Reaction of Alkaline Sodium Picrate with Creatinine: 
I. Kinetics and Mechanism of Formation of the 
Mono-Creatinine Picric Acid Complex

John Vasiliades

Spectrophotometric, kinetic, and nuclear magnetic resonance studies indicate that alkaline sodium picrate and creatinine react to form a 1/1 adduct between picric and creatinine, with a stability constant of log K = 4.26. Kinetic studies indicate that the forward reaction is first order with respect to picric acid, hydroxide, and creatinine concentration. The reverse reaction, the dissociation of the 1/1 complex, shows a complex dependence on hydroxide concentration. The expression for the observed pseudo-first-order rate constant in the presence of excess picric acid is:

\[ k_{\text{obsd}} = k_1 K_0 [P] [OH] + k_2 [OH]^x \]

A value of \( k_1 K_0 \) = 5.0 (mol/liter)^{-2} s^{-1} is obtained. For accurate analytical results with this reaction, hydroxide concentration must be maintained at a constant value for both samples and standards.

Additional Keyphrases: spectrophotometric, proton NMR, and ^13^C NMR evidence for structures • analytical implications

Alkaline sodium picrate has been used as an analytical reagent in the clinical laboratory for the determination of creatinine since the color reaction was first described by Jaffé (1). Serum and urine contain other substances besides creatinine that react with alkaline picrate to give a color (2–5), the most common such interferants being acetone and acetoacetic acid. Early efforts to make the reaction more selective were directed at separating creatinine from the interfering substances. Adsorption chromatography (6) and extraction (7) were the two most common separation methods used, but oxidation of creatinine to guanidine was also used as a technique to eliminate interferences (8). More recently, enzymatic (9, 10) and kinetic methods have been applied (11–13). Differences in the rate of the reaction at two pH values have been used to selectively determine creatinine in the presence of interferences (14).

In spite of the voluminous literature on the Jaffé reaction, little has been reported on its mechanism. I report here the reaction of alkaline sodium picrate and creatinine to form a 1/1 complex, as investigated by conventional spectrophotometry, carbon nuclear magnetic resonance, and proton nuclear magnetic resonance.

Materials and Methods

Chemicals

An aqueous picric acid solution (53 mmol/liter) was prepared from reagent-grade picric acid (B & A Chemicals, Morristown, N. J. 07960). The solution was standardized with a 0.1 mol/liter sodium hydroxide solution, with phenolphthalein as indicator, and all solutions for the spectral and kinetic studies were made from this solution after appropriate dilutions. Creatinine aqueous solution (10 mmol/liter) was prepared from pure creatinine (Eastman Organic Chemicals, Rochester, N. Y. 14650). Dilutions of this stock creatinine solution were prepared as needed. A 0.1 mol/liter solution of sodium hydroxide, was prepared and standardized against potassium hydrogen phthalate (phenolphthalein indicator). Picric acid was standardized with this solution (phenolphthalein indicator). De-ionized distilled water was used throughout.

NMR Studies

NMR studies were done in ^2^H_2O and in deuterated dimethyl sulfoxide, both of greater than 99% purity (Merck Sharp and Dohme, Montreal, Canada). For proton NMR, a saturated solution of picric acid was prepared in ^2^H_2O. This solution was exchanged with ^2^H_2O to decrease the amount of H_2O present. A 0.7 mol/liter creatinine solution was prepared in ^2^H_2O. For ^13^C NMR, the reaction was run with picric acid dis-
solved in deuterated dimethyl sulfoxide and creatinine dissolved in $^2$H$_2$O. Creatinine is insoluble in deuterated dimethyl sulfoxide at high concentrations (>0.5 mol/liter). However, by dissolving the picric acid in deuterated dimethyl sulfoxide and the creatinine in $^2$H$_2$O, the reaction could be followed without precipitation of creatinine. NaO$_2$H added to the above mixture keeps the reactants in solution.

For proton NMR studies I used $^2$H$_2$O with a Varian EM-390 90 MHz NMR spectrometer, with trimethylsilane as internal standard. To a solution of picric acid in $^2$H$_2$O (~50 mmol/liter), NaO$_2$H and creatinine in $^2$H$_2$O were added. The solutions were mixed and the spectra recorded at different time intervals. Final concentrations were: picric acid ~50 mmol/liter, creatinine 50 mmol/liter, and O$^2$H$^-0.5$ mol/liter. All spectra were taken at ambient probe temperature, 30 °C.

Pulse Fourier transform spectra (22.63 MHz) for $^{13}$C NMR studies were obtained with a Bruker HX-90 (45-cm magnet) spectrometer and a Nicolet-1085 Computer (Nicolet Instrument Corp., Madison, Wis.). All spectra were measured at ambient probe temperature, 28 °C, relative to trimethylsilane.

To a 0.5 mol/liter solution of picric acid in deuterated dimethyl sulfoxide was added NaO$_2$H and creatinine in $^2$H$_2$O. Final concentrations were: picric acid 0.25 mol/liter, creatinine 0.25 mol/liter, and NaO$_2$H 0.25 mol/liter.

Spectroscopic Studies

Ultraviolet–visible spectra of the reaction of picric acid and creatinine were obtained with an Acta IV spectrophotometer (Beckman Instruments, Fullerton, Calif. 92634). Differential spectra of the reaction with time were obtained by placing picric acid and sodium hydroxide in the 1-cm sample and reference cells, starting the reaction by rapidly adding creatinine to the sample cell with a plexiglass plunger (reactions could therefore be followed within 15 s of mixing) and using the repetitive automatic scanning mode. I studied the effect of sodium hydroxide, picric acid, and creatinine concentrations on the spectra by independently varying the concentration of each species while keeping the others constant.

Kinetics

The reaction of alkaline sodium picrate and creatinine was followed at the maximum absorption (vs. water) of the product, 480 nm, by using a 1-cm thermostated cell at 25 ± 0.1 °C. Potassium nitrate was used to control the ionic strength to 0.1 mol/liter. The reaction was studied with various picric acid, creatinine, and sodium hydroxide concentrations and also under pseudo-first-order conditions with use of excess picric acid. Excellent first-order plots were obtained by plotting $-\ln(A_0 - A)$ vs. time, indicating that the rate of the reaction is first order in creatinine concentration.

$$\text{Rate} = \frac{d[PC]}{dt} = k_{obsd}[C]$$

Fig. 1. Identification of the 1:1 complex formed at 480 nm, by use of the method of continuous variation

Conditions: 480 nm, picric acid = 10 mmol/liter, creatinine = 10 mmol/liter, sodium hydroxide = 20 mmol/liter

The effect of varying picric acid and sodium hydroxide concentrations on $k_{obsd}$ was investigated. The observed first-order rate constant, $k_{obsd}$ was first order in picric acid concentration and showed a complex dependence on sodium hydroxide concentration.

Results and Discussion

Spectrophotometry

Alkaline sodium picrate and creatinine react to form a product that absorbs maximally at 480 nm. The final product is a 1/1 complex of picrate and creatinine with an effective stability constant of $\log K = 4.26$ (Figure 1). A differential scan of the reaction of picric acid and creatinine with time in the presence of 37 mmol/liter sodium hydroxide is shown in Figure 2. The first dashed line at zero absorbance is at time zero (no creatinine added). On addition of creatinine there is an increase in absorbance at 480 nm and 280 nm and a decrease in absorbance at 350 nm. Two isosbestic points are observed, 310 and 378 nm, indicating that at least three substances must be in equilibrium during the course of...
protons. On addition of NaOD, the absorption at 4.07 ppm disappears, owing to the rapid exchange of the protons by deuterium, which indicates that creatinine is in rapid equilibrium between the enol and keto forms. Picric acid (II) in 2H2O shows a singlet at 8.9 ppm relative to trimethylsilane. Addition of NaOD and creatinine in 2H2O to a solution of picric acid in 2H2O results in the slow decrease in the intensity of the 8.9 ppm resonance, with appearance of a new proton resonance (broad triplet) at 5.4 ppm. In addition, absorptions are observed at 3.06 and 2.70 ppm.

NMR spectra taken at different time intervals reveal that there is a decrease in the 8.9 ppm absorption with the appearance of the 5.4 ppm resonance. However, there is not a one to one correlation between the decrease of the 8.9 ppm absorption and the appearance of the resonance at ~5.4 ppm. In addition, there is a decrease in the absorption at 3.06 ppm with an increase in the absorption at 2.7 ppm. Finally, there is a complete loss of the 8.9 ppm absorption as the reaction progresses and the two absorptions at 3.06 and 2.70 ppm are of the same intensity. The 3.06 ppm absorption is due to the methyl group on the creatinine. Thus the picric acid ring protons are shifted upfield from 8.9 to 5.4 ppm while only one half of the methyl groups on the creatinine appear to be shifted upfield from 3.06 to 2.70 ppm. The proton NMR spectrum of the isolated Jaffé reaction product in deuterated dimethyl sulfoxide was reported to be consistent with a 1/1 adduct, with absorptions in the final product at 8.59, 4.19, and 3.08 ppm caused by the benzene ring protons of picric acid, methylene protons (CH2), and N-methyl protons of creatinine, respectively (11). However, an absorption at 2.6 ppm in the spectrum was not accounted for (11). The addition of NaOD to picric acid in 2H2O shows a similar upfield shift of the resonance at 8.9 ppm. At NaOD concentrations greater than 1.0 mol/liter the 8.9 ppm resonance is completely lost. A small absorption at ~6.1 ppm is observed.

Carbon-13 NMR

The 13C NMR spectrum of creatinine in 2H2O shows four absorptions (Figure 4). That at 190.03 ppm was assigned to the carbonyl (C=O) group, the 170.53 ppm absorption to the (C=NH) group, and the 57.58 and 31.32 ppm absorptions to the (—CH2—) and (—CH3) groups, respectively. Addition of NaOD results in the disappearance of the absorption at 190.03 (C=O) and 57.58 ppm (—CH2—), while the other two are still ob-

---

Proton NMR

Proton NMR studies were done in 2H2O, to elucidate the mechanism of this reaction. Creatinine (I) in 2H2O has two absorptions, at 3.06 and 4.07 ppm, relative to trimethylsilane, owing to the methyl and methylene groups. The reaction. The final spectrum (dotted line) shows that the substance absorbing at 280 nm is completely converted to the substance absorbing at 350 nm, which is still in equilibrium with the substance absorbing at 480 nm, and that the reaction can be represented by the following equilibria:

\[
A \overset{k_0}{\longrightarrow} B \overset{k_1}{\longrightarrow} C
\]

with the conversion of B to C, the final product. Increasing the sodium hydroxide concentration to 0.62 mol/liter results in the separation of the absorption bands at 400–500 nm into two distinct absorption bands (Figure 3). Again, three species are in equilibrium during the time of the reaction, with isosbestic points observed at 305 and 370 nm. In this case there is a rapid equilibrium between A and B, first scan, with B finally being in equilibrium with C. The final product (Figure 3, dotted line) has absorption maxima at 390 and 490 nm. Thus the sodium hydroxide concentration influences the final product that is formed. With conventional spectrophotometric similar results have been reported (15)—isosbestic points at 314 and 370 nm—for the reaction of alkaline sodium picrate and creatinine. A 1/1 picric/creatinine complex was reported as the final product in the reaction of alkaline sodium picrate and creatinine at constant hydroxide concentration (16), with a stability constant \( K = 3.68 \). Differences in the reported values of the stability constants between this study and the previous study are most likely due to differences in the conditions of the two studies.

---

2 "Absorptions" refers to the resonances at which these compounds absorb.
served, at 171.83 and 31.45 ppm. (Minor shifts would be expected because of solvent changes.) $^{13}$C NMR confirms that the methylene (—CH$_2$—) protons are very rapidly exchanged by $^2$H$_2$O in the presence of O$_2$H$^-$. Loss of the 57.58 ppm absorption is due to line width broadening because of deuterium attack on that carbon. The loss of the peak at 190.03 suggests, although it does not prove, that there must be some carbon–deuteron coupling between the carbonyl carbon and the deuterium on the adjacent carbon. Examination of molecular models suggests that this is unlikely, although the possibility exists.

Picric acid in deuterated dimethyl sulfoxide shows four absorptions, at 160.00, 142.60, 128.81, and 126.60 ppm (Figure 5). The absorption at 160.00 ppm was assigned to the carbon bearing the hydroxyl group, that at 142.6 ppm to the equivalent carbons having an attached nitrogen group, that at 128.8 ppm to the carbon having an attached nonequivalent nitro group para to carbon one, and the absorption at 126.60 ppm to the two equivalent meta carbons with attached hydrogens.

The addition of NaO$^{2}$H to the picric acid/deuterated dimethyl sulfoxide solution results in a decrease of the absorption at 160.00 ppm, the hydroxyl-containing carbon C$_1$, and a decrease in the intensity of the line at 128.8 ppm (Figure 6). Absorptions at 142.60 and 126.60 ppm caused by the equivalent nitro- and hydrogen-containing carbons are unchanged. Picric acid has been reported to form a first and second complex with hydroxide ions (17). The first of these reversible reactions in aqueous alkali is thought to be the picrate ion and is complete even in water. At sodium hydroxide concentrations exceeding 1 mol/liter a second type of complex has been reported to form with hydroxide (17). $^{13}$C NMR spectra indicate that there is an interaction between the picrate ion and the NaO$^{2}$H at low NaO$^{2}$H concentrations. Evidence for this comes from the fact that the resonance lines at 160 and 128 ppm are affected by addition of NaO$^{2}$H. A possible explanation for this interaction is hydrogen binding to the benzene ring at carbon one and carbon four. Thus, in the presence of NaO$^{2}$H, deuterium coupling between the carbon and deuterium can result in line broadening of the resonance of these carbons, resulting in a loss of the absorptions.

Complexes containing a hydrogen at the ortho and para position of picric acid and trinitrobenzene have been suggested as possible intermediates in the presence of sodium hydroxide (18, 19).

A postulated intermediate in the reaction of 1,3,5-trinitrobenzene with sodium hydroxide to give a red color having structure III has been reported (25). The stability constant of this 1/1 trinitrobenzene/hydroxide complex is 2.7 (mol/liter)$^{-1}$ (25), with its wavelength maximum reported at 280 nm. That picric acid in the presence of hydroxide can form such an intermediate species therefore seems likely. Tetryl (2,4,6-trinitrophenylmethylnitramine) in the presence of sodium hydroxide also forms a red color, which slowly changes to yellow. The final spectrum is identical to the spectrum of picric acid (26). $^{13}$C NMR data are consistent with the above findings and strongly support the fact that hydroxide interacts with picric acid to form some intermediate reactive species.

![Diagram](https://via.placeholder.com/150)

The $^{13}$C NMR spectrum of the product of the reaction of picric acid and creatinine is given by Figure 7. Absorption peaks owing to creatinine at 31.84, 170.0, and
Table 1. Effect of Picric Acid Concentration on the Observed Forward Rate Constants

<table>
<thead>
<tr>
<th>[P]_1 mol/liter</th>
<th>k_{obsd} s^{-1} × 10^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine concentration, 0.164 μmol/liter</td>
<td></td>
</tr>
<tr>
<td>9.85 × 10^{-4}</td>
<td>0.289 ± 0.01</td>
</tr>
<tr>
<td>1.64 × 10^{-4}</td>
<td>0.410 ± 0.008</td>
</tr>
<tr>
<td>3.28 × 10^{-3}</td>
<td>0.631 ± 0.006</td>
</tr>
<tr>
<td>4.92 × 10^{-3}</td>
<td>0.706 ± 0.007</td>
</tr>
<tr>
<td>8.20 × 10^{-3}</td>
<td>1.26 ± 0.13</td>
</tr>
<tr>
<td>Creatinine concentration, 82 μmol/liter</td>
<td></td>
</tr>
<tr>
<td>1.64 × 10^{-3}</td>
<td>0.346 ± 0.010</td>
</tr>
<tr>
<td>2.46 × 10^{-3}</td>
<td>0.402 ± 0.012</td>
</tr>
<tr>
<td>3.28 × 10^{-3}</td>
<td>0.493 ± 0.050</td>
</tr>
<tr>
<td>4.1 × 10^{-3}</td>
<td>0.638 ± 0.12</td>
</tr>
</tbody>
</table>

*Conditions: 25 ± 0.1 °C, 0.1 mol/liter KOH (ionic strength adjustment), 33 mmol/liter NaOH, 480 nm. [ ] represents “total analytical concentration of substance in solution.”

Kinetic Study

The reaction of alkaline sodium picrate and creatinine is first order in creatinine and first order in picric acid concentration (Figure 8 and Table 1). A positive intercept is observed because of the dissociation of the complex. The effect of sodium hydroxide on the observed first-order rate constant is given in Figure 9 and Table 2. Sodium hydroxide affects the observed forward rate constant (k_{obsd}) as well as the intercept reverse reaction (Figure 9). A plot of the observed second-order rate constant at various hydroxide concentrations indicates that the forward reaction is first order in hydroxide concentration (Table 3). Thus the expression for the observed pseudo-first-order rate constant is:

\[ k_{obsd} = k_{forward}[P][OH] + k_{rev}[OH]^x \]

In the above expression, x is the order of the reverse reaction with respect to hydroxide concentration.

Proposed Mechanism

Alkaline sodium picrate and creatinine under the conditions of the Jaffé reaction, excess picric acid, forms a 1/1 complex with an absorption maximum at 480 nm.

If the sodium hydroxide concentration is less than 0.70 mol/liter spectroscopic evidence indicates that there are at least three species in equilibrium during the reaction. Kinetic evidence shows that the forward reaction is first order in picric acid, creatinine, and hydroxide concentration up to a hydroxide concentration of 0.7 mol/liter. Spectroscopic evidence shows a rapid first equilibrium (isosbestic point, 310 nm) between the first two complexes in solution, which is followed by a second equilibrium between the second and third complex (isosbestic point at 378 nm). 13C NMR data show that there is an interaction between sodium hydroxide and picric acid in deuterated dimethyl sulfoxide, suggesting that some activated picric/hydroxide complex (POH*) forms in solution, resulting in hydro-

188.21 ppm are observed with a small absorption at 57.32 ppm. Absorptions at 57.32, owing to (—CH2—), and 188.21, owing to (C=O), of creatinine suggest that the creatinine has not completely enolized in the solvent mixture. Slight shifts from solvent changes are expected. Absorptions at 127.60 and 142.29 ppm are observed because of the two equivalent meta (C3 and C5) and ortho (C2 and C6) carbons of picric acid. An absorption owing to the para carbon on picric acid is not observed and has probably shifted under the peak at 127.60 ppm. An absorption peak owing to carbon 1 of picric acid is also not observed. The presence of the two unchanged absorption peaks for picric acid give the first indication that the reaction of picric acid and creatinine to form the 1/1 complex does not involve attack at the meta positions. Thus the aromatic structure of the ring is maintained in the final product. A possible site of attack may be at carbon 1 or (an interpretation that is more consistent with the data) an adduct may be formed between the enolized creatinine and picrate ion in the presence of sodium hydroxide.
Table 2. Effect of Sodium Hydroxide Concentration on the Observed Forward Rate Constants

<table>
<thead>
<tr>
<th>[P]_T mmol/l</th>
<th>k_{obed} s^{-1} \times 10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide concn 0.09 mol/l</td>
<td>1.81 1.52 ± 0.02</td>
</tr>
<tr>
<td>2.71 2.36 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>3.61 2.92 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>4.51 3.24 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide concn 0.31 mol/l</td>
<td>1.77 3.76 ± 0.05</td>
</tr>
<tr>
<td>2.66 5.57 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>3.54 7.10 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>4.43 8.34 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide concn 0.52 mol/l</td>
<td>1.74 8.80 ± 0.2</td>
</tr>
<tr>
<td>2.61 12.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>3.48 15.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>4.35 17.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide concn 0.74 mol/l</td>
<td>1.71 10.5 ± 0.5</td>
</tr>
<tr>
<td>2.56 13.6 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>4.27 19.5 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

* Conditions: [C] = 1.77 \times 10^{-4} mol/liter, 480 nm, 25 ± 0.1 °C, ionic strength not controlled. [ ]_T total analytical concentration of substance in solution.

Table 3. Second-Order Rate Constants from the Effect of Sodium Hydroxide on the Observed Forward Rate Constants

<table>
<thead>
<tr>
<th>[NaOH]_T, mol/liter</th>
<th>Slope (k/mol/liter){-1} s^{-1}</th>
<th>Intercept (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.033</td>
<td>0.115 ± 0.015</td>
<td>1.41 ± 0.2 \times 10^{-4}</td>
</tr>
<tr>
<td>0.033</td>
<td>0.128 ± 0.03</td>
<td>1.71 ± 0.51 \times 10^{-4}</td>
</tr>
<tr>
<td>0.09</td>
<td>0.64 ± 0.12</td>
<td>5.02 ± 1.3 \times 10^{-4}</td>
</tr>
<tr>
<td>0.20\textsuperscript{a}</td>
<td>1.29 ± 0.06</td>
<td>1.32 ± 0.06 \times 10^{-3}</td>
</tr>
<tr>
<td>0.31</td>
<td>1.72 ± 0.30</td>
<td>8.50 ± 1.42 \times 10^{-4}</td>
</tr>
<tr>
<td>0.52</td>
<td>3.15 ± 0.37</td>
<td>3.81 ± 0.46 \times 10^{-3}</td>
</tr>
<tr>
<td>0.74</td>
<td>3.51 ± 0.03</td>
<td>4.55 ± 0.05 \times 10^{-3}</td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\) Symbolizes total analytical concn of NaOH.

\(\textsuperscript{b}\) With excess creatinine.

Fig. 9. Sodium hydroxide dependence of the observed pseudo-first-order rate constant in the presence of excess picric acid

Conditions: creatinine 0.177 mmol/liter, 25 °C. No ionic strength control.

Numbers in parentheses designate sodium hydroxide concentrations.

The presence of sodium hydroxide causes a decrease in absorbance at 480 nm, suggesting that picric acid reacts with sodium hydroxide to form a complex. There is also a decrease in \(A_\text{max}\) and a decrease in \(\Delta A\) of the reaction with an increase in hydroxide concentration. However, the rate of the reaction increases with an increase in hydroxide concentration.\(^3\) Thus sodium hydroxide appears to be involved in the formation and dissociation of the picric/creatinine complex at 480 nm.

NMR studies indicate that creatinine is rapidly converted to the enol form and that this is the reactive form of creatinine in solution.

A proposed mechanism that is consistent with the above data is given by equations 1 and 2.

\[ \begin{align*}
\text{P} + \text{OH} & \rightleftharpoons \text{POH}^\ast \text{ (rapid)} \quad (1) \\
\text{POH}^\ast + \text{C} & \rightleftharpoons \text{PC} + x\text{OH} \quad (2)
\end{align*} \]

In the above mechanism the rate of the reaction is given by the second equilibrium.

\[ \text{Rate} = k_1[\text{POH}^\ast][\text{C}] - k_2[\text{PC}][\text{OH}]^x = k_{obed}[\text{C}]_T \quad (3) \]

Substituting in terms of creatinine and rearranging, the following expression can be derived,

\[ k_{obed} = k_1[\text{POH}^\ast] + k_2[\text{OH}]^x \quad (5) \]

or, substituting in terms of the reactants,

\[ k_{obed} = k_1K_0[\text{P}][\text{OH}] + k_2[\text{OH}]^x \quad (6) \]

The above mechanism is consistent with the observed kinetic data. A plot of the observed pseudo-first-order rate constant is linear with respect to picric acid concentration (Figures 8 and 9) at constant hydroxide

\(^3\) At high sodium hydroxide concentrations (1 mol/liter), picric acid forms a colored species identical to the Jaffé reaction. The structure of this species has been suggested to be that of a Meisenheimer complex. Also, at high hydroxide concentrations there is no picric/creatinine complex formed with a maximum absorbance at 480 nm. NMR studies indicate that this complex is probably a bis complex of picrate and hydroxide resulting from attack of \(\text{OH}^\ast\) at the meta positions of picrate.
concentration, with the reverse rate constant corresponding to the intercept. The forward and reverse rate constants are dependent on hydroxide concentration, as shown by Figure 9 and Table 3. A value for \( k_1K_0 = 5 \) (mol/liter)\(^{-2} \) s\(^{-1} \) is obtained from a plot of the observed second-order rate constant at different sodium hydroxide concentrations (Table 3). If one assumes a value of \( K_0 = 2.7 \) (mol/liter)\(^{-1} \) (25), a value for \( k_1 = 1.85 \) (mol/liter)\(^{-1} \) s\(^{-1} \) is obtained. From the above data and the equilibrium constant for the 1/1 complex, a value for \( k_2 \) of \( 1 \times 10^{-4} \) s\(^{-1} \) is obtained.

The effect of hydroxide on the reverse rate constant \( k_2 \) is very complex, as shown by Table 3. The final product, which is the 1/1 complex, slowly decomposes to unreactive substances. The above mechanism is consistent with the observed NMR and kinetic findings at low hydroxide concentrations. At higher hydroxide concentrations the reactions become more complex. In a previous study of this reaction the formation of POH* was proposed as the rate determining step and that the reaction was independent of creatinine concentration (15). The forward reaction can be made pseudo-first-order by using excess creatinine, and the observed first-order rate constant is definitely first order in creatinine concentration (24). Thus, the rate limiting step is the second step as proposed in this reaction mechanism.

Identification of the 1/1 Complex

The proton NMR spectrum of the isolated product of the Jaffè reaction shows absorptions at 8.59, 4.19, and 3.08 ppm, which are the ring protons of picric acid and the methylene and methyl absorptions of creatinine (11). \(^{13}\)C NMR studies indicate that the product formed does not involve attack at the meta position of sodium picrate and that the aromatic nature of the ring is preserved. Thus, under the conditions of this study the most likely structure is a 1/1 adduct of picrate/creatinine, as shown by structures IV and V (21).

The above structures are consistent with the \(^{13}\)C NMR data of this study and the proton NMR data of the previous study (11). Macrocyclic polyethers are also reported to form ion pairs with picrate salts (28). The 1/1 adduct must then rearrange over a longer time period to form the bis complex. This would be consistent with proton NMR data obtained in this laboratory. On reacting sodium picrate and creatinine in \(^2\)H\(_2\)O and NaO\(_2\)H a reddish-orange substance immediately forms. Proton NMR shows an absorption for the ring protons of picrate at 8.9 ppm. This absorption at 8.9 ppm decreases slowly with time and is shifted upfield. Thus the initial adduct reacts further to give the more stable bis complex. The 1/1 adduct is therefore the reddish-orange complex observed initially, which is slowly converted to a more stable form. At equal concentrations of picrate and creatinine about 10% of the final product is a 2/1 complex of creatinine and picrate, as shown by polarography (22). Our spectroscopic studies with use of excess creatinine show that the reddish-orange product 1/1 complex slowly changes to a yellow color, the change following first-order kinetics. Similar results have been reported by Butler (24). Thus, under the analytical conditions used in the clinical laboratory (excess picrate), the final product formed is the 1/1 adduct as proposed. Under conditions of excess creatinine the reaction will proceed to form the yellow bis complex.

The picrate/creatinine complex has been proposed as a Janovsky complex (20), with creatinine attacking the benzene ring at the meta (C—H) position through the methylene carbon of creatinine (16, 24).

Proton NMR studies indicate a shift of the ring protons upfield upon reacting sodium picrate and creatinine. (This reaction is very slow.) In addition there are the two absorptions at 3.06 and 2.70 ppm from the methyl group of creatinine. Attack at the meta position would be expected to show an upfield shift of the ring proton at the site of attack from 8.9 ppm to 6 ppm (20), which is consistent with our data. However, in this study we did not see a smaller upfield shift of the resonance at 8.9 ppm owing to the undisturbed proton of picric acid, where attack does not occur, to the vicinity of 8.5 ppm (20, 23). The fact that a Janovsky-type complex was not observed in the deuterated dimethyl sulfoxide during the \(^{13}\)C NMR studies indicates that the 1/1 adduct is probably stabilized by deuterated dimethyl sulfoxide and does not involve attack at the meta position of picrate (27). The creatinine attacking the picrate at position 1 (hydroxyl group) is an alternative possibility. Bis complex formation involving attack at the meta positions of picrate could explain the shift of the ring protons with time (22, 23) as indicated by the proton NMR data. This bis complex is perhaps of the Janovsky type and involves attack by the creatinine via the methylen or the enolized carbonyl carbon.

Analytical Implications

Kinetic and spectroscopic evidence on the reaction of alkaline sodium picrate and creatinine shows that the forward reaction is first order in picric acid concentra-
tion, first order in creatinine concentration, and first order in hydroxide concentration. Under the pseudo-first-order conditions for analysis (excess picric acid) the rate of the reaction is very hydroxide dependent for the forward and reverse reactions. Therefore very close hydroxide control is imperative for accurate analysis (15, 21). As the hydroxide concentration is increased there is an increase in both the forward and reverse reactions, resulting in a decrease in absorbance change ($\Delta A$). In addition there is a shift of the maximum absorbance of the product to longer wavelengths as the hydroxide concentration is increased. Thus one must check the maximum absorbance of the product as sodium hydroxide is changed. Interferences from acetone, hydantoin, and other substances are an ever-present problem with this reaction.

I especially thank Phil Pitner, the Core Cancer Research Facility, University of Alabama in Birmingham, for running the $^{13}$C NMR spectra, and Charles Walkins, of the Chemistry Department, for running the $^{1}$H NMR spectra.

References


