this and established an apparent K_I of 20 μM.[17] Incubation of the enzyme with 250 μM **2** for 45 min, followed by removal of the inhibitor, gives no recovery of activity.[18] Furthermore, treatment with 4 M urea and inhibitor removal also fails to restore activity,[18] thereby demonstrating that the inactivation is irreversible and probably not due to a slow-binding inhibitor with a high dissociation constant. Preincubation of PHM with substrate (D-Phe-L-Phe-Gly) (**1**)) and **2** shows protection of the enzyme in proportion to the amount of added substrate, which indicates that **2** acts at the active site of the enzyme. Treatment of PHM with **2** in the absence of Cu^{II} and ascorbate for 1 h followed by removal of the inhibitor results in no significant loss of activity. The requirement that all cofactors be present for inactivation to occur illustrates that **2** is a mechanism-based inhibitor.

Possible mechanisms of action for **2** are depicted in Scheme II. Abstraction of the α-hydrogen from the D-vinylglycine residue produces a conjugated radical at the α-carbon. Direct hydroxylation (path A) would generate a vinylglycine carbinol amide that can cleave to give the peptide amide and the α-keto acid, a potential alkylating species. Oxidative electron transfer (path B) could form a very reactive *N*-acyl iminium species that could result in the covalent attachment of the inhibitor to the enzyme. Path C involves interaction of the resonance-stabilized radical at the distal primary carbon with amino acid residues in the enzyme active site (e.g., hydrogen abstraction). To gain further insight, the inhibitor was resynthesized with a fluorescent 5-(dimethylamino)-1-naphthalenesulfonyl (dansyl) group attached to the amino terminus. Compound **5** also irreversibly inhibits PHM. A large sample of the enzyme was inactivated with this fluorescent tripeptide, a small amount of active PHM was then added to aid in isolation, and the inactivated enzyme was separated from **5** by gel filtration chromatography. Fractions containing amidating activity were pooled and concentrated as were those which had a UV absorption at 250 nm (λ_{max} of **5**). Spectrofluorimetric analysis of these samples showed no fluorescence associated with the PHM fractions above the minimum level of detection (ca. 5 pmol). A single late-eluting fraction which did fluoresce was further analyzed by HPLC and found to contain the tripeptide **5**.

These results indicate that the substrate analogues **2** and **5** inactivate PHM by a process that results in cleavage of the terminal portion of the peptide substrate (e.g., path A, Scheme II).[19] Current work on the detailed mechanism of this inactivation, along with studies on a variety of other inhibitors of PHM, will be described later.

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Supplementary Material Available: Schemes for the syntheses of compounds **2-5** (4 pages). Ordering information is given on any current masthead page.

(17) The enzyme was isolated as described previously.[7a] Preincubation mixtures (250 μL) contained 50 μL of enzyme and varying amounts of **2** in a cocktail of 1 mM ascorbate, 5 mM $CuSO_4$, 25 mM KI, 0.125 mg/mL catalase, and 1 mg/mL BSA in 50 mM Na_2HPO_4/NaH_2PO_4 and 200 mM NaCl, pH 6.8 buffer. Aliquots (25 μL) of the preincubated mixtures were diluted into 475-μL solutions containing 1.26 μM D-Phe-L-Phe-[1,2-^{14}C]Gly (specific activity 113 μCi/μmol) and 83 μM unlabeled **1** (10 × K_M) in the same cocktail and incubated for 1 h. The percent of substrate converted was determined as described previously.[9]

(18) The inhibitor was removed by three dilution/concentration steps using an Amicon (Beverly, MA) Centricon-30 ultrafiltration device. Uninhibited PHM remains active during this procedure.

(19) Path B is also possible if cleavage of the *N*-acyl imine bond occurs.

A Novel FeC_{60} Adduct in the Solid State

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The 7-Å diameter cavity in buckminsterfullerene has long been considered as a possible host for various atoms and molecules.[1] There have been several attempts to incorporate metal atoms such as La into this cavity, and these studies[2-6] have mainly employed mass spectrometry for characterization. The question of whether the metal atom is inside or outside the spheroidal hollow cage in these species has not been fully resolved. Freiser and co-workers[4] have generated an FeC_{60} complex in the gas phase by means of a ligand-exchange reaction and have also characterized NiC_{60} and $Ni(C_{60})_2$ in the gas phase. One example where an atom has been entrapped in C_{60} is that where a high-energy beam of C_{60} is made to interact with helium to give HeC_{60} in the gas phase.[5] Chai et al.[6] have recently prepared fullerenes with La trapped in the cages by laser vaporization. Metal complexes of C_{60} where the metal is present outside the cavity have been prepared by solution methods and characterized.[7,8] We investigated an iron adduct of C_{60}, FeC_{60} (I), where iron atoms were introduced during the contact-arc vaporization of graphite, expecting that this would result in the entrapment of Fe in the cavity. In order to understand some of the unique features of this adduct, we have compared its properties with those of solid FeC_{60} (II), where Fe is clearly bound externally to the fullerene.

In our effort to obtain the Fe adduct of C_{60} with the metal inside the cage, we carried out the contact-arc vaporization of graphite in an atmosphere of $Fe(CO)_5$. The arc chamber was pumped to a base vacuum of $\sim 10^{-6}$ Torr, and He gas was admitted to about 50 Torr through a needle valve. $Fe(CO)_5$ was admitted to the system from a reservoir at the same rate as He. The soot[9] was washed with diethyl ether, the residue was Soxhlet-extracted with toluene for about 3 h, and the extract was vacuum-dried at 380 K to remove any volatile iron compounds. The mass spectrum of the product of solvent extraction[10] showed the presence of mass peaks other than those due to C_{60} and C_{70}, which could be ascribed to FeC_{60}. The sample showed an additional spot in the TLC other than those due to C_{60} and C_{70} with an R_f value close to that of C_{70}. After C_{60} was separated out by column chromatography, the new product was eluted along with a small proportion of C_{70}.

The mass spectrum of the new product (in mixture with C_{70}) obtained after column chromatography (Figure 1) showed peaks at 776, 752, and 728, ascribable to FeC_{60}, FeC_{58}, and FeC_{56}, besides peaks at 720 and 840;[11] a peak at 388 was seen due to

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(9) The ^{57}Fe Mössbauer spectrum of the soot showed 6-finger patterns attributable to magnetic Fe_3C and α-Fe along with a singlet due to a new species.
(10) The mass spectrum of the residue left over after the solvent extraction did not show any volatile species up to 400 °C.
(11) An intense peak at m/z 720 is found in the mass spectrum of pure C_{70} as well.

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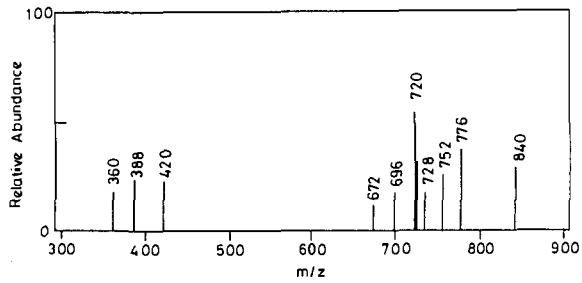

Figure 1. Mass spectrum of FeC_{60} (I) (in mixture with C_{70}) from the gas-phase reaction. Peaks at 840, 720, 696, and 672 arise from the parent fullerenes.

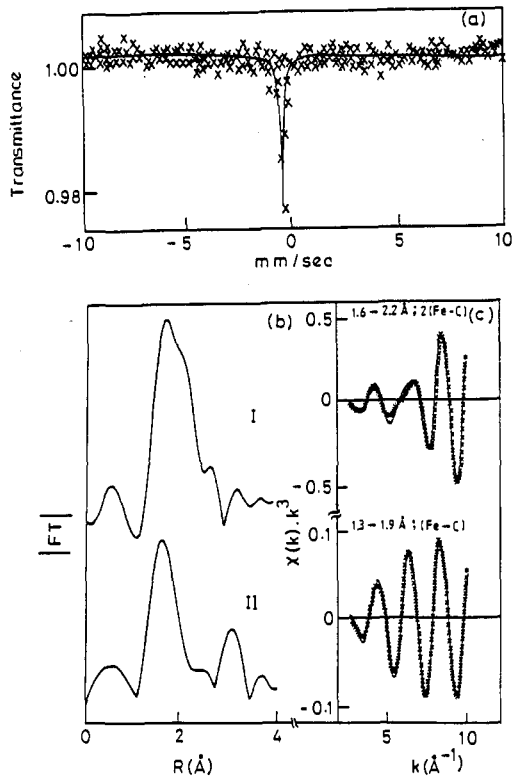

Figure 2. ^{57}Fe Mössbauer spectrum of FeC_{60} (I) (a); Fourier transforms of the Fe-K EXAFS of FeC_{60} (I and II) (b); inverse-transformed EXAFS data (c).

the half-mass of FeC_{60}. The presence of peaks due to C_2 and C_4 losses in the decreasing order of intensity suggests that Fe may be internally bound to the C_{60} frame. Similar C_2 losses found in HeC_{60} and LaC_{60} have been attributed to the entrapment of He and La in the hollow cage.[5,6] The ^{57}Fe Mössbauer spectrum[9] of the adduct[12] shows a singlet with an isomer shift of -0.083 mm/s with respect to α-Fe (Figure 2a), characteristic of iron in a near-zero oxidation state. The sharpness of the spectrum suggests a strongly bound Fe species. The compound was paramagnetic over the 10-300 K range, and its electronic absorption spectrum showed a slight red shift relative to that of C_{60}. The IR spectrum showed little change from that of C_{60}.

Unlike the FeC_{60} (I) prepared by the gas-phase reaction, solid FeC_{60} (II) where Fe was bound externally to the fullerene[13] showed a doublet in the Mössbauer spectrum with an isomer shift of 0.45 mm/s due to Fe(III); magnetic susceptibility measurements showed evidence for antiferromagnetic interaction. Both the electronic and infrared spectra were different from those of C_{60}. Furthermore, the mass spectrum did not show C_2 losses.[14]

We have examined the structures of the two FeC_{60} adducts (I and II) by means of Fe-K EXAFS measurements by employing a Rigaku spectrometer. Figure 2b shows the Fourier transforms of the EXAFS data obtained with k_{min} 3.0 and k_{max} 12.0 Å^{-1} after weighing the data by k^3. Analysis of the EXAFS data, including curve fitting of the inverse-transformed data (employing ferrocene as the reference), gave a good fit (Figure 2c) with two coordinations for Fe at Fe-C distances of 2.06 and 2.34 Å for FeC_{60} (I) with lower Debye-Waller factors ($\Delta\sigma^2$ = 0.0002 Å^2). Two such short Fe-C distances can only be reconciled if Fe is in the cage. Any adduct with Fe bound externally would show a single short Fe-C distance, as indeed found by us from the analysis of the EXAFS of FeC_{60} (II) prepared from solution reaction (Figure 2b). In this solid, where Fe is externally bound, we find Fe-C distances of 2.03 and 3.46 Å with $\Delta\sigma^2$ of 0.0003 and 0.001 Å, respectively.

The near-zero oxidation state of Fe, C_2 losses in the mass spectrum, the occurrence of the two Fe-C distances close to each other, as well as some of the other features indicate that FeC_{60} (I) obtained by the gas-phase reaction during arc vaporization of graphite is likely to be an endohedral species with the Fe atom inside the cage of C_{60}. Further studies on the various FeC_{60} adducts are in progress.

Registry No. Fe^IC_{60}, 137433-48-8; $Fe(CO)_5$, 13463-40-6; C_{60}, 99685-96-8; $FeCl_2$, 7758-94-3; NaC_{60}^+, 137718-24-2; Na, 7440-23-5; graphite, 7782-42-5.

(12) The sample gave a single spot in TLC and satisfactory analysis for Fe (7.2%) and C (92%). A preliminary study of the powder X-ray diffraction pattern of FeC_{60} (I) suggests that a primitive tetragonal structure (a = 15.43 Å, c = 9.08 Å) may be preferable over the FCC structure. The strongest (221) reflection could be simulated with Fe atoms in the cage 8 Å apart.

(13) The solid Na compound of C_{60} prepared by the reaction of Na with C_{60} in toluene solution was reacted with $FeCl_2$ in THF solution to obtain solid FeC_{60} (anal. Fe 7.2%).

(14) We have sought to prepare $(C_{60})_2Fe$ by reacting the monosodium salt of C_{60} with $FeCl_2$ in THF solution. A brown solid thus obtained has a different electronic spectrum. Electron-impact mass spectroscopy unfortunately seems to fragment the species. Fe-C distances in this ferrocene analogue cannot be distinguished from those of FeC_{60} (II).

Nanoarchitectures. 1. Controlled Synthesis of Phenylacetylene Sequences

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Well-defined oligomeric sequences are valuable models for investigating how physical properties depend on molecular size and chemical structure. For example, controlled sequences have recently been used to study cooperative motions[1] and microphase separation[2] of copolymers. Monodisperse sequences have also been used to study the electronic properties of conjugated chains, such as optical nonlinearity[3] and band gap.[4] We have found that well-defined oligomers are valuable for preparing very large two- and three-dimensional molecular frameworks.[5] Outside the realm

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