JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 5, 644–648 (1992)

THE PHOTOCHEMISTRY OF PYRENE–CYTOSINE CONJUGATES: MODELLING THE CARCINOGENIC ACTION OF AROMATIC HYDROCARBONS

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Photoreduction, but not the corresponding photooxidation, of cytosine can be sensitized by a covalently appended pyrene molecule in a process that may have some importance for understanding the known carcinogenic activity of polynuclear aromatic hydrocarbons.

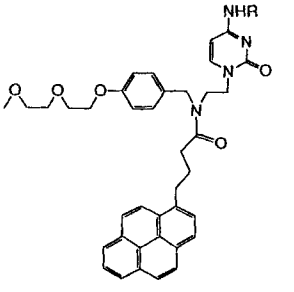
INTRODUCTION

Ubiquitous environmental carcinogens of the polynuclear aromatic hydrocarbon family, such as pyrene, are metabolized by cellular enzyme systems to yield reagents which bind covalently to DNA. However, despite detailed investigation, the mechanisms of subsequent mutagenic and carcinogenic processes remain unknown. These hydrocarbons absorb strongly in the near-UV region and, therefore, it is likely that, under illumination, the carcinogenic activity arises from photo-induced strand scission. Even under such conditions, there is considerable uncertainty about the reaction mechanism, although the intermediacy of singlet molecular oxygen and/or redox ions should be considered.

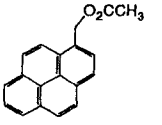
Here, we describe the photochemistry and radiation chemistry of a covalently linked pyrene–cytosine compound in organic solvents and consider the possibility of light-induced intramolecular electron transfer processes in this new multicomponent system.

EXPERIMENTAL

General synthetic aspects. Synthesis of the pyrene–cytosine supramolecular conjugate **1** and its 'blocked' trityl-protected analogue **2** was accomplished as shown in Scheme 1. The first step involved reaction of 1-pyrene-4-butyric acid (Aldrich Chemical) with *N*-hydroxysuccinimide to give the activated carboxylic acid derivative **5**. Condensation of **5** with the known[1] trityl-protected, cytosine-containing secondary amine **6** in dry 1,2- dimethoxyethane gave the 'blocked' tertiary amine **2** in 67% isolated yield after chromatographic purification (silica gel, 2% methanol in chloroform as eluent). Deprotection with trifluoroacetic acid gave the target molecule **1** in 32% yield after work-up and chromatographic purification (silica gel, 5% methanol in chloroform as effluent). The solubilized control molecule **3** was prepared by reduction of 1-pyrenecarboxaldehyde with $NaBH_4$ in dry



1. R = H
2. R = $C(C_6H_5)_3$



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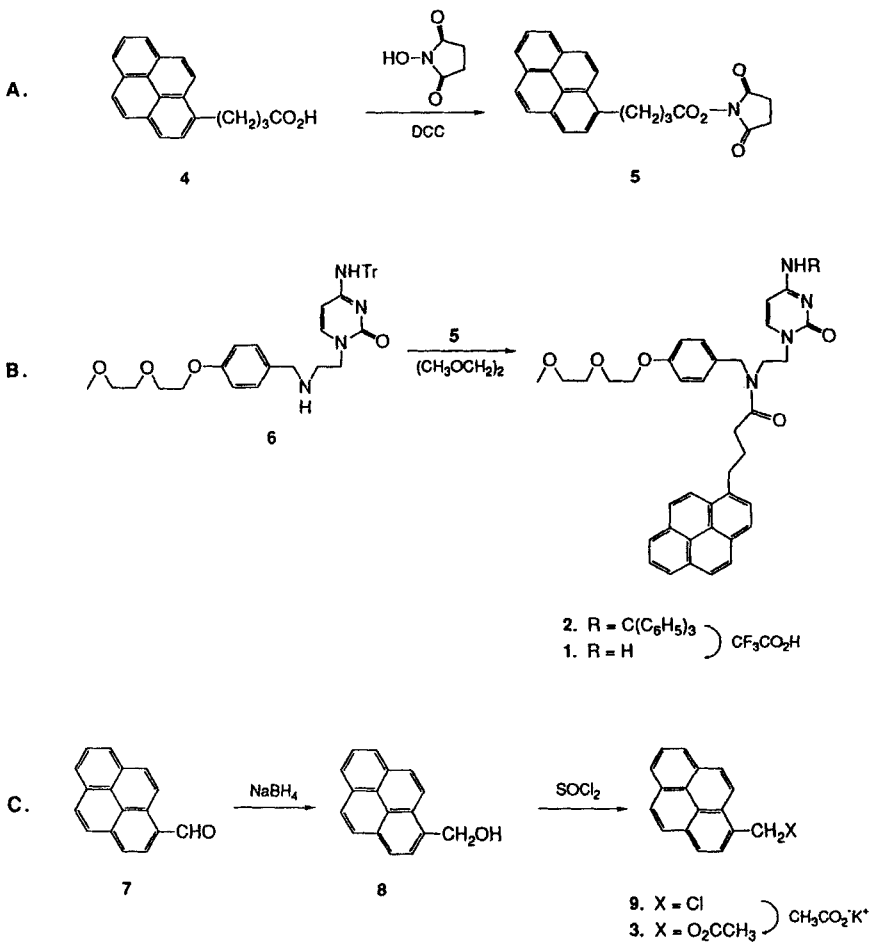
0894–3230/92/100644–05$07.50

Received 6 March 1992
Revised 30 April 1992



Scheme 1. Synthetic route used to produce the compounds studied

tetrahydrofuran (giving **8**), followed by treatment with thionyl chloride (giving **9**) and, subsequently, potassium acetate in acetic acid; it was obtained in 45% overall yield. All new compounds gave ^{1}H NMR and high-resolution (HR) mass spectrometric (MS) results consistent with their proposed structures.

Succinimidyl-1-pyrenebutyrate (**8**). 1-Pyrenebutyric acid (2·25 g, 7·8 mmol) and *N*-hydroxysuccinimide (1·2 g, 10·4 mmol) were dissolved in DMF (20 ml). After cooling to 0 °C, 1,3-dicyclohexylcarbodiimide (DCC) (2·69 g, 13·1 mmol) was added to the solution and the resulting mixture was stirred for 20·5 h. It was then filtered and the filtrate was collected and taken to dryness on a rotary evaporator. The resulting product was recrystallized from 95% ethanol to give 2·52 g of **5** (83·7%). ^{1}H NMR ($CDCl_3$), δ 2·27–2·33 (2H, m, $-CH_2CH_2CH_2-$), 2·73 (2H, t, CH_2CO), 2·84 (4H, s, succinimide H), 3·47 (2H, t, pyrene CH_2), 7·86–8·30 (9H, m, pyrene H). Chemical ionization (CI) MS, *m/z* 386 $[M + H]^+$.

N-4-[2-(2-Methoxyethoxy)ethoxy] phenylmethyl-N-2-[1-(4-triphenylmethylamino)pyrimidin-2-one] ethyl-1-pyrenebutylamide (**2**). Compounds **6** (300 mg, 0·50 mmol) and **5** (0·29 g, 0·75 mmol) were dissolved in 1,2-dimethoxyethane (50 ml). The resulting solution was then heated at reflux under a nitrogen atmosphere for 24 h. After removal of the solvent on a rotary evaporator, chloroform (50 ml) was added and the resulting solution washed with water (2 × 50 ml). The chloroform solvent was then removed under reduced pressure. The crude material obtainer was purified by column chromatography on silica gel using 2% CH_3OH–$CHCl_3$ as the eluent. In this way, 292 mg of **2** were obtained (67%). ^{1}H NMR ($CDCl_3$), δ 2·16–2·21 (2H, m, $-CH_2CH_2CH_2-$), 2·49 (2H, t, CH_2CO), 3·38 (3H, s, OCH_3), 3·39–3·49 (4H, m,

pyrene CH_2 and pyrimidine CH_2CH_2), 3·56 (2H, t, $CH_2CH_2OCH_3$), 3·58 (2H, s, NCH_2PhO), 3·70 (2H, t, $CH_2CH_2OCH_3$), 3·82 (2H, t, $PhOCH_2CH_2$), 3.89 (2H, t, pyrimidine CH_2CH_2), 4·11 (2H, t, $PhOCH_2CH_2$), 5·10 (1H, d, C_5H), 6·87 (2H, d, $J = 6·63$ Hz, PhH), 7·02 (1H, d, C_6H), 7·08–7·44 (17H, m, PhH and TrH) 7·84–8·30 (9H, m, pyrene H). CI-MS, *m/z* 876 $[M + 2]^+$.

N-*4-[2-Methoxyethoxy)ethoxy§phenylmethyl*-N-*2-(4-amino)pyrimidin-2-one] ethyl-1-pyrenebutylamide* (**1**). Compound **2** (100 mg, 0·11 mmol) was dissolved in CF_3CO_2H (14 ml) and stirred at room temperature for 9 h. After removing the solvent on a rotary evaporator, chloroform (50 ml) was added. The solution was then made basic (pH 9) by adding K_2CO_3 solution and washed with water (2 × 100 ml) before being taken to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using 5% CH_3OH–$CHCl_3$ as the eluent. In this way, 23 mg of **1** were obtained (32%). ^1H NMR ($CDCl_3$), δ 2·07–2·14 (2H, m, $—CH_2CH_2CH_2—$), 2·35 (2H, t, CH_2CO), 3·27 (2H, t, pyrimidine CH_2CH_2), 3·36 (3H, s, OCH_3), 3·51–3·56 (4H, m, $CH_2CH_2OCH_3$ and NCH_2PhO), 3·68 (2H, t, $CH_2CH_2OCH_3$), 3·71 (2H, t, pyrimidine CH_2CH_2), 3·77 (2H, t, $PhOCH_2CH_2$), 3·95 (2H, t, pyrene CH_2), 4·21 (2H, t, $PhOCH_2CH_2$), 5·66 (1H, d, $J = 6·91$ Hz, C_5H), 6·63 (2H, d, $J = 8·35$ Hz, PhH), 6·78 (2H, d, $J = 8·35$ Hz, PhH), 7·73–8·25 (10H, m, C_6H and pyrene H). ^{13}C NMR ($CDCl_3 + CD_3OD$), δ 26·07 ($COCH_2CH_2CH_2—$), 28·74 ($COCH_2CH_2CH_2—$), 31·76 ($COCH_2CH_2CH_2—$), 43·80 (pyrimidine CH_2CH_2), 46·39 (pyrimidine CH_2CH_2), 50·53 (OCH_3), 57·84 ($PhOCH_2CH_2$), 66·43 (NCH_2Ph), 68·75 ($PhOCH_2CH_2$), 69·61 ($CH_3OCH_2CH_2$), 70·90 ($CH_3OCH_2CH_2$), 93·63 (C_5), 114·02, 122·42, 123·90, 123·96, 124·02, 125·03, 125·78, 126·42, 126·48, 126·58, 126·63, 127·07, 128·77 and 130·50 (Ph and pyrene), 146·91 (C_6), 157·51 (C_2), 161·00 (C_4), 170·65 ($NCOCH_2—$). CI-MS, *m/z* 633 $[M + H]^+$. HRMS, $C_{38}H_{40}N_4O_5$; $M^+ = 632·29987$; found, 632·30423.

1-Pyrenemethanol (**8**). 1-Pyrenecarboxyaldehyde (**7**) (5 g, 21·7 mmol) was dissolved in dried tetrahydrofuran (25 ml) and solid $NaBH_4$ (0·41 g, 10·8 mmol) was added in small portions over about 30 s. The resulting mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction mixture was poured into water (200 ml) and the resulting white solid was collected and dried *in vacuo*. The crude material so obtained was recrystallised from ethyl acetate and dried to give 3·48 g (69%) of **8**. Melting point 115–118 °C (lit.[2] m.p., 121·5–122·5 °C). ^1H NMR ($CDCl_3$), δ 5·83 (2H, s, CH_2OH), 8·72–8·40 (9H, m, pyrene H). EI-MS, *m/z* 232 (M^+).

1-Chloromethylpyrene (**9**). 1-Pyrenemethanol (7·37 g, 31·8 mmol) and pyridine (4·3 ml) were dissolved in 150 ml of CH_2Cl_2 and cooled to 0 °C. Thionyl chloride (4·3 ml) was then added dropwise and the resulting solution was stirred overnight at room temperature before being poured into water (500 ml). A further 150 ml of CH_2Cl_2 were added. The CH_2Cl_2 layer was then collected and washed with first a solution of 5% $NaHCO_3$ (150 ml) and then a saturated solution of NaCl (150 ml) before being dried (Na_2SO_4) and evaporated to give 6·87 g of **9** (86%). Melting point 143–146 °C (lit.[3] m.p., 144–145 °C). ^1H NMR ($CDCl_3$), δ 5·33 (2H, s, pyreneCH_2Cl), 8·00–8·39 (9H, m, pyrene H). EI-MS, *m/z* 250 (M^+).

1-Pyrenemethyl acetate (**3**). Compound **9** (3 g, 12 mmol) was added to a stirred suspension of potassium acetate (2·20 equiv.) in glacial acetic acid at room temperature. The mixture was stirred for about 2·5 h under reflux. After cooling, the solvent was removed on a rotary evaporator and the residue was partitioned between chloroform and water. The chloroform layer was collected and taken to dryness under reduced pressure. The material so obtained was purified by column chromatography on silica gel using chloroform as the eluent. In this manner, 2·48 g of **3** were obtained (75%). ^1H NMR ($CDCl_3$), δ 2·13 (3H, s, O_2CCH_3), 5·83 (2H, s, pyrene CH_2OOCH_3), 8·00–8·26 (9H, m, pyrene H). EI-MS, *m/z* 274 (M^+).

Photophysical measurements. Dilute solutions of the various pyrene derivatives in freshly distilled *N,N*-dimethylformamide (DMF) or spectroscopic-grade methanol were used for all photophysical measurements. Absorption and fluorescence spectra were recorded with a Hitachi U3210 spectrophotometer and a fully-corrected Perkin-Elmer LS5 spectrofluorimeter, respectively. Fluorescence lifetimes were measured with a mode-locked, frequency-doubled Nd:YAG CW laser synchronously pumped, cavity-dumped pyridine-1 dye laser. The output was frequency-doubled to give 6 ps pulses at 342 nm. Emitted photons were detected with a Hamamatsu microchannel plate photo tube (FWHM 60 ps) operated in the time-correlated, single-photon counting mode. Transient absorption studies were made with a frequency-tripled, Q-switched Nd:YAG laser giving 8 ns pulses at 355 nm. Solutions were bubbled continuously with nitrogen and spectra were recorded point-by-point with five separate laser shots being averaged at each wavelength. Kinetic measurements were made at fixed wavelength with 50 individual laser shots being averaged, baseline-corrected and analysed by non-linear least-squares iterative procedures. Pulse radiolysis studies were made with the CFKR 4 MeV Van de Graaff electron beam accelerator.

RESULTS AND DISCUSSION

Photooxidation of pyrene

Photophysical properties of **1** and **3**, measured in dilute methanol or DMF solution for selective excitation into the pyrene absorption bands at 355 nm, were comparable and there was no indication that the appended cytosine molecule quenched the excited states of the pyrene subunit. Similarly, UV–visible, fluorescence, circular dichroism and ^{1}H NMR spectra showed the absence of any significant interaction between the pyrene and cytosine subunits in **1**.

Excitation of the pyrene subunit in **1** and **3** in oxygen-saturated DMF with a 10 ns laser pulse at 355 nm resulted in generation of singlet molecular oxygen, as detected by time-resolved luminescence-spectroscopy.[4] The quantum yields, however, were extremely low. Further, the lifetime of $O_2(^1\Delta_g)$ was independent of the concentration of substrate, indicating that the bimolecular rate constant for quenching by cytosine is $<10^6$ dm^3 mol^{-1} s^{-1}.

Time-resolved spectroscopic studies showed that methylviologen (MV^{2+}) quenched both excited singlet and triplet states of **1** and **3** in DMF solution.[5,6] Laser flash photolysis studies showed the intermediate formation of the π-radical cations derived from pyrene (λ_{max} = 450 nm) and MV^{2+} (λ_{max} = 395 and 605 nm):

$$*(\text{Pyr})\text{—Cyt} + MV^{2+} \rightarrow {}^{\cdot+}(\text{Pyr})\text{—Cyt} + MV^{+\cdot} \quad (1)$$

For both compounds, the pyrene π-radical cation decayed rapidly via first-order kinetics (typical lifetimes being *ca* 2 μs) but the rate constant was found to be markedly dependent on the initial concentration of the pyrene subunit. Based on earlier work,[7–9] this process is attributed to association between a pyrene π-radical cation and a ground-state pyrene molecule:

$$^{\cdot+}(\text{Pyr})\text{—Cyt} - \text{Cyt} + \text{Pyr—Cyt} \rightarrow [(\text{Pyr—Cyt})_2]^{+\cdot} \quad (2)$$

The rate of formation of the pyrene dimer π-radical cation, which was monitored at 750 nm, matched exactly the rate of disappearance of the monomer π-radical cation, as monitored at 450 nm, at any given concentration of pyrene. By varying the concentration of substrate, the bimolecular rate constant for reaction (2) was found to be $(1{\cdot}0 \pm 0{\cdot}6) \times 10^9$ dm^3 mol^{-1} s^{-1}. The dimer π-radical cation decayed slowly via electron transfer to $MV^{+\cdot}$ although, for both **1** and **3**, a small amount of reduced viologen persisted over prolonged times. Since identical behaviour was observed for these two compounds, it is clear that neither the pyrene π-radical cation nor its corresponding diver abstract an electron from the appended cytosine on these time scales. This hypothesis was confirmed by pulse radiolysis studies carried out with **1** (5×10^{-4} mol dm^{-3}) in oxygen saturated acetone, where it was found that the decay kinetics of the intially formed pyrene π-radical cation ($\tau = 1{\cdot}4$ μs) and that of the subsequent dimer (π-radical cation; $\tau = >200$ μs) remained unaffected by the presence of cytosine ($<1 \times 10^{-3}$ mol dm^{-3}).

Photoreduction of pyrene

It was observed that triethylamine quenched[10] the excited singlet and triplet states of **1**, **2** and **3** in DMF, resulting in intermediate formation of the pyrene π-radical anion (λ_{max} = 490 nm):

$$*(\text{Pyr})\text{—Cyt} + (C_2H_5)_3N \rightarrow {}^{\cdot-}(\text{Pyr})\text{—Cyt} + (C_2H_5)^3N^{+\cdot} \quad (3)$$

The π-radical anion of **3** decayed very slowly ($t_{1/2} \approx 1{\cdot}2$ ms) via first-order kinetics due to protonation by trace amounts of water present in the solvent:[11]

$$Pyr^{-\cdot} + H_2O \rightarrow PyrH^{\cdot} + OH^- \quad (4)$$

The resulting neutral radical undergoes slow disproportionation.

Identical behaviour was observed following one-electron reduction of **3** in nitrogen-saturated propan-2-ol under pulse radiolytic conditions; here, the lifetime of the π-radical anion was (100 ± 10) μs:

$$Pyr^{-\cdot} + C_3H_7OH \rightarrow PyrH^{\cdot} + C_3H_7O^- \quad (5)$$

For both **1** and **2**, the pyrene π-radical anion, generated via laser flash photolysis in the presence of triethylamine or by pulse radiolysis in nitrogen-saturated propan-2-ol, decayed rapidly via first-order kinetics with typical lifetimes of a few microseconds. The rate constants for these decay processes were slightly dependent on the concentration of **1** or **2** and, following extrapolation to zero concentration, were found to be $(4{\cdot}2 \pm 0{\cdot}7) \times 10^5$ and $(2{\cdot}8 \pm 0{\cdot}5) \times 10^5$ s^{-1} for **1** and **2**, respectively. We attribute this process to intramolecular electron transfer to the appended cytosine moiety; the bimolecular component being due to electron transfer to cytosine attached to a different pyrene molecule:

$$^{\cdot-}(\text{Pyr})\text{—Cyt} \rightarrow \text{Pyr—}(\text{Cyt})^{-\cdot} \quad (6)$$

$$^{\cdot-}(\text{Pyr})\text{—Cyt} + \text{Pyr—Cyt} \rightarrow \text{Pyr—Cyt} + \text{Pyr—}(\text{Cyt})^{-\cdot} \quad (7)$$

Indeed, electron transfer to cytosine was found to compete with protonation and subsequent bimolecular decay of the π-radical anion of **3** in DMF solution. The cytosine π-radical anion undergoes deprotonation and bimolecular processes which result in its ultimate destruction.[12]

CONCLUSION

This study has demonstrated that pyrene can photosensitize the reduction of cytosine by electron donors such as triethylamine. Similar processes, occurring *in vivo*, could result in photochemical strand scission of DNA with ribose acting as the electron donor. Since pyrene functions only as a light absorbing relay, and is not destroyed, it is likely that one molecule could cause extensive damage to the host polynucleotide. As such, this mechanism may explain some of the carcinogenic activity of polynuclear aromatic hydrocarbons. It is also conceivable that such processes could initiate cancer of the skin or other exposed regions. Further studies are in progress and are aimed at refining our understanding of this type of reductive process.

ACKNOWLEDGEMENTS

J.L.S. and Y.K. thank the Sloan Foundation, the National Institutes of Health (GM41657) and the Robert A. Welch Foundation for financial assistance. The Center for Fast Kinetics Research is supported jointly by the Biotechnology Resources Division of the National Institutes of Health (RR00886) and by The Univesity of Texas at Austin.

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