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Short Communications

Note on the Preparation of Phenyl Thiohydantoins from Glutamine, S-Carboxymethyl Cysteine and Cysteic Acid

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In an earlier paper [1] the preparation of the phenyl thiohydantoins (PTH) from most of the natural amino acids has been described. Some of these amino acids did not yield the expected thiohydantoins owing to decomposition during the preparation, which involved prolonged refluxing of the phenyl thiocarbamyl (PTC) amino acids in N hydrochloric acid. Under these conditions PTC-cystine and PTC-cysteine gave off hydrogen sulfide and formed undefined products. The PTC derivatives of the β-hydroxyamino acid lost water and became α-β-unsaturated which at least for serine led to polymerization. However, since then Ingram [2] has described a satisfactory synthesis of PTH-serine and PTH-threonine using milder conditions for the cyclization. It has now been found that two derivatives of cysteine, cysteic acid and S-carboxymethyl cysteine, form normal thiohydantoins of sufficient stability for identification purposes in the amino acid sequence determination [3]. By appropriate treatment of the peptide under investigation it should be possible to form these derivatives [4,5]. The preparation of PTH-glutamine is also described *.

To avoid side reactions ring closure of the PTC-amino acids was brought about under mildest possible conditions in respect to concentration of acid and temperature. It was then helpful to follow the ring closure by measuring the ultraviolet absorption (in ethanol) of a sample, since the PTC-amino acids have two maxima at approx. 248 mμ and 268 mμ whereas the PTH-amino acids show only one maximum at approx. 268 mμ. At pH 1—2 and room temperature the ring closure was generally completed in less than 48 hours.

Experimental. Elementary analyses except micro-Kjeldahl were made at the Analytical Laboratory, Department of Chemistry, University of Lund and at the Chemical Laboratory, University of Copenhagen.

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

Paper chromatography was carried out as described by Sjöquist [6]. All R_F-values are referred to PTH-glycine, R_F for PTH-X / R_F for PTH-glycine = q.

3-Phenyl-2-thio-5-hydantoinpropionamide. A suspension of L-glutamine (1.46 g) in pyridine-water (3:2, 30 ml) was brought to pH 8.6 by the addition of N sodium hydroxide. Under stirring phenyl *iso*thiocyanate (5 ml) was added and pH was kept at 8.6 by the continuous addition of N sodium hydroxide. After two hours the reaction mixture was extracted three times with 1 vol. of benzene, which was then discarded. The aqueous solution was brought to pH 1 by the addition of 4 N hydrochloric acid and left overnight at room temperature. The crystals (1.9 g) were filtered off and crystallized and recrystallized from boiling glacial acetic acid. M. p. 201—211° (decomp.).

Paper chromatography: Solvent A, q=0.23; solvent B, q = 0.08; solvent C, q = 0.82.

Analyses. N (Kjeldahl) 15.87; S (Zimmermann [7]) 12.2. $C_{12}H_{13}O_2N_3S$ requires: N 15.95; S 12.2.

5-(Carboxymethylmercaptomethyl)-3-phenyl-2-thiohydantoin. L-β-(Carboxymethylmercapto)-alanine (S-carboxymethyl cysteine) was prepared essentially as described by Dickens [5]. This preparation (1.79 g) was dissolved in

* Recently Ramachandran *et al.*[9] have reported the preparation of this compound.

pyridine-water (3:2, 30 ml) and pH was adjusted to 8.4 by the addition of N sodium hydroxide. Phenyl *iso*thiocyanate (4 ml) was added with stirring and pH was kept at 8.4 by the addition of N sodium hydroxide. After seven hours the reaction mixture was extracted three times with equal volumes of benzene, which were then discarded. The aqueous phase was brought to pH 2.7 and exhaustively extracted with ethyl acetate (90 ml). The extracts were combined and the ethyl acetate was evaporated *in vacuo* (temperature not above 30° C). To the oily residue water (20 ml) was added and pH was adjusted to 8 by the addition of N sodium hydroxide. A small precipitate was removed and the filtrate then extracted with ethyl acetate (30 ml), which was discarded. The solution was acidified to pH 2 by the addition of 4 N hydrochloric acid which resulted in an oily precipitate. After 24 hours the oil had crystallized (1.8 g). Recrystallization twice from ethanol-petroleum ether. M. p. 164°.

Paper chromatography: Solvent A, decomposition with the appearance of several spots; solvent B, $q = 0.23$; solvent C, $q = 0.98$.

Analyses. N (Dumas) 9.30; S (Cuck and Grim [8]) 21.3. $C_{12}H_{12}O_3N_2S_2$ requires: N 9.45; S 21.6.

5 - (Potassiummethylsulfonate) - 3 - phenyl - 2-thiohydantoin. A solution of L-cysteic acid (1.7 g) in pyridine-water (3:2, 30 ml) was brought to pH 8.6 by the addition of N potassium hydroxide. Under stirring phenyl *iso*thiocyanate (4 ml) was added and pH was then kept at 8.6 by the continuous addition of N potassium hydroxide. After three hours the reaction mixture was extracted three times with equal volumes of benzene, which were then discarded. The aqueous phase was brought to pH 1 by the addition of 4 N hydrochloric acid and the solution was left overnight at room temperature. The solution was then brought to pH 4.2 with N potassium hydroxide and concentrated *in vacuo* to dryness. The residue was dried in a desiccator over phosphorus pentoxide and the dry product extracted with aqueous acetone which left the potassium chloride undissolved. The acetone solution was concentrated *in vacuo* to a viscous solution. On addition of acetone crystallization set in and was completed in the ice box (2.2 g). The product was taken up in a small volume of water and pH adjusted to 5 with potassium hydroxide. On the addition of abs. ethanol the preparation crystallized in fine needles. Recrystallization twice from water-ethanol. The product was highly hygroscopic and was dried *in vacuo* over phosphorus pentoxide at 110°. M. p. 193—7° (decomp.).

Paper chromatography: Solvent A, $q = 0$; solvent B, $q = 0$; solvent C, $q = 0.11$.

Analyses: N (Dumas) 8.62; S (Cuck and Grim [8]) 19.4. $C_{10}H_9O_4N_2S_2K$ requires: N 8.64; S 19.8.

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Note on the Reaction between Linear Polyamides and Ethylene Carbonate

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In connection with an investigation on the interaction between polyamides and cyclic esters the observation was made, contrary to earlier statements elsewhere [1] that ethylene carbonate may act, under particular conditions, as a hydroxyethylating agent upon homopolymers of ε-aminocaproic acid or hexamethylenediammonium adipate. The reaction is observable just in the temperature range, where the polymers are dissolved by the cyclic ester (approximately 180—185° C). At still higher temperatures (200—220° C) *water soluble* derivatives are obtained by heating the polymer in a tenfold excess of ethylene carbonate for 4 hours. An inert gas, such as dry carbon dioxide, should be present to prevent side reactions (oxidation, depolymerization).

In a system, containing an unstabilized polymer, no simple kinetic scheme can be