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REVIEWS

PROPERTIES OF PLANT AMINOTRANSFERASES

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Abstract—The occurrence and properties of plant aminotransferases are considered in relation to the known characteristics of corresponding animal and bacterial aminotransferases. Development of aminotransferase systems during seed germination and plant development is examined and changes in the activity of various systems are discussed in relation to environmental factors and endogenous hormone changes. Purification and substrate specificity of various plant aminotransferases are considered and the evidence for substrate multispecificity shown by certain enzymes is related to similar findings with some animal and bacterial aminotransferases. The physical and kinetic properties of plant aminotransferases such as their molecular weight, sedimentation coefficient, subunit composition, pyridoxal phosphate requirement, effect of pH and cations on activity, and their mechanism of action are reviewed and compared to similar observations from animal and bacterial aminotransferases. Finally, the intracellular location and functions of plant aminotransferases and their isoenzyme composition are discussed and compared to those of corresponding animal enzymes.

INTRODUCTION

Since the first demonstration of enzymic transamination was made by Braunstein and Kritzmann [1] using pigeon muscle preparations, considerable information has accumulated concerning the occurrence and properties of aminotransferases (or transaminases, E.C. 2.6.1) in bacteria, animal and plant tissues. Early work with a number of plant preparations [2–5, 178] established the widespread occurrence of the following reversible transamination reactions:

Glutamate + oxaloacetate ⇌ α-ketoglutarate + aspartate
Glutamate + pyruvate ⇌ α-ketoglutarate + alanine
Aspartate + pyruvate ⇌ oxaloacetate + alanine.

Similar reversible reactions between α-ketoglutarate and the branched-chain amino acids, valine, leucine, isoleucine, have also been demonstrated in extracts from several plant species and plant organs [6–8] and transamination of the three aromatic amino acids with various keto acid acceptors has been shown to occur in extracts from young plants of *Pisum sativum* [9–11], *Phaseolus aureus* [12–16], *Ph. vulgaris* [17] and *Lycopersicon vesculentum* [18, 19]. Glycine has also been shown to undergo transamination with a number of keto-acids when supplied as a substrate to cell-free extracts of *Phaseolus aureus*, *Triticum aestivum* and *Avena sativa* seedlings [20–22].

Transamination reactions have also been found to play an important role in the metabolism of 'non-protein' amino acids. For example, the transamination of γ-methylglutamic acid was demonstrated in *Arachis* seedlings [23] and γ-aminobutyric acid and citrulline in *Pisum sativum* and *Alnus* spp. [24] when α-ketoglutarate was used as the amino group acceptor. In more recent work, enzymes present in the particulate fraction of germinating *Phaseolus vulgaris* seeds were found to catalyse transamination reactions between β-alanine and oxaloacetate or pyruvate, but not with α-ketoglutarate [25].

Detailed examination of the potential amino acid and keto acid substrates for the different aminotransferases present in a single species have been carried out in only a few cases. Using a homogenate of white lupin seedlings, Wilson *et al.* [26] demonstrated the occurrence of transamination reactions with 17 free 'protein' amino acids when α-ketoglutarate was used as the amino group acceptor. More recently, we investigated the potential transamination reactions of 22 free 'protein' amino acids in soluble fractions prepared from cotyledons, roots and shoots of *Phaseolus vulgaris* seedlings, when either α-ketoglutarate, oxaloacetate, pyruvate or glyoxylate was provided as amino group acceptor [17]. The results indicated that both the non-growing and growing seedling tissues exhibited a similar pattern of aminotransferase activities. With the exception of proline, hydroxyproline and cystine, which did not appear to undergo transamination, all the other 19 amino acids were found to be transaminated, though to different extents, when each of the four keto acids was supplied as the amino group acceptor. These results with bushbean extracts and those obtained earlier with white lupin extracts [26]

only indicate, however, the variety of aminotransferases that may occur in these seedling plants. Since neither set of data provides information on the degree of substrate specificity shown by the different aminotransferases present in each plant extract, no conclusion can be drawn about the actual number of these enzymes operating in the two species.

The results of early studies of the transamination reactions in plants have already been reviewed [27–30]. This paper will consider the more recent advances in the field which have been mainly concerned with studies on the development of aminotransferase systems during seed germination and plant growth, the isolation and characterization of the different enzymes and their intracellular location and function. The results obtained in these recent studies will be compared to related findings with animal and bacterial aminotransferases.

DEVELOPMENT OF AMINOTRANSFERASE SYSTEMS DURING SEED GERMINATION AND PLANT DEVELOPMENT

The development of aminotransferase systems during seed germination has been studied in a variety of plants. In early investigations, a marked increase in activity of the glutamate-oxaloacetate (GOT) and glutamate-pyruvate (GPT) aminotransferases was found in the embryo during the first few days of germination in *Hordeum vulgare*, *Zea mays*, *Avena sativa* and *Cucurbita* seeds [4, 31] and also in the entire seedlings of *Phaseolus vulgaris*, *Pisum sativum* and *Triticum vulgarae* during the first week of growth [32]. In *Pisum* cotyledonary tissue on the other hand, the specific activity of glutamate-oxaloacetateaminotransferase was found to reach a peak by the 4th day of germination, after which it steadily decreased [33]. In *Cucurbita* seedlings grown in light or darkness, the total activity of this aminotransferase was found to increase for the first 8 days after germination, but beyond this stage of growth the total activity of the enzyme was found to decrease rapidly [34]. Alanine aminotransferase activity was also found to show a similar pattern of development [35]. Forest and Wightman [36] studied changes in the activities of alanine-α-ketoglutarate, aspartate-α-ketoglutarate and asparagine-α-ketoglutarate aminotransferases in the roots, shoots and cotyledons of *Phaseolus vulgaris* seedlings grown for 2 weeks in complete darkness or under daily photoperiods of 16 hr at 32300 lux. The patterns of development of the three enzymes were found to be very similar under both experimental conditions: aspartate and alanine aminotransferases developed high levels of activity that were comparable in the different organs of the two kinds of seedlings, whereas asparagine aminotransferase activity developed to only about one-fifth of that shown by the other two enzymes in the different organs. In the cotyledons, activity of the three aminotransferases was found to be high within 24 hr of the start of germination, but all three activities decreased steadily thereafter. In the shoots, the activity of each enzyme increased dramatically during the first 10 days of germination while in roots, the three activities increased steadily during the first 8 days of growth and then diminished slowly. This latter finding is in agreement with the results of other investigations with developing roots which showed a decrease in the activity of aminotransferase enzymes in older roots of *Lens culinaris* and in ageing root protoplasts [37–38]. No correlation was found by Forest and Wightman [36] between the development of alanine, aspartate and asparagine aminotransferase activity in the different seedling organs of *P. vulgaris* and changes in amounts of the corresponding amino acids in the soluble nitrogen fractions from plants grown under light or dark conditions.

After studying the effect of changes in length of photoperiod on the activity of alanine-α-ketoglutarate aminotransferase in developing leaves of the photosensitive monocotyledon, *Lolium temulentum*. Hedley and Stoddart [39] reported that two phases could be discerned in the development of this enzyme under different photoperiods. The first phase was found to be related to leaf emergence and expansion and was characterised by a steadily increasing aminotransferase activity which was completely insensitive to changes in photoperiod, a situation similar to that observed by Forest and Wightman [36] with *Phaseolus* seedlings. In the fully expanded leaf, however, the level of alanine aminotransferase activity stabilised under short-day conditions but the maintenance of activity was found to be sensitive to changes in the photoperiod. Transfer of short-day treated leaves to long-day conditions resulted in an immediate reduction of enzyme activity and light-break treatments established that the repression was not directly related to floral initiation, but was rather a response to increased incident light energy. It was later shown that gibberellin could maintain alanine aminotransferase activity under long-day conditions while treatment of the leaves with the growth retardant, 2-chloroethyltrimethylammonium chloride (CCC) increased the enzyme level under both long and short days conditions [40]. Under the same conditions, the activity of the related enzyme, aspartate-α-ketoglutarate aminotransferase remained almost constant and was found to be insensitive to changes in the photoperiod or to growth-regulator treatments [39–41].

On further investigation of the effect of light stimulation on the activity of alanine and aspartate-α-ketoglutarate aminotransferases in dark-grown first leaves of *Lolium* seedlings, Hedley and Stoddart [42] found that the activity of the alanine enzyme was increased, after an initial lag-phase of 4–6 hr, by more than 130% during the first 24 hr of light exposure. In comparison, activity of the aspartate enzyme rose by only 18%. Red light treatments of up to 60 min duration also produced increases in alanine aminotransferase activity, but the effects were too small to indicate a phytochrome-mediated response and return of the plants to darkness after light exposure arrested the light-stimulated increase of alanine aminotransferase activity. In these investigations, Hedley and Stoddart observed that treatments which increased or decreased the chlorophyll content of the *Lolium* first leaf had similar effects upon the level of alanine aminotransferase activity, but were without effect on aspartate aminotransferase activity. For example, pretreatment with cycloheximide caused either stimulatory or inhibitory effects depending upon the concentration applied, but in general, chlorophyll formation and alanine aminotransferase activity responded in a similar manner, while aspartate aminotransferase activity showed virtually no change. By using light-grown leaves of a wide range of ages, it was found that a highly significant correlation coefficient could be derived for the relationship between alanine-α-ketoglutarate aminotransferase

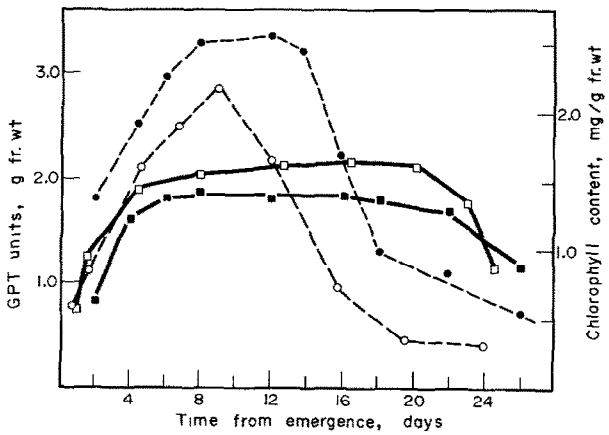

Fig. 1. Changes in alanine aminotransferase (GPT) activity and chlorophyll content in the fifth leaves of *Lolium temulentum* growing either in 8 or 16 hr photoperiods. ●——● Chlorophyll content (16-hr day-length); ■——■ Chlorophyll content (8-hr day-length); ○——○ GPT activity (16-hr day-length); □——□ GPT activity (8-hr day-length) (redrawn from data in ref. [41]).

(GPT) activity and chlorophyll content (Fig. 1). Based on these observations and on the close similarity between the reaction catalysed by alanine-α-ketoglutarate aminotransferase and that catalysed by alanine-α-ketoglutaraldehyde aminotransferase (δ-aminolevulinic acid aminotransferase), Hedley and Stoddart [42] proposed a scheme for the replication of alanine-α-ketoglutarate aminotransferase in chlorophyll biosynthesis.

In a recent study, Hatch and Mau [43] have demonstrated increases of ten-fold or more in the activity of both aspartate and alanine aminotransferases during greening of the leaves of *Panicum milaceum*, a plant with the C_4 dicarboxylic acid pathway of photosynthesis. This increased activity was due specifically to the two quantitatively major isoenzymes of these aminotransferases associated with the mesophyll and the bundle sheath cells in green leaves of this plant, which suggests a specific role for the two enzymes in the C_4 pathway of photosynthesis.

In an investigation of enzyme and protein changes occurring during seed development in *Lolium temulentum*, Hedley and Stoddart [44] reported that alanine-α-ketoglutarate and asparte-α-ketoglutarate amino-transferases showed similar patterns of activity which corresponded closely to the changes observed in protein synthesis during maturation of the seed. Both enzymes showed two peaks of high activity at the 2- and 4-week stages after anthesis and these coincided with the periods of maximum protein synthesis. In view of this close relationship, which was also observed in a similar study of enzyme and protein changes in developing *Lolium* leaves [41], these workers have suggested that alanine and aspartate aminotransferases may exert an important role in the regulation of protein synthesis during organ development [44].

Studies on the development of aromatic aminotransferase systems in seedling plants have so far been carried out with only two species, namely, *Phaseolus aureus* [45] and *Ph. vulgaris* [46]. Phenylalanine-pyruvate aminotransferase activity was found to increase gradually in shoots of light-grown *P. aureus* seedlings during the first 12 days after germination and the high level of activity reached by the 12th day was maintained for at least the next 10 days of growth. In the cotyledons of germinating *P. aureus* seeds, aromatic aminotransferase

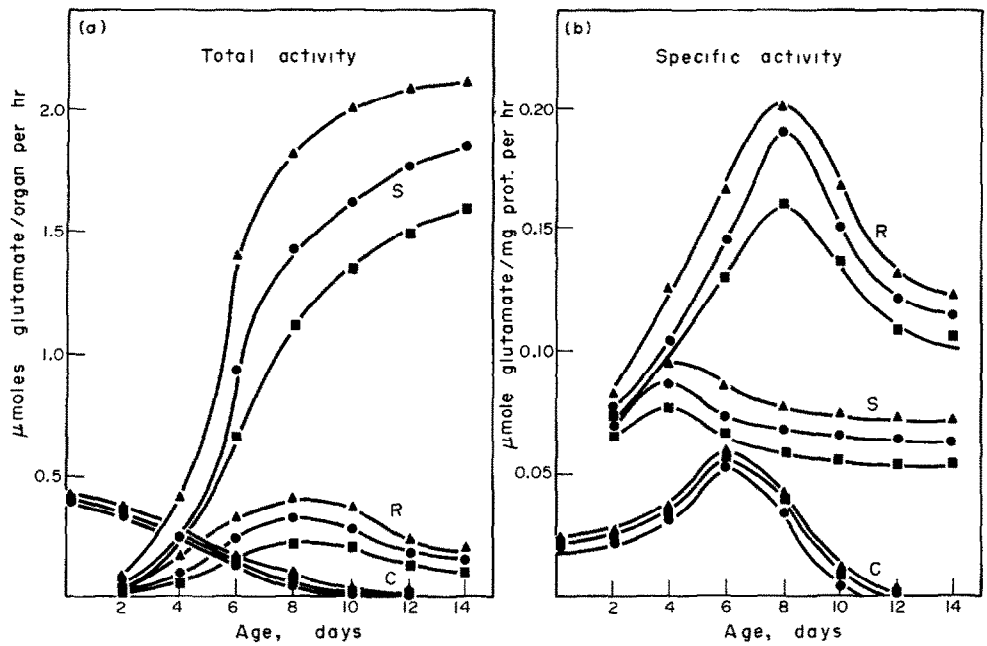

Fig. 2. Development of total activity and changes in specific activity of the three aromatic amino acid-α-ketoglutarate aminotransferase activities in the shoots (S), roots (R) and cotyledons (C) of light-brown bushbean seedlings during the first 2 weeks of growth. (▲) L-Phenylalanine-α-ketoglutarate activity; (●) L-Tyrosine-α-ketoglutarate activity; (■) L-Tryptophan-α-ketoglutarate activity (from ref. [46]).

activity also increased but at a much slower rate and when the cotyledons senesced at about the 9th day, the phenylalanine-pyruvate activity was only one quarter of that found in the attached shoot tissues [45]. In *P. vulgaris* seedlings, development of the tyrosine-, phenylalanine-, and tryptophan-α-ketoglutarate aminotransferase activities was examined in the cotyledons, roots and shoots of plants growing under light or dark conditions for the first 2 weeks after germination [46]. All three aminotransferases showed similar patterns of activity in comparable organs grown under the two environmental conditions, and changes in the levels of activity appeared unrelated to variations in the endogenous amounts of free aromatic amino acids found in the organs of both types of seedlings. The highest total activity for all three enzymes, expressed on an organ basis, was found in shoots of light-grown seedlings after 14 days, whereas the highest specific activity was shown by the enzymes extracted from the roots of both types of seedlings after 8 days of growth (Fig. 2). In cotyledons, the total activity of the aromatic aminotransferases was found to be highest within 24 hr of the onset of germination and thereafter it decreased more rapidly in light-grown than in dark-grown seedlings. This pattern of activity was similar to the changes previously observed for aspartate-α-ketoglutarate aminotransferase activity in cotyledons of *P. vulgaris* seedlings grown under light or dark conditions [36].

In a study of the biochemical events occurring during the development of apple fruits, Hulme and coworkers showed that the level of glutamate-oxaloacetate aminotransferase activity in the mitochondria increased some 6–8 fold coincident with the climateric rise in respiration during maturation of the fruit [47]. Since the level of oxaloacetate in developing apples appears to control respiratory metabolism through its regulatory effect (via succinic dehydrogenase activity) on the operation of the Krebs cycle [48], Hulme and coworkers have suggested that the increased activity of mitochondrial glutamate aminotransferase during the later phase of fruit development could be an important factor controlling the onset of maturation, since increased activity of the enzyme would decrease the level of oxaloacetate in the tissue which would then allow development of the respiratory climacteric. During maturation of tomato fruit, on the other hand, the total cytoplasmic and mitochondrial activity of aspartate-α-ketoglutarate aminotransferase and alanine-α-ketoglutarate aminotransferase has been shown to decline from the immature green stage to the full red condition [49, 50].

Certain enzyme changes associated with bud development were examined by Bachelard and Wightman [51] who followed the changes in aspartate-, alanine-, leucine-, and phenylalanine-α-ketoglutarate aminotransferase activity in overwintering vegetative buds of the tree, *Populus balsamifera*, as they developed from the stage of mid-winter dormancy to the spring flush of growth. All four enzymes were found to show essentially the same rise in activity during the late winter and spring period of growth, and this increase in activity appeared to be closely related to a decreased level of inhibitor substances in the buds [52].

With regard to other aminotransferases that are likely to occur in plant tissues, few investigations have so far been carried out to study the development of these enzymes in germinating seeds and young plants. Mazelis and Fowden [53] have examined the increase in activity of an ornithine-α-ketoglutarate aminotransferase in germinating peanut and found that the specific activity of the enzyme in the cotyledons increased steadily up to the 6th day of germination. In one of the few investigations of glyoxylate aminotransferases in germinating seeds, Cossins and Sinha [54] reported that glutamate-glyoxylate aminotransferase activity decreased rapidly in cotyledons of *Helianthus annus* during the first 4 days of germination. Alanine-glyoxylate aminotransferase activity was found to increase during the first 2 days and then declined over the next 3 days.

From the above considerations, it may be concluded that while the activity of different aminotransferases may vary quantitatively from one species to another during seed germination and seedling growth, in general these enzymes all increase significantly in total activity in the developing organs of the young plant during the first two weeks after germination. The exception to this generalization is found in the non-growing cotyledonary tissue where an initial high level of activity appears to decline steadily as germination proceeds, a situation which is presumably due to a low and declining rate of protein synthesis in this tissue once seedling growth is underway. There is some evidence, mainly from the work of Hedley and Stoddart [39, 40, 42] and that of Hatch and Mau [43] that environmental conditions, such as the length of the daily photoperiod, may influence the development and maintenance of activity of certain aminotransferases. Further studies are needed to examine the influence of environmental factors on these enzyme systems. Some animal aminotransferases, such as aspartate aminotransferase [55] and tyrosine aminotransferase [56–60] have been shown to be under the control of endogenous hormones and the daily rhythms of the organism [61, 62]. Similar regulation of plant aminotransferases by naturally-occurring hormones may also occur, but this possibility has not yet received much attention. In this connection, however, Jones and Stoddart [63] demonstrated that in a mutant of *Trifolium pratense* which remained vegetative in normally inductive long-day conditions, application of gibberellin A_3 produced normal flowering under long-day conditions and this growth response was accompanied by two phases of protein synthesis. The second and most major phase of protein synthesis, which appeared about 15 days after GA_3 application, was associated with an almost complete repression of alanine-α-ketoglutarate aminotransferase, whereas other enzymes such as aspartate-α-ketoglutarate aminotransferase, acid phosphatase, amylase and peroxidase showed no appreciable changes in activity. More recently, Pilet [64] has demonstrated that abscisic acid accelerates the fall of aspartate aminotransferase activity in senescent root cells of *Lens culinaris* while kinetin counteracts the effect of abscisic acid on this transamination reaction. The interpretation of these results at this point could be equivocal and more information is required on the primary reaction of the different plant hormones before these mechanisms of regulation can be understood.

ISOLATION AND SUBSTRATE SPECIFICITY OF PLANT AMINOTRANSFERASES

Aspartate and aromatic amino acid aminotransferases

The most extensively studied aminotransferase in plants is the enzyme responsible for catalysing the transamination of aspartic acid. Smith and Williams [178] were the first workers to present clear evidence for the occurrence of this aminotransferase in plants when they demonstrated appreciable levels of aspartate-α-ketoglutarate transaminase activity in the embryos from several types of germinating monocot and dicot seeds. High activity for this enzyme was later demonstrated in wheat germ [65]. Since these early studies, preparations of aspartate-α-ketoglutarate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, E.C. 2.6.1.1.) have now been purified to various levels from a range of plant tissues, such as cauliflower buds [66, 67], cotton seeds [68], germinating pea seeds [33], wheat germ [69], and bushbean roots [45, 70], oat leaves [71] and spinach leaves [72]. The enzyme extracted from cauliflower buds was purified about 200-fold by Ellis and Davies [66] and was found to utilize α-ketoglutarate or oxaloacetate with equal facility as amino group acceptor, depending on which of the two dicarboxylic amino acids was provided as the amino donor, but the enzyme could not utilize pyruvate as an amino acceptor. This aminotransferase from cauliflower was also found to catalyse the transamination of cysteic acid and certain substituted glutamic and aspartic acids whose molecular structure was closely similar to those of the normal substrates, but a full examination of substrate specificity was apparently not carried out since several amino acids, such as those of the aromatic series, were not listed as possible substrates.

A soluble and a mitochondrial aspartate-α-ketoglutarate aminotransferase were extracted from pea cotyledons and partially purified (60-fold) by Wong and Cossins [33, 73], but a full investigation of their substrate specificity was not performed. Reed and Hess [71] isolated the aspartate aminotransferase of oat leaves and showed that the enzyme could be separated electrophoretically into two forms. Form I, which was more anionic, was purified to a specific activity of 120 μmol min^{-1} mg $protein^{-1}$ (1100-fold) and comprised 80–90% of the total activity. Form II was purified 300-fold to a specific activity of 6 μmol min^{-1} mg $protein^{-1}$. The substrate specificities of the two forms were not investigated. Four isoenzymes of aspartate aminotransferase, associated respectively with the cytosol, chloroplasts, mitochondria and peroxisomes have been isolated from spinach leaves [72]. In each case α-ketoglutarate was preferred to pyruvate or glyoxylate as the amino group acceptor. The reaction was freely reversible and with glutamate as the amino group donor, oxaloacetate was the preferred acceptor except in the peroxisomes where greater activity was shown towards pyruvate and glyoxylate. Forest and Wightman [46] purified an aspartate aminotransferase 600-fold from the cytosol fraction of bushbean roots and demonstrated that the single protein remaining in the purified fraction was able to catalyse the transamination of five L-amino acids, aspartic, glutamic, phenylalanine, tyrosine and tryptophan when α-ketoglutarate or oxaloacetate was provided as the amino group acceptor. The enzyme was also found to catalyse transamination of the two amides, asparagine and glutamine, although the rates of these reactions were only about one-fifth of the rates observed with the corresponding dicarboxylic amino acids. The additional ability of this multispecific bushbean aspartate aminotransferase to transaminate both asparagine and glutamine contrasts with the finding that the activity of highly purified rat liver glutamine transaminase towards asparagine is very low [74].

The first study of the aromatic aminotransferase system in plants was reported by Gamborg and Wetter [12] and by Gamborg [13] who examined an aromatic aminotransferase extracted from acetone powders of mungbean seedlings. They purified the preparation 40–60 fold and showed that in addition to transaminating all three aromatic amino acids, the enzyme could transaminate several other amino acids, such as aspartate, glutamate and methionine, and that pyruvate, rather than α-ketoglutarate, was the best amino group acceptor. Gamborg [13] also demonstrated competitive inhibition of the phenylalanine-pyruvate aminotransferase activity by other amino acid substrates, such as lysine, glutamate and methionine, the latter two acids being the most effective inhibitors. In subsequent work, Red'Kina *et al.* [75] purified (50–80 fold) a glutamate-phenylpyruvate aminotransferase from pea seedlings and demonstrated that this preparation also showed very appreciable aspartate-α-ketoglutarate aminotransferase activity, which in fact was about 15–30 times greater than that of the phenylalanine aminotransferase activity. Gadal and coworkers [76] similarly examined an aromatic aminotransferase extracted from the leaves of *Quercus pedunculata*, and showed that this enzyme could utilize either *p*-hydroxyphenylpyruvate or phenylpyruvate as the amino group acceptor when aspartate or glutamate was provided as the amino group donor. The reversibility of these reactions was also demonstrated. In a study of an aromatic aminotransferase partially purified (about 50 fold) from pea shoots [77], the enzyme fraction was found to transaminate tryptophan, tyrosine and phenylalanine, as well as the aliphatic amino acids, alanine, methionine and leucine when α-ketoglutarate was used as the amino group acceptor. It seems likely, however, that in this investigation as in the earlier ones reported above, the partially purified enzymic fraction contained more than one aminotransferase.

Nevertheless, the suggestion was apparent from these early studies of the aromatic aminotransferase system in plants that the enzyme(s) comprising this system might be capable of catalysing the transamination of aspartic and glutamic acids, as well as the three aromatic amino acids. The substrate multispecificity of the aromatic aminotransferase in a plant was finally confirmed by Forest [78] who purified the enzyme from the cytosol fraction of bushbean seedlings to a single protein and demonstrated that the enzyme could indeed effect the transamination of aspartic and glutamic acids, in addition to phenylalanine, tyrsoine and tryptophan, when α-ketoglutarate or oxaloacetate was provided as the amino group acceptor. The pure enzyme was shown to catalyse the transamination of its aliphatic substrates at a much higher rate (10–12 times) than those determined for the three aromatic amino acids [46]. Inhibition studies showed that L-aspartate was able to competitively inhibit the aromatic aminotransferase activity of the enzyme, which strongly suggests that both the aliphatic and aromatic amino acid substrates compete for the same active site on the enzyme [70].

Although the findings of Forest and Wightman [46, 70] provide the first clear demonstration of a multispecific aspartate aminotransferase in higher plants,

detailed studies have been made by Munkres of a multifunctional protein from *Neurospora crassa* which possesses both aspartate aminotransferase and malate dehydrogenase activity [79–81]. Purified *Neurospora* malate dehydrogenase is a tetramer with sequentially non-identical monomers in the form $\alpha\alpha\alpha\beta$ [81]. Since isolated α-subunits bind pyridoxal phosphate stoichiometrically, while isolated β-subunits do not bind this coenzyme but instead are associated with NADH, Munkres [80] suggests that the active centre of the aminotransferase may be associated with the α-chains and that of the dehydrogenase with the β-chain. In presence of an excess of glutamate, the overall forward reaction proceeds in a concerted manner without exchange of the intermediate, oxaloacetate. Pyridoxal phosphate and pyridoxamine phosphate, co-factors of the aminotransferase, are non-competitive, reversible inhibitors of the dehydrogenase. Low concentrations of aspartate release the dehydrogenase from pyridoxal phosphate inhibition, suggesting that the small ligands of one of the reactions are serving as allosteric effectors of the other.

Table 1. Comparison of the amino acid substrate specificity shown by a multispecific aspartate aminotransferase from the cytosol of bushbean roots [46], a multispecific tyrosine aminotransferase from rat liver mitochondria [82] and a multispecific glutamate-oxaloacetate aminotransferase from *E. coli* [88] when α-ketoglutarate was provided in all reaction systems as the amino group acceptor

Amino acid substrate	Relative activities: Soluble aspartate amino transferase from bushbean roots* (%)	Mitochondrial tyrosine amino-transferase from rat liver† (%)	Glutamate amino transferase from *E. coli*‡ (%)
L-Aspartic acid	100	100	100
L-Asparagine	17	2.4	22
L-Tryptophan	6	10	181
L-Phenylalanine	9.5	26	104
D-Phenylalanine	0	0	—
L-β-3,4-Dihydroxyphenylalanine	1.4	13	—
L-Tyrosine	7	16	39
D-Tyrosine	0	0	—
3-Iodo-L-tyrosine	6	13	—
3,5-Diiodo-L-tyrosine	—	0.1	—
L-Alanine	0	0	0
L-Glycine	0	0	0
L-Serine	0	0	0
L-Threonine	0	—	0
L-Valine	0	0	0
L-Leucine	0	0	0
L-Isoleucine	0	0.6	0
L-Methionine	0	3.2	28
L-Cystine	0	—	—
L-Cysteine	—	20	—
L-Proline	0	0	0
L-Histidine	2	1.1	0
L-Arginine	0	—	0
L-Lysine	0	0	0
Erythro-β-hydroxy-aspartic acid	70	—	—

* Amino acids were tested at a final concentration of 40 mM, pyridoxal-5'-phosphate at 0.02 mM, and α-ketoglutarate at 8 mM. Incubation was at 35° in Tris–HCl 0.05 M buffer at pH 8.5 [46].

† Amino acids were tested at a final concentration of 9 mM, pyridoxal-5-phosphate at 0.03 mM and α-ketoglutarate at 0.12 mM. Incubation was at 37° in 0.2 M potassium phosphate buffer at pH 8.1 [82].

‡ Amino acids were tested at a final concentration of 41 mM except for tyrosine (8 mM) and tryptophan (21 mM) and α-ketoglutarate at 1 mM. Incubation was at 30° in phosphate buffer at pH 7.5 [88].

There have also been several reports during the last few years indicating the occurrence of similar multispecific aspartate aminotransferases in animal and bacterial cells (Table 1). From evidence based on substrate specificity, competitive inhibition by common substrates, inhibition of activity by specific antibodies and from physical chemical properties, Miller and Litwack [82] have concluded that the mitochondrial tyrosine aminotransferase from rat liver is identical with mitochondrial aspartate aminotransferase from the same tissue. Similarly, it has been shown that transamination of phenylalanine is catalysed by the same protein that catalyses transamination of aspartic acid in mitochondrial preparations from beef kidney [83] and pig heart [84]. A soluble aspartate aminotransferase from pig heart was also shown to possess aromatic aminotransferase activity, but to a much lesser extent than the mitochondrial enzyme [84]. In an examination of the substrate specificity of the soluble and mitochondrial tyrosine (aromatic) aminotranferase from rat liver, it was found that the mitochondrial enzyme readily transaminated several ring-substituted L-phenylalanines, such as *m*-tyrosine and *p*-chlorophenylalanine when α-ketoglutarate, oxaloacetate or pyruvate were provided as amino group acceptors, whereas none of the synthetic amino acids served as a substrate for the soluble enzyme [85]. In contrast to these results with animal enzymes, a recent study in the first author's laboratory of the substrate specificity of the soluble and mitochondrial aromatic aminotransferases from bushbean seedlings to a range of ring-substituted L-phenylalanines and L-tryptophans has shown that the soluble enzyme had the greatest capacity to transaminate these synthetic substrates, although the mitochondrial enzyme showed some activity with many of the substituted amino acids [86].

Bacterial aromatic aminotransferases have been poorly investigated until recently. Rudman and Meister [87] were the first to report the presence of three aminotransferases (A, B & C) in *Escherichia coli*. The multispecific aromatic-α-ketoglutarate (oxaloacetate) aminotransferase (transaminase A) of *E. coli* was reported to catalyse the transamination of aspartate or glutamic acids, tryptophan, tyrosine, phenylalanine and methionine when α-ketoglutarate or oxaloacetate was provided as the amino group acceptor. In another investigation of the glutamate: oxaloacetate aminotransferase in bacteria, Chesne and Pelmont [91] purified the enzyme from *E. coli* K12 250-fold by means of hydroxyapatite and DEAE-Sephadex chromatography followed by electro-focussing. Polyacrylamide gel electrophoresis showed only one major protein band in the purified extract, which was found to catalyse all the reactions cited above for transaminase A.

Recently, however, it has been shown that transaminase

A was in fact made up of two proteins [89, 90]. Mavrides and Orr [90] demonstrated that the major enzyme (Form 1B) was 90% repressed by growing the organism in the presence of L-tyrosine, while the minor form (1A) was unaffected. The two forms (now called A and B rather than 1A and 1B) have been purified to homogeneity [91]. Enzyme A shows activity towards aspartic acid and the three aromatic amino acids, while enzyme B is only an aromatic aminotransferase. The two enzymes are similar in their amino acid composition and enzyme B may be converted to enzyme A by controlled proteolysis.

Alanine aminotransferase

Alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase E.C. 2.6.1.2) has been isolated and partially purified from cotton seeds [92], leaves of *Atriplex spongiosa* [93], tomato fruit [50, 94] and pumpkin cotyledons [35]. Kinetic studies have been carried out with the partially purified enzyme (Table 3), but no comprehensive examination of its substrate specificity has been made.

Glyoxylate aminotransferases

L-Amino acid-2-glyoxylate aminotransferase activity has been detected in tobacco leaves [26], pea leaves [54], *Phaseolus radiatus* seeds [95], wheat leaves [21], oat leaves [22], *Atriplex* and sorghum leaves [96], kidney bean leaves [97, 98] and spinach leaves [99–101]. Alanine-glyoxylate and serine-glyoxylate aminotransferases from oat leaves were purified 134-fold by Brock *et al.* [22]. Using this partially purified preparation it was found that only alanine, glutamate and serine could act as amino group donors when glyoxylate was the keto acceptor. When serine was used as an amino group donor, only glyoxylate could be used as the keto acceptor. These results suggested that the glyoxylate aminotransfer reactions are distinct from those of other aminotransferases, as they are in animal tissues [102], but it was not clear whether the three glyoxylate reactions were catalysed by a single enzyme. However, during purification of the glyoxylate aminotransferases of kidney bean leaves, Smith [97] obtained a 100-fold purification of serine-glyoxylate aminotransferase giving a preparation which was essentially devoid of alanine-glyoxylate and glutamate-glyoxylate aminotransferase activity. During purification, there was a change in the ratio of the three aminotransferase activities, indicating that each is catalysed by a separate protein. The serine-glyoxylate aminotransferase was also able to catalyse the serine-pyruvate reaction, with the formation of hydroxypyruvate and alanine. Similar results were reported by Rehfeld and Tolbert [101] who found that the peroxisomes of spinach leaves contained a serine-glyoxylate and a glutamate-glyoxylate aminotransferase which could be separated by isoelectric focussing. Both enzymes also catalysed an alanine-glyoxylate reaction, and the serine-glyoxylate aminotransferase could also use pyruvate as a keto acceptor. Rehfeld and Tolbert found that the glutamate-, serine- and alanine-glyoxylate reactions were irreversible, but Carpe and Smith [98] found that the serine-glyoxylate aminotransferase of kidney bean leaves could also catalyse the reverse reaction (hydroxypyruvate-glycine) as well as a reversible serine-pyruvate transamination.

Branched-chain amino acid aminotransferases

Transamination of the branched-chain amino acids, valine, leucine and isoleucine has been reported to occur in various plant organs [8, 17] but no detailed investigation of the enzyme(s) responsible for these activities has yet been carried out. These preliminary studies indicated that the most suitable keto acid substrate for optimum activity of this aminotransferase is α-ketoglutarate. In animal tissues, three isoenzymes showing branched-chain amino acid aminotransferase activity, and referred to as enzymes I, II and III (E.C. 2.6.1.6), have been detected in rat and pig tissues [103–106]. Isoenzymes I and III were shown to be equally active with all three amino acids and their properties were similar, but their distribution was found to be different. While enzyme I was widely distributed in rat and pig tissues, enzyme III was only found in brain tissues of the two animals. Enzyme II was shown to be quite different from enzymes I and III. It was found only in rat liver and was specific for leucine; its K_m value was 30 times higher than that of enzyme I [105]. Furthermore, the activity of enzyme II was found to be rapidly induced in rat liver by cortisol or a high protein diet [107, 108], while these treatments did not influence the induction of enzyme I. On the other hand, alloxon diabetes induced enzyme I activity in various rat tissues, including kidney, but did not induce enzyme II activity in the liver [107]. It would appear from these studies that the isoenzymes of mammalian branched-chain aminotransferase are under separate genetic control. In contrast to these findings with mammalian enzyme, with the bacteria *Pseudomonas aeruginosa* [109] and *Salmonella typhimurium* [110], only one protein was found to have aminotransferase activity with branched-chain amino acids. This enzyme catalysed the transamination of leucine and isoleucine with equal facility, but its rate of activity with valine was only 60% of that observed with the other two amino acids.

Ornithine aminotransferase

Ornithine aminotransferase activity has been detected in both cytosol and mitochondrial fractions from a range of plant seedlings [111–115] and an L-orthinine-α-ketoglutarate aminotransferase (E.C. 2.6.1.13) has been partially purified by Mazelis and Fowden [53] from acetone powders of germinating peanut cotyledons. The enzyme was shown to catalyse the δ-transamination of L-orthinine to glutamic-γ-semialdehyde and specifically required α-ketoglutarate as the amino group acceptor. The addition of L-canavanine or L-valine produced substantial inhibition of enzyme activity, and this finding is consistent with earlier reports that the activities of ornithine-δ-aminotransferases purified from rat liver [116] and *Tetrahymena pyriformis* [117] were strongly inhibited by these two amino acids. Kinetic studies with the partially purified peanut enzyme showed that its optimum pH of 8.0, and Michaelis constants for L-ornithine and α-ketoglutarate were quite similar to those reported for the ornithine-δ-aminotransferases of *Chlamydomonas* [118] and *Tetrahymena* [117], but differed from those of rat liver [116] and *Neurospora crassa* [119]. Recently, the ornithine-α-ketoglutarate aminotransferase of squash cotyledons has been purified over 400-fold [120]. The enzyme was very specific for L-ornithine and α-ketoglutarate, as was the enzyme

from mungbean mitochondria [111]. Leucine, isoleucine and valine were found to be inhibitory.

Other plant aminotransferases

Although most free 'protein' amino acids have been shown to be transaminated by plant extracts when either α-ketoglutarate, oxaloacetate, pyruvate or glyoxylate is provided as the amino group acceptor [17, 20, 26], no detailed studies of many of these enzymes, other than those discussed above, have been carried out. An enzyme catalysing the transamination of the non-protein amino acid γ-aminobutyric acid has been detected in peanut cotyledons [121], various succulent plants [122], radish leaves [123] and the carpophores of *Agaricus bisporus* [124]. The radish and peanut enzymes showed much greater activity towards pyruvate than α-ketoglutarate [121, 123], but the enzymes found in succulent plants [122] and *Agaricus* [124] were specific for α-ketoglutarate, as were the enzymes of *Pseudomonas fluroescens* [125], brain and liver [126]. The partially purified *Agaricus* enzyme was not specific for γ-aminobutyric acid but could also transaminate aspartate, alanine and a higher homologue of γ-aminobutyric acid, δ-aminovaleric acid [124].

PHYSICAL AND KINETIC PROPERTIES OF PLANT AMINOTRANSFERASES

Molecular weight, sedimentation coefficient and subunit composition

Table 2 shows the values obtained in determinations of the MW, sedimentation coefficient and subunit composition of aspartate aminotransferase isolated from various plant and animal tissues. The soluble and mitochondrial isoenzymes of aspartate aminotransferase from animal tissues were found to have almost identical MWs, most of which fell in the range of 90000 to 100000 daltons [82, 83, 127–129]. The soluble aspartate aminotransferase purified from bushbean roots was found to have a MW of 128000 daltons, as determined by gel filtration on Sephadex G-200 [46], while the same enzyme isolated from wheat germ has a MW of 75000 daltons when estimated by gel filtration on Sephadex G-100 [69]. In bacteria, this enzyme appears to have a MW in the range of 82000–88000 [90]. The sedimentation coefficients of the two main forms of animal aspartate aminotransferase were found to be of the order of 5.5 (Table 1) and both forms were found to be composed of two subunits, each half the MW of the dimer [83, 128, 129]. The dimeric enzyme could be dissociated into its subunit monomers by treatment with guanidine hydrochloride [128], or by dilution and increased pH [129]. A comparable examination of a plant aspartate aminotransferase has not yet been made, but since the purified bushbean enzyme was shown by Forest and Wightman [46] to have a MW in the same order of magnitude as the isoenzymes examined from animal tissues, it seems possible that the plant enzyme will have a comparable subunit composition.

Alanine aminotransferase isolated from tomato fruit was shown to have a MW of 100000 as estimated by gel chromatography [50]. This result is in agreement with the values found for alanine aminotransferase purified from pig heart (100000) [130], and rat liver (114000) [131].

Pyridoxal phosphate requirement

Mammalian aminotransferases have been resolved into their apoenzyme and coenzyme components by several workers [132] and a quantitative determination of the pyridoxal phosphate content per molecule of holoenzyme has been made [83, 127, 129, 133, 134]. Aspartate aminotransferase extracted from beef kidney [83] and the aminotransferase for branched-chain amino acids isolated from *Pseudomonas aeruginosa* [109] were found to contain 2 moles of pyridoxal phosphate per mole of holoenzyme, while a soluble tyrosine aminotransferase purified from rat liver was found to bind approximately 4 moles of pyridoxal phosphate per mole of protein [135]. Wong and Cossins [73] partially

Table 2. Comparison of the properties of aspartate aminotransferase isolated from bacteria, plant and animal tissues

Enzyme source	Isoenzymes	MW	$S_{20,w}$	Subunits	Pyridoxal-phosphate content (moles/mole enz)	pH optimum	Amino acid substrate when α-ketoglutarate was provided as amino group acceptor	Reference
Whole wheat germ	—	75000	—	—	—	8.0–8.5	Aspartate	69
Bushbean roots	1 (anionic)	128000	—	—	—	8.5	Aspartate, phenylalanine, tyrosine, tryptophan	46
Rat liver		90000	4.5	—	—	-	Aspartate, phenylalanine, tyrosine, tryptophan, cysteine	82
mitochondria	1 (cationic)	114000						
Beef kidney		93000	5.5	2	2	8.0	Aspartate, Phenylalanine	83
mitochondria	1 (cationic)							
Pig heart		91000	5.75	2	—	8.3	Aspartate, phenylalanine, tyrosine, tryptophan	84, 128, 186
mitochondria	3 (cationic)							
soluble fraction	4 (anionic)	93000	5.7	2	2	8.3	Aspartate, phenylalanine, tyrosine, tryptophan	
Chicken heart		95000 100000	5.4	—	—	—	Aspartate	127, 129
mitochondria	1 (cationic)							
soluble fraction	3 (anionic)	95000 100000	5.5	2	2		Aspartate	
Escherichia coli	2	82000	—	—	—	7.5	Aspartate phenylalanine	88, 90, 91, 153
		84000	—	—	—		tyrosine, tryptophan,	
		88888	—	—	—		methionine	

resolved an aspartate aminotransferase from germinating pea cotyledons and demonstrated its requirement for pyridoxal phosphate and pyridoxamine phosphate. Complete resolution of the multispecific aspartate aminotransferase purified from bushbean roots into its protein and coenzyme moieties was not achieved by Forest and Wightman [46]. The pure enzyme did not show any appreciable increase in activity when pyrixodal phosphate was added to the reaction mixture, which suggested that sufficient coenzyme was tightly bound to the apoenzyme moiety. This finding was confirmed by Reed and Hess [71] who observed no activation by pyridoxal phosphate during a 1100-fold purification of aspartate aminotransferase from oat leaves. A similar result was also obtained with partially-purified aromatic aminotransferase preparations from tomato shoots [18] and mung bean seedlings [16], and no requirement for exogenous pyridoxal phosphate was found for an ornithine aminotransferase partially purified from peanut cotyledons [53]. After partial purification of the glutamate and aspartate aminotransferases from wheat germ [65], cauliflower [66] and pea cotyledons [136], a partial dependence upon pyridoxal phosphate for optimum activity could be demonstrated, although considerable transamination was still observed in the absence of added coenzyme. Thus, it appears that plant aminotransferases differ from the animal enzymes in the much tighter binding encountered between protein and coenzyme moiety.

pH *optima of plant aminotransferases*

Investigations of the pH optima of several plant aminotransferases have indicated that these enzymes all show optimum activity within the pH range 8.0–8.9. For example, the pH optimum for soluble aspartate-α-ketoglutarate aminotransferase extracted from pea cotyledons [33] was found to be pH 8.0; for the multispecific aspartate aminotransferase purified from bushbean roots it was found to be pH 8.5 [46]; for the tryptophan-α-ketoglutarate aminotransferase in mung bean seedlings and pea shoot tips it was shown to be pH 8.5 [15, 16, 77] and for the same enzyme in tomato shoots it was found to be pH 8.0 [18]. The pH optimum for γ-aminobutyrate-pyruvate aminotransferase of radish leaves was 8.9 [123]. These results with plant aminotransferases are quite comparable to the pH optima obtained with similar enzymes from animal tissues [137, 138].

Effect of metal ions and buffers on aminotransferase activity

Although the role of metal ions in transamination reactions is not clear, there have been reports of the stimulation of aminotransferase activity by various cations [139, 140]. Patwardhan [141] showed the participation of Fe^{++} ion as a cofactor in promoting the activity of the glutamate-oxaloacetate aminotransferase isolated from *Dolichos lablab*, but this effect could not be replicated by Verjee and Evered [69], and Forest [78] found that Fe^{++} had no effect on the activity of a multispecific aspartate aminotransferase from bushbean seedlings. On the other hand, Wong [136] showed that the addition of Mg^{++} and Mn^{++} stimulated aspartate aminotransferase activity in cell-free extracts of pea cotyledons and Forest [78] demonstrated a 20% stimulation of the activity of this aminotransferase from bushbean roots when Ca^{++} or Mg^{++} was added to the reaction mixture. Other divalent ions such as Zn^{++} and Cu^{++} were found to have no effect on aspartate aminotransferase activity [78].

The role of anions in regulating the activity of aminotransferases has been investigated and it has been shown that certain anions, such as PO_4^{---}, SO_4^{--} and citrate have an inhibitory effect (up to 40%) on the mitochondrial (cationic) isoenzyme of animal aspartate aminotransferase, whereas these anions have slight stimulatory effect (up to 14%) on the corresponding soluble (anionic) isoenzyme [142]. It has also been shown that Michaelis constants for both isoenzymes depend on the ionic concentration of the reaction medium, being approximately proportional to PO_4^{---} concentration over considerable range [143]. With a soluble (anionic) aspartate aminotransferase from bushbean roots, Forest [78] demonstrated that activity of the enzyme was not significantly influenced by the presence of PO_4^{---}, SO_4^{--} or citrate anions at various concentrations in the reaction medium. However, in another study with the corresponding animal enzyme, Cheng and coworkers [144] showed that anions can act as competitive inhibitors of aspartate transamination reactions and suggested that anions occupy the positively charged sites at the active center of the aminotransferase where the substrate's negatively charged carboxyl group(s) bind. These workers also demonstrated that anions can act as competitive inhibitors of the second half of the aspartate transamination reaction, during conversion of the pyridoxamine enzyme to pyridoxal enzyme. These findings may explain the observation that since mitochondrial aspartate aminotransferase generally shows a higher anion affinity than the corresponding soluble isoenzyme, whether in the pyridoxal or pyridoxamine form, anion competition with the normal substrate of the enzyme at any given concentration is greater for the mitochondrial isoenzyme [144].

Kinetic studies and the mechanism of action of plant aminotransferases

Kinetic studies of the effect of changes in concentration of the two primary substrates on the initial velocity of a transamination reaction together with spectrophotometric analysis of changes in the molecular characteristics of the enzyme, have made possible investigations on the mechanism of this biochemical reaction. Two kinds of mechanism have been postulated which are known respectively as the ternary and binary complex mechanisms and these have been well discussed in previous reviews [145–149]. It is possible to distinguish between these two mechanisms in which it is proposed that the two substrates either bind to the enzyme simultaneously (ternary complex mechanism), or bind successively, one at a time (binary complex mechanism). In the former case, double reciprocal plots of the results obtained when initial relative velocities of the enzyme are determined using varying concentrations of one substrate and several fixed concentrations of the other substrate, should give a family of converging lines. In the latter case, Lineweaver and Burk plots for varying concentrations of one substrate against several fixed concentrations of the other, should give a series of parallel lines [92]. The results obtained from several kinetic studies with animal aminotransferases [84, 138,

150–152], with plant aminotransferases [22, 33, 46, 50, 97] and with bacterial aminotransferases [153] all fully support the binary complex mechanism as the mode of action of these enzymes. The mechanism can be illustrated by the following two equations:

$$\text{Amino acid}_1 + \text{PLP-enzyme} \rightleftharpoons \text{keto acid}_1 + \text{PMP-enzyme} \quad \text{(Equation I)}$$

$$\text{Keto acid}_2 + \text{PMP-enzyme} \rightleftharpoons \text{amino acid}_2 + \text{PLP-enzyme} \quad \text{(Equation II)}$$

This mechanism implies that the overall reaction is the sum of two separate binary reactions catalysed by the holoenzyme oscillating between the pyridoxal-5′-phosphate (PLP) form and the pyridoxamine-5′-phosphate (PMP) form. The reaction pattern between the pyridoxal phosphate enzyme and the amino acid (Equation I) can be stated as follows: formation of a coenzyme-substrate aldimine, tautomerization of the aldimine to a ketimine and hydrolysis of the ketimine to yield the aminic coenzyme and the keto acid product [154]. This reaction sequence can be referred to as the Ping Pong Bi Bi mechanism in the terminology of Cleland [155]. From spectral studies, it can be shown that the amino acid reacts with the pyridoxal form of the enzyme (absorbance, 350–360 nm) to generate the pyridoxamine form of the enzyme (absorbance, 330 nm). This form reconverts into the pyridoxal form upon addition of the keto acid [84, 149, 150]. In this connection, exchange transamination reactions between amino acids and their keto acid analogues, like those catalysed by mammalian aminotransferase [150, 156] have been demonstrated with plant aspartate aminotransferase [157].

The dissociation constant for each substrate of an aminotransferase can be calculated from double reciprocal plots of the initial reaction velocities obtained when the concentration of one substrate is varied at several fixed concentrations of the second substrate. Plotting the ordinate intercept values against reciprocals of the second substrate concentration produces a straight line which intercepts the ordinate at a value equivalent to the true dissociation constant of the second substrate. The data in Table 3 show the dissociation constants obtained by the above procedure for various animal and plant aminotransferases for their different substrates. A few conclusions can be drawn from a comparison of these dissociation constants for aminotransferases isolated from various sources. It is evident that the supernatant and mitochondrial isoenzymes of aspartate aminotransferase have different kinetic properties in most tissues investigated. Aspartate aminotransferase appears to be a multispecific enzyme in both plant and animal tissues and in bacteria. The dissociation constants for its aromatic amino acid substrates are comparable. The mitochondrial isoenzyme, however, seems to have more affinity for aromatic amino acids than has the supernatant enzyme, although the affinity of both isoenzymes for the aromatic substrates is generally much less than that for the aliphatic substrates. The biological significance of these findings remains unclear and further studies on the mechanism of action of these isoenzymes, and on their regulation, are necessary before

Table 3. Kinetic parameters of some aminotransferases isolated from various plant and animals sources

Enzyme	Source	Buffer employed	Dissociation constant (M) of different substrates tested				References
Aspartate aminotransferase	Bushbean roots	Tris–HCl (0.05 M)	Aspartate	α-Ketoglutarate	Glutamate	Oxaloacetate	70
	soluble fraction	pH 8.5	4.3×10^{-2} Phenylalanine 5.7×10^{-2} Tyrosine 6.3×10^{-2} Tryptophan 6.8×10^{-2}	4.5×10^{-2} 2.5×10^{-4}	5.6×10^{-2} 3.3×10^{-3}	5.0×10^{-2} Phenylpyruvate 5.6×10^{-2} p-Hydroxyphenylpyruvate 6.3×10^{-2}	
	Pig heart	Sodium pyrophosphate (0.10 M)	Phenylalanine			Phenylpyruvate	84, 150
	mitochondria	pH 8.2	2.5×10^{-2}	7.0×10^{-4}	1.3×10^{-2}	6.0×10^{-4}	
	soluble fraction	Tris–acetate (0.10 M) pH 8.2	1.8×10^{-2}	4.0×10^{-4}	1.4×10^{-2}	4.0×10^{-4}	
	Beef kidney	Potassium phosphate (0.5 M)	Aspartate			p-Hydroxyphenylpyruvate	83
	mitochondria	pH 8.0	6.8×10^{-4}	2.5×10^{-3}	5.0×10^{-3}	3.5×10^{-2}	
	Atriplex spongiosa	Hepes–NaOH (0.025 M)					
	(1) Mesophyll cells		Aspartate	α-Ketoglutarate	Glutamate	Oxaloacetate	93
	soluble fraction	pH 8.0	8.5×10^{-4}	8.0×10^{-5}	1.6×10^{-3}	1.0×10^{-4}	
	(2) Bundle sheath cells *mitochondria*	pH 8.0	5.0×10^{-4}	1.2×10^{-4}	4.5×10^{-5}	7.5×10^{-4}	
Alanine aminotransferase		Tris–HCl (0.05 M)	Alanine	α-Ketoglutarate	Glutamate	Pyruvate	50
	Tomato fruit	pH 7.25	2.8×10^{-3}	2.8×10^{-4}	2.3×10^{-3}	9.0×10^{-5}	
	Rat liver	Sodium pyrophosphate (0.10 M)	3.4×10^{-2}	1.1×10^{-3}	1.5×10^{-2}	0.9×10^{-3}	187
	Atriplex spongiosa	Hepes–NaOH (0.025 M)					
	(1) Mesophyll cells		Alanine	α-Ketoglutarate	Glutamate	Pyruvate	
	supernatant	pH 7.5	3.0×10^{-3}	9.0×10^{-5}	1.15×10^{-3}	4.0×10^{-5}	93
	(2) Bundle sheath cells *supernatant*	pH 7.5	3.1×10^{-3}	2.8×10^{-5}	8.0×10^{-4}	2.2×10^{-5}	

any general statements can be made on this topic. The variations observed between results obtained for the dissociation constant for each substrate of aspartate aminotransferase isolated from various tissues may partially be explained by the utilization of different buffers, ionic concentration and pH by the different investigators, but it is most likely mainly due to genetic variations between the tissues employed.

THE INTRACELLULAR LOCATION AND FUNCTIONS OF AMINOTRANSFERASES

The intracellular distribution of aspartate-α-ketoglutarate aminotransferase has been extensively studied in several mammalian and avian tissues, where in each case a soluble anionic form and a mitochondrial cationic form have been found [127, 133, 158–163]. Aspartate aminotransferase was also found in the mitochondrial and peroxisomal fractions of the protozoan, *Tetrahymena*, but the ionic properties of these two forms of the enzyme were not examined [164]. The mitochondrial and soluble forms of aspartate aminotransferase from pig heart were found to have different kinetic properties and immunological reactions [84, 134] and studies on the concentration of these isoenzymes at various stages of growth [160, 162, 165], on induction of the enzymes [55, 166] and of their amino acid sequences [167] suggest that the soluble and mitochondrial forms of this aminotransferase in mammalian and chicken tissues are under separate genetic control. The location of the three aromatic aminotransferases has also been studied in animal tissues. Semba and Civen [168] investigated the distribution of the aminotransferases for L-tyrosine, 3,4-dihydroxy-L-phenylalanine (DOPA), L-tryptophan and 5-hydroxy-L-tryptophan in rat brain tissue and found that 60–80% of the total activity of each enzyme resided in the mitochondrial fraction. The soluble and mitochondrial forms of liver tyrosine aminotransferase appear to have different amino acid compositions [82, 135] which suggests that at least in mammals, formation of the two forms of tyrosine aminotransferase are under separate genetic control [60].

In plant tissues, much of the aminotransferase activity is found in the cytosol. In bushbean roots, more than 90% of the aspartate aminotransferase activity is in the soluble fraction [46] and in pumpkin roots and hypocotyls 98% of this enzyme is found in the cytosol [34]. Soluble aminotransferases have also been found in pea cotyledons [33], pumpkin cotyledons [35], soybean root nodules [169], tomato fruit [8, 50], *Atriplex* leaves [43], *Lolium* leaves [170, 179], oat leaves [71], spinach leaves [72] and castorbean endosperm [171]. The percentage of the enzyme in the soluble fraction varies from 26% in the case of aspartate-α-ketoglutarate aminotransferase of spinach leaves [72] to 98% in the case of the alanine aminotransferase of pumpkin cotyledons [35]. In contrast to the results found in animals, the main intracellular location of the three L-aromatic amino acid-α-ketoglutarate aminotransferase activities in roots of bushbean seedlings was found to be in the cytosol fraction, with only about 6% of the total activity associated with the plastids and 4% with the mitochondria [46]. Thus, it appears that in many cases the soluble enzyme is the quantitatively predominant form. Where an aminotransferase is present in both soluble and particulate form, there may be a specific isoenzyme present in the soluble fraction. Such isoenzymes have been found in the aspartate-α-ketoglutarate aminotransferase of pumpkin cotyledons [34], soybean root nodules [169], spinach leaves [72] and cucumber cotyledons [198], and in the alanine aminotransferase of *Lolium* leaves [170, 179].

As in the case of the animal enzymes, many plant aminotransferases are also found in the mitochondria. Mukerji and Ting [172] detected glutamate-oxaloacetate aminotransferase in mitochondria from phylloclades of the cactus *Opuntia*, and γ-aminobutyric acid-α-ketoglutarate aminotransferase is present in the mitochondria of *Agaricus* [124]. In germinating peanut cotyledons, the specific activity of mitochondrial ornithine-α-ketoglutarate aminotransferase was more than eight times that of the soluble enzyme, although the total activity of the enzyme in the cytosol was greater [53]. Yu and Spencer [8, 94] found a low level of leucine-α-ketoglutarate aminotransferase and a higher level of alanine aminotransferase activity in the mitochondria of tomato fruit. The alanine aminotransferase of tomato fruit mitochondria has also been studied by Gazeau-Reyjal and Crouzet [173], who conclude that their data on chromatographic patterns, stability, pH optimum, specificity and apparent MW show that the mitochondrial and soluble enzymes are the same and not isoenzymes, which is contrary to the findings with this enzyme in rat heart [174]. However, in the case of aspartate-α-ketoglutarate aminotransferase of spinach leaves, a specific mitochondrial isoenzyme is present, accounting for about 19% of the total activity [72]. An isoenzyme of aspartate aminotransferase has also been found to be associated with mitochondria in the bundle sheath cells of the C_4 plants *Atriplex songiosa* and *Panicum milaceum* [43]. This mitochondrial isoenzyme may play an important role in C_4 photosynthesis, as discussed below.

Considerable interest has been expressed, in the presence of aminotransferases in the chloroplasts and their possible role in amino acid formation, and hence protein synthesis in this organelle. As discussed below, aminotransferase activity may increase during the greening of etiolated leaves on exposure to light [42, 43, 170]. Earlier workers, using non-aqueous methods of isolation, demonstrated the presence of glutamate-oxaloacetate aminotransferase in the chloroplasts of *Opuntia* phylloclades [172] and *Vicia* and *Nicotiana* leaves [175]. The latter workers also found glutamate-pyruvate aminotransferase activity in the chloroplast, although in smaller amounts; e.g. in *Vicia*, 38% of the glutamate-oxaloacetate and 20% of the glutamate-pyruvate activity was in this organelle. Aspartate aminotransferase activity was associated with the mesophyll chloroplasts of maize [176], although little alanine aminotransferase was found in the chloroplasts of this species or in those of *Amaranthus palmeri*, a C_4 species with high aminotransferase activity. In spinach leaves, 17% of the total aspartate aminotransferase activity was found in the whole chloroplast fraction after aqueous isolation. The enzyme was easily lost from broken chloroplasts, suggesting that it was located in the stroma [100]. The isoenzyme of aspartate transaminase found in the chloroplasts was distinct from two peroxisomal forms of this enzyme, but appeared at the same position as the mitochondrial enzyme [101]. However, Huang and coworkers [72] found that the aspartate aminotransferase of spinach

leaves could be separated into four distinct isoenzymes, associated respectively with the chloroplasts, mitochondria, peroxisomes and cytosol; the chloroplast isoenzyme accounted for 45% of the total activity and like the other isoenzymes was freely reversible and preferred α-ketoglutarate to pyruvate or glyoxylate as the amino-group acceptor.

The most comprehensive study of the aminotransferases of the chloroplast is that of Kirk and Leech [177] using aqueously isolated chloroplasts from *Vicia faba*. The aminotrasferase activity of whole chloroplasts was examined using 21 amino acids and three keto-acid acceptors, α-ketoglutarate, pyruvate and oxaloacetate. High activity for aspartate-α-ketoglutarate, glutamate-oxaloacetate and glutamate-pyruvate aminotransferases were found, and it was shown that all the common protein amino acids could be synthesized in the intact chloroplast by aminotransfer reactions from alanine or aspartate. The activity of glutamate-oxaloacetate aminotransferase, on a mg chlorophyll basis, was higher in the purified chloroplast than in the crude chloroplast fraction. Aminotransferase activity was found to be confined to the stroma, confirming the results of Yamazaki and Tolbert [100]. Kirk and Leech concluded that glutamate, which forms 33% of the free amino acid pool of the chloroplasts, is the primary product of photosynthetic amino acid synthesis. Secondary transfer from glutamate results in the formation of aspartate and alanine, and from those two compounds all the other protein amino acids can be synthesized.

In contrast to this well known view of the central role of glutamate in ammonia assimilation and amino acid synthesis in chloroplasts, investigations by O'Neal and Joy [180] and by Miflin and Lea [181, 182] have shown that glutamine rather than glutamate must be regarded as the primary product of ammonia assimilation in leaf chloroplasts, and probably also in most plant cells. The assimilated amide group can be readily transferred to α-ketoglutarate in a reductive transamination reaction catalysed by the enzyme, glutamine (amide)-α-ketoglutarate aminotransferase oxidoreductase (trivial name; glutamate synthetase) which in pea chloroplasts was found to be dependent on reduced ferredoxin and inactive with reduced pyridine nucleotides [183]. The same Fd-dependent enzyme has been reported to occur in green algae and blue-green bacteria [181] as well as in the leaves of several plant species [184–186]. A different form of the enzyme dependent on NADPH for activity was first demonstrated in extracts of carrot cell cultures [187] and was subsequently found in many other non-green tissues such as pea roots [188], legume nodules [189] and developing seeds [190, 191]. The significance of the demonstration of this amidotransferase in chloroplasts and leaves, in proplastids [192] and in a range of non-green tissues is that in cooperation with glutamine synthetase activity, it provides an alternative route in plants for the net synthesis of glutamate from α-ketoglutarate and ammonia not involving the enzyme, glutamic dehydrogenase.

In related investigations on the metabolism of asparagine, enzyme studies have shown that the amide group of glutamine is the preferred donor in the formation of asparagine from aspartic acid [190, 193], a reaction which involves an ATP-dependent transfer of the amide group from glutamine catalysed by the enzyme, asparagine synthetase. The primary role of glutamine in asparagine biosynthesis has been recently confirmed by Bauer *et al.* [194] in ^{15}N-labelling studies on the rates of formation and utilization of the two amides in pea leaves. This investigation also showed that nitrogen in asparagine can be readily transferred to a range of compounds and in particular to pyruvate in the synthesis of alanine. The transamination of asparagine, does not appear to be catalysed by an enzyme which transfers the amide group to a suitable acceptor, but by an aminotransferase which utilizes pyruvate or glyoxylate as its primary keto-acid substrate [195]. The main characteristics of this enzyme isolated from soybean leaves and root nodules are thus very similar to the asparagine aminotransferase purified from rat liver [196].

The peroxisomes in spinach leaves have been shown by Rehfeld and Tolbert [101] to contain two specific aminotransferases which utilise glyoxylate as the amino acceptor in the synthesis of glycine. These are a serine-glyoxylate aminotransferase, which also catalyses the reactions serine-pyruvate and alanine-glyoxylate, and a glutamate-glyoxylate aminotransferase which also carries out the alanine-glyoxylate reaction. The peroxisomes also contain an aspartate-α-ketoglutarate aminotransferase, the activity of which is low compared to that found in the chloroplasts and mitochondria. The peroxisomes contain three isoenzymes of this enzyme; isoenzyme 1 is also found in the chloroplasts and mitochondria, while isoenzymes 2 and 3 appear to be unique to the peroxisome. However, Huang *et al.* [72] found that spinach peroxisomes contain only a single isoenzyme of aspartate aminotransferase, which is distinct from those found in the cytosol, chloroplasts or mitochondria, and represents 3–10% of the total cell activity of this enzyme.

The other 'microbody' of plant cells, the glyoxysome, is also known to contain certain aminotransferases. The presence of an aspartate-α-ketoglutarate aminotransferase in glyoxysomes from castorbean endosperm was first demonstrated by Cooper and Beevers [197] and confirmed by Briedenbach [198]. These workers reported that the enzyme was able to catalyse the aspartate-α-ketoglutarate reaction with five times greater activity than the reverse, glutamate-oxaloacetate, reaction. They also investigated but found no evidence for a glutamate-glyoxylate aminotransferase in this organelle. These findings were confirmed and extended by Wightman [171] in a comprehensive study of the aminotransferase activity exhibited by glyoxysomal fractions prepared from castorbean endosperm tissue. The purified fractions were tested against 20 common amino acids, each in presence of one or other of the 4 keto-acid substrates normally involved in plant transamination reactions. High activity was again found for the aspartate-α-ketoglutarate enzyme and also for alanine-α-ketoglutarate and alanine-glyoxylate aminotransferases and moderate activity was shown by the enzymes catalysing the leucine-α-ketoglutarate, phenylalanine-α-ketoglutarate, ornithine-α-ketoglutarate and glutamate-pyruvate transamination reactions. Although the significance of this range of aminotransfer reactions in glyoxysomal metabolism is not yet understood, it is interesting to see that Liu and Huang [199] have recently demonstrated the presence of six isoenzymes of aspartate-α-ketoglutarate aminotransferase in the cotyledons of cucumber seedlings and have shown that two of these

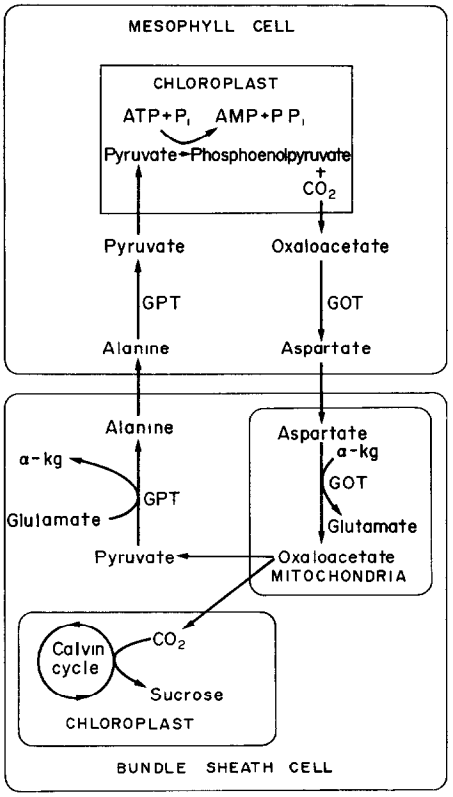

Fig. 3. Postulated involvement of alanine aminotransferase and aspartate aminotransferase in the transport of aspartate from a mesophyll cell to a bundle sheath cell for decarboxylation in the C_4 pathway of photosynthesis (redrawn from figure in ref. [43]).

isoenzymes are found only in the glyoxysomal fraction. As might be expected, the developmental pattern of these two isoenzymes during germination closely follows that of the glyoxysomes.

Thus it is clear that plant cells contain many aminotransferase activities, and when one of these activities is located in more than one subcellular component, a different isoenzymic form is often present in each organelle. Detailed knowledge of the function of many of these aminotransferase activities is still lacking. It is known that transamination is the final step in the biosynthesis of about half of the protein amino acids [30, 200], and this may be the function of many of the soluble and chloroplast enzymes. One difficulty in determining the roles of plant aminotransferases is their multispecificity. For example, Hedley and Stoddart [42] have suggested that the glutamate-pyruvate aminotransferase of *Lolium* leaves may function *in vivo* as the alanine-α-ketoglutaraldehyde enzyme in the production of δ-aminolevulinic acid, an intermediate in chlorophyll biosynthesis. Again, Wightman and coworkers have shown that the transamination of tryptophan and phenylalanine by a multispecific aspartate aminotransferase is the first step in the main pathway for the biosynthesis of the growth hormones, 3-indoleacetic acid and phenylacetic acid, in shoot tissues of higher plants [18, 19, 46, 201, 202]. Another proposed role of aminotransferases is in the transfer of C_3 and C_4 units between the mesophyll and bundle sheath cells of plants carrying out C_4 photosynthesis [43]. Some C_4 plants have very low malic enzyme activity, but show 10–50 times the normal activity of aspartate and alanine aminotransferases [176]. In addition, different isoenzymes of each of these two enzymes are present in each tissue [43, 93, 176]. On the basis of these enzyme distribution studies, Hatch and coworkers propose that in these species, the oxaloacetate formed in the mesophyll cells by the PEP carboxylase reaction is transaminated by the mesophyll isoenzyme of aspartate aminotransferase with the formation of aspartate. This aspartate is then transferred to the bundle sheath cells, where it enters the mitochondria in which the bundle sheath isoenzyme of aspartate aminotransferase is located. The oxaloacetate regenerated in the mitochondria is then decarboxylated and the resulting pyruvate is transaminated in the bundle sheath cytoplasm by an isoenzyme of alanine aminotransferase, with the formation of alanine. The alanine may then return to the mesophyll cells, where pyruvate is regenerated by the mesophyll alanine aminotransferase. Finally, this pyruvate can be converted to PEP and so the cycle continues, as illustrated in Fig. 3. Although this carbon-transfer scheme is attractive, Heber [203] points out that the proposal rests almost entirely on enzyme distribution studies and that before it is fully accepted, direct measurements of the rates of transfer of the metabolites between the various compartments are required. Transfer of aspartate across the chloroplast membrane is very rapid [204], but transfer of pyruvate and alanine proceeds much more slowly [203]. Heber has suggested another role for aminotransferases in an electron shuttle between the chloroplast and the cytoplasm, involving a cyclic transfer of malate out of the organelle with a corresponding movement of aspartate inwards. As mentioned earlier, glutamate-oxaloacetate aminotransferase, the enzyme required in this reaction, is found in both the chloroplast and the cytoplasm [175, 177].

Lastly, the aminotransferases of the peroxisome play an important role in the process of photorespiration, a gluconeogenic pathway requiring co-operation between peroxisomes, mitochondria, chloroplasts and the cytosol [101, 205]. During this process (Fig. 4), carbon from photosynthesis in the form of glycolate passes into the peroxisome, where it is converted to glyoxylate by the enzyme glycolate oxidase. The glyoxylate is then transaminated by either glutamate-glyoxylate or serine-glyoxylate aminotransferase with the formation of glycine. The glycine is transferred to the mitochondria and used in the biosynthesis of serine, which then returns to the peroxisome. Here the amino acids are functioning as mobile compounds which can readily move between intracellular compartments. Serine is transaminated by the peroxisomal serine-glyoxylate aminotransferase with the formation of hydroxypyruvate, which can then be reduced to glycerate, transferred to the chloroplast and used in the manufacture of sugars. The peroxisomal aspartate-α-ketoglutarate aminotransferase is thought to function together with malate dehydrogenase in the shuttle of reducing equivalents into the peroxisome.

Thus, the activity of aminotransferases provide an essential link between carbohydrate and amino acid metabolism in plant cells. They play a role in the synthesis

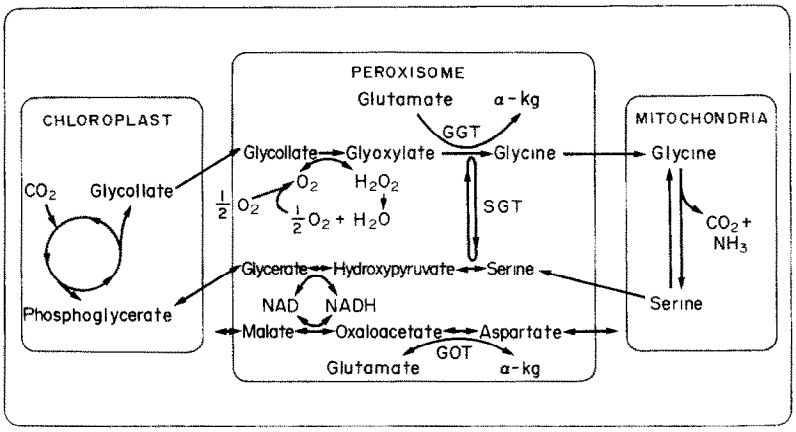

Fig. 4. Role played by aminotransferases in leaf peroxisome metabolism of glycollate. GGT: glutamate-glyoxylate aminotransferase; SGT: serine-glyoxylate-aminotransferase; GOT: glutamate-oxaloacetate-aminotransferase (redrawn from figure in ref. [185]).

of amino acids, which may be used for the biosynthesis of important compounds such as proteins and growth hormones, or they may serve as carbon transporting compounds which can readily move between intracellular compartments [208] or between tissues. They may also form part of electron shuttle systems which transport reducing equivalents between the cytosol and other compartments such as the chloroplast and the peroxisome. In addition to their major role in amino acid biosynthesis, aminotransferases have been implicated in the formation of aliphatic amines in higher plants [209] where the amino group of L-alanine has been shown to be transferred to a range of aldehydes from methanol to octanol [210]. The involvement of aminotransferases in the biosynthesis of alkaloids has also been indicated from studies with cell-free extracts of several alkaloid-containing plants. Such extracts have been shown to utilise appropriate amino acids in the formation of expected intermediates in alkaloid biosynthesis [211, 212] and in recent work, Roberts [213–215] has demonstrated the presence of an L-alanine aminotransferase in *Conium maculatum* which readily utilises the aldehyde, 5-ketooctanol, in the formation of the simple alkaloid, γ-coniceine, from which other complex alkaloids in hemlock are derived.

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REFERENCES

1. Braunstein, A. E. and Kristzmann, M G. (1937) *Enzymologia* **2**, 129.
2. Virtanen, A. I. and Laine, T. (1938) *Nature* **141**, 748.
3. Kritzmann, M. G. (1939) *Nature* **143**, 603.
4. Albaum, H. G. and Cohen, P. P. (1943) *J. Biol. Chem.* **149**, 19
5. Leonard, M. J. K. and Burris, R. H. (1947) *J. Biol. Chem.* **170**, 701.
6. Stumpf, P. K. (1951) *Fed. Proc.* **10**, 256.
7. Kagan, K. S., Kretovich, W. L. and Dronov, A. S. (1966) *Biochemistry* (translated from Russian) **31**, 615.
8. Yu, M. H. and Spencer, M. S. (1969) *Phytochemistry* **8**, 1173.
9. Kretovich, W. L. and Polyanovskii, O. L. (1959) *Biochemistry* (translated from Russian) **24**, 917.
10. Kretovich, W. L. and Uspensakaia, Z. W. (1958) *Biochemistry* (translated from Russian) **23**, 232.
11 Moore, T. C. and Shaner, C. A. (1968) *Arch. Biochem. Biophys.* **127**, 613.
12. Gamborg, O. L. and Wetter, L. R. (1963) *Can. J. Biochem. Physiol.* **41**, 1733.
13. Gamborg, O. L. (1965) *Can. J. Biochem.* **43**, 723.
14. Wightman, F. and Fowden, L. (1966) in *Abstr. I.U.P A C Symp. on the Chemistry of Natural Products*, pp. 166–167 Stockholm.
15. Wightman, F. and Cohen, D. (1968) in *Biochemistry and Physiology of Plant Growth Substances* (Wightman, F. and Setterfield, G., eds.) pp. 273–288. Runge Press, Ottawa.
16. Truelsen, T. A. (1972) *Physiol. Plant.* **26**, 289.
17. Forest, J. C. and Wightman, F. (1972) *Can. J. Biochem.* **50**, 538.
18. Gibson, R. A., Barrett, G. and Wightman, F. (1972) *J. Exp Botany* **23**, 775.
19. Wightman, F. (1973) in *Nitrogen Metabolism in Plants*, Biochem. Soc. Symposium **38**, 247.
20. Bone, D. H. and Fowden, L. (1960) *J. Exp. Botany* **11**, 104.
21. King, J and Waygood, E. R. (1968) *Can. J Biochem.* **46**, 771
22. Brock, B. L. W., Wilkinson, D. A. and King, J. (1970) *Can. J. Biochem.* **48**, 486.
23. Fowden, L and Done, J. (1953) *Nature* **171**, 1068
24. Virtanen, A. I. and Miettinen, J. K. (1953) *Biochem. Biophys. Acta* **12**, 181.
25. Stinson, R. A. and Spencer, M. S. (1969) *Biochem Biophys. Res. Commun.* **34**, 120.
26. Wilson, D. G., King, K. W. and Burris, R. H. (1954) *J. Biol. Chem.* **208**, 863.
27. Sanwal, B. D., Zink, M. W. and Din, G. (1964) in *Modern Methods of Plant Analysis* (Linkens, H. F., Sanwal, B. D. and Tracey, M V., eds.) Vol 7, pp. 361–391. Springer, Berlin.
28. Kretovich, W. L (1965) *Ann. Rev Plant Physiol.* **16**, 141.

29. Fowden, L. (1965) in *Biosynthetic Pathways in Higher Plants* (Pridham, J. B. and Swain, T., eds.) pp. 73–79. Academic Press, London.
30. Fowden, L. (1967) *Ann. Rev. Plant Physiol.* **18**, 85.
31. Smith, B. P. and Williams, H. H. (1951) *Arch. Biochem. Biophys.* **31**, 366.
32. Bevillacqua, L. R. and Gardella, M. P. (1964) *Atti Accad. Ligure Sci. Lett.* **20**, 234.
33. Wong, K. F. and Cossins, E. A. (1969) *Phytochemistry* **8**, 1327.
34. Splittstoesser, W. E. and Steward, S. A. (1970) *Physiol. Plant.* **23**, 1119.
35. Splittstoesser, W. E., Chu, M. C., Stewart, S. A. and Splittstoesser, S. A. (1976) *Plant Cell Physiol.* **17**, 83.
36. Forest, J. C. and Wightman, F. (1971) *Can. J. Biochem.* **49**, 709.
37. Pilet, P. E. and Athanasiades-Mercanton, M. (1966) *C. R. Acad. Sci. (Paris) Ser. D* **262**, 1090.
38. Pilet, P. E., Pratt, R. and Roland, J. C. (1972) *Plant Cell Physiol.* **13**, 297.
39. Hedley, C. L. and Stoddart, J. L. (1971) *J. Exp. Botany* **22**, 239.
40. Hedley, C. L. and Stoddart, J. L. (1971) *J. Exp. Botany* **22**, 249.
41. Hedley, C. L. and Stoddart, J. L. (1972) *J. Exp. Botany* **23**, 490.
42. Hedley, C. L. and Stoddart, J. L. (1971) *Planta* **100**, 309.
43. Hatch, M. D. and Mau, S.-L. (1973) *Arch. Biochem. Biophys.* **156**, 195.
44. Hedley, C. L. and Stoddart, J. L. (1972) *J. Exp. Botany* **23**, 502.
45. Gamborg, O. L. and Keeley, F. W. (1966) *Biochem. Biophys. Acta* **115**, 65.
46. Forest, J. C. and Wightman, F. (1972) *Can. J. Biochem.* **50**, 813.
47. Hulme, A. C., Rhodes, M. J. C. and Wooltorton, L. S. C. (1967) *Phytochemistry* **6**, 1343.
48. Hulme, A. C., Rhodes, M. J. C. and Wooltorton, L. S. C. (1967) *J. Exp. Botany* **18**, 277.
49. Besford, R. T. and Hobson, G. E. (1973) *Phytochemistry* **12**, 1255.
50. Rech, J. and Crouzet, J. (1974) *Biochim. Biophys. Acta* **350**, 392.
51. Bachelard, E. P. and Wightman, F. (1974) in *Mechanisms of Regulation of Plant Growth* (Bieleski, R. L., Ferguson, A. R. and Cresswell, M. M., eds.) pp. 703–708. Bulletin 12, The Royal Society of New Zealand, Wellington.
52. Bachelard, E. P. and Wightman, F. (1975) *Can. J. Botany* **52**, 1483.
53. Mazelis, M. and Fowden, L. (1969) *Phytochemistry* **8**, 801.
54. Cossins, E. A. and Sinha, S. K. (1965) *Can. J. Biochem.* **43**, 495.
55. Katunuma, N., Okada, M., Katsunuma, T., Fujimo, A. and Matsuzawa, T. (1968) in *Pryidoxal Catalysis: Enzyme and Model Systems* (Snell, E. E., Braunstein, A. E., Severin, E. S. and Torchinsky, Y. M., eds.) p. 225. Interscience, New York.
56. Litwack, G. and Diamondstone, T. I. (1962) *J. Biol. Chem.* **237**, 469.
57. Martin, D., Tomkins, G. T. and Granner, D. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **62**, 248.
58. Tompkins, G. M., Gelehrter, T. D., Granner, D., Martin, D., Samuels, H. H. and Thompson, E. B. (1969) *Science* **166**, 1474.
59. Blake, R. L. (1970) *Biochem. Gen.* **4**, 215.
60. Litwack, G., Lichtash, E. and Diamondstone, T. I. (1972) *Nature New Biol.* **237**, 149.
61. Black, I. B. and Axelrod, J. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61**, 1287.
62. Lee, D. J. W. and Reaper, I. (1972) *Int. J. Biochem.* **3**, 73.
63. Jones, T. W. A. and Stoddart, J. L. (1970) *J. Exp. Botany* **21**, 452.
64. Pilet, P. E. (1971) *Experientia* **27**, 880.
65. Cruickshank, D. H. and Isherwood, F. A. (1958) *Biochem. J.* **69**, 189.
66. Ellis, R. J. and Davies, D. D. (1961) *Biochem. J.* **78**, 615.
67. Davies, D. D. and Ellis, R. J. (1961) *Biochem. J.* **78**, 623.
68. Fasella, P., Bossa, F., Turano, C. and Fanelli, A. R. (1966) *Enzymologia* **30**, 198.
69. Verjee, Z. H. M. and Evered, D. F. (1969) *Biochem. Biophys. Acta* **185**, 103.
70. Forest, J. C. and Wightman, F. (1973) *Can. J. Biochem.* **51**, 332.
71. Reed, R. E. and Hess, J. L. (1975) *J. Biol. Chem.* **250**, 4456.
72. Huang, A. H. C., Liu, K. D. F. and Youle, R. J. (1976) *Plant Physiol.* **58**, 110.
73. Wong, K. F. and Cossins, E. A. (1966) *Biochem. Biophys. Res. Commun.* **25**, 651.
74. Cooper, J. L. and Meister, A. (1972) *Biochemistry* **11**, 661.
75. Red'kina, T. V., Uspenskaya, J. V. and Kretovich, L. (1969) *Biokhimiya* **34**, 312.
76. Gadal, P., Bouyssou, J. and Barthe, J.-P. (1969) *Physiol. Veg.* **7**, 69.
77. Matheron, M. E. and Moore, T. C. (1973) *Plant Physiol.* **52**, 63.
78. Forest, J. C. (1971) Ph.D thesis, Carleton University, Ottawa.
79. Munkres, K. D. (1968) *Ann. N.Y. Acad. Sci.* **151**, 294.
80. Munkres, K. D. (1970) *Biochem. Biophys. Acta* **220**, 149.
81. Munkres, K. D. (1965) *Biochemistry* **4**, 2186.
82. Miller, J. E. and Litwack, G. (1971) *J. Biol. Chem.* **246**, 3234.
83. Scandurra, R. and Canelli, C. (1972) *Eur. J. Biochem.* **26**, 196.
84. Shrawder, E. and Martinez-Carrion, M. (1972) *J. Biol. Chem.* **247**, 2486.
85. Tong, J. H., Stoochnoff, B. A., D'Iorio, A. and Benoiton, N. L. (1973) *Can. J. Biochem.* **51**, 407.
86. Wightman, F. and Starr, A. (1977) Unpublished data.
87. Rudman, D. and Meister, A. (1953) *J. Biol. Chem.* **200**, 591.
88. Chesne, S. and Pelmont, J. (1973) *Biochimie* **55**, 237.
89. Collier, R. H. and Kohlaw, G. (1972) *J. Bacteriol.* **16**, 395.
90. Mavrides, C. and Orr, W. (1974) *Biochem. Biophys. Acta* **336**, 70.
91. Mavrides, C. and Orr, W. (1975) *J. Biol. Chem.* **250**, 4128.
92. Turano, C., Bossa, F., Fasella, P. and Fanelli, A. R. (1966) *Enzymologia* **30**, 186.
93. Hatch, M. D. (1973) *Arch. Biochem. Biophys.* **156**, 207.
94. Yu, M. H. and Spencer, M. (1970) *Phytochemistry* **9**, 341.
95. Sastry, L. V. S. and Ramakrishnan, T. (1961) *J. Sci. Ind. Res. (India)* **20C**, 277.
96. Osmond, C. B. and Harris, B. (1971) *Biochem. Biophys. Acta* **234**, 270.
97. Smith, I. K. (1973) *Biochem. Biophys. Acta* **321**, 156.
98. Carpe, A. I. and Smith, I. K. (1974) *Biochem. Biophys. Acta* **370**, 96.
99. Kisaki, T. and Tolbert, N. E. (1969) *Plant Physiol.* **44**, 242.
100. Yamazaki, R. K. and Tolbert, N. E. (1970) *J. Biol. Chem.* **245**, 5137.
101. Rehfeld, D. W. and Tolbert, N. E. (1972) *J. Biol. Chem.* **247**, 4803.
102. Thompson, J. S. and Richardson, K. E. (1967) *J. Biol. Chem.* **242**, 3614.
103. Ichihara, A. and Koyama, E. (1966) *J. Biochem. (Japan)* **59**, 160.
104. Aki, K., Yokojima, A. and Ichihara, A. (1969) *J. Biochem. (Japan)* **65**, 539.
105. Aki, K., Ogawa, K. and Ichihara, A. (1968) *Biochim. Biophys. Acta* **159**, 276.
106. Ogawa, K., Yokojima, A. and Ichihara, A. (1970) *J. Biochem. (Japan)* **68**, 901.
107. Ichihara, A., Takahashi, H., Aki, K. and Shirai, A. (1967) *Biochem. Biophys. Res. Commun.* **26**, 674.
108. Shirai, A. and Ichihara, A. (1971) *J. Biochem. (Japan)* **70**, 741.
109. Norton, J. E. and Sokatch, J. R. (1970) *Biochim. Biophys. Acta* **206**, 261.

110. Coleman, M. S. and Armstrong, F. B. (1971) *Biochim. Biophys. Acta* **227**, 56.
111. Bone, D H (1959) *Plant Physiol.* **34**, 171.
112. Kleczkowski, K. and Kretovich, W. L. (1960) *Biochemistry* (translated from Russian) **25**, 164.
113. Smith, J. E. (1962) *Biochim. Biophys. Acta* **57**, 183.
114. Seneviratne, A. S. and Fowden, L. (1968) *Phytochemistry* **7**, 1047.
115. Splittstoesser, W. E. and Fowden, L. (1973) *Phytochemistry* **12**, 785.
116. Strecker, H. J. (1965) *J. Biol. Chem.* **240**, 1225.
117. Hill, D. L. and Chambers, P. (1967) *Biochim. Biophys. Acta* **148**, 435.
118. Sudi, J. and Denes, G. (1967) *Acta Biochim. Biophys. Acad. Sci Hung* **2**, 291.
119. Vogel, R. H. and Kopac, M. J. (1960) *Biochim. Biophys. Acta* **37**, 539.
120. Lu, T.-S. and Mazelis, M. (1975) *Plant Physiol.* **55**, 502.
121. Dixon, R. O. D. and Fowden, L. (1961) *Ann. Botany* **25**, 513.
122. Sanchez-Medina, F. and Mayor, F. (1970) *Rev. Exp. Fisiol.* **26**, 217.
123. Streeter, J. G. and Thompson, J. F. (1972) *Plant Physiol.* **49**, 579.
124. Baldy, P. (1976) *Planta* **130**, 275.
125. Scott, E. M. and Jakoby, W. B. (1959) *J. Biol. Chem.* **234**, 932.
126. Roberts, E. and Bregoff, H. M. (1953) *J. Biol. Chem.* **201**, 393.
127. Bertland, L. H. and Kaplan, N. O. (1970) *Biochemistry* **9**, 2653.
128. Feliss, N. and Martinez-Carrion, M. (1970) *Biochem. Biophys. Res. Commun.* **40**, 932.
129. Bertland, L. H. and Kaplan, N. O. (1968) *Biochemistry* **7**, 134.
130. Saier, M. H. and Jenkins, W. T. (1967) *J. Biol. Chem.* **242**, 91.
131. Gatehouse, P. W., Hopper, S., Schatz, L. and Segal, H. (1967) *J. Biol. Chem.* **242**, 2319.
132. Wada, H. and Snell, E. E. (1962) *J. Biol. Chem.* **237**, 127.
133. Wada, H. and Morino, Y. (1964) *Vitamins and Hormones* **22**, 411.
134. Martinez-Carrion, M. and Timeir, D. (1967) *Biochemistry* **6**, 1715.
135. Valeriote, F. A., Tomkins, G. M. and Riley, D. (1969) *J. Biol. Chem.* **244**, 3618.
136. Wong, K. F. (1968) Ph.D. thesis, University of Alberta, Edmonton.
137. Scandurra, R., Cannella, C. and Feretti, M. G. (1967) *Eur. J. Biochem.* **3**, 219.
138. George, H. and Gabay, S. (1968) *Biochim. Biophys. Acta* **167**, 555.
139. Happold, F. C. and Turner, J. M. (1957) *Nature* **179**, 155.
140. Nadkarni, S. R. and Sohoni, K. (1963) *Indian J. Chem.* **1**, 220.
141. Patwardhan, M. V. (1960) *Biochem. J.* **75**, 401.
142. Nisselbaum, J. S. (1968) *Anal. Biochem.* **23**, 173.
143. Boyde, T. R. C. (1968) *Biochem. J.* **106**, 581.
144. Cheng, S., Michunda-Kozak, C. and Martinez-Carrion, M. (1971) *J. Biol. Chem.* **246**, 3623.
145. Jenkins, W. T. (1963) in *Chemical and Biological Aspects of Pyridoxal Catalysis* (Snell, E. E., Fasella, P. M., Braunstein, A. and Fanelli, A. R., eds.) pp. 139-148. MacMillan, New York.
146. Velick, S. F. and Vavra, J. (1962) *J. Biol. Chem.* **237**, 2109.
147. Snell, E. E. (1962) *Brookhaven Symp. Biol* **15**, 32.
148. Ivanov, V. I. and Karpeisky, M. Y. A. (1969) *Adv. Enzymol.* **32**, 21.
149. Fasella, P. and Turano, C. (1970) *Vitamins and Hormones* **28**, 157.
150. Michuda, C. M. and Martinez-Carrion, M. (1969) *J. Biol. Chem.* **244**, 5920.
151. Shrawder, R. and Martinez-Carrion, M. (1973) *J. Biol. Chem.* **248**, 2140.
152. Aunis, D., Mark, J. and Mandel, P. (1971) *Life Sci.* **10**, 617.
153. Chesne, S. and Pelmont, J. (1974) *Biochimie* **56**, 631.
154. Hinds, L. de C. and Fasella, P. (1970) *Abstr. 8th Int. Cong. Biochem., Switzerland*, pp. 95–107.
155. Cleland, W. W. (1963) *Biochim. Biophys. Acta* **67**, 104.
156. Jenkins, W. T. and Sizer, I. W. (1959) *J. Biol. Chem.* **234**, 1179.
157. Abbadi, S. and Shannon, L. M. (1969) *Bot. Gaz.* **130**, 23
158. Boyd, J. W. (1961) *Biochem. J.* **81**, 434.
159. Fleischer, G. A., Potter, C. S. and Wakin, K. G. (1960) *Biol. Med.* **103**, 229.
160. Scheid, B., Morris, H. P. and Roth, J. S. (1965) *J. Biol. Chem.* **240**, 3016
161. John, R. and Jones, R. (1974) *Biochem. J.* **141**, 401.
162. Shrawder, R. and Martinez-Carrion, M. (1973) *J. Biol. Chem.* **248**, 2147.
163. Bodansky, O., Schwartz, M. K. and Nisselbaum, J. S. (1966) *Adv Enzyme Regul.* **4**, 299.
164. Porter, P., Blum, J. J. and Elrod, H. (1972) *J. Protozool.* **19**, 375.
165. Nakata, Y., Suematzu, T. and Sakamoto, T. (1964) *J. Biochem. (Japan)* **55**, 199.
166. Katanuma, N., Okada, M. and Nishii, Y. (1966) *Adv. Enzyme Regul.* **4**, 311.
167. Morino, Y. and Watanabe, T. (1969) *Biochemistry* **8**, 3412.
168. Semba, T. and Civen, M. (1970) *J. Neurochem.* **17**, 795.
169. Ryan, E., Bodley, F. and Fottrell, P. F. (1972) *Phytochemistry* **11**, 957.
170. Thomas, H. and Stoddart, J L. (1974) *Phytochemistry* **13**, 1053.
171. Wightman, F. (1976) *Proc. Can. Soc. Plant Physiol.* **16**, 51
172. Mukerji, S. K. and Ting, I. P. (1968) *Phytochemistry* **7**, 903.
173. Gazeau-Reyjal, M. and Crouzet, J. (1976) *Phytochemistry* **15**, 1619.
174. Ziegenbein, R. (1966) *Nature* **212**, 935.
175. Santarius, J. A. and Stocking, C. R. (1969) *Z. Naturforsch.* **24B**, 1170.
176. Andrews, T. J., Johnson, H. S., Slack, C. R. and Hatch, M D. (1971) *Phytochemistry* **10**, 2005
177. Kirk, P. R. and Leech, R. M. (1972) *Plant Physiol* **50**, 228.
178. Smith, B. P. and Williams, H H (1951) *Arch. Biochem. Biophys.* **31**, 366.
179 Thomas, H. (1975) *Z. Pflanzenphysiol.* **74**, 208.
180. O'Neal, D. and Joy, K. W. (1973) *Nature* **246**, 61.
181. Miflin, B. J and Lea, P. J. (1976) *Phytochemistry* **15**, 873.
182. Miflin, B. J. and Lea, P. J. (1977) *Ann. Rev. Plant Physiol.* **28**, 299.
183. Lea, P. J. and Miflin, B. J. (1974) *Nature* **251**, 614.
184. Rhodes, D., Rendon, G. A. and Stewart, G. R. (1976) *Planta* **129**, 203.
185. Rathnam, C. K. M. and Edwards, G. E. (1976) *Plant Physiol.* **57**, 881.
186. Wallsgrove, R. M., Harel, E., Lea, P. J. and Miflin, B J. (1977) *J. Exp. Botany* **28**, 588.
187. Dougall, D. K. (1974) *Biochem. Biophys. Res. Commun.* **58**, 639.
188. Miflin, B. J. and Lea, P. J. (1975) *Biochem. J.* **149**, 403.
189. Robertson, J. G., Warburton, M. P. and Farnden, K. J. (1975) *F.E.B.S. Lett.* **55**, 33.
190. Lea, P. J. and Fowden, L. (1975) *Proc. Roy. Soc. Lond. B* **192**, 13.
191. Beevers, L. and Storey, R. (1976) *Plant Physiol.* **57**, 862.
192. Washitani, I. and Sato, S. (1977) *Plant and Cell Physiol.* **18**, 505.
193. Lea, P J. and Fowden, L. (1975) *Biochem. Physiol Pflanzen.* **168**, 3.
194. Bauer, A., Joy, K. W. and Urquhart, A. A. (1977) *Plant Physiol.* **59**, 920.
195. Streeter, J. G. (1977) *Plant Physiol.* **60**, 235.
196. Cooper, A. J. L. (1977) *J. Biol. Chem.* **252**, 2032.

197. Cooper, T. G. and Beevers, H. (1969) *J. Biol. Chem.* **244**, 3507.
198. Breidenbach, R. W. (1969) *Ann. N.Y. Acad. Sci.* **168**, 342.
199. Liu, K. D. F. and Huang, A. H. C. (1977) *Plant Physiol.* **59**, 777.
200. Joy, K. W. and Folkes, B. F. (1965) *J. Exp. Botany* **16**, 646.
201. Gibson, R. A., Schneider, E. A. and Wightman, F. (1972) *J. Exp. Botany* **23**, 381.
202. Wightman, F. and Rauthan, B. (1974) in *Plant Growth Substances* 1973, pp. 15–27. Hirokawa, Tokyo.
203. Heber, U. (1974) *Ann. Rev. Plant Physiol.* **25**, 393.
204. Heldt, H. W., Sauer, F. and Rapley, L. (1972) in *Proc. 2nd Int. Conf. for Photosynthetic Research* (Forti, G., Avron, M. and Melandri, A., eds.) pp. 1345–1355. W. Junk, The Hague.
205. Tolbert, N. E. (1971) *Ann. Rev. Plant Physiol.* **22**, 45.
206. Martinez-Carrion, M., Turano, C., Chiancone, E., Bossa, F., Giartosia, A. Riva, F. and Basella, P. (1967) *J. Biol. Chem.* **242**, 2397.
207. Hopper, S. and Segal, H. L. (1962) *J. Biol. Chem.* **237**, 3189.
208. Richardson, M. (1974) *Sci. Prog. Oxf.* **61**, 41.
209. Hartmann, T., Ilert, H. I. and Steiner, M. (1972) *Z. Pflanzenphysiol.* **68**, 11.
210. Hartmann, T., Donges, D. and Steiner, M. (1972) *Z. Pflanzenphysiol.* **67**, 404.
211. Hasse, K., Ratych, O. T. and Salnikow, J. (1967) *Hoppe-Seyler's Z. Physiol. Chem.* **348**, 843.
212. Jindra, A. (1967) *Acta Facult. Pharm. Bohemoslov* **13**, 7.
213. Roberts, M. F. (1971) *Phytochemistry* **10**, 3057.
214. Roberts, M. F. (1977) *Phytochemistry* **16**, 1381.
215. Roberts, M. F. (1977) *Phytochemistry* **17**, 107.