On Cyclic Intermediates in Hydrolytic Reactions. I. The Alkaline Hydrolysis of Dinitro-2-pyridylalanylglycine

ANGELO SIGNOR, LAURA BIONDI, AND EMILIO BORDIGNON

Centro Nazionale di Chimica delle Macromolecole and Istituto di Chimica Organica, Universita' di Padova, Padova, Italy

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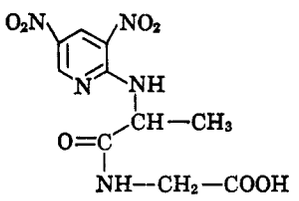
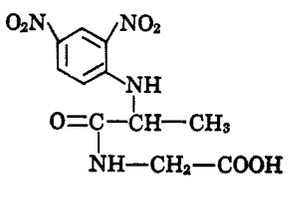
The rates of aqueous alkaline hydrolysis of 3,5-dinitro-2-pyridylalanylglycine (I) and 2,4-dinitrophenylalanylglycine (II) have been determined at 30°. The reactions have been carried out with large excesses of hydroxide ion and pseudo-first-order kinetics are obtained. The kinetics of hydrolysis were followed by measurement of the appearance of glycine and spectrophotometrically by the change of the intermediate 6,8-dinitroimidazo[1,2-*a*]-pyridin-3(2H)-one at 440 mμ; the rate law followed by I is $k_1 = k_2[OH^-] + k_3[OH^-]^2$. A mechanism of hydrolysis is proposed involving attack of the amide by pyridyl anion, giving the cyclic intermediate, 6,8-dinitroimidazo[1,2-*a*]pyridin-3(2H)-one and glycine.

In a previous investigation[1] it was noted that the rate of acid hydrolysis of 3,5-dinitro-2-pyridylalanylglycine (I) was markedly dependent on acid concentration; the rate associated with the loss of glycine was approximately 10^2–10^3 times as fast as that of 2,4-dinitrophenylalanylglycine (II). Furthermore, the rate of hydrolysis is proportional to the concentration of the conjugate acid of I; that is, the catalysis is mediated by the protonated pyridine species; these results add credence to the four-center nucleophilic–electrophilic mechanism proposed by Bender, *et al.*,[2] for general acid intramolecular catalysis of amide hydrolysis. In other words, these earlier experiments demonstrate the possibility of intramolecular catalysis by pyridine.

It is well known that the most important and extensive studies of nucleophilic catalysis have involved tertiary amines such as imidazole and pyridine. Recently we have observed that the hydrolysis of I is strongly catalyzed by concentrated alkali: for example, in 1.0 *M* sodium hydroxide the specific rate constant of hydrolysis of I is about 50 times as fast as that of II.



I



II

The present investigation was undertaken to evaluate the effect of the aza function on the rate of alkaline hydrolysis of peptide bond; furthermore, a mechanism for the reaction has been proposed.

Results

All experiments were carried out with at least a tenfold excess (generally much larger) of hydroxide ion over amide and at constant ionic strength; under these conditions pseudo-first-order kinetics are obtained. Constant ionic strength has been achieved by addition of suitable amounts of sodium chloride; although a constant salt mixture is obviously not attainable at different hydroxyl ion concentrations, the effect on the activity coefficient function of changing the relative amounts of hydroxyl ion and neutral salt may be assumed to be slight.

The accuracy of the first-order constants obtained from duplicate experiments is ±5% and the reactions were followed from 25 to 85% of complete reaction. A typical plot of log $a/(a - x)$ for the hydrolysis of I and II is shown in Figure 1. In Table I we report

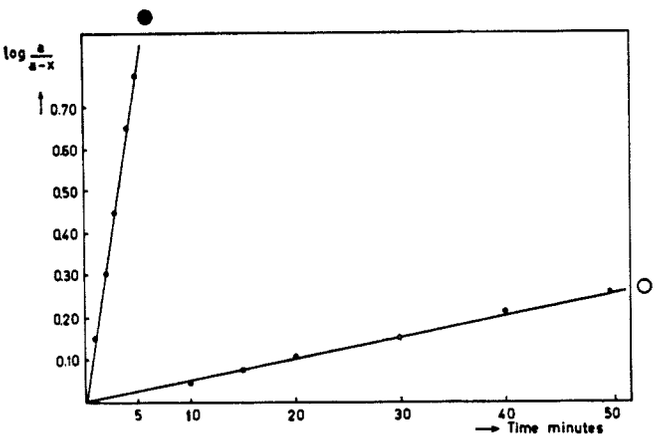


Figure 1.—Typical alkaline hydrolysis of dinitro-2-pyridylalanylglycine (●) and of dinitrophenylalanylglycine (○) in 0.50 *M* sodium hydroxide; ionic strength constant 1.00 *M*.

the observed pseudo-first-order rate constants, k_1, obtained at 30° both for I and II as determined by measurements of the rate of appearance of glycine. A few runs without any base showed that the reaction with water of both substrates was negligible compared to the reaction with hydroxide ion. From the data of Table I a linear plot of k_1 *vs.* hydroxide ion concentration is permitted for the hydrolysis of dinitrophenylalanylglycine (Figure 2). On the contrary, a linear plot of k_1 *vs.* hydroxide ion concentration for the hydrolysis of dinitro-2-pyridylalanylglycine is not possible, the rate of hydrolysis increasing most rapidly as the concentration of hydroxide ion increases (Figure 3); in this case the first-order constants are not proportional to hydroxide ion concentration, but instead the quantity $k_1/[OH^-]$ increases markedly with increasing $[OH^-]$, and contrast with the behavior shown by dinitrophenylalanylglycine. The first-order constants fit satisfactorily the rate equation (1).

$$k_1 = k_2[OH^-] + k_3[OH^-]^2 \quad (1)$$

Dividing through by $[OH^-]$

$$k_1/[OH^-] = k_2 + k_3[OH^-] \quad (2)$$

(1) A. Signor and E. Bordignon, *J. Org. Chem.*, **30**, 3447 (1965).

(2) M. L. Bender, Y. Chow, and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).

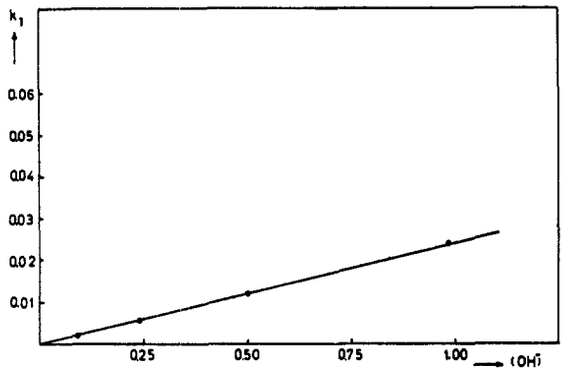


Figure 2.—The observed pseudo-first-order rate constants for the hydrolysis of dinitrophenylalanylglycine plotted against hydroxide ion concentration at 30°.

TABLE I

RATE CONSTANTS FOR ALKALINE HYDROLYSIS IN WATER AT 30°

$[OH^-]$, M	k_1	$k_1/[OH^-]$	k_1 (calcd)
	Dinitro-2-pyridylalanylglycine		
0.252	0.098	0.390	0.099
0.252	0.103	0.412	...
0.404	0.220	0.550	0.234
0.404	0.224	0.555	...
0.489	0.360	0.735	0.333
0.489	0.352	0.715	...
0.581	0.445	0.768	0.458
0.581	0.450	0.775	...
0.702	0.630	0.900	0.653
0.702	0.645	0.922	...
0.795	0.860	1.080	0.811
0.795	0.848	1.070	...
0.905	1.040	1.150	1.055
0.905	1.044	1.160	...
	Dinitrophenylalanylglycine		
0.097	0.0020	0.0206	
0.097	0.0022	0.0227	
0.240	0.0057	0.0237	
0.240	0.0055	0.0228	
0.503	0.0130	0.0258	
0.503	0.0128	0.0254	
0.985	0.0240	0.0243	
0.985	0.0230	0.0233	

A plot of $k/[OH^-]$ against $[OH^-]$ gives a straight line of slope k_3 and intercept k_2 (Figure 4). The fit of the data to eq 1 is shown in Table I by a comparison of observed and calculated values of k_1. The values of k_2 and k_3 obtained from experiments carried out at ionic strength of 1.00 M are 0.10 l. mole^{-1} min^{-1} and 1.19 l.^{2} mole^{-2} min^{-1}, respectively. In addition to the studies mentioned above it was also possible to follow the kinetics by measurements of the rate of appearance of an intermediate. The spectral changes which occur in the alkaline hydrolysis of I make this system particularly suitable for the detection of possible intermediates in its hydrolytic reaction by spectroscopic means; the initial rise and slow fall of the optical density at 440 mμ are indicative of the formation and decomposition of an intermediate species (Figure 5). The hydrolytic reaction of I may be followed spectrophotometrically by the change of the optical density at this wavelength; on the contrary, no reactive intermediate has been found for the alkaline hydrolysis of II. In Figure 6 are shown the spectra of dinitro-2-pyridylalanylglycine (I) and of dinitrophenylalanylglycine (II) in 1% $NaHCO_3$.

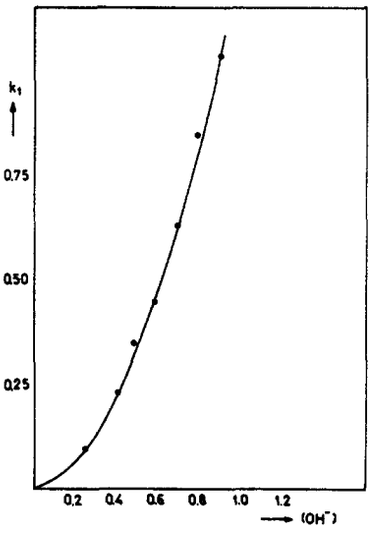


Figure 3.—The observed pseudo-first-order rate constants for the hydrolysis of dinitro-2-pyridylalanylglycine at 30° plotted against $[OH^-]$. The curve is calculated on the basis of the rate law $k_1 = k_2[OH^-] + k_3[OH^-]^2$.

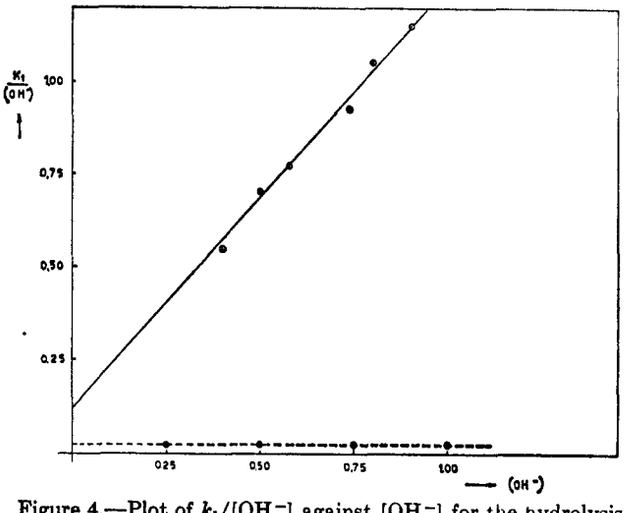


Figure 4.—Plot of $k_1/[OH^-]$ against $[OH^-]$ for the hydrolysis of dinitro-2-pyridylalanylglycine (solid line) and of dinitrophenylalanylglycine (broken line) in water at 30°.

We have determined experimentally the rates of formation and decomposition of the intermediate at 30° and at various concentrations of base; the rate constants of hydrolysis of I are reported in Table II together with the values obtained measuring the appearance of glycine.

TABLE II

RATE CONSTANTS OF THE HYDROLYSIS OF DINITRO-2-PYRIDYLALANYLGLYCINE IN WATER AT 30°

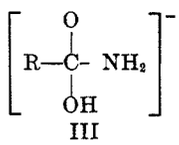
	k_1, min^{-1}	
$[OH^-]$, M	Appearance of glycine (amide) = 2×10^{-3} M	Appearance of intermediate, 440 mμ (amide) = 5×10^{-3} M
0.243	0.095	0.084
0.386	0.213	0.186
0.537	0.390	0.345

The intermediate is presumed to be 6,8-dinitroimidazo[1,2-a]pyridin-3(2H)-one as will be discussed later; its rate of disappearance in 0.537 M NaOH is $k_{obsd} = 0.020$ min^{-1}.

Discussion

Hitherto relatively little work has been done on the kinetics of the alkaline hydrolysis of amides, peptides, or peptide derivatives.

Bender[3-5] has obtained convincing evidence from the relative rates of hydrolysis and oxygen-isotope exchange that the alkaline hydrolysis of amides, like that of esters, proceeds through an intermediate hydroxide ion addition complex, III, and not through a simple displacement reaction.

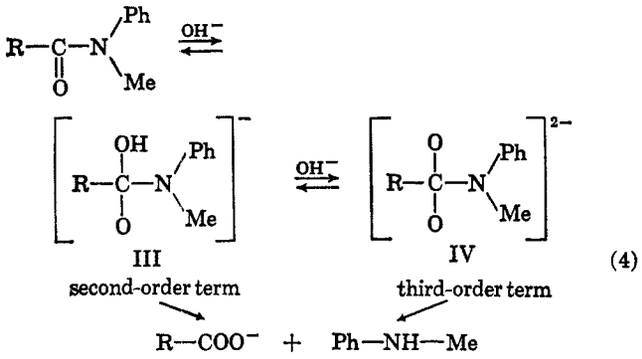


III

Recently Biechler and Taft[6] have investigated the kinetics and mechanism of the alkaline hydrolysis of some anilides and found that in concentrated alkali the rate law followed by N-methylanilides is eq 3.

$$\text{rate} = k_2[OH^-][\text{anilide}] + k_3[OH^-]^2[\text{anilide}] \qquad (3)$$

The observed rate equation is explained in terms of a reaction sequence involving two reactive intermediates and suggests that these hydrolyses proceed by two paths. The second-order term correspond to the ordinary hydrolysis through an intermediate analogous to III, whereas the third-order term probably results from hydrolysis proceeding through conjugate base IV, the concentration of which is proportional to $[OH^-]$. The following scheme (4) satisfactorily explains the "nonclassical" kinetics established for the alkaline hydrolysis of N-methylanilides.



(4)

The experimental data of Table I and Figure 2 show that the hydrolysis of dinitrophenylalanylglycine (II) are first order in $[OH^-]$ and can be ascribed to specific base catalysis; the average value of the second-order constant, k_2, is 0.024 l. mole^{-1}. min^{-1}. It is reasonable to assume that the hydrolysis of II proceeds through a tetrahedral intermediate such as III. In the case of the hydrolysis of dinitro-2-pyridylalanylglycine (I) we have an enhanced second-order term (0.100 l. mole^{-1} min^{-1} against 0.024 l. mole^{-1} min^{-1}) in addition to a third-order term (Figure 4).

The third-order term cannot be explained by means of an intermediate such as IV in that the parallel runs performed for dinitrophenylalanylglycine are first order in $[OH^-]$ throughout.

(3) M. L. Bender and R. D. Ginger, *J. Am. Chem. Soc.*, **77**, 348 (1955).
(4) M. L. Bender, R. D. Ginger, and K. C. Kemp, *ibid.*, **76**, 3350 (1954).
(5) M. L. Bender, *ibid.*, **75**, 5986 (1953).
(6) S. S. Biechler and R. W. Taft, Jr., *ibid.*, **79**, 4927 (1957).

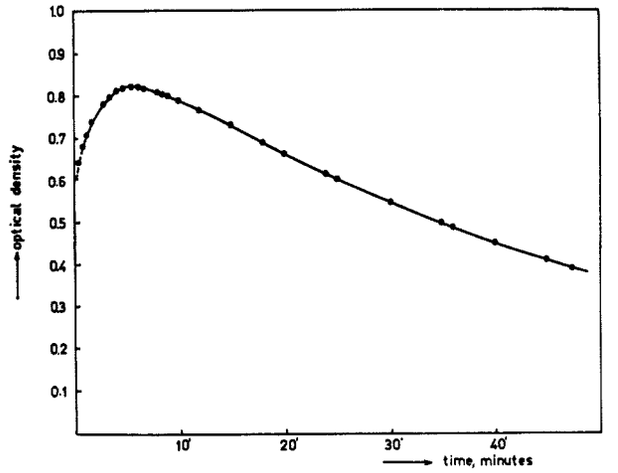


Figure 5.—Formation and decomposition of the intermediate in the hydrolysis of dinitro-2-pyridylalanylglycine.

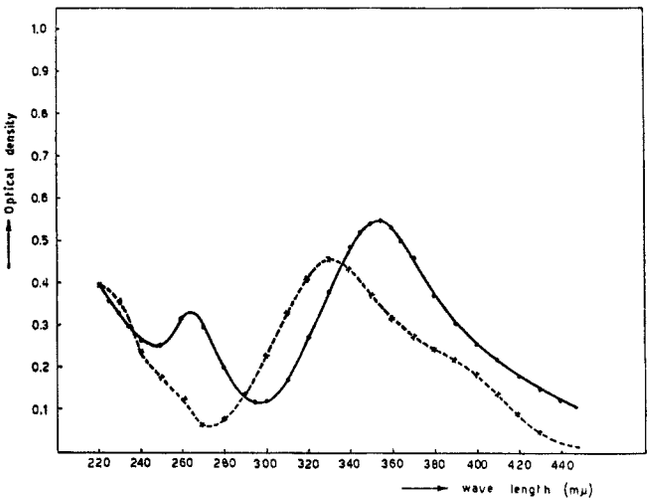
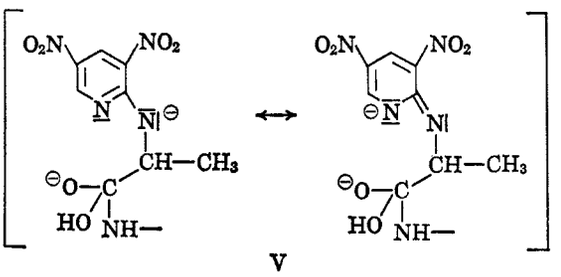


Figure 6.—Light absorption curves for dinitro-2-pyridylalanylglycine (solid line) and dinitrophenylalanylglycine (broken line) in 1% $NaHCO_3$.

It may be assumed that the presence of the aza function, in addition to the two nitro groups conjugated with the acidic –NH group, affect by a resonance interaction the ease with which the proton is removed from the acidic group.

We strongly favor the formation of the conjugate base V as the explanation of the kinetics observed for I.



V

It is to be expected that intramolecular nucleophilic displacement of glycine will give the cyclic intermediate 6,8-dinitroimidazo[1,2*a*]pyridin-3(2H)-one (VII) which has been independently synthetized from dinitro-2-pyridylalanine (VI) and PCl_3. We have found that the ultraviolet spectra of VII possessed the same shape of the intermediate in the alkaline hydrolysis of I, that is,

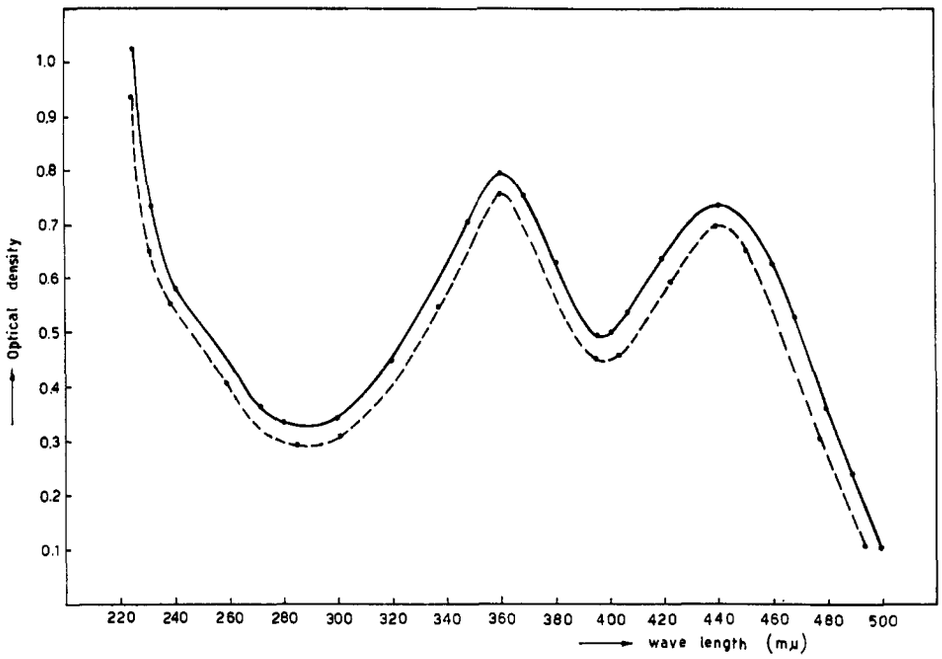
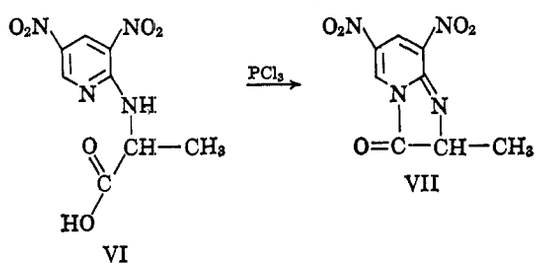


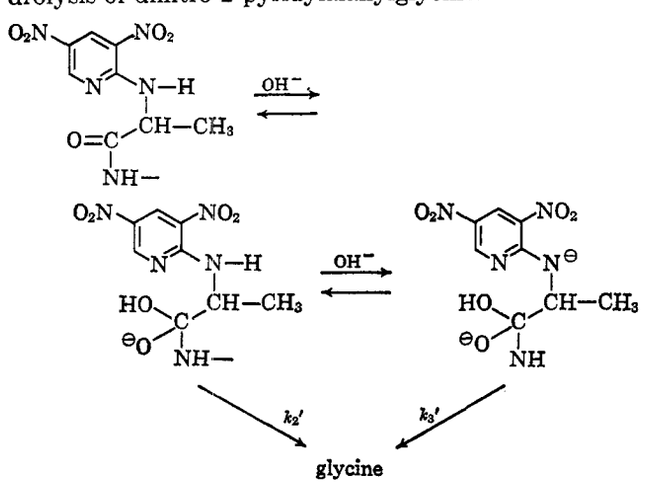
Figure 7.—Light absorption curves in 1% $NaHCO_3$ for 6,8-dinitroimidazo[1,2*a*]pyridin-3(2H)-one (broken line) and hydrolytic intermediate (solid line).

two nearly symmetrical maxima in the 440- and 360-mμ regions, a minimum around 270 mμ, and a monotonic increase from 270 to 220 mμ (Figure 7).



The rate of decomposition of VII at 30° in 0.54 *M* NaOH is in excellent agreement with the observed rate constant of decomposition for the hydrolytic intermediate.

The following scheme satisfactorily explains the "nonclassical" kinetics established for the alkaline hydrolysis of dinitro-2-pyridylalanylglycine.



Both neutral pyridine nitrogen and pyridyl mesomeric anion give a nucleophilic displacement of glycine anion; subsequently fast acid–base equilibria give glycine and the cyclic ketone in hydrated form.

In order for the dianion intermediate V, present at smaller concentration, to compete favorably with III, its rate of decomposition must be favored. The following rate equation for the hydrolysis of I is derived on the basis of the proposed reaction scheme (5) where a =

$$\frac{d[Gly]}{dt} = k_2[OH^-](a - x) + k_3[OH^-]^2(a - x) \qquad (5)$$

initial stoichiometric concentration of amide, b = fraction of amide hydrolyzed at time t, $[OH^-]$ = hydroxide ion concentration ($[OH^-] >> a$). Rearranging and integrating, we have eq 6. Hence the pseudo-first-

$$\ln \frac{a}{a - x} = (k_2[OH^-] + k_3[OH^-]^2)t = k_1 t \qquad (6)$$

order constant k_1, is given by

$$k_1 = k_2[OH^-] + k_3[OH^-]^2$$

which is the rate equation (1).

On the basis of the above formulation it is to be expected that the presence in the aryl system of groups capable of electron withdrawal would enhance the acidity of the N–H group and thereby increase the observed rate constant k_1. On the other hand, the basicity of the pyridyl anion would be lowered by the presence of electron-withdrawing groups and thus cause a decrease in k_3 and consequently the experimentally observed constant k_1.

Experimental Section

Materials.—Alanylglycine was prepared according to Sheenan and Hess[7] from carbobenzoxyalanine and glycineethyl ester; after hydrolysis of the ester and hydrogenolysis of the carbobenzoxy group, the free dipeptide had mp *ca.* 222°. Its purity was tested by chromatography on paper in at least two different solvent systems. The samples of dinitropyridyl derivatives used in this investigation were prepared as described previously.[8]

(7) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(8) A. Signor, L. Biondi, M. Terbojevich, and P. Pajetta, *Gazz. Chim. Ital.*, **94**, 619 (1964).

6,8-Dinitroimidazo[1,2-a]pyridin-3(2H)-one was obtained, according to Knott,[9] in the following manner. Dinitro-2-pyridylalanine (10 g) and phosphorus trichloride (50 ml) were heated under reflux on a steam bath for 15 min. The solid slowly dissolved and a transitory yellow-brown coloration occurred; the mixture was then allowed to cool at room temperature and the product was completely precipitated by addition of ether (100 ml). The solid was filtered off, dissolved in methyl ethyl ketone, and precipitated with ether; the yellow grains were difficult to purify.

Anal. Calcd for $C_8H_6N_4O_5$: C, 40.33; H, 2.52; N, 23.52. Found: C, 39.97; H, 2.40; N, 23.28.

Kinetics of Hydrolysis.—The kinetics of hydrolysis of I and II were determined (a) by following the appearance of glycine and (b) by observing the intermediate at or very nearly its absorption maximum.

Rate of Appearance of Glycine.—Aqueous solutions of substrate (2×10^{-3} *M*) were diluted to two volumes with appropriate NaOH solutions; the hydrolysis experiments were performed in a thermostated water bath whose temperature was held constant to ±0.05°, and 1.0-ml portions were withdrawn for analysis at predetermined times. The extent of hydrolysis was measured by analysis of samples neutralized by 30% acetic acid, according to the ninhydrin procedure of Moore and Stein.[10] Portions of each hydrolysis mixture were subjected to complete hydrolysis by heating in 1.0 *M* NaOH for 1 hr at 60°. Spectrophotometric readings were made with a Beckman Model DU spectrophotometer at wavelength 570 mμ.

Rate of Change of Intermediate.—The stock solutions of I and sodium hydroxide for all runs were equilibrated in a water bath (±0.05°) prior to mixing; the temperature of the thermostated compartment of the Beckman DU spectrophotometer was maintained to within 0.1° by circulating water from a constant-temperature bath. The following general procedure was used in the kinetic determinations. To 50 μl of 2×10^{-3} *M* aqueous solution of I placed in the cell was rapidly added 3.0 ml of NaOH solution. The time lapse between removal of the alkali solution from the water thermostat and placement in the spectrophotometer was never longer than a few seconds. The reactions were followed by continuous recording of the optical density at 440 mμ.

Beer's law was found to be obeyed within the concentration and wavelength range employed.

Acknowledgments.—Many thanks are due to Professor A. Indelli and Professor E. Scoffone for helpful discussions and to Dr. A. Fotia for competent technical assistance.

(9) E. B. Knott, *J. Chem. Soc.*, 1360 (1956).

(10) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

Optical Resolution and Absolute Configuration of *trans*-β-Phenylglycidic Acid[1,2]

KAORU HARADA

Institute of Molecular Evolution and Department of Chemistry, University of Miami, Coral Gables, Florida 33134

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β-Phenylglycidic acid prepared by the Darzens' method was resolved by the use of (*S*)-(−)- and (*R*)-(+)-methylbenzylamine. The ammonolysis of these resolved (−)- and (+)-β-phenylglycidic acids resulted in mixtures of *erythro*-(+)-phenylserine and *erythro*-(+)-phenylisoserine and of *erythro*-(−)-phenylserine and *erythro*-(−)-phenylisoserine. The configurations of (−)- and (+)-glycidic acid were identified as follows: (−) isomer, (2*R*),(3*S*); (+) isomer, (2*S*),(3*R*). The configurations of (+)- and (−)-phenylisoserine were determined as follows: (+) isomer, (2*R*),(3*R*); (−) isomer, (2*S*),(3*S*). The ethyl β-phenylglycidate, prepared by the Darzens' method, was confirmed as being mostly or entirely composed of the *trans* isomer. The SN2 reaction in the ammonolysis of glycidic acid is discussed.

Many studies have been made on the chemistry of epoxides. These have been reviewed by Winstein and Henderson[3] and by Parker and Isaacs.[4] The Darzens' reaction in the synthesis of the glycidic acids has been reviewed.[5] Several ammonolysis and aminolysis reactions of the glycidic acids have been reported.[6-15]

A previous study[16] showed that ammonolysis of potassium (±)-β-phenylglycidate yielded a mixture of *erythro*-β-phenylserine and β-phenylisoserine (low melting point) in a ratio of 25:75.

The optical resolution of the glycidic acids has not been studied and their absolute configuration has not yet been directly clarified. Therefore it seemed worthwhile to resolve the glycidic acids and to characterize their optically active isomers stereochemically (Scheme I).

In this study, ethyl β-phenylglycidate was synthesized by the Darzens' method, and the ester was converted to its potassium salt (I) by treatment with alcoholic potassium hydroxide. The salt was liberated and the resulting unstable free β-phenylglycidic acid (II) (G acid) was resolved by (*S*)-(−)- and (*R*)-(+)-methylbenzylamine [(−)-amine, (+)-amine][17,18] in an ether solution. (−)-Amine and (±)-G acid resulted in crystallization of (−)-amine–(−)-G acid salt (IIIa). The mother liquor was treated with an equimolar proportion of hydrochloric acid and the free G acid was crystallized by addition of (+)-amine to form (+)-amine–(+)-G acid salt (IIIb). These amine salts

(1) Stereochemistry of Glycidic Acid. I.

(2) Aided by Grant No. NsG-689 of the National Aeronautics and Space Administration. Contribution No. 053 of the Institute of Molecular Evolution, University of Miami.

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(4) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, **59**, 737 (1959).

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(6) P. Melikow, *Ber.*, **13**, 956 (1880).

(7) E. Erlenmeyer, *ibid.*, **13**, 1077 (1880).

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(9) O. von Schickh, German Patent 583,243 (1933); *Chem. Abstr.*, **28**, 260 (1934).

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(12) V. F. Martynov, *Dokl. Akad. Nauk SSSR*, **89**, 869 (1953).

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(14) T. Inui and T. Kaneko, *ibid.*, **82**, 747 (1961).

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(16) T. Kaneko and K. Harada, presented at the 8th Annual Meeting of the Japanese Chemical Society, Kyoto, 1956.

(17) W. Theilacker, *Chem. Ber.*, **87**, 690 (1954).

(18) W. Leithe, *ibid.*, **64**, 2831 (1931).