AMINO ACIDS

IV. THE REACTION OF GLYCINE AND β-ALANINE WITH CARBON DISULPHIDE[1]

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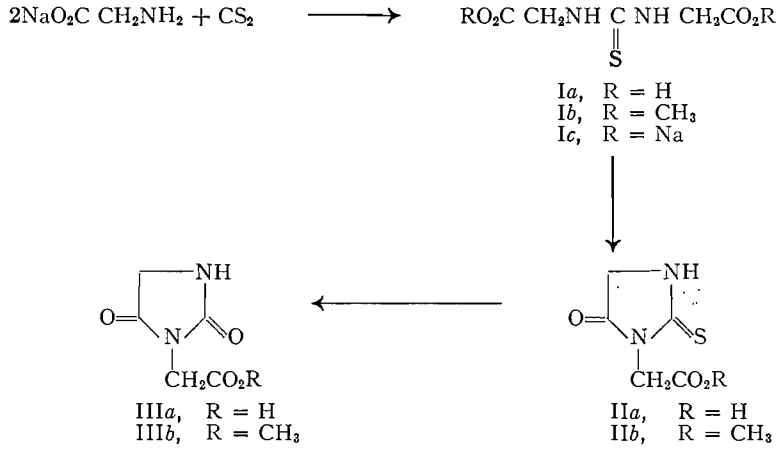
ABSTRACT

Glycine and β-alanine on condensation with carbon disulphide gave the corresponding 1,3-di-(carboxyalkyl) thioureas. 1,3-Di-(carboxymethyl) thiourea is rearranged to 2-thio-3-(carboxymethyl) hydantoin by warming with hydrochloric acid solution or heating above its melting point. Evidence is presented for the reversible condensation of 2 mole equivalents of the sodium salt of 2-thio-3-(carboxymethyl) hydantoin by the elimination of water. This condensation product is obtained as well-defined pink crystals. 1,3-Di-(β-carboxyethyl) thiourea was oxidized with an alkaline solution of hydrogen peroxide or sodium hypochlorite solution to 1,3-di-(β-carboxyethyl) urea. 1,3-Di-(β-carboxyethyl) thiourea and 1,3-di-(β-carboxyethyl) urea have been cyclized by heating with *p*-toluenesulphonic acid. The products were 2-thio-3-(β-carboxyethyl) hydrouracil and 3-(β-carboxyethyl) hydrouracil respectively.

The reaction of several α-amino acids and carbon disulphide in aqueous sodium bicarbonate solution has been studied earlier by Kodama (1, 2). In most cases, he found that the only identifiable products were substituted thiohydantoins, which presumably were formed by cyclization of the intermediate 1,3-di-(carboxymethyl) thioureas. In the reaction with glycine, however, no distinct products were obtained although the copious evolution of hydrogen sulphide indicated that condensation had occurred. The reaction has now been reinvestigated in conjunction with the condensation of glycine and its homologue, β-alanine, with carbon disulphide.

Sodium glycinate and carbon disulphide were condensed in aqueous solution to yield the crude disodium salt of 1,3-di-(carboxymethyl) thiourea (I*c*). Reaction of this disodium salt with methanolic hydrogen chloride at room temperature gave 1,3-di-(carbomethoxymethyl) thiourea (I*b*) in 25% yield. The combined mother liquors on treatment with concentrated hydrochloric acid gave 2-thio-3-(carboxymethyl) hydantoin (II*a*) (m.p. 214°–215° C. dec.) in 17% yield. The latter compound was identified by analysis and by desulphurization with aqueous chloroacetic acid, to the known 3-(carboxymethyl) hydantoin (III*a*) and methyl ester of 3-(carboxymethyl) hydantoin (III*b*) (3). The methyl ester of 2-thio-3-(carboxymethyl) hydantoin (II*b*) melted at 113°–114° C.



[1]*Manuscript received November 20, 1957.*
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Careful saponification of the dimethyl ester (I*b*) gave 1,3-di-(carboxymethyl) thiourea (I*a*) which melted at 164°–165° C. with loss of 1 mole of water and resolidified to form 2-thio-3-(carboxymethyl) hydantoin which then melted at 214°–215° C. with decomposition. 1,3-Di-(carboxymethyl) thiourea was previously reported (4) as melting at 170° C. with decomposition. In concurrence with previous work (4, 5) this thiourea diacid (I*a*), its dimethyl ester (I*b*), and disodium salt (I*c*) all cyclize in acid solution to give 2-thio-3-(carboxymethyl) hydantoin. As a preparative method, the reaction mixture of crude disodium 1,3-di-(carboxymethyl) thiourea (I*c*) on brief treatment with concentrated hydrochloric acid gave the thiohydantoin (II*a*) in 48% yield.

When the condensation of sodium glycinate and carbon disulphide was conducted in the usual manner and the reaction mixture was acidified at room temperature to a pH of 2 with hydrochloric acid, no precipitation of product occurred. However, the solution yielded an orange-colored product (V)[2] melting at 287°–290° C. with decomposition. A 23% yield of 2-thio-3-(carboxymethyl) hydantoin (II*a*) was obtained from the mother liquors. In another experiment, the high melting condensation product (V) appeared in 31% yield on acidification of the reaction mixture with 2.2 moles of hydrochloric acid, followed by subsequent evaporation and extraction. In this case, the residue from the mother liquors gave on methylation 2-thio-3-(carbomethoxymethyl) hydantoin (II*b*) in 7% yield. The colored condensation product (V) proved to contain chemically bound sodium but it gave a negative halogen test. Potentiometric titration of the compound in aqueous solution with standard alkali showed it to be an acid with a primary pK_a of 3.4 and a neutralization equivalent of 373, which agrees with the acid equivalent (374.31) of the empirical formula $C_{10}H_8N_4O_5S_2Na_2$. Attempts to methylate this product with methanolic hydrogen chloride gave 2-thio-3-(carbomethoxymethyl) hydantoin (II*b*) in 81% yield. Brief treatment of the condensation product (V) with concentrated hydrochloric acid gave an 84% yield of 2-thio-3-(carboxymethyl) hydantoin (II*a*), while desulphurization with aqueous chloroacetic acid followed by methylation gave 3-(carbomethoxymethyl) hydantoin (III*b*) in 25% yield. Other attempted alkylation experiments with benzyl chloride or dimethyl sulphate in the presence of aqueous base led to the isolation of 2-thio-3-(carboxymethyl) hydantoin (II*a*) and not to a derivative of the condensation product (V). Empirically, these observations indicate the condensation product (V) to be the result of an easily reversible condensation of 2 moles of the sodium salt of 2-thio-3-(carboxymethyl) hydantoin with the elimination of 1 mole of water in such a way as to leave a titratable acid function in the product. Potentiometric titration data on 2-thio-3-(carboxymethyl) hydantoin (II*a*) and the colored condensation product add additional evidence to this hypothesis.

The titration of 2-thio-3-(carboxymethyl) hydantoin (II*a*) (Fig. 1, curve A) with 0.1 *N* sodium hydroxide solution gave a typical titration curve for an acid (pK_a 3.2) with an observed neutralization equivalent of 173 (calculated, 174). At the neutralization point (n_1) the color of the solution changed from yellow to red-orange. The solution was then back-titrated with standard hydrochloric acid (Fig. 1, curve B). Again, at the neutralization point (n_2), a color change of red-orange to orange occurred. Curves A and B do not coincide and it will be observed that if the amount of sodium hydroxide solution (0 to n_1) required to neutralize 2-thio-3-(carboxymethyl) hydantoin (II*a*) is 1 mole, then the difference between n_1 and n_2 is 0.5 mole. This can be explained by consideration of the following reaction sequence:

[2]*This product is referred to hereafter as condensation product.*

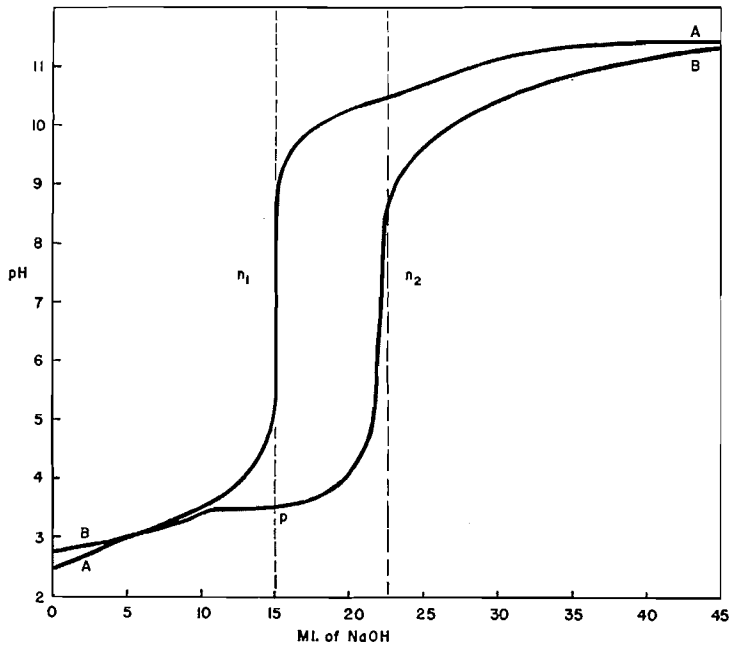


FIG. 1. Titration (*A*) and back-titration (*B*) of 2-thio-3-(carboxymethyl) hydantoin (II*a*) in aqueous solution.

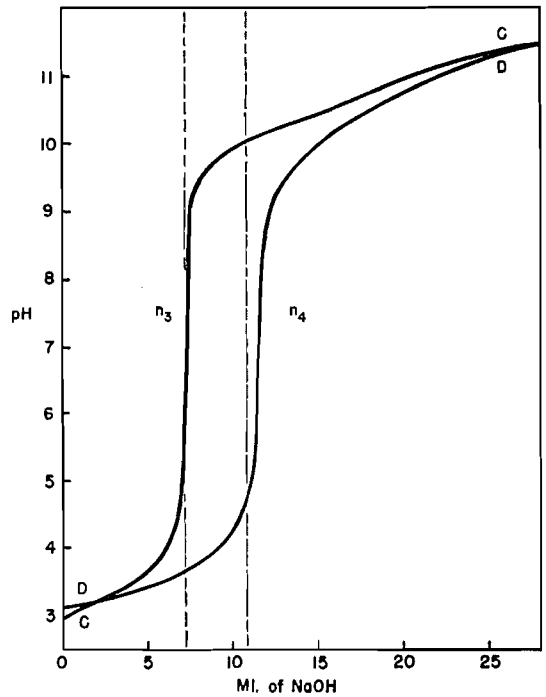
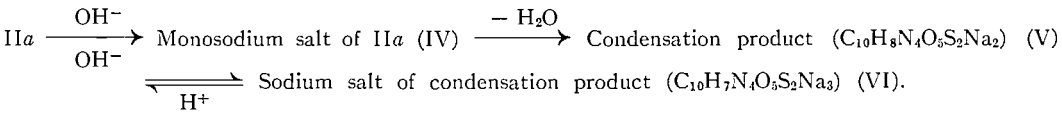


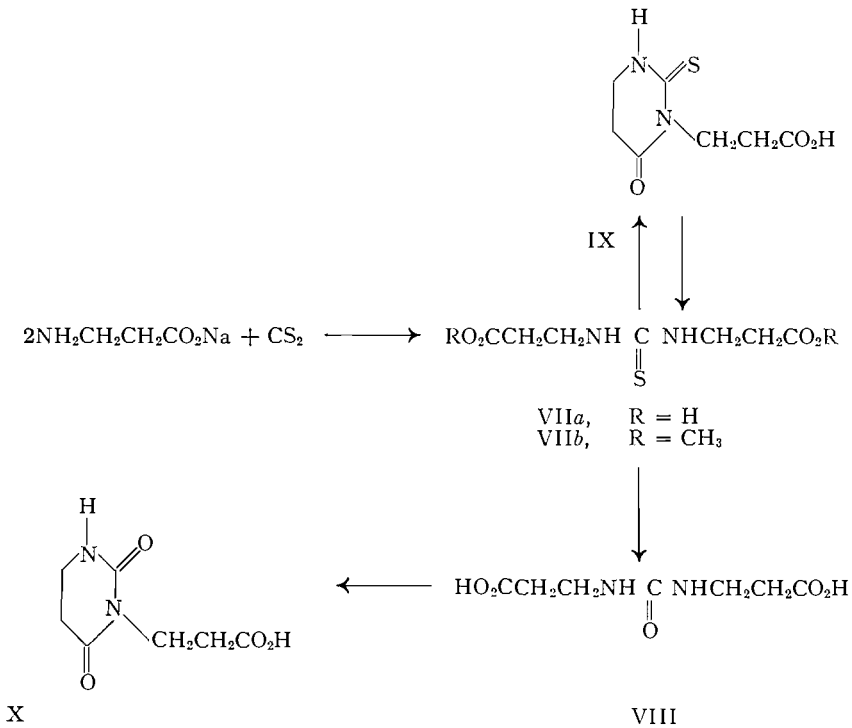
FIG. 2. Titration (*C*) and back-titration (*D*) of condensation product (V) in aqueous solution.



Following curve A, the conversion of 2-thio-3-(carboxymethyl) hydantoin (II*a*) into its monosodium salt (IV) is complete at n_1; at n_1, IV undergoes self-condensation with elimination of water to give the condensation product (V), which is in turn neutralized to give VI. The neutralization of V to VI requires 1 mole of base per mole of condensation product or *one-half the amount of base required to neutralize IIa originally*. On the back-titration curve B, the conversion of VI to V begins at n_2 and should be complete at p. The validity of this interpretation rests on the isolation of the condensation product (V) from the solution used in the titration and back-titration of II*a*. Experimentally, this method gave an 80% conversion of II*a* into V. The analytical values obtained for the condensation products prepared by the two methods agreed within experimental error. However, in both cases the values obtained for hydrogen were higher than the value calculated for the empirical formula $C_{10}H_8N_4O_5S_2Na_2$.

The titration and back-titration of the condensation product (V) gave the same result (Fig. 2). Again, the difference between the two curves (C and D) at n_3 and n_4 represents approximately one-half the amount of base required to reach n_3. In analogy with the titration experiments on 2-thio-3-(carboxymethyl) hydantoin (II*a*), it appears that the condensation process has again occurred.

Although there is not sufficient evidence to formulate the structure of the condensation product (V) with its two combined sodium atoms, the data suggest that the product has a free carboxyl group and that the sodium atoms are bound as salts of an enolic structure. Furthermore, the structure of the initial condensation product (V) must be such that the same mode of self-condensation can be repeated.



The condensation of sodium β-alaninate and carbon disulphide proceeded normally. The product, 1,3-di-(β-carboxymethyl) thiourea (VII*a*), proved to be very water soluble and hence was isolated in the form of its dimethyl ester (VII*b*) in 56% yield. Saponification of the diester (VII*b*) in aqueous base readily gave the diacid (VII*a*). This thiourea–diacid (VII*a*) was oxidized with 4 moles of aqueous sodium hypochlorite or hydrogen peroxide in the presence of 4 moles of base to 1,3-di-(β-carboxyethyl) urea (VIII).

There are numerous examples in the literature of the cyclization of β-thioureido and β-ureido alanines to hydrouracil and its derivatives. This was accomplished by the cyclizing action of acetic anhydride, hydrochloric acid, or simply heat (6, 7, 8, 9, 10, 11) on the β-ureido alanines, while others (12, 13, 14) found that the corresponding esters undergo ring closure in the presence of sodium alkoxides. When 1,3-di-(β-carboxyethyl) thiourea (VII*a*) was heated with *p*-toluenesulphonic acid at 160°–175° C., 2-thio-3-(β-carboxyethyl) hydrouracil (IX) was obtained in 85% yield. In accordance with observations on other hydrouracil derivatives (7, 15) this process was easily reversed by the action of warm aqueous base on IX. Similarly, the action of hydrochloric acid or acetic anhydride on 1,3-di-(β-carboxyethyl) urea (VIII) gave 3-(β-carboxyethyl) hydrouracil (X).

As a preparative method, the 2-thio-3-(β-carboxyethyl) hydrouracil (IX) can be prepared in 46% yield from the crude 1,3-di-(β-carboxyethyl) thiourea (VII*a*) by cyclization with *p*-toluenesulphonic acid. It is of interest to note that the cyclization of 1,3-di-(carboxymethyl) thiourea (I*a*) to yield 2-thio-3-(carboxymethyl) hydantoin (II*a*) is more facile than the ring closure of its homologue (VII*a*) to the thiohydrouracil (IX). This is demonstrated by the behavior of the two compounds on fusion. As noted above, 1,3-di-(carboxymethyl) thiourea (I*a*) loses water readily at its melting point while VII*a* remains unchanged under these conditions.

During the investigation of the cyclization of 1,3-di-(β-carboxyethyl) thiourea (VII*a*) with acetic anhydride under reflux, desulphurization as well as cyclization occurred. The product, 3-(β-carboxyethyl) hydrouracil (X), was obtained in 26% yield. To our knowledge, the desulphurization of a thiohydrouracil with acetic anhydride has not been observed previously.

EXPERIMENTAL[3]

1,3-Di-(carboxymethyl) Thiourea and 2-Thio-3-(carboxymethyl) Hydantoin

A stirred mixture of glycine (20 g., 0.26 mole), carbon disulphide (10.2 g., 0.13 mole), and water (20 ml.) was treated dropwise with a solution of sodium hydroxide (10.68 g., 0.26 mole) in water (27 ml.) over a period of 15 minutes. After the initial heat effect had abated additional carbon disulphide (2 g.) was added and the solution was held at the reflux temperature of carbon disulphide for 30 minutes. The excess carbon disulphide was evaporated and the remaining solution was refluxed for 7 hours. During this latter heating stage copious evolution of hydrogen sulphide occurred. The solution was cooled, filtered, and divided into two equal portions.

One portion of the solution was evaporated to dryness *in vacuo* on the steam bath and the residual viscous red oil was dried *in vacuo* over potassium hydroxide pellets. This residue was treated with 10% methanolic hydrogen chloride solution (100 ml.) at room temperature. A pale yellow solution containing some insoluble crystals of sodium chloride was obtained. Methylation was allowed to proceed at room temperature overnight. The filtered solution was evaporated to dryness *in vacuo* and the residue was dissolved in

[3]*All melting points are uncorrected. Microanalyses were performed by Micro Tech Laboratories, Skokie, Illinois.*

chloroform. The chloroform solution was extracted with water, dried, and then evaporated to dryness. The residue on treatment with benzene gave 3.7 g. (25%) of crude crystalline 1,3-di-(carbomethoxymethyl) thiourea (m.p. 97°–98° C.). Several crystallizations from benzene–hexane solution gave colorless platelets melting at 99°–100° C. Anal. Calc. for $C_7H_{12}N_2O_4S$: C, 38.17; H, 5.49; N, 12.72; S, 14.56%. Found: C, 38.59; H, 5.61; N, 12.81; S, 14.10%.

Part of the 1,3-di-(carbomethoxymethyl) thiourea (0.5 g., 0.002 mole) in a solution of sodium hydroxide (0.3 g., 0.007 mole) in water (7 ml.) was heated on a steam bath for 30 minutes. The solution was cooled to 0° C. and acidified with cold concentrated hydrochloric acid solution. The crystals (m.p. 157°–158° C.; resolidifies and remelts with decomposition at 214°–215° C.) were recovered by filtration, yield 0.38 g. (89%). This material was purified by solution in cold methanol and precipitation with benzene. The pure sample gave a double melting point of 164°–165° C. and 214°–215° C. with decomposition. Anal. Calc. for $C_5H_8N_2O_4S$: C, 31.25; H, 4.20; N, 14.58; S, 16.69%. Found: C, 31.49; H, 4.23; N, 14.43; S, 16.61%.

The mother liquors and washings from the preparation of 1,3-di-(carbomethoxymethyl) thiourea were combined and evaporated to dryness. The residue in concentrated hydrochloric acid solution (35 ml.) was warmed on a steam bath for 20 minutes. When the solution was cooled, 2-thio-3-(carboxymethyl) hydantoin (m.p. 210°–212° C. with decomposition) separated as pale yellow crystals, yield 2 g. (17%). Recrystallization from methanol–benzene solution raised the melting point to 214°–215° C. with decomposition. The melting points reported in the literature are 212° C. with decomposition (4) and 210°–212° C. (5). Anal. Calc. for $C_5H_6N_2O_3S$: C, 34.47; H, 3.47; N, 16.09; S, 18.41%. Found: C, 34.55; H, 3.54; N, 16.10; S, 18.21%.

A solution of 2-thio-3-(carboxymethyl) hydantoin (0.5 g., 0.003 mole) in 10% methanolic hydrogen chloride solution (10 ml.) was allowed to stand at room temperature overnight. The solvent was removed *in vacuo* at room temperature and the crystalline residue was crystallized from benzene (3 ml.) to yield 0.49 g. (91%) of 2-thio-3-(carbomethoxymethyl) hydantoin melting at 112°–113° C. One crystallization from benzene–hexane solution raised the melting point to a constant value of 113°–114° C. Anal. Calc. for $C_6H_8N_2O_3S$: C, 38.29; H, 4.28; N, 14.89; S, 17.04%. Found: C, 38.51; H, 4.37; N, 14.46; S, 16.74%.

The methyl ester of 2-thio-3-(carboxymethyl) hydantoin on hydrolysis with either 6% potassium hydroxide solution or 37% hydrochloric acid solution gave a 72% yield of 2-thio-3-(carboxymethyl) hydantoin (m.p. 214°–215° C. with decomposition). The product was identified by a mixture melting point determination with an authentic sample of 2-thio-3-(carboxymethyl) hydantoin.

The second portion of solution from the original reaction was acidified to a pH of 2 with concentrated hydrochloric acid solution. This solution was evaporated to dryness *in vacuo* and the residue was dried *in vacuo* over potassium hydroxide pellets. The dried residue was extracted with boiling methanol (2×100 ml.) and the insoluble inorganic salts were removed by filtration. After the methanolic filtrate was taken to dryness, the residue was crystallized from water (15 ml.). Orange-red crystals of condensation product (m.p. 270°–275° C. with decomposition) were obtained, yield 2.9 g. (24%). One crystallization from water (7 ml.) together with treatment with Nuchar raised the melting point to 287°–290° C. with decomposition. The product gave a negative halogen test and it gave a neutralization equivalent of 373 on titration with 0.1 *N* sodium hydroxide solution. Anal. Calc. for $C_{10}H_8N_4O_5S_2Na_2$ (mol. wt. 374.31): C, 32.09; H, 2.15; N, 14.97%. Found: C, 32.50; H, 3.13; N, 15.11; Ash, 17.53%.

The mother liquors from the condensation product were evaporated to dryness and the residue was heated with 37% hydrochloric acid solution (25 ml.) for 1 hour on the steam bath. The solution was cooled and a crystalline precipitate of 2-thio-3-(carboxymethyl) hydantoin (m.p. 210°–212° C. with decomposition) was obtained, yield 2.6 g. (23%). This product was identified by a mixed melting point determination with a known sample of 2-thio-3-(carboxymethyl) hydantoin (m.p. 214°–215° C. with decomposition).

In another experiment, after the stage of the condensation reaction, the reaction mixture was acidified with 1.1 mole of hydrochloric acid per mole of sodium hydroxide originally used. The colored condensation product (m.p. 285°–290° C. with decomposition) was obtained in 32% yield. The dry residue from the mother liquors was methylated with 10% methanolic hydrogen chloride to yield 7% of the methyl ester of 2-thio-3-(carboxymethyl) hydantoin (m.p. 112°–113° C.). This product gave no depression in melting point on admixture with an authentic sample of 2-thio-3-(carbomethoxymethyl) hydantoin (m.p. 113°–114° C.).

Direct Preparation of 2-Thio-3-(carboxymethyl) Hydantoin

A mixture of glycine (150 g., 2 moles), carbon disulphide (76 g., 1 mole), and sodium hydroxide (80 g., 2 moles) in water (350 ml.) was condensed in the above-described manner. After complete reaction, the solution was filtered and evaporated to dryness. The residue in concentrated hydrochloric acid solution (500 ml.) was heated on a steam bath for 45 minutes. The yellow solid which separated was collected and dried to give 84 g. (49%) of 2-thio-3-(carboxymethyl) hydantoin (m.p. 214°–215° C. with decomposition). This product was identified by a mixture melting point determination.

3-(Carboxymethyl) Hydantoin

2-Thio-3-(carboxymethyl) hydantoin (1.3 g., 0.007 mole) was converted into 3-(carboxymethyl) hydantoin (m.p. 183°–191° C.) in 37% yield by heating with chloroacetic acid under the conditions described by Johnson and Renfrew (5). Two crystallizations from ethanol–hexane solution raised the melting point to 197°–198° C. The melting points reported in the literature are 190°–191° C. (5), 195°–196° C. (16), and 199°–201° C. (17). Anal. Calc. for $C_5H_6N_2O_4$: C, 37.98; H, 3.83; N, 17.72%. Found: C, 38.28; H, 3.94; N, 17.95%.

A solution of 3-(carboxymethyl) hydantoin (0.1 g., 0.0006 mole) in 10% methanolic hydrogen chloride solution (8 ml.) was allowed to stand overnight. The solvent was removed *in vacuo* and the residue was crystallized from benzene–hexane solution to give 91 mg. (84%) of the methyl ester of 3-(carboxymethyl) hydantoin melting at 88°–89° C. One crystallization from acetone–hexane solution raised the melting point to 90°–91° C. The previously (3) reported melting point is 91° C.

Chemistry of the Condensation Product

(*a*) *Conversion into 2-Thio-3-(carboxymethyl) Hydantoin*

The condensation product (0.5 g., 0.0013 mole) in 37% hydrochloric acid solution (4 ml.) was heated at 100° C. for 7 minutes. The mixture was cooled and the precipitate of 2-thio-3-(carboxymethyl) hydantoin (m.p. 212°–213° C. with decomposition) was removed by filtration, yield 0.39 g. (84%). One crystallization from glacial acetic acid raised the melting point to 214°–215° C. with decomposition. This product was identified by a mixture melting point determination with a known sample of 2-thio-3-(carboxymethyl) hydantoin.

(*b*) *Conversion into 2-Thio-3-(carbomethoxymethyl) Hydantoin*

A solution of the condensation product (0.5 g., 0.0013 mole) in 10% methanolic hydrogen chloride solution (10 ml.) was allowed to stand at room temperature overnight. The solvent was removed *in vacuo* and the residue was extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated to dryness. The crystalline residue was crystallized from benzene, yield 0.4 g. (81%). It melted at 112°–113.5° C. alone and on admixture with a known sample of 2-thio-3-(carbomethoxymethyl) hydantoin (m.p. 113°–114° C.).

(*c*) *Conversion into 3-(Carbomethoxymethyl) Hydantoin*

An aqueous solution (2 ml.) of the condensation product (0.5 g., 0.0013 mole) and chloroacetic acid (0.5 g., 0.005 mole) was refluxed for 1 hour. The solution was taken to dryness *in vacuo* and the residue was methylated with 10% methanolic hydrogen chloride solution (15 ml.) at room temperature overnight. The solvent was removed *in vacuo* and the residual oil was dissolved in chloroform. The chloroform solution was washed with 5% sodium bicarbonate solution and water. It was dried and the chloroform evaporated. The residue was crystallized from benzene–hexane solution (3 ml.) to give 116 mg. (25%) of 3-(carbomethoxymethyl) hydantoin melting at 89°–90° C. A sample crystallized from acetone–hexane solution melted at 90°–91° C. alone and on admixture with a known sample of 3-(carbomethoxymethyl) hydantoin.

(*d*) *Condensation Product from 2-Thio-3-(carboxymethyl) Hydantoin*

A solution of 2-thio-3-(carboxymethyl) hydantoin (5.0 g., 0.0287 mole) in water (100 ml.) was titrated with 0.1 *N* sodium hydroxide solution. After each addition of base, the pH of the solution was determined by means of a Beckman Model G pH meter equipped with external glass and calomel electrodes. At the neutralization point (288 ml. of 0.1 *N* NaOH, 0.0288 mole), the color of the solution changed from pale yellow to red. After addition of an equal amount of base, the solution was back-titrated with 118 ml. of 0.244 *N* hydrochloric acid (0.0288 mole). Again, a color change from red to orange at the equivalence point was observed when approximately half of the hydrochloric acid solution had been added. The solution was evaporated *in vacuo* until crystals began to appear. These were collected and dried to give 1.28 g. (m.p. 287°–292° C. with decomposition). A second crop (3.02 g., m.p. 270°–285° C. with decomposition) was obtained on further concentration of the mother liquor to give a total crude yield of 80%. Recrystallization of the product from water (10 ml.) together with Nuchar treatment gave orange-red crystals (2.04 g., m.p. 287°–291° C. with decomposition) which gave no depression in melting point on admixture with the condensation product (above). It gave a neutralization equivalent of 378 on titration with 0.1 *N* sodium hydroxide solution. Anal. Calc. for $C_{10}H_8N_4O_5S_2Na_2$: C, 32.09; H, 2.15; N, 14.97%. Found: C, 32.61; H, 3.36; N, 15.55; Ash, 17.47%.

(*e*) *Titration Experiments*

The titration curves (Fig. 1 and 2) were obtained by titration of aqueous solutions (1 g./100 ml.) of 2-thio-3-(carboxymethyl) hydantoin and the condensation product with 0.1 *N* sodium hydroxide solution and back-titration with standardized hydrochloric acid solution. The pH of the solution was determined by means of a Beckman Model G pH meter equipped with external glass and calomel electrodes.

1,3-Di-(β-carboxyethyl) Thiourea

A mechanically stirred mixture of β-alanine (15 g., 0.16 mole), carbon disulphide (6.4 g., 0.08 mole), and water (10 ml.) was treated dropwise with a solution of sodium

hydroxide (6.7 g., 0.17 mole) in water (17 ml.) over a period of 5 minutes. The temperature gradually rose to 30° C. and in 30 minutes all of the carbon disulphide had been consumed in the reaction. Additional carbon disulphide (2 g.) was added and the solution was heated at the reflux temperature for 30 minutes. The excess carbon disulphide was removed by distillation and the clear solution was refluxed for 7 hours. It was cooled and then filtered to remove a small amount of insoluble material. The filtrate was acidified to pH 2 with 10% hydrochloric acid solution and then evaporated to dryness *in vacuo*. The residue was extracted with hot methanol to remove the inorganic salts. The dry residue from the methanolic extract was treated with 5% methanolic hydrogen chloride solution (50 ml.) and allowed to stand at room temperature overnight. The residue from evaporation of the methanolic solution was dissolved in chloroform and the chloroform solution was washed with 5% hydrochloric acid solution, 5% sodium bicarbonate solution, and water. The dried chloroform solution was evaporated to dryness *in vacuo* and the residue was crystallized from cold benzene (60 ml.), yield 11.6 g. (56%). The melting point was raised from 69°–73° C. to 73°–74° C. by one crystallization from methanol–water solution, yield 10.4 g. Anal. Calc. for $C_9H_{16}N_2O_4S$: C, 43.53; H, 6.50; N, 11.28; S, 12.92%. Found: C, 43.93; H, 6.65; N, 11.10; S, 12.65%.

The dimethyl ester of 1,3-di-(β-carboxyethyl) thiourea (5.5 g., 0.02 mole) in a solution of potassium hydroxide (3.7 g.) in water (65 ml.) was heated on a steam bath for 45 minutes. The solution was cooled, acidified to pH 2 with 10% sulphuric acid solution, and then evaporated to dryness. The residue was extracted with acetone (2×100 ml.). After the acetone was evaporated from the combined extracts, the residue was crystallized from acetone–chloroform solution. This procedure gave 4.5 g. (93%) of 1,3-di-(β-carboxyethyl) thiourea melting at 137°–139° C. A further crystallization from the same solvent pair raised the melting point to 138°–139° C. Anal. Calc. for $C_7H_{12}N_2O_4S$: C, 38.18; H, 5.50; N, 12.73; S, 14.56%. Found: C, 38.18; H, 5.66; N, 12.45; S, 14.68%.

1,3-Di-(β-carboxyethyl) Urea

Method A

A solution of 1,3-di-(β-carboxyethyl) thiourea (1.4 g., 0.006 mole) and sodium hydroxide (1.1 g., 0.028 mole) in water (10 ml.) was treated at 20°–25° C. with 32 ml. (0.028 mole) of 0.891 *M* sodium hypochlorite solution. The addition was complete in 5 minutes and the oxidation was allowed to proceed at room temperature overnight. A small amount of insoluble sulphur was removed by filtration, and the filtrate was acidified with 10% hydrochloric acid to pH 2. It was evaporated *in vacuo* on the steam bath to a volume of 20 ml. or until white crystals began to appear. The solution was cooled and the crystals were collected, washed, and dried, yield 1.07 g. (80%). The melting point was raised from 183°–185° C. to 185°–185.5° C. by crystallization from methanol. Anal. Calc. for $C_7H_{12}N_2O_5$: C, 41.17; H, 5.93; N, 13.72%. Found: C, 41.17; H, 5.98; N, 13.68%.

Method B

A mixture of the dimethyl ester of 1,3-di-(β-carboxyethyl) thiourea (10 g., 0.04 mole) and sodium hydroxide (6.4 g., 0.16 mole) in water (70 ml.) was dissolved by slight warming on a steam bath. The solution was cooled and treated with 7.9 *M* hydrogen peroxide solution (20.4 ml., 0.16 mole) at 15°–20° C. The oxidation was allowed to proceed at room temperature overnight, after which the pH of the solution was adjusted to 2 with 37% hydrochloric acid solution. This solution was concentrated *in vacuo* until crystals began to appear. The crystals (m.p. 183°–185° C.) were removed by filtration and dried, yield 7.1 g. (86%). These crystals did not depress the melting point of 1,3-di-(β-carboxyethyl) urea (m.p. 185°–185.5° C.) prepared by Method A.

2-Thio-3-(β-carboxyethyl) Hydrouracil

β-Alanine (75 g., 0.8 mole) and carbon disulphide (35 g., 0.46 mole) in a solution of sodium hydroxide (33.7 g., 0.8 mole) in water (135 ml.) were condensed in the usual manner by refluxing for 7 hours. This solution was cooled, acidified to a pH of 2 with concentrated hydrochloric acid, and evaporated to dryness. The residue was extracted with hot methanol and the methanol extract was taken to dryness *in vacuo*. The residual oil was heated with *p*-toluenesulphonic acid monohydrate (8 g.) at 160°–175° C. *in vacuo* (15 mm.) for 30 minutes. This melt on crystallization from water (200 ml.) gave 38.8 g. (46%) of 2-thio-3-(β-carboxyethyl) hydrouracil (m.p. 163.5°–164.5° C.). Crystallization from acetone–water solution raised the melting point to 166°–167° C. Anal. Calc. for $C_7H_{10}N_2O_3S$: C, 41.57; H, 4.98; N, 13.86; S, 15.86%. Found: C, 41.80; H, 4.89; N, 14.00; S, 15.90%.

In another experiment a mixture of 1,3-di-(β-carboxyethyl) thiourea (0.5 g., 0.002 mole) and *p*-toluenesulphonic acid monohydrate (0.05 g., 0.0003 mole) was heated at 160°–175° C. for 10 minutes. The melt was cooled and crystallized from water (4 ml.) to give 0.4 g. (85%) of 2-thio-3-(β-carboxyethyl) hydrouracil melting at 166°–167° C.

2-Thio-3-(β-carboxyethyl) hydrouracil (0.5 g., 0.002 mole) was heated on a steam bath for 35 minutes in a solution of 85% potassium hydroxide (0.48 g., 0.007 mole) in water (8 ml.). This solution on acidification to pH 2 and evaporation to a small volume gave 1,3-di-(β-carboxyethyl) thiourea (m.p. 137°–138° C.) in 75% (0.41 g.) yield. The product was identified by a mixture melting point determination.

3-(β-Carboxyethyl) Hydrouracil

Method A

A mixture of 1,3-di-(β-carboxyethyl) urea (1 g., 0.005 mole) and acetic anhydride (10 ml.) was refluxed for 15 minutes. The solution was cooled, diluted with 100 ml. of water, and the excess acetic anhydride allowed to hydrolyze. Evaporation of the aqueous solution *in vacuo* on the steam bath gave a crystalline residue. The residue was crystallized from ethanol–benzene solution (10 ml.) to yield 0.59 g. of product. Concentration of the mother liquors gave another 0.1 g. of crystalline product. The total yield of 3-(β-carboxyethyl) hydrouracil (m.p. 178°–184° C.) was 76%. One crystallization raised the melting point to 183°–184° C. A sample of this compound mixed with the starting material, 1,3-di-(β-carboxyethyl) urea (m.p. 185°–185.5° C.), gave a depressed melting point. Anal. Calc. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05%. Found: C, 45.50; H, 5.48; N, 15.23%.

Method B

A solution of 1,3-di-(β-carboxyethyl) urea (0.5 g., 0.0025 mole) in 37% hydrochloric acid solution (3 ml.) was evaporated to dryness in an open beaker on a steam bath. This procedure was repeated twice and the final dry residue was extracted with hot acetone (25 ml.). After the solvent was removed by evaporation, the residue was crystallized from acetone–chloroform solution. The crystals melted at 172°–175° C., yield 0.38 g. (83%). One crystallization from methanol–benzene solution raised the melting point to 181°–182° C. A mixture melting point determination with a known sample of 3-(β-carboxyethyl) hydrouracil gave no depression.

Method C

1,3-Di-(β-carboxyethyl) thiourea (0.27 g., 0.001 mole) in acetic anhydride (5 ml.) was refluxed for 30 minutes. The solution was cooled, poured into ice-water (25 ml.), and the excess acetic anhydride was allowed to hydrolyze at room temperature. The oil remaining after evaporation of the solution was crystallized from acetone–chloroform solution. The

crude crystalline product (m.p. 179°–181° C.) was obtained in 26% (0.059 g.) yield. After two recrystallizations from the same solvent pair it melted at 183°–184° C. alone and on admixture with a known sample of 3-(β-carboxyethyl) hydrouracil (m.p. 183°–184° C.).

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