Tetrahedron Letters No.38, pp. 2697-2700, 1964. Pergamon Press Ltd.
Printed in Great Britain.

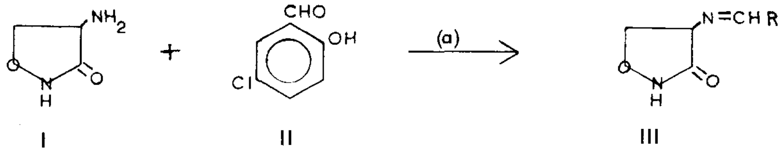
ON THE MECHANISM OF CYCLOSERINE DIMERIZATION IN THE PRESENCE OF 5-CHLOROSALICYLALDEHYDE

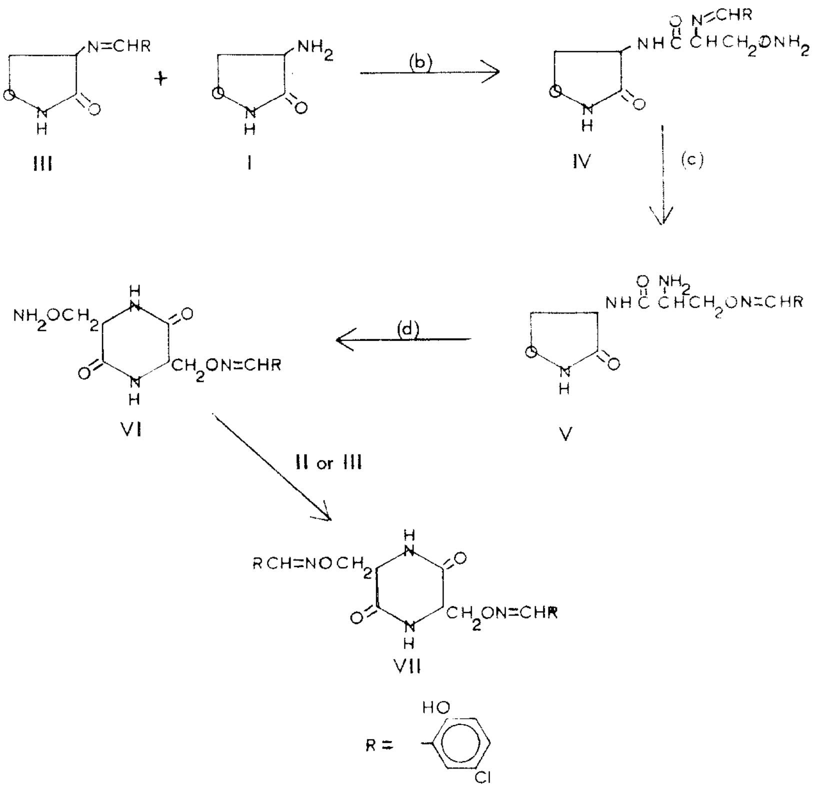
Charles H. Stammer and James D. McKinney
University of Georgia
Athens, Georgia

(Received 27 July 1964)

A cycloserine-pyridoxal Schiff base has been implicated (1, 2, 3) in the mechanism by which cycloserine inhibits certain pyridoxal-dependent enzyme systems. In our previous work (4), we described the first synthesis of a Schiff base (III) of D-cycloserine (I) (5) and established that it does not rearrange into the dimer derivative (VII) as suggested earlier by Michalsky and coworkers (3). These workers showed that DL-cycloserine reacts with several aldehydes in boiling ethanol giving dimer derivatives and we confirmed this result using 5-chlorosalicylaldehyde. We concluded (4) that VII was formed by the dimerization of cycloserine prior to its reaction with aldehyde, since other pathways, including that through the Schiff base, seemed unlikely.

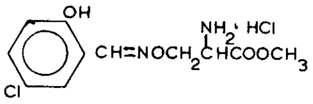
Contrary to this conclusion, our more recent studies indicate that the Schiff base is an intermediate leading to dimer; not by rearrangement, but via reaction with cycloserine itself. The postulated mechanism is outlined in the following scheme.





Rapid formation of Schiff base (III) followed by the attack of cycloserine on the isoxazolidone ring of III would form IV. Rearrangement of IV to V followed by intramolecular isoxazolidone ring opening would yield the partially derivatized dimer (VI). This intermediate can afford VII by reaction with the aldehyde *or* the Schiff base.

We know (4) that step (a) of this sequence occurs quite readily. We have found that the key step (b) of this mechanism occurs very rapidly when cycloserine and III are heated together in aqueous N, N-dimethyl formamide (DMF). An 80% yield of the dimer derivative (VII) was formed. Possible precedent for step (c) is seen in the methanolysis of III which gives the β-aminoxyalanine derivative VIII. This conversion



VIII

may well proceed through ring opening followed by an intramolecular rearrangement similar to steps (b) and (c) (6). Intermediate V would be expected to rearrange rapidly into the aminoxydiketopiperazine VI, since the very facile dimerization of cycloserine itself must proceed by a similar route. Conversion of VI into VII might be accomplished by reaction of VI with either II or III. Indeed, we have found that cycloserine dimer (3, 6-bis[aminoxymethyl]-2, 5-diketopiperazine) reacted with III very rapidly in aqueous DMF yielding VII quantitatively.

We prepared the dimer derivative (VII) three different ways (7,8) in aqueous DMF at 70°. The properties of these products are summarized in Table I. The infrared spectra of these products differed only in the intensities of bands at 6.05, 9.6, 12.0 and 12.2μ (9).

TABLE I

Reactants	Yield	Melting Point	Specific Rotation
(A) I + I (followed by addition of II)	70	240-243°	+150°
(B) I + II	94	224-231°	+94°
(C) I + III	80	234-244°	+122°

Assuming the product of reaction A to be essentially one optical isomer (7), partial racemization apparently occurred during both reactions B and C. This is consistent with the mechanism just proposed for reaction B. We observed that Schiff base (III) was 46% racemized in one hour at 70° in aqueous DMF and thus we expected some racemization when *preformed* Schiff base (reaction C) was used. Since racemization also occurred in reaction B the Schiff base is very probably an intermediate also in this reaction (10). On kinetic grounds, we might expect that *more* racemization would occur in B than in C, since the Schiff base molecules would have a longer lifetime during the former reactions The results bear this out.

The mechanism by which cycloserine inhibits pyridoxal-dependent enzyme systems *in vivo* may or may not be that suggested here. Our present studies with pyridoxal may clarify this.

We very gratefully acknowledge the financial support of the National Institutes of Health, Grant No. AI 05539-01.

REFERENCES

1. J. L. Strominger, *Fed. Proc.*, **22**, 423 (1963).
2. R. M. Khomutov, et. al. *Doklady Akad. Nauk.*, **140**, 492 (1961).
3. J. Michalsky, J. Opichal and J. Ctvrtnik, *Monatsh.*, **93**, 618 (1962).
4. C. H. Stammer, *Experientia*, 1964, in press.
5. The natural *D*-Isomer of cycloserine was used throughout this work.
6. We are investigating the possibility that alcoholysis of the azomethine linkage *precedes* ring opening. An intermediate of type IV would not then occur in the conversion of III to VIII.
7. "Authentic" dimer derivative prepared by treating cycloserine with ethanolic acetic acid [F. C. Neuhaus and J. L. Lynch, *Biochem.*, **3**, 471 (1964)] followed by 5-chlorosalicyladehyde had $[\alpha]_D^{22}$ + 152^0 (c, 1 in DMF), m. 244-246^0, and showed neither 9.6 nor 12.0µ infrared bands.
8. We previously reported (4) m. 226-229^0, $[\alpha]_D^{22}$ + 43.6^0 (c, 1 in DMF), for the dimer derivative. This was obtained from reaction B in boiling ethanol in which cycloserine is insoluble. The lifetime of the Schiff base is much greater under these conditions than in aqueous DMF which dissolves cycloserine.
9. H. Brockmann and H. Musso [*Ber.*, **89**, 241 (1956)] showed that among the *DL* and *meso* amino acid "anhydrides" investigated, the major infrared spectral differences occurred in the 8.6-9.75µ and 11.6-14.0µ regions.
10. The dimer derivative (VII) is optically stable under the reaction conditions.