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ROLE OF THE AZOMETHINE IN THE DIMERIZATION OF CYCLOSERINE BY ALDEHYDES[1]

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Abstract—Further elucidation of the mechanism by which an aldehyde converts cycloserine (I) into its dimer is reported. A "prior dimerization" mechanism is eliminated and a carbonolamine is shown to be a possible intermediate when "electrophilic" aldehydes react. Azomethine formation is shown to be a necessary step and an aminal intermediate nicely explains the results of some experiments involving racemic reactants. Both the medium and the nature of the aldehyde determine the preferred pathway of dimerization. Attempts to prepare a D-cycloserine pyridoxal Schiff base are described.

IN OUR earlier report on the dimerization of cycloserine in the presence of aldehydes[2] we postulated the reaction path shown in Scheme I in which the aldehyde was 5-chlorosalicylaldehyde (5-CSA). The Schiff base **3** was considered a probable intermediate in this process. We also showed that **3** reacts rapidly with cycloserine in aqueous DMF giving the dimer derivative **7a**. We suggested that this same pathway might be responsible for the inhibition of pyridoxal-dependent enzyme systems by the antibiotic. This report describes research indicating that **3** may be a necessary intermediate in this conversion, at least when the aldehyde is 5-CSA and the reaction is carried out in aqueous DMF at 70°.

In an attempt to obtain evidence for the formation of the intermediate **4** we prepared[2] the "reduced Schiff base" **8** and allowed it to react with cycloserine hoping to obtain a dihydro-**4** which could not rearrange further to **5**. To our surprise, there was no reaction and **8** was recovered unchanged in 89% yield. This unexpected result suggested that the azomethine linkage, absent in **8**, was required for the reaction of the isoxazolidone ring with cycloserine. This is consistent with an earlier finding that N-acetylcycloserine also did not react with cyloserine to form a peptide like **4**.

One reaction path (Scheme II) which requires the azomethine for the formation of **7** invokes the prior dimerization† of cycloserine to form **9** which in turn reacts with the Schiff base **3** giving cycloserine and **7** by aldehyde transfer. This readily explains the unreactivity of dihydro-**3** (**8**) since it is incapable of acting as an aldehyde donor.

Using the 5-CSA Schiff base of 2-trityl-DL-cycloserine[3] (**10**), this "prior dimerization" path was eliminated from consideration. No dimer derivative (**7a**) was obtained when **10** was allowed to react with cycloserine, but, when treated with the dimer **9** under the same conditions, **10** gave an 88% yield of **7a**. We concluded that if cycloserine had been

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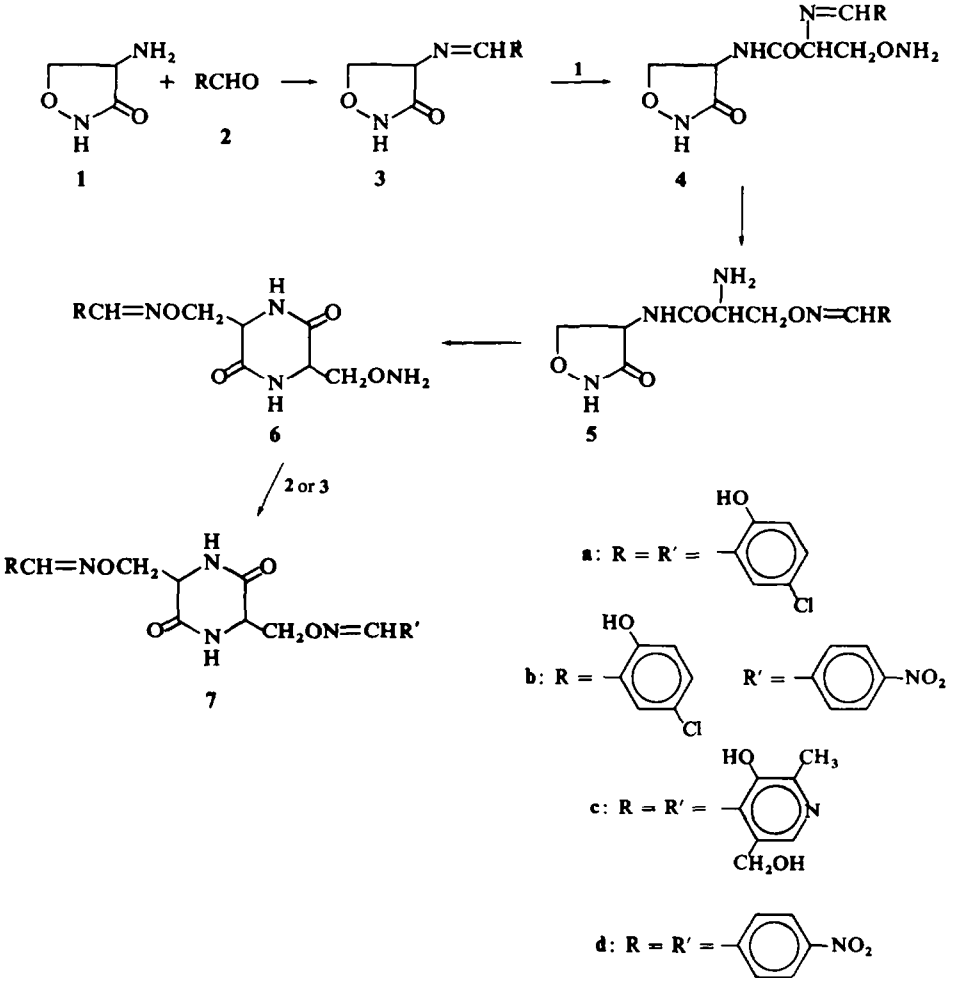
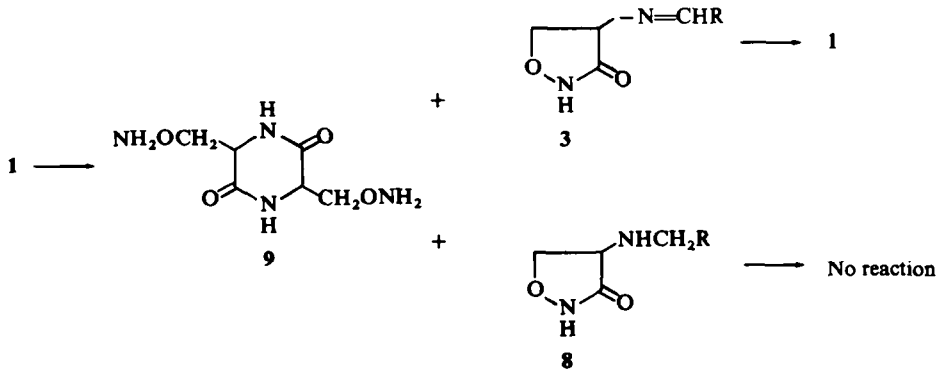
† Since cycloserine solutions rapidly become antibacterially inactive, it has long been assumed that dimerization is responsible. Much of our work indicates that dimer (**9**) is probably *not* the major component in these solutions.

Scheme I

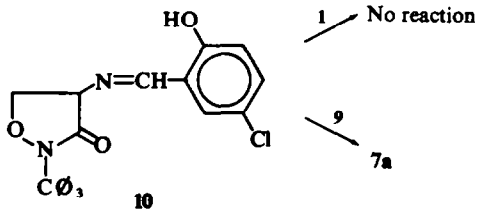


Scheme II



dimerizing rapidly, **7a** should have been formed in the first reaction also (Scheme III). Since no reaction occurred between **1** and **10**, no dimer **9** could have been present. The bulky trityl group at the 2-position in **10** was expected[4,*] to prevent direct nucleophilic attack of the amino group in **1** on the ring carbonyl thus forcing the reaction to proceed by the "prior dimerization" pathway if at all possible.

SCHEME III



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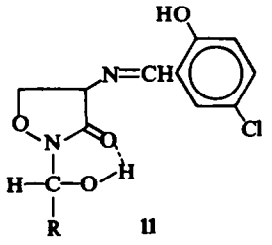
Since "prior dimerization" was apparently not occurring, alternative pathways were considered. Some hydrolysis† of **3** was known to occur in aqueous DMF and it seemed possible that the 5-CSA so liberated might be reacting with the ring nitrogen of cycloserine giving a small concentration of a carbinolamine (**11**, R = 2-hydroxy-5-chlorophenyl) which was the actual actylating agent.‡ Hydrogen-bonding of the OH group to the ring CO should increase its electrophilicity and possibly stabilize



11

the molecule. Attempts to prepare a stable carbinolamine of **3** using 5-CSA failed, but *p*-nitrobenzaldehyde (*p*-NBA) did indeed form a stable crystalline carbinolamine (**11**, R = *p*-nitrophenyl) having all the expected spectral properties.§ This carbinolamine was indeed a better acylating agent than the parent Schiff base **3** since it reacted with cycloserine even in boiling ethyl acetate‖ forming the mixed dimer derivative **7b**,

* Even the azomethine group feels the effect of the trityl as shown by an upfield shift of the azomethine proton upon tritylation of **3**.

† A detectable amount of cycloserine is always present in aqueous DMF solutions of the Schiff base **3**.

‡ The possibility that aldehydes add to the ring rather than the amino nitrogen was first suggested by Michalsky.

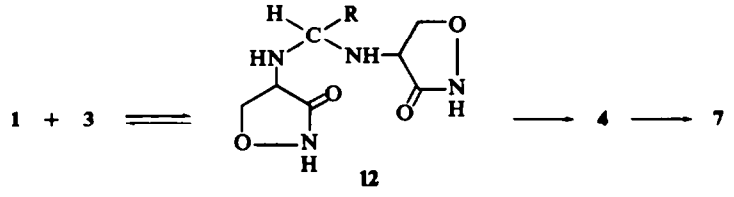
§ The synthesis and properties of several carbinolamines of this type will be reported later.

‖ D-cycloserine is insoluble in ethyl acetate.

while the Schiff base **3** was unchanged under these conditions.* NMR studies of solutions containing Schiff base **3** and 5-CSA, however, gave no indication that a carbinolamine was forming. We feel that adducts like **11** may play a part in the formation of the pyridoxal†(**7c**) and *p*-nitrobenzaldehyde (**7d**) dimer derivatives when these aldehydes react with cycloserine. Both have more electrophilic carbonyl functions than 5-CSA and, thus, are more capable of reacting at the ring nitrogen atom. 5-Chlorosalicylaldehyde, however, most probably converts cycloserine into its dimer via the Schiff base without intermediacy of a carbinolamine.

The fact that **3**, the 5-CSA Schiff base, reacted with cycloserine in *anhydrous* DMF to give **7a** indicated that another pathway not involving Schiff base hydrolysis and carbolamine formation was indeed available. If one considers the high reactivity of azomethine linkages toward nucleophiles,‡ it is reasonable that cycloserine might add to the azomethine linkage giving an aminal structure **12**, which collapses rapidly into the acylated cycloserine§**4** (Scheme IV). If the formation of an animal intermediate such as **12** does occur, the Schiff base (**3**) should react with amines other than **1**

SCHEME IV



giving β-aminoxy-D-alanyl amides. Other primary amines did react with **3**, but we have not succeeded in identifying the numerous products. The racemization experiments described below can also be explained by the **1** + **3** ⇌ **12** equilibrium, making this hypothesis more attractive.

In a further investigation of the reaction between cycloserine (**1**) and its Schiff base (**3**) we determined the yields and optical purities of the dimer derivative (**7a**) which was formed when D-cycloserine reacted with both active and racemic Schiff base and when racemic cycloserine reacted with active Schiff base. The stoichiometry of the overall reaction required two molecules of Schiff base for each of cycloserine (see Scheme **1**) however, the reactions were carried out using a 1 : 1 ratio of the reactants so that relative rates of competitive reactions might be observable. Table 1 shows the results of these reactions:

* The carbinolamine (R = *p*-nitrophenyl) was stable to boiling methanol for twelve hours indicating that alcoholysis does not occur. It had been suggested earlier by Michalsky[5] that alcoholysis of such a carbinolamine might lead to an α-amino ester which could dimerize forming a piperazinedione derivative like **7**. In the light of our earlier work[2] on α-amino esters of this type, these present results conclusively rule out that mechanism.

† Schiff base **3** did react with both pyridoxal and pyridoxal hydrochloride giving crude products having the infra spectra expected of carbinolamines like **11**. We were unable to purify and characterize these materials.

‡ Azomethines are known to be intermediates in amine-catalyzed carbonyl derivatizations.[6]

§ Dreiding models show a very closer approach of the amino group to the ring carbonyl in **12**. The participation of a similarly placed OH group has been postulated.[7]

TABLE 1. RACEMIZATION DURING FORMATION[a] OF DIMER 7a

	$[\alpha]_D$ of 7a[b]	Optical purity of 7a	Yield of[c] 7a
(1) D − 1 + DL − 3	+43°	28%	85%
(2) DL − 1 + D − 3	+34	22	82
(3) D − 1 + D − 3	+94	62	94

[a] Reactant ratio 1:1. Reactions carried out in DMF—H_2O (2:1) at 70° for 1 hr.

[b] Pure 7a shows $[\alpha]_D^{25}$ +152°.

[c] Based on Schiff base 3.

In our previous report[2] on reaction (3), we proposed that the Schiff base was partially thermally racemized before its reaction with cycloserine thus leading to optically impure 7a. Thus the optically purity of 7a obtained in reaction (3) where both reactants were optically pure was established as the maximum optical purity obtainable in any reaction between 1 and 3. The Schiff base racemization hypothesis could be used to explain the low optical purity of a 7a obtained from reaction (2), but it cannot be invoked to explain the results of reaction (1) because the Schiff base used in (1) was already completely racemic. Another process leading to the predominate formation of *meso*-7a must therefore be occurring.

If we examine carefully the steps in the overall process (Scheme I) which involved 1 and 3, two possible explanations for these results present themselves. Scheme V shows the pertinent reactions, intermediates and assigns rate constants to the various steps. As is readily seen, DL-cycloserine was liberated in the second step of reaction (1),

SCHEME V

$$\text{(1) D} - 1 + \text{D} - 3 \xrightarrow{k_1} \text{DD} - 6^* + \begin{cases} \text{D} - 3 \xrightarrow{k_3} \text{DD} - 7 + D - 1 \\ \text{L} - 3 \xrightarrow{k_4} \text{DD} - 7 + \text{L} - 1 \end{cases}$$

$$\text{D} - 1 + \text{L} - 3 \longrightarrow \text{DL} - 6^\dagger + \begin{cases} \text{D-3} \xrightarrow{k_5} \\ \text{L-3} \xrightarrow{k_6} \end{cases} meso - 7 + \text{DL} - 1$$

$$\text{(2) D-1} + \text{D-3} \xrightarrow{k_1} \text{DD-6} + \text{D-3} \xrightarrow{k_3} \text{DD-7} + \text{D-1}$$

$$\text{L-1} + \text{D-3} \xrightarrow{k_2} \text{DL-6}\dagger + \text{D-3} \xrightarrow{k_7} meso\text{-7} + \text{D-1}$$

while only D-cycloserine was obtained in reaction (2). It is reasonable to expect this liberated cycloserine to recycle by reacting with Schiff base (3) to form dimer derivative (7a). If this occurred to a considerable extent, we would expect the 7a formed in reaction (1) to be of lower optical purity than that obtained in reaction (2) because the recycling cycloserine in reaction (1) is racemic. Experimentally, however, the opposite order of

* DD-6 has the 3- and 6-substituents in the *cis* relationship.

† DL-6 has the 3- and 6-substituents in the *trans* relationship. This compound is optically active.

‡ Constants k_1, k_2 and k_3 are equal to those used in reaction (1).

§ This DL-6 is the enantiomer of that formed in reaction (1).

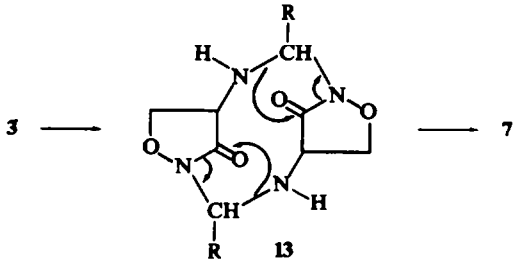
optical purity was observed (Table 1) and we can infer that recycling is not an important process. The absence of recycling also implies that k_1 and k_2 are considerably larger than the other rate constants, since apparently the Schiff base (3) had been consumed before reaction with the liberated cycloserine could occur.

It seems clear that only a considerable difference between k_1 and k_2 can explain the low optical purity of the dimer (**7a**) obtained in these reactions. In other words asymmetric induction caused D-cycloserine to "prefer" reaction with L-Schiff base rather than D-Schiff base. It is the ratio of DD-**6** to DL-**6** in both of these reactions which ultimately determines the optical purity of the final product. In reaction (1), where Schiff base racemization cannot be a factor, the ratio of *meso*- to DD-**7a** is 2·5 to 1 and thus, the k_2 to k_1 ratio must be approximately the same. The first step as shown for both reactions (1) and (2) in Scheme V is actually a composite of three reactions (Scheme I); the first is a bimolecular combination of **1** and **3** followed by two unimolecular rearrangements to give the piperazinedione (**6**). The two rearrangement reactions are reasonably expected to be faster* than the first, so that the k_2/k_1 ratio is a measure of the first bimolecular rate-controlling combination of cycloserine (**1**) and Schiff base (**3**). The second step, conversion of (**6**) into **7** by aldehyde transfer from **3**, may be slower than the first (as inferred from the lack of cycloserine recycling) and thus rate determining. We would expect, however, that this step might be configurationally insensitive; i.e. $k_3 \simeq k_5 \simeq k_6 \simeq k_7$, since the asymmetric centers are further removed from the reaction sites than in step one and that very little effect on the product composition would obtain in this step.

If, indeed, the above considerations are correct, the manner in which cycloserine and its Schiff base first combine determines the composition of the dimer even though there is a series of reactions occurring after the primary one. The fact that *meso*-dimer (**7a**) was preferred by a factor of 2·5 gives some support for the equilbrium formation of an animal as the first intermediate in the reaction. The *meso*-animal which has greater symmetry (σ-plane) than the active animal, should be favored entropywise and the equilibrium constant for its formation should be larger than that for the formation of the active compound. This equilibrium preference for the *meso* compound could be the determining factor in product composition.

Two other reactions paths available especially to "reactive" Schiff bases; viz., those with very electrophilic azomethine functions, have not been eliminated. As shown in Scheme VI, an intermediate **13** formed by direct dimerization of the Schiff

SCHEME VI

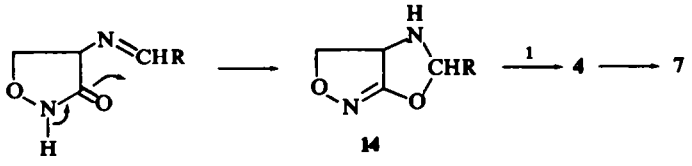


* We know from work with cycloserine peptides that the conversion of **4** into **5** is extremely fast.[3]

base itself through addition of the ring nitrogen atoms to the azomethine linkages is possible. This, of course, would occur most readily when the azomethine group was activated by an electronegative function on the aldehyde moiety, as in *p*-NBA and pyridoxal.* Decomposition of **13** as shown would lead directly to **7**.

Another interesting possibility available most probably to "reactive" Schiff bases is the formation of a cyclic imino ester intermediate (**14**) formed by intramolecular cyclization† as shown in Scheme VII. An intermediate such as **14** should react rapidly‡

SCHEME VII



3 **14**

with cycloserine giving **4** and ultimately **7**. The formation of **14** would require the enolization of the isoxazolidone ring; consequently, if **14** were a necessary intermediate, 2-substituted Schiff bases should be unreactive toward nucleophiles. As discussed earlier in this report, the 2-trityl Schiff base (**10**), which cannot enolize, does not react with cycloserine. We have also found that a 2-methyl Schiff base does not react with cycloserine to give the expected methylated dimer.§ This, of course, is not evidence for the formation of **14** but does suggest that enolization of the ring may facilitate reaction with the ring. On the basis of this evidence, it would seem that *both* the azomethine linkage *and* an enolizable isozazolidine ring are required for dimer formation.

All of this work has been directed toward the elucidation of a reaction path by which cycloserine might react with the aromatic aldehyde pyridoxal in a living system. Our attempts to prepare the crucial pyridoxal Schiff base of cycloserine have given rather inconclusive results. Two major products, one of which showed clearly azomethine absorption in the infrared, were obtained in varying yields. Elemental analysis indicated that the ratio of pyridoxal to cycloserine was greater than one in these adducts.‖ The adduct showing azomethine absorption reacted rapidly in aqueous solution with cycloserine giving the optically inactive dimer derivative **7c** which was identical to that formed when cycloserine and pyridoxal reacted. The optically active form of **7c** was also prepared and characterized so that an authentic sample was available for comparison with these products. Thus, pyridoxal can be irreversibly captured by reaction with cycloserine through a Schiff base intermediate.

In a further attempt to prepare the pure N-pyridoxylidene cycloserine, we examined the reaction of pyridoxal with 2-trityl-DL-cycloserine[3] (**15**) (Scheme VIII). The crystalline trityl compound **16** was readily obtained but detritylation to the desired Schiff

* We found that solutions of the salicyladehyde and 5-nitrosalicylaldehyde Schiff bases of cycloserine slowly deposit dimer derivatives (**7**), while the 5-CSA Schiff base (**3**) did not.

† A similar intramolecular cyclization has been reported for a β-hydroxyimine.[8]

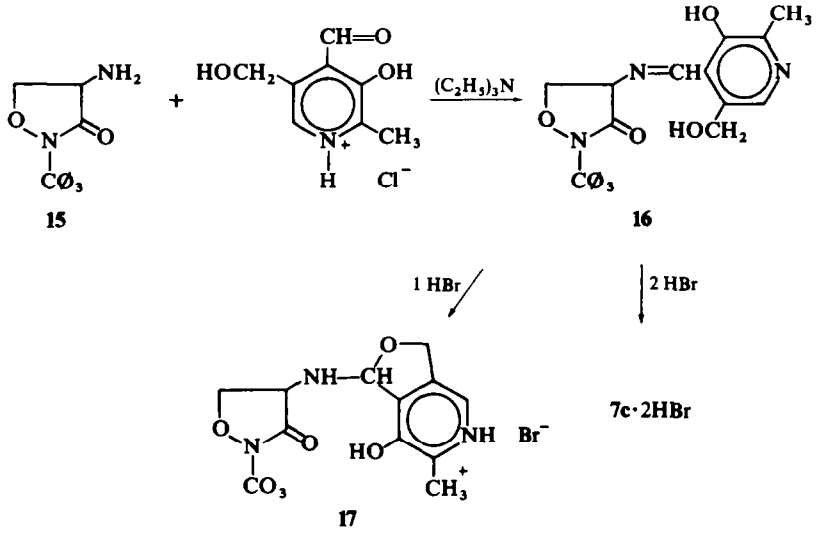
‡ The use of cyclic imino esters to increase the reactivity of the pyrrolidone ring toward nucleophiles has been reported.[9]

§ In this case, a reaction does take place giving some **7a** and other unidentified products.

‖ K. Folkers *et al.*, have reported the synthesis of the pure Schiff base.[10]

base was unsuccessful. When **16** was treated with one equivalent of hydrogen bromide–acetic acid solution, an amorphous solid was obtained which had infrared absorption characteristic of $-NH_3^+$ and trityl groups but none in the 6·0–6·5 μ region indicating the azomethine function. This material was tentatively assigned structure **17** on the basis of this data.* When **16** reacted with two equivalents of hydrogen bromide, the amorphous hydroscopic product obtained had many of the infrared absorption bands characteristic of **7c** and, in particular, showed no azomethine absorption. The NMR spectrum of this product showed absorption at δ 8·82 ppm characteristic of oxime azomethine protons and no absorption in the trityl aromatic region. If this product was indeed crude **7c**·2HBr, it could have been formed by direct Schiff base dimerization as postulated in Scheme VI. This product was not investigated further.

SCHEME VIII



In summary, we feel that in the presence of aldehydes cycloserine may undergo dimerization through Schiff base and/or carbinolamine intermediates. The predominating pathway will depend on the reaction conditions and the "reactivity" of the aldehyde carbonyl group. Aldehydes such as 5-CSA direct dimerization through the Schiff base-aminal path while *p*-NBA and pyridoxal, the more reactive aldehydes, probably use the carbinolamine pathway. Direct Schiff base dimerization and cyclic imino ester formation are also reasonable alternatives, but there is very little evidence for their formation.

EXPERIMENTAL

All m.ps were taken on a Nalge hot stage and are corrected. IR spectra were determined on a Perkin-Elmer Infracord, Models 137 and 237. UV spectra were determined on a Perkin–Elmer spectrophotometer, Model 202. NMR Spectra were determined on Varian Associates A-60 and HA-100 NMR spectrometers.

* Pyridoxal itself undergoes an intramolecular hemiacetal formation similar to this ring closure.[11]

2-(4-*Nitrophenylhydroxymethyl*)-N-(5-*chlorosalicylidene*)-D-*cycloserine* (**11**).

To a soln of 238 mg (0·99 mmole) of (III) in 15 ml of dry EtOAc (dried over Linde molecular sieves, Type 3A) was added 152 mg (1·0 mmole) *p*-nitrobenzaldehyde. The resulting soln was refluxed for 2·5 h and evaporated to dryness. The residue was extracted with two 3-ml portions of hot benzene and the insoluble solid product weighed 285 mg (73%), m.p. 130–133°. An analytical sample was obtained by recrystallization from EtOAc, m.p. 132–134°; $[\alpha]_D^{25}$ 107° (*c* 1·0, EtOAc); IR ($CHCl_3$) 2·95–3·65 (bonded OH), 5·93 (broad C=O), 6·15 (C=N), 6·23 (ϕ; 7·43 μ (NO_2); NMR (acetone-d_6) δ 4·62 (m, 3, isoxazolidone ring protons), 6·69 (s, –CHOH), 7·18 (multiplet-5-CSA aromatics), 8·13 (four doublets two from *p*-NBA of **11**, J = 1·2 Hz; two from free *p*-NBA, J = 1·2 Hz) 8·64 ppm (s, CH=). Found: C, 52·23; H, 3·83; N, 10·78; Cl, 9·24. Calcd. for $C_{17}H_{14}N_3O_6Cl$: C, 52·12; H, 3·60; N, 10·73; Cl, 9·05%).

The formation of 3-(N-5-*chlorosalicylideneaminoxymethyl*)-6-(N-4-*nitrobenzylideneaminoxymethyl*)-2,5-*piperazinedione* (**7b**).

A soln of 204 mg (0·52 mmole) of (**11**) and 56 mg (0·54 mmole) D-cycloserine in 6 ml 95% EtOAc was refluxed for 4 h. The reaction soln was then diluted with 3 ml distilled water and cooled to 10°. The partially crystalline solid ppt was filtered off and washed with one 3-ml portion of a 1:1 95% EtOAc–water. The solid was dried *in vacuo*, wt 128 mg (54%), m.p. 100–130°. An analytical sample was obtained by recrystallization from 1:1 95% EtOAc–water, m.p. 148–152°; IR (Nujol) 3·15 (NH), 5·98 (C=O), 6·60 (NO_2), 12·15 μ (ϕ-Cl). (Found: C, 51·00; H, 4·28; N, 14·35; Cl, 8·15. Calcd. for $C_{20}H_{18}H_5O_7Cl$: C, 50·48; H, 3·81; N, 14·72; Cl, 7·45%).

The formation of 3,6-*bis*-(N-5-*chlorosalicylideneaminoxymethyl*)-2,5-*piperazinedione* (**7a**)

A. *The reaction of* D-*cycloserine with* N-5-*chlorosalicylidene*-D-*cycloserine* (**3**). A mixture of 241 mg (1·0 mmole) of **3** and 101 mg (1·1 mmoles) D-cycloserine was dissolved in 3 ml of a 2:1 DMF–water soln. The reaction soln 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; centrifuged and the supernatant was decanted from the ppt. The solid, which was washed with two 5-ml portions of distilled water and dried under vacuum over P_2O_5, weighed 230 mg (94%), m.p. 224–231°, $[\alpha]_D^{27}$ 94° (*c* 1·00, DMF). The IR spectrum (Nujol) was essentially identical with the other samples of **7a**.

B. *The reaction of* D-*cycloserine with* N-(5-*chlorosalicylidene*)-DL-*cycloserine*. A mixture of 130 mg (0·54 mmole) of **3** and 59 mg (0·58 mmole) D-cycloserine was dissolved in 3 ml of a 2:1 DMF–water soln.

The reaction was carried out as described in part A giving **7a**, wt 110 mg (85%), $[\alpha]_D^{28}$ 43° (c 1·0, DMF). The IR spectrum was indicative of a mixture of optically active and inactive dimer derivatives. Similar experiments using one-half equivalent and one-fourth equivalent of D-cycloserine gave mixtures of dimer derivatives weighing 65 mg (100%), $[\alpha]_D^{25}$ 18° (*c* 1·0, DMF) and 28 mg (94%), $[\alpha]_D^{28}$ 5·6° (*c* 1·0, DMF), respectively.

C. *The reaction of* DL-*cycloserine with* N-(5-*chlorosalicylidene*)-D-cycloserine (VIII). When DL-cycloserine was treated with **3** in a 1:1 molar ratio, the mixture of dimer derivatives isolated by the procedure described above weighed 49 mg (82%), $[\alpha]_D^{28}$ 34° (*c*, 1·0 in DMF).

The formation of (+)-3,6-*bis*-(N-4-*nitrobenzylideneaminoxymethyl*)-2,5-*piperazinedione* (**7d**)

A. *From* (+)-3,6-*bis*(*aminoxymethyl*)-2,5-*piperazinedione* (**9**) *and* p-*nitrobenzaldehyde*. A soln of 603 mg (4·0 mmole) *p*-nitrobenzaldehyde and 406 mg (2·0 mmole) of **9** in 7 ml DME containing 2 ml water was refluxed for 2 hr. The suspension was cooled to room temp, filtered, and the solid was washed with two 20-ml portions water, wt 260 mg (28%) m.p. 230–232° dec. An analytical sample was obtained by recrystallization from a DMF–water m.p. 231–232° dec; $[\alpha]_D^{22}$ 94° (*c* 0·73, DMF). (Found: C, 50·85; H, 4·20; N, 17·45. Calcd. for $C_{20}H_{18}N_6O_8$: C, 51·06; H, 3·86; N, 17·87%).

B. *From* D-*cycloserine and* p-*nitrobenzaldehyde*. A soln of 0·85 g (5·65 mmole) *p*-nitrobenzaldehyde in 20 ml EtOAc was added to a suspension of 0·50 g (4·90 mmole) D-cycloserine in 30 ml EtOAc and 5 ml MeOH. The reaction soln was stirred at room temp for 2·5 hrs (all the cycloserine had dissolved) and then evaporated to a solid residue. The solid was slurried with 75 ml anhydrous ether giving 0·88 g (77%) of an ether insoluble solid, m.p. 228–231° dec. The IR spectrum of this solid was identical to an authentic sample of **7d**.

N-Pyridoxylidene-D-cycloserine

To a soln of 421 mg (2·1 mmole) pyridoxal hydrochloride in 8 ml of anhydrous EtOAc (dried over Linde molecular sieves, Type 4A) was added 1·6 ml (2·1 mmole) 1·3 N NaOEt. The resulting soln was stirred rapidly at room temp resulting in a ppt of NaCl. The solid was removed by centrifugation and washed with one 1-ml portion EtOH. The combined supernatants were mixed with 209 mg (2·1 mmole) D-cycloserine and the reaction soln was cooled to 0° in an ice bath for 3·5 hrs. The resulting solid ppt was collected by centrifugation and extracted with one 2-ml portion of EtOH. The solid, dried *in vacuo*, weighed 368 mg (70%), m.p. 132–134° dec; $[\alpha]_D^{21}$ 119° (*c*, 0·47, MeOH), IR (Nujol) (—OH), 3·80 (bonded OH), 5·91 (C=O), 6·12 μ (C=N). (Found: C, 51·04, 51·06; H, 5·56, 5·61; N, 16·67, 16·74. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52·59; H, 5·21; N, 16·73%).

2-(*Triphenylmethyl*)-N-(*pyridoxylidene*)-DL-*cycloserine* (**16**).

To a soln of 202 mg (0·52 mmole) 2-triphenylmethyl-DL-cycloserine hydrochloride and 107 mg (0·52 mmole) pyridoxal hydrochloride in 15 ml EtOH and 5 ml MeOH was added 0·15 ml (1·1 mmole) Et_3N. The soln was stirred at room temp for 1·25 hs and then evaporated *in vacuo* to a solid residue. Trituration of the residue with 25 ml EtOAc gave 166 mg (100%) insoluble material which was identified as Et_3NHCl. The EtOAc soln was evaporated to a residue which solidified *in vacuo*, wt 241 mg (97%), m.p. 102–105°. analytical sample which contained one molecule EtOAc of crystallization was obtained by recrystallization from EtOAc, m.p. 111–114°; IR (Nujol) 2·65 (OH), 3·80 (bonded OH), 5·80 (C=O), 6·15 (C=N), 13·35–14·40 μ (ϕ_3-c); UV (MeOH), 218 (ε 33,850), 258 (12,950), 340 mμ (6080); NMR ($CDCl_3$), δ 0·55 (s, OH), 1·20 (t, EtOAc), 1·95 (s, EtOAc), 2·40 (s, Me of pyridoxal), 4·05 (q, EtOAc), 4·35 (m, isoxazolidone ring), 7·23 (m-ϕ_3c), 7·68 (s, pyridoxal aromatic), 8·74 ppm (s, C<u>H</u>=). (Found: C, 70·12; H, 5·95; N, 7·44. Calcd. for $C_{30}H_{27}N_3O_4 \cdot C_3H_8O_2$: C, 70·21; H, 6·07; N, 7·22%).

The formation of (−)3,6-*bis*[N-*pyridoxylideneaminoxymethyl*]-2,5-*piperazinedione Dihydrochloride* (**7c**)

A. From (+)3,6-*bis*(*aminoxymethyl*)-2,5-*piperazinedione* (**9**) *and pyridoxal hydrochloride.* To a suspension of 104 mg (0·51 mmole) of **9** in 5 ml MeOH was added 209 mg (1·03 mmole) pyridoxal hydrochloride. The suspension was stirred at room temp for 1·5 hrs. A crystalline solid was precipitated from the reaction soln by the addition of 10 ml anhydrous ether and the solid was filtered off and dried, wt 242 mg (84%), m.p. 225–235° dec. An analytical sample, m.p. 228–230°, $[\alpha]_D^{20}$ −62° (*c*, 0·74, 1N HCl) was obtained by recrystallization from water–EtOH. (Found: C, 45·85; H, 5·16; N, 14·69; Cl^-, 12·03. Calcd. for $C_{22}H_{28}N_6O_8Cl_2$: C, 45·92; H, 4·90; N, 14·61; Cl^-, 12·32%).

B. *From* D-*cycloserine and* N-*pyridoxylidene*-D-*cycloserine.* To a slurry of 152 mg (0·61 mmole) N-pyridoxylidene-D-cycloserine in 1 ml distilled water was added a soln of 64 mg (0·63 mmole) D-cycloserine in 0·5 ml water. The soln was adjusted to pH5 with AcOH and heated at 60° until all the solid had dissolved (0·5 hr). The soln was then evaporated under a N_2 stream and pumped to dryness *in vacuo*. The residue was extracted with two 0·5-ml portions of water followed by two 1-ml portions EtOH and one 2-ml ether. The dried insoluble solid weighed 133 mg (88%), m.p. 170–180°, $[\alpha]_D^{24}$ 0°. Conversion of this solid into the dihydrochloride gave a solid material, m.p. about 180°, having an IR spectrum containing the characteristic bands of optically inactive 3,6-bis-aminoxymethyl-2,5-piperazinedione derivatives.

C. *From* D-*cycloserine and pyridoxal.* A mixture of 167 mg (1·0 mmole) pyridoxal and 211 mg (2·0 mmole) D-cycloserine was diluted to 3 ml with water. The reaction soln was heated at 65° for 0·5 hr. The soln was then evaporated to a total volume of 0·5 ml under a N_2 stream which gave a glassy residue under vacuum. Trituration of the residue with 3 ml EtOH followed by extraction of the EtOH insolubles with two 3-ml portions of ether gave 196 mg sold residue. Addition of ether to the combined extracts afforded another 73 mg solid. The combined solid product was converted into its dihydrochloride which had identical spectral properties to the product to obtained in part B.

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