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SHORT COMMUNICATION

PEPTIDES FROM *BLIGHIA SAPIDA* SEED

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Abstract—Four peptides have been isolated from a seed extract of *Blighia sapida* and characterized as glycylglycine, diglycylglycine, glycyl-L-alanine and γ-L-glutamyl-*trans*-α-L-(carboxycyclopropyl)glycine.

INTRODUCTION

RECORDS of the existence of low molecular weight peptides in plants are rare with the exception of a considerable number of γ-glutamyl di- and tri-peptides. Recent compilations of the γ-glutamyl derivatives can be found in the reviews of Waley[1] and Synge.[2] The former reviewer gives the plant source from which each peptide was derived initially; seeds and fleshy storage organs form the principal tissues used for isolation. Other small peptides have been characterized as cell constituents of certain algae, e.g. L-pyrrolidonoyl-L-glutaminyl-L-alanine (eisenin) from *Eisenia bicyclis*,[3] L-pyrrolidonoyl-L-glutaminyl-L-glutamine from *Pelvetia fastigiata*,[4] and L-arginyl-L-glutamine from a *Cladophora* sp.;[5] peptide-like materials rich in arginine also accumulate in *Chlorella*.[6,7] Synge[2] has summarized the most recent evidence relating to the structures of oligopeptides such as evolidine (from *Evodia xanthoxyloides*), pandamine (the peptide alkaloid of *Panda oleosa*) and similar substances isolated from the genera, *Waltheria, Zizyphus* and *Ceanothus*, whilst Waley[1] has provided an account of phalloidin and other toxic peptides of *Amanita phalloides*.

This paper describes the isolation of another γ-glutamyl peptide from seeds, as well as three small glycyl peptides. Although this is apparently the first unequivocable demonstration of glycyl peptides in plants, the isolations can be rationalized with earlier work showing that plants contain a transpeptidase able to catalyse the synthesis of glycyl peptides.[8]

RESULTS AND DISCUSSION

The peptides were isolated from the extract of *Blighia sapida* (Sapindaceae) seed yielding *trans*-α-L-(carboxycyclopropyl)glycine, whose characterization was described in an earlier

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paper.[9] γ-Glutamyl-*trans*-α-(carboxycyclopropyl)glycine was retained on a Dowex-1 column together with the other acidic amino acids and was eluted after all other ninhydrin-positive substances during displacement with acetic acid. The three glycyl peptides, which were eluted from cation-exchange resin columns in the same small group of fractions, were separated from other amino acids by preparative paper chromatography.

γ-L-Glutamyl-trans-α-L-(*Carboxycyclopropyl*)*Glycine*

Fractions containing this compound were pooled, concentrated and decolorized with charcoal, before being freeze-dried to recover the peptide. Elementary analysis agreed with the formula, $C_{11}H_{16}N_2O_6 \cdot 1H_2O$, i.e. a monohydrate form as found for several previously isolated γ-glutamyl dipeptides.[10,11] Hydrolysis with 2 N HCl at 100° for 2 hr resulted in complete breakdown to equimolar amounts of glutamic acid and *trans*-α-(carboxycyclopropyl)glycine: this lability to dilute acid is typical of a γ-glutamyl, rather than an α-glutamyl, peptide. Dinitrophenylation of the peptide followed by acid hydrolysis gave *N*-dinitrophenylglutamic acid and *trans*-α-(carboxycyclopropyl)glycine, indicating that the peptide-*N* atom was derived from the latter amino acid. Optical rotation measurements on an acid hydrolysate of the peptide indicated that the constituent amino acids were both L-forms.

Glycyl Peptides

The three glycyl peptides were recognized initially as yellow spots on chromatograms developed with ninhydrin but, as with all glycyl peptides, the colour of each spot rapidly changed to blue-purple on standing at laboratory temperature for a few hours. (The isomeric alanylglycine gives an initial blue-purple colour after ninhydrin treatment.) The nature of each peptide was indicated by identifying the products of acid hydrolysis, and structures were confirmed by comparisons of the i.r. spectra with those of authentic materials.

The characterization of γ-glutamyl-*trans*-α-(carboxycyclopropyl)glycine as a seed component is not unexpected because it it now common experience to find that a particular non-protein amino acid [in this instance *trans*-α-(carboxycyclopropyl)glycine], if present in a storage organ in relatively high concentration, is accompanied by the corresponding γ-glutamyl peptide. Although the isolation of the glycyl peptides is novel, their presence in plants in very low amounts may be more commonplace, for they were only recognized as components of the *Blighia* seed extract after preliminary separations had concentrated them into a small group of fractions. It is unlikely that these glycyl peptides are artefacts of proteolysis formed after maceration of the seeds in aqueous ethanol, because the extract apparently did not contain the many other types of small peptide that would result from a degradation of seed protein.

EXPERIMENTAL

Paper and ion-exchange resin chromatographic procedures used for the identification and isolation of the peptides were similar to those described earlier for the other components of *Blighia* seed.[9]

γ-Glutamyl-trans-α-(*Carboxycyclopropyl*)*Glycine*

This peptide moved slightly slower than aspartic acid in 75% (w/w) phenol–NH_3 solvent, and between aspartic and glutamic acids when butan-1-ol–acetic acid–water was the solvent.

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After lyophilization of fractions containing the peptide, 41 mg of white needle-like crystals were obtained. Found: C, 42·7; H, 5·8; N, 9·0; loss at 105°, 6·4 per cent. $C_{11}H_{16}N_2O_6.1H_2O$ required: C, 43·1; H, 5·9; N, 9·2; H_2O, 5·9 per cent. The $[\alpha]_D^{20}$ values were +61° (*c*, 2 in H_2O) and +65° (*c*, 1 in 5 N HCl). After hydrolysing a 1% solution of the peptide in 5 N HCl, the $[\alpha]_D^{20}$ value measured was +84°. The equimolar mixture of L-glutamic acid and *trans*-α-L-(carboxycyclopropyl)glycine arising after hydrolysis should show an $[\alpha]_D^{20}$ value of +90° (calculated using literature values[9]), and so the two constituent amino acids present in the peptide must be L-forms.

Glutamic acid and *trans*-α-(carboxycyclopropyl)glycine were identified in hydrolysis mixtures by chromatographic comparison with authentic materials using three solvent systems.[9] In addition, glutamic acid was converted into pyrrolidone-2-carboxylic acid by heating in solution at pH 3 for 3 hr at 120° (chromatographic identification using aniline-xylose[12] and the starch-chloroimide[13] spray reagents), while *trans*-α-(carboxycyclopropyl)glycine was hydrogenated using Adam's Pt catalyst to give a mixture of *erythro*-β- and *threo*-γ-methylglutamic acids, products characteristic of the *trans* form of this cyclopropyl amino acid.[9]

Dinitrophenylation of the peptide (2 mg) in 1% $NaHCO_3$ (2 ml) was effected with 1-fluoro-2,4-dinitrobenzene (5 μl). The dinitrophenylpeptide was hydrolysed with 6 N HCl at 100° for 3 hr, when *N*-dinitrophenylglutamic acid and *trans*-α-(carboxycyclopropyl)glycine were formed: no free glutamic acid was detected.

Glycyl Peptides

Glycylglycine (205 mg) and diglycylglycine (192 mg) were crystallized from aqueous ethanol after elution from paper chromatograms. Each gave a positive Rydon-Smith test[13] and glycine was formed as the only product of acid hydrolysis. The i.r. spectra of the two isolates exactly matched those of the authentic peptides.

Glycylalanine formed the minor glycyl peptide (24 mg crystalline material). $[\alpha]_D^{20}$ −46° (*c*, 2 in H_2O) compares with a literature value[14] of −50° for glycyl-L-alanine. The i.r. spectrum of the isolate (nujol mull) was identical with that of glycyl-L-alanine.

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