Acridine Adduct of [60]Fullerene with Enhanced DNA-Cleaving Activity

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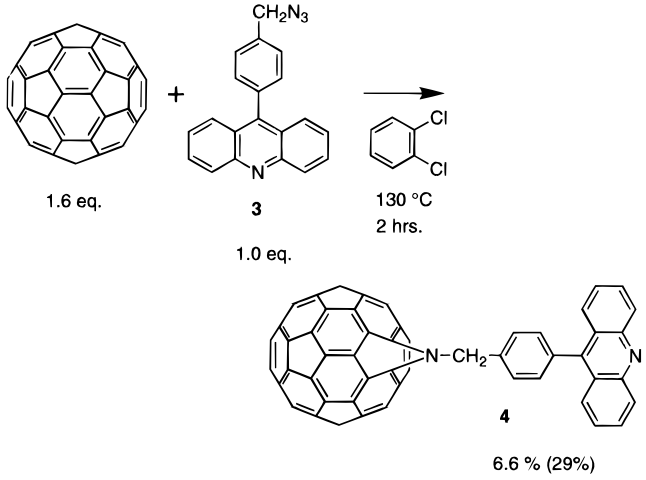
[60]Fullerene (C_{60}) has attracted considerable attention since it was discovered in 1985, and many studies on fulleroids have been conducted in the fields of physics, chemistry, *etc.*[1] It has been expected that C_{60} has a biological action caused by its unique shape and/or characteristic physicochemical properties, such as in a facial redox reaction[2−4] and photosensitization.[5−7] One of the difficulties in studying the biological properties of C_{60} is its insolubility in water,[8] but part of this problem has been solved by using poly(vinylpyrrolidone) (PVP) as a detergent.[9] We have recently reported that C_{60} solubilized in water with PVP has a unique condrogenesis-promoting effect.[10,11] Moreover, we have found that an aqueous C_{60} solution expresses several biological activities under irradiation by visible light. These include mutagenicity in *Salmonella* tester strains TA102, TA104, and YG3003,[12] hemolytic activity in rabbit red blood cells,[13] and initiation activity in a BALB/3T3 cell transformation assay.[14] These biological activities may be caused by singlet oxygen ($^1O_2$) generated by C_{60} under photoirradiation. In this paper, we report the photoinduced DNA-cleaving activities of C_{60} itself and of a C_{60} derivative with an acridine group.

Scheme 1. Reaction of C_{60} and 3



An acridine moiety was chosen because of its intercalating activity with DNA double strands. Among the many methods that have been reported for C_{60} modification, we selected aziridino addition onto C_{60}.[15,16] To link an acridine moiety to the C_{60} molecule, we synthesized a benzyl azide derivative of acridine. 9-(4-Methylphenyl)-acridine (**1**), prepared by the Bernthsen reaction[17] of diphenylamine with 4-methylphenyl benzoate, was brominated to give a bromomethyl derivative (**2**), which was then converted to 9-[4-(azidomethyl)phenyl]acridine (**3**) in a yield of 13% from **1**. The addition reaction of C_{60} with **3** was carried out in *o*-dichlorobenzene at 130 °C, as shown in Scheme 1. The progress of the reaction was monitored by reversed-phase HPLC to obtain the expected monoaddition product in the best yield. The peak of the monoadduct, which was detected at a retention time of 3.5 min, reached a maximum in 2.5 h and then gradually decreased on prolonged heating, while the peak of C_{60} remained. After 2.5 h of heating, the reaction was stopped and the reaction mixture was purified by silica gel column chromatography, which was developed with a *n*-hexane−benzene−CH_2Cl_2−EtOAc solvent system under gradient conditions. The eluate with EtOAc was collected and concentrated to give a dark brownish powder (yield, 6.6%: conversion yield, 29%).

The structure of the product (compound **4** in Scheme 1) was confirmed using liquid secondary ionization mass spectrometry (LSI-MS) and ^{13}C-NMR and ^{1}H-NMR spectrometry.[18,19] The spectrum in LSI-MS showed that the product was the monoadduct based on its observed *m*/*z*

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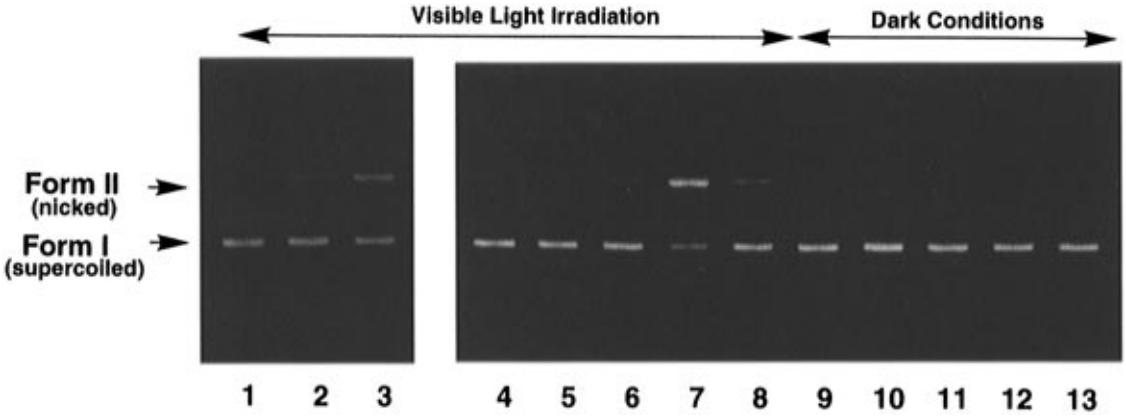

Figure 1. Photocleavage of DNA by C_{60} and an acridine adduct of C_{60} (**4**). Lanes 1, 4, and 9: no chemicals in distilled water (DW). Lanes 2, 5, and 10: no chemicals with 1% PVP. Lanes 3, 6, and 11: C_{60} (1.4×10^{-4} M with 1% PVP). Lanes 7 and 12: **4** (1.4×10^{-4} M with 1% PVP). Lanes 8 and 13: **1** (1.4×10^{-4} M with 1% PVP). Lanes 1−8: incubated under visible light irradiation (lanes 1−3, 35−40 °C, 1 h; lanes 4−8, 0−5 °C, 4 h). Lanes 9−13: incubated under dark conditions (0−5 °C, 4 h).

peak at 1003 ($[M + H]^+$). The molecular formula of this adduct was determined to be $C_{80}H_{14}N_2$ (*m*/*z* 1003.122 500, $[M + H]^+$ calcd for $C_{80}H_{15}N_2$ 1003.123 524) based on high-resolution LSI-MS data. Thirty-seven signals were observed for the sp^2 carbons with no proton by ^{13}C-NMR and were assigned to be 32 carbons of the C_{60} skeleton and five carbons of the 9-phenylacridine moiety. No sp^3 signals derived from the C_{60} skeleton were observed. These data indicate that this adduct has C_2 symmetry with a 5,6-opened azaannulene structure. These results are also supported by the results from LSI-MS and do not contradict the results of ^{1}H-NMR analysis. The adduct (**4**) was then reacted with saturated HCl solution in EtOH to be converted to the HCl salt and then incorporated into a PVP micellar system to give a completely transparent aqueous solution that could be used in a DNA-cleaving test.

The DNA-cleaving activities of C_{60} and **4**, both solubilized in water with PVP (final concentration of PVP was 1.0% in each system), were tested under visible light irradiation using a pBR322 supercoiled plasmid. The final concentrations of the chemicals were 1.4×10^{-4} M. To clarify the effect of visible light, all chemicals were also tested under dark conditions. The HCl salt of **1** was tested as a reference compound to clarify the effect of the phenyl acridinyl group itself. The results are shown in Figure 1. Under dark conditions, none of the chemicals showed any DNA-cleaving activity (lanes 9−13). Under visible light irradiation, C_{60} itself showed weak but significant DNA-cleaving activity at 0−5 °C within 4 h of photoirradiation (lane 6). C_{60} showed stronger DNA-cleaving activity at a higher temperature. Within 1 h of visible light irradiation at 35−40 °C, about 25% of supercoiled DNA (form I) was converted to nicked DNA (form II) (lane 3). This is the first demonstration of the DNA-cleaving activity of C_{60} itself under photoirradiated conditions. The previously reported result by Nakamura *et al.*[20] that C_{60} itself had no DNA-cleaving activity may have been due to the insolubility of C_{60} under their experimental conditions. While C_{60} had weak DNA-cleaving activity, **4** showed stronger activity even at 0−5 °C within 4 h of visible light irradiation (lane 7). About 50% of form I was cleaved in 2 h and more than 90% of form I was converted to form II after 6 h of photoirradiation (data not shown). The HCl salt of **1** used as a reference showed only weak activity under these conditions (lane 8). The minimum dose of **4** for DNA scission was 17.5 μM at 0−5 °C for 4 h of visible light irradiation. A dose−response relationship was observed between 17.5 and 140 μM under these conditions. DNA scission by **4** may be caused by the singlet oxygen generated from the C_{60} part of **4**, and the enhanced activity may be due to the affinity of the acridine moiety for DNA molecules.

In summary, the DNA-cleaving activity of C_{60}, which is closely related to the mutagenic and transforming activities of C_{60}, was demonstrated using a pBR322 supercoiled plasmid. A C_{60} derivative (**4**) with a DNA-intercalating moiety showed even stronger DNA-cleaving activity than C_{60}. The genotoxicity of active oxygen species, including $^1O_2$, has been well studied.[21,22] Our current efforts are directed toward clarifying the molecular mechanisms of DNA damage caused by the photosensitization reaction of C_{60} and its derivatives.

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Supporting Information Available: Experimental details and spectroscopic data for compound **4** (9 pages).

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