A $^{15}$N NMR Study on D-Lysine Metabolism in *Neurospora crassa*  

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The metabolic relationship of d-lysine, l-lysine, and l-pipecolic acid has been investigated in *Neurospora crassa*. Kinetic experiments show that radioactivity from d-lysine is efficiently incorporated into l-pipecolic acid and that this metabolite is converted to l-lysine. The $\alpha$-amino group from d-[a-$^{15}$N]lysine is lost in the course of its conversion to l-pipecolic acid and is trapped by pyruvate and $\alpha$-keto glutarate to give l-[a-$^{15}$N]alanine and l-[a-$^{15}$N]glutamic acid.

These amino acids are devoid of any label, however, when d-[e-$^{15}$N]lysine is applied to the fungus. As determined by mass and $^{15}$N NMR spectrometry the label from d-[e-$^{15}$N]lysine migrate via l-pipecolic acid into the $\alpha$ position of l-lysine, i.e. d-[e-$^{15}$N]lysine is converted to l-[a-$^{15}$N]lysine. l-Pipecolic acid functions as an intermediate in this conversion.

$\delta$-Amino acids and some of their derivatives occur rather widely in living organisms (1). A number of reactions, such as racemization (2), reversible transamination (3), oxidation (4, 5), N-acetylation (6), and N-malonylation (7) are available for their biosynthesis and metabolism. N-Malonylation, however, does not occur in d-lysine metabolism (1). Instead, when administered to such diverse organisms as Hordeum vulgare (8), Zea mays (9), Rattus sp. (10, 11), Pseudomonas putida (12-14), *Neurospora crassa* (15), Nicotiana glauca, Sedum sarmentosum, Sedum acre (16), and Triglochin maritima (17), d-lysine (1, Fig. 1) is converted with high efficiency to pipecolic acid (4, Fig. 1). This amino acid (4, Fig. 1) has been shown in most cases to have the $\alpha$ configuration (1, 15).

Catabolism of l-pipecolic acid (4, Fig. 1) is initiated by oxidation at C atom 6, a reaction which does not affect the chiral center at C atom 2 (Fig. 1). This reaction occurs in bacteria (18, 19), fungi (15, 20, 21), and possibly also in higher plants (22). While in bacteria $\Delta^1$-piperideine-6-carboxylic acid (5, Fig. 1) is further degraded to glutamic acid (18, 19), this reaction is not observed in *Neurospora* (15) and *Rhodotorula glutinis* (21) where l-pipecolic acid is metabolized to l-lysine. Thus, pipecolic acid is an intermediate in the conversion of d-lysine to l-lysine (Fig. 1). This finding is derived from experiments with *N. crassa* Shear and Dodge wild type strain SY 7 A No. 622 from the Fungal Genetics Stock Center in Hanover, N. H. (15).

An alternative model for the metabolic relationship between d-lysine, l-lysine, and l-pipecolic acid has been proposed (Fig. 2) based on experiments with *Rhizoctonia leguminicola* (23) and a lysine auxotroph strain of *N. crassa* No. 33933 (24). According to this model d-lysine is racemized to l-lysine which then gives rise to l-pipecolic acid (Fig. 2). This reaction sequence has been postulated as part of a scheme which is "peculiar to and generalized among yeasts and fungi" (24).

In an attempt to distinguish between these two schemes we have prepared d-[a-$^{15}$N]lysine and d-[e-$^{15}$N]lysine and applied each substrate to our *N. crassa* wild type strain. If the scheme depicted in Fig. 1 were operative, $^{15}$N from the $\alpha$ position of d-lysine would be removed (step 1 $\rightarrow$ 2) during the conversion to l-lysine, whereas if the scheme shown in Fig. 2 were operative, the $^{15}$N should be retained in the $\alpha$ position of l-lysine (step 1 $\rightarrow$ 7) (25). $^{15}$N label from the $\epsilon$ position of d-lysine, however, should migrate into the $\alpha$ position of l-lysine in the first case (Fig. 1), whereas in the second case (Fig. 2) it should be found in the $\epsilon$ position of l-lysine. Since $^{15}$N NMR spectrometry of amino acids became available recently (26), this technique was used to investigate this problem.

### MATERIALS AND METHODS

The source of this strain of *N. crassa* has been described previously (15). Growth Curves and Dry Weight Determination—This was carried out as described previously (15). Chemicals and Isotopes—Radioactive compounds were purchased from companies as described previously (15). d-[a-$^{15}$N]lysine was synthesized by the method of Neuberger and Sanger (27) with one slight modification. Instead of ammonia the L-a-bromo-c-benzaminoacrylic acid was treated with $^{15}$NH$_3$NO$_3$ and an equimolar amount of NaOH in a gas-tight flask. Since this reaction involves a Walden inversion, d-[e-$^{15}$N]lysine was the product. Yield was 54% with reference to the $^{15}$NH$_3$NO$_3$ employed.

d-[e-$^{15}$N]lysine was prepared according to Mizon and Mizon (28). Yield was 32% with reference to the potassium salt of [1-$^{15}$N]phthalalimide. The racemic amino acid was dissolved in phosphate buffer (50 ml), 0.2 M, pH 6.0, and L-lysine decarboxylase (500 mg, type I, Sigma Chemical Co.) added. Incubation was carried out for 2 h at 37°C. The reaction mixture was then passed over a column (2 x 10 cm) of Dowex 50-H$^+$, the column washed (H$_2$O), and the resulting d-[e-$^{15}$N]lysine eluted with aqueous ammonia (2 M). The eluate was evaporated to dryness and the treatment with L-lysine decarboxylase was repeated as before. d-[e-$^{15}$N]lysine was again isolated using an ion exchange column. The amino acid was crystallized as the monohydrochloride.

Assay of Enantiomeric Purity of d-[1-$^{15}$N]Lysine Samples—d-Lysine (1 mg) was incubated (37°C, 1 h) with L-lysine decarboxylase (10 mg) and decreasing amounts of l-lysine (1.0, 0.5, 0.2, 0.1, and 0.08% of d-lysine) in phosphate buffer (0.2 M, pH 6.0, 1 ml). The incubation mixtures were then freeze-dried and the residues applied to thin layer plates (silica gel, 0.25 mm). The chromatogram was developed in chloroform:methanol:ammonia (25%) (22:1) (lysine $R_f$ = 0.21; cadaverine $R_f$ = 0.44). d-Lysine and cadaverine (derived from L-lysine by decarboxylation) were made visible by spraying with ninhydrin. Cadaverine was still detectable when the d-lysine employed contained 0.1% of L-lysine. When the $^{15}$N-labeled samples of l-lysine which had been synthesized as described above were checked for the presence of l-lysine by this method no cadaverine was detectable. Thus, the synthetically prepared samples of d-[a-$^{15}$N]- and d-[e-$^{15}$N]lysine contained if at all less than 0.1% L-lysine.

Mass Spectrometry of Amino Acids—This was carried out on a Varian MAT SM17 (70 eV). Before measuring $^{15}$N-labeled amino acids (L-glutamic acid, L-alanine, L-lysine, and L-pipecolic acid) the
mass spectra of unlabeled specimens isolated from the Neurospora mycelium (L-glutamic acid, L-alanine, L-lysine) or culture broth (L-pipecolic acid) were determined as a reference to calculate \(^{15}\text{N}\) enrichments. While spectra of L-pipecolic acid, L-alanine, and L-glutamic acid were determined directly, D- or L-lysine was analyzed as the tetramethyl derivative (29). The \(^{15}\text{N}\) enrichment was calculated from the ratio of the following peak heights: \(m/e\) 89 and 90 (L-alanine, molecular ion); \(m/e\) 102 and 105 (L-glutamic acid, base peak and amine fragment); \(m/e\) 84 and 85 (L-pipecolic acid, base peak, and imine fragment); \(m/e\) 102 and 103 (fragment containing the \(\alpha\)-\(^{15}\text{N}\) of tetramethyllysine); 58 and 59 (base peak and fragment containing the \(\epsilon\)-\(^{15}\text{N}\) of tetramethyllysine). The peaks were determined amounts \(4.59\) \((4.62\) times, alanine, and L-glutamic acid were isolated from the protein after separation of the mycelium from the medium. Free amino acids were determined directly, D- or L-lysine was analyzed as the tetramethyl derivative (29). The \(^{15}\text{N}\) enrichments was calculated with dilution of known amounts of unlabeled D-lysine, a good correlation between the calculated and the measured (in parentheses) \(^{15}\text{N}\) enrichments was observed: \(17.61\) \((17.34 \pm 0.63)\); \(9.65\) \((9.82 \pm 0.44)\); \(6.67\) \((6.72 \pm 0.38)\); \(4.59\) \((4.62 \pm 0.38)\); \(2.28\) \((2.19 \pm 0.29)\). Natural abundance of \(^{15}\text{N}\): 0.37%.

**Isolation of Amino Acids**—L-Pipecolic acid and D-lysine were isolated from the medium as described previously (15). L-Lysine, L-alanine, and L-glutamic acid were isolated from the protein after separation of the mycelium from the medium. Free amino acids were removed by extraction of the mycelium with hot water (100°C, 2 times, 1 h). The extracted mycelium was stirred in NaOH (1 M, room temperature, 24 h) and the NaOH separated by filtration. Trichloroacetic acid (3 M) was added to the filtrate until the protein precipitated. The precipitate was centrifuged off, the pellet resuspended in water, again centrifuged, and the pellet suspended in HCl (6 M). The suspension was transferred to glass tubes which were sealed under N\(_2\) and kept at 110°C for 24 h. The hydrolysate was diluted with water and filtered and amino acids were isolated from the filtrate by ion exchange (Dowex H\(^+\)) and paper chromatography (15).

**RESULTS**

In a set of pilot experiments conditions were found under which the \(^{15}\text{N}\)-labeled precursors were successfully applied to the fungus. Increasing amounts of D- and L-lysine were added to liquid cultures of the fungus at the beginning of the logarithmic growth phase (15). The incubations were terminated after 22 h by separation of the mycelium from the medium. The mycelium was dried (24 h, 80°C) and weighed. It was shown that growth of the fungus was essentially unaffected by increasing amounts of D- or L-lysine in the medium. When the concentration of D-lysine reached 5 g/liter of medium, however, the mycelium turned red. For all further experiments a concentration of 500 mg of D- or L-lysine/liter of medium was chosen.

L-Pipecolic acid (4) was isolated from the media in which N. crassa had been incubated with increasing amounts of D- or L-lysine, respectively. Isolation and quantitative determination of L-pipecolic acid with ninhydrin showed that the imino acid was detectable in the culture broth from those experiments where D-lysine had been applied but not when L-lysine had been used as a potential precursors (Fig. 3). In another experiment a mixture (500 mg) of D-[6-\(^{14}\text{C}\)]lysine and D-[6-H\(^3\)]lysine was applied to the culture (Table I). While retention of tritium activity in L-lysine would be expected if the pathway shown in Fig. 2 were operative, 50% loss of

![Fig. 1. Lysine conversion. Conversion of D-lysine to L-lysine via L-pipecolic acid.](image)

![Fig. 2. Metabolic relationships. Metabolic relationship between D-lysine, L-lysine, and L-pipecolic acid according to Guengerich and Broquist (24).](image)

![Fig. 3. L-Pipecolic acid isolation. L-Pipecolic acid isolated 22 h after application of increasing amounts of D- or L-lysine to the culture medium of N. crassa.](image)

**Table I**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Amount (µmol)</th>
<th>Radioactivity (dpm)</th>
<th>Specific radioactivity (dpm/µmol)</th>
<th>Specific incorporation (%)</th>
<th>(^{3}\text{H})/(^{14}\text{C}) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-[6-(^3\text{H})]lysine</td>
<td>342.47 (500.0)</td>
<td>579.4 × 10(^2) (H)</td>
<td>16.9 × 10(^2) (H)</td>
<td>18.4 (i.e. 100%)</td>
<td>(18.4) (i.e. 100%)</td>
</tr>
<tr>
<td>D-[6-(^{14}\text{C})]lysine</td>
<td>101.4 (14.8)</td>
<td>0.9 × 10(^2) (H)</td>
<td>0.9 × 10(^2) (H)</td>
<td>5.8 (H)</td>
<td>9.5 (i.e. 51.6%)</td>
</tr>
<tr>
<td>L-Lysine from protein</td>
<td>101.4 (14.8)</td>
<td>0.1 × 10(^2) (H)</td>
<td>0.1 × 10(^2) (H)</td>
<td>11.2 (H)</td>
<td>(11.2) (H)</td>
</tr>
</tbody>
</table>

**Application to N. crassa (1 liter medium)** of a mixture of D-[6-\(^{14}\text{C}\)]lysine and D-[6-\(^3\text{H}\)]lysine and isolation of L-lysine from the protein.
tritium (step 4→5, see Fig. 1) would be expected if the alternate scheme were valid.

A $^{13}H/^{14}C$ ratio of the isolated L-lysine which was neither 50% nor 100% of that of the administered lysine would indicate that during the metabolism of d-$[6^{-13}H,6^{-14}C]$lysine a nonspecific transamination of the $\varepsilon$-nitrogen atom had occurred. If so, experiments with $\varepsilon$-$^{15}N$-labeled lysine would be unsuitable to distinguish between the two schemes.

Results listed in Table I show that nonspecific transamination of the $\varepsilon$-amino group did not occur, that the reaction sequence in Fig. 1 rather than that in Fig. 2 was operative, and that a high specific incorporation (i.e. specific radioactivity in product/specific radioactivity in precursor) × 100) (11.2%) of label from d-$[6^{-14}C]$lysine into L-lysine took place.

Evidence for the scheme depicted in Fig. 1 was also obtained from kinetic experiments (Fig. 4). When d-$[6^{-14}C]$lysine (500 mg/liter) was applied to the culture, the radioactive amino acid disappeared gradually from the medium. Radioactivity simultaneously appeared in L-pipecolic acid and reached a maximum about 20 h after application of the tracer. The maximum radioactivity in L-lysine isolated from cellular protein appeared, however, 50 h after application of the precursor. This is in agreement with a sequence D-lysine $\rightarrow$ L-pipecolic acid $\rightarrow$ L-lysine. When, L-$[4.5^{-2}H]$lysine (500 mg/liter) was applied to the cultures, only negligible activity was detected in L-pipecolic acid (Fig. 4). As expected, the L-amino acid applied was incorporated into protein from which it was isolated after hydrolysis (Fig. 4).

When values of specific incorporation (i.e. specific radioactivity in product/specific radioactivity in precursor) × 100) rather than incorporation (i.e. (total radioactivity in product/total radioactivity in precursor) × 100) alone are recorded, it can be seen that at any time during the experiment the specific activity of the L-lysine isolated from protein was lower than the specific activity of L-pipecolic acid (Fig. 5, diagram D). This is in agreement with the expectation that L-pipecolic acid is a precursor of L-lysine (Fig. 1) and not vice versa (Fig. 2).

Experiments were also carried out with $^{15}N$-enriched precursors. d-$[\alpha^{-15}N]$lysine was administered to the fungus and after 60 h of incubation L-pipecolic acid was isolated from the medium whereas L-lysine, L-glutamic acid, and L-alanine were isolated from the protein after hydrolysis. No $^{14}N$ label was found in L-pipecolic acid, and neither the $\varepsilon$- nor the $\alpha$-nitrogen atom of L-lysine showed significant enrichment in $^{15}N$. L-Alanine and L-glutamic acid, however, were enriched in $^{15}N$ indicating that the corresponding $\alpha$-keto acids, pyruvic acid, and $\alpha$-ketoglutaric acid, respectively, served as acceptors for the $\alpha$-amino group of D-lysine (Table 11). It follows that according to Fig. 2 $^{15}N$ enrichment in the $\alpha$-nitrogen atom of L-lysine was to be expected and that according to Fig. 5 more than 10% enrichment should have been observed. The very minor enrichment (0.94 ± 0.09 atom % excess, Table II) shows again that the scheme depicted in Fig. 2 is at best insignificant.

Further support of this view emerged from an experiment with d-$[\varepsilon^{-15}N]$lysine (Table III). L-Pipecolic acid was highly enriched in $^{15}N$ (almost 50%) as predicted by Figs. 1 and 2.

Table II

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount isolated</th>
<th>$^{15}N$ enrichment</th>
<th>$^{15}N$ atom% excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Pipecolic acid</td>
<td>2.8</td>
<td>$-0.3 \pm 0.01$</td>
<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>8.9</td>
<td>$11.2 \pm 0.85$</td>
<td></td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>14.2</td>
<td>$4.9 \pm 0.68$</td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>14.1</td>
<td>$0.94 \pm 0.09$</td>
<td>$\alpha-N$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$-0.18 \pm 0.01$</td>
<td>$\varepsilon-N$</td>
</tr>
</tbody>
</table>

Table III

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount isolated</th>
<th>$^{15}N$ enrichment</th>
<th>$^{15}N$ atom% excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Pipecolic acid</td>
<td>4.2</td>
<td>$47.3 \pm 1.84$</td>
<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>8.7</td>
<td>$-0.77 \pm 0.04$</td>
<td></td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>11.9</td>
<td>$0.19 \pm 0.01$</td>
<td></td>
</tr>
<tr>
<td>L-Lysine</td>
<td>20.0</td>
<td>$10.63 \pm 0.85$</td>
<td>$\alpha-N$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$9.07 \pm 0.003$</td>
<td>$\varepsilon-N$</td>
</tr>
</tbody>
</table>
Alanine and L-glutamic acid isolated from the experiment with D-[α-15N]lysine were not enriched in 15N, indicating that the 15N was not randomized. According to Fig. 2 entry of label obtained (Table III) and thus the scheme in Fig. 1 is clearly demonstrated more easily by 15N NMR spectrometry. Fig. 6 shows the proton-decoupled 15N NMR spectra of synthetic DL-[α-15N]lysine (b) and DL-[ε-15N]lysine (c). The signal due to ε-15N has a chemical shift, relative to NH4NO3, of 19.31 ppm. When D-[ε-15N]lysine was applied to N. crassa, the L-lysine isolated after incubation showed a proton-decoupled 15N NMR spectrum (Fig. 1) resulting from deamination of D-lysine (Fig. 1) undergoing ring closure to give Δ1-piperideine-2-carboxylic acid (3, Fig. 1), a compound which is stereospecifically reduced to L-pipeolic acid (4, Fig. 1) (33).

L-Pipecolic acid does not seem to be degraded in mutants of N. crassa (24) but in other organisms it has been shown to be oxidized. An enzyme has been isolated from Pseudomonas which catalyzes step 4 to 5 (Fig. 1) (18, 19). This reaction has also been observed in R. glutinis (21), N. crassa wild type strain (15), and in Acacia plants (22). The oxidation product Δ1-piperideine-6-carboxylic acid (5) is in equilibrium with α-aminoacidic-δ-semialdehyde (6) (34). This compound is a known intermediate of the α-aminoacidic acid pathway for the biosynthesis of L-lysine (7) (21). Conversion of L-pipecolic acid to L-lysine is also indicated by the observation (21) that L-pipecolic acid supports the growth of lysine auxotrophs of R. glutinis.

The data presented here clearly show that L-pipecolic acid is an intermediate in the conversion of D-lysine to L-lysine. Enantiomers of amino acids are usually interconverted by enzymic racemization or reversible transamination. The reaction sequence depicted in Fig. 1 constitutes yet another mechanism for epimerization, but whereas racemization and transamination are reversible, the process described here is not. This is evident from the fact that D-lysine, but not L-lysine serves as a precursor of L-pipecolic acid.

**Fig. 6.** 15N NMR spectra. a, L-[15N]lysine (22 mg, 10,100 scans) isolated after incubation of N. crassa with D-[ε-15N]lysine, b, synthetic DL-[α-15N]lysine (1.2 mg, 5,000 scans); and c, synthetic DL-[ε-15N]-lysine (8.5 mg, 1,300 scans). Chemical shifts are given relative to external 15NH4NO3 in D2O/H2O. The spectra were recorded on a Bruker WH 270 pulsed Fourier transform spectrometer operating at 27.36 MHz at ambient temperature, with broad band proton noise decoupling. Lysine spectra were obtained from aqueous solutions (3.5 ml) at pH 2.6. Repetition time 4 s, pulse time, 35 μs.

Alanine and L-glutamic acid isolated from the experiment with D-[ε-15N]lysine can be demonstrated more easily by 15N NMR spectrometry. Fig. 6 shows the proton-decoupled 15N NMR spectra of synthetic DL-[ε-15N]lysine (b) and DL-[ε-15N]lysine (c). The signal due to ε-15N has a chemical shift, relative to 15NH4NO3, of 19.31 ppm. The signal due to ε-15N appears at 11.95 ppm. When D-[ε-15N]lysine was applied to N. crassa, the L-lysine isolated after incubation showed a proton-decoupled 15N NMR spectrum (a) with a signal corresponding to that of synthetic [ε-15N]lysine. Thus, D-[ε-15N]lysine had been converted to L-[ε-15N]lysine. This observation is predicted by the scheme shown in Fig. 1 but cannot be explained by the scheme in Fig. 2.

**DISCUSSION**

D-Lysine is a naturally occurring amino acid (30). Race-masses generating D-lysine from L-lysine are known (2). D-Lysine is converted efficiently into L-pipecolic acid in animals (10, 11), bacteria (12-14), N. crassa (15), and various higher plants (16, 17).

It has been reported (23), however, that in a fungus (R. leguminicola) the incorporation of L-lysine into L-pipecolic acid was higher than the incorporation of D-lysine into L-pipecolic acid. This was taken as evidence that L-lysine is the precursor of L-pipecolic acid. Incorporation efficiencies, however, are unsuitable (16) to characterize dynamic processes like uptake of a labeled precursor, biosynthesis, and metabolism of a product. We have therefore used kinetic experiments (Ref. 15 and "Results") to elucidate the relative contribution of D- and L-lysine to L-pipecolic acid biosynthesis. These data clearly show that it is D-lysine and not L-lysine which gives rise to L-pipecolic acid. Pitfalls in the interpretation of incorporation efficiencies have also been encountered in experiments designed to elucidate the biosynthesis of the antibiotic pyrrolnitrin from D- or L-tryptophan (31).

The conclusion that L-lysine is the precursor of L-pipecolic acid had also been drawn from the results of experiments where a mixture of doubly labeled isomers of lysine was applied to a mutant of N. crassa (24). While this feeding technique was shown (16) to be the method of choice in attempts to establish precursor configuration in precursor product relationships, in the experiments under discussion (24) lysine-less mutants of N. crassa were used (24) which require for growth an excess of L-lysine, i.e. one of the isomers to be tested as a possible precursor of L-pipecolic acid. In spite of this, D-lysine was still found to contribute significantly to the biosynthesis of L-pipecolic acid (24).

The first enzyme involved in the catabolism of D-lysine may be an amino acid oxidase or a specific D-amino acid transaminase. In N. crassa both the L- and the D-amino acid oxidases are present. Their activity depends on the developmental stage and the growth conditions of the fungus. However, D-lysine was shown to be the precursor of L-pipecolic acid in this mold (15) regardless of the developmental stage of the fungus and conditions of growth. This may suggest that it is not a D-amino acid oxidase which catalyzes deamination of D-lysine but a specific D-transaminase. Such enzymes are known to use pyruvate and α-ketoglutarate as amino group acceptors (32). The fact that label from D-[α-15N]lysine was incorporated into L-alanine and L-glutamic acid (Table II) is consistent with this view.

This observation also confirms previous findings (20, 24) that in the course of conversion of lysine to L-pipecolic acid the ε-amino group is retained whereas the α-amino group of lysine is removed. The α-keto-ε-aminocaproic acid (3, Fig. 1) resulting from deamination of D-lysine (Fig. 1) undergoes ring closure to give Δ1-piperideine-2-carboxylic acid (3, Fig. 1), a compound which is stereospecifically reduced to L-pipecolic acid (4, Fig. 1) (33).
The proposition that D-lysine is converted to L-lysine via L-pipecolic acid finds its strongest support in the observation that D-[L-\(^{15}\)N]lysine is converted to L-[\(^{15}\)N]lysine. This evidence confirms that the scheme depicted in Fig. 1 is operative and proves conclusively that the scheme in Fig. 2 cannot be "peculiar to and generalized among yeast and fungi," as had been claimed earlier (24).

Finally, it should be mentioned that we cannot exclude the possibility that a racemase capable of catalyzing the interconversion of the enantiomers of lysine may be present in *Neurospora*. The fact that a very minor incorporation of label from D-[\(^{15}\)N]lysine into L-pipecolic acid (15) is observed could be attributed to the presence of such a racemase. Although the metabolic relations depicted in Fig. 1 are not only detectable in our *N. crassa* stain we take into account that they may be operative in other organisms including *R. leguminicola* (23) and *N. crassa* No. 33933 (24). We have demonstrated, however, that the metabolic steps shown in Fig. 1 are not only detectable in our *N. crassa* wild type strain but also in intact plants and cell suspension cultures of *N. glauca*.\(^1\)

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REFERENCES


\(^1\) N. Fangmeier and E. Leistner, manuscript in preparation.