STUDIES OF MULTIVALENT AMINO ACIDS AND PEPTIDES

I. THE SYNTHESIS OF CERTAIN TETRAVALENT AMINO ACIDS AND THEIR DERIVATIVES

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In the study of the physicochemical properties of the proteins, it is often convenient to consider the behavior of the simpler ampholytes which reflect to some degree the properties of the more complex protein molecules. The investigation of simpler synthetic amphoteric models, usually amino acids and peptides, has illuminated such diverse properties of proteins as electrolytic dissociation (9, 13), apparent molal volume (7), solubility (6), and behavior toward enzymes (1).

From the researches of Herrick and Northrop on pepsin (14) and of Freudenberg and Eyer (12), Jensen (15), and du Vigneaud (21) on insulin, it seems likely that the apparently specific properties of many proteins may inhere in certain groups or linkages in the molecule. Many of these groups or linkages may be reproduced in synthetic models. Whether these groups may completely function dissociated from the colloid moiety itself cannot as yet be answered. It would seem of interest, however, to prepare a series of complex synthetic ampholytes and their derivatives and to examine several of their properties. These substances will resemble the protein in bearing several free amino, carboxyl, and other characteristic groups upon a relatively small molecular area.

The present communication is concerned with the synthesis of three new tetravalent amino acids: \(\alpha,\gamma,\delta\)-triamino-\(\Delta^{7,8}\)-pentenic acid, \(\alpha\)-aminotricarballylic acid, and \(\epsilon,\epsilon'\)-diamino-di(\(\alpha\)-thio-\(n\)-caproic acid). The latter substance was further characterized by the formation of its \(\epsilon,\epsilon'\)-diguanido and \(\epsilon,\epsilon'\)-diphenylureido.
derivatives. The glycylpeptide of α-aminotricarballylic acid was also prepared. Triamino acids possessing three amino groups and one carboxyl group, such as the dipeptides lysyllysine and α,β-diaminopropionic acid dipeptide, have been synthesized by Fischer and Suzuki (11). The peptide glutamylglutamic acid, containing one amino group and three carboxyl groups and hence analogous to the above α-aminotricarballylic acid or its peptide, has been prepared by Bergmann and Zervas (2). The triamino acid and the tricarboxylic acid, whose syntheses are described below, were chosen for a proposed later synthesis of a hexapole dipeptide.

The synthesis of the dipeptide tetrapole, lysylglutamic acid, by Bergmann, Zervas, and Greenstein (3) has yielded a compound fairly soluble in cold water and a few of its electrochemical properties have been described by the author (13). In the endeavor to prepare a tetrapole which in addition to the property of being highly soluble in water would also contain the characteristic disulfide linkage of cystine, the synthesis of ε,ε'-diamino-di(α-thion-caproic acid) was undertaken. The wide spacing of the charged groups in the latter compound results in its highly polar character and consequent aqueous solubility.

Certain physicochemical and physiological properties of these substances and their derivatives will be described in later communications.

\[
\begin{align*}
\text{a-Aminotricarballylic Acid} \\
\text{CHCOOC}_2\text{H}_5 & \quad \text{NH}_2\text{OCCH}_2 & \quad \text{NH}_2\text{CHCOOH} \\
\text{CCOOC}_2\text{H}_5 & \quad \text{→} \quad \text{NH}_2\text{OCCH} & \quad \text{→} \quad \text{CHCOOH} \\
\text{CH}_3\text{COOC}_2\text{H}_5 & \quad \text{OCCHNH} & \quad \text{CH}_3\text{COOH} \\
\text{CH}_3\text{CONH}_2 & \quad \text{HNCHCO} & \quad \text{CH}_3\text{CONH}_2 \\
\text{I} & \quad \text{II} & \quad \text{III}
\end{align*}
\]

When β-hydroxy acids are treated with a dehydrating agent, they pass over into α,β unsaturated acids. On treatment of the
latter with ammonia, the corresponding amino acid should result, as was first indicated by Engel (10). The yields, however, were quite poor and later Morsch (18) and Dunn and Fox (8) employed for the syntheses of β-aminobutyric acid and of aspartic acid respectively not the unsaturated acid, but its corresponding ester. The latter method was followed in the synthesis of α-aminotricarballylic acid, starting with aconitic acid triethyl ester (I) derived from citric acid.

**EXPERIMENTAL**

Anhydroaminotricarballylic Acid Tetraamide (II)—Samples of 16 gm. of triethyl aconitate (19) were placed in each of six 200 cc. pressure flasks and 150 cc. of absolute alcohol saturated with dry NH₃ gas at 0° were added to each flask. The flasks were then kept at 100° for 6 hours and allowed to cool slowly to room temperature. The anhydro-α-aminotricarballylic acid tetraamide crystallized out on the sides of the flasks in yellowish clumps. This was filtered off, washed with cold water several times, then with alcohol, and finally dried in vacuo. The yield of crude substance, melting at 217°, was 19 gm. The alcoholic mother liquor yielded on evaporation approximately 11 gm. of impure tetraamide. For analysis a small amount was dissolved in lukewarm water, shaken with norit, and filtered. To the filtrate was added absolute alcohol to turbidity. On cooling in the ice chest, long colorless needles separate. M. p., 232° with decomposition.

C₁₂H₁₈N₄O₆ (342.14)

Calculated. C 42.08, H 5.30, N 24.55, amide N as NH₃ 19.8

Found. " 42.22, " 5.40, " 24.48, " " 19.2

α-Aminotricarballylic Acid (III)—175 gm. of the combined crude anhydroaminotricarballylic acid tetraamide were dissolved in 600 cc. of 5 N NaOH and boiled under a reflux in an oil bath for 4 hours. At the end of this time the solution was chilled, diluted, and neutralized to methyl red with 10 N HCl. At this point a precipitate amounting to a few hundred mg. appeared. This was filtered off and not further investigated. To the filtrate, decolorized with norit, was added a hot solution of 250 gm. of copper acetate in 2500 cc. of water. A voluminous precipitate of the blue Cu salt immediately appeared, which was filtered off and
washed with water. Inasmuch as this salt was extremely insoluble in all solvents, it was thoroughly dried and ground up to a powder, and shaken on the machine with large volumes of water until the decanted wash water yielded a negative test for chloride ion. The yield of dried copper salt amounted to 90.0 gm. It was suspended in water and decomposed with H$_2$S and the clear colorless filtrate achieved by the use of norit was evaporated in vacuo. In concentrated solution the separation of the amino acid began. It was removed to a filter and washed several times with water. The yield of crude α-aminotricarballylic acid, m.p. 185°, was 30 gm. The acid was dissolved in hot water and hot alcohol added slowly to turbidity. On cooling there crystallize beautiful flat gleaming plates. The yield of the pure substance melting at 196°, with decomposition, was 24.5 gm. This is around 12 per cent of the theory, based on the aconitic acid triethyl ester.

The amino acid is slightly soluble in cold water, easily in warm water, acids, and alkalies, and insoluble in organic solvents. In water solution it turns Congo red paper blue.

C$_6$H$_{12}$NO$_6$ (191.07). Calculated. C 37.68, H 4.75, N 7.32, NH$_2$-N 7.32
Found. " 37.95, " 5.19, " 7.11, " 7.58

Carbobenzoxyglycyl-α-Aminotricarballylic Acid—A sample of 9.3 gm. of carbobenzoxyglycine (2) was suspended in 70 cc. of dry ether and treated with 10 gm. of fresh PCl$_3$. The mixture was shaken at 0° for about 15 minutes and then filtered. The filtrate was evaporated in vacuo to a syrup, the latter shaken out several times with dry cold petroleum ether to remove the oxychloride, and then taken up in 40 cc. of dry ether and immediately employed for the synthesis.

An amount of 7.3 gm. of α-aminotricarballylic acid was dissolved in 60 cc. of 2 N NaOH and chilled in an ice bath. With vigorous shaking, the above ether solution of carbobenzoxyglycyl chloride was added alternately with 40 cc. of 2 N NaOH to the alkaline solution of the amino acid. When the reaction after 20 minutes was at an end, 5 N HCl was added to Congo blue, the ether layer removed, and the aqueous layer shaken out several times with ethyl acetate. The ethyl acetate extracts were combined, washed well with water, dried, and evaporated in vacuo at a low temperature to dryness. The residue was a thick colorless syrup. After
thorough drying in a vacuum desiccator, it set to a white solid. The yield amounted to 7.0 gm.; m.p., 72° with decomposition.

\[ \text{C}_{12}\text{H}_{18}\text{O}_2\text{N}_2 \text{ (382.14). Calculated, N 7.33; found, N 7.20} \]

**Glycyl-α-Aminotricarballylic Acid**—A sample of 6.5 gm. of carboxyglycyl-α-aminotricarballylic acid in 20 cc. of methyl alcohol and 10 cc. of water plus a few drops of acetic acid was catalytically hydrogenated in the presence of palladium. After about 20 minutes the reaction was at an end and the catalyst plus the crystallized peptide was filtered off. The material was separated from the palladium by washing with warm water and the combined filtrates evaporated in vacuo to dryness. The residue was taken up in the minimum amount of warm water, filtered, and treated with an equal volume of absolute alcohol. An oil separated which after standing at 0° for several hours crystallized into beautiful sheaves of prisms. The yield, amounting to 4.1 gm., was practically quantitative; m.p., 195° with foaming. The peptide is insoluble in cold water and in organic solvents, but soluble in hot water. Its aqueous solution is acid to Congo red paper.

\[ \text{C}_8\text{H}_{12}\text{O}_2\text{S}_1 \text{ (248.1). Calculated, C 38.64, H 4.88, N 11.28; found, C 38.31, H 5.04, N 11.35} \]

**α, γ, δ-Triatino-Δγδ-Pentenic Acid**

\[
\begin{array}{c}
\text{CHNHCOCH}_3 \\
| \text{CHNH}_3 \\
\text{CNHCOCH}_3 \\
| \text{CH}_2 \\
\text{CHNHCOCH}_3 \\
| \text{CHNH}_2 \\
\text{COOCH}_3 \\
\text{COOH}
\end{array}
\]

It was found by Kossel and Edelbacher (17) that treatment of histidine ester with an excess of benzoyl chloride resulted in an opening of the imidazole ring with the formation of a tribenzoyl unsaturated aliphatic ester (I). These authors did not go beyond the formation of this derivative of histidine. It was found that
simple splitting off of the benzoyl groups from the \(\alpha,\gamma,\delta\)-tribenzoyltriamino-\(\Delta^\gamma,\Delta^L\) -pentenic acid methyl ester (I) by means of concentrated hydrochloric acid resulted in the formation of the corresponding free triamino acid (II) as the trihydrochloride salt.

To 9.4 gm. of \(\alpha,\gamma,\delta\)-tribenzoyltriamino-\(\Delta^\gamma,\Delta^L\) -pentenic acid methyl ester was added a mixture of 150 cc. of concentrated HCl and 20 cc. of water and the entire mass was boiled for 5 hours under a reflux condenser. The solution is chilled, filtered from the benzoic acid, and the filtrate shaken out twice with ether.

The aqueous acid solution yields a negative reaction with diazotized sulfanilic acid. It was taken down to dryness in vacuo and the dark colored residue taken up several times with water and evaporated each time to dryness. Finally the solution was taken to dryness in a vacuum desiccator over \(\mathrm{P}_2\mathrm{O}_5\) and solid \(\mathrm{NaOH}\). The residue was a black, gummy mass. On treatment with acetone it solidified and was ground up to a fine powder in a mortar with absolute alcohol. The black color is completely extracted by the alcohol, leaving the substance a dirty gray. This was filtered off and washed several times with alcohol. The yield of the crude material was 2.2 gm., or 60 per cent of the theory. The substance is dissolved in an excess of hot methyl alcohol and filtered. To the filtrate dry ether was added drop by drop until precipitation was complete. The triamino acid separated as the trihydrochloride salt in large rhomboid prisms. The melting point was 171–173°, the substance turning brown and foaming.

The substance decolorizes bromine water. It yielded a copious precipitate with phosphotungstic acid.

\[
\begin{align*}
\text{C}_9\text{H}_{16}\text{O}_2\text{N}_3\text{Cl}_3 + 14\text{H}_2\text{O} & \quad (281.6) \\
\text{Calculated.} & \quad \text{C} 21.30, \text{H} 6.06, \text{N} 14.92, \text{Cl} 37.7 \\
\text{Found.} & \quad " 21.16, " 6.48, " 15.16, " 37.2
\end{align*}
\]

The substance decolorizes bromine water. It yielded a copious precipitate with phosphotungstic acid.

\[
\begin{align*}
\text{NHCOC}_2\text{H}_4 & \quad \text{NHCOC}_2\text{H}_4 \\
\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHCOOH} & \quad \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHCOOH}
\end{align*}
\]

\[
\begin{align*}
\text{Br} & \quad \rightarrow \\
\text{I} & \quad \text{II}
\end{align*}
\]
The starting material for the synthesis of this substance was $\epsilon$-benzoylamino-\(\alpha\)-bromo-\(n\)-caproic acid (I) (5). Several methods are available for the replacement of the \(\alpha\)-halogen by sulfur and after several trials the technique of Biilmann (4) in which potassium ethyl xanthogenate is used was adopted.

\(\epsilon\)-Benzoylamino-\(\alpha\)-Xanthogenate-\(n\)-Caproic Acid (II)—A sample of 48 gm. (3 M) of potassium ethyl xanthogenate dissolved in 50 cc. of water was treated with 31.4 gm. (1 M) of \(\epsilon\)-benzoylamino-\(\alpha\)-bromo-\(n\)-caproic acid, and the mixture shaken for about an hour in an open flask until in solution. The flask was stoppered and allowed to stand for 24 hours at room temperature. The solution was then shaken out once with ether to remove some oil, and 5 N HCl added until the reaction of the mixture was acid to Congo red paper. A brown oil appeared which after standing for several days in the ice chest crystallized. The material was filtered off, ground up in a mortar with water, filtered, and washed thoroughly with water. The yield of crude dried xanthogenate acid was 32 gm. or 94 per cent of the theory. It was crystallized twice from hot ethyl acetate. The substance separates from the solvent in short stout prisms and is slightly yellow in color. M. p., 112-114° to a clear yellow liquid.

\[\text{C}_{16}\text{H}_{21}\text{NO}_{4}\text{S}_{2} (355.29)\]. Calculated. N 3.94, S 18.1

Found. "4.35," "18.7"
**e-Benzoylamino-α-Thiol-n-Caproic Acid (Ⅲ)—**A sample of 26.0 gm. of the xanthogenate acid was dissolved with warming in 150 cc. of absolute alcohol, cooled, and then treated with 100 cc. of 27 per cent NH₃ solution in water. Enough alcohol was then added to bring the bulk of the solution nearly to the capacity of the flask. The latter was allowed to stand at room temperature for 3 days. The solution was then taken down to an acid-reacting syrup on the water bath, made slightly alkaline by addition of ammonia water, and shaken out twice with ether. To the cooled aqueous solution, concentrated HCl was added until the reaction was acid to litmus and then several pieces of Zn granules were added. The hydrochloric acid was now added further until the final reaction was acid to Congo red paper. A heavy oil separates which on continuous rubbing solidifies. The solid mass was broken up to a fine powder and allowed to stand in contact with the Zn-HCl mixture overnight in order to reduce any disulfide present. The mass is then filtered off, washed thoroughly with water, and then extracted several times with boiling absolute alcohol. The combined alcoholic extracts are filtered hot from the Zn particles and treated with hot water. The e-benzoylamino-α-thiol-n-caproic acid crystallizes out rapidly on cooling in large white prisms. It is filtered off, washed with water, then with dilute alcohol, and dried. The yield was 14 gm. or 80 per cent of the theory. M. p., 158° sharply to a clear liquid. It yielded a positive nitroprusside reaction.

\[ \text{C}_{11}H_{17}NO_5S \ (251.2) \]  
Calculated. \( N \ 5.57 \), \( S \ 12.6 \)  
Found. \( \bar{N} \ 5.36 \), \( \bar{S} \ 12.2 \)

**ε-Amino-α-Thiol-n-Caproic Acid-HCl (Ⅳ)—**An amount of 95 gm. of e-benzoylamino-α-thiol-n-caproic acid was dissolved in a liter of hot concentrated HCl and boiled under a reflux condenser for fully 12 hours. The solution was allowed to cool overnight, the benzoic acid filtered off, and the yellowish colored filtrate shaken out twice with ether. The aqueous solution was then evaporated in vacuo to a syrup, taken up in water several times, and evaporated each time to remove excess acid. The entire syrup plus a few crystals of unattacked benzoyl compound was taken up in about a liter of water, heated with norit, and filtered. The filtrate was then treated with 8 liters of saturated HgCl₂
solution with stirring. A white oil first appears which in a few
minutes turns crystalline. The mixture was allowed to stand
overnight. The mercury salt is then filtered off, washed with
dilute reagent, and dissolved with slight warming in 500 cc. of 10
per cent HCl solution. The latter was treated with H₂S, the
mercuric sulfide removed with the aid of norit, and the clear
filtrate evaporated in vacuo to a thick syrup. The latter is taken
up several times in water to remove excess acid. On standing in
the ice chest for several weeks the syrup becomes crystalline.
With previously prepared seed crystals, complete crystallization
is effected within an hour. The crystal mass is thoroughly dried
in vacuo over NaOH. It is then dissolved in hot absolute ethyl
alcohol, filtered, and treated with dry ether. On cooling, there
crystallize large macroscopic prisms of the hydrochloride of
ε-amino-α-thiol-n-caproic acid. The yield amounted to 36 gm.
The substance began to soften at 117° and melted to a clear liquid
at 123°. The nitroprusside reaction was positive.

C₆H₁₄K₀₂SCl (199.62). Calculated. C 36.07, H 7.07, N 7.01, S 16.2
Found. " 35.92, " 6.99, " 7.03, " 16.9

ε,ε-Diamino-di(α-Thio-n-Caproic Acid) (V)—A sample of 35
gm. of the ε-amino-α-thiol-n-caproic acid hydrochloride was dis-
solved in 240 cc. of water. With cooling, enough 10 per cent NH₃
solution was added to make the mixture slightly alkaline, and then
3.5 cc. of FeCl₃ solution (1:6). The color became deep purple-red.
A stream of air was blown through the solution for 10
hours at the end of which time the purple color had disappeared.
The solution was filtered and the filtrate evaporated in vacuo at a
bath temperature of 35° to a syrup. The remainder of the drying
occurred in a vacuum desiccator over P₂O₅. On alternate rubbing
and decantation with absolute alcohol, the entire mass is trans-
formed into a fine amorphous and hygroscopic powder. The
material is suspended in 95 per cent alcohol and shaken on the
machine for several hours to remove as much ammonium chloride
as possible. The shaking is then repeated, 80 per cent alcohol
being used with alternate decantation and renewal of diluted
alcohol until the material yields a negative test for chloride ion.
The material was then filtered off, washed with diluted alcohol,
and then dissolved in the minimum amount of water. The latter
was shaken with norit, filtered, and to the clear colorless filtrate absolute ethyl alcohol was added in excess. An oil separates which after several days standing in the ice chest crystallizes into large feathery needles. The supernatant mother liquor is decanted and the precipitation procedure repeated two or three times. The amino acid is then filtered off, washed with alcohol and ether, and dried in vacuo. The yield of pure substance was 23.6 gm., or 84 per cent of the theory. M. p., 207° with decomposition. The compound is easily soluble in water due most probably to its highly polar nature. It is insoluble in organic solvents. The aqueous solution is acid to litmus. On warming the aqueous solution with basic lead acetate the molecule is disrupted and PbS is formed.

\[ \text{C}_{12}\text{H}_{24}\text{O}_{4}\text{N}_{2}\text{S}_{2} \text{ (324.31). Calculated. C 44.40, H 7.46, N 8.63, S 19.8} \]

\[ \text{Found. C 44.33, H 7.58, N 8.37, S 20.2} \]

\( \epsilon,\epsilon'-\text{Di}guanido-\text{di}(\alpha-\text{Thio-}n-\text{Caproic Acid}) \) — An amount of 4.418 gm. of O-methylisourea hydrochloride (16, 20) in about 20 cc. of dry methyl alcohol solution was chilled in a freezing mixture and then 18.9 cc. of sodium methylate solution (2.11 N) were added. The sodium chloride was filtered off through a layer of infusorial earth and to the filtrate were added 2.78 gm. of \( \epsilon,\epsilon'-\text{diamino-di}(\alpha-\text{thio-}n-\text{caproic acid}) \). The mixture was heated to about 50° and small portions of water added until in solution. The solution was filtered clear and placed in the ice chest. Crystallization of the guanidine derivative began after 24 hours and was apparently complete in 5 days. The yield was 1.5 gm. or 43 per cent of the theory. It was dissolved in a little hot water and treated with an excess of absolute ethyl alcohol. An oil appears which after standing in the ice chest for 48 hours crystallizes into aggregates of tiny needles. M.p., 178–180° with foaming. The aqueous solution of the guanido acid was alkaline to litmus. On prolonged boiling with basic lead acetate the molecule is disrupted, yielding PbS. Otherwise it is quite stable even in hot water. The acid yields a positive Sakaguchi reaction.

\[ \text{C}_{14}\text{H}_{20}\text{O}_{4}\text{N}_{2}\text{S}_{2} \text{ (408.40). Calculated. C 41.13, H 6.91, N 20.58, S 15.70} \]

\[ \text{Found. C 40.91, H 7.34, N 18.60, S 15.70} \]

\[ \text{S 18.42} \]
The nitrogen analysis values are too low by approximately 10 per cent. In view of the excellent agreement between the theoretical and calculated values for the other elements, this discrepancy is inexplicable.

\( \epsilon, \epsilon'-\text{Diphenylureido-di(}\alpha\text{-Thio-n-Caproic Acid}) \) — An amount of 1.62 gm. of \( \epsilon, \epsilon'-\text{diamino-di(}\alpha\text{-thio-n-caproic acid}) \) was dissolved in 10 cc. of \( \text{N} \) KOH and the solution chilled in the ice bath. With continual shaking, 1.19 gm. of phenyl isocyanate were added in small portions. The solution was filtered and to the clear filtrate 5 N HCl was added to Congo blue. The phenylureido derivative crystallized out immediately. It was filtered off, washed thoroughly with large quantities of water, and then dried in \( \text{vacuo} \). The yield amounted to about 95 per cent of the theory. The substance began to melt at 81°, then became solid above this temperature, and finally decomposed at 140°.

\[ \text{C}_{26}\text{H}_{34}\text{O}_{4}\text{N}_{5}\text{S}_2 \ (562.3) \] Calculated. C 55.48, H 6.09, N 9.98

Found. " 55.24, " 6.09, " 10.38

SUMMARY

The syntheses of three new tetravalent amino acids have been described; namely, \( \alpha\)-aminotricarballylic acid, m. p. 196°, \( \alpha, \gamma, \delta\)-triamino-\( \Delta\gamma, \delta\)-pentenic acid, m. p. 171–173°, and \( \epsilon, \epsilon'-\text{diamino-di-(}\alpha\text{-thio-n-caproic acid}) \), m. p. 207°. To further characterize the latter substance, its \( \epsilon, \epsilon'-\text{diguanido derivative, m. p. 178–180°, and its } \epsilon, \epsilon'-\text{diphenylureido derivative, m. p. 140°, were prepared. The peptide of } \alpha\text{-aminotricarballylic acid, namely glycyl-}\( \alpha\text{-aminotricarballylic acid, m. p. 195°, was also prepared. The intermediate steps are described.}

BIBLIOGRAPHY

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