Phytochemistry, Vol. 25, No. 3, pp. 679–682, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

γ-GLUTAMYL PEPTIDES OF *VIGNA RADIATA* SEEDS

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(*Revised received* 31 *July* 1985)

Key Word Index—*Vigna radiata*; Leguminosae; seeds; γ-glutamyl peptides; γ-glutamyl-*S*-methylcysteinyl-β-alanine (*S*-methylhomoglutathione); γ-glutamyl-N^{δ}-acetylornithine; γ-glutamyl-γ-glutamyl-*S*-methylcysteine; homoglutathione; γ-glutamyl derivatives.

Abstract—It has been reported elsewhere that *Vigna radiata* seeds contain a high concentration of γ-glutamyl-*S*-methylcysteine and its sulphoxide and that these compounds serve as index compounds for the chemotaxonomy of *V. radiata*. Beside these two γ-glutamyl peptides, nine other γ-glutamyl derivatives, of which three are new compounds, have been isolated and identified from *V. radiata* seeds. The structures of the new peptides, γ-glutamyl-*S*-methylcysteinyl-β-alanine (*S*-methylhomoglutathione), γ-glutamyl-N^{δ}-acetylornithine and γ-glutamyl-γ-glutamyl-*S*-methylcysteine, were confirmed by direct comparison with synthetic specimens. The other six γ-glutamyl derivatives were homoglutathione, γ-glutamyl glutamic acid, -aspartic acid, -phenylalanine, -leucine and -isoleucine.

INTRODUCTION

Fifty to sixty thousand tons of *Vigna radiata-mungo* complex are imported annually and consumed mostly as bean sprouts in Japan [1]. Most of them come from Southeast Asia; they contain large amounts of γ-glu-met and its sulphoxide [2] and are identified as *V. mungo* on the basis of their γ-glutamyl peptide pattern [3]. According to Otoul *et al.* [3], mature seeds of *V. mungo* (black gram) contain a high concentration of γ-glu-met and its sulphoxide, while large amounts of their lower homologues, γ-glu-*S*-Me-cys and its sulphoxide, are contained in *V. radiata* (mung bean) seeds. Recently, the use of beans imported mainly from China for the production of bean sprouts has increased. These beans are nearly the same size and weight as *V. mungo*, but their seed coat is lighter in colour (green) than that of *V. mungo* (nearly black). In this paper, we report the identification of this bean as *V. radiata*, L. from the high concentration of γ-glu-*S*-Me-cys and its sulphoxide in its seeds. Nine other γ-glutamyl derivatives, including three hitherto unknown compounds, have also been isolated from the same plant material. The γ-glutamyl peptides isolated from *V. radiata* seeds in this experiment are given in Table 1 along with the isolation method, yield and chromatographic properties. Data for synthetic substances are also included in the Table for comparison.

Abbreviations: glu-met, glutamylmethionine; glu-*S*-Me-cys, glutamyl-*S*-methylcysteine; glu-*S*-Me-cys-β-ala, glutamyl-*S*-methylcysteinyl-β-alanine; glu-cys-β-ala, glutamylcysteinyl-β-alanine; glu-*N*-Ac-orn, glutamyl-*N*-acetylornithine; glu-leu, glutamylleucine; glu-ileu, glutamylisoleucine; glu-phe, glutamylphenylalanine; glu-glu, glutamylglutamic acid; glu-glu-*S*-Me-cys, glutamylglutamyl-*S*-methylcysteine; glu-asp, glutamylaspartic acid.

RESULTS AND DISCUSSION

2D-PC of the acidic amino acid fraction of the bean seeds showed the presence of several ninhydrin positive spots besides aspartic and glutamic acid. The compounds responsible for the colours were separated using ion exchange and cellulose column chromatography and preparative PC. The most abundant compound was identified as γ-L-glu-*S*-Me-L-cys by ^{1}H NMR, FDMS, PC, HVE, R_f in an amino acid analyser and specific rotation [4–6]. The second major compound was identified as γ-glu-*S*-Me-cys sulphoxide by application of the same methods. A high concentration of γ-glu-*S*-Me-cys and its sulphoxide in seeds indicated clearly that the bean seeds analysed were those of *V. radiata* [3].

Other γ-glutamyl derivatives isolated from *V. radiata* seeds are described in their order of elution from a Dowex 1 × 4 (AcO^-) column, rather than in order of quantity.

The ^{1}H NMR spectrum of γ-glu-*S*-Me-cys-β-ala, a hitherto unknown γ-glutamyl peptide, was very similar to that of γ-glu-*S*-Me-cys except for the signals corresponding to the β-ala residue. The PC, HVE and ^{1}H NMR properties of this tripeptide were identical to those of synthetic γ-L-glu-*S*-Me-L-cys-β-ala (see Experimental for syntheses of α- and γ-glu-*S*-Me-cys-β-ala). The configurations of the glu and *S*-Me-cys residues of this isolated tripeptide are unknown.

The disulphide form of γ-L-glu-L-cys-β-ala (homoglutathione) was identified on the basis of experimental data described in the Experimental. It was first detected in the leaves of *Phaseolus vulgaris* [7] and isolated later from seedlings of *P. aureus* [8].

The ^{1}H NMR spectrum of the new γ-glutamyl derivative, γ-glu-N^{δ}-Ac-orn, in D_2O soln revealed glu and orn residues and an NAc group. The chemical shift (δ 3.76) of the glutamyl α-H indicated a γ-glutamyl linkage [4]. To determine whether the C-terminal residue is N^{α}- or N^{δ}-Ac-orn, the peptide was heated in aq. soln [9]. The C-terminal amino acid obtained had the same R_f as that of N^{δ}-Ac-orn

Table 1. γ-Glutamyl derivatives isolated from *Vigna radiata* seeds and synthesized materials and their behaviour on PC, HVE and an amino acid analyser

Peptide	Amount (mg)*	PC		HVE		Amino acid analyser elution time† (min)
		R_{Glu} in solvent 1†	R_f in solvent 2†	Relative mobility to glu in buffer 1†	Relative mobility to glu in buffer 2†	
Natural peptides						
γ-Glutamyl-*S*-methylcysteine(CC)‡	1610	1.45	0.45	2.72	0.72	20.2
γ-Glutamyl-*S*-methylcysteine sulphoxide(CC)	402	0.42	0.46	2.72	0.72	8.2
γ-Glutamylglutamic acid	240	0.80	0.11	2.68	1.29	13.5
γ-Glutamylphenylalanine	66.5	2.60	0.59	1.99	0.63	71.3
γ-Glutamyl-γ-glutamyl-*S*-methylcysteine(CC, PPC1)	55.3	1.00	0.23	3.84	1.09	10.5
γ-Glutamylaspartic acid(CC)	29.7	0.61	0.04	3.32	1.33	10.5
Disulphide form of γ-glutamylcysteinyl-β-alanine	14.7	0.20	0.24	0.48	0.78	51.9
γ-Glutamyl-N^{δ}-acetylornithine(PPC1, 2)	6.7	0.94	0.61	1.84	0.60	13.6
γ-Glutamyl-*S*-methylcysteinyl-β-alanine(PPC1)	5.1	1.58	0.67	0.48	0.58	n.d.
γ-Glutamylleucine + γ-glutamylisoleucine(PPC1)	9.3	2.17	n.d.§	1.67	0.70	n.d.
Synthetic peptides						
γ-Glutamyl-γ-glutamyl-*S*-methylcysteine		1.02	0.23	n.d.	1.05	10.2
α-Glutamyl-γ-glutamyl-*S*-methylcysteine		1.37	n.d.	n.d.	n.d.	26.7
γ-Glutamyl-N^{δ}-acetylornithine		0.93	0.62	1.77	n.d.	14.8
α-Glutamyl-N^{δ}-acetylornithine		1.61	0.68, 0.55‖	0.36	n.d.	43.0, 58.8‖
γ-Glutamyl-N^{α}-acetylornithine		1.00	0.66	1.88	0.59	16.2
α-Glutamyl-N^{α}-acetylornithine		1.34	0.66	0.18	0.61	54.2
γ-Glutamyl-*S*-methylcysteinyl-β-alanine		1.60	0.66	n.d.	0.57	n.d.
α-Glutamyl-*S*-methylcysteinyl-β-alanine		1.97	0.67	n.d.	0.57	n.d.

*From 3 kg seeds.

†See Experimental for composition of solvents and buffers and conditions for amino acid analysis.

‡Method of isolation from other coexisting components after Dowex 1 × 4 (AcO^-) column chromatography. PPC1, PPC2, Prep. PC with solvents 1 and 2, respectively; CC, cellulose column chromatography with solvent 1.

§n.d., Not determined.

‖See text for comments about double spots and peaks.

(43 min) in an amino acid analyser, while N^{α}-Ac-orn was eluted at 100 min. The PC, HVE, ^{1}H NMR and analyser properties agreed with those of synthetic γ-L-glu-N^{δ}-Ac-L-orn. The configurations of both residues of the isolated material are not known (see Experimental for syntheses of N^{α}- and N^{δ}-Ac-orn, α- and γ-glu-N^{δ}-Ac-orn and α- and γ-glu-N^{α}-Ac-orn). Synthetic α-glu-N^{δ}-Ac-orn gave double spots on PC (solvent 2) and was eluted as double peaks in the analyser. The ratio of peak areas of the faster to slower moving components was *ca* 1 : 5. The double peaks may indicate that the synthetic α-glu-N^{δ}-Ac-orn is a mixture of diastereoisomers (L,D- and L,L-) in the ratio of *ca* 1 : 5, because an α-L-glu-D-amino acid is eluted faster than an α-L-glu-L-amino acid on an analyser [10]. Synthetic α-glu-N^{α}-Ac-orn was eluted as a single peak in the analyser.

γ-Glu-leu and γ-glu-ileu could not be separated from each other as in the case of the same peptides from *Fagus silvatica* seeds [11] and seedlings [12] and *Rumex obtusifolius* roots [13]. The characterization of these peptides was based on a comparison of the ^{1}H NMR and FDMS with those of a mixture of γ-glu-leu and γ-glu-ileu obtained from bulbs of *Allium cepa* [14]. The ratio of γ-glu-ileu to γ-glu-leu was *ca* 1 : 2 from the ratio of glu, ileu and leu after hydrolysis.

Identification of γ-glu-phe was based on a comparison of the PC, HVE, ^{1}H NMR and analyser properties with those of an authentic specimen isolated from *Trifolium repens* seed [15].

Isolated γ-glu-glu gave the same chromatographic and spectroscopic properties as those of synthesized γ-L-glu-L-glu, but it seems that one glu residue of the isolated γ-glu-glu is in the D-form or the isolated material is a mixture of diastereoisomers judging from specific rotations of isolated peptide and its hydrolysate as shown in Experimental.

Partial hydrolysis of γ-L-glu-γ-L-glu-*S*-Me-L-cys, gave five ninhydrin positive substances on PC and on the analyser which corresponded to unchanged material, γ-glu-glu, γ-glu-*S*-Me-cys, glu and *S*-Me-cys. The specific rotation and spectral and chromatographic properties of the isolated peptide were consistent with those of synthesized γ-L-glu-γ-L-glu-*S*-Me-L-cys (see Experimental for syntheses of α- and γ-glu-γ-glu-*S*-Me-cys). Two tripeptides having a γ-glu-γ-glu linkage have been found. They are, γ-glu-γ-glu-met from *V. mungo* [16] and γ-glu-γ-glu-phe from *F. silvatica* var *purpurea* seeds [12].

γ-Glu-asp was identified by comparison (^{1}H NMR, PC, HVE and analyser) with an authentic specimen isolated from *T. repens* seeds [15].

The γ-glutamyl peptides found in mature seeds of *V. radiata* could not be detected in seedlings grown for 3 days at 40° in the dark (see ref. [17] for distribution of γ-glutamyl derivatives in plant kingdom). Besides the 11 γ-glutamyl derivatives described above, three non-protein amino acids, α-amino adipic acid and saccharopine, which are widely distributed in the plant kingdom, and *S*-(1,2-

dicarboxyethyl)cysteine, which has been isolated from *Asparagus officinalis* shoots [18], were isolated from the acidic amino acid fraction of *V. radiata* seeds. γ-Glu-met and its sulphoxide which are contained in quantity in *V. mungo* seeds [2, 3] could not be detected in *V. radiata* seeds. Classification of *V. radiata-mungo* complex has also been achieved on the basis of the difference in anthocyanin composition [1].

EXPERIMENTAL

General methods. PC: *n*-BuOH–HOAc–H_2O (4:1:2) (solvent 1) and PhOH–H_2O–conc. NH_4OH (120:30:1, w/v/v) (solvent 2); 2D-PC: solvents 1 and 2; HVE: pH 3.6 (C_5H_5N–HOAc–H_2O, 1:20:200, 55 v/cm) (buffer 1) and pH 6.5 (C_5H_5N–HOAc–H_2O, 25:1:500, 100 v/cm) (buffer 2); amino acid analysis: Model 835 Hitachi high speed amino acid analyser, Hitachi custom ion-exchange resin # 2619 (2.6 mm i.d. × 250 mm), MCI® Buffer 835-PF-Kit under the conditions for physiological fluids analysis.

Plant material and extraction. Bean seeds (3 kg) which had been purchased at a local market were pulverized and extracted with 70% EtOH (8 l.) for 5 days. After filtration, the residue was extracted continuously with 32 l. of 70% EtOH. The extracts were combined, concd *in vacuo* and washed with $CHCl_3$.

Isolation and identification of each compound. The aq. extract was centrifuged and the supernatant was applied to a column of Amberlite IR-120 (H^+, 1 l.), which was thoroughly washed with H_2O (10 l.). The amino acid fraction was eluted with 2 M NH_4OH. Fractions of 1 l. each were collected. Fr. 2 was concd *in vacuo* and applied on a column of Dowex 1 × 4 (AcO^-, 300 ml). The column was washed with H_2O (3 l.) and eluted with 2 M HOAc (3 l.) to obtain an acidic amino acid fraction. The eluate was applied again to the column of Dowex 1 × 4 (AcO^-, 300 ml). The column was eluted as follows and fractions of 13.5 ml each were collected: 0.2 M HOAc, fr. 1–170; 0.5 M HOAc, fr. 171–250; 1 M HOAc, fr. 251–366 and 2 M HOAc fr. 367–415. The column was finally eluted with 2 M HCl and fractions of 13.5 ml each were also collected. Crystalline α-aminoadipic acid was isolated from fr. 18 (6.3 mg) (PC, HVE, behaviour in amino acid analyser and IR same as an authentic specimen). A small amount of crystalline material (1.6 mg) isolated from fr. 27–32 was identified as saccharopine (PC and HVE [19]). A relatively strong positive Cotton effect at 202 nm (H_2O) in the CD spectrum suggested that the two chiral centres of the compound have the L-configuration [20] (CD Δελ max (202 nm) + 18.4 (*c* 0.01; H_2O). The disulphide of γ-L-glu-L-cys-β-ala (homoglutathione) which is slightly soluble in H_2O crystallized out of fr. 61–73 (14.7 mg). ^{1}H NMR (100 MHz, DCl, pD < 1): δ 2.27 (*t*, *J* = 6 Hz, 2H), 2.62 (2 × *t*, *J* = 7 Hz, 2H, each overlapped completely), 3.00 (*dd*, *J* = 9, 14 Hz, 1H), 3.17 (*dd*, *J* = 6, 14 Hz, 1H), 3.48 (*t*, *J* = 6 Hz, 2H), 4.13 (*t*, *J* = 6 Hz, 1H), 4.60 (*dd*, *J* = 6, 9 Hz, 1H); FDMS *m/z* (rel. int.): 641 $[M+1]^+$ (28.8), 623 (35.8), 578 (19.4), 321 (94.3), 227 (100). Hydrolysis of this compound (6 M HCl, 110°, overnight in a sealed tube) gave peaks of glu, cystine and β-ala with the ratio of *ca* 2:1:2 on an analyser. $[\alpha]_D^{21}$ −50.7° (*c* 0.15; H_2O) (lit. [8] −59.5°; *c* 0.5; supersatd in H_2O). CD Δελ max (206 nm) + 8.74 (*c* 0.01; H_2O). γ-Glu-*S*-Me-cys-β-ala was isolated from fr. 25–28 as a chromatographically pure evaporation residue (5.1 mg). ^{1}H NMR (100 MHz, D_2O): δ 2.11 (*s*, 3H), 2.13 (2H, overlap with a big singlet at 2.11), 2.41 (*t*, *J* = 7 Hz, 2H), 2.52 (*t*, *J* = 8 Hz, 2H), 2.79 (*dd*, *J* = 8, 11 Hz, 1H), 2.98 (*dd*, *J* = 6, 11 Hz, 1H), 3.40 (*t*, *J* = 7 Hz, 2H), 3.76 (*t*, *J* = 6 Hz, 1H), 4.46 (*dd*, *J* = 6, 8 Hz, 1H); FDMS *m/z* (rel. int.): 336 $[M+1]^+$ (48.5), 318 (13.9), 207 (51.8), 130 (100). Synthesis of this new tripeptide is described later. γ-Glu-N^δ-Ac-orn was isolated as a chromatographically pure evaporation residue (6.7 mg) from fr. 81–95. ^{1}H NMR (100 MHz, D_2O): δ 1.7 (*m*, 4H), 1.96 (*s*, 3H), 2.17 (*t*, *J* = 7 Hz, 2H), 2.49 (*t*, *J* = 8 Hz, 2H), 3.17 (*t*, *J* = 6 Hz, 2H), 3.76 (*t*, *J* = 6 Hz, 2H), 4.13 (*dd*, *J* = 5, 6 Hz, 1H); FDMS *m/z* (rel. int.): 304 $[M+1]^+$ (6.0), 175 (100), 130 (93.8), CD Δελ max (209 nm) + 4.36 (*c* 0.01; H_2O). An aq. soln of this γ-glutamyl peptide (1.0 mg in 0.5 ml) was heated in a sealed tube for 1 hr at 110° to obtain the C-terminal amino acid [9]. The elution time of the C-terminal amino acid on an analyser was the same as that of synthetic N^δ-Ac-orn, but not with N^α-Ac-orn. The syntheses of N^α- and N^δ-Ac-orn and γ-glu-N^α- and -N^δ-Ac-orn and α-glu-N^α- and -N^δ-Ac-orn are described later. A mixture of γ-glu-ileu and γ-glu-leu was obtained from fr. 92–103 as an evaporation residue (9.3 mg). Although they could not be separated from each other by PC or ion exchange chromatography, the hydrolysate (3 M HCl, 120°, 3 hr in a sealed tube) gave peaks of glu, ileu and leu with the ratio of *ca* 3:1:2 on an analyser. The FDMS spectrum gave a $[M+1]^+$ ion at *m/z* 261. Therefore the evaporation residue giving one spot on PC was characterized as a mixture of γ-glu-ileu and γ-glu-leu in the ratio of *ca* 1:2. The PC and HVE properties were consistent with those of a mixture of γ-glu-ileu and γ-glu-leu obtained from bulbs of *Allium cepa* [14]. γ-L-glu-*S*-Me-L-cys crystallized out of fr. 219–282 (1.61 g). ^{1}H NMR (90 MHz, D_2O): δ 2.17 (*s*, 3H), 2.20 (2H, overlap with a large singlet at 2.17), 2.53 (*m*, 2H), 2.83 (*dd*, *J* = 7, 13 Hz, 1H), 2.99 (*dd*, *J* = 5, 13 Hz, 1H), 3.76 (*t*, *J* = 7 Hz, 1H), 4.36 (*dd*, *J* = 5, 7 Hz, 1H); FDMS *m/z* (rel. int.): 265 $[M+1]^+$ (100), 135 (49.8), 130 (43.8). $[\alpha]_D^{20}$ −22.0° (*c* 1.1; H_2O) (lit. [6] −20.5°, *c* 2.2; H_2O). γ-Glu-*S*-Me-cys sulphoxide was obtained as a chromatographically pure evaporation residue (402 mg) from fr. 219–309. A small amount of crystalline material was obtained from EtOH–H_2O. ^{1}H NMR (90 MHz, D_2O): δ 2.17 (*m*, 2H), 2.48 (*m*, 2H), 2.74 (*s*, 3H), 3.20 (*dd*, *J* = 8, 13.5 Hz, 1H), 3.30 (*dd*, *J* = 5, 13.5 Hz, 1H), 3.80 (*t*, *J* = 7 Hz, 1H), 4.3 (partial overlap with a spinning side band of DOH); FDMS *m/z* (rel. int.): 281 $[M+1]^+$ (100), 130 (23.1). Strong absorption at 1000 cm^{-1} in the IR spectrum (KBr) also gave an evidence for sulphoxide group. γ-Glu-phe crystallized out of fr. 311–333 (66.5 mg). ^{1}H NMR, PC and HVE were identical to those of an authentic specimen isolated from *T. repens* seeds [15]. γ-Glu-glu crystallized out of fr. 379–402 (240 mg). PC and HVE agreed with those of synthetic γ-L-glu-L-glu, but the specific rotation values differed. $[\alpha]_D^{19}$ of isolated peptide, −6.7° (*c* 2.4; H_2O) (lit. [21] < 1°; *c* 1.6; H_2O). Moreover, the specific rotation of glu obtained by hydrolysis of the isolated peptide (3 M HCl, 120°, 4 hr in a sealed tube) was 0 in H_2O. It was likely, therefore, that one glu residue of γ-glu-glu isolated from *V. radiata* seeds is in the D-form or both glu residues are racemic. γ-L-Glu-γ-L-glu-*S*-Me-L-cys was isolated as a chromatographically pure evaporation residue from fr. 433–443 (55.3 mg). Hydrolysate (3 M HCl, 100°, 3 hr in a sealed tube) gave peaks of glu and *S*-Me-cys with the ratio of *ca* 2:1 on an analyser. Partial hydrolysis (1 M HCl, 100°, 20 min in a sealed tube) gave glu, *S*-Me-cys, γ-glu-glu, γ-glu-*S*-Me-cys and unchanged material on PC and an analyser. ^{1}H NMR (90 MHz, D_2O): δ 2.13 (*s*, 3H), 2.16 (4H, overlap with a big singlet at 2.13), 2.40 (*m*, 4H), 2.74 (*dd*, *J* = 8, 12 Hz, 1H), 2.93 (*dd*, *J* = 5, 12 Hz, 1H), 3.74 (*t*, *J* = 6 Hz, 1H), 4.07 (*dd*, *J* = 4, 8 Hz, 1H), 4.36 (*dd*, *J* = 4, 8 Hz, partially overlap with a spinning side band of DOH). FDMS *m/z* (rel. int.): 376 $[M+1-H_2O]^+$ (13.6), 358 (35.9), 265 (100). $[\alpha]_D^{20}$ −15.6° (*c* 1.0; 3 M HCl) (synthetic γ-L-glu-γ-L-glu-*S*-Me-L-cys, −20.5°; *c* 1.3; 3 M HCl). The synthesis of this new tripeptide is described later. γ-Glu-asp crystallized out of fr. 441–449 (29.7 mg); ^{1}H NMR, PC and HVE agreed with those of authentic γ-glu-asp isolated from *T. repens* seeds [15]. A small amount of *S*-(1,2-dicarboxyethyl)cysteine was isolated as a chromatographically pure evaporation residue (1.4 mg) from fr. 441–445. It showed the same behaviour on PC, HVE and an

analyser as authentic *S*-(1,2-dicarboxyethyl)cysteine isolated from *Asparagus officinalis* shoots [18].

Syntheses of α- and γ-L-glu-S-Me-L-cys-β-ala. Ethylchlorocarbonate (10 mmol) was added to a soln of *N*-Boc-*S*-Me-L-cys prepared by the method of ref. [22] in THF (3.5 mmol in 10 ml with 9.6 mmol of Et_3N). After stirring for 30 min, an aq. soln of β-ala (20 mmol in 5 ml with 20 mmol of Et_3N) was added and stirred for another 2 hr. The Boc group was removed with 2 M HCl (20 ml). ^{1}H NMR (90 MHz, D_2O) of *S*-Me-L-cys-β-ala: δ 2.11 (*s*, 3H), 2.35 (*t*, *J* = 7 Hz, 2H), 2.87 (*dd*, *J* = 8, 14 Hz, 1H), 2.90 (*dd*, *J* = 6, 14 Hz, 1H), 3.36 (*t*, *J* = 7 Hz, 2H), 3.97 (*dd*, *J* = 6, 8 Hz, 1H). FDMS *m/z* (rel. int.): 207 $[M+1]^+$ (100). An aq. soln of *S*-Me-L-cys-β-ala (1.9 mmol in 1.5 ml with 7.8 mmol of Et_3N) was mixed with *N*-Cbz-L-glu anhydride soln in DMF (2.5 mmol in 3 ml) and stirred for 30 min. The Cbz group was removed by hydrogenolysis over Pd–C (400 mg) in 50% EtOH (20 ml). The α- and γ-L-glu-*S*-Me-L-cys-β-ala formed were separated by elution from Dowex 1 × 4 (AcO^-) column with 0.2 M HOAc giving 0.15 mmol of the α-isomer and 0.2 mmol of the γ-isomer. *α-Isomer.* ^{1}H NMR (100 MHz, D_2O): δ 2.13 (*s*, 3H), 2.18 (*m*, 2H, overlap with a large singlet at 2.13), 2.46 (*m*, 2H), 2.54 (*t*, *J* = 6 Hz, 2H), 2.85 (*dd*, *J* = 6, 13 Hz, 1H), 2.93 (*dd*, *J* = 8, 13 Hz, 1H), 3.47 (*t*, *J* = 6 Hz, 2H), 4.11 (*t*, *J* = 6 Hz, 1H), 4.50 (*dd*, *J* = 6, 8 Hz, 1H); FDMS *m/z* (rel. int.): 318 $[M+1-H_2O]^+$ (100), 317 (85.2); $[\alpha]_D^{23}$ −30.4° (*c* 1.0; H_2O). *γ-Isomer.* ^{1}H NMR (100 MHz, D_2O): δ 2.11 (*s*, 3H), 2.16 (*m*, 2H, overlap with a big singlet at 2.11), 2.52 (*m*, 2H), 2.59 (*t*, *J* = 6 Hz, 2H), 2.78 (*dd*, *J* = 8, 14 Hz, 1H), 2.96 (*dd*, *J* = 6, 14 Hz, 1H), 3.47 (*t*, *J* = 6 Hz, 2H), 3.78 (*t*, *J* = 6 Hz, 1H), 4.44 (*dd*, *J* = 6, 8 Hz, 1H); FDMS *m/z* (rel. int.): 336 $[M+1]^+$ (90), 318 (19.4), 291 (24.6), 272 (32.0), 207 (100), 130 (66.5); $[\alpha]_D^{22}$ −27.8° (*c* 1.0; H_2O).

Syntheses of α- and γ-L-glu-N$^\delta$-Ac-L-orn. An aq. soln of L-orn and $NaHCO_3$ (20 mmol and 50 mmol, respectively, in 50 ml) was mixed with Ac_2O (25 mmol) and stirred for 2 hr in an ice bath to obtain N^α- and N^δ-Ac-orn, which were separated from each other by prep. HVE (1 M HOAc, 100 v/cm) giving, 9.7 mmol of N^α- and 1.2 mmol of N^δ-Ac-L-orn. N$^\alpha$*-Isomer.* ^{1}H NMR (90 MHz, D_2O): δ 1.73 (*m*, 4H), 2.02 (*s*, 3H), 2.97 (*t*, *J* = 7 Hz, 2H), 4.13 (*t*, *J* = 5 Hz, 1H). N$^\delta$*-isomer.* ^{1}H NMR (90 MHz, D_2O): δ 1.60 (*m*, 2H), 1.76 (*m*, 2H), 1.98 (*s*, 3H), 3.16 (*t*, *J* = 7 Hz, 2H), 3.69 (*t*, *J* = 5 Hz, 1H). *N*-Cbz-L-glu anhydride soln in DMF (1.3 mmol in 0.7 ml) was added to an aq. soln of N^δ-Ac-L-orn (1.0 mmol in 1 ml with 4.0 mmol of Et_3N) and stirred for 30 min at room temp. The Cbz group was removed by hydrogenolysis over Pd–C (600 mg) in 50% EtOH (15 ml). α- and γ-L-Glu-N^δ-Ac-L-orn were separated by elution from Dowex 1 × 4 (AcO^-) column with 0.2 M HOAc giving 0.37 mmol of the α-isomer and 0.49 mmol of the γ-isomer. The α-isomer gave two spots on PC with solvent 2 and two peaks on the analyser. Each of two components, which were separated to each other by prep. PC with solvent 2, gave nearly the same ^{1}H NMR spectrum, but considerably different specific rotation. ^{1}H NMR (100 MHz, D_2O): δ 1.65 (*m*, 4H), 1.97 (*s*, 3H), 2.14 (*m*, 2H), 2.36 (*m*, 2H), 3.18 (*t*, *J* = 7 Hz, 2H), 4.06 (*t*, *J* = 6 Hz, 1H), 4.17 (*dd*, *J* = 5, 5.5 Hz, partially overlap with triplet at 4.06). $[\alpha]_D^{23}$ of the component with higher R_f value on PC with solvent 2 and longer R_t on an analyser, + 16.5° (*c* 3.4; H_2O). $[\alpha]_D^{22}$ of other component, −28.4° (*c* 0.8; H_2O). *γ-Isomer.* ^{1}H NMR (100 MHz, D_2O): δ 1.73 (*m*, 4H), 1.97 (*s*, 3H), 2.18 (*m*, 2H), 2.51 (*m*, 2H), 3.18 (*t*, *J* = 6 Hz, 2H), 3.83 (*t*, *J* = 6 Hz, 1H), 4.30 (*dd*, *J* = 5, 6 Hz, 1H); FDMS *m/z* (rel. int.): 304 $[M+1]^+$ (47.7), 175 (100), 130 (98.2). $[\alpha]_D^{22}$ −6.7° (*c* 2.3; H_2O).

Syntheses of α- and γ-L-glu-N$^\alpha$-Ac-L-orn. Those two glutamyl peptides were synthesized by the same procedure as those used for the syntheses of α- and γ-glu-N^δ-Ac-orn described above starting from *N*-Cbz-L-glu anhydride and N^α-Ac-L-orn. *α-Isomer.* ^{1}H NMR (90 MHz, D_2O): δ 1.6 (*m*, 4H), 2.00 (*s*, 3H), 2.11 (*m*, 2H), 2.40 (*m*, 2H), 3.18 (*t*, *J* = 6 Hz, 2H), 3.90 (*t*, *J* = 7 Hz, 1H), 4.13 (poor resolution, 1H); FDMS *m/z* (rel. int.): 286 $[M+1-H_2O]^+$ (100); $[\alpha]_D^{22}$ + 6.7° (*c* 1.6; H_2O). *γ-Isomer.* ^{1}H NMR (90 MHz, D_2O): δ 1.6 (*m*, 4H), 2.00 (*s*, 3H), 2.10 (*m*, 2H), 2.36 (*m*, 2H), 3.13 (*t*, *J* = 6 Hz, 2H), 3.71 (*t*, *J* = 6 Hz, 1H), 4.23 (overlap with a spinning side band of DOH); FDMS *m/z* (rel. int.): 304 $[M+1]^+$ (11.9), 175 (69.2), 130 (100); $[\alpha]_D^{22}$ −1.5° (*c* 3.1, H_2O).

Syntheses of α- and γ-L-glu-γ-L-glu-S-Me-L-cys. *N*-Cbz-L-glu anhydride soln in DMF (0.91 mmol in 0.6 ml) was mixed with an aq. soln of γ-L-glu-*S*-Me-L-cys isolated from *V. radiata* seeds as described above (0.76 mmol in 0.5 ml with 3.0 mmol of Et_3N) and stirred for 1 hr at room temp. The Cbz group was removed by hydrogenolysis over Pd–C (400 mg) in 50% EtOH (20 ml). The α- and γ-isomers formed were separated by elution from Dowex 1 × 4 (AcO^-) column with 2 M HOAc followed by 8 M HOAc. FDMS *m/z* (rel. int.): 394 $[M+1]^+$ (21.8), 376 (100), 137 (96.4). *γ-Isomer.* ^{1}H NMR: (90 MHz, D_2O): δ 2.15 (*s*, 3H), 2.19 (*m*, 4H, overlap with a big singlet at 2.15), 2.44 (*m*, 4H), 2.86 (*dd*, *J* = 9, 15 Hz, 1H), 3.04 (*dd*, *J* = 5, 15 Hz, 1H), 3.86 (*t*, *J* = 6 Hz, 1H), 4.35 (overlap with a spinning side band of DOH), 4.57 (*dd*, *J* = 5, 9 Hz, partial overlap with DOH). FDMS *m/z* (rel. int.): 394 $[M+1]^+$ (48.3), 135 (37.7), 130 (100). $[\alpha]_D^{22}$ −20.5° (*c* 1.3; 3 M HCl).

Acknowledgement—We express our gratitude to Mr. K. Watanabe, Department of Agricultural Chemistry, Hokkaido University, for measurements of the FDMS.

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