Phytochemistry, 1978, Vol 17, pp 1911–1915 ©Pergamon Press Ltd. Printed in England. 0031-9422/78/1101-1911 $02.00/0

FREE AMINO ACIDS AND γ-GLUTAMYL PEPTIDES IN FAGACEAE

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(*Received* 4 *April* 1978)

Key Word Index—*Fagus*; *Quercus*; *Castanea*; Fagaceae; non-protein amino acids; γ-glutamyl peptides; γ-L-glutamyl-L-tryptophan; γ-L-glutamyl-γ-L-glutamyl-L-phenylalanine; willardiine; 4-hydroxypipecolic acid.

Abstract—Amino acids have been investigated in seeds and fresh parts of members of the Fagaceae. Seeds from the genus *Fagus* contain willardiine, 5-hydroxy-6-methylpipecolic acids, *N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]azetidine-2-carboxylic acid and γ-glutamyl peptides, mainly γ-glutamylphenylalanine. These compounds are nearly or totally absent from leaves of *F. silvatica* and from seedlings and immature seeds of *F. silvatica* var. *purpurea*; instead, the seedlings contain large amounts of γ-L-glutamyl-L-isoleucine and γ-L-glutamyl-L-leucine. γ-L-Glutamyl-L-tryptophan and γ-L-glutamyl-γ-L-glutamyl-L-phenylalanine, not previously known from nature, have been isolated from seeds of *F. silvatica* var. *purpurea*. The structures have been confirmed by syntheses. 4-Hydroxypipecolic acid (with *trans*-configuration) has been identified in seeds of *F. japonica* Maxim. and *F. sieboldii* Endl. None of the above compounds was found in *Quercus* or *Castanea* species whereas argininosuccinic acid was identified in *Castanea sativa*.

INTRODUCTION

Seeds of *Fagus silvatica* L. (beechnuts) have previously been shown to contain a large number of non-protein amino acids and γ-glutamyl peptides [1–4]. In continuation of these studies, we now report on the free amino acids in fresh parts of *F. silvatica* and *F. silvatica* var. *purpurea* and in various other species of Fagaceae.

METHODS AND RESULTS

In Table 1 are listed the different species and parts of plants investigated. Fagaceae according to traditional classifications contains five genera, *Nothofagus*, *Fagus*, *Castanea*, *Pasania* and *Quercus* [5], but so far material from only three of these has been available. In the table are also recorded the occurrence of amino acids and γ-glutamyl peptides in the various samples. Only non-protein amino acids (and asparagine) are recorded, but as usual, most of the protein amino acids were also present. For comparison, the results obtained previously with seeds of *F. silvatica* [1–4] are included. Not included are compounds previously found in these seeds but not identified in any of the new samples investigated, i.e. γ-glutamyl dervatives of alanine, glutamic acid, methionine sulfoxide, phenylalanylwillardiine, and valine, glutathione, ornithine, N^5-acetylornithine, β-alanine, *N*-(3-amino-3-carboxypropyl)-azetidine-2-carboxylic acid, 3-(2-furoyl) alanine and hydroxyproline.

The methods used for identification and isolation are the same (see Experimental) as those used previously, [3]. For compounds new to the Fagaceae, more detailed information is now given.

γ-L-Glutamyl-L-tryptophan was obtained by chromatography of the total amino acid from *F. silvatica* var. *purpurea* on a strongly basic ion-exchange resin in the acetate form. Hydrolysis of the pure crystalline, material with 6 N HCl for 20 hr at 110° provided only glutamic acid, but hydrolysis with 1 N HCl for 1 hr at 100° gave both glutamic acid and tryptophan. γ-Glutamyl derivatives have low pK_2-values in comparison to α-glutamyl derivatives [6] just as pK_1 for glutamine is 2.17, whereas pK_1 for isoglutamine is 3.81. [7] Therefore, γ-glutamyl derivatives have greater mobility than the corresponding α-glutamyl derivatives in paper electrophoresis at pH 4. [8, 9] Correspondingly γ-glutamyl derivatives in high voltage paper electrophoresis (HVE) at pH 3.6 move fast towards the anode, whereas the α-glutamyl derivatives behave like neutral amino acids at this pH. The pK_2-values also determine the chromatographic behaviour of the compounds on strongly basic ion-exchange resins, permitting establishment of γ-glutamyl linkage [6]. Finally, PMR and IR spectra are indicative for γ-glutamyl derivatives [10–12]. The structure of the natural compound was supported by evidence obtained by all these methods, the PMR spectrum in D_2O exhibiting signals at 2.3 ppm (*m*, 4H), 3.35 (pair of *dd*, 2H), 3.8 (*t*, 1H), 4.8 (overlap with DOH) and 7.0–7.8 (*m*, 5H). The structure was confirmed by synthesis using standard methods [13,14], the synthetic compound being identical with the natural product in all respects, including optical rotation.

γ-L-Glutamyl-γ-L-glutamyl-L-phenylalanine was likewise obtained by chromatography of the total amino acid fraction for *F. silvatica* var. *purpurea* on a strongly basic ion-exchange resin. Strong acid hydrolysis gave glutamic acid and phenylalanine; mild hydrolysis (1 N HCl, 100°, 20 min) resulted in a mixture of unreacted starting material, γ-glutamylglutamic acid, [3], γ-glu-

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Table 1. Free amino acids and γ-glutamyl peptides in Fagaceae

Species	Part of plant	Non Protien Amino Acids																
		Acidic										Neutral						
		γ-L-Glutamyl-γ-L-glutamyl-L-phenylalanine	γ-L-Glutamyl-L-isoleucine + γ-L-glutamyl-L-leucine	γ-Glutamylphenylalanine	γ-L-Glutamyl-L-tryptophan	γ-Glutamyltyrosine	γ-Glutamylwillardine	γ-Aminobutyric acid	N-[N-(3-Amino-3-carboxypropyl)-3-amino-3-carboxypropyl]axetidine-2-carboxylic acid (nicotianamine)	Argininosuccinic acid	Asparagine	(2S,5R,6S)- and (2S,5S,6S)-5-hydroxy-6-methylpipecolic acid	*trans*-4-Hydroxypipecolic acid	Pipecolic acid	Willardiine (3-(1-uracil) alanine
Fagus silvatica L	seeds*		I X	I,C XXX		I X	I,C X	P (X)	I,C X		P X	I,C XX		I,C X	I,C XXX
	leaves and stems							P XXX	P (X)		P (X)				
F. silvatica var. *purpurea* Ait	seeds	I,C X		I,C XXX	I,C X		I XX	P X	P X		P XX	P XX			I XX
	immature seeds with husks							P XXX	P X		I XX	P (X)		P X	
	seedlings		I,C XX					P X	P (X)		I XXX				I (X)
F. silvatica var. *pendula* Loud	seeds			P XXX		P (X)	P X	P XX	P (X)		P X	P (X)	P (X)		I XX
F. japonica Maxim	seeds			P XXX		P (X)	P X	P XX	P (X)		P XX		E X	P (X)	I XX
F. orientalis Lipsky	seeds			P XXX		P (X)	P (X)	P XX			P (X)	P (X)			P X
F. sieboldii Endl	seeds			P XXX		P (X)		P X	P (X)		P (X)	P X	E XX		P (X)
Castanea sativa Miller	seeds							P XXX		I X	P XX			P X	
Quercus cerris L.	seeds							P XXX			P XX				
Q. robur L	seeds							P XXX			P XX				

*From Refs. 1–4.

I: Identification by isolation; C: Configuration determined; P: Identification by ion-exchange fractionation and paper chromatography; E: Identification by ion-exchange chromatography.
Relative concentrations of compounds indicated by XXX, XX, X and (X)

tamylphenylalanine, [3] glutamic acid, and phenylalanine. The PMR spectrum of the original compound in D_2O + NaOD showed signals at 2.16 ppm (*m*, 8H), 3.26 (*t*, 1H, pair of *dd*, 2H), 4.15 (*dd*, 1H), 4.55 (overlap with DOH) and 7.38 (*brs*, 5H) in agreement with the proposed structure [3, 10, 11]. Again, the structure was confirmed by synthesis, the synthetic compound being identical with the natural product in all respects including optical rotation.

γ-L-Glutamyl-L-isoleucine and γ-L-glutamyl-L-leucine were obtained as a 1:2 mixture by chromatography of the total amino acid fraction of *F. silvatica* var. *purpurea* seedlings on a strongly basic ion-exchange resin. The γ-glutamyl linkage of both compounds was demonstrated by the ion-exchange behaviour, HVE and by PMR spectroscopy in D_2O, displaying the α-proton in the glutamic acid moiety at 3.8 ppm. Acid hydrolysis gave a mixture which by amino acid analysis was shown to contain glutamic acid, isoleucine and leucine in the ratio 3:1:2. The hydrolysis mixture was fractionated on an ion-exchange resin to give glutamic acid and a mixture of isoleucine and leucine. L-Configurations of all three compounds were demonstrated by CD, showing a positive Cotton effect in H_2O at 200 nm [15]. The intensity of the peak from the isoleucine–leucine mixture was the same as that found for an authentic mixture of the two amino acids and also as that found for either isoleucine or leucine alone.

Argininosuccinic acid was obtained from seeds of *Castanea sativa* by ion-exchange fractionation, preparative paper chromatography and final ion-exchange purification (see Experimental). The PMR spectrum in D_2O exhibited signals at 1.89 ppm (*m*, 4H), 2.90 (*m*, 2H), 3.45 (*t*, 2H), 3.85 (*t*, 1H) and 4.58 (overlap with DOH). The ^{13}C NMR spectrum in D_2O exhibited signals at 24.6, 28.3, 37.8, 42.3, 55.0, 57.9, 175.0, 176.4 and 179.8 ppm. No signal was observed for the guanidine-carbon atom, probably because of the long relaxation time for this atom. The identity of the compound was established by comparison on an amino acid analyser with authentic material.

4-Hydroxypipecolic acid was identified in the fraction of neutral and basic amino acids from *F. sieboldii*. The amino acid was partly purified by paper and ion-exchange chromatography. This step removed most of the asparagine, glutamine and serine present, facilitating the subsequent identification on the amino acid analyser at pH 3.25, where 4-hydroxypipecolic acid is eluted very close to these compounds. 4-Hydroxypipecolic acid was identified on the amino acid analyser by the high ratio between the 440 and 570 nm absorption and by co-chromatography with authentic (2*S*, 4*S*)-4-hydroxypipecolic acid (the *trans*-compound). (2*S*, 5*R*)-5-Hydroxypipecolic acid was also used as reference material, giving a distinct peak of its own. The separation of the diastereoisomers of 4- and 5-hydroxypipecolic acid has previously

been accomplished by this procedure [16]. The identification was further substantiated by paper chromatography, where the natural compound and the authentic material had identical R_f-values and the same blue ninhydrin reaction. The absolute configuration of the natural compound has not been determined.

The same procedure was used for identification of the amino acid in *F. japonica*, whereas the identification in *F. silvatica* var. *pendula* Loud is based only on paper chromatography.

DISCUSSION

In previous communications, it was demonstrated that seeds of *F. silvatica* contain a large variety of non-protein amino acids and γ-glutamyl peptides [1–4]. Even with the restricted number of species investigated here, it seems likely that this pattern is characteristic of the genus *Fagus* but not of the whole family. In all the *Fagus* seeds investigated γ-glutamylphenylalanine is the main compound. Previously a large number of other γ-glutamyl derivatives were identified in *F. silvatica* L., but some of these were present in small concentrations and only found because of the large amount of seeds used and the very detailed analysis. [3] It is likely that most or all of these compounds are present in the other *Fagus* species in low concentrations. On the other hand, there is some variability, reflected in the identification of two new γ-glutamyl derivatives in *F. silvatica* var. *purpurea*. The two compounds are either absent or present only in minute concentrations in the original *F. silvatica* sample investigated.

Other substances besides γ-glutamyl derivatives are characteristic of the genus *Fagus*. Willardiine, previously found only in Leguminosae, is present in a high concentration in the species; also *N*-[*N*-(3-amino-3-carboxypropyl)- 3-amino-3-carboxypropyl] azetidine-2-carboxylic acid occurs in detectable quantities in *Fagus* species. The two isomers of 5-hydroxy-6-methylpipecolic acid are also present in all *Fagus* species, except *F. japonica*. The paper chromatographic identification used here does not permit their differentiation, but in *F. silvatica* L. the (2*S*, 5*S*, 6*S*)- and (2*S*, 5*R*, 6*S*)-compound were present in a ratio of about 2:1 [4]. 4-Hydroxypipecolic acid has previously been encountered in various plant families including Leguminosae [17]. The configuration of the amino acid from *Acacia* species is (2*S*, 4*S*) [18] whereas additional stereoisomers may be present in *Strophanthus scandens* [19]. At first sight, there might be a biogenetic relationship between the above 5-hydroxy-6-methylpipecolic acids and 4-hydroxypipecolic acid. However, as discussed previously, the basic carbon skeletons are rarely changed in non-protein plant amino acids, and it seems therefore unlikely that the methyl-substituted pipecolic acids are closely related to pipecolic acid itself and its hydroxylated derivatives [4].

Most of the compounds found in these seeds are either absent or present in small concentrations in other parts of the plants. Instead, asparagine and γ-aminobutyric acid are the major constituents. It is, however, remarkable that γ-glutamylisoleucine and γ-glutamylleucine are present in fairly high concentrations in seedlings of *F. silvatica* var. *purpurea*. Thus during germination in this species, the γ-glutamylphenylalanine disappears, whereas substantial concentrations of two new γ-glutamyl derivatives are formed.

γ-L-Glutamyl-γ-L-glutamyl-L-phenylalanine is a new example of a γ-glutamyl based tripeptide, being structurally related to the γ-glutamyl-γ-glutamylmethionine reported from *Phaseolus aureus* [20]. In both cases, these tripeptides occur together with large amounts of the corresponding dipeptides. The identification of argininosuccinic acid in *Castanea sativa* is not surprising. This amino acid is an intermediate in the biosynthesis of arginine and it has previously been identified in *Vicia faba* [21].

EXPERIMENTAL

General methods. Methods for determination of IR, CD, PMR, ^{13}C NMR and for preparative PC have previously been described [3]. PC was performed in *n*-BuOH–HOAc–H_2O (12:3:5) (solvent 1) and PhOH–H_2O–conc NH_3 (120:30:1) (w/v/v) (solvent 2) by descending technique on Whatman No. 1 paper. HVE was performed using a flat-plate unit (Shandon 1.24) on Whatman No. 3 MM paper at pH 3.6 (Py–HOAc–H_2O) (1:20:200) (buffer 1) and pH 6.5 (Py–HOAc–H_2O) (25:1:500) (buffer 2). Amino acid analysis was performed on a Beckman Model 120 C instrument.

Plant material. Seeds, immature seeds with husks and seedlings of *F. silvatica* var. *purpurea* were collected in natural habitats in Denmark. Leaves and stems of *F. silvatica* L. and seeds of *Q. cerris* and *Q. robur* were obtained from natural habitats in Denmark and seeds of *F. silvatica* var. *pendula*, *F. orientalis*, and *C. sativa* from the Arboretum, Royal Veterinary and Agricultural University, Denmark, through the assistance of Dr. B. Søegaard. Seeds of *F. japonica* were collected in natural habitats on Honshu, Japan by Prof. I. Murakoshi, Chiba University, and seeds of *F. sieboldii* were collected in natural habitats on Hokkaido, Japan, by Professor S. Sakamura, Hokkaido University.

Reference compounds. (2*S*,4*S*)-4-Hydroxypipecolic acid was obtained from Professor J. W. Clark-Lewis, University of Adelaide, Australia. Argininosuccinic acid was obtained from Calbiochem. Other reference compounds have been described in the previous publications.

Standard procedure. The plant material (*ca* 25 g) was disintegrated and extracted with 150 ml of 70% EtOH at room temp. The extract was concd to near dryness, the residue suspended in H_2O, the suspension filtered, and the filtrate applied to an Amberlite IR 120 (H^+) column. After flushing with H_2O, the total fraction of amino acids was eluted with 2N NH_3. An aq. soln of the amino acids was subsequently applied to a Dowex 1 × 8 (AcO^-) column. Neutral and basic amino acids occurred in the effluent and acidic compounds were eluted with 2N HOAc. The various fractions were subjected to 2D PC. Both from the total amino acid fraction and from the fraction of neutral and basic amino acids crystalline ppts of asparagine or willardiine were isolated. These were identified as willardiine or asparagine by IR spectroscopy. The IR spectra of optically active and racemic willardiine are distinctly different [2]. The willardiine samples isolated represented optically active material, presumably the L-form.

Isolation of γ-L-glutamyl-L-tryptophan and γ-L-glutamyl-γ-L-glutamyl-L-phenylalanine from seeds of F. silvatica *var.* purpurea. The isolation was performed according to the standard procedure starting with 330 g of seeds. The residue from the EtOH–H_2O extract (32 g) gave an amino acid fraction (5.4 g) which was applied to a Dowex 1 × 8 column (200–400 mesh, AcO^-, 1 × 38 cm). Neutral and basic amino acids were washed out with H_2O, and the column eluted with 0.5 N HOAc (0.55 l.) and 2 N HOAc (1 l.). Fractions of 12 ml were collected. Fractions 1–15 (1170 mg) contained aspartic acid, glutamic acid and γ-glutamylwillardiine, fractions 16–45 (1180 mg) contained γ-glutamylphenylalanine and γ-glutamylwillardiine, fractions 61–78 (90 mg) contained γ-glutamyltryptophan, and fractions 94–130 (65 mg) γ-glutamyl-γ-glutamylphenlalanine The residue from fractions

61–78 was subjected to preparative PC in solvent 1 with subsequent purification on small Dowex 1 × 8 column, to give a crystalline evapn residue of γ-L-glutamyl-L-tryptophan (55 mg), pure according to PC and HVE. Recrystallization was performed from H_2O. R_f in solvent 1, 0.38; in solvent 2, 0.58. Migration ratio to glutamic acid in HVE in buffer 1, 1.75; in buffer 2, 0.54. IR ν_{max}^{KBr} cm^{-1}: 3440 (s), 3320 (m), 3080 (m), 2960 (m), 1700 (s), 1620 (s), 1540 (s), 1470 (m), 1450 (m), 1430 (m), 1390 (m), 1370 (m), 1330 (m), 1300 (w), 1290 (w), 1270 (m), 1240 (s), 1200 (m), 1160 (w), 1140 (w), 1120 (w), 1105 (m), 1080 (w), 1020 (w), 1000 (w), 950 (w), 870 (w), 835 (w), 820 (m), 775 (w), 760 (s), 670 (m), 620 (m), 590 (m), 570 (m), 550 (w), 530 (m), 505 (m), 500 (m), 470 (m), 440 (m), 370 (w), 340 (m), 320 (w), $[\alpha]_D^{23}$ + 23.0° (*c* 0.9, 1 N HCl). For PMR data in D_2O see Results.

The residue from fractions 94–130 was subjected to preparative PC in solvent 1 and purification on a small Dowex 1 × 8 column to give a crystalline evapn residue of γ-L-glutamyl-γ-L-glutamyl-L-phenylalanine (35 mg), pure according to PC and HVE. Recrystallization was performed from H_2O. R_f in solvent 1, 0.33; in solvent 2, 0.33. Migration ratio to glutamic acid in buffer 1, 3.76; in buffer 2, 1.01. IR ν_{max}^{KBr} cm^{-1}: 3400 (s), 3360 (s), 2960 (m), 2600 (w), 1730 (s), 1670 (s), 1650 (s), 1580 (m), 1530 (s), 1460 (w), 1450 (w), 1420 (w), 1410 (m), 1350 (m), 1305 (m), 1285 (m), 1260 (m), 1240 (m), 1220 (s), 1130 (w), 1120 (w), 1100 (w), 1065 (w), 1040 (w), 970 (w), 930 (w), 920 (w), 895 (w), 880 (w), 855 (w), 790 (w), 780 (w), 765 (w), 755 (w), 710 (m), 640 (w), 600 (w), 575 (m), 540 (w), 505 (w), 440 (w), 340 (w), 300 (w). $[\alpha]_D^{23}$ + 13.4° (*c* 0.4, 1 N HCl). For PMR data in D_2O + NaOD, see Results.

Isolation of γ-L-glutamyl-L-isoleucine and γ-L-glutamyl-L-leucine from seedlings of F. silvatica *var.* purpurea. The isolation was performed according to the standard procedure using H_2O instead of EtOH–H_2O and starting with 28 g of seedlings. The total amino acid fraction was passed through a strongly acid ion-exchange resin in the NH_4^+ form (Amberlite IR 120, 1.5 × 28 cm), and acidic and neutral amino acids (450 mg) washed out with H_2O. Asparagine (270 mg) was removed from this fraction by crystallization, and the amino acids remaining in soln applied to a Dowex 1 × 8 (AcO^-) column. Neutral amino acids (105 mg) were washed out with H_2O, and acidic compounds (37 mg) eluted with 2 N HOAc. The fraction of acidic compounds were again applied to a Dowex 1 × 8 (200–400 mesh, 0.7 × 2.6 cm, AcO^-) resin and the column eluted with 0.2 N HOAc, fractions being collected. Fractions containing the 2 γ-glutamyl derivatives were pooled, and final purification accomplished by preparative PC and ion-exchange purification to give 4.6 mg of crystalline material. The material behaved as a single compound in PC and HVE (migration ratio to glutamic acid in buffer 1, 1.81) but PMR spectroscopy demonstrated the presence of both the isoleucine and the leucine moiety. Quantitative amino acid analysis of an acid hydrolysate showed glutamic acid, isoleucine and leucine (3:1:2). 2 mg of acid hydrolysate was applied to a Dowex 1 × 8 (AcO^-) column to give isoleucine and leucine in the effluent and glutamic acid in the HOAc eluate. The 2 fractions were used for CD measurements.

Isolation of argininosuccinic acid from Castanea sativa. Argininosuccinic acid behaves as a very weakly acidic amino acid, moving towards the cathode at pH 3.6. It is only partly retained on a strongly basic ion-exchange resin in the AcO^- form and easily eluted with 0.2 N HOAc. 640 g of seeds were extracted giving a total amino acid fraction of 12.8 g. The amino acid was obtained from this fraction by repeated passages through a Dowex 1 × 8 (200–400 mesh, AcO^-, 1 × 38 cm) column, collecting the first fractions in the 0.2 N HOAc eluate. Further purification was accomplished on strongly acidic ion-exchange resin, preparative PC in solvent 1, and Dowex 1 × 8 resin to give crystalline material (32 mg), pure according to PC and HVE. R_f in solvent 1, 0.13, in solvent 2, 0.26. Migration ratio to glutamic acid in HVE in buffer 1, −5.7 (glutamic acid moves towards the anode at this pH); in buffer 2, 0.61. For PMR and ^{13}C NMR data, see Results. Automatic amino acid analysis was performed at pH 3.25 and 4.20. A symmetric peak was obtained both for the prepn itself and for a mixture with authentic argininosuccinic acid.

Synthesis of γ-L-glutamyl-L-tryptophan. N-Benzyloxycarbonyl-L-glutamic anhydride [13] (0.015 mol) in DMF (7.8 g) was condensed with L-tryptophan (0.015 mol) in the presence of NEt_3 (0.045 mol) in H_2O (80 ml) for 20 min at room temp. [14]. Pd black (1.2 g) was added, and after hydrogenation for 7.5 hr, the catalyst was removed by filtration, and the amino acids and peptides isolated from the reaction mixture by adsorption to a strongly acid ion-exchange resin with subsequent elution with 2 N NH_4. Separation of the components was accomplished on a Dowex 1 × 8 (200–400 mesh, AcO^-, 1 × 38 cm) column. After flushing with H_2O, elution was performed with 2 N HOAc fractions of 18 ml being collected. α-L-Glutamyl-L-tryptophan was found as a crystalline residue (0.003 mol) by concn of fractions 3–6, and γ-L-glutamyl-L-tryptophan as a crystalline residue (0.004 mol) by concn of fractions 12–17. Final purification of the γ-glutamyl derivative was obtained by recrystallization from H_2O to give a product identical in all properties with the natural compound $[\alpha]_D^{23}$ + 23.5° (*c* 0.9, 1 N HCl).

Synthesis of γ-L-glutamyl-γ-L-glutamyl-L-phenylalanine. N-Benzyloxycarbonyl-L-glutamic anhydride (0.004 mol) in DMF (2.1 g) was condensed with γ-L-glutamyl-L-phenylalanine (0.004 mol) in the presence of NEt_3 (0.016 mol) in H_2O (5 ml) for 20 min. Catalytic hydrogenation, isolation of a fraction of amino acids and peptides, and chromatography on a basic ion-exchange resin was performed as described above. α-L-Glutamyl-γ-L-glutamyl-L-phenylalanine and unreacted γ-L-glutamyl-L-phenylalanine were eluted in fractions 3–8. γ-L-Glutamyl-γ-L-glutamyl-L-phenylalanine was obtained as a crystalline residue (0.9 mmol) from fractions 26–55. Recrystallization from H_2O afforded a sample identical in all properties with the natural compound. $[\alpha]_D^{23}$ + 15.0° (*c* 0.4, N HCl).

Acknowledgements—The authors are greatly indebted to the persons mentioned above for their help. The work was performed while one of us (TK) was on leave of absence from Hokkaido University, Sapporo, Japan, supported by grants from the Danish Ministry of Education as part of the Japanese–Danish Cultural Agreement, and by a grant from the Danish National Science Research Council. We are indebted for this support and also for support from the Research Council for instrumentation.

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