AMINO ACIDS

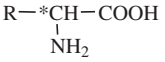
1. Introduction

Amino acids are the main components of proteins. Approximately twenty amino acids are common constituents of proteins (1) and are called protein amino acids, or primary protein amino acids because they are found in proteins as they emerge from the ribosome in the translation process of protein synthesis (2), or natural amino acids. In 1820 the simplest amino acid, glycine, was isolated from gelatin (3); the most recently isolated, of nutritional importance, is L-threonine which was found (4) in 1935 to be a growth factor of rats. The history of the discoveries of the amino acids has been reviewed (5,6).

Hydroxylated amino acids (eg, 4-hydroxyproline, 5-hydroxylysine) and *N*-methylated amino acids (eg, *N*-methylhistidine) are obtained by the acid hydrolysis of proteins. γ-Carboxyglutamic acid occurs as a component of some sections of protein molecules; it decarboxylates spontaneously to L-glutamate at low pH. These examples are formed upon the nontranslational modification of protein and are often called secondary protein amino acids (1,6).

The presence of many nonprotein amino acids has been reported in various living metabolites, such as in antibiotics, some other microbial products, and in nonproteinaceous substances of animals and plants (7). Plant amino acids (8) and seleno amino acids (9) have been reviewed.

The general formula of an α-amino acid may be written:



The asterisk signifies an asymmetric carbon. All of the amino acids, except glycine, have two optically active isomers designated D- or L-. Isoleucine and threonine also have centers of asymmetry at their β-carbon atoms (1,10). Protein amino acids are of the L-α-form (1,10) as illustrated in Table 1.

Amino acids are important components of the elementary nutrients of living organisms. For humans, ten amino acids are essential for existence and must be ingested in food. The nutritional value of proteins is governed by the quantitative and qualitative balance of individual essential amino acids.

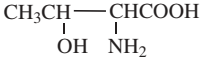
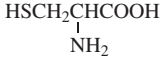
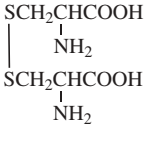
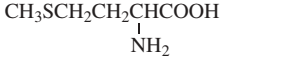
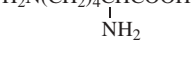
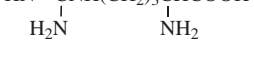
The nutritional value of a protein can be improved by the addition of amino acids of low abundance in that protein. Thus the fortification of plant proteins such as wheat, corn, and soybean with L-lysine, DL-methionine, or other essential amino acids (L-tryptophan and L-threonine) is expected to alleviate some food problems (11). Such fortification has been widespread in the feedstuff of domestic animals.

Proteins are metabolized continuously by all living organisms, and are in dynamic equilibrium in living cells (6,12). The role of amino acids in protein biosynthesis has been described (2). Most of the amino acids absorbed through the digestion of proteins are used to replace body proteins. The remaining portion is metabolized into various bioactive substances such as hormones and purine and pyrimidine nucleotides, (the precursors of DNA and RNA) or is consumed as an energy source (6,13).

Table 1. **α-Am89ino Acids**

Common name	CAS Registry Number	Abbreviation	Systematic name	Formula	Molecular weight
			Monocarboxylic		
Aliphatic					
glycine	[56-40-6]	Gly	aminoacetic acid	H_2NCH_2COOH	75.07
alanine	[6898-94-8]	Ala	2-amino-propanoic acid	$CH_3CH(NH_2)COOH$	89.09
L-alanine	[56-41-7]				
D-alanine	[338-69-2]				
DL-alanine	[302-72-7]				
valine[a]	[7004-03-7]	Val	2-amino-3-methyl-butanoic acid	$(CH_3)_2CHCH(NH_2)COOH$	117.15
L-valine	[72-18-4]				
D-valine	[640-68-6]				
DL-valine	[516-06-3]				
leucine[a]	[7005-03-0]	Leu	2-amino-4-methyl-pentanoic acid	$(CH_3)_2CHCH_2CH(NH_2)COOH$	131.17
L-leucine	[61-90-5]				
D-leucine	[328-38-1]				
DL-leucine	[328-39-2]				
isoleucine[a]	[7004-09-3]	Ileu	2-amino-3-methyl-pentanoic acid	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$	131.17
L-isoleucine	[73-32-5]				
D-isoleucine	[319-78-8]				
DL-isoleucine	[443-79-8]				
Aliphatic containing —OH, —S—, —NH— group					
serine	[6898-95-9]	Ser	2-amino-3-hydroxy-propanoic acid	$HOCH_2CH(NH_2)COOH$	105.09
L-serine	[56-45-1]				
D-serine	[312-84-5]				
DL-serine	[302-84-1]				

Table 1 (*Continued*)

Common name	CAS Registry Number	Abbreviation	Systematic name	Formula	Molecular weight
threonine[a]	[36676-50-3]	Thr	2-amino-3-hydroxy-butanoic acid		119.12
L-threonine	[72-19-5]				
D-threonine	[632-20-2]				
DL-threonine	[80-68-2]				
cysteine	[4371-52-2]	Cys	2-amino-3-mercapto-propanoic acid		121.16
L-cysteine	[52-90-4]				
D-cysteine	[921-01-7]				
DL-cysteine	[3374-22-9]				
cystine	[24645-67-8]	$(Cys)_2$	3,3′-dithio-bis-(2-amino-propanoic acid)		240.30
L-cystine	[56-893]				
D-cystine	[349-46-2]				
DL-cystine	[923-32-0]				
methionine[a]	[7005-18-7]	Met	2-amino-4-methyl-thio-butanoic acid		149.21
L-methionine	[63-68-3]				
D-methionine	[348-67-4]				
DL-methionine	[59-51-8]				
lysine[a]	[6899-06-5]	Lys	2,6-diamino-hexanoic acid		146.19
L-lysine	[56-87-1]				
D-lysine	[923-27-3]				
DL-lysine	[70-54-2]				
arginine[b]	[7004-12-8]	Arg	2-amino-5-guani-dopen-tanoic acid		174.20

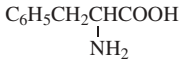
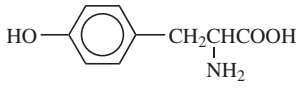
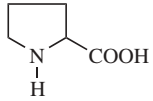
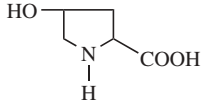
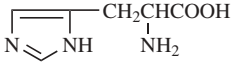
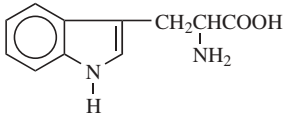
L-arginine	[74-79-3]				
D-arginine	[157-06-2]				
DL-arginine	[7200-25-1]				
Aromatic					
phenylalanine[a]	[3617-44-5]	Phe	2-amino-3-phenyl-propanoic acid		165.19
L-phenylalanine	[63-91-2]				
D-phenylalanine	[673-06-3]				
DL-phenylalanine	[150-30-1]				
tyrosine	[55520-40-6]	Tyr	2-amino-3-(4-hydroxy-phenyl)-propanoic acid		181.19
L-tyrosine	[60-18-4]				
D-tyrosine	[556-02-5]				
DL-tyrosine	[556-03-6]				
Heterocyclic					
proline	[7005-20-1]	Pro	2-pyrrolidine-carboxylic acid		115.13
L-proline	[147-85-3]				
D-proline	[344-25-2]				
DL-proline	[609-36-9]				
hydroxyproline	(cis)	Hypro	4-hydroxy-2-pyrrolidine-carboxylic acid		131.13
L-hydroxyproline	[618-27-9]				
D-hydroxyproline	[2584-71-6]				
DL-hydroxyproline	[49761-17-3]				
hydroxyproline	(trans)				
L-hydroxyproline (trans)	[51-35-4]				
D-hydroxyproline (trans)	[3398-22-9]				
DL-hydroxyproline (trans)	[618-28-0]				
histidine[b]	[7006-35-1]	His	2-amino-3-imidazole-propanoic acid		155.16
L-histidine	[71-00-1]				
D-histidine	[351-50-8]				

Table 1 (*Continued*)

Common name	CAS Registry Number	Abbreviation	Systematic name	Formula	Molecular weight
DL-histidine	[4998-57-6]				
tryptophan[a]	[6912-86-3]	Trp	2-amino-3-indoyl-propanoic acid		204.22
L-tryptophan	[73-22-3]				
D-tryptophan	[153-94-6]				
DL-tryptophan	[54-12-6]				
			Dicarboxylic		
aspartic acid	[6899-03-2]	Asp	2-amino-butane-dioic acid	$HOOCCH_2CHCOOH$ NH_2	133.10
L-aspartic acid	[56-84-8]				
D-aspartic acid	[1783-96-6]				
DL-aspartic acid	[617-45-8]				
glutamic acid	[6899-05-4]	Glu	2-amino-pentane-dioic acid	$HOOCCH_2CH_2CHCOOH$ NH_2	147.13
L-glutamic acid	[56-86-0]				
D-glutamic acid	[6893-26-1]				
DL-glutamic acid	[617-65-2]				
asparagine	[7006-34-0]	Asn	2-amino-3-carbamoyl-propanoic acid	$H_2NCOCH_2CHCOOH$ NH_2	132.12
L-asparagine	[70-47-3]				
D-asparagine	[2058-58-4]				
DL-asparagine	[3130-87-8]				
glutamine	[6899-04-3]	Gln	2-amino-4-carbamoyl-butanoic acid	$H_2NCOCH_2CH_2CHCOOH$ NH_2	146.15
L-glutamine	[56-85-9]				
D-glutamine	[5959-95-5]				
DL-glutamine	[585-21-7]				

[a] Essential amino acid.
[b] Arginine and histidine are also essential for children (6).

The history of the discovery of amino acids is closely related to advances in analytical methods. Initially, quantitative and qualitative analysis depended exclusively upon crystallization from protein hydrolysates. The quantitative precipitation of several basic amino acids including phosphotungstates, the separation of amino acid esters by vacuum distillation, and precipitation by sulfonic acid derivatives were developed successively during the last century.

After World War II, analytical methods for amino acids were improved and new methods were introduced. The first was microbial assay using lactic acid bacteria which require all of the regular amino acids for growth. Manometric determination (by use of a Warburg manometer) of CO_2 liberated by the action of amino acid decarboxylase is now also classical. However, these methods are still used for the microdetermination of amino acids. Later, chromatographic separations using filter paper, ion-exchange resins, and other adsorbants were rapidly developed. Automatic analyzers of amino acids using ion-exchange resin chromatography (14) are now used widely in routine analyses of amino acid mixtures. More recently, high performance liquid chromatography (hplc) has been extensively developed and the separate determination of L- and D-amino acids has been possible by this method. Amino acid sensors have been studied (15,16). The contribution of various biosynthetic pathways for amino acids has been analyzed by ^{13}C-nmr with glucose, labeled with carbon-13 at C-1 or C-6, as substrate (17).

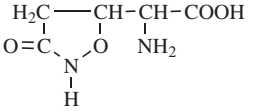
The determination of amino acids in proteins requires pretreatment by either acid or alkaline hydrolysis. However, L-tryptophan is decomposed by acid, and the racemization of several amino acids takes place during alkaline hydrolysis. Moreover, it is very difficult to confirm the presence of cysteine in either case. The use of methanesulfonic acid (18) and mercaptoethanesulfonic acid (19) as the protein hydrolyzing reagent to prevent decomposition of L-tryptophan and L-cysteine is recommended. Enzymatic hydrolysis of proteins has been studied (20).

In 1950 all L-amino acids were manufactured by isolation from protein hydrolyzates or by separation of L-amino acids from the synthesized racemic mixtures. Since the mid-1950s, methods of production of L-amino acids have changed extensively. The first important change was made by the invention of a new fermentation process using so-called glutamic acid bacteria (eg, *Corynebacterium glutamicum*, *Brevibacterium flavum*) to produce L-glutamic acid (21). Thereafter, fermentation processes were developed to produce many other amino acids. Most amino acids (except for glycine, L-methionine, L-cysteine, and L-serine) are now economically produced by fermentation (22,23). Subsequently, enzymatic processes were developed to produce L-aspartic acid, L-alanine, L-tryptophan, L-cysteine, L-serine, L-lysine, L-phenylalanine, and some D-amino acids from chemically synthesized substrates (24). Glycine, DL-alanine, DL-methionine, and DL-cysteine, and some other amino acids are still produced by chemical synthesis. Chemical manufacturing procedures for amino acids have been discussed (25).

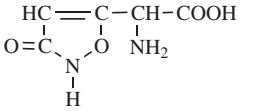
All of the protein amino acids are currently available commercially and their uses are growing. Amino acids and their analogues have their own characteristic effects in flavoring, nutrition, and pharmacology.

In the food industries a number of amino acids have been widely used as flavor enhancers and flavor modifiers (see FLAVORS AND SPICES). For example,

monosodium L-glutamate is well-known as a meat flavor-enhancer and an enormous quantity of it is now used in various food applications (see L-MONOSODIUM GLUTAMATE (MSG)). Protein, hydrolyzed by acid or enzyme to be palatable, has been used for a long time in flavoring agents. The addition of L-glutamate, L-aspartate, glycine, DL-alanine, and other palatable amino acids can improve flavoring by these protein hydrolyzates. In addition, some nucleotides, such as 5′-inosinic acid [131-99-7] and 5′-guanylic acid [85-32-5], have a synergistic effect on the meat flavor enhancing effects of L-glutamate and L-aspartate. Tricholomic acid [2644-49-7] (**1**) and ibotenic acid [2552-55-8] (**2**), nonprotein amino acids found in mushrooms, have 4 to 25 times stronger umami taste than L-glutamic acid (26). However, they have not been used in food.



(**1**)



(**2**)

Some peptides have special tastes. L-Aspartyl phenylalanine methyl ester is very sweet and is used as an artificial sweetener (see SWEETENERS). In contrast, some oligopeptides (such as L-ornithinyltaurine·HCl and L-ornithinyl-β-alanine·HCl), and glycine methyl or ethyl ester·HCl have been found to have a very salty taste (27).

Amino acids are also used in medicine. Amino acid infusions prepared from crystalline amino acids are used as nutritional supplements for patients before and after surgery. Some amino acids and their analogues are used for treatment of major diseases. L-DOPA, L-3-(3,4-dihydroxyphenyl)alanine, [59-92-7] is an important drug in the treatment of Parkinson's disease, and L-glutamine and its derivatives are used for treatment of stomach ulcers. α-Methyl-DOPA [555-30-6] is an effective antidepressant (see PSYCHOPHARMACOLOGICAL AGENTS). Some peptides, eg, oxytocin [50-56-6], angiotensin [1407-47-2], gastrin, and cerulein, have hormonal effects which have medical utility. The physiological effect of glutathione [70-18-8] (L-glutamyl-L-cysteinyl glycine) has been reviewed (28).

Amino acid polymers like poly(γ-methyl-L-glutamate) [29967-97-3] have been developed as raw materials for artificial leathers (see LEATHERLIKE MATERIALS). Derivatives of amino acids are now finding new applications in industry and agriculture.

2. Physical Properties

2.1. Melting Point. Amino acids are solids, even the lower carbon-number amino acids such as glycine and alanine. The melting points of amino acids generally lie between 200 and 300°C. Frequently amino acids decompose before reaching their melting points (Table 2).

2.2. Crystalline Structures. Crystal shape of amino acids varies widely, for example, monoclinic prisms in glycine and orthorhombic needles in L-alanine. X-ray crystallographic analyses of 23 amino acids have been described

Table 2. **Physical Constants of Amino Acids**[a]

Amino acid		Melting point, °C	Density, d_{t1}^{t2}	Specific rotation			
				$[\alpha]_D$	t, °C	c, %	Solvent
Ala	L-	297 (dec)	1.401	+2.8	25	6	H_2O
		314 (dec)	1.432^{23}	+2.8	25	6	H_2O
	L-·HCl	204 (dec)		+8.5	26	9.3	
	D-	314 (dec)		−13.6	25	1	6 *N* HCl
	DL-	264 (dec)	1.424				
		295 (dec)	1.424				
Arg	L-	244 (dec)		+12.5	20	3.5	H_2O
	L-·HCl	235 (dec)		+12.0	20	4	
	DL-	217–218					
Asn	L-·H_2O	234–235	$1.543^{15}{}_4$	−5.42	20	1.3	
	D-·H_2O	215		+5.41	20	1.3	
		234.5	$1.543^{15}{}_4$	+5.41	20	1.3	
	DL-·H_2O	182–183	$1.4540^{15}{}_4$				
Asp	L-	270–271	$1.661^{12.5}{}_4$	+25.0	20	1.97	6 *N* HCl
		324 (dec)	$1.6613^{13}{}_{13}$	+24.6	24	2	6 *N* HCl
	D-			−23.0	27	2.30	6 *N* HCl
		269–271	$1.6613^{13}{}_{13}$	−25.5	20		HCl
	DL-	338–339	$1.6632^{13}{}_{13}$				
Cys	L-			+6.5	25		5 *N* HCl
		240 (dec)		+9.8	30	1.3	H_2O
	L-·HCl	175–178		+5.0	25		5 *N* HCl
$(Cys)_2$	L-	260–261 (dec)	1.677	−223.4	20	1	1 *N* HCl
	D-			+223	20		1 *N* HCl
		247–249		+224	20	1	1 *N* HCl
	DL-	260					
Glu	L-	247–249 (dec)	$1.538^{20}{}_4$	+31.4	22.4		6 *N* HCl
		224–225 (dec)	$1.538^{20}{}_4$	+31.4	22	1	6 *N* HCl
	L-·HCl	214 (dec)		+24.4	22	6	
	D-			−30.5	20	1.0	6 *N* HCl
		213 (dec)	$1.538^{20}{}_4$	−31.7	25		1.7 *N* HCl
	DL-	225–227 (dec)	1.4601^{20}				

Table 2 (*Continued*)

Amino acid		Melting point, °C	Density, d_{t1}^{t2}	Specific rotation			
				$[\alpha]_D$	t, °C	c, %	Solvent
		199 (dec)	$1.4601^{20}{}_4$				
Gly		233 (dec)	1.1607				
		262 (dec)	0.828^{17}				
His	L-	287 (dec)		−39.74	20	1.13	
		287 (dec)		−39.7	20	1.13	H_2O
	L-·HCl·H_2O	259 (dec)		+8.0	26	2	3 *N* HCl
	D-	287 (dec)		+40.2	20		H_2O
	DL-	285 (dec)					
Ileu	L-	284 (dec)		+11.29	20	3	
				+40.61	20	4.6	6.1 *N* HCl
		285–286 (dec)		+12.2	25	3.2	H_2O
				+36.7		4	1 *N* HCl
	D-	283–284 (dec)		−12.2	20	3.2	H_2O
				−40.7		1	5 *N* HCl
	DL-	280 (dec)					
Leu	L-	293–295 (dec)		−10.8	25	2.2	
		293–295	$1.293^{18}{}_4$	−10.42	25	22	H_2O
	D-	293		+10.34	20		
	DL-	332 (dec)					
		293–295	$1.293^{18}{}_4$				
Lys	L-	224.5 (dec)		+25.9	23	2	6 *N* HCl
		224–245 (dec)		+14.6	20	6	H_2O
	L-·HCl	263–264		+14.6	25	2	0.6 *N* HCl
	L-·2HCl	193		+15.3	20	2	
		201–202		+15.29	20		H_2O
	DL-·HCl	260–263					
	DL-·2 HCl	187–189					
Met	L-	280–282 (dec)		−8.2	25		
		283 (dec)		−8.2	25	1	H_2O
	DL-	281 (dec)	1.340				
Phe	L-	283 (dec)		−35.1	20	1.94	
	D-	285 (dec)		+35.0	20	2.04	

	DL-	271–273 (dec)					
Pro	L-	220–222 (dec)		−52.6	20	0.58	0.5 *N* HCl
		220–222 (dec)		−80.9	20	1	H_2O
	DL-	205 (dec)					
Hyp	L-	274		−76.5		2.5	H_2O
Ser	L-	228 (dec)		−6.83	20	10	H_2O
	D-	228 (dec)		+6.87	20	10	H_2O
	DL-	246 (dec)	1.537				
		246 (dec)	$1.603^{22.5}$				
Thr	L-	255–257 (dec)		−28.3	26	1.1	
	DL-	229–230 (dec)					
Trp	L-	289 (dec)		−31.5	23	1	
				+2.4	20		0.5 *N* HCl
				+0.15	20	2.43	0.5 *N* NaOH
		290–292 (dec)		−31.5	20	0.5	H_2O
				+6.1	20	11	1 *N* NaOH
	D-	281–282		+33	20		H_2O
	DL-	282					
Tyr	L-	342–344 (dec)	1.456	−10.6	22	4	1 *N* HCl
				−13.2	18	4	3 *N* NaOH
	D-	310–314 (dec)		+10.3	25	4	1 *N* HCl
	DL-	316 (dec)					
		340 (dec)					
Val	L-	315	1.230	+22.9	23	0.8	20% HCl
		93–96(?)	1.230	+22.9	23	0.8	20% alc
	D-	156–157.5		−29.4	20		20% alc
	DL-	298 (dec)	1.310				

[a]From refs. 29 and 30.

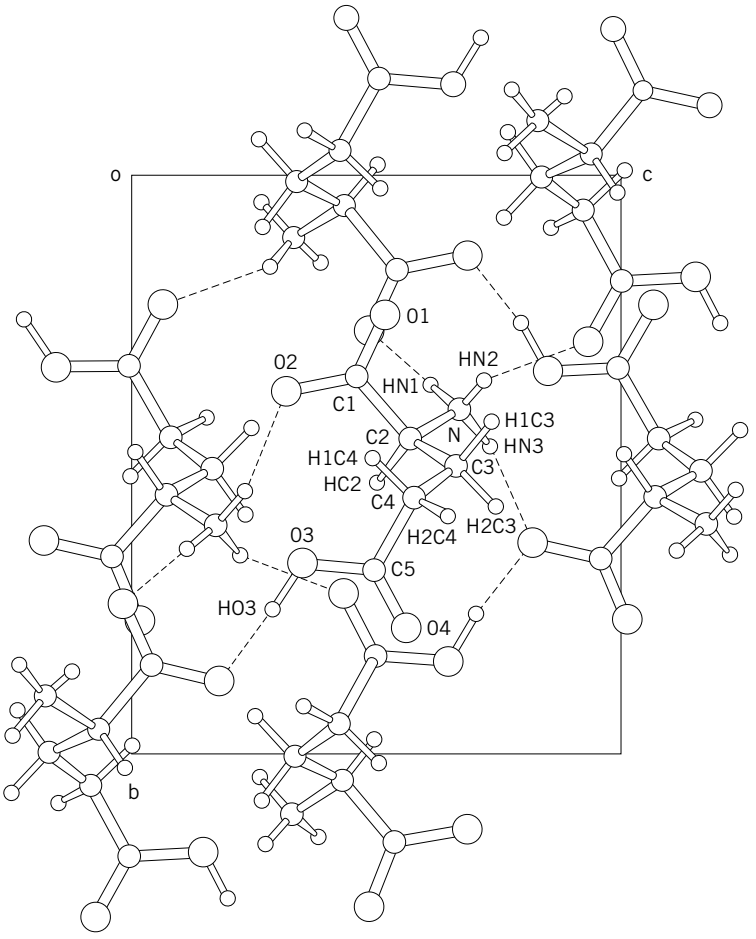


Fig. 1. A crystal structure of L-glutamic acid (α-form).

(31). L-Glutamic acid crystallizes in two polymorphic forms (α and β) (32), and the α-form is more facilely handled in industrial processes. The crystal structure has been determined (33) and is shown in Figure 1.

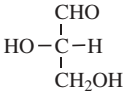
2.3. Dipole. Every amino acid molecule has two equal electric charges of opposite sign caused by the amino and carboxyl groups on the α-carbon atom.

$$\mathrm{R{-}\underset{\displaystyle \underset{\displaystyle NH_3^+}{|}}{CH}{-}COO^-}$$

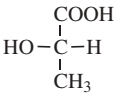
The dielectric constants of amino acid solutions are very high. Their ionic dipolar structures confer special vibrational spectra (Raman, ir), as well as characteristic properties (specific volumes, specific heats, electrostriction) (34).

2.4. Optical Configuration. With the exception of glycine, all α-amino acids contain at least one asymmetric carbon atom and may be characterized

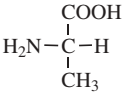
by their ability to rotate light to the right (+) or to the left (−), depending on the solvent and the degree of ionization. Specific rotations are given in Table 2. They are also characterized by the stereochemical configuration of the asymmetric carbon based on the configuration of glyceraldehyde; D,L-notation is popular for amino acids, but *R,S*-notation is a more precise designation of chirality.



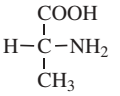
L-glyceraldehyde;
(*S*)-2,3-dihydroxypropanal



L-lactic acid;
(*S*)-2-hydroxypropanoic acid



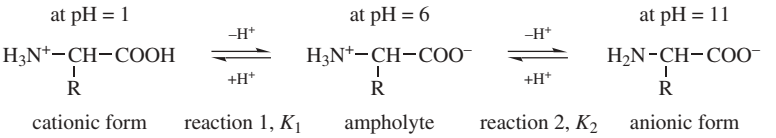
L-alanine;
(*S*)-2-aminopropanoic acid



L-alanine;
(*R*)-2-aminopropanoic acid

2.5. Solubility. Solubility data of amino acids are given in Table 3. In all instances there are at least two polar groups, acting synergistically on the solubility in water. The solubility of amino acids having additional polar groups, eg, −OH, −SH, −COOH, $-NH_2$, is even more enhanced.

2.6. Dissociation. In aqueous solution, amino acids undergo a pH-dependent dissociation (37):



where

$$K_1 = \frac{[H^+]\,[H_3N^+CH(R)COO^-]}{[H_3N^+CH(R)COOH]} \qquad K_2 = \frac{[H^+]\,[H_2NCH(R)COO^-]}{[H_3N^+CH(R)COO^-]}$$

These are the definitions of the two characteristic dissociation constants normally expressed in terms of pK. When three dissociating groups are present in a molecule there are three pK values, ie, pK_1, pK_2, pK_3. A knowledge of these pK values is important in the separation or isolation of each amino acid by ion-exchange chromatography.

A large part of the dissolved amino acid exists as the ampholyte (zwitterion). The isoelectric point (pI) is the pH at which the net electric charge of a dissolved amino acid molecule is zero. pI is expressed as

$$pI = \frac{pK_1 + pK_2}{2}$$

Table 3. **p*K* and p*I* at 25 °C and Solubility of Amino Acids**[a]

Amino acid		pK_1 (COOH)	pK_2 (NH_3^+)[b]	pK_3 (NH_3^+)	pI	Solubility in water, g/L				
						0°C	25°C	50°C	75°C	100°C
					Divalent acids					
Gly		2.34	9.60		5.97		250	391	544	672
Ala	L-	2.34	9.69[c]		6.00	127.3	166.5	217.9	285.1	373.0
	DL-	2.35	9.87			121	167	231	319	440
Val	L-	2.32	9.62[c]		5.96	83.4	88.5	96.2	102.4 (65°C)	
	DL-	2.29	9.72			59.8	70.9	91.1	126.1	188.1
Leu	L-				5.98	22.7	24.26	28.87	38.23	56.38
	DL-	2.36	9.60			7.97	9.91	14.06	22.76	42.06
Ileu	L-	2.26	9.62[c]		5.94	37.9	41.2	48.2	60.8	82.6
	DL-	2.32	9.76			18.3	22.3	30.3	46.1	78.0
Ser	L-	2.21	9.15[c]		5.68			soluble		
	DL-	2.21	9.15			22.04	50.23	103	192	322
Thr	L-	2.15	9.12		5.64			freely soluble		
Pro	L-	1.99	10.60		6.30	1272	1623	2067	2509 (70°C)	
Hyp	L-	1.82	9.65		5.74	288.6	361.1	451.8	516.7 (65°C)	
Phe	L-	1.83	9.13[c]		5.48	19.8	29.6	44.3	66.2	99.0
	DL-	2.58	9.24			9.97	14.11	21.87	37.08	68.9
Trp	L-	2.38	9.39		5.89	0.23	11.4	17.1	27.95	49.9
Met	DL-	2.28	9.21		5.74	18.18	33.81	60.70	105.2	176.0
					Trivalent acids					
Asp	L-	1.88	3.65 (COOH)	9.60	2.77	2.1	5.0	12.0	28.8	68.9
Glu	L-	2.19	4.25 (COOH)	9.67	3.22		8.64	21.86	55.32	140.0
	DL-						20.54	49.34	118.6	284.9
Tyr	L-	2.20	9.11	10.07 (OH)	5.66	0.196	0.453	1.052	2.438	5.65
	DL-					0.147	0.351	0.836		
Cys	L-	1.71	8.33	10.78				freely soluble		
His	L-	1.78	5.97	8.97	7.47		41.9			
Arg	L-	2.18	9.09	13.2	11.15		the satd aq soln contains 15% (w/w) at 21°C			
Lys	L-	2.20	8.90	10.28 (38°C)	9.59			freely soluble		
					Tetravalent acids					
$(Cys)_2$	L-	<1	2.1 (COOH)	8.02[d]	5.03	0.05	0.112	0.239	0.523	1.142

[a] Refs. 29 and 35.
[b] Unless indicated (COOH).
[c] Ref. 36.
[d] pK_4 (NH_3^+) = 8.71.

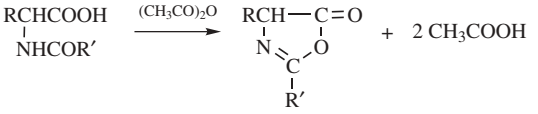
The solubility of each amino acid is minimal at its p*I*. p*K* and p*I* values are given in Table 3.

3. Chemical Properties

α-Amino acids are ampholytic compounds. The chemical reactions of amino acids can be classified according to their carboxyl, amino, and side-chain groups. Most of the reactions have been well known for a long time; the details of these reactions have been reviewed (38).

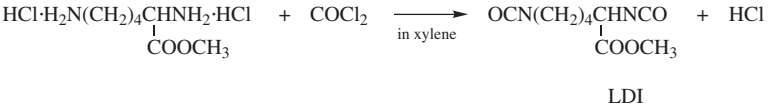
3.1. Reactions of the Amino Group

N-Acylation. *N*-Acylation and related reactions are brought about in straightforward ways with acyl chloride or acid anhydride, although the proximity of the carboxyl group may produce other reactions, eg, oxazolinone formation, under some conditions.



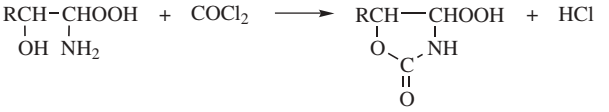
In these cases, it is better to protect the carboxyl group. Optimized conditions for *N*-acetylation have been studied (39). *N*-Acylation can be utilized for protecting the amino group in the reaction of amino acids, for example in peptide synthesis.

Reaction with Phosgene. This reaction of amino acid esters is used for preparing the corresponding isocyanates, especially lysine diisocyanate [4460-02-0] (LDI). LDI is a valuable nonyellowing isocyanate with a functional side group for incorporation in polyurethanes.

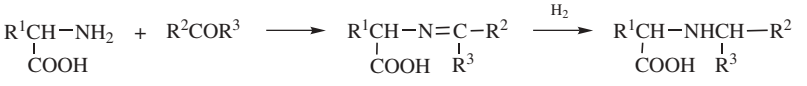


LDI

In the case of β-hydroxy-α-amino acids, oxazolidinone derivatives are formed with retention of configuration.



Formation of Schiff-Bases. Reaction of an amino acid and an aldehyde or ketone gives a Schiff-base in neutral or alkaline solution, and following reduction gives the corresponding *N*-alkylamino acid.



Schiff-base

Maillard Reaction (Nonenzymatic Glycation). Browned reaction products are formed by heating amino acid and simple sugar. This reaction is important in food science relating to coloring, taste, and flavor enhancement (40), and is illustrated as follows:

$$\text{sugar} + \text{amino acid (or protein)} \longrightarrow \underset{(\text{aldimine})}{\text{Schiff base}} \longrightarrow \underset{(\text{ketoamine})}{\text{Amadori compound}} \longrightarrow \text{browned material}$$

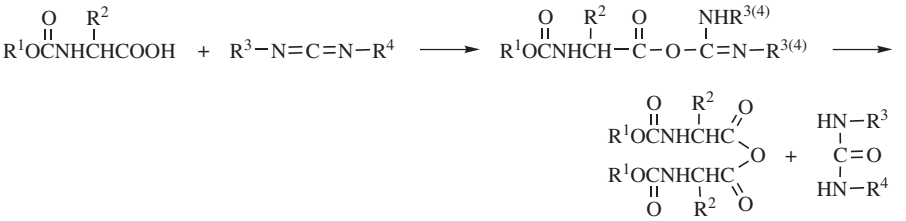
Substitution Reactions. Reaction with nitrous acid in dilute aqueous solutions yields the corresponding hydroxy acid or in solution containing a hydrohalic acid, the corresponding α-halo acid, with inversion in many cases.

3.2. Reactions of the Carboxyl Group

Esterification, Amidation, and Acid Chloride Formation. Amino acids undergo these common reactions of the carboxyl group with due regard for the need for *N*-protection.

Reduction to Amino Alcohols. Reduction can be brought about using diborane–dimethyl sulfide in THF (41). $NaBH_4$ in ethanol is also effective, but requires that the carboxyl group be esterified first (42). $LiAlH_4$ is inferior in terms of yield and practical convenience to $LiAlH_2(OCH_3)_2$ for the reduction of amino acid esters (43). Solvent effects have been reported in the case of $LiAlH_4$ (44).

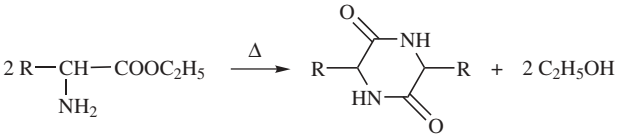
Anhydride Formation. The carboxyl group in *N*-protected amino acids is converted into the symmetrical anhydride on treatment with the carbodiimide (45).



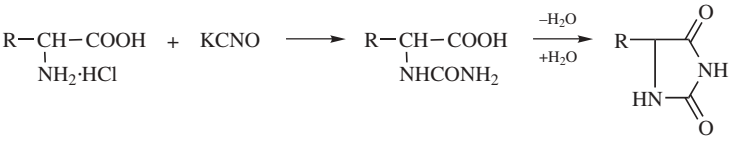
Cyclic anhydrides are formed readily from *N*-protected aspartic and glutamic acids.

3.3. Reactions Depending on Both Amino and Carboxyl Groups.

Formation of Diketopiperazines. Esters of α-amino acids can be readily prepared by refluxing anhydrous alcoholic suspensions of α-amino acids saturated with dry HCl. Diketopiperazines are formed by heating the alcoholic solution of the α-amino acid ester.



Formation of Hydantoin



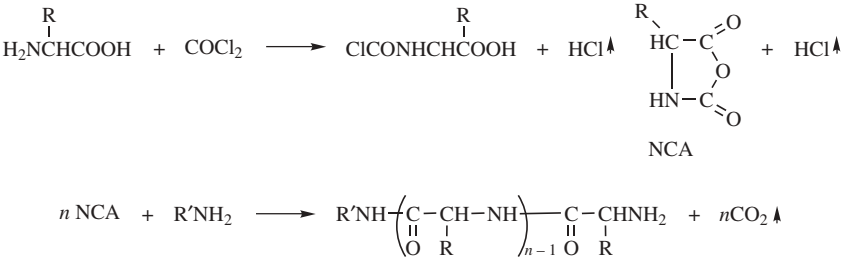
The use of an organic isocyanate instead of potassium isocyanate gives a *N*-substituted hydantoin.

Strecker Degradation (Oxidative Deamination). Mild oxidizing agents such as aqueous sodium hypochlorite or aqueous *N*-bromosuccinimide, cause decarboxylation and concurrent deamination of amino acids to give aldehydes.

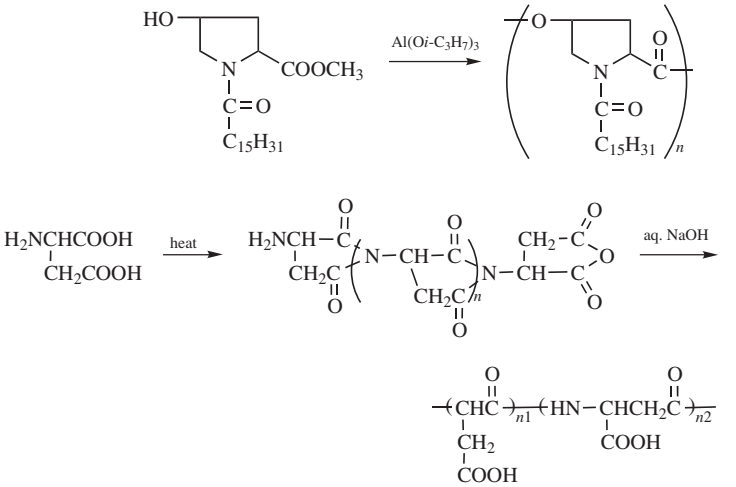
$$H_3N^+CHRCO_2^- \xrightarrow{[O]} H_2N^+ = CR + CO_2 + H_2O \xrightarrow{H_2O} RCHO + NH_3$$

Similarly, silver(II) picolinate and lead tetraacetate can be used to produce carbonyl compounds.

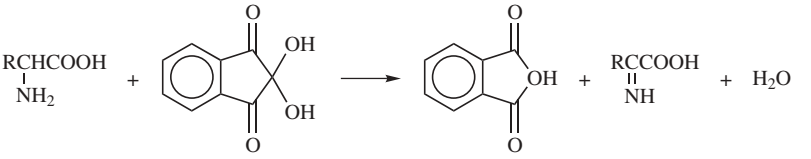
Formation of N-Carboxy-α-Amino Acid Anhydride (NCA). NCAs are important as starting materials for amino acid polymers. They are prepared by the reaction of amino acids with phosgene in an aprotic solvent (46).



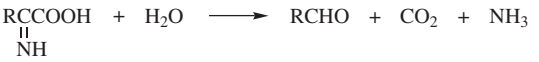
Although polymerization of NCA is popular, other types of amino acid polymerization have also been reported, for example (47,48):

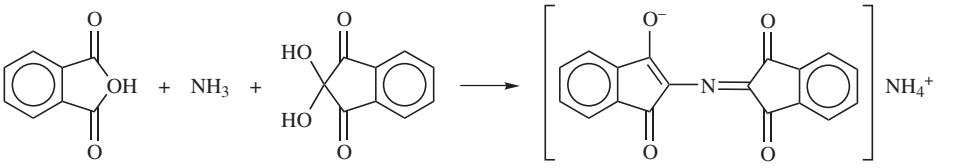


Ninhydrin-Color Reaction. This reaction is commonly used for qualitative analysis of α-amino acids, peptides, and proteins.



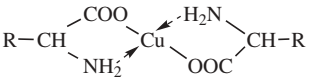
ninhydrin





Ruhemann's Purple

3.4. Other Reactions. *Salt Formation and Metal Chelation.* Most α-amino acids form salts in alkaline and acidic aqueous solutions (49). For example, α-amino acids form inner complex salts with copper.



Benzenesulfonate compounds yield very insoluble salts which have been used for separation and identification of amino acids (50). Similarly, phosphotungstic acid forms insoluble salts with basic amino acids such as lysine, arginine, and cysteine.

Synthesis of Peptide. There is continual progress in the improvement of instruments and reagents for peptide synthesis, especially "solid phase polymerization" (51) (see PROTEINS). This method is suitable for the synthesis of peptides with 20 ∼ 30 amino acid units.

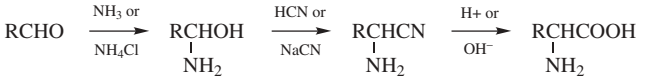
Induction of Asymmetry by Amino Acids. No fewer than six types of reactions can be carried out with yields of 75–100% using amino acid catalysts, ie, catalytic hydrogenation, intramolecular aldol cyclizations, cyanhydrin synthesis, alkylation of carbonyl compounds, hydrosilylation, and epoxidations (52).

4. Synthesis of α-Amino Acids

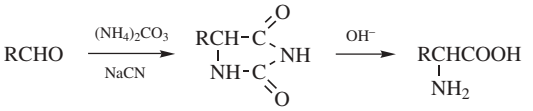
Many methods for chemical synthesis of α-amino acids have been established. Because excellent reviews have been published (53), well-known reactions are introduced here only by their names and synthetic pathways.

4.1. Synthetic Pathways

Strecker Synthesis.



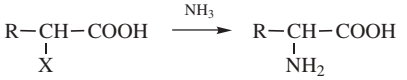
Bucherer Synthesis.



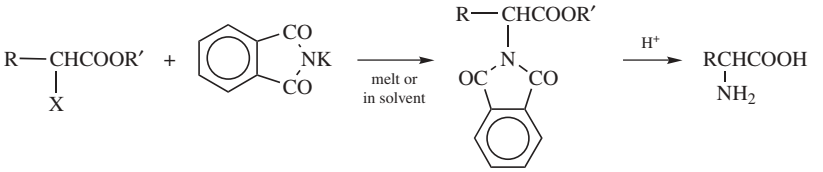
These two methods are popular for α-amino acid synthesis, and used in the industrial production of some amino acids since raw materials are readily available.

Amination of α-Halogeno Carboxylic Acids

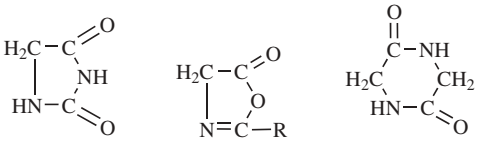
Original Method



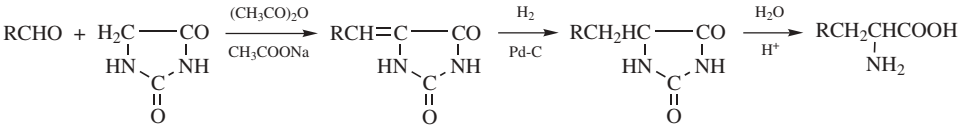
Gabriel's Modification



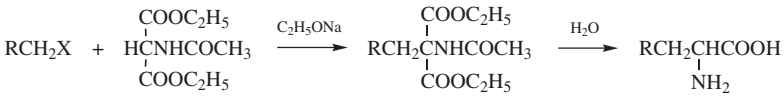
Alkylation of Active Methylene Compounds Erlenmeyer Synthesis and Others. Hydantoin [461-72-3] (**3**), azlactone (**4**), diketopiperazine [106-57-0] (**5**), etc, are readily available, so that these methods are often utilized. In structure (4), R=CH_3 [24474-93-9] or R=C_6H_5 [1199-01-5].



(**3**) (**4**) (**5**)

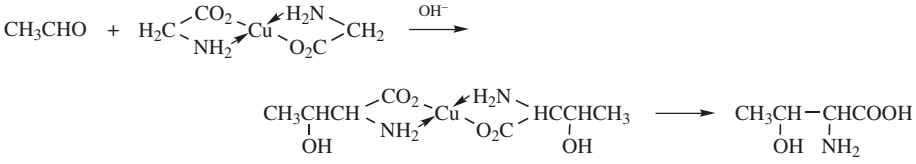


Reaction with Alkyl Halide. The active methylene group of an *N*-acylamino-malonic acid ester or *N*-acylamino cyanoacetic acid ester condenses readily with primary alkyl halides.

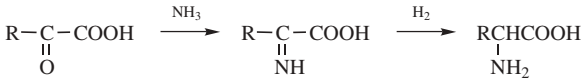


Also, Michael addition reactions occur between *N*-acylaminomalonic acid esters and unsaturated compounds, ie, acrolein [107-02-8], acrylonitrile [107-13-1], acrylic acid esters, and amino acids result from hydrolysis of the addition products.

Reaction of Bisglycinatocopper(II). Bisglycinatocopper(II) [13479-54-4] condenses with aliphatic aldehydes. Removal of copper from the condensate results in β-hydroxy-α-amino acid. This is a classical synthetic method of DL-threonine, but the formation of *allo*-isomer is unavoidable.

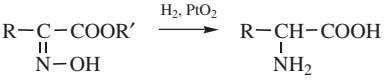


Amination of α-Keto Acids. α-Keto acids are catalytically reduced

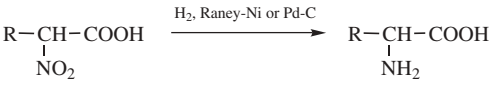


in the presence of ammonia. α-Keto acids are readily prepared by hydrolysis of substituted hydantoins or double carbonylation of benzyl halide in the case of phenylpyruvic acid [156-06-9]. Enzymatic amination of α-keto acids has been developed by many research groups (54).

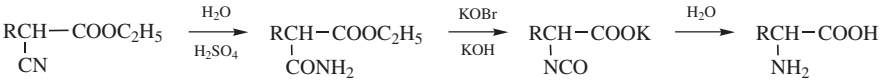
Reduction of α-Ketoxime



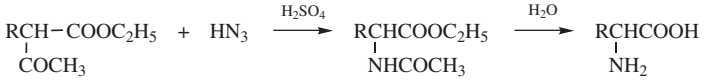
Reduction of α-Nitro Carboxylic Acid

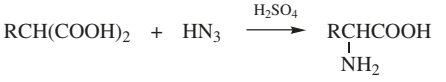


Hofmann Degradation

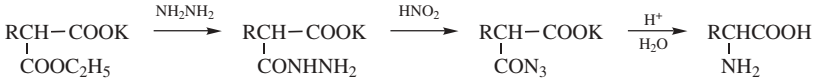


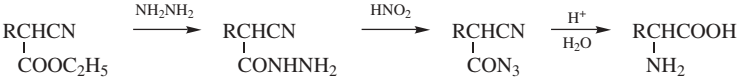
Schmidt Reaction



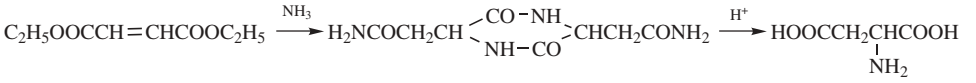


Curtius Degradation

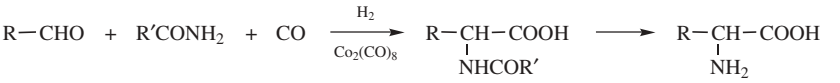




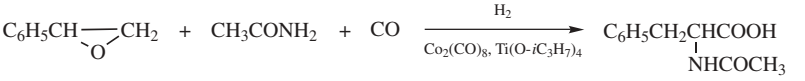
Amine Addition to Double Bond. Production of D,L-aspartic acid from maleic acid ester or fumaric acid ester is a typical example.



Carbonylation of Aldehyde. This method (55, 56) is noteworthy as an efficient one-step synthesis. *Wakamatsu Reaction.*



Modified Method



4.2. Optical Resolution. In many cases only the racemic mixtures of α-amino acids can be obtained through chemical synthesis. Therefore, optical resolution (57) is indispensable to get the optically active L- or D-forms in the production of expensive or uncommon amino acids. The optical resolution of amino acids can be done in two general ways: physical or chemical methods which apply the stereospecific properties of amino acids, and biological or enzymatic methods which are based on the characteristic behavior of amino acids in living cells in the presence of enzymes.

Crystallization Method. Such methods as mechanical separation, preferential crystallization, and substitution crystallization procedures are included in this category. The preferential crystallization method is the most popular. The general procedure is to inoculate a saturated solution of the racemic mixture with a seed of the desired enantiomer. Resolutions by this method have been reported for histidine (58), glutamic acid (59), DOPA (60), threonine (61), *N*-acetyl phenylalanine (62), and others. In the case of glutamic acid, the method had been used for industrial manufacture (63).

Diastereoisomeric Salts. The formation of salts of optically active bases with racemic acids or of optically active acids with racemic bases leads to diastereomeric mixtures which may be resolved by the differential solubility of the components of such mixtures (64), ie,

$$(+)\mathrm{B} + (\mathrm{DL})\mathrm{A} \longrightarrow (+)\mathrm{B}\cdot(\mathrm{L})\mathrm{A} + (+)\mathrm{B}\cdot(\mathrm{D})\mathrm{A}$$

or

$$(+)\mathrm{A} + (\mathrm{DL})\mathrm{B} \longrightarrow (+)\mathrm{A}\cdot(\mathrm{L})\mathrm{B} + (+)\mathrm{A}\cdot(\mathrm{D})\mathrm{B}$$

The salts in turn may be decomposed by a metathetical reaction involving a stronger base than (+)B or stronger acid than (+)A.

Typical examples of optically active materials for resolution are as follows:

Name	Configuration	CAS Registry Number
	Acidic	
camphorsulfonic acid	D	[3144-16-9]
camphoric acid	*R*	[124-83-4]
camphoric acid	*S*	[560-09-8]
tartaric acid	D	[147-71-7]
tartaric acid	L	[87-69-4]
dibenzoyltartaric acid	*R*	[2743-38-6]
dibenzoyltartaric acid	*S*	[17026-42-5]
malic acid	*R*	[636-61-3]
malic acid	*S*	[97-67-6]
mandelic acid	*R*	[611-71-2]
mandelic acid	*S*	[17199-29-0]
glutamic acid	L	[56-86-0]
	Basic	
brucine		[357-57-3]
cinchonidine	*R*	[485-71-2]
ephedrine	*R*	[299-42-3]
strychnine		[57-24-9]
morphine		[52-27-2]
α-methylbenzylamine	*R*	[3886-69-9]
α-methylbenzylamine	*S*	[2627-86-3]
1-(1-naphthyl)ethylamine	*R*	[3886-70-2]
1-(1-naphthyl)ethylamine	*S*	[10420-89-0]
1-phenyl-2-(*p*-toly)ethylamine	*R*	[30339-32-3]
1-phenyl-2-(*p*-toly)ethylamine	*S*	[30339-30-1]

This procedure is restricted mainly to aminodicarboxylic acids or diaminocarboxylic acids. In the case of neutral amino acids, the amino group or carboxyl group must be protected, eg, by *N*-acylation, esterification, or amidation. This protection of the racemic amino acid and deprotection of the separated enantiomers add stages to the overall process. Furthermore, this procedure requires a stoichiometric quantity of the resolving agent, which is then difficult to recover efficiently. Practical examples of resolution by this method have been published (65,66).

Enzymatic Method. L-Amino acids can be produced by the enzymatic hydrolysis of chemically synthesized DL-amino acids or derivatives such as esters, hydantoins, carbamates, amides, and acylates (24). The enzyme which hydrolyzes the L-isomer specifically has been found in microbial sources. The resulting L-amino acid is isolated through routine chemical or physical processes. The D-isomer which remains unchanged is racemized chemically or enzymatically and the process is recycled. Conversely, enzymes which act specifically on D-isomers have been found. Thus various D-amino acids have been produced (see Table 10).

In another procedure, D-amino acid oxidase (67) is useful to produce L-amino acids from DL-amino acids. α-Ketocarboxylic acids which are formed by the action of enzymes on D-amino acids, are aminated to form L-amino acids by coupling through the action of amino acid aminotransferases (68).

In these procedures, the choice of derivatives and enzyme is important. Sometimes it is possible to get a D-amino acid which remains in the microbial culture supplemented with DL-amino acids (69).

Chromatographic Method. Progress in the development of chromatographic techniques (70), especially, in high performance liquid chromatography, or hplc, is remarkable (71). Today, chiral separations are mainly carried out by three hplc methods: chiral hplc columns, achiral hplc columns together with chiral mobile phases, and derivatization with optical reagents and separation on achiral columns. All three methods are useful but none provides universal application.

Chiral Hplc Columns. There are about 40 commercially available chiral columns which are suitable for analytical and preparative purposes (72). In spite of the large number of commercially available chiral stationary phases, it is difficult and time-consuming to obtain good chiral separation. In order to try a specific resolution meaningfully, a battery of chiral hplc columns is necessary and this is quite expensive.

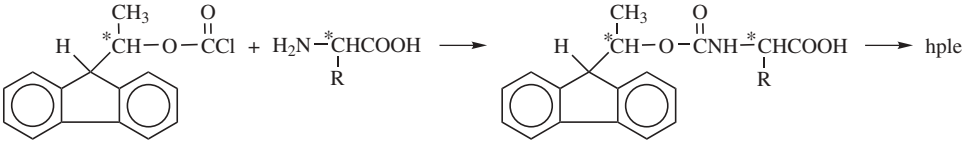
Among various types of chiral stationary phases, the host-guest type of chiral crown ether is able to separate most amino acids completely (73).

Achiral Columns Together with Chiral Mobile Phases. Ligand-exchange chromatography for chiral separation has been introduced (74), and has been applied to the resolution of several α-amino acids. Prior derivatization is sometimes necessary. Preparative resolutions are possible, but the method is sensitive to small variations in the mobile phase and sometimes gives poor reproducibility.

The principle of this method depends on the formation of a reversible diastereomeric complex between amino acid enantiomers and chiral addends, by coordination to metal, hydrogen bonding, or ion–ion mutual action, in the presence of metal ion if necessary. L-Proline (75), L-phenylalanine (76), *N*-(*p*-toluene

sulfonyl)-L-phenylalanine (77), L-histidine methyl ester (78), *N*-acetyl L-valine *t*-butyl amide (79), etc, are used as chiral addends.

Derivatization with Optically Active Reagents and Separation on Achiral Columns. This method has been reviewed (80); a great number of homochiral derivatizing agents (HDA) are described together with many applications. An important group is the chloroformate HDAs. The reaction of chloroformate HDAs with racemic, amino-containing compounds yields carbamates, which are easily separated on conventional hplc columns, eg, Ref. (81).

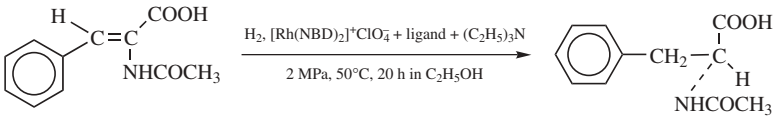


Gas chromatography (gc) is inferior to hplc in separating ability. With gc, it is better to use capillary columns and the application is then limited to analysis (82). Resolution by thin layer chromatography or tlc is similar to lc, and chiral stationary phases developed for lc can be used. However, tlc has not been studied as extensively as lc and gc. Chiral plates for analysis and preparation of micro quantities have been developed (83).

A new technique referred to as the liquid membrane method has been developed (84). Immobilized liquid membranes are expected to become practical because of many advantages.

Enzymatic hydrolysis of *N*-acylamino acids by amino acylase and amino acid esters by lipase or carboxy esterase (85) is one kind of kinetic resolution. Kinetic resolution is found in chemical synthesis such as by epoxidation of racemic allyl alcohol and asymmetric hydrogenation (86). New routes for amino acid manufacturing are anticipated.

Asymmetric Synthesis. Asymmetric synthesis is a method for direct synthesis of optically active amino acids and finding efficient catalysts is a great target for researchers. Many excellent reviews have been published (87). Asymmetric syntheses are classified as either enantioselective or diastereoselective reactions. Asymmetric hydrogenation has been applied for practical manufacturing of L-DOPA and L-phenylalanine, but conventional methods have not been exceeded because of the short life of catalysts. An example of an enantioselective reaction, asymmetric hydrogenation of α-acetamidoacrylic acid derivatives, eg, *Z*-2-acetamidocinnamic acid [55065-02-6] (6), is shown below and in Table 4 (88).



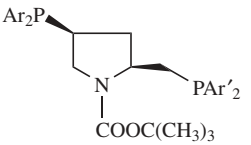
(**6**) (R-) (**7**)

Table 4. **Asymmetric Hydrogenation of (*Z*)-2-Acetamidocinnamic Acid (6) to (*R*)-*N*-Acetylphenylalanine**[a,b]

Ligand	$\frac{[\text{Substrate}]}{[\text{Rh}]}$	Conversion, %	Enantiomeric excess, %
(2*S*, 4*S*)-BPPM	1000	100	78.0
	10000	11	79.7
(2*S*, 4*S*)-BCPM	10000	100	37.0
(2*S*,4*S*)-*o*-methoxy-BPPM	1000	100	98.0
	10000	64	98.9
(2*S*,4*S*)-*m*-methoxy-BPPM	1000	100	84.8
	10000	7	
(2*S*,4*S*)-*p*-methoxy-BPPM	1000	100	90.4
	10000	100	89.8

[a] Ref. 7.
[b] The *R* enantiomer is D-*N*-acetylphenylalanine [10172-89-1].

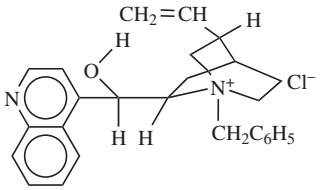
The ligands are phosphino derivatives of *N-tert*-butoxycarbonyl pyrrolidine, BPPM and BCPM:



where, Ar = Ar′ = C_6H_5 in BPPM
Ar = cyclo-C_6H_{11}, Ar′ = C_6H_5 in BCPM

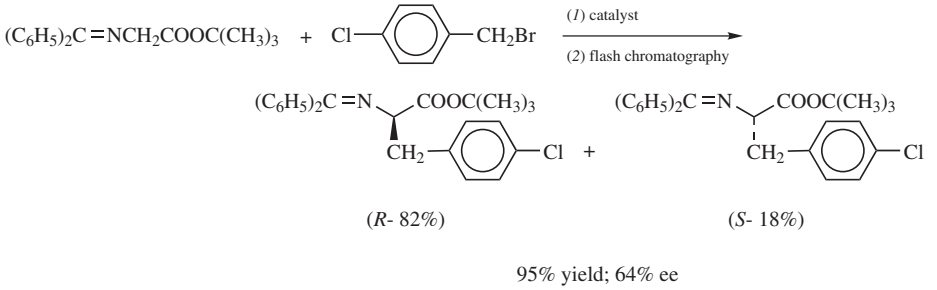
Recent developments in asymmetric synthesis include asymmetric amplifying effects and the phenomenon of a nonlinear effect in which the asymmetric reaction gives a product with very high enantiomeric excess (ee) by a chiral auxiliary of low ee (89). Such new techniques will hopefully be applied to the asymmetric synthesis of amino acids in the near future.

Alkylation of protected glycine derivatives is one method of α-amino acid synthesis (90). Asymmetric synthesis of a D-α-amino acid from a protected glycine derivative by using a phase-transfer catalyst derived from the cinchona alkaloids (8) has been reported (91).



(**8**)

This catalyst (0.2 equivalents) is used in 50% aq NaOH in CH_2Cl_2 at 25°C for 15 h. The reaction may be represented



The initial product is recrystallized and filtered. The filtrate contains a 65% yield (99% ee) of the *R*-isomer. The crystals are racemic (32% yield; 8% ee).

5. Manufacture and Processing

Since the discovery of amino acids in animal and plant proteins in the nineteenth century, most amino acids have been produced by extraction from protein hydrolyzates. However, there are many problems in the efficient isolation of the desired amino acid in the pure form.

DL-Alanine is the first amino acid which was synthesized chemically (92). Glycine and DL-methionine have also been supplied by this method (20). However, amino acids formed by the chemical method are racemic, and it is necessary to resolve the mixture to get the L- or D-form amino acid which is usually demanded.

In the 1950s, a group of coryneform bacteria which accumulate a large amount of L-glutamic acid in the culture medium were isolated (21). The use of mutant derivatives of these bacteria offered a new fermentation process for the production of many other kinds of amino acids (22). The amino acids which are produced by this method are mostly of the L-form, and the desired amino acid is singly accumulated. Therefore, it is very easy to isolate it from the culture broth. Rapid development of fermentative production and enzymatic production have contributed to the lower costs of many protein amino acids and to their availability in many fields as economical raw materials.

5.1. Direct Fermentation Process. In this process, the microorganisms are cultured in the medium containing carbohydrates (eg, cane molasses, sucrose, glucose, starch hydrolyzate), acetic acid, alcohols (eg, ethanol, methanol) or hydrocarbons (eg, *n*-paraffin) as carbon sources, and nitrogen sources (eg, liquid ammonia, urea, ammonium salt), and minor nutrients [phosphate, sulfate, metal ions (K^+, Mg^{2+}, Fe^{2+}, Ca^{2+} etc.), and if necessary, other growth factors (eg, vitamins, amino acids)]. The amino acid-producing microorganisms metabolize the carbon source and the nitrogen source via the pathways shown in Figure 2 to overproduce and accumulate amino acids in the culture medium. Figure 3 exemplifies the time course of L-glutamic acid production with Corynebacterium glutamicum. A flow sheet of fermentative production of amino acids is shown in Figure 4. The amount and the kinds of amino acids accumulated depend on the trait of microbial strains used and the culture conditions (such as medium composition, pH, temperature, aeration, agitation) (22). At present, cane molasses

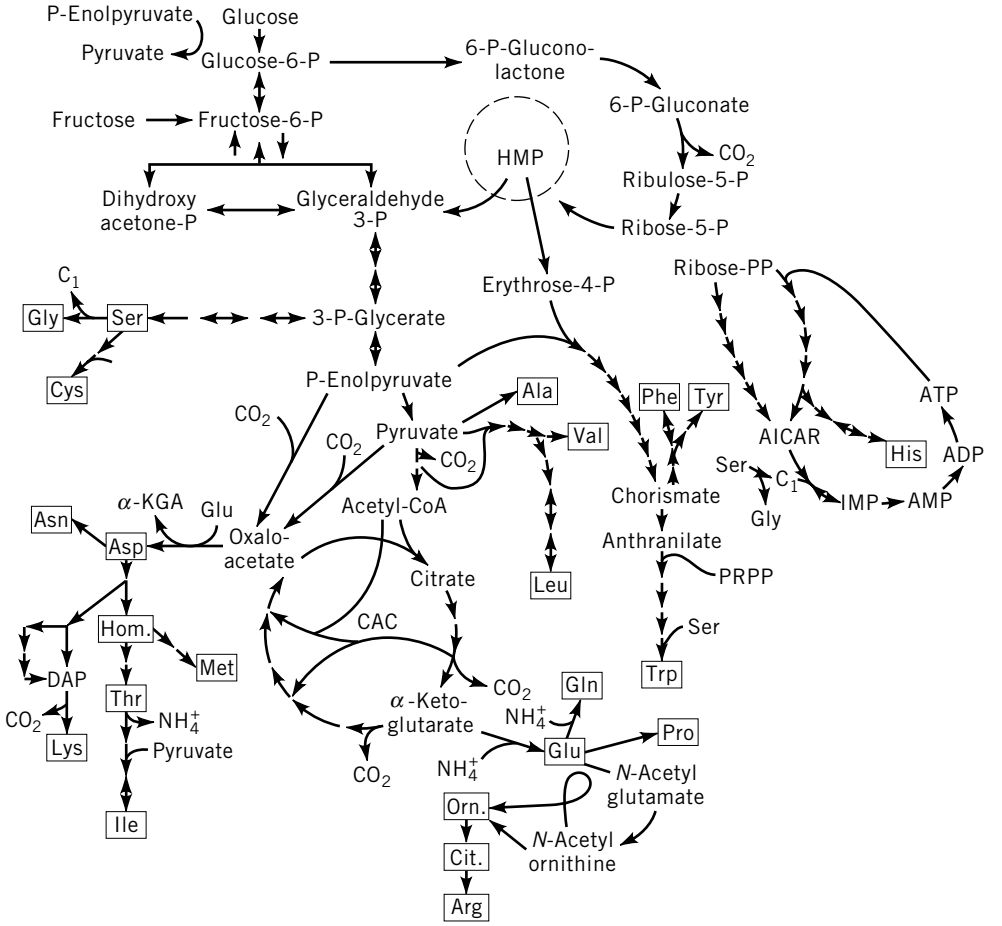


Fig. 2. Biosynthetic pathways for amino acids. HMP = hexose monophosphate pathway; CAC = citric acid cycle; P = phosphate; PP = pyrophosphate; AMP, ADP, and ATP = adenosine mono-, di-, and triphosphate; IMP = inosine 5′-monophosphate; AICAR = 5′-phosphoribosyl-5-amino-4-imidazolecarboxamide; DAP = diaminopimelic acid; PRPP = phosphoribosyl pyrophosphate; α-KGA = α-ketoglutaric acid; Orn = ornithine; Cit = citrulline; C_1 represents the one carbon unit lost to tetrahydrofolate as serine is converted to glycine.

and starch hydrolyzate have been used as carbon sources in the manufacture of amino acids. As can be seen in Table 5 which lists, the amino acid production from carbohydrate, the microorganisms which produce amino acids in high amounts (enough to use for manufacture), are very few: (*1*) So called "glutamic acid bacteria" (95) (eg, *Corynebaterium glutamicum*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*); (*2*) Enteric bacteria (eg, *Serratia marcescens*, *E. coli*); and (*3*) *Bacillus* sp. (eg, *Bacillus subtilis*). Among these, the glutamic acid bacteria can produce some amino acids from acetic acid and ethanol in high amount and these have been used widely as the carbon source (96). Ethanol alone has not been used. The bacteria which can assimilate hydrocarbons (C_{11-14} kerosenes are favorable) have been isolated independently of the ones described

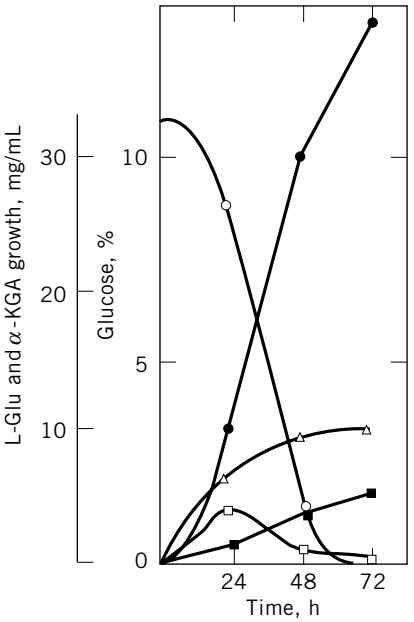


Fig. 3. L-Glutamic acid (and α-ketoglutaric acid, α-KGA) production by a wild type *Corynebacterium glutamicum* (93). The fermentation was carried out with a medium containing 10% glucose, 0.05% KH_2PO_4, 0.05% K_2HPO_4, 0.025% $MgSO_4 \cdot 7\ H_2O$, 0.001% $FeSO_4 \cdot 7\ H_2O$, 0.001% $MnSO_4 \cdot 4\ H_2O$, 0.5% urea, and 2.5 μg/L biotin under aeration and agitation. The pH of the medium was controlled by adding urea during fermentation. ●, L-glutamic acid; ○, glucose; □, lactic acid; △, growth (dry cell weight); ■, α-ketoglutaric acid.

above. As shown in Table 6, the mutant derivatives of *Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *and Nocardia* species have been known to produce a large amount of many kinds of amino acids (111). However, these have not been used in actual production at present. The mutant derivatives of methanol assimilating bacteria belonging to *Pseudommonas*, *Arthrobacter*, *Methylomonas*, *Protaminobacter*, *Microcylus* (most of these are recently named *Methylobacillus glycogenes* (115)) have been isolated and their accumulation of L-glutamic acid (116), L-leucine, L-valine, L-isoleucine, and L-phenylalanine (115) have been reported. An L-lysine producing mutant of Bacillus sp., a thermophilic methylotroph, has been reported (117).

The pathway for amino acid biosynthesis is regulated at the key enzyme mainly by two regulation mechanisms—feedback inhibition (inhibition of enzyme activity usually by the end product of the pathway) and repression (repression of enzyme formation usually by the end product) (118). These prevent the overproduction of amino acids in the wild-type microbial cells. Amino acid production is attained by selecting favorable culture conditions, and/or changing the bacterial trait through mutational or other genetic treatment to overcome the feedback regulations and induce the overproduction and excretion of amino acids outside the cells (22). Production of L-glutamic acid (112), L-glutamine (119), and L-proline (120) by wild-type glutamic acid bacteria are typical examples of the

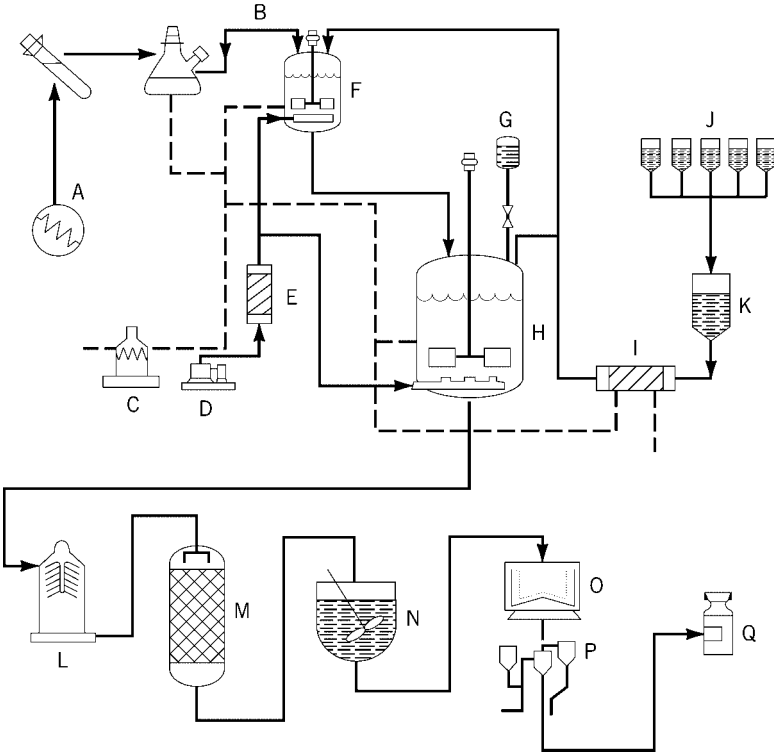


Fig. 4. Fermentative production of amino acids (94). A, pure culture; B, inoculation; C, boiler; D, air compressor; E, air filter; F, seed tank; G, ammonia water for pH control; H, fermenter; I, sterilizer; J, culture media; K, preparation tank; L, centrifugal separator; M, ion-exchange column; N, crystallizing tank; O, crystal separator; P, dryer; Q, amino acid production.

effect of controlling the culture condition. The limited addition of biotin [58-85-5], a growth factor, or the addition of penicillin (or other β-lactam antibiotics) to the culture induce the accumulation of a large amount (80 mg/mL or more) of L-glutamic acid in the culture medium. Other strains of glutamic acid bacteria accumulate L-proline or L-glutamine when NaCl is added to the medium in a high concentration (eg, 6%).

Many kinds of amino acids (eg, L-lysine, L-ornithine, L-phenylalanine, L-threonine, L-tyrosine, L-valine) are accumulated by auxotrophic mutant strains (which are altered to require some growth factors such as vitamins and amino acids) (Table 5, Primary mutation) (22). In these mutants, the formation of regulatory effector(s) on the amino acid biosynthesis is genetically blocked and the concentration of the effector(s) is kept low enough to release the regulation and induce the overproduction of the corresponding amino acid and its accumulation outside the cells (22).

The amino acids (eg, L-arginine, L-histidine) that are synthesized by a straight line biosynthetic pathway are overproduced by the regulatory mutants in which the feedback regulations are genetically released. These mutants are easily selected among the mutants that are resistant to the growth inhibition

Table 5. **Fermentative Production of Amino Acids and Their Related Substances from Carbohydrates**[a]

Microorganisms	Primary mutation[b]	Genetic markers[b] or culture conditions which enhance productivity	Yield, mg/mL (and or %)	References
L-Glutamic acid				
"glutamic acid bacteria" such as *Coryneb. glutamicum, Brevib. flavum, Brevib. lactofermentum, Microb. ammoniaphilum*	wild	penicillin is added, or biotin is limited	>100, 50%	21
Brevib. thiogenitalis	oleic acid$^-$	production from acetic acid and Cu^{2+} is effective	66, 50%	
Coryneb. alkanolyticum	glycerol$^-$		40%	
B. lactofermentum	temperature^s		50%	
C. glutamicum	lysozyme^s			
B. flavum	vitamin P^r			97
(*N*-acetyl-L-glutamic acid) *C. glutamicum*	arg$^-$		7.5	
(*N*-acetylglutamic-γ-semialdehyde) *C. glutamicum*	arg$^-$		2.7	
(L-glutamic-γ-semialdehyde) *B. flavum*	pro$^-$	sulfite is effective	13.2	98
L-Glutamine				
"glutamic acid bacteria"	wild	high concentration of NaCl is effective	>44	
Flavobacterium rigens	ser$^-$	ammonium fumarate is added	25	
B. flavum (*N*-acetyl-L-glutamine)	sulfaguanidine^r		40	
"glutamic acid bacteria"	wild		20%	
L-Proline				
B. flavum	ile$^-$; 3,4-dehydro-Pro^r			
Brevibacterium sp.	his$^-$		25.5	
C. glutamicum	wild	high concentration of NH_4Cl is effective	40	
Kurthia cathenaforma	ser$^-$	Asp is added	30	
Serratia marcescens	3,4-dehydro-Pro^r	prodegradation$^-$, thiazoline-4-carboxylate^r, azetidine-2-carboxylate^r (transduction)	75	99
Coryneb. melassecola	tyr$^-$, phe$^-$			
L-Arginine				

B. flavum	2-thiazole-3-Ala^r	Guanine$^-$	35	
C. glutamicum	D-Ser^s	D-Arg^r, Arg·hydroxamate^r, 2-thiazole-3-Ala^r; contenious culture is effective	60	100
B. subtilis	Arg·hydroxamate^r	6-azauracil^r	28	
S. marcescens	Arg · degradation$^-$, argR$^-$, argA$^-$	succinate nonassimilating, 6-azauracil^r (transduction)	100	
L-Citrulline				
C. glutamicum, B. flavum	arg$^-$		16	
B. subtilis	arg$^-$	Arg-analogue^r, 6-azauracil^r	26	
L-Ornithine				
C. glutamicum, B. flavum, B. subtilis (N^δ-acetyl-L-ornithine)	arg$^-$		36%	
Paracolobactrum coliforme	arg$^-$, uracil$^-$			
B. subtilis	Arg·hydroxamate^r			
Streptomyces virginae (N^α-acetyl-L-ornithine)	lys$^-$		11	
C. glutamicum	arg$^-$		1.2	
L-Lysine				
C. glutamicum	hom$^-$/(Thr$^-$, Met$^-$)	leu$^-$, AEC^r, pyrimidine-analogue^r, Asp-analogue^r; contenious culture is effective	100	101
B. flavum	hom$^-$/(Thr$^-$, Met$^-$)	AEC^r, 2-fluoropyruvic acid^s, pyruvate kinase$^\pm$	40%	102
B. lactofermentum	AEC^r	leu$^-$, ala$^-$, 2-fluoropyruvic acid^s	50, 30%	
B. lactofermentum	AHV^r		16%	
S. marcescens	Lys decarboxylase$^-$, hydroxy-Lys^r, Lys·hydroxamate^r		6.5	
Candida pelliculossa (N^ϵ-acetyl-L-lysine)	AEC^r		3.2	
C. hydrocarboclastus (ε-Polylysine)	AEC^r	phe$^+$	23	
Streptomyces albus, S. noursei	wild	AEC^r, hom$^-$/(Met$^-$, Thr$^-$); addition of Lys is effective	20.3	103
α-ε-*Diaminopimelic acid (DAP)*				
E. coli, C. glutamicum, B. subtilis, Brevib. ammoniagenes (*N*-succinyl-DAP)	lys$^-$		25	

Table 5 (*Continued*)

Microorganisms	Primary mutation[b]	Genetic markers[b] or culture conditions which enhance productivity	Yield, mg/mL (and or %)	References
A. aerogenes, S. marcescens	DAP$^-$		20	
L-Homoserine				
C. glutamicum, E. coli, B. flavum (*O*-acetyl-L-homoserine)	thr$^-$		15	
Bacillus sp.	met$^-$		1	
L-Threonine				
E. coli	met$^-$, DAP$^-$	ile$^-$, AHV^r, *Thr – degradation$^-$, rifampicin^r, Lys^r, Met^r, Hom.^{r}, Asp^r*	76	104
E. coli	AHV^r	AEC^r, *S-methyl-Cys* · hydroxa-hydroxamate^r, *ile$^-$, leu$^-$*	55	
B. lactofermentum	AHV^r	AEC^r, *S – methyl – Cys hydroxamate^r, ile$^-$, leu$^-$*	25	
C. glutamicum	AHV^r	met$^-$	14	
S. marcescens	AHV^r	Thr – degradation$^-$, ile$^-$, AEC^r *(transduction)*	40	
Providencia rettgerii	ile$^-$, AHV^r	ethionine^r, *Asp·hydroxamate^r, leu$^-$, Thr – degradation$^-$, Thia-Ile^r*	82	105
B. flavum	AHV^r	dihydropicolinate synthase$^-$	16.7	
Proteus rettgerii	ile$^-$, AHV^r		13	
Candida guilliermondii	ile$^-$, met$^-$, trp$^-$		4	
L-Methionine				
C. glutamicum	thr$^-$, ethionine^r		2	
L-Aspartic acid				
B. flavum	diaminopurine^r		10	
B. flavum	glu$^{\pm}$			
D,L-Alanine				
C. gelatinosum, B. pentosaminoacidicum, Fusarium moniliforme etc	wild		40	
M. ammoniaphilum	Arg·hydroxamate	addition of lactic acid is effective	60	

(L-*Alanine)*				
Pseudomonas sp.	wild			
C. glutamicum	Ala racemase$^-$			
(D-*Alanine)*				
Coryneb. fasciens	wild			
B. lactofermentum	D-*cyclo-Ser*^{r}	addition of DL-*Ala is effective*	48	106
L-*Isoleucine*				
S. marcescens	Ile·hydroxamate^r	α-aminobutyric acid^r, *lys C*$^-$, *thr A*$^-$	25	
B. flavum	AHV^r	*O-methyl-Thr*^{r}, *ethionine*^{r}	34	
C. glutamicum	Thia-Ile^r	AHV^r, *Aza-Leu*^{r}, α-aminobutyric acid^r	10	
L-*Leucine*				
S. marcescens	α-aminobutyric acid^r	ile$^-$	15	
B. lactofermentum	2-thiazole-3-Ala^r	met$^-$, ile$^-$	30	
C. glutamicum	AEC^r		20	
L-*Valine*				
P. coliforme, E. coli, A. aerogenes, B. ammoniagenes	wild		7–12, 23%	
C. glutamicum	ile$^-$, leu$^-$		30	
S. marcescens	α−aminobutyric acid$^-$		8	
B. lactofermentum	2-thiazole-3-Ala^r		31	
C. glutamicum	AEC^r		20	
L-*Tryptophan*				
B. flavum	5-fluoro-Trp^r	*m-fluoro-Phe*^{r}, *phe*$^-$, *tyr*$^-$, *Aza-Ser*^{r}	10	
C. glutamicum	phe$^-$, tyr$^-$, 5-methyl-Trp^r	Trp·hydroxamate^r, 4-methyl-Trp^r, *ppc*$^-$; *gene technology is effective*	45	107
B. subtilis	5-fluoro-Trp^r	indolemycin^r, *Aza-Ser*^{r}, *6-diazo-5-oxonorleucine*^{r}	21.5	
E. coli	5-fluoro-Trp^r	gene technology is effective; addition of detergent is effective	40	108
L-*Tyrosine*				
C. glutamicum	phe$^-$	3-amino-Tyr^r, *p-amino-Phe*^{r}, *p-fluoro-Phe*^{r}, *Tyr·hydroxamate*^{r}	17.6	
B. flavum	phe$^-$	*m-fluoro-Phe*^{r}		

Table 5 (*Continued*)

Microorganisms	Primary mutation[b]	Genetic markers[b] or culture conditions which enhance productivity	Yield, mg/mL (and or %)	References
L-Phenylalanine				
B. lactofermentum	tyr$^-$	*p-fluoro-Phe^r, 5-methyl-Trp^r, decoinine^r*	25	
C. glutamicum	tyr$^-$	*p-fluoro-Phe^r*	9.5	
L-Serine				
C. glycinophilum	sulfaguanidine^r		4.5	
L-Histidine				
C. glutamicum	2-thiazole-3-Ala^r*; triazole-Ala^r; 2-fluoro-His^r*	purine and pyrimidine analogues^r	15	
B. flavum	2-thiazole-3-Ala^r	sulfa drugs^r, AEC^r	10	
S. marcescens	2-methyl-His^r, *Triazole-Ala^r*	His degradation$^-$ (transduction)	23	
B. subtilis	5-fluoro-Trp^r, tri-	dimethyltriazaindolidine^r, 2-thiazole-3-Ala^r	13.6	
Streptomyces coelicolor (L-*Histidinol)*	his$^-$		3.5	
B. flavum	his$^-$		8	
C. glutamicum	his$^-$		10	

[a] Refs. (21, 22, 109).

[b] Abbreviations: AHV, α-amino-β-hydroxyvaleric acid; Hom, L-homoserine; AEC, (S-(2-aminoethyl)-L-cysteine; ppc, phosphoenolpyruvate carboxylase; the strain improvement largely depends on the transduction technology; ^{s}, sensitive; ^{r}, resistant; −, *auxotroph or deficient;* ±, *leaky auxotroph;* +, *prototrophic revertant.*

Table 6. **Amino Acid Production from Hydrocarbons**[a]

Amino acid produced	Microorganisms	Characteristics[b] of amino acid producers	Amount of accumulation, mg/mL
L-glutamic acid[c]	*Arthrobacter paraffineus, Coryneb. hydrocarboclastus, Coryneb. alkanolyticum etc*	wild	84
	C. alkanolyticum	glycerol$^-$	
L-ornithine	*C. hydrocarboclastus, A. paraffineus*	arg$^-$/cit$^-$	9
L-citrulline	*Corynebacterium sp.*	arg$^-$	8
L-lysine (*N*-acetyl-L-lysine)[d]	*Brevibacterium ketoglutamicum, Nocardia sp.*	hom$^-$	75
	C. hydrocarboclastus	AEC^r, his$^-$	41
diaminopimelic acid	*A. paraffineus*	lys$^-$	10
L-serine	*A. paraffineus*	ile$^-$	3
L-threonine	*A. paraffineus*	ile$^-$	27
L-homoserine	*Corynebacterium sp.*	thr$^-$	12
DL-alanine	*C. hydrocarboclastus*	wild	4
L-valine	*A. paraffineus*	ile$^-$	5
L-isoleucine	*Microbacterium paraffinolyticum*	val$^-$, leu$^-$	1.6
L-tyrosine[e]	*A. paraffineus*	phe$^-$	18
L-phenylalanine[e]	*A. paraffineus*	tyr$^-$	15

[a] Ref. (23, 111).
[b] Abbreviations: Hom, homoserine; AEC, *S*-(2-aminoethyl)-L-cysteine; r, resistant; −, auxotroph.
[c] Ref. 112.
[d] Ref. 113.
[e] Ref. 114.

of the amino acid analogue (whose chemical structure closely resembles the amino acid and falsely regulates the amino acid biosynthesis) (22). Many other amino acids (eg, L-isoleucine, L-leucine, L-lysine, L-proline, L-threonine, L-tryptophan, L-valine) are also overproduced by the analogue resistant mutants as well as the auxotrophic mutants (22).

Amino acid producing strains do not always produce amino acids efficiently enough to be useful for manufacture. Mutant strains are usually improved by combined additions of various genetic markers including other kinds of auxotrophy, analogue resistance, etc (Table 5). These mutations, which increase the amino acid productivity, not only include those which cause deregulation of biosynthesis, but also mutations which make the metabolic flow more efficient to produce the desired amino acid (22,102). The mutations which cause elimination of degradation enzymes and a permeability barrier for the amino acid are, if these are serious, important to improve the amino acid producers. L-Glutamic acid, L-glutamine, L-proline, L-arginine, L-ornithine, L-lysine, L-isoleucine, L-threonine, L-leucine, L-valine, L-tyrosine, L-phenylalanine, L-tryptophan, and L-histidine have been manufactured by fermentation processes.

Advanced biotechnologies such as cell fusion and gene technology are powerful tools offering improvements (121,122). By cell fusion technology, L-lysine and L-isoleucine production of glutamic acid bacteria has been improved

(123). The transduction method is very useful for strain breeding because it is very easy to introduce excellent genetic markers to the amino acid producers (124). The L-histidine, L-arginine, L-threonine, L-proline, and L-isoleucine producers of Serratia marcescens have been skillfully improved by this method using phage PS 20. The bred strains have been used in the manufacture of those amino acids.

Since 1982, the gene multiplication method with plasmid vectors has been applied to the breeding of amino acid producers. To the plasmid vector which can multiply in the amino acid producers, the DNA fragment carrying the genetic information of the other strains of the same or other microorganisms is joined enzymatically, and the resultant recombinant DNA is transferred to the amino acid producers to introduce the new markers due to the donor microorganisms (121,122,125). As exemplified in Table 7, great advances have been made in *C. glutamicum, B. lactofermentum, S. marcescens, B. subtils,* and *E. coli.*

Table 7. **Breeding of Amino Acid Producers by Gene Technology**[a]

Amino acid produced	Microorganisms	Gene donor	Cloned gene[b] or enzyme	Yield mg/mL	Reference
L-alanine	*E. coli*	*B. stearothermophilus*	Ala dehydrogenase		
D-alanine	*E. coli*	*Ochrobactrum anthropi*	D-aminopeptidase	200	128
L-aspartic acid	*E. coli*	*E. coli*	Asp A	[c]	129
	S. marcescens	*S. marcescens*	Asp A		
L-arginine	*C. glutamicum*	*E. coli*	Arg E, C, B, H		
	E. coli	*E. coli*	Arg A		
L-glutamic acid	*C. melassecola*	*C. melassecola*	Glu A, citrate dehydrogenase,ppc, aconitate dehydratase		
	C. glutamicum	*C. glutamicum*	Glu A		
L-histidine	*C. glutamicum*	*C. glutamicum*	His G, D, C, B	15	
	B. subtilis	*B. subtilis*	His D	8.8	
	S. marcescens	*S. marcescens*	His G, D, B	43	
L-isoleucine	*C. glutamicum*	*C. glutamicum*	Hom dehydrogenase	11	
	S. marcescens	*S. marcescens*	ilv A		
	B. flavum	*E. coli*	ilv A	21	
	C. glutamicum	*B. lactofermentum*	ilv B		
L-lysine	*E. coli*	*B. lactofermentum*	lys A, asd-1		
	E. coli	*Achromob. obae*	α-Amino-ε-caprolactum racemase		
	B. subtilis	*B. subtilis*	Lys A		
	C. glutamicum	*C. glutamicum*	Lys A, dap A,B,D,Y		
	B. flavum	*B. flavum*	ppc		
	C. glutamicum	*E. coli*	Asp A		

Table 7 (*Continued*)

Amino acid produced	Microorganisms	Gene donor	Cloned gene[b] or enzyme	Yield mg/mL	Reference
L-phenylalanine	*C. glutamicum*	*C. glutamicum*	aro F, chorismate mutase, PRDH	28	
	C. glutamicum	*E. coli*	aro G, Phe A		
	B. lactofermentum	*B. lactofermentum*	aro F, E, L, PRDH	21	
	E. coli	*E. coli*	aro F, Phe A	28.5	
	E. coli	*E. coli*	transaminase		
	E. coli	*C. freuindii*	transaminase		
	E. coli	*Paracoccus denitrificans*	transaminase		
	E. coli	*B. stearothermophilus, Sporosarcina ureae, B.sphaeroides*	Phe dehydrogenase		
	E. coli	*Rhodotorula rubra*	Phe ammonialyase		130
L-proline	*S. marcescens*	*S. marcescens*	Pro A, B	75	99
L-serine	*E. coli*	*E. coli*	Gly A		
	E. coli	*E. coli*	Ser A, B, C	1.2	
	C. glutamicum	*C. glutamicum*	Gly A		131
L-threonine	*E. coli*	*E. coli*	Thr A, B, C	55	132
	C. glutamicum	*E. coli*	asp kinase, hom dehydrogenase,hom kinase, Thr C	21	
	C. glutamicum	*C. glutamicum*	hom dehydrogenase, hom kinase,Thr C	51	133
	B. lactofermentum	*B. lactofermentum*	ppc, hom dehydrogenase, homkinase	33	134
	B. flavum	*E. coli*	Thr B, C	27	
	S. marcescens	*E. coli*	ppc	60	
L-tryptophan	*B. subtilis*	*B. subtilis*	Trp B, C, F		
	E. coli	*E. coli*	Trp A, B, E, Ser B		
	E. coli	*E. coli*	Trp A, E, R, tna A	40	108
	B. lactofermentum	*B. lactofermentum*	ant-PR transferase, aro B, L, E;Trp A, B, C, D, E, G	7.5	135
	C. glutamicum	*C. glutamicum*	Trp E, aro F, chorismate mutase,PRDH	45	107
	E. coli	*Enterob. aerogenes*	Tna A		
	E. coli	*Alcalig. faecalis*	Tna A		
L-tyrosine	*C. glutamicum*	*E. coli*	Aro F	9	
	B. lactofermentum	*B. lactofermentum*	Aro A		

[a] Ref. (121, 122, 126).

[b] Gene symbols are according to those of *E. coli*. (127). Abbreviations: Hom, Homoserine; Ant, Anthranilic acid; PR, Phosphoribosyl; ppc, Phosphoenolpyruvate carboxylase; PRDH, prephenate dehydrogenase.

[c] 80-fold activity.

Table 8. **Amino Acid Production by Semifermentation Process**[a]

Amino acid produced	Precursor added to the medium	Amount, mg/mL	Reference
D-alanine	DL-alanine	48.8	106
L-histidine	L-histidinol	4	
L-isoleucine	D-threonine	15	
	DL-α-aminobutyric acid	15.7	138
	DL-α-hydroxybutyric acid		
	DL-α-bromobutyric acid		
L-homoisoleucine	L-isoleucine	0.5	
L-methionine	L-hydroxy-4-methylthiobutyric acid	10.9	
	DL-5-(2-methylthioethyl)hydantoin	34	139
L-norleucine	L-norvaline	3	
L-norvaline	L-α-aminobutyric acid	5.5	
	D-threonine		
L-phenylalanine	acetoamidocinnamic acid	75.9	140
L-proline	L-glutamic acid	108.3	141
L-serine	glycine	16	142
	glycine + methanol	54.5	143
L-threonine	L-homoserine	16	
L-tryptophan	anthranilic acid	40	108
	indole	16.7	144

[a] Refs. 23,109.

5.2. Semifermentation Process. In this process, the metabolic intermediate in the amino acid biosynthesis or the precursor thereof is added to the medium, which contains carbon and nitrogen sources, and other nutrients required for growth and production, and the metabolite is converted to the amino acid during fermentation. Some part of the carbon skeleton, the amino donor, and the energy required to complete the amino acid formation are supplied *de novo*. L-Serine production from glycine and methanol (by methylotrophic bacteria, *Hyphomicrobium* sp. and *Pseudomonas* sp.), L-tryptophan production from anthranillic acid [118-92-3] (or indole [120-72-9]) (by *E. coli* and *B. subtilis*) and L-isoleucine production from DL-α-aminobutyric acid and ethanol (by *Brevibacterium* sp.) have been done commercially by this process (22) (Table 8).

5.3. Enzymatic Process. Chemically synthesized substrates can be converted to the corresponding amino acids by the catalytic action of an enzyme or the microbial cells as an enzyme source. L-Alanine production from L-aspartic acid, L-aspartic acid production from fumaric acid, L-cysteine production from DL-2-aminothiazoline-4-carboxylic acid, D-phenylglycine (and D-*p*-hydroxyphenylglycine) production from DL-phenylhydantoin (and DL-*p*-hydroxyphenylhydantoin), and L-tryptophan production from indole and DL-serine have been in operation as commercial processes. Some of the other processes shown in Table 9 are at a technical level high enough to be useful for commercial production (24). Representative chemical reactions used in the enzymatic process are shown in Figure 5.

Nonenzymatic fluorinated amino acids (136) have been developed by enzymatic and chemical methods as bioactive compounds since the antiviral effect of fluorinated alanine was found (137).

Table 9. **Enzymatic Production of Amino Acids**[a]

Amino acid produced	Substrate	Enzyme	Enzyme source	Reference
		L-Form amino acids		
L-amino acids	DL-acetylamino acids	L-aminoacylase	*Asp. oryzae*	25
	DL-amino acid carbamates		*Pseud. fluorescens*	145
L-alanine	L-aspartic acid	Aspartic-β-decarboxylase	*Pseud. dacunhae* etc[b]	146
	pyruvic acid + NH_4^+ +NADH	Ala dehydrogenase		
L-aspartic acid	fumaric acid + NH_4^+	aspartase	*E. coli*[b]	
L-citrulline	L-arginine	Arg deiminase		
L-cysteine (derivatives)	β-chloro-DL-alanine + Na_2S (thiols)	Cys desulfhydrase	*Enterobactor cloacae*	147
	L-serine (derivatives) + H_2S	Trp synthase	*E. coli*[b]	
L-cysteine	DL-2-aminothiazoline-4-carboxylic acid	hydrolase + racemase	*Pseud. thiazolinophilum*[b]	
	L-acetylserine + H_2S	acetylserine sulfhydrylase		
L-cystathionine(derivatives)	L-homoserine + L-cysteine (derivatives)	cystathionine-γ-synthase	*Streptomyces phaeochromogenes*	
		cystathionine-α-lyase	*Ervi carotovora*	168
L-homoserine (derivatives)	L-homoserine + thiol(s)	L-methionine-γ-lyase	*Clostridium, Pseudomonas*	168
L-leucine	α-ketoisocaproic acid + NH_4^+ + NADH	Leu dehydrogenase	*Bacillus sphaericus*[b]	148,149
	DL-leucine amide		*Coryneb. metharica*	
	α-aminoisocapronitril		*Nocardia* sp.	150
L-3-methylvaline	3,3-dimethyl-2-oxobutyric acid + Asp	transaminase		151
L-lysine	DL-α-aminocaprolactum	hydrolase+ Racemase	*Cryptococcus leurentii* *Achromobactor obae*[b]	
	α,ε-diaminopimelic acid	DAP decarboxylase		
L-methionine	DL-methionine + Asp	D-amino acid oxidase + transaminase	*Trigonopsis variabilis*	
	α-keto-γ-methylthiobutyric acid + NH_4 + formic acid	Phe dehydrogenase+formate dehydrogenase	*Sporosarcina ureae*	149
L-ethionine	L-methionine + Na_2S	methioninase		
L-phenylalanine	*trans*-cinnamic acid +NH_4^+	Phe ammonialyase	*Rhodotolura glutinis, Endomyces linderi* etc.[b]	152

Table 9 (*Continued*)

Amino acid produced	Substrate	Enzyme	Enzyme source	Reference
	phenylpyruvic acid + Asp(or NH_4-fumarate)	transaminase	*Citrobactor freuindii, E. coli*[b]	
	phenylpyruvic acid +-NH_4^+ + formic acid	Phe dehydrogenase +formate dehydrogenase	*Brevibacterium sp., Rodccussp., Sporosarcina ureae*	149,153
	DL-phenylalanine amide		*Coryneb. metharica*	
	DL-phenyllactic acid + NH_4^+	D- and L-hydroxyisocaproate dehydrogenase + Phe dehydrogenase dehydrogenase	*Lactobacillus casei*	
	DL-5-phenylhydantoin	hydantoinase + hydrolase	*Flavob. sp.*	
	acetoamidocinnamic acid	acylase + Phedehydrogenase	*Brevib. sp.*	
L-2-amino-4-phenylbutyric acid	2-keto-4-phenylbutyric acid (KPBA) + Asp	transaminase		
	KPBA + formic acid +NH_4^+	Phe dehydrogenase + formate dehydrogenase		
L-α-methylphenylalanine	DL-α-methylphenylalanineamide		*Micobacterium sp.*	154
fluoro-L-phenylalanine	fluorophenylpyruvic acid +Asp	transaminase etc		
	fluoro-*trans*-cinnamic acid	Phe ammonialyase		
L-serine	glycine + formaldehyde	serine hydroxymethyltransferase[b]	*E. coli*	155
	DL-2-oxo-oxazolidine-4-carboxylic acid	hydrolase + racemase	*Pseud. testeroni*	
	D-glyceric acid + NH_4^+	glycerate dehydrogenase +Ala dehydrogenase		156
selenium amino acids	amino acid(s) + selenol(s)	methionine-γ-lyase		
L-tryptophan	indole+ pyruvic acid+NH_4^+	tryptophanase	*Proteus rettgerii*	
	indole + DL-serine	tryptophanase+ Ser racemase	*E. coliPseud. putida*[b]	157
	indole + L-serine	Trp synthase	*E. coli*	
	DL-5-indorylmethylhydantoin	hydantoinase + hydrolase	*Flavobacterium sp.*[b]	158
	indole-3-pyruvic acid + NH_4^+ + NADH	Phe dehydrogenase		149
5-hydroxy-L-tryptophan	5-hydroxyindole + pyruvic acid + NH_4^+	tryptophanase		

fluro-L-tryptophan	fluoroindole + pyruvic acid+NH_4^+	tryptophanase		
L-tyrosine	phenol + pyruvic acid +NH_4^+	β-tyrosinase	*Erwinia herbicola*[b]	
3,4-dihydroxy-L-phenylalanine	cathecol + pyruvic acid +NH_4^+	β-tyrosinase		
L-valine	α-ketoisovaleric acid + NH_4^+ + formic acid	Phe dehydrogenase +formate dehydrogenase		144
		D-Form amino acids[c]		
D-amino acids	DL-acylamino acids	D-aminoacylase		160
D-alanine	DL-alanine hydantoin	D-hydantoinase + D-N-carbamylamino acid amidohydrolas	*Arth. crystallopoietes*	
	DL-alanine amide		*Arthrobactor sp.*	
D-alanine (peptides)	DL-alanine (peptide) amide(s)	D-amino acidaminopeptidase	*Ochrobactrum anthropi; Rhodococcus erythropolis*[b]	128
D-aspartic acid	DL-aspartic acid	asp-β-decarboxylase		
D-cysteine (derivatives)	3-chloro-DL-alanine + NaHS (derivatives)	3-chloro-D-Aladehydrochlorinaseor D-cysteinedesulfhydrase	*Pseudomonas putida E. coli etc*	
D-glutamic acid	L-glutamic acid	Glu decarboxylase + Gluracemase	*Lactobac. brevis*	161
D-methionine	DL-acetylmethionine	D-aminoacylase + acylamino acidracemase	*Alcaligenes denitrificans-Streptomyces sp.*	162
D-phenylalanine	DL-phenylalanine amide	amidase	*Ochrobactrum anthropi, Rhodococcus erythropolis*	
D-phenylglycine	DL-phenylglycine amide	amidase		
N-carbamyl-D-phenylglycine	DL-5-phenylglycine hydantoin	hydantoinase	*Pseud. putida*	
N-carbamyl-D-*p*-hydroxyphenylglycine	DL-*p*-hydroxyphenylglycinehydantoin	hydantoinase	*Pseud. putida*[b]	
p-hydroxy-D-phenylglycine	*p*-hydroxy-DL-phenylglycinehydantoin	hydantoinase	*Pseud. putida*	163
N-carbamyl-D-valine	DL-valine hydantoin	hydantoinase		

[a] Refs. 23,24.

[b] Chemical reactions are shown in Figure 6.

[c] Refs. 24,168,159.

L-Alanine

$HOOCCH_2CH(NH_2)COOH \xrightarrow{\text{aspartic acid } b\text{-decarboxylase}} CH_3CH(NH_2)COOH + CO_2$

L-Asp L-Ala

D-Alanine

$CH_3CH(NH_2)CONH_2 \xrightarrow{\text{D-alanine aminopeptidase}} CH_3CH(NH_2)COOH + CH_3CH(NH_2)CONH_2$

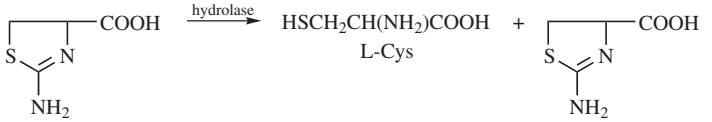
DL-Alanine amide D-Ala L-Alanine amide

L-Aspartic acid

$HOOCHC{=}CHCOOH + NH_3 \xrightarrow{\text{aspartase}} HOOCCH_2CH(NH_2)COOH$

Fumaric acid L-Asp

L-Cysteine



DL-2-Aminothiazoline-4-carboxylic acid $\xleftarrow{\text{racemase}}$ D-2-Aminothiazoline-4-carboxylic acid

$ClCH_2CH(NH_2)COOH + Na_2S \xrightarrow{\text{cysteine desulfhydrase}} HSCH_2CH(NH_2)COOH$

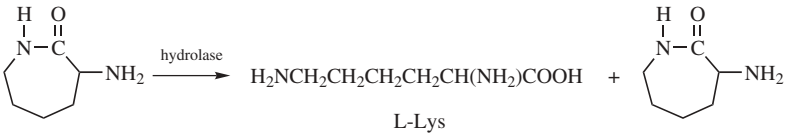
b-Chloro-L-alanine L-Cys

L-Leucine

$(CH_3)_2CHCH_2COCOOH + NH_3 + NADH \xrightarrow{\text{leucine dehydrogenase}} (CH_3)_2CHCH_2CH(NH_2)COOH + NAD$

a-Ketoisocaproic acid L-Leu

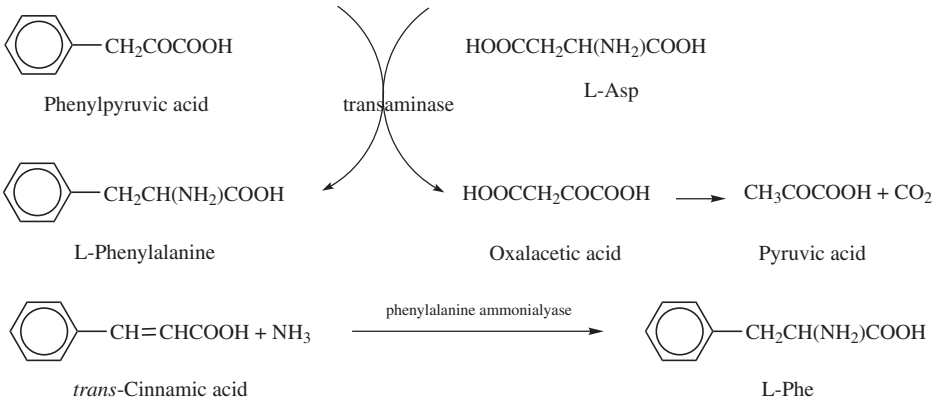
L-Lysine



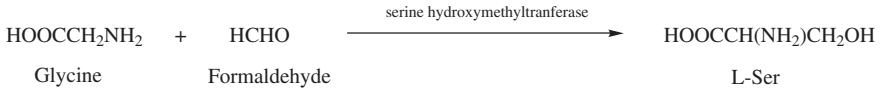
DL-*a*-Amino-caprolactam $\xleftarrow{\text{racemase}}$ DL-*a*-Amino-caprolactam

Fig. 5. Representative chemical reactions in the enzymatic production of amino acids.

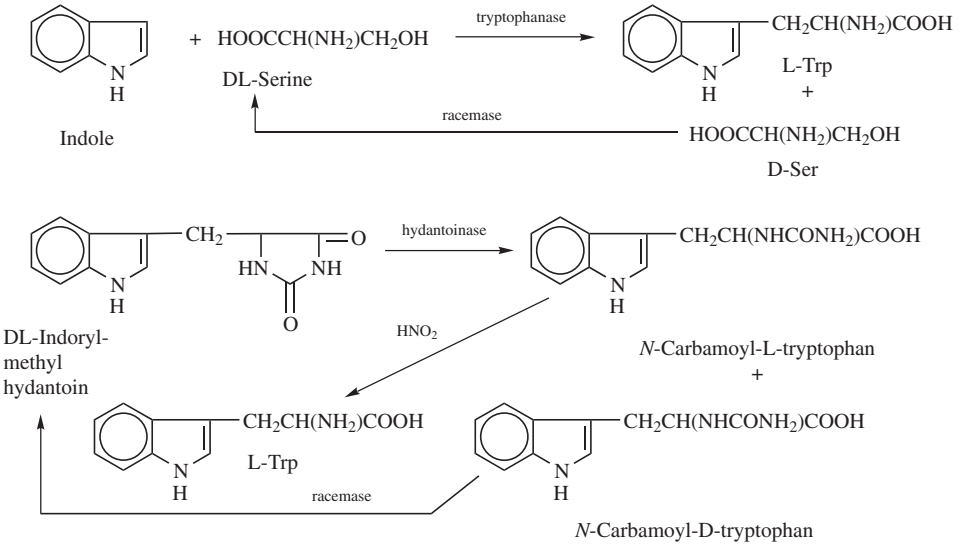
L-Phenylalanine



L-Serine



L-Tryptophan



L-Tyrosine

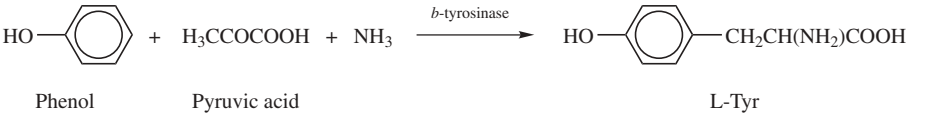
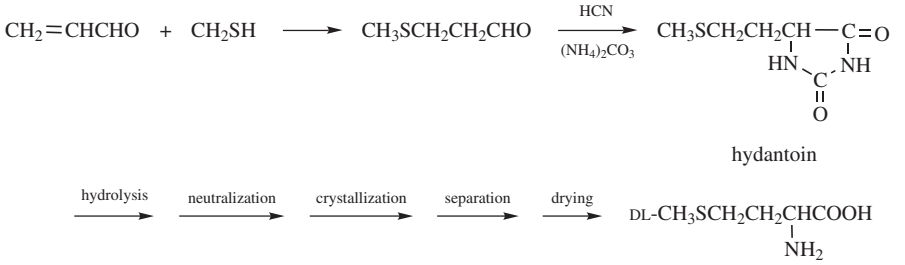


Fig. 5 (*Continued*)

5.4. Chemical Production. Glycine, DL-methionine, and DL-alanine are produced by chemical synthesis. From 1964 to 1974, some glutamic acid was produced chemically (48). The synthetic amino acid with the largest production is DL-methionine from acrolein (see ACROLEIN AND DERIVATIVES). The industrial production method is shown in the following (164).



For glycine (165), two production methods have been employed; Strecker's process and amination of monochloroacetic acid.

Strecker's Process

$$CH_2O + HCN + NH_3 \longrightarrow H_2NCH_2CN \longrightarrow H_2NCH_2COOH$$

Monochloroacetic Acid Process

$$ClCH_2COOH + NH_3 \longrightarrow H_2NCH_2COOH$$

In some cases, Bucherer's process is employed also, but strict control of reaction conditions is needed because the reactivity of formaldehyde is different from other aldehydes. DL-Alanine (166) is produced by either Strecker's or Bucherer's process from acetaldehyde.

5.5. Production by Isolation. Natural cysteine and cystine have been manufactured by hydrolysis and isolation from keratin protein, eg, hair and feathers. Today the principal manufacturing of cysteine depends on enzymatic production that was developed in the 1970s (167).

6. Economic Aspects

The United States amino acid market was estimated at 400×10^6 kg (885×10^6 lb) valued at $11.2 billion in 1999. Key products were methionine and lysine. Both are used as additives in animal feed. Demand should reach 0.5×10^9 kg (1.1×10^9 lb) in 2004, representing an increase in demand of 3.8%.

Table 10 lists the U. S. market demand of various amino acids by type (169). Table 11 lists the U. S. amino market demand by end use (169).

7. Analytical Methods

Methods have been developed for analysis or determination of free amino acids in blood, food, and feedstocks (170). In proteins, the first step is

Table 10. **U. S. Demand for Amino Acids by Type, $\times$ 10^6 kg (10^6 lb)**[a]

Amino acids	1999	2004[b]	2009[b]
methionine	200(440)	231(510)	268(590)
lysine	133(293)	169(372)	203(448)
glutamic acid	49(107)	54(120)	59(130)
aspartic acid	7.7(17)	11(25)	19(42)
threonine	4.1(9)	6.3(14)	8.6(19)
phenylalanine	2.7(6)	3.2(7)	3.6(8)
other	5.9(13)	8.2(18)	11(24)
total	*401(885)*	*483(1065)*	*572(1260)*

[a] Ref. 169.
[b] Estimated.

hydrolysis, then separation if necessary, and finally, analysis of the amino acid mixture.

7.1. Protein Hydrolysis. Acid hydrolysis of protein by 6 *M* HCl in a sealed tube is generally used (110°C, 24-h). During hydrolysis, slight decomposition takes place in serine (ca 10%) and threonine (ca 5%). Cystine and tryptophan in protein cannot be determined by this method because of complete decomposition.

For determination of tryptophan, 4 *M* methanesulfonic acid hydrolysis is employed (18). For cystine, the protein is reduced with 2-mercaptoethanol, the resultant cysteine residue is carboxymethylated with iodoacetic acid, and then the protein sample is hydrolyzed. Also, a one-pot method with mercaptoethanesulfonic acid has been developed for tryptophan and cystine (19).

$$R'—S—S—R'' \xrightarrow{\text{reduction}} R'—SH + R''—SH$$

$$R—SH + ICH_2COOH \xrightarrow{\text{carboxymethylation}} R—S—CH_2COOH$$

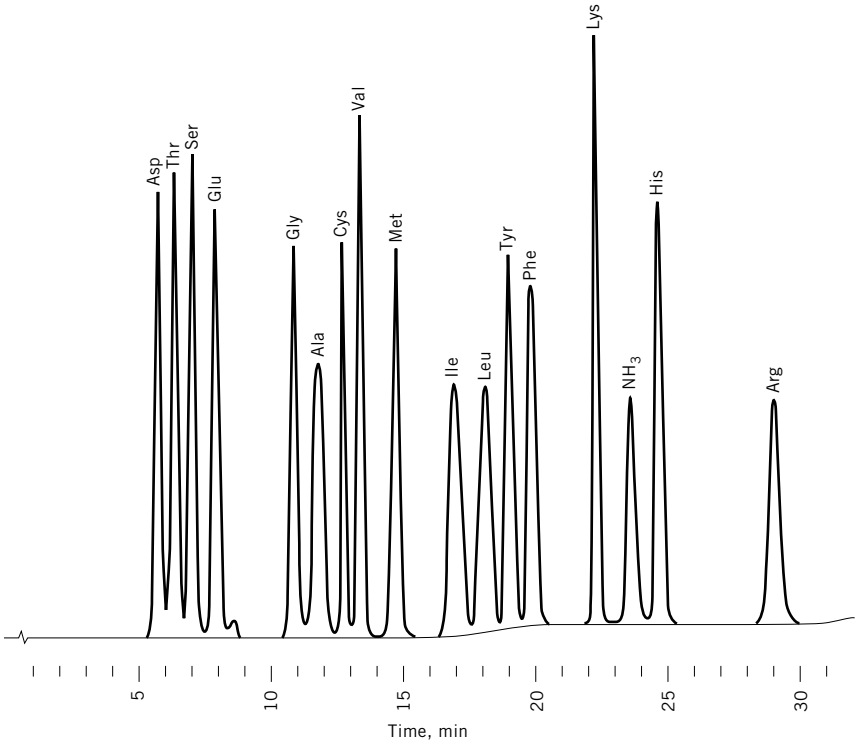
The automated amino acid analyzer depends on ion-exchange chromatography (171) and is now a routine tool for the analysis of amino acid mixtures (172). This most advanced machine can detect as little as 10 pmol in ninhydrin reaction analysis. One-half to two hours are required for each analysis. An analysis chart is shown in Figure 6.

Individual analyses for each amino acid have also been established (173), in particular, Edelhoch spectrometric analysis for tryptophan (174) and Ellman colormetric analysis (175) for cysteine are often used.

Table 11. **U. S Demand for Amino Acids by Market, $\$10^6$**[a]

Market	1999	2004[b]	2009[b]
animal feed additives	1195	1545	1176
food and beverage additives	274	321	378
pharmaceuticals and neutracals	178	244	331
other	20	36	65

[a] Ref. 109.
[b] Estimated.



Name	RT	Height	Area	Ratio
Asp	5.81	32764	556900	1699.7
Thr	6.44	34036	592872	1741.8
Ser	7.10	35493	591477	1666.4
Glu	8.02	31380	568744	1812.4
Gly	10.92	28436	574689	2020.9
Ala	11.85	20527	529589	2579.9
Cys	12.81	28942	313874	1084.4
Val	13.45	38240	555963	1453.8
Met	14.77	28544	586978	2056.3
Ile	17.02	18485	538596	2913.6
Leu	18.17	17819	530384	2976.5
Tyr	19.08	27625	519627	1881.0
Phe	19.93	25061	538429	2148.4
Lys	22.33	44070	612254	1389.2
NH_3	23.69	16393	376220	2295.0
His	24.73	31333	586159	1870.7
Arg	29.13	16421	484608	2951.1

Fig. 6. Amino acid analysis by automated ion-exchange chromatography. Standard column, 4.6 mm ID $\times$ 60 mm; Ninhydrin developer. Computer print out indicates retention time (RT), height and area of peaks, and the ratio of the height of an amino acid in the sample to the height of a standard amino acid. The number of ng in a 2 nmol sample of each amino acid is also tabulated (not shown here).

7.2. Chromatographic Methods. *High Performance Liquid Chromatography (hplc).* Hplc is currently the fastest growing analytical method and is now available in many laboratories. DL-Analysis by hplc has already been described and hplc methods have been reviewed (176).

Gas Chromatography (gc). A principal advantage of gas chromatography has been the facility with which it can be combined with mass spectrometry for amino acid identification and confirmation of purity. The gc-mass spectrometry combination offers the advantage of obtaining structural information rather than the identification by retention time in hplc.

Successful analysis of amino acids with gas chromatography is dependent on the synthesis of derivatives that are stable, yet volatile (177). The first step is esterification. A variety of alcohols have been used for esterification, including methanol, *n*-propanol, 2-propanol, *n*-butanol, and isobutyl alcohol, as well as some optically pure alcohols, eg, (+)butan-2-ol, (+)octan-2-ol. The next step is *N*-acylation by the addition of acetic anhydride, trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA) or heptafluorobutyric anhydride (HFBA), along with an appropriate solvent. Alkylsilylation which has the advantage of being a fast, one-step derivatization for all groups commonly encountered (NH, OH, SH, COOH) is a useful tool for mass spectrometry. Trimethyl-silylation also is well-suited to chromatographic studies.

The synthesis and the quantitative gas chromatographic analysis of stable, yet volatile, *N*-trifluoroacetyl-*n*-butyl esters of amino acids has been established (178). An extensive review of subsequent advances in gas chromatographic instrumentation has been provided (179).

Thin-Layer Chromatography (tlc). Tlc (180) is used widely for qualitative analysis and micro-quantity separation of amino acid mixtures. The amino acids detected are developed by ninhydrin coloring, except for proline and hydroxyproline. Isatin has been recommended for specific coloring of proline (181).

7.3. Colorimetric and Fluorimetric Analysis. The functional groups of amino acids exhibit little absorption of uv light from 210 to 340 nm where uv absorption spectrometry is most conveniently conducted. Thus color or fluorescence formation reactions are employed for amino acid detection (182).

The most widely applied colorimetric assay for amino acids relies upon ninhydrin-mediated color formation (183). Fluorescamine [38183-12-9] and *o*-phthalaldehyde [643-79-8] are popular as fluorescence reagents. The latter reagent, in conjunction with 2-mercaptoethanol, is most often used in post-column detection of amino acids separated by conventional automated amino acid analysis. More recently, determination by capillary zone electrophoresis has been developed and it is possible to determine attomole quantities of amino acids (184).

7.4. Spectrometric Analysis. Remarkable developments in mass spectrometry (ms) and nuclear magnetic resonance methods (nmr), eg, secondary ion mass spectrometry (sims), plasma desorption (pd), thermospray (tsp), two or three dimensional nmr, high resolution nmr of solids, give useful structure analysis information (185). Because nmr analysis of ^{13}C- or ^{15}N-labeled amino acids enables determination of amino acids without isolation from organic samples, and without destroying the sample, amino acid metabolism can be dynamically analyzed (186). Protein metabolism and biosynthesis of many important

metabolites have been studied by this method. Preparative methods for labeled compounds have been reviewed (168).

7.5. Enzymatic Determination and Microbial Assay. In these methods, only the desired amino acid is detected in spite of the presence of other amino acids. No expensive tools are needed for these determinations. The required nutrients for microorganisms and practical operations for the microbial assay of amino acids have been reviewed (187,188).

Manometric determination of L-lysine, L-arginine, L-leucine, L-ornithine, L-tyrosine, L-histidine, L-glutamic acid, and L-aspartic acid has been reviewed (189). This method depends on the measurement of the carbon dioxide released by the L-amino acid decarboxylase which is specific to each amino acid.

A kit for the enzymatic determination of L-glutamic acid has been commercialized. Hydrogen peroxide, which is formed through the L-glutamic acid oxidase reaction, is determined by coupling with the peroxidase reaction which forms a blue color complex in the presence of 4-aminoantipyrine [83-07-8] and *N*-hydroxysulfopropylate. The oxidase reactions are applied to amino acid sensors, which have been commercialized recently for the determination of L-glutamic acid, L-lysine, or mixtures of L-amino acids. The amount of oxygen consumed by the reaction is electrically determined. It is possible to determine amino acids through the NAD-dependent dehydrogenase reaction which is specific to the corresponding amino acid (190). For the details of the enzymatic determination of amino acids, see reference 189.

8. Health and Safety Factors

8.1. Nutrition. Protein amino acids, which are not synthesized by the body and should be supplied as nutrients to maintain life, are called essential amino acids (6). For humans, L-arginine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-valine, L-threonine, and L-tryptophan are essential amino acids. However, in adults, L-arginine and L-histidine are somewhat synthesized in cells. For histidine, there is evidence that it is dietetically essential for the maintenance of nitrogen balance (191). On the other hand, those amino acids which are synthesized in apparently adequate amounts are nonessential amino acids: L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-proline, L-serine, and L-tyrosine. Of these, L-tyrosine and L-cysteine are essential for children. Recent advances in nutritional studies of amino acids have led to development of amino acid transfusion (192).

The nutritional value of a protein can be improved by the addition of amino acids which are short in the protein (11). The amino acid score has been used to evaluate the nutritive value of food proteins. The reference pattern or scoring pattern of essential amino acids presented by the Food and Agriculture Organization of the World Health Organization (FAO/WHO) in 1973 (193) and by FAO/WHO/of the United Nations in 1985 (194) has been used to calculate the amino acid score. The proportion of each essential amino acid in a particular protein to that of the reference pattern is calculated and an essential amino acid that has a score of less than 100% is called the limiting amino acid of the protein. If there

are two or more essential amino acids of this kind, they are called the first limiting amino acid, the second limiting amino acid, and so on in ascending order of their percentage. The amino acid scores and the limiting amino acids of many foods have been calculated (195). Almost all of the plant proteins have limiting amino acids. The nutritionally important cereal proteins are particularly deficient in L-lysine and are also low in L-threonine and L-tryptophan. The limiting amino acid in soybean meal is methionine. No animal proteins, except for those of shellfish, mollusks, and crustaceans, have limiting amino acids.

Before the reference pattern was established, it was usual to use the amino acid composition of egg protein as a standard. The ratio of the amount of limiting amino acid present in a protein to that in egg protein is the chemical score of that protein. For practical purposes, the chemical score and the amino acid score are quantitatively similar. Because these are based on the chemical analysis of protein, both ignore the biological availability of the essential amino acids. The biological value (BV, the ratio of nitrogen retained in the body to that absorbed), the Net Protein Utilization (NPU, the BV of the protein multiplied by the digestibility), and the Protein Efficiency Ratio (PER, the gain in weight per gram of dietary protein) are biological measures. For details of protein quality, see references 6 and 195.

Feeding standards, which have been instituted nationally, indicate the amount of the essential amino acids (together with other nutrients) for the rational breeding of domestic animals. The feeding standards of the National Research Council (NRC) of the United States and Agricultural Research Council (ARC) of the United Kingdom are well known (the former indicates the minimal amount and the latter shows the recommended amount). Japanese Feeding Standards have been instituted (196).

Amino acids essential for young rats (197) and fishes (198) have been reviewed. Rats preferably eat a diet with sufficient amounts of essential amino acids rather than one that is deficient (199). Each essential amino acid, consumed in self-selection, has been reviewed (200). A protein diet with an excess of essential amino acids has been described as a poor protein diet from investigations that showed remarkable growth inhibition and occurrence of fatty liver disease in rats (201). This is called amino acid imbalance (202).

8.2. Biosynthesis of Protein. The dynamic equilibrium of body protein was confirmed by animal experiments using ^{15}N-labeled amino acids in 1939 (203). The human body is maintained by a continuous equilibrium between the biosynthesis of proteins and their degradative metabolism where the nitrogen lost as urea (about 85% of total excreted nitrogen) and other nitrogen compounds is about 12 g/d under ordinary conditions. The details of protein biosynthesis in living cells have been described (2,6) (see also PROTEINS).

8.3. Toxicity of α-Amino Acid. LD_{50} values of α-amino acids are listed in Table 12. L-Lysine and L-arginine are mutually antagonistic. The addition of an excess of one reduces the biological value of protein and only the addition of the other overcomes the effect. The other antagonism occurs between the branched-chain amino acids (L-isoleucine, L-leucine, and L-valine). Pellagra is caused by L-leucine inhibition of niacin formation from L-tryptophan. People living on low protein nutrition and with only a marginally adequate intake of

Table 12. **Toxicity[a] of Amino Acids**

	LD_{50}[b]	
Amino acid	Oral	Intraperitoneal
L-Arg·HCl	12 g	
L-Cys	5580 mg	1620 mg
L-Cys·HCl		1250 mg[c]
L-$(Cys)_2$	25 g	
L-His	7930 mg	
L-Ileu		6822 mg
L-Leu		5379 mg
D-Leu		6429 mg
L-Lys·HCl	10 g	4019 mg
L-Met	36 g	4328 mg
L-Phe		5287 mg
D-Phe		5452 mg
DL-Thr		3098 mg
L-Trp		1634 mg
D-Trp		4289 mg
L-Val		5390 mg
D-Val		6093

[a] Rat, unless otherwise noted.
[b] Ref. 203.
[c] Mouse.

L-tryptophan and niacin, eg, living on sorghum as a dietary staple, are at a risk of developing pellagra if they have an excess intake of L-leucine (6).

In the case of hyperphenylalaninaemia, which occurs in phenylketonuria because of a congenital absence of phenylalanine hydroxylase, the observed phenylalanine inhibition of protein synthesis may result from competition between L-phenylalanine and L-methionine for methionyl-*t*RNA. Patients suffering from maple syrup urine disease, an inborn lack of branched chain oxo acid decarboxylase, are mentally retarded unless the condition is treated early enough. It is possible that the high level of branched-chain amino acids inhibits uptake of L-tryptophan and L-tyrosine into the brain. Brain injury of mice within ten days after their birth was reported as a result of hypodermic injections of monosodium glutamate (MSG) (0.5–4 g/kg). However, the FDA concluded that MSG is a safe ingredient, because mice are born with underdeveloped brains regardless of MSG injections (205).

Furthermore, the Joint Expert Committee on Food Additives (206) (JECFA) of the WHO and FAO of the United Nations issued the evaluation of the safety, stating that on the basis of the available data, the total dietary intake of glutamates arising from their use at the levels necessary to achieve the desired technological effect and from their acceptable background in food does not, in the opinion of the committee, represent a hazard to health.

The nephrotoxic amino acid, lysinoalanine [18810-04-3], formed upon alkaline treatment of protein, was reported in 1964 (207). Its toxicity seems to be mitigated in protein in that it is not released by normal digestion (208). Naturally occurring new amino acids, which can be classified as proteinaceous or

non-proteinaceous, can, as in the case of those from some legumes, show a remarkable toxicity (209). For the details of amino acid toxicity, see reference 6. Enzyme inhibition by amino acids and their derivatives have been reviewed (210).

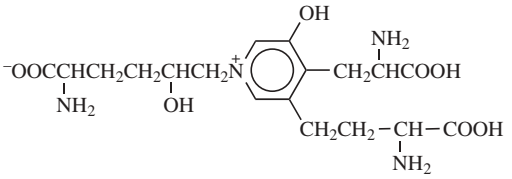
8.4. Metabolism of Amino Acids. The amino acids are metabolized principally in the liver to a variety of physiologically important metabolites, eg, creatine (creatinine), purines, pyrimidines, hormones, lipids, amino sugars, urea, ammonia, carbon dioxide, and energy sources. The reactions of transamination, deamination, and decarboxylation are important for amino acid metabolism. Glutamate–oxalacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT) are the most important transaminases. NAD-dependent glutamic acid dehydrogenase, amino acid oxidases, and aspartic acid ammonia-lyase catalyze the deamination of amino acids. Amino acid decarboxylase is important to amine formation. The products of these enzyme reactions are metabolized finally to carbon dioxide and water via the pathways for sugar metabolism or fatty acid metabolism. The amino acids which are degraded via the former or the latter pathway are called glucogenic (or glycogenic) amino acids or ketogenic amino acids, respectively (13).

8.5. As Neurotransmitters. Several amino acids serve as specialized neurotransmitters in both vertebrate and invertebrate nervous systems. These amino acids can be classified as inhibitory transmitters, such as γ-aminobutyric acid [56-12-2] (GABA) and glycine, and excitatory amino acids, examples of which are L-glutamic acid and L-aspartic acid. A number of other amino acids and their related substances occur in the brain and have some physiological activity. These include taurine [107-35-7], serine, proline, pipecolic acid [535-75-1], *N*-acetyl-aspartic acid [997-55-7], α- and β-alanines, and L-cysteine sulfinic acid [2381-08-0]. For more details about neurotransmitter amino acids, see reference 211.

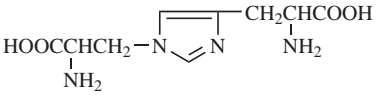
8.6. Modification of Amino Acid in Protein Molecules. Protein kinases, whose activities are regulated by secondary messengers, such as cyclic nucleotide and Ca^{2+}, modify physiologically important proteins by phosphorylating the hydroxy moiety of serine, threonine, and tyrosine in protein molecules. Consequently, various cellular functions, cell growth, and cell differentiation are seriously affected (212). Because the intracellular pool of secondary messengers is under the control of hormones, growth factors, and neurotransmitters, protein phosphorylation is also regulated by these signal compounds.

The participation of protein kinases in oncogenesis has been suggested (213). Thus some oncogenes (src, fps, yes, mos) are known to encode for protein kinases. Formation of some cross-links within molecules of proteins which are physiologically important and have a slow turnover rate (such as collagen) is believed to correlate seriously with aging of animal tissue. Nonenzymatic glycation of protein by the Maillard reaction possibly acts by such cross-link formation. L-Lysine and L-hydroxylysine moieties in a collagen molecule are often oxidized to their corresponding aldehydes by the action of lysyloxidase. Aldehydes react with the amino groups of L-lysine or L-hydroxylysine in the adjacent collagen molecules to form Schiff-base cross-links among the different collagen molecules. β-Aminopropionitrile [151-18-8] inhibits the oxidase reaction. Pyridinoline [63800-01-1] (**9**) and histidinoalanine [65428-77-5] (10) are also found as

cross-linkers in collagen. The relationship between the aging of animal tissues and these cross-linking agents has been discussed (214).



(**9**)



(**10**)

9. Uses

Amino acids are used in feeds (215), food (215), parenteral and enteral nutrition (192), medicine (216), cosmetics (217), and raw materials for the chemical industry (218).

9.1. In Feeds. The agricultural products which are used as feedstuff for domestic animals are different, depending on the areas where they are used. These feedstuffs do not always meet the essential amino acid requirements for the economical growth of the animals and usually require DL-methionine and L-lysine supplements as the first and/or second limiting amino acid as shown in Table 13. The addition of these amino acids to the feeds saves the use of feed protein without affecting the growth response of animals. L-Tryptophan is the second limiting amino acid of maize and as the feed protein level becomes lower, its requirement increases. When the protein level decreases further, L-threonine becomes another limiting amino acid. In Western Europe where wheat and barley are the basis of feeds, L-threonine is the second limiting

Table 13. **Limiting Amino Acids of Some Common Feedstuffs for Pig and Chicken**[a].

Ingredient	Crude protein, %	Pig		Chicken	
		First	Second	First	Second
maize	8.9	Lys	Trp	Lys	Trp
sorghum	9.5	Lys	Thr	Lys	Arg
barley	11.1	Lys		Lys	Met
wheat	12.6	Lys	Thr	Lys	Thr
soybean meal	46.2	Met	Thr	Met	Thr
fish meal	64.3			Arg	
rapeseed meal	35.3	Met		Lys	Arg
peanut meal	47.4	Lys		Met	Lys
sunflower seed meal	31.7	Lys		Met	Thr
meat and bone meal	48.6	Lys		Trp	Met
cottenseed meal	64.3	Lys	Thr	Lys	Met
corn gluten meal	63.6	Lys	Trp	Lys	Trp

[a] Ref. 216

amino acid after L-lysine and both are usually added to the feedstuff. The issue most focused on at present is development of protected amino acids for ruminants (219). Protected methionine has already been commercialized. A method for supplying methionine to cows (220) and an aqueous lysine-containing animal feed supplement (221) have been described.

9.2. In Foods. Each amino acid has its characteristic taste of sweetness, sourness, saltiness, bitterness, or "umami" as shown in Table 14. Umami taste, which is typically represented by L-glutamic acid salt (and some 5′-nucleotide salts), makes food more palatable and is recognized as a basic taste, independent of the four other classical basic tastes of sweet, sour, salty, and bitter (222).

The existence of protein receptors in the tongues of mice and cows have been shown. Monosodium L-glutamate MSG [142-47-2] is utilized as a food flavor enhancer in various seasonings and processed foods. D-Glutamate is tasteless. L-Aspartic acid salt has a weaker taste of umami. Glycine and L-alanine are slightly sweet. The relationship between taste and amino acid structure has been discussed (223).

Aspartame (L-aspartyl-L-phenylalanine methyl ester [22839-47-0]) is about 200 times sweeter than sucrose. The Acceptable Daily Intake (ADI) has been

Table 14. **Taste Profiles of L-Amino Acids**[a]

L-Amino acid	Threshold-value, mg/dL	Taste quality[b]				
		Sweet	Sour	Bitter	Salty	Umami
Gly	110	++				
Hyp	50	++		++		
β-Ala	60	++				+
Thr	260	++				
Pro	300	++		+++		
Ser	150	++				+
Lys·HCl	50	++		++		+
Gln	250	+				+
Phe	150			+++		
Trp	90			+++		
Arg	10			+++		
Arg·HCl	30	+		+++		
Ile	90			+++		
Val	150	+		+++		
Leu	380			+++		
Met	30			+++		+
His	20			++		
His·HCl	5		+++	+	+	
Asp	3		+++			+
Glu	5		+++			++
Asn	100		++	+		
MSG	30	+			+	+++
monosodium aspartate	100				++	++

[a] Ref. 215.

[b] Profiles of each basic taste intensity are expressed as follows: (+ + +) strongest, (++) stronger, and (+) detectable.

established by JECFA as 40 mg/kg/day. Structure-taste relationship of peptides has been reviewed (224). Demand for L-phenylalanine and L-aspartic acid as the raw materials for the synthesis of aspartame has been increasing. D-Alanine is one component of a sweetener "Alitame" (225). Derivatives of aspartame are also described as flavor modifiers (226) and sweeteners in chewing gum (227).

In traditional cooking of proteinaceous foods, the fundamental difference between Western and Oriental cultures is that the former cooks proteins with unseasoned fats and the latter cooks with many kinds of traditional seasonings that have tastes of amino acids. Western cultures have some traditional foods with amino acid taste such as cheese. Protein hydrolysates are popular as seasonings (228).

The enzymatic hydrolysates of milk casein and soy protein sometimes have a strong bitter taste. The bitter taste is frequently developed by pepsin [9001-75-6], chymotrypsin [9004-07-3], and some neutral proteases and accounted for by the existence of peptides that have a hydrophobic amino acid in the carboxylic terminal (229). The relation between bitter taste and amino acid constitution has been discussed (230).

Amino acids play a role in food processing in the development of a cooked flavor as the result of a chemical reaction called the nonenzymatic browning reaction (231).

Currently available proteins are all deficient to greater or lesser extent in one or more of the essential amino acids. The recently advanced plastein reaction (232) has made it possible to use protein itself as substrate and to attach amino acid esters to the protein with high efficiency. By this method, soy bean protein (which is deficient in methionine) has been improved to the extent of having covalently attached L-methionine at 11%.

9.3. In Parenteral and Enteral Nutrition. Amino acid transfusion has been widely used since early times to maintain basic nitrogen metabolism when proteinaceous food cannot be eaten. It was very difficult to prepare a pyrogen-free transfusion from protein hydrolysates. Since the advances in L-amino acid production, the crystalline L-amino acids have been used and the problem of pyrogen in transfusion has been solved. The formulation of amino acid transfusion has been extensively investigated, and a solution or mixture in which the ratio between essential and nonessential amino acid is 1:1, has been widespread clinically. Special amino acid mixtures (eg, branched chain amino acids-enriched solution) have been developed for the treatment of several diseases (192). An enteral nutritional composition containing methionine for clinical or dietary use has been described (233).

9.4. In Medicine. Many amino acids have been used or studied for pharmaceutical purposes. L-Glutamine has been used as a remedy for gastric and duodenal ulcers. L-DOPA [L-3-(3,4-dihydroxyphenyl)alanine] has been widely employed as an antiparkinsonism agent. L-α-MethylDOPA is an effective antihypertensive drug. L-Tryptophan and 5-hydroxy-L-tryptophan [4350-09-8] are effective as antidepressants. In animal experiments it was demonstrated that L-tryptophan induces sleep. Potassium aspartate [14007-45-5] is widely used for improving disturbances in electrolyte metabolism. Calcium aspartate [10389-09-0] is known as a calcium supplement. Glutamic acid hydrochloride is

a gastric acidifier which acts to counterbalance a deficiency of hydrochloric acid in the gastric juice. L-Arginine and L-ornithine are used for ammonia detoxification. *p*-Hydroxy-D-phenylglycine, D-phenylglycine, D-cysteine (234), D-aspartic acid (235) are important as the side chains of β-lactam antibiotics (see ANTIBIOTICS, β-LACTAMS). D-Homophenylalanine (236) is a raw material for chemical synthesis of enalapril [75847-73-3], an inhibitor of angiotensin converting enzymes. D-Valine (237) is the raw material for the chemical synthesis of pyrethroid agricultural chemicals. For the details of the use of amino acids in medicine, see reference 138. Some of the recently reported medical applications include: glutamic acid decarboxylase for treating type I diabetes (238); compositions based on proline, glycine, and lysine for treating lesions and wounds (239); modulation of Bruton's tyrosine kinease and intermediates for the treatment of osteoporosis (240); and the regulation of T cell mediated immunity by tryptophan (241).

9.5. In Cosmetics. Amino acids and their derivatives occur in skin protein, and they exhibit a controlling or buffering effect of pH variation in skin and a bactericidal effect (217). Serine is one component of skin care cream or lotion. *N*-Acylglutamic acid triethanolamine monosalt is used for shampoo. Glucose glutamate is a moisturizing compound for hair and skin (242). New histidine derivatives as free antiradical agents in cosmetics has been described (243).

Cysteine is used as a reductant for cold wave treatment in place of thioglycolic acid. *N*-Lauroylarginine ethyl ester [48076-74-0] is applied as the hydrochloride as a preservative. Urocanic acid [104-98-3] which is derived from histidine is used in skin cream as a uv absorber (244).

9.6. In Industrial Chemicals. Recently, as some amino acids (eg, L-glutamic acid, L-lysine, glycine, DL-alanine, DL-methionine) have become less expensive chemical materials, they have been employed in various application fields. Poly(amino acid)s are attracting attention as biodegradable polymers in connection with environmental protection (245).

Surfactants. *N*-Acylglutamates, sodium *N*-lauroyl sarcosinate [137-16-6], and *N*-acyl-β-alanine Na salt are used in the cosmetic field as nontoxic surfactants (246). Some of them (eg, *N*-acylglutamic acid dibutylamide) are used as oil gellating agents to recover effluent oil in seas and rivers (247).

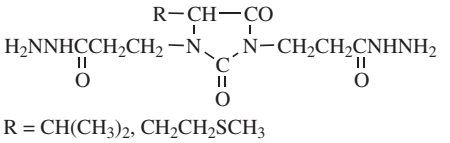
Liquid Crystals. Ferroelectric liquid crystals have been applied to LCD (liquid crystal display) because of their quick response (248). Ferroelectric liquid crystals have chiral components in their molecules, some of which are derived from amino acids (249). Concentrated solutions (10–30%) of α-helix poly(amino acid)s show a lyotropic cholesteric liquid crystalline phase, and poly(glutamic acid ester) films display a thermotropic phase (250). Their practical applications have not been determined.

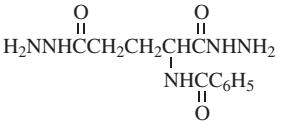
Artificial Leather. Poly(γ-methyl glutamate) [29967-97-3] that has excellent weatherability, nonyellowing, high moisture permeability, and heat resistance, was developed as the original coating agent for artificial leather (85). To improve flexibility and stretch, a block copolymer with polyurethane was developed. Poly(L-leucine) [25248-98-0] is being tested as artificial skin or wound dressing (251).

Protected Amino Acids. Various types of protected amino acids for peptide synthesis are available commercially (252).

Isocyanate. Lysine has two amino groups in the molecule and diisocyanate is prepared by reaction with phosgene. Lysine triisocyanate [69878-18-8] (LTI) is developing on a commercial scale in Japan (253).

Hardeners and Vulcanizing Agents. For epoxy resins, acylhydrazide derivatives of amino acids are used (254).





As vulcanizing agents, amino acids with or without sulfur are used for nipple rubber of babies' bottles and rubbers used in medical applications (255).

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