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Lysine Catabolism in Barley (*Hordeum vulgare* L.)

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ABSTRACT

Lysine catabolism in seedlings of barley (*Hordeum vulgare* L. var. Emir) was studied by direct injection of the following tracers into the endosperm of the seedlings: aspartic acid-3-^{14}C, 2-aminoadipic acid-1-^{14}C, saccharopine-^{14}C, 2,6-diaminopimelic acid-1-(7)-^{14}C, and lysine-1-^{14}C. Labeled saccharopine was formed only after the administration of either labeled 2,6-diaminopimelic acid or labeled lysine to the seedlings. The metabolic fate of the other tracers administered also supported a catabolic lysine pathway via saccharopine, and apparently proceeding by a reversal of some of the biosynthetic steps of the 2-aminoadipic acid pathway known from lysine biosynthesis in most fungi. Pipecolic acid seems not to be on the main pathway of L-lysine catabolism in barley seedlings.

A considerable diversity of pathways exists not only for the biosynthesis but also for the catabolism of lysine. At least three catabolic routes have been proposed to operate in fungi (17) and animals (4), aerobic bacteria (12, 34, 36), and anaerobic bacteria (35), respectively. Studies on lysine catabolism in higher plants are scarce and no general pathway has been established. The conversion of lysine to alkaloids occurs in a limited number of plants (10, 22). However, the following observations and results on the presence of possible products of lysine catabolism and on catabolic pathways have been reported. 2-Aminoadipic acid is a common constituent of higher plants (11, 18). Saccharopine, N^6-(2'-glutaryl)lysine, has been isolated from the seed kernels of *Fagopyrum esculentum* (buckwheat) (32), and recently from the inflorescence of *Reseda odorata* (H. Sørensen, personal communication). The high amount isolated from *Reseda odorata* establishes saccharopine as a constituent of higher plants. Both 2-aminoadipic acid and saccharopine are intermediates of lysine biosynthesis in fungi (25), but are also known as products of lysine catabolism in animals (3, 4, 13). Lysine biosynthesis in those higher plants hitherto examined proceeds via the 2,6-diaminopimelic acid pathway (2, 29, 30). The operation of the 2-aminoadipic acid pathway at a very low rate has not been excluded because 2-aminoadipic acid and saccharopine are incorporated to a low extent into lysine (29, 31). Thus the position of saccharopine and 2-aminoadipic acid in lysine metabolism of plants is not clear. Observations in *Phaseolus vulgaris* (8, 23) indicate a catabolic pathway of lysine with pipecolic acid as a key intermediate. Δ^1-Piperideine-2-carboxylic acid is reduced to L-pipecolic acid by *Pisum sativum* and *Phaseolus radiatus* (26). An amine oxidase in *Pisum sativum* converting L-lysine to 2-amino-6-oxocaproic acid has also been reported (24). In *Acacia phyllodes*, 2-aminoadipic acid and pipecolic acid are formed from lysine via 2-amino-6-oxocaproic acid (5). Unfortunately, all these studies are conducted with plants belonging to the Leguminosae where pipecolic acid and its derivatives are unusually common (9). This plant family might possess methods for pipecolic acid biosynthesis not characteristic for other plant families and not necessarily related to lysine catabolism. Recent experiments with *Nicotiana glauca* and *Sedum acre* conducted by use of double labeled precursors indicate that more than 90% of the labeled pipecolic acid formed in these plants originate from D-lysine (22). In order to clarify some of these points, a study of lysine catabolism in a nonleguminous plant, barley, was undertaken. The different routes of lysine biosynthesis and catabolism which are discussed in this paper are shown in Figure 1.

MATERIALS AND METHODS

Radioisotopic Chemicals. DL-Lysine-1-^{14}C, L-[UL-^{14}C]lysine and 2,6-diaminopimelic acid-1-(7)-^{14}C were obtained from Calatomic, Los Angeles. DL-Aspartic acid-3-^{14}C was obtained from Mallinckrodt Radiochemical Department, St. Louis, Missouri.

Synthesis of Labeled Saccharopine (N^6-(2'-glutaryl)-L-lysine-[1,2,3,4,5,6]-^{14}C). Chemical synthesis of labeled saccharopine was performed by a Strecker synthesis (19). The reaction was carried out in a 500-μl ampoule, and the reaction mixture consisted of 0.730 mg of α-ketoglutaric acid, 0.822 mg of L-lysine, HCl, 2.207 mg of KCN, 20 μl of L-[UL-^{14}C]lysine (8.11 μCi, 10 mCi/mmole) and 50 μl of 1 N NaOH. The clear solution was kept at 50 to 52 C for 24 hr. The reaction mixture was lyophilized, dissolved in H_2O, and applied to a strongly acidic ion exchange resin (Dowex 50W × 8, 200-400 mesh, H^+, 0.2 × 1.5 cm). After washing with 8 ml of H_2O, labeled saccharopine was eluted with 8 ml of 1 N pyridine. After lyophilization and solution in H_2O, paper chromatography of the pyridine eluate on Whatman No. 3MM followed by autoradiography showed two strongly labeled spots corresponding to saccharopine and pyrosaccharopine and several much weaker spots. Co-chromatography of authentic saccharopine and pyrosaccharopine with aliquots of the eluted two major compounds proved the identity. Radiochemical yields were 0.44 μCi of saccharopine and 0.35 μCi of pyrosaccharopine.

To increase the yield of saccharopine, untransformed L-lysine was recovered from the ion exchange resin by elution with 8 ml of 1 N NH_3. After evaporation to dryness *in vacuo*, the Strecker synthesis was repeated. This was done twice, and the total radiochemical yields were 1.04 μCi of saccharopine (13%) and 0.80 μCi of pyrosaccharopine (10%).

Germination and Growth of Plant Material. Seeds of *Hordeum vulgare* L. var. Emir were sterilized in absolute ethyl alcohol for 5 min, rinsed in three changes of redistilled H_2O, and kept in redistilled H_2O overnight with constant aeration. After imbibition, seeds were resterilized in absolute ethyl alcohol for 30 sec, washed with sterile H_2O as described above, and grown 3 days on moist, sterile filter paper in the dark. After this period seedlings were placed in a water culture medium of Hoagland solution (14) with constant aeration and subjected to a regime consisting of 20 hr of light and 4 hr of dark in a growth chamber at 19 C and 65% humidity.

Tracer Experiments. Each tracer was dissolved in H_2O to give

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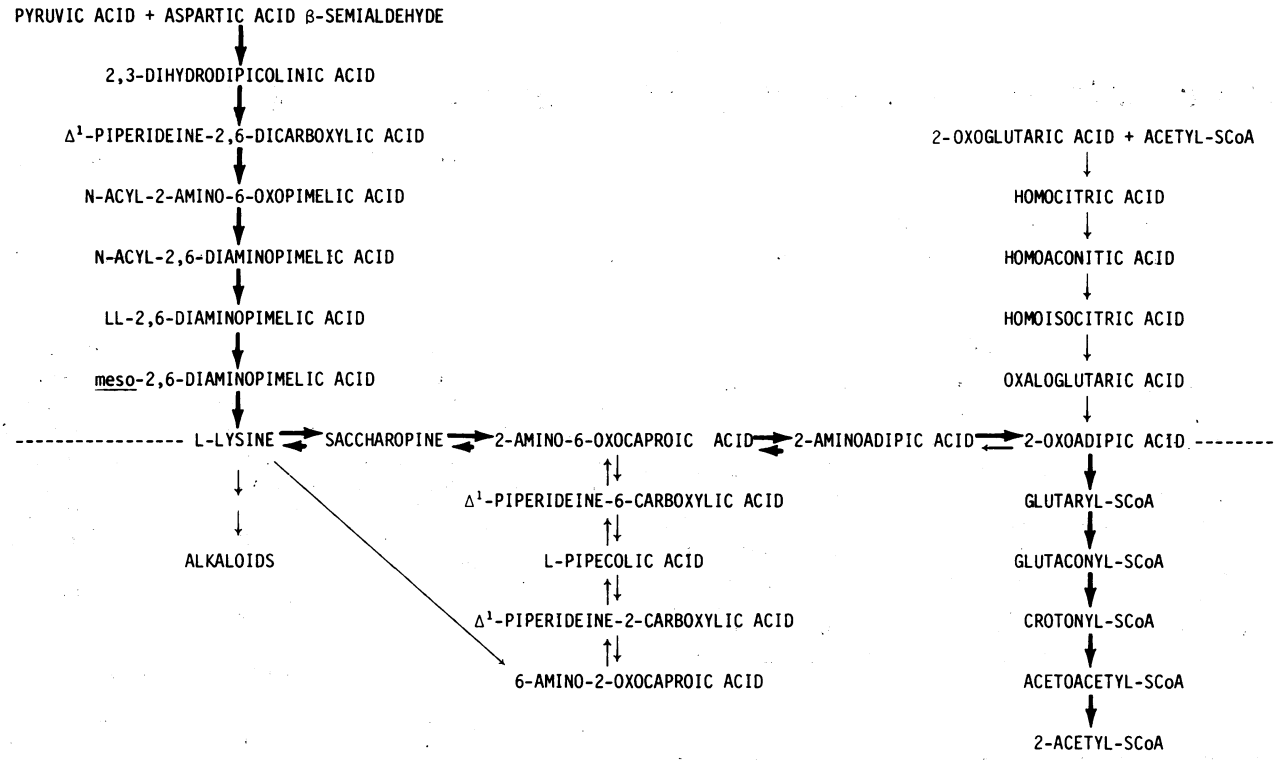

FIG. 1. Proposed biosynthetic and catabolic lysine pathways in higher plants. Reactions marked with heavy arrows are shown in this and previous work (29, 30) to be operating in barley seedlings.

a solution containing 0.2 μCi/μl. The solution of saccharopine contained only 6 nCi/μl due to the low total amount synthesized. A Terumo microsyringe was used to inject 1 μl of tracer solution into the endosperm of 4-day-old seedlings grown as above. Eighty seedlings were employed for each experiment. Two days after injection, the seedlings were harvested and separated into primary leaf, endosperm, and root. Each part was dried and hydrolyzed in 6 N HCl, and ion exchange resins were used to obtain a water eluate containing sugars and organic acids, a pyridine eluate containing neutral and acidic amino acids, and to obtain pure lysine (29).

Isolation of Acidic Amino Acids by Ion Exchange Chromatography. The pyridine eluate containing neutral and acidic amino acids were evaporated to dryness in a rotary evaporator, dissolved in H_2O, and applied to a strongly basic ion exchange resin (Dowex 1 × 8, 50–100 mesh, acetate form, 1 × 15 cm). After elution of neutral amino acids with 150 ml of H_2O, acidic amino acids were eluted with 250 ml of 1 N acetic acid. The acetic acid eluate was evaporated to dryness and applied to a strongly basic ion exchange resin (Dowex 1 × 8, 200–400 mesh, acetate form, 0.9 × 60 cm). After the column was washed with 100 ml of H_2O, elution of acidic amino acids was performed with 250 ml of 0.1 N acetic acid followed by 600 ml of 0.3 N acetic acid. Fractions of 10 ml were collected. Aliquots (300 μl) of each fraction were counted in a liquid scintillator. Saccharopine emerged in fractions 18 to 21, 2-aminoadipic acid in fractions 21 to 24, glutamic acid in fractions 30 to 35, aspartic acid in fractions 47 to 50, and pyrosaccharopine in fractions 50 to 60.

Paper Chromatography. Two-dimensional paper chromatography (Whatman No. 1 or 3MM) was performed with butanol-acetic acid-water (12:3:5, v/v/v) as the first solvent and phenol-water-concentrated ammonia (120:30:1, w/v/v) as the second. R_F values obtained are given in Table I.

Thin Layer Chromatography. Two-dimensional TLC (Merck DC-Fertigplatten, Kieselgel 60 F-254) was performed with methanol-chloroform-concentrated ammonia (5:5:1, v/v/v) as the first solvent, and isopropanol-formic acid-water (20:1:5, v/v/v) as the second. R_F values obtained are given in Table I.

High Voltage Electrophoresis. High voltage electrophoresis was carried out on Whatman No. 3MM paper using a Shandon high voltage apparatus Model L.24. Electropherograms were obtained by 1-hr electrophoresis at 3.0 kv and 0.80 mamp with a volatile buffer solution of pH = 3.6 (pyridine-acetic acid-water [5:50:945, v/v/v]). Electrophoretic mobilities are given in Table I.

Isolation and Identification of Labeled Intermediates. Isolation and identification of labeled intermediates were performed by use of the four separation methods described above.

Ion exchange chromatography of acidic amino acids gave no sharp separation of saccharopine and 2-aminoadipic acid, and of aspartic acid and pyrosaccharopine, respectively. The respective fractions containing more than one amino acid were therefore pooled and evaporated to dryness in a rotary evaporator. After being dissolved in a small volume of water, two-dimensional paper chromatography gave a distinct separation of saccharopine, 2-aminoadipic acid, and pyrosaccharopine, the latter formed from saccharopine during the evaporation step, and of pyrosaccharopine and aspartic acid, respectively. The compounds were located on the chromatograms by autoradiography or scanning, and the R_F values observed were identical to those of authentic standards. The labeled areas were cut out and eluted with water, and fractions containing identical amino acids were pooled. The paper chromatographic purification procedure was repeated applying aliquots of the aspartic and glutamic acid fractions, and the total amounts of the fractions containing 2-aminoadipic acid, saccharopine and pyrosaccharopine. The labeled areas were again located on the chromatograms by autoradiography and showed R_F values identical to authentic standards. The labeled compounds were recovered from the chromatograms and examined by two-dimensional TLC. All compounds gave R_F values identical to authentic standards. After recovery from the thin layer chromatograms final identification was ob-

Table I. *R_F Values and Electrophoretic Mobilities of Authentic Standards*

Amino acids were visualized on the chromatograms by spraying with a 0.2% solution of ninhydrin in acetone and heating to 80 C for 2 min. Piperidine-2,6-dicarboxylic acid was visualized by use of a 1.9% solution of ninhydrin in glacial acetic acid. Lactams were visualized either by the Rydon-Smith reagent or by iodine vapor. Electrophoretic mobilities are given in cm, negative values indicating a migration towards the anode, positive values a migration towards the cathode. HVE: high voltage electrophoresis.

Standard	HVE	Paper Chrom. 1.	Paper Chrom. 2.	Thin Layer 1.	Thin Layer 2.
β-Alanine	-12.0	0.37	0.73	0.14	0.45
4-Aminobutyric Acid	-14.8	0.46	0.86	0.15	0.60
5-Aminovaleric acid	-15.4	0.52	0.92	0.18	0.66
N-Acetyl-2,6-diaminopimelic Acid	+ 4.6	0.26	0.40	0.14	0.28
N-Succinyl-2,6-diaminopimelic acid	+ 4.6	0.26	0.20	0.05	0.30
2-Amino-6-hydroxycaproic acid	- 2.2	0.33	0.78	0.36	0.45
Piperidine-2,6-dicarboxylic acid	+12.4	0.31	0.46	0.20	0.16
Pipecolic Acid	-	0.44	0.92	0.33	0.37
Aspartic Acid	+ 4.8	0.17	0.17	0.07	0.21
Glutamic Acid	+ 1.3	0.26	0.23	0.11	0.34
2-Aminoadipic Acid	+ 0.3	0.31	0.28	0.13	0.42
2-Aminopimelic Acid	- 0.2	0.35	0.36	0.15	0.52
Saccharopine	+ 1.2	0.14	0.62	0.07	0.07
Pyrosaccharopine	+ 7.5	0.27	0.68	0.21	0.28
Pyroglutamic Acid	+10.0	0.59	0.70	0.49	0.58
Pyroaminoadipic Acid	+11.4	0.67	0.75	0.59	0.65

tained by high voltage electrophoresis as described above. The electrophoretic mobilities obtained were identical to those of co-chromatographed authentic standards. The identification of saccharopine was further supported by the fact that chromatography of the compound in all four separation systems invariably gave two spots, one corresponding to saccharopine, the other corresponding to pyrosaccharopine formed from saccharopine during the evaporation step involved. Similarly, repeated chromatography of 2-aminoadipic acid, a minor spot which on thin layer chromatograms and on high voltage electrophoresis behaved identically to pyroaminoadipic acid.

In order to examine the formation of labeled neutral intermediates, the two water eluates from the strongly basic ion exchange resin were combined and evaporated to dryness in a rotary evaporator. After being dissolved in a small volume of water, aliquots were examined by high voltage electrophoresis. Labeled compounds were recovered and examined by paper and TLC, consecutively. No radioactivity was found in 5-aminopentanoic acid and 2-amino-6-hydroxycaproic acid.

Pipecolic acid was separated from other neutral amino acids by two-dimensional paper chromatography. Two identical chromatograms were made by use of aliquots from the eluate containing neutral amino acids and by addition of an unlabeled pipecolic acid standard. Labeled compounds on the chromatograms were visualized by autoradiography. Both chromatograms showed two labeled spots in the area of pipecolic acid. One of the chromatograms was developed with ninhydrin and the two labeled spots were found superimposable with a yellow and a clear blue ninhydrin spot, characteristic for proline and pipecolic acid, respectively. The labeled compound corresponding to pipecolic acid was cut out and recovered from the second chromatogram. Two dimensional TLC showed only one spot corresponding to pipecolic acid.

Determination of Radioactivity. Radioactivity on paper or thin layer chromatograms and on electropherograms was determined either by scanning (Bertold Dünnschicht Scanner II) or by autoradiography (Kodirex x-ray film, 18 × 24 cm). To obtain an accurate determination of radioactivity in labeled spots on the chromatograms, the labeled areas were cut out, eluted with H_2O, and aliquots counted in a Packard Tri-Carb scintillation spectrometer Model 3320. Aqueous samples were made up to 1 ml with H_2O. Ten milliliters of scintillation fluid (toluene-Triton X-100 with 2,5-diphenyloxazole and 1,4-bis[2,5-(phenyloxazolyl)]benzene) were added to each sample (37).

RESULTS AND DISCUSSION

The overall metabolic fate of the tracers used to study lysine metabolism in barley are shown in Table II. The tracers were administered to the seedlings by injection into the endosperm and the radioactivity was readily transported to the primary leaf (Table II). Also, the distribution of radioactivity indicates none of the administered tracers were metabolically inert (Table II).

The results of the studies on lysine metabolism are shown on Table III and IV. The lysine and the aspartic and 2-aminoadipic acids administered were racemic. The labeled 2,6-diaminopimelic acid used was a mixture of 23.5% LL-, 23.5% DD-, and 53.5% *meso* compound (29), while the labeled saccharopine, N^6-(2'-glutaryl)-lysine-[1,2,3,4,5,6]-^{14}C, presumably was a mixture of equal parts of the two diastereoisomers L-saccharopine and D-*allo*saccharopine (16). 2-Aminopimelic acid was included as a standard because the original 2,6-diaminopimelic acid precursor was found to contain 0.3% of an impurity which in all separation systems used behaved identically to this compound. In none of the experiments with other precursors was radioactivity found in 2-aminopimelic acid. Besides the labeled intermedi-

Table II. *Distribution of Radioactivity on Various Groups of Compounds in Primary Leaf of Barley*

The total radioactivity present in primary leaf is given as total dpm and as percentage of the whole seedling. Incorporation percentages in the various fractions are based on total radioactivity present in the primary leaf.

Precursor	Total Activity		Distribution of Radioactivity			
	dpm x 10^{-3}	%	Organic Acids & Sugars	Acidic Amino Acids	Neutral Amino Acids	Basic Amino Acids
Aspartic Acid-3-^{14}C	4080	44.2	18.3	53.0	12.4	9.5
2-Aminoadipic Acid-1-^{14}C	4110	33.9	11.2	78.2	3.2	0.7
Saccharopine-^{14}C	48	6.7	10.1	65.0	12.1	5.2
2,6-Diaminopimelic Acid-1-(7)-^{14}C	3490	22.1	6.4	8.8	18.6	53.4
Lysine-1-^{14}C	8050	49.8	1.5	14.8	18.3	60.6

Table III. *Distribution of Radioactivity in Various Amino Acids in Primary Leaf of Barley*

The data present total dpm and incorporation percentages based on the total amount of radioactivity present in the primary leaf at the end of the biosynthetic experiment.

	Lysine		Pipecolic Acid		Aspartic Acid		Glutamic Acid		2-Aminoadipic Acid		Saccharopine		Pyrosaccharopine		Total Saccharopine	
Precursor	dpm	%	dpm	%	dpm	%	dpm	%	dpm	%	dpm	%	dpm	%	dpm	%
Aspartic Acid-3-^{14}C	157100	3.85	370	0.009	1236000	30.3	457000	11.2	3390	0.083	0	0	0	0	0	0
2-Aminoadipic Acid-1-^{14}C	6160	0.15	0	0	14000	0.35	11500	0.28	1443000	35.1	0	0	0	0	0	0
Saccharopine-^{14}C	770	1.60	0	0	3500	7.3	1890	3.9	2540	5.3	6670	13.9	10940	22.8	17610	36.7
2,6-Diaminopimelic Acid-1-(7)-^{14}C	1843000	52.8	0	0	6260	0.18	3330	0.10	17590	0.50	475	0.014	300	0.008	775	0.022
Lysine-1-^{14}C	3188000	39.6	66000	0.82	10450	0.12	24300	0.30	39000	0.48	780	0.010	315	0.004	1095	0.014

Table IV. *Distribution of Radioactivity in Amino Acids in Root, Endosperm, and Primary Leaf of Barley Seedlings with Saccharopine-^{14}C as Precursor*

The data present total dpm in the individual amino acid and the incorporation percentage based on the total amount of saccharopine injected into the endosperm.

Products	Root		Endosperm		Primary Leaf	
	dpm	%	dpm	%	dpm	%
Lysine	60	0.006	2470	0.23	770	0.073
Aspartic Acid	460	0.043	4360	0.41	3500	0.33
Glutamic Acid	490	0.046	2350	0.22	1890	0.18
2-Aminoadipic Acid	875	0.082	1730	0.16	2540	0.24
Saccharopine	5220	0.49	177400	16.7	6670	0.63
Pyrosaccharopine	6920	0.65	237300	22.3	10940	1.03
Total Saccharopine	12140	1.14	414700	39.0	17600	1.66

ates indicated in Tables III and IV, a low amount of radioactivity was also found in pyrrolidone-5-carboxylic acid and piperidone-6-carboxylic acid. However, these two compounds, like pyrosaccharopine, were formed during the procedure of acid hydrolysis from glutamic and 2-aminoadipic acids, respectively (7, 19). It appears from the data in Table I that the consecutive use of the four separation methods described gave a distinct separation of all identified compounds.

Saccharopine, like 2-aminoadipic acid, is a poor lysine precursor in barley (Table III and IV). The aspartic acid-3-^{14}C administered to the plants is, via the citric acid cycle, easily transformed to α-ketoglutaric acid-2-^{14}C and acetyl-CoA-1,3-^{14}C, both initial

lysine precursors of the 2-aminoadipic acid pathway known from fungi. Radioactivity from aspartic acid-3-^{14}C can therefore be incorporated into lysine via both the 2-aminoadipic acid pathway and via the 2,6-diaminopimelic acid pathway. However, if the 2-aminoadipic acid pathway was functioning, aspartic acid-3-^{14}C might give rise to label in both 2-aminoadipic acid and saccharopine. No radioactivity was observed in saccharopine. The radioactivity found in 2-aminoadipic acid corresponds to 2.1% of the radioactivity found in lysine. The similar percentages found when 2,6-diaminopimelic acid and lysine were used as tracers were 1.0 and 1.2%, respectively. In the experiment with aspartic acid as tracer, labeled 2-aminoadipic acid may also originate from degradation of the labeled tryptophan formed (6). Thus the labeled 2-aminoadipic acid formed in this experiment may originate from lysine and tryptophan catabolism. In the experiment with labeled 2-aminoadipic acid as tracer, no activity was found in saccharopine at all. It appears that barley seedlings, to a low extent, can convert saccharopine and 2-aminoadipic acid to lysine. However, it has not been shown that the seeedlings can synthesize lysine from simple precursors of the 2-aminoadipic acid pathway. The previously reported result (29, 31), that this pathway is of no quantitative importance for the lysine production of the seedlings, is confirmed.

The discussion above indicates that saccharopine has no major role in lysine biosynthesis in barley seedlings. Labeled saccharopine is formed when labeled 2,6-diaminopimelic acid-1-(7)-^{14}C or lysine-1-^{14}C are administered to the plants (Table III). 2,6-Diaminopimelic acid is incorporated very efficiently into lysine. The formation of labeled saccharopine from both 2,6-diaminopimelic acid and lysine therefore establishes saccharopine as a lysine catabolite in barley. The amounts of labeled saccharopine isolated are small. Although the plant material was sterilized before the biosynthetic experiments were conducted and no fungal infection was detectable (15, 33), the saccharopine isolated might be claimed to be of fungal origin. This possibility seems unlikely because neither the experiment with labeled aspartic acid nor the experiment with labeled 2-aminoadipic acid showed any activity in saccharopine. This would have been expected if fungal infection had occurred and the saccharopine isolated was formed via operation of the 2-aminoadipic acid pathway. It is not surprising that the amounts of saccharopine which can be detected are low. No characteristic precursors or catabolites of lysine, except 2-aminoadipic acid, have hitherto been reported in higher plants, and it is remarkable that none of the numerous nonprotein amino acids which are known from plants can be easily derived from lysine precursors (20, 30). Thus lysine metabolism in plants seems to be strictly controlled with resulting very low levels of free intermediates.

It has previously been reported that lysine catabolism in animals proceeds via saccharopine and 2-aminoadipic acid and results in the production of 2 molecules of each of acetyl-CoA and CO_2 (4, 6). If this pathway is also operating in plants, saccharopine should be metabolized to 2-aminoadipic acid. The data show that this conversion actually occurs (Tables III and IV). 2-Aminoadipic acid should be further metabolized to acetyl-CoA. Acetyl-CoA is readily incorporated into glutamic and aspartic acids via the citric acid cycle. Therefore, besides saccharopine and 2-aminoadipic acid, glutamic and aspartic acids are formed by the catabolism of lysine. In agreement with this, labeled saccharopine (N^6-(2'-glutaryl)-lysine-[1,2,3,4,5,6]-^{14}C) is seen to give high labeling of both glutamic and aspartic acids. The 2,6-diaminopimelic acid, 2-aminoadipic acid, and lysine precursors employed were labeled in carbon 1. This carbon atom is lost as CO_2 in the degradation pathway and should therefore give much weaker labeling of glutamic and aspartic acids. This is observed.

Another possibility for lysine catabolism would be by a reversal of all of the steps of the 2-aminoadipic acid pathway with α-ketoglutaric acid and acetyl-CoA as end products. The conversion of oxaloglutaric acid to α-ketoadipic acid is hardly reversible. α-Ketoglutaric acid-2,3,4,5-^{14}C and acetyl-CoA-1,2-^{14}C would be formed in the experiment with labeled saccharopine, and a strong labeling of glutamic acid compared to aspartic acid would be expected. This is not observed. In the experiments with 2,6-diaminopimelic acid-1-(7)-^{14}C, lysine-1-^{14}C, and 2-aminoadipic acid-^{14}C as precursors, acetyl-CoA-1-^{14}C would be formed. The observed low labeling of glutamic and aspartic acids compared with the saccharopine experiment would then not be expected. It is concluded that lysine catabolism in barley seedlings may proceed according to the pathway known from animals and as shown with heavy arrows in Figure I.

In the experiment with labeled DL-lysine-1-^{14}C as precursor, formation of labeled pipecolic acid was observed (Table III). This label is thought to originate from the D-lysine administered (22, 28). No experiments were done with commercial claimed optical pure D- or L-lysine because it was found difficult to establish the degree of optical purity, and therefore to obtain unequivocal results (22). This difficulty was surmounted by the use of 2,6-diaminopimelic acid-1-(7)-^{14}C as precursor. From this precursor no labeled pipecolic acid was formed, suggesting that the labeled 2,6-diaminopimelic acid administered gave rise to only labeled L-lysine and this was not catabolized via pipecolic acid.

The metabolism of lysine is remarkable in the sense that different organisms may use different pathways for biosynthesis or catabolism of the compound. A previous study (29) showed that barley seedlings can synthesize lysine via the 2,6-diaminopimelic acid pathway also used by bacteria. In plants amino acid biosynthesis seems to proceed at least partly in the chloroplasts (21, 27) and these are also likely to be sites of lysine biosynthesis. Animals cannot synthesize lysine, but catabolize lysine via an apparent reversal of some of the biosynthetic steps of the 2-aminoadipic acid pathway (4). This study shows that this catabolic lysine pathway can also operate in barley seedlings. The catabolism of lysine in animals proceeds in the mitochondria (4), and also in fungi, at least, some steps of the 2-aminoadipic acid pathway takes place in the mitochondria (1). Catabolism of lysine in barley seedlings may also take place in the mitochondria.

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