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PROCEEDINGS OF THE PHYTOCHEMICAL SOCIETY

A meeting of the Society was held at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, on 11 and 12 September 1969 when the following papers were presented, under the general title

NON-PROTEIN AMINO ACIDS, PEPTIDES AND ANALOGOUS METABOLITES IN HIGHER PLANTS AND FUNGI

General Survey of Amino Acid Types in Higher Plants

P. OLESON LARSEN, Royal Veterinary and Agricultural College, Copenhagen, Denmark.

The number of amino acids from higher plants known today is more than 200 and is rapidly increasing. An exact enumeration, however, requires a precise definition of the term "amino acid", a definition which has not yet been given and which may not be very practicable. Whereas the free natural amino acids first discovered were those also occurring generally in proteins ("protein amino acids") most of the amino acids found in recent years do not enter into proteins.

The amino acids contain both acidic and basic groups. The resulting common chemical properties are exploited in the procedures used for qualitative and quantitative determination and for isolation of the amino acids and this is one of the main reasons for the treatment of the group of amino acids as an entity. In this connexion, the traditional chemical classification of the amino acids into acidic, neutral, or basic is useful. The large variations in side-chains and additional functional groups mean, however, that there are large differences in many of the chemical properties of the amino acids.

Whereas most alkaloids are large and complicated molecules, the naturally occurring non-protein amino acids, like the protein amino acids, are usually small molecules with 4, 5, or 6 carbon atoms. Yet the structural variability within these limits is great. Biosynthetic considerations can be used for the grouping of the amino acids. Some of the guiding principles for such a classification are the carbon skeleton, the positions of the functional groups and finally the nature of the functional groups. A very large part of the amino acids have carbon skeletons identical with those of protein amino acids. The extent to which structural similarities reflect biogenetic relationships can only be ascertained experimentally.

Many non-protein amino acids are intermediates in the synthesis and degradation of protein amino acids and other compounds. Others serve as storage and transport compounds. Supposedly, some non-protein amino acids are formed by the action of enzymes with low specificity, normally producing protein amino acids. Also the production of amino acids by non-enzymatic processes may take place in some plants. In a few cases, amino acids are known to be end-products of metabolism. However, in most instances, information about the biological role of non-protein amino acids is lacking.

The free amino acid contents of plants vary not only from one species to another but also among organs of the same species. Variation occurs also with variations in external conditions as well as during growth. Free amino acids are also not evenly distributed within a given

cell but are located in distinct pools giving different possibilities for metabolic transformations.[1]

Some of the amino acids from higher plants also occur in micro-organisms, especially in peptide antibiotics, or in animals, but most of them have not been observed elsewhere in nature. Many reviews have appeared on the free plant amino acids, only the most recent being cited here.[1–4]

References

[1] F. C. STEWARD and D. J. DURZAN, in *Plant Physiology* (edited by F. C. STEWARD), Vol. IV A, p. 379, Academic Press, New York and London (1965).
[2] A. I. VIRTANEN, *Phytochem.* **4**, 207 (1965).
[3] E. A. BELL, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 195, Academic Press, London (1966).
[4] L. FOWDEN, *Ann. Rev. Plant Physiol.* **18**, 85 (1967).

Unusual Amino Acids of the Sapindaceae: Biosynthesis and Analogue Function

L. FOWDEN, J. W. ANDERSON and MAY-LIN SUNG, Botany Department, University College, London.

This family of plants, together with the related *Aesculus* genus, are characterized by a group of unusual amino acids, based on branched-chain C_6 and C_7 structures, which accumulate in their seeds. Twelve amino acids and two related γ-glutamyl peptides have been identified to date. Several of the acids contain ethylenic or acetylenic linkages whilst others include cyclopropane ring systems in their structures. Isoleucine may represent the common biogenetic precursor of many of these compounds, being converted initially into homoisoleucine during the elaboration of the C_7 group of amino acids, which include the toxic β-(methylenecyclopropyl) alanine (hypoglycin A) of *Blighia sapida* and 2-amino-4-methylhex-4-enoic acid (Amha) from *A. californica*.

The possibility that certain of these new compounds act as analogues of the protein amino acids, leucine, isoleucine or phenylalanine has been examined, especially in relation to specific aminoacyl-sRNA synthetase activities. Synthetase preparations, obtained from four different *Aesculus* species, have been compared in respect of their amino acid substrate specificities. Amha was accepted as a substrate by all four phenylalanyl-sRNA synthetase preparations, but species differences were apparent in K_m values and $V_{\text{Amha}}/V_{\text{Phe}}$ ratios. Hypoglycin A was activated by leucyl-sRNA synthetase and acted as a competitive substrate when supplied together with leucine.

Sulphur and Selenium containing Amino Acids

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There are a number of toxic plants confined to seleniferous soils from which they characteristically accumulate up to 15,000 ppm selenium.[1] Selenium in these plants occurs predominantly in the soluble fraction from which two principal seleno-amino acids have been isolated, namely Se-methyl-L-selenocysteine and L-selenocystathionine,[2,3] which have been implicated as the causal agents in this toxicity.[4] The former compound has been shown to be present in six accumulator species of the legume genus *Astragalus*, e.g. *A. bisulcatus* (Hook.) Gray (Papilionaceae) as well as in *Haplopappus fremontii* Gray subsp. *wardii* (Gray) Hall (syn.

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Oonopsis condensata A. Nels.) (Compositae) and in *Stanleya pinnata* var *bipinnata* (Greene) Rollins (Cruciferae) while the latter compound has been isolated from the legume *Neptunia amplexicaulis* Domin. (Mimosaceae) and from *Lecythis ollaria* Piso (Myrtales) and *S. pinnata*. No decisive chemotaxonomic conclusions emerged from a study of the selenium-accumulating *Astragalus* spp. for they fell into six of the twenty-nine morphologically based sections of the genus, while in four of these, non-accumulators also occurred.

Low levels of selenium are usually toxic to non-accumulator plants. Those examined synthesized L-selenomethionine which is largely incorporated into proteins[5,6] We have postulated that the exclusion from protein accounts for the tolerance of selenium-accumulators to high levels of selenium.[3] Current biosynthetic pathways leading to the formation of *S*-methyl-L-cysteine and L-cystathionine in plants will be outlined and comparisons made with the biosynthesis of the selenium analogues.

Another seleno-amino acid is also encountered in some plants, namely Se-methyl-L-selenomethionine. This compound is characteristic of several pasture plants[6] and also occurs in non-accumulator *Astragalus* spp., while it is absent or present in trace amounts only, in selenium-accumulator *Astragalus* spp.[7] The occurrence of various selenopeptides and other selenium-containing amino acids will be described.

A considerable number of sulphur-amino acids have been isolated and characterized in recent years from various plant genera and these will be listed in structurally related series. Chemotaxonomic studies in various legumes containing L-djenkolic acid and its *N*-acetyl- and γ-glutamyl-derivatives, S-(2-carboxyethyl)-L-cysteine and S-(carboxyisopropyl)-L-cysteine and their sulphoxides, and dichrostachinic acid will be described[8] and compared with the distribution of other S-substituted cysteines, their sulphoxides and γ-glutamyl-derivatives in *Allium* spp. and *Brassica* spp.[9]

References

[1] ROSENFELD and O. A. BEATH, in *Selenium*, Academic Press, New York (1964).
[2] A. SHRIFT and T. K. VIRUPAKSHA, *Biochem. Biophys. Acta* **100**, 65 (1965).
[3] P. J. PETERSON and G. W. BUTLER, *Nature (Lond.)* **213**, 599 (1967).
[4] L. ARONOW and F. KERDEL-VEGAS, *Nature (Lond.)* **205**, 1185 (1965).
[5] G. W. BUTLER and P. J. PETERSON, *Australian J. Biol. Sci.* **20**, 77 (1967).
[6] P. J. PETERSON and G. W. BUTLER, *Australian J. Biol. Sci.* **15**, 126 (1962).
[7] T. K. VIRUPAKSHA and A. SHRIFT, *Biochim. Biophys. Acta* **107**, 69 (1965).
[8] A. S. SENEVIRATNE and L. FOWDEN, *Phytochem.* **7**, 1039 (1968).
[9] A. I. VIRTANEN, *Phytochem.* **4**, 207 (1965).

Acyl Amino Acids and Related Compounds

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The occurrence of acyl amino acids in plants is reviewed. Those known so far are mainly acetyl, oxalyl and malonyl derivatives. The widespread occurrence of amines acylated with aromatic acids suggests that more amino acids thus acylated will be found. Special mention is made of material not included in the review of Synge.[1]

When plant material is fractionated with a view to isolation of acyl amino acids as a group, complicated mixtures of acidic substances which yield amino acids on hydrolysis are invariably found. Besides acylation, coupling of amino acids with carbonyl compounds and with quinones[2] can give rise to such products. This last reaction is important when plant material is wilted, in the curing of tea and tobacco and in humic acid formation. The extent

to which such reactions occur in the living plant and during extractions of fresh plant material is a problem deserving of more detailed study.

References

[1] R. L. M. SYNGE, *Ann. Rev. Plant Physiol.* **19**, 113 (1968).
[2] W. S. PIERPOINT, *Biochem. J.* **112**, 609, 619 (1969).

Non-Protein Amino Acids in Crown-Gall Tumor Tissue

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Among the various tumors known on plants, such as virus tumors, genetic tumors, the crown-gall tumors have been the most extensively studied, since they provide a very useful model to investigate the tumor transformation phenomenon.

In the case of crown-gall, the transformation is easily brought about by inoculation of a specific bacterium, *Agrobacterium tumefaciens*; 70 hr after inoculation, the tissue is entirely transformed and shows considerable changes in its biochemical properties. It has been shown that the crown-gall tissue, in contrast to normal tissue, is able to grow *in vitro* without any growth regulator. The terminal oxidases seem to be different. The arginase activity is considerably lower and finally new amino acids are synthesized and accumulate in the tissue.

The first one, discovered by Lioret and investigated by K. Biemann, C. Lioret, J. Asselineau, E. Lederer and J. Polonsky, appears to be the condensation product of lysine and pyruvate; it has been called lysopine.

The use of a specific reagent for monosubstituted guanidines, such as the Sakaguchi or Rosenberg reagent, has shown that crown-gall tumor tissue of almost any species of plant, either primary tumor *in situ* or tumor tissue cultivated *in vitro*, always contains one or several guanidines never found in normal tissue. The first isolated appeared to be octopine, the condensation product of arginine and pyruvate, already known in some invertebrates. Besides octopine, we have found in these tumors its deaminination product, octopinic acid. The tumors induced by some other strains of *Agrobacterium* do not contain octopine, but another unknown guanidine that we extracted and determined as the condensation product of arginine and α-keto-glutaric acid. This amino acid has been called nopaline, since it was extracted first from a cactus tumor (*Opuntia vulgaris*) whose French name is nopal.

We have found that the various strains of *Agrobacterium* show rather strict specificity as far as the induction of non-protein amino acids is concerned: some, such as A_6, B_6, always induce octopine and lysopine synthesis in bacteria free tumor tissue, others always nopaline. The strains inducing octopine are able to metabolize this compound but not nopaline and vice versa. The biosynthesis of these new amino acids has been investigated in tissue cultures with labelled precursors.

The significance of these metabolic changes during tissue transformation will be discussed.

Biologically-Active Cyclodepsipeptides

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The continued search for new antibiotics during the last 30 years has been largely responsible for the studies that have led to the characterization of natural cyclodepsipeptides. The

class now includes members ranging in complexity from a compound with one α-amino- and one α-hydroxy-acid residue in a six-membered ring to macrocyclic structures with a variety of α- or β-hydroxyacid units combined with unusual, as well as common, amino acids of both D- and L-configuration. The unusual amino acids include several which have not been observed in other classes of natural products. In this survey the characteristics of the structures of the various main types of natural cyclodepsipeptides will be reviewed.

The methods used for the elucidation of structures of cyclodepsipeptides will be illustrated by reference to the monamycins, a new family of antibiotics produced by a *Streptomyces* species. These compounds contain one hydroxyacid residue and five amino acid residues per molecule. The application of partial hydrolysis, mass spectrometry and nuclear magnetic resonance techniques to the determination of structure will be discussed. The chemistry of novel constituent amino acid residues, piperidazine-2-carboxylic acid together with 5-hydroxy- and 5-chloro-derivatives will be mentioned.

The synthesis of particular cyclodepsipeptides has been achieved by twinning, acyl insertion and, most commonly, cyclization of linear systems, which have themselves been derived by modern methods of peptide synthesis. These methods will be briefly reviewed as will studies on simple synthetic model systems which have contributed to knowledge of the stereochemical factors influencing the cyclization process. The stereochemistry of the ring systems themselves will be discussed.

In addition to the case of monamycin, there are various examples of families of cyclodepsipeptides being produced by micro-organisms. The individual members differ only with respect to particular amino acid and hydroxyacid side-chains; the ring system of each member is the same. This, the common occurrence of "non-protein" amino acids, and some studies on the biosynthesis of cyclic peptides have led to the suggestion that the biosynthesis of cyclodepsipeptides and cyclic peptides proceeds by a pathway different from that of proteins. These aspects of the biosynthesis of cyclodepsipeptides will be reviewed.

Peptides of the Higher Fungi

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As far as we know in the kingdom of higher fungi, peptides have only been isolated from *Amanita* species and even here this class of substances seems to accumulate only in *A. phalloides* and *A. verna.* In the former there are present together with some common amino acids and uncommon ones (which have not been specified) mainly cyclic peptides. From several dozens of biologically inert cyclopeptides, Phallin A has been isolated in pure form. It is a cyclohexapeptide, c-Gly-Pro-Val-Phe-Phe-Ala (exclusively L-enantiomers). The toxic components of *A. phalloides* (and *A. verna*) are bicyclic peptides whose peptide rings consisting of seven (phallotoxines) or eight (amatoxines) amino acids are divided into two parts by a bridge originating from an oxidative coupling of a cysteine SH with an adjacent tryptophan-indole nucleus. Other uncommon features of these phytotoxines are γ-hydroxylated amino acids of the leucine type (phallotoxines) and isoleucine type (amatoxines). The stereochemical structure of all of them has been elucidated.

In order to learn something about structure toxicity relationships the molecule of phalloidin has been altered chemically at its periphery in different ways. There is a wide tolerance as to the nature of the side-chains, but not of the shape of the bicyclic system. Opening of one of the rings will destroy the toxic effect. In contrast to these findings, in the amanitin family

already the presence of a γ-hydroxyl group in a side-chain is essential; amanullin, an amanitin-like bicyclic peptide of *A. phalloides* which differs only by absence of this γ-OH-group is non-toxic.

Finally, a cyclopeptide has been discovered in *A. phalloides*, which is able to counteract the toxic action of the mushroom venoms in the white mouse. Antamanid, as it has been named, has the structure of c-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro-Phe-Phe (L-amino acids). This macrocycle seems to form a rather stable complex with Na^+ ions as can be deduced from i.r., mass spectroscopic, and ORD studies.

Alcaloides Peptidiques

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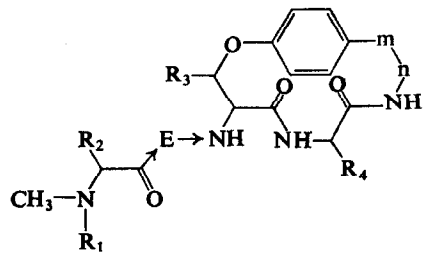
La plupart des alcaloides peptidiques répondent à un schéma commun, comportant un cycle à 14 chaînons, dans lequel m-n- —CH=CH—, cas le plus fréquent ou —CHOH—CH_2— (pandamine), out —CO—CH_2— (hymenocardine).



$R_1 = H$ ou CH_3, le caractère basique de l'alcaloide étant dûe au groupe libre méthylamino ou diméthylamino d'un acide aminé. R_2, R_3 et R_4 sont des restes aliphatiques ou aromatiques correspondant aux acides aminés les plus divers. E représente un amino-acide et n'est pas toujours présent. Un autre type est represénté par les alcaloides peptidiques "ouverts", tels que la zizyphine et la lasiodine A. Les méthodes de détermination des structures de ces composés mettent en oeuvre des techniques d'hydrolyse et surtout la spectrométrie de masse.

Pharmacologically Active Polypeptides from Plants of the Loranthaceae

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The European Mistletoe, *Viscum album* L., contains several small, basic polypeptides with a molecular weight around 5000. These substances were first isolated as a mixture which was given the name Viscotoxin. By chromatography on cellulose phosphate and sulpho-ethyl Sephadex the pure substances Viscotoxin A2, Viscotoxin A3 and Viscotoxin B have been isolated. Parenterally administrated, these substances produce reflex bradycardia, negative inotropic effect on the heart and, in high doses, vascoconstriction of vessels in skin and skeletal muscle. The intravenous lethal dose of Viscotoxin is about 0·1 mg/kg of bodyweight (cat). The amino acid sequences of Viscotoxin A2 and Viscotoxin A3 have been determined. Both substances contain 46 amino acids and the sequences are identical for amino acids 1–14, 29–36 and 38–46. Each polypeptide contains three disulfide bridges.

Viscotoxin A2: Lys-Ser-Cys-Cys-Pro-Asx-Thr-Thr-Gly-Arg-Asx-Ile-
„ A3: „ „ „ „ „ Asn „ „ „ „ Asn „
1 2 3 4 5 6 7 8 9 10 11 12
Tyr-Asx-Thr-Cys-Arg-Phe-Gly-Gly-Gly-Ser-Arg-Gly-Val-Cys-Ala-Ser-
„ Asn-Ala „ „ Leu-Thr „ Ala-Pro „ Pro-Thr „ „ -Lys-
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Leu-Ser-Gly-Cys-Lys-Ile-Ile-Ser-Ala-Ser-Thr-Cys-Pro-Ser-Tyr-Pro-
„ „ „ „ „ „ „ „ Gly „ „ „ „ „ „ „
29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
Asx-Lys (Asx means Asp or Asn)
Asp „
45 46

Screening of forty-one mistletoe species has revealed the presence of Viscotoxin-like substances in the following plants:

Dendrophtora clavata, Notothixos subaureus, Phoradendron villosum and *Viscum verrucosum. Phoradendron tomentosum* has been further studied and shown to contain a polypeptide with the same pharmacological effects as the Viscotoxins. This substance—Phoratoxin—has a molecular weight around 5000 and the amino acid sequence of the first four amino acids is identical with that of Viscotoxin A2 and A3.

Biogenesis of Aliphatic Monoamines in Plants

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Aliphatic monoamines (e.g. isoamylamine, isobutylamine) are widely distributed in the plant kingdom. They are generally recognized as arising by decarboxylation of amino acids. However our knowledge of carboxy-lyases of neutral aliphatic amino acids is very scanty. The only enzyme of this type so far studied in detail is the bacterial L-valine carboxylyase (EC 4.1.1.14). In the course of investigations on the biogenesis of simple plant amines, we have now discovered a substrate-unspecific L-leucine carboxylyase with high catalytic activity in marine red algae. In addition, an alternative way of amine synthesis could be demonstrated in higher plants, namely the synthesis of aliphatic amines by amination of the corresponding aldehydes catalysed by an amino acid: aldehyde aminotransferase. Both systems were studied in detail. The leucine carboxylyase was found to occur in thirteen of twenty-seven species of marine red algae so far tested. The very stable enzyme appears to firmly particle-bound. For enzymatic analyses, acetone-treated preparations further purified by exhaustive aqueous extraction were used. The enzymic reactions have distinct pH optima in the range of 4·25 to 6·0. The precise values depend on the algal species the enzymes are derived from. In addition to L-leucine, the algal preparations decarboxylate the following eleven amino acids to the corresponding amines: isoleucine, norleucine, valine, norvaline, 2-amino-*n*-butyric acid, alanine, 2-aminoheptanoic acid, phenylalanine, methionine, cysteine, homocysteine. It has been confirmed that all the substrates listed are decarboxylated by a single enzyme. In each case leucine was found to be the main substrate. The holoenzymes contain firmly bound pyridoxal phosphate (PALP) as coenzyme. Under certain conditions, partly resolved enzymes could be obtained. Several carbonyl compounds, especially 2-oxo-carboxylic acids, and PALP in a concentration far exceeding that needed for coenzyme saturation activate the enzymes in some unspecific manner. A close relationship was found to exist between the

natural occurrence of amines and the presence of the decarboxylases in algae. The presence of the leucine carboxylyase explains the biogenetic origin of at least six steam-volatile amines identified from red algae.

An amino acid:aldehyde aminotransferase which catalyses the transamination between amino acids and aliphatic aldehydes was found to occur in *Mercurialis perennis* (dog's mercury) and other higher plants. The enzyme in *Mercurialis* is in the soluble cytoplasmic fraction. Using a partially purified preparation obtained by precipitation with acetone at 80% saturation, some properties of the enzyme were investigated. The enzymic reaction has a pH optimum of approx. 8·5. Alanine appeared to be the most efficient amino donator. Substitution of alanine by glutamic acid, γ-amino-butyric acid or ϵ-aminocaproic acid resulted in much lower activity. Other amino compounds were ineffective. All aldehydes of the homologous series ethanal to undecanal were found to be active amino acceptors. No requirement of the enzyme for PALP could be demonstrated but the enzymic reaction was inhibited by carbonyl agents. Certain keto acids such as pyruvate, 2-oxo-glutarate and oxaloacetate were strong probably competitive inhibitors of the aldehyde amination reaction. The physiological role of the transaminase is discussed with reference to amine biogenesis in higher plants.

Biogenesis of Xanthones in Gentianaceae

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The incorporation of phenylalanine (^{14}C) into gentisein and its methylated counterparts will be discussed in relation to the elaboration of the C_6C_1 residue of the xanthone ring system.

Earlier studies on the biogenesis of xanthones have established the incorporation of "acetate" and "phenylalanine" into gentisein and the intermediate role played by 2,3',4,6-tetrahydroxybenzophenone in this pathway. More detailed experiments have revealed that metabolites of phenylalanine (cinnamic acid and coumaric acid) are incorporated, as is shikimic acid and protocatechuic acid.

Aspects of the Biosynthesis of Amino Acids in Higher Plants

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It is usual in reviewing aspects of the metabolism of amino acids in higher plants to examine as two separate groups those amino acids found in protein hydrolysates on the one hand and the so called "non-protein" amino acids on the other. However, in biosynthetic studies especially, the close biochemical relationship between the two groups is becoming more apparent. Many of the non-protein amino acids are structural homologues or analogues of "protein" amino acids; others are simple substituted forms of them.

Although much less well investigated in higher plants, the evidence presently available indicates that amino acid biosynthesis follows a similar pattern to that established for microbial systems. Each family of amino acids is derived from a product or intermediate of carbohydrate metabolism and thus, in higher plants, is connected directly with the fixation of CO_2 through photosynthesis. The family concept of amino acid biosynthesis offers, however, an explanation of the biogenesis of the carbon skeleton alone and the source of the amino group must be considered separately. The evidence indicates that ammonia is the ultimate source of the amino group of all amino acids *via* glutamic acid and its amide. In higher plants, the latter two compounds are probably the only amino acids aminated directly; all other

amino acids obtain their amino groups through transamination with glutamic acid. Little is known of the number or specificity of the enzymes involved in higher plants.

The biogenesis of many of the non-protein amino acids can be explained by the entry of homologues or analogues of the more usual intermediates into the metabolic pathways of the amino acid families. This is made possible by the low substrate specificity exhibited by some of the enzyme systems involved and is exemplified by the formation of β-pyrazol-1-ylalanine[1] in which pyrazole is introduced as an indole analogue in the tryptophan synthetase reaction. The extent to which the tryptophan synthetase mechanism accounts for the formation of other heterocyclic β-substituted alanines remains to be seen although more examples are already known. Theoretical considerations and experiments in our laboratories with model pyridoxal systems indicate that it is unlikely however that either of the isomeric uracilyl alanines[2–4] arise in this way.

Several amino acids are simply substituted forms of others, e.g. 4-hydroxyproline, N^4-ethylasparagine and asparagine. Whether the location of the substitutive step is early or late in the biosynthetic sequence of a particular family appears to vary from one compound to another: the hydroxylation of proline can probably even occur either pre- or post-cyclization, depending on the biological conditions.

Although the mechanisms by which amino acid biosynthesis is regulated are fairly clear in microbial systems, little is known of these in higher plants. End-product inhibition of the first reaction specific to a particular sequence has, however, been demonstrated in a few plant tissues.

References

[1] P. M. DUNNILL and L. FOWDEN, *J. Exp. Botany* **14**, 237 (1963).
[2] R. GMELIN, *Z. Physiol. Chem.* **316**, 164 (1959).
[3] F. LAMBEIN and R. VAN PARIJS, *Biochem. Biophys. Res. Commun.* **32**, 474 (1968).
[4] E. G. BROWN and B. S. MANGAT, *Biochim. Biophys. Acta* **177**, 427 (1969).

Mass Spectrometry in Peptide Chemistry

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Conventional methods employed for the determination of amino acid sequences in oligopeptides are time-consuming and tedious. Mass spectrometry, in recent years, has found promising applications in the determination of the sequence of amino acid residues in N-acyloligopeptide derivatives. The minute quantity needed for obtaining a mass spectrum and the rapidity of measurements compete favourably with other established methods.

Poor volatility of higher N-acyloligopeptide methyl esters has been a major problem in the mass spectrometric investigation of these compounds. An important factor limiting the volatility of peptide derivatives is hydrogen bonding due to the presence of —CO—NH— groups. Efforts to extend the scope of mass spectrometry for sequence determination of longer peptide chains have led to the development of methods for permethylation of the —CO—NH— groupings of oligopeptide derivatives, thus eliminating hydrogen bonding. Free carboxyl groups are also methylated in the same operation.

Because of their enhanced volatility, as well as their simplified mass spectral fragmentations, the resulting O,N-permethylated derivatives are especially suited to sequence determination by mass spectrometry. This method has been used to confirm or correct the structures proposed for a number of natural and synthetic peptides.

Occurrence of Diaminopropionic Acid and Derivatives in the Genus Schrankia

E. A. BELL and P. B. NUNN, The Department of Botany, The University of Texas at Austin, U.S.A.

Free diaminopropionic acid was detected in the seeds of five species of *Schrankia* and in each of these it was accompanied by an unidentified ninhydrin-reacting compound which moved fractionally faster than diaminopropionic acid on ionophoresis at pH 3·6 and pH 1·9. On acid hydrolysis, the "unknown" gave a single ninhydrin-reacting spot which moved markedly faster than diaminopropionic acid on ionophoresis under the same conditions.

By co-chromatography and co-ionophoresis, the "unknown" and its hydrolysis product have now been identified as acetylethylenediamine and ethylenediamine respectively. The presence of β-acetyldiaminopropionic acid has also been shown in *S. roemeriana* and it is suggested that the acetylethylenediamine may be formed by decarboxylation of the acetyl amino acid.

We thank Professor L. Fowden for a gift of authentic β-acetyldiaminopropionic acid.

Separation and Characterization of Two New Diasterioisomeric Amino Acids from Gymnocladus dioicus

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A 70% alcohol extract of seeds and leaves of *Gymnocladus dioicus* (Leguminosae), when chromatographed two-dimensionally in *n*-BuOH–HCO_2H–H_2O (75:15:10, v/v) and in PhOH buffered at pH 4·2, gave two unknown spots, G1 and G2, between aspartic and glutamic acids. They were isolated by chromatography on Dowex 1 × 8, acetate form, eluted with 0·5 N HOAc and then on Dowex 50 W × 8, Na^+ form, eluted with pyridine–HOAc (16–250 ml/l.); fractions 2 to 20 contained G2 and fractions 21 to 37 G1. After purification on Dowex 1 × 8, recrystallization was from aqueous alcohol. Both amino acids contained water of crystallization but analysed correctly for $C_6H_{11}O_5N$ after drying. G1 and G2 are α-amino acids and on electrophoresis behave like dicarboxylic acids, G2 being more acidic than G1. After spraying with alkaline periodate, the release of NH_3 could be demonstrated with Nessler's reagent. This indicates the presence of a hydroxyl group and an amino group at adjacent carbon atoms.

On hydrolysis with 5 N NaOH, both gave some glycine and γ-methylglutamic acid. On reduction with HI and red P, they yielded γ-methylglutamic acid. From these results, it is clear that G1 and G2 are two diasterioisomeric forms of a novel amino acid, namely β-hydroxy-γ-methylglutamic acid.* Their configurations have not yet been established.

* Dr. E. A. Bell (University of Texas, Austin, U.S.A.) has informed us that he has independently characterized these novel amino acids from the same source.

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October, 1969

J. B. HARBORNE
Honorary Secretary