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Electrochemistry of Brucine. 2. The Brucine-Based Determination of Nitrate

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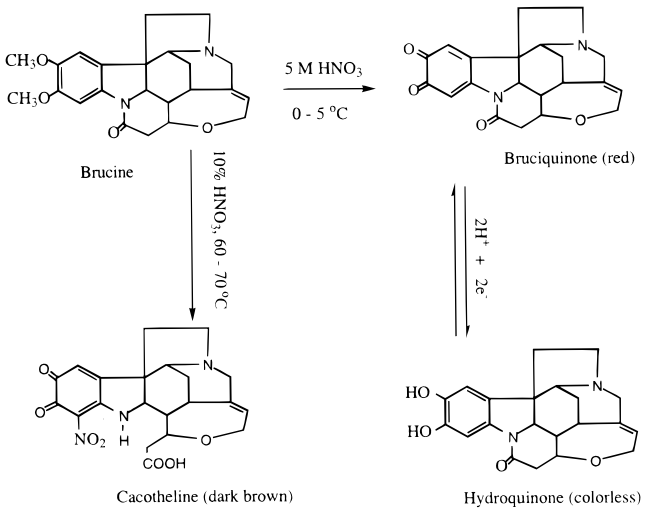
The electrochemical and chemical reactions of brucine following an oxidation step were investigated by cyclic voltammetry, chronocoulometry, bulk electrolysis, visible spectrophotometry, and electrospray mass spectrometry with the goal of better understanding the brucine-based determination of nitrate. The experiments were performed in acidic solution (0.010−7.0 M sulfuric acid) using carbon paste, glassy carbon, and platinum electrodes. Following a two-electron oxidation (E_p = 0.90−1.15 V vs Ag/AgCl), brucine^{2+} reacts with water to form an aminochrome (brucichrome) plus methanol. Brucichrome undergoes a reversible two-electron reduction ($E^{o\prime}$ = 0.54 V, 0.01−7.0 M sulfuric acid) to form a hydroquinone and also slowly dimerizes. The dimer is also an aminochrome, and it, too, undergoes a reversible two-electron reduction ($E^{o\prime}$ = 0.39 V). The primary evidence for a dimeric final product comes from electrospray mass spectrometry during electrolysis, elemental analysis, and H-NMR on a product isolated from bulk electrolysis in HCl.

Despite an array of techniques available for the analysis of nitrate,[1,2] the United States Environmental Protection Agency (EPA)[3] and the Association of Official Analytical Chemists (AOAC)[4] procedures are still based on the brucine method.[5−7] Brucine, an indole alkaloid, is oxidized in a 7 M sulfuric acid solution kept at 100 °C (boiling water) for 20 min. The final oxidation product is a yellow compound of unknown structure with high absorptivity at 410−430 nm. As with many spectrophotometric determinations, it is not necessary to know the structure of the absorbing product. This is a "black box" approach, and clearly it would be useful if the underlying reactions and the final product were known.

Scheme 1. Reported Brucine Reactions[8−11]



Scheme 1 illustrates the redox chemistry of brucine. Mild oxidation (5 M nitric acid, 0−5 °C) is said to convert brucine into a bright red compound (bruciquinone) that can be isolated as a perchlorate salt.[8,9] Bruciquinone can be reversibly reduced to a colorless hydroquinone.[10] Moderate oxidizing conditions (10% nitric acid, 60−70 °C) convert brucine to cacotheline,[11] a compound in which electron deficiency of the quinone is alleviated by nitration. Without nitric acid, the chemical oxidation of brucine under strongly oxidizing conditions proceeds via a bright red intermediate to a yellow unknown compound.[12−14] Electrochemical oxidation of brucine (E_p = 0.80 V vs SCE, 2 M sulfuric acid) also produces a red intermediate which slowly reacts to form a final yellow compound.[15] Both the red intermediate, and the final compound show reversible cyclic voltammograms ($E^{o\prime}$ = 0.47 and 0.27 V respectively).

When two methoxy groups are on the same benzene ring, oxidation occurs at both sites only when they are either ortho or para to each other. The quinones formed from oxidation of dimethoxy (or dihydroxy) sites are electron deficient and, as a result, undergo numerous addition and substitution reactions.[16] In many of these reactions, the net effect is a substitution reaction

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by an by electron-donating species, as illustrated by 6-hydroxydopamine[17,18] and epinephrine.[19] In both 6-hydroxydopamine and epinephrine, an intramolecular reaction with the electron-donating amine site leads to the formation of a dihydroxyindole. Oxidation of dihydroxyindole leads to an electron-deficient quinone, and in the absence of substitution reagents, the quinone dimerizes.[20] It is particularly noteworthy that indole itself dimerizes when oxidized.[21,22] Under some oxidation conditions[23] (e.g., silver oxide in methanol containing some formic acid), dihydroxyindole is oxidized into an aminochrome. The very high solubility of aminochromes and their tendency to decompose when the solution is concentrated prohibits their isolation in the majority of cases. The physical and chemical properties of the solution, however, suggest that the aminochrome has the zwitterionic form.[24−26]

To better understand the brucine-based determination of nitrate, we have reexamined these reactions and reached the conclusion that the red intermediate dimerizes into the yellow compound. A structure of the yellow compound is proposed.

EXPERIMENTAL SECTION

Instrumentation. Electrochemical measurements were performed on a Bioanalytical Systems (West Lafayette, IN) BAS 100BW electrochemical analyzer. Visible spectra were obtained using a Hewlett Packard HP 8452A diode array spectrophotometer, and IR spectra were obtained in a KBr pellet matrix using a Perkin Elmer 1610 FT-IR. Electrospray mass spectrometry was performed on a Kratos Profile HVR 2500 instrument using a 1:1 mixture of methanol and water with 1% acetic acid with a flow rate of 4 μL/min. ^{1}H NMR spectra were obtained on a Bruker AC-200 FT-NMR.

The platinum and gold electrodes were 1.5 mm in diameter, and the glassy carbon electrode was 3.0 mm in diameter; all were purchased from Bioanalytical Systems. The carbon paste electrodes were made by mixing 2.0 g of carbon with 1.5 g of hexadecane. Electrolysis was performed with the aid of a Bioanalytical Systems electrolysis cell. A Ag/AgCl reference electrode was used in all cases.

Materials and Procedures. Analytical-grade reagents and distilled water were used to prepare all aqueous solutions, and except for cacotheline (Fluka Chemica-Biochemica, Ronkonkoma, NY), all reagents were obtained from Aldrich (Milwaukee, WI). Cerium ammonium sulfate solutions were standardized by titration with ferrous ammonium sulfate using ferroin as the indicator. Brucine solutions were prepared fresh on a daily basis.

Bulk electrolysis was performed at various potentials indicated in the text, and in all cases the procedure was terminated when the current was 1% of its initial value. Bulk electrolysis performed to isolate the final product (yellow compound) was performed in 5.6 M HCl. After the electrolysis, the water and HCl were removed on a vacuum line. Elemental analysis of the isolated product by Desert Analytics (Tucson, AZ) yielded the following results: C, 69.1; H, 5.48; N, 7.71; Cl, 0.09; O, 17.60 (by difference). Expected for $C_{42}H_{40}N_4O_8$: C, 69.25; H, 5.49; N, 7.69; O, 17.56.

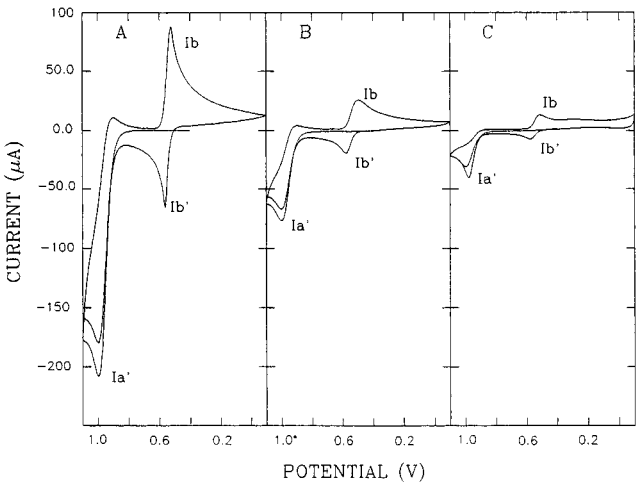

Figure 1. Cyclic voltammograms obtained at 100 mV/s in 5.0 × 10^{-3} M brucine, 1.8 M sulfuric acid. (A) CPE, (B) GCE, and (C) PE.

The titration of brucine with Ce(IV) was performed using 5.0 mL of a 5.0 mM brucine solution and a 10.0 mM Ce(IV) solution with both reagents in 1.8 M sulfuric acid. The titration was monitored by measuring the cyclic voltammetry oxidation current at 0.80 V and also by measuring the absorbance of the solution at 420 nm.

The rate of the conversion of the red intermediate (produced after oxidation of brucine) to the final yellow compound was investigated in the following manner: 0.50 mL of a 9.8 × 10^{-3} M solution of Ce(IV) was added to 3.0 mL of a nearly saturated solution of brucine in 1.8 (or 7.0) M sulfuric acid in a cuvette, and the diode array spectrophotometer (kinetics mode) was started. Under these conditions, the oxidation of brucine was complete before the first measurement. The rate of reaction of the red intermediate was then monitored in the range 350−700 nm every 1 or 2 s.

RESULTS AND DISCUSSION

Figure 1A shows a cyclic voltammogram (CV) of a 5.0 mM solution of brucine in 2.0 M sulfuric acid at a carbon paste electrode (CPE). With the CV starting at 0.40 V and going positive, an irreversible oxidation wave with a peak potential of 1.00 V (peak Ia′) is observed. On the second (negative potential) scan, a reversible reduction occurs with a peak potential of 0.52 V (peak Ib). Figure 1B,C shows CVs at the glassy carbon electrode (GCE) and the platinum electrode (PE), respectively. In all three CVs, peak Ia′ occurs over a smaller potential range; as a consequence, peak current Ia′ is larger than peak current Ib.

Figure 2 shows CVs in 7.0 M sulfuric acid at the GCE and PE. On going from 2.0 to 7.0 M sulfuric acid (7.0 M is used in the brucine nitrate method), the following changes are observed at the PE: peak Ia′ shifts in the negative direction by 0.10 V, peaks Ib and Ib′ shift in the positive direction by about 0.05 V, peak current Ia′ is approximately equal to peak current Ib, and notably, there is now evidence of two new peaks (Ic, Ic′). The GCE CV

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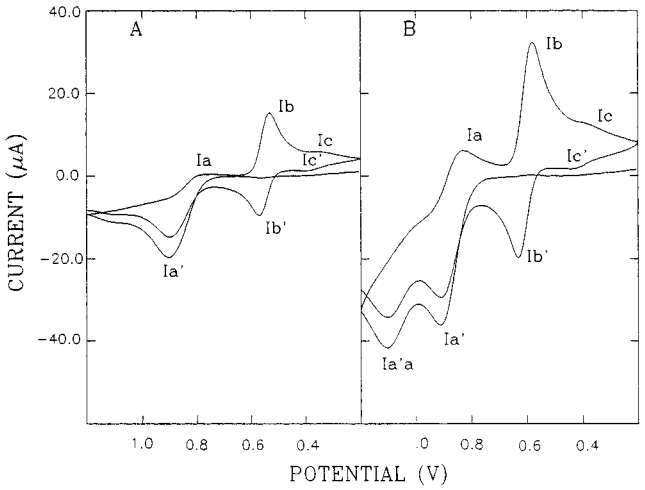


Figure 2. Cyclic voltammograms obtained at 100 mV/s in 2.4 × 10^{-3} M brucine, 7.0 M sulfuric acid. (A) PE and (B) GCE.

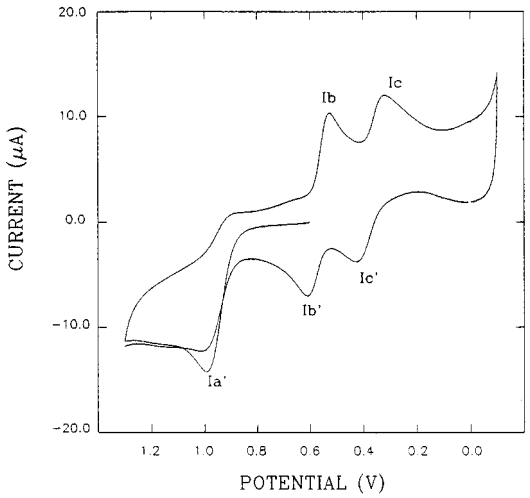


Figure 3. Platinum electrode cyclic voltammograms obtained at 100 mV/s in a brucine solution partially oxidized by Ce(IV) in 1.8 M sulfuric acid.

in 7.0 M sulfuric acid is similar to that at the PE for peaks Ib, Ib′, Ic, and Ic′ but differs by showing a double-oxidation wave: peak Ia′ (quasireversible, E_p = 0.90 V) and peak Ia″ (irreversible, E_p = 1.15 V). Oxidizing beyond peak Ia′ at the GCE and switching the direction of scan before the second oxidation (peak Ia″) is reached leads to a much smaller peak Ib. At both GCE and PE, high scan rates (>1500 mV/s) lead to virtual reversibility for peak Ia′, and with such reversibility, peaks Ib and Ib′ are much smaller. When the 7.0 M sulfuric acid brucine solution used to obtain the cyclic voltammograms in Figure 2B was diluted to 2.0 M, the original behavior shown in Figure 1 was observed. Except for a significant increase in background current at potentials greater than 0.85 V, the electrochemistry of brucine at a CPE in 7.0 M acid is similar to that at a GCE. Low concentrations of acid (0.01−0.2 M) gave the same results at all three electrodes. Peak Ia was found to be relatively insensitive to acid concentration, while the reversibility of peaks Ib and Ib′ decreased with decreasing acid concentration.

Bulk electrolysis at 1.0 V (2.0 M sulfuric acid) resulted initially in a bright red solution that turned dark yellow with time. Results indicated the transfer of 1.78−1.87 electrons per brucine molecule. Ce(IV) titrations of brucine yielded 1.87−1.89 oxidizable sites per brucine molecule, in agreement with the bulk electrolysis results.

The double-oxidation wave in concentrated acid (peaks Ia′ and Ia″), the bulk electrolysis, and the titration results all suggest a two-electron process. Brucine^{2+} reacts with water to produce a quinone (or aminochrome) plus methanol. Detailed mechanistic information is not available for this process; thus, it is not known if the methoxy residues are removed together or sequentially. As noted earlier, many previous studies suggest that bruciquinone is the intermediate in the oxidation of brucine.[8−15] The following observations, while not contradicting the orthoquinone proposal, suggest that the intermediate is better represented by the zwitterionic form (brucichrome): (1) lack of extractability into organic solvents, (2) poor extraction onto an "organic" electrode (carbon plus hexadecane), and (3) visible spectra that are similar to known aminochromes (see below).

An attempt to determine the number of electrons for peak Ib by first oxidizing brucine at 1.2 V and thereafter performing bulk electrolysis at 0.50 V failed to yield meaningful results. The failure can be attributed to the reaction converting the red intermediate into the yellow final product. Starting with a saturated solution of brucine in 2.0 M sulfuric acid, the potential was held at 1.2 V for 5 s to oxidize brucine and then stepped to 0.5 V for 5 s to reduce the bruciquinone. The current and charge (Q) were monitored as functions of time for both the oxidation (1.2 V) and reduction (0.5 V). Q was plotted against the square root of the time, from which Q_{red}/Q_{ox} and $\text{slope}_{red}/\text{slope}_{ox}$ were calculated. Both values were found to be between 0.94 and 1.00.

Assuming that the diffusion coefficients and the concentrations of both forms are the same, the integrated Cottrell equation ($Q = 2nFAD^{0.5}Ct^{0.5}/\pi^{0.5}$) predicts that

$$\frac{Q_{red}}{Q_{ox}} = \frac{\text{slope}_{red}}{\text{slope}_{ox}} = \frac{n_{red}}{n_{ox}}$$

With $Q_{red}/Q_{ox} = \text{slope}_{red}/\text{slope}_{ox} = 1$ and $n_{ox} = 2$, peak Ib is a two-electron reduction process.

Figure 3 shows a CV recorded in a solution made by mixing 5.0 mL of 5.0 mM brucine and 4.0 mL of 2.00 mM Ce(IV) diluted to 20.0 mL with 2.0 M sulfuric acid. The CV was recorded after 1 h of mixing to allow the reacting mixture to come to equilibrium. Since brucine is in excess, we see peaks Ia′, Ib, and Ib′ as before (Figure 1), but in addition we see reversible behavior (peaks Ic and Ic′) with $E^{o\prime}$ = 0.39 V. As has been shown earlier, peak Ib does not generate a significant peak Ic within the time frame of an ordinary cyclic voltammogram. Consequently, peak Ic in Figure 3 comes from a product already present in solution before the cyclic voltammogram. CVs recorded in brucine solutions to which an excess of Ce(IV) had been added show that peaks Ic and Ic′ increase at the expense of peaks Ib and Ib′ with increasing acid concentration. In fact, in 7.00 M acid there is barely a trace of peak Ib. The number of electrons involved in peak Ic was determined by bulk electrolysis. After brucine was oxidized at 1.10 V overnight in 4.0 M sulfuric acid, another bulk electrolysis was performed at 0.00 V. Before bulk electrolysis at 0.00 V, peak Ib was found to be negligible compared to peak Ic. The reduction at 0.00 V requires 0.95 electrons per initial brucine molecule. This result suggests either a one-electron process or a two-electron process for a dimeric product.

Figure 4 shows the spectrophotometric monitoring of a reaction between excess brucine (3.0 mL of 7 mM brucine) and

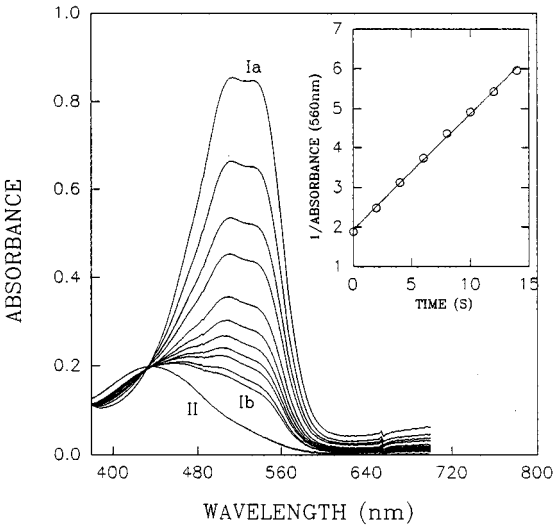
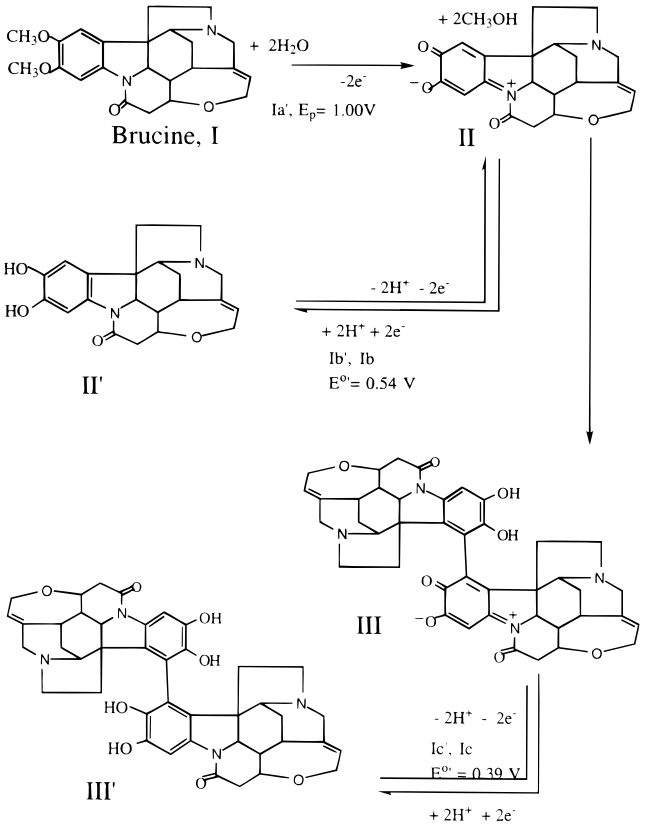


Figure 4. Kinetics of the red intermediate disappearance. Spectra labeled II were obtained at equilibrium. Graph of (absorbance (520 nm))$^{-1}$ versus time shown has equation $y = 0.292x + 1.93$, correlation coefficient = 0.9995.

0.5 mL of 1.0 mM Ce(IV), with both reagents dissolved in 2.0 M sulfuric acid at 23 °C. The reaction was monitored in the range 350−750 nm at 2.0 s intervals. The initial reaction (brucine + Ce(IV)) is very fast, producing brucichrome, a red intermediate with comparable absorptions at 513 and 537 nm. (This broad plateau is typical of many aminochromes.[27,28]) The initial reaction is much faster than the subsequent reaction (brucichrome to final product). It is interesting to note that brucichrome has a higher molar absorptivity than the final product (λ_{max} = 420−430 nm), a feature that would have been of considerable benefit in the determination of nitrate had this been the final product. Figure 4 also shows a plot of $1/A_t$ versus time, where A_t is the absorbance at time t (λ = 520 nm). The calculated second-order rate constant (k) is 0.025 ± 0.001 M^{-1} s^{-1} in 7.0 M sulfuric acid at 23 °C. Interestingly, the reaction is actually faster in 2.0 M sulfuric acid (k = 0.161 ± 0.004 M^{-1} s^{-1}). Since all the reagents involved in this reaction (H_2O, H^+) are in large excess compared to aminochrome, the second-order behavior points to either a very complex mechanism or a dimerization reaction.

In an attempt to isolate the final product from the oxidation of brucine with an excess of Ce(IV), extraction into various organic solvents (e.g., ethyl acetate and dichloromethane) was attempted. Unlike cacotheline and dimethoxyindole oxidation products, which extract easily, no extraction was observed in the pH range 0−13. In an effort to isolate the product, we electrochemically oxidized brucine (bulk electrolysis) in a 1:1 HCl water medium and then removed the water and HCl on a vacuum line. That product was light brown (quite similar to cacotheline) and was highly water soluble at all pHs. A CV of a sample showed only peaks Ic and Ic′, indicating formation of the final product (λ_{max} = 420−430 nm). Electrospray mass spectrometry of this product had m/z = 365.2. IR spectra on the dried final product showed absorptions at 3446 (strong, broad), 1616 (strong, broad), 1448, 1380 and 1290 (broad, weak), and 1109 cm^{-1} (strong, sharp). H-NMR showed uncoupled protons at 7.7 and 6.2 ppm. Electrospray mass spectroscopy and

Scheme 2. Brucine Electrochemical Scheme



visible absorption spectrometry were used to monitor the course of two further bulk electrolyses. In the first trial, bulk electrolysis was performed in 0.001 M HCl, and in the second, it was done in 3.5 M HCl. The solution was neutralized with sodium bicarbonate prior to the measurement. Brucine has a mass of 394.1 amu and so shows an electrospray mass peak at 395.1 amu (M + H)—a result which indicates that only the tertiary amine is protonated. During the first 35 min of electrolysis, mass peaks were observed at 365.2 and 395.1 amu in both trials. The spectral characteristics of the red solution in the first trial matched those of the red intermediate in Figure 4 (λ_{max} = 513 and 537 nm), while the spectral characteristics in the second trial matched those of the final product (λ_{max} = 420 nm). Upon acidification of the first trial solution to 5 M HCl, the spectral peaks at 513 and 537 nm disappeared and were replaced by the peak at 420 nm.

In the first trial (0.001 M H^+), visible spectrophotometry showed that the rate of reaction of the red intermediate is low. Therefore, the electrospray mass at 365.2 amu is that of the intermediate. The rate of disappearance of the red intermediate in the second trial (3.5 M H^+) is quite high; hence, the increased intensity at 365.2 amu is due to the final product (λ_{max} = 420 nm). This suggests that the intermediate and the final product have the same m/z ratio. Postulating a dimerization reaction gives us a product with two amine sites, which would explain why the m/z values of the intermediate and final product are the same. Additional support for a dimeric final product comes from (1) one electron per initial brucine molecule for Ic, (2) second-order rate law, (3) hypsochromic shift, which can be predicted from a final product that is less electron deficient, (4) two H-NMR peaks with no splitting at 7.7 and 6.4 ppm (aromatic and quinone protons, respectively), and (5) elemental analysis data. Collectively, these provide strong support for a dimeric product. A proposed

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structure of the dimer is shown in Scheme 2 (structure **III**). Allowing the electrolysis solutions to stand for extended periods leads to further unknown reactions, as evidenced by the appearance of additional electrospray peaks. The proposed electrochemical scheme for brucine is shown in Scheme 2.

As mentioned in the introduction, the brucine method for determination of nitrate is performed with an excess of brucine in 7 M sulfuric acid. Bubbling nitrogen dioxide gas through a solution of brucine in sulfuric acid rapidly oxidizes it. When a solution of 1.0 mM nitrate in 7 M sulfuric acid is placed in boiling water for 20 min, cooled to room temperature, and added to a solution of brucine, the reaction is fast and comparable to oxidation of brucine by Ce(IV). We therefore surmise that the 100 °C/7 M treatment converts nitrate to nitrogen dioxide by the following reaction:[29]

$$4NO_3^- + 4H^+ \rightarrow 4NO_2(g) + O_2(g) + 2H_2O$$

CONCLUSIONS

The electrochemistry of brucine has been elucidated (Scheme 2), and a structure for the final product, a dimer, is proposed. The following experimental conditions employed in the brucine nitrate method may now be explained: (1) The 20 min of heating at 100 °C specified in the procedure is necessary to convert nitrate to nitrogen dioxide. With parts per million levels of nitrate, this reaction is slow and is the reason why it is important that standards and sample be treated in a common bath to ensure the same degree of reaction. It is the nitrogen dioxide that oxidizes brucine. The oxidation of brucine and the subsequent reaction of the bruciquinone intermediate are much faster than the generation of nitrogen dioxide. (2) The standards and sample need to be measured as soon as the solution cools because the final product undergoes further reactions that lead to lower absorptivity.

The present study also suggests that voltammetric detection may be used to determine nitrate by the brucine method. Such a procedure is currently being evaluated in our laboratory.

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