mately optimum as indicated by paper chromatographic studies. The structure of III was confirmed by its hydrogenolysis to D-serine and its conversion to an isopropylidene derivative.

The antibacterial activity of III was considerably lower than that of cycloserine.

Experimental

All melting points were taken on a Kofler micro hot stage. Paper chromatography data is reported using the system previously described.[6] Radial chromatograms using the methyl ethyl ketone–pyridine–water system (MPW, 20:5:8) were run and the spots were detected with ninhydrin (N) reagent.

β-Aminoxy-D-alanine Methyl Ester Dihydrochloride (II).—Hydrogen chloride was passed for 20 min. into 300 ml. of methanol contained in a 500-ml. round-bottomed flask immersed in an ice bath. To the resulting solution was added 25 g. of cycloserine and the solution was refluxed 3 hr. After standing overnight at room temperature, the solution was poured slowly into 1500 ml. of ethyl acetate. The precipitated oil crystallized when the mixture was cooled in an ice bath for about 1 hr. The solid was collected on a filter and washed with ethyl acetate and ether. The crude β-aminoxy-D-alanine methyl ester dihydrochloride, which weighed 39.9 g., was dissolved in 150 ml. of hot methanol. The solution was filtered and 200 ml. of ethyl acetate was added to the filtrate. The mixture was placed in the refrigerator for several hours. Twenty-nine grams (52%) of β-aminoxy-D-alanine methyl ester dihydrochloride, m.p. 135–139°, $[\alpha]^{25}D$ −11.7° (*c*, 2 in 1 *N* HCl), was obtained.

Anal. Calcd. for $C_4H_{12}N_2O_3Cl_2$: C, 23.2; H, 5.84; N, 13.5; Cl^-, 34.3. Found: C, 23.4; H, 5.71; N, 13.7; Cl^-, 34.3.

β-Aminoxy-D-alanine Monohydrochloride (III). (a) **By Ester Hydrolysis.**—A solution of 24 g. of β-aminoxy-D-alanine methyl ester dihydrochloride in 120 ml. of 6 *N* hydrochloric acid was heated at 85–90° in an oil bath for 4 hr. The solution was then evaporated *in vacuo*. The residue was dissolved in absolute ethanol and evaporated to remove residual hydrochloric acid. This operation was repeated twice. After 4 hr. at *ca.* 0.1 mm., the residue was dissolved in 100 ml. of boiling absolute ethanol, and the solution was cooled to room temperature. Ten milliliters of pyridine was added slowly to the stirred solution. The precipitated product, which weighed 24.3 g., was collected on a filter, washed with absolute ethanol, and dried. Elemental analysis of this material, m.p. 134–136°, R_f^{MPW} 0.74 (N), indicated it to be a mixture of pyridine hydrochloride and the desired aminoxy compound.

A solution of 23.8 g. of this mixture in 50 ml. of water (pH 2) was filtered and then diluted slowly with 125 ml. of absolute ethanol. After 6 hr. at 5°, the solution was filtered giving 12.5 g. (68%) of β-aminoxy-D-alanine monohydrochloride, m.p. 144–145°. The analytical sample, m.p. 146–148°, $[\alpha]^{25}D$ −19.5° (*c*, 2 in 1 *N* HCl), R_f^{MPW} 0.78 (N), was dried at 52° for 5 hr. *in vacuo*.

Anal. Calcd. for $C_3H_9N_2O_3Cl$: C, 23.0; H, 5.79; N, 17.9, Cl, 22.7. Found: C, 23.5; H, 5.35; N, 17.3; Cl, 23.7.

(b) **By Cycloserine Hydrolysis.**—A solution of 20 g. of D-cycloserine in 100 ml. of 6 *N* hydrochloric acid was heated at 60° in an oil bath for 3 hr. The solution was evaporated to dryness *in vacuo*. The residue was twice dissolved in absolute ethanol and evaporated to dryness to remove residual hydrochloric acid. After 3 hr. *in vacuo*, the residue was dissolved in 150 ml. of boiling absolute ethanol, and the solution was allowed to cool to room temperature. Twelve milliliters of pyridine was added dropwise to the stirred solution. The pink precipitate was collected on a filter, washed with ethanol, and dried. The product, which weighed 32.9 g., m.p. 124–128° dec., was apparently the same pyridine hydrochloride–aminoxyalanine complex isolated in part a. This material was dissolved in 60 ml. of water and, after filtration, the solution was slowly (2 hr.) diluted with 150 ml. of absolute ethanol. After standing at 5° overnight, the β-aminoxy-D-alanine monohydrochloride, 12.5 g. (41%), m.p. 143–145°, $[\alpha]^{25}D$ −18.3° (*c*, 2.1 in 1 *N* HCl), was collected on a filter and dried. Its paper chromatographic behavior and infrared spectrum were the same as that of the product obtained in part a.

β-Aminoxy-D-alanine.—A solution of 500 mg. of β-aminoxy-D-alanine monohydrochloride in 3.5 ml. of 3 *N* ammonium hydroxide was cooled in an ice bath and 10 ml. of a 1:1 2-propanol–ethanol mixture was added. The dropwise addition of acetic acid to the solution caused crystallization of the zwitter ion. Another 10 ml. of the 2-propanol–ethanol solution was added and the mixture stood at 5° for 1 hr. The β-aminoxy-D-alanine, m.p. 166–168° dec., $[\alpha]^{25}D$ −24.4° (*c*, 2.13 in 1 *N* HCl), weighed 296 mg. (77%). A sample was dried for analysis at 52° *in vacuo*.

Anal. Calcd. for $C_3H_8N_2O_3$: C, 30.0; H, 6.71; N, 23.3. Found: C, 30.2; H, 6.48; N, 23.2.

Hydrogenation of β-Aminoxy-D-alanine Monohydrochloride.—A solution of 250 mg. of β-aminoxy-D-alanine monohydrochloride in 10 ml. of water was shaken under hydrogen for 16 hr. at 40 p.s.i. and room temperature using 0.25 g. of platinum oxide as catalyst. After removal of the catalyst, the solution was evaporated to a 1-ml. volume and diluted slowly to 5 ml. with absolute ethanol. The crystalline precipitate weighed 145 mg., R_f^{MPW} 0.46 (N); $[\alpha]^{25}D$ −13.2° (*c*, 5 in 1 *N* HCl). L-Serine, R_f^{MPW} 0.46 (N), $[\alpha]^{25}D$ +14.45° (*c*, 8.9 in 1 *N* HCl).

Reaction of Acetone with β-Aminoxy-D-alanine Monohydrochloride.—A solution of 500 mg. of β-aminoxy-D-alanine monohydrochloride in 50 ml. of boiling acetone containing 1 ml. of water was refluxed 4 hr. and evaporated to dryness. The residue was dissolved in a few drops of water and acetone was added to a total volume of 10 ml. After 16 hr. in the refrigerator, the solution yielded 265 mg. of crystalline β-(isopropylideneaminoxy)-D-alanine; m.p. 160–162° dec., R_f^{MPW} 0.69 (N), $[\alpha]^{25}D$ +4.1° (*c*, 2.2 in 1 *N* HCl). A sample was dried for analysis at 52°, *in vacuo* for 2 hr.

Anal. Calcd. for $C_6H_{13}N_2O_3Cl$: C, 36.6; H, 6.66; N, 14.3; Cl^-, 18.0. Found: C, 37.0; H, 6.76; N, 14.1; Cl^-, 18.4.

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N-Carbobenzoxyamino Acyl Derivatives of D-Glucosamine[1,2]

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Prior to our attempt to synthesize "model glycoproteins," we carried out a preliminary study of

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the behavior of simple systems containing *N*-carbobenzoxyamino acids and D-glucosamine. The first *N*-carbobenzoxyamino acyl derivatives of D-glucosamine were prepared by Bergman and Zervas[4] by treating the *N*-carbobenzoxyamino acid chloride with the tetra-*O*-acetyl derivative of D-glucosamine. This procedure cannot readily be applied to the coupling of proteins with polysaccharides and therefore, a new procedure had to be employed. Sheehan and Hess[5] had shown that when *N,N'*-dicyclohexylcarbodiimide was employed as the condensing agent for peptide synthesis, it was not necessary to protect hydroxyl groups. Glycopeptides have been synthesized by treating an aldonic acid with the ethyl esters of amino acids using *N,N'*-dicyclohexylcarbodiimide as the condensing agent.[6] If no free amino group is present, esterification of the primary hydroxyl of glucose with an *N*-carbobenzoxy amino acid was shown to occur in the presence of *N,N'*-dicyclohexylcarbodiimide.[7]

In the present work, in addition to carbobenzoxyglycine, two *N*-carbobenzoxyamino acids with different types of —OH groups were chosen as reactants. In both instances, esterification was not shown to occur in the condensation reaction. The product gave a positive Morgan-Elson reaction, indicative of an *N*-acyl hexosamine. The purple color produced on the chromatogram faded within a few minutes. The possibility of *O*-glycoside formation was eliminated by the positive test for reducing sugars and the *N*-acylation of glucosamine was further indicated by the absence of ninhydrin-positive material in the isolated product.

Experimental[8]

Capillary melting points were determined for all compounds and are corrected.

Chromatographic analyses were performed on Whatman No. 1 paper using a butanol–acetic acid–water (4:1:5, v./v.) solvent system. The acylated amino sugars were detected by the use of a modified Morgan-Elson procedure[9] and by alkaline permanganate containing sodium metaperiodate.[10] The acylated glucosamine derivatives were chromatographically pure. When the glucosamine derivatives were hydrolyzed and the hydrolyzates chromatographed, only the constituent amino acid and the amino sugar could be detected by ninhydrin.

N-(**Carbobenzoxyglycyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to a solution composed of D-glucosamine (free base,[11] 0.01 mole, 1.79 g.) and carbobenzoxyglycine[12] (0.01 mole, 2.09 g.) dissolved in 50 ml. of 25% aqueous ethanol and then stirred overnight at room temperature. The insoluble dicyclohexylurea was removed by filtration and the ethanol was removed at 60° *in vacuo*. The aqueous residue was filtered and the filtrate was evaporated *in vacuo* over potassium hydroxide and phosphorus pentoxide. The residue was extracted with boiling acetone, the solution filtered while hot, and the filtrate was evaporated to dryness. The solid was crystallized from absolute ethanol; yield 1.97 g. (53%); m.p. 178–178.5°; $[\alpha]^{25}D$ +50 (*c* 0.20, methanol); $[\alpha]^{25}D$ +25.0° (*c* 0.23, water, final) downward mutarotation, R_f = 0.60 (lit.,[4] m.p. 181; $[\alpha]^{20}D$ +40.0° (methanol).

Anal. Calcd. for $C_{16}H_{22}O_8N_2$: C, 51.9; H, 6.0; N, 7.6. Found: C, 51.4; H, 6.3; N, 7.2.

N-(*N'*-**Carbobenzoxy**-L-**tyrosyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to 50 ml. of 25% aqueous ethanol containing D-glucosamine (0.01 mole, 1.79 g.) and *N*-carbobenzoxy-L-tyrosine.[13] The solution was stirred overnight and then filtered. The filtrate was evaporated *in vacuo* and the residue was washed with 50 ml. of boiling water and then with 50 ml. of boiling ethyl acetate. The solid residue was crystallized from absolute ethanol; yield 2.4 g. (48%); m.p. 189–190°; $[\alpha]^{26}D$ +26.6° (*c* 0.21, ethanol), R_f = 0.75.

Anal. Calcd. for $C_{23}H_{28}O_9N_2 \cdot H_2O$: C, 55.9; H, 6.1; N, 5.7. Found: C, 56.1; H, 5.6; N, 5.6.

N-(*N'*-**Carbobenzoxyhydroxy**-L-**prolyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to 50 ml. of 25% aqueous ethanol containing D-glucosamine (free base, 0.01 mole, 1.79 g.) and *N*-carbobenzoxyhydroxy-L-proline[14] (0.01 mole, 2.65 g.) and the solution stirred at room temperature overnight. The remainder of the procedure was similar to that described for the preparation of *N*-(*N'*-carbobenzoxyglycyl)-D-glucosamine. Yield 2.04 g. (46%); m.p. 155–156°; $[\alpha]^{31}D$ −13.5° (*c* 0.25, water, final) downward mutarotation, R_f = 0.56.

Anal. Calcd. for $C_{19}H_{26}O_9N_2 \cdot H_2O$: C, 51.4; H, 6.3; N, 6.3. Found: C, 51.8; H, 6.2; N, 6.4.

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A New Synthesis of *threo*-β-Methylaspartic Acid

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For a study of the stereospecificity of a certain bacterial deaminase,[1] we required a sample of β-methylaspartic acid, preferably the *erythro* isomer. Although syntheses of the amino acid were reported in the literature,[2] the methods seemed

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