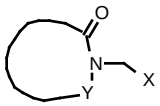
Examination of the Mechanism of Base Hydrolyses of *N*-(*p*-Nitrophenoxymethyl)-Substituted Imides

Eric J. Ginsburg, J. Ramon Vargas and Xiqiang Yang
Imaging Research and Advanced Development, Eastman Kodak Company
Rochester, New York

Abstract

The *N*-(CH_2PR)-phthalimide and *N*-(CH_2PR)-saccharin series of compounds (PR = photographic reagent) are of interest for their utility in color instant photographic systems. The acidic *p*-nitrophenol is a good model for the released photographic reagents. The release of *p*-nitrophenol from *N*-(*p*-nitrophenoxymethyl)phthalimide in base has been proposed by others to proceed via direct S_N2 displacement at the methylene carbon. We have reexamined the mechanistic aspects of base hydrolysis of this molecule and other substituted imides in mixed aqueous/organic solutions. Our findings and the new mechanism they suggest is described.

Introduction



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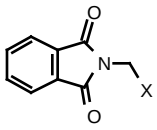
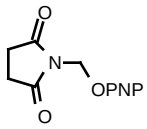
X = leaving group
Y = CO, SO_2



2: X = PMT
3: X = OPNP

Compounds having the general structure **1** have been examined for photographic,[1] agricultural,[2] pharmaceutical applications,[3-5] and as a means of identifying alcohols.[6-8] The linked electrophilic functionalities of these molecules have been exploited to either protect and then liberate a particular molecule, or to trap multiple nucleophilic groups of an enzyme active site. In color instant film applications, **2** (PMT = phenylmercaptotetrazole) has been used to control fog. While the mechanism of hydrolysis of imides and amides has been well studied[9-16] less attention has been devoted to the alkaline hydrolysis of compounds such as **1**. In the mechanism proposed for active-site inactivation of serine protease by saccharin-based inhibitors, the inhibitor forms two covalent bonds to residues at the active site.[3,4] The first bond is formed when a serine residue attacks the carbonyl, leading to ring-opening and release of the leaving group. The resulting imine is then intramolecularly trapped by a histidine residue. An analogous sequence of hydrolytic ring-opening followed by elimination was suggested as the mode of action of the compounds examined for photographic applications.[1] More recently, Sloan and coworkers, who have demonstrated the use of this class of molecules as prodrugs, suggested these compounds release a leaving group via direct S_N2 displacement at the methylene carbon by hydroxide, based on studies in alkaline aqueous methanol solution.[17] Given the uncertainty regarding the mechanism of hydrolysis and the general interest in this class of compounds, we have carefully examined the mechanism. Our results support a ring-opening/elimination mechanism and *not* S_N2 displacement.

Results



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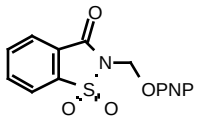

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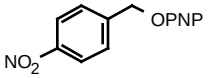

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Kinetic Data

Sloan and coworkers report that the phthalimide (**3**), succinimide (**4**), and saccharin (**5**) derivatives (PNP = *p*-nitrophenyl) release *p*-nitrophenolate ($^-$OPNP) by first-order kinetics in buffered 1:4 MeOH/H_2O solution over a pH range of 7-11. They proposed a one-step S_N2 displacement mechanism. They reason that substituents on both the imide and phenol affect the rates of S_N2 displacement by making the methylene a more reactive electrophilic center. We have confirmed their experimental results, but disagree with their mechanism. A ring-opening/elimination mechanism is also viable. We chose to examine the kinetics of an electronically similar substrate that cannot ring open. Thus, *p*-nitrophenoxymethyl *p*-nitrophenyl ether (**6**) was chosen. Ab initio calculations indicate *p*-nitrobenzene has a high-lying occupied molecular orbital of π-symmetry close in energy to a similar orbital of phthalimide. Kost and Aviram found that

the perturbation of an S_N2 transition state by a heteroatom adjacent to the central carbon atom is dominated by π, rather than σ effects,[18] so that **3** and **6** would be expected to undergo S_N2 reactions with similar rates. However, when **6** was dissolved in 4:1 pH 9 buffer/MeOH, no reaction to release *p*-nitrophenolate was observed.

Steric Effects

A series of 4-substituted saccharin derivatives **7**-**10** was prepared with PMT as the leaving group. Their pseudo first-order hydrolysis rate constants were measured and are given in Table 1. We reasoned that increased steric bulk near the carbonyl would have a greater effect on carbonyl attack than any steric or electronic effect on the methylene carbon. As is evident from the data, rates of hydrolysis slowed with increasing steric bulk. These results support the ring-opening/elimination pathway, however, they do not exclude an S_N2 pathway.

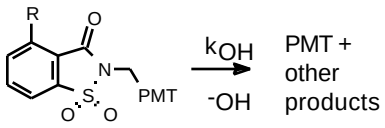


Table 1. Pseudo first-order hydrolysis rate constants for 4-substituted saccharin derivatives 7-10

R	compound	k_{OH} (L mol^{-1} min^{-1})
H	**7**	1.30×10^4
Me	**8**	2.21×10^3
Et	**9**	90.1
i-Pr	**10**	74.4

In 3% Triton X-100

Effect of Nucleophile

Release rates for the PMT and OPNP saccharin derivatives were measured at pH 8.2 with 1 mM added nucleophile (sulfite or hydroxylamine). These results are given in Table 2. Note that while the release rates were unaffected by sulfite, added hydroxylamine produced a greater than two-fold increase. Sulfite is known to have higher nucleophilicity in S_N2 reactions than hydroxylamine.[19] Although hydroxylamine is well known to react with amides,[20] sulfite is not. In reactions with esters, the rates of neutral hydroxylamine and dianionic sulfite are comparable.[21] The fact that the release rates were only increased by hydroxylamine and not sulfite supports the ring-opening/elimination pathway.

Table 2. k_{obs} (min^{-1}) in pH 8.2 buffer with and without added nucleophiles

compound	buffer only	SO_3^{2-}	NH_2OH
5	1.81×10^{-3}*	1.67×10^{-3}	4.30×10^{-3}
7	2.03×10^{-3}	2.04×10^{-3}	4.59×10^{-3}

In 3% Triton X-100, [Nu] = 1mM, * pH = 8.1

Product Studies

These hydrolyses are well suited to product analyses. Figure 1 shows the expected products from base hydrolysis of 3 via either an S_N2 displacement or a ring-opening/elimination sequence. Hydroxymethylphthalimide 11 and phthalimide 12 are slow to hydrolyze under basic conditions[13,22] therefore, the mechanism should be discernible by product analysis. Detection of hydroxymethyl-phthalimide 11 and phthalimide 12 (which with formaldehyde is in equilibrium with 11) would imply S_N2 reaction, while phthalamic acid 13 detection would indicate a ring-opening/elimination pathway.

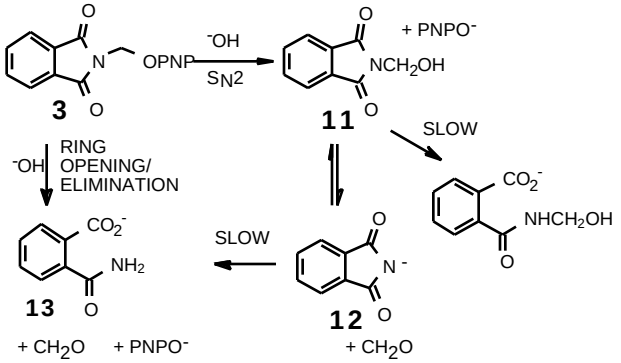


Figure 1. Expected products from ring-opening and SN2 reactions of 3.

We examined the reaction products both by ^{1}H NMR and HPLC; however, different reactants and conditions were used for each, as necessitated by instrument sensitivity, solubility limitations and the need for a UV-active chomophore for HPLC detection. In the NMR experiment, the succinimide derivative **4** was dissolved in 5:3 D_2O (pD = 9.3 phosphate buffer):CD_3CN. After 60 h (*ca.* 50% conversion), neither succinimide nor hydroxymethylsuccinimide were observed. Instead, resonances consistent with ring-opened product(s) were observed. Control experiments showed that the ring-opening rates of both succinimide and hydroxymethylsuccinimide were slower than the rate of *p*-nitrophenol release from **4**. A *ca.* 1:1 mixture of the reaction and control samples clearly indicated that no succinimide nor hydroxymethylsuccinimide are present in this hydrolysis of **4**. Resonances associated with ring-opened compounds are observed at *ca.* 2.40 ppm. When the reaction was run in 1:1 D_2O (pD = 9.21 buffer):CD_3OD, however, resonances consistent with a mixture of ring-opened product(s), succinimide, and hydroxy-methylsuccinimide were observed. Exactly analogous results were obtained when the products of hydrolysis of the phthalimide derivative **3** were examined by HPLC. In 4:1 H_2O (pH 9 buffer):CH_3CN, no ring-closed products (phthalimide or hydroxymethylphthalimide) are observed at the end of the reaction. Again, these compounds were found to be stable to the reaction conditions. In 4:1 H_2O (pH 9 buffer):MeOH, though, both ring-closed and ring-opened products were formed during the course of the reaction, including

methoxymethylphthalimide (Fig. 2). Also, the reaction of **3** in a third medium, 4:1 buffer: CF_3CH_2OH was monitored, and gave only ring-opened products.

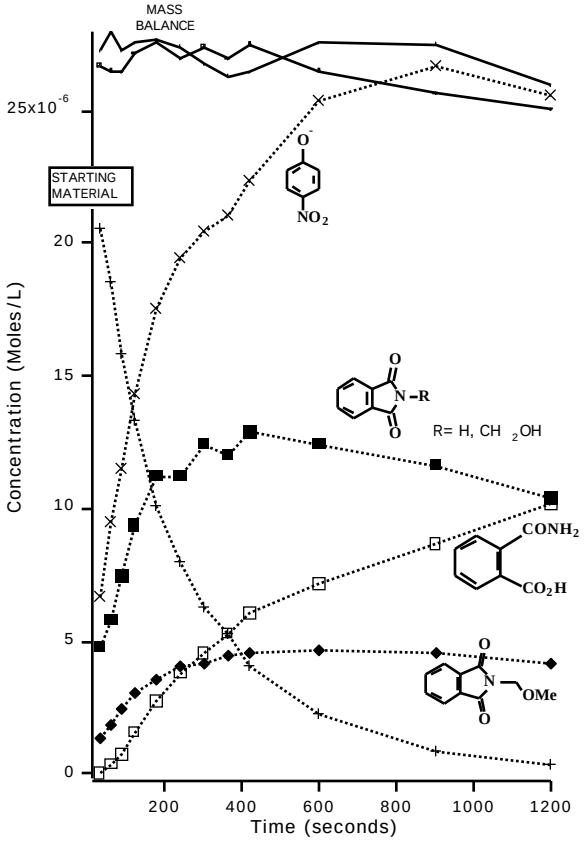


*Figure 2. Products of the reaction of **3** in 4:1 H_2O (pH 9):MeOH as monitored by HPLC.*

Discussion

The finding that the reaction products change when only 20% of the solvent is changed from acetonitrile (or trifluoroethanol) to methanol might be considered surprising. Since all three organic cosolvents have high dielectric constants (ε = 36.4, 26.3, and 32.3, respectively)[23] and both trifluoroethanol and methanol are protic, a dramatic change in mechanism would not be expected. However, of all three solvent systems, only aqueous methanol contains a nucleophile other than hydroxide. This leads us to propose that in aqueous methanol both methoxide and hydroxide can attack the carbonyl to form a tetrahedral intermediate, which then ring-opens. Note that the product formed from methoxide attack should be very unstable under the reaction conditions. Shafer and Morawetz found that methyl *N*-methylphthalamate was, at the time, "the most reactive known methyl ester." Cyclization to *N*-methylphthalimide occurs with a half life of approximately 6 seconds at pH 9 in water.[24]

We propose that these reactions all proceed via stepwise ring-opening/elimination as outlined in Fig. 3. In basic aqueous acetonitrile, methanol, or trifluoroethanol, attack by hydroxide at the carbonyl leads to ring-opening followed by elimination of *p*-nitrophenolate. In basic aqueous methanol, however, a second pathway is available in which methoxide opens the ring, *p*-nitrophenolate is released, and the ring then recloses after trapping of the imide by hydroxide or methoxide.

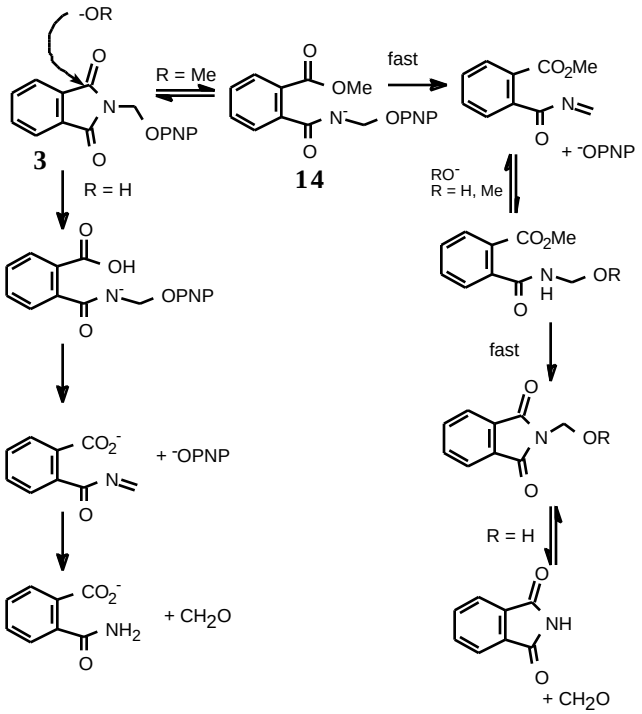
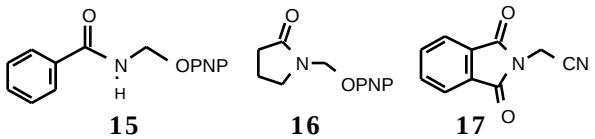


Figure 3. Proposed mechanism.

This conclusion is further supported by some additional literature data on the rates of *p*-nitrophenolate release. Compound **15** was shown to eliminate *p*-nitrophenolate more rapidly than do the cyclic imides that were examined (for example, **15** releases *p*-nitrophenolate ten times faster than does **3**).[17,25] Note that compound **15** is analogous to the ring-opened phthalamate derivative **14** proposed here as an intermediate. Compound **16** releases *p*-nitrophenolate approximately 1000 times slower than does **3**. This is consistent with a rate-determining step preceding elimination of *p*-nitrophenolate. Other workers have found, in slightly different solvent systems, that the ring-opening of *N*-methylpyrrolidone is approximately five to six orders of magnitude slower than the rate of ring-opening of *N*-methylphthalimide.[26,27]



The earlier investigation by others that concluded that the mechanism was via S_N2 displacement relied primarily on

the kinetic data for the appearance of the phenolic leaving groups. Although their experimental data was good, they failed to consider the ring-opening/elimination mechanism. Their study lacked necessary product analysis. In a trapping experiment they performed, **3** was treated with a 100-fold excess of cyanide in pH 8 buffer:methanol (10:1). Cyanomethyl phthalimide **17** was detected by HPLC (and NMR) but in only 1% yield based on the yield of *p*-nitrophenol. No other product analyses were performed. This small amount of **17** is readily accountable in the ring-opening/elimination mechanism (Fig. 3), via capture of the intermediate acylimine by cyanide.

Conclusion

Our observations indicate that these cyclic imides release *p*-nitrophenol via ring-opening and elimination upon alkaline hydrolysis. This corrects the erroneous previously published mechanism. While this study does not prove that these reactions in aqueous methanol cannot proceed in part via an S_N2 process, it demonstrates that the reactions do proceed via ring-opening/elimination in all aqueous organic solvent mixtures examined. Futhermore, in non-nucleophilic organic cosolvent mixtures, there is no evidence for any S_N2 displacement. These findings also lend some support to the enzyme inactivation mechanism proposed by others.

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