Stabilities and reactivities of buckminsterfullerene radicals, $(Bu^tO)_nC_{60}^{\bullet}$, towards dioxygen, nitric oxide and spin trapping agents

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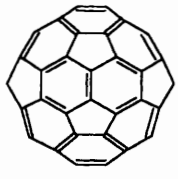
$(Bu^tO)_nC_{60}^{\bullet}$ radicals have been conveniently produced by addition of photochemically generated $Bu^tO^{\bullet}$ radicals to buckminsterfullerene (C_{60}). Radical adducts (where $n > 2$) are very persistent under N_2 at room temperature and can be observed for as long as nine days. Reactions between the radical adducts and dioxygen (O_2) are slow and reactivities vary among $(Bu^tO)_nC_{60}^{\bullet}$ radicals. However, nitric oxide ($^{\bullet}NO$) reacts more rapidly with $(Bu^tO)_nC_{60}^{\bullet}$ radicals. Among the product components are stable dibuckminsterfullerene aminoxyl (nitroxide) radicals, $[(Bu^tO)_nC_{60}]_2N{-}O^{\bullet}$, which have been observed by EPR spectroscopy at concentrations of *ca.* 10^{-7} M for the first time. Surprisingly, $(Bu^tO)_nC_{60}^{\bullet}$ radicals are not reactive towards several typical radical-trapping agents (spin traps), *e.g.* DMPO (5,5-dimethyl-1-pyrroline *N*-oxide), PBN (*C*-phenyl *N*-*tert*-butylnitrone†), NB (nitrosobenzene) and MNP (2-methyl-2-nitrosopropane), when used at typical concentrations (10–100 mM) at room temperature.

Introduction

Hypotheses about the structure of buckminsterfullerene (C_{60}) and theoretical studies on this soccer-ball shaped molecule were made between 1966 to 1985[1–9] before the experimental discovery of fullerenes by Kroto *et al.* with time-of-flight mass spectrometry in 1985.[10] Establishment of the methods for preparation of macroscopic quantities of C_{60} in 1990 by Krätschmer *et al.*[11] greatly stimulated developments in fullerene physics,[12] fullerene chemistry[13,14] and fullerene biochemistry.[15–19] The atomic resolution X-ray crystallographic characterization of $C_{60}(OsO_4)(4\text{-}tert\text{-butylpyridine})_2$ by Hawkins *et al.*[20] in 1991 provided the first definitive proof of the buckminsterfullerene structure.



C_{60}

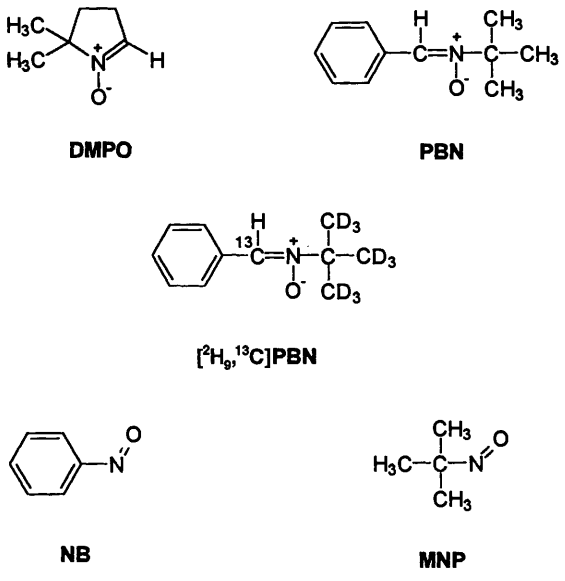
The cage-like structure of C_{60} and the presence of 30 carbon–carbon double bonds define the radical sponge property of this unusual molecule. The investigation into the chemical reactivity of C_{60} towards various free radicals was pioneered by Krusic and co-workers[21–30] and is still in progress.[21–31] C_{60} reacts with benzyl radicals producing paramagnetic and diamagnetic adducts $(C_6H_5CH_2)_nC_{60}$ with $n = 1$ to at least 15.[21,22] With methyl radicals, the reaction of C_{60} forms Me_nC_{60} with $n = 1$ to at least 34.[21] For perfluoroalkyl radicals, up to 16 radicals can add to C_{60} to afford isolable fluoroalkylated derivatives.[30] Multiple additions of *tert*-butoxyl radicals ($Bu^tO^{\bullet}$) to C_{60} have also been proposed.[22] These results indicate that fullerenes such as C_{60} and C_{70} warrant characterization as radical sponges. This property further suggests that fullerenes and/or their specifically designed derivatives are potentially useful for the treatment of some diseases where free radicals are involved. Some interesting progress in the application of C_{60} derivatives to biological systems has been made,[15–19] *e.g.* fullerene carboxylic acids exhibited photoinduced guanine-selective DNA cleaving ability.[15] Several substituted fullerenes were able to inhibit HIV-1 protease.[16,17] Fine aqueous suspensions of C_{60} have been successfully achieved and used in cultured human cells.[18] Studies showed that C_{60} rapidly accumulates in human cells (human keratinocytes or human fibroblasts) and acute toxicity is not found.[18] A fullerene–oligonucleotide conjugate binds in a sequence-specific manner to single- or double-stranded DNA and upon UV irradiation, cleavage of the nucleic acid at guanine residues close to the binding site occurs.[19]

An interesting question is whether fullerenes and/or some fullerene derivatives can be found which have some useful applications in the therapies of free radical-related diseases by combining their radical sponge properties with their biological activities assuming prices become reasonable. In order to begin research in this area, reactions between fullerenes and free radicals, and the stabilities and reactivities of the radical adducts produced, namely R_nC_{60}, are the initial focus of this study. We selected $(Bu^tO)_nC_{60}^{\bullet}$ radicals as the initial topic because most of the free radicals associated with biological disorders are oxygen-centred free radicals.[32,33]

It has recently been found that nitric oxide is a very important small biomolecule involved in a variety of biological processes, including inhibition of cytochrome P-450,[34] antitumour action *in vivo*,[35,36] physiological control of blood pressure and inhibition of DNA synthesis.[37] Therefore, it might be interesting to investigate the reactivity of $(Bu^tO)_nC_{60}^{\bullet}$ radicals towards $^{\bullet}NO$.

Several nitrones and nitroso compounds with special structures have been used as spin traps for almost 30 years to detect and identify reactive free radicals.[38–41] Therefore, several common spin traps were employed to test the reactivity

† IUPAC name: *N*-benzylidene-*tert*-butylamine *N*-oxide.



of $(Bu^tO)_nC_{60}^{\bullet}$ radicals. The spin traps in this study are DMPO (5,5-dimethyl-1-pyrroline *N*-oxide), PBN (*C*-phenyl *N*-*tert*-butylnitrone), [$^2H_9$, ^{13}C]PBN, NB (nitrosobenzene) and MNP (2-methyl-2-nitrosopropane). Results concerning the stabilities and reactivities of $(Bu^tO)_nC_{60}^{\bullet}$ radicals towards nitric oxide ($^{\bullet}NO$), dioxygen (O_2) and some spin-trapping agents will be described.

Experimental

EPR spectra were measured on a Bruker ESP 300E spectrometer at room temperature. Buckminsterfullerene (C_{60}, ≥99%) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The concentration of C_{60} in C_6H_6 was 2×10^{-4} M and the solution had a purple colour. Nitric oxide gas (99.9%) was the product of Air Liquide Electronics (Morrisville, PA). An aminoxyl (nitroxide) impurity was detected when commercial nitric oxide was bubbled into pure C_6H_6 solutions (EPR spectrometer set at 5×10^5 level receiver gain). The aminoxyl impurity was removed by use of a cold-trap cooled with dry ice–acetone (−78 °C) as monitored by EPR spectrometry. A blue liquid of unknown structure was condensed in the cooling trap which became a brown gas upon warming to room temperature. Removal of the paramagnetic impurity was crucial when EPR spectroscopy was the main method of study. A 75 W high pressure mercury UV lamp (ORIEL Corp. of American, Stamford, Conn.) was used for photolysis of free radical precursors such as di-*tert*-butyl peroxide. Nitrone spin traps (DMPO, PBN and [$^2H_9$, ^{13}C]PBN) were produced in the laboratory of OMRF Spin Trap Source (Oklahoma Medical Research Foundation, 825 N.E. 13th St, Oklahoma City, Oklahoma). Nitrosobenzene was purchased from Aldrich Chemical Company, Inc.

Results and discussion

Stability of $(Bu^tO)_nC_{60}^{\bullet}$ radicals under N_2

A solution of C_{60} (2×10^{-4} M) in C_6H_6 with or without UV irradiation, and $(Bu^tO)_2$ solution (0.13 M) in C_6H_6 before or after UV photolysis did not show any EPR signal at room temperature. A purple solution of C_6H_6 containing C_{60} and $(Bu^tO)_2$ also did not show any EPR signal before UV irradiation. After UV irradiation for 1 min under N_2, the colour of the solution changed to brown and a single EPR signal of significant intensity resulted (Fig. 1). The peak-to-peak linewidth was 1.9 G (1 G = 0.1 mT) and became 1.5 G, as reported,[22] if the solution was bubbled again with N_2 after irradiation. Two experimental conditions influenced the signal

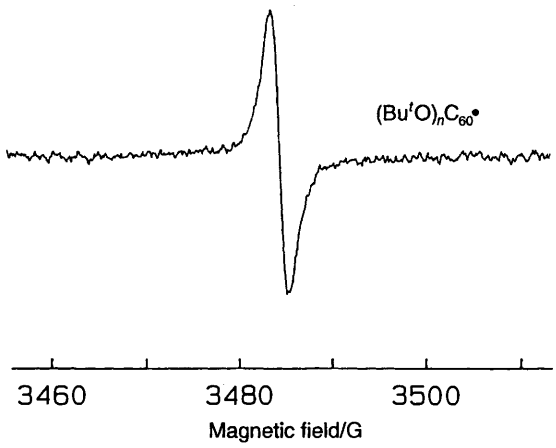


Fig. 1 EPR spectrum of $(Bu^tO)_nC_{60}^{\bullet}$ radicals generated by 1 min intensive irradiation of a N_2-bubbled solution of C_{60} (2×10^{-4} M) and $(Bu^tO)_2$ (0.13 M) in C_6H_6 at room temperature. The peak-to-peak linewidth is 1.9 G (1 G = 0.1 mT). EPR conditions: microwave power = 19.9 mW, receiver gain = 5.0×10^5, modulation frequency = 100.0 kHz, sweep time = 84 s, scale 17 and centre field = 3483.0 G.

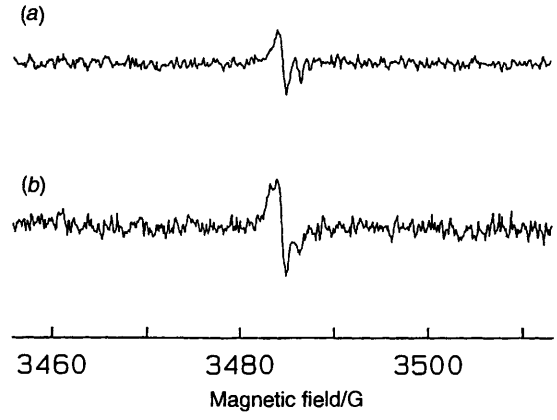


Fig. 2 EPR spectra recorded during the initial stage of UV irradiation of a N_2-bubbled solution of C_{60} (2×10^{-4} M) and $(Bu^tO)_2$ (0.013 M) in C_6H_6 at room temperature, indicating the generation of more than one adduct [$(Bu^tO)_nC_{60}^{\bullet}$, $n \geq 1$]. (*a*) The solution was photolysed for several seconds in the EPR cavity, EPR receiver gain = 6.3×10^5. (*b*) The solution was photolysed for more than 60 s in the EPR cavity. EPR receiver gain = 1×10^6. For other EPR conditions, see the caption to Fig. 1.

intensity and shape. One was the concentration of $(Bu^tO)_2$. Low concentrations resulted in a low signal intensity, *e.g.* when 0.24 vol% of $(Bu^tO)_2$ (0.013 M) was used instead of 0.13 M $(Bu^tO)_2$, the signal intensity decreased by 3.5 times with no change in linewidth. The second factor was the duration of irradiation. At the initial stage of UV irradiation, the EPR spectra showed multiple peaks (Fig. 2). These spectra indicate that different radical adducts of $Bu^tO^{\bullet}$ radicals and C_{60} have been generated [reactions (1) and (2)]. There is the possibility that an even

$$(Bu^tO)_2 \xrightarrow{h\nu} 2\,Bu^tO^{\bullet} \quad (1)$$

$$n Bu^tO^{\bullet} + C_{60} \longrightarrow (BuO)_nC_{60}^{\bullet}\ (n \geq 1) \quad (2)$$

number of $Bu^tO^{\bullet}$ radicals attach covalently to the surface of a single C_{60} molecule producing triplet radicals,[22] but it is unlikely that these species contribute to the room temperature EPR spectra observed.

Stabilities of $(Bu^tO)_nC_{60}^{\bullet}$ radicals are strongly dependent on their structure. Initially generated adduct signals [*e.g.* in Fig. 2(*b*)] disappear in 5 min under N_2. However, radical adducts (see Fig. 1) generated by extensive irradiation were remarkably

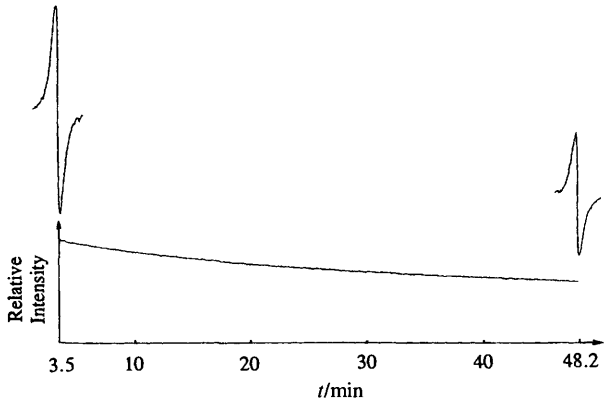


Fig. 3 Curve for the decay of the EPR signal of $(Bu^tO)_nC_{60}^{\bullet}$ radicals under N_2 in C_6H_6 at room temperature from 3.5 to 48.2 min. The EPR signals before and after the decay are shown on the top of the curve. The decay curve was recorded directly on the EPR spectrometer. EPR conditions: receiver gain = 2.5 × 10^5, sweep time = 2684.4 s, scale = 21, centre field = 3483.7 G and sweep width = 0.00 G.

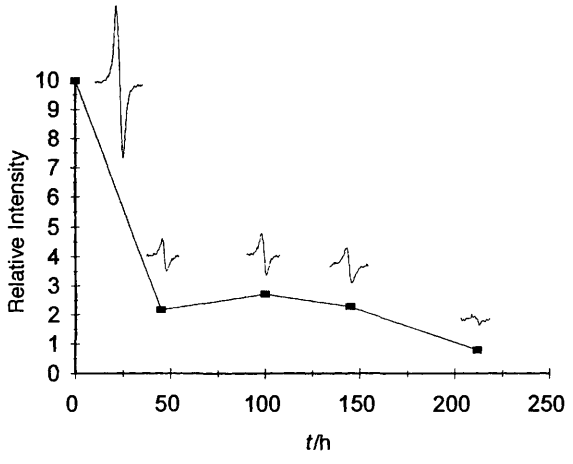


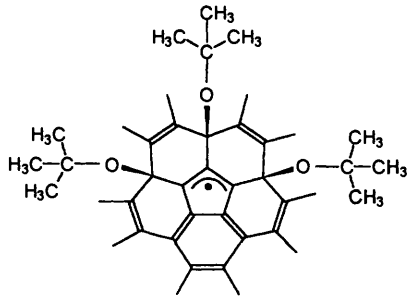
Fig. 4 Decay of the EPR signal of $(Bu^tO)_nC_{60}^{\bullet}$ radicals over 212 h (*ca.* 9 days) under N_2 in C_6H_6 at room temperature. The EPR signals are also presented beside or above the points of the data to show the shapes of the signals. EPR conditions: receiver gain = 2.5 × 10^5 and scale = 16. For other EPR parameters, see the caption to Fig. 1.

persistent under N_2 and detectable even after nine days. A curve for the decay of $(Bu^tO)_nC_{60}^{\bullet}$ EPR signal from 3.5 to 48.2 min is shown in Fig. 3. The radical was generated by 1 min UV irradiation of a C_6H_6 solution containing 2 × 10^{-4} M C_{60} and 0.13 M $(Bu^tO)_2$ under N_2 and the solution was re-bubbled with N_2 immediately after UV irradiation. It took 62 min to reach the half-height of the starting signal. The decay rate gradually reduced as the decay process continued. The long-range decay of the $(Bu^tO)_nC_{60}^{\bullet}$ EPR signal is illustrated in Fig. 4. The signal was still detectable even after nine days in the dark at room temperature. Radicals $(Bu^tO)_nC_{60}^{\bullet}$ (n = 1, 2) may be more reactive than $(Bu^tO)_nC_{60}^{\bullet}$ (n = 3, 5, 6, 8, 9, 10, *etc.*). Because allyl-radical-type structures in radical adduct **1** and cyclopentadienyl-radical-type structures in radical adduct **2** may stabilize the radical adducts[21] (see structures **1** and **2**), we suggest that the radical adducts with an allyl radical unit, a cyclopentadienyl radical unit or their combinations might correspond to the EPR signals which last many days under N_2. Experimental evidence is not available at this time for the determination of the fate of $(Bu^tO)_nC_{60}^{\bullet}$ radicals, but dimerization reactions might take place between mono-radical adducts.[25,30] Reversion of $(Bu^tO)_nC_{60}^{\bullet}$ radicals to C_{60} and $Bu^tO^{\bullet}$ radicals is not likely because the solutions remained brown in colour and did not change back to the purple colour of C_{60} even after the signal disappeared.

Table 1 EPR signal decay of $(Bu^tO)_nC_{60}^{\bullet}$ radicals under O_2 and N_2[a]

	% Decay after 15 min	% Decay after 32 min
O_2	44[b]	54[c]
N_2	23	37

[a] The percentage given in this table stands for the decrease in the EPR signal height divided by the height of the starting signal. The EPR spectra of the radical were recorded at room temperature in C_6H_6. [b] Bubbling with O_2 for 5 min, then with N_2. [c] Bubbling with O_2 for 10 min, then with N_2.



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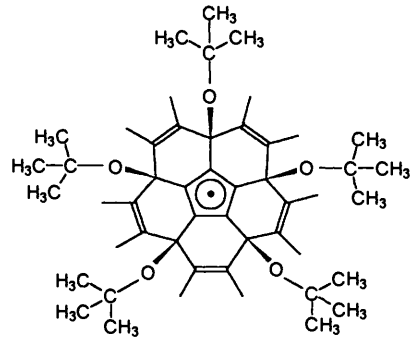


2

Stability of $(Bu^tO)_nC_{60}^{\bullet}$ radicals under dioxgen (O_2)

As shown in Table 1, after 15 and 32 min decay of the $(Bu^tO)_nC_{60}^{\bullet}$ radicals, the signal intensity decreased by 44 and 54% when bubbled with O_2 for 5 and 10 min, respectively, whereas only 23 and 36% decreases were observed if under N_2. It should be mentioned that dioxygen can broaden EPR signals resulting in a decrease in the intensity. Therefore, bubbling of the solution with N_2 is required after O_2 treatment. Thus O_2 slightly promotes the decrease in the EPR signal intensity of $(Bu^tO)_nC_{60}^{\bullet}$ radicals.

The chemical reactions proposed in reactions (3)–(5) may

$$(Bu^tO)_nC_{60}^{\bullet} + O_2 \xrightarrow{\text{slow}} (Bu^tO)_nC_{60}\text{–}OO^{\bullet} \quad (3)$$

$$(Bu^tO)_nC_{60}\text{–}OO^{\bullet} \longrightarrow (Bu^tO)_nC_{60}^{\bullet}\text{–}OO \quad (4)$$

$$(Bu^tO)_nC_{60}\text{–}OO^{\bullet} + (Bu^tO)_nC_{60}^{\bullet} \longrightarrow [(Bu^tO)_nC_{60}]_2O_2 \quad (5)$$

account for these observations. Reaction (3) may be slow because the $(Bu^tO)_nC_{60}^{\bullet}$ radical signal decreases slowly in the presence of O_2. It was found that photolysis of an air-saturated C_6H_6 solution containing 2 × 10^{-4} M C_{60} and 0.13 M $(Bu^tO)_2$ only generated a very small EPR signal where the intensity is only 3% of that generated under N_2. The reaction of $Bu^tO\text{–}C_{60}^{\bullet}$ radicals with O_2 to form $Bu^tO\text{–}C_{60}\text{–}OO^{\bullet}$ and $(Bu^tO\text{–}C_{60})_2O_2$

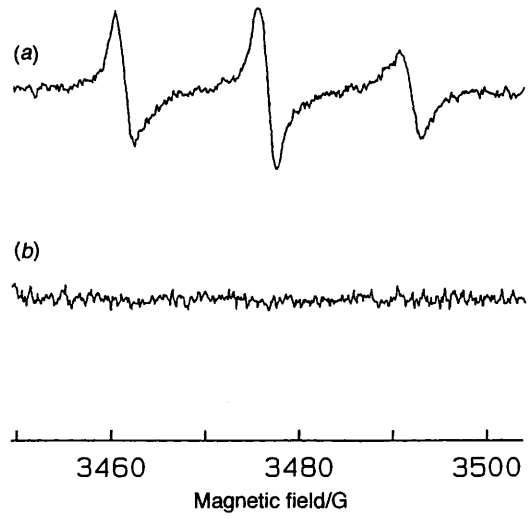


Fig. 5 (*a*) EPR spectrum of aminoxyl impurities (a_N = 15.2 G) in a control solution of C_6H_6 bubbled with nitric oxide ('NO) for 2 min. EPR receiver gain = 5 × 10^5 and centre field = 3477.0 G. (*b*) EPR spectrum of a solution of 'NO in C_6H_6, indicating the removal of the EPR impurities in (*a*). The 'NO gas was passed through a trap cooled with dry ice–acetone (−78 °C). EPR receiver gain = 8 × 10^5 and centre field = 3477.0 G. For other EPR conditions, see the caption to Fig. 1.

would explain this result. Intra- and inter-molecular reactions [reactions (4) and (5)] are possible further reactions.

The stability of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in the presence of H_2O was also tested. Small amounts of H_2O (0.5 vol%) were added to a C_6H_6 solution of the radicals and the mixture was agitated for *ca.* 10 min. No decrease in the EPR signal intensity was found.

Reactivity of $(Bu^tO)_nC_{60}^{\bullet}$ radicals towards nitric oxide ('NO)

As shown in Fig. 5(*a*), the control spectrum of a C_6H_6 solution, bubbled with 'NO for 2 min and then N_2 for several minutes, exhibited a three-line spectrum which is assigned to a mixture of aminoxyls (nitroxides) (a_N = 15.2 G). The lines are not sharp and the high-field peak is only 56% of the height of the middle peak. After being passed through a cold trap cooled with dry ice–acetone (−78 °C), 'NO gas did not show the aminoxyl impurity as illustrated in Fig. 5(*b*).

UV irradiation of a mixture of C_{60} and 'NO in C_6H_6 did not provide any EPR signal and the purple colour still remained. Reactions between C_{60} and 'NO did not occur under these conditions. However, reactions between $(Bu^tO)_nC_{60}^{\bullet}$ radicals and 'NO have been observed. Bubbling of 'NO gas for 2–3 min through a C_6H_6 solution containing freshly generated $(Bu^tO)_nC_{60}^{\bullet}$ radicals [see Fig. 6(*a*)] immediately eliminated the strong single peak and generated new aminoxyl signals at a concentration of *ca.* 10^{-7} M [see Fig. 6(*b*)]. The N hyperfine splitting (a_N) is 13.0 G, which is much smaller than found for normal di-*tert*-alkyl aminoxyls such as 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, a_N = 15.6 G in C_6H_6). Another difference is the EPR signal shape. In C_6H_6, the three peaks in the EPR spectrum of TEMPO have the same height at room temperature. The spectrum in Fig. 6(*b*) is unique. The middle peak is much higher and sharper than the high- and low-field peaks. The spectrum must be due to large aminoxyl radicals because this phenomenon results from slow tumbling rates of large molecules. These results suggest that stable dibuckminsterfullerene aminoxyl radicals, $[(Bu^tO)_nC_{60}]_2N$–O$^{\bullet}$ **3**, have been generated.

Strong interactions between the nitroxyl functional group and two C_{60} balls are expected resulting in a delocalization of the electron spin density and a smaller a_N value. No decay of these $[(Bu^tO)_nC_{60}]_2N$–O$^{\bullet}$ signals was found over 5 days. It was observed that only *ca.* 5% of the $(Bu^tO)_nC_{60}^{\bullet}$ radicals were converted into the stable aminoxyl radicals **3** based on relative EPR spectral intensities.

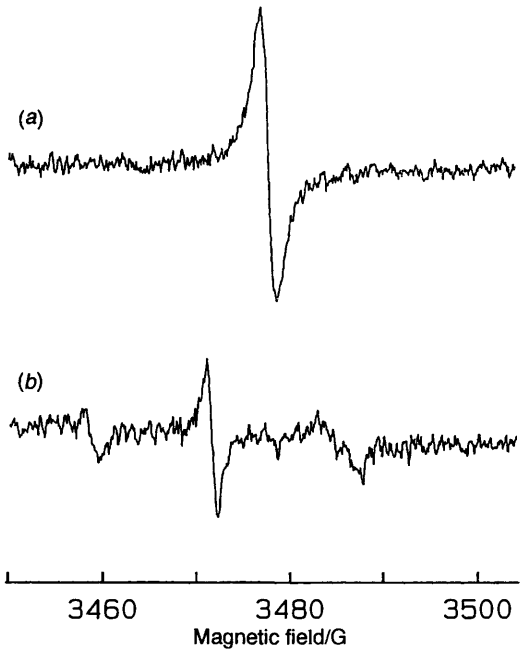
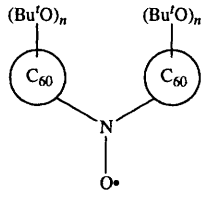


Fig. 6 (*a*) EPR spectrum of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in C_6H_6 before the bubbling of 'NO gas. EPR receiver gain = 5 × 10^5, scale = 16 and scan number = 1. (*b*) EPR spectrum of dibuckminsterfullerene aminoxyl radicals $[(Bu^tO)_nC_{60}]_2N$–O$^{\bullet}$ (a_N = 13.0 G) obtained from 3 min bubbling of 'NO gas through a N_2-bubbled C_6H_6 solution of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in (*a*). EPR receiver gain = 2 × 10^6, scale = 19 and scan number = 5. For other EPR conditions, see the caption to Fig. 1.



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The aminoxyl signals probably result from double additions of $(Bu^tO)_nC_{60}^{\bullet}$ radicals to 'NO very much like *tert*-butyl radicals and 'NO[38] [reactions (6) and (7); see later]. The

$$(Bu^tO)_nC_{60}^{\bullet} + {}^{\bullet}NO \longrightarrow (Bu^tO)_nC_{60}\text{–}N{=}O \quad (6)$$

$$(Bu^tO)_nC_{60}^{\bullet} + (Bu^tO)_nC_{60}\text{–}N{=}O \longrightarrow [(Bu^tO)_nC_{60}]_2N\text{–}O^{\bullet} \quad (7)$$

reaction between $(Bu^tO)_nC_{60}^{\bullet}$ and 'NO might be much faster than the reaction between $(Bu^tO)_nC_{60}^{\bullet}$ and $(Bu^tO)_nC_{60}$–N=O thus generating $(Bu^tO)_nC_{60}$–N=O as a major product and $[(Bu^tO)_nC_{60}]_2N$–O$^{\bullet}$ aminoxyl radicals as a minor product. This prediction is consistent with another observation that $(Bu^tO)_nC_{60}^{\bullet}$ radicals are not very reactive towards the reactive nitroso spin trap, 2-methyl-2-nitrosopropane (MNP, Me_3C–N=O), as discussed below.

Stabilities of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in the presence of DMPO, PBN, [$^2H_9$, ^{13}C]PBN, NB and MNP spin traps

DMPO is a very reactive spin trap. It can react with many kinds of free radicals such as C-centred, S-centred and O-centred free radicals; the rate constants for the reaction of DMPO with C-centred free radicals, *e.g.* methyl and hexen-5-yl radicals, are 1.3–26 × 10^5 dm^3 mol^{-1} s^{-1} at 40 °C in C_6H_6.[40]

For $(Bu^tO)_nC_{60}^{\bullet}$ radicals, 10 mM DMPO was used to test the

Table 2 EPR signal decay of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in the presence of DMPO[a]

Time (t/min)	% Decrease with DMPO	% Decrease without DMPO
10	35	20
25	48	35
45	57	45
60	64	50

[a] The percentage given in this table stands for the decrease of the EPR signal height divided by the height of the starting signal. The radical was decayed under N_2 at room temperature in C_6H_6. The concentration of the DMPO spin trap was 10 mM.

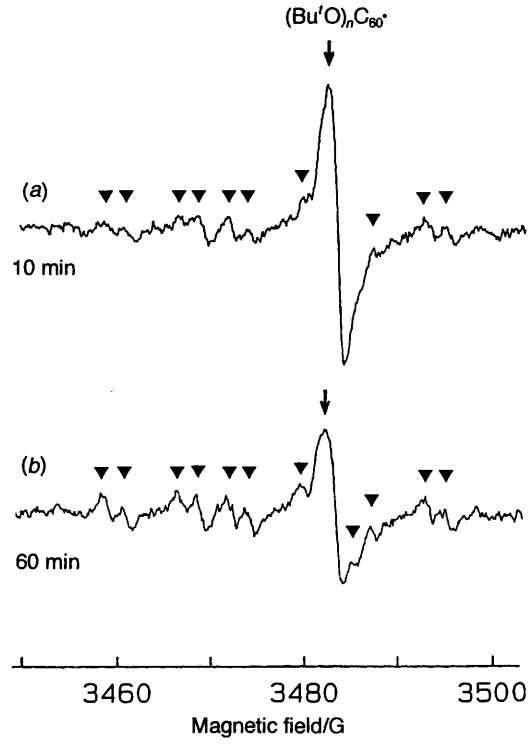

Fig. 7 EPR spectra of a solution of DMPO spin trap (10 mM) and $(Bu^tO)_nC_{60}^{\bullet}$ radicals (labelled with '↓') in C_6H_6. The signals, labelled with '▼' are due to the $Bu^tO^{\bullet}$ adduct of DMPO generated *in situ* from DMPO and excess $(Bu^tO)_2$. There is an overlap between the signals of $(Bu^tO)_nC_{60}^{\bullet}$ (one strong peak) and DMPO–OBu^t (twelve weak peaks). (*a*) Spectrum recorded 10 min after the addition of DMPO to the solution of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in C_6H_6. (*b*) Spectrum recorded 60 min after the addition of DMPO. EPR conditions for both (*a*) and (*b*): receiver gain = 5×10^5, scale = 17 and for other parameters, see the caption to Fig. 1.

reactivity of the radicals. Signal decay data for $(Bu^tO)_nC_{60}^{\bullet}$ radicals are presented in Table 2 and two EPR spectra are shown in Fig. 7 to exhibit the decay process. Comparison of the data in Table 2 indicates that the presence of DMPO does not mediate the decrease of $(Bu^tO)_nC_{60}^{\bullet}$ radicals significantly. It is concluded that $(Bu^tO)_nC_{60}^{\bullet}$ radicals are not very reactive towards DMPO as a spin trap.

Two acyclic nitrone spin traps, PBN (10 mM) and [$^2H_9$, ^{13}C]PBN (10 mM), were also used to test their reactivities with $(Bu^tO)_nC_{60}^{\bullet}$ radicals. During 30 min observation, no significant decrease in the EPR signal for $(Bu^tO)_nC_{60}^{\bullet}$ radicals was observed. This result is not surprising because PBN-type nitrones are more sterically hindered and less reactive than DMPO.[40]

Nitrosobenzene (C_6H_5–N=O) is a typical aromatic nitroso spin trap. But this compound is also not reactive with $(Bu^tO)_nC_{60}^{\bullet}$ radicals. No significant decrease in the EPR signal was found during 30 min monitoring with the EPR spectrometer.

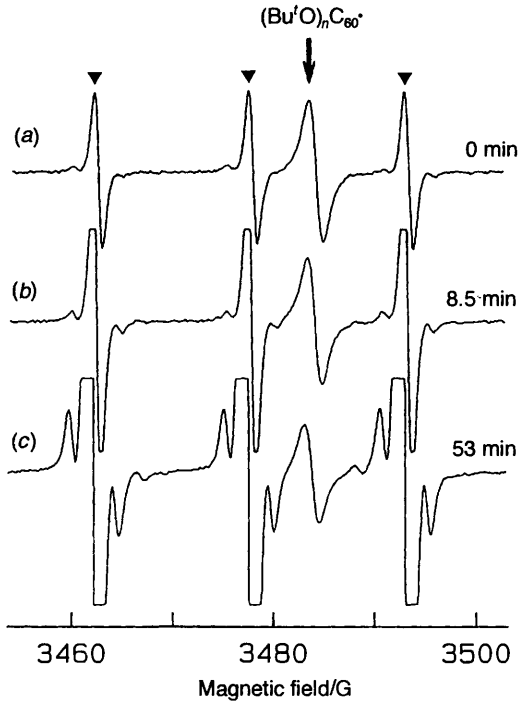

Fig. 8 EPR spectra from a solution of MNP spin trap (*ca.* 10 mM) and $(Bu^tO)_nC_{60}^{\bullet}$ radicals (labelled with '↓') in C_6H_6. The signals, labelled with '▼' are due to di-*tert*-butyl aminoxyl radicals generated *in situ* from MNP. (*a*) Spectrum recorded immediately after the addition of MNP to the solution of $(Bu^tO)_nC_{60}^{\bullet}$ radicals in C_6H_6. (*b*) Spectrum recorded 8.5 min after the addition of MNP. (*c*) Spectrum recorded 53 min after the addition of MNP. EPR conditions for these three spectra: receiver gain = 2×10^5, scale = 17 and for other parameters, see the caption to Fig. 1.

MNP (Me_3C–N=O) is a *tert*-alkyl nitroso spin trap which is more reactive than NB. Surprisingly, $(Bu^tO)_nC_{60}^{\bullet}$ radicals still decay very slowly in the presence of MNP as shown in Fig. 8. Under laboratory light, an increase in di-*tert*-butyl aminoxyl [$(Me_3C)_2$N–O$^{\bullet}$] was found. This aminoxyl radical corresponds to the triplet EPR signal (a_N = 15.4 G, C_6H_6) in Fig. 8. It is well known that reactions (8) and (9) take place under laboratory

$$Me_3C\text{–}N{=}O \longrightarrow Me_3C^{\bullet} + {}^{\bullet}NO \quad (8)$$

$$Me_3C^{\bullet} + Me_3C\text{–}N{=}O \longrightarrow (Me_3C)_2N\text{–}O^{\bullet} \quad (9)$$

light.[38] Therefore, MNP, $(Me_3C)_2$N–O$^{\bullet}$ and the MNP dimer are present in this C_6H_6 solution of MNP. Fig. 8 clearly indicates that $(Bu^tO)_nC_{60}^{\bullet}$ radicals are remarkably persistent in such solutions.

Conclusions

The buckminsterfullerene radical, $(Bu^tO)_nC_{60}^{\bullet}$ ($n > 2$), is very persistent under N_2. The reaction of $(Bu^tO)_nC_{60}^{\bullet}$ radicals with $^{\bullet}NO$ takes place for several minutes to generate stable dibuckminsterfullerene aminoxyl radicals [$(Bu^tO)_nC_{60}]_2$N–O$^{\bullet}$ (a_N = 13.0 G in C_6H_6) probably *via* nitroso adducts $(Bu^tO)_nC_{60}$–N=O as intermediates. Reactions of $(Bu^tO)_nC_{60}^{\bullet}$ radicals with O_2, H_2O and several spin traps, including DMPO, PBN, [$^2H_9$, ^{13}C]PBN, NB and MNP, are relatively slow in C_6H_6 at room temperature. The low reactivity of $(Bu^tO)_nC_{60}^{\bullet}$ radicals suggests that these radicals might not be harmful to biological macromolecules.

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