that it undergoes a facile quasi-reversible one-electron oxidation (cyclic voltammetry in DMF; $E_{1/2}$ = −587 mV vs Ag/Ag^+; n = 1). Using ferricinium ion as oxidant, we were able to isolate a brownish-green mixed-valence compound $[Cu^ICu^{II}(UN\text{-}O^-)]^{2+}$ (**3**).[30] This $S = {}^1/_2$ species has a magnetic moment μ_{eff}/Cu_{RT} = 2.0 ± 0.1 μ_B, no low-energy intervalence charge-transfer band is observed,[31] and it exhibits a four-line EPR spectrum ($g_\parallel$ = 2.25, $A_\parallel$ = 155 × 10^{-4} cm^{-1}; 77 K, CH_2Cl_2/C_7H_8) (Figure 1a). This latter behavior suggests a localized valence-trapped Cu(I)/Cu(II) structure for **3**, similar to that seen for a number of other RO^- bridged mixed-valence dicopper complexes.[32]

As followed by UV-vis spectroscopy at −80 °C, bubbling $[Cu^ICu^{II}(UN\text{-}O^-)]^{2+}$ (**3**) with O_2 causes a change to bright green (spectrum e; λ_{max} = 404 nm, ϵ = 5400 M^{-1} cm^{-1} (Figure 2); $O_2^- \rightarrow$ Cu(II) (LMCT?)). The product is formulated as a superoxodicopper(II) complex $[Cu_2(UN\text{-}O^-)(O_2^-)]^{2+}$ (**4**), consistent with manometric measurements indicating $3/O_2$ = 1.1 ± 0.1. The binding of O_2 to **3** is reversible; via the application of a vacuum (with brief warming), several oxygenation/deoxygneation cycles can be effected and followed spectrophotometrically. An EPR spectrum (77 K, CH_2Cl_2/C_7H_8) of $[Cu_2(UN\text{-}O^-)(O_2^-)]^{2+}$ (**4**) is shown in Figure 1b. The g = 1.91-2.20 absorptions occur over a broader range than those seen for free O_2^-,[33] superoxocobalt(III) and O_2^- bridged dicobalt(III) compounds,[34-36] or other MO_2^- species.[37] This may reflect delocalization and coupling to the two $I = {}^3/_2$ Cu(II) ions, but further EPR spectroscopic and electronic structural studies are required.[38] A further indication for the presence of the superoxo radical anion in $[Cu_2(UN\text{-}O^-)(O_2^-)]^{2+}$ (**4**) is that, when the complex is reacted with the spin-trapping agent M_4PO (M_4PO = 3,3,5,5-tetramethyl-1-pyrroline N-oxide),[39] a mixture[40] which includes a sharp triplet centered at g = 2.006 (A_N = 20 G) (Figure 1c, 77 K, CH_2Cl_2/C_7H_8) is generated, indicating the formation of a superoxo-M_4PO adduct which may or may not be coordinated to the coppers. Spin-trapping agents have been used similarly in detecting adducts with superoxocobalt[41] and -iron[42] complexes.

Further proof for the formulation and superoxo nature of $[Cu_2(UN\text{-}O^-)(O_2^-)]^{2+}$ (**4**) comes from its observed relationship to peroxo complex $[Cu_2(UN\text{-}O^-)(O_2^{2-})]^+$ (**2**). As is the case in the well-established $\{Co^{III}(O_2)Co^{III}\}^{4+,5+}$ compounds,[34] oxidation of the peroxo complex **2** directly produces **4**. A spectrophotometric titration where ${}^1/_4$ mol equiv of $Ag(CF_3SO_3)$ as oxidant is added successively (spectra b-e) shows that the 510-nm band associated with **2** (spectrum a) decreases with concomitant formation of the 404-nm absorption of **4**; $[Cu_2(UN\text{-}O^-)(O_2^-)]^{2+}$ (**4**) generated in this manner is spectroscopically identical to that obtained by addition of O_2 to $[Cu^ICu^{II}(UN\text{-}O^-)]^{2+}$ (**3**) (Figure 2).

In summary, we have described here still another type of dioxygen adduct of copper ion, a one-electron-reduced species formed at a dicopper center. Further elaboration of this type of chemistry is in progress. Fundamental structural and spectroscopic interest in such moieties is also relevant to O_2 reduction chemistry in proteins such as laccase and ascorbate oxidase, where binding of reduced O_2 intermediates occurs at a dicopper segment of a tricopper cluster.[6a,12a,b]

Acknowledgment. We thank the National Institutes of Health (GM 28962) for support of this research.

(30) (a) $[Cu^ICu^{II}(UN\text{-}O^-)](PF_6)_2\cdot{}^1/_4DMF$ (**3**): IR (Nujol) $\nu(PF_6)$ = 840 cm^{-1}, ν(C=O; DMF) = 1645 cm^{-1}; UV-vis [CH_2Cl_2; λ_{max}, nm (ϵ, M^{-1} cm^{-1})] 348 (4000, sh), 680 (360). Anal. Calcd for $C_{35.75}H_{38.75}Cu_2F_{12}N_{6.25}O_{1.25}P_2$: C, 43.24; H, 3.90; N, 8.82. Found: C, 43.55; H, 3.97; N, 8.56.

(31) (a) The existence or intensity of intervalence charge-transfer bands (IT) can be quite variable.[32] For a recent example of a mixed-valence copper complex with an intense near-IR band, see: Harding, C.; McKee, V.; Nelson, J. *J. Am. Chem. Soc.* **1991**, *113*, 9684-9685.

(32) (a) Long, R. C.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1983**, *105*, 1513-1521. (b) Hendrickson, D. N.; Long, R. C.; Hwang, Y. T.; Chang, H. R. In *Biological and Inorganic Copper Chemistry*; Karlin, K. D., Zubieta, J., Eds.; Adenine Press: Guilderland, New York, 1985. (c) Gagné, R. R.; Koval, C. A.; Smith, T. J.; Comolino, M. C. *J. Am. Chem. Soc.* **1979**, *101*, 4571-4580. (d) Gagné, R. R.; Henling, L. M.; Kistenmacher, T. J. *Inorg. Chem.* **1980**, *19*, 1226-1230. (e) Gagné, R. R.; Koval, C. A.; Smith, T. J. *J. Am. Chem. Soc.* **1977**, *99*, 8367-8368.

(33) (a) Green, M. R.; Hill, H. A. O.; Turner, D. R. *FEBS Lett.* **1979**, *103*, 176-180. (b) Valentine, J. S. In *Oxygen: Biochemical and Clinical Aspects*; Caughey, W. S., Ed.; Academic Press: New York, 1979; pp 659-677.

(34) (a) Niederhoffer, E. C.; Timmons, J. H.; Martell, A. E. *Chem. Rev.* **1984**, 137-203. (b) Wilkens, R. G. In *Oxygen Complexes and Oxygen Activation by Transition Metals*; Martell, A. E., Sawyer, D. T., Eds.; Plenum: New York, 1988; pp 49-60. (c) Fallab, S.; Mitchell, P. R. *Adv. Inorg. Bioinorg. Mech.* **1984**, *3*, 311-377. (d) Jones, R. D.; Sommerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, *2*, 139.

(35) Smith, T. D.; Ruzic, I. M.; Tirant, S.; Pilbrow, J. R. *J. Chem. Soc., Dalton Trans.* **1982**, 363-372.

(36) (a) Mori, M.; Weil, J. A.; Kinnaird, J. K. *J. Phys. Chem.* **1967**, *71*, 103-108. (b) Duffy, D. L.; House, D. A.; Weil, J. A. *J. Inorg. Nucl. Chem.* **1969**, *31*, 2053-2058.

(37) (a) Bear, J. L.; Yao, C.-L.; Capdevielle, F. J.; Kadish, K. M. *Inorg. Chem.* **1988**, *27*, 3782-3785. (b) Hasegawa, K.; Imamura, T.; Fujimoto, M. *Inorg. Chem.* **1986**, *25*, 2154-2160. (c) Raynor, J. B.; Gillard, R. D.; Pedrosa de Jesus, J. D. *J. Chem. Soc., Dalton Trans.* **1982**, 1165-1166. (d) Wayland, B. B.; Newman, A. R. *Inorg. Chem.* **1981**, *20*, 3093-3097.

(38) (a) Solomon and co-workers[6a,12d] have recently discussed electronic interactions occurring in a cluster with three $S = {}^1/_2$ spins (i.e., three Cu(II)), possibly analogous to that seen here, i.e., with two Cu(II) plus O_2^-. (b) As one possibility, Figure 1b may be the spectrum of a superoxide radical (sharp spike at $g \approx 2.0$) which is essentially uncoupled to the dicopper(II) center, i.e., with its broader signals plus a weak half-field line near g = 4. The latter weak $g \approx 4$ signal is indeed observed.

(39) Janzen, E. G.; Shetty, R. V.; Kunanec, S. M. *Can. J. Chem.* **1981**, *59*, 756-758.

(40) We speculate that the four-line signal observed in Figure 1c may be due to the presence of $[Cu^ICu^{II}(UN\text{-}O^-)]^{2+}$ (**3**), an M_4PO adduct with **3** ($g_\parallel$ = 2.26, $A_\parallel$ = 162 × 10^{-4} cm^{-1}, independently determined), or a dicopper(II) derivative if a superoxo-TEMPO adduct dissociates.

(41) Hamilton, D. E.; Drago, R. S.; Telser, J. *J. Am. Chem. Soc.* **1984**, *106*, 5353-5355.

(42) Herron, N.; Schammel, W. P.; Jackels, S. C.; Grzybowski, J. J.; Zimmer, L. L.; Busch, D. H. *Inorg. Chem.* **1983**, *22*, 1433-1440.

(*Z*)-3-Fluorophosphoenolpyruvate as a Pseudosubstrate of EPSP Synthase: Enzymatic Synthesis of a Stable Fluoro Analog of the Catalytic Intermediate

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The critical plant enzyme EPSP synthase[1,2] (5-enolpyruvoylshikimate-3-phosphate synthase) catalyzes an unusual transfer of a carboxyvinyl moiety derived from phosphoenolpyruvate (PEP) to the 5-OH of shikimate 3-phosphate (S3P). The reaction proceeds through a single, kinetically competent tetrahedral intermediate[3] (I) which has been previously isolated.[4] While a variety of PEP analogs have been examined as alternate substrates

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(1) Sikorski, J. A.; Anderson, K. S.; Cleary, D. G.; Miller, M. J.; Pansegrau, P. D.; Ream, J. E.; Sammons, R. D.; Johnson, K. A. In *Chemical Aspects of Enzyme Biotechnology: Fundamentals*; Baldwin, T. O., Raushel, F. M., Scott, A. I., Eds.; Plenum Press: New York, 1991; pp 23-39.

(2) Anderson, K. S.; Johnson, K. A. *Chem. Rev.* **1990**, *90*, 1131-1149.

(3) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 7395-7406.

(4) Anderson, K. S.; Sikorski, J. A.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.* **1988**, *110*, 6577-6579.

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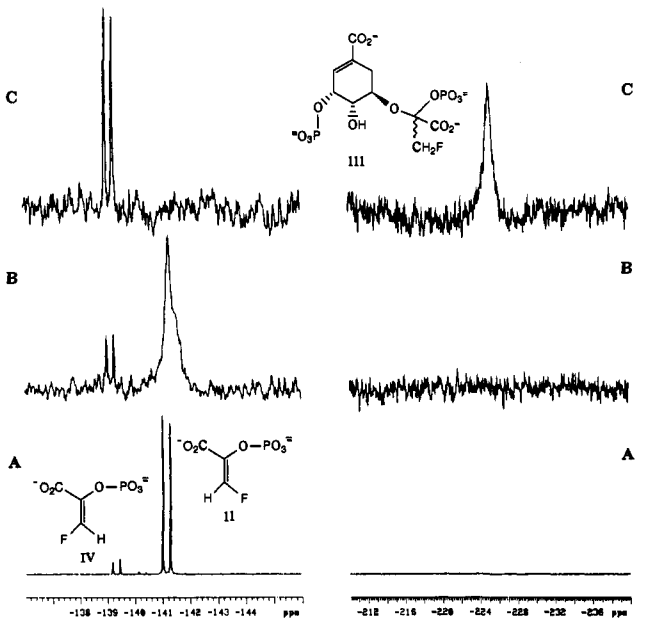
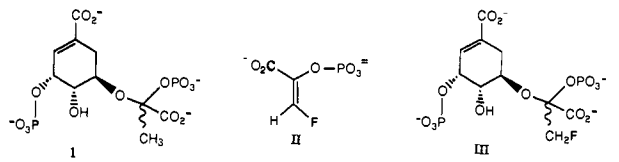


Figure 1. ^{19}F-NMR spectra of F-PEP in the presence of EPSP synthase. Spectra were acquired on a Varian XL-300 operating at 282.2 MHz and are referenced externally to Freon-11. (A) 3-F-PEP (1 mM) in 50 mM HEPES, pH 7.0, with 50 mM KCl and 25% D_2O for lock. Integration indicates a 10:1 mixture of (*Z*) and (*E*) isomers of 3-F-PEP (δ = −141.2 and −139.3, respectively), which was independently confirmed by ^{1}H-NMR. Insets indicate corresponding structural assignments. (B) 3-F-PEP (1.5 mM) in the presence of a stoichiometric amount of EPSP synthase. Conditions are identical to those of panel A. (C) Acquired following the addition of 12 mM S3P to the same sample represented in panel B.

and/or inhibitors of EPSP synthase,[5-7] none to date has demonstrated turnover to EPSP-like products. We report here the first evidence that (*Z*)-3-fluoro-PEP (II) functions as a pseudosubstrate for EPSP synthase, producing in one step the unexpected monofluoro analog (III), which remains tightly bound at the enzyme active site.



As illustrated in Figure 1, the ^{19}F-NMR resonance of free II (δ = −141.15 ppm) is significantly broadened upon binding to a stoichiometric amount of enzyme. This resonance moves upfield 84 ppm (δ = −225.4 ppm) following the addition of excess S3P, consistent with a change in hybridization at the terminal carbon of II. No such change in ^{19}F chemical shift was observed when 5-deoxy-S3P[8] was substituted for S3P, despite evidence from ^{31}P-NMR indicating the formation of an enzyme·5-deoxy-S3P·II complex. No evidence for the binding of (*E*)-3-fluoro-PEP (IV, δ = −139.3 ppm) to EPSP synthase was observed, either alone or with excess S3P.

Incorporation of radiolabel from [^{14}C]S3P into a new species (III) was demonstrated by anion-exchange HPLC analysis of chemically quenched[4] reaction mixtures. Each enzyme molecule undergoes a single turnover; intermediate III does not accumulate in solution, and no evidence for enzymatic conversion of this species to an EPSP-like product has been observed. The half-time for the formation of III is less than 1 min at 30 °C under pseudo-first-order conditions.[9] The appearance of ^{14}C-label into III in a preequilibrated reaction mixture occurs with a $t_{1/2}$ of approximately 35 min,[9] demonstrating that the enzyme·III binary complex is in slow equilibrium with the enzyme·S3P·II ternary complex. Readdition of a stoichiometric amount of III to EPSP synthase results in the slow formation of S3P ($t_{1/2}$ = 34 min). Increased acid stability of III ($t_{1/2}$ = 1.5 and 48 h at pH 2.5 and 5.0, respectively) relative to I[13] may account for the enzyme's inability to facilitate the elimination of phosphate and the ultimate formation of a fluoro-EPSP product. Preliminary data[10] suggests that III exhibits a K_d for EPSP synthase of approximately 600 pM. This result is consistent with that for I[12,13] as well as the trend to increased potency with decreased fluorine content exhibited by the previously synthesized[11] difluoromethyl and trifluoromethyl analogs.

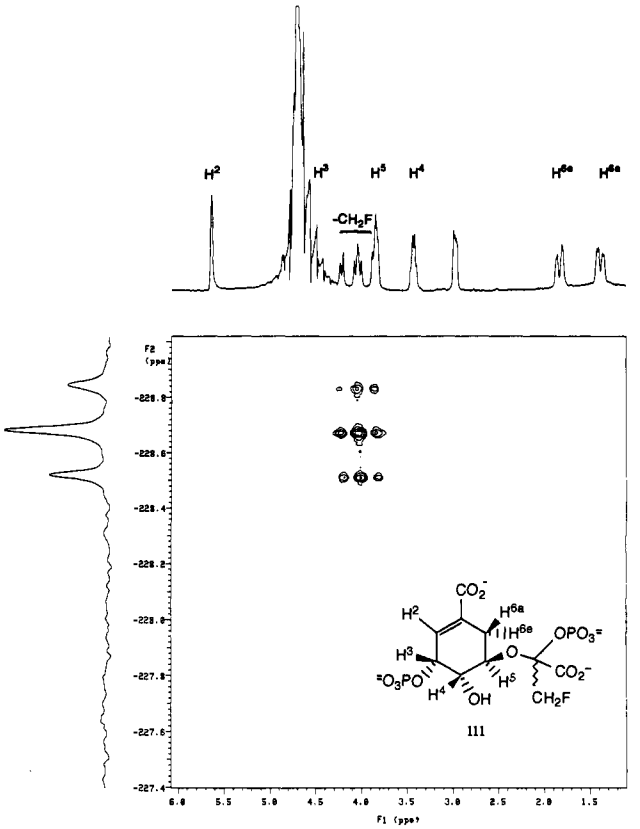


Figure 2. ^{19}F–^{1}H 2-D HETCOR NMR spectrum of the isolated monofluorotetrahedral intermediate. The HETCOR spectrum was run fully coupled in both the ^{19}F and ^{1}H dimensions. The peaks corresponding to the ^{19}F resonances on the vertical axis correlate to two doublets within the ^{1}H spectrum (horizontal axis) which are slightly overlapping to give a triplet-like appearance. Each doublet is due to one of the two diastereotopic CH_2F protons split by the 45.8 Hz coupling to the ^{19}F. The geminal ^{1}H–^{1}H coupling is not resolved in the 2-D spectrum. This analysis was confirmed by collecting a second HETCOR which was decoupled in both dimensions (data not shown). The broad resonance appearing at 2.95 ppm in the ^{1}H-NMR spectrum is attributed to buffer contaminants (ref 4).

(5) Walker, M. C.; Ream, J. E.; Sammons, R. D.; Logusch, E. W.; O'Leary, M. H.; Somerville, R. L.; Sikorski, J. A. *BioMed. Chem. Lett.* **1991**, *1*, 683–688.

(6) Gruys, K. J.; Walker, M. C.; Sikorski, J. A. *Biochemistry* **1992**, *31*, 5534–5544.

(7) Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1776–1783.

(8) Pansegrau, P. D.; Anderson, K. S.; Widlanski, T.; Ream, J. E.; Sammons, R. D.; Sikorski, J. A.; Knowles, J. R. *Tetrahedron Lett.* **1991**, *32*, 2589–2592.

(9) Assays were performed in the presence of 60 μM enzyme and 1.0 mM each of S3P and 3-F-PEP in 100 mM HEPES, pH 7.0, with 50 mM KCl.

(10) Catalytic amounts of enzyme (3 nM) were incubated with 60 μM each S3P and PEP in the presence of purified III (0.09–25 nM) using buffer conditions described in ref 9. Reactions were quenched and analyzed as described in ref 3. An estimated K_d was obtained by computer fitting of the data to a model for a random kinetic mechanism (ref 6).

(11) Alberg, D. G.; Lauhon, C. T.; Nyfeler, R.; Fässler, A.; Bartlett, P. A. *J. Am. Chem. Soc.* **1992**, *114*, 3535–3546.

(12) Cleland, W. W. *Biochemistry* **1990**, *29*, 3194–3197.

(13) Anderson, K. S.; Johnson, K. A. *J. Biol. Chem.* **1990**, *265*, 5567–5572.

Quantities of III sufficient for structural characterization have been prepared by quenching 500–700 mg of enzyme. The ^{1}H-NMR spectrum (Figure 2) confirms the presence of a single shikimate species, as well as the CH_2F protons which are nonequivalent (δ = 3.94 and 4.14 ppm; 9.6 Hz geminal coupling); coupling of these methylene protons to fluorine is also demonstrated. The ^{19}F-NMR resonance appears as a triplet (δ = −230.88 ppm; J_{F-H} = 45.8 Hz). The ^{31}P-NMR spectrum (not presented; δ = 0.38 and −5.54 ppm) is essentially identical to that reported previously for I.[4] The ES-MS spectrum was dominated by a single peak with the molecular mass of 439 amu expected for the (M^+) − 1 ion of III ($C_{10}H_{15}F_1O_{14}P_2$).

These observations demonstrate that (*Z*)-3-fluoro-PEP serves as a pseudosubstrate of EPSP synthase, resulting in the formation of a novel enzyme-bound fluoro intermediate III, which does not proceed further toward product. Thus, (*Z*)-3-fluoro-PEP is unique in its ability to support incomplete enzymatic catalysis. Intermediate III also provides a new tool to probe the mechanistic and structural details of EPSP synthase. Studies are underway to define the geometry of this intermediate when bound at the enzyme active site.

Acknowledgment. We thank Mr. David Zeigler for assisting in the collection of NMR data and Dr. Kevin Duffin for providing mass spectral data.

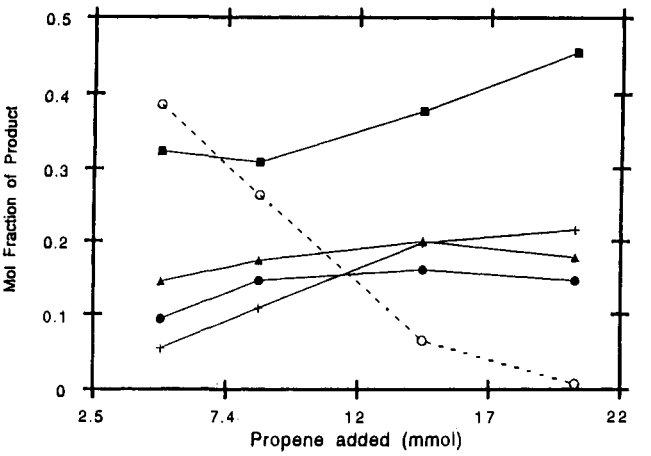


Figure 1. Product yields as a function of added propene: (●) $C_{14}H_{10}$, (▲) $C_{15}H_{10}$, (■) total $C_{16}H_{10}$, + total $C_{18}H_{10}$, (○) C_{60}. The horizontal axis refers to the total amount of propene added during a 1-h reaction. Total yields of product ranged from 9.4×10^{-3} to 4.1×10^{-1} mmol.

On the Mechanism of Fullerene Formation. Trapping of Some Possible Intermediates

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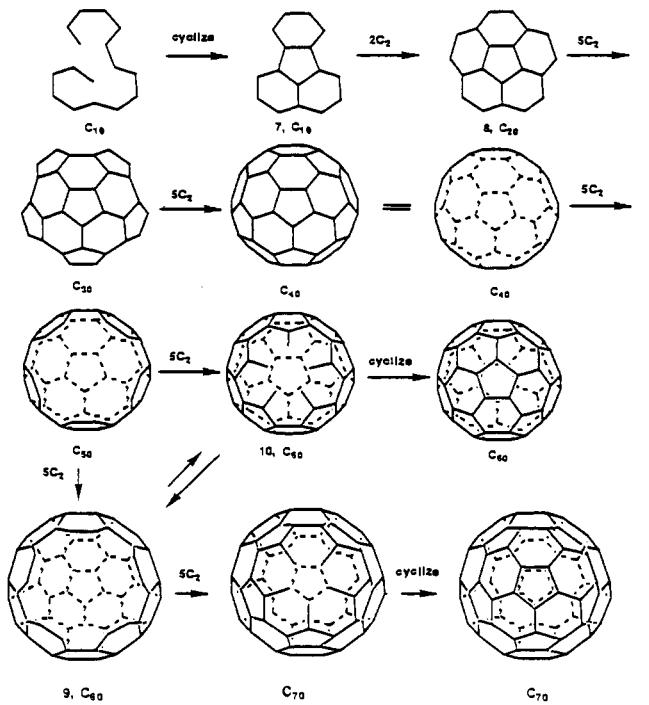
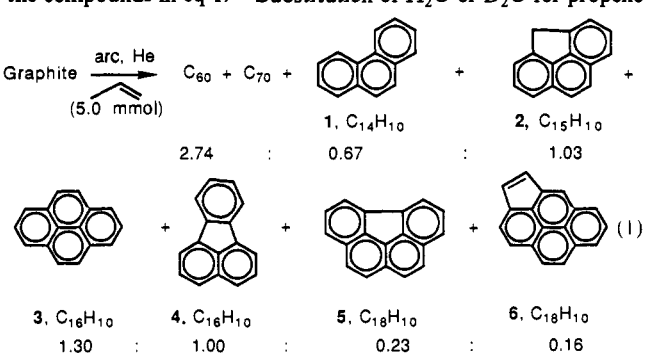
Of the many interesting scientific questions generated by the synthesis and isolation of the fullerenes,[1] one of the most intriguing concerns the mechanism of the remarkable reactions which bring small carbon molecules together to form large hollow cages. Although several ingenious mechanistic schemes have been proposed,[2] they suffer from the fact that intermediates have not been trapped. We now report that addition of hydrogen donors to systems in which C_{60} and C_{70} are generated results in the formation of polycyclic aromatic hydrocarbons whose carbon skeleton may represent intermediates in fullerene formation.[3]

We have modified the standard conditions for fullerene synthesis by evaporating carbon from an arc in an atmosphere of He to which have been added propene and other H donors. Analysis of the benzene-soluble portion of the carbonaceous products by mass spectrometry reveals, in addition to C_{60} and C_{70}, a series of peaks corresponding to $C_{12}H_8$, C_nH_{10} (n = 14–18), and, in lower yield, C_nH_{12} (n = 20, 22, 24). GC/MS analysis identifies the $C_{12}H_8$ as acenaphthylene and shows that the C_nH_{10} series contains the compounds in eq 1.[5] Substitution of H_2O or D_2O for propene

Scheme I. Formation of C_{60} and C_{70} by a Series of C_2 Additions





(1) (a) Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354. (b) Haufler, R. E.; Conceicao, J.; 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.; Ciufolini, M. A.; Hauge, R. H.; Margrave, J. L.; Wilson, L. J.; Curl, R. F.; Smalley, R. E. *J. Phys. Chem.* **1990**, *94*, 8634–6. (c) Taylor, R.; Hare, J. P.; Abdul-Sada, A. K.; Kroto, H. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1423–5. (d) Ajie, H.; et al. *J. Phys. Chem.* **1990**, *94*, 8630.

(2) (a) Kroto, H. W. *Science* **1988**, *242*, 1139–45. (b) Kroto, H. W.; McKay, K. G. *Nature* **1988**, *331*, 328–31. (c) Curl, R. F.; Smalley, R. E. *Science* **1988**, *242*, 1017–22. (d) Curl, R. F.; Smalley, R. E. *Sci. Am.* **1991**, *265* (October) 54–63. (e) Heath, J. R. In *Fullerenes—Synthesis, Properties, and Chemistry of Large Carbon Clusters*; Hammond, G. S., Kuck, V. J., Eds.; ACS Symposium Series 481; American Chemical Society: Washington, DC, 1992; pp 1–21. (f) Wakabayashi, T.; Achiba, Y. *Chem. Phys. Lett.* **1992**, *190*, 465. (g) Smalley, R. E. *Acc. Chem. Res.* **1992**, *25*, 98.

(3) Mass spectral studies of the addition of H_2 to the He carrier gas of a laser vaporization cluster source reveal the formation of polyacetylenes.[4]

(4) (a) Heath, R. J.; Zhang, Q.; O'Brien, S. C.; Curl, R. F.; Kroto, H. W.; Smalley, R. E. *J. Am. Chem. Soc.* **1987**, *109*, 359. (b) Rohlfing, E. A. *J. Chem. Phys.* **1990**, *93*, 7851-62.

(5) Yields of C_{60} were determined by ^{13}C NMR using hexamethylbenzene as internal standard. The polycyclic aromatics, which could not be detected in the absence of H donor, were determined by GC using the same hexamethylbenzene as internal standard. Fullerenes and polycyclic aromatics constitute ~20% of the carbonaceous residue. Propene was bled into the reactor through a calibrated valve.

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