radical–dimer equilibrium process analogous to reaction 2. Absolute values of the termination rate constant for this radical obeyed the equation

$$2k_t = 10^{12}e^{-8000/RT}\ M^{-1}\ \text{sec}^{-1}$$

with the activation energy in cal mol$^{-1}$.

Trimethylplumbylperoxy radicals prepared in the cryostat in cyclopropane were stable up to the boiling point of the solvent, and there was again no evidence for a radical–dimer equilibrium.

From this rather cursory kinetic investigation, it would appear that radical stabilities decrease in the order $(CH_3)_3PbOO\cdot > (CH_3)_3SnOO\cdot > (CH_3)_3SiOO\cdot$ with the position of $(CH_3)_3GeOO\cdot$ in some doubt. A more comprehensive study of the kinetics of the self-reaction of group IVb organometallic peroxy radicals is being carried out and will be presented in a subsequent paper.

**Acknowledgments.** We thank Messrs. C. P. Rimmer and R. Summers for assistance with the experimental work.

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## Reactions of Coordinated Nucleophiles. Formation of an Amide from a Nitrile

*Sir:*

The extraordinary reactivity of coordinated hydroxide ion and water for intramolecular hydrolysis has been established for coordinated glycine esters[1] and amides.[2] This communication deals with a related addition process at an unsaturated C atom, namely the hydration of a nitrile to an amide.

The treatment of the kinetically robust and purple complex *cis*-$[Co(en)_2(NH_2CH_2CN)Br]^{2+}$ [3] with $Hg_{aq}^{2+}$ rapidly gave an orange solution of $[Co(en)_2glyNH_2]^{3+}$ (~95%). Spectrophotometric studies showed that the rate was first order in $[Hg^{2+}]$ and was independent of $[H^+]$ between 0.09 and 0.89 *M* $HClO_4$ ($k$ = 0.14 $M^{-1}$ sec$^{-1}$, 25°, $\mu$ = 1.2–1.6 *M*, $ClO_4^-$ = 1.1 *M* at 560, 470, and 280 nm). The visible spectrum of the product agreed with that of authentic $[Co(en)_2glyNH_2](NO_3)_2ClO_4\cdot 2H_2O$ [4] ($\epsilon_{487}$ = 104 $M^{-1}$ cm$^{-1}$, 25°, 0.5 *M* $Hg(ClO_4)_2$, 0.1 *M* $HClO_4$) as did the ion-exchange behavior (Dowex 50W-X2, $NaClO_4$ eluent). Two fractions of the product were isolated in the manner described for the authentic material.[4]

*Anal.* Calcd for $[Co(en)_2(gly\text{-}NH_2)](NO_3)_2ClO_4\cdot 2H_2O$: C, 14.05; H, 5.11; N, 21.86. Found: C, 13.8; 14.1; H, 4.8, 5.1; N, 21.7, 21.7.

The product was also characterized by its pmr spectrum and optical rotatory power. The chelated amide was generated *in situ* using $Hg(ClO_4)_2$ (1 *M* in 0.1 *M* $DClO_4$, 0.3 *M* in complex). Under these conditions $Br^-$ removal was complete in <3 min and the first pmr spectrum (~5 min) agreed with that for the authentic amide under the same conditions, and remained unchanged for several hours. Similarly the rotatory dispersion curve of $(-)_{589}$-$[Co(en)_2(NH_2CH_2CN)Br](ClO_4)_2$ ($[M]_{589}$ −580°, $[M]_{436}$ +1250° in 0.1 *M* $HClO_4$) in $Hg(ClO_4)_2$ (1.0 *M*, $HClO_4$ 0.1 *M*) also agreed (within 5%) with that of the amide complex under the same conditions ($[M]_{465}$ +5860°). The rate constants obtained polarimetrically also agreed with the spectrophotometric data. Any question of a coincidence of properties between the chelated amide and aquonitrile complexes was removed by tlc experiments. The free nitrile was separated from ethylenediamine and glycinamide on silica gel plates using *n*-butyl alcohol–HCl (12 *M*)–$H_2O$ (8:1:1) as eluent. With the cobalt complexes, the ligands were released by reducing Co(III) to Co(II) with $NaBH_4$ in acidic solution; $[Co(en)_2(NH_2CH_2CN)Br]^{2+}$ gave a positive test for nitrile under these conditions but the reaction product showed no nitrile. Both experiments were conducted with suitable blanks containing free nitrile and amide. Clearly the amide is produced rapidly after $Br^-$ is removed. The possibility that *cis*-$[Co(en)_2(glyNH_2)(H_2O)]^{3+}$ was initially formed was also excluded. In 2 *M* HBr, no *cis*-$[Co(en)_2(glyNH_2)Br]^{2+}$ was formed after 6 days at 25°. Further, it is known that *cis*-$[Co(en)_2(glyNH_2)(H_2O)]^{3+}$ forms $[Co(en)_2(gly)]^{2+}$ ($t_{1/2}$ ~ 120 sec at 25°) and not chelated $[Co(en)_2glyNH_2]^{3+}$.

Since the actual chelation step was not observed with the aminoacetonitrile complex the analogous aminopropionitrile complex was examined. The expectation was that formation of the larger chelate would be a slower process. Preliminary kinetic studies in the presence of 0.1 *M* $Hg^{2+}$ show that there is a slow reaction ($t_{1/2}$ ~108 min, 25°, $[H^+]$ = 0.1 *M*) following the rapid removal of $Br^-$. This process shows an inverse acid dependence and a term in $[Hg^{2+}]^1$ concentration. The observed spectral change is characteristic of a cis aquo complex going to a chelated product ($\epsilon_{488}$ ~80 to $\epsilon_{492}$ 123, 0.1 *M* $Hg^{2+}$, 0.1 *M* $H^+$). Ion exchange analysis indicates that the final product has a 3+ charge and the visible spectrum is characteristic of the type of product expected ($\epsilon_{max}$ at 492 and 351 nm). The products at various stages of the reaction were identified by tlc. When the initial product from the $Hg^{2+}$-catalyzed reaction was analyzed, no amide was found, only nitrile. However, after several days the product contained only amide and no nitrile. Heating this product with HBr gave no bromo complex which also indicates the chelate had formed. In concentrated HBr, however, the bromonitrile complex was slowly converted to the bromomonodentate-β-alaninamide complex (~2 days for completion).

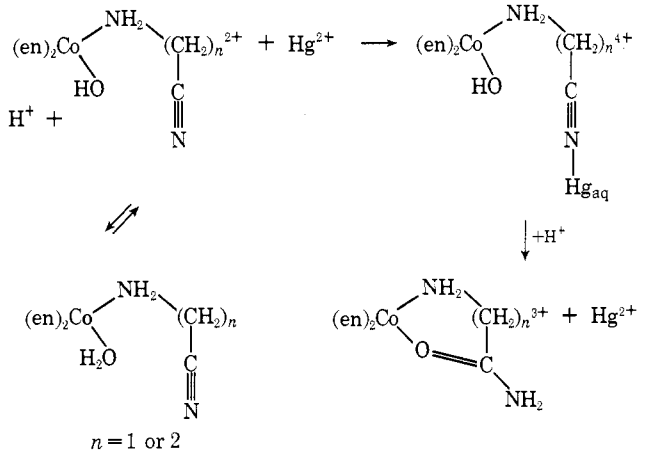
The order of events for the overall process for both complexes appears to be the following: (1) $Hg^{2+}$-assisted removal of $Br^-$; (2) rapid entry of solvent into the vacant coordination site; (3) $Hg^{2+}$ assisted nucleophilic attack by bound $OH^-$ at the nitrile to give the chelated amide.

Subsequently the *cis*-aquoaminopropionitrile complex was prepared and its kinetic behavior is in agreement with the data obtained from the cis bromo complex $[Co(en)_2(NH_2CH_2CH_2CN)(H_2O)](ClO_4)(NO_3)_2$. *Anal.*

(1) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **91**, 4102 (1969).
(2) D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *ibid.*, **92**, 6151 (1970).
(3) Abbreviations used are: glycinamide ($glyNH_2$); chelated glycinate anion (gly); ethylenediamine (en).
(4) D. A. Buckingham, C. E. Davis, D. M. Foster, and A. M. Sargeson, *J. Amer. Chem. Soc.*, **92**, 5571 (1970).

Calcd: C, 17.13; H, 4.93; N, 22.84; Cl, 7.22. Found: C, 16.79; H, 4.99; N, 22.71; Cl, 7.47.

The results are rationalized in the following mechanism where the electrophile $Hg^{2+}$ adds to the nitrile N atom and thereby assists the attack of the nucleophile–Co–OH.



$n = 1$ or 2

From published data[5] for propionitrile the specific rates for base-catalyzed hydration of free $NH_2CH_2CN$ and $NH_2CH_2CH_2CN$ are estimated as $\sim 10^{-6}$ $M^{-1}$ $sec^{-1}$; the specific rates for the corresponding hydroxocobalt(III) complexes assisted by $Hg^{2+}$ (0.1 $M$) are estimated as $>10^5$ $M^{-1}$ $sec^{-1}$ and $10^2$ $M^{-1}$ $sec^{-1}$ at 25°, respectively.[6] Since there is a linear dependence upon $Hg^{2+}$ up to at least 0.5 $M$ $Hg^{2+}$, the $[Co(en)_2(OH)(RCNHg]^{4+}$ species is far from saturation and its specific rate must therefore be greater than the estimates above. This implies that there is a rate enhancement of $>10^{18}$ and $>10^{15}$ for the complexed aminonitriles at pH 7 relative to the uncoordinated nitriles. These enhancements might be compared with those in previous studies ($\sim 10^8$) using labile metal ion complexes.[7-9] Part of this acceleration is attributed to the $Hg^{2+}$ addition, which enhances the attack of the nucleophile, and part must be attributed to the "intramolecular chelation." Factors of $10^8$ or more have been observed for the latter effect relative to uncoordinated substrates. Moreover the formation of five-membered rings appears to be generally faster than their six-membered counterparts in analogous circumstances.[1,2,10] This last pattern is also preserved in this instance.

(5) B. S. Rabinovitch and C. A. Winkler, *Can. J. Res., Sect. B*, **20**, 185 (1942).

(6) $k_{obsd} = kK_a[OH^-][Hg^{2+}]/K_w$; $pK_a \simeq 6$; $[H]^+ = 0.1$ $M$; and $[Hg^{2+}] = 0.1$ $M$; $k_{obsd} > 0.14$ $sec^{-1}$ for the aminoacetonitrile complex and $10^{-4}$ $sec^{-1}$ for the aminopropionitrile complex.

(7) R. Breslow, R. Fairweather, and J. Keana, *J. Amer. Chem. Soc.*, **89**, 2135 (1967).

(8) P. F. B. Barnard, *J. Chem. Soc. A*, 2140 (1969).

(9) K. Sakai, T. Ito, and K. Watanabe, *Bull. Chem. Soc. Jap.*, **40**, 1660 (1967).

(10) D. A. Buckingham, E. Baraniak, and A. M. Sargeson, unpublished work.

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## Hollow Fiber Enzyme Reactors

*Sir:*

Enzymes have been physically adsorbed on surfaces by electrostatic interactions, cross-linked around a carrier, entrapped within a gel lattice, covalently attached to porous and nonporous solids,[1-4] and encapsulated within semipermeable microcapsules.[5,6] Such procedures facilitate enzyme separation from products, both in batch and continuous operation, and in certain cases partially stabilize the enzyme against thermal denaturation. We wish to report what are apparently the first successful experiments in which enzymes are contained in (and, in effect, "insolubilized" within) semipermeable hollow fibers.[7,8]

In experiments designed to demonstrate the feasibility of the concept, the fiber bundle within a Dow Chemical Co. beaker dialyzer b/HFD-1 was filled with a 0.1 mg/ml of solution of alkaline phosphatase (Worthington Biochemical Corp. BAPC) in 0.05 $M$ Tris at pH 8.0 and then sealed with a pair of eyedropper bulbs. A solution of disodium 4-nitrophenyl phosphate (100 ml) in 0.05 $M$ Tris at pH 8.0 was then rapidly added to the well-stirred dialyzer. The initial rate of reaction was monitored at 410 nm with the aid of a Beckman DB-G uv–visible spectrophotometer and a Technicon pump that recirculated the external solution through a 1-cm flow-through cell. After the initial rate of reaction was determined, the 100 ml of solution external to the fiber bundle was rapidly poured into a separate 150-ml beaker and continuously monitored at 410 nm for indications of enzyme leakage. At the completion of each experimental run, the hollow fiber bundle was dialyzed with copious amounts of deionized water and 4 × 100 ml of 0.05 $M$ Tris at pH 8.0. In separate experiments, the activity of the alkaline phosphatase was determined to be 5.4 μmol of 4-nitrophenol liberated per minute per milligram of enzyme for a solution that was $1.06 \times 10^{-3}$ $M$ in substrate and 0.90 $M$ in Tris at pH 8.0 (approximately 5.4 units/mg according to the Worthington assay).

The initial reaction rate was directly proportional to the substrate concentration over the entire range of substrate concentrations studied—36–640 μ$M$. The pseudo-first-order "rate constant" determined from a plot of initial rates *vs.* substrate concentration was $1.19 \times 10^{-2}$ $min^{-1}$. This result is in distinct contrast to the behavior of alkaline phosphatase in the absence of hollow fibers, under which conditions the reaction is normally zero order at substrate concentration levels above 180 μ$M$.

The limiting rate of permeation of the substrate–product system through the fiber walls was determined by increasing the amount of alkaline phosphatase within the fiber bundle to 20 mg. At such high enzyme concentrations, the hollow fiber beaker functioned as a *reactor dialyzer*, with the measured pseudo-first-order rate constant being approximately[9] equal to the permeation rate of the substrate under normal dialysis condi-

(1) L. Goldstein in "Fermentation Advances," D. Perlman, Ed., Academic Press, New York, N. Y., 1969, p 391.

(2) R. G. Carbonell and M. D. Kostin, *AIChE J.*, **18**, 1 (1972).

(3) L. Goldstein and E. Katchalski, *Fresenius Z. Anal. Chem.*, **243**, 375 (1968).

(4) I. H. Silman and E. Katchalski, *Annu. Rev. Biochem.*, **35**, 873 (1966).

(5) T. M. S. Chang, *Science*, **146**, 524 (1964).

(6) T. M. S. Chang, *Sci. J.*, **3** (7), 62 (1967).

(7) P. R. Rony, *Biotechnol. Bioeng.*, **13**, 431 (1971).

(8) P. R. Rony, U.S. patent pending.

(9) In the experiments reported in this paper, the increase in product concentration was monitored spectrophotometrically as a function of time. For the b/HFD-1 beaker to accurately function as a reactor dialyzer, the substrate concentration must instead be monitored.