# Kinetic Studies on the Alkaline Decomposition of Cystine Derivatives and Peptides[1]

**Oscar Gawron and Gerald Odstrchel[2]**

*Contribution from the Department of Chemistry, Duquesne University, Pittsburgh, Pennsylvania 15219.* *Received January 25, 1967*

**Abstract:** Alkaline decomposition of endopeptides of cystine takes place *via* $\beta$ elimination to give a persulfide moiety and a dehydroalanyl residue containing moiety. Rates of decomposition of N,N′-dicarbobenzyloxy-L-cystinyldiamide, N,N′-dicarbobenzyloxy-L-cystinyldiglycine, N,N′-dicarbobenzyloxy-L-cystinyldi-L-alanine, and oxidized glutathione are first order in substrate and hydroxyl ion concentrations with bimolecular rate constants at 90.5° of 2.0, 0.95, and 1.43 l. mole$^{-1}$ sec$^{-1}$, respectively, for the first three compounds and 0.11 l. mole$^{-1}$ sec$^{-1}$ for oxidized glutathione at 80.5°. Rates of decomposition at 90.5° of L-cystine bishydantoin are first order in substrate and show a pH dependency, interpretable in terms of a single ionization with a p$K_A$ of 7.56. Initial elimination from oxidized glutathione is followed by addition of the amino group of glutamic acid residues to the dehydroalanyl residue. This secondary reaction observed with oxidized glutathione does not occur in the presence of morpholine. Similarly, in the presence of morpholine, alkaline decomposition of ribonuclease yields only one lysinoalanine residue, four normally being observed.

Recent work of Bohak[3] on alkaline decomposition of cystinyl residues of proteins establishes $\beta$ elimination as the route for such decompositions, the primary products being a dehydroalanyl residue[4] and a thiocysteinyl residue.[5] These decompositions of cystinyl peptides are similar, in principle, to the alkaline decomposition of L-cystine bisphenylhydantoin first noted by Bergmann and co-workers[6] and attributed by them to an elimination reaction. A similar elimination was noted by Andrews and Andrews[7] with L-cystine bishydantoin, pyruvic acid being found on treatment of the reaction mixture with acid. Tarbell and Harnish[8] proposed a $\beta$-elimination mechanism for such decompositions of disulfides, suggesting that removal of a proton from carbon $\beta$ to sulfur gives a carbanion which undergoes elimination to yield olefin and persulfide anion.

To further explore this mode of alkaline decomposition of cystine endopeptides and analogous cystine derivatives, a kinetic study of the alkaline decomposition of N,N′-dicarbobenzyloxy-L-cystinyldiamide, the corresponding bisglycine and bis-L-alanine, and L-cystine bishydantoin was undertaken and the results are reported herein.

## Experimental Section

**Compounds.** N,N′-Dicarbobenzyloxy-L-cystine,[9] mp 123°, after two recrystallizations from chloroform, was used for synthesis of the following compounds: N,N′-dicarbobenzyloxy-L-cystinyldiamide, mp 181–182°, after two recrystallizations from methanol, was prepared according to Boehringer, *et al.*;[10] N,N′-dicarbobenzyloxy-L-cystinyldiglycine was prepared by the method of Du Vigneaud and Miller;[11] N,N′-dicarbobenzyloxy-L-cystinyl bis-L-alanine, mp 158–159°, after recrystallization from dioxane, was prepared by adaptation of the Du Vigneaud and Miller[11] procedure for the preparation of the corresponding glycine peptide.

*Anal.* Calcd for $C_{28}H_{34}N_4O_{10}S_2$: C, 51.70; H, 5.25; N, 9.85; S, 8.63. Found: C, 51.89; H, 5.38; N, 10.03; S, 8.34.

L-Cystine bishydantoin was synthesized from L-cysteine hydantoin by oxidation with iodine.[12,13]

**Kinetic Runs.** Substrate sufficient for 25.0 ml of approximately $3.00 \times 10^{-3}$ *M* solution was placed in a 25.0-ml volumetric flask, and at zero time, 25.0 ml of buffer or sodium hydroxide solution at bath temperature was added. The flask was stoppered, inverted several times to dissolve the substrate, and returned to the constant temperature bath. Aliquots (1 ml) were removed at suitable intervals and analyzed for pyruvic acid by a modification of the enzymatic method.[14] The aliquots were quenched with 2.0 ml of 3 *N* hydrochloric acid and then refluxed for 2.5 hr to liberate, by hydrolysis,[8,15] pyruvic acid from the dehydroalanyl peptide formed by the decomposition. After cooling, the pH of the hydrolysis mixture was adjusted to 8.3–8.5 with 10 *M* sodium hydroxide and the volume was adjusted to 4.0 ml. An aliquot of this solution was then analyzed for pyruvic acid with reduced diphosphopyridine nucleotide and lactate dehydrogenase.

**Persulfide Analysis.** Kinetic runs were also monitored for alkyl persulfide, the analyses being carried out by modification of the procedure of Cavallini, *et al.*[16] A 0.1-ml aliquot of the reaction mixture was added to a mixture of 1.0 ml of 0.2 *M* borate buffer, pH 9.0, and 0.5 ml of 0.1 *M* sodium cyanide (adjusted to pH 9.0 with acid), and cyanolysis of the persulfide was permitted to proceed for 10 min at room temperature. Under these conditions (pH 9.0–9.5 of the cyanolysis reaction mixture), thiocyanate was not obtained from starting disulfide. At the conclusion of the cyanolysis period thiocyanate was estimated with ferric ion.

**Inorganic Sulfide, Sulfur, and Thiol Analyses.** Persulfide formed during the course of a kinetic run was not stable, and after its disappearance, decomposition products at the −2 and 0 oxidation level were analyzed for, as follows. Inorganic sulfide was determined by the method of Fogo and Popowsky[17] on a 0.2-ml aliquot of the reaction mixture for a kinetic run. Sulfur was

(1) Presented in part at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966; Abstracts, p 70C.
(2) Abstracted in part from the Ph.D. Thesis of G. Odstrchel, Sept 1966, Duquesne University.
(3) Z. Bohak, *J. Biol. Chem.*, **239**, 2878 (1964).
(4) In the case of ribonuclease and other proteins, condensation of $\epsilon$-amino groups of lysine with the newly formed dehydroalanine residues occurs to yield N-(DL-2-amino-2-carboxyethyl)-L-lysine residues.[3]
(5) Z. Bohak, personal communication.
(6) M. Bergmann and F. Stather, *Z. Physiol. Chem.*, **152**, 189 (1926); M. Bergmann and D. Delis, *Ann.*, **458**, 76 (1927).
(7) J. C. Andrews and K. C. Andrews, *J. Biol. Chem.*, **105**, IV (1934).
(8) D. S. Tarbell and D. P. Harnish, *Chem. Rev.*, **49**, 11 (1951).
(9) Nutritional Biochemicals Corp.
(10) A. Boehringer, E. Boehringer, I. Liebnecht, and J. Liebnecht, British Patent 693,524; *Chem. Abstr.*, **49**, 1782 (1955).
(11) V. Du Vigneaud and G. L. Miller, *Biochem. Prepn.*, **2**, 74 (1952).
(12) J. V. Karabinos and J. L. Szobo, *J. Am. Chem. Soc.*, **66**, 649 (1944).
(13) W. C. Hess, *ibid.*, **56**, 1421 (1934).
(14) S. Shifrin, N. O. Kaflan, and M. M. Ciotti, *J. Biol. Chem.*, **234**, 1555 (1959).
(15) A. Patchornik and M. Sokolovski, *J. Am. Chem. Soc.*, **86**, 1206 (1964).
(16) D. Cavallini, C. De Marco, B. Mondoni, and B. G. Mali, *Enzymologia*, **22**, 161 (1960).
(17) J. K. Fogo and M. Popowsky, *Anal. Chem.*, **21**, 732 (1949).

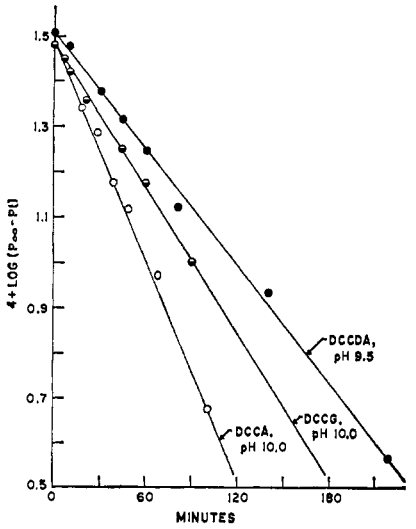


Figure 1. Typical first-order plots for the decomposition at 90.5° and several pH values, of DCCDA (N,N′-dicarbobenzyloxy-L-cystinyldiamide), DCCG (N,N′-dicarbobenzyloxy-L-cystinyldiglycine), and DCCA (N,N′-dicarbobenzyloxy-L-cystinyldi-L-alanine). Reactions followed by estimation of pyruvic acid after hydrolysis.

determined by the method of Meister, *et al.*,[18] the sulfur in a suitable aliquot being reduced to hydrogen sulfide with cysteine and the hydrogen sulfide formed being removed with nitrogen and trapped in alkaline zinc acetate solution. The sulfide ion was then estimated by the procedure given above. Inorganic sulfur was calculated as the difference between hydrogen sulfide before and after the reduction treatment. Thiol was determined by Ellman's method[19] on a 0.2-ml aliquot of the reaction mixture by addition of 3.0 ml of a 1 *M* phosphate buffer, sufficiently acid to give a final pH of 8, followed by 0.05 ml of the Ellman reagent [5,5′-dithiobis-(2′-nitrobenzoic acid)].

## Results and Discussion

The base-catalyzed $\beta$ elimination observed with symmetrical disulfides derived from cystine may be written as

$$\text{CHCH}_2\text{SSCH}_2\text{CH} + \text{OH}^- \rightleftharpoons \overset{(-)}{\text{C}}\text{CH}_2\text{SSCH}_2\text{CH} + \text{H}_2\text{O} \quad (1)$$

$$\overset{(-)}{\text{C}}\text{CH}_2\text{SSCH}_2\text{CH} \rightleftharpoons \text{CH}_2{=}\text{C} + {}^{(-)}\text{SSCH}_2\text{CH} \quad (2)$$

with the rate-controlling step either reaction 1 or reaction 2. With reaction 1 rate controlling, a first-order rate dependence on hydroxide ion concentration is expected and with reaction 2 rate controlling, a sigmoid curve is the expected relationship between rates and hydroxide ion concentrations. In both cases the reaction is expected to be first order in disulfide concentration.

The compounds studied showed the expected first-order dependency on disulfide concentration. With respect to hydroxide ion concentration, both possible dependencies were observed, rates of the three dicarbobenzyloxycystinylbisamides showing first-order dependency while rates of cystine bishydantoin decompositions showed a sigmoid curve relationship.

Figure 1 presents several typical first-order plots at a given pH (0.2 *M* borate buffers) for the decomposition at 90.5° of N,N′-dicarbobenzyloxy-L-cystinyldiamide (DCCDA), N,N′-dicarbobenzyloxy-L-cystinyldiglycine (DCCG), and N,N′-dicarbobenzyloxy-L-cystinyldi-L-alanine (DCCA). Initial concentrations were $3 \times 10^{-3}$ *M* and first-order kinetics were obeyed for some 70% of the reaction. The slope of each of the first-order plots yielded $k'$, the apparent first-order rate constants for the reaction, at a given pH where

$$v = k(\text{OH}^-)(\text{RSSR}) \quad (3)$$

and

$$k' = k(\text{OH}^-) \quad (4)$$

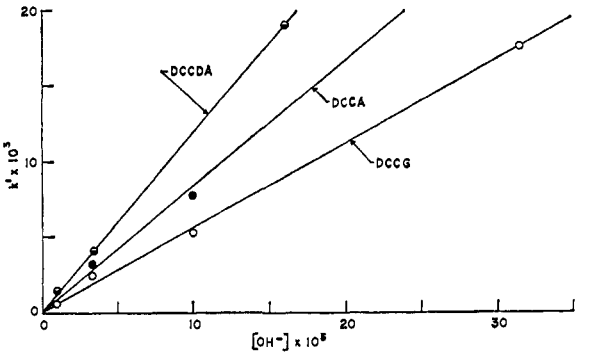


Figure 2. Plots of $k'$, apparent first-order rate constants, at 90.5° *vs.* hydroxide ion concentration for the several indicated compounds.

Plots of $k'$ *vs.* hydroxide ion concentration (Figure 2) for the individual compounds gave straight lines passing through the origin, in agreement with eq 4, and $k$, the bimolecular rate constant, was calculated from the slopes of these lines. The values of $k$ are 2.0, 0.95, and 1.43 l. mole$^{-1}$ sec$^{-1}$ for N,N′-dicarbobenzyloxy-L-cystinyldiamide, N,N′-dicarbobenzyloxy-L-cystinyl-diglycine, and N,N′-dicarbobenzyloxy-L-cystinyldi-L-alanine, respectively.

As might be expected on the basis of structural similarities of the three N,N′-dicarbobenzyloxy-L-cystinyl-bisamides, and without regard to charge differences, $k$ values are of the same order of magnitude. With regard to charge differences between the three compounds, it is apparent the negatively charged carboxylate groups are sufficiently removed from the reaction site so as not to interfere with approach of hydroxide ion. In this connection it may be noted that N,N′-dicarbobenzyloxy-L-cystine with its adjacent $\alpha$-carboxylate group is unreactive to hydroxide under the reaction conditions employed. It would seem that in this case electrostatic repulsion would be mainly responsible for lack of attack by hydroxide.

Although not encountered in the limited series investigated, it is possible that substitution of the glycine of carbobenzyloxycystinylglycine by other amino acids would lead to relatively larger rate effects. Such effects from amino acid residues with sufficiently large side chains could conceivably arise from steric hindrance and/or alteration in the solvent environment around the site of the reaction and, assuming a *trans* conformation as would be expected for the elimination, from hindrances to attainment of this conformation. These effects would be expected to be greater at lower temperatures. Preliminary investigation of temperature effects yielded bimolecular rate constants of $2.83 \times 10^{-3}$ and $0.31 \times 10^{-3}$ l. mole$^{-1}$ sec$^{-1}$ for N,N′-dicarbobenzyloxy-L-cystinyldiglycine and N,N′-dicarbobenzyloxy-L-cystinyldi-L-alanine, respectively, at 31° in sodium hydroxide solution. At this temperature $k_{\text{glycine}}/k_{\text{L-alanine}}$

(18) A. Meister, P. E. Fraser, and S. U. Tice, *J. Biol. Chem.*, 206, 561 (1954).
(19) G. L. Ellman, *Arch. Biochem. Biophys.*, 82, 70 (1959).

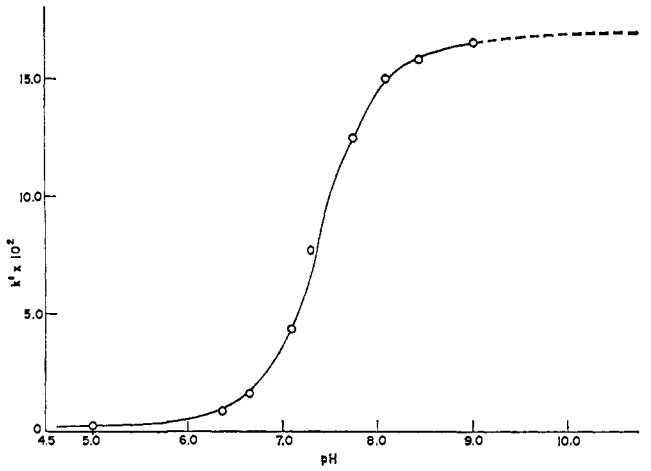


Figure 3. Plot of $k'$ at 90.5° *vs.* pH for L-cystine bishydantoin. Reaction followed by estimation of pyruvic acid after hydrolysis.

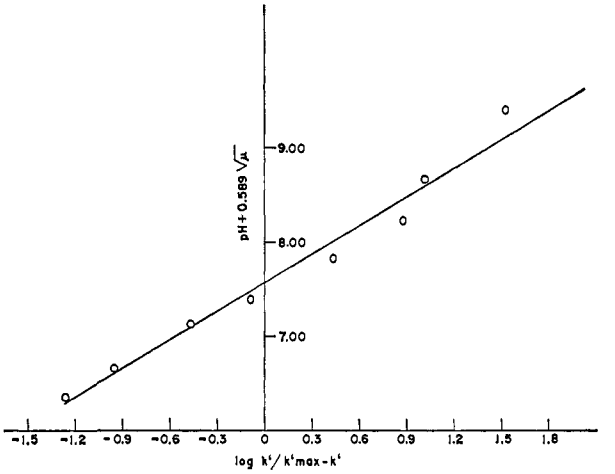


Figure 4. Plot of (pH − 0.589/$u$) *vs.* log $k'/(k'_{max} - k')$ for the data of Figure 3. Slope, 1.02, and intercept, 7.56, for the solid line were calculated by the least-squares method from the experimental points.

is 9.1 while at 90.5° the corresponding ratio is 0.67 and it is clear that the rate effect of substitution of alanine for glycine is greater at the lower temperature.

Figure 3 presents a plot of $k'$ *vs.* pH for the bishydantoin of L-cystine. The shape of the curve is that of an ionization curve with a p$K$ in the vicinity of 7.5. A Henderson–Hasselbach plot of pH *vs.* log $k'/(k'_{max} - k')$ gives a straight line with a slope of 0.82 rather than 1.00 as expected for a single ionization. However, inclusion of a correction term[20] for activity of the anionic species, the ionic strength of the buffer varying with the pH, gives the modified Henderson–Hasselbach equation, and a plot (Figure 4) of pH − log $\gamma_{A^-}$

$$\text{pH} - \log \gamma_{A^-} = \text{p}K + \log [k'/(k'_{max} - k')] \quad (5)$$

*vs.* log $k'/(k'_{max} - k')$ gives a straight line with a least-squares slope of 1.02 and intercept (p$K_A$) of 7.56. Buffer effects were not noted, variation at pH 9.02 of total buffer concentration from 0.1 to 0.2 $M$ not affecting rates. Accordingly the reaction rate may be viewed as dependent on a single ionization albeit secondary ionizations are possible and racemization may also be occurring.[24] A possible reaction mechanism is given in Figure 5, hydrogen attached to imide nitrogen being considered the most acidic. Tautomerization to a form such as that given in the figure would then provide an intermediate for the elimination. The p$K_A$ value, 7.56 at 90° found for L-cystine bishydantoin, is considerably less than that, 9.12 at 25°, for hydantoin itself,[26] the imide group of the latter ionizing.[27] The lower value for the cystine hydantoin may be due in part to a temperature effect and in part to an acid-strengthening effect in the L-cystine hydantoin.

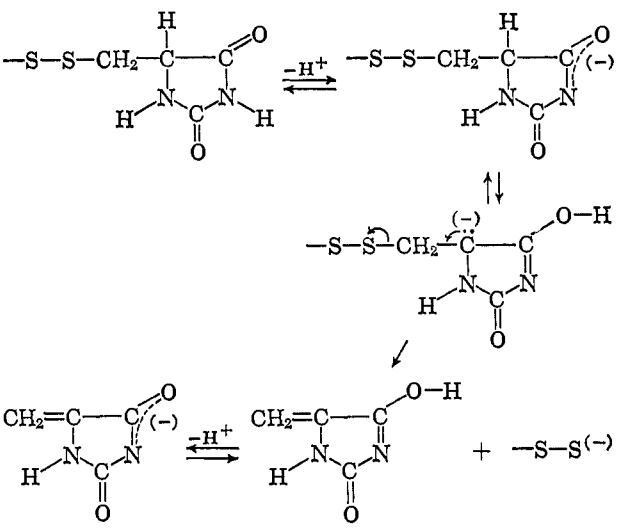


Figure 5. Possible mechanism for $\beta$ elimination from L-cystine bishydantoin. Ionization from imide nitrogen followed by tautomerization and elimination.

Figure 6 presents plots at several pH values of the time course of decomposition of dicarbobenzyloxy-L-cystinyldiglycine at 100°, the reactions being followed by pyruvic acid and alkyl persulfide analyses. Comparison of the kinetic curves for persulfide and pyruvic acid indicates a similar pH dependence for initial rates of appearance of the two products and alkyl persulfide instability under the reaction conditions. The mode of decomposition of the particular alkyl persulfides encountered is not known. Investigation of persulfide decomposition products after disappearance of persulfide from reaction mixtures gave results demonstrating the presence of alkyl mercaptan and sulfide ion but not sulfur. At 100°, pH 10.0 (0.2 $M$ borate), the stoichiometric ratios of products per mole of dicarbobenzyloxy-L-cystinyldiglycine are 0.67 mercaptan, 0.67 sulfide ion, and 1.0 pyruvate, while at 30° in 0.1 $M$ sodium hydroxide, the corresponding ratios are 0.73, 0.1, and 0.97. The data obtained at 100°, assuming completeness of transformations subsequent to persulfide de-

$$\begin{matrix} 3(-2) & 2(-2) & 2(-2) & (\text{net } +2) \\ 3\text{RSS}^- & \longrightarrow & 2\text{RS}^- + 2\text{S}^{2-} + & (\text{X}) \end{matrix} \quad (6)$$

(20) −Log $\gamma_{A^-}$ was evaluated as $0.589/u$,[21] the numerical value at 90° being that given by Manov, *et al.*[22] The ionic strength at a given pH was calculated from the buffer anion concentration; total buffer concentration 0.2 $M$, p$K_A$ = 8.88 for the first ionization of boric acid. The $K_A$ value was obtained by extrapolation to 90° of a log $K_A$ *vs.* $1/T$ plot, $K_A$ values at several temperatures being obtained from the literature.[23]

(21) E. S. Amis, "Kinetics of Chemical Change in Solution," The Macmillan Co., New York, N. Y., 1949, p 25.

(22) G. C. Manov, R. G. Bates, W. J. Hamer, and J. F. Acree, *J. Am. Chem. Soc.*, **65**, 1765 (1943).

(23) "Handbook of Chemistry and Physics," 47th ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1966, p D88.

(24) The hydantoin of S-benzyl-L-cysteine racemizes rapidly in alkali.[25]

(25) O. Gawron and A. J. Glaid, III, *J. Am. Chem. Soc.*, **71**, 3232 (1949).

(26) M. Zief and J. T. Edsall, *ibid.*, **59**, 2245 (1937).

(27) L. W. Pickett and M. McLean, *ibid.*, **61**, 423 (1939).

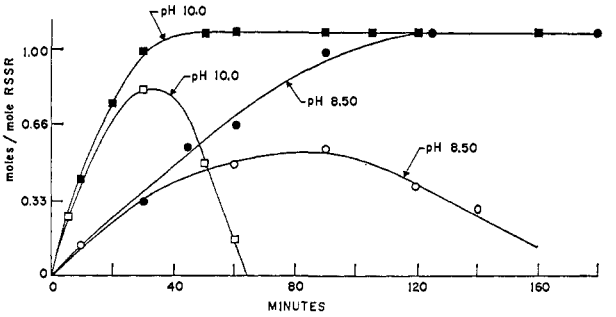


Figure 6. Rate curves for formation of pyruvic acid and persulfide by the decomposition of dicarbobenzyloxy-L-cystinyldiglycine at 100° at several pH values in 0.2 *M* borate buffers: ●, pyruvate; ○, persulfide.

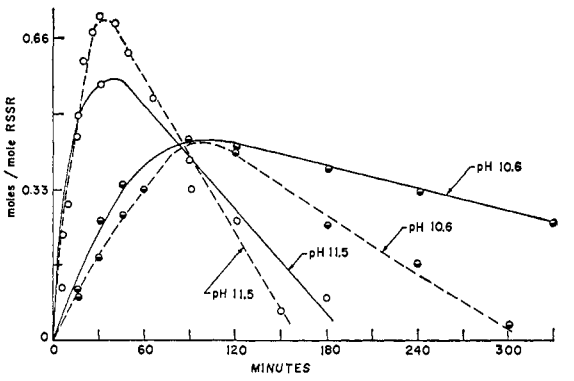


Figure 7. Product-time curves for decomposition of oxidized glutathione at 80.5° at several pH values in 0.2 *M* phosphate: ——, pyruvate; – – – –, persulfide.

composition, suggest the decomposition stoichiometry shown in eq 6, where (X) would contain one R and two sulfur atoms with a net oxidation number of +2. On this basis the decomposition reaction might be written as

$$3RSS^- + 2OH^- \longrightarrow 2RS^- + 2SH^- + RS_2O_2^-$$

Since a search for a decomposition product containing sulfur at a higher oxidation level than zero was not attempted, further discussion is inappropriate albeit it may be stated that $RSSO_2^-$ fits the requirements for such a product and a mechanism may be written similar to that proposed by Danehy[28] and by Danehy and Hunter[29] for the alkaline decomposition of certain symmetrical alkyl disulfides. On the above basis, the low yield of sulfide ion in 0.1 *M* sodium hydroxide at 30° may signifiy a different pathway or destruction of sulfide ion.

To further explore the scope of $\beta$ elimination from cystine peptides, oxidized glutathione was investigated, the decompositions being carried out at 80.5° in 0.2 *M* phosphate buffers. Both products, the dehydroalanyl residue and alkyl persulfide, were unstable (Figure 7) and could not be used to follow the reaction. The reaction was followed by analysis for residual oxidized glutathione with reduced triphosphopyridine nucleotide in the presence of glutathione reductase.[30] Disappearance of oxidized gutathione followed first-order kinetics and a plot (Figure 8) of $k'$, the first-order rate constants for individual runs, *vs.* hydroxyl ion concentration gives a straight line passing through the origin. Decomposition of oxidized glutathione is, therefore, similar to the other cystine peptides, the reaction being bimolecular with a bimolecular rate constant at 80.5° of 0.11 l. mole$^{-1}$ sec$^{-1}$. Disappearance of dehydroalanyl residue (pyruvic acid) was accompanied by loss of glutamic acid and appearance of a new amino acid as evidenced by amino acid analysis[31] following acid hydrolysis. While the structure of the new amino acid was not determined, its mode of formation is analogous to that of lysinoalanine and, presumably in this case, the amino group of the glutamic acid adds across the double bond of the dehydroalanine residue to give N-(DL-2-amino-2-carboxyethyl)-L-glutamic acid. Oxidized glutathione in its behavior toward alkali is, thus, entirely similar to the several cystine-containing proteins that undergo alkaline decomposition[3] and can be used as a model for such protein studies.

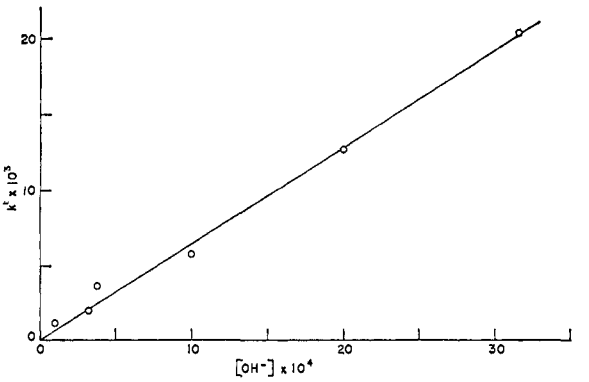


Figure 8. Apparent first-order rate constants, $k'$, for oxidized glutathione *vs.* hydroxyl ion concentration; 80.5°, 0.2 *M* phosphate buffers.

In connection with alkaline decomposition of the four cystine residues of ribonuclease, it has been noted[3] that one of the four dehydroalanyl residues produced by the elimination reaction is rapidly converted by a consecutive reaction to a lysinoalanine residue while the other three are converted to lysinoalanine residues at a slower rate. In this laboratory we have noted[32] the appearance of only one lysinoalanine residue per mole of ribonuclease when the alkaline decomposition is allowed to proceed in the presence of 0.1 *M* morpholine, the other three being replaced by the corresponding morpholine adduct. This remaining lysinoalanine residue is most likely the one formed at the fastest rate and its formation indicates an intrachain proximity of the dehydroalanyl residue and $\epsilon$-amino group involved, such that competition from morpholine is precluded. On this basis location in the amino acid sequence of the particular lysine and particular cystine residue involved is of interest with regard to their spatial relation. While a lysine-adjacent cystine pair is a likely lysinoalanine precursor,[3] it is also possible that the single polypeptide chain is so looped as to bring together a lysine residue and a cystine residue that are removed one from the other in the sequence. Lysozyme with its four cystine residues, three of which, at least,

(28) J. P. Danehy in "The Chemistry of Organic Sulfur Compounds," Vol. 2, N. Kharasch, Ed., Pergamon Press Inc., New York, N. Y., 1966, p 337.

(29) J. P. Danehy and W. E. Hunter, Abstracts, 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 1966, p 42K.

(30) E. Racker and B. Vennesland, *Methods Enzymol.*, 2, 719 (1955).

(31) Analyses were performed on the Technicon automatic amino acid analyzer.

(32) O. Gawron and G. Odstrchel, unpublished observations.

being subject to alkaline decomposition,[3] and its known sequence and three-dimensional structure[33] is a particularly good single polypeptide chain for investigating lysine–cystine proximity in this manner. It is also possible that a lysine ε-amino group close to a cystine residue acts as a base catalyst for the elimination.

(33) C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Nature*, **206**, 756 (1965).

**Acknowledgments.** Support of this investigation by Grant GB-2512 from the National Science Foundation and the assistance of David Ford and Mary Gallagher are gratefully acknowledged.

# Novel Analgesics and Molecular Rearrangements in the Morphine–Thebaine Group. I. Ketones Derived from 6,14-*endo*-Ethenotetrahydrothebaine[1]

**K. W. Bentley and D. G. Hardy**

*Contribution from the Research Laboratories, Reckitt and Sons Ltd., Kingston-upon-Hull, England.* *Received September 26, 1966*

**Abstract:** Several ketones II derived from 6,14-*endo*-ethenotetrahydrothebaine have been prepared by the Diels–Alder addition of certain α,β-unsaturated ketones to thebaine, by the action of cadmium alkyls on the corresponding acid chloride II (R = Cl), and, in some cases, by the action of Grignard reagents on the corresponding ester II (R = OEt) which is the adduct of thebaine and ethyl acrylate. Both 7α and 7β forms of the ketone II (R = Me), the ester II (R = OEt), and the nitrile III have been isolated. Attempts to convert the 7α ketone II (R = Me) into the 7β isomer have resulted only in the formation of a ketol X (R = H). Many of the bases prepared in this work are potent analgesics. The reaction product obtained from thebaine methiodide and *p*-benzoquinone has been shown to be a charge-transfer complex and not a true Diels–Alder adduct.

For many years determined efforts have been made to produce an analgesic markedly superior to morphine by minor and major modifications of the molecule of the alkaloid. The major modifications have all been in the direction of making simpler structures, representing what are believed to be the features of the molecule responsible for its analgesic action.[2] Structural simplifications of this type, however, have not so far resulted in any marked separation of the desirable and undesirable effects. It has been postulated that the action of such compounds is due to the fit of their molecules onto receptor surfaces, which triggers off their physiological effects.[3] Compounds of structure more simple than that of morphine, however, being more flexible, would be expected to fit at least as easily as the alkaloid to each of the similar receptor surfaces associated with the different effects, thus reproducing all of the physiological effects of the alkaloid. These considerations led us to examine bases more complex and, in particular, more rigid than morphine in the hope that the reduced flexibility and the differences in peripheral shape between such compounds and other known analgesics would result in the new bases being unacceptable at some of the receptor surfaces, and thus to a separation of the various effects.

Several series of such complex derivatives of codeine and thebainone, which are themselves derivatives of morphine, are accessible by the Diels–Alder addition of dienophiles to thebaine[4] and by chemical transformations of the resulting adducts,[5] and in these compounds the new two-carbon bridge across ring C renders the molecule rigid. Many of the compounds derived from thebaine in this way contain also a new reactive center, derived from the dienophile, at which further chemical reactions may be effected to yield bases of still greater peripheral complexity. Some of our early work along these lines has already been reported,[6] but the work was suspended owing to the difficulty experienced in securing adequate pharmacological appraisal of the compounds prepared. During the course of this work, however, it was found that the ketonic base I, obtained by the catalytic reduction of the adduct of thebaine and *p*-benzoquinone, is an analgesic with about one-seventh the potency of morphine, when tested in rats by the conventional techniques.

In further pursuit of this idea, the other known Diels–Alder adducts of thebaine, namely the aldehyde II (R = H), the ketones II (R = Me and Ph), the nitrile III, and the diester IV were prepared, together with a number of new analogous bases, and these were tested in animals for analgesic properties.

(1) (a) A preliminary report of part of this work has been made by K. W. Bentley and D. G. Hardy, *Proc. Chem. Soc.*, 220 (1960). (b) Parts of this work are covered by British Patents 905,659 and 925,723.

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(6) K. W. Bentley and A. F. Thomas, *J. Chem. Soc.*, 1863 (1956).