

VOLUME 1

oooooooooooooooooooooooooooo

*Chemistry
of the
Amino Acids*

JESSE P. GREENSTEIN

and MILTON WINITZ

Laboratory of Biochemistry,
National Cancer Institute,
National Institutes of Health,
Public Health Service,
United States Department of Health,
Education, and Welfare

**NEW YORK • LONDON
JOHN WILEY & SONS, INC.**

Copyright © 1961 by John Wiley & Sons, Inc.

All Rights Reserved. This book or any part thereof must not be reproduced in any form without the written permission of the publisher.

Library of Congress Catalog Card Number: 61-6474

Printed in the United States of America

oooooooooooooooooooooooooooo

To Our Wives and Children



Preface

Few products of natural origin are as versatile in their behavior and properties as are the amino acids, and few have such a variety of biological duties to perform. They are at once: water-soluble and amphoteric electrolytes, with the ability to form acid salts and basic salts and thus act as buffers over at least two ranges of pH ; dipolar ions of high electric moment with a considerable capacity to increase the dielectric constant of the medium in which they are dissolved; compounds with reactive groups capable of a wide range of chemical alterations leading readily to a great variety of degradation, synthetic, and transformation products, such as esters, amides, amines, anhydrides, polymers, polypeptides, diketopiperazines, hydroxy acids, halogenated acids, keto acids, acylated acids, mercaptans, shorter- or longer-chained acids, and pyrrolidine and piperidine ring forms; indispensable components of the diet of all animals including man; participants in crucial, metabolic reactions on which life depends and substrates for a variety of specific enzymes *in vitro*; binders of metals of many kinds; absorbers of ultraviolet and infrared radiation within specific ranges of wavelength; possessors with one exception of optical rotatory power related to the configuration of asymmetric centers; and essential constituents of protein molecules whose biological and chemical specificities are determined in part by the number, distribution, and spatial interrelations of the amino acids of which they are composed. They reveal at once uniformity and diversity; uniformity, in that with rare exceptions they are α -amino acids with all the physical consequences which flow from this fact, and, for those that are constituents of proteins and hence of living tissues, the same optical configuration at the α -carbon atom is common to all; diversity, because each possesses a different side chain which confers upon it unique properties distinguishing it physically, chemically, and biologically from the others. In this duality, the array of the amino acids is a partial reflection of the larger biological world which is always the same and always different.

Let it be admitted that we have been entranced by the spectacle of these many and diverse phenomena. In attempting to transfer these pleasurable emotions to paper, and to portray accurately the work of so many able investigators, we were well aware of the magnitude and difficulty of the task which we set for ourselves, and of the impossibility of perfection. With filial piety and in justice to those who have built this field, as well as to convey a sense of perspective, we have stressed where we could the historical development of each subject. The literature of science is generally curt and colorless, except when enlivened at times by the statement of controversial opinion; on occasion an expression of notable and graceful generosity or of far-seeing imagination finds its way into this literature, and such examples have been quoted where pertinent in the pages to follow. The topics covered in this work have generally been those wherein a certain stability has been attained and are thereby suitable for comprehensive treatment—not that progress has ceased in such topics, but rather that nothing has so far emerged to render any substantial portion of them obsolete or questionable.

Emphasis has been placed on the organic chemistry and the physical chemistry of the amino acids, and on the role these compounds play in the nutrition of a variety of living forms. The pertinent literature has been covered to the close of 1958. The design of the treatise embraces both the intellectual development of the subject and its corollary, experiment; and a large number of illustrative experimental procedures, many of them either tested or developed in our own Laboratory, have been interpolated in the text. The subject of the metabolism of individual amino acids has been omitted because of its brilliant coverage by our former colleague, Dr. Alton Meister, in his recent book on the Biochemistry of the Amino Acids.

Volumes 1 and 2 have been designed to embrace the study of the amino acids as a class of chemical compounds, including aspects more or less general to these compounds. Volume 3, on the other hand, takes into consideration the history, reactions, syntheses, physical characteristics, and optical resolution of each individual amino acid. Only the α -amino acids and their derivatives have been treated in detail in these volumes. A comparable treatment of the ω -amino acids would require more space than appears desirable at this time.

We have read thousands of papers in preparation for this treatise, and despite our best efforts it is likely that some reports equal in significance to those cited may have inadvertently eluded our notice. Much of the modern Russian scientific literature is still accessible with difficulty, and it is not improbable that some areas of value in this literature have also been overlooked. On the other hand, it has been deemed necessary to omit consideration of many reports of doubtful interest. It requires skill and constant

vigilance to keep afloat in the flood of contemporary scientific reports, and although we can lay claim to little of the former quality and only to a modicum of the latter, we take a certain assurance in having rarely referred to any work without the original before us at the moment.

In drawing on the accumulated experience of several years in the writing of this treatise, it is only proper to express our appreciation to our colleagues, past and present, who have labored with us and contributed so much to the various programs of the Laboratory, namely, Drs. Shiro Akabori, Carl G. Baker, Leo Benoiton, Louis Berlinguet, Sanford M. Birnbaum (to whom we are additionally indebted for help on various phases of this treatise), Leah Bloch-Frankenthal, Charles E. Carter, Harold W. Chalkley, Jerome Cornfield, Mariano de Mingo, Jean P. DuRuisseau, Irene Z. Eiger, Maurice Errera, Paul J. Fodor, William S. Fones, Shoucheng J. Fu, James B. Gilbert, Jose M. Gonçalves, Robert E. Greenfield, Piero Gullino, Douglas Hamer, Nobuo Izumiya, Gerson Kegeles, Robert J. Koegel (to whom we are additionally indebted for the infrared spectra given in volume 3), Guy Letellier, Leon Levintow, Robin Marshall, Alton Meister, Kimiyo Michi, Vithal B. Mitbander, Toru Miyaji, Thomas Moore, Jo Nordmann, Einosuke Ohmura, Theodore Otani, M. Clyde Otey, W. K. Paik (to whom we are additionally indebted for the translation of a large number of papers from the Japanese chemical literature), Jekishan R. Parikh, Elbert A. Peterson, Vincent E. Price, Krishnarau R. Rao, Miloslav Rechcigl, Donald S. Robinson, Irene Rosenfeld, Joseph Shack, Herbert A. Sober, Matthys Staehelin, Gerassimos Stelakatos, Takashi Sugimura, Roy Wade, Ellinor Weiss, Elizabeth Work, and Leonidas Zervas. It is also a pleasure to thank our administrative assistant, Mrs. Betty Ann Mitchell, and her staff (especially Mrs. Anna Elizabeth Stotler), for their devotion and skill in handling the numerous and varied duties involved in the office of the Laboratory. To the Director, and Associate Director, of the National Cancer Institute, Drs. J. R. Heller, Jr., and G. B. Mider, respectively, we are especially grateful for establishing a milieu conducive to scientific effort. To Dr. Hubert B. Vickery of the Connecticut Agricultural Experiment Station, who generously devoted a considerable amount of his time to reading these volumes in their entirety, and whose constructive criticism and invaluable suggestions contributed greatly to the readability, accuracy, and proper historical perspective of their contents, we express our heartfelt gratitude and warmest thanks.

In venturing upon a pleasant but thorny task, we have been consoled by the unknown author of the Second Maccabees, whose delightful description of his mutual problems and aspirations may well close *this* long prologue:

For considering the multitude of books . . . and the multitude of the matter, we have taken care for those indeed that are willing to read, that it might be a

pleasure of mind; and for the studious that they may more easily commit to memory; and that all that read may receive profit. As to ourselves indeed, in undertaking this work . . . we have in hand no easy task . . . but as they that prepare a feast, and seek to satisfy the will of others, for the sake of many we willingly undergo the labour. For as the master builder of a new house must have care of the whole building, but he that taketh care to paint it, must seek out fit things for the adorning of it, so must it be judged for us. To stand upon every point . . . and to be curious in particulars, belongeth to the first author of the story. But to use brevity, and avoid much labouring of the work, is to be granted to him that will make an abridgement. . . . If we have done well, and as is fitting the story, it is that which we desired, but if slenderly and meanly, it is that which we could attain unto. For as it is hurtful to drink always wine or always water, but pleasant to use sometimes the one, and sometimes the other, so if the speech be *always* nicely framed, it will not be grateful to the readers. Here then we will begin the narration. Let this be enough of a preface, for it is a foolish thing to make a long prologue, and to be short in the story itself.

JESSE P. GREENSTEIN
MILTON WINITZ

Bethesda, Maryland

oo

In Memoriam *

The scientific community lost one of its most gifted investigators when, during the early morning hours of February 12, 1959, Dr. Jesse P. Greenstein suddenly succumbed to a massive cerebral hemorrhage suffered two days earlier. Thus was terminated the career of one of the century's most brilliant and colorful chemists, whose many contributions enriched the field of biochemistry and whose extraordinary productivity continued unabated until his death. Over the past two decades, Dr. Greenstein's Laboratory at the National Cancer Institute had become an outstanding center of research on amino acids and peptides, on nutrition, and on cancer. His numerous comparative studies on the enzyme patterns of normal and neoplastic tissues were among the first to bring modern biochemistry to bear on the cancer problem, and his masterful synthesis of the then current knowledge in the cancer field, published as his monograph, *Biochemistry of Cancer*, in 1947 (and extensively revised in 1954), constituted a milestone in cancer research. His equally numerous investigations on amino acids, peptides, and nucleoproteins enhanced our knowledge of biochemistry, nutrition, enzymology, and organic and physical chemistry. Particularly noteworthy was his development of general enzymic methods for the resolution of amino acids, which permitted ready access to the optically pure isomers of these substances on the large scale, and which will undoubtedly lead to a greatly increased use of these formerly rare materials in investigations of animal and human nutrition; the importance of these optically pure amino acids is evidenced by the virtually staggering number of requests for samples received from scores of investigators

* Taken in the main from a biographical sketch of Jesse P. Greenstein which was written in collaboration with Drs. C. G. Baker, S. M. Birnbaum, R. E. Greenfield, V. E. Price, and H. A. Sober (*The Jesse P. Greenstein Memorial Issue, J. Natl. Cancer Inst.*, **24**, vii (1960)).

throughout the world—requests that were honored by Dr. Greenstein in his characteristically generous fashion. The indefatigable energy and selfless devotion with which he pursued his scientific researches, and the high quality and permanent value of his results, have earned him the lasting respect and admiration of all who knew him.

Probably the most characteristic traits of Jesse Greenstein were his deep respect for the individual and his intense devotion and dedication to his chosen field. His mind was a massive vault filled with scientific knowledge, yet he was humble when he viewed the living miracles of the world about him while possessing a never-ending curiosity to unlock the mystery of their elusively concealed secrets. To his contemporaries he was a living dynamo, primed with a source of energy that permitted him to work at a vigorous pace for sixteen to eighteen hours a day, seven days a week, and the only waste product of which was the smoke which emerged from a seemingly endless chain of cigars. This boundless energy, coupled with a firm yet understanding and jovial nature, served as a continual source of inspiration to his younger scientific associates, whose eager compulsion to follow his lead from example often led them to scientific achievements of which they had believed themselves incapable. Of his scientific findings he imparted freely, in a written style that was elegant in its simplicity. Either alone or in collaboration with his scientific colleagues, he published some 300 original papers and his now classical book on *Biochemistry of Cancer*. Yet, despite this prodigious output of work, he was modest and unassuming about his own accomplishments while displaying an overt pride in the achievements of his younger collaborators, in whom he took a sincere personal interest, on whom he would freely lavish praise, and to whom he served as a leader in the finest sense. And despite his vigorous and rigorous scientific activity, he was invariably friendly and good humored and was always certain to give a warm and generous welcome to the visitors who frequented his Laboratory in a never-ending stream. His door was always open, too, to a constant flow of scientific associates who sought his counsel and advice, and those who were privileged to work closely with him remember the many pleasant conversations often held in the early morning hours, conducted while he was actively performing an organic synthesis or inducing the crystallization of a new compound, while a reaction mixture or two were refluxing in the background. Because of his personal characteristics—his high moral courage, his great knowledge of and insight into science, his insistence on excellence, his patient good humor—and because of his extraordinary scientific accomplishments, he attracted to his Laboratory a stream of capable young scientists—from England, France, Japan, Germany, Canada, Israel, China, Korea, Greece, Italy, Belgium, Switzerland, India, and Brazil—eager to

follow his lead. And indeed, it is the scientific legacy he left to these young scientists—in whom he imbued his zeal, his enthusiasm for science, his high standards, and his penchant for hard work—that may be ranked among the greatest of his contributions. The present treatise, which was begun in 1955 and very nearly complete at the time of his death, represents his last major writing effort.

The hiatus created by the death of an individual such as Jesse P. Greenstein can never be adequately filled, and for this we are much the poorer, but for the very reason that he was with us, we have been left a good deal richer and perhaps a little bit wiser.

MILTON WINITZ

10. Optical Superposition	80
11. Rotational Shifts Induced by Ionization	83
12. Rotational Shifts Induced by Substitution	93
13. Rotatory Dispersion	105
14. Dispersions of Complex Copper Salts	118
15. Theories of Optical Activity	125

BIOLOGICAL METHODS

16. General Considerations	130
17. Utilization or Degradation by Micro-organisms	132
18. Metabolism by Higher Animals	139
19. Asymmetric Oxidation	142
20. Asymmetric Hydrolysis	147
21. Asymmetric Synthesis	148
22. Miscellaneous Biological Procedures	150

Configurational Correlations between α -Amino Acids and Other Classes of Compounds

GENERAL CONSIDERATIONS

23. Walden Inversion	152
24. Effect of Structural Alteration on Configuration	154

METHODS EMPLOYED

25. Rule of Shift	157
26. Kinetic Methods	159
27. Chemical Interconversions Involving 2-Amino-Sugars	167
28. Correlations Involving Quasi-racemic Compounds	171
29. Enzymic Methods	175

Stereochemical Configuration of Diasymmetric Amino Acids

30. Threonine	176
31. Isoleucine	183
32. Hydroxyproline	191
33. γ -Hydroxyglutamic Acid	199
34. 5-Hydroxyproline	203
35. Octopine	205
36. Aminotricarballylic Acid	206
37. β -Hydroxyglutamic Acid	211
38. β -Hydroxynorleucine	213
39. β -Hydroxyaspartic Acid	214
40. β -Phenylserine	215
41. α -Amino Acids Related to Sphingosine	223
References	232

CHAPTER 3 AMINO ACIDS IN NUTRITION

245

1. Introduction 245
2. Food 247
3. Protein Degradation Products 249
4. The Use of Protein Hydrolysates to Satisfy Protein Needs 254
5. The Biological Evaluation of Proteins—The Early Concept of Dispensable and Indispensable Amino Acids 258
6. The Use of Artificial Mixtures of Amino Acids and the Problem of Distinguishing Dispensable and Indispensable Amino Acids 266
7. Repletion 282
8. Other Criteria of Adequacy 287
9. Amino Acid Requirements of Various Animal Species—Mice 290
10. Dogs 291
11. Chicks 292
12. Honeybee 295
13. Salmon Fingerlings 297
14. Summary 297
15. The Study of Man 298
16. Amino Acids Essential for the Adult Human Subject 299
17. The Replaceability of Natural, L-Amino Acids in Diets by Their D-Antipodes and by Various Derivatives 311
18. Phenylpyruvic Oligophrenia 326
19. Parenteral Feeding of Amino Acids; Toxicity and the Effect of Arginine—Metabolism of Amino Acids Given Intravenously 329
20. Maintenance of Nitrogen Balance 332
21. Amino Acid Mixtures in the Growth of Animal Cells in Tissue Culture 339
22. Renal Clearance of Amino Acids 354
23. Toxicity of Ammonia and the Protective Effect of Arginine 367
24. Water-Soluble Diets Composed Entirely of Chemically Defined Constituents: Further Criteria of Dispensable and Non-dispensable Amino Acids 384
25. Pregnancy and Lactation on Water-Soluble Diets 397
26. Soluble Diets and Mice 403
27. Further Aspects of "Non-essential" Nitrogen 403

28. Maintenance and Growth	407
29. The Effect of Arginine	410
30. Repletion	413
31. Considerations of Active Transport	416
32. Economic Aspects of the Problem	416
References	421

Part II Physicochemical Aspects

CHAPTER 4 AMINO ACIDS AS DIPOLAR IONS 435

1. Introduction	435
2. Development of the Dipolar Ion Concept	441
3. Further Aspects of the Development of the Dipolar Ion Concept	449
4. Evidence of the Dipolar Ion Nature of Amino Acids from Dielectric Constant Measurements	456
5. Molecular Volumes	467
6. Volume Changes on Ionization	473
7. Surface Tension of Solutions of Amino Acids	473
8. Raman Spectra Compatible with Dipolar Ion Structure	474
9. The Apparent Dissociation Constants of Amino Acids	475
10. Method of Calculation—Illustrative Examples	476
11. Dissociation Constants from Solubility Measurements	479
✓ 12. The Isoelectric Point	482
13. Tables of Dissociation Constants	484
14. Cells without Liquid Junction	484
15. Heats of Ionization and Assignment of Groups	492
16. The Acid Strength of the Sulfhydryl Group	494
✓ 17. The Phenolic Hydroxyl	496
18. Chemical Structure and pK'	497
19. The Effect of Distance between Groups on pK' Values	501
20. Apparent Dissociation Constants of Diastereomeric Amino Acids and Peptides	505
21. Apparent Dissociation Constants of Cystine and of Peptides of Cystine	506

22. The Apparent Dissociation Constants in Non-aqueous Mixtures 509
23. Formaldehyde, Alcohol, and Acetone Titrations 515
- ✓ 24. Titration in Glacial Acetic Acid Solution 517
25. Ionization in Absolute Sulfuric Acid 518
- References 519

CHAPTER 5 THERMODYNAMICS AND SOLUBILITY 523

1. Thermal Data 523
2. Heat of Formation 523
3. Heats of Combustion 524
4. Entropy, and Entropy of Formation 525
5. Free Energy of Formation 527
6. Free Energy of Solution and of Formation of Ionic Species at 298.1° 528
7. Heats of Ionization 531
8. Heats of Solution and Dilution 533
9. Heat of Hydration of L-Asparagine 538
10. Temperature Coefficients 539
11. Solubility of Racemic and Optically Active Isomers 544
12. Solubility of Amino Acids and Peptides in Alcohol-Water Mixtures 545
13. Solubility of Amino Acids in the Presence of Salts 550
14. Peptide Bond Energy 558
15. Appendix 565
- References 566

CHAPTER 6 METAL AND SALT COMBINATIONS WITH AMINO ACIDS 569

1. Metal Chelates 569
2. Early Studies on Stability and Ease of Formation of Metal Complexes 573
3. Steric Aspects of the Metal Complexes 575
4. Note on Nomenclature 584
5. Metal Complexes of the Higher Amino Acids and Derivatives 585
6. The Cotton Effect 591
7. Metal Complexes in Reactions with Pyridoxal and Amino Acids 593
8. Composition and Stability of Metal-Amino Acid Complexes 610
9. Peptides 627

10. Oxygen Consumption by Metal Complexes 635
11. Complexes of Cysteine 639
12. Metal Complexes with Histidine 646
13. Mercury and Silver Complexes with Amino Acids 646
14. Salts of the Lighter Metals with Amino Acids 647
15. Salt Combinations with Amino Acids 648
16. Separation of L-Amino Acids from Natural Sources as Metal and Salt Complexes 664
17. Compounds of Neutral Salts with Amino Acids 666
18. Amino Acid-Sugar Combinations 672
- References 675

CHAPTER 7 THE EFFECT OF RADIATION 683

- ✓ 1. Ultraviolet Light 683
2. X-Rays and Other Forms of Radiation 686
- References 692

Part III Organic Chemical Aspects

CHAPTER 8 SYNTHESIS OF α -AMINO ACIDS 697

1. Introduction 697
2. Strecker Synthesis 698
3. Amination of α -Halogen Acids 700
4. Reductive Amination 702
5. Amination via Molecular Rearrangement 703
6. Condensation of an Aldehyde with an Active Methylene Group 706
7. Oxidation of Aminoalcohols 709
8. Condensations with N-Substituted Aminomalonic Esters 709
- References 712

CHAPTER 9 RESOLUTION OF THE AMINO ACIDS 715

1. Definition 715
2. Crystallization Procedures 715
3. Diastereoisomeric Salts 716
4. Diastereoisomeric Derivatives 728
5. Biological Procedures 728
6. Plant Acylases 742
7. Resolution of α -Methylamino Acids 743

CONTENTS

xxi

8. Paper Chromatographic Approaches	750
9. Separation of Steric Isomers	751
References	755

TRANSLATIONS OF FOREIGN QUOTATIONS	760a
------------------------------------	------

INDEX (see Volume 3)
