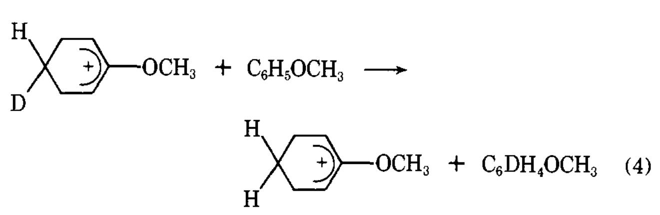
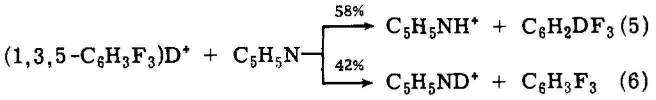


proton transfer processes (e.g., reaction 4 which proceeds to completion)[9] and a comparison of the relative extent of proton and deuteron transfer to stronger bases such as pyridine (e.g., reactions 5 and 6). With sufficiently acidic donors (e.g., D_3O^+ reacting with benzonitrile) protonation occurs on both the ring and the substituent. In this case reaction 4 is observed but does not proceed to completion. In the case of reactions 5 and 6 this situation is clarified by using an equilibrated population whereby several collisions with the aromatic species lead to deuterium transfer to the more basic site in the molecule.



$$(1,3,5\text{-}C_6H_3F_3)D^+ + C_5H_5N \begin{cases} \xrightarrow{58\%} C_5H_5NH^+ + C_6H_2DF_3 & (5) \\ \xrightarrow{42\%} C_5H_5ND^+ + C_6H_3F_3 & (6) \end{cases}$$

While necessary, it is evident from the data in Table I that ring protonation is not a sufficient condition to observe exchange. The exchange mechanism most likely involves transfer of the labile proton to D_2O forming an activated complex of D_2OH^+ with the aromatic compound. This species dissociates to regenerate the isotopically exchanged reactants. We are currently investigating a range of aromatic compounds including naphthalene, anthracene, and biphenyl as well as a variety of other saturated and unsaturated carbonium ions where the extent of exchange may clarify additional features of the reaction mechanism and provide other interesting structural information.

Acknowledgments. This work was supported in part by the Energy Research and Development Administration under Grant No. AT(04-3)767-8 and by the National Science Foundation under Grant No. NSF-GP-18383.

References and Notes

(1) K. Biemann, "Mass Spectrometry", McGraw-Hill, New York, N.Y., 1962, Chapter 5.

(2) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. I, Holden-Day, San Francisco, Calif., 1964, Chapter 2.

(3) D. F. Hunt, C. N. McEwen, and R. A. Upham, *Anal. Chem.*, **44**, 1292 (1972).

(4) J. L. Beauchamp, *Annu. Rev. Phys. Chem.*, **22**, 527 (1971).

(5) Only a single deuterium is exchanged per reaction as evidenced by the total disappearance of a product ion upon ejection of the ion having one less mass unit. Thus in reaction 3, H_2O is never a product.

(6) The decay of $C_6H_6D^+$ in Figure 1 is complicated, with the ion being more abundant at long times than expected for exponential decay. This behavior is attributed to the symmetrical proton transfer process analogous to reaction 4.

(7) F. W. McLafferty, "Interpretation of Mass Spectra", 2nd ed, W. A. Benjamin, New York, N.Y., 1973, p 109.

(8) L. D. Smithson, A. K. Bhattacharya, and C. Tamborski, *Org. Mass Spectrom.*, **4**, 1 (1970).

(9) Protonation of anisole on the ring is in accord with the proton affinity of phenol and anisole differing by only ~0.3 kcal/mol (R. T. McIver, private communication). Protonation on oxygen accompanied by a slow intramolecular hydrogen rearrangement might also lead to the observation of reaction 4. The rate of such a process would be very dependent on the internal energy of the ion and thus lead to a distribution of rearranged (reactive) and nonrearranged (nonreactive) species. The kinetics of reaction 4 were not consistent with a distribution of reactive and nonreactive species.

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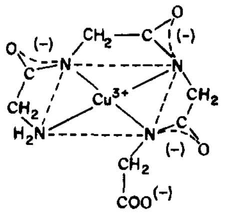
Characterization of a Readily Accessible Copper(III)-Peptide Complex

Sir:

The tripositive oxidation state of copper occurs in a number of compounds, many of which are not stable in aqueous solution. Thus, crystalline $NaCuO_2$ can be prepared[1] but it decomposes in solution in a few seconds.[2] Pulse radiolytic studies[3] have generated Cu(III)-aquo and Cu(III)-amine complexes, which are transient species with rapid rates of decay. Electrochemical preparation of Cu^{III} complexes of macrocyclic amines has been possible in acetonitrile solution but the complexes are unstable, undergoing spontaneous reduction to Cu^{II}.[4] Copper(III) intermediates have been proposed in the chloroiridate oxidation of copper(II)-oligopeptide complexes which leads to peptide oxidation and fragmentation.[5,6] Crystalline, highly insoluble Cu(III)-bis(biuret) and Cu(III)-bis(oxamide) compounds have been characterized.[7,8] Alkyl-substituted bis(biuretato) complexes of Cu^{III} were sufficiently soluble and stable in DMSO to permit measurements of their electronic spectra, NMR spectra, and polarographic properties,[8] but aqueous solutions were not prepared. Recent studies in this laboratory[9,10] of the autoxidation of copper(II)-peptide complexes suggested that relatively long-lived Cu(III)-peptide complexes could be formed in aqueous solution. In the present work we confirm that this is the case and show that with peptide complexes Cu^{III} is a much more accessible oxidation state than had been realized.

Cu(II)-tetraglycine can be oxidized quantitatively to $[Cu^{III}(H_{-3}G_4)]^-$ (I) by $IrCl_6{}^{2-}$. This oxidation is reversible with pH variations. If the iridium species are removed by anion exchange separation, the resulting solutions of $[Cu^{III}(H_{-3}G_4)]^-$ are slow to decompose in weakly acidic media. There are several types of evidence which show that the complex does indeed contain Cu^{III}. (1) The Cu^{II} electronic absorption spectrum is lost and an intense absorption band at 365 nm, characteristic of Cu^{III},[8] is formed as a result of the oxidation. (2) The EPR spectrum characteristic of the d^9 Cu^{II} disappears upon oxidation as expected for a diamagnetic d^8 Cu^{III} complex. The EPR signal slowly reappears as the Cu^{III} complex decomposes. (3) The oxidized complex is sluggish in its substitution reactions. This is characteristic of d^8 square-planar complexes such as the proposed $[Cu^{III}(H_{-3}G_4)]^-$ species. In solution the oxidized complex passes through a Chelex 100[11] ion exchange column which, under the same conditions, will quantitatively remove Cu^{II} from its tetraglycine complex. Similarly, the rate of reaction of acid with the oxidized complex is much slower than with $[Cu^{II}(H_{-3}G_4)]^{2-}$. (4) The proposed Cu^{III} complex is capable of oxidizing ferrocyanide ion, iodide ion, and sulfite ion. (5) The acid decomposition of the oxidized complex regenerates 50-65% of the tetraglycine that was initially present and releases small amounts of O_2. The above experimental observations strongly support the proposal that the metal center rather than the peptide is initially oxidized by $IrCl_6{}^{2-}$. In the decomposition reactions, however, some of the peptide is oxidized.



I

The molar absorptivity of the $[Cu^{III}(H_{-3}G_4)]^-$ complex

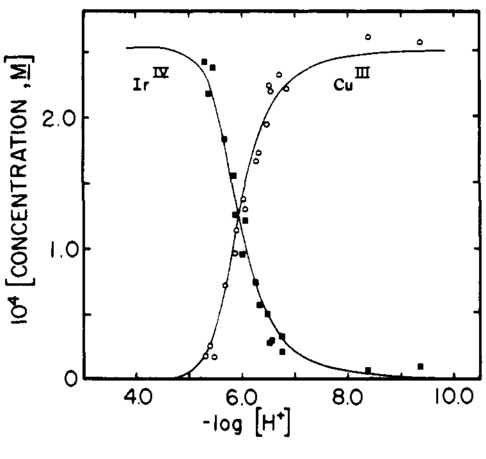


Figure 1. Formation of $[Cu^{III}(H_{-3}G_4)]^-$ (○) and corresponding loss of $Ir^{IV}Cl_6^{2-}$ (■) vs. $-\log [H^+]$. Solid lines are calculated on the basis of eq 1 and $K_{ox} = 2.7 \times 10^4$. $[CuG_4]_T = [Ir]_T = 2.51 \times 10^{-4}$ M, $\mu = 0.1$ M ($NaClO_4$), 25°C.

at 365 nm was determined by three independent methods as shown in Table I. The value determined after the separation of Cu^{II} by Chelex ion exchange indicates that the oxidized Cu(II)-tetraglycine complex contains a Cu^{III} center. The values of the molar absorptivity when determined by the other methods agree with this. Both the $IrCl_6^{2-}$ oxidation and the $(t\text{-}Bu)_2NO$ reduction experiments indicate one electron redox reactions of the copper-tetraglycine complexes. $IrCl_6^{2-}$ is a well-known one-electron oxidant while $(t\text{-}Bu)_2NO$ acts as a one-electron reductant.[12]

Figure 1 shows the redox-pH curve for the hexachloroiridate reaction with copper-tetraglycine. The concentration of $IrCl_6^{2-}$ is calculated from the absorbance at 490 nm (ϵ 4075 M^{-1} cm^{-1}) with corrections for the small overlap due to the 365-nm Cu^{III} peak, while the concentration of $[Cu^{III}(H_{-3}G_4)]^-$ is calculated from the absorbance at 365 nm (ϵ 7100 M^{-1} cm^{-1}) again with corrections from overlapping Ir^{IV} absorbance. The Ir^{III} and Cu^{II} absorbances are negligible. There is a 1:1 correlation between the loss of Ir^{IV} and the gain of Cu^{III}. The pH dependence is a result of the variable degree of protonation of the Cu(II)-tetraglycine complexes, while the Cu^{III} complex is present only as the triply deprotonated peptide complex, as shown in eq 1. The

$$\begin{array}{l} Cu^{II}(H_{-3}G_4)^{2-} + IrCl_6^{2-} \overset{K_{ox}}{\rightleftharpoons} Cu^{III}(H_{-3}G_4)^{-} + IrCl_6^{3-} \qquad (1) \\ \left[\begin{array}{c} \upharpoonleft\downharpoonright +H^+ \\ Cu(H_{-2}G_4)^- \\ \upharpoonleft\downharpoonright +H^+ \\ Cu(H_{-1}G_4) \\ \upharpoonleft\downharpoonright +H^+ \\ CuG_4^+ \\ \upharpoonleft\downharpoonright +H^+ \\ Cu^{2+} + HG_4 \end{array}\right] \end{array}$$

redox and the protonation equilibria are rapidly reversible and hence the ratio of Cu^{III}/Ir^{IV} can be shifted back and forth with change of pH. The solid curves in Figure 1 are calculated from eq 1 using the equilibrium constants for copper(II)-tetraglycine[13] and a redox equilibrium constant, K_{ox}, calculated to be $(2.7 \pm 0.2) \times 10^4$ from the experimental data. It was not possible to obtain a theoretical fit of the experimental data if any other Cu(III)-peptide complexes with a lower degree of deprotonation, such as $[Cu^{III}(H_{-2}G_4)]^{2-}$, were assumed to be present. The fortuitous situation of the pH equilibria off-setting a redox equilibrium permits an exact determination of the redox potential for $[Cu^{III,II}(H_{-3}G_4)]^{-,2-}$, provided the $[Ir^{IV,III}Cl_6]^{2-,3-}$ potential is known.

Table I. Molar Absorptivity of $[Cu^{III}(H_{-3}G_4)]^-$ at 365 nm

Method	Basis	ϵ_{365}, M^{-1} cm^{-1}
$IrCl_6^{2-}$ oxidation of $[Cu^{II}(H_{-3}G_4)]^{2-}$	Ir^{IV} used (1 equiv)	7100 ± 320
Separation of Cu^{II} by Chelex ion exchange[a]	Cu_T present[b]	7220 ± 310
Reaction $[Cu^{III}(H_{-3}G_4)]^-$ with $(t\text{-}Bu)_2NO$[a]	Loss of absorption at 365 nm	7120 ± 200

[a] After removal of $Ir^{III,IV}$ by anion exchange. [b] Determined after Cu^{III} decay by the Zincon method (R. M. Rush and J. H. Yoe, *Anal. Chem.*, 26, 1345 (1954)).

Latimer[14] lists a potential of 1.017 V for the $[Ir^{IV,III}Cl_6]^{2-,3-}$ couple (all values given here are in terms of the standard electrode potential, i.e., reduction potentials) which was from the early work of Dwyer, McKenzie, and Nyholm.[15] The E^0 value obtained by George and coworkers[16] was 0.867 V at 25° and $\mu = 0$, and at $\mu = 0.1$ the interpolated value was 0.904 V. The discrepancy between these results has been explained by Kravtsov and Petrova[17] as due to the hydrolysis of Ir^{III} in earlier studies. More recently Jackson and Pantony[18] have reported a potential of 0.899 V at 25° and $\mu = 0.1$. Because of the importance of the potential for the hexachloroiridate couple in our work we have reevaluated its value for our conditions by two methods. Using cyclic voltammetry we obtain a value of 0.892 ± 0.009 V at 25°, $\mu = 0.1$ M $NaClO_4$. Using a potentiometric titration with $[Fe(CN)_6]^{4-}$ we obtain a value of 0.892 ± 0.002 V at 25°C and $\mu = 0.1$ M $NaClO_4$. The reference potential taken for the hexacyanoferrate couple was 0.4164 ± 0.0005 V.[19] The latter redox couple was used to calibrate the electrode system for the cyclic voltammetry and potentiometric titrations. Hence, we are in general agreement with the lower values for the $[Ir^{IV,III}Cl_6]^{2-,3-}$ potential.

Using the equilibrium constant, K_{ox}, in eq 1 determined from Figure 1 and the E^0 value of 0.892 V for $Ir^{IV,III}$ gives an E^0 value of 0.631 V for eq 2.

$$Cu^{III}(H_{-3}G_4)^- + e \rightleftharpoons Cu^{II}(H_{-3}G_4)^{2-} \quad E^0 = 0.631 \text{ V} \quad (2)$$

Thus, this $Cu^{III,II}$ potential is 0.140 V less than the $Fe^{III,II}$(aquo) potential, so that Cu^{III} is an oxidation state which can be attained more readily than had been realized. The E^0 value is undoubtedly lowered by the strong electron-donor properties of the deprotonated-peptide groups and the favorable electronic configuration (d^8) for square-planar complexes. In neutral solution the $[Cu^{III}(H_{-3}G_4)]^-$ complex decomposes slowly with a half-life of about 2 hr at 25°. The complex decomposes faster in acid and in base.

The low potential and relatively high stability in aqueous solution for this Cu(III)-peptide complex is of special interest in biological redox reactions, because copper coordination to deprotonated peptide groups occurs readily. Molecular oxygen reacts with Cu(II)-tetraglycine in neutral solutions to generate $[Cu^{III}(H_{-3}G_4)]^-$, provided photochemical inhibition is avoided.[9] This reaction gives a pathway for the activation of molecular oxygen in its oxidation of peptides. In addition, the attractive possibility of two-electron transfer reactions between Cu^I and Cu^{III} is of interest as a biological redox pathway which would avoid high energy free radical intermediates. A Cu^{III}/Cu^I couple has been proposed in the reactions of galactose oxidase.[20]

Acknowledgment. This investigation was supported by Public Health Service Grant No. GM-19775 from the National Institute of General Medical Sciences.

References and Notes

(1) R. Scholder and U. Voelskow, *Z. Anorg. Allg. Chem.*, **266**, 256 (1951).
(2) J. S. Magee and R. H. Wood, *Can. J. Chem.*, **43**, 1234 (1965).
(3) D. Meyerstein, *Inorg. Chem.*, **10**, 638, 2244 (1971).
(4) D. C. Olson and J. Vasilekskis, *Inorg. Chem.*, **10**, 463 (1971).
(5) A. Levitzki, M. Anbar, and A. Berger, *Biochemistry*, **6**, 3757 (1967).
(6) A. Levitzki and A. Berger, *Biochemistry*, **10**, 64 (1971).
(7) J. J. Bour and J. J. Steggerda, *Chem. Commun.*, 85 (1967).
(8) J. J. Bour, P. J. M. W. L. Birker, and J. J. Steggerda, *Inorg. Chem.*, **10**, 1202 (1971).
(9) G. L. Burce, E. B. Panlago, and D. W. Margerum, *J. Chem. Soc., Chem. Commun.*, 261 (1975).
(10) G. L. Burce, Ph.D. Thesis, Purdue University, 1975.
(11) G. Shmuckler, *Talanta*, **12**, 281 (1965).
(12) A. K. Hoffman and A. T. Henderson, *J. Am. Chem. Soc.*, **83**, 4671 (1961).
(13) A. Kaneda and A. E. Martell, *J. Coord. Chem.*, **4**, 137 (1975).
(14) W. M. Latimer, "Oxidation Potentials", 2nd ed, Prentice-Hall, Englewood Cliffs, N.J., 1952, p 217.
(15) F. P. Dwyer, H. A. McKenzie, and R. S. Nyholm, *Proc. R. Soc. N.S. Wales*, **81**, 216 (1947).
(16) P. George, G. I. H. Hanna, and D. H. Irvine, *J. Chem. Soc.*, 3048 (1957).
(17) V. I. Kravtsov and G. M. Petrova, *Russ. J. Inorg. Chem.*, **9**, 552 (1964).
(18) E. Jackson and D. A. Pantony, *J. Appl. Electrochem.*, 1, 113 (1971).
(19) I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem.*, **39**, 945 (1935).
(20) G. A. Hamilton, R. D. Libby, and C. R. Hartzell, *Biochem. Biophys. Res. Commun.*, **55**, 333 (1973).

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Low Temperature Photochemical Studies on Acetyl Benzoyl Peroxide. The Observation of Methyl and Phenyl Radicals by Matrix Isolation Infrared Spectroscopy

Sir:

Attempts to photochemically produce radicals in rare gas matrices are usually frustrated by their tendency to recombine. This severely limits the spectroscopic techniques that can be used to detect the presence of a radical. In particular, relatively large concentrations of radicals are required for a reliable infrared identification. Here we report on matrix isolation studies which are capable of producing large concentrations of radicals. The method is related to that used by Box,[1] and Karch and McBride[2] in their elegant EPR and ENDOR studies. These authors generated radical pairs by photoelimination of a small stable molecule from a suitable precursor.

As an initial system, we have chosen diacyl peroxides which when irradiated with ultraviolet light cleave into radical pairs and carbon dioxide. The latter isolates the two simultaneously generated radicals from one another and suppresses their recombination. Warming the argon matrix from liquid helium temperatures to approximately 30 K is expected to "soften" the environment enough to remove the obstruction created by the CO_2 molecules thus allowing the radicals to recombine and act as their own trapping reagents.

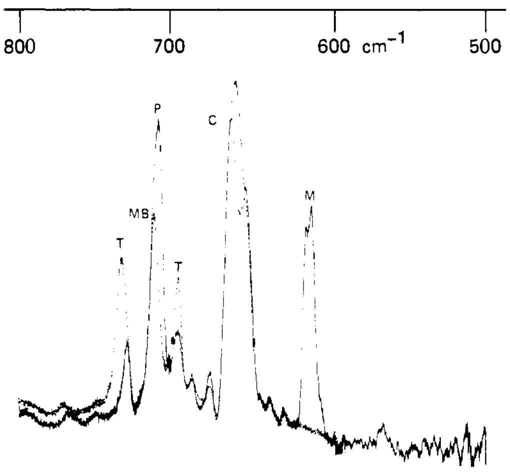


Figure 1. (a) The infrared spectrum of acetyl benzoyl peroxide after extensive irradiation with light longer than 3000 Å (—). (b) The infrared spectrum after warm-up to 30 K (- - -): legend, MB = methyl benzoate, C = carbon dioxide, M = methyl radical, P = phenyl radical.

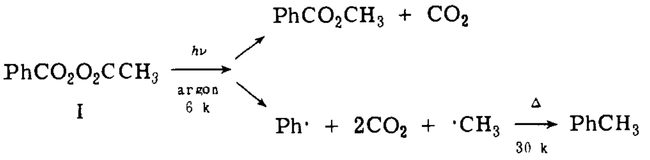
Table I. Fundamental Vibrational Frequencies of the Methyl Radical[a]

		ν_2	ν_3	ν_4
CH_3	*b*	611		
	c	617	1396	3162
	d	612	1385	3150
CD_3	*b*	463		
	c	463	1026	2381
	d	461	1029	2369

[a] ν_2 = out of plane bend, ν_3 = asymmetric in plane bend, ν_4 = asymmetric CH stretch. [b] Reference 9. Produced by the vacuum uv photolysis of methane. Spectra obtained in an argon matrix. [c] Reference 6. Pyrolysis of CH_3I, CD_3I, and $(CH_3)_2Hg$ in neon gas. Spectra obtained in a neon matrix. [d] Present study. Photolysis of $C_6H_5CO_2O_2CCH_3$, $C_6H_5CO_2O_2CCD_3$, and $C_6D_5CO_2O_2CCH_3$ in an argon matrix.

Acetyl benzoyl peroxide (I) was isolated in an argon matrix at 6 K and subsequently irradiated with a medium pressure mercury lamp in conjunction with a 10-cm water filter and a Corning No. 053 UV filter with no transmission below 2900 Å.[3] During the irradiation bands due to CO_2, methyl benzoate,[4] two very intense bands at 710, 612 cm^{-1} and weaker absorptions at 3150, 1385 cm^{-1} appeared. Upon warming the matrix to 30 K all of the bands produced during the irradiation (except those due to CO_2 and methyl benzoate) disappeared after several seconds with the simultaneous appearance of bands due to toluene.[4,5] These results are shown in Figure 1. In Table I literature values of matrix isolation experiments on the methyl radical are given for comparison. From these it follows that the absorptions observed in our experiments at 612, 1385, and 3150 cm^{-1} agree in band center and intensity assignment with the methyl radical. In support of the above claim the matrix photolysis experiments of I with deuterium substitution on the acetyl group produced bands at 461, 1029, and 2369 cm^{-1} due to CD_3 in complete accord with the independent studies of Snelson.[6] Thus, our results and Snelson's firmly establish the ground state vibrational spectrum of the methyl radical. Since it is known[2] that the irradiation of I yields methyl and phenyl radicals simultaneously and since we observe that upon warming the sample to 30 K methyl combines with an other species to produce toluene, we conclude that the intense band at 710 cm^{-1} which disappears synchronously with those assigned to the methyl radical is due to the phenyl radical.[7] We assign the 710-cm^{-1} band of the phenyl radical to a CH out of plane bending mode which are characteristically very intense in the infrared. Scheme I

Scheme I



$$\underset{\text{I}}{PhCO_2O_2CCH_3} \xrightarrow[\text{argon, 6 k}]{h\nu} \begin{cases} PhCO_2CH_3 + CO_2 \\ Ph\cdot + 2CO_2 + \cdot CH_3 \xrightarrow[30\ \text{k}]{\Delta} PhCH_3 \end{cases}$$