Reactions of Plant Proteins with Oxidation Products of Polyphenols

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Plants contain *o*-diphenols, liable to enzymic or spontaneous oxidation to *o*-quinones or semiquinone-radicals. Besides undergoing oxidative polymerization, these may react with thiol, amino, thio-ether, indole and perhaps other functional groups of proteins. Since nutritionally important cyst(e)ine, lysine, methionine and tryptophan residues carry such groups, damage to nutritive quality seems likely, a possibility so far largely neglected by nutritionists. In protein-rich supplements to basal cereal diets for pigs and poultry, lysine is critical; quinone reactions can damage it in leaf-protein concentrates and oilseeds. My colleague R. Davies is studying reactions of *o*-quinones with aliphatic amines; R. Davies, W. M. Laird and I are hydrogenating model compounds and proteins over rhodium in 70% (*v*/*v*) aqueous acetic acid, for stabilizing lysine-quinone adducts as cyclohexane derivatives.

Oxidative polymerization of quinones seems important in the formation of soil organic matter; simultaneous coupling to proteins provides a "bank" of slowly mineralizing N and S. ^{14}C-Dating shows the protein moiety to be degraded faster than the aromatic "core"; lysine residues are important for attaching "protein" to "core". Such reactions may begin in senescent subaerial parts of plants before they enter the soil. A major evolutionary determinant of massive polyphenol accumulation by plants could be the *post mortem* establishment of a humus favourable for growth of later generations.

The Metabolism of N-containing Fungicides in Plants

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Foreign compounds in plants are metabolized by a variety of processes including oxidation, hydrolysis, dealkylation and conjugation with sugars, amino acids and other plant constituents. Each of these processes is illustrated by reference to the pattern of metabolism of fungicides. Dimethyldithiocarbamate salts are metabolized in plants to three fungicidally active compounds, namely a glucoside, an L-alanine conjugate and an unidentified material. An inactive compound thiozolidine-2-thion-4-carboxylic acid is also formed but by a non-biological process. The related ethylene *bis*-dithiocarbamates give rise to ethylenethiourea which because of its toxicity has given rise to some concern.

The metabolism of the systemic pyrimidine fungicides, ethirimol and dimethirimol has been studied in detail. Dealkylation, conjugation, hydroxylation and ring cleavage are all important in producing a wide range of metabolites and degradation products. The conversion of a range of broad spectrum fungicides such as benomyl and thiophanate-methyl to a common fungicidally active metabolite, methyl benzimidazole carbamate is discussed. This metabolite is also further metabolized.

The anilide fungicides, carboxin and 2,3-dimethyl-3-furancarboxylic acid anilide are metabolized by oxidation, the former to a sulphoxide and sulphone and the latter to hydroxymethyl derivatives. There is no evidence for hydrolysis of the amide linkage in these compounds. The metabolism of dodecylguanidine, triforine and other newer fungicides is mentioned briefly.

SHORT PAPERS

The Occurrence of D-*alanyl*-D-*alanine in* Phalaris tuberosa—(*Gramineae*)

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D-Alanine is one of the few D-isomers of protein amino acids known to be present in plant tissues. It probably occurs in the free state in corn roots [1] and it has been isolated in conjugated form as *N*-malonyl-D-alanine and as γ-L-glutamyl-D-alanine [3] from pea seedlings.

We have now found that the dipeptide, D-alanyl-D-alanine, occurs consistently in the pasture grass *Phalaris tuberosa* L, growing in South Australia, its content being *ca* 0·02% of the dry wt of the grass. The dipeptide has a characteristic electrophoretic mobility on paper impregnated with borate buffer (pH 9·2) on which it separates cleanly from all common amino acids present in the extracts. Its preparative isolation was achieved by electrophoresis on cellulose thin layers using dilute acetic acid as the electrolyte. The sample was hydrolyzed and yielded α-alanine as the only detectable amino acid, and this was shown to be of D-configuration by enzymic assay.

D-Alanyl-D-alanine is known to be the terminal dipeptide in the peptide-glycan precursor of bacterial cell-wall material [4] and it has been isolated as a freely-occurring dipeptide in *Streptococcus faecalis* [5]; but as far as we know, its occurrence in plant material has not been reported previously.

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Experiments with Stereospecifically-labelled Amino Acids: Convenient Synthesis of (2SR, 3SR)-[3-$^2H_1$]*Phenylalanine*

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Amino acids, stereospecifically-labelled at the β-centre, have been used to provide valuable information on the stereochemistry and mechanism of action of enzymes (e.g. phenylalanine

ammonia lyase) which operate with the removal of β-protons. A general method has been investigated for the synthesis of the required stereospecifically-labelled substrates which involves the stereospecific ring fission of an aziridine intermediate. This approach is illustrated by a new method for the synthesis of (2SR, 3SR)-[3-$^2H_1$]phenylalanine.

Penmacric Acid: a New Dicarboxylic Amino Acid from Seeds of the tropical Legume Pentaclethra macrophylla*

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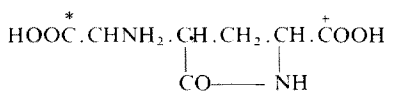
Aqueous extract of endosperm subjected to analysis by Moore and Stein cation-exchange chromatography showed a novel ninhydrin-reactive zone close to aspartic acid. This is a major free amino acid *ca* 0·03% of endosperm dry matter. Successive displacement (ammonia on Dowex-50) and gradient-elution (Dowex-1 with acetic acid gradient) chromatography of extract on a preparative scale yielded a crystalline product. The proposed structure (**1**)



is supported by evidence as follows. High-resolution mass spectrometry of the *N*-acetyl dimethyl ester indicated M = $C_{11}H_{16}N_2O_6$. The low-resolution MS of the free acid had common features with that of pyrrolidonecarboxylate. Permethylation introduced two further methyl groups. IR spectra of the free amino acid and pyrrolidonecarboxylic acid showed similarities. Electrophoretic behaviour and PMR spectrum (D_2O) of the free amino acid were consistent with above structure. On acid–alkali titration, chemical shifts for the α-H atoms changed with ionization of $-COOH$ (pK ~ 2·4) and $-\overset{+}{C}OOH$ (pK ~ 3·4). Accompanying $[\alpha]_D$ changes suggest L-configuration of both α-C atoms.

* See also following abstract.

*New Free Amino acids from Leguminosae**

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From the legume, *Pentaclethra macrophylla*, a Mimosaceae from Central Africa, we have isolated a new acid with a very unexpected structure: 3(R) [1′(S)-aminocarboxymethyl]-2-pyrrolidone-5(S)-carboxylic acid [1]. Some years ago, Krauss and Reinbothe detected only by TLC a large quantity of dichrostachinic acid in these seeds. As we wanted to isolate this amino acid, an extract of the seeds was subjected to 2D chromatography on paper and to HVE. On a 2D chromatogram, we found at the same place as dichrostachinic acid a grey–blue ninhydrin spot but this compound reacted only weakly with the reagent for sulfur amino acids. Also, on electrophoresis at pH 3·6, the substance moved between asparatic and dichrostachinic acid added as markers. From the results, we decided to isolate this compound on a basic ion-exchange resin. The structure has been established by chemical and spectroscopic methods (IR, ^{13}C and ^1H NMR). The absolute configuration at C1′ is −S and is inferred from the results of the circular dichroism measurements. The absolute configuration of C3 and C5 was established by X-ray diffraction [2].

Treatment of this amino acid with HCl transforms it to three new compounds which have been isolated. Their structures are: 2,5-diamino-3-carboxyadipic acid, 3-amino-4,6-carboxypiperidone carboxylic acid and 3-amino-5,6-carboxy-2-piperidone carboxylic acid.

Two years ago, we isolated from leaves of *Calliandra haematocephalla* (Mimosaceae) a disubstituted derivative of pipecolic acid: 2(S)-carboxy-4(R),5(S)-dihydroxypyperidine or *cis*-4,5-dihydroxypipecolic acid [3]. Its absolute configuration was determined by X-ray diffraction [4]. This imino acid coexists with proline, pipecolic acid, *trans*-4-hydroxypipecolic acid and with a new imino acid which has now been isolated in relatively large quantities. This compound gives a red ninhydrin color and it moves on a 2D chromatogram just near 4-hydroxypipecolic acid. By HCl hydrolysis, this neutral compound is transformed to acetic acid and to a basic imino acid which has now been separated and identified as 4-aminopipecolic acid, by 2-DPC and NMR spectroscopy. On deamination, this pipecolic acid derivative gives 4-hydroxypipecolic acid. From these results, this new compound was identified as 4-acetylaminopipecolic acid. The configuration at C(4) has been tentatively assigned as *trans* by NMR and IR spectroscopy. This is the fourth amino derivative of an imino acid found in the plant kingdom.

Recently we have identified a large number of imino acids in the leaves of the legume, *Derris elliptica*. This legume contains proline, pipecolic acid, *trans*-5-hydroxypipecolic acid, *trans*-4-hydroxypipecolic acid and two other isatin-reacting compounds which give a blue–green color with ninhydrin. The mixture of imino acids has been isolated through the ether soluble *N*-nitroso acids. Hydrolysis of the mixture following by separation on cation exchange resin gives first the two new imino acids. One of them has been identified as *cis*-4,5-dihydroxypipecolic acid isolated also from *Calliandra*. The second compound was expected to be a diastereoisomer. In order to confirm this hypothesis we synthesized the four stereoisomers of 4,5-dihydroxypipecolic acid. This was achieved by specific hydroxylation of L-baikaine (4,5-dehydropipecolic acid). We used for the *cis* isomers: H_2O_2–OsO_4 and for the *trans* isomers H_2O_2–performic acid. These four isomers have been studied by IR and NMR spectroscopy. One of the *trans* isomers has been studied by X-ray diffraction. Comparisons with the synthetic compounds showed that the second new natural imino acid was 2(S)-carboxy-4-(S)-dihydroxypiperidine or *trans*-4,5-dihydroxypipecolic acid.

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* See also preceding abstract.