SECOND EDITION
Seventh Printing, November, 1950

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE TO THE SECOND EDITION

The purpose, plan, and scope of this treatise are given in the accompanying preface to the first edition.

This second edition, which represents a significant expansion of the first, contains twenty-six chapters, of which the following eight are new: the reactions of aliphatic hydrocarbons; synthetic polymers; catalytic hydrogenation and hydrogenolysis; organic sulfur compounds; aliphatic fluorides; the chemistry of the porphyrins; chlorophyll; and the redistribution reaction. All the chapters carried over from the first edition have been revised. In some chapters the literature has been reviewed up to September, 1942.

Corrections and suggestions will again be cordially welcomed. The editors are grateful to many friends for the examination of the manuscripts. Particular thanks are due to Messrs. R. K. Abbott, R. W. Leeper, D. S. Melstrom, G. J. O'Donnell, S. M. Spatz, J. R. Thirtle, and L. A. Woods.

H. G.

AMES, IOWA
October, 1942

PREFACE TO THE FIRST EDITION

Organic chemistry is richly endowed with excellent textbooks. However, there is a need for a general treatise of organic chemistry suitable for instruction at the graduate level. Such a book must focus attention upon new developments. At best, it can but serve the purpose of the moment and provide a point of departure for unceasing revision.

The idea of a collaborative work by specialists in the several branches of the science was developed in 1934. Each author was asked to prepare a chapter dealing with a subject of particular interest to himself. It was hoped to obtain, in this way, an authoritative treatise which would cover most of the important phases of organic chemistry. The execution of this plan has resulted in the present volumes.

For the sake of convenience in revising and expanding the book, the rapidly developing fields of natural products, relationship between physical properties and chemical constitution, valence, and resonance have been grouped together in the second volume. It is planned to revise both volumes at intervals, not only in order to bring the present material up to date, but also to permit the inclusion of new chapters to fill the more conspicuous gaps. For example, chapters on polymerization and chlorophyll will be included in the next edition. Corrections and suggestions will be heartily welcomed.

The contents have been integrated and the accessibility of the information increased by cross references, by individual tables of contents for each chapter, and by a comprehensive subject index which is repeated in each of the two volumes. The inordinate wealth of the literature has made it necessary to restrict references, in general, to a relatively few selected original articles. Researches are cited, as a rule, by reference to the most recent publications; however, sufficient references to early work are given to provide an historical background. Occasional chapters, particularly those in the field of natural products, have abundant citations to original articles, and should be especially useful to research workers. In some chapters the literature has been reviewed up to September, 1937. There is, in addition, occasional mention of work hitherto unpublished. The section General References at the end of each chapter includes mention of some of the more important review articles and books as a guide to collateral reading.

The editors gratefully acknowledge the assistance of many friends in the examination of the manuscripts. Valuable aid was provided by the late Dr. W. H. Carothers, who served on the Editorial Board. Special thanks are due to Drs. G. E. Hilbert, J. F. Nelson, P. T. Parker, A. M. Patterson, G. F. Wright, and Messrs. J. C. Bailie, R. L. Bebb, L. C. Cheney, E. J. Crane, W. Harber, A. L. Jacoby, and J. Swislowsky.

H. G.

AMES, IOWA
December, 1937

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NATURAL AMINO ACIDS

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CONTENTS

AMINO ACIDS FROM PROTEINS

The term protein connotes an ill-defined group of complex nitrogenous organic substances which form an important part of animal and vegetable tissues. The separation and characterization of individual "simple" proteins depend mainly on solubility relations, in accordance with which they are classified as albumins, globulins, and so forth. All the simple proteins yield ammonia and mixtures of amino acids on hydrolysis by acids, alkalies, or enzymes. "Conjugated" proteins also exist; these yield, besides amino acids, other products such as purines, pyrimidines, porphyrins, carbohydrates (or their derivatives), lipoidal substances, and phosphoric acid. Invariably, however, the principal products of hydrolysis consist of amino acids.

The most convenient method of hydrolysis involves treatment with

hot aqueous mineral acids. The action of hot alkalies, though it readily brings about the desired hydrolysis, is less satisfactory, for during the process a notable proportion of the amino acids, which preëxist in pure optically active form, become racemized. This objection applies in a far less degree to acid hydrolysis. The action of proteolytic enzymes, though offering the practical disadvantage of slow and often incomplete action, induces neither racemization nor decomposition of the more sensitive amino acids.

In proteins the constituent amino acids are united by peptide linkages (—CO–NH— or, with the prolines (pp. 1118, 1120), —CO—N <), which on hydrolysis are opened with liberation of carboxyl and amino or imino groups. To follow the progress of hydrolysis, three methods are available: (1) titration of carboxyl groups, (2) titration of amino groups, (3) estimation of primary amino groups by treatment with nitrous acid. In the first two, conditions are so selected that the titration end points are influenced only by the groups to be estimated; in the third, a specific reaction is involved. The principles underlying the various procedures will be discussed later. On completion of hydrolysis, the resulting amino acids may be separated into three broad classes, which depend upon the preponderatingly acidic, basic, or neutral character of their members.

The predominantly acidic group consists of the monoamino dicarboxylic acids. These may be separated from the others by taking advantage either of the sparing solubility of their calcium or barium salts in aqueous alcohol, or of their selective tendency to migrate toward the positive pole when subjected in solution at suitable *p*H levels to the influence of an electric current.[1]

The members of the predominantly basic group, comprising the diamino monocarboxylic acids, are characterized by their precipitability with phosphotungstic acid and by their tendency to migrate towards the negative pole in neutral solution.[2] The essentially neutral monoamino monocarboxylic acids, which constitute the major portion of most protein hydrolysates, differ from the members of the other groups * by the fact that they can be extracted from neutral solution by butyl alcohol.[3] The majority of the members of this group, though appreciably soluble in butyl alcohol saturated with water, are insoluble in the anhydrous alcohol. Two amino acids of protein origin (proline and hydroxyproline), however, are distinguished by their solubility in pure

[1] Foster and Schmidt, *J. Biol. Chem.*, **56**, 545 (1923).

[2] Foster and Schmidt, *J. Am. Chem. Soc.*, **48**, 1709 (1926).

* Histidine (p. 1151), in which the imidazole group possesses extremely weakly basic properties, forms an exception as it accompanies the monoamino monocarboxylic acids.

[3] Dakin, *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**, 499 (1920).

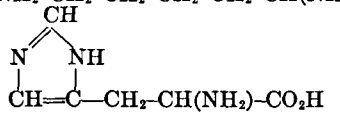
alcohols; these also differ from all others in being not primary, but cyclic secondary amines. The group of "natural" monoamino monocarboxylic acids also includes a few which may be separated by virtue of their low solubility in water.

The following list, arranged on the basis of the above practical classification, enumerates the amino acids which have been demonstrated to be products of the hydrolysis of proteins.[4]

I. *Monoamino Dicarboxylic Acids.*

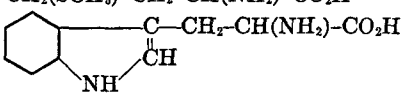
Aspartic acid	$HO_2C–CH_2–CH(NH_2)–CO_2H$
Glutamic acid	$HO_2C–CH_2–CH_2–CH(NH_2)–CO_2H$
Hydroxyglutamic acid	$HO_2C–CH_2–CHOH–CH(NH_2)–CO_2H$

II. *Basic Amino Acids.*

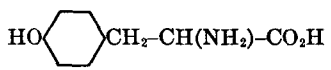
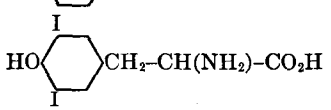
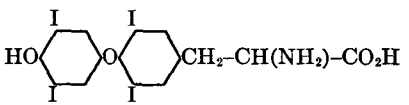
Arginine	$NH_2C(=NH)–NH–CH_2–CH_2–CH_2–CH(NH_2)–CO_2H$
Lysine	$NH_2–CH_2–CH_2–CH_2–CH_2–CH(NH_2)–CO_2H$
Histidine	

III. *Monoamino Monocarboxylic Acids.*

1. Extractable by wet butyl alcohol; readily soluble in water; insoluble in anhydrous alcohols.

Glycine	$CH_2(NH_2)–CO_2H$
Alanine	$CH_3–CH(NH_2)–CO_2H$
Valine	$(CH_3)_2CH–CH(NH_2)–CO_2H$
Leucine	$(CH_3)_2CH–CH_2–CH(NH_2)–CO_2H$
Norleucine	$CH_3–CH_2–CH_2–CH_2–CH(NH_2)–CO_2H$
Isoleucine	$CH_3–CH_2–CH(CH_3)–CH(NH_2)–CO_2H$
Phenylalanine	$C_6H_5–CH_2–CH(NH_2)–CO_2H$
Serine	$CH_2OH–CH(NH_2)–CO_2H$
Threonine	$CH_3–CHOH–CH(NH_2)–CO_2H$
Cysteine	$CH_2(SH)–CH(NH_2)–CO_2H$
Methionine	$CH_2(SCH_3)–CH_2–CH(NH_2)–CO_2H$
Tryptophan	

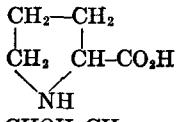
2. Sparingly soluble in water.

Tyrosine	
Diiodotyrosine	
Thyroxine	
Cystine	$[–S–CH_2–CH(NH_2)–CO_2H]_2$

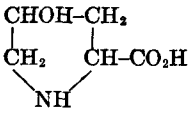
[4] Vickery and Schmidt, *Chem. Rev.*, **9**, 169 (1931).

3. Soluble in alcohols.

Proline



Hydroxyproline



In addition to the above compounds of protein origin, certain other amino acids have been isolated from natural sources. Some of these will be discussed later.

The inclusion of cysteine in the above list is unconventional, as during the customary processes of isolation the sulfhydryl becomes oxidized to the disulfide, so that cysteine finally appears as cystine. The unquestionable presence of sulfhydryl groups in many proteins,[5] however, points to the probability of the existence of cysteine as a component amino acid.

The character, both physical and chemical, of proteins and peptides is largely determined by the nature and relative abundance of the various types of constituent amino acids. The polypeptides synthesized by Fischer contained only monoamino monocarboxylic acids, and the only free acidic and basic groups present were those terminating the peptide chain. Proteins and natural polypeptides contain polar groups, situated at the uncombined ends of the acid and basic amino acids distributed throughout the molecule; the properties of the proteins represent a resultant of the individual and mutual effects of these.

By acid hydrolysis, proteins yield considerable amounts of ammonia. There is reason to believe that this is derived from acid amide groups associated with the combined dicarboxylic acids, semi-amides of which have been isolated from the products of enzymatic hydrolysis of proteins.

Separation of the individual members of the first and second groups is effected by special methods involving selective precipitation of salts formed with metals or with acids. The quantitative aspects of these separations have been most completely developed for the basic amino acids (p. 1138). The separation of the relatively simple monoamino monocarboxylic acids is rendered particularly difficult by the familial similarity of members of homologous series, and it has not yet been found possible to develop quantitatively reliable methods for all. The original procedure of Fischer, fractional distillation of the ethyl esters under reduced pressure, involves notable losses due to formation of diketopiperazines.[6] Attempts have been made to avoid this difficulty by acylation

[5] Mirsky and Anson, *J. Gen. Physiol.*, **18**, 307 (1935).

[6] Fischer, *Ber.*, **34**, 433 (1901); *Z. physiol. Chem.*, **33**, 151 (1901); Foreman, *Biochem. J.*, **13**, 378 (1919).

of the esters before fractionation,[7] but these modifications await development to a state of practical utility.

Partial separation of the amino acids of a protein hydrolysate can be effected by taking advantage of the differential solubilities of their copper salts in water and in methyl alcohol.[8]

Advantage can also be taken [9] of the constancy of the solubility product of sparingly soluble salts of amino acids and certain strong acids in order to determine the amounts of the former in protein hydrolysates. For this purpose, complexes, such as potassium trioxalatochromiate,[10] sodium dioxpyridate,[11] or ammonium rhodanilate,[12] which precipitate specific amino acids, or simple aromatic sulfonic acids [13, 14] which yield suitable salts with a wide variety of amino acids, offer special promise.

The nutritional significance of the individual amino acids has received much study. It has long been known that rats from whose dietary protein certain amino acids were absent failed to grow normally until the missing compound was added to the ration as a supplement. Until 1935 such experimentation was hampered by the fact that normal growth could not be maintained on diets in which proteins were entirely replaced by pure amino acids. This was made possible, however, by the isolation of threonine by Rose, and his recognition of its importance as an indispensable dietary component. As a result of experiments with diets containing carbohydrates, fats, inorganic salts, glucosamine, vitamins, and various mixtures of amino acids, Rose [15] was able to establish the following classification of the amino acids according to their growth effects on immature albino rats:

INDISPENSABLE	DISPENSABLE
Arginine	Aspartic
Lysine	Glutamic
Histidine	Hydroxyglutamic
Valine	Glycine
Leucine	Alanine
Isoleucine	Norleucine
Phenylalanine	Serine
Threonine	Cystine
Methionine	Tyrosine
Tryptophan	Proline
	Hydroxyproline

[7] Cherbuliez and collaborators, *Helv. Chim. Acta*, **12**, 317 (1929); **13**, 1390 (1930).
[8] Town, *Biochem. J.*, **22**, 1083 (1928); Brazier, *ibid.*, **24**, 1188 (1930).
[9] Bergmann and Stein, *J. Biol. Chem.*, **128**, 217 (1939).
[10] Bergmann and Niemann, *ibid.*, **122**, 577 (1938).
[11] Bergmann, *ibid.*, **122**, 569 (1938).
[12] Bergmann, *ibid.*, **110**, 471 (1935).
[13] Bergmann and Stein, *ibid.*, **129**, 609 (1939).
[14] Doherty, Stein, and Bergmann, *ibid.*, **135**, 487 (1940).
[15] Rose, *Physiol. Rev.*, **18**, 109 (1938).

The indispensable amino acids, listed above, fall into two further categories, namely, those of which only the stereochemically natural (p. 1085) varieties meet the needs of growth, and those of which either spatial form fulfills growth requirements:

Only Natural Variety Promotes Growth	Either Configurational Variety Promotes Growth
Lysine	Histidine
Valine	Phenylalanine
Leucine	Methionine
Isoleucine	Tryptophan
Threonine	

Information relative to the unnatural variety of arginine is not yet available. The natural form appears to be synthesized in the body, but at too slow a rate to support growth.

It has also been found that the nutritional effect of the indispensable amino acids of the second class can be secured not only with the unnatural varieties but with certain N-acyl derivatives and α-hydroxy acids configurationally related to the natural amino acids, and with the corresponding α-keto acids. These relations, outlined for tryptophan on p. 1162, indicate that in such instances the essential portion of the molecule, i.e., that not synthesized in the animal organism, is that represented by the group R in the general formula $RCH(NH_2)CO_2H$. On the other hand, the α-amino grouping may be synthesized by normal metabolic processes from α-keto or other groupings (*cf.* pp. 1102, 1106) at a rate sufficient for the needs of the growing animal.

It seems probable that similar biochemical changes also proceed with the indispensable amino acids of the first class, though at rates too slow for growth requirements. Leucine of unnatural configuration is rapidly converted, in adult rats, into the natural variety. This has been demonstrated by the administration of "unnatural" leucine containing deuterium in the alkyl group and "heavy" nitrogen in the amino group; the leucine then isolated from the tissue proteins consists entirely of the natural variety and contains deuterium but practically no heavy nitrogen. However, after the administration of the corresponding isotopically labeled natural leucine, the tissue leucine contains both isotopes, the ratio of which indicates that only a minor proportion of the nitrogen had become detached in the process.[16]

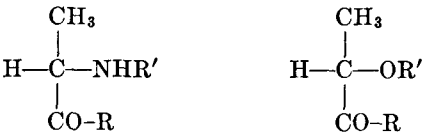
[16] Schoenheimer, Ratner, and Rittenberg, *J. Biol. Chem.*, **130**, 703 (1939); Ratner, Schoenheimer, and Rittenberg, *ibid.*, **134**, 653 (1940).

GENERAL PROPERTIES AND REACTIONS OF NATURAL AMINO ACIDS

With the exception of proline and hydroxyproline, all the amino acids isolated from protein hydrolysates contain a primary amino group in the α position to the carboxyl. The exceptions may be regarded also as α-amino acids in which the amino group is involved in ring formation; however, as may be judged from the solubility of proline in alcohol, this departure from the common form has a marked effect on physical as well as chemical properties.

With the exception of glycine, which contains no center of asymmetry, all the amino acids of protein origin occur in optically active form. The sign of rotation by which they are distinguished is conveniently that observed in hydrochloric acid solution; an amino acid which rotates to the left when dissolved in four or more equivalents of acid is stated to be the levorotatory or (−) variety, and vice versa.

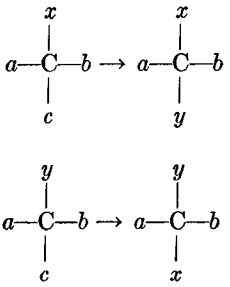
Among the amino acids of protein origin some are dextrorotatory and some are levorotatory, but evidence is accumulating that all possess the same spatial configuration. From approximately quantitative regularities in the molecular rotatory powers of corresponding derivatives of lactic acid and alanine, Freudenberg and his collaborators have concluded [17] that natural alanine possesses the same configuration as *l*(+)-lactic acid. Analogous displacements of rotation are observed when groups (R) combined with the acid radical are varied in compounds containing the same substituents (R′) on the amino and the hydroxyl group, respectively.



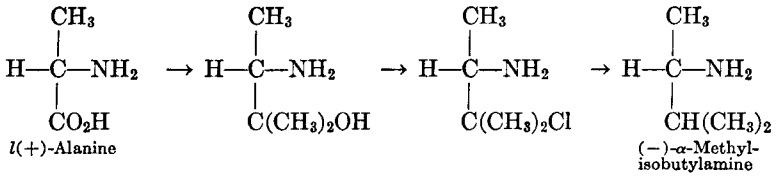
Similarly, the introduction of various acyl groups into natural leucine and valine and their esters causes parallel changes in optical rotation.[18]

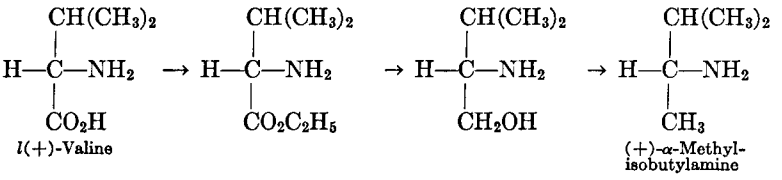
More direct evidence for the identity of the configurations of two "natural" amino acids has been secured by Barrow and Ferguson.[19] If two optically active compounds *Cabcx* and *Cabcy*, having the same configuration, each be converted into *Cabxy* by replacement of a common group *c* by *y* and *x*, respectively, the respective products, provided that no Walden inversion has occurred, will possess opposite configurations.

[17] Freudenberg and collaborators, *Ber.*, **57**, 1547 (1924); *Ann.*, **518**, 86 (1935).
[18] Karrer and Veer, *Helv. Chim. Acta*, **15**, 746 (1932).
[19] Barrow and Ferguson, *J. Chem. Soc.*, 410 (1935).



This principle has been applied to the natural, dextrorotatory forms of alanine and valine.



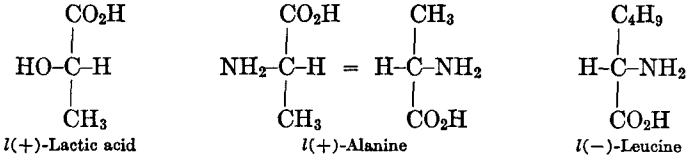


The α-methylisobutylamine from the natural alanine formed a levorotatory hydrochloride; that from the valine was found to be dextrorotatory. During the syntheses some loss of activity occurred in each case, but since none of the atoms directly attached to the asymmetric carbon atoms was replaced during the processes, Walden inversions (p. 264) were not to be anticipated. Natural alanine and valine therefore possess the same configuration.

The rotatory power of an amino acid is often entirely different in neutral, acid, and alkaline solution. With the natural compounds the values pass through a negative maximum at the isoelectric point and invariably become less levo- (or more dextro-) rotatory with increasing molar proportions of either alkali or acid.[20] The reverse holds for the unnatural varieties.

[20] Wood, *J. Chem. Soc.*, **105**, 1988 (1914); Clough, *ibid.*, **107**, 1509 (1915); Levene and collaborators, *J. Biol. Chem.*, **81**, 687 (1929); Lutz and Jirgensons, *Ber.*, **63**, 448 (1930): **64**, 1221 (1931).

Natural amino acids of protein origin are accordingly believed to possess universally the same configuration as *l*(+)-lactic acid, and this spatial relationship is expressed by the use of the prefix *l*-, which is employed without regard to the direction of the observed rotation. The latter is indicated by the sign (+) or (−).



The solubility relations of the simple α-amino monocarboxylic acids have been subjected to a critical study by Cohn and his collaborators.[21] With increasing length of chain, the solubility in water decreases and the solubility in aqueous alcohol increases. In the homologous series, the difference between the logarithms of the solubility ratios for water and for absolute alcohol decreases by a constant amount for each additional methylene group. The substantial insolubility of amino acids, in general, in absolute alcohol and other organic liquids reflects the charged condition of the molecule. In alcohol-water systems containing small proportions of alcohol the logarithm of the molar solubility diminishes inversely as the dielectric constant. The effect of inorganic salts, and the mutual effect of different amino acids, present in the same solution, upon their individual solubilities are ascribable to their influence upon the dielectric constant of the solvent.

For every amino acid there is a definite value of *p*H at which it fails to migrate in solution to either pole when subjected to an electric current. This value, termed the isoelectric point, is that at which the molecule as a whole carries no unbalanced positive or negative charge. The isoelectric point coincides with the point of minimum solubility.

According to the modern theory,[22] an aliphatic amino acid in solution at its isoelectric point exists in its most highly charged condition with respect to its acidic and basic groups alike. This theory alone explains, for example, the effect of formaldehyde on the titration curves of amino acids. Addition of increasing amounts of formaldehyde to a solution of glycine causes a downward displacement of the curve in the region of higher *p*H but no change in that of lower *p*H; a similar effect is observed with ammonium acetate. Since, according to generally accepted views, the effect of the addition of alkali to ammonium salts is the suppression

[21] Cohn, McMeekin, Edsall, and Weare, *J. Am. Chem. Soc.*, **56**, 2270 (1934).
[22] Bjerrum, *Z. physik. Chem.*, **104**, 147 (1923); Harris, *Biochem. J.*, **24**, 1080 (1930).

of basic ionization, it follows that in glycine, as in ammonium acetate, the upper portion of the titration curve relates to the basic function. In each case, therefore, the formaldehyde similarly suppresses the dissociation of the basic groups. With amino acids containing more than one amino group (e.g., lysine) the number of constituent curves characteristically shifted by addition of formaldehyde is equal to the number of basic groups present in the amino acid molecule; conversely, with monoamino dicarboxylic acids (e.g., aspartic acid) only one segment of the original titration curve is displaced, the two attributable to the carboxyl groups remaining unaltered. On the other hand, formaldehyde brings about little or no displacement in the upper (higher *p*H) portion of the titration curve of *p*-aminobenzoic acid, from which it is concluded that the aromatic amino group is only slightly dissociated.

Aliphatic amino acids are therefore regarded as existing, in aqueous solution, largely in the form of molecules containing both positive and negative charges.

$$NH_3^+\text{–}CHR\text{–}CO_2^-$$

Such molecules, the net charge of which is zero at the isoelectric point, have received the infelicitous name "Zwitterion" (from the German word *Zwitter*, meaning hermaphrodite). The expression "dipolar ion" is more acceptable to the linguistically sensitive than the hybrid term currently employed.

A solution of any given amino acid in pure water has not necessarily the *p*H corresponding to the isoelectric point of the amino acid; this would be true only if the acid and basic functions had exactly the same tendency to assume the charged condition. In the simple α-amino acids, the carboxyl groups have a slightly greater tendency to part with their protons than the amino groups to accept them; as a result the hydrogen-ion concentration of their solutions is higher than that of water, but not sufficiently high to bring the total number of positive and negative charges on all the amino acid molecules into exact balance. This condition can be reached only by the addition to the solution of more hydrogen ions in the form of some acid. For the monoamino monocarboxylic acids, the isoelectric points of which lie at approximately $p\text{H} = 6$, the discrepancy between the *p*H value of pure aqueous solutions and isoelectric point is but slight; it is much greater, of course, with the monoamino dicarboxylic acids. Conversely, the isoelectric point of the diamino monocarboxylic acids lies above $p\text{H} = 7$, and hydroxyl ions (in the form of alkali) must be added to their pure solutions to render them isoelectric.

Addition of increasing amounts of mineral acid to a solution of an amino acid causes the suppression of the negative charge, until finally

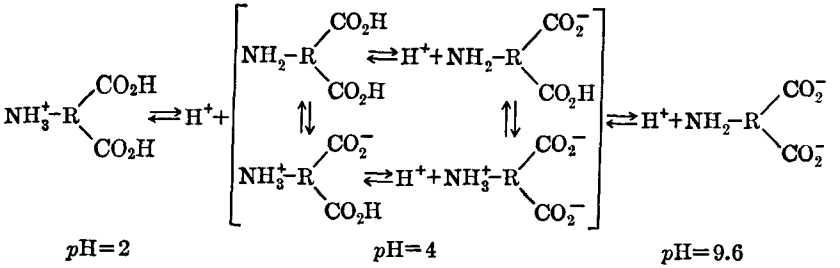
the equilibrium mixture contains the amino acid in its purely cationic form.

$$NH_3^+\text{–}CHR\text{–}CO_2^- + H^+ \rightleftarrows NH_3^+\text{–}CHR\text{–}CO_2H$$

Addition of alkali causes the suppression of the positive charge, with production of the anionic form.

$$NH_3^+\text{–}CHR\text{–}CO_2^- + OH^- \rightleftarrows NH_2\text{–}CHR\text{–}CO_2^- + H_2O$$

The equilibria involved at different *p*H levels are illustrated by the case, discussed by Cohn in his admirable review,[23] of the monoamino dicarboxylic acids.



That amino acids in their isoelectric range exist mainly in the dipolar ionic form is indicated by their Raman spectra.[24] Fatty acids in aqueous solution (in which they are but weakly ionized) exhibit a line at about 1720 cm.$^{-1}$ characteristic of the carbonyl group; on the addition of sufficient alkali to cause almost complete ionization, this line vanishes. Amino acids fail to exhibit a line at this frequency, but do so when converted into their hydrochlorides. Conversely, free primary amines show strong Raman lines between 3300 and 3400 cm.$^{-1}$; lines in this region are not displayed by amino acids in their isoelectric zone, but appear on the addition of alkali. Similar conclusions may be drawn from the behavior of amino acids towards water containing isotopic oxygen (H_2O^{18}). In this medium, simple carboxylic acids acquire "heavy" oxygen atoms at *p*H 1, but do not so exchange when in the form of their potassium salts; glycine fails to exchange at *p*H 7, but does so at *p*H 1.9.[25] The production of betaines from ethereal diazomethane with solid amino acids also points to their existence in dipolar ionic form;[26] in most cases the simultaneous production of amino acid methyl ester demonstrates the presence

[23] Cohn, *Ergeb. Physiol.*, **33**, 781 (1931).
[24] Edsall, *J. Chem. Phys.*, **4**, 1 (1936); **5**, 225 (1937).
[25] Mears, *ibid.*, **6**, 295 (1938).
[26] Kuhn and Brydówna, *Ber.*, **70**, 1333 (1937).

of some of the uncharged form. Only the ester is formed from compounds like anthranilic acid in which the amino group is inherently of weakly basic character.

Dipolar ions possess a large electric moment,[27] particularly those of lysine and arginine, which exist in solution largely in the form of ions containing positive and negative charges at opposite ends of relatively long chains. Dicarboxylic amino acids, in isoelectric solution, exist mainly as less polar ions, resembling those of the simple α-amino acids, for their terminal carboxyl groups are less highly dissociated than those contiguous to the amino group. In solvents of low dielectric constant, such as 90 per cent alcohol, the concentration of highly polar ions is smaller, and that of uncharged molecules greater, than in water. For this reason it is possible, by the use of suitable indicators, to titrate independently either the acidic [28] or the basic [29] function of amino acids in aqueous alcohol, acetone, or dioxan [30] solution.

The basic groups of amino acids can be quantitatively titrated in glacial acetic acid solution with perchloric acid in the same solvent.[31] The titration may be carried out either potentiometrically by the method of Hall and Conant,[32] or with the aid of a suitable indicator such as crystal violet.[33] The amino group behaves as a strong base, as in all aliphatic amines,[34] while the dissociation of the carboxyl group is completely suppressed by the solvent.

The dipolar character of the amino acids is reflected in their relative infusibility and low volatility. When strongly heated, they melt with profound decomposition [35] at temperatures well above 200°; some show a tendency to sublime below the decomposition point.[36]

Since the negative character of the carboxyl group is suppressed by esterification, the amino acid esters are far more volatile than the amino acids. On distillation, they undergo some condensation with loss of alcohol. This reaction occurs more readily with the methyl and ethyl esters

$$2\ \begin{array}{l}\mathrm{RCHNH_2}\\ |\\ \mathrm{CO_2C_2H_5}\end{array} \rightarrow \begin{array}{l}\mathrm{RCH{-}NH{-}CO}\\ |\qquad\quad |\\ \mathrm{CO{-}NH{-}CHR}\end{array} + 2\mathrm{C_2H_5OH}$$

[27] Edsall and Blanchard, *J. Am. Chem. Soc.*, **55**, 2337 (1933).
[28] Foreman, *Biochem. J.*, **14**, 451 (1920); **22**, 208, 222 (1928).
[29] Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32; **174**, 275 (1928).
[30] Popovici and Radulescu, *Bull. soc. chim. biol.*, **20**, 73 (1938).
[31] Harris, *Biochem. J.*, **29**, 2820 (1935); *J. Biol. Chem.*, **84**, 296 (1929); Nadeau and Branchen, *J. Am. Chem. Soc.*, **57**, 1363 (1935).
[32] Hall and Conant, *ibid.*, **49**, 3047 (1927).
[33] Conant and collaborators, *ibid.*, **49**, 3062 (1927); **52**, 4436 (1930).
[34] Hall and collaborators, *ibid.*, **50**, 2367 (1928); **52**, 5115 (1930).
[35] Dunn and Brophy, *J. Biol. Chem.*, **99**, 221 (1932).
[36] Brown, *Trans. Roy. Soc. Can.*, Sect. III, **26**, 173 (1932) [*C.A.*, **27**, 1617 (1933)].