

At the critical point the inverse matrix M_{θ, k_c}^{-1} becomes singular. But the above equation can be solved by recognizing that $1 = \cos 0x$ and when inverse matrix is applied to the RHS, the subscript k_c should be replaced by 0 for the first term and by $2k_c$ for the second term as in eq.(2.19).

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The Photoaddition Reaction of 1,4-Diphenyl-1,3-butadiyne with 5-Fluorouracil

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Diacetylene compound, 1,4-diphenyl-1,3-butadiyne, was photolyzed with 5-fluorouracil as a model reaction of the phototoxic conjugated poly-yne with DNA or RNA and obtained a [2+2] photocycloadduct. The structure of the photoadduct was determined by spectral methods and compared with the [2+2] photoadducts of 1,4-diphenyl-1,3-butadiyne with tetramethylethylene and dimethyl fumarate.

Introduction

Certain naturally occurring conjugated poly-yne (polyacetylenes) have been reported to be phototoxic to a variety of microorganisms, human skin fibroblasts, Syrian hamster cells, mosquito larvae and Paramecium.¹⁻⁶ For example, 1-phenylhepta-1,3,5-triyne (PHT) which is a conjugated poly-yne occurring in high concentration in the leaves of the tropical weed *Bidens pilosa* L. is phototoxic toward various organisms containing membranes, including eukaryotic cells, fungi, bacteria, and viruses in the presence of long wave UV.⁷ PHT, unlike the naturally occurring photoactive psoralens, does not form interstrand cross-links with calf thymus DNA^{8,9} while furanocoumarins inactivate viruses by cross-linking the DNA.⁹ Instead, PHT exerts its phototoxic action via the

viral membrane damage which inactivates DNA or RNA to replicate.⁸ However, nothing is known about the photochemistry of poly-yne even though it is essential to understand the molecular mechanism of phototoxicity of the compounds. This membrane damage may involve the modification of cellular DNA or RNA.

The conjugated di-yne are reported to be less toxic than the conjugated tri-yne¹ but the cross-addition products were isolated and characterized in the photochemical reaction of diacetylene compound 1,4-diphenyl-1,3-butadiyne (DPB) and some olefins.¹⁰

We now report for the first time the photocycloaddition of DPB with 5-fluorouracil (5-FU) as a model photoreaction of conjugated poly-yne with DNA or RNA possibly in the membranes of various organisms.

Materials and Methods

DPB and 5-FU were purchased from Aldrich Chemical Co. Ltd. and used without further purification. Kiesel Gel GF₂₅₄(Merck) was used for silica gel thin layer chromatography. Extra pure diethyl ether (Wako Chemical Co.), extra pure n-hexane (Wako Chemical Co.) and methanol(Merck) were used without further purification.

Infrared spectra were recorded on a Perkin-Elmer 283 B spectrophotometer using potassium bromide pellets or NaCl cells. Ultraviolet-visible spectra were observed on a Cary-17 spectrophotometer. ¹H NMR spectra were obtained on a Varian FT-80A Spectrometer in chloroform-d. Fluorescence spectra were measured on a Aminco-Bowman spectrofluorometer with Aminco-XY recorder. Mass spectra were determined with Hewlett Packard 5985A GC/MS system. Elemental analyses were carried out on a F&M Scientific Cooperation-C.H.N. Analyzer Model 180. To analyze the photoreaction mixtures, high performance liquid chromatography was also performed on a Waters Associates HPLC system with μ -Porasil column.

Irradiation was carried out in a Rayonet Photochemical Reactor (The Southern New England Ultraviolet Company) Model RPR-208 or RPR-100 equipped with 300 nm UV lamps.

Irradiation of DPB with 5-FU. 1.1 mM (150 mg) of DPB and 8.2 mM (750 mg) of 5-FU were dissolved in 700 ml of methanol. The resulting solution was deoxygenated and irradiated with 300 nm UV lamps for 40 h.

After irradiation the reaction mixtures were concentrated in vacuo. The excess 5-FU was removed by filtering the mixture with diethyl ether. The photoreaction mixtures were analyzed by silica gel thin layer chromatography utilizing n-hexane - diethyl ether (20:1 v/v) as an eluting solvent. Analytical liquid chromatography was also used to analyze the photoproduct under the following condition: column; μ -Porasil, solvent; n-hexane-diethyl ether(20:1 v/v), flow rate; 1.0 ml/min, detector; UV(254nm).

The photoreaction mixtures were isolated by the silica gel preparative thin layer chromatography utilizing n-hexane - diethyl ether (20:1 v/v) as an eluting solvent.

Results and Discussion

When a deoxygenated methanol solution of DPB and 5-FU is irradiated with 300 nm UV light for 40 h, a cross-cycloaddition product is obtained (yield 14 mg, 4.1%). The structure of the adduct was determined by elemental analysis, UV, IR, ¹H NMR, mass spectra, and fluorescence spectra, and it was proven to be a [2+2] photoadduct (Figure 1). In this reaction, several regioisomers are possible but only isomer is isolated

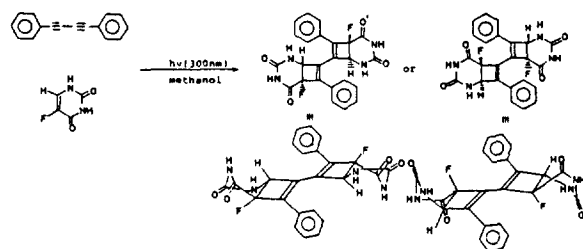


Figure 1. Photocycloaddition reaction of DPB with 5-FU in methanol.

due to the stability of intermediate biradical. The absolute configuration of the photoadduct was not determined. The elemental analyses data are consistent with the molecular formula of 1:2 photoadduct, C₂₄H₁₆N₄O₄F₂, as shown below; Calculated for C₂₄H₁₆N₄O₄F₂ - C 62.34, H 3.46, N 12.12, found - C 62.25, H 3.40, N 12.01.

Conjugated poly-yne exhibit characteristic electronic absorption spectra with the most prominent feature being a very high intensity band with well-defined vibrational fine structure.¹¹ DPB exhibits λ_{max} at 326, 304, 286, 268, 257, 244 nm. The UV absorption spectrum of the adduct in methanol does not show the poly-yne vibrational bands. It gives λ_{max} at 331, 317, and 230 nm. The photoadducts of DPB with tetramethylethylene(TME) and dimethyl fumarate (DMFU) showed the longest absorption maxima at 345 nm and 341 nm, respectively.¹⁰ The blue shift in DPB-5-FU adduct compared to DPB-TME or DPB-DMFU adduct is probably due to the electron withdrawing effect of the fluorine atom on the cyclobutene rings (Figure 2).

The infrared spectrum shows the carbonyl stretching band at 1735 cm⁻¹ indicating the saturation of C5-C6 double bond of 5-FU (1710 cm⁻¹). The N-H bending band appears at 1630 cm⁻¹ instead of 1660 cm⁻¹ in 5-FU due to the formation of the rigid cyclobutene ring. The ¹H NMR spectrum shows up-field shift of 6-H peak in 5-FU from 7.74 (doublet) to 4.07 (singlet).

The dihedral angle between the C-H and C-F bond is about 90° in this arrangement. The singlet peak at 4.07 ppm proves the proposed structure of [2+2] photoadduct. A plausible structure for the [2+2] photoadduct is suggested as shown in Figure 1 from the steric factor of the fluorine and the oxygen atoms.

Mass spectra obtained by EI method(15 eV) show a base

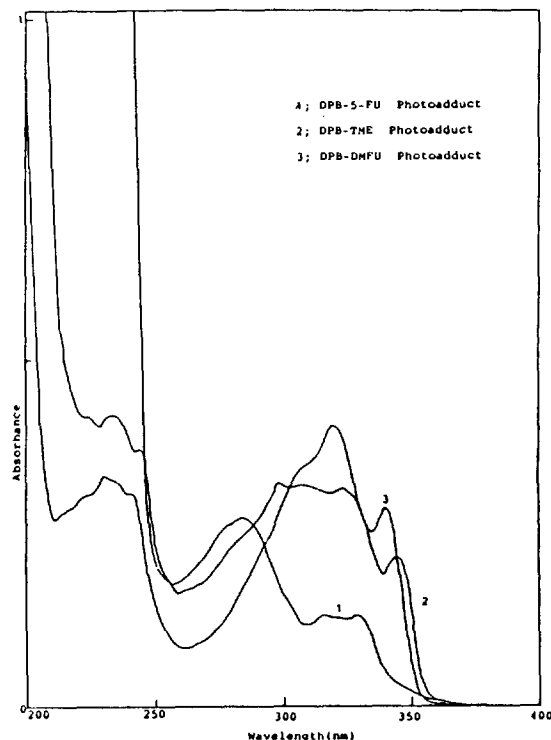


Figure 2. UV spectra of [2+2] photoadducts of DPB with 5-FU, TME, and DMFU in methanol.

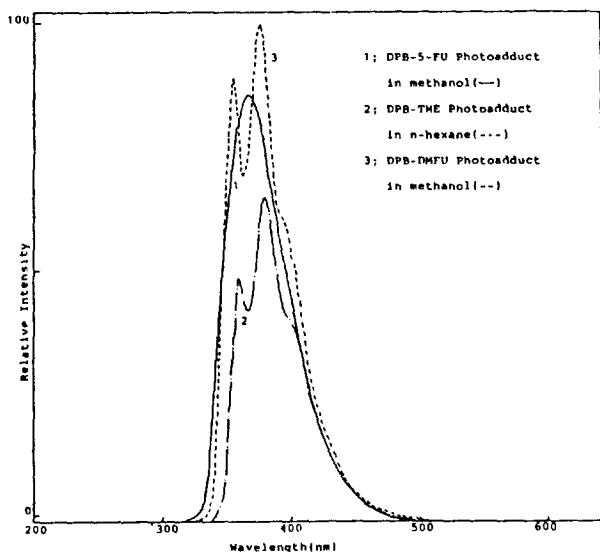


Figure 3. Fluorescence spectra of [2+2] photoadducts of DPB with 5-Fu, TME, and DMFU. λ_{ex} 300 nm.

peak at m/e 149 which can result from the cleavage of 5-FU moiety from the molecular ion and the addition of the free fluorine radical to the 5-FU moiety. The fragment ion deprived of the fluorine radical was observed at m/e 111. The peaks at m/e 130 and m/e 202 are good evidences for the existence of DPB and 5-FU moiety. No molecular ion peak was observed. The [2+2] photoadduct fluoresces, while DPB does not. The fluorescence spectrum in methanol at room temperature is shown along with those of DPB-TME and DPB-DMFU photoadducts in Figure 3.

Ingold has shown that the first excited singlet state of acetylene has the *trans*-bent structure.¹² The species are regarded as a *trans*-vinyl biradical. The photoreduction of simple acetylenes is accounted for by successive molecular hydrogen abstractions from the solvent by the excited singlet alkyne and then by the vinyl radical produced in the first step.¹³ In the photoreaction of DPB and 5-FU, the same mechanism seems to be valid and the excited $C\equiv C$ triple bond initially

attacks 5-FU to give the vinyl biradical followed by coupling of the biradical to form cyclobutene ring.

The photocycloaddition of DPB to various olefins including 5-FU suggests that phototoxic poly-yne cycloadd to pyrimidine bases in membrane DNA or RNA on irradiation with long wave UV and results in inactivation of RNA or DNA to replicate. The photochemistry of poly-yne with DNA bases will be a next target for elucidating the mechanism of poly-yne phototoxicity. These studies are under progress in this laboratory.

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