ALKALINE HYDROLYSIS OF PHENYLTHIOHYDANTOINS OF AMINO ACIDS

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A previous communication from this laboratory (Van Orden and Carpenter, 1964) described the results obtained on the hydrolysis of a number of 3-phenyl-2-thiohydantoins under various acidic and alkaline conditions. The best recoveries of the constituent amino acids were obtained under alkaline conditions (0.1 N NaOH for 12 hours at 120°) similar to those used by Stark and Smyth (1963) for the hydrolysis of hydantoins of amino acids. The recoveries for most of the alkali-stable amino acids ranged from 70 to 100% of the theory. In the performance of these experiments 1 to 1.5 μmole of each of as many as 11 different thiohydantoins were admixed before being subjected to alkaline hydrolysis. As a result, the actual hydrolysis mixture contained as much as a total of 16.5 μmoles of thiohydantoins per 2 ml of 0.1 N NaOH. Recently, the increased sensitivity of the amino acid analyzer has allowed us to perform the alkaline hydrolysis on decreased amounts of thiohydantoins (less than 1 μmole of total thiohydantoin per 2 ml of 0.1 N NaOH). In these latter experiments the recoveries of amino acids from the thiohydantoins were much inferior to those previously reported, indicating that the recovery of amino acid was dependent on the amount of phenylthiohydantoin in the hydrolysis tube. The investigations reported here were undertaken to establish this possibility and to ascertain the reasons for it.

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EXPERIMENTAL

Aliquots of solutions of the 3-phenyl-2-thiohydantoins (0.5 μmole/ml in ethyl acetate or aqueous acetone) (Van Orden and Carpenter, 1964) were transferred to constricted ignition tubes and the solvent was removed under a stream of nitrogen at 40°. Hydrolysis was effected by 2 ml of 0.1 N NaOH for 12 hours at 120°. Preparation for hydrolysis was performed in two different ways. Regular. The alkali was taken from a bottle from which no attempt had been made to exclude oxygen. The contents of the tubes were frozen in a dry ice bath. The tubes were evacuated on a mechanical pump and then sealed under vacuum. Oxygen-free. Nitrogen was bubbled through the alkali for four hours and the alkali was stored under an atmosphere of nitrogen using a three bottle system similar to that of Moore and Stein (1948) with the exception that the third bottle in the train contained Feiser's solution (Feiser, 1924). The ignition tube containing the solid phenylthiohydantoin was chilled in a dry ice bath and flushed with nitrogen. The oxygen-free alkali was introduced into the tube under a constant stream of N_2 which was maintained until the alkali had frozen. The tube was evacuated immediately on a mechanical pump and sealed.

After hydrolysis the contents of each tube were acidified with 3 ml of 0.2 N HCl and taken to dryness on a rotatory evaporator at 40°. The residues were dissolved in pH 2.2 citrate buffer (Moore and Stein, 1954) and subjected to amino acid analysis on a Beckman/Spinco analyzer in which the recorder was equipped with an expanded range card allowing measurement of amino acids down to about 0.005 μmole per sample.

RESULTS

Effect of Amount Hydrolyzed. A series of hydrolyses was performed under the regular conditions in which a constant amount (1.01 μmole) of

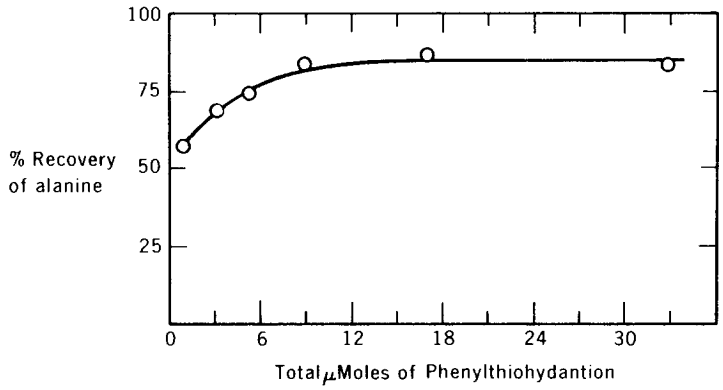

Fig. 1 1.01μMoles of phenylthiohydantoin of alanine plus sufficient phenylthiohydantoin of α-amino butyric acid to bring the total amount to that shown were hydrolyzed in 2 ml of 0.1 N NaOH under Regular conditions.

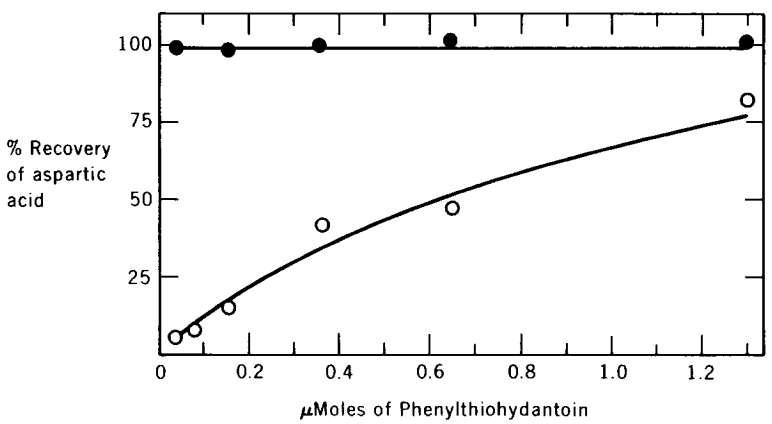

Fig. 2 Increasing amounts of the phenylthiohydantoin prepared from aspartic acid were subjected to alkaline hydrolysis under Regular (O—O) and Oxygen-Free (●—●) conditions.

the phenylthiohydantoin prepared from alanine was admixed with increasing amounts (2 to 32 μmoles) of the phenylthiohydantoin prepared from α-amino-n-butyric acid. The results (Fig. 1) show that the recovery of alanine increased from 55 to 85% of the theoretical amount as the total amount of phenylthiohydantoin was increased.

Effect of Oxygen. Paired sets of tubes containing increasing amounts of the phenylthiohydantoin prepared from aspartic acid were

hydrolyzed by the regular and oxygen-free procedures with the results shown in Figure 2. When hydrolysis was performed by the regular procedure, the recovery of aspartic acid was virtually nil at levels below 0.1 μmole/tube. The recoveries gradually improved with increasing amounts of phenylthiohydantoin in the ignition tube, reaching a value of about 85% when the tube contained 1.3 μmole of phenylthiohydantoin. When hydrolysis was performed by the oxygen-free technique, virtually 100% recovery of aspartic acid was obtained over the range 0.04 to 1.3 μmole per tube.

Regular versus Oxygen-free Hydrolysis. Paired hydrolysis tubes each containing 0.5 μmole of one of the phenylthiohydantoins were subjected to hydrolysis under the regular or oxygen-free conditions. The results obtained on a number of phenylthiohydantoins are shown in Table I.

Under either condition of hydrolysis, cysteic acid, cystine and serine were not recovered. Arginine was converted to ornithine and threonine was recovered in part as glycine. Methionine was recovered as such, not as the sulfoxide. For the alkali-stable amino acids the oxygen-free conditions yielded recoveries of 60-100% of the constituent amino acid with the majority approaching the theoretical yield. These were much superior to the recoveries obtained upon hydrolysis of the same amounts of phenylthiohydantoin by the regular procedure.

In the alkaline hydrolysis of all of the phenylthiohydantoins, the amino acid analyzer detected small amounts of material appearing at elution volumes identical with threonine, glycine, and alanine. Since these peaks were also detected when phenylthioisocyanate was hydrolyzed, they are probably due to some decomposition products of the phenylthiocarbamyl group. Because these peaks amounted to no more than 1% of the main amino acid peak, they did not give rise to any ambiguities in the interpretation of most of the chromatograms. However, in calculating

TABLE I

HYDROLYSIS OF PHENYLTHIOHYDANTOINS

Phenylthiohydantoin Hydrolyzed	Amino Acid Recovered,%		Phenylthiohydantoin Hydrolyzed	Amino Acid Recovered,%	
	Regular	O_2-Free		Regular	O_2-Free
Tryptophan	36	83	Glutamine as Glutamic Acid	4	59
Lysine	31	81	Proline	61	91
Histidine	14	100	Glycine	45	77
Arginine	8	0	Alanine	32	83
as Ornithine	11	45			
			Cystine	-	0
Cysteic Acid	< 1	< 1			
			Valine	34	102
Aspartic Acid	47	99			
			Methionine	20	95
Asparagine as Aspartic Acid	11	88	Isoleucine	14	41
			as Alloisoleucine	17	53
Threonine as Glycine	17	64	Leucine	26	88
Serine	-	< 1	Tyrosine	37	96
Glutamic Acid	13	66	Phenylalanine	47	103

the results shown in Figure 1 where in some instances a small amount of alanine derivative was hydrolyzed in the presence of a large amount of the derivative of α-amino-n-butyric acid, it was necessary to correct the alanine recoveries for the amount of artifactual material chromatographing as alanine that is formed upon hydrolysis of the derivative from α-amino-n-butyric acid.

DISCUSSION

In the previous report of Van Orden and Carpenter (1964) it was suggested that, since most of the phenylthiohydantoins could be hydro-

lyzed to their constituent amino acids in good yields, this hydrolysis procedure could be used in determination of the N-terminal amino acid released in the Edman (1950) degradation procedure. This in fact proved to be the case when it was applied to the Edman degradation of insulin through five consecutive steps (Van Orden and Carpenter, unpublished). In these investigations, however, we were largely interested in obtaining the degraded insulin for other studies and the degradation was performed on a large scale. As a consequence 10 to 15 μmoles of the cyclization products were subjected to alkaline hydrolysis which resulted in excellent recoveries of the constituent amino acids. It was only when the degradation was performed on a scale comparable to that used in protein structure studies that the poor recoveries on the hydrolysis of small amounts of phenylthiohydantoins were encountered.

The fact that the recovery of amino acid in the hydrolysis under regular conditions is a function of the amount of phenylthiohydantoin subjected to hydrolysis (Fig. 2) indicates that the hydrolysis mixture contains some ingredient that reacts with a fixed amount of the phenylthiohydantoin either destroying the constituent amino acid or converting the hydantoin into a nonhydrolyzable compound. We are indebted to W. F. Benisek for suggesting that this constituent of the hydrolysis medium might be oxygen.

In the regular procedure for hydrolysis, the contents of the tubes are frozen and evacuated on a mechanical pump which should remove all but the dissolved oxygen. Attempts to remove the latter by allowing the contents to thaw under vacuum (the standard procedure for acid hydrolysis) encountered considerable difficulty in that the alkaline solutions tended to bump very badly. We were only able to obtain consistent results by using the deoxygenated alkali as described under the oxygen-free conditions.

With the oxygen-free conditions, excellent recoveries of the

constituent amino acids can be obtained upon alkaline hydrolysis of as little as 0.05 μmole of phenylthiohydantoins of the alkali-stable amino acids. This modified direct procedure has proved a useful adjunct to the subtractive method in determining the N-terminal amino acids of peptides by the Edman degradation.

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REFERENCES

Edman, P. (1950). Acta Chem. Scand., 4, 283.

Fieser, L. F., Jr. (1924). J. Am. Chem. Soc., 46, 2639.

Moore, S., and Stein, W. H. (1948). J. Biol. Chem., 176, 367.

Moore, S., and Stein, W. H. (1954). J. Biol. Chem., 211, 893.

Stark, G. R., and Smyth, D. G. (1963). J. Biol. Chem.,238, 214.

Van Orden, H. O., and Carpenter, F. H. (1964). Biochem. and Biophys. Research Comm., 14, 399.