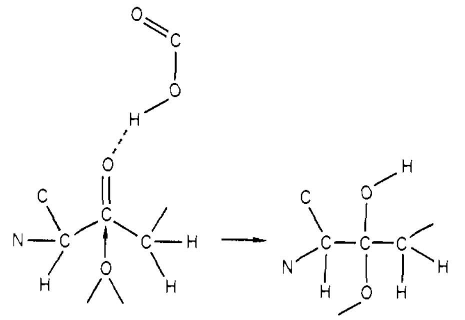
Scheme I



isotopically enriched carbonyl peak at 204.2 ppm (Figure 1a). When 1 equiv of ketone **2** is added to a solution of porcine pepsin in ${}^{2}H_2O$, the low-field signal disappears and a new peak appears at 99 ppm (Figure 1b). With the natural abundance peptide **2a** instead of **2** the spectrum appears to be missing only the peak at 99 ppm (Figure 1c). This is confirmed by the difference spectrum 1b minus 1c (Figure 1d), which contains only the 99-ppm peak. Addition of pepstatin A (**1**) to a sample containing ketone **2** plus pepsin yielded a spectrum with the inhibitor signal at 207.2 ppm (Figure 1e). Pepstatin A, which binds to pepsin 1000-fold tighter than ketone **2**,[10] displaces the keto Sta analogue, which is then observed as the free species in solution.

A shift of 105–108 ppm upfield for the C_3 resonance when ketone **2** binds to pepsin is direct evidence that the carbonyl carbon is converted from trigonal to tetrahedral geometry or very close to it, due to nucleophilic addition to the carbonyl. An analogous shift occurs for fructose, whose ketone carbon chemical shift of 217.4 ppm in pyridine-d_6 (Me_4Si) moves to 107.5 and 105.0 in the α- and β-furanose forms or 99.8 and 100.6 ppm in the α- and β-pyranose structures.[15] Baillargeon et al.[11] examined chemical shifts in a series of constrained model compounds where a carbonyl was undergoing partial nucleophilic attack. They found that even, for a nitrogen–carbonyl carbon bond distance of only 1.6 Å (compared to 1.48 Å for a covalent bond) the carbonyl chemical shift remained at about 200 ppm. These data would suggest that nucleophilic addition to form a tetrahedral intermediate has occurred when **2** is bound to pepsin. Oxygen is the most likely nucleophile since only O ligands or water would be close to the inhibitor carbonyl group in the pepsin active site.[7]

When an α-dimethyl sulfonium derivative of ketone **2** was examined, the results were quite different. Sulfonium ylide **3a** displays a single peak for the isotopically enriched carbon at 188 ppm in $CHCl_3$-d (data not shown). Bound to pepsin, the peak splits into two very broad resonances near 191 and 192 ppm. No other inhibitor peaks were found in the spectrum of the complex. Preliminary experiments showed that the peaks become narrower at lower temperature, so it is most likely that the two broad peaks represent two forms of bound **3**, probably the keto and enol forms (**3b** and **3c**), exchanging at a rate on the order of the chemical shift difference between them. In either case the carbon remains trigonal in the complex.

Conclusion. The carbonyl group in keto Sta of peptide **2** undergoes a major chemical change when the peptide binds to the pepsin active site. A tetrahedral species is formed whose properties are consistent with Scheme I. Further experiments are aimed at determining the nature of the nucleophile and the constraints on peptide structure leading to development of the intermediate.

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(14) ${}^{13}C$ NMR spectra were taken at 75.5 MHz with a Cryomagnet Systems CMS 300/50 magnet and 10 mM probe (Cryomagnet Systems, Indianapolis, IN). In-house rf electronics and a Nicolet 1180 computer complete the system. Temperatures were maintained with a thermostated N_2 stream and were measured by insertion of a thermocouple directly in the NMR sample. Porcine pepsin was prepared by activation of pepsinogen as previously described (Schmidt, P. G. In "Frontiers in Protein Chemistry"; Liu et al., Eds.; Elsevier: New York, 1980; pp 63–87). Peptides were added to protein solutions as 10 mM stock solutions in CH_3OH-d_4. Generally this resulted in a 5% solution of the alcohol, a level that does not affect binding of pepstatin analogues to pepsin.

(15) Funcke, W.; Klemer, A. *Carbohydr. Res.* **1976**, *50*, 9–13.

Picosecond Dynamics of Solution-Phase Photofragmentation of $[Mn_2(CO)_{10}]$

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The identification and characterization of primary photochemical pathways are central to understanding the photochemistry of organo–transition metal complexes. Picosecond flash photolysis allows direct spectroscopic observation of transients following photoexcitation and is therefore ideal for this purpose. We report the first application of this technique to the study of an important photochemical organo–transition metal reaction, the solution photolysis of $[Mn_2(CO)_{10}]$. These experiments provide the first direct evidence for two primary chemical pathways following optical excitation of $[Mn_2(CO)_{10}]$.

The picosecond absorption spectrometer has been described previously.[1] $[Mn_2(CO)_{10}]$, purchased from Alfa Products, was purified by sublimation and added to the sample cell as a 0.05 M solution in ethanol, which was flowed through the cell to avoid buildup of photoproducts. Previous solution flash photolysis studies[2-4] of $[Mn_2(CO)_{10}]$ and $[Re_2(CO)_{10}]$ were carried out on longer time scales on which secondary bimolecular processes are frequently rapid, and the results had to be analyzed in terms of a complex pattern of photochemical and thermal reactions. Picosecond experiments allow direct observation of primary photoproducts under conditions where subsequent bimolecular reactions do not occur. Photolysis employed a 355-nm, 1-mJ, 25-ps excitation pulse and absorption spectra of the products were recorded between 400 and 850 nm with probe delays of 0–250 ns. There is no change in the spectra within this delay range.

A typical spectrum (Figure 1) shows two absorption maxima with λ_{max} 780 and 480 nm. The first of these can be assigned to $[Mn(CO)_5]\cdot$, presumably produced by homolysis, by comparison with the spectrum of $[Mn(CO)_5]\cdot$ observed in a pulse radiolysis[5] as well as a recent matrix isolation experiment.[6] The species generated in ethanol by pulse radiolysis is reported[5] to have a red absorption between 680 and 1030 nm, while $[Mn(CO)_5]\cdot$ synthesized[6] by UV photolysis of $[HMn(CO)_5]$ in solid CO matrices at 10–20 K displays a spectrum with λ_{max} 798 nm and a continuous UV absorption at $\lambda < 340$ nm. In the present experiment, as in the previous pulse radiolysis work, the UV region ($\lambda < 400$ nm) is obscured by absorptions from the parent and other species, and the continuous UV absorption at $\lambda < 340$ nm[6] could not be recorded. The spectrum presently reported for $[Mn(CO)_5]\cdot$ constitutes the first direct observation of this species in solution flash photolysis. No band similar to the 480-nm one observed in the present experiment has been reported for $[Mn(CO)_5]\cdot$ and the

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[†] A. P. Sloan Fellow.

(1) Peters, K. S.; Freilich, S. C.; Schaeffer, C. G. *J. Am. Chem. Soc.* **1980**, *102*, 5701.

(2) Geoffroy, G. L.; Wrighton, M. S. "Organometallic Photochemistry"; Academic Press: New York, 1978.

(3) Wegman, R. W.; Olsen, R. J.; Gard, D. R.; Faulkner, L. R.; Brown, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 6089.

(4) Hughey, J. L.; Anderson, C. P.; Meyer, T. J. *J. Organomet. Chem.* **1977**, *125*, C49.

(5) Waltz, W. L.; Hackelberg, O.; Dorfman, L. M.; Wojcicki, A. *J. Am. Chem. Soc.* **1978**, *100*, 7259.

(6) Church, S. P.; Poliakoff, M.; Timney, J. A.; Turner, J. J. *J. Am. Chem. Soc.* **1981**, *103*, 7515.

0002-7863/82/1504-3536$01.25/0

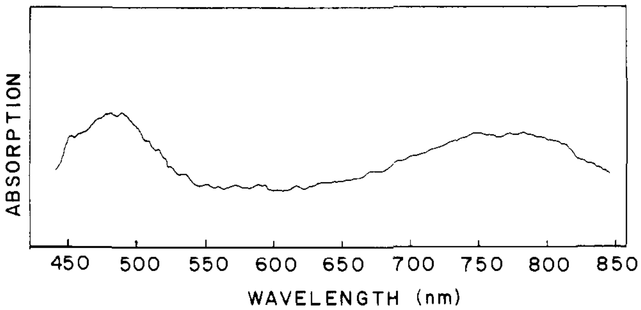

Figure 1. Transient absorption spectra following photolysis of $[Mn_2(CO)_{10}]$ in ethanol. The transient absorption spectra are probed 25 ps after photolysis. The absorbance at 480 nm is 0.15.

band cannot therefore be the result of $[Mn_2(CO)_{10}]$ homolysis; since it is formed in 25 ps, and is therefore unlikely to be a secondary product, we conclude that it must be assigned to a second primary photoproduct. One possible assignment is to a dimanganese species such as $[Mn_2(CO)_9(EtOH)]$, which could be formed by loss of CO and coordination of a solvent molecule.[7] The absorption spectrum of the related molecule $[Mn_2(CO)_9(pyr)]$ (pyr = pyridine) has recently been reported to contain a band at 450 nm,[8] and the difference in λ_{max} values is not unreasonable given the range of values reported for the $d\pi \rightarrow \sigma^*$ transition for other $[Mn_2(CO_9)L]$ complexes.[9,10] It is, however, entirely possible that the band observed in the present experiment is due to a carbonyl bridged species such as that proposed by Pöe.[11]

The solution photolyses of both $[Mn_2(CO)_{10}]$ and $[Re_2(CO)_{10}]$ have been studied previously by a number of groups, and they are generally taken as prototypical of the photoreactions of organometallic compounds containing metal–metal bonds.[2-4,11-14] It has been reported that irradiation of the metal–metal $\sigma \rightarrow \sigma^*$ transition results in homolysis of the metal–metal bond,[13] but kinetic analysis of the photolysis of $[Mn_2(CO)_{10}]$ in the presence of CCl_4 has led to the suggestion that there are in fact two primary photoprocesses.[11] One of these is the homolysis reaction, while the second was proposed to be isomerization of $[Mn_2(CO)_{10}]$ to a form containing one or more bridging carbonyl ligands. The most recent flash photolysis studies,[3] however, have not supported these conclusions and were interpreted in the terms of exclusive homolysis. We were unable to reproduce earlier observations[4] of a second long-lived intermediate in addition to $[Mn(CO)_5]\cdot$. Our results indicate that there are in fact two primary photoproducts as suggested by Pöe,[11] but it is unclear whether we are observing the same species reported by Hughey et al.[4] since that species was only observed after repeated irradiation. Our suggestion that the second species is formed by CO loss is consistent with recent results on photolysis of other metal–metal bonded species containing carbonyl ligands, which indicated that two primary photoprocesses were occurring and were interpreted in terms of competitive cleavage and CO loss.[15,16]

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Registry No. $Mn_2(CO)_{10}$, 10170-69-1.

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(7) Experiments carried out in these laboratories have shown that coordination of solvent molecules to photogenerated coordinatively unsaturated metal carbonyl fragments is fast on a picosecond time scale: Peters, K. S., unpublished results.

(8) McCullen, S. B.; Brown, T. L. *Inorg. Chem.* **1981**, *20*, 3528.

(9) For example, L = CO, λ_{max} (in 3-PIP at 77 K) 375 nm; L = PPh_3, λ_{max} 426 nm: Levenson, R. A.; Gray, H. B. *J. Am. Chem. Soc.* **1975**, *97*, 6042.

(10) Attempts to obtain the spectra immediately after photolysis of $[Mn_2(CO)_{10}]$ in pyridine were unsuccessful due to formation of gas bubbles within the sample.

(11) Fox, A.; Pöe, A. *J. Am. Chem. Soc.* **1980**, *102*, 2497.

(12) Hudson, A.; Lappert, M. F.; Nicholson, B. K. *J. Chem. Soc., Dalton Trans.* **1977**, 551.

(13) Wrighton, M. S.; Ginley, D. S. *J. Am. Chem. Soc.* **1975**, *97*, 2065.

(14) Kidd, D. R.; Brown, T. L. *J. Am. Chem. Soc.* **1978**, *100*, 4095.

(15) Caspar, J. V.; Meyer, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 7794.

(16) Hughey, J. L.; Bock, C. R.; Meyer, T. J. *J. Am. Chem. Soc.* **1975**, *97*, 4440.

Chemical Effects of Ultrasound on Aqueous Solutions. Evidence for ·OH and ·H by Spin Trapping

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Ultrasound is widely used in diagnosis[1] and therapy[2] in medicine, and the chemical effects of ultrasound on aqueous solutions have long been of interest.[3] Intense ultrasound has been reported to cause chemical damage through the phenomenon of cavitation.[4-9] Cavitation produces high local instantaneous temperatures and pressures and sonoluminescence.[3,5] In sonolysis studies of aqueous solutions, it has been proposed that hydroxyl radicals (·OH) and hydrogen atoms (·H) are produced by ultrasound.[10-19] In the present spin-trapping and ESR study, we report conclusive evidence for the formation of ·OH and ·H during sonolysis of aqueous solutions. Hydroxyl radicals are known to be responsible for the reproductive death of mammalian cells exposed to ionizing radiation.[20,21]

In spin trapping[22-24] a diamagnetic nitroso or nitrone compound (the spin trap) is used to convert short-lived radicals into relatively longer lived nitroxyl radicals that are ESR observable. Recently, it has been demonstrated[25] that free radicals produced by sonolysis of carbon tetrachloride, but not of aqueous solutions, can be spin trapped by 2-methyl-2-nitrosopropane and identified by ESR.

In the present work, nonvolatile nitrone spin traps[26]

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(1) J. I. Distasio, "Ultrasonics as a Medical Diagnostic Tool", Noyes Data Corporation, Park Ridge, NJ, 1980.

(2) W. Kremkau, *J. Clin. Ultrasound*, **7**, 287 (1979).

(3) I. E. Elpiner, "Ultrasound, Physical Chemistry and Biological Effects", Consultants Bureau, New York, 1964.

(4) A. Weissler, *J. Accoust. Soc. Am.*, **25**, 651 (1953).

(5) A. Weissler in "Encyclopedia of Chemical Technology", P. E. Kirk and D. F. Othmer, Eds., Wiley-Interscience, New York, 1978.

(6) A. Weissler, H. W. Cooper, and S. Snyder, *J. Am. Chem. Soc.*, **72**, 1769 (1950).

(7) A. Weissler, I. Pecht, and M. Anbar, *Science (Washington, D.C.)*, **150**, 1288 (1965).

(8) S. Moon, L. Duchin, and J. V. Cooney, *Tetrahedron Lett.*, **41**, 3917 (1979).

(9) C. M. Sehgal and S. Y. Wang, *J. Am. Chem. Soc.*, **103**, 6606 (1981).

(10) M. Anbar and I. Pecht, *J. Chem. Phys.*, **40**, 608 (1964).

(11) M. Anbar and I. Pecht, *J. Phys. Chem.*, **68**, 352 (1964).

(12) M. Anbar and I. Pecht, *J. Phys. Chem.*, **68**, 1460 (1964).

(13) M. Anbar and I. Pecht, *J. Phys. Chem.*, **68**, 1462 (1964).

(14) M. Anbar and I. Pecht, *J. Phys. Chem.*, **71**, 1246 (1967).

(15) A. Weissler, *J. Am. Chem. Soc.*, **81**, 1077 (1959).

(16) A. Weissler, *Nature (London)*, **193**, 1070 (1962).

(17) S. B. Reifsneider and L. A. Spurlock, *J. Am. Chem. Soc.*, **95**, 299 (1973).

(18) E. L. Mead, R. G. Sutherland, and R. E. Verrall, *Can. J. Chem.*, **53**, 2394 (1975).

(19) J. R. Mckee, C. L. Christman, W. D. O'Brien, Jr., and S. Y. Wang, *Biochemistry*, **16**, 4651 (1977).

(20) E. J. Hall, "Radiobiology for the Radiologist", 2nd ed., Harper and Row, New York, 1978, Chapter 1, p 11.

(21) It should be noted that in clinical applications the ultrasound frequency used is above 0.5 MHz. Although the threshold for cavitation at that frequency is orders of magnitude higher than that in the kHz range used in the present work (C. R. Hill, *J. Accoust. Soc. Am.*, **52**, 667 (1972)), cavitation can occur at above 0.5 MHz depending on the ultrasound intensity. See, for example, E. P. Armour and P. M. Corry, *Radiat. Res.*, **89**, 369 (1982); G. R. ter Haar and S. Daniels, *Phys. Med. Biol.*, **26**, 1145 (1981); R. E. Apfel, *Methods Exp. Phys.*, **19**, 355 (1981).

(22) E. G. Janzen in "Free radicals in biology", W. A. Pryor, Ed., Vol. *4*, Academic Press, New York, 1980, Chapter 4, p 115; M. J. Perkins, in *Adv. Phys. Organ. Chem.*, **17**, 1 (1980).

(23) P. Riesz and S. Rustgi, *Radiat. Phys. Chem.*, **13**, 21 (1979).

(24) K. Makino, *J. Phys. Chem.*, **84**, 1968 (1980).

(25) I. Rosenthal, M. M. Mossoba, and P. Riesz, *J. Magn. Reson.*, **45**, 359 (1981).

(26) Abbreviations: DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; PYBN, α-*tert*-butyl-α-(1-methyl-4-pyridinio)nitrone; POBN, α-4-pyridyl-*N*-*tert*-butylnitrone 1-oxide.