Mechanism of Interference with the Jaffé Reaction for Creatinine

Martin H. Kroli, Neil A. Roegh, Brent Poe, and Ronald J. Elin

We investigated the mechanism of the Jaffé reaction for determination of creatinine by studying the spectrophotometric, kinetic, and equilibrium properties of the reaction of picrate with creatinine and with cyclic and aliphatic ketones. Absorbance spectra for the reaction products of picrate with all the ketones were superimposable with that of creatinine ($A_{\text{max}}$, 490 nm). Cyclic ketones not containing nitrogen had a molar absorptivity less than half that of creatinine and equilibrium constants approximately 0.01 that of creatinine. Aliphatic ketones, except for benzylacetone, had molar absorptivities similar to that of creatinine, but all of these compounds had equilibrium constants approximately a tenth or less of that of creatinine. The common structure for all of the compounds reacting with picrate is the carbonyl group. The variable magnitude of interference for aliphatic and cyclic ketones is ascribable to the different rate constants, molar absorptivities, and equilibrium constants as compared with creatinine. Structures adjacent to the carbonyl group significantly affect the absorptivity and equilibrium constant, but steric hindrance is the major factor affecting the rate of reaction. We postulate that the carbonyl group is required for the Jaffé reaction, and we suggest a mechanism for the reaction.

The requisite structure for interference with the Jaffé method for creatinine has not been determined. Alkaline picrate, the chromogen in the Jaffé reaction, reacts with creatinine and consequently shifts its spectrum to show an absorbance maximum at 490 nm (1). The cepha antibiotics and the hypoglycemic agent, acetohexamide, both interfere in the Jaffé reaction, but are structurally dissimilar ketones. The structure of the cepha antibiotics is cyclic, like creatinine, but acetohexamide is an aliphatic ketone (2, 3). We investigated the reaction of cyclic and aliphatic ketones with picrate, to further elucidate the nature of interference with the Jaffé reaction.

Materials and Methods

Instruments

We used a Model 25 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA 92634) for all absorbance measurements.

Reagents

We used the following reagents:

Creatinine (Standard Reference Material no. 914; National Bureau of Standards, Gaithersburg, MD 20234).

Picric acid (99%, "Gold Label"; Aldrich Chemical Co., Milwaukee, WI 53201). The concentration of the stock solution must be determined, because picric acid is stored wetted with water (for safety reasons). We determined the concentration by use of its molar absorptivity and titration with NaOH.

Acetohexamide (pure dried powder "Dymelor," M, 324, lot no. 6DF63; Eli Lilly & Co., Indianapolis, IN).

Acetophenone (99%, Aldrich).

Acetone (99%+, spectrophotometric grade, Gold Label, Aldrich).

Propiophenone (99%+, Aldrich).

Benzenesulfonic acid (Technical grade, 90%, Aldrich) filtered before use.

Benzyaceton (98%, Aldrich).

Cyclobutanone (99%, Aldrich).

Cyclopentanone (99%+, Aldrich).

Cyclohexanone (99.8%, gold label, Aldrich).

1,3-Dimethyl-2-imidazolidinone (98%, Aldrich).

4-Aminoantipyrine (98%, "Amyprone"; Eastman Kodak Co., Rochester, NY 14650).


Fenchone (98%+, Aldrich), dissolved in reagent-grade ethanol.

Experimental Design

We reacted all the compounds listed above with 10 millimolar picrate and 150 millimolar NaOH (final concentrations) at 37 °C, and recorded the spectra and absorbance at 490 nm as a function of time. For studies at equilibrium we varied the concentration of picrate from 2 to 20 mmol/L and measured the absorbance at 490 nm, incubating all samples at 37 °C. We used 0.05 mmol of creatinine per liter, 0.1 mmol of acetohexamide, acetophenone, and propiophenone per liter, 97 mmol of benzenesulfonic acid per liter, and 10 mmol of each of the rest of the compounds per liter (all final concentrations).

Statistical Analysis

We plotted the natural logarithm of $A_w - A_t$ vs time, where $A_w$ is the absorbance at equilibrium and $A_t$ the absorbance at some time $t$, to calculate the rate constants. If such a plot is linear, then the reaction is pseudo-first-order with respect to the reacted compound and $k$ is the observed rate constant (2). We plotted $[C]/A_w$ vs $[picrate]^{-1}$ (where $[C]$ is the initial concentration of compound and $[picrate]^{-1}$ is the reciprocal of the initial concentration of picrate) to calculate the molar absorptivity and equilibrium constants ($K$), according to the Benesi-Hildebrand equation (4). We analyzed all plots by linear regression, then calculated the mean and standard deviations from multiple analyses for each constant. We calculated the forward rate constant, $k_1$, using the formula $k_1 = k/(1 + K^{-1})$, comparing the results by use of Student's $t$-test.

Results

All the compounds except benzenesulfonic acid formed products with picrate that had spectra superimposable with that formed by creatinine, with an absorbance maximum at 490 nm (Figure 1). The molar absorptivities of acetohexa-
mide, acetophenone, acetone, and propiophenone were similar: 11 600 to 16 400 L/mol cm at 490 nm; those for benzylacetone and the three cyclic ketones were lower, ranging from 2090 to 5200 L/mol cm (Table 1). The equilibrium constants varied greatly, being largest for the picrate product with creatinine (3500 L/mol), one-tenth that of creatinine for acetohexamide, acetophenone, and propiophenone, and one-hundredth that of creatinine for cyclobutanone, cyclopentanone, cyclohexanone, benzylacetone, and acetone (Table 1). Creatinine and cyclopentanone react with picrate at the same rate: 0.73 min⁻¹. Also, acetohexamide and propiophenone react with picrate at the same rate, 0.28 min⁻¹; acetophenone reacts a little slower, 0.15 min⁻¹. The rest of the compounds react at variable rates (Table 1).

The forward rate constants, kₚ, for creatinine, acetone, benzylacetone, and cyclopentanone (Table 1) were not significantly different by Student's t-test. Acetohexamide and propiophenone have essentially the same kₚ while that for acetophenone is significantly slower (P <0.05). The kₚ for cyclohexanone is considerably slower for creatinine (P <0.02). The average kₚ of creatinine, acetone, benzylacetone, and cyclopentanone is 0.70 ± 0.11 (2 SD), and the kₚ for cyclobutanone differs significantly (P <0.01) from this mean.

The compounds dimethylimidazolidinone, aminooantipyrine, ninhydrin, and fenchone all react with picrate to form light-absorbing species with spectra identical to that formed by the reaction of picrate with creatinine. Molar absorptivities, equilibrium constants, and rate constants were not determined for these compounds.

Discussion

The mechanism for the reaction between a ketone, such as creatinine, and picrate is unclear (5, 6). Kimura and Butler have suggested that hydroxide ion removes the hydrogen from the alpha carbon (next to the carbonyl group) to form a carbanion, which in turn attacks picrate at either the 3 or 5 carbon positions to form a Janovsky complex (7, 8). This mechanism requires an available acidic carbon, that can have its hydrogen removed by aqueous base (8-10). A key piece of evidence for this mechanism is the failure of compound IV (a derivative of creatinine that lacks a methylene group) to react with picrate (8). But picrate does react with dimethylimidazolidinone, aminooantipyrine, ninhydrin, and fenchone to form products with peaks near 490 nm (Figure 1). These four compounds lack acidic carbons adjacent to the carbonyl group (Figure 2B). Further, picrate does not form a product with benzenesulfonic acid (the backbone of acetohexamide) that absorbs at 490 nm (Figure 2D). The activity of ketones with picrate in the Jaffé reaction appears to center on the carbonyl group and not the adjacent carbon.

The alternative proposed mechanism is the interaction of the enolate of creatinine (or a ketone) with the nitro groups of picrate through ion-ion interactions (11-14). This mechanism, also, should be impossible with dimethylimidazolidinone, aminooantipyrine, ninhydrin, and fenchone. Thus, the mechanism of the Jaffé reaction may not involve ion formation and interaction.

The reactivity of a ketone with picrate is dependent on the carbonyl and its neighboring atoms. We can make predic-

---

**Table 1. Kinetic and Equilibrium Data**

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Molar absorptivity, L/mol cm, 480 nm</th>
<th>Equilibrium constant, L/mol</th>
<th>Rate constant, min⁻¹</th>
<th>kₚ, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>12000 (180)</td>
<td>3500 (460)</td>
<td>0.73 (.05)</td>
<td>0.73 (.11)</td>
</tr>
<tr>
<td>Acetohexamide</td>
<td>13500 (480)</td>
<td>346 (27)</td>
<td>0.28 (.006)</td>
<td>0.28 (.02)</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>16400 (250)</td>
<td>500 (22)</td>
<td>0.15 (.003)</td>
<td>0.15 (.01)</td>
</tr>
<tr>
<td>Acetone</td>
<td>15800 (200)</td>
<td>1.80 (0.20)</td>
<td>0.97 (.15)</td>
<td>0.62 (.12)</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>11600 (83)</td>
<td>488 (19)</td>
<td>0.28 (.02)</td>
<td>0.28 (.02)</td>
</tr>
<tr>
<td>Benzylacetone</td>
<td>2090 (87)</td>
<td>8.99 (45)</td>
<td>0.83 (.08)</td>
<td>0.75 (.08)</td>
</tr>
<tr>
<td>Cyclobutanone</td>
<td>5000 (270)</td>
<td>37.1 (2.4)</td>
<td>0.52 (.009)</td>
<td>0.51 (.03)</td>
</tr>
<tr>
<td>Cyclopentanone</td>
<td>5200 (800)</td>
<td>18.5 (3.7)</td>
<td>0.73 (.04)</td>
<td>0.69 (.14)</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>4100 (125)</td>
<td>51.1 (3.0)</td>
<td>0.21 (.0008)</td>
<td>0.21 (.01)</td>
</tr>
</tbody>
</table>

Standard deviations are shown in parentheses. n was three for the determinations of molar absorptivities and equilibrium constants, and seven for the determinations of the rate constants, except for acetohexamide and acetone, where it was eight, benzylacetone and cyclobutanone, where it was six, and creatinine, where it was five. kₚ is the forward rate constant.

---

Fig. 1. Absorbance spectra for the products of different compounds reacted with picrate (10 mmol/L) at 37°C, with picrate as the blank. All the compounds tested, except benzenesulfonic acid, reacted with picrate to form products that absorb light with spectra nearly identical to that for the product of reaction of creatinine with picrate.
ure 2A) support this supposition, because they have similar equilibrium constants, approximately one-hundredth less than creatinine. The carbonyl for all of these compounds is bracketed by methyl groups. Thus, the chemical groups adjacent to the carbonyl group also appear to be of great significance for determining the equilibrium constant.

Steric hindrance seems to be the most important for determining the rate constant. Creatinine, cyclopananon, acetone, and benzyloacetone have the largest rate constants. The carbonyl group of acetone and benzyloacetone is readily accessible compared with the other three compounds in Figure 2C, which have a phenyl group adjacent to the carbonyl group. The slower rate constant for cyclopananon and cyclohexanon is also due to steric hindrance. Cyclohexanon usually assumes a chair or boat configuration, which could retard the reactivity of the carbonyl group. On the other hand, the five-membered rings of creatinine and cyclopananon are almost flat, which would decrease steric hindrance.

We propose that a co-planar charge transfer complex is formed between picrate and creatinine (or other ketones) in the Jaffé reaction. The steric effects on the rate of reaction suggest that the complex in the Jaffé reaction is in the form of two parallel plates, one plate being picrate and the other the carbonyl group with its adjacent atoms (Figure 3).

Butler has shown that dicyclohexyl-18-crown-6 ether in benzene reacts with picric acid to form a product that absorbs light similar to compounds in the Jaffé reaction (15). Picric acid and dibenzo-24-crown-8 (a similar crown ether) form a similar red complex (15). The x-ray crystal structure of the picric acid and dibenzo-24-crown-8 complex shows the planes of the two molecules in parallel forming a charge-transfer complex, the pi-electron-rich catechol units of the dibenzo-24-crown-8 donating electron density to the pi-electron-deficient picric acid (16). Thus, the product formed in the Jaffé reaction may be a co-planar charge-transfer complex.

Ketones demonstrate high reactivity with picrate, especially if they are four- or five-membered cyclic rings, contain nitrogens, or have a phenyl group adjacent to the carbonyl. Properties that increase the likelihood of interference are an equilibrium constant greater than 100 L/mol cm, a high molar absorptivity (at least 1000 L/mol cm), and an observable rate constant near that for creatinine (0.73 min⁻¹).

![Fig. 3. Proposed structure of the picrate-creatinine complex](Image)

O means oxygen and N means nitrogen, all other atoms are carbon. The plane of picrate (open bars) is above and parallel to the plane of creatinine (solid bars).
We thank Eli Lilly & Co. for the donation of acetoheptamide.

References