Amino Acids, Peptides, Proteins

Chapter · December 2008

DOI: 10.1007/978-3-540-69934-7_2

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1 Amino Acids, Peptides, Proteins

1.1 Foreword

Amino acids, peptides and proteins are important constituents of food. They supply the required building blocks for protein biosynthesis. In addition, they directly contribute to the flavor of food and are precursors for aroma compounds and colors formed during thermal or enzymatic reactions in production, processing and storage of food. Other food constituents, e. g., carbohydrates, also take part in such reactions. Proteins also contribute significantly to the physical properties of food through their ability to build or stabilize gels, foams, emulsions and fibrillar structures. The nutritional energy value of proteins (17 kJ/g or 4 kcal/g) is as high as that of carbohydrates.

The most important sources of protein are grain, oilseeds and legumes, followed by meat and milk. In addition to plants and animals, protein producers include algae (*Chlorella, Scenedesmus, Spirulina* spp.), yeasts and bacteria (single-cell proteins [SCP]). Among the C sources we use are glucose, molasses, starch, sulfite liquor, waste water, the higher n-alkanes, and methanol. Yeast of the genus *Candida* grow on paraffins, for example, and supply about 0.75 t of protein per t of carbohydrate. Bacteria of the species *Pseudomonas* in aqueous methanol produce about 0.30 t of protein per t of alcohol. Because of the high nucleic acid content of yeasts and bacteria (6–17% of dry weight), it is necessary to isolate protein from the cell mass. The future importance of single-cell proteins depends on price and on the technological properties.

In other raw materials, too, protein enrichment occurs for various reasons: protein concentration in the raw material may be too low for certain purposes, the sensory characteristics of the material (color, taste) may not be acceptable, or undesirable constituents may be present. Some products rich in protein also result from other processes, e. g., in oil and starch production. Enrichment results from the extraction of the constituents (protein concentrate) or from extraction and subsequent separation of protein from the solution, usually through thermal coagulation or isoelectric precipitation (protein isolate). Protein concentrates and protein isolates serve to enhance the nutritional value and to achieve the enhancement of the above mentioned physical properties of foods. They are added, sometimes after modification (cf. 1.4.6.1), to traditional foods, such as meat and cereal products, but they are also used in the production of novel food items such as meat, fish and milk substitutes. Raw materials in which protein enrichment takes place include:

- Legumes such as soybeans (cf. 16.3.1.2.1) and broad beans;
- Wheat and corn, which provide gluten as a by-product of starch production;
- Potatoes; from the natural sap left over after starch production, proteins can be isolated by thermal coagulation;
- Eggs, which are processed into different whole egg, egg white and egg yolk products (cf. 11.4);
- Milk, which supplies casein (cf. 10.2.9 and whey protein (cf. 10.2.10);
- Fish, which supplies protein concentrates after fat extraction (cf. 13.1.6.13 and 1.4.6.3.2);
- Blood from slaughter animals, which is processed into blood meal, blood plasma concentrate (cf. 12.6.1.10) and globin isolate.
- Green plants grown for animal fodder, such as alfalfa, which are processed into leaf protein concentrates through the thermal coagulation of cell sap proteins.

1.2 Amino Acids

1.2.1 General Remarks

There are about 20 amino acids in a protein hydrolysate. With a few exceptions, their general

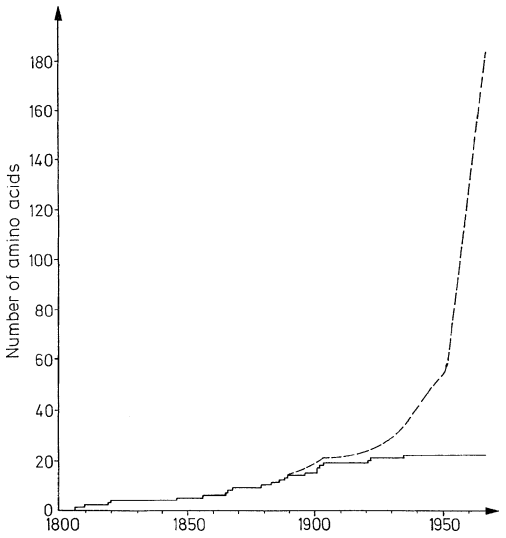
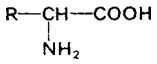


Fig. 1.1. Discovery of naturally occurring amino acids (according to *Meister*, 1965).--- Amino acids, total; — protein constituents

structure is:



(1.0)

In the simplest case, R=H (aminoacetic acid or glycine). In other amino acids, R is an aliphatic, aromatic or heterocyclic residue and may incorporate other functional groups. Table 1.1 shows the most important "building blocks" of proteins. There are about 200 amino acids found in nature (Fig. 1.1). Some of the more uncommon ones, which occur mostly in plants in free form, are covered in Chap. 17 on vegetables.

1.2.2 Classification, Discovery and Occurrence

1.2.2.1 Classification

There are a number of ways of classifiying amino acids. Since their side chains are the deciding factors for intra- and intermolecular interactions in proteins, and hence, for protein properties, amino acids can be classified as:

- Amino acids with nonpolar, uncharged side chains: e. g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine.
- Amino acids with uncharged, polar side chains: e. g., serine, threonine, cysteine, tyrosine, asparagine and glutamine.
- Amino acids with charged side chains: e. g., aspartic acid, glutamic acid, histidine, lysine and arginine.

Based on their nutritional/physiological roles, amino acids can be differentiated as:

- Essential amino acids:
  Valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine (essential for infants), lysine and arginine ("semi-essential").
- Nonessential amino acids:
  Glycine, alanine, proline, serine, cysteine, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid.

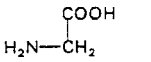
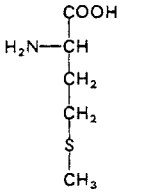
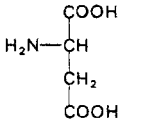
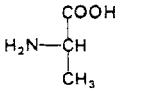
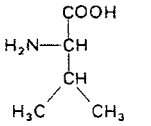
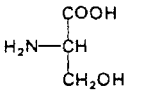
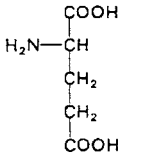
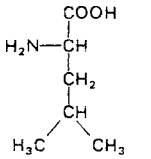
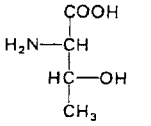
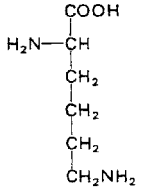
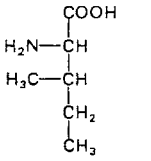
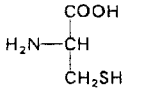
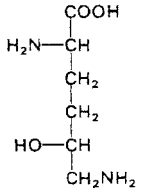
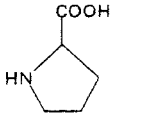
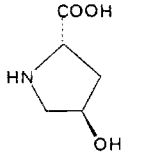
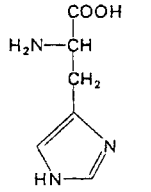
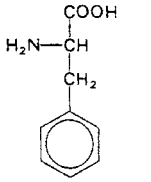
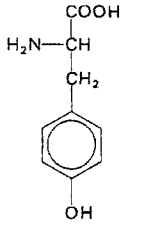
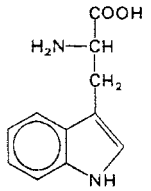
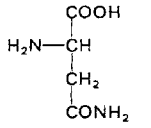
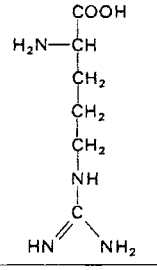
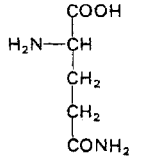
1.2.2.2 Discovery and Occurrence

Alanine was isolated from silk fibroin by *Weyl* in 1888. It is present in most proteins and is particularly enriched in silk fibroin (35%). Gelatin and zein contain about 9% alanine, while its content in other proteins is 2–7%. Alanine is considered nonessential for humans.

Arginine was first isolated from lupin seedlings by *Schulze* and *Steiger* in 1886. It is present in all proteins at an average level of 3–6%, but is particularly enriched in protamines. The arginine content of peanut protein is relatively high (11%). Biochemically, arginine is of great importance as an intermediary product in urea synthesis. Arginine is a semi-essential amino acid for humans. It appears to be required under certain metabolic conditions.

Asparagine from asparagus was the first amino acid isolated by *Vauguelin* and *Robiquet* in 1806. Its occurrence in proteins (edestin) was confirmed by *Damodaran* in 1932. In glycoproteins the carbohydrate component may be bound N-glycosidically to the protein moiety through the amide group of asparagine (cf. 11.2.3.1.1 and 11.2.3.1.3).

Table 1.1. Amino acids (protein building blocks) with their corresponding three ond one letter symbols

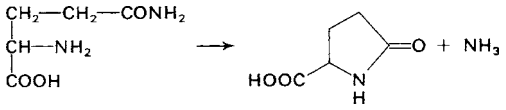
	Glycine (Gly. G)		L-Methionine (Met. M)		L-Aspartic acid (Asp. D)
	L-Alanine (Ala. A)				
	L-Valine (Val. V)		L-Serine (Ser. S)		L-Glutamic acid (Glu. E)
	L-Leucine (Leu. L)		L-Threonine (Thr. T)		L-Lysine (Lys. K)
	L-Isoleucine (Ile. I)		L-Cysteine (Cys. C)		L-5-Hydroxy-lysine
	L-Proline (Pro. P)		L-4-Hydroxy-proline		L-Histidine (His. H)
	L-Phenylalanine (Phe. F)		L-Tyrosine (Tyr. Y)		
	L-Tryptophan (Trp. W)		L-Asparagine[a] (Asn. N)		L-Arginine (Arg. R)
			L-Glutamine[a] (Gln. Q)		

[a] When no distinction exists between the acid and its amide then the symbols (Asx, B) and (Glx, Z) are valid.

Aspartic Acid was isolated from legumes by *Ritthausen* in 1868. It occurs in all animal proteins, primarily in albumins at a concentration of 6–10%. Alfalfa and corn proteins are rich in aspartic acid (14.9% and 12.3%, respectively) while its content in wheat is low (3.8%). Aspartic acid is nonessential.

Cystine was isolated from bladder calculi by *Wolaston* in 1810 and from horns by *Moerner* in 1899. Its content is high in keratins (9%). Cystine is very important since the peptide chains of many proteins are connected by two cysteine residues, i. e. by disulfide bonds. A certain conformation may be fixed within a single peptide chain by disulfide bonds. Most proteins contain 1–2% cystine. Although it is itself nonessential, cystine can partly replace methionine which is an essential amino acid.

Glutamine was first isolated from sugar beet juice by *Schulze* and *Bosshard* in 1883. Its occurrence in protein (edestin) was confirmed by *Damodaran* in 1932. Glutamine is readily converted into pyrrolidone carboxylic acid, which is stable between pH 2.2 and 4.0, but is readily cleaved to glutamic acid at other pH's:



(1.1)

Glutamic Acid was first isolated from wheat gluten by *Ritthausen* in 1866. It is abundant in most proteins, but is particularly high in milk proteins (21.7%), wheat (31.4%), corn (18.4%) and soya (18.5%). Molasses also contains relatively high amounts of glutamic acid. Monosodium glutamate is used in numerous food products as a flavor enhancer.

Glycine is found in high amounts in structural protein. Collagen contains 25–30% glycine. It was first isolated from gelatin by *Braconnot* in 1820. Glycine is a nonessential amino acid although it does act as a precursor of many compounds formed by various biosynthetic mechanisms.

Histidine was first isolated in 1896 independently by *Kossel* and by *Hedin* from protamines occurring in fish. Most proteins contain 2–3% histidine. Blood proteins contain about 6%. Histidine is essential in infant nutrition.

5-Hydroxylysine was isolated by *van Slyke et al.* (1921) and *Schryver et al.* (1925). It occurs in collagen. The carbohydrate component of glycoproteins may be bound O-glycosidically to the hydroxyl group of the amino acid (cf. 12.3.2.3.1).

4-Hydroxyproline was first obtained from gelatin by *Fischer* in 1902. Since it is abundant in collagen (12.4%), the determination of hydroxyproline is used to detect the presence of connective tissue in comminuted meat products. Hydroxyproline is a nonessential amino acid.

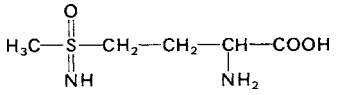
Isoleucine was first isolated from fibrin by *Ehrlich* in 1904. It is an essential amino acid. Meat and ceral proteins contain 4–5% isoleucine; egg and milk proteins, 6–7%.

Leucine was isolated from wool and from muscle tissue by *Braconnot* in 1820. It is an essential amino acid and its content in most proteins is 7–10%. Cereal proteins contain variable amounts (corn 12.7%, wheat 6.9%). During alcoholic fermentation, fusel oil is formed from leucine and isoleucine.

Lysine was isolated from casein by *Drechsel* in 1889. It makes up 7–9% of meat, egg and milk proteins. The content of this essential amino acid is 2–4% lower in cereal proteins in which prolamin is predominant. Crab and fish proteins are the richest sources (10–11%). Along with threonine and methionine, lysine is a limiting factor in the biological value of many proteins, mostly those of plant origin. The processing of foods results in losses of lysine since its ε-amino group is very reactive (cf. *Maillard* reaction).

Methionine was first isolated from casein by *Mueller* in 1922. Animal proteins contain 2–4% and plant proteins contain 1–2% methionine. Methionine is an essential amino acid and in many biochemical processes its main role is as a methyl-donor. It is very sensitive to oxygen and heat treatment. Thus, losses occur in many food processing operations such as drying, kiln-drying, puffing, roasting or treatment with oxidizing agents. In the bleaching of flour

with NCl_3 (nitrogen trichloride), methionine is converted to the toxic methionine sulfoximide:



(1.2)

Phenylalanine was isolated from lupins by *Schulze* in 1881. It occurs in almost all proteins (averaging 4–5%) and is essential for humans. It is converted *in vivo* into tyrosine, so phenylalanine can replace tyrosine nutritionally.

Proline was discovered in casein and egg albumen by *Fischer* in 1901. It is present in numerous proteins at 4–7% and is abundant in wheat proteins (10.3%), gelatin (12.8%) and casein (12.3%). Proline is nonessential.

Serine was first isolated from sericin by *Cramer* in 1865. Most proteins contain about 4–8% serine. In phosphoproteins (casein, phosvitin) serine, like threonine, is a carrier of phosphoric acid in the form of O-phosphoserine. The carbohydrate component of glycoproteins may be bound O-glycosidically through the hydroxyl group of serine and/or threonine [cf. 10.1.2.1.1 (κ-casein) and 13.1.4.2.4].

Threonine was discovered by *Rose* in 1935. It is an essential amino acid, present at 4.5–5% in meat, milk and eggs and 2.7–4.7% in cereals. Threonine is often the limiting amino acid in proteins of lower biological quality. The "bouillon" flavor of protein hydrolysates originates partly from a lactone derived from threonine (cf. 5.3.1.3).

Tryptophan was first isolated from casein hydrolysates, prepared by hydrolysis using pancreatic enzymes, by *Hopkins* in 1902. It occurs in animal proteins in relatively low amounts (1–2%) and in even lower amounts in cereal proteins (about 1%). Tryptophan is exceptionally abundant in lysozyme (7.8%). It is completely destroyed during acidic hydrolysis of protein. Biologically, tryptophan is an important essential amino acid, primarily as a precursor in the biosynthesis of nicotinic acid.

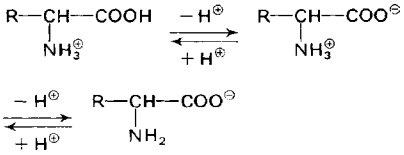
Tyrosine was first obtained from casein by *Liebig* in 1846. Like phenylalanine, it is found in almost all proteins at levels of 2–6%. Silk fibroin can have as much as 10% tyrosine. It is converted through dihydroxyphenylalanine by enzymatic oxidation into brown-black colored melanins.

Valine was first isolated by Schutzenberger in 1879. It is an essential amino acid and is present in meat and cereal proteins (5–7%) and in egg and milk proteins (7–8%). Elastin contains notably high concentrations of valine (15.6%).

1.2.3 Physical Properties

1.2.3.1 Dissociation

In aqueous solution amino acids are present, depending on pH, as cations, zwitterions or anions:



(1.3)

With the cation denoted as ^{+}A, the dipolar zwitterion as $^{+}A^{-}$ and the anion as A^{-}, the dissociation constant can be expressed as:

$$\frac{[^{\oplus}A^{\ominus}][H^{\oplus}]}{[^{\oplus}A]} = K_1 \qquad \frac{[A^{\ominus}][H^{\oplus}]}{[^{\oplus}A^{\ominus}]} = K_2 \tag{1.4}$$

At a pH where only dipolar ions exist, i. e. the isoelectric point, pI, $[^{+}A] = [A^{-}]$:

$$[^{\oplus}A] = \frac{[^{\oplus}A^{\ominus}][H^{\oplus}]}{K_1} = [A^{\ominus}] = \frac{[^{\oplus}A^{\ominus}]K_2}{[H^{\oplus}]}$$

$$[H^{\oplus}] = (K_1 \cdot K_2)^{0,5}$$

$$pI = 0{,}5\,(pK_1 + pK_2) \tag{1.5}$$

The dissociation constants of amino acids can be determined, for example, by titration of the acid. Figure 1.2 shows titration curves for glycine, histidine and aspartic acid. Table 1.2 lists the dissociation constants for some amino acids. In amino acids the acidity of the carboxyl group is higher and the basicity of the amino group lower than in the corresponding carboxylic acids and amines (cf. pK values for propionic acid, 2-propylamine and alanine). As illustrated by the comparison of pK values of 2-aminopropionic acid (alanine) and 3-aminopropionic acid (β-alanine), the pK is influenced by the distance between the two functional groups.

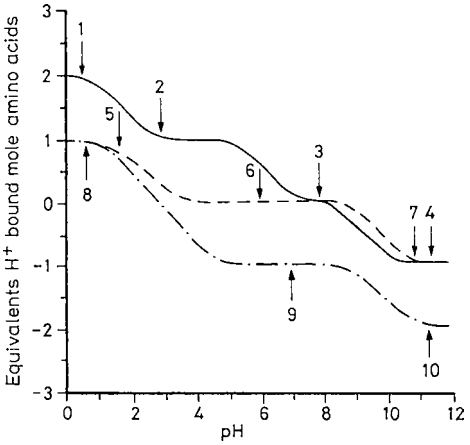
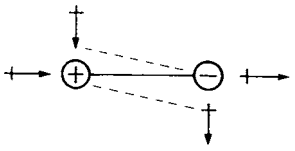


Fig. 1.2. Calculated titration curves for glycine (---), histidine (—) and aspartic acid (-·-·-). Numerals on curves are related to charge of amino acids in respective pH range: 1 $^{++}$His, 2 $^{++}$His^{-}, 3 $^{+}$His^{-}, 4 His^{-}, 5 $^{+}$Gly, 6 $^{+}$Gly^{-}, 7 Gly^{-}, 8 $^{+}$Asp. 9 $^{+}$Asp^{--}, 10 Asp^{--}

Table 1.2. Amino acids: dissociation constants and isoelectric points at 25 °C

Amino acid	pK_1	pK_2	pK_3	pK_4	pI
Alanine	2.34	9.69			6.0
Arginine	2.18	9.09	12.60		10.8
Asparagine	2.02	8.80			5.4
Aspartic acid	1.88	3.65	9.60		2.8
Cysteine	1.71	8.35	10.66		5.0
Cystine	1.04	2.10	8.02	8.71	5.1
Glutamine	2.17	9.13			5.7
Glutamic acid	2.19	4.25	9.67		3.2
Glycine	2.34	9.60			6.0
Histidine	1.80	5.99	9.07		7.5
4-Hydroxyproline	1.82	9.65			5.7
Isoleucine	2.36	9.68			6.0
Leucine	2.36	9.60			6.0
Lysine	2.20	8.90	10.28		9.6
Methionine	2.28	9.21			5.7
Phenylalanine	1.83	9.13			5.5
Proline	1.99	10.60			6.3
Serine	2.21	9.15			5.7
Threonine	2.15	9.12			5.6
Tryptophan	2.38	9.39			5.9
Tyrosine	2.20	9.11	10.07		5.7
Valine	2.32	9.62			6.0
Propionic acid	4.87				
2-Propylamine	10.63				
β-Alanine	3.55	10.24			6.9
γ-Aminobutyric acid	4.03	10.56			7.3

The reasons for this are probably as follows: in the case of the cation → zwitterion transition, the inductive effect of the ammonium group; in the case of the zwitterion → anion transition, the stabilization of the zwitterion through hydration caused by dipole repulsion (lower than in relation to the anion).



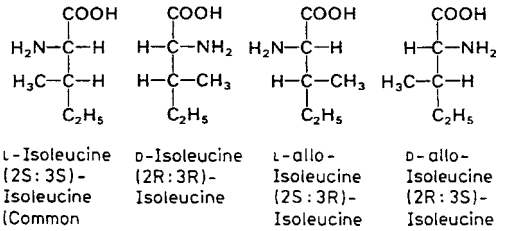
(⊕—⊖, zwitterion; +⟷ water dipole) (1.6)

1.2.3.2 Configuration and Optical Activity

Amino acids, except for glycine, have at least one chiral center and, hence, are optically active. All amino acids found in proteins have the same configuration on the α-C-atom: they are considered L-amino acids or (S)-amino acids* in the *Cahn-Ingold-Prelog* system (with L-cysteine an exception; it is in the (R)-series). D-amino acids (or (R)-amino acids) also occur in nature, for example, in a number of peptides of microbial origin:

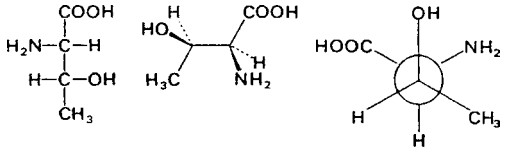
(1.7)

Isoleucine, threonine and 4-hydroxyproline have two asymmetric C-atoms, thus each has four isomers:



(1.8)

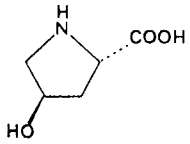
* As with carbohydrates, D,L-nomenclature is preferred with amino acids.



(Fischer-projection) (dotted line-wedge) (Newman-projection)

L-Threonine, (2S : 3R)-Threonine (Common in proteins)

(1.9)



L-4-Hydroxyproline, (2S: 4R)-Hydroxyproline (Common in proteins)

(1.10)

The specific rotation of amino acids in aqueous solution is strongly influenced by pH. It passes through a minimum in the neutral pH range and rises after addition of acids or bases (Table 1.3). There are various possible methods of separating the racemates which generally occur in amino acid synthesis (cf. 1.2.5). Selective crystallization of an over-saturated solution of racemate after seeding with an enantiomer is used, as is the fractioned crystallization of diastereomeric salts or other derivatives, such as (S)-phenylethylammonium salts of N-acetylamino acids. With enzymatic methods, asymmetric synthesis is used, e. g., of acylamino acid anilides from acylamino acids and aniline through papain:

$$\text{D,L—R—CO—NH—CHR}^1\text{—COOH} \xrightarrow[\text{Papain}]{\text{Aniline}}$$

$$\text{L—R—CO—NH—CHR}^1\text{—CO—NH—C}_6\text{H}_5$$

$$+\ \text{D—R—CO—NH—CHR}^1\text{—COOH} \qquad (1.11)$$

or asymmetric hydrolysis, e. g., of amino acid esters through esterases, amino acid amides through amidases or N-acylamino acids through amino-acylases:

$$\text{D,L—H}_2\text{N—CHR—COOR}^1 \xrightarrow{\text{Esterase}}$$

$$\text{L—H}_2\text{NCHR—COOH} + \text{D—H}_2\text{N—CHR—COOR}^1$$

$$\text{D,L—H}_2\text{N—CHR—CONHR}^1 \xrightarrow{\text{Amidase}}$$

$$\text{L—H}_2\text{N—CHR—COOH} + \text{D—H}_2\text{N—CHR—CONHR}^1$$

$$\text{D,L—R—CO—NH—CHR}^1\text{—COOH} \xrightarrow{\text{Acylase}}$$

$$\text{L—H}_2\text{N—CHR}^1\text{—COOH}$$

$$+\ \text{D—R—CO—NH—CHR}^1\text{—COOH} \qquad (1.12)$$

The detection of D-amino acids is carried out by enantioselective HPLC or GC of chiral amino acid derivatives. In a frequently applied method, the derivatives are produced in a precolumn by reaction with o-phthalaldehyde and a chiral thiol (cf. 1.2.4.2.4). Alternatively, the amino acids can be transformed into trifluoroacetylamino acid-2-(R,S)-butylesters. Their GC separation is shown in Fig. 1.3.

Table 1.3. Amino acids: specific rotation ($[\alpha]_D^t$)

Amino acid	Solvent system	Temperature (°C)	$[\alpha]_D$
L-Alanine	0.97 M HCl	15	+14.7°
	water	22	+ 2.7°
	3 M NaOH	20	+ 3.0°
L-Cystine	1.02 M HCl	24	−214.4°
L-Glutamic acid	6.0 M HCl	22.4	+31.2°
	water	18	+11.5°
	1M NaOH	18	+10.96°
L-Histidine	6.0 M HCl	22.7	+13.0°
	water	25.0	−39.01°
	0.5 M NaOH	20	−10.9°
L-Leucine	6.0 M HCl	25.9	+15.1°
	water	24.7	−10.8°
	3.0 M NaOH	20	+7.6°

1.2.3.3 Solubility

The solubilities of amino acids in water are highly variable. Besides the extremely soluble proline, hydroxyproline, glycine and alanine are also quite soluble. Other amino acids (cf. Table 1.4) are significantly less soluble, with cystine and tyrosine having particularly low solubilities. Addition of acids or bases improves the solubility through salt formation. The presence of other amino acids, in general, also brings about

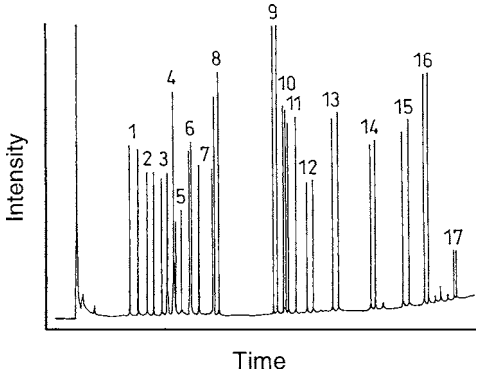


Fig. 1.3. Gas chromatogram of N-pentafluoropropanoylDL-amino acid isopropylesters on Chirasil-Val (N-propionyl-L-valine-tert-butylamide-polysiloxane) (1: D-, L-Ala, 2: D-, L-Val, 3. D-, L-Thr, 4: Gly, 5: D-, L-Ile, 6: D-, L-Pro, 7: D-, L-Leu, 8: D-, L-Ser, 9: D-, L-Cys, 10: D-, L-Asp, 11: D-, L-Met, 12: D-, L-Phe, 13: D-, L-Glu, 14: D-, L-Tyr, 15: D-, L-Orn, 16: D-, L-Lys, 17: D-, L-Trp; according to *Frank* et al., 1977)

Table 1.4. Solubility of amino acids in water ($g/100$ g H_2O)

	Temperature (°C)				
Amino acid	0	25	50	75	100
L-Alanine	12.73	16.51	21.79	28.51	37.30
L-Asparatic acid	0.209	0.500	1.199	2.875	6.893
L-Cystine	0.005	0.011	0.024	0.052	0.114
L-Glutamic acid	0.341	0.843	2.186	5.532	14.00
Glycine	14.18	24.99	39.10	54.39	67.17
L-Histidine	–	4.29	–	–	–
L-Hydroxy-proline	28.86	36.11	45.18	51.67	–
L-Isoleucine	3.791	4.117	4.818	6.076	8.255
L-Leucine	2.270	2.19	2.66	3.823	5.638
D,L-Methionine	1.818	3.381	6.070	10.52	17.60
L-Phenyl-alanine	1.983	2.965	4.431	6.624	9.900
L-Proline	127.4	162.3	206.7	239.0	–
D,L-Serine	2.204	5.023	10.34	19.21	32.24
L-Tryptophan	0.823	1.136	1.706	2.795	4.987
L-Tyrosine	0.020	0.045	0.105	0.244	0.565
L-Valine	8.34	8.85	9.62	10.24	–

an increase in solubility. Thus, the extent of solubility of amino acids in a protein hydrolysate is different than that observed for the individual components.

The solubility in organic solvents is not very good because of the polar characteristics of the amino acids. All amino acids are insoluble in ether. Only cysteine and proline are relatively soluble in ethanol (1.5 g/100 g at 19 °C). Methionine, arginine, leucine (0.0217 g/100 g; 25 °C), glutamic acid (0.00035 g/100 g; 25 °C), phenylalanine, hydroxy-proline, histidine and tryptophan are sparingly soluble in ethanol. The solubility of isoleucine in hot ethanol is relatively high (0.09 g/100 g at 20 °C; 0.13 g/100 g at 78–80 °C).

1.2.3.4 UV-Absorption

Aromatic amino acids such as phenylalanine, tyrosine and tryptophan absorb in the UV-range of the spectrum with absorption maxima at 200–230 nm and 250–290 nm (Fig. 1.4). Dissociation of the phenolic HO-group of tyrosine shifts the absorption curve by about 20 nm towards longer wavelengths (Fig. 1.5).

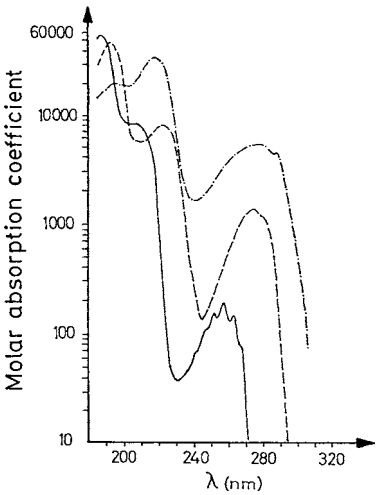


Fig. 1.4. Ultraviolet absorption spectra of some amino acids. (according to *Luebke, Schroeder* and *Kloss*, 1975). -----Trp. ---Tyr. —Phe

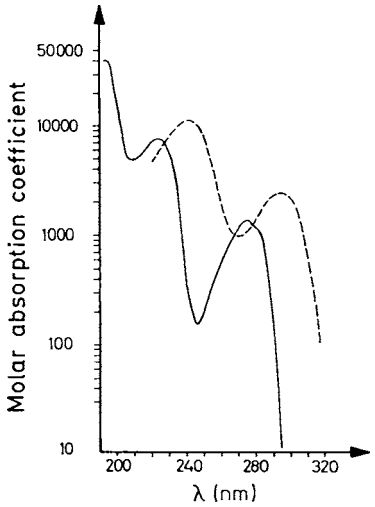


Fig. 1.5. Ultraviolet absorption spectrum of tyrosine as affected by pH. (according to *Luebke, Schroeder* and *Kloss*, 1975) — 0.1 mol/l HCl, --- 0.1 mol/l NaOH

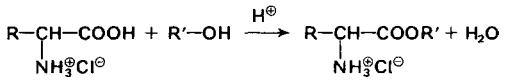
Absorption readings at 280 nm are used for the determination of proteins and peptides. Histidine, cysteine and methionine absorb between 200 and 210 nm.

1.2.4 Chemical Reactions

Amino acids show the usual reactions of both carboxylic acids and amines. Reaction specificity is due to the presence of both carboxyl and amino groups and, occasionally, of other functional groups. Reactions occurring at 100–220 °C, such as in cooking, frying and baking, are particularly relevant to food chemistry.

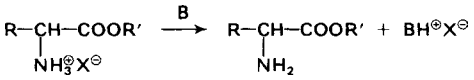
1.2.4.1 Esterification of Carboxyl Groups

Amino acids are readily esterified by acid-catalyzed reactions. An ethyl ester hydrochloride is obtained in ethanol in the presence of HCl:



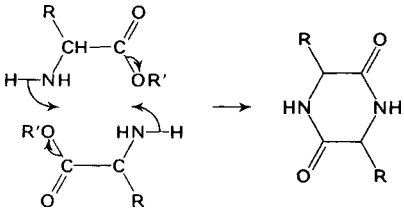
(1.13)

The free ester is released from its salt by the action of alkali. A mixture of free esters can then be separated by distillation without decomposition. Fractional distillation of esters is the basis of a method introduced by *Emil Fischer* for the separation of amino acids:

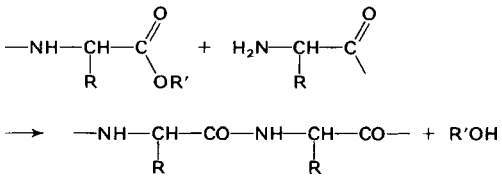


(1.14)

Free amino acid esters have a tendency to form cyclic dipeptides or open-chain polypeptides:



(1.15)



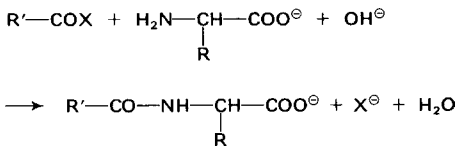
(1.16)

tert-butyl esters, which are readily split by acids, or benzyl esters, which are readily cleaved by HBr/glacial acetic acid or catalytic hydrogenation, are used as protective groups in peptide synthesis.

1.2.4.2 Reactions of Amino Groups

1.2.4.2.1 Acylation

Activated acid derivatives, e. g., acid halogenides or anhydrides, are used as acylating agents:

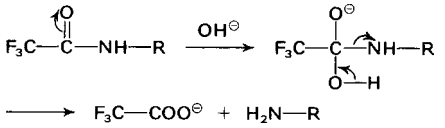


(1.17)

N-acetyl amino acids are being considered as ingredients in chemically-restricted diets and for fortifying plant proteins to increase their biological value. Addition of free amino acids to food which must be heat treated is not problem free. For example, methionine in the presence of a reducing sugar can form methional by a *Strecker* degradation mechanism, imparting an off-flavor to food. Other essential amino acids, e. g., lysine or threonine, can lose their biological value through similar reactions. Feeding tests with rats have shown that N-acetyl-L-methionine and N-acetyl-L-threonine have nutritional values equal to those of the free amino acids (this is true also for humans with acetylated methionine). The growth rate of rats is also increased significantly by the α- or ε-acetyl or α,ε-diacetyl derivatives of lysine.

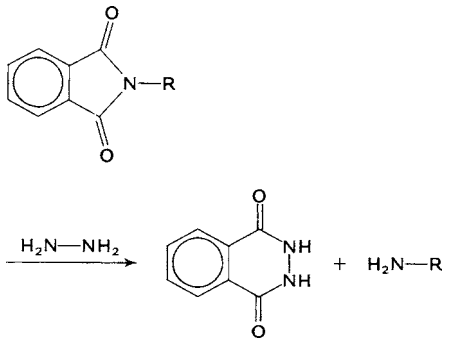
Some readily cleavable acyl residues are of importance as temporary protective groups in peptide synthesis.

The trifluoroacetyl residue is readily removed by mild base-catalyzed hydrolysis:



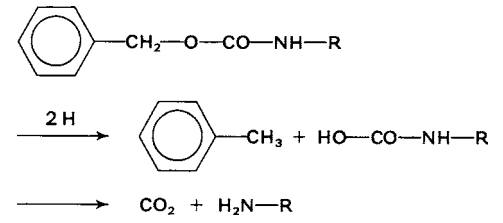
(1.18)

The phthalyl residue can be readily cleaved by hydrazinolysis:

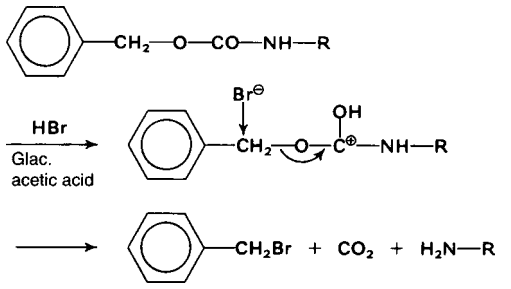


(1.19)

The benzyloxycarbonyl group can be readily removed by catalytic hydrogenation or by hydrolysis with HBr/glacial acetic acid:

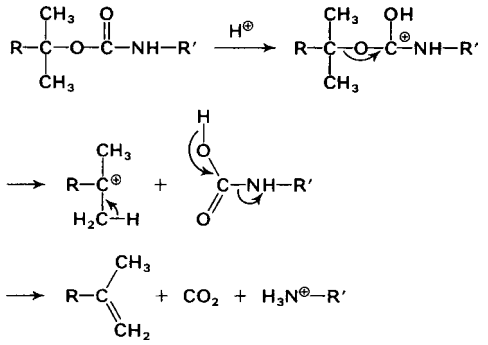


(1.20)



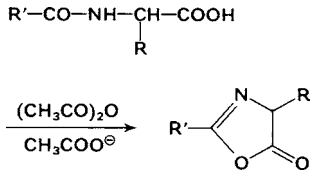
(1.21)

The *tert*-alkoxycarbonyl residues, e. g., the *tert*-butyloxycarbonyl groups, are cleaved under acid-catalyzed conditions:



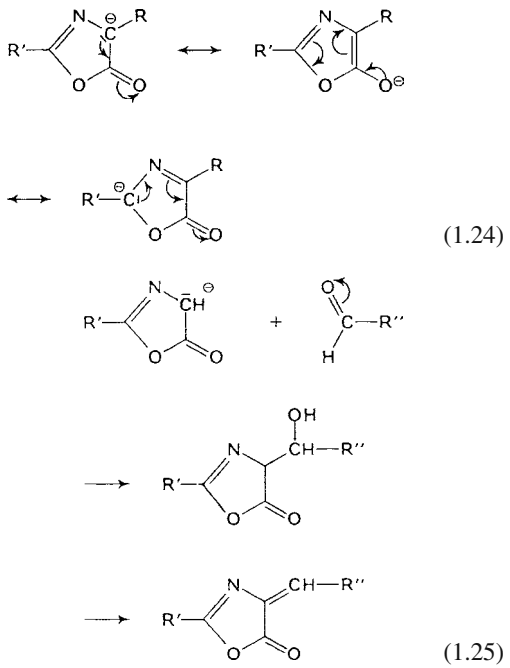
(1.22)

N-acyl derivatives of amino acids are transformed into oxazolinones (azlactones) by elimination of water:



(1.23)

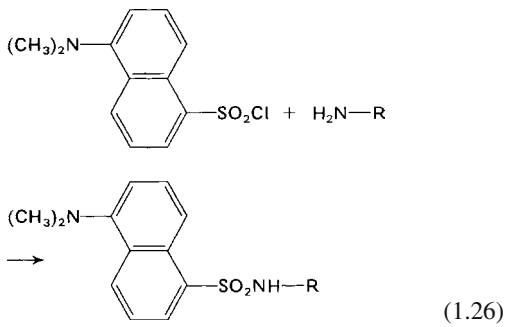
These are highly reactive intermediary products which form a mesomerically stabilized anion. The anion can then react, for example, with aldehydes. This reaction is utilized in amino acid synthesis with glycine azlactone as a starting compound:



(1.24)

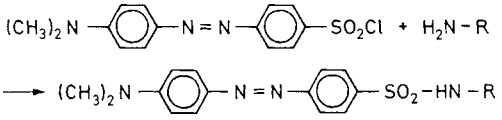
(1.25)

Acylation of amino acids with 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride, DANS-Cl) is of great analytical importance:

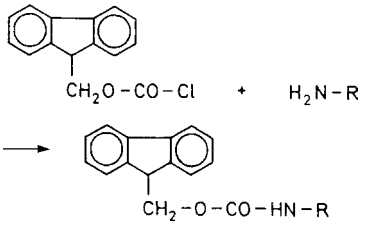


(1.26)

The aryl sulfonyl derivatives are very stable against acidic hydrolysis. Therefore, they are suitable for the determination of free N-terminal amino groups or free ε-amino groups of peptides or proteins. Dansyl derivatives which fluoresce in UV-light have a detection limit in the nanomole range, which is lower than that of 2,4-dinitrophenyl derivatives by a factor of 100. Dimethylaminoazobenzenesulfonylchloride (DABS-Cl) and 9-fluoroenylmethylchloroformate (FMOC) detect amino acids (cf. Formula 1.27 and 1.28) including proline and hydroxyproline. The fluorescent derivatives can be quantitatively determined after HPLC separation.



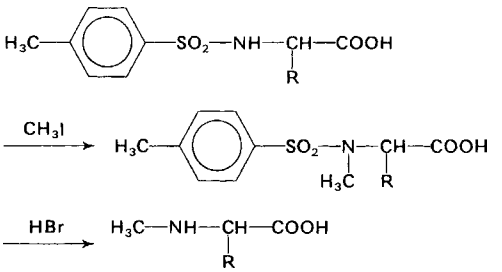
(1.27)



(1.28)

1.2.4.2.2 Alkylation and Arylation

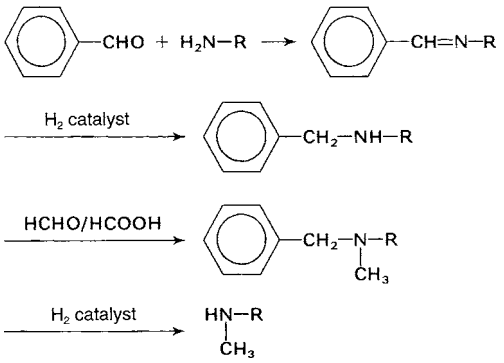
N-methyl amino acids are obtained by reaction of the N-tosyl derivative of the amino acid with methyl iodide, followed by removal of the tosyl substituent with HBr:



(1.29)

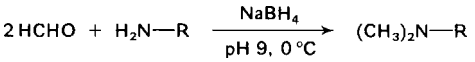
The N-methyl compound can also be formed by methylating with HCHO/HCOOH the benzylidene derivative of the amino acid, formed initially by reaction of the amino acid with benzaldehyde. The benzyl group is then eliminated

by hydrogenolysis:



(1.30)

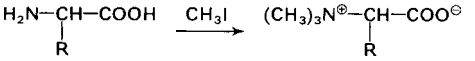
Dimethyl amino acids are obtained by reaction with formaldehyde, followed by reduction with sodium borohydride:



(1.31)

The corresponding reactions with proteins are being considered as a means of protecting the ε-amino groups and, thus, of avoiding their destruction in food through the *Maillard* reaction (cf. 1.4.6.2.2).

Direct reaction of amino acids with methylating agents, e. g. methyl iodide or dimethyl sulfate, proceeds through monomethyl and dimethyl compounds to trimethyl derivatives (or generally to N-trialkyl derivatives) denoted as betaines:



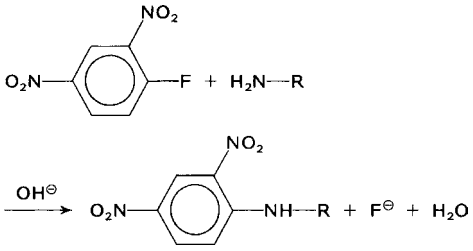
(1.32)

As shown in Table 1.5, betaines are widespread in both the animal and plant kingdoms.

Derivatization of amino acids by reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) yields N-2,4-dinitrophenyl amino acids (DNP-amino acids), which are yellow compounds and crystallize readily. The reaction is important for labeling N-terminal amino acid residues and free ε-amino groups present in peptides and proteins; the DNP-amino acids are stable under conditions of acidic hydrolysis (cf. Reaction 1.33).

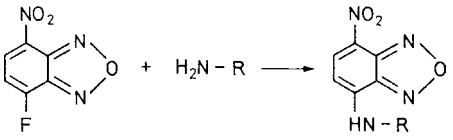
Table 1.5. Occurrence of trimethyl amino acids $(CH_3)_3N^+$-CHR-COO^- (betaines)

Amino acid	Betaine	Occurrence
β-Alanine	Homobetaine	Meat extract
γ-Amino-butyric acid	Actinine	Mollusk (shell-fish)
Glycine	Betaine	Sugar beet, other samples of animal and plant origin
Histidine	Hercynine	Mushrooms
β-Hydroxy-γ-amino-butyric acid	Carnitine	Mammals muscle tissue, yeast, wheat germ, fish, liver, whey, mollusk (shell-fish)
4-Hydroxy-proline	Betonicine	Jack beans
Proline	Stachydrine	Stachys, orange leaves, lemon peel, alfalfa, Aspergillus oryzae



(1.33)

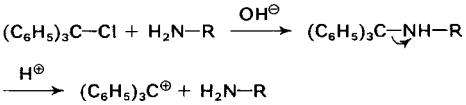
Another arylation reagent is 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazol (NBD-F), which is also used as a chlorine compound (NBD-Cl) and which leads to derivatives that are suited for an amino acid analysis through HPLC separation:



(1.34)

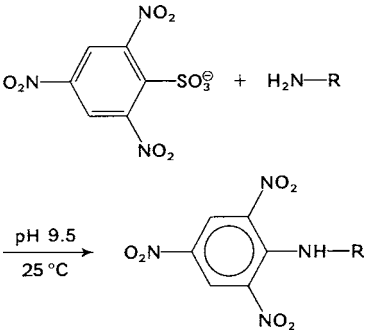
Reaction of amino acids with triphenylmethyl chloride (tritylchloride) yields N-trityl derivatives, which are alkali stable. However, the derivative is cleaved in the presence of acid,

giving a stable triphenylmethyl cation and free amino acid:



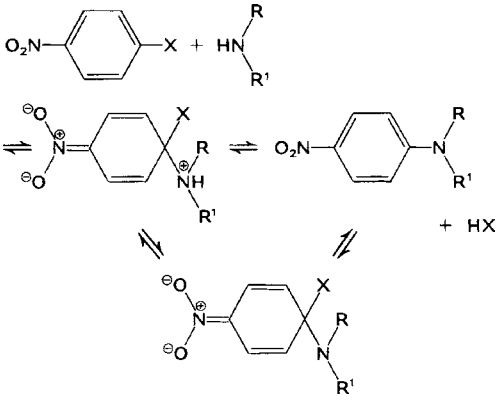
(1.35)

The reaction with trinitrobenzene sulfonic acid is also of analytical importance. It yields a yellow-colored derivative that can be used for the spectrophotometric determination of protein:



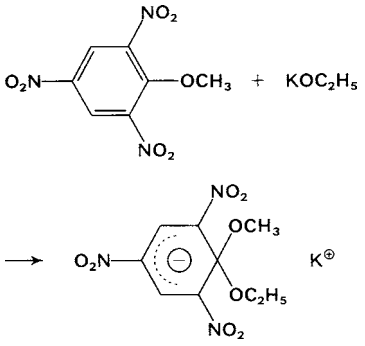
(1.36)

The reaction is a nucleophilic aromatic substitution proceeding through an intermediary addition product (*Meisenheimer* complex). It occurs under mild conditions only when the benzene ring structure is stabilized by electron-withdrawing substituents on the ring (cf. Reaction 1.37).



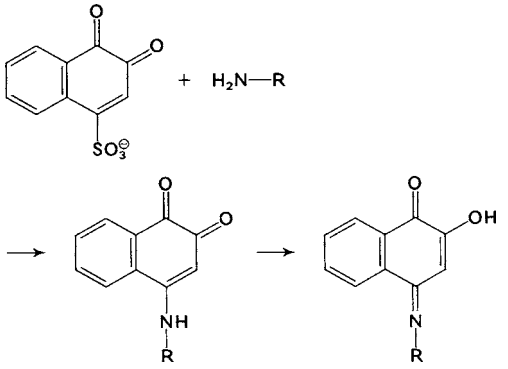
(1.37)

The formation of the *Meisenheimer* complex has been verified by isolating the addition product from the reaction of 2,4,6-trinitroanisole with potassium ethoxide (cf. Reaction 1.38).



(1.38)

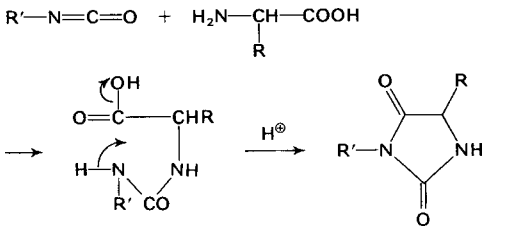
An analogous reaction occurs with 1,2-naphthoquinone-4-sulfonic acid (*Folin* reagent) but, instead of a yellow color (cf. Formula 1.36), a red color develops:



(1.39)

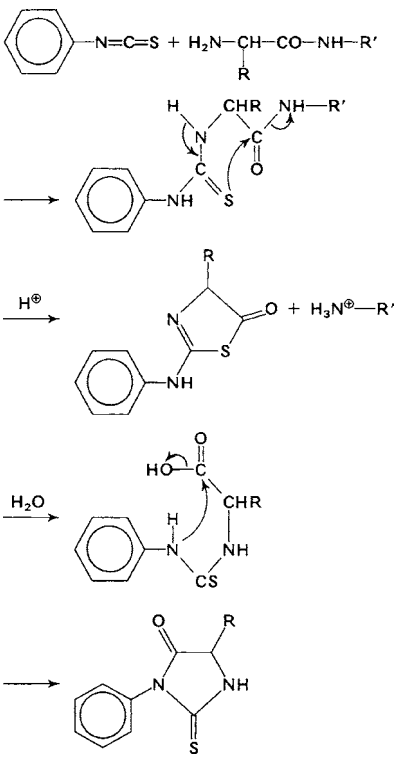
1.2.4.2.3 Carbamoyl and Thiocarbamoyl Derivatives

Amino acids react with isocyanates to yield carbamoyl derivatives which are cyclized into 2,4-dioxoimidazolidines (hydantoins) by boiling in an acidic medium:

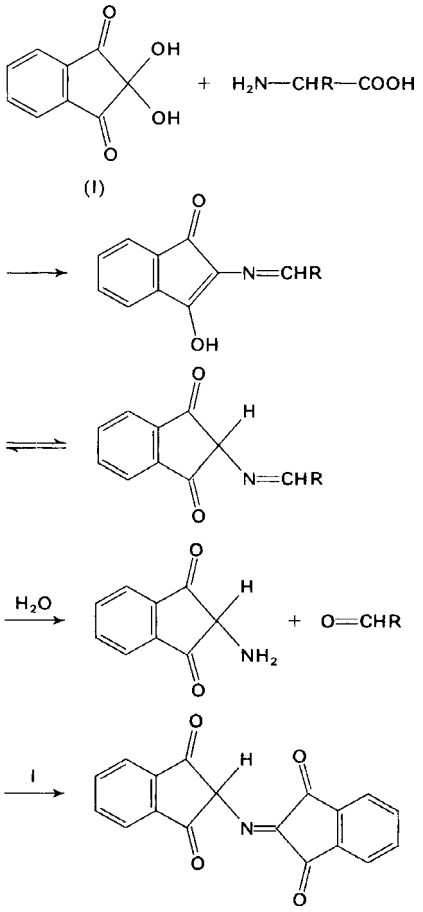


(1.40)

A corresponding reaction with phenylisothiocyanate can degrade a peptide in a stepwise fashion (*Edman* degradation). The reaction is of great importance for revealing the amino acid sequence in a peptide chain. The phenylthiocarbamoyl derivative (PTC-peptide) formed in the first step (coupling) is cleaved non-hydrolytically in the second step (cleavage) with anhydrous trifluoroacetic acid into anilinothiazolinone as derivative of the N-terminal amino acid and the remaining peptide which is shortened by the latter. Because of its instability, the thiazolinone is not suited for an identification of the N-terminal amino acid and is therefore – after separation from the remaining peptide, in the third step (conversion) – converted in aqueous HCl via the phenylthiocarbamoylamino acid into phenyl-thiohydantoin, while the remaining peptide is fed into a new cycle.



(1.41)

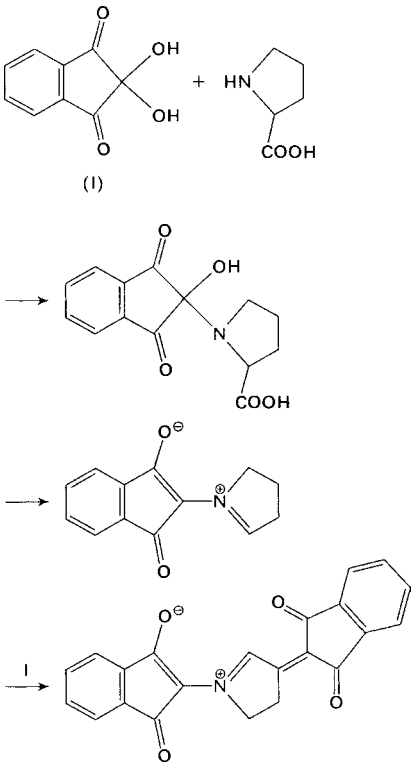


(1.42)

1.2.4.2.4 Reactions with Carbonyl Compounds

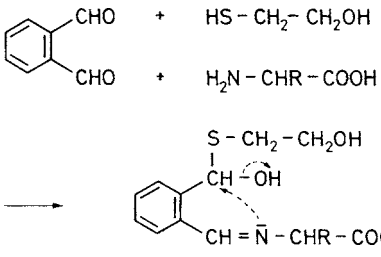
Amino acids react with carbonyl compounds, forming azomethines. If the carbonyl compound has an electron-withdrawing group, e. g., a second carbonyl group, transamination and decarboxylation occur. The reaction is known as the *Strecker* degradation and plays a role in food since food can be an abundant source of dicarbonyl compounds generated by the *Maillard* reaction (cf. 4.2.4.4.7). The aldehydes formed from amino acids (*Strecker* aldehydes) are aroma compounds (cf. 5.3.1.1). The ninhydrin reaction is a special case of the *Strecker* degradation. It is an important reaction for the quantitative determination of

amino acids using spectrophotometry (cf. Reaction 1.42). The detection limit lies at 1–0.5 nmol. The resultant blue-violet color has an absorption maximum at 570 nm. Proline yields a yellow-colored compound with $\lambda_{max} = 440$ nm (Reaction 1.43):

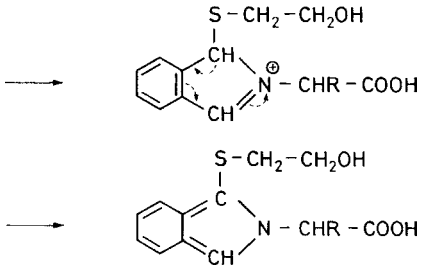


(1.43)

The reaction of amino acids with o-phthaldialdehyde (OPA) and mercaptoethanol leads to fluorescent isoindole derivatives ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 455$ nm) (Reaction 1.44a).



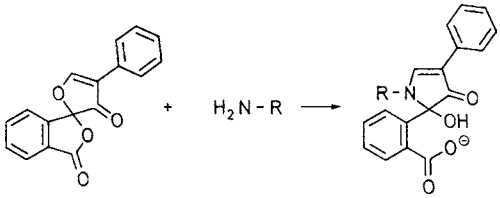
(1.44a)



(1.44b)

The derivatives are used for amino acid analysis via HPLC separation. Instead of mercaptoethanol, a chiral thiol, e. g., N-isobutyryl-L-cysteine, is used for the detection of D-amino acids. The detection limit lies at 1 pmol. The very fast racemizing aspartic acid is an especially suitable marker. One disadvantage of the method is that proline and hydroxyproline are not detected. This method is applied, e. g., in the analysis of fruit juices, in which high concentrations of D-amino acids indicate bacterial contamination or the use of highly concentrated juices. Conversely, too low concentrations of D-amino acids in fermented foods (cheese, soy and fish sauces, wine vinegar) indicate unfermented imitations.

Fluorescamine reacts with primary amines and amino acids – at room temperature under alkaline conditions – to form fluorescent pyrrolidones ($\lambda_{ex} = 390$ nm, $\lambda_{em} = 474$ nm). The detection limit lies at 50–100 pmol:



(1.45)

The excess reagent is very quickly hydrolyzed into water-soluble and non-fluorescent compounds.

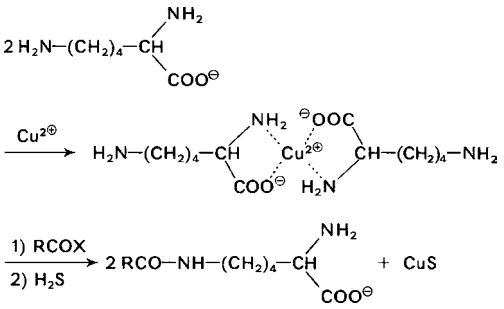
1.2.4.3 Reactions Involving Other Functional Groups

The most interesting of these reactions are those in which α-amino and α-carboxyl groups are

blocked, that is, reactions occurring with peptides and proteins. These reactions will be covered in detail in sections dealing with modification of proteins (cf. 1.4.4 and 1.4.6.2). A number of reactions of importance to free amino acids will be covered in the following sections.

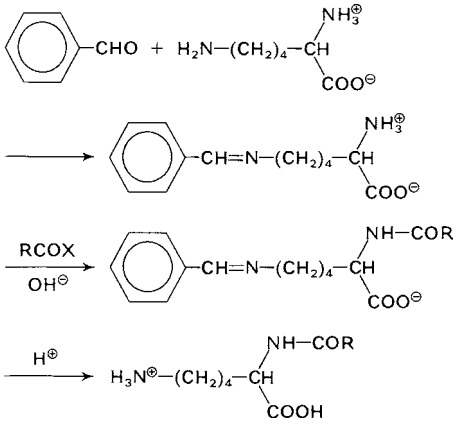
1.2.4.3.1 Lysine

A selective reaction may be performed with either of the amino groups in lysine. Selective acylation of the ε-amino group is possible using the lysine-Cu^{2+} complex as a reactant:



(1.46)

Selective reaction with the α-amino group is possible using a benzylidene derivative:

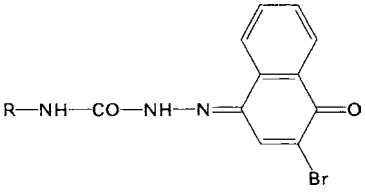


(1.47)

ε-N-benzylidene-L-lysine and ε-N-salicylidene-L-lysine are as effective as free lysine in growth feeding tests with rats. Browning reactions of these derivatives are strongly retarded, hence they are of interest for lysine fortification of food.

1.2.4.3.2 Arginine

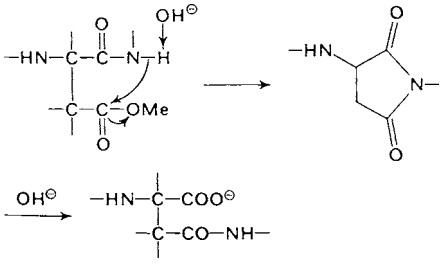
In the presence of α-naphthol and hypobromite, the guanidyl group of arginine gives a red compound with the following structure:



(1.48)

1.2.4.3.3 Aspartic and Glutamic Acids

The higher esterification rate of β- and γ-carboxyl groups can be used for selective reactions. On the other hand the β- and γ-carboxyl groups are more rapidly hydrolyzed in acid-catalyzed hydrolysis since protonation is facilitated by having the ammonium group further away from the carboxyl group. Alkali-catalyzed hydrolysis of methyl or ethyl esters of aspartic or glutamic acids bound to peptides can result in the formation of isopeptides.

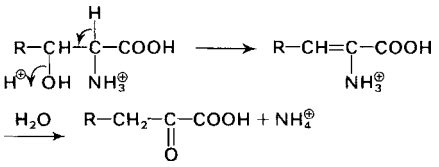


(1.49)

Decarboxylation of glutamic acid yields γ-aminobutyric acid. This compound, which also occurs in wine (cf. 20.2.6.9), tastes sour and produces a dry feeling in the mouth at concentrations above its recognition threshold (0.02 mmol/l).

1.2.4.3.4 Serine and Threonine

Acidic or alkaline hydrolysis of protein can yield α-keto acids through β-elimination of a water molecule:

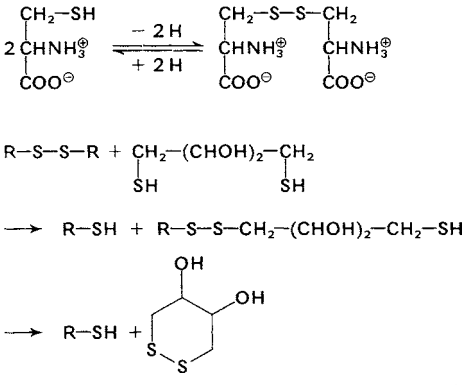


(1.50)

In this way, α-ketobutyric acid formed from threonine can yield another amino acid, α-aminobutyric acid, via a transamination reaction. Reaction 1.51 is responsible for losses of hydroxy amino acids during protein hydrolysis.
Reliable estimates of the occurrence of these amino acids are obtained by hydrolyzing protein for varying lengths of time and extrapolating the results to zero time.

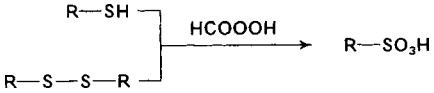
1.2.4.3.5 Cysteine and Cystine

Cysteine is readily converted to the corresponding disulfide, cystine, even under mild oxidative conditions, such as treatment with I_2 or potassium hexacyanoferrate (III). Reduction of cystine to cysteine is possible using sodium borohydride or thiol reagents (mercaptoethanol, dithiothreitol):



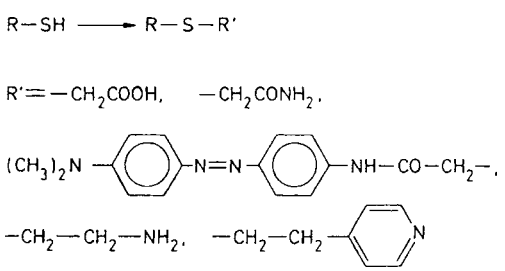
(1.51)

The equilibrium constants for the reduction of cystine at pH 7 and 25 °C with mercaptoethanol or dithiothreitol are 1 and 10^4, respectively.
Stronger oxidation of cysteine, e. g., with performic acid, yields the corresponding sulfonic acid, cysteic acid:



(1.52)

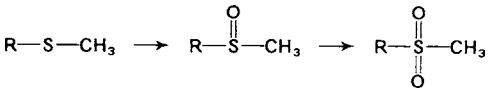
Reaction of cysteine with alkylating agents yields thioethers. Iodoacetic acid, iodoacetamide, dimethylaminoazobenzene iodoacetamide, ethylenimine and 4-vinylpyridine are the most commonly used alkylating agents:



(1.53)

1.2.4.3.6 Methionine

Methionine is readily oxidized to the sulfoxide and then to the sulfone. This reaction can result in losses of this essential amino acid during food processing:

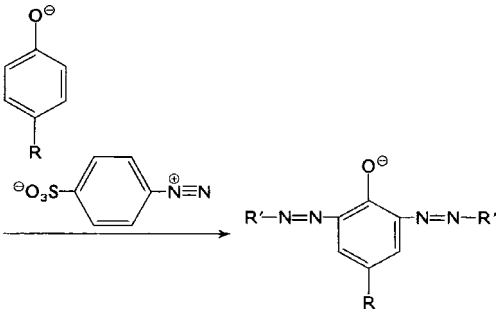


(1.54)

1.2.4.3.7 Tyrosine

Tyrosine reacts, like histidine, with diazotized sulfanilic acid (*Pauly* reagent). The coupled-

reaction product is a red azo compound:



(1.55)

1.2.4.4 Reactions of Amino Acids at Higher Temperatures

Reactions at elevated temperatures are important during the preparation of food. Frying, roasting, boiling and baking develop the typical aromas of many foods in which amino acids participate as precursors. Studies with food and model systems have shown that the characteristic odorants are formed via the *Maillard* reaction and that they are subsequent products, in particular of cysteine, methionine, ornithine and proline (cf. 12.9.3).

1.2.4.4.1 Acrylamide

The toxic compound acrylamide is one of the volatile compounds formed during the heating of food (cf. 9.7.3). Model experiments have shown that it is produced in reactions of asparagine with reductive carbohydrates or from the resulting cleavage products (e. g., 2-butanedione, 2-oxopropanal).

The formation is promoted by temperatures >100 °C and/or longer reaction times. Indeed, model experiments have shown that the highest yields based on asparagine are ca. 0.1–1 mol%. Cysteine and methionine also form acrylamide in the presence of glucose, but the yields are considerably lower than those from asparagine. The thermal reaction of acrolein with ammonia also produces acrylamide, but again only in small amounts.

Although from a purely stoichiometric standpoint, it would be possible that the degradation of asparagine by the cleavage of CO_2 und NH_3 directly produces acrylamide, the course of formation is quite complex. Indeed, various proposals exist for the mechanism of this formation. It was shown that considerable amounts of 3-aminopropionamide are produced in the reaction of asparagine with α-dicarbonyl compounds with the formation of the *Schiff* base and subsequent decarboxylation and hydrolysis in the sense of a *Strecker* reaction (Fig. 1.6). It could be shown in model studies and in additional experiments with foods (cocoa, cheese) that the splitting-off of ammonia from 3-aminopropionamide occurs relatively easily at higher temperatures and even in the absence of carbohydrates results in very high yields of acrylamide (>60 mol%). Therefore, 3-aminopropionamide, which is to be taken as the biogenic amine of asparagine, represents a transient intermediate in the formation of acrylamide in foods. In the meantime, this compound has also been identified in different foods.

Another mechanism (Fig. 1.7, right) starts out from the direct decomposition of the *Schiff* base obtained from a reductive carbohydrate and asparagine via instable analytically undetectable intermediates. It is assumed that the ylide formed by the decarboxylation of the *Schiff* base directly decomposes on cleavage of the

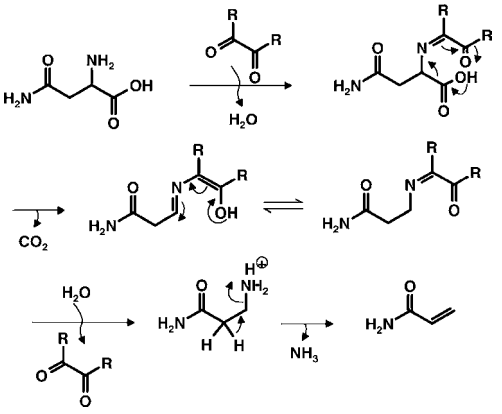


Fig. 1.6. Formation of 3-aminopropionamide (3-APA) from the Strecker reaction of asparagine and subsequent deamination to acrylamide (according to *Granvogl* et al., 2006)

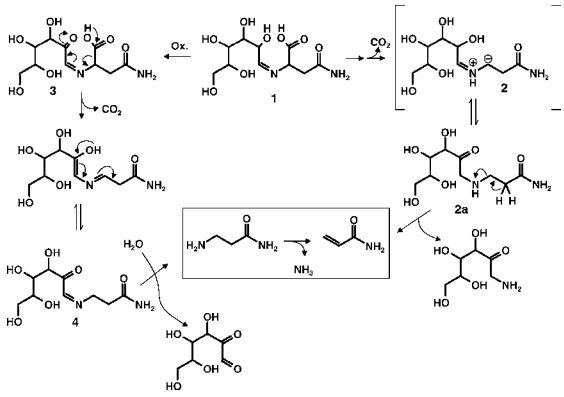
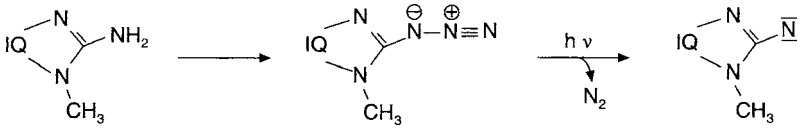


Fig. 1.7. Reaction paths from the *Schiff* base of asparagine and glucose which result in acrylamide (according to *Stadler* et al., 2004 and *Granvogl* et al., 2006)

C-N bond to give acrylamide and a 1-amino-2-hexulose. Another proposed mechanism (Fig. 1.7, left) is the oxidation of the *Schiff* base and subsequent decarboxylation. Here, an intermediate is formed which can decompose to 3-aminopropionamide after enolization and hydrolysis. 3-Aminopropionamide can then be converted to acrylamide after the splitting-off of ammonia.



(1.56)

1.2.4.4.2 Mutagenic Heterocyclic Compounds

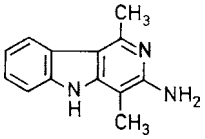
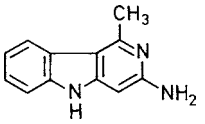
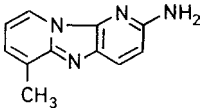
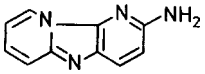
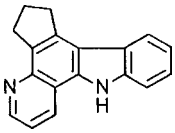
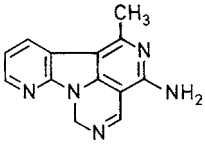
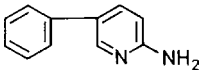
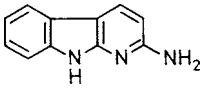
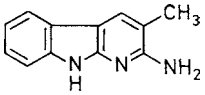
In the late 1970s it was shown that charred surface portions of barbecued fish and meat as well as the smoke condensates captured in barbecuing have a highly mutagenic effect in microbial tests (*Salmonella typhimurium* tester strain TA 98). In model tests it could be demonstrated that pyrolyzates of amino acids and proteins are responsible for that effect. Table 1.6 lists the mutagenic compounds isolated from amino acid pyrolyzates. They are pyridoindoles, pyridoimidazoles and tetra-azafluoroanthenes.

At the same time, it was found that mutagenic compounds of amino acids and proteins can also be formed at lower temperatures. The compounds listed in Table 1.7 were obtained from meat extract, deep-fried meat, grilled fish and heated model mixtures on the basis of creatine, an amino acid (glycine, alanine, threonine) and glucose. For the most part they were imidazoquinolines and imidazoquinoxalines. The highest concentrations (μ/kg) were found in meat extract: IQ (0−15), MeIQ (0−6), MeIQx (0−80). A model experiment directed at processes in meat shows that heterocyclic amines are detectable at temperatures around 175 °C after only 5 minutes. It is assumed that they are formed from creatinine, subsequent products of the *Maillard* reaction (pyridines, pyrazines, cf. 4.2.4.4.3) and amino acids as shown in Fig. 1.8.

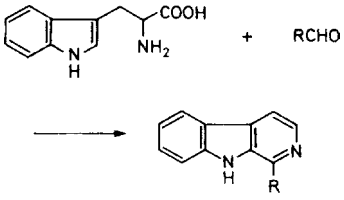
The toxicity is based on the heteroaromatic amino function. The amines are genotoxic after oxidative metabolic conversion to a strong electrophile, e. g., a nitrene. Nitrenes of this type are synthesized for model experiments as shown in Formula 1.56. According to these experiments, MeIQ, IQ and MeIQx have an especially high genotoxic potential. The compounds listed in Table 1.6 can be deaminated by nitrite in weakly acid solution and thus inactivated.

The β-carbolines norharmane (I, R=H) and harmane (I, R=CH_3) are well known as components

Table 1.6. Mutagenic compounds from pyrolysates of amino acids and proteins

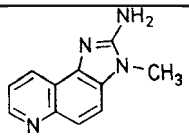
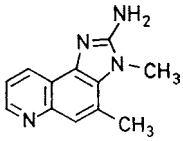
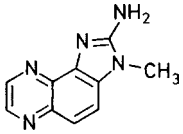
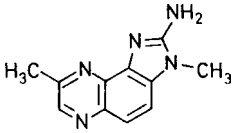
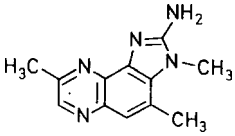
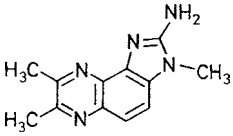
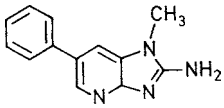
Mutagenic compound	Short form	Pyrolized compound	Structure
3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole	Trp-P-1	Tryptophan	
3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole	Trp-P-2	Tryptophan	
2-Amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole	Glu-P-1	Glutamic acid	
2-Aminodipyrido[1,2-*a*:3',2'-*d*]imidazole	Glu-P-2	Glutamic acid	
3,4-Cyclopentenopyrido[3,2-*a*]carbazole	Lys-P-1	Lysine	
4-Amino-6-methyl-1H-2,5,10,10*b*-tetraazafluoranthene	Orn-P-1	Ornithine	
2-Amino-5-phenylpyridine	Phe-P-1	Phenylalanine	
2-Amino-9H-pyrido[2,3-*b*]indole	AαC	Soya globulin	
2-Amino-3-methyl-9H-pyrido[2,3-*b*] indole	MeAαC	Soya globulin	

of tobacco smoke. They are formed by a reaction of tryptophan and formaldehyde or acetaldehyde:



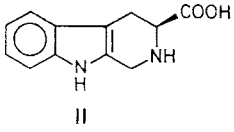
I (1.57)

Table 1.7. Mutagenic compounds from various heated foods and from model systems

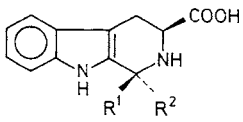
Mutagenic compound	Short form	Food Model system[a]	Structure
2-Amino-3-methylimidazo-[4,5-*f*]quinoline	IQ	1,2,3	
2-Amino-3,4-dimethylimidazo-[4,5-*f*]quinoline	MeIQ	3	
2-Amino-3-methylimidazo-[4,5-*f*]quinoxaline	IQx	2	
2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline	MeIQ2x	2,3	
2-Amino-3,4,8-trimethyl-imidazo-[4,5-*f*]quinoxaline	4,8-Di MeIQx	2,3,5,6	
2-Amino-3,7,8-trimethyl-imidazo-[4,5-*f*]quinoxaline	7,8-Di MeIQx	4	
2-Amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine	PhIP	2	

[a] 1: Meat extract; 2: Grilled meat; 3: Grilled fish; 4: Model mixture of creatinine, glycine, glucose; 5: as 4, but alanine; 6: as 4, but threonine

Tetrahydro-β-carboline-3-carboxylic acid (II) and (1S, 3S)-(III) and (1R, 3S)-methyltetrahydro-β-carboline-3-carboxylic acid (IV) were detected in beer (II: 2–11 mg/L, III + IV: 0.3–4 mg/L) and wine (II: 0.8–1.7 mg/L, III + IV: 1.3–9.1 mg/L). The ratio of diastereomers III and IV (Formula 1.58) was always near 2:1:



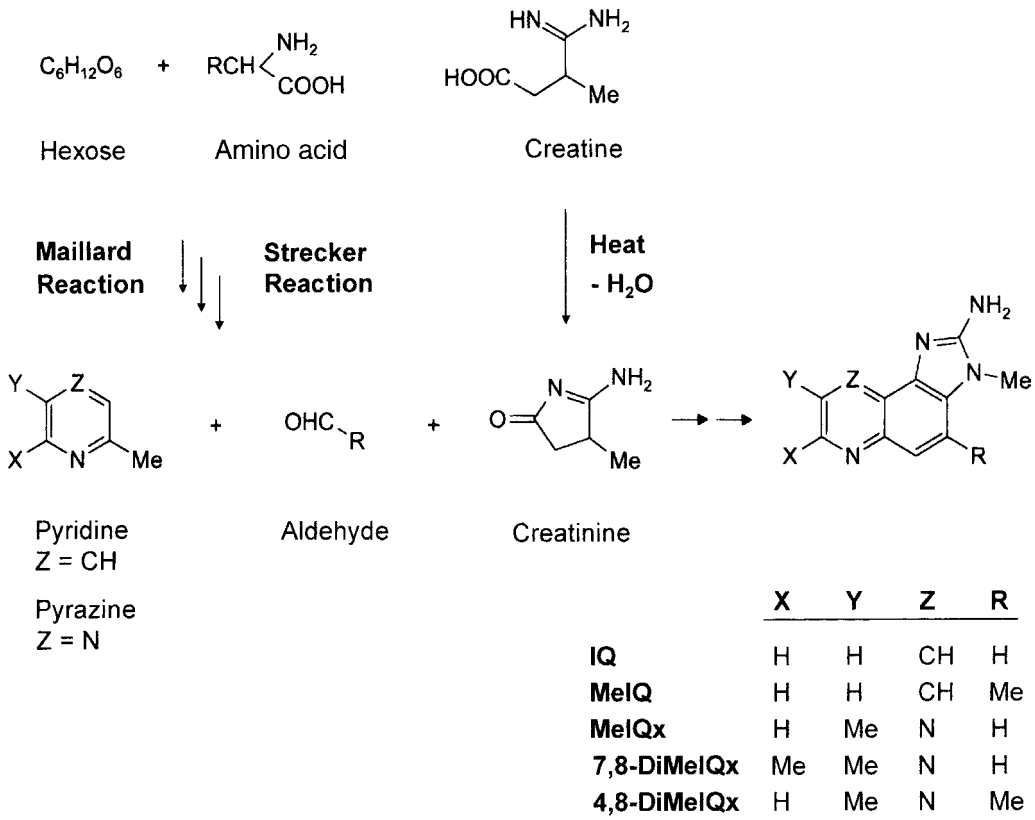
II



III $R^1 = CH_3$, $R^2 = H$
IV $R^1 = H$, $R^2 = CH_3$

(1.58)

The compounds are pharmacologically active.



	X	Y	Z	R
IQ	H	H	CH	H
MeIQ	H	H	CH	Me
MeIQx	H	Me	N	H
7,8-DiMeIQx	Me	Me	N	H
4,8-DiMeIQx	H	Me	N	Me

Fig. 1.8. Formation of heterocyclic amines by heating a model system of creatine, glucose and an amino acid mixture corresponding to the concentrations in beef (according to *Arvidsson* et al., 1997). For abbreviations, see Table 1.7

1.2.5 Synthetic Amino Acids Utilized for Increasing the Biological Value of Food (Food Fortification)

The daily requirements of humans for essential amino acids and their occurrence in some important food proteins are presented in Table 1.8. The biological value of a protein (g protein formed in the body/100 g food protein) is determined by the absolute content of essential amino acids, by the relative proportions of essential amino acids, by their ratios to nonessential amino acids and by factors such as digestibility and availability. The most important (more or less expensive) *in vivo* and *in vitro* methods for determining the biological valence are based on the following principles:

- Replacement of endogenous protein after protein depletion.
  The test determines the amount of endogenous protein that can be replaced by 100 g of food protein. The test person is given a non-protein diet and thus reduced to the absolute N minimum. Subsequently, the protein to be examined is administered, and the N balance is measured. The biological valence (BV) follows from

$$\mathrm{BV} = \frac{\text{Urea-N(non-protein diet)} + \text{N balance}}{\text{N intake}} \times 100, \qquad (1.59)$$

  "Net protein utilization" (NPU) is based on the same principle and is determined in animal experiments. A group of rats

Table 1.8. Adult requirement for essential amino acids and their occurrence in various food

Amino acid	1	2	3	4	5	6	7	8	9
Isoleucine	10–11	3.5	4.0	4.6	3.9	3.6	3.4	5.0	3.5
Leucine	11–14	4.2	5.3	7.1	4.3	5.1	6.5	8.2	5.4
Lysine	9–12	3.5	3.7	4.9	3.6	4.4	2.0	3.6	5.4
Methionine + Cystine	11–14	4.2	3.2	2.6	1.9	2.1	3.8	3.4	1.9
Methionine		2.0	1.9	1.9	1.2	0.9	1.4	2.2	0.8
Phenylalanine + Tyrosine	13–14	4.5	6.1	7.2	5.8	5.5	6.7	8.9	6.0
Phenylalanine		2.4	3.5	3.5	3.1	3.3	4.6	4.7	2.5
Threonine	6–7	2.2	2.9	3.3	2.9	2.7	2.5	3.7	3.8
Tryptophan	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Valine	11–14	4.2	4.3	5.6	3.6	3.3	3.8	6.4	4.1
Tryptophan[a]			1.7	1.4	1.4	1.5	1.1	1.0	1.3

1: Daily requirement in mg/kg body weight.
2–8: Relative value related to Trp = 1 (pattern).
2: Daily requirements, 3: eggs, 4: bovine milk, 5: potato, 6: soya, 7: wheat flour, 8: rice, and 9: *Torula*-yeast.
[a] Tryptophan (%) in raw protein.

is fed a non-protein diet (Gr 1), while the second group is fed the protein to be examined (Gr 2). After some time, the animals are killed, and their protein content is analyzed. The biological valence follows from

$$\text{NPU} = \frac{\text{Protein content Gr 2} - \text{protein content Gr 1}}{\text{Protein intake}} \times 100$$

- Utilization of protein for growth.
  The growth value (protein efficiency ratio = PER) of laboratory animals is calculated according to the following formula:

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Available protein (g)}}$$

- Maintenance of the N balance.
- Plasma concentration of amino acids.
- Calculation from the amino acid composition.
- Determination by enzymatic cleavage *in vitro*.

Table 1.9 lists data about the biological valence of some food proteins, determined according to different methods.

The highest biological value observed is for a blend of 35% egg and 65% potato proteins. The biological value of a protein is generally limited by:

- Lysine: deficient in proteins of cereals and other plants
- Methionine: deficient in proteins of bovine milk and meat

Table 1.9. Biological valence of some food proteins determined according to different methods[a]

Protein from	Biological valence			Limiting amino acid
	BV	NPU	PER	
Chicken egg	94	93	3.9	
Cow's milk	84	81	3.1	Met
Fish	76	80	3.5	Thr
Beef	74	67	2.3	Met
Potatoes	73	60	2.6	Met
Soybeans	73	61	2.3	Met
Rice	64	57	2.2	Lys, Tyr
Beans	58	38	1.5	Met
Wheat flour (white)	52	57	0.6	Lys, Thr

[a] The methods are explained in the text.

Table 1.10. Increasing the biological valence (PER[a]) of some food proteins through the addition of amino acids

Protein from	Addition(%)					
	without	0.2 Lys	0.4 Lys	0.4 Lys 0.2 Thr	0.4 Lys 0.07 Thr	0.4 Lys 0.07 Thr 0.2 Thr
Casein (Reference)	2.50					
Wheat flour	0.65	1.56	1.63	2.67		
Corn	0.85		1.08		2.50	2.59

[a] The method is explained in the text.

- Threonine: deficient in wheat and rye
- Tryptophan: deficient in casein, corn and rice.

Since food is not available in sufficient quantity or quality in many parts of the world, increasing its biological value by addition of essential amino acids is gaining in importance. Illuminating examples are rice fortification with L-lysine and L-threonine, supplementation of bread with L-lysine and fortification of soya and peanut protein with methionine. Table 1.10 lists data about the increase in biological valence of some food proteins through the addition of amino acids. Synthetic amino acids are used also for chemically defined diets which can be completely absorbed and utilized for nutritional purposes in space travel, in pre-and post-operative states, and during therapy for maldigestion and malabsorption syndromes.

The fortification of animal feed with amino acids (0.05–0.2%) is of great significance. These demands have resulted in increased production of amino acids. Table 1.11 gives data for world production in 1982. The production of L-glutamic acid, used to a great extent as a flavor enhancer, is exceptional. Production of methionine and lysine is also significant.

Four main processes are distinguished in the production of amino acids: chemical synthesis, isolation from protein hydrolysates, enzymatic and microbiological methods of production, which is currently the most important. The following sections will further elucidate the important

Table 1.11. World production of amino acids, 1982

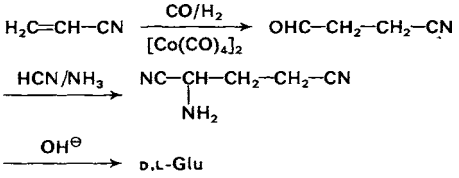
Amino acid	t/year	Process[a]				Mostly used as
		1	2	3	4	
L-Ala	130		+		+	Flavoring compound
D,L-Ala	700	+				Flavoring compound
L-Arg	500			+	+	Infusion Therapeutics
L-Asp	250		+		+	Therapeutics Flavoring compound
L-Asn	50				+	Therpeutics
L-CySH	700				+	Baking additive Antioxidant
L-Glu	270,000			+		Flavoring compound flavor enhancer
L-Gln	500			+		Therapeutics
Gly	6.000	+				Sweetener
L-His	200			+	+	Therapeutics
L-Ile	150			+	+	Infusion
L-Leu	150			+	+	Infusion
L-Lys	32,000		+	+		Feed ingredient
L-Met	150		+			Therapeutics
D,L-Met	110,000	+				Feed ingredient
L-Phe	150		+	+		Infusion
L-Pro	100			+	+	Infusion
L-Ser	50			+	+	Cosmetics
L-Thr	160			+	+	Food additive
L-Trp	200		+	+		Infusion
L-Tyr	100			+		Infusion
L-Val	150		+		+	Infusion

[a] 1: Chemical synthesis, 2: protein hydrolysis, 3: microbiological procedure, 4: isolation from raw materials.

industrial processes for a number of amino acids.

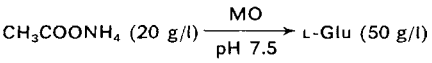
1.2.5.1 Glutamic Acid

Acrylnitrile is catalytically formylated with CO/H_2 and the resultant aldehyde is transformed through a *Strecker* reaction into glutamic acid dinitrile which yields D,L-glutamic acid after alkaline hydrolysis. Separation of the racemate is achieved by preferential crystallization of the L-form from an oversaturated solution after seeding with L-glutamic acid:



(1.60)

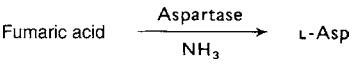
A fermentation procedure with various selected strains of microorganisms (*Brevibacterium flavum, Brev. roseum, Brev. saccharolyticum*) provides L-glutamic acid in yields of 50 g/l of fermentation liquid:



(1.61)

1.2.5.2 Aspartic Acid

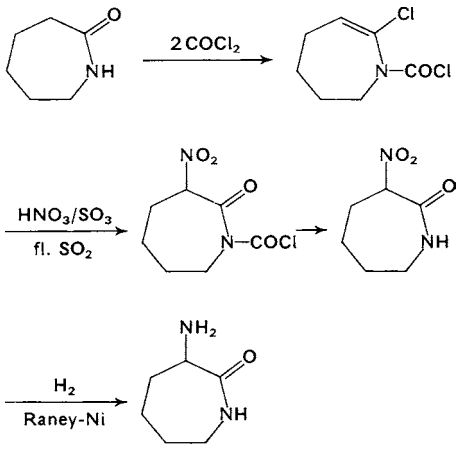
Aspartic acid is obtained in 90% yield from fumaric acid by using the aspartase enzyme:



(1.62)

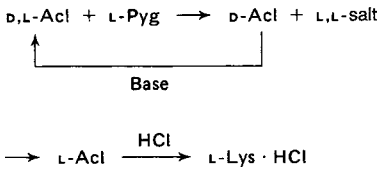
1.2.5.3 Lysine

A synthetic procedure starts with caprolactam, which possesses all the required structural features, except for the α-amino group which is introduced in several steps:



(1.63)

Separation of isomers is done at the α-amino caprolactam (Acl) step through the sparingly soluble salt of the L-component with L-pyrrolidone carboxylic acid (Pyg):

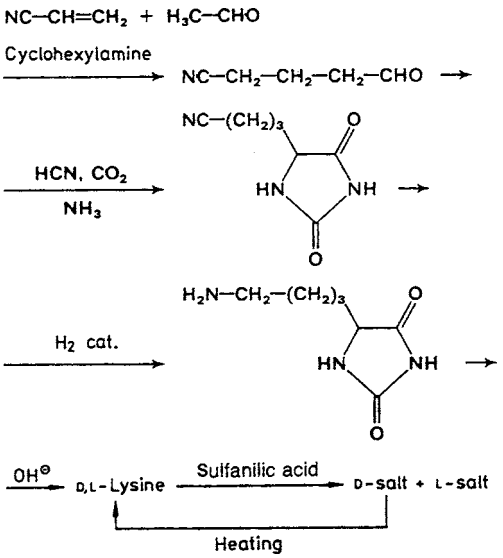


(1.64)

More elegant is selective hydrolysis of the L-enantiomer by an L-α-amino-ε-caprolactamase which occurs in several yeasts, for example in *Cryptococcus laurentii*. The racemization of the remaining D-isomers is possible with a racemase of *Achromobacter obae*. The process can be performed as a one-step reaction: the racemic aminocaprolactam is incubated with intact cells of *C. laurentii* and *A. obae*, producing almost 100% L-lysine.

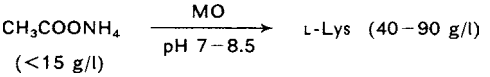
In another procedure, acrylnitrile and ethanal react to yield cyanobutyraldehyde which is then transformed by a *Bucherer* reaction into cyanopropylhydantoin. Catalytic hydrogenation of the nitrile group, followed by alkaline hydrolysis yields D,L-lysine.

The isomers can be separated through the sparingly soluble L-lysine sulfanilic acid salt:



(1.65)

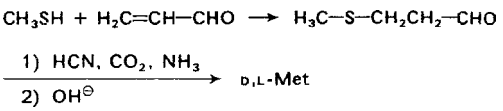
Fermentation with a pure culture of *Brevibacterium lactofermentum or Micrococcus glutamicus* produces L-lysine directly:



(1.66)

1.2.5.4 Methionine

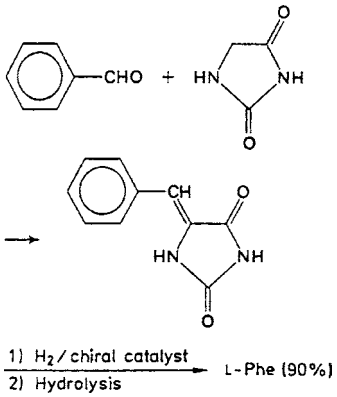
Interaction of methanethiol with acrolein produces an aldehyde which is then converted to the corresponding hydantoin through a *Bucherer* reaction. The product is hydrolyzed by alkaline catalysis. Separation of the resultant racemate is usually not carried out since the D-form of methionine is utilized by humans via transamination:



(1.67)

1.2.5.5 Phenylalanine

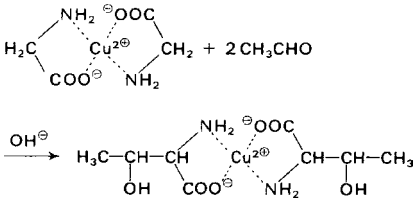
Benzaldehyde is condensed with hydantoin, then hydrogenation using a chiral catalyst gives a product which is about 90% L-phenylalanine:



(1.68)

1.2.5.6 Threonine

Interaction of a copper complex of glycine with ethanal yields the *threo* and *erythro* isomers in the ratio of 2:1. They are separated on the basis of their differences in solubility:



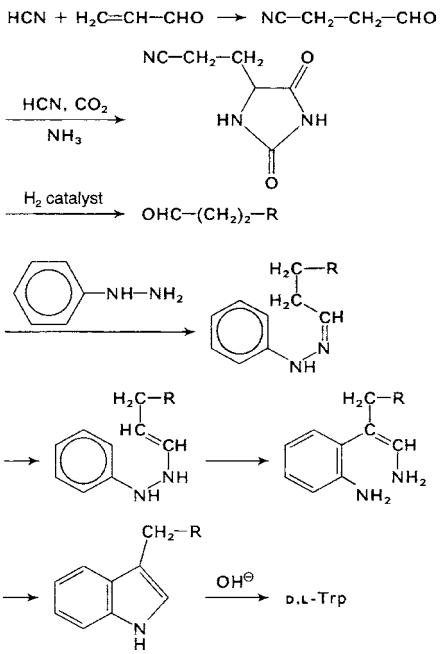
(1.69)

D,L-threonine is separated into its isomers through its N-acetylated form with the help of an acylase enzyme.
Threonine is also accessible via microbiological methods.

1.2.5.7 Tryptophan

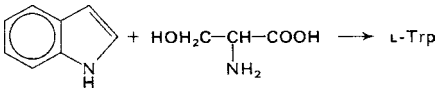
Tryptophan is obtained industrially by a variation of the *Fischer* indole synthesis. Addition of hydrogen cyanide to acrolein gives 3-cyanopropanal which is converted to hydantoin through a *Bucherer* reaction. The nitrile group is then

reduced to an aldehyde group. Reaction with phenylhydrazine produces an indole derivative. Lastly, hydantoin is saponified with alkali:



(1.70)

L-Tryptophan is also produced through enzymatic synthesis from indole and serine with the help of tryptophan synthase:



(1.71)

1.2.6 Sensory Properties

Free amino acids can contribute to the flavor of protein-rich foods in which hydrolytic processes occur (e. g. meat, fish or cheese).

Table 1.12 provides data on taste quality and taste intensity of amino acids. Taste quality is influenced by the molecular configuration: sweet amino acids are primarily found among members of the D-series, whereas bitter amino acids are generally within the L-series. Consequently amino acids with a cyclic side chain (1-aminocycloalkane-1-carboxylic acids) are sweet and bitter.

The taste intensity of a compound is reflected in its recognition threshold value. The recognition threshold value is the lowest concentration needed to recognize the compound reliably, as assessed by a taste panel. Table 1.12 shows that the taste intensity of amino acids is dependent on the hydrophobicity of the side chain.

L-Tryptophan and L-tyrosine are the most bitter amino acids with a threshold value of $c_{t\,bitter} = 4-6\,mmol/l$. D-Tryptophan, with $c_{t\,sweet} = 0.2-0.4\,mmol/l$, is the sweetest amino acid. A comparison of these threshold values with those of caffeine ($c_{t\,bi} = 1-1.2\,mmole/l$) and sucrose ($c_{t\,sw} = 10-12\,mmol/l$) shows that caffeine is about 5 times as bitter as L-tryptophan and that D-tryptophan is about 37 times as sweet as sucrose.

L-Glutamic acid has an exceptional position. In higher concentrations it has a meat broth flavor, while in lower concentrations it enhances the characteristic flavor of a given food (flavor enhancer, cf. 8.6.1). L-Methionine has a sulfur-like flavor.

The bitter taste of the L-amino acids can interfere with the utilization of these acids, e. g., in chemically defined diets.