

*Anal.*—Calcd. for  $C_{15}H_{15}N_3O_2$ : C, 67.40; H, 4.90. Found: C, 67.47; H, 5.04,

$\alpha, \alpha'$ -Dicyano- $\alpha'$ - $\beta$ -dimethyl- $\beta$ -phenyl-N-methylglutarimide.—Using the above procedure, but using 0.15 Gm. atom of sodium and 0.165 mole of methyl iodide to 0.05 mole of the imide, yielded 10 Gm. (71%) of the product, m.p. 190–192°.

*Anal.*—Calcd. for  $C_{15}H_{15}N_3O_2$ : C, 68.32; H, 5.38. Found: C, 68.29; H, 5.37.

Similar results were obtained with a 2:1 ratio of methyl iodide to each active hydrogen and the solution refluxed for 21 hours. Assignment of the second entering methyl group to the  $\alpha'$ -position rather than the  $\alpha$  is an arbitrary one.

**Reaction of the Ammonium Salt of  $\alpha, \alpha'$ -Dicyano- $\beta, \beta$ -dimethylglutarimide with Dimethyl Sulfate.**—The ammonium salt of  $\alpha, \alpha'$ -dicyano- $\beta, \beta$ -dimethylglutarimide, 52.0 Gm. (0.25 mole), was suspended in a mixture of 100 ml. of anhydrous benzene and 100 ml. of absolute ethanol contained in a 500 ml., three necked, round-bottom flask equipped with a stirrer, dropping funnel, and reflux condenser. The mixture was stirred and heated under reflux for 1 hour after which time 37.8 Gm. (0.3 mole) of dimethyl sulfate was added with stirring. After the addition was completed, the mixture was stirred

and heated under reflux for 2 hours. Within the first 15 minutes of this reflux period, the solid had all dissolved. The solution was cooled, diluted with an equal volume of water and refrigerated overnight. The solid that had separated (31.8 Gm.), was collected, and recrystallized from 60% ethanol. This material melted between 139–155°. A mixed melting point of  $\alpha, \alpha'$ -dicyano- $\beta, \beta$ -dimethylglutarimide and  $\alpha, \alpha'$ -dicyano- $\beta, \beta$ -dimethyl-N-methylglutarimide melted at 144–155°.

#### SUMMARY

1.  $\beta$ -Aryl Guareschi-like imides failed to undergo bicyclization with  $\alpha, \Omega$ -dihalides.

2. The failure of  $\beta$ -aryl Guareschi-like imides to undergo bicyclization is attributed to a favored enolic system in the presence of alkali which in turn resists the expected reaction.

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## Mercury(II) Complex of *d*-Cycloserine

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The apparent formation constant of the 2:1 complex of *d*-cycloserine and mercury (II) has been measured and found to be  $10^{17.5}$  at 25° in solutions of ionic strength of  $\sim 0.4$ . Evidence is presented to substantiate the conclusion that chelation contributes little to the structure of the complex.

AS PART of a study dealing with the influence of metal ions on the mode of degradation of the antibiotic *d*-cycloserine (*d*-4-amino-3-isoxazolidone) in aqueous solution, the apparent formation constant of the mercury(II) complex of cycloserine was measured. Neilands (1) and Neuzil and Breton (2) have indicated that cycloserine formed 1:1 and 2:1 complexes with copper(II), zinc(II), cadmium(II), cobalt(II), and nickel(II). No significant complex formation was observed with beryllium(II), magnesium(II), manganese(II), iron(II), and iron(III). It was of interest to compare the stability of the cycloserine-mercury(II) complex with the stabilities reported for similar complexes with other metal ions, as well as with several of the metal ion complexes of other ligands.

#### EXPERIMENTAL

A potentiometric technique was employed (3, 4). The potential of a mercury electrode in equilibrium with a solution of the mercury(II)-cycloserine complex in the presence of excess cycloserine was measured as a function of pH. Solutions were prepared containing from 0.01 to 0.05 *M* cycloserine, equimolar amounts of sodium hydroxide,

from 0.0004 to 0.001 *M* mercuric acetate,<sup>1</sup> and sufficient potassium nitrate to adjust their ionic strength to 0.4. The potential of the mercury electrode (Sargent No. S-30438) vs. a saturated calomel electrode (S.C.E.) isolated from the solution through a 1.5 *M* potassium nitrate agar salt bridge was measured using a Fisher student potentiometer. The pH of the solution was varied by titration under nitrogen with 1 *M* perchloric acid. The resulting volume changes amounted to less than 8 per cent of the initial volume at the end of the titration. Corrections were made for concentration changes owing to dilution when significant. A Beckman model G pH meter was employed, using an external glass electrode (type 1190-80) to measure the pH. Since the complex was observed to precipitate at pH values greater than 8, the potential measurements were restricted to the pH range 2–8. The temperature of the solution and calomel electrode was maintained at  $25 \pm 0.5^\circ$ .

The apparent dissociation constants of the cycloserine were also measured in 0.4 *M* potassium

<sup>1</sup> The acetate and nitrate ions present in the system would have a negligible effect (other than that due to alteration in ionic strength) on the measured potential, owing to the much greater concentration of cycloserine than acetate and the very weak tendency of mercury(II) to complex nitrate.

nitrate, by titration with sodium hydroxide and perchloric acid.

The cycloserine<sup>2</sup> had a melting point of 155° (decompn.) after recrystallization from alcohol. The other chemicals used were of reagent grade.

#### CALCULATIONS AND RESULTS

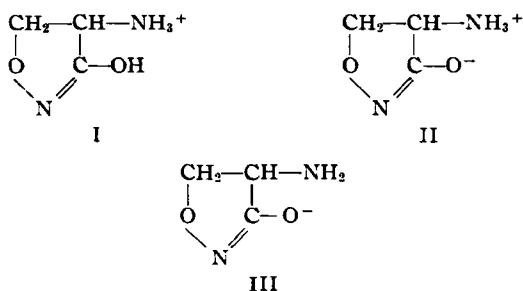
Table I summarizes the equilibria describing the state of a solution containing cycloserine and

TABLE I.—SUMMARY OF EQUILIBRIA AND APPARENT EQUILIBRIUM CONSTANTS DESCRIBING THE MERCURY(II)-CYCLOSERINE SYSTEM<sup>a</sup>

Equilibria	K'	Log K'
H <sub>2</sub> L <sup>+</sup> = HL + H <sup>+</sup>	K <sub>a1</sub>	-4.57
HL = L <sup>-</sup> + H <sup>+</sup>	K <sub>a2</sub>	-7.40
Hg <sup>++</sup> + HL = HgL <sup>+</sup>	K <sub>10</sub>	...
+ H <sup>+</sup>		
Hg <sup>++</sup> + HL = Hg(HL) <sup>++</sup>	K <sub>11</sub>	...
Hg <sup>++</sup> + 2HL = HgL <sub>2</sub>	K <sub>20</sub>	2.73
+ 2H <sup>+</sup>		
Hg <sup>++</sup> + 2HL = HgL(HL) <sup>+</sup> + H <sup>+</sup>	K <sub>21</sub>	8.67
Hg <sup>++</sup> + 2HL = Hg(HL) <sub>2</sub> <sup>++</sup>	K <sub>22</sub>	14.07
Hg <sup>++</sup> + HL + L <sup>-</sup> = HgL(HL) <sup>+</sup>	K <sub>23</sub> = K <sub>21</sub> /K <sub>a2</sub>	16.06
Hg <sup>++</sup> + 2L <sup>-</sup> = HgL <sub>2</sub>	β <sub>2</sub> = K <sub>20</sub> /(K <sub>a2</sub> ) <sup>2</sup>	17.53
HgL(HL) <sup>+</sup> = HgL <sub>2</sub> + H <sup>+</sup>	K <sub>31</sub> = K <sub>20</sub> /K <sub>21</sub>	-5.93
Hg(HL) <sub>2</sub> <sup>++</sup> = HgL(HL) <sup>+</sup> + H <sup>+</sup>	K <sub>32</sub> = K <sub>21</sub> /K <sub>22</sub>	-5.44

<sup>a</sup> In aqueous solution at 25°C. and μ ~ 0.4.

mercury(II) and the equilibrium constants computed from the experimental data. In aqueous solution cycloserine may exist as a cation (H<sub>2</sub>L<sup>+</sup>), dipolar ion (HL), or anion (L<sup>-</sup>), as indicated by structures I, II, and III. The observed values of K<sub>a1</sub>' and K<sub>a2</sub>', describing the equilibria between these forms, agreed with those reported by Neilands (1).



The experimental data were analyzed in a manner similar to that described by Brooks and Davidson (5). In order to simplify the calculations, it was assumed that the 1:1 complexes (equilibria 3 and 4) did not significantly contribute to the complexation of mercury(II) by cycloserine. This assumption was justified since measurements were made in the presence of excess ligand. It was substantiated by the subsequent analysis. K<sub>10</sub> and K<sub>11</sub> were not measurable under the conditions employed in this study.

<sup>2</sup> The cycloserine employed in this study was a sample of Seromycin, Lot No. 61, generously supplied by Eli Lilly & Co.

The total concentration of ligand species, therefore, is

$$\Sigma L = [L^-] + [HL] + [H_2L^+] + 2[HgL_2] + 2[HgL(HL)^+] + 2[Hg(HL)_2^{++}] \quad (\text{Eq. 1})$$

while the total concentration of mercury(II) species is

$$\Sigma \text{Hg(II)} = (\text{Hg}^{++}) + [\text{HgL}_2] + [\text{HgL(HL)}^+] + [\text{Hg(HL)}_2^{++}] \quad (\text{Eq. 2})$$

Since  $\Sigma \text{Hg(II)} \gg (\text{Hg}^{++})$ , the concentration of uncomplexed ligand species,  $[L^-] + [HL] + [H_2L^+]$ , may be taken as  $\Sigma L - 2\Sigma \text{Hg(II)}$ . It is convenient to define the following function which may be derived from Eq. 2 and equilibria 5, 6, and 7

$$\frac{\text{Hg(II)}}{(\text{Hg}^{++})} - 1 = \frac{K_{20}}{(\text{H}^+)^2} + \frac{K_{21}}{(\text{H}^+)} + K_{22} = \bar{K} \quad (\text{Eq. 3})$$

Also

$$[\text{HL}] = \frac{\Sigma L - 2\Sigma \text{Hg(II)}}{\alpha} \quad (\text{Eq. 4})$$

where

$$\alpha = 1 + \frac{(\text{H}^+)}{K_{a1}} + \frac{K_{a2}}{(\text{H}^+)}$$

The mercuric ion activity was calculated from the measured potential of the mercury electrode vs. the S.C.E. using the Nernst equation (3)

$$E = 0.612 - 0.0296 \log (\text{Hg}^{++}) \quad (\text{Eq. 5})$$

where 0.612 volts<sup>3</sup> is the standard potential of the Hg/Hg<sup>++</sup> couple vs. the S.C.E. Corrections owing to the reduction of Hg<sup>++</sup> by Hg<sup>0</sup> to Hg<sub>2</sub><sup>++</sup> were negligible because of the excess ligand present. The mercury (I) complex of cycloserine is not stable, readily disproportionating.

Equation 3 was used to evaluate the constants K<sub>20</sub>, K<sub>21</sub>, and K<sub>22</sub>. Values of the function ( $\bar{K}$ ) between pH 6.5-8 were used to compute K<sub>20</sub>; values between pH 4.5-6.5 were used to compute K<sub>21</sub>; and values between pH 4.5-2 were used to compute K<sub>22</sub>. The consistency of the experimental data with the proposed model is illustrated by Fig. 1 which shows a plot of log  $\bar{K}$  as a function of pH. Values of the function on the left-hand side of Eq. 3 computed from the experimental data are compared to  $\bar{K}$  calculated using the tabulated values of K<sub>20</sub>, K<sub>21</sub>, and K<sub>22</sub>.

The apparent formation constant (β<sub>2</sub>') and dissociation constants (K<sub>31</sub>' and K<sub>32</sub>') of the complex were calculated as indicated in Table I.

#### DISCUSSION

A comparison of the apparent formation constants reported for some metal ion (1, 6) complexes of *dl*-serine and *d*-cycloserine is made in Table II. As expected, the stability of the 2:1 cycloserine-mercury(II) complex is considerably greater than

<sup>3</sup> No attempt was made to correct the standard potential to a value corresponding to μ ~ 0.4, because of a lack of information concerning the effect of ionic strength on the potential of the Hg/Hg<sup>++</sup> couple. This meant that the value of the computed formation constant was high, probably by not more than 0.3 log K unit.

that observed for other metals. The known tendency of cycloserine to chelate copper(II), zinc(II), and cobalt(II) is a great deal less than that observed for serine. Neilands (1) has pointed out that this is probably due to the less favorably fixed

TABLE II.—COMPARISON OF APPARENT FORMATION CONSTANTS ( $\text{LOG } \beta_2'$ ) OF *d*-CYCLOSERINE AND *dl*-SERINE METAL COMPLEXES

Ligand	Temp., °C.	Hg(II)	Cu(II)	Zn(II)	Co(II)
<i>d</i> -Cycloserine	25	17.5	9.7	6.0	5.7
<i>dl</i> -Serine	20	17.5	14.6	8.6	8.0

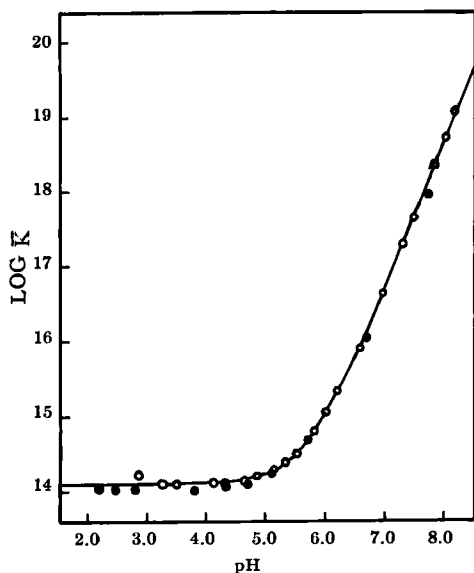
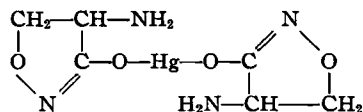


Fig. 1.— $\text{Log } \bar{K}$  vs. pH: the solid line represents  $\bar{K}$  calculated from Eq. 5 using values of  $K_{20} = 10^{2.72}$ ,  $K_{21} = 10^{8.67}$ , and  $K_{22} = 10^{14.07}$ ; the open circles represent values calculated from measured mercuric ion activities where  $\Sigma L$  is 0.04 *M* and  $\Sigma \text{Hg(II)}$  is 0.0004 *M*; the solid circles represent values calculated from measured mercuric ion activities where  $\Sigma L$  is 0.01 *M* and  $\Sigma \text{Hg(II)}$  is 0.001 *M*.

positions of the potential chelating groups in cycloserine as contrasted to the flexibility of the amino acid molecule. On the other hand, the stabilities of the mercury(II) complexes are of the same order of magnitude. This observation, as well as the experimental data, supports the view that chelation does not significantly contribute to the structure of the complex, which may be represented at pH  $\sim 8$  as



The dissociation constants of the mercury(II) complex ( $K_{21}'$  and  $K_{22}'$ ) are on the order of  $10^8$  greater than the corresponding ionization constant ( $K_{a2}'$ ) of cycloserine. If chelation through the amino-group was significant, a much greater increase should have been observed. The fact that the formation constants  $K_{22}'$ ,  $K_{23}'$ , and  $\beta_2'$  increase successively in value may be accounted for by charge effects as well as by chelation. Similar observations were made by Brooks and Davidson (5) in their analysis of the histidine-mercury(II) system.

Some observations have been made concerning the stability of the cycloserine-metal complexes to hydrolysis. They appear to degrade by a mechanism different from that involved in the decomposition of cycloserine (7). Particularly striking changes may be observed in a solution of the copper (II) chelate upon aging. The solution changes from a green to a deep blue color and deposits a violet precipitate. These observations are the subject of studies now in progress and will be reported on in more detail at a later date.

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