

THE PHOTOCYCLOADDITION REACTION OF 8-METHOXYPSORALEN WITH OLEFINS

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Summary

The photocycloaddition reactions of 8-methoxypsoralen (8-MOP) to the π -electron-rich olefin 2,3-dimethyl-2-butene (DMB) and the π -electron-deficient olefins fumaronitrile (FN) and dimethylfumarate (DF) were studied as a model for the photosensitizing 8-MOP-pyrimidine base photocycloaddition reaction. The direct and sensitized photoreactions were carried out in a liquid methanol solution and a frozen benzene solution. The major photoadducts 8-MOP \diamond DMB, 8-MOP \diamond FN and 8-MOP \diamond DF were monitored by silica gel thin layer chromatography (TLC) and were isolated by preparative TLC and silica gel column chromatography. The structures of the photoadducts were determined by physical methods to be 1:1 C₄ cycloadducts formed through the pyrone double bond of excited 8-MOP. The same photoadducts were obtained on irradiation of the frozen solution. The fluorescence of 8-MOP was quenched by DMB, FN, DF and tetracyanoethylene. Linear Stern-Volmer plots with k_q values of $7.27 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$, $2.36 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$, $8.65 \times 10^8 \text{ l mol}^{-1} \text{ s}^{-1}$ and $2.91 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$ respectively were obtained. A singlet exciplex formation mechanism is proposed for the photocycloaddition reaction of 8-MOP to olefins. However, the triplet mechanism cannot be ruled out, particularly for 8-MOP \diamond DMB.

1. Introduction

In our previous publications we have described the C₄ photocycloaddition of 5,7-dimethoxycoumarin (DMC) to 2,3-dimethyl-2-butene (DMB) [1], thymine [2] and thymidine [3] as a model for the biologically important DMC-deoxyribonucleic acid (DNA) and furocoumarin-DNA photoreactions. Furocoumarins have two photochemical functional groups, the 3,4-pyrone and 4',5'-furan double bonds, both of which are necessary for the photosensitizing activity. DMC has only one photochemical functional group, the pyrone double bond. The photobiological activities of furocoumarins are generally attributed to an interaction of furocoumarins with

DNA, mainly [2+2] photocycloaddition to pyrimidine bases [4]. The 5,6-pyrimidine double bond reacts with the 3,4 double bond and/or the 4',5' double bond to form cyclobutane rings which can lead to the cross-linking of two strands of DNA [5, 6]. These photoreactions can be divided into three steps, intercalation, monoadduct formation and biadduct formation, which lead to the cross-linking of DNA [7 - 12]. The interstrand cross-linking is believed to be largely responsible for the photosensitizing effects of furocoumarins although some activity appears to be associated with the monoadducts [13, 14]. The monoadducts can be formed on either the 3,4-pyrone double bond or the 4',5'-furan double bond, and they are expected to show different photochemical reactivities towards biadduct formation because of their different UV absorption characteristics. Therefore a knowledge of the order and regioselectivity of the furocoumarin photoaddition to DNA is necessary in order to understand the photosensitization of the compound. Some of the monoadducts formed between furocoumarins and thymine have been isolated and characterized. The 3,4-pyrone double-bond monoadducts were formed between 8-methoxypsoralen (8-MOP) and 1,3-dimethylthymine [15] or thymidine [16]. However, the 4',5'-furan double-bond monoadduct is isolated from 4'-hydroxymethyl-4,5',8-trimethylpsoralen and thymidine and also from 8-MOP \diamond thymine [17] and 8-MOP \diamond thymidine [18]. Thus the order and regioselectivity of the photoaddition of furocoumarin to pyrimidine bases are dependent on the reaction conditions and the olefins. The physical and chemical data and the results of systematic studies are inadequate for the determination of the regioselectivity or geometry of the photoadducts even though a knowledge of the reaction mechanism and the structure of furocoumarin \diamond pyrimidine photoadducts is essential in understanding the molecular mechanism of photosensitization by furocoumarins. Therefore we have investigated the photoaddition of furocoumarins to various simple olefins with different electron affinities. Electron-rich and electron-poor olefins are expected to form exciplexes, which are the precursors of the cycloadducts, on different sites of the furocoumarins leading to 3,4- or 4',5'-C₄ cycloadducts.

2. Experimental details

2.1. Materials

8-MOP (Sigma) and DMB (Aldrich) were used without further purification. Tetracyanoethylene (TCNE) and benzophenone (Merck) were recrystallized in ethanol (melting points, 200 °C for TCNE and 48.5 °C for benzophenone). Fumaronitrile (FN) and dimethylfumarate (DF) were synthesized from fumaric acid by standard methods [19] and were purified by sublimation (melting points, 97 °C for FN and 102 °C for DF). Benzene and methanol (Wako Chemical Co.) were purified immediately before use by standard methods [20]. The other solvents were obtained from the Wako Chemical Co. and were used without further purification. Kiesel Gel G

(Merck) and GF₂₅₄ (Merck) were used for the thin layer chromatography (TLC) and Kiesel Gel 60 (Merck, 230 mesh) was used for the column chromatography. Tris(oxalato)ferrate(III) actinometry was used to measure the light intensity.

2.2. Spectroscopic measurements

The UV-visible absorption spectra were recorded using a Cary 17 spectrophotometer. The nuclear magnetic resonance (NMR) spectra were measured in acetone-*d*₆ and chloroform-*d* using a Varian T-60A spectrometer with tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer model 267 spectrophotometer using KBr pellets. The mass spectra were determined using a Hewlett-Packard 5985A gas chromatography-mass spectrometry system. Fluorescence spectra were recorded using an Aminco-Bowman spectrofluorometer equipped with an Aminco α - γ recorder. The elemental analysis was carried out at the Korea Institute of Science and Technology on an F&M Scientific Corporation C.H.N. analyser model 180.

2.3. Irradiation apparatus

The irradiations were performed using a Rayonet photochemical reactor (Southern New England Ultraviolet Company) model RPR 208 equipped with 350 nm fluorescent lamps. Two modules of model PRP-208 were stacked together and arranged in a horizontal position to allow the photolysis of the frozen benzene solutions. A medium pressure 450 W Hanovia mercury arc lamp was used to measure the quantum yield.

2.4. Fluorescence quenching study of 8-methoxypsoralen

Fluorescence quenching of 8-MOP by DMB, DF, FN and TCNE was carried out in ethanol solutions. The fluorescence spectra of 8-MOP (50 μ M) containing 0.1, 0.125, 0.150 and 0.175 M DMB in ethanol were measured at room temperature and an excitation wavelength of 350 nm. The fluorescence spectra of 8-MOP (50 μ M) containing 0.20, 0.40, 0.60, 0.80 and 1.00 M DF, 0.10, 0.20, 0.30, 0.40 and 0.50 M FN, and 0.10, 0.20, 0.30, 0.40 and 0.50 M TCNE in ethanol were measured by the same method at room temperature and an excitation wavelength of 350 nm.

2.5. Irradiation of 8-methoxypsoralen in the presence of olefins

2.5.1. 8-methoxypsoralen \diamond 2,3-dimethyl-2-butene

216 mg of 8-MOP and 2.5 ml of DMB (mole ratio, 1:10) were dissolved in 500 ml of methanol. The resulting solution was deoxygenated by bubbling nitrogen through for 30 min and was then irradiated for 20 h using an RUL-3500 Å lamp. 43.2 mg of 8-MOP and 0.5 ml of DMB (mole ratio, 1:10) were dissolved in 1 ml of chloroform and diluted to 250 ml with benzene. The solution was then poured into Petri dishes and frozen in a refrigerator. The thickness of the resulting frozen solutions was less than 5 mm. The Petri dishes containing the frozen benzene solutions were placed 15 cm from the

RUL-3500 Å lamps and irradiated for 20 h. Dry ice was used to maintain the temperature of the frozen benzene solution below 0 °C during the irradiation.

2.5.2. 8-methoxypsoralen \diamond fumaronitrile

216 mg of 8-MOP and 780 mg of FN (mole ratio, 1:10) were dissolved in 500 ml of methanol. The solution was deoxygenated and was then irradiated for 50 h. 43.2 mg of 8-MOP and 156 mg of FN (mole ratio, 1:10) were dissolved in 1 ml chloroform and diluted to 250 ml with benzene. The solution was poured into Petri dishes and frozen. It was then irradiated for 50 h by the same method as was used for 8-MOP \diamond DMB.

2.5.3. 8-methoxypsoralen \diamond dimethylfumarate

216 mg of 8-MOP and 1440 mg of DF (mole ratio, 1:10) were dissolved in 500 ml of methanol. The solution was deoxygenated and was then irradiated for 50 h. 43.2 mg of 8-MOP and 288 mg of DF (mole ratio, 1:10) were dissolved in benzene. The solution was frozen and was then irradiated for 50 h by the same method as was used for 8-MOP \diamond DMB.

2.6. Analysis of the photoreaction products

The 8-MOP \diamond DMB and 8-MOP \diamond FN photoreaction mixtures were analysed by silica gel TLC using a 55vol.%cyclohexane–45vol.%acetone mixture as the developing solvent. The chromatograms were exposed using a Mineral lamp (model UVS-11; wavelengths, 254 and 350 nm) or in an iodine vapour chamber. The 8-MOP \diamond DF photoreaction mixtures were analysed by silica gel TLC using a 45vol.%benzene–55vol.%acetone mixture as the developing solvent. The chromatograms were exposed using a Mineral lamp or in an iodine vapour chamber.

2.7. Isolation of the photoaddition products

The major photoadducts 8-MOP \diamond FN and 8-MOP \diamond DMB were separated from the photoreaction mixtures by silica gel column chromatography and preparative TLC. The 8-MOP \diamond DF photoadduct could only be isolated with difficulty.

2.8. Photodisintegration of the photoadducts

The purified photoadducts were dissolved in methanol (5×10^{-5} M). The solutions were irradiated at 254 nm in a quartz UV cell at room temperature. The UV spectra were recorded at 10 min intervals. The light source was an RPR-2537 Å lamp with a Rayonet photochemical reactor (model RPR-100). After irradiation, the solutions were analysed by TLC.

2.9. Quantum yield measurements of 8-methoxypsoralen \diamond 2,3-dimethyl-2-butene photoadduct formation and the benzophenone sensitization of the 8-methoxypsoralen \diamond 2,3-dimethyl-2-butene photoreaction

34 mg of 8-MOP (1.5×10^{-4} mol) and 1.5 ml (1.5×10^{-2} mol) of DMB were dissolved in 1 ml of chloroform and diluted to 15 ml. 3 ml of the

solution were poured into an ampoule made from a Pyrex test tube of dimensions 13 mm × 10 mm and were degassed on a vacuum line and sealed. 34 mg of 8-MOP, 1.5 ml of DMB and 273 mg of benzophenone were dissolved in chloroform and diluted to 15 ml. 3 ml of the solution were poured into a Pyrex ampoule, degassed and sealed. The samples prepared in this way were irradiated at 366 nm for 310 min using a 450 W medium pressure Hanovia mercury arc lamp and were then analysed.

3. Results and discussion

3.1. Characterization of the photoadducts

The photolysis products of 8-MOP and DMB were analysed by TLC and only one product was detected. The frozen benzene solution gave a better photoproduct yield than was obtained in the methanol solution. The product was isolated by silica gel column chromatography and preparative TLC. The melting point of the product was 179 - 179.5 °C. The elemental analysis data were consistent with the molecular formula of a 1:1 adduct of 8-MOP and DMB, *i.e.* C₁₈H₁₈O₄; the calculated composition of C₁₈H₁₈O₄ is 72.0% C, 6.7% H and 21.3% O; the observed composition was 72.8% C, 6.17% H and 21.02% O.

The electron impact (EI) mass spectra of the product show a molecular ion peak corresponding to a 1:1 8-MOP-DMB adduct at $m/e = 300$. The fragmentation patterns show a base peak at $m/e = 216$, indicating the efficient formation of 8-MOP and DMB by splitting of the photoadduct as shown in Table 1. The results suggest that the photoadduct is a C₄ photocycloaddition product of 8-MOP and DMB formed through [2+2] addition of the 3,4 or 4',5' double bonds of 8-MOP to the double bond of DMB. The low intensity of the molecular ion peak strongly supports this proposition since the molecular ions of C₄ cyclodimers of pyrimidine bases and C₄ cyclo-

TABLE 1

Fragmentation patterns of the electron impact mass spectrum of the photoadduct of 8-methoxypsoralen and 2,3-dimethyl-2-butene

m/e	Fragment ion	Relative intensity
69	DMB - CH ₃	18.0
83	DMB - H	17.5
84	DMB	18.0
145	8-MOP - CO ₂ - CO + H	8.5
173	8-MOP - CO ₂ + H	10.0
201	8-MOP - CH ₃	12.5
216	8-MOP	100
217	8-MOP + H	21.0
300	8-MOP + DMB (M ⁺)	19.5
301	M + 1	1.0

adducts of 5,7-dimethoxycoumarin and DMB are known to fragment easily into ions of low mass [1]. The UV absorption spectra of the photoadduct show a blue shift in λ_{\max} from 300 to 250 nm, indicating a loss of conjugation in 8-MOP. It is known [9, 10] that the UV absorption spectra of the 3,4 photoadduct of psoralen and thymine show a large blue shift in λ_{\max} compared with psoralen and that the UV spectra of the 4',5' photoadduct of psoralen and thymine show a slight red shift of λ_{\max} compared with psoralen. Therefore the large blue shift of λ_{\max} in the photoadduct formed here indicates the disappearance of the 3,4 double bond of 8-MOP through [2+2] cycloaddition with DMB. The IR spectra show that the strong carbonyl stretching band of 8-MOP at 1705 cm^{-1} is shifted to 1740 cm^{-1} which is similar to the behaviour of the C_4 cycloadduct of DMC with DMB [11]. This blue shift also indicates that the pyrone double bond of 8-MOP is broken during the photoreaction. A characteristic cyclobutane ring deformation band at 860 cm^{-1} which is not present in the 8-MOP spectra is also observed. The proton NMR spectrum of the photoadduct taken in acetone- d_6 shows that the olefinic protons of the pyrone double bond of 8-MOP at $\delta = 6.50$ (H(3)) ppm and $\delta = 7.90$ (H(4)) ppm are shifted upfield, indicating loss of conjugation. As a result of this the chemical shifts of the protons of the phenyl ring and the furan double bond are also shifted slightly upfield. New signals at $\delta = 3.61$ ppm (H(4)) (doublet, 1 H) and $\delta = 3.08$ ppm (H(3)) (doublet, 1 H) are observed in a spectral region which is typical of cyclobutane protons [1, 2]. The remainder of the spectrum is consistent with the cyclobutane adduct structure of 8-MOP and DMB. When a methanol solution of the 8-MOP \diamond DMB photoadduct was irradiated with UV light at 254 nm for 20 - 50 min, 8-MOP and DMB were detected by UV and TLC. Thus the product is a C_4 cycloadduct of 8-MOP and DMB. From these results, it is evident that the 8-MOP \diamond DMB photoadduct is formed through the [2+2] cycloaddition of the pyrone double bond of 8-MOP to DMB (Table 2).

TABLE 2

Proton magnetic resonance data for the photoadduct of 8-methoxypsoralen and 2,3-dimethyl-2-butene

Chemical shift δ (ppm)	Multiplicity ^a	Coupling constant J (Hz)	Relative intensity
3.61 (H(4))	d	10	1
3.08 (H(3))	d	10	1
7.00 (H(5))	s	—	1
7.80 (H(5'))	d	2	1
6.83 (H(4'))	d	2	1
4.04 (OCH ₃)	s	—	3
0.68 - 1.67 (4CH ₃)	s	—	12

^as, singlet; d, doublet.

The photolysis product of 8-MOP and FN was isolated and characterized by the same method as was used for the 8-MOP \diamond DMB photo-product. The frozen benzene solution yielded the photoaddition product but the photoreaction in the methanol solution was very inefficient. The major photoadduct was isolated and recrystallized in methanol with a 10% yield (based on 8-MOP). It became dark brown on standing in air and decomposed on heating first slowly and then rapidly and completely at temperatures above 100 °C. The EI mass spectra did not show a molecular ion peak corresponding to a 1:1 8-MOP-FN adduct. However, the fragmentation pattern shows a base peak at $m/e = 216$ (8-MOP) and a strong peak at $m/e = 78$ (FN), indicating the efficient formation of 8-MOP and FN by splitting of the photoadduct. This suggests that the photoadduct is a C₄ photocycloaddition product of 8-MOP and FN. The UV absorption spectra indicate that the photoadduct was formed through the pyrone double bond. A large blue shift of λ_{\max} (300 to 250 nm) shows the loss of conjugation of the pyrone double bond. The IR spectra show that the strong carbonyl stretching band of 8-MOP at 1705 cm⁻¹ is shifted to 1740 cm⁻¹ as in the case of 8-MOP \diamond DMB. A characteristic cyclobutane ring vibrational band (860 cm⁻¹) and a -CN stretching band (2240 cm⁻¹) were also observed. The proton NMR data shown in Table 3 are consistent with the cyclobutane adduct structure formed through [2+2] photoaddition of the pyrone double bond of 8-MOP to FN. When a methanol solution of the photoadduct was irradiated at 254 nm for 20 - 50 min, 8-MOP and FN were detected by UV ($\lambda_{\max} = 300$ nm) and TLC analyses. This also suggests that the product is a C₄ cycloadduct of 8-MOP and FN. All the results conclusively indicate the product to be a C₄ cycloaddition product of 8-MOP and FN through the pyrone double bond.

The photolysis product of 8-MOP and DF was monitored by silica gel TLC and the major photoadduct was isolated by silica gel column chromatography and preparative TLC (the yield was 4% based on 8-MOP). The product was characterized by UV, IR, proton NMR and mass spectrometry and by the photosplitting of the product into 8-MOP and DF as in the case

TABLE 3

Proton magnetic resonance data for the photoadduct of 8-methoxypsoralen and fumaronitrile

Chemical shift δ (ppm)	Multiplicity ^a	Coupling constant J (Hz)	Relative intensity
7.70 (H(5'))	d	2	1
6.60 (H(4'))	d	2	1
6.75 (H(5))	s	—	1
3.95 (OCH ₃)	s	—	3
4.10 - 4.60 (cyclobutyl protons)	m	—	4

^as, singlet; d, doublet; m, multiplet.

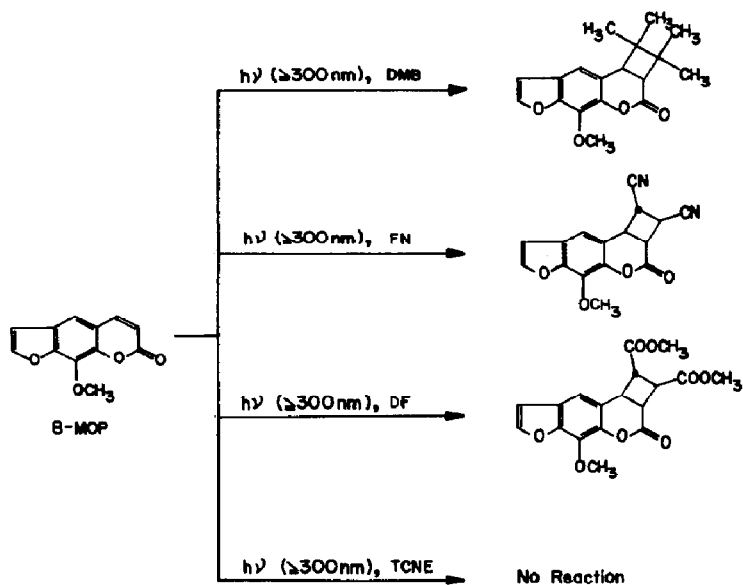


Fig. 1. Photoreaction of 8-methoxypsoralen with olefins.

TABLE 4

Proton magnetic resonance data for the photoadduct of 8-methoxypsoralen and dimethylfumarate

Chemical shift δ (ppm)	Multiplicity ^a	Coupling constant J (Hz)	Relative intensity
7.40 (H(5'))	d	2	1
6.90 (H(5))	s	—	1
6.60 (H(4'))	d	2	1
3.67 (C(=O)-OCH ₃)	s	—	3
3.65 (C(=O)-OCH ₃)	s	—	3
4.00 (O-CH ₃)	s	—	3
3.25 - 4.50 (cyclobutyl protons)	m	—	4

^as, singlet; d, doublet; m, multiplet.

of 8-MOP \diamond DMB and 8-MOP \diamond FN. The pyrone double bond of excited 8-MOP reacted with DF to give a C₄ cycloadduct, exactly as observed with the other olefins (Table 4 and Fig. 1).

3.2. The mechanism of the photoreaction between 8-methoxypsoralen and the olefins

No ground state complex is formed between 8-MOP and the olefins because the UV spectra of the mixtures exactly match the sum of individual spectra of 8-MOP and olefins. The fluorescence of 8-MOP is quenched by the

olefins as shown in Table 5, and it is clear that the quenching becomes more efficient as the π electron density of the olefins decreases. A singlet exciplex is expected to be formed between 8-MOP and the olefins, especially with the π -electron-deficient olefins.

The electron densities on the 3,4 and 4',5' double bonds are different in the excited states of the furocoumarins. An exciplex on the pyrone double bond leading to the 3,4 C₄ cycloadduct is expected with electron-rich olefins such as DMB. With electron-deficient olefins such as FN and DF, exciplexes on the 4',5' double bond of furan leading to the 4',5' C₄ cycloadduct are expected. However, only 3,4 C₄ cycloadducts were obtained with all the olefins investigated, suggesting that only the 3,4 exciplex is formed or that only the 3,4 exciplex leads to the C₄ cycloadduct. The fluorescence quenching shows that the exciplex formation is very efficient for the electron-poor olefins, which indicates that excited state 8-MOP is the only electron donor. Even though TCNE is the most efficient fluorescence quencher, no adduct is formed. The more polar charge transfer exciplexes are formed as the electron affinity of the olefins decreases and the yield of the cycloaddition reaction becomes less.

The fluorescence quenching results suggest that C₄ photocycloaddition of 8-MOP to olefins proceeds through a singlet exciplex formation between 8-MOP and the olefins. However, the triplet mechanism cannot be ruled out since 8-MOP populates the excited singlet state very inefficiently (Φ_p/Φ_t is much greater than 10 at 77 K). In the case of C₄ photocycloaddition of 8-MOP to DMB it is possible that the photocycloaddition reaction proceeds through a triplet mechanism. Comparison of the quantum yields of C₄ photocycloaddition of 8-MOP to DMB by direct irradiation and by benzophenone sensitization (Table 6) suggests that the triplet mechanism is more likely.

The best yield of 8-MOP-olefin photoadducts is obtained by irradiating frozen solutions of the compounds of interest with UV light at a wavelength

TABLE 5

Quenching of 8-methoxypsoralen fluorescence by olefins in ethanol

Olefins	DMB	DF	FN	TCNE
k_q (l mol ⁻¹ s ⁻¹)	7.27×10^8	8.65×10^8	2.36×10^9	2.91×10^9

TABLE 6

Quantum yields of the C₄ photocycloaddition of 8-methoxypsoralen to 2,3-dimethyl-2-butene by direct irradiation and by benzophenone sensitization in chloroform

	Direct irradiation	Benzophenone sensitization
Quantum yield	2.90×10^{-3}	1.01×10^{-2}

of 350 nm. The photoreaction also takes place when the solution is irradiated in the liquid state but lower yields are obtained. It is known that the formation of aggregates in the solid state leads to a structure which restricts the relative orientation of neighbouring molecules. Thus the photo-addition of 8-MOP to olefins is more efficient in the solid state.

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