Technical Report No. IRL-1035 September 21, 1965

# AN INVESTIGATION OF THE MASS SPECTRA OF TWENTY-TWO FREE AMINO ACIDS

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# FOREWORD

The latent power of mass spectrometry for ultramicroanalysis is widely recognized, but has only begun to be applied to biochemical problems, e.g., sequence analysis of proteins. Our laboratory is engaged in a program of comprehensive automation, under computer control, of analytical systems. In a preliminary study to provide insight into operational problems for computer control, Miss Martin has collected a comprehensive set of spectra of amino acids, as observed with solid samples in the Bendix time-of-flight instrument. One of the most important problems of data acquisition, the calibration of mass numbers, has only begun to be handled by the computer system at this stage of development, and the assignments given here must be regarded as tentative. Furthermore, no attempt was made to assess the ultimate sensitivity of the assay. Nevertheless, these data show the potentialities of the technique, especially when the distinctive temperature characteristics of each amino acid are considered.

This work has been supported jointly by National Aeronautics and Space Administration grant no. NsG 81-60, National Institute of Neurological Diseases and Blindness grant no. NB-04270, and Air Force grant no. AF-AFOSA-886-65.

Joshua Lederberg
Professor of Genetics

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## I. Introduction

### A. Existing Fractionation and Identification Techniques for the Amino Acids (Excluding Mass Spectrometry)

There are many existing techniques for the fractionation and identification of amino acids. An excellent review of the current state of the field may be found in "The Proteins," Vol. I, Chap. I, by Light and Smith, edited by Neurath, Academic Press, New York, 1963, pp. 20-32.

Progressing from the early fractionation on potato starch to the present elegant, automated systems utilizing ion exchange resins (usually of the sulfonated polystyrene types) for the separation of amino acids, column chromatography as an analytical technique for amino acids has now become the method of choice. Recent developments in the state of the art have produced analysis times of about two hours and sensitivities below 10 nanomoles for any of the members of a mixture: it is considered feasible that within the next year, the former value will be reduced by one half and the latter by one fourth.

The separation of the 2-4-dinitrophenyl as well as the phenylthiocarbamate derivatives of the amino acids by column chromatography is also common. Separation, although less quantitative precision, is obtained by chromatography on paper and thin-layer media. The times involved may be anywhere from forty-eight hours for the free amino acids to about four hours for either free amino acids or their derivatives on cellulose acetate in a two-dimensional chromatographic electrophoretic separation. The PTC amino acids and the DNP amino acids have been separated by paper chromatography. Perhaps the greatest sensitivity is obtained by a scheme utilizing $H^3$ DNP:$C^{14}$ amino acids in which quantities of less than 0.4 nanomole are detected.

Vapor phase chromatographic schemes have been reported for a large number of amino acid derivatives. In these systems, it is necessary to block one or both of the reactive sites in order to suppress their zwitterion properties and usually to attach a radical to lower the vapor pressure

of the compounds. The following derivatives represent only some of those used with some success in gas chromatography: conversion to the aldehydes with ninhydrin; decarboxylation to the amines; conversion to the methyl or ethyl esters with methyl or ethyl alcohol-hydrochloric acid; formation of the doubly substituted N-alkyl and carboxyl esters; the N-acetyl, n-amyl esters; the trimethylsilyl ethers or esters; the triflouroacetyl derivatives; and the triflouroacetyl, methyl or ethyl esters. Sensitivities reported for the detection of the N-acetyl, n-amyl esters and the DNP derivatives (using an electron capture detector) are of the order of 0.1 nanomole.

There are microbiological assays for the amino acids which have been in use for many years, but these are among the most tedious and lengthy of all the techniques.

B. Mass Spectrometric Investigations of the Amino Acids

Many attempts have been reported in which amino acids or their derivatives have been subjected to mass spectrometric investigation. A recent review of the work in this field may be found in "Mass Spectrometry of Organic Ions" by Biemann, edited by McLafferty, Academic Press, New York, 1963, pp. 529-596.

Of the work on individual free amino acids, that by Junk and Svec in J.A.C.S. 85, 839 (1963) is most complete. They report (partial) information on the mass spectra of 15 alpha amino acids. Biemann, Seibl and Gapp in Biochem. and Biophys. Res. Comm., 1, 307 (1959) have published the results of the mass spectrometry of 20 of the ethyl esters of amino acids (including the ethyl ester of gamma-amino butyric acid). In 1962, Teeter reported the mass spectra of 13 trimethylsilyl esters of the amino acids (A.S.T.M. Committee E-14). In none of these reports (or any others I have been able to find) is any analytical scheme proposed for complete mixtures of the class.

Junk and Svec have reported elsewhere (Anal. Chem. Acta 28, 164 (1963) on mass spectrometric identification of mixtures of small numbers of free amino acids. Biemann and Vetter, in Biochem. and Biophys. Res. Comm. 11, 93 (1960), determined, with reasonable quantitation (± 5% of theory), the composition of synthetic mixtures of amino acid ethyl esters, the most members in any mixture being ten.

Some work has been done with small peptides, notably that of Junk and Svec on a large number of free dipeptides, published in Anal. Biochem. 6: 199 (1963). A paper has recently appeared by Barber, Jolles, Vilkas and Lederer, in Biochem. and Biophys. Res. Comm. 18: 469 (1965) in which they report the stunning accomplishment of a whole structure and sequence determination for "fortuitine". This is a natural peptidolipid (M=1359) of nine amino acid residues; it was determined as the acyl nonapeptide methyl ester. Despite the lack of future success in mass spectrometric sequence analysis suggested by the name of this compound, the description of the experimental results is one of the nicest things since insulin's sequence

was first reported.

In the course of an unsuccessful attempt to obtain the mass spectra of the PTC amino acids, introduced as solids into the Bendix time-of-flight mass spectrometer, I decided to look at the free amino acids. The fact that their vapor pressures are higher than the PTC amino acids, that no conversion steps are necessary for their investigation, and that some work had already been published by Junk and Svec made the possibility of their systematic analysis quite attractive.

## II. Materials Used in this Investigation

Whenever possible the free amino acids were employed. The use of the hydrochloride and/or hydrate form resulted in an apparent depression of the transition temperature of the amino acid, as well as a marked increase in the decomposition products in the spectrum; in some cases, only ions of the m/e 14 to 32 range and of the m/e 35 to 38 (from Cl) appeared in any significant abundance above the background spectrum.

In most cases the amino acids used were either California Biochemical Products' or Nutritional Biochemical Company's "A" grade or "Reagent" grade. In no case was a sample subjected to further purification or treatment of any kind before the mass spectrometric analysis.

One to two milligrams of material was more than sufficient to obtain a spectrum for any of the compounds investigated.

## III. Experimental Conditions

### A. Description of the System

A Bendix time-of-flight mass spectrometer, Model 12-107, was used throughout the investigation. The instrument was equipped with a 180 cm. drift tube. A Bendix, Model 843A, solid sample inlet system was used, which permits the sample to be inserted directly into the source to a distance of 5 mm. from the ionizing electron beam; it is constructed so that the sample crucible may be heated during an experiment and the temperature monitored continuously. An analog scanner, supplied by Bendix, enables the high data rate at the detector (which is of the order of 50 megacycles) to be reduced sufficiently so that the output may be applied to a conventional strip chart recorder or to an analog-to-digital converter for on-line evaluation by a digital computer. The output of the detector, a crossed-field Wiley electron multiplier, was monitored continuously by means of a Hewlett-Packard, Model 462A, pulse amplifier (which have 7 nanosecond risetime and a bandpass to 50 megacycle). Thus, it was possible to observe at what temperature sample ions began to arise and what mass spectral changes occurred with increasing temperature.

### B. Experimental Conditions

In the investigation of the free amino acids the mass spectrometer was operated with both a pulsed electron beam and a pulsed ion beam. The basic frequency, i.e. the repetition rate, was 10 kilocycles, a complete spectrum being produced every 100 microseconds. The electron beam was operated at 70 electron volts, the current regulated at 0.25 microamperes and the beam admitted to the ionizing region for 0.25 microseconds of each cycle. Immediately after the elctron pulse, the ions formed were admitted to the accelerating region which was maintained at -2.8 kilovolts. The pressure in the system during a run was maintained at about $10^{-6}$ mm Hg by means of a liquid nitrogen-filled trap and a refrigerated, optically dense, baffle placed above the mercury diffusion pump.

C. Thermal Reactions of the Free Amino Acids in Vacuo

At the pressures obtaining in the source of the mass spectrometer, the free amino acids examined pass to the gaseous state beginning at about 40°C and extending beyond 600°C. Some of the amino acids, when heated over a broad range of temperature continue to evolve from the solid to the gaseous state in a controllable manner; others, in response to a small temperature increment, suddenly "explode" from the crucible, in rather violent and uncontrollable exothermic reactions. These "explosions" have almost always resulted in various source elements, particularly the repeller and the ion grids, becoming visibly coated with sample. This causes a serious reduction in the resolving power of the spectrometer, as well as spurious information about the transition temperature of the sample. Frequently, the sample charge was completely exhausted by the "explosion". It was sometimes possible to clean off the source elements by merely heating the probe tip, in situ, for an hour or so at 400°C or more, but this was not always sufficient to remove the contamination. In these cases, the source had to be either removed and chemically cleaned or the entire system submitted to a baking treatment.

The problem described above could be avoided in several ways. In the case of a compound whose "volatilization" properties are unknown, a series of small temperature increments (separated by periods during which the temperature stabilizes) is employed, until the spectrum begins to show contributions from the sample.

One might construct a fairly simple apparatus which would allow determination of the transition temperature of an unknown compound. It could consist of a vacuum envelope of relatively small volume with a valve for isolation of the system after it had been pumped to a pressure of about one micron of mercury, or less. Within the envelope might be placed a heating filament surrounding a sample crucible and a thermocouple probe for temperature measurement, similar to the tip of the existing Bendix 843A probe. A thermocouple gauge might be located above the crucible in order to monitor both the pressure in the isolated system (before the

sample was heated) and also the increase in pressure which would result from sample molecules evolving into the gas phase at the appropriate temperature. This system would not require large amounts of sample, would not require the operation of the mass spectrometer, would not require an elaborate pumping system and would certainly reduce the time presently necessary to obtain mass spectrometric data for solid samples.

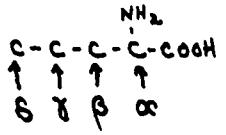
Alternatively, if there were a control loop established between the detector (measuring changes in total multiplier current, for example) and the variable transformer which drives the sample crucible oven, the time required to bring a sample to the proper temperature might be considerably reduced.

At the present time, it may take twenty minutes to two hours to attain the appropriate temperature for an unknown compound with the manually controlled heat source (whose time-constant seems to be quite large). If, however, the transition temperature of the sample is known, it is possible to approach the appropriate temperature in fairly large increments and then to slowly attain it from slightly below the final temperature. In either case, it is necessary to control the sample temperature, thus the sample pressure in the source, with some care while recording a spectrum.

D. Differential Temperature Analysis

The fact that the various free amino acids examined exhibit such a wide range of temperatures at which they pass into the gas phase should make it possible to distinguish among those whose mass spectra are quite similar. The possibility of differential temperature analysis of the free amino acids in the mass spectrometer will be discussed at length in the body of this report.

# A Comparison of Mass Spectral




(M=molecular ion)

| | 1. Monoamino-monocarboxylic | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | a. Aliphatic | | | | | b. Aromatic | | | c. β-hydroxy-α-NH2 | | |
| | gly | ala | val | leu | ileu | phe | tyr | try | ser | thr | |
| M | 6 | | | | | 5 | 5 | 5 | | 1 | |
| M-COOH | 100 | 100 | 100 | 99 | 100 | 57 | 3 | 2 | 17 | 29* | |
| (M-COOH)+H | | | | 6 | 7 | | | | | | |
| α×β | | | 28 | 31 | 25 | 100 | | | 9 | 29* | |
| α×β+H | | | 14 | 4 | 37 | | | | 5 | 90 | |
| α×β | | | | 10 | 36 | 57 | 100 | 100 | 4 | 32 | |
| α×β+H | | | | 8 | 8 | 18 | 13 | 10 | | | |
| β×γ | | | | | | | | | | | |
| β×γ-H | | | | | | | | | | | |
| β×γ | | | | 54 | 35 | 10 | | | | | |
| β×γ+H | | | | 100 | 37 | | | | | | |
| γ×δ+H | | | | | | | | 9 | | | |
| [+CHNH2] | | 38 | | | | | | | | 86 | |
| M-NH3 | | | | | | | | | | | |
| M-NH3-COOH | | | | | | 8 | | | | 90* | |
| M-H2O | | | | | | | | | | | |
| M-H2O-COOH | | | | | | | | | | 90* | |
| M-2·H2O | | | | | | | | | | | |
| (M-2·H2O)-CO2 or COOH | | | | | | | | | | | |
| M-H2O-NH3 | | | | | | | | | | | |
| (M-H2O-NH3)-CO2 or COOH | | | | | | | | | | | |
| M-2·NH3 | | | | | | | | | | | |
| (M-2·NH3)-CO2 or COOH | | | | | | | | | | | |

N.B. Numbers in the table represent the per cent abundance for which the identification is equivocal. A fragment a supporting peak for the 'identifier'.

# Fragments Among the Amino Acids



| d.S" Containing | | | II. Monoamino-di-carboxyl | | III. Diamino-monocarboxyl | | IV. Secondary amino acids | | V. $\alpha$-$NH_2$ + sec. NH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| cys/2 cys | | met | asp | glu | asp-N | glu-N lys-NH | pro | hypro | his | $\gamma$-abu | |
| 4 | see | 38 | | | | 1 | 1 | | | 2 | M |
| 44 | section IV, B, d), 2. | 16 | | | | | 100 | 100 | 8 | See Section IV, B, 6. | M-COOH |
| | | | | | | | | 18 | | | (M-COOH)+H |
| 40 | | 27 | | | | | | | | | $\alpha \times \beta$ |
| 23 | | 37* | | | | | | | | | $\alpha \times \beta + H$ |
| 11 | | 37* | | | | 34 | | | 26 | | $\alpha \times \beta$ |
| | | | | | | | | | 42 | | $\alpha \times \beta + H$ |
| | | 9 | | | | | | | | | $\beta \times \gamma$ |
| | | 8 | | | | | | | | | $\beta \times \gamma - H$ |
| 3 | | 100 | | | | | 1 | | | | $\beta \times \gamma$ |
| 3 | | | | | | | | | | | $\beta \times \gamma + H$ |
| | | | | | | | | | | | $\gamma \times \delta + H$ |
| | | | | | | | | | | | $[{}^{+}CHNH_2]$ |
| | | | | | 11 | 6 | | | | | $M-NH_3$ |
| 21 | | | | | 100 | 49 | | | | | $M-NH_3-COOH$ |
| | | 18 | | 9 | | | | | | 2 | $M-H_2O$ |
| | | | | 88 | | | | 48 | | | $M-H_2O-COOH$ |
| | | | 9*, 5* | | | | | | | | $M-2\cdot H_2O$ |
| | | | 37* | | | | | | | | $(M-2\cdot H_2O)-$ CO2 or COOH |
| | | | 9* | | 20 | | | | | | $M-H_2O-NH_3$ |
| | | | 37* | | 38* | | | | | | $(M-H_2O-NH_3)-$ CO2 or COOH |
| | | | | | 7 | | | | | | $M-2\cdot NH_3$ |
| | | | | | 38* | | | | | | $(M-2\cdot NH_3)-$ CO2 or COOH |

of the Base Peak for the indicated fragment. Asterisks denote fragments with a straight underlining indicates the identifying peak: a wavy underlining.

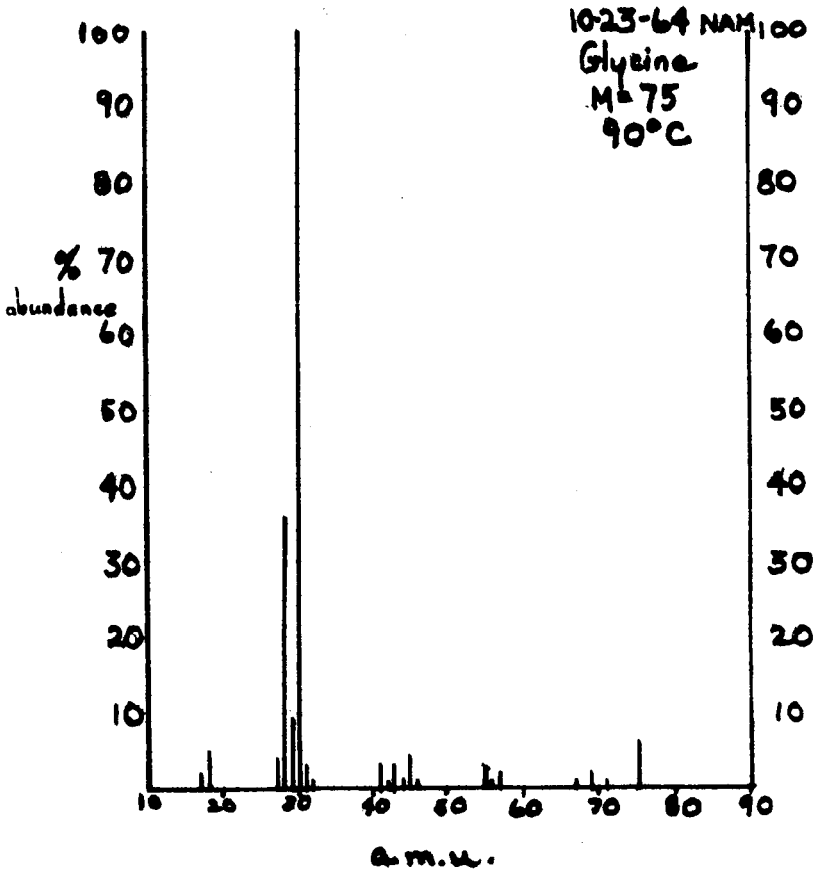
IV. Fragmentation Patterns of the Amino Acids in the Mass Spectrometer

B. Fragmentation of the Individual Amino Acids

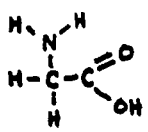
1. Monoamino-monocarboxyl Acids

a. Aliphatic acids

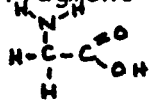
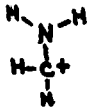
1) Glycine: M.Wt. = 75: Recording Temperature = 90° C




Structure:



Spectrum Analysis:

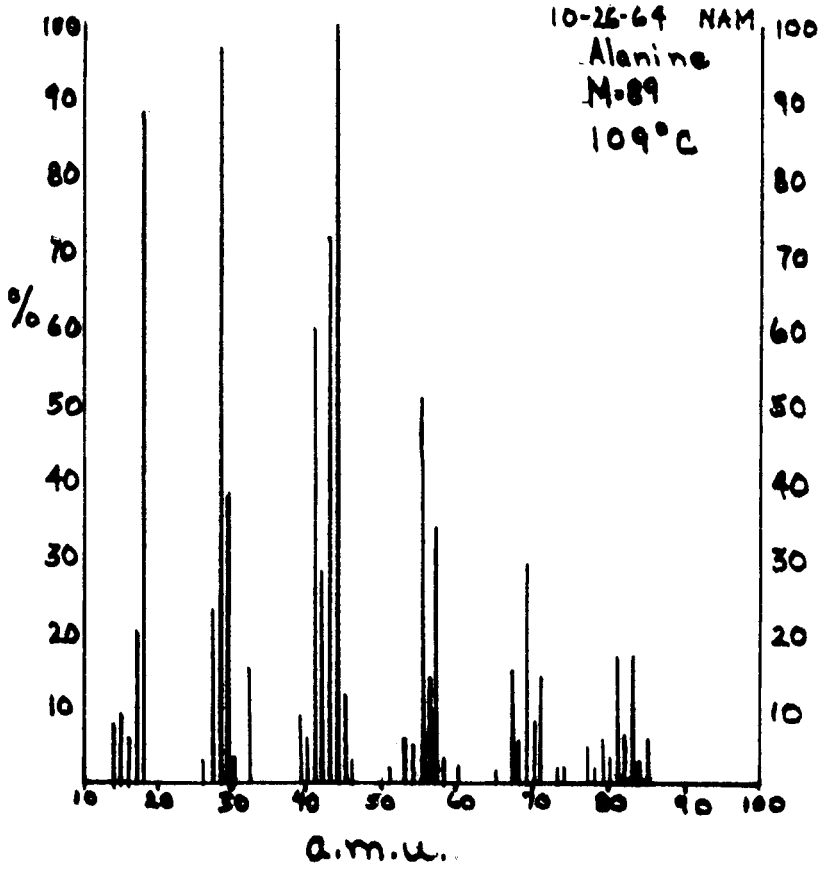
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 75 | 6 |  | M |
| 30 | 100 |  | M-45, Bpk. |

Glycine - continued:

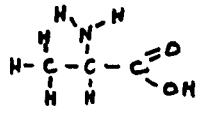
Identifying Peaks:

The ratio of m/e 30/31 of 100%/3% is the most unique feature of the spectrum of glycine at 90° C.

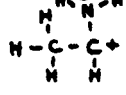
2) Alanine: M.Wt. = 89: Recording Temperature = 109° C




Structure:



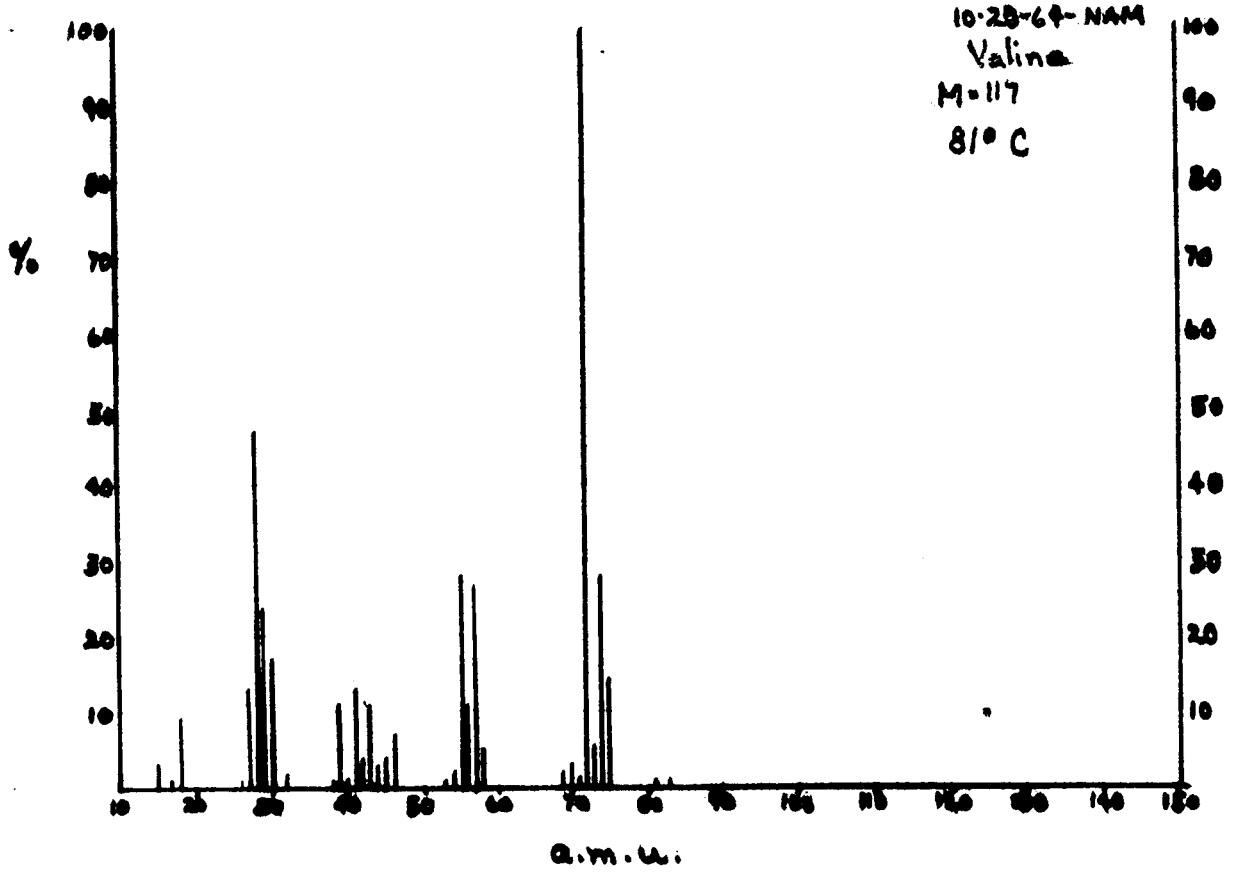
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 44 | 100 |  | M-45, Bpk. |
| 29 | 38 |  | |

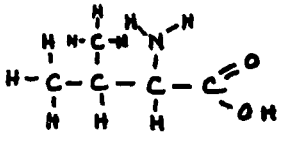
Identifying Peaks:

The fact that no other amino acid investigated has as large an m/e 44 at 109° C makes this the identifying peak for alanine.

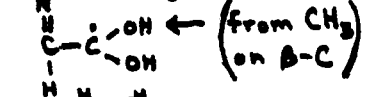
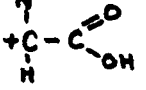
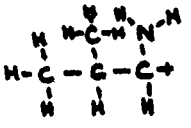
3) Valine: M.Wt. = 117: Recording Temperature = 81° C




Structure:



Spectrum Analysis:

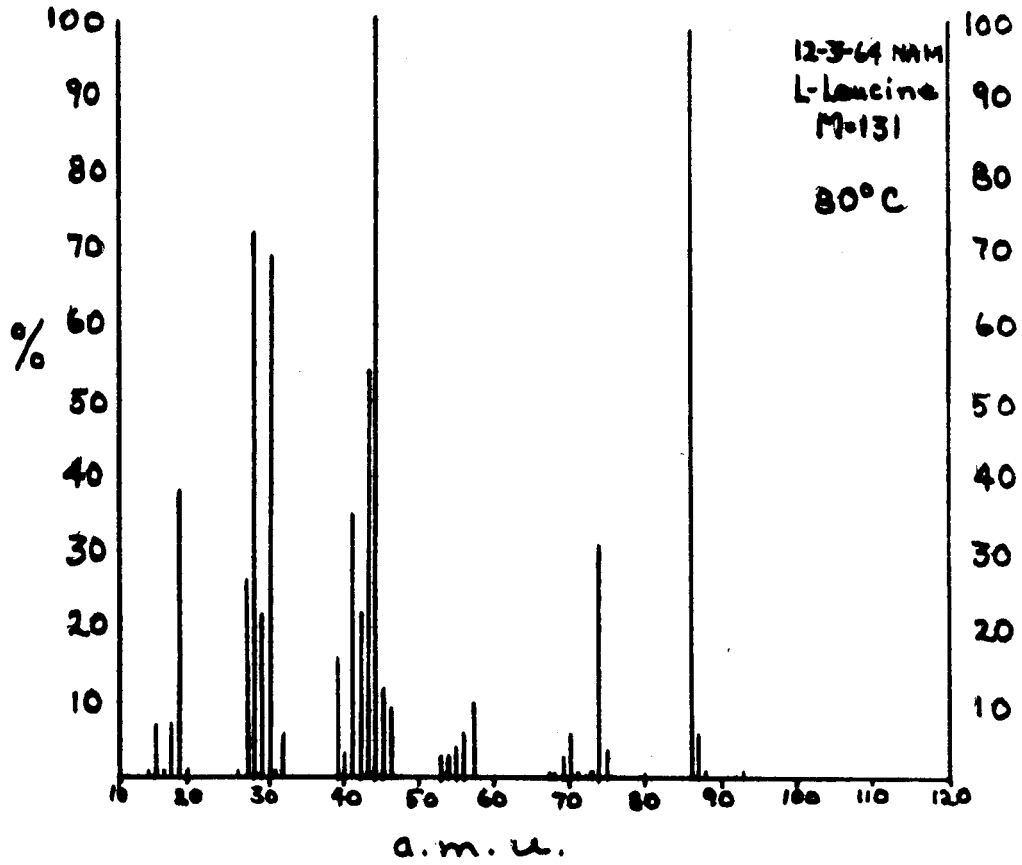
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 75 | 14 |  | $\underline{\alpha}$ x β + H |
| 74 | 28 |  | $\underline{\alpha}$ x β |
| 72 | 100 |  | M-45, Bpk. |

Valine - continued:

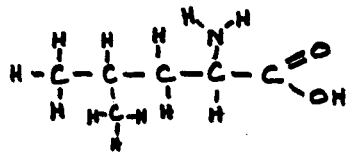
Identifying Peaks:

The identifying peak for valine is m/e 72 at 81° C.

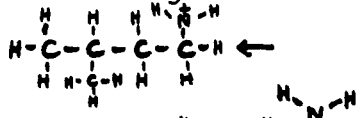
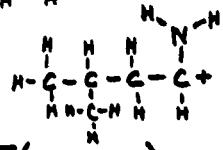
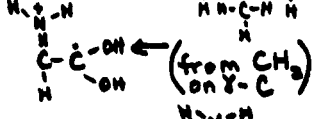
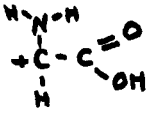
4) Leucine: M.Wt. = 131: Recording Temperature = 80° C




Structure:

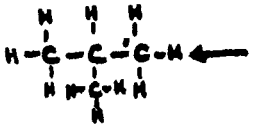
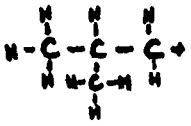
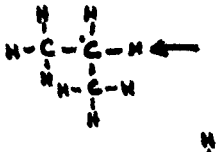
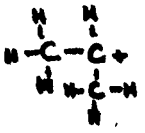


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 87 | 6 |  | (M-45) + H |
| 86 | 99 |  | M-45 |
| 75 | 4 |  | α × β + H |
| 74 | 31 |  | α × β |

Leucine - continued:

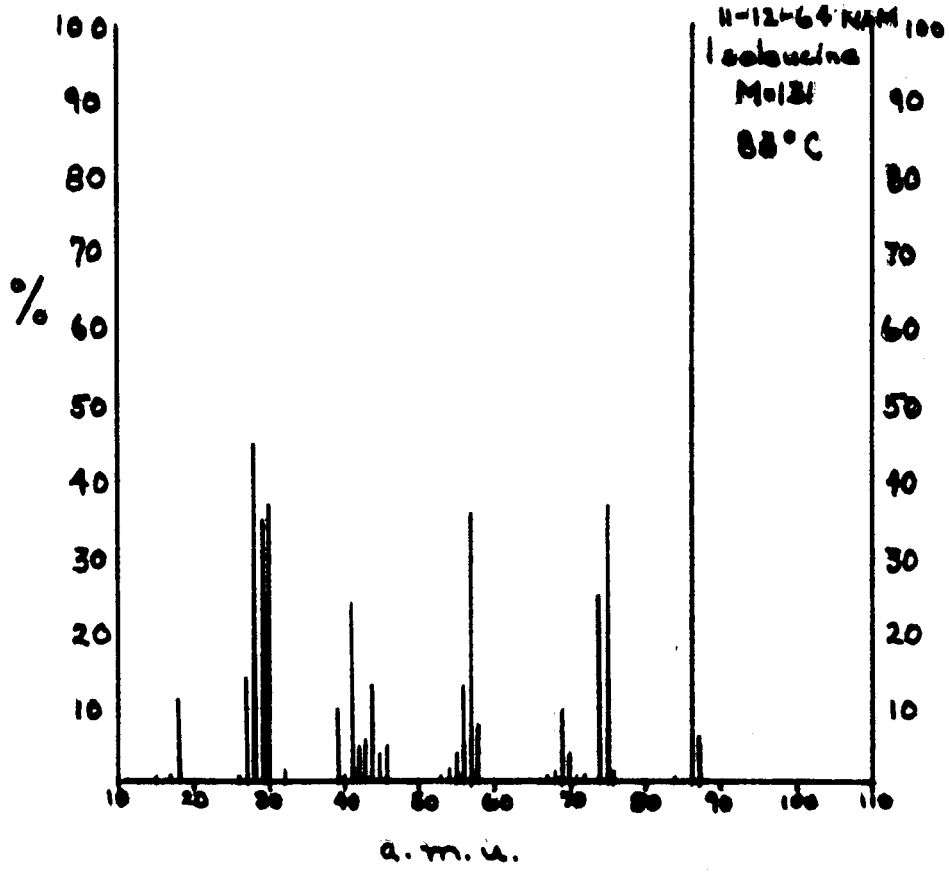
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 58 | 8 |  | $\alpha \times \underline{\beta} + H$ |
| 57 | 10 |  | $\alpha \times \underline{\beta}$ |
| 44 | 100 |  | $\beta \times \underline{\chi} + H$, Bpk. |
| 43 | 54 |  | $\beta \times \underline{\chi}$ |

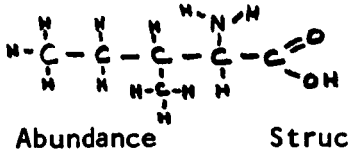
Identifying Peaks:

The abundance of the m/e 86 ion at a temperature below 100° C distinguishes leucine from every other amino acid but isoleucine. Leucine may be distinguished from isoleucine by its larger m/e 44 ion at this temperature (80° C) and by the fact that the leucine spectrum degenerates as the sample temperature is increased whereas isoleucine remains stable up to 100° C.

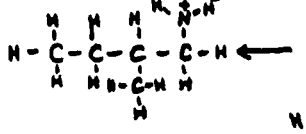
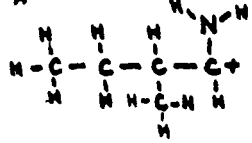
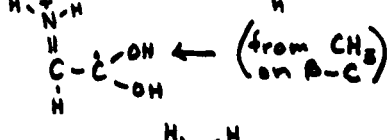
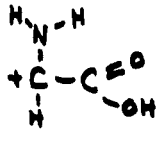
5) Isoleucine: M.Wt. = 131: Recording Temperature = 83° C




Structure:

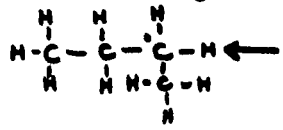
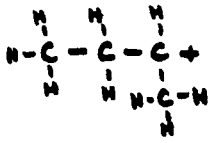
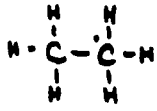
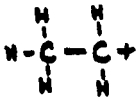


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragments | Code |
|---|---|---|---|
| 87 | 7 |  | (M-45) + H |
| 86 | 100 |  | M-45, Bpk. |
| 75 | 37 |  | $\underline{\alpha}$ x β + H |
| 74 | 25 |  | $\underline{\alpha}$ x β |

Isoleucine - continued:

Spectrum Analysis:

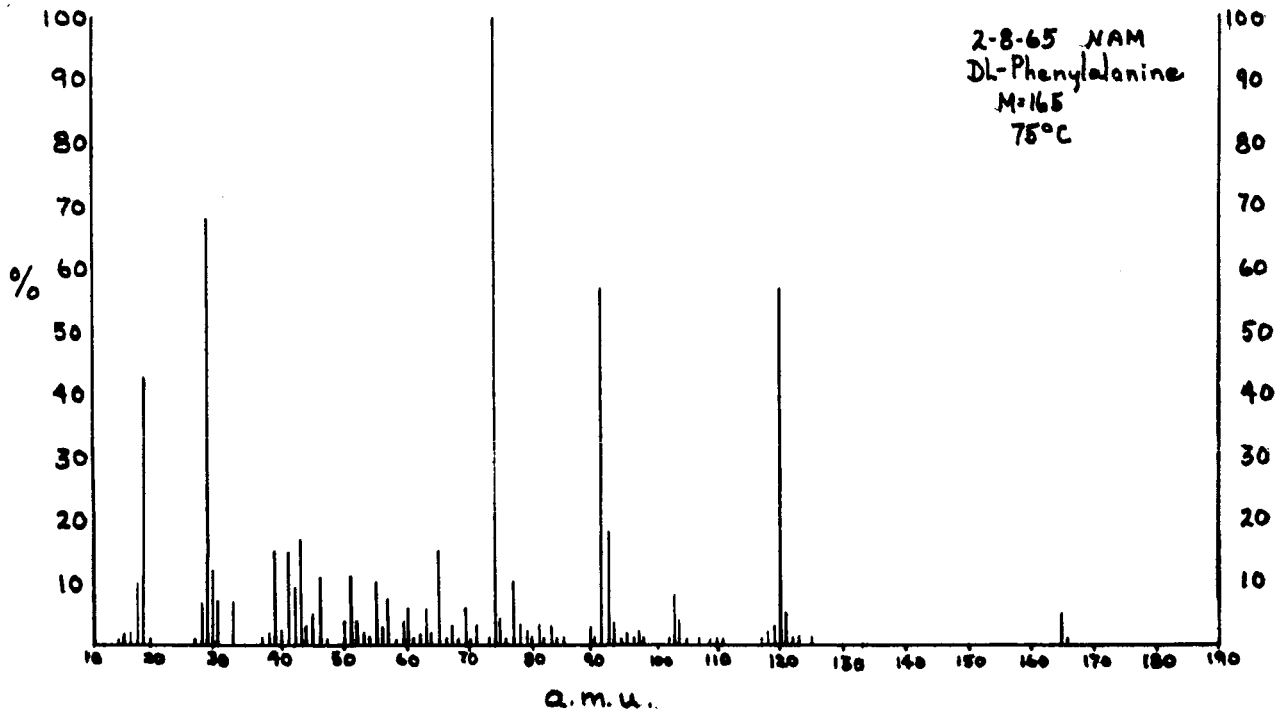
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 58 | 8 |  | $\alpha \times \underline{\beta} + H$ |
| 57 | 36 |  | $\alpha \times \underline{\beta}$ |
| 30 | 37 |  | $\beta \times \underline{\chi} + H$ |
| 29 | 35 |  | $\beta \times \underline{\chi}$ |
| 44 | 13 | -------- | Background |

Identifying Peaks:

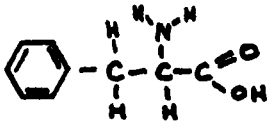
The abundance of the m/e 86 ion at a temperature below 100° C distinguishes isoleucine from every other amino acid but leucine. Isoleucine may be distinguished from leucine by its smaller, almost negligible, m/e 44 peak at this temperature (83° C) and by the fact that the isoleucine spectrum remains stable up to 100° C whereas leucine appears to degenerate above about 85° C.

b. Aromatic Amino Acids

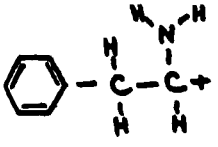
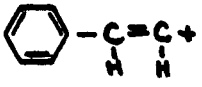
1) Phenylalanine: M.Wt. = 165: Recording Temperature = 75° C




Structure:

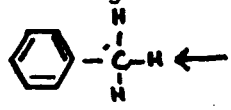
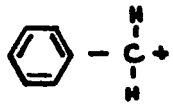
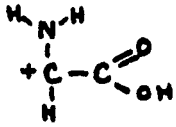


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 165 | 5 | see above | M |
| 120 | 57 |  | M-45 |
| 103 | 8 |  | (M-45)-$NH_3$ |

Phenylalanine - continued:

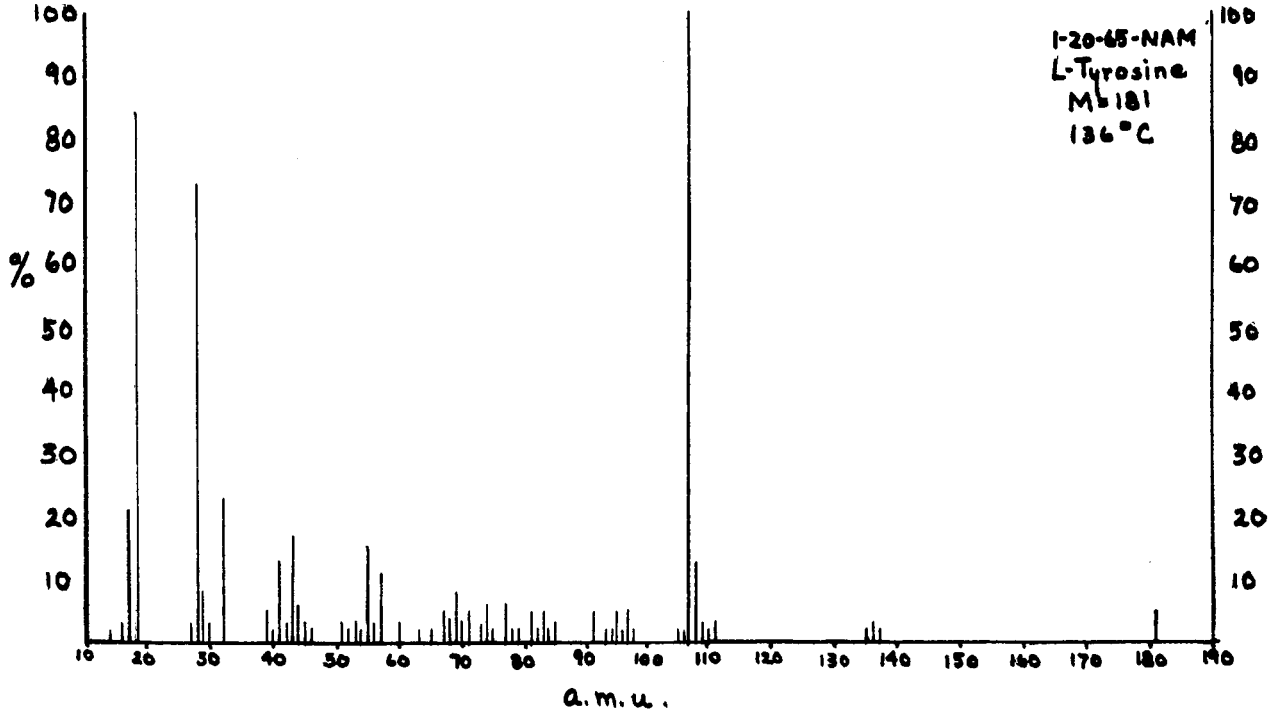
Spectrum Analysis:

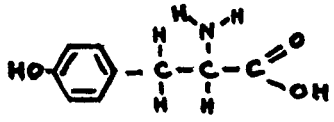
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 92 | 18 |  | $\alpha \times \underline{\beta} + H$ |
| 91 | 57 |  | $\alpha \times \underline{\beta}$ |
| 77 | 10 |  | $\beta \times \underline{\gamma}$ |
| 74 | 100 |  | $\underline{\alpha} \times \beta$, Bpk. |

Identifying Peaks:

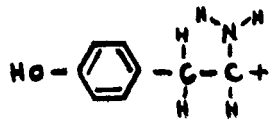
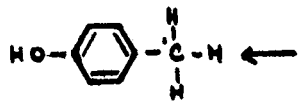
The peak at m/e 120 is unique to phenylalanine.

2) Tyrosine: M.Wt. = 181: Recording Temperature = 136° C



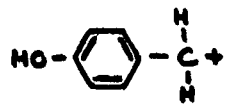

Structure: 

Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 181 | 5 | see above | M |
| 136 | 3 |  | M-45 |
| 108 | 13 |  | $\alpha \times \beta + H$ |

Tyrosine - continued:

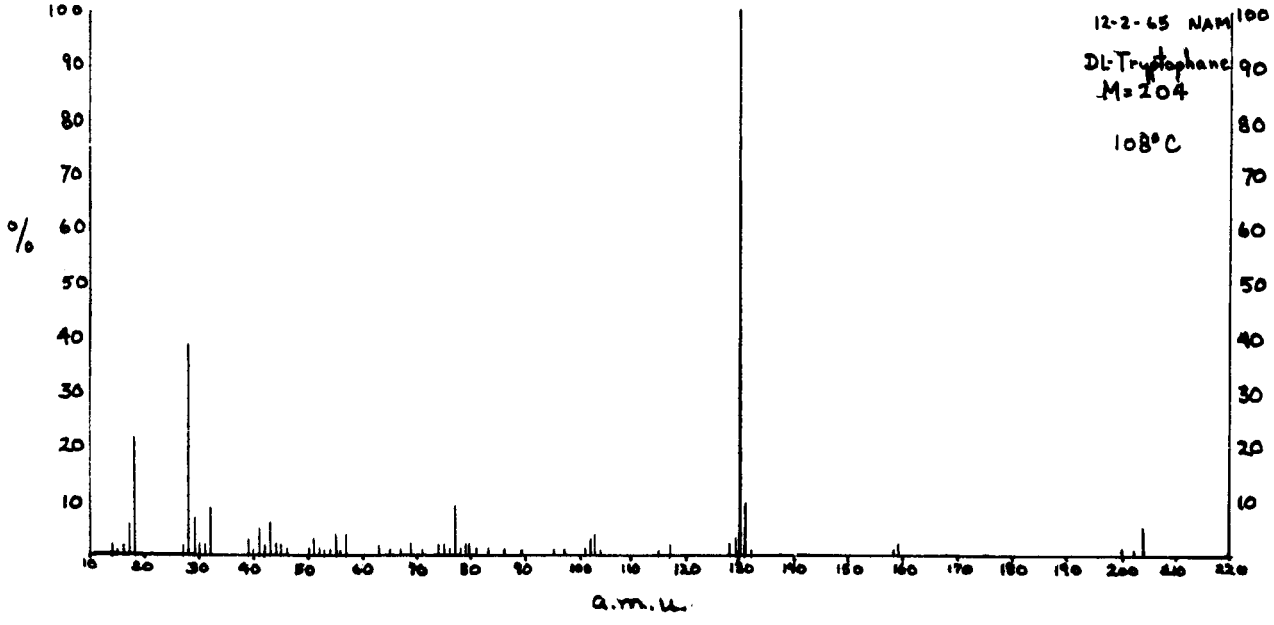
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 107 | 100 |  | $\alpha$ x $\underline{\beta}$, Bpk. |

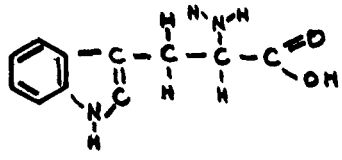
Identifying Peaks:

The peak at m/e 107 is unique to tyrosine.

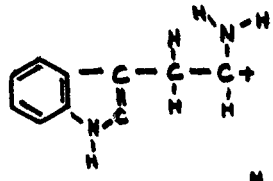
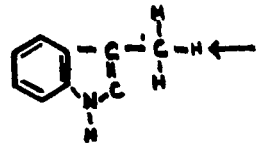
3) Tryptophane: M.Wt. = 204: Recording Temperature = 108° C




Structure:

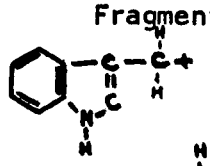
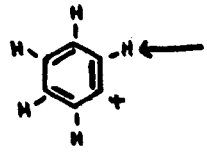


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 204 | 5 | see above | M |
| 159 | 2 |  | M-45 |
| 131 | 10 |  | $\alpha \times \underline{\beta}$ + H |

Tryptophane - continued:

Spectrum Analysis:

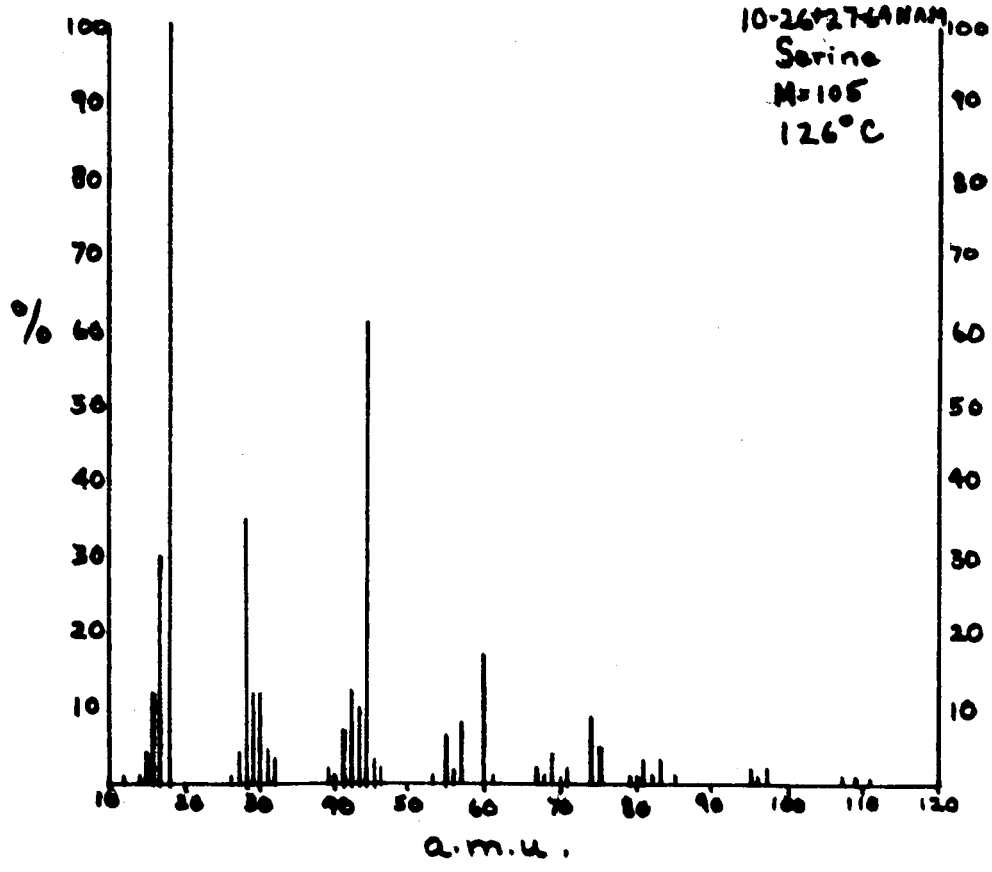
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 130 | 100 |  | $\alpha \times \underline{\beta}$, Bpk. |
| 77 | 9 |  | $\gamma \times \underline{\delta} + H$ |

Identifying Peaks:

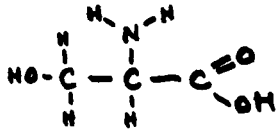
The peak at m/e 130 is unique to tryptophane.

c. β-hydroxy-α-amino acids

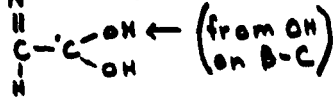
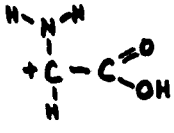
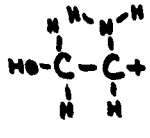
1) Serine: M.Wt. = 105: Recording Temperature = 126° C




Structure:

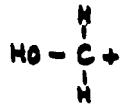


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 75 | 5 |  | α x β + H |
| 74 | 9 |  | α x β |
| 60 | 17 |  | M-45 |

Serine - continued:

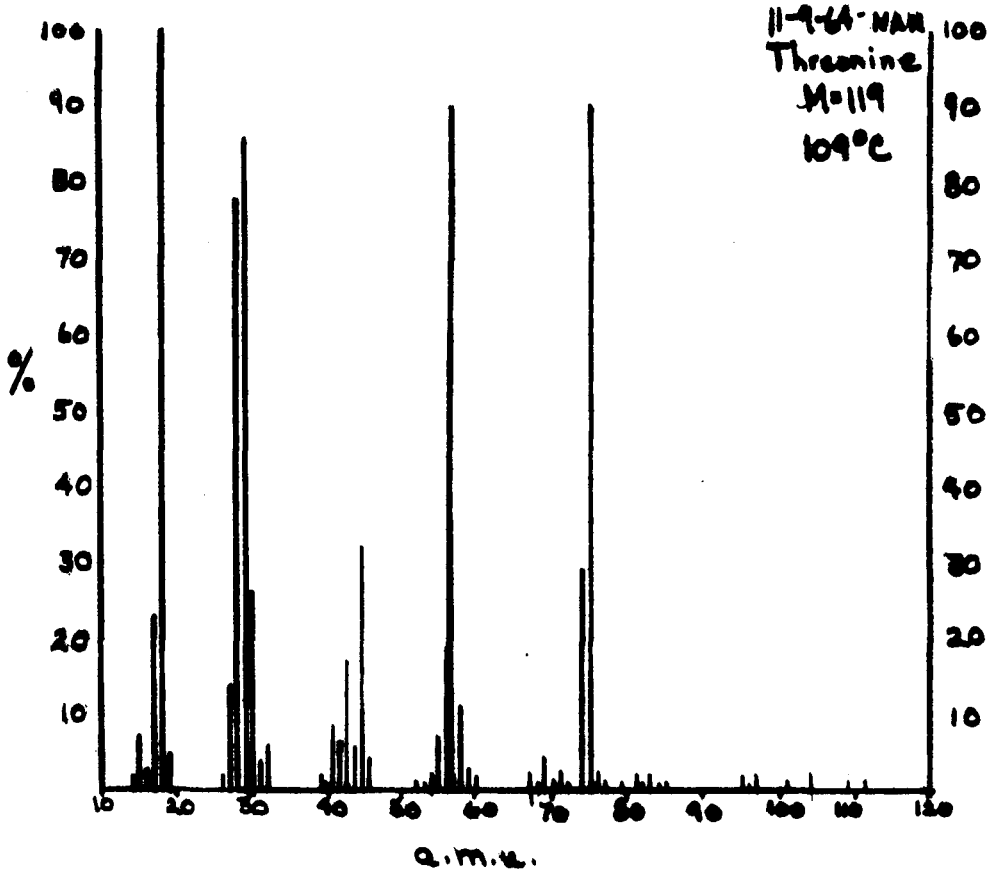
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 31 | 4 |  | $\alpha \times \underline{\beta}$ |

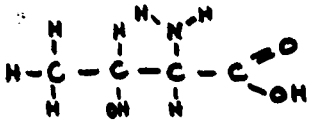
Identifying Peaks:

The peak at m/e 60 appearing at 126°C may serve to identify serine.

2) Threonine: M.Wt. = 119: Recording Temperature = 109°C




Structure:



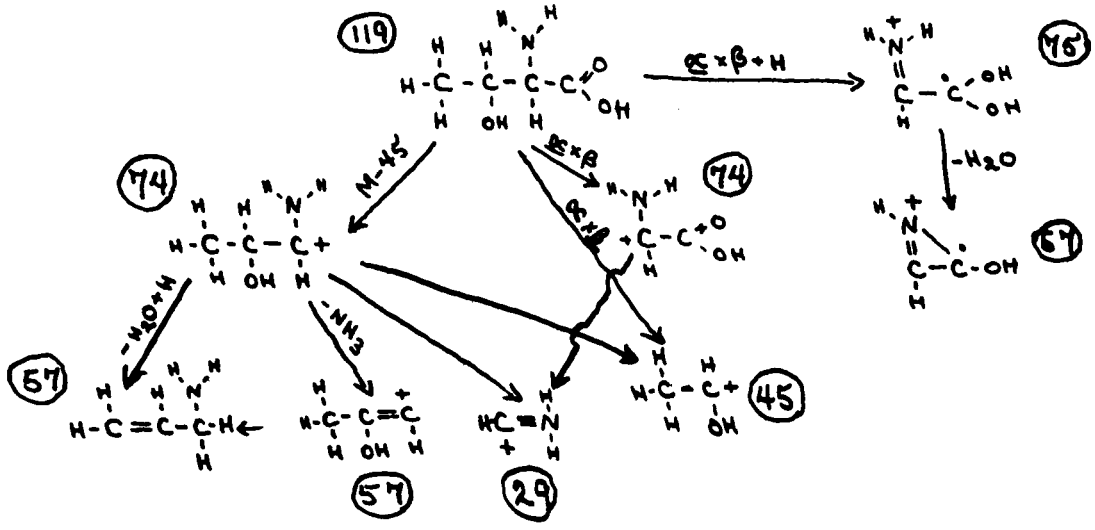
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 119 | 1 | see above | M |
| 75 | 90 | see page 27 | $\underline{\alpha} \times \beta + H$ |
| 74 | 29 | " " " | $\underline{\alpha} \times \beta$, or M-45 |
| 57 | 90 | " " " | $(\underline{\alpha} \times \beta + H) - H_2O$, or $(M-45) - NH_3$ or $-H_2O+H$ |

Threonine - continued:

Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 45 | 32 | see below | $\alpha$ x $\beta$ |
| 29 | 86 | " " | |

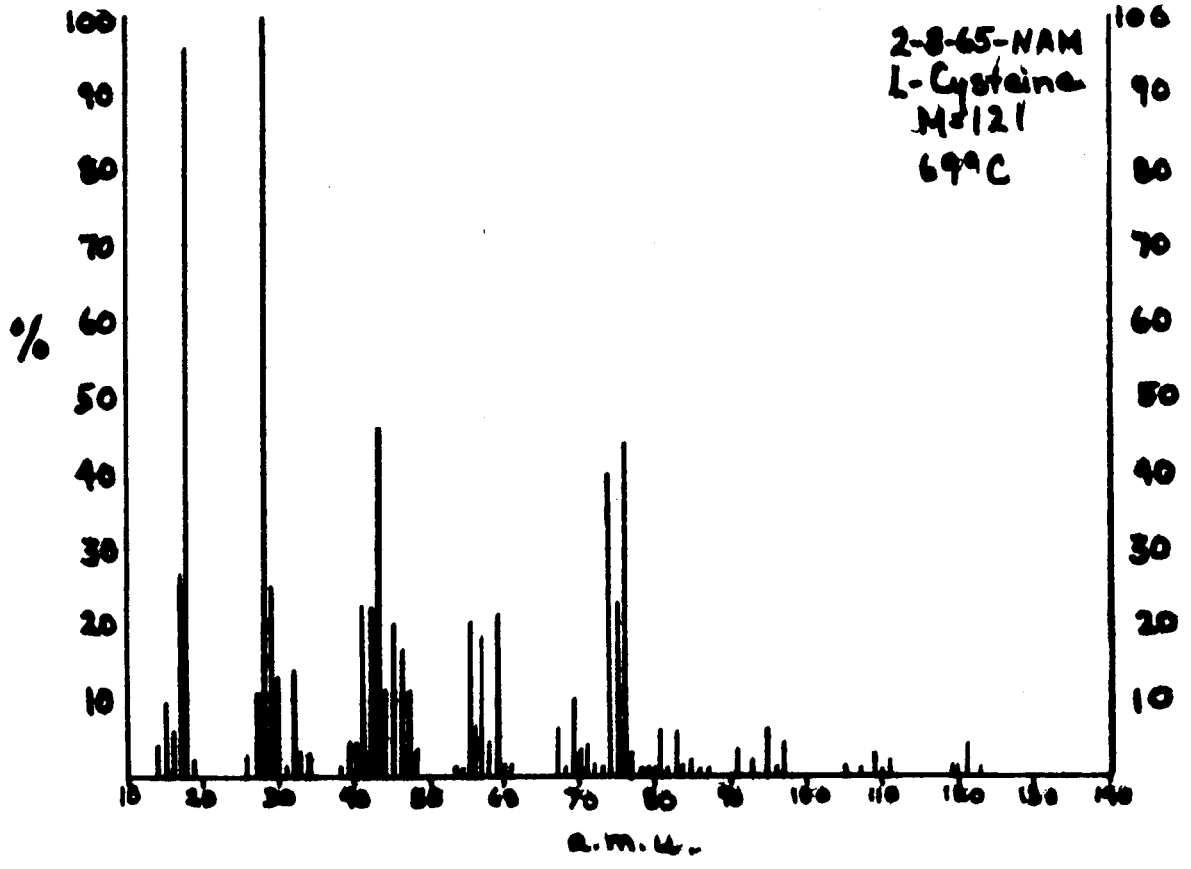



Identifying Peaks:

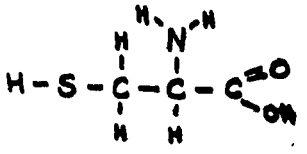
The peak at m/e 75, appearing in large abundance (and persisting up through 500°C), identifies threonine.

d. Sulfur-containing amino acids

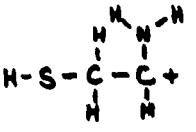
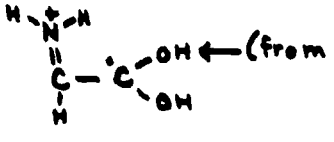
1) Cysteine: M.Wt. = 121: Recording Temperature = 69°C



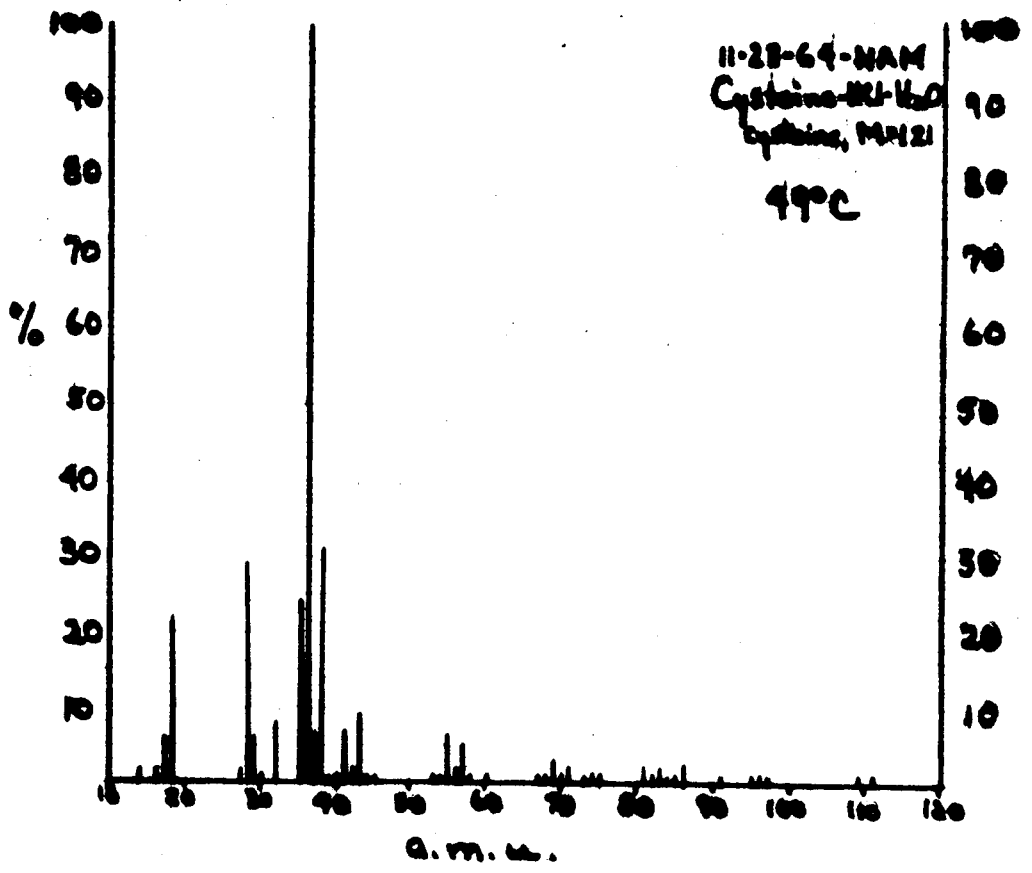

Structure:



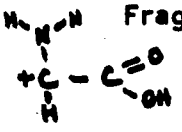
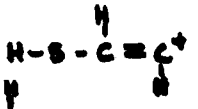
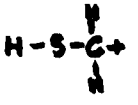
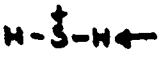
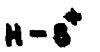
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 121 | 4 | see above | M |
| 76 | 44 |  | M-45 |
| 75 | 23 |  | α × β + H |

Cysteine - continued:



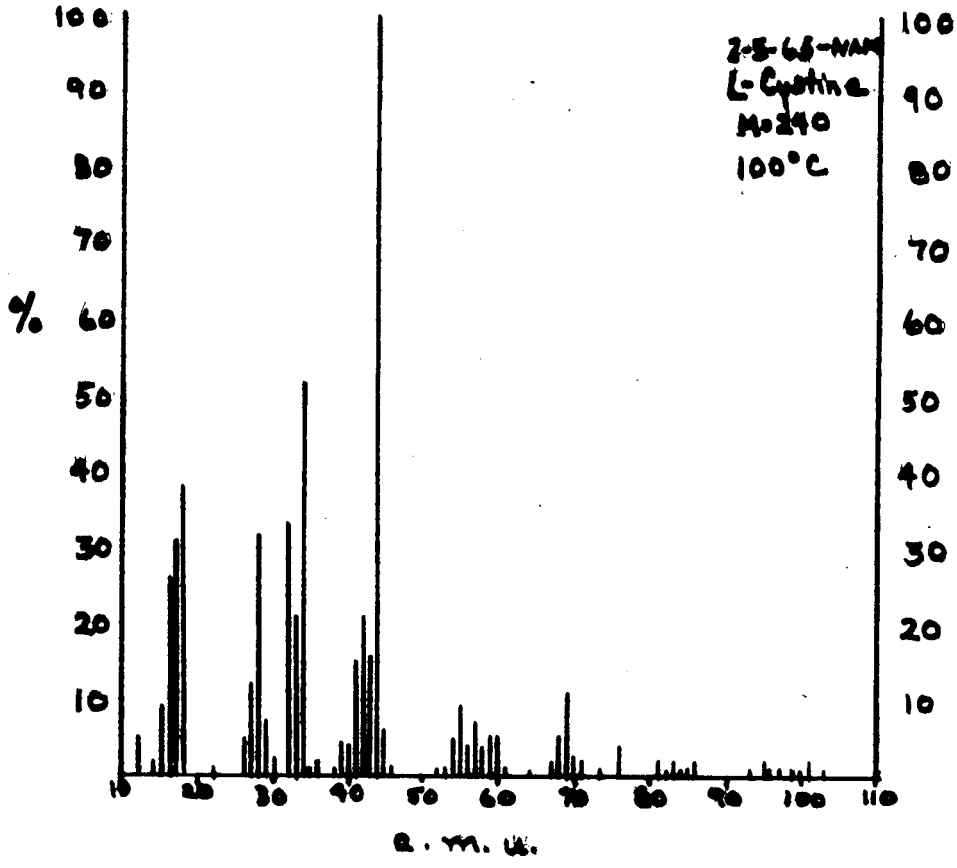

Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 74 | 40 |  | $\alpha \times \beta$ |
| 59 | 21 |  | $(M-45)-NH_3$ |
| 47 | 11 |  | $\alpha \times \underline{\beta}$ |
| 34 | 3 |  | $\beta \times \underline{\gamma} + H$ |
| 33 | 3 |  | $\beta \times \underline{\gamma}$ |

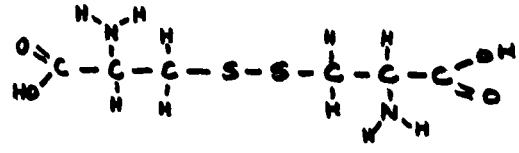
Identifying Peaks:

The appearance of a peak at m/e 76 indicates cysteine, and an m/e 76/34 ratio which is high will eliminate the possibility of the m/e 76 arising from cystine (whoce 76/34 ratio is low).

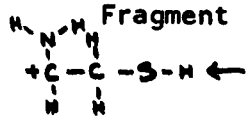
2) Cystine: M.Wt. = 240: Recording Temperature = 100°C




Structure:



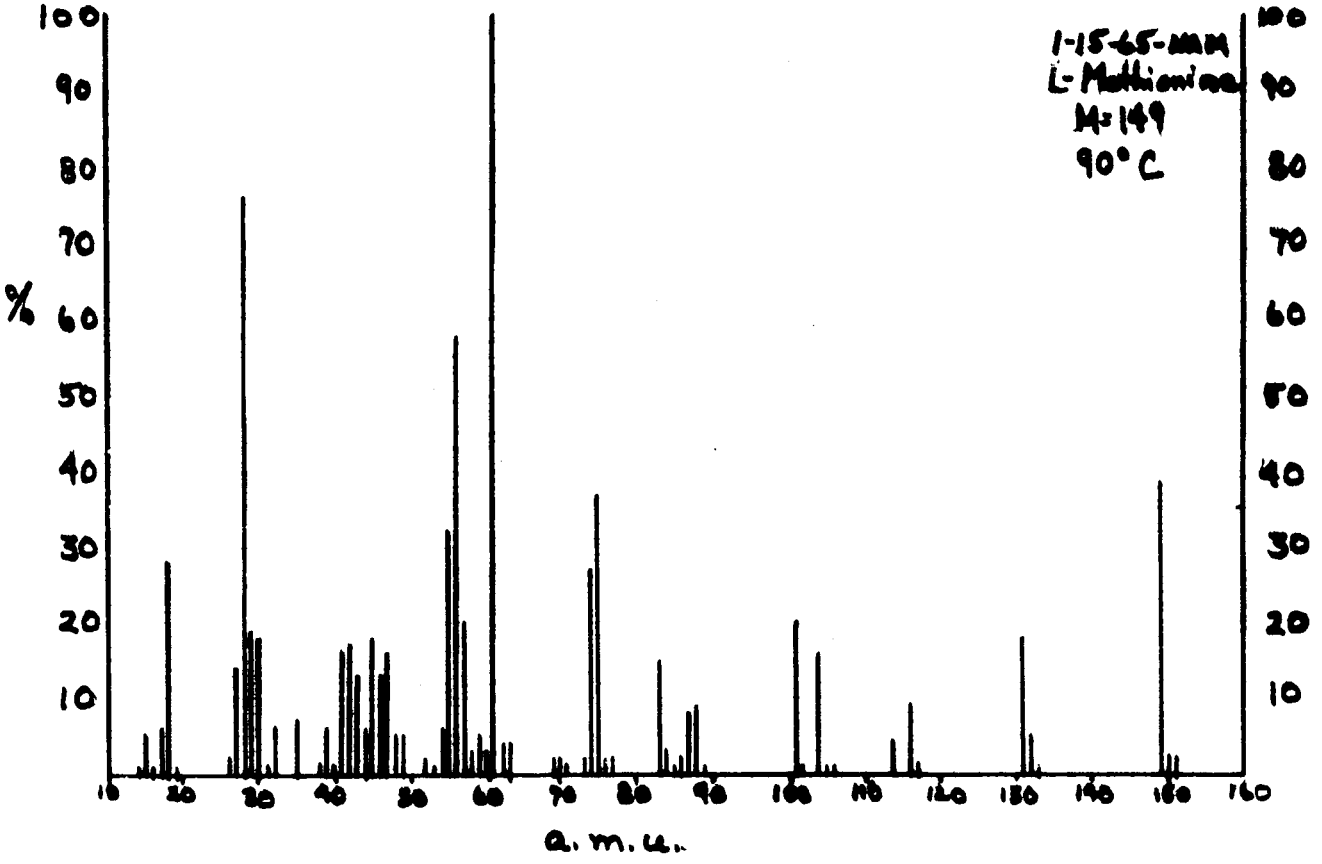
Spectrum Analysis:

| Characteristic Peaks m/e | % Abuncance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 76 | 4 |  | $(M/2_s)-45 + H$ |
| 34 | 52 | $H-\overset{+}{S}-H$ | |
| 33 | 21 | $H-S^+$ | |
| 32 | 33 | S | |

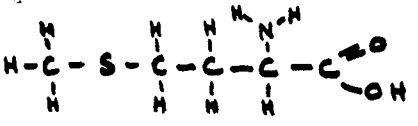
Identifying Peaks:

The appearance of both m/e 34 and 33 peaks of more than a few percent abundance in a ratio of about two or three to one indicates the presence of cystine.

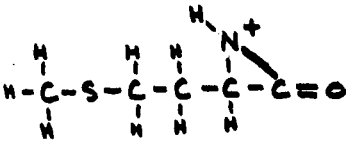
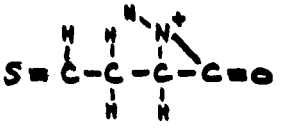
3) Methionine: M.Wt. = 149: Recording Temperature = 90°C




Structure:

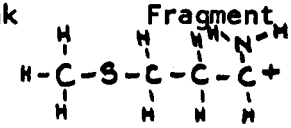
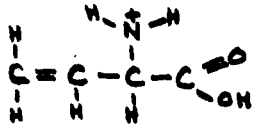
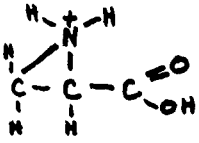
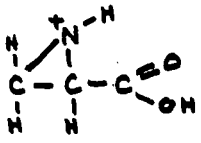
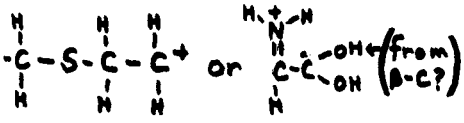
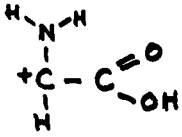
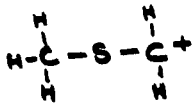
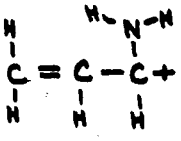


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 149 | 38 | see above | M |
| 131 | 18 |  | $M-H_2O$ |
| 116 | 9 |  | $(M-H_2O)-CH_3$ |

Methionine - continued:

Spectrum Analysis:

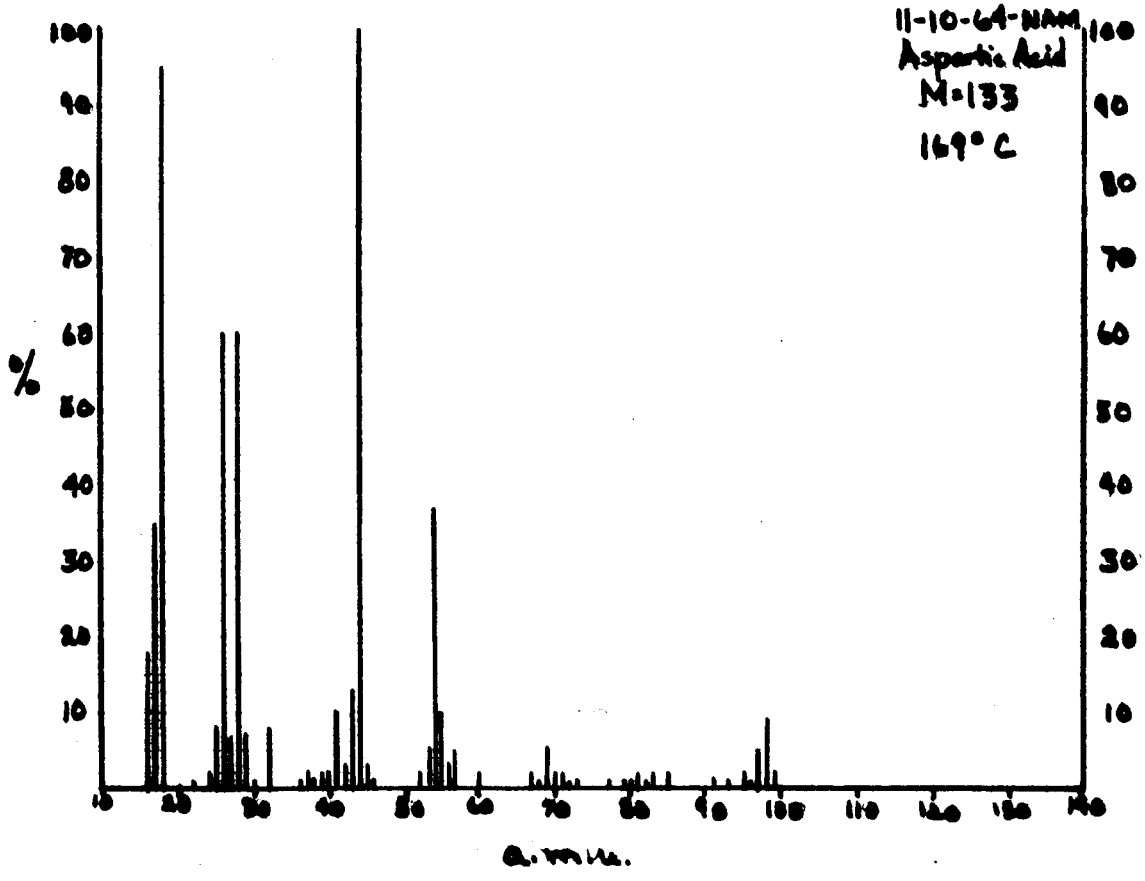
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 104 | 16 |  | M-45 |
| 101 | 20 |  | M-$CH_3S$-H; $\gamma \times \delta$-H |
| 88 | 9 |  | $\underline{\beta} \times \gamma$ |
| 87 | 8 |  | $\underline{\beta} \times \gamma$ -H |
| 75 | 37 |  | $\alpha \times \underline{\beta}$ or $\underline{\alpha} \times \beta$ + H |
| 74 | 27 |  | $\underline{\alpha} \times \beta$ |
| 61 | 100 |  | $\beta \times \gamma$, Bpk. |
| 56 | 58 |  | (M-45)-$CH_3S$-H |

Identifying Peaks:

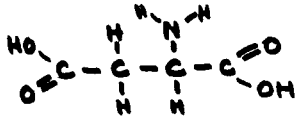
The m/e 61 peak is unique to methionine.

2. Monoamino-dicarboxylic Acids

a. Aspartic acid: M.Wt. = 133: Recording temperature = 169°C



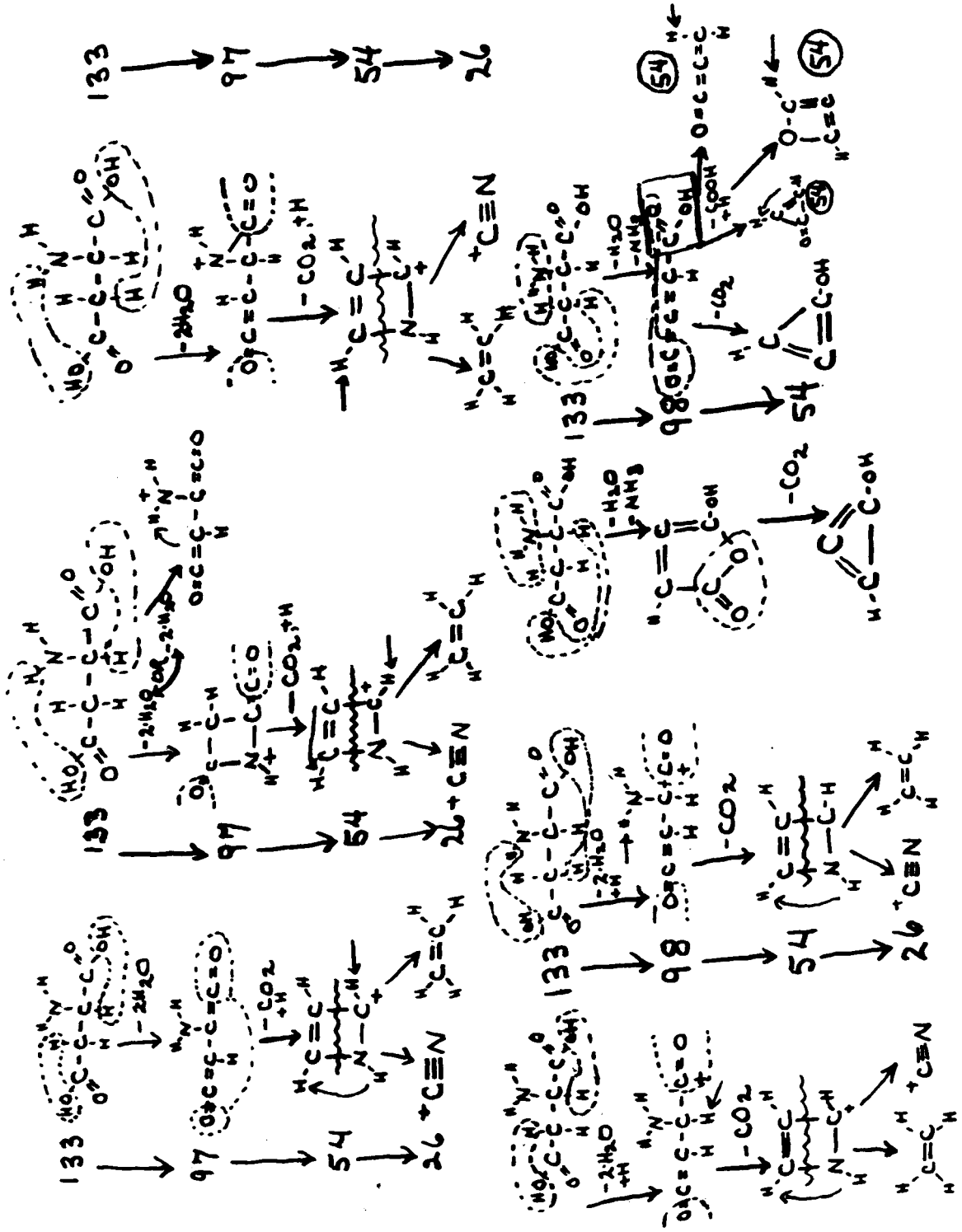

Structure:



Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 98 | 9 | See page 34. | |
| 97 | 5 | See page 34. | |
| 54 | 37 | See page 34. | |
| 26 | 60 | See page 34. | |

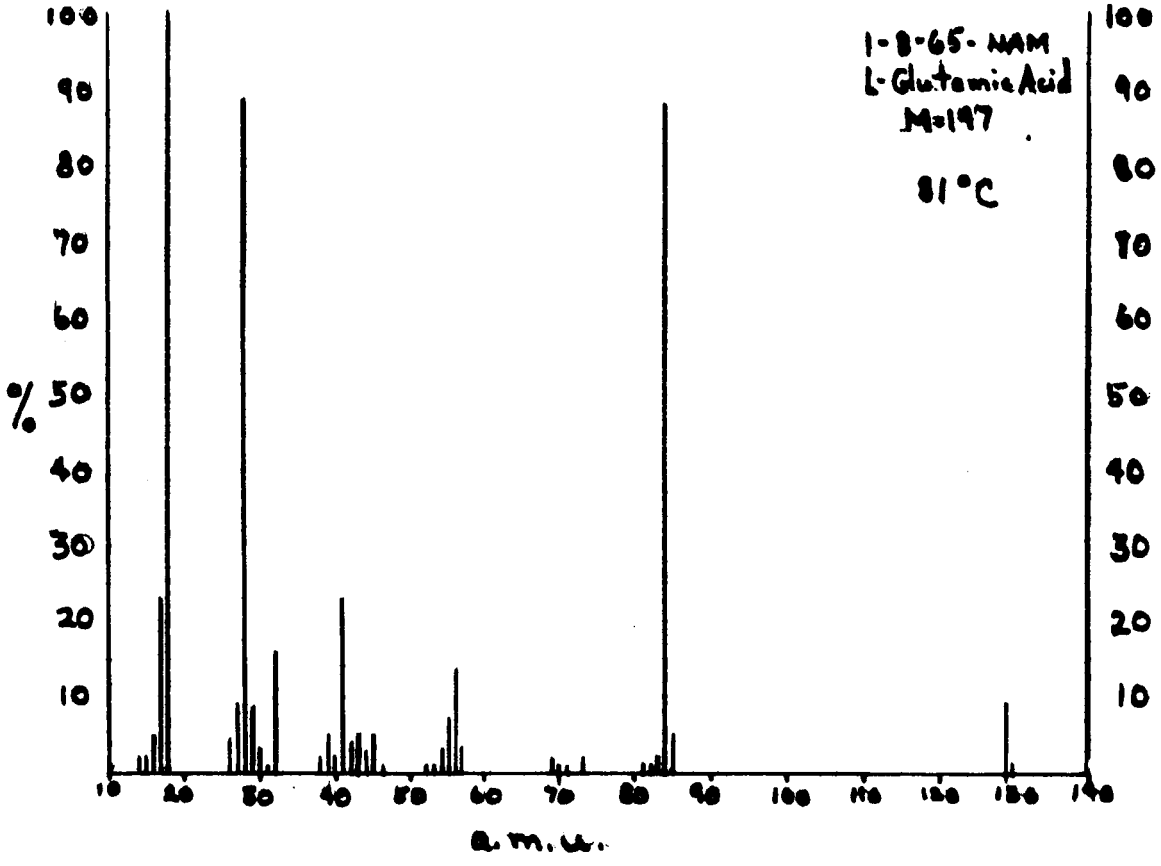
Aspartic Acid - continued:



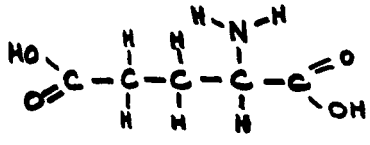
Identifying Peaks:

The presence of an m/e 54 of significant abundance indicates either aspartic acid or asparagine. Aspartic acid may be distinguished from asparagine by an m/e 97/98 ratio of less than unity and by the stability of its spectrum over a temperature range which is higher than that in which asparagine is stable; aspartic acid may be observed from 150°C to 320°C, but asparagine is stable only from 100° C to 150° C.

b. Glutamic Acid: M.Wt. = 147: Recording Temperature = 81°C



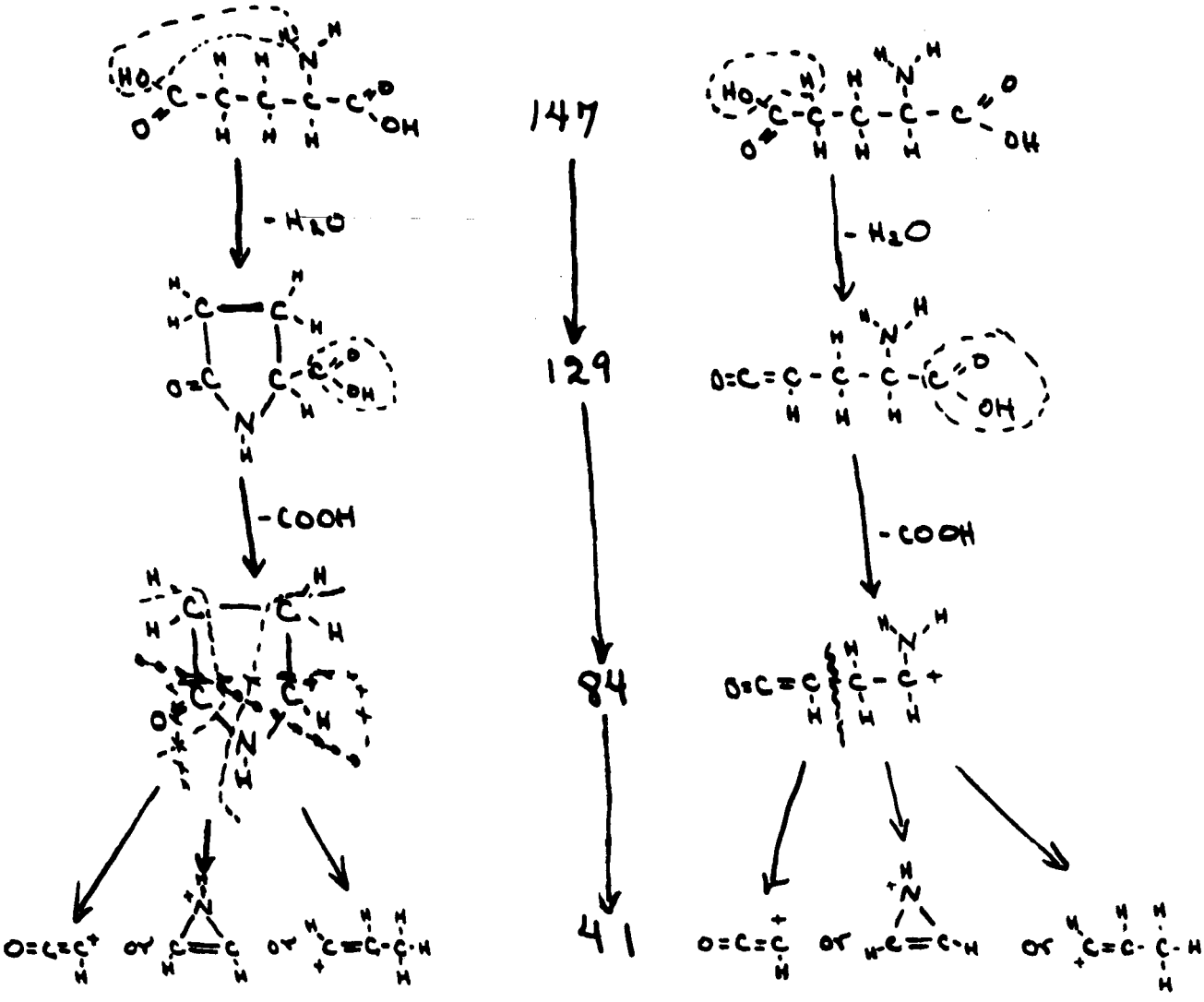

Structure:



Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 129 | 9 | See page 37. | $M-H_2O$ |
| 84 | 88 | See page 37. | $(M-H_2O)-45$ |
| 41 | 23 | See page 37. | |

Glutamic acid - continued:

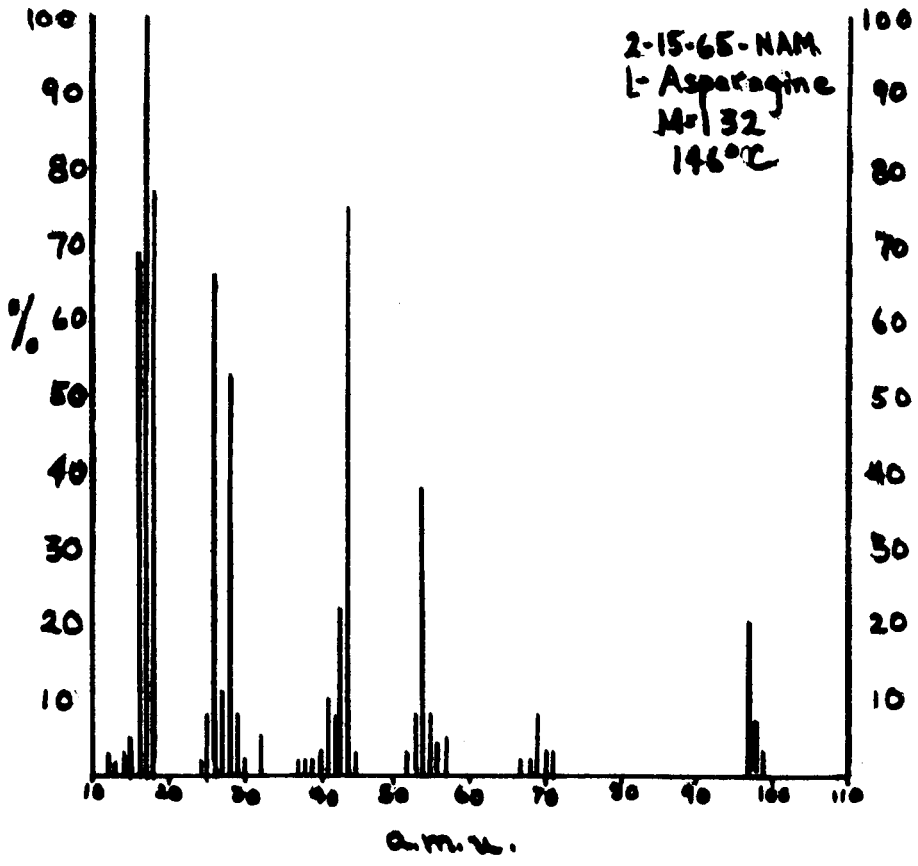



Identifying Peaks:

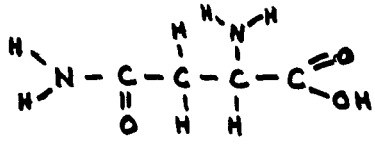
The presence of a peak at m/e 84 in significant abundance and in the temperature range of 75°C to 82°C indicates the presence of glutamic acid: glutamine exhibits a strong peak at m/e 84 beginning only at 85°C and persisting until 330°C and lysine only in the range of 110°C to 630°C.

3. Diamino-monocarboxylic Acids

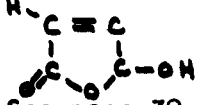
a. Asparagine: M.Wt. = 132: Recording Temperature = 146°C



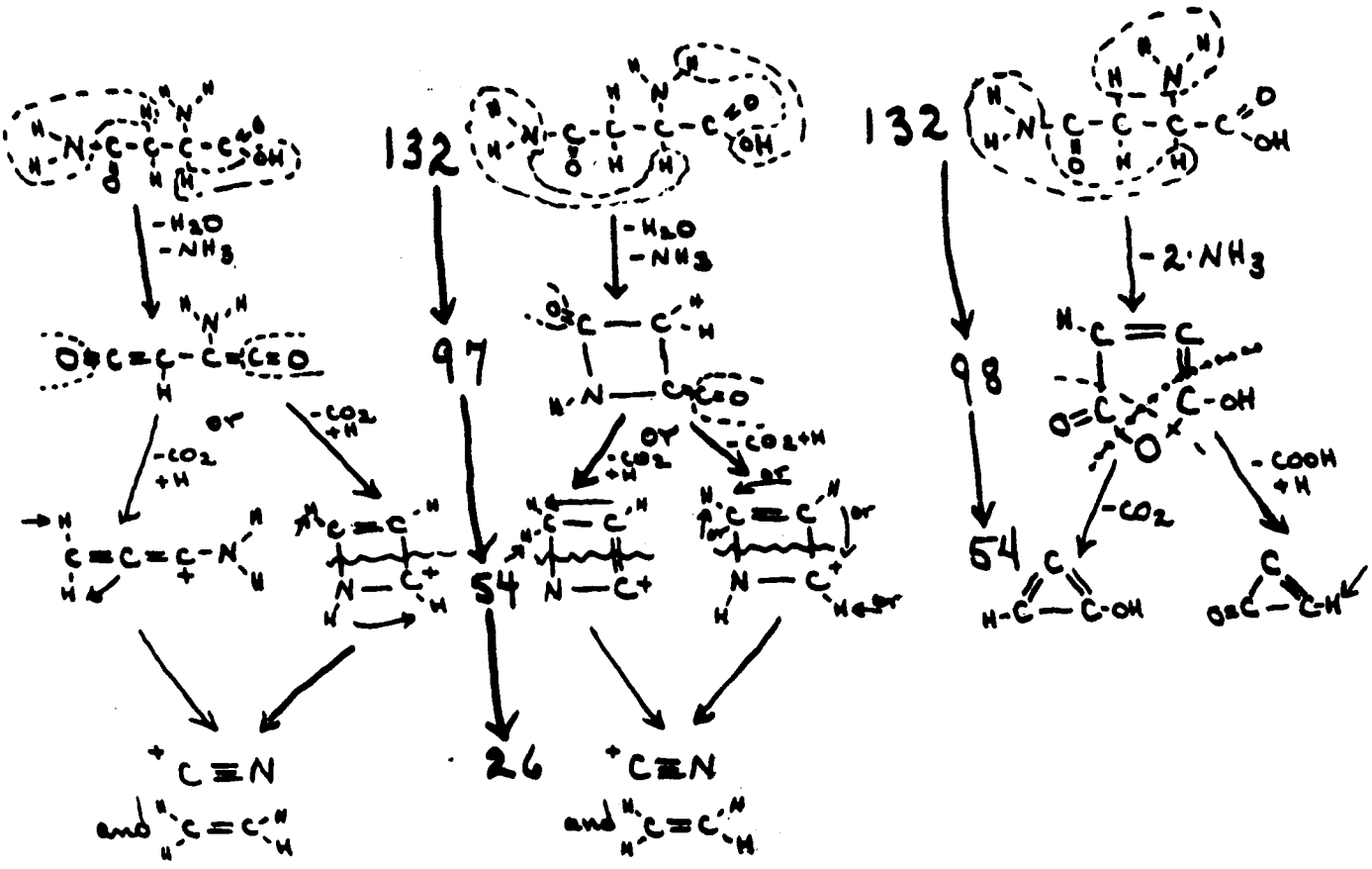

Structure:



Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 98 | 7 |  | M-2 x $NH_3$ |
| 97 | 20 | See page 39. | M-$H_2O$-$NH_3$ |
| 54 | 38 | See page 39. | |
| 26 | 66 | +C ≡ N | +C ≡ N |
| 17 | 100 | $NH_3$ | $NH_3$, Bpk. |

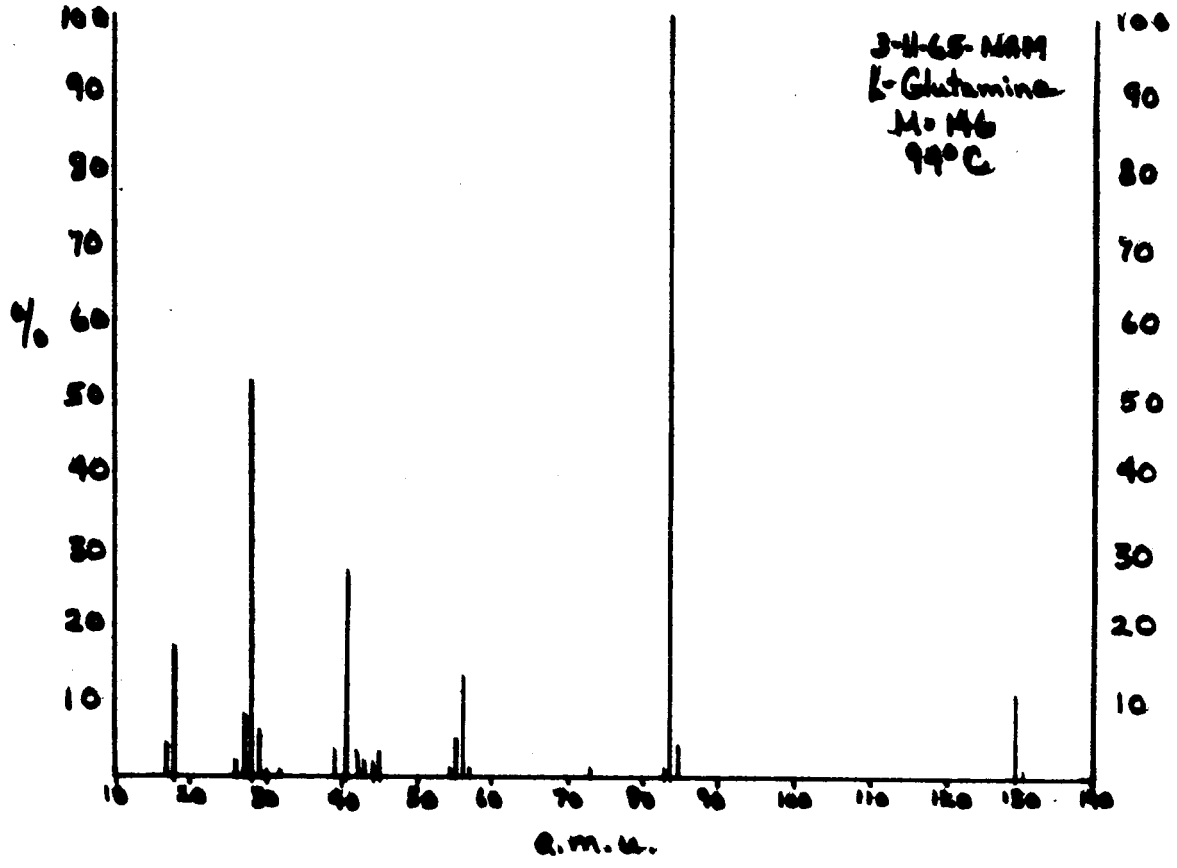
Asparagine - continued:



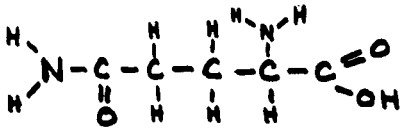

Identifying Peaks:

The presence of an m/e 54 of significant abundance indicates either asparagine or aspartic acid. Asparagine may be distinguished from aspartic acid by an m/e 97/98 ratio of greater than two and by the stability of its spectrum over a temperature range which is lower than that in which aspartic acid appears: asparagine may be observed from 100°C to 146°C and aspartic acid only from 150°C up to 320°C.

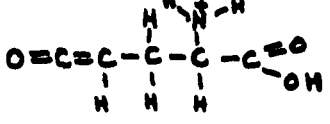
b. Glutamine: M.Wt. = 146: Recording Temperature = 94°C



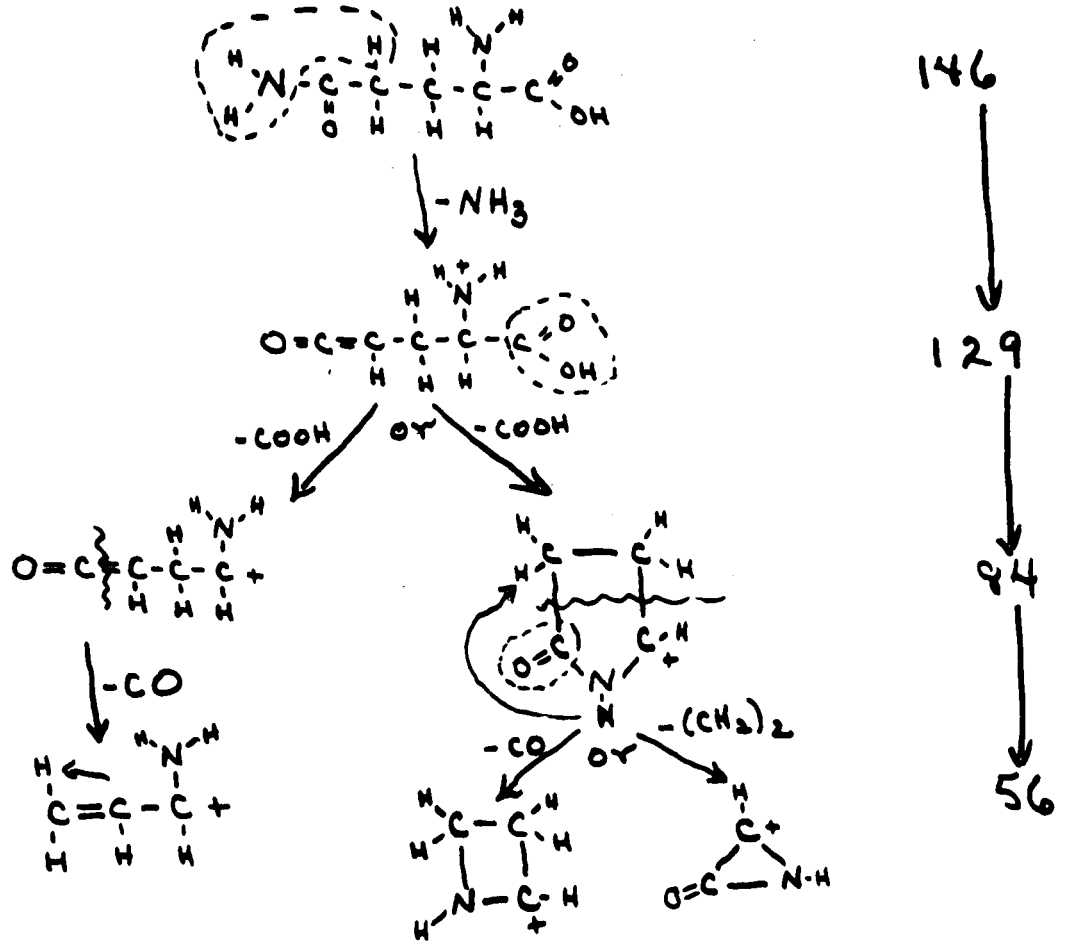

Structure:



Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 129 | 11 |  | $M-NH_3$ |
| 84 | 100 | See page 41. | $(M-NH_3)-45$, Bpk. |
| 56 | 13 | See page 41. | See page 41. |

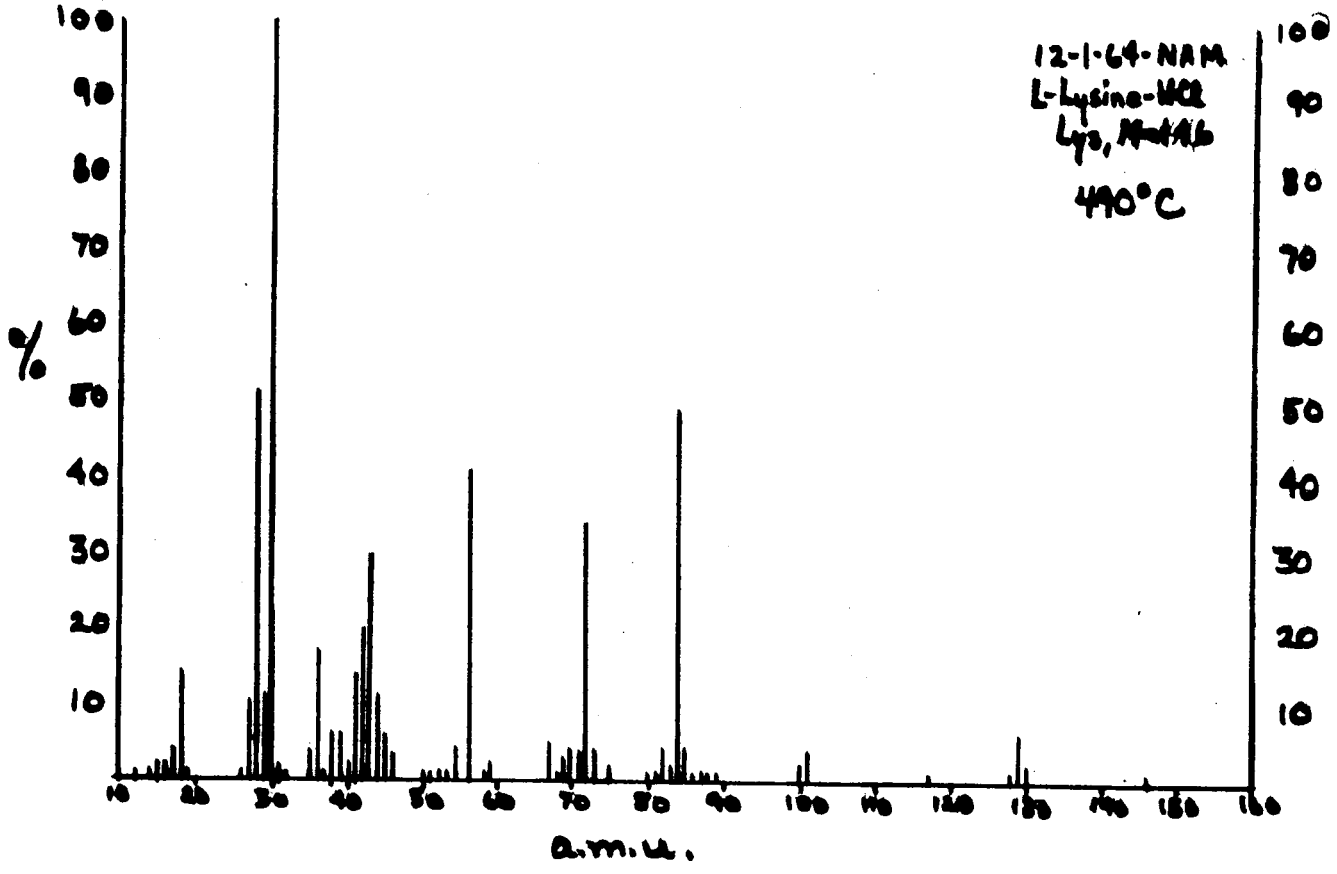
Glutamine - continued:



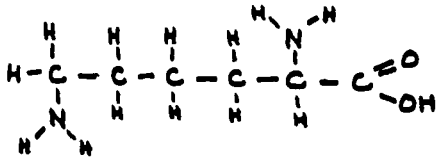

Identifying Peaks:

The presence of a peak at m/e 84 in significant abundance and in the temperature range of 85°C to 330°C indicates the presence of glutamine: glutamic acid exhibits a strong peak at m/e 84, but in the lower range of 75°C to 82°C, and lysine has an m/e 84 peak from 110°C to 630°C.

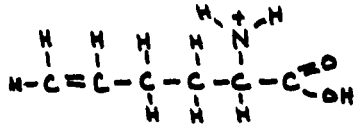
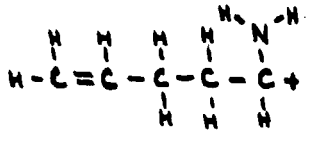
c. Lysine (HCl): M.Wt. = 146: Recording Temperature = 490°C




Structure:

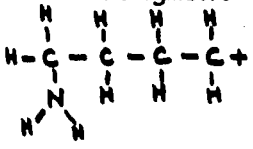
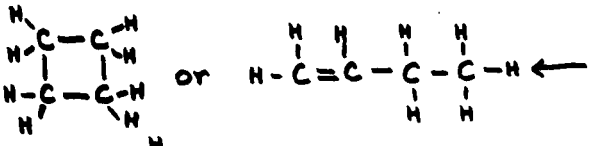
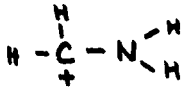


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 146 | 1 | See above | M |
| 129 | 6 |  | $M-NH_3$ |
| 84 | 49 |  | $(M-NH_3)-45$ |

Lysine (HCl) - continued:

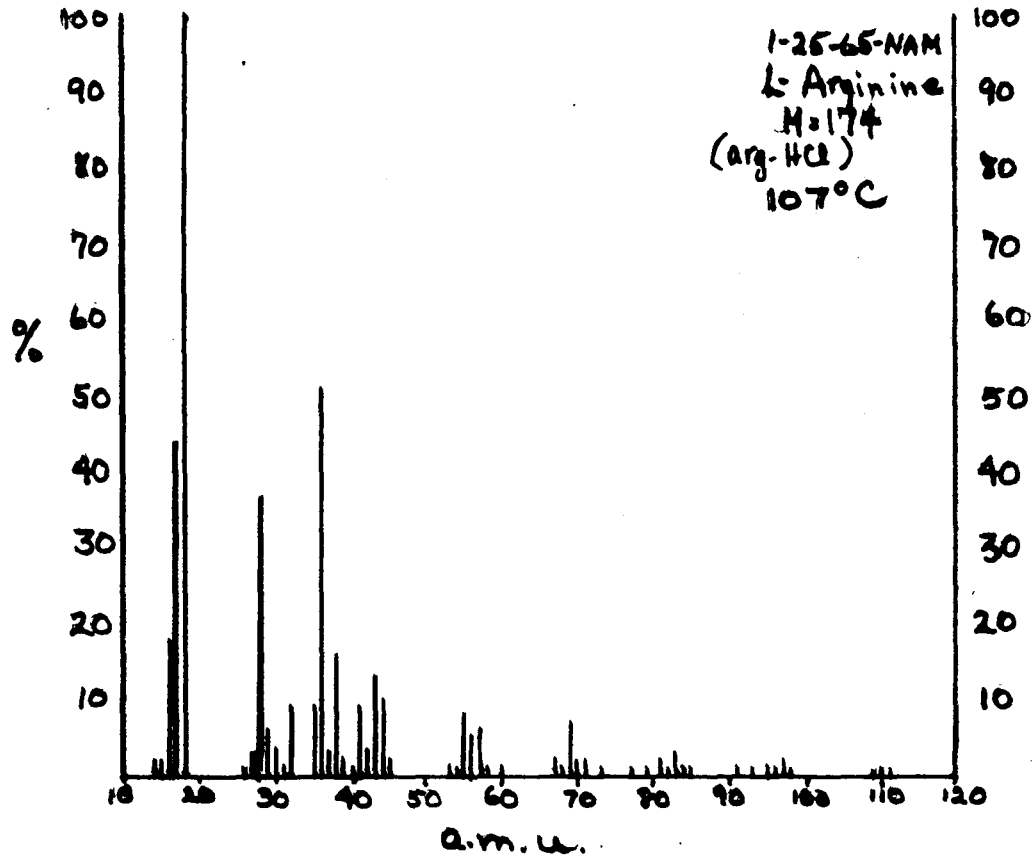
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 72 | 34 |  | $\alpha \times \underline{\beta}$ |
| 56 | 41 |  | |
| 30 | 100 |  | |

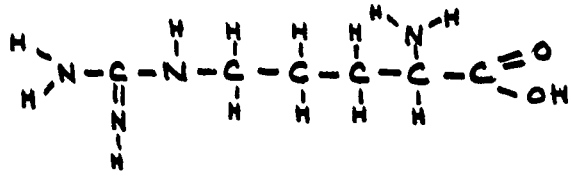
Identifying Peaks:

A peak at m/e 72 of significant abundance, arising at 110°C and persisting to 630°C indicates the presence of lysine: valine, which has its base peak at m/e 72, decomposes above about 82°C.

d. Arginine: M.Wt. = 174




Structure:



Spectrum Analysis:

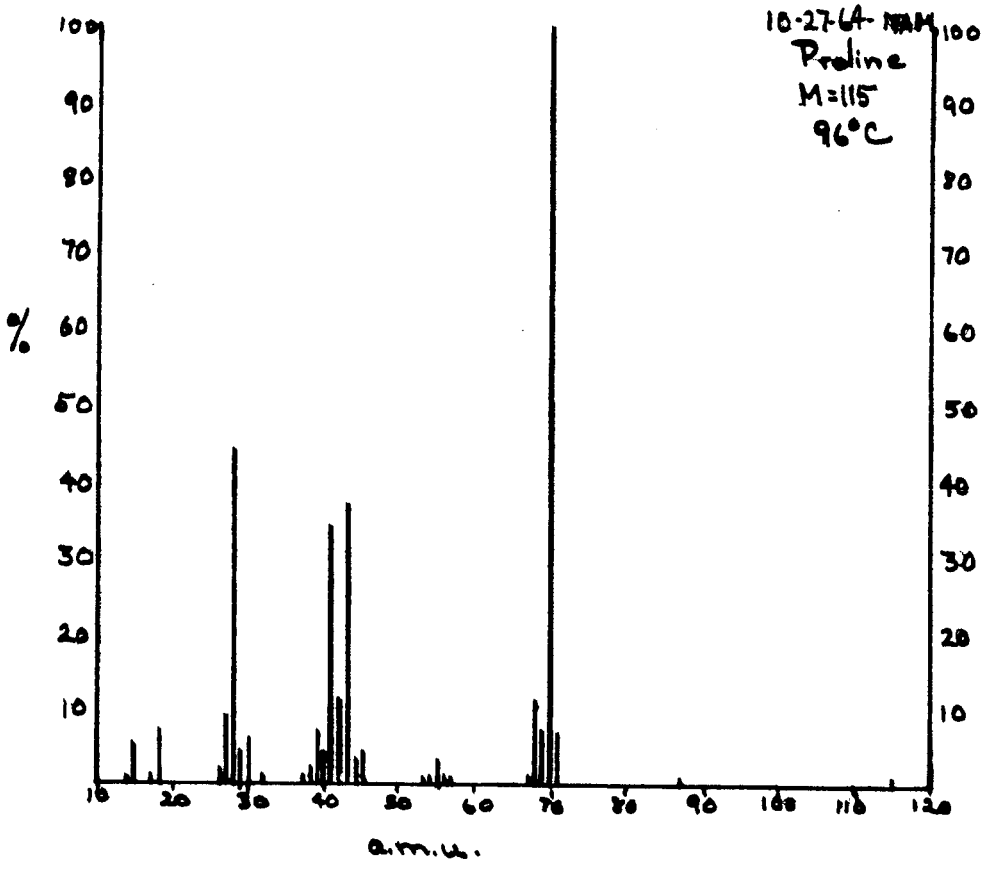
The spectrum of arginine-HCl shows only decomposition fragments at low mass numbers.

On 5-13-'65 an attempt was made to obtain the mass spectrum of free arginine: this also was unsuccessful.

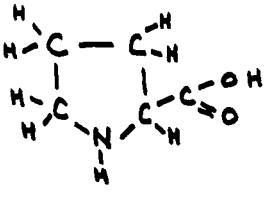
There are no identifying peaks.

4. Secondary Amino Acids

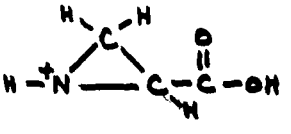
a. Proline: M.Wt. = 115: Recording Temperature = 96°C




Structure:

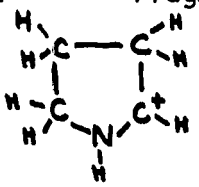
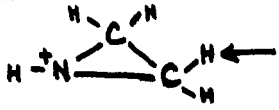
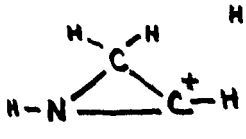
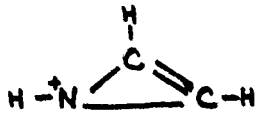


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 115 | 1 | See above | M |
| 87 | 1 |  | M-2 x 2($CH_2$) β x γ |

Proline - continued:

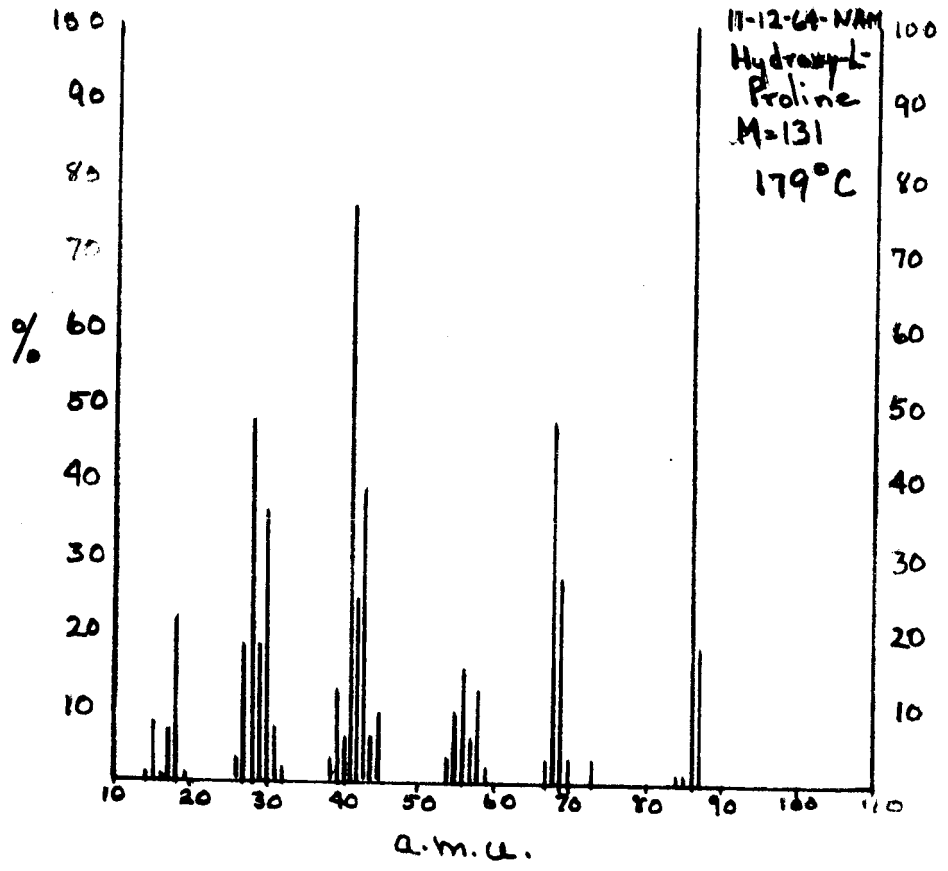
Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 70 | 100 |  | M-45, Bpk. |
| 43 | 37 |  | |
| 42 | 11 |  | |
| 41 | 34 |  | |

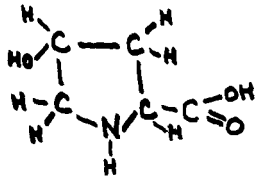
Identifying Peaks:

A peak of significant abundance at m/e 70 in the temperature range 92°C to 96°C indicates proline.

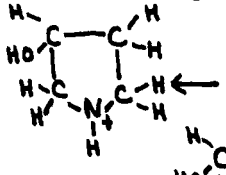
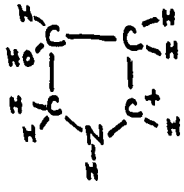
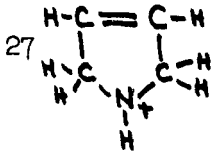
b. Hydroxyproline: M.Wt. = 131: Recording Temperature = 179°C




Structure:

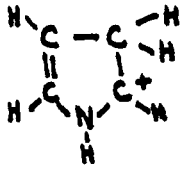
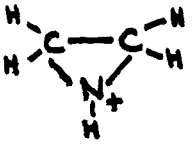
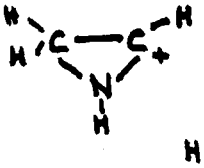
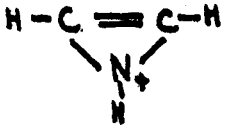


Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 87 | 18 |  | (M-45) + H |
| 86 | 100 |  | M-45, Bpk. |
| 69 | 27 |  | $[(M-45)+H] - H_2O$ |

Hydroxyproline - continued:

Spectrum Analysis:

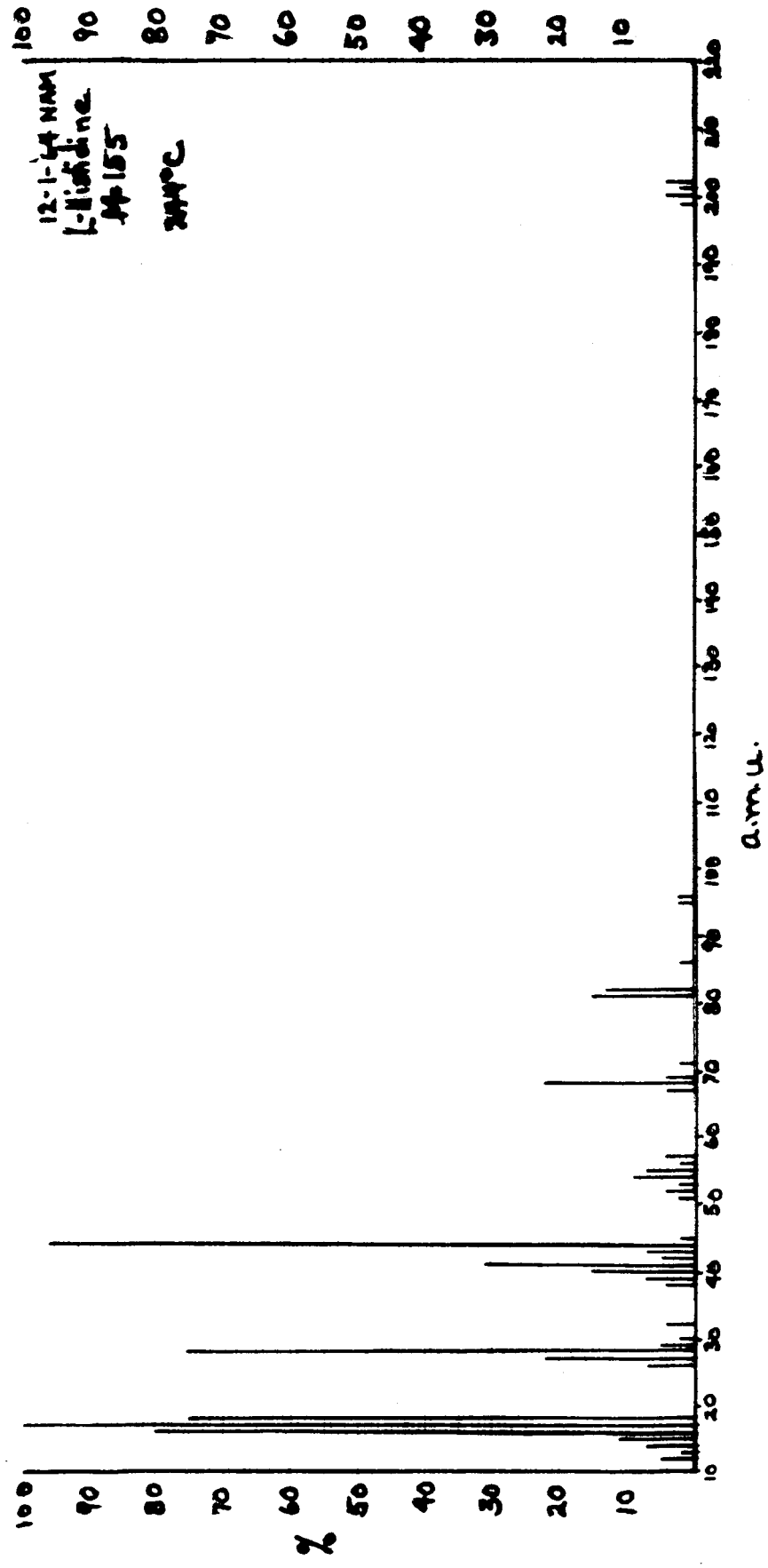
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 68 | 48 |  | (M-45) $-H_2O$ |
| 43 | 39 |  | |
| 42 | 25 |  | |
| 41 | 76 |  | |

Identifying Peaks:

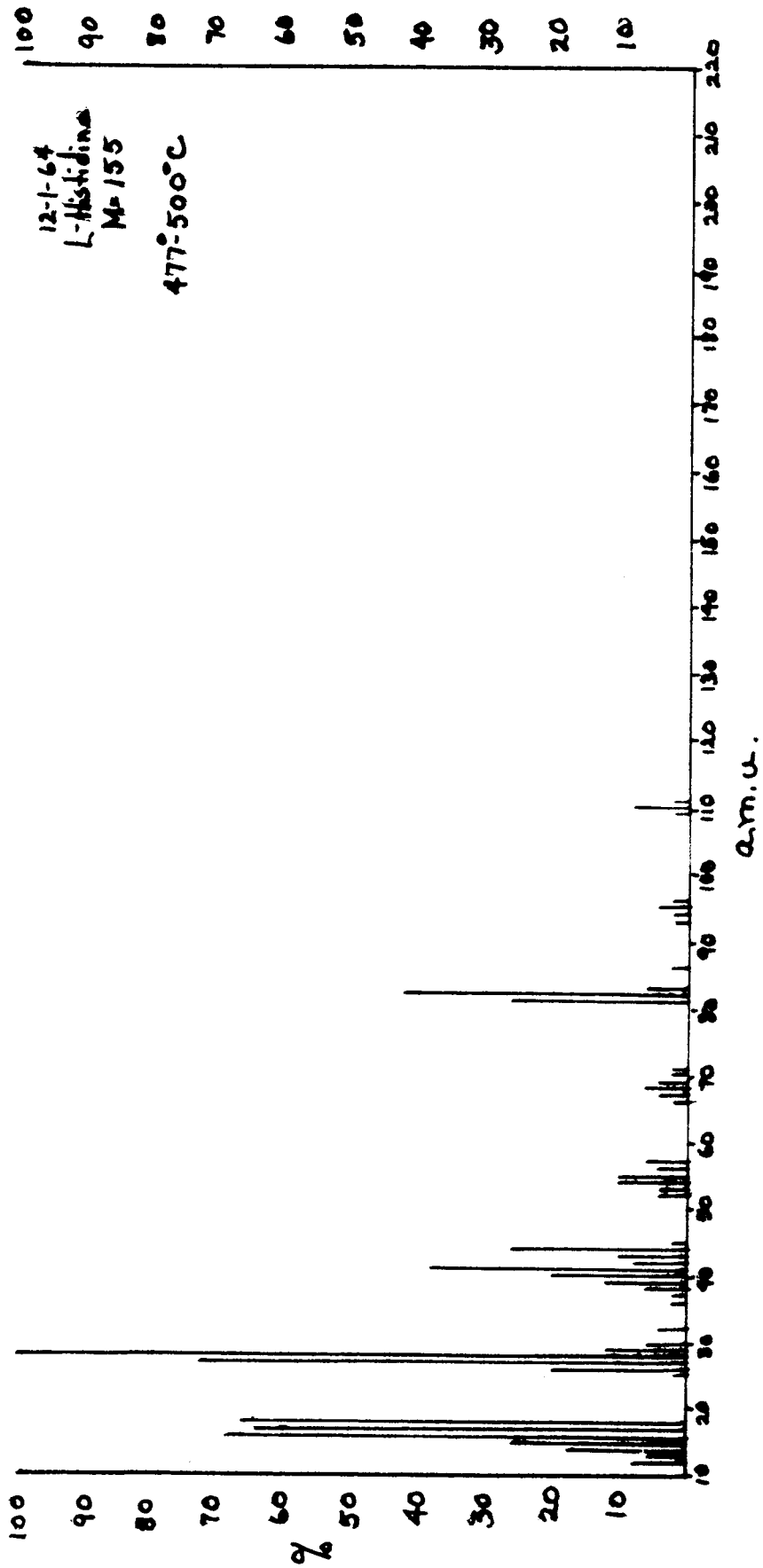
A peak appearing in significant abundance at m/e 86 in the temperature range of 136°C to 179°C indicates hydroxyproline: both leucine and isoleucine have prominent m/e 86 peaks, but neither is stable above 100°C.

5. α-amino and secondary amino acid

Histidine: M.Wt. = 155: Recording Temperature = 477°C - 500°C

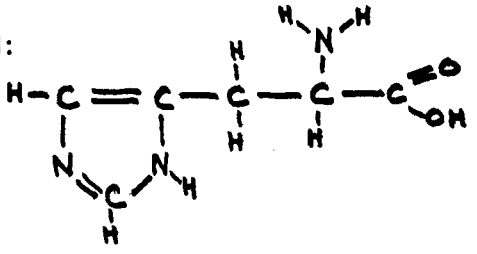


Histidine - continued:

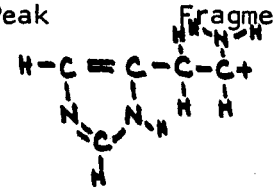
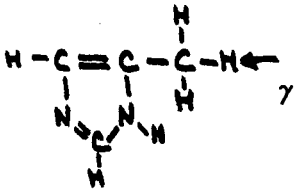
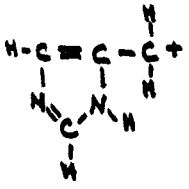
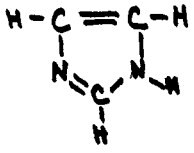


Histidine - continued:

Structure:



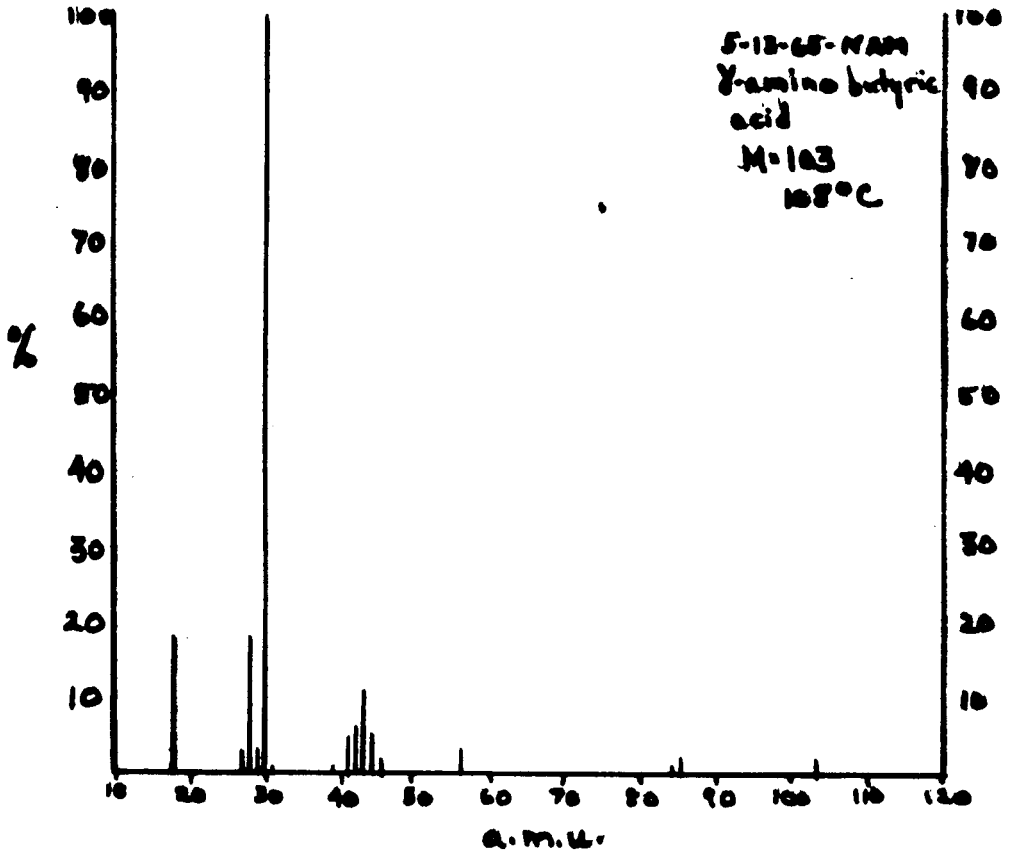
Spectrum Analysis:

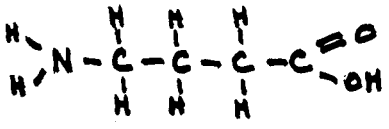
| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 110 | 8 |  | M-45 |
| 82 | 42 |  | $\gamma \times \underline{\beta} + H$ |
| 81 | 26 |  | $\gamma \times \underline{\beta}$ |
| 68 | 6 |  | $\beta \times \underline{\gamma} + H$ |

Identifying Peaks:

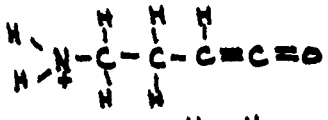
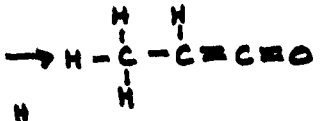
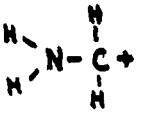
The appearance of a peak of significant abundance at m/e 82 and another at m/e 110, with a ratio of m/e 82/110 of about 5 in the temperature range of 477°C to 500°C indicates the presence of histidine.

6. γ-amino butyric acid: M.Wt. = 103: Recording Temperature = 108°C




Structure: 

Spectrum Analysis:

| Significant Peaks m/e | % Abundance of Base Peak | Structure of Fragment | Code |
|---|---|---|---|
| 103 | 2 | See above | M |
| 85 | 2 |  | $M-H_2O$ |
| 56 | 3 |  | $(M-H_2O)$ β×γ+H |
| 30 | 100 |  | $(M-H_2O)$ β×γ Bpk. |

γ-amino butyric - continued:

Identifying Peaks:

The presence of a peak in significant abundance at m/e 30, appearing between 100°C and 105°C indicates the presence of γ-aminobutyric acid.

## V. A Schema for the Identification of the Members of a Mixture of Free Amino Acids

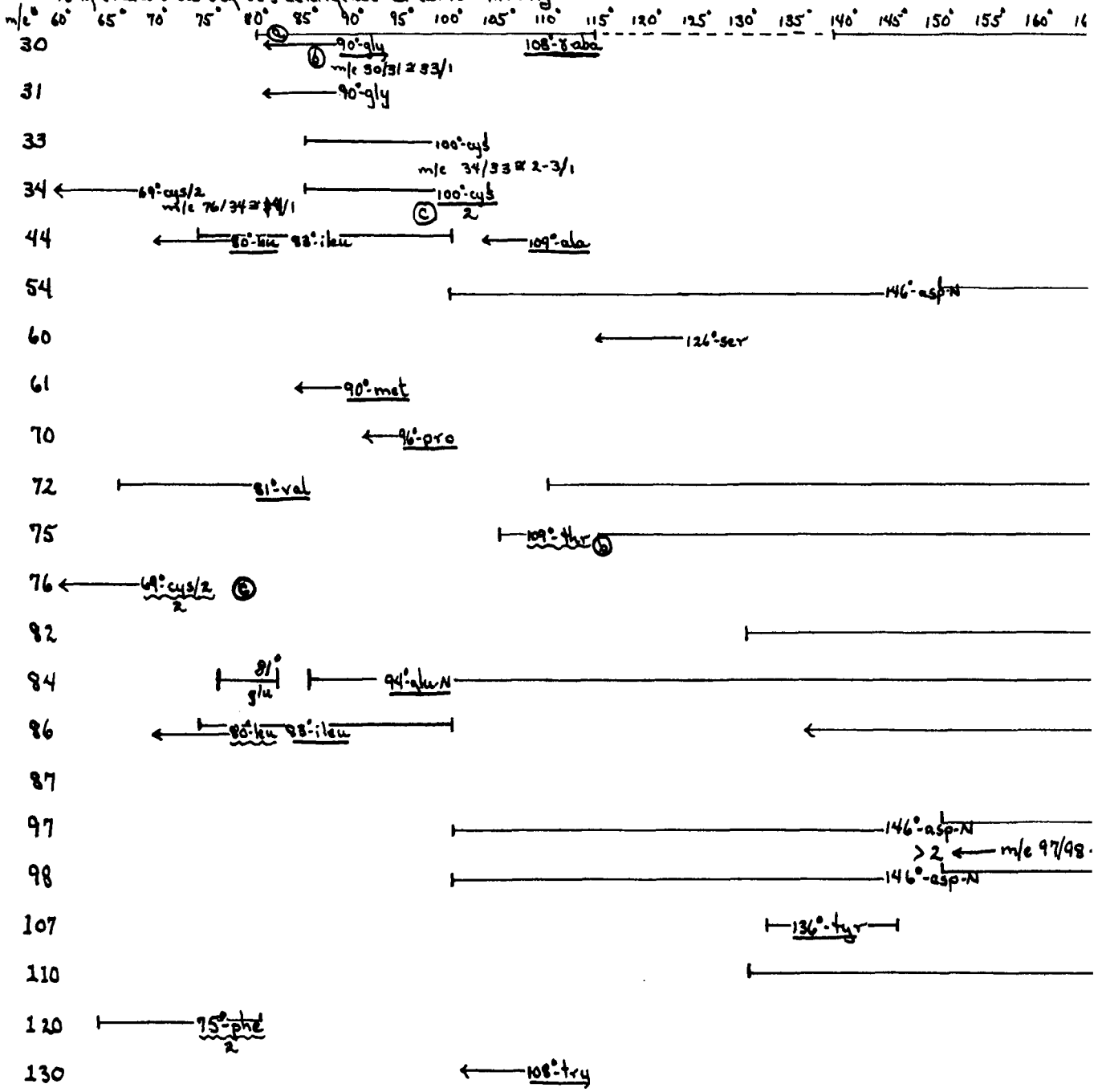
It is proposed that, from the information on the mass spectra of single free amino acids discussed in section IV, B, it should be possible to identify the members of a mixture of free amino acids. By utilizing the identifying m/e peaks as well as the apparently discrete temperature ranges over which they exist as molecular gases, any of the twenty-two amino acids investigated should be easily detected.

Because none of the experiments in this series was performed with mixtures of free amino acids, it is impossible to state that the schema is actually sound. The possibility cannot be ignored that molecular interaction among dissimilar free amino acids may occur when they are placed in the vacuum existing at the mass spectrometer's source region and the temperature is then incrementally elevated: this might well make it difficult, if not impossible, to obtain correct information about the original mixture. (See Section I, B)

The following table lists the identifying peaks and the temperature ranges at which individual amino acids may be detected.

## The Appearance of Identifying Peaks from the

Temperature in degrees Centigrade at ca. $10^{-6}$ mm Hg



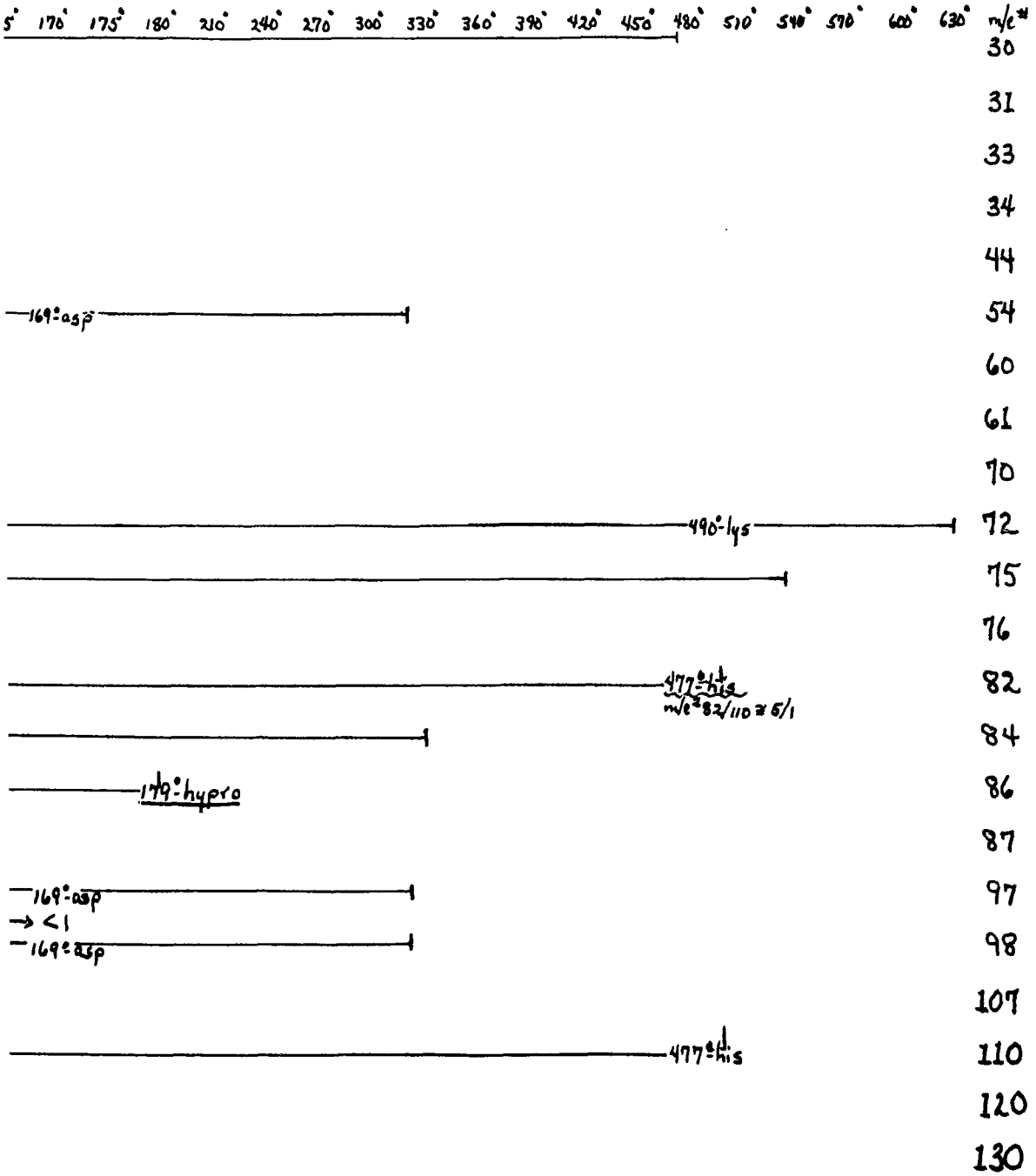

ⓐ These horizontal lines indicate the temperature range over which an amino acid has been observed t
range is indicated; where terminated by an arrow, the end of the range was not observed.

ⓑ A straight horizontal line under a compound's name indicates that the m/e peak is also the b
is within a few percent of the base peak's abundance.

ⓒ A number "2" beneath a straight horizontal line indicates that the m/e peak i
horizontal line indicates that the m/e peak is within ten percent of half of

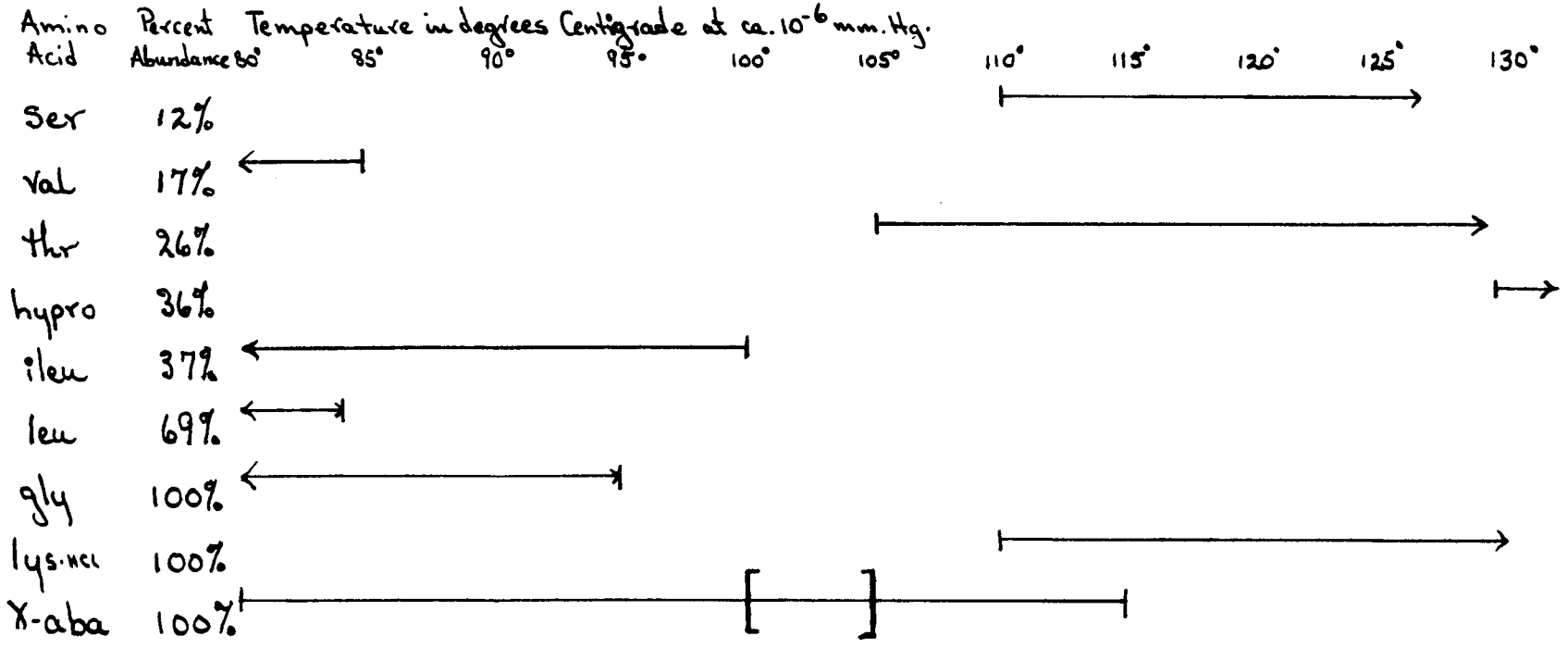
For additional information consult Section IV.B.

Mass Spectra of Some Amino Acids at Various Temperatures — Section V.




o exist in a molecular gas state: where terminated by a vertical line, an extreme of the ase peak for that compound. When the underline is wavy, the m/e peak indicated within a few percent of half of the base peak. A number "2" beneath a wavy the base peak.

# Spectral Contributions at Mass *30 by Some Amino Acids at Various Temperatures



## VI. Conclusions

From the work included in this report it seems certain that single free amino acids may be identified by their characteristic mass spectra. Although a number of experiments have been repeated on particular amino acids, there have not been multiple runs on all of them. Where repeat experiments have been performed, the qualitative agreement has been very good. Because no attempt has been made to obtain quantitative information in this series of experiments, no further statement may be made than that milligram quantities of sample (calculated from solid volume) have been sufficient to maintain sample gases for periods from twenty minutes to three hours: a spectrum may be recorded on film in a fraction of a second: in the LINC computer in fifty seconds or less; and on a strip chart recorder in five minutes or less.

The agreement between this work and the published work is very close, where experimental conditions are similar.

The work of Junk and Svec (see Section I. B) on limited mixtures of free amino acids suggests that the scheme proposed here for complete mixtures might well be sound.