied to all other tables in the text. The parent ions and those m/e ion currents whose magnitude is 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.

As mentioned in the discussion of molecule-ions, the presence of an unsaturated group or a hetero-atom in the R group leads to

molecule-ions in which the charge is not localized on the nitrogen atom. Because this complicates the fragmentation patterns the major fragment ions of the saturated aliphatic amino acids will be considered first, that is, where R is alkyl. Table III gives the comparative size of the amine fragment $(P - \text{COOH})^+$ as well as all the fragments produced by rupture of bond a and/or b,

The listed values illustrate the preferred rupture of bond a with the charge remaining on the nitrogen fragment. The systematic decrease in the intensity of the amine ion fragment 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 $(P - \text{COOH})^+$ fragment. The decrease in the relative amounts of the total number of ions produced by the rupture of a and/or b with the charge going to all possible fragments produced, also illustrates the larger number of favorable reaction paths for the molecules having greater numbers of atoms.

Several ionic species can be positively identified by comparing the normal acid spectra to the deuterated acid spectra (see Table II). However, exact comparisons of the spectra should not be made in all cases because of the complexity of some of the processes which occur and because of the possible differences in the fragmentation patterns of molecules in which deuterium is substituted for protium.¹³ In some instances, the process by which the fragment ion is formed

TABLE III^a
 FRAGMENTS FROM RUPTURE OF BOND a AND/OR b.
 PERCENT OF TOTAL ION CURRENT

Sample	(P - COOH) ⁺	(R) ⁺	(P - R) ⁺	(COOH) ⁺	(P - R - COOH) ⁺	Total %
glycine	68.9	0.0	0.1	2.2	0.5	72
alanine	50.5	2.6	1.2	1.9	1.7	58
butyric	33.8	3.9	5.9	1.1	- ^b	45
isobutyric	39.2	2.6	1.9	1.7	2.0	47
valine	29.2	2.0	9.7	0.8	5.7	47
norvaline	29.0	2.3	6.3	1.0	3.6	42
leucine	24.5	1.0	8.2	0.3	2.1	36
norleucine	28.7	1.0	5.9	0.9	3.3	40
isoleucine	22.1	1.0	6.9	0.5	3.3	34

^aThis table contains estimates when obvious interfering processes have occurred to produce the same m/e value.

^bSame m/e as R⁺.

has been indicated by the observed metastable ion current. Several discussions of the fragment ions from the saturated aliphatic amino acids which are tabulated in Table II are given below.

Mass 75. - The ionic species which are responsible for this ion current can be either (CH₂NH₂COOH)⁺ [A] or (CH-NH₂C(OH)₂)⁺ [B]. Arguments in favor of the latter structure will be given later. The observed ion current results from a specific single rearrangement

as defined by McLafferty.¹⁴ The process proceeds by an odd-electron ion going to an odd-electron ion plus an even-electron molecule. Its formation could be analogous to the process by which the mass 60^+ ion current is produced from the aliphatic acids.¹⁵ The shift of the mass 75^+ ion current to the mass 78^+ ion current in the deuterated amino acid spectra confirms the identity of the ion. Similar to what has been observed for the 60^+ ion current from propionic acid,¹⁵ alanine has no 75^+ rearrangement ion current. A large number of 60^+ ions due to rearrangement has been observed with butyric acid. However α -amino-n-butyric acid has only an insignificant number of 75^+ ions. This observation indicates that the rearrangement process in the amino acids may be different than in the aliphatic acids. A further consideration of why only valine and isoleucine have appreciable amounts of this ion fragment will be given below in the discussion of the major rearrangements.

Mass 69. - Ion currents of the same relative order of magnitude at mass 69 for isoleucine and isoleucine- d_3 indicate that this species contains neither the amino group nor the acid group. Also, the metastable ion currents observed from isoleucine and isoleucine- d_3 give evidence that the ionic species is a hydrocarbon rather than a keto type fragment such as $(CH_3-CH=CH-C=O)^+$. The observed metastable ion current from isoleucine is at mass 55.5. This mass corresponds to part of the large number of 86^+ ions decomposing unimolecularly after ion acceleration to 69^+ ions. Equation (2) shows the proposed reaction.

The corresponding decomposition after acceleration of part of the 88^+ ions from isoleucine- d_3 should also give mass 69^+ ions as shown in Eq. (3). However, the metastable ion current should occur at

mass 54.1 in this case. An unresolved tail was observed on the high mass side of the 54^+ ion current which suggests that a metastable ion current at approximately mass 54 contributes to the normal 54^+ ion current. Thus the 69^+ fragment is a hydrocarbon formed by loss of the elements of NH_3 from the amine fragment. This observation agrees with the results of Biemann et al.¹⁶

The failure of leucine to show a significant amount of this fragment is attributed to a different activation energy for the unimolecular decomposition and hence a different rate of reaction due to the change in structure of the hydrocarbon end of the molecule. It is significant, though possibly fortuitous, that the sum of the relative intensities of the 69^+ and 86^+ ion currents from isoleucine equals the relative intensity of the 86^+ ion current from leucine. This suggests that the activation energy for the process in Eq. (2) is higher in the case of leucine and enough higher that the reaction did not occur during the average lifetime of the 86^+ ion in the mass spectrometer.

Mass 57. - The appearance of a meta ion current at mass 43.3 for isoleucine is caused by the unimolecular decomposition of the 75^+ ion, after acceleration, to the 57^+ ion and a neutral fragment or

fragments equal to mass 18. The isoleucine- d_3 spectra shows one meta ion current at 44.6^+ and a second one at 43.1^+ . The 44.6^+ ion current is formed by the process given in Eq. (4) where the accelerated 78^+ decomposes to $59^+ + 19$ before it gets into the magnetic field.

The 43.1^+ ion current forms by the process given in Eq. (5) where 78^+ yields $58^+ + 20$ after acceleration. The explanation deduced from a consideration of the observed meta ion currents is further supported by the shift of the 57^+ ion fragment from isoleucine to masses 58^+ and 59^+ in isoleucine- d_3 .

Valine also shows a large 57^+ ion current but the meta ion currents from valine and valine- d_3 are so diffuse that it is impossible to assign accurate fractional mass numbers in all cases. However, the similar behavior of the 57^+ ion fragment when the valine spectra is compared to the valine- d_3 spectra suggests that the same process associated with isoleucine occurs with valine. It is noteworthy that isoleucine and valine, both of which have large 75^+ ion currents, also have large 57^+ ion currents, while all the other acids, with the exception of glycine, have small 75^+ and 57^+ ion currents. Glycine has a 75^+ ion current of a relative intensity (3.9%) which is comparable to that observed for valine (4.2%), but glycine has no significant 57^+ ion current. Its absence can be attributed to the different

molecular structures shown in Eqs. (6), (7) and (8). Equation (6)

shows the process whereby the 75^+ ion is formed from glycine and Eqs. (7) and (8) show the process whereby the 75^+ ion is formed from valine and isoleucine, respectively. The 75^+ ionic structure given in (6) refers to structure [A] and those given in (7) and (8) refer to structure [B], given earlier under the consideration of the 75^+ ion currents. The A.P. of the 75^+ ion from isoleucine is 9.9 ev. This value can be used to develop arguments to support either structure [A] or [B] given earlier. However, the failure of glycine to show a 57^+ ion current leads us to support structure [B].

Biemann et al.¹⁶ published an interpretation of the mass spectra of the ethyl esters just as this work with the acids was being completed. Their results with the N¹⁵ labelled leucines indicate that the 57⁺ is a hydrocarbon formed by the decomposition of the amine fragment.

The reaction is $86^+ \rightarrow 57^+ + 29$ with a meta peak at 37.8. This mechanism is not supported by the evidence presented here since the 57^+ ion current shifted about equally to 58^+ and 59^+ in the deuterated acids. Also, the meta ion current at 43.3 from isoleucine and valine and at 44.6 and 43.1 from isoleucine- d_3 confirms the process $75^+ \rightarrow 57^+ + 18$.

The absence of a 37.8 metastable ion current and the behavior of the deuterated spectra eliminates the possibility that the 57^+ could be the amine type fragment $(CH_3CHCH-NH_2)^+$ formed by the process given in Eq. (9).

This process should lead to the probable observation of a meta ion current at 37.8 with isoleucine and 45.1 with valine. In addition a complete shift of the 57^+ ion current to 59^+ in the deuterated spectra of valine and isoleucine should have been observed. Neither of the meta ion currents nor the mass shift was observed.

Mass 55 . - This species is present in all the acids and with only a few obvious exceptions it is primarily a hydrocarbon. Valine has the only large mass 55^+ ion current and the process whereby it is formed is the elimination of NH_3 from the amine fragment as shown in Eq. (10).

No meta ion current was positively identified to confirm the above process but the failure of the 55^+ ion current to shift in valine- d_3 shows that the species did not contain the nitrogen atom. Also Biemann et. al. observed considerable elimination of the elements of ammonia from the amine fragments of the ethyl ester of valine which supports this interpretation.

Mass 46 . - All the 46^+ ion currents of the normal acids are undoubtedly due to rearrangements. The ionic 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 could account for the observed mass shift. These are shown in Eqs. (11) and (12). The process in (11) is preferred because this process 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.

Mass 41 . - This species was observed to a considerable extent in all the amino acids and its intensity did not shift in the deuterated acids. The ionic mass is most certainly a keto type fragment from glycine and probably from alanine also. With the other acids the observed

ion current is probably due to the sum of keto and hydrocarbon type fragments. No meta ion currents are available to check the fragment designations and the modes of production. Butyric and isobutyric amino acids show the most intense mass 41^+ ion currents. This is due to the process whereby the amine fragment loses the elements of ammonia.

Masses 28 and 30 . - The shift of most of the 28^+ ion current to mass 29 in the deuterated acids suggests that the major species which causes the 28^+ ion current in the normal acids is $(\text{HC}=\text{NH})^+$. This interpretation is somewhat tenuous because of the 28^+ due to CO^+ from glycine and alanine and CO^+ plus hydrocarbon from all the other amino acids.

The shift of most of the 30^+ fragment to mass 32 in the deuterated acids indicates that the species is either $(\text{H}_2\text{C}=\text{NH}_2)^+$ or $(\text{CH}-\text{NH}_3)^+$ formed by a rearrangement process. The 30^+ fragment could be formed by fragmentation of the parent ion at bonds a and b with rearrangement of one hydrogen. This process would be similar to that observed by Collin¹⁷ with some amines substituted on the α carbon atom. The 30^+ fragment could also arise by a process of ring formation¹⁸ made possible by the increase in the valence of the nitrogen atom when it is ionized. The simple rupture of two bonds in the ring compound would produce a mass 30^+ fragment directly. However, the formation and fragmentation of the ring requires considerable bond-making and bond-breaking processes and therefore the more direct

route of two bond ruptures and a single hydrogen rearrangement seems the more probable.

Masses 18 through 22 . - The appearance of m/e values of 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 acids¹⁵ and NH_4^+ , formed by rearrangement, occurs in the amines.¹⁷ The resolution of the mass spectrometer was not high enough 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 given under the discussion of the 57^+ ion current. The only change in Eqs. (4) and (5) involves the charge going with the water fragment. Very probably some of the water 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.

$(P - COOH - NH_3)^+$ 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 69^+ , 55^+ and 41^+ ions from isoleucine, valine and α -amino-n-butyric acid have already been discussed. Table IV summarizes the results for these and the other aliphatic amino acids which have been studied. The general reaction for the formation of the hydrocarbon ion-currents is

TABLE IV
TENDENCY TO ELIMINATE THE ELEMENTS OF AMMONIA

	$(P - \text{COOH})^+$	$(P - \text{COOH} - \text{NH}_3)^+$	$(P - \text{COOH} - \text{NH}_2)^+$
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
norval	29.0	2.0	1.2
leucine	24.6	0.5	0.8
norleu	28.4	1.7	0.2
isoleu	22.1	2.5	0.1

Since this fragment comes from the abundant $(P - \text{COOH})^+$ fragment, the relative intensity of this fragment has been included in the table for comparative purposes. As is illustrated in the table it is not possible to draw universally consistent correlations between structure and the extent of hydrocarbon ion formation by the process in (13). Biemann et al. state that the intensity of these ion currents is generally low unless the beta carbon atom has a branch or contains an aryl substituent as is the case in valine, isoleucine and phenyl-alanine. Their ion currents at 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 -amino-isobutyric

acid are 28 and 19% and neither of these acids has a substituent on the beta carbon atom.

(P - COOH - NH₂)⁺ Fragments. - Table IV also lists the intensity of these fragments. Where possible the deuterated spectra have been compared to the normal spectra to get an estimate of the intensity of this fragment. The intensity is low in all cases except α -amino-isobutyric acid. Here the greater intensity could be due to the increased stability of a hydrocarbon ion which has the charge on an inner rather than a terminal carbon atom. For example, the ion (CH₃-C⁺-CH₃) from α -amino-isobutyric acid has an intensity of 16.3% and the charge is on an inner carbon atom, whereas α -amino-n-butyric acid yields the fragment (CH₃-CH₂-CH⁺) with an intensity of only 0.6% and the charge is on the terminal carbon atom. The charge is localized on the terminal carbon atom with all the other amino acids which accounts for the low observed intensities.

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 the 30⁺, 44⁺, 57⁺ and 75⁺ ion currents from a number of aliphatic amino acids. The 30⁺, 44⁺ and 75⁺ ion currents cannot be formed by simple bond ruptures and must be the result of hydrogen migration. The 57⁺ arises by the elimination of the elements of water from the 75⁺. It is included in the discussion because its intensity reflects the tendency to form 75⁺. Alanine is included in the table to show the limited tendency of this acid to become involved in 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
norval	0.4	1.2	3.3	21.8	26.7
leucine	1.1	1.7	16.3	14.1	33.2
norleu	0.5	1.3	4.9	18.0	24.7
isoleu	9.6	9.3	2.7	8.6	30.2

It is tempting to attribute the large 75⁺ ion currents from valine and isoleucine to the availability of the labile tertiary hydrogen on the beta carbon atom. However, olefin elimination probably occurs in the process accounting for the low A.P. (9.9 ev) of the 75⁺ fragment from isoleucine. If the labile beta hydrogen migrates to the charged fragment then the additional concurrent migration of a second hydrogen in the neutral fragment is necessary before an olefin can be formed. This dual hydrogen migration process does not seem reasonable. The variation in the intensity of the 75⁺ and the 44⁺ rearrangement ion currents is best explained on the basis of steric effects. It is proposed that the large 75⁺ ion currents from valine and isoleucine are formed by a cyclic rearrangement process as suggested by McLafferty¹⁴ for the aliphatic esters. The valine and isoleucine examples were given earlier in Eqs. (7) and (8). Note that both valine and isoleucine have methyl substituents on the beta carbon atom.

The process proposed by Biemann et al. for the appearance of the large 44^+ ion current from leucine is reproduced in Eq. (14). Hydrogen rearrangement with olefin elimination occurs. Note that the same

steric effects exist here as in the mechanism proposed for 75^+ formation. The methyl substituent in both processes is on the carbon atom twice removed from the atom which has the substituent that accepts the migrating hydrogen. An olefin is readily eliminated in both cases.

The 30^+ from the leucines and valines could be formed by loss of CH_2 from the 44^+ fragment but this requires the rupture of too many bonds to occur to a considerable extent. The moderate 30^+ ion current of the amino-n-butyric acid, valine and isoleucine and the large 30^+ for leucine, norleucine and norvaline arises by olefin elimination as proposed by McLafferty¹⁴ and used by Biemann et al. to explain the occurrence of the 30^+ from the esters of the amino acids. The reaction is

The weak 30^+ ion current from alanine is attributed by Biemann et al. to be due to the lack of available γ hydrogens. However, it is difficult to give reasons for the relatively high 30^+ ion intensities from norleucine, norvaline and leucine and the moderate 30^+ ion current

from α -amino-n-butyric acid using this criteria. The extent of the 30^+ rearrangement ion current cannot be readily explained by considering the number of γ hydrogens available. Norleucine and norvaline, which have two γ hydrogens available, give intense 30^+ ion currents while α -amino-n-butyric acid which has three γ hydrogens gives a much weaker 30^+ ion current. The extent of the rearrangement apparently does not depend upon the lability of the γ hydrogen since the value for leucine which has a tertiary γ hydrogen is lower than for norvaline and norleucine which have less mobile secondary hydrogens. An explanation based upon the steric factors used to explain the 75^+ and 44^+ is subject to question since the energetically favorable olefin elimination cannot readily occur unless one of the γ hydrogens is involved in the rearrangement. Still, the possibility that the hydrogen which migrates need not necessarily be from the γ carbon position must be admitted. The mass spectra of leucines selectively deuterated on the γ position would prove fruitful in tagging the hydrogen which migrates to give the 30^+ ion fragment. A study of the rearrangement ions in the mass spectra of several deuterium labelled leucines or valines would clarify the interpretation of these rearrangements.

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^+ rearrangement ion currents; 2) γ methyl substituents encourage 44^+ ion formation and 3) 30^+ ion formation predominates in straight chain isomers.

The results of the 75^+ , 44^+ and 30^+ ion currents from 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 Stewart¹⁵ observed selective rearrangement ion currents from the aliphatic acids which were of a higher relative intensity than has been indicated by Sharkey et al.,⁵ Beynon et al.,¹⁹ and Asselineau et al.²⁰ in the investigations of the aliphatic esters.

TABLE VI
COMPARATIVE REARRANGEMENTS OF THE AMINO ACIDS
AND ESTERS

PERCENT OF THE AMINE FRAGMENT						
Sample	Acid 75^+	Ester 103^{+a}	Acid 44^+	Ester 44^{+a}	Acid 30^+	Ester 30^{+a}
leucine	1	1 ^b	66	50	58	37
norleu	1	0 ^b	18	10	64	45
isoleu	10	2 ^b	12	10	39	31
valine	4	2 ^b	2	2 ^b	12	10
norval	1	0 ^b	11	4 ^b	77	54

^aValues taken from Biemann et al.¹⁶ The 103^+ ion rearrangement from the esters is analogous to the 75^+ ion from the acids.

^bValues not listed in text and roughly estimated from fragmentation graphs.

It has been shown by Happ and Stewart¹⁵ that the carboxyl hydrogen is not involved in the formation of large rearrangement ion currents. The present results with deuterated amino acids also indicate that the carboxyl hydrogen is not involved in the formation of 30^+ , 44^+ and 75^+ rearrangement ion currents.

(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 higher than the loss of H from glycine. Evidently substitution on the α carbon atom, as in α -amino-isobutyric acid, lowers the probability of methyl loss and favors the rupture of bond a to produce the amine fragment.

The (P - R)⁺ fragment has an m/e value of 88 for α -amino-isobutyric acid. 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 an additional alkyl substituent. The evidence is not positive however since an alkyl substituent on the nitrogen atom could cause the same mass shift. Biemann et al. discuss more fully 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⁺ is small even if R is the stable ethyl, propyl or butyl radicals.

$(P - R - COOH)^+$ Fragment. - This fragment appears at mass 29 from all the amino acids given in Table IV 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 intensity of these fragments are offered other than to say that their general low intensity is due to the necessary rupture of two bonds to produce the $(CH-NH_2)^+$ fragments.

$(COOH)^+$ Fragments. - The intensity of this fragment is low 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. It is also additional evidence for localizing the charge on the nitrogen and α carbon atom rather than on one of the carboxyl oxygens.

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 intensities of this fragment with valine and norvaline are 29.2 and 29.0%, respectively, while the intensity with proline is 45.6% of the total ion yield. The intensities with isoleucine, norleucine and leucine are 22.1, 28.4 and 24.6% while the intensity with cycloleucine is 39.9%.

Fragmentation Where R Contains a Substituent or Hetero Atom

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 has been discussed earlier

under the consideration of the molecule-ions and can be summarized by stating that the electron removed upon electron impact is not 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 tryptophane. The mass designations for the fragments from 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 at 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 behavior in tyrosine is not readily apparent.

A significant observation is the high 74^+ ion current (27%) from phenylalanine while tyrosine and tryptophane have low intensity 74^+ ion currents. The reason for this is the greater tendency of the $(R-CH_2)^+$ fragments from tyrosine and tryptophane to accommodate the positive charge. Biemann et al. 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 increased π electron density in the ring through the series phenylalanine, tyrosine and tryptophane. 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		
	Phenyl	Tyro	Trypto	Phenyl	Tyro	Trypto
amine peak	74	3.5	3.4	100	51	8.5
ester peak	100	6.3	2.0	83	52	4
(ArCH ₂) ⁺	55	100	100	23	100	100

^aTaken from Biemann et al.¹⁶

Hydroxy Substituents. - Two hydroxy substituted acids, serine and threonine, were studied. 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 favors hydrogen rearrangements and the formation of H₂O⁺ ions. This tendency is reflected by the high relative intensity of the 18⁺ and 75⁺ ion currents. The amine fragment is of moderate intensity (12.8%) from serine. On the other hand this fragment is much less intense from threonine (5.7%) and it includes the intensity of the [CH(NH₂)COOH]⁺ fragment. Certainly some of this latter fragment is formed which makes the intensity of the (P - COOH)⁺ fragment somewhat less than the observed 5.7%. The elimination of H₂O from the amine fragments occurs to form significant amounts of mass 56⁺ fragments from threonine and mass 42⁺ from serine. Also, H₂O is eliminated from the 75⁺ fragments from both acids by the same process as that described for isoleucine and valine.

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. Table VIII gives the relative intensities of the fragments discussed.

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

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 suggested¹⁶ 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 proposed cyclic structures can readily be formed 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

Energetic considerations for this type of mechanism are favorable due to the formation of the carbon-nitrogen bond during the decomposition. The difficulty in measuring accurately and interpreting A.P. data of the di-acids rules out this as a test of the validity of the proposed ionic structures. However, it was possible to check the mechanism by replacing the carboxyl and amino hydrogens with deuteriums. The mass spectra of aspartic acid and glutamic acid were then compared to the mass spectra of the tagged acids. The intensity of the mass 70^+ fragment from aspartic acid shifted to mass 71^+ in aspartic acid- d_4 . The intensity of the mass 84^+ fragment shifted to mass 85^+ in glutamic acid- d_4 . The general reaction given in (17) 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 $(\text{P} - \text{COOH} - \text{H}_2\text{O})^+$ ions in the cyclic state.

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

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

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

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

Table IX gives the relative intensity of the $(\text{P} - \text{COOH})^+$, $(\text{P} - \text{H}_2\text{O})^+$, $(\text{P} - \text{COOH} - \text{H}_2\text{O})^+$ and $(\text{H}_2\text{O})^+$ fragments from the two di-acids. The

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

values listed reflect the greater tendency which glutamic acid has to eliminate water. This is reasonable in view of the stability of the 5-atom cyclic structure from glutamic acid compared to the 4-atom cyclic structure from aspartic acid.

The 60^+ ion current due to acetic acid ions is less than 1% of the total ion intensity from both dicarboxylic amino acids. This observation is somewhat surprising in view of the strong tendency for formation of this ion from the aliphatic acids.¹⁵

Sulfur Substituents. - The two acids studied were methionine and cystine. The mass spectrum of cystine is not unique and reproducible. Observations indicate that cystine thermally decomposes and that the ion currents observed are those from 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. A meta ion current at an m/e of 115.2 is observed to confirm the process $131^+ \rightarrow 83^+ + 48$. The appearance of the most intense ion current at mass 61, $(CH_3-S-CH_2)^+$, shows that most of the parent ions are produced by removal of one of the sulfur electrons. The observed 74^+ and 75^+ ion currents are $(CH_3-S-CH_2-CH)^+$ and $(CH_3-S-CH_2-CH_2)^+$ fragments rather than $(CH-(NH_2)COOH)^+$ and $(CH-(NH_2)C(OH)_2)^+$ fragments. The 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. However, methionine has a 58^+ ion current and the $58^+/56^+$ ratio is sufficiently close to the 34/32 ratio of the sulfur isotopes to suggest that the 56^+ fragment contains a sulfur atom. The 57^+ $(CH_2-CH_2-CH=NH_2)^+$ is formed by 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 $35^+/37^+$ is in the sulfur 32/34 ratio which indicates 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 show 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 tryptophane, 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^{19,21} 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.¹⁶ who worked with the ester derivatives. Reference is made to their publication for the use of the mass spectrometer in amino acid studies.

A satisfying similarity exists between the intensity 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 with the acids; 2) the more extensive

rearrangement ion currents from the acids; 3) minor differences in the intensity of the fragments resulting from the rupture of the amine fragment, may be attributed to the different operating conditions of the mass spectrometers used. One exception to this is the process whereby the 57^+ ion currents were formed from the leucines and valines. Acceptable evidence that the species was a hydrocarbon from the esters and a keto-amino fragment from the acids has been given. Evidently the destabilization of the charge on the α carbon atom and the amino nitrogen referred to by Biemann et al. is not as effective in the amino acids as in their esters. The general greater intensities of the 74^+ fragments from the acids compared to the analogous 102^+ fragments from the esters also indicate less destabilization of the charge due to the adjacent carboxyl group.

To a large extent, the mass spectra of the amino acids can be best explained by accepting the A.P. evidence that the charge is localized on one of the functional substituents.

The low vapor pressure of the amino acids has classically been attributed to their zwitter-ion character. Close examination of the mass spectra of the amino acids and deuterated amino acids reveals that the amino acids do not exist as an inner salt in the vapor state. The $(P - COO)^+$ ion currents serve as the best indication of the amounts of 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.^{22, 23} 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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