The author expresses his gratitude to Dr. H. Kurata of this Laboratory for his kind encouragement and guidance. Thanks are due to Dr. S. Sakai and Dr. I. Kitagawa of the Faculty of Pharmaceutical Sciences, University of Tokyo, for elementary analyses, and to Dr. T. Oba of this Institute for spectral measurements.

(Received September 24, 1960)

UDC 547.747

Takuo Kosuge and Hiroko Kamiya : L-Leucyl-L-proline from Peptone.

(*Shizuoka College of Pharmacy*[*1])

During investigation of antifungal principles derived from culture of a *Bacillus subtilis* strain, a crystalline matter was obtained as one of the by-products. It was necessary to decide whether this substance was a metabolite of the organism or originally contained in peptone used as a dietary essential. The same procedure employed as above afforded the same crystals from commercial peptone, Mikuni Pepton, proving the crystals as a component of peptone. This peptone was extracted with hot chloroform and chromatography over silica gel column gave a crystalline substance.

Purification was effected by recrystallization from benzene-petroleum ether mixture. The purified colorless crystals needles melted at 159.5° and were readily soluble in alcohols, chloroform, and benzene, and insoluble in water, ether, and hydrocarbons.

Elemental analysis and molecular weight measurement by the Rast method indicated molecular formula of $C_{11}H_{18}O_2N_2$. The infrared spectrum showed absorptions at 3260, 1670, and 1630 cm^{-1}, no absorption at 1550 cm^{-1} region, which is characteristic of cyclic amides. Significant optical rotation in methanol solution was observed at $[\alpha]_D$ −132.8°.

Quantitative analysis of the amino acids was carried out by paper chromatographic method. Hydrolysis with 6*N* hydrochloric acid in sealed tube at 110° for 20 hours yielded amino acids and the quantitative analysis by paper chromatography indicated equimolar quantities of L-proline and L-leucine.

All data are in agreement for cyclic dipeptide composed of two amino acids, L-proline and L-leucine. The isolated dipeptide was identical with synthesized L-leucyl-L-proline[1] in mixed melting point and infrared spectrum. This would be identical with the dipeptide reported by Abderhalden[2] as probably L-prolyl-L-leucine anhydride obtained from a tryptic digest of gliadin. The same isolation procedure was employed on milk casein, but the same substance was not obtained.

Experimental

Extraction of Dipeptide——To 100 g. of peptone 300 cc. of water was added and the mixture was extracted with three 150-cc. portions of $CHCl_3$. $CHCl_3$ extract was dried over anhyd. Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was submitted to column chromatography on silica gel with $CHCl_3$. A part of $CHCl_3$ eluates contained crude dipeptide. Its recrystallization from benzene-petr. ether mixture gave 100 mg. of a pure compound, m.p. 159.5°; mol. wt., 215(camphor). *Anal.* Calcd. for $C_{11}H_{18}N_2O_2$: C, 62.85; H, 8.56; N, 13.32. Found : C, 62.79; H, 8.59; N, 13.16.

*1 160 Oshika, Shizuoka (小菅卓夫, 神谷弘子).

1) R. E. Neuman, E. L. Smith : J. Biol. Chem., **193**, 97 (1951).
2) E. Abderhalden : Z. physiol. Chem., **128**, 123 (1923).

Identification of Amino Acids by Paper Chromatography——Twenty mg. of the dipeptide was hydrolyzed with 2 cc. of 20% HCl for 20 hr. in a sealed tube at 110° and the solution was evaporated to dryness *in vacuo*. Water was added to the residue and the solution was again evaporated to dryness *in vacuo* to remove HCl. The residue was submitted to one-dimentional ascending paper chromatography. The solvent systems used were BuOH-AcOH-H_2O (4:1:2) and *tert*-AmOH saturated with phthalic acid buffer solution (pH 6). The filter paper used was Toyo Roshi No. 51, 2×50 cm. The chromatograms were developed for 5 hr. at room temperature. The results are shown in Tables I and II.

Table I.

		Rf	
Standard Amino Acids	L-Hydroxyproline	0.266	
	L-Proline	0.366	
	L-Leucine	0.693	
	L-Valine	0.550	
Sample	No. 1	0.052	0.239
	No. 2	0.050	0.218

Solvent System : BuOH-AcOH-H_2O=4:1:2

Table II.

		Rf	
Standard Amino Acids	L-Proline	0.051	
	L-Leucine	0.219	
	L-Isoleucine	0.175	
Sample	No. 1	0.052	0.239
	No. 2	0.050	0.218

Solvent system : *tert*-AmOH saturated with phthalic acid buffer solution.

Quantitative Analysis by Paper Chromatography——In a sealed tube, 7 mg. of the dipeptide was hydrolyzed with 2 cc. of 20% HCl for 20 hr. at 110° and the solution was evaporated to dryness *in vacuo* several times to remove HCl. To the residue, 0.4 cc. of water was added and this solution was used for analysis. The solvent system used was BuOH-AcOH-H_2O (4:1:2) and the filter paper was Toyo Roshi No. 51. The hydrolyzed solution was spotted in a range of 0.002~0.003 cc. (50~60 γ). The Rf area of L-proline and L-leucine was cut off and extracted with 1 cc. of water. The weight of the both amino acids was determined by the Yemm and Cocking's colorimetric method[3] and the result is shown in Table III.

Table III.

Sample (γ)	Leucine (γ)	Proline (γ)
1 (43.0)	25.2	20.0
2 (59.3)	30.0	31.5

The authors are deeply indebted to Dr. E.L. Smith who supplied synthesized L-leucyl-L-proline. The authors are also indebted to Misses K. Saito and S. Tanaka for elemental analysis.

Summary

L-Leucyl-L-proline anhydride was isolated from peptone and identified with synthesized L-leucyl-L-proline.

(Received February 2, 1961)

3) E.W. Yemm, E.C. Cocking : Analyst, **80**, 209 (1953).