Conversion of α-Aminoadipic Acid to L-Pipecolic Acid by *Aspergillus nidulans**

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Two mutants of *Aspergillus nidulans*, which exhibit a growth response to L-lysine, but not to L-α-aminoadipic acid, accumulated L-pipecolic acid when grown in media containing growth-limiting concentrations of L-lysine. No accumulation occurred when these mutants were grown on optimal concentrations of L-lysine, nor was pipecolic acid accumulation observed with other types of lysine-requiring mutants of *A. nidulans*. L-Pipecolic acid accumulation, but not growth, was delayed by addition of *o*-aminobenzaldehyde to the medium. Catalytic hydrogenation of extracts of cells grown in the presence of *o*-aminobenzaldehyde gave DL-pipecolic acid, whereas catalytic hydrogenation of D-α-aminoadipic-δ-semialdehyde after prolonged treatment with *o*-aminobenzaldehyde gave D-pipecolic acid. The findings are consistent with the accumulation of Δ^1-piperideine-2-carboxylic acid in the presence of *o*-aminobenzaldehyde. Studies with C^{14}- and N^{15}-labeled α-aminoadipic acid and C^{14}-lysine indicate that the carbon chain of α-aminoadipic acid rather than that of lysine is the major precursor of pipecolic acid, and that the nitrogen atom of α-aminoadipic acid becomes the nitrogen atom of pipecolic acid.

The participation of α-aminoadipic acid in lysine biosynthesis in certain yeasts and fungi is suggested by the observation that several mutant microorganisms are able to utilize either lysine or α-aminoadipic acid for growth (Mitchell and Houlahan, 1948; Bergström and Rottenberg, 1950), and by tracer studies on such a mutant of *Neurospora crassa*, which showed that C^{14}-aminoadipic acid was converted to C^{14}-lysine without significant change in specific radioactivity (Windsor, 1951). Although there is evidence that certain microorganisms can convert α-aminoadipic acid to lysine, the enzymatic reactions involved are not yet understood. A number of plausible intermediates may be considered, including α-keto-ε-aminocaproic acid (Δ^1-piperideine-2-carboxylic acid), α-aminoadipic-δ-semialdehyde (Δ^1-piperideine-6-carboxylic acid), and pipecolic acid; the possibility that *N*-acyl derivatives of these compounds and of lysine may be involved must also be considered.

In the course of studies on the biosynthesis of lysine via the α-aminoadipic acid pathway, we observed the accumulation of an imino acid when two mutants of *Aspergillus nidulans*[1] were grown on media containing limiting amounts of lysine. This imino acid has been identified as L-pipecolic acid.[2] The present studies have also shown that the pipecolic acid produced by such mutants arises mainly from α-aminoadipic acid. Previous investigations have demonstrated that the precursor of pipecolic acid in the rat (Rothstein and Miller, 1953, 1954; Boulanger and Osteux, 1954) and in plants (Grobbelaar and Steward, 1953; Lowy, 1953) is lysine; the finding that α-aminoadipic acid is the precursor of pipecolic acid in certain mutants of *Aspergillus nidulans* is therefore unique, and appears to bear a relationship to the biosynthetic pathway of lysine in this organism.

Experimental

Materials.—DL-Pipecolic acid was obtained by catalytic hydrogenation of picolinic acid hydrochloride as described by Stevens and Ellman (1950). L-Pipecolic acid was generously provided by Dr. F. C. Steward. L-Lysine was purchased from Schwarz BioResearch, Inc. D-Lysine, L-α-

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[1] The authors thank Dr. H. Gloor, Genetisch Laboratorium der Rijksuniversiteit, Leiden, The Netherlands, for generously providing us with these lysine-requiring mutants. We are also indebted to Dr. Gloor for valuable advice concerning the conditions of culture.

[2] Accumulation of pipecolic acid by certain lysine-requiring mutants of *A. nidulans* has been observed independently by Miss Elly Pees, Genetisch Laboratorium der Rijksuniversiteit, Leiden, The Netherlands (personal communication).

aminoadipic acid, and D-α-aminoadipic acid were obtained from the corresponding racemates by enzymatic resolution (Greenstein *et al.*, 1950, 1953). ε-Hydroxy-DL-α-aminocaproic acid was a gift of E. I. duPont-de Nemours and Company, Inc. ε-*N*-Acetyl-L-lysine and α-*N*-acetyl-L-lysine were prepared according to Neuberger and Sanger (1943). α-Keto-ε-aminocaproic acid (Meister, 1954), α - keto - *N* - acetyl - ε - aminocaproic acid (Meister, 1952), α-ketoadipic acid (Gault, 1912), α-aminoadipic-δ-semialdehyde and the *N*-acetyl derivative of this compound (Aspen and Meister, 1962), piperidone carboxylic acid (Greenstein *et al.*, 1953), and o-aminobenzaldehyde (Smith and Opie, 1955) were prepared as described. D-Amino acid oxidase was isolated from hog kidney according to Negelein and Brömel (1939). N^{15}-Labeled aminoadipic acid was obtained by reductive amination of α-ketoadipic acid by the procedure of Schoenheimer and Ratner (1939). Randomly-labeled L-lysine C^{14} was obtained from the New England Nuclear Corporation. 6-C^{14}-DL-α-Aminoadipic acid was synthesized by the procedure of Rothstein (1961).

Conditions of Culture.—Mutants of *Aspergillus nidulans* were grown on the following medium. Sodium nitrate, 6.0 g, potassium chloride, 0.52 g, magnesium sulfate·$7H_2O$, 0.52 g, potassium dihydrogen phosphate, 1.52 g, zinc chloride, 0.5 mg, ferric chloride, 4.2 mg, biotin, 20 μg, sodium thiosulfate·$5H_2O$, 1.24 g, and glucose, 10.0 g; final volume, 1 liter. The *p*H was adjusted to 6.5 by addition of potassium hydroxide. The cultures were grown at 26° in test tubes or small Erlenmeyer flasks, which were shaken reciprocally at about 40 excursions per minute. The basal medium given above was supplemented with lysine, aminoadipic acid, or other compounds as described below.

Isolation of Pipecolic Acid.—The media or cell extracts were evaporated to low volume *in vacuo* and passed through a Dowex 50 column (1.5 × 15 cm; H^+ form). After the column was washed with water, the amino acids were eluted with 3 M ammonium hydroxide, and the column effluent (after concentration by evaporation *in vacuo*) was chromatographed on paper as described below. Extraction of the cells was carried out by grinding the cells obtained from 50 ml of medium in a mortar with 1.5 parts of sand, followed by extraction with 20 ml of water. The mixture was centrifuged and the supernatant solution was evaporated *in vacuo* to approximately 0.5 ml.

Pipecolic acid was isolated from the solutions obtained above by large-scale ascending paper chromatography on Whatman No. 1 paper with a solvent consisting of *n*-butanol–acetic acid–water (4:1:1). The pipecolic acid was eluted with water and the eluate was evaporated to a known volume; the imino acid was determined as described by Schweet (1954). Radioactivity was determined in an automatic gas flow counter.

Studies with D-Amino Acid Oxidase.—Samples of authentic L-pipecolic acid, DL-pipecolic acid, and the isolated pipecolic acids were treated with D-amino acid oxidase as follows: Samples containing between 0.2 and 0.7 μmole of pipecolic acid were incubated in a reaction mixture (final volume, 0.3 ml) containing flavin adenine dinucleotide (2.5 μg), sodium pyrophosphate buffer (*p*H 8.2; 30 μmoles), and 1 mg of purified D-amino acid oxidase. The mixture was shaken in air for 2 hours at 37°, and then placed in a boiling water bath for 3 minutes, cooled, and centrifuged to remove denatured protein. The supernatant solutions were analyzed for pipecolic acid. Each series of determinations included controls with authentic DL-pipecolic acid and reaction mixtures in which heat-inactivated D-amino acid oxidase was employed. It was established in each series of experiments that within experimental error, 50% of racemic pipecolic acid disappeared.

Catalytic Hydrogenation.—Catalytic hydrogenation was carried out in a Parr hydrogenation apparatus, essentially as described previously (Meister and Abendschein, 1956). In general, 0.5–5 ml of solution was mixed with 3–10 mg of platinum oxide catalyst and hydrogenation was carried out at 30 lb per square inch for 3–4 hours at 26°.

Growth Requirements of Mutants of Aspergillus nidulans.—The ability of six mutants to grow on basal media supplemented with various compounds related to lysine was determined with the medium and conditions of growth described above (Table I). One mutant (no. 50) grew to some extent in unsupplemented media, but grew more rapidly when either α-aminoadipic acid or lysine was added. None of the other mutants grew on unsupplemented media; two of these (nos. 7 and 51) grew equally well when the basal medium was supplemented with equivalent concentrations of either L-α-aminoadipic acid or L-lysine. One mutant (no. 6) exhibited a slight growth response to α-aminoadipic acid, but grew much more rapidly when provided with lysine. Two of the mutants (nos. 30 and 14) grew only when the medium was supplemented with L-lysine.

On the basis of these growth characteristics we tentatively conclude that mutants 14 and 30 are blocked between α-aminoadipic acid and lysine, and that mutants 7, 6, 51, and 50 are blocked prior to α-aminoadipic acid. Most of the studies

TABLE I

GROWTH RESPONSES OF *A. nidulans* MUTANTS[a]

Supplement to Basal Medium[b]	Mutant Number					
	14	30	7	6	51	50
None	0	0	0	0	0	[+]
L-lysine	+	+	+	+	+	+
L-α-aminoadipic acid	0	0	+	[+]	+	+

[a] Conditions of culture are given in the text. + = optimal growth; 0 = no growth; [+] = less than optimal growth. [b] 0.001 M.

reported in this paper were carried out with mutants 14, 30, and 7. We have found no significant differences between mutants 14 and 30 in the present studies. Neither mutant 14 nor mutant 7 grew on media supplemented with *N*-acetyl-α-aminoadipic-δ-semialdehyde (0.01 M), α-aminoadipic-δ-semialdehyde (0.01 M), α-keto-ε-aminocaproic acid (0.005 M), D-lysine (0.005 M), DL-pipecolic acid (0.01 M), or L-piperidone carboxylic acid (0.01 M). Both mutants grew on media containing α-*N*-acetyl-L-lysine (0.005 M) or ε-*N*-acetyl-L-lysine (0.005 M). Mutant 7 responded equally well to both L-lysine and L-aminoadipic acid in various concentrations (0.0001–0.01 M), but no growth was observed with D-α-aminoadipic acid (0.005 M) or α-ketoadipic acid (0.005 M); this mutant, in contrast to mutant 14, grew on media supplemented with ε-hydroxy-DL-α-aminocaproic acid (0.01 M).

Optimal growth (0.3–0.4 g of wet cells per 100 ml of culture medium) was observed when media were supplemented with 0.001 M L-lysine (or L-α-aminoadipic acid with mutant 7); approximately 20% of this weight of wet cells was obtained with 0.0001 M L-lysine).

Accumulation of L-Pipecolic Acid by Mutants 14 and 30.—The culture media after growth of the several mutants were examined for the presence of ninhydrin-reacting materials. None of these organisms accumulated detectable quantities of amino acids when grown on media containing an optimal concentration (0.001 M) of lysine. When a suboptimal concentration of lysine (0.0001 M) was used, only mutants 14 and 30 accumulated a compound in the medium which gave the ninhydrin color reaction on paper chromatograms. No color developed when the chromatograms were heated for 10–15 minutes at temperatures less than 60°. Pipecolic acid reacts relatively slowly with ninhydrin on paper chromatograms as compared to most of the α-amino acids, and indeed the compound was readily identified as pipecolic acid by paper chromatography in four solvent systems (Aspen and Meister, 1962).

A study of the accumulation of pipecolic acid by mutants 14 and 30 revealed that virtually no pipecolic acid appeared in the medium until 3–5 days after inoculation; after this time, the accumulation of pipecolic acid increased appreciably (Fig. 1). Separate examination of the cells and the medium indicated that about 97% of the accumulated pipecolic acid was present in the medium. Pipecolic acid was isolated from 50–200 ml batches of spent culture media as described above, and the isolated imino acid was treated with D-amino acid oxidase. Since virtually none of the isolated pipecolic acid disappeared under these conditions, it may be concluded that the accumulated pipecolic acid is of the L-configuration (Table II). Catalytic hydrogenation of the medium, treatment with 3 N hydrochloric acid (100°, 3 hours), or such treatment with hydrochloric acid followed by catalytic hydrogenation

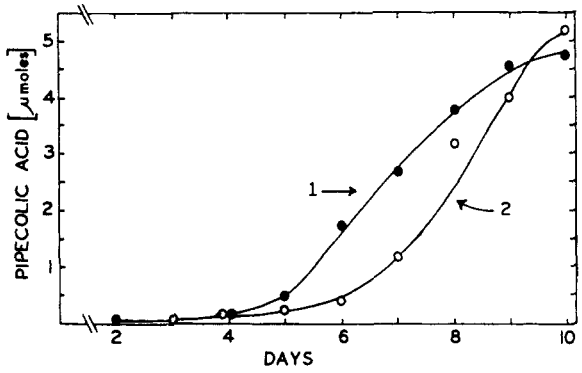

FIG. 1.—Accumulation of pipecolic acid in the medium by *Aspergillus nidulans*, mutant 30, in the presence (curve 2) and absence (curve 1) of 0.05% *o*-aminobenzaldehyde. The organism was grown on basal medium supplemented with 0.0001 M L-lysine. Ordinate, μmoles of pipecolic acid per 100 ml of medium.

did not increase the yield of pipecolic acid. Addition of L-α-aminoadipic acid (0.0025 M) did not affect the yield of pipecolic acid.

When mutants 14 or 30 were grown on media containing 0.0001 M lysine and 0.05% *o*-aminobenzaldehyde, the rate of growth was about the same as that observed in the absence of *o*-aminobenzaldehyde; however, the accumulation of pipecolic acid was delayed by approximately one day (Fig. 1). Catalytic hydrogenation of extracts of the cells 5 days after inoculation (at which time very little pipecolic acid could be detected) led to the formation of pipecolic acid. This pipecolic acid was isolated as described above and subjected to the D-amino acid oxidase test. One half of the pipecolic acid disappeared under these conditions, indicating that the pipecolic acid formed by catalytic hydrogenation was racemic. In the control experiment (Fig. 1, curve 1; growth in the absence of *o*-aminobenzaldehyde) catalytic reduction of the medium after 4 or 5 days of growth did not lead to the formation of pipecolic acid. Several experiments of this design were carried out; although accumulation of pipecolic acid began after 3 or 4 days of growth in some experiments (presumably due to use of a larger inoculum), the effects of *o*-aminobenzaldehyde and of catalytic hydrogenation were the same as observed in the experiment described in Figure 1.

When mutants blocked prior to α-aminoadipic acid were grown in media containing 0.0001 M L-lysine and 0.05% *o*-aminobenzaldehyde, catalytic reduction of the cell extracts did not yield pipecolic acid. When 5 ml of a solution containing 0.008 M D-α-aminoadipic acid-δ-semialdehyde and 0.05% *o*-aminobenzaldehyde (adjusted to *p*H 6.0) was shaken at 26° for one day and then catalytically hydrogenated, the pipecolic acid subsequently isolated was almost completely oxidized by D-amino acid oxidase (Table II)

TABLE II
OXIDATION BY D-AMINO ACID OXIDASE OF PIPECOLIC ACID OBTAINED IN VARIOUS WAYS[a]

Source of Pipecolic Acid	Oxidation by D-Amino Acid Oxidase: Initial Value (μmole)	Oxidation by D-Amino Acid Oxidase: Final Value (μmole)	% Pipecolic Acid Oxidized	Conclusion Concerning Configuration of Pipecolic Acid
DL-Pipecolic acid[b]	0.588	0.325	45	DL
L-Pipecolic acid[c]	0.360	0.348	3	L
L-Pipecolic acid[c] after treatment with PtO_2+H_2	0.822	0.802	2	L
Accumulated by *A. nidulans*, mutants 14 or 30	0.252	0.234	7	L
By hydrogenation of extracts of mutants 14 or 30 grown in presence of *o*-aminobenzaldehyde	0.456	0.228	50	DL
By hydrogenation of D-α-aminoadipic-δ-semialdehyde + *o*-aminobenzaldehyde[d]	0.666	0.042	94	D

[a] Carried out as described under Methods. [b] Synthesized by catalytic reduction of picolinic acid. [c] Isolated from plants; kindly donated by Dr. F. C. Steward. [d] Treated with *o*-aminobenzaldehyde prior to catalytic reduction (see text).

TABLE III
CONVERSION OF α-AMINOADIPIC ACID AND LYSINE CARBON TO L-PIPECOLIC ACID[a]

Supplements to Basal Media	Specific Activity (cpm/μmole): C^{14}-Precursor Added (A)	Specific Activity (cpm/μmole): Pipecolic Acid Isolated (B)	Ratio $\frac{B}{A}$
C^{12}-L-lysine (10 μmoles) + C^{14}-DL-α-aminoadipic acid (50 μmoles)	20,800	5,380	0.26
C^{12}-L-lysine (10 μmoles) + C^{14}-DL-α-aminoadipic acid (100 μmoles)	9,000	4,100	0.46
C^{12}-L-lysine (10 μmoles) + C^{14}-DL-α-aminoadipic acid (200 μmoles)	7,300	4,260	0.58
C^{14}-L-lysine (10 μmoles)	39,600	1,400	0.035
C^{14}-L-lysine (10 μmoles)	36,100	1,550	0.043

[a] *Aspergillus nidulans* (mutant 14) was grown in 100 ml of basal media supplemented as indicated above. After 5 days of growth, the pipecolic acid (3–4 μmoles) was isolated from the medium and its specific radioactivity was determined.

This result indicates that under these conditions neither treatment with *o*-aminobenzaldehyde nor catalytic reduction caused appreciable racemization. L-Pipecolic acid was not racemized by the procedure employed for catalytic hydrogenation (Table II).

Conversion of α-Aminoadipic Acid Carbon and Nitrogen to Pipecolic Acid.—Mutant 14 was grown on the basal medium supplemented with a minimal concentration (0.0001 M) of L-lysine and concentrations of C^{14}-DL-α-aminoadipate varying from 0.0005 to 0.002 M; after growth, the accumulated pipecolic acid was isolated from the medium and its specific radioactivity was determined. The results of experiments carried out with three different initial concentrations of C^{14}-α-aminoadipic acid are given in Table III; the specific radioactivity of the isolated pipecolic acid was relatively high as compared to that of the α-aminoadipate added to the medium. Some dilution of the added C^{14}-aminoadipic acid would be expected because of endogenous synthesis of α-aminoadipic acid from unlabeled precursors. Nevertheless, the specific activity of the isolated pipecolic acid attained a value that was greater than 50% of that of the added C^{14}-aminoadipic acid in one experiment; lower specific activity values were obtained when smaller quantities of C^{14}-α-aminoadipic acid were added. When this mutant was grown in media supplemented with C^{14}-L-lysine, the amount of pipecolic acid that accumulated was about the same as in the experiment in which C^{14}-α-aminoadipic acid was added; however, the specific radioactivity of the isolated pipecolic was very much lower than that of the lysine added initially to the medium (Table III).

Evidence that the amino nitrogen atom of α-aminoadipic acid is the precursor of the nitrogen atom of pipecolic acid was obtained by experiments with N^{15}-labeled α-aminoadipic acid. Mutant 14 was grown on 100 ml of basal medium supplemented with 10 μmoles of L-lysine and 50 μmoles of DL-N^{15}-α-aminoadipic acid, and a parallel experiment was carried out with 50 μmoles of

TABLE IV
CONVERSION OF α-AMINOADIPIC ACID NITROGEN TO PIPECOLIC ACID[a]

Supplement to Basal Media	Initial Atom % Excess or Specific Activity (cpm/μmole)	Pipecolic Acid Atom % Excess or Specific Activity (cpm/μmole)
N^{15}-α-Aminoadipic acid	57.5	9.8
C^{14}-α-Aminoadipic acid	13,000	1,840

[a] The experimental details are given in the text.

C^{14}-DL-α-aminoadipic acid. After 7 days, the pipecolic acid was isolated from the media and its content of isotopes was determined.[3] As indicated in Table IV, the pipecolic acid contained about 17% as much isotope as the N^{15}-α-aminoadipic acid added initially. In the experiment with C^{14}-aminoadipic acid, the specific activity of the isolated pipecolic acid was 14.2% of the original C^{14}-aminoadipic acid. These values are in reasonable agreement; that they are not identical may be attributed to experimental error and to the possibility that the inocula used in the two experiments may have been slightly different. The dilution of α-aminoadipic acid is somewhat greater in these experiments than in those described in Table III; however, the cells were permitted to grow for a longer period of time (7 days) in order to facilitate the isolation of larger quantities of pipecolic acid.[4]

DISCUSSION

Although previous studies have shown that lysine is a precursor of pipecolic acid in the rat and in certain plants, the present findings indicate that α-aminoadipic acid rather than lysine is the major precursor of the L-pipecolic acid accumulated by certain *Aspergillus nidulans* mutants. The data (Table III) indicate that some conversion of lysine to pipecolic acid takes place, but the contribution of lysine to pipecolic acid is much smaller than that of α-aminoadipic acid. The mutants accumulate L-pipecolic acid only when grown on media containing suboptimal concentrations of L-lysine. This suggests that the formation of one or more of the enzymes that are required for the synthesis of L-pipecolic acid is repressed by optimal concentrations of L-lysine. The findings are consistent with the belief that one or more of the enzymes required for L-pipecolic acid formation are also involved in the synthesis of lysine. The present studies suggest that pipecolic acid itself or closely related compounds are intermediates in lysine biosynthesis from α-aminoadipic acid. The compound that accumulated during the early phases of growth of mutants 14 and 30 in the presence of *o*-aminobenzaldehyde gave pipecolic acid on catalytic hydrogenation, and may be either a precursor or a product of pipecolic acid. It did not accumulate in the absence of *o*-aminobenzaldehyde, nor was it found in comparable studies with other mutants. Although the present data do not permit definite conclusions as to the nature of this compound, two logical possibilities are Δ^1-piperideine-2-carboxylic acid and Δ^1-piperideine-6-carboxylic acid; these compounds exist in solution in equilibrium with their respective open-chain forms, α-keto-ε-aminocaproic acid and α-aminoadipic-δ-semialdehyde. Both react with *o*-aminobenzaldehyde to form complexes (presumably the corresponding dihydroquinazolinium derivatives), and both yield pipecolic acid on catalytic hydrogenation. As indicated in Figure 2, nonenzymatic hydrogenation of Δ^1-piperideine-6-carboxylic acid would be expected to yield L-pipecolic acid, while catalytic reduction of Δ^1-piperideine-2-carboxylic acid would give DL-pipecolic acid. In the present studies, the finding of L-pipecolic acid after catalytic reduction of extracts of mutants 14 and 30 grown on media containing *o*-aminobenzaldehyde would have provided evidence that L-α-aminoadipic-δ-semialdehyde (presumably derived from L-α-aminoadipic acid) was accumulated. On the other hand, the finding of DL-pipecolic acid after catalytic hydrogenation indicates that Δ^1-piperideine-2-carboxylic acid was accumulated, provided that no racemization occurred during growth of the cells or as a result of the procedures employed. Such racemization cannot be unequivocally excluded; however, we obtained D-pipecolic acid after prolonged treatment of D-α-aminoadipic-δ-semialdehyde with *o*-aminobenzaldehyde followed by catalytic hydrogenation, and we also observed that L-pipecolic acid was not racemized under these conditions.

As indicated in the scheme given in Figure 3, pipecolic acid may be an intermediate between Δ^1-piperideine-6-carboxylic acid and Δ^1-piperideine-2-carboxylic acid (as has been suggested in the degradative metabolism of lysine in mammals [Rothstein and Miller, 1954]), or it may be formed in the *Aspergillus nidulans* mutant from either Δ^1 - piperideine - 6 - carboxylic acid (reaction 3, Figure 3) or Δ^1-piperideine-2-carboxylic acid (reaction 4a, Figure 3). The latter reaction has been

[3] We thank Dr. David Rittenberg, Miss Laura Pontecorvo, and Mr. Irving Sucher of Columbia University for the N^{15} determinations.

[4] When preparations of α-ketoadipic-δ-semialdehyde (Aspen and Meister, 1962) were incubated with crude extracts of *A. nidulans*, followed by catalytic hydrogenation, DL-pipecolic acid was obtained. No pipecolic acid was formed in control studies with heat-inactivated extracts. This result suggests the occurrence of enzymatic amination or transamination of the aldehyde group to yield α-keto-ε-aminocaproic acid. Although it is conceivable that α-ketoadipic-δ-semialdehyde is an intermediate in lysine biosynthesis, its intermediate participation in pipecolic acid formation appears to be excluded by the studies with N^{15}-α-aminoadipic acid.

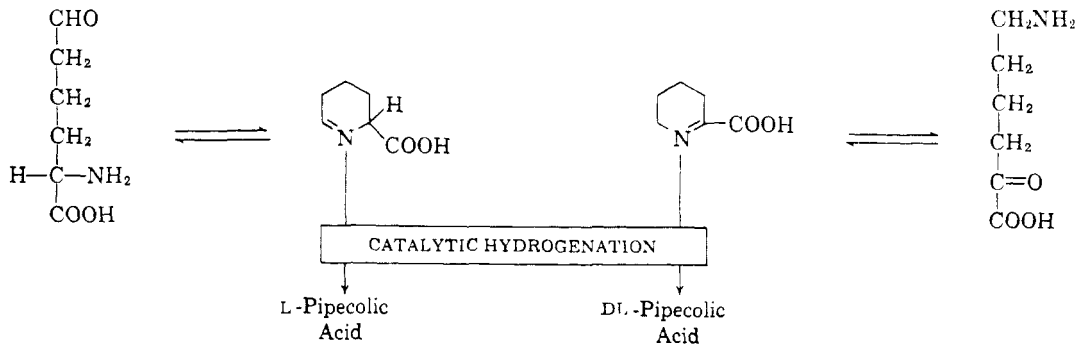

Figure 2

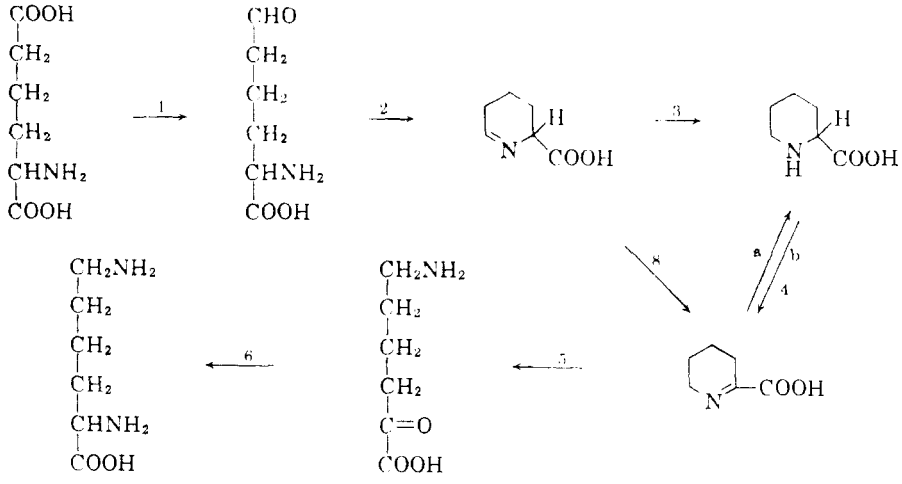

Figure 3

shown to be catalyzed by a pyridine nucleotide–dependent enzyme present in mammalian tissues, higher plants, and *Neurospora crassa* (Meister *et al.*, 1957), and we have also been able to demonstrate this activity in *A. nidulans*. If Δ^1-piperideine-6-carboxylic acid is the immediate precursor of lysine, then the formation of both pipecolic acid and Δ^1-piperideine-2-carboxylic acid would represent side reactions. On the other hand, if Δ^1-piperideine-2-carboxylic acid is the immediate precursor of lysine, and pipecolic acid is not on the direct pathway, then a mechanism for isomerizing Δ^1 - piperideine - 6 - carboxylic acid and Δ^1 - piperideine - 2 - carboxylic acid must exist. These considerations—the possibility that both Δ^1-piperideine-2-carboxylic acid and Δ^1-piperideine-6-carboxylic acid are precursors of lysine and that *N*-acyl derivatives[5] are involved in lysine biosynthesis from α-aminoadipic acid—amply justify further investigation.

[5] We have found that extracts of *A. nidulans* (mutants 14 and 51) catalyze transamination between glutamine and α-keto-ϵ-*N*-acetylaminocaproic acid to yield ϵ-*N*-acetyllysine.

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Occurrence of β-Aspartyl and γ-Glutamyl Oligopeptides in Human Urine*

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By means of column and paper chromatography 16 aspartyl di- and tripeptides and 3 glutamyl dipeptides have been isolated from pooled collections of human urine. All but one of the aspartyl peptides have the β linkage and the glutamyl peptides have the γ linkage. β-L-Aspartylglycine is the most abundant of these, and by isotopic means it was shown that it does not arise by isomerization of α-aspartylglycine during the isolation. The more abundant of these peptides, β-aspartylglycine and β-aspartylserine, were also found in 24-hour urine collections from fasted patients.

It has long been known that the amino acid content of urine increases considerably on acid hydrolysis (Henriques and Sörensen, 1909; Sauberlich and Baumann, 1946; Steele *et al.*, 1947; Hier, 1948; Woodson *et al.*, 1948; Stein, 1953). Hippuric acid and phenylacetylglutamine (Stein *et al.*, 1954) account for sizable fractions of the increase in glycine (Stein, 1953) and glutamic acid, but more of other amino acids, especially aspartic acid (Dent, 1947; Steele *et al.*, 1947; Woodson *et al.*, 1948; Hier, 1948; Uzman and Hood, 1952; Stein, 1953), are liberated on hydrolysis than exist free. That urinary peptides are involved has been demonstrated by several studies (Dent, 1947, 1948; Boulanger *et al.*, 1952; Carsten, 1952; Uzman and Hood, 1952; Bode *et al.*, 1953; Stein, 1953; Westall, 1955; Hanson and Fittkau, 1958; Ansorge *et al.*, 1961; Sarnicka-Keller, 1961) in which substances have been isolated that give free amino acids on total hydrolysis. Nearly all workers have found aspartic and glutamic acids to be among the more abundant of these.

Although many of the isolated peptides have been partially characterized, few have been completely identified. The present report describes the isolation and identification of several β-aspartyl oligopeptides and of three γ-glutamyl dipeptides. Preliminary experiments reported in abstract form (Haley *et al.*, 1961; Buchanan *et al.*, 1961) indicated that the urinary dipeptides, β-aspartylglycine and β-aspartylserine, might have been artifacts, produced by isomerization of the corresponding α peptides (*cf.* John and Young, 1954; Swallow and Abraham, 1958; Bryant *et al.*, 1959). Data presented here demonstrate that L-aspartylglycine is excreted predominantly in the β form. With this finding, and the recent identification of β-L-aspartyl-L-histidine as a normal urinary constituent (Kakimoto and Armstrong, 1961), it is likely that the fourteen other β-aspartyl di- and tripeptides described here are actually excreted with the β structure and are not artifacts.

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Materials and Methods

Urine Collections.—Urine collections (24 hour) were from male hospital patients or laboratory employees, and all voidings were refrigerated immediately and desalted the day that the collection ended. Patients were selected who had no known metabolic or debilitating disease, but two of these had been subjected to gastrointestinal surgery and had received neither oral nor parenteral nitrogen-containing nutriments for 3 and 4 days prior to the start of the collection. In some instances α-L-aspartylglycine-1-C^{14} (10 mg, 50,000 dpm) was added to the urine before desalting or to the urine vessel prior to the collection.

Paper Chromatography.—The solvents used were: (I), *n*-butanol–acetic acid–water (4:1:1); (II), methylethylketone – propionic acid – water (15:5:6); (III), *n*-butanol–acetic acid–pyridine–water (15:3:10:12); (IV), methanol–water–pyridine (20:5:1); (V), methylethylketone–*t*-butanol–