cation rearranges to protonated 4-substituted nortricyclenes on warming.

The formation of protonated 4-substituted nortricyclenes from each of the precursors may be visualized as occurring by the pathways shown in Scheme I.

The Wagner–Meerwein shift of the C_1–C_6 bond to form bridgehead-substituted norbornanes has been used as the basis for the synthesis of 1-substituted norbornanes.[7] At present, 4-substituted nortricyclenes are prepared by multistep syntheses.[8] The ease of formation of 4-substituted nortricyclenes under stable ion conditions could lead to a synthetically useful method for the preparation of 4-substituted nortricyclenes *via* deprotonation by tertiary bases (a method proved successful in the case of protonated nortricyclene itself[2]).

Acknowledgment. Support of this work by a grant of the National Science Foundation is gratefully acknowledged.

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[9] (a) NIH Postdoctoral Research Fellow; (b) Postdoctoral Research Investigator.

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Observation of Coordinated Free Radicals Formed in the Reaction of Hydroxyl Radicals with Benzoatopentaamminecobalt(III) in Aqueous Solution

Sir:

The existence of radical transient species with finite lifetimes coordinated to the metal center has been proposed[1] to account for the kinetic behavior of pentaamminecobalt(III) complexes undergoing reaction with one-electron oxidizing agents such as Ce(IV). Despite efforts[2] to observe such species by esr, their transient nature and the presence of paramagnetic ions has apparently prevented their direct observation. We wish to report here the observation of a coordinated free radical transient formed in the reaction of OH radicals with $(NH_3)_5Co(O_2CPh)^{2+}$ using the pulse radiolysis technique.

A 30-nsec pulse of 2.3-MeV electrons[3] caused the radiolytic decomposition of water: $H_2O \rightsquigarrow 2.8e_{aq}^- + 2.8OH + 0.6H$. Saturation of the solution with N_2O (25 m*M*) resulted in the conversion of e_{aq}^- to OH: $e_{aq}^- + N_2O \rightarrow OH + N_2 + OH^-$. $Co(NH_3)_5(O_2CPh)(ClO_4)_2$ was prepared[4] from carbonatopentaamminecobalt(III) and benzoic acid in DMF according to a modification of the literature procedure.[5] Comparison of the absorption spectrum of the complex after recrystallization with that reported in the literature[6] indicated <5% impurities, presumably aquopentaamminecobalt(III) and free benzoic acid.

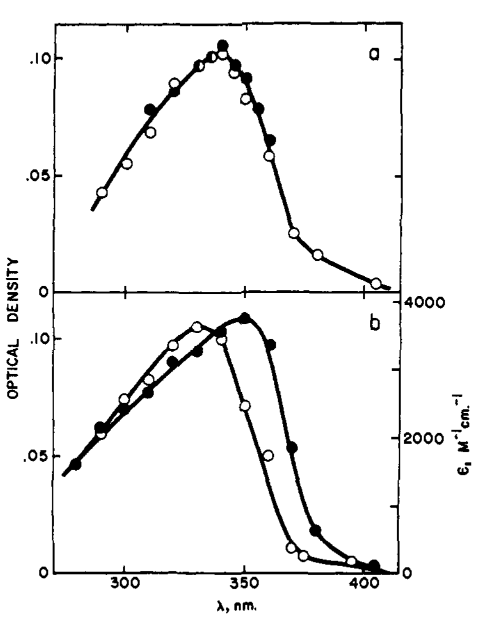

Figure 1. Absorption spectra from the pulse radiolysis of N_2O-saturated solutions: (a) 0.2 m*M* $(NH_3)_5Co(O_2CPh)^{2+}$ at pH 3.1, ●; pH 6.7, O; dose/pulse, ~2.4 krads; (b) 1 m*M* NaO_2CPh at pH 3.1, ●; pH 9.0, O; dose/pulse ~2.4 krads. These pH-dependent spectra are interconvertible at pH 4.4.

The transient absorption spectrum shown in Figure 1a results from the reaction of OH radicals with $(NH_3)_5Co(O_2CPh)^{2+}$. In comparison to this pH-independent spectrum (pH 3–7) with λ_{max} 340 nm, the spectra resulting from OH attack on free benzoate (λ_{max} 330 nm) and benzoic acid (λ_{max} 350 nm) are shown in Figure 1b. These latter two spectra have been assigned to the $\cdot C_6H_5(OH)COO^-$ and $\cdot C_6H_5(OH)COOH$ radicals,[7] respectively, with $pK_a = 4.4$ for the deprotonation of the carboxylate group.[8]

The reaction of OH radicals with the complex can result in three possible modes of attack: (1) addition of OH to the benzoato ligand; (2) dehydrogenation of an ammonia ligand; or (3) direct electron transfer from the metal center to form Co(IV). Comparison of the spectra from coordinated and free benzoate strongly indicates that process 1 predominates. The differences in the spectra indicate that reaction does not immediately cause labilization of the benzoato ligand in the form of its OH adduct. With regard to process 2, OH radicals apparently do not attack NH_4^+ in aqueous solution although NH_3 can be oxidized in alkaline medium;[9] the reaction of OH with free benzoate is very fast.[10] There is no reason to expect a drastic reversal of these rates due to complexation with Co(III). Furthermore, reaction of OH with $Co(NH_3)_6^{3+}$ generates a weak absorption tail in the 250–350-nm region.[11]

The complex radical transient decays *via* second-order kinetics with $2k = 9.3 \times 10^7\ M^{-1}\ sec^{-1}$ (pH independent) compared with $1.2 \times 10^9\ M^{-1}\ sec^{-1}$ for the OH adduct to benzoic acid and $4.4 \times 10^8\ M^{-1}\ sec^{-1}$

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for that of free benzoate. These rate constants are in agreement with those expected from the Debye relationship for the bimolecular reaction of +2, 0, and −1 charged species, respectively, in aqueous solution. The direct electron transfer from Co(III) to OH to generate Co(IV) can be ruled out since such a step would result in a +3 charged transient, the second-order disappearance of which would be expected to have a rate constant of $\sim 10^6\ M^{-1}\ \mathrm{sec}^{-1}$. If the observed decay of the complex radical transient contained a substantial first-order component, $t_{1/2}$ would be greater than 200 μsec in agreement with Taube's conclusion on the basis of his kinetic data[1] that internal electron transfer between the ligand radical and the metal center is not a rapid process. It should be noted that we find that the free benzoate OH adduct is unreactive toward $\mathrm{Co(NH_3)_6}^{3+}$.

On the basis of all the evidence we conclude that the OH radical adds to the benzoato ligand to form $\mathrm{(NH_3)_5Co^{III}(O_2CC_6H_5OH)^{2+}}$. The absorption spectrum of this transient species, resulting from transitions localized on the benzoato OH radical, is intermediate between those for the protonated and anionic forms of the OH adduct to free benzoate in keeping with the intermediate nature of the carboxylate group coordinated to the metal center.

It is of interest to note that $\mathrm{(NH_3)_5Co(O_2CPh)^{2+}}$ reacts readily with H atoms (at pH 1 in the presence of 1 *M* *tert*-butyl alcohol as an OH scavenger) to form an H atom adduct ($\lambda_{\max}$ 350 nm) which appears to be identical with that formed from H atom attack on benzoic acid.[7,8] On the other hand, reaction of the complex with e_{aq}^- does not give the transient with $\lambda_{\max}$ 310 nm obtained from e_{aq}^- addition to free benzoate at pH 7–11;[8] e_{aq}^- evidently reduces Co(III) to Co(II), directly or indirectly, in less than 1 μsec. Substitution of an $\mathrm{NO_2}$ group para on the ligand ring increases the lifetime of the e_{aq}^- adduct sufficiently for its spectrum to be recorded.

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The Use of Proflavin as an Indicator in Temperature-Jump Studies of the Binding of a Competitive Inhibitor to Trypsin

Sir:

The acridine dye proflavin is a strong competitive inhibitor for specific substrates of trypsin[1] and chymotrypsin.[1–4] The binding of dye to either enzyme is accompanied by a substantial shift in the visible absorption spectrum[1,3,5] which serves as a convenient means of detecting complexes between trypsin or chymotrypsin and nonchromophoric substrates and competitive inhibitors. In particular, the displacement of proflavin has been employed to follow the appearance and disappearance of transient intermediates in enzymic catalysis. To date such studies have been limited to stopped-flow measurements far from chemical equilibrium.[1,3,6,7]

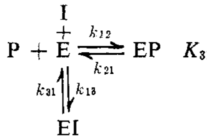
It would also be of interest to use proflavin as an indicator for investigating systems in a state of true or pseudoequilibrium by relaxation techniques. No applications of this kind have yet appeared in the literature, however. In the present communication we report thermodynamic and kinetic parameters for the binding of proflavin to trypsin as measured with the temperature-jump technique.[8] In addition, proflavin has been employed as an indicator to estimate thermodynamic and kinetic parameters for the binding to trypsin of the nonchromophoric competitive inhibitor benzamidine.

Kinetic Relationships. By means of the temperature-jump technique, formation and dissociation rate constants for the enzyme–proflavin complex can in principle be evaluated from the linear relationship of eq 2.[8]

$$\mathrm{E} + \mathrm{P} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{EP} \quad K_{21} = \bar{c}_\mathrm{P}\bar{c}_\mathrm{E}/\bar{c}_\mathrm{EP} \tag{1}$$

$$1/\tau_{12} = k_{12}(\bar{c}_\mathrm{E} + \bar{c}_\mathrm{P}) + k_{21} \tag{2}$$

In the presence of a second competitive inhibitor, I, the minimal reaction scheme which must be considered is



$$\begin{tikzcd} & \mathrm{I} \\ & + \\ \mathrm{P} + & \mathrm{E} \arrow[r, shift left, "k_{12}"] \arrow[d, shift left, "k_{13}"] & \mathrm{EP} \arrow[l, shift left, "k_{21}"] \\ & \mathrm{EI} \arrow[u, shift left, "k_{31}"] \end{tikzcd}$$

$$K_{31} = \bar{c}_\mathrm{E}\bar{c}_\mathrm{I}/\bar{c}_\mathrm{EI} \tag{3}$$

Mechanism 3 is characterized by two relaxation times

$$1/\tau_{1,2} = S/2(1 \mp \sqrt{1 - 4P/S^2}) \tag{4}$$

S and *P* are the trace and determinant of the matrix[8] associated with the linearized rate equations: $S = a_{11} + a_{22}$, $P = a_{11}a_{22} - a_{12}a_{21}$. Choosing δc_EP and δc_EB as variables x_1 and x_2, respectively,[8] the a_{ij} for mechanism **3** are: $a_{11} = k_{12}(\bar{c}_\mathrm{E} + \bar{c}_\mathrm{P}) + k_{21}$, $a_{12} = k_{12}\bar{c}_\mathrm{P}$, $a_{21} = k_{13}\bar{c}_\mathrm{I}$, $a_{22} = k_{13}(\bar{c}_\mathrm{E} + \bar{c}_\mathrm{I}) + k_{31}$. If proflavin binding is rapid enough to equilibrate independently (*i.e.*, $a_{11} = 1/\tau_1 \gg a_{22}$), eq 4 may be expanded,[9] yielding expression 2 for $1/\tau_1$ and

$$\begin{gathered} 1/\tau_2 = k_{13}(\bar{c}_\mathrm{E} + \alpha\bar{c}_\mathrm{I}) + k_{31} \\ \alpha = (\bar{c}_\mathrm{E} + K_{21})/(\bar{c}_\mathrm{E} + \bar{c}_\mathrm{P} + K_{21}) \end{gathered} \tag{5}$$

In a practical application the $\bar{c}$'s would be calculated using K_{21} and K_{31}, and then k_{13} and k_{31} could be obtained graphically from the plot of $1/\tau_2$ *vs.* $(\bar{c}_\mathrm{E} + \alpha\bar{c}_\mathrm{I})$.

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