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REVIEW

RECENT ADVANCES IN THE BIOCHEMISTRY OF PLANT AMINES

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Key Word Index—Plants; amines; transaminase; carboxy-lyase; oxidase; alkaloids; indole; indol-3yl-acetic acid; mescaline; review.

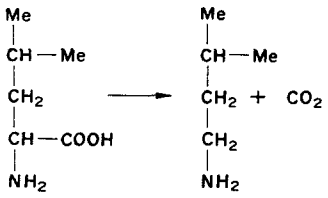
Abstract—Simple aliphatic monoamines are formed in the red algae by a non-specific amino acid carboxy-lyase. An aldehyde–amino acid transaminase with broad substrate specificity is the mechanism for the formation of these amines in many higher plants. Putrescine accumulates in response to potassium and magnesium deficiency, and the amount of this diamine is greatly increased on supplying nitrogen as ammonium salts. Putrescine is also found at high levels in rapidly growing tissues. Cadaverine occurs in several leguminous plants. Putrescine, spermidine and spermine have been detected in many different species of plants, and they are probably ubiquitous. The polyamines are probably synthesized in plants by the pathways already demonstrated in animals and micro-organisms. Several new polyamine alkaloids have been characterized. Amine oxidases are probably involved in the formation of nicotine and indol-3yl-acetic acid. The amine oxidases from the Leguminosae and Gramineae have been further characterized. Carboxy-lyases have been demonstrated for tryptophan, tyrosine and DOPA. Cacti contain a large variety of phenethylamine derivatives related to mescaline.

INTRODUCTION

Since an earlier review of the biochemistry of the plant amines covering the period from 1958 to 1970 [1] was published, our knowledge of plant amine metabolism has been greatly enlarged. It is difficult to cover all aspects of this subject in a brief review, and the present survey is consequently selective, with emphasis on the recent major advances in this field. Many studies on the incorporation of amines into alkaloids are not considered in detail, as they are reviewed in refs. [2] and [3].

SIMPLE ALIPHATIC AMINES

A number of simple amines occur in marine algae, and these are especially widespread in the Rhodophyceae [4]. Although no enzymes responsible for the formation of these amines could be detected in the Phaeophyceae, Chlorophyceae, Diatomeae or in the Cyanophyceae, a highly active pyridoxal phosphate dependent amino acid carboxy-lyase (decarboxylase) occurred in 13 species of 27 members of the Rhodophyceae [5]. Since simultaneous presentation of the different substrates did not result in additive CO_2 production, a single enzyme with a wide substrate specificity was thought to be involved. Leucine was most readily attacked, but norleucine, *iso*leucine, valine, norvaline, 2-aminobutyric acid, phenylalanine, methionine, cysteine and homocysteine were also decarboxylated. With the exception of the mercapto amino acids this decarboxylation was stoichiometric [6]. The spontaneous reaction between pyridoxal phosphate and cysteine and homocysteine reduced the apparent activity with these substrates. Only the L-forms of the amino acids were attacked, and a further 25 amino acids would not function as substrates. Six of the amines which



Leucine (1) *iso*-Amylamine (2)

Scheme 1. Decarboxylation of leucine by an enzyme from red algae.

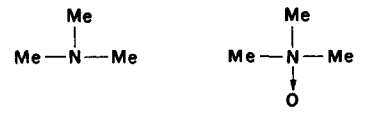
occur in the Rhodophyceae [4] can be produced by this amino acid carboxy-lyase. L-Leucine (**1**) supplied exogenously to living algae is converted predominantly to *iso*amylamine (**2**) (Scheme 1) [7]. Very high activity was found in *Ceramium deslongchampsii* (opt pH 4·25) and *Cystoclonium purpureum* (opt pH 6·0). The pH optima for each substrate was similar with an extract from a single species and the pH optima varied from 4·25 to 6·0 according to species. In several species the enzyme activity depended on the developmental stage. In *Ceramium rubrum* and *Polysiphonia urceolata*, two species with an isomorphic alternation of generations, there was a higher activity in the female than in the male thallus or in the tetra-sporophyll [5].

Using a variety of techniques, including washing in solutions of various inorganic salts, organic solvents and detergents, the enzyme could not be solubilized. Its properties were therefore investigated in acetone powders which had been washed in phosphate buffer at pH 7. The enzyme gained activity appreciably on storage in a deep-freeze [5]. The K_m values for the amino acid carboxy-lyase of *Polysiphonia urceolata* were 8×10^{-4} and $1{\cdot}7 \times 10^{-3}$ M for leucine and norvaline respectively, and the K_m values for the remaining substrates were within this range. The enzyme activity was optimal between 50 and 60°, but 80% of the activity was lost on heating at 80° for 10 min. Activity was increased up to 60% by phosphate and sulphate ions at 0·1 to 1 M. The enzyme was inhibited by carbonyl reagents, by *p*-chloromercuribenzoate, and by heavy metals, though not by iodoacetate or iodoacetamide. A series of aliphatic mono-carboxylic acids (butyrate, valerate, capronate and *iso*capronate) inhibited competitively, presumably by binding to the carbonyl acceptor on the enzyme. Neither Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Fe^{2+}, Fe^{3+} nor EDTA inhibited the enzyme [6].

Pyridoxal phosphate present in the enzyme cannot be dissociated by prolonged dialysis, nor by heating with various buffers. However, addition of substrate causes loss of enzyme activity due to the displacement of the pyridoxal phosphate from the enzyme. Almost pure apodecarboxylase may be obtained on centrifugation of the particles after incubation with leucine for 2 hr at 50° [8]. The enzyme extracted from the plant was fully saturated with pyridoxal phosphate. The displacement of pyridoxal phosphate may be prevented by addition of excess pyridoxal phosphate, pyridoxal or by several other carbonyl compounds, notably the 2-oxo monocarboxylic acids. On incubation with leucine alone for 1 hr, the carboxy-lyases from *Cystoclonium* and *Polysiphonia* are inactivated, and full reactivation is achieved on adding pyridoxal phosphate. Addition of glyoxylate in the absence of pyridoxal phosphate reactivated only the enzyme from *Polysiphonia*. Pyruvate, which also activates the enzyme, is not metabolized and its effect is purely catalytic. Only those amino acids which act as substrates cause coenzyme dissociation, and the effectiveness as dissociator or substrate was correlated. Dissociation therefore probably occurs as an amino acid pyridoxal phosphate complex. Inhibition by semicarbazide, hydroxylamine and cyanide is reversible and does not cause pyridoxal phosphate dissociation; a direct reaction between the carbonyl reagent and the enzyme-bound pyridoxal phosphate is unlikely. Pyridoxamine will not reactivate the enzyme by itself, but on adding glyoxylate, pyridoxal phosphate is regenerated from the pyridoxamine non-enzymically, and activation will then take place [6, 8].

In the intact algae the *iso*amylamine formed from leucine by the carboxy-lyase is probably bound to the acidic polysaccharides in the cell walls, though some is also released to the sea water. There is no indication that the amine is further metabolized. Endogenous amino acids are not available to the enzyme. It is likely that the enzyme decarboxylates amino acids arising from decomposition of organic material in the surrounding sea water, and the amines may serve to protect the algae against micro-organisms [7] for which certain amines are known to be toxic [9, 10].

The widespread occurrence of trimethylamine (**3**) in sea-weed has now been confirmed [11]. In the Rhodophyceae, Phaeophyceae and Chlorophyceae the mean content was 24, 18 and 37 μmol/g dry wt respectively. The trimethylamine-*N*-oxide (**4**) level was generally high in the Rhodophyceae and low in the Phaeophyceae (25 and 4 μmol/g dry wt respectively). In the Chlorophyceae the level was variable, ranging from 0 to 30 μmol/g dry wt.



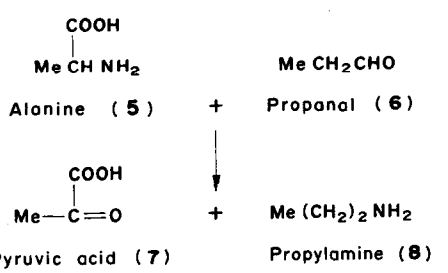
Trimethylamine is present at high levels in the lichens *Lobaria laetevirens*, *Sticta sylvatica*, *S. fuliginosa* and *S. limbata* at 100–200 μmol/g dry wt, i.e. at a higher concentration than the free amino acids. Di- and mono-methylamine were also found, though at much lower levels [12]. The incorporation of label into mono-, di- and tri-methylamine has been studied on feeding glycine-[2-^{14}C] to the lichen *Lobaria laetevirens* [13]. After 5 min feeding, most label was found in monomethylamine, and this is therefore probably formed before trimethylamine. Incorporation into dimethylamine was very low. Even after 5 min feeding, label in methionine was over 10 times that in the methylamines, and after 24 hr only 3% of the total radioactivity was present in the methylamines. In a similar study on sea lavender (*Limonium vulgare*; Plumbaginaceae) [14], radioactivity from glycine-[2-^{14}C] was incorporated into monomethylamine before di- and trimethylamine. Again, the label in dimethylamine was very weak. However, the origin of the amines by the breakdown of other methylamino compounds during alkaline distillation, or the possibility that methylamine could be formed by amination of formaldehyde [15], and the existence of other metabolic pathways of glycine [16], are possible complications in the interpretation of the results of these experiments. Reliable evidence for the formation of methylamine by the decarboxylation of Gly in higher plants has still not been obtained.

Using a system of amine separation based on steam distillation, derivatization with dinitrofluorobenzene and separation by TLC, about half of the 52 species of flowering plants investigated in 30 families were shown to contain steam-volatile amines [17]. On feeding the substrates to intact plants, only one species (*Lychnis coronaria*; Caryophyllaceae) decarboxylated valine significantly, and none attacked leucine. By contrast, all the plants aminated propanal (**6**) and nearly half aminated hexanal $Me(CH_2)_4CHO$. No correlation could be found between the amine content and the ability to transaminate aldehydes. The following 10 naturally-occurring amines were identified (with the formula and no. of species in brackets):

iso-amylamine [$MeCH(Me)(CH_2)_2NH_2$; 14],
*iso*butylamine [$MeCH(Me)CH_2NH_2$; 8],
hexylamine [$Me(CH_2)_5NH_2$; 7],
2-methylbutylamine [$MeCH_2CH(Me)CH_2NH_2$; 5],
ethylamine ($MeCH_2NH_2$; 5),
*iso*propylamine [$MeCH(Me)NH_2$; 3],
octylamine [$Me(CH_2)_7NH_2$; 3],
n-propylamine [$Me(CH_2)_2NH_2$; 2],
heptylamine [$Me(CH_2)_6NH_2$; 2],
and diethylamine [$(MeCH_2)_2NH$; 1].

Methylamine occurred in all extracts, but this amine may be formed by the breakdown of unstable aminomethyl compounds on distillation. This is the first record of the natural occurrence of heptylamine, octylamine and 2-methylbutylamine. The propanal–alanine transaminase was detected in cell-free extracts of six plants, each from a different family, and the enzyme was particularly active in the spadix of *Arum* [18].

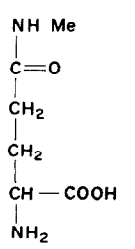
The amino acid–aldehyde transaminase of *Mercurialis perennis* (Euphorbiaceae) has a pH optimum of 8·5 and an optimum temperature of 30°, with alanine (**5**) and propanal (**6**) as substrates (Scheme 2). Pyruvate (**7**) and propylamine (**8**) were



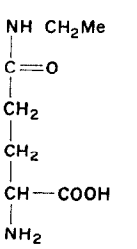
Scheme 2. Transamination of propanal with alanine as amino group donor by an enzyme from *Mercurialis*.

the products of this transamination. The enzyme purified by acetone precipitation and then freeze-dried was stable for more than 1 month, though in solution the enzyme was rather unstable. In decreasing order of efficiency, out of 29 amino acids investigated, only L-alanine, ϵ-aminocaproic acid, γ-aminobutyric acid and L-glutamic acid acted as donors. For the amination of the aldehydes, amines and D-amino acids were ineffective as amino-group donors. In the presence of alanine (**5**) all members of the homologous series of aldehydes from ethanal to undecanal were aminated. Hexanal was the best substrate, but this may be due to the low solubility of the higher homologues, which were used as saturated solutions. The *n*-butanal and *n*-pentanal were better substrates than their branched isomers. A clear pyridoxal phosphate dependency could not be demonstrated, though the enzyme was inhibited by carbonyl reagents as well as by heavy metals. It is unlikely that the enzyme is dependent on metal ions for activation. The enzyme is inhibited by several keto acids, notably by pyruvate, oxaloacetate, α-ketoglutarate and glyoxylate. Pyruvate inhibits most strongly when alanine is the amino donor and, conversely, α-ketoglutarate inhibits most strongly when glutamate is the amino donor. Using *iso*amylamine-[^{14}C] with pyruvate as acceptor, *iso*pentanal-[^{14}C] was detected, and it was calculated that the reaction favours the formation of the amine with an equilibrium of 10:1 for amine:aldehyde [19].

In steam distillates of alkaline extracts of *Mercurialis perennis*, methylamine, ethylamine, *iso*propylamine, *iso*butylamine, *iso*amylamine and hexylamine were detected. Steam distillation of acid extracts of *M. perennis* indicated the presence of ethanal, propanal, butanal and decanal, and at least four of the six amines found in this plant could be formed *in vitro* by the transaminase. The branch chain aldehydes are probably derived by metabolism of the respective amino acids and the higher aldehydes from degradation of fatty acids. The origin of methylamine and of *iso*propylamine is still in doubt. It is possible that the aldehyde amination occurs through the activity of a normal amino acid–keto acid transaminase, which also reacts with aldehydes. The presence of the amines may therefore depend on the occurrence of the aldehydes [19].



γ-Glutamylmethylamide (9)



Theanine (10)

γ-Glutamylmethylamide (GMA) (**9**), which is found together with theanine (**10**) (γ-glutamylethylamide) in the tea plant (*Thea sinensis*; Theaceae), is formed by combination of methylamine with glutamic acid, by analogy with the formation of glutamine. The methyl group of γ-glutamylmethylamide is utilized in caffeine biosynthesis [20, 21]. In a study of the metabolism of methylamine-[^{14}C] by the shoot tips of the tea plant [22], during a 10-hr feeding experiment 57% of the label was utilized, and the main products were serine, GMA, theobromine, caffeine and CO_2. Smaller fractions of the label were found in glutamate, aspartate and RNA purine nucleotides. Although label was also found in *S*-adenosylmethionine, none was detected in methionine, probably due to the small pool size and rapid turnover of this amino acid. Oxidation to formaldehyde by an amine oxidase is apparently the first step in methylamine utilization and the labelled formaldehyde is incorporated into metabolites on the C_1 pathways. The C_1 intermediates are utilized in the synthesis of the purines. The formaldehyde is further oxidized to HCO_2H and to CO_2. Methylamine could be regenerated from GMA, and other products of this amide were serine, theobromine, caffeine, glutamate and aspartate. GMA probably acts as a means of storage or transport of methylamine in a non-toxic form in the tea plant. It is likely that GMA, like theanine, is formed in the roots and transported to the leaves [23]. Theanine content was especially high in the first leaf of 2-yr-old tea plants [24]. The ethylamine moiety of theanine was formed more efficiently from L-alanine than from acetaldehyde [25].

In sunflower (*Helianthus annuus*; Compositae), methylamine-[^{14}C] is probably incorporated into tetra-hydrofolic acid, via methanol and formate [26]. In banana (*Musa sapientum*; Musaceae) fruit, methylamine, ethylamine, *iso*butylamine, *iso*amylamine, propanolamine and dimethylamine have

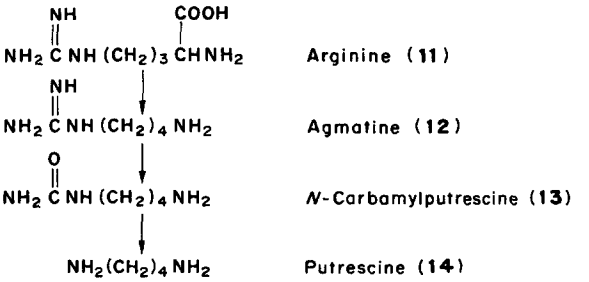
been detected, together with a variety of aromatic amines [27].

DI- AND POLYAMINES

The recent interest in the di- and polyamines, which occur in animals, micro-organisms and plants, is illustrated by the large number of reviews and books devoted to their study [28–36]. This results primarily from the demonstration of their ability to interact with nucleic acids, and the consequent implication which this has for the control of growth processes.

Using TLC with the ninhydrin chromogenic reagent, the putrescine (**14**) content of 6 species of higher plants was shown to be increased by potassium (K) deficiency [37]. Later work using the dansyl reagent indicated that the putrescine level of K-deficient barley (*Hordeum vulgare*; Gramineae) leaves was 5–15 times greater than the control. Mg deficiency also enhances putrescine content (× 5–10), while the putrescine level was reduced by P, S and N deficiencies. K and Mg deficiencies also increased agmatine (**12**) (× 5 and × 2·5) and arginine (**11**) (× 2 and × 1·4 respectively). Compounds tentatively identified as diamino-propane (**26**) and 1(3-aminopropyl)pyrroline (**27**) were increased significantly by Ca deficiency and by high salt. Mg deficiency also caused putrescine (**14**) accumulation in bean (*Vicia faba*) and pea (*Pisum sativum*) (Leguminosae) leaves [38, 39].

Radioactivity from L-arginine-[U-^{14}C] (**11**) fed to 16-day-old shoots was detected in agmatine (**12**) within 2 hr of feeding [39], and probably also in the hordatines (**44**) [40] on feeding for 24 hr. After 2 hr the label in the agmatine was greatest in the K-deficient shoots, but after 24 hr the level declined to that found in the agmatine of the shoots of the Mg-deficient and control seedlings. The results are consistent with the operation of the pathway of putrescine synthesis from arginine shown in Scheme 3. However, label could not be detected in *N*-carbamylputrescine (**13**), and this compound is probably rapidly degraded to putrescine. The rate of putrescine synthesis was high in both K and Mg deficiencies. On feeding putrescine-[1,4-^{14}C] to barley shoots, incorporation of radioactivity was higher in spermidine (**21**) and lower in spermine (**22**) in the K-deficient plants than in the controls.



Scheme 3. Formation of putrescine from arginine in barley.

In Italian rye grass (*Lolium multiflorum*; Gramineae), putrescine was increased from 0·72 μmol (control) to 14·2 μmol/g dry wt (K-deficient) [41]. Agmatine levels increased from 0·12 μmol (control) to 0·72 μmol/g dry wt (K-deficient). The K-deficient leaves contained about 50% of the K found in the controls on a dry wt basis. Reduction of leaf K similarly induced a 10- to 20-fold rise in the putrescine content of apricot, apple and grape vine leaves, and it was concluded that putrescine is a more sensitive indicator of K status than the actual leaf potassium content [42]. However, K deficiency in apple (*Malus*; Rosaceae) trees failed to induce putrescine accumulation when root temperatures were supra-optimal [43].

The spermidine, cadaverine (pentamethylene diamine; **15**) and especially the putrescine content was increased in soybean (*Glycine max*; Leguminosae) seedlings grown on an ammonium medium as compared with seedlings grown in a medium with NO_3 or urea as the N source. The increase in putrescine was 25- to 100-fold on a dry wt basis [44, 45]. The concentrations of putrescine and cadaverine were also increased in tobacco (*Nicotiana tabacum*; Solanaceae) (× 5 and × 4 respectively) and in tomato (*Lycopersicon esculentum*; Solanaceae) by application of ammonium sulphate to the soil medium. Application of KCl decreased the level of putrescine in tobacco. However, KCl application increased the cadaverine in tobacco and especially in tomato to the surprisingly high level of 140 μmol/g fr. wt. Lesions similar to those found with excess ammonium were formed on feeding putrescine to tomato [46]. Putrescine was detected only in leaves of apple (*Malus*), pear (*Pyrus*), prune (*Prunus*) (Rosaceae) and grape (*Vitis*; Vitaceae) which showed symptoms of K deficiency [47].

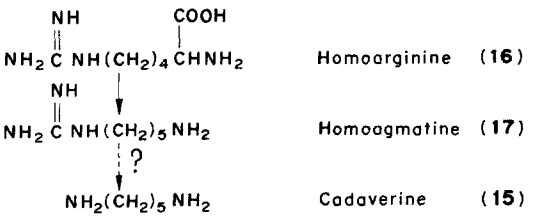
Salt concentrations high enough to cause leaf necrosis induced a 3- to 10-fold increase in putres-

cine, spermidine and spermine in *Oenothera biennis* (Onagraceae) [48]. Saline conditions caused high levels of putrescine in broad bean (*Vicia faba*; Leguminosae) plants, and treatment with 0·6% NaCl increased activity of the diamine oxidase 2-fold. However, with 1·5% NaCl no diamine oxidase activity could be detected [49]. Spraying broad bean plants grown in normal and saline conditions with 100–200 ppm putrescine increased leaf area and dry wt significantly [50]. However, in this study it is possible that the putrescine applied as a foliar spray was stimulating growth by acting as a source of N.

On feeding L-arginine-[U-^{14}C] to rice (*Oryza sativa*; Gramineae) callus cultures, ornithine and citrulline were detected after 2 hr, and agmatine and putrescine after 10 hr and 24 hr respectively [51]. Putrescine was more effective than glutamate as a source of organic nitrogen for the growth of soybean (*Glycine max*; Leguminosae) cells in suspension culture, but less effective than $(NH_4)_2SO_4$, glutamine or alanine. Spermidine, spermine and cadaverine were completely ineffective [52].

Pea (*Pisum*) seedling ornithine carbamyl transferase will also carbamoylate putrescine, agmatine, spermidine and spermine at 10, 4, 0·05 and 0·05% respectively of the rate with ornithine [53]. Since putrescine (1%), unlike *N*-carbamylputrescine (**13**), was toxic to pea seedlings, the possibility that putrescine carbamoylation might provide a mechanism for putrescine detoxication was investigated. However, on feeding putrescine-[^{14}C] for 1 hr to pea seedlings, 26% of the total radioactivity was found in γ-aminobutyric acid, and no label could be found in *N*-carbamylputrescine [54].

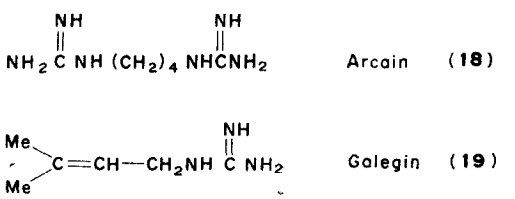
Cadaverine (**15**) has now been detected in a number of leguminous plants. This diamine was found in 13-day-old pea seedlings at 69 nmol/g fr. wt [55], and at 7·1 μmol/g dry wt in the roots of soybean (*Glycine max*) seedlings grown for 15 days in an ammonium medium in the light [56]. Cadaverine has also been characterized in soybean flour by MS and NMR [57]. Putrescine, spermidine and spermine were estimated to occur at 76, 113 and 28 nmol/g flour respectively [58]. Cadaverine has been demonstrated in *Sedum acre* (Crassulaceae) and the incorporation of this amine into the piperideine alkaloid sedamine has been studied [59]. In view of the non-symmetrical incorporation of



Scheme 4. Formation of homoagmatine by decarboxylation of homoarginine in *Lathyrus sativus*.

cadaverine derived from lysine, it was proposed that the lysine is decarboxylated as a pyridoxal phosphate adduct, which is then transferred intact as a cadaverine pyridoxal phosphate adduct to the apo-diamine oxidase. Equilibrium would favour the bound form of cadaverine. Cadaverine-[1,5-^{14}C] was also incorporated into alkaloids of *Goebelia pachycarpa* (Leguminosae) [60].

Homoagmatine (**17**), which has been characterized from seedlings of *Lathyrus sativus* (Leguminosae) [61] is formed by the decarboxylation of L-homoarginine (**16**) (Scheme 4). In the entire seedling, homoarginine declined by about 20% after 3 days germination and then remained stable, while the homoagmatine progressively increased and this amine accounted for 20% of the homoarginine lost in 10 days' growth. The axis appeared to be the major site of decarboxylation. A system analogous to the established arginine–agmatine–putrescine pathway (Scheme 3) may occur in *Lathyrus*, since a compound tentatively identified as cadaverine was also found.



On feeding ornithine-[5-^{14}C] for 1 hr to shoots of *Limonium vulgare* (Plumbaginaceae), putrescine was strongly labelled and radioactivity was also found in arginine, citrulline and agmatine [62]. Feeding unlabelled agmatine to the shoots for 3 hr prior to ornithine-[5-^{14}C] feeding significantly reduced the label in putrescine, indicating that the main pathway for putrescine formation is via arginine and agmatine.

Radioactivity from arginine-[^{14}C] fed to fruit bodies of the fungus (*Panus tigrinus* (Agaricaceae)

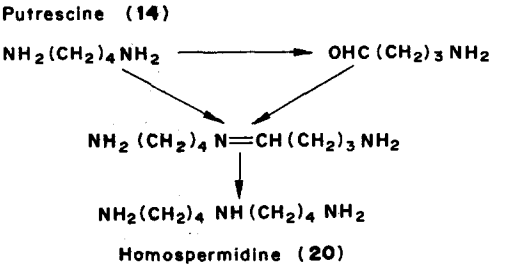
was incorporated into agmatine and arcain. Agmatine, which may be formed both directly from arginine by decarboxylation or by arcain hydrolysis, is further hydrolysed to putrescine and then oxidized to γ-aminobutyric acid. L-Arginine carboxy-lyase (opt pH 5·3), which was demonstrated in crude extracts, would not decarboxylate D-arginine, L-homoarginine, L-arginic acid, γ-guanidinobutyric acid, L-glutamic acid or L-ornithine, but L-canavanine was attacked at 32% of the rate with L-arginine. Diamine oxidase activity was also detected. In addition to arginine decarboxylation, agmatine may be formed by transamidination from arginine to putrescine; arcain (**18**) is similarly formed by transamidination to agmatine (**12**). The enzyme functions with a wide range of donors and acceptors. *In vivo* the transamidination of putrescine is unimportant quantitatively in comparison with the oxidation of putrescine to γ-aminobutyric acid [63]. The relative activities of the enzymes which hydrolyse respectively agmatine and arcain to urea depended on the developmental stage and on the growth medium. The two ureohydrolases could be separated by Me_2CO precipitation [64].

Guanidino acetic acid and arginine are efficient precursors for the isoprenoid guanidine, galegin (**19**) which is found in *Galega officinalis* (Leguminosae) [65]. In *Oldenlandia affinis* (Rubiaceae) tetramethylputrescine comprised 0·1% of the dry wt of the aerial parts [66].

In Japan, *Chlorella* is the source of spermidine used for stimulating lactic acid bacteria in the dairy industry, and a study [67] of the polyamines of *Scenedesmus* has shown that the latter alga is equally suitable for this purpose. In *C. fusca* and *S. acutus*, cadaverine and spermine were absent, but ethanolamine, methylamine and dimethylamine, putrescine and spermidine were identified; the mean spermidine content was 13 μmol/g dry wt. Methylamine and dimethylamine were also found in the nutrient medium. On the basis of dry wt, the spermidine content was independent of light when the intensity was increased 3-fold. Since the yield varied roughly in proportion to the light intensity, a direct relationship between growth rate, as affected by light, and spermidine content could not be established. For *C. fusca* and *S. acutus* in general, nitrate and ammonium were equally good, and superior to urea, for elevating the spermidine content. On the basis of dry wt, increasing the temperature from 5–30° (optimum) increased the spermidine content from 3 μmol/g dry wt to 13 μmol/g dry wt. The spermidine content on a dry wt basis was reduced by half after 8 days growth, though this decline could be reduced by changing the medium every 2 days.

Many aliphatic and cyclic amines have been tested as potential inhibitors of fungal spore germination. Spermidine (**21**) and spermine (**22**) were particularly effective against spores of *Penicillium digitatum* [9, 10]. In screening a homologous series of polyamines for fungitoxicity against spores of *Penicillium expansum* and *Neurospora crassa*, the spermine series was more effective than the spermidine series, though greatest activity was found with the natural polyamines [68]. Spores of wheat stem rust uredosporelings contained spermidine (4 μmol/g fr. wt) which increased (× 1·8) after germination. Putrescine was apparently absent [69].

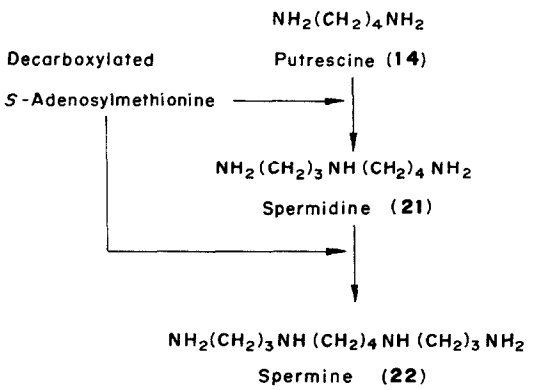
From the leaves of the sandalwood tree (*Santalum album*; Santalaceae) a higher homologue of spermidine, *sym*-homospermidine (**20**) has been isolated [70, 71]. This polyamine occurs at a much higher concentration than spermidine and comprises 0·5–1·5% of the dry wt (i.e. up to 100 μmol/g dry wt). Isotope dilution experiments showed that arginine was a better precursor than ornithine by a factor of 7. Putrescine feeding gave rise to γ-aminobutyric acid, though dilution experiments showed that γ-aminobutyraldehyde may not be a free intermediate. In view of the molecular symmetry of homospermidine, the metabolic pathway shown in Scheme 5 was suggested for homospermidine formation. Diaminobutyrate was a better precursor of spermidine than methionine, and both of the triamines may be formed by an analogous pathway in the sandalwood leaves. The discovery of homospermidine moieties in the alkaloids solapalmitine and solapalmitenine in



Scheme 5. Formation of homospermidine from putrescine in the leaves of *Santalum album*.

Solanum tripartitum [72] indicates that this polyamine could occur widely. It may hitherto have been confused with spermidine in chromatography.

In animals and micro-organisms the polyamines spermidine (**21**) and spermine (**22**) are derived from putrescine by the consecutive addition of propylamino residues donated by decarboxylated *S*-adenosylmethionine (SAM) (Scheme 6).



Scheme 6. Pathway for the formation of the polyamines from putrescine in animals and microorganisms, and probably also in higher plants.

Yeast has an especially active SAM carboxy-lyase (SAM–CL), which has an absolute requirement for putrescine, though Mg^{2+} is not required. This enzyme therefore resembles the SAM–CL from the rat ventral prostate. In the presence of putrescine, the pH optimum was about 7, but in its absence the activity increased at least up to pH 8·3 [73]. The carboxy-lyase has been purified 400-fold (yield 2%) by ammonium sulphate fractionation (40–65% saturation) and by chromatography on DEAE cellulose, hydroxylapatite, DEAE Sephadex and Biogel P-200. Spermidine synthetase, the enzyme which transfers the propylamino group from decarboxylated SAM to putrescine to form spermidine, was reduced by a factor of only 10 in the course of the purification of the SAM–CL, indicating the close association of these two enzymes [74]. Yeast should be an excellent source of the enzymes involved in polyamine biosynthesis [73].

On treatment of an extract of mung bean (*Phaseolus aureus*) seedlings with $(NH_4)_2SO_4$, a fraction of the SAM carboxy-lyase (SAM–CL) which precipitated at 0–40% saturation was not enhanced by Mg^{2+}, while the fraction precipitated at 40–65% saturation was stimulated (×2·3) (opt at 10 mM Mg^{2+}). No activity could be detected in the fraction precipitated from 65 to 80% ammonium sulphate saturation. Stoichiometry for the SAM–CL precipitated at 40–65% ammonium sulphate saturation could be established between CO_2 and the decarboxylated SAM in the presence of Mg^{2+}, but not in its absence. This enzyme fraction was not stimulated by putrescine, and therefore more closely resembled the bacterial than the yeast and rat enzymes. However, SAM–CL from carrot or cabbage leaves was not stimulated by Mg^{2+} or by putrescine [73], and the SAM–CL in extracts of *Vinca rosea* (Apocynaceae) seedlings is not stimulated by Mg^{2+} but activity is enhanced 8-fold by putrescine [75]. Spermidine synthetase was also detected in the *Vinca* extracts, together with an enzyme hydrolysing the decarboxylated SAM after propylamino transfer (5-*S*-methyl-5-thioadenosine), to give 5-*S*-methyl-5-thio-D-ribose and adenine.

Scorzonera hispanica (Compositae) tumours contain spermidine and putrescine in larger amounts than the normal tissue [76]. In the habituated tumour, which is not induced by *Agrobacterium tumefaciens*, spermidine was increased ×1·5 and putrescine ×10. In crown gall tumour, which is induced by *A. tumefaciens* but in which the bacterium is no longer present, spermidine was increased ×3 and putrescine ×100 on a dry wt basis. Spermine was decreased (×0·5) in both tumours by comparison with normal tissue. The increase in polyamines appears to be correlated with the growth rate, as has also been found in many animal tumours [33]. In habituated tissue of *Nicotiana glauca* 2 months after transplantation, spermine was again lower than in the normal tissue, and putrescine showed a considerable increase [77]. Moreover, tumours on *Sesamum* (Pedaliaceae) plants caused by *Agrobacterium tumefaciens* showed maximal growth in potassium-deficient media which is known to induce high putrescine levels in plants [78].

*t*RNA and *r*RNA isolated from spinach (*Spinacia*; Chenopodiaceae) and pea (*Pisum*; Leguminosae) leaves and from *Neurospora crassa* mycelia contained polyamines in a ratio differing from the ratio in the entire tissues. The putrescine content

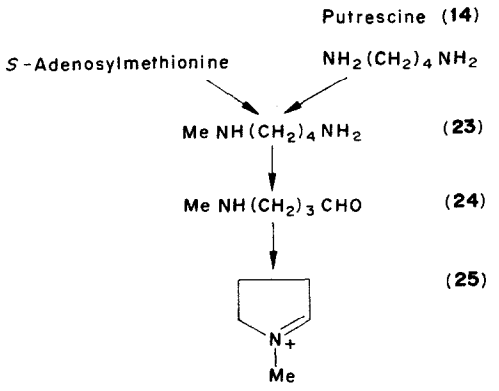
of spinach *r*RNA was greater than that of spermidine (× 1·5), while in the entire tissue the spermidine is greater than putrescine (× 3); in pea *r*RNA the spermine content is higher (× 3) than that of spermidine, while in the entire tissue the converse is true. Only spermidine could be found in the RNAs of *Neurospora*, and the consistent occurrence of spermidine in *t*RNA and *r*RNA fractions indicates the importance of this polyamine for RNA function. The number of polyamine residues was considerably less than the theoretical capacities of the RNA fractions. The extent of the redistribution of the polyamines on extraction is difficult to assess, though this was minimized by isolation in media of low ionic strength [79].

Treatment of *Helianthus tuberosus* (Compositae) tissue with polyamines or indol-3yl-acetic acid (IAA) stimulated growth, and treatment with IAA induced polyamine accumulation. It was therefore proposed that at least one of the processes by which IAA stimulates growth may be mediated by the polyamines [80]. RNA synthesis induced by polyamines and by IAA is similar, and these substances appear to act at the transcription stage [81]. A greater stimulation of RNA synthesis was found with both spermidine and IAA in the tubers at the beginning of dormancy (November) than in the tubers studied 6 months later. Inhibition due to actinomycin D ($1{\cdot}5 \times 10^{-6}$M) of ^{32}P incorporation in DNA and RNA was reversed by spermidine (10^{-4} M). Since actinomycin D is known to bind specifically with DNA, spermidine probably forms complexes with DNA, thus displacing the antibiotic.

The polyamines have similar functions to Mg^{2+} ions in nucleic acid metabolism, and the relationship between these cations has now been studied in mycelia of the fungus *Neurospora crassa* [82]. The Mg^{2+}, spermidine and spermine levels of mycelium grown on a standard medium were 16, 16 and 0·22 μmol/g fr. wt respectively. The putrescine level was less than half that of the spermine. In Mg-deficient mycelium, the molar levels of both spermidine and spermine were increased 2·5-fold on the basis of RNA nucleotides, and it appears that the polyamines may partially substitute for Mg^{2+} on depletion of this ion. The increase in the polyamines occurs before any effect on growth or RNA content can be detected. The increase in putrescine level in Mg-deficient barley, bean and pea leaves [39] cited earlier may also result from the interchangeability of Mg and the di- and polyamines in nucleic acid metabolism.

Spermidine interacts with a chromatin-bound RNA polymerase (polymerase 1) from the hypocotyls of soybean seedlings which requires Mg^{2+} or Mn^{2+}. Optimal concentrations of these ions were about 1 mM and 10 mM respectively, but on addition of 200 mM KCl or 1 mM spermidine, the effect of Mn^{2+} was increased 3- to 4-fold and maximal activity was obtained when the Mg^{2+} was reduced to 1 mM. The high KCl concentration or the spermidine were thought to reverse a secondary inhibitory effect due to the binding of Mn^{2+} to DNA [83]. The effect of polyamines on the oxidation of NADPH by spinach flavoprotein–iron–sulphur protein complexes has also been studied. Cadaverine (**15**) and spermine (**22**) stimulated the plant system markedly (× 9 and × 4 respectively). The polyamines probably affect the interaction between the flavoprotein and the iron–sulphur protein [84]. The levels of the phytoalexin pisatin and phenylalanine ammonia lyase activity were increased by treatment of pea pods with a variety of natural and synthetic basic polypeptides and also by putrescine, cadaverine, spermidine and especially spermine [85, 86]. The conformation of multiple segments of nuclear DNA may be changed by these inducers and rendered accessible to RNA polymerase. At suboptimal levels of Mg^{2+}, bean leaf cytoplasmic valyl *t*RNA synthetase was stimulated optimally by spermidine at 1 mM. With 10 mM spermidine, activity was negligible. However, for the chloroplastic enzyme the optimal spermidine concentration was broad, ranging from 0·5–10 mM. The optimum for both the cytoplasmic and chloroplastic valine dependent PP–ATP exchange was 0·5 mM spermidine [87]. In germinating maize seed, histone formation was inhibited 70–80% by putrescine at 10^{-4} M during 48 hr incubation. However, after 72 hr the length of the lateral roots was stimulated 2-fold by this treatment [88].

Recent studies have demonstrated the mechanism by which putrescine is incorporated into the pyrrolidine ring of nicotine in tobacco. Putrescine *N*-methyl transferase (PMT), forming *N*-methylputrescine (**23**) from putrescine and *S*-adenosylmethionine (Scheme 7), an enzyme which is found only in the roots of the tobacco plant, has



Scheme 7. Formation of *N*-methylpyrrolinium from putrescine in the biosynthesis of nicotine in tobacco roots.

been purified 30-fold with a yield of 27%. The enzyme has a MW of 60000 and an optimum pH between 8 and 9. Methylation of *N*-methyl-putrescine was about 7% of the rate found with putrescine. Neither diaminopropane nor cadaverine acted as acceptors [89]. An enzyme capable of oxidising the primary amino groups of putrescine (*N*-methylputrescine oxidase, MPO) (Scheme 7) is also found in tobacco roots. (For the properties of this enzyme, see below under amine oxidase). The product of the oxidation of *N*-methylputrescine, 4-methylaminobutanal (**24**), undergoes spontaneous ring closure to give *N*-methyl-pyrrolinium (**25**) which may be incorporated into the nicotine molecule [90]. The demonstration of PMT and MPO in the root of the tobacco plant is strong evidence for the implication of *N*-methylputrescine in nicotine biosynthesis, which is known to take place most actively in this organ. Moreover, activities of ornithine carboxy-lyase, PMT and MPO were strongly stimulated on decapitation of the tobacco plants, and the activities reached a peak concurrently 24 hr later, a time when the nicotine content was also at its peak. Indol-3yl-acetic acid increased the activity of these enzymes and application of nicotine decreased them in unison, suggesting that they are under a common regulatory control. The sharp decline in enzyme activity after 24 hr from decapitation may be due to nicotine accumulation causing enzyme repression. Ornithine carboxy-lyase activity was detected in leaves, roots and callus of *Nicotiana tabacum*, roots of *Datura stramonium*, *Atropa belladonna*, *Lycopersicon esculentum* (Solanaceae) and in barley seedlings. PMT and MPO were detected only in the roots of the plants forming alkaloids with the *N*-methyl-pyrrolidine moiety and were absent from tomato and barley [91]. In *D. stramonium*, *δ*-*N*-methylornithine is probably the precursor of *N*-methylputrescine in the biosynthesis of the tropine moiety of hyoscyamine and hyosine [92].

AMINE OXIDASE

The amine oxidases are of considerable importance in the physiology of animals, plants and micro-organisms, and work on these enzymes has been the subject of two recent reviews [93, 94].

The tobacco root *N*-methylputrescine oxidase has been purified 150-fold with a yield of 38%. The pH optimum was 8·0. The enzyme oxidized putrescine and cadaverine at a rate about 40% of that found for *N*-methylputrescine (Scheme 7), but spermine, *n*-hexylamine, histamine, tyramine and phenethylamine were not oxidized. The enzyme was insensitive to aminoguanidine, isoniazid or iproniazid, and it was effectively inhibited by hydroxylamine, hydrazine, potassium cyanide, mercuric chloride and diethyldithiocarbamate (DIECA). On dialysis against DIECA, the inactivated enzyme could be regenerated with cupric ions at 10^{-6} M [90].

Amine oxidase in red clover (*Trifolium pratense*; Leguminosae) which was first detected in 2-day-old seedlings, showed maximal activity in the roots at 7 days and in the leaves at 15 days from germination on a dry wt basis [95]. On the basis of the whole plant, activity increased at least up to 42 days. Amine oxidase was reduced even in 9-day-old clover plants grown in Cu-deficient media, compared with the controls of the same age. The difference increased with age, and at 42 days old activity was 5–12 times greater in control than in the deficient plants. Most of the amine oxidase was recovered in the supernatant fraction remaining after centrifugation at 10^4 *g* [95], confirming previous work [96].

A very sensitive method for the detection of Cu, based on the reactivation of the apo-enzyme of the pea seedling amine oxidase has been developed, which is specific for this metal. The increase in activity was linear for up to 10 ng Cu^{2+} added. On application to the determination of the Cu in plant material, the results obtained agreed well with

those found for the diethyldithiocarbamate method, though with a sensitivity *ca* 50 times greater [95].

Using tryptamine (**58**) as substrate, the affinity of the purified pea seedling amine oxidase for O_2 was shown to be dependent on amine concentration [97]. The mechanism for the reaction was therefore "ping-pong". The amine is converted to the corresponding aldehyde, and the enzyme is reduced in the first step. In a subsequent and independent step, the reduced enzyme reacts with molecular O_2 to give H_2O_2. The NH_3 may be released at either of these steps, or even at another step. At 10^{-3} M tryptamine, the K_m for O_2 was *ca* 10^{-4} M. In confirmation of the reaction mechanism, aldehyde formation from tryptamine was demonstrated on incubation with the enzyme in the absence of O_2. One mol of aldehyde was formed per mol of enzyme. β-Hydroxyethylhydrazine, 1,1-dimethylhydrazine and hydrazine were irreversible competitive inhibitors, giving maximum inhibition after *ca* 2 hr. Substrates can delay this, but do not prevent the inhibition by hydrazines.

The K_m for tryptamine in air with pH 8 phosphate–borate buffer was 4×10^{-4} M [97]. Although the pH optimum for tryptamine oxidation is at pH 9, the enzyme is unstable at that pH. Using a bioassay for measurement of "oxidation products", the K_m for tryptamine was $1{\cdot}6 \times 10^{-3}$ M [98]. The results obtained with this bioassay are complicated by the effect of the substrate, tryptamine, on the *Avena* curvature test. Using a more reliable spectrophotometric method at pH 7, the K_m was found to be $3{\cdot}2 \times 10^{-3}$ M. K_m values were greater for the oxidation of tryptamine [97, 98] than for the oxidation of the polyamines [98, 99]. For these reasons it is doubtful if the pea seedling amine oxidase is concerned with IAA formation. The formation of cadaverine may coincide with the greatest amine oxidase activity in pea seedlings [55], and the reason for this may be of importance physiologically.

Using a new procedure, the pea seedling amine oxidase has been purified by a factor of 420-fold [98]. Cellulose phosphate chromatography in the final step revealed two peaks, one with a high and the other a low specific activity. The latter may be an amine oxidase which has been partially modified by the purification procedure. On electrophoresis and ultra-centrifugation of the final preparation, the enzyme appeared homogeneous. In other work, purified pea seedling amine oxidase gave two protein fractions on disk electrophoresis, only one of which was active [100].

Using benzylamine as substrate, the phosphate ion was shown to be necessary for maximal activity in glycine, bicarbonate and borate buffers (K_m 0·9 mM for phosphate), but this requirement could not be demonstrated in Tris buffer [98]. The phosphate requirement would be compatible with the possibility that this amine oxidase is concerned in the oxidation of amines associated with nucleic acids, notably the polyamines for which the enzyme shows relatively low K_m values.

As determined by sedimentation equilibrium centrifugation, the MW of the enzyme was found to be $1{\cdot}85 \times 10^5$ [98] compared with an estimate of $9{\cdot}6 \times 10^4$ from electron microscopy [101]. It therefore appears that the enzyme can dimerize.

Pea seedling diamine oxidase catalyses the oxidation of 3-hydroxycadaverine stoichiometrically to 4-hydroxy-1-piperideine with a pH optimum of 6·5 [102]. Sulphur-containing analogues of lysine and cadaverine are also oxidized by this enzyme [103].

Diamine oxidase activity is absent from ungerminated pea seed cotyledons but increases significantly within a few hours on germination, and antibiotics have been used to determine whether protein synthesis is involved. Actinomycin D, present initially, completely inhibited diamine oxidase activity, but addition 24 hr after imbibition resulted in 80–90% of normal activity. It is therefore possible that the amine oxidase depends on *de novo* protein synthesis, the *m*RNA for which is synthesized in the first 24 hr after germination [104]. Pea seedlings from which the cotyledons had been removed showed a higher diamine oxidase activity, and also a higher content of the substrates for this enzyme on a fr. wt basis. These responses may be related to the depletion of K^+ [105].

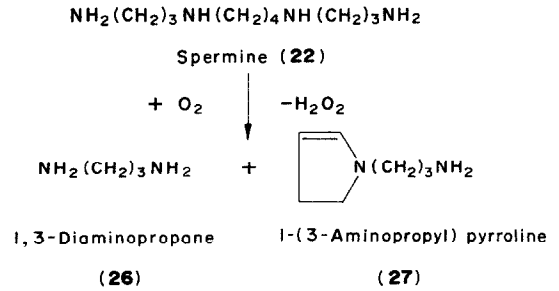
Two amine oxidases were isolated from cucumber seedlings, one of which was more active with tryptamine than with putrescine as substrate [106]. The pea seedling diamine oxidase could be inhibited by 1,4-diamino-2-butanone and 1,5-diamino-3-pentanone. The second substrate analogue, which was the most efficient inhibitor, reduced growth only at relatively high concentrations, which again suggests that the pea dia-

mine oxidase is not involved in the formation of IAA from tryptamine [107]. The amine oxidase inhibitors safrazine and nialamide inhibited shoot growth of rice seedlings. However, at 10^{-4} to 10^{-3} M nialamide, rice root growth was almost doubled [108]. At 10^{-3} and 10^{-2} M safrazine, IAA at 10^{-5} M reversed the shoot growth inhibition. These results are compatible with the suggestion that IAA is formed from tryptamine by an amine oxidase in *rice* plants. Using the tetrazolium technique with tryptamine as substrate, the pea seedling amine oxidase was shown to be present in the epidermis and sieve tubes [108]. This enzyme therefore has a similar location to that of the polyamine oxidase of maize [109].

The properties of the amine oxidase from soybean are similar to those from pea [110]. The substrate range and sensitivity to inhibitors was similar for the enzyme from both sources, and as for pea, Cu^{2+} ions were needed for the reactivation of the soybean apoenzyme. Unlike the pea enzyme, no activity was found in the cotyledons until at least 6 days from germination, while activity in hypocotyl and root reached peaks 3 and 1 day respectively after germination. The ratio of activity putrescine–spermidine remained constant with age; the ratio putrescine–spermine at first declined and then increased.

The discovery of 1,3-diaminopropane (**26**) in the leaves of barley seedlings and the increased levels of this compound on feeding unlabelled spermine suggested the presence of a polyamine oxidase in this tissue [109]. An additional compound formed on spermine feeding was later identified as 1(3-aminopropyl)pyrroline (**27**) and peaks attributable to this cyclic amine were found on GLC of extracts of normal barley tissue. The putrescine peak was unchanged on spermine feeding. Spermidine feeding enhanced the putrescine level. This finding could result from the degradation of spermidine to putrescine, but it is more likely to be due to a mass action effect since the synthesis of spermidine from putrescine might be inhibited. The peaks attributable to 1,3-diaminopropane and 1(3-aminopropyl)pyrroline were only slightly enhanced on feeding spermidine. The increased levels of diaminopropane in wheat grain on storage [111] could be attributed to this enzyme.

The polyamine oxidase effecting spermine and spermidine oxidation is found in the leaves of many Gramineae [112], though not in the leaves of many other mono- or dicotyledonous plants investigated, nor even in the leaves of a sedge, a member of the Cyperaceae, which is a family closely related to the Gramineae [113]. In maize shoots the enzyme is apparently associated with the vascular system [109]. The enzyme from barley leaves oxidizes spermine to 1,3-diaminopropane (**26**) and 1(3-aminopropyl)pyrroline (**27**) (Scheme 8) (opt pH 4·8), and spermidine is oxidized to 1,3-diaminopropane and 1-pyrroline (opt pH 8·0). H_2O_2 is formed in both cases. Stoichiometry of the amine products of spermine oxidation was established by GLC [112] and by the *N*-methyl-2-benzothiazolone hydrazine reagent [114]. The enzyme was associated with a particle which sedimented in low centrifugal fields (1000 *g*) and the enzyme could be solubilized by washing the particles in 0·5 M NaCl. In this step a 24-fold purification was achieved.



Scheme 8. Oxidation of spermine by an enzyme from barley leaves.

The properties of the enzyme were further studied by a spectrophotometric assay based on the utilization of the H_2O_2 for the oxidation of guaiacol in the presence of peroxidase to a brown chromogen. The K_m for the barley enzyme with spermidine as substrate was 2×10^{-5} M (pH 7·5) and for spermine the K_m was 3×10^{-5} M (pH 4·5). At the respective pH optima, spermine was oxidized 14 times faster than spermidine. At pH values above 7 the enzyme was unstable; at pH 7·5, 50% of the activity was lost in 15 min at 25°. Activity against spermidine (**21**) and spermine (**22**) was lost simultaneously, but 1 fraction (*ca* 20%) of the enzyme was not destroyed under these conditions. For the barley enzyme at pH 4·5, spermine oxidation was 580-fold greater than at pH 7·5, and for spermidine at pH 7·5 activity was 35-fold greater than at pH 4·5 [115].

The enzyme was not sensitive to Cu chelating reagents, but 2-hydroxyethylhydrazine at 5×10^{-4} M caused 50% inhibition. At pH 7·5, 50% inhibition of activity with spermidine as substrate was given with $3{\cdot}5 \times 10^{-6}$ M spermine. A number of polyamine analogues were tested as potential inhibitors or substrates of the polyamine oxidase. $NH_2(CH_2)_3NH(CH_2)_{10}NH_2$ was particularly effective as an inhibitor of spermine oxidation at pH 4·5 (K_i 5×10^{-6} M). Methylglyoxal-*bis*-guanylhydrazone, an established inhibitor of polyamine metabolism in animals [116], was relatively ineffective. As substrates, the polyamine analogues were less than 10% as effective as spermine.

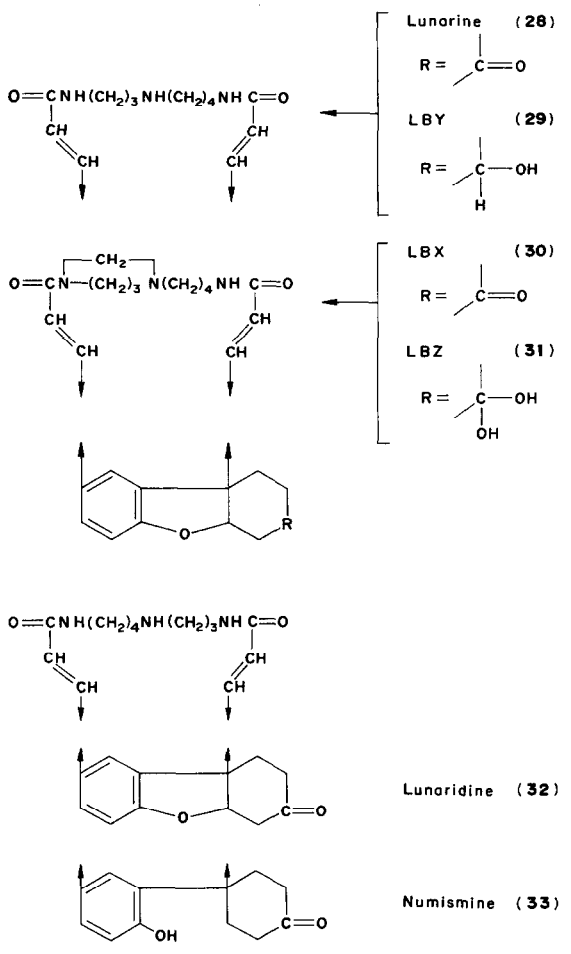
Using an O_2 electrode, the K_m for 100% O_2 was 3×10^{-4} M, and in air activity was about half that at 100% O_2. The K_m for spermine oxidation was independent of the O_2 concn, suggesting a sequential mechanism for the polyamine oxidation. Activity in the leaves increased with age up to 4 weeks, but in the leaves of 11-week-old plants activity was lower than in the leaves of 1-week-old plants [114].

The polyamine oxidase of maize [117, 118] was similar in many respects to the enzyme from barley. The enzyme was eluted from the particles with KH_2PO_4 (0·5 M). The pH optimum was 6·3 with spermidine and the K_m was 6×10^{-4} M. The pH optimum for spermine oxidation was 5·5, and the activity with spermine was about 0·75 of that with spermidine. The enzyme was inhibited by the acridine compounds quinacrine, 6,9-diamino-2-ethoxyacridine and acriflavin, and the quinacrine inhibition was reversed by FMN or FAD. The inactive apoenzyme prepared by acid ammonium sulphate precipitation was reactivated 50% by FAD, but no activity was detected on addition of FMN or riboflavin. An FAD dehydrogenase found in *Serratia* [119] effects the same reaction as the barley enzyme. The bacterial enzyme also contains a haem prosthetic group, and this may be expected in the enzyme from the Gramineae.

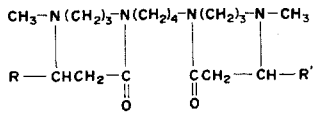
DI- AND POLYAMINE CONJUGATES

Cinnamic acid polyamine conjugates

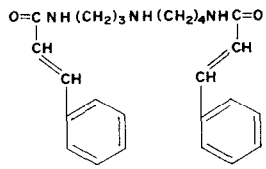
Lunarine (**28**), which is found in the seeds of *Lunaria annua* and *L. rediviva* (Cruciferae) is derived from spermidine and two cinnamic acid residues. The structures of the five alkaloids related to



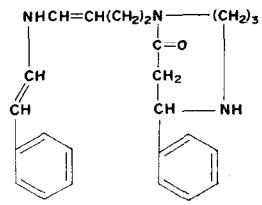
lunarine—LBX (**30**), LBY (**29**), LBZ (**31**), lunaridine (**32**) and numismine (**33**) (of which the pale yellow tautomer has been named lunariamine) —have now been established [120–124]. The hexahydrobenzofuran moiety of lunarine is formed from phenylalanine via cinnamic acid and *p*-coumaric acid: tyramine is not readily incorporated [120]. It was possible to chemically synthesize tetrahydrolunaridine by a process in which 2 *p*-coumaric acid units were first coupled oxidatively, and subsequent addition of spermidine (mechanism a). Alkaline ferricyanide was more efficient than horseradish peroxidase in effecting this oxidation. Oxidative coupling of *p*-coumaric acid with prior attachment to the primary amino groups of spermidine (mechanism b) was not successful [124]. However, the co-occurrence *in vivo* of lunarine and lunaridine, which differ only in the mode of attachment of spermidine, can be most



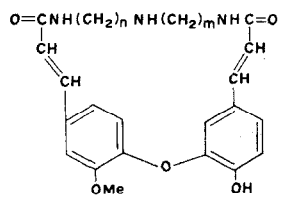
(34) Homaline R = R′ = Phenyl
(35) Hopriminol R = —$CH_2CHOH(CH_2)_5Me$; R′ = —$(CH_2)_4Me$
(36) Hopromalinol R = —$CH_2CHOH(CH_2)_5Me$; R′ = —Phenyl
(37) Hopromine R = —$(CH_2)_6Me$; R′ = —$(CH_2)_4Me$



Maytenin (38)



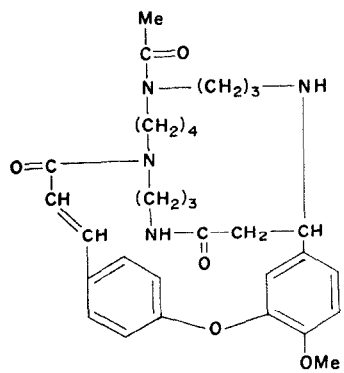
Periphylline (39)



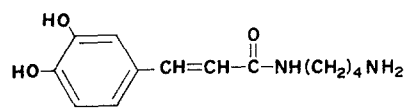
Codonocarpine (40)
n = 4 m = 3
or
n = 3 m = 4

easily explained on the basis of mechanism b. Moreover, the structures of the polyamine alkaloids homaline (**34**), maytenin (**38**) and periphylline (**39**) suggests that these are formed by pathways analogous to mechanism b.

Several lunarine-like alkaloids have been identified from the leaves of *Homalium pronyense* (Homaliaceae) from New Caledonia. The structure of homaline (**34**) has been confirmed by synthesis. Other related alkaloids are hoprominol (**35**), hopromalinol (**36**) and hopromine (**37**) [125, 126]. Maytenin (di-*trans*-cinnamylspermidine) (**38**) was found in an extract (35 yr old) of the South-American *Maytenus chuchuhuasha* (Celastraceae). The crude preparation was a weak antipyretic and vasodilator [127]. The structure of maytenin has now been confirmed by synthesis [128, 129]. Two similar alkaloids have been found in *Peripterygia marginata* (Celastraceae) [130]. The structure of the main alkaloid, periphylline (**39**) has now been established [131]. Codonocarpine (**40**) extracted from the bark of *Codonocarpus australis* (Phytolaccaceae) is structurally very similar to lunarine [132]. Of the two isomers expected ($n = 3$, $m = 4$ and $n = 4$, $m = 3$), experimental methods used would not establish which occurred naturally. Chaenorhine (**41**) is a spermine-based macrocylic alkaloid obtained from the dried aerial parts of *Chaenorhinum origanifolium* (Scrophulariaceae) [133].



Chaenorhine (41)

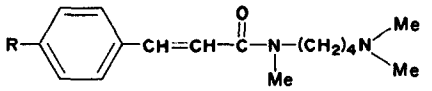


Paucin (42)

Cinnamic acid diamine conjugates

Caffeylputrescine (paucin, **42**) has been found in seeds of *Pentaclethra macrophylla* (Leguminosae) [134, 135]. It was the main ninhydrin positive component in this material, the hydrochloride dihydrate comprising 1·4% of the dry wt. *p*-Coumaryl-, caffeyl- and ferulyl-putrescine were isolated from callus tissue cultures of *Nicotiana tabacum* [136], and ferulyl-putrescine and di-ferulyl-putrescine have now been identified in the leaves of virus-infected tobacco [137]. Di-ferulyl-putrescine, which was detected as three interconverting *cis–trans* isomers on chromatography, is located mainly in the stalk and veins of the leaves. It was suggested that ferulyl-putrescine is formed in tobacco on virus-infection as a protective mechanism. In tobacco, caffeyl-putrescine was also found in the apex and flowers but it was absent

from the fully-expanded leaves [138]. *N,N,N'*-Trimethyl(4-hydroxy-*cis*-cinnamyl)putrescine (**43**)

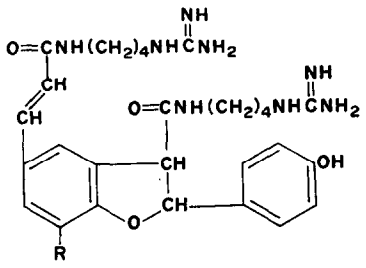


(**43**)

R = H or OMe

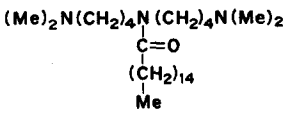
and the corresponding *O*-methyl compound have been found in leaves of *Kniphofia flavovirens* and *K. tuckii* (Liliaceae) [139, 140].

The antifungal dimers of coumarylagmatine, hordatines A and B (**44**) and hordatine M which is a mixture of their glucosides, have been characterized from barley shoots. At 10 ppm, hordatines A, B and M completely inhibit *Monilinia fructicola* germination, and hordatine A similarly inhibited five other pathogenic fungi at this concentration [40].

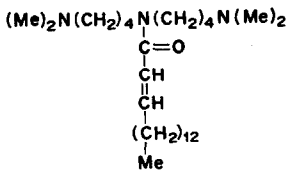


(**44**) Hordatine A R = H

Hordatine B R = OMe



Solapalmitine (**45**)

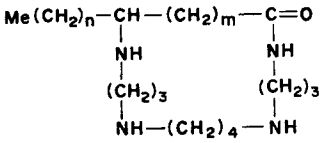


Solapalmitenine (**46**)

Fatty acid–polyamine conjugates

Two tumour inhibitory homospermidine derivatives have been isolated from *Solanum tripartitum* [72]. An extract of this plant inhibited growth of cells derived from human carcinoma of the nasopharynx (KB), and the 2 component alkaloids solapalmitine (**45**) and solapalmitenine (**46**) showed significant inhibition against Walker 256 rat carcinosarcoma. Free homospermidine has been found in the leaves of *Santalum* [71].

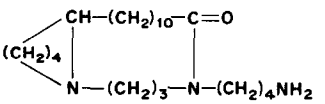
The pithecolobines (**47**) from *Samanea saman* (Leguminosae) occur as a mixture of spermine–fatty acid alkaloids with three main components.



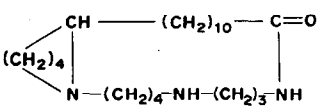
Pithecolobine (**47**)

These are: (a) $m = 3$ and $n = 6$; (b) $m = 1$ and $n = 8$; and (c) $m = 1$ and $n = 10$. The proportions of these components were (a) 24–30%; (b) 40–49%; and (c) 13·5–16·5%. These components could not be separated intact, and the proportions were determined after degradation of the mixture [141].

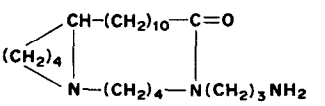
The macrocyclic spermidine alkaloids oncinotine (**48**), *iso*-oncinotine (**49**) and neo-oncinotine (**50**) have been isolated from the bark of *Oncinotis*



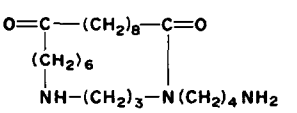
Oncinotine (**48**)



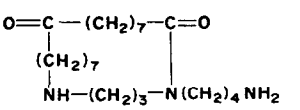
iso - Oncinotine (**49**)



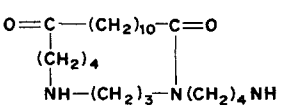
Neo - Oncinotine (**50**)



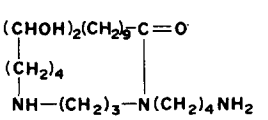
Inandenine A (**51**)



Inandenine B (**52**)

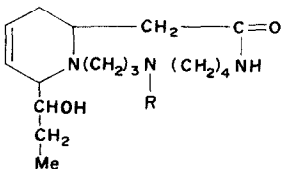


Inandenine-10-One (**53**)



Inandenine-10,11-Diol (**54**)

nitida (Apocynaceae) from West Africa [142]. The spermidine alkaloids inandenines A (**51**) and B (**52**) were isolated from the leaves of *Oncinotis inandensis* [143]. Inandenine-10-one (**53**) and -10,11-diol (**54**) were found in the bark of this plant. The diol may occur as the epoxide within the plant. Palustrine (**55**) and palustridine (**56**) are toxic spermidine alkaloids isolated from *Equisetum palustre* [144].



Palustrine (**55**)
R = H

Palustridine (**56**)
R = CHO

The structural chemistry of the widely distributed macrocyclic alkaloids has been reviewed in ref. [145]. The function of the amine conjugates is still largely unknown, though they may have evolved as a protective mechanism against pathogens or predators.

AROMATIC AMINES

Certain aromatic amines found in plants are physiologically active in animals, and ingestion under certain conditions may even be fatal. The occurrence of these harmful amines in food plants has been recently reviewed [146–148].

Indoles

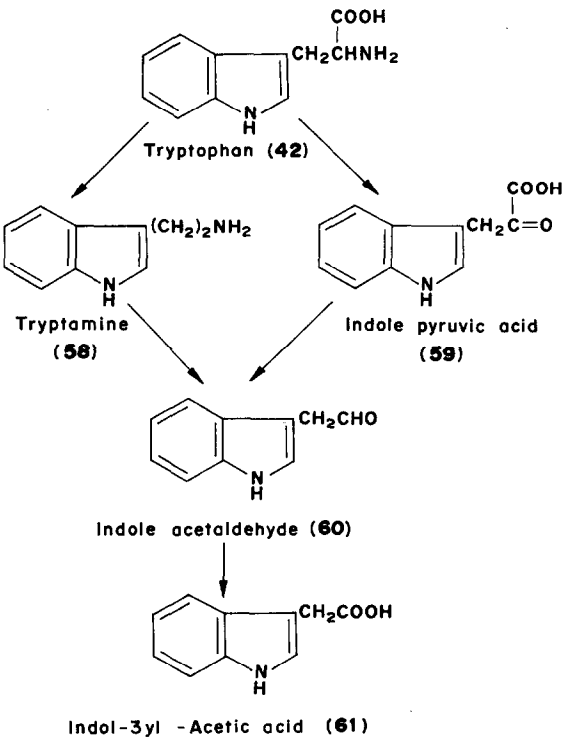
A number of insect-attracting volatile amines are produced by several members of the Araceae in flowers at anthesis. Amongst these, indole (**62**) has now been characterized in *Sauromatum guttatum*, and is probably also found in *Arum dioscoridis*, *Dracunculus vulgaris* and *Lysichitum americanum*, though none could be found in *Arum maculatum*, *A. orientale* or *Helicodiceros muscivorum* [149]. In the appendix of *Sauromatum guttatum* indole is produced for 24 hr at flowering, commencing at 6–8 p.m. Output was maximal in the cortex in a region 10–20 cm above the male flowers (total spadix length 40 cm) at 12 noon on the following day, when 200–300 nmol indole were present per g fr. wt. About 10% of the indole formed is released in the volatile fraction and the residue is probably metabolized. Indole formation is stimulated by an inducer named calorigen secreted by the staminate flowers. This inducer has a similar function in several members of the Araceae. The calorigen was assayed by determination of the indole synthesized or the heat produced on application to excised appendix tissue segments [150]. Calorigen induced a temperature rise of nearly 4° in the bioassay, compared with a rise of 5° in the intact appendix at flowering. The time course of indole synthesis and heat formation was independent of the amount of calorigen applied. The sequence was similar in both the bioassay and in the natural flowering cycle of the intact inflorescence. On reapplication of calorigen, no indole or heat is produced. However, even 9 days from excision of the mature appendix a single response may still be obtained, though the amount of indole formed is correlated with the amount of calorigen applied. Calorigen is stable for at least 7 days at room temperature and in the dried state for up to 4 months. It is stable both to autoclaving and to extremes of pH. On DEAE Sephadex chromatography the calorigen was recovered in 2 fractions.

A tryptophan carboxy-lyase demonstrated in crude extracts of cucumber hypocotyls has a pH optimum of 7 [151]. The product, although not rigorously characterized, was almost certainly tryptamine. Octanol was added to the assay to inhibit amine oxidase, which otherwise destroys the tryptamine. In the absence of octanol, the activity, determined by tryptamine formation, was insignificant. Other amine oxidase inhibitors (parnate and isoniazid) apparently inhibited the carboxy-lyase. Neither NaF nor NaN_3 inhibited the reaction in the presence of octanol, and peroxidase was therefore probably not an additional cause of tryptamine loss. Ammonium sulphate precipitation caused partial resolution of apoenzyme, and activity was restored with pyridoxal phosphate. Since bacteria are present within cucumber tissues, the possibility that the enzyme could be attributed to these was thoroughly investigated, but considered to be most unlikely. It was concluded that auxin synthesis proceeds mainly via the tryptamine pathway in cucumber. However, tryptophan carboxy-lyase activity does not parallel IAA level in cucumber seedlings [152].

In tomato shoots the tryptophan carboxy-lyase (opt pH 8) was shown to be absolutely dependent on pyridoxal phosphate. By a combination of ammonium sulphate precipitation and Sephadex

chromatography the enzyme was purified 86-fold, with a recovery of 20%. The tryptophan carboxy-lyase was precipitated at between 25 and 35% $(NH_4)_2SO_4$ saturation, while a tryptophan transaminase was precipitated at between 40 and 70% saturation. The K_m for tryptophan decarboxylation was 3×10^{-3} M and the activity was maximal with 2×10^{-2} M tryptophan; higher tryptophan concentrations caused inhibition. The K_m for pyridoxal phosphate was 3×10^{-4} M. The carboxy-lyase was strongly inhibited by Co^{2+} (90% inhibition at 2 mM), Fe^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+}, but Mg^{2+} and Mn^{2+} had little effect. The enzyme was rather unstable during purification and lost 50% of its activity on dialysis against phosphate buffer, though inclusion of pyridoxal phosphate in the buffer prevented this loss. At pH 6, 80% of the activity was lost within 6 hr. The enzyme would decarboxylate only L-tryptophan or 5-hydroxy-L-tryptophan, the latter at about half the rate of the former, irrespective of the degree of purification. D-Tryptophan, L-phenylalanine and L-tyrosine were not able to act as substrates [153].

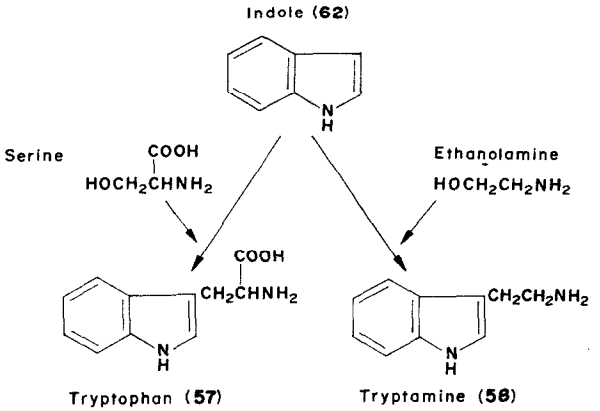
In an intensive study [154] of the biosynthesis of indol-3yl-acetic acid (IAA) (**61**) in tomato and barley plants, tryptophan-[3-^{14}C] (**57**) and tryptamine-[2-^{14}C] (**58**) was fed to excised shoots. Tryptophan-[3-^{14}C] gave label in tryptamine, in addition to indol-3yl-pyruvic acid (**59**) (IPA), indol-3yl-acetaldehyde (IAAl) (**60**) and IAA; tryptamine-[2-^{14}C] feeding gave rise to labelled IAAl and IAA but not to labelled IPA. Both the IPA and the tryptamine pathways (Scheme 9) appear to operate in both species, though the limitations of compartmentation prevented any quantitative assessment of the relative contribution of the two pathways. IAAl would be an intermediate in both pathways in the formation of IAA. Radioactivity from tryptophan and tryptamine was also detected in 5-hydroxytryptamine (5-HT, serotonin, **63**), especially in tomato, but no radioactive or native 5-hydroxytryptophan (5-HTP) was detected. Although 5-HT is usually considered to be a product of 5-HTP, in barley and tomato hydroxylation of tryptamine can also apparently take place. The activity of tryptophan transaminase (forming IPA), tryptophan carboxy-lyase (forming tryptamine) and indolacetaldehyde dehydrogenase (giving IAA) were demonstrated in cell-free extracts of barley and tomato. IPA carboxy-lyase was found only in



Scheme 9. Probable pathway of IAA formation in higher plants.

tomato tissue. The enzyme for converting tryptamine to IAAl was the only one in these two pathways which could not be demonstrated. Although this enzyme may be unstable, it is possibly a transaminase for which the acceptor was not present in the assay. Radioactivity from tryptophan and tryptamine was incorporated into IAA with little dilution in both species [154]. Conversion of tryptamine to IAA in sterile pea stem sections was reduced by addition of bisulphite and dimedon, and IAAl was detected chromatographically on liberation from its bisulphite addition product [155].

Zinc deficiency induced the accumulation of both tryptophan and tryptamine in maize seedlings, though the mechanism for this is unknown, and this result is surprising since Zn is needed by tryptophan synthetase (Scheme 10). Application of IAA did not overcome the growth repression caused by Zn deficiency, and growth reduction is therefore unlikely to be due to a Zn-requiring step in the formation of IAA from tryptamine. Tryptamine was also found in normal maize seedlings in



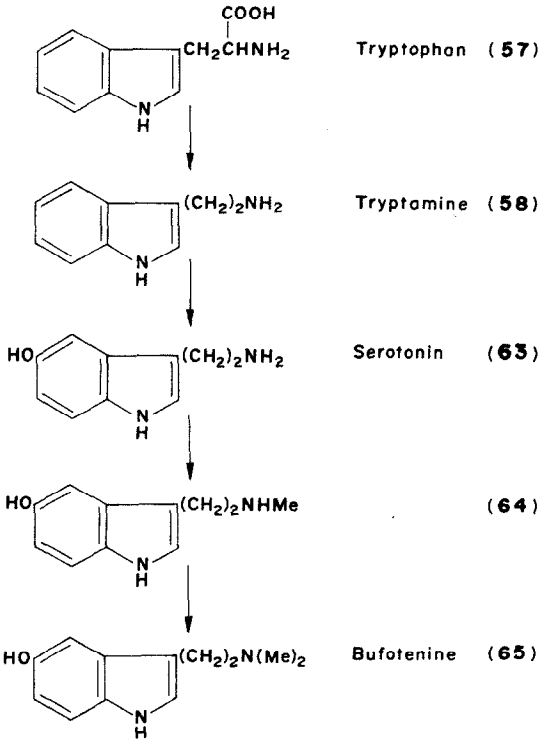
Scheme 10. Probable pathway for the synthesis of tryptophan, and an analogous pathway postulated for the synthesis of tryptamine.

very small amounts [156]. Deficiencies of trace elements other than Zn had no effect on tryptamine levels, and on adding Zn to Zn-deficient plants the tryptamine content was reduced, though the resultant plants had very small leaves and elongated internodes [157]. Radioactivity from L-tryptophan-[3-^{14}C] was found in tryptamine on being fed to maize seedlings [158].

A synergistic effect of indole and ethanolamine has been observed in the promotion of growth in pea stem sections (optima 10^{-4} M each) [159], and tryptamine may be formed from these precursors by an enzyme resembling tryptophan synthetase (Scheme 10). This step would then obviate the necessity for tryptophan decarboxylation in tryptamine formation.

Tryptamine (**58**) and 5-HT (**63**) are found in barley [160, 161] and tomato shoots [161], and tryptamine occurs in the legumes *Desmodium tiliaefolium* [162], *D. gangeticum* [163], *Acacia baileyana* [164] and *Prosopis alba* [165]. A useful review of the occurrence of these indoles is given in ref. [161]. 5-HTP found in mature seeds of *Griffonia simplicifolia* (Leguminosae) is formed by the hydroxylation of tryptophan. In the pods, 5-HT (**63**) comprised 0·2% of the dry weight, and lower concentrations occurred in the leaves, in which an inverse relationship was found between 5-HTP and 5-HT, suggesting that 5-HT is derived from 5-HTP [166]. In seeds of *Piptadenia peregrina* (Leguminosae) used by S. American Indians to make an intoxicating snuff, tryptamine is hydroxylated directly to 5-HT on germination. It is suggested that bufotenine (**65**) is formed from tryptophan in this plant, with tryptamine, 5-HT and *N*-methyl-5-HT as intermediates (Scheme 11). *N,N*-Dimethyltryptamine was not a precursor of bufotenine, and 5-HTP fed to seedling tissue was decarboxylated very slowly [167]. *N,N*-Dimethyltryptamine (**67**) together with its N_b oxide was detected in the leaves of *Banistereopsis argentea* (Malpighiaceae) [168] and in *Desmodium gangeticum* [163], *D. gyrans* [169] and *D. pulchellum* [170] (Leguminosae).

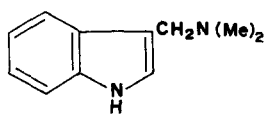
Phalaris arundinacea and *P. tuberosa*, which are major pasture grasses in N. America and Australia, are sometimes highly toxic to sheep, causing "Phalaris staggers". This may be attributed to the content of tryptamine alkaloids, notably gramine (**66**), *N,N*-dimethyltryptamine (**67**), 5-methoxy-*N,N*-dimethyltryptamine (**68**) and 5-hydroxy-*N,N*-dimethyltryptamine (**65**, bufotenine) [171, 172]. Variation in the levels of these indoles is due to both seasonal and genetic factors [173–175]. Feeding labelled tryptophan to *P. tuberosa* gave tryptamine (**58**), 5-HT (**63**) and 5-methoxytryptamine



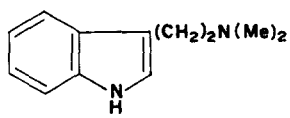
Scheme 11. Formation of bufotenine in *Piptadenia peregrina*.

(**69**). Enzyme activities for *N*-methylation, *O*-methylation and tryptophan decarboxylation were detected in cell free extracts. In seedlings of *Phalaris tuberosa*, activity of the carboxy-lyase was negligible in the first 3 days of germination, was highest on the 4th day and declined progressively to very low levels on the 20th day after germination. The enzyme, which was assayed with tryptophan-[^{14}C] as substrate, was purified 20-fold by $(NH_4)_2SO_4$ fractionation and DEAE cellulose chromatography. The optimum pH was 7·6 and the K_m 2 × 10^{-4} M, and pyridoxal phosphate was necessary for maximal activity. D-Tryptophan was inactive as a substrate; 5-HTP was decarboxylated to 5-HT at 70% of the rate of L-tryptophan. In the absence of carrier 5-HT, the apparent rate fell to 6% due to the lability of the amine. It is possible that two enzymes are present, one for each substrate. *N*,*N*-Dimethyltryptamine (1 mM), tryptamine (1 mM), 5-HTP (1 mM), IAA (0·5 mM) and IAAl (1 mM) all inhibited *ca* 50% at the concentration given in brackets [176].

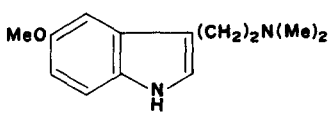
In a study of the biosynthesis of gramine (**66**) from tryptophan-[β-^{14}C] by barley shoots, it was concluded that the main site of synthesis is in the basal region of the seedling leaf [177]. The gramine is then translocated to the tip of the leaf where it accumulates. Gramine and its N_b-oxide have also been found in the flowers of *Arundo donax* (Gramineae), together with bufotenine, *N*,*N*-dimethyltryptamine and 5-methoxy-*N*-methyltryptamine [178].



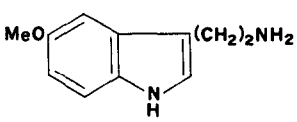
Gramine (**66**)



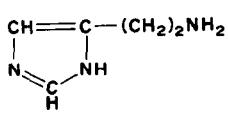
(**67**)



(**68**)



(**69**)



Histamine (**70**)

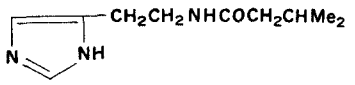
5-HT was detected in the stinging hairs of *Mucuna pruriens* (Leguminosae). In other parts of this plant, *N*,*N*-dimethyltryptamine (**67**), its N_b oxide, bufotenine and 5-methoxy-*N*,*N*-dimethyltryptamine (**68**) also occurred and explain the pharmacological properties of this plant [179]. *N*,*N*-dimethyltryptamine (**67**), and not 5-HT (**63**), was thought to be the precursor of the methylated indoles. It has been noted that when 5-HT occurs in the plant kingdom it is usually unaccompanied by other indoles, and conversely, when a variety of indoles are synthesized, 5-HT is not produced [180]. 5-HT (**63**) has also been found in the leaves, bark and cotyledons of *Elaeagnus umbellata* (Elaeagnaceae), and it was demonstrated histochemically in the lower epidermis of the leaf and in the cortex of the stem [181]. 5-HT [182] and bufotenine [183] were also found in the leaves and stems of *Urtica pilulifera* (Urticaceae). 5-HT was located mainly in the stings, in which it is probably one of the active principles. The presence of 5-HT and histamine (**70**) in the hairs of *Urtica dioica* has recently been confirmed by histochemical methods [184]. 5-HT also occurs in the venoms of octopus, social wasps, scorpions and spiders, and it is one of the most active pain inducers which occurs naturally [185]. 5-HT was found in *Albizzia julibrissin*, *Phaseolus multiflorus*, *Samanea saman*, *Pisum sativum* (Leguminosae) and *Passiflora quadrangularis* (Passifloraceae). In addition, noradrenaline (**88**) was found in all of these plants and also in *Mimosa pudica* [186].

Histamine

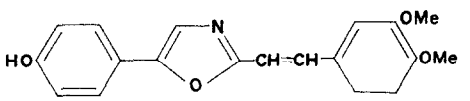
Histamine (**70**) applied to pea seedlings through the roots at 10^{-3} M increased root length and fr. wt by 35%, but at 10^{-2} M the histamine inhibited the growth of the tops [187].

In the biosynthesis of dolichotheline (**71**), an alkaloid from the cactus *Dolichothele sphaerica*, in which it comprises 1% of the dry wt, histidine is decarboxylated to histamine, which condenses with *iso*valeric acid, probably as *iso*valeryl CoA which originates from leucine [188, 189]. The

Dolichotheline (71)



Annuloline (72)



enzyme effecting this condensation is quite versatile, since several unnatural alkaloids were formed on injecting *iso*caproic acid and other precursors related to histamine into this cactus [190].

Phenethylamines and other aromatic amines

Tyrosine carboxy-lyase activity in the roots of barley seedlings was investigated in crude extracts and in extracts concentrated by ammonium sulphate precipitation (30–60% saturated) [191]. In the latter preparations using tyrosine-[U-^{14}C] as substrate, the products were shown to be tyramine and *p*-coumaric acid, indicating the presence of both tyrosine carboxy-lyase and tyrosine ammonia-lyase. DEAE cellulose chromatography was not successful in separating these enzymes. The pH optimum for decarboxylation was about 7, and addition of pyridoxal phosphate increased activity about 3-fold. Hydroxylamine and *p*-chloromercuribenzoate inhibited almost completely at 10^{-7} M. Highest activity was found in the roots of 2-day-old seedlings, and activity was insignificant 8 days after germination. The content of tyramine reached a peak on the 4th day after germination and decreased to negligible amounts on the 8th day. *N*-Methyltyramine was highest on the 5th day after germination. The enzyme was purified 25-fold and its presence demonstrated in wheat (*Triticum aestivum*) and maize (*Zea mays*) (Gramineae) [192]. In other work [193] the barley root tyrosine carboxy-lyase was purified 80-fold using ammonium sulphate precipitation, Sephadex G-50 and DEAE cellulose. The enzyme was specific for L-tyrosine and the purified enzyme yielded stoichiometric amounts of CO_2 and tyramine. The enzyme was stimulated by *S*-adenosylmethionine (opt 100 μM) and it was completely inhibited by *p*-hydroxyphenylpyruvate. The latter result indicated that the enzyme is active only when the roots are dependent on tyrosine from reserve protein, and that when tyrosine synthesis is active *via* the keto acid, tyrosine carboxy-lyase is inhibited.

Cytisus scoparius (Scots broom: Leguminosae) which contains 3,4-dihydroxyphenethylamine (dopamine, **76**) has now been shown to contain DOPA carboxy-lyase, the enzyme responsible for the formation of this amine. The optimum pH was 8 and activity at pH 7 and 9 was about 90% of the level at pH 8. $^{14}CO_2$ was formed from labelled DOPA at about 15× the rate found with tyrosine. The activity with tyrosine may be due to the hydroxylation of that amino acid followed by decarboxylation of the resulting DOPA. The enzyme decarboxylated D- or L-DOPA at about the same rates. Since the decarboxylation was O_2-dependent, it is possible that phenolase is implicated, and the high sensitivity to diethyldithiocarbamate supports this view. The amine product of the reaction was not characterized. Highest activity was found in the youngest defoliated branches, though activity was also quite high in the leaves. After prolonged dialysis in an attempt to resolve a coenzyme, pyridoxal phosphate stimulation was very small. A 20-fold purification was achieved by $(NH_4)_2SO_4$ fractionation [194].

A similar enzyme has been found in the alga *Monostroma fuscum* (Chlorophyta) in which dopamine comprises 3% of the fr. wt. Both D- and L-DOPA are decarboxylated, but neither tyrosine nor phenylalanine act as substrates. The optimum pH was between 7 and 8, and O_2 greatly stimulated activity but was not essential. Like the enzyme from *Cytisus*, Cu appears to be required for activity [195].

An enzyme isolated from the pulp of the banana fruit (*Musa sapientum*) oxidised tyramine to dopamine, an amine known to occur in the peel at high levels (6–12 μmol/g fr. wt). The tyramine hydroxylase was purified 15-fold (recovery 50%), and the optimum pH found to be 6·0. Dopamine was the main product, though at least one other product (possibly noradrenaline) was also formed. The production of unknown(s) was reduced in aged preparations. The initial lag in activity was eliminated by adding ascorbate, and also by dopamine, and the reaction is apparently autocatalytic. Tyrosine, tyramine and dopamine were oxidised by the

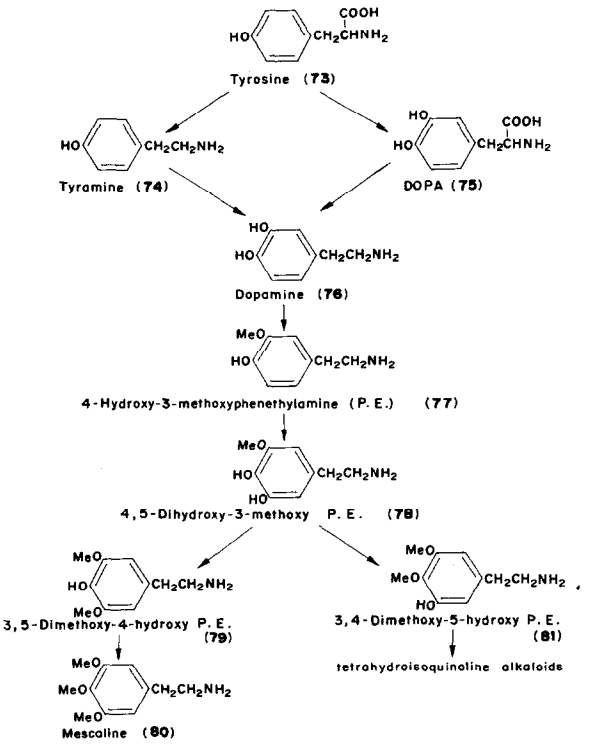
enzyme preparations [196]. A related enzyme oxidizing tyrosine to DOPA was characterized in the pulp of the banana fruit [197]. Tyramine and phenethylamine, together with 5-HT and histamine (**70**), have been detected in banana fruit by the dansyl method [27].

Varieties of sugar beet (*Beta vulgaris*, Chenopodiaceae) with high levels of dopamine are resistant to the fungal pathogen *Cercospora beticola*, and this amine increases on infection or in response to injury. The level of this amine is determined by at least 4 genes, and it appears to be difficult to breed for disease resistance on this basis [198].

Tyramine is found in raspberries (*Rubus idaeus*, Rosaceae) (0·1–0·8 μmol/g fr. wt) [199], and tyramine and phenethylamine (**82**) occur in *Prosopis alba* (Leguminosae) [165]. Tyramine has also been detected in *Phaseolus radiatus* var *typicus* [200], and dopamine occurs as its *O*-glucoside in seeds of *Entada pursaetha* (Leguminosae) [201]. Phenethylamine (**82**) has been detected in 11 species of higher plants occurring in five families [18]. *Desmodium tiliaefolium* (Leguminosae) contains tyramine and 3,4-dimethoxyphenethylamine (**92**) [162], and *D. gyrans* [169], *D. gangeticum* [202], *D. triflorum* [203] and *D. cephalotes* [204] contain phenethylamine and other *N*-methylated aromatic amines. *N*-methylmescaline has been detected in *Alhagi pseudalhagi* (Leguminosae) and this is the first recorded occurrence of a tri-oxygenated phenethylamine other than in a cactus. Phenethylamine and other *N*-methylated amines were also found. Some of the pharmacological properties of this plant may be attributed to these alkaloids [205]. *N*-methyltyramine and *N*-methylhomotyramine were detected in *Croton humilis* (Euphorbiaceae) [206].

Annuloline (**72**), an alkaloid found in *Lolium multiflorum* (Gramineae) is formed from phenylalanine and tyrosine, with tyramine as an intermediate. Tyramine condenses with caffeic acid (derived from phenylalanine) to give an oxazole ring, with subsequent methylation of the hydroxyls [207]. Tyramine (**74**), *N*-methyltyramine (**84**) and hordenine (**85**) have been detected in millet (*Panicum*, Gramineae) seed [208].

The excretion by humans of 3,4-dimethoxyphenethylamine (**92**), the "pink spot", which has been associated with schizophrenia, has also been attributed to a high intake of tea [209]. However,

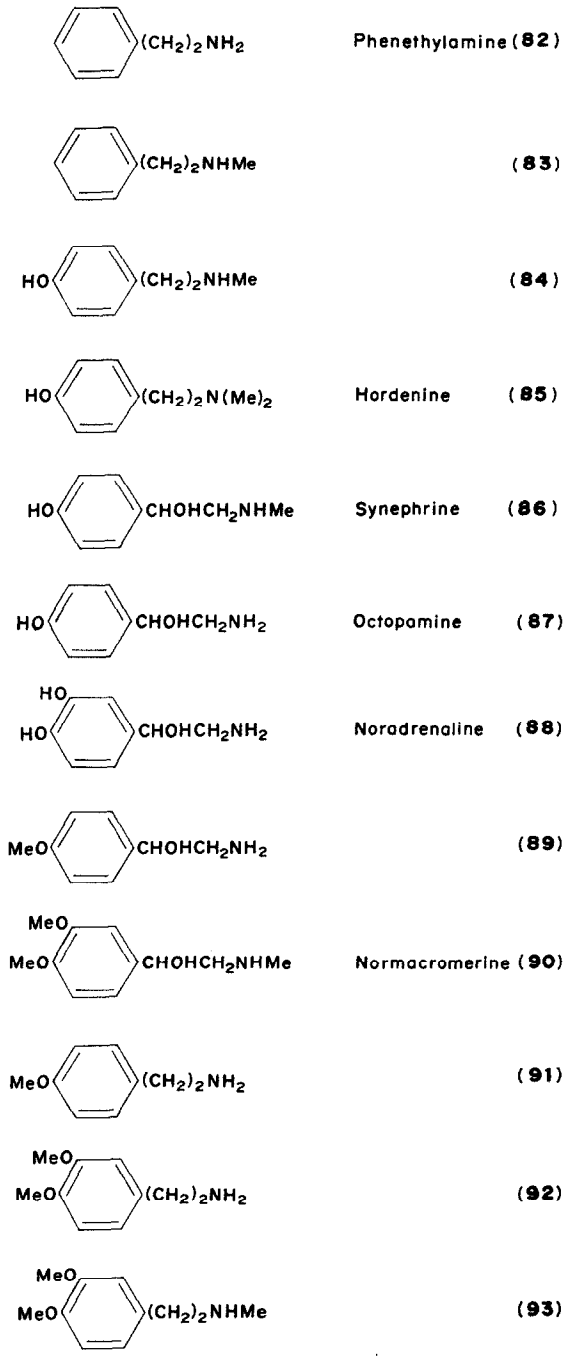


Scheme 12. Formation of mescaline in the cactus *Lophophora williamsii*.

in a recent study this substance could not be found in the urine of 13 normal or 9 schizophrenic patients, nor could it be detected in subjects having a high tea intake [210]. There is no evidence at present for the occurrence of this amine in the tea plant.

A variety of cactus species have now been shown to contain *N*-methylated and ring hydroxylated and methoxylated phenethylamines, of which perhaps the best-known is the hallucinogen mescaline (**80**) (3,4,5-trimethoxyphenethylamine) found principally in the peyote cactus *Lophophora williamsii* [211].

The work which has led to the formulation of the biosynthetic pathway for mescaline and for the phenolic tetrahydroisoquinolines in peyote (Scheme 12) is reviewed in refs. [212–216]. Phenylalanine is first hydroxylated to tyrosine (**73**) which is then either hydroxylated to give DOPA (**75**) or decarboxylated to tyramine (**74**). Dopamine (**76**), formed either from DOPA or tyramine, is further converted to 4-hydroxy-3-methoxyphenethylamine (**77**) and then to 4,5-dihydroxy-3-methoxyphenethylamine (**78**). Further *O*-methylation at



position 4 gives the precursor (**81**) of the tetrahydroisoquinoline alkaloids. Alternatively, *O*-methylation first at position 5 to give **79**, then position 4 gives mescaline (**80**). The methylation is catalysed by an *O*-methyltransferase with *S*-adenosylmethionine as donor [217]. An identical scheme has been proposed for the biosynthesis of mescaline in the Peruvian cactus *Trichocereus pachanoi* [218]. In this cactus, the amount of mescaline (0·3% of dry wt) approaches that found in the peyote cactus (1% of dry wt). 4-Hydroxy-3-methoxyphenethylamine (77) was the major phenolic alkaloid [219]. However, in *Pelecyphora aselliformis* which contains 3,4-dimethoxyphenethylamine (**92**), together with *N*-methyl-3,4-dimethoxyphenethylamine (**93**) and *N*-methylmescaline, the mescaline content was only 0·00002% of the dry wt [220].

In *Echinocereus merkeri* both tyrosine and dopamine are incorporated into 3,4-dimethoxyphenethylamine (**92**). 4-Hydroxy-3-methoxyphenethylamine (77), which has been detected in this cactus, is more likely to be a natural intermediate than 3-hydroxy-4-methoxyphenethylamine which is absent [221]. 4-Methoxy-β-hydroxyphenethylamine (**89**) was recorded for the first time as a natural product in species of *Coryphantha* together with 4-methoxyphenethylamine (**91**) [222]. All species of *Coryphantha* studied contained *N*-methylated phenethylamines, including hordenine (**85**), *N*-methyltyramine (**84**) and synephrine (**86**) [222, 223]. Normacromerine (**90**), which is the major alkaloid of *Coryphantha macromeris* var. *runyonii* is formed from tyramine (**74**), either *via* octopamine (**87**) or *N*-methyltyramine (**84**) [224]. Tyramine was detected in this cactus, in addition to several *N*-methylphenethylamines [225]. Tyramine has also been found in cacti of the genera *Cereus*, *Echinopsis*, *Gymnocalycium*, *Mammillaria*, *Obregonia*, *Opuntia*, *Stetsonia* and *Trichocereus* [226–232]. In many cases *N*-methyltyramine, hordenine and other related *N*-methylated amines were also found.

N-methyltyramine (**84**) and *N*-methylphenethylamine (**83**) occur in *Gymnocactus* spp. [233], and together with synephrine (**86**) in *Dolichothele surculosa* [234] and in *D. sphaerica* [235]. *N*-Methyl-3,4-dimethoxy-phenethylamine (**93**) was found in 3 *Ariocarpus* spp [236–238], though this was not detected in *A. kotschoubeyanus* in which only hordenine (**85**) and *N*-methyltyramine (**84**) could be demonstrated [239].

Glucosinolates and glucosides of *o*-hydroxybenzylamine, *p*-hydroxybenzylamine, benzylamine and 3,4-dihydroxyphenethylamine are present in various members of the Moringaceae, Resedaceae and Leguminosae [240]. Benzylamine has been

detected in *Sinapis alba* (Cruciferae) [18] and *p*-hydroxybenzylamine occurs in whole ungerminated barley seed [241].

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