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Preparation of Phenyl Thiohydantoins from Some Natural Amino Acids

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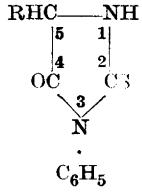
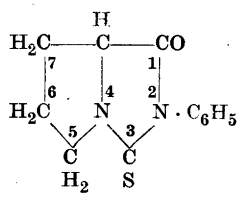
In the development of a method for determination of the amino acid sequence in peptides[1] it became necessary for reference purposes to prepare the 3-phenyl-2-thiohydantoin derivatives (I.) of the natural amino acids. Some of these are described in the literature. Aschan [2] was the first to report the preparation of phenyl thiohydantoins from amino acids. Later Brautlecht [3] described the analogous derivatives from an additional number of amino acids.



(I.)

$HOOC \cdot RHC \cdot NH \cdot CS \cdot NH \cdot C_6H_5$

(II.)



(III.)

(R = amino acid side chain)

In the present work the intermediate phenylthiocarbamyl (PTC) derivatives (II.) were obtained by allowing the amino acids to react with phenyl isothiocyanate in alkaline solution. It was found that some desulfuration of the PTC-derivative took place if the total amount of alkali required was added at the beginning of the reaction and if the temperature was too high. For this reason alkali was added continuously and the temperature was not allowed to exceed 40° C. The crude PTC-derivatives were not purified but transferred directly into the phenyl thiohydantoins by refluxing in acid.

EXPERIMENTAL

Preparation of phenylthiocarbamyl (PTC) amino acids

One hundredth of a mole of the amino acid was dissolved in a mixture of 25 ml of water and 25 ml of pyridine. The pH of the solution was adjusted to about 9 as shown by an indicator paper by the addition of *N* NaOH. The solution was heated to 40° C and kept at that temperature during the reaction. 2.4 ml of phenyl isothiocyanate was added with vigorous stirring. Small portions of *N* NaOH were added to keep the pH at about 9. The reaction was completed when the alkali consumption ceased. This as a rule required less than 30 minutes.

Pyridine and excess phenyl isothiocyanate were then removed by repeated extractions with equal volumes of benzene. Subsequently an amount of *N* HCl equivalent to the total addition of sodium hydroxide was added. This usually brought about precipitation of the PTC-amino acid and if necessary the mother liquor was concentrated to increase the yield. In the cases of arginine and histidine the PTC-derivatives were soluble at an acidic reaction. It was found that the PTC-arginine could be precipitated isoelectrically at pH ~ 7 and the PTC-histidine at pH 3.5.

Preparation of 3-phenyl-2-thiohydantoins

Unless otherwise stated for the particular hydantoin the following procedure was employed.

The PTC-amino acid was suspended or dissolved in 30 ml of *N* HCl and refluxed for two hours. The reaction mixture was then repeatedly concentrated to dryness *in vacuo* in order to remove hydrochloric acid.

The yield of hydantoin calculated on the amino acid was 80—90 %.

Recrystallizations were carried out from mixtures of glacial acetic acid and water. Preparations were dried *in vacuo* (1 mm Hg) over P_2O_5 and pellets of KOH for 48 hours prior to analyses.

Melting points were determined on a heating block (Fisher-Johns) and are uncorrected.

Optical rotations of the hydantoins were determined in absolute ethanol. A 0.5 dm tube was used. Owing to the limited solubility of the hydantoins the accuracy of these measurements is not higher than $\pm$ 2°.

Nitrogen analyses were carried out by the micro-Kjeldahl method. Sulfur analyses were done according to Zimmermann [4].

3-Phenyl-2-thiohydantoin. Prepared from glycine. M. p. 245—48° (d.). M. p. acc. to Brautlecht [3] 240—42° (d.).
Found: N, 14.53; S, 16.81.
Calc. for $C_9H_8ON_2S$: N, 14.58; S, 16.69.

5-Methyl-3-phenyl-2-thiohydantoin. Prepared from DL-alanine. M. p. 185°. Acc. to Brautlecht [3] 180—84°.
Found: N, 13.45; S, 15.36.
Calc. for $C_{10}H_{10}ON_2S$: N, 13.57; S, 15.53.

5-Ethyl-3-phenyl-2-thiohydantoin. Prepared from DL-α-amino-*n*-butyric acid. M. p. 192°. Acc. to Brautlecht[3] 190–92°.
Found: N, 12.68; S, 14.62.
Calc. for $C_{11}H_{12}ON_2S$: N, 12.72; S, 14.54.

5-Isopropyl-3-phenyl-2-thiohydantoin. Prepared from DL-valine. M. p. 206°. Acc. to Brautlecht[3] 206–08°.
Found: N, 11.94; S, 13.55.
Calc. for $C_{12}H_{14}ON_2S$: N, 11.97; S, 13.70.

5-Isobutyl-3-phenyl-2-thiohydantoin. Prepared from L-leucine. Recryst. from ethanol. M. p. 178°. Acc. to Brautlecht[3] 176–79°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 11.18; S, 12.83.
Calc. for $C_{13}H_{16}ON_2S$: N, 11.28; S, 12.91.

5-[Sec-butyl]-3-phenyl-2-thiohydantoin. Prepared from L-isoleucine. Recryst. from ethanol. M. p. 173–75°. $[\alpha]_D^{20} = +12°$ ($c = 1$ % $^W/_V$).
Found: N, 11.25; S, 12.81.
$C_{13}H_{16}ON_2S$ requires N, 11.28; S, 12.91.

5-[2-Methylmercaptoethyl]-3-phenyl-2-thiohydantoin. Prepared from DL-methionine. M. p. 132°.
Found: N, 10.53; S, 24.26.
$C_{12}H_{14}ON_2S_2$ requires N, 10.51; S, 24.05.

1-Oxy-2-phenyl-3-thio-1-imidazolidino-[1,5-a]-pyrrolidine. (Formula III.) Prepared from L-proline. M. p. 179°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 11.94; S, 13.62.
$C_{12}H_{12}ON_2S$ requires N, 12.07; S, 13.80.

1-Oxy-2-phenyl-3-thio-1-imidazolidino-[1,5-a]-6-hydroxy-pyrrolidine. Prepared from L-hydroxyproline. Recryst. from water. M. p. 145–48°.
Found: N, 11.35; S, 12.60.
$C_{12}H_{12}O_2N_2S$ requires N, 11.28; S, 12.88.

5-Benzyl-3-phenyl-2-thiohydantoin. Prepared from DL-phenylalanine. M. p. 187°. Acc. to Brautlecht[3] 187°.
Found: N, 10.07; S, 11.47.
Calc. for $C_{16}H_{14}ON_2S$: N, 9.93; S, 11.36.

5-p-Hydroxybenzyl-3-phenyl-2-thiohydantoin. Prepared from L-tyrosine. M. p. 216°. Acc. to Brautlecht[3] 214–16°. $[\alpha]_D^{20} = -15°$ ($c = 1$ % $^W/_V$).
Found: N, 9.35; S, 10.69.
Calc. for $C_{16}H_{14}O_2N_2S$: N, 9.39; S, 10.76.

5-[3'-Indolylmethyl]-3-phenyl-2-thiohydantoin. Prepared from DL-tryptophan. When an attempt was made to form the hydantoin in the ordinary way by refluxing the PTC-derivative with *N* HCl, a sticky, amorphous, dark brown product resulted. It was then

found that refluxing with glacial acetic acid for two hours was sufficient to bring about the reaction. The resulting product showed a yellow discoloration which could be removed by repeated crystallizations from mixtures of glacial acetic acid and water. On exposure to light the dry product slowly took on a reddish color. M. p. 177°.
Found: N, 13.11; S, 10.06.
$C_{18}H_{15}ON_3S$ requires N, 13.08; S, 9.97.

3-Phenyl-2-thio-5-hydantoinacetic acid. Prepared from DL-aspartic acid. Recryst. from ethanol-water. M. p. 229°. Acc. to Brautlecht [3] 233–34°.
Found: N, 11.16; S, 12.75.
Calc. for $C_{11}H_{10}O_3N_2S$: N, 11.19; S, 12.81.

3-Phenyl-2-thio-5-hydantoinacetamide. Prepared from DL-asparagine. Recryst. from ethanol-water. M. p. 234°. Acc. to Brautlecht [3] 234°.
Found: N, 16.75; S, 12.93.
Calc. for. $C_{11}H_{11}O_2N_3S$: N, 16.87; S, 12.86.

3-Phenyl-2-thio-5-hydantoinpropionic acid. Prepared from L-glutamic acid. Recryst. from ethanol-water. This compound crystallized with 1 mole of water which for removal required drying at 110°. M. p. 166–67°. Acc. to Brautlecht [3] 169–70°. $[\alpha]_D^{20} = 0$ ($c = 1$ % $^W/_V$).
Found: N, 10.52; S, 12.05.
Calc. for $C_{12}H_{12}O_3N_2S$: N, 10.60; S, 12.13.

5-[4-(β-Phenylthioureido)butyl]-3-phenyl-2-thiohydantoin. Prepared from L-lysine. Recryst. from ethanol-water. M. p. 162–64°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 13.97; S, 15.95.
$C_{20}H_{22}ON_4S_2$ requires N, 14.07; S, 16.09.

5-(3-Guanidopropyl)-3-phenyl-2-thiohydantoin hydrochloride. Prepared from L-arginine. Recryst. from boiling water. M. p. 189°. $[\alpha]_D^{20} = 0$ ($c = 1$ % $^W/_V$).
Found: N, 21.41; S, 9.55.
$C_{13}H_{18}ON_5ClS$ requires N, 21.35; S, 9.77.

5-(4'-Imidazolylmethyl)-3-phenyl-2-thiohydantoin hydrochloride. Prepared from L-histidine monohydrochloride. Recryst. from abs. ethanol. M. p. 200–06° (d.). $[\alpha]_D^{20} = +6°$ ($c = 0.5$ % $^W/_V$).
Found: N, 18.10; S, 10.43.
$C_{13}H_{13}ON_4ClS$ requires N, 18.14; S, 10.38.

DISCUSSION

All the amino acids investigated with the exception of lysine formed 3-phenyl-2-thiohydantoin derivatives with their unmodified side chains occupying position 5 in the hydantoin ring. Lysine after reaction with phenyl isothiocyanate became substituted with phenylthiocarbamyl groups at both

the α-amino and ε-amino positions and the latter substituent was retained when the hydantoin was formed. The α-imino acids in consequence of their cyclic structures formed fused ring systems completely analogous with the hydantoins formed from the α-amino acids.

Attempts have also been made to prepare the phenyl thiohydantoin derivatives of cystine, serine and threonine. Only the threonine derivative formed a crystalline compound of definite composition. Elementary analyses of the threonine derivative showed a discrepancy from the expected composition indicating the loss of one molecule of water. During the formation of the hydantoin from cystine a considerable evolution of hydrogen sulfide was noticed. These facts taken in conjunction with earlier observations on the hydantoins and thiohydantoins of serine [5] and cystine [6,7] suggest that the elements of water or in the latter case hydrogen sulfide are split off from the side chains during the reaction.

The optical rotations were determined of those hydantoins which had been prepared from optically active amino acids. The specific rotations were generally low or nil demonstrating again the marked tendency for racemization during the formation of hydantoins [8,9]. The electron attracting phenyl group in position 3 of the ring appears to augment this tendency [10].

SUMMARY

The 3-phenyl-2-thiohydantoin derivatives of the following amino acids have been prepared: Glycine, alanine, α-amino-*n*-butyric acid, valine, leucine, *isoleucine*, *methionine*, *proline*, *hydroxyproline*, phenylalanine, tyrosine, *tryptophan*, aspartic acid, asparagine, glutamic acid, *arginine*, *lysine*, *histidine* *. Some of their properties are described.

The 3-phenyl-2-thiohydantoins display a marked tendency for racemization.

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* The 3-phenyl-2-thiohydantoin derivatives of the italicized amino acids have not been described earlier.

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