On evaporation of the actone, the aldehyde was converted to the 2,4-dinitrophenylhydrazone, which was identical in spectrum with that of aldehyde VIIF. Imine VIIC was unchanged on similar reirradiation.

In Table III is a summary of the experimental conditions used in following the rates of disappearance of the unstable oxaziridines in the dark. The rate data were found to fit first-order kinetics at several concentrations. The rate of disappearance of the oxaziridine was approximately the same as the rate of formation of products. When treated with acid, the 2,3-diaryloxaziridines show absorption in the 400-mμ region.[58] This absorption rapidly disappears but is useful in following the rates of disappearance of oxaziridine VB in acetone and IVB in ethanol.

The following procedure was used to estimate the $t_{1/2}$ of reaction for oxaziridine VIB in ethanol. Five portions of nitrone VIA in ethanol were irradiated as described in Table III, immediately analyzed at 268 mμ for VIE and at 382 mμ for VIA, and then combined. After standing for 10 min. to allow for completion of reaction, 5 drops of 0.1 N H_2SO_4 were added. Five other portions were irradiated, but immediately after irradiation (at approximately 7 sec.) a drop of 0.1 N H_2SO_4 was added to each portion with shaking. These portions were combined, and after standing about 1 hr., spectra of both combined solutions were compared at 268 mμ for VIE and 340 mμ for VIF. The difference was consistent with about 89% completion of reaction at the time (7 sec.) of addition of the acid. By extrapolation, the $t_{1/2}$ was estimated to be about 2 sec. This also was in agreement with the $t_{1/2}$ estimated by irradiating portions of nitrone VIA, optical density 0.6, for 2 sec. (photospots 3 in. apart) and analyzing at 385 mμ for VIA and 280 mμ for VIB.

The rates of disappearance of the unstable oxaziridines in ethanol and acetone were little affected by traces of acid[59] on the cell wall. However, in benzene there was a catalytic effect. With the same cell treatment, the values of $t_{1/2}$ were found to be IVB, 14 hr.; VB, 60 hr.; VIB, 15 min., 80% yield of VIE; VIIB, 31 min. The values of $t_{1/2}$ reported in Table II were determined in glassware that had not been in contact with sulfuric acid. The rates of disappearance of oxaziridines VIB and VIIB were greatly decreased when benzene saturated with water was used.

Acknowledgment.—The authors are indebted to Dr. E. M. Voigt for helptul discussions of this work. The preparation of this paper was sponsored in part by the U. S. Atomic Energy Commission.

(58) This reaction will be reported in detail in the next communication.

(59) Glassware used had been in contact with cleaning solution or dilute sulfuric acid, but had been repeatedly rinsed with dilute NH_4OH, or NaOH and distilled water.

Cycloserine. III. A Schiff Base and Its Reactions[1]

CHARLES H. STAMMER AND JAMES D. MCKINNEY

Department of Chemistry, University of Georgia, Athens, Georgia

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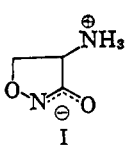
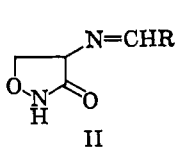
The first Schiff base of D-cycloserine has been prepared and its chemical reactions have been examined. The first conversion of D-cycloserine into its racemate *via* the Schiff base is reported. Hydrolysis, borohydride reduction, acetylation, and methanolysis of the Schiff base are described. Evidence indicating the probability that the Schiff base is intermediate in the conversion of cycloserine into its dimer derivative is presented. Possible biochemical implications are discussed.

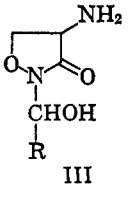
Numerous investigators have established that cycloserine (I) inhibits pyridoxal-dependent transaminase, decarboxylase, and racemase enzyme systems.[2] Speculation about the mechanism of this inhibition has led to sugggestions[3] that cycloserine reacts with pyridoxal forming a Schiff base (II) or possibly a carbinolamine of type III. Michalsky and co-workers found that when DL-cycloserine was allowed to react with pyridoxal



I

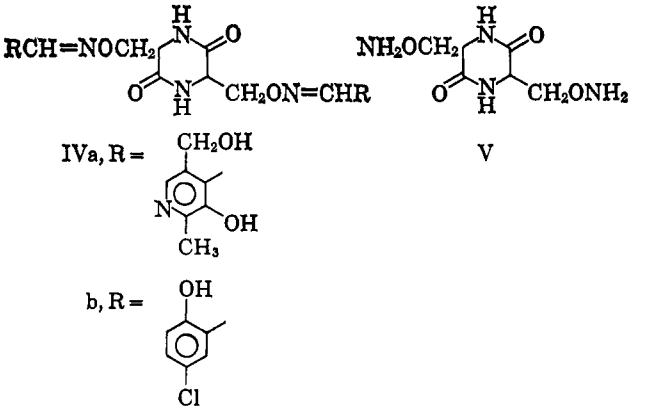


II



III

and various aromatic aldehydes, the 2,5-diketopiperazine derivatives (IV) were the only products formed. These workers reasoned that either II or III might be intermediates in the formation of IV or that cycloserine might first dimerize forming the 3,6-bis(aminoxymethyl)-2,5-diketopiperazine (V) which was rapidly derivatized giving IV.



If we make the biochemically naive assumption that the abstraction of pyridoxal from an enzyme system by cycloserine occurs through its conversion to IVa, the chemical pathway of this conversion becomes of considerable biochemical importance. The essence of the problem is to determine which of the intermediates II, III, or V is most likely.

As outlined in preliminary reports,[4] we have approached this problem by synthesizing an authentic Schiff base of cycloserine and examining its chemical properties. A more detailed discussion of its preparation, reactions, and probable implication in the formation of IV is the subject of this paper.

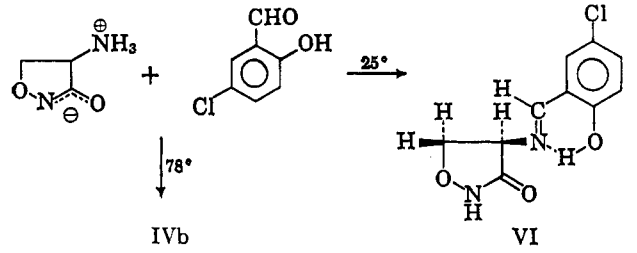
(1) We gratefully acknowledge the financial assistance of National Institutes of Health, Grant No. AI 05539-02.

(2) J. L. Strominger, *Physiol. Rev.*, **40**, 87 (1960); F. Cedrangolo, *I. U. B. Symp. Ser.*, **30**, 343 (1962); O. T. Dann and C. E. Carter, *Biochem. Pharmacol.*, **13**, 677 (1964); M. R. Alioto, *Biochim. Appl.*, **9**, 238 (1962); *Chem. Abstr.*, **58**, 13015 (1963); G. D. Pretra, F. DeLorenzo, and G. Illiano, *Biochim. Appl.*, **10**, 123 (1963); *Chem. Abstr.*, **60**, 6105 (1964).

(3) (a) J. Michalsky, J. Opichal, and J. Ctvrtnk, *Monatsh.*, **93**, 618 (1962); (b) N. K. Kotschetkow, *Oesterr. Chemiker-Ztg.*, **62**, 276 (1961).

(4) (a) C. H. Stammer, *Experientia*, **20**, 417 (1964); (b) C. H. Stammer and J. D. McKinney, *Tetrahedron Letters*, **No. 38**, 2607 (1964).

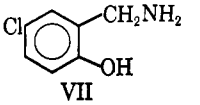
We prepared N-(5-chlorosalicylidene)-D-cycloserine (VI) by allowing 5-chlorosalicylaldehyde[5] (5-CSA) to react with D-cycloserine in alcohol at room temperature or below during a 10–16-hr. period. The cycloserine slowly dissolved and a crystalline optically active Schiff base (VI) was obtained from the solution in 84% yield. In the absence of aldehyde, cycloserine is essentially insoluble in ethanol and remained unchanged. At reflux temperature, a quantitative yield of dimer derivative IVb was obtained from aldehyde and cycloserine as reported by Michalsky.[3a] This surprising difference in product composition will be discussed later.



IVb VI

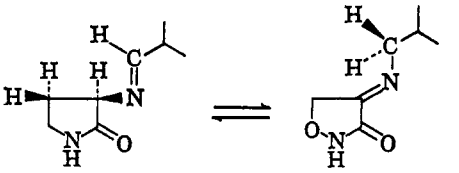
Spectral data are consistent with the Schiff base structure VI.[6] The solid state spectrum of cycloserine is that typical of an amino acid zwitterion showing a band at 2200 cm.$^{-1}$ assigned to $-NH_3^+$ and broad absorption in the 1660–1550-cm.$^{-1}$ region assigned to the resonance-stabilized hydroxamate anion (see I). The solid state infrared spectrum of VI showed the expected broad absorption at 3100 cm.$^{-1}$ indicating a bonded hydroxyl group[7] and sharp bands at 1710 and 1625 cm.$^{-1}$ which can be assigned to the ring carbonyl and azomethine groups, respectively. Apparently, the isoxazolidone ring exists predominantly in the keto rather than the enol form when the amino function is masked. The ultraviolet spectrum of VI differed very little in peak positions from the aldehyde, but the peak intensities were approximately doubled. Both the infrared and ultraviolet data are consistent with those found by Witkop[8] for a series of amino acid Schiff bases. The n.m.r. spectrum of VI shows the expected[9] downfield peak at τ 1.34 for the azomethine hydrogen atom, the aromatic proton peaks in the τ 2.5–3.0 region, and a complex group of six peaks centered around τ 5.32 expected of the three magnetically nonequivalent isoxazolidone ring protons.[10] These data, along with elemental analysis, are all consistent with the Schiff base structure VI and eliminate the possibility that our product might have structure III (R = 5-chloro-2-hydroxyphenyl).

The reactions of VI confirmed its Schiff base structure. Most diagnostic was its rapid hydrolysis in 0.3 *N* hydrochloric acid, giving a 76% yield of aldehyde and a 48% yield of crystalline[11] D-cycloserine of 84% optical purity. A paper chromatography study of the hydrolysis showed it to be essentially complete in 1 hr. and indicated that a significant proportion of a second amine, identified as 2-hydroxy-5-chlorobenzylamine (VII), was formed.[12] An authentic sample of VII was prepared by lithium aluminum hydride reduction of 5-chlorosalicylaldoxime.



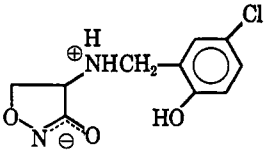
VII

Since the Schiff base VI was formed at room temperature and the dimer derivative IV was formed at 78°, it seemed plausible, even though mechanistically obscure, that the former was an intermediate in the formation of the latter. However, only *racemic Schiff base* was obtained when an ethanolic solution of the optically active compound was refluxed for 16 hr. Both its infrared spectrum and facile hydrolysis to racemic cycloserine confirmed this conclusion. The racemization undoubtedly occurred *via* the tautomerism shown below.

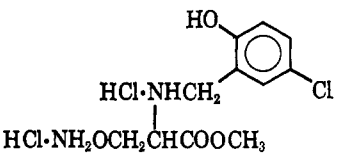


Although Ingold[13] showed that azomethine tautomerism occurred at measurable rates only in the presence of a strong base like sodium ethoxide, Herbst[14] and Baddar[15] found that an α-carboxyl group facilitated the prototropic shift. Azomethine tautomerism is now considered to be an integral part of the mechanism of enzymic transamination and racemization.[16] The absence of dimer in the product seemed to eliminate the possibility that Schiff base was intermediate in the formation of dimer derivative IV.

Sodium borohydride reduction of VI in ethanol afforded a dihydro derivative which had no 1625-cm.$^{-1}$ band in its infrared spectrum and which showed the zwitterionic properties of aminoisoxazolidones. These data are consistent with structure VIII. Both



VIII



IX

(5) This aldehyde was chosen because of reports by F. C. McIntyre [*J. Am. Chem. Soc.*, **69**, 1377 (1947)] and J. C. Sheehan [*ibid.*, **84**, 2417 (1962)] that it formed crystalline amino acid Schiff bases very readily.

(6) We draw the *trans* configuration about the azomethine linkage primarily for steric reasons. See H. A. Staab, F. Vogtle, and A. Mannschreck, *Tetrahedron Letters*, **No. 12**, 697 (1965).

(7) O. A. Osepov, V. I. Minkin, and V. A. Kogan [*Chem. Abstr.*, **59**, 11218 (1963)] reported that the hydroxyl group of 5-chlorosalicyladehyde anil is highly hydrogen bonded.

(8) B. Witkop and T. W. Beiler, *J. Am. Chem. Soc.*, **76**, 5589 (1954).

(9) The spectrum of salicylaldoxime shows the azomethine proton at τ 1.82: N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962.

(10) It is interesting to note that cycloserine shows a similar complex absorption in the τ 5.0–5.7 and 5.5–6.2 regions in acidic and basic solutions, respectively.

(11) The crystallization of cycloserine from solution rarely gives better than a 60% recovery. See C. H. Stammer, A. N. Wilson, C. F. Spencer, F. W. Bachelor, F. W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, **79**, 3236 (1957).

(12) Both VII and racemic cycloserine are formed owing to tautomerism of the azomethine system discussed later in this paper.

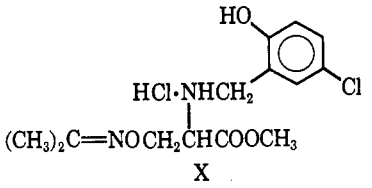
(13) S. K. Hsu, C. K. Ingold, and C. L. Wilson, *J. Chem. Soc.*, 1778 (1935); C. K. Ingold and C. W. Shoppee, *ibid.*, 1199 (1929).

(14) S. D. Brewer and R. M. Herbst, *J. Org. Chem.*, **6**, 867 (1941); R. M. Herbst, *Advan. Enzymol.*, **4**, 75 (1944).

(15) F. G. Baddar and S. A. M. Sherif, *J. Chem. Soc.*, 4292 (1956), and previous papers.

(16) A. F. Wagner and K. Folkers, "Vitamins and Coenzymes," Interscience Publishers, Inc., New York, N. Y., 1964, p. 176.

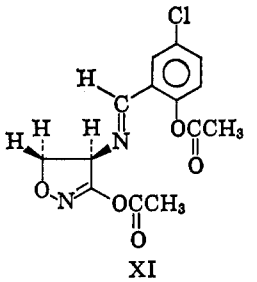
VI and VIII gave the nitroprusside color test described by Jones[17] characteristic of the 3-isoxazolidone ring. To confirm further the structure VIII, the reduction product was converted by acidic methanolysis to the expected[18] aminoxy ester dihydrochloride IX, which was nitroprusside negative and reacted rapidly with aqueous acetone giving an isopropylidene derivative, X, as expected of an O-alkylhydroxylamine such as



X

IX.[18b,19] The presence of the aminoxy function showed both that the N–O bond survived the borohydride treatment and that the azomethine group had definitely been reduced. If this reduction had not occurred, the salicylidene moiety would have rearranged and blocked the aminoxy group (*vide infra*).

Acetylation of the Schiff base VI with acetic anhydride in pyridine gave surprising results. We found that VI racemized only slowly in pyridine, but that the addition of acetic anhydride to the solution caused racemization too rapid to measure. The crystalline diacetate isolated was completely racemic and was generally accompanied in variable yield by an amorphous compound which, even when purified chromatographically, gave undecipherable elemental analyses. The crystalline diacetate undoubtedly has one acetyl on the phenolic hydroxyl, but the position of the second cannot be rigorously assigned. We favor structure XI although we have no compelling evidence[20] for O-acetylation on the isoxazolidone ring. The crystalline diacetyl derivative was readily hydrolyzed in dilute aqueous ethanol giving two ninhydrin-positive prod-

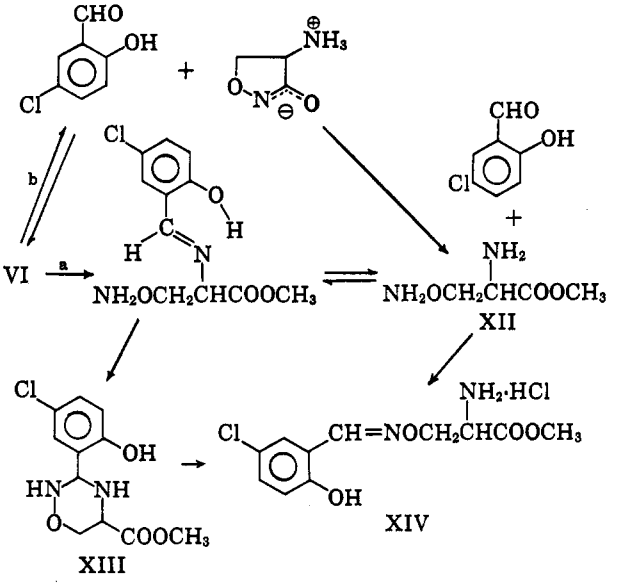


XI

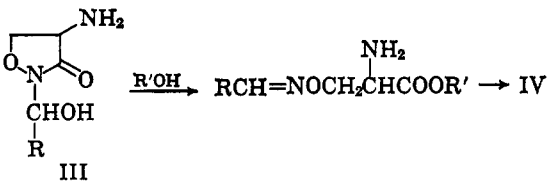
ucts. After treatment with sodium hydroxide, one was identified by paper chromatography as cycloserine and the second was identified as the previously prepared phenolic amine VII, a product of the azomethine tautomer of XI.

The amorphous product may be the result of addition of acetic anhydride to the azomethine linkage[21] followed by ring closure[21b,22] or rearrangement.[23] Certainly some kind of rapid reaction occurred between acetic anhydride and the azomethine linkage which led to racemization. The amorphous product was not a Schiff base since it was inert in dilute acid.

SCHEME I



The Schiff base reacted with methanolic hydrogen chloride forming the O-alkyl oxime XIV. The various reaction paths which may lead to XIV are outlined in Scheme I. The azomethine linkage might be cleaved either before (path b) or after (path a) ring opening leading to a mixture of aminoxy ester XII and aldehyde which rapidly reacted, forming XIV. Indeed, an authentic sample of XIV was prepared by allowing XII, prepared from cycloserine,[19] to react with 5-chlorosalicylaldehyde. A third path *via* the interesting 1,2,4-tetrahydrooxadiazine (XIII) is also a possibility. Work is underway to determine whether the free aldehyde is an intermediate in this reaction. The properties of ester XIV have further significance. As mentioned before, Michalsky[2] proposed earlier that if attack by aldehyde occurred at the ring nitrogen of cycloserine, an intermediate carbinolamine III might be formed. Ring alcoholysis might then lead to an α-amino ester like XIV which might then dimerize giving IV. To examine the possibility that XIV might lead to IV, we prepared the crystalline free base of XIV and found that on heating it with or without solvent



III

only 5-chlorosalicylaldehyde oxime (and tar) was formed. Most probably, β elimination of the oxime occurred giving an α-aminoacrylic ester which polymerized. This, of course, is evidence contrary to Michalsky's proposal.

We had previously concluded that Schiff base was not intermediate in the formation of dimer derivative

(17) L. R. Jones, *Anal. Chem.*, **28**, 39 (1956).

(18) (a) F. A. Kuehl, *et al.*, *J. Am. Chem. Soc.*, **77**, 2344 (1955); (b) C. H. Stammer, *J. Org. Chem.*, **27**, 2957 (1962).

(19) P. H. Hidy, *et al.*, *J. Am. Chem. Soc.*, **77**, 2346 (1955).

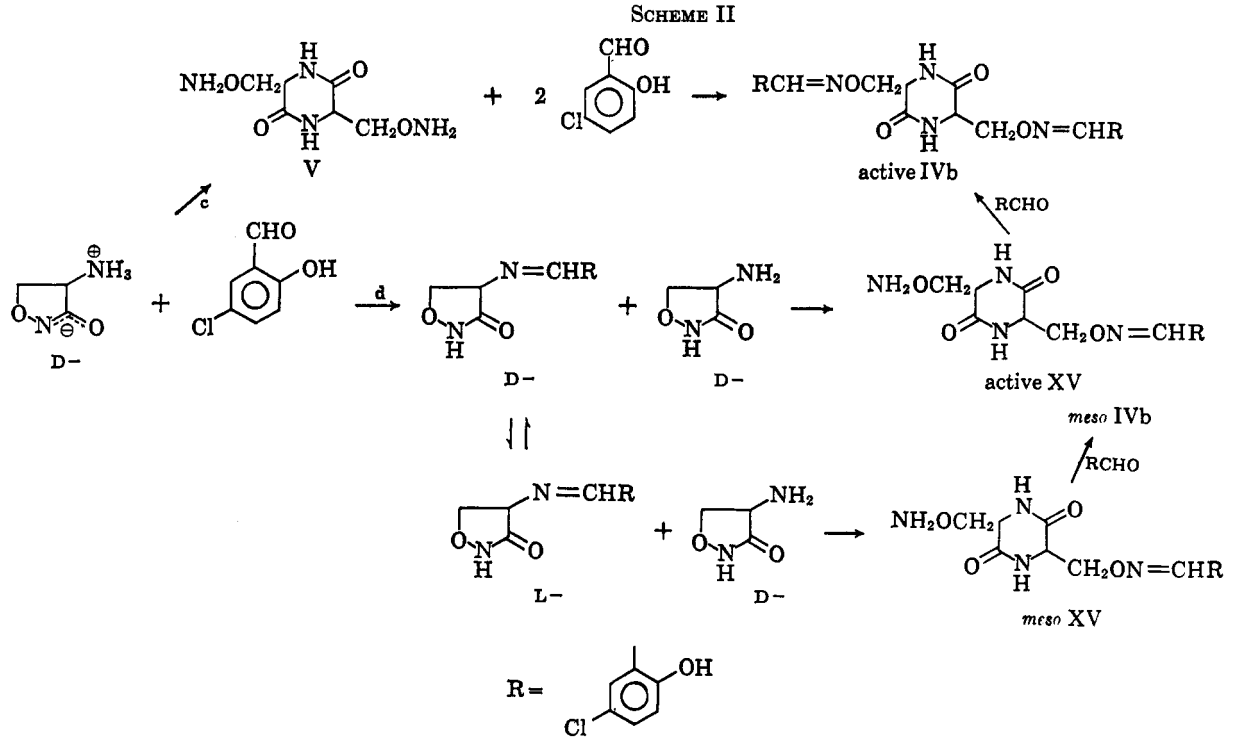
(20) A. J. Boulton, A. R. Katritzky, A. M. Hamid, and S. Oksne [*Tetrahedron*, **20**, 2835 (1964)] reported that O-methyl-3-hydroxyisoxazoles absorb at 218 mμ, whereas the corresponding N-methyl compound absorbs at 230 mμ. Our diacetyl compound XI absorbs at 220 mμ.

(21) (a) H. R. Snyder, R. H. Levin, and P. F. Wiley, *J. Am. Chem. Soc.*, **60**, 2025 (1958); (b) G. N. Walker and M. A. Moore, *J. Org. Chem.* **26**, 432 (1961).

(22) M. Bergmann, H. Enselin, and L. Zervas, *Ber.*, **58**, 1034 (1925).

(23) R. Fryer and L. H. Sternbach, *J. Org. Chem.*, **30**, 524 (1965).

SCHEME II



IVb from aldehyde and cycloserine.[3a] However, a spectral study of the reaction between cycloserine and pyridoxal led to a suggestion by Khomutov[24] that a Schiff base was formed which acylated a second molecule of cycloserine giving a product which rearranged into an "oxime derivative." Indeed, we found that our Schiff base reacted rapidly with I in aqueous N,N-dimethylformamide (DMF) giving the dimer derivative IVb, which is an "oxime derivative." This key piece of information led us to postulate[3b] the following course (Scheme II) of events during the reaction of cycloserine with 5-chlorosalicylaldehyde in boiling ethanol, the Michalsky reaction conditions, and in aqueous DMF.[25]

Schiff base which was formed in path d reacted with another molecule of cycloserine giving *partially* derivatized optically active dimer derivative XV which was rapidly converted to the isolated product IVb. If this sequence is correct, partial racemization of the Schiff base will occur giving some *meso* XV which would afford *meso* IVb. Thus the dimer derivative isolated should have a lower optical rotation than that formed *via* route c. This is confirmed by the experiments shown in Table I.[26]

Actually, whether path c or d was followed predominately depends on the relative rates of Schiff base and dimer V formation. Quantitative measurements of these rates are presently underway. We can speculate at this time, however, that an aldehyde (possibly pyridoxal in an enzyme system) might be removed from solution by formation of a cycloserine Schiff base which then acylates a second molecule of cycloserine giving dimer derivative. An alternative mechanism of enzyme inhibition in which the Schiff base acylates an amino group on the enzyme surface instead of a second cycloserine molecule has been suggested by Khomutov[24] and by Karpeiskii.[27] The path which actually obtains, assuming the formation of Schiff base, depends on whether a "surface amino group" or a second molecule of cycloserine, which might be enzyme bound, is closer to the Schiff base.

TABLE I

FORMATION OF CYCLOSERINE DIMER DERIVATIVE

Expt.	Conditions	% yield	Specific rotation, deg.
A	I + heat, followed by 5-CSA	70	+150
B	I + 5-CSA	94	+94
C	I + Schiff base	80	+122

The pathway by which the reaction between Schiff base and cycloserine affords the dimer derivative is probably as shown in Scheme III.

The intermediate XVI is the product of cycloserine acylation by ring opening of VI and, by analogy with the methanolysis of VI, would be expected to rearrange to the oxime derivative XVII. An intramolecular ring opening by attack of the free amino group then gives the diketopiperazine XV. We have not attempted to isolate XVI or XVII, but attempts to capture XVI by derivatizing the aminoxy group with a second carbonyl compound have failed. We have been unable to synthesize XV, but the underivatized

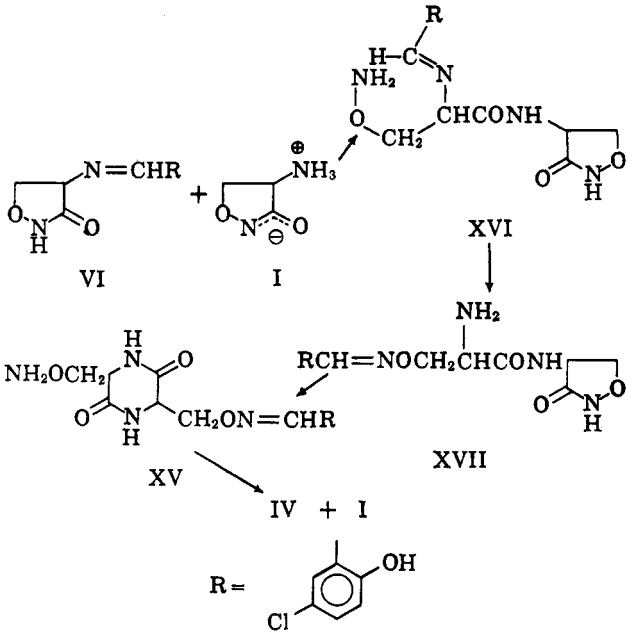
(24) R. M. Khomutov, M. Ya. Karpeiskii, E. S. Severin, and N. V. Gunchev, *Dokl. Akad. Nauk. SSSR*, **140**, 492 (1961); *Chem. Abstr.*, **56**, 704 (1962); R. M. Khomutov, M. Ya. Karpeiskii, and E. S. Severin, *Biokhimiya*, **26**, 772 (1961); *Biochemistry* (USSR), **26**, 667 (1961).

(25) This medium was chosen because it allowed the reaction to occur under homogeneous conditions. Cycloserine is insoluble in boiling ethanol.

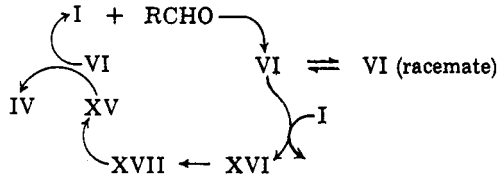
(26) The infrared spectra of the dimer derivatives from expt. B and C differed from "optically pure" dimer derivative at 6.05, 9.6, 12.0, and 12.2 μ. We converted DL-cycloserine into the expected mixture of dimers (DL and *meso*) using boiling ethanolic acetic acid. One of the products afforded a 5-chlorosalicylidene derivative which showed the expected bands in the infrared and thus confirmed the thesis that the spectral differences among the products of expt. A, B, and C were due to the presence of inactive dimer derivative and not some other compound.

(27) M. Ya. Karpeiskii, Yu. N. Breusov, R. M. Khomutov, E. S. Severin, and O. L. Polyanovskii, *Biokhimiya*, **28**, 345 (1963); *Biochemistry* (USSR), **28**, 280 (1963).

SCHEME III



dimer V made from cycloserine,[28] did react rapidly with the Schiff base to form IVb. Thus it is very likely that, in the absence of excess aldehyde, XV reacted with Schiff base forming IVb and liberating a molecule of cycloserine which recycled. This is reminiscent of cyclic biochemical mechanisms involving a carrier molecule and can be written as below. All of the aldehyde and cycloserine (except for one molecule) would be converted to dimer derivative *via* the Schiff base. The possibility that this occurs with pyridoxal under physiological conditions is under investigation.



Experimental Section

All melting points were taken on a Nalge hot stage and are corrected. Infrared spectra were determined on a Perkin-Elmer Infracord, Model 137. Ultraviolet spectra were determined on a Perkin-Elmer spectrophotometer, Model 202. N.m.r. spectra were determined on a Varian Associates A-60 n.m.r. spectrometer. All paper chromatography was carried out on Whatman No. 1 32-cm. circles having a 0.5-in. center hole. The procedure and solvent systems are those previously described[29]: solvent systems were BAW, the upper phase of butanol–acetic acid–water (4:1:5), and MPW, methyl ethyl ketone–pyridine–water (4:1:1.6); the compounds were located on the paper by means of ninhydrin reagent (N).

N-5-Chlorosalicylidene-D-cycloserine (VI).—A suspension of 3.0 g. (0.029 mole) of D-cycloserine,[30] $[\alpha]^{23}D$ +114° (*c* 1.25, H_2O), in a solution of 6.6 g. (0.042 mole) of 5-chlorosalicylaldehyde in a mixture of 795 ml. of absolute ethanol and 54 ml. of methanol was stirred magnetically 20 hr. at room temperature. The homogeneous solution was evaporated to dryness *in vacuo* and 100 ml. of ether was added to the solid residue. The crude yellow product, weighing 5.7 g. (81%), m.p. 145–146°, was collected on a filter and washed with two 25-ml. portions of ether. This compound was soluble in aqueous sodium hydroxide and gave a positive nitroprusside test.[17] An analytical sample of N-5-chlorosalicylidene-D-cycloserine was obtained by recrystallization from ethyl acetate–petroleum ether (b.p. 60°): m.p. 148–150°; $[\alpha]^{25}D$ + 130° (*c* 1.05, CH_3OH); λ^{Nujol}, 5.85 (C=O), 6.12 (C=N), triplet 12.05, 12.4, 12.75 μ; λ_{max}^{MeOH} 222 mμ (ϵ 31,280), 258 (13,010), 332 (4525); τ (acetone-d_6) 5.32 (six bands), 2.45–3.15 (seven bands), 1.34 (one band).

Anal. Calcd. for $C_{10}H_9ClN_2O_3$: C, 49.91; H, 3.77; Cl, 14.73; N, 11.64. Found: C, 50.21; H, 4.14; Cl, 14.72; N, 11.69.

Hydrolysis of N-5-Chlorosalicylidene-D-cycloserine (VI).—To a suspension of 508 mg. (2.12 mmoles) of N-5-chlorosalicylidene-D-cycloserine in 5 ml. of water was added 2.30 ml. of 1.0 *N* hydrochloric acid. The suspension was stirred magnetically 1.25 hr. at room temperature during which time the suspended solid changed from yellow to white. The mixture was extracted with three 10-ml. portions of ethyl acetate and the combined extracts were dried (anhydrous magnesium sulfate) and evaporated to dryness, yielding 252 mg. (76%) of 5-chlorosalicylaldehyde (infrared spectrum identical with that of an authentic sample). The aqueous layer was evaporated to dryness *in vacuo* at temperatures less than 35°. The amorphous residue was dissolved in 1.4 ml. of 7% ammonium hydroxide and 5.6 ml. of a 1:1 mixture of ethanol and 2-propanol was added to the solution. A small amount of red oily precipitate was removed by centrifugation and the solution was cooled in an ice bath. Acidification of the cold solution with glacial acetic acid afforded 102 mg. (48%) of D-cycloserine, $[\alpha]^{23}D$ +95° (*c* 1.57, H_2O); infrared spectrum (Nujol) was identical with that of an authentic sample, R_f^{MPW} 0.46 (N); D-cycloserine had R_f 0.45.

A mixture of 1 ml. of 1 *N* hydrochloric acid, 3 ml. of distilled water, 1 ml. of ethanol, and 250 mg. of N-5-chlorosalicylidene-D-cycloserine was stirred 1.25 hr. The reaction mixture was evaporated to dryness and the residue dissolved in water. Paper chromatography using BAW and MPW systems showed the presence of two amines: R_f^{MPW} 0.77 (N, D-cycloserine), 0.98 (N, 5-chlorosalicylamine); R_f^{BAW} 0.39 (N, D-cycloserine), 0.88 (N, 5-chlorosalicylamine). It is notable that in MPW both cycloserine and its hydrochloride had the same R_f value (0.76), but in BAW cycloserine had R_f 0.68 and its hydrochloride gave R_f 0.38.

2-Hydroxy-5-chlorobenzylamine (VII).—To a solution of 1.0 g. (5.83 mmoles) of 5-chlorosalicylaldoxime in 50 ml. of tetrahydrofuran was added 0.5 g. (13.2 mmoles) of lithium aluminum hydride and the mixture was refluxed for 28 hr. After cooling the solution, 10 ml. of water was added followed by 10 ml. of 10% sulfuric acid solution giving a solution of pH *ca.* 5. The precipitate was filtered and identified as inorganic material. The addition of 2.0 *N* sodium hydroxide solution to the filtrate (pH 7–8) gave a second precipitate which was also identified as inorganic. The pH was adjusted to pH 8–9 with 2.0 *N* sodium hydroxide, and the crystalline organic precipitate, weighing 456 mg., m.p. 176–180°, was dried *in vacuo*. The crude product was placed on a 1 × 28 cm. column of Woelm silica gel TLC prepared in ethyl acetate, and the column was eluted with 120 ml. of ethyl acetate to remove an impurity. The column was then eluted with tetrahydrofuran. After evaporation of the eluate (25 ml.), the resulting solid product was extracted with five 5-ml. portions of 10:1 ether–ethyl acetate. The insoluble solid weighed 231 mg. (25%), m.p. 172–176°, R_f^{MPW} 0.96 (N), R_f^{BAW} 0.88 (N). (The column chromatography could probably be eliminated by doing the extraction procedure first.) An analytical sample melting at 172–176° was obtained by recrystallization of this material from 4:1 ethanol–water.

Anal. Calcd. for C_7H_6ClNO: C, 53.35; H, 5.12; N, 8.89. Found: C, 53.77; H, 5.40; N, 8.98.

Racemization of N-5-Chlorosalicylidene-D-cycloserine (VI).—A solution of 506 mg. of N-5-chlorosalicylidene-D-cycloserine in 50 ml. of ethanol was refluxed for 16 hr. At this time the solution showed no optical rotation. The solution was evaporated to dryness and the residue was dissolved in 30 ml. of boiling chloroform. The solution was filtered and the filtrate was evaporated by boiling to 10 ml. After standing at 5° for several hours, the crystalline precipitate, 213 mg., m.p. 156–157° dec., was

(28) D-Cycloserine dimer V was prepared according to F. C. Neuhaus and J. L. Lynch [*Biochemistry*, **3**, 471 (1964)]. The bisoxime (IVb) prepared from it showed $[\alpha]^{23}D$ +152° (*c* 1, DMF). This is the highest rotation for IVb we obtained. Attempts to determine the optical purity of dimer V by hydrogenation to the known active 2,5-bis(hydroxymethyl)-3,6-diketopiperazine failed. Nitrous acid oxidation gave only a 58% yield of fully active hydroxymethyl compound, so that the absolute optical purity of the dimer V was not established.

(29) C. H. Stammer, *J. Org. Chem.*, **26**, 2556 (1961).

(30) The D-cycloserine used in this work was very kindly supplied by Dr. Wallace F. Runge of Commercial Solvents Corp., Terre Haute, Ind.

collected on a filter. The infrared spectrum of N-5-chlorosalicylidene-DL-cycloserine differed only slightly from that of the D compound in the 3000–3200-, 1700-. and 1000-cm.$^{-1}$ regions.

Anal. Calcd. for $C_{10}H_9ClN_2O_3$: C, 49.91; H, 3.77; N, 11.64. Found: C, 49.65; H, 3.80; N, 11.50.

DL-Cycloserine.—A suspension of 306 mg. (1.27 mmoles) of N-5-chlorosalicylidene-DL-cycloserine in 2 ml. of water and 1.4 ml. of 1 *N* hydrochloric acid was stirred at room temperature for 1.5 hr. The remaining solid, weighing 210 mg. (100%), was filtered and identified as 5-chlorosalicylaldehyde by its infrared spectrum. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in 0.87 ml. of 7% concentrated ammonium hydroxide. The cold solution was diluted with 7 ml. of a 1:1 ethanol–isopropyl alcohol mixture and acidified dropwise at 0° with glacial acetic acid. The crystalline DL-cycloserine, 44 mg. (35%), m.p. 142–143°, was collected and dried *in vacuo*. It was identical with D-cycloserine in both BAW and MPW paper chromatography systems and differed from D-cycloserine only in the 10–12-μ region of its infrared spectrum.

Conversion of N-5-Chlorosalicylidene-D-cycloserine into N$^{\omega}$-5-Chlorosalicylidene-β-aminoxy-D-alanine Methyl Ester Dihydrochloride (XIV).—A solution of 267 mg. (1.1 mmoles) of N-5-chlorosalicylidene-D-cycloserine in 5 ml. of methanol containing excess hydrogen chloride was allowed to stand 4.5 hr. at room temperature. The solution was evaporated to about 1-ml. volume and 5 ml. of ether was added. The crystalline product, 333 mg. (97%), m.p. 157–159°, was identified as N$^{\omega}$-5-chlorosalicylidene-β-aminoxy-D-alanine methyl ester dihydrochloride by comparison of its infrared spectrum (Nujol) with that of an authentic sample.

N$^{\omega}$-(5-Chlorosalicylidene)-β-aminoxy-D-alanine Methyl Ester (XIV).—A solution of 162 mg. (1.04 mmoles) of 5-chlorosalicylaldehyde and 216 mg. (1.04 mmoles) of β-aminoxy-D-alanine methyl ester dihydrochloride[18] in 5 ml. of methanol was allowed to stand 4 hr. at room temperature. Evaporation of the solution under a nitrogen stream to 0.5-ml. volume followed by addition of about 5 ml. of ether gave 310 mg. (91%) of N$^{\omega}$-(5-chlorosalicylidene)-β-aminoxy-D-alanine methyl ester dihydrochloride: m.p. 156–159°; λ^{Nujol} 5.85 (C=O), 6.05, 6.12, 7.9, 8.3 μ; λ_{max}^{MeOH} 220 mμ (ϵ 16,610), 258 (9980), 323 (5170).

Decomposition of N$^{\omega}$-(5-chlorosalicylidene)-β-aminoxy-D-alanine Methyl Ester.—A saturated aqueous solution of potassium bicarbonate was added dropwise to a solution of 1.4 g. (4.3 mmoles) of N$^{\omega}$-(5-chlorosalicylidene)-β-aminoxy-D-alanine methyl ester dihydrochloride in 7 ml. of water until precipitation was complete. The oil was extracted with one 40-ml. and two 15-ml. portions of ethyl acetate and the dried (anhydrous magnesium sulfate) extracts were evaporated to dryness under a nitrogen stream. The oily residue crystallized on standing overnight in the refrigerator under petroleum ether. The crystalline amino ester, 1.02 g. (81%), m.p. 75–76°, $[\alpha]^{26}D$ −51° (*c* 1.19, methanol), was centrifuged and dried *in vacuo*. The analytical sample was obtained by recrystallization of the product from ether.

Anal. Calcd. for $C_{11}H_{13}ClN_2O_4$: C, 48.45; H, 4.80; Cl, 13.00; N, 10.28. Found: C, 48.46; H, 4.89; Cl, 13.28; N, 10.10.

Without solvent, 802 mg. (2.9 mmoles) of the above amino ester was heated in an oil bath at 90–95° for 1.5 hr. The dark red liquid was extracted with three 5-ml. portions of boiling ether. The extracts, on evaporation, gave a partially crystalline residue which was extracted with three 10-ml. portions of a 4:1 petroleum ether–ether mixture. When these combined extracts were evaporated, 344 mg. (98%) of 5-chlorosalicylaldoxime, m.p. 109–119°, was obtained. Recrystallization of this material gave oxime, m.p. 122–124°, with an infrared spectrum and melting point identical with an authentic sample.

N-(2-Hydroxy-5-chlorobenzyl)-D-cycloserine (VIII).—To a solution of 2.0 g. (8.35 mmoles) of Schiff base in 120 ml. of absolute ethanol was added 0.70 g. (5.20 mmoles) of sodium borohydride. The reaction solution was stirred at 10° for 17 hr. The reaction solution was acidified to pH 5 with acetic acid after allowing it to warm up to room temperature. Crystallization began and, after the mixture was cooled, 1.56 g. (78%) of crude product, m.p. 180–184°, was collected on a filter. The infrared spectrum showed absorption in the 6.1–6.6-μ region similar to that characteristic of cycloserine. An analytical sample of N-(2-hydroxy-5-chlorobenzyl)-D-cycloserine [m.p. 184–187°; $[\alpha]^{25}D$ +14° (*c* 1.0, 1.0 *N* NaOH); λ_{max}^{MeOH} 205 mμ (ϵ 8800), 227 (8460), 287 (1825)] was obtained by recrystallization of the crude product from 25:1 methanol–water mixture. This product gave a positive test with the Jones reagent.[17]

Anal. Calcd. for $C_{10}H_{11}ClN_2O_3$: C, 49.49; H, 4.57; N, 11.55. Found: C, 49.32; H, 4.68; N, 11.33.

N$^{\alpha}$-(2-Hydroxy-5-chlorobenzyl)-β-aminoxy-D-alanine Methyl Ester Dihydrochloride (IX).—A solution of 10 ml. of methanol saturated with hydrogen chloride gas was stirred with 200 mg. (0.83 mmole) of reduced Schiff base (VIII) for 1.25 hr. This solution was evaporated under nitrogen, washed with two 5-ml. portions of ether, and dried *in vacuo* yielding 280 mg. (98%) of crude product. Crystallization from a methanol–ether mixture yielded 151 mg. of the dihydrochloride derivative, m.p. 109–113°. An analytical sample which contained one molecule of methanol of crystallization and melting at 93–97° was obtained by two recrystallizations from a methanol–ether mixture.

Anal. Calcd. for $C_{11}H_{17}Cl_3N_2O_4 \cdot CH_3OH$, C, 37.96; H, 5.58; Cl, 27.25; N, 7.38. Found: C, 37.92; H, 5.66; Cl, 26.95; N, 7.39.

N$^{\omega}$-Isopropylidene-N$^{\alpha}$-(2-hydroxy-5-chlorobenzyl)-D-alanine Methyl Ester Hydrochloride (X).—A solution of 225 mg. (0.65 mmole) of IX in 10 ml. of acetone and 1 ml. of distilled water was refluxed for 1.25 hr. The reaction solution was evaporated to dryness *in vacuo* yielding 201 mg. (88%) of the isopropylidene derivative, m.p. 168–174°. An analytical sample melting at 168–174° was obtained by recrystallization from ethyl acetate.

Anal. Calcd. for $C_{14}H_{19}Cl_2N_2O_4$: C, 47.87; H, 5.74; N, 7.98. Found: C, 48.12; H, 5.82; N, 8.25.

Diacetyl Derivative (XI) of N-(5-Chlorosalicylidene)-D-cycloserine.—To a solution of 502 mg. (2.12 mmoles) of the Schiff base VI in 20 ml. of dry pyridine (dried over Type 4A Molecular Sieves) at 0° was added 0.80 ml. (8.7 mmoles) of acetic anhydride. The reaction mixture had an observed optical rotation of 0.09° at 5 min., 0.03° at 15 min., and 0.01° at 35 min. after mixing. [A solution of the Schiff base in pyridine lost only 5% of its optical activity in 3 hr. and a similar solution containing 8% acetic acid (by volume) lost 10% of its activity in the same length of time.] The reaction solution was stirred for 2.75 hr. at 0° and then diluted to a total volume of 75 ml. with distilled water. The mixture was extracted with two 60-ml. portions of ethyl acetate and the combined ethyl acetate extracts were washed with two 75-ml. portions of distilled water, one 75-ml. portion of 5% potassium bicarbonate solution, and then two 75-ml. portions of distilled water. The ethyl acetate layer was dried (anhydrous magnesium sulfate) and evaporated yielding 630 mg. (85%) of diacetyl N-(5-chlorosalicylidene)-D-cycloserine: m.p. 131–137°; $[\alpha]^{26}D$ 0°; λ_{max}^{MeOH} 200 mμ (ϵ 18,460), 252 (15,090), 297 (2430); τ ($CDCl_3$) 7.65 (one band, 3H), 7.58 (one band, 3H), 1.95–2.95 (eight bands), 1.60 (one band, 1H). An analytical sample melting at 143–146° was obtained by recrystallization of the crude product from cyclohexane.

Anal. Calcd. for $C_{14}H_{13}ClN_2O_5$: C, 51.78; H, 4.04; N, 8.63. Found: C, 51.47; H, 4.22; N, 8.68.

Repetition of this acetylation reaction gave varying yields of the diacetyl derivative plus varying quantities of an amorphous material which was purified by precipitation from chloroform with cyclohexane and silica gel column chromatography. The purified material was homogeneous by thin layer chromatography and did not hydrolyze in 0.3 *N* hydrochloric acid.

The Formation of 3,6-Bis[N-(5-chlorosalicylidene)aminoxymethyl)]-2,5-diketopiperazine (IVb). 1. From D-Cycloserine and 5-Chlorosalicylaldehyde. In Boiling Ethanol.—A mixture of 204 mg. (2.0 mmoles) of D-cycloserine, 322 mg. (2.1 mmoles) of 5-chlorosalcylaldehyde, and 10 ml. of ethanol was refluxed 5 hr. and the resulting solution was evaporated to dryness under a stream of nitrogen. The crystalline residue, m.p. 205–220° dec., weighed 500 mg. (100%). The crude product was recrystallized from DMF and water giving 137 mg. of the bis-(5-chlorosalicylidene) derivative of cycloserine, m.p. 226–229°, $[\alpha]^{22}D$ +44° (*c* 1.26, DMF). Its infrared (Nujol) spectrum differed from that of "optically pure" derivative at 3.1–3.3, 6.0, 9.5, and 11.2 μ, and showed the diketopiperazine bands of Brockmann and Musso[31] at 6.8 and 7.5 μ.

Anal. Calcd. for $C_{20}H_{18}ClN_4O_6$: C, 49.91; H, 3.77; Cl, 14.73; N, 11.64. Found: C, 49.82; H, 4.08; Cl, 14.77; N, 11.96.

A. The Dimerization of D-Cycloserine Followed by the Introduction of 5-Chlorosalicylaldehyde (5-CSA).—A solution of 100 mg. (1.1 mmoles) of D-cycloserine in 2 ml. of DMF and 1 ml. of distilled water was heated at 70° for 1 hr. At the end of this time, 157 mg. (1.0 mmole) of 5-chlorosalicylaldehyde was

(31) H. Brockmann and H. Musso, *Ber.*, **89**, 250 (1956).

added to the reaction solution and heated at 70° for 10 min. The work-up, which followed the same procedure as in part C, gave 171 mg. of water insolubles. After extraction of this solid with ether and ethyl acetate to remove starting materials, the remaining product weighed 130 mg. (70%), m.p. 240–243°, $[\alpha]^{28}D$ +150° (*c* 1.0, DMF). The infrared spectrum (Nujol) was identical with that of "optically pure" dimer derivative.

B. The Dimerization of D-Cycloserine in the Presence of 5-CSA.—A mixture of 201 mg. (2 mmoles) of D-cycloserine and 158 mg. (1 mmole) of 5-chlorosalicylaldehyde was dissolved in 2 ml. of dimethylformamide and 1.5 ml. of distilled water. The reaction solution was heated at 70° for 1 hr. Crystals separated at the end of 0.5 hr. and the crude product was obtained as in part C. It weighed 194 mg., m.p. 200–240°. The solid was extracted with two 5-ml. portions of hot ether and two 5-ml. portions of hot ethyl acetate. A small amount of unreacted reactants was removed by these extractions. The solid remaining after these extractions weighed 154 mg. (80%), m.p. 234–244°, $[\alpha]^{31}D$ +122° (*c* 1.00, DMF). The infrared spectrum (Nujol) was essentially identical with that of other samples of cycloserine dimer derivative (IVb).

C. The Reaction of D-Cycloserine with N-5-Chlorosalicylidene-D-cycloserine (VI).—A mixture of 241 mg. (1.0 mmole) of Schiff base and 101 mg. (1.1 mmoles) of D-cycloserine was dissolved in 2 ml. of DMF and 1 ml. of distilled water. The reaction solution was heated at 70° for 1 hr. and crystals began to separate at the end of 0.5 hr. The reaction mixture was then diluted to a total volume of 10 ml. with distilled water. The suspension of product was centrifuged and the mother liquor was decanted from the solid precipitate. The solid was washed with two 5-ml. portions of distilled water. The solid was dried under vacuum over phosphorus pentoxide and weighed 230 mg. (94%), m.p. 224–231°, $[\alpha]^{27}D$ +94° (*c* 1.00, DMF). The infrared spectrum (Nujol) was essentially identical with the other samples of cycloserine dimer derivative (IVb).

2. From the D Dimer of Cycloserine.—A mixture of 200 mg. (1 mmole) of D-cycloserine dimer[28] V, 315 mg. (2 mmoles) of 5-chlorosalicylaldehyde, and 10 ml. of ethanol was stirred magnetically at room temperature for 27 hr. and the solvent was evaporated under a nitrogen stream giving a solid residue which was washed twice with 10-ml. portions of ether and twice with 3-ml. portions of water. It had a melting point of 245–247°, $[\alpha]^{30}D$ +149° (*c* 1.33, DMF), and weighed 428 mg. (84%). This product was recrystallized from 2 ml. of a 1:1 DMF–water mixture giving 284 mg., m.p. 244–245°, $[\alpha]^{22}D$ +152° (*c* 1.35, DMF), of analytically pure 3,6-bis[N-(5-chlorosalicylidene)-aminoxymethyl]-2,5-diketopiperazine (IVb). Its infrared spectrum (Nujol) showed bands at 3.05 (N–H), 5.9, 6.0 (C=O), 6.2 (C=N), 9.75, and 12.0 μ, and the ultraviolet spectrum showed absorption at λ_{max}^{MeOH} 220 mμ (ϵ 39,900), 258 (22,280), and 323 (10,330).

Anal. Found: C, 49.97; H, 3.99; Cl, 14.67; N, 11.82.

3. From the *meso* Dimer of Cycloserine.—A mixture of 103 mg. (0.5 mmole) of *meso* dimer, 160 mg. (1 mmole) of 5-chlorosalicylaldehyde, and 7 ml. of ethanol was stirred magnetically at room temperature for 16 hr. and the resulting slurry was evaporated to dryness under a nitrogen stream. The residue was washed three times with 5 ml. of ether and the resulting product, m.p. 211–220°, weighed 202 mg. (88%). The crude product was recrystallized from 2 ml. of a 2:1 DMF–water mixture giving 125 mg., m.p. 221–223°, of analytically pure 3,6-bis[N-(5-chlorosalicylidene)aminoxymethyl]-2,5-diketopiperazine. Recrystallization caused no change in its infrared spectrum. Along with the expected infrared bands (Nujol) at 3.1 (N–H), 5.95, 6.05 (C=O), 6.1–6.25 (C=N), 6.8, and 7.55 μ, the bands characteristically appearing in partially racemized dimer derivatives (IVb) at 9.6, 9.8, and 12.2 μ were also evident.

Anal. Found: C, 49.63; H, 3.99; N, 11.78.

***meso*-3,6-Bis(aminoxymethyl)-2,5-diketopiperazine (*meso* Dimer).**—A suspension of 501 mg. of DL-cycloserine in 25 ml. of absolute ethanol containing 1 ml. of acetic acid was refluxed 40 min. and the insoluble solid was filtered and washed with ethanol and ether successively. The product weighed 299 mg., m.p. 335°. The combined filtrate and washings were evaporated to 0.5-ml. volume and the mixture was stirred with a little ether. The precipitated product, 135 mg., differed considerably in its infrared spectrum from first crop obtained above. The first crop was recrystallized by solution in 0.7 ml. of water and slow addition of 5 ml. of ethanol. The crystalline precipitate, 204 mg., m.p. 300°, had an infrared spectrum (Nujol) differing from the D dimer by a band at 7.7 μ and by the absence of bands at 10.2, 11.9, and 12.9 μ. This product is tentatively assigned the *meso* configuration because its bis-5-chlorosalicylidene derivative had an infrared spectrum having bands which appear in the spectra of the mixtures of *meso* and *active* dimer derivatives obtained from reactions A, B, and C.

D-3,6-Bis(hydroxymethyl)-2,5-diketopiperazine from D-3,6-Bis(aminoxymethyl)-2,5-diketopiperazine (V).—To a solution of 870 mg. (4.4 mmoles) of D-cycloserine dimer V in 6 ml. of 10% hydrochloric acid was added dropwise 15 ml. of 1 *N* sodium nitrite solution (15 mmoles) with stirring over a 30-min. period at room temperature. The reaction solution was heated 30 min. at 50°, cooled, and put on a column containing 25 mequiv. of Dowex 50-X8 ion-exchange resin. The column was eluted with 200 ml. of distilled water over a 4-hr. period and the eluate was lyophylized. The solid residue was dissolved in 6 ml. of hot water and evaporated under a nitrogen stream to a volume of 3 ml., and 0.5 ml. of absolute ethanol was added. Upon cooling crystallization occurred giving 319 mg. of D-3,6-bis(hydroxymethyl)-2,5-diketopiperazine, m.p. 245–248°, $[\alpha]^{26}D$ +55.1° (*c* 0.98, water). A second crop weighed 106 mg. bringing the total yield to 58%. The infrared spectrum was identical with that of an authentic sample of serine anhydride obtained by the method of Brockmann and Musso.[31]