Oxygen and Methylene Adducts of C_{60} and C_{70}

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Received March 18, 1991

Ions of the carbon clusters C_{60} and C_{70} derived from mixtures of fullerenes[1] extracted from soot[2] are accompanied by satellite ions in low abundance. Changes in the relative ion abundances of these peaks with the method of sample preparation and as a function of evaporation into the mass spectrometer suggest that there are several different fullerene adducts, corresponding to the addition of 14, 15, 16, and 17 daltons (Da). The mass increases are assigned to CH_2, CH_3,[3] O,[4,5] and OH, respectively.[6]

The carbon clusters C_{60}, C_{70}, and their derivatives were extracted from soot prepared in a graphite arc operating under an ac current of 200 A.[2] The satellite ions occur for C_{60}^-, C_{60}^+, C_{70}^-, and C_{70}^+. Their presence in different types of mass spectra (electron attachment using NH_3 as the chemical-ionization reagent and electron impact to generate singly and doubly charged ions) discounts their production via ionic processes occurring in the mass spectrometer.[7] The omnipresence of the adducts of C_{60} and C_{70} indicates that they are prepared concurrently with the fullerenes in either the arc-welding synthesis or the continuous extraction process, or both.

Displayed in Figure 1 is a small portion of the electron-attachment mass spectrum of a fullerene mixture. It shows a group of peaks that appear as a weak (3% relative abundance) satellite of the C_{70}^- signal. The spectrum demonstrates that there are at least four discrete C_{70} adducts with negative ions corresponding to the masses 854, 855, 856, and 857 Da with contributions from naturally occurring ^{13}C accounting for the overall profile.[8] An equivalent spectrum was recorded for the C_{60} adducts. The evaporation profiles (temporal dependence of ion abundance during sample evaporation into the mass spectrometer) of the +16 and +17 adducts of C_{60} had different shapes, providing further evidence for the existence of two compounds.

Collision activated dissociation (CAD) experiments[9,10] with xenon at 40 eV and 1.8 mTorr revealed that $(M + 17)^-$ ($M = C_{60}$ and C_{70}) species fragment to yield C_{60}^- and C_{70}^-, respectively.

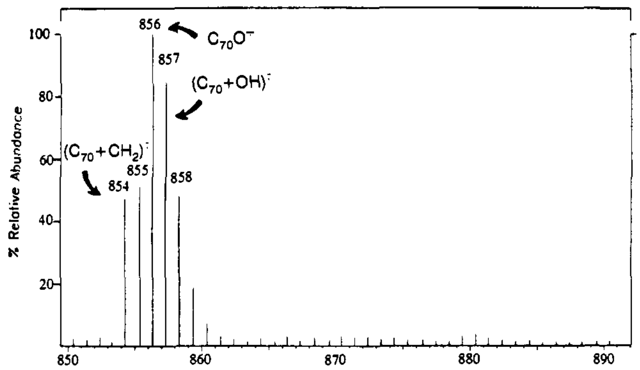

Figure 1. Electron-attachment mass spectrum of a soot extract in benzene showing C_{70} satellite peaks.

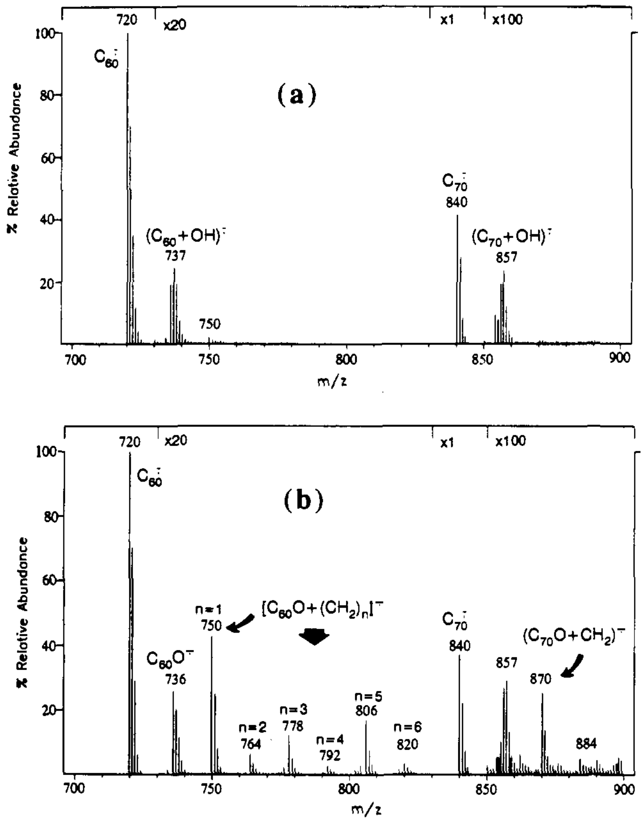

Figure 2. (a) Electron-attachment mass spectrum of raw soot extract in 1:1 CD_2Cl_2/hexanes; (b) after 3-h irradiation, showing the $(C_{60}O+(CH_2)_n)^-$ and $(C_{70}O+(CH_2)_n)^-$ adducts.

The $(M + 16)^-$ adduct of C_{60} fragmented to C_{60}^- in the course of more energetic collisions (100 eV). The vigorous conditions needed for CAD suggest that the adducts are covalently bonded. The $(M + 17)^-$ adducts are ascribed to the addition of the elements O and H, and the $(M + 16)^-$ ions are assigned as O adducts. The $(M + 14)^-$ and $(M + 15)^-$ peaks are presumably CH_2 and CH_3 adducts, respectively. These observations were corroborated by the positive-ion, electron-impact spectra, which confirmed the existence of the four types of adducts. However, signal-to-noise characteristics were no longer adequate for CAD experiments.

Results of a series of experiments under various conditions of arcing, extraction, and treatment of the extract suggest that photochemical processes are involved in derivatization of fullerenes. The $C_{60}O$ adduct was enhanced, relative to C_{60} and the C_{60} + OH adduct, by 1 order of magnitude upon UV irradiation of solutions in air.[11] A similar but less dramatic change occurred for the $C_{70}O$

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[4] The oxide $C_{70}O$ has been observed previously. See: Diederich, F.; Ettl, R.; Rubin, Y.; Whetten, R. L.; Beck, R.; Alvarez, M.; Anz, S.; Sehsharma, D.; Wudl, F.; Khemani, K. C.; Koch, A. *Science* **1991**, *252*, 548.

[5] The ion *m/z* 736, generated by fast-atom bombardment, was shown to have the formula $C_{60}O$ (Gross, M. L.; Caldwell, K., personal communication, March 1991).

[6] Confirmation by exact mass was not possible due to low ion abundance and short evaporation time.

[7] Mass spectra were recorded by using a Finnigan triple quadrupole Model 700. Samples were introduced by desorption from a probe which was ramped from ambient temperature to 800 °C in 10 s. Electron attachment was performed by using NH_3 as a gas to moderate electron energies and to allow nondissociative electron attachment. See: (a) von Ardenne, M.; Steinfelder, K.; Tummler, R. *Elekronenanlagerungs-massenspektrometrie Organischer Substanzen*; Springer-Verlag: Berlin, 1971. (b) Hunt, D. F.; Sethi, S. K. *J. Am. Chem. Soc.* **1980**, *102*, 6953. (c) Dillard, J. G. *Chem. Rev.* **1973**, *73*, 589.

[8] If the ^{13}C contribution from *m/z* 856 is subtracted from the peak at *m/z* 857, an ion, identified as $C_{70}OH^-$, at 19% relative abundance remains.

[9] Busch, K. L.; Glish, G. L.; McLuckey, S. A. *Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; VCH Publishers: New York, 1988.

[10] Luffer, D. R.; Schram, H. K. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 555.

[11] The solutions in soft glass or quartz vials were irradiated with a 150-W mercury arc lamp for 1-4 h.

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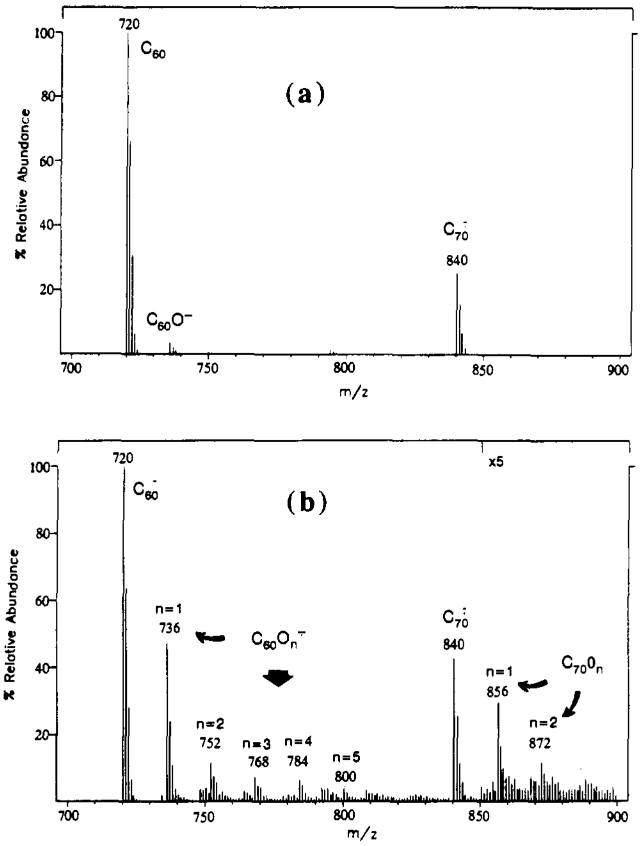

Figure 3. (a) Electron-attachment mass spectrum of ether washings of raw soot extract; (b) after 45-min irradiation, showing the $(C_{60}O_n)^-$ and $(C_{70}O_n)^-$ adducts.

adduct. These results clearly indicate that the oxides are created outside the mass spectrometer.

Even more significant is the observation that the M + 16 adduct is the first member of a series of derivatives we interpret as sequential cyclopropanation and epoxidation products of monoxides. For example, Figure 2a shows the electron-attachment spectrum of benzene extracts of graphitic soot. Ultraviolet irradiation for 1 h (Figure 2b) not only increased the $(M + O)^-$ peaks substantially (with appropriate isotopic peaks at higher mass) but also produced peaks that correspond to $(C_{60}O + (CH_2)_n)^-$ where n = 1, 2, 3, 4, 5, and 6 and $(C_{70}O + (CH_2)_n)^-$ where n = 1 and 2. However, these products were not increased upon irradiation of fullerene mixtures that were thoroughly washed with ether. This suggests that the ether washing removed an unidentified compound that participated in the photochemical reaction of the fullerenes.

In an attempt to concentrate the reactive compound we irradiated ether washings that contained small amounts of C_{60} and C_{70} and discovered a new photochemical reaction. As expected, we saw increased yields of $C_{60}O$ and $C_{70}O$. However, rather than sequential CH_2 additions we observed sequential oxygen atom additions; the monoxides were accompanied by peaks corresponding to $C_{60}O_n$, where n = 2, 3, 4, and 5, and $C_{70}O_n$, where n = 2. Mass spectra of these ether washings recorded before and after irradiation are shown in Figure 3. Similar spectra were obtained on irradiation of ether washings redissolved in benzene, thus discounting ether as the oxygen source.

Low-mass hydrogen containing compounds, relative to C_{60}, are produced in the arc-welding synthesis, and their effect on the aforementioned photochemistry is being investigated. Isolation and characterization of the adducts themselves are also underway.

Acknowledgment. This work was supported by the Purdue University Department of Chemistry, the Exxon Education Foundation, and the National Science Foundation (CHE 87-21768). We appreciate the assistance of Jason C. Gunderson.

The Electron Input to Cytochrome *c* Oxidase from Cytochrome *c*[†,‡]

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Received April 4, 1991
Revised Manuscript Received May 30, 1991

The intermolecular electron transfer (ET) between cytochrome *c* and cytochrome *c* oxidase (CcO) has attracted considerable attention in recent years.[1-6] It is still controversial, however, which one of the two low-potential centers (Cu_A and cytochrome *a*) is the primary electron acceptor in the native form of the enzyme. The determination of the initial electron acceptor is of particular interest because of the possible involvement of one of these two centers in proton pumping.[7,8]

In this communication, we report kinetic studies of the intracomplex ET between cytochrome *c* and CcO in both the native and Cu_A-depleted forms using the laser flash photolysis technique recently developed by Hazzard et al.[5] In this experiment, cytochrome *c* is rapidly reduced by flavin semiquinone generated by the laser excitation of 5-deazariboflavin (5-DRF) in the presence of EDTA and the ET from ferrocytochrome *c* to CcO is followed by optical spectroscopy.

Figure 1 shows the kinetic data observed for the intracomplex ET between bovine cytochrome *c* and fully oxidized native bovine CcO at 1:1 molar ratio and 110 mM ionic strength.[9] The reduction of ferricytochrome *c* by 5-DRF semiquinone and its subsequent reoxidation by CcO were monitored at 550 nm (Figure 1A). The kinetic trace is biphasic and fits well to a sum of two exponentials. A rate constant of 1250 ± 63 s^{-1} is obtained for the fast phase with an amplitude corresponding to 75% of the total signal change. The reduction of cytochrome *a* was followed at 604 nm (Figure 1B). This reduction is also biphasic with a rate constant of 1300 ± 45 s^{-1} for the fast phase. Thus, there is excellent correspondence between the reoxidation of the ferrocytochrome *c* and the reduction of cytochrome *a* in the fast phase.

Under otherwise identical conditions, the Cu_A-depleted CcO[9] also displays biphasic kinetics for the reoxidation of ferrocytochrome *c* and reduction of cytochrome *a* (Figure 2). Whereas the rate constants for the slow phase are the same for both the native and the Cu_A-depleted enzymes (slower than 80 s^{-1}), the rate constant for the fast phase for the Cu_A-depleted protein is approximately 25% that of the native enzyme. The fitting gives 300 ± 20 s^{-1} (at 604 nm) and 320 ± 18 s^{-1} (at 550 nm).

The rate constant for the fast kinetic phase (k_{obsd}) is dependent on the concentration of CcO.[5] This dependence is hyperbolic for

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[†] Contribution No. 8421 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, CA 91125. This work was supported by NIH Grants GM 22432 (to S.I.C.) and DK 15057 (to G.T.).

[‡] Abbreviations: ET, electron transfer; CcO, cytochrome *c* oxidase; 5-DRF, 5-deazariboflavin.

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