The Reactions of 3,6-Bis(aminoxymethyl)-2,5-piperazinedione with Acid and Alkali. A Kinetic Study

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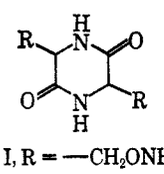
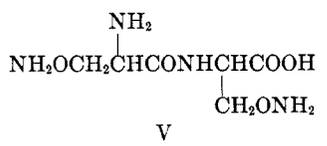
A detailed kinetic study of the reactions of D-cycloserine dimer, (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione (I), in aqueous base and acid are described. A facile elimination of hydroxylamine giving 3,6-dimethylene-2,5-piperazinedione (II) occurred upon treatment of I with base, whereas hydrolysis of I in acid solution gave the dipeptide, β-aminoxy-D-alanyl-β-aminoxy-D-alanine. The reaction paths of each of these processes are discussed.

Our interest in the chemistry and biological activities of cycloserine and its congeners has lead us to examine, in some detail, the reactions of the D-cycloserine dimer, (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione, with aqueous sodium hydroxide and hydrochloric acid. In 1962, Michalsky[1] proposed that the active form of D-cycloserine *in vivo* is the dimer (I) and also reported[2] that the racemic dimer is as effective as DL-cycloserine against *Mycobacterium tuberculosis*. Consequently, in any studies on the mode of action of cycloserine, it became imperative to remove the dimer as a contaminant and of considerable interest to examine its chemical properties. In 1955, Hidy,[3] *et al.*, reported that the cycloserine dimer underwent a base-catalyzed elimination reaction giving hydroxylamine and 3,6-dimethylene-2,5-piperazinedione (II). We report here a kinetic study of this reaction and show that it passes through a discrete intermediate, 3-methylene-6-aminoxymethyl-2,5-piperazinedione. The nmr spectrum of II is also described.



I, R = —CH_2ONH_2
II, R = =CH_2
III, R = —CH_2OH
IV, R = —H

Our interest in the acid-catalyzed hydrolysis of 2,5-piperazinediones was aroused by the facile mutarotation of I in aqueous acid. Subsequent investigation showed this mutarotation to result from the hydrolytic cleavage of the (+)-2,5-piperazinedione (I) yielding the (−)-dipeptide, aminoxy-D-alanylaminoxy-D-alanine (V).



V

The presence of the aminoxymethyl groups in I led us to anticipate the possibility of intramolecular participation by this highly nucleophilic function. We chose III, the 2,5-piperazinedione derived from serine, as a model compound having similar substitution to I for comparison.

Experimental Section

Reaction of I with Aqueous Base. Materials. (+)-3,6-Bis(aminoxymethyl)-2,5-piperazinedione (I).—To 5.41 g (0.045 mol) of D-cycloserine in a 500-ml round-bottomed flask equipped with a reflux condenser and drying tube was added 250 ml of absolute ethanol, and the mixture was stirred magnetically. Glacial acetic acid (10.3 ml) was then added, and the suspension was refluxed for 45 min. The white flocculent solid was removed by vacuum filtration, washed with three 50-ml portions of cold ethanol, and dried at room temperature overnight under vacuum giving 4.55 g of crude dimer. The crude solid product was dissolved in a minimum amount of hot distilled water and this solution was centrifuged while hot to remove insoluble contaminants. Boiling ethanol was added slowly until the solution became cloudy and 1 drop of hot water was added. The solution was allowed to cool slowly; the white needles were removed by vacuum filtration and washed with 10 ml of 4:1 ethanol–water solution followed by two 10-ml portions of absolute ethanol. The crystals were dried overnight under vacuum giving 3.60 g (67%) of cycloserine-free I: $[\alpha]^{23}D$ +70° (*c* 1, H_2O), +57° (*c* 1, 1 *N* HCl); mp >340°; ir (KBr), 3310, 3200 (NH), 1650 (C=O), and 1365, 1175, and 1018 cm^{-1} (characteristic of NH_2O—); nmr (D_2O + CF_3COOH), δ 4.55 (singlet), (D_2O), 4.37 (multiplet), and 4.04 ppm (multiplet).

We are indebted to Dr. O. K. Behrens, Eli Lilly Co., and Dr. W. F. Runge, Commercial Solvents Corp., for generous samples of D-cycloserine and to Mrs. Nonja Bisgard for β-aminoxy-D-alanine prepared by the method of Stammer.[4]

3,6-Dimethylene-2,5-piperazinedione (II).[5]—A solution of 0.75 g of 3,6-bis(aminoxymethyl)-2,5-piperazinedione in 148 ml of 0.008 *M* sodium hydroxide was maintained at 25° for 2 hr. After 30 min the product began to crystallize giving 0.27 g (53%) of II. The 3,6-dimethylene-2,5-piperazinedione was recrystallized from water: uv max (0.05 *M* NaOH), 284 mμ (ε 15,200),[6] (H_2O), 270; ir (Nujol), 3.01 (N—H), 5.90 (CONH), 6.07 (C=C), 11.2 μ (C=CH_2); nmr (DMSO-d_6), δ 5.11 (s) and 5.29 ppm (s).

Anal. Calcd for $C_6H_6N_2O_2$: C, 52.17; H, 4.83; N, 20.28. Found: C, 52.03; H, 4.43; N, 20.09.

Kinetic Measurements.—Unless otherwise stated, the kinetics of the elimination reaction were measured with a Cary 14 recording spectrophotometer equipped with a thermostated cell compartment at 25°. The reaction was initiated by adding the 3,6-bis(aminoxymethyl)-2,5-piperazinedione to a cuvette containing NaOH. The ionic strength (0.5) was established with NaCl. The activation and fluorescence spectra were measured with the use of an Aminco–Bowman spectrophotofluorometer. Stand-

(1) J. Michalsky, J. Opichal, and J. Ctvrtnik, *Monatsh. Chem.*, **93**, 618 (1962).

(2) J. Michalsky, J. Ctvrtink, A. Horakova, and V. Bydzovsky, *Experientia*, **18**, 217 (1962).

(3) 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 (1965).

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

(5) F. C. Neuhaus and J. L. Lynch, *Biochemistry*, **3**, 471 (1964).

(6) During the course of this work, E. Svatek and J. Vaehek [*Csek. Farm.*, **12**, 509 (1963), and *Chem. Abstr.*, **61**, 9359 (1964)] reported that the absorption maxima for I and II are 270 and 295 mμ, respectively. This is inconsistent with the work described in this paper.

ard calibrations were performed with 3,6-dimethylene-2,5-piperazinedione to ensure linearity of relative intensity with concentration.

Chromatography.—(+)-3,6-Bis(aminoxymethyl)-2,5-piperazinedione and 3,6-dimethylene-2,5-piperazinedione were chromatographed on Whatman 3MM paper in the following descending solvent system: methanol–sodium acetate (μ 0.05), pH 4.6 (80:20). R_f values were obtained from 3,6-bis(aminoxymethyl)-2,5-piperazinedione (0.42) and 3,6-dimethyl-2,5-piperazinedione (0.74).

Reaction of I with Aqueous Acid. Materials.—The L-serine was obtained from Aldrich Chemical Co. and was not further purified. (−)-3,6-Bis(hydroxymethyl)-2,5-piperazinedione (III) was prepared from L-serine by the method of Brockmann and Musso[7] giving a 60% yield of serine-free III after recrystallization from 1:1 water–ethanol: $[\alpha]^{23}D$ −64° (*c* 1, H_2O); mp 247–251° (lit. $[\alpha]D$ −64° (*c* 1, H_2O); mp 248–249°). Fisher certified reagent 1 *N* hydrochloric acid was used in the hydrolysis reactions.

Kinetic Studies.—Rates of hydrolysis for both I and III in 1 *N* HCl were determined at 50, 60, 65, 70, and 75°. The disappearance of the 2,5-piperazinediones was followed by the change in optical rotation using a Rudolph Model 80 Precision polarimeter. Thermodynamic data and rate constants were determined from kinetic plots which were least-squared on an IBM 1620 computer. The time was measured on a Precision Scientific Time-It electric clock. The hydrolysis reactions were carried out in a water-jacketed all glass 200-mm polarimeter tube. The temperature of the polarimeter tube was maintained by a Sargent constant temperature bath. Before each hydrolysis, the cell was allowed to equilibrate overnight. The substrate was weighed into a tared 10-ml class A volumetric flask and made up to volume with 1 *N* HCl. This solution was centrifuged and injected into the polarimeter cell using a 10 ml syringe equipped with a 10-cm Teflon needle. Measurements $[\alpha_0]$ were started when thermal convection currents were no longer observed in the light path.[8] The rotation at infinity $[\alpha_\infty]$ was the lowest reading which did not change appreciably over at least 2–3 half-lives.

Product Studies. 1. Chromatographic Studies.—The products formed during the hydrolyses were examined on circular paper chormatograms on which samples were spotted at various times during and at the completion of the reaction. The chromatograms were eluted by two mixed solvent systems: MPW (methyl ethyl ketone–pyridine–H_2O, 20:5:8, by volume) and BAW (*n*-butyl alcohol–acetic acid–H_2O, 5:1:1, by volume). Authentic samples of aminoxy-D-alanine and L-serine were used as reference standards, and the chromatograms were developed by spraying them with a 0.2% ninhydrin solution in 5% acetic acid–ethanol.

2. Isolation and Identification of D-Aminoxyalanyl-D-aminoxyalanine (V) from the Hydrolysis of I.—One mmole (0.250 g) of 3,6-bis(aminoxymethyl)-2,5-piperazinedione was dissolved in 14 ml of 2 *N* HCl, and the solution was heated with stirring at 60° for 4.5 hr ($t_{1/2}$ at 60° in 2 *N* HCl = 60 min). The reaction was immediately diluted with water to 400 ml, and the solvent mixture was removed by lyophilization. Circular paper chromatography and ir spectra showed the solid product to be the same as that obtained in the kinetic experiments (R_f 0.84 in MPW). The ir spectrum of this compound after three precipitations from ethanol–ether showed bands at 1740 (-COOH), 1680 (-CONH-), 1560 (amide II) and bands characteristic of the aminoxy groups at 1390, 1190, and 1015 cm^{-1}. Comparison of this ir spectrum with that of β-aminoxyalanine dihydrochloride showed that they were different.

The methyl ester of the dipeptide (V) was prepared by the Fischer method using dry methanol and dry HCl and showed infrared bands at 1740 ($COOCH_3$), 1680 (CONH), 1540 (amide II), and a broad C–O–C band at 1220 with a shoulder at 1030 cm^{-1}. The nmr spectrum of the methyl ester of V in DMSO-d_6 showed δ 3.73 (s, 3 H, CH_3O-), 4.50 (complex multiplet, 6 H), and 8.25 ppm (broad multiplet, 11 H, NH). These data showed this ester (V) to be different from β-aminoxyalanine methyl ester dihydrochloride and identical with the product of HCl-catalyzed methanolysis of the dimer I. The dipeptide ester (V) was also converted back into optically active I when treated with IRA-400 ion-exchange resin (OH^- cycle) in 1:1 aqueous methanol solution. Because of the ease of the loss of HCl from the weakly basic aminoxy groups and the hygroscopic nature of this material, acceptable elemental analyses were not obtained.

3. The Isolation and Optical Rotation of I from the Hydrolysis Reaction.—One mmole (0.205 g) of I was heated with stirring at 70° in 10 ml of 1 *N* HCl for 48 min (1 half-life). The solution was diluted to 400 ml with distilled water, and the solvent was removed by lyophilization. The residue was dissolved in 10 ml of dry methanol; 0.318 g (2 mmol) of 5-chlorosalicyladehyde was added to the solution and the mixture was stirred overnight at room temperature. After the solvent was removed 20 ml of distilled water was added to the pink solid, and the mixture was stirred magnetically. The insoluble dimer derivative was removed by centrifugation and was washed with three additional 10-ml portions of water to remove all ninhydrin positive material. The crude solid product weighed 0.225 g, $[\alpha]^{23}D$ +152° (*c* 1, DMF), and had an ir spectrum identical with that of N,N′-bis(5-chlorosalicylidene)-3,6-bis(aminoxymethyl)-2,5-piperazinedione,[9] $[\alpha]^{23}D$ +152° (*c* 1, DMF).

4. Product of Hydrolysis of III.—Circular chromatography (MPW and BAW) of the hydrolysis products of III showed only one ninhydrin positive spot: R_f (MPW) 0.40; R_f (BAW) 0.35; L-serine, R_f (MPW) 0.41 and R_f (BAW) 0.37. The ir spectrum of the product showed three important peaks at 1740 (COOH), 1680 (CONH) and 1560 cm^{-1} (amide II) indicating it to be the dipeptide, L-seryl-L-serine. Comparison of the ir spectrum with that of L-serine hydrochloride[10] showed that the compounds were different. Esterification of the dipeptide using dry HCl–methanol gave the extremely hygroscpic dipeptide methyl ester dihydrochloride which showed bands at 3320 (-OH), 1748 (-$COOCH_3$), 1670 (-CONH-), 1580 (amide II), 1235 and 1050 cm^{-1} (C–O–C) in its ir spectrum. Its nmr spectrum showed δ (DMSO-d_6, D_2O) 4.18 (s, 3 H, CH_3O-) and 4.41 (complex multiplet, 6 H). Comparison of these data with those of serine methyl ester hydrochloride showed these compounds to be different.

Anal. Calcd for $C_7H_{15}ClN_2O_5$: C, 34.34; H, 6.23; N, 11.55. Found: C, 34.35; H, 6.45; N, 11.49.

The dipeptide methyl ester was converted back into optically active III using IRA-400 ion-exchange resin (OH^- cycle) in 1:1 aqueous methanol solution.

Results and Discussion

When treated with alkali, (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione (I) was converted into 3,6-dimethylene-2,5-piperazinedione (II). The kinetics of the α,β elimination have been investigated in increasing concentrations of NaOH at constant ionic strength. The amount of product formed was determined from the increase in absorption of 284 mμ, and these results are summarized in Figure 1A and Table I. The first-order plot is linear for the terminal phase of the reaction. An expanded plot reveals a pronounced nonlinearity in

TABLE I

PSEUDO-FIRST-ORDER RATE CONSTANTS AND SECOND-ORDER RATE CONSTANTS FOR 3,6-DIMETHYLENE-2,5-PIPERAZINEDIONE FORMATION[a]

NaOH, *M*	$k_{obsd} \times 10^3$, sec^{-1}	$k \times 10^1$, M^{-1} sec^{-1}
a, 0.075	19.1 ± 0.4	2.55 ± 0.06
b, 0.050	15.5 ± 0.5	3.11 ± 0.13
c, 0.025	10.1 ± 0.2	4.05 ± 0.70[b]
d, 0.010	4.22 ± 0.20	4.22 ± 0.11[b]
e, 0.008	3.51 ± 0.09	4.39 ± 0.11[b]
f, 0.005	2.46 ± 0.35	4.92 ± 0.70[b]
g, 0.003	1.24 ± 0.08	4.13 ± 0.29[b]

[a] Four reactions [μ 0.5] were performed for each concentration of NaOH [two with 3.93×10^{-5} *M* and two with 7.94×10^{-5} *M* 3,6-bis(aminoxymethyl)-2,5-piperazinedione]. The pseudo-first-order rate constants were calculated from the terminal phase of the reaction as illustrated in Figure 2. [b] Average is 4.34.

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

(8) The time required for equilibrium was approximately 25 min.

(9) C. H. Stammer and J. D. McKinney, *J. Org. Chem.*, **30**, 3436 (1965).

(10) The crude product showed $[\alpha]^{23}D$ 10.5° (*c* 1, HCl). J. S. Fruton [*J. Biol. Chem.*, **146**, 463 (1962)] found $[\alpha]^{23}D$ 14.2 (*c*, 1 in H_2O).

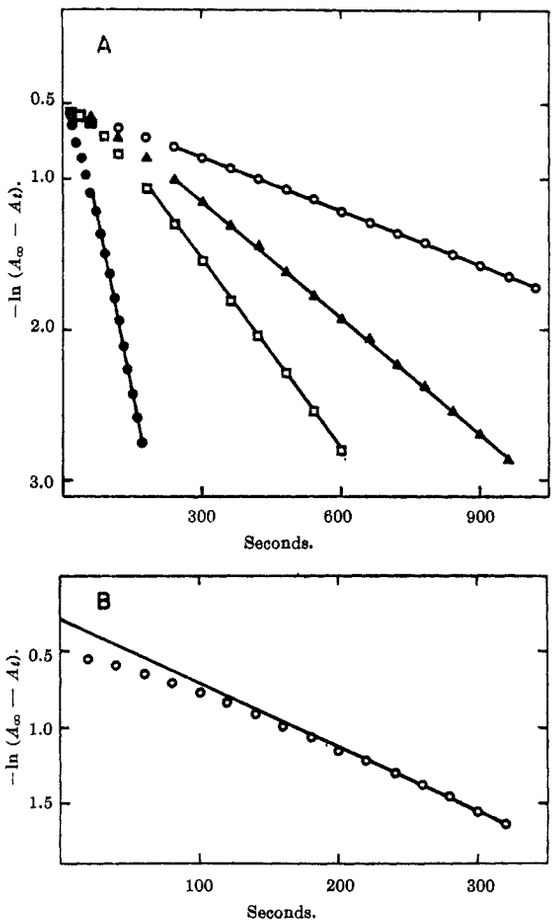


Figure 1.—A. First-order plot for the base-catalyzed formation of 3,6-dimethylene-2,5-piperazinedione from 3,6-bis(aminoxymethyl)-2,5-piperazinedione. The concentrations of NaOH are ○—○, 0.003 *M*; ▲—▲, 0.005 *M*; □—□, 0.001 *M*; and ●—●, 0.05 *M*. The reaction mixtures (μ 0.5) contained 3.61 × 10^{-5} *M* 3,6-bis(aminoxymethyl)-2,5-piperazinedione and NaOH as indicated. B. Expanded plot from □—□ in A.

the initial phase of the reaction (Figure 1B). A summary of the pseudo-first-order rate constants obtained from the terminal phase is presented in Table I, and the second-order rate constant established from these results was found to be 0.434 M^{-1} sec^{-1}.

The instantaneous pseudo-first-order rate constants calculated during the course of the reaction approach that calculated from the linear phase of the reaction plot. These results are consistent with the formation of an intermediate, *e.g.*, 3-methylene-6-aminoxymethyl-2,5-piperazinedione. Although this intermediate has not been isolated in large amounts, evidence for its presence has been observed on paper chromatograms. 3,6-Dimethylene-2,5-piperazinedione (II) was detected on chromatograms by its fluorescence. The (+)-3,6-bis(aminoxymethyl)-2,5-piperazinedione (I) was easily located on paper by spraying the chromatogram with NaOH. When aged preparations of I were chromatographed, a new ultraviolet absorbing nonfluorescing spot (R_f 0.58) was observed. On spraying with NaOH, this spot became fluorescent. The ultraviolet absorbing nonfluorescing material was eluted from preparative chromatograms, and when treated with 0.05 *M* NaOH, its ultraviolet absorption spectrum was identical with that observed for II. The kinetics of elimination from the intermediate are illustrated in Figure 2 and are compared with the formation of II from I. In contrast to the latter reaction, the first-order plot for the formation II from the intermediate is linear and the pseudo-first-order rate constant is 12.4 × 10^{-3} sec^{-1}. Under the same conditions the pseudo-first-order rate constant from the linear phase of the elimination reaction from I is 15.5 × 10^{-3} sec^{-1}. From the results in Figure 2, we can establish $A_\infty - A_0$ is equal to 0.412 for the reaction of the intermediate. The observed absorbance, A_∞, is 0.722 and therefore A_0 is 0.310. Thus, elimination of the second molecule of hydroxylamine results in the formation of II with approximately twice the ϵ of the intermediate. As a control, I was chromatographed and eluted from the paper. The kinetics of elimination are identical with those reported in Figure 1, and reaction mixtures similar to those described in Figure 1 were examined by chromatography. In every case, an ultraviolet absorbing, nonfluorescing spot was detected which corresponded to the intermediate described above. Thus, on the basis of the kinetic experiments and the detection of the intermediate, we propose the following sequence.

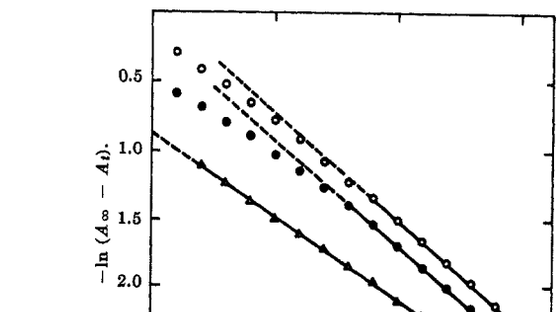
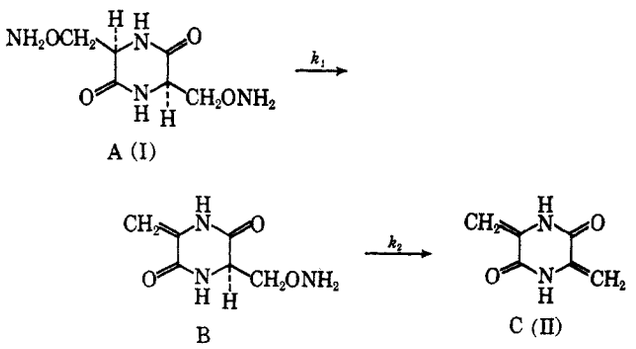


Figure 2.—First-order plot for the conversion of intermediate into 3,6-dimethylene-2,5-piperazinedione. The intermediate (R_f 0.58) and 3,6-bis(aminoxymethyl)-2,5-piperazinedione were eluted from a preparative chromatogram and added to a reaction mixture which contained 0.05 *M* NaOH. In △—△ the formation of 3,6-dimethylene-2,5-piperazinedione from the intermediate is compared with the formation of 3,6-dimethylene-2,5-piperazinedione (●—●) from 3,6-bis(aminoxymethyl)-2,5-piperazinedione eluted from a preparative chromatogram. The latter reaction is compared with a sample of 3,6-bis(aminoxymethyl)-2,5-piperazinedione which had not been chromatographed (○—○).



The other product, H_2NOH, has not been rigorously defined.

The fluorescence emission spectrum of II provided a method for the estimation of product during the course

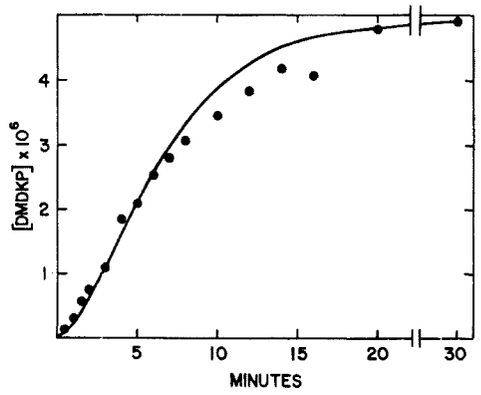


Figure 3.—Formation of 3,6-dimethylene-2,5-piperazinedione from 3,6-bis(aminoxymethyl)-2,5-piperazinedione. The reaction mixture (μ 0.5) contained 0.01 M NaOH and 4.9 $\times$ 10^{-6} M 3,6-bis(aminoxymethyl)-2,5-piperazinedione. The activation wavelength is 300 mμ and the fluorescence wavelength is 420 mμ. The solid line is calculated from eq 1 (k = 4.22 $\times$ 10^{-3} sec^{-1}; see Table I).

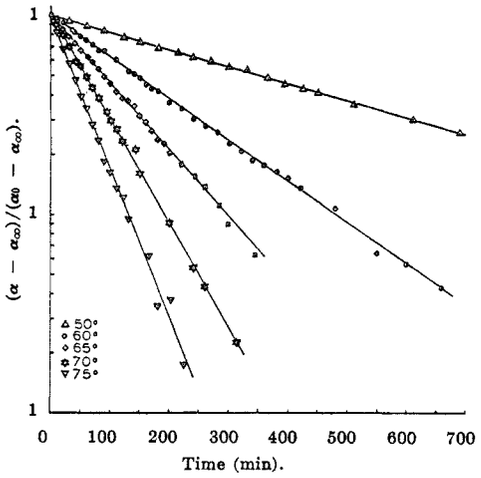


Figure 4.—Kinetic plots for the hydrolysis of 3,6-bis(aminoxymethyl)-2,5-piperazinedione (I) in 1 N HCl at various temperatures. Experimental points and least-square lines are shown.

of the reaction. This is in contrast to the absorbance which measures both the intermediate and the product. In Figure 3 the concentration of product is presented as a function of time. If we assume that $k_1 = k_2$ in the sequence shown above, the integrated rate equation is (1) where A, B, and C are defined in the sequence above.

$$C = A_0[1 - e^{-kt} - kte^{-kt}] \quad (1)$$

The calculated curve is in good agreement with the experimental data. When 3,6-bis(hydroxymethyl)-2,5-piperazinedione (III) was treated with 1 N sodium hydroxide for 15 min under conditions identical with those used for I, no spectral changes were observed from 250 to 400 mμ.

Surprisingly, the nmr spectrum of the bismethylene compound (II) consists of only two sharp singlets at δ 5.11 and 5.29 ppm. Since the two methylene protons are magnetically nonequivalent, an AX or AB spectrum was expected. In order to explain the observed spectrum we must assume that the geminal coupling constant is zero,[11] thus the angle between the methylene protons is about 125°.[12] We can assign the downfield peak to that proton adjacent to and in the plane of the carbonyl group.[13]

The results of these studies have established that the elimination of hydroxylamine from I proceeds *via* two consecutive pseudo-first-order elimination reactions. This conclusion is based on the kinetics of 3,6-dimethylene-2,5-piperazinedione (II) formation and evidence has been presented for the intermediacy of 3-methylene-6-aminoxymethyl-2,5-piperazinedione (B). It is known that optically active 2,5-piperazinediones racemize in very dilute alkali and that appropriately substituted compounds of this type will undergo elimination giving methylene-2,5-piperazinediones.[14] Both of these reactions probably occur by the removal of a proton from the "α carbon atom" since there is considerable evidence for the acidity of this proton.

Michalsky[2] proposed that I may inhibit pyridoxal dependent enzyme systems by forming an N,N'-bispyridoxylidene derivative of I, thus irreversibly removing the coenzyme from solution. The facile elimination described in this work, however, indicates that hydroxylamine, II, and the intermediate B may also be present and may contribute to enzyme inhibition. These compounds, however, have not been tested as inhibitors.

The base-catalyzed elimination of hydroxylamine from I provided a sensitive method for detecting this compound in the presence of cycloserine and was used to detect I in the *in vitro* studies of D-ala-D-alasynthetase inhibition.[5]

First-order kinetics were observed for the acid hydrolysis of both I and III and the rate constants are given in Table II. The kinetic plots remained linear in all cases for 3.5 half-lives and in most cases for 4–5 half-lives. Some slight deviation after approximately 3.5 half-lives was observed for reactions of I carried out at 65° and above. Rate constants were calculated from least-square kinetic plots according to the integrated first-order rate law

$$\ln \frac{(\alpha_\infty - \alpha)}{(\alpha_\infty - \alpha_0)} = -kt$$

Kinetic plots for $(\alpha - \alpha_\infty)/(\alpha_0 - \alpha_\infty)$ *vs.* time are shown in Figures 4 and 5. Entropy and heat of acti-

Table II

Pseudo-First-Order Rate Constants for Hydrolysis of I and III in 1 N HCl

Temp, °C	$k \times 10^3$, min^{-1}	$t_{1/2}$, min	$k \times 10^3$, min^{-1}	$t_{1/2}$, min
50	1.95 ± 0.05[a]	355	1.45 ± 0.05[b]	475
60	4.76 ± 0.05[b]	146	3.78 ± 0.01[c]	183
65	7.78 ± 0.09[b]	89	5.51 ± 0.07[b]	126
70	12.1 ± 0.1[c]	57	8.80 ± 0.07[b]	79
75	17.5 ± 0.4[d]	40	13.3 ± 0.4[d]	52

[a] Average of five runs. [b] Average of two runs. [c] One run with standard deviation. [d] Average of three runs.

(11) Alantolactone, which also has a methylene group conjugated with a carbonyl function, shows J_{AX} = 0; *cf.* J. A. Marshall and N. Cohen, *J. Org. Chem.*, **29**, 3727 (1964).

(12) H. Conroy, *Advan. Org. Chem.*, **2**, 310 (1960).

(13) The structure of II was established by Hidy,[3] *et al.*, in 1955 by hydrogenation to V. The ir spectrum and a mass spectrum of II, kindly supplied by Dr. C. Aldridge and Mr. D. R. McAdams, Esso Research Laboratories, Baton Rouge, La., also confirm the structure of II.

(14) R. C. Elderfield, "Heterocyclic Compounds," Vol. 6, John Wiley & Sons, Inc., New York, N. Y., 1957, p 441.

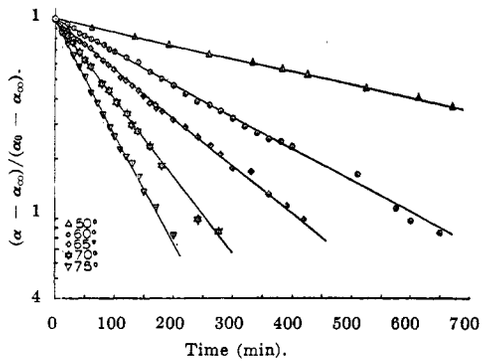


Figure 5.—Kinetic plots for the hydrolysis of 3,6-bis(hydroxymethyl)-2,5-piperazinedione (III) in 1 *N* HCl at various temperatures. Experimental points and least-square lines are shown.

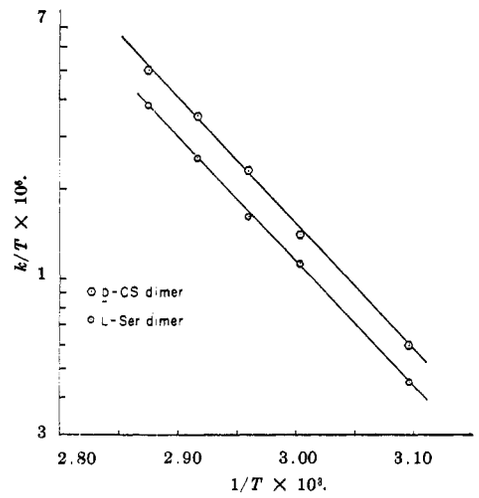


Figure 6.—Plot of k/T *vs.* $1/T$.

vation were calculated from the least-squares plot of log k/T *vs.* $1/T$ (Figure 6) according to the equation

$$\log \frac{k_r}{T} = -\frac{H^{\pm}}{2.303R}\frac{1}{T} + \frac{S^{\pm}}{2.303R} + \log \frac{k}{h}$$

The Arrhenius energy of activation was calculated from a least-squares plot of ln k *vs.* $1/T$ as shown in Figure 7.

Activation parameters are given in Table III.

TABLE III

ACTIVATION PARAMETERS FOR HYDROLYSIS OF I AND III IN 1 *N* HCl

	E_a, kcal	$H^{\pm}$, kcal	$S^{\pm}$, eu
D-3,6-Bis(aminoxymethyl)-2,5-piperazinedione (I)	19.9 ± 0.6	19.2 ± 0.6	−19.7 ± 1
L-3,6-Bis(hydroxymethyl)-2,5-piperazinedione (III)	19.7 ± 0.6	19.0 ± 0.6	−19.8 ± 1

The main products of the hydrolyses of I and III were found to be β-aminoxy-D-alanyl-β-aminoxy-D-alanine (V) and L-seryl-L-serine, respectively. Paper chromatography of samples taken at various times during the hydrolyses showed that the dipeptides were formed almost exclusively during the first 3–4 half-lives of the reaction.

It was necessary to show that the polarimetric data obtained in this study was not complicated by the racemization of products and/or substrates. Unreacted I was isolated from the hydrolysis mixture

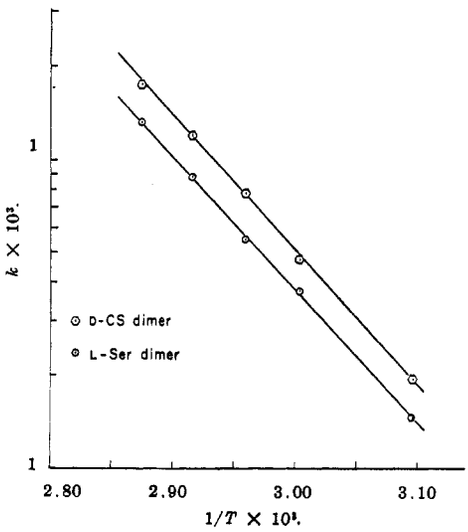


Figure 7.—Plot of k *vs.* $1/T$.

as the very water-insoluble 5-chlorosalicylaldehyde derivative after 1 half-life and showed no loss in optical activity. The optical activity of the end products from hydrolysis of both I and III do not change at an appreciable rate after reaching a final value. This, along with the excellent linearity of the kinetic plots (Figures 4 and 5), is convincing evidence that racemization does not appreciably effect the reliability of the kinetic procedure.

The hydrolysis of various 2,5-piperazinediones has been used extensively as a method for the preparation of dipeptides and a review on this subject is available.[15] Although most of the kinetic investigations concerning the hydrolysis of 2,5-piperazinediones have involved basic hydrolysis, some kinetic studies in aqueous acid have been reported. Edward and Meacok[16] studied the effect of hydrochloric acid concentration upon the rate of hydrolysis of unsubstituted 2,5-piperazinedione (IV) and found that a linear relationship existed for acid concentrations up to 10 *M*, and that no rate maximum was reached. It is of interest that in most cases studied the hydrolysis appeared to take place stepwise and that in the hydrolysis of IV, k_1 was 50 times greater than k_2, *i.e.*

$$\text{IV} \xrightarrow{k_1} \text{Gly-Gly} \xrightarrow{k_2} 2\text{Gly}^{17}$$

The fact that we obtained only the dipeptides as products of the hydrolysis of I and III is consistent with this large k_1/k_2 ratio. This dipeptide stability is also consistent with reports[18] that N-terminal serine and threonine moieties appear to stabilize these linkages toward acid hydrolysis and suggest that the aminoxy function has a similar stabilizing effect.

The possibility that the aminoxy and hydroxyl functions of I and III were both participating intramolecularly in the hydrolysis of these compounds has been carefully considered in the light of the experimental

(15) Reference 10, p 435.
(16) J. T. Edward and S. C. Meacok, *J. Chem. Soc.*, 2000 (1957).
(17) E. F. Hammel, Jr., and S. Glasstone, *J. Amer. Chem. Soc.*, **17**, 3741 (1954).
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results. While no definite statement can be made about this, we are presently making a more complete study of the hydrolyses of I, III, and other 2,5-diketopiperazinediones in order to clarify further the hydrolysis mechanism.

Registry No.—I, 17393-47-4; II, 15996-22-2; III, 17393-48-5; IX, 17393-49-6; L-seryl-L-serine hydrochloride, 17393-50-9.

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Studies Relating to the Synthesis of (+)-Valeranone

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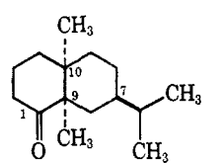
Several synthetic schemes leading from ketol **9**, the condensation product of (−)-dihydrocarvone (**8**) and methyl vinyl ketone, to (+)-valeranone (**19**) are described. The first of these employs norvaleranone (**18**), prepared from ketol **9** by sequential reduction (H_2/Pt, then $LiAlH_4$), acetylation, hydrogenolysis (Li, $EtNH_2$), hydroboration, and oxidation ($NaOH$–H_2O_2, then CrO_3). Angular methylation of norvaleranone was effected *via* the *n*-butylthiomethylene derivative **22**. A second route to valeranone involved conversion of ketol **9** into 2-oxovalerane (**39**). This conversion involved reduction (H_2/Pt, then $LiAlH_4$), selective mesylation, fragmentation (KO-*t*-Bu), addition of methyllithium, cation-initiated olefin cyclization (HCO_2H), saponification of the resulting formate, and oxidation (CrO_3) of the alcohol thus obtained. Transposition of the ketone grouping to the adjacent C-1 position completed the synthesis. Two methods are described for effecting this transposition. Both employ the 1-acetoxy derivative **50**, obtained from ketone **39** by bromination, dehydrobromination [$CH_3CON(CH_3)_2$, $CaCO_3$], acetoxylation [$Pb(OAc)_4$, BF_3], and hydrogenation. Conversion of ketone **50** into the thioketal derivative **51** followed by removal of the acetyl grouping ($LiAlH_4$) and desulfurization (Raney nickel) afforded alcohol **53** which yielded (+)-valeranone (**19**) upon oxidation (CrO_3). Alternatively, reduction ($NaBH_4$) of keto acetate **50** followed by mesylation and base treatment yielded the 1β,2β-oxide **56** which was converted into (+)-valeranone through reduction ($LiAlH_4$) followed by oxidation (CrO_3).

Valeranone, a naturally occurring sesquiterpene ketone first isolated by Stoll and coworkers[2] in 1957, was identified as 9α,10α-dimethyl-7β-isopropyl-1-decalone (**1**)[3] after extensive chemical investigation[4] and an initial erroneous structure proposal. Subsequently, several other natural products[5] were related to valeranone, and together these compounds comprise the valerane (**2**) family of sesquiterpenes. The valeranes are unusual among sesquiterpenes in two respects. Their carbon skeleton, although formally divisible into isoprene units (*cf.* **2**), cannot be derived from farnesol since two of the isoprene units must be linked in a tail to tail arrangement. Thus the valeranes differ from their more commonly found hydronaphthalene relatives, the eudesmanes (**3**) and the cadinanes (**4**) which may be formulated as head to tail linked isoprenoids.[6]



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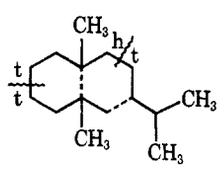

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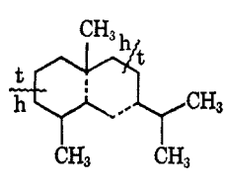

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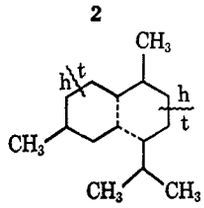

4

A second distinctive feature of the valeranes is their C-10 absolute configuration which is opposite that of most eudesmanes.[7] However, both families have the same isopropyl side-chain orientation. A biogenetic scheme which accomodates these points has been presented.[8]

The unusual structural features that confounded the structure elucidation of valeranone make this substance an interesting target for synthetic studies. An attractive starting point for such studies was suggested by the work of Howe and McQuillin[9] who found that (+)-dihydrocarvone (**5**) underwent annelation with ethyl vinyl ketone to give an 85:15 mixture of epi-α-cyperone (**6**) and α-cyperone (**7**) in about 60% yield. This sequence offers a direct route to bicyclic materials with the correct C-10 to C-7 stereochemical relationship of valeranone. Moreover, intermediates of known

(1) (a) Fellow of the Alfred P. Sloan Foundation, 1966–1968; (b) National Institutes of Health Predoctoral Fellow, 1965–1967.

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(4) J. Křepinský, M. Romaňuk, V. Herout, and F. Šorm, *Tetrahedron Lett.*, **5**, 169 (1962), and previous papers; E. Hõhne, *Collect. Czech. Chem. Commun.*, **28**, 3128 (1963); T. R. Govindachari, B. R. Pai, K. K. Purushothaman, and S. Rajadurai, *Tetrahedron*, **12**, 105 (1961); C. Djerassi, *Tetrahedron Lett.*, **6**, 226 (1961); H. Hikino, T. Hikino, Y. Takeshita, K. Meguro, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **11**, 1207 (1963); W. Klyne, S. C. Bhattacharyya, S. K. Paknikar, C. S. Narayanan, K. S. Kulkarni, J. Křepinský, M. Romaňuk, V. Herout, and F. Šorm, *Tetrahedron Lett.*, 1443 (1964); K. S. Kulkarni, S. K. Paknikar, and S. C. Bhattacharyya, *Tetrahedron*, **20**, 1289 (1964).

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