CANADIAN THESES

THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE UNIVERSITY OF ALBERTA

Application of High Resolution EIMS to the Study of Fragmentation of 1-(amino acid)-1-deoxy-D-Fructoses and its Implication to the Mechanism of the Maillard Reaction

by

Varoujan Yaylayan

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Doctor of Philosophy

IN

Food Chemistry

Department of Food Science

EDMONTON, ALBERTA

Fall 1986

ISBN 0-315-32416-3

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR

Varoujan Yaylayan

TITLE OF THESIS

Application of High Resolution EIMS to the Study of Fragmentation of 1-(amino acid)-1-deoxy-D-Fructoses and its Implication to the Mechanism of the Maillard Reaction

DEGREE FOR WHICH THESIS WAS PRESENTED **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED **Fall 1986**

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) ..... V. Yaylayan .....

PERMANENT ADDRESS:

.....

.....

.....

DATED .....

19

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **Application of High Resolution EIMS to the Study of Fragmentation of 1-(amino acid)-1-deoxy-D-Fructoses and its Implication to the Mechanism of the Maillard Reaction** submitted by **Varoujan Yaylayan** in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Food Chemistry**.

.....

Supervisor

.....

.....

.....

.....

.....

Date...June 25, 1986

Abstract

The following Amadori rearrangement products were synthesized by a general procedure wherein L-amino acids and an excess of D-glucose were refluxed in methanol and then the product was isolated by column chromatography on ion-exchange resin followed by cellulose powder:

- 1-[(carboxymethyl)amino]-1-deoxy-D-fructose;
- 1-[(carboxyethyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-3'-methylbutyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-2'-methylbutyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-2'-hydroxylethyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose;
- 1-{[1'-carboxy-3'-(methylthio)propyl]amino}-1-deoxy-D-fructose;
- 1-[1'-carboxy-3'-(methylcarboxypropyl)amino]-1-deoxy-D-fructose;
- 1-[1'-carboxy-2'-imidazolylethyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose;
- 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose;
- 1-[(2'-carboxy-4'-hydroxy)pyrrolidinyl]-1-deoxy-D-fructose.

In addition, the following derivatives of lysine were synthesized according to known procedures:

- 1-[(5'-aminofructosyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose;

- 1-[(5'-aminoformyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose;
- 1-[(5'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose;
- 1-[(1'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose.

All the Amadori rearrangement products were subjected to electron-impact mass spectroscopy and their fragmentation patterns were studied.

Generally, the fragmentations of Amadori rearrangement products were triggered either by the ionization of the amino acid nitrogen or by the ionization of the ring oxygen. In the former case, the fragments obtained were termed "amino acid fragmentation" products and in the latter case "sugar fragmentation" products.

Basic amino acid nitrogens, having lower ionization energies in the gas phase, tended to give predominantly "amino acid fragmentation" products by 2,3-dehydration of the sugar ring and less basic amino acids tended to produce predominantly "sugar fragmentation" products by dehydroxylation and formation of oxonium ions.

According to the fragments identified, a modified mechanism of decomposition of Amadori rearrangement products was suggested, based on cyclic structures rather than the accepted open-chain structures.

Some of the important fragments identified were correlated with the products isolated in browning model systems and in the pyrolysates of the Amadori rearrangement products reported in the literature.

Acknowledgements

The author wishes to acknowledge Dr. Peter Sporns for his guidance and advice, the Mass Spectrometer Laboratory of the Department of Chemistry, Mr. Gour Choudhury, Mr. Len Steele for his "interactive" typing of the manuscript and, finally, NSERC for financial assistance.

Table of Contents

Chapter — Page

1. INTRODUCTION 1

1.1 Maillard Reaction 1

1.1.1 An Overview 1

1.1.2 Early Maillard reaction 5

1.1.2.1 Carbonyl-amine interactions 5

1.1.2.2 Formation of glycosylamine 9

1.1.2.3 Amadori and Heyns rearrangements 11

1.1.2.4 Formation of diketosamines 16

1.1.3 Advanced Maillard reaction 16

1.1.3.1 1,2- and 2,3-enolizations (pathways a and b) 16

1.1.3.2 Strecker degradation (pathway c) 21

1.1.3.3 Other pathways (pathways d and e) 21

1.1.4 Formation of Heterocyclic Compounds during the Advanced Maillard Reaction, and Their Role in the Production of Flavor, Aroma and Color in Food Systems ..24

1.1.4.1 Introduction 26

1.1.4.2 Formation of color 27

1.1.4.3 Mechanism of formation of heterocyclic flavor compounds ..29

1.1.5 Final Maillard reaction 34

1.1.6 Parameters that affect the Maillard reaction 35

1.1.6.1 Temperature and time 35

1.1.6.2 Water activity (a_w) 36

1.1.6.3 pH 39

1.1.6.4 The nature of the reactants 40

1.1.7 *In vivo* Maillard reaction 41

1.1.8 Technological aspects 44

1.2 Electron Impact Mass Spectroscopy (EIMS) 46

1.2.1 Introduction 46

1.2.2 Unimolecular ion decompositions 47

1.2.2.1 Mechanistic approach 47

1.2.2.2 Theoretical approach 49

1.2.2.3 Ionization by electron impact ..50

1.2.2.4 General properties of mass spectroscopy 55

1.3 The Rationale Behind Studying the Fragmentation of Amadori Rearrangement Products by EIMS 57

1.3.1 Introduction 57

1.3.2 The analogy between mass spectral reactions and the ground state or solution chemistry 60

1.3.2.1 Fragmentation mechanisms 60

1.3.2.2 McLafferty rearrangement and Norrish type II photochemical decomposition of ketones 61

1.3.2.3 Retro-Diels-Alder reaction 62

1.3.2.4 Correlation of ionization energies and reaction rates in mass spectrometer with Hammett σ values 63

1.3.2.5 "Retro mass-spectral synthesis" 65

1.3.2.6 Analogies to photolytic and radiolytic reactions 68

1.3.2.7 "Steric acceleration" 68

1.3.2.8 Measurement of acid dissociation constants by Fast Atom Bombardment Mass Spectroscopy (FABMS) 70

1.3.3 Conclusion 71

2. SUGGESTED FRAGMENTATION PATHWAYS OF AMADORI REARRANGEMENT PRODUCTS 73

2.1 Introduction 73

2.2 Treatment of the Mass Spectra 75

2.3 General Remarks 77

2.3.1 Definitions 77

2.3.2 Nomenclature 78

2.3.3 Experimental 79

2.4 Mass Spectra of Amadori Rearrangement Products ..79

2.4.1 Strategy of analysis of the mass spectra 79

2.4.2 Mass spectrum of D-fructose 85

2.4.2.1 Fragmentations of the pyranoid form 85

2.4.2.2 Fragmentations of the furanoid form 91

2.4.3 Mass spectra of amino acid ethyl esters ..96

2.4.4 General rearrangements and fragmentations observed in the mass spectra of Amadori rearrangement products 107

2.4.5 1-[(carboxymethyl)amino]-1-deoxy-D-fructose 117

2.4.5.1 "Sugar fragmentations" of the pyranoid form 117

2.4.5.2 Fragmentations of the furanoid form 123

2.4.5.3 "Amino acid" fragmentations ...126

2.4.6 1-[(1'-carboxy-2'-methylpropyl) amino]-1-deoxy-D-fructose 127

2.4.6.1 "Amino acid" fragmentations ...127

2.4.6.2 Specific fragmentations 131

2.4.7 1-[(1'-carboxy-2'-hydroxyethyl) amino]-1-deoxy-D-fructose 133

2.4.7.1 "Amino acid" fragmentations ...133

2.4.7.2 Specific fragmentations 135

2.4.8 1-[(1'-carboxy-2'-hydroxypropyl) amino]-1-deoxy-D-fructose 137

2.4.8.1 "Amino acid" fragmentations ...137

2.4.8.2 Specific fragmentations 142

2.4.9 1-{[(1'-carboxy-3'-(methylthio)propyl] amino}-1-deoxy-D-fructose 145

2.4.9.1 "Amino acid" fragmentations ...145

2.4.9.2 Specific fragmentations 150

2.4.10 1-[(1'-carboxy-3'-methylcarboxypropyl) amino]-1-deoxy-D-fructose 152

2.4.10.1 "Amino acid" fragmentations ...152

2.4.10.2 Specific fragmentations 154

2.4.11 Lysine derivatives 155

2.4.11.1 1-[(5'-amino fructosyl-1'-carboxypentyl) amino]-1-deoxy-D-fructose 155

2.4.11.2 "Amino acid" fragmentations ...155

2.4.11.3 Specific fragmentations 158

2.4.11.4 1-[(5'-amino formyl-1'-carboxypentyl) amino]-1-deoxy-D-fructose 159

2.4.11.5 "Amino acid" fragmentations ...159

2.4.11.6 Specific fragmentations 161

2.4.11.7 1-[(5'-amino-1'-carboxy-pentyl)amino]-1-deoxy-D-fructose 162

2.4.11.8 "Amino acid" fragmentations ...162

2.4.11.9 Specific fragmentations 164

2.4.11.10 1-[(1'-amino-1'-carboxy-pentyl)amino]-1-deoxy-D-fructose 166

2.4.11.11 "Amino acid" fragmentations ...166

2.4.11.12 Specific fragmentations 168

2.4.11.13 1-[(1'-carboxy-2'-imidazolyl-ethyl)amino]-1-deoxy-D-fructose 170

2.4.11.14 "Amino acid" fragmentations ...170

2.4.11.15 Specific fragmentations 173

2.4.11.16 1-[(1'-carboxy-2'-phenethyl) amino]-1-deoxy-D-fructose 174

2.4.11.17 "Amino acid" fragmentations ...174

2.4.11.18 Specific fragmentations 177

2.4.11.19 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)amino]-1-deoxy-D-fructose 178

2.4.11.20 "Amino acid" fragmentations ...178

2.4.11.21 Specific fragmentations 182

2.4.11.22 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose 189

2.4.11.23 "Amino acid" fragmentations ...189

2.4.11.24 Specific fragmentations 191

2.4.11.25 1-[(2'-carboxy-4'-hydroxy)-pyrrolidinyl]-1-deoxy-D-fructose 194

2.4.11.26 "Amino acid" fragmentations ...194

2.4.11.27 Specific fragmentations 197

2.4.12 Discussion 200

2.4.13 Amadori rearrangement products with alkyl side chains 203

2.4.14 Amadori rearrangement products with side chains containing carboxyl, hydroxyl or sulfur groups 205

2.4.15 Amadori rearrangement products with basic side chains 208

2.4.16 Amadori rearrangement products with aromatic side chains 213

2.4.17 Amadori rearrangement products of imino acids 217

3. IMPLICATIONS OF THE FRAGMENTATION PATTERNS OF AMADORI COMPOUNDS TO THE MECHANISM OF THE MAILLARD REACTION 219

3.1 Effect of amino acid moiety on the mechanism of the Maillard reaction 220

3.2 Comparison of the Fragments obtained by EIMS to those of Solution and Pyrolytic Reactions ...236

3.3 Implications of the Fragmentation Patterns of Amadori Compounds to the Formation of Early Maillard Polymers 244

4. SYNTHESIS OF AMADORI REARRANGEMENT PRODUCTS 248

4.1 General Procedure for the Synthesis of Amadori Products 248

4.2 Chemical Tests 250

5. CONCLUSION 252

6. REFERENCES 254

7. APPENDIX I -- MASS SPECTRA 274

8. APPENDIX II -- "SUGAR FRAGMENTATIONS" 275

List of Tables

Table		Page
1.1	IE (eV) of some monofunctional aromatic compounds C_6H_5X.	66
1.2	Relative efficiencies of β-cleavage with H-transfer in photolytic, radiolytic and electron impact reactions.	69
2.1	.....	105
3.1	Relative amounts of m/z 144 and $^{+}NH_2=CH-R_1$ as $\%\Sigma_{50}$.	231
8.1	A. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose (valine-ARP).	285
8.2	B. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-hydroxyethyl)amino]-1-deoxy-D-fructose (serine-ARP).	286
8.3	C. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose (threonine-ARP).	287
8.4	D. 'Sugar fragmentations' of 1-{[(1'-carboxy-3'-(methylthio)propyl]-amino}-1-deoxy-D-fructose (methionine-ARP).	288
8.5	E. 'Sugar fragmentations' of 1-[(1'-carboxy-3'-methylcarboxypropyl)-amino]-1-deoxy-D-fructose (glutamic acid methyl ester-ARP).	289
8.6	F. 'Sugar fragmentations' of 1-[(5'-aminofructosyl-1'-carboxypentyl)-amino]-1-deoxy-D-fructose (difructosyl-lysine-ARP).	290
8.7	G. 'Sugar fragmentations' of 1-[(5'-aminoformyl-1'-carboxypentyl)-amino]-1-deoxy-D-fructose.	291
8.8	H. 'Sugar fragmentations' of 1-[(5'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose.	292

Table Page

8.9 I. 'Sugar fragmentations' of 1-[(1'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose. 293

8.10 J. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-imidazolylethyl) amino]-1-deoxy-D-fructose (histidine-ARP). 294

8.11 K. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose (phenylalanine-ARP). 295

8.12 L. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)-amino]-1-deoxy-D-fructose (trypto-phan-ARP). 296

8.13 M. 'Sugar fragmentations' of 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose (proline-ARP). 297

8.14 N. 'Sugar fragmentations' of 1-[(2'-carboxy-4'-hydroxy)pyrrolidinyl]-1-deoxy-D-fructose (hydroxyproline-ARP). 298

List of Figures

Figure		Page
1.1	Aldose-amino acid interaction.	2
1.2	Effect of pH on the pseudo first-order rate constant for the reaction of 5 x 10^{-4} M acetone with 0.0167 M hydroxylamine (Jencks, 1969).	7
1.3	Condensation of glucose in its open-chain form with the free amino group of an α-amino acid.	10
1.4	Amadori and Heyns rearrangments.	12
1.5	Lobry de Bruyn-Alberda van Ekenstein transformation.	13
1.6	Decomposition of diketosamines in water.	17
1.7	Some proposed pathways for the advanced Maillard reaction.	18
1.8	1,2- and 2,3-enolizations (pathways a and b).	20
1.9	Strecker degradation.	22
1.10	Pathway proposed by Høltermand (1966).	23
1.11	Pathway proposed by Namiki and Hayashi (1983).	25
1.12	Mechanism for the production of N-substituted pyrrole-2-aldehydes (Kato and Fujimaki, 1968).	31
1.13	Formation of oxazolines from Strecker degradation products (Rizzi, 1969).	33
1.14	Yield (%) of Amadori rearrangement products (glucose with different amino acids) versus % water after 1 hour at 100°C (Godefridus *et al.*, 1978).	38
1.15	Schematic diagram of basic components of a mass spectrometer.	51
1.16	McLafferty rearrangement.	61

Figure		Page
1.17	Reaction of cyclohexenes in the mass spectrometer.	62
1.18	Retro-Diels-Alder reaction.	63
1.19	Reaction of substituted benzophenones.	64
1.20	IE (eV) versus Hammett σ value for mono-substituted toluene.	67
2.1	Ionization energy in molecules containing noninteracting functional groups.	81
2.2	Prefered cleavages of amino acid ethyl esters.	96
3.1	Proposed mechanism for the Maillard reaction.	226
3.2		234
3.3		236
3.4		242
7.1	Mass spectrum of 1-[(carboxymethyl)-amino]-1-deoxy-D-fructose (glycine-ARP).	275
7.2	Mass spectrum of 1-[(carboxyethyl)amino]-1-deoxy-D-fructose (alanine-ARP).	275
7.3	Mass spectrum of 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose (valine-ARP).	276
7.4	Mass spectrum of 1-[(1'-carboxy-3'-methylbutyl)amino]-1-deoxy-D-fructose (leucine-ARP).	276
7.5	Mass spectrum of 1-[(1'-carboxy-2'-methylbutyl)amino]-1-deoxy-D-fructose (isoleucine-ARP).	277
7.6	Mass spectrum of 1-[(1'-carboxy-2'-hydroxyethyl)amino]-1-deoxy-D-fructose (serine-ARP).	277
7.7	Mass spectrum of 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose (threonine-ARP).	278

Figure Page

7.8 Mass spectrum of 1-{[(1'-carboxy-3'-(methylthio)propyl]amino}-1-deoxy-D-fructose (methionine-ARP). 278

7.9 Mass spectrum of 1-[(1'-carboxy-3'-methylcarboxypropyl)amino]-1-deoxy-D-fructose (glutamic acid methyl ester-ARP). 279

7.10 Mass spectrum of 1-[(5'-aminofructosyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose (difructosyllysine-ARP). 279

7.11 Mass spectrum of 1-[(5'-aminoformyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose. 280

7.12 Mass spectrum of 1-[(5'-amino-1'-carboxy-pentyl)amino]-1-deoxy-D-fructose. 280

7.13 Mass spectrum of 1-[(1'-amino-1'-carboxy-pentyl)amino]-1-deoxy-D-fructose. 281

7.14 Mass spectrum of 1-[(1'-carboxy-2'-imidazolylethyl)amino]-1-deoxy-D-fructose (histidine-ARP). 281

7.15 Mass spectrum of 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose (phenylalanine-ARP). 282

7.16 Mass spectrum of 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)amino]-1-deoxy-D-fructose (tryptophan-ARP). 282

7.17 Mass spectrum of 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose (proline-ARP). 283

7.18 Mass spectrum of 1-[(2'-carboxy-4'-hydroxy)pyrrolidinyl]-1-deoxy-D-fructose (hydroxyproline-ARP). 283

1. INTRODUCTION

1.1 Maillard Reaction

1.1.1 An Overview

The interaction between a reducing sugar and an amino group initiates a sequence of consecutive and parallel reactions known as the Maillard reactions, after the French scientist L.C. Maillard, who was the first to observe this facile transformation (Maillard, 1912) and was the first to describe the production of darkly-colored compounds when solutions of glucose and lysine were heated. He then extended these studies to different sugars and amino acids and found that it was the reducing group (aldehyde or ketone) in the sugar that was important and that different sugars and different amino acids reacted at different rates (Maillard, 1916). Nearly two decades later, it was shown that proteins (Ramsey *et al.*, 1933) reacted similarly, and another one and one half decades passed before the free amino groups of the proteins were found to be the main reacting groups (Mohammed *et al.*, 1949).

The Maillard reaction occupies an important position in food chemistry since it has far reaching implications on several aspects of food research: at a sensory level it is responsible for the production of flavors and aromas (Nursten, 1980); at a nutritional level it causes substantial reduction of nutritional value of proteins

(Hurrell and Carpenter, 1981); at a toxicological level it has been implicated in the production of certain mutagenic substances (Aeschbacher *et al.*, 1981); and at a technological level it causes the production of undesirable browning and large changes in solubility during food processing (Sullivan, 1981).

The complex network of steps involved in the Maillard reaction is not yet completely understood and our present knowledge of this reaction is still quite fragmentary.

Aldose-amino acid interaction results in the formation of aldosylamines in equilibrium with their Schiff bases. The aldosylamines thus formed subsequently undergo Amadori rearrangement, giving rise to 1-amino-1-deoxy-2-ketoses, known as Amadori rearrangement products (ARP) (Hodge, 1953), see Figure 1.1.

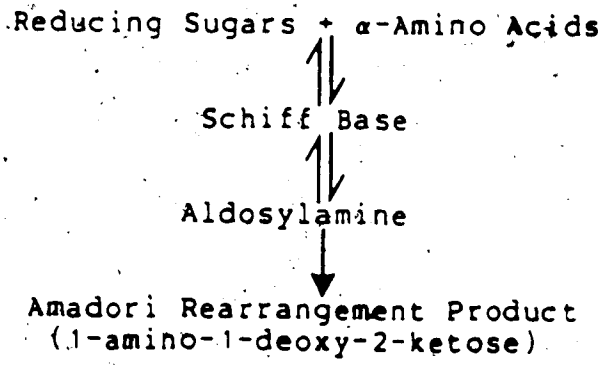


Figure 1.1 Aldose-amino acid interaction.

It appears that this rearrangement vastly reduces the sugar's stability so that the resulting sugar derivative

ultimately decomposes through chain splitting, oxidation, dehydration, retro-aldolization, etc. The resulting breakdown products also react with each other so that a multiplicity of reactive compounds is produced with strong flavor and aroma properties. On further decomposition these compounds can polymerize to form brown pigments known as melanoidins.

The first step in the Maillard reaction is part of a broader spectrum of reactions known as carbonyl-amine reactions. This is a key reaction in many enzymatic (Snell and Di Mari, 1970) and other biological processes such as vision (Dartnall, 1972) and it is the onset of many natural deterioration processes of tissue, such as aging.

The second step, the Amadori rearrangement, is also a well documented process in natural products (Anet and Reynolds, 1957) and *in vivo* (Mester *et al.*, 1981). ARPs have been isolated from freeze-dried peaches and apricots, tomato powder, black tea, wine, etc.

Haemoglobin HbA_{Ic} is an ARP of glucose with NH_2-terminal of valine in the β-chain of HbA (Mester, 1981).

In *Diabetes Mellitus* the high glucose concentration can change, through an Amadori type reaction, not only haemoglobin but also the structural proteins of the lens and of the blood vessels, producing cataracts and thickening of the basal membrane, respectively (Cerami *et al.*, 1979; Shyh-Horng Chiou, *et al.*, 1980).

Amadori rearrangment products have also been identified as the site of protein-glucose linkage in atopic allergens (Berrens, 1967). Recent studies (Mester *et al.*, 1981) indicate that serotonin also forms an Amadori rearrangement product with glucose *in vivo* and this exhibits a great deal of specific physiological effects by its action on platelets and tryptaminergic neurons.

The Maillard reaction can be divided into three stages: early, advanced and final.

Early Maillard reaction corresponds to the reversible formation of a glycosylamine and its Amadori rearrangement.

Advanced Maillard reaction corresponds to the degradation of the Amadori rearrangement products, leading to heterocyclic compounds.

Final Maillard reaction corresponds to the polymerization of the reactive intermediates of the advanced stage, leading to insoluble melanoidins.

It is worth mentioning here that the Maillard reaction should be distinguished from the caramelization reaction occurring when pure sugars are heated (Heyns and Klier, 1968). However, most reactions that occur during the thermal degradation of sugars are also observed in the Maillard reaction. In fact, many chemical reactions that occur in sugar alone only at very high temperatures take place at much lower temperature once the sugars have reacted with amino acids.

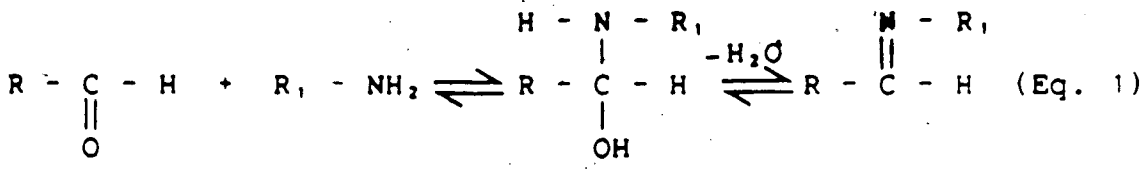
1.1.2 Early Maillard reaction

1.1.2.1 Carbonyl-amine interactions

Reaction of a carbonyl group with an amino group is one of the most frequent natural reactions. Since the Maillard reaction is initiated by such an interaction, some of the basic chemistry will be reviewed.

A wide variety of substances with NH_2-groups condense with carbonyl compounds.

Strongly-basic amines, like aliphatic amines, react according to equation 1 (Patai, 1968; Jencks, 1969):

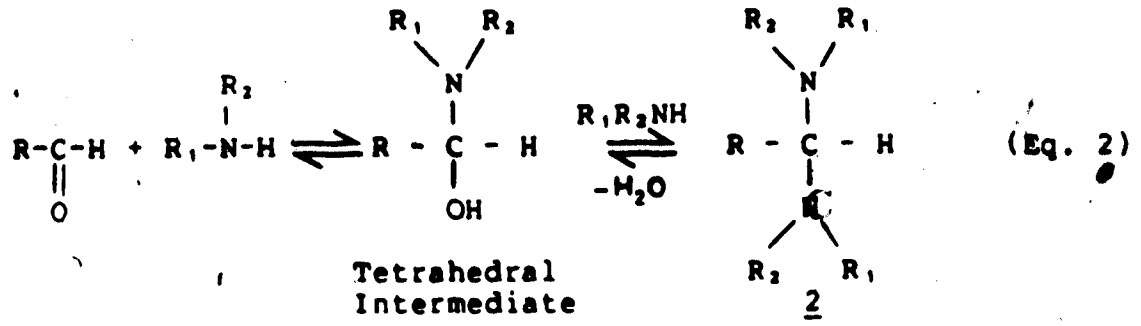


Tetrahedral Intermediate

1

This condensation of primary amines with aldehydes and ketones to give imines (1) was first described by Schiff (1900). The overall equilibrium greatly favors the hydrolysis in aqueous solutions for aliphatic aldehydes; with aromatic aldehydes, the equilibrium is shifted in favor of Schiff's base formation due to the stabilization of the product by conjugation. It is important to note that increasing the nucleophilic strength of the amine will increase the rate of carbonyl-amine reaction but will have almost no effect on the position of the equilibrium.

On the other hand, the weak bases, such as amides and secondary amines, can undergo reaction according to equation 2:



The tetrahedral intermediate formed in the initial addition cannot undergo intramolecular dehydration, but it reacts with another molecule of amine to form an alkylidene-diamine or gem-diamine (2).

The rate of the carbonyl-amine reaction usually shows a characteristic pH dependence that results in a bell-shaped curve (see Figure 1.2). At neutral pH, amine addition to the carbonyl compound is fast and the loss of water from the tetrahedral intermediate is rate determining. With decreasing pH, the rate of acid-catalyzed dehydration continues to increase, but as the free amine becomes protonated, the equilibrium concentration of the addition compound decreases. At pH values well below the pKa of the amine, these two effects (the increase in the rate of dehydration and the decrease of the equilibrium concentration of the tetrahedral intermediate) offset each other and the calculated rate becomes independent of pH. However, since the observed rate does not reach a plateau with

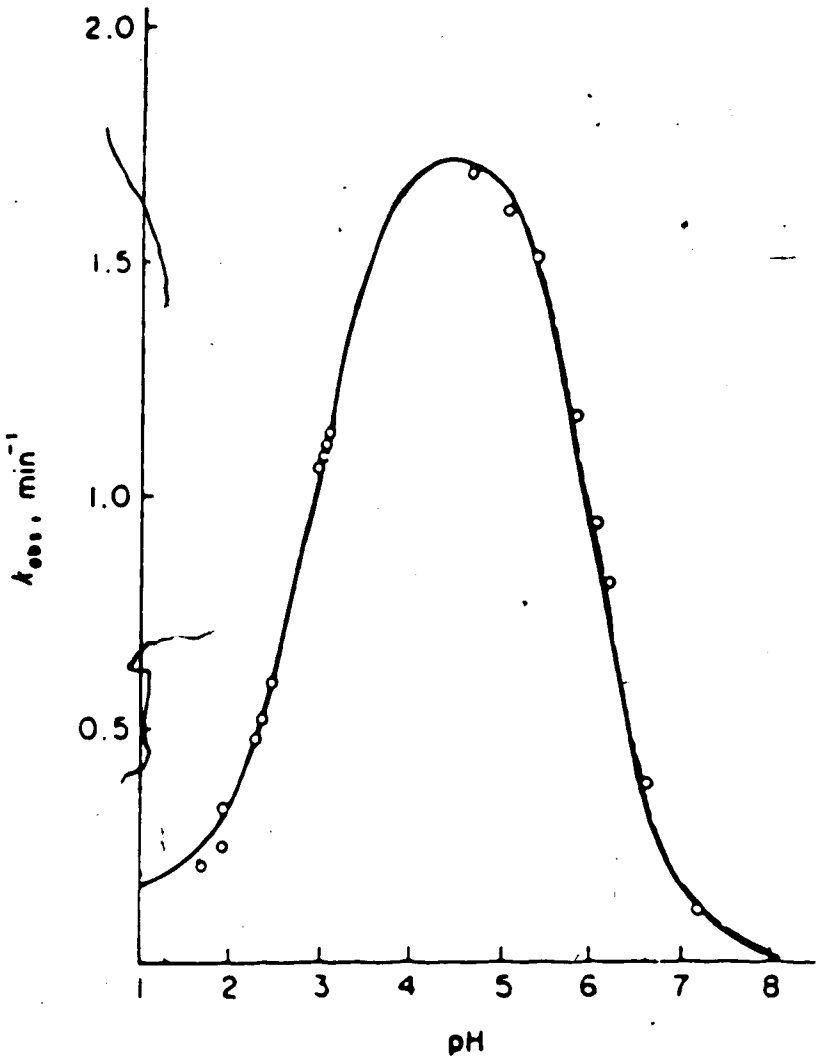
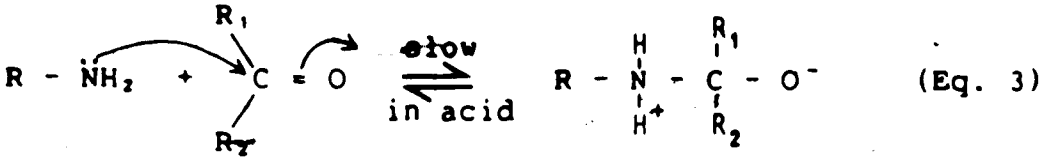


Figure 1.2 Effect of pH on the pseudo first-order rate constant for the reaction of 5 x 10^{-4} M acetone with 0.0167 M hydroxylamine (Jencks, 1969).

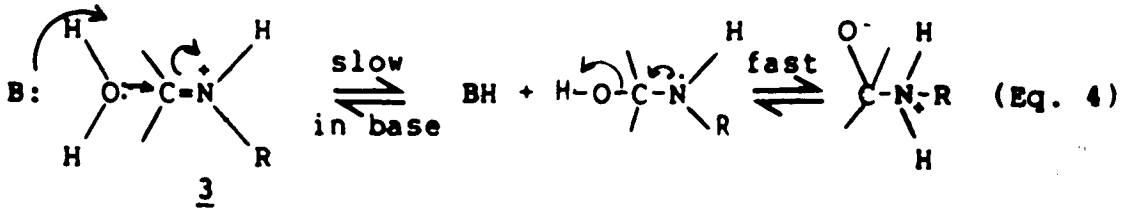
decreasing pH, but decreases after going through a maximum, another step in the reaction sequence must have become rate determining. The rate of acid-catalyzed dehydration becomes so fast that the rate of formation of the tetrahedral intermediate can no longer keep up with the rate of its dehydration, therefore, the attack of free amine on the carbonyl group becomes rate determining. The rate of this reaction is proportional to the concentration of the amine present. The rate of amine attack at the carbonyl group therefore increases with increasing pH, as shown in Figure 1.2.

For strongly basic amines, the change in the rate-determining step generally occurs between pH 2-5; below this pH the attack and loss of water is fast and the attack and loss of free amine is rate determining (equation 3);



(Eq. 3)

above this pH, the attack and loss of free amine is fast and the attack and loss of water is rate determining (equation 4):



(Eq. 4)

3

The protonated imine (3) is the reactive species. The attack of water on the protonated imine is subject to general base catalysis. The expelling of hydroxide ion from

the intermediate is generally acid catalyzed.

In the reaction of weakly-basic amines, the change in the rate-determining step occurs at the same or slightly higher pH as in the reaction of the more basic amines. The addition and loss of water follows the same mechanism as with the more basic amines. The smaller driving force of the less basic nitrogen atom results in a higher degree of acid catalysis for the dehydration step. As the amine becomes more acidic, base catalysis for water attack and expulsion becomes more significant.

1.1.2.2 Formation of glycosylamine

Glycosylation is initiated by condensation of glucose in its open-chain form with the free amino group of the amino acid, since, principally, carbonyl-amine interactions have their highest rate under weakly-acidic conditions and, since amino acids provide their own acid catalysts, the initial reaction is rapid, even in the absence of added acid; the product formed is a Schiff base (see Figure 1.3). However, the effect of pH on the rate of glycosylation is a little more complicated than on simple carbonyl-amine interaction due to the effect of pH on the mutarotation of sugars (Burton and McWeeny, 1963). At higher pHs, the concentration of the open-chain form of hexoses increases, hence the maximum in Figure 1.2 is shifted towards higher pHs. The equilibrium constant for the glycosylation reaction is unfavorable, but the glycosylamino acid (4) slowly undergoes

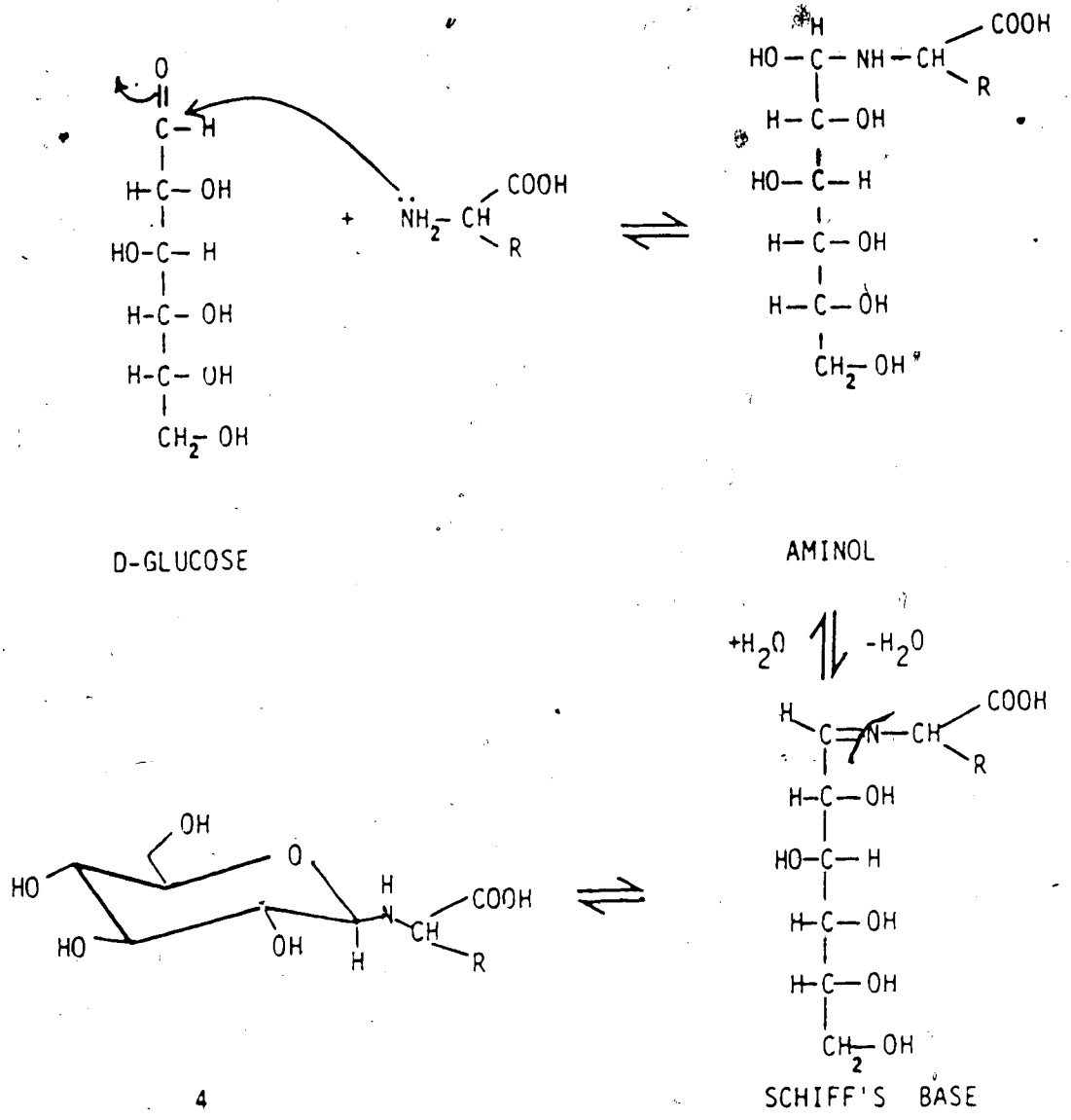


Figure 1.3 Condensation of glucose in its open-chain form with the free amino group of an α-amino acid.

Amadori rearrangement to yield a relatively stable derivative. The N-substituted glycosylamino acid (4) formed readily hydrolyzes in dilute acetic acid, many even hydrolyze relatively fast when dissolved in water at room temperature.

1.1.2.3 Amadori and Heyns rearrangements

The next step in the Maillard sequence is uncommon to most carbonyl-amine reactions. This is either the Amadori rearrangement (Amadori, 1931) or Heyns rearrangement (Reynolds, 1965). These closely related rearrangements are key reactions in the Maillard sequence and are shown in Figure 1.4.

The glycosylamino acid (4) is converted to 1-amino-1-deoxy-2-ketose (7) via the Amadori rearrangement, while the ketosamine (8) is transformed to 2-amino-2-deoxy-1-aldose (9) via the Heyns rearrangement. Both rearrangements are acid catalyzed, the carboxyl groups of the amino acids providing the internal acid catalyst.

This transformation of an aldose into a ketose and *vice versa*, via the formation of N-glycosides, is analogous to the Lobry de Bruyn-Alberda van Ekenstein transformation (Speck, 1958), which occurs when a reducing sugar is dissolved in water containing an alkaline catalyst. The transformation is illustrated in Figure 1.5.

Amadori (1931) showed that, depending on the manner of heating D-glucose with a primary arylamine, two different isomers, one labile and the other stable, could be isolated.

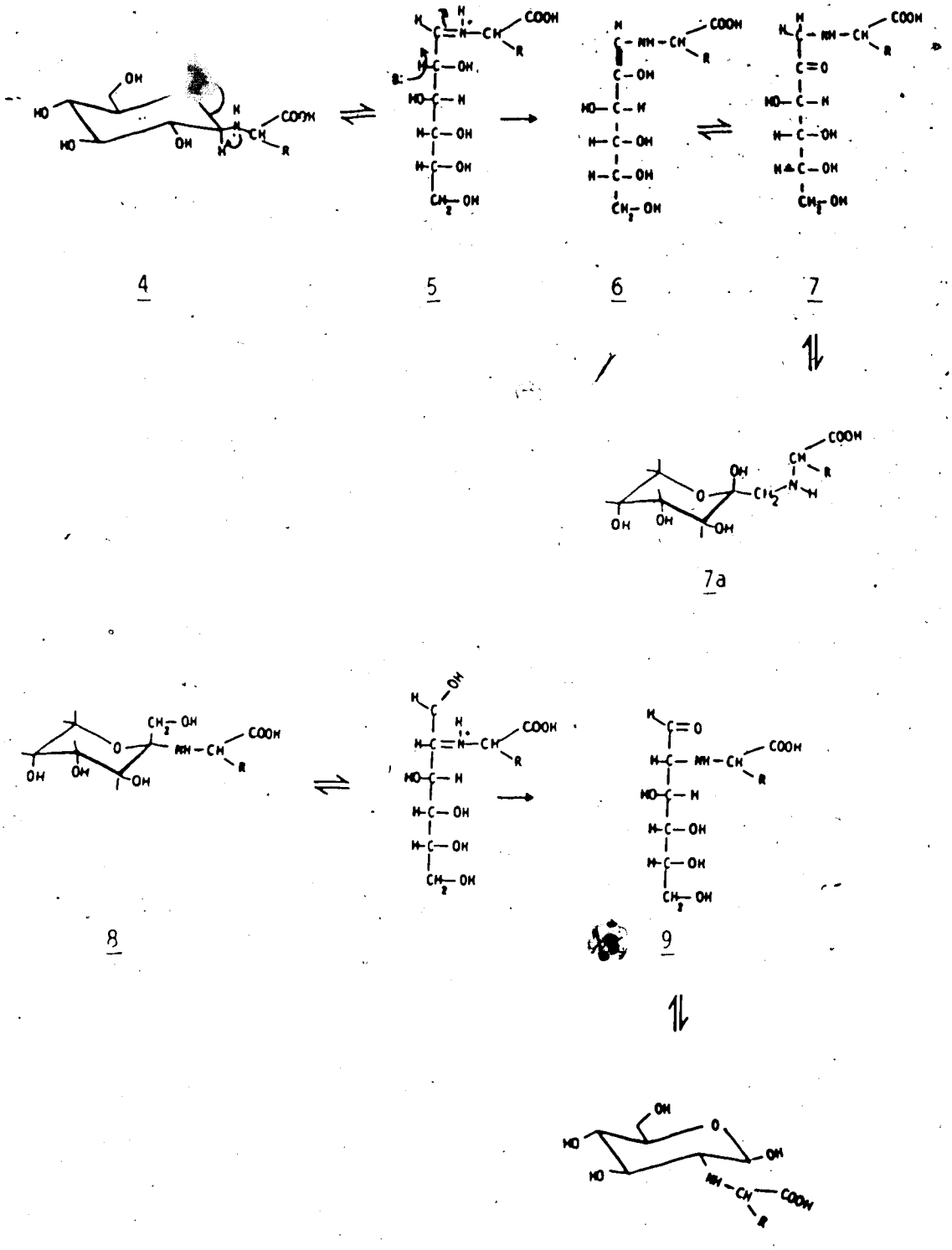
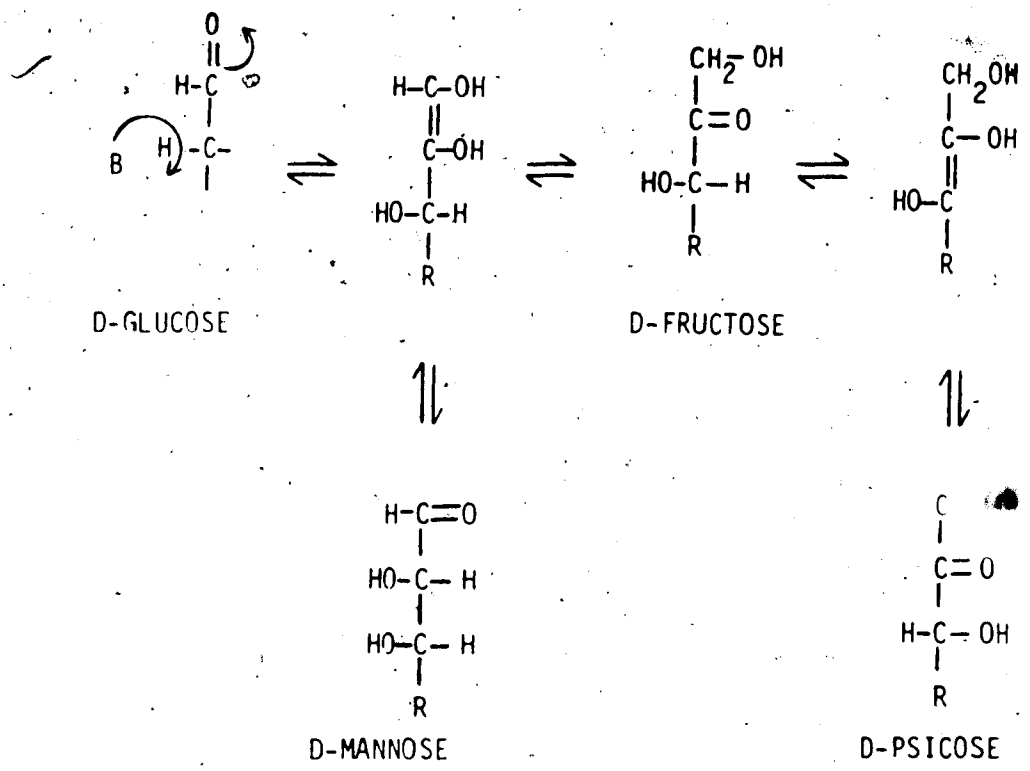


Figure 1.4 Amadori and Heyns rearrangments.



R=

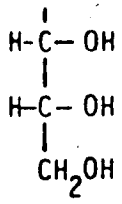


Figure 1.5 Lobry de Bruyn-Alberda van Ekenstein transformation.

Initially, Amadori thought that both isomers were D-glucose derivatives; later, Kuhn and Weygand (1937) identified Amadori's more labile isomer as the N-glycoside and the more stable one as a 1-amino-1-deoxy-2-ketose derivative and named it Amadori rearrangement product. Weygand (1940), in a critical review of the rearrangement conditions, was unable to obtain 1-amino-1-deoxy-2-ketose derivatives consistently from glycosylamines unless catalytic amounts of acid were present. It is now known that the Amadori rearrangement of glycosylamine derivatives to 1-amino-1-deoxy-2-ketose occurs slowly in the solid state on storage at 25°C and rapidly in hot alcoholic solution in the presence of compounds containing active methylenic hydrogen atoms.

Glycosylamino acids (4) in the furanose form rearrange ten times faster than the corresponding pyranose form; the ring opening appears to be rate limiting for the course of this reaction (Heyns *et al.*, 1970). Apparently, a base abstracts a proton from C-2 of the sugar residue to give enaminol (6) which is stabilized by rearranging into the hemiacetal form (7a). Because decomposition reactions run parallel to the rearrangement, the Amadori compounds can often be isolated only in small yields.

The first synthesis and isolation of 1-(amino acid)-1-deoxy-D-fructose was achieved by Gottschalk (1952) by refluxing a solution of DL-phenylalanine and glucose in methanol. Abrams *et al.* (1955) synthesized nine members of this series by the same method, purifying small quantities

by elution chromatography on a cation-exchange resin.

The Schiff bases and Amadori compounds exist largely in cyclic furanose and pyranose conformations. High resolution ^1H and ^{13}C-NMR spectra of the mutarotated Amadori products of L-amino acids in D_2O were reported by Röper *et al.* (1983). The ^1H-spectra allow unambiguous assignment of the signals of the major constituent (β-pyranose form); signals of the other forms, however, were not well resolved. The ^{13}C-spectra of all the compounds show ≃64% of β-pyranose, ≃15% α-pyranose, ≃15% β-furanose and ≃6% α-furanose forms and ≃2% of the open chain keto form. The proportions of the various components of the equilibria were not significantly altered by change in pH and were not dependent on the kind of amino acid. Röper *et al.* (1983) also recorded the ^{13}C-NMR spectra of fructose-alanine in the pH range 0.7-11.9 in D_2O-DCl or D_2O-NaOD. The compound in question was stable in this pH range for several hours. The pKa values obtained by this method were 1.7 and 8.5, respectively, which shows the variation in pKa values of the amino group compared with that of 9.69 for alanine; the acidic pKa was not very much different from that of 2.35 for alanine.

Amadori rearrangement products are relatively stable compounds which in some food systems, like milk, and under mild heating conditions represent the end stage of Maillard reaction. Although they convey neither brown pigmentation nor flavor to the food, their formation has been implicated in reducing the nutritive value of foods by depressing the

bioavailability of essential amino acids, especially the basic amino acid, lysine.

1.1.2.4 Formation of diketosamines

1-amino-1-deoxy-2-ketoses (Amadori compounds) derived from a primary amine can react with another molecule of a reducing sugar, which then can undergo another Amadori rearrangement to give a diketosamine (10). Apparently, the only crystalline compound of this type isolated is difructose-glycine (10) (Burton and MacWeeny, 1964). The reactions of diketosamines are very similar to those of Amadori product, with the notable exception that diketosamines readily decompose in water (Gottschalk, 1972), as shown in Figure 1.6.

1.1.3 Advanced Maillard reaction

Several pathways have been proposed for the advanced Maillard reaction, some of which are summarized in Figure 1.7. As is shown, pathways a and b start directly from the Amadori product, pathway c indirectly via the dicarbonyls produced in pathways a and b, whereas pathways d and e bypass the Amadori stage and are less significant.

1.1.3.1 1,2- and 2,3-enolizations (pathways a and b)

One of the more marked characteristics of Amadori compounds appears to be their tendency to produce enediol forms which arise from ring opening (Heyns and Paulsen, 1960; Hodge, 1953; Hodge *et al.*, 1963a). Enol formation can

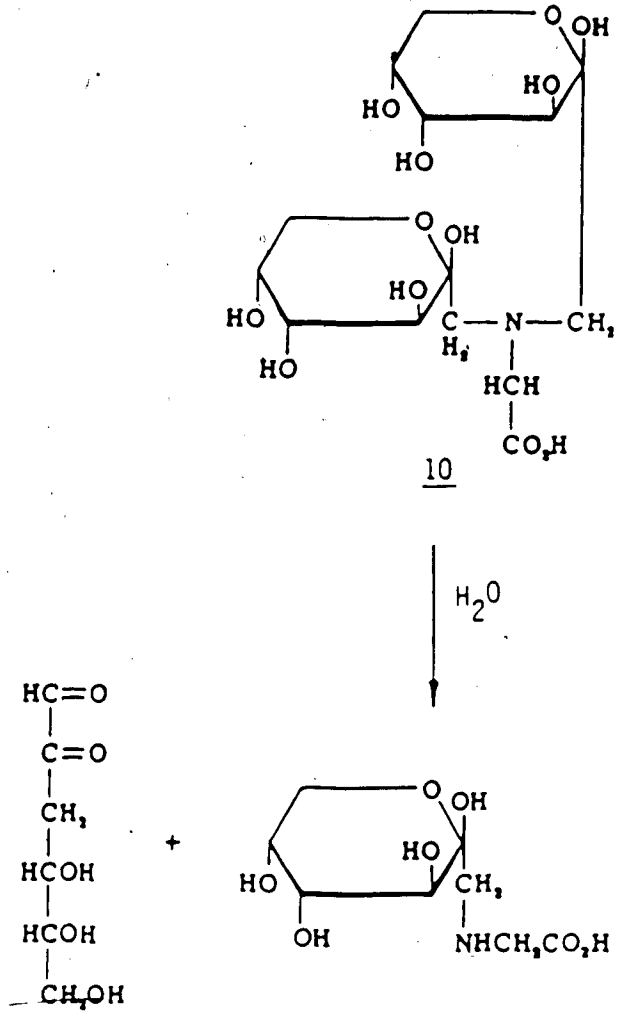


Figure 1.6 Decomposition of diketosamines in water.

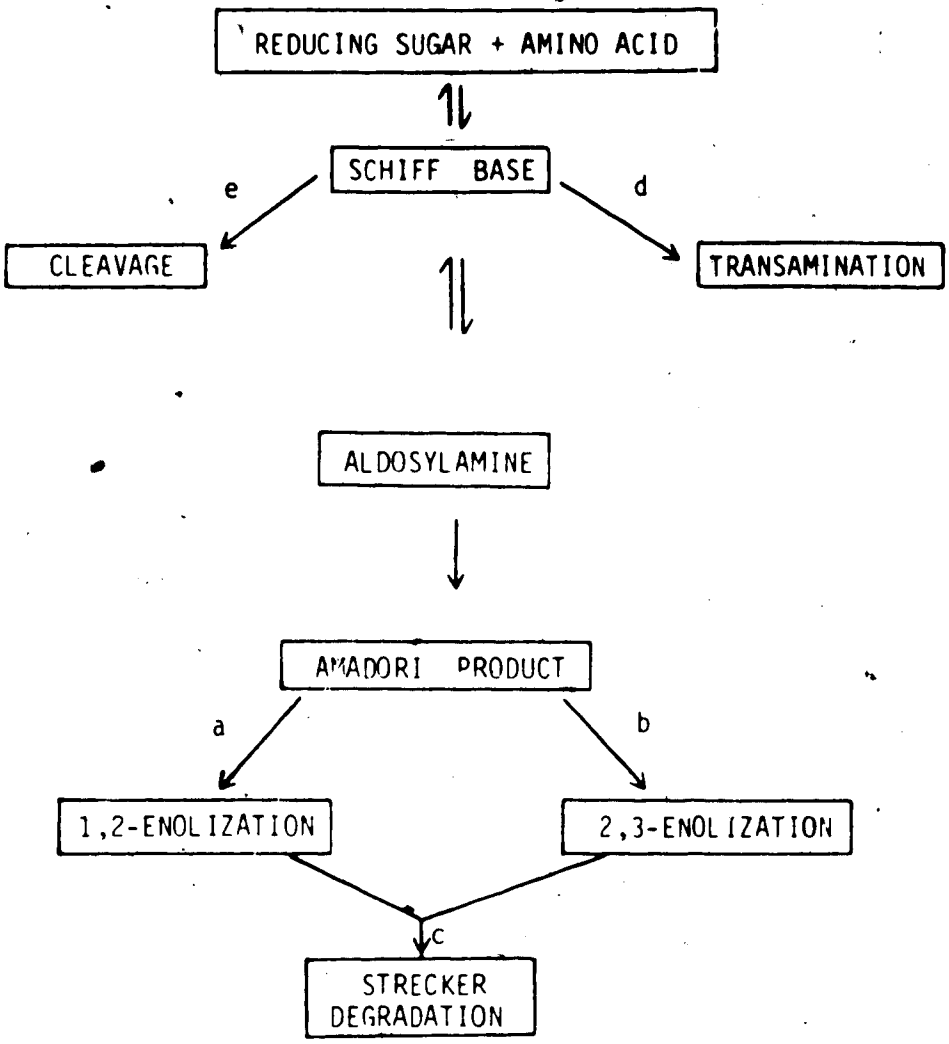
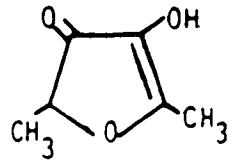


Figure 1.7 Some proposed pathways for the advanced Maillard reaction.

occur between C-1 and C-2 of the sugar residue as well as between C-2 and C-3, and in both cases characteristic decomposition sequences can be observed (Figure 1.8). In the first case, the allyl hydroxyl group on C-3 of 11 is eliminated, thereby creating a double bond between C-2 and C-3. This promotes a hydrolytic scission of the previously very tightly attached amino-acid residue, thus causing the formation of 3-deoxy-hexosulose (13) (Anet, 1964), a relatively stable compound found in various browning products such as soy sauce and dried fruits. The reaction continues through further dehydration and by translocation of the double bond until, finally, compound 14 yields hydroxymethylfurfural (15).

In the second case, an enediol is first formed between C-2 and C-3 of 16. This double bond facilitates the elimination of the allylamino acid residue and leads to the formation of 1-deoxyhexosulose (18), which decomposes further, ultimately yielding pyruvaldehydes, acetylfuran and, predominantly, 2,5-dimethyl-3-2(H)-4-hydroxy-furanone (20) (Hodge, 1967).



20

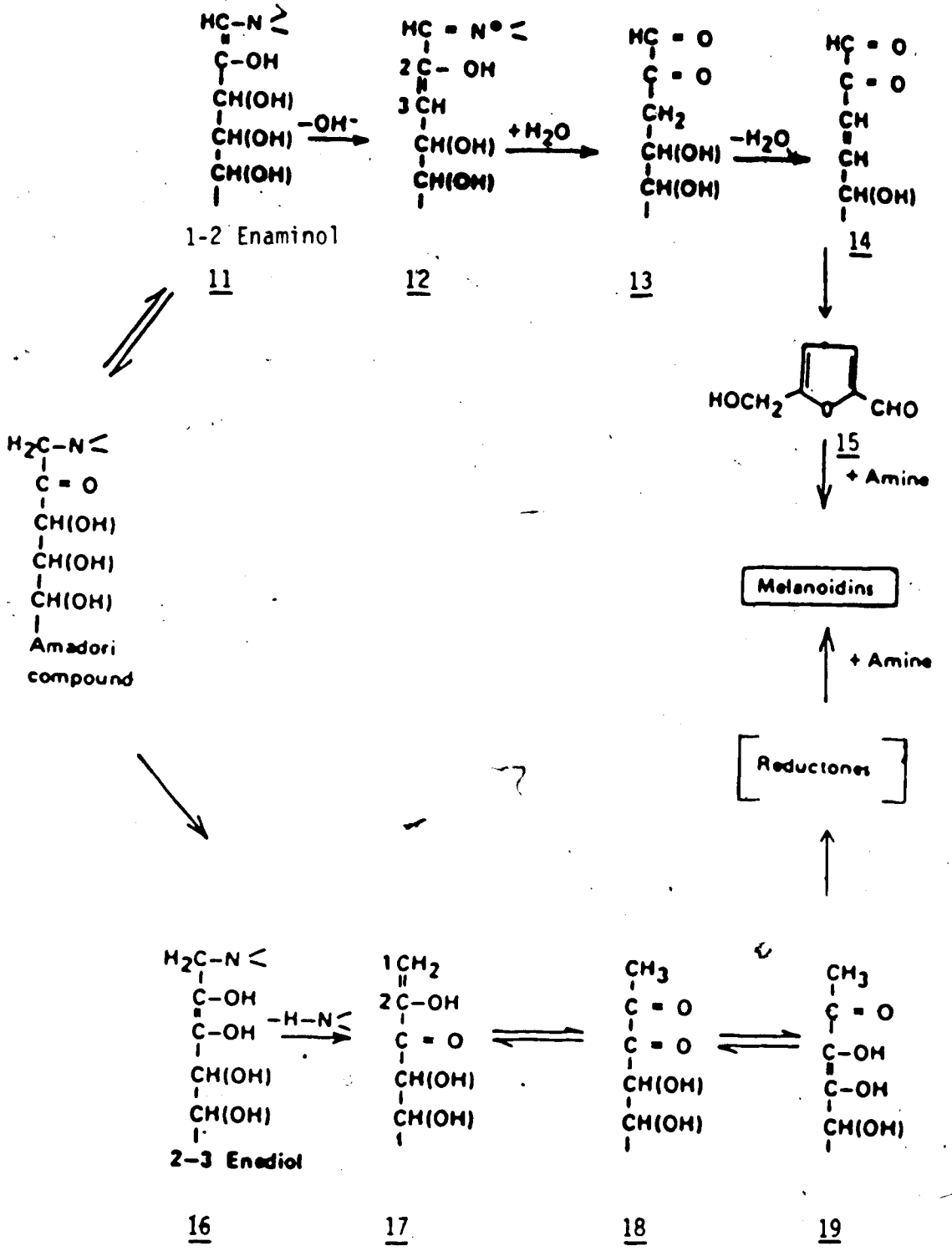
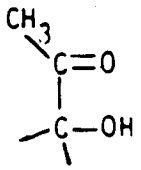


Figure 1.8 1,2- and 2,3-enolizations (pathways a and b).

1.1.3.2 Strecker degradation (pathway c)

Strecker degradation (Schönberg and Moubacher, 1952) involves the oxidative degradation of α-amino acids by α-dicarbonyl compounds or their vinyl analogs (Figure 1.9), but a recent study (Nyhammar *et al.*, 1983) showed that an α-acyloin (25) can also undergo such a degradation with α-amino acids.



25

In this reaction, α-amino acids react with α-dicarbonyl compounds to form the Schiff base (21), which is easily decarboxylated into enol 22 and then subsequently hydrolyzed into a β-keto amine (23) and an aldehyde (24) which corresponds to the original amino acid with one less carbon atom. The Strecker aldehyde (24) is a very important auxiliary flavor compound (Hodge *et al.*, 1972) which might participate in the production of melanoidins.

1.1.3.3 Other pathways (pathways d and e)

In addition to the above mentioned pathways, a fourth has been advanced, by Høltermand (1966), which bypasses the the Amadori stage (see Figure 1.10).

According to Høltermand (1966), this pathway starts with the Schiff base (26) and involves isomerization of the imine bond into 27, which undergoes hydrolysis in which the amino acid is converted into the corresponding oxo-acid (29)

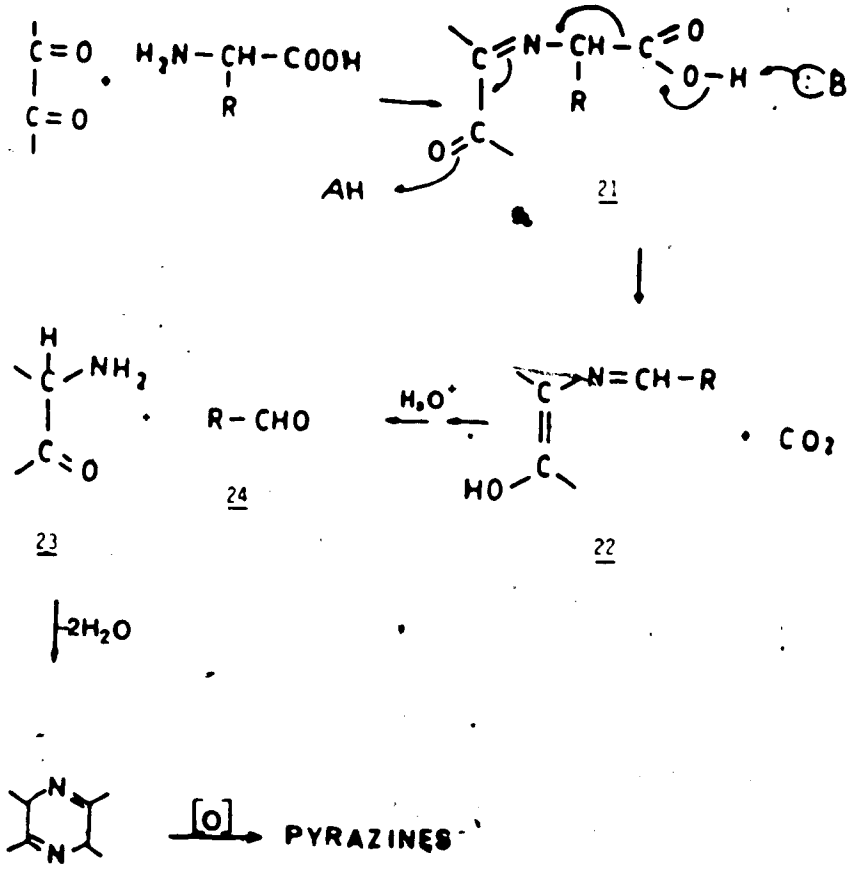


Figure 1.9 Strecker degradation.

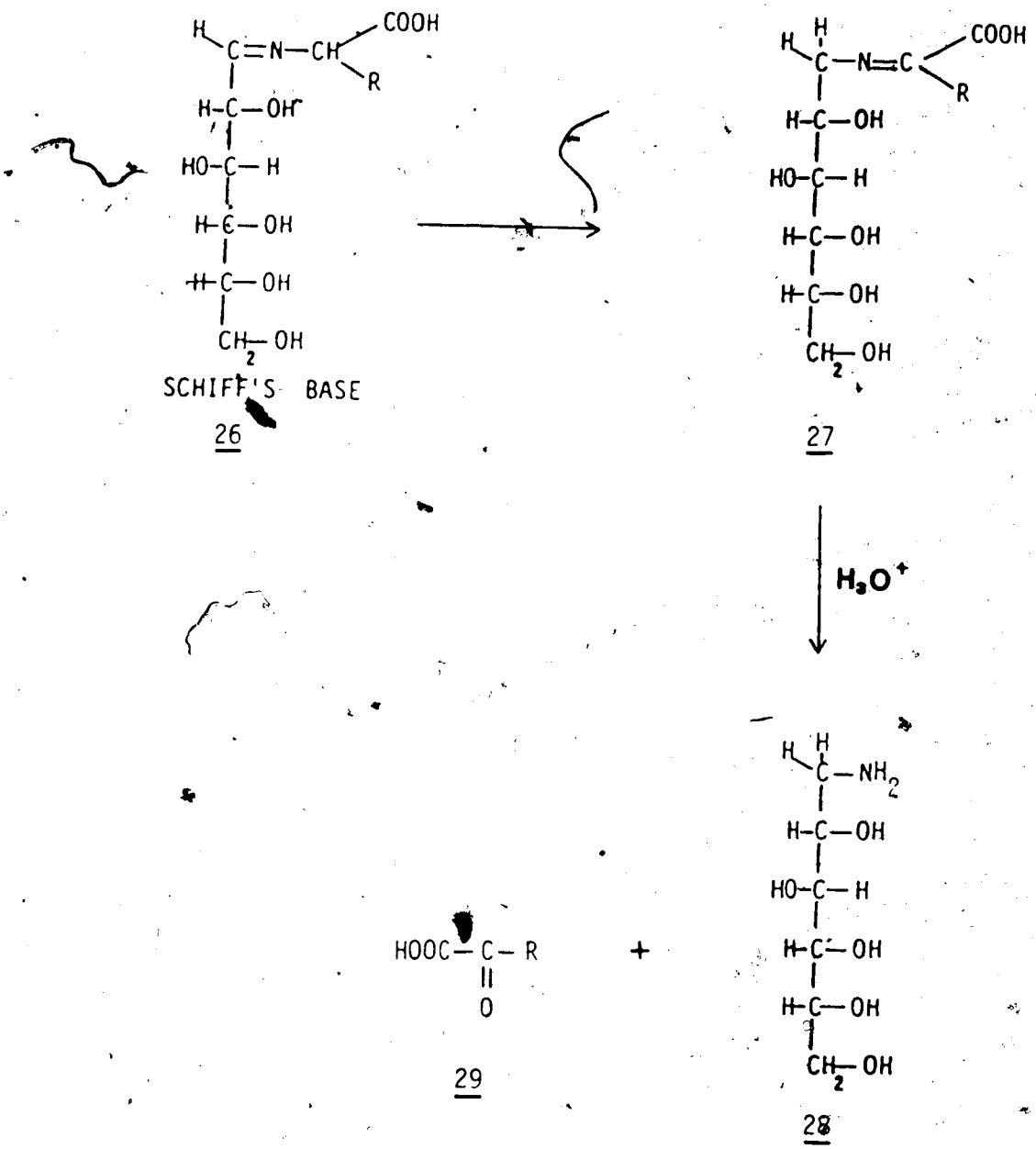


Figure 1.10 Pathway proposed by Høltermand (1966).

and the sugar into a non-reducing amino-sugar (28). This transformation is erroneously termed "transamination" in the literature. The oxo acid (29) reacts further with an intact amino acid, resulting in decarboxylation of the oxo acid and liberation of an aldehyde by Strecker degradation.

A second pathway (e) that also bypasses the Amadori stage was proposed by Namiki and Hayashi (1983) (Figure 1.11).

It was shown that there exists a new pathway in the Maillard reaction involving sugar fragmentation and free radical formation at an early stage prior to the Amadori rearrangement. This was demonstrated by the use of ESR spectroscopy. Analysis of the hyperfine structures for various sugar-amino compounds led to the conclusion that the radical products were N,N'-disubstituted pyrazine cation radicals (31). These new pyrazine derivatives are assumed to be formed by bimolecular condensation of a two-carbon enaminol compound (30) involving the amino residue. The presence of such a two-carbon product was demonstrated by isolation and identification of glyoxal dialkylimine (32) by use of TLC, GLC, NMR, MS and IR.

1.1.4 Formation of Heterocyclic Compounds during the Advanced Maillard Reaction, and Their Role in the Production of Flavor, Aroma and Color in Food Systems

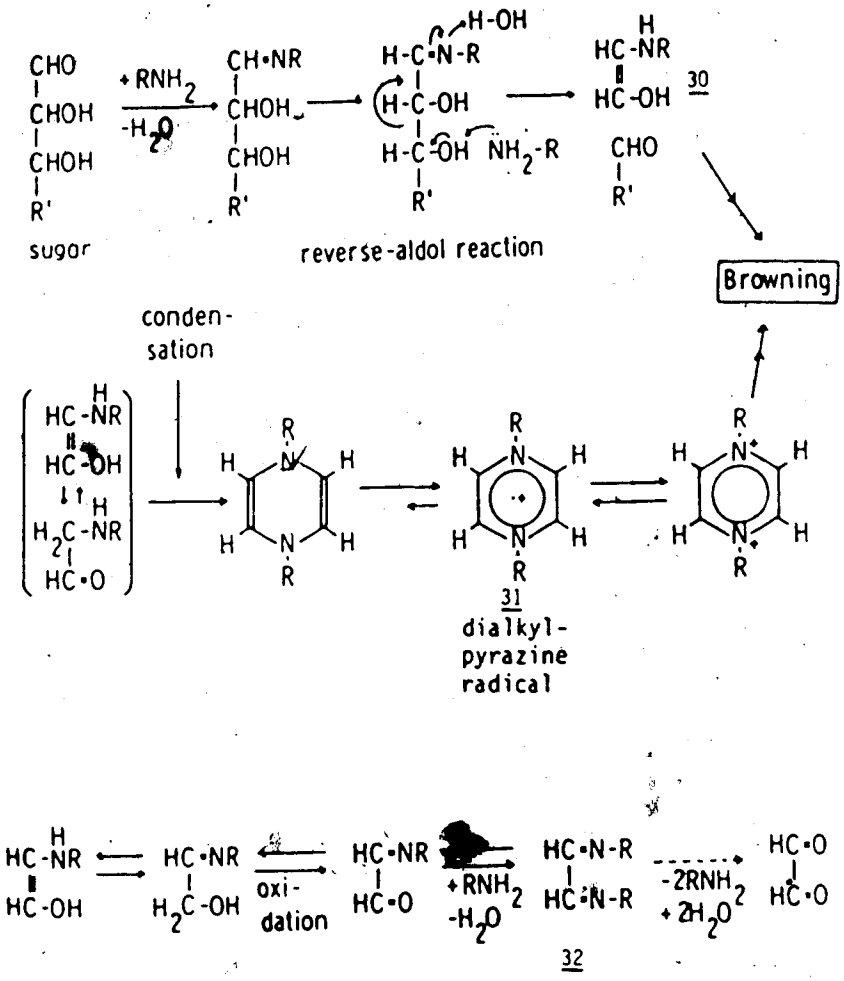
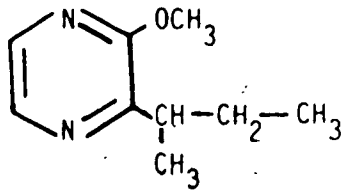


Figure 1.11 Pathway proposed by Namiki and Hayashi (1983).

1.1.4.1 Introduction

Among the volatile components of flavor, up to a thousand heterocyclic compounds have been identified in various processed foods. During the last twenty years, our knowledge of the chemical composition of food aroma has made considerable progress mainly because of GC/MS coupling (Land and Nursten, 1979). Among a total of about ten thousand substances occurring in aromas, heterocyclic compounds occupy a prominent position as a result of their quite exceptional olfactory properties. Indeed, some of these compounds exhibit such a low olfactory perception threshold that it is possible to detect the equivalent of one milligram in five hundred tons of water. An example is the compound 2-isobutyl-2-methoxypyrazine (33):



33

The origin of these compounds can be divided into two processes:

1. The enzymatic and microbiological processes to which fermentation can be linked.
2. The non-enzymatic process (Maillard reaction) resulting from thermal treatments, such as cooking, roasting, frying, baking, boiling, etc.

The first of these two processes plays a significant role in the formation of aromas in fermented foods; the second plays an important role in the formation of aromas from meat, coffee, nuts, etc.

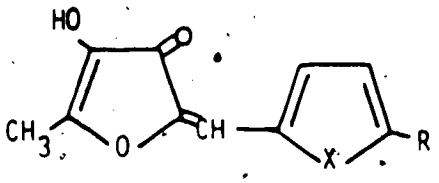
In considering the production of aroma, the initial precursors found in the food play an important role since aroma results from the biotransformation and/or complex chemical reactions of these initial precursors, whose partial or total destruction leads to reactive intermediates directly responsible for the formation of aroma.

Two kinds of precursors are to be considered. Primary precursors are those that already exist in the fresh food, e.g. nucleosides, nucleotide-sugars, nucleic acids, etc. Intermediate precursors are the hydrolysis products of primary precursors, e.g. monosaccharides, amino acids, etc. In the Maillard reaction the intermediate precursors are involved in the production of aroma.

1.1.4.2 Formation of color

Although the formation of color is an important characteristic of Maillard reaction, very little is known of the structures of the chromophores present.

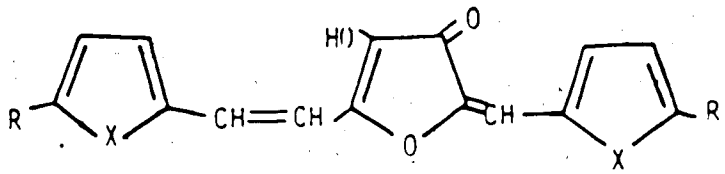
Severin and Kröning (1972) reported the isolation of the yellow compound 34a from a mixture produced by heating xylose with isopropylammonium acetate and found that 34a was also formed when xylose was replaced by arabinose or isopropylammonium acetate by amino acids, such as lysine or glycine.



34a R = H, X = O
b R = CH_3, X = N-CH_3
c R = CH_3, X = N-CH_2-COOH

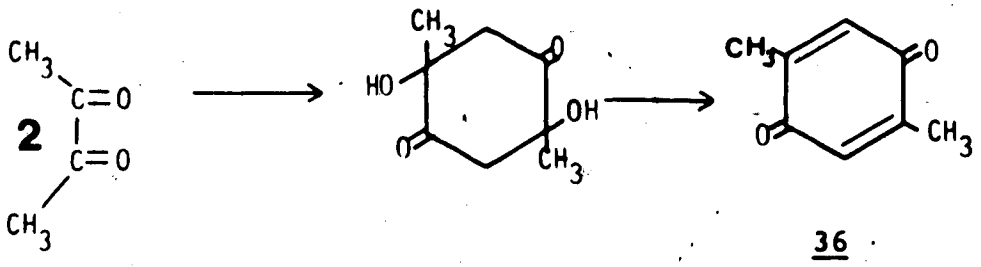
In addition, Ledl and Savarin (1978) also reported that pentoses and methylammonium acetate produce 34b besides 34a and that xylose and glycine similarly give the orange compound 34c.

The latter authors also found that the methyl group of compounds such as 34 is sufficiently reactive to condense with carbonyls to form compounds such as 35 (R = H, X = O; deep orange; R = CH_3, X = O; R = H, X = NCH_3).



35 R = H, CH_3
X = O, N-CH_3

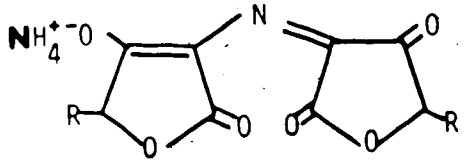
Another possible route to a chromophore lies in the formation of quinones (36) by double aldol condensation and dehydration of 1,2-diones (Nursten, 1980).



36

Amines can readily add to quinones and, after an oxidation step, can lead to colored compounds.

Another model chromophore for Maillard reaction products was reported by Kurata *et al.* (1973). It has the structure 37, obtained from the interaction of amino acids with dehydroascorbic acid.



37 R = =CH-OH, CH_2-OH

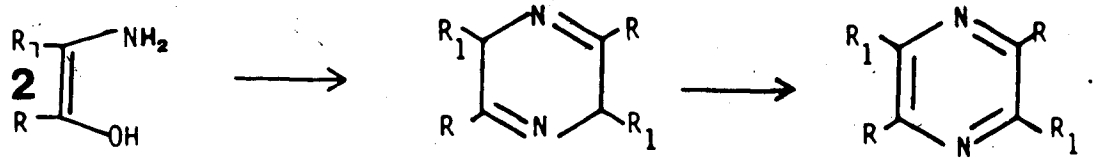
1.1.4.3 Mechanism of formation of heterocyclic flavor compounds

According to Nursten (1980), the hundreds of aroma compounds produced in processed food can be classified into three groups:

1. "simple" sugar dehydration/fragmentation products;
2. "simple" amino acid degradation products; and
3. compounds produced by further interactions.

The last group constitutes the Maillard reaction products.

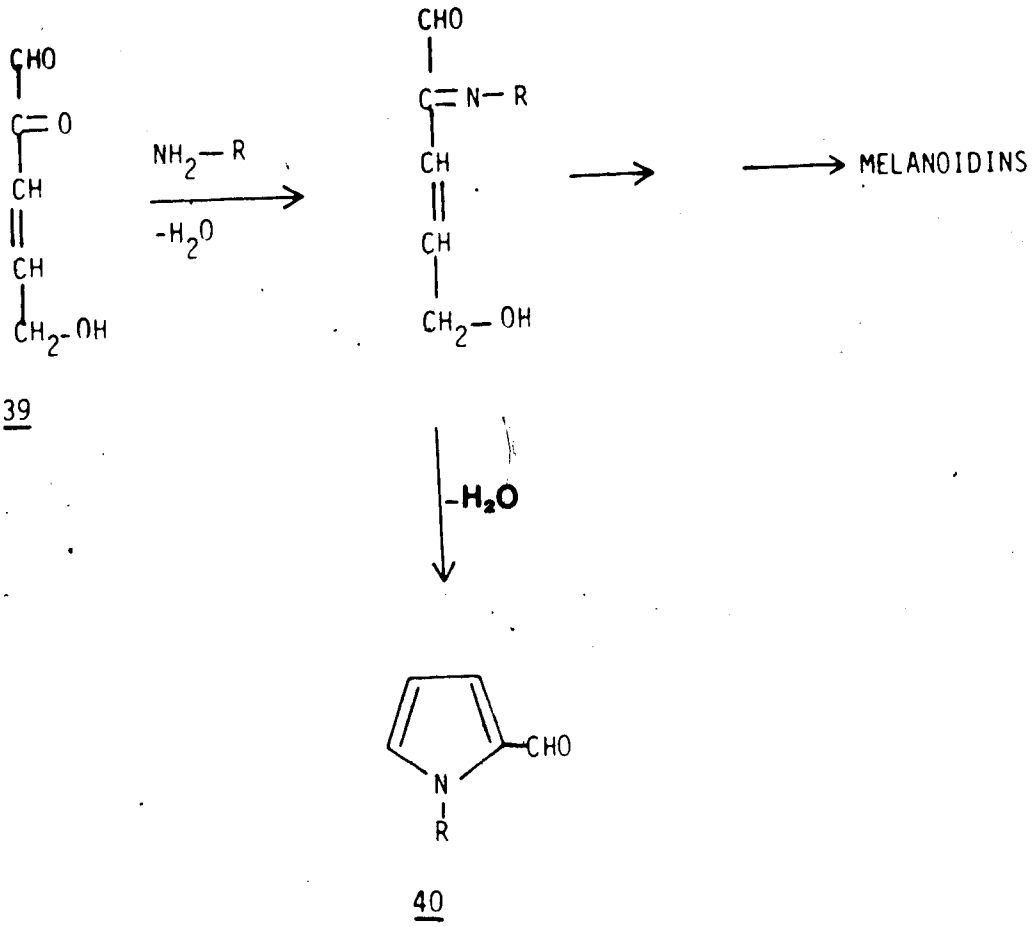
• pyrazines are one of the most important discoveries in flavor chemistry (Maga and Sizer, 1973). During the past decade evidence has indicated that pyrazines contribute directly to the roasted or cooked flavor of various foodstuffs. They are typical products of the advanced Maillard reaction and have been reported in many heated food systems, including beef products, cocoa, coffee, and peanuts. In their review, Maga and Sizer (1973) listed many pyrazines that have been isolated from food. Pyrazines (38) can be formed through the condensation of Stecker degradation products.



38

• pyrroles, pyrrolidines and pyridines are another class of N-heterocycles. They have been observed in a wide variety of heated foods.

Kato and Fujimaki (1968) proposed the mechanism outlined in Figure 1.12 for the production of N-substituted pyrrole-2-aldehydes (40), where 3,4-dideoxypentosulose-3-ene (39), in the presence of amino acid, can form a Schiff base at C-2 and then cyclize to 40. Another mechanism, proposed



39

40

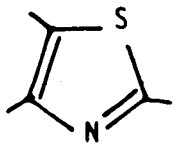
Figure 1.12 Mechanism for the production of N-substituted pyrrole-2-aldehydes (Kato and Fujimaki, 1968).

by Rizzi (1974), explains the formation of acyl-alkyl pyrroles from the corresponding furans. Since proline and 4-hydroxyproline already contain a pyrrolidine ring, it is possible that the Maillard reactions involving those amino acids can lead to derivatives of pyrrolidine or pyrrole.

Pyridines and alkyl-substituted pyridines have also been isolated from heated food systems and they appear to result from Maillard reactions, although their formation has not yet been investigated. They have been detected in the flavor components of coffee (Goldman *et al.*, 1967), barley (Wang *et al.*, 1969), etc.

• oxazolines (42) may also be formed from the Strecker degradation products, as shown in Figure 1.13 by Rizzi (1969). The oxazoline formation is favored when the electron distribution after the decarboxylation of 41 enables cyclization before enolization occurs. In the latter case formation of pyrazines is favored.

• thiazoles (43) are formed in the Maillard reaction involving the sulfur amino acids, but the mechanism of their formation is not well understood.



43

• O-heterocycles are responsible for the caramel aroma and can be formed by sugar pyrolysis in the absence of amino

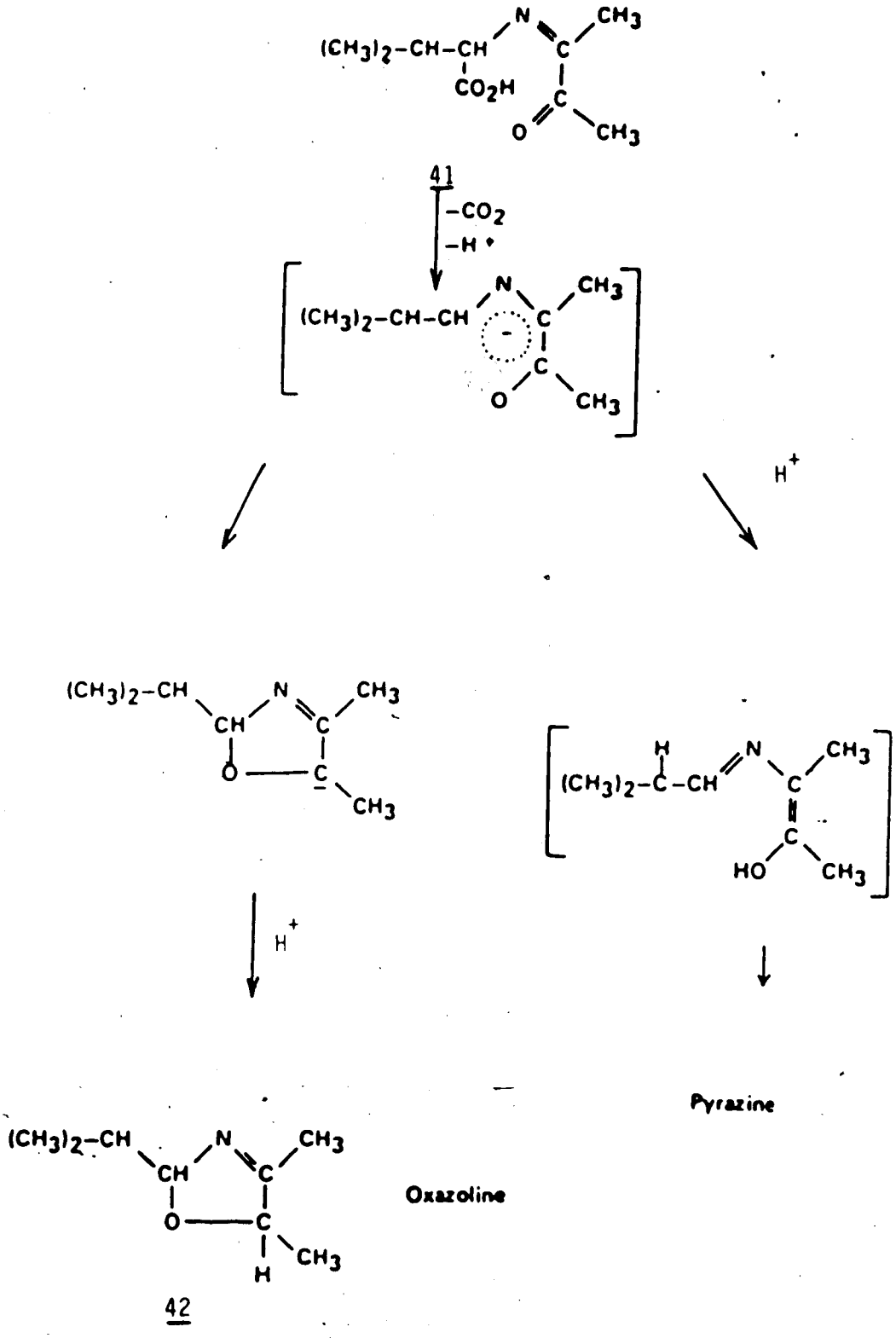
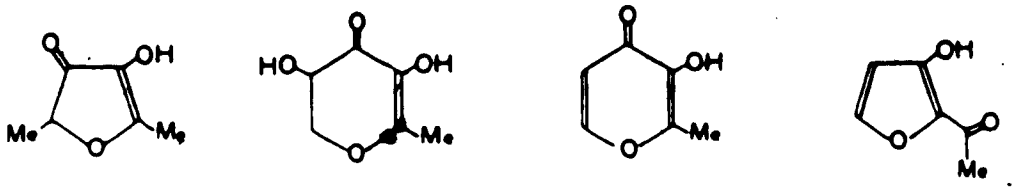


Figure 1.13 Formation of oxazolines from Strecker degradation products (Rizzi, 1969).

acids, but they can be similarly formed at a faster rate as a result of the Maillard reaction. The best known O-heterocycles are maltol (44) and isomaltol (45).



44 45

1.1.5 Final Maillard reaction

The brown melanoidin pigments are produced in the third and final phase of the Maillard reaction. The pigments isolated from the reaction between aldoses and amines contain nitrogen. Some are readily soluble in water, some are slightly soluble, and others are insoluble. Soluble pigments were found to be not dialyzable. Melanoidin formation is the result of the polymerization of the many highly reactive compounds that are formed during the advance Maillard reaction, especially the unsaturated carbonyl compounds and furfural, the latter yielding water-insoluble brown pigments (Reynolds, 1965).

In addition to the brown color formation, these polymerization reactions definitely lead to toughening of stored food (Labuza *et al.*, 1977). Not much is really known about the chemistry of the formation of these polymers, however, recently Feather and Nelson (1984) reported the

preparation of water-soluble, nondialyzable Maillard polymers, having molecular weights in excess of 16,000, from 5-(hydroxymethyl)-2-furaldehyde (HMF), D-glucose or D-fructose, and glycine. In all cases, the polymers showed no absorption maximum in the 220-230 nm range. Elemental analysis suggested that the polymer was composed of 1 mole of sugar and 1 mole of glycine minus about 3 moles of water.

Studies using 90 atom % enriched D-glucose-1-^{13}C, glycine-1-^{13}C and glycine-2-^{13}C as precursors in the reactions and ^{13}C-NMR as a probe showed that both carbon atoms of glycine were incorporated into the polymer and that C-1 of D-glucose appears as a substituted methyl group. The NMR data further suggested that the main monomeric (dialyzable) units are unreacted sugar or amino acid and Amadori rearrangement product derivatives.

1.1.6 Parameters that affect the Maillard reaction

Temperature, time of reaction, moisture content, concentration and nature of reactants are important factors in the Maillard reaction.

1.1.6.1 Temperature and time

Temperature and duration of the Maillard reaction was first studied by Maillard (1916) who reported that the rate of the reaction increases with temperature. Many workers have since confirmed this observation. Lea and Hannan (1949) showed that, in a casein-glucose mixture, the rate of the loss in amino acid nitrogen increases 40,000-fold when the

temperature is raised from 0°C to 80°C. The term Q_{10}, defining the increase in rate for every 10 C° increase in temperature, has been shown (Labuza and Saltmarch, 1981) to range from 2 to 8.

Duration is also an important factor controlling the extent of browning. For example, Hurrell and Carpenter (1974) reported that almost as many ε-aminolysine groups had reacted in an albumin-gluco[redacted] mix after 30 days storage at 37°C (76%) as had react[redacted]n the same mix was heated for 15 min at 121°C (85%).

Very little is known about the effect of heat on melanoidins, however, it has been reported that heating melanoidins for long periods causes discoloration and fragmentation (Gomyo *et al.*, 1972). The effect of temperature on the structure of melanoidins has been reported by Benzing-Purdie *et al.* (1985). They concluded that an increase in reaction temperature causes a major increase in aromaticity in the melanoidins, with only a slight decrease in the carbonyl character.

1.1.6.2 Water activity (a_w)

Browning reactions in food and model systems of low moisture content occur over a wide range of water activities (Karel, 1960; Heiss, 1968). Maximum browning reaction occurs in most foods between water activities 0.3 and 0.7. The position of this maximum depends on the type of food, however, in some model experiments of glucose with amino acids (Godefridus *et al.*, 1978) it was found that optimal

conversion into the Amadori rearrangement product takes place at a water content of 23-30%, as illustrated in Figure 1.14.

Although water activity reflects the effect of water being bound to specific polar groups in the food and to other factors limiting the availability of water molecules for chemical reaction, it cannot be used to predict optimum browning conditions (Karel and Eichner, 1972) because the rate of browning is not simply related to water activity but depends on the extent to which various conflicting influences, like water activity, state of the bound water and mobility of the reactants, affect the reaction.

Karel and Eichner (1972) studied browning due to reaction between reducing sugars and glycine in systems containing varying amounts of water, glycerol and hydrophilic polymers. They observed that the browning rate decreased with increasing water content, except in systems in which mobility of reactants became substantially impeded in high viscosity solutions of low water activity. Partial restoration of the mobility through the plasticizing effect of glycerol increased the browning rate at the same low moisture contents.

The effect of water is complex and depends on the presence of various water-binding agents, among others. The inhibitory effect of high water contents could be due to the fact that water is a product of several condensation steps and also to the fact that it acts as a diluent.

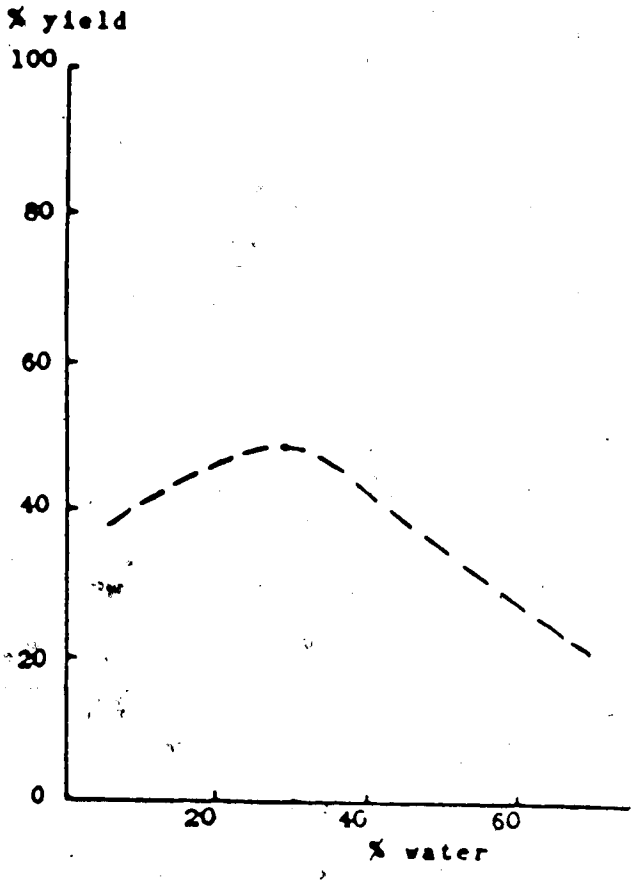


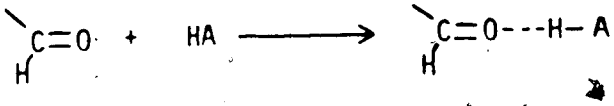
Figure 1.14 Yield (%) of Amadori rearrangement products (glucose with different amino acids) versus % water after 1 hour at 100°C (Godefridus *et al.*, 1978).

1.1.6.3 pH

Although the effect of pH on the rate of carbonyl-amine reaction has been discussed, some generalizations are pertinent.

The rate of the overall Maillard reaction increases linearly with increasing pH from 3 up to as high as pH 8 (Lea, 1950). Such data, however, also include a multitude of reactions from which it is difficult to pinpoint the rate of the initial carbonyl-amine interaction.

Many stages in the Maillard reaction (glycosylation, Amadori rearrangement) are catalyzed by acid or base. The acid catalysis increases the polarity of the oxo group of the open chain form (46).



46

In the case of base catalysis, the concentration of the open-chain tautomer (Burton and McWeeny, 1963) and the amount of the free amino group increase. The amino group cannot take part in acid-catalyzed reactions because of the loss of its free electron pair. Schroeder *et al.* (1955) attributed the browning that takes place between pH 3 and 5 to the decomposition products of sugars. Between pH 6.5 to 9, besides browning, a considerable decomposition of amino acids was also observed.

Phosphate buffers have been observed to affect the rate of browning. Reynolds (1963) reported that the rate of

formation of glycine Amadori product with glucose increases proportionally to the square root of the $H_2PO_4^-$ ion concentration. According to Burton and McWeeny (1963), this effect is due to the decreased stability of the ring form of the sugar and hence increased concentration of the open-chain form.

1.1.6.4 The nature of the reactants

Since the initial process in the Maillard reaction is essentially attributed to the nucleophilic addition of the free amine to the carbonyl group, only reducing sugars take part in the Maillard reaction as they provide the necessary free carbonyl group. In this respect, aldopentoses are more reactive than aldohexoses, and reducing disaccharides are still less reactive (Ellis, 1959). The higher reactivity of aldopentoses is due to a higher concentration of open-chain form compared with that of aldohexoses.

Regarding the amino acids, every factor which decreases the basic character of the amino group exerts a negative effect on the intensity of the Maillard reaction. In the case of α-amino acids, the existence of the zwitterionic form impedes the reaction (Ellis, 1959). The electron donating character of the side chains of the amino acid influences the nucleophilicity of the amino group (Taufel *et al.*, 1956).

1.1.7 In vivo Maillard reaction

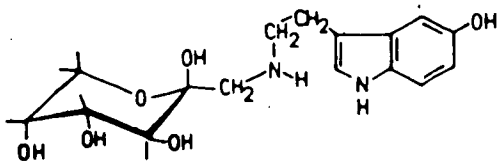
L.C. Maillard was the first to attribute some biological significance to the reaction that bears his name. Paradoxically, this aspect was completely neglected until recently, whereas the role of the reaction in food chemistry generated a paramount interest.

The occurrence of Amadori compounds *in vivo* was first observed by Boorsook *et al.* (1955) and later confirmed by Heyns and Paulson (1959). They identified 1-deoxy-N-(amino acid)-D-fructose derivatives in hog liver extract as being the stimulating factor for the *in vivo* incorporation of amino acids in the proteins of rabbit reticulocytes. Nearly twenty years later Tanzer (1973) observed the presence of the reduced form of the Amadori compounds formed with hydroxylysine and reducing sugars in aged connective tissues.

In 1976, Flückiger and Winterhalter observed that in haemoglobin A_{1c} fraction the N-terminal α-amino groups of the β-chains are blocked by a carbohydrate residue. Chemical investigation showed that haemoglobin A_{1c} is an Amadori-type 1-deoxy-D-fructose derivative. The amount of this haemoglobin fragment in the erythrocytes of diabetics is 2-3 times larger than in normal individuals.

Food consumption can affect basic physiological processes and the formation *in vivo* of Amadori compounds can interfere with these.

The metabolic chain: tryptophan → 5-hydroxy-tryptophan → serotonin, is well established, showing that tryptophan in food has a direct effect on the serotonin level in the brain (Costa *et al.*, 1974). A great deal of information confirms the dietary control of brain serotonin, affecting such complex processes as aggressiveness, depression, and sexual behavior. Other amino acids resulting from the ingestion of food compete with tryptophan for uptake into the brain. In 1971, Fernstrom and Wurtman reported that carbohydrates increase the brain serotonin level and, in 1975, Mester *et al.* reported the formation of serotonin Amadori rearrangement product with glucose (47) in the blood.



47

Later, Mester *et al.* (1980) showed that compound 47 has a decisive role not only in the passage of serotonin through the blood-brain barrier, but also in its physiological effects.

Although the early steps of the Maillard reaction have been shown to occur *in vivo*, it is not yet established whether non-enzymatic browning also occurs *in vivo*. However, two observations point to the possible formation of browning

products under physiological conditions. First, in analogy to model food systems, some tissues are characterized by having high contents of protein with little or very slow turnover; these proteins are in effect "stored" for months or years without any appreciable proteolytic degradation. Second, Mohammad *et al.* (1949) showed that non-enzymatic browning can occur in solutions of albumin and glucose incubated at 37°C and physiological pH. Thus, it is likely that the Amadori products which are formed *in vivo* can also undergo further rearrangements to form pigments. It was previously noted that with increasing age the human lens accumulates yellow-brown fluorescent pigments which can act as covalent cross-links between proteins. It has been suggested that the non-enzymatic browning products could account in part for the fluorescent cross-links found in old lens (Monnier *et al.*, 1981).

Studies on the chemical and spectral properties of purified atopic allergens have suggested that these antigens are characterized by the incorporation of N-substituted 1-amino-1-deoxy-2-ketoses in the molecular structure. These structural units, embracing the site of N-glycosidic protein-sugar linkage, give rise to characteristic fluorescence spectra. Since the literature on the Maillard reaction contains some indications of formation of fluorescent compounds (Burton *et al.*, 1962), it was suggested that such linkages may be formed by the Maillard reaction between free protein amino groups and reducing sugars under

conditions of natural decomposition, and indeed Berrens (1967) reported that N-substituted 1-amino-1-deoxy-2-ketoses and their enolic tautomers exhibit fluorescence characteristics similar to that of purified atopic allergens. These results offer independent evidence for the incorporation of N-glycosidically-linked sugar in atopic allergens.

While considering the *in vivo* Maillard reaction it is worth mentioning the proposition of Bunn and Higgins (1981) regarding the evolutionary significance of the Maillard reaction. Based on studies showing that glucose was the least reactive of a series of aldohexoses in the formation of Schiff base linkages with haemoglobin, they suggested that the emergence of glucose as the primary metabolic fuel may be due in part to the relatively high stability of its ring structure. This would allow high concentrations of glucose and proteins to coexist with the least interaction.

1.1.8 Technological aspects

The Maillard reaction can be involved in the manufacture of foods in at least three quite different ways. First, there is the role played in the development of flavor in such traditional processes as the roasting of coffee and cacao beans, and the baking of bread and cake. Second, the deliberate use of the reaction in the production of artificial flavors, such as improving the smoking flavor and taste of tobacco by addition of the Amadori rearrangement products to the tobacco leaves before processing (Morihita

et al., 1973). Third, the efforts to control the undesirable effects of the reaction in food processing.

Physical preservation methods for food, such as heat sterilization and drying, are associated with the application of heat. In these cases, because of its high temperature coefficient, the Maillard reaction becomes the dominant deteriorative reaction (Hodge, 1953). During the progress of the Maillard reaction, the great variety of compounds formed causes undesirable browning and sensory changes, hence, the control of undesirable browning becomes important in food industry.

Many procedures have been suggested for the control or prevention of undesirable browning, including:

1. Blocking the carbonyl groups on the sugar by addition of sulfites (Wedzicha, 1984);
2. Oxidizing the glucose into gluconic acid by glucose oxidase (Scott, 1953);
3. Blocking the amino groups by acylation or methylation (Lee *et al.*, 1978);
4. Compartmentalization of sugars and the amino acids by starch (Mohammed, 1979);
5. Addition of a reagent to compete with the amino groups in reaction with sugars, such as cysteine to form thiazolidine (Kline and Fox, 1946);
6. Removal of carbohydrates by dialysis or by enzymatic reaction (Kline *et al.*, 1951); and
7. Changing the water activity and/or pH (Lineweaver

and Feeney, 1950-51).

1.2 Electron Impact Mass Spectroscopy (EIMS)

1.2.1 Introduction

In recent years, the application of mass spectrometric techniques has increased enormously, especially in fields dealing with quantification (Markey, 1981) and identification, which had been exploited not only in identification of complex organic compounds (Waller, 1972) but also for its ability to pick out for special study particular ion species which may only be present in small amounts in a complex mixture (Gilbert, 1984). As a result of developments introduced over the years, the mass spectrometer has become perhaps the most versatile of all the analytical techniques available. It can be coupled with separation methods such as chromatography (McFadden, 1973); it can handle solids and liquids, as well as gases; it can be used with various ionization techniques to highlight particular properties of the sample; it can produce positive or negative ions as well as ions carrying more than one charge; it can separate the ions according to their masses, momenta or kinetic energies; it can study specific properties of separated ions such as their unimolecular fragmentation behavior (McLafferty, 1980) or specific reactions induced by collision with neutral gases or a laser beam; and it can be computer controlled.

The modern mass spectrometer is in many ways a complete chemical laboratory. The ion source provides the ions for separation and the mass separation stage completes the "purification". The isolated ionic species can then be identified on the basis of its characteristic reactions just as in conventional chemistry.

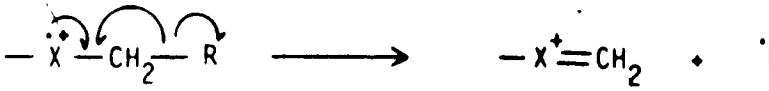
1.2.2 Unimolecular ion decompositions

It is important to stress that mass spectral reactions are unimolecular; the sample pressure in the ion source is kept sufficiently low that bimolecular reactions are usually negligible.

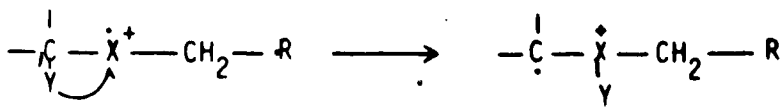
For a thorough understanding of mass spectrometry, it is necessary to recognize the basis of interpretation of unimolecular ion decompositions. There are two approaches, one *mechanistic*, the other *theoretical*.

1.2.2.1 Mechanistic approach

The mechanistic approach to the interpretation of fragments formed in the ion source is concerned with the rationalization of how and why certain fragmentations occur (McLafferty, 1980). It relies on the idea that a localized charge and/or radical site trigger reactions, that either results in the pairing of unpaired electrons by bond cleavage (equation 5) or result in rearrangements that transfer a radical site to a better-stabilized locus (equation 6).



(Eq. 5)



(Eq. 6)

For a molecule containing noninteracting functional groups, the localized charge is mostly on the functional group of lowest ionization energy.

For product stability as a reaction-path determinant, it is generally more important that the product provide a stable environment for the charge rather than for the unpaired electron; the initial charge site in the molecular ion has, in general, the most favorable environment for stabilization of the positive charge.

There are great differences in the overall ability of a particular functional group to influence the fragmentation of a molecular ion. Each functional group triggers a different pattern of decomposition and there is an exponential increase in the number of possible fragmentation pathways with an increase in the number of functional groups.

The real significance of the localized charge concept becomes evident only when one considers the most important aspect of rationalizing mass spectral fragmentations, namely the ability to predict which bonds are most likely to break in a given molecular ion. Fortunately, these predictions can be based on ground state solution chemistry as supported by a wide body of experimental evidence.

1.2.2.2 Theoretical approach

The best known among theories that attempt to provide a physical description of mass-spectral behavior is the quasi-equilibrium theory (QET), originally formulated by Rosenstock *et al.* (1952) and subsequently modified several times. The basic idea underlying QET is that, if, in a system of competing and consecutive reactions (as in the ion source of a mass spectrometer), all the rate constants are known, the abundance of any product after a given time can be calculated. The problem, however, is to obtain reliable values for the various rate constants. The QET departs from the idea that ionization of the molecule, which takes place in approximately 10^{-13} sec., initially yields the excited molecular ion in the lowest electronic state and without change in bond length (a Frank-Condon process). The transitions between all accessible vibrational and rotational degrees of freedom of this ion are sufficiently rapid so that a "quasi-equilibrium" among these energy states is established before ion decomposition takes place. Bond cleavage therefore occurs whenever enough energy is accumulated in a given bond. Calculations however proved to be extremely difficult since, in order to calculate rate constants, the structure of an ion has to be known, hence a vicious circle develops.

At first glance, the mechanistic approach is in direct contrast to the main idea of QET: a triggering localized charge or radical site versus a free fluctuation of energy

causing fragmentation. It was not until the early 1970's that Williams and Howe (1972) tried to reconcile the contradicting ideas by pointing out that preferred fragmentation should occur from a highly populated low-lying electronic state which corresponds broadly to ions where an electron has been removed from a non-bonding pair or from a π system.

1.2.2.3 Ionization by electron impact

Ionization by electron impact, as the name implies, is the ionization caused by an electron impinging on a molecule, however the impact of an electron may also lead to electronic excitation in the molecule by promoting an electron from a lower to a higher orbital (the same effect as in UV spectroscopy).

The term "electron impact" is misleading because an electron is so small in molecular terms that if would have difficulty "hitting" any part of a molecule. It is better to think of the electron as passing close to or even through a molecule rather than of any "impact" taking place. The above definition of electron impact implies two important considerations. First, it is assumed that the molecules are in the gas phase. That means in many cases that a substance needs energy in the form of heat of evaporation in order to vaporize. Additionally, the ion source (see figure 1.15), in which this evaporation process takes place, must be heated to a high temperature in order to avoid an immediate condensation of the sample. Therefore, thermal energy is supplied to the molecules. Second, it is assumed that

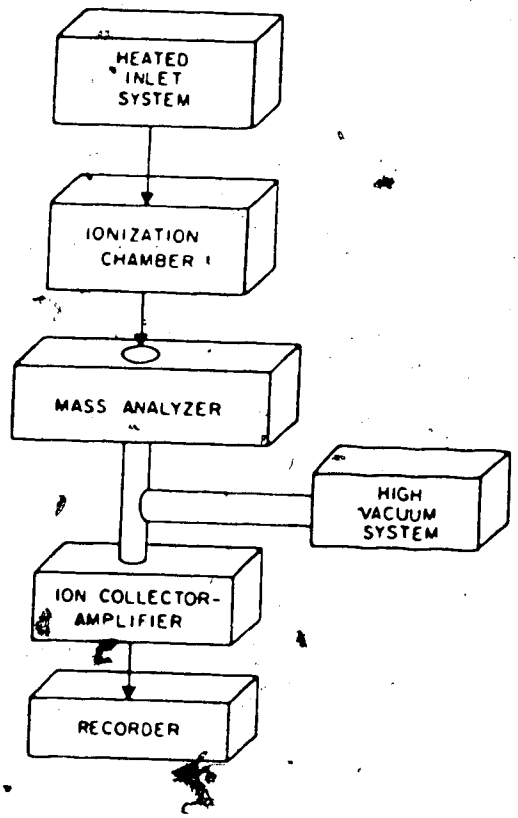
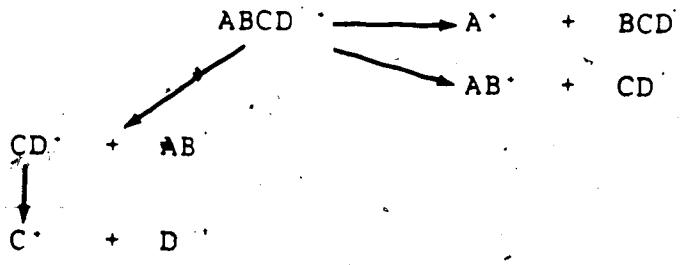


Figure 1.15 Schematic diagram of basic components of a mass spectrometer.

relatively energetic electrons are used for ionization by impact. Usually, the mean energy of these electrons is standardized at 70 eV since a flat maximum is observed at this value in the ionization efficiency curves of most organic compounds. However, ionization of an organic molecule only needs about 10 eV (230 kcal/mole). This means that at the moment of impact not only is an electron extruded from the molecule, but at the same time a rather high amount of energy is imparted to the molecule. However, experimental evidence suggests that only about 5-6 eV of energy above the ionization energy of a compound are transferred by 70-eV electrons (Rose and Johnstone, 1982), thus, after the ionization, the ions contain additional energy: thermal as well as transmitted energy from the impact. Such ions therefore are in an excited state and, according to QET, this additional energy is approximately regularly spread over the whole ion.

Due to high vacuum in the ion source, there is no equilibrium distribution of energy, therefore it can be assumed that impact of electrons with M molecules produces $M^{\cdot}$ ions with a variety of internal energies, ranging between 0-10 eV or about 0-230 kcal/mole. Only a small fraction of molecular ions is formed with internal energies in excess of 10 eV. The molecular ions, therefore, tend to get rid of this excess energy and to stabilize themselves. In general the chemical stability of the molecule parallels the stability of $M^{\cdot}$ and so is reflected in the abundance of $M^{\cdot}$.

The molecular ions tend to stabilize themselves by bond cleavages and fragmentations. The energy needed for such fragmentation can be evaluated from the energy of the bonds themselves (C-C, 93 kcal/mole; C=O, 86 kcal/mole; etc.). Accordingly, the energy needed for fragmentation is much smaller than the energy of the ionizing electrons. It is highly probable that such an amount of energy is accumulated during electron impact to form an excited ion and that this energy induces bond cleavage. Product ions ($A^{\cdot}$, $AB^{\cdot}$, $CD^{\cdot}$) are thereby formed with less energy and therefore are more stable.

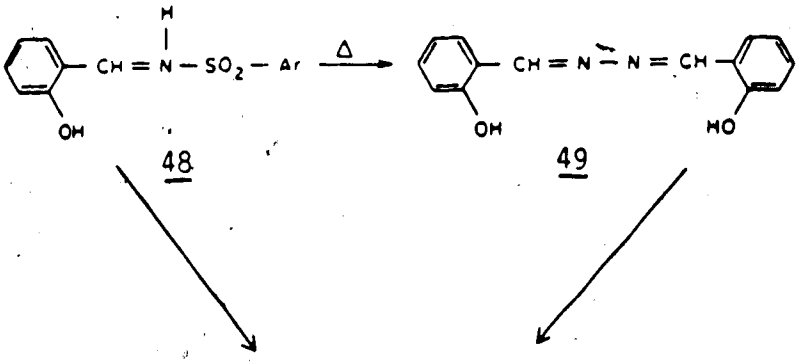


If such ions still contain excess energy, they will undergo a further fragmentation step ($CD^{\cdot} \rightarrow C^{\cdot}$). In this way whole series of fragmentations may result. If it is kept in mind that a series of fragmentations and reactions of rearrangements can start from different parts of the molecular ion ($ABCD^{\cdot}$), it is possible to understand the complexity of a mass spectrum.

One of the problems encountered in the interpretation of mass spectra is the differentiation between thermal reactions prior to ionization and authentic electron-impact promoted reactions. At high temperatures, volatilization and decomposition are competing processes. Although the use of

direct insertion probes eliminated the need for excessive heating of samples for introduction into the ion source, the hot filament affects the temperature of the ion source, which usually ranges from 150-250°C. Thus, once volatilized, a molecule can escape the region around the direct insertion probe which is dense with sample molecules and where bimolecular interactions can occur. The molecule then enters the low pressure region of the ion source where the chances of decomposition are lower because collisions are rare.

The thermal energy imparted to a sample by high temperatures in the inlet system or ion source may have profound effects on the mass spectrum. The effects observed may result from actual changes in molecular structure or may be caused by the excess of vibrational or rotational energy gained by the molecule before it is ionized. It is often necessary to heat a sample to achieve a great enough vapor pressure in the ion source. On heating, a compound may react uni- or bimolecularly to give a new compound, as was observed in the mass spectrum of sulfonylhydrazide (48) which was complicated by the presence of a thermally-formed azine (49) (Johnstone, 1972).



In general, however, this thermal effect is more evident in the mass spectra of aliphatics than of aromatics, which possess fairly rigid structures.

1.2.2.4 General properties of mass spectroscopy

1. Most chemical compounds have their own distinctive pattern of fragmentation, known as a "fingerprint".
2. Fragmentation patterns are remarkably constant as long as experimental parameters are unchanged. However, in practice, the situation is complicated by the influence of the apparatus and experimental conditions. As it is hardly feasible to always obtain comparable measurements, the use of various apparatus gives rise to great variations in intensities, but the overall aspect of the spectrum is usually very well preserved. The main influence on the change in experimental conditions comes from the source temperature, the current used in the filament, insertion mode of the sample, ionization

energy, etc. In practice, it is impossible to maintain strictly similar conditions from one measurement to another or even during only one recording. Thus, the difference in reproducibility, whether it is due to technology or to the presence of the compound in a mixture, always comes down to more or less important intensity variations that should be considered when comparing two spectra.

3. When two or more components are present at the same time, each will produce its own fragmentation pattern and the resultant spectrum is produced by linear addition of the components. If one component can be correctly identified, then the reference spectrum of that compound can be subtracted from that of the mixture ("spectrum stripping") and the residual spectrum can be analyzed.

These and other properties of mass spectroscopy can be utilized for a variety of purposes, including:

- Qualitative identification.
- Quantitative analysis.
- Investigation of ions from electric discharges, flames, shock tubes, etc.
- High temperature chemistry -- unimolecular reaction studies.

This last property of mass spectroscopy was utilized in the present study of unimolecular fragmentation patterns of Amadori rearrangement products.

1.3 The Rationale Behind Studying the Fragmentation of Amadori Rearrangement Products by EIMS

1.3.1 Introduction

Certain factors inherent to the Maillard reaction limit the methods of analysis that can be employed; among them are the following:

- During the advanced Maillard reaction, a diversity of products obtained at low concentrations make the isolation and identification quite difficult.
- Many of the products are unstable and will either polymerize or decompose prior or during analysis.
- There is no rigorous or unified definition for the Maillard reaction in terms of temperature, pH, time of reaction, etc. Each researcher defines his own and, since the kind and range of products obtained depend on the above mentioned parameters, the results obtained are not comparable.
- Many products have been isolated from different model systems, without ascertaining whether those products are genuine Maillard reaction products or due to sugar or amino acid decomposition -- a systematic approach is essential.

Since the initial products formed in the advanced Maillard reaction are mainly the result of the unimolecular decompositions of Amadori rearrangement products which subsequently will undergo bimolecular reactions or

polymerizations and since under electron impact conditions those initial products of unimolecular decompositions can be observed at temperatures corresponding to real situations (frying, broiling, boiling, etc.), the study of fragmentation patterns of different Amadori rearrangement products under electron impact conditions should give a good indication as to their chemical behavior. Eventually, a generalized pattern should emerge which describes their chemical reactivities and bond labilities and, subsequently, based upon the fragments observed, it should be possible to predict their reaction pathways considering their known chemical properties.

However, the following question arises: Can we compare the fragmentation of a molecule by EI to the fragmentations occurring in solution or ground state?

The mass spectrum of a compound provides three types of information: the molecular weight of the compound, the mass of the various fragments produced from the molecular ion, and the chemical properties as evident in the mechanism of fragmentation. Fragmentation is a chemical process that results in bond cleavages; the energetic considerations that are applicable to classical chemical reactions are also applicable to these fragmentation processes. The fragmentation patterns are best interpreted based on the known chemistry of carbonium ions in solution. The fragmentation of a molecule in the ion source may be considered as the reactions of "carbonium ions without solvent". The rate of

production, and hence the intensity observed for any fragment ion, appears to correlate with the stability of that ion in the ground state and with the nature of the leaving group. Such ion-decomposition reactions can be viewed as another field of chemistry but, fortunately, there are many close similarities to pyrolytic, photolytic, radiolytic, and other energetic reactions and there are even more general similarities to solution organic reactions. The wide variety of detailed studies which have been published offers persuasive evidence that these ion-decomposition reactions take place by means of chemically-reasonable processes since bond labilities within the decomposing ion often parallel reactivities known for chemical processes in solution. However, it should be emphasized that much wider variety of reactions is possible under mass spectral conditions than in usual solution phase reactions, as is evident by the presence of literally hundreds of product ion peaks in the mass spectra of complex molecules. This might be compared to running a solution phase reaction under a wide range of temperatures. For example, the types of products obtained by the pyrolysis of an n-alkane change quite dramatically with increasing temperature. This is an important characteristic of EIMS related to this study since EIMS can give an indication of possible fragments of Amadori rearrangement products obtained over a wide range of temperatures.

1.3.2 The analogy between mass spectral reactions and the ground state or solution chemistry

The analogy of mass spectral fragmentation processes to the ground state or solution chemistry is overwhelming, keeping in mind that the chemical changes induced by EI are quite similar to high energy thermal reactions at ground state rather than at lower temperatures. Therefore, similarities have been sought and found between mass spectral reactions and solution or ground state reactions (Budzikiewicz *et al.*, 1967). Among these are the following:

1.3.2.1 Fragmentation mechanisms

The fact that fragmentation mechanisms -- which can explain most of the peaks and their intensities -- are essentially modelled after reactions in solutions taking into account ion and radical stabilities, make it difficult to attribute this to pure coincidence since the most intense peak corresponds to the formation of the most stable product. Here, stability is estimated from the known ground state solution or gas phase reactions.

The validity of such a thermodynamic approach to kinetically-controlled reactions lies in the assumption that the transition state begins to reflect product stabilities, thus processes leading to carbonium ions are favored in the order 3°>2°>1° and a similar order is observed for the case of elimination of alkyl radicals.

1.3.2.2 McLafferty rearrangement and Norrish type II photochemical decomposition of ketones

Under electron impact conditions, in compounds containing an unsaturated functionality, such as a carbonyl group, the γ-hydrogen atom is transferred by a sterically favorable six-membered ring transition state (Figure 1.16).

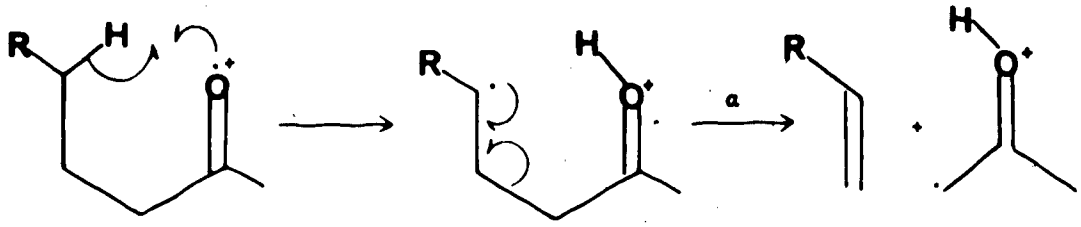
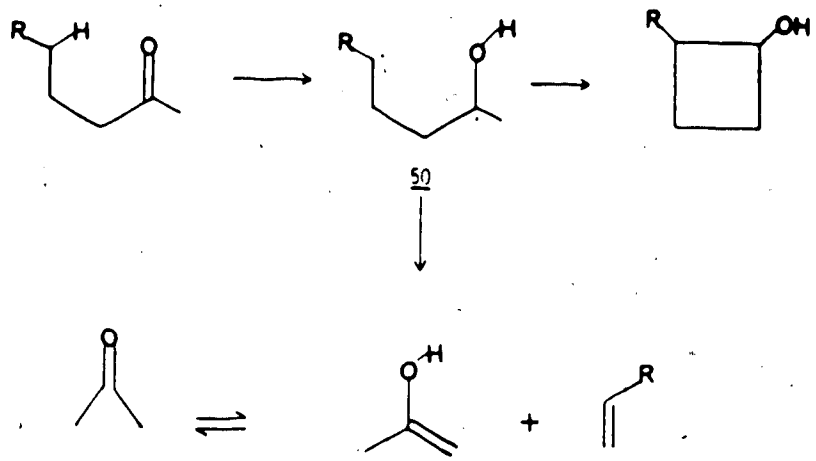


Figure 1.16. McLafferty rearrangment.

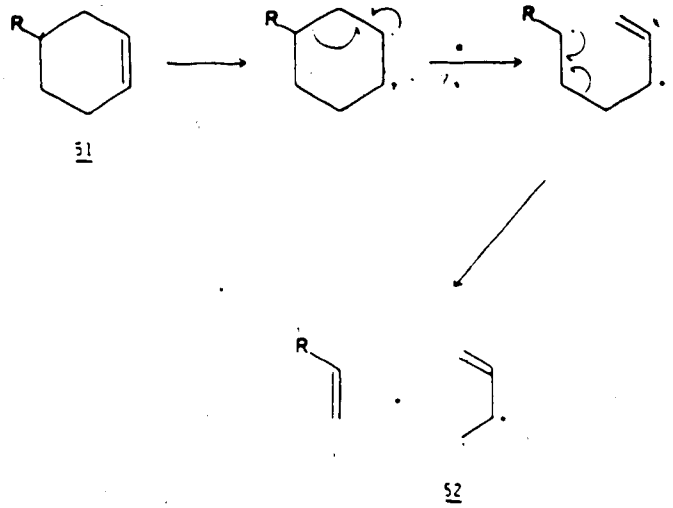
This is referred to as "McLafferty rearrangement" (McLafferty, 1956). In this process the initial cleavage does not result in the loss of part of the ion but only in a change in the position of the radical site. The new radical site can initiate an α-cleavage reaction, resulting in fragmentation of the carbon-carbon bond which is beta to the carbonyl group, with loss of an olefin. The obvious analogy to this process is the Norrish type II photochemical decomposition of ketones (Wagner, 1971) in which an aldehyde or ketone possessing a γ-hydrogen undergoes intramolecular hydrogen abstraction (see below) via a six-membered ring transition state. The resulting 1,4-biradical (50) may either cleave or cyclize to give the Norrish type II products. Nicholson (1954) was the first to point out the strong parallels between the two reactions.



50

1.3.2.3 Retro-Diels-Alder reaction

In cyclohexenes (51), the π-electrons provide a favored site for the initial charge and radical formation in the mass spectrometer. Donation of this unpaired electron produces an acyclic isomer by α-cleavage (Figure 1.17).



51 52

Figure 1.17

A second such reaction eliminates neutral C_2H_4. The other product is an ionized 1,3-butadiene (52), so that this process corresponds to a retro-Diels-Alder reaction (Figure 1.18) (Diels and Alder, 1928), commonly observed in solution

chemistry of cyclohexene derivatives at high temperatures.

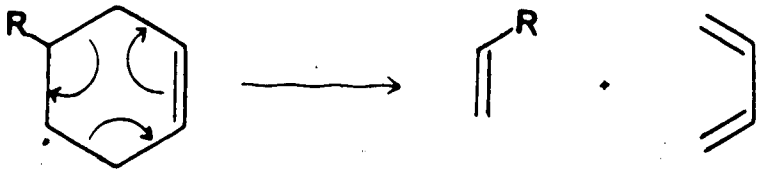


Figure 1.18 Retro-Diels-Alder reaction.

1.3.2.4 Correlation of ionization energies and reaction rates in mass spectrometer with Hammett σ values

The analogy of mass spectral fragmentation processes to ground state chemistry is further pressed by the correlation of reaction rates with Hammett σ values (Jaffé, 1953).

Hammett and Deyrup (1932) set up the equation log $k/k_0 = \sigma\rho$, which is a linear free-energy relationship, for m- and p-X-C_6H_4Y undergoing a particular reaction, where k_0 is the rate constant or equilibrium constant for X=H, k is the constant for the group X, ρ is a constant for a given reaction under a given set of conditions, and σ is a constant characteristic of group X. σ values are numbers which sum up the total electronic effects (resonance plus induction) of a group X when attached to a benzene ring. The treatment usually fails for the ortho position because of steric effects. σ is a number (+ or -) indicating the relative electron-withdrawing or electron-releasing effect of a particular substituent. ρ is a number (+ or -) indicating the relative need of a particular reaction for electron withdrawal or electron release.

For a series of substituted benzophenones (53) undergoing the reaction 53 → 54 (Figure 1.19) it has been shown (Budzikiewicz *et al.*, 1967) that electron-withdrawing substituents usually enhance the abundance of acyl ion 54 relative to molecular ion, while electron-donating substituents cause the reverse effect. This behavior is understandable in terms of delocalization of the positive charge in the molecular ion over the aromatic ring.

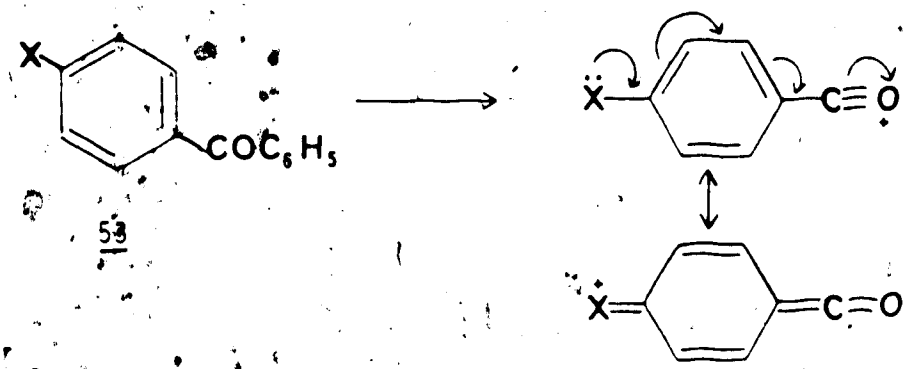


Figure 1.19. Reaction of substituted benzophenones.

The existence of a correlation with Hammett σ values indicates that, with respect to the effect of the substituent, the transition state for the gas phase reaction is similar to that for solution phase, i.e. the rate of formation of 54 is enhanced due to resonance effect.

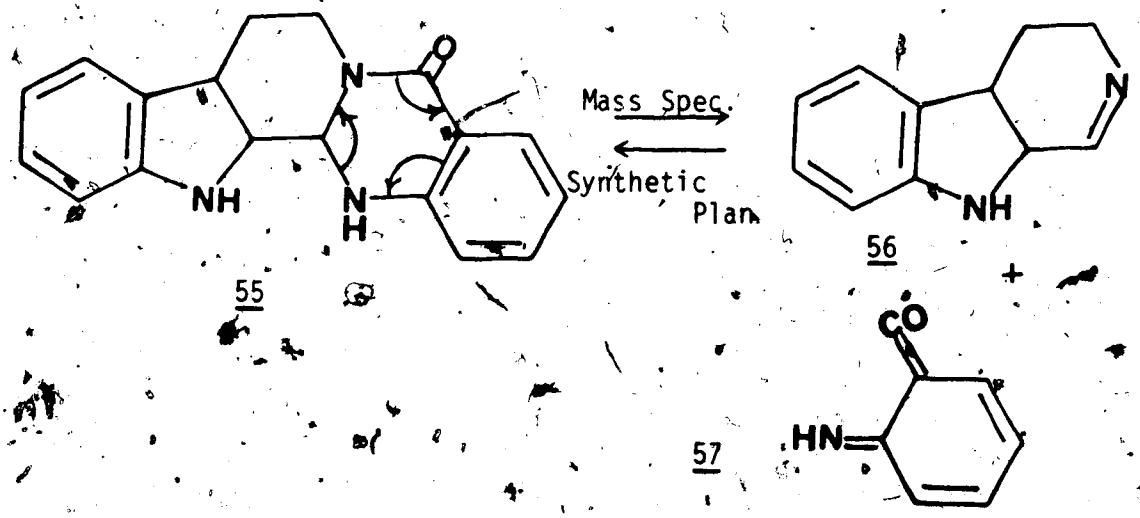
The correlation of Hammett ρ values with the ion abundance also provides strong support for the postulate that product ion stability is an important driving force in ion decomposition reactions in the mass spectrometer.

A relationship is also found between the ionization energy and molecular structure. A correlation is found for

monosubstituted aromatic compounds (Table 1.1) (Howe *et al.*, 1981). These data show that substituents that have strong electron-withdrawing effects (NO_2, CN, CHO) increase the ionization energy relative to benzene. In contrast, those substituents which have the largest electron-donating effects (OH, OCH_3, NH_2) cause a reduction in the ionization energy relative to benzene. A correlation is found between the ionization energy and the Hammett σ values for the substituents (Crable and Kearns, 1962) (Figure 1.20) in the cases of both monosubstituted benzenes and toluenes.

1.3.2.5 "Retro mass-spectral synthesis"

An effective method of synthesizing complex organic molecules was proposed by Kametani *et al.* (1976) and was termed "retro mass-spectral synthesis", where the fragmentations observed in the mass spectrum are utilized in a useful synthetic strategy, for example evodiamine (55) was synthesized from synthons 56 and 57, which correspond to the fragment ions in the mass spectrum of 55.



55 56 + 57

Table 1.1 IE (eV) of some monofunctional aromatic compounds C_6H_5X.

X	EI
NO_2	10.18
CN	10.09
CHO	9.70
H	9.56
OH	9.16
OCH_3	8.83
NH_2	8.32

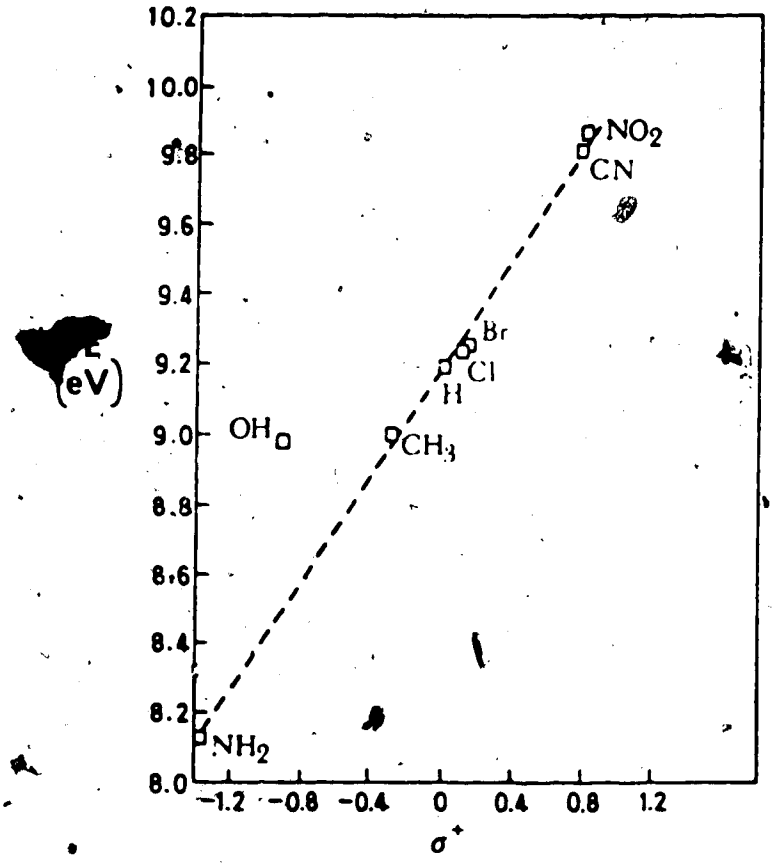
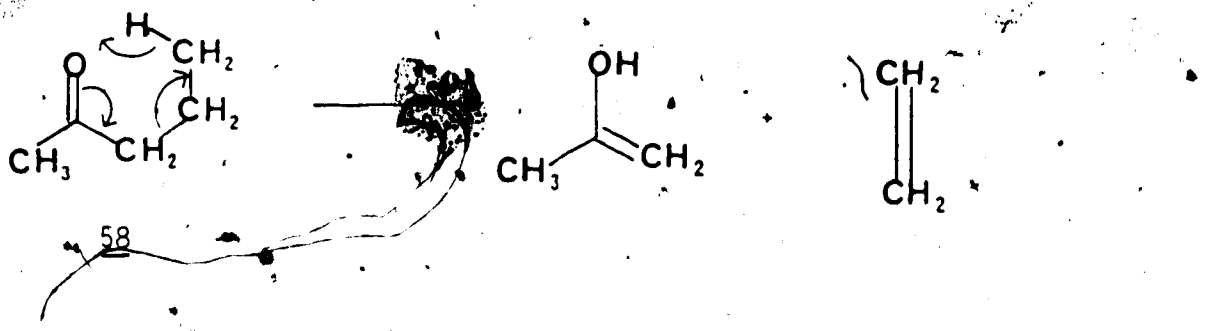


Figure 1.20 IE (eV) versus Hammett σ value for monosubstituted toluene.

1.3.2.6 Analogies to photolytic and radiolytic reactions

An impressive example of the analogous chemical reactions which may be induced by electron impact is illustrated by data accumulated for methyl-alkyl ketones (Table 1.2) by Pitts and Osborne (1961). All data refer to fragmentation by β-cleavage with γ-hydrogen transfer as typified for methylpropyl ketone (58).

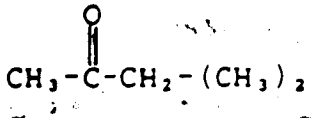
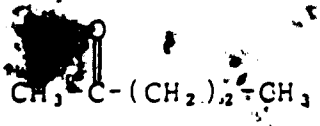
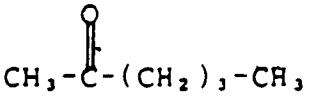
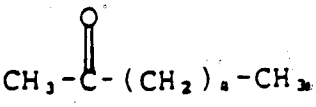


An approximate proportionality exists between quantum yields (for vaporphase photolysis at 3130 A and 120°C), G values (for 3 mev rays in the liquid phase at 25°C) and the relative abundance of m/z 58.

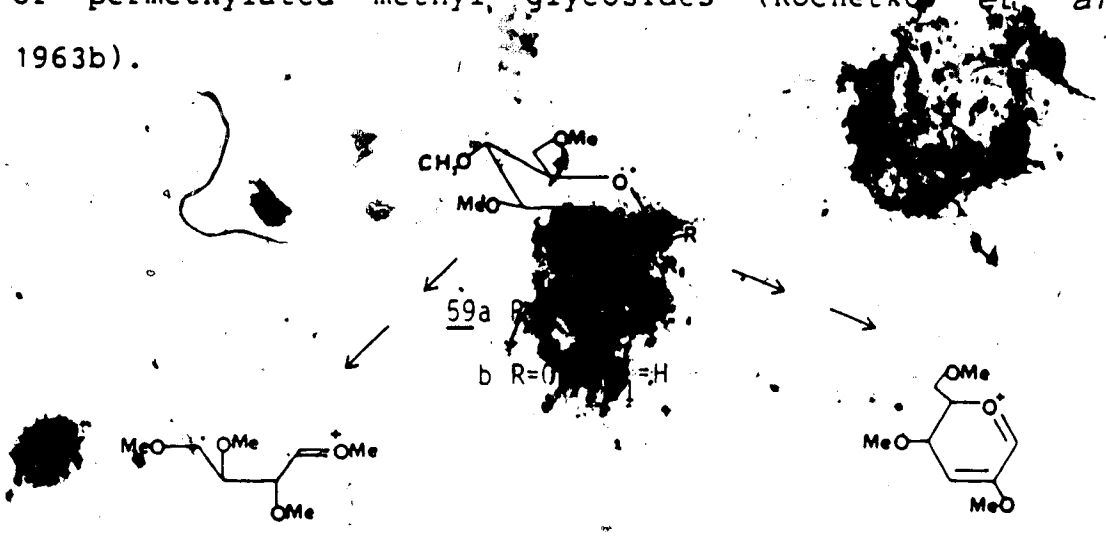
1.3.2.7 "Steric acceleration"

The mass spectra of compounds 59a and 59b contain identical sets of peaks (Kochetkov *et al.*, 1963a). However, the marked difference between them consists of the different ratio of intensities of peaks m/z 176 (B_1) and m/z 187 (A_2). The α-D-anomer (59a) produces the A_2 peak about twice the intensity of B_1, a reversed relationship being found with β-D-anomer (59b). This may be explained by the fact that, in the α-D-anomer, the methoxyl group at C-1 occupies a trans-axial position with respect to the lone pair electrons of

Table 1.2 Relative efficiencies of β-cleavage with H-transfer, in photolytic, radiolytic and electron impact reactions.

Compound	Quantum Yield	G value	m/z 58 (%)
	0.00	0.00	0.1
	0.27	0.15	7
	0.40	0.29	42
	0.40	0.16	50

the ring oxygen, so that its elimination is facilitated due to the participation of the oxygen lone pair electrons in the process. This effect is called "steric acceleration". An analogous regularity is observed with other anomeric pairs of permethylated methyl glycosides (Kochetkov *et al.*, 1963b).



m/z 176(B_1) m/z 187(A_2)

1.3.2.8 Measurement of acid dissociation constants by Fast Atom Bombardment Mass Spectroscopy (FABMS)

Work with aqueous solutions containing ionic solutes in a 1:1 mixture of water and glycerol showed that factors, such as the pH of the solution and the salt content, had significant and reproducible effects on the distribution of ionic species, as measured by the mass spectrometer. Using the Henderson-Hasselbalch equation under simplified conditions (low ionic strength with acid components whose pKa's lie between 3 and 10), it was shown that the pKa of an acid could be accurately determined knowing the pH of the solution and the concentration of acidic and basic species

(Caprioli, 1983). With respect to the measurement of this constant by FABMS, the following equation has been used:

$$pKa = pH + \log \frac{(HA + H)^{\cdot}}{(ANa+H)^{\cdot} + (ANa+Na)^{\cdot}}$$

where $(HA+H)^{\cdot}$ is the ion intensity of the undissociated acid HA, and $(ANa+H)^{\cdot}$ and $(ANa+Na)^{\cdot}$ are the ion intensities of the corresponding conjugate base A^{-}.

In addition, FABMS has been successfully applied in determining the equilibrium constants for enzyme-catalyzed reactions, metal-ligand association constants (Johnstone *et al.*, 1983) and measurements of reaction rates for specific substrate-enzyme reactions (Smith and Caprioli, 1984).

1.3.3 Conclusion

From the preceding discussion, it can be concluded that the study of fragmentations of Amadori rearrangement products by EI mass spectroscopy may reflect actual decompositions taking place in food systems. Since the fragmentation patterns are of prime importance in determining the course of the advanced Maillard reaction, the analysis of the mass spectra of a number of Amadori rearrangement products should give a wealth of information concerning the progress of the advanced Maillard reaction.

Amadori rearrangement products are the key intermediates in any real or model system, irrespective of the parameters involved -- in order for a reaction to qualify as

a Maillard reaction, the intermediates should pass through the Amadori rearrangement stage, whatever the temperature or the pH or any other parameter -- the difference that those parameters will make is only in the extent to which the decomposition will proceed. Since mass spectral conditions resemble those when using a wider range of temperatures, all the possible modes of decomposition will be exhibited through the study of mass spectra.

General patterns common to all derivatives and specific patterns due to a specific amino acid present can give important clues as to what types of intermediate products can be expected due to a specific amino acid and how those intermediates can interact and give further bimolecular or polymeric products in the final Maillard reaction stage. Furthermore, it can give important clues as to the relative predominance of important flavor compounds produced from the initial intermediates.

2. SUGGESTED FRAGMENTATION PATHWAYS OF AMADORI REARRANGEMENT PRODUCTS

2.1 Introduction

During the past two decades the mass spectrometric fragmentation behavior of virtually all common functional groups in organic chemistry has been examined, especially the field of carbohydrates. The study of the behavior of organic molecules upon electron impact and its comparison with other organic chemical reactions of the same molecule has become a very important and active field of research. Since the fragment ions are not isolated, only indirect support can be presented to describe their nature, and the evidence is by no means as rigorous as in many other organic chemical reaction mechanisms. Nevertheless, the circumstantial evidence is now overwhelmingly in favor of discussing the mass spectrometric reactions in terms of standard and simplified language of organic chemistry and, if the compound being investigated has a known structure, the mass spectrum is often interpreted by assuming a minimum of structural change at each fragmentation step.

The mass-spectral technique has become a useful supplement to chemical methods, and provides a ready solution of a variety of problems which were stumbling blocks using chemical approaches as is the case with studies related to the Maillard reaction.

The mass spectral method was first applied to carbohydrate chemistry in 1958, when Reed *et al*. reported the mass spectra of D-glucose, D-galactose, methyl α- and β-D-glucopyranosides and a number of disaccharides. Since then, the applications of the technique in the carbohydrate area have been prolific.

The early studies of carbohydrates using low-resolution electron-impact were not encouraging. The spectra of the compounds studied exhibited molecular ions of low intensity and a myriad of fragment ions, which suggested that breakdown pathways were complex. The introduction of high-resolution mass spectrometers with modifications necessary for analysis of organic compounds and the determination of fragmentation composition which became possible, added a new dimension to mechanistic studies.

The recognition that important structural features of monosaccharide derivatives could be determined from their mass spectra encouraged the development of mass spectrometry in carbohydrate chemistry. Although mass spectra have been obtained for simple sugars, the limitations imposed due to low volatility and thermal instability of free sugars rendered this procedure difficult. The availability of mass spectrometer with inlet systems, however, allowed direct introduction of the sample into the ion source, instead of introduction of more volatile derivatives such as methyl esters, acetates and trimethyl silyl ethers.

On the other hand, the amino acids, too, are of relatively low volatility. It has been shown (Gross and Grodsky, 1955), however, that most of the protein amino acids, with the exception of lysine, can be sublimed in a good vacuum at temperatures of 150-240°C without appreciable decomposition.

Almost all the work dealing with mass spectral analysis of amino acids has been done using electron impact mass spectrometry. This type of ionization induces very strong fragmentation in the molecular ions of the free amino acids and their derivatives, frequently rendering the M peaks difficult to detect, even at low electron energies.

The mass spectra of the esters of amino acids probably reflect more clearly the structural features of the amino acids than those of all other derivatives and of the free amino acids themselves. In addition, the fragmentation reactions occurring in the esters are common to most N-substituted derivatives and to the free amino acids (Svec and Junk, 1967). The most remarkable difference, however, occurs in the spectra of those amino acids that contain a hydrogen atom in the γ-position of the chain.

2.2 Treatment of the Mass Spectra

The peaks of the mass spectrum often differ by a factor of several hundred in their intensity. On the other hand, the intensity of the peaks is also dependent on the amount of substance introduced into the ion source. Hence, it is

necessary to subject the spectrum obtained to additional treatment, which consists of normalizing the peak heights with respect to the most intense peak in the spectrum, which is called the base peak, as follows:

$$\% \text{ relative intensity} = \frac{\text{peak height (div.)}}{\text{peak height (div.) of base peak}} \times 100$$

The units of the peak height are in chart divisions (div.) because, when a mass spectrum is recorded, ion currents are normally measured in arbitrary units of peak heights (chart divisions) rather than in units of current. Therefore, the percent relative intensity is independent of the amount of substance, and all the peaks can be expressed in one scale.

The peak intensities may also be expressed in percent of the total ion current (%TIC or %Σ) of all the peaks of the mass spectrum. This form is preferred when the mass spectra of several related compounds are to be compared (as in the present study) because the total peak intensity of the mass spectra of related compounds varies insignificantly, whereas the intensity of the base peaks changes markedly. The $\%\Sigma_m$ values are obtained by summing all peak heights or normalized values from a selected mass m (usually 50) to the molecular ion peak or the highest peak in the spectrum, in case the spectrum does not show the molecular ion peak, and then calculating the percent contribution of the various peaks to this total. When the only large peak in the spectrum is the base peak, its $\%\Sigma_m$ value is large. When several large peaks are present, the $\%\Sigma_m$ value of each is

small. This technique is also useful in studying the significance of individual peaks in a fragmentation process.

2.3 General Remarks

2.3.1 Definitions

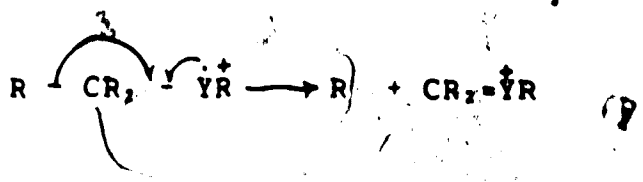
- Molecular ion ($M^{+\cdot}$), the ion produced from the intact molecule by expulsion of one electron.
- Daughter ion, the product of an ionic reaction.
- Parent or precursor ion, the decomposing ion in any reaction.
- Ion generation, all the daughter ions generated directly from one parent ion.
- $^{+\cdot}$: Radical cation, odd-electron ion.
- ↷ (full arrow), transfer of an electron pair.
- ↶ (fishhook), transfer of a single electron.
- rH, rearrangement involving hydrogen transfer.
- m/z, the mass of the ion divided by its charge.
- σ (sigma electron ionization), a simple cleavage reaction visualized as taking place through initial ionization at the sigma bond cleaved in the reaction:

$$R{:}CR_3 \longrightarrow R^{+} + {\cdot}CR_3$$

- i (inductive effect), inductive initiation of a reaction through electron withdrawing by the charge site:

$$R \curvearrowleft \ddot{Y} - R \longrightarrow R^{+} + {\cdot}YR \qquad Y = O,S,N,C$$

- α (alpha cleavage), cleavage of a bond on an atom adjacent to the atom bearing the odd electron:



2.3.2 Nomenclature

The nomenclature used by Chizhov and Kochetkov (1966) for the different fragmentation series of carbohydrate molecules will be adopted, with some modifications to take into account the fragmentation series originating in the amino acid moiety, thus an ion generation related by a common origin in the sugar moiety, will be denoted by capital letters (A_i, B_i, C_i, ...). Ion generation related by a common origin in the amino acid moiety will be denoted by double capital letters (AA_i, BB_i, CC_i, ...). The subscript numeral (i) corresponds to the number of steps needed for the transformation of the parent ion to the given fragment. The symbol asterix (*) will refer to the fact that the fragment ion was not observed in the mass spectrum; a lower case letter on the left hand side of the symbol will indicate an alternate route of fragmentation from the same molecular ion. All other fragment ions not originating from known carbohydrate or amino acid fragmentation routes will be designated by their m/z values. For example, the designation of A_3 indicates that the corresponding fragment originated from the sugar moiety by an established pathway, A, with three consecutive steps.

Amadori rearrangement products should be named as derivatives of D-arabino-2-hexuloses, however, the common name D-fructose will be used, due to its widespread

acceptance in the literature related to the Maillard reaction.

2.3.3 Experimental

The high resolution electron impact mass spectra of Amadori rearrangement products were determined on an Associated Electrical Industries (AEI) MS-50, high performance double-focusing mass spectrometer with Nier-Johnson geometry. The ionization energy was 70 eV and the peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15,000.

The temperature in the ion source was varied between 150-250°C depending on the volatility of the particular compound. The samples were introduced directly into the ion source (quartz probe) through a vacuum lock system, the pressure inside was 2 x 10^{-7} torr, and the accelerating voltage was 8,000 volts.

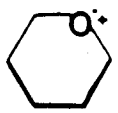
The data were analyzed by a DS-55 (Kratos), a computer-based data acquisition and analysis system for mass spectrometry. It consists of a powerful minicomputer with a custom, high speed data acquisition interface and a set of programs for collecting, analyzing and reporting mass spectrometric data.

2.4 Mass Spectra of Amadori Rearrangement Products

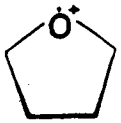
2.4.1 Strategy of analysis of the mass spectra

The rationalization and eventually a prediction of preferred decomposition pathways and bond cleavages in molecules is most conveniently effected by assuming that the reactions are initiated by preferential localization of the charge at a favored site in the molecular ion, as well as in fragment ions undergoing further decompositions. Such a site is viewed as providing the driving force for specific types of reactions which are characteristic of the chemical nature of that site.

The most favored radical and charge sites in the molecular ion are assumed to arise from the loss of the molecule's electron of lowest ionization energy. Relative energy requirements are similar to those for the electronic transitions affecting the ultraviolet spectra. Favorability for ionization generally is in the order of η-electrons>π>σ.



60



61

Thus, all the data available on the mass spectra of carbohydrates may be reasonably interpreted on the basis of the assumption that the molecular ion is formed by the removal of an electron from the ring-oxygen atom (60, 61), however, more complex molecules containing several possible sites for the localized charge and unpaired electrons require weighting of the relative importance of each site in

order to rationalize the relative intensities of ions produced from each site.

For molecules containing noninteracting functional groups, the ionization energy usually corresponds to the value characteristic of the functional group of lowest ionization energy (IE) (Svec and Junk, 1963). This is illustrated in Figure 2.1.

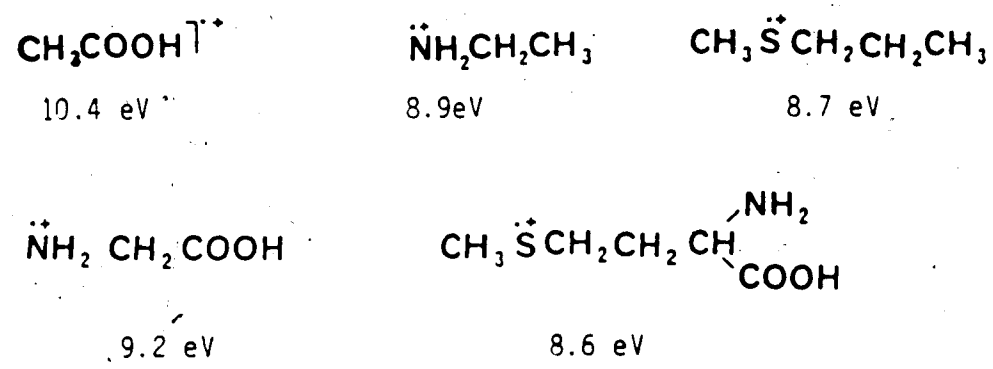


Figure 2.1

Apparently, the electronegative carboxyl group does influence the IE of the amine in glycine (Svec and Junk, 1967).

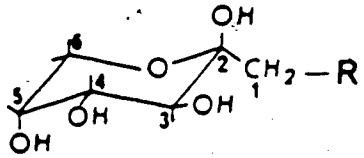
Amadori rearrangement products, being complex, polyfunctional molecules, contain several possible sites for the localized charge. However, keeping in mind that they can be considered as either N-substituted amino acids or as derivatives of D-fructose, it can be assumed that both the ring oxygen and amino acid nitrogen can trigger fragmentation characteristics of fructose and the specific amino acid. The relative importance of each fragmentation pattern is determined by the ionization energies of the ring-oxygen compared to that of amino acid nitrogen, and by the

complexity of the side chain of the amino acids. Therefore, by studying the fragmentation patterns of D-fructose and the different amino acids, many of the peaks observed in the mass spectra of Amadōri rearrangement products can be rationalized, based on the known fragmentation patterns of fructose and amino acids.

Since the compounds being investigated have known structures, the fragment ions, as well as the neutral species, can be identified by assuming or postulating structures with minimum charge from the original molecule at each fragmentation step.

The above approach is similar to that of "Shift technique" (Biemann, 1962), which states that the addition of a small functional group to a large molecule, such as an alkaloid, changes the spectrum by merely increasing the masses of the specific ion fragments which contain this added functional group.

N-substituted amino acids generally have the same pattern of fragmentation as unsubstituted ones and, in general, the substitution at C-1 of the fructose moiety



is not expected to affect the overall mass spectral pattern of the molecule (this is especially true with amino acids having alkyl side chains) because the principal bond fissions in fructose are not governed by the substituents at

C-1 but rather at C-2. It follows that most cleavages of the fructose moiety and that of the amino acids will be found to some extent in the mass spectra of the Amadori rearrangement products. Which pattern will dominate the mass spectrum will largely depend on the stability of the molecular ions originating in either nucleii and the nature of the side chain of the amino acids.

There are two principal reasons why Amadori rearrangement products lend themselves so readily to mass spectroscopic investigation. First, the two moieties -- the sugar and the amino acid -- have a great capability for stabilizing a positive charge through the formation of oxonium and imminium ions. Second, in most cases the amino acid moiety contains certain bonds that are especially prone to cleavage, thus giving rise to intense fragment ions.

Generally, in the analysis of fragmentation patterns the consecutive degradation of a parent ion is studied by detecting structural subunits eliminated from the precursor ion, with minimum change in its structure. It is then necessary to simply look for $M^{+\cdot}$-R peaks, where R is the mass corresponding to the expelled functionality. The most specific assignments that can be made in this respect are for small, neutral fragment losses, especially those that are formed directly from the molecular ion. For example, important ions at masses $(M-1)^+$, $(M-15)^+$ and $(M-18)^+$ almost always represent the losses of H, CH_3 and H_2O, respectively, from the molecular ion. In this connection, the analysis of

fragmentation pathways also represents an elucidation of ion structures. The ion in question is characterized as much as possible by its precursor ions and, in addition, by the greatest possible number of product ions in order to disclose its structure. This procedure allows deduction of the structure of fragment ions using mass spectroscopic ion fragmentation mechanisms explained in detail by McLafferty (1980).

In the following sections, a homolytic cleavage of a bond will be indicated by one fishhook arrow and, when convenient, by two.

The ion fragments will be identified by their molecular weights and, in parentheses, by % relative intensity.

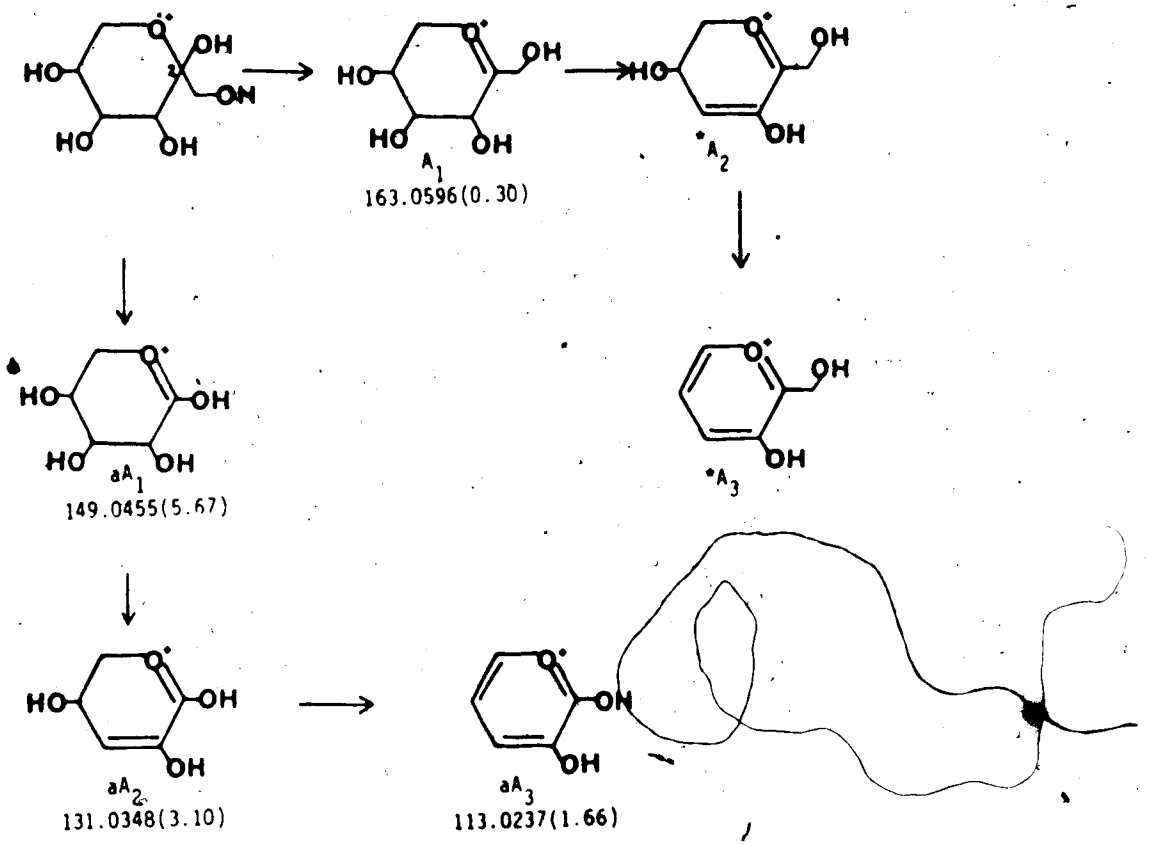
The fragmentation patterns of fructose are based on the work of Chizhov and Kochetkov (1966).

2.4.2 Mass spectrum of D-fructose

2.4.2.1 Fragmentations of the pyranoid form

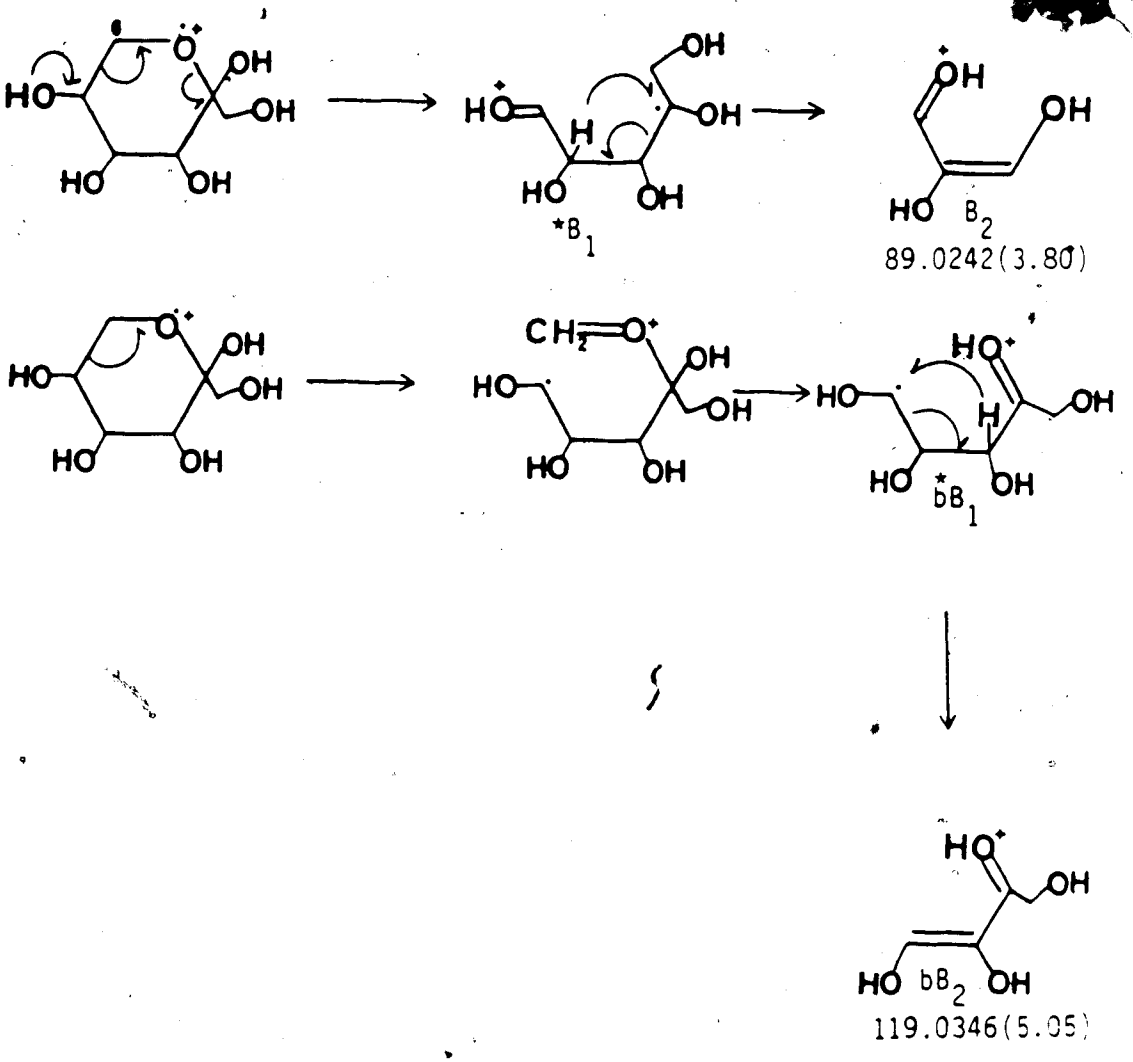
Fragmentations of the A series

Ions of the series A are produced by loss of substituents from C-2, with subsequent and stepwise elimination of other substituents.



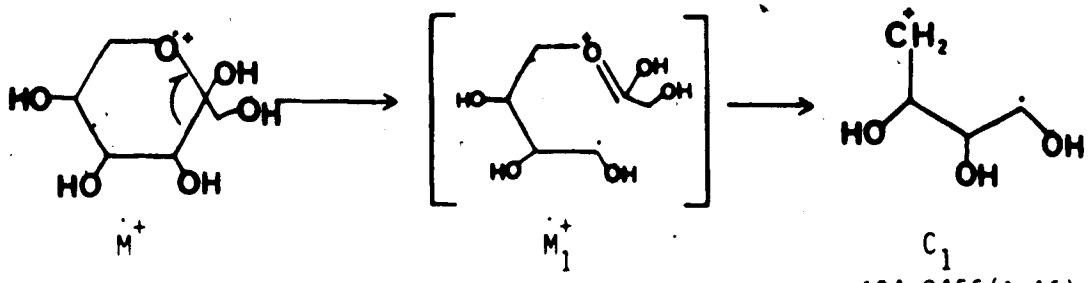
Fragmentations of the B series

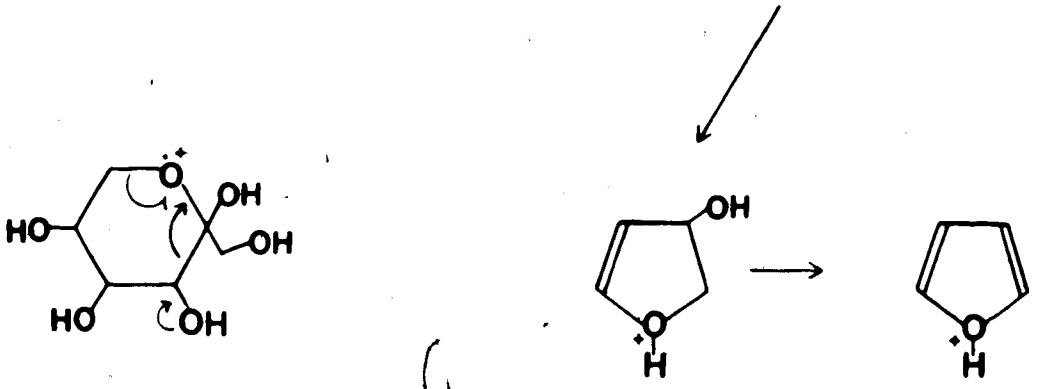
Ions of the B series are formed by elimination of C-6 and the ring oxygen as formaldehyde.

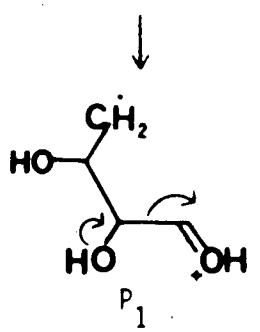


Fragmentations of the C and P series

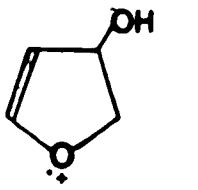
Ions of the C and P series are initiated by cleavage of the C-2 to C-3 bond.

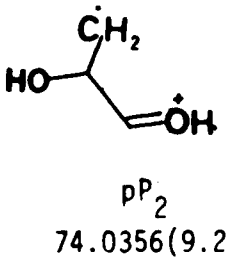




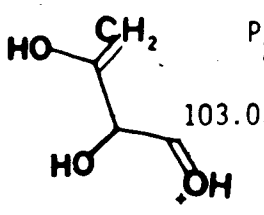


104.0456(1.16)



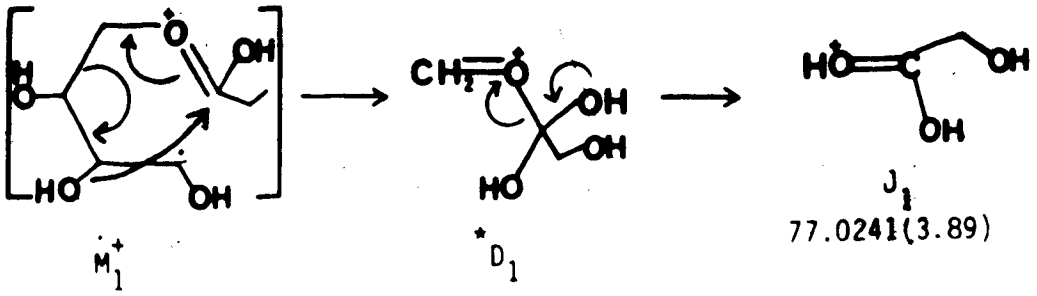


-H·



Fragmentations of the D, F and J series

Another pathway of fragmentation of the $M_1^{\cdot +}$ produced in series C leads to the ion D_1. Subsequent decomposition of D_1 gives rise to a new type of ion, the J series.

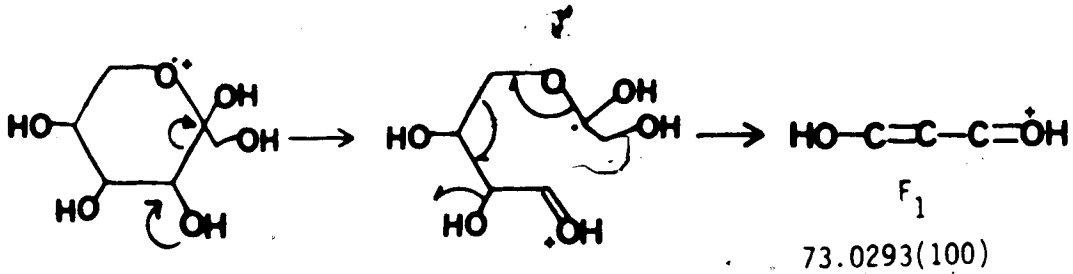


M_1^{+} D_1 J_1

77.0241(3.89)

The ion D_1, initially formed, is not traced in the mass spectrum. Being extremely unstable, it must immediately lose a formaldehyde group.

The fragmentation of the $M_1^{\cdot +}$ ion with cleavage of the same bondings, but with different distribution of charges on the fragments produced, leads to ion F_1, the base peak.



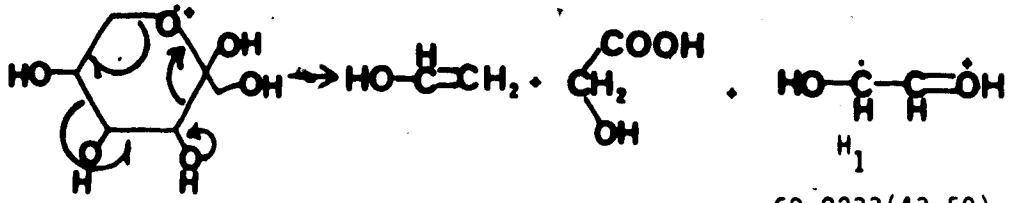
F_1

73.0293(100)

In F_1 the positive charge is distributed over the entire molecule.

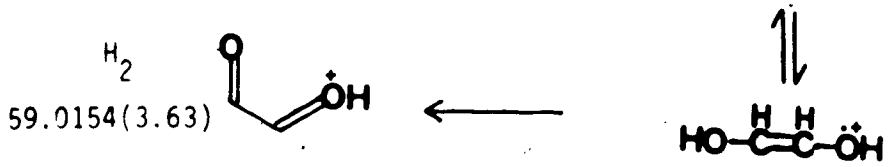
Fragmentations of the H series

Conjugated shift in the molecular ion gives rise to the H series.



H_1

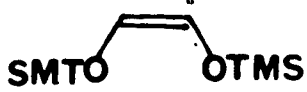
60.0233(43.50)



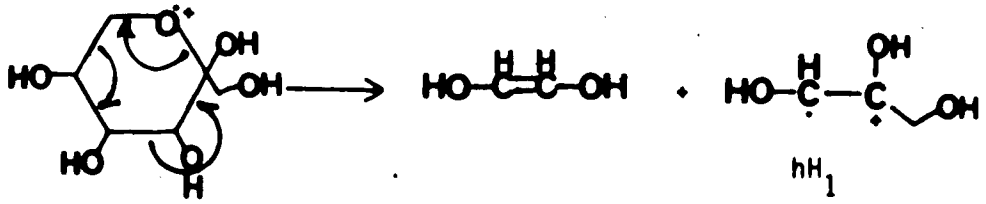
H_2

59.0154(3.63)

In trimethylsilylated fructose the corresponding peak at m/z 204 is the base peak.



m/z 204

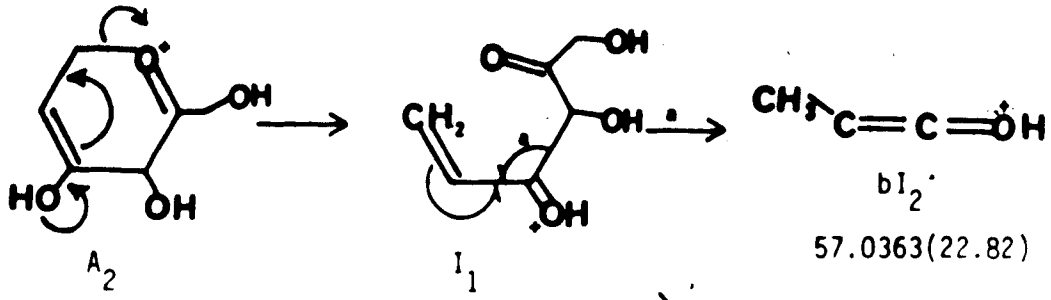


hH_1

90.0318(3.20)

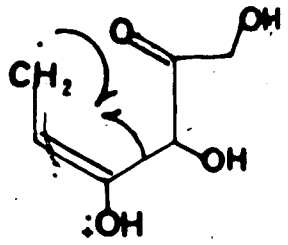
Fragmentations of the I series

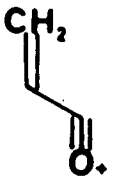
Ions of the I series are produced by cleavage of C-6 to ring-oxygen bond of A_2 ion.



A_2 I_1 bI_2

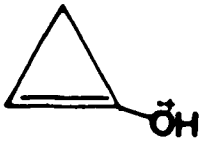
57.0363(22.82)





I_2

55.0202(9.24)

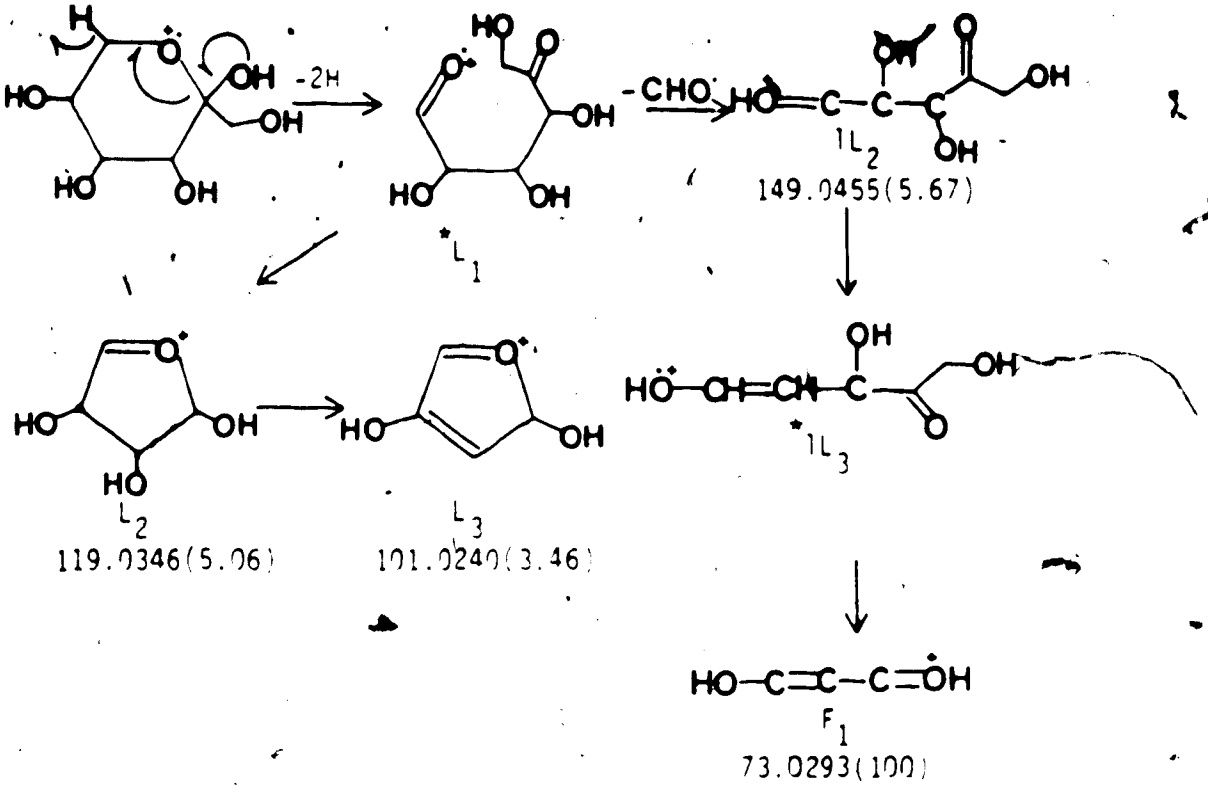


aI_2

56.0282(7.15)

Fragmentations of the L series.

The decomposition of compounds having a free hydroxyl group (like fructose) is essentially different from that of the corresponding glycosides, and the fragmentations assisted by the free hydroxyl group are termed L series.

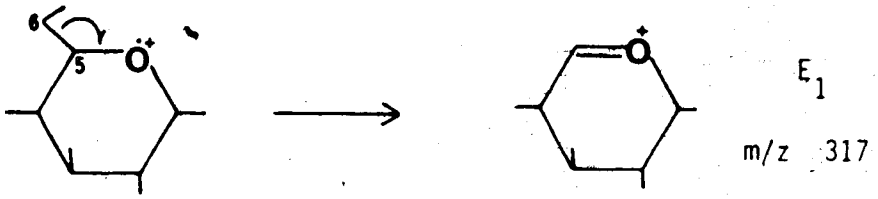


2.4.2.2 Fragmentations of the furanoid form

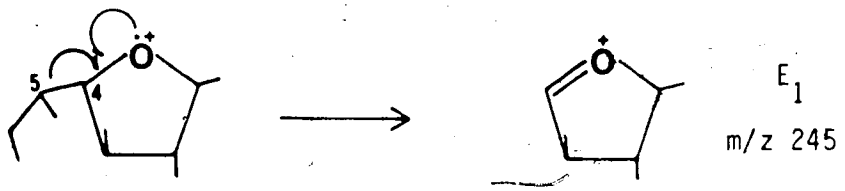
Introduction

The size of the ring in a monosaccharide bears strongly upon its mass spectrum, the characteristic differences providing a firm basis for distinguishing between pyranoid and furanoid forms (Biemann and DeJongh, 1963). The most marked are the differences in the positions and intensities of peaks of the E series; these peaks are due to ions formed by fission of the side chain at C-6. Generally, peaks of

this series in the mass spectra of hexopyranoses are of low intensity. The corresponding fragments arise after cleavage of the C-5 to C-6 bond. For example, in β-D-galactopyranose pentacetate the peak is at m/z 317:



The isomeric β-D-galactofuranose pentacetate gives rise to fragment E_1 by cleavage of the C-4 to C-5 bond, and therefore the ion has a lower mass number (m/z 245):



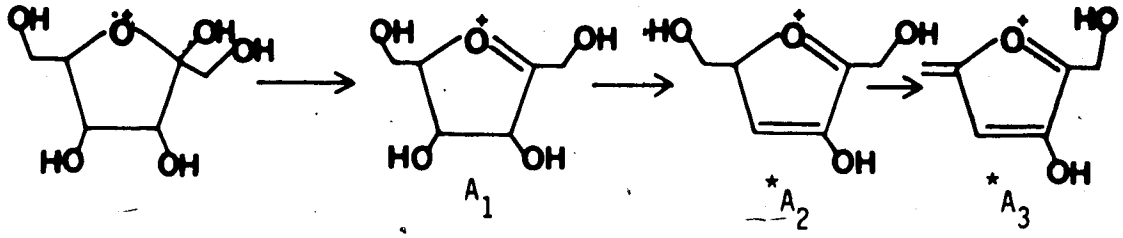
Moreover, the peaks of the E series in the mass spectra of furanoses have an increased intensity, the fission of the side chain from the five-membered ring leading to a planar oxonium ion, thermodynamically favored over the analogous ion having a six-membered ring (this is comparable with the rates of hydrolysis of furanosides and pyranosides). In addition, furanosides contain no D and B_1 ions, their formation being structurally impossible.

Although the characteristic differences permit distinction between furanose and pyranose derivatives, their fragmentation patterns have much in common. The fragments of

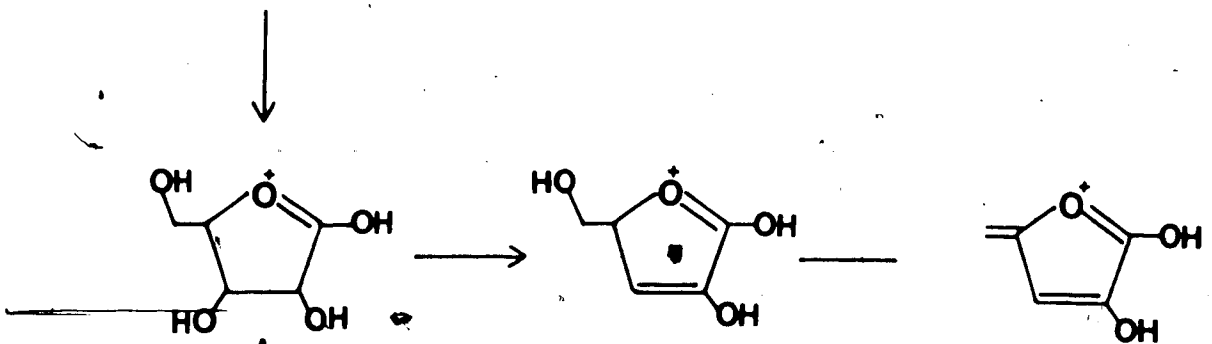
the A, C and J series are formed in a similar manner from both of the two types of derivatives.

Since fructose and ARPs have no side chain at C-6 in the pyranose form, the ions of the E series are not observed. However, since they can exist in furanose form, they show the E series of the furanose form.

Fragmentations of the A series



A_1 163.0596(0.30)

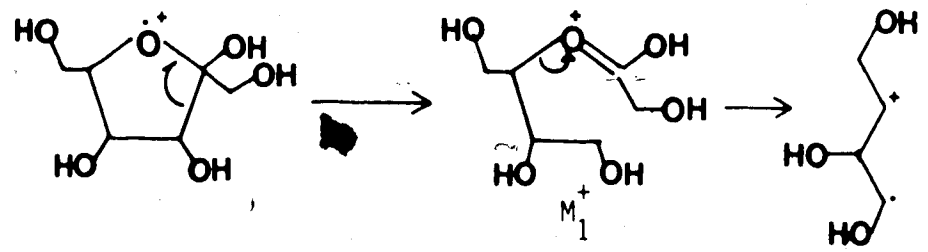


aA_1 149.0455(5.67)

aA_2 131.0348(3.10)

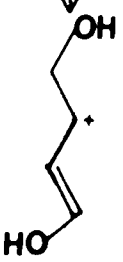
aA_3 113.0239(1.66)

Fragmentations of the C series



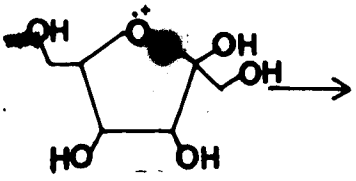
M_1^+

C_1 104.0456(1.16)



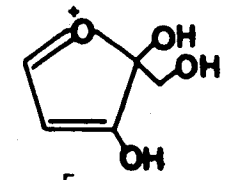
C_2 87.0437(0.51)

Fragmentations of the E series



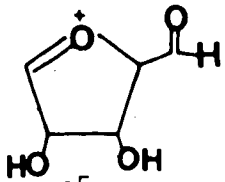
E_1

149.0455(5.67)



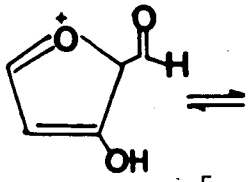
E_2

131.0348(3.10)



eE_2

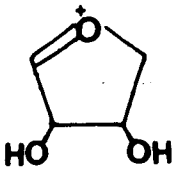
131.0348(3.10)



E_3

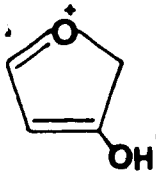
113.0239(1.66)

-CO



eE_3

103.0397(11.91)



eE_4

85.0293(8.61)

According to NMR studies, fructose and ARPs, exist in tautomeric equilibrium between furanoid and pyranoid forms, therefore, it will be assumed that the m/z values are contributed by the two conformers in the same proportion as indicated by C^{13}-NMR studies (Röper *et al.*, 1983).

2.4.3 Mass spectra of amino acid ethyl esters

Since the mass spectra of the esters of amino acids reflect more clearly the structural features of the amino acids than those of any other derivatives and, in addition, the fragmentation reactions occurring in the esters are common to most N-substituted derivatives, the mass spectra of amino esters will be discussed.

The peaks comprising the spectra of ethyl esters of amino acids are due to fragmentation of the molecule by prefered cleavages of those bonds which lead to energetically-favored, i.e. best stabilized, positive ions. The characteristic peaks are due to breaking of bond AA, bond BB or a bond of the side chain R, CC (Figure 2.2), particularly at highly substituted carbon atoms or at those bearing heteroatoms.

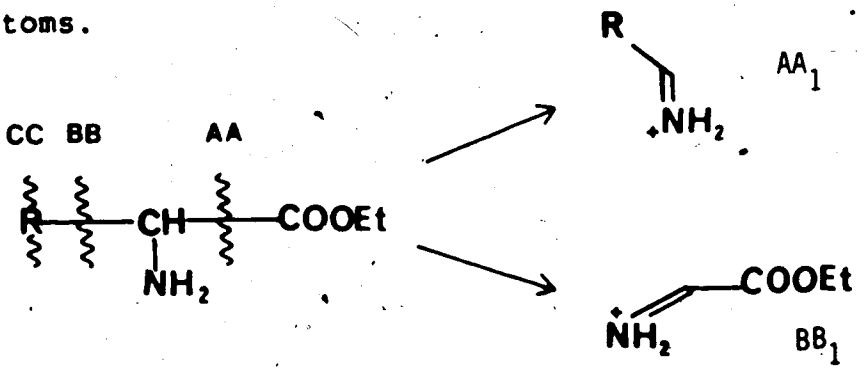
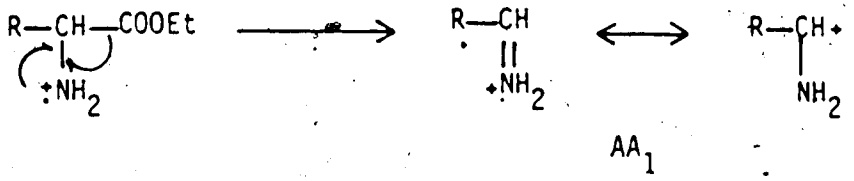


Figure 2.2.

Fragmentations of the AA series

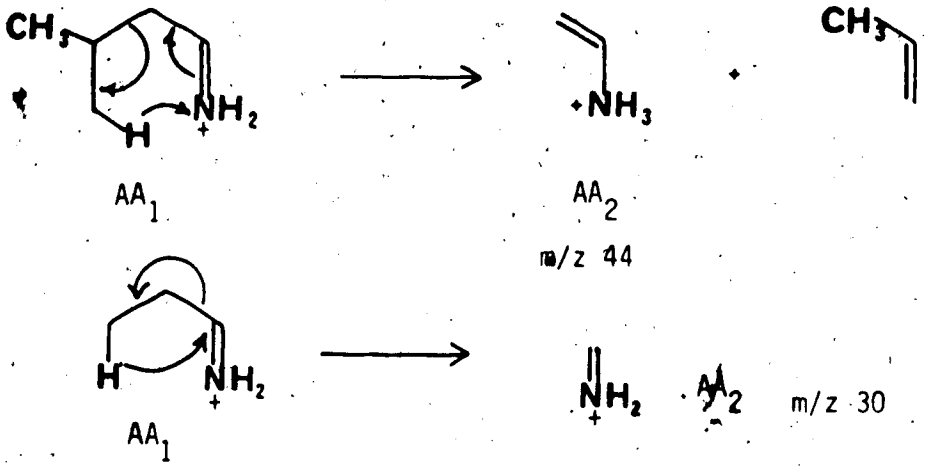
In all amino acid ethyl esters, the cleavage of one of the C-C bonds next to the amino group is a very prominent fragmentation reaction because retention of the positive charge on the nitrogen-containing fragment results in a resonance-stabilized ion, AA_1. Cleavage of bond BB, however, gives rise to a much smaller peak (Andersson, 1958) because the positive charge in the resulting ion is destabilized by the neighboring carbethoxy group.



Further decomposition of the fragment AA_1 may proceed from either one of the two extreme resonance forms.

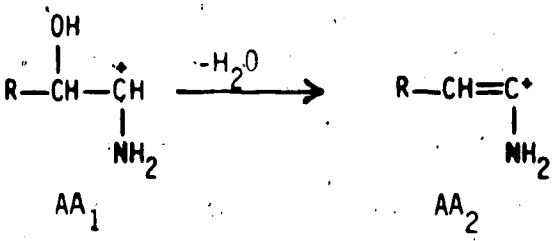
In the spectra of α-amino esters lacking additional functional groups, the AA_1 fragment is the most prominent one. Introduction of a heteroatom or aromatic system into the R group increases the tendency for cleavage of other bonds, either in the original molecular ion or in the AA_1 fragment. Both factors lead to a lower abundance of the latter. Such further decomposition of the amine-fragment (AA_1) arises from the elimination of neutral molecules like olefins, water, ammonia, hydrogen sulfide, mercaptans or ethanol. In almost all such cases the positive charge is

retained on the nitrogen-containing fragment. If the side chain R consists of more than two carbon atoms, considerable secondary fragmentation of AA_1 ions occurs. Two reactions generally occur: cleavage of the bonds β or γ to the $-CH=NH_2$ fragment, both with simultaneous migration of a hydrogen to the ionic center.

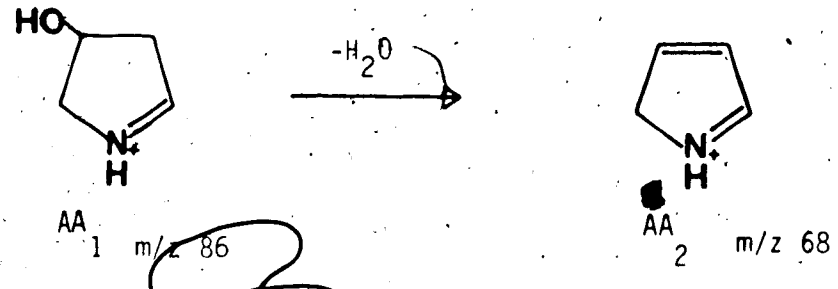


Such reactions are accompanied by strong metastable peaks. The mechanism of the first reaction is well established. It requires a hydrogen atom in the γ-position and occurs only if that condition is fulfilled (McLafferty rearrangement). In contrast, the mechanism of the second process is not well understood. Hydrogen atoms are rearranged from all positions, however, both reactions are sensitive to branching in the side chain.

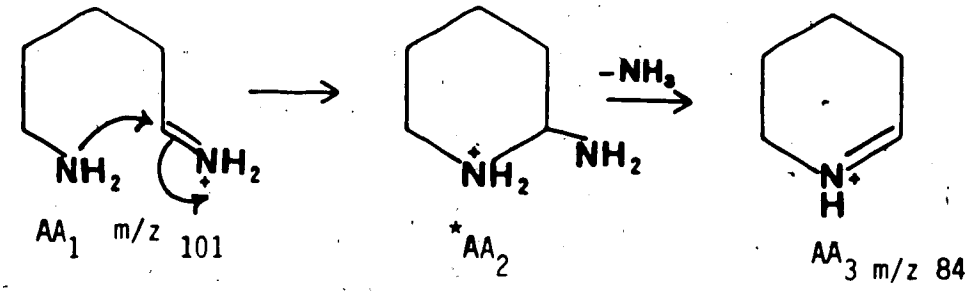
Serine and threonine esters exhibit peaks at m/z 42 and 56, respectively (AA_2):



Hydroxyproline also undergoes the elimination of water from the AA_1 fragment at m/z 86 to give a peak at m/z 68:

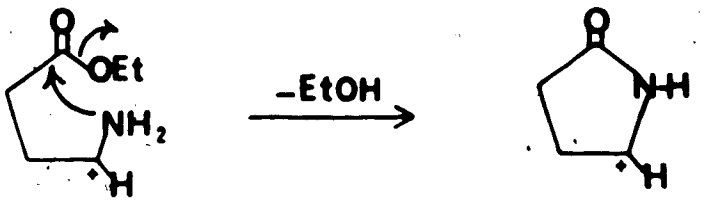


In the case of lysine esters, contrary to all other amino acids, the fragment AA_3 gives the most intense peak in the spectrum, whereas the AA_1 fragment is rather small:



This facile elimination of ammonia must, therefore, be assisted by the second amino group present.

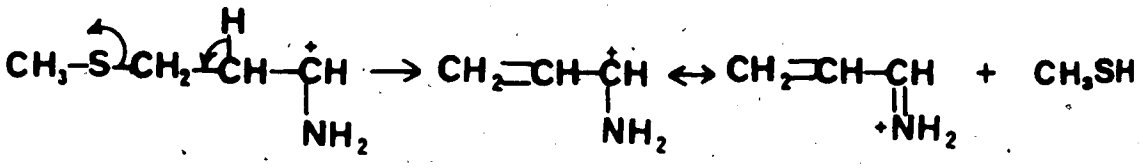
A somewhat similar behavior is shown by the ethyl ester of glutamic acid:



AA_1 m/z 130 AA_2 m/z 84

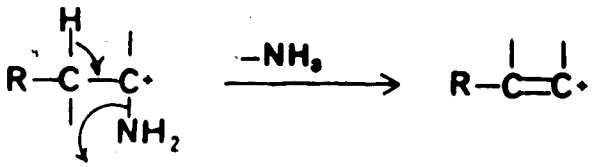
The amine fragment AA_1 loses the elements of ethanol to give the peak at m/z 84. Although some of the fragments of mass m/z 84 arise via prior thermal cyclization of the ester to ethyl-2-pyridone-5-carboxylate in the inlet system of the mass spectrometer, most of the fragments come from the original ester. The thermal cyclization is rather slow and can be followed by plotting the intensity of m/z 130 versus time.

Methionine ester gives a rather intense peak at m/z 56 AA_2 (Met), a metastable peak at m/z 30.3, supports the depicted course of the reaction:



AA_1 AA_2 m/z 56

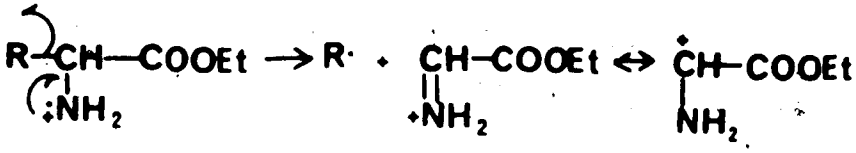
In addition to the above mentioned fragmentation of the AA_1 ions, they can also eliminate ammonia, leading to olefin-type fragments. These fragments are of low intensity, unless the β-carbon is highly substituted or bears an alkyl group.



AA_1 AA_2

Fragmentations of the BB series

If the bond BB is cleaved, a fragment is formed in which the positive charge is again stabilized by nitrogen, but less so compared to AA_1 fragment, due to the proximity of the positive charge to the carboxyl group.



BB_1 m/z 102

This fragment BB_1 at m/z 102 is characteristic of all ethyl esters of α-amino acids. Obviously, it is absent in the spectra of proline and hydroxyproline esters and negligible in glycine and alanine esters because hydrogen or methyl radical are less stable and are not cleaved as easily as

larger groups.

A high degree of branching or an aryl group on the β-carbon, as in valine, isoleucine, phenylalanine and tyrosine, further facilitates the cleavage of the C -C bond and gives rise to more intense peaks at m/z 102 (14, 17, 83 and 100%, respectively, of the intensity of the AA_1 peak at the corresponding ester).

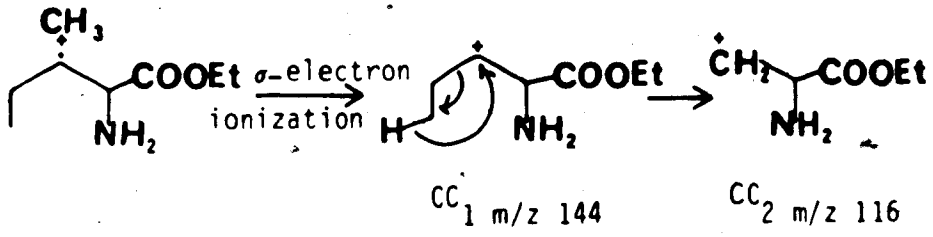
Two facts should be mentioned about the BB_1 fragment. First, it is relatively low in intensity compared to the AA_1 peak, in which similarly a carbon-carbon bond next to nitrogen is broken. Second, unsubstituted ethyl esters do not give the corresponding fragment but instead undergo McLafferty rearrangement. This rearrangement is operative in the ethyl esters of serine and threonine also, due to the acidity of hydrogens attached to oxygen compared with those attached to a carbon.

Fragmentations of the CC series

The majority of the ions in the mass spectra of α-amino esters are formed through the AA and BB series, however, if these bonds remain intact and others present in the molecule are cleaved instead, a third group of fragments is formed. These peaks are particularly useful if one wants to obtain detailed information about the structure. The abundance of such peaks will depend on the ease of fragmentation of those bonds versus the C-C bond α to the amino group and is particularly aided by the presence of highly substituted

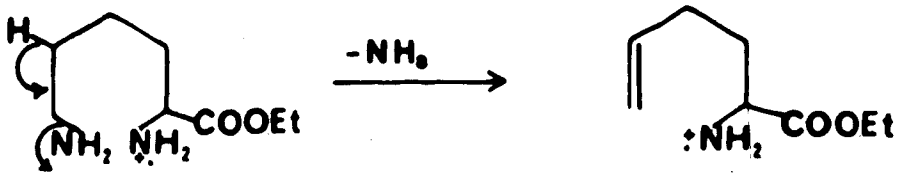
carbon atoms or of heteroatoms in the side chain of the amino ester. Proline ester, lacking any such groupings, gives only very few fragments of low intensity besides the very strong AA peaks. In methione ester, on the other hand, many peaks of considerable intensity are found, among which the AA_1 peak is far from being the highest.

Branching in the side chain favors fragmentation at that point. For example, the peak CC_1 at m/z 144 is much higher in isoleucine compared to norleucine.

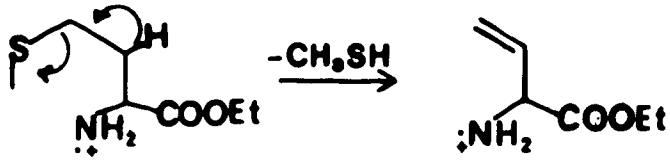


The peak at m/z 116 is small since it corresponds to the loss of three carbon atoms which cannot be derived by simple cleavage of a C-C bond but form a two-step process which is less probable and leads, therefore, only to a small peak.

The presence of functional groups in the side chain of the amino acids gives rise to more intense peaks, either by their elimination in the form of water, ammonia, ethanol, etc. in a manner similar to that of AA_1 fragment, or by direct cleavage of a C-C bond. Examples of the first case are the peaks in lysine ester at m/e 167 and in methionine at m/z 129:



CC_1 m/z 167

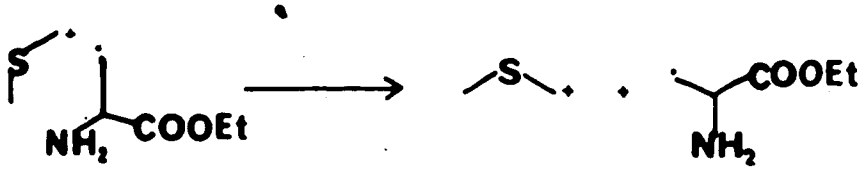


CC_1

m/z 129

More significant are peaks due to direct cleavage of a bond at a carbon atom to which a heteroatom is attached. If this functional group is at the β-carbon atom, as in serine or threonine, the C_α-C_β bond is cleaved with particular ease, giving rise to an enhanced ester peak at m/z 102.

A functional group at the γ-carbon atom, as in the case of methionine ester, leads to cleavage of C_β-C_γ bond, positive charge being maintained almost exclusively on the sulfur-containing fragment, where it is stabilized by the sulfur atom:



Its retention at the β-carbon would lead to a primary carbonium ion lacking any additional stabilization.

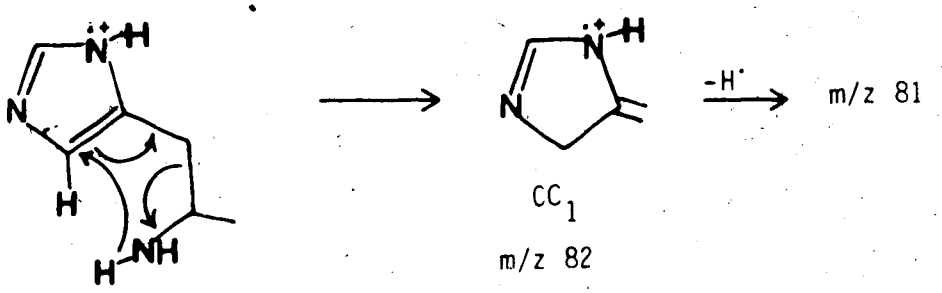
The ethyl esters of phenylalanine, tryosine and tryptophan provide examples in which the substituent at the β-carbon atom is able to accomodate the positive charge so well that the peaks corresponding to cleavage of C_α-C_β bond are rather intense, particularly the one containing the β-carbon.

The increase in the π-electrón density in the aromatic nuclei in the series phenylalanine, tyrosine and tryptophan is expressed in the increased intensity of the peak due to the $Ar-CH_2^+$ fragment. The probability of formation of this ion versus the amine peak, AA_1, and the BB_1 increases remarkably in this series (Table 2.1).

Table 2.1

	Phe	Tyr	Trp
AA1	100	51	85
BB1	83	52	4
Ar-CH2	23	100	100

Histidine ethyl ester appears to be the least aromatic in this respect. The AA_1 peak at m/z 110 is much higher than the BB_1 peak at m/z 102. The peak CC_1 at m/z 82 is formed by the rearrangement depicted below, and is the most intense peak in the spectrum.



Remarks on the molecular ion

The presence in a molecule of a number of bonds easily broken decreases the number of molecular ions able to survive for about 10^{-4} sec, the time required to be fully accelerated. For this reason, the peak at the molecular weight of the amino esters is always very small or nonexistent unless there is present in the molecule a grouping which can tolerate the loss of an electron particularly well. Such is the case in the aromatic amino acids. The thio-ether group of methionine also adds to the stability of the molecular ion, the intensity of which is 12% of the highest peak at m/z 61. However, it is more realistic to compare the intensities of peaks in different spectra if expressed in percentage of total ion yield (%Σ) instead of the highest peak. This value is 2.3% for tryptophan, 3.4% for methionine and 0.025% for leucine.

The low intensity of the molecular weight peak sometimes makes it difficult to identify or distinguish it from other small peaks due to impurities. However, the peak one mass unit above the molecular weight can be used to recognize the latter (McLafferty, 1957). The "M+1" peak

arises through hydrogen abstraction from another molecule by the molecular ion, during a collision process in a second order reaction:

$$\underset{(M^{+\cdot})}{X^{\cdot}} + RH \rightarrow \underset{(M^{+\cdot}+1)}{XH^{\cdot}} + R^{\cdot}$$

The abundance of $XH^{\cdot}$ ions is not directly proportional to the concentration of $X^{\cdot}$ in the ion source, like all other ions, but to the product $[X^{\cdot}][RH]$.

Hydrogen abstraction to form $(M^{+\cdot}+1)$ ion is the only bimolecular reaction observed in the mass spectrum.

2.4.4 General rearrangements and fragmentations observed in the mass spectra of Amadori rearrangement products

Amadori rearrangement products of the following amino acids were synthesized:

1. Amino acids with aliphatic side chains. Glycine, alanine, valine, leucine and isoleucine.

2. Amino acids with side chains containing hydroxyl and carboxy groups or sulfur atoms. Serine, threonine, methionine and glutamic acid methyl ester.

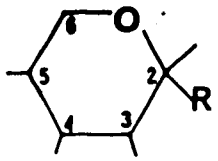
3. Amino acids with side chains containing basic groups, Lysine (three possible Amadori products) and histidine.

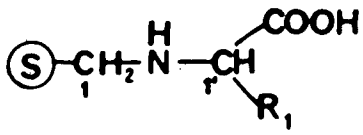
4. Amino acids containing aromatic rings. Phenylalanine and tryptophan.

5. Imino acids. Proline and 4-hydroxyproline.

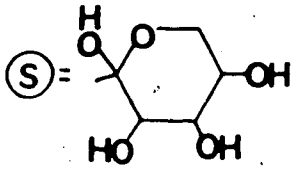
Since most of the fragmentation patterns of Amadori rearrangement products are essentially based on the known

fragmentation patterns of D-fructose and the specific amino acids, the following designations of the Amadori rearrangement products will be used:

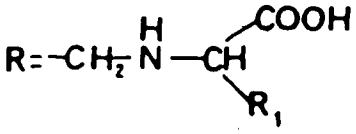




where



R_1 = Side chain of the specific amino acid.

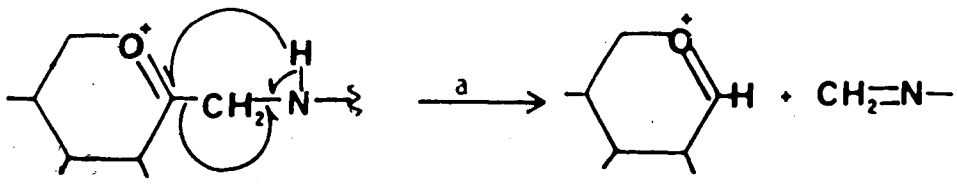


— = —OH

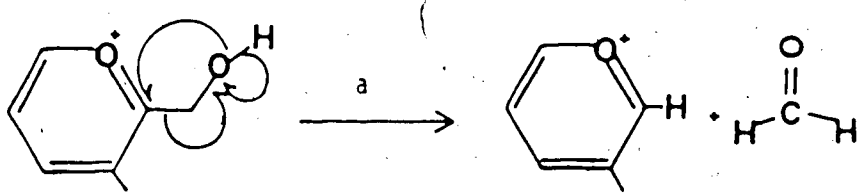
The following rearrangements and conversions were observed in the mass spectra of the Amadori rearrangement products.

• Rearrangement of R→H (a)

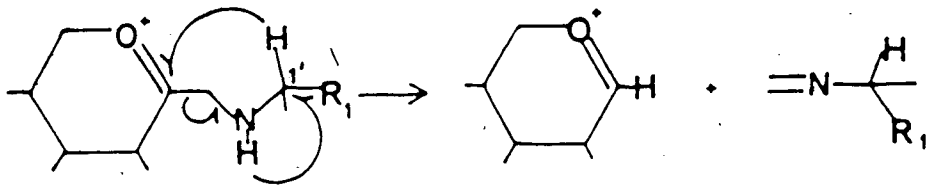
Partially dehydrated carbohydrate ring derivatives (pyran derivatives) were observed to undergo the following rearrangement:



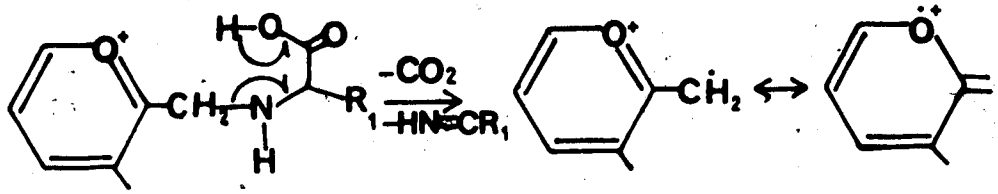
Saturated carbohydrate ring systems do not undergo this rearrangement because rearrangements are entropically unfavorable. The ion is in the conformation necessary for reaction only a very small fraction of the time, thus this "tight activated complex", characteristic of rearrangements, requires an offsetting energetic favorability for significant product-ion formation. The dehydroxylation step renders C-2 of the fructose moiety more electrophilic and the ring planar, hence, N- of amino acid and C-1 and C-2 of fructose are coplanar, which enhances the rearrangement. The same type of rearrangement is also observed in the mass spectrum of D-fructose:



Alternatively, the hydrogen atom can be abstracted from the C-1' as follows:

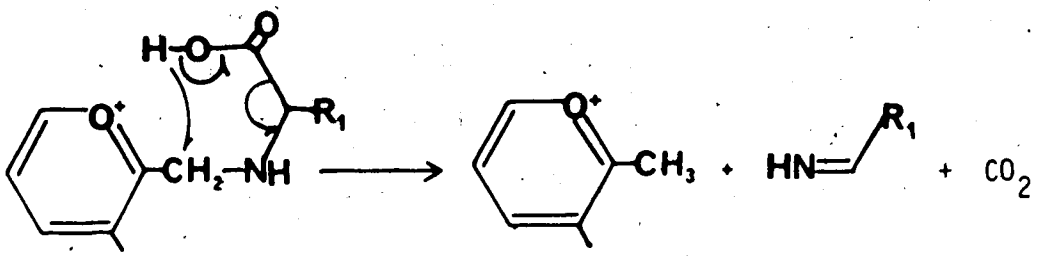


• Conversion of R→CH_2 (b)



This conversion is facilitated by the loss of stable neutral species, CO_2 and the imine.

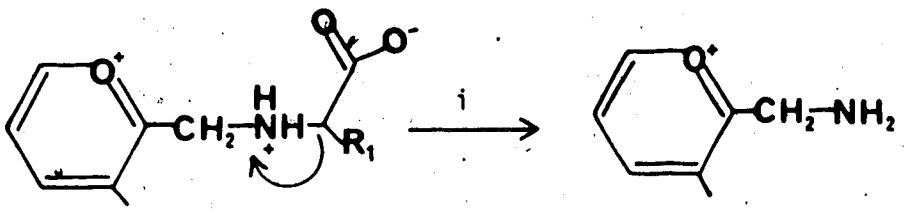
• Rearrangement of R→CH_3 (c)



In this rearrangement, which passes through a six-membered ring transition state, a rigid ring structure of the dehydrated sugar moiety prevents conformational changes that would otherwise occur and thus move the site of hydrogen migration away from the carboxyl moiety. There is indirect evidence for the above observation: the mass spectra of all ARPs lack peaks that correspond to R→CH_3 rearrangement prior to any dehydration in the ring, and the intensity of the peaks corresponding to R→CH_3 increases with

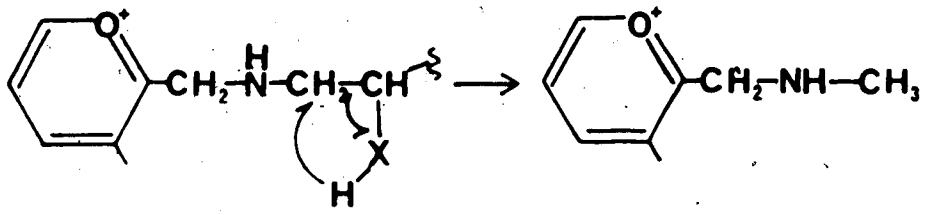
increasing dehydration in the ring. Again, the loss of stable neutral molecules favors this rearrangement.

- Rearrangement of R→-CH_2-NH_2 (d)



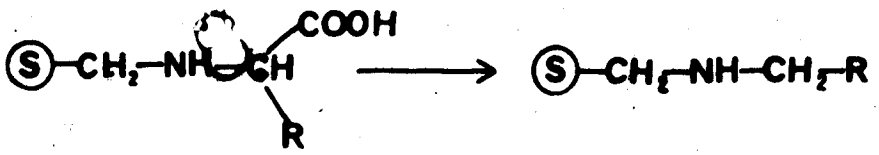
The zwitterionic form of the amino acid moiety undergoes an inductive cleavage, giving rise to the free amine attached to the sugar.

- Rearrangement of R→-CH_2-NH-CH_3 (e)

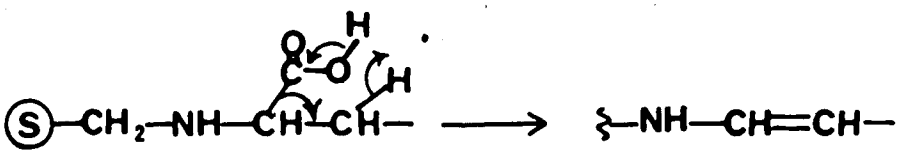


A 1,3-migration of the hydrogen atom produces the secondary amine side chain on the sugar. This rearrangement is specially enhanced when x=0 (serine and threonine) due to electrophilicity of oxygen atom compared to carbon.

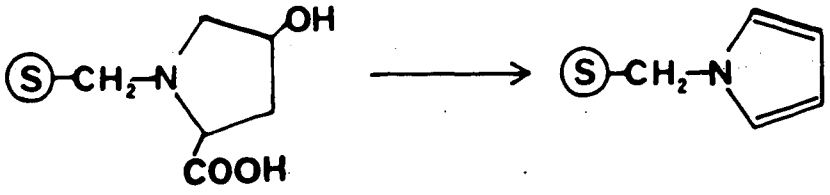
- Decarboxylation (g) SNi



- Decarboxylation + dehydrogenation (f) cis-elimination

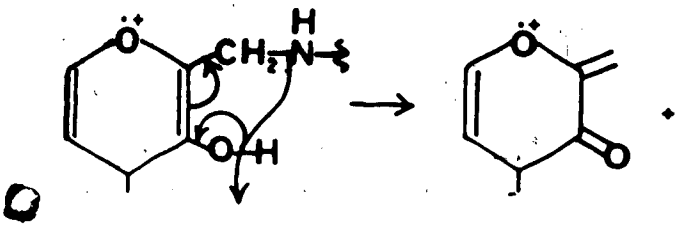


- Decarboxylation + dehydrogenation + dehydration (k)

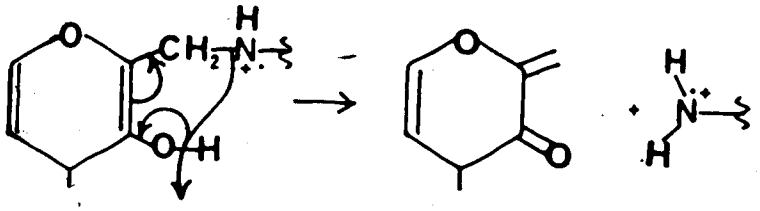


This conversion is specific to hydroxyproline.

• Ortho-elimination (o-e)

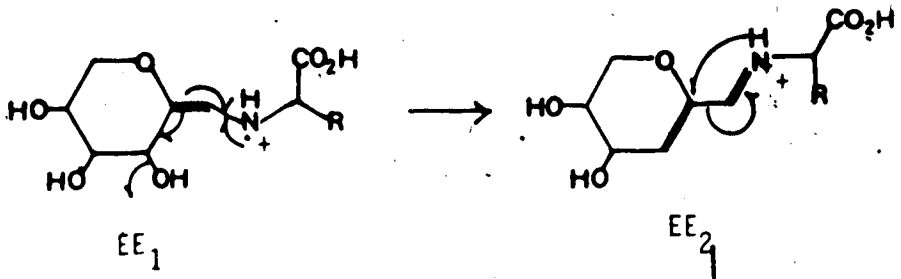


m/z 126

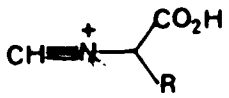


Stable neutral molecules are eliminated in this rearrangement reaction, in which a more stable ion is formed from the precursor. The ion at m/z 126 is detected in the majority of ARPs.

- Fragmentations of the EE series:

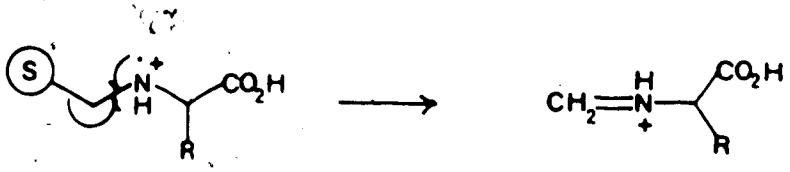


EE_1 EE_2



EE_3

- Fragmentations of the DD series:

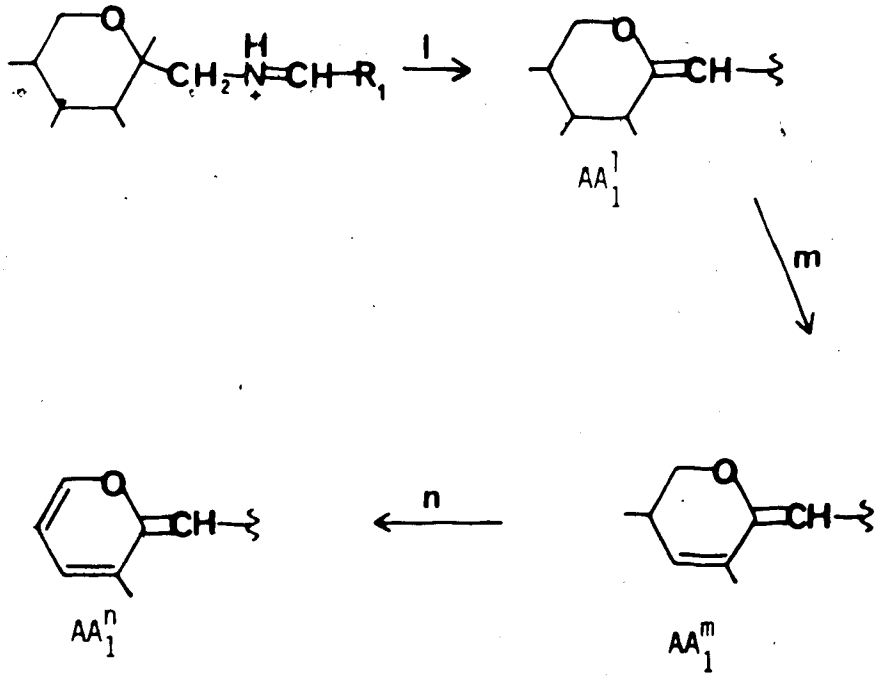


DD_1

• Notes on the nomenclature

1. The rearrangements/conversions a to k will be incorporated into the symbols devised previously, for identification of ion peaks, as a superscript. Thus, the designation A_3^a indicates that the corresponding fragment ion originated from the sugar moiety by pathway A, with three consecutive steps from the parent molecule and it had undergone rearrangement a.

2. The dehydration sequence of the sugar ring or the amino acid side chain, if applicable, of ions originating in the amino acid moiety will be denoted by the following letters as superscript: l,m,n.



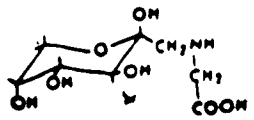
3. All the symbols representing rearrangments, conversions and dehydrations are one step processes, except m and n, which are two and three step processes,

respectively. Consequently, to calculate the total number of steps from the symbols representing a fragment ion, one has to add the value of the subscript to that of the superscript. For example, the fragment ion A_3^a represents a four step process, whereas AA_3^m represents a five step process.

- The mass spectra of all Amadori rearrangement products are reproduced in Appendix I.
- Since the products formed by the "sugar fragmentation" pattern follow the same mechanism, only the "amino acid fragmentation" mechanisms are reported for individual ARPs, except for glycine; however, "sugar fragmentation" products are listed in Appendix II.

2.4.5 1-[(carboxymethyl)amino]-1-deoxy-D-fructose

T = 225°C



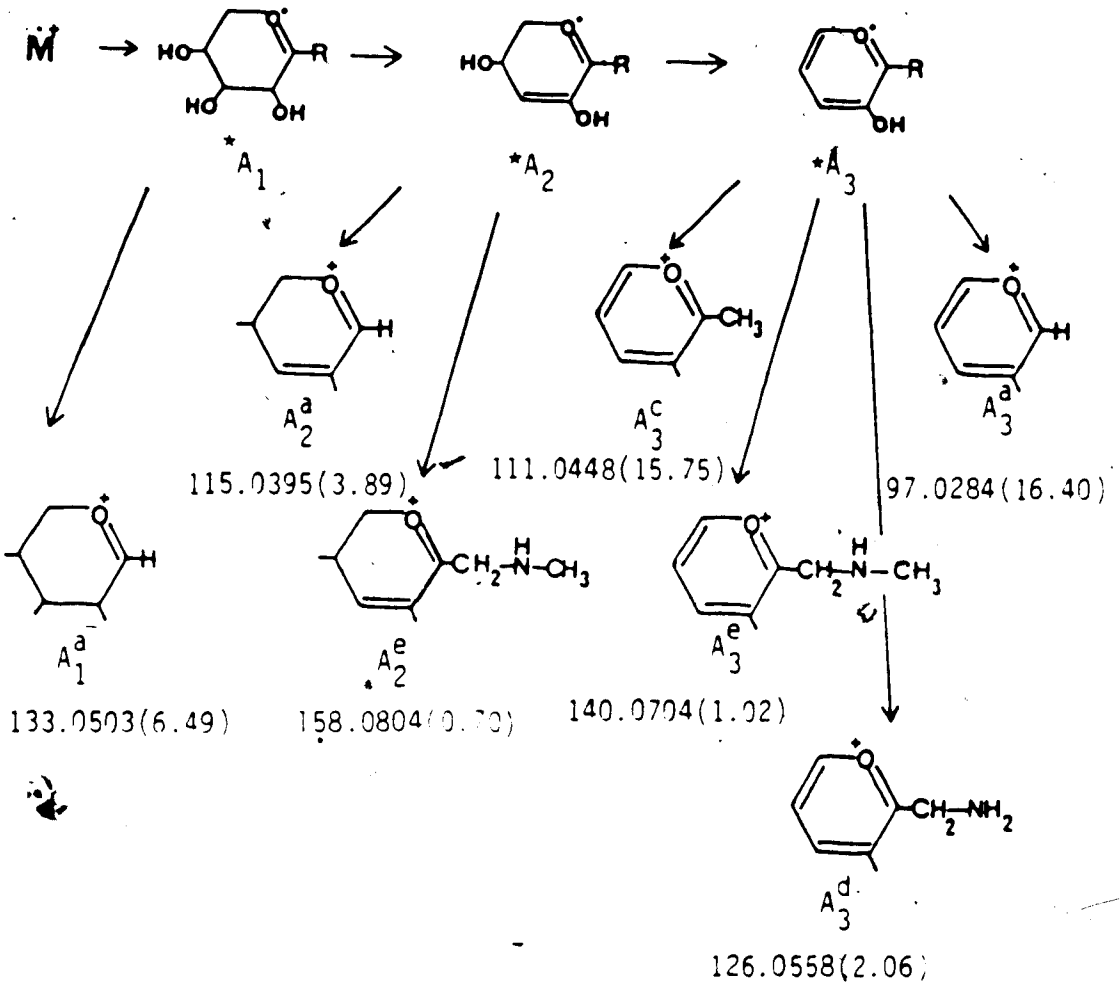
No. of peaks = 130

Mass range = 50.0235-158.0804

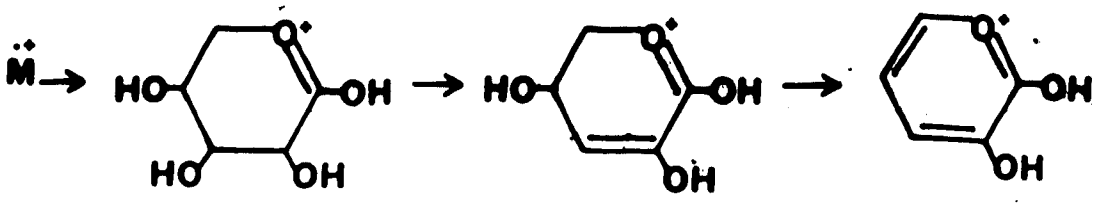
TIC: 49,047 (7,334) (contribution from the base peak).

2.4.5.1 "Sugar fragmentations" of the pyranoid form

• Fragmentations of the A series:



• **Fragmentations of the aA series:**

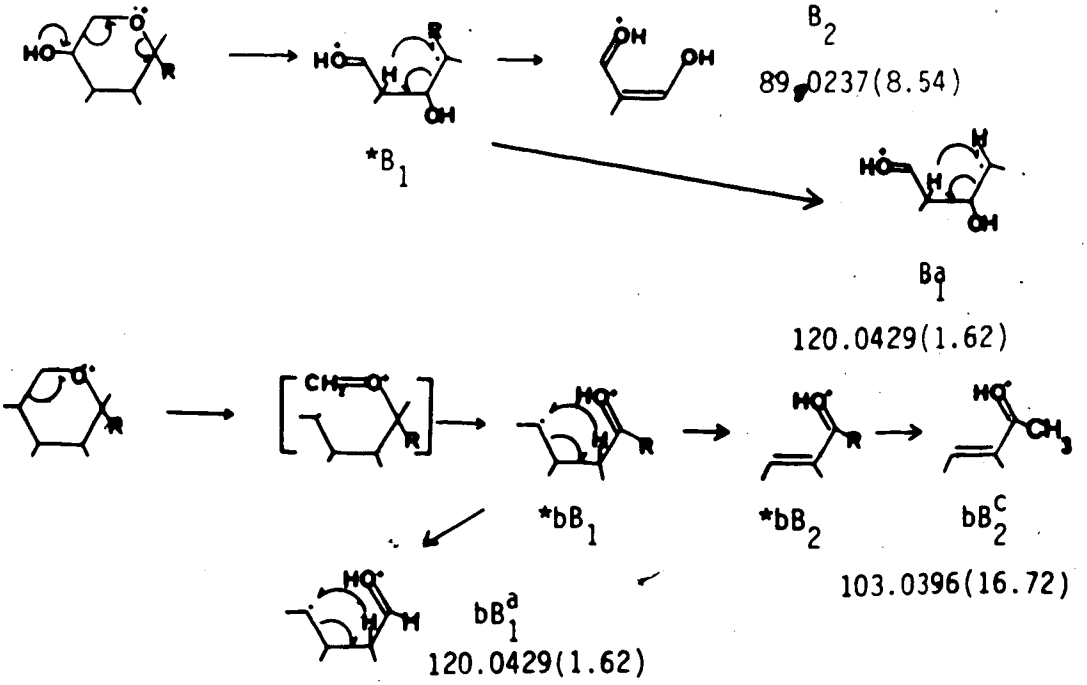


aA_1 aA_2 aA_3

149.0451(1.55) 131.0346(12.41) 113.0241(3.82)

This is the only Amadori product that shows this series since the side chains of the other amino acids can initiate reactions that can stabilize the ion product, whereas in glycine it is easily cleaved because of the lack of functionality.

• **Fragmentations of the B series:**



B_2

89.0237(8.54)

$^*B_1$

Ba_1

120.0429(1.62)

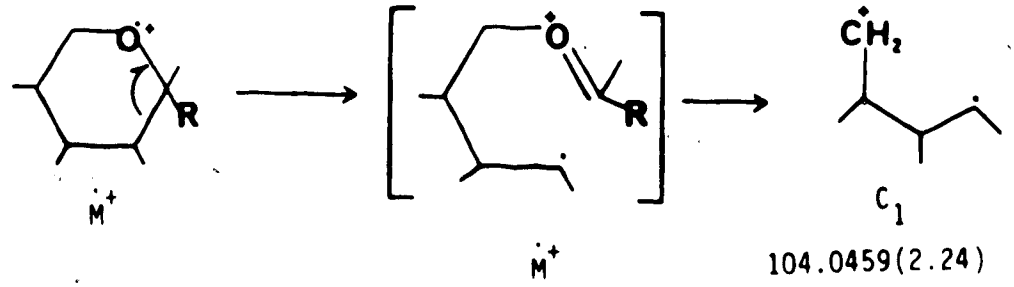
$^*bB_1$ $^*bB_2$ bB_2^c

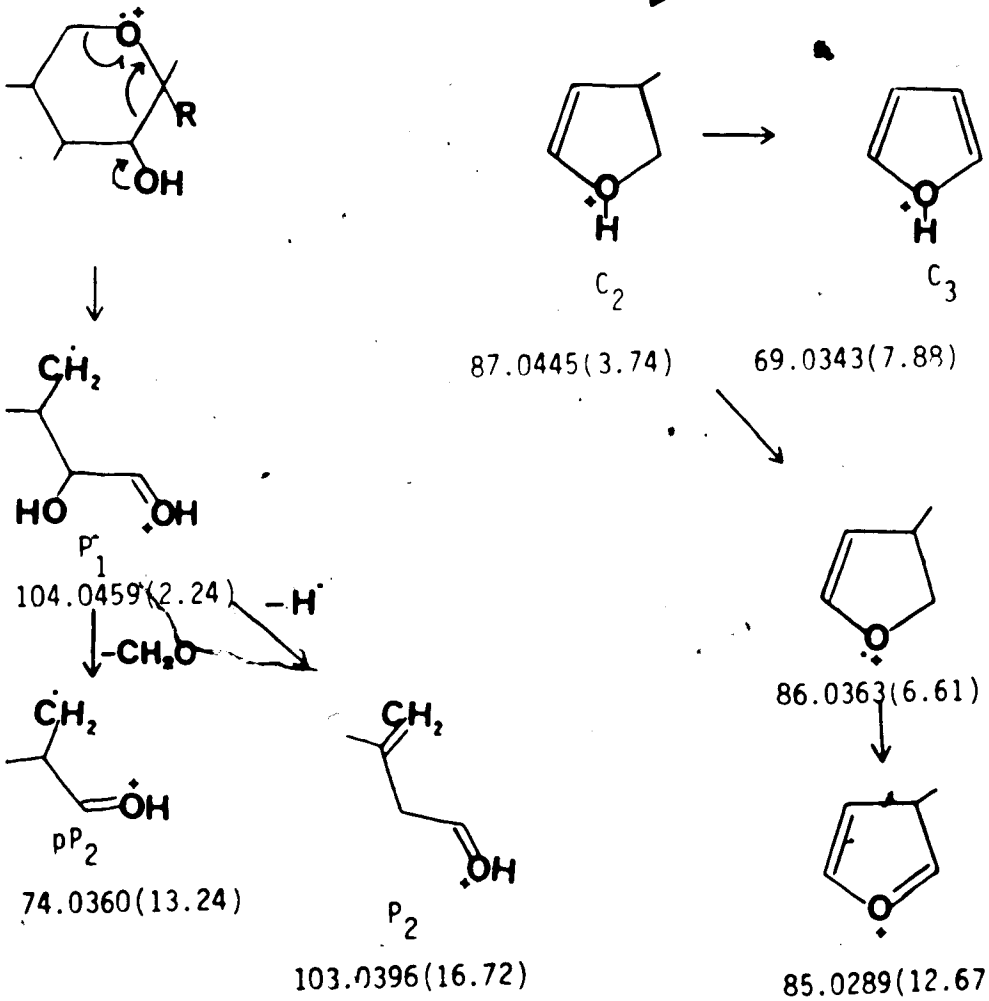
103.0396(16.72)

bB_1^a

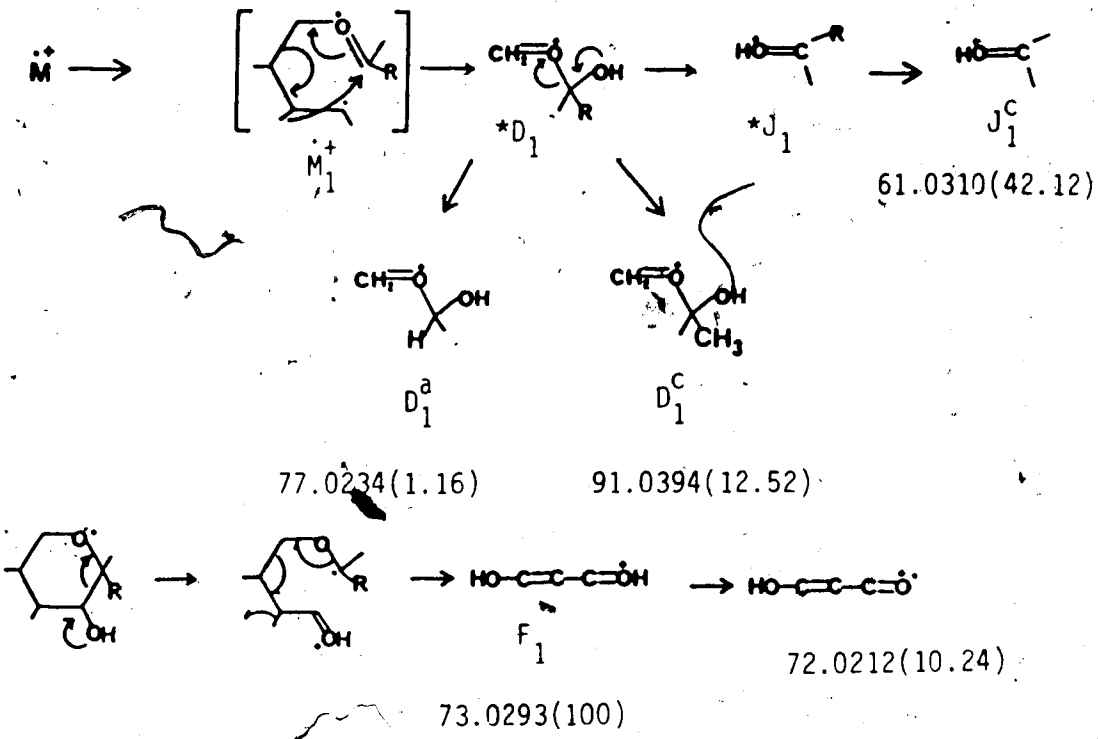
120.0429(1.62)

- **Fragmentations of the C and P series:**

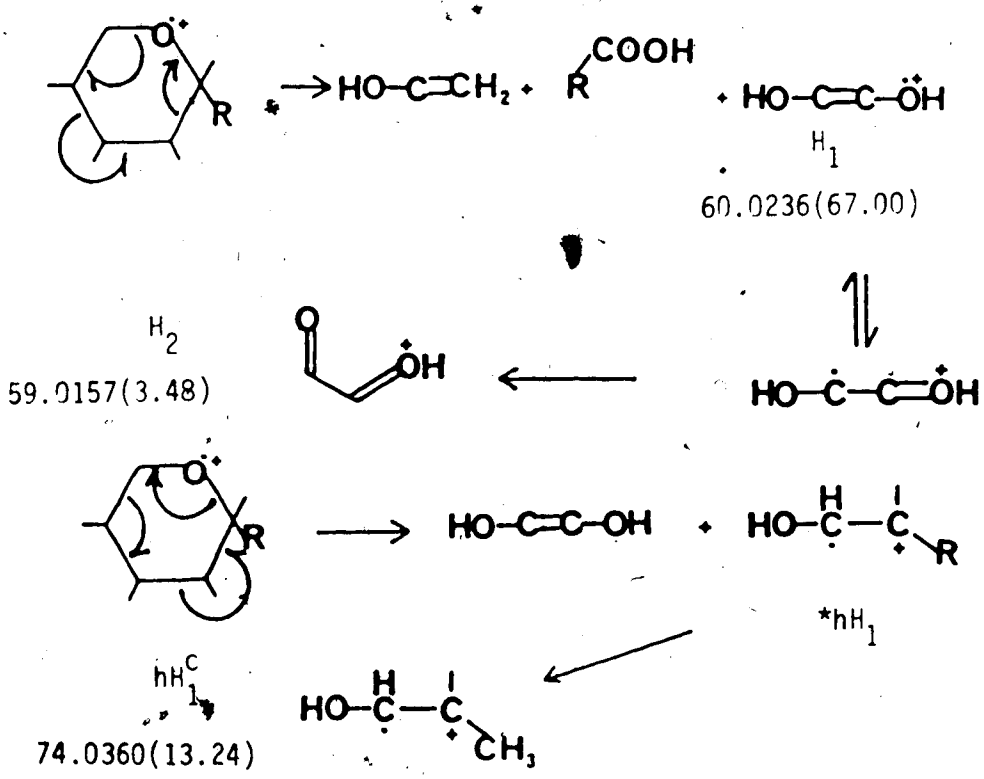




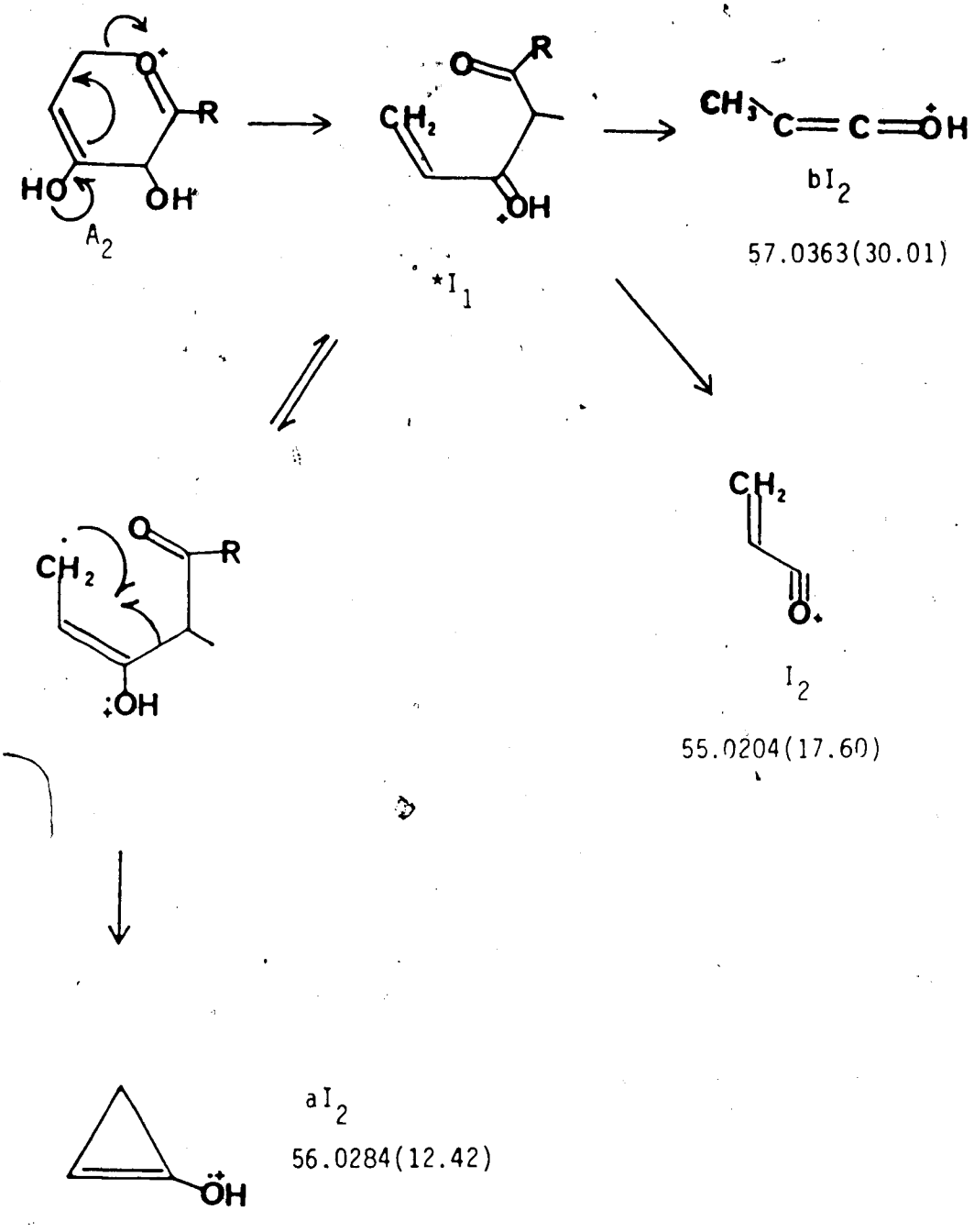
- Fragmentations of the D, E and J series:



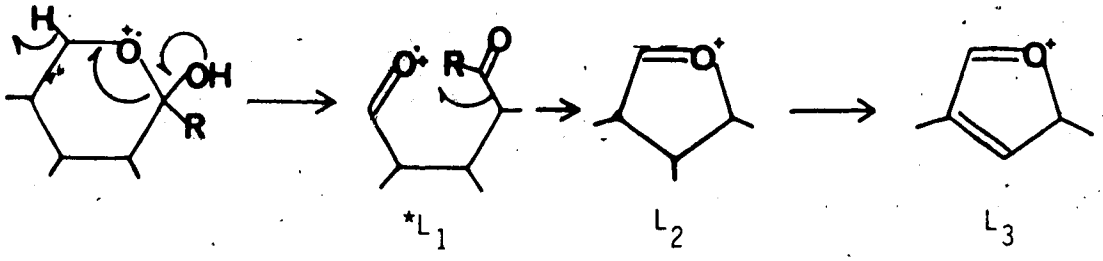
- Fragmentations of the H series:



• Fragmentations of the I series:

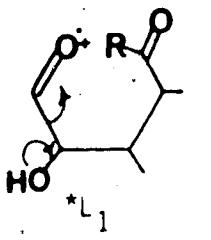


• Fragmentations of the L series:

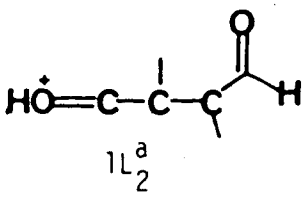


$^{*}L_1$ L_2 L_3

119.0342(9.82) 101:0293(16.42)

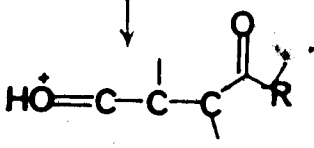


$^{*}L_1$

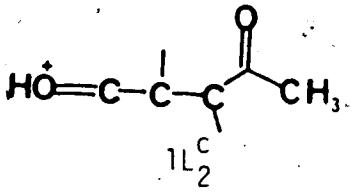


$1L_2^a$

119.0342(9.82)

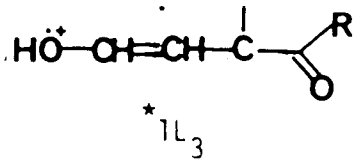


$^{*}1L_2$

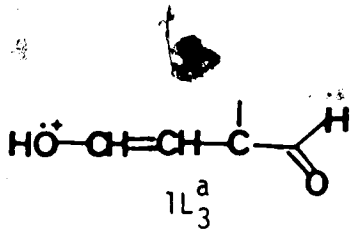


$1L_2^c$

133.0503(6.49)

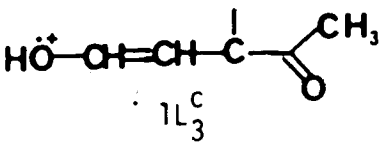


$^{*}1L_3$



$1L_3^a$

102.0315(7.91)

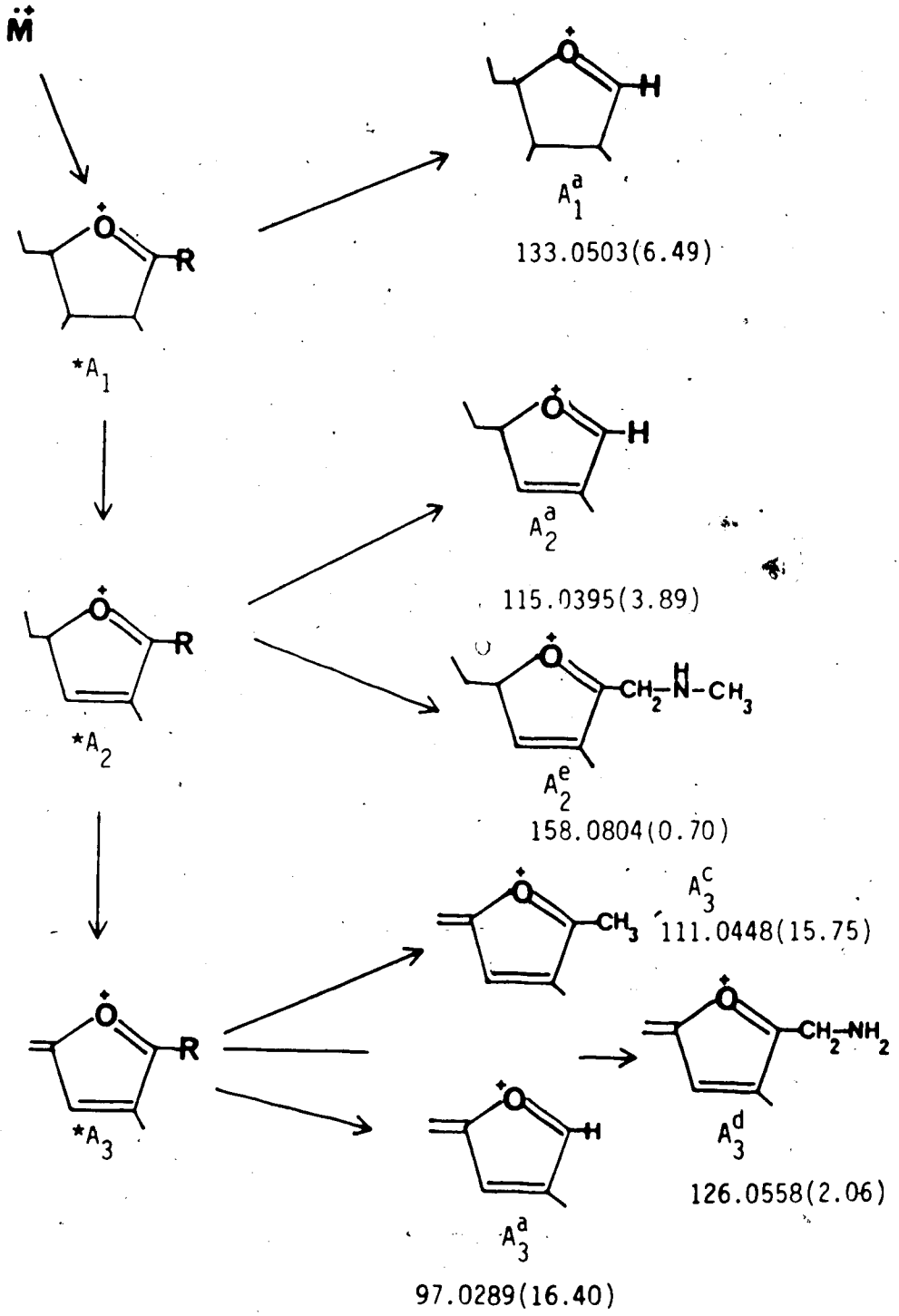


$1L_3^c$

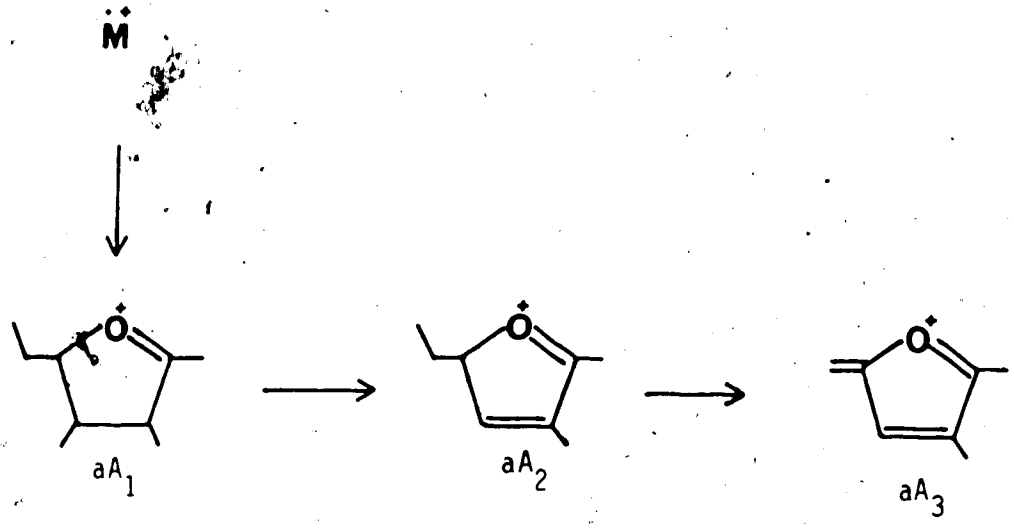
116.0470(1.68)

2.4.5.2 Fragmentations of the furanoid form

• Fragmentations of the A series:

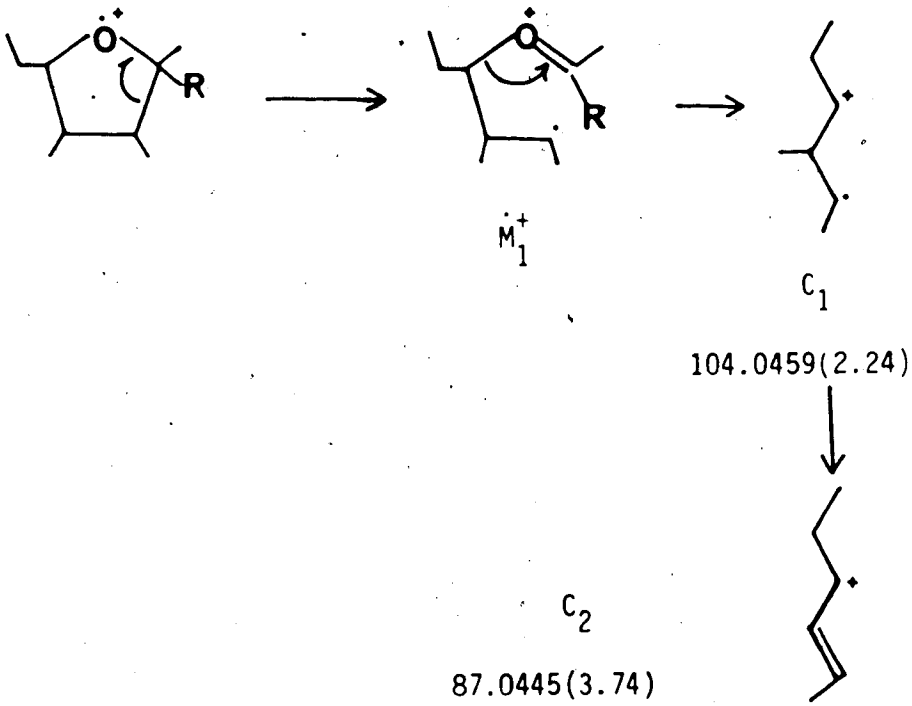


- Fragmentations of the aA series:



149.0451(1.55) 131.0346(12.41) 113.0241(3.82)

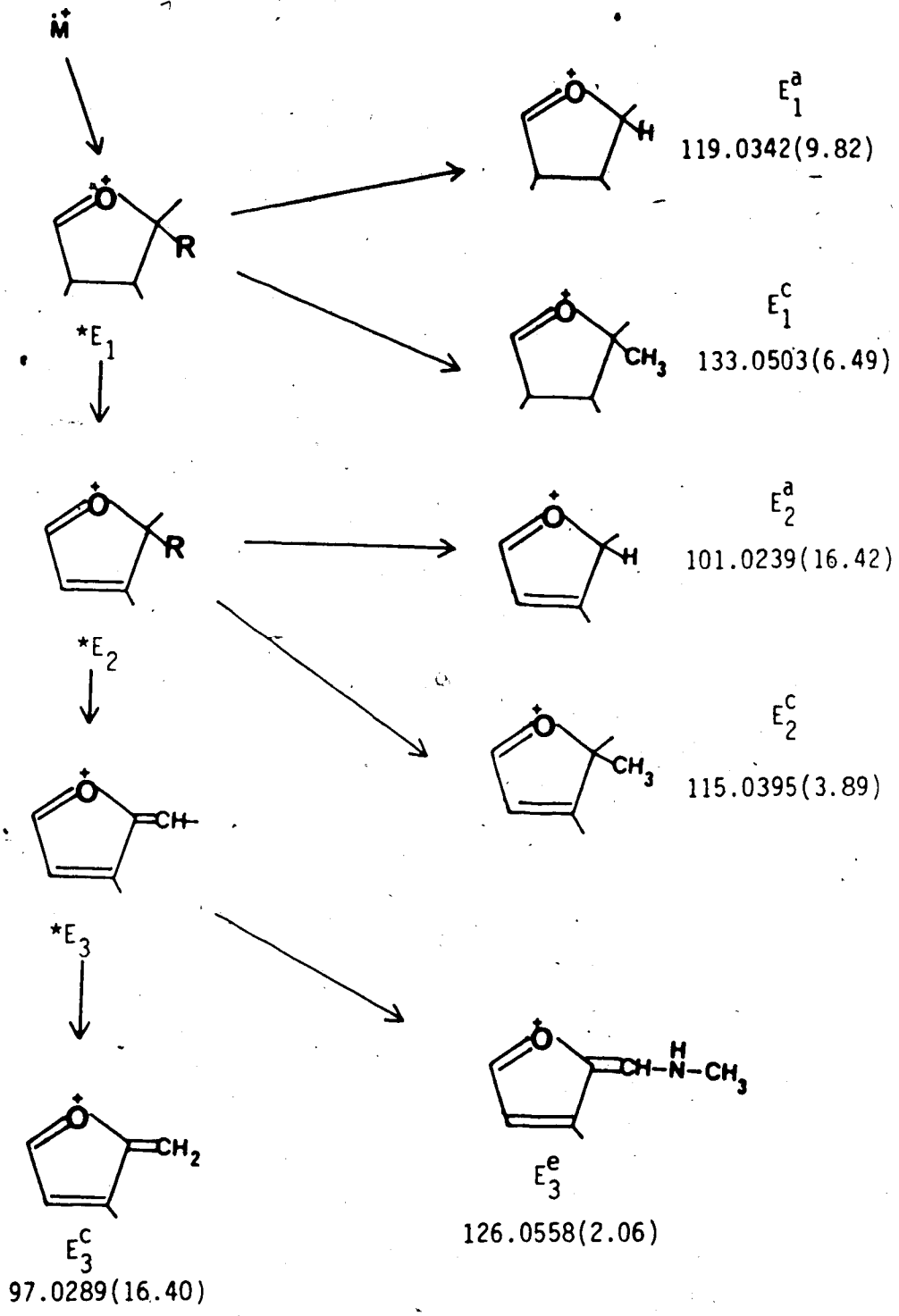
- Fragmentations of the C series:



104.0459(2.24)

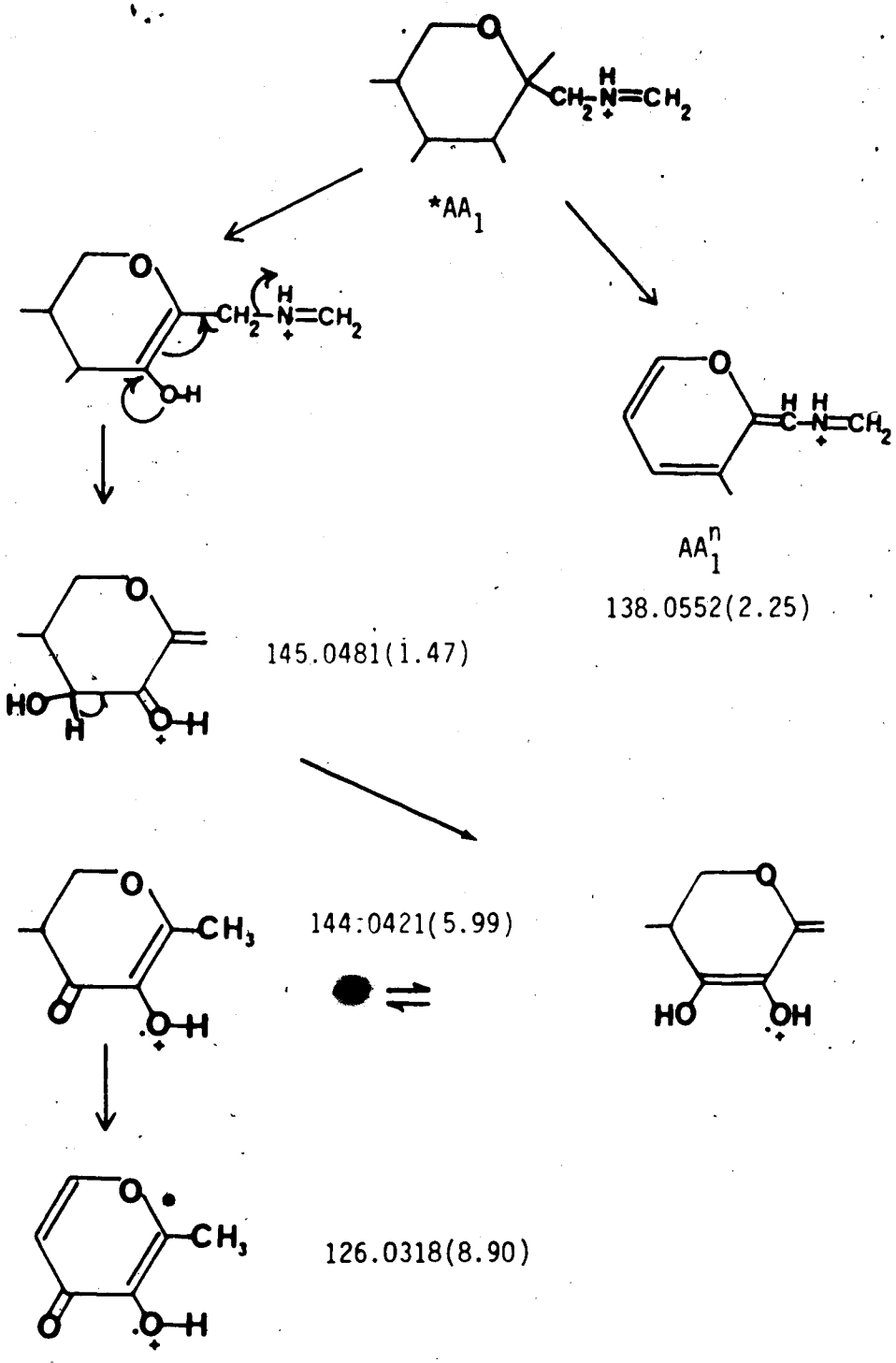
87.0445(3.74)

- **Fragmentations of the E series:**

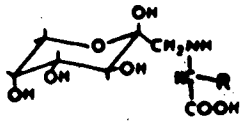


2.4.5.3 "Amino acid" fragmentations

• Fragmentations of the AA series:



2.4.6 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose

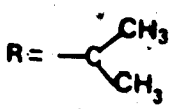


T = 125°C

No. of peaks = 253

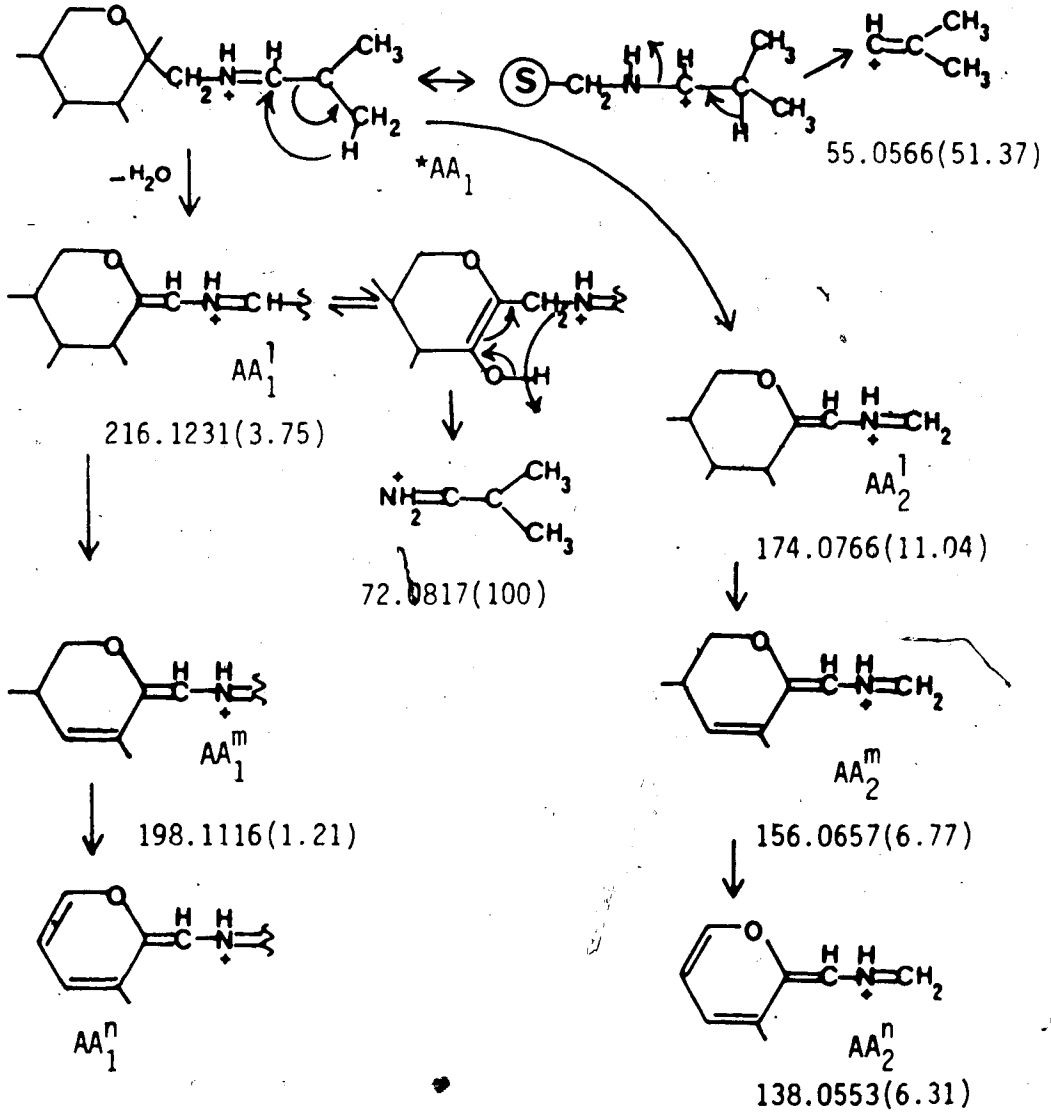
Mass range = 51.0237-262.1272

TIC: 66,638 (7,191)

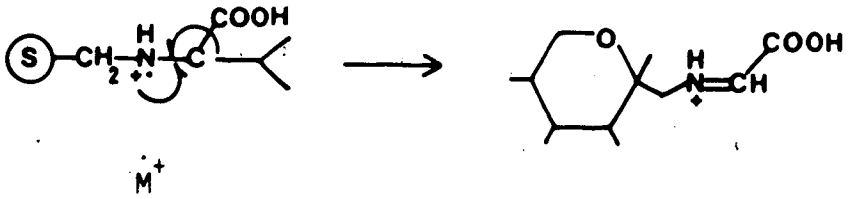


2.4.6.1 "Amino acid" fragmentations

• Fragmentations of the AA series:

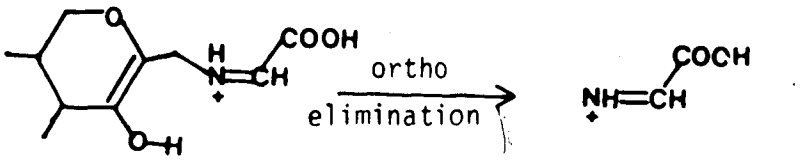


• Fragmentations of the BB series:



M^{+}

$^{*}BB_1$



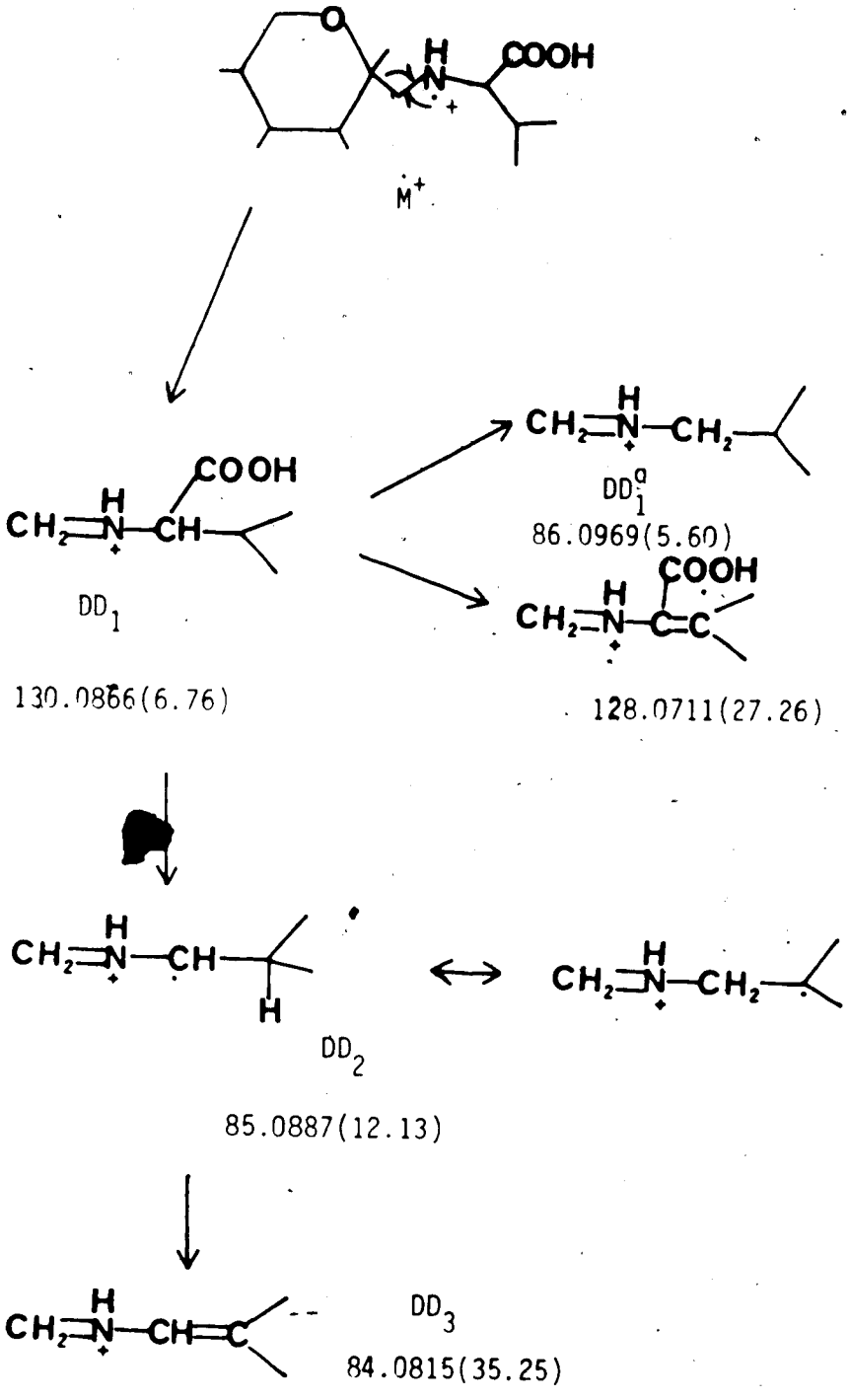
74.0246(28.13)

BB_1

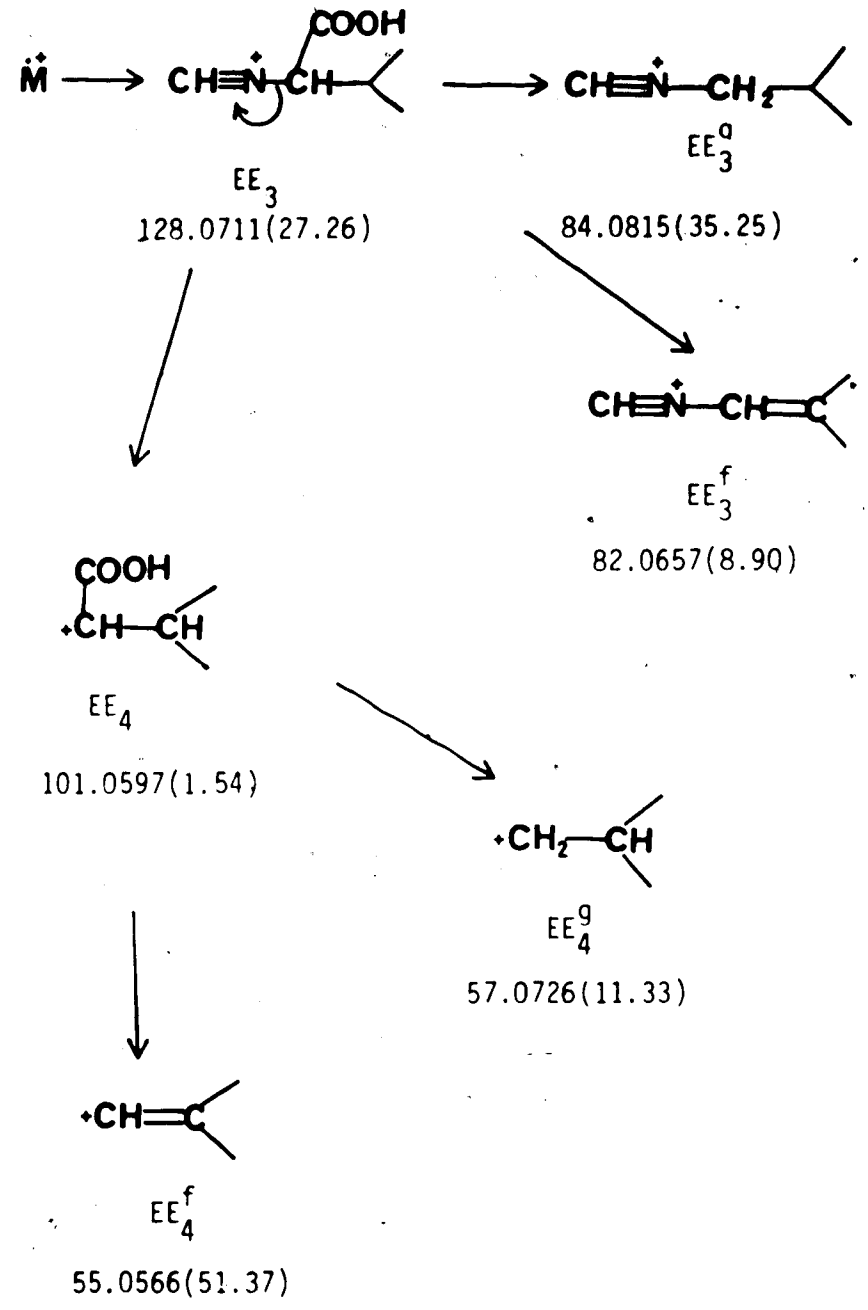
• Fragmentations of the CC series:

No CC series.

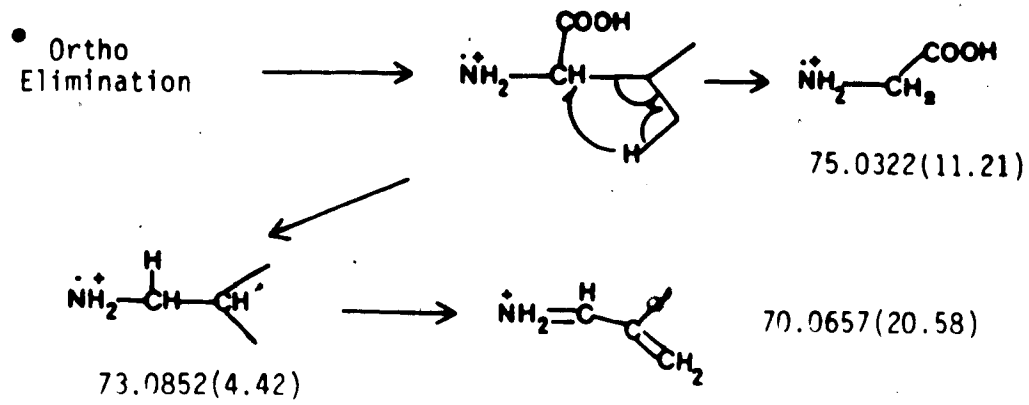
• Fragmentations of the DD series:

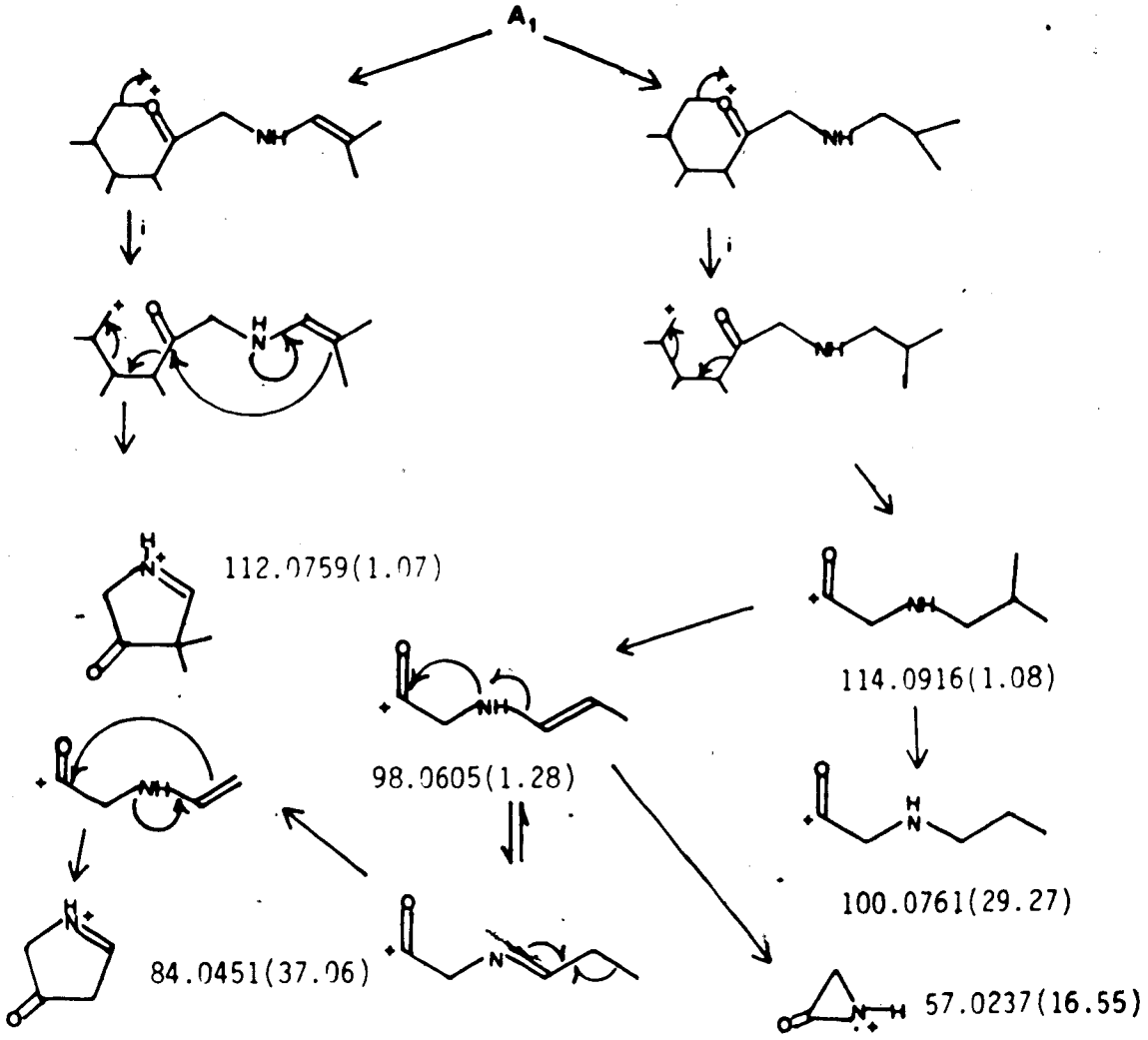


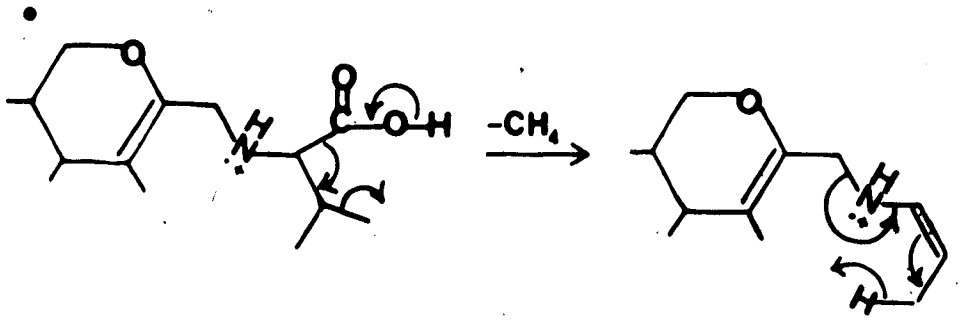
• Fragmentations of the EE series:

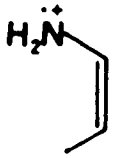


2.4.6.2 Specific fragmentations

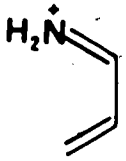




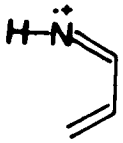




57 .0601(5.28)

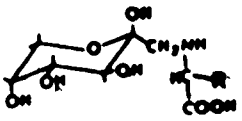


56.0532(12.81)



54.0443(1.07)

2.4.7 1-[(1'-carboxy-2'-hydroxyethyl)amino]-1-deoxy-D-fructose



T = 300°C

No. of peaks = 93

Mass range = 51.0233-155.0585

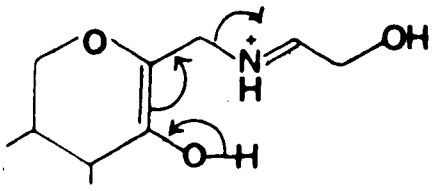
TIC: 75,008 (41,346)

R = $-CH_2OH$

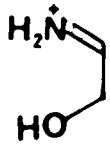
2.4.7.1 "Amino acid" fragmentations

• Fragmentations of the AA series:

AA_1



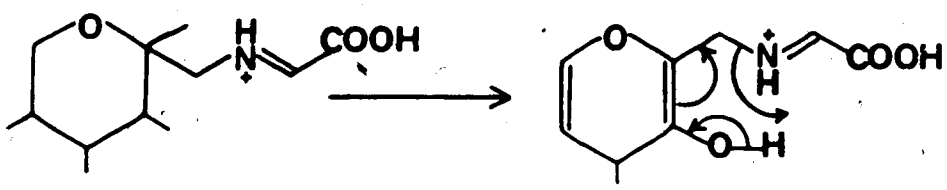
$^*AA_1^1$



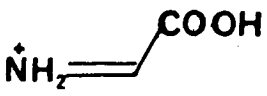
AA_2^1

60.0466(3.92)

• Fragmentations of the BB series:



$*BB_1$ $*BB_1^m$

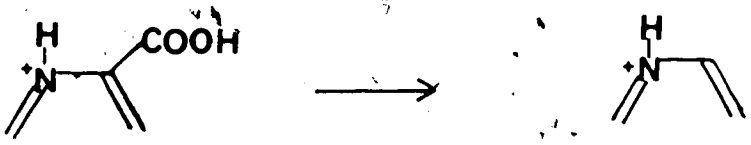


74.0254(100)

• Fragmentations of the CC series:

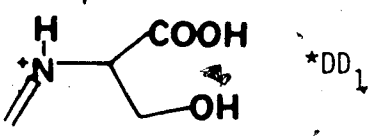
No CC series.

• Fragmentations of the DD series:



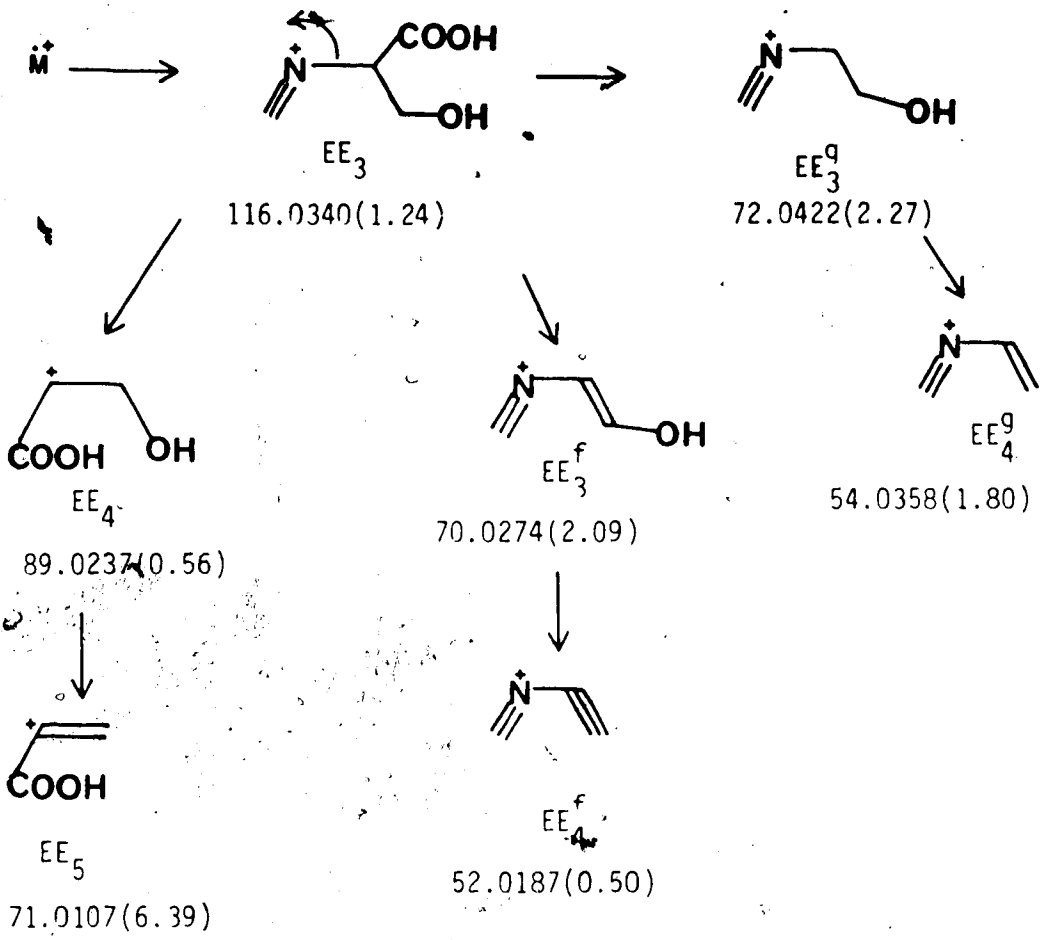
DD_2 DD_2^a

100.0394(1.09) 56.0518(3.65)

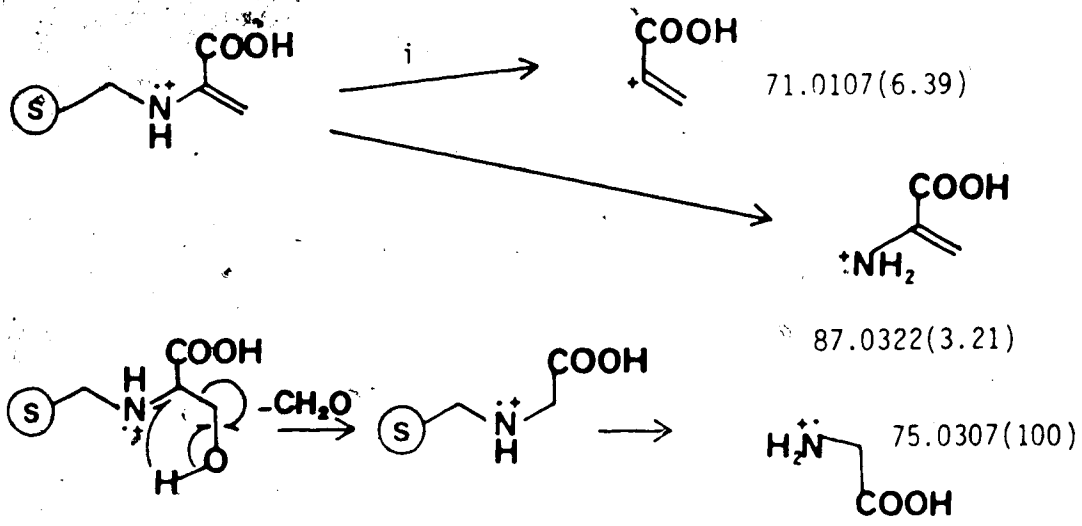


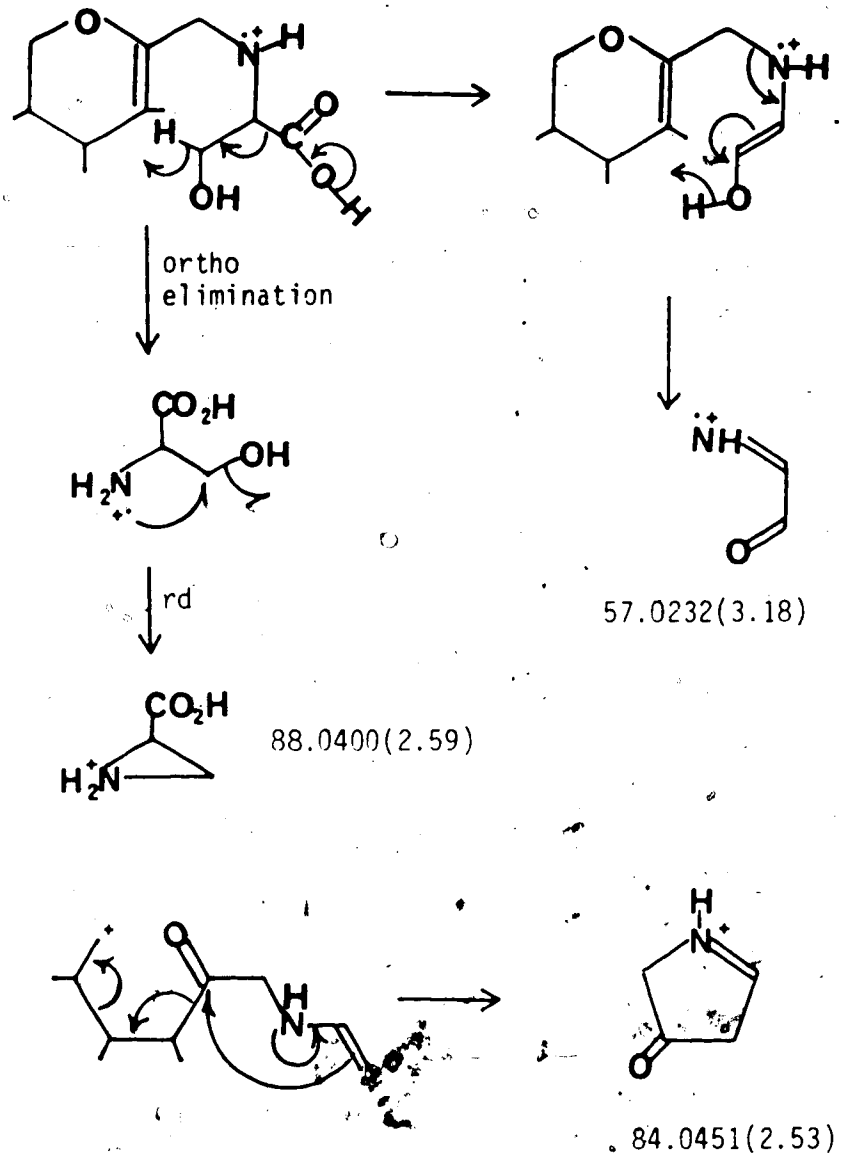
$*DD_1$

• Fragmentations of the EE series:



2.4.7.2 Specific fragmentations




ortho
elimination
rd
57.0232(3.18)
88.0400(2.59)
84.0451(2.53)

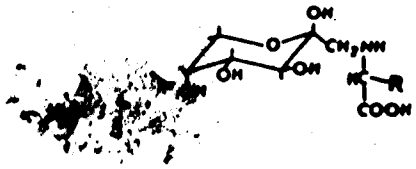
2.4.8 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose

T = 220°C

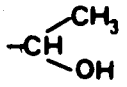
No. of peaks = 177

Mass range = 51.0235-201.0768

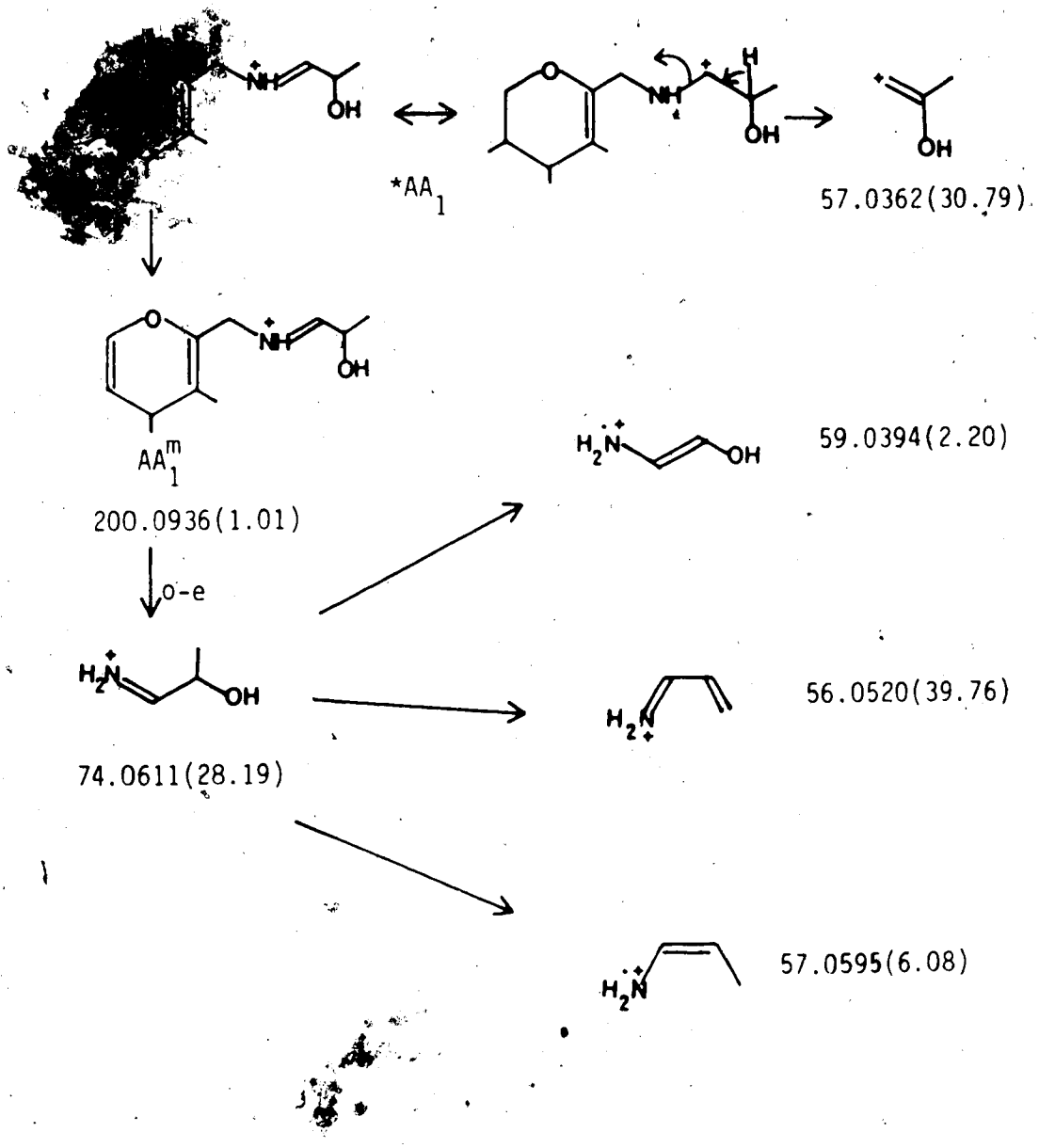
TIC: 54,728



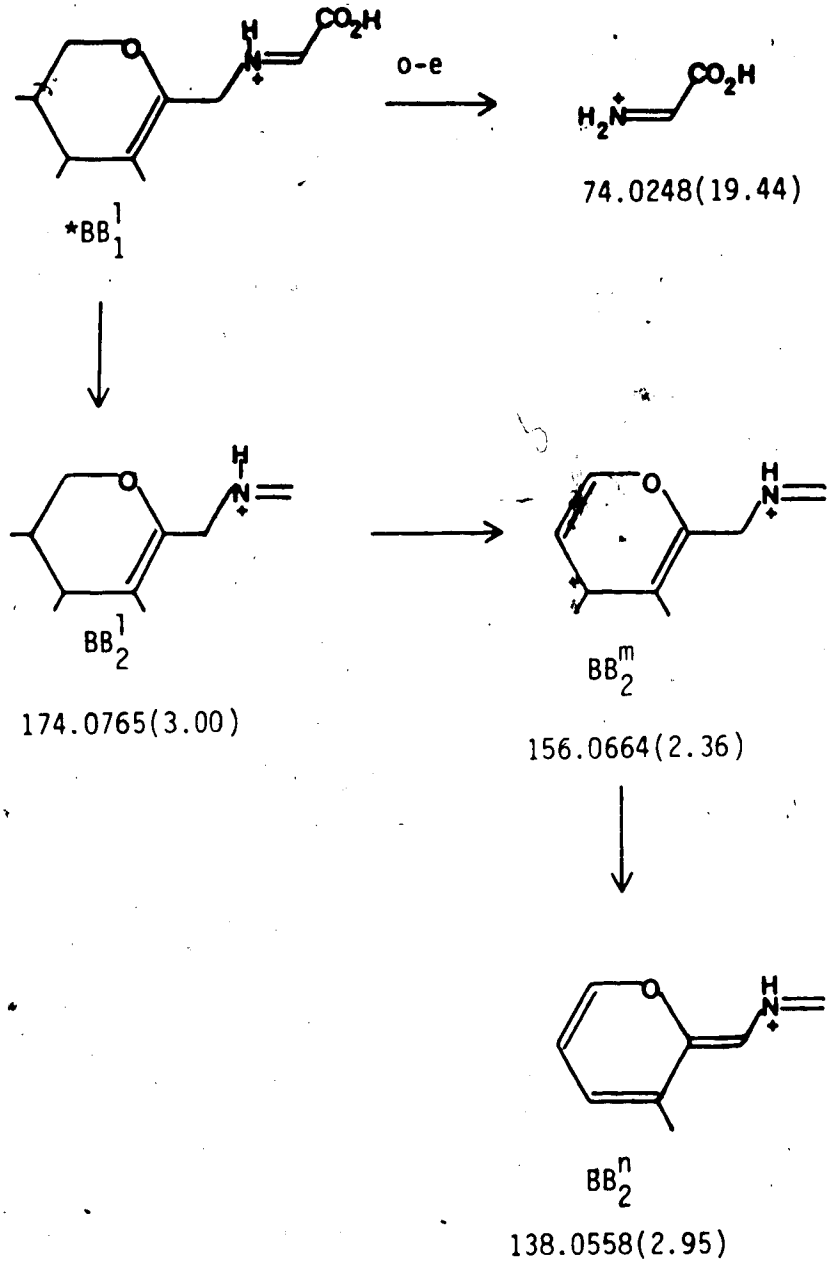
2.4.8.1 "Amino acid" fragmentations

R= 

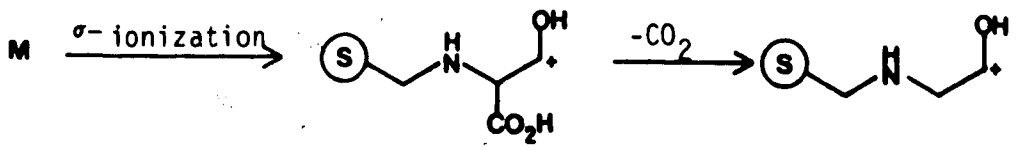
• Fragmentations of the AA series:



• **Fragmentations of the BB series:**

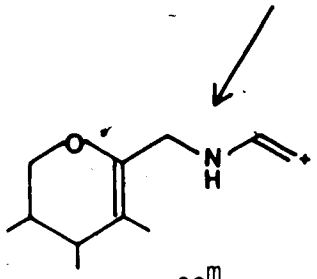


• **Fragmentations of the CC series:**



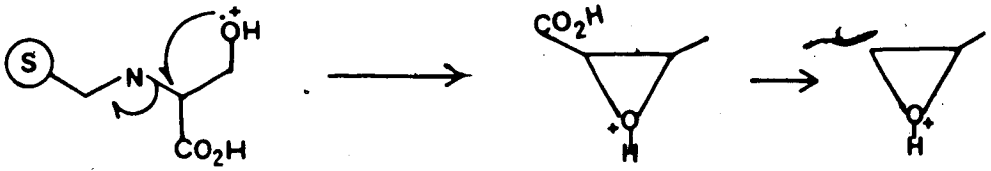
$*CC_1$

$*CC_2$



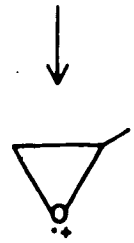
CC_2^m

186.0744(1.28)



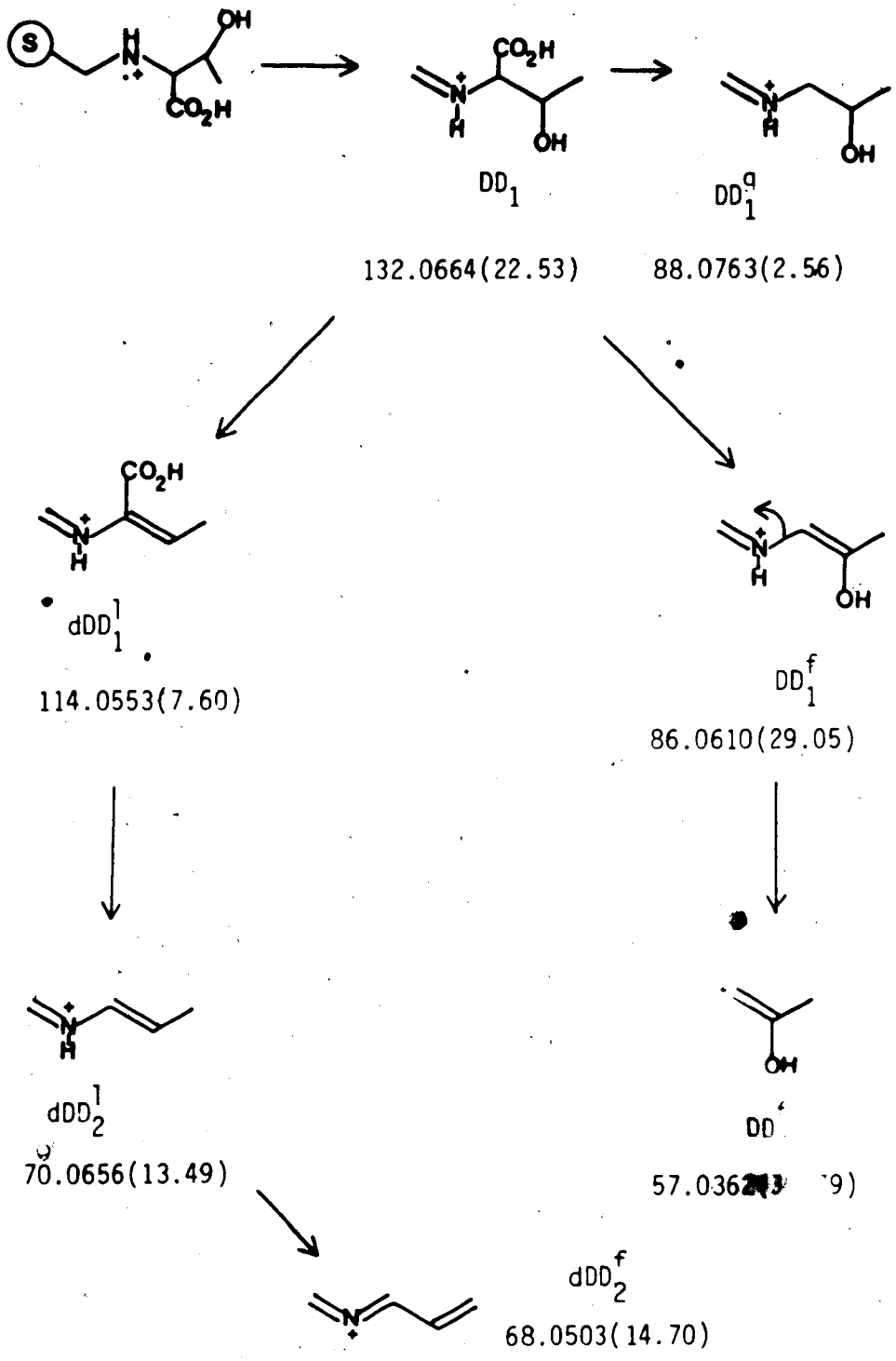
103.0396(3.68)

59.0518(3.57)

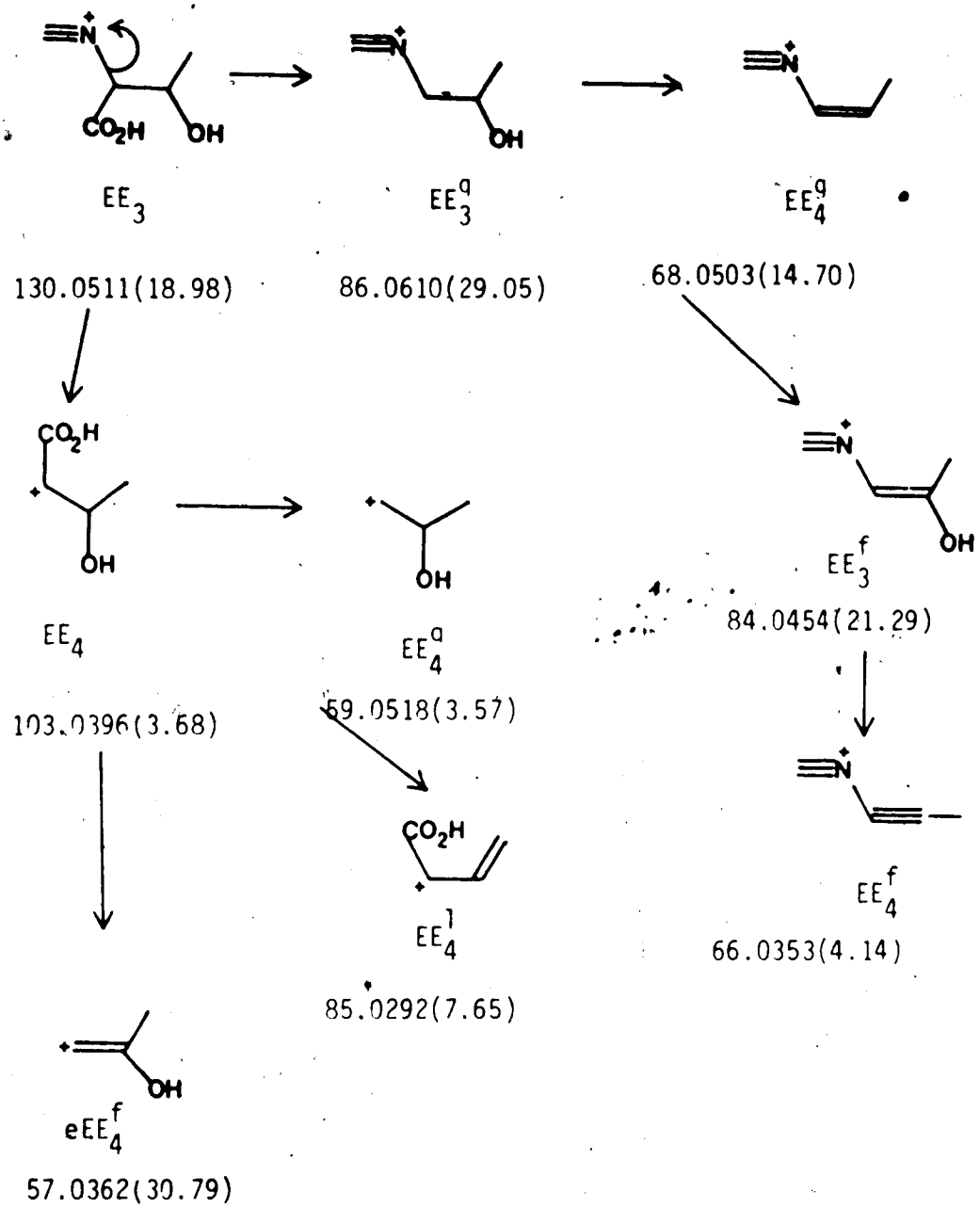


58.0437(14.19)

• **Fragmentations of the DD series:**

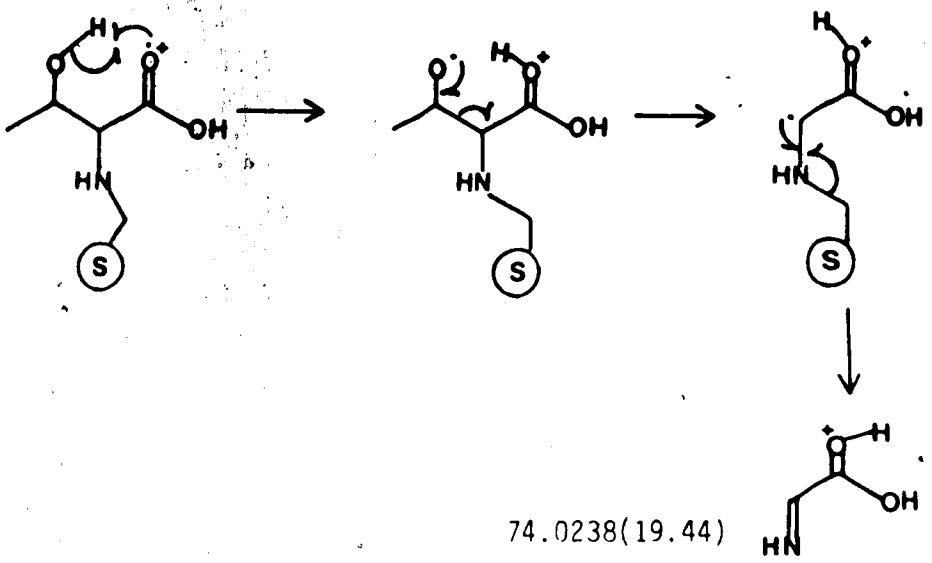


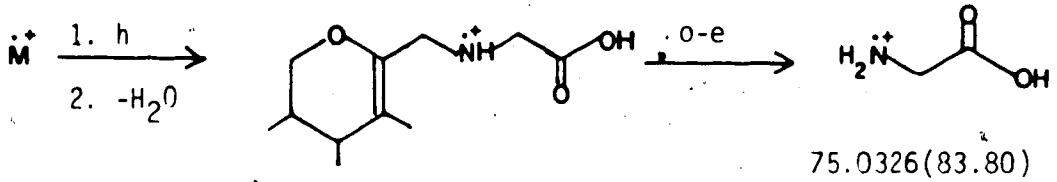
• **Fragmentations of the EE series:**

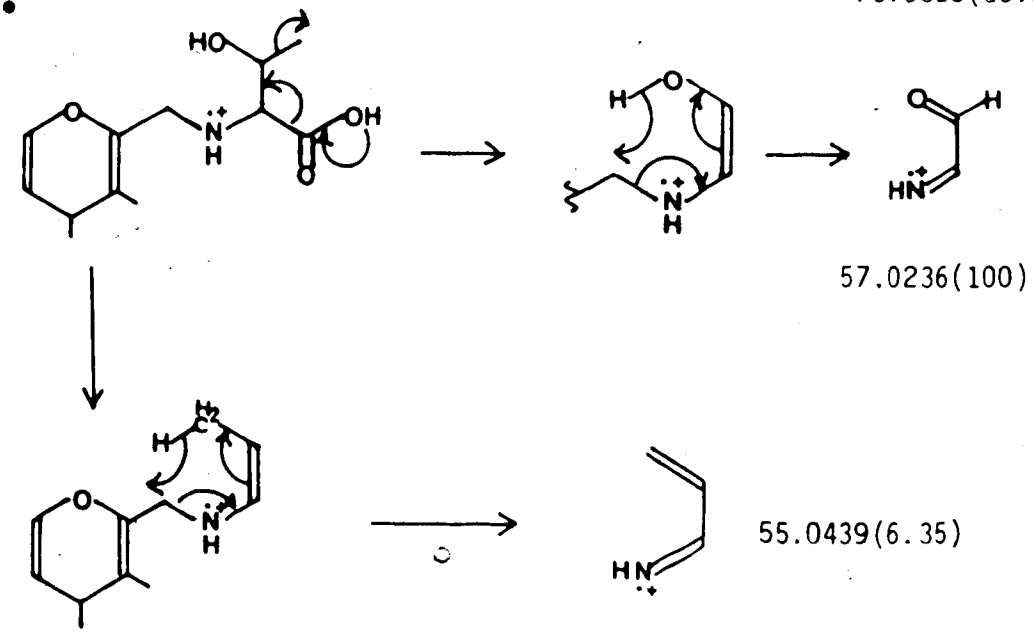


2.4.8.2 Specific fragmentations

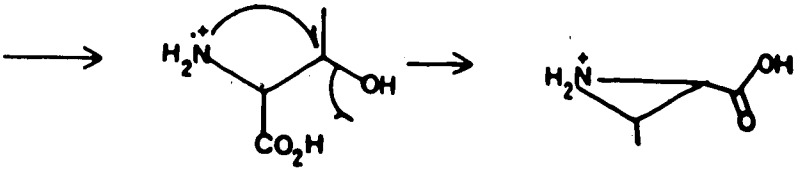
- McLafferty Rearrangment:





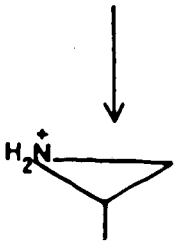


Ortho
Elimination

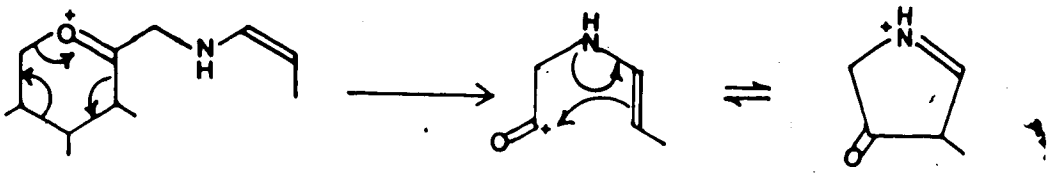


102.0556(5.33)

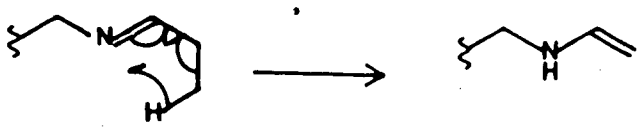
56.0520(39.76)

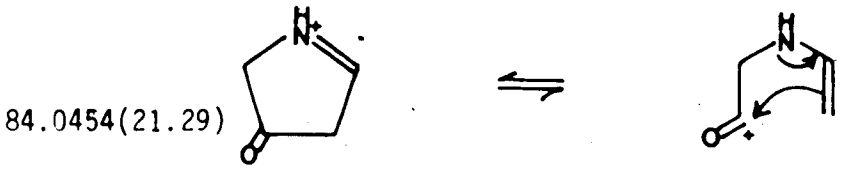


58.0675(7.56)

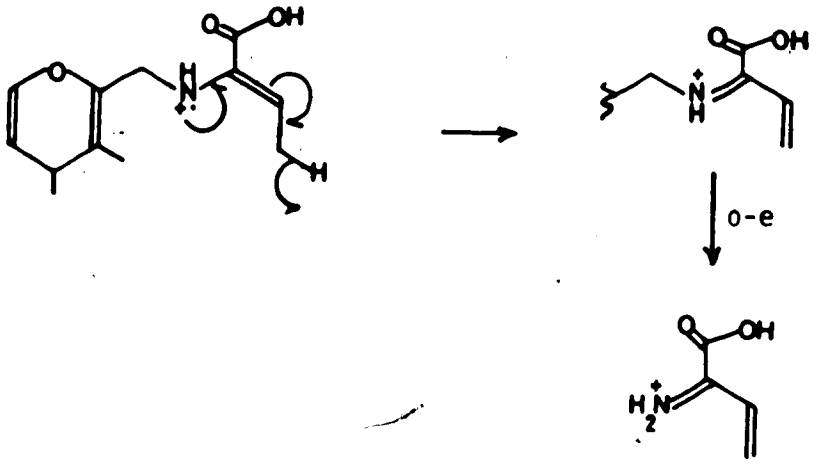


98.0603(9.19)



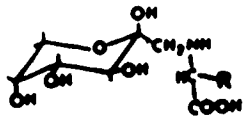


84.0454(21.29)



88.0400(18.73)

2.4.9 1-{[(1'-carboxy-3'-(methylthio)propyl]amino}-1-deoxy-D-fructose



T = 220°C

No. of peaks = 199

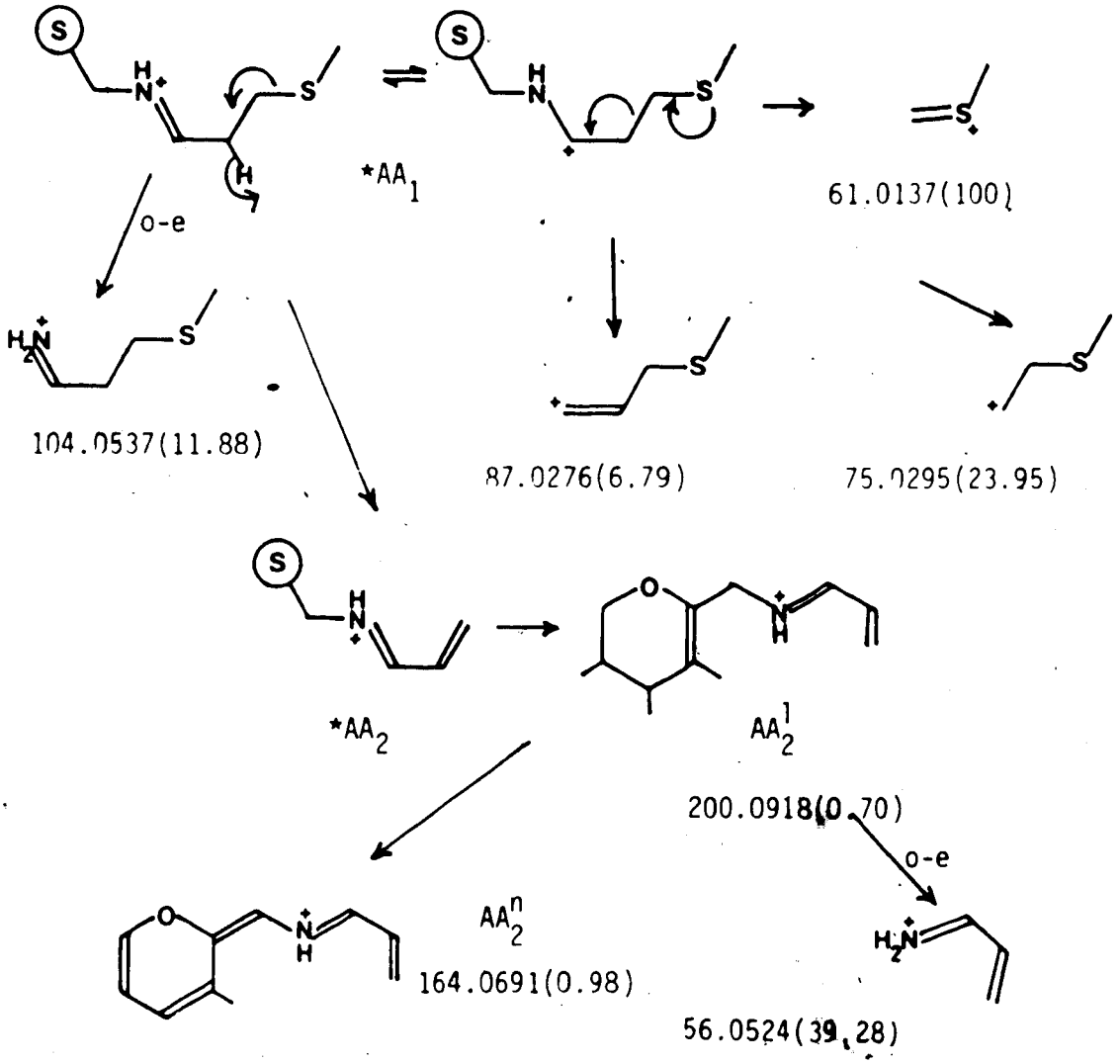
Mass range = 51.0234-303.1167

TIC: 66,580 (8,907)

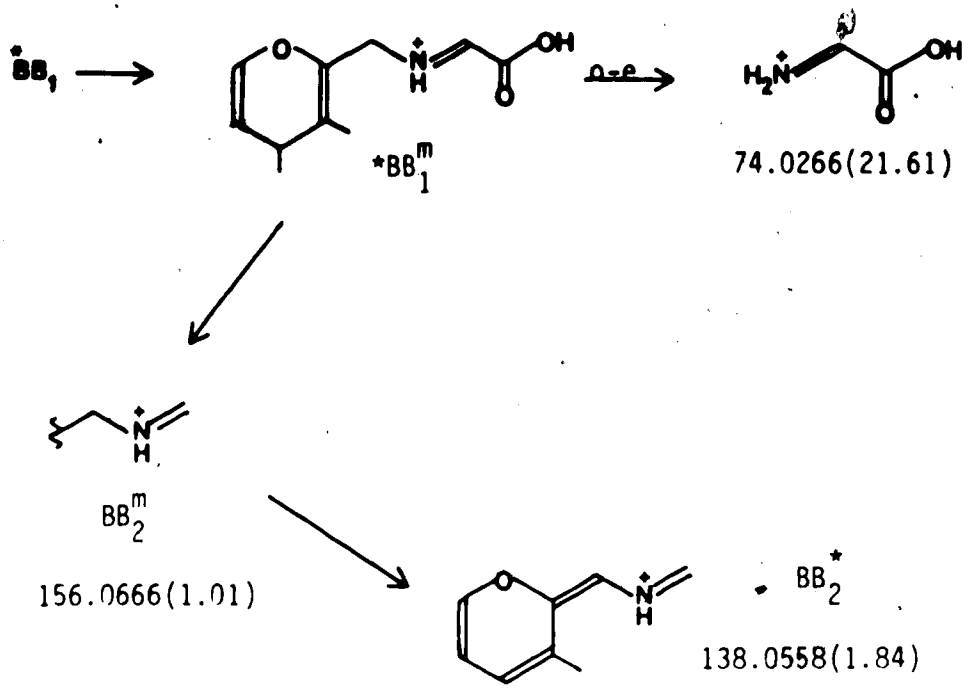
R = $-CH_2CH_2S\,CH_3$

2.4.9.1 "Amino acid" fragmentations

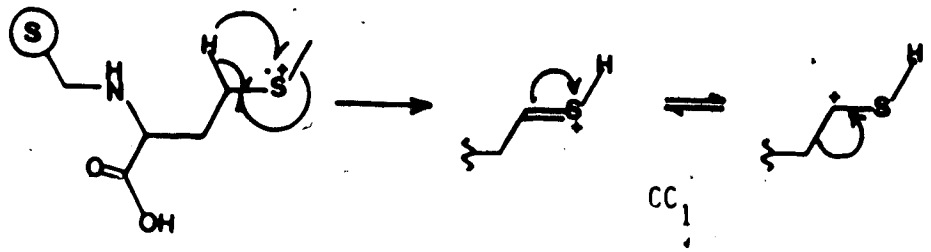
• Fragmentations of the AA series:



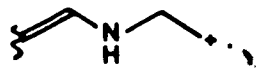
• **Fragmentations of the BB series:**



• **Fragmentations of the CC series:**

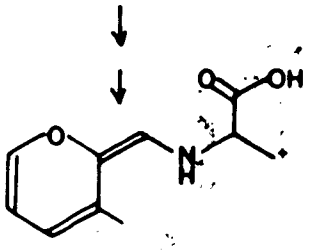


CC_1



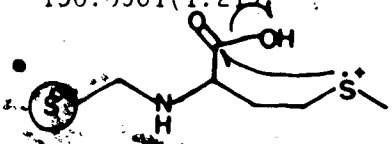
CC_3^n

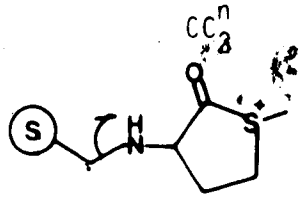
152.0691(0.99)



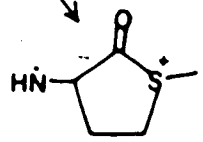
cCC_3^n

150.0561(1.21)

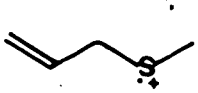




CC_2^n

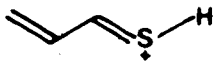


131.0406(21.20)

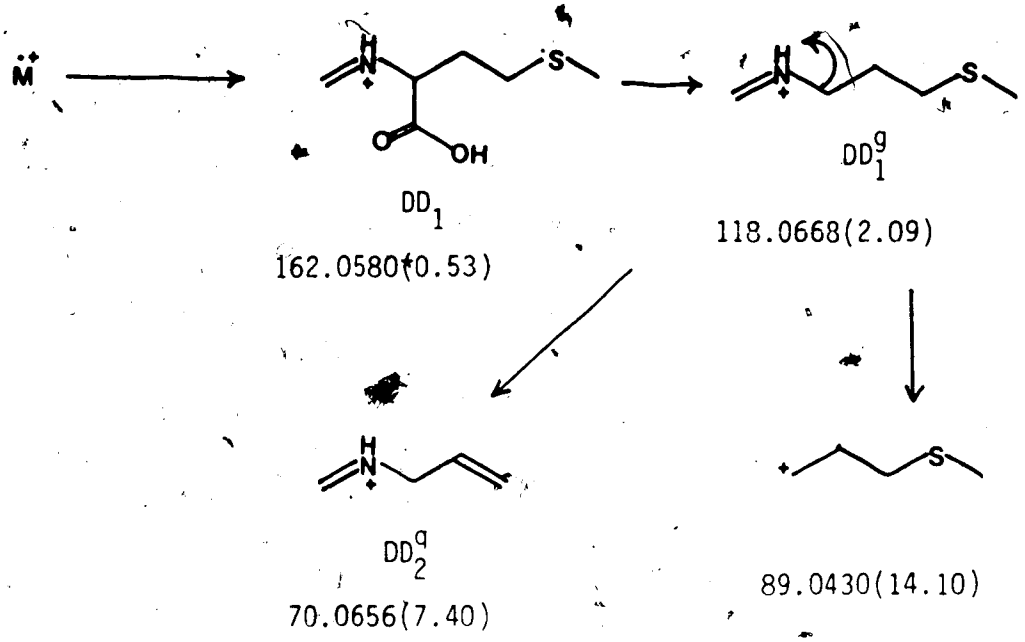


88.0317(10.13)

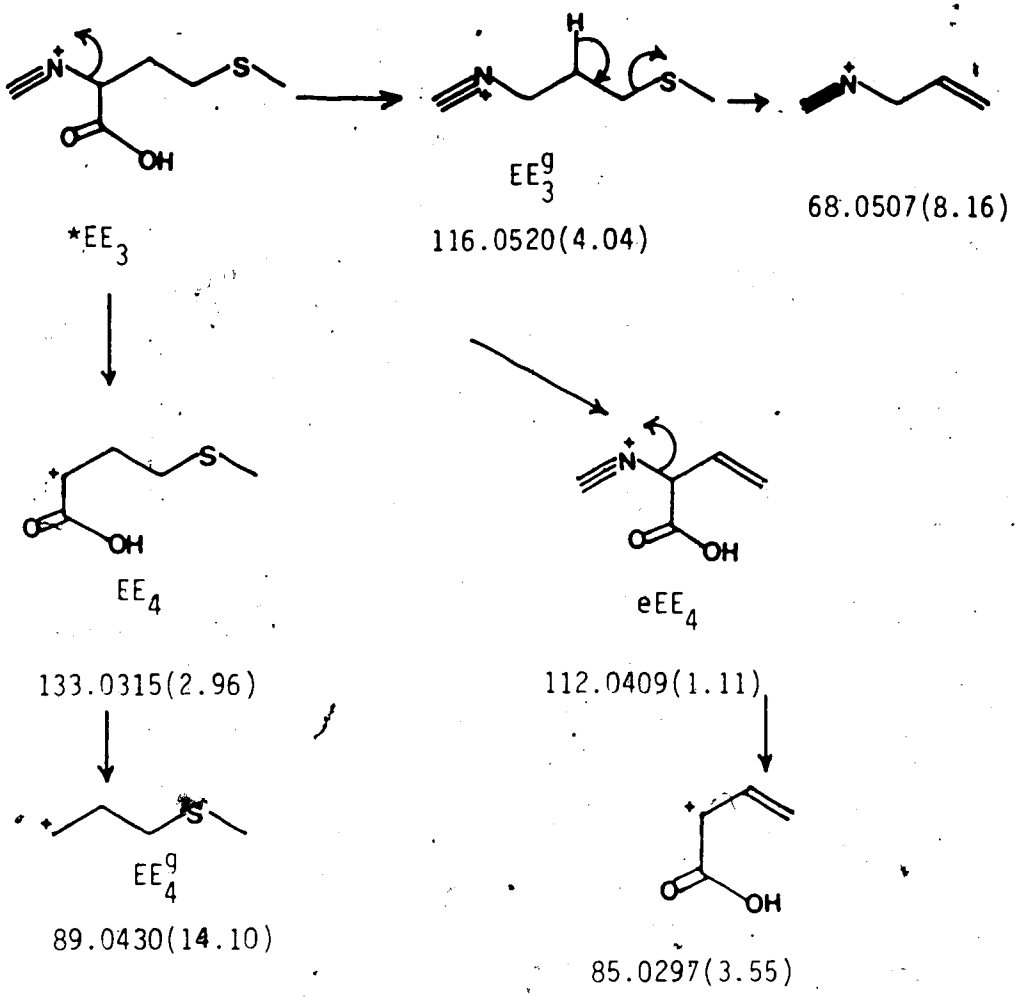
73.0122(2.30)



• Fragmentations of the DD series:

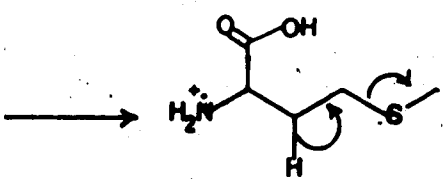


• Fragmentations of the EE series:

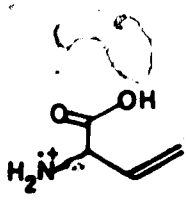


2.4.9.2 Specific fragmentations

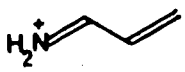
Ortho Elimination



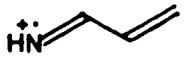
149.0524(9.33)



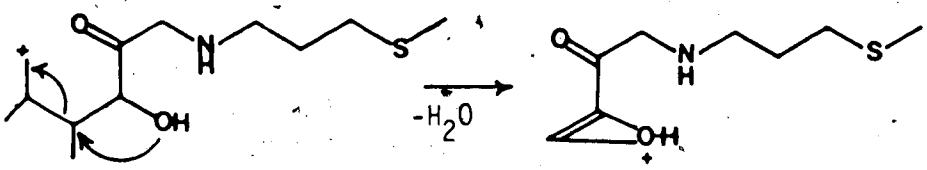
101.0482(14.79)



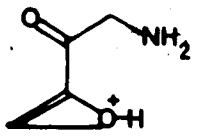
56.0524(39.28)



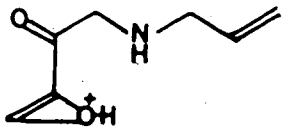
55.0443(14.05)



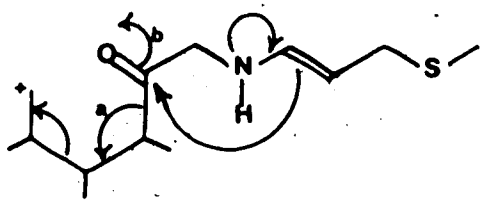
188.0760(9.13)

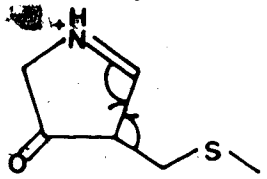


100.0404(4.86)

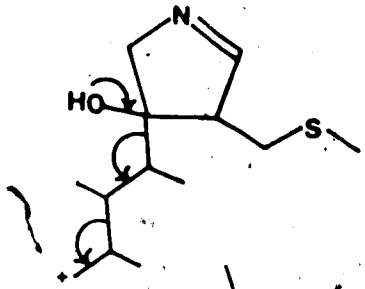


140.0715(2.26)

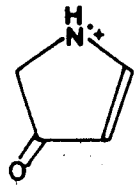




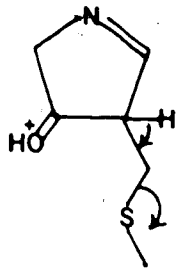
144.0436(3.30)



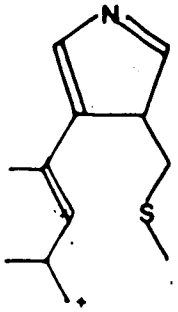
$-2H_2O$



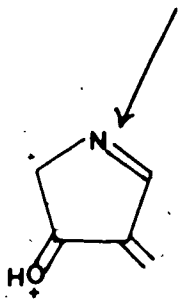
83.0384(10.18)



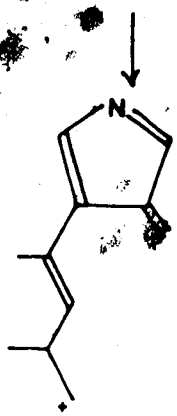
144.0436(3.30)



212.0713(0.70)



96.0452(2.75)



164.0691(0.98)

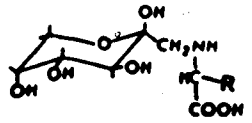
2.4.10 1-[(1'-carboxy-3'-methylcarboxypropyl)amino]-1-deoxy-D-fructose

T = 200°C

No. of peaks = 40

Mass range = 52.0084-144.0419

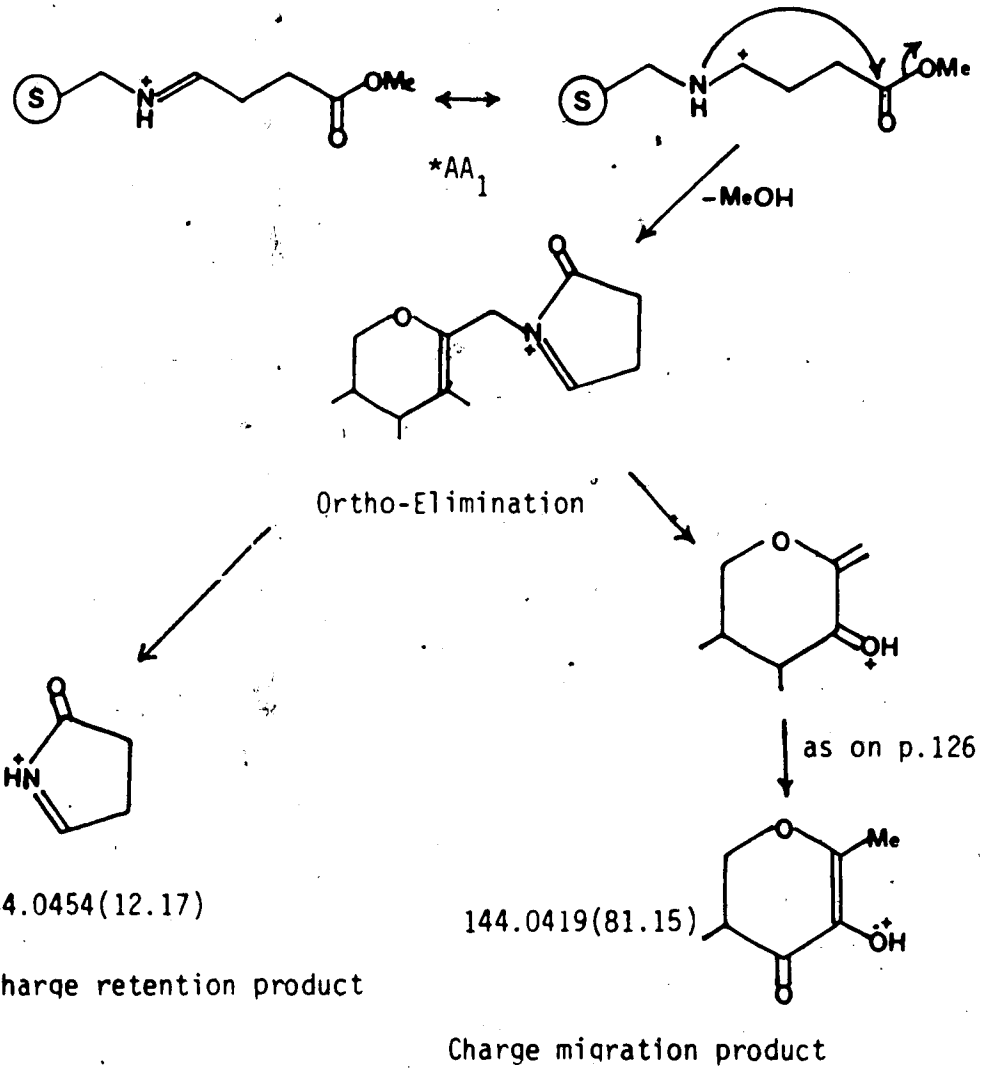
TIC: 4,287 (419)



R=CH_2CH_2COOMe

2.4.10.1 "Amino acid" fragmentations

• Fragmentations of the AA series:



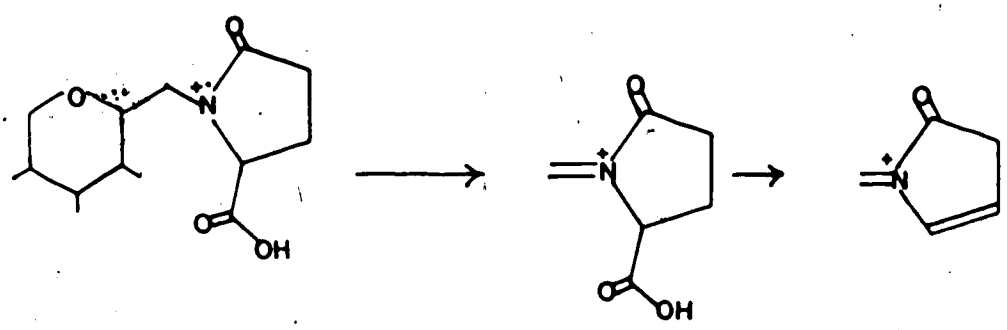
- Fragmentations of the BB series:

  No BB series.

- Fragmentations of the CC series:

  No CC series.

- Fragmentations of the DD series:



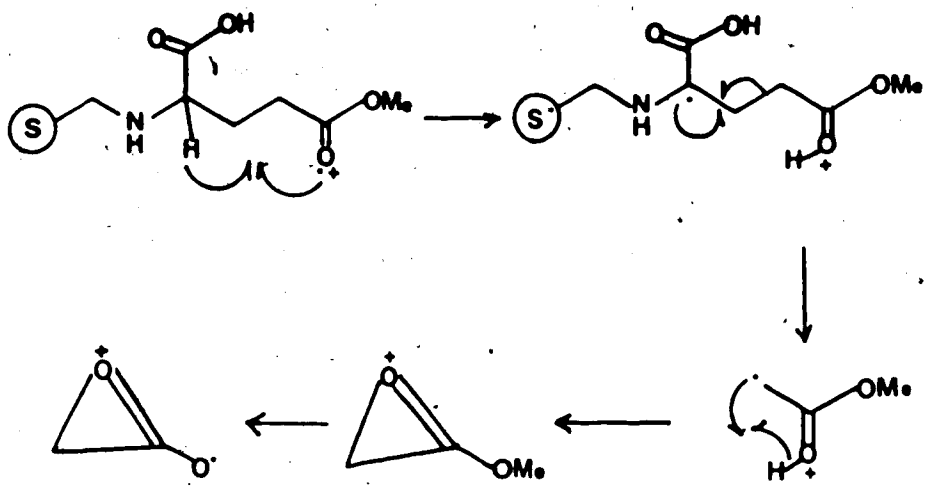
$^{*}DD_1$ DD_1^f

96.0437(8.59)

- Fragmentations of the EE series:

  No EE series.

2.4.10.2 Specific fragmentations



58.0072(13.13) 73.0295(82.58) 74.0370(26.97)

2.4.11 Lysine derivatives

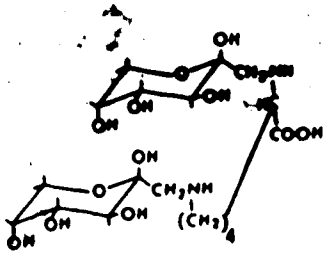
2.4.11.1 1-[(5'-aminofructosyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose

T = 200°C

No. of peaks = 42

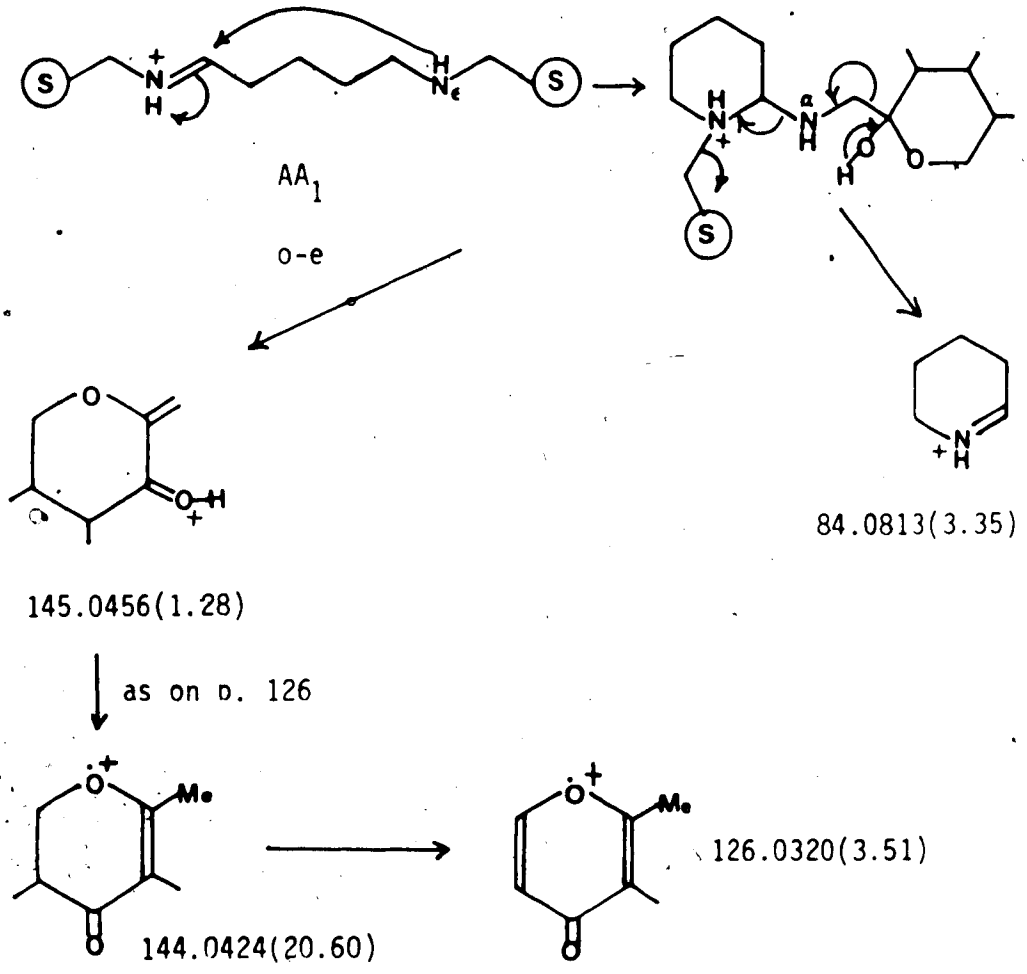
Mass range = 51.0231-145.0456

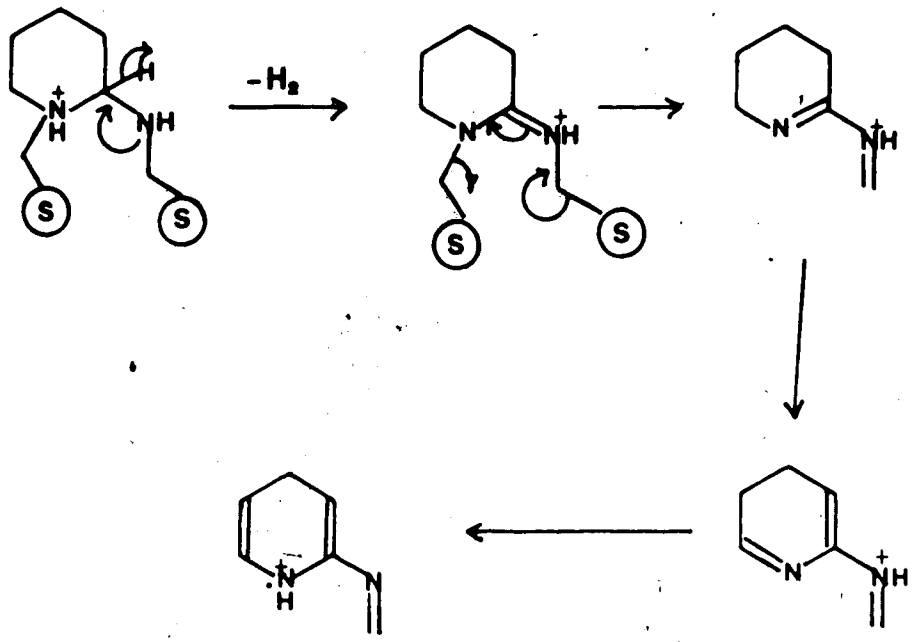
TIC: 20,275 (7,587)



2.4.11.2 "Amino acid" fragmentations

• Fragmentations of the AA series:





108.0686(0.70)

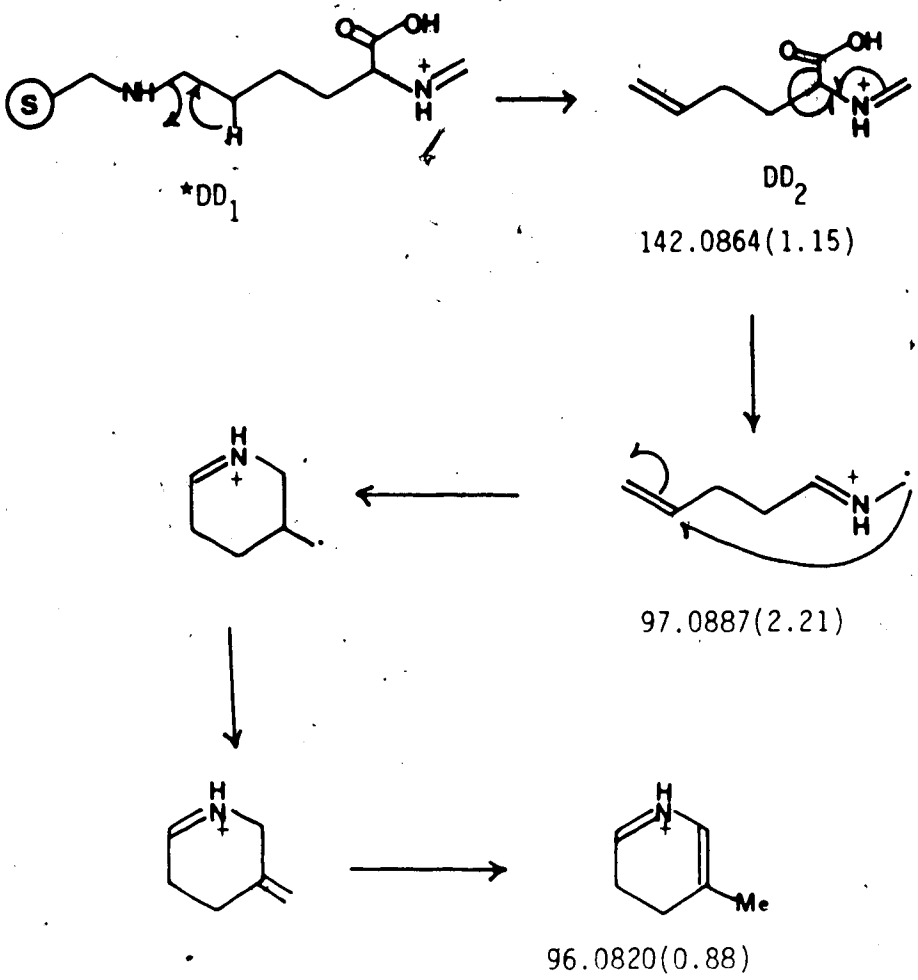
- Fragmentations of the BB series:

  No BB series.

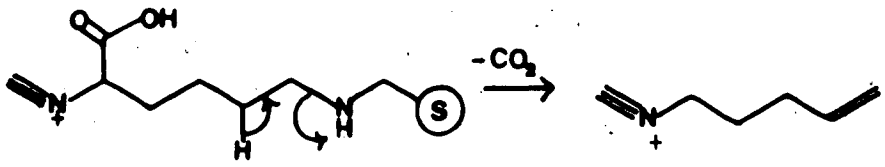
- Fragmentations of the CC series:

  No CC series.

• **Fragmentations of the DD series:**



• **Fragmentations of the EE series:**



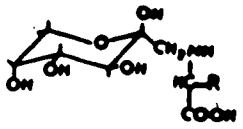
EE_3 EE_3^g

96.0820(0.88)

2.4.11.3 Specific fragmentations

No specific fragmentations.

2.4.11.4 1-[(5'-aminoformyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose



T = 100°C

No. of peaks = 51

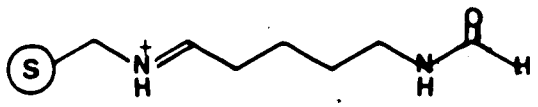
Mass range = 51.0235-149.0256

TIC: 15,888 (4,259)

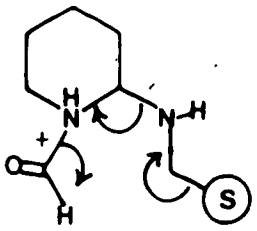
R = $-(CH_2)_4NHCHO$

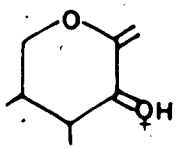
2.4.11.5 "Amino acid" fragmentations

• Fragmentations of the AA series:



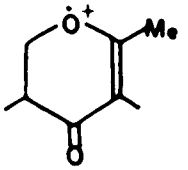
AA_1





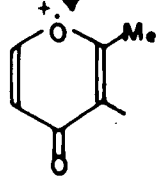
145.0455(1.22)

84.0820(4.81)



144.0424(19.51)

83.0738(0.86)



126.0331(1.24)

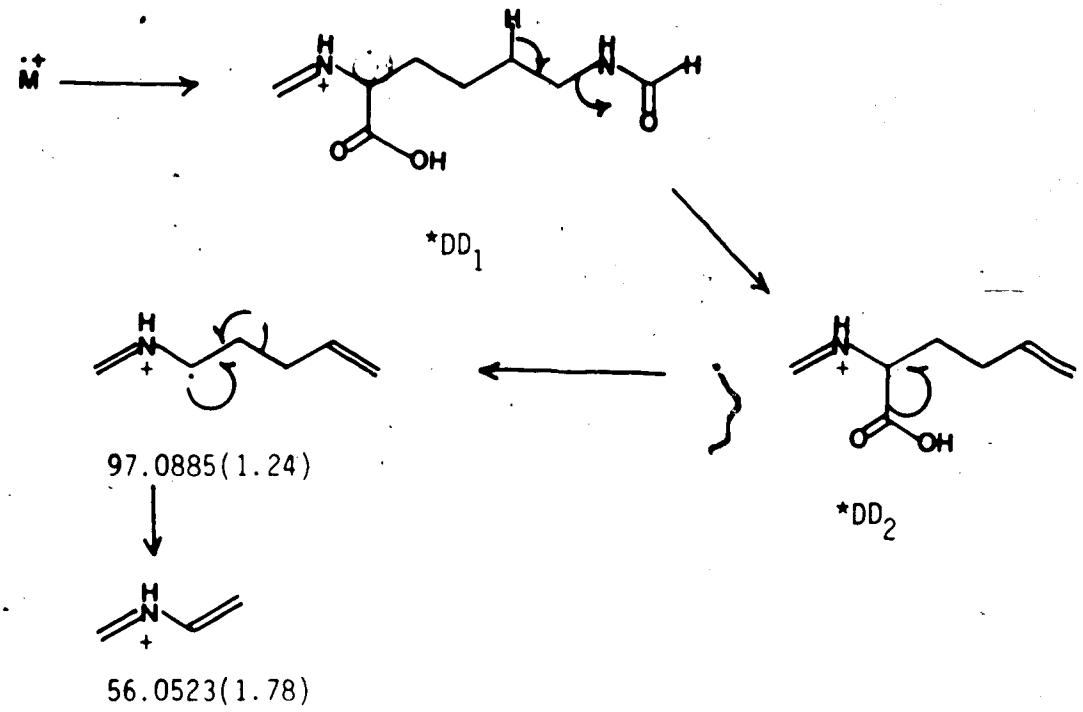
• **Fragmentations of the BB series:**

No BB series.

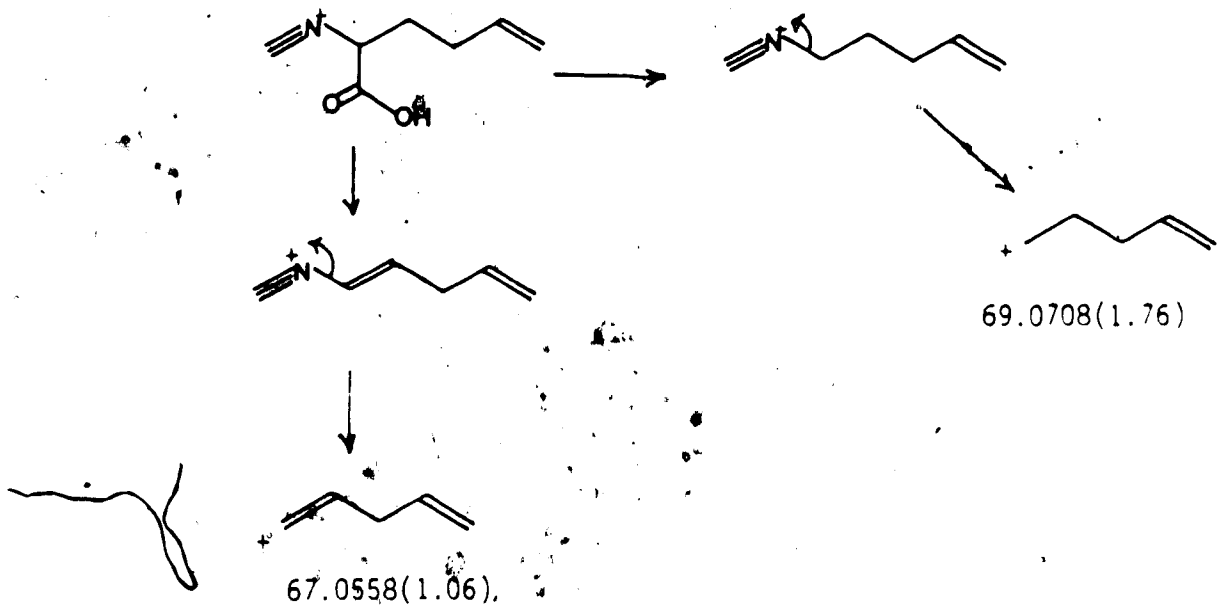
• **Fragmentations of the CC series:**

No CC series.

• **Fragmentations of the DD series:**

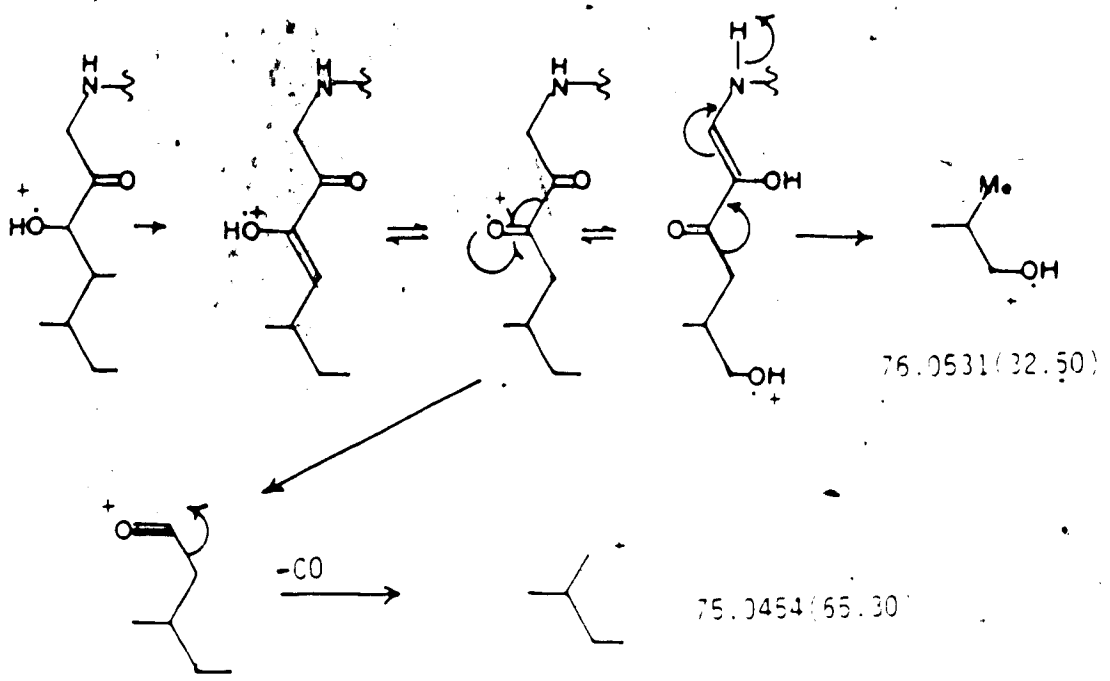


• Fragmentations of the EE series:



2.4.11.6 Specific fragmentations

• Open chain fragmentation.



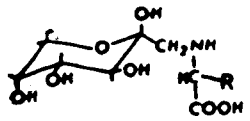
2.4.11.7 1-[(5'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose

T = 200°C

No. of peaks = 36

Mass range = 53.0404-130.0864

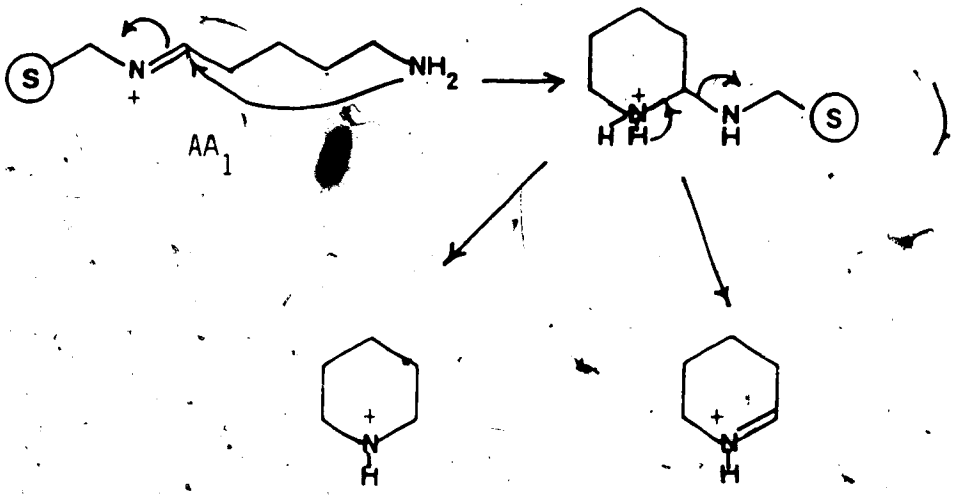
TIC: 9,372 (2,266)



R=-$(CH_2)_4NH_2$

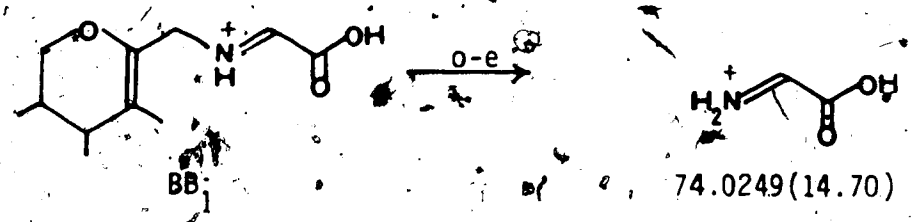
2.4.11.8 "Amino acid" fragmentations

• Fragmentations of the AA series:



85.0861(5.74) 84.0817(100)

• Fragmentations of the BB series:



74.0249(14.70)

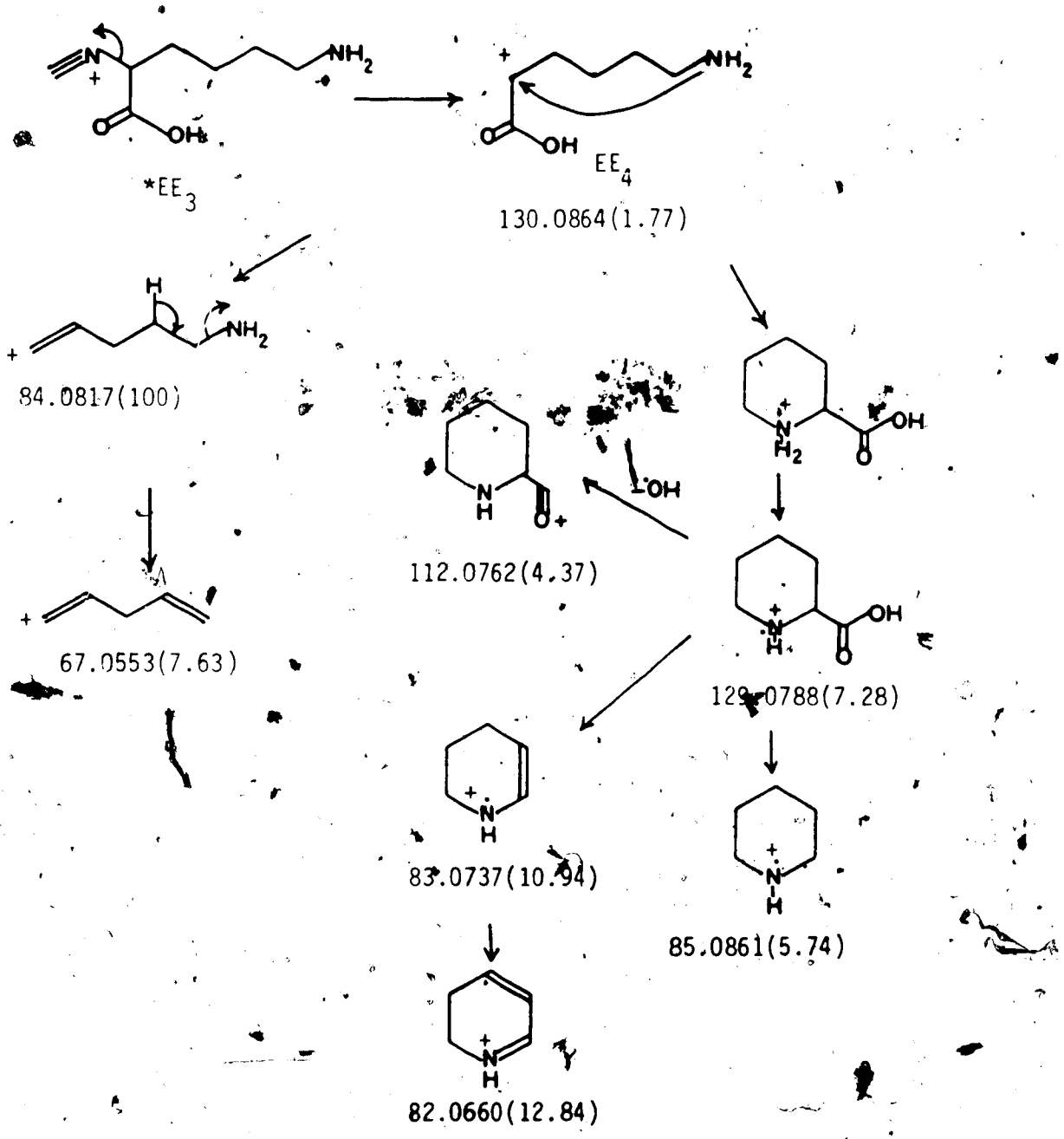
- Fragmentations of the CC series:

  No CC series.

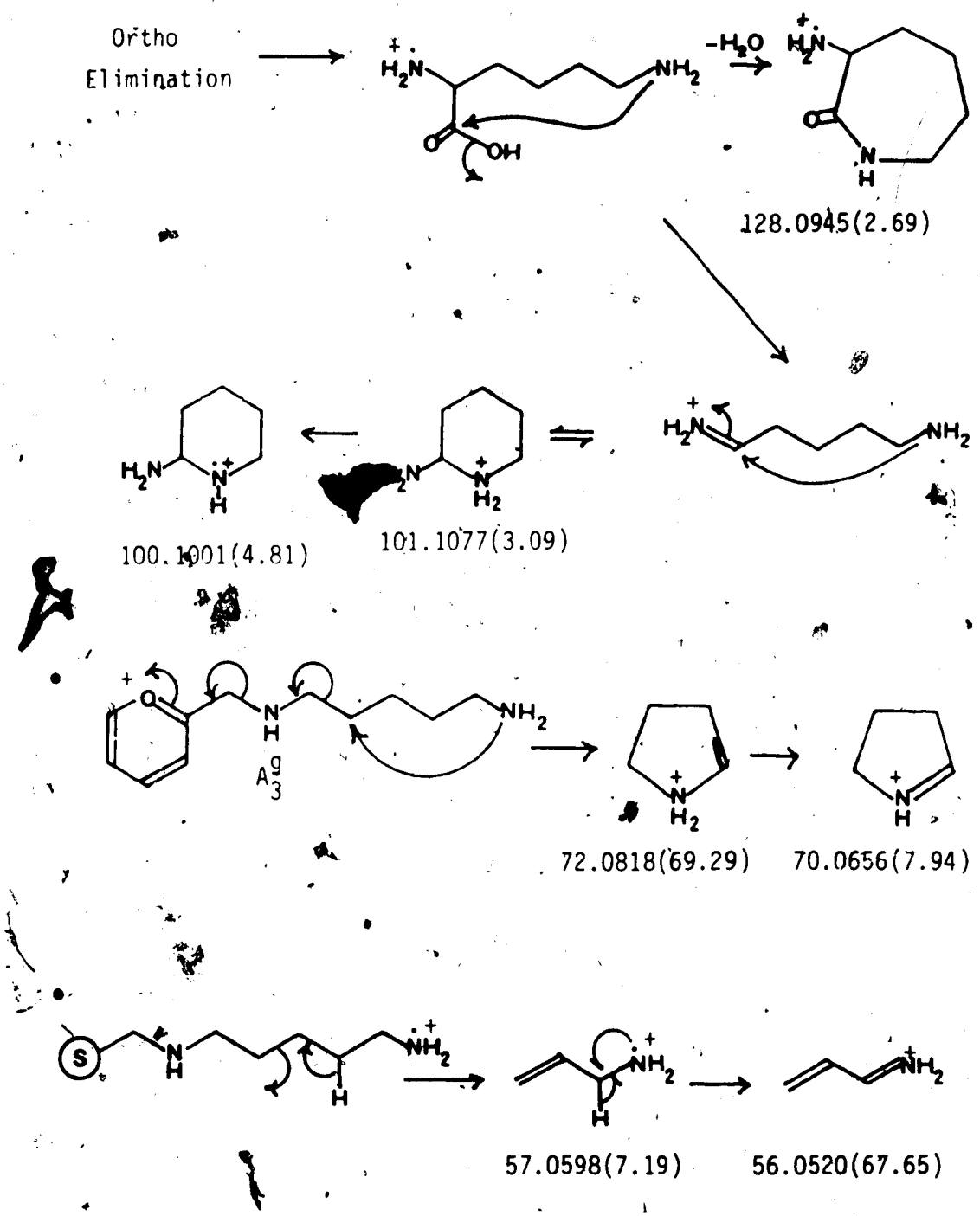
- Fragmentations of the DD series:

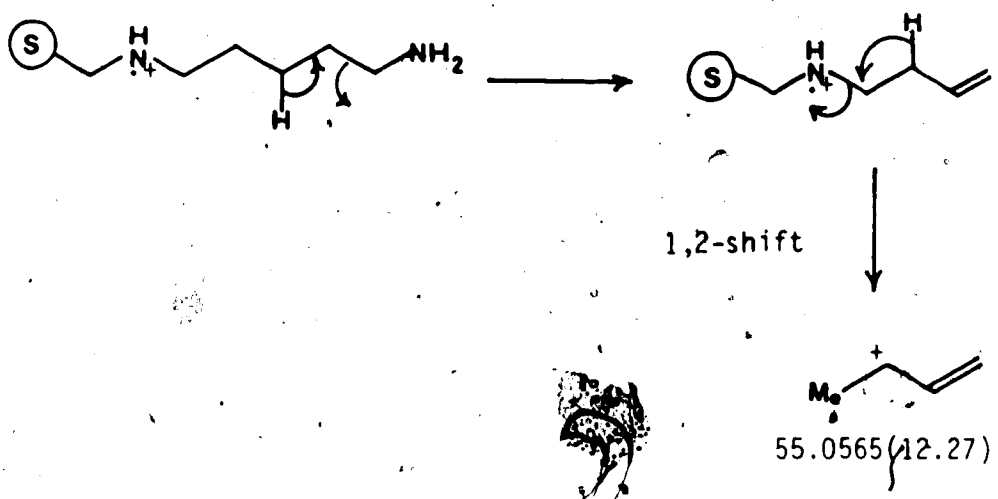
  No DD series.

- Fragmentations of the EE series:

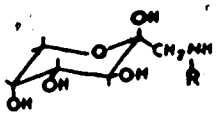


2.4.11.9 Specific fragmentations




S
H
N
NH2
H
S
H
N
H
1,2-shift
+
M
55.0565(12.27)

2.4.11.10 1-[(1'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose

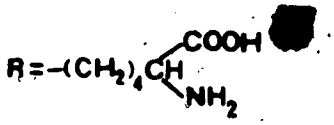


T = 200°C

No. of peaks = 94

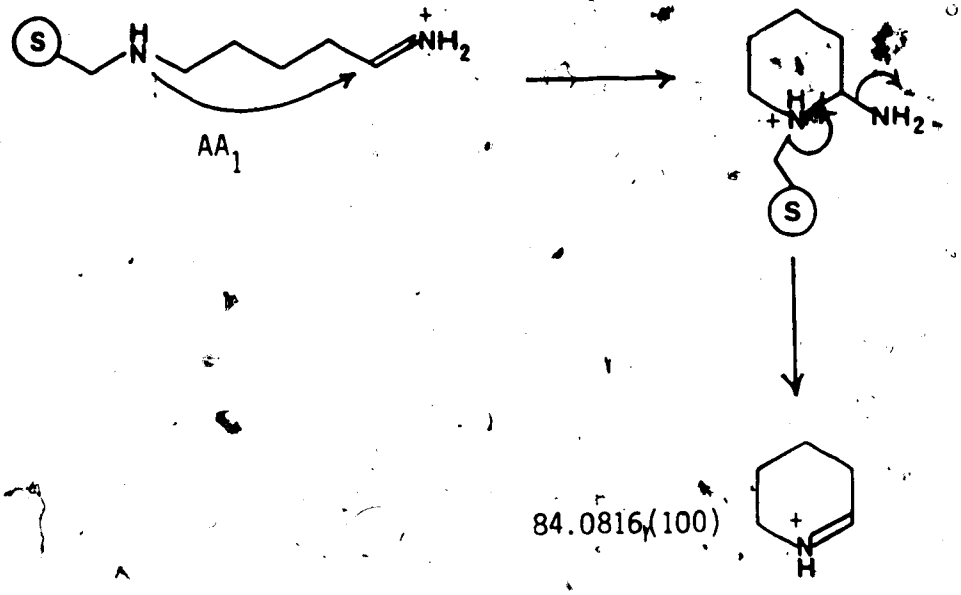
Mass range = 51.0232-177.1019

TIC: 25,190 (4,852)

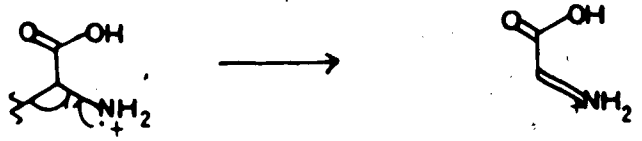


2.4.11.11 "[redacted]o acid" fragmentations

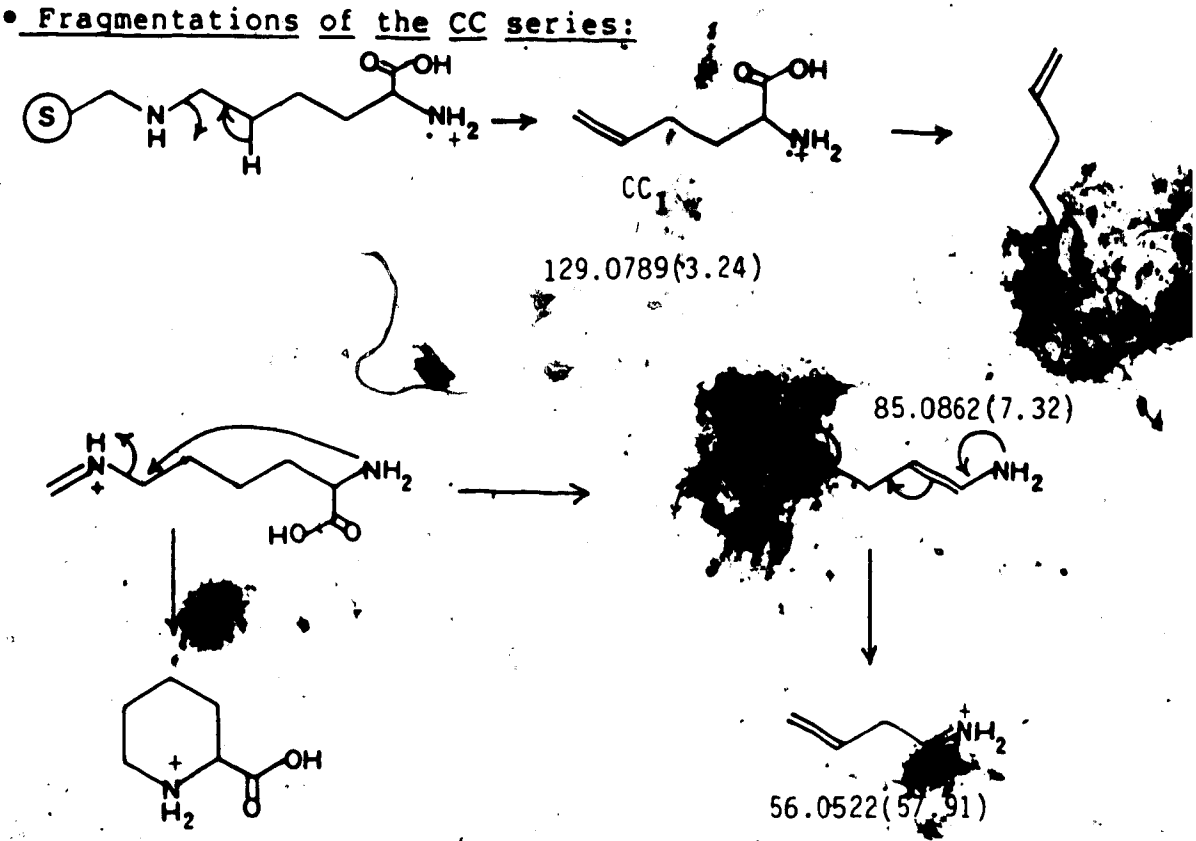
• Fragmentations of the AA series:



• Fragmentations of the BB series:



• Fragmentations of the CC series:



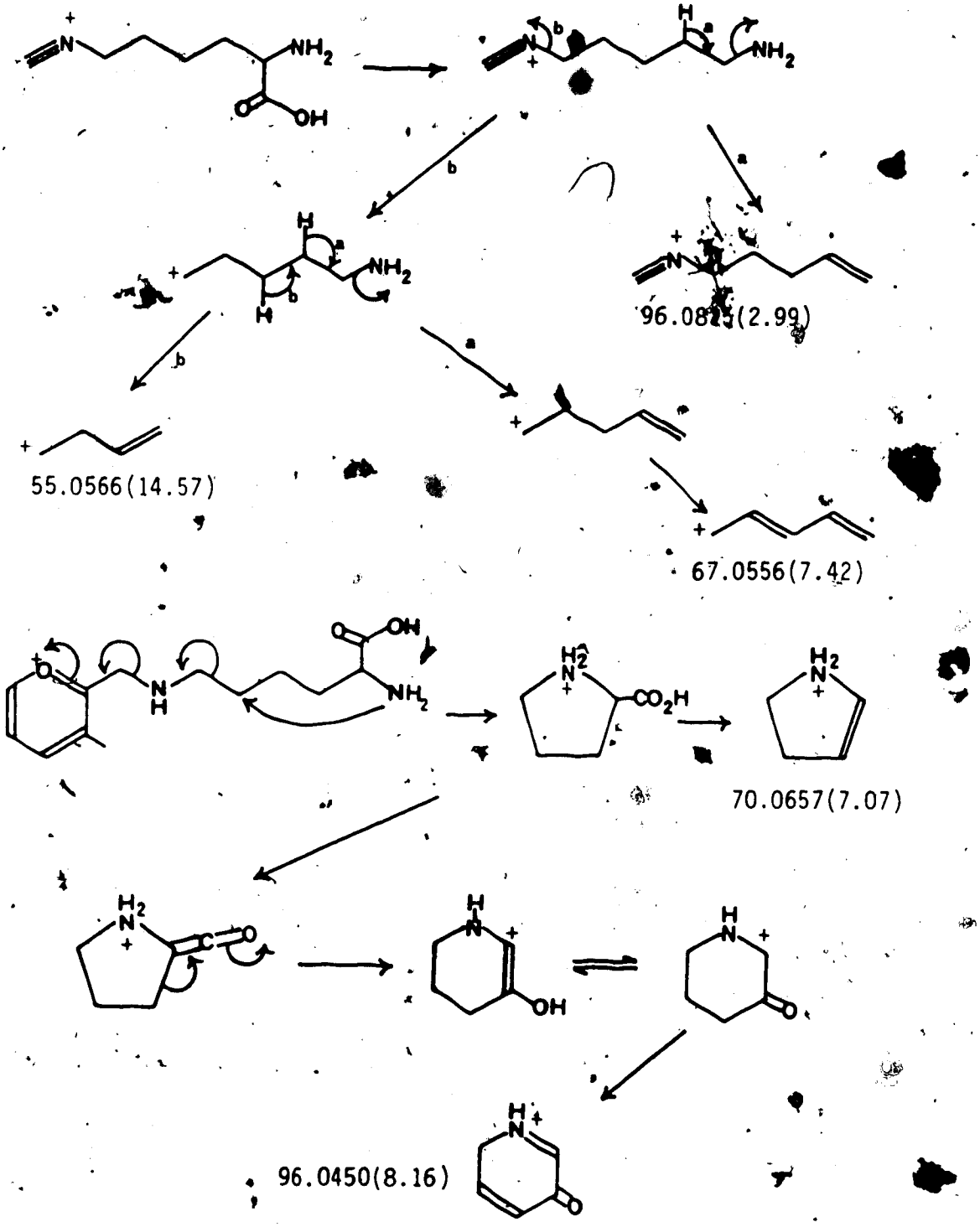
• Fragmentations of the DD series:

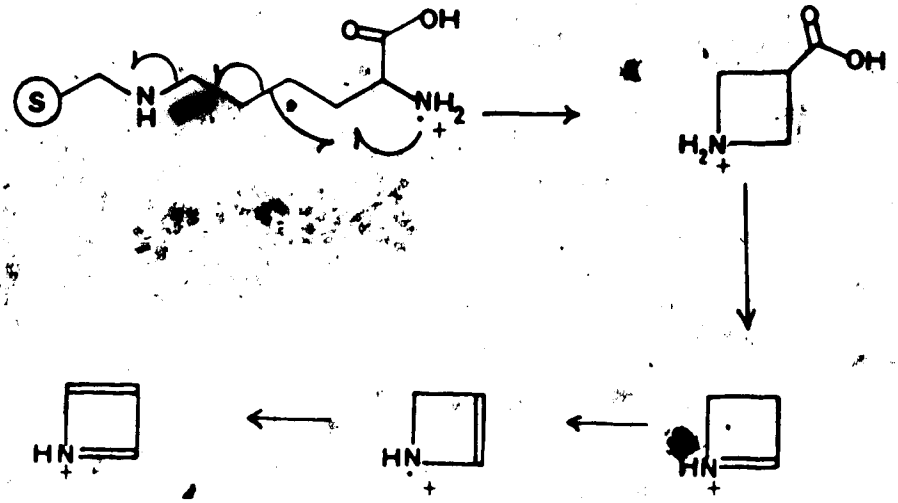
Not possible.

• Fragmentations of the EE series:

Not possible.

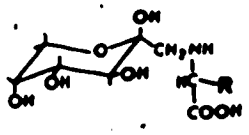
2.4.11.12 Specific fragmentations





54.0361(11.27) 55.0440(.61)

2.4.11.13 1-[(1'-carboxy-2'-imidazolylethyl)amino]-1-deoxy-D-fructose

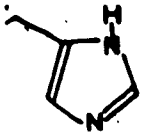


T = 200°C

No. of peaks = 118

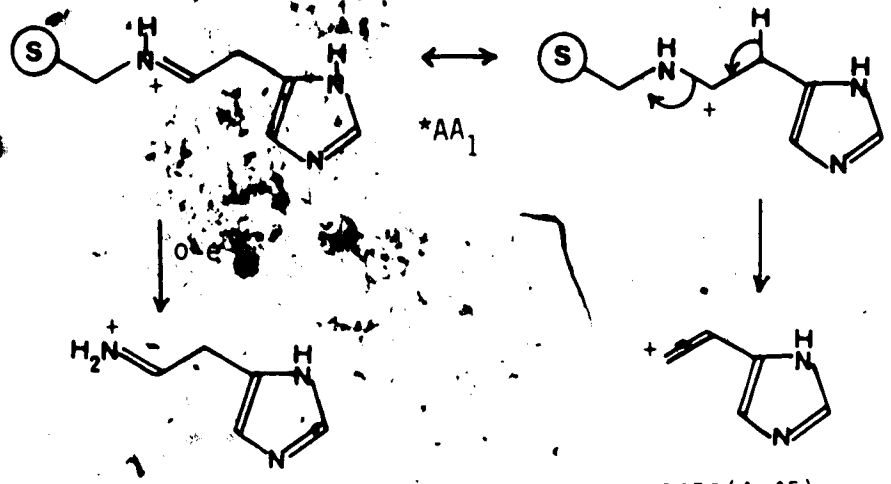
Mass range = 51.0109-274.1183

TIC: 65,477 (24,236)

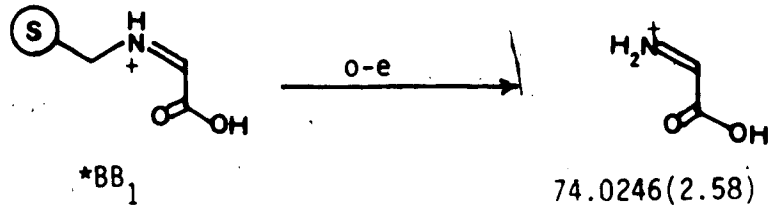
R= 

2.4.11.14 "Amino acid" fragmentations

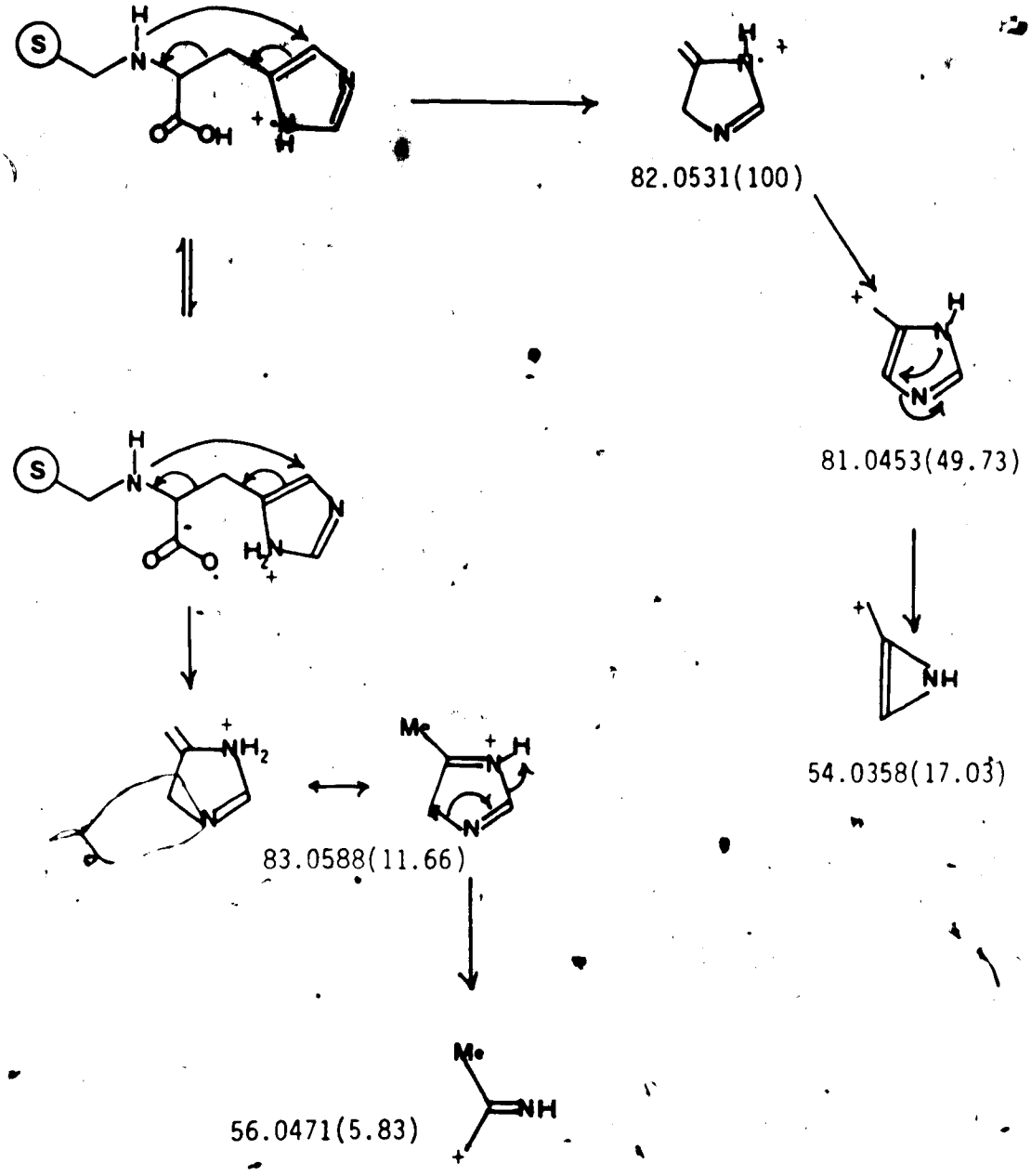
• Fragmentations of the AA series:



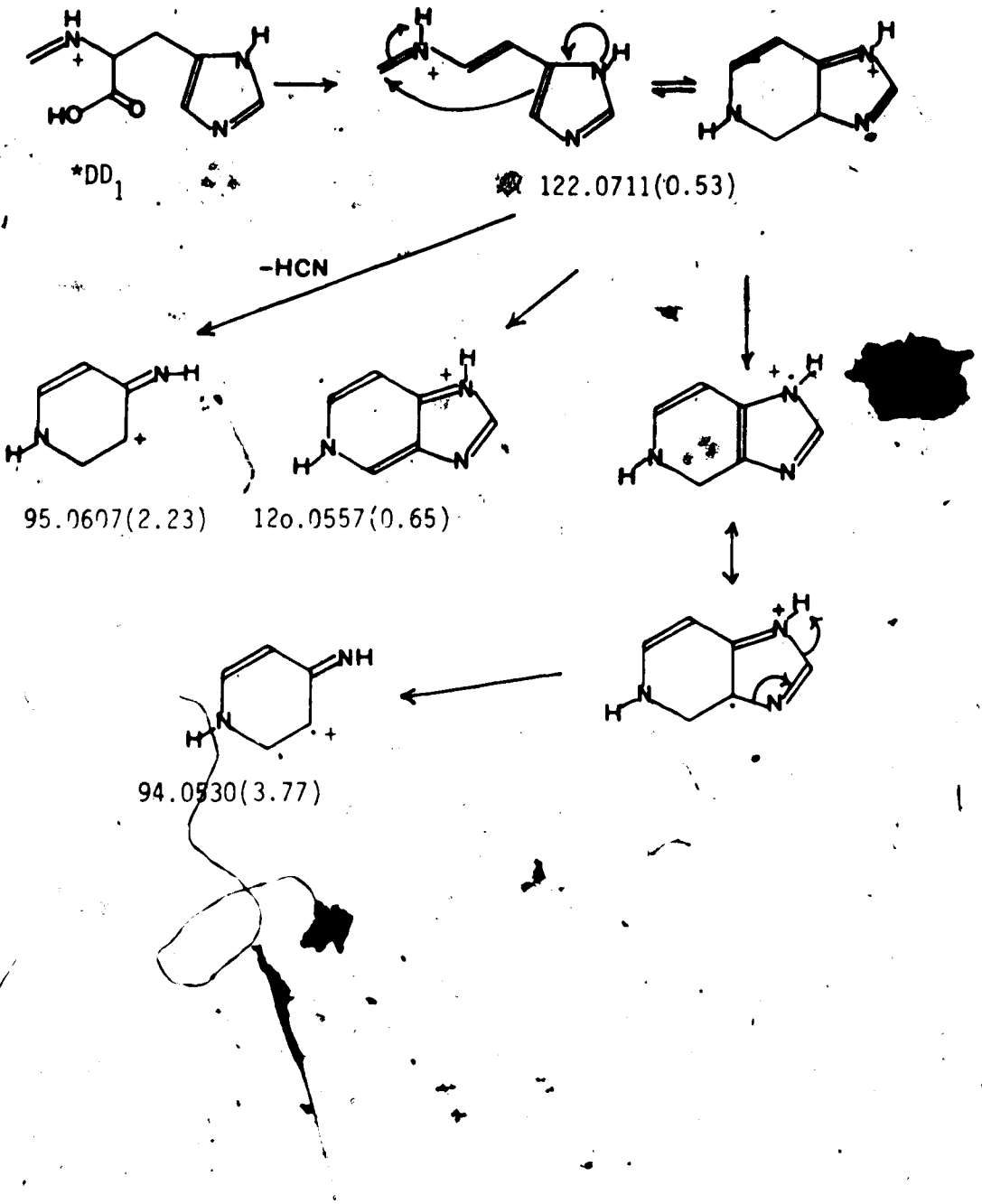
• Fragmentations of the BB series:



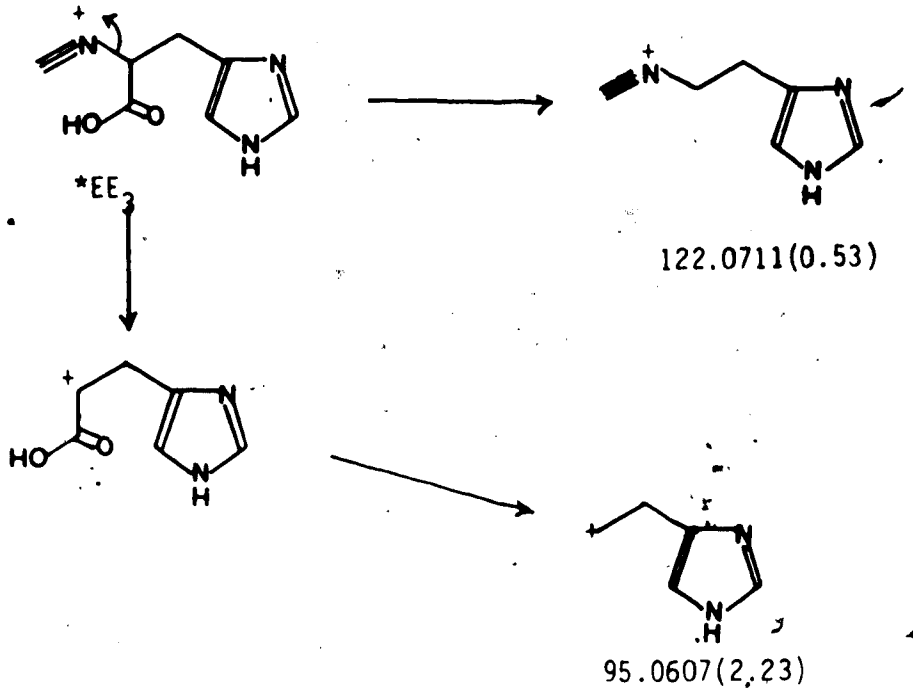
• Fragmentations of the CC series:



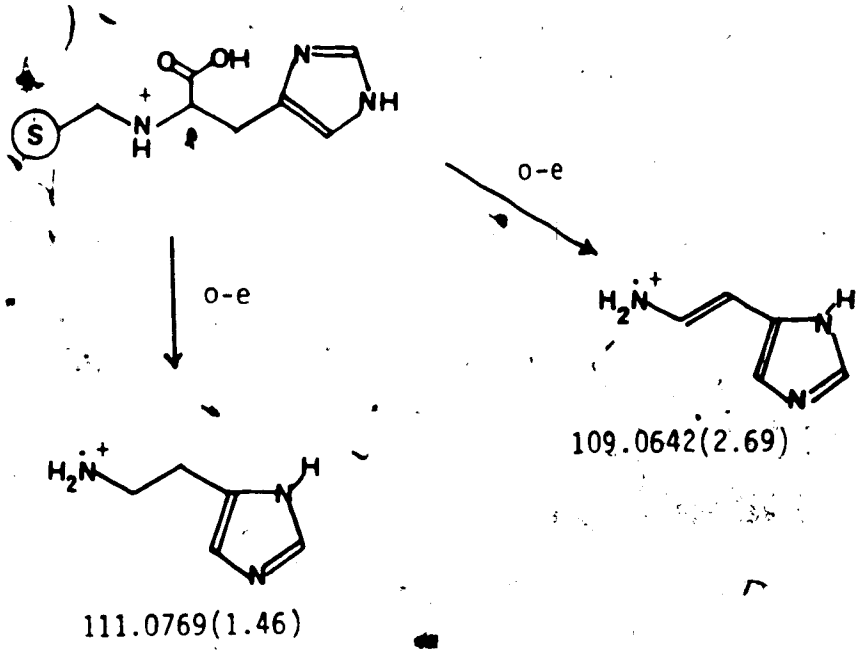
• **Fragmentations of the DD series:**



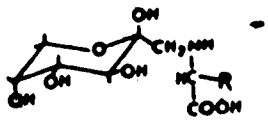
• Fragmentations of the EE series:



2.4.11.15 Specific fragmentations



2.4.11.16 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose



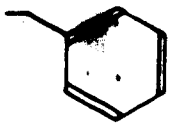
T = 250°C

No. of peaks = 69

Mass range = 51.0235-206.1072

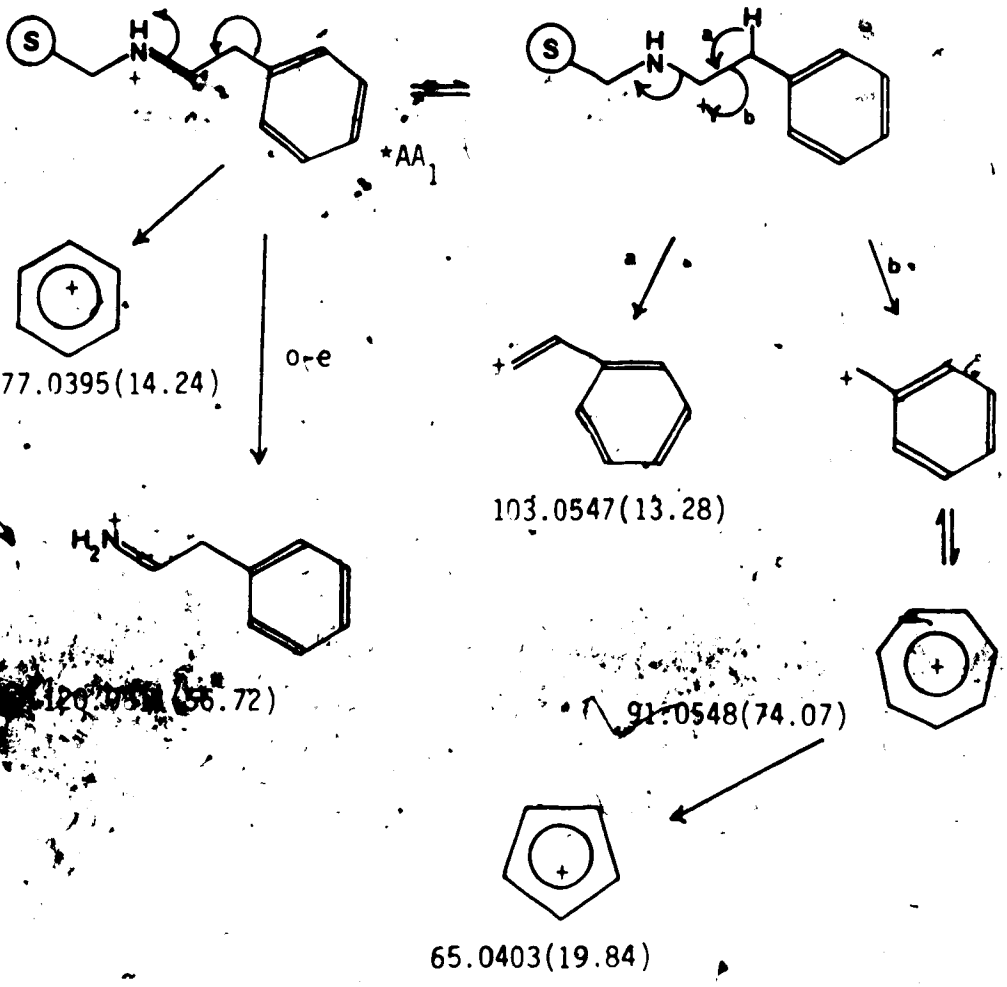
TIC: 23,971 (9,076)

R=

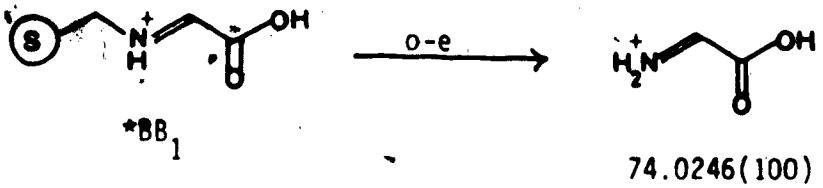


2.4.11.17 "Amino acid" fragmentations

• Fragmentations of the AA series:



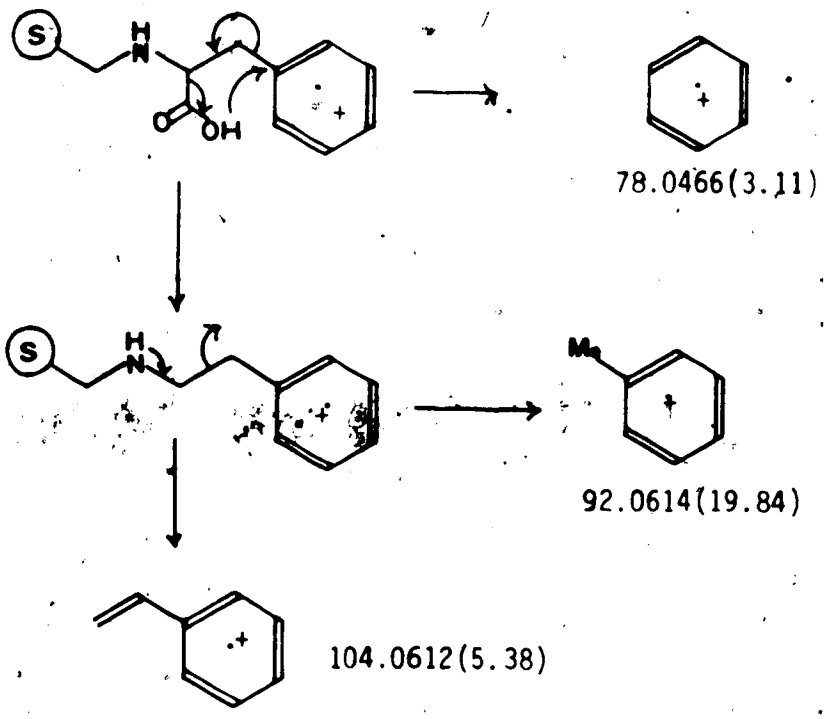
• Fragmentations of the BB series:



*BB_1

74.0246(100)

• Fragmentations of the CC series:

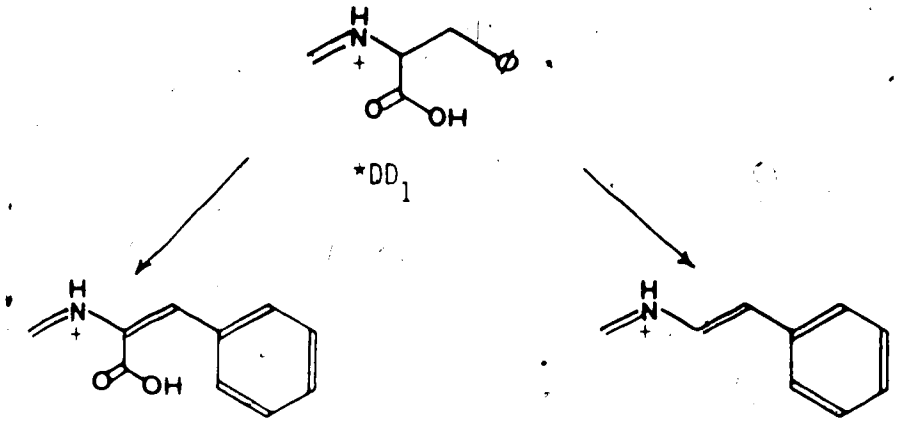


78.0466(3.11)

92.0614(19.84)

104.0612(5.38)

• Fragmentations of the DD series:

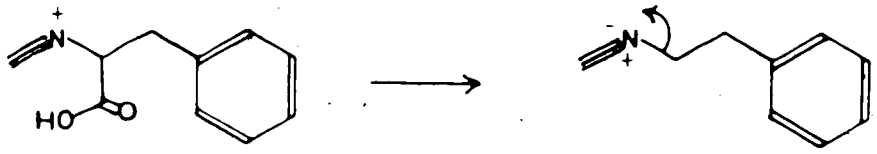


*DD_1

176.0707(1.75)

132.0809(2.74)

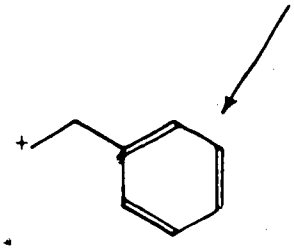
• Fragmentations of the EE series:



EE_3

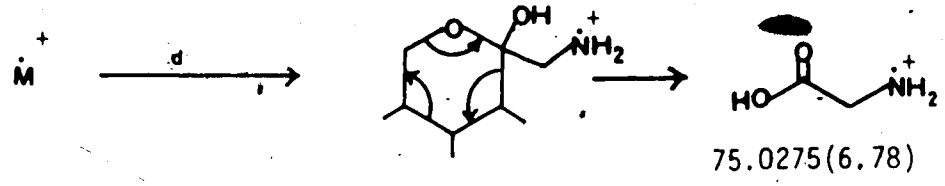
176.0707(1.75)

132.0809(2.74)



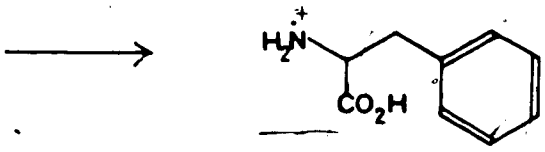
105.0702(4.18)

2.4.11.18 Specific fragmentations



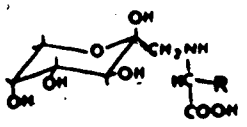
75.0275(6.78)

Ortho
Elimination



165.0783(3.31)

2.4.11.19 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)amino]-1-deoxy-D-fructose



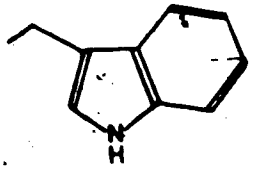
T = 250°C

No. of peaks = 212

Mass range = 51.0234-298.1297

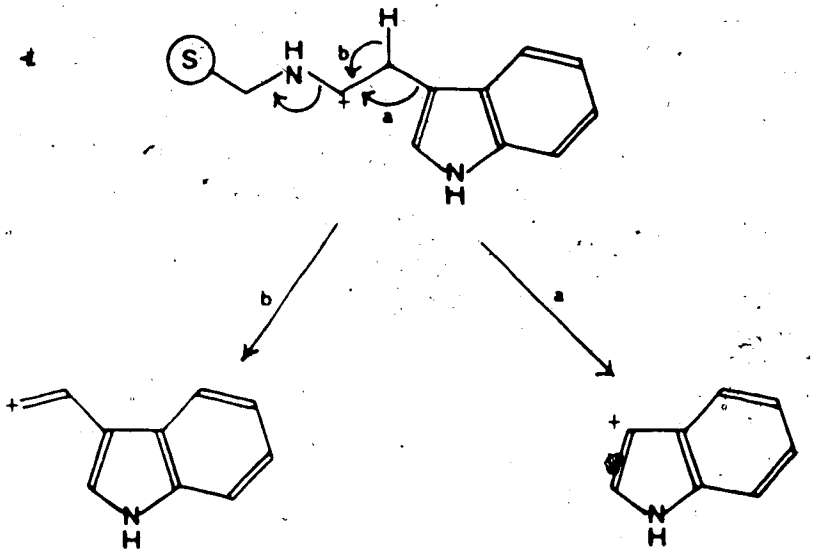
TIC: 30,214 (2,404)

R=



2.4.11.20 "Amino acid" fragmentations

• Fragmentations of the AA series:



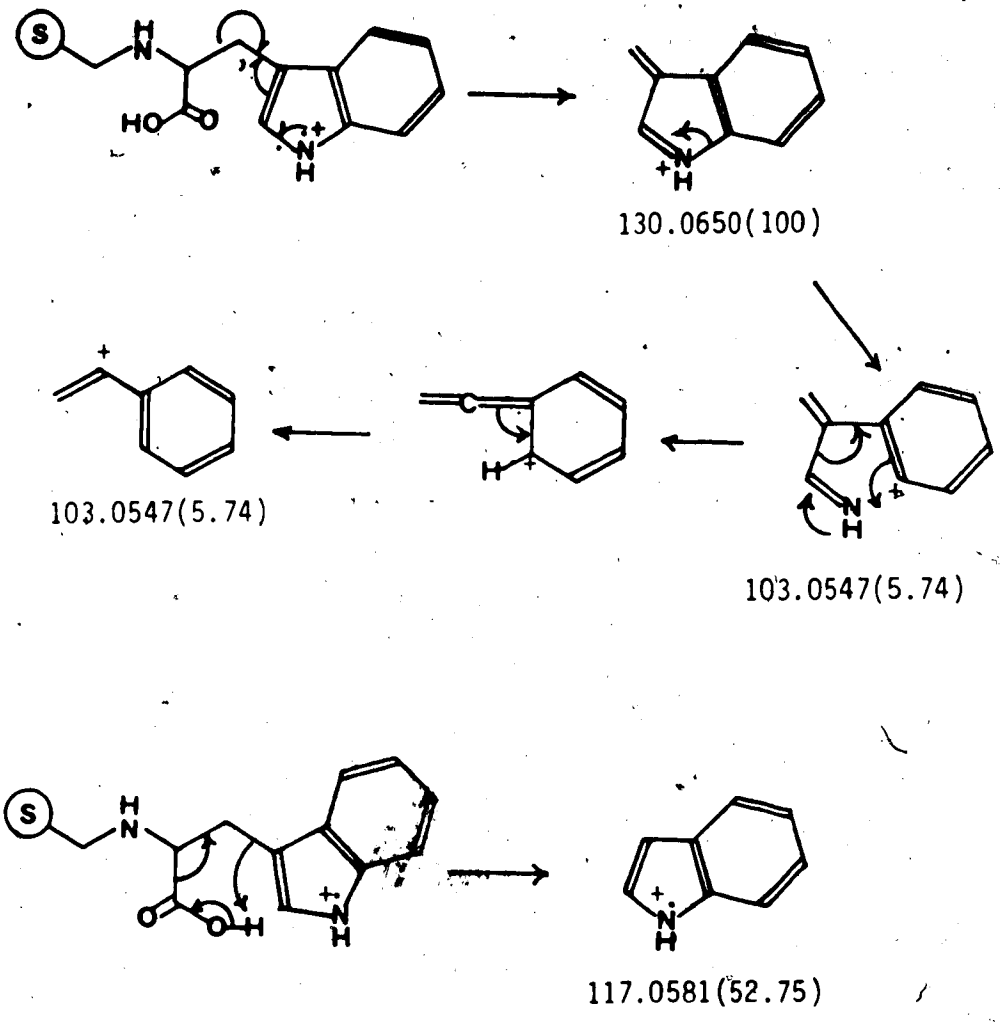
142.0645(3.16)

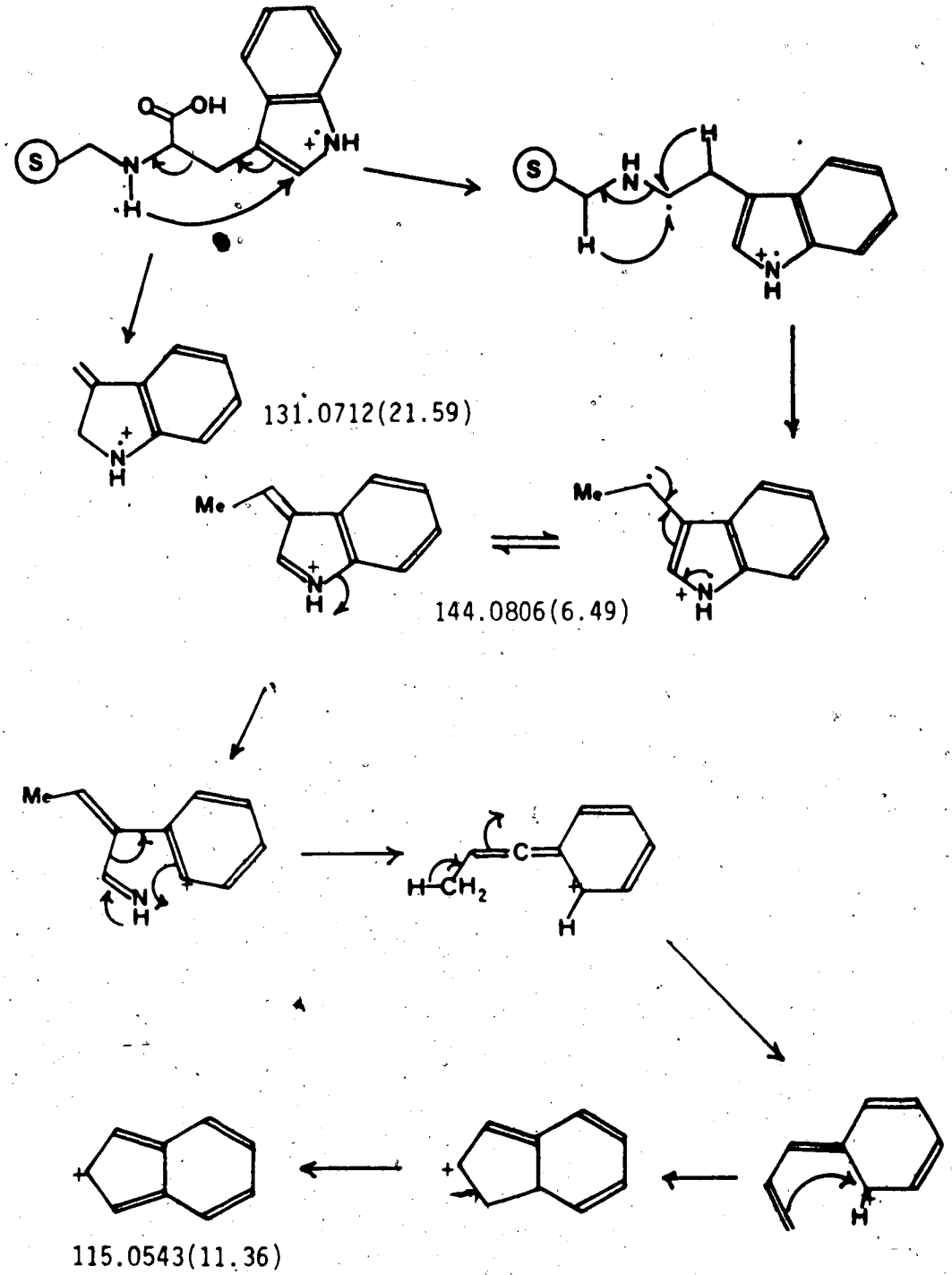
116.0503(4.45)

• **Fragmentations of the BB series:**

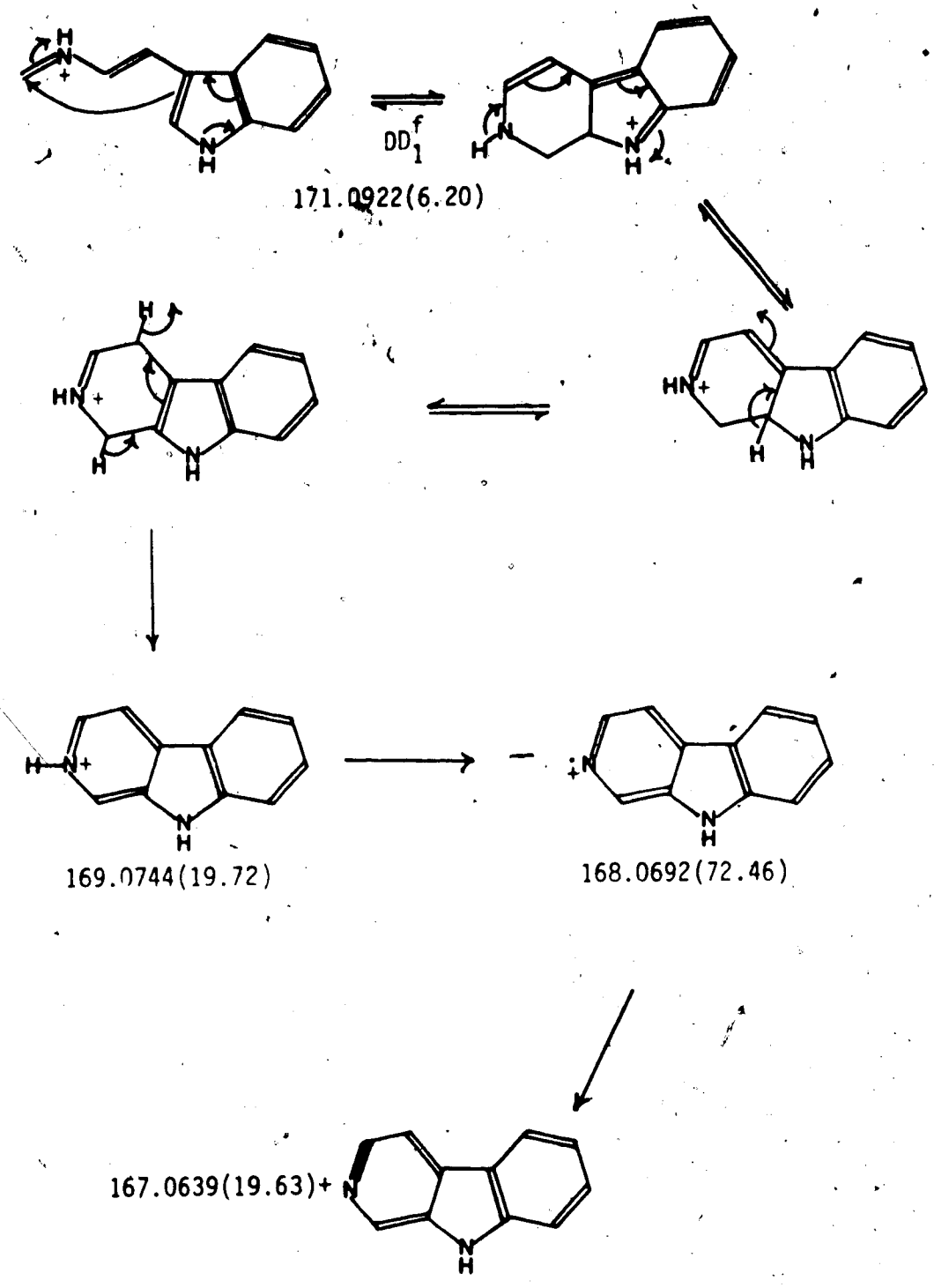
No BB series.

• **Fragmentations of the CC series:**

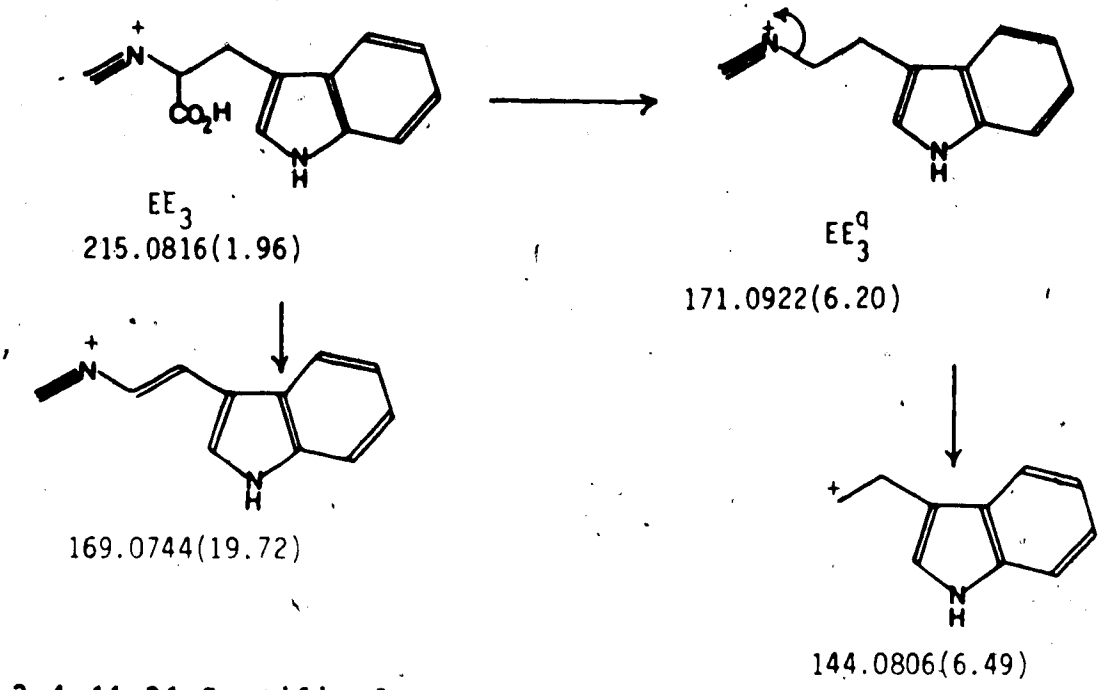



131.0712(21.59)
144.0806(6.49)
115.0543(11.36)

• **Fragmentations of the DD series:**

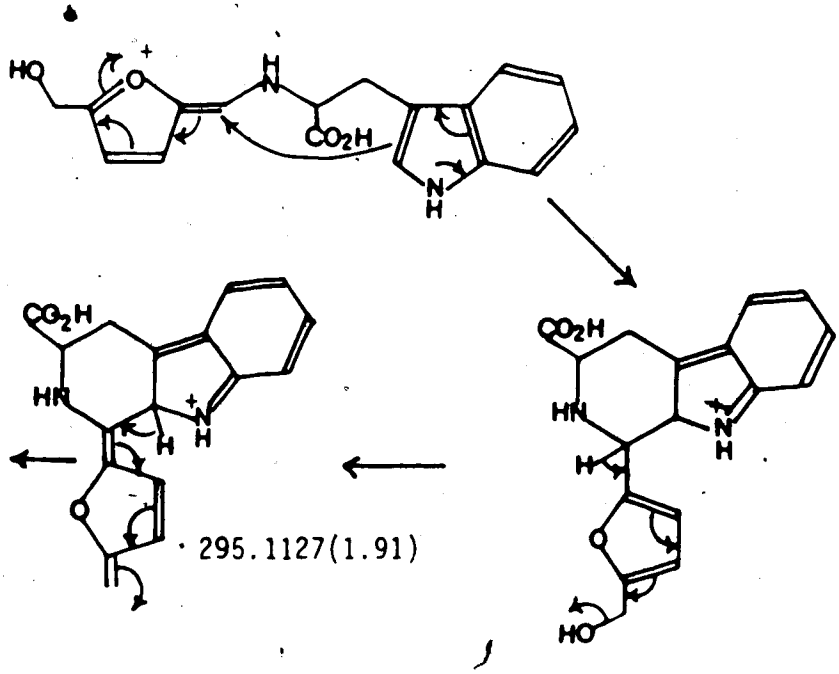


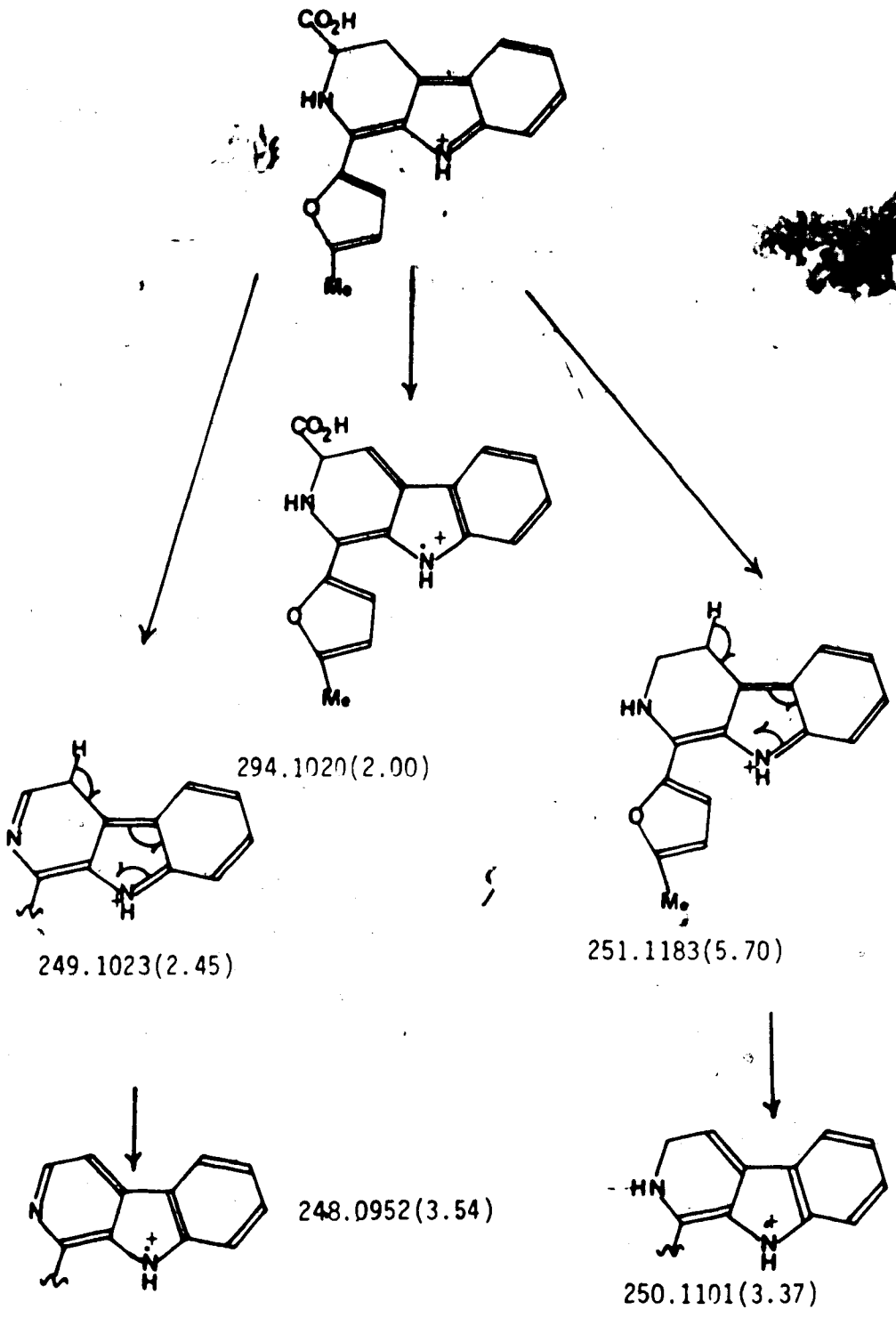
• **Fragmentations of the EE series:**

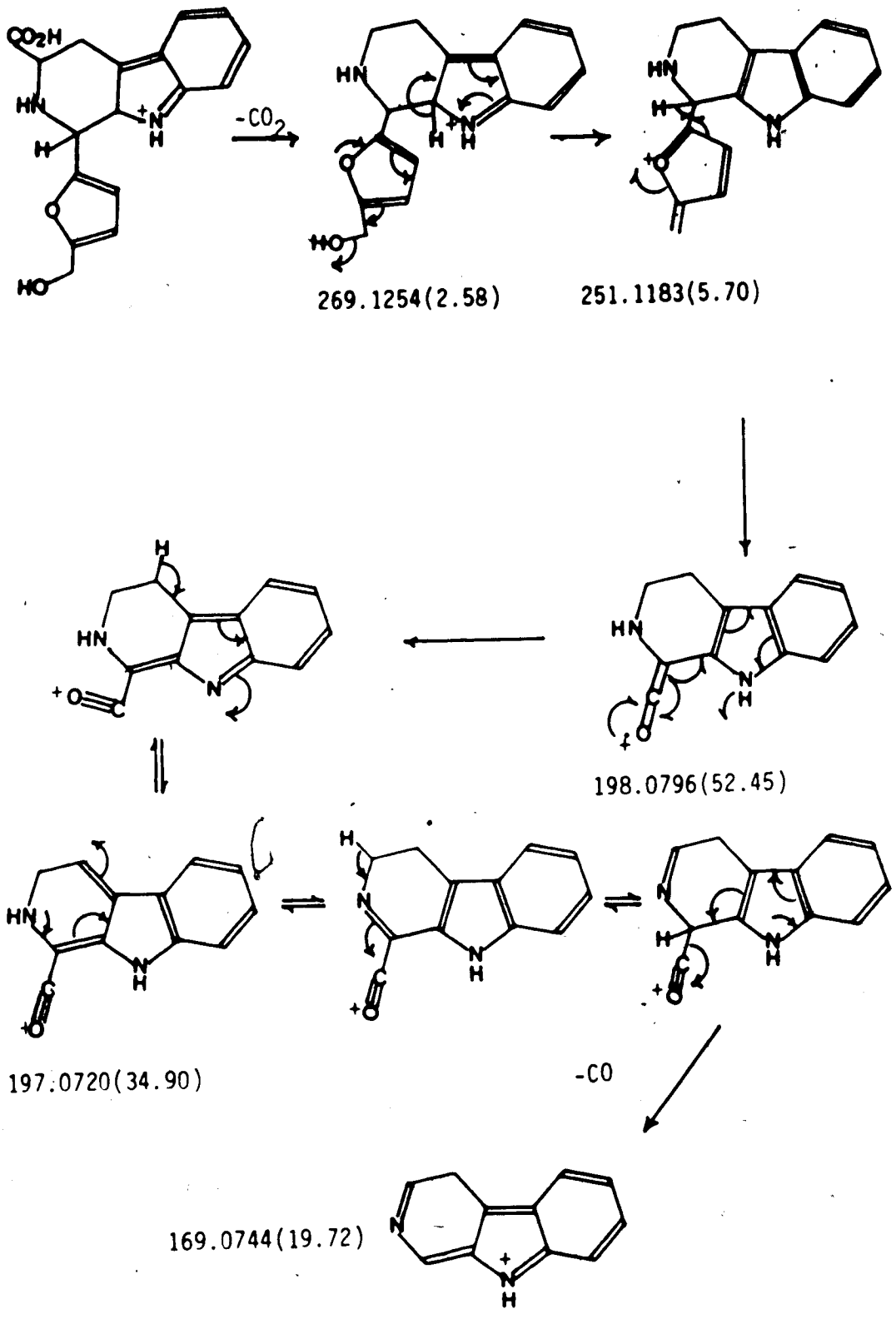


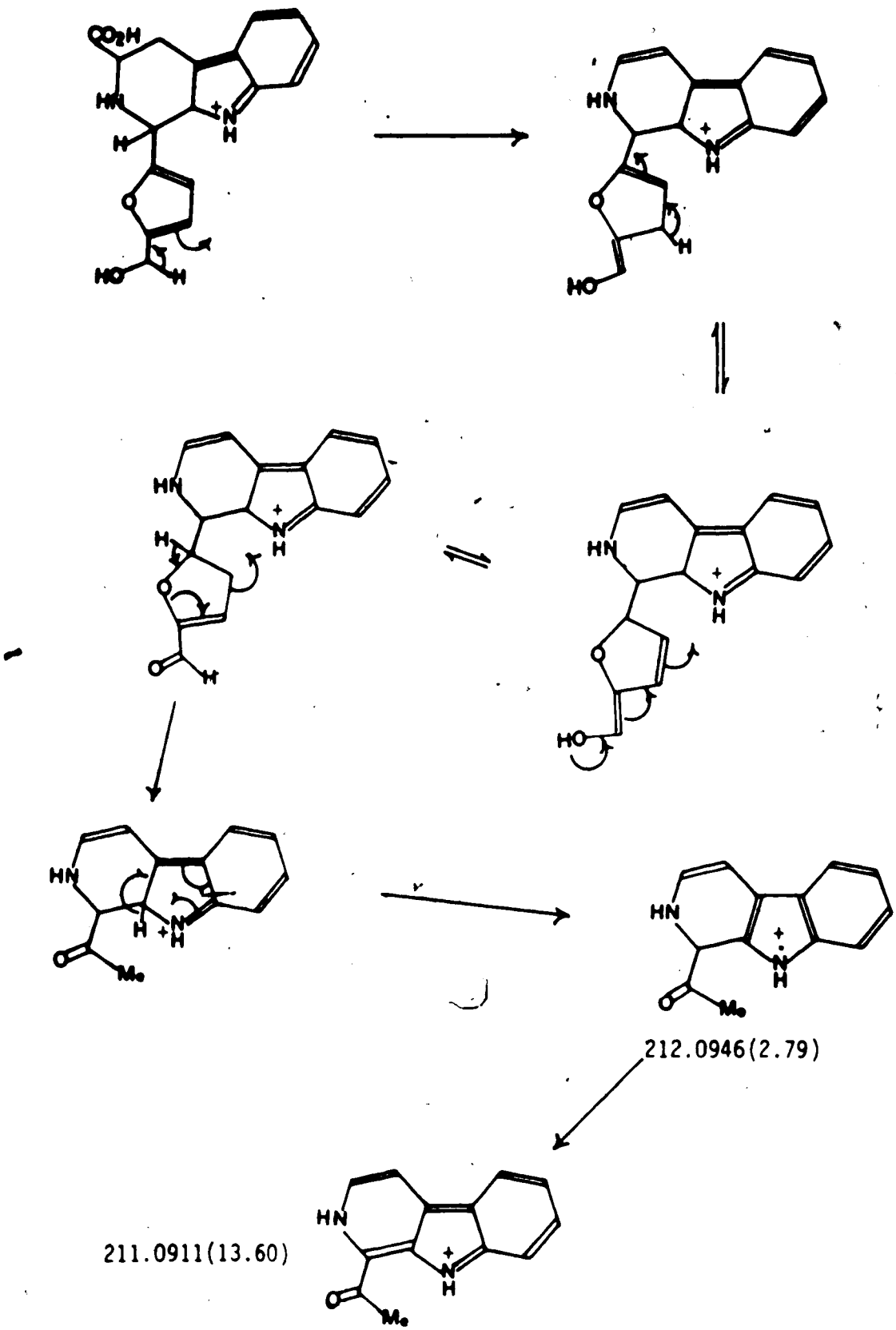
2.4.11.21 Specific fragmentations

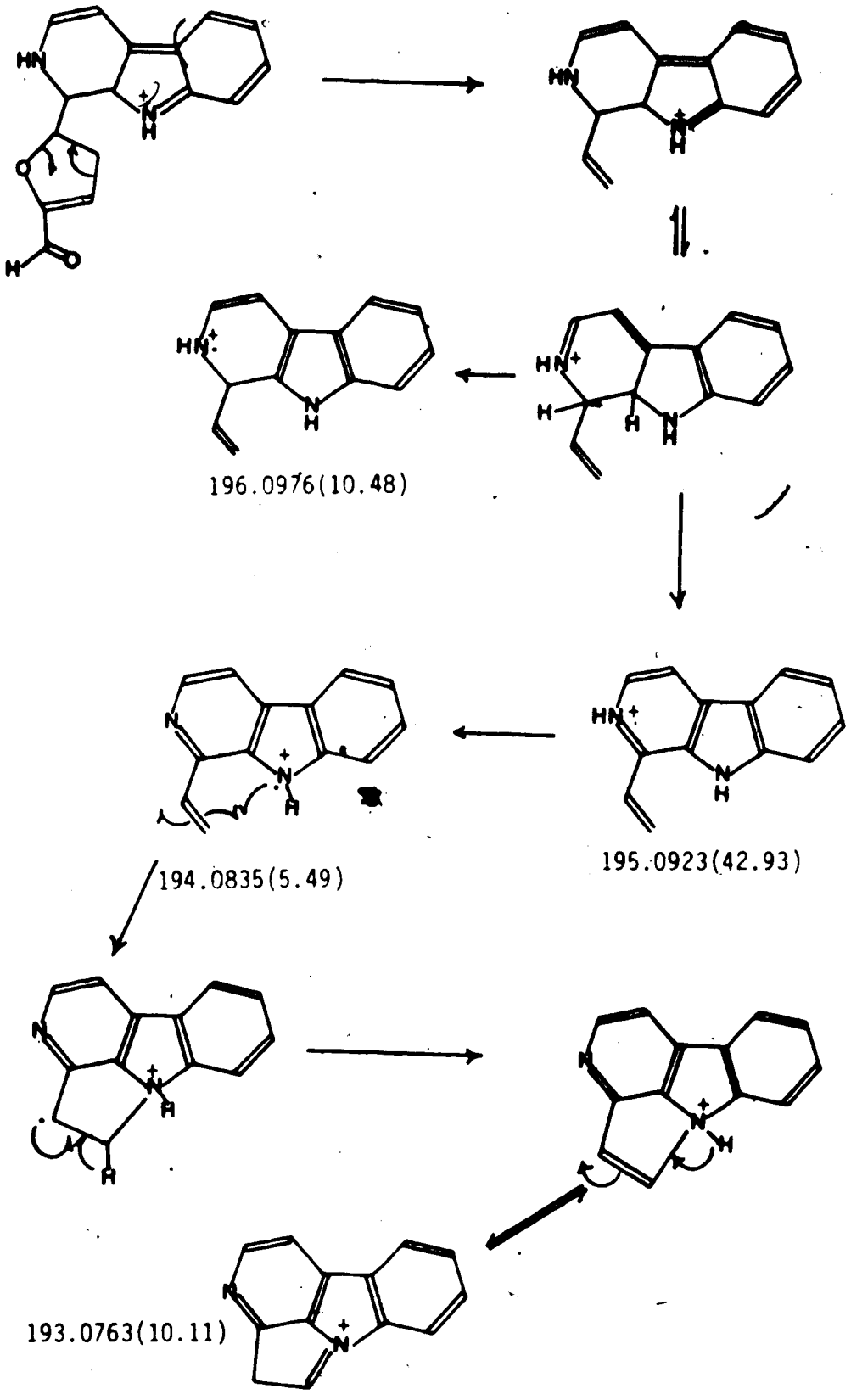
Another route to β-carbolines, besides the DD series:

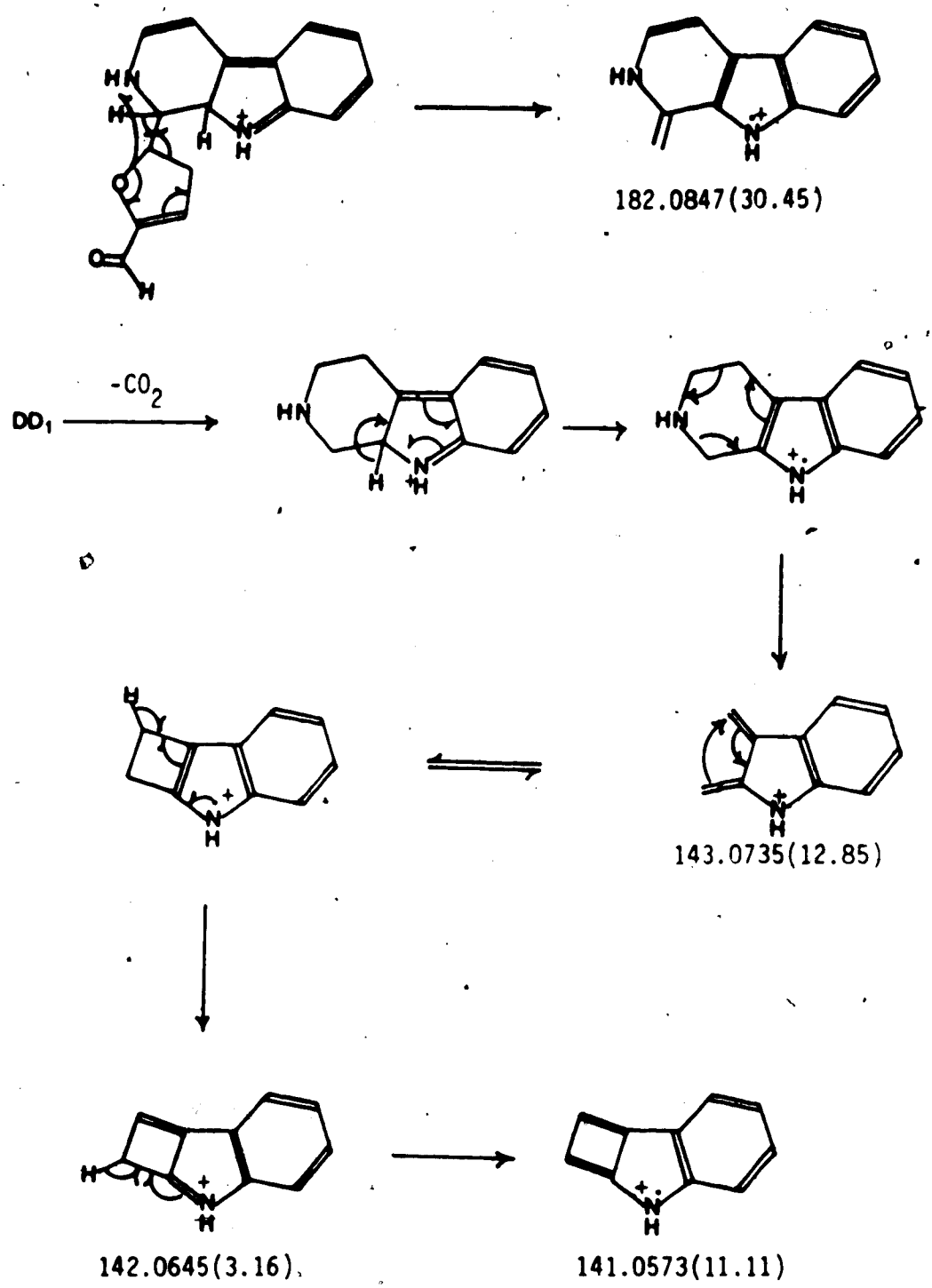


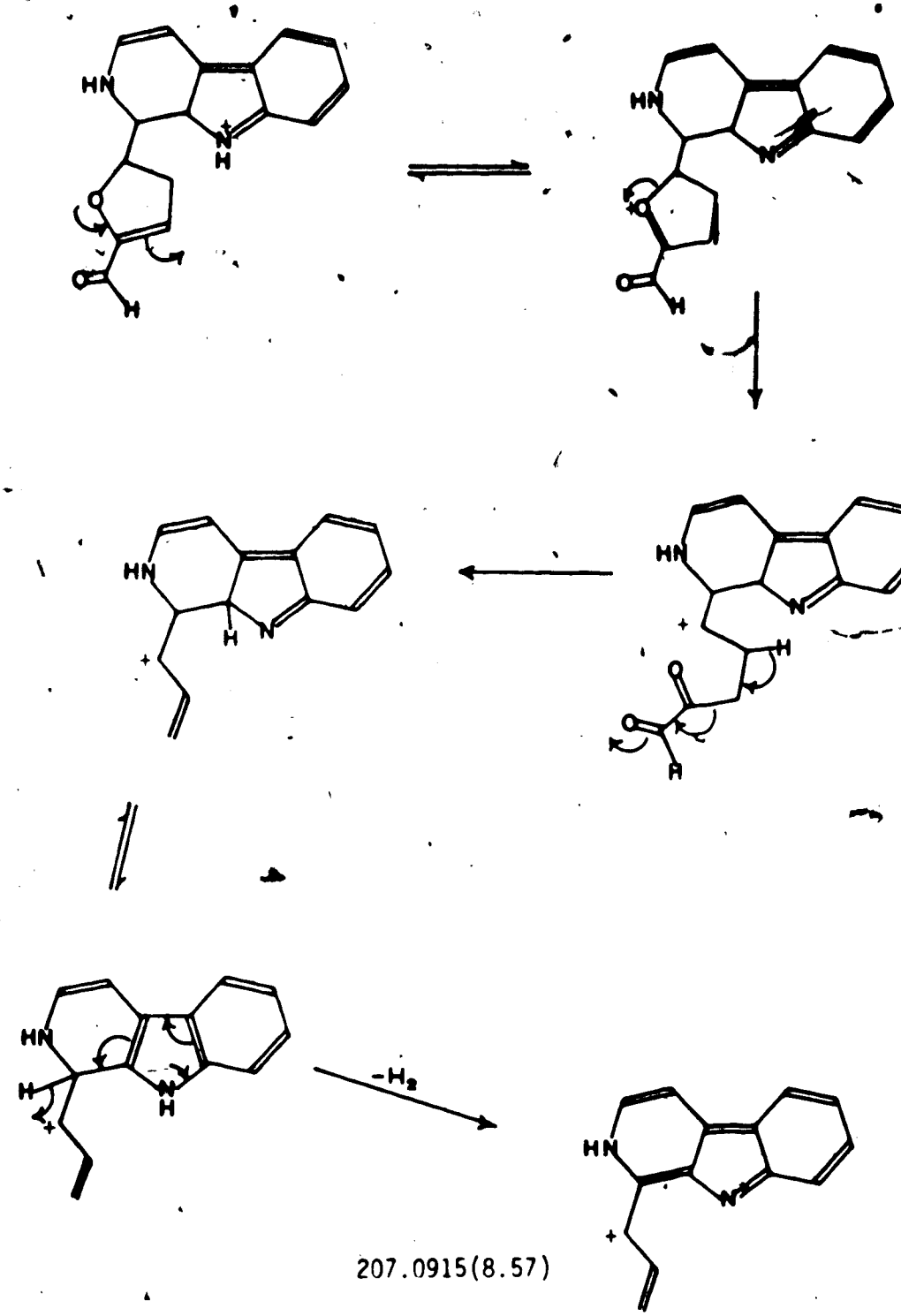

294.1020(2.00)
249.1023(2.45)
251.1183(5.70)
248.0952(3.54)
250.1101(3.37)


-CO2
269.1254(2.58)
251.1183(5.70)
198.0796(52.45)
197.0720(34.90)
-CO
169.0744(19.72)


212.0946(2.79)
211.0911(13.60)


196.0976(10.48)
194.0835(5.49)
195.0923(42.93)
193.0763(10.11)

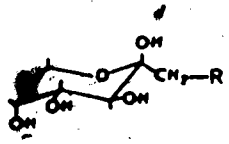

182.0847(30.45)
$-CO_2$
DD_1
143.0735(12.85)
142.0645(3.16)
141.0573(11.11)



207.0915(8.57)

2.4.11.22 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose

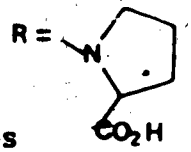
T = 180°C



No. of peaks = 142

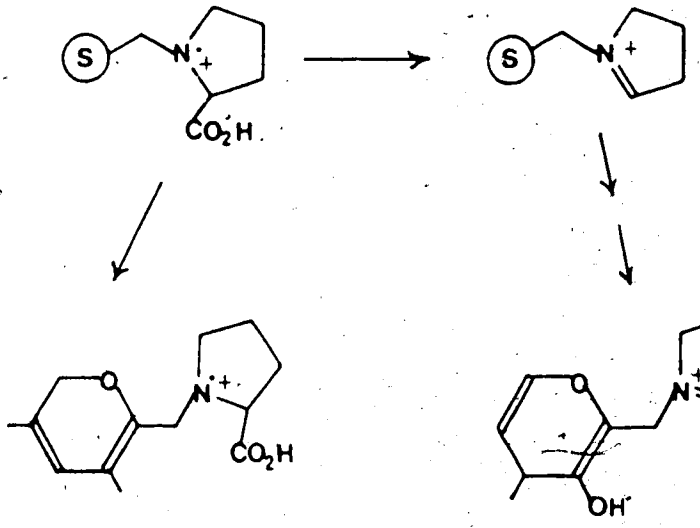
Mass range = 51.0231-241.0954

TIC: 67,763 (24,128)



2.4.11.23 "Amino acid" fragmentations

• Fragmentations of the AA series:

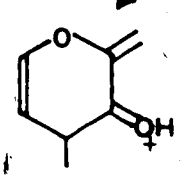


241.0954(2.04)

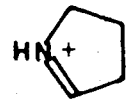
196.0971(6.65)

o-e

o-e



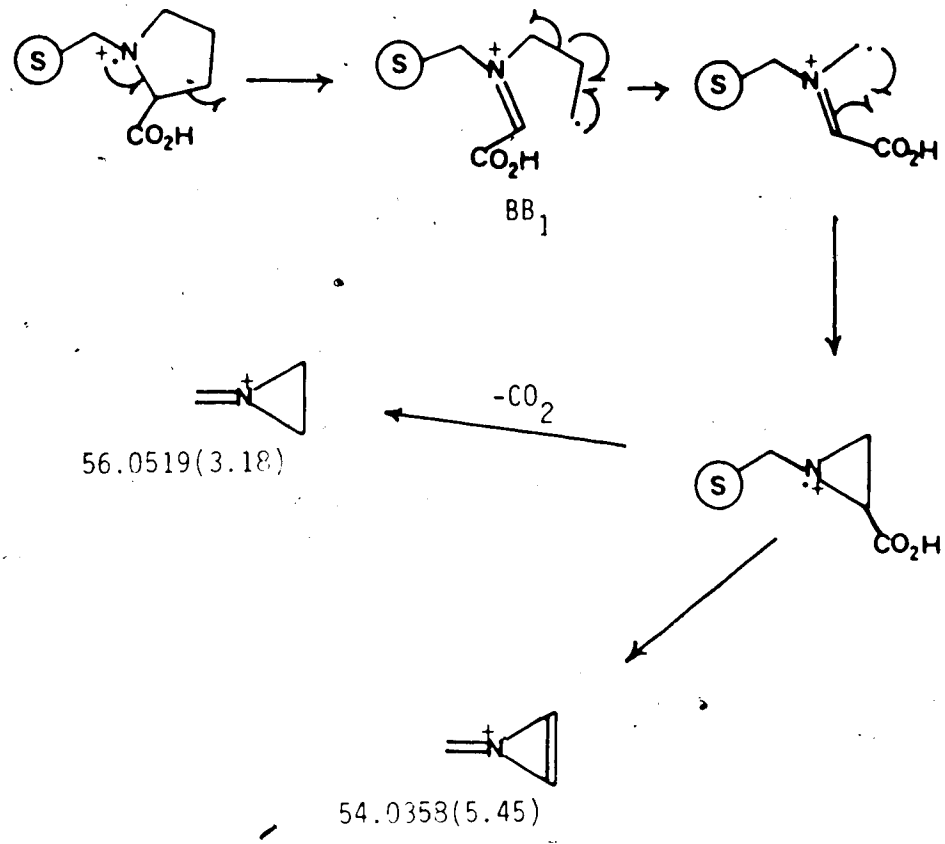
127.0382(3.84)



70.0657(100)

Before Renormalization

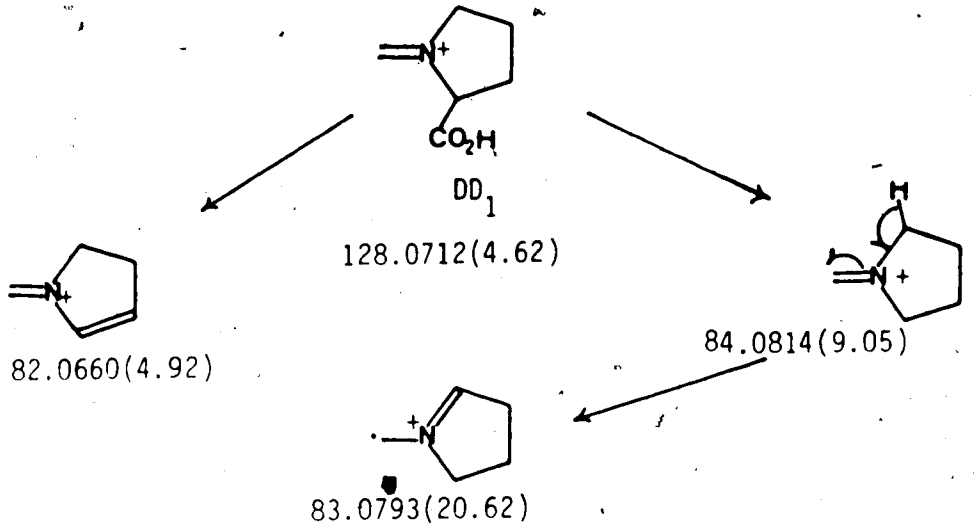
• Fragmentations of the BB series:



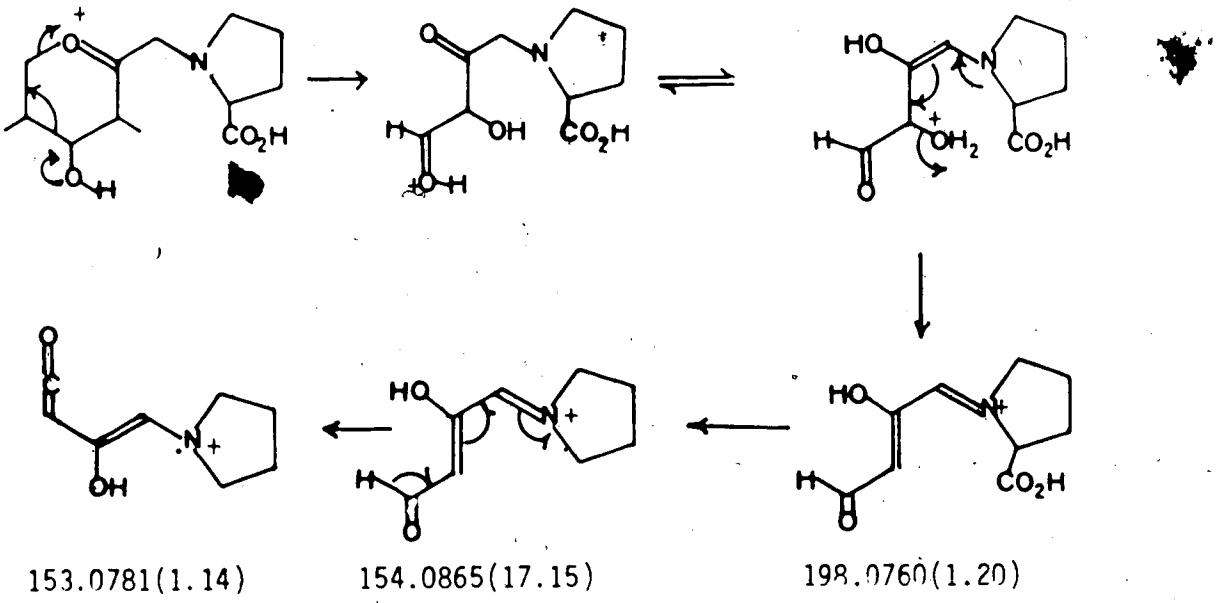
• Fragmentations of the CC series:

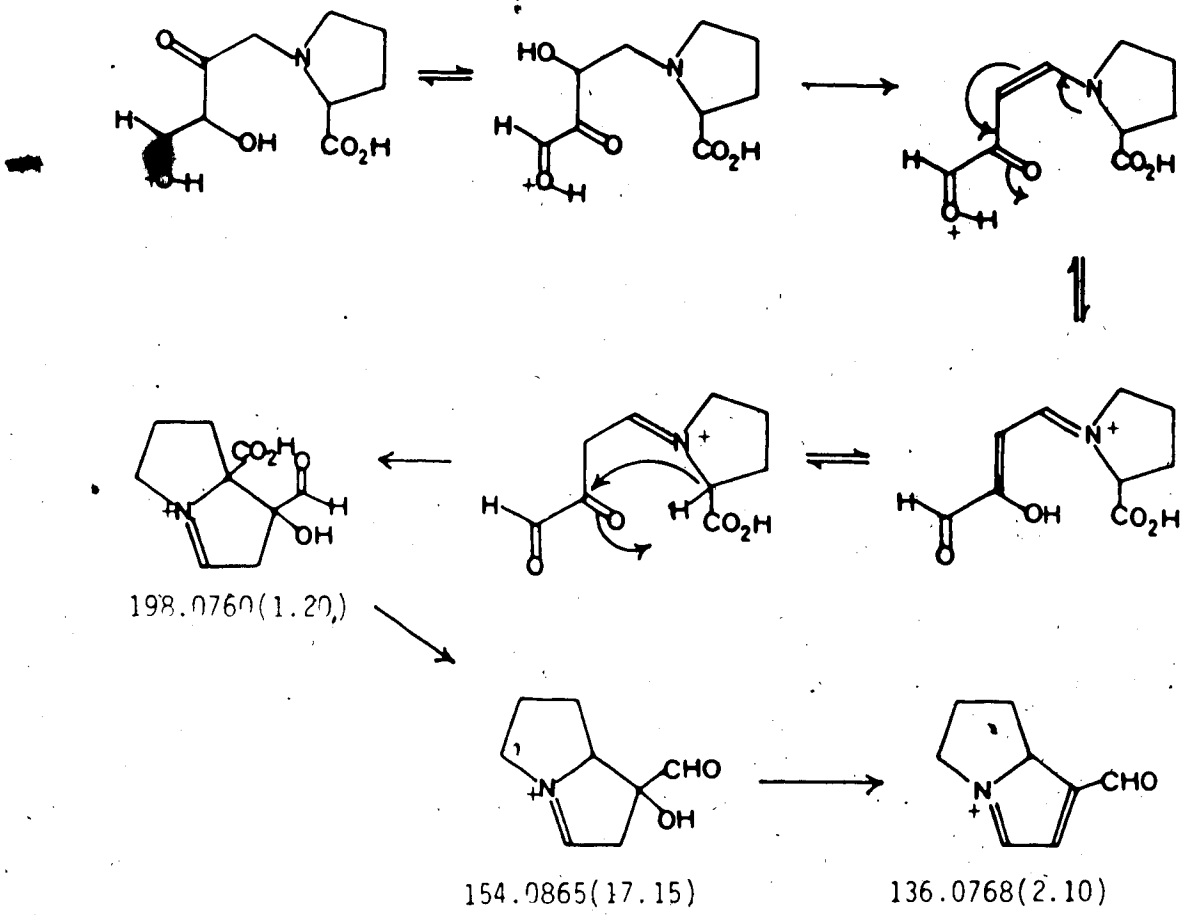
No CC series.

• **Fragmentations of the DD series:**

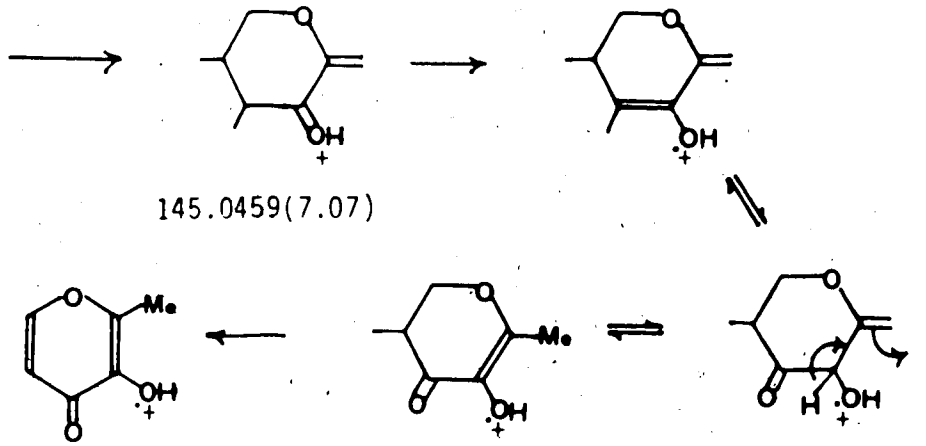


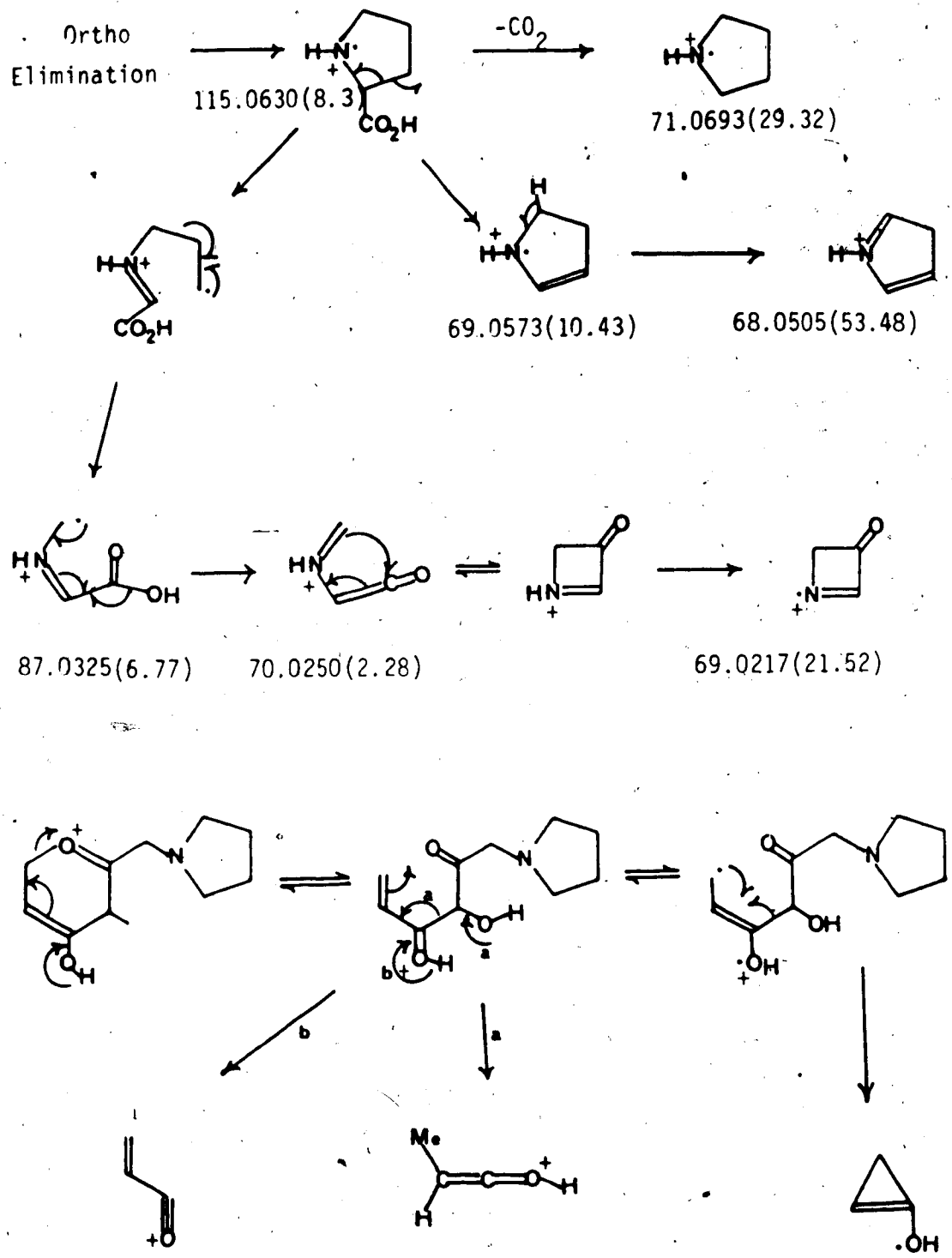
2.4.11.24 Specific fragmentations



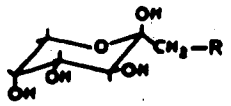


Ortho Elimination




Ortho
Elimination
115.0630(8.3)
-CO2
71.0693(29.32)
69.0573(10.43)
68.0505(53.48)
87.0325(6.77)
70.0250(2.28)
69.0217(21.52)
55.0202(56.17)
57.0362(12.23)
56.0279(20.32)+

2.4.11.25 1-[(2'-carboxy-4'-hydroxy-pyrrolidinyl]-1-deoxy-D-fructose

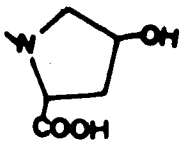


T = 250°C

No. of peaks = 101

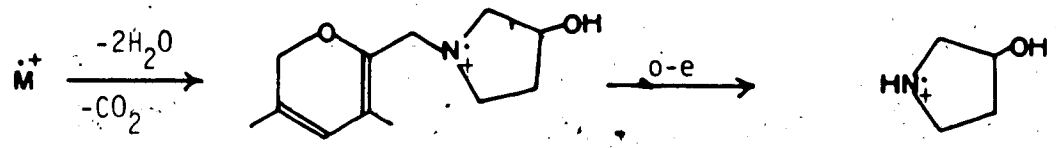
Mass range = 51.0235-213.1001

TIC: 53,606 (6,709)

R= 

2.4.11.26 "Amino acid" fragmentations

• Fragmentations of the AA series:

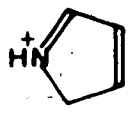


219.1001(6.89)

87.0657(8.30)

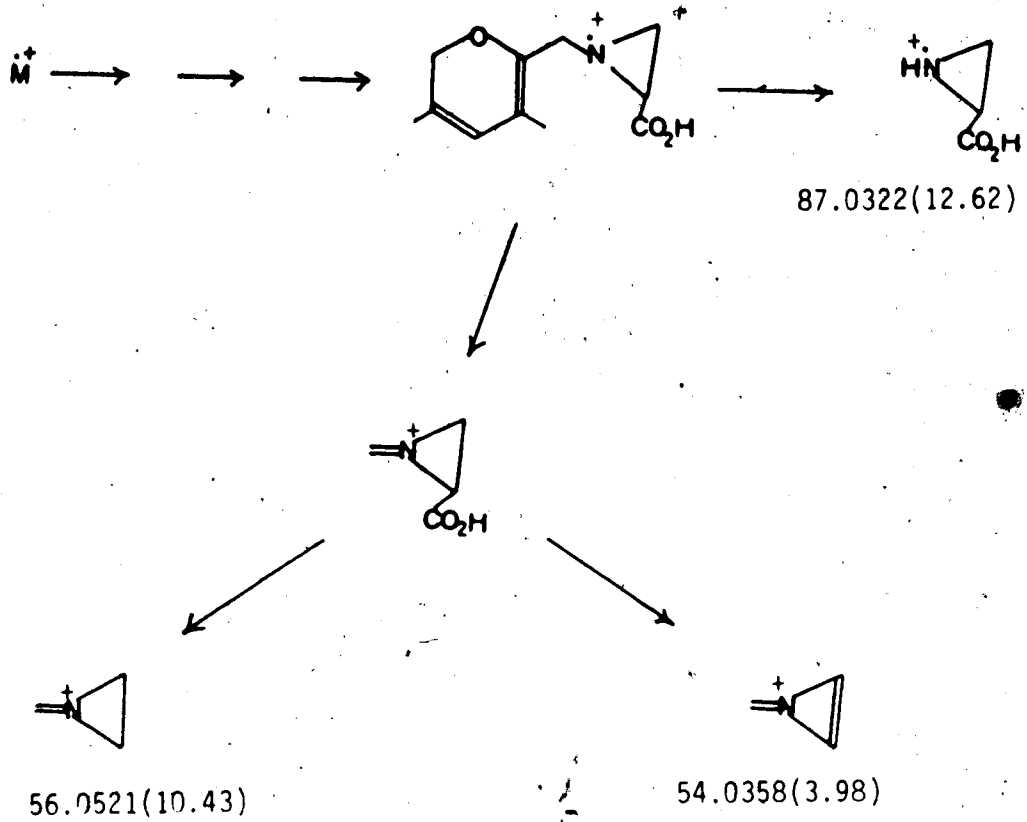
195.0893(3.91)

86.0608(100)



68.0505(62.05)

• Fragmentations of the BB series:

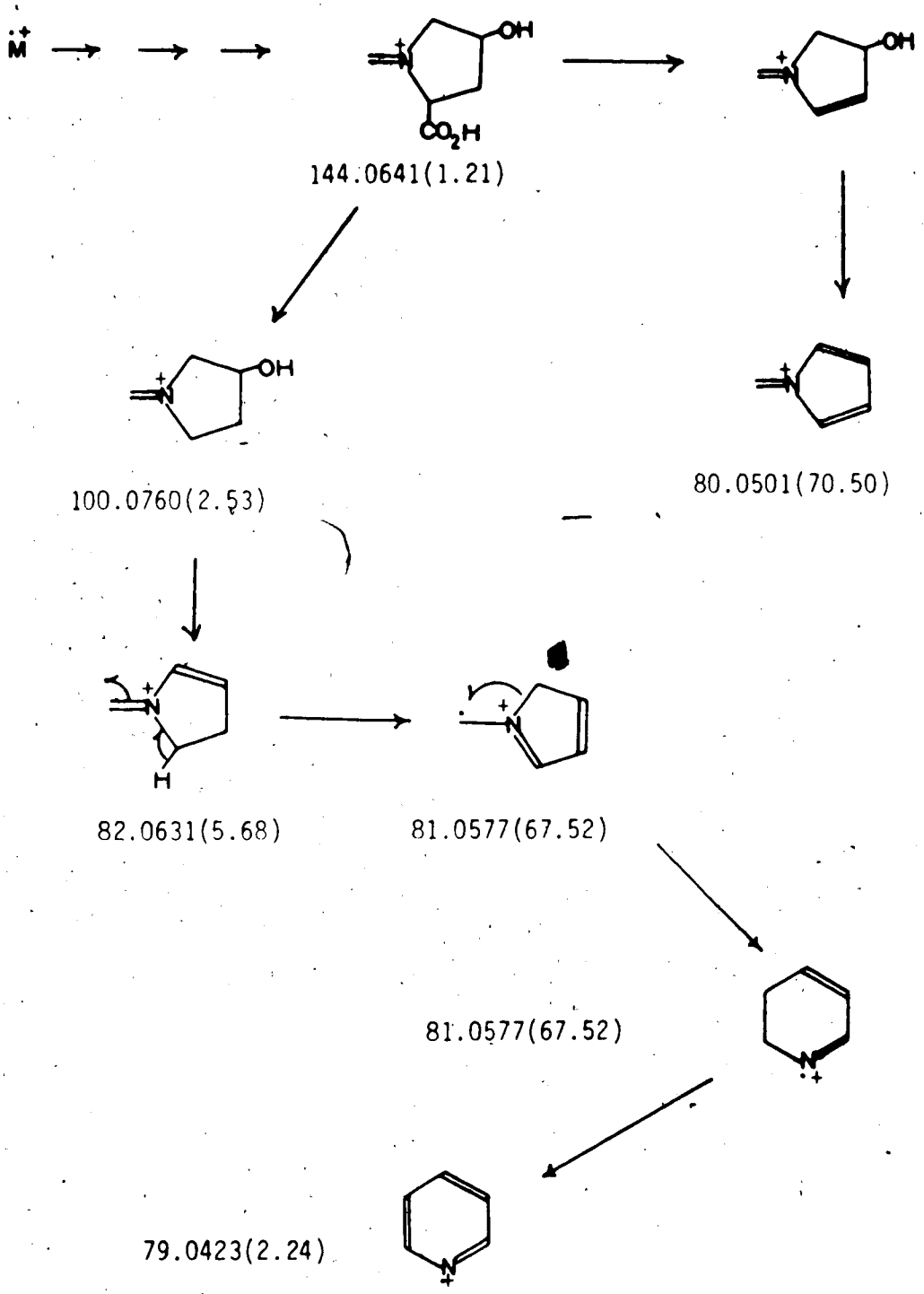


Similar mechanism to that of page 190

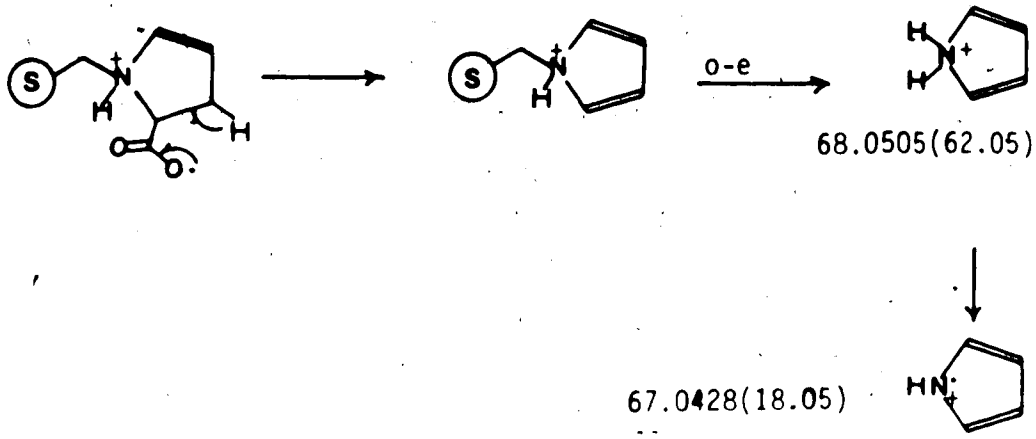
• Fragmentations of the CC series:

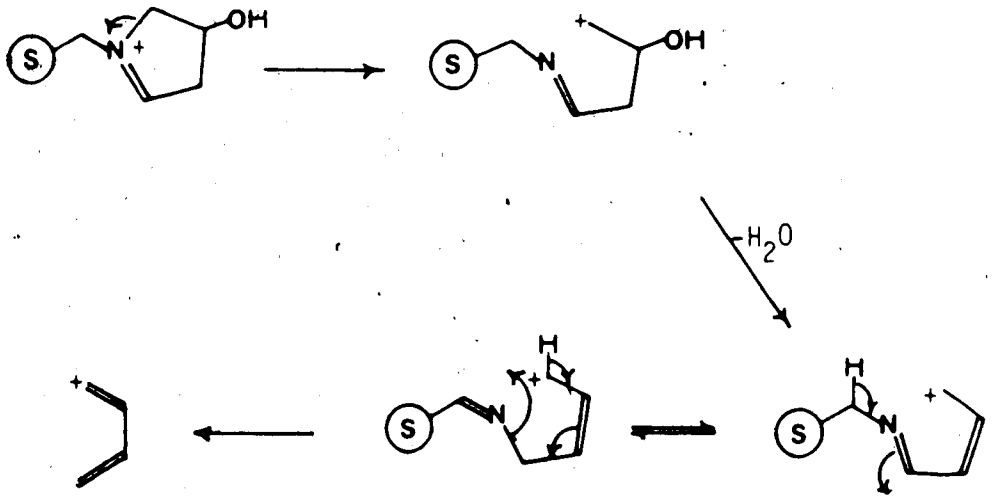
No CC series.

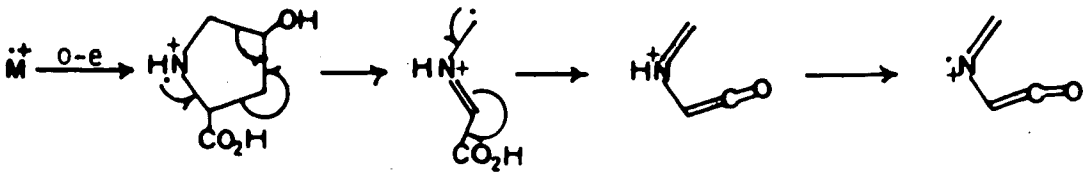
• **Fragmentations of the DD series:**

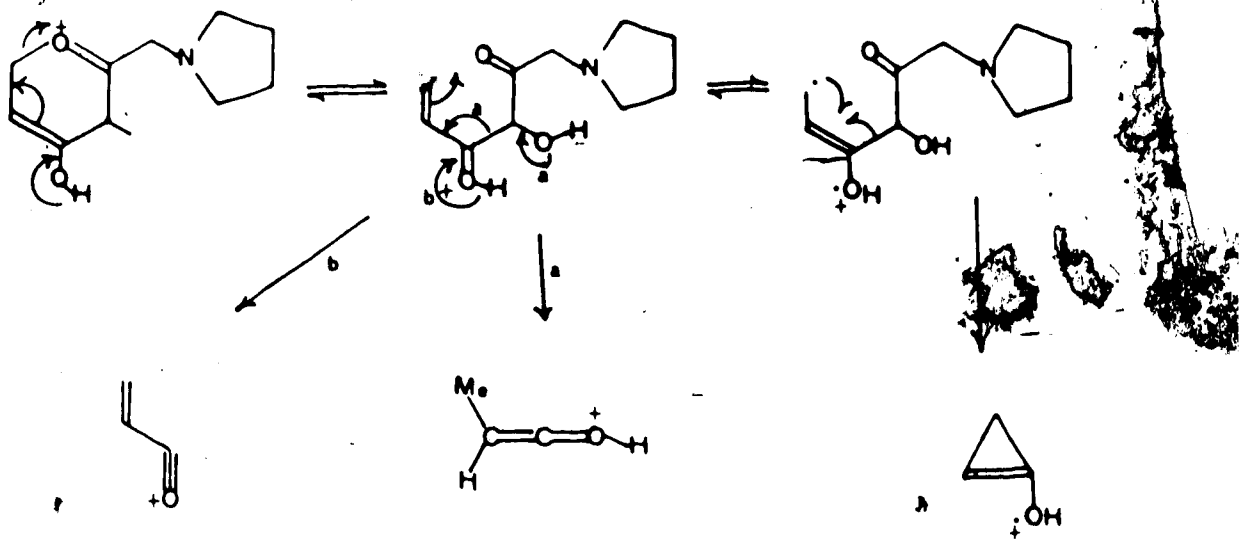


2.4.11.27 Specific fragmentations





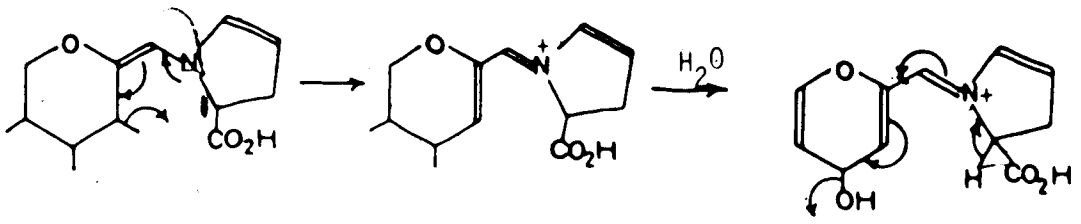




55.0201(19.91)

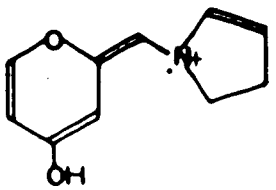
57.0362(10.05)

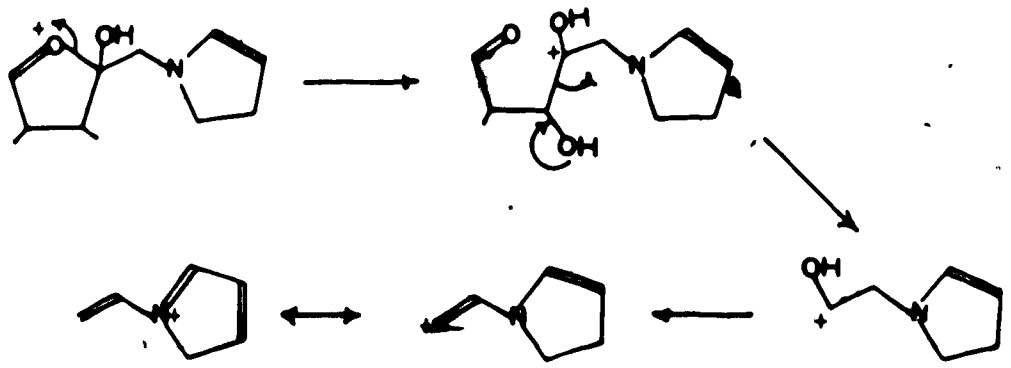
56.0282(6.20)



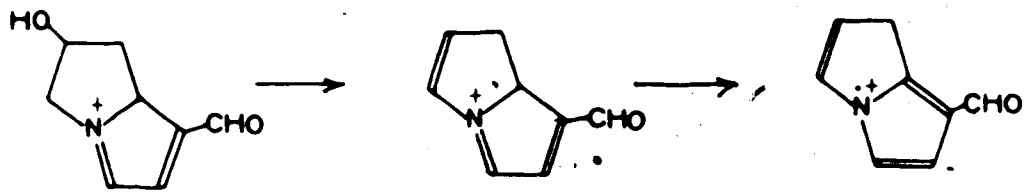
161.0829(0.75)

177.0787(5.23)





94.0656(17.75)



152.0715(1.46) 134.0602(3.92) 133.0502(9.99)

2.4.12 Discussion

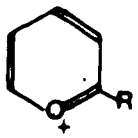
In the following section, some of the most important aspects of the fragmentation patterns of Amadori rearrangement products will be discussed. At this point it should be emphasized that, to some of the assigned structures, other possibilities also exist and some of the stipulated mechanisms can be envisaged to take place in more than one way. However, as was mentioned in the beginning of this chapter, structural assignments were made by assuming a minimum change from the known molecular ion.

The behavior of Amadori rearrangement products under electron impact conditions reflects the structural considerations mentioned earlier. As expected, the two moieties, sugar and amino acid, more or less control the fragmentation patterns if there are no interactions between the two moieties, as in the case of tryptophan and proline.

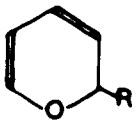
Fragmentations initiated by the fructose moiety are termed "sugar fragmentation" patterns (A, B, C, D, F, H, I, J, L, P series in the pyranoid form; A, C, E series in the furanoid form). On the other hand, fragmentations initiated by the amino acid moiety are termed "amino acid fragmentation" patterns (AA, BB, CC, DD and EE series). DD and EE series are specific to Amadori rearrangement products since these fragments incorporate C-1 of the sugar ring. In addition, the fragmentation patterns not observed in the mass spectra of fructose or the amino acids are termed "specific fragmentations".

A quick look at sugar and amino acid fragmentation patterns will follow some detailed discussion on specific fragmentations.

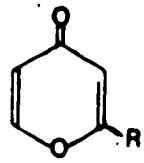
Sugar fragmentation pattern A (pyranoid form) is mainly responsible for the production of substituted pyrylium cations, pyrans, pyrons and dihydropyrans.



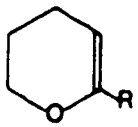
pyrylium Cation



Pyran



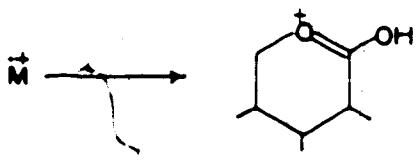
Pyrone



Dihdro-Pyran

The substituents on the ring are hydroxyl groups, originating from the sugar, and the side chain R at C-2 is H, CH_2, CH_3, CH_2-NH_2, CH_2NH-CH_3 or any other fragment of the particular amino acid, produced by the general rearrangements discussed earlier or by known cleavage reactions.

The aA (pyranoid) pattern is absent in all Amadori rearrangement products except for the case of glycine ARP.



aA_1

This can be explained by the fact that the side chain of the glycine is hydrogen, whereas the other amino acids can

initiate reactions that might stabilize the ion product, but in glycine it is easily cleaved due to lack of functionality.

Fragmentations of the B series are mainly responsible for the production of straight-chain fragments of the sugar.

Furan derivatives are produced by C and L series of the pyranoid form and A and E series of the furanoid form. These include hydroxy-, dihydro- and dihydroxyfurans, and furans with alkyl or aminoalkyl groups.

The α-cleavage of the carboxyl group of the amino acid produces the AA series. The secondary fragmentations of the AA_1 ion are either due to dehydration of the sugar ring or fragmentations with neutral losses from the amino acid side chain. The peaks of this series can give clues as to the kind of amino acid in the Amadori rearrangement product.

The BB series is due to α-cleavage of the side chain of the amino acid. BB_1 ions can undergo secondary fragmentations with loss of water molecules from the sugar ring or by ortho elimination, giving a prominent peak at m/z 74 in many mass spectra of Amadori products.

m/z 74

The CC series is indicative of the reactivity of the side chain, especially if it contains a functional group or atom with low ionization energy, for example, sulfur atom in

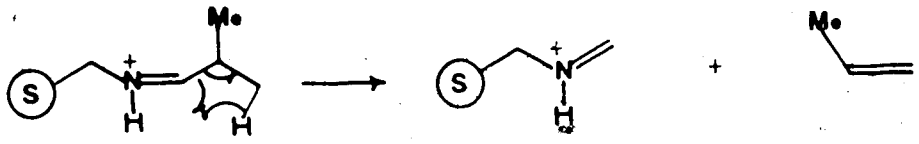
methionine.

The DD series is formed by α-cleavage of the sugar ring. The resulting DD_4 ion has one carbon atom more than the amino acid, hence, DD ions can be used as a diagnostic series for identification of Amadori rearrangement products. The same conclusion also applies to the EE series.

2.4.13 Amadori rearrangement products with alkyl side chains

Amadori rearrangement product of glycine represents an extreme case in which only the sugar fragmentation pattern is present. All the peaks of fructose are found in glycine ARP; the base peak, F_1, is the same as in fructose.

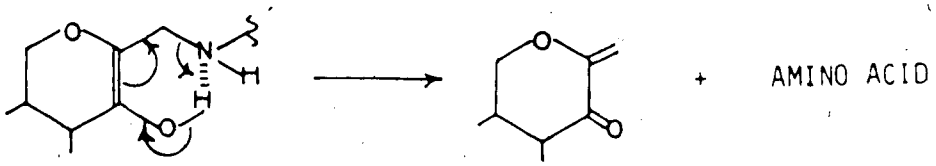
Among other ARPs with alkyl side chains, valine will be discussed as a representative compound. The secondary fragmentations of the AA_1 peak of valine include, in addition to the dehydrations of the sugar ring, a 1,3-shift of the methyl hydrogen, resulting in the loss of a neutral molecule:



AA_1

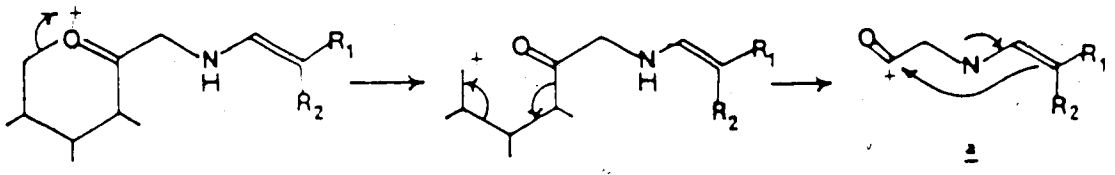
It is important to mention that the alkyl side chains in the Amadori rearrangement products do not initiate σ-ionization at the branch points (hence no CC series).

Ortho elimination is an important process in all Amadori rearrangement products. This process allows the identification of the amino acid part of the molecule. Before ortho elimination, the sugar should dehydrate to acquire a coplanar conformation with respect to the ortho substituents,



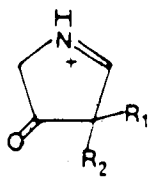
which then passes through a six-membered activated complex. This elimination can occur before or after the decarboxylation of the amino acid or its α-cleavage.

Another important fragmentation is the inductive cleavage of the oxonium ion A_1 and subsequent fragmentation of the sugar chain, giving fragment a, which can subsequently cyclize into substituted pyrolidinone b.



A_1 a

		%Int.	%Σ
GLYCINE	$R_1=R_2=H$	34(29%)	1.01
VALINE	$R_1=R_2=CH_3$	112(1%)	0.13
LEUCINE	$R_1=H$, $R_2=CH(CH_3)_2$	126(0%)	0

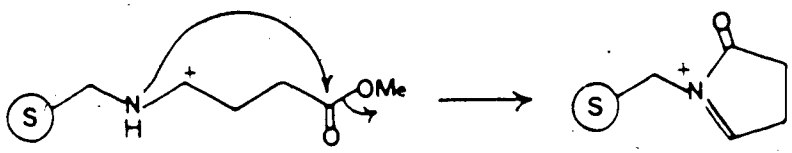


b

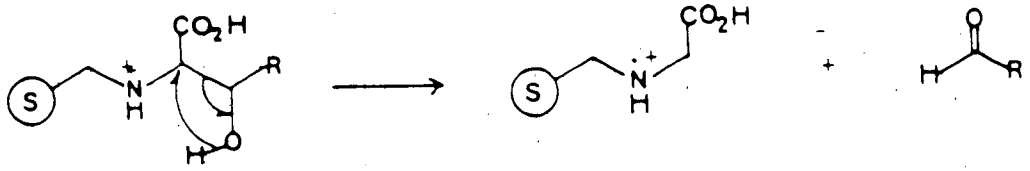
A cyclic structure is stipulated for the fragment a, after comparison of $\%\Sigma_{s,o}$ values of a for glycine, valine and leucine, since an sp^3 hybridized carbon atom in a five-membered ring (b) possesses a much smaller bond angle than an sp^2 hybridized carbon atom (a) and, hence, a larger angular strain. This will be more pronounced with larger groups, as in leucine, which does not show the peak for a.

2.4.14 Amadori rearrangement products with side chains containing carboxyl, hydroxyl or sulfur groups

The Amadori rearrangement product of glutamic acid methyl ester shows the known cyclization of the glutamic acid into a 5-membered lactam ring:



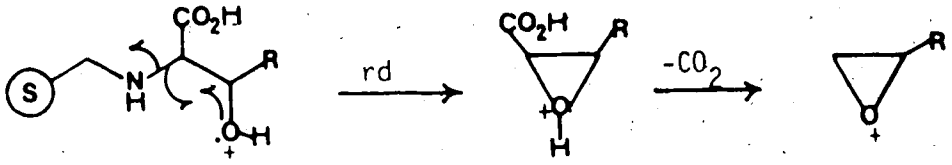
Serine and threonine ARPs show the following rearrangement:



in which the acidic hydroxy hydrogen undergoes a 1,3-shift, producing a glycine residue. This rearrangement is facilitated by the loss of a stable neutral molecule,

formaldehyde in the case of serine and acetaldehyde in the case of threonine.

Unlike th Ps with alkyl side chains, this group shows CC series:

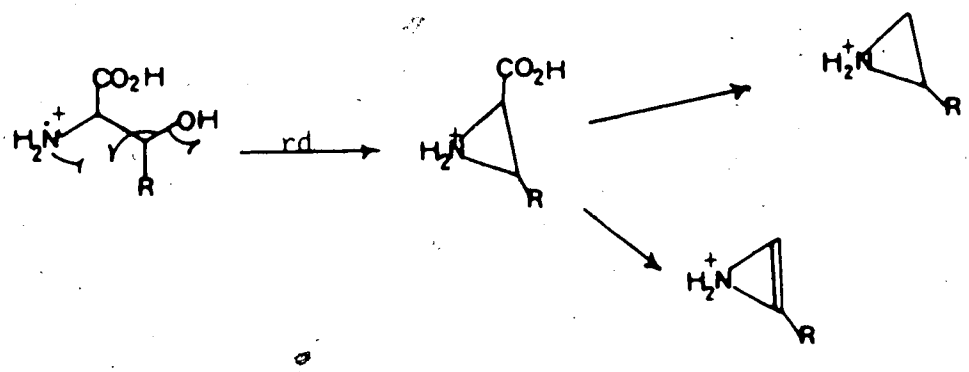


R=H, Serine

R=CH_3, Threonine

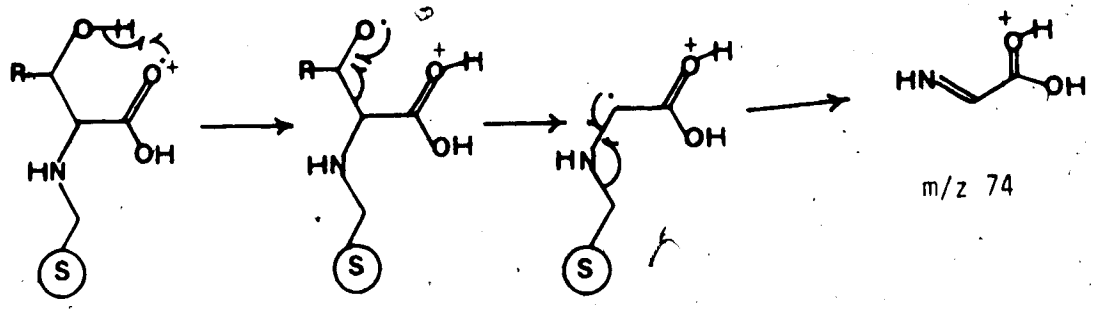
The M·, formed by ejection of an electron from the side chain hydroxyl group, undergoes a rearrangement with dispacement (rd) which involves a bond-forming step between two parts of the ion in combination with the cleavage of a bond to expel an exterior part of the ion.

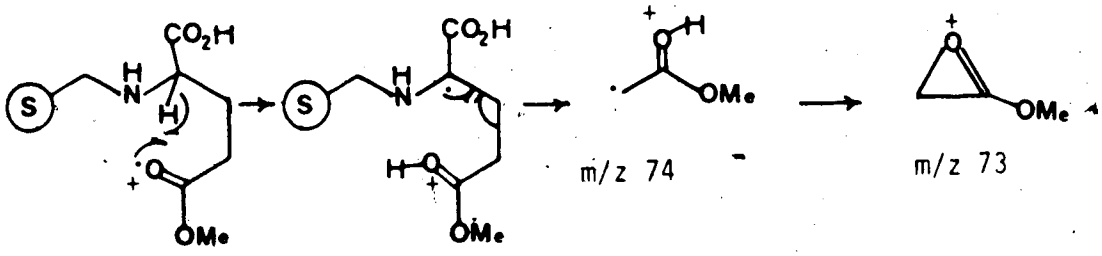
The rearrangement with displacement (rd) is also possible in the opposite direction in the fragment formed by ortho elimination in serine and threonine.



this produces aziridines and azirins, while the former gives oxiranes.

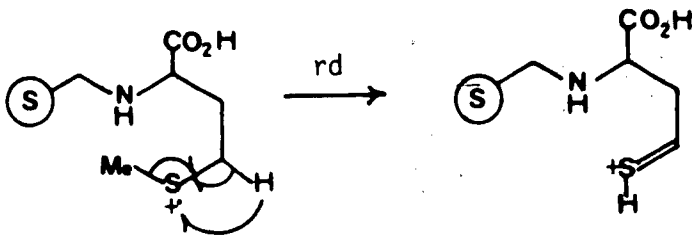
The McLafferty rearrangement is observed in serine and threonine due to the acidity of the hydroxyl hydrogen, and in glutamic acid methyl ester due to the ester group:



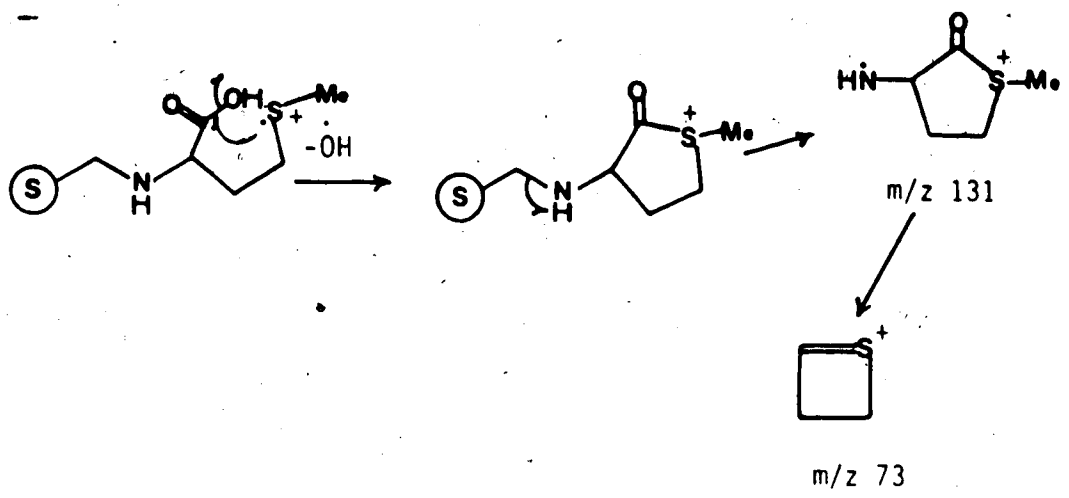


The inductive ring opening of fragment A_1 is also observed in this group.

Another example of rearrangement with displacement (rd) is observed in methionine ARP in the CC series.

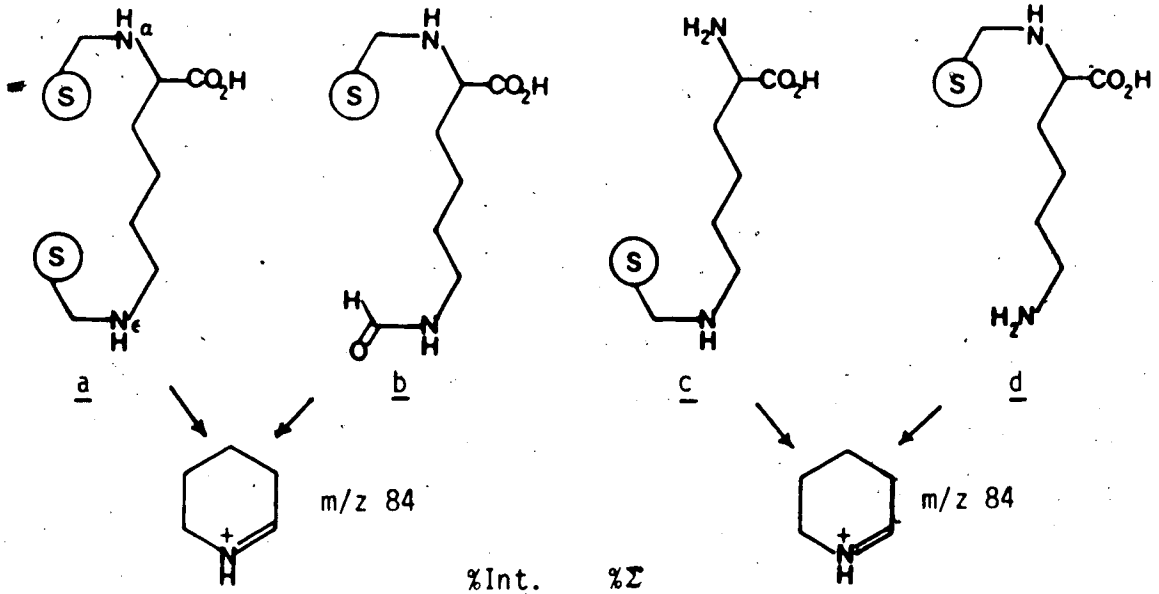


A cyclization, similar to that of glutamic acid methyl ester ARP, is observed in methionine ARP, giving rise to thiolactone at m/z 131.



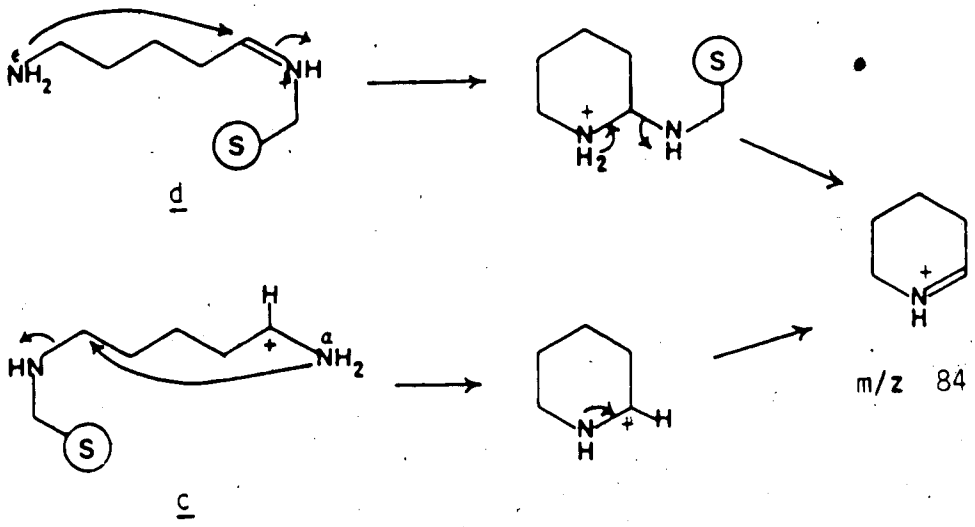
2.4.15 Amadori rearrangement products with basic side chains

The following four derivatives of lysine were synthesized:

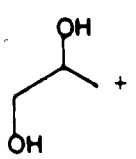


	%Int.	%Σ
a	3.35	1.25
b	4.81	1.30
c	100	19.26
d	100	24.18

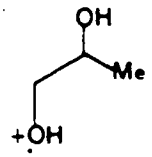
The prominent reaction in the lysine derivatives having a free amino group (c and d) is the cyclization into the piperidinum cation at m/z 84. From the above results, it can be concluded that this intramolecular cyclization can occur by two different mechanisms involving either Nα or Nε as the nucleophilic atom, and that the bulky sugar group prevents this cyclization to a great extent due to steric hindrance.



When Nε is formylated (b), the open-chain conformation of the sugar predominates, contrary to all other ARPs, giving rise to unique peaks at m/z 75 and m/z 76.



m/z 75(65%)

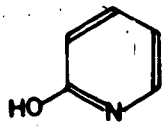


m/z 76(32%)

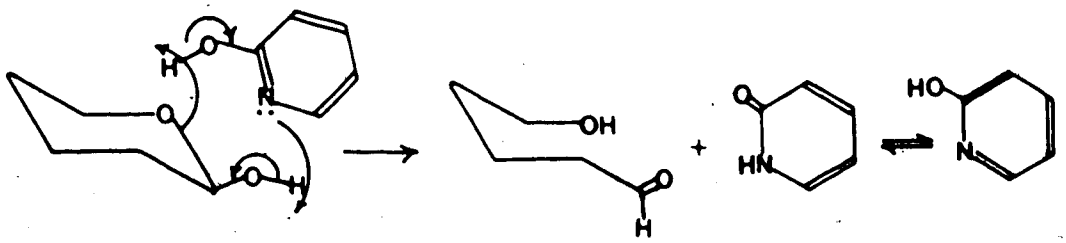
These two peaks are completely absent, not only from the other three derivatives of lysine but also from all other

mass spectra of ARPs.

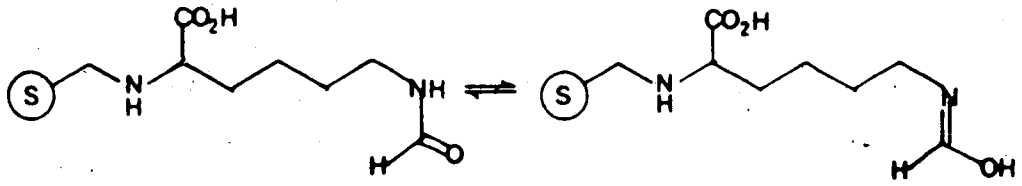
This phenomenon can be explained by analogy to the catalytic effect of α-hydroxy pyridine on mutarotation of sugars (Lemieux *et al.*, 1971).



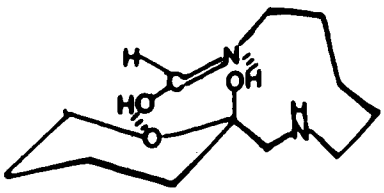
α-Hydroxypyridine



α-hydroxy pyridine can act as an acid through the hydroxyl group and as a base through the nitrogen atom and thus assist in opening the sugar ring, as shown above.

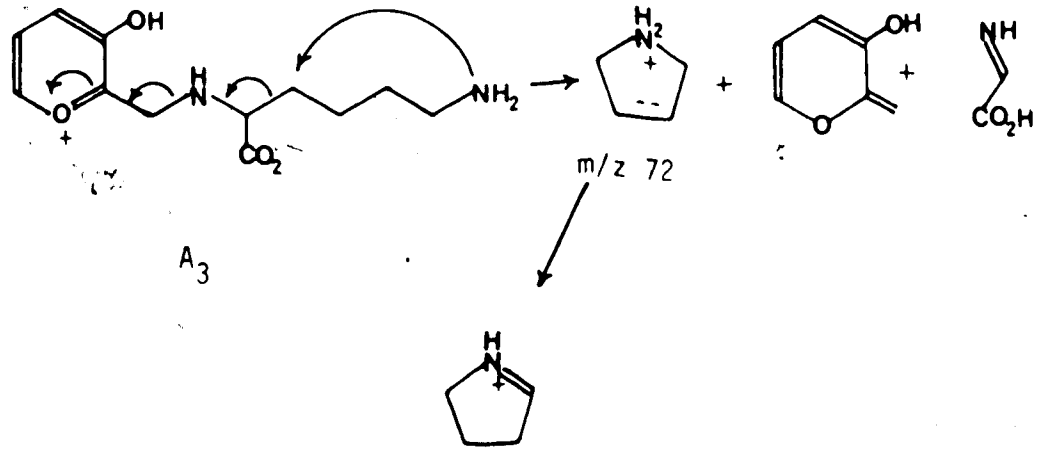


The tautomeric equilibrium between the hydroxy imine and the formyl amine can catalyze, like α-hydroxy pyridine, the mutarotation of the sugar which is attached to it. A model of the molecule shows the proximity of the hydroxy imine to the catalytic site, as shown below:

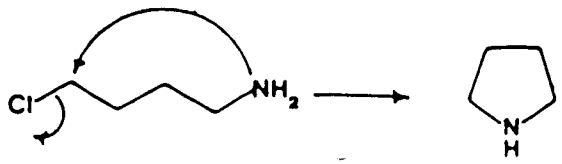


Due to the predominance of the open-chain form of the sugar in the Nε-formyl ARP of lysine, the A series is almost absent.

Another key feature of the mass spectra of lysines with free amino group is the "domino effect", which is an intramolecular nucleophilic substitution, followed by two concerted trans-elimination reactions.



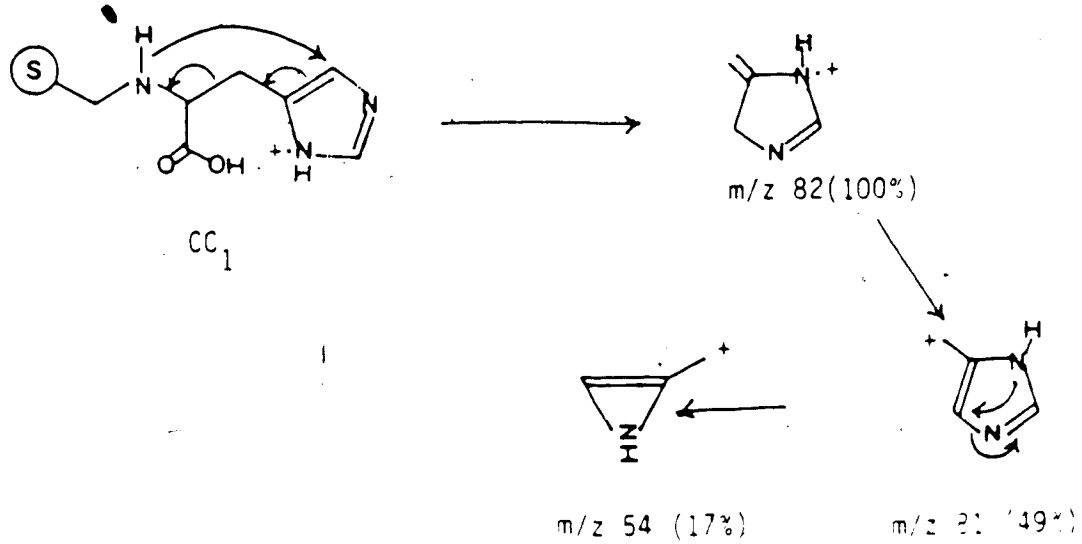
Intramolecular nucleophilic substitution reactions are used for preparation of cyclic compounds, like pyrrolidine from 4-chlorobutylamine.



4-Chlorobutylamine Pyrrolidine

The driving force for the "domino effect" is the positive charge on the oxonium ion and the loss of two stable neutral molecules.

On the other hand, the most prominent peaks in the mass spectrum of histidine ARP are due to the CC series and to the rearrangement shown below:



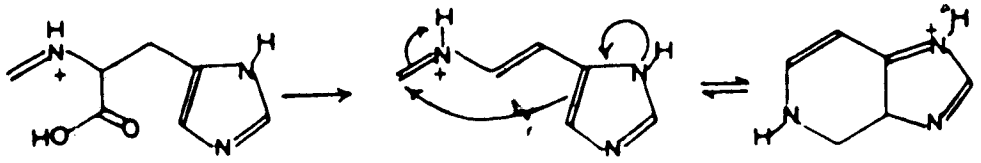
CC_1

m/z 82(100%)

m/z 54 (17%) m/z 81 (49%)

This rearrangement is facilitated by the fact that the activated complex passes through a six-membered ring.

An interesting intramolecular cyclization of DD_1 fragment in the mass spectrum of histidine ARP produces imidazo-pyridine derivatives:

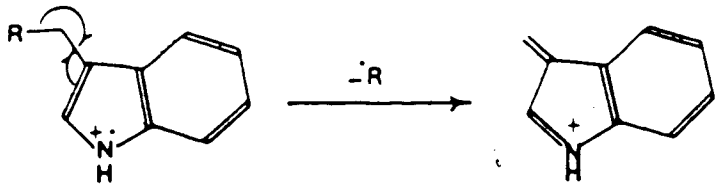


DD_1

m/z 122

2.4.16 Amadori rearrangement products with aromatic side chains

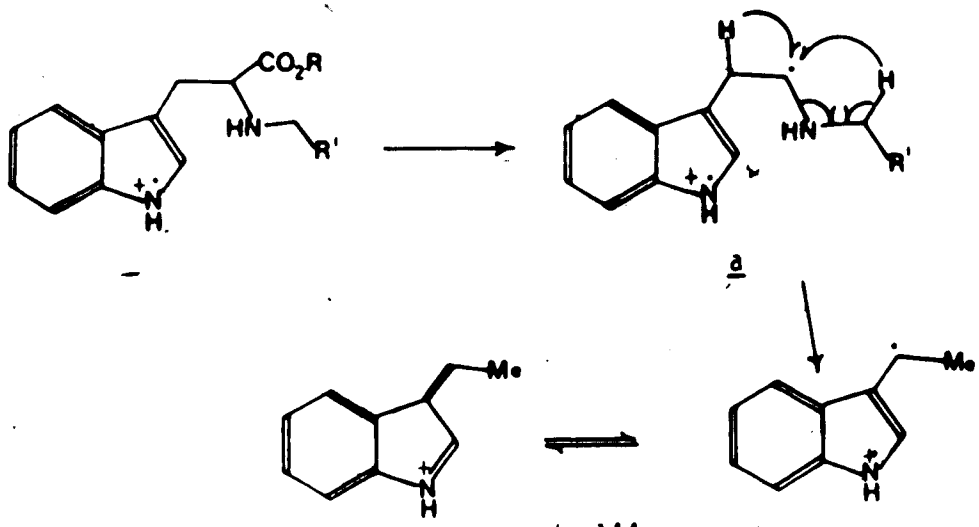
The base peak at m/z 130 in the mass spectrum of tryptophan ARP is produced by ejecting an electron from the indole nitrogen.



m/z 130

In fact, many of the fragmentations of naturally occurring derivatives of indoles can be rationalized in this way (Djerassi *et al.*, 1964), and the peak at m/z 130 is by far the most abundant ion in all derivatives of indole alkaloids.

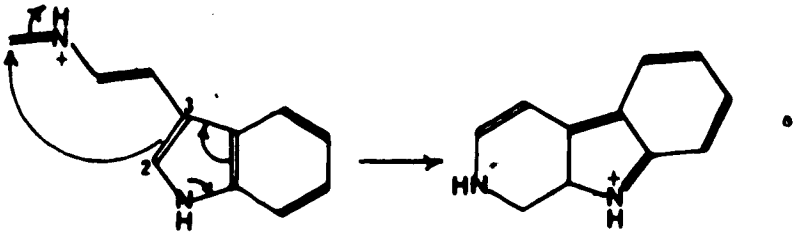
Another characteristic fragmentation of indole derivatives merits comment:



The driving force for this multiple bond scission and hydrogen rearrangement from an intermediate such as a would have to be ascribed to the stability of the ion at m/z 144 and the favorable nature of the expelled neutral species (H-N=CH-R').

However, the most prominent feature of the mass spectrum of tryptophan ARP is the peaks due to derivatives of β-carbolines. These can be formed by two distinct mechanisms.

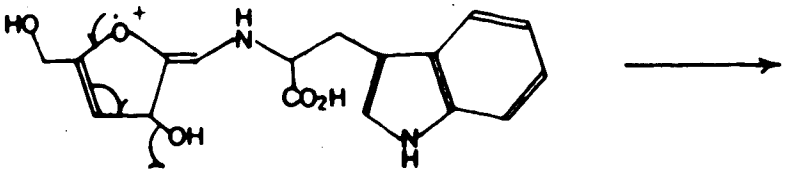
The first mechanism involves intramolecular cyclization of the DD' ion, in a manner similar to that observed in the case of histidine.

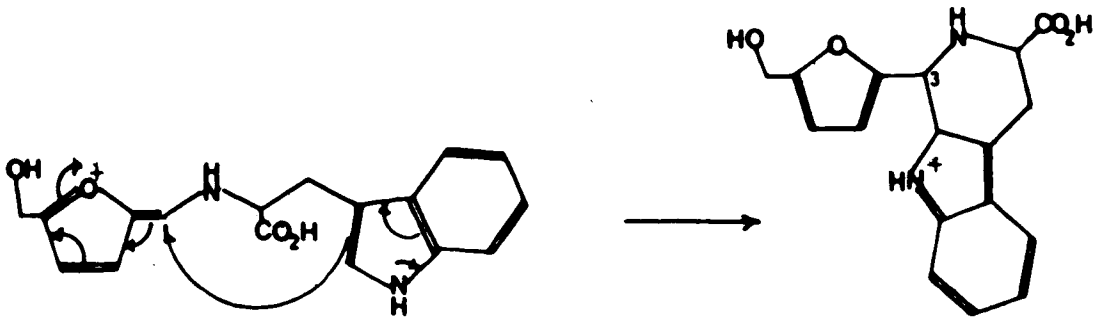


DD_1^1 m/z 171

In general, the electophilic substitution of indoles yields C_3-derivatives, however, if this position is blocked, as in this case, the substituent enters position 2 (position 3 has the highest π-electron density).

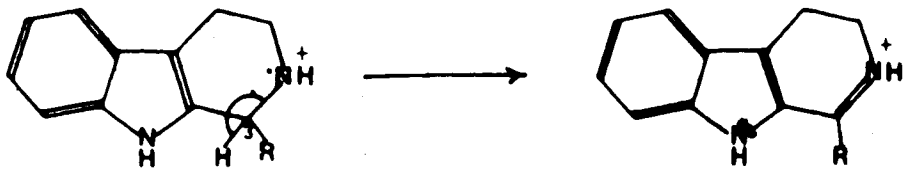
The second mechanism involves the A series of the furanoid form:





In the first mechanism, the driving force for the intermolecular cyclization is the electrophilic carbon atom of the immonium ion (DD^{+}), whereas in this mechanism it is the electrophilic carbon in the oxonium ion of the sugar moiety. This mechanism produces substituted β-carbolines at C-3, mostly furan derivatives.

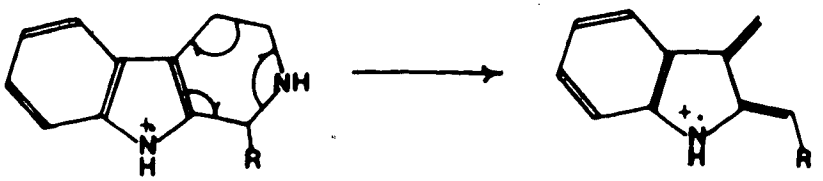
β-carbolines undergo very characteristic decompositions. One of the chief diagnostic features of their mass spectra is a pronounced M-1 peak.



b

This peak is predominantly due to the loss of the hydrogen attached to C-3, with formation of species b, in which the positive charge is stabilized by conjugation with the indole system.

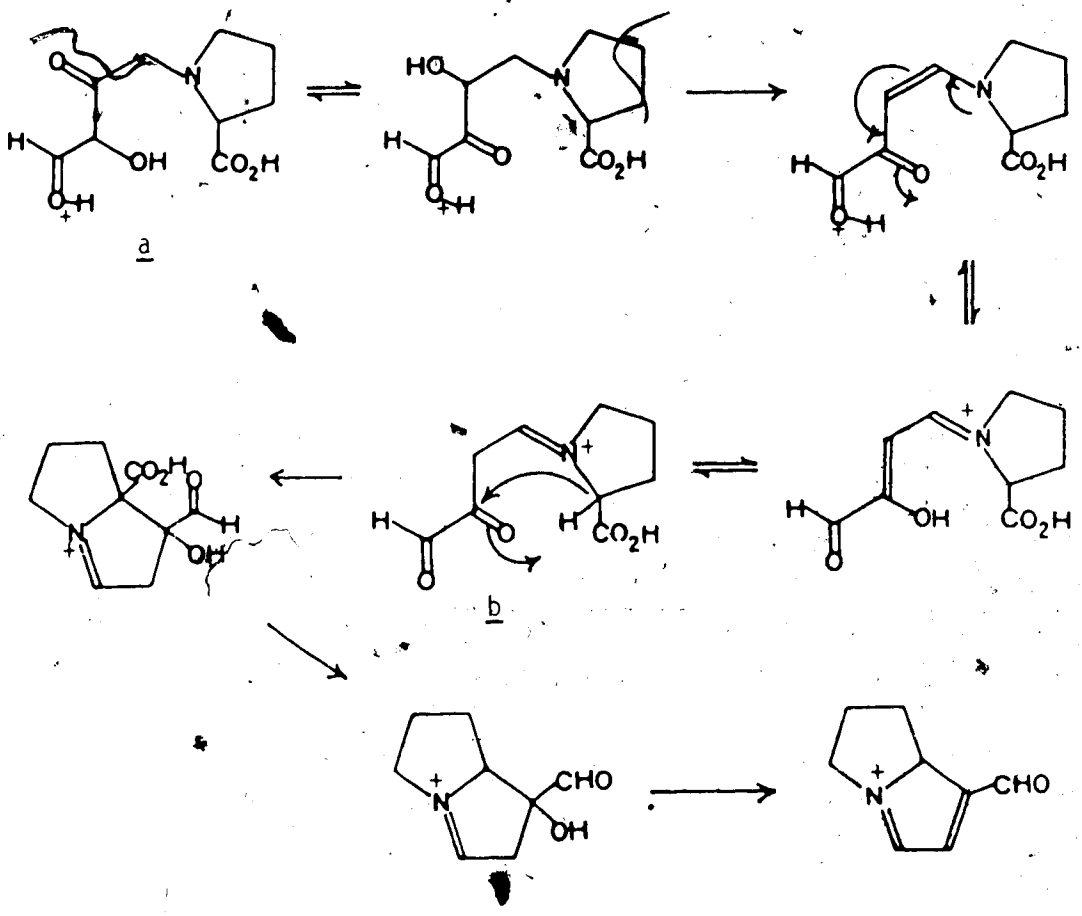
Another characteristic decomposition of β-carbolines is retro-aldolization:



m/z 143 (R=H)

2.4.17 Amadori rearrangement products of imino acids

Proline and hydroxy-proline are other examples in which the interaction between amino acid and sugar moieties produces fused heterocyclic compounds.



The inductive cleavage of the A_1 ion gives rise to the aldehyde (a) which then undergoes a series of isomerizations after a dehydration step, to give the diketone (b) which subsequently cyclizes due to the acidity of the hydrogen at C-2, thus giving rise to substituted pyrrolizines.

3. IMPLICATIONS OF THE FRAGMENTATION PATTERNS OF AMADORI COMPOUNDS TO THE MECHANISM OF THE MAILLARD REACTION

The established importance of the Maillard reaction for food acceptance led us to investigate the fragmentation of Amadori compounds under electron impact, since, in doing so, the effect of changing amino acid moiety would be evident on the products and the degradation pathways and would provide us with important insights into their chemical behavior. Since the fragment ions are not isolated, only indirect evidence can be presented in the course of the analysis of mass spectra, and, in order to compensate for this deficiency, some of the structures assigned to the important fragments will be compared to products isolated from similar amino acid-sugar model systems or to the products obtained from the pyrolysis of the Amadori compound.

Browning model systems have routinely been applied to the study of complex food systems. Numerous chemicals have been isolated (Shibamoto, 1983) and identified in the reaction mixtures from model systems consisting of an amine or amino acid and a sugar. The major constituent of the organic solvent extracts prepared from these model systems have been identified as heterocyclic compounds, such as furans, thiophenes, pyrroles, pyrazines, imidazoles and other heterocyclic compounds.

Another approach to the browning reaction has been the study of pyrolysis products of Amadori compounds at different temperatures. This approach eliminates the

possibility of the identified products originating from the sugar or the amino acid alone.

Studies on the pyrolysis of Amadori compounds indicate that they are precursors of many organoleptically important volatile compounds (Hodge *et al.*, 1970a) in addition to being intermediates in the formation of brown color. The intermediacy of Amadori compounds in the Maillard reaction provides a low-energy route to the thermal degradation of their amino acid and sugar moieties (Birch *et al.*, 1980).

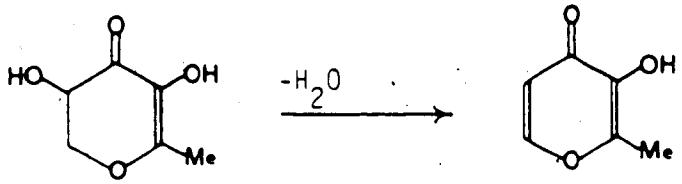
Evidence suggests that degradation through 1,2-enolization is the main pathway to brown color, whereas 2,3-enolization is more important in flavor production (Reynolds, 1970).

It has been proposed (Reynolds, 1963) that the basicity of the amino moiety influences the pathway by which Amadori compounds undergo degradation. According to this hypothesis, the relative amounts of 1,2- and 2,3-enolizations occurring during degradation depend on the basicity of the amino substituent.

3.1 Effect of amino acid moiety on the mechanism of the Maillard reaction

There are conflicting reports in the literature regarding the influence of the amino acid moiety, in terms of enolizations, on the future direction of the Amadori compounds. According to Birch *et al.* (1984), there is no overall relationship between the basicity of the amino acid

moiety and the quantities of the expected products from the pyrolysate of the Amadori product. On the other hand, according to Hodge and Mills (1976), weakly basic amines will prefer 1,2-enolization and strongly basic amines will undergo 2,3-enolization (Hodge and Mills, 1976). According to the former view, the primary event in the decomposition of Amadori compounds is the scission of the carbon-nitrogen bond at C-1 of the sugar, releasing the free amine, then the resultant fructosyl moiety may degrade via 1,2- or 2,3-enol intermediates, without the influence of the amino acid moiety. However, Hodge and Mills (1976) concluded that the Amadori products undergo 1,2- or 2,3-enolizations while the amino acid moiety is attached. Then the formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran--4-one (62)



62 Maltol

in these systems is evidence for the operation of the 2,3-enolization pathway, whereas the isolation of 2-furaldehydes, N-substituted pyrroles, indicates that 1,2-enolization has occurred. However, it is very difficult to determine the relative importance of the two pathways since some products can be formed by either route. One reason for this confusion is the use of pk values as basicity

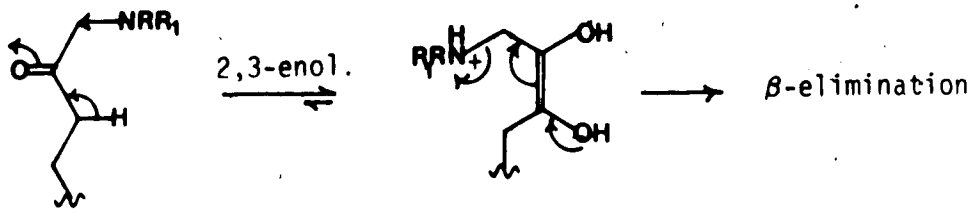
indicators while studying pyrolytic reactions, which are gas phase reactions. In aqueous solutions (2° amine > 3° amine), the basicities of amines are different from in the gas phase due to solvent effect (Brauman *et al.*, 1971).

According to Anet (1964), a 2-ketose may enolize in two ways: by the loss of a proton from either C-1 or C-3. A β-elimination can then take place from C-3, in the first case, and, in the second, from either C-1 or C-4. Under alkaline conditions, the enolizations are rapid and reversible, so that the direction of the overall reaction depends on the relative ease of elimination of the functional groups at C-1, C-3 and C-4. Under acidic conditions, the elimination from D-fructose is nearly all from C-3, that is, with 1,2-enolization, since the main product is 5-(hydroxymethyl)-2-furaldehyde. However, a small proportion of 2-hydroxyacetylfuran (Miller and Cantor, 1952) is obtained, indicating 2,3-enolization with the elimination of the hydroxyl group from C-4. Therefore, the ratio of the products indicates that, in acid, a proton is more easily removed from C-1 than from C-3.

Amadori compounds in <u>acidic solution</u> decompose by 1,2-enolization followed by the elimination of the functional group from C-3. However, <u>under weakly alkaline</u> conditions those compounds derived from strongly basic secondary amines, such as morpholine, proline, etc., eliminate the amine from C-1, with 2,3-enolization (Simon, 1962). Degradation products arising from 2,3-enolization

with elimination from C-4 have not been observed with Amadori compounds.

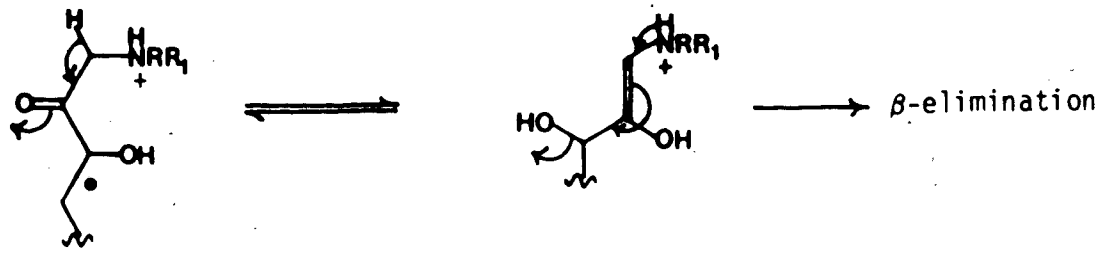
For enolization to take place, Amadori compounds must be in one of the keto forms. In the free-base form (63),



63 64

the flow of electrons from the nitrogen atom increases the electron density at C-1 and makes 1,2-enolization more difficult. This effect is more important with strongly basic amines and may be negligible with weakly basic amines. Therefore, under alkaline conditions Amadori compounds derived from strong bases undergo 2,3-enolization. Hence, the degradations involving 2,3-enolization with amine elimination are best carried out in the presence of amine salts with excess amine (Hodge and Nelson, 1961).

Under acidic conditions, the Amadori product is in the salt form (65);



65 66

1,2-enolization is assisted by the withdrawal of electrons from C-1 by the positively charged nitrogen atom. This effect is strongest with derivatives of weak bases. Protonation of the carbonyl group would accelerate the reaction. The elimination from C-3, as shown in 66, is assisted by the amines being in the free-base form.

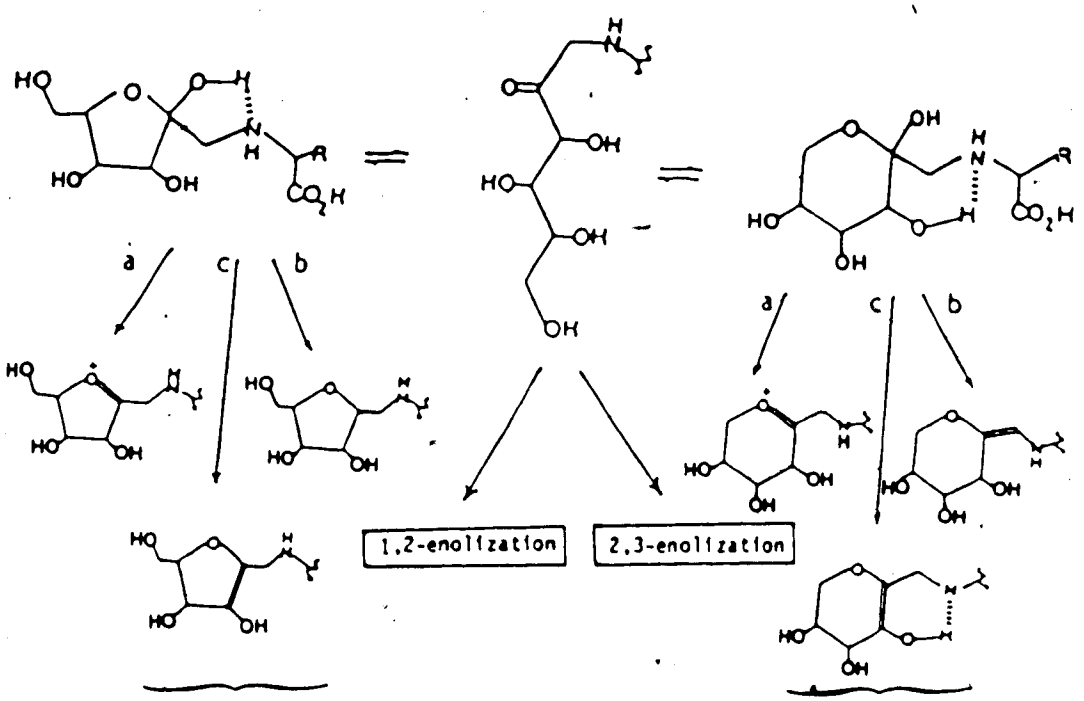
At a low pH, strongly basic, as well as weakly basic derivatives, decompose, although slowly, by 1,2-enolization and C-3 elimination. In the decompositions that go most smoothly, both the salt and the base forms of Amadori product are required, one form assisting the enolization and the other the elimination. Therefore, the determining factor for the future course of the degradation of Amadori compounds is the pH of the medium. At alkaline pH's, strongly basic ARP's will undergo 2,3-enolization, whereas at highly acidic pH's ARP's with weakly basic amino acid will mostly undergo 1,2-enolization.

In order to ascertain the effect of the basicity of amino acid moieties in the degradation of Amadori compounds, irrespective of the pH of the medium, studies of the fragmentation of these products should be done in the gaseous phase (pyrolysis, electron impact). Since the method of choice was electron impact mass spectroscopy, a relationship should be found between gas-phase basicities of amino acids and their ionization energies. According to Morishima *et al.* (1974), a relationship does exist between ionization energies, basicities and the hybridized nature of the lone

pair electrons in amines. According to this study, as the percent S character of the lone pair electrons of nitrogen increases, the ionization energy increases with subsequent decrease in basicity. Theoretically, therefore, those Amadori compounds that show dominant "amino acid fragments" should have relatively lower ionization energies for the nitrogen atom and, hence, will have a higher gas phase basicity and *vice versa*.

The assumption that Amadori compounds undergo decomposition by 1,2- or 2,3-enolizations is based on Lobrey de Bruyn-Alberda van Ekenstein transformation (Speck, 1958). This transformation is subject to general acid-base catalysis and, although its highest rate is in alkaline solution, it also takes place under neutral and acidic conditions, but at very slow rates. The open-chain form of the compound must be present for 1,2- and 2,3-enolizations.

According to ^{13}C-NMR studies, 99% of the Amadori compounds (regardless of the kind of amino acid) exist in cyclic forms and only 1% are in open chain form. Therefore, it is not realistic to evoke acyclic, open chain forms in proposing a mechanism for the Maillard reaction. High pH conditions are required for attaining reasonable reaction rates, especially with 1,2- and 2,3-enolizations, and in food systems this is seldom achieved. Therefore, the new reaction mechanism shown in Figure 3.1 is proposed. It is based on fragmentations observed in the mass spectra of Amadori compounds.



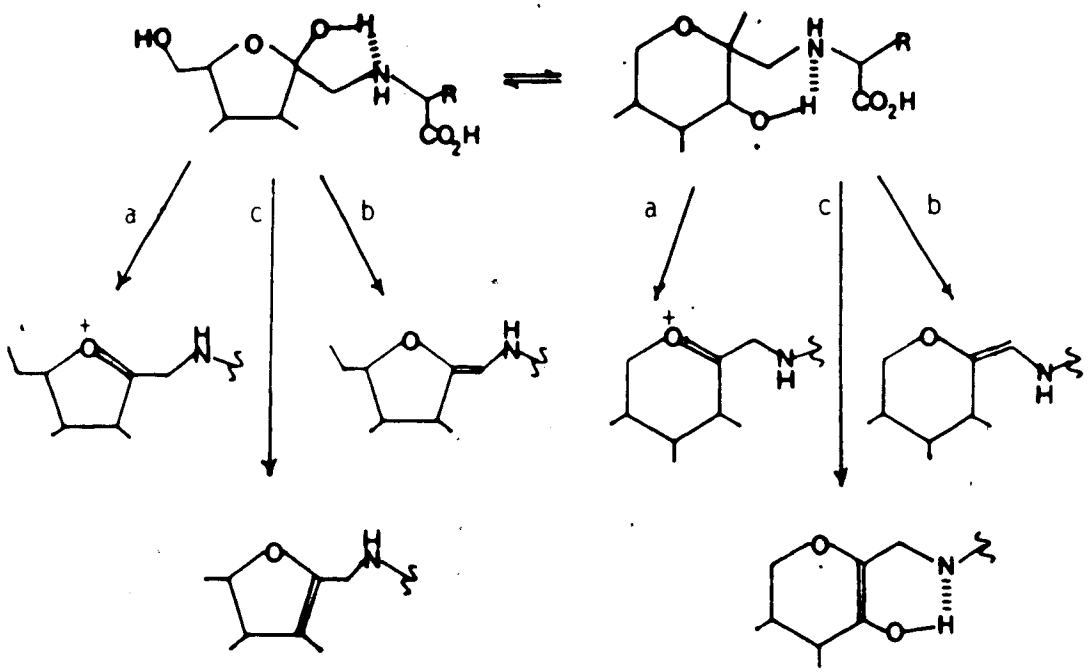
-Dehydrations of the furanoid ring.
-Secondary fragmentations of the amino acid moiety.
-Ring opening.
-Etc.

-Dehydrations of the pyranoid ring.
-Secondary fragmentations of the amino acid moiety.
-Ortho elimination.
-Ring opening.
-Etc.

a 0,2-dehydroxylation
b 1,2-dehydration
c 2,3-dehydration

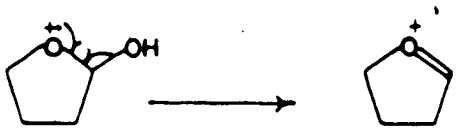
Figure 3.1 Proposed mechanism for the Maillard reaction.

Acyclic 2-ketoses may decompose by enolization at two positions, 1,2 and 2,3, whereas cyclic forms decompose by 1,2- or 2,3-dehydrations and O,2-dehydroxylations at the anomeric hydroxyl group since the presence of two oxygen-containing groups on the same carbon provides a powerful reaction initiating site.



a O,2-dehydroxylation
b 1,2-dehydration
c 2,3-dehydration

The driving force for path a is the creation of a stable oxonium ion (O,2-dehydroxylation). In the mass spectrometer this path becomes dominant when the molecular ion is formed at the ring oxygen and, since five-membered oxonium ions are more planar than six-membered ones, it is



expected that the furanoid oxonium ion will be formed at a faster rate. However, the ratio of furanoid to pyranoid forms is 38/61, so the final concentrations of each form might be comparable. In food systems, this path might be preferred in cases of high ionic strength or polar conditions.

Paths b and c cannot be distinguished by mass spectroscopy.

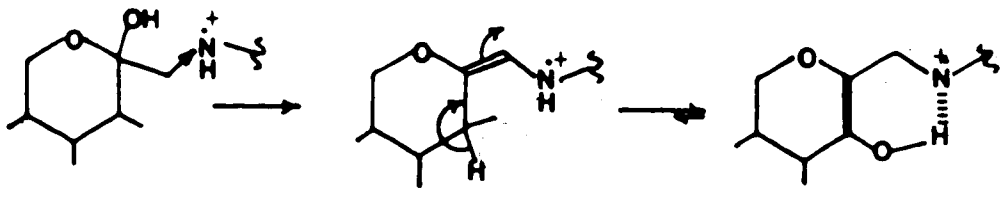
68 67

However, the 2,3-dehydration path is preferable over 1,2-dehydration for two reasons. First, compound 67 is more stable than 68, since it is a product of Saytzef elimination (more substituted double bond). Second, ^{13}C-NMR, circular dichroism and X-ray analysis of a large number of 1-deoxy-2-ketose sugar derivatives (Mester *et al.*, 1979b) indicate the presence of a hydrogen bond between the C-3 hydroxyl and the nitrogen of the amino acid (in the case of the furanoid form, the hydrogen bond is between the anomeric hydroxyl of

C-2 and the nitrogen of the amino acid). Therefore, 2,3-dehydration preserves this hydrogen bond, whereas 1,2-dehydration breaks it by freezing the -NH-CH(COOH)-R, group away from the C-3 hydroxyl hydrogen. In the furanoid form, both 1,2- and 2,3-dehydrations are unable to preserve the hydrogen bonding, therefore both paths may be operative in certain cases.

It follows that 0,2-dehydroxylation (path a) can be initiated in the mass spectrometer by the formation of the molecular ion at the ring oxygen, and in food systems by highly polar conditions. Path a corresponds to the fragmentations of the A series (sugar fragmentation pattern).

1,2- and 2,3-dehydrations might be initiated in the mass spectrometer by the formation of the molecular ion at the amino acid nitrogen due to the electron withdrawing effect of positively-charged nitrogen atom. However, it will isomerize into the more stable 2,3-dehydration product to restore the six-membered ring hydrogen bond. Paths b and c constitute AA and BB series of the "amino acid fragmentation" pattern.



In the mass spectrometer, amino acid nitrogens with relatively low ionization potentials (having high gas phase basicities and low solution phase basicities) and protonated amino acid nitrogen species might prefer the 2,3-dehydration path.

According to the proposed mechanism in Figure 3.1, Maillard reactions operating under kinetic control (frying, baking, broiling, etc.) might decompose from cyclic structures following 0,2-dehydroxylation or 2,3-dehydration, whereas under conditions of thermodynamic control and mild conditions 1,2- and 2,3-enolization might lead to the decomposition of Amadori products, especially in foods stored for longer times at room temperature.

In this scheme, the 2,3-dehydration path produces compound 62 by ortho elimination (similar to β-elimination in the open-chain counterpart) since compound 62 appears (at m/z 144) in the majority of the mass spectra of Amadori products. Therefore, we can consider percent total ionization of m/z 144 as a measure of the tendency of Amadori products to undergo 2,3-dehydration path (see Table 3.1).

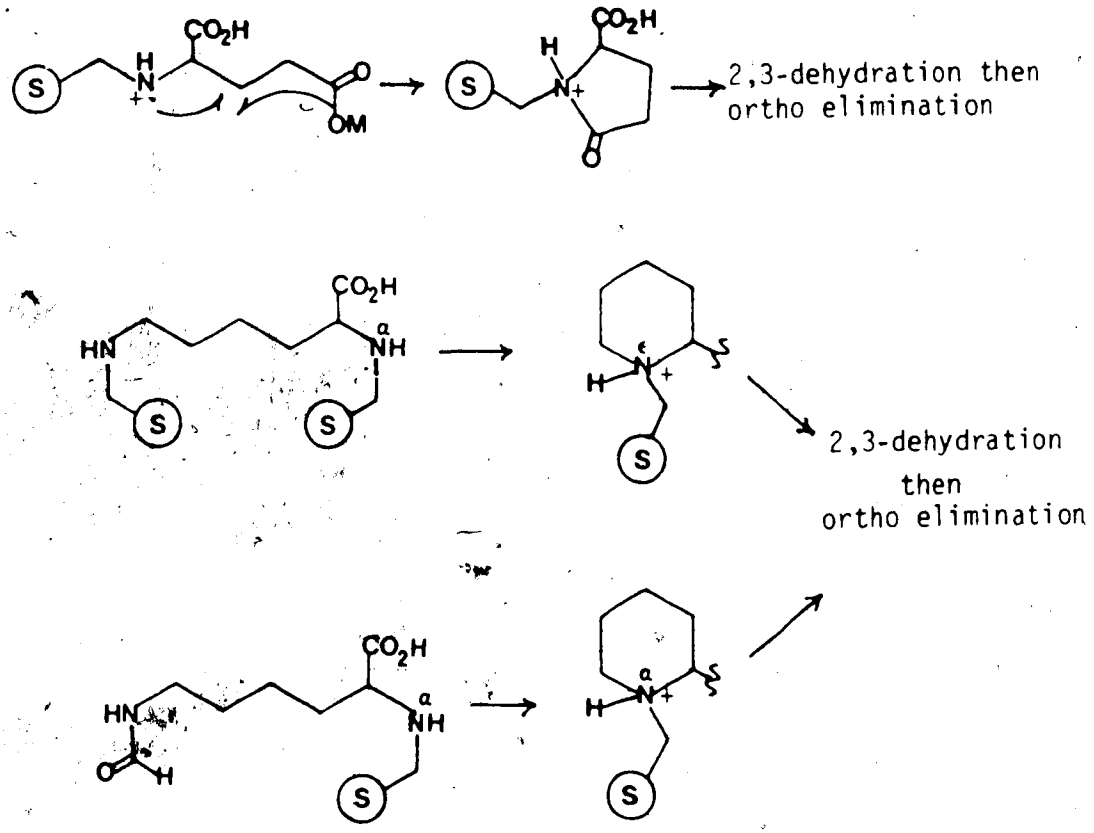
There is a significant difference in relative amounts of m/z 144 in the first four Amadori compounds, relative to the rest, in Table 3.1.

The presence of fructosyl-proline in the top four Amadori compounds can be explained by the fact that it is a 3° amine and, hence, possesses a high gas phase basicity and

Table 3.1 Relative amounts of m/z 144 and $^{+}NH_2=CH-R_1$ as $\%\Sigma_{50}$.

Amino acid moiety	$\%\Sigma_{50}$ m/z 144	$\%\Sigma_{50}$ $^{+}NH_2=CH-R_1$
Glutamic acid methyl ester	8.06	0
Difructosyl lysine	7.74	0
Proline	6.27	37.00
Lysine-CHO	5.25	0
Alanine	3.56	--
Valine	1.18	11.82
Glycine	0.90	--
Leucine	0.84	15.15
Threonine	0.71	--
Methionine	0.45	--
Hydroxyproline	0.28	12.51
Isoleucine	0.23	--
Nα-lysine	0	0
Nε-lysine	0	0
Serine	0	0
Histidine	0	0
Phenylalanine	0	0
Tryptophan	0	0

low ionization energy at the amino acid nitrogen. As a result, the 2,3-dehydration path will dominate. However, the three remaining Amadori compounds are 2° amines. The common property underlying these compounds is their ability to cyclize into five- or six-membered nitrogen-containing rings, thus converting them into 3° amines, as follows:



The cyclized structures contain an ammonium ion which is more electron withdrawing than the radical ion of the secondary amine, thus favoring 2,3-dehydration. Cyclization is faster than the dehydration step. Amadori compounds that cannot cyclize and contain 2° amines (valine, alanine, glycine) produce less of the 2,3-dehydration product because

the radical cation that initiates 2,3-dehydration is less electron withdrawing than the ammonium ion.

Mills (1979) subjected N-methylglycine Amadori compound (3° amine) to thermal degradation and isolated compound 62 in 63% yield from the distillate, whereas glycine Amadori compound (Birch *et al.*, 1980) gave, as the major component in the pyrolysate, compound 69,

69

which is an aA series compound formed by "sugar fragmentation pattern", and was observed only in the glycine ARP mass spectrum.

Ortho elimination proceeds by the movement of two electrons. Since the resulting peak at m/z 145 is an even electron species, this can happen either by charge migration or charge retention (see Figure 3.2).

In the case of proline, charge migration and charge retention products have similar stabilities (oxonium ion vs. immonium ion), hence both products were obtained in appreciable amounts (see Table 3.1), whereas in cyclized structures the charge migration produces the more stable oxonium ion.

Although the other two lysine derivatives can also cyclize to produce protonated 3° amines, they do not yield the ion at m/z 144, the indicator ion for 2,3-dehydration. The reason for this "anomaly" is the presence of free amino

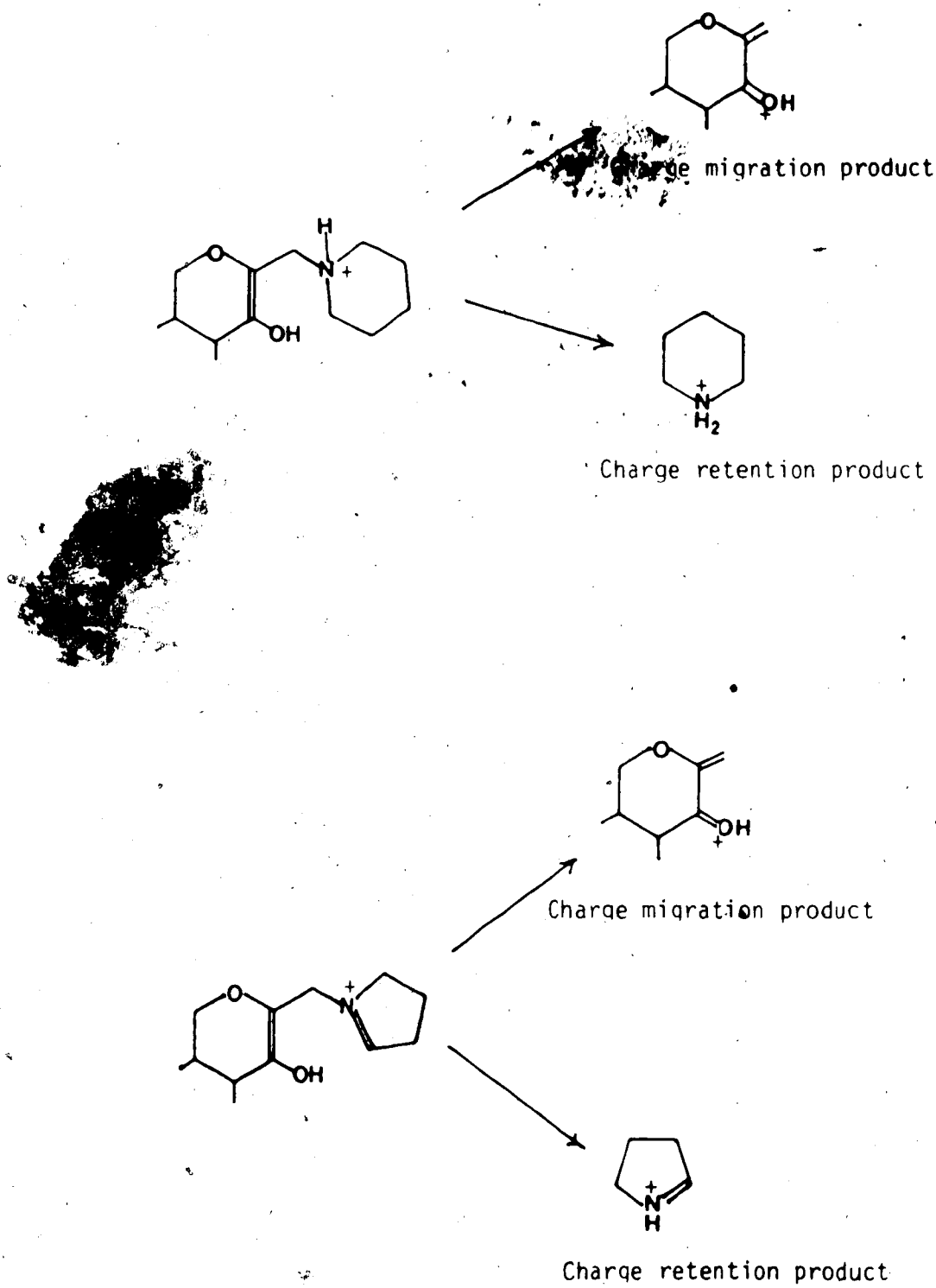
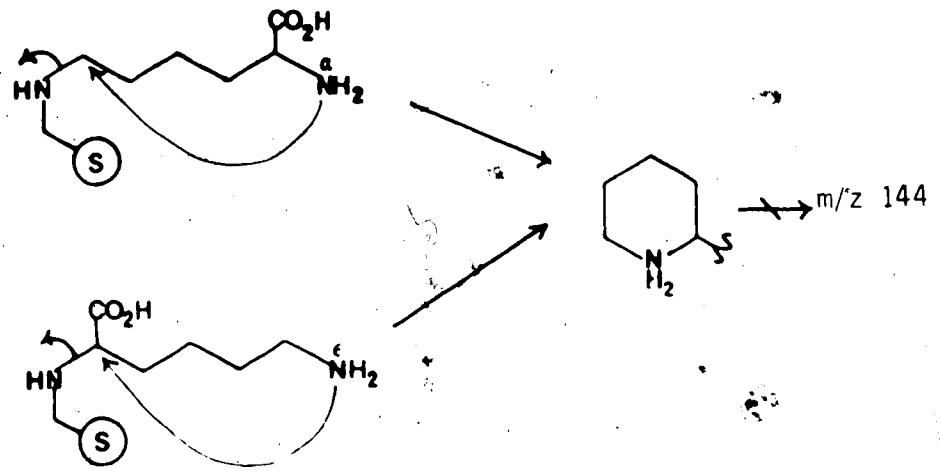

Charge migration product
Charge retention product
Charge migration product
Charge retention product

Figure 3.2

groups in the two derivatives, thereby removing the competition between the two nitrogens (Nα, Nε) to act as a nucleophile in the cyclization reaction. The free amino group will always act as a nucleophile, so the molecule will cyclize in such a way that the sugar moiety is always displaced after cyclization because they are on the opposite sides of the molecule (see below).



There is another apparent "anomaly" in Table 3.1, the case of hydroxy-proline, which is a 3° amine in the ARP, and yet, does not yield the ion at m/z 144 in the expected quantities (7.74 for proline vs 0.28 for hydroxy-proline).

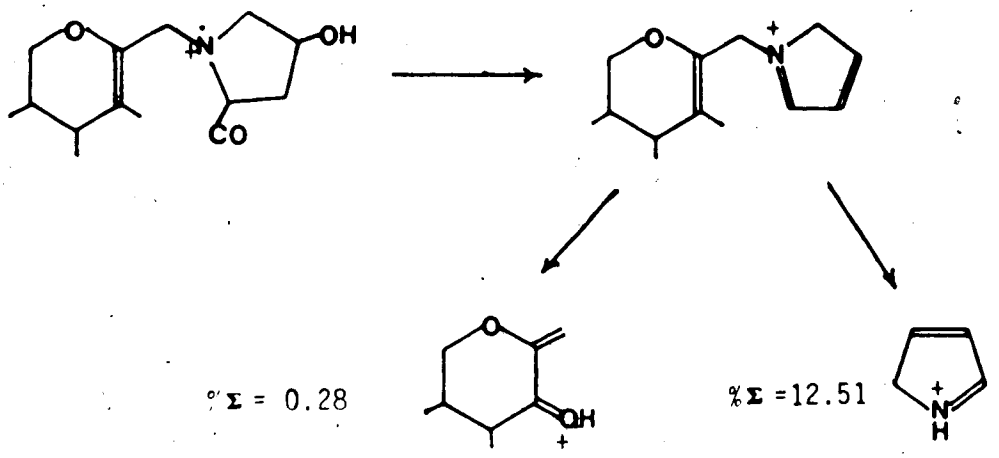


Figure 3.3.

The presence of the hydroxyl group, however, on the five-membered ring causes aromatization and hence the charge retention product is prefered extensively over the charge migration product (see Figure 3.3).

3.1 Comparison of the Fragments obtained by EIMS to those of Solution and Pyrolytic Reactions

A few selected examples of important fragment ions identified in the mass spectra will be compared to the products isolated in browning model systems or in the pyrolysates of Amadori rearrangement products.

The initial identification of a peak in the mass spectra was done according to the strategy outlined in Chapter 2. Since even high resolution mass spectrometers cannot differentiate between two structural isomers, the

final distinction was made by comparison with literature fragmentation patterns and relative intensities of the particular compound. The "best match" was chosen according to the highest number of common peaks and highest number of common acceptable ratios of any two intense peaks. The emphasis was placed on the number of common peaks rather than on the ratios of intensities for several reasons:

a. Different instruments with different operating temperatures and repeller voltages will give different relative intensities.

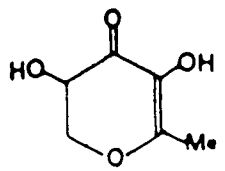
b. Only odd electron fragment ions are expected to produce comparable relative intensities.

c. Relative intensities are reduced if the compound in question is in a mixture.

d. Contributions from other sources to the same peak make the intensities higher.

Because of the above mentioned reasons, two ratios of the same two peaks in the standard and the unknown are considered acceptable if they are within 20% range. This value is based on the Crawford-Morrison method of comparing mass spectra (Crawford and Morrison, 1968).

Hodge *et al.* (1970b) identified the compound (62)



62

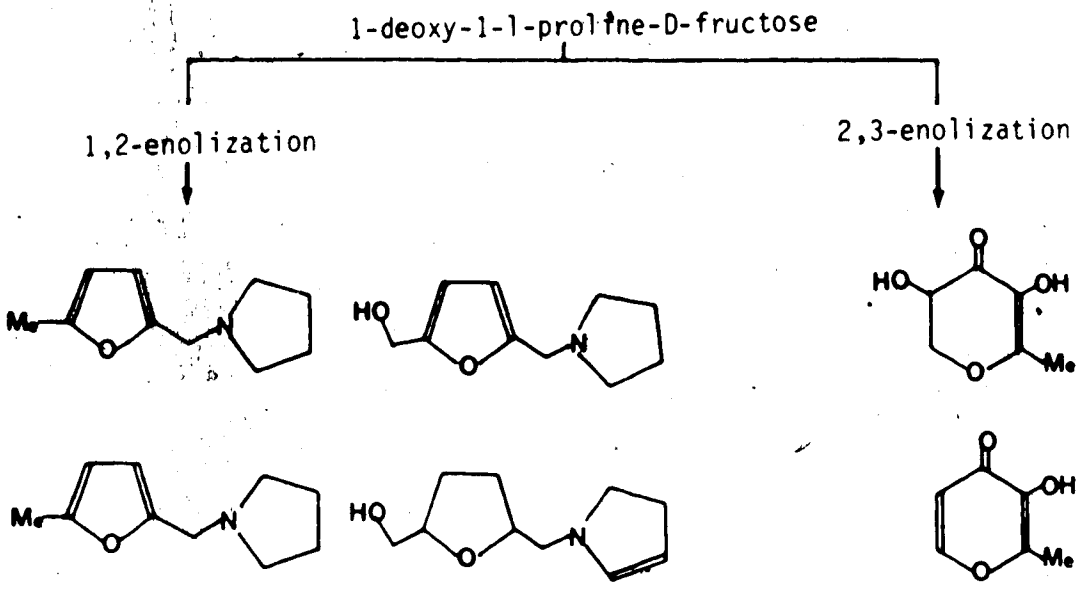
that is the major product which is volatilized during the pyrolysis of 1-deoxy-1-(proline)-D-fructose at 138°/1 mm. 62 is important in the production of characteristic browned cereal flavor and is the precursor for maltol.

The same compound was also identified by Shigematsu *et al.* (1977) in the pyrolysates of alanine and valine Amadori products, with glucose.

Kato *et al.* (1982) incubated a mixture of D-glucose and L-lysine at 75°C for 48 hr, then the mixture was extracted with ethyl acetate and the concentrate was analyzed by GC-MS. One of the main products was compound 62. In the above mixture the possible lysine-Amadori products are difructosyl lysine and Nϵ-fructosyl lysine.

Both pyrolytic and solution reactions, therefore, support the findings in Table 3.1 both qualitatively and quantitatively.

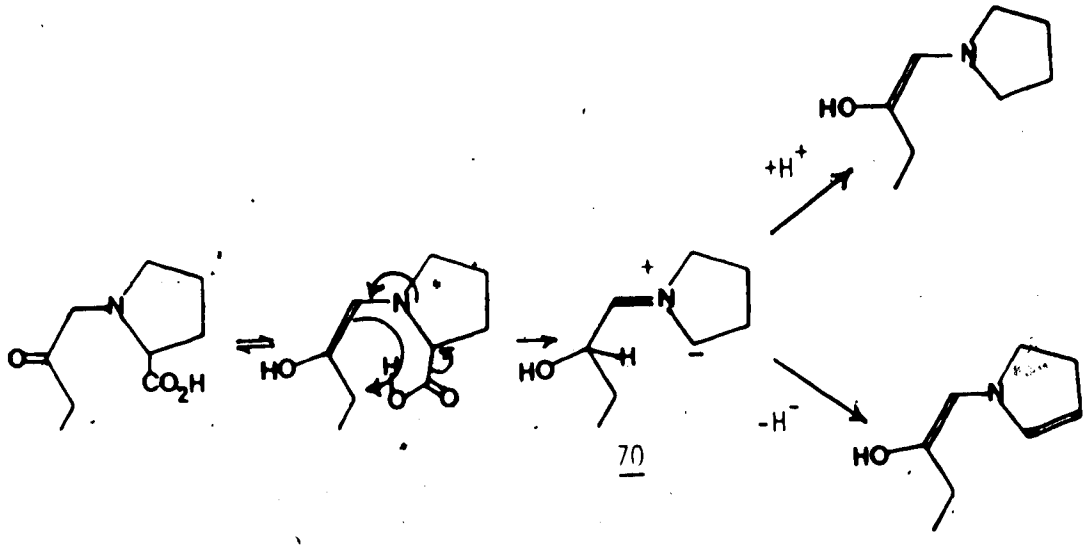
Hodge and Mills (1976) proposed the following scheme for the decomposition of the proline Amadori product under pyrolytic conditions.



After heating the Amadori compound, the distillate was condensed and the above compounds were identified. All the furan derivatives were also identified by Tressl *et al.* (1985a) in a browning model system.

The electron impact mass spectrum of proline Amadori product also reveals the cationic counterparts of all the above shown products.

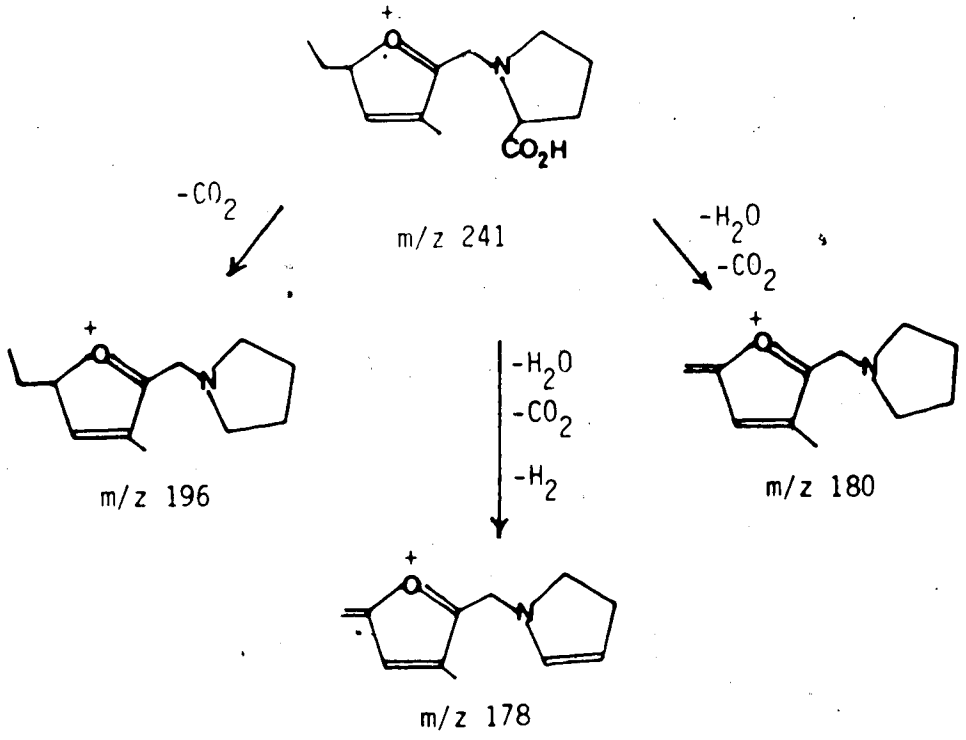
Hodge and Mills (1976), however, proposed a 1,2-enolization mechanism for the formation of the furan derivatives, in which C-2 of the sugar moiety aids in the decarboxylation of the amino acid during the process of 1,2-enolization.



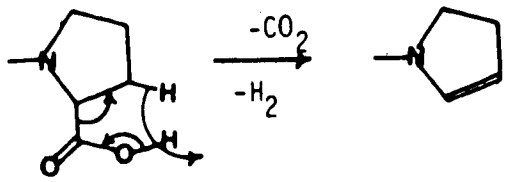
70

Proposed 1,2-enolization mechanism of Hodge and Mills (1976)

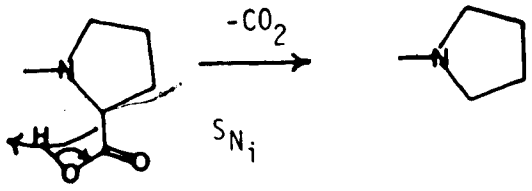
The identification of the following fragments disputes that claim since the proposed mechanism claims decarboxylation before cyclization of the sugar moiety.



It is more likely that it proceeds by cis-elimination reaction, in the case of formation of pyrroline



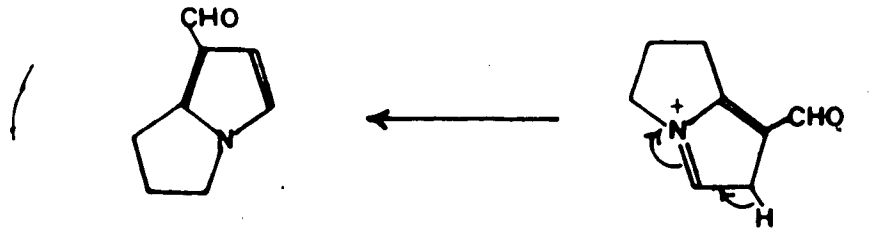
Cis-elimination



and by S_{N_i} (substitution nucleophilic internal) in the case of the formation of pyrollidines.

S_{N_i} is an intramolecular (unimolecular) nucleophilic interchange in which the attacking nucleophile is part of the substrate.

In 1975 Shigematsu *et al.* characterized several 1H-pyrrolizines, among them compound 71,



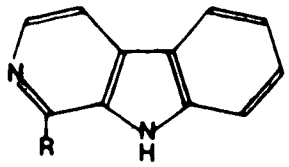
71 m/z 154 (17.55%)

on heating equimolar amounts of L-proline and D-glucose at 200°C. These compounds, which possess smokey, roasty aromas, were also determined in beer. Compound 71 and other

pyrrolizine derivatives were also identified in proline-glucose model systems by Tressl *et al.* (1985b).

The peak at m/z 154, which is a cationic precursor of compound 71, was characterized in the mass spectrum of proline-Amadori product in 17.55% relative intensity.

Brautigam and Severin (1974) isolated the following β-carboline derivatives from a model system consisting of tryptophan and glucose or zylose.



R=H m/z 168(72%)

R=CH_2—OH m/z 198(52%)

R=$COCH_3$ m/z 212(14%)

R=CH(OH)—CH_2—CH=CH_2 m/z 238(2%)

m/z 234(2%)

Figure 3.4.

The fragmentation pattern of tryptophan Amadori product was dominated by the formation of β-carbolines. Figure 3.4 shows the relative intensities of the β-carbolines identified in the mass spectrum that corresponds to the structures identified in the model system.

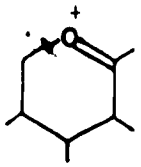
In the last few years, interest in β-carbolines has greatly increased due to the detection of these compounds in human tissue (Airaksinen and Kari, 1981). 1,2,3,4-tetrahydro-β-carboline (THBC) is a normal constituent of human plasma and platelets, however, 1-methyl-THBC is normally not present in human plasma in detectable amounts, but only after acute intake of ethanol was this compound found to occur in widely varying concentrations. The maxiumum level seems to coincide with the time of hangover. The effects assumed for β-carbolines in man correspond quite well to the symptoms observed during alcohol withdrawal. In addition, β-carbolines were found to act physiologically on GABA-receptors, being possibly endogenous ligands at benzodiazeprine receptors (Braestrup and Nielson, 1980).

It has been shown that β-carbolines are formed in tobacco smoke (Poindexter and Carpenter, 1962) and it is possible that, after inhalation, traces of these compounds may diffuse into the blood.

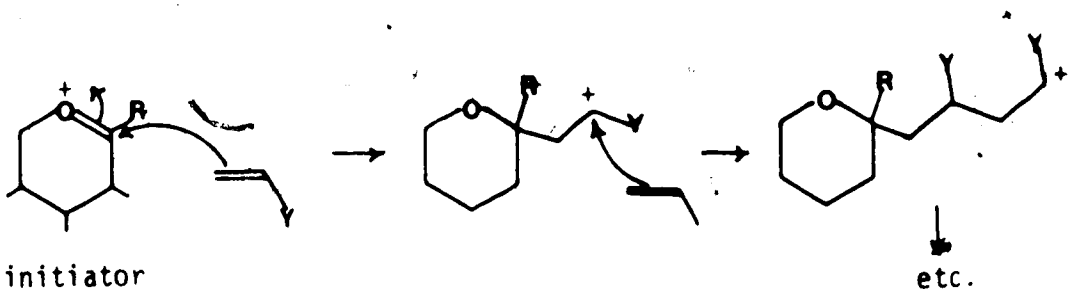
All the above mentioned examples demonstrate the physiological importance of β-carbolines and, since they can be formed abundantly in tryptophan-rich food products during processing, their importance in the so called "mood altering foods" becomes evident.

3.2 Implications of the Fragmentation Patterns of Amadori Compounds to the Formation of Early Maillard Polymers

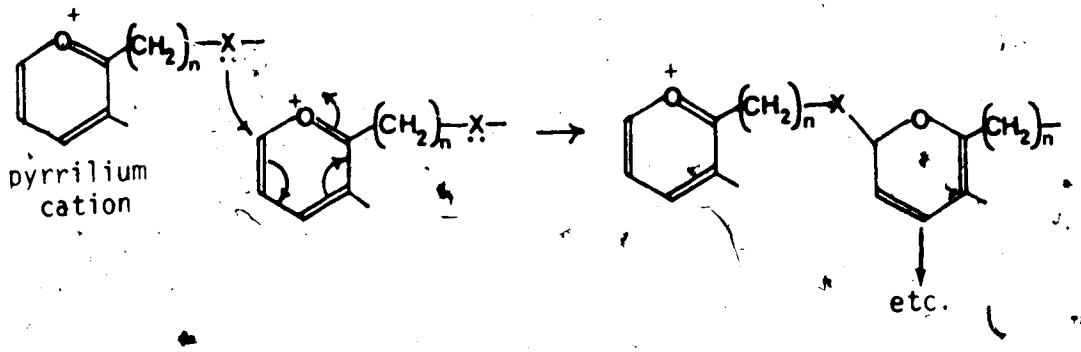
The importance of O,2-dehydroxylation pathway of Amadori products to the formation of early Maillard polymers becomes evident if we consider the ionic nature of the products which serve as an initiator for chain polymerization or as a monomer for condensation or addition polymers.



Chain polymerizations require an initiator species with a reactive center which can be either a free radical, cation or and anion. Polymerization occurs by the propagation of the reactive center by the successive additions of large numbers of monomers in a chain reaction happening in a matter of a second or so.



Alternatively, if the pyrrilium ion contains a nucleophilic center, it can act as a monomer in a condensation or addition polymerizations.

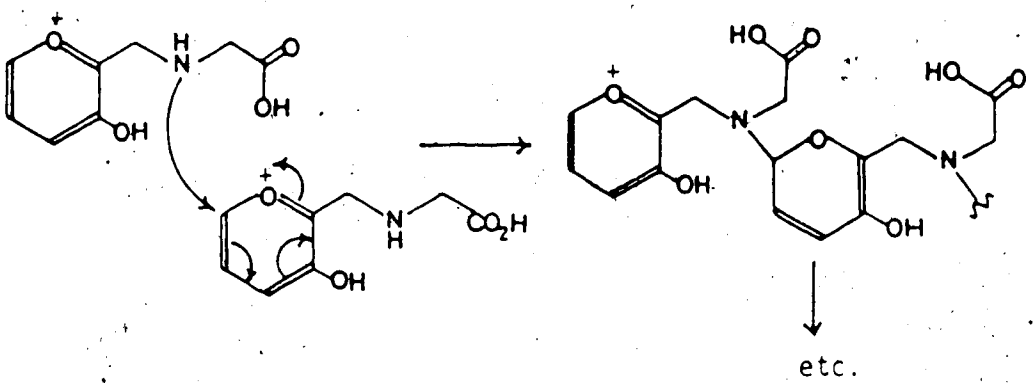


Feather and Nelson (1984) isolated a water soluble Maillard polymer (M.Wt. = 16,500) from a mixture of glucose and glycine, after refluxing for eight hours. Elemental analysis suggested that the polymer is composed of 1 mole of sugar, 1 mole of glycine, minus three moles of water. studies using 90 atom % enriched D-glucose-1-^{13}C, glycine-^{13}C and glycine-2-^{13}C as precursors in the reaction and ^{13}C-NMR probe show that both carbon atoms of glycine are incorporated into the polymer and that C-1 of D-glucose appears as a substituted methyl group. The NMR data further suggested that the main monomeric units are unreacted sugar and amino acid or Amadori derivatives.

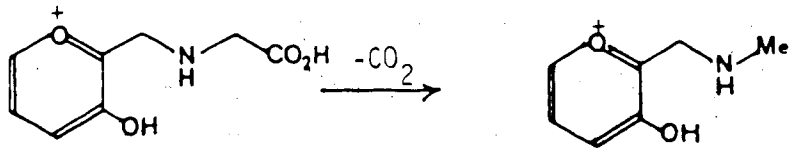
Olsson *et al.* (1982) isolated the same polymer and reported that it showed no UV absorbance at 230 or 280 nm, and that the ^{13}C-NMR spectrum of the polymer resembled that of Amadori compound derived from glucose and glycine.

Similar results were obtained when glycine was replaced with methionine.

All the above data about the polymers can be incorporated into the following polymerization reaction:

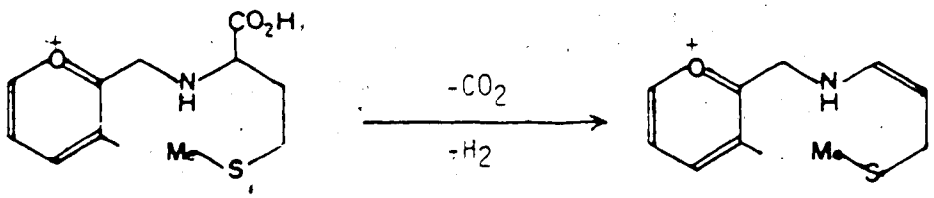


where the monomer is the pyrylium ion 72.



72

72 does not appear in the mass spectrum, but the decarboxylated product does, at m/z 140.

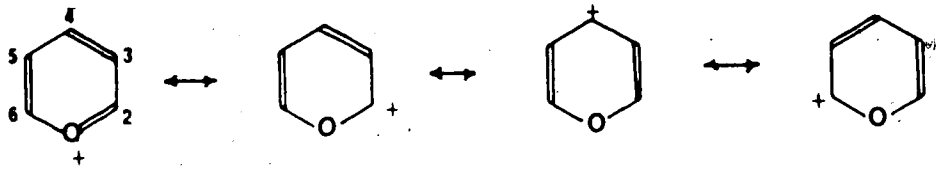


73

The same holds true in the methionine case, where the monomeric unit 73 does not show in the mass spectrum, but

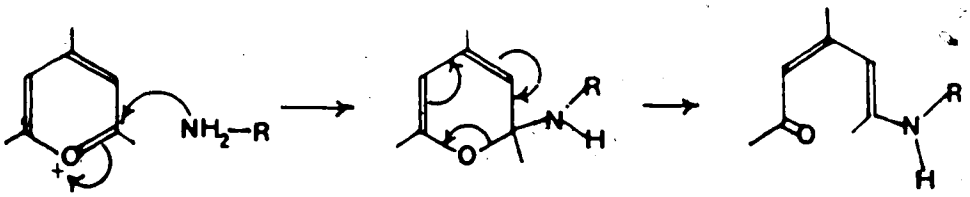
its product at m/z 212 does.

The repeating units in the early Maillard polymers might be attributed to the products obtained in the A_3 series after O,2-decarboxylation pathway. The A_3 series constitutes a series of substituted pyrylium cations that are known to possess pronounced electrophilic reactivity



A_3

at position 2, 4 and 6, which enables it to add nucleophile in these postions. With basic nucleophiles (Pearson, 1963), such as amines and cyanide, the addition occurs at position 2 or 4 or 6 and a pyran is formed. The nucleophilic reactivity of 2- or 6-positions usually exceeds that of the 4-position (see below).



pyran

The pyrans thus formed are susceptible to electrocyclic ring opening, giving rise to the conjugated product.

4. SYNTHESIS OF AMADORI REARRANGEMENT PRODUCTS

The synthesis of Amadori rearrangement products was first conducted by Amadori (1931) with D-glucose and aromatic amines. Kuhn and Wegand (1937) were the first to correctly interpret the reaction and named it the Amadori rearrangement; then the reaction had been extended to condensation products of a variety of sugars with most classes of amines, including the naturally occurring amino acids.

The first synthesis and isolation of 1-(N-amino acid)-1-deoxy-D-fructose was achieved by Gottschalk (1952) by refluxing a solution of DL-phenylalanine and glucose in methanol. Abrams, Lowy and Borsook (1955) synthesized nine members of this series by the same method, purifying small quantities by elution chromatography on cation-exchange resin.

Roper *et al.* (1983) synthesized fourteen Amadori products of D-glucose with amino acids following the method of Hodge and Fisher (1963b).

The present method is a general procedure of synthesis based on the methods of Sgarbieri *et al.* (1973) and Lee and Liau (1971).

4.1 General Procedure for the Synthesis of Amadori Products

1.0 g of the L-amino acid and 15.0 g of D-glucose were refluxed for 3 h in 500 mL of anhydrous methanol in a 1 L round bottom flask. Unreacted D-glucose (if any) was

filtered and the filtrate was evaporate *in vacuo* at 40°C. The residue was dissolved in a minimum amount of water and directly applied on a column (6 cm x 12 cm) of Dowex 50X4-400 ion exchange resin (H^+-form) and eluted with water until the eluent was negative to TTC test.

After the separation of excess sugar, the column was eluted with 5 N NH_4OH solution until the eluent was negative to Elson-Morgan test (a specific test for amino sugars). The eluted NH_4OH solution was evaporated under the fume hood in an evaporating dish. The residue was dissolved in 25 mL water and decolorized (room temp.) with charcoal several times until the solution was colorless. The filtrate from the last decolorization was evaporated again under the fume hood.

To further separate the Amadori product from the free amino acid contamination, the amorphous solid was dissolved in a minimum amount of water and then loaded on a cellulose column (2 cm x 20 cm). The column was packed with Whatman CF_{11} fibrous powder suspended in water-saturated *n*-butanol and eluted with the same solvent. The fractions were collected with an automatic fraction collector (1 mL/min) until the eluent was negative to Elson-Morgan test. The purity of the fractions was tested by TLC (cellulose; solvent system of *n*-butanol:H_2O:acetic acid, 12:5:3 v/v; ninhydrin spray).

All the fractions that showed one spot on TLC were combined, treated with charcoal and the filtrate evaporated.

The remaining white solid was crystallized several times from solvents recommended in the literature. Melting points and H'-NMR data were compared with those reported in the literature.

Synthesis of Nα,Nε-di(deoxy-1-D-fructosyl)-L-lysine

General procedure was followed, except that a catalytic amount of ethyl malonate was added to the refluxing mixture of lysine and D-glucose.

Synthesis of Nε-(deoxy-1-D-fructosyl)-L-lysine, Nα-(deoxy-D-fructosyl)-L-lysine and Nε-formyl,Nα-(deoxy-D-fructosyl)-L-lysine

Same procedure as that of Finot and Mauron (1969) was followed.

4.2 Chemical Tests

TTC test for carbohydrates, (Horn *et al.*, 1968)

To 1 drop of sample were added 4 drops of TTC reagent (3% methanol solution of 2,3,5-triphenyl-2H-tetrazolium chloride) and 3 drops of 6 N NaOH. The mixture was shaken and allowed to stand at room temperature. A pink to brick red color was a positive test.

Ninhydrin test for amino acids (Horn *et al.*, 1968)

To 1 drop of sample were added [illegible] drops of [illegible]drin reagent (4% methanol solution of ninhydrin) and [illegible] of propionate buffer (199.2 mL of propionic acid added slowly

with stirring to a cool solution of 67.2 g NaOH in 150 mL water, then diluted to 400 mL with water). The mixture was shaken and heated to 100°C for 30 min. A positive test was a blue-violet color.

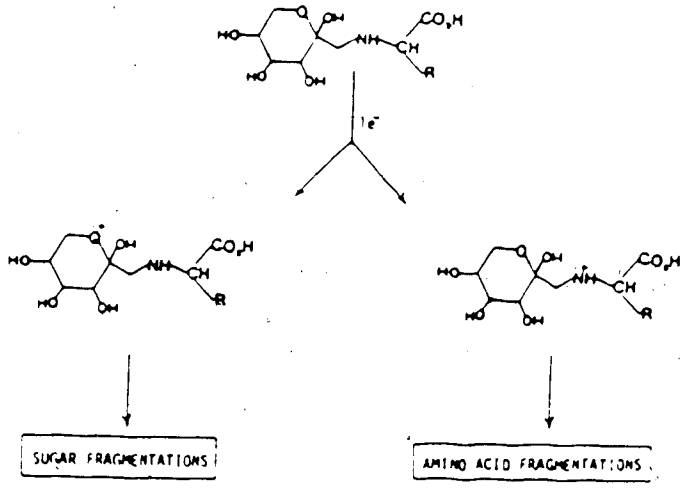
Morgan-Elson test for Amadori products (Elson and Morgan, 1933)

To 1 mL of sample was added one mL of acetyl acetone solution (1 mL of acetyl acetone was dissolved in 50 mL of 0.5 N Na_2CO_3 solution; prepared fresh daily), followed by heating for 15 min in a boiling water bath. After heating, the tube was cooled and 1 mL ethanol and 1 mL of Ehrlich reagent (1% acidified methanol solution of 4-dimethylamino benzaldehyde) were added. The appearance of red color and evolution of CO_2 was a positive test.

5. CONCLUSION

The application of electron impact mass spectrometry to the study of the fragmentation of 1-(amino acid)-1-deoxy-D-fructoses provides a useful technique to identify the possible fragments of Amadori rearrangement products formed in food systems, especially during the use of Maillard reaction in flavour production, since this method allows the recognition of different heterocyclic compounds associated with specific amino acids in the production of a particular flavour compound. In addition, it can be used as an identification tool for Amadori rearrangement products (the DD and EE diagnostic series).

The behavior of Amadori rearrangment products under electron impact conditions reflects the structural characteristics of the molecule. The two moieties, sugar and amino acid, more or less control the fragmentation patterns (see below), if there are no interactions between the two moieties, as in the case of tryptophan.



Based on the fragmentations observed in the mass spectra of Amadori rearrangement products and ^{13}C-NMR studies, a new mechanism for the Maillard reaction is proposed (see page 227) in which a greater role is allocated to the furanose and pyranose forms of the Amadori rearrangement products in the production of intermediates and polymers, in relation to the open-chain form.

Therefore, in addition to its importance in the flavour industry, such a study provides important insights into the mechanism of Maillard reaction.

6. REFERENCES

Abrams, A., Lowy, P.H., Borsook, H. 1955. Preparation of 1-amino-1-deoxy-2-ketohexoses from aldohexoses and α-amino acids. J. Amer. Chem. Soc. 77:4794-6.

Aeschbacher, H.U., Chappuis, C.M., Manganel, M. and Aeschbach, R. 1981. Investigation of Maillard products in bacterial mutagenicity test systems. Prog. Fd. Nutr. Sci. 5:279-94.

Airaksinen, M.M. and Kari, I. 1981. β-carbolines, psychoactive compounds in the mammalian body. Med. Biol. 59:21-34.

Amadori, M. 1931. Condensation products of glucose with p-toluidine. Atti reale Accad. nazl. Lincci. 13:72.

Andersson, C.O. 1958. Mass spectrometric studies on amino acid and peptide derivatives. Acta. Chem. Scand. 12:1353.

Anet, E.F.L.J. and Reynolds, T.M. 1957. Chemistry of non-enzymic browning. I. Reactions between amino acids, organic acids, and sugars in freeze-dried apricots and peaches. Aust. J. Chem. 10:182.

Anet, E.F.L.J. 1964. 3-deoxyglycosuloses and the degradation of carbohydrates. Adv. Carbohydr. Chem. 19:71-122.

Benzing-Purdie, L.M., Ripmeester, J.A. and Ratcliffe, C.I. 1985. Effects of temperature on Maillard reaction products. J. Agr. Food Chem. 33:31-3.

Berrens, L. 1967. Fluorescence of atopic allergins. Immunochemistry 4:81-90.

Biemann, K. 1962. Mass Spectrometry: Organic Chemical Applications. McGraw Hill.

Biemann, K. and Dejongh, D.C. 1963. Application of mass spectrometry to structure problems. XIV. Acetates of partially methylated pentoses and hexoses. J. Amer. Chem. Soc. 85:2289-94.

Birch, E.J., LeLievre, J. and Richards, E.L. 1980. Thermal analysis of 1-deoxy-1-glycino-D-fructose and 1-β-alanino-1-deoxy-D-fructose. Carbohydrate Res. 83:263-72.

Birch, E.J., LeLievre, J. and Richards, E.L. 1984. Influence of the amine moiety on the thermal degradation of N-substituted 1-amino-1-deoxyfructoses. Carbohydrate Res. 128:351-55.

Borsook, M., Abrams, A. and Lowy, P.M. 1955. Fructose-amino acids in liver: stimuli of amino acid incorporation *in vitro*. J. Biol. Chem. 215:111.

Bowers, M.T., Aue, D.M., Webb, H.M. and McIver, R.T. 1971. Equilibrium constants for gas-phase ionic reactions. Accurate determination of relative proton affinities. J. Amer. Chem. Soc. 93:4314-5.

Braestrup, C. and Nielson, M. 1980. Searching for endogenous benzodiazepine receptor ligands. Trends. Pharmacol. Sci. 1:424-7.

Brauman, J.I., Riveros, J.M. and Blair, L.K. 1971. Gas-phase basicities of amines. J. Amer. Chem. Soc. 93:3914-6.

Brautigam, K.M. and Severin, Th. 1974. Untersuchungen zur Maillard-Reaktion. Z. Lebensm. Unters.-Forsch. 154:80-3.

Budzikiewicz, H., Djerassi, C. and Williams, D.H. 1964. Interpretation of Mass Spectra of Organic Compounds. p. 271. Holden-Day Inc., San Francisco, CA.

Budzikiewicz, H., Djerassi, C. and Williams, D.H. 1967. Mass Spectrometry of Organic Compounds. Holden-Day Inc., San Francisco, CA.

Bunn, H.F. and Higgins, P.J. 1981. Reaction of monosaccharides with proteins: possible evolutionary significance. Science 213:222.

Burton, H.S., McWeeny, D.J., Pandhi, P.N. and Biltcliffe, D.O. 1962. Fluorescent compounds and non-enzymatic browning. Nature (London) 196:948-50.

Burton, H.S. and McWeeney, D.J. 1963. Consideration of sugar stability. Nature (London) 197:266.

Burton, H.S. and McWeeny, D.J. 1964. Non-enzymatic browning: routes to the production of melanoidins from aldoses and amino-compounds. Chem. Ind. 462-3.

Caprioli, R.M. 1983. Fast atom bombardment mass spectrometry for determination of dissociation constants of weak acids in solution. Anal. Chem. 55:2387-91.

Cerami, A., Stevens, V.J. and Monnier, V.M. 1979. Role of non-enzymatic glycosylation in the development of the sequence of diabetes mellitus. Metabolism 28;431.

Chizhov, O.S. and Kochetkov, N.K. 1966. Mass spectrometry of carbohydrate derivatives. Adv. Carbohydr. Chem. 21:39.

Commoner, B., Vithayathil, A. and DoLara, P. 1980. Mutagenic analysis as a means of detecting carcinogens in food. J. Food Protection 41:996-1003.

Costa, E., Gessa, g.L. and Sandler, M. 1974. Serotonin: New Vistas. Advances in Biochemistry and Psychopharmacology. Vol. 10 and 11. Raves Press, NY.

Crable, G.F. and Kearns, G.L. 1962. Effect of substituent groups on the ionization potentials of benzene. J. Phys. Chem. 66:436-9.

Crawford, L.R. and Morrison, J.D. 1968. Computer methods in analytical mass spectrometry -- identification of an unknown compound in a catalog. Anal. Chem. 40:1464-9.

Dartnall, M>J.A. (ed). 1972. Handbook of Sensory Physiology. Vol. 6. Springer-Verlag, Berlin and New York.

Diels, O. and Alder, K. 1928. Synthesen in der hydroaromatischen Reihe; I. Mitteilung: Anlagerungen von "Di-en"-Kohlen-Wasserstoffen. Justus Liebigs Ann. Chem. 460:98-122.

Djerassi, C., Budzikiewicz, H. and Willians, D.H. 1964. Structure Elucidation of Natural Products by Mass Spectrometry. Vol. 1. p. 42.

Ellis, G.P. 1959. The Maillard reaction. Adv. Carbohydr. Chem. 14:63-134.

Elson, L.A. and Morgan, W.J. 1933. CCXLVIII. A calorimetric method for the determination of glucosamine and chondrosamine. Biochem. J. 27:1824-6.

Feather, M.S. and Nelson, D. 1984. Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde, and glycine and methionine. J. Agric. Food Chem. 32:1428-32.

Fernstrom, J.D. and Wurtman, R.J. 1971. Brain serotonin content. Increase following ingestion of carbohydrate diet. Science 174:1023.

Finot, P.A. and Mauron, J. 1969. Le blocage de la lysine par la réaction de Maillard. I. Synthèse de N-(déoxy-1-D-fructosyl-1-)- et N-(déoxy-1-D-lactosyl-1-)-L-lysines. Helv. Chim. Acta 52:1488-95.

Flückiger, R. and Winterhalter, K.H. 1976. *In vivo* synthesis of haemoglobin A FEBS Lett. 71:356.

Gilbert, J. 1984. Confirmation and quantification of trace organic food contaminants by mass spectrometry -- selected ion monitoring. In: "Analysis of Food Contaminants". J. Gilbert (ed.). p. 265. Elsevier Applied Science Publ., London.

Godefridus, A.M., Ouweland, V.D., Peer, H.G. and Tjan, S.B. 1978. Occurrence of Amadori and Heyns rearrangement products in processed foods and their role in flavor formation. In: "Flavor of Food and Beverages". G.

Charalambous (ed.). pp. 131-43. Academic Press, NY.

Goldman, I.M., Seibl, J., Flament, I., Gautschi, F., Winters, M., Willhalm, B. and Stoll, M. 1967. Recherches sur les arômes sur l'arôe de café. II. pyrazines et pyridines. Helv. Chim. Acta 50:694-705.

Gomyo, T., Kato, M., Udaka, K., Horikoshi, M. and Fujimaki, M. 1972. Effects of heating on chemical properties of melanoidin prepared from glycine-xylose system. Agric. Biol. Chem. 36:125-32.

Gottschalk, A. 1952. Some biochemically relevant properties of N-substituted fructosamines derived from N-glycosyl-amino-acids and N-arylglucosylamines. Biochem. J. 52:455-60.

Gottschalk, A. 1972. Glycoproteins. Vol. 5b (2nd ed.). A. Gottschalk (ed.). pp. 141-57. Elsevier, Amsterdam.

Gross, D. and Grodsky, G. 1955. On the sublimation of amino acids and peptides. J. Amer. Chem. Soc. 77:1678-80.

Hammett, L.P. and Deyrup, A.J. 1932. A series of simple basic indicators. I. The acidity functions of mixtures of sulfuric and perchloric acids with water. J. Amer. Chem Soc. 54:2721-39.

Heiss, R. 1968. Haltbarkeit und Sorptionsverhalten Wasser-armer Lebensmittel. Springer-Verlag.

Heyns, K. and Paulson, M. 1959. Über "Fructose-Amino Säuren" und "Glucose-Aminosäuren" in Leberextraktem. Leibigs Ann. Chem. 622:160.

Heyns, K. and Paulson, H. 1960. Veränderungen der Nahrung durch industrieele und haushaltmässige Verarbeitung. Wissenschaftl. Veröffentl. der Deutschen Gessellschaft f. Ernährung. p. 15. Verlag, Darmstadt, Steinkopff.

Heyns, K. and Klier, K. 1968. Bräunungsreaktionen und Fragmentierungen von Kohlenhydraten. Teil 4, Flüchtige Abbauproduckte bei der pyrolyse von Mono-, Oligo- und Polysacchariden. Carb. Res. 6:436-48.

Heyns, K., Chiemprasert, T. and Baltes, W. 1970. Über den Effeckt W-Ständiger Substituenten auf N-Glycosidbildung und Amadori-Umlagerung bei D-glucohexosen. Chem. Ber. 103:2877-84.

Hodge, J.E. 1953. Chemistry of browing reactions in model systems. J. Agric. Food Chem. 1:928-43.

Hodge, J.E. and Nelson, E.C. 1961. Preparation and properties of galactosylisomaltol and isomaltol. Cereal Chem. 38:207-21.

Hodge, J.E., Fisher, B.E. and Nelson, E.C. 1963a. Dicarbonyls, reductones and heterocyclics produced by reactions of reducing sugars with secondary amine salts. Proc. Amer. Soc. Brew. Chem. 84-92.

Hodge, J.E. and Fisher, B.E. 1963b. Methods in Carbohydrate Chemistry. Vol. 2. pp. 99-107.

Hodge, J.E. 1967. Origin of flavors in food. Nonenzymatic browning reactions. In: "Chemistry and Physiology of Flavors". H.W. Schulz, E.A. Day and L.M. Libbey (eds.). Avi Publ. Co., Westport, CT.

Hodge, J.E., Mills, F.D. and Baker, B.G. 1970a. Thermal degradation of 1-deoxy-1-piperidino--D-fructose. Carb. Res. 15:205-13.

Hodge, J.E., Mills, F.D. and Weisleder, D. 1970b. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. A novel nonenzymic browning product. Tetrahedron Lett. No. 15, 1243-6.

Hodge, J.E., Mills, F.D. and Fisher, B.E. 1972. Compounds of browned flavour derived from sugar-amine reactions. Cereal Sci. Today 17:34-8.

Hodge, J.E. and Mills, F.D. 1976. Amadori compounds: vacuum thermolysis of 1-deoxy-1-L-prolino-D-fructose. Carb. Res. 51: [illegible]21.

Høltermand, A. 1966. The browning reaction. Die Stärke [illegible]:319-28.

Horn, M.J., Lichtenstein, H. and Womack, M. 1968. Availability of amino acids. J. Agric. Food Chem. 16:741-5.

Howe, I., Williams, D.H. and Bowen, R.D. 1981. Mass Spectrometry, Principles and Applications. 2nd ed. p. 33. McGraw-Hill.

Hurrell, R.f. and Carpenter, K.J. 1974. Mechanism of heat damage in proteins. 4. The reactive lysine content of heat-damaged material as measured in different ways. Brit. J. Nutr. 32:589-604.

Hurrell, R.F. and Carpenter, K.J. 1981. The estimation of available lysine in foodstuffs after Maillard reactions. Prog. Food Nutr. Sci. 5:159-76.

Jaffe, H.H. 1953. A reexamination of the Hammett equation. Chem. Rev. 53:191-261.

Jayasimhula, K. and Day, R.A. 1980. Mass spectrometric determination of N-terminal tryptophane and N-terminal histidine in peptides. Biomed. Mass Spectrom. 7:321-6.

Jencks, W.P. (ed.). 1969. Catalysis in Chemistry and Enzymology. McGraw-Hill, NY.

Johnstone, R.A.W. 1972. Mass Spectrometry for Organic Chemists. Cambridge Univ. Press.

Johnstone, R.A.W., Lewis, I.A.S. and Rose, M.E. 1983. Fast atom bombardment of Crown ether/cation complexes in solution. Tetrahedron 39:1597-1603.

Kametani, T., Higa, T., Loc, C.V., Ihara, M.K., Oizumi, M. and Fukumoto, K. 1976. Iminoketene cycloaddition. 1. A facile synthesis of quinazolone system by condensation of iminoketene with imine -- a total synthesis of evidiamine and rutecarpine by retro mass-spectral synthesis. J. Amer. Chem. Soc. 98:6186.

Karel, M. 1960. Ph.D. Thesis. Massachusetts Inst. of Technology.

Karel, M. and Eichner, K. 1972. The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. J. Agric. Food Chem. 20:218-23.

Kato, H. and Fujimaki, M. 1968. Formation of N-substituted pyrrole-2-aldehydes in the browning reaction between D-xylose and amino compounds. J. Food Sci. 33:445.

Kato, H., Nakayama, T., Sugimoto, S. and Hayase, F. 1982. Volatile and nonvolatile Maillard reaction products between L-lysine and D-glucose. Agric. Biol. Chem. 46:2599-2600.

Kline, [illegible] and Fox, S.W. 1946. Iowa State Coll. J. Sci. 20[illegible]6.

Kline, L., Hanson, H.L., Sonoda, T.T., Gegg, J.E., Feeney, R.E. and Lineweaver, H. 1951. Role of glucose in the storage deterioration of whole egg powder. III. Effect of glucose removal before drying on organoleptic, baking, and chemical changes. Food Technol. 5:323-31.

Kochetkov, N.K., Wulfson, N.S., Chizhov, O.S. and Zolatarev, B.M. 1963a. Mass spectrometry of carbohydrate derivatives. Tetrahedron 19:2209-24.

Kochetkov, N.K., Wulfson, N.S., Chizhov, O.S. and Zolatarev, B.M. 1963b. Investigation of carbohydrates by mass spectroscopy -- methyl ethers and acetates of glycosides. Dokl. Akad. Nauk SSSR 151:543-6.

Kuhn, R. and Weygand, F. 1937. Die Amadori-Unlagerung. Chem. Ber. 70:769-72.

Kurata, T., Fujimaki, M. and Sakurai, Y. 1973. Red pigment produced by the reaction of dehydro-L-ascorbic acid with α-amino acid. Agric. Biol. Chem. 37:1471-7.

Labuza, T.P., Warren, R.M. and Warmbier, H.C. 1977. In: "Protein-Linking". M. Friedman (ed.). pp. 379-418. Plenum Publ. Corp., NY.

Labuza, T.P. and Saltmarch, M. 1981. Water Activity Influences on Food Quality. L.B. Rockland and G.F. Stewart (eds.). p. 605. Academic Press, NY.

Land, D.G. and Nursten, H.E. 1979. Progress Research. Applied Science Publ. Ltd., London.

Lea, C.M. and Hannan, R.S. 1949. ect of activity of water, of pH and of tempera the primary reaction between casein and glucose. chim. Biophys. Acta. 3-25.

Lea, C.H. 1950. The role of amino acids in the deterioration of food: the browning reaction. Chem. Ind. (London), 155-8.

Ledl, F. and Severin, T. 1978. Bräunungsreaktionen von Pentosen mit Aminen. Untersuchungen zur Maillard-Reaktion. XIII. Z. Lebensm.-Unters.-Forsch. 167:410-3.

Lee, C.C. and Liau, Y.M. 1971. Studies with radiation tracers. XVII. Model browning reactions between glycine and D-glucose. Cereal Chem. 48:238-46.

Lee, H.S., Sen, L.C., Clifford, A.J., Whitaker, J.R. and Feeny, R.E. 1978. Effect of reductive alkylation of the ε-amino group of lysyl residues of casein on its nutritive value in rats. J. Nutr. 108:687-97.

Lemiux, R.U., Anderson, L. and Conner, A.H. 1971. The mutarotation of 2-deoxy-β-D-erythro-pentose. Conformations, kinetics and equilibria. Carb. Res. 20:59-72.

Lineweaver, H. and Feeny, R.E. 1950-51. Crops in Peace and War. A. Stefferud (ed.). Yearbook of Agriculture, USDA. Govmt. Printing Office. p. 642. Washington, DC.

Maga, J.A. and Sizer, C.E. 1973. Pyrazines in food CRC Crit. Rev. Food Technol. 4:39-115.

Maillard, L.C. 1912. Action des Acides Aminés sur les sucres: Formation des Mélanoidines par voie Méthodologique. C.R. Acad. Sci. 154:66-8.

Maillard, L.C. 1916. Synthèses des Matière humiques par action des Acide Aminés sur les sucres réducteurs. Ann. Chim. 6:258-317.

Markey, S.P. 1981. Quantitative mass spectrometry. Biomed. Mass Spect. 8:426.

McFadden, W.H. 1973. Techniques of Combined Gas Chromatography/Mass Spectrometry. Wiley-Interscience, NY.

McLafferty, F.W. 1956. Mass spectrometric analysis, broad applicability to chemical research. Anal. Chem. 28:306-16.

McLafferty, F.W. 1957. Mass spectrometric analysis, aliphatic ethers. Anal. Chem. 29:1782-9.

McLafferty, F.W. 1959. Mass spectrometric analysis, molecular rearrangements. Anal. Chem. 31:82-7.

McLafferty, F.W. 1980. Interpretation of Mass Spectra. University Science Books, Mill Valley, CA.

Mester, L., Kraska, B., Crisba, J. and Mester, M. 1975. Sugar-amine interactions in the blood clotting system and their effects on haemostasis. Proc. 5th Intern. Congr. Thrombosis, Haemostasis. Paris.

Mester, M., Kraska, B., Crisba, J. and Mester, L. 1976. Effect of Amadori-type sugar derivatives of poly-L-lysine on platelet aggregation. Haemostasis 5:115.

Mester, L., Mester, M., Bhargava, K.P., Labrid, C., Dureng, G. and Cheucle, M. 1979a. Sugar derivatives of indolamines, part V: cardiovascular effects and "anti-stress" activity of desoxyfructose derivative of serotonin and 5-methoxy-tryptamine. Thromb. Res. 15:245-53.

Mester, L., Amaya, A., Berenger, G. and Mester, M. 1979b. Correlation between steric structure and chiroptical properties of 1-desoxy-2-keto sugar derivatives of serotonin. J. Carbohydrates, Nucleosides, Nucleotides 6:247-54.

Mester, L., Mester, M., Jayaraman, P. and Mahadevan, P.R. 1980. Inhibition of the incorporation of 3H DOPA in *Mycobacterium leprea* by desoxyfructo-serotonin. Biochem. Pharm. 27.

Mester, L., Szabados, L., Mester, M. and Yadov, N. 1981. Maillard type carbonyl-amine reactions *in vivo* and their physiological effects. Prog. Food Nutr. Sci.

5:295-314.

Meyerson, S. 1963. Organic ions in the gas phase. XIII. Cycloheptatrienyl-7-d; decomposition of $C_7H_8^+$ and $C_7H_8^{++}$ from cycloheptatriene and toluene. J. Amer. Chem. Soc. 85:3[illegible]-4.

Miller, R.E. and Cantor, S.M. 1952. 2-Hydroxyacetylfuran from sugars. J. Amer. Chem. Soc. 74:5236-7.

Mills, F.D. 1979. Vacuum thermolysis of 1-deoxy-1-sarcosino-D-fructose. J. Agric. Food Chem. 27:1136-8.

Mohammed, A., Olcott, H.S. and Fraenkel-Conrat, H. 1949. The "browning" reaction of proteins with glucose. Archs. Biochem. 24:157-63.

Mohammed, A. 1979. U.S. Patent 4,144,357.

Monnier, V.M., Stevens, V.J. and Cerami, A. 1981. Maillard reactions involving proteins and carbohydrates *in vivo*: relevance to diabetes mellitus and aging. Prog. Food Nutr. Sci. 5:315-27.

Morihita, I., Takahara, H. and Shinkai, T. 1973. Japanese Patent 73 03,398. Japan Monopoly Corp.

Morishima, I., Hashimoto, M. and Yoshikawa, K. 1974. Photoelectron spectroscopic study of cyclic amines. The relation between ionization potentials, basicities and S character of the nitrogen lone pair electrons. J. Amer. Chem. Soc. 96:288-9.

Namiki, M. and Hayashi, T. 1983. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In: "The Maillard Reaction in

Foods and Nutrition". G.R. Walker and M.S. Feather (eds.). pp. 21-46. ACS Symposium Series. Washington, DC.

Nicholson, A.J.C. 1954. The photochemical decomposition of the aliphatic methyl ketones. Trans. Faraday Soc. 50:1067-73.

Nursten, H.E. 1980. Recent developments in studies of the Mailaird reaction. Food Chem. 6:263-77.

Nyhammar, T., Olsson, K. and Pernemalm, P.A. 1983. Strecker degradation products from (1-^{13}C)-D-glucose and glycine. In: "The Maillard Reaction in Foods and Nutrition". G.R. Walker and M.S. Feather (eds.). pp. 71-82. ACS Symposium Series. Washington, DC.

Olsson, K., Pernemalm, P.A. and Theander, O. 1981. Reaction products and mechanism in some simple model systems. Prog. Food Nutr. Sci. 5:47-55.

Patai, S. (ed.). 1968. The Chemistry of the Amino Group. Wiley, Interscience, NY.

Pearson, R.G. 1963. Hard and soft acids and bases. J. Amer. Chem. Soc. 85:3533-9.

Pitts, J.N. and Osborne, A.D. 1961. Symposium on Chemical Reaction in the Upper and Lower Atmosphere. p. 129. Wiley, NY.

Poindexter, E.H. and Carpenter, R.D. 1962. The isolation of harmane and norharmane from tobacco and cigarette smoke. Phytochemistry 1:215-21.

Ramsey, R.J., Tracy, P.M. and Ruehe, H.A. 1933. J. Dairy Ind. 16:17.

Reed, R.I., Finan, P.A. and Snedden, W. 1958. The application of the mass spectrometer to carbohydrate chemistry. Chem. Ind. (London), 1172.

Reynolds, T.M. 1963. Chemistry of nonenzymic browning. The reaction between aldoses and amines. Adv. Food Res. 12:1-52.

Reynolds, T.M. 1965. Chemistry of nonenzymic browning. II. Adv. Food Res. 14:167-283.

Reynolds, T.M. 1970. Flavors from nonenzymic browning reactions. Food Tech. Aust. 22:610-9.

Rizzi, G.P. 1974. Formation of N-alkyl-2-acylpyrroles and aliphatic aldimines in model nonenzymic browning reaction. J. Agric. Food Chem. 22:279-82.

Rizzi, G.P. 1969. The formation of tetramethyl pyrazine and 2-isopropyl-4,5-dimethyl-3-oxazoline in the Strecker degradation of DL-valine with 2,3-butadione. J. Org. Chem. 34:2002.

Rogers, D., King, T.E. and Sheldelin, V.H. 1953. Growth stimulation of *Lactobacillus gayoni* by N-D-glucosyl-glycine. Proc. Soc. Exp. Biol. Med. 82:140-4.

Rose, M.E. and Johnstone, R.A.W. 1982. Mass Spectrometry for Chemists and Biochemists. Cambridge Univ. Press, Cambridge.

Rosenstock, H.M., Wallenstein, M.B., Wahrhaftig, A.L. and Eyring, H. 1952. Absolute rate theory for isolated systems and the mass spectra of polyatomic molecules. Proc. Nat. Acad. Sci. U.S. 38:667-78.

Röper, H., Röper, S., Heyns, K. and Meyer, B. 1983. NMR spectroscopy of N-(1-deoxy-D-fructos-1-yl)-L-amino acids ("fructose-amino acids"). Carb. Res. 116:183-95.

Schiff, H. 1900. Uber Methylenasparagine. Justus Liebig's Ann. Chem. 310:25.

Schönberg, A., Moubacher, R. 1952. The Strecker degradation of α-amino acids. Chem. Rev. 50:261-77.

Schroeder, L.J., Jacobellis, M. and Smith, A.H. 1955. The influence of water and pH on the reaction between amino compounds and carbohydrates. J. Biol. Chem. 212:973.

Scott, D. 1953. Glucose conversion in preparation of albumin solids by glucose oxidase-catalase system. J. Agric. Food Chem. 1:727-30.

Schwarz, H. 1978. Some newer aspects of mass spectrometric *ortho* effect. Topics Current Chem. 73:231-63.

Severin, T. and Krönig, V. 1972. Studien zur Maillard-Reaktion. IV. Struktur eines farbigen Producktes aus Pentosen. Chem. Mikrobiol. Technol. Lebensm. 1:156-7.

Sgarbieri, V.C., Amaya, J., Tanaka, M. and Chichester, C.O. 1973. Nutritional consequences of the Maillard reaction. Amino acid availability from fructose-leucine and fructose-tryptophan in the rat. J. Nutr. 103:657-63.

Shibamoto, T. 1983. Heterocyclic compounds in browning and browning/nitrite model systems: occurrence, formation mechanisms, flavor characteristics. and mutagen activity. In: "Instrumental Analysis of Foods -- Recent Progress". G. Charalambous and G. Inglett (eds.). pp. 229-278. Academic Press, NY.

Shigematsu, H., Shibata, S., Kurata, T., Kato, H. and Fujimaki, M. 1975. 5-aeetyl-2,3-dihydro-1H-pyrollizines and 5,6,7,8-tetrahydroindolizine-8-ones, odor constituents formed on heating L-proline with D-glucose. J. Agric. Food Chem. 23:233.

Shigematsu, H., Shibata, S., Kurata, T., Kato, H. and Fujimaki, M. 1977. Thermal degradation products of several Amadori compounds. Agric. Biol. Chem. 41:2377-85.

Shyh-Horng Chiou, Chylack, L.T., Bunn, H.F. and Kinoshita, J.H. 1980. Role of non-enzymatic glycosylation in experimental cataract formation. Biochem. Biophys. Res. Commun. 95(2):894-901.

Simon, H. 1962. Zum Bildungsmechanismus des Piperidino-hexose-reduktons. Chem. Ber. 95:1003-8.

Simon, H. and Heubach, G. 1965. Alicyclic, open chain, N-containing reductones by the action between secondary amine salts and monosaccharides. Chem. Ber. 98:3703-11.

Smith, L.A. and Caprioli, R.M. 1984. Enzyme reaction rates determined by fast atom bombardment mass spectrometry. Biomed. Mass Spectrom. 11:392-5.

Snell, E.E. and Di Mari, S.J. 1970. The Enzymes. Vol. 2. D.D. Boyer (ed.), p. 335. Academic Press, NY.

Speck, J.C. 1958. The Lobry de Bruyn-Alberda van Ekenstein transformation. Adv. Carbohydr. Chem. 13:63-103.

Sullivan, J.F. 1981. Control of non-enzymatic browning in the dehydration of fruits and vegetables. Prog. Food Nutr. Sci, 5:377-93.

Svec, H.J. and Junk, G.A. 1963. The mass spectra of α-amino acids. J. Amer. Chem. Soc. 85:839-45.

Svec, H.J. and Junk, G.A. 1967. Electron-impact studies of substituted alkanes. J. Amer. Chem. Soc. 89:790-6.

Tanzer, M.L. 1973. Cross-linking of collagen. Science 180:561.

Taufel, K., Iwainsky, M. and Bergner, H. 1956. Analytische und chromatographische studien zur Maillard-Reaktion. III. Ernaehrungsforschung 1:719.

Tressl, R., Rewicki, D., Helak, B. and Kamperschroer, H. 1985a. Formation of pyridines and piperidines on heating L-proline with reducing sugar. J. Agric. Food Chem. 33:924-8.

Tressl, R., Rewicki, D., Helak, B., Kamperschroer, H. and Martin, N. 1985b. Formation of 2,3-dihydro-1H-pyrrolizines as proline specific Maillard products. J. Agric. Food Chem. 33:919-23.

Verstraeten, L.M.J. 1967. D-fructose and its derivatives. Adv. Carbohydr. Chem. 22:229-305.

Wagner, P.J. 1971. Type II photoelimination and photocyclization of ketones. Accts. Chem. Res. 4:168-77.

Waller, G.R. (ed.). 1972. Biochemical Applications of Mass Spectrometry. Wiley-Interscience, NY.

Wang, P., Kato, H. and Fujimaki, M. 1969. Studies on flavor components of roasted barley. Part III. The major volatile basic compounds. Agric. Biol. Chem. 33:1775-81.

Wedzicha, B.L. 1984. A kinetic model for the sulphite inhibited Maillard reaction. Food Chem. 14:173-87.

Weygand, F. 1940. Uber N-Glycoside. II. Amadori-Umlagerungen. Ber. deutsch. Chem. Ges. 73:1259.

Williams, D.H. and Howe, I. 1972. Principles of Organic Mass Spectrometry. McGraw-Hill, London.

7. APPENDIX I -- MASS SPECTRA

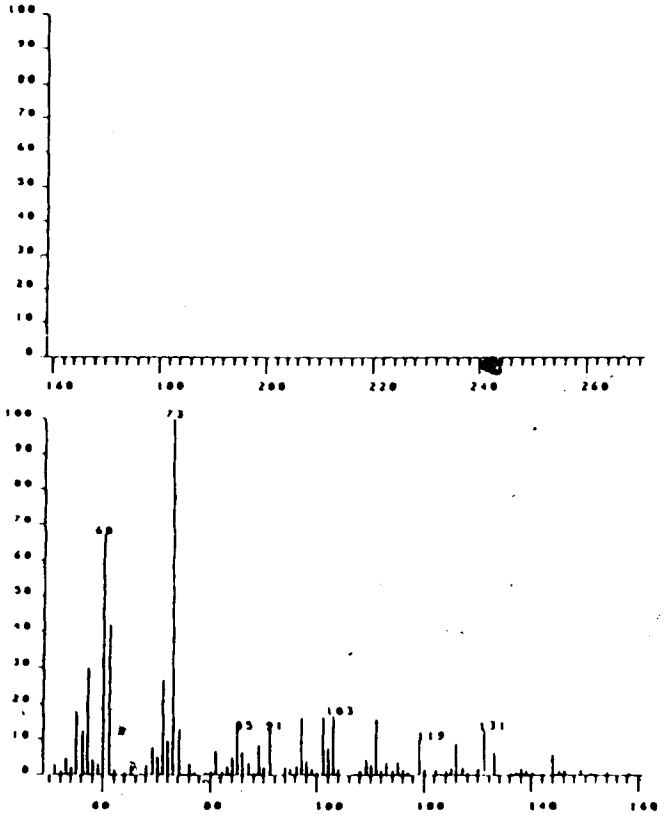


Figure 6.1 Mass spectrum of 1-[(carboxymethyl) amino]-1-deoxy-D-fructose (glycine ARP)

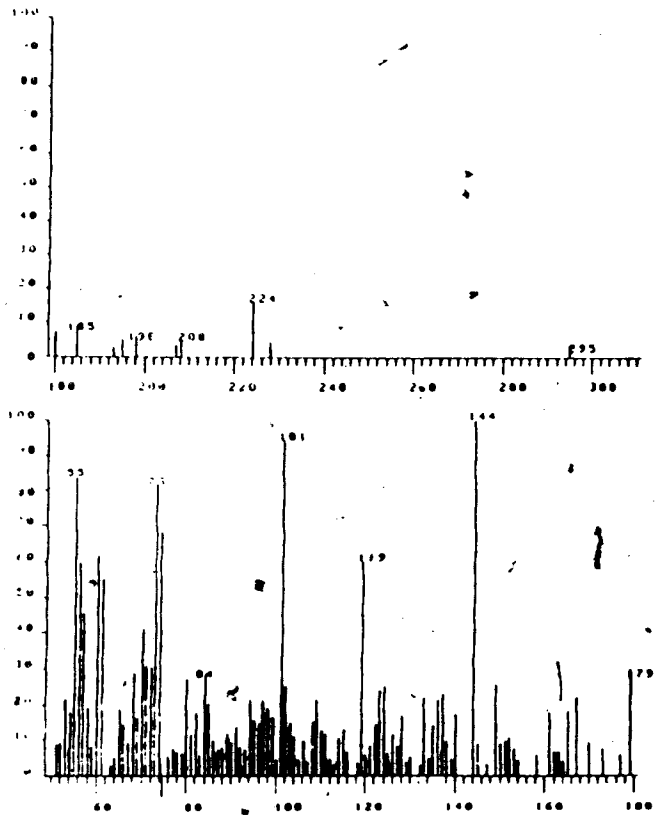


Figure 6.2 Mass spectrum of 1-[(carboxyethyl) amino]-1-deoxy-D-fructose (alanine-ARP)

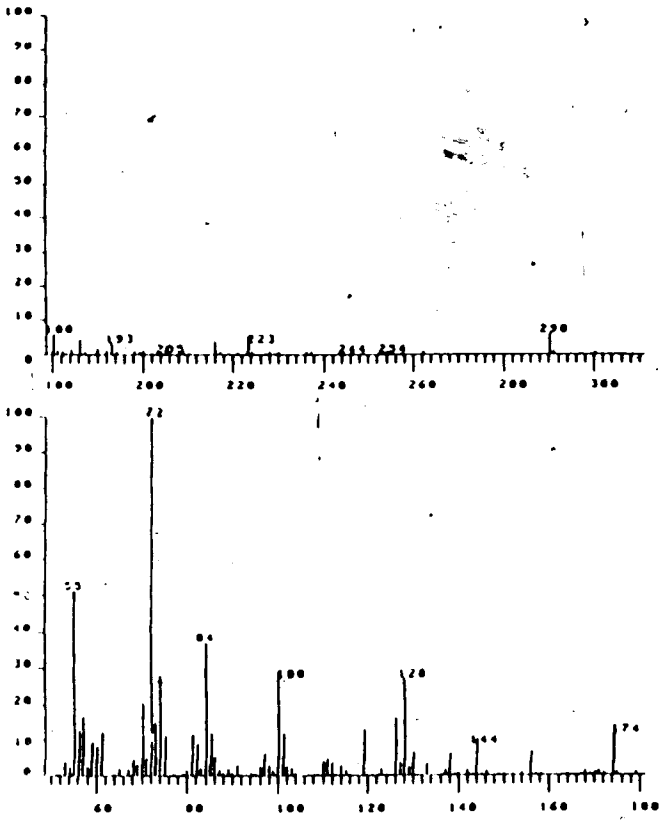


Figure 6.3. Mass spectrum of 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose (valine-ARP)

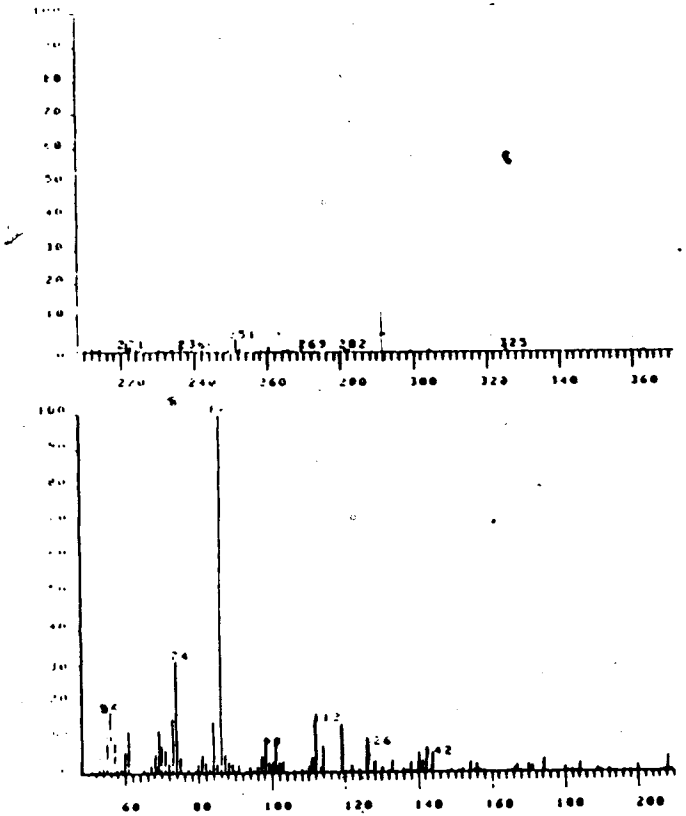


Figure 6.4. Mass spectrum of 1-[(1'-carboxy-3'-methylbutyl)amino]-1-deoxy-D-fructose (leucine-ARP)

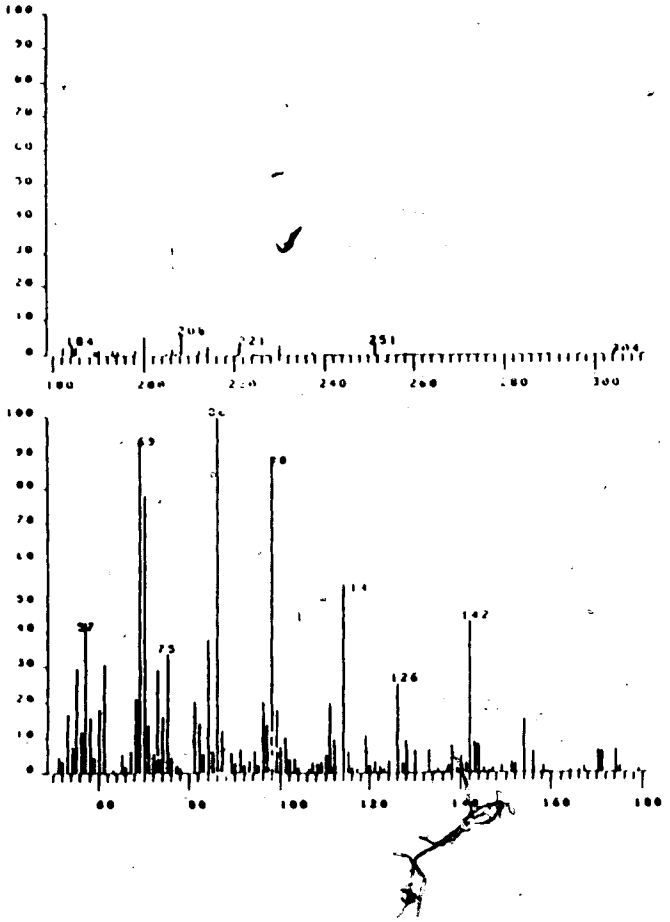


Figure 6.5 Mass spectrum of 1-[(1'-carboxy-2'-methylbutyl)amino]-1-deoxy-D fructose (isoleucine ARP)

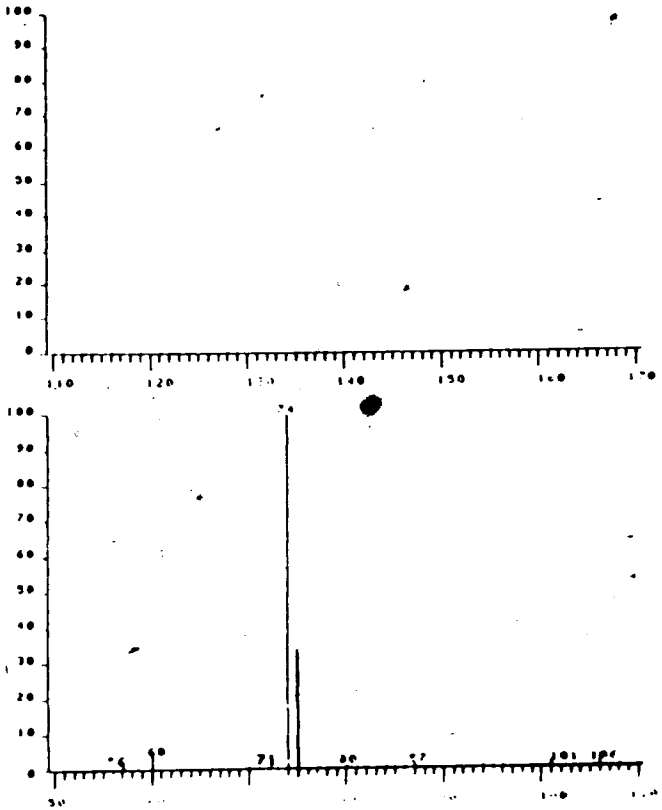


Figure 6.6 Mass spectrum of 1-[(1'-carboxy-2'-hydroxyethyl)amino]-1-deoxy-D-fructose (serine-ARP)

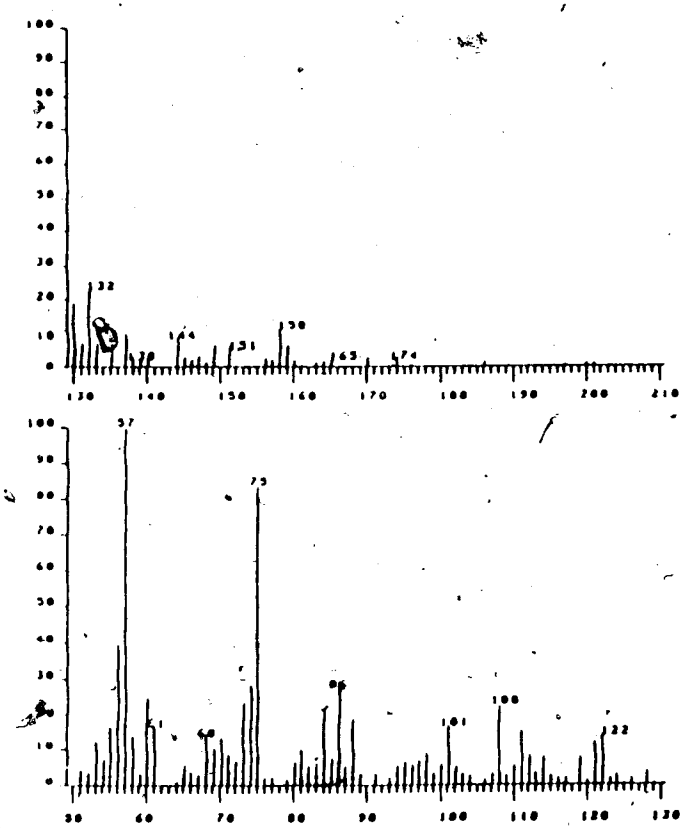


Figure 6.7 Mass spectrum of 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose (threonine ARP)

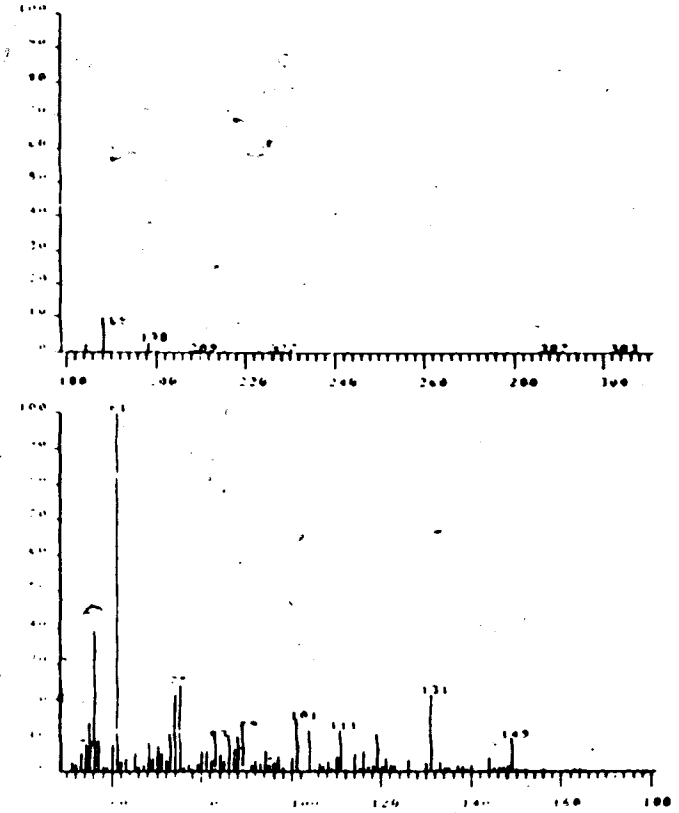


Figure 6.8 Mass spectrum of 1-[[(1'-carboxy-3'-(methylthio)propyl]amino)-1-deoxy-D-fructose (methionine ARP)

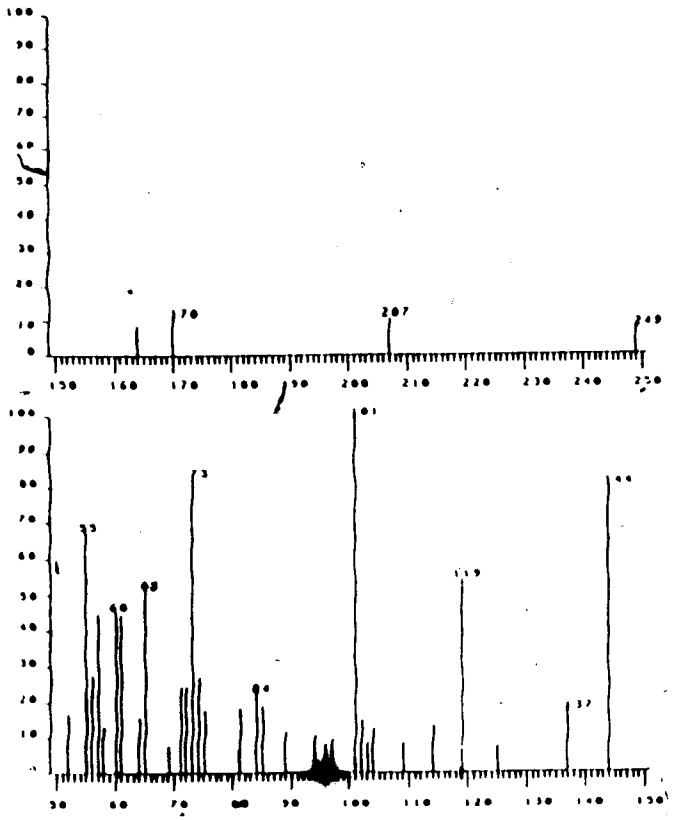


Figure 6.9 Mass spectrum of 1-[(1'-carboxy-3'-methylcarboxypropyl)amino]-1-deoxy-D-fructose (glutamic acid methyl ester ARP)

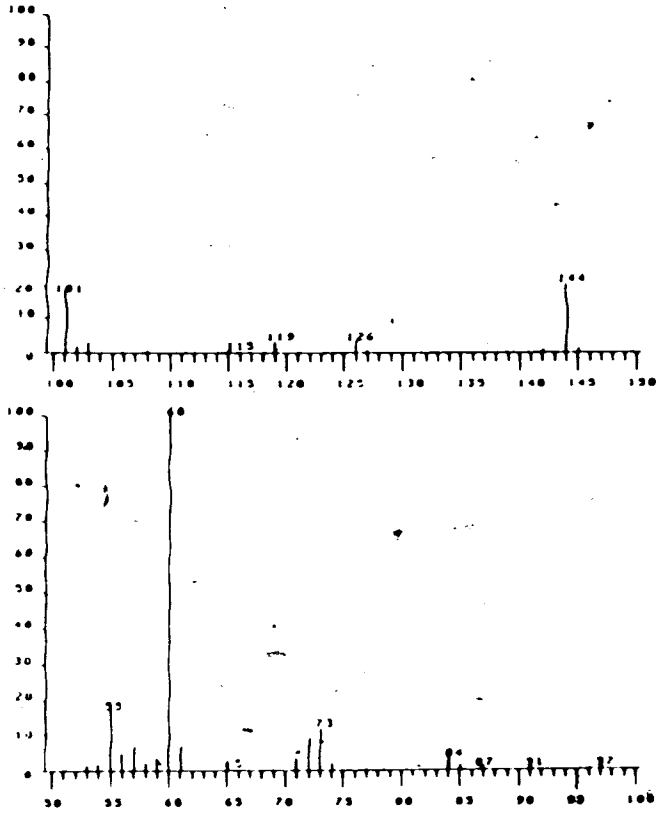


Figure 6.10 Mass spectrum of 1-[(5'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose (difructosyllysine ARP)

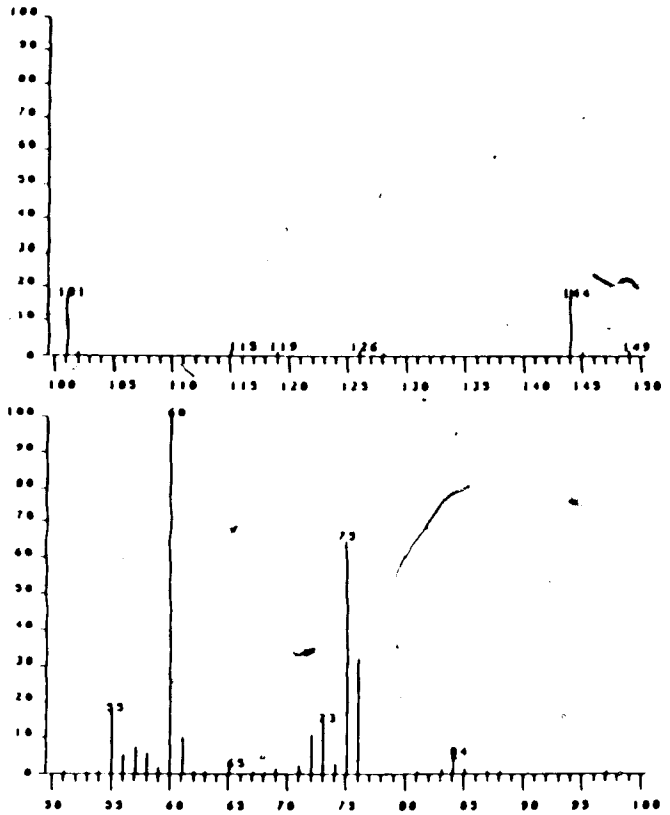


Figure 6.11 Mass spectrum of 1-[(5'-aminoformyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose -

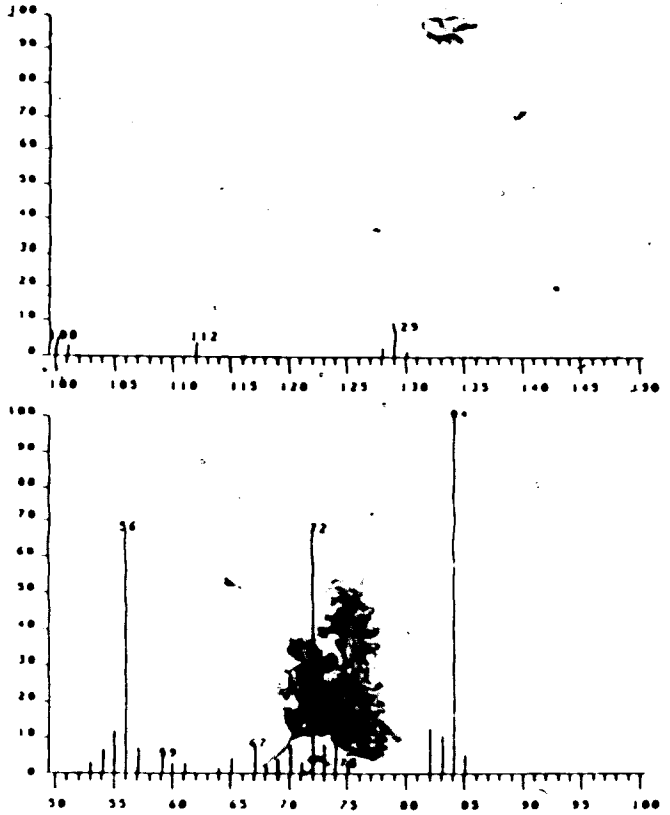


Figure 6.12 Mass spectrum of 1-[(5'-amino-1'-carboxypentyl)a[illegible]-1-deoxy-D-fructose

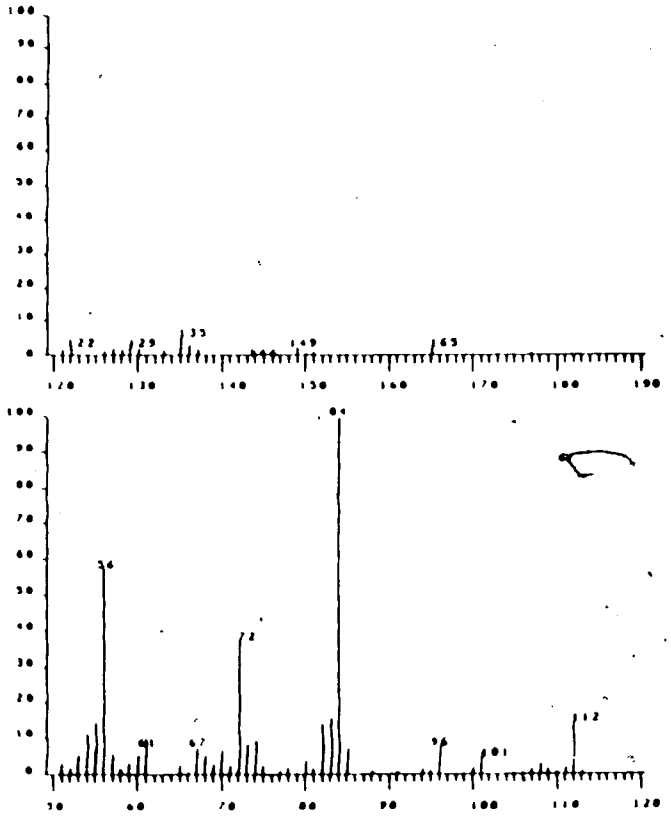


Figure 6.13 Mass spectrum of 1-[(1-amino 1-carboxypentyl)amino]-1-deoxy-D-fructose

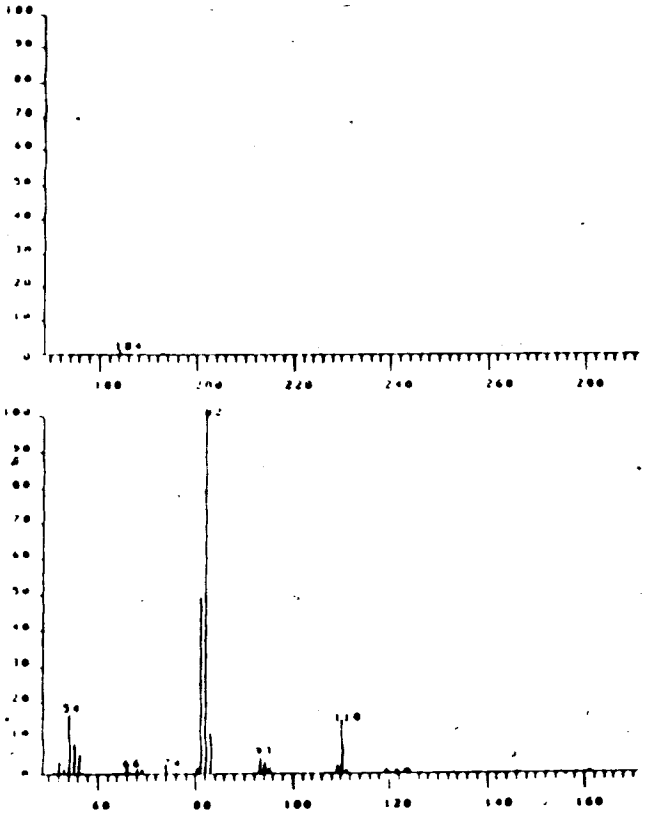


Figure 6.14 Mass spectrum of 1-[(1-carboxy 2-imidazolylethyl)amino]-1-deoxy-D-fructose (histidine ARP)

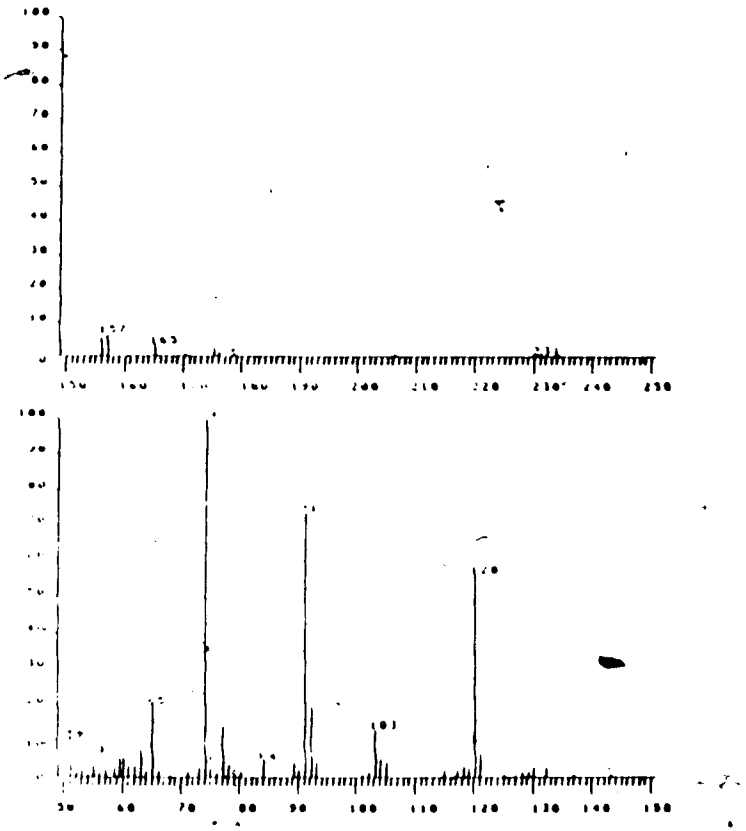


Figure 6.15 **Mass spectrum of 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose (phenylalanine ARP)**

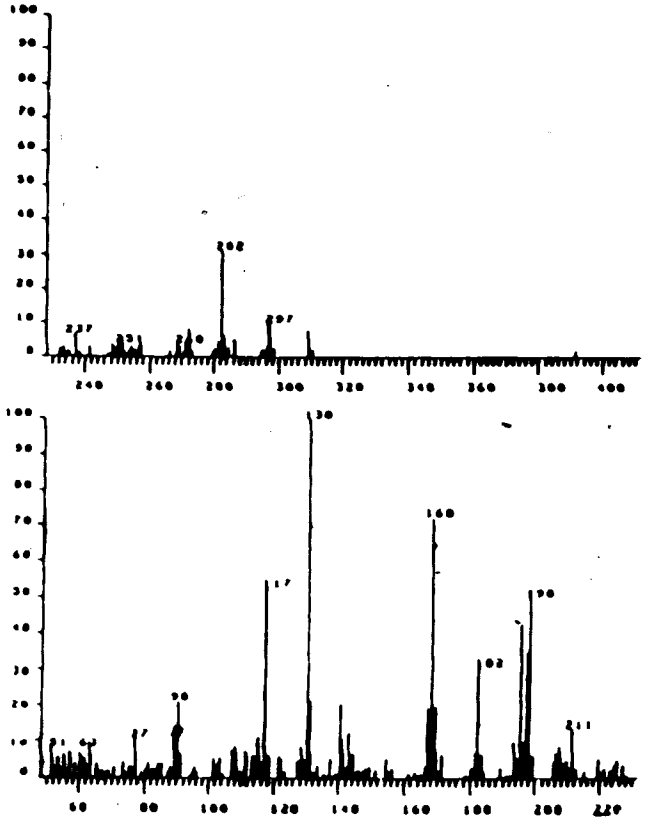


Figure 6.16 **Mass spectrum of 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)amino]-1-deoxy-D-fructose (tryptophan ARP)**

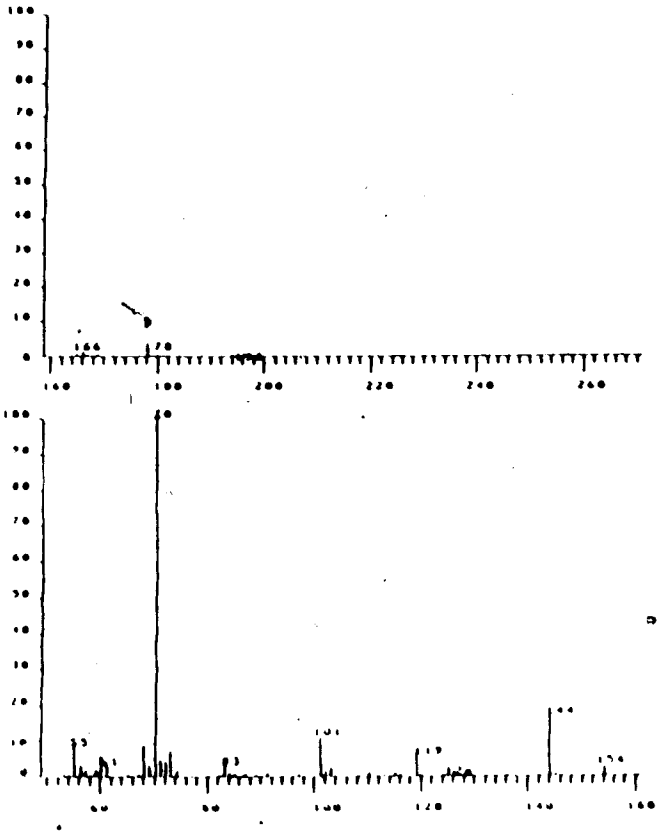


Figure 6.17 Mass spectrum of 1-[(2-carboxy) pyrrolidinyl]-1-deoxy-D-fructose (proline ARP)

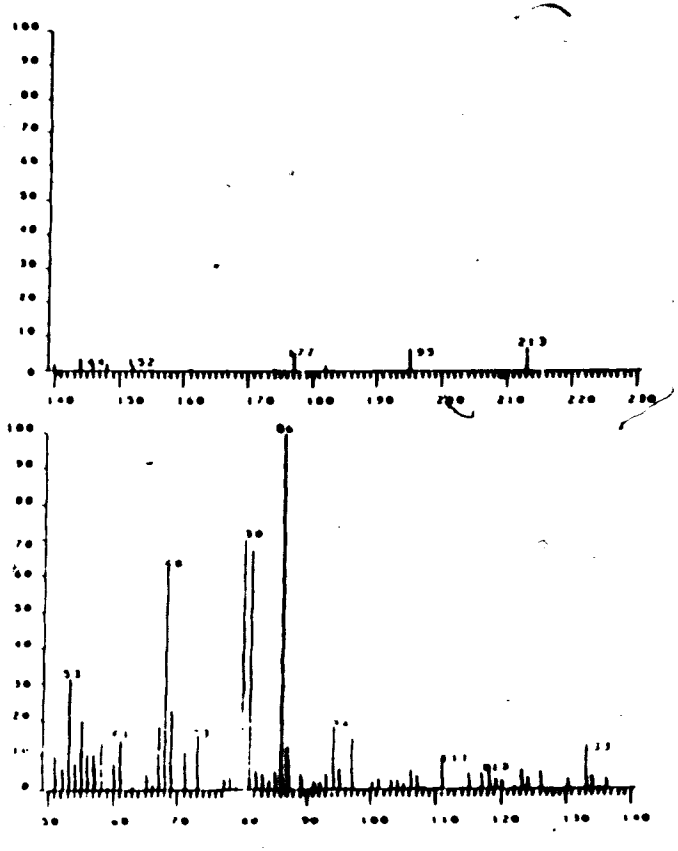


Figure 6.18 Mass spectrum of 1-[(2-carboxy-4-hydroxy)pyrrolidinyl]-1-deoxy-D-fructose (hydroxyproline ARP)

8. APPENDIX II -- "SUGAR FRAGMENTATIONS"

Table 7.1. A. 'Sugar' fragmentations' of 1-[(1'-carboxy-2'-methylpropyl)amino]-1-deoxy-D-fructose (valine-ARP).

Fragment	Mol. wt (% int)	Fragment	Mol. wt (% int)	Fragment	Mol. wt (% int)
A_1 (p,f)	262.1272 (0.96)	A_1^a (p,f)	133.0560 (1.57)	A_2 (p,f)	244.1175 (1.88)
A_2^a (p,f)	115.0392 (1.91)	A_2^d (p,f)	148.0655 (1.71)	A_2^q (p,f)	200.1276 (1.62)
A_3^a (p,f)	97.0289 (6.36)	A_3^c (p,f)	111.0445 (5.03)	A_3^d (p,f)	126.0556 (16.45)
A_3^q (p,f)	182.1175 (1.00)	B_2	89.0239 (2.48)	bo_2^c	103.0395 (2.62)
C_1 (p,f)	104.0472 (1.18)	C_2 (p,f)	87.0437 (1.77)	C_3 (p,f)	69.0342 (5.71)
P_1	104.0472 (1.18)	pP_2	74.0356 (9.25)	D_1^c	91.0394 (3.30)
J_1^c	61.0310 (12.10)	F_1	73.0294 (15.36)	H_1	60.0234 (8.44)
H_2	59.0157 (1.22)	hH_1^c	74.0371 (4.77)	hH_2^c	73.0294 (15.36)
I_2	55.0203 (10.96)	aI_2	56.0282 (5.97)	bI_2	57.0363 (9.32)
L_2	119.0344 (13.03)	L_3	101.0238 (11.68)	IL_2^a	119.0344 (11.03)
IL_2^c	133.0500 (3.57)	IL_3^q	102.0312 (2.93)	E_2^q	186.1122 (4.91)
E_2^c	115.0392 (1.91)	E_2^a	101.0238 (11.68)	E_3^c	97.0289 (6.36)
E_3^d	112.0398 (2.14)	E_3^q	168.1024 (1.58)		

* In this and subsequent tables, p = pyranose form and f = furanose form

Table 7.2. B. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-hydroxyethyl)amino]-1-deoxy-D-fructose (serine-ARP).

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt (% int)
A_2^b (p,f)	128.0493 (0.26)	A_3^a (p,f)	97.0282 (0.71)	A_3^d (p,f)	126.0529 (0.62)
A_4^c (p,f)	138.0534 (0.35)	B_2	89.0237 (0.56)	bB_2^a	89.0237 (0.56)
bB_2^e	132.0663 (0.65)	C_3 (p)	69.0343 (0.53)	J_1^b	60.0227 (1.12)
D_1^d	106.0517 (9.54)	F_1	73.0278 (6.21)	H_1	60.0227 (1.12)
bH_1^b	73.0278 (6.21)	hH_2^b	72.0202 (1.88)	I_2	55.0200 (1.53)
bI_2	57.0350 (1.09)	L_3	101.0239 (5.45)	LL_3^d	131.0584 (0.51)
E_2^a	101.0239 (5.45)	E_3^c	97.0282 (0.71)	E_3^e	126.0529 (0.62)
E_4^n	138.0534 (0.35)				

Table 7.3. C. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-hydroxypropyl)amino]-1-deoxy-D-fructose (threonine-ARP).

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_1^e (p,f)	176.0956 (0.75)	A_1^b (p,f)	146.0566 (1.68)	A_1^a (p,f)	133.0512 (1.64)
A_2^e (p,f)	158.0818 (10.54)	A_2^d (p,f)	144.0662 (5.42)	A_2^b (p,f)	128.0471 (2.01)
A_3^e (p,f)	140.0711 (4.01)	A_3^d (p,f)	111.0443 (15.45)	A_3^b (p,f)	110.0366 (2.01)
A_3^a (p,f)	97.0290 (6.96)	B_2	89.0242 (3.60)	B_1^e	163.0856 (1.43)
B_1^d	149.0713 (6.13)	B_1^b	133.0512 (1.64)	bB_1^e	163.0856 (1.43)
bB_1^d	149.0713 (6.13)	bB_1^b	133.0512 (1.64)	bB_2^e	132.0664 (22.51)
bB_2^q	176.0956 (0.75)	bB_2^b	102.0318 (4.32)	C_1 (p,f)	104.0469 (2.71)
C_2 (p,f)	87.0448 (5.69)	C_3	69.0342 (10.45)	P_1	104.0469 (2.71)
pP_2	74.0366 (5.91)	P_2	103.0376 (1.68)	D_1^c	120.0683 (1.10)
D_1^c	91.0395 (3.50)	J_1^d	76.0356 (1.72)	J_1^c	61.0308 (16.86)
J_1^b	60.0233 (24.44)	F_1	73.0294 (23.01)	H_1	60.0233 (24.44)
hH_1^c	74.0366 (5.91)	hH_1^b	73.0294 (23.01)	hH_1^a	60.0233 (24.44)
H_2	59.0150 (2.38)	I_2	55.0203 (16.53)	aI_2	56.0280 (7.87)
bI_2	57.0362 (30.79)	L_2	119.0342 (7.85)	L_3	101.0235 (17.17)
IL_2^c	133.0512 (3.64)	IL_2^a	119.0342 (7.85)	IL_3^d	131.0586 (6.92)
IL_3^a	102.0318 (4.32)	E_1^c	133.0572 (3.64)	E_1^a	119.0342 (7.85)
E_2^e	144.0662 (5.42)	E_2^d	130.0511 (18.98)	E_1^c	97.0290 (6.96)

Table 7.4. D. 'Sugar fragmentations' of 1-[[[1'-carboxy-3'-(methylthio)propyl]amino]-1-deoxy-D-fructose (methionine-ARP).

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_1^a (p,f)	133.0315 (2.96)	A_1^b (p,f)	146.0596 (0.81)	A_2^a (p,f)	115.0404 (1.45)
A_2^h (p,f)	230.1004 (1.28)	A_3^a (p,f)	97.0292 (4.72)	A_3^b (p,f)	110.0365 (2.01)
A_3^c (p,f)	111.0452 (11.72)	A_3^d (p,f)	126.0550 (3.36)	A_3^e (p,f)	140.0715 (2.26)
B_2	89.0245 (3.65)	B_1^a	120.0428 (1.26)	B_1^b	133.0315 (2.96)
B_1^c	134.0596 (1.00)	B_1^d	149.0693 (1.43)	B_2^b	115.0404 (1.45)
B_1^e	163.0842 (1.23)	bB_1^a	120.0428 (1.26)	bB_1^b	133.0315 (2.96)
bB_1^c	134.0596 (1.00)	bB_1^d	149.0693 (1.43)	bB_1^e	163.0842 (1.2y)
bB_2^a	89.0245 (3.65)	bB_2^c	132.0650 (1.02)	C_2 (p,f)	87.0444 (1.90)
C_3	69.0343 (3.72)	F_1	73.0291 (10.82)	P_2	74.0373 (0.94)
D_1^c	91.0395 (2.57)	D_1^e	120.0659 (0.56)	D_2^h	148.0946 (0.31)
J_1^c	61.0313 (12.50)	J_1^b	60.0235 (7.79)	D_1^b	90.0318 (0.64)
H_1	60.0235 (7.79)	H_2	59.0151 (1.47)	hH_1^d	89.0430 (14.10)
hH_1^c	74.0373 (0.94)	hH_2^c	72.0216 (3.49)	hH_1^a	60.0235 (7.79)
hH_1^b	73.0298 (10.02)	hH_2^a	59.0157 (1.47)	I_2	55.0205 (11.07)
bI_2	57.0366 (8.61)	aI_2	56.0286 (4.86)	L_2	119.0345 (10.74)
L_3	101.0745 (6.84)	IL_2^d	148.0632 (0.30)	IL_3^e	145.0748 (1.34)
IL_3^c	116.0520 (4.04)	IL_2^c	133.0315 (2.96)	IL_3^b	115.0404 (1.45)
E_1^a	119.0345 (10.74)	E_1^d	148.0632 (0.30)	E_2^a	101.0245 (6.84)
E_2^c	115.0404 (1.45)	E_1^c	97.0292 (4.72)	E_3^e	126.0550 (3.36)
E_3^d	112.0409 (1.88)				

Table 7.5. B. 'Sugar fragmentations' of 1-[(1'-carboxy-3'-methylcarboxypropyl)amino]-1-deoxy-D-fructose (glutamic acid methyl ester-ARP).

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_2^a (p,f)	97.0282 (9.79)	B_2	89.0238 (11.69)	bB_2^a	89.0238 (11.69)
bB_2^b	102.0307 (15.04)	bB_2^c	103.0392 (9.07)	C_1 (p,f)	104.0463 (12.65)
C_3	69.0340 (8.11)	P_2	103.0392 (9.07)	pP_2	74.0370 (26.97)
J_1^b	60.0232 (45.35)	J_1^c	61.0306 (84.63)	F_1	73.0295 (82.58)
H_1	60.0232 (45.35)	hH_1^a	60.0232 (45.35)	hH_1^b	73.0295 (82.38)
hH_1^c	74.0370 (26.97)	hH_2^b	72.0211 (24.58)	I_2	55.0202 (67.78)
aI_2	56.0280 (27.45)	bI_2	57.0360 (44.87)	L_2	119.0345 (54.18)
L_3	101.0240 (100)	LL_2^a	119.0345 (54.18)	LL_1^a	102.0307 (15.04)
F_1	73.0295 (82.58)	E_1^a	119.0345 (54.18)	E_2^a	101.0240 (100)
E_3^c	97.0283 (9.79)				

Table 7.6. F. 'Sugar fragmentations' of 1-[(5'-aminofructosyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose (difructosyllysine-ARP)

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_2^a (p,f)	115.0396 (1.20)	bB_2^b	102.0304 (2.20)	bB_2^c	103.0396 (3.03)
C_3	69.0334 (0.50)	P_2	103.0396 (3.03)	pP_2	74.0365 (2.25)
J_1^c	61.0300 (7.24)	J_1^b	60.0234 (100)	F_1	73.0292 (11.86)
H_1	60.0234 (100)	H_2	59.0157 (1.50)	hH_1^c	74.0365 (2.25)
hH_1^b	73.0292 (11.86)	hH_2^b	72.0213 (9.28)	I_2	55.0204 (19.21)
aI_2	56.0281 (5.23)	bI_2	57.0362 (7.26)	L_2	119.0344 (3.49)
L_3	101.0238 (18.44)	F_1	73.0292 (11.88)	lL_2^a	119.0344 (3.49)
lL_3^a	102.0304 (2.20)	lL_3^b	115.0396 (1.20)	E_1^a	119.0344 (3.49)
E_2^a	101.0238 (18.44)	E_2^c	115.0396 (1.20)		

Table 7.7. G. 'Sugar fragmentations' of 1-[(5'-aminoformyl-1'-carboxypentyl)amino]-1-deoxy-D-fructose.

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_2^a (p,f)	115.0391 (1.46)	A_2^b (p,f)	128.0473 (0.82)	bB_2^b	102.0305 (1.46)
C_2 (p,f)	87.0444 (0.99)	pP_2	74.0373 (3.05)	J_1^c	61.0307 (12.61)
J_1^b	60.0234 (100)	F_1	73.0295 (16.46)	H_1	60.0234 (100)
hH_1^c	74.0373 (3.05)	hH_1^b	73.0295 (16.46)	hH_2^c	73.0295 (16.46)
hH_2^b	72.0215 (11.22)	L_2	119.0348 (1.48)	L_3	101.0219 (18.34)
lL_2^a	119.0348 (1.48)	aL_3^a	102.0305 (1.46)	lL_3^b	115.0391 (1.46)
I_2	55.0204 (18.17)	aI_2	56.0283 (5.66)	bI_2	57.0163 (7.54)
ε_1^a	119.0348 (1.48)	ε_2^a	101.0239 (18.34)	ε_2^c	115.0391 (1.46)

Table 7.8. H. 'Sugar fragmentations' of 1-[(5'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose.

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
J_1^c	61.0311 (3.18)	J_1^b	60.0231 (3.31)	F_1	73.0296 (8.41)
H_1	60.0231 (3.31)	hH_1^b	73.0296 (8.43)	I_2	55.0197 (2.29)

Table 7.9. 1. 'Sugar fragmentations' of 1-[(1'-amino-1'-carboxypentyl)amino]-1-deoxy-D-fructose.

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_3^d (p,f)	126.0554 (1.22)	C_3	69.0347 (1.77)	pP_2	74.0372 (2.93)
J_1^c	61.0311 (9.77)	J_1^b	60.0231 (5.46)	F_1	73.0295 (8.18)
H_1	60.0231 (5.46)	hH_1^c	74.0372 (2.93)	hH_1^b	73.0295 (8.18)
I_2	55.0202 (7.19)	bI_2	57.0362 (5.75)	L_1	101.0238 (5.50)

Table 7.10. J. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-imidazolylethyl)amino]-1-deoxy-D-fructose (histidine-ARP).

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
bB_2^b	102.0326 (10.09)	bB_2^c	103.0420 (0.14)	bB_2^d	118.0501 (0.16)
bB_2^e	132.0683 (0.13)	C_1 (p,f)	104.0501 (0.18)	P_2	103.0420 (0.14)
J_1^c	61.0312 (0.30)	J_1^b	60.0228 (0.30)	F_1	73.0290 (0.12)
H_1	60.0228 (0.30)	hH_1^b	73.0290 (0.32)	IL_3^d	131.0594 (0.22)
IL_3^e	102.0326 (0.09)	I_2	55.0199 (0.56)	aI_2	56.0280 (0.10)
bI_2	57.0364 (0.23)	E_2^d	130.0519 (0.17)		

Table 7.11. K. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-phenethyl)amino]-1-deoxy-D-fructose (phenylalanine ARP)

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt (% int)
C_1 (p,f)	104.0509 (1.10)	J_1^c	61.0311 (3.11)	J_1^b	60.0231 (3.76)
F_1	73.0293 (3.09)	H_1	60.0231 (3.76)	NH_1^b	73.0291 (3.09)
L_2	199.0345 (1.18)	L_3	101.0226 (1.24)	IL_2^a	119.0345 (1.18)
I_2	55.0202 (3.51)	aI_2	56.0278 (1.65)	bI_2	57.0161 (2.48)
E_1^a	119.0345 (1.18)	E_2^a	101.0226 (1.24)		

Table 7.12. L. 'Sugar fragmentations' of 1-[(1'-carboxy-2'-indol-3'-yl-ethyl)amino]-1-deoxy-D-fructose (tryptophan-ARP)

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt (% int)
A_2^a (p.f)	115.0413 (1.83)	A_2^b (p.f)	128.0498 (8.82)	A_2^c (p.f)	129.0579 (5.41)
A_3^c (p.f)	111.0447 (7.15)	A_3^b (p.f)	110.0364 (1.79)	B_1^e	163.0864 (1.71)
B_1^d	149.0707 (3.24)	bB_2^b	102.0344 (1.33)	bB_2^c	103.0415 (2.58)
bB_2	146.0846 (2.41)	C_1 (p.f)	104.0482 (1.50)	C_3	69.0342 (2.16)
P_2	103.0415 (2.58)	J_1^c	61.0310 (5.07)	J_1^b	60.0230 (12.31)
F_1	73.0294 (4.37)	H_1	60.0234 (12.31)	hH_1^b	73.0294 (4.37)
L_3	101.0238 (5.24)	IL_3^e	145.0780 (2.16)	IL_3^c	116.0503 (4.45)
IL_3^a	102.0309 (1.96)	IL_3^b	115.0413 (1.83)	bI_2	37.0359 (66.95)
E_2^a	101.0238 (5.24)	E_2^b	114.0349 (2.28)	E_2^c	115.0413 (1.83)

Table 7.13. M. 'Sugar fragmentations' of 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D fructose (proline-ARP)

Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)	Fragment	Mol. wt. (% int)
A_2^1 (p,f)	196.0971 (6.65)	A_2^b (p,f)	128.0465 (1.08)	A_2^a (p,f)	115.0394 (5.04)
A_3^q (p,f)	180.1019 (1.62)	A_3^1 (p,f)	178.0867 (7.37)	A_3^a (p,f)	97.0289 (1.38)
B_2	89.0242 (5.27)	B_1^a	120.0385 (4.02)	bB_1^a	120.0385 (4.02)
bB_2^a	89.0242 (5.27)	bB_2^b	102.0368 (11.68)	bB_2^c	103.0395 (16.25)
C_1 (p,f)	104.0441 (1.86)	C_2 (p,f)	87.0450 (2.64)	C_3	69.0343 (1.06)
P_2	103.0395 (16.25)	pP_2	74.0371 (13.25)	D_1^b	90.0316 (1.06)
D_1^c	91.0395 (9.23)	J_1^1	128.0712 (4.62)	J_1^c	61.0308 (19.66)
J_1^b	60.0232 (35.80)	F_1	73.0294 (44.84)	H_1	60.0232 (35.80)
H_2	59.0155 (2.30)	hH_1^c	74.0371 (13.25)	hH_2^c	73.0294 (44.84)
hH_1^b	73.0294 (44.84)	hH_2^b	72.0215 (26.80)	bH_1^a	60.0232 (35.80)
hH_2^a	59.0155 (2.30)	L_2	119.0344 (41.49)	L_3	101.0239 (66.01)
IL_2^a	119.0344 (41.49)	IL_3^a	102.0308 (11.63)	IL_3^b	115.0394 (5.04)
IL_3^c	116.0471 (4.68)	E_1^a	119.0344 (41.49)	E_2^a	101.0239 (66.01)
E_3^c	97.0289 (1.38)	E_3^q	166.0858 (9.05)	E_2^b	114.0114 (2.46)
E_2^c	115.0374 (5.04)	E_2^q	184.0967 (1.86)		

Table 7.14. M. 'Sugar fragmentations' of 1-[(2'-carboxy-4'-hydroxy)pyrrolidinyl]-1-deoxy-D-fructose (hydroxyproline-ARP)

Fragment	Mol. wt. (% int)	Fragment	Mol. wt (% int)	Fragment	Mol. wt. (% int)
A_1^b (p.f)	146.0600 (1.76)	A_1^a (p.f)	133.0502 (9.99)	A_2^a (p.f)	115.0395 (4.68)
A_3^c (p.f)	111.0447 (9.[illegible])	A_3^a (p.f)	97.0289 (14.74)	B_2	89.0238 (4.38)
B_1^c	134.0602 (3.92)	B_1^b	133.0502 (9.99)	bB_2^a	101.0235 (2.98)
bB_1^c	134.0602 (3.92)	bB_1^b	133.0502 (9.99)	bB_2^c	103.0397 (2.95)
C_1 (p.f)	104.0500 (2.77)	C_3	69.0341 (6.04)	P_2	103.0397 (2.95)
P_1	104.0500 (2.97)	J_1^c	61.0309 (14.34)	J_1^b	60.0232 (7.63)
D_1^c	91.0399 (2.61)	J_1^a	126.0555 (5.07)	F_1	73.0295 (15.41)
H_1	60.0232 (7.63)	hH_2	73.0292 (15.41)	L_2	119.0346 (3.06)
L_3	101.0256 (2.98)	$1L_3^1$	183.0892 (0.66)	$1L_3^b$	115.0395 (4.68)
$1L_2^c$	133.0502 (9.99)	$1L_2^a$	119.0346 (3.06)	E_1^a	119.0346 (3.06)
E_1^c	133.0502 (9.99)	E_2^a	101.0235 (2.98)	E_2^c	115.0395 (4.68)
E_3^c	97.0289 (14.74)				