

## Synthetic Studies on Thiothreonines. II

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Several investigations that have been carried out recently in our laboratory were motivated by a desire to study the method for the preparation of certain thiothreonine derivatives via threonines. In a previous paper<sup>(1)</sup>, it was shown that ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate, m. p. 84.5-86.5°, was obtained from DL-threonine, when the methyl ester of the latter was chlorinated with phosphorus pentachloride in acetyl chloride, hydrolyzed, benzoylated and finally esterified with ethanol. On the other hand, from DL-allothreonine another diastereoisomer of ethyl DL-benzamido- $\beta$ -chlorobutyrate, m. p. 99-100°, was obtained by an analogous process. Recently D. F.

Elliot<sup>(2)</sup> reported on the formation of these substances from ethyl  $\alpha$ -benzamido- $\beta$ -hydroxybutyrate. The present study<sup>(3)</sup> deals with the chlorination of DL-N-benzoylallothreonine ethyl ester and the formation of thiothreonine derivatives.

Chlorination of DL-N-benzoylallothreonine ethyl ester with thionyl chloride in benzene in the presence of pyridine afforded the lower-melting isomer of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate in a yield of 57%, while on the contrary, chlorination with phosphorus pentachloride in chloroform afforded the higher-melting isomer. Thus we found<sup>(4)</sup> that

(2) D. F. Elliott, *J. Chem. Soc.*, **1949**, 589-594.

(3) The present paper was read by one of the authors (M. K.) at the 4th Annual Meeting of the Chemical Society of Japan in Tokyo held on April 6, 1951.

(1) M. Kinoshita and S. Umezawa, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **72**, 382 (1951).

one of the two diastereoisomers of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate was obtained depending on the experimental conditions, apparently one of these chlorinating agents causing the Walden inversion.

Replacement of the chlorine atom of the two diastereoisomers of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate by the sulfhydryl group could be accomplished by the action of benzyl mercaptan. We found that the reaction of the two isomers of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate with benzyl mercaptan in absolute ethanol in the presence of sodium ethylate gave a separable mixture of the two isomers of  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyric acid. The benzene-soluble isomer melted at 136-141° and gave  $\beta$ -phenylethylamine salt, m. p. 166-168°, which afforded a pure free acid, melting at 145-147°, namely, N-benzoyl-S-benzylthiothreonine A. The benzene-insoluble isomer melted at 181-185° and this corresponded to the N-benzoyl-S-benzylthiothreonine B. These preparations showed no mixed melting point depression with the specimens prepared by the azlactone synthesis as reported by Carter *et al.*<sup>(4)</sup>. The replacement reaction under the above conditions caused apparently some inversion. In contrast, a single racemic thiothreonine derivative was obtained when the reaction was carried out in such a non-polar or less polar solvent as benzene or ether. The reaction of benzyl mercaptan with the two isomers of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate in benzene or ether led to the same N-benzoyl-S-benzylthiothreonine A as the sole product isolated. From the two isomers of  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyric acid were obtained thiothreonine A and B as Carter has described already.

### Experimental

**DL-N-Benzoylallothreonine Ethyl Ester.**—A suspension of DL-N-benzoylallothreonine (5 g., m. p. 176°) in a solution of absolute ethyl alcohol (100 cc.) and conc. sulfuric acid (0.76 cc.) was refluxed for 3 hours. After the addition of water (0.7 cc.) solid sodium bicarbonate was added until the mixture became neutral to Congo red. The filtered solution was concentrated to a syrup under reduced pressure, and the residue was taken up in benzene. The benzene extract was concentrated again to a viscous oil. The crude ethyl ester of DL-N-benzoylallothreonine was dissolved in a small amount of dry benzene, and petroleum ether was added to this solution. The separated oil solidified after standing for several hours,

yielding crude ester (5.4 g.). The recrystallization of the crude material from benzene-petroleum-ether yielded colourless needles, m. p. 84-86° (2.7 g.). Recrystallization twice from benzene raised the melting point up to 87-89.3°. By further repetition of recrystallization the melting point could not be raised further, elementary analysis showing a fair coincidence with the theoretical value (Found: N, 5.54. Calculated for  $C_{15}H_{17}O_4N$ : N, 5.58%). J. Attemburrow *et al.*<sup>(5)</sup> have described this substance as having a higher melting point of 98.5-101.5°.

**Ethyl  $\alpha$ -Benzamido- $\beta$ -chlorobutyrate.** (i) Chlorination of N-benzoyl-DL-allothreonine ethyl ester with thionyl chloride.—A solution of dry pyridine (0.49 cc.) and thionyl chloride (0.74 cc.) in dry benzene was added to a suspension of DL-N-benzoylallothreonine (1.7 g.) ethyl ester in dry benzene (11 cc.). The mixture was heated on a boiling water-bath for 20 minutes. After cooling, the mixture was washed with water, dil. hydrochloric acid, a saturated sodium bicarbonate solution in turn, and finally with water. The benzene was removed under reduced pressure. A red-brown crystalline mass (1.3 g.) was obtained. Recrystallization from petroleum ether with charcoal gave colourless plates of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate, m. p. 84.5-86.5° (1 g., yield 57%); the mixed melting point with an authentic sample (cf. the previous report<sup>(1)</sup>) showed no depression.

(ii) Chlorination of DL-N-benzoylallothreonine ethyl ester with phosphorus pentachloride.—Phosphorus pentachloride (0.85 g.) was dissolved in a solution of DL-N-benzoylallothreonine ethyl ester (1 g.) in chloroform (10 cc.) cooled with ice-water. After refluxing several minutes on a water-bath, the mixture was cooled and then washed with water, dil. hydrochloric acid, a sodium bicarbonate solution in turn, and finally with water again. The chloroform was distilled off, leaving a syrup. The syrup was extracted with hot petroleum ether. When the extract was cooled, colourless prisms, m. p. 98-99°, were obtained, and recrystallization from the same solvent gave colourless prisms of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate, m. p. 99-100°. The melting point was not depressed on admixture with the higher-melting specimen obtained from DL- $\alpha$ -benzamido- $\beta$ -chlorobutyric acid (cf. the previous report<sup>(1)</sup>).

### $\alpha$ -Benzamido- $\beta$ -benzylmercaptobutyric Acids.

(A) The reaction of the two isomers of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate with sodium benzyl mercaptide in absolute alcohol.—(i) A solution of benzyl mercaptan (0.19 g.) in a 12% sodium ethoxide solution (0.86 cc.) was added to a solution of the higher-melting ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate (0.4 g., m. p. 99-100°) in absolute alcohol (0.6 cc.). After heating on a water-bath for 30 minutes, the precipitated sodium chloride

(4) H. E. Carter, C. M. Stevens and L. F. Ney, *J. Biol. Chem.*, **139**, 247-254 (1941).

(5) J. Attemburrow, D. F. Elliott and G. F. Penny, *J. Chem. Soc.*, **1948**, 310-18.

was filtered off, and the alcohol was distilled under reduced pressure. The residue was taken up in ether and evaporated, leaving ethyl  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyrate as a syrup. The syrup was dissolved in a hot mixture of glacial acetic acid (3.8 cc.), conc. hydrochloric acid (1.1 cc.), and water (2.3 cc.). The solution was refluxed for 50 minutes and allowed to cool overnight. The crystalline precipitate was filtered and washed with 50% acetic acid and then with water, yielding a mixture of the two isomers of  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyric acid (230 mg., yield 47%). The mixture was extracted with two portions of boiling benzene (1.8 cc. and 1 cc.). From the residue was obtained the crude material melting at 176-183° (25 mg.). Recrystallization from benzene-alcohol gave colourless crystals, m. p. 181-185°, not depressed by mixing with the S-benzyl-N-benzoylthiothreonine B obtained by Carter's azlactone method. (Found: N, 3.95. Calculated for  $C_{18}H_{19}O_3NS$ : N, 4.26%). The combined benzene extract was cooled and a material melting at 136-141° was obtained (100 mg.). This material was converted into the  $\beta$ -phenylethylamine salt, m. p. 166-8°, by treating with  $\beta$ -phenylethylamine in hot ethyl acetate. By the decomposition of the salt we obtained, colourless plates, m. p. 145-7°, which were not depressed by mixing with S-benzyl-N-benzoylthiothreonine A obtained by Carter's method (Found: N, 3.95. Calculated for  $C_{18}H_{19}O_3NS$ : N, 4.26%).—(ii) The lower-melting ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate (m. p. 84-86°) was treated with benzyl mercaptan in sodium ethoxide under the same conditions as described above, giving crude S-benzyl-N-benzoylthiothreonine A melting at 136-141° (107 mg.) and S-benzyl-N-benzoylthiothreonine B melting at 176-183° (20 mg.).

(B) The reaction of the two isomeric ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate with sodium benzylmercaptide in absolute benzene. — (i) Sodium (0.02 g.) in absolute methanol (0.18 cc.) was treated with benzyl mercaptan (0.098 g.) and the solvent was removed *in vacuo*. The white sodium benzylmercaptide was covered with dry benzene, and a solution of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate (0.2 g., m. p. 99-100°) in dry benzene was added. The mixture was refluxed for 2 hours on a water-bath. The mixture was filtered and the benzene was distilled under reduced pressure. The residue was extracted with ether, and ether evaporated, leaving ethyl  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyrate as a syrup. The syrup was dissolved in a hot solution of glacial acetic acid (1.87 cc.), conc. hydrochloric acid (0.57 cc.) and water (1.13 cc.). The solution was refluxed for 50 minutes and allowed to cool overnight. The crystalline precipitate was washed with 50% acetic acid and with water, yielding a crude material melting at 134-141°

(110 mg., 45%). Almost all portions of this product were soluble in boiling benzene. From the benzene extract crystals melting at 136-141° were obtained (70 mg.), and the melting point was not depressed by mixing with the crude S-benzyl-N-benzoylthiothreonine A (m. p. 136-141°) described in (A). (ii) The lower-melting ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate (0.2 g., m. p. 84-6°) was treated with sodium benzylmercaptide in dry benzene under the same conditions, and hydrolyzed as described above, giving a crude product melting at 130-136° (65 mg.). S-benzyl-N-benzoylthiothreonine A melting at 136-141° (41 mg.) was obtained from the benzene-soluble part.

(C) The reaction of ethyl  $\alpha$ -benzamido- $\beta$ -chlorobutyrate with sodium benzylmercaptide in absolute ether. — Sodium benzylmercaptide, which was prepared by the same method as described in (B), was covered with dry ether and an ethereal solution of lower-melting ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate (0.2 g., m. p. 84-86°) was added. The mixture was refluxed in a water-bath at 60° for 15 minutes, and the subsequent treatment was accomplished as mentioned above. A crude product (112 mg.) melting at 128-138° was obtained. From benzene extract, S-benzyl-N-benzoylthiothreonine A (75 mg.) melting at 136-141° was obtained.

### Summary

(1) Chlorination of DL-N-benzoylallothreonine ethyl ester with thionyl chloride in benzene in the presence of pyridine gave the lower-melting isomer of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate, m. p. 84.5-86.5°, in a yield of 57%. On the other hand, chlorination with phosphorus pentachloride in chloroform gave the higher-melting isomer, m. p. 99-100°.

(2) Two isomers of  $\alpha$ -benzamido- $\beta$ -benzylmercaptobutyric acid (derivatives of thiothreonine A and B) were synthesised. The chlorine atom of ethyl DL- $\alpha$ -benzamido- $\beta$ -chlorobutyrate could be replaced by a sulfhydryl group by the action of benzyl mercaptan. A remarkable effect of different solvents on this replacement reaction was described.

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