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SHORT REPORTS

γ-GLUTAMYLPEPTIDES FROM *RHYNCHOSIA ALBIFLORA*

BERNARD WATHELET, MICHEL MARLIER,* GASTON DARDENNE and JEAN CASIMIR

Laboratoires de Chimie organique et biologique; *Chimie générale et organique, Faculté des Sciences Agronomiques de l'Etat, 5800, Gembloux, Belgium

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Key Word Index—*Rhynchosia albiflora*; Phaseolinae; γ-glutamylpeptides; unsaturated amino acid; ester of amino acid.

Abstract—Two new γ-glutamylpeptides (γ-L-glutamyl-α-methylene-β-aminopropionic acid, γ-L-glutamyl-ethyl-β-aminoisobutyrate) together with γ-L-glutamyl-β-aminoisobutyric acid have been isolated from seeds of Rhynchosia albiflora. The structures were determined by chemical and physical methods.

INTRODUCTION

In the course of our investigations of the free-amino acids and peptides in higher plants and fungi, we have identified in the seeds of *Rhynchosia albiflora* Lour. several glutamylpeptides. γ-L-Glutamyl-β-aminoisobutyric acid (**1**), previously isolated from *Iris tingitana* [1] and *Lunaria annua* [2, 3], is present in large amounts together with two new γ-glutamylpeptides: γ-L-glutamyl-α-methylene-β-aminopropionic acid (**2**) and γ-L-glutamyl-ethyl-β-aminoisobutyrate (**3**). This is the first report of a natural γ-glutamylamino acid ester.

RESULTS AND DISCUSSION

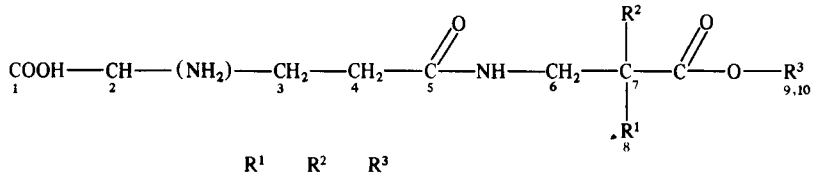
2D-PC surveys revealed that seeds of *R. albiflora* contained six or seven unusual or new amino acids or peptides. Compounds **1–3** gave a purple colouration with ninhydrin. **1** and **2** were slow moving and **3** was fast moving. On high voltage electrophoresis at pH 3.6, **1** and **2** were less acidic than aspartic acid. **2** was more acidic than **1** and **3** was a neutral compound.

Seeds (50 g) were extracted with 75% ethanol–water, the extract treated with Amberlite CG 120, H^+ form and the amino acids eluted with 2 M NH_4OH. The eluate was concentrated, dissolved in water and applied to a column of Dowex 1 × 8, acetate form, washed with water. Elution with water gave neutral and basic amino acids. Acidic compounds were separated with 0.125–2 M HOAc. **1** was eluted before glutamic acid with some impurities but it was pure after several crystallizations (320 mg). **2** was eluted pure after glutamic acid (20 mg). **3** was separated from basic and neutral amino acids on a column of Amberlite CG 120, H^+ form, elution with 1 M pyridine and then preparative PC (80 mg). **3** was pure and was eluted at the same place as glutamic acid on an automatic amino acid analyser. From elementary analysis and MS, the molecular formulae were estimated as: **1**, $C_9H_{16}N_2O_5$; **2**, $C_9H_{14}N_2O_5$; **3**, $C_{11}H_{20}N_2O_5$. The unsaturation of **2** was confirmed by its instability to treatment with acidic permanganate and bromine. The IR spectra showed absorption bands characteristic of dicarboxylic amino acids or γ-glutamylpeptides.

1–3 were completely hydrolysed by heating with 2 M HCl for 3 hr at 100°; this lability to dilute acid was typical of γ-glutamylaminoacids. **1** gave glutamic acid and β-aminoisobutyric acid (β AIB). This was confirmed by comparison of the IR, MS, ^1H and ^{13}C NMR spectra and optical rotation of these compounds with those of authentic materials isolated from *Lunaria annua* [2]. **1** is therefore γ-L-glutamyl-β-aminoisobutyric acid.

Hydrogenation of **2** gave a compound which on 2D–PC and HVE behaved as **1**. Mild hydrolysis of hydrogenated **2** gave L-glutamic acid. The MS of **2** showed a pseudomolecular peak at *m/z* 231 $[M+1]^+$ and other major peaks at *m/z* 147 $[glu]^+$, 130 $[glu-OH]^+$ and 102 [C terminal amino acid + 1]$^+$. The MS of the ditrimethylsilylpeptide showed $M^+ = m/z$ 374. The ^1H NMR spectrum in D_2O containing 2,2,3,3-tetradeutero-3-trimethylsilylpropionate as an internal standard showed a triplet at δ3.78 (1H, H-2), multiplets at δ2.15 (2H, H_2-3) and 2.47 (2H, H_2-4) and a singlet at δ4.02 (2H, H_2-6). The two isolated olefinic protons, H_2-8, showed two singlets at δ5.27 and 6.25. **2** was therefore deduced to be γ-glutamyl-α-methylene-β-aminopropionic acid.

The IR of **3** supported the presence of an ester group (1740 cm^{-1}). On treatment with 2 M HCl it gave L-glutamic acid, βAIB (2DPC, HVE and amino acid analyser) and ethanol (detected by GLC). The mass spectrum of **3** showed a weak $[M+1]^+$ ion at *m/z* 261 and ions at *m/z* 243, 132 and 86 interpreted as $[M-OH]^+$, [ethyl βAIB + 1]$^+$ and [terminal amino acid–OEt]$^+$. The ^1H NMR spectrum showed a triplet at δ3.74 (1H, H-2) and multiplets at δ2.10 (2H, H_2-3), 2.38 (2H, H_2-4) attributed to glutamic acid as well as multiplets at δ3.34 (2H, H_2-6) and δ2.72 (1H, H-7), and a doublet at δ1.06 (3H, Me-8). The ethyl group of the ester function was observed as the quadruplet–triplet sequence δ4.11 (2H,



	R^1	R^2	R^3
1	Me	H	H
2	CH_2	–	H
3	Me	H	Et

H_2-9) and δ1.22 (3H, Me-10). All these assignments were confirmed by double irradiations. The ^{13}C NMR spectra of **1** and **3** in NaOD 5% and D_2O were in agreement with the proposed structures. The γ-glutamyl structure of **1** and **3** was confirmed by ion-exchange chromatography and NMR at different pHs [4]. The similar $[\alpha]_D^{20°}$ (H_2O) values obtained for **1** and **3** showed that they had the same configuration. **3** is therefore γ-L-glutamyl-ethyl-β-aminoisobutyrate. β-aminoisobutyric acid and β-alanine were also present in the extract (from amino acid analyser).

The isolation of two γ-glutamylpeptides with the second amino acid in the acidic or ester forms is unusual. **3** does not seem an artefact since it is obtained with different extraction procedures, e.g. aqueous *n*-butanol, ethanol and methanol. The three peptides are present at variable concentrations in different *Rhynchosia* species. A chemiotaxonomic study is now in progress.

EXPERIMENTAL

Material. Seeds of *Rhynchosia albiflora* were collected in Kumania (Katanga, Zaire) and identified by F. Malaisse. A voucher specimen is deposited in the Department of Chimie organique et biologique.

General. IR: KBr; ^1H NMR: 300 MHz; ^{13}C NMR: 22.63 MHz with dioxane as int. standard (5% v/v), δTMS = δdioxane + 67.4 ppm. MS: 70 eV with DCI mode (NH_3). Amino acids were determined by means of an amino acid analyser equipped with the resin buffer systems described previously [5].

Chromatography and electrophoresis. 2D-PC were carried out using *n*-BuOH–HFo–H_2O (15:3:2, solvent 1) and PhOH saturated with buffer pH 4.2 [5], solvent 2. RA1 values in solvent 1 were: 1.03 (**1**), 0.58 (**2**), 1.67 (**3**); in solvent 2:1.22 (**1**), 1.20 (**2**), 1.74 (**3**). High voltage electrophoresis was carried out at pH 3.6, 70 V/cm, 90 min. – 0.1 cm (**1**), – 0.5 cm (**2**), + 1.5 cm (**3**), – 0.5 cm for glut. acid.

Determination of peptides. γ-L-Glutamyl-β-aminoisobutyric acid: $[\alpha]_D^{20°}$ – 18.6° (H_2O; *c* 0.75); lit: $[\alpha]_D^{22°}$ – 18.5 (H_2O; *c* 1.00) [2]. β-Aminoisobutyric acid from peptide: $[\alpha]_D^{20°}$ – 14.9° (H_2O; *c*1.30); lit: $[\alpha]_D^{17°}$ – 14.2 (H_2O; *c* 0.42) [6]. The amino acid configuration was therefore R. γ-Glutamyl-α-methylene-β-aminopropionic acid: Found C, 47.12; H, 6.07; N, 12.10. $C_9H_{14}N_2O_5$ requires C, 46.95; H, 6.13; N, 12.17%. γ-Glutamyl-ethyl-β-aminoisobutyrate: Found C, 50.68; H, 7.71; N, 10.80 $C_{11}H_{20}N_2O_5$ requires C, 50.75; H, 7.75; N, 10.76%. $[\alpha]_D^{22°}$ – 14.0° (H_2O; *c* 0.6). ^{13}C NMR: δ178.1, 175.4 and 174.8 (*s*, C-1, C-5, C-9), 56.6 (*d*, C-2), 32.6 (*t*, C-3), 35.0 (*t*, C-4), 46.3 (*t*, C-6), 40.9 (*d*, C-7), 17.8 (*q*, C-8), 62.7 (*t*, C-9), 14.7 (*q*, C-10).

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