[CONTRIBUTION FROM PARKE, DAVIS AND COMPANY'S MULTIPLE FELLOWSHIP IN MEDICINAL CHEMISTRY, MELLON INSTITUTE]

Some New Esters of Serine with Various Acids[1]

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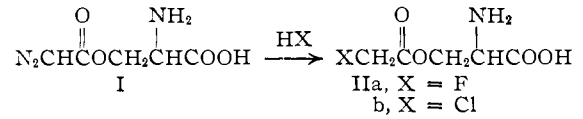
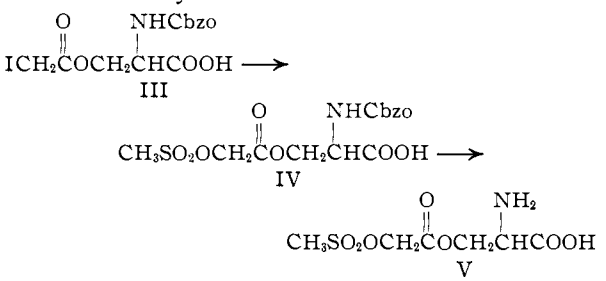
A series of new amino acid esters has been prepared in which several hydroxyamino acids, serine, homoserine and 6-hydroxynorleucine, serve as the alcohol function.

The related paper[2] described compounds in which the serine part of the azaserine (I) molecule was replaced with various hydroxyamino acids. The effect of replacing the diazoacetic portion of the azaserine molecule with various acids has also been studied. The present paper discusses the preparation of the esters of serine with fluoro (chloro and azido)-acetic acids; with acetic, propionic, butyric, succinic, bromosuccinic and maleic acids; and with (methylsulfonyloxy)-acetic acid. *O*-Methylsulfonyl-6-hydroxy-DL-norleucine was synthesized for comparison with *O*-(methylsulfonyloxy)-acetylserine.

O-Fluoroacetylserine (IIa), which contains the fluoroacetate radical attached by an easily hydrolyzed linkage, was prepared because of the known potent effect of fluoroacetate in carbohydrate metabolism. *O*-Chloroacetylserine (IIb) features an active halogen atom; the compound was prepared in an attempt to relate the antitumor

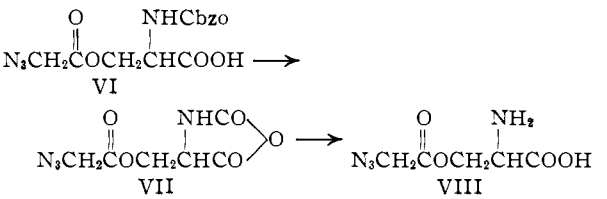


properties of azaserine, which contains the uncommon and highly reactive diazo group, to the structures of other anticancer agents, TEM,[3a] Myleran[3b] and HN_2,[3c] all of which contain highly reactive centers and have been described as "biological alkylating agents." Compound IIa and IIb were prepared by the action of anhydrous hydrogen fluoride and hydrogen chloride, respectively, on *O*-diazoacetylserine.



A Myleran-type of structure in this series was exemplified by *O*-(methylsulfonyloxy)-acetylserine (V). Compound V was synthesized by the reaction of *O*-iodoacetyl-*N*-carbobenzoxy-DL-serine with the silver salt of methanesulfonic acid in acetonitrile, followed by removal of the protecting carbobenzoxy group by hydrogenolysis. For comparison, the methanesulfonic ester of 6-hydroxy-DL-norleucine was prepared by the hydrogenolysis of *O*-methylsulfonyl-*N*-carbobenzoxy-6-hydroxy-DL-norleucine.

Of particular interest was the terminal, cumulative unsaturated system of the azido group ($\bar{N}=\overset{+}{N}=N$), the geometry of which resembles that of the diazomethyl group ($\bar{N}=\overset{+}{N}=CH-$) of azaserine. The azidoacetic ester VIII was prepared by treatment of *O*-azidoacetyl-*N*-carbobenzoxy-DL-serine[4] (VI) with phosphorus tribromide[5] to yield the *N*-carboxyamino acid anhydride (VII). In water, the anhydride decomposed to yield the amino acid.



The maleic ester of *N*-carbobenzoxy-DL-serine (XIII) was prepared by the reaction of maleic anhydride with *N*-carbobenzoxy-DL-serine in acetone and *N*,*N*-dimethylaniline. In order to maintain the carbon–carbon unsaturation, the protecting carbobenzoxy group was removed by the same mild hydrolysis procedure described above for *O*-azidoacetylserine, namely *via* the *N*-carboxyamino acid anhydride XIV.

Hydrogenation of XIII gave the succinic ester XI of DL-serine. Compound XI was prepared also by hydrogenolysis of the succinic ester of *N*-carbobenzoxy-DL-serine (X). The anhydrous hydrogen bromide glacial acetic acid reagent, used by Ben-Ishai,[6] removed the carbobenzoxy group of XIII without hydrolysis of the ester; however, addition of hydrogen bromide to the α,β-unsaturated ester took place to yield a bromosuccinic ester (XII) of serine.

An attempt to prepare the crotonic ester of serine *via* *N*-carbobenzoxy-DL-serine was not successful. However, when the free carboxy of *N*-carbobenzoxy-DL-serine was protected as the benzyl ester, a reaction with crotonic anhydride was effected. Hydrogenation of this crotonic ester

(1) Presented before the Division of Medicinal Chemistry at the 129th Meeting of the American Chemical Society, Dallas, Tex., April 9, 1956.

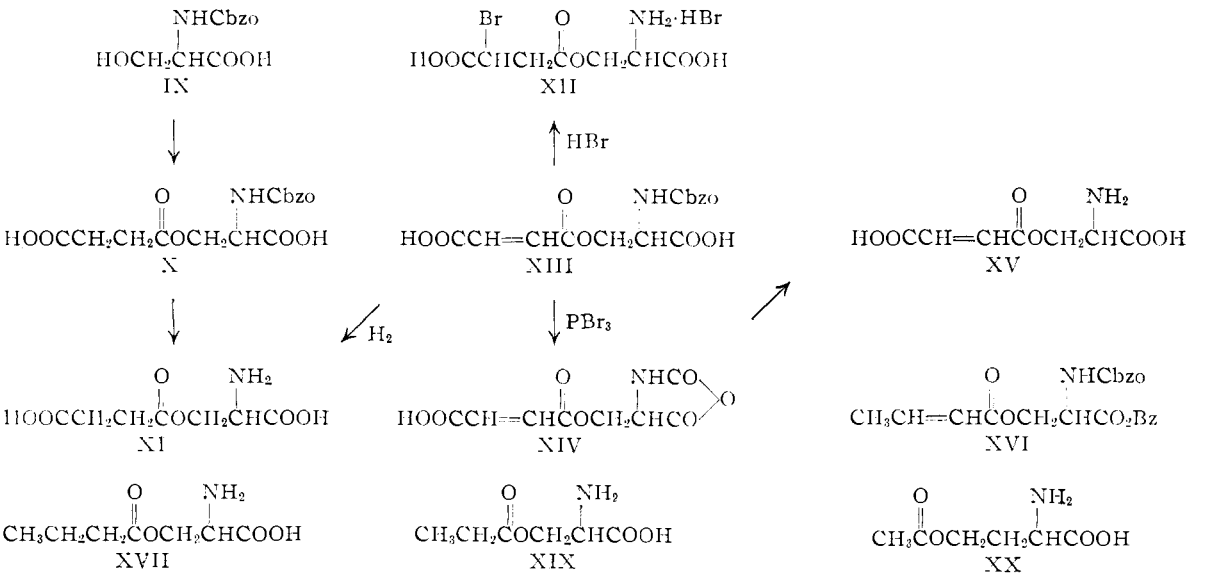
(2) H. A. DeWald, D. C. Behn and A. M. Moore, THIS JOURNAL, **81**, 4364 (1959).

(3) Systematic names, as listed in the indexes of *C.A.* are: (a) 2,4,6-tris-(1-aziridinyl)-*s*-triazine, (b) methanesulfonic ester, tetramethylene ester, (c) 2,2,-dichloro-*N*-methyldiethylamine.

(4) E. D. Nicolaides, R. D. Westland and E. L. Wittle, THIS JOURNAL, **76**, 2887 (1954).

(5) D. Ben-Ishai and E. Katchalski, *ibid.*, **74**, 3688 (1952).

(6) D. Ben-Ishai, *J. Org. Chem.*, **19**, 63 (1954).



intermediate XVI gave the butyric ester XVII of DL-serine.

Finally, *O*-propionyl-DL-serine (XIX) and *O*-acetyl-DL-homoserine (XX) were prepared by the direct action of propionic and acetic anhydrides, respectively, in a perchloric acid medium,[7] on DL-serine and DL-homoserine, respectively.

Experimental[8]

O-Fluoroacetyl-DL-serine (IIa).—Anhydrous hydrogen fluoride was introduced into a suspension of 1.1 g. of *O*-diazoacetyl-DL-serine in 75 ml. of anhydrous ether to a weight increase of 3 g. The polyethylene reaction bottle was stoppered and stored for 24 hours with occasional swirling. The colorless powder was collected by filtration and washed with dry ether. A sample showed no ultraviolet absorption due to unchanged diazoester. The hygroscopic powder melted at 85–87° with decomposition.

Anal. Calcd. for $C_3H_5FNO_4 \cdot {}^1/_2H_2O$: C, 35.06; H, 5.72; N, 8.04; F, 10.92. Found: C, 34.75; H, 5.84; N, 7.89; F, 11.24.

O-Chloroacetyl-L-serine, Monohydrochloride (IIb).—Azaserine (0.5 g., kindly furnished by Dr. Rieveschl, Parke, Davis and Co.) was suspended in 200 ml. of absolute ether, the suspension cooled to 0–3° and an excess (12 to 15 g.) of dry hydrogen chloride passed into the stirred suspension. The colorless solid (0.61 g.) was filtered off. Since an infrared spectrum disclosed the presence of a weak absorption band at 4.85 μ (diazo group), the product was resuspended in ether and retreated with additional hydrogen chloride; yield 500 mg., m.p. 150–157° dec. The ultraviolet absorption spectrum revealed an $E^{1\%}_{1cm}$ 2.3 at 250 mμ which is equivalent to 99.8% conversion of the diazo group. In the infrared, the 4.85 μ band was now absent, along with the appearance of 5.7 and 5.86 μ bands (carbonyl group).

Anal. Calcd. for $C_5H_8ClNO_4 \cdot HCl$: C, 27.54; H, 4.16; N, 6.42; Cl, 32.52. Found: C, 27.33; H, 4.11; N, 6.12; Cl, 31.75.

O-Iodoacetyl-N-carbobenzoxy-DL-serine (III).—*O*-Chloroacetyl-*N*-carbobenzoxy-DL-serine, (6.2 g., 0.02 mole) was added to 21 ml. of a normal solution of sodium iodide in acetone. The mixture was allowed to stand at room temperature with occasional swirling for 7 hours as sodium chloride began to separate almost immediately. The mixture was filtered and the filtrate was concentrated *in vacuo*. The oil was taken up in dry ether, treated with Darco and the solvent was evaporated *in vacuo*. The oil was dissolved in 12–14 ml. of benzene for crystallization. The colorless solid weighed 4.8 g., 60% yield, m.p. 84–86°. A sample was recrystallized twice from benzene, m.p. 85–86°.

Anal. Calcd. for $C_{13}H_{14}INO_6$: C, 38.34; H, 3.47; N, 3.44. Found: C, 38.59; H, 3.67; N, 3.67.

O-(Methylsulfonyloxy)-acetyl-DL-serine (V).—*O*-Iodoacetyl *N*-carbobenzoxy-DL-serine (4.1 g., 0.01 mole) was dissolved in 30 ml. of acetonitrile and 2 g. (0.01 mole) of the silver salt of methanesulfonic acid was added. The mixture was refluxed gently for two hours, and then was filtered to remove 1.95 g. of silver iodide. The filtrate was evaporated *in vacuo*, the residue was taken up in absolute ethanol and another 0.15 g. of salt was removed by filtering.

The alcoholic solution of *O*-(methylsulfonyloxy)-acetyl-*N*-carbobenzoxy-DL-serine was hydrogenated for two hours in the presence of 1 g. of 10% Pd-C catalyst. After standing overnight, the mixture was filtered using Filter-cel. The filter cake was extracted with 40 ml. of water and the extract was frozen and lyophilized. The crude amino acid weighed 0.7 g., 30% yield. It was crystallized readily from a small volume of water by the addition of absolute ethanol, m.p. 128–130° dec. An analytical sample melted with decomposition at 133–134°. The infrared spectrum showed bands at 1375 and 1170 cm.$^{-1}$ for RSO_2OR'.

Anal. Calcd. for $C_6H_{11}NO_7S$: C, 29.87; H, 4.60; N, 5.81. Found: C, 29.78; H, 4.65; N, 5.69.

***N*-Carbobenzoxy-6-hydroxy-DL-norleucine Methyl Ester.**—A suspension of 10 g. (0.035 mole) of *N*-carbobenzoxy-6-hydroxy-DL-norleucine[2] in 150 ml. of anhydrous ether was treated portionwise with a solution of diazomethane in ether prepared from 7 g. of *N*-nitrosomethylurea. The clear yellow solution was allowed to stand for 15 minutes and the excess diazomethane was destroyed by the addition of a few drops of acetic acid. After evaporation of the solvent, the oil weighed *ca.* 10.5 g.

***O*-Methylsulfonyl-*N*-carbobenzoxy-6-hydroxy-DL-norleucine Methyl Ester.**—This methyl ester above was dissolved in 15 ml. of dry pyridine. The solution was cooled to 0° and 3.7 g. (0.032 mole) of methanesulfonyl chloride was added in one portion. The mixture was allowed to stand at 0° for 18 hours and then was poured into ice and 150 ml. of normal hydrochloric acid. The insoluble oil was extracted into ether. The ether solution was dried over anhydrous magnesium sulfate and evaporated *in vacuo*.

***O*-Methylsulfonyl-6-hydroxy-DL-norleucine.**—The crude ester above was redissolved in 150 ml. of methanol. The solution was cooled to 0° and 31 ml. of normal sodium hydroxide was added. After 18 hours at 0°, 31 ml. of normal hydrochloric acid was added and the methanol was distilled *in vacuo*. The water-insoluble oil was extracted into ethyl acetate. The organic solution was in turn extracted

(7) W. Sakami and G. Toennies, *J. Biol. Chem.*, **144**, 203 (1942). When serine was dissolved in the acetous perchloric acid system and treated with other anhydrides, such as chloroacetic, acrylic, etc., the product isolated in high yield was *always* *O*-acetylserine.

(8) Microanalyses were performed by Mr. C. Childs and associates, Parke, Davis and Co.; Dr. C. Tiedke, Laboratory of Microchemistry, Teaneck, N. J. and Mr. C. W. Beazley, Micro-Tech Laboratory, Skokie, Ill.

with 150 ml. of water containing 0.1 mole of sodium bicarbonate. The aqueous extracts were acidified to congo red with concentrated hydrochloric acid, and the oil was extracted into ethyl acetate. After drying and evaporating the solvent *in vacuo* the residual yellow oil weighed *ca.* 9.5 g. The infrared spectrum of the oil was consistent with the structure for *O*-methylsulfonyl-*N*-carbobenzoxy-6-hydroxy-DL-norleucine.

The carboxylic acid (*ca.* 0.03 mole) was dissolved in 60 ml. of absolute ethanol and hydrogenated in the usual manner in the presence of 1.5 g. of 10% Pd–C catalyst. The mixture was cooled well and filtered. The filter cake was extracted with 40 ml. of hot water and the aqueous extract of the amino acid was frozen and lyophilized to yield 4 g. of hygroscopic solid. This solid was crystallized from aqueous ethanol, 1.8 g., m.p. 132–135° dec. (27% yield). An analytical sample melted with decomposition at 136–137°. The infrared spectrum exhibited bands at 1340 and 1165 cm.$^{-1}$ which were assigned to RSO_2OR.

Anal. Calcd. for $C_7H_{15}NO_5S$: C, 37.32; H, 6.71; N, 6.22. Found: C, 37.50; H, 6.73; N, 6.46.

O-Azidoacetyl-DL-serine (VIII).—A suspension of 3.2 g. (0.01 mole) of *O*-azidoacetyl-*N*-carbobenzoxy-DL-serine[4] in 10 ml. of anhydrous ether was treated with 0.38 ml. of phosphorus tribromide and allowed to stand at room temperature for 8 hours. The solid slowly dissolved and an oil began to separate. Petroleum ether (20 ml.) was added and the mixture was stored at 0° overnight. The mother liquors were decanted from the oil and the residual oil was triturated repeatedly with dry petroleum ether to wash out most of the benzyl bromide. The infrared spectrum of this oil had bands at 1750 and 1830 cm.$^{-1}$ characteristic of the *N*-carboxyamino acid anhydride. The oil dissolved readily in a small volume of water. The solution was extracted with ether to remove traces of benzyl bromide and then the aqueous portion was frozen and lyophilized. The colorless powder was precipitated from aqueous ethanol; 0.9 g. (50% yield) of amorphous solid, m.p. 120–135°. The infrared spectrum had a strong band for the azide group at 2100 cm.$^{-1}$

Anal. Calcd. for $C_3H_3N_4O_4$: C, 31.92; H, 4.29; N 29.78. Found: C, 30.76; H, 4.02; N, 28.69; ash, 3.2.

O-Succinyl-*N*-carbobenzoxy-DL-serine (X).—A solution of 4.8 g. (0.02 mole) of *N*-carbobenzoxy-DL-serine and 2.3 g. (0.023 mole) of succinic anhydride in 75 ml. of acetone was cooled to 0–5° in an ice-bath and a solution of 2.8 g. (0.023 mole) of *N,N*-dimethylaniline in 20 ml. of acetone was added dropwise with stirring. After 1.5 hours at 5°, the reaction mixture was allowed to stand at room temperature for three hours. The solvent was evaporated *in vacuo*, the oil was redissolved in ether and washed well with dilute hydrochloric acid, then water and dried over magnesium sulfate. The oil which remained after evaporation of the solvent *in vacuo* was crystallized by dissolving it in a small volume of benzene and adding petroleum ether to cloudiness; 4.2 g., yield 62%. An analytical sample was recrystallized from ethyl acetate–petroleum ether, m.p. 96–97°.

Anal. Calcd. for $C_{15}H_{17}NO_8$: C, 53.09; H, 5.05; N, 4.13. Found: C, 53.02; H, 5.13; N, 4.17.

O-Succinyl-DL-serine (XI).—A solution of 4.7 g. (0.014 mole) of *O*-succinyl-*N*-carbobenzoxy-DL-serine in 75 ml. of absolute ethanol was hydrogenated for two hours in the presence of 1.2 g. of 10% Pd–C catalyst. After two hours, the mixture was filtered and the filter cake was extracted with 70 ml. of boiling water. The aqueous extract was concentrated *in vacuo* to a small volume and absolute ethanol added. The precipitated amino acid weighed 2.3 g., m.p. 170–173° dec., yield 80%. A sample was recrystallized from aqueous ethanol as thick curling needles, m.p. 171° dec.

Anal. Calcd. for $C_7H_{11}NO_6$: C, 40.97; H, 5.40; N, 6.83. Found: C, 40.73; H, 5.62; N, 6.86.

O-Maleyl-*N*-carbobenzoxy-DL-serine (XIII) was prepared by the same procedure used to make the succinic acid ester above. From 4.8 g. (0.02 mole) of *N*-carbobenzoxy-DL-serine, 2.3 g. (0.023 mole) of maleic anhydride and 2.8 g. (0.023 mole) of *N,N*-dimethylaniline in acetone there was isolated 6.0 g. of crude product as an oil. The compound crystallized very slowly from an ethyl acetate-hexane mixture as a colorless powder, m.p. 140–143°. An analytical sample was recrystallized from water, m.p. 144–145°.

Anal. Calcd. for $C_{15}H_{15}NO_8$: C, 53.62; H, 4.46; N, 4.01. Found: C, 53.41; H, 4.48; N, 4.15.

O-Maleyl-DL-serine (XV).—The crude *O*-maleyl-*N*-carbobenzoxy-DL-serine (6.0 g.) from above was dissolved in 20 ml. of anhydrous ether and 0.75 ml. of phosphorus tribromide was added and let stand at room temperature four hours. A silky precipitate formed and was filtered off—this was unchanged *O*-maleyl-*N*-carbobenzoxy-DL-serine, 1.5 g., m.p. 135–138°. Petroleum ether (40 ml.) was added and the mixture was stored overnight. The semi-solid was separated by decantation of the mother liquors. The crude Leuchs anhydride was triturated with petroleum ether and then was treated with 25 ml. of hot water. The water-soluble portion was lyophilized. The product was crystallized from water; 0.7 g., m.p. 185° dec. An analytical sample melted at 186–187° with decomposition.

Anal. Calcd. for $C_7H_9NO_6$: C, 41.38; H, 4.47; N, 6.90. Found: C, 41.24; H, 4.60; N, 7.10.

O-Succinyl-DL-serine (X) from Reduction of O-Maleyl-*N*-carbobenzoxy-DL-serine (XIII).—*O*-Maleyl-*N*-carbobenzoxy DL-serine, (6.0 g.) was hydrogenated in absolute ethanol in the presence of 1.0 g. of 10% Pd–C. The mixture was filtered and the filter cake was extracted with 50 ml. of hot water. The water extract was concentrated to yield 2.6 g. (63% yield) of the succinic ester, m.p. 170–172° dec.

O-Bromosuccinyl-DL-serine Hydrobromide (XII) from O-Maleyl - *N* - carbobenzoxy - DL - serine.—*O*-Maleyl - *N*-carbobenzoxy-DL-serine (6.0 g.) was dissolved in 30 ml. of 33% anhydrous hydrogen bromide in glacial acetic acid and allowed to stand at room temperature overnight. The solid was collected by filtration and washed well with dry ether. The hygroscopic solid (3.1 g., yield 42%) was recrystallized by solution in glacial acetic acid and adding ether to cloudiness. The colorless solid now weighed 2.8 g., m.p. 122–124° dec.

Anal. Calcd. for $C_7H_{11}Br_2NO_6$: C, 23.03; H, 3.04; N, 3.85; Br, 43.79. Found: C, 22.91; H, 3.24; N, 4.08; Br, 43.24.

***N*-Carbobenzoxy-DL-serine Benzyl Ester.**—Following the general method of Ben-Ishai and Berger,[9] a suspension of 4.8 g. (0.02 mole) of *N*-carbobenzoxy-DL-serine in 75 ml. of dry benzene was refluxed during three hours with an excess (0.03 mole) of benzyl alcohol in the presence of 0.5 g. of *p*-toluenesulfonic acid. The water was removed azeotropically in a Dean–Stark trap. The benzene solution was washed twice with 5% potassium bicarbonate and then dried over anhydrous sodium sulfate. Evaporation of the benzene filtrate to dryness resulted in a mixture of low-melting solid and an oil. This product was dissolved in ether, pentane added to turbidity and cooled to yield a crop (2.66 g., 41% of theory) of colorless crystals, m.p. about 60–62° (indefinite). Extraction of a 2.0-g. aliquot with two 300-ml. portions of boiling hexane resulted in the recovery of 0.57 g. of colorless crystals, m.p. 72–73.5°.

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.65; H, 5.82; N, 4.25. Found: C, 65.65; H, 5.90; N, 4.38.

O-Crotonyl-*N*-carbobenzoxy-DL-serine Benzyl Ester (XVI).—Crude *N*-carbobenzoxy-DL-serine benzyl ester (6.5 g.) which was prepared as described above was dissolved in 15 ml. of pyridine and cooled to 0°. Crotonic anhydride (3.2 g., 0.021 mole) was added in one portion and the mixture was stored at 0° for 30 hours. The mixture was poured into ice and excess hydrochloric acid. The oil was extracted into ether, washed again with dilute hydrochloric acid and dried over magnesium sulfate.

O-Butyryl-DL-serine (XVII) by Reduction of Crotonic Ester (XVI).—The crude oil (6.0 g.) from above was hydrogenated in the usual manner in absolute ethanol. The catalyst, with some solid product, was collected by filtration using Filter-cel. The filter cake was extracted with hot water and the extract was concentrated to a small volume. The amino acid was crystallized by the addition of absolute ethanol; 1.0 g. (yield 30% based on *N*-carbobenzoxy-DL-serine). The product melted at 157–159° with decomposition and a strong odor of butyric acid. An analytical sample melted at 163–165° dec.

Anal. Calcd. for $C_7H_{13}NO_4$: C, 47.97; H, 7.48; N, 7.99. Found: C, 47.81; H, 7.74; N, 7.92.

O-Propionyl-DL-serine.—Pulverized serine (5.0 g.) was dissolved in 100 ml. of a propionic acid solution of perchloric

(9) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

acid (0.6 molar). The solution was swirled in an ice-bath and propionic anhydride (3.0 gram) was added from a dropping funnel during 20 minutes. After 2.5 hours at room temperature, the solution was decanted from a gummy placque and treated with 2 ml. of water during 2–3 minutes. After 90 minutes, isoamylamine (6.8 ml.) was added slowly. The solution was treated with a total of 350 ml. of anhydrous ether. After 18 hours in the ice-box a crude product (5.3 g., m.p. 145–147°) was collected on a filter. The recrystallized product melted at 157–158° with decomposition.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.72; H, 6.83; N, 8.69. Found: C, 44.71; H, 6.90; N, 8.94.

O-Acetyl-DL-homoserine was prepared from homoserine and acetic anhydride in perchloric acid by the same procedure[7] as described above in 75% yield, glistening plates, m.p. 183–185° dec.

Anal. Calcd. for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.50; H, 6.89; N, 8.48.

PITTSBURGH, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

*p*H Titration Studies of Polypeptidyl Proteins[1]

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*p*H titration curves of bovine and rabbit albumin which had been modified by the chemical attachment of polypeptides of glycine, leucine, phenylalanine, glutamic acid or lysine were determined by a micro continuous *p*H titration method. The number of polypeptide chains added and the average chain length were calculated. Unusual titration behavior of polylysyl bovine albumin was interpreted as an interaction of the polylysyl residues with tyrosine groups of the protein.

Changes in the number and types of ionic groups of a protein are best indicated by a study of its *p*H titration curve. Ionizations within certain ranges of *p*H may be assigned to certain amino acid residues[3]; from the number of hydrogen ions bound within a certain *p*H range, a quantitative estimate of the number of such groups may be made. This paper will report changes in the *p*H titration behavior of bovine and rabbit albumins which had been modified by the addition of extra amino acid residues linked as polypeptide chains to the original protein.

Stahmann and Becker[4] developed the preparation of water-soluble polypeptidyl proteins by reaction of N-carboxyamino acid anhydrides with proteins in buffered aqueous solution. This procedure yields proteins containing new amino acid residues attached through peptide bonds to the α- and ϵ-amino groups of the original protein.

On the basis of *p*H titration or treatment with 2,4-dinitrofluorobenzene, Becker and Stahmann[5] concluded that approximately one-third of the ϵ-amino groups of bovine albumin had reacted with N-carboxyglycine anhydride to form glycine polypeptides terminated with α-amino groups. The methods of Tsuyuki, *et al.*,[6] permitted the preparation of proteins with additional residues of the ionic amino acids glutamic acid and lysine. Greater changes in the *p*H titration behavior would be expected in these preparations as compared to modifications with neutral amino acids.

A careful study of the *p*H titration curve of bovine albumin was made by Tanford, Swanson and Shore.[7] We have used a rapid, albeit less accurate method of *p*H titration developed by R. M. Bock to determine the titration curve of bovine albumin and rabbit albumin as well as both albumins which had been modified by the addition of polypeptides of glycine, leucine, phenylalanine, glutamic acid or lysine.

Experimental

Preparation of Polypeptidyl Proteins.—Most of the polypeptidyl proteins were prepared by treating the protein with the N-carboxyamino acid anhydride in bicarbonate buffer at 4° as described by Tsuyuki, Van Kley and Stahmann.[6] The polyglycyl proteins were prepared by Dr. R. R. Becker[5] in phosphate buffer. Protein preparations were stored at −20° as a lyophilized powder. The increase in amino acid content was determined by microbiological assay of an HCl hydrolyzate.[8] Calculations for bovine albumin were based on a molecular weight of 69,000 and the amino acid analysis of Stein and Moore.[9] In the absence of physical measurements of the molecular weight of rabbit serum albumin, we have assumed its molecular weight to be 69,000. Calculations were based on the amino acid analysis of Schneiderman, Greene, Schieler, McClure and Dunn[10] except for the lysine values. These were obtained from the Wisconsin Alumni Research Foundation. This was done since the titration studies in the lysine range did not agree with the lysine content reported in the previously cited paper. The microbioassay value for lysine reported in our paper was performed on a sample obtained from the same source as the titration sample.

Apparatus used for Titration.—A titration apparatus requiring only 1 ml. of solution was used; this titrator was designed by Dr. R. M. Bock of the Biochemistry Department, University of Wisconsin. The sample is placed in a plastic centrifuge tube which is placed inside a turbine stirrer. A Leeds and Northrop all-purpose glass electrode fits inside the titration tube. Two polyethylene capillary tubes are taped onto the side of the electrode; one filled with

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(2) National Heart Institute Fellow, 1956–1957.

(3) R. A. Alberty in H. Neurath and K. Bailey, "The Proteins," Vol. 1, Part A, Academic Press, Inc., New York, N. Y., 1953.

(4) M. A. Stahmann and R. R. Becker, THIS JOURNAL, **74**, 2695 (1952).

(5) R. R. Becker and M. A. Stahmann, *J. Biol. Chem.*, **204**, 745 (1953).

(6) H. Tsuyuki, H. Van Kley and M. A. Stahmann, THIS JOURNAL, **78**, 764 (1956).

(7) C. Tanford, S. A. Swanson and W. S. Shore, *ibid.*, **77**, 6414 (1955).

(8) Several of the microbiological assays were performed by Drs. G. Lewis, F. Hepburn and J. Gupta in the laboratory of Prof. Elvehjem in the Biochemistry Department of the University of Wisconsin; the remainder of the assays were carried out by Dr. Maria Berger and Dr. B. V. Kline of the Wisconsin Alumni Research Foundation. We express our appreciation to these groups for the assays.

(9) W. H. Stein and S. Moore, *J. Biol. Chem.*, **178**, 79 (1949).

(10) A. Schneiderman, M. Greene, L. Schieler, L. McClure and M. Dunn, *Proc. Soc. Exp. Biol. and Med.*, **82**, 53 (1953).