mass extracted with benzene–pentane (3 × 100 ml. of 1:2 v./v.). The mixture was dissolved in 100 ml. of water and the resulting solution extracted thrice with 1:2 benzene–pentane to remove remaining traces of acid. The aqueous solution was then extracted continuously with warm ether until no further material was removed. On concentration, the ethereal extract afforded crude crystalline product (2.6 g., 13%). Recrystallization from either ethyl acetate, acetone or 1,2-dimethoxyethane gave, with little loss, pure material melting at 165–166° and rotating $[\alpha]^{20}D$ +72.4° (H_2O, *c* 0.5), no mutarotation being observed within 1 hr. Rotations in other solvents were observed as follows: 0.01 *N* HCl, $[\alpha]^{20}D$ +72.5° (*c* 0.5); methanol, $[\alpha]^{20}D$ +74° (*c* 0.49); pyridine, $[\alpha]^{20}D$ +53° (*c* 1.1).

Anal. Calcd. for $C_{13}H_{16}O_7$: C, 54.93; H, 5.67. Found: C, 54.84; H, 5.78.

A sample (36.5 mg.) of the substance was treated with lead tetraacetate in glacial acetic acid as described by Hockett and McClenahan.[20] After 355, 1340 and 1867 minutes, analyses indicated the consumption of 1.98, 2.09 and 2.07 molar equivalents of oxidant.

D-Talose from 1-*O*-Benzoyl-α-D-talopyranose (III).—A sample (0.1616 g.) of the ester was dissolved in absolute methanol to make a total volume of 10 ml. and the rotations observed in a 1.5-dm. tube. Over a period of 8 min. at 20° no change was observed, the rotation corresponding to $[\alpha]^{20}D$ +72.8°. Four drops of 1.3 *N* barium methoxide was then added. After 43 min. the rotation had become constant at a value of +0.34°, corresponding to $[\alpha]^{20}D$ +22.7°, based on the theoretical yield of hexose. Barium ions were removed with Amberlite IR-120 and the solution concentrated *in vacuo* to a sirup. From ethanol the handsome prisms characteristic of D-talose were deposited: 0.065 g. (64%), m.p. 130–131°, $[\alpha]^{20}D$ +20.1° (H_2O, *c* 1.2). Isbell and Pigman[21] recorded m.p. 133–134° and $[\alpha]^{20}D$ +20.8° (H_2O, *c* 4) for pure D-Talose.

2,3,4,6-Tetra-*O*-acetyl-1-*O*-benzoyl-α-D-talose (V). (a) **From 1-*O*-Benzoyl-α-D-talopyranose (III).**—To a mixture of 2 ml. of pyridine and 10 ml. of acetic anhydride cooled to 0° was added 1.0 g. of 1-*O*-benzoyl-α-D-talopyranose. After 3 days at 0° the reaction mixture was poured into cold water and worked up in the usual fashion. The sirup (1.41 g., 89%) which resisted all attempts at crystallization was dissolved in 4:1 hexane–benzene and adsorbed on a column of 56 g. of alumina.[22] Successive elution with 4:1 hexane–benzene, benzene and 1:1 benzene–ether eluted no material from the adsorbent. With ether a well-defined "peak" was eluted; several of the amorphous fractions before and at the top of the peak showed $[\alpha]^{20}D$ +84° in chloroform (*c* 0.3).

Anal. Calcd. for $C_{21}H_{24}O_{11}$: C, 55.75; H, 5.35. Found: C, 56.18; H, 5.51.

(b) **From 2,3,4,6-Tetra-*O*-acetyl-α-D-talosyl Bromide (IV).**—A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-talosyl bromide (6.5 g., m.p. 83–84°[23]), silver benzoate (10 g.) and benzene (50 ml.) was stirred at room temperature for 1 hr. and then filtered. Removal of solvent from the filtrate gave a sirup (6.5 g.) which could not be induced to crystallize. The sirupy product was, therefore, chromatographed on alumina as described in part (a) above. Elution with ether afforded a number of fractions showing $[\alpha]^{20}D$ +83° in chloroform; these were combined and rechromatographed to give a clear, colorless sirup showing $[\alpha]^{20}D$ +83° in chloroform (*c* 0.66).

Anal. Calcd. for $C_{21}H_{24}O_{11}$: C, 55.75; H, 5.35. Found: C, 55.96; H, 5.54.

Infrared absorption spectra of solutions of the products from (a) and (b) above appeared to be identical in all respects.

Acknowledgments.—We wish to express our indebtedness to Dr. H. S. Isbell for his helpful cooperation and interest in this research. We also wish to thank Mr. H. W. Diehl for the preparation of a quantity of D-galactal. Analytical determinations and absorption spectra measurements were carried out in the Institutes' microanalytical laboratory under the direction of Dr. W. C. Alford.

(20) R. C. Hockett and W. S. McClenahan, THIS JOURNAL, **61**, 1667 (1939).

(21) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

(22) "Alcoa" alumina, Grade F-20, mesh 80–200 manufactured by the Aluminum Company of America, Pittsburgh, Pa., was washed thoroughly with 5% hydrochloric acid and then with water until neutral. After activation at 200° the material was deliberately exposed to atmospheric humidity before use. The adsorptive capacity of alumina thus treated is inferior to that which has been kept under rigorously anhydrous conditions but its reproducibility is superior and the use of such partially hydrated alumina has the marked advantage of obviating the use of specially dried eluents.

(23) Pigman and Isbell (ref. 1) reported m.p. 84–84.5° for this substance.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC.]

Synthesis of Cycloserine and a Methyl Analog

BY CHARLES H. STAMMER, ANDREW N. WILSON, CLAUDE F. SPENCER, FRANK W. BACHELOR, FREDERICK W. HOLLY AND KARL FOLKERS

RECEIVED DECEMBER 29, 1956

DL-4-Carbomethoxy-2-phenyl-2-oxazoline (V) was converted into the corresponding hydroxamic acid VI. Dry hydrogen chloride in dioxane opened the oxazoline ring to a β-chloropropionohydroxamic acid which in aqueous alkali formed DL-4-benzamido-3-isoxazolidone (VIII). Treatment of VIII with ethanolic hydrogen chloride gave the aminoxy ester IX which cyclized in potassium hydroxide solution to DL-4-amino-3-isoxazolidone (X) (DL-cycloserine). The L-form of VIII was synthesized by the same series of reactions from L-serine. Both the benzamido (VIII) compound and DL-cycloserine were resolved. DL-4-Amino-5-methyl-3-isoxazolidone (XII) was synthesized from DL-threonine by the same series of reactions.

Cycloserine,[1] a broad-spectrum antibiotic, has been shown to be D-4-amino-3-isoxazolidone.[2,3] Preliminary reports of syntheses[3,4] of the racemate DL-4-amino-3-isoxazolidone (X) and a resolution[4] of the racemate have been published. The details of our synthesis and resolution together with the synthesis of an analog, DL-4-amino-5-methyl-3-isoxazolidone (XII), are described in this paper.

Since 3-isoxazolidone (I) had been synthesized[5]

(1) Cycloserine has been accepted as the generic name for this antibiotic. Oxamycin is now the trademark of Merck & Co., Inc., for cycloserine.

(2) F. A. Kuehl, F. J. Wolf, N. R. Trenner, R. L. Peck, E. Howe, B. D. Hunnewell, G. Downing, E. Newstead, R. P. Buhs, I. Putter, R. Ormond, J. E. Lyons, L. Chaiet and K. Folkers, THIS JOURNAL, **77**, 2344 (1955).

(3) P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz and H. R. Sullivan, *ibid.*, **77**, 2345 (1955).

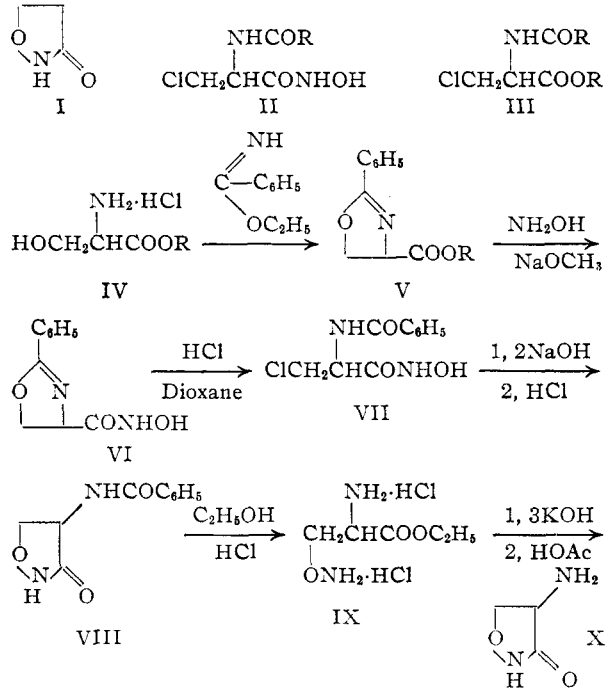
(4) C. H. Stammer, A. N. Wilson, F. W. Holly and K. Folkers, *ibid.*, **77**, 2346 (1955).

(5) C. H. Shunk, F. W. Bachelor and K. Folkers, *J. Org. Chem.*, **22**, in press (1957).

by cyclization of β-chloropropionohydroxamic acid, a key intermediate for synthesis of cycloserine appeared to be a β-chloro-α-acylaminopropionohydroxamic acid (II). The preparation of such a hydroxamic acid from the corresponding ester III was complicated by side reactions occurring in the necessarily alkaline reaction medium. Consequently, a structure was needed which was stable to alkali and convertible to the β-chloro-α-acylaminopropionohydroxamic acid. An oxazoline fulfils both of these requirements and was used in this synthesis.

DL-Serine methyl ester hydrochloride (IV, R = CH_3) was converted into DL-4-carbomethoxy-2-phenyl-2-oxazoline (V, R = CH_3) by the procedure of Elliott.[6] This ester yielded 4-carbohydroxamido-2-phenyl-2-oxazoline (VI) when treated with one equivalent each of hydroxylamine and sodium methoxide in methanol at room temperature. When the hydroxamic acid VI was heated on a steam-bath for 15 minutes in dioxane containing one equivalent of hydrogen chloride, α-benzamido-β-chloropropionohydroxamic acid (VII) was formed.[7] The β-chloropropionohydroxamic acid (VII) was cyclized to 4-benzamido-3-isoxazolidone (VIII) by aqueous alkali.[8]

Removal of the benzoyl group from the benzamidoisoxazolidone VIII by either acid or alkaline hydrolysis was not feasible. The amide VIII is

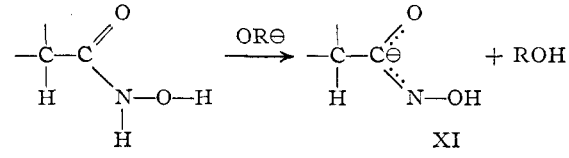


stable to boiling 2 *N* sodium hydroxide, while aqueous acids open the ring in addition to cleaving the amide. However, boiling ethanol saturated with hydrogen chloride removed the benzoyl group and also opened the isoxazolidone ring giving the hydrochloride of DL-β-aminoxyalanine ethyl ester (IX).[2] When this ester was treated with potassium hydroxide in aqueous solution, it cyclized rapidly to DL-cycloserine (X).

The resolution of DL-4-amino-3-isoxazolidone was accomplished with tartaric acid. Treatment of DL-cycloserine with D-tartaric acid gave D-4-amino-3-isoxazolidone-D-tartrate. This salt was converted by Amberlite IR-120 resin into D-4-amino-3-isoxazolidone, which was identical in all respects to cycloserine. Thus, the structure of cycloserine, deduced from degradation studies,[2,3] has been confirmed by synthesis. Using L-tartaric acid as resolving agent, we were able to prepare the unnatural L-cycloserine by the same procedure.

DL-4-Benzamido-3-isoxazolidone (VIII) was also resolved. This compound formed a crystalline cinchonidine metho-salt which on treatment with acid gave D-4-benzamido-3-isoxazolidone.

L-Serine ethyl ester hydrochloride (IV, R = C_2H_5) was prepared and converted into the optically active oxazoline ester (V, R = C_2H_5). When this ester was converted into the oxazoline hydroxamic acid VI by the procedure used on the racemic ester, about 50% racemization occurred. Variation of the reaction time, temperature or order of addition of the reactants to each other did not decrease the amount of racemization. It was interesting to note that the optically active hydroxamic acid VI did not racemize on standing in 0.1 *N* methanolic sodium methoxide overnight, but that the optically active ester V racemized so rapidly that its rotation in 0.1 *N* methanolic sodium methoxide when taken immediately was zero.[9] It is well known[10]



that optically active carboxylic acids racemize only with difficulty in alkali, whereas the corresponding esters racemize readily. Thus, the optical stability of the oxazoline hydroxamic acid in alkali may indicate that in the salt the negative charge resides next to the α-carbon (XI) just as the charge on a carboxylic acid salt does. This interpretation conflicts with the view[11] that hydroxamic acid salts have the charge on the hydroxyl oxygen.

The L-oxazoline hydroxamic acid VI was separated from its racemate by fractional crystallization. The 4-benzamido-3-isoxazolidone (VIII) formed from the L-hydroxamic acid VI by the reactions shown in the flow sheet was the optically pure L-4-benzamido-3-isoxazolidone. The conversion of this compound to L-cycloserine was not carried out,

(6) D. F. Elliott, *J. Chem. Soc.*, 589 (1949).

(7) Rearrangements of oxazoline hydrochlorides to β-haloalkylamides have been discussed by E. M. Fry, *J. Org. Chem.*, **14**, 887 (1949); **15**, 438, 802 (1950).

(8) There is a surprisingly large effect of temperature on the direction of the reaction between the β-chloropropionohydroxamic acid (VII) and aqueous alkali. At room temperature these reactants gave the oxazoline hydroxamic acid VI in 79% yield, but at 60–65° the isoxazolidone VIII was isolated in 82% yield. The oxazoline VI was not converted into VIII by aqueous alkali at 60–65°.

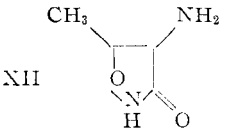
(9) In the light of our work, a report by Fry, *J. Org. Chem.*, **15**, 438 (1950), that the oxazoline ester (V, R = CH_3) is hydrolyzed in aqueous alkali to the corresponding acid *without racemization* was surprising. We confirmed this result. Apparently, in this case the rate of saponification is much greater than that of racemization.

(10) C. L. Bickel, THIS JOURNAL, **60**, 927 (1938).

(11) F. Mathis, *Bull. soc. chim. France*, D9 (1953).

because the resolution of DL-cycloserine had been accomplished previously.

DL-4-Amino-5-methyl-3-isoxazolidone (XII), an analog of cycloserine, was synthesized from DL-



XII

threonine by the reaction series already described. This compound has two asymmetric centers and thus may exist as two DL-pairs. Our synthesis gave only one DL-pair since DL-threonine was used as starting material.[12]

Acknowledgment.—We are indebted to Dr. N. R. Trenner and Mr. R. W. Walker for infrared analyses and to Mr. R. N. Boos and associates for microanalyses.

Experimental

All melting points were taken on a Kofler micro hot-stage.

DL-4-Carbomethoxy-2-phenyl-2-oxazoline (V).—This compound was prepared by allowing ethyl benzimidate[13] and serine methyl ester hydrochloride[6] to react according to the procedure of Elliott.[6] A 45% yield of the oxazoline ester, b.p. 118–122° (0.15 mm.), $n^{24}D$ 1.5480, was obtained.

DL-4-Carbohydroxamido-2-phenyl-2-oxazoline (VI).—To a cooled solution of 4 g. (0.06 mole) of hydroxylamine hydrochloride in 100 ml. of absolute ethanol was added 35 ml. of 1.82 *N* (0.06 mole) sodium methoxide in methanol. The mixture was cooled to 0°, the sodium chloride was filtered and a solution of 10 g. (0.05 mole) of 4-carbomethoxy-2-phenyl-2-oxazoline in 20 ml. of ethanol was added to the filtrate. This solution was cooled to 0°, and 25 ml. of 1.82 *N* (0.05 mole) sodium methoxide was added slowly. The reaction mixture was allowed to stand overnight. The solution was concentrated *in vacuo* at room temperature to about 75 ml. When about 100 ml. of water was added to the solution, rapid and exothermic crystallization of what was probably the sodium salt of the oxazoline hydroxamic acid occurred. Further addition of water caused the solid to redissolve. To this solution, 43.3 ml. of 1.16 *N* (51.5 mmoles) hydrochloric acid was added dropwise, and the product crystallized. The product was filtered and dried *in vacuo* yielding 8 g. (80%) of 4-carbohydroxamido-2-phenyl-2-oxazoline, m.p. 174–176°. A sample, recrystallized from ethanol, melted at 176–179° dec.

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.20; H, 4.88; N, 13.60. Found: C, 58.38; H, 5.05; N, 13.41.

Our subsequent work indicated that the reaction of the oxazoline ester V with hydroxylamine and sodium methoxide is complete after 15 minutes at 0°.

DL-α-Benzamido-β-chloropropionohydroxamic Acid (VII).—To a mixture of 4 g. (0.02 mole) of 4-carbohydroxamido-2-phenyl-2-oxazoline and 50 ml. of dry dioxane, was added 44 ml. of 0.46 *N* hydrogen chloride (0.02 mole) in dry dioxane, and the resulting mixture was heated on a steam-bath for 15 minutes. During the heating period, the solution became pink and then colorless, and the solid dissolved. The hot solution was filtered and evaporated *in vacuo* to a thick mass of crystals. These crystals were dissolved in approximately 25 ml. of hot isopropyl alcohol, and 175 ml. of low-boiling petroleum ether was added slowly to the solution. Crystallization occurred and, after two days at 5°, the DL-α-benzamido-β-chloropropionohydroxamic acid, 4.4 g., m.p. 148–152°, was filtered. A second crop, weighing 0.2 g., brought the total yield to 94%. A small sample, recrystallized from isopropyl alcohol–petroleum ether, melted at 153–155°.

Anal. Calcd. for $C_{10}H_{11}N_2O_3Cl$: C, 49.40; H, 4.56; N, 11.54; Cl, 14.60. Found: C, 48.94; H, 4.49; N, 11.77; Cl, 14.31.

DL-4-Benzamido-3-isoxazolidone (VIII).—To a solution of 4.0 g. (0.017 mole) of α-benzamido-β-chloropropionohydroxamic acid in about 30 ml. of hot methanol was added dropwise 31.5 ml. of 1.0 *N* sodium hydroxide (0.032 mole, 1.9 equivalents). When 16 ml. of the sodium hydroxide solution had been added, a phenolphthalein end-point was reached; the solution was heated to 60°, the remaining alkali was added and the end-point became permanent. To the cold basic solution, 4.2 ml. of 1.16 *N* hydrochloric acid (0.017 mole) was added, and the solution was immediately extracted with one 100-ml. portion and five 50-ml. portions of chloroform. The combined chloroform extracts were concentrated to about a 225-ml. volume, filtered and further concentrated to a 200-ml. volume. Crystallization began and, after the mixture was cooled, 2.1 g. of DL-4-benzamido-3-isoxazolidone, m.p. 167–170°, was filtered. A second crop weighed 0.5 g., m.p. 158–168° (total yield 75%). An analytical sample melting at 165–168° was obtained by recrystallization of the crude product from ethyl acetate.

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.24; H, 4.89; N, 13.59. Found: C, 58.45; H, 4.70; N, 13.37.

DL-β-Aminoxyalanine Ethyl Ester Dihydrochloride (IX).—A rapid stream of hydrogen chloride was passed for 3 hr. into 2500 ml. of absolute ethanol cooled in an ice-bath. To this solution was added 100 g. (0.48 mole) of DL-4-benzamido-3-isoxazolidone, and this solution was refluxed for 3 hr. After standing at 3–5° overnight, the solution was concentrated at reduced pressure giving 83 g. of DL-β-aminoxyalanine ethyl ester dihydrochloride, m.p. 128–135°. An additional 3.6 g. of product was obtained by concentrating the mother liquor to about a 300-ml. volume and diluting it with ethyl acetate. The yield was 87 g. (81%). A sample of the ester dihydrochloride, recrystallized twice from ethanol, melted at 123–130°.

Anal. Calcd. for $C_5H_{14}N_2O_3Cl_2$: C, 27.16; H, 6.38; N, 12.67; Cl, 32.07. Found: C, 28.09; H, 6.35; N, 12.63; Cl, 32.00.

DL-4-Amino-3-isoxazolidone (X).—A solution of 73.3 g. (0.33 mole) of DL-β-aminoxyalanine ethyl ester dihydrochloride in 100 ml. of water was stirred and cooled. During a 30-minute period, a solution of 65.6 g. (1.17 moles) of potassium hydroxide in 100 ml. of water was added. Between *p*H 7 and 10.5, a red color appeared in the reaction mixture; and when the solution reached *p*H 11–11.5, it had changed to a light yellow color. The solution was allowed to stand at room temperature for 0.5 hr. at this *p*H and then was added to 1800 ml. of 1:1 ethanol–isopropyl alcohol. The precipitated salts were filtered and the filtrate was cooled to 5°. To the cold well-stirred solution was added dropwise over a 35-minute period sufficient glacial acetic acid to bring the alcohol solution to *p*H 6.0. When the *p*H of the solution had reached 7–7.5, the solution was seeded; and no further acetic acid was added until crystallization of the oil had definitely begun. The crystalline precipitate was filtered, washed twice with 1:1 ethanol–isopropyl alcohol and twice with ether. The yield of DL-4-amino-3-isoxazolidone was 22.7 g. (65%), m.p. 140–143°.

The product was dissolved in 152 ml. of 7% ammonium hydroxide. To this solution was added 1215 ml. of 1:1 ethanol–isopropyl alcohol. This mixture was filtered and the filtrate was cooled to 5°; 21 ml. of glacial acetic acid was added dropwise while the solution was seeded and scratched. The final solution was at *p*H 6.0. The crystals were filtered and washed with ethanol and ether giving 17.7 g. (52%) of DL-cycloserine, m.p. 137–140°. The infrared spectrum showed absorption in the 6.1–6.6 μ region characteristic of the zwitterion form of cycloserine. The spectra of the racemic and natural compounds differed in the 10–12 μ region.

Anal. Calcd. for $C_3H_6N_2O_2$: C, 35.29; H, 5.90; N, 27.45. Found: C, 35.11; H, 5.70; N, 27.14.

Resolution of DL-4-Amino-3-isoxazolidone.—A solution of 7.5 g. of D-tartaric acid in 25 ml. of water was cooled to 3°. To this solution was added rapidly 5.0 g. (0.05 mole) of DL-cycloserine and the mixture was stirred vigorously. Crystallization began almost immediately. After ten minutes, the crystals were filtered and washed with 5 ml. of cold water and three 20-ml. portions of acetone yielding D-

(12) The oxazoline ring opening and isoxazolidone ring closure are most probably SN2 reactions, each of which inverts the β-carbon atom. Since the starting amino acid had the *threo* configuration, the aminoisoxazolidone XII should also have the *threo* configuration. The methyl and amino groups of XII will thus be *cis*.

(13) H. Gilman and A. H. Blatt, "Organic Syntheses," Coll. Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1941, p. 6

cycloserine-D-tartrate, 5.2 g. (84%), m.p. 166–168° dec., $[\alpha]^{26}D$ +35.7° (*c* 0.7 in H_2O).

The D-cycloserine-D-tartrate was dissolved in 100 ml. of water, and the solution was passed through a column of 200 g. of Amberlite IR-120 (Na^+ cycle). The column was washed free of sodium tartrate (eluate tested with lead acetate) with water, and D-cycloserine was eluted from the column with 2% ammonium hydroxide. The eluate was collected and lyophilized. The residue, 2.1 g., was dissolved in 14 ml. of 7% ammonium hydroxide, and 112 ml. of 1:1 ethanol–isopropyl alcohol was added. Super-Cel and Darco were added to the cloudy solution and the mixture was filtered. The clear filtrate was cooled to 5° and acetic acid was added dropwise to *p*H 6.0. The crystalline precipitate (1.1 g.) was filtered, washed with ethanol and ether and dried, m.p. 151–153° dec., $[\alpha]^{29}D$ +112° (*c* 1.0 in H_2O).

Recrystallization from 7 ml. of 7% ammonium hydroxide and 28 ml. of 1:1 ethanol–isopropyl alcohol in the manner described above gave 0.9 g. (39%) of D-cycloserine, m.p. 153–154° dec., $[\alpha]^{29}D$ +109° (*c* 1.0 in H_2O).

Anal. Calcd. for $C_3H_6N_2O_2$: C, 35.29; H, 5.92; N, 27.45. Found: C, 35.65; H, 5.78; N, 27.30.

The infrared spectrum of the synthetic D-cycloserine was identical with that of the natural compound.

L-Cycloserine-L-tartrate, $[\alpha]^{30}D$ −36.6° (*c* 0.79 in H_2O), was prepared as described above for the D-isomer. This tartrate was converted into L-cycloserine, $[\alpha]^{33}D$ −110° (*c* 0.83 in H_2O), as described above for the D-isomer.

Resolution of DL-4-Benzamido-3-isoxazolidone.—A solution of cinchonidine methohydroxide was prepared by dissolving 8.0 g. (18.4 mmoles) of cinchonidine methiodide in a few milliliters of water and shaking the solution for 4 hr. with 2.3 g. (10 mmoles) of silver oxide. Titration of an aliquot with 0.01 *N* hydrochloric acid showed that the solution contained 12.3 mmoles of the methohydroxide. To this solution was added 5.0 g. (24.3 mmoles) of DL-4-benzamido-3-isoxazolidone. The solution was kept overnight at room temperature. A small amount of undissolved isoxazolidone was filtered and the filtrate was evaporated *in vacuo* to a sirupy residue. The residue was dissolved in 500 ml. of hot methyl ethyl ketone, and the solution was cooled slowly to room temperature. The cinchonidine metho-salt of D-4-benzamido-3-isoxazolidone separated as small white needles. After two days at room temperature, the crystals were filtered, washed with ice-cold methyl ethyl ketone and dried *in vacuo* over phosphorus pentoxide yielding 2.0 g. of product, m.p. 192–195° dec., $[\alpha]^{25}D$ −80° (*c* 1.0 in H_2O).

A sample of this salt was recrystallized from water and dried at 100° (0.5 mm.).

Anal. Calcd. for $C_{30}H_{34}N_4O_4$: C, 70.01; H, 6.66; N, 10.89. Found: C, 69.82; H, 7.25; N, 10.81.

A sample of the cinchonidine metho-salt of D-4-benzamido-3-isoxazolidone, prepared from natural D-cycloserine, melted at 192–195° dec., $[\alpha]^{25}D$ −78° (*c* 0.9 in H_2O).

D-4-Benzamido-3-isoxazolidone.—A solution of 1.90 g. of the cinchonidine metho-salt of D-4-benzamido-3-isoxazolidone in 25 ml. of ice-water was acidified to *p*H 2 with concentrated hydrochloric acid. The solution was extracted with four 20-ml. portions of ether. The combined ether extracts were dried and evaporated *in vacuo*, giving a residue weighing 0.62 g. (86%). Crystallization from chloroform–petroleum ether gave 0.37 g. of D-4-benzamido-3-isoxazolidone, m.p. 172–175° dec., $[\alpha]^{25}D$ +16° (*c* 2.5 in pyridine). A sample, after recrystallization from water, melted at 173–175° dec.

Anal. Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.24; H, 4.89; N, 13.59. Found: C, 58.42; H, 5.11; N, 13.09.

D-4-Benzamido-3-isoxazolidone, prepared from natural D-cycloserine, melted at 174–176° dec., $[\alpha]^{25}D$ +17° (*c* 2.6 in pyridine). The infrared absorption spectrum of this compound was identical with that of the resolved product.

L-Serine Ethyl Ester Hydrochloride.—Dry hydrogen chloride was passed into a suspension of 95 g. of L-serine in 950 ml. of ethanol until the amino acid dissolved. About 200 ml. of ethanol was distilled from the solution and the residue was concentrated *in vacuo* to a thick sirup. When triturated with ether the product crystallized. It was filtered, washed with ether and dried, yielding 148 g. (97%) of L-serine ethyl ester hydrochloride, m.p. 126–127°, $[\alpha]^{25}D$ −5.5° (*c* 9.9 in 95% ethanol). After three recrystallizations from ethanol–ether, the product showed m.p. 130–131°, and $[\alpha]^{25}D$ −5.7° (*c* 10.2 in 95% ethanol).

Anal. Calcd. for $C_5H_{12}NO_3Cl$: C, 35.40; H, 7.13; N, 8.26. Found: C, 35.00; H, 6.96; N, 7.82.

L-4-Carbethoxy-2-phenyl-2-oxazoline.—This compound was prepared from L-serine ethyl ester hydrochloride and ethyl benzimidate in 68% yield by the procedure described for synthesis of the racemate. After distillation at 100–130° (1 mm.), the product crystallized, m.p. 42–47°. A small sample, recrystallized from ether–petroleum ether, melted at 47–48°, $[\alpha]^{25}D$ +134.5° (*c* 10.0 in 95% ethanol).

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 66.34; H, 5.14; N, 6.05.

A 2.5% solution of this ester in 0.2 *N* sodium methoxide showed no optical rotation when examined immediately.

L-4-Carboxy-2-phenyl-2-oxazoline.—To 5 ml. of 2 *N* sodium hydroxide was added 2.2 g. (0.01 mole) of L-4-carbethoxy-2-phenyl-2-oxazoline. The mixture became warm and was stirred intermittently for 3 hr. After dilution with about 20 ml. of water, 200 ml. of acetone was added slowly and the mixture was cooled in an ice-bath. The product was filtered and dried, giving 2.0 g. of L-4-carboxy-2-phenyl-2-oxazoline sodium salt, $[\alpha]^{27}D$ +82.4° (*c* 5.0 in H_2O).

A suspension of 1 g. of the sodium salt in 5 ml. of water was cooled and 4.4 ml. of 1 *N* hydrochloric acid was added. The precipitated acid was filtered and washed with cold water, giving 0.5 g. of L-4-carboxy-2-phenyl-2-oxazoline, m.p. 131–135°, $[\alpha]^{27}D$ +219° (*c* 1.0 in pyridine). Insoluble products were formed and the melting point was lowered when the acid was recrystallized from benzene. Recrystallization from a 1:2 mixture of ether–petroleum ether gave the acid, m.p. 135–138°, $[\alpha]^{27}D$ +200° (*c* 1.0 in pyridine).

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.81; H, 4.75; N, 7.33. Found: C, 62.68; H, 4.75; N, 7.29.

L-4-Carbohydroxamido-2-phenyl-2-oxazoline.—This compound was prepared in 81% yield from L-4-carbethoxy-2-phenyl-2-oxazoline essentially by the procedure used for the preparation of the racemic compound. However, the reaction mixture was allowed to stand at 0° overnight in order to lessen the amount of racemization. The product, m.p. 167–168°, had $[\alpha]^{25}D$ +65° (*c* 2.0 in 0.2 *N* sodium hydroxide).

One gram of this partially racemized hydroxamic acid was crystallized from 350 ml. of boiling 6:1 methyl isobutyl ketone–ethanol giving 0.3 g. of material, m.p. 180–181°, $[\alpha]^{27}D$ +2.4° (*c* 2.0 in 0.2 *N* sodium hydroxide). When the filtrate was cooled, 0.4 g. of a second crop was obtained, m.p. 179–180°, $[\alpha]^{29}D$ +97° (*c* 2.0 in 0.2 *N* sodium hydroxide). The filtrate from the second crop, when concentrated to 50 ml., yielded 0.15 g. of a third crop, m.p. 170–171°, $[\alpha]^{28}D$ +94.3° (*c* 2.0 in 0.2 *N* sodium hydroxide). The combined second and third crops were again recrystallized from 117 ml. of 6:1 methyl isobutyl ketone–ethanol giving 0.2 g. of product, m.p. 179–180°, $[\alpha]^{27}D$ +119.5° (*c* 2.0 in 0.2 *N* sodium hydroxide). Further recrystallization gave no increase in rotation.

L-4-Benzamido-3-isoxazolidone.—To a solution of 0.57 (2.8 mmoles) of L-4-carbohydroxamido-2-phenyl-2-oxazoline, $[\alpha]^{26}D$ +113° (*c* 2.0 in 0.2 *N* sodium hydroxide), in 20 ml. of dry dioxane was added 1.4 ml. of 2.2 *N* hydrogen chloride in dioxane. The solution was heated for 15 minutes on a steam-bath and was evaporated *in vacuo*. The residue was dissolved in 20 ml. of water, and the solution was heated at 55–65°, while 51.5 ml. of 0.1 *N* sodium hydroxide was added dropwise to a permanent phenolphthalein end-point. The solution was cooled to 10° and, after addition of 2.8 ml. of 1 *N* hydrochloric acid, was extracted with four 50-ml. portions of ethyl acetate. The extracts were dried and evaporated to 10 ml. This solution was cooled giving 0.24 g. of L-benzamido-3-isoxazolidone, m.p. 163–171°, $[\alpha]^{27}D$ −17.6° (*c* 2.5 in pyridine). The filtrate gave an additional 0.12 g., $[\alpha]^{27}D$ −17.5° (*c* 2.5 in pyridine), which brought the yield to 0.36 g. (63%).

DL-4-Carbohydroxamido-5-methyl-2-phenyl-2-oxazoline.—The procedure for the preparation of 4-carbohydroxamido-5-methyl-2-phenyl-2-oxazoline is similar to that described for the 4-carbohydroxamido-2-phenyl-2-oxazoline. From 187 g. of the oxazoline ester,[14] 149 g. (79%) of the oxazoline

(14) D. F. Elliott, *J. Chem. Soc.*, 62 (1950).

hydroxamic acid, m.p. 152–154°, was obtained. A sample, recrystallized from isopropyl alcohol, melted at 148–150°.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.73. Found: C, 59.98; H, 5.31; N, 12.91.

DL-α-Benzamido-β-chlorobutyrohydroxamic Acid.—To a stirred refluxing solution of 9.6 g. (0.044 mole) of 4-carbohydroxamido-5-methyl-2-phenyl-2-oxazoline in approximately 500 ml. of dry dioxane (distilled from sodium three times) was added dropwise over a period of 1 hr. 120 ml. of dioxane containing 1.8 g. (0.05 mole) of hydrogen chloride. During the addition of the hydrogen chloride solution, the reaction mixture became pink and a small quantity of a flocculent precipitate appeared. The solution was cooled, treated with Darco G-60 and filtered. The residue obtained from lyophilization of the filtrate was extracted with one 400-ml. and three 50-ml. portions of boiling ethyl acetate, and the combined extracts were diluted with an equal volume of chloroform. This solution was cooled and 6.6 g. (59%) of α-benzamido-β-chlorobutyrohydroxamic acid, m.p. 139–143°, was obtained. A sample, recrystallized from chloroform, melted at 141–142.5°. The ethyl acetate-insoluble portion of the residue was apparently unrearranged oxazoline hydrochloride and was not further investigated.

Anal. Calcd. for $C_{11}H_{13}N_2O_3Cl$: C, 51.47; H, 5.10; N, 10.89; Cl, 13.81. Found: C, 51.72; H, 5.40; N, 10.94; Cl, 13.72.

DL-4-Benzamido-5-methyl-3-isoxazolidone.—To a stirred slurry of 6.4 g. (0.025 mole) of α-benzamido-β-chlorobutyrohydroxamic acid in 100 ml. of water at 65–75°, 46 ml. (1.83 equivalents) of 1 *N* sodium hydroxide was added over a period of 1 hr. A permanent phenolphthalein end-point was obtained. The solution was cooled in an ice-bath and 21.4 ml. of 1.16 *N* (0.025 mole) hydrochloric acid was added. The acidic solution was extracted with three 250-ml. portions of chloroform, and the combined dried extracts were concentrated to approximately 225 ml. The DL-4-benzamido-5-methyl-3-isoxazolidone, 3.3 g., m.p. 188–194°, was filtered. The filtrate was concentrated and yielded another 1.0 g., m.p. 187–192°, bringing the total yield to 4.3 g. (79%). A small sample, recrystallized from chloroform, melted at 187–190°.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.62; H, 5.14; N, 12.56.

Methyl DL-α-Amino-β-aminoxybutyrate Dihydrochloride.—A rapid stream of hydrogen chloride was passed into 250 ml. of dry methanol for approximately 40 minutes. To this solution was added 5 g. (0.02 mole) of 4-benzamido-5-methyl-3-isoxazolidone, and the solution was refluxed for 2 hr. The solvent was removed *in vacuo*. The crystalline residue was extracted with 150 ml. of boiling isopropyl alcohol leaving 2.1 g. (42%) of crystalline methyl α-amino-β-aminoxybutyrate dihydrochloride, m.p. 139–145°. A small sample, triturated with boiling isopropyl alcohol, melted at 136–138°.

Anal. Calcd. for $C_5H_{14}N_2O_3Cl_2$: C, 27.16; H, 6.38; N, 12.67; Cl, 32.07. Found: C, 27.44; H, 6.46; N, 12.46; Cl, 31.27.

In some runs only amorphous ester dihydrochloride was obtained. The amorphous product was used successfully in the next step.

DL-4-Amino-5-methyl-3-isoxazolidone (XII).—To a cold solution of 2.0 g. (9.1 mmoles) of methyl α-amino-β-aminoxybutyrate dihydrochloride in 2 ml. of water was added dropwise a cold solution of 1.8 g. (>3 equivalents) of potassium hydroxide in 2 ml. of water. During the addition of the alkali, the reaction mixture became pink and then colorless as the solution reached *p*H 11. The precipitate, after removal of the supernatant solution, was washed twice with 0.5 ml. of water. The washings and supernatant solution were combined and then diluted with 40 ml. of 1:1 ethanol–isopropyl alcohol. The resulting precipitate was filtered and was washed with a small volume of the 1:1 alcohol solution. The filtrate was cooled to 0–5° and was acidified to *p*H 6 by the dropwise addition of glacial acetic acid. The crystalline precipitate, 923 mg. (88%), was recrystallized in the same manner as DL-4-amino-3-isoxazolidone giving 761 mg. of DL-4-amino-5-methyl-3-isoxazolidone, m.p. 170–173°.

Anal. Calcd. for $C_4H_8N_2O_2$: C, 41.36; H, 6.94; N, 24.13. Found: C, 41.31; H, 6.68; N, 23.88.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES DIVISION OF MERCK & CO., INC.]

Some Reactions of *erythro-* and *threo-β-p-*Nitrophenylserine

BY ARTHUR F. WAGNER

RECEIVED DECEMBER 29, 1956

Ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) was converted to the corresponding 2-oxazoline, 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II), by an SNi displacement using *p*-toluenesulfonyl chloride in pyridine solution. The 4-carbethoxy-2-oxazoline II was converted to the 4-carbohydroxamido-2-oxazoline IV which was opened to the *erythro*-β-chlorohydroxamic acid V. Among the products resulting from the alkaline cyclization of the *erythro*-β-chlorohydroxamic acid V was the 4-carbohydroxamido-2-oxazoline IV. Ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) reacted with thionyl chloride to yield the corresponding β-chloro compound III with retention of configuration. The corresponding *threo* isomer VI in the same reaction underwent nitrogen-to-oxygen acyl migration and inversion at the β-carbon to yield ethyl *erythro*-α-amino-β-benzoyloxy-β-*p*-nitrophenylpropionate hydrochloride (VII).

The characterization and synthesis of chloramphenicol[1] did much to stimulate interest in the stereochemistry and reactivity of the β-*p*-nitrophenylserines. More recently publications from these laboratories[2] described the synthesis of the antibiotic cycloserine[3] using serine as the precursor for the 3-isoxazolidone nucleus. This synthesis used a 4-carbethoxy-2-oxazoline intermediate to protect the α- and β-positions of the original serine molecule while the carbethoxy group was converted to a carbohydroxamido group. In contrast to the alternative of converting a β-chloroester to a β-chlorohydroxamic acid directly, the alkaline stability of the 2-oxazoline ring effectively protected the α- and β-positions of serine from undergoing any reaction during the conversion of the ester to a hydroxamic acid. The carbohydroxamido-2-oxazoline was opened to a β-chlorohydroxamic acid which was then cyclized to a 3-isoxazolidone.

This paper describes the behavior of *erythro-* and *threo-β-p-*nitrophenylserine in a similar sequence of reactions designed to synthesize 2-oxazolines and

(1) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) C. H. Stammer, A. N. Wilson, F. W. Holly and K. Folkers, *ibid.*, **77**, 2346 (1955); C. H. Stammer, A. N. Wilson, C. F. Spencer, F. W. Holly and K. Folkers, *ibid.*, **79**, 3236 (1957).

(3) D-4-Amino-3-isoxazolidone. Our generic name for this antibiotic has been changed from oxamycin to cycloserine. Oxamycin is now the registered trade-mark of Merck & Co., Inc., for this antibiotic.