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The Synthesis of DL-Threonine. II. Interconversion of DL-Threonine and DL-Allothreonine[1]

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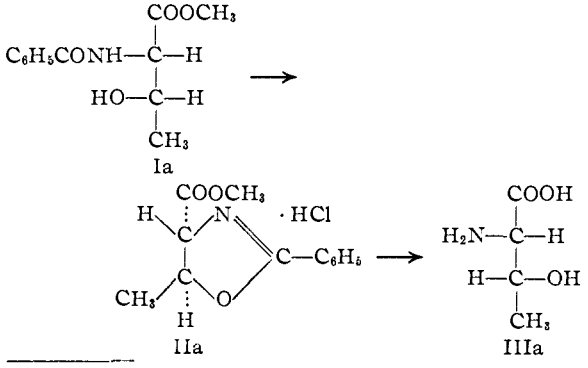
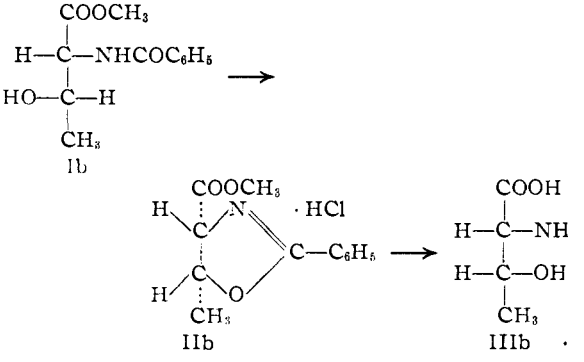
In connection with a search for more practical syntheses of DL-threonine, useful methods of converting the more readily available DL-allothreonine into its epimer were sought. A method of effecting the inversion was discovered and announced in a preliminary communication.[3] While that paper was in press a report[4a] in the recent British literature on the same subject reached this country, and was followed later by a second article.[4b] We wish to elaborate at this time our previous communication on the method for the interconversion of DL-allothreonine and DL-threonine, which is also the basis of a higher yielding synthesis of the latter.

The reaction of α-acylamido-β-hydroxy compounds with dehydrating agents such as thionyl chloride and phosphorus chlorides to give oxazolines is of general application.[5] Accordingly, 2-phenyl-5-methyl-4-carbomethoxyoxazoline hydrochloride,[6] IIa, was prepared in quantitative yield by treatment of the methyl ester of N-benzoyl-DL-allothreonine, Ia, with thionyl chloride. The oxazoline, IIa, on treatment with dilute mineral acids gave DL-threonine, IIIa, in 96.7% yield as determined by microbial assay and in 70% yield as isolated pure material.



The hydrolysis of the oxazoline, IIa, can be carried out stepwise. When a water solution is refluxed one hour, the methyl ester of O-benzoyl-DL-threonine hydrochloride is obtained in excellent yield. The latter is converted into DL-threonine by acid hydrolysis and is rearranged to N-benzoyl-DL-threonine by treatment with alkali.

The inversion described can be utilized to convert DL-threonine into DL-allothreonine. By the same series of reactions, DL-threonine was converted via N-benzoyl-DL-threonine methyl ester, Ib, and the isomeric 2-phenyl-5-methyl-4-carbomethoxyoxazoline hydrochloride, IIb, into DL-allothreonine, IIIb. The yield of isolated product from the oxazoline, IIb, was 81%.



We overlooked the important observation made by Elliot[4b] that the oxazoline made from threonine, IIb, can be converted into the more stable isomeric oxazoline, IIa, by treatment with alkali. We did note that both isomers were sensitive to alkali; since on heating with 2.5 *N* sodium hydroxide for four hours, the yield of DL-threonine in each case was about 27% as determined by microbial assay. In agreement with Elliot, we also observed that when the ethyl ester of DL-allothreonine hydrochloride was treated with benzylimido ethyl ether and the resulting oxazoline subjected to acid hydrolysis, no threonine was formed.

Masking of the carboxyl group during oxazoline formation is necessary, since otherwise azlactonization occurs. Thus, after treatment of N-benzoyl-DL-allothreonine with thionyl chloride, only the azlactone, 2-phenyl-4-ethylidene-5-oxazolone, could be isolated. Hydrolysis of the reaction mixture from such treatment followed by microbial assay showed DL-threonine in less than 1.7% of the theoretical yield, indicating that oxazoline formation was entirely suppressed.

The formation of an oxazoline hydrochloride from N-acyl derivatives of DL-serine and some chemical properties of this oxazoline have been

(1) For the preceding paper of the series, see Pfister, Howe, Robinson, Shabica, Pietrusza and Tishler, THIS JOURNAL, **71**, 1096 (1949).

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(3) Pfister, Robinson, Shabica and Tishler, THIS JOURNAL, **70**, 2297 (1948), (D-threonine in line 11 paragraph 3 of this communication is a typographical error which should read DL-threonine). Additional data are recorded by Pfister and Tishler, U. S. Patent 2,446,196; *C. A.*, **42**, 811 (1948).

(4) (a) Attenburrow, Elliot and Penny, *J. Chem. Soc.*, 310 (1948); (b) Elliot, *Nature*, **162**, 657 (1948).

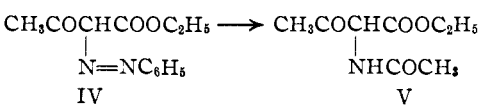
(5) Bergmann and Brand, *Ber.*, **56**, 1280 (1923).

(6) All configurational formulations in this paper are in accord with Fischer's conventions. To effect economy of space only one enantiomorph is given although it should be understood that the second enantiomorph is included when the text refers to the DL-form. The stereochemical formulations for the oxazolines (IIa, IIb and VII) are those suggested by the English workers.[4]

described by Bergmann.[7] Since serine contains only one asymmetric carbon atom, the full significance of this series of reactions was not realized. Nagai and Kanao,[8] however, did convert *dl*-N-benzoyl-norephedrine through the oxazoline into the diastereomeric *dl*-N-benzoyl-norisoephedrine. The latter report and the additional data we shall present in a later publication indicate the method is a general one for the interconversion of diastereoisomers containing an amino and a hydroxyl group on adjacent asymmetric carbon atoms.

The successful utilization of the oxazoline inversion reaction for a practical, total synthesis of DL-threonine requires an easy method for the preparation of esters of N-acyl derivatives of allothreonine. Acetoacetic ester proved to be an excellent starting point for this synthesis.

Ethyl α-acetamidoacetoacetate, V, was obtained in 88% yield from acetoacetic ester by the preparation and reductive acetylation of ethyl α-phenylazoacetoacetate, IV.



IV V

The preparation of this acetamido compound by the reductive acetylation of ethyl α-oximinoacetoacetate has been reported recently.[9] The present method is preferred, since the use of the unstable oximino compound is avoided, and a better over-all yield is obtained.[10]

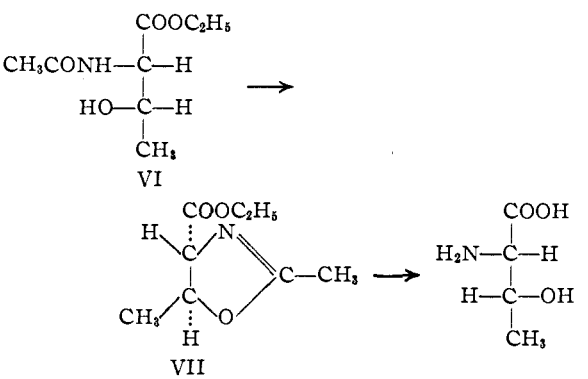
The behavior of ethyl α-acetamidoacetoacetate toward catalytic hydrogenation was studied, since Adkins and Reeve[11] reported that a mixture of DL-threonine and DL-allothreonine was obtained on catalytic hydrogenation and subsequent hydrolysis of ethyl α-oximinoacetoacetate. A variety of hydrogenation procedures were tried and appraised by hydrolysis of the reaction mixture and determination of the DL-threonine content microbiologically. The results, listed in Table I, are similar to those reported by Albertson and co-workers[9] and establish the fact that the ethyl ester of N-acetyl-DL-allothreonine is formed predominantly.

TABLE I

Catalyst	Solvent	DL-Threonine, % yield
Platinum oxide	Water	15–20
Platinum oxide	Glacial acetic acid	19
Platinum oxide	Alcohol	26
Raney nickel[a]	Water	30
Raney nickel	Water	34

[a] This run was made at high pressure; all others at 15–40 p.s.i.

By the use of platinum oxide in aqueous solution, ethyl α-acetamidoacetoacetate was readily hydrogenated at room temperature and low pressure to give in theoretical yield a colorless oil having an 80–85% content of N-acetyl-DL-allothreonine ethyl ester. An identical product was also obtainable by catalytic reductive acetylation of ethyl α-phenylazoacetoacetate, removal of acetanilide and further hydrogenation. Repeated crystallization gave pure N-acetyl-DL-allothreonine ethyl ester (VI). Hydrolysis of this material and microbial assay of the solution showed DL-threonine in only a 0.7% yield. When the purified N-acetyl-DL-allothreonine ester was treated with thionyl chloride, the oxazoline VII was obtained. Acid hydrolysis of VII produced DL-threonine in 94.6% yield as established by microbial assay.



VI VII

When the entire hydrogenation product was treated with thionyl chloride and the oxazoline-containing reaction solution was hydrolyzed, crude DL-threonine of 83.2% purity (bioassay) was isolated in 88.7% yield. Simple crystallization in this case failed to yield DL-threonine free from contaminating DL-allothreonine produced by inversion of the N-acetyl-DL-threonine ethyl ester in the hydrogenation product. In order to avoid wasteful recrystallization of the hydrogenation product, it was necessary to devise a separation of the diastereoisomeric amino acids. Although a number of procedures are available for separating various derivatives of DL-threonine and DL-allothreonine,[12] none have been reported for the unsubstituted amino acids. We have found that the sodium salt of DL-threonine is far less soluble in ethanol than that of DL-allothreonine and is, in fact, well-suited to separation of these isomers. By this method the crude DL-threonine obtained without purification of the hydrogenation product was converted into pure DL-threonine sodium salt in 83.5% yield. Reconversion into DL-threonine in 91% yield followed by a recrystallization (96.8% recovery)

(7) Bergmann and Miekeley, *Z. physiol. Chem.*, **140**, 128 (1924); Bergmann, Miekeley, Weinmann and Kann, *ibid.*, **143**, 108 (1925).

(8) Nagai and Kanao, *Ann.*, **470**, 160, 175 (1929).

(9) Albertson, Tullar, King, Fishburn and Archer, THIS JOURNAL, **70**, 1151 (1948); Wiley and Borum, *ibid.*, **70**, 1666 (1948).

(10) An attractive procedure for preparing ethyl α-benzamidoacetoacetate from hippuric acid and acetic anhydride is described by Attenburrow, Elliot and Penny.[4a]

(11) Adkins and Reeve, THIS JOURNAL, **60**, 1328 (1938).

(12) West and Carter, *J. Biol. Chem.*, **119**, 109 (1937); Carter and Risser, *ibid.*, **139**, 255 (1941).

gave pure DL-threonine in 57% over-all yield from acetoacetic ester.

The oxazoline, VII, was found to be similar in chemical reactivity to the phenylated oxazoline, IIa. Its hydrochloride was converted into O-acetyl-DL-threonine ethyl ester hydrochloride on exposure to moist air. On treatment with alkali, the latter was transformed into N-acetyl-DL-threonine. Other reactions are described in the experimental section.

A study of the inversion mechanism using optically active compounds is under way. Our data support the views of the English investigators[4] that inversion occurs during ring closure involving the β-carbon atom. The oxazoline, convertible into DL-threonine by hydrolysis, has the same *trans* configuration as threonine, whereas the isomeric oxazoline has the *cis* configuration.

Experimental

I. Conversion of DL-Allothreonine into DL-Threonine

N-Benzoyl-DL-allothreonine Methyl Ester, I.—N-Benzoyl-DL-allothreonine[13] was treated with an ether solution containing an excess of diazomethane. After evaporation of solvent and reagent, the crystalline ester, m. p. 109–110°, remained. A sample crystallized from benzene in rosettes of needles, m. p. 110–111°.

Anal. Calcd. for $C_{12}H_{15}O_4N$: C, 60.76; H, 6.37. Found: C, 60.86; H, 5.99.

2-Phenyl-5-methyl-4-carbomethoxyoxazoline Hydrochloride, IIa.—The crude methyl ester from above (2.37 g.) was added in portions to 6.0 cc. of ice-cold thionyl chloride. A little heat was evolved, and the solution was allowed to stand at room temperature for two hours. The solution was poured into 150 cc. of absolute ether; the precipitated gum crystallized when scratched. The yield after filtering, washing with ether and drying was 2.55 g. (quantitative); m. p. 117–118° with HCl evolution.

Solution in cold dry chloroform (5 cc./g.) and precipitation with absolute ether (15 cc./g.) gave material that melted at 118–119° on rapid heating from 110°.

Anal. Calcd. for $C_{12}H_{13}O_3N \cdot HCl$: C, 56.36; H, 5.52. Found: C, 56.50; H, 5.71.

DL-Threonine.—Two grams of crude oxazoline IIa in 20 cc. of 10% hydrochloric acid was refluxed three hours. After the solution was cooled in ice for one-half hour the benzoic acid was removed by filtration (73.4% yield, m. p. 120–121°), and the filtrate was concentrated to dryness on an aspirator. The residue was again dissolved in water and concentrated, and the residue was taken up in water for microbial assay. The assay showed the presence of DL-threonine in 96.7% yield (0.90 g.).

Two-thirds of the bioassay solution was concentrated to dryness as described above, taken up in alcohol and again concentrated. The residue was dissolved in 15 cc. of warm anhydrous alcohol and the solution made alkaline to litmus with ammonium hydroxide. The crystalline product (0.75 g.) was dissolved in 4 cc. of water, and the solution was diluted with 9.3 cc. of alcohol. After four hours at 5°, the crystalline DL-threonine was filtered, washed with alcohol and ether, and dried. The product weighed 0.435 g. (70.1% yield) and melted at 229–230°.

Anal. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.35; H, 7.70; N, 11.46.

Bioassay showed this material was 100% DL-threonine.

O-Benzoyl-DL-threonine Methyl Ester Hydrochloride.—Crude oxazoline hydrochloride IIa (1.28 g.) was refluxed for one hour in 10 cc. of water. The solution was concentrated under reduced pressure and the residue dissolved in chloroform was again concentrated to ensure the absence of water. A solution of the residue in 10 cc. of chloroform was treated with Norit, filtered and diluted with 20 cc. of absolute ether. The nicely crystalline precipitate weighed 1.2 g. (87.5%) and melted at 151–153°. Reprecipitation of a sample as above gave a product sintering at 145° and melting at 155–156° with gas evolution.

Anal. Calcd. for $C_{12}H_{15}O_4N \cdot HCl$: C, 52.66; H, 5.89; N, 5.12. Found: C, 52.60; H, 6.06; N, 5.37.

A solution of 0.25 g. in 3 cc. of 10% hydrochloric acid was boiled three hours, concentrated to dryness, and the residue was dissolved in water for microbial assay. Found: 0.105 g. of DL-threonine (88.3% yield).

N-Benzoyl-DL-threonine was obtained in 69% yield from the methyl ester of O-benzoyl-DL-threonine hydrochloride by storing a solution of the latter (0.5 g.) in 4.75 cc. of normal sodium hydroxide for fifteen hours. The resulting mixture was made acid to congo paper with hydrochloric acid and refrigerated for four hours. The product was obtained as needles, m. p. 145–146°; wt. 0.28 g., and was identified as N-benzoyl-DL-threonine by a mixed m. p. determination with an authentic sample.

Benzoyl-α-aminocrotonic Azlactone.—A mixture of 2.33 g. of N-benzoyl-DL-allothreonine and 6 cc. of thionyl chloride was held at room temperature for two hours. The mixture was diluted with 50 cc. of ether, and the crystalline solid (0.35 g., m. p. 85–86°) was collected. After recrystallization from dilute alcohol, the product melted at 89–92° and did not depress the m. p. of an authentic sample of the "Azlactone I" described by Carter, Handler and Melville.[14]

A similar run was worked up by subjecting the completed reaction mixture to hydrolysis with 10% hydrochloric acid, separation of benzoic acid and microbial assay. The yield of DL-threonine was less than 1.7%.

II. Conversion of DL-Threonine into DL-Allothreonine

N-Benzoyl-DL-threonine Methyl Ester.—A sample of N-benzoyl-DL-threonine treated with ethereal diazomethane and concentrated to dryness gave a viscous oil, which slowly crystallized, m. p. 82–84°. Recrystallization of a sample from benzene–ether did not change the melting point.

Anal. Calcd. for $C_{12}H_{14}O_4N$: C, 60.76; H, 6.37. Found: C, 60.97; H, 6.50.

The product is very sparingly soluble in ether or hot ligroin (b. p. 80–115°), but dissolves readily in benzene, alcohols or hot water.

2-Phenyl-5-methyl-4-carbomethoxyoxazoline Hydrochloride, IIb.—Treatment of N-benzoyl-DL-threonine methyl ester exactly as described for the N-benzoyl-DL-allothreonine ester gave material of m. p. 130–131° (2.45 g., 96%). A sample purified by precipitation from chloroform with ether melted at 131–132° (capillary inserted at 120°).

Anal. Calcd. for $C_{12}H_{13}O_3N \cdot HCl$: C, 56.36; H, 5.52. Found: C, 56.58; H, 4.91.

A mixture of IIb with pure oxazoline IIa (m. p. 118–119°) melted lower than the latter alone.

The oxazoline was converted into pure DL-allothreonine in 80.6% yield in the same way as the oxazoline IIa was transformed into DL-threonine. Microbial assays of a solution of the hydrolyzed oxazoline indicated less than 0.8% yield of DL-threonine.

III. Synthesis of DL-Threonine from Acetoacetic Ester

Ethyl α-Phenylazoacetoacetate, IV.—This intermediate was prepared in 92.7% yield from acetoacetic ester essentially by the method of Bülow and Neber.[15]

Ethyl α-Acetamidoacetoacetate, V.—A mixture of glacial acetic acid (513 cc.) and acetic anhydride (257 cc.

(13) West and Carter, *J. Biol. Chem.*, **119**, 109 (1937).

(14) Carter, Handler and Melville, *ibid.*, **129**, 359 (1939).

(15) Bülow and Neber, *Ber.*, **45**, 3732 (1912).

was cooled to 10°, and 390 g. of zinc dust was added to the vigorously stirred solution. While the temperature was kept at 10–15°, a solution of 217.4 g. (0.927 mole) of ethyl α-phenylazoacetoacetate in 232 cc. of glacial acetic acid was added over one and one-half to two hours. The mixture was then allowed to warm to 25–30°, and stirring was continued for three hours. The slurry was filtered and the cake of zinc dust and zinc acetate washed well with glacial acetic acid. Concentration of the filtrate and washings under reduced pressure gave a residue to which was added 500 cc. of water at 55°. After being stirred for five minutes the mixture was cooled to 10°, and the remaining acetic acid was neutralized with solid sodium bicarbonate. The mixture was held at 0° for one-half hour, and the acetanilide (113.5 g., 91% yield) was collected.

The filtrate was extracted with four 250-cc. portions of chloroform, and the combined extracts were concentrated to dryness under reduced pressure. The viscous residue weighed 164.2 g. (94.6% yield) and solidified on standing; m. p. 39–42°.

A sample recrystallized from toluene melted at 47.5–48.5°.

Anal. Calcd. for $C_8H_{13}O_4N$: C, 51.34; H, 6.95. Found: C, 51.56; H, 7.08.

Ethyl α-Acetamido-β-hydroxybutyrate, VI. (a) From Ethyl α-Acetamidoacetoacetate.—A solution of 65.5 g. (0.35 mole) of ethyl α-acetamidoacetoacetate in 525 cc. of water was made barely alkaline by adding 5 cc. of 1 *N* sodium hydroxide and hydrogenated at 25° and 30–40 p. s. i. in the presence of 0.65 g. of platinum oxide catalyst. The theoretical amount of hydrogen was absorbed in four hours, and a ferric chloride test at this point was negative. After separation of the catalyst the filtrate was concentrated to dryness on the water aspirator and the residue dissolved in benzene and reconcentrated to constant weight. The thick, colorless oil (33.2 g.) is readily soluble in water, chloroform and alcohol, somewhat less soluble in benzene and ethyl acetate, and sparingly soluble in ether, toluene and carbon tetrachloride.

A considerable number of such reductions were made, and aliquots were hydrolyzed with hydrochloric acid and assayed microbiologically for DL-threonine. The assays fixed the DL-threonine yield as 15 to 20%, corresponding to a 15–20% content of N-acetyl-DL-threonine ethyl ester in the hydrogenation product.

The hydrogenation of ethyl α-acetamidoacetoacetate under other conditions was studied. As indicated in Table I, the yield of N-acetyl-DL-threonine ethyl ester does vary with the catalyst, solvent and temperature. Raney nickel at low pressure favors the formation of threonine. The yield of DL-threonine was established by microbial assays.

(b) **From Ethyl α-Phenylazoacetoacetate.**—A solution of ethyl α-phenylazoacetoacetate (36.5 g.) in 156 cc. of glacial acetic acid was treated with 0.5 g. of Darco and filtered. After the addition of 36.8 cc. of acetic anhydride and 4.0 g. of 5% palladium on Darco the solution was hydrogenated at room temperature and 25–40 p. s. i. Two moles of hydrogen were absorbed in six hours. The solution was filtered and concentrated to dryness. Two additions of water followed by concentration to dryness (reduced pressure) removed most of the acetic acid. The reddish oil was treated with 117 cc. of water, stirred at 0° for one-half hour, and the crystalline acetanilide was collected; wt. 19.3 g. (92% yield).

The filtrate was neutralized with sodium hydroxide, treated with charcoal and hydrogenated at room temperature and 25–40 p. s. i. in the presence of 0.3 g. of platinum oxide. The hydrogenation was slow, and only after fifteen hours was the ferric chloride test negative. Filtration and concentration of the reduced mixture gave the same type of product as above with acetanilide as a contaminant. Microbial assay of a hydrolyzed aliquot established a 15.8% yield of N-acetyl-DL-threonine ethyl ester.

DL-Threonine. (a) From Partially Purified N-Acetyl-DL-allothreonine Ethyl Ester.—The residue obtained from the reduction of 32.8 g. of ethyl α-acetamidoacetoacetate carried out and worked up in the manner described above was dissolved in 25 cc. of hot ethyl acetate and allowed to crystallize at 5° for sixteen hours. The product was filtered, washed with 5 cc. of cold ethyl acetate and dried; wt. 17.5 g. (52.8% recovery), m. p. 68–74°. (According to the microbial assay procedure, the product contained 3.6% of the DL-threonine precursor.) Repeated recrystallizations from ethyl acetate gave pure ester as large rectangular prisms melting at 76–77°.

Anal. Calcd. for $C_8H_{15}O_4N$: C, 50.78; H, 7.99. Found: C, 50.54; H, 8.04.

A solution of 16.0 g. of the partially purified ester (m. p. 68–74°) in 17.3 cc. of benzene was cooled to 10° and stirred; thionyl chloride (14 cc.) was added dropwise while the temperature was kept at 13–15°. After the addition (three-fourths hour) the temperature was raised to 30° and held there for an hour. The solution was cooled to below 10° while 43 cc. of cold water was added slowly. The layers were separated and the benzene layer extracted twice with 12-cc. portions of water. The combined aqueous extracts were washed with 5 cc. of benzene and refluxed for two and one-half hours. After treatment with charcoal, an aliquot was subjected to the microbial assay procedure. The assay yield of DL-threonine was 88.5%.

The remainder of the reaction mixture was concentrated to dryness under reduced pressure. The colorless residue was dissolved in 62 cc. of hot isopropyl alcohol, and 15 cc. of aniline was added to the solution. The mixture was agitated at room temperature for fourteen hours and filtered. The product (8.65 g., 89.4% yield) was found to be 98.6% DL-threonine by microbial assay. Recrystallization of 6.1 g. from 30.5 cc. of water and 71 cc. of absolute ethanol gave pure product; 5.5 g., 90.2% recovery, 37.6% yield from ethyl acetoacetate, m. p. 229–230°, bioassay 100%.

Anal. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62. Found: C, 40.46; H, 7.64.

When the pure ester (m. p. 76–77°) was subjected to the cyclization reaction, the yield of DL-threonine was 94.6% as established by the microbial assay.

(b) **From Crude Ethyl α-Acetamido-β-hydroxybutyrate.**—Ethyl α-acetamidoacetoacetate (29.1 g.) was hydrogenated as described above, and the residual oil in 32 cc. of benzene was treated with 25.9 cc. of thionyl chloride. The reaction mixture was worked up and hydrolyzed in the manner just described. The solid residue of threonine and allothreonine hydrochloride was dissolved in 116 cc. of hot isopropyl alcohol and precipitated by the addition of 28.2 cc. of aniline. The crystalline product weighed 16.4 g. (88.7% yield) and contained 83.2% DL-threonine (microbial assay).

For elimination of DL-allothreonine, 15.6 g. (0.131 mole) of this product was added to a hot solution of sodium ethoxide prepared from 3.02 g. (0.131 mole) of sodium and 60.4 cc. of anhydrous alcohol. The mixture was refluxed for five minutes to ensure complete solution. Shortly thereafter the crystalline sodium salt of DL-threonine separated. After storage for sixteen hours at room temperature the salt was collected, washed with 15 cc. of alcohol and dried at 50–55°; wt. 15.43 g.; 83.5% yield. Fifteen grams (0.106 mole) of sodium salt was added to 18.5 cc. (0.222 mole) of ice-cold concentrated hydrochloric acid, and while the solution was stirred 120 cc. of isopropyl alcohol was added. The mixture was heated to 60° to ensure completion of reaction and then refrigerated for twelve hours. The sodium chloride was filtered, and the filtrate was treated with 20.4 cc. of aniline and held at 5° for sixteen hours. The product (11.5 g., 91% yield) was recrystallized by solution in 28.8 cc. of hot water and dilution with 86 cc. warm ethanol. After refrigeration for twelve hours, the pure DL-threonine was collected; wt. 11.14 g.; 96.8% recovery; yield from ethyl acetoacetate, 57.2%.

Anal. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.42; H, 7.32; N, 11.82.

This material was 100% pure by microbial assay and 99.6% pure by solubility analysis.

Hydrolysis of N-Acetyl-DL-allothreonine Ethyl Ester.—For complete identification of this compound, hydrolysis to N-acetyl-DL-allothreonine was carried out. A mixture of 1.69 g. of the ester (m. p. 76–77°) and 10 cc. of normal sodium hydroxide was held at room temperature for one hour. The mixture was acidified with hydrochloric acid to *p*H 3.5, and the solution was extracted with *n*-butyl alcohol. The extract was washed with a little water and concentrated to dryness. A solution of the residue in 10 cc. of absolute alcohol was filtered and concentrated to a pale yellow oil, which was extracted three times with 25 cc. portions of boiling ethyl acetate. The combined extracts on cooling deposited the product as small dense prisms. The combined yield of the first and second crops was 65.2%; m. p. 133–135°. Recrystallization from ethanol and ether gave pure N-acetyl-DL-allothreonine melting at 134–135°.

Anal. Calcd. for $C_6H_{11}O_4N$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.81; H, 7.04; N, 8.77.

2,5-Dimethyl-4-carbethoxyoxazoline Hydrochloride, VII.—The crude hydrogenation product (33.2 g.) from ethyl α-acetamidoacetoacetate was dissolved in 40 cc. of benzene and treated with 25.4 cc. of thionyl chloride at 10–15°. After being stirred an additional hour without cooling, the solution was poured into 965 cc. of anhydrous ether, and the mixture was refrigerated for sixteen hours. The crystalline product was collected, washed with ether and dried *in vacuo* over concentrated sulfuric acid; wt. 31.0 g.; m. p. 95–98°. An analytically pure sample was prepared by precipitation of a solution of 5.0 g. in 15.5 cc. of dry chloroform with 90 cc. of dry ether; m. p. 105–106°.

Anal. Calcd. for $C_8H_{13}O_3N\cdot HCl$: C, 46.27; H, 8.79; N, 6.75; N.E., 207.7. Found: C, 46.11; H, 6.87; N, 6.97; N. E., 202.2.

Very careful drying of all solvents used in this reaction is absolutely essential to obtain pure oxazoline, since this compound hydrolyzes rapidly. The pure oxazoline hydrochloride gave DL-threonine on hydrolysis in 92% yield (bioassay).

O-Acetyl-DL-threonine Ethyl Ester Hydrochloride.—A sample of 2,5-dimethyl-4-carbethoxyoxazoline hydrochloride (m. p. 102–103°) exposed to the air for twenty-four hours had a melting point of 128–130°. After drying at 56° and 5 mm. for one hour the weight loss was only 1.8% (calculated for one mole of water is 8.08%), and the melting point was 130–131°.

Anal. Calcd. for $C_8H_{15}O_4N\cdot HCl$: C, 42.57; H, 7.15; N. E., 225.7. Found: C, 42.33; H, 7.45; N. E., 225.3.

Hydrolysis of a sample with 10% hydrochloric acid gave DL-threonine in 91% yield (bioassay).

N-Acetyl-DL-threonine.—A solution of O-acetyl-DL-threonine ethyl ester hydrochloride (2.25 g.) in 26 cc. of 1 *N* sodium hydroxide was kept at room temperature overnight. After filtration to remove a trace of insoluble material, the solution was made acid to congo paper with 17 cc. of 1 *N* hydrochloric acid, and extracted with five 25-cc. portions of *n*-butyl alcohol. The extract was concentrated to dryness and the residue taken up in benzene and reconcentrated. A solution of this residue in 20 cc. of alcohol was filtered from a little inorganic material and concentrated to give a colorless oil (1.55 g., 96.3% yield), which crystallized on standing in the refrigerator; m. p. 126–128°. Two recrystallizations from ethyl acetate gave clusters of prisms melting at 129–130°.

Anal. Calcd. for $C_6H_{11}O_4N$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.73; H, 6.65; N, 8.65.

A mixture of this material and N-acetyl-DL-allothreonine (m. p. 134–135°) melted at 105–113°. Hydrolysis of this compound with hydrochloric acid gave a 96% yield of DL-threonine (microbial assay).

N-Acetyl-DL-threonine Ethyl Ester.—Oxazoline hydrochloride, VII (3.11 g.) was added to a solution of 1.4 g. of sodium bicarbonate in 20 cc. of water. After storage for fourteen hours at room temperature, the solution was extracted with eight 10-cc. portions of chloroform. The extract was washed with water, dried over anhyd. magnesium sulfate, and concentrated to an oil, which crystallized on refrigeration, wt. 1.75 g., m. p. 84–85°. Recrystallization from isopropyl alcohol gave flat prisms of the same melting point.

Anal. Calcd. for $C_8H_{15}O_4N$: C, 50.78; H, 7.99. Found: C, 50.98; H, 7.93.

The same ester was prepared in almost quantitative yield from N-acetyl-DL-threonine by treatment with ethereal diazoethane. When mixed with a sample of the above ester, the melting point remained unchanged.

N-Acetyl-DL-threonine methyl ester was also prepared from N-acetyl-DL-threonine and ethereal diazomethane. When recrystallized from benzene, it is obtained as lustrous needles, m. p. 105–106°.

Anal. Calcd. for $C_7H_{13}O_4N$: C, 47.99; H, 7.48. Found: C, 48.18; H, 7.40.

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Summary

1. The interconversion of DL-threonine and DL-allothreonine in high yields has been described. The interconversion is based upon the inversion of the configuration about one of the asymmetric carbon atoms

2. By utilization of this inversion, the synthesis of DL-threonine from acetoacetic ester can be accomplished in an over-all yield of 57%.

3. A practical method for the separation of DL-threonine from DL-allothreonine has been developed. This method depends upon the difference in solubility of the sodium salts in ethanol.

4. A number of new derivatives of DL-threonine and DL-allothreonine have been prepared and reported.

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