Figure 1. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, nucleotide no. 138-5238, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min warming at 100 °C, gel electrophoresis, and autoradiography. Lanes 1–4, Sanger G, C, A, and T sequencing reactions; lanes 5–8, (+)-CI-CDPI$_2$ [(5.6 × 10^{-4}) – (5.6 × 10^{-7} M)]; lanes 9–12, (+)-CPI-CDPI$_2$ [(5.6 × 10^{-5}) – (5.6 × 10^{-8} M)]; lanes 13–16, (+)-CBI-CDPI$_2$ [(5.6 × 10^{-5}) – (5.6 × 10^{-8} M)]; lane 17, control DNA. The origin of the double bands observed for a single adenine N-3 alkylation with 5'-end-labeled double-stranded DNA has been detailed elsewhere (ref 5 and 6).

The resultant DNA was treated with the agents at 4 or 37 °C (24 h) at a range of concentrations. Removal of the unreacted agent through ethanol precipitation of the DNA, redissolution of the alkylated DNA in aqueous buffer, thermally induced cleavage of the DNA at the sites of alkylation (100 °C, 30 min),[2,5] gel electrophoresis of the resultant DNA alongside Sanger dideoxynucleotide sequencing reactions, and subsequent autoradiography revealed the agent sites of alkylation and their relative intensities, Figure 1.[6] As illustrated in Figure 1, the most stable and least reactive agent [(+)-4] exhibits the most intense DNA alkylation at 4 °C, and the exceptionally reactive agent [(+)-2] exhibits the least intense DNA alkylation. Thus, an inverse (versus direct) relationship between the intensity of DNA alkylation and the chemical solvolytic reactivity of the agents was observed. In addition, the most reactive agent [(+)-2] exhibits slightly less selectivity among the available alkylation sites than (+)-4 or (+)-3. As detailed elsewhere, this more selective and productive covalent modification of DNA by the more stable agent presumably arises in part from the enhanced agent availability (stability, selectivity).[16] However, when the DNA alkylation reactions were taken to >90% completion (37 °C, 24 h) under conditions where excess DNA was present and the alkylation would be expected to follow first-order kinetics with respect to the agent concentration (10^{-7}–10^{-8} M agent), each of the three agents exhibited a comparably intense DNA alkylation, Table I. Under such conditions where the agents are effectively sequestered by double-stranded DNA,[19] the results suggest that the nonproductive solvolysis[20] and alkylation selectivity differences account for only part of the distinctions in the observed DNA alkylation intensity at 4 °C. In particular, the comparisons of (+)-4 and (+)-3 made at 4 °C (24 h)[20] additionally reflect the relative rates of DNA alkylation

by the two agents, which follow the unexpected order[19] of (+)-CBI-CDPI$_2$ > (+)-CPI-CDPI$_2$, Table I. The precise origin of this relationship and its generality are under investigation.[21]

Although limited to the three classes of agents presently available for comparison,[17] the inverse correlation between the cytotoxic potency of the agents and the solvolytic reactivity of the cyclopropane suggests that the direct relationship between the agent stability and cytotoxic activity may constitute a relevant feature in the further design of functional analogues, Table I. Potentially contributing to the distinctions in the properties of the agents examined herein[17] may be the unexpected observation of the more rapid (rate), more discriminate (selectivity), and more productive (intensity) DNA alkylation by the more stable agent.

Supplementary Material Available: Table of IC$_{50}$, k, $t_{1/2}$, and DNA cleavage data and graph of $-\log k$ vs $\log$ IC$_{50}$ for various agents and listing of agents and their IC$_{50}$ values (2 pages). Ordering information is given on any current masthead page.

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(20) Both **3** and **4** are stable to the conditions of assay in the absence of double-stranded DNA while **2** is of limited stability. In the case of **3** and **4**, the adenine N-3 alkylation has been shown to account for ≥85–95% of the consumption of the agent in the presence of double-stranded DNA.
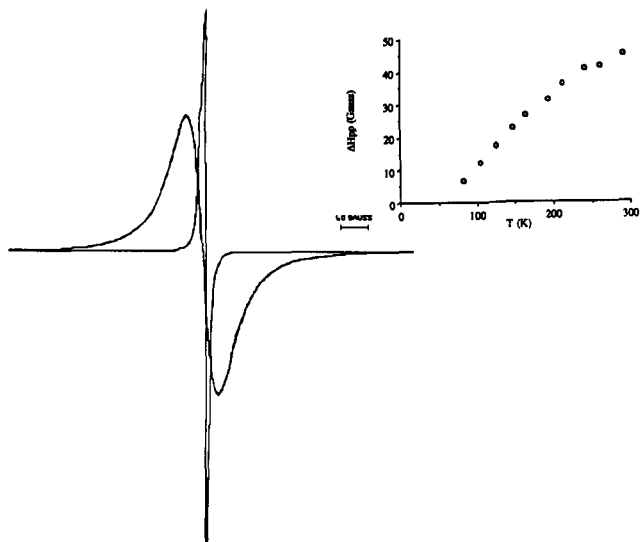
(21) Other agent properties may correlate with the DNA alkylation intensity. Such correlations are under examination.

The Unusual Electron Spin Resonance of Fullerene C$_{60}$$^{•-}$

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The success in the preparation of the fullerenes[1–3] and the confirmation[4,5] of the theoretical prediction[6] that C$_{60}$ is a relatively electronegative molecule prompted us to prepare the first fulleride salt and evaluate its condensed matter properties. Here we report on electron spin resonance and transport properties.

Samples of the pure fullerene C$_{60}$ were obtained as described previously.[3] We discovered five interesting features:

1. Upon bulk electrolysis, fullerene C$_{60}$ deposits the C$_{60}$$^{•-}Ph_4P^+$·(Ph$_4$PCl)$_2$ salt as a microcrystalline powder[7] at a platinum

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(7) X-ray powder pattern determination indicates a pure, crystalline phase, with a pattern that is unrelated to, and much simpler than, the powder pattern of Ph$_4$P$^+$Cl$^-$. Attempts to index and obtain unit cell parameters are in progress.

Communications to the Editor

J. Am. Chem. Soc., Vol. 113, No. 7, 1991 2781

Figure 1. Electron spin resonace spectrum of C_{60}`*-`. The broad line (ca. 45 G) is the 300 K line; the narrow line is the same sample at 80 K. The inset is the change in line width with temperature.

Figure 2. The reciprocal of the spin magnetic susceptibility of fullerene C_{60} as a function of temperature. The width of the data points does not reflect the experimental error.

electrode.[8] Even though the results of elemental analysis[8] can be reconciled better with the empirical formula shown above (or HC_{60}^{2*}`-`Ph_4P^+·$(Ph_4PCl)_2$),[10] the data could also correspond to a trianionic compound C_{60}`*` $^{3-}$$(Ph_4P^+)_3$·$C_6H_4Cl_2$; however, infrared spectroscopy shows the absence of diagnostic $C_6H_4Cl_2$ bands ($C_6H_4Cl_2$ = o-dichlorobenzene, the solvent of electrolysis) in a sample of the C_{60} tetraphenylphosphonium salt. Besides, the observed atmosphere stability of our solid is more in accord with a monoanion, which should be relatively stable;[5] the trianion (C_{60}`*`$^{3-}$) would be expected to be very reactive, particularly toward

atmospheric oxygen and moisture due to its relatively high reduction potential ($E_3^{1/2}$ = −1.21 V vs Ag/AgCl).[5]

2. The solid-state electron spin resonance signal at 300 K is a 45-G, symmetric line with g = 1.9991 (±0.0002), to be contrasted with the typical, free-electron value of 2.0023.

3. There is a dramatic temperature effect on the line width, as shown in Figure 1.

4. The temperature dependence of the signal intensity (spin susceptibility) follows the Curie–Weiss law,[9] as shown in Figure 2. Careful integration of the signal at 300 K and comparison with DPPH (diphenylpicrylhydrazyl) gives 1.5 S = $^1/_2$ spins per C_{60}, a value corroborated by static magnetic susceptibility measurements in the temperature range 300–150 K; θ = 0 and C = $\chi_P T$ = 0.5. Since

$$C = \chi T = Ng^2\mu_B^2 S(S + 1)/3k_B$$

for our sample mass of 14.5 mg, C = 0.125$g^2 S(S + 1)$; if $g \approx 2$, then C = 0.375 for 1 paramagnetic center with S = $^1/_2$ per molecule, yet the experimental value is 0.5.[10] The origin of the ~0.5 "excess spin" is unknown at this stage.[10] The unusual g value is consistent with the high degree of degeneracy of the LUMO[6] and the results of photophysics studies.[11]

5. Samples of C_{60}`*-`Ph_4P^+·$(Ph_4PCl)_2$ salt, in the form of a pressed pellet, exhibit room temperature conductivities in the range of 10^{-5}–10^{-7} S cm^{-1}.

In conclusion, we discovered that C_{60} can be reduced to a stable radical anion salt that is a semiconductor. The most unusual feature is the highly shifted g value of the ESR line, into the range usually observed in transition-metal and rare-earth compounds. This could be due to a large spin–orbit coupling resulting from the electronic structure (triply degenerate LUMO) of the fullerene. Another unusual feature is the dramatic decrease in ESR line width with temperature in the range 300–81 K. A possible explanation is a combination of motional narrowing[12] and an increase in intermolecular hopping with decreasing temperature and exchange narrowing by the same process; further experiments are necessary to better understand this phenomenon.

Other fullerene salts as well as covalent derivatives are being prepared, and their properties will be reported in due course.

(8) An H cell with a graphite anode and Pt cathode was employed; a mixture of o-dichlorobenzene (2 × 10^{-3} M C_{60}) and Ph_4PCl (0.7 M) was the electrolyte. The current was 0.6 μA. Further details will be presented in a separate publication. Exactly the same material can be prepared by chemical reduction in the presence of excess Ph_4PCl. Elemental anal. Calcd for $C_{132}H_{60}Cl_2P_3$: C, 87.61; H, 3.32; Cl, 3.93; P, 5.14. Found: C, 87.54; H, 3.22; Cl, 3.80; P, 5.15. Different samples were analyzed by two independent laboratories (Galbraith and Spang) with the same result. IR (KBr, ν, cm^{-1}; (*) = frequency common to both C_{60} and the salt, (#) = frequency common to both the salt and Ph_4PCl): 1585 w, 1485 w, 1440*# s, 1390 m, 1335 w, 1315 w, 1180 w, 1110# s, 1000# m, 755# m, 725# s, 690# m, 575 w, 530*# s. UV–vis (reflectance, λ_{max}, nm): 260, 350, 460 br, 580 br increased absorption above 800 nm, our spectrometer cuts off at 800 nm. UV–vis C_{60} (reflectance, λ_{max}, nm): 260, 350, 500 br, 625, 675.

(9) ESR spectra of powdered [$C_{60}Ph_4P(Ph_4PCl)_2$] (1.0 mg) were recorded with a Bruker ER200D-SRC operating at the X band (9.6 GHz) with 100-kHz modulation and equipped with an Air Product LTD-3-110 variable temperature cryostat. While a ruby was used as intensity standard for the temperature dependence of the ESR signal, the spin concentration at room temperature was determined vs solid DPPH and g values were also determined relative to DPPH. Observation of a larger than the spin-only calculated magnetic susceptibility value is not unprecedented in the case of transition-metal salts (West, A. R. *Solid State Chemistry and its Applications*; Wiley: New York, 1984; Chapter 16). There was a barely significant change in g value when it was determined both at room temperature (1.9991) and at ~96 K (1.9978). Powdered samples were placed in a Delrin bucket whose magnetization was measured (SQUID magnetometer SHE 200 using a field of 10 kG) over the entire temperature range and subtracted from the total magnetization; C_{60}, χ_{dia} = −259 × 10^{-6} emu/mol. There appears to be an anomaly at ~50 K which is much larger than expected from oxygen contamination. More data in this region is being collected to establish if it is due to a phase transition.

(10) A plausible approach to obtain higher than the theoretical C = 0.375 spin value is to invoke HC_{60}^{2*}`-`Ph_4P^+·(Ph_4PCl) as the formula of the salt. In fact, the calculated values for the elemental analysis[8] give an even closer fit with the experimentally found values.[8] However, analogous treatment of the data assuming two independent $^1/_2$ electrons per molecule and MW = 1809 gives C = 0.750, which is now 0.25 above the experimentally found value. On the other hand, the same equation yields C = 1 for MW = 3616 (~2 × 1809), implying a triplet (S = 1) dimer. However, a triplet dimer would require C_{60}`-`C_{60}^{2-} in order to maintain electroneutrality and stoichiometry. There are two results of cyclic voltammetry mitigating against this formulation: (a) the disproportionation (2C_{60}^- ⇌ C_{60} + C_{60}^{2-}) would be highly endothermic, and (b) C_{60}^{2-} is formed at ~−0.8 V vs Ag/AgCl, making it highly reactive toward oxygen and moisture.
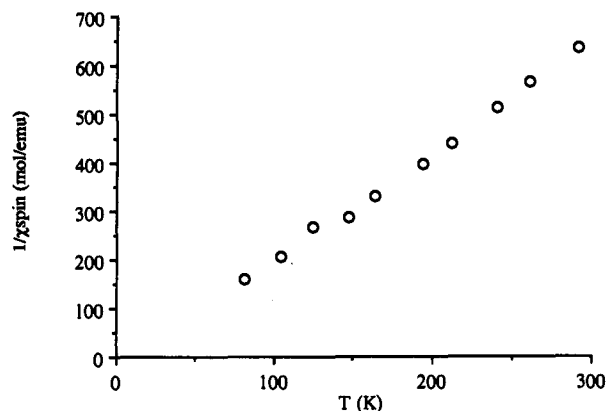
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(12) Molecular rotation and/or oscillation at room temperature is possible, if the salt behaves like neutral C_{60}.[11] As the temperature is lowered, the rotations and/or oscillations may slow down, and the intermolecular distances will decrease, making an increase in the intermolecular interactions and electron exchange (with concomitant line narrowing) possible.

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