Electron Paramagnetic Resonance Study of the Reaction of Ag Atoms with C_{60} in Cyclohexane on a Rotating Cryostat[†]

James A. Howard,* Mauro Tomietto, and Della A. Wilkinson

Contribution from the Steacie Institute for Molecular Sciences, National Research Council of Canada, Ottawa K1A 0R9, Canada.

Received May 14, 1991

Abstract: An EPR study at 175 K of the paramagnetic products from reaction of vapor-deposited ^{107}Ag atoms with vapor-deposited C_{60} at 77 K, matrix isolated in cyclohexane, revealed the presence of a species with the isotropic magnetic parameters a_{107} = 228 ± 5 MHz and g = 1.9913 ± 0.0005. These parameters are consistent with the formation of the mononuclear silver(0) complex, AgC_{60}, which has most of the unpaired spin on the C_{60} ligand.

Introduction

The isolation and purification of buckminsterfullerene, C_{60}, in gram quantities[1-3] has allowed for more detailed studies of the physical and chemical properties of this new form of carbon. Several papers have been published illustrating the wide-ranging reactivity of this unusual molecule.[3-5] The addition of a functional group to the C_{60} molecule without causing disruption to the carbon framework has recently been shown by osmylation of C_{60}.[4] The hydrogenation by Birch reduction[3] to generate $C_{60}H_{36}$ and its dehydrogenation back to C_{60} illustrates the rich chemistry associated with the surface of this truncated icosahedron. In addition, the formation of $C_{60}La$,[5] where the lanthanum is thought to occupy the central cavity of the cage, presents the possibility of generating a wide range of endohedral C_{60} compounds where the atom or small molecule would occupy the inner cavity of the C_{60} molecule.[6] C_{60} has potential commercial use as a lubricant and as a catalyst support and when doped with alkali metals gives the first three-dimensional high-temperature organic superconductor.[7] The study of the possible interactions of C_{60} with metals is therefore of prime interest.

We report here the first electron paramagnetic resonance (EPR) spectroscopic evidence for the formation of a mononuclear transition metal–C_{60} complex, AgC_{60}, which has been prepared by reaction of vapor-deposited Ag atoms with the vapor-deposited C_{60}/C_{70} mixture and with vapor-deposited pure C_{60} in a cyclohexane matrix on a rotating cryostat at 77 K.

Experimental Section

Silver powder enriched to 98.2% in the 107 isotope was purchased from Oak Ridge National Laboratory, Oak Ridge, TN, and natural silver powder was a gift from Dr. C. M. Hurd (NRCC). Cyclohexane (Anachemia) was degassed prior to use. After an initial gift of C_{60}/C_{70} from Yan Chai (Rice University), the C_{60}/C_{70} soot was purchased from Texas Fullerenes Corporation. Soxhlet extraction from the soot gave a mixture of C_{60}/C_{70} (ratio 4:1) from which the pure C_{60} was obtained by chromatography according to published procedures.[8]

The rotating cryostat used for these experiments has previously been described.[9,10] Silver was evaporated from a molybdenum pouch suspended from the molybdenum electrodes of a furnace resistively heated to 1000–1100 °C, and C_{60}/C_{70} was sublimed from a graphite furnace resistively heated to 500–550 °C. These furnaces were situated in the outer housing of the cryostat so that there was no danger of pyrolysis of C_{60}/C_{70} even though we have shown that C_{60} is stable in the absence of oxygen to 1000 K.[11]

EPR spectra were recorded at 77 K on a Bruker ESP 300 spectrometer. The microwave frequency was measured with a Systron-Donner Model 6016 frequency counter and the magnetic field with a Varian NMR gaussmeter. Spectra were recorded at higher temperatures by use of a variable-temperature accessory. All EPR parameters were calculated from exact solutions of the spin Hamiltonian with use of computer programs made available by Drs. J. R. Morton and K. F. Preston (NRC, Ottawa).

Results and Discussion

A mixture of C_{60} and C_{70}, in the ratio of 4:1 according to FAB mass spectrometry, gave two isotropic lines centered at g = 2.0023 and 2.0005, with peak-to-peak line widths (ΔH_{pp}) of 3.6 and 2.2 MHz and relative intensities of 3.5:1. Since the fullerenes C_{60} and C_{70} are closed-shell compounds and are expected to be diamagnetic, these features must result from impurities within the C_{60}/C_{70} sample. The signal-to-noise ratio (S:N) of the most intense line was 40:1 without computer enhancement. Sublimation of this C_{60}/C_{70} mixture onto an excess of cyclohexane at 77 K gave an EPR spectrum consisting of the same two lines but with a reduced S:N of 8:1. Removal of the paramagnetic impurities required column chromatography since sublimation resulted in only limited success. The EPR spectrum of purified C_{60} showed no signal that was consistent with the proposed "soccerball" structure.

In contrast, Figure 1a shows the EPR spectrum produced by reacting vapor-deposited ^{107}Ag atoms ($I = {}^1/_2$) with vapor-deposited C_{60}/C_{70} in cyclohexane. The spectrum had two areas of interest that were not observed in the spectrum from vapor-deposited C_{60}/C_{70} in cyclohexane: (i) a doublet with transitions at 3042 and 3649 G (ν = 9450 MHz) and (ii) a broad central anisotropic feature centered at 3375 G. The doublet observed in the spectrum at 77 K was attributed to ^{107}Ag atoms trapped in the cyclohexane matrix. The EPR parameters obtained, a_{107} = 1690 ± 5 MHz and g = 2.0017 ± 0.0005, can be compared with the parameters of ^{107}Ag atoms in adamantane (a_{107} = 1681 MHz and g = 2.0018) as well as those in cyclohexane (a_{107} = 1723.2 MHz and g = 2.0021).[12]

On warming the sample from 77 to 175 K the central region resolved into the three distinct features shown in Figure 1b. These were (i) a doublet of sharp almost isotropic lines with transitions at 3349 and 3431 G, spectrum **A**, (ii) a narrow singlet at 3381 G, labeled **B**, and (iii) a broad central feature at 3363 G, labeled **C**. We conclude that the doublet structure of **A** arose because of interaction of an unpaired electron with a single ^{107}Ag nucleus, and the magnetic parameters a_{107} = 228 ± 5 MHz and g = 1.9913 ± 0.0005 were obtained from an exact solution of the Breit–Rabi

---

[†] Issued as NRCC No. 32919.

(1) Kratschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354.
(2) Taylor, R.; Hare, J. P.; Adult-Sad, a, A. K.; Kroto, H. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1423.
(3) Haufler, R. E.; Conceicao, R.; Chibante, L. P. F.; Chai, Y.; Byrne, N. E.; Flanagan, S.; Haley, M. M.; O'Brien, S. C.; Pan, C.; Xiao, Z.; Billups, W. E.; Clufolini, M. A.; Hauge, R. H.; Margrave, J. L.; Wilson, L. J.; Curl, R. F.; Smalley, R. E. *J. Phys. Chem.* **1990**, *94*, 8634.
(4) Hawkins, J. M.; Lewis, T. A.; Loren, S. D.; Meijer, A.; Heath, J. R.; Shibato, Y.; Saykally, J. R. *J. Org. Chem.* **1990**, *55*, 6250.
(5) Heath, J. R.; O'Brien, S. C.; Zhang, Q.; Liu, Y.; Curl, R. F.; Kroto, H. W.; Tittel, K. F.; Smalley, R. E. *J. Am. Chem. Soc.* **1985**, *107*, 7779.
(6) Cioslowski, J. *J. Am. Chem. Soc.*, **1991**, *113*, 4139.
(7) Hebard, A. F.; Rosseinsky, M. J.; Haddon, R. C.; Murphy, D. W.; Glarum, S. H.; Palstra, T. T. M.; Ramirez, A. P.; Kortan, A. R. *Nature* **1991**, *350*, 600.
(8) Allemand, P.-M.; Koch, A.; Wudl, F.; Rubin, Y.; Diederich, F.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 1050.
(9) Bennett, J. E.; Thomas, A. *Proc. R. Soc. London, Ser. A* **1964**, *280*, 123.
(10) Bennett, J. E.; Mile, B.; Thomas, A. L.; Ward, B. *Adv. Phys. Org. Chem.* **1970**, *8*, 1.
(11) Tse, J. S.; Klug, D. D.; Wilkinson, D. A. Submitted for publication.
(12) Buck, A. J.; Mile, B.; Howard, A. J. *J. Am. Chem. Soc.* **1983**, *105*, 3381.

0002-7863/91/1513-7870$02.50/0 Published 1991 by the American Chemical Society

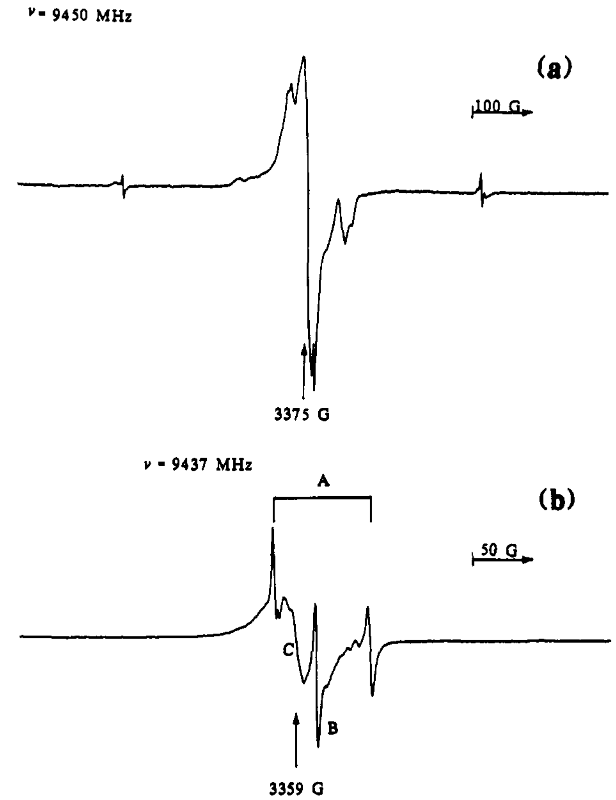

Figure 1. EPR spectra of ^{107}Ag and C_{60}/C_{70} in cyclohexane at 77 K (a) and at 175 K (b).

equation. Features **B** and C showed no resolved hyperfine interaction and were centered at g = 1.9942 ± 0.0005 and 2.0048 ± 0.0005, respectively.

The unpaired spin population in the 5s orbital of the Ag nucleus of A, ρ_{5s}, was calculated to be 0.13 and was obtained by dividing a_{107} by A^0 = 1831 MHz, the parameter for unit spin population in a Ag 5s orbital.[13] Most of the remainder of the unpaired spin population, approximately 0.87, probably resides in the orbitals of a ligand which we conclude to be C_{60} although there could be some unpaired spin in a Ag 5p. This would not necessarily produce an anisotropic spectrum because of the low value of P^0 for ^{107}Ag.[14] Consequently the carrier of spectrum A was identified as AgC_{60}. The negative value of Δg (−0.0011) is consistent with this assignment since C_{60}^- has a negative g shift of −0.0032[15] from an intramolecular spin–orbit interaction between the ground state and a low-lying empty excited state although a matrix contribution to the spin–orbit coupling cannot be ruled out.

The silver atom of this novel organometallic derivative of C_{60} could be bound to the organic molecule in one of three ways. The silver could form a bond to a single carbon, C_α, in which case the unpaired spin population would be delocalized in the region near C_α. Alternatively the silver atom could be located on one of the "benzene" rings in a manner previously observed for the species $Ag(C_6H_6)$.[12] This situation has been dismissed since the hyperfine interaction observed for the C_{60} complex of 228 MHz bears no similarity to that seen in the Ag–benzene species which has a hyperfine interaction of 1698 MHz.[12] Finally, the silver atom could be migrating over the entire surface of the C_{60} molecule. The unique geometry of the C_{60} molecule means that the truncated icosahedron can be considered as a sphere of aromaticity with the unpaired electron delocalized over the surface of the sphere. If this fluxional description were true it should be possible to observe the hyperfine interactions of the ^{13}C nuclei of the C_{60} molecule with Ag since $^{12}C_{59}\,^{13}C$ represents ~54% of C_{60}. These satellites were not detected either because of anisotropic line broadening or because the structure of AgC_{60} contains a single Ag–C bond.

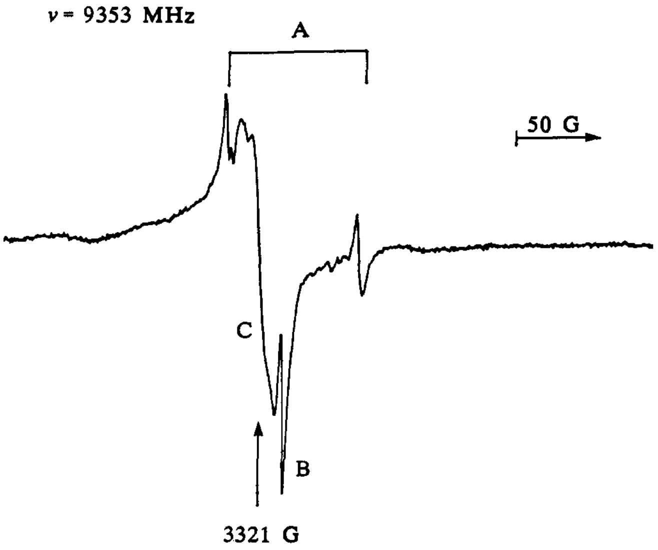

Figure 2. EPR spectrum of ^{107}Ag and C_{60} in cyclohexane at 175 K.

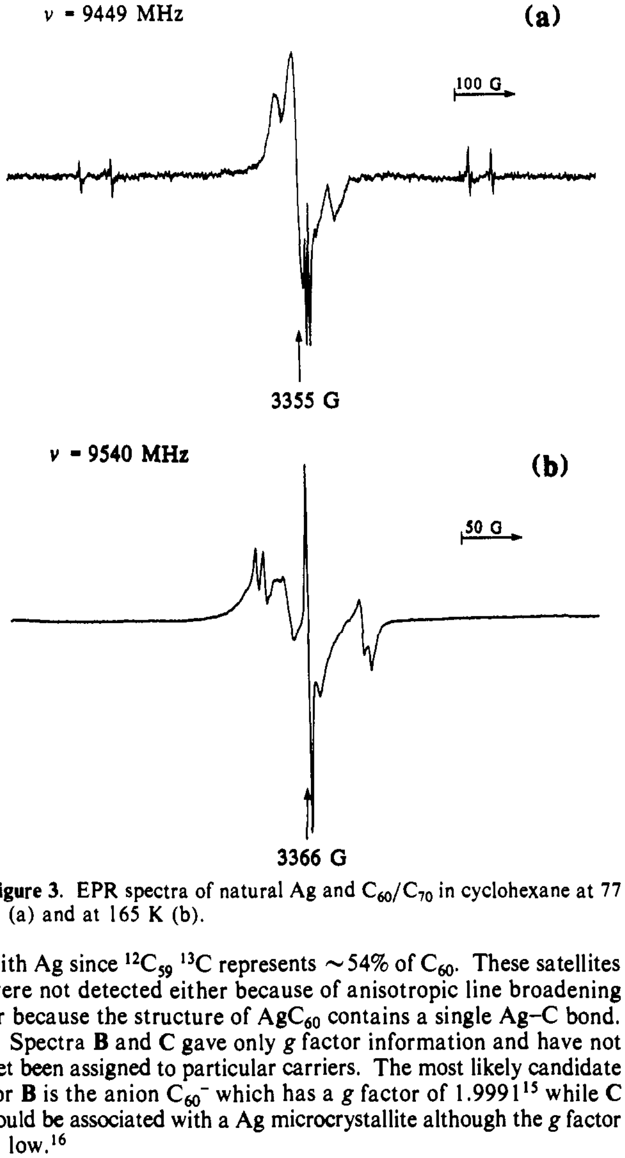

Figure 3. EPR spectra of natural Ag and C_{60}/C_{70} in cyclohexane at 77 K (a) and at 165 K (b).

Spectra **B** and C gave only g factor information and have not yet been assigned to particular carriers. The most likely candidate for **B** is the anion C_{60}^- which has a g factor of 1.9991[15] while C could be associated with a Ag microcrystallite although the g factor is low.[16]

The reaction of Ag atoms with C_{60} was repeated with use of a pure sample of C_{60}, and the EPR spectrum shown in Figure 2

---

(13) Morton, J. R.; Preston, K. F. *J. Magn. Reson.* **1978**, *30*, 577.

(14) Howard, J. A.; Mile, B.; Morton, J. R.; Preston, K. F. *J. Phys. Chem.* **1986**, *90*, 2027.

(15) Allemand, P.-M.; Srdanov, G.; Koch, A.; Khemani, K.; Wudl, F.; Rubin, Y.; Diederich, F.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 2780.

(16) Chenier, J. H. B.; Joly, H. A.; Howard, J. A.; Mile, B. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 3329.

was obtained on warming the sample to 175 K. This spectrum has the same doublet and singlet features that were observed with a C_{60}/C_{70} mixture, confirming that the spectral features discussed above are due to the interaction of Ag atoms with C_{60} rather than any other carbon cluster present in the mixed sample.

Reaction of natural silver (51% ^{107}Ag and 49% ^{109}Ag) with C_{60} in cyclohexane at 77 K gave the EPR spectrum shown in Figure 3a and has two isotropic doublets from trapped Ag atoms with $a_{109} = 1947 \pm 5$ MHz and $g = 2.0017 \pm 0.0005$ and $a_{107} = 1690 \pm 5$ MHz and $g = 2.0017 \pm 0.0005$ as well as a complex central feature. Each of the lines of the Ag doublets had satellites that were attributed to forbidden proton "spin-flip" lines from simultaneous flipping of the unpaired electron and the protons of the cyclohexane matrix.[17] On warming this sample to 165 K two doublets became apparent with the parameters $a_{109} = 263 \pm 5$ MHz and $g = 1.9913 \pm 0.0005$ and $a_{107} = 228 \pm 5$ MHz and $g = 1.9913 \pm 0.0005$ (Figure 3b). The ratio of these hfi, 1.154, was close to the ratio of the magnetic moments of ^{109}Ag and ^{107}Ag, and they were attributed to the formation of species A, AgC_{60}, from natural Ag atoms. In addition the lines corresponding to species B and C were also present.

All the spectral lines assigned to AgC_{60} disappeared in the temperature range 175–185 K, and all samples when warmed to 280 K gave identical EPR spectra consisting of a single isotropic line with $g = 2.0023 \pm 0.0002$.

As a result of the successful preparation of AgC_{60} by low-temperature matrix-isolation techniques we are investigating the reaction of C_{60} with other metal atoms in inert hydrocarbon matrices on a rotating cryostat. Preliminary results suggest that Au atoms give an EPR spectrum that can be assigned to AuC_{60} while Cu atoms do not appear to give CuC_{60}. The results of these investigations will be published in a subsequent paper.

Acknowledgment. We thank Dr. H. W. Kroto (University of Sussex) and Yan Chai (Rice University) for the kind donation of 10 mg of pure C_{60} and for a sample of C_{60}/C_{70}, respectively. We also thank Lise Hughes (NRCC) for help with the chromatography and Dr. F. P. Cooper (NRCC) for performing FAB mass spectroscopy. An award from the Summit Fellowship program is gratefully acknowledged for providing financial support (D. A.W).

---

[17] Narayana, M.; Li, A. S. W.; Kevan, L. *Chem. Phys. Lett.* **1981**, *30*, 208.

Vanadate Interactions with Bovine Cu,Zn-Superoxide Dismutase As Probed by ^{51}V NMR Spectroscopy

Lisa Wittenkeller,[†] Aida Abraha,[†] Ravichandran Ramasamy,[†,1] Duarte Mota de Freitas,[*,†] Lisa A. Theisen,[‡] and Debbie C. Crans[*,‡]

Contribution from the Departments of Chemistry, Loyola University of Chicago, Chicago, Illinois 60626, and Colorado State University, Fort Collins, Colorado 80523.
Received October 30, 1990

Abstract: The interaction of various vanadate anions with native and chemically modified derivatives of bovine Cu,Zn-superoxide dismutase (Cu,Zn-SOD) was studied by ^{51}V NMR spectroscopy to obtain information on the substrate selectivity of Cu,Zn-SOD. Using quantitative ^{51}V NMR measurements and speciation analysis, we determined the concentrations of the rapidly equilibrating vanadate derivatives that form in solutions containing Cu,Zn-SOD. As the concentration of native Cu,Zn-SOD increased, the total visible vanadium concentration decreased and the intensity of a broad signal assigned to a vanadate–protein (V-SOD) complex increased. Because of the quadrupolar nature of the ^{51}V nucleus, most of the bound vanadium was invisible and only 15–30% of the V-SOD complex was visible in the ^{51}V NMR spectra. Significant changes in the line widths of the ^{51}V NMR resonances of vanadate anions were observed. Several quantitative models were applied to the intensity changes to determine which anions formed V-SOD complexes. Native Cu,Zn-SOD bound four vanadium atoms per subunit (or eight vanadium atoms per enzyme molecule). Bovine Cu,Zn-SOD has the greatest affinity for the vanadate tetramer and forms two specific complexes, V_4·SOD and $(V_4)_2$·SOD. The affinity constants for these vanadate–protein complexes are 2×10^7 M^{-1} and 5×10^6 M^{-1}, respectively. The affinities of the vanadate dimer and monomer for native Cu,Zn-SOD were less than that of the vanadate tetramer but more than that of phosphate. Addition of vanadate to native Cu,Zn-SOD was also monitored by difference UV/vis spectroscopy. No changes in absorbance maxima were observed, indicating that vanadate does not bind directly to Cu(II) in the enzyme. When Cu,Zn-SOD was modified at arginine-141, the vanadate tetramer still bound to the enzyme with drastically reduced affinity; much weaker interactions were observed between the vanadate tetramer and Cu,Zn-SOD modified at lysines-120 and -134. Our data suggest that the vanadate tetramer binds to the two lysine residues in the solvent channel of Cu,Zn-SOD. The vanadate tetramer is the first large anion that is reported to have such a high affinity for native bovine Cu,Zn-SOD.

Introduction

Cu,Zn-superoxide dismutase (Cu,Zn-SOD) is an abundant protein in most eukaryotic cells, with high water solubility and thermal stability.[2] Apo-SOD (the metal-free protein) can bind several metal ions, in place of the native Cu^{2+} and Zn^{2+} ions, in distorted square-planar and tetrahedral configurations.[3,4] Despite extensive biophysical studies of Cu,Zn-SOD, the relationship between its physical properties and its physiological role in vivo is not well understood.[5,6] The proposed enzymatic function of Cu,Zn-SOD is based on the observation that this protein catalyzes superoxide disproportionation.[6]

The putative substrate of Cu,Zn-SOD, superoxide, is an anion. Anion-binding studies of Cu,Zn-SOD have provided key infor-

---

[†] Loyola University of Chicago.

[‡] Colorado State University.

[1] Present address: Department of Chemistry, University of Texas at Dallas, Richardson, TX 75083.

[2] Valentine, J. S.; Mota de Freitas, D. *J. Chem. Educ.* **1985**, *62*, 990.

[3] Ming, L.-J.; Valentine, J. S. *J. Am. Chem. Soc.* **1990**, *112*, 4256.

[4] Ming, L.-J.; Valentine, J. S. *J. Am. Chem. Soc.* **1990**, *112*, 6374.

[5] Fee, J. A. *Trends Biochem. Sci.* **1984**, *7*, 84.

[6] Fridovich, I. *Acc. Chem. Res.* **1982**, *15*, 200.

0002-7863/91/1513-7872$02.50/0