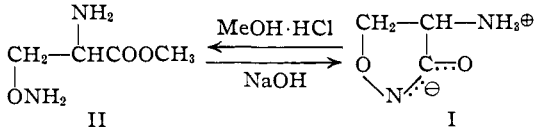
H, 5.50; N, 13.35; OCH_3, 14.46. Treatment of the ester II with alkali converted it to the original antibiotic.

Upon the basis of these degradation reactions, structure I, D-4-amino-3-isoxazolidone (oxamycin) is assigned to this new antibiotic.



II I

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RECEIVED MARCH 3, 1955

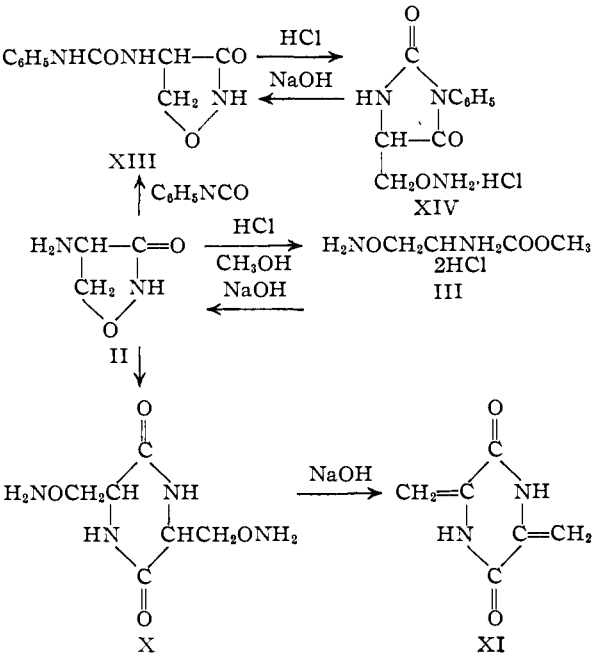
STRUCTURE AND REACTIONS OF CYCLOSERINE[1]

Sir:

The soil organism *Streptomyces orchidaceus* elaborates a new broad spectrum antibiotic which has been given the generic name cycloserine.[1-4] Isolation from culture filtrates was accomplished by absorption on anion exchange resins, elution with dilute mineral acid, and formation of a crystalline silver salt (I) [Calcd. for $C_3H_5N_2O_2Ag$: C, 17.2; H, 2.40; N, 13.4; Ag, 51.6. Found: C, 17.4; H, 2.83; N, 13.1; Ag, 49.9] from which the crystalline antibiotic was obtained as fine white needles from aqueous alcohol, m.p. 156° (dec.), $[\alpha]^{25}_{5461}$ 137 ± 2° (*c*, 5 in 2*N* NaOH), $[\alpha]^{25}D$ 112°, (*c*, 5 in 2*N* NaOH) [Calcd. for $C_3H_6N_2O_2$: C, 35.3; H, 5.92; N, 27.4; mol. wt., 102. Found: C, 35.4; H, 5.98; N, 26.9; equiv. wt., 104]. Potentiometric titration (pK'_a 4.4 and 7.3) indicates that cycloserine exists in aqueous solution as a dipolar ion. These data, together with the infrared spectrum, are consistent with structure II, D-4-amino-3-isoxazolidinone, for cycloserine.

Reaction of II with methanol and hydrogen chloride gave methyl D-α-amino-β-aminoxypropionate dihydrochloride (III), m.p. 163–164° (dec.) [Calcd. for $C_4H_{10}N_2O_3 \cdot 2HCl$: C, 23.2; H, 5.84; N, 13.5; Cl, 34.2. Found: C, 23.0; H, 5.94; N, 13.5; Cl, 33.9; $[\alpha]^{25}D$ −12.5° (*c*, 1 in methanol); pK'_a 2.3 and 6.9] which was recyclized in good yield to II by means of base.

In the Van Slyke amino nitrogen analysis about one half of the total nitrogen was found. Prolonged acid hydrolysis yielded DL-serine, while under milder conditions D-serine was isolated. These were identified by paper chromatography, rotation, and the identity of their infrared spectra with those of authentic specimens. Hydroxylamine was isolated from the hydrolysate as *m*-nitrobenzaldoxime (IV), m.p. and m.m.p. 122°.



On catalytic reduction, one mole of hydrogen was consumed and D-serine amide was isolated as the hydrochloride (V), m.p. 188–189° [Calcd. for $C_3H_8O_2N_2 \cdot HCl$: C, 25.6; H, 6.45; N, 19.9; Cl, 25.2. Found: C, 26.1; H, 6.70; N, 19.7; Cl, 25.1] which had the same infrared spectrum as authentic L-serine amide hydrochloride, but equal and opposite rotation. Acetylation yields both a monoacetyl derivative (VI), m.p. 179–180°, pK'_a 5.80 [Calcd. for $C_5H_8O_3N_2$: C, 41.7; H, 5.59; N, 19.4; mol. wt., 144. Found: C, 41.9; H, 5.61; N, 18.8; equiv. wt., 139] and a diacetyl derivative (VII), m.p. 120–121° [Calcd. for $C_7H_{10}N_2O_4$: C, 45.2; H, 5.41; N, 15.0. Found: C, 45.3; H, 5.67; N, 15.2]. Alkaline hydrolysis of VI yields cycloserine. Methylation of VI with diazomethane followed by chromatography on alumina gave an O-methyl derivative (VIII), m.p. 140–142°, and an N-methyl derivative (IX), m.p. 111–113° [Calcd. for $C_6H_{10}O_3N_2$: C, 45.6; H, 6.37; N, 17.7: OCH_3, 19.6; NCH_3, 9.5. Found (VIII): C, 45.7; H, 6.34; N, 17.4; OCH_3, 18.9; NCH_3, 0.0. Found (IX): C, 45.6; H, 6.26; N, 17.4; OCH_3, 0.0; NCH_3, 9.2].

In solution cycloserine dimerizes to 2,5-bis-(aminoxymethyl)-3,6-diketopiperazine (X), m.p. 190–200° (dec.) [Calcd. for $O_6H_{12}N_4O_4$; C, 35.3; H, 5.92; N, 27.5. Found: C, 35.1; H, 5.80; N, 25.9]. Catalytic reduction of X leads to ammonia and either D-serine anhydride or DL-serine anhydride, identified by the analyses, m.p., and comparison with the infrared spectra of authentic specimens. Alkaline degradation of X yields hydroxylamine and 2,5-dimethylene-3,6-diketopiperazine (XI), m.p. > 300° [Calcd. for $C_6H_6N_2O_2$: C, 52.2; H, 4.35; N, 20.3. Found: C, 52.1; H, 4.40; N, 20.0]. Catalytic hydrogenation of XI affords DL-alanine anhydride (XII), m.p. 286–287°

(1) Comparison of oxamycin (Merck) and cycloserine indicates that these two products are identical.

(2) R. L. Harned, P. H. Hidy and E. A. Kropp, *Antibiotics & Chemotherapy*, in press.

(3) H. Welch, Fourteenth Veterans Administration-Army-Navy Conference on the Chemotherapy of Tuberculosis, Atlanta, Georgia, February 7–10 (1955).

(4) I. Epstein, K. G. S. Nair and L. J. Boyd, *Antibiotic Med.*, **1**, 80 (1955).

[Calcd. for $C_6H_{10}N_2O_2$: C, 50.7; H, 7.10; N, 19.7. Found: C, 50.9; H, 7.11; N, 19.4].

Reaction of cycloserine with phenyl isocyanate provides the mono derivative (XIII), m.p. 197–198° [Calcd. for $C_{10}H_{11}N_3O_3$: C, 54.3; H, 5.01; N, 19.0. Found: C, 54.8; H, 5.4; N, 18.4]. Hydrochloric acid converts XIII to the hydrochloride of 5-aminoxymethyl-3-phenylhydantoin (XIV), m.p. 124–126°, $[\alpha]^{25}_{5461}$ 93° (*c*, 1 in H_2O) [Calcd. for $C_{10}H_{11}N_3O_3 \cdot HCl \cdot CH_3OH$: C, 45.5; H, 5.52; N, 14.5; Cl, 12.2. Found: C, 45.8; H, 5.42; N, 14.3; Cl, 12.4] whose infrared spectrum and properties are consistent with the hydantoin structure proposed. In alkali XIV is reconverted to the optically active derivative XIII.

3-Isoxazolidinone (XV), the parent ring of II, was prepared as follows: acid hydrolysis of 3-(isopropylideneaminoxy)-propionitrile[5] (XVI) to 3-aminopropionic acid hydrochloride (XVII), m.p. 150–151° [Calcd. for $C_3H_7NO_3 \cdot HCl$: C, 25.5; H, 5.70; N, 9.90; Cl, 25.1. Found: C, 25.3; H, 5.65; N, 9.98; Cl, 25.0]; esterification to ethyl 3-aminoxypropionate (XVIII), b.p. 87° (10 mm.), $n^{25}D$ 1.4328 [Calcd. for $C_5H_{11}NO_3$: C, 45.1; H, 8.33; N, 10.5. Found: C, 45.4; H, 8.43; N, 10.5]; and cyclization in base to XV, isolated as the hygroscopic potassium salt (XIV) [Calcd. for $C_3H_4NO_2K$: C, 28.8; H, 3.22; N, 11.2, mol. wt., 125. Found: C, 27.6, H, 3.59; N, 10.6, pK'_a 6.70, equiv. wt., 135] and also the silver salt (XX) [Calcd. for $C_3H_4NO_2Ag$: C, 18.6; H, 2.06; Found: C, 18.5; H, 2.17].

The infrared spectra of cycloserine (II) and the silver salts of cycloserine (I) and 3-isoxazolidinone (XX) are given in Fig. 1. The bands at 3.03, 3.09, 3.23, 6.17, 8.73, 9.04, 9.69, 10.16 and 12.12 microns, related to $-NH_2$ by deuteration studies, are absent in the 3-isoxazolidinone silver salt spectrum.

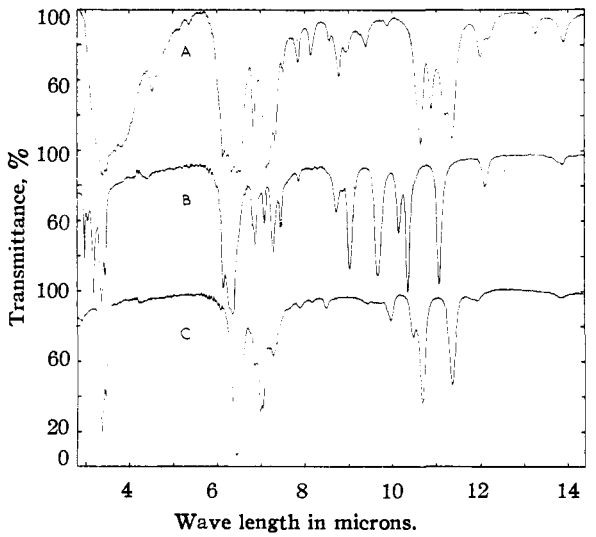


Fig. 1.—Infrared absorption spectra in mineral oil: A-cycloserine (II); B-cycloserine silver salt (I); C-3-isoxazolidinone silver salt (XX).

Additional confirmation has been obtained by syntheses by two independent routes and will be reported later.

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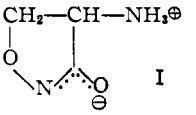
SYNTHESIS OF D-4-AMINO-3-ISOXAZOLIDONE

Sir:

A new antibiotic, oxamycin, has been isolated and shown by degradation to be D-4-amino-3-isoxazolidone (I).[1]



I

The synthesis of D-4-amino-3-isoxazolidone is described herein; the synthetic compound and oxamycin are identical.

DL-Serine was converted to its methyl ester hydrochloride (II) by Fischer esterification. On treatment of the ester II with ethyl iminobenzoate, DL-2-phenyl-4-carbomethoxy-2-oxazoline[2] (III) was obtained. The oxazoline ester III was then allowed to react with hydroxylamine and sodium ethoxide. Acidification of the reaction mixture afforded DL-2-phenyl-4-carbohydroxamido-2-oxazoline (IV), m.p. 176–179°. *Anal.* Calcd.: C, 58.20; H, 4.88; N, 13.60. Found: C, 58.38; H, 5.05; N, 13.41. Treatment of this hydroxamic acid IV with hydrogen chloride in dry dioxane yielded DL-α-benzamido-β-chloropropionohydroxamic acid (V), m.p. 153–155°. *Anal.* Calcd.: C, 49.40; H, 4.56; N, 11.54; Cl, 14.61. Found: C, 48.94; H, 4.49; N, 11.77; Cl, 14.31. When the hydroxamic acid was treated with 1*N* alkali followed by acidification, DL-4-benzamido-3-isoxazolidone (VI), m.p. 165–168° was formed. *Anal.* Calcd.: C, 58.24; H, 4.89; N, 13.59. Found: C, 58.45; H, 4.70; N, 13.37. The isoxazolidone VI was treated with a concentrated solution of methanolic hydrogen chloride to give DL-β-aminoxyalanine methyl ester dihydrochloride (VII), m.p. 128–131°. *Anal.* Calcd.: C, 23.20; H, 5.85; N, 13.53; Cl, 34.24. Found: C, 22.95; H, 6.19; N, 13.43; Cl, 32.27. (The ester VII and 4-acetamido-3-isoxazolidone were first obtained in the D series during structural investigation.)[1] Reaction of the ester VII with potassium hydroxide formed DL-4-amino-3-isoxazolidone (I), m.p. 138–141°, the racemate of oxamycin. *Anal.* Calcd.: C, 35.29; H, 5.92; N, 27.45. Found: C, 35.27; H, 6.04; N, 27.01.

This racemate was resolved with D-tartaric acid

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

(2) D. F. Elliot, *J. Chem. Soc.*, 589 (1949).

(5) H. Bruson, THIS JOURNAL, **65**, 23 (1943).