4′, 5′-디히드로소랄렌과 테트라메틸에틸렌의 광고리화 첨가반응에 관한 연구

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Photocycloaddition Reaction of 4′, 5′-Dihydropsoralen to Tetramethylethylene

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요 약. 4′, 5′-디히드로소랄렌(DHP)을 합성하여 상온 및 77 K 에서 이 화합물의 형광 양자수율은 0.08, 인광양자수율은 0.013, 형광수명은 0.95ns, 인광수명은 0.039s 임을 알았다. 이 결과로부터 형광수명은 5, 7-디메톡시쿠마린보다 작은반면 소랄렌보다 크고 반면 인광수명은 DMC 보다 크고 소랄렌보다 작음을 알았다. 소랄렌 유도체들의 광독성을 분자적 차원에서 규명하고 DNA 에의 광부가 반응을 알아내기 위하여 DHP 와 TME 의 광부가 반응을 연구하였다. 주 생성물은 TLC 로 분석하고 Preparative TLC 와 용매추출로 분리해냈다. 분리된 생성물의 구조는 질량분석기, UV, IR, NMR 그리고 원소분석결과에 의해 DHP 와 TME 의 광고리화첨가반응에 의한 생성물 임을 알았다. DHP 는 형광의 자체소광 효과를 볼 수 없으며 TME, 에틸푸마레이트, 푸마로니트릴에 의해 형광이 효과적으로 소광된다. 광부가 반응의 양자수율과 소광실험에서 DHP의 광첨가반응은 단일상태와 삼중상태에서 다 일어나는 것으로 추측된다.

ABSTRACT. A psoralen-DNA monoadduct analogue, 4′, 5′-dihydropsoralen (DHP) is synthesized and its photophysical properties such as fluorescence quantum yield (0.08), phosphorescence quantum yield (0.013), fluorescence lifetime (0.95ns), and phosphorescence lifetime (0.039s) were measured in ether solution and in frozen matrix state at 77 K. The photocycloaddition reaction of DHP with tetramethylethylene (TME) was carried out in solution and in the frozen state. The major photoaddduct was isolated and characterized by elemental analysis and physical methods such as UV, IR, NMR, and mass spectrometry. The major DHP-TME photoadduct was proved to be an 1 : 1 C_4-cycloadduct formed through 2+2 cycloaddition of the pyrone double bond of DHP to TME.

INTRODUCTION

Furocoumarins have been investigated with respect to their skin photosensitizing activity and various photobiological actions. These photobiological actions such as skin erythema on human and guinea pig skin, mutagenic and lethal effects in bacteria, inactivation of DNA virus, and inhibition of tumor cells have been correlated with photoreactivity of furocoumarins in the presence of long-wavelength UV light[1~4].

Furocoumarins intercalate between the base pairs in duplex DNA and these intercalated molecules can successively photoreact with two pyrimidine bases causing a crosslinkage between the separated strands of DNA [5~7]. The cross-linkage is attributed to the formation of cyclobutane adducts resulting from the psoralen 3,4-pyrone and 4′,5′-furan double bonds photoreacting with 5,6-double bonds of two pyrimidine bases[8~9].

It is well known that the molecular structure of furocoumarins are related to the photobiological actions of the compounds. Monofunctional compounds such as 4′,5′-dihydropsoralen(DHP) and 3,4-dihydropsoralen, and xanthyletin have no photosensitizing effects in contrast to psoralen and 8-methoxypsoralen. However, 5,7-dimethoxycoumarin (DMC) which has only one functional group, a pyrone double bond, has been shown to have photochemically lethal effects for *B. Substillis rec* and *her* strains[10].

The photoreaction of furocoumarins have been extensively investigated but chemical and physical evidences for cyclobutane adducts formed between furocoumarins and DNA are not sufficient. DMC photoreacts with pyrimidine bases giving C_4-cycloadducts,[11] although the quantum yield is quite low. No sufficient physical and chemical data of photoadducts are available for studying regioselectivity or geometry of the photoadducts even though the reaction mechanism and the structure of furocoumarins-thymine photoadducts are essential in understanding the molecular mechanism of skin sensitization and UV carcinogenesis by furocoumarins. For this reason, we determined the photophysical properties of DHP and undertook the study of the photocycloaddition of DHP to TME as a model for photocycloaddition reaction of 4′,5′-dihydropsoralen (DHP) with thymine to elucidate the mechanism of

DHP-thymine C_4-photocycloaddition reaction.

EXPERIMENTAL

Materials. 4′,5′-Dihydropsoralen(DHP) was synthesized according to the method reported by E. C. Horning and D. B. Reisener and recrystallized from methanol[12,13]. TME and thymine were obtained from Aldrich and Sigma Chemicals, respectively. Kiesegel G was used for silicagel thin layer chromatography. 2,5-Diphenyloxazole and benzil were obtained from Aldrich Chemicals and Fisher Co., respectively. Other common solvents were obtained from Wako Chemical Company.

Analytical Measurements. Fluorescence spectra were recorded on an Aminco-Bowman spectrofluorometer with XY-recorder. Infrared spectra were measured on a Perkin-Elmer Model 267 spectrophotometer using potassium bromide pellets. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer. NMR spectra were taken on a Varian T-60A spectrometer in chloroform-d and pyridine-d_5 against tetramethylsilane internal standard. Mass spectra were determined with a Hewlett Packard 5985 A GC/MS system. Gas chromatogram were recorded on a Varian Aerograph 2800 gas chromatograph.

Determination of Fluorescence Quantum Yield. Fluorescence quantum yield(Φ_f^s) of dilute solution of DHP was calculated by eq. 1

$$\Phi_f^s = \Phi_f^r \times \frac{\int I_f^s(\bar\nu)d\bar\nu}{\int I_f^r(\bar\nu)d\bar\nu} \frac{0 \cdot D^r}{0 \cdot D^s} \frac{n_s^2}{n_r^2} \quad (1)$$

where Φ_f^r is the fluorescence quantum yield of the reference compound, $I_f(\bar\nu)$ and n are correlated fluorescence intensity and refractive index, respectively ($\bar\nu$ in cm^{-1}). The reference compound, 2,5-diphenyloxazole, was dissolved in benzene[14]. Absolute quantum yield of the

reference 2,5-diphenyloxazole in benzene is known to be 0.58. All sample compounds were dissolved in ether and the fluorescence spectra were recorded on a Perkin-Elmer spectrophotometer Model MPF-3.

Determination of Phosphorescence Quantum Yield. Phosphorescence quantum yield (Φ_p') of DHP in ether was determined in the same way as shown above. The phosphorescence spectra were recorded in EPA glass matrix at 77 K with liquid nitrogen in Dewar flask on an Aminco-Bowman spectrophotofluorometer attached with phosphoroscope in the sample compartment. The phosphorescence quantum yield of reference compound, benzil, was taken to be 0.67 in EPA solvent[15].

Measurement of Fluorescence Lifetime. The fluorescence lifetime was measured at room temperature with SLM-Model 480 phase modulation spectrophotofluorometer utilizing continuous light that had been sinusoidally modulated.

Measurement of Phosphorescence Lifetime. The phosphorescence lifetime of DHP was measured at 77 K with Aminco-Bowman spectrophotofluorometer with a shutter installed in exactly the same way as the phosphoroscope. The phosphorescence decay curve was obtained on an oscilloscope which was triggered by relesasing microswitch. The decay curve was analyzed to get phosphorescence lifetime from eq. 2.

$$I = I_0 \exp(-t/\tau_p) \qquad (2)$$

I_0: initial phosphorescence intensity, I: phosphorescence intensity at time t after releasing microswitch, τ_p: phosphorescence lifetime

Irradiation Apparatus. Irradiations were carried out in a Rayonet Photochemical Reactor (The Southern New England UV Company) Model RPR 208 or RPR-100 equipped with 350 nm fluorescent lamps.

Two modules of model RPR-100 were arranged in a horizontal position, allowing the photolysis of frozen solution state. A Rayonet reactor Model RPR-100 equipped with MGR-100 Merry-Go-Round was used for comparative studies.

Irradiation of DHP in the Presence of TME at 350 nm

In Acetonitrile Solution. Photolysis of DHP (1 mM) in the presence of 5 mM TME in acetonitrile was carried out at room temperature for 39 h in a RPR-100 Rayonet reactor after evacuating oxygen with nitrogen gas.

In Frozen Solution State. The solution of DHP and TME in cyclohexane-dichloromethane (10:1 v/v) was poured into a petri dish and frozen in refrigerator. The thickness was less than 7 mm. The perti dishes containing the frozen solution were placed at 15 cm distance from the RUL-3500 Å lamps arranged in a horizontal position and irradiated for 15 h. The temperature of the reaction chamber was maintained below 0 °C during the photolysis in order to keep the frozen solution from melting.

Analysis of Photoproducts. The photoadducts were mointored by silica gel thin layer chromatography utilizing cyclohexane-actone (45:55 v/v) as an eluent. The DHP-TME major photoadduct was isolated with preparative thin layer chromatography. The isolated major photoproduct was recrystallized from methanol.

Fluorescence Quenching Study of DHP with Olefins

The fluorescence spectra of DHP (1.0×10^{-6} M) containing 1.34×10^{-1}, 5.36×10^{-1}, 8.04×10^{-1}, and 1.07 M TME, 3.12×10^{-2}, 6.24×10^{-2}, 1.25×10^{-1}, and 2.50×10^{-1} M fumaronitrile were measured in chloroform at room temperature with excitation at 350 nm. Absorbance

vs. relative intensity of fluorescence was plotted.

The Measurement of photocycloaddition Quantum Yields. The solution of DHP ($5 \times 10^{-3} M$) and TME ($2.5 \times 10^{-2} M$) in chloroform was prepared, The same solution with benzophenone sensitizer was also prepared. Thus prepared solutions (3m*l*) were poured into the ampoule and were degassed by three freeze-pump-thaw cycle on a vacuum line using liquid nitrogen as a coolant, and were sealed. All sealed samples were irradiated with 366 nm UV light. A Hanovia 450 W medium pressure mercury arc lamp was used as a light source and filters were utilized to get monochromatic light. Light intensity was measured using potassium ferrioxalate actinometry reported by Hatchard and Parker. The major product was analyzed quantitatively with Varian Aerograph 2800 gas chromatograph. OVE 101 column was used and the temperatures of detector, injector, and column were 275 °C, 265 °C, and 210 °C, respectively.

Photosplitting of the Adduct. The purified photoproduct was dissolved in methanol and irradiated with 254 nm UV light in a Rayonet photoreactor after degassing. After irradiation, photoproducts were analyzed with silica gel thin layer chromatography and visualized with mineral lamp.

RESULTS AND DISCUSSION

The λ_{max} of the fluorescence of DHP in methanol appears at 402 nm which indicates that the lowest singlet (π, π^*) state is higher than that of psoralen ($\lambda_{max}^{fl} = 410$nm). The other physical properties which were determined in ether solution are shown in *Table* 1.

If we compare DHP with other coumarins such as DMC and psoralen, fluorescence quantum yield of DHP is higher than that of psoralen ($\Phi_f = 0.02$) but lower than that of DMC ($\Phi_f = 0.65$). The ratio of Φ_p to Φ_f of DHP is higher than that of DMC and lower than that of psoralen ($\Phi_p/\Phi_f = 10$). Thus intersystem crossing quantum yield ($\Phi_{isc} = 0.068$) is lower than that of psorlen[16]. These results indicate that DHP has the photophysical properties which lie between those of psoralen and DMC.

Characterization of DHP-TME Products. The photoadducts of DHP-TME were analysed by thin layer chromatography. The photoreactivity is higher in frozen state than that of the solution state. One major and several side products were detected in trace amounts. The major product was isolated by preparative silica gel thin layer chromatography and recrystallized from methanol. When 1 mM DHP and 10 mM TME were irradiated in the frozen state, about 50 mg of the major product was obtained after recrystallization. It has a melting point of 129~130 °C and the elemental analysis data are consistent with the molecular formula of an 1:1 adduct of DHP-TME, $C_{17}H_{20}O_3$, as shown below

	C	H
Calcd.	75.00	7.35
exp.	75.35	7.10

The mass spectra of the photoadduct were

Table 1. Photophysical properties of DHP.

Solvent	$\overset{*}{\Phi_{fl}}$	Φ_p^+ at 77K	Fluorescence lifetime $\tau_{f(s)}^{*+}$				Phosphorescence lifetime $\tau_p(s)$
			τ_M	τ_θ	τ_M	τ_θ(nsec)	
ether	0.08±0.02	0.013±0.001	1.05±0.2	0.87±0.2	1.00±0.2	0.87+0.2	0.03±0.001

*λ_{ex}=310nm; +λ_{ex}=325nm; **τ_M=by modulation; τ_θ=by phase shift.

determined by electron impact (EI) and chemical ionization (CI) method. A molecular ion peak corresponding to an 1:1 DHP-TME adduct was observed at m/e 272 from both CI and EI method. The peaks of M+1, DHP, and TME were observed at m/e 273, 188, and 84, respectively. This suggests that the photoproduct is a C_4-photocycloaddition product of DHP and TME formed through 2+2 addition of the 3,4-double bond of DHP to double bond of TME.

The UV absorption spectra of the photoproduct show the loss of 3,4-double bond of DHP. The λ_{max} blue shifted from 332nm to 292 nm in methanol indicating the loss of conjugation.

IR spectra show the strong carbonyl stretching band of DHP at 1702cm^{-1} shifted to 1739 cm^{-1} in the photoproduct. This along with the blue shift of λ_{max} in UV spectra strongly supports the hypothesis that the 3,4-double bond is lost by the photoreaction. Doublet peak caused by geminal methyl group and a characteristic cyclobutane ring deformation band which is not observed in DHP and TME, were observed at 1370 cm^{-1} and 841 cm^{-1}, respectively[17].

In proton nuclear magnetic resonance spectra of the photoproduct taken in chloroform-d, pyrone double bond vinyl proton peaks which were observed at $\delta6.1$ and $\delta7.5$ in DHP, were not observed as shown in *Fig.* 1. This also indicates the loss of the 3,4-double bond of DHP through 2+2 cycloaddition with TME on photolysis. New signals at $\delta3.35$ $(d, 1H)$ and $\delta3.15$ $(d, 1H)$ having coupling constant of 9Hz, typical for syn cyclobutane protons, were observed. Four methyl protons were observed at $\delta1.32$, $\delta1.25$, $\delta1.07$, and $\delta0.78$, respectively.
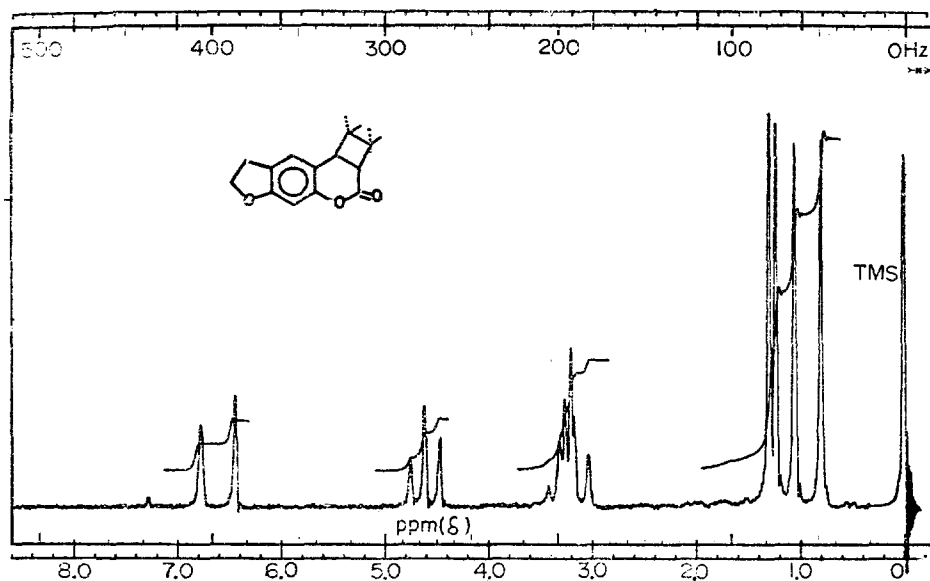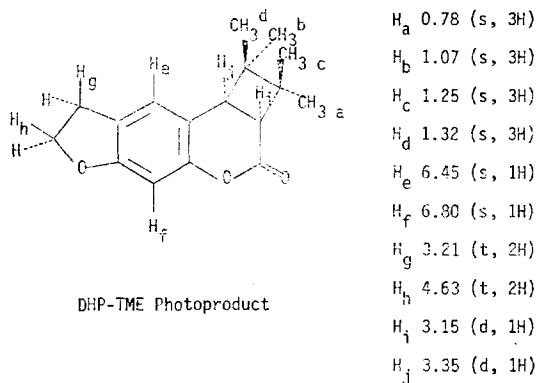
H_a	0.78 (s, 3H)
H_b	1.07 (s, 3H)
H_c	1.25 (s, 3H)
H_d	1.32 (s, 3H)
H_e	6.45 (s, 1H)
H_f	6.80 (s, 1H)
H_g	3.21 (t, 2H)
H_h	4.63 (t, 2H)
H_i	3.15 (d, 1H)
H_j	3.35 (d, 1H)

DHP-TME Photoproduct

Fig. 1. NMR spectrum of the DHP-TME photoadduct.

H_c and H_d were observed at the lower field than H_a and H_b due to the deshielding by aromatic ring currents. H_j is generally observed at the lower field than H_i as benzyl proton is observed at the lower field than the DMC. All the spectral data indicate the product to be an 1:1 DHP TME C_4-cycloadduct as shown below.
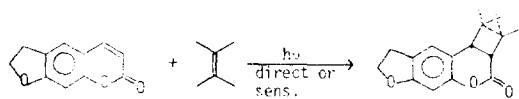
Photosplitting of the DHP–TME Adduct. The photoproduct of DHP with TME is very sensitive to 254 nm light as expected. When the methanol solution of the photoproduct is irradiated with 254 nm light for 1∼2 min, the photosplitting products are detected as DHP and TME on TLC visualized with mineral lamp. UV spectra also show that one of the products is DHP indicating the product to be an 1:1 DHP–TME C_4-cycloadduct.

Flourescence Quenching Study of DHP with TME, Ethylfumarate, and Fumaronitrile. The fluorescence of DHP was quenched very efficiently by TME, ethylfumarate, and fumaronitrile. The fluorescence quenching constants, k_q's, are obtained from the Stern–Volmer

Fig 2. A Stern–Volmer plot for the DHP fluorescence quenching by TME.

plot (*Fig.* 2) and they are 1.52×10^9, 2.80×10^9 and 7.41×10^{-9} $M^{-1}s^{-1}$ for TME, ethylfumarate, and fumaronitrile, respectively. The quenching constants are a little smaller than the diffusion controlled rate (k_{diff}) of 1.1×10^{10} $M^{-1}s^{-1}$ but k_q of fumaronitrile is almost same as k_{diff}.

Electron deficient olefins quench the fluorescence of DHP more effectively than the electron rich olefins. These results suggest that the first singlet excited state of DHP forms an exciplex with ground state olefins as other furocoumarins and DHP–TME photoadduct seems to be formed through this kind of exciplex.

Studies on the Quantum Yields. Quantum yield of photocycloaddition reaction between DHP and TME was measured in chloroform both with and without a sensitizer (benzophenone). The quantum yield were 0.056 without a sensitizer and 0.186 with a sensitizer. This indicates that DHP which has $\Phi_{isc} = 0.068$ undergoes 2+2 photocycloaddition with TME from both excited singlet and triplet state. But the triplet reactivity is higher than that of singlet state as shown by the higher quantum yield when sensitized by benzophenone.

This result is in contrast to that of DMC which has high singlet reactivity,[18] but is similar to that of coumarin and other substituted DHP[19,20].

CONCLUSION

The fluorescence maximum of DHP appears at the long–wavelength UV which is similar to that of 4′,5′-monoadducts of psoralen to thymine reported by Musajo, *et al.*, suggesting that only 4′,5′-monoadduct of psoralen to DNA can cross–link DNA by 2+2 photocycloaddition to another pyrimidine base of DNA on further irradiation of the monoadduct with long–wavelength UV light. The fluorescence of DHP
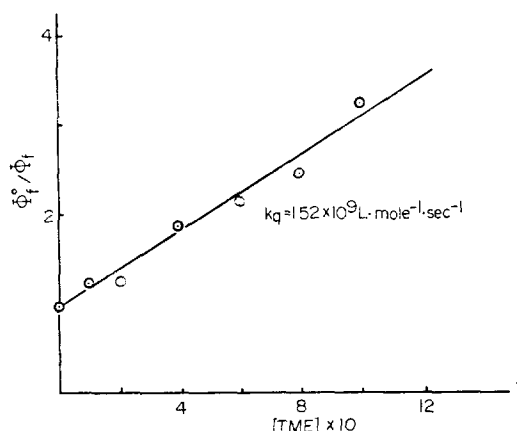
was quenched efficiently by olefins such as TME, ethylfumarate, and fumaronitrile with the quenching constants of 1.52×10^9, 2.84×10^9, 7.41×10^9 $M^{-1}s^{-1}$, respectively. The electron defient olefins quench the fluorescence of DHP more efficiently than the electron rich olefins.

The cycloaddition reaction of DHP with TME occurs through both triplet and singlet excited states giving C_4-cycloadduct as a major product. The reaction mechanism of DHP photocycloaddition to TME is very similar to that of other furocoumarins. DHP has no photosensitizing effects in spite of this similarity in reaction mechanism with other furocoumarins. The results suggest that molecular structure is also very important in photosensitizing activity of furocoumarins, much more so than the reaction mechanism.

ACKNOWLEDGEMENTS

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