An 0.360-g (1.25 mmol) portion of **26** was added to 35 ml of dichloromethane and 1 g (5 mmol) of pyridine–chromium trioxide complex[31] was added with stirring. After 3 min the reaction was terminated by addition of 50 ml of hexane. The suspension was chromatographed on a silica gel column; 10% ether and hexane eluted 0.315 g (88%) of aldehyde **27**: τ 9.17 and 8.98 (both s, 3 H), 6.40 (m, 1 H), 8.26 (d, 3 H, J = 1.5 Hz), and 0.27 (s, 1 H). The glpc retention time was different from that of the starting material.

The aldehyde **27** (0.315 g, 1.1 mmol) was dissolved in 30 ml of diethylene glycol and 5 ml of 99% hydrazine hydrate was added. The solution was heated under nitrogen for 5 hr at 120–130°, 3.5 g (90 mmol) of sodium hydroxide was added, and the hydrazine and water were distilled from the reaction. The solution was then heated at 185° for 15 hr. The product was isolated by ether extraction and chromatographed on silica gel. Hexane eluted 0.280 g (90%) of an unsaturated hydrocarbon (**28**) which was crystallized from methanol: mp 81.5–82.5° (lit.[7] mp 84–85°); τ 9.17, 9.13, and 9.03 (all s, 3 H), 8.26 (d, 3 H, J = 1.5 Hz), 4.40 (m, 1 H); $[\alpha]^{23}D$ −75° (*c* 9.0) (lit.[7] −73.99°); ν_{max} 3025 cm^{-1} (C=CH). The spectral data correspond to those reported for natural isoatiserene.[7]

Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 88.26; H, 11.77.

ent-16-Antisene (Atisirene, 25).—The same reduction procedure as described above was followed using 0.353 g (1.1 mmol) of unsaturated ester **21** and 0.5 g of lithium aluminum hydride. Alcohol **23** was recrystallized from hexane (0.330 g, 99%): mp 139–140°; τ 9.02 (s, 6 H), 5.29 and 5.46 (both quartets with $J \sim 2$ Hz, 1 H), 6.28 and 6.53 (AB, 1 H, J = 11 Hz); ν_{max} 3390 (OH), 3070, 1650, and 870 cm^{-1} (C=CH$_2$).

Anal. Calcd for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 82.71; H, 11.21.

Alcohol **23** (0.245 g, 0.84 mmol) was then added to 30 ml of dry dichloromethane and 2 g of chromium trioxide–dipyridine complex[31] was added. After 3 min, the suspension was diluted with 50 ml of hexane and then poured on to a column of silica gel; elution with hexane containing 10% ether gave 0.230 g (95%) of aldehyde **24** (glpc retention time different from that of **23**).

The aldehyde **24** (0.230 g, 0.8 mmol) was subjected to Wolff–Kishner reduction as above, with 15 ml of diethylene glycol and 3 ml of 99% hydrazine hydrate. Column chromatography of the product on silica gel using hexane as eluent gave 0.083 g (38%) of atisirene (**25**), which after crystallization from methanol had mp 58–58.5° [lit.[7,27a] mp 57–58°; 60–61° (for enantiomer)]; τ 9.16, 9.14, and 9.02 (all s, 3 H), 5.29 and 5.46 (both m, 2 quartet, 1 H, J = 2 Hz); $[\alpha]^{23}D$ −41.20° (*c* 5.0) (lit.[7] −40.46°]; ν_{max} 3080, 1648, 880, and 870 cm^{-1} (C=CH$_2$). The spectral data are in reasonable agreement with the corresponding literature data for natural atiserene,[7] its enantiomer,[27a] and synthetic racemic antiserene.[32] In addition, the complete ir and nmr spectra are superimposable upon those of natural atiserene.[7]

Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 87.92; H, 11.82.

Registry No.—**1**, 27975-19-5; **2**, 30217-41-5; **3**, 30217-42-6; **4**, 21682-55-3; **5b**, 30288-12-1; **6b**, 30217-44-8; **7a**, 30217-45-9; **7b**, 21682-20-2; **9**, 23963-60-2; **10**, 30217-48-2; **12**, 30217-49-3; **13a**, 24022-50-2; **13b**, 24022-51-3; **14a**, 30217-52-8; **14b**, 23963-18-0; **14c**, 23963-59-9; **15a**, 30217-54-0; **15b**, 23963-19-1; **15c**, 30288-14-3; **16a**, 23963-20-4; **17a**, 30217-57-3; **17b**, 30217-58-4; **18a**, 30217-59-5; **18b**, 23963-23-7; **19**, 23963-24-8; **20**, 19898-49-8; **21**, 23963-21-5; **22**, 23963-22-6; **23**, 30217-65-3; **25**, 20230-48-2; **26**, 30217-67-5; **28**, 5975-29-1.

Acknowledgment.—We are grateful to Dr. Sukh Dev for the spectra of natural atiserene, to the National Institutes of Health for financial assistance, and to the National Science Foundation for funds used in the purchase of a 100-MHz nmr spectrometer.

Cycloserine Dimer Hydrolysis and Its Equilibration with Cycloserine

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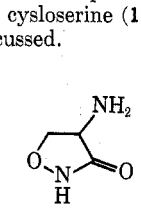
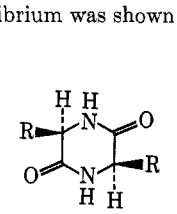
Received January 26, 1971

A kinetic investigation of the hydrolysis of three cis-3,6-disubstituted 2,5-piperazinediones (**2**, **3**, and **4**) at several HCl concentrations has been completed. The relative rates are compared to the unsubstituted 2,5-piperazinedione (**5**). In solutions of pH 1–2, a cycloserine dimer **2** ⇆ cysloserine (**1**) equilibrium was shown to be rapidly established. The mechanism of this interconversion is discussed.

It has been well established[2] that the broad-spectrum antibiotic cycloserine (**1**) is converted into its dimer, (+)-*cis*-3,6-bis(aminoxymethyl)-2,5-piperazinedione (**2**) even in the solid state.[3] This present investigation indicates that in solution **2** is also converted into cycloserine and that the establishment of an equilibrium between **1** and **2** is pH dependent. Our recent kinetic study[4] of the acid-catalyzed hydrolysis of **2** indicated that the side-chain aminoxy groups do not anchimerically assist the reaction at low pH. Such participation would necessarily lead to the intermediate formation of



1



2, R = CH_2ONH_2
3, R = CH_2OH
4, R = CH_3
5, R = H

a cycloserine peptide which further hydrolysis would convert into the observed product, β-aminoxy-D-alanyl-β-aminoxy-D-alanine (see Scheme I). Table I summarizes the results of more recent studies on the hydrolysis of **2** and analogous 2,5-piperazinediones **3** and **4** at various concentrations of HCl. The results confirm the previous hypothesis that the aminoxy groups do not participate at low pH.

Table II shows the activation parameters for the hydrolyses of **2**, **3**, **4**, and the unsubstituted compound **5**. It was primarily the small differences in hydrolysis rates

(1) Abstracted from the Ph.D. thesis of F. O. Lassen, submitted to the Graduate School of the University of Georgia, June 1969.

(2) (a) P. H. Hidy, E. B. Hodge, V. V. Young, R. L. Harned, G. A. Brewer, W. F. Phillips, W. F. Runge, H. E. Stavely, A. Pohland, H. Boaz, and H. R. Sullivan, *J. Amer. Chem. Soc.*, **77**, 2345 (1955); (b) J. M. Nielands, *Arch. Biochem. Biophys.*, **62**, 151 (1956); (c) R. M. Khomutov, M. Ya. Karpeskii, and E. S. Severin, "Chemical and Biological Aspects of Pyridoxal Catalysis," I. U. B. Symposium Series, Pergamon, New York, N. Y., 1963.

(3) M. Ya. Karpeskii, Yu N. Brensov, R. M. Khomutov, E. S. Severin, and O. L. Polyanovskii, *Biochemistry*, **28**, 280 (1963).

(4) J. L. Miller, F. C. Neuhaus, F. O. Lassen, and C. H. Stammer, *J. Org. Chem.*, **33**, 3908 (1968).

SCHEME I

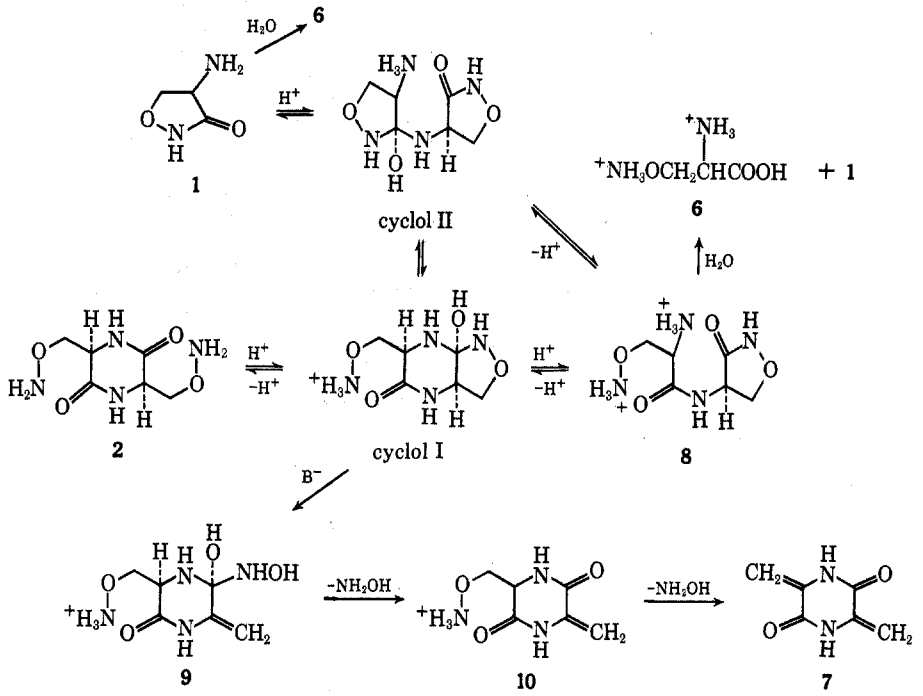


TABLE I

HYDROLYSIS RATE CONSTANTS OF 2, 3, AND 4 IN HCl AT 60°

[HCl], M	$k \times 10^3$ 2	3	4
1.00	4.76 ± 0.05[a]	3.78 ± 0.01	10.7 ± 0.07[a]
2.00	10.6 ± 0.1[b]		26.3 ± 0.3[b]
3.99	33.9 ± 0.9[c]	19.4 ± 0.01	58.0 ± 2.1[c]
5.93	55.1 ± 0.9[a]	33.9 ± 0.01	

[a] Average of three runs. [b] Average of two runs. [c] Average of four runs.

TABLE II

ACTIVATION PARAMETERS FOR HYDROLYSIS OF CIS-SUBSTITUTED 2,5-PIPERAZINEDIONES IN 1 N HCl AT 60°

R	No.	Relative rates	E_a, kcal	$H^{\ddagger}$, kcal	$S^{\ddagger}$, eu
CH_2ONH_2	2	1.2	19.9[a]	19.2[a]	−19.7[a]
CH_2OH	3	1	19.7[a]	19.0[a]	−20.8[a]
CH_3	4	4.5	18.3	17.6	−22.9
H	5	2.0[c]	21.3[b]	20.6[b]	−14.7[b]

[a] From ref 4. [b] B. O. Sykes, E. B. Robertson, H. B. Dunford, and D. Konuswich, *Biochemistry*, **5**, 697 (1966). [c] Calculated from the data of J. T. Edward and S. C. Meacock, *J. Chem. Soc.*, 2000 (1957).

and activation parameters which led us to conclude that the aminoxy groups were not participating in the hydrolysis of **2**. The fact that the dimethyl compound **4** hydrolyzes faster than **2** is strong evidence against aminoxy participation, which should increase the rate. Furthermore, there are no really significant differences in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ among **2**, **3**, and **4**. Further evidence against aminoxy participation in the hydrolysis was obtained when we found that the rate of hydrolysis of the dimethyl compound **4** was somewhat decreased when methoxyamine was added (Table III). Apparently the only effect of methoxyamine was to slow the reaction by decreasing the HCl concentration.

When attempts were made to examine the hydrolysis of **2** at pH >1, both polarimetry and paper chromatography showed that other reactions were occurring.

TABLE III

EFFECT OF METHOXYAMINE ON THE HYDROLYSIS RATE OF 4

	$k \times 10^2$	Relative rates
2 *M* HCl	2.63	1
0.4 *M* $^+NH_3OCH_3Cl^-$, 1.6 *M* HCl	2.1	0.80
0.4 *M* NaCl, 1.6 *M* HCl	2.2	0.83
1.6 *M* HCL	2.1	0.80

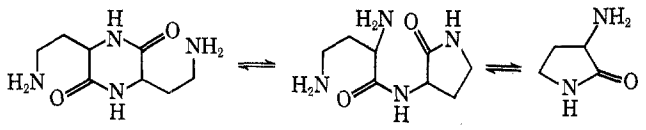
Potentiometric titration of **2** showed the pK_a of the aminoxy groups[5] to be *ca.* 3.5, with no differentiation between the two groups. Thus at pH >1, a finite concentration of aminoxy groups becomes available for intramolecular attack on the adjacent ring carbonyl functions. Paper chromatography studies of solutions of **2** in pH 1.2 phosphate buffer and in 1 *N* acetic acid (*ca.* pH 2.2) showed that not only was cycloserine (**1**) being formed, but also β-aminoxylalanine (**6**) (hydrolysis product of **1**) and 3,6-dimethylene-2,5-piperazinedione (**7**) (the product of hydroxylamine elimination from **2**). Scheme I shows a reasonable explanation for these results.

Considering the high nucleophilicity of hydroxylamines and the ease with which the cycloserine ring closes,[6] the formation of cyclol I seems a reasonable first step. Opening of the six-membered ring in I gives the dipeptide **8** which can be hydrolyzed to cycloserine (**1**) and β-aminoxy-D-alanine (**6**) or rearrange to cyclol II. The formation of the bismethylene compound **7** can be explained by a second path through which cyclol I might decompose. Attack by a general base on the α proton would convert I into the unstable hydroxylamine **9** which leads directly to the monomethylene compound **10** by the loss of hydroxylamine. The formation of **10** from **2** has been observed previously

(5) R. W. A. Oliver, T. Viswanatha, and W. J. D. Whish, *Biochem. Biophys. Res. Commun.*, **27**, 107 (1967), report pK_a = 4.0 for these aminoxy groups.

(6) C. H. Stammer, A. N. Wilson, C. F. Spencer, F. W. Bachelor, F. W. Holly, and K. Folkers, *J. Amer. Chem. Soc.*, **79**, 3236 (1957).

during the base-catalyzed decomposition of **2** to **7**. Further elimination from **10** then leads to **7**. Cyclol formation was also postulated by Rudinger and coworkers[7] to explain the formation of the dipeptide *N*-(α,γ-diaminobutyryl)-α-aminopyrrolidone (**12**) and α-aminopyrrolidone (**13**) from the piperazinedione **11** in dilute ammonia. These workers also showed that the dipeptide **12** was converted into both **11** and **13** under these same conditions showing that an equilibrium was probably established. Similarly, solutions of high pH probably convert the dimer **2** into cyclol I, but this intermediate eliminates hydroxylamine much more



11 **12** **13**

rapidly than it undergoes hydrolysis. Thus, the behavior of the dimer **2** in both weakly acidic and basic media can be explained by assuming the formation of cyclol I.

The behavior of cycloserine (**1**) under strongly and weakly acidic[8] conditions can also be explained using Scheme I. Under strongly acidic conditions (3 *N* HCl) the isoxazolidone ring is hydrolyzed[9] giving the alanine derivative **6**, but dimerization predominates when **1** is treated[4] with 4% ethanolic acetic acid. Under the latter conditions, the acid catalyzes the condensation of two molecules of **1** to give cyclol II which can go to cyclol I either directly or through the dipeptide[10] **8**. The dimer **2** is then formed from I and the equilibrium between **1** and **2** is established. Our experiments showed that, in aqueous 1 *M* acetic acid solutions, cycloserine was converted into both **2** and **7** and that under these same conditions dimer **2** was converted into both cycloserine and **7**, indicating the presence of an equilibrium. Weakly acidic (pH 2–6) conditions are expected to favor establishment of this equilibrium since the amino groups are not so completely protonated as in strong acid. This allows the condensation of two molecules of **1** giving cyclol II and the conversion of **8** into cyclol I to occur at reasonable rates.

The presence of an equilibrium between cycloserine and its dimer in solution makes the assignment of any specific biological activity to either of these compounds difficult. Certainly, future biological assessments of these and related substances should take these facts into account.

Experimental Section

Cycloserine,[11] serine,[12] and alanine[13] were used without further purification. The (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione (**2**) and the (−)-3,6-bis(hydroxymethyl)-2,5-piperazinedione (**3**) were prepared by previously published procedures.[5,13]

(−)-**3,6-*cis*-Dimethyl-2,5-piperazinedione** (**4**).—*tert*-Butoxycarbonyl-L-alanine[14] (24.23 g, 0.13 mol) was coupled *via* the isobutyl chloroformate mixed anhydride procedure[15] with L-alanine methyl ester[16] (17.88 g, 0.13 mol) yielding, after two recrystallizations from anhydrous ether–petroleum ether (bp 40–60°), 28.62 g (88%) of *N*-*tert*-butoxycarbonyl-L-alanyl-L-alanine methyl ester: mp 112–113°; $[\alpha]^{24}D$ −63.7° (*c* 2, MeOH); ir (KBr) 3255 (NH), 1738 (ester C=O), 1675 (amide C=O), 1650 cm^{-1} (*tert*-BOC C=O); nmr ($CDCl_3$) δ 1.32 (d, 3, H, C-terminal CH_3, J = 8 Hz), 1.34 (d, 3 H, N-terminal CH_3, J = 8 Hz), 1.42 (s, 3 H, *tert*-BOC CH_3), 3.80 (s, 3 H, OCH_3), 4.30 (m, 1 H, C-terminal CH, J = 8 Hz), 5.86 (d, 1 H, peptide NH, J = 8 Hz), 7.47 ppm (broad s, 1 H, *tert*-BOC NH). *Anal.* Calcd for $C_{21}H_{22}N_2O_5$: C, 52.54; H, 8.08; N, 10.21. Found: C, 52.52; H, 8.19, N, 10.14.

The *tert*-butoxycarbonyl group was removed from 27.46 g (0.1 mol) of the dipeptide ester using 200 ml of glacial acetic acid and 350 ml of 1 *M* HBr in glacial acetic acid. A yield of 23.16 g (91.4%) of crude hygroscopic L-alanyl-L-alanine methyl ester hydrobromide was obtained: $[\alpha]^{20}D$ −40.2° (*c* 1, 0.1 *M* HBr); ir (KBr) 1985 (NH_3^+), 1735 (ester C=O), 1670 cm^{-1} (amide C=O).

The cyclization of the above ester hydrobromide into **4** was effected by treating a 2:1 methanol–water solution of the crude hydrobromide in a batchwise manner with Amberlite IRA 400 (OH^- cycle) until the solution was shown to be bromide free by silver nitrate. The solution was filtered and evaporated to 30 ml. After standing overnight at room temperature, 8.19 g (65%) of crude (−)-3,6-dimethyl-2,5-piperazinedione (**4**) precipitated and was collected on a filter. After recrystallization from ethanol–water (10:1), 5.52 g (44%) of **4** was obtained: mp 282–285° (lit.[16] 288–290): $[\alpha]^{20}D$ −31.0° (*c* 1, H_2O) [lit.[17] $[\alpha]^{21}D$ −29.6° (*c* 1.9, H_2O)]; ir (KBr) identical with that published by Brockmann and Musso;[17] nmr (D_2O) δ 1.90 (d, 6 H, ($CHCH_3$), J = 8 Hz), 4.60 ppm (q, 2 H, $CHCH_3$, J = 8 Hz). *Anal.* Calcd for $C_6H_{10}N_2O_2$: C, 50.68; H, 7.09; N, 19.71. Found: C, 50.21; H, 7.20; N, 19.80.

Kinetic Studies.—The kinetic studies were carried out using the polarimetric procedures previously described.[4] The kinetic data for the hydrolysis of **4** in 1 *M* HCl is shown in Table IV.

TABLE IV

Temp, °C	$k \times 10^3$, min^{-1}	$t_{1/2}$, min
50	4.77 ± 0.02	145
55	7.79 ± 0.02	89
60	10.7 ± 0.7	65
65	17.6 ± 0.1	39
70	25.0 ± 0.4	27

Rates of hydrolysis of **2**, **3**, and **4** were determined at 60 ± 0.05° using various concentrations of hydrochloric acid. The hydrolysis of **2** was carried out in 1.00, 2.00, 3.99, and 5.94 *M* HCl, **3** in 1.00, 3.99, and 5.94 *M* HCl, and **4** in 1.00, 1.60 (0.40 *M* NaCl), 1.60 (0.40 *M* methoxyamine), 2.00, and 3.99 hydrochloric acid.

Product Studies.—The amino acids and dipeptides formed during the hydrolysis were examined on Whatman No. 1 circular paper chromatograms to which samples were applied at various times during the reactions. The chromatograms were eluted by one of two solvents systems: MPW (methyl ethyl ketone–pyridine–water, 20:5:8 by volume) and BAW (1-butanol–acetic acid–water, 5:1:4 by volume). Authentic samples of D-cycloserine (**1**), D-aminoxyalanine (**6**), L-alanine, L-serine, and 3,6-dimethylene-2,5-piperazinedione (**7**) were used as reference standards. The components were visualized by spraying with a solution of 0.2% ninhydrin in 5% acetic acid–ethanol. The elimination product **7** was detected by its uv florescence. The presence of (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione (**2**) was detected chromatographically by treating a small amount of solution with hot 2 *M* sodium hydroxide for 1 min followed by circular paper chromatography using MPW as eluent and observ-

(7) K. Poduska, C. A. Naturkha, A. B. Silaer, and J. Rudinger, *Collect. Czech. Chem. Commun.*, **30**, 2410 (1965).

(8) In basic solution, the cyclic hydroxamic acid group in **1** is converted into a salt which protects the ring from further attack.

(9) C. H. Stammer, *J. Org. Chem.*, **27**, 2957 (1962).

(10) We know from previous work that dipeptides of the type **8** rearrange rapidly into 2,5-piperazinediones; see R. A. Payne and C. H. Stammer, *ibid.*, **33**, 2421 (1968).

(11) We thank Dr. Wallace F. Runge of Commercial Solvents Corp., Terre Haute, Ind., for generous gifts of D-cycloserine.

(12) Sigma Chemical Co., St. Louis, Mo.

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

(14) Prepared from *tert*-BOC azide according to the procedure used by R. A. Payne and C. H. Stammer, *J. Org. Chem.*, **33**, 2421 (1968).

(15) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **89**, 5012 (1967).

(16) M. Brenner and W. Haber, *Helv. Chim. Acta*, **36**, 1109 (1953).

(17) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 2, Wiley, New York, N. Y., 1961, p 930.

ing the dimethylene compound **7** at R_f 0.91 under ultraviolet light. Cycloserine and its derivatives were visualized on chromatograms by the following procedure. Equal volumes of 4 *M* sodium hydroxide and a fresh 4% aqueous solution of sodium nitroprusside were mixed and sprayed on the dry chromatogram. When dry, it was sprayed with 1 *M* aqueous acetic acid until the yellow background was almost gone. 3-Isoxazolidone and cycloserine and its derivatives gave blue spots which faded to brown.[18]

An authentic sample of L-alanyl-L-alanine was prepared by the hydrolysis of 0.260 g (0.94 mol) of *tert*-butoxycarbonyl-L-alanyl-L-alanine methyl ester in 15 ml of 1 *M* HCl at 85°. The progress of the reaction was followed by thin layer chromatography (tlc) using Eastman Chromagram No. 6060 silica gel plates and solvent system, methyl ethyl ketone–acetic acid–water (2:5:8 by volume). When the alanylalanine methyl ester (R_f 0.83) had disappeared, the solution was cooled, diluted to 50 ml with distilled water, and lyophilized giving 0.167 g (90%) of crude L-alanyl-L-alanine. The crude dipeptide was recrystallized from isopropyl alcohol–ether yielding 0.144 g (77%) of L-alanyl-L-alanine hydrochloride: $[\alpha]^{23}D$ −36.9° (*c* 1.1, H_2O) [lit.[19] $[\alpha]^{24}D$ −37.3° (*c* 2, 0.5 *N* HCl)]; ir (KBr) 3460 (NH), 1995 (NH_3^+), 1730 (COOH), 1680 cm^{-1} (amide 1).

Isolation and Identification of the Hydrolysis Products.—The isolation and identification of the major hydrolysis product of **2**, β-aminoxy-D-alanyl-β-aminoxy-D-alanine, and of **3**, L-seryl-L-serine, was described in a previous publication.[5] Lyophilization of a sample taken after complete hydrolysis of **4** afforded a residue which was dissolved in 2 ml of hot isopropyl alcohol. The crude dipeptide was precipitated by addition of anhydrous ether. The product, L-alanyl-L-alanine, isolated by centrifugation and dried under reduced pressure, was identical with the authentic sample.

Nmr Study of the Hydrolysis of 4.—Samples (0.5 ml) taken at various time intervals during the hydrolysis were placed in nmr tubes and immediately frozen in a Dry Ice–acetone freezing mixture for storage. Nmr spectra between 1.79 and 2.13 ppm of these samples were obtained using a Varian HA-100 nmr spectometer. The dissappearance of the doublet centered at δ 1.960 which corresponded to the piperazinedione methyl groups and the appearance of two new doublets, one centered at δ 1.945, and the other at 2.056 which corresponded to the dipeptide methyl groups was observed. The nmr sample taken after 410 min showed no doublet corresponding to the piperazinedione methyl groups. After 500 min a new doublet appeared at δ 2.078 which was shown to be that of alanine by its increased intensity when authentic alanine was added.

The nmr spectrum of (−)-3,6-dimethyl-2,5-piperazinedione was obtained in D_2O. After the addition of one drop of 1 *M* hydrochloric acid, the sample was heated at 60° after 72 hr and the nmr spectrum was again determined. Hydrolysis had occurred as shown by the dipeptide methyl signals, but no collapse of the methyl doublets was observed indicating that no deuterium exchange (racemization) had occurred at the asymmetric centers of either the products or the substrate.

Reactions of (+)-3,6-Bis(aminoxymethyl)-2,5-piperazinedione (2) in Buffer Systems.—Two 0.031 *M* solutions of **2** in pH 1.2 phosphate buffer and in 1 *M* hydrochloric acid were prepared. These solutions were heated at 60° and chromatographic samples (MPW system) were taken at various time intervals; the results are shown in Table V.

TABLE V

PAPER CHROMATOGRAPHY STUDY OF 2 IN SOLUTION AT 60°

	Time, min		
	30	60	240
pH 1.2 (phosphate buffer)	0.52 (N, NP)[a,b]	0.52 (N, NP)	0.52 (N, NP)
1 *M* HCl	0.78 (N)	0.78 (N)	0.78 (N)
	0.63 (N)	0.63 (N)	0.63 (N)

[a] N indicates a positive test with ninhydrin; NP indicates a positive test with the nitroprusside reagent. [b] Cycloserine control, R_f 0.54 (N, NP); β-aminoxy-D-alanine control, R_f 0.63 (N).

Solutions of **2** (0.004 *M*) in pH 1 and 2 buffer solutions (0.05 *M* KCl–HCl) were prepared and were tested at timed intervals with the nitroprusside reagents and ninhydrin. The solutions gave negative ninhydrin and nitroprusside tests when first prepared, but after 1 hr at room temperature, both gave positive tests.

Cycloserine (1) and 3,6-Bis(aminoxymethyl)-2,5-piperazinedione (2) in 1 *M* Acetic Acid.—Two solutions, one containing 27 mg/ml of cycloserine-free **2** and the other containing 54 mg/ml of **1** in 1 *M* aqueous acetic acid (pH 2.5) were prepared and a timed study (MPW system) was carried out (Table VI).

TABLE VI

PAPER CHROMATOGRAPHY STUDY OF 1 AND 2 IN 1 *M* ACETIC ACID

	Time, hr		
	0	16	28
1	0.52 (N, NP)[a]	0.54 (N, NP)	0.54 (N, NP)
		0.71 (N)	0.71 (N)
2		0.54 (N, NP)	0.54 (N, NP)
		0.71 (N)	0.71 (N)

[a] Controls: 0.54 (N, NP), cycloserine; β-aminoxy-D-alanine, 0.69 (N).

A white precipitate was observed in both of the above solutions after standing 4 days at room temperature. The precipitates were centrifuged and washed twice with distilled water. Approximately 7 mg of solid (R_f 0.91) was obtained from each solution. The ir spectra of the two materials were identical with each other and with that of an authentic sample of 3,6-dimethylene-2,5-piperazinedione (**7**).

Registry No.—**1**, 339-72-0; **2**, 17393-47-4; **3**, 15996-17-5; **4**, 30428-16-1; **5**, 106-57-0; *N*-*tert*-butoxycarbonyl-L-alanyl-L-alanine methyl ester, 19794-10-6; L-alanyl-L-alanine methyl ester hydrobromide, 30378-33-7.

(18) The procedure given here was adapted from the work of L. R. Jones, *Anal. Chem.*, **28**, 39 (1956).

(19) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 2, Wiley, New York, N. Y., report a melting point of 154–155°.