SYNTHESIS OF HYDROXYLYSINE

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Hydroxylysine was first recognized as a constituent of protein hydrolysates by Van Slyke and Hiller (1) who in later work (2) established a procedure whereby the relatively pure amino acid could be separated from the lysine fraction by fractional precipitation of the picrates. Van Slyke, Hiller, and MacFadyen (3) studied the reaction of hydroxylysine with periodate in alkaline solution and developed a method for determining hydroxylysine in protein hydrolysates. The periodate reaction, which is specific to vicinal glycols and ethanolamines (4), was shown by Van Slyke, Hiller, MacFadyen, Hastings, and Klemperer (5) to liberate 1 molecule of formaldehyde and 1 molecule of ammonia from hydroxylysine, thus establishing that one of the vicinal ethanalamine carbons was terminal. The influence of the hydroxyl group on the basicity of the amino groups as compared with lysine was studied by Klemperer, Hastings, and Van Slyke (6) and observed to agree with the assumption that the hydroxyl group was vicinal to the more basic (non-α) amino group. These findings established the structure of hydroxylysine as either 2,5-diamino-6-hydroxycaproic acid or as 2,6-diamino-5-hydroxycaproic acid.

Recently Bergström and Lindstedt (7) have isolated natural hydroxylysine and prepared the dibenzoyl derivative in which they demonstrated the hydroxyl group to be in the 5 position by oxidation with chromic acid. 2 equivalents of chromic acid were utilized to yield 5-ketolysine rather than the 4 equivalents required by the structural isomer to give 2,5-diaminoadipic acid. They also demonstrated the reduction of hydroxylysine to lysine by the action of hydriodic acid and phosphorus. The present work confirms this structure by synthesis of the diastereoisomers and separation of a derivative of the L isomer having identical properties with the same derivative prepared from hydroxy-L-lysine of natural origin.

The introduction of four functional groups into the 6-carbon structure of hydroxylysine requires reactions which do not affect irreversibly any of these groups. The synthesis has been carried out by introducing the hydroxyl and terminal amino groups as a final step under sufficiently mild conditions to restrict secondary reactions. The intermediary com-
pounds can be purified by crystallization or by counter-current distribution, thus avoiding the high temperatures required for distillation.

**DISCUSSION**

The fundamental reaction in the synthesis is the opening of an epoxide ring by the reaction with ammonia.

\[
\text{CO}_2\text{C}_2\text{H}_4
\]

\[
\text{CH}_3-\text{CH}-\text{CH}_2-\text{CH}_3-\text{C}-\text{CO}_2\text{C}_2\text{H}_4 \rightarrow \text{NH}_3
\]

\[
\text{NH}-\text{CHO}
\]

\[
\text{CO}_2\text{C}_2\text{H}_4
\]

\[
\text{CH}_2-\text{CH(CH}_2)_2-\text{C}-\text{CO}_2\text{C}_2\text{H}_4
\]

\[
\text{NH}_2 \quad \text{OH} \quad \text{NH}-\text{CHO}
\]

Krassousky (8) studied the opening of simpler epoxide rings by ammonia and established that the amine group became attached to the carbon carrying the larger number of hydrogen atoms. Thus both 1,2-epoxy-3-methylbutane and 2,3-epoxy-3-methylpentane reacted with ammonia to give 1-amino-2-hydroxy-3-methylbutane and 2-amino-3-hydroxy-3-methylpentane respectively. The properties of the products were identical with those of the derivatives prepared through methyl magnesium bromide addition to glycine and alanine esters. No evidence of the addition of ammonia to the more substituted carbon atom was observed. Gabriel and Ohle (9) found similar results in the reaction of substituted ethylene oxides with phthalimide. Of the reactions of ethylene oxides with ammonia which have been reported in the literature, no deviations from Krassousky's rule have been reported. The predominant product of the amination reaction is therefore assumed to be the 2,6-diamino-5-hydroxycaproic acid.

The presence of 2 asymmetric carbon atoms in hydroxylysine should result in synthesis of four diastereoisomers. The ratio in which they are formed by the present synthesis has not been determined. Stein and Moore (private communication) have studied the rate of appearance of natural and synthetic hydroxylysine hydrochlorides (not carried through the benzoylation procedure but prepared directly from the pic-

1 Chinard has studied the color developed by the reaction of ninhydrin with amino acids in acid solution and has found that synthetic and natural hydroxyllysine give the same color. The nature of this reaction has not been elucidated but appears to depend upon the formation of ninhydrin derivatives of 5- and 6-membered nitrogen heterocyclic rings in which the color of the derivatives is determined by the size of the ring and the substitution with hydroxyl groups.
rate) in the propanol-0.5 N HCl eluate of the starch column (10). Their results revealed identical rates of travel of the two components and absence in the synthetic product of any other components which give color with the ninhydrin reagent. Their findings do not establish the absence of diastereoisomers unless the diastereoisomers could be shown to travel at a significantly different rate. However, the \( N,N' \)-dibenzoylhydroxy-DL-lysine and its sodium salt have the properties expected of pure DL compounds. The failure to obtain a crystalline acid from the mother liquor of the sodium \( N,N' \)-dibenzoylhydroxy-DL-lysine suggests that a mixture of acids is present.

The asymmetric synthesis of the anilide under the influence of papain has been used to show the identity of the dibenzoylhydroxy-DL-lysine. The difficult hydrolysis of the product does not result in analytically pure hydroxyllysine for comparison of rotation with the natural product. Both natural and synthetic products had low positive rotation but not in satisfactory agreement. However, the isolation of dibenzoylhydroxy-D-lysine and its sodium salt, which agree in melting point with the \( \alpha \) derivatives, and the reconstitution of dibenzoylhydroxy-DL-lysine and its sodium salt from the pure optical enantiomorphs leave no question as to identity. This is further established by preparation of the dibenzoylhydroxy-L-lysine from the hydrolysis product of the synthetic anilide.

2 The rate of travel of any constituent on the starch column is thought to depend upon its distribution coefficient between the mobile and fixed phases as well as adsorptive forces. Under the conditions of the procedure, these forces are but little affected by the presence of other constituents. Thus, in contrast to the determination of melting point and solubility, in which case one or more solid phases are present, optical enantiomorphs travel at the same rate on the starch column. However, as diastereoisomers are different compounds, they should have different physical and chemical properties, although the differences are not ordinarily as great as those of structural isomers. In the present case, one pair of isomers may have been separated in the picrate recrystallization and conversion to the hydroxyllysine monohydrochloride, which would explain the absence of asymmetry to be expected with diasterioisomers.

3 Acyl amino acids having the natural \( \alpha \) configuration of the \( \alpha \)-carbon atoms have been found by Fruton, Irving, and Bergmann (11) to react more rapidly than the optical enantiomorphs with aniline under the enzymatic action of papain. In the present case the purity of the residual dibenzoylhydroxy-D-lysine and of the anilide of the \( \alpha \) modification indicates markedly different rates of reaction.

4 The analytical methods applied to hydroxyllysine have not given satisfactory results. Although impurities which are difficult to remove may be present, the consistency of deviations from theoretical results on many preparations of the picrate and hydrochloride suggests either a lability of the amino acid or that side reactions in the analytical methods do not permit recovery of theoretical amounts of carbon dioxide, ammonia, or nitrogen. The observed positive specific rotations of less than 1 for both natural and synthetic hydroxy-L-lysine in neutral solution must remain tentative until absolute analytical and optical purity can be demonstrated.
The reactions used are the following.

**Reaction I**

\[
\text{CH}_2\text{=CH-CH}_2\text{CH}_2\text{Br} + \text{OCH-N-CH} \xrightarrow{\text{NaOCl}_2\text{H}_5} \text{CH}_2\text{=CH-CH}_2\text{CH}_2\text{-C-CO}_2\text{H}_4
\]

A. Buten-3-yl bromide  
B. Ethyl formamidomalonate  
C. Ethyl-2-formamido-2-carbethoxyhexen-5-oate

**Reaction II**

\[
\text{C} + \text{CH}_3\text{CO}_2\text{H} \xrightarrow{\text{CHCl}_3} \text{CH}_2\text{-CH-CH}_2\text{-CH}_2\text{-CH}_2\text{-C-CO}_2\text{H}_4
\]

Peracetic acid  
D. Ethyl-2-formamido-2-carbethoxy-5,6-epoxycaproate

**Reaction III**

\[
\text{D} + \text{NH}_2, \text{ then } \xrightarrow{\text{HCl}} \Delta \text{NH}_2\text{-CH}_3\text{-CHOH-CH}_3\text{-CH}_2\text{-C-CO}_2\text{H}_4
\]

E. 2,6-diamino-5-hydroxycaproic acid  
(hydroxylysine)

**Reaction I**—The condensation was effected to give a 55 per cent yield of pure crystalline product. Attempted condensation with a commercially available sample of the acetyl rather than the formyl derivative of aminomalonic ester did not result in a crystalline product containing unsaturation. The difference in results with the two acyl derivatives may be attributable to either a difference in steric hindrance to condensation, a difference in inductive effect of the two acyl groups, to some impurity of the commercial material, or to better crystallization of the formyl product from the reaction mixture. Perhaps even better yields with the formyl derivative might have been obtained by slow addition of the sodium ethoxide.

Formamidomalonic ester (B) was prepared by the method of Galat (12). Buten-3-yl bromide (A) was prepared by the procedure of Linstead.
and Rydon (13). The purity of this bromide and of the alcohol from which it is formed is essential to a satisfactory synthesis.

Reaction II—Findley, Swern, and Scanlan (14) have studied the conditions for formation of epoxide rings of the D type and for avoiding opening of the rings. The conditions used in the present synthesis are those of Bøseken and Schneider (15) in which the epoxide is formed by reaction with peracid in chloroform solution. The vinyl derivative (C) reacts relatively slowly with peracetic acid, as might be expected of a compound in which two methylene groups separate the vinyl group from strongly inductive groupings. Peracetic acid has several desirable properties. The concentrated chloroform solution required to effect epoxidation at a significant rate can be readily prepared from the commercially available 40 per cent solution in acetic acid. Secondly, the excess of peracid and acetic acid can be partially separated from the final reaction mixture by extraction with water, thus avoiding the local heating effects resulting from extraction with alkali.

To obtain maximum yields of D, it was essential to prolong Reaction II until it approached completion, but not so long that large losses of D could occur from opening of the epoxide ring. The conditions described in the experimental section were designed accordingly. Separation of D from unchanged C was accomplished with the aid of Craig's (16) counter-current distribution procedure with negligible losses. The recovered C could be treated again with peracetic acid.

Reaction III—The epoxide (D) reacts readily with ammonia in alcohol solution. The separation of crystalline hydroxylysine picrate from the reaction mixture was carried out by conventional procedures. Hamilton and Chinard5 have found that proline and other secondary α-amino acids liberate carbon dioxide on reaction with ninhydrin subsequent to reaction with nitrous acid, while the primary α-amino acids are deaminated and liberate no carbon dioxide under these conditions. The amount of such secondary α-amino nitrogen and of the difference between the total and primary amino (by nitrous acid reaction) nitrogen in the reaction mixture are nearly equivalent, indicating that little or no disubstitution of ammonia by the epoxide occurs. A sample of the secondary α-amino acid was separated from picrate mother liquors by precipitation with Reinecke salt and converted to the free amino acid. The most probable structure of this contaminant, which could result from solvolysis of the formamido group followed by intramolecular reaction with the epoxide group, is that of the next higher homologue of hydroxyproline (6-membered ring). The picrate of this material is sufficiently insoluble to cause loss of hydroxylysine when the two substances are separated by crystallization of

5 Hamilton, P. B., and Chinard, F. P., personal communication.
hydroxylysine picrate. Since this work was done, new ion exchange resins have appeared which greatly facilitate the separation.\(^6\)

**Reaction I.** Ethyl-2-formamido-\(\beta\)-carbethoxyhexen-6-oate—23 gm. of sodium metal were dissolved in 600 ml. of absolute alcohol. 203 gm. (1 mole) of diethyl formamidomalonate (12) were added, followed by 135 gm. (1 mole) of buten-3-yl bromide (13). The flask was heated gently on the steam cone for 18 hours. After cooling, the now neutral supernatant solution was decanted from the precipitate. The precipitate was partially dissolved in water, and the residue was extracted into chloroform, from which 18 gm. of the desired product separated on concentration. The major portion of the product in the alcoholic supernatant was obtained by removing about half of the alcohol *in vacuo* and adding water to a volume of 500 ml. The crystalline product which readily separated was collected on a Büchner funnel and washed with water. After being dried in air, the material was dissolved in chloroform, dried with sodium sulfate, filtered, and crystallized by slow evaporation of the chloroform. The slightly yellow crystals were washed white on the filter with 15 ml. of water. Combined yield 130 gm. The crystals were colorless and soluble in alcohol, chloroform, and ether. A pure sample was obtained by seeding a concentrated chloroform solution and evaporating the solvent at room temperature until a number of large hexagonal plates had formed. M.p. 84°.

\[ \text{C}_{12}\text{H}_{19}\text{NO}_3 \]  
Calculated. C 56.05, H 7.45, N 5.45  

**Reaction II.** Ethyl-\(\beta\)-formamido-\(\beta\)-carbethoxy-5,6-epoxycaproate—A chloroform solution of peracetic acid free of mineral acid was obtained by mixing 200 ml. of 40 per cent commercial peracetic acid (in glacial acetic acid)\(^7\) with 100 ml. of chloroform and adding 20 ml. of water to separate the phases. The chloroform layer was separated and the aqueous residue was extracted with four 100 ml. portions of chloroform, thereby reducing the volume of the aqueous-acetic acid phase to 85 ml. The combined chloroform extracts were washed with 10 ml. of water and dried over sodium sulfate. This solution was 1.92 M in peracetic acid determined iodometrically. The total acid, measured by titrating to a phenolphthalein end-point with constant shaking of the phases, was 2.84 M, while to phenol red it was 2.23 M.

500 ml. of this chloroform solution of peracetic acid and 207 gm. (0.80

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\(^6\) At the suggestion of Stein and Moore, the separation of hydroxylysine on a column of the ammonium form of Dowex 50 developed by 0.3 M ammonia has been studied and found to be effective. The natural hydroxylysine used in this study was separated in this way from the basic amino acids of gelatin.

\(^7\) Supplied by the Buffalo Electro-Chemical Company, Inc., Buffalo 7, New York.
mole) of ethyl-2-formamido-2-carbethoxyhexen-5-oate dissolved in 200 ml. of chloroform were mixed and allowed to react in the ice chest at 4°. The reaction was followed by iodometric determination of residual peracetic acid and by epoxide analysis of the products by the method of Nicolet and Poulter (17). The reaction had slowed to such an extent after 3 days that the solution was allowed to warm to room temperature overnight. Nearly the theoretical amount of peracetic had been used, although 20 per cent of unchanged starting material remained, as estimated by measurement of unsaturation with IBr by the method of Hanus (18). To remove acetic acid and residual peracetic acid from the chloroform solution, the latter was first extracted with three 100 ml. portions of water, in which the acids are preferentially distributed. Finally sufficient 5 N sodium hydroxide was added drop by drop to a fourth 100 ml. of water above the chloroform to extract the last traces of acid and render the aqueous phase alkaline to phenolphthalein. The chloroform phase was further washed with distilled water, separated, dried over sodium sulfate, and concentrated to dryness in vacuo. Crystals suddenly formed from the residue on cooling. Residual chloroform was removed in a vacuum desiccator. Yield 215 gm. Determination of epoxide by the method of Nicolet and Poulter (17) and of unsaturation by the method of Hanus (18) showed that the mixture consisted of 73 per cent epoxide and 18 per cent of original hexenoate. A sample prepared after the reaction had proceeded for 3 days at 4° contained 54 per cent epoxide and 37 per cent hexenoate. The IBr reagent of Hanus has not been found to react quantitatively with this hexenoate.

To separate the epoxide from residual hexenoate, the counter-current distribution procedure, the theory and practice of which have been described by Craig et al. (16), was applied with benzene and a 50:50 methanol-water mixture as the solvents. The distribution coefficients are approximately 12 for the hexenoate and 2 for the epoxide. 85 gm. of the mixture of products were distributed by the Craig method through four funnels each containing 390 ml. of the methanol-water phase and 80 ml. of the benzene phase. The first benzene and the last methanol-water phases were then separated as “high hexenoate” and “high epoxide” respectively, and the distribution continued by removing pure material from end phases and replacing with fresh phases until further distribution was impractical. The epoxide was obtained in pure crystalline form by concentrating the methanol-water phases in vacuo until crystals had formed. 46 gm. of pure epoxide were obtained in this manner. A further 4.2 gm. were obtained by extracting the mother liquor with chloroform and removing the chloroform in vacuo. M.p. 75°.

C₉H₁₉NO₄. Calculated, C 52.80, H 7.01; found, C 52.77, H 6.96
The recovered hexenoate was retreated with peracetic acid. The losses by this procedure were minor.

Reaction III. (Hydroxylysine)—82 gm. of the purified epoxide were dissolved in 200 ml. of 95 per cent alcohol and 300 ml. of 28 per cent ammonia were added. The suspension was warmed gently until solution was complete. After 2 days at room temperature, the solution was concentrated in vacuo to remove excess ammonia. The concentration was repeated with the addition of 200 ml. of alcohol to reduce further the concentration of free ammonia. To the 250 ml. volume of concentrated reaction mixture were added 250 ml. of concentrated hydrochloric acid, and the solution was refluxed for 20 hours. The solution was concentrated to dryness. Water was then added and the solution concentrated in vacuo three times successively to remove excess hydrochloric acid.

77 gm. of silver oxide were added and stirred to remove chloride ion. The precipitate of silver chloride was removed by centrifugation and washed with water. The washings and supernatant were concentrated in vacuo to remove ammonia. An additional 45 gm. of silver oxide were required to obtain a chloride-free solution. The excess of silver ion was removed by precipitation with hydrogen sulfide. The filtered solution, concentrated to remove H₂S, was made up to a volume of 500 ml. and analyzed for total nitrogen by the Kjeldahl method (19), nitrogen liberated by nitrous acid (20), carbon dioxide liberated by the ninhydrin reaction (21), and carbon dioxide liberated by the ninhydrin reaction after previous reaction with nitrous acid (secondary α-nitrogen of the proline type). Total nitrogen (micro-Kjeldahl), 6.60 gm.; α-amino nitrogen by ninhydrin reaction, 3.59 gm.; primary amino nitrogen by long nitrous acid reaction, 6.065 gm.; secondary α-nitrogen (as determined by an unpublished method of Hamilton and Chinard), 0.473 gm.; secondary and tertiary nitrogen (difference of micro-Kjeldahl and nitrous acid nitrogen), 0.535 gm.

To crystallize the hydroxylysine as picrate, 447 ml. of the chloride-free solution were stirred with 0.195 mole of picric acid and allowed to stand for 1 hour. The suspension was then warmed to 60° for 30 minutes with stirring to complete the solution of picric acid. After standing overnight in the ice chest, the crystals were collected on a Büchner funnel and washed with a small amount of cold distilled water. The picrate was recrystallized from 2 liters of boiling water containing 3 gm. of acid-washed norit. After drying in a vacuum desiccator, the product weighed 46 gm. (0.103 mole). A sample dried in vacuo at 100° was analyzed.

<table>
<thead>
<tr>
<th>Theory</th>
<th>Found</th>
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<tr>
<td>Amino nitrogen by nitrous acid method</td>
<td>7.164</td>
</tr>
<tr>
<td>α-Amino nitrogen by ninhydrin method</td>
<td>3.58</td>
</tr>
<tr>
<td>Nitrogen freed by periodate (3)</td>
<td>3.58</td>
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An additional yield of slightly less pure picrate was obtained by concentrating the mother liquors. A contaminant having the properties of the next higher homologue of hydroxyproline was separated from the picrate mother liquors and purified through the Reinecke salt. This amino acid contained no nitrogen liberated by nitrous acid but 9.7 per cent carboxyl nitrogen.

Hydroxylysine Hydrochloride—30 gm. (7.68 mm) of hydroxylysine picrate were stirred while heating in 50 ml. of \( \text{N} \) HCl. After cooling, the precipitated picric acid was removed and washed with 10 ml. of \( \text{N} \) HCl. The picric acid was again heated to boiling with 30 ml. of \( \text{N} \) HCl, cooled, and again collected. The combined filtrates were extracted with ether and filtered with norit. The clear filtrate was concentrated \textit{in vacuo} to dryness, taken up in distilled water, and stirred with silver oxide until slightly alkaline. The precipitate was collected on the filter and washed with distilled water. The pH of the filtrate was adjusted to approximately 7 by addition of dilute HCl and filtered with norit through a retentive filter paper. The filtrate was concentrated to an oil \textit{in vacuo} and transferred to a 100 ml. beaker with washing. Boiling alcohol was added until the solution became turbid. The oil which separated was rubbed with a glass rod until crystalline. Additional absolute alcohol was added to promote further crystallization. After standing in the ice chest overnight, the crystals were collected and washed with absolute alcohol. Yield 10 gm.; m.p. 225°. Additional material can be recovered from the mother liquors.

C₆H₁₂N₂O₅Cl. Calculated. \( N_2 \) by HONO (20) 14.10, \( N_2 \) by ninhydrin (21) 7.05


\( N,N'\)-Dibenzoylhydroxy-\( dl \)-lysine—9.9 gm. (50 mm) of hydroxylysine hydrochloride were dissolved in 10 ml. of 5 \( \text{N} \) sodium hydroxide and 20 ml. of water. The solution was shaken with 1 ml. of benzoyl chloride and 2.85 ml. of 5 \( \text{N} \) sodium hydroxide at room temperature. 11.5 ml. of benzoyl chloride in 1 ml. lots, together with proportional amounts of sodium hydroxide, were added in 1 hour. After the seventh addition, the entire volume was filled with crystals of the sodium salt. The reaction mixture was allowed to stand for 3 hours at room temperature to insure hydrolysis of esters. The sodium salt of dibenzoylhydroxy-\( dl \)-lysine was collected on the filter and transferred to a beaker in which it was stirred thoroughly with 50 ml. of saturated sodium chloride. The salt was again collected and washed with an additional 50 ml. of saturated sodium chloride in small portions. A sample of the sodium salt recrystallized from absolute alcohol melted at 228–230°. The salt was dissolved in a minimal volume of water, and hydrochloric acid was added to precipitate dibenzoylhydroxy-\( dl \)-lysine. Crystallization of the oil occurred readily.
when rubbed with a glass rod. The white acid was collected and washed with water. The yield of air-dried material was 13.9 gm. A sample recrystallized from absolute alcohol melted at 129-141°. Additional material can be recovered from the mother liquors after precipitation of the acids, removal of benzoic acid, and reprecipitation as the sodium salt.

**Synthetic N,N'-Dibenzylyhydroxy-L-lysine Anilide**—13.9 gm. (37 mM) of dibenzoylethanolamines were added to 28.5 ml. of N sodium hydroxide. Then the extract of 1 gm. of papain with 10 ml. of M citrate buffer at pH 5.2, 0.25 gm. of cysteine hydrochloride, and 1.65 ml. of redistilled aniline were added (11). The volume was made up to 80 ml., the pH was roughly adjusted to 5.2, and the suspension of the sodium dibenzyloxycodysine was stirred frequently while standing at 38°. The reaction mixture rapidly became solid as a result of anilide formation. The anilide was collected after 18 hours and washed with water. The precipitate was repeatedly broken up with a glass rod in 0.1 N sodium hydroxide and collected until free from unchanged sodium dibenzylohydroxylysine, which is strongly held in the precipitate. The precipitate was washed with water, N HCl, and water and dry ether. The precipitate was then dissolved in 250 ml. of hot 80 per cent alcohol and filtered. The filtrate was taken to dryness in vacuo; m.p. 223-225°. After washing with 15 ml. of 95 per cent alcohol, the product melted at 230-232°. Yield 4.55 gm.; [α]D25 +9.14° (82 mg. in 10 ml. of 80 per cent alcohol).

**C32H22N4O4.** Calculated, C 70.10, H 6.11; found, C 69.51, H 6.08

**Natural Dibenzyloxycodysine and Anilide**—Hydroxy-L-lysine hydrochloride of natural origin was similarly converted to the dibenzyloxylsine hydrochloride (m.p. 115°) and to the free dibenzyloxocodysine (m.p. 172°); [α]D25 = +3.75° (100 mg. in 5 ml. of absolute alcohol).

**C32H22N4O4.** Calculated. C 64.85, H 5.96, N 7.54

**Found.** C 64.45, H 6.01, N 7.43

Reaction with papain and aniline gave the anilide, which melted at 230-232° and was undepressed in melting point on mixing with the synthetic anilide; [α]D25 = +9.05° ± 0.50 (83 mg. in 10 ml. of 80 per cent alcohol).

**Dibenzyloxycodysine-d lysine**—The filtrate and washings from the reaction of dibenzyloxycodysine-d L-lysine with aniline and papain were acidified with hydrochloric acid. The crude oil crystallized upon rubbing with a glass rod. After collection and washing with water on the filter, the acid was dissolved in hot alcohol and filtered from the insoluble residue. The solvent was removed on the steam bath and the residue was dissolved in sodium hydroxide, filtered, and acidified. A sample recrystallized from alcohol melted at 172°; [α]D25 = -3.75° (100 mg. in 5 ml. of absolute alcohol). A sample in alcohol was converted to the sodium salt with
5 N NaOH and evaporated to dryness. The salt, recrystallized from alcohol, melted at 115°. Equal weights of the D acid and L acid crystallized together from alcohol melted at 140°, while the sodium salt (from alcohol) melted at 228–230°.

**Synthetic Dibenzoylhydroxy-L-lysine**—4 gm. of dibenzoylhydroxy-L-lysine anilide prepared from the synthetic amino acid were refluxed with 100 ml. of 95 per cent alcohol and 20 ml. of 6 N HCl until solution was complete. The alcohol was distilled and the residue refluxed with 50 ml. of 6 N HCl for 24 hours. The benzoic acid was removed by ether extrac-tion and the solution taken to dryness *in vacuo*. Silver oxide was added with stirring until the solution was slightly alkaline in reaction. The highly colored solution was extracted with ether, filtered with norit to remove the color, and adjusted to pH 7 with dilute hydrochloric acid. The hydrochloride was precipitated as described above; m.p. 225°.

\[ \text{C}_4\text{H}_6\text{N}_2\text{O}_2\text{Cl}. \text{Calculated. } \text{C } 36.35, \text{H } 7.63, \text{N } 14.10 \text{ (21)} \]

\[ \text{Found. } \text{“ } 36.25, \text{ “ } 7.62, \text{ “ } 14.10 \]

450 mg. of this hydrochloride were benzyolated. The alkaline solution was filtered and the residue on the filter washed with water until all the sodium salts had dissolved. The filtrate and washings were acidified with HCl. The aqueous phase was decanted from the precipitate, which was then washed with dry ether. The ether was decanted and the washing repeated. Rubbing the oil under dry ether resulted in crystallization. The crystals were collected and recrystallized from 95 per cent alcohol; m.p. 172–172.5° (micro stage).

The author wishes to thank Dr. D. D. Van Slyke for suggesting the problem and for his constant encouragement and advice. He acknowledges the helpful advice of Dr. Alma Hiller and Dr. Lyman C. Craig. He is indebted to Dr. William H. Stein and Dr. Stanford Moore for the starch column studies, to Dr. Francis P. Chinard for spectral comparisons of the ninhydrin reaction products of synthetic and natural hydroxylysine, and to Mr. D. Rigakos for microanalyses for carbon and hydrogen. He is grateful to the Buffalo Electro-Chemical Company, Inc., for the gift of peracetic acid solution.

**SUMMARY**

Hydroxylysine has been synthesized.

The \( N,N' \)-dibenzoyl derivative of hydroxy-DL-lysine has been separated as its crystalline sodium salt.

The anilide of dibenzoylhydroxy-L-lysine has been prepared and shown to be identical with the anilide of synthetic origin.
The identity of dibenzoylhydroxy-di-lysine has been established by reconstitution from its optical enantiomorphs.

BIBLIOGRAPHY