that the optical activity at 260 mμ was induced by denaturation. The native state might thus be a configuration in which the chromophores participate in dipole-dipole interaction, hydrogen bridging, or vicinal effects which give rise to the optically active groups absorbing in the 180 mμ region. A primary shift in the effective electronic perturbated states[4] would result in the 260 mμ activity once the monomers were set free from the restraints exercised by the native configurations. The labile bonds between the purines and pyrimidines in complementary polymer chains may be the reason for this shift[5,6,7]. Old data on the optical activity of the purine and pyrimidine nucleosides suggest that their activity is of opposite sign[8]. Associations of these two monomer types would give rise to an activity highly sensitive to rearrangements. Undoubtedly the optical behavior of this molecule is very complex but the above argument finds a counterpart in the recent observations of LINDERSTØRM-LANG AND SCHELLMAN on the optical behavior of proteins[9] and is supported by our own measurements on adenosine which shows a $\lambda_0$ of 265 mμ.

The preliminary work reported in this note is being expanded in our laboratory to include detailed experiments on the effect of pH and ionic strength on the rotary dispersion of DNA and its component parts. The effect of enzyme depolymerization is at present being investigated by this method. Because of the similarities between DNA and RNA we have begun similar measurements on the RNA molecule and its component parts.

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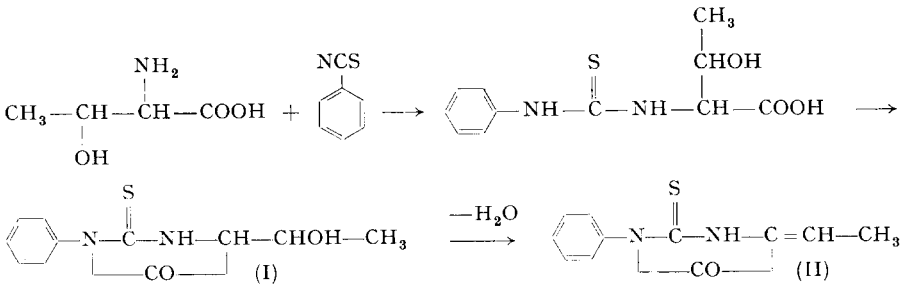
* The senior author of this note was decided by the flip of a coin.

## Preparation and properties of 3-phenyl-2-thiohydantoins of serine, threonine and cystine

In the reaction proposed by EDMAN[1] for the stepwise degradation of peptides, 3-phenyl-2-thiohydantoins (PTH) are formed from the reaction of phenyl*iso*thiocyanate with the N-terminal amino acid residues. While the preparation of the PTH's of some of the amino acids has been achieved by EDMAN[2], the synthesis of the PTH's of serine, threonine and cystine heretofore have not been reported*. In this note, the preparation of these phenylthiohydantoins will be described, and their molar extinction coefficients and absorption maxima will be presented. The absorption characteristics of the PTH's of other natural amino acids have also been determined.

In the course of the investigation, it was observed that when the PTH of threonine (I) was dissolved in 1.0 *N* NaOH, the colorless solution became pink and then changed to yellow within approximately 20 minutes. On acidification of the solution with glacial acetic acid, an amorphous precipitate formed. A crystalline product, which elementary analysis indicates is probably the PTH of *A*-threonine (II), was obtained from ethanol; the final proof for the inferred structure remains to be established.

* Since the completion of this investigation by the late Dr. LEVY, a report by V. M. INGRAM (*J. Chem. Soc.* 3717 (1953)) of the synthesis of the PTH's of serine and threonine has appeared. It is felt that since the present report describes a simplified procedure, publication of the details is justified. It should be noted that the values reported in both instances for the m.p. of serine are in agreement (176–178° given by INGRAM), whereas there is a considerable divergence between the value reported for threonine by the British author (194°) and that reported here (infra). [C. H. LI.]



In *iso*propyl ether, the absorption maximum of I is at 269.5 mμ, whereas that of II is at 321.5 mμ. Similarly, when the PTH of serine was allowed to stand in 1.0 *N* HCl at 40° C, a new absorption maximum at 320 mμ gradually appeared (see Fig. 1), indicating that the PTH of serine had been converted into the PTH of *Δ*-serine, in a fashion similar to what occurs in the case of threonine, although the PTH of *Δ*-serine has not yet been isolated and crystallized.

These absorption characteristics of the PTH's of serine and threonine have been very helpful in the identification of serine and threonine by the EDMAN procedure*. Table I gives the molar extinction coefficients ($E$) and the absorption maxima ($\lambda$ max.) of the PTH's of 19 amino acids; $E$ was defined (optical density) (volume in ml) (molecular weight of PTH)/(weight of PTH in mg).

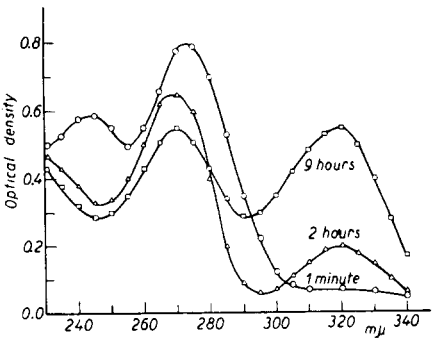



Fig. 1. The changes of absorption characteristic of serine phenylthiohydantoin with time in *N* HCl at 40° C. (After 24 h, curve is essentially same as the 9 h curve.)

* See for an example[3].

TABLE I

MOLAR EXTINCTION COEFFICIENTS ($E$) AND ABSORPTION MAXIMA ($\lambda$ max) OF THE PHENYLTHIOHYDANTOINS OF NATURAL AMINO ACIDS

| *Phenylthiohydantoins** | $E$ | $\lambda$ *max* mμ |
|---|---|---|
| DL-Aspartic acid | 16,100 | 268.5 |
| L-Glutamic acid | 16,000 | 269.0 |
| L-Cystine | 27,600 | 271.5 |
| DL-Serine | 15,500 | 269.0 |
| DL-Threonine | 16,400 | 269.5 |
| DL-*Δ*-Threonine | 25,700 | 321.5 |
| Glycine | 13,300 | 267.5 |
| DL-Alanine | 16,100 | 268.5 |
| L-Proline | 16,000 | 271.5 |
| DL-Valine | 18,000 | 270.0 |
| DL-Methionine | 17,900 | 270.5 |
| L-Leucine | 17,800 | 269.0 |
| DL-Isoleucine | 18,100 | 269.5 |
| DL-Phenylalanine | 16,000 | 270.5 |
| L-Tyrosine | 16,400 | 271.0 |
| DL-Tryptophan | 20,100 | 269.5 |
| L-Asparagine | 18,000 | 269.5 |
| L-Histidine | 15,100 | 265.5 |
| L-Arginine | 14,600 | 265.5 |
| L-Lysine, ε-PTC | 29,100 | 270.0 |

* All dissolved in *iso*propyl ether except histidine and arginine which were dissolved in water.

*PTH of serine.* 3.0 g of DL-serine and 2.1 g of KOH were dissolved in 5.0 ml of water; 4.5 ml of phenyl*iso*thiocyanate (ØNCS) in 150 ml of ethanol was added. When this solution had stood in the cold for approximately 3 h, the K salt of phenylthiocarbamyl serine crystallized out. The crystals were dissolved in 125 ml of $H_2O$, and then 15 ml of conc. HCl were added. When the solution was scratched and allowed to stand overnight in the cold, the PTH of serine crystallized out. Yield: 3.5 g (55%). Recrystallized from glacial acetic acid and then from hot ethanol. M.p.: 178° C.*

| | | | |
|---|---|---|---|
| Calculated for $C_{10}H_{10}N_2O_2S$: | C, 54.05% ; | H, 4.50% ; | N, 12.61%. |
| Found: | C, 54.08% ; | H, 4.71% ; | N, 12.87%. |

*PTH of threonine.* 3.0 g of DL-threonine and 1.6 g of KOH were dissolved in 5.0 ml of $H_2O$. Then 3.5 ml of ØNCS in 50 ml of ethanol was added and the mixture was stirred frequently for approximately 2 h at room temperature. 100 ml of glacial acetic acid and 15 ml of conc. HCl were added and the mixture was warmed until it became homogeneous. On cooling, the PTH of threonine formed. Recrystallized from ethanol. M.p., 212° C.

| | | | |
|---|---|---|---|
| Calculated for $C_{11}H_{12}N_2O_2S$: | C, 55.93% ; | H, 5.08% ; | N, 11.86%. |
| Found: | C, 56.56% ; | H, 5.12% ; | N, 11.98%. |

*PTH of A-threonine.* The PTH of DL-threonine was suspended in *N* NaOH. The colorless solution changed to pink and then to yellow within approximately 20 min. The solution was acidified with glacial acetic acid and the precipitate was filtered. Recrystallized from ethanol and then from hot glacial acetic acid as prismatic yellow needles. M.p.: 237° C.

| | | | |
|---|---|---|---|
| Calculated for $C_{11}H_{10}N_2O_2S$: | C, 60.55% ; | H, 4.59% ; | N, 12.84%. |
| Found: | C, 61.26% ; | H, 4.53% ; | N, 13.00%. |

*PTH of cystine.* 3.6 g of L-cystine and 2.0 g of KOH were dissolved in 5.0 ml of water; 4.3 ml of ØNCS in 50 ml of ethanol was added. After frequent stirring at room temperature for 2 h, the solution was evaporated to a small volume and glacial acetic acid-conc. HCl (1:1) was added until an oil separated. When the mixture was warmed gently, the oil dissolved. The PTH of cystine crystallized out after the solution was allowed to stand overnight in the cold. Recrystallized from hot glacial acetic acid. M.p.: 154° C, dec.

| | | | |
|---|---|---|---|
| Calculated for $C_{20}H_{18}N_4O_2S_4$: | C, 50.60% ; | H, 3.82% ; | N, 11.81%. |
| Found: | C, 50.16% ; | H, 4.23% ; | N, 11.40%. |

*Molar extinction coefficients.* The extinction coefficients of the phenylthiohydantoins were measured from a solution composed of approximately 2 mg of each PTH, weighed accurately into 250 ml volumetric flasks, in peroxide-free *iso*propyl ether**. The PTH's of histidine and arginine were isolated as their monochlorides, and in this form were determined in water. The absorption spectra were obtained by triplicate determinations on a Beckman Model DU spectrophotometer, using standard 1 cm quartz cells.

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* All melting points are uncorrected.

** The crystalline PTH's of lysine, cystine, *A*-threonine, threonine, tyrosine, and asparagine were difficult to dissolve in *iso*propyl ether. They were first dissolved in 1.0 ml of dioxane and then the volume was brought up to 250 ml with *iso*propyl ether. The PTH's of histidine and arginine were insoluble in *iso*propyl ether and hence they were dissolved in water for the determination of their extinction coefficients.

*** This investigation was completed before the death of Dr. LEVY [C. H. LI].