

at C-5. For the specific case of **16** → **17**, we have obtained second-order rate constants  $k = 1.95 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$  (measured over several half-lives,  $t_{1/2} \sim 8 \text{ min}$ ) at pH 12.5, 25 °C. At pH 7 the reaction is considerably slower; no reaction is observed at pH 3.5. The kinetics at pH 12.5 showed a significant temperature dependence (Figure 2) with an apparent  $E_a = 18 \text{ kcal/mol}$ ,  $\Delta H^\ddagger = 17.5 \text{ kcal/mol}$ ,  $\ln A = 28$ ,  $\Delta S^\ddagger = -5 \text{ eu}$ , and  $\Delta G^\ddagger = 19 \text{ kcal/mol}$ . A solvent deuterium isotope effect  $K_{\text{H}_2\text{O}}/K_{\text{D}_2\text{O}} \sim 2.4$  is indicative of proton transfer in the rate-limiting step, and offers further support that tautomeric ring opening is obligate.

Our findings are consistent with the hypothesis that the bicyclic ring system is capable of ring opening to an  $\alpha,\beta$ -unsaturated ketone that requires a minimal structure containing (1) a C-5 *exo*-methylene, (2) a C-6-OH, (3) N-H amide at N-10, and (4) a hydroxyalkyl group at C-1. The irreversibility of the reaction may reflect a small energy gain from conjugating the newly formed ketone at C-6 with the C-5 olefin that is not enjoyed by the corresponding sulfide adducts. This is evidenced by the lack of  $^{18}\text{O}$  incorporation in the sulfide adducts such as **17** (vide infra).

The compounds reported in this study have been evaluated for antimicrobial activity;<sup>3</sup> only **1** and **10** displayed activity. The lack of correlation between simple thiolate susceptibility and biological activity indicates that this interesting reaction alone cannot be used as the biomechanistic template. An alternative mechanism we have proposed<sup>3</sup> involving suicide inactivation of bacterial proteases by the bicyclic system is presently under scrutiny in our laboratories.

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### Reaction of *o*-Phthalaldehyde with Alanine and Thiols: Kinetics and Mechanism

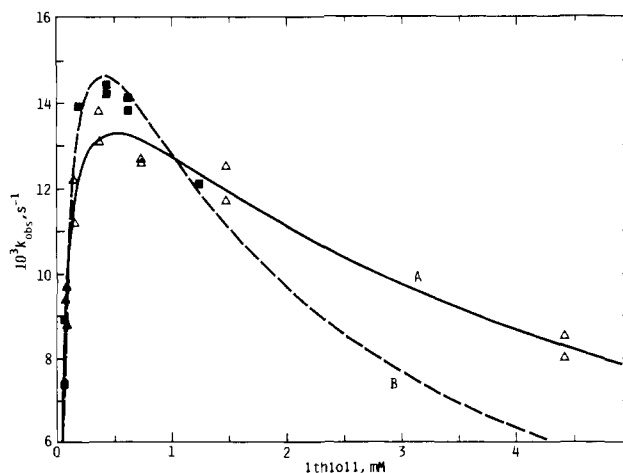
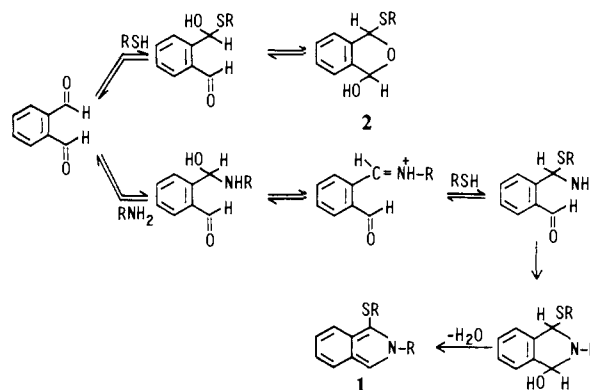
Osborne S. Wong,\*<sup>†‡</sup> Larry A. Sternson,<sup>†‡</sup> and Richard L. Schowen<sup>§</sup>

Department of Pharmaceutical Chemistry, Department of Chemistry, and Center for Bioanalytical Research  
University of Kansas, Lawrence, Kansas 66045

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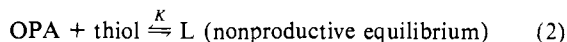
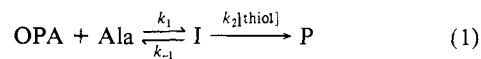
The reaction of OPA with a primary amine ( $\text{RNH}_2$ ) in the presence of a thiol (RSH) produces 1-(alkylthio)-2-alkylisoindole **1** which is intensely fluorescent.<sup>1-4</sup> This unique reaction provides the basis for a highly sensitive and specific method for the detection of low levels of primary amines.<sup>5-10</sup> In this report we present

Scheme I. Probable Reaction Paths for the Reaction of OPA with Amines and Thiols



**Figure 1.** Plot of first-order rate constants for the reaction of OPA with Ala vs. 2ME and 3MPA concentrations in 80 mM borate buffer (pH 9.3) at 25 °C. Reaction progress determined by monitoring fluorescence intensity at 450 nm ( $\lambda_{\text{ex}} = 340 \text{ nm}$ ). Solid lines through the data points were generated by using best-fit parameters obtained from nonlinear least-squares analysis. Line A (thiol = 2ME) [OPA] = 0.144 mM, [Ala] = 3.2  $\mu\text{M}$ . Line B (thiol = 3MPA) [OPA] = 0.179 mM, [Ala] = 2.1  $\mu\text{M}$ .

results from kinetic studies of the reaction of *o*-phthalaldehyde (OPA) with alanine (Ala) in the presence of 2-mercaptoethanol (2ME) and 3-mercaptopropionic acid (3MPA). Our results show that, under the conditions studied, this OPA reaction follows the kinetic model shown below (eq 1 and 2), and the overall reaction



can be described by the mechanism depicted in Scheme I. Despite some speculation, to date the mechanism for the formation of the isoindole has not been established.<sup>4,11,12</sup> As such, the findings presented here are important not only for the fundamental understanding of the reaction mechanisms of carbonyl groups but also for the application of OPA chemistry for analysis and design

\* Present address: Oread Laboratories, Inc., Lawrence, KS 66046.

<sup>†</sup> Present address: Smith Kline and French Laboratories, Philadelphia, PA 19101.

<sup>‡</sup> Department of Pharmaceutical Chemistry.

<sup>§</sup> Department of Chemistry.

<sup>+</sup> Center for Bioanalytical Research.

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**Table I.** Best-Fit Parameters for Eq 5 from a Nonlinear Least-Squares Method<sup>13</sup>

thiol	$k_1, \text{mM}^{-1} \text{s}^{-1}$	$k_{-1}/k_2, \text{mM}$	$K, \text{mM}$
2ME	$113 \pm 4$	$0.053 \pm 0.005$	$4.64 \pm 0.55$
3MPA	$127 \pm 13$	$0.100 \pm 0.021$	$1.58 \pm 0.40$

of new phthalaldehyde-like fluorogenic reagents.

Figure 1 shows the dependence of  $k_{\text{obsd}}$  on 2ME and 3MPA concentrations (line A and B, respectively) for the formation of the isoindole product. In both cases, the initial increase in  $k_{\text{obsd}}$  is followed by a gradual decrease as the thiol concentration increases. Under similar conditions, with the exception that 3MPA concentration was kept constant ( $[3\text{MPA}] = 0.186 \text{ mM}$ ) and the OPA concentration was varied, a plot of  $k_{\text{obsd}}$  vs.  $[\text{OPA}]$  showed good linear correlation (correlation coefficient  $r > 0.97$ ) with best-fit slope =  $70.4 \pm 5.6 \text{ mM}^{-1} \text{ s}^{-1}$ . The dependence of  $k_{\text{obsd}}$  on  $[\text{Ala}]$  was also examined in mixtures in which thiol was in excess ( $[2\text{ME}] = 2.0 \text{ mM}$  or  $[3\text{MPA}] = 1.83 \text{ mM}$ ). Good linear correlations were observed in these studies with  $r > 0.99$  in all cases; linear regressions gave slopes of  $75.5 \pm 0.8$  and  $60.2 \pm 1.9 \text{ mM}^{-1} \text{ s}^{-1}$  for 2ME and 3MPA, respectively.

According to the kinetic model described above, OPA reacts with Ala to form an intermediate I which further reacts with the thiol to give the fluorescent isoindole product. In addition, OPA also reacts reversibly with the thiol to form an adduct L, with apparent dissociation constant  $K$ . Applying the steady-state approximation for the concentration of I and under the condition where  $[\text{OPA}] < [\text{thiol}]$ , the rate expression for the formation of P is given by

$$d[\text{P}]/dt = \frac{k_1[\text{OPA}][\text{thiol}][\text{Ala}]}{k_{-1}/k_2 + [\text{thiol}]} \frac{K}{K + [\text{thiol}]} \quad (3)$$

where  $[\text{OPA}] =$  total OPA concentration. Under the condition where  $[\text{Ala}] \ll [\text{OPA}]$  and  $[\text{thiol}]$  (as in the case with the analytical reaction),

$$d[\text{P}]/dt = k_{\text{obsd}}[\text{Ala}] \quad (4)$$

where

$$k_{\text{obsd}} = \frac{k_1[\text{OPA}][\text{thiol}]}{k_{-1}/k_2 + [\text{thiol}]} \frac{K}{K + [\text{thiol}]} \quad (5)$$

The data shown in Figure 1 were fitted to eq 5 by a nonlinear least-squares method<sup>13</sup> and the results are shown in Table I. The  $k_1$  values are identical within the error limits, which is in agreement with the proposed mechanism because the  $k_1$  step should be thiol-independent. The reverse/forward partition ratio ( $k_{-1}/k_2$ ) for 3MPA is approximately 2-fold larger than that for 2ME. Since the  $k_{-1}$  step represents the breakdown of I to give the reactants, it should also be thiol-independent; therefore, it follows that  $k_2(2\text{ME})/k_2(3\text{MPA}) \sim 2$ , which implies that 2ME is more effective in trapping the intermediate I under these conditions. This may very well be the result of a larger fraction of 2ME mercaptide anion than 3MPA anion at pH 9.3 because of the higher basicity of 3MPA ( $\text{p}K = 10.2-10.3$ )<sup>14,15</sup> relative to that of 2ME ( $\text{p}K = 9.4-9.5$ ).<sup>15,16</sup>

The observed rate suppression at high thiol concentration is probably due to the formation of one or more OPA-thiol adducts which decrease the free OPA concentration. The addition reaction of water or thiol to an aldehyde to give the hydrate or hemithioacetal is well-known,<sup>16-20</sup> and for this system, the proposed

adduct L may be the cyclic hemithioacetal 2 (Scheme 1). Similar thiol rate suppression was observed by Trepman and Chen<sup>11</sup> in their study of the reaction of OPA with alanine and 2ME, but the rate suppression was attributed to the formation of an OPA-(2ME)<sub>2</sub> adduct, and a mechanism involving the reaction of the amino acid with an OPA-2ME adduct to give the isoindole product was proposed. The formation constant for the OPA-2ME adduct was determined spectrophotometrically by these workers to be approximately  $164 \text{ M}^{-1}$  at pH 9.0 which corresponds to a dissociation constant of 6.1 mM. However, examination of the profile of  $\log k_0$  vs.  $\log [2\text{ME}]$  presented in the same report shows a change in slope from zero to -1 at  $[2\text{ME}] \sim 6 \text{ mM}$ , suggesting that the OPA-2ME adduct does not lead to the isoindole product. Although our kinetic results do not exclude the formation of other intermediates or more complex pathways that are kinetically indistinguishable, the kinetic model presented in this report is consistent with data recorded in this work and also with data observed by Trepman and Chen.<sup>11</sup>

In conclusion, analysis of the kinetic results presented above suggests (a) a mechanism for the formation of the isoindole product involving the reaction of an OPA-amine intermediate, probably an imine, with the thiol to give the fluorescent isoindole product, and (b) the formation of the OPA-thiol adduct decreases the free OPA concentration and brings about a reduction in the rate of formation of the isoindole product.

**Registry No.** OPA, 643-79-8; Ala, 56-41-7; 2ME, 60-24-2; 3MPA, 107-96-0.

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## Design of DNA-Binding Peptides: Stabilization of $\alpha$ -Helical Structure by DNA

Lee Walters and E. T. Kaiser\*

*The Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University  
New York, New York 10021*

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Several lines of evidence have been recently converging to demonstrate the fundamental importance of  $\alpha$ -helical elements in determining the binding specificity of sequence-specific DNA-binding proteins. Attention was first focused on the role of the  $\alpha$ -helix when the crystal structures of  $\lambda$  repressor,<sup>1</sup> cro,<sup>2</sup> and *E. coli* CAP<sup>3</sup> were each found to contain a protruding bihelical unit that appeared to be complementary to the surface of B DNA. Amino acid sequence homologies tentatively identified 20-30 other DNA-binding proteins that contained a similar bihelical unit.<sup>4</sup> Structure determinations by X-ray on cocrystals of DNA with phage 434 repressor<sup>5</sup> and with the enzyme EcoRI<sup>6</sup> have now shown that an  $\alpha$ -helix from each protein does indeed lie in the DNA major groove, presumably making sequence-specific contacts. In size and shape the  $\alpha$ -helix (typical cylindrical radius 4-6 Å) is complementary to the B DNA major groove (cylindrical radius 6 Å), where the base pairs differ in hydrogen bond donor/acceptor patterns and hydrophobicity. Similar conclusions about the role of  $\alpha$ -helical elements were supported by genetic analyses of DNA-binding proteins and have recently been extended using the

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