Notes on Amino acids

By
Dr. Gangutri Saikia

Amino acids are organic molecules that consist of a basic amino group ($-NH_2$), an acidic carboxyl group (-COOH) and a side chain that is unique for each amino acid. When hydrolysed by acids, alkalis or enzymes, proteins yield a mixture of amino acids.

❖ The number of amino acids so far obtained from proteins appears to be about twenty-five.

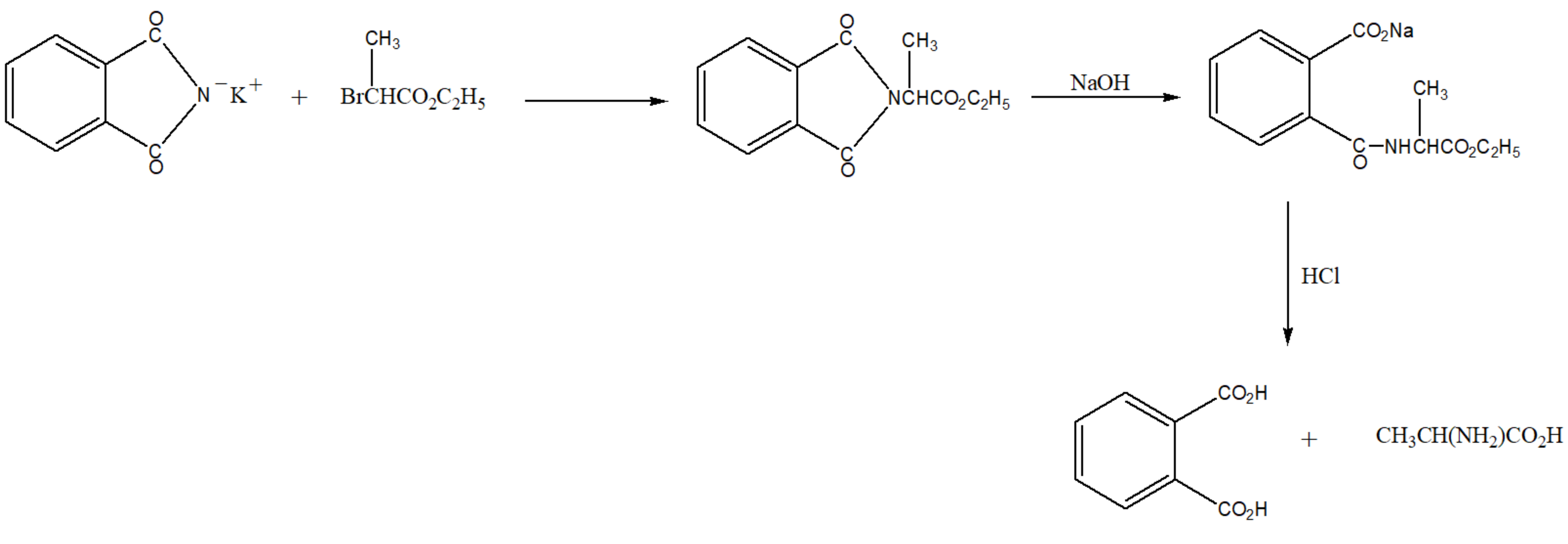
❖ Ten of the amino acids are essential acids, i.e. a deficiency in any one prevents growth in young animals and may even cause death.

❖ There are twenty amino acids of general occurrence, i.e., these are usually found in all proteins.

Preparation of amino acids

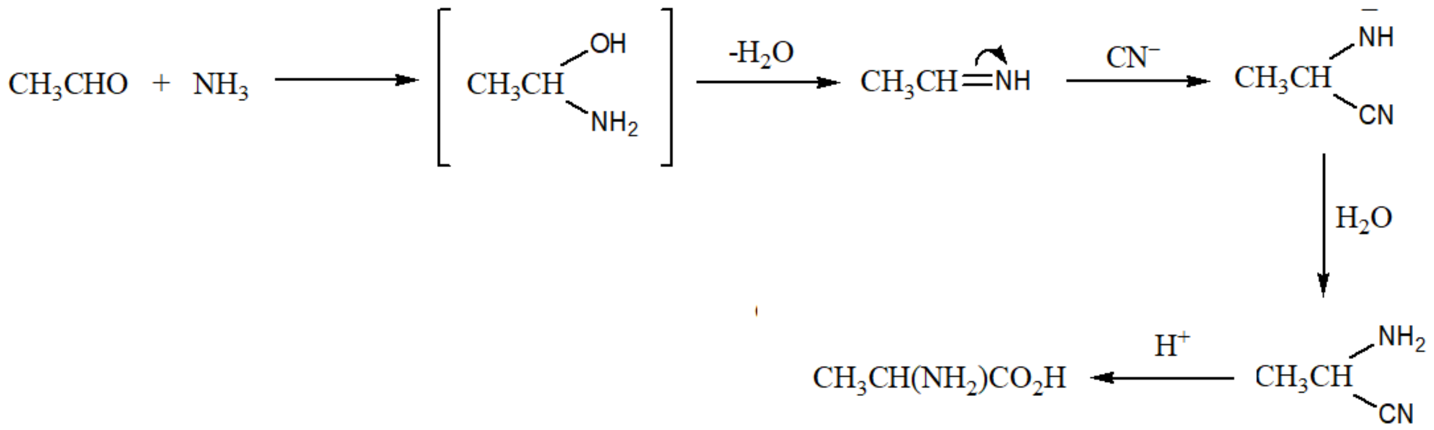
1) Gabriel phthalimide synthesis

The Gabriel phthalimide synthesis for converting halides to primary amines is based on the following reaction. The halide is treated with potassium phthalimide and the product got hydrolyzed.



2) **Strecker synthesis**

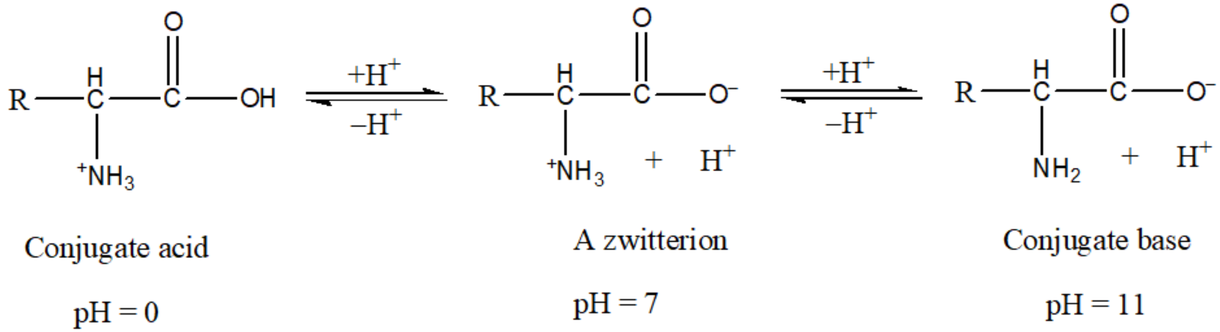
A cyanohydrin is treated with concentrated ammonia, and the resulting amino-nitrile is then hydrolysed with acid to obtain amino acid. In practice, amino-nitrile is usually prepared from the oxo compound in one step by treating the latter with equimolar mixture of ammonium chloride and potassium cyanide. This method is useful for preparing the following amino acids: glycine, alanine, serine, valine, leucine, glutamic acid, phenylalanine etc.



Acid- base properties of amino acids

Every amino acid has a carboxyl group and an amino group and each group can exist in an acidic or a basic form depending on the pH of the solution in which the amino acid is dissolved. The carboxyl group of the amino acids have pKa values of approximately 2 and the protonated amino groups have pKa values near 9. Therefore, an amino acid can never exist as an uncharged compound regardless of the pH of the solution. For this reason, at physiological pH (~7) an amino acid exists as a dipolar ion, called zwitterion. A zwitterion is a compound that has a negative charge on one atom and a positive charge on a nonadjacent atom.

This dipolar ion structure also accounts for the absence of acidic and basic properties of an amino acid.

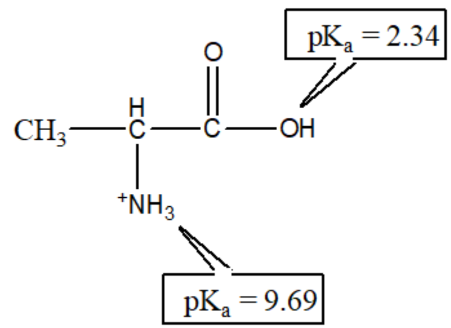


Isoelectric point

The isoelectric point (pI) of an amino acid is the pH at which it has no net charge. In other words, it is the pH at which the amount of negative charge on an amino acid exactly balances the amount of positive charge.

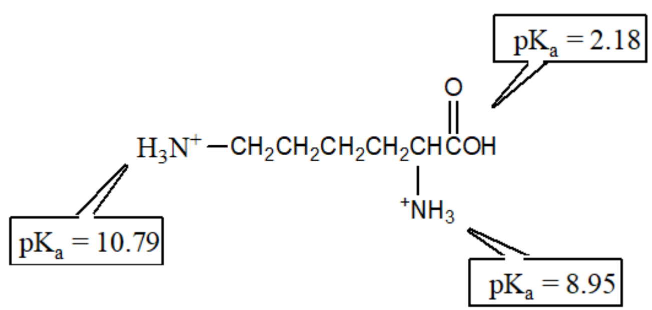
pI (Isoelectric pont) = pH at which there is no net charge

In case of an amino acid that does not have an ionizable side chain, the isoelectric point is the average value of pK$_a$s of both amino and carboxyl group.



$$pI = \frac{2.34 + 9.69}{2} = 6.02$$

In case of an amino acid with an ionizable side chain, the isoelectric point is the average value of pK$_a$s of the two groups that are positively charged in their acidic form and uncharged in their basic form.



$$pI = \frac{8.95 + 10.79}{2} = 9.87$$

Electrophoresis

Electrophoresis separates amino acids on the basis of their pI values.

❖ **A few drops of an amino acid mixture are applied in the middle of a piece of filter paper or a gel.**

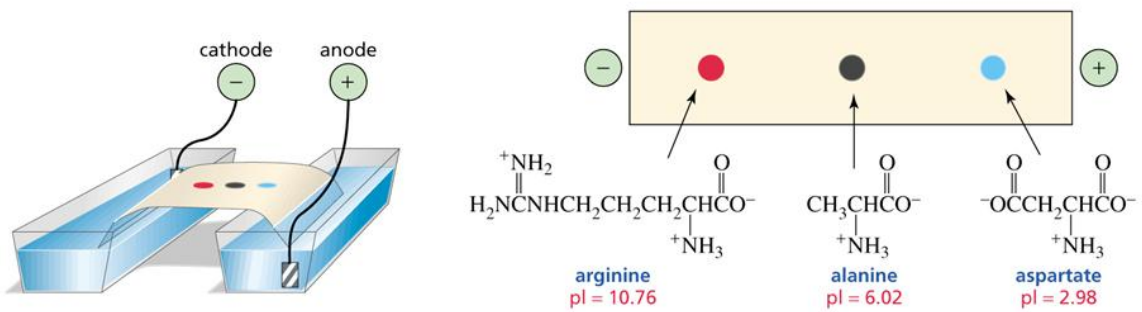
❖ **When the paper or the gel is placed in a buffered solution between two electrodes and an electric field is applied, an amino acid with a pI greater than the pH of the solution will have an overall positive charge and will migrate toward the cathode.**

❖ **The farther the pI is from the pH of the buffered solution, the more positive it will be and the farther it will migrate toward the cathode in a given time.**

❖ **An amino acid with a pI less than the pH of the solution will have an overall negative charge and will migrate toward the anode.**

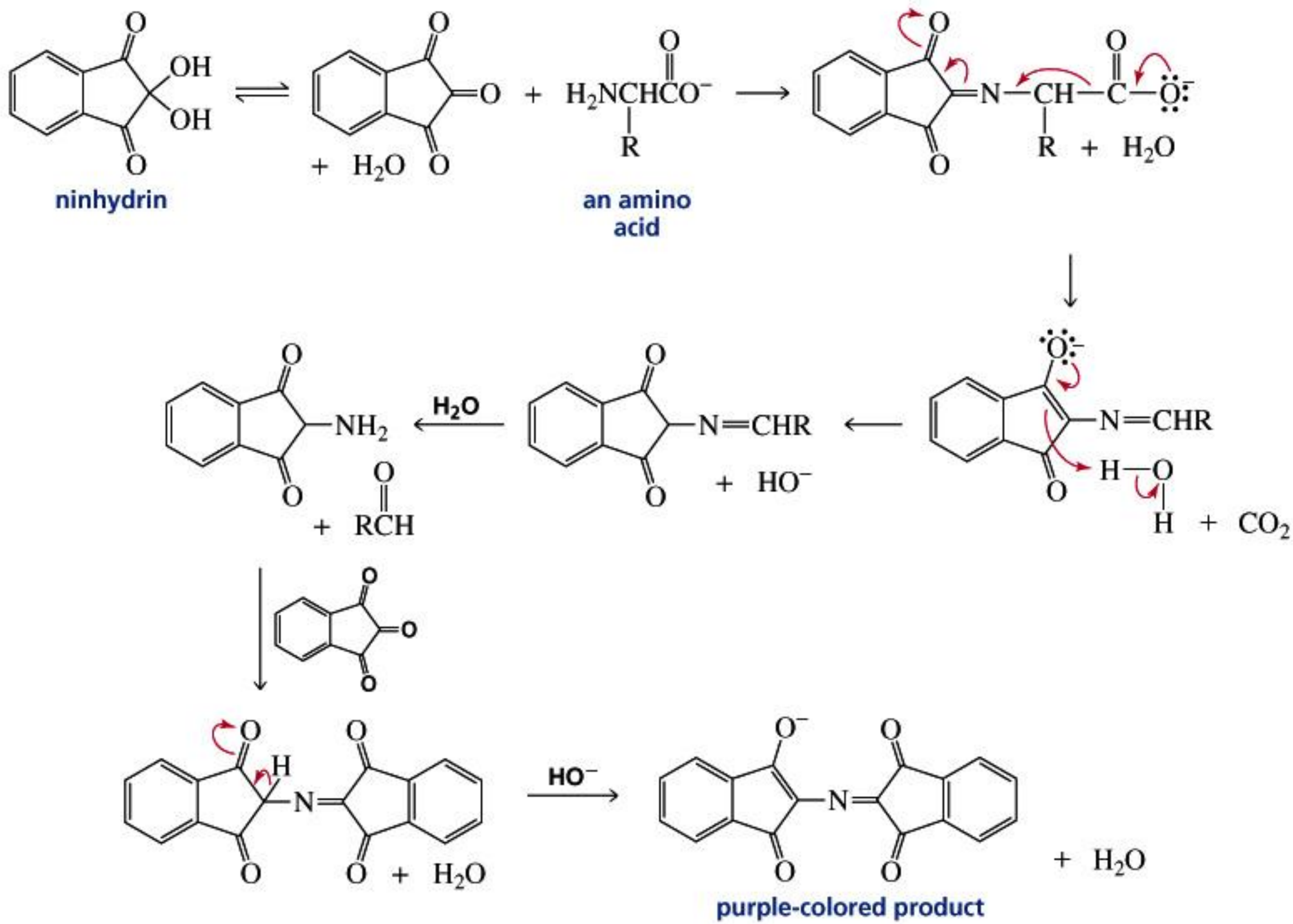
❖ **If two molecules have the same charge, the larger one will move more slowly during electrophoresis.**

Arginine, alanine and aspartic acid separated by electrophoresis at pH = 5



❖ **After electrophoretic separation of amino acids, the filter paper is sprayed with ninhydrin and dried in warm oven. Most amino acids form purple products. The individual amino acids are identified by their location on the paper.**

mechanism for the reaction of an amino acid with ninhydrin to form a colored product



Reactions of amino acids

(i) Amino acid when heated with an alcohol in presence of dry hydrogen chloride, they form ester hydrochlorides, e.g.,

$$NH_2CH_2CO_2H + C_2H_5OH + HCl \longrightarrow Cl^-\{NH_3^+CH_2CO_2C_2H_5\} + H_2O$$

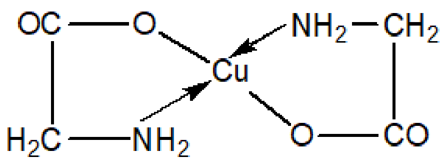
The free ester can be obtained by the action of aqueous sodium carbonate on the ester salt.

(ii) Amino acids may be acetylated by means of acetyl chloride or acetic anhydride.

$$RCH(NH_2)CO_2H + (CH_3CO)_2O \longrightarrow RCH(NHCOCH_3)CO_2H + CH_3CO_2H$$

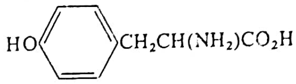
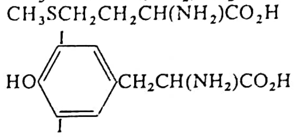
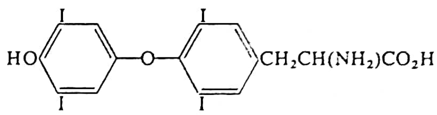
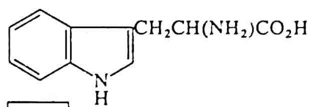
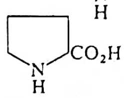
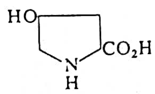
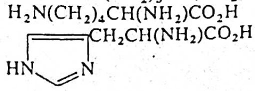
These acetylated derivatives are acidic, the basic character of the amino group being effectively eliminated by the presence of –I group attached to the nitrogen.

(iii) Amino acids form salts when complexation with heavy metals. Copper salt of glycine (deep blue needles) is formed by heating copper oxide with an aqueous solution of glycine.



(iv) Ninhydrin test: The ninhydrin reaction is used as spraying reagent in identification and quantitative estimation of amino acids. All α-amino acids give the same blue product, while proline and hydroxyproline give a yellow product.

Table 13.1

Name	*Systematic name*	*Formula*
Neutral Amino-acids (one amino-group and one carboxyl group)		
1. Glycine (*g*)	Aminoacetic acid	$CH_2(NH_2)CO_2H$
2. Alanine (*g*)	α-Aminopropionic acid	$CH_3CH(NH_2)CO_2H$
3. Valine (*g, e*)	α-Aminoisovaleric acid	$(CH_3)_2CHCH(NH_2)CO_2H$
4. Leucine (*g, e*)	α-Aminoisocaproic acid	$(CH_3)_2CHCH_2CH(NH_2)CO_2H$
5. Isoleucine (*g, e*)	α-Amino-β-methyl-n-valeric acid	$CH_3CH_2CH(CH_3)CH(NH_2)CO_2H$
6. Norleucine* (*l*)	α-Amino-n-caproic acid	$CH_3(CH_2)_3CH(NH_2)CO_2H$
7. Phenylalanine (*g, e*)	α-Amino-β-phenylpropionic acid	$C_6H_5CH_2CH(NH_2)CO_2H$
8. Tyrosine (*g*)	α-Amino-β-(*p*-hydroxyphenyl)propionic acid	
9. Serine (*g*)	α-Amino-β-hydroxypropionic acid	$HOCH_2CH(NH_2)CO_2H$
10. Cysteine (*g*)	α-Amino-β-mercaptopropionic acid	$HSCH_2CH(NH_2)CO_2H$
11. Cystine (*g*)	Bis-(α-aminopropionic acid)-β-disulphide	$[-SCH_2CH(NH_2)CO_2H]_2$
12. Threonine (*g, e*)	α-Amino-β-hydroxy-n-butyric acid	$CH_3CHOHCH(NH_2)CO_2H$
13. Methionine (*g, e*)	α-Amino-γ-methylthio-n-butyric acid	$CH_3SCH_2CH_2CH(NH_2)CO_2H$
14. Iodogorgic acid† (*l*)	3,5-Di-iodotyrosine	
15. Thyroxine† (*l*)	β-3,5-Di-iodo-4-(3′,5′-di-iodo-4′-hydroxy)-phenyl-α-aminopropionic acid	
16. Tryptophan (*g, e*)	α-Amino-β-indolepropionic acid	
17. Proline (*g*)	Pyrrolidine-α-carboxylic acid	
18. Hydroxyproline (*g*)	γ-Hydroxypyrrolidine-α-carboxylic acid	
Acidic Amino-acids (one amino-group and two carboxyl groups)		
19. Aspartic acid (*g*)	α-Aminosuccinic acid	$HO_2CCH_2CH(NH_2)CO_2H$
20. Asparagine (*l*)	α-Aminosuccinamic acid	$H_2NOCCH_2CH(NH_2)CO_2H$
21. Glutamic acid (*g*)	α-Aminoglutaric acid	$HO_2CCH_2CH_2CH(NH_2)CO_2H$
22. β-Hydroxyglutamic acid‡	α-Amino-β-hydroxyglutaric acid	$HO_2CCH_2CHOHCH(NH_2)CO_2H$
23. Glutamine (*l*)	α-Aminoglutaramic acid	$H_2NOCCH_2CH_2CH(NH_2)CO_2H$
Basic Amino-acids (two amino-groups and one carboxyl group)		
24. Ornithine §	α,δ-Diamino-n-valeric acid	$H_2NCH_2CH_2CH_2CH(NH_2)CO_2H$
25. Arginine (*g, e*)	α-Amino-δ-guanidino-n-valeric acid	$NH{=}C(NH_2)NH(CH_2)_3CH(NH_2)CO_2H$
26. Lysine (*g, e*)	α,ε-Diaminocaproic acid	$H_2N(CH_2)_4CH(NH_2)CO_2H$
27. Histidine (*g, e*)	α-Amino-β-imidazolepropionic acid	

* The occurrence of norleucine in proteins is uncertain.
† See also §5.
‡ The occurrence of β-hydroxyglutamic acid in proteins is uncertain.
§ Ornithine is probably not present in proteins, but is formed by the hydrolysis of arginine.