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A Photochemical Precursor for Carbon Monoxide Release in Aerated Aqueous Media

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Interest in therapeutic applications of carbon monoxide release to physiological targets has led us to explore a photochemical strategy for such CO delivery. Here, we describe the photoactivated carbon monoxide releasing moiety (photoCORM), W(CO)$_5$(TPPTS)$^{3-}$ (**1**), an air-stable, water-soluble tungsten(0) carbonyl complex of the trianionic ligand tris(sulphonatophenyl)phosphine. Near-UV photolysis of **1** in an aqueous buffer solution leads to the high quantum yield release of a single CO, the formation of which has been verified by three analytical methodologies. Furthermore, in aerated media, additional CO is slowly released from the W(CO)$_4$(H$_2$O)(TPPTS)$^{3-}$ photoproduct owing to autoxidation of the tungsten center. Thus, **1** serves as a carbon monoxide releasing moiety both in the primary photochemical reaction and in the secondary reactions of the initially formed photoproduct. The three methodologies for quantifying CO release under these physiologically relevant conditions are also described.

Introduction

The potential therapeutic effects of carbon monoxide, demonstrated to be cytoprotective during inflammation, to promote wound healing, and to reduce transplant rejection,[1] have generated considerable interest in CO delivery mechanisms.[2] In mammals, CO production is estimated to be comparable to that of nitric oxide,[3] for which the biological signaling roles are better understood. The primary endogenous sources of CO are the heme oxygenases that play key roles in heme catabolism.[4] Demonstrations that exogenously applied CO is therapeutic prompted the synthesis of new compounds that slowly release CO once introduced into the organism, and such carbon monoxide releasing molecules (CORMs)[2] have indeed been shown in animal studies to alleviate damage in ischemia/reperfusion injury in various organs and tissues.[5]

A fundamental problem with any drug delivery system is specific, quantitative delivery, that is, how to trigger the bioactive substance in the targeted tissues and how to control the amount released. These issues can be addressed by employing a photochemical strategy, since photoinduced release allows one to control the location, timing, and dosage of the therapeutic agent generated.[6] We and others are pursuing similar strategies with respect to NO release.[7] The problems encountered in those studies should be similar, and

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(1) (a) Otterbein, L. E. *Antioxid. Redox Signaling* **2002**, *4*, 309–319. (b) Zuckerbraun, B. S.; Billiar, T. R.; Otterbein, S. L.; Kim, P. K. M.; Liu, F.; Choi, A. M. K.; Bach, F. H.; Otterbein, L. E. *J. Exp. Med.* **2003**, *198*, 1707–1716. (c) Kim, H. P.; Ryter, S. W.; Choi, A. M. K. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 411–449. (d) Seda Neto, J.; Nakao, A.; Kimizuka, K.; Romanosky, A. J.; Stolz, D. B.; Uchiyama, T.; Nalesnik, M. A.; Otterbein, L. E.; Murase, N. *Am. J. Physiol.* **2004**, *287*, F979–F98982. (e) Nakao, A.; Kaczorowski, D. J.; Sugimoto, R.; Billiar, T. R.; McCurry, K. R. *J. Clin. Biochem. Nutr.* **2008**, *42*, 78–88. (f) Bilban, M.; Haschemi, A.; Wegiel, B.; Chin, B. Y.; Wagner, O.; Otterbein, L. E. *J. Mol. Med.* **2008**, *86*, 267–279.

(2) (a) Motterlini, R.; Clark, J. E.; Foresti, R.; Sarathchandra, P.; Mann, B. E.; Green, C. *J. Circulation Res.* **2002**, *90*, e17–e24. (b) Johnson, T. R.; Mann, B. E.; Clark, J. E.; Foresti, R.; Green, C. J.; Motterlini, R. *Angew. Chem., Int. Ed.* **2003**, *42*, 3722–3729. (c) Sandouka, A.; Balogun, E.; Foresti, R.; Mann, B. E.; Johnson, T. R.; Tayem, Y.; Green, C. J.; Fuller, B.; Motterlini, R. *Cell. Mol. Biol.* **2005**, *51*, 425–432. (d) Alberto, R.; Motterlini, R. *Dalton Trans.* **2007**, 1651–1660. (e) Foresti, R.; Bani-Hani, M. G.; Motterlini, R.. *Intensive Care Med.* **2008**, *34*, 649–658.

(3) Watts, R. N.; Ponka, P.; Richardson, D. R. *Biochem. J.* **2003**, *369*, 429–440.

(4) (a) Ryter, S. F.; Alam, J.; Choi, A. M. K. *Physiol. Rev.* **2006**, *86*, 583–650. (b) Abraham, N. G.; Kappas, A. *Pharmacol. Rev.* **2008**, *60*, 79–127.

(5) Examples are: (a) Zimmermann, A.; Leffler, C. W.; Tcheranova, D.; Fedinec, A. L.; Parfenova *Am. J. Physiol.* **2007**, *293*(4, Pt. 2), H2501–H2507. (b) Bagul, A.; Hosgood, S. A.; Kaushik, M.; Nicholson, M. L. *Transplantation* **2008**, *85*, 576–581. (c) Cepinskas, G.; Katada, K.; Bihari, A.; Potter, R. F. *Am. J. Physiol.* **2008**, *294*, G184–G191. (d) *Neuroscience* **2008**, *151*, 1104−1111.(e) Lancel, S.; Hassoun, S. M.; Favory, R.; Decoster, B.; Motterlini, R.; Neviere, R. *J. Pharmacol. Exp. Therapeutics* **2009**, *329*, 641–648. (f) Sun, B. W.; Chen, X. *Cell. Mol. Life Sci.* **2009**, *66*, 365–369.

(6) Ford, P. C. *Acc. Chem. Res.* **2008**, *41*, 190–200.

(7) (a) Ford, P. C.; Bourassa, J.; Lee, B.; Lorkovic, I.; Miranda, K.; Laverman, L. *Coord. Chem. Rev.* **1998**, *171*, 185–202. (b) Pavlos, C. M.; Xu, H.; Toscano, J. P. *Curr. Top. Med. Chem.* **2005**, *5*, 637–647. (c) Tfouni, E.; Ferreira, K. Q.; Doro, F. G.; Santana da Silva, R.; Novais da Rocha, Z. *Coord. Chem. Rev.* **2005**, *249*, 405–418. (d) Rose, M. J.; Mascharak, P. K. *Curr. Opin. Chem. Biol.* **2008**, *12*, 238–244.

(8) (a) Arregui, B.; A.; Pez, B. L.; Garća Salom, M.; Valero, F.; Navarro, C. N.; Fenoy, F. J. *Kidney Int.* **2004**, *65*, 564–574. (b) Koneru, P.; Leffler, C. W. *Am. J. Physiol.: Heart Circ. Physiol.* **2004**, *286*, H304–H309. (c) Barkoudah, E.; Jaggar, J. H.; Leffler, C W. *Am. J. Physiol.: Heart Circ. Physiol.* **2004**, *287*, H1459–H1465.

there have been several reports of photochemical CO delivery to biological targets.[2a,8,9] Desirable properties for such a photoactivated CO releasing moiety (photoCORM) would be aqueous solubility, air and water stability, tissue specificity, and reasonable photolability preferably at wavelengths where tissue penetration is optimal. PhotoCORMs having such properties would allow photochemical triggering to induce a dose-dependent CO response (eq 1).

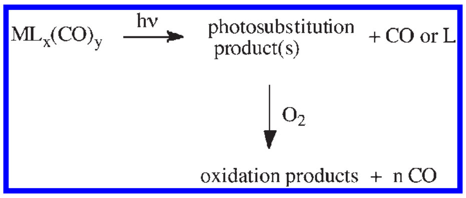
$$ML_x(CO)_y \xrightarrow{h\nu} ML_x(CO)_{y-1} + CO \qquad (1)$$

Metal carbonyls tend to be photoactive toward CO release,[10] but quantitative studies have been largely confined to anhydrous, anaerobic environments.[11-13] However, there are several examples of studies utilizing $Mn_2(CO)_{10}$ as activated by light to deliver CO to tissue and cells in aqueous media[2a,8] and a recent report that DMSO solutions can deliver the complex salt $[Mn(CO)_3(tpm)]PF_6$ directly to HT29 colon cancer cells where CO release is triggered by 365 nm light, resulting in significant photoinduced toxicity.[9] These developments indicate genuine promise for the physiological applications of photoCORMS. In this context, our efforts at developing photoactive CO donors have focused on investigating the effects of an aerated aqueous medium on the properties and photochemical reactivities of prospective photoCORMs and the resulting photochemically generated intermediates. Reported here are quantitative photochemical studies of the water-soluble salt $Na_3[W(CO)_5(TPPTS)]$ (**1**, $TPPTS^{3-}$ is the tris(sulfonatophenyl)phosphine trianion), a species first prepared by Darensbourg and Bischoff.[14]

Also described are quantitative methods for determining the CO released in the primary photoreactions as well as that resulting from the subsequent decay of photoreaction products. The reaction of CO with reduced myoglobin (Mb) followed by spectroscopic evaluation of the resulting Mb(CO) is often used as a quantitative measure of carbon monoxide release.[2,9] However, this methodology is not compatible with photolyses in aerated media, so there is a need for refining quantitative methods that avoid that limitation. The procedures described here for quantifying CO are compatible with photolyses in aerated solutions, with varying degrees of convenience, and demonstrate that the photolysis of **1** in an aerated aqueous solution results in immediate, high quantum yield labilization of one CO and that the subsequent aerobic oxidation of the initial photoproduct slowly releases additional CO.

The latter observation that photolysis-generated intermediates continue to release CO in aerated media suggests an alternative strategy for photochemical carbon monoxide delivery, namely, photoinduced conversion of an air-stable CO carrier into another species that is easily oxidized. Since CO binds more strongly to lower-valent metal centers, oxidation would increase its lability (Scheme 1), potentially generating additional CO equivalents per photochemical event.

Scheme 1



Experimental Section

Materials. All materials were used as received unless otherwise stated. Carbon monoxide and argon were purchased from Praxair and passed through an oxygen scrubbing solution prior to addition to aqueous samples using vacuum line techniques. CO pressures were calculated using a mercury monometer. The water-soluble iron(III) porphyrinato complex $Na_3[Fe^{III}(TPPS)]$ (TPPS = tetrakis(sulfonatophenyl)porphyrinato) was purchased from MidCentury Chemicals. All solutions were prepared with Millipore deionized and Milli-Q filtered water. The anionic ligand tris(sulfonatophenyl)phosphine was synthesized by T. Matson of UCSB as the sodium salt $Na_3[TPPTS]$ according to a literature procedure[15] or was purchased from Sigma Aldrich Co. Material from both sources was found to contain both tri- and disulfonated triphenylphosphine as well as the respective oxides. These were purified by aqueous gel filtration chromatography on Sephadex G-15 to give, after isolation, a material displaying only one ^{31}P NMR peak at −5.1 ppm[15] (versus phosphoric acid; see the Supporting Information for more details).

The complex salt $Na_3[W(CO)_5(TPPTS)]$ was prepared from tungsten hexacarbonyl via a procedure modified slightly from that described by Darensbourg and Bischoff.[14] Briefly, $W(CO)_6$ was dissolved in methanol, and the solution was irradiated under argon using a 150 W medium pressure mercury lamp, giving a solution of the $W(CO)_5(MeOH)$ precursor for the formation of other pentacarbonyl derivatives. The solution volume was reduced in vacuo and was then added in the dark to a deaerated reaction flask containing aqueous $Na_3[TPPTS]$, and the resulting mixture was allowed to stir in the dark for two days. The product solution contained some unreacted $W(CO)_5$(solvent), which was removed by exposing the solution to the air for several days, leading to precipitation of an unidentified solid. The resulting solution was filtered, then chromatographed over Sephadex G-15 with nanopure water as the eluant. Those fractions containing **1**, as identified by ^{31}P NMR, were combined, and a solid product was obtained by rotary evaporation. The ^{31}P NMR spectrum of the purified solid displayed a single phosphorus resonance at 24.8 ppm relative to phosphoric acid. This peak showed two side bands at 25.4 and 24.2 ppm due to coupling of the ^{31}P nucleus to the spin 1/2 ^{183}W isotope (natural abundance 14%), indicative of a phosphorus coordinated to tungsten. The IR spectrum agreed with that reported[14] (see Supporting Information Figure S-1), and negative ion electrospray-ionization mass spectrometry showed the principal anionic species to have the parent peak for **1** .

Instrumentation. Optical absorption spectra were recorded for solutions in 1.0 cm quartz cuvettes on an OLIS upgraded Cary 118 spectrophotometer. A Matson Research Series FTIR spectrometer was used to record infrared spectra of solid

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[9] (a) Niesel, J.; Pinto, A.; Peindy N'dongo, H. W.; Merz, K.; Ott, I.; Gust, R.; Schatzschneider, U. *Chem. Commun.* **2008**, 1798–1800. (b) Pfeiffer, H.; Rojas, A.; Niesel, J.; Schatzschneider, U. *Dalton Trans.* **2009**, 4292–4298.

[10] e.g.,(a) Wrighton, M. S. *Chem. Rev.* **1974**, *74*, 401–30. (b) Ford, P. C.; Massick, S. *Coord. Chem. Rev.* **2002**, *226*, 39–49.

[11] For example: (a) Wrighton, M. S.; Abrahamson, H. B.; Morse, D. L. *J. Am. Chem. Soc.* **1976**, *98*, 4105–9. (b) Darensbourg, D, J.; Nelson, H. H., III; Murphy, M. A. *J. Am. Chem. Soc.* **1977**, *99*, 896–903. (c) Kolodziej, R. M.; Lees, A. J. *Organometallics* **1986**, *5*, 450–5.

[12] Among limited exceptions is: Kunkely, H.; Vogler, A. *Inorg. Chem. Commun.* **2003**, *6*, 830–832.

[13] (a) Kayran, C.; Richards, M.; Ford, P. C. *Inorg. Chem. Acta* **2004**, *357*, 143–148. (b) Diz, E. L.; Ford, P. C. *Inorg. Chem. Acta* **2008**, *361*, 3084−3088 and references therein.

[14] Darensbourg, D. J.; Bischoff, C. J. *Inorg. Chem.* **1993**, *32*, 47–53.

[15] Bartik, T.; Bartik, B.; Hanson, B. E.; Glass, T.; Bebout, W. *Inorg. Chem.* **1992**, *31*, 2667–2670.

samples in KBr pellets and as solutions. Bulk photolysis reactions used for syntheses were carried out in an ACE Glass photochemical safety UV cabinet, model 7836−20 with a Hanovia 150 W medium pressure mercury lamp immersed in a quartz well filled with flowing water for cooling and for IR filtering. A Varian UNITY INOVA 500 MHz NMR Spectrometer was used to record NMR spectra. Gas chromatography of gas samples containing CO was accomplished using a programmable Agilent model 6890 gas chromatograph with thermal conductivity detection.

Photochemical Procedures and Apparatus. The solutions studied by continuous and laser flash photolysis techniques were contained in a gastight flask fused to a quartz cell, or in a CaF_2 IR cell sealed with serum caps. The sample concentrations were typically 1−3 mM. The solutions were deaerated by three freeze−pump−thaw cycles, then entrained with argon or CO.

Continuous photolysis experiments were conducted using standard procedures[16] in a darkroom. The excitation source was a 200 W high-pressure mercury lamp mounted on an Oriel optical train with intensity regulation by an internal feedback loop. The light from this source was first passed through an IR filter, then an interference filter to isolate single mercury lines at 313, 366, or 405 nm. A lens was used to collimate the light entering the sample, and the solution was stirred continuously. Ferrioxalate actinometry was used to evaluate light intensities.[17] The sample was irradiated for a specific period, and the photoreaction progress was monitored by UV−visible and FTIR spectroscopy. Quantum yields were determined by fitting plots of incremental quantum yields (first 10−20% of the reaction) for absorbance changes versus the irradiation time to a linear equation, the *y* intercept being the apparent photoreaction quantum yield, $\Phi_{apparent}$[16] (see the Supporting Information).

The flash photolysis system at UCSB has been described previously,[18] and the current configuration is described in the Supporting Information.

Results and Discussion

Photolysis of $W(CO)_5(TPPTS)^{3-}$ (1). The homologous series of complex ions $M(CO)_5(TPPTS)^{3-}$ (M = Cr, Mo, and W) was previously shown by Darensbourg and Bischoff[14] to be stable in solutions of mixed aqueous tetrahydrofuran and acetonitrile under a CO atmosphere, even at elevated temperatures. We have confirmed these observations for $W(CO)_5(TPPTS)^{3-}$ and also demonstrated **1** to be stable for a period of hours in the dark in *aerated* aqueous buffer solutions (pH 7.4, 15 mM phosphate) at ambient temperature. Photolysis of an argon deaerated solution led to spectral changes, as illustrated in Figure 1 for the 313 nm irradiation of aqueous $Na_3[W(CO)_5(TPPTS)]$, principally the appearance of a strong, broad absorption band at λ_{max} ~410 nm (shoulder at ~450 nm) with an isosbestic point at 357 nm. The photolysis-induced spectral changes were similar regardless of whether the solution was deaerated or aerated or whether the solution contained added CO or not. However, the subsequent temporal absorption changes differed considerably, as a function of the reaction conditions (see Figure S-3, Supporting Information).

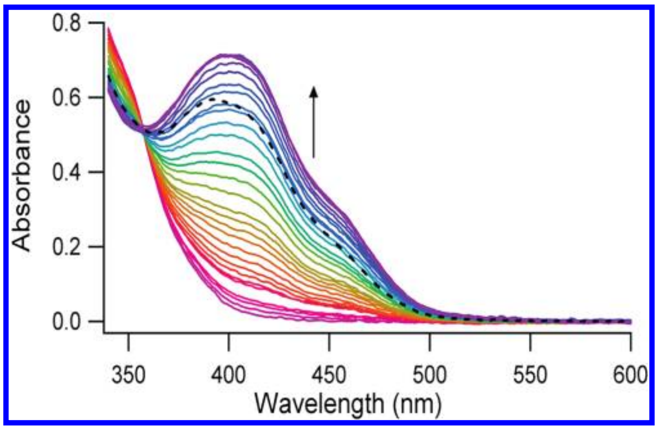

Figure 1. Photolysis-induced changes in the spectrum of $Na_3[W(CO)_5(TPPTS)]$ dissolved in an argon deaerated pH 7.4 phosphate buffer solution following long-term 313 nm irradiation (3000 s) in a closed vessel at ambient temperature. The dotted line shows the spectrum after the photolysis product solution was left in the dark overnight (T = 25 °C; $[\mathbf{1}]_{initial}$ = 8.07 μM).

The apparent quantum yield of the photoreaction was measured from the initial spectral changes, leading eventually to the final spectrum shown in Figure 1 after exhaustive photolysis. On the basis of absorbance changes over the first 10% of the reaction, $\Phi_{apparent}$ was calculated to be 0.90 ± 0.05 and 0.6 ± 0.1 for 313 and 405 nm irradiation, respectively. For the latter wavelength, product absorption led to strong inner filter effects; however, the overall spectral changes after exhaustive photolysis were the same as for 313 nm irradiation (Supporting Information Figure S-2). As noted below, the principal photoreaction is ligand dissociation, primarily of CO, as seen previously for the uncharged complex $W(CO)_5(PPh_3)$ in a tetrahydrofuran solution.[19] The quantum yields under these conditions are comparable to those determined for the hexacarbonyl analog $W(CO)_6$ (**2**) in various media (e.g., the Φ_{CO} for CO photolabilization from **2** was reported as 0.79 in *n*-heptane).[20]

For deaerated solutions, a slow and incomplete back reaction in the direction of the original spectrum of the prephotolysis solution was observed (Figure 1). Under a CO atmosphere, the back reaction was markedly accelerated, although it remained relatively slow. After a day, the resulting spectrum was quite similar to that of the initial solution. Figure 2 illustrates the absorption spectrum of a solution initially, $W(CO)_5(TPPTS)^{3-}$ under CO (1.0 atm), the spectrum after exhaustive photolysis using the repetitive 308 nm pulses from a XeCl excimer laser, and that seen after back-reaction in the dark for 24 h. The third spectrum is very close, but not identical, to that of the starting material. The lifetime of the back reaction decay was ~4 h under 1 atm of CO, giving a second-order rate constant $k_{CO} \sim 3 \times 10^{-2}$ M^{-1} s^{-1} (the temporal profile of this decay is shown in the Supporting Information, Figure S-3). Rephotolyzing the solution leads

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(16) (a) Malouf, G.; Ford, P. C. *J. Am. Chem. Soc.* **1977**, *99*, 7213–7221. (b) Works, C. F.; Jocher, C. J.; Bart, G. D.; Bu, X.; Ford, P. C. *Inorg. Chem.* **2002**, *41*, 3728–3736.

(17) Calvert, J. G.; Pitts, J. N. *Photochemistry*; J. Wiley and Sons: New York, 1967; pp 783−786.

(18) (a) Crane, D. R.; Ford, P. C. *J. Am. Chem. Soc.* **1991**, *113*, 8510–8516. (b) Dethlefsen, J. W.; Hedegård, E. D.; Rimmer, R. D.; Ford, P. C.; Døssing, A. *Inorg. Chem.* **2009**, *48*, 231–238.

(19) Schwenzer, G.; Darensbourg, M. Y.; Darensbourg, D. J. *Inorg. Chem.* **1972**, *11*, 1967–1970.

(20) Wieland, S.; Van Eldik, R. *J. Phys. Chem.* **1990**, *94*, 5865–70.

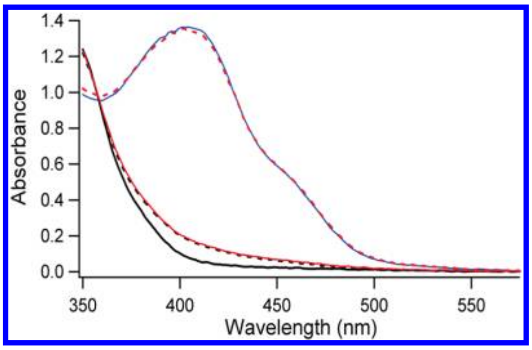
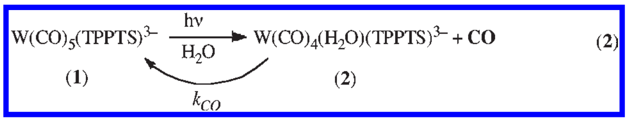


Figure 2. Irradiation of a pH 7.4 phosphate buffer solution of $Na_3[W(CO)_5(TPPTS)]$ (solid black line) under a CO atmosphere (P_{CO} = 1.0 atm) generates a photoproduct (solid blue) which decays nearly completely back (dashed black) to the original spectrum over a period of 24 h. The photoproduct spectrum was regenerated by further photolysis (dashed red), and reaction with CO again nearly restored the starting spectrum (red line).

again to the same transient spectrum, and subsequent decay again nearly regenerates the initial spectrum (Figure 2).

These data clearly indicate CO rather than phosphine labilization as the principal photoreaction under these conditions (eq 2).



$$\underset{(\mathbf{1})}{W(CO)_5(TPPTS)^{3-}} \underset{k_{CO}}{\overset{h\nu}{\underset{H_2O}{\rightleftarrows}}} \underset{(\mathbf{2})}{W(CO)_4(H_2O)(TPPTS)^{3-}} + \mathbf{CO} \qquad (\mathbf{2})$$

This conclusion is substantiated by comparing the ^{31}P NMR spectra of the starting and final solutions for the photolysis of a deaerated aqueous solution of **1** using the third harmonic of the Nd/YAG laser at 355 nm as the excitation source. The initial solution shows only the resonance at 24.8 ppm (with side bands due to the presence of the ^{183}W isotope). Photolysis leads to diminished peak intensity at 24.8 ppm and to growth of a new peak at 32.7 ppm with side bands due to ^{183}W coupling, consistent with formation of the aquo product $W(CO)_4(H_2O)(TPPTS)^{3-}$ (**2**). At the same time, a very weak resonance at −5.3 ppm (identified above as the free ligand) appears. Comparing the integrated peak intensity of the resonance at 24.8 ppm to that at −5.3 ppm shows the former to be about 20 times as large (Supporting Information Figure S-4). Thus, all but ~5% of the photochemistry occurs via eq 2 under these conditions.

More qualitatively, the FTIR spectrum of a D_2O solution of **1** before, during, and after continuous photolysis at 366 nm shows the disappearance of the ν_{CO} bands at 1943, 1989, and 2074 cm^{-1}, which are characteristic of the starting material, together with the appearance of new ν_{CO} bands at 1807, 1905, and 2021 cm^{-1}, consistent with the formation of a new metal carbonyl (Figure 3).

For aerated aqueous solutions of **1**, analogous photolysis initially induced absorption changes equivalent to those measured in a deaerated solution. Thus, the primary photoreaction process appears to be the same for both aerated and deaerated systems, principally CO labilization. Subsequent to the photolysis, the absorption spectra of the aerated product solution underwent changes over a period of a few hours that were different from the changes seen for the deaerated solutions or for those under a CO atmosphere. This suggests that the photoproducts, primarily $W(CO)_4(H_2O)(TPPTS)^{3-}$ plus any $W(CO)_5(H_2O)$ formed, undergo irreversible oxidative processes not seen under a CO or argon atmosphere. Figure 4 illustrates the changes in the optical spectra. Moreover, under aerated conditions, the eventual product of the secondary reaction does not undergo subsequent photoreactions, again confirming that it is not the species that was reformed under CO.

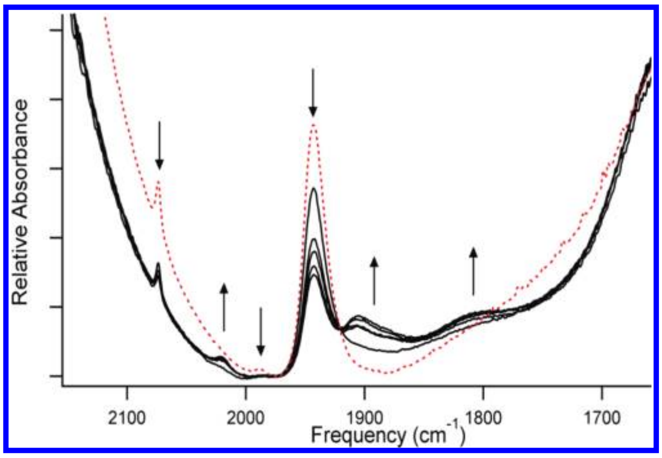


Figure 3. Infrared spectral changes accompanying the 366 nm CW irradiation of an Ar-saturated D_2O solution of **1**.

Direct Detection and Quantification of CO Release. The photolabilization of CO from **1** led to two questions in terms of potential applications of photoCORMs for carbon monoxide delivery to physiological targets: (1) How much CO is actually released in the primary photochemical event? (2) Is additional CO released in subsequent thermal reactions of the initial photochemical product under physiologically relevant conditions, and, if so, how much?

With regard to the first question, while CO release is indeed implied by the spectral changes noted upon photolysis of aqueous **1**, direct observation of CO is needed to confirm this premise and to quantify the amount released. Interestingly, relatively few established procedures for quantitatively measuring CO under such conditions have been reported, especially for CO released in aerated media. As noted above, use of the spectral changes upon the reaction of deoxymyoglobin (Mb) with CO to form carboxymyoglobin Mb(CO) is a very sensitive method for CO detection owing to the strong Soret and Q bands of the Mb heme chromophore but is unlikely to be quantiative with a mixture of CO and air. To address this question, we have compared three additional analytical procedures for quantifying photochemically generated CO. (More complete details are presented in the Supporting Information.)

In each methodology, the technique involves sampling the gas phase in equilibrium with the photolyzed solution sample enclosed in a Schlenk cuvette. This device consists of a 1.0 cm path-length fluorescence cell fused to a glass bulb and tubing designed so that the cuvette can be attached to a vacuum line for the purpose of degassing or introducing a specific atmosphere. In this case, the Schlenk cuvette has a port sealed by a rubber serum cap for the purpose of withdrawing a sample using a gastight

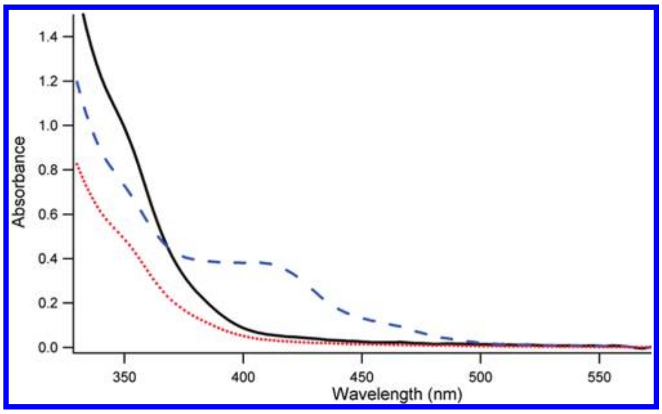


Figure 4. The 405 nm photolysis of **1** (solid black line) in an aerated aqueous solution (pH 7.4 in 15 mM phosphate buffer), generating a photoproduct (dashed blue line), then decaying to an oxidized product (dotted red line).

syringe (see Supporting Information Figure S-5). It has an internal volume of 30 mL and typically would contain 4.0 mL of the photolysis solution. Given that CO has a solubility of 0.92 mM/atm in aqueous solution at 303 K[21] (the temperature used in these experiments), one can calculate a partition coefficient of 45/1 for CO between the gas and liquid phases at equilibrium. The product of the partition coefficient times the ratio of gas and liquid volumes (26/4) indicates that, at equilibrium, more than 99.5% of the CO generated will be in the gas phase of the Schlenk cuvette. Once a gas sample was analyzed, the total CO released was calculated by taking into account the partitioning into the gas and liquid volumes.

The experiments were carried out as follows. A sample (typically about 7 mg) of $Na_3[W(CO)_5(TPPTS)]$ in a buffered aqueous solution under 1 atm of argon was photolyzed exhaustively using 366 nm light from the continuous photolysis train until there were no further changes in the absorption spectrum. The solution was allowed to equilibrate with the gas phase by shaking repeatedly for 2−3 min. A 500 μL sample of the gas phase in the Schlenk cell was then removed by gastight syringe and the amount of CO determined by one of the techniques described below. The remaining gas phase in the sample was removed by evacuating the Schlenk cell, and then the cell was refilled with air and sealed. This solution was allowed to react for 24 h with regular shaking to ensure equilibration of the gas and liquid phases. The gas phase was then again sampled and analyzed. In this manner, we were able to determine first the quantity of CO released in the primary photochemical event, then the quantity released in the subsequent thermal reactions of the initial photoproduct.

FTIR Analysis. The first, and seemingly most simple, method would be to use infrared spectroscopy to interrogate the gas phase above solutions that have been subjected to photolysis. The FTIR spectrum of CO gas shows the very characteristic R and P branched rotational vibrational spectrum of a diatomic molecule centered at 2142 cm^{-1}, and the spectrum of a sample drawn from a photolysis cell is illustrated in Figure S-6 of the Supporting Information. However, it was found that the intensity of this band is markedly dependent on the gas pressure in the cell, so in order to obtain quantitative reproducibility, all samples were measured at the same total pressure, 1 atm. The gas sample withdrawn from the photolysis cell was introduced to a 7.1 cm path-length IR cell under a vacuum, and then air was added to bring the pressure to 1 atm (UCSB air contains CO_2 but no CO) and the IR spectrum was recorded. The intensity of the R(6) band at 2168.1 cm^{-1} was then compared to a calibration curve obtained from pure CO in exactly the same manner, in order to determine the amount of CO in the sample.

By using this method, it was determined that an aqueous buffered solution of **1**, photolyzed exhaustively at 366 nm, releases 0.93 ± 0.04 equiv of CO per mole of complex in the primary photochemical step (three independent experiments). This result agrees well with the ^{31}P NMR spectra of analogous solutions, which showed nearly complete conversion of **1** to another tungsten carbonyl phosphine, presumably **2**, plus traces of free TPPTS (< 5%). Subsequent oxidative-induced release of CO from the product solution gave an additional 1.64 ± 0.11 equiv after 24 h, confirming the principle that was articulated above in the Introduction (Scheme 1).

GC Analysis. The head space of the Schlenk cuvette was sampled (500 μL via gastight syringe) and the sample transferred to the injection port of a programmable gas chromatograph with a 10 m carbosieve packed column and a thermal conductivity detector using helium as the carrier gas. Using a customized heating program, CO and CO_2 had the respective elution times ~13.3 and ~22.5 min. Quantification was achieved by comparing integrated peak intensities to calibration curves determined for each gas (see the Supporting Information).

By the GC method, it was determined that an aqueous buffered solution of **1**, photolyzed exhaustively at 366 nm, releases 0.84 ± 0.04 CO equivalents per mole of complex in the primary photochemical step (three independent experiments). Subsequent oxidative-induced release of CO from the product solution was an additional 1.39 ± 0.04 equiv after 24 h. The GC method also indicated that ~0.02 ± 0.01 equiv of CO_2 per mole of complex was present after the primary photochemical step, but the amount present after evacuating the photolysis solution and then allowing it to react with air was a much higher 0.20 ± 0.02 equiv (corrected for any CO_2 initially present in the air).

Analysis by Flash Photolysis. Flash photolysis of the ferrous porphyrin complex Fe^{II}(TPPS) solutions (TPPS = tetrakis(sulphonatophenyl)porphyrinato) containing excess CO leads to disruption of the equilibrium shown in eq 3 followed by exponential decay back to the equilibrium state. The k_{obs} obtained fits the relationship $k_{obs} = k_{on}[CO] + k_{off}$, and the plot of k_{obs} versus [CO] is linear with a slope k_{on} and an intercept k_{off}.[22] In this context, one can measure by flash photolysis the k_{obs} for a sample of Fe^{II}(TPPS) exposed to an unknown quantity of CO by transferring a fraction of the head space from the photolysis cell into a second Schlenk cuvette containing a solution of aqueous Fe^{II}(TPPS). Comparison to a calibration curve determined with known quantities of CO

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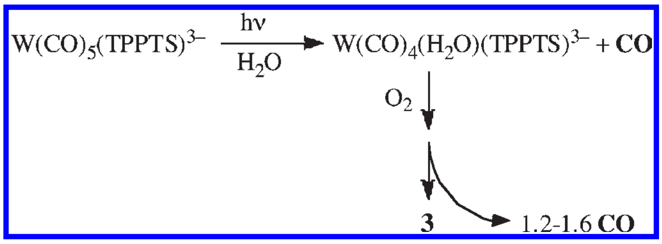
(21) Bartino, R. In *IUPAC Solubility Data Series*; Cargill, R. W., Ed.; Pergamon Press: Oxford, U. K., 1990; pp 1−2.

(22) Laverman, L. E.; Hoshino, M.; Ford, P. C. *J. Am. Chem. Soc.* **1997**, *119*, 12663–12664.

Scheme 2



allows one to calculate the amount of CO present in the unknown (see the Supporting Information for more details.)

$$\mathrm{Fe^{II}(TPPS) + CO} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{Fe^{II}(TPPS)(CO)} \qquad (3)$$

Using this method, we determined that an aqueous buffered solution of **1**, photolyzed exhaustively at 366 nm, releases 0.94 ± 0.08 equiv of CO per mole of complex in the primary photochemical step (three independent experiments). Since the Fe^{II}(TPPS) is sensitive to the presence of oxygen, the gas phase above the photoproduct exposed to air and allowed to react for some time could not be introduced directly into the flash photolysis cell. It was first necessary to expose the gas sample to an aqueous solution of Cr(II), an effective trap for O_2, then transfer this deoxygenated gas to the Fe^{II}(TPPS) solution for flash experiments. The result of this treatment gave a value of 1.2 ± 0.1 equiv per mole of complex as the oxidative-induced release of CO from product of the exhaustive photolysis of **1**.

Overview and Summary

The carbonyl complex salt $Na_3[W(CO)_5(TPPTS)]$ is very stable in aerated aqueous media unless subjected to photolysis. Under irradiation, **1** demonstrates high quantum yield photolability, leading to the release of approximately one CO (eq 2), as verified by the three analytical techniques developed for this purpose. NMR data confirm that phosphine photolabilization is at most a minor pathway ($< 5\%$). Thus, **1** is an effective photoCORM.

In a deaerated aqueous solution, the aquo complex $W(CO)_4(H_2O)(TPPTS)^{3-}$ formed as the other photoproduct of eq 2 is thermally stable, although it does undergo a slow back-reaction with excess CO to regenerate (largely) **1**. Notably, **2** is not stable in aerated solution but undergoes a slow thermal reaction. This oxidative process leads to the release of additional CO, approximately 1.2 to 1.6 CO per tungsten, as verified by the three analytical procedures, as well as the formation of an unknown tungsten product (or products), denoted in Scheme 2 as **3**. Some CO_2 was also formed, as seen in the GC traces. Since it is stable in deaerated media, but is converted by air oxidation to another species that is a CO-releasing moiety, **2** can be viewed as a proCORM.

The oxidative-induced CO release confirms the strategy described by Scheme 1, although less than half the CO's that might potentially be released in this manner appeared as free CO. This implies that the oxidative product(s) **3** not only continues to retain CO but is relatively stable. In order to explore this possibility, the solutions resulting from the sequential exhaustive photolysis, followed by 24 h of aerobic oxidation, were concentrated to dryness under a vacuum and the IR spectra of the residue was recorded. As seen in Figure 5, this residue displayed ν_{CO} bands at 2075, 2020, ∼1925 (broad), and ∼1890 (broad) cm^{-1}, indicative of metal carbonyl(s). We have not tried to characterize this product further. Since a variety of W(II) carbonyls have been characterized,[23] this result should not have been surprising, although earlier studies have indicated that long-term photolysis of $W(CO)_6$ in noncoordinating media containing O_2 eventually progresses completely to tungsten oxides, perhaps by multiple photoexcitation events.[24]

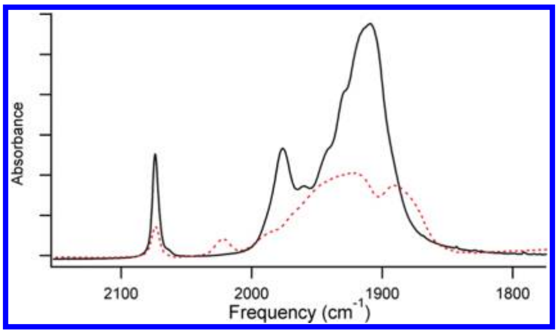


Figure 5. FTIR spectrum of solid $Na_3[W(CO)_5(TPPTS)]$ (black line) and of the solid **3** recovered after the aerobic oxidation of the product of exhaustive photolysis of aqueous **1** (dotted line), both in KBr.

In summary, the present studies demonstrate that the sodium salt of the water-soluble tungsten carbonyl complex $W(CO)_5(TPPTS)^{3-}$ meets several key goals for the design of a photochemical precursor for bioactive carbon monoxide. This photoCORM is soluble in aqueous media and is air- and water-stable, yet it displays high photolability toward the release of CO, as verified by three quantitative techniques developed and tested as described. Furthermore, the product of the primary photoreaction undergoes subsequent aerobic oxidation, leading to the release of *additional* CO equivalents. As such, **1** is potentially useful for photoinduced CO release in topical applications with a UVA excitation source. However, for internal physiological applications, it will likely be necessary to develop photochemical CO precursors that are effective with longer wavelength excitation. Ongoing studies are focused on preparing and fully characterizing the photochemical properties of new materials with this and other properties desirable for physiological applications.

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Supporting Information Available: Figures S-1 to S-6, showing the IR spectrum of aq. **1**, optical spectral changes during 405 nm photolysis, temporal decay of photoproduct absorbances under CO and under air, ^{31}P NMR spectral changes upon the photolysis of **1**, a photograph of the Schlenk cuvette for photolysis studies, and the gas phase IR spectrum of a CO sample. Also included are expanded versions of the Experimental Section describing compound purifications and CO analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) Baker, P. K. *Adv. Organomet. Chem.* **1996**, *40*, 45–115.
(24) Crayston, J. A.; Almond, M. J.; Downs, A. J.; Poliakoff, M.; Turner, J. S. *Inorg. Chem.* **1984**, *23*, 3051–6.