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DIAMINO ACID METABOLISM IN PLANTS WITH SPECIAL REFERENCE TO α,β-DIAMINOPROPIONIC ACID

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Abstract—The ability of various seedling extracts to catalyse transamination between diamino acids and α-ketoglutarate or pyruvate is recorded. Normally, the homologous series of diamino acids undergo transamination at decreasing rates in the series: ornithine > α,β-diaminopropionate > α,γ-diaminobutyrate > lysine. During transamination, ornithine and diaminobutyrate preferentially lose their terminal amino group, whereas diaminopropionate more readily donates its α-amino group. Several pieces of evidence indicate that more than one enzyme is involved in this series of transamination reactions. When ^{14}C-labelled diaminopropionate was supplied to intact seedlings of *Acacia*, acetylation of the β-amino group formed a more important process quantitatively than transamination. Formation of β-acetyldiaminopropionic acid was demonstrated to proceed in extracts of *Acacia* seedlings and *N*-acetylglutamic acid was shown to be an effective acetyl group donor. Attempts to establish an enzyme-catalysed formation of albizziine from diaminopropionate and carbamyl phosphate were unsuccessful, but the reaction was shown to proceed chemically.

INTRODUCTION

THE PREVIOUS paper[1] outlined the occurrence of a series of α,ω-diamino acids in plants, which included members having unbranched carbon skeletons of 3 to 6 carbon atoms. Acetyl, oxalyl and carbamyl derivatives of the diamino acids sometimes coexist with the parent compound: the substituent usually is found attached to the ω-nitrogen atom. The existence of such structurally-related compounds, many in members of the family Leguminosae, suggests that common metabolic pathways, and perhaps even common enzymes, may be implicated in their biosynthesis and biodegradation.

The present paper describes experiments to investigate the ability of the diamino acids to participate in transamination reactions catalysed by enzyme preparations from legume seedlings, and to elucidate which of the two amino groups is transferred preferentially from each diamino acid. In another approach, the metabolism of ^{14}C-labelled diamino acids by intact seedlings was investigated; in particular, the conversion of α,β-diaminopropionic acid into a variety of related compounds was observed in seedlings of certain *Acacia* species. This last study was supplemented by other experiments in which the synthesis of acetyl and carbamyl derivatives, especially those of diaminopropionic acid, was examined using soluble enzyme preparations. From the amounts of the different labelled products formed in the intact seedlings, it was possible to calculate approximately the ratio of α- to ω-amino group loss, and so correlate the results of *in vivo* experiments with those obtained in the enzymic transamination studies.

RESULTS

Studies of Transamination

There are numerous studies relating to the transaminase enzymes of plants, but few have involved the use of diamino acids as substrates. Bone[2] claimed to have demonstrated the

[1] A. S. SENEVIRATNE and L. FOWDEN, *Phytochem.* 7, 1039 (1968).
[2] D. H. BONE, *Plant Physiol.* 34, 171 (1959).

conversion of ornithine into glutamic-γ-semialdehyde by a transaminase system present in mitochondria isolated from mung bean seedlings. Although the mitochondria seem to represent the main sub-cellular site of transaminases present in these seedlings,[3] work with other plant species has indicated that considerable proportions of the total transaminase activity may be associated with the non-particulate cytoplasmic fraction of cells.

In our transamination experiments with enzyme preparations from a variety of legume seedlings and with diamino acids as substrates, it was easier to demonstrate and assay transamination when mitochondrial extracts were used as enzyme sources, i.e. the transamination rates (expressed on a mg protein basis) measured for mitochondrial preparations were considerably higher than those determined for unfractionated cell extracts of the same seedling. In practice, mitochondria isolated from seedlings were disrupted by transferring them to a dilute buffer solution containing a non-ionic detergent. The solublized enzyme preparation resulting was frequently used directly as a source of transaminases; alternatively, a 25–50 per cent saturated ammonium sulphate protein fraction was prepared from the solublized enzymes. Small amounts of endogenous amino acids, and residual ammonium sulphate, were removed from the enzyme preparations either by dialysis or by gel-filtration using Sephadex G-25.

Transamination rates. Most of the experiments to compare the rates of transamination of lysine, ornithine, α,γ-diaminobutyric and α,β-diaminopropionic acids were performed at pH 8·4 using mitochondrial enzymes prepared from 2-day-old seedlings of mung bean (*Phaseolus aureus*). When α-ketoglutarate served as the amino group acceptor, the progress of transamination was followed by assaying the glutamate formed and correcting the value obtained for any transamination due to endogenous amino acids. Initial reaction rates showed a maximum value when ornithine or α,β-diaminopropionic acid and α-ketoglutarate were present at 0·05 M concentrations. Higher substrate concentrations gave slightly lower transamination rates. When α,γ-diaminobutyric acid was used as a substrate, maximum reaction rates were measured in mixtures containing about 0·1 M-amino acid. Pyruvic acid usually was less efficient than α-ketoglutarate as an amino group acceptor.

When freshly-prepared mitochondrial enzyme fractions of mung bean seedlings were employed (i.e. endogenous amino acids removed by Sephadex treatment and not by the more prolonged dialysis procedure), the initial rates of transamination observed for the individual amino acids gave the following sequence: ornithine > α,β-diaminopropionate > α,γ-diaminobutyrate > lysine (Table 1). Ornithine was utilized at about one-tenth the rate observed for aspartic acid,[3] while diaminopropionate and diaminobutyrate reacted at about 8 and 3 per cent, respectively, of the ornithine rate. Negligible amounts of glutamic acid were formed when lysine was the amino acid substrate. The relative substrate efficiencies of the diamino acids remained unchanged when seedlings of *Acacia willardiana*, *Albizzia lophantha* and *Leucaena leucocephala* were used as sources of mitochondrial transaminase preparations, but when enzymes were obtained from either *Lathyrus latifolius* or *Phaseolus vulgaris*, transamination proceeded slightly faster when α,γ-diaminobutyrate, rather than α,β-diaminopropionate, formed the amino acid substrate.

Transamination rates tended to fall when measured at 25° over a period of time. With ornithine as the amino acid substrate, the production of glutamate reached a maximum value after about 2–3 hr and incubation of reaction mixtures for longer periods led to a fall in glutamic acid levels. Although reaction rates measured with diaminopropionate showed the

[3] D. H. BONE and L. FOWDEN, *J. Exptl Botany* **11**, 104 (1960).

same decrease with time, there was a continuing production of glutamate beyond the 3 hr period. These observations can be explained by assuming that the ornithine transaminase was quite labile under the experimental conditions used, and that the mitochondrial extracts also contained enzyme(s) effecting glutamate degradation: the diaminopropionate transaminase activity is assumed to exhibit greater stability. This idea is supported by the results obtained in transamination experiments using mitochondrial enzyme preparations that had been dialysed overnight at 0–4°: the initial rate of diaminopropionate transamination was not affected by dialysis, but now was higher than that measured with ornithine.

TABLE 1. TRANSAMINATION RATES MEASURED FOR REACTIONS BETWEEN DIAMINO ACIDS, USED SINGLY AND TOGETHER, AND 0·05 M-α-KETOGLUTARATE CATALYSED BY MUNG BEAN MITOCHONDRIAL ENZYMES

Substrate	Reaction rate measured*	
	With single substrate	With substrate + 0·05 M-ornithine
0·05 M-L-α,β-diaminopropionic acid	8	55
0·1 M-L-α,γ-diaminobutyric acid	3	25
0·05 M-δ-aminovaleric acid	0	65

* Transamination rates based on that measured for ornithine = 100. The initial rate of glutamic acid formation from ornithine varied from 0·3–0·55 μg/ml/min in the different experiments.

Transamination of individual diamino acids was affected by the presence of a second member of the series, e.g. glutamic acid formation was less when a mixture of ornithine and either diaminopropionate or diaminobutyrate were used than when ornithine alone formed the amino donor (Table 1). If δ-aminovaleric acid was added to assay mixtures containing ornithine as substrate, a similar partial inhibition of ornithine utilization was observed, although δ-aminovalerate itself did not act as a substrate. In a comparable experiment, transamination rates were measured for 0·05 M-α,β-diaminopropionate and 0·05 M-β-alanine, used singly and as a mixture; in this case, the initial reaction rate measured for the mixture of the two amino acids was approximately equal to the sum of the rates measured for each substrate alone.

The nature of amino group transfer. The products of the transamination reaction (other than glutamate) were examined to determine which amino group was involved in the transamination process. ^{14}C-Labelled ornithine, diaminobutyrate and diaminopropionate were employed to confer sensitivity upon the methods used to distinguish between the two possible types of product, i.e. α-keto-ω-amino acids after α-amino group loss, or α-amino-ω-aldehydo acids following ω-amino transfers. After the products of transamination had been separated on paper chromatograms, radioactive bands in positions likely to represent either the α-keto or ω-aldehydo derivatives of the diamino acid used were eluted. These labelled materials either were oxidized with hydrogen peroxide or reduced with sodium borohydride. Oxidation of α-keto derivatives would yield an ω-amino acid having one less carbon atom than the

original diamino acid, while ω-aldehydo compounds would give the corresponding dicarboxylic amino acid. Isomeric α-hydroxy-ω-amino and α-amino-ω-hydroxy acids would represent the reduction products obtained after α- and ω-amino group transfer respectively. For example, α-transamination from ornithine would lead to γ-aminobutyric acid (after oxidation) and α-hydroxy-δ-aminovaleric acid (after reduction), while δ-transamination would yield glutamic acid and δ-hydroxynorvaline, respectively. The two types of oxidation product can be separated effectively on paper chromatograms developed with butanol–acetic acid–water as solvent, while the isomeric reduction products can be resolved using an ethyl acetate–pyridine–water solvent system. Therefore, all the compounds could be identified by "fingerprinting" techniques after chromatography admixed with authentic materials.

In practice, transamination between ^{14}C-diaminopropionate (2 μC) or ^{14}C-ornithine (2 μC) and α-ketoglutarate was effected by a 25–50 per cent satd. ammonium sulphate fraction of mung bean mitochondria, while in a similar reaction between ^{14}C-diaminobutyrate (2 μC) and pyruvate a mitochondrial preparation from *Lathyrus latifolius* seedlings provided the enzyme source (in this instance pyruvate acted as a better amino group acceptor than α-ketoglutarate). The radioactive products arising from ^{14}C-diaminobutyrate included a heavily-labelled product behaving like aspartic-β-semialdehyde and two weakly-labelled compounds identified by fingerprinting as aspartic acid and β-alanine: the heavily-labelled product was converted into aspartic acid by peroxide treatment. The initial products of α- and β-transamination of α,β-diaminopropionate would each yield glycine on oxidation (α-aminomalonic acid undergoes spontaneous decarboxylation), so the presence of labelled glycine among the products formed from ^{14}C-diaminopropionate was not surprising. In addition, ^{14}C-label was associated with substances yielding either serine or isoserine on reduction: the isoserine contained about four times more radioactivity than the serine. These findings suggest that it is the γ-amino group that is most readily lost from α,γ-diaminobutyrate, whereas α-transamination predominates when α,β-diaminopropionate is used as the amino donor.

The transamination experiments using ^{14}C-ornithine (2 μC) and 0·05 M-α-ketoglutarate were performed first and, to these reaction mixtures only, pyridoxal phosphate was added. Subsequently a facile chemical reaction was shown to occur between ornithine and the cofactor yielding pyridoxamine phosphate, which could be detected readily on paper chromatograms by its characteristic orange-coloured ninhydrin chromaphore. The other product of the chemical reaction was converted into γ-aminobutyric acid on oxidation and so α-amino group loss from ornithine was implicated. No glutamic acid was formed under these conditions of chemical reaction indicating that the further transfer of the amino group from pyridoxamine phosphate to α-ketoglutarate did not occur. When transamination occurred in the presence of freshly-prepared mung bean enzyme, labelled products formed from ^{14}C-ornithine included both α-keto-δ-aminovaleric acid (oxidized to γ-aminobutyric acid and reduced to α-hydroxy-δ-aminovaleric acid) and glutamic-γ-semialdehyde (oxidized to glutamic acid), together with a compound tentatively identified as Δ^1-pyrroline-2-carboxylic acid (the product of spontaneous cyclization of α-keto-δ-aminovaleric acid). If the enzyme preparation was subjected to prolonged dialysis before use, glutamic-γ-semialdehyde formation was negligible, in agreement with the earlier observation that ornithine transaminase activity was quite labile. The enzyme therefore catalysed δ-transamination from ornithine.

Lysine reacted chemically with pyridoxal phosphate as readily as ornithine, but little pyridoxamine phosphate was formed from α,γ-diaminobutyric acid. Chemical reaction between α,β-diaminopropionic acid and pyridoxal phosphate was scarcely detectable.

Acetylation and Carbamylation Processes

In these experiments, attention was largely centred on the metabolism of α,β-diaminopropionic acid in *Acacia* species. Several substances that may be regarded as β-substituted derivatives of diaminopropionate occur in members of the complex *Acacia* genus[1]: these include β-acetyldiaminopropionic acid, albizziine (β-carbamyldiaminopropionic acid or β-ureido-α-aminopropionic acid) and willardiine (β-uracil-1-yl-α-aminopropionic acid). The metabolic relationships existing between the β-substituted derivatives and the parent compound have been investigated (i) by determining the fate of ^{14}C-diaminopropionate supplied to intact *Acacia* seedlings, and (ii) by assaying the formation of acetyl and carbamyl derivatives of ^{14}C-diaminopropionate in cell-free extracts prepared from *Acacia* seedlings. The experimental approach was very similar to that described earlier in regard to ornithine metabolism in legumes,[4] and features of metabolism common to the two diamino acids emerge from the studies.

Pathways in intact seedlings. Sainfoin (*Onobrychis viciifolia*) is a legume containing large amounts of δ-acetylornithine,[5] while *A. armata* contains β-acetyldiaminopropionic acid as one of its principal free amino acids.[1] The selectivity of the acetylation process was investigated by supplying 0·2 per cent diaminopropionic acid to sainfoin seedlings and 0·2 per cent ornithine to *A. armata* seedlings. Initially, seedlings having radicles just protruding through the testas were allowed to absorb the solutions for 2 days and then they were grown in moistened vermiculite for a further 4 days before analysis. Under these conditions, sainfoin seedlings synthesized considerable amounts of β-acetyldiaminopropionic acid, although none could be detected in normal seedlings. Another product of feeding diaminopropionate to sainfoin was characterized tentatively as a γ-glutamyldiaminopropionic acid (it is not certain whether the γ-glutamyl moiety was attached at the α- or β-nitrogen atom). The *A. armata* seedlings were capable of synthesizing δ-acetylornithine from ornithine, but the percentage acetylation was low.

The metabolism of ^{14}C-diaminopropionic acid and ^{14}C-serine were compared in seedlings of *Acacia podalyriaefolia*: serine is considered to be a precursor of albizziine (and diaminopropionate) in *Albizzia* seedlings by Reinbothe.[6] Comparable groups of 2-day-old seedlings were allowed to absorb 2 μC of either labelled diaminopropionate or serine and the distribution of ^{14}C within the amino acid pool of the seedlings was examined after two days. Table 2 summarizes the results obtained. The most striking difference lay in the degrees to which radioactivity entered the soluble amino acid pool from the two precursors. In the case of ^{14}C-diaminopropionate, about half the activity supplied to the seedlings remained in the amino acid fraction after 2 days, whereas with serine this figure was less than 1 per cent. The predominant feature of diaminopropionate metabolism was its conversion into the β-acetyl derivative, but albizziine (which probably accounted for most of the activity associated with the albizziine-asparagine spot) and willardiine also appeared as labelled products. Serine did not act as a precursor of β-acetyldiaminopropionic acid or willardiine, and probably not of albizziine (activity in the combined spot was present largely in asparagine), indicating that the pathway from serine to diaminopropionate indicated by Reinbothe was not operative in the young *Acacia* seedlings used. Other labelled substances arising from serine included *S*-carboxyethylcysteine sulphoxide, djenkolic acid and γ-glutamyldjenkolic acid, products probably arising from cysteine as a common precursor. Some radioactivity

[4] D. H. Brown and L. Fowden, *Phytochem.* **5**, 887 (1966).
[5] D. H. Brown and L. Fowden, *Phytochem.* **5**, 881 (1966).
[6] H. Reinbothe, *Flora* **152**, 545 (1962).

was introduced into the sulphur-containing amino acids after ^{14}C-diaminopropionic acid feeding but the biosynthetic pathways probably again involved serine and cysteine as intermediates (serine itself was labelled after ^{14}C-diaminopropionate feeding).

TABLE 2. THE DISTRIBUTION OF RADIOACTIVITY WITHIN THE AMINO ACID FRACTION OF *Acacia podalyriaefolia* SEEDLINGS TWO DAYS AFTER SUPPLYING EITHER [U-^{14}C] DIAMINOPROPIONIC ACID (2 μC) OR [U-^{14}C] SERINE (2 μC)

Labelled product	Activity present in seedling constituents			
	^{14}C-Diaminopropionate supplied		^{14}C-serine supplied	
	Counts/100 sec*	% of total counts†	Counts/100 sec*	% of total counts†
β-Acetyldiaminopropionic acid	109,000	95·4	—	—
Serine	587	0·51	913	43·0
Aspartic acid	14	—	33	1·55
Glutamic acid	43	0·03	37	1·74
Glutamine	650	0·57	—	—
Albizziine/asparagine	1549	1·36	231	10·9
Willardiine	325	0·28	—	—
S-Carboxyethylcysteine sulphoxide	62	0·05	116	5·46
Djenkolic acid	51	0·04	149	7·0
γ-Glutamyldjenkolic acid	—	—	24	1·13

* Radioactivity measured by Geiger–Müller scanning of spots on chromatograms; efficiency about 3 per cent.

† Counts present in each constituent expressed as a percentage of total counts measured for labelled substances present on chromatograms.

When ^{14}C-diaminopropionic acid was supplied to seedlings of *Acacia melanoxylon* in a similar manner, β-acetyldiaminopropionic acid was again the most heavily-labelled product, representing 78 per cent of the total radioactivity recovered in the amino acid fraction.

Diaminopropionic acid metabolism by seedling extracts. (a) *Acetylation.* The ability of enzymes to acetylate diaminopropionic acid in the presence of various possible acetyl group donors was investigated using a 0–60 per cent saturated ammonium sulphate protein fraction (Sephadex G-25 treated) obtained from an extract of 7-day-old seedlings of *Acacia mollissima*. The results of one such experiment are outlined in Table 3. Small proportions of the ^{14}C-diaminopropionate added to reaction mixtures were converted into the β-acetyl derivative. Some acetylation occurred in the absence of added acetyl derivatives, indicating the presence of endogenous acetyl donors in the enzyme preparation. β-Acetyldiaminopropionic acid synthesis was stimulated by the addition of *N*-acetylglutamic acid. The addition to reaction mixtures of coenzyme A failed to stimulate acetylation.

In another experiment using a non-fractionated extract of *A. mollissima* seedlings (Sephadex G-25 treated to remove low molecular weight constituents), coenzyme A was found to stimulate acetyl transfer from *N*-acetylglutamate and acetyl coenzyme A also acted as an acetyl group donor. δ-Acetylornithine, α-acetylornithine and *N*-acetyldjenkolic acid did not serve as acetyl donors, and there was no evidence that the presence of diaminobutyric acid, ornithine or lysine (in amounts approximately equimolar to the diaminopropionate used) reduced the amount of β-acetyldiaminopropionic acid formed from *N*-acetylglutamic acid as donor.

TABLE 3. THE FORMATION OF β-ACETYLDIAMINOPROPIONIC ACID AND OTHER LABELLED AMINO ACIDS FROM ^{14}C-DIAMINOPROPIONATE BY EXTRACTS OF *Acacia mollissima* SEEDLINGS

Additions to standard reaction mixture*	Percentage of total recovered activity‡						
	Glycine	Diamino-propionic acid	β-Acetyl-diamino-propionic acid	Glutamic acid	Aspartic acid	Alanine	Serine
None	2·13	72·5	2·43	2·88	1·10	18·6	0·39
Coenzyme A (0·01 μmoles)	2·98	62·9	2·63	3·57	1·89	25·2	0·85
N-Acetylglutamic acid (0·5 μmoles)	2·30	73·3	5·41	0·27	1·84	15·4	1·49
N-Acetylglutamic acid (0·5 μmoles) + Coenzyme A (0·01 μmoles)	2·76	73·7	1·83	2·22	0·73	17·5	1·25
N-Acetylglutamic acid (0·5 μmoles) + Coenzyme A (0·01 μmoles)†	0	100	0	0	0	0	0

* Standard reaction mixture contained: [U-^{14}C] diaminopropionate (0·2 μC, 38 μC/mg), potassium phosphate buffer, pH 7·4 (10 μmoles) and enzyme; final volume, 0·1 ml. Incubation was for 3 hr at 30°.

† This mixture contained boiled enzyme.

‡ Radioactivity in each compound is expressed as a percentage of the total activity recovered in labelled amino acids.

(b) *Carbamylation.* The possibility of enzyme-catalysed formation of albizziine from diaminopropionate and carbamyl phosphate was investigated using extracts of 7-day-old *Acacia cyclopsis* seedlings. The extract was prepared in 0·2 M-phosphate buffer, pH 7·4, and low molecular weight substances were removed by running through a Sephadex G-25 column: the two substrates were added at final concentrations of 0·04 M. Paper chromatographic methods indicated the presence of albizziine in reaction mixtures incubated for 3 hr at 30°; however, albizziine was formed in mixtures containing either boiled enzyme or no enzyme, and there was no suggestion that increased levels of albizziine were present in mixtures containing non-denatured protein. Indeed, less albizziine was found in the latter than in those having boiled enzyme indicating that enzyme(s) degrading albizziine were active in the extract of *A. cyclopsis* seedlings.

When ornithine (0·04 M) was added to the reaction mixture instead of diaminopropionic acid, the synthesis of citrulline was detected chromatographically and in this instance the presence of an ornithine transcarbamylase, rather than a chemical reaction, was responsible.

DISCUSSION

The very low rates of transamination measured for the diamino acids when seedling extracts were used as a source of transaminases have a parallel in the earlier studies of Roberts[7] with animal and fungal enzymes. Although β-alanine, γ-aminobutyric acid and δ-aminovaleric acid acted as good substrates for mouse brain and liver enzymes, the introduction of an α-amino group into these molecules led to the complete loss of substrate activity as seen

[7] E. ROBERTS, *Arch. Biochem. Biophys.* **48**, 395 (1954).

in α,β-diaminopropionic and α,γ-diaminobutyric acids, and to a much lower activity in ornithine as compared with δ-aminovalerate. However, the situation in respect of the C_5 compounds is the reverse of that observed with our transaminase preparations from mung bean, for δ-aminovalerate showed no substrate behaviour—rather it acted as an inhibitor of ornithine transamination.

The intrinsically low activities, coupled with an inherent instability of the ornithine transaminase activity, did not allow fully satisfactory kinetic experiments to be performed. For this reason it has not proved possible to decide with certainty whether one or several distinct enzymes are involved in the catalysis of transamination between the diamino acids and appropriate keto acid molecules. The observation that the enzyme active in ornithine transamination is much more labile than that utilizing diaminopropionate strongly suggests that distinct protein species are involved. The existence of at least two separate transaminase enzymes also follows logically from the fact that positionally different amino groups are involved in the transfer, i.e. transamination from diaminopropionate involves the loss of the α-amino group whereas ornithine and diaminobutyrate preferentially donate their non-α-amino group. The results of experiments in which the rates of transamination observed with single diamino acid substrates were compared with those determined for mixtures of two substrates (see Table 1), unfortunately fail to provide support for the view that more than one enzyme is involved: indeed, the effect upon transamination rates of introducing diaminopropionate and diaminobutyrate into reaction mixtures containing ornithine is that normally encountered when a single enzyme is responsible for catalysing the reactions of homologous substrates. However, diaminobutyric acid, and to a lesser extent diaminopropionic acid, may function only as inhibitors of the ornithine transaminase, and not as competitive substrates, thereby resembling δ-aminovalerate in action.

The transamination experiments performed with ^{14}C-diaminobutyric acid indicated that either of the two amino groups could be lost, although the examination of the labelled reaction products suggested the more facile removal of the γ-amino group. A similar quantitative discrimination between the two groups is suggested by the analysis of radioactive metabolic products formed from ^{14}C-diaminobutyrate supplied to intact seedlings of *Lathyrus latifolius*: products likely to have arisen following γ-amino group loss (e.g. aspartic acid, asparagine and homoserine) represented about double the amount of radioactivity found in β-alanine, the only product clearly arising after α-amino group loss.

The chemical reaction (α-transamination) between ornithine and pyridoxal phosphate occurred under conditions that were similar to those described by Wickremasinghe and Swain,[8] who showed that a bean callus tissue preparation could convert ornithine, in the presence of pyridoxal phosphate, into γ-aminobutyric acid by way of α-keto-δ-aminovaleric acid as an intermediate. Although there seems no doubt that the oxidative decarboxylation of the latter compound to yield γ-aminobutyric acid was enzymic in nature, our observation would suggest that the initial reaction producing pyridoxamine phosphate and α-keto-δ-aminovaleric acid proceeded chemically.

Acetylation appears as a far more predominant mechanism in the metabolism of the diamino acids than transamination, especially as an *in vivo* process. The present study with *Acacia* seedlings has established an extensive conversion of diaminopropionic acid into its β-acetyl derivative, a finding compatible with the earlier observation[4] that when ^{14}C-ornithine was introduced into sainfoin seedlings nearly 80 per cent of the radioactivity associated with the free amino acid fraction was present in δ-acetylornithine. Diaminobutyric apparently

[8] R. L. WICKREMASINGHE and T. SWAIN, *Phytochem.* **4**, 687 (1965).

may undergo a similar reaction for in unrelated experiments in which labelled material was supplied to either seedlings of *Lathyrus latifolius* or developing seeds of *Cucumis melo* (Frisch and Fowden, unpublished experiments), acetyldiaminobutyric acid was formed as a major labelled product. The fact that acetylation of the terminal amino group forms a common feature of diamino acid metabolism in a number of unrelated species (see review by Reuter[9]) suggests that an enzyme of low substrate specificity and wide distribution may be responsible for catalysing the process. Certainly, the ability of sainfoin seedlings to elaborate β-acetyldiaminopropionate if supplied diaminopropionic acid exogenously is in agreement with this view.

When acetyl phosphate was tested as a possible acetyl donor in the *in vitro* systems used to study β-acetyldiaminopropionic acid biosynthesis, it was noticed that a rapid chemical reaction occurred. This was analogous to the formation of δ-acetylornithine chemically from ornithine and acetyl phosphate,[4] and in fact the reaction could form the basis of a useful method for synthesizing β-acetyldiaminopropionate, since the β-amino residue was far more reactive than the α-amino group.

The amounts of albizziine and, to a lesser extent, *S*-carboxyethylcysteine and its sulphoxide tended to fall during the early stages of growth of *Acacia* seedlings; in contrast, the concentration of β-acetyldiaminopropionic acid increased in many species following germination. The failure to effect more than a slight labelling of albizziine and *S*-carboxyethylcysteine sulphoxide in seedlings of *A. podalyriaefolia* fed ^{14}C-diaminopropionic acid or ^{14}C-serine then reflects this pattern of degradative metabolism: presumably, significant incorporation of diaminopropionate into albizziine (or willardiine) would be observed only in developing seeds of *Acacia* during the period of albizziine accumulation.

EXPERIMENTAL

Materials

Radioactive compounds. L-[U-^{14}C]Diaminopropionic acid was prepared from L-[U-^{14}C]asparagine (Radiochemical Centre, Amersham) following the method of Karrer and Schlosser,[10] adapted to a micro scale: the final product had a specific activity of 3·96 mC/mmole. DL-[4-^{14}C]Diaminobutyric acid (10 mC/mmole) was purchased from Commissariat a l'Energie Atomique, Gif-sur-Yvette, France, and DL-[2-^{14}C]-ornithine (1·93 mC/mmole) from Volk Radiochemical Company, Illinois.

Plant materials. Seedlings were grown at 25° in moistened vermiculite. For the transamination experiments, the following species were used (age of seedling in parentheses): *Phaseolus aureus* (2 days), *P. vulgaris* (3 days), *Acacia willardiana* (3 days), *Albizzia lophantha* (5 days), *Leucaena leucocephala* (14 days). *Acacia* species used in other experiments were grown similarly, but for the periods indicated under each experiment.

Chromatographic methods. Paper chromatograms were prepared using the techniques and solvents described in the previous paper.[1]

Counting and radioautographic procedures. Radioautographs were prepared from paper chromatograms by placing them in contact with Kodirex X-ray film for appropriate periods, and subsequently the radioactivity present in each amino acid spot was determined by surface counting using a Geiger–Müller system.

Transamination. The significant transamination experiments were performed using mitochondrial enzymes. Mitochondria were isolated by fractional centrifugation of extracts of seedlings made in 0·1 M-potassium phosphate or 0·1 M-tris-HCl buffers containing 0·4 M-sucrose and 0·01 M-EDTA. After washing the mitochondrial pellet twice with further quantities of the grinding medium, the mitochondria normally were ruptured by transferring to 0·1 M-buffer containing only 0·1 per cent O.P.C. 45 (a non-ionic detergent, Shell Chemical Co., London). Mitochondrial debris was removed by centrifuging, and the solublized preparation of mitochondrial enzymes then could be dialysed or run through a small Sephadex G-25 (fine) column to remove endogenous low molecular weight components. Alternatively, the soluble enzyme fraction was subjected to ammonium sulphate fractionation: transaminase activity was found to be associated mainly with a 25–50 per cent saturated ammonium sulphate protein fraction.

[9] G. Reuter, *Flora* **145**, 326 (1957).
[10] P. Karrer and A. Schlosser, *Helv. Chim. Acta* **6**, 411 (1923).

The normal reaction mixture used to study transamination contained: 0·05 M-amino acid, 0·05 M-keto acid (usually α-ketoglutarate), and enzyme preparation in 0·1 M-phosphate or tris-HCl buffer (pH 8·4). Reaction was allowed to proceed at 25° for various periods of time, and then stopped by addition of two volumes of ethanol. Precipitated protein was removed by centrifuging and aliquots of the supernatant were used to determine the glutamic acid formed. Glutamic acid was separated from other amino acids on chromatograms developed in butan-1-ol-acetic acid-water, and was assayed quantitatively using the ninhydrin-cadmium acetate reagent of Atfield and Morris.[11]

In the transaminations employing ^{14}C-labelled amino acids as substrates, labelled reaction products were separated chromatographically in butanol-acetic acid and located by radioautographic techniques. These substances were eluted from appropriate areas of the chromatogram and either oxidized with an equal volume of 30 per cent (w/v) H_2O_2 (1 hr, 20°) or reduced with $NaBH_4$ in the presence of 0·1 N-HCl (1 hr, 20°).

Acetylation and carbamylation studies. When the metabolism of ^{14}C-labelled amino acids was studied in intact *Acacia* seedlings, amino acid fractions were separated from the seedling extracts using small Zeokarb 225 cation-exchange resin columns[12] before paper chromatography.

For the *in vitro* studies of acetylation and carbamylation, whole seedling, rather than mitochondrial, extracts were used as a source of enzymes. The details of reaction mixtures appear under the description of individual experiments.

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[11] G. N. ATFIELD and C. J. O. R. MORRIS, *Biochem. J.* **81**, 606 (1961).
[12] P. M. DUNNILL and L. FOWDEN, *Phytochem.* **4**, 933 (1965).