Phytochemistry, 1978, Vol. 17, p. 2027 © Pergamon Press Ltd. Printed in England. 0031-9422/78/1101-2027 $02.00/0

SHORT REPORTS

γ-L-GLUTAMYL-L-LATHYRINE FROM *LATHYRUS JAPONICUS*

Shin-Ichi Hatanaka and Setsuko Kaneko
Department of Biology, College of General Education, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

(*Received* 1 *May* 1978)

Key Word Index—*Lathyrus japonicus*; Leguminosae; γ-L-glutamyl-L-lathyrine.

INTRODUCTION

Lathyrine, β-(2-aminopyrimidin-4-yl)alanine, is a nonprotein amino acid, which was isolated from the seeds of *Lathyrus tingitanus* [1] and shown to be distributed in some other species of *Lathyrus* [2]. Previously we reported the occurrence of *cis*-5-hydroxy-L-pipecolic acid in the seeds of *Lathyrus japonicus* Willd. [3]. During this work we noticed the presence of another acidic ninhydrin-positive substance. We report now the isolation and characterization of γ-L-glutamyl-L-lathyrine, which has not been reported previously. L-*erythro*-γ-methyl-glutamic acid has already been isolated from the same species [4].

RESULTS

γ-Glutamyl-L-lathyrine was eluted before glutamic acid from a column of Dowex 1 in acetate form. The results of elementary analysis and the determination of water of crystallization were in good agreement with the formula $C_{12}H_{17}N_5O_5 \cdot 2H_2O$. Mild hydrolysis gave L-glutamic acid and L-lathyrine in the ratio 1:1. Analysis by TLC of the hydrolysates of the dansyl-derivative [5, 6] and the determination of CO_2 and NH_3 evolved in the reaction with ninhydrin [7] indicated that the γ-glutamyl residue is attached to the amino group of the side chain of lathyrine.

EXPERIMENTAL

Mps were determined in capillary tubes and uncorr. Solvents were evapd in a rotary evaporator below 40°.

Plant. The material was the same as that used for the isolation of *cis*-5-hydroxy-L-pipecolic acid [3].

Isolation of γ-glutamyllathyrine. Seeds (360 g) were ground in a mill, defatted with Et_2O (3 l.), and dried in air. They were then extracted with 80% EtOH ×4 and the residue was soaked in the same solvent. The combined extract (10.6 l.) was concd and passed through Amberlite IR-120 (H^+) (300 ml). The amino acids were eluted with 2 M NH_4OH (3 l.), concd, and fractionated on a column of Dowex 1 × 4 (AcO^-, 90 × 4.2 cm) with 0.2 M HOAc. The relevant fractions were combined, concd and treated with activated charcoal. On further concn of the filtrate the crystals were obtained (260 mg). They were recrystallized ×3 from H_2O, mp 162.5° (decomp). (Found: C, 41.65; H, 5.77; N, 20.33. $C_{12}H_{17}N_5O_5 \cdot 2H_2O$ requires: C, 41.50; H, 6.09; N, 20.16%). H_2O of crystallization (loss of the weight at 110° ± 1°, 2 mmHg). (Found: 11.2: Calc.: 10.4%). $\lambda_{\max}^{\mathrm{pH}1}$ nm (log ε): 222 (4.1), 300 (3.6), $\lambda_{\max}^{\mathrm{pH}13}$: 228 (4.0), 293 (3.6). CO_2 and NH_3 evolved in the reaction with ninhydrin [7]: 0.85 and 1.17 mol/mol, respectively.

Hydrolysis. The peptide (230 mg, 0.66 mmol) was dissolved in M HCl (12 ml) and heated at 100° for 3 hr. HCl was removed by evapn *in vacuo* and fractionated on a Dowex 1-column (AcO^-, 23 × 0.8 cm) with 0.2 M HOAc. Concn of the relevant fractions gave the crystals of free L-lathyrine (120 mg, 0.66 mmol) and L-glutamic acid (91 mg, 0.62 mmol), respectively. L-Lathyrine hydrochloride was prepared for analysis. mp >*ca* 170° (decomp). $[\alpha]_D^{25}$ −6.6° (H_2O: *c* 1.6) (Found: C, 30.61; H, 5.19; N, 20.53; Cl, 25.36. Calc. for $C_{17}H_{10}N_4O_2 \cdot 2HCl \cdot H_2O$: C, 30.78; H, 5.17; N, 20.51; Cl, 25.96%). H_2O of crystallization (loss of the weight at 110–115°, 4 mmHg). (Found: 6.6; Calc.: 6.6%). $\lambda_{\max}^{\mathrm{pH}1}$ nm (log ε): 224 (4.2), 298 (3.7), $\lambda_{\max}^{\mathrm{pH}13}$ 226 (4.1), 292 (3.6). L-Glutamic acid: mp 186° (decomp), $[\alpha]_D^{25}$ +11.7° (H_2O: *c* 1.5), +30.0° (3 M HCl: *c* 0.75). (Found: C, 40.60; H, 6.18; N, 9.62. Calc. for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52%).

Comparison of the IR and TLC (*n*-BuOH–HOAc–H_2O, PhOH–H_2O) with those of the authentic L-lathyrine and L-glutamic acid were also satisfactory.

Chromatographic data. R_f values of γ-glutamyl-lathyrine on cellulose TLC with *n*-BuOH–HOAc–H_2O (63:10:27) and PhOH–H_2O (25:9) are 0.07 and 0.67, respectively and those of free lathyrine under the same conditions 0.12 and 0.83, respectively.

Acknowledgment—We wish to thank Prof. F. M. Strong, University of Wisconsin, for his kind supply of the authentic L-lathyrine.

REFERENCES

1. Bell, E. A. (1961) *Biochim. Biophys. Acta* **47**, 602.
2. Bell, E. A. (1962) *Biochem. J.* **83**, 225.
3. Hatanaka, S.-I. and Kaneko, S. (1977) *Phytochemistry* **16**, 1041.
4. Przybylska, J. and Strong, F. M. (1968) *Phytochemistry* **7**, 471.
5. Gray, W. R. (1967) *Methods Enzymol.* **11**, 139.
6. Weiner, A. M., Platt, T. and Weber, K. (1972) *J. Biol. Chem.* **247**, 3242.
7. Linko, P. (1955) *Suom. Kemistil.* **28B**, 96.