Synthesis, Isolation, and Equilibration of 1,9- and 7,8-$C_{70}H_2$

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Equilibration of 1,9- and 7,8-$C_{70}H_2$ has allowed the relative free energy of these isomers to be measured. These "simplest hydrocarbon derivatives of C_{70}" are formed by hydroboration of C_{70} at room temperature. Analysis of the platinum-catalyzed equilibration of these isomers yielded a relative free energy at 295 kelvin of 1.4 ± 0.2 kilocalories per mole, with the 1,9 isomer being more stable. This value is in excellent agreement with the ab initio HF/6-31G* calculated energy difference of 1.3 kilocalories per mole, whereas semiempirical calculations gave poor agreement.

Questions concerning the regiochemistry of both the kinetic and thermodynamic products of addition to C_{60} and C_{70} are highly relevant to the chemical modification of fullerenes for applications ranging from pharmaceuticals to materials science. Regioselective addition of "XY" across a "double" bond (across a 6,6-ring fusion bond) is a general feature of addition to fullerenes (*1*). In C_{70}, 1,2-addition to the two 6,6-ring fusion bonds closest to the poles of this molecule yields the lowest energy products by both theory and experiment (*2–4*). However, the relative importance of thermodynamic and kinetic effects on the product distribution has not been determined. We report here the synthesis, isolation, and equilibration of 1,9- and 7,8-$C_{70}H_2$ (Fig. 1), from which we have obtained the difference in the free energy, ΔG, between the two species. The experimental value is in excellent agreement with the energy difference obtained from high-level ab initio computations.

A full description of these computational results has been reported (*2*). Geometry optimizations of C_{70} and all 143 distinct isomers of $C_{70}H_2$ (*5*) using the semiempirical MNDO Hamiltonian were performed with the MOPAC program and PM3 parameters (*6*). The four lowest energy isomers of $C_{70}H_2$ were then chosen as candidates for further investigation with ab initio methods within the GAUSSIAN 92 series of programs (*7*). Complete geometry optimizations were performed at the Hartree-Fock (HF) level with the 3-21G and 6-31G* basis sets, the latter containing one set of spherical harmonic *d* functions on each carbon. A summary of the results, including those from a previous MNDO/AM1 study (*3*), is given in Table 1.

Products resulting from 1,2-addition of H_2 to 6,6-ring fusions and from 1,4-addition across 6-rings are calculated to be lowest in energy at all levels of theory for both C_{70} and C_{60}. In C_{70}, four products may result from 1,2-addition to 6,6-ring fusions and six products may result from 1,4-addition across a 6-ring. All levels of theory used here predict that the two lowest energy isomers of $C_{70}H_2$ are products of 1,2-addition to 6,6-ring fusions. The 7,8 isomer is the lowest energy isomer in the semiempirical treatments. However, this ordering is reversed at ab initio levels: the 1,9-$C_{70}H_2$ isomer is favored by 1.3 kcal/mol over the 7,8 isomer at the most rigorous level of calculation.

The product mixture that results from the reaction of two equivalents of BH_3 [1 M in tetrahydrofuran (THF)] with C_{70} in toluene (0.5 mg/ml) at 22° to 25°C for 1 hour and hydrolysis with water (*8*) contains a 1:2 mixture of 7,8- and 1,9-$C_{70}H_2$ in 20% overall yield (*9*). The order of elution on a Buckyclutcher I stationary phase (*10*) with a 60:40 toluene:hexane isocratic mobile phase is C_{60} (impurity), C_{70}, 7,8-$C_{70}H_2$, and 1,9-$C_{70}H_2$. Minor, uncharacterized products are also observed at longer elution times. Isolation, concentration, and reseparation of the $C_{70}H_2$ products has led to pure samples of each isomer. The high-resolution fast atom bombardment mass spectrum (FAB MS) of these compounds confirmed the assignment of the molecular formula (*11*).

The ^1H nuclear magnetic resonance (NMR) spectrum of the first-eluting isomer in toluene-d_8 consists of a singlet at δ 3.91 ppm (*12*). The spectrum of the corresponding $C_{70}HD$ isomer formed by hydrolysis of the intermediate organoborane with D_2O shows a $^3J_{HD}$ of 2.3 ± 0.2 Hz and a small upfield isotope shift $^3\Delta H(D)$ of 8.4 ppb, consistent with vicinal equivalent hydrogens. Of the possible products resulting from addition to 6,6-ring fusions, only the 7,8-, 21,22-, and 23,24-$C_{70}H_2$ isomers would have spectra consistent with these data. The significantly lower heat of formation calculated at the MNDO level for the 7,8 isomer led to the final structure assignment (*13*).

The ^1H NMR spectrum of the second-eluting isomer in toluene-d_8 consists of an AB quartet centered at δ 4.00 ppm (*12*). Simulation of the spectrum leads to a ($\upsilon_A - \upsilon_B$) of 204 Hz and a $^3J_{HH}$ of 16.1 Hz (*14*). The spectrum of the corresponding $C_{70}HD$ isomer also shows a $^3J_{HD}$ of 2.2 ± 0.2 Hz and a small upfield isotope shift $^3\Delta H(D)$ of 7.8 ppb, all consistent with vicinal, nonequivalent hydrogens. Only the 1,9 structure can be assigned to this product on the basis of calculated heats of formation.

The 7,8 and 1,9 isomers do not interconvert on standing at room temperature in solution for several weeks, or on heating to

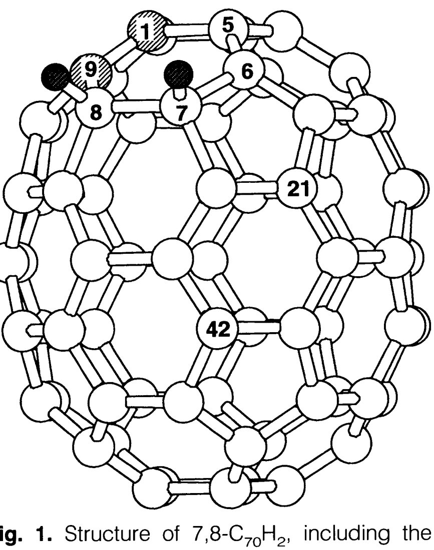

Fig. 1. Structure of 7,8-$C_{70}H_2$, including the labeling scheme (*4*). The 1 and 9 carbons are shaded to indicate the positions of hydrogen addition in the 1,9 isomer. Note that the 21,42 isomer, which has the third lowest calculated heat of formation (but which is not detected), spans the equatorial layer.

Table 1. Relative energies (kilocalories per mole) for the four lowest energy dihydride isomers of C_{70}, based on fully optimized geometries at each level of theory. The measured relative energy for the 7,8 isomer in toluene is 1.4 ± 0.2 kcal/mol.

Isomer	MNDO/AM1 (*3*)	MNDO/PM3	HF/3-21G	HF/6-31G*
1,9-$C_{70}H_2$	0.0	0.0	0.0	0.0
7,8-$C_{70}H_2$	−1.0	−1.0	0.2	1.3
21,42-$C_{70}H_2$	0.7	0.3	2.1	4.5
1,7-$C_{70}H_2$	2.2	1.4	5.8	6.4

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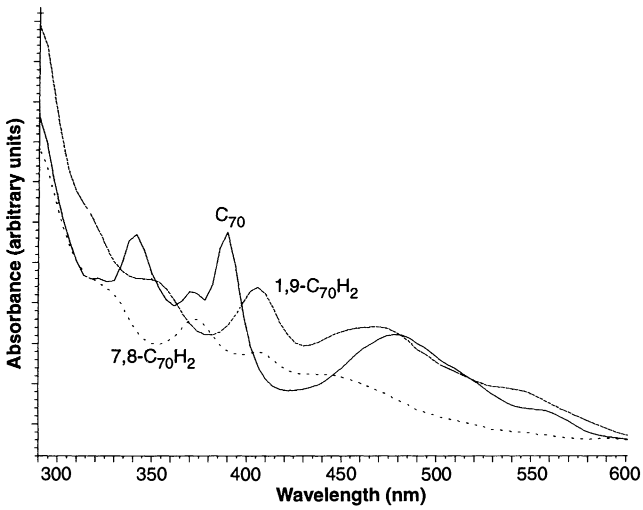
Fig. 2. Ultraviolet-visible spectra of C_{70} and both $C_{70}H_2$ isomers recorded on an HPLC photodiode array detector in 60:40 toluene:hexane. Relative intensities do not imply relative molar absorptivities.



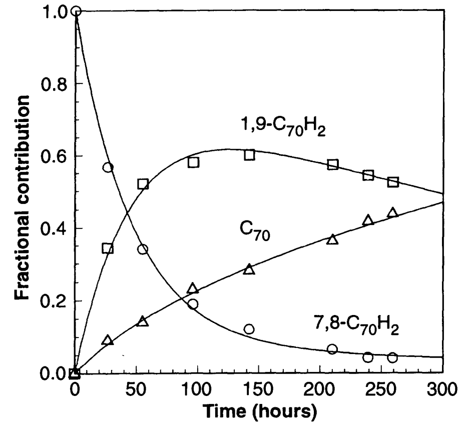


Fig. 3. Equilibration (and decomposition to C_{70}) of 7,8-$C_{70}H_2$ and 1,9-$C_{70}H_2$ over a platinum-on-silica catalyst. The symbols represent the experimental data, and the curves are the optimal numerical fits that were calculated on the basis of the proposed kinetic scheme.

100°C for 1 hour, although minor decomposition to C_{70} was noted. The 7,8 product is qualitatively less stable toward decomposition than the 1,9 isomer, but both are indefinitely stable in toluene or hexane at −20°C. Both compounds are more sensitive toward the combination of light and air than is the only observed isomer of $C_{60}H_2$. Infrared spectra of the $C_{70}H_2$ compounds are complex, but their electronic spectra (Fig. 2) show significant features that may be characteristic of the addition pattern to the C_{70} cage and therefore useful in future structural assignments.

Conversion of pure 7,8-$C_{70}H_2$ to a mixture of 7,8- and 1,9-$C_{70}H_2$ (isomerization) and C_{70} (decomposition) was accomplished at room temperature in toluene over a platinum-on-silica catalyst (*15*). The composition of the mixture versus time obtained by high-performance liquid chromatography (HPLC) with detection at 340 nm is shown in Fig. 3. If the kinetic scheme given by

$$C_{70} \xleftarrow{k_3} 7{,}8\text{-}C_{70}H_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} 1{,}9\text{-}C_{70}H_2 \xrightarrow{k_2} C_{70}$$

accurately describes the dominant chemical reactions, it should be possible to obtain values for all four rate constants which lead to time-dependent concentration curves consistent with the experimental data. By letting *A*, *B*, and *C* represent the fractional composition of 7,8-$C_{70}H_2$, 1,9-$C_{70}H_2$, and C_{70}, respectively, simple kinetic analysis gives

$$\frac{dA}{dt} = -(k_1 + k_3)A + k_{-1}B$$

$$\frac{dB}{dt} = k_1A - (k_{-1} + k_2)B$$

$$\frac{dC}{dt} = k_2B + k_3A$$

Trial curves (not shown) drawn through the data show that the derivative of curve $C(dC_{70}/dt)$ drops slowly and smoothly with time. Because this derivative depends only on the two rate constants k_2 and k_3, estimates of the derivative at several times (k_3 is directly available from the initial slope) together with estimates of *A* and *B* from the trial curves allowed initial estimates of both k_2 and k_3. In a similar fashion, the derived value of k_2 and estimated derivatives of the trial *B* curve at several different times allowed initial estimates of k_1 and k_{-1}. Numerical methods were then used to generate theoretical concentration curves, and the parameters were optimized to arrive at both the optimum fit and error estimates. The results are $k_1 = 0.0165 \pm 0.0015$ hour^{-1}, $k_{-1} = 0.0015 \pm 0.0004$ hour^{-1}, $k_2 = 0.00166 \pm 0.0002$ hour^{-1}, and $k_3 = 0.0035 \pm 0.0005$ hour^{-1}.

The high quality of the numerical fits (Fig. 3) indicates that the proposed kinetic scheme is consistent with the experimental results. The equilibrium constant (k_1/k_{-1}) calculated from these parameters is 11 ± 2, which yields a $\Delta G_{295} = -1.4 \pm 0.1$ kcal/mol. Consideration of possible sources of systematic error, for example, from the approximation of equal molar absorptivities for all three species (*16*), leads to a larger error estimate in the ΔG_{295} than is required by the fit to the data. Therefore, a conservative estimate of the free energy difference between the 1,9 and 7,8 isomers is $\Delta G_{295} = 1.4 \pm 0.2$ kcal/mol, with the 1,9 isomer lying lower in energy. This value is in excellent agreement with the energy difference of 1.3 kcal/mol obtained from the ab initio HF/6-31G* calculations.

We note that MNDO calculations are neither qualitatively nor quantitatively in agreement with experiment and should only be used with extreme caution in fullerene studies involving small energy differences (*17*). The C_{70} dihydrides, which are the "simplest hydrocarbon derivatives of C_{70}," have provided fundamental kinetic data and initial thermodynamic information about addition to the fullerenes.

REFERENCES AND NOTES

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4. For nomenclature, see R. Taylor, *J. Chem. Soc. Perkin Trans. 2*, 813 (1993).
5. Only exo isomers of $C_{70}H_2$ are considered in the count of 143 isomers: calculations suggest that hydrogen addition to the interior of C_{70} is not favorable.
6. J. J. P. Stewart, *J. Comput. Chem.* **10**, 209 (1989).
7. M. J. Frisch *et al.*, GAUSSIAN 92 (Gaussian, Inc., Pittsburgh, PA, 1992).
8. No significant reaction occurs between C_{70} and BH_3:THF at 0°C, conditions that yielded 1,2-$C_{60}H_2$ in 20 to 30% yield [C. C. Henderson and P. A. Cahill, *Science* **259**, 1885 (1993)]. The previously reported reaction of C_{60} with BH_3:THF yielded, after hydrolysis, 1,2-$C_{60}H_2$ as the major product (H_2 addition to a 6,6-ring fusion). For reference, hydrolysis with D_2O yielded $C_{60}HD$ with a coupling constant, $^3J_{HD}$, of 2.4 Hz. More recent NMR spectroscopic data on $C_{60}H_2$ has revealed coupling constants due to natural abundance ^{13}C. $^1J_{CH}$ = 142 Hz and $^3J_{HH}$ = 15.7 Hz (consistent with the HD data). These data demonstrated that 1,2-$C_{60}H_2$ is static on the NMR time scale and that the hybridization of the C–H carbons is intermediate between sp^2 and sp^3. See also L. Becker, T. P. Evans, J. L. Bada, *J. Org. Chem.* **58**, 7630 (1993).
9. This ratio is similar to the 1:2.1 ratio observed in the osmylation of C_{70} [J. M. Hawkins, A. Meyer, M. A. Solow, *J. Am. Chem. Soc.* **115**, 7499 (1993)].
10. C. J. Welch and W. H. Pirkle, *J. Chromatogr.* **609**, 89 (1992).
11. The high-resolution FAB MS was calculated for

$^{13}C^{12}C_{69}H_2$ to be 843.0190 and found for both the 1,9 and 7,8 isomers to be 843.0192. Mass peaks were broadened by the isotope mixture. Unlike $C_{60}H_2$, signals for the parent fullerene ions were not observed by FAB MS.

12. The chemical shifts of the $C_{70}H_2$ isomers are ~2 ppm upfield from that of $C_{60}H_2$ in the same solvent, δ 5.93 ppm. This result may indicate that $C_{70}H_2$ is less acidic than $C_{60}H_2$, the pK_a of which is 4.8 ± 0.3. D. H. Evans, paper presented at the Electrochemical Society Meeting, New Orleans, LA, 13 October 1993.
13. MNDO/PM3 heats of formation, relative to that of 7,8-$C_{70}H_2$, for the 21,22 and 23,24 isomers are 7.08 and 48.45 kcal/mol [corresponding data from (*3*) for the 21,22 isomer is 6.80 kcal/mol]. Addition to the equatorial carbons is predicted to be extremely unfavorable. The calculated reaction of H_2 with C_{70} to give 23,24-$C_{70}H_2$ is endothermic by 11.20 kcal/mol.
14. For comparison, $^3J_{HH}$ = 14.3 Hz for $C_{60}H_2O$ (dihydride epoxide) and $^3J_{HH}$ = 14.1 ± 0.5 Hz for 1,2,3,4-$C_{60}H_4$ for the 6,6-ring fusion (only 9.8 ± 0.5 Hz for the 5,6 fusion). Other $C_{60}H_4$ isomers have coupling constants at 6,6-ring fusions of 15.5 to 15.8 Hz (C. C. Henderson, C. M. Rohlfing, R. A. Assink, P. A. Cahill, *Angew. Chem.*, in press).
15. The support is a Buckyclutcher I phase in which an unusually large amount of platinum remained from synthesis of the ligand.
16. Tighter error limits might result if (i) precise molar absorptivity data becomes available or if (ii) a catalyst that does not lead to hydrogen loss could be found. Such a catalyst would also be useful for studies of the isomerization of the multiple $C_{60}H_4$ isomers formed on reduction of C_{60} or $C_{60}H_2$.
17. Data on relative energies of $C_{60}H_4$ isomers derived from semiempirical calculations is therefore suspect [see, for example, N. Matsuzawa, T. Fukunaga, D. A. Dixon, *J. Phys. Chem.* **96**, 10747 (1992)]. We have repeated the $C_{60}H_4$ calculations at the HF/3-21G and HF/6-31G* levels and have found a markedly different energy ordering (C. C. Henderson, C. M. Rohlfing, R. A. Assink, P. A. Cahill, *Angew. Chem.*, in press).

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A Mass Spectrometric Solution to the Address Problem of Combinatorial Libraries

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The molecular weights of femtomole quantities of small peptides attached to polystyrene beads have been determined with imaging time-of-flight secondary ion mass spectrometry. The analysis is made possible by the selective clipping of the bond linking the peptide to a bead with trifluoroacetic acid vapor before the secondary ion mass spectrometry assay. The approach can be applied to large numbers of 30- to 60-micrometer polystyrene beads for the direct characterization of massive combinatorial libraries.

Combinatorial synthetic methods, which entail a series of chemical steps with multiple reagent choices for each step, can provide large repertoires of compounds with extensive molecular variation (*1–8*). Collections or libraries containing more than a million members have been created through synthesis on solid supports such as Merrifield beads (*5, 7*). The chemical identity of an oligonucleotide or peptide attached to a single bead can often be elucidated by microsequencing methods. For other families of compounds, the problem of identification has been solved by the attachment of tag molecules to the bead, thus encoding a history of the chemical operations. In the first of two recent examples, an oligonucleotide was cosynthesized alternating with each reagent addition. The resulting deoxynucleotide strand was then amplified and sequenced to determine the identity of the compound attached to the support bead (*8*). In the second example, a set of tagging molecules that encode a given reagent added in that step was attached to the supporting bead, and the process was repeated with additional sets of tagging molecules added during subsequent reagent addition steps. The collection of tags attached to a single supporting bead was liberated chemically and then identified by electron capture, capillary gas chromatography (*9*).

In this report, we propose a direct mass spectrometric assay that is generally applicable to the identification of the chemical nature of the compound on a single supporting bead in a large combinatorial library. This method does not depend on the synthesis of a tagging molecule or the attachment of sets of tagging molecules after each chemical separation. Although we illustrate the method for trimer peptides, the assay should ultimately prove useful in identifying a variety of pharmaceutically active agents.

We use imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) to identify the molecular weights of molecules bound to polystyrene bead surfaces (Fig. 1). This technology is potentially suited to such a problem (*10, 11*) because (i) molecular ions from a wide variety of precursors may be desorbed intact (*12, 13*); (ii) the parallel detection and high mass resolution associated with TOF detection provide a 10^4- to 10^6-fold improvement in sensitivity over scanning mass spectrometric methods (*14*); and (iii) the primary ion beam may be focused to a spot size of <150 nm, so that the concentration of molecules can be mapped over small spatial domains (*10, 11*). Extreme sensitivity for an assay of the kind proposed is obviously necessary. For example, a 40-μm sphere covered with one layer of Phe will only have 50 fmol of surface molecules available for sampling.

To test the feasibility of this approach, we examined the TOF-SIMS spectrum of a 40-μm polystyrene bead coated with about one molecular layer of Phe. This sample exhibits large peaks at mass-to-charge ratios (m/z) of 120 $(M-CO_2H)^+$, 166 $(M + H)^+$, 188 $(M + Na)^+$, and 210 $(M-H + Na_2)^+$ (Fig. 2A). Other peaks characteristic of bulk polystyrene (*15*) and Cu are also assignable. Phenylalanine was deposited on the bead by a simple physisorption procedure whereby the bead was immersed in a 10^{-4} M methanol solution of Phe, removed after several minutes, allowed to air-dry, and then placed on a Cu surface for analysis. For these measurements, the dose of incident 25-keV Ga^+ ions was controlled by limiting the sample exposure to 200,000 pulses (20 ns in duration each) of a 500-pA current. This exposure corresponds to 10^7

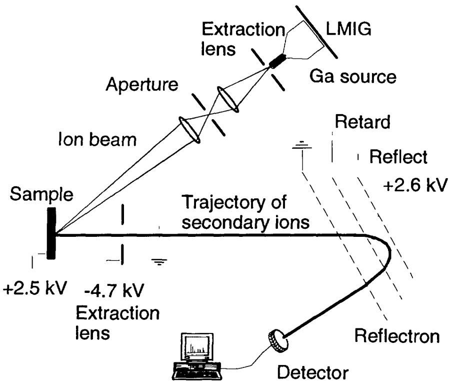

Fig. 1. Schematic diagram of the TOF-SIMS apparatus. Gallium ions from a liquid metal ion source (LMIG) are accelerated to 25 kV and are focused onto the sample with a spot size of ~150 nm. The beam is pulsed by rapid electrical deflection through an aperture typically for ~20 ns. Molecular ions sputtered from the sample are extracted through a field of 7.2 kV into a 2-m reflectron TOF analyzer. Ions are counted at a channel plate detector and processed by an online computer.

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