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Formation of Thiohydantoin Derivative of Proline from C-Terminal of Peptides

Sequential analysis of peptides or proteins with stepwise degradation from C-terminal amino acid has recently been reported by several authors.[1,2] However the reaction conditions were so vigorous and resulted in poor thiohydantoin formation, and the methods have not been applicable for proline and hydroxyproline.

We found a more mild condition which led to quantitative ring formation and further was successful to obtain a thiohydantoin derivative of proline.

Acetylamino acids were employed for quantitative investigation of thiohydantoin formation and the following condition was selected: The 10^{-6} mole of acetylamino acid is dissolved in a mixture of 10 μl of trifluoroacetic acid and 200 μl of acetylchloride and the solution is kept for 15 min at 30°. Then to the solution is added with 200 μl of 3% thiocyanic acid in dioxane and the solution is kept for 60 min at 30°. The solution of thiocyanic acid is prepared by ion exchange resin (Amberlyst-15 H^+ form) treatment from a dioxane solution of triethylamine thiocyanate. The solution is stable for some weeks when sealed and stored in a refrigerator. After evaporation of solvent *in vacuo*, acetylthiohydantoin is determined by gas chromatography after treatment with N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane in pyridine, using a glass tube packed with 2% XF-1105 on Gaschrom P.

Many acetylamino acids were quantitatively converted to the 1-acetyl-2-thiohydantoin derivatives as shown in Table I.

Under this experimental condition acetylproline gave also thiohydantoin derivative in 81% yield. This material had an mp 171—173° and elementally analytical data (*Anal.* Calcd. for $C_6H_8ON_2S$: C, 46.15; H, 5.16; N, 17.94. Found: C, 45.96; H, 5.28; N, 17.96) to be proved as deacetylated thiohydantoin derivative of proline. All the other data on infrared (IR) spectrum and ultraviolet (UV) spectrum supported thiohydantoin structure as shown in Fig. 1 and 2.

These results suggest that C-terminal prolyl residue is directly converted to thiohydantoin

1) G.R. Stark, *Biochemistry*, **7**, 1796 (1968).
2) L.D. Cromwell and G.R. Stark, *Biochemistry*, **8**, 4735 (1969).

Table I. Formation of Acetylthiohydantoin Derivatives from Acetylamino Acids

Acetylamino acid	Yield	Acetylamino acid	Yield
Alanine	100%	methionine	100%
Glycine	100%	phenylalanine	100%
Glutamic acid	100%	proline[a)]	81%
Histidine	100%	tryptophan	100%
Isoleucine	100%	tyrosine	100%
Leucine	100%	valine	100%
Lysine	100%		

a) deacetylated thiohydantoin derivative

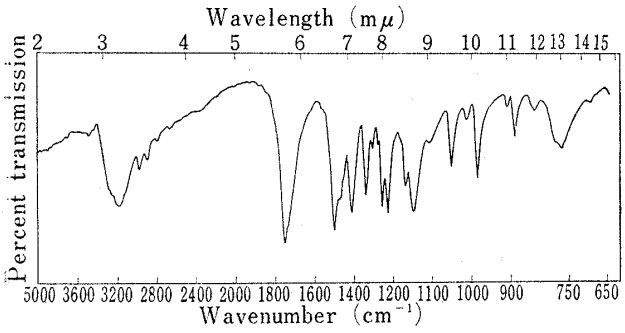

Fig. 1. Infrared Spectrum of Thiohydantoin Derivative of Proline (KBr)

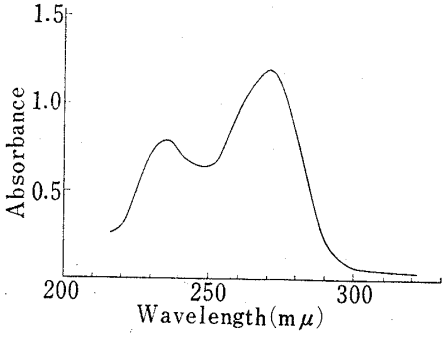

Fig. 2. Ultraviolet Spectrum of Thiohydantoin Derivative of Proline

concentration: 7×10^{-5}M solvent: EtOH

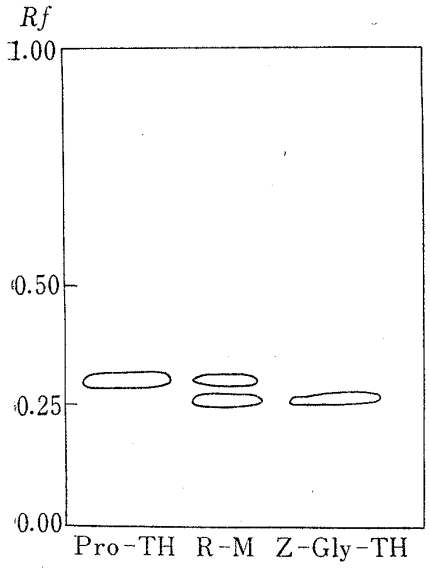

Fig. 3. A Chromatogram of Thiohydantoin Derivatives on a Thin-Layer of "Wakogel B-5 UV"

Z-Gly-TH: Z-glycylthiohydantoin
R-M : reaction mixture
Pro-TH : prolylthiohydantoin
[solvent, $CHCl_3$: HCOOH=100:5(v/v)]

derivative during the first step without any treatment of hydrolysis. In fact Z-glycylproline showed two spots of prolylthiohydantoin and Z-glycylthiohydantoin on a thin-layer containing the mixed fluorescent substance,[3)] (Fig. 3) and other peptide such as Z-glycylprolylleucylproline also showed the spot of prolylthiohydantoin on the thin-layer.

Selective liberation of a thiohydantoin from a peptidylthiohydantoin except proline thiohydantoin was occured in a good yield by treatment with 0.5M triethylamine. Even 10^{-7}—10^{-8} g of thiohydantoin derivatives were detected on a thin-layer containing the mixed fluorescent substance.

The details and applications of the method for peptides will be published in the near future.

3) Z. Tamura, C.S. Kim, N. Hosoda, S. Takitani, M. Suzuki, M. Suzuki and M. Inoue, *Bunseki Kagaku*, **19**, 518 (1970).

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