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 non-equivalent (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.

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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

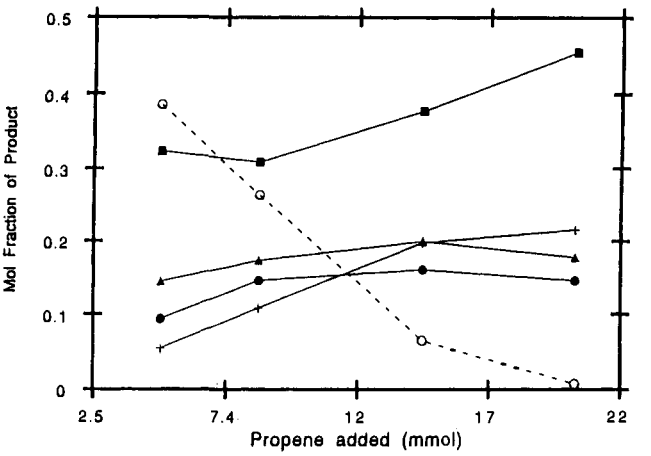
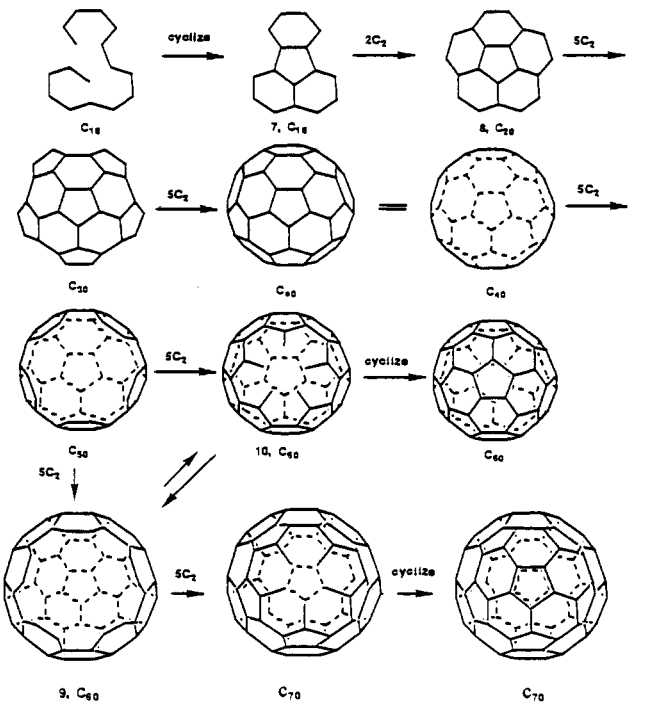
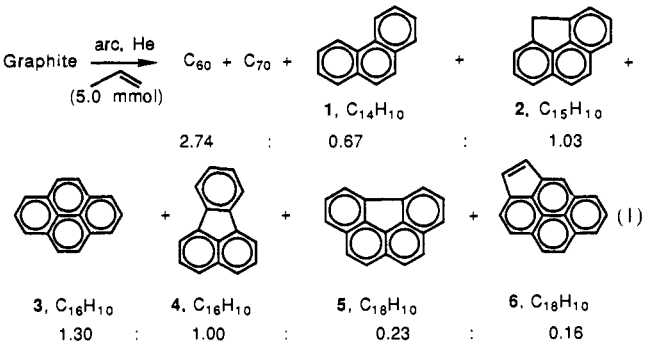


Figure 1. Product yields as a function of added propene: (●) $C_{14}H_{10}$, (▲) $C_{15}H_{10}$, (■) total $C_{16}H_{10}$, + total $C_{18}H_{10}$, (○) C_{60}. The horizontal axis refers to the total amount of propene added during a 1-h reaction. Total yields of product ranged from 9.4×10^{-3} to 4.1×10^{-1}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

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



$C_{12}H_8$ 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



(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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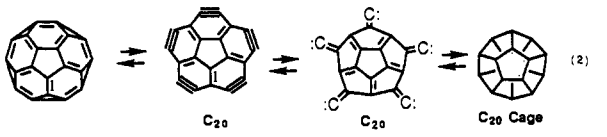
in this reaction generates the same polycyclic aromatics with H replaced by D in the case of D_2O. When CH_3OD is used as a trap, the products contain both D and H with H predominating as expected from a consideration of homolytic bond strengths.

The relationship between H donor concentration and yields is displayed graphically in Figure 1. Since compounds **4** and **5** contain remnants of the ring skeletons found in C_{60}, it is tempting to postulate that the decadehydro derivatives of these compounds are intermediates on the way to the fullerenes and are trapped by H abstraction. The data in Figure 1, which show an increase in trapping products with a corresponding decrease in C_{60} as H donor concentration is increased, are consistent with this hypothesis. A mechanism for the conversion of a C_{16} fragment, corresponding to **4**, to the fullerenes by a series of C_2 additions is proposed in Scheme I. Ring closure and cyclization of a C_{16} chain under the energetic conditions of fullerene formation could lead to dehydrofluoranthrene, **7**. Subsequent additions of two C_2 molecules to the free valences in **7** would generate the dehydrocorannulene, **8**. Stepwise addition of molecular C_2 then builds up the carbon clusters, eventually resulting in the fullerenes. In the steps leading from C_{16} to C_{50}, the growing carbon cluster adds to C_2 in a 1,2 fashion always generating intermediates with 10 free valences or five benzyne units. Once C_{50} is reached, continued addition to C_2 in a 1,2 fashion generates open C_{60}, **9**, which could rearrange to **10**, a C_{60} with five cyclopentadienylidene carbenes.[6] Cyclization of **10** yields fullerene-60. Alternately, C_{50} could add to C_2 molecules in a 1,1 fashion generating **10** directly. Addition of more C_2 molecules to **9** would lead to fullerene-70 or to tubules.[8]

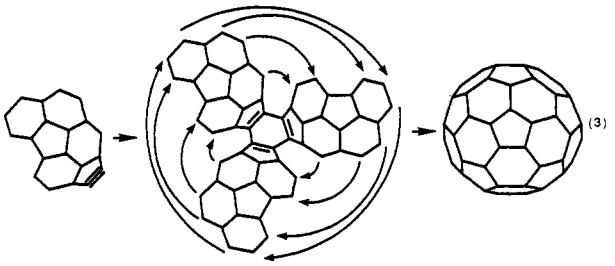
Smalley[2g] has proposed a "pentagon road" route to C_{60} in which carbon sheets with as many nonadjacent pentagons as possible reduce the number of dangling bonds to 10 during a large portion of the cluster buildup. The mechanism in Scheme I, which also involves intermediates with 10 free valences, may represent a route to the fullerenes along the "pentagon road". This mechanism, in which clusters grow by the addition of C_2 molecules, builds up the carbon clusters in even-numbered units, as is observed in mass spectral studies of clusters arising from laser-evaporated graphite.[9,10]

That the trapping experiments do not show C_nH_{10} with $n > 18$ may be due to the fact that once C_{20} is reached, free valences may be satisfied by formation of cages which are not trapped (eq 2).[11]



(2)

An interesting alternative explanation for our failure to trap clusters above C_{18} is a rapid trimerization of a C_{20} to fullerene-60 (eq 3). Although such a mechanism is not consistent with mass spectral studies of laser-evaporated graphite, C_{30} has been observed to dimerize to C_{60} in the gas phase,[12] and it seems possible that


(3)

a trimerization may play a role when C_{60} is generated in an arc.

These investigations implicate a mechanism of fullerene formation in which linear, monocyclic, and polycyclic carbon clusters precede fullerene synthesis.[13]

Acknowledgment. Support of this work through National Science Foundation Grants CHE-9013240 and CHE-9101252 is gratefully acknowledged.

(6) The rearrangement of **9** to **10** is an example of the benzyne to cyclopentadienylidene carbene rearrangement which has been calculated to be endothermic by 31 kcal/mol.[7] In the case of fullerene formation, this endothermicity would be compensated by cage formation.

(7) Burton, N. A.; Quelch, G. E.; Gallo, M. M.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **1991**, *113*, 764-9.

(8) (a) Lijima, S. *Nature* **1991**, *354*, 56-8. (b) Lijima, S.; Ichihishi, T.; Ando, Y. *Nature* **1992**, *354*, 776-8. (c) Ebbesen, T. W.; Ajayan, P. M. *Nature* **1992**, *358*, 220-2.

(9) (a) Rohlfing, E. A.; Cox, D. M.; Kaldor, A. *J. Chem. Phys.* **1984**, *81*, 3322-30. (b) Kroto, H. W.; Heath, R. J.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E. *Nature* **1985**, *318*, 162. (c) McElvany, S. W.; Ross, M. M.; Callahan, J. H. *Acc. Chem. Res.* **1992**, *25*, 162 and references cited therein.

(10) (a) Labeling studies have shown that, if C_2 is involved in the formation of the fullerenes, it must be formed from atomic carbon after evaporation.[10b-e] (b) Meijer, G.; Bethune, D. S. *J. Chem. Phys.* **1990**, *93*, 6900. (c) Yannoni, C. S.; Bernier, P. P.; Bethune, D. S.; Meijer, G.; Salem, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 3190. (d) Hawkins, J. M.; Meyer, A.; Loren, S.; Nunlist, R. *J. Am. Chem. Soc.* **1991**, *113*, 9394. (e) Ebbesen, T. W.; Tabuchi, T.; Tanigaki, K. *Chem. Phys. Lett.* **1992**, *191*, 336.

(11) A recent calculation indicates that fullerene-20 is more stable than the monocyclic C_{20}; Parasuk, V.; Almöf, J. *Chem. Phys. Lett.* **1991**, *184*, 187-90.

(12) Rubin, Y.; Kahr, M.; Knobler, C. B.; Diederich, F.; Wilkens, C. L. *J. Am. Chem. Soc.* **1991**, *113*, 495-500.

(13) Helden et al. have obtained evidence for isomeric cyclic clusters prior to fullerene formation: Helden G. v.; Hsu, M.-T.; Kemper, P. R.; Bowers, M. T. *J. Chem. Phys.* **1991**, *95*, 3835-7.

$\psi[PO_2^-CH_2N^+]$, a New Amide Bond Replacement: Potent, Slow-Binding Inhibition of the HIV Protease

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The design and synthesis of peptidomimetic enzyme inhibitors continue to be active areas of research. Such compounds have proven useful in elucidating mechanisms of catalysis and as therapeutic agents.[1] The discovery that the human immunodeficiency virus encodes an aspartic protease (HIV PR) vital for its propagation has brought this protein under intense scrutiny.[2,3] In this regard, the development of compounds which inhibit the HIV PR has been particularly rapid.[4]

It seemed rational that an effective modification of the phosphonamidate structure **1**, well-known in protease inhibition,[5] would be to include additional features along the reaction coordinate for amide hydrolysis. The insertion of a methylene spacer between phosphorus and nitrogen produces the nonhydrolyzable moiety **2**, which is likely a zwitterion near physiological pH. This construct could be representative of a late transition state/early

(1) (a) Rich, D. H. In *Comprehensive Medicinal Chemistry*; Sammes, P. G., Ed.; Pergamon: Oxford, 1990; Vol. 2, pp 391-441. (b) Dingle, J. T., Gordon, J. L., Eds. *Research Monographs in Cell and Tissue Physiology*; Barrett, A. J., Salvesen, G., Eds.; Elsevier: Amsterdam, 1986; Vol. 12 Proteinase Inhibitors.

(2) Kramer, R. A.; Schaber, M. D.; Skalka, A. M.; Ganguly, K.; Wong-Staal, F.; Reedy, E. P. *Science* **1986**, *231*, 1580-1584.

(3) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686-4690.

(4) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305-2314. It should be noted that structure **15** in Table III is depicted incorrectly and is actually a secondary carbocyclic phosphinate.

(5) (a) Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, *235*, 569-571. (b) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618-4624. (c) Thorsett, E. D.; Harris, E. E.; Peterson, E. R.; Greenlee, W. J.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2176-2180. (d) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654-657.

0002-7863/92/1514-7604$03.00/0