'UNCOMMON' AMINO ACIDS IN PLANTS

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1. Introduction

For the purposes of this letter I shall refer to those 25–30 amino acids regularly encountered in living organisms as protein constituents (e.g. alanine and lysine) or as metabolic intermediates (e.g. ornithine and homoserine) as the 'common' amino acids and the remainder which are much more numerous, but enjoy a more restricted distribution, as the 'uncommon' amino acids. For the sake of simplicity I have used the term amino acid to include proline and the other imino acids discussed.

While the *N*-methylated lysines and arginines of mammalian protein and the halogenated aromatic amino acids of the molluscs provide examples of 'uncommon' amino acids in the animal kingdom, most compounds of this class have been isolated from micro-organisms and plants. In micro-organisms 'uncommon' amino acids have long been known as components of bacterial cell walls and are frequently encountered in the bound form as constituents of small polypeptides of the 'antibiotic' type. The 'uncommon' amino acids of plants, however, of which over 200 have been isolated [1], are usually found in the free state or as simple condensation products such as the γ-glutamyl, acetyl and oxalyl derivatives. They have been found in both lower and higher plants and the rate at which they are being discovered shows no sign of decreasing.

Relatively little is known of the biosynthesis and metabolism of most of the 'uncommon' plant amino acids and little effort has yet been made to determine their role or roles in the plants which synthesise them. In this necessarily restricted survey I have attempted to present data which give some indication of how these compounds may have arisen, how their distribu-
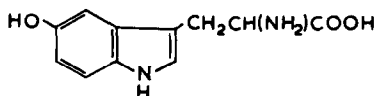
tion is of significance in studying plant evolution and how their accumulation may confer a selective advantage on the species in which they are found. Their roles as potential toxins or nutrients in man and higher animals are also mentioned.
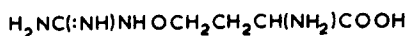
2. The accumulation of 'uncommon' amino acids

Plants which synthesise 'uncommon' amino acids frequently accumulate them in very high concentrations. This is well illustrated by the amino acid content of certain legume seeds. 5-Hydroxy-L-tryptophan (I) can account for 14% of the seed weight of *Griffonia simplicifolia* [2], canavanine (II) for 7–10% of the seed weight of *Dioclea megacarpa* [3] and L-3,4-dihydroxyphenylalanine (III) for more than 8% of the seed weight of *Mucuna mutisiana* [4]. High concentrations of 'uncommon' amino acids are not only found in seeds however and the leaves of the legume *Leucaena leucocephala* [5] contain 8% (dry wt.) of mimosine (IV) while the shoots of one Lilliaceous species *Convallaria majalis* and the rhizomes of another, *Polygonatum multiflorum* [6] contain respectively over 3% and 6% of azetidine-2-carboxylic acid (V).

No general rules can be made concerning the stage or stages at which 'uncommon' amino acids are synthesised during the life cycles of plants, nor concerning the organs in which they are accumulated. Canavanine stored in the seeds of *Medicago sativa* disappears rapidly during germination [7] while albizine (VI) which is found in high concentrations in the seeds of *Albizia julibrissin* is also found as a major component of the free amino acid pool in the developing seedlings of this species (unpublished
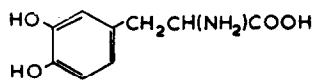
observation). Azetidine-2-carboxylic acid (V), already mentioned as a constituent of *Convallaria* and *Polygonatum,* is also found as the major free amino acid in seeds of the legume *Bussea massaiensis,* while in another legume, *Delonix regia,* this imino acid is absent from the seed but can be detected in the developing seedlings [8].
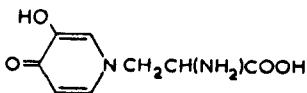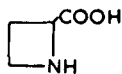

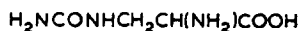HO⎯CH$_2$CH(NH$_2$)COOH

[I]

H$_2$NC(:NH)NH OCH$_2$CH$_2$CH(NH$_2$)COOH

[II]

HO⎯CH$_2$CH(NH$_2$)COOH
HO

[III]

HO⎯N CH$_2$CH(NH$_2$)COOH
O=

[IV]

COOH
NH

[V]

H$_2$NCONHCH$_2$CH(NH$_2$)COOH

[VI]

3. Biosynthetic origins

Structurally the 'uncommon' amino acids can be divided into two categories; those which are close chemical analogues of the 'common' amino acids and those which are not. There are three possible ways in which the close analogues may arise: they may be formed by the modification of 'common' amino acids; they may arise as a result of modifications to the biosynthetic pathways normally associated with the synthesis of 'common' amino acid; or they may be synthesised by novel routes. Examples of 'uncommon' amino acids arising in each of these ways are known. Modifications of either the 'common' amino acids themselves or the biosynthetic pathways leading to them may reflect relatively minor changes in the genomes of species already equipped with enzymes required for the synthesis of a full complement of 'common' amino acids. When however an 'uncommon' amino acid is structurally unlike any 'common' amino acid or when it is formed by an unexpected or novel route then its presence probably reflects more extensive changes in the genes controlling amino acid biosynthesis.

3.1. *Modifications of 'common' amino acids*

The hydroxylation of tryptophan to form 5-hydroxyl-L-tryptophan (I) in *Griffonia simplicifolia* [9], the acetylation of ornithine to δ-acetylornithine (VII) in *Onobrychis viciifolia* [10] and the formation of O-oxalylhomoserine (VIII) from homoserine and oxalylcoenzyme A in *Lathyrus sativus* [11] provide examples of ways in which 'common' amino acids can be modified to form 'uncommon' ones.
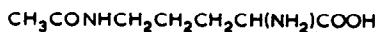
3.2. *Modifications of 'common' pathways*

Examples of ways in which 'uncommon' amino acids may have arisen by modifications of general biosynthetic pathways leading to 'common' amino acids are provided by the *m*-carboxy-substituted aromatic amino acids, in species of *Reseda* and *Iris* [12]. The biosynthetic pathways to 3-(3-carboxyphenyl)alanine (IX) to 3-(3-carboxy-4-hydroxyphenyl) alanine (X) to phenylalanine and to tyrosine follow a common route from shikimic acid to chorismic acid. In the formation of phenylalanine and tyrosine however, chorismic acid undergoes rearrangement to prephenic acid which then gives rise to tyrosine, or,

alternatively, to phenylalanine if the hydroxyl group is lost. In the formation of the corresponding *m*-carboxy compounds, chorismic acid is transformed successively to isochorismic acid and then to isoprephenic acid which, unlike prephenic acid itself, can give rise to an aromatic system without the loss of the original carboxyl group of shikimic acid.
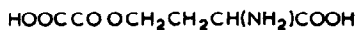
3.3. *Novel pathways*

The transfer of a methyl group from a donor such as methionine to glutamic acid is the simplest reaction which one can envisage for the biosynthesis of *erythro*-γ-methylglutamic acid (XI). In *Gleditsia triacanthus,* however, this substituted glutamic acid is formed not from glutamic acid but from L-leucine by the oxidation of a methyl group [13].
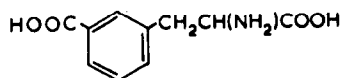
The nearest analogues which lathyrine (XII) has among the 'common' amino acids are histidine, tryptophan and phenylalanine. In the biosynthesis of each of these however the formation of the aromatic nucleus precedes the addition or completion of the alanine side chain. The biosynthesis of lathyrine is a fundamentally different process which involves the cyclisation and subsequent removal of hydrogen from γ-hydroxyhomoarginine (XIII). A novel route not only to an aromatic amino acid but also to the pyrimidine nucleus [14].
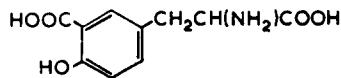
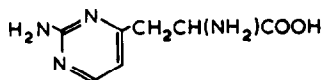$$CH_3CONHCH_2CH_2CH_2CH(NH_2)COOH$$

[VII]

$$HOOCCOOCH_2CH_2CH(NH_2)COOH$$

[VIII]

[IX]

[X]

$$HOOCCH(CH_3)CH_2CH(NH_2)COOH$$

[XI]

[XII]

$$H_2NC(:NH)NHCH_2CH_2CH(OH)CH_2CH(NH_2)COOH$$

[XIII]

4. Phylogenetic significance of 'uncommon' amino acids

As the 'common' amino acids are present in all living organisms their use as taxonomic 'markers' or in establishing phylogeny is limited. A difference in the concentrations of a 'common' amino acid in two species may reflect a genuine genetic difference between them, but variations in environmental factors or in the stages of the plants' development may make of such a quantitative difference extremely difficult to interpret unless elaborate precautions to ensure uniform conditions of growth are maintained.

The presence of an 'uncommon' amino acid, which is restricted to a few limited species themselves related in other respects, provides a much less equivocal indication that the species concerned have evolved from a common ancestral form. If several such

'uncommon' amino acids, which are unrelated bio-synthetically, are common to these species the simultaneous presence of these compounds provides even more certain evidence of common ancestry, for while it is possible to visualise one 'uncommon' biosynthetic pathway arising independently in different species, the only likely explanation of a common pattern of several independent unusual pathways in a number of species is that all these species are descended from a common ancestor in which these pathways were already established.

Fowden has shown that certain 'uncommon' amino acids, which were thought at one time to be restricted to a few plant families, are widely distributed in the plant kingdom as trace components [15]. It appears, nevertheless, that the ability of a plant to synthesise and accumulate an 'uncommon' amino acid in readily detectable concentrations is a genetically controlled characteristic and the occurrence of such compounds in different species can provide clear evidence of an evolutionary relationship.

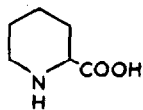### 4.1. *Distribution of 'uncommon' amino acids*

Studies of amino acids in the seeds of the Leguminosae have provided a number of examples of taxa which can be characterised by the accumulation of one such 'uncommon' amino acid or by the accumulation of an association of such compounds. The genus *Griffonia* contains four species, it has been possible to obtain seeds of three of these. In all three, 5-hydroxy-L-tryptophan (I) is the major free amino acid, no species from the other 270 genera of the Leguminosae which we have examined to date contains detectable concentrations of this amino acid. It is probable then that this small West African genus is unique biochemically with respect to its ability to synthesise and accumulate this compound [2]. Similarly all the species of *Mucuna* which have been examined and no species from other genera contain high concentrations of L-3,4-dihydroxyphenylalanine [4]. Examples of associations of 'uncommon' amino acids which characterise certain plant taxa are pipecolic acid (XIV) and γ-glutamyl-*S*-methylcysteine (XV) which are found together in many species of *Phaseolus* and homoarginine (XVI) and α-amino-β-oxalylaminopropionic acid (XVII), which occur in characteristic association in a number of species of *Lathyrus* [16]. It is possible to use the presence or

absence of such compounds or associations of compounds in the same way that one uses the presence or absence of morphological characters to establish relationships between species. On the other hand if the biosynthetic relationships between different 'uncommon' amino acids of the same species and related species can be established, then we may obtain a clearer idea of the degree of relationship in terms of evolutionary history. If an observed difference in amino acid pattern between species involves many differences in their biosynthetic pathways, then it is likely that the species diverged much earlier than would be the case if the difference in pattern could be attributed to changes in one or a few enzyme systems. This type of approach has been used in studying relationships within *Lathyrus* and *Vicia* [16].
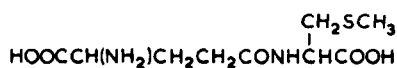
4.2. *Discrimination against 'uncommon' amino acids*

As far as we know none of the 'uncommon' amino acids found in plants are incorporated into proteins by the plants which synthesise them. If however an 'uncommon' amino acid is introduced into species to which it is normally foreign incorporation into protein may occur. The protein of *Convallaria majalis* does not contain residues of azetidine-2-carboxylic acid (V) which occurs in high concentrations in the free state in this plant. If however azetidine-2-carboxylic (V) acid is introduced into *Phaseolus aureus*, replacement of proline by its lower homologue occurs in the protein of the bean. The proline tRNA synthetase of *Phaseolus aureus* unlike that of *Convallaria majalis* is unable to discriminate against azetidine-2-carboxylic acid [17]. In experiments designed to test the effect of 3-hydroxymethylphenylalanine (XVIII) and 3-hydroxymethyl-4-hydroxyphenylalanine (XIX) on root uptake of phenylalanine and tyrosine it was found [1] that these two 'uncommon' amino acids antagonised the uptake of phenylalanine and tyrosine less in *Caesalpinia tinctoria,* the species from which they were isolated, than in other species which do not synthesise them.
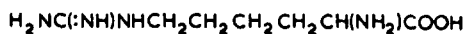
These and similar results suggest that the ability of some species to discriminate against 'uncommon' amino acids, no less than the ability of some species to synthesise and accumulate them, may provide valuable evidence as to the similarities and differences in enzyme systems of nearby and distantly related species or taxa.
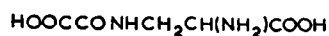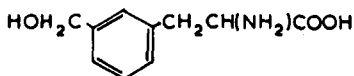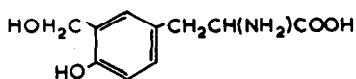
[XIV]

CH_2SCH_3

HOOCCH(NH$_2$)CH$_2$CH$_2$CONHCHCOOH

[XV]

H$_2$NC(:NH)NHCH$_2$CH$_2$CH$_2$CH$_2$CH(NH$_2$)COOH

[XVI]

HOOCCONHCH$_2$CH(NH$_2$)COOH

[XVII]

HOH$_2$C—⟨benzene⟩—CH$_2$CH(NH$_2$)COOH

[XVIII]

HOH$_2$C—⟨benzene⟩—CH$_2$CH(NH$_2$)COOH
HO

[XIX]

cation of α-amino-β-oxalylaminopropionic acid (XVII) of *Lathyrus sativus* as the cause of classical human lathyrism in the Indian sub-continent [18,19]; the effects of mimosine (IV) from *Leucaena leucophala* as a depilatory agent [20], and of indospicine (XX) from *Indigofera spicata* as a teratogen and hepato-toxin in sheep [21] are well known, as are the effects of the seleno amino acids of certain *Astragalus* species (the 'loco weeds' of the American West) on horses [22]. It is indeed to be expected that some at least of these close chemical analogues of the 'common' amino acids will compete with the corresponding 'common' amino acids at one or more enzyme sites if introduced into species to which they are normally foreign. Not all 'uncommon' amino acids are harmful to mammals however and L-homoarginine (XVI) of *Lathyrus* and *Lotus* species will support the growth of rats on a lysine-free diet as lysine is liberated from the 'uncommon' guanidino amino acid by the action of arginase. The possible value of other 'uncommon' amino acids as replacements for 'essential' amino acids is an area which remains to be investigated. In contrast to its role as a nutrient in mammals, L-homoarginine has been found toxic to algae [23], bacteria [24] and yeasts [24] suggesting possible uses for 'uncommon' amino acids in medicine.

The massive scale on which some plant species synthesise 'uncommon' amino acids such as L-3,4-dihydroxyphenylalanine and L-5-hydroxytryptophan (which are precursors of physiologically active amines in man) is also of interest, and may well account for the reputations enjoyed by certain of these species among primitive peoples.

H$_2$NC(:NH)CH$_2$CH$_2$CH$_2$CH$_2$CH(NH$_2$)COOH

[XX]

6. Possible ecological significance of 'uncommon' amino acids

One of the most interesting questions about 'uncommon' amino acids is, 'Why do plants make them at all?' A plant which diverts as much as 10% of its resources, biosynthetic capacity and storage space to the accumulation of a compound which is not

5. Physiological effects of 'uncommon' amino acids

Interest in the physiological activity of 'uncommon' amino acids has been primarily an interest in their effects on man and his domestic animals. The impli-

required for its primary metabolic processes is not going to survive in competition with seemingly less prodigal plants in the same environment unless the presence of the 'uncommon' amino acid confers some selective advantage on the plant which contains it.

Some clues as to possible advantages which 'uncommon' amino acids may confer on the plants which synthesise them have been provided in the previous sections, where reference has been made to the toxicity of various of these compounds to man, domestic animals, other plants, fungi, bacteria, and yeasts.

Most plants lack mobility and are unable either to escape from potential predators by 'running away' or to drive out potential competitors by physical means. Consequently, plants have evolved diverse and sophisticated chemical mechanisms both to protect themselves and to discourage competition from other species. I suggest that many if not all the 'uncommon' amino acids accumulated in plants are weapons in this chemical armoury.

6.1. Plant animal relationships

The toxicity of some 'uncommon' amino acids to higher animals (including man) has already been referred to. Others for which information is available include α,γ-diaminobutyric acid (XXI) from *Lathyrus* species [25] which exerts its toxicity in mammals by inhibiting the action of the urea cycle enzyme ornithine transcarbamylase [26] . β-Cyanoalanine (XXII) from *Vicia* species which acts as a cystathionase inhibitor [27] and canavanine which has been shown to be toxic to mice when fed at concentrations of 200 mg/kg mouse [28] .

6.2. Plant–insect relationships

Insect predators exercise a major selectionary pressure on plants, and mutational changes leading to the synthesis of compounds which are toxic or repellent to insects in a particular environment will increase a plant's 'fitness' in that environment. It is therefore of interest to note that canavanine (II) and β-hydroxy-γ-methylglutamic acid (XXIII) from *Gymnocladus dioicus* are repellent to the larvae of *Prodenia eridania* (the southern army worm) while 5-hydroxytryptophan and 3,4-dihydroxyphenyl-alanine are toxic to them [29,30] . Preliminary results also indicate that these and other 'uncommon'

amino acids are toxic to the larvae of bruchid beetles which feed on legume seeds (Janzen, D. H., personal communication).

6.3. Plant–plant interrelationships

The toxicity of azetidine-2-carboxylic acid to *Phaseolus aureus* has already been referred to [17] and it has been known for many years that canavanine is toxic to some higher plants [24] . More recently [31] N-dimethyltryptophan (XIV) has been isolated from seeds of *Abrus precatorius* and this newly discovered amino acid has been found to inhibit the growth of lettuce seedlings. It is possible then that some 'uncommon' amino acids may also act as plant-growth inhibitors in a natural environment. For example, a free amino acid leached from the seed of one species may provide that species with a selective advantage by inhibiting seed germination or seedling growth in competitive species within its immediate environment.
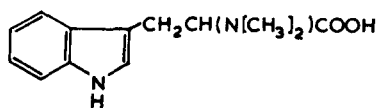
$$H_2NCH_2CH_2CH(NH_2)COOH$$

[XXI]

$$NCCH_2CH(NH_2)COOH$$

[XXII]

$$HOOCCH(CH_3)CH(OH)CH(NH_2)COOH$$
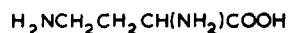
[XXIII]

[XXIV]

7. Conclusion

It is hoped that this brief review may have drawn attention to the importance of 'uncommon' plant amino acids, not only to the plant biochemist but also to others working in diverse fields of biology and medicine.

Acknowledgements

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