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The Formation of Polypeptides by Rearrangement of α-Carboxy Hydroxamic Acids

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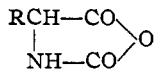
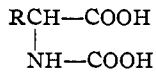
Two α-carboxy benzoylhydroxamic acids were synthesized in order to determine the effect of an α-carboxy group on the course of the Lossen rearrangement. The products of the rearrangement included polypeptides, whether carried out in water, benzene or ethyl alcohol. The formation of these products is attributed to the intermediary transformation of the hydroxamic acids to azasuccinic anhydrides followed by the Leuchs polymerization. The polypeptides were hydrolyzed to the corresponding amino acids.

In the present study of the reactions of α-carboxy hydroxamic acids, compounds of a polypeptide nature were obtained which seemed comparable to those[1-5] obtained by decomposition of anhydrides of structure (I). The corresponding acids (II)



I



II

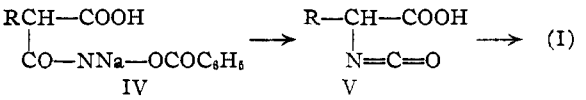
have been named with an N-carboxy prefix, *e.g.*, N-carboxyglycine for II (R = H), but such a name fails to conform with the principle of treating like groups (carboxyl) alike. To meet this objection, the name azasuccinic acid (or azabutanedioic acid) is suggested, following recent I. U. C. rulings sanctioning the extension of "a" nomenclature (*e.g.*, oxa, aza, thia) to open chain compounds of sufficient complexity. The cyclic compound would then be named azasuccinic anhydride.

These azasuccinic anhydrides have been prepared by heating 2-(N-carbethoxyamino)-alkanoyl chlorides,[1] by the rearrangement of 2-carboxyalkanoyl azides,[2] or by the action of phosgene on α-amino acids.[3]

The polypeptides, which have been obtained by heating the azasuccinic anhydrides in water,[1] ethyl alcohol[1,2] or ordinary reagent grade benzene,[4] can be hydrolyzed to the corresponding amino acids and appear to be linear polymers of peptide units. The fine structure of the synthetic polypeptides has been the subject of several investigations.[6]

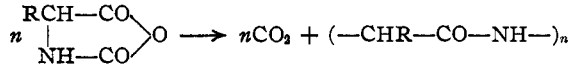
Carboxyacetohydroxamic acid, $HOOCCH_2$-CONHOH, and α-carboxycaprohydroxamic acid, HOOC-CH(C_4H_9)-CONHOH (II), were the parent compounds selected for study. In the Lossen rearrangement of hydroxamic acids a salt of an acylated hydroxamic acid is heated in water to yield an amine or urea, by way of an isocyanate: R—CO—NNa—OCOR′ → R′COONa + RN=C=O. To test such a rearrangement, III was benzoylated. Its salt, sodium α-carboxycapro-(benzoylhydroxamate) (IV), was heated both in water solution and in benzene suspension. The products formed were sodium benzoate, carbon dioxide, and an insoluble powder which could be hydrolyzed to D,L-norleucine.

The sequence of steps below provides an explanation for this behavior. The isocyanate group of V reacts with the carboxyl group to yield the azasuccinic anhydride (I).



IV V

At elevated temperatures, at least in the presence of even traces of water, the anhydride would be expected to be unstable, decomposing to a polypeptide with elimination of carbon dioxide



The polypeptide is nearly insoluble in water, alco-

(1) H. Leuchs, *Ber.*, **39**, 857 (1906); H. Leuchs and W. Geiger, *ibid.*, **41**, 1721 (1908).

(2) T. Curtius and W. Sieber, *ibid.*, **54**, 1430 (1921); **55**, 1543 (1922).

(3) F. Fuchs, *ibid.*, **55**, 2943 (1922).

(4) R. B. Woodward and C. H. Schramm, THIS JOURNAL, **69**, 1551 (1947).

(5) A. C. Farthing and R. J. W. Reynolds, *Nature*, **165**, 647 (1950), and references therein cited.

(6) References may be obtained in one of the most recent of such reports: A. Elliot and E. J. Ambrose, *ibid.*, **165**, 921 (1950).

hol, and dilute acidic and alkaline solutions. It did not dissolve completely in refluxing phenol, formamide, aniline, nitrobenzene or molten camphor. The material was also unaffected by refluxing in concentrated hydrochloric acid, although at higher temperatures in a sealed tube, hydrolysis was effected with this reagent. The polypeptide gives a positive biuret reaction and becomes colored blue when heated with the ninhydrin test solution, although the solution remains colorless in the latter test.

As noted earlier, Curtius and Sieber[2] obtained several azasuccinic anhydrides by allowing 2-carboxyalkanoyl azides to rearrange in ether. To test whether they could be prepared similarly from the hydroxamic acid derivatives, a suspension of IV in ether was refluxed for several hours. A small amount of polypeptide was formed, but most of the unchanged IV was recovered. No 3-butyl-2-azasuccinic anhydride was found.

When a suspension of the previously dried (IV) in ethyl alcohol was heated, carbon dioxide was evolved. The yield of the polypeptide was lower, however, than obtained from the rearrangement in water or benzene. In spite of this, a good yield of benzoic acid was isolated. The mixture of other products, which are soluble in alcohol, was not resolved. These observations compare with those of Curtius and Sieber,[2] who reported, for example, that the major products from the rearrangement of α-carboxybutyryl azide in a mixture of ethyl alcohol and ether were ethyl (1-carboxypropyl)-carbamate and 3,6-diethyl-2,5-diketopiperazine.

When an aqueous solution of sodium carboxyacetobenzoylhydroxamate (IV, R = H) was heated, carbon dioxide was evolved, but the solution remained clear. On cooling, the glycine polypeptide separated in a semi-colloidal form and could not be readily separated by filtration. The product could be coagulated to some extent by heating. The amount of separable polymer was increased by adding ethyl alcohol and this was divided into water-soluble and water-insoluble fractions. The rather large amount of water-soluble component would indicate a comparatively low molecular weight for the product prepared in this way.[7] The polymer from the rearrangement of the salt (IV) in benzene was somewhat less soluble in water. In one experiment, the entire amount of polymer, after separation of the benzoic acid, was subjected to hydrolysis. Glycine was isolated as the hydrochloride of its ethyl ester in 41% yield based on the amount of IV employed.

The α-carboxy hydroxamic acids were prepared by treatment of the appropriate malonic half esters with hydroxylamine in the presence of sodium ethoxide in ethyl alcohol. The hygroscopic disodium salts separated but no suitable methods for their purification were found. The free hydroxamic acids were readily obtained from the salts. The free acids yielded deep-red complexes with aqueous ferric chloride. Alcohol insoluble ammonium acid salts were also prepared.

Preliminary attempts to obtain benzoyl derivatives of the α-carboxy hydroxamic acids by the action of benzoyl chloride on a suspension of their disodium salts in benzene resulted in the formation of dibenzohydroxamic acid among other products. The crude salts could be benzoylated satisfactorily, however, by the Schotten–Baumann technique. The quality and yields of the benzoylated products were improved somewhat by the addition of an emulsifying agent to the reaction mixture. The stronger acidity of diacylhydroxylamines than monoacylhydroxylamines is exemplified by the fact that carboxyacetohydroxamic acid is monobasic in a titration to the phenolphthalein end-point, whereas carboxyaceto-(benzoylhydroxamic) acid is dibasic.

(7) For instance, the glycine pentapeptide requires 700 parts of water to effect solution at 15° and 60 parts at 100°: E. Abderhalden and A. Weil, *Z. physiol. Chem.*, **109**, 296 (1920).

Experimental

Ethyl Hydrogen Butylmalonate.[8]—Ethyl butylmalonate was partially hydrolyzed to ethyl hydrogen butylmalonate according to the procedure of Easson and Pyman,[9] who did not state the yield of the product obtained, but reported the neutral equivalent and bismuth salts. From 71.3 g. of ethyl butylmalonate, 49.0 g. of ethyl hydrogen butylmalonate (dried over Drierite, $n^{20}D$ 1.430) was obtained. This represents 78% of the theoretical yield. In addition, 6.0 g. of unreacted ethyl butylmalonate was recovered.

Derivative.[8]—By treatment of ethyl hydrogen butylmalonate first with thionyl chloride and then with *p*-bromoaniline, α-carbethoxycapro-(*p*-bromoanilide) was prepared. This compound melted at 107–108° after recrystallization from dilute ethyl alcohol.

Anal. Calcd. for $C_{15}H_{20}BrNO_3$: C, 52.64; H, 5.89. Found: C, 52.71; H, 5.97.

Salts of α-Carboxy Hydroxamic Acids

Disodium α-Carboxycaprohydroxamate.[8]—A solution of sodium ethoxide was prepared by dissolving 7.5 g. of sodium in 150 ml. of absolute ethyl alcohol. To a solution of 8.0 g. of hydroxylammonium chloride in 100 ml. of warm, absolute ethyl alcohol was added 58 ml. of the sodium ethoxide solution. The precipitated sodium chloride was separated by filtration. The hydroxylamine solution was then added slowly to 18.8 g. of ethyl hydrogen butylmalonate. The remainder of the sodium ethoxide solution was then added slowly with stirring. Disodium α-carboxycaprohydroxamate precipitated during the addition of the last sodium ethoxide. After the mixture was stirred for an additional 30 minutes, the salt was collected on a filter. It was hygroscopic and gave a red-violet color with aqueous ferric chloride. The product was dried in a vacuum desiccator over sulfuric acid; yield 18.0 g.; 83%. This contained 19.3% sodium. Analytical samples were prepared by twice precipitating the solid from water by addition of acetone and drying *in vacuo* over sulfuric acid before analysis.

Anal. Calcd. for $C_7H_{11}Na_2NO_4$: Na, 21.00; N, 6.40. Found: Na, 20.85, 20.74; N, 6.54.

Potassium Sodium Carboxyacetohydroxamate.—Similarly, potassium ethyl malonate[10] (17.0 g.), suspended in 100 ml. of absolute ethyl alcohol, was treated with a solution of hydroxylamine (from 6.95 g. of the hydrochloride) in 80 ml. of absolute ethyl alcohol. Then a solution of sodium ethoxide (from 2.30 g. of sodium) in 50 ml. of absolute ethyl alcohol was added dropwise with stirring. The mixture was stirred until the relatively large crystals of potassium ethyl malonate had disappeared (one hour). The potassium sodium carboxyacetohydroxamate, which precipitated as a fine powder, was separated by filtration and dried over sulfuric acid; yield 13.2 g. The K:Na ratio in this salt was approximately 1:2 as judged by the fact that 0.3333 g. of the salt, on heating with sulfuric acid, yielded 0.2724 g. of mixed sodium and potassium sulfate which, in turn, was converted into 0.4121 g. of barium sulfate.

(8) This experiment was first carried out in this Laboratory by John Longfellow.

(9) A. P. T. Easson and F. L. Pyman, *J. Soc. Chem. Ind.*, **52**, 97 (1933).

(10) M. Freund, *Ber.*, **17**, 780 (1884).

α-Carboxy Hydroxamic Acids

α-Carboxycaprohydroxamic Acid.—To 4.38 g. of crude disodium α-carboxycaprohydroxamate in 15 ml. of ice-cold water was added concentrated hydrochloric acid until the solution was just acid to congo red. α-Carboxycaprohydroxamic acid (1.75 g., m.p. 133–134.5°) precipitated as lustrous plates when the solution was allowed to stand in an ice-bath and these crystals were separated by filtration. A second crop (0.77 g.) was obtained after concentration of the filtrate in an air stream. The melting point of the acid was raised to 134–135° by recrystallization from water. A deep-red color was produced when the acid was treated with aqueous ferric chloride.

Anal. Calcd. for $C_7H_{13}NO_4$: N, 7.99; neut. equiv. (monobasic), 175.2. Found: N, 8.30, 8.22; neut. equiv. (to phenolphthalein end-point), 173.

Ammonium α-Carboxycaprohydroxamate precipitated when a solution of 0.25 g. of α-carboxycaprohydroxamic acid in 10 ml. of absolute ethyl alcohol was treated with 0.3 ml. 27% ammonia. The mixture was cooled in an ice-bath and the ammonium acid salt was then collected by filtration. The product (0.26 g., 95%) melted at 155–158° with gas evolution. The salt was recrystallized from 90% ethyl alcohol and thus obtained as colorless prisms. The melting range of the salt remained at 155–158°.

Anal. Calcd. for $C_7H_{16}N_2O_4$: N, 14.58. Found: N, 14.52.

Carboxyacetohydroxamic Acid.—To a suspension of 1.79 g. of potassium sodium carboxyacetohydroxamate in 10 ml. of absolute alcohol was added slowly with stirring a solution of 0.73 g. of dry hydrogen chloride (weight determined by titration) in 25 ml. of absolute ethyl alcohol. Inorganic salts were separated and alcohol was removed under reduced pressure. Benzene was added to the residue. Fine needles of carboxyacetohydroxamic acid (0.82 g., 69%, m.p. 139–140°) separated. The melting point of the acid was not raised by recrystallization from a mixture of ethyl acetate and benzene.

Anal. Calcd. for $C_3H_5NO_4$: neut. equiv. (monobasic), 119.1. Found: neut. equiv., 116 (to phenolphthalein).

Ammonium Carboxyacetohydroxamate was prepared in 62% yield by a method similar to that described above for ammonium α-carboxycaprohydroxamate. This product melted at 178–181° (dec.). This compound was prepared previously by another method and reported to melt at 181°.[11]

α-Carboxy Benzoylhydroxamic Acids

α-Carboxycapro-(benzoylhydroxamic) Acid.—A solution of 4.38 g. of disodium α-carboxycaprohydroxamate in 20 ml. of ice-cold water in a 3-neck flask was surrounded with an ice-bath. The solution was stirred rapidly while 2.62 g. of benzoyl chloride was added in small portions. The solution was kept just alkaline to litmus by the addition of approximately 2 *N* sodium hydroxide as necessary. A small amount of gummy material separated. In previous experiments, it had been found that this material did not dissolve when a large excess of alkali was added, but that some of the desired benzoyl derivative could be isolated from this gum. The mixture was stirred for 30 minutes after the addition of the benzoyl chloride. It was then acidified with hydrochloric acid and shaken vigorously with 20 ml. of ethyl acetate until two clear layers were obtained. The ethyl acetate solution was separated and the aqueous solution was washed with another portion of ethyl acetate. The combined organic layer, washed with a small amount of water, was then evaporated in an air stream and the residue was crystallized from benzene. The yield of α-carboxycapro-(benzoylhydroxamic) acid thus obtained as fine needles (m.p. 117–120°) was 2.95 g. (53%). The melting point was raised to 123–124° by recrystallization from benzene.

Anal. Calcd. for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.14; N, 5.02; neut. equiv. (dibasic), 139.6. Found: C, 60.25; H, 6.04; N, 5.11, 5.19; neut. equiv., 138 (to phenolphthalein).

Carboxyaceto-(benzoylhydroxamic) Acid.—To 9.80 g. of potassium sodium carboxyacetohydroxamate in 60 ml. of ice-cold water was added approximately 50 mg. of saponin. The mixture was treated with 9.0 g. of benzoyl chloride as described in the preceding experiment. The small amount of insoluble material, from which some dibenzohydroxamic acid was isolated, was finally discarded. The filtrate was acidified. Carboxyaceto-(benzoylhydroxamic) acid separated as colorless plates and was recrystallized from a mixture of ethyl acetate and benzene, yielding 4.78 g. (m.p. 122–125°). The aqueous filtrate was extracted twice with ethyl acetate. By working up the extracts and mother liquor, an additional 2.43 g. of product was obtained. The total yield was thus 59% of the theoretical amount. The melting point was raised to 128–129° by recrystallization from a 50–50 mixture of ethyl acetate and benzene.

Anal. Calcd. for $C_{10}H_9NO_5$: neut. equiv. (dibasic), 111.6. Found: neut. equiv., 113 (to phenolphthalein).

Sodium Salts

Sodium α-Carboxycapro-(benzoylhydroxamate).—A solution of sodium ethoxide was prepared and a portion equivalent to 1.15 g. of sodium was used in the following experiment. It was added slowly with vigorous stirring to a solution of 13.96 g. of α-carboxycapro-(benzoylhydroxamic) acid in 350 ml. of absolute ethyl alcohol. The sodium salt separated as a gelatinous precipitate, occluding most of the solvent. The solvent was readily separated, however, by filtration. The salt was dried over sulfuric acid; yield 10.95 g. An additional 1.15 g. was obtained by the addition of anhydrous ether to the filtrate; total yield 12.27 g., 81%.

Anal. Calcd. for $C_{14}H_{16}NaNO_5$: Na, 7.63. Found: Na, 7.51, 7.63.

This salt, which is completely soluble in water when freshly prepared, slowly becomes contaminated with a water-insoluble material similar to the polypeptides described later when exposed to moist air.

Sodium Carboxyaceto-(benzoylhydroxamate) was prepared from carboxyaceto-(benzoylhydroxamic) acid in 75% yield by the procedure just described. This salt, which is somewhat soluble in ethyl alcohol, is readily precipitated by the addition of anhydrous ether.

Anal. Calcd. for $C_{10}H_8NaNO_5$: Na, 9.38. Found: Na, 9.63.

Rearrangement of Sodium α-Carboxycapro-(benzoylhydroxamate) in Water.—A solution of 3.50 g. of sodium α-carboxycapro-(benzoylhydroxamate) in 50 ml. of water was heated. When 80° was reached, evolution of carbon dioxide became apparent, as tested with barium hydroxide solution. At the same time, an amorphous powder began to separate. The mixture was heated on a steam-bath for one hour. After being cooled, the insoluble material was separated by filtration and washed with absolute ethyl alcohol. The yield of the dried product was 1.06 g., which corresponds to 81% of the theoretical amount of the polypeptide, $(-CH(C_4H_9)CONH-)_x$. When heated in a sealed capillary tube, a sample of this material began to darken at approximately 250°. At 335–337°, the material melted partially with extensive decomposition. The substance was colored red by the biuret test solution. When heated with an aqueous solution of ninhydrin, it acquired a blue color, although the solution remained colorless.

The filtrate which was separated from the polypeptide was acidified. Benzoic acid (1.16 g., m.p. 119–120°) separated.

Five milliliters of concentrated hydrochloric acid and 0.85 g. of the polypeptide were heated in a sealed tube at 150° for 20 hours. After being cooled, the contents of the tube were filtered of a small amount of carbonaceous material and the filtrate was then evaporated to dryness. The crystalline residue was dissolved in 20 ml. of water. When the solution was neutralized to congo red, slightly brown crystals of DL-norleucine separated. The amino acid dissolved when the mixture was heated and the resulting solution was clarified with activated carbon. The carbon was separated by filtration while hot and, on being cooled, 0.64 g. of DL-norleucine (m.p. 290–292° dec.) precipitated from the filtrate. A second crop (0.19 g.) was obtained after the mother liquor was concentrated; total yield, 0.83 g., 85%. Samples of the amino acid were converted to the *p*-toluenesulfonyl derivative (m.p. 123–124°)[12] and the formyl derivative (m.p. 113–114°).[12]

(11) A. Hantzsch, *Ber.*, **27**, 804 (1894).

(12) E. W. McChesney and W. K. Swann, Jr., THIS JOURNAL, **59**, 1116 (1937) report the melting point of the *p*-toluenesulfonyl derivative of DL-norleucine as 124°, while D. Marko, *Ann.*, **362**, 333 (1908), reports the melting point of the corresponding formyl derivative as 113–115°.

In Benzene.—Freshly prepared, dried sodium α-carboxycapro-(benzoylhydroxamate) was pulverized and 2.37 g. of it was suspended in 30 ml. of benzene. The suspension was refluxed for two hours, during which time carbon dioxide was evolved. The solvent was filtered while hot. The residue (1.93 g.) was triturated with water and filtered. The water-insoluble part was washed with ethyl alcohol and dried in a vacuum desiccator over sulfuric acid. The yield of polypeptide, which darkened at 250° and melted at 335–337° with decomposition, was 0.88 g. (99%).

Anal. Calcd. for $(C_6H_{11}NO)_x$: C, 63.68; H, 9.80; N, 12.38. Found: C, 63.06; H, 9.93; N, 11.99 (average of four determinations).

When the aqueous filtrate was acidified, 0.77 g. of benzoic acid (m.p. 115–117°) separated. A sample of the polypeptide was hydrolyzed by the same method described above. From 0.72 g. of the polypeptide a yield of 0.66 g. (80%) of DL-norleucine was obtained.

In Ethyl Alcohol.—A 0.87-g. quantity of sodium α-carboxycapro-(benzoylhydroxamate) was powdered and suspended in 20 ml. of absolute ethyl alcohol. The mixture was refluxed for two hours, during which time tests for carbon dioxide in the exit gases were positive. The insoluble material which had formed was separated by filtration while hot and washed with water. The residue (m.p. 335° dec.) was dried and found to weigh 0.15 g. (46%). The alcoholic filtrate was evaporated to dryness and the residue was washed with water. There remained 0.17 g. of a gummy material which was not identified. The aqueous filtrate were combined and acidified. Benzoic acid (m.p. 115–117°) was obtained.

In another experiment, the freshly precipitated sodium α-carboxycapro-(benzoylhydroxamate) (from 1.90 g. of α-carboxycapro-(benzoylhydroxamic) acid in 50 ml. of absolute ethyl alcohol and an equimolar quantity of sodium ethoxide) was not separated but the mixture was heated immediately on a steam-bath. The precipitated salt was first dissolved, but on further heating, some insoluble polypeptide and sodium benzoate separated. The mixture was processed as before to obtain 0.12 g. of the polypeptide (16%), 0.38 g. of alcohol-soluble, water-insoluble material and 0.59 g. of crude benzoic acid (m.p. 114–116°).

In **Ether.**—To 20 ml. of ethyl ether, dried over sodium, was added 0.79 g. of freshly prepared powdered sodium α-carboxycapro-(benzoylhydroxamate). The mixture was refluxed for ten hours and the ether was then separated by filtration. Only a trace of ether-soluble material was found after evaporation of the ether. The residue was extracted with water. A small amount of the insoluble polypeptide was separated and the resulting filtrate was acidified with hydrochloric acid. A 0.48-g. quantity of α-carboxycapro-(benzoylhydroxamic) acid (m.p. 121–122°, 66%) was recovered. A mixed melting point sample with benzoic acid melted at 95–105°.

Rearrangement of Sodium Carboxyaceto-(benzoylhydroxamate) in Water.—(A) A solution of 1.60 g. of sodium carboxyaceto-(benzoylhydroxamate) in 10 ml. of water was heated under reflux for 15 minutes. The solution remained clear until it was cooled. This material was not readily filterable. The mixture was heated to 100° and the insoluble material was coagulated to some extent. After cooling, a small amount of material (20 mg.) was separable by filtration. This product gave positive biuret and ninhydrin tests. Another portion of the polypeptide separated when ethyl alcohol was added. This mixture was concentrated to approximately 5 ml. and then acidified. A 0.42-g. quantity of benzoic acid (m.p. 119–120°) precipitated. The benzoic acid fraction was completely soluble in ethyl alcohol, showing that no glycine polypeptide separated with it. The filtrate was then evaporated to dryness and the residue was combined with the small amounts of polymer that had separated and 1 ml. of concentrated hydrochloric acid. This mixture was heated in a sealed tube at 140° for 20 hours. The contents of the tube were evaporated to dryness. The residue was extracted with 30 ml. of refluxing, absolute ethyl alcohol in which anhydrous hydrogen chloride had been dissolved. After separation of the sodium chloride and other insoluble material, the filtrate was refluxed for one hour, then treated with activated carbon, filtered while hot and concentrated to approximately 10 ml. On cooling, 0.287 g. of glycine ethyl ester hydrochloride (m.p. 141–142°) separated. A second crop (0.089 g., m.p. 135–138°) was obtained by concentration of the filtrate. A mixed melting point sample of the first crop and authentic ethyl aminoacetate hydrochloride also melted at 141–142°; total yield 0.376 g., 41%.

(B) In another experiment, a solution of 1.80 g. of sodium carboxyaceto-(benzoylhydroxamate) in 10 ml. of water was refluxed for ten minutes and then cooled. To the cloudy mixture was added 30 ml. of 95% ethyl alcohol and the mixture was left overnight. The insoluble product was separated by filtration through Whatman No. 50 filter paper, dried on the filter paper and found to weigh 0.21 g. The material, an amorphous powder, was then washed with 10 ml. of water in the Büchner funnel. In this process 0.17 g. (42% of the theoretical yield) dissolved leaving only 0.04 g. (10%) of water-insoluble glycine polypeptide. The filtrate was still cloudy. The soluble polypeptide was reprecipitated with ethyl alcohol, separated by filtration and dried; yield of water-soluble polymer, which began to darken at 220°, 0.09 g.

In **Benzene.**—Sodium carboxyaceto-(benzoylhydroxamate) was pulverized and 3.68 g. of it was suspended in 50 ml. of benzene. The suspension was heated under reflux at 100° for one hour. The insoluble material was then separated by filtration. The filtrate was evaporated to dryness but only a trace of a glassy material remained. The insoluble powder was mixed with 50 ml. of boiling water, stirred well and filtered hot through Whatman No. 50 filter paper. The insoluble polypeptide was washed with water and placed in a vacuum desiccator over sulfuric acid while still moist. It was thus obtained as a glassy mass; yield 0.53 g., 61%. This material darkened when heated above 250° but did not melt when heated to 350°.

The filtrate was then concentrated to 10 ml. and 40 ml. of 95% ethyl alcohol was then added. An additional quantity of glycine polypeptide was obtained therefrom which, after being dried in air, was found to weigh 0.19 g. (22%). This product was a powder which darkened when heated above 235°.

A sample of the water-insoluble glycine polypeptide was prepared for analysis by washing with ether, suspending in benzene, distillation of part of the benzene and drying in a vacuum desiccator.

Anal. Calcd. for $(C_2H_3NO)_x$: C, 42.10; H, 5.29; N, 24.56. Found: C, 40.11; H, 5.61; N, 21.30, 20.69, 21.04.

The sample was further dried by heating at 170° under a pressure of 2 mm. of mercury for 30 minutes and then reanalyzed.

Anal. Found: C, 41.10; H, 5.18.

Adsorption of Water Vapor by the Polypeptides.—A 0.1311-g. sample of the glycine polypeptide dried at 170° *in vacuo* was exposed to the atmosphere and the weight was recorded from time to time. The percentage increase in weight was observed at the following intervals: 16 hours, 6.8; 24, 6.9; 40, 7.6; 64, 8.2; 96, 9.5; 216, 10.8.

The weight of the DL-norleucine polypeptide similarly dried increased only 1.2% at the end of 70 hours.

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EVANSTON, Ill. RECEIVED DECEMBER 2, 1950