as in the above rearrangement. Diazotization of the L-compound gave 106 mg. of crude O-diazoacetyl-L-serine (V), $E^{1\%}_{1\,cm}$ 1065 at $\lambda^{H_2O}_{max}$ 250 mμ. After recrystallization from aqueous alcohol, a value of $E^{1\%}_{1\,cm}$ 1140 at $\lambda^{H_2O}_{max}$ 250 mμ was obtained. The infrared spectrum and microbiological assay (330 times standard) were identical with those of azaserine.

DETROIT 32, MICHIGAN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS & COMPANY]

Azaserine, Synthetic Studies. II

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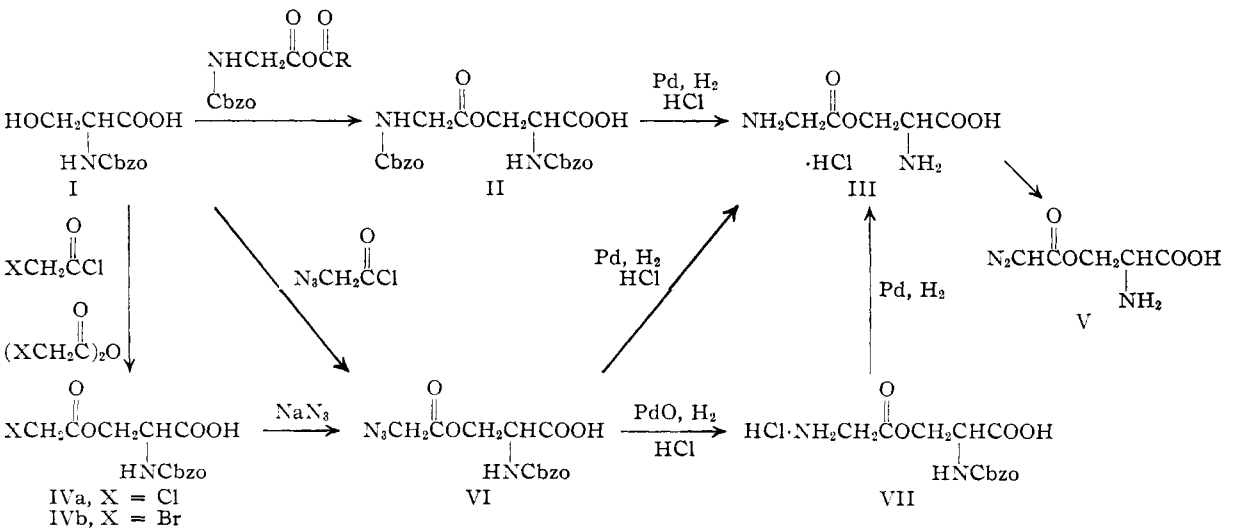
Two methods for the preparation of O-glycyl-L-serine monohydrochloride, the intermediate in the synthesis of the antibiotic azaserine, are presented. The esterification of N-carbobenzoxy-L-serine with a haloacetyl halide or anhydride and an azide displacement of the halide group followed by hydrogenation and debenzylation has given a moderate yield of the desired intermediate. Alternately, the esterification of N-carbobenzoxy-L-serine with mixed anhydrides of carbobenzoxyglycine and various acids followed by debenzylation, gave satisfactory yields of O-glycyl-L-serine monohydrochloride. Selective diazotization of O-glycyl-L-serine has produced azaserine. These reactions have also been carried out with DL- and D-serine.

In the preceding paper,[1] the preparation of O-glycyl-L-serine monohydrochloride (III) by two methods and its diazotization to the antibiotic azaserine (V) has been described. Since a more suitable method for the preparation of this intermediate was desired, other potential syntheses were investigated. This paper describes two additional methods for the preparation of the intermediate. As with the previous work, all the reactions were first carried out with DL-serine. For comparative purposes O-glycyl-D-serine monohydrochloride also was prepared and converted to O-diazoacetyl-D-serine.

While the direct introduction of the amino acyl group into a serine derivative has been successful, as described in the previous paper, in the present work it has been found more advantageous first to introduce an amine precursor or protected amino group and subsequently generate the amine.



One of the methods developed for the synthesis of O-glycylserine monohydrochloride (III) involved the preparation of O-haloacetyl-N-carbobenzoxyserine (IV), a nucleophilic displacement of the halide group by the azide ion and a subsequent hydrogenation of the azide and carbobenzoxy groups in the presence of hydrogen chloride.

The reported instances of serine derivatives in which the hydroxyl group of serine is esterified are limited,[2-5] and the O-halo- or O-azidoacetylserine derivatives have not been previously reported.

In the present work the reactions of chloroacetyl chloride, chloroacetic anhydride and bromoacetyl bromide with N-carbobenzoxy-L- or DL-serine (I) have been studied. A diversity of anhydrous solvents have been used in the presence or absence of a tertiary base. In the DL-series, crystalline products could be obtained in every case, while in the L-series, due to increased solubility and lower melting points, nearly all of the reaction products were oils. It was possible in the L-series, however, to obtain small amounts of crystalline product from three of the reactions which enabled characterization of every intermediate. The reaction of chloroacetic anhydride with N-carbobenzoxy-DL-serine in refluxing benzene or ethyl acetate without a base

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(3) M. Frankel and M. Halmann, *J. Chem. Soc.*, 2735 (1952).

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(5) M. Bergmann and A. Miekeley, *Z. physiol. Chem.*, **140**, 128 (1924).

gave a 30–35% yield of crystalline product. The highest yield of O-chloroacetyl-N-carbobenzoxy-DL-serine (60%) was obtained using chloroacetic anhydride with one equivalent of dimethylaniline in acetone solution. Chloroacetyl chloride gave results comparable to those with the anhydride.

The use of chloroacetic anhydride in the absence of a base gave a quantitative amount of crude oily O-chloroacetyl-N-carbobenzoxy-L-serine (IVa) which was used for the synthesis of azaserine without purification, and consequently the absolute yield was not determined. Using dimethylaniline in acetone with this reaction, a 10% yield of crystalline IVa was obtained, while dimethylformamide in ethyl acetate gave a 25% yield of crystalline product.

Bromoacetyl bromide, which reacts quite readily at room temperature with N-carbobenzoxy-DL-serine gave good yields (70%) of O-bromoacetyl-N-carbobenzoxy-DL-serine (IVb) using a partial vacuum to remove hydrogen bromide as it was formed. Failure to remove the hydrogen bromide results in a decreased yield due to cleavage of the carbobenzoxy group.[6] This method in the L-series gave O-bromoacetyl-N-carbobenzoxy-L-serine only as an oil. A crystalline product was obtained in only one reaction (1.6% yield) by using pyridine in ethyl acetate.

The reaction of either O-chloro- or O-bromoacetyl-N-carbobenzoxy-L- or DL-serine (IV) with sodium azide in aqueous dioxane proceeded smoothly and the desired O-azidoacetyl-N-carbobenzoxy-L- or DL-serine (VI) was isolated in good yield. An alternate method for the preparation of the azido compound VI consists in the reaction of azidoacetyl chloride with N-carbobenzoxy-DL-serine in methyl ethyl ketone solution in the presence of pyridine; however, the hazardous nature of azidoacetyl chloride and the impurities which accompany it definitely limited the use of this reagent.

Catalytic reduction of O-azidoacetyl-N-carbobenzoxy-L- or DL-serine (VI) with palladium and hydrogen in aqueous alcohol containing one equivalent of hydrogen chloride gave crystalline O-glycyl-L- or DL-serine monohydrochloride (III). In one case a selective reduction of the azide group occurred, and the compound which was isolated was found to be O-glycyl-N-carbobenzoxy-DL-serine hydrochloride (VII). On further reduction the carbobenzoxy group was removed.

In the large-scale preparation of O-glycyl-L-serine monohydrochloride using the above described method, several difficulties were encountered. The O-chloro- and O-azidoacetyl derivatives were obtained as oils, thus accumulating impurities, and the complete reduction of the azido compound was hindered due to the accumulation of nitrogen and carbon dioxide. This necessitated that the hydrogenation flask be swept out intermittently with fresh hydrogen. The O-glycyl compound also appeared as an oil which was resistant to crystallization. The oil was therefore diazotized directly to azaserine which was then purified by carbon chromatography. The over-all yields from N-carbobenzoxy-L-serine were in the order of 5–10%.

The most satisfactory method for the preparation of O-glycyl-L-serine monohydrochloride, and thus also azaserine, was found in a reaction utilizing mixed anhydrides of N-carbobenzoxyglycine. Various mixed anhydrides of N-carbobenzoxyamino acids have been prepared and used extensively in the preparation of peptides,[7] but there has been no report on their reaction with hydroxyl groups to form esters.[8]

The mixed anhydride of N-carbobenzoxyglycine with isovaleric acid, 2-ethylbutyric acid, or ethyl acid carbonate was allowed to react with the triethylamine salt of N-carbobenzoxyserine in methylene chloride solution at low temperatures. The product, O-(N-carbobenzoxyglycyl)-N-carbobenzoxy-L- or DL-serine (II), was isolated in yields of 80–95%, but invariably as an oil. Reduction of this oil with palladium and hydrogen in aqueous alcohol containing one equivalent of hydrogen chloride gave crystalline O-glycyl-L- or DL-serine monohydrochloride in a 60% over-all yield. Large runs gave slightly lower yields, but consistently gave crystalline material. Diazotization of pure O-glycyl-L-serine monohydrochloride was found to give yields of azaserine from 15 to 20%.

The mixed anhydride method also was used in the preparation of O-diazoacetyl-D-serine. This compound was obtained as a yellow crystalline solid, m.p. 153–155° dec., E_1^1 1140 at $\lambda_{max}^{H_2O}$ 250 mμ. While the infrared spectrum was identical with that of azaserine, O-diazoacetyl-D-serine was found to be inactive in the *Kloeckera brevis* microbiological assay.

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Experimental

O-Chloroacetyl-N-carbobenzoxy-DL-serine (IVa). (a) **From Chloroacetic Anhydride.**—To a cold, well-stirred solution of 10 g. (0.042 mole) of N-carbobenzoxy-DL-serine and 9.5 g. (0.055 mole) of chloroacetic anhydride in 100 ml. of reagent grade acetone was added a solution of 6.65 g. (0.055 mole) of dimethylaniline in 50 ml. of acetone over a period of 3.5 hours. The acetone was removed *in vacuo* and the remaining purple oil was shaken with 100 ml. of 1.2 *N* hydrochloric acid. This mixture was extracted once with 100 ml. of benzene and twice with 100-ml. portions of ether. The combined ether–benzene solution was washed with 50 ml. of 1 *N* hydrochloric acid and several times with water. The solution was dried and concentrated until a small amount of benzene remained. The product crystallized as colorless platelets, 9.3 g. (70%), m.p. 115–118°. Recrystallization from dilute ethanol gave 7.9 g. (60%), m.p. 121–122°.

Anal. Calcd. for $C_{13}H_{14}O_6NCl$: C, 49.46; H, 4.46; N, 4.43. Found: C, 49.65; H, 4.69; N, 4.49.

(6) This gave rise to a method for the removal of the carbobenzoxy group which has since been disclosed by D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(7) J. R. Vaughan, THIS JOURNAL, **73**, 3547 (1951); J. R. Vaughan and R. L. Osato, *ibid.*, **73**, 5553 (1951); R. A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951); T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(8) Two reports on the preparation of penicillin esters using mixed anhydrides have recently appeared: D. A. Johnson, THIS JOURNAL, **75**, 3636 (1953); R. L. Barnden, *et al.*, *J. Chem. Soc.*, 3733 (1953).

A mixture of 4.8 g. of N-carbobenzoxy-DL-serine, 4 g. of chloroacetic anhydride and 30 ml. of benzene was refluxed on the steam-bath for 15 minutes. The solution was cooled and seeded; the product crystallized and was separated and recrystallized from dilute ethanol to yield 2.4 g. (37%), m.p. 113–118°. Using ethyl acetate instead of benzene and with a small amount of concd. sulfuric acid as catalyst a 3.2% yield of product, m.p. 109–116°, was obtained.

(b) **From Chloroacetyl Chloride.**—To a solution of 10 g. (0.042 mole) of N-carbobenzoxy-DL-serine and 4.6 g. of triethylamine in 150 ml. of ethyl acetate cooled in an ice-bath was added with stirring over a 45-minute period a solution of 5.6 g. (0.05 mole) of chloroacetyl chloride in 50 ml. of ethyl acetate. A white solid precipitated after a few minutes. The solution was allowed to stir at 0° for one-half hour and then at 25° for 3 hours. The solution was washed three times with water, and the ethyl acetate layer was separated, dried and reduced to a small volume. The addition of petroleum ether gave 12.5 g. of slightly gummy solid which was recrystallized from ethylene dichloride to yield 7 g. (52%) of crystalline product, m.p. 120–121°.

When this reaction was run in dioxane using dimethylaniline as the base the yields were comparable. When run in benzene or ethyl acetate without a base and refluxing one-half hour or less the yields were lower (20–25%).

O-Chloroacetyl-N-carbobenzoxy-L-serine (IVa). (a).—To a stirred solution of 10 g. (0.042 mole) of N-carbobenzoxy-L-serine (m.p. 117–119°) and 9.4 g. (0.055 mole) of chloroacetic anhydride in 100 ml. of reagent grade acetone cooled in an ice-bath was added a solution of 6.7 g. (0.055 mole) of dimethylaniline in 40 ml. of acetone. Stirring was continued for one-half hour longer at ice temperature. The acetone was removed *in vacuo* and the oil was taken up in 100 ml. of ether; the ethereal solution was washed three times with dilute hydrochloric acid and three times with water. The ether layer was dried, 50 ml. of benzene was added, and the solution was concentrated to a small volume in a stream of air. The insoluble oil which formed in the benzene solution was separated and by treatment with ethylene dichloride and ligroin gave 3.3 g. (25%) of a crystalline product. The mother liquor was taken up in ether and the ethereal solution was extracted with dilute sodium bicarbonate solution. The bicarbonate extract was acidified with dilute hydrochloric acid and the oil which separated was dissolved by the addition of ether. The ether layer was separated, dried and concentrated to an oil which gave a further crop of crystals from a ligroin–ethylene dichloride–benzene mixture. This crop was recrystallized by allowing an ethylene dichloride solution to evaporate slowly at room temperature to yield 1.3 g. (10%) as large plates, m.p. 93–96°, $[\alpha]^{28}D$ +28.8° (*c* 5 in chloroform).

Anal. Calcd. for $C_{13}H_{14}O_6NCl$: C, 49.46; H, 4.46; N, 4.43. Found: C, 49.36; H, 4.67; N, 4.31.

(b).—A mixture of 10 g. of N-carbobenzoxy-L-serine, 10 g. of chloroacetic anhydride, 15 ml. of dimethylformamide and 100 ml. of ethyl acetate was allowed to stand overnight at 25°. The solution was heated for 6 hours at 40–50° and again allowed to stand at 25° overnight. Water (10 ml.) was added and the solution was concentrated to an oil at 40° *in vacuo*. The oil was washed thoroughly with 500-, 100-, 50-, 50-ml. portions of water, the aqueous washings were filtered to collect droplets of oil, and the oil then taken up with acetone. The acetone solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 50 ml. of benzene and partially evaporated. A sticky white solid formed in this solution after one week, yield 6.4 g. (50%). Six recrystallizations from benzene gave 3.2 g. (25%) as colorless plates, m.p. 92–94°, identical with the product above.

O-Bromoacetyl-N-carbobenzoxy-DL-serine (IVb).—A mixture of 4.8 g. (0.02 mole) of N-carbobenzoxy-DL-serine, 120 ml. of ethyl acetate and 8 g. (excess) of bromoacetyl bromide was stirred in a three-necked flask under a partial vacuum. With slight heating this vacuum was sufficient to evaporate ethyl acetate from the stirred solution and maintain the temperature at 30–32° for 2 hours. The solution was cooled to 22°, 10 ml. of water was added, and the ethyl acetate solution was separated and washed twice with 10-ml. portions of water. The ethyl acetate solution was dried and evaporated to a small volume giving a crystalline solid, 6.84 g. (95%), m.p. 98–107°. Recrystallization from benzene–ethyl acetate gave 5 g. (70%), m.p. 109–111°.

Anal. Calcd. for $C_{13}H_{14}O_6NBr$: C, 43.35; H, 3.92; N, 3.89. Found: C, 43.51; H, 4.13; N, 3.68.

This process in the L-series gave O-bromoacetyl-N-carbobenzoxy-L-serine only as an oil. The use of ethylene oxide to remove the hydrogen bromide gave yields lower than above. When the hydrogen bromide was not removed, the carbobenzoxy group was cleaved as shown by infrared absorption.

O-Bromoacetyl-N-carbobenzoxy-L-serine (IVb).—To a solution of 5 g. (0.021 mole) of N-carbobenzoxy-L-serine in 50 ml. of ethyl acetate was added 5.5 g. (0.027 mole) of bromoacetyl bromide. The reaction was allowed to stand at 25° for 20 minutes with occasional shaking. The solution was cooled in an ice-bath and 1.5 g. (0.019 mole) of pyridine was added dropwise over a period of 20 minutes. The solution was kept in the ice-bath for 45 minutes and then at 25° overnight. The ethyl acetate solution was filtered, washed with water, dried and concentrated. The residual oil crystallized on long standing, and the crystals were separated by careful washing with benzene to yield 120 mg. (1.6%), m.p. 95–97°, $[\alpha]^{26}D$ +30.1° (*c* 4.7 in chloroform).

Anal. Calcd. for $C_{13}H_{14}O_6NBr$: C, 43.35; H, 3.92; N, 3.89. Found: C, 43.60; H, 3.79; N, 4.08.

O-Azidoacetyl-N-carbobenzoxy-DL-serine (VI). (a).—To a solution of 5 g. (0.014 mole) of O-bromoacetyl-N-carbobenzoxy-DL-serine in 100 ml. of dioxane was added 2.3 g. (0.035 mole) of sodium azide and sufficient water to effect solution of the salt. The solution was kept at 25° for three days. The dioxane and water were removed *in vacuo* and the residual oil was dissolved in 100 ml. of water and acidified to congo red paper with concentrated hydrochloric acid; the oil which separated was extracted with benzene. The benzene solution was washed with water, dried and concentrated to a small volume. On cooling, the product crystallized and was recrystallized from benzene to yield 2.7 g. (60%) of product, m.p. 91–92°. A second fraction of 0.92 g., m.p. 87–91°, also was obtained. The infrared spectrum had a strong band at 4.70 mμ.

Anal. Calcd. for $C_{13}H_{14}O_6N_4$: C, 48.45; H, 4.38; N, 17.39. Found: C, 48.48; H, 4.58; N, 17.65.

(b).—From 1.9 g. of O-chloroacetyl-N-carbobenzoxy-DL-serine treated with 0.78 g. of sodium azide as above for 6 hours at 40° and 18 hours at 25° was obtained 1.4 g. (73%) of product, m.p. 89–90°, identical with the product above.

O-Azidoacetyl-N-carbobenzoxy-L-serine (VI).—To a solution of 1.0 g. (3.2 millimoles) of crystalline O-chloroacetyl-N-carbobenzoxy-L-serine in 20 ml. of purified dioxane was added 0.42 g. (6.4 millimoles) of sodium azide and sufficient water to dissolve the salt. The solution was allowed to stand at 25° for three days. The solution was concentrated *in vacuo* to an oil which was dissolved in 25 ml. of water, ice added, and the solution acidified to congo red paper with 7 ml. of 1 *N* hydrochloric acid. The solution was extracted with two 50-ml. portions of ether. The ether extract was dried and concentrated to an oil which crystallized after standing for a week without solvent. The crystals were washed carefully with benzene and recrystallized from benzene to give 150 mg., m.p. 82–84°, $[\alpha]^{26}D$ +33.6° (*c* 1.92 in chloroform). The infrared spectrum had a strong band at 4.73 mμ.

Anal. Calcd. for $C_{13}H_{14}O_6N_4$: C, 48.45; H, 4.38; N, 17.39. Found: C, 48.36; H, 4.49; N, 17.18.

Azidoacetyl Chloride.—To a stirred suspension of 11.3 g. (0.08 mole) of potassium azidoacetate in 100 ml. of dry ether was added 15 g. (0.08 mole) of oxalyl chloride in 20 ml. of dry ether, with external cooling in an ice–salt-bath. The reaction mixture was allowed to stand at 25° overnight. The potassium chloride was removed by filtration and the ether and excess oxalyl chloride were removed *in vacuo* below 35° to yield 10 g. (theory) of liquid. In an earlier reaction this material was distilled *in vacuo*, but the residue in the distillation flask exploded violently. No subsequent distillations were run.

O-Azidoacetyl-N-carbobenzoxy-DL-serine.—To a stirred solution of 10 g. (0.042 mole) of N-carbobenzoxy-DL-serine in 70 ml. of methyl ethyl ketone at 10° was added dropwise 10 g. of the above crude azidoacetyl chloride. Dry pyridine (3 ml.) was added, and stirring and cooling were continued for 2 hours. Most of the methyl ethyl ketone was removed *in vacuo* and the resulting oil was taken up in 200 ml. of

ethyl acetate. The ethyl acetate solution was washed with four 50-ml. portions of water, dried and concentrated *in vacuo* to a yellow oil, 13 g. (96%). The product was used as such.

O-Glycyl-N-carbobenzoxy-DL-serine Hydrochloride (VII).—A solution of 13 g. of the oily O-azidoacetyl-N-carbobenzoxy-DL-serine in 200 ml. of 60% ethanol and 3.4 ml. of concd. hydrochloric acid was hydrogenated over 1 g. of regenerated palladium oxide catalyst at 3 atm. of hydrogen in an Adkins hydrogenator for 6 hours. The catalyst and the solvent were removed, leaving an oil. The oil was dissolved in water, the aqueous solution was washed with ether and again concentrated *in vacuo* to an oil. The oil was dissolved in a small volume of ethanol, an insoluble oil was removed, and the ethanol solution was diluted with ether giving a white solid, 4.8 g. (35%), m.p. 176–179° dec. The infrared spectrum showed that this compound was identical with O-glycyl-N-carbobenzoxy-DL-serine previously prepared[1] and that only the azido group had reduced.

O-Glycyl-DL-serine Monohydrochloride (III). (a).—A solution of 3.1 g. of VII in 75 ml. of water was hydrogenated at 3 atm. for 2 hours over 0.5 g. of palladium black. The aqueous solution was filtered and concentrated *in vacuo* to an oil which was crystallized by the addition of 25 ml. of ethanol and sufficient water to effect solution on warming, affording 1.7 g. of solid which on recrystallization from water–ethanol gave 1.2 g., m.p. 168–170° dec., identical with O-glycyl-DL-serine monohydrochloride previously prepared.[1]

Anal. Calcd. for $C_5H_{11}O_4N_2Cl$: C, 30.23; H, 5.58; N, 14.11. Found: C, 30.36; H, 5.85; N, 14.02.

(b).—The O-azidoacetyl-N-carbobenzoxy-DL-serine (VI) could be reduced completely in one step. A solution of 1.5 g. (4.7 millimoles) of VI in 150 ml. of 50% methanol and 4.7 ml. of 1 *N* hydrochloric acid was hydrogenated at 3 atm. for 3.5 hours over 0.16 g. of palladium black. The solution was filtered and concentrated *in vacuo* to an oil which on crystallization from water–ethanol gave 0.75 g. (82%) of O-glycyl-DL-serine monohydrochloride, m.p. 168–170° dec., identical with that above and also described in the previous paper.

Azaserine (V), Azide Process without Isolation of Intermediates.—The esterification of 24 g. (0.1 mole) of N-carbobenzoxy-L-serine with chloroacetic anhydride gave the theoretical amount (32 g.) of oily O-chloroacetyl-N-carbobenzoxy-L-serine. Treatment of this oil with sodium azide in the usual manner produced the azido compound also as a crude oil (34 g.). Hydrogenation of this material in the manner described above led to an oily O-glycyl-L-serine monohydrochloride which was not crystallized but was diazotized directly.

Diazotization.—The crude O-glycyl-L-serine monohydrochloride from above (21 g.) was dissolved in 500 ml. of water and the *p*H of the solution was adjusted to 3.5–4 by the addition of a 5% sodium bicarbonate solution. To the aqueous solution was added 10.4 g. (0.15 mole) of sodium nitrite in a small volume of water. The *p*H of the solution shifted to 4.7–4.8 and remained there throughout the diazotization. The reaction was allowed to proceed at 25° until the ultraviolet absorption at λ250 mμ ceased to increase (4–5 hours). The solution was shell-frozen and lyophilized leaving a yellow powder, 34 g., $E^{1\%}_{1\ cm.}$ 195 at $\lambda^{H_2O}_{max}$ 250 mμ.

The 34 g. of solid was dissolved in 300 ml. of water and the solution was passed through a Darco G-60 (300 g.)–Celite 545 (300 g.) column having a holdup volume of 2 l. The column was washed with 3 l. of water and was eluted with water containing 2% acetone. After 2.5 l. of eluate had been collected, a strong ultraviolet absorption and a positive ninhydrin reaction was obtained. A 1-l. fraction was collected and evaporated to a small volume at 40°, finally freeze-drying to give 3.6 g. of yellow solid, $E^{1\%}_{1\ cm}$ 995 at $\lambda^{H_2O}_{max}$ 250 mμ. The solid was recrystallized from water–ethanol to yield 1.7 g. of azaserine, $E^{1\%}_{1\ cm}$ 1145, identical with the product from fermentation and showing complete activity in the *Kloeckera brevis* assay. The over-all yield from N-carbobenzoxy-L-serine was 9%.

O-(N-Carbobenzoxyglycyl)-N-carbobenzoxy-L-serine (II).—To a stirred solution of 44 g. (0.21 mole) of carbobenzoxyglycine and 21 g. (0.21 mole) of triethylamine in 300 ml. of methylene chloride cooled to −5° in an ice–salt-bath was added dropwise over a period of one-half hour a solution of 25 g. (0.21 mole) of isovaleryl chloride in 50 ml. of methylene chloride. The solution was stirred at 0 to −5° for 2 hours during which time triethylamine hydrochloride precipitated. To this mixture was added in one portion a cold (0°) solution of 50 g. (0.21 mole) of N-carbobenzoxy-L-serine and 21 g. of triethylamine in 200 ml. of methylene chloride. The solution was stirred at 0° for 8 hours and then allowed to stand overnight at 25°. To this solution was added 21 g. (0.21 mole) of triethylamine and the solution was washed with two 200-ml. portions of water. The methylene chloride solution was evaporated to an oil which was dissolved in 200 ml. of ethyl acetate. The ethyl acetate solution was extracted with three 150-ml. portions of water. The combined aqueous extracts were acidified with 2 *N* hydrochloric acid to congo red paper and the colorless oil which precipitated was extracted with three 150-ml. portions of ethyl acetate. The ethyl acetate solution was dried and the solvent evaporated to yield 86 g. (96%) of a colorless oil. This bis-carbobenzoxy compound II has not been obtained crystalline.

O-(N-Carbobenzoxyglycyl)-N-carbobenzoxy-DL-serine (II).—This compound, prepared in the same manner as the L-compound above was obtained as a colorless oil in 95% yield.

O-Glycyl-L-serine Monohydrochloride (III).—To 5 g. of 5% palladium on carbon catalyst was added 180 ml. of 1 *N* hydrochloric acid followed by a solution of 86 g. (0.20 mole) of O-(N-carbobenzoxyglycyl)-N-carbobenzoxy-L-serine in 250 ml. of absolute ethanol. The solution was hydrogenated for 3.5 hours at 50 lb. pressure. The bottle was vented every one-half hour and filled with fresh hydrogen, and at the end of 1.5 hours 5 g. of fresh catalyst (first wetted with water) was added. The catalyst was removed and the filtrate was concentrated under reduced pressure to 50–75 ml. Absolute ethanol was added until the solution became turbid and a small amount of oil separated. The oil was redissolved by warming the solution to 40–50°, and on standing overnight a white solid formed. Absolute ethanol was added in small portions over a period of several hours until no more solid precipitated. The O-glycyl-L-serine monohydrochloride was obtained as white platelets, yield 25 g. (60% from N-carbobenzoxy-L-serine), m.p. 163° dec., $[\alpha]^{26}D$ +11.8° (*c* 5 in water).

O-Glycyl-DL-serine Monohydrochloride.—This substance was prepared from the oily O-(N-carbobenzoxyglycyl)-N-carbobenzoxy-DL-serine in a reduction as described above in comparable yield, m.p. 168–170° dec.

Diazotization of the L-material and purification of the product over a carbon column as described in the previous paper and above, gave a 16% yield of azaserine, m.p. 157° dec., $E^{1\%}_{1\ cm}$ 1140 at $\lambda^{H_2O}_{max}$ 250 mμ.

N-Carbobenzoxy-D-serine.—This compound was prepared from D-serine by the procedure described earlier[1] in 82% yield, m.p. 115–118°, $[\alpha]^{26.5}D$ −5.1° (*c* 4 in glacial acetic acid).

Anal. Calcd. for $C_{11}H_{13}O_5N$: C, 55.22; H, 5.48; N, 5.86. Found: C, 55.01; H, 5.50; N, 5.80.

O-(N-Carbobenzoxyglycyl)-N-carbobenzoxy-D-serine.—This compound was prepared in an identical manner as its L-enantiomorph from 75 g. of N-carbobenzoxy-D-serine to yield 140 g. (theoretical) of a viscous oil.

O-Glycyl-D-serine Monohydrochloride.—To a solution of 140 g. of the oil from the previous reaction in 1500 ml. of ethanol was added 118 ml. of 2 *N* hydrochloric acid. The resulting solution was hydrogenated at 3 atm. pressure over 10 g. of 5% palladium on carbon catalyst for 4 hours. The apparatus was flushed out with fresh hydrogen at one-half hour intervals to remove accumulated carbon dioxide and nitrogen. After removal of the catalyst by filtration, the solution was concentrated *in vacuo* to a small volume. The product was crystallized by the slow addition of ethanol to the concentrated aqueous solution, affording 33 g. (53%), m.p. 139–140° dec.

Recrystallization from water–ethanol gave 19 g. (30%), m.p. 160–162° dec., $[\alpha]^{26.5}D$ −8.3° (*c* 1.8 in water). The infrared spectrum was identical with that of O-glycyl-L-serine monohydrochloride.[1]

Anal. Calcd. for $C_5H_{12}O_4N_2Cl$: C, 30.23; H, 5.58; N, 14.11. Found: C, 30.52; H, 5.68; N, 14.11.

O-Diazoacetyl-D-serine.—To a solution of 15 g. (0.075 mole) of O-glycyl-D-serine monohydrochloride in 1500 ml. of water and ice was added 13.1 g. (0.14 mole) of sodium nitrite. The resulting solution was allowed to stand overnight at 25°, *p*H 4.7–5.2. The solution was degassed under vacuum and put on a column consisting of 100 g. of Darco G-60 and 100 g. of Celite 545. The column was washed with 2 l. of water and eluted with water containing 2% acetone and the eluate, having a yellow color and positive ninhydrin, was collected (500 ml.), shell-frozen and lyophilized. This gave a yellow solid, 2.6 g., $E^{1\%}_{1\ cm}$ 1124 at $\lambda^{H_2O}_{max}$ 250 mμ. It was recrystallized from water–ethanol yielding yellow crystals, 1.7 g. (13%), m.p. 153–155° dec., $[\alpha]^{26}D$ +0.4° (*c* 5.57 in water), $E^{1\%}_{1\ cm}$ 1140 at $\lambda^{H_2O}_{max}$ 250 mμ. The infrared spectrum was identical with that of azaserine.[1]

Anal. Calcd. for $C_5H_7O_4N_3$: C, 34.69; H, 4.08; N, 24.27. Found: C, 34.94; H, 4.34; N, 24.42.

DETROIT 32, MICHIGAN

[CONTRIBUTION FROM WALLACE LABORATORIES, DIVISION OF CARTER PRODUCTS, INC.]

Aminomethyloxazolidones Derived from Substituted Diamino-2-propanols

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A series of substituted 1,3-diamino-2-propanols and the aminomethyloxazolidones obtained from them have been prepared for pharmacological evaluation. The methods of preparation, evidence for the chemical structure of the oxazolidones and the physical properties of the compounds are described.

Pharmacological evaluation of compounds structurally related to the muscle paralyzing drug mephenesin (3-*o*-toloxy-1,2-propanediol) has revealed that substitution of the ether oxygen with an –NH– linkage gives a compound completely devoid of paralyzing action but possessing moderate convulsant activity.[1] Extension of this modification to substituted diamino-2-propanols has led to the synthesis of a group of compounds possessing striking convulsant properties.[2]

This paper describes the synthesis and physical properties of a number of substituted 1,3-diamino-2-propanols which have been prepared for pharmacological evaluation. It also describes the preparation, properties and structural proof of some substituted 5-aminomethyl-2-oxazolidones obtained by ethyl carbonate cyclization of these diaminopropanols. The oxazolidones were of interest because of the favorable enhancement of pharmacological activity observed earlier on carbamylation of certain anticonvulsant and muscle paralyzing propanediols and toloxypropanols.[3]

The symmetrically substituted 1,3-diamino-2-propanols were prepared by condensation of epichlorohydrin with an excess of the appropriate primary or secondary amine. The unsymmetrical members were obtained by stepwise amination of epichlorhydrin, usually without isolation of the intermediate 1-amino-3-chloro-2-propanol. Conversion of the diaminopropanols to the cyclic carbamates was accomplished by distilling a mixture of the compound and an excess of ethyl carbonate with a catalytic amount of sodium methylate until the theoretical volume of ethanol had been removed.

Cyclization of the symmetrically monosubstituted diaminopropanols in this manner leads to the formation of a single oxazolidone (I, R = R′). However, the unsymmetrical monosubstituted diaminopropanols under the same conditions are theoretically convertible to two isomeric oxazolidones, I and II. The high yield and relative homogeneity of the product obtained from the condensation of ethyl carbonate and alkylaminoarylaminopropanols definitely indicated the formation of only one isomer. Proof for the identity of this isomer was obtained from another set of reactions.

The product obtained from the reaction of ethyl carbonate and 1-anilino-3-*n*-butylamino-2-propanol was *n*-butylated to give either III or IV, where R = *n*-butyl and R′ = phenyl. The butylated adduct was compared to samples of III and IV prepared by the unambiguous condensation of ethyl carbonate with 1-*n*-butylamino-3-N-*n*-butylanilino-2-propanol and 1-anilino-3-di-*n*-butylamino-2-propanol, respectively, and was found to be identical with III. Also, on acid hydrolysis, the butylated product yielded 1-*n*-butylamino-3-N-*n*-butylanilino-2-propanol rather than the isomeric compound 1-anilino-3-di-*n*-butylamino-2-propanol which would result from the hydrolysis of IV. In the condensation of ethyl carbonate with the trifunctional diaminopropanol, cyclization occurs exclusively through the hydroxyl group and the more basic alkylamino group. It is probable that the cyclization of unsymmetrical bis-(alkylamino)- or bis-(arylamino)-propanols, where little if any difference in base strength existed, would lead to a mixture of the two possible isomers.

It is of interest that no evidence was found to indicate the formation of a cyclic ureide through condensation of ethyl carbonate with both amino groups. This condensation would lead to the formation of the 1,3-disubstituted-4-hydroxytetrahydro-2-pyrimidone (V).

The substituted diaminopropanols prepared in this study are low melting crystalline solids. Except for the two lowest members of the series, these compounds are relatively insoluble in water. They are readily convertible to their soluble hydrochloride salts. The substituted aminomethyloxazolidones also possess limited solubility in water, and are readily cleaved by strong acid or alkali. The strik-

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