THE SYNTHESIS OF D-ERYTHRO- AND D-THREO-\(\alpha\)-AMINO-\(\beta,\gamma\)-DIHYDROXY-\(n\)-BUTYRIC ACIDS*

BY CARL NIEMANN AND PETER L. NICHOLS, JR.

(From the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena)

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In 1931 Klenk and Diebold (1) reported that sphingosine, upon oxidative degradation, gave rise to myristic acid and an optically active dihydroxyaminobutyric acid, \(\left[\alpha\right]_D^{13} = -33.4^\circ\), which these authors characterized as an \(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid. 5 years later Fischer and Feldmann (2), starting from \(D\)-glyceric aldehyde, synthesized an optically active \(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid, \(\left[\alpha\right]_D^{20} = -13.7^\circ\), and, in order to explain the difference in the specific rotation of their amino acid and that of Klenk and Diebold, suggested that the amino acid with \(\left[\alpha\right]_D^{20} = -13.7^\circ\) was a mixture of the two expected diastereoisomers, one of which was identical with, or an antipode of, the amino acid obtained from sphingosine.

We repeated the synthesis of Fischer and Feldmann and obtained, as they did, the amino acid with \(\left[\alpha\right]_D^{24} = -13.7^\circ\) in good yield. However, in addition another \(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid, with \(\left[\alpha\right]_D^{24} = 16.0^\circ\), was isolated from the reaction mixture. It is the purpose of this communication to show that the amino acid with \(\left[\alpha\right]_D^{24} = -13.7^\circ\) is not a mixture of diastereoisomers, but is \(D\)-threo-\(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid, and that the amino acid with \(\left[\alpha\right]_D^{24} = 16.0^\circ\) is \(D\)-erythro-\(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid.

An \(\alpha\)-benzamido-\(\beta,\gamma\)-dihydroxy-\(n\)-butyrolactone, m.p. 210–211°, was obtained when the \(\alpha\)-amino-\(\beta,\gamma\)-dihydroxy-\(n\)-butyric acid with \(\left[\alpha\right]_D^{24} = -13.7^\circ\) was benzoylated. This lactone when treated with phenylhydrazine gave an \(\alpha\)-benzamido-\(\beta,\gamma\)-dihydroxy-\(n\)-butyr-(N-phenyl)hydrazide, \(\left[\alpha\right]_D^{24} = -15.9^\circ\). The partial hydrolysis of this phenylhydrazide led to the regeneration of the \(\alpha\)-benzamido-\(\beta,\gamma\)-dihydroxy-\(n\)-butyrolactone, m.p. 210–211°, and when this lactone was hydrolyzed the original \(\alpha\)-amino-

* The prefixes erythro and threo define the relative configuration about the 2 asymmetric carbon atoms bearing the amino and hydroxyl groups; the letters \(D\) and \(L\) relate the configuration about the asymmetric carbon atom bearing the hydroxyl group with the configuration about the asymmetric carbon atom present in \(D\)- or \(L\)-glyceric aldehyde.
\(\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) was recovered. If the \(\alpha\text{-amino-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) with \([\alpha]_D^{24} = -13.7^\circ \) were a mixture of diastereoisomers, one would expect that the above transformations would bring about at least a partial resolution, and a concomitant change in specific rotation. As this did not occur, we concluded that the \(\alpha\text{-amino-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) with \([\alpha]_D^{24} = -13.7^\circ \) is not a mixture but is one of the two expected diastereoisomers. The correctness of this conclusion was demonstrated when the same series of reactions was applied to the \(\alpha\text{-amino-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) with \([\alpha]_D^{24} = 16.0^\circ \). In this instance benzylation of the amino acid led to the formation of an \(\alpha\text{-benzamido-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) with a melting point of 135–136\(^\circ\). Thus we have a situation in which benzylation of one diastereoisomer results in the formation of a stable benzamido lactone and benzylation of the other diastereoisomer results in the formation of a stable benzamido acid.\(^1\)

Since the physical and chemical properties of the benzamido acid and the benzamido lactone are so different, it is clear that any diastereoisomeric impurity in one or the other amino acid would not survive the benzylation reaction and since the two benzyol derivatives could be converted into the amino acids from which they were formed without altering the original specific rotations of the amino acids it is obvious that the latter compounds are pure diastereoisomers.\(^2\)

Bergmann and coworkers (3) have shown that the enzymatic synthesis of the amides and phenylhydrazides of acylated amino acids by purified papain proceeds only when the acylated amino acid has the \(L\) configuration around the carbon atom bearing the potential amino group. The incubation of sodium \(\alpha\text{-benzamido-}\beta,\gamma\text{-dihydroxy-}n\text{-butyrate} \), \([\alpha]_D^{24} = 31.3^\circ \), prepared from the \(\alpha\text{-amino-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \) with \([\alpha]_D^{24} = -13.7^\circ \) with phenylhydrazine, purified papain, and cysteine hydrochloride led to the formation of an \(\alpha\text{-benzamido-}\beta,\gamma\text{-dihydroxy-}n\text{-butyryl-(N-phenyl)hydrazide} \) which was identical with the phenylhydrazide prepared by condensing phenylhydrazine with the \(\alpha\text{-benzamido-}\beta,\gamma\text{-dihydroxy }n\text{ butyro-}

1 Scale models of the two diastereoisomeric benzamido lactones revealed that in the case of the erythro compound some of the possible positions, produced by rotation around carbon-nitrogen and carbon-oxygen single bonds, resulted in absurd interatomic distances. This was not the case with the threo compound and considering the possibility of restricted rotation around the various single bonds it is understandable why one diastereoisomer should form a stable benzamido acid and the other a stable benzamido lactone. It is clear that these considerations also lead to the assignment of the erythro configuration to the amino acid \([\alpha]_D^{24} = 16.0^\circ \) and the threo configuration to the amino acid \([\alpha]_D^{24} = -13.7^\circ \).

2 The benzamido acid, derived from the \(\alpha\text{-amino-}\beta,\gamma\text{-dihydroxy-}n\text{-butyric acid} \), \([\alpha]_D^{24} = 16.0^\circ \), was also converted into the corresponding phenylhydrazide, \([\alpha]_D^{24} = 87.8^\circ \), from which the original amino acid, \([\alpha]_D^{24} = 15.7^\circ \), was obtained by hydrolysis.
lactone derived from the \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid with \([\alpha]_b^{24} = -13.7^\circ\). Furthermore the enzymatically synthesized phenylhydrazide was hydrolyzed stepwise to give the \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyrolactone with a melting point of 210–211\(^\circ\), and the \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid with \([\alpha]_b^{24} = -13.7^\circ\). These experiments not only offer another demonstration of the homogeneity of the \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid but also provide substantial evidence that this amino acid is the \(D\)-threo-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid.

Lutz and Jirgensons (4) have shown that the specific rotation of an amino acid in aqueous solution is dependent, within limits, upon the pH of the solution and that with increasing acid concentration the specific rotation changes in a positive sense for \(L\) antipodes and in a negative sense for \(D\) antipodes. When these principles were applied to the two diastereoisomeric \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acids, it was found that the specific rotation of the amino acid with \([\alpha]_b^{24} = -13.7^\circ\) changed in a positive sense and the specific rotation of the amino acid with \([\alpha]_b^{24} = 16.0^\circ\) changed in a negative sense, with increasing acid concentration. Thus we have additional evidence consistent with the interpretation that the amino acid with \([\alpha]_b^{24} = -13.7^\circ\) is \(D\)-threo-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid and the one with \([\alpha]_b^{24} = 16.0^\circ\) is \(D\)-erythro-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid.

The results of the investigations of Krebs (5) on \(D\)-amino acid oxidase were utilized in a study of the action of this enzyme upon the two diastereoisomeric \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acids. As the rate of oxygen consumption, in c.mm. per hour at 30\(^\circ\), for the \(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid with \([\alpha]_b^{24} = 16.0^\circ\) was 27.6 and that for the acid with \([\alpha]_b^{24} = -13.7^\circ\) was 0.0, we can conclude that this experiment provides still further evidence that the amino acid with \([\alpha]_b^{24} = 16.0^\circ\) is \(D\)-erythro-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid and that the other acid is \(D\)-threo-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid.

\(D\)-Erythro-\(\alpha\)-benzamido-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid was refluxed with anhydrous butanol to give a reaction product which was composed of 2 parts of \(D\)-erythro-\(\alpha\)-benzamido-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyrolactone and 1 part of \(D\)-threo-\(\alpha\)-benzamido-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyrolactone. The mixture was dissolved in aqueous alkali and acidification of this solution led to the isolation of \(D\)-erythro-\(\alpha\)-benzamido-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acid and \(D\)-threo-\(\alpha\)-benzamido-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyrolactone. This conversion of one diastereoisomer into the other is still further proof of the correctness of our conclusions regarding the homogeneity and configuration of the two diastereoisomeric \(D\)-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxy-\(n\)-butyric acids.

The synthesis and characterization of the two theoretically possible
diastereoisomeric $D$-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acids have made it possible to compare the properties of these structurally unambiguous amino acids, or those of their antipodes, with those exhibited by the aminodihydroxy-$n$-butyric acid which Klenk and Diebold (1) obtained from sphingosine. As the specific rotation of this latter amino acid differs markedly from those of the known $\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acids, we conclude that the amino acid obtained by Klenk and Diebold (1) cannot be an $\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acid, if the specific rotation that they report is correct. On the basis of our present knowledge we must therefore reject the structural formula which Klenk and Diebold (1) proposed for sphingosine and proceed to consider other possible structures. Such investigations are now in progress in this laboratory.

**EXPERIMENTAL**

$D$-Threo-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric Acid—To a solution of 200 gm. of 1,2,5,6-diacetone mannitol, m.p. 120–121°, in 2 liters of anhydrous ethyl acetate, were added, in ten portions, with vigorous stirring at 25°, 337.6 gm. of lead tetraacetate. The precipitated lead salts were discarded, and the filtrate freed of solvent by distillation at atmospheric pressure. The residue was dissolved in 450 ml. of methanol and 41 gm. of anhydrous hydrogen cyanide added to the chilled solution, contained in a pressure bottle. The reaction mixture was maintained at 37° for 2 days, saturated, at 0°, with anhydrous ammonia, and again allowed to stand at 37° for 2 days. The excess ammonia was removed by evaporation in vacuo at 30°, the residue dissolved in 700 ml. of methanol, and 1600 ml. of concentrated hydrochloric acid added to the chilled solution. After standing at 37° for 2 days the solution was saturated, at 0°, with hydrogen chloride and allowed to stand at 37° for 1 day. The precipitated ammonium chloride was discarded and the filtrate freed of excess hydrogen chloride by repeated evaporation in vacuo at 25°. The residue was dissolved in 2.5 liters of water and sufficient crystalline barium hydroxide added to allow the complete removal of ammonia upon subsequent evaporation of the solution. The filtered ammonia-free solution was successively treated with lead carbonate, silver carbonate, hydrogen sulfide, and norit, and the colorless solution evaporated to dryness in vacuo at 35°. The residue was dissolved in 300 ml. of hot water, and after the addition of 400 ml. of methanol, the solution was allowed to stand for 16 hours at 5°. The crystalline product was collected, dried, and recrystallized first from 70 per cent aqueous

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*As the specific rotation of the known $\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acids is practically independent of the temperature, a direct comparison of specific rotations is permissible.*

*Microanalyses by Dr. G. Oppenheimer and Mr. G. A. Swinehart.*
methanol and then from water to give 41.5 gm. of $D$-threo-$\alpha$-amino-$\beta$, $\gamma$-dihydroxy-$n$-butyric acid, hexagonal platelets, m.p. 215°, with decomposition.

**Analysis**—$C_{13}H_{15}O_5N$ (135.1). Calculated. C 35.6, H 6.7, N 10.4

**Found.** C 35.4, H 6.7, N 10.4

**Specific Rotation**—$[\alpha]_D^24 = \frac{-0.02\times 2}{1 \times 0.0906} = -13.7^\circ$ (in water)

**D-Erythro-$\alpha$-amino-$\beta$, $\gamma$-dihydroxy-$n$-butyric Acid**—Methanol (300 ml.) was added to the mother liquor remaining after the separation of the $D$-threo-$\alpha$-amino-$\beta$, $\gamma$-dihydroxy-$n$-butyric acid and after standing at 5° for 2 days the oil, which had separated on the addition of the methanol, solidified. The solid was collected and recrystallized twice from 60 per cent aqueous methanol, and then from water, to give 7.6 gm. of a crystalline product, m.p. 188–190°, with decomposition, $[\alpha]_D^24 = 6.8^\circ$. As microscopic examination of this product revealed the presence of two types of crystals, hexagonal platelets and short thick needles, it was subjected to three recrystallizations from 15 parts of 45 per cent aqueous methanol and one recrystallization from water, to give 3.1 gm. of $D$-erythro-$\alpha$-amino-$\beta$, $\gamma$-dihydroxy-$n$-butyric acid, short thick needles, m.p. 192–194°, with decomposition.

**Analysis**—$C_{13}H_{15}O_5N$ (135.1). Calculated. C 35.6, H 6.7, N 10.4

**Found.** C 35.6, H 6.7, N 10.4

**Specific Rotation**—$[\alpha]_D^N = \frac{0.44\times 2}{1 \times 0.055} = +16.0^\circ$ (in water)

**D-Threo-$\alpha$-benzamido-$\beta$, $\gamma$-dihydroxy-$n$-butyrolactone**—Benzoyl chloride (28 gm.) and 230 ml. of 2 N sodium hydroxide were added, at 5°, in ten equal portions at 30 minute intervals, to 6.76 gm. of $D$-threo-$\alpha$-amino-$\beta$, $\gamma$-dihydroxy-$n$-butyric acid, $[\alpha]_D^24 = -13.7^\circ$, dissolved in 12.5 ml. of water and 37.5 ml. of 2 N sodium hydroxide (6). The reaction mixture was acidified, filtered, the filtrate extracted with ether, the aqueous phase concentrated in vacuo to 100 ml., and the concentrate allowed to stand at 5° for 5 days. The solid was collected, and recrystallized from 50 per cent aqueous ethanol to give 4.7 gm. of $D$-threo-$\alpha$-benzamido-$\beta$, $\gamma$-dihydroxy-$n$-butyrolactone, needles, m.p. 210–211°, with decomposition.

**Analysis**—$C_{13}H_{11}O_5N$ (221.1). Calculated. C 59.7, H 5.0, N 6.3

**Found.** C 59.9, H 5.1, N 6.3

**Specific Rotation**—The lactone, 37.7 mg., was dissolved in 1.92 ml. of 0.0876 N sodium hydroxide and the solution made up to 2.0 ml.; $[\alpha]_D^N = \frac{0.59\times 2}{1 \times 0.0377} = +31.3^\circ$

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5 An additional 1.1 gm. were obtained on working up the mother liquors.
D-Threo-α-benzamido-β,γ-dihydroxy-n-butyrolactone, m.p. 210–211°, (250 mg.) was refluxed for 4 hours with 5 ml. of 8 per cent hydrochloric acid, the hydrolysate extracted with ether, the aqueous phase freed of chloride ion, and evaporated to dryness. 1.5 ml. of methanol were added to the residue dissolved in 1 ml. of water, and after standing for 16 hours at 5° the precipitate was collected and dried to give 84.2 mg. (55 per cent) of D-threo-α-amino-β,γ-dihydroxy-n-butyric acid, hexagonal platelets, m.p. 215°, with decomposition.

\[ \text{Specific Rotation} - [\alpha]_D^{25} = \frac{-0.56\times 2}{1 \times 0.084} = -13.4^\circ \text{ (in water)} \]

D-Erythro-α-benzamido-β,γ-dihydroxy-n-butyric Acid—D-Erythro-α-amino-β,γ-dihydroxy-n-butyric acid, \([\alpha]_D^{24} = 16.0^\circ\), was benzoylated in a manner similar to that described above and from 0.8 gm. of the amino acid we obtained 1.04 gm. of D-erythro-α-benzamido-β,γ-dihydroxy-n-butyric acid, needles, m.p. 135–136°, after recrystallization from water. 6

**Analysis—**C_{11}H_{10}O_4N (239.1). Calculated. C 55.2, H 5.4, N 5.9  Found. " 55.4, " 5.8, " 5.8

**Specific Rotation—**The acid, 37.7 mg., was dissolved in 1.80 ml. of 0.0876 N sodium hydroxide and the solution made up to 2.0 ml.; \([\alpha]_D^{24} = \frac{-0.44\times 2}{1 \times 0.0377} = -23.3^\circ\)

A solution of 1 gm. of D-erythro-α-benzamido-β,γ-dihydroxy-n-butyric acid in 25 ml. of anhydrous butanol was refluxed for 3 hours. The solvent was removed and the residue recrystallized from 7 ml. of absolute ethanol to give 0.6 gm. of lactone, platelets, m.p. 135–138°.

**Analysis—**C_{11}H_{10}O_4N (221.1). Calculated. C 59.7, H 5.0, N 6.3  Found. " 59.8, " 5.4, " 6.5

**Specific Rotation—**The lactone, 37.8 mg., was dissolved in an equivalent amount of 0.0876 N sodium hydroxide and the solution made up to 2.0 ml.; \([\alpha]_D^{24} = \frac{-0.11\times 2}{1 \times 0.0378} = -5.8^\circ\)

A mixture of 2 parts of D-erythro-α-benzamido-β,γ-dihydroxy-n-butyrolactone and 1 part of D-threo-α-benzamido-β,γ-dihydroxy-n-butyrolactone, when converted into the sodium salts of the corresponding acids, would be expected to have a specific rotation of \([\alpha]_D^{24} = -5.8^\circ\). The presence of the two diastereoisomers in the above product was demonstrated as follows: 200 mg. of the lactone, \([\alpha]_D^{24} = -5.8^\circ\), were dissolved in 2 ml. of N sodium

6 D-Erythro-α-benzamido-β,γ-dihydroxy-n-butyric acid is readily soluble in cold aqueous potassium bicarbonate. As D-threo-α-benzamido-β,γ-dihydroxy-n-butyrolactone is insoluble in this reagent, it is obvious that we have here a second and more elegant method for separating the two diastereoisomers.
hydroxide and the solution cautiously acidified, at 0°, with 12 N hydrochloric acid. The precipitate that had formed was collected to give 142 mg. of D-erythro-α-benzamido-β,γ-dihydroxy-n-butyric acid, m.p. 135-136°, and was soluble in cold aqueous potassium bicarbonate. The filtrate (above) was kept at 5° for 1 day, and the crystalline precipitate collected, and recrystallized from water, to give 20 mg. of D-threo-α-benzamido-β,γ-dihydroxy-n-butyrolactone, m.p. 210-211°, insoluble in cold aqueous potassium bicarbonate.

D-Erythro-α-benzamido-β,γ-dihydroxy-n-butyric acid (500 mg.) was refluxed for 5 hours with 10 ml. of 8 per cent hydrochloric acid, the hydrolysate extracted with ether, and the aqueous phase freed of chloride ion and evaporated to dryness. 7 ml. of methanol were added to a hot filtered solution of the residue in 2 ml. of water, and after standing overnight at 0°, the precipitate was collected, dried, and recrystallized from 7 ml. of 70 per cent methanol, to give 42 mg. of D-erythro-cr-amino-β,γ-dihydroxy-n-butyric acid, needles, m.p. 192-194°, with decomposition.

\[
\begin{align*}
\text{Specific Rotation} - [\alpha]_D^{2} &= \frac{0.33\times 2}{1 \times 0.042} = +15.7^\circ \text{ (in water)} \\

D-Threo-α-benzamido-β,γ-dihydroxy-n-butyr-(N-phenyl)hydrazide \quad \text{— A} \\
\text{mixture of 200 mg. of D-threo-α-benzamido-β,γ-dihydroxy-n-butyr-} \\
\text{lactone, m.p. 210–211°, and 1.5 ml. of phenylhydrazine was heated, at 100°} \\
\text{in an atmosphere of nitrogen, for 3 hours. 5 ml. of ether were added to} \\
\text{the clear solution, and the latter allowed to stand, at 5°, for 16 hours.} \\
\text{The crystalline precipitate was collected and recrystallized from 95 per} \\
\text{cent ethanol to give 168 mg. (57 per cent) of the phenylhydrazide of D-threo-} \\
\text{α-benzamido-β,γ-dihydroxy-n-butyric acid, platelets, m.p. 169°.}
\end{align*}
\]

Analysis—C_{17}H_{15}O_{3}N_{5} (329.2). Calculated. C 61.9, H 5.9, N 12.8

\[
\begin{align*}
\text{Found. } \quad &\text{C } 61.9, \text{ H } 5.9, \text{ N } 13.0 \\

\text{Specific Rotation} - [\alpha]_D^{2} &= \frac{-0.48\times 2}{1 \times 0.0604} = -15.9^\circ \text{ (in pyridine)}; \quad [\alpha]_D^{2} = \frac{-0.14\times 2}{1 \times 0.0308} = -9.1^\circ \text{ (in glacial acetic acid)}
\end{align*}
\]

In order to provide evidence regarding the configuration around the asymmetric carbon atom bearing the benzamido group the phenylhydrazide was synthesized enzymatically according to the procedure of Bergmann and coworkers (3). 4.10 gm. of D-threo-α-benzamido-β,γ-dihydroxy-n-butyr- lactone were dissolved in the minimum amount of N sodium hydroxide, and the resulting solution adjusted to pH 4.7 with glacial acetic acid. After the addition of 15 ml. of citrate buffer (pH 5.0), 20 ml. of papain-buffer solution,\(^7\) 1.85 ml. of phenylhydrazine, and 0.2 gm. of

\(^7\) Prepared by dissolving 180 mg. of purified papain (3) in 10 ml. of water and 10 ml. of citrate buffer (pH 5.0).
cysteine hydrochloride to the above solution, it was adjusted to pH 4.7, made up to 100 ml., and incubated at 40° for 1 week. After the solution was cooled to 0°, the phenylhydrazide was collected and recrystallized from 95 per cent ethanol to give 2.96 gm. (49 per cent) of D-threo-α-benzamido-β, γ-dihydroxy-n-butyr-(N-phenyl)hydrazide, platelets, m.p. 169°, mixed melting point with the non-enzymatically prepared phenylhydrazide 169°.

Specific Rotation—\[\alpha\]_D^{\text{\scriptsize (pyridine)}} = \frac{-0.45^\circ \times 2}{1 \times 0.0603} = -15.9^\circ

In accordance with a reaction observed by Hann and Hudson (7) 2.3 gm. of D-threo-α-benzamido-β, γ-dihydroxy-n-butyr-(N-phenyl)hydrazide, m.p. 169°, were refluxed for 2 hours with 1.76 gm. of cupric sulfate pentahydrate dissolved in 23 ml. of water. The cuprous oxide was removed and the filtrate placed in the cold room overnight. The solid was collected and recrystallized from 50 per cent ethanol to give 0.5 gm. of D-threo-α-benzamido-β, γ-dihydroxy-n-butyrolactone, needles, m.p. 210–211°, with decomposition.

Analysis—C_{11}H_{14}O_{2}N (221.1). Calculated. C 59.7, H 5.0, N 6.3
Found. " 59.4, " 5.1, " 6.3

Specific Rotation—\[\alpha\]_D^{\text{\scriptsize (water)}} = \frac{0.60^\circ \times 2}{1 \times 0.0377} = +31.8^\circ (in an equivalent amount of sodium hydroxide)

The filtrate remaining after the separation of D-threo-α-benzamido-β, γ-dihydroxy-n-butyrolactone was freed of cupric and sulfate ions and evaporated to dryness. The residue dissolved in 8 ml. of 8 per cent hydrochloric acid was refluxed for 5 hours and upon working up the hydrolysate we obtained 224 mg. of D-threo-α-amino-β, γ-dihydroxy-n-butyc acid, hexagonal platelets, m.p. 215°, with decomposition.

Specific Rotation—\[\alpha\]_D^{\text{\scriptsize (water)}} = \frac{-0.66^\circ \times 2}{1 \times 0.100} = -13.2^\circ (in water)

D-Erythro-α-benzamido-β, γ-dihydroxy-n-butyr-(N-phenyl)hydrazide — A mixture of 100 mg. of D-erythro-α-benzamido-β, γ-dihydroxy-n-butyc acid, m.p. 135–136°, and 2 ml. of phenylhydrazine was heated, at 100° in an atmosphere of nitrogen, for 5 hours, 10 ml. of ether were added to the chilled solution, and the latter allowed to stand in an ice bath for 2 hours. The precipitate was collected, washed with ether, and recrystallized from 95 per cent ethanol to give 84 mg. (57 per cent) of the phenylhydrazide of D-erythro-α-benzamido-β, γ-dihydroxy-n-butyc acid, needles, m.p. 203–204°, with decomposition.
Analysis—C₁₇H₁₅O₄N₃ (329.2). Calculated. C 61.9, H 5.9, N 12.8
Found. " 61.9, " 5.9, " 12.8

Specific Rotation—$[\alpha]_D^{\text{p}} = \frac{0.72^\circ \times 2}{1 \times 0.0164} = +87.8^\circ$ (in pyridine)

A mixture of 500 mg. of the phenylhydrazide of $D$-erythro-$\alpha$-benzamido-$\beta,\gamma$-dihydroxy-$n$-butyric acid, m.p. 203–204°, and 5 ml. of 8 per cent hydrochloric acid was refluxed for 3 hours, the hydrolysate extracted with ether, and the aqueous phase freed of chloride ion and evaporated to dryness. The residue was dissolved in 1 ml. of hot water, 3 ml. of methanol added, and the solution placed in the cold room overnight. The solid was collected and recrystallized from 45 per cent methanol to give 49 mg. of $D$-erythro-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acid, short thick needles, m.p. 192–194°, with decomposition.

Specific Rotation—$[\alpha]_D^{\text{p}} = \frac{0.36^\circ \times 2}{1 \times 0.049} = +14.7^\circ$ (in water)

Relation between Acidity and Specific Rotation of Aqueous Solutions of $D$-Threo-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric Acid—In all experiments relating to $D$-threo-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric acid, 270 mg. of the amino acid, $[\alpha]_D^{\text{p}} = -13.7^\circ$, were dissolved in varying amounts of N hydrochloric acid and the solutions made up to 10 ml. (Table I).

In all experiments relating to $D$-erythro-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
$\frac{\text{m hydrochloric acid}}{\text{m amino acid}}$ & $[\alpha]_D^{\text{p}}$ \\
\hline
0.0 & -13.7 \\
0.50 & -8.7 \\
1.00 & -3.5 \\
1.50 & -2.8 \\
1.75 & -2.4 \\
2.00 & -1.8 \\
3.00 & -1.8 \\
4.80 & -1.7 \\
\hline
\end{tabular}
\caption{Relation between Acidity and Specific Rotation of Aqueous Solutions of $D$-Threo-$\alpha$-amino-$\beta,\gamma$-dihydroxy-$n$-butyric Acid}
\end{table}

* The low rotation observed in this case is probably due to the presence of decomposition products produced by the action of hydrochloric acid on the amino acid. Because of the small amount of material available, no attempt was made to raise the rotation by further recrystallization.
SYNTHESIS OF AMINO ACIDS

TABLE II
Relation between Acidity and Specific Rotation of Aqueous Solutions of D-Erythro-α-amino-β,γ-dihydroxy-n-butyric Acid

<table>
<thead>
<tr>
<th>m hydrochloric acid</th>
<th>m amino acid</th>
<th>[α]_&lt;sub&gt;D&lt;/sub&gt;°</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td>16.0</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>-11.1</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>-18.5</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>-23.0</td>
</tr>
</tbody>
</table>

TABLE III
Oxygen Uptake at 30°

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rate of oxygen consumption (c.mm. per hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-Alanine</td>
<td>237.0</td>
</tr>
<tr>
<td>DL-Serine</td>
<td>145.0</td>
</tr>
<tr>
<td>D-Erythro acid, [α]_&lt;sub&gt;D&lt;/sub&gt; = 16.0°</td>
<td>27.6</td>
</tr>
<tr>
<td>D-Threo acid, [α]_&lt;sub&gt;D&lt;/sub&gt; = -13.7°</td>
<td>0.0</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
</tr>
</tbody>
</table>

TABLE IV
Enzymatic Synthesis of Amides and Hydrazides (Acidic Component Varied)

| Acidic component       | Basic component | M.p. of anilide or phenyl-
|                        |                 | hydrazone* | Yield of anilide or phenyl-
|                        |                 | °C.        | hydrazone | per cent |
| Carbobenzyloxyglycine  | Aniline         | 144        | 80        |
| Benzoylglycine         | "               | 212        | 70        |
| N-Phenylcarbamylglycine| "               | 214        | 10        |
| Benzenesulfonylglycine | "               | 151        | 5         |
| Carbobenzyloxyglycine  | Phenylhydrazine | 144        | 90        |
| Benzoylglycine         | "               | 185        | 95        |
| p-Toluenesulfonylglycine| "            | 169        | 80        |
| Carbethoxyglycine      | "               | 123        | 65        |
| Benzenesulfonylglycine | "               | 165        | 60        |

* Determined on recrystallized products giving satisfactory analyses.

butyric acid, 27.0 mg. of the amino acid, [α]_<sub>D</sub> = 16.0°, were dissolved in varying amounts of N hydrochloric acid and the solutions made up to 2 ml. (Table II).
Action of D-Amino Acid Oxidase upon D-Threo- and D-Erythro-α-amino-β,γ-dihydroxy-n-butyric Acid—The D-amino acid oxidase was prepared according to the directions given by Negelein and Bromel (8); 300 gm. of dried product were obtained from 1.6 kilos of lamb kidney. 70 gm. of the above preparation were stirred, for 45 minutes, at 38°, with 1.4 liters of 0.0167 M sodium pyrophosphate, pH 8.3. Disodium phosphate was then added until the solution was 0.0167 M in respect to sodium pyrophosphate and 0.2 M in respect to disodium phosphate. In each experiment, 2 ml. of enzyme solution, 0.3 ml. of water, and 0.37 mM of amino acid were used, and the oxygen uptake, at 30°, was determined in the usual manner (Table III).

Some Observations on Enzymatic Synthesis of Amides and Hydrazides—In one series of experiments 50 ml. of solution, adjusted to pH 4.7, containing 1 gm. of the acidic component, a 10 per cent molar excess of aniline or phenylhydrazine, 50 mg. of cysteine hydrochloride, and 25 ml. of papain-buffer solution, were incubated for 10 days at 40° (Table IV).

In a second series of experiments 50 ml. of solution, adjusted to pH 4.7, containing a 10 per cent molar excess of the basic component, 1 gm. of carbobenzoxyglycine or hippuric acid, 50 mg. of cysteine hydrochloride, and 25 ml. of papain-buffer solution, were incubated for 10 days at 40° (Table V).

**Table V**

<table>
<thead>
<tr>
<th>Basic component</th>
<th>Acidic component</th>
<th>M.p. of amide or hydrazide</th>
<th>Yield of amide or hydrazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Toluidine</td>
<td>Carbobenzoxyglycine</td>
<td>162</td>
<td>90</td>
</tr>
<tr>
<td>Benzhydrazide†</td>
<td>&quot;</td>
<td>162</td>
<td>80</td>
</tr>
<tr>
<td>m-Phenylenediamine†</td>
<td>&quot;</td>
<td>155</td>
<td>40</td>
</tr>
<tr>
<td>α-Methylphenylhydrazine</td>
<td>Benzoylglycine</td>
<td>163</td>
<td>45</td>
</tr>
<tr>
<td>Hydrazine†</td>
<td>&quot;</td>
<td>256</td>
<td>20</td>
</tr>
</tbody>
</table>

* Determined on recrystallized product giving satisfactory analyses.
† In these cases diacyl amides or hydrazides are formed.

BIBLIOGRAPHY


We wish to thank Mr. Erik Heegaard for his assistance in connection with these experiments.