spectrum with stepwise addition of $Eu(fod)_3$ reagent. At various compound-reagent ratios, the complex bands between δ 6.5 and 8.3 became resolved into one-proton bands and were interrelated by spin-decoupling. Interpretation of these data allows the following assignments to be made for the unshifted spectrum: δ 2.16, 3 H, s (C-1); 6.02, 1 H, d, J = 15 Hz (C-3); 7.00, 1 H, dd, J = 15, 9 (C-4); ~6.3, 1 H, dd, J = 14, 9 (C-5); ~6.3, 1 H, dd, J = 14, 11 (C-6); ~6.3, 1 H, dd, J = 14, 11 (C-7); ~6.3, 1 H, dd, J = 14, 10 (C-8); 6.79, 1 H, dd, J = 14, 10 (C-9); 6.50, 1 H, d, J = 14 (C-10); 8.45, 1 H, s, (C-2'); 8.29, 1 H, d, J = 5 (C-6'); 7.09, 1 H, dd, J = 8, 5 (C-5'); 7.59, 1 H, d, J = 8 (C-4'). Since all olefin protons exhibit couplings of 14 or 15 Hz, the olefins of the conjugated tetraene system in **1** must all be *E*. Also, the multiplicities and coupling constants characteristic of an aromatic ABXY system rigorously show the side chain to be β-substituted (C-3') on the pyridine ring.

Navenone B (**2**), mp 125–140 °C dec (CH_2Cl_2), analyzed for $C_{16}H_{16}O$ by mass spectrometry (obsd 224.1204; calcd 224.1201) and illustrated spectral features which related this metabolite to navenone A: UV $\lambda_{max}^{CH_3OH}$ 389 nm, ϵ 18 000; IR ($CHCl_3$) 1670, 1640–1550 cm^{-1}. The ^{13}C NMR spectrum was also in close accord with **1**, showing bands at 175.5, 143.1, 141.5, 137.5, 135.4, 132.3, 130.6, 129.9, 128.8, 128.6, 128.2, 127.9, 127.6, 126.7, 125.6, and 27.4 ppm. The proton NMR spectrum showed bands analogous to **1** which could be partially assigned by spin-decoupling experiments on the unshifted spectrum: δ 2.25, 3 H, s (C-1); 6.14, 1 H, d, J = 15 Hz (C-3); 7.17, 1 H, dd, J = 15, 11 Hz (C-4); 6.32–6.70, 4 H, m (C-5–C-8); 6.86, 1 H, dd, J = 15, 10 Hz (C-9); 6.66, 1 H, d, J = 15 (C-10); 7.18–7.45, 5 H, m (aromatics).

Navenone C (**3**), $C_{16}H_{16}O_2$, M^+ *m/e* 240, UV $\lambda_{max}^{CH_3OH}$ 412 nm, ϵ 11 400, base shift $\lambda_{max}^{CH_3OH}$ 432 nm, ϵ 12 500, was difficult to fully purify as the free phenol. Acetylation (Ac_2O/py) gave navenone C acetate, mp 135–137 °C (Bz), which was easily purified and gave the following spectral features: IR ($CHCl_3$) 1735 (OAc), 1630, 1620–1560 cm^{-1}, proton NMR (220 MHz, $CDCl_3$) δ 2.16, 3 H, s (OAc); 2.26, 3 H, s (C-1); 6.14, 1 H, d, J = 15 Hz (C-3); 7.27, 1 H, dd, J = 15, 11 Hz (C-4); 6.3–6.7 (m, C-5–C-8); 6.79, 1 H, dd, J = 15, 10 Hz (C-9); 6.61, 1 H, d, J = 15 Hz (C-10); 7.02, 2 H, d, J = 8 Hz (C-3', C-5'); 7.32, 2 H, d, J = 8 Hz (C-2', C-6'). Para disubstitution was clearly indicated for the acetate of **3** by the pair of two-proton doublets (a simplified AA'BB' pattern) at 7.02 and 7.32 ppm, respectively.

For navenones B and C, olefin-proton coupling constants for C-5 through C-8 could not be measured, and hence the stereochemistries of olefins of the central diene cannot be rigorously assigned. However, the complex bands for these protons are virtually superimposable on those of **1** and indicate similar couplings from an all *E* configuration, as in navenone A.

Acknowledgments. This work is a result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant no. 04-6-158-44021, RM/P-7. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. We wish to gratefully acknowledge our use of the NMR facility, Chemistry Department, University of California, San Diego, Calif., supported under NIH Grant RR-708.

References and Notes

(1) J. G. Cooper, *Proc. Calif. Acad. Nat. Sci.*, **2**, 202 (1862).
(2) R. T. Paine, *Veliger* **6** (1), 1 (1963).
(3) A. M. Keen, "Marine Molluscan Genera of Western North America", Stanford University Press, Stanford, Calif., 1963.
(4) R. T. Paine, *Ecology*, **46** (5), 603 (1965).
(5) M. J. Murray, and E. R. Lewis, *Veliger*, **17** (2), 156 (1974).
(6) G. M. Blair, and R. R. Scapy, *Veliger*, **15** (2), 119 (1972).
(7) An encompassing discussion of marine allelochemics is given in: J. S. Kittredge, F. T. Takahashi, J. Lindsey, and R. Lasker, *Fish. Bull.*, **72** (1), 1 (1974).
(8) The avoidance response of *Navanax* is most effectively produced from a 4:2:1 mixture of navenones A–C, which is the ratio found in the natural secretion. Complete details of the bioassay results for individual compounds will be published elsewhere.
(9) D. J. Pasto and C. R. Johnson, "Organic Structure Determination", Prentice-Hall, Englewood Cliffs, N.J., 1969, p 106.

Howard L. Sleeper, William Fenical*
Institute of Marine Resources
Scripps Institution of Oceanography
La Jolla, California 92093
Received January 11, 1977

Biosynthesis of *Cephalotaxus* Alkaloids. 3. Specific Incorporation of Phenylalanine into Cephalotaxine[1]

Sir:

Conifers of the genus *Cephalotaxus* elaborate a group of novel alkaloids, the most abundant of which is cephalotaxine (**1**) (Scheme I).[2] We recently reported experiments indicating that two molecules of the amino acid tyrosine (**2**) are incorporated into **1** in an unusual manner.[3] We now describe the results of additional experiments which further clarify the mode of tyrosine incorporation into cephalotaxine and demonstrate that phenylalanine is also a specific precursor of this alkaloid.

It has been previously shown[3] that administration of side-chain-labeled tyrosine to *C. harringtonia* plants for an 8-week period yields radioactive cephalotaxine. Degradations of the labeled samples of **1** obtained from these experiments disclosed a labeling pattern consistent with the derivation of carbons 10–17[4] of cephalotaxine from one molecule of tyrosine. However, the labeling pattern also indicated that tyrosine was incorporated into the C/D ring system of the alkaloid in an unexpected manner. In order to account for these observations, it was hypothesized that a second molecule of tyrosine served as a precursor of the C/D ring system of the alkaloid via a route involving cleavage of the aromatic ring of the amino acid. This hypothesis predicted that carbon atoms 1–3 and 6–8 of **1** should be derived from the aromatic ring of tyrosine. This prediction has now been tested, and the results are summarized in Table I (expt 1, 2).

[*ring*-^{14}C]-L-Tyrosine[5] was administered to *C. harringtonia*[6] for 8 weeks and radioactive cephalotaxine was obtained. Permanganate oxidation[3] of the labeled alkaloid gave 4,5-methylenedioxyphthalic acid which was degraded[7] to the an-

Scheme I

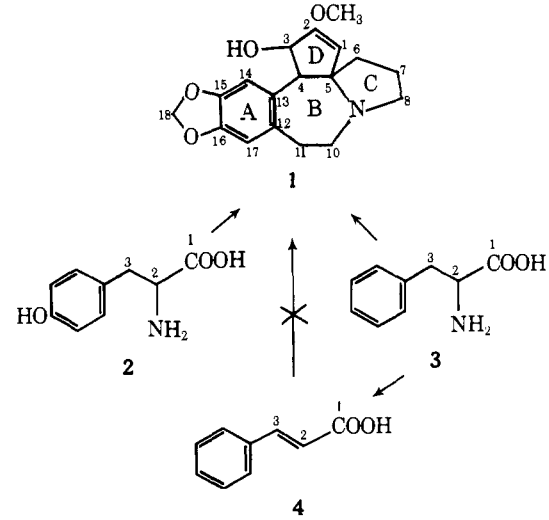

Table I. Feeding Experiments with *Cephalotaxus harringtonia*

Expt no.	Precursor fed to *Cephalotaxus*	Feeding period (weeks)	% incorporation	Dist. of activity in cephalotaxine
1	[*ring*-^{14}C]-L-Tyrosine	8	0.11	90% in C-11(4) to C-18
2	[*p*-^{14}C]-L-Tyrosine	8	0.06	93% in C-4, C-11 to C-18
3	[2-^{14}C]-DL-Tyrosine	2	0.04	49% at C-10[9]
4	[2-^{14}C]-DL-Tyrosine	1	0.003	63% at C-10[9]
5	[1-^{14}C]-DL-Phenylalanine	2	0.02	84% at C-8
6	[1-^{14}C]Cinnamic acid	2	0.004	9% at C-8
7	[1-^{14}C]Cinnamic acid	2	0.006	3% at C-8
8	3(*RS*)-[3-^{3}H-2,^{14}C]-DL-Phenylalanine, ^{3}H:^{14}C = 4.55	2	0.01	^{3}H:^{14}C = 2.12

thranilic acid. The anthranilic acid carried 90% of the total radioactivity of **1**. A second experiment with [*p*-^{14}C]-L-tyrosine[5] produced a similar result: 93% of the total radioactivity of the cephalotaxine was present in the 4,5-methylenedioxyphthalic acid obtained by permanganate oxidation. These results are inconsistent with the previously suggested hypothesis. However, they do support an alternative explanation for the unusual labeling pattern observed when side-chain-labeled tyrosine is incoporated into cephalotaxine. The alternative explanation requires that tyrosine be catabolized in *Cephalotaxus* plants in a manner analogous to that observed in some microorganisms,[8] namely, by cleavage of the amino acid between the side chain and the aromatic ring to give a C_6 unit and a C_3 unit. If such a cleavage occurs and if the C_3 unit, but not the C_6 unit, is incorporated into rings C and D of **1**, then the results observed with both side-chain- and ring-labeled forms of tyrosine can be understood.

Additional evidence favoring the above explanation has been obtained by administration of [2-^{14}C]-DL-tyrosine to *Cephalotaxus* plants for periods of less than 8 weeks, followed by degradation[3] of the radioactive samples of cephalotaxine to isolate C-10. The results of these experiments (Table I, expt 3, 4) clearly show that decreasing the length of the feeding period has two effects: it lowers the levels of tyrosine incorporation into **1** and it *raises* the percent of the total radioactivity in the alkaloid which is present at C-10. Thus, the cephalotaxine obtained from [2-^{14}C]-DL-tyrosine after a 1-week feeding period carries 63% of the total label at C-10 (expt 4).[9] In contrast, the radioactive alkaloid obtained from the same form of labeled tyrosine after an 8-week feeding carries only 37% of the label at C-10.[3,9] Presumably, the use of still shorter feeding periods would further increase the specificity of C-10 labeling of **1** by [2-^{14}C]tyrosine, but such experiments are not likely to be practical due to extremely low levels of tyrosine incorporation.

When it became apparent that the incorporation of radioactivity from tyrosine into the C/D ring system of **1** was the result of catabolism of the amino acid, it was then necessary to consider possible alternative precursors of that portion of the cephalotaxine skeleton. The most likely precursor appeared to be phenylalanine (**3**) (Scheme I) since homerythrina alkaloids occur in *Cephalotaxus*.[10-12] During the early stages of our investigations, we had examined the potential role of **3** as a cephalotaxine precursor and had obtained very low incorporation figures. In the interim, however, we had developed better techniques for the administration of precursors to *Cephalotaxus*, and the possible role of phenylalanine in the biosynthesis of **1** was therefore reexamined. If cephalotaxine is a modified phenethylisoquinoline alkaloid, one might expect the alkaloid to be formed from phenylalanine in such a manner that C-8 of the alkaloid is derived from C-1 of the amino acid.[11,12] The results obtained by administration of [1-^{14}C]-DL-phenylalanine to *C. harringtonia* confirm this expectation (Table I, expt 5): degradation[3] of the radioactive alkaloid revealed that 84% of the total radioactivity was present at C-8. This observation provides substantial evidence that cephalotaxine is a member of the family of phenethylisoquinoline alkaloids which includes the *Schelhammera* and *Colchicum* alkaloids, and it suggests that ring D of **1** is derived from the aromatic ring of phenylalanine by loss of one carbon atom.[10]

It has been established[11] that the C_6C_3 unit of colchicine is derived from phenylalanine via cinnamic acid (**4**) (Scheme I). Consequently, additional proof that cephalotaxine is derived from phenylalanine in the manner anticipated for a phenethylisoquinoline alkaloid was sought by administration of [1-^{14}C]cinnamic acid to *Cephalotaxus*. Radioactive alkaloid was obtained in these experiments (Table I, expt 6, 7), but, to our surprise, very little radioactivity was present at C-8, where expected. The apparent inability of cinnamate to serve as a specific precursor of **1** raised the question of the nature of the steps associated with the loss of the amino group from the side chain of phenylalanine during its conversion to **1**. If the amino group were removed by elimination of ammonia, as occurs in the conversion of phenylalanine to cinnamic acid,[13] then a stereospecific removal of one hydrogen atom from C-3 of the amino acid should take place.[13] Administration of 3(*RS*)-[3-^{3}H,2-^{14}C]-DL-phenylalanine to *Cephalotaxus* demonstrated that such a process indeed occurs (Table I, expt 8): the tritium to carbon-14 ratio in the radioactive alkaloid corresponded to a 54% loss of tritium (expected loss equals 50%). This result suggests that an intermediate with α,β-unsaturation probably lies on the pathway between phenylalanine and cephalotaxine. On the other hand, the possibility that hydrogen is lost from C-3 of the amino acid by an exchange process[14,15] which is unrelated to cephalotaxine biosynthesis cannot be ruled out at present. Investigations of these and other aspects of cephalotaxine biosynthesis are continuing.

Acknowledgment. We wish to thank Dr. Robert E. Perdue for supplying us with *Cephalotaxus* plants and Dr. B. E. Ellis for a culture of *Escherichia intermedia*. Financial support from the National Institutes of Health (GM-19220) is gratefully acknowledged as is support from the National Science Foundation (GU 3852, GP 37156) which allowed purchase of a Bruker WH-90 NMR spectrometer used in this work.

References and Notes

(1) For the previous paper in this series, see R. J. Parry, D. D. Sternbach, and M. D. Cabelli, *J. Am. Chem. Soc.*, **98**, 6380 (1976).
(2) See S. M. Weinreb and M. F. Semmelhack, *Acc. Chem. Res.*, **8**, 155 (1975), and references cited therein.
(3) R. J. Parry and J. M. Schwab, *J. Am. Chem. Soc.*, **97**, 2555 (1975).
(4) The numbering used in this communication conforms to that now accepted for the cephalotaxane ring system.
(5) B. E. Ellis, G. Major, and M. H. Zenk, *Anal. Biochem.*, **53**, 470 (1973).
(6) Feeding experiments were conducted in a Lab-line Biotronette Mark III environmental chamber.
(7) E. Leete, *J. Am. Chem. Soc.*, **85**, 473 (1963).
(8) H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi, and K. Ogata, *J. Biol. Chem.*, **245**, 1767 (1970).
(9) The figures for the [2-^{14}C]tyrosine feedings have been corrected for dilution of the radioactive formaldehyde derived from C-10 by radioinactive formaldehyde which is formed from *N*-methyl groups of the methine during osmate–periodate oxidation; see A. R. Battersby and R. J. Parry, *Chem. Commun.*, 901 (1971).
(10) R. G. Powell, *Phytochemistry*, **11**, 1467 (1972).
(11) A. R. Battersby, R. Binks, J. J. Reynolds, and D. A. Yeowell, *J. Chem. Soc.*,

4257 (1964).
(12) A. R. Battersby, E. McDonald, J. A. Milner, S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Tetrahedron Lett.*, 3419 (1975).
(13) R. H. Wightman, J. Staunton, A. R. Battersby, and K. R. Hanson, *J. Chem. Soc. Perkin Trans. 1*, 2355 (1972).
(14) N. Johns, G. W. Kirby, J. D. Bu'Lock, and A. P. Ryles, *J. Chem. Soc. Perkin Trans. 1*, 383 (1975).
(15) G. W. Kirby and S. Narayanaswami, *J. Chem. Soc. Perkin Trans. 1*, 1564 (1976).
(16) Recipient of a Public Health Servvice Research Career Development Award (5 K04-GM-00143) from The National Institute of General Medical Sciences, 1975–1980.

John M. Schwab, Michael N. T. Chang, Ronald J. Parry*[16]
Department of Chemistry, Brandeis University
Waltham, Massachusetts 02154
Received December 6, 1976

Addition of Superoxide Radical Anion to Cobalt(II) Macrocyclic Complexes in Aqueous Solution

Sir:

The superoxide radical anion, $\cdot O_2^-$, is apparently of immense consequence in biological systems, its elimination being catalyzed by superoxide dismutase, SOD, a metalloenzyme.[1] The uncatalyzed disproportionation reaction

$$2\cdot O_2^- \xrightarrow{2H^+} H_2O_2 + O_2$$

is very slow ($k \sim 10^2\ M^{-1}\ s^{-1}$) although the corresponding reaction of $\cdot O_2^-$ with its conjugate acid, $\cdot O_2H$ (pK_a 4.8), is quite rapid ($k = 8.5 \times 10^7\ M^{-1}\ s^{-1}$).[2] The reported aqueous chemistry of $\cdot O_2^-$ has been exclusively oxidation-reduction[3,4] although there remains a controversy about the possible deleterious effects of $\cdot O_2^-$ in complex biochemical systems inasmuch as simple reversible redox reactions cannot lead to biological damage.[3] To date, no evidence has been advanced for nonredox reactions of $\cdot O_2^-$ in aqueous solution although some have been proposed in the past.[5] In this paper we report the addition of $\cdot O_2^-$ to some Co(II) complexes containing macrocyclic ligands as demonstrated by use of the fast kinetics pulse radiolysis technique.

The pulse radiolysis apparatus (time resolution < 1 μs) at the U.S. Army Natick Laboratories[6] and the radiolysis technique for generating selected free radicals[7] have been amply described in detail. The macrocyclic complexes used in this study, $[Co(4,11\text{-dieneN}_4)(H_2O)_2^{2+}$, $Co(1,3,8,10\text{-tetraeneN}_4)(H_2O)_2^{2+}$, $Co(1,3,8,10\text{-tetraeneN}_4)(H_2O)_2^{3+}]$,[8] were available from our laboratory reserves.[9]

In contrast to many Co(II) chelates which are sensitive to O_2, the Co(II) macrocyclic complexes used in this study are stable for modest periods of time in oxygenated neutral solution; the spectra of the complexes were not affected by the presence of O_2 during the time required (~30 min) to perform the experiments. The radiolysis of these solutions generates $\cdot O_2^-$ from the fast reactions of e_{aq}^- and H atoms with O_2; the presence of *tert*-butyl alcohol ensures the scavenging of OH radicals. Alternatively, all the primary radicals can be converted to $\cdot O_2^-$ in the presence of HCO_2^- and O_2.[3]

$$e_{aq}^- + O_2 \rightarrow \cdot O_2^- \qquad (k = 2.2 \times 10^{10}\ M^{-1}\ s^{-1})^{10}$$

$$H + O_2 \rightarrow \cdot O_2H \qquad (k = 2.2 \times 10^{10}\ M^{-1}\ s^{-1})^{11}$$

$$OH + (CH_3)_3COH \rightarrow \cdot CH_2C(CH_3)_2OH + H_2O \qquad (k = 5.2 \times 10^8\ M^{-1}\ s^{-1})^{12}$$

The reaction of both Co^{II} macrocyclic complexes with $\cdot O_2^-$ at pH 7–8 yielded the spectra of transient intermediates (Figure 1). From the formation kinetics of the transient spectra, the rate constants for reaction of $\cdot O_2^-$ with $Co^{II}(4,11\text{-dieneN}_4)$ and $Co^{II}(1,3,8,10\text{-tetraeneN}_4)$ $(CoN_4)^{2+}$ were determined to be 1.4×10^9 and $1.6 \times 10^9\ M^{-1}\ s^{-1}$, respectively. The magnitude of these rate constants rules out the participation of $\cdot O_2H$ in these reactions at the pH values of the experiments. The same spectral intermediates were obtained irrespective of the method of generating the $\cdot O_2^-$ radical. The spectrum of $\cdot O_2^-$ is also given[2] in Figure 1 for comparison. The spectra of the transient species are unquestionably not those of the corresponding Co(I)[9] or Co(III)[13] complexes thereby ruling out $\cdot O_2^-$ as a simple electron transfer agent in its reaction with Co(II). We conclude that $\cdot O_2^-$ adds to the metal center at a labile axial site[14] giving rise to a complex possessing the observed intense charge transfer bands.

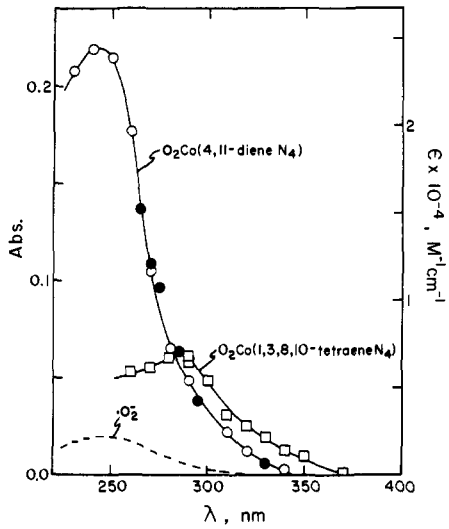

Figure 1. Transient absorption spectra produced in the pulse radiolysis of: ○, 5×10^{-5} M $Co^{II}(4,11\text{-dieneN}_4)$, 1.3×10^{-3} M O_2, 0.25 M *tert*-butyl alcohol at pH 7.1 ($Co(II) + \cdot O_2^-$ reaction): □, 4×10^{-5} M $Co^{II}(1,3,8,10\text{-tetraeneN}_4)$, 1.3×10^{-3} M O_2, 0.25 M *tert*-butyl alcohol at pH 8.0 ($Co(II) + \cdot O_2^-$ reaction); ●, 4×10^{-4} M $Co^{II}(4,11\text{-dieneN}_4)$, 5.6×10^{-5} M O_2, 0.5 M *tert*-butyl alcohol at pH 7.1 ($Co(I) + O_2$ reaction). Dose/pulse = 1.6 krad; optical path = 2 cm. The spectrum of $\cdot O_2^-$ is shown for comparison (- - -).

$$\cdot O_2^- + CoN_4^{2+} \rightarrow O_2CoN_4^+ \qquad (1)$$

It is not possible at present to establish if the products of reaction 1 are Co(II)-superoxy or Co(III)-peroxy complexes. In any event, the spectra of the adduct intermediates are not stable; they decay into more weakly but similarly absorbing species. The $O_2Co(4,11\text{-dieneN}_4)$ complex decays with $t_{1/2} \sim 1$ min with kinetics that are uncharacterizable due to the slow rate and small absorbance changes. The $O_2Co(1,3,8,10\text{-tetraeneN}_4)$ complex decayed more rapidly in a decidedly second-order manner ($k = 2.1 \times 10^3\ M^{-1}\ s^{-1}$); the formation of a binuclear μ-complex as the final product is a distinct possibility.

Figure 1 also shows that the spectrum of the $O_2Co(4,11\text{-dieneN}_4)$ complex also arises from the reaction of $Co^I(4,11\text{-dieneN}_4)$ with O_2. Under the experimental conditions shown, e_{aq}^- reduces Co(II) to Co(I) ($k = 4.4 \times 10^{10}\ M^{-1}\ s^{-1}$) and O_2 subsequently reacts with the Co(I) complex ($k_2 = 1.7 \times 10^9\ M^{-1}\ s^{-1}$).[9] Thus, the O_2-addition mechanism suggested previously[9] for Co^I-macrocyclic complexes is now unequivocally established:

$$O_2 + CoN_4^+ \rightarrow O_2CoN_4^+ \qquad (2)$$

The magnitude of k_2 and the time frame for reaction 2 (10–30 μs) rule out dimer formation as a primary process even if it is assumed that $k \sim 10^9\ M^{-1}\ s^{-1}$ for the dimerization of the monomer.

In contrast, O_2 does not form an adduct to Co_{aq}^+, but rather oxidizes it, and $\cdot O_2^-$ does not react with Co_{aq}^{2+}.[15] We have