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Photocycloaddition of 5,7-Dimethoxycoumarin to 5-Fluorouracil

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C_4 -Photocycloaddition of 5,7-dimethoxycoumarin(DMC) to 5-fluorouracil(5-FU) was studied in frozen aqueous solution. The major photoproduct was diagnosed and isolated by TLC and column chromatography. The structure of isolated photoproduct was identified as a C_4 -cycloaddition product of DMC and 5-FU by the characteristics of its UV, IR, NMR, mass spectra, elemental analysis, and photosplitting.

Introduction

Psoralens, naturally occurring coumarin derivatives in plants of the families *Umbelliferae* and *Rutaceae*, are known to photoreact with pyrimidine bases in DNA with near UV light. These biological effects have been attributed to this photoreaction.^{1,2}

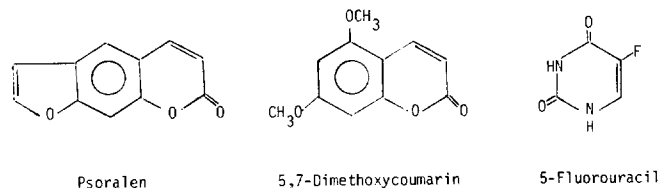
5,7-Dimethoxycoumarin(DMC) which has only one photochemical functional group (without the furan ring) in the molecule also intercalate with DNA and photobinds covalently to DNA bases causing the same biological effects as furocoumarins, unlike most other coumarins.³⁻⁵ Since DMC is as reactive as psoralens and since fewer photoproducts are formed due to the lack of bifunctionality compared to furocoumarins, the photoreaction of DMC with pyrimidine bases has been studied as a model reaction for the molecular basis of skin-photosensitization. The photoadducts of DMC to thymine⁶ and thymidine⁷ were isolated by chromatography and characterized.

The incorporation of 5-fluorouracil(5-FU) into the RNA of tobacco mosaic virus sensitizes both the intact virus^{8,9} to UV light. The sensitivity is proportional to the incorporation of 5-FU in place of uracil. The photosensitization by acetone or N-methylglutidone yields a C_4 -cycloaddition product from 5-FU in aqueous solution.¹⁰

5-FU has been shown to be the most reactive among the pyrimidine bases tested for photoreactivity with coummaryl compounds¹¹ and highly active with DMC.⁶

To see the base specificity of psoralens and DMC in the photoaddition of the compounds to DNA and as a model for

the photosensitization of psoralens, the photoaddition reaction of DMC to 5-FU is studied.



Experimental

Materials. DMC and 5-FU obtained from Aldrich chemicals gave only one spot on the silicagel thin layer chromatogram. Kieselgel G (Typ 60) and Kieselgel GF 254 were used for silicagel thin layer chromatography (TLC). Kieselgel 60 (Merck, 230 mesh) was used for column chromatography. Other common solvents were from Wako Chemical Company and used without further purification.

Spectroscopic Measurements. UV spectra were measured on a Cary 17 spectrophotometer and IR spectra were recorded in potassium bromide pellets on a Perkin-Elmer Model 267 grating spectrophotometer. NMR spectra were measured on a Varian T-60A spectrometer against tetramethylsilane internal standard in perdeuteriated pyridine solvent. Mass spectra were determined with Hewlett Packard 5985 A GC/MS system. Fluorescence spectra were recorded on an Aminco-Bowman spectrofluorometer with Aminco-XY-recorder. Elemental analysis were carried out at ADD with a C.H.N. analyzer.

Irradiation Apparatus. Irradiations were carried out in a Rayonet Photochemical Reactor (The Southern New England

Ultraviolet Company) Model RPR-208 or RPR-100 equipped with 350 nm fluorescent lamps. Two modules of RPR-208 were stalked together and arrayed in a horizontal position allowing the photolysis of frozen aqueous solutions.

Frozen Aqueous Solution Irradiation of 5,7-Dimethoxycoumarin in the Presence of 5-Fluorouracil. The solutions of 5-FU (0.1 M) and DMC (0.01 M) in water-dioxane (9:1 v/v) were poured into petri dishes and frozen in the refrigerator. The thickness of the resulting frozen solutions was less than 5 mm. The petri dishes containing the frozen aqueous solutions were placed at 15 cm distance from the RUL-3500 Å lamps and irradiated for 20 hr. The temperature of reaction chamber was maintained below 0°C during the photolysis to prevent melting of the frozen aqueous solution.

Analysis of Photoproducts. Irradiated samples were analyzed by silicagel TLC utilizing cyclohexane-ethylacetate (30:70 v/v) as a developing solvent and visualized by Mineral light (Model UVS 11; 254 nm shortwave UV; Model UVS 12; 360 nm longwave UV) or iodine vapour.

Fluorescence Quenching Study of 5,7-Dimethoxycoumarin with 5-Fluorouracil. The fluorescence spectra of DMC (12 μM) containing 0.2, 0.4, 0.6, 0.8, and 1 mM 5-FU were measured in methanol at room temperature with excitation at 370 nm.

Photosplitting of DMC-5-FU Photoadducts. Purified photoadduct was dissolved in methanol and irradiated with 254 nm UV light in a quartz UV cell at room temperature. The light sources were RPR-2537 Å lamps in a Rayonet Photoreactor (Model RPR-100). After irradiation, the products were separated by silicagel TLC and UV absorbance at 324 nm was monitored.

Results and Discussion

Characterization of DMC-5-FU Photoadduct. The photolysis products of DMC and 5-FU were diagnosed by TLC and two cross addition products, one major and another in minor quantity, were detected. In both products, DMC and 5-FU were formed upon irradiation with 254 nm mineral light followed by developing on TLC. The major product was isolated by silica gel column chromatography. It decomposed thermally above 160°C without melting. The elemental analysis data are consistent with the molecular formula of a 1:1 adduct of DMC and 5-FU as shown below.

Anal. Calcd. for C₁₅H₁₃N₂O₆ C, 53.6%; H, 3.87%; N, 8.33%. Found C, 52.9%; H, 4.1%; N, 8.1%.

The mass spectra of the product were recorded by electron impact (EI) and chemical ionization (CI) method. A quasi-molecular ion peak corresponding to a 1:1 DMC-5-FU was observed at *m/e* 337 with the relative intensity of 21.6 from the methane gas CI method. The fragmentation patterns show the base peak at *m/e* 207 (DMC) and are very similar to those of a DMC and 5-FU mixture, indicating efficient DMC and 5-FU formation by splitting of the photoadduct (Table 1). The EI mass spectrum does not show a peak corresponding to

the molecular ion of a 1:1 DMC-5-FU adduct and the conspicuous absence of molecular ion peak in EI mass spectra of cyclobutane type dimers of DMC¹², and pyrimidine bases^{10,13-15} are well established and the product fragments readily into ions of lower mass in EI method as well as decomposing thermally prior to electron impact.

The large blue shift of λ_{max} 324 nm to 270 nm in the UV absorption spectrum of the photoadduct shows the loss of conjugation in DMC. When the aqueous solution of the photoadduct was irradiated with 254 nm light for 3 min, DMC and 5-FU were obtained as detected by UV and TLC analyses. The infrared spectra show the strong carbonyl stretching band of DMC at 1,710 cm⁻¹ shifted to 1,775 cm⁻¹ indicating the loss of the pyrone double bond which is identical with that of the DMC C₄-cyclodimer,¹² C₄-cycloadduct of DMC and tetramethylethylene,⁷ and thymine.⁶ Another ν_{C=O} band at 1,710 cm⁻¹ is due to the blue shifted 5-FU carbonyl (1,650 cm⁻¹) caused by the saturation of the 5,6-double bond of 5-FU, which is consistent with the infrared data of many pyrimidine base C₄-photocyclodimers. These evidences suggest that the photoadduct is a C₄-photocycloaddition product of DMC and 5-FU formed through 2+2 addition of the 3,4-double bond of DMC to the 5,6-double bond of 5-FU.

Nuclear magnetic resonance spectral data show neither pyrone double bond protons of DMC at δ 6.09 and 7.90 nor the 5-FU olefinic proton at δ 7.61, while cyclobutyl protons appear at δ 5.1 4.2, indicating the product to be a

TABLE 1: Fragmentation Patterns of CI Mass Spectrum of the Photoadduct of DMC to 5-FU

<i>m/e</i>	Fragmentation	Relative intensity
113		19.6
131	5FU + H	76.1
206	DMC	20.7
207	DMC + H	100.0
317	(M + H)-HF	13.3
337	M + H	21.6

TABLE 2: The Ratio of Absorbance Decrease at 324 nm of DMC in the Presence and Absence of Various Pyrimidine Derivatives upon Irradiation at 365 nm. The Concentration of DMC was 80 μM and that of the Pyrimidine Bases was 8 mM in Methanol Solution

Derivatives	Absorbance decrease of DMC-pyrimidine mixtures	
	Absorbance decrease of DMC	
Control (DMC alone)	1.0	
5-Fluorouracil	1.47 ± 0.03	
Thymine	1.350 ± 0.002	
Uracil	1.005 ± 0.002	
1,3-Dimethylthymine	1.64 ± 0.04	
1,3-Diethylthymine	1.248 ± 0.002	

TABLE 3: Relative Rates of the Photoreaction between Skin-Sensitizers and Pyrimidine Bases

Reaction mixture	Initial rate. $\Delta O.D.$ ($s^{-1} \times 10^5$)	Relative rate*
Coumarin	1.38	1.5
Coumarin + Thymine	0.90	1.0
Coumarin + Uracil	0.76	0.8
Coumarin + 5-Fluorouracil	1.66	1.8
8-Methoxypsoralen	0.60	0.7
8-Methoxypsoralen + Thymine	0.60	0.7
8-Methoxypsoralen + Uracil	0.68	0.8
8-Methoxypsoralen + 5-Fluorouracil	0.93	1.0
<i>cis</i> -Benzodipyrene	6.73	7.5
<i>cis</i> -Benzodipyrene + Thymine	5.19	5.8
<i>cis</i> -Benzodipyrene + Uracil	0.04	6.7
<i>cis</i> -Benzodipyrene + 5-Fluorouracil	7.93	8.8
<i>trans</i> -Benzodipyrene	48.70	54.1
<i>trans</i> -Benzodipyrene + Thymine	34.10	37.9
<i>trans</i> -Benzodipyrene + Uracil	46.20	51.3
<i>trans</i> -Benzodipyrene + 5-Fluorouracil	54.40	60.4

* Relative to the rate of the coumarin-thymine reaction

photocycloadduct of DMC and 5-FU formed through 2+2 cycloaddition.

The Mechanism of the Photoaddition Reaction of DMC to 5-FU. DMC has no self-quenching effect on fluorescence and fluorescence of DMC was not quenched by 5-FU under the experimental conditions employed. The photocycloaddition reaction of DMC to 5-FU occurs probably via excited singlet state, since DMC has very low efficiency in populating the triplet state ($\Phi_{F1} = 0.65$ at 298 °K, $\Phi_P / \Phi_{F1} 0.05$ at 77 °K) and the photocyclodimerization of DMC proceeds via excited singlet state exclusively in direct irradiation.¹² The relative photoreactivity of DMC and other coumarins toward several pyrimidine bases⁶ and other coumaryl compounds¹¹ were investigated as shown in Table 2 and 3. 5-FU is the most reactive base to excited coumarins. Only exception among many pyrimidine bases tested is 1,3-dimethylthymine.

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Photocycloaddition Reaction of 1,2-Bispyrazylethylene to Tetracyanoethylene

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Benzene solution of *trans*-1,2-bispyrazylethylene and electron deficient olefin, tetracyanoethylene, as a π -acceptor gave 1,2-bispyrazyl-3,3,4,4-tetracyanocyclobutane, a $2\pi+2\pi$ cycloaddition product, on irradiation with 350 nm UV light. Fluorescence studies revealed the reaction to proceed through a singlet exciplex. The fluorescence of *trans*-1,2-bispyrazylethylene was quenched very efficiently by tetracyanoethylene with the quenching constant of $1.6 \times 10^{10} M^{-1} s^{-1}$ while electron rich olefin, tetramethylethylene, did not quench the fluorescence of bispyrazylethylene.