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Warren C. Jones, Jr., T. Michael Rothgeb, Frank R. N. Gurd*
Department of Chemistry, Indiana University
Bloomington, Indiana 47401
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The Two-Step Mechanism of Noncatalytic Aromatic Bromination. Controlled Variation of the Rate-Controlling Step

Sir:

By a systematic variation of the initial conditions, the rate-controlling step in an aromatic bromination has been changed from that of arenium ion formation to that of proton removal from arenium ion. The results support the simple two-step mechanism, eq 1 and 2, with no significant contribution by mechanisms involving more than one bromine molecule.

The well-known kinetic complications that often arise in noncatalytic aromatic bromination in the absence of added excess Br^- have been expressed in the form of eq 5.[1,2] Such behavior has been interpreted literally; that is, as meaning that the first term is due to an activated complex containing the elements of one Br_2, the second term due to an activated complex containing the elements of two Br_2, and so on. Various mechanisms have been assigned to each literally interpreted term. However, the *same* kinetic behavior will be shown by the simple two-step mechanism of eq 1 and 2 *alone*, provided only that $v_{-1}/v_2 = k_{-1}[Br^-]/k_2$ grows to significance during a kinetic run as the result of Br^- production. Equation 4, the general rate expression for the simple two-step mechanism, can be shown to be the *equivalent* of the power series equation, 5. Starting with $[Br_2]_{stoich} + [Br^-]_{stoich} = [Br_2]_0$, one expresses the variable $[Br^-]$ in terms of $[Br_2]$. The denominator of eq 4 then takes the form $C_1(1 + C_2[Br_2])^{-1}$ which then is expandable as a power series in $C_2[Br_2]$. This leads to eq 5, in which the constants k_I, k_{II}, and k_{III} have the complex form shown in eq 6.

$$ArH + Br_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ar^{+}(H)Br + Br^- \quad (1)$$

$$Ar^{+}(H)Br + B_i \longrightarrow ArBr + HB_i^+;\ k_2 = \Sigma k_{2_i}[B_i] \quad (2)$$

$$Br^- + Br_2 \rightleftharpoons Br_3^-;\ K_3 = [Br_3^-]/[Br^-][Br_2] \quad (3)$$

$$-d[ArH]/dt = \frac{k_1k_2[ArH][Br_2]}{k_{-1}[Br^-] + k_2} \quad (4)$$

Table I. Rate Constants and Kinetic Isotope Effects in the Para-Bromination of *N*-Methylacetanilide in 50% HOAc at 25°

$[Br_2]_0$	$[NaBr]_0$	10^2k_H[a]	$10^2k_H^{(corr)}$[b]	k_H/k_D[c]
$\sim 2 \times 10^{-4}$	0	8.59	8.59	0.93 ± 0.02[d]
$\sim 2 \times 10^{-3}$	0	(7.90)[e]		
$(2-20) \times 10^{-4}$	0.050[f]	2.23	5.31	1.41 ± 0.05
$(2-20) \times 10^{-4}$	0.150[g]	0.654	3.36	1.85 ± 0.04
$(2-20) \times 10^{-4}$	0.300	0.248	2.30	2.14 ± 0.02
$(2-20) \times 10^{-4}$	0.500	0.151	2.23	2.27 ± 0.02
$(2-20) \times 10^{-4}$	0.050[h]	1.96	4.66	1.18 ± 0.01

[a] $k_H = k_{obsd}(sec^{-1})/[ArH]$, average of several runs. [b] $k_H^{(corr)} = k_H[Br_2]_{stoich}/[Br_2] = k_H(1 + K_3[Br^-])$; $K_3 = 27.6$.[6] [c] k_D is the rate constant for *N*-methylacetanilide-*2,4,6-d*$_3$. [d] Standard deviation. [e] Based on initial slope, 20% reaction, of a curved rate plot. [f] Contained 0.250 *M* $NaClO_4$. [g] Contained 0.150 *M* $NaClO_4$. [h] Contained 0.250 *M* NaOAc.

$$-d[ArH]/dt = [ArH](k_I[Br_2] + k_{II}[Br_2]^2 + k_{III}[Br_2]^3) \quad (5)$$

$$k_I = \frac{k_1k_2}{k_{-1}[Br_2]_0 + k_2}$$

$$k_{II} = \frac{k_I(k_{-1} + 2k_{-1}K_3[Br_2]_0)}{k_{-1}[Br_2]_0 + k_2} \quad (6)$$

$$k_{III} = \frac{k_{II}(k_{-1} - 2k_2K_3)}{k_{-1}[Br_2]_0 + k_2}$$

Consider the effect of added excess Br^-, which is experimentally that of simplifying the kinetics to straight first order in Br_2. The literal interpretation of eq 5 requires that this be due to enough of a reduction in $[Br_2]$, as the result of equilibrium 3, to make negligible the probability of forming activated complexes containing the elements of more than one Br_2. On the basis of just the simple two-step mechanism, the kinetic simplification is due to the constancy of $k_{-1}[Br^-]$ and does not require a reduction in $[Br_2]$. Indeed, in the bromination of benzene in 78% of CF_3CO_2H, kinetically complex in the absence of added Br^- ($[Br_2]_0 \sim 3 \times 10^{-3}$ *M*), the addition of excess NaBr reduced the kinetics to cleanly first order in Br_2 under conditions that did *not* significantly decrease $[Br_2]$.[3] This result is contrary to the multimechanistic interpretation, but consistent with the simple two-step mechanism.

Given a suitable value of k_{-1}/k_2 it should be possible to change at will the rate-controlling step of the two-step mechanism by controlling $[Br^-]$. This has been realized in the para bromination of *N*-methylacetanilide in 50.1% acetic acid at 25°.[4] Rates were determined by following Br_2 absorption at 410 nm, an excess of the aromatic being employed ($[ArH] = 0.01$-0.1 *M*).

Without added excess Br^-, kinetic complications arose when a 1-cm Beckman cell was employed as the reaction vessel ($[Br_2]_0 \sim 2 \times 10^{-3}$ M). First-order plots were noticeably curved (decreasing apparent rate constant), especially beyond about 20% reaction. The curvature was substantially greater than could be attributed to an increasing tie-up of Br_2 in the form of Br_3^-,[5] and is consistent with $v_{-1}/v_2 = k_{-1}[Br^-]/k_2$ growing to significance during the production of Br^-. However, by the use of 10-cm cells, the amount of Br^- produced could be reduced tenfold ($[Br_2]_0 = 2 \times 10^{-4}$ *M*). Under these conditions, the reaction was cleanly first order for three half-lives of observation. Thus v_{-1} was throughout negligible relative to v_2, making the first step essentially completely rate controlling, with $k_{obsd} = k_1[ArH]$. Also consistent with rate control by the first step was the observation of a slightly smaller rate constant for 2,4,6-trideuterio-*N*-methylacetanilide under the same conditions (Table I).

The addition of excess NaBr to 1-cm cells runs also pro-

duced the expected effect of reducing the kinetics to cleanly first order. Furthermore, the decrease in values of $k_H \approx k_{obsd}/[ArH]$ with increasing added NaBr was steeper than the calculated fall-off of $[Br_2]/[Br_2]_{stoich}$ (see Table I), consistent with an increasing significance of the $k_{-1}[Br^-]$ term of eq 4, i.e., increasing rate-control by the second step.[7] This is verified by the concomitant increase in the observed kinetic isotope effect, k_H/k_D. The fact that k_H/k_D levels off in solutions of highest $[Br^-]$ indicates that essentially maximum rate control by the second step has been achieved in 0.5 *M* NaBr.[8] Finally, in 0.05 *M* NaBr, k_H/k_D was significantly reduced by the replacement of $NaClO_4$ (0.25 *M*) with NaOAc (last row, Table I). The result is consistent with the expected effect of the basic salt, increasing v_2/v_{-1}, thus leading to greater rate-control by the first step. It is to be noted that k_{obsd}, rather than being increased by the substitution of NaOAc for $NaClO_4$, was decreased slightly, indicating the action of significant specific salt effects on individual rate constants[10] and/or K_3.

References and Notes

(1) Further complexity can arise if the fraction of stoichiometric Br_2 tied up as Br_3^- (eq 3) grows to significance during a kinetic run.
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(7) A detailed analysis of the data indicates underlying salt effects, either a positive salt effect on the aromatic substitution per se and/or a negative salt effect on K_3.
(8) The maximum isotope effect is comparable to that found by Zollinger and Christen, 2.1, in the actual step of proton removal from the bromoarenium ion of 2-naphthol 6,8-disulfonate in water. In that instance, the arenium ion was not a steady state intermediate but was "instantly" and reversibly formed to greater than 90% from the naphthol plus Br_2 or HOBr.[9]
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W. M. Schubert,* Jeffrey L. Dial

Department of Chemistry, University of Washington
Seattle, Washington, 98195

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Direct Observation of Sulfur Coordination in Bean Plastocyanin by X-Ray Photoelectron Spectroscopy

Sir:

The ligand environment of "blue" (or type 1) copper proteins has not been established.[1-3] Recent studies in our laboratory on colbalt(II) derivatives of stellacyanin, French bean (*Phaseolus vulgaris*) plastocyanin, and *Pseudomonas aeruginosa* azurin, have suggested that cysteine is a ligand, and that a ligand-to-metal charge transfer (LMCT) transition in the Cu(II)-S(Cys) unit is responsible for the intense absorption band at about 600 nm in each of the native proteins.[2] Resonance Raman spectral experiments on several "blue" proteins have also been interpreted in terms of Cu(II)-S(Cys) coordination.[3]

A direct test of the proposed sulfur-copper coordination in "blue" copper proteins is afforded by X-ray photoelectron spectroscopy (XPS). The sulfur 2p (S2p) binding energy is approximately 164 eV, which is well separated from the core levels associated with the other atoms present in these proteins. The effect of metal incorporation on the sulfur atoms can then be observed by comparing the S2p region of the copper (or cobalt) derivatives with that of the apoprotein. If a sulfur, in fact, coordinates to the metal, its electron density will decrease, thereby increasing the S2p binding energy. We have chosen to perform this experiment on bean plastocyanin, as it contains only one copper and three potential sulfur donor atoms (two methionines and one cysteine).[4] This simplicity allows a number of important conclusions to be drawn about the "blue" copper site.

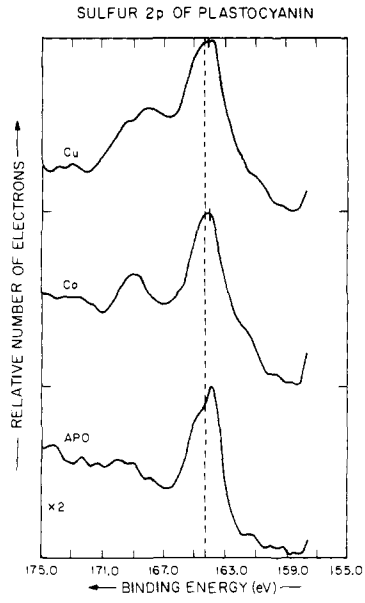

Figure 1. X-Ray photoelectron spectra of copper, cobalt, and apo plastocyanins in the S2p region at 250 K.

The X-ray photoelectron spectra reported here were measured on a Hewlett-Packard 5950 A ESCA spectrometer equipped with a low energy electron source for neutralization of charging effects. This spectrometer utilizes a monochromatic aluminum Kα X-ray source and has an instrumental resolution of 0.55 eV full width at half maximum (FWHM). All metalloprotein spectra reported were taken at 250 K, and 800-W X-ray power. The residual gas pressure in the analyzer chamber was 9×10^{-10} Torr, and the ambient gas consisted primarily of hydrogen, helium, nitrogen, carbon monoxide, and water. All binding energies are referenced to the protein aliphatic carbon 1s signal at 285.4 eV.

The extraction, purification, and metal substitution methods for bean plastocyanin have been described previously.[2] The copper sample had an absorbance ratio A_{278}/A_{597} of 1.1. Protein radiation damage was monitored by following C1s, N1s, and O1s regions. Two 126-sec scans were taken of each region. All sulfur 2p spectra were taken in sets of 30 scans, followed by the carbon, nitrogen, and oxygen sequence. No significant change was found in these regions (including S2p) after continual irradiation for several hours.

The samples were prepared by evaporating a small amount of protein solution (~50 μl) on rigorously clean, gold-plated stainless steel platens in a dry-nitrogen-flushed drybox connected directly to the inlet chamber of the spectrometer. The samples were gradually cooled to 250 K before subjecting them to the X-ray beam. The data were subjected to noise removal procedures using a low pass filter calculated on the basis of 0.8 eV line width.[5]

Figure 1 presents the sulfur 2p binding energy region for copper, cobalt, and apo plastocyanins. One sulfur peak is shifted by approximately 5 eV to higher binding energy (relative to the apo sample) in both the copper and cobalt