Novel Water-soluble Hexa(sulfobutyl)fullerenes as Potent Free Radical Scavengers

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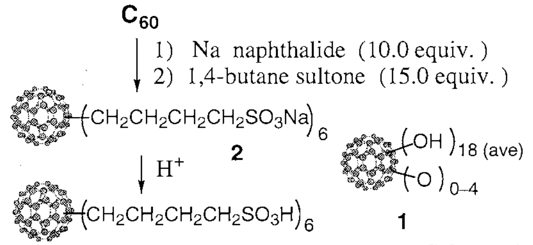
Novel highly water-soluble hexa(sulfobutyl)fullerenes were synthesized via electron reductions of C_{60}. It showed an enhanced superoxide radical ($O_2^{-\cdot}$) scavenging activity at a dose level of 100 μM.

The use of chemopreventive agents with antioxidation activity becomes a recognized approach against the oxidative stress caused by the reactive oxygen species (ROS) in animals and humans.[1] We have recently reported the free-radical scavenging activities of water-soluble fullerenols **1** in eliminating ROS under physiologic conditions[2] and the related biological studies, including inhibition on the H_2O_2-induced oxidative damage of brain neurons[3] and attenuation of the exsanguination-induced bronchoconstriction.[4] Similar free-radical scavenging behavior was also observed using carboxylated fullerene as an antioxidant.[5] Other water-soluble derivatives of fullerenes were noted to be effective in the inhibition of HIV protease[6] and in the generation of singlet oxygen for the DNA cleavage.[7,8]



Scheme 1.

By variation of the averaged conjugation length of olefins around the C_{60} cage, the electron affinity of the resulting adducts can be modified accordingly. That may allow one to alter the reactivity of water-soluble fullerenes toward ROS. Here we report the synthesis of hexa(sulfo-butyl)fullerenes **2** and **3**, as novel water-soluble fullerene derivatives, with a number of addends per C_{60} cage differing much from that of fullerenols[9,10] **1**.

The sodium salt of hexa(sulfobutyl)fullerenes **2** were synthesized in a yield of 80–85% by the treatment of C_{60} in dimethoxyethane (DME) with sodium naphthalide (10.0 equiv.) at 25 °C, followed by reacting the resulting hexaanionic fullerene intermediates with an excess of 1,4-butane sultone (15.0 equiv.), as shown in Scheme 1. After purification by filtration and repeated reprecipitations in MeOH from an aqueous solution, the product **2** gave a single peak in its HPLC chromatogram using a reverse-phase C–18 column with H_2O as eluent. Acidification of **2** with 4N HCl afforded the corresponding hexasulfonic acid **3** in a quantitative yield. The former reaction was carried out under N_2 in a glove-box which was conditioned to under 50 ppm of O_2 to minimize the partial oxidation[11] of fullerenic anions by dissolved O_2. Electron oxidation of anionic C_{60} intermediates by O_2 may produce the corresponding fullerenic radicals. Highly conjugated radical of a multi-sulfobutylated fullerene derivative was found to be rather stable at ambient temperatures. Its *e.s.r* resonance is readily detectable in aqueous solution. Even under rigorously deoxygenated conditions, the persistence of fullerenic radicals in the resulting products was evident from repeated reactions. Therefore, an excess of sodium naphthalide was found to be necessary to achieve the generation of hexaanionic fullerene intermediates. The solution of sodium naphthalide in DME was titrated by succinic acid prior to use. A systematic evaluation carried out using 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, or 16.0 equiv. of sodium naphthalide in a reaction with C_{60} afforded the corresponding **2** with a sulfur/carbon atomic ratio of 0.011, 0.010, 0.029, 0.049, 0.067, 0.063, or 0.063, respectively.[12] Apparently, a further increase in quantity of sodium naphthalide to more than 10.0 equiv. of C_{60} did not lead to the corresponding increase of the sulfur content in the final products. That indicated conceivable saturation of the reduced fullerenes in a hexaanionic state, as major reaction intermediates.

Infrared spectrum of **2** displayed a broad absorption band centered at 3444 cm^{-1} (owing to a hydrated molecule) along with two strong absorptions centered at 1178 and 1050 cm^{-1}, corresponding to the stretching bands of the sulfonic acid salt with a $C{-}SO_2{-}O$ linkage. The ^1H NMR spectrum of **2** in D_2O showed two broad peaks centered at roughly δ 1.92 and 3.10, corresponding to the chemical shift of CH_2–C and CH_2–S protons, respectively. The lack of sharp multiplet proton peaks in this NMR spectrum revealed the presence of a fullerenic radical in **2**, consistent with the detection of an absorption peak (g = 2.0046) in its *e.s.r.* spectrum (H_2O).

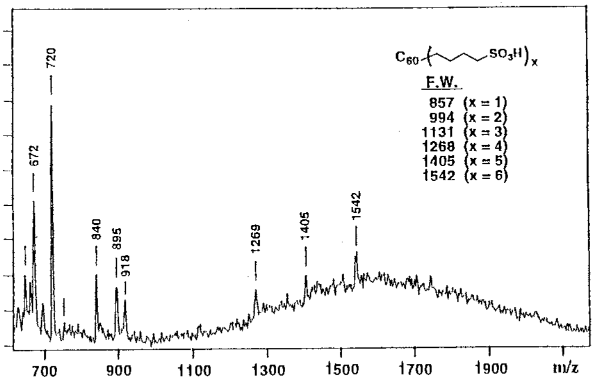


Figure 1. Negative ion matrix-assisted laser desorption ionization (MALDI) mass spectrum of **3** prepared from a mixture of C_{60} and C_{70}. A mixture of sinapinic acid and H_2O or methanol was used as a matrix material.

The mass spectroscopic studies were carried out using both the desorption chemical ionization (DCI) and the matrix-assisted laser desorption ionization (MALDI) techniques. The negative ion DCI mass spectrum of **2** showed a maximum relative intensity at a peak of the C_{60} ion fragmentation (*m/z* 720). It was followed by groups of peaks at *m/z* 776, 832, 887, and 944 having a consecutive weight increase of 56 mass units, which matches with the gain of a butyl group to the preceding ion fragment, corresponding to $C_{60}Bu_1$, $C_{60}Bu_2$, $C_{60}Bu_3$, and $C_{60}Bu_4$, respectively. In the higher mass region of the spectrum, a series of the fragmentation groups with a consecutive weight increase of 136 mass units, corresponding to the mass of a sulfobutyl group ($CH_2CH_2CH_2CH_2SO_3$), were observed. The mass of these fragmented ions agrees with that of di(sulfobutylated), tri(sulfobutylated), tetra(sulfobutylated), penta(sulfobutylated), and hexa(sulfo-butylated) fullerenes. In the case of the negative ion MALDI mass spectrum of **3** (Figure 1), a conceivable molecular ion of $C_{60}(CH_2CH_2CH_2–CH_2SO_3H)_6$ at *m/z* 1542 was detected. It was followed by two groups of the ion fragments with a maximum intensity of peak at *m/z* 1405 and 1269, corresponding to the mass of $C_{60}(CH_2CH_2CH_2CH_2SO_3H)_5$ and $C_{60}(CH_2CH_2CH_2CH_2–SO_3H)_4$, respectively. These mass spectroscopic data provided clear evidence of the chemical composition for these hexa(sulfobutylated) fullerene compounds.

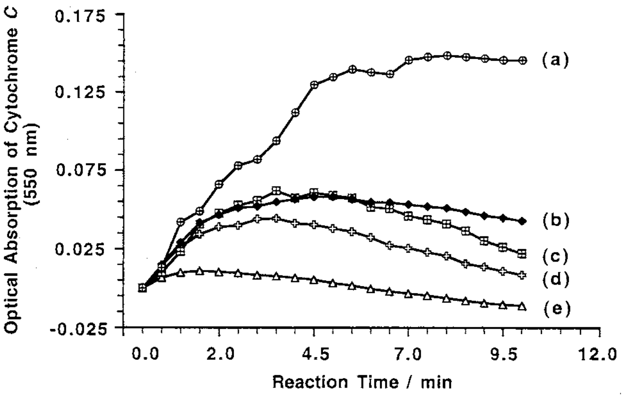


Figure 2. Suppression activity of **1** and **2** on the $O_2^{-\cdot}$-induced ferricytochrome *c* reduction at a dose level of (a) 0.0 μM, (b) 50 μM of **2**, (c) 50 μM of **1**, (d) 100 μM of **1**, and (e) 100 μM of **2**, as measured by the optical absorbance of ferrocytochrome *c* at a constant wavelength of 550 nm.

High reactivity of fullerenols **1** toward ROS was demonstrated by employing a xanthine/xanthine oxidase enzymatic system for the generation of superoxide radicals ($O_2^{-\cdot}$).[3] Conversion of xanthine to uric acid by xanthine oxidase in the presence of oxygen is accompanied by the production of one equiv. of $O_2^{-\cdot}$. Capture of $O_2^{-\cdot}$ *in situ* in a buffer solution is possible using ferricytochrome *c* (Fe^{+3}), resulting in the formation of the reduced cytochrome *c* which absorbs optically at 550 nm. Experimentally, xanthine (0.05 mM), ferricytochrome *c* (10 μM), and **1** or **2** (0–100 μM) were mixed with EDTA (0.1 mM) in a buffer solution of KH_2PO_4–K_2HPO_4 (50 mM, *pH* = 7.8) prior to the addition of xanthine oxidase ($7x10^{-3}$ unit). In the most of cases, the intensity of optical absorbance at 550 nm increased promptly at a reaction period of less than 4 min and then leveled off within 10 min of reaction, as shown in Figure 2a of a control experiment. A progressive decrease in intensity of the optical absorbance of ferrocytochrome *c* was observed as the dose of **2** being increased from 0 to 100 μM. The phenomenon was interpreted as the response of increasing effectiveness of the dose-dependent suppression by **2** on the superoxide-induced cytochrome reduction. At a dose level of 50 and 100 μM, a profound suppression efficiency of 60 and 96% was detected at a reaction period of 4.5 min, as shown in Figure 2b and 2e, respectively. When fullerenol **1** was utilized as a $O_2^{-\cdot}$-scavenger under similar physiologic conditions, a suppression efficiency of 59% (Figure 2c) and 70% (Figure 2d) was detected at a dose level of 50 and 100 μM, respectively. These results indicated a comparative suppression efficiency of **1** and **2** at a lower dose level below 50 μM and a more than 25% higher in suppression efficiency for **2** than that of **1** at a dose level of 100 μM. The observed high reactivity of water-soluble hexa(sulfobutylated) fullerenes toward ROS at enzymatic sites revealed potential utilization of these molecules as the potent free-radical scavengers in physiologic media.

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11 Y. Murata, K. Komatsu, and T. S. M. Wan, *Tetrahedron Lett.*, **39**, 7061 (1996).
12 Elemental analyses of hydrated **2** were found as follows. Anal. Calcd for $C_{84}H_{88}O_{38}S_6Na_6$ as $C_{60}(CH_2CH_2–CH_2CH_2SO_3Na)_6–20H_2O$: C, 50.45; H, 4.20; O, 28.82; S, 9.60; Na, 6.91. Found: C, 49.56; H, 4.33; S, 9.44; Na, 6.78.