The resin was then slurried with 1.5 *N* hydrochloric acid and poured into a Pyrex column 50 cm. long with a 9 mm. inside diameter. After settling by gravity, the resin was conditioned by the percolation of 1.5 *N* acid for 48 hours at the rate of 4 ml./hour.

To prepare the charge, 0.6 ml. of the stock solution of streptolin AB hydrolysate (12 mg. of streptolin hydrochloride/ml.) was evaporated to dryness under a stream of nitrogen at 100°. The residue was taken up in 0.6 ml. of 1.5 *N* hydrochloric acid and the resulting solution placed on the column. As the charge reached the resin bed level, the column was filled with 1.5 *N* acid; then a 125-ml. dropping funnel of acid was fitted onto the top. Collection of the eluate in 1.0 ml. fractions was started just as the charge disappeared below the resin bed. The rate of flow was controlled by slight air pressure so as to yield four 1-ml. fractions per hour. After 75 tubes had been collected, the remaining acid was removed from the column and replaced with 2.5 *N* acid. After 325 fractions had been collected, the 2.5 *N* acid was replaced with 4.0 *N* acid, and fractions were collected to tube 500.

The fractions were grouped in batches of 25 consecutive tubes, and the batches were evaporated at 15–20 mm. in a vacuum desiccator immersed in a boiling water-bath. This operation required about four hours, after which time they were allowed to stand eight to ten hours at 0.2 mm. over a mixture of solid potassium hydroxide and calcium chloride. Each batch of collected fractions was then analyzed by the photometric ninhydrin method of Moore and Stein.[5] Since traces of ammonia can be detected by this procedure, it is important that the blanks and analytical samples be handled under strictly parallel conditions. Two leucine standards and two blanks were run with each batch, and the color yield was determined directly in terms of optical density with a Coleman 6A Junior Spectrophotometer. One milliliter of ninhydrin reagent was pipetted into a tube containing the sample. The tube was shaken, capped with a 10-ml. beaker and placed in a vigorously boiling water-bath for 20 minutes. The tube was removed, 5 ml. of diluent added and the tube shaken. The sample was allowed to stand 15 minutes, and then readings were taken at 570 mμ. Readings were essentially constant for one hour after the reaction was completed. The optical density readings were converted to micromoles of leucine by reference to a previously prepared standard curve and the results plotted as a function of tube number (Fig. 1).

Preparative Fractionation.—A glass chromatographic tube 60 cm. long and having a 28-mm. inside diameter was filled with 300 g. of Dowex-50 resin which had been washed with 4.0 *N* hydrochloric acid six times and centrifuged each time. The column was placed on the fraction cutter, and 2.5 *N* acid was used to equilibrate the column as described above. No pressure was used, and the column ran at the rate of 27 ml. per hour. Three hundred milligrams of dry, hydrolyzed streptolin AB was dissolved in 1 ml. of 2.5 *N* acid and the solution placed on the column. The tube was filled with 2.5 *N* acid and a one-liter reservoir mounted on top. Ten-ml. fractions were collected. After the 200th fraction had drained, the 2.5 *N* acid was replaced with 4.0 *N* acid and 165 five-ml. fractions collected. The unit volume of fractions 365 to 450 was raised to 10 ml. Every third fraction was analyzed photometrically as described for the analytical separation. In preparative runs, the ninhydrin procedure was omitted, and the material was isolated by grouping the tubes in accordance with the initially obtained ninhydrin data. The combined tube contents were reduced to dryness *in vacuo*, taken up in distilled water and finally evaporated to dryness in a jet of nitrogen.

Fraction No. 4.—The dry residue was identified as ammonium chloride by conversion to benzamide with benzoyl chloride by the Schotten–Baumann method.

Fraction No. 6.—Evaporation of the combined tubes afforded, on standing, a crystalline hydrochloride, m.p. 215–216°. The *p*-hydroxyazobenzene-*p'*-sulfonate, after repeated recrystallization from water, decomposed sharply at 258–260°.

Anal. Calcd. for $C_4H_8N_2O_3(C_{12}H_{10}N_2O_4S)$: C, 46.82; H, 4.42; N, 13.65. Calcd. for $C_4H_{10}N_2O_3(C_{12}H_{10}N_2O_4S)$: C, 46.60; H, 4.89; N, 13.59. Found: C, 46.62; H, 4.72; N, 13.62.

Fraction No. 7.—About 50 mg. of crude fraction no. 7 was dissolved in 2 ml. of water. Two hundred milligrams of *p*-hydroxyazobenzene-*p'*-sulfonic acid was dissolved in 2 ml. of hot water, and the two solutions were mixed. The fine, golden needles of the di-(*p*-hydroxyazobenzene-*p'*-sulfonate) which formed on cooling were recrystallized repeatedly from water until the dec. point 243.5–244°[8] was reached.

Anal. Calcd. for $C_6H_{14}N_2O_2{\cdot}2C_{12}H_{10}N_2O_4S$: C, 51.28; H, 4.87; N, 11.95. Found: C, 50.91; H, 5.08; N, 11.56.

The dipicrate of fraction no. 7 was prepared by adding 4 ml. of saturated, aqueous picric acid solution to a solution of 50 mg. of crude fraction no. 7 in 4 ml. of 95% ethanol. The yellow precipitate which formed was recrystallized from ethanol until the melting point 200–201° was attained.

Anal. Calcd. for $C_6H_{14}N_2O_2(C_6H_3N_3O_7)_2$: C, 35.78; H, 3.34; N, 18.55. Found: C, 36.38; H, 3.63; N, 18.06.

Acknowledgment.—The authors gratefully acknowledge grants from the National Institute of Health and Parke, Davis and Co.

(8) Taken in a melting point bath and corrected.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Streptolin. The Structure and Synthesis of Isolysine

BY EUGENE E. VAN TAMELEN AND EDWARD E. SMISSMAN

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Isolysine ($C_6H_{14}N_2O_2$), an acid-hydrolysis product derived from streptolin AB,[1] viomycin[3] and streptothricin,[4] possesses a carboxyl group and two primary amino groups, but no C-methyl groups, exhibits optical activity, but gives no copper or cobalt complex. It is oxidized to succinic acid by potassium permanganate, but is not attacked by potassium periodate. Of the structures consistent with these characteristics (β,ε-diaminocaproic acid, γ,ε-diaminocaproic acid and α-aminomethyl-δ-aminovaleric acid), the first was confirmed as that of isolysine by a synthesis involving the Arndt–Eistert homologation sequence starting with L-ornithine. Syntheses of the two remaining structures are also presented.

In a recent communication[1] we described in detail the chromatographic separation of the hydrolysis products derived from streptolin AB, an antibiotic produced by a strain of Streptomyces.[2] The last major fraction (no. 7) to be eluted by acid on a Dowex-50 column was a base, $C_6H_{14}N_2O_2$, which we regarded as a position isomer of lysine. This substance, which we designate as "isolysine," has also been obtained from viomycin by Haskell, *et al.*,[3] and from streptothricin by Carter, *et al.*[4] We now wish to record pertinent chemical characteristics

(1) E. E. Smissman, R. W. Sharpe, B. F. Aycock, E. E. van Tamelen and W. H. Peterson, THIS JOURNAL, **75**, 2029 (1953).

(2) R. W. Rivett and W. H. Peterson, *ibid.*, **69**, 3006 (1947).

(3) T. H. Haskell, S. A. Fusari, R. P. Frohardt and Q. R. Bartz, *ibid.*, **74**, 599 (1952).

(4) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers," 119th Meeting, American Chemical Society, Cleveland, Ohio, April, 1951. p 25A.

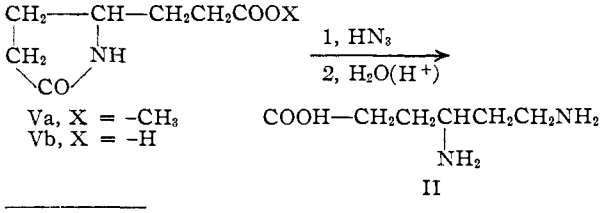
of isolysine as well as a rational synthesis which establishes its structure.[5]

First of all, two primary amino groups in isolysine are indicated by existing analytical data,[1] *viz.*, the dibasic nature of the molecule and the absence of N-alkyl groups. The Van Slyke nitrogen value[6] as well as the failure to liberate volatile base upon treatment with sodium hydroxide at 100°, is consistent with this conclusion.

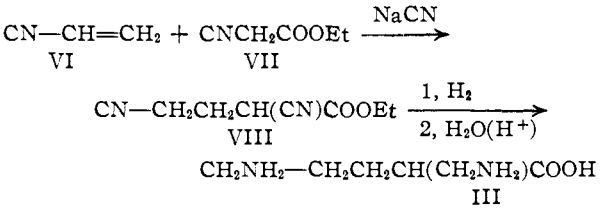
Valuable initial information was obtained by performing a number of simple tests on the richer portions of fraction no. 7 or on one of the pure salts previously described.[1] Isolysine gave a positive hydroxamic acid test,[6] indicative of a carboxyl group. The substance was ninhydrin positive, but gave no copper or cobalt complex; therefore it is probably not an α-amino acid. No potassium periodate was consumed on standing with the amino acid; this observation demonstrates that the amino groups are not vicinal. These data, together with the optical activity of isolysine di-(*p*-hydroxyazobenzene-*p'*-sulfonate) ($[\alpha]^{25}D$ +6.5 ± 1°, alc.) and the absence of C-methyl groups, allow only four structural possibilities: β,ε-diaminocaproic acid (I), γ,ε-diaminocaproic acid (II), α-aminomethyl-δ-aminovaleric acid (III) and β-aminomethyl-δ-aminovaleric acid (IV).

In an effort to gain information regarding the carbon skeleton, we carried out a degradation with potassium permanganate. The oxidation appeared to be sluggish at 40°; and at 80–90°, apparently the only material formed was succinic acid, which was characterized by suitable derivatives. This finding excludes possibility IV, since a substance with that structure should give rise to, not succinic acid, but the permanganate-resistant tricarballylic acid, $CH_2(COOH)CH(COOH)CH_2COOH$.

Because of the paucity of natural material, attention was turned at a very early stage to proof of structure by synthesis. Accordingly, synthetic programs leading to all three remaining possibilities were simultaneously initiated. The straight chain γ,ε-isomer (II) was obtained first, starting with 5-(β-carbomethoxyethyl)-2-pyrrolidone (Va). This heterocycle was conveniently obtained through condensation of one mole of nitromethane with two moles of methyl acrylate, followed by catalytic reduction and spontaneous lactamization of the reduction product.[7] Selective hydrolysis at the ester carbonyl with one mole of sodium hydroxide afforded the crystalline free acid (Vb). This substance was treated with sodium azide and concentrated sulfuric acid under the usual conditions of the Schmidt reaction. The intermediate 5-(β-aminoethyl)-2-pyrrolidone was not isolated but was hydrolyzed directly to the desired *dl*-diamino acid (II), which was isolated and purified as the dipicrate (m.p. 210–211°). The over-all yield from Vb was 29%. Non-identity of isolysine and the γ,ε-isomer was established readily by a comparison of paper strip chromatograms: the R_f value of the natural acid is 0.65, whereas II gave the value 0.58. All chromatograms were developed with phenol–water–formic acid and color-developed with ninhydrin.



One of the two remaining isomers, α-aminomethyl-δ-aminovaleric acid (III), would appear to be easily obtainable by condensing cyanoacetic ester (VI) and acrylonitrile (VII) equimolecularly and reducing the resulting dinitrile to the diamine. Under the usual conditions of condensation, however, the two components VI and VII give an excellent yield of only the 1:2 product, γ-cyano-γ-carbethoxypimelonitrile.[8] By carrying out the condensation at 150–165° with a sodium cyanide catalyst,[9] we have been able to obtain a 33% yield of pure ethyl α,γ-dicyanobutyrate (VIII). Sulfuric acid hydrolysis to glutaric acid proved the structure of the product. Platinum-catalyzed hydrogenation of VIII proceeded smoothly in the presence of an equimolar amount of concentrated sulfuric acid, affording the diamino ester (IX). The mineral acid was added to prevent, presumably through salt



formation, intramolecular condensation of intermediate reduction products and ultimate reduction to piperidine-3-acetic acid. The diamino ester (IX) was not isolated, but was hydrolyzed instead with dilute hydrochloric acid. The *dl*-acid III formed only one salt which crystallized, the di-(*p*-hydroxyazobenzene-*p'*-sulfonate) (DHABS), which decomposed sharply at 233–233.5°. Paper strip chromatographic studies indicated the R_f value 0.65, the same as that of isolysine; mixtures of III and isolysine showed no tendency to separate on the chromatogram.

The third possibility, β,ε-diaminocaproic acid (I), was obtained concurrently by the homologation of DL-ornithine (IXa) according to the Arndt–Eistert method.[10] The basic groups of the starting amino acid were protected[10] by conversion to the di-(N-phthalyl) derivative (XIa) by treatment with phthalic anhydride. The acid chloride, elegantly obtained through the use of oxalyl chloride, was not isolated but was transformed immediately into the crystalline diazoketone (XIIa) by standing with ethereal diazomethane for some hours. The rearrangement to the ester of the homologated acid

(5) This work first appeared in a Communication to the Editor, THIS JOURNAL, **74**, 3713 (1952). *Cf.* H. E. Carter, *ibid.*, **74**, 3704 (1952).

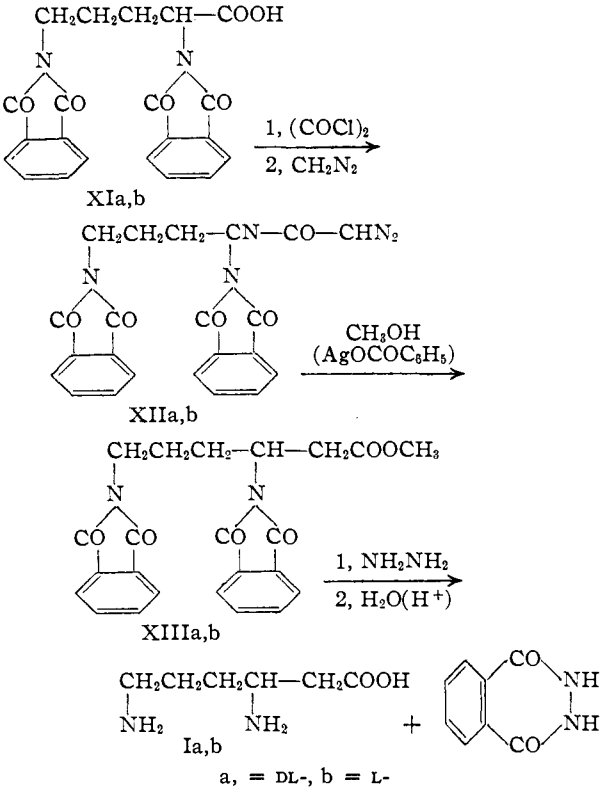
(6) F. Feigl, "Qualitative Analysis by Spot Tests," Elsevier Publishing Co., Inc., New York, N. Y., 1946, p. 355.

(7) N. J. Leonard, L. R. Hruda and F. L. Long, THIS JOURNAL, **69**, 690 (1947).

(8) H. A. Bruson and T. W. Riener, *ibid.*, **65**, 23 (1943).

(9) These reaction conditions have been described by A. O. Rogers, U. S. Patent 2,460,536; *C. A.*, **43**, 3446 (1949).

(10) K. Balenović and D. Fleš, *J. Org. Chem.*, **17**, 347 (1952).



(XIIIa) proceeded smoothly when catalyzed by silver benzoate at room temperature[11]; on standing, almost the entire yield (53%) of pure material crystallized from the excess methanol. The single transformation remaining was removal of the phthalyl protecting groups. High temperature hydrolysis with concentrated hydrochloric acid yielded a mixture of products, but the cleavage with alcoholic hydrazine[12] proceeded smoothly. Hydrochloric acid hydrolysis of methyl β,ϵ-diaminocaproate without isolation was advantageous, and the free acid Ia was then isolated and purified by recrystallization as the DHABS (dec. 243–244°). The R_f value of the acid (0.65) was no different than that of isomer III or isolysine itself, and therefore no choice between structures I and III could be made on this basis.

However, application of the Arndt–Eistert method to the production of an optically active β,ϵ-diaminocaproic acid resulted in a definite decision as to the structure of isolysine. A parallel series of transformations, starting with L-ornithine, led to an optically active ($[\alpha]^{25}D$ −13.5 ± 0.5°, chf.) form (XIIIb), melting at 156–157°, of methyl β,ϵ-diphthalimidocaproate. Hydrazinolysis and subsequent hydrolysis afforded the acid, which was characterized as the DHABS ($[\alpha]^{25}D$ +6.0 ± 1°, alc.) (dec. 242.2–243°). The infrared spectra (Fig. 1, I) of the synthetic and natural dye salts were essentially identical, whereas each differed in several significant respects from the spectrum of α-aminomethyl-δ-aminovaleric acid DHABS (Fig. 1, II). For example, the synthetic and natural isolysine salts exhibited carbonyl absorption at 5.82 μ, but the corresponding band of isomer III was shifted to 5.94 μ. Furthermore, fusion of natural isolysine (as a constituent of crude streptolin hydrolysate) with phthalic anhydride followed by esterification of the product with diazomethane, yielded the di-(N-phthalyl) methyl ester (m.p. 155.5–156.5°); the melting point of this substance did not depress that of the synthetic ester XIIIb. The infrared spectra of these compounds, too, were indistinguishable. Thus, this synthesis establishes the structure of isolysine as β,ϵ-diaminocaproic acid.

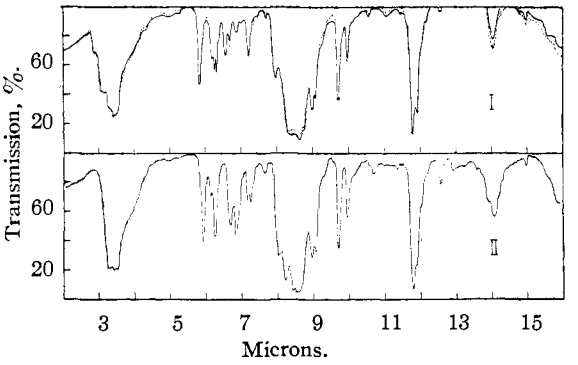


Fig. 1.—I, —, di-(p-hydroxyazobenzene-p'-sulfonate) (DHABS) of authentic isolysine; - - - - -, DHABS of synthetic L-β,ϵ-diaminocaproic acid. II, DHABS of DL-α-aminomethyl-δ-aminovaleric acid. All spectra were taken in Standolind mull with a compensating Stanolind cell.

In addition, it appears likely that isolysine belongs to the same stereochemical series (L) as the bulk of the more common amino acids. The only synthetic step which can affect the asymmetric center is the rearrangement of the diazoketone XIIb to the methyl ester of the homolog, XIIIb. Lane and Wallis[13] have demonstrated in model cases that the Wolff rearrangement occurs *without* inversion, and the presence of the α-phthalimido function in XIIb would not appear to warrant regarding the present case as atypical.[14] Since the ornithine used in this investigation was the L-variety, we assign the same configuration to natural isolysine.

Because of the lack of primary interest in isomers II and III, conditions for optimum yields in the synthetic steps were not worked out. The over-all yield of synthetic isolysine, on the basis of L-ornithine dihydrochloride as the starting material, was 15%. All the yields appeared to be normal for the type of steps involved,[10] except for the hydrazinolysis—concomitant hydrazide formation decreased the yield and made purification of the final product costly (see Experimental).

NOTE ADDED IN PROOF.—Prof. Carter and the present authors have agreed to suggest adaptation of "β-lysine" as the common name for β,ϵ-diaminocaproic acid.

Experimental[15]

Chemistry of Natural Isolysine.—Qualitative tests were run on the richer portions of fraction no. 7 (see Fig. 2, ref. 1)

(11) M. S. Newman and P. F. Beal, THIS JOURNAL, **72**, 5163 (1950).

(12) J. H. Sheehan and V. S. Frank, *ibid.*, **71**, 1856 (1949).

(13) J. F. Lane and E. S. Wallis, *ibid.*, **63**, 1674 (1941).

(14) Balenović and Flês (ref. 10) have expressed a like opinion regarding similar cases.

(15) Melting points, except those noted, were taken in a melting point bath and are corrected. Infrared spectra were recorded on a Baird double beam automatic recording infrared spectrophotometer.

or on material purified as the picrate or the di-(*p*-hydroxyazobenzene-*p'*-sulfonate) (DHABS).[1] The acid was allowed to stand overnight with standard potassium periodate in aqueous solution; titration of excess periodate showed no uptake of the reagent. On treatment with 6 *N* sodium hydroxide at 100° under conditions previously described,[1] no volatile base was liberated. β-Alanine also failed to eliminate ammonia under similar conditions. No acetic acid was formed in either of two Kuhn–Roth determinations.[16] The optical rotation of isolysine DHABS was $[\alpha]^{25}D$ +6.5° (alc.).

Permanganate Oxidation of Isolysine.—One hundred and twenty milligrams of crude isolysine was placed in an 8-inch test-tube fitted with a screw-type stirrer. After the addition of 10 ml. of water, the solution was heated to approximately 80°, and a solution of 200 mg. of potassium permanganate in 10 ml. of water was added dropwise with stirring over a period of one hour. The reaction mixture was then cooled to room temperature, hydrochloric acid was added and the mixture was filtered. The solution was taken to dryness under nitrogen, leaving a solid residue. Extraction with ether and subsequent evaporation afforded 41 mg. of crystals which melted at 157–162°.[17] The anilide (m.p. 255–256°[17]), *p*-phenylphenacyl ester (m.p. 207–208.5°[17]) and *p*-bromophenacyl ester (m.p. 209–210°[17]) were prepared. The corresponding derivatives of succinic acid melt, respectively, at 260, 208 and 211°. The mixed melting point between each derivative of the oxidation product and the corresponding authentic derivative was not depressed.

The melting point of the oxidation product was greatly depressed on admixture with authentic tricarballylic acid (m.p. 160–161°).[18] The latter material was unattacked by permanganate under the conditions of the oxidation described above.

5-(β-Carboxyethyl)-2-pyrrolidone (Vb).—5-(β-Carbomethoxyethyl)-2-pyrrolidone (Va) (m.p. 52–54°, lit. 52–53°[7]) was prepared according to the directions of Leonard, *et al.*[7] A solution of Va (15.16 g., 0.088 mole) in 50 ml. of methanol was refluxed for two hours after the addition of 3.64 g. (0.088 mole) of sodium hydroxide dissolved in 200 ml. of methanol. All the solvent was then removed *in vacuo;* the sodium salt remaining failed to crystallize. This residue was dissolved in water and the resulting solution acidified with hydrochloric acid. Evaporation to dryness *in vacuo* and dissolution of the residue in a small amount of methanol left the sodium chloride undissolved; it was removed by filtration. Evaporation yielded a viscous oil which was induced to crystallize by slow evaporation from ethanol. Recrystallization from ethanol–benzene gave material melting at 126–127°.

Anal. Calcd. for $C_7H_{11}O_3N$: C, 53.50; H, 7.01; methoxyl, 0.00. Found: C, 53.52; H, 7.07; methoxyl, 0.00.

DL-γ,ε-Diaminocaproic Acid (II).—A suspension of 0.52 g. (0.0033 mole) of Vb in a mixture of 4 ml. of chloroform and 8 ml. of concentrated sulfuric acid was placed in a round-bottomed flask and heated in a water-bath to 45–47°. After solution of the acid was complete, 0.20 g. (0.0031 mole) of sodium azide was added in small portions over a 45-minute period. During this time the mixture was stirred mechanically and the internal temperature was maintained at 45–47°. After the addition was complete, stirring and heating at 45–50° were continued for four hours. The cooled mixture was then poured, with stirring, onto 40 g. of ice. After the initial, exothermic reaction was over, the chloroform layer was separated. The aqueous portion (50 ml.) was refluxed for 12 hours. After cooling, the *p*H was adjusted to 4 by careful addition of hot, concentrated barium hydroxide solution. The precipitate of barium sulfate was filtered off and washed with water. The combined filtrate (*p*H 4) amounted to about 80 ml. A solution of 1.6 g. of picric acid in 175 ml. of ether was added and the resulting mixture shaken. After standing several hours, the mixture deposited a yellow, crystalline solid, which was filtered off and dried. The melting point (210–211°) of the dipicrate (0.60 g., 29%) was not raised by two recrystallizations from water.

Anal. Calcd. for $C_6H_{14}N_2O_2(C_6H_3N_3O_7)_2$: C, 35.78; H, 3.33. Found: C, 36.01; H, 3.22.

The acid gave an R_f value of 0.58 on a paper chromatogram developed with phenol–water–formic acid and color-developed with ninhydrin. Isolysine gave the value 0.65.[19]

Ethyl α,γ-Dicyanobutyrate (VIII).—One gram of sodium cyanide and three drops of water were added to 250 g. (2.52 moles) of cyanoacetic ester in a 500-ml. three-necked flask equipped with a condenser, stirrer, thermometer and dropping funnel. After the internal temperature was raised to about 150°, 26.5 g. (0.5 mole) of acrylonitrile was added dropwise with stirring over a period of 20 minutes; during this time 0.5-g. portions of sodium cyanide were added at five-minute intervals. The internal temperature rose to 165°, and a slight decrease in external heating was required to prevent the temperature from rising above this point. After the addition of the nitrile was finished, stirring and heating at 150–160° were continued for 0.5 hour. The cooled, red suspension was washed twice with 50-ml. portions of saturated salt solution, then with dilute hydrochloric acid–salt solution and finally again with two small portions of the salt solution. Distillation afforded 34.8 g. of colorless liquid, b.p. 165–180° (8 mm.). Twenty-seven and two-tenths grams (33%) of material boiling at 168–171° (9 mm.) was obtained on redistillation.

Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.06. Found: C, 57.93; H, 6.00.

A small amount of ethyl dicyanobutyrate was allowed to stand overnight in concentrated sulfuric acid. The reaction mixture was poured into water and the resulting solution was extracted with ether. Evaporation of the solvent and recrystallization of the residue from water yielded glutaric acid, melting at 97–98° (lit. 97°). The acid was converted by thionyl chloride to glutaric anhydride (m.p. 55–57°, lit. m.p. 56–57°).

DL-α-Aminomethyl-δ-aminovaleric Acid (III).—Ethyl α,γ-dicyanobutyrate (1.66 g., 0.01 mole) was dissolved in a solution of 1.0 g. of concentrated sulfuric acid previously mixed with 15 ml. of glacial acetic acid. Hydrogenation was carried out in a shaker-type apparatus, using 0.2 g. of platinum oxide (American Platinum Works) catalyst. The reduction was complete (calcd. p.s.i. uptake: 10.2; found, 10.5) in about five hours. A few crystals suspended in the medium were filtered off along with the catalyst and the filtrate was evaporated to dryness *in vacuo*. Twelve ml. of 20% hydrochloric acid was added and the resulting solution was heated for two hours on the steam-bath. Evaporation *in vacuo* gave a thick sirup, which was diluted to 10 ml. with water. An aliquot (0.5 ml., 0.5 mmole) was added to a hot solution of 0.278 g. (1 mmole) of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 5 ml. of water. On cooling, golden crystals formed, which were collected by filtration and recrystallized twice from water. The yield of analytical material, which decomposed sharply at 233.0–233.5°, was 24%.

Anal. Calcd. for $C_6H_{14}N_2O_2(C_{12}H_{10}N_2O_4S)_2$: C, 51.28; H, 4.87. Found: C, 51.33; H, 4.92.

The R_f value, obtained as previously described, was 0.65.

DL-Di-(N-phthalyl)-ornithine (XIa).—To 15 ml. of glacial acetic acid was added 1.0 g. (0.0059 mole) of DL-ornithine monohydrochloride, 0.82 g. (0.010 moles) of sodium acetate and 2.0 g. (0.013 mole) of phthalic anhydride. After the mixture had refluxed for 45 minutes, the acetic acid was removed under reduced pressure. The residue was taken up in dilute sodium hydroxide; the resulting solution was immediately filtered and then acidified with dilute hydrochloric acid. The solid which precipitated was washed with water and recrystallized wet from 95% ethanol. The phthalyl derivative melted at 192–194° and amounted to 1.45 g. (62%).

Anal. Calcd. for $C_{21}H_{16}N_2O_6$: C, 64.28; H, 4.11; N, 7.15. Found: C, 64.18; H, 4.24; N, 7.39.

DL-1-Diazo-3,6-diphthalimidohexanone-2 (XIIa).—Two grams (0.0052 mole) of XIa was mixed with 3 ml. of oxalyl chloride. After the initial reaction had subsided, the mixture was refluxed for several minutes (calcium chloride drying tube attached). Seven and one-half ml. of dry (distilled from lithium aluminum hydride) dimethyl cellosolve was then added and refluxing was continued for two hours.

(16) The authors are indebted to Mr. E. Eisenbraun for the Kuhn–Roth determinations.

(17) Melting point was taken on a micro hot-stage and is corrected.

(18) H. T. Clarke and T. F. Murray, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1932, p. 508.

(19) The authors wish to thank Mr. G. Brewer for the execution of this work.

After removal of solvent *in vacuo*, dry benzene was added twice and removed *in vacuo* each time. The residue was dissolved in 30 ml. of dimethyl cellosolve by swirling at about 50°. The solution was cooled to about 30° (not lower, or the acid chloride precipitates) and rapidly added dropwise with swirling, to a 250-ml. ethereal solution of diazomethane at 0–5° (the latter reagent was prepared from 3.7 g. of N-methyl-N-nitroso-N′-nitroguanidine). After standing for one hour at 0–5° and then two hours at room temperature, the solution had deposited a crop of crystals. The supernatant ether was decanted and evaporated to dryness. The residue, after combination with the original material, was triturated with 50 ml. of methanol; the diazoketone (1.62 g.) was collected by filtration. Concentration of the filtrate to 10 ml. afforded an additional 160 mg. The final yield of diazoketone (m.p. 138.5–139.0°, with dec.) was 84%. The diazoketone was not analyzed but was used directly for the preparation of XIIb.

Methyl DL-β,ε-Diphthalimidocaproate (XIIIa).—The diazoketone XIIa (1.62 g.) was suspended in 50 ml. of pure methanol and the mixture placed in a round-bottomed flask with a sidearm that could be attached to an azotometer. The rearrangement, catalyzed by 0.2 g. of dry silver benzoate dissolved in 3 ml. of triethylamine, was carried out according to Newman and Beal.[11] Ninety-two ml. of nitrogen at 750 mm. and 25° was evolved (calcd., 99 ml.). The mixture was heated to boiling with a little charcoal and then was filtered. On cooling to room temperature, the clear solution slowly deposited well-formed nodules, which were filtered off and dried. The ester melted at 146–147° and weighed 0.85 g. (54%). One additional recrystallization raised the melting point to 147–148°.

Anal. Calcd. for $C_{23}H_{20}N_2O_6$: C, 65.70; H, 4.80; N, 6.67. Found: C, 65.58; H, 4.78; N, 6.57.

DL-β,ε-Diaminocaproic Acid (Ia).—The diphthalimido ester XIIIa (105 mg., 0.25 mmole) was refluxed for one hour with 0.50 ml. of 1 *M* alcoholic hydrazine hydrate and 1 ml. of 95% ethanol. The resulting suspension was cooled and then was evaporated to dryness on the steam-bath. The mixture resulting from the addition of 5 ml. of 5% hydrochloric acid to the solid residue was filtered; heating on the steam-bath for one hour ensured hydrolysis of the ester. After filtration, the material was again taken to dryness on the steam-bath. To a solution of the residue in 1 ml. of water was added 140 mg. of *p*-hydroxyazobenzene-*p*′-sulfonic acid; the mixture was heated until solution was complete. Cooling resulted in the formation of delicate, orange crystals of the DHABS (dec. 239–240°). Two recrystallizations from water left 80 mg. (46%) of the salt, which decomposed sharply at 243–244°. The R_f value was 0.65.

Anal. Calcd. for $C_6H_{14}N_2O_2(C_{12}H_{10}N_2O_4S)_2$: C, 51.28; H, 4.87. Found: C, 50.95; H, 4.70.

L-Di-(N-phthalyl)-ornithine (XIb).—Ornithine dihydrochloride (2.05 g., 0.01 mole), anhydrous sodium acetate (1.64 g., 0.02 mole) and phthalic anhydride (2.96 g., 0.02 mole) were intimately mixed in a 50-ml. round-bottomed flask. The reagents were carefully dried and finely powdered before using. The flask was stoppered loosely and heated at 135–140° for one hour; the stopper was removed occasionally to release the acetic acid and water formed. Finally, after heating for an additional 0.5 hour at 155°, the residue was cooled and then was dissolved in 50 ml. of hot absolute ethanol. The oil thrown out on pouring the alcoholic solution into water was extracted with one 40-ml. portion, then two 20-ml. portions of benzene. Evaporation to about 30 ml. and subsequent cooling resulted in the deposition of 5.22 g. (67%) of colorless crystals (m.p. 184.5–187°). Recrystallization from hot, 95% ethanol raised the melting point to 187–188.5°, $[\alpha]^{25}D$ −31.5 ± 0.5° (alc.).

Anal. Calcd. for $C_{21}H_{16}N_2O_6$: C, 64.28; H, 4.10. Found: C, 63.95; H, 4.09.

Methyl L-β,ε-Diphthalimidocaproate (XIIIb).—Starting with 1.0 g. of XIb, the directions above for the corresponding members in the DL-series were followed. After the reaction of the acid chloride with diazomethane was finished, the ethereal solution was evaporated to near dryness. The crystalline residue was triturated with 100 ml. of ether and then filtered. The diazoketone weighed 0.80 g. (76%) and melted at 135.5–136.5° (dec.).

Using 50 ml. of methanol, the 0.80 g. of the diazoketone was transformed into L-β,ε-diphthalimidocaproate as described above. Crystallization was accomplished from the methanolic reaction medium, the product (m.p. 155.5–157°) weighing 0.36 g. Thirty additional milligrams, of comparable purity, was obtained from the mother liquor, giving a total yield of 53%. For analysis the needles were recrystallized again from methanol (m.p. 156–157°, $[\alpha]^{25}D$ −13.5 ± 0.5°, chf.).

Anal. Calcd. for $C_{23}H_{20}N_2O_6$: C, 65.70; H, 4.80. Found: C, 65.33; H, 4.64.

Methyl Ester of Di-(N-phthalyl)-isolysine.—The aqueous hydrolysate[1] derived from 150 mg. of streptolin AB was treated with Norite A to remove suspended humin, and the resulting solution was evaporated under a nitrogen atmosphere. The residue was thoroughly dried in a vacuum desiccator. Six hundred and seventy milligrams of phthalic anhydride was mixed with the dry solid and the material was heated at 175° for 0.5 hour. Excess phthalic anhydride was then removed *in vacuo* at the same temperature. After cooling, a mixture of dilute hydrochloric acid and ether was added to the residue. A black, insoluble tar was removed by filtration. The aqueous solution was extracted thoroughly with ether and the extracts were combined with the initial portion. Evaporation of the ether gave a gum, which was thoroughly extracted with hot water to remove phthalic acid. The remaining material was dissolved by triturating in the minimum amount (about 30 ml.) of ether. To this solution was added, until the yellow color persisted, an ethereal solution of diazomethane. After spontaneous evaporation of the ether solution to about one-half its original volume, a pattern of intricate crystals formed which tenaciously adhered to the flask walls; at the same time, well-defined needles precipitated on the bottom. The latter (*ca.* 5 mg.) were filtered off after their formation was apparently complete. The melting point, 155.5–156.0°, was not raised by recrystallization from methanol.

Anal. Calcd. for $C_{23}H_{20}N_2O_6$: C, 65.70; H, 4.80. Found: C, 65.82; H, 5.02.

The melting point of this derivative was not depressed by admixture with methyl L-β,ε-diphthalimidocaproate. The infrared spectra of the two substances were identical and exhibited characteristic peaks at 3.30, 3.38, 5.66, 5.80–5.86, 6.20, 6.80 and 6.93 μ.

L-β,ε-Diaminocaproic Acid (Ib).—Repetition of the directions described above for the DL-acid, using 0.105 g. (0.25 mmole) of XIIIb, afforded 130 mg. of crude DHABS. Two recrystallizations from hot water gave 95 mg. (54%) of the DHABS of L-β,ε-diaminocaproic acid (dec. 242.2–243.0°, $[\alpha]^{25}D$ +6 ± 1°, alc.). The infrared spectrum was, within experimental error, identical with that of authentic isolysine DHABS (Fig. 1).

Anal. Calcd. for $C_6H_{14}N_2O_2(C_{12}H_{10}N_2O_4S)_2$: C, 51.28; H, 4.87. Found: C, 51.31; H, 5.09.

Cleavage of the phthalyl groups by hydrazine was seemingly accompanied by hydrazide formation. In attempts to obtain a picrate from the crude hydrolysis product, only hydrazine picrate could be obtained crystalline. The base could only have arisen by hydrolysis of a hydrazide, because excess hydrazine is removed in the evaporation steps and because phthalhydrazide, the cleavage product from XIII, is inert under the conditions used for the hydrolysis. This complication undoubtedly explains the low yield of the hydrazinolysis step, which contrasts with the nearly quantitative conversion[12] in other, comparable cases.

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