Isolation and Physical Properties of Photochemical 8-Methoxypsoralen-thymidine 4′, 5′-C_4-Monoadducts

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The 8-methoxypsoralen $\langle^{4',5}_{5',6}\rangle$thymidine monoadducts are isolated from the irradiation mixture of 8-methoxypsoralen and thymidine in a dry film state by a flash column followed by lobar column chromatography. Some physical propeties of the adducts were determined. The fluorescence maximum and quantum yield of the monoadduct are dependent on the solvent polarity and the phosphorescence to fluorescence quantum yield ratio was 2.10 which was significantly increased by external heavy atoms. The phosphorescence lifetime was 1.2s which is relatively large compared to other coumarin derivatives. Accurate spectral data of the monoadducts are presented.

Introduction

8-Methoxypsoralen (8-MOP), which has been the most used furocoumarin in the clinical treatment of psoriasis, is a well known photosensitizing agent. However, possible deleterious effects such as carcinoma development in hairless mice as well as possible liver injury from the use of 8-MOP have been reported[1]. The photosensitivity of furocoumarins has been generally correlated with their photoreactivity toward pyrimidine bases of DNA [2-5]. One psoralen molecule can successively photoreact with two pyrimidine bases causing a cross-linkage between two separate strands of DNA. The crosslinks are caused by the formation of cyclobutane adducts which arise from [2+2] cycloaddition of the psoralen 3,4- and 4′,5′-double bonds with 5,6-double bonds of two pyrimidine bases. The 4′,5′-monoadducts absorb light up to 380 nm whereas the 3,4-monoadducts do not absorb light above 320 nm. It is thus obvious that after the first quantum of near UV-light has been absorbed by the psoralen itself to form a monoadduct, the second quantum has to be absorbed by a 4′,5′-monoadduct, rather than a 3,4-monoadduct, to form a crosslink. It is, therefore, very important to understand the physical and chemical properties of 4′,5′-monoadducts for the elucidation of photobiological activities of furocoumarins.

As an important model for these monoadducts of psoralens and DNA, the major photoadducts of DMC*-thymine[6] and DMC-thymidine[7] have been previously isolated and characterized. In this work, the preparative scale isolation of the major 4′,5′-monoadduct of 8-MOP and thymidine and its physical properties are reported.

Materials and Methods

Materials. 8-MOP and thymidine were purchased from Sigma and used without further purification. Deionized water, acetonitrile (Merck, LC grade) and tetrahydrofuran (Merck, LC grade) were used for high performance liquid chromatograhpy and low pressure *semi*-preparative column chromatography. Extra pure methanol (Wako Chemical Co.) and common solvents were used without further purification. Kiesel Gel GF_{254} (Merck) and Kiesel Gel G (Merck, all 230 mesh) were used for thin layer and flash column chromatography, respectively. D_2O (100.0 %, Aldrich) and ethanol (Analytical Grade, 99.5%) were used for ^1H NMR and total emission spectra, respectively.

Spectroscopic Measurements. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer. Infrared spectra were recorded on potassium bromide pellets on a Perkin-Elmer 283 B spectrophotometer. Proton nuclear magnetic resonance and circular dichroism spectra were recorded on a Varian FT-80A spectrometer and a JASCO J-20 spectrophotopolarimeter, respectively. Fluorescence and phosphorescence spectra were recorded on an Aminco-Bowman spectrophotofluorometer with an Aminco-XY recorder. The phosphorescence lifetime was measured with this instrument in conjunction with a Tektronix 5115 storage oscilloscope.

HPLC and Lobar Column Chromatography. High performance liquid chromatography was performed on a Waters Associates Model 244 liquid chromatograph equipped with Model 600A solvent delivery system, Model 440 UV absorbance detector, Model U6K universal injector and Model 660 solvent programmer.

In the analysis of photoproducts, primarily analytical liquid chromatography was utilized under the following conditions: column; Radial-pak cartridge 5μ-C_{18} (8.0mm ID $\times$10cm), solvent; CH_3OH/H_2O gradient, flow rate; 2.0ml/min., detector; UV (254nm).

To isolate 8-MOP$\langle^{4',5}_{5',6}\rangle$thymidine monoadducts, liquid chromatography was carried out under the following conditions: for flash column chromatography[8], solvent; ethanol/ether/n-hexane=2/1/1 (v/v), flow rate; about 1.5cm/min., for low pressure preparative liquid chromatography, column; Lobar LiChroprep RP-8 (2.5cm ID$\times$30cm), solvent; $H_2O/CH_3CN/THF$=20/2/1 (v/v), flow rate; 5.0 ml/min.

*DMC: 5,7-Dimethoxycoumarin

(with a Model 600A solvent delivery system), detector; UV (254nm).

Irradiation Procedure. Two modules of a Rayonet photochemical reactor (The Southern New England Ultraviolet Co.) Model RPR-100 were stacked together and arranged in a horizontal position allowing the photolysis of solid samples. 8-MOP (216mg) and 2.42g of thymidine (molar ratio 1:10) were dissolved in 500m*l* of hot methanol. The resulting solution was poured into Petri dishes and heated and concentrated quickly to dryness to form dry films. The transparent substances in the Petri dishes were placed at 15cm distance from RUL-350nm lamps, arranged in a horizontal position. After irradiation for 20 minutes, the dry substances were dissolved in methanol and the solvent was evaporated off to form a dry film again and irradiated. This procedure was repeated several times to get a better yield of the photoadducts.

Analysis and Isolation of 8-MOP $\langle^{4',5}_{5',6}\rangle$ *thymidine Monoadducts.* After irradiation the reaction mixtures were analyzed by TLC on silica gel utilizing a ethylacetate: ethanol (5:1, v/v) as a developing solvent and visualized by mineral light (Model VUS-11:254nm short wave UV and 350nm long wave UV). Analytical liquid chromatography was used to analyze the photoreaction mixures. After irradiation of the mixture of 8-MOP and thymidine in the dry film state, the reaction mixtures were dissolved in methanol and concentrated to nearly dryness. The excess unreacted thymidine was removed by extracting the mixture with chloroform at reflux for about an hour. The 4',5'-monoadducts were isloated by flash column chromatography followed by low pressure preparative liquid chromatography.

Measurement of Photophysical Properties. The concentration employed in measuring luminescence spectra was always less than 10^{-4} *M*. Fluorescence excitation (λ_{em}=450 nm) and emission (λ_{ex}=330 nm) spectra of 4',5'-monoadducts at room temperature were measured using methanol solution of the monoadducts. Phosphorescence (λ_{ex}=330 nm) and total emission spectra (λ_{ex}=320 nm) were measured using H_2O:CH_3OH(7:3, v/v) and ethanol solutions of the 4',5'-monoadduct at 77K, respectively. Ethanol-ethyl iodide (2:1, v/v) solution of the monoadducts was used to estimate the external heavy atom effect on their excited states. The phosphorescence lifetime was determined using a mechanical shutter to cut the excitation light. Polarized excitation and emission spectra were obtained with a Glan-Prism polarizer and the polarized spectra were corrected by the Azumi-McGlynn formulation [9]. The spectra were corrected for the response characteristics of the photomultiplier tube (IP21, S-4 spectral response) and monochromator of this instrument as a function of wavelength. Corrected spectra subsequently permitted the determination of the phosphorescence to fluorescence quantum yields ratio and fluorescence quantum yields relative to that of 5,7-dimethoxycoumarin. The ratio of the phosphorescence to fluorescence quantum yields (Φ_p/Φ_f) was estimated by the following correlation: Φ_p/Φ_f= (area of phosphorescence)/(area of fluorescence). The Aminco-Bowman instrument was also employed in measurement of fluorescence quantum yields relative to DMC (Φ_F (298°K)=0.65) by following the relationship, $\Phi_F=\Phi_F^R\times(I_S/I_R)\times(A_R/A_S)$ where Φ_F^R represents fluorescence quantum yield of reference and I_S, I_R and A_S, A_R are fluorescence intensities and absorbances of sample and reference, respectively. In quantum yield determinations, absorbance was kept as low as possible, usually below 0.3, in order to minimize errors due to the front surface imprisonment and inner-filter effects.

Results and Discussion

Isolation and Characterization of 8-MOP $\langle^{4',5}_{5',6}\rangle$ *thymidine Monoadducts.* The photoreaction mixture showed a number of spots on TLC visualized by means of mineral light. Among the cross-addition products of 8-MOP and thymidine, the spot of R_f 0.37 (F-2) is strongly fluorescent at 350nm light. F-2 containing small amount of thymidine was isolated by a flash silica gel column chromatography. The excess thymidine of this fraction was efficiently removed by a lobar Li-Chroprep RP-8 column chromatograph and only F-2 fraction was recycled several times collecting only the outer parts of F-2-A and F-2-B peaks (Figure 1). Each fraction of F-2-A and F-2-B was lyophillized to remove water. In a previous paper, the isolation and characterization of 8-MOP $\langle^{4',5}_{5',6}\rangle$ thymidine monoadducts were reported but they were all diastereomeric mixtures [10]. In this work, about 100 mg of one pair of enantiomer F-2-A and F-2-B were isolated in pure form by the procedure described. The UV absorption, fluorescence and phosphorescence spectra of F-2-A and F-2-B are coincident and similar to those of 4',5'-dihydropsoralen. The infrared spectra of F-2-A and F-2-B show a strong carbonyl stretching band at 1710 cm^{-1} caused by the unsaturated lactone of 8-MOP and amide carbonyl of thymidine moiety. A characteristic cyclobutane ring vibration band

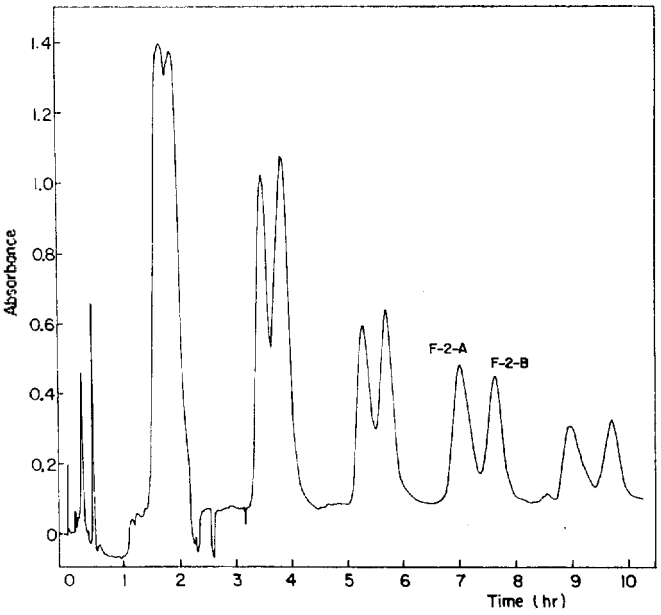


Figure 1. Recycled preparative liquid chromatogram of 4',5'-monoadducts.

TABLE 1: Chemical Shift (δ) and Coupling Constants of F-2-A and F-2-B

Moiety*	F-2-A	F-2-B
C-4 H (8-MOP)	7.96 *d*	7.94 *d*
$J_{3,4}$ (Hz)	9.5	9.5
C-5 H (8-MOP)	7.20 *s*	7.17 *s*
C-3 H (8-MOP)	6.42 *d*	6.36 *d*
C-1'H (dRib)	6.29 *t*	6.31 *t*
C-5'H (8-MOP)	5.64 *dd*	5.70 *dd*
$J_{5',4'}$ (Hz)	5.5	5.5
$H_{5',6}$ (Hz)	5.8	5.6
C-6 H (Thy)	4.55 *m*	4.55 *m*
C-3'H (dRib)	4.50 *m*	4.50 *m*
C-4'H (8-MOP)	4.26 *d*	4.25 *d*
C-8 OCH_3 (8-MOP)	4.00 *s*	4.05 *s*
C-4'H (dRib)	3.85 *m*	3.90 *m*
C-5'H (dRib)	3.70 *m*	3.85 *m*
C-2', 2''H (dRib)	2.40 *m*	2.35 *m*
C-5 CH_3 (Thy)	1.80 *s*	1.78 *s*

*dRib=2'-deoxyribose; Thy=Thymine.

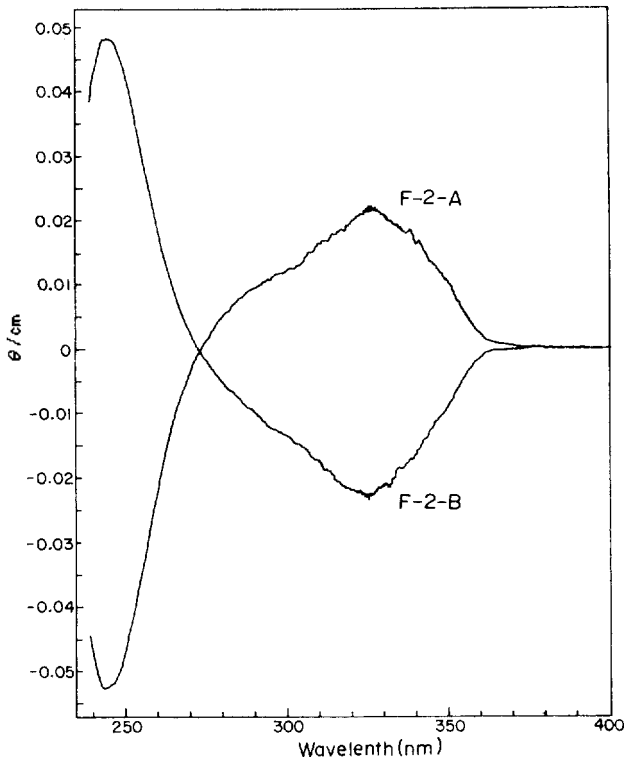


Figure 2. Circular dichroism spectra of F-2-A and F-2-B in $H_2O/CH_3CN/THF$ (20/2/1, v/v).

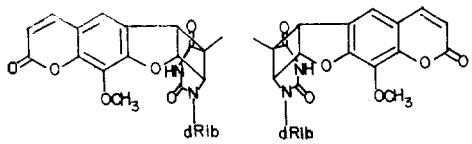
at 875 cm^{-1} is apparent and the remainder of the spectrum is consistent with a 1:1 cycloadduct of 8-MOP and thymidine through 4',5'-double bond of 8-MOP and 5,6-double bond of thymidine. The data also indicate that F-2-A and F-2-B are stereoisomers. The ^1H NMR spectra of F-2-A and F-2-B were taken in D_2O and they show very small differences of chemical shifts. The chemical shifts and coupling constants of F-2-A and F-2-B were consistent with a cyclobutane structure derived from a [2+2] cycloaddition of a thymidine residue to the 4',5'-double bond of 8-MOP. The 5'-H of

TABLE 2: Solvent and Temperature Dependence of Fluorescence Quantum Yield and Phosphorescence to Fluorescence Quantum Yield Ratio

	λ_{max}^{fl} (nm)	Φ_F^*	Φ_P/Φ_F
H_2O	465	0.044	
CH_3OH	446	0.007	
C_2H_5OH	435	0.005, 0.012[a]	2.10[d], 1.83[e]
		0.017[b], 0.018[c]	1.51[f]
i-PrOH	425	0.003	
$(C_2H_5)_2O$	410	0.001	
EtOH-Et_2O (2:5 v/v)			0.81[d]

[a] at 20°C, [b] at 14°C, [c] at 10°C, [d] λ_{ex}=320 nm, [e] λ_{ex}=290 nm. [f] λ_{ex}=260 nm, *at 25°C, λ_{ex}=320 nm.

8-MOP moiety shows an AB quartet because of the coupling with 4'-H of 8-MOP ($J_{4',5'}$=5.5 Hz) and 6-H of thymidine ($J_{5',6}$=5.6−5.8 Hz). This pattern is consistent with *cis-syn* orientation of the cyclobutane system. The saturation of the 4',5'-double bond caused a small upfield shift of 5-H, 3-H, 4-H, and 8-OCH_3 protons of 8-MOP. All the spectral data are summarized in Table 1. Circluar dichroism spectra of F-2-A and F-2-B clearly show that they are in an enantiomeric relationship to each other if sugars are removed from F-2-A and F-2-B (Figure. 2). The following structural relationship of F-2-A and F-2-B is derived from the data obtained.



Photophysical Properties. After the monoadducts are formed from the singlet excited state rather than the triplet state of 8-MOP, absorption of a second photon may lead to the formation of crosslinks [11]. In this second photoreaction, the relative importance of excited singlet and triplet states of 8-MOP has yet to be completely resolved. Some of the photophysical properties of F-2, therefore, were investigated.

The fluorescence of F-2 was not quenched by solutions of 6m*M*, 12m*M*, 18m*M*, 30m*M* and 64m*M* thymidine. The fluorescence maxima of F-2 at room temperature were greatly red-shifted as the polarity of solvents was increased and the fluorescence quantum yields increase along the polarity of solvents as shown in Table 2. The absorption and excitation spectra at room temperature, however, remain essentially unchanged as the solvent is changed from water to ethyl ether. Absorption and fluorescence spectra at room temperature are shown in Figure 3. Furthermore, the phosphorescence of F-2 in solid solutions was not quenched by thymidine concentrations of up to 0.24*M*. The total emission spectra of F-2 and their polarization results with respect to λ_{ex}=320 nm in ethanol at 77K are shown in Figure 4.

On the basis of the positive values of polarization, the fluorescence state is assigned to the $^1(\pi, \pi^*)$ state. The highly positive value of polarization is internally consistent

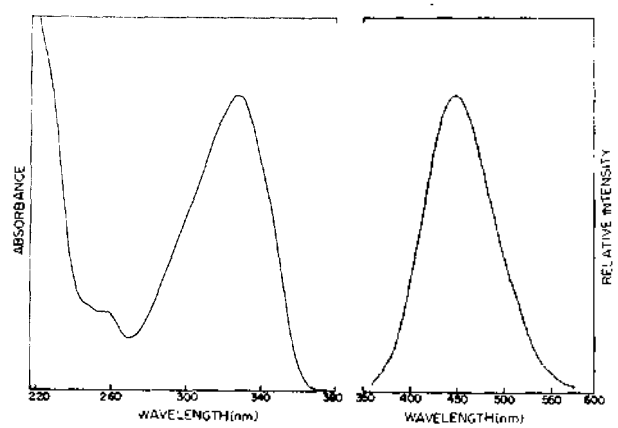


Figure 3. Absorption (left) and fluorescence (right) spectra of 4′,5′–monoadduct in methanol at room temperature.

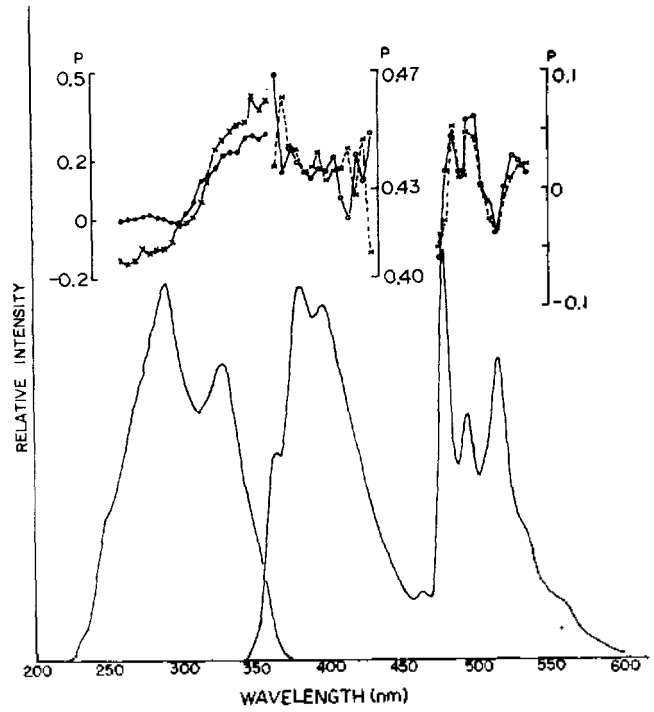
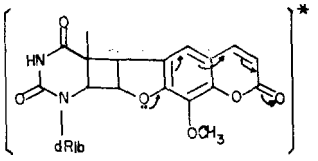


Figure 4. Fluorescence excitation (left), emission (middle), phosphorescence (right), and polarized fluorescence excitation (● : in ethanol, x: in ethanol–ethyl ether, λ_{em}=450 nm), polarized fluorescence and phosphorescence (F–2–A; o, F–2–B; x, λ_{ex}= 320 nm) spectra of 4′,5′–monoadducts in ethanol at 77°K.

with the polarized fluorescence excitation spectrum, particularly the highly positive value at 320 nm. In fluorescence excitation spectrum, two bands at 290 nm and 327 nm have negative and positive polarization with respect to λ_{em}=450 nm, respectively. In absorption spectra, the former is buried under the latter band.

The ratio of the phosphorescence to fluorescence quantum yields is 2.10 in ethanol which is smaller than that of 8–MOP itself (13.05) but greater than that of DMC (<0.05) [12]. The ratio for the monoadduct is greatly increased to 11.90 by external heavy atom solvents. This suggests that the two states which intersystem–cross between excited singlet and triplet states have the same configuration. Its phosphorescence lifetime (τ_p=1.2s), however, is greater than that of DMC (0.85s) [10] and rather similar to that of psoralens having the $-OCH_3$ or –OH groups at 5– or 7–position. These 5,7–OH or $-OCH_3$ psoralens have much longer phosphorescence lifetime than their parent compounds. Based on this longer phosphorescence lifetime and negative value (–0.06) of polarization of the O–O band of phosphorescence as shown in Figure 3, the lowest excited triplet state is assigned to the $^3(\pi, \pi^*)$ state.

Since the furan double bond is saturated in the 4′,5′–monoadduct, the following charge–transfer resonance form may contribute to the delocalization of the $^3(\pi, \pi^*)$ state as in the case of 5–MOP. The big contribution of this resonance form to the $^3(\pi, \pi^*)$ state is accompanied by the large red shift of the O–O phosphorescence band and a long phosphorescence lifetime.



The fluorescence quantum yields of the monoadduct are very small as shown in Table 2. These low quantum yields are ascribed to the effective vibronic coupling between the lowest excited singlet (π, π^*) and (n, π^*) states. It is generally observed that $^1(n, \pi^*)$ states are not usually fluorescent, and the $^1(n, \pi^*)$ state is ruled out as the lowest singlet and fluorescent state. However, the lowest $^1(\pi, \pi^*)$ and $^1(n, \pi^*)$ states of the 4′,5′–monoadduct may be very close in energy and vibronic states of the two electronic states may be very much overlapped. Theoretically, the extended HMO predicts the lowest $n \rightarrow \pi^*$ transition to be higher in energy by 0.126eV (31nm) than the lowest $\pi \rightarrow \pi^*$ in coumarin [13]. The energy difference between these states may be smaller in the 4′,5′–monoadduct than coumarin itself because of the thymidine moiety in the adduct. It is thus possible that inversion of configuration between the two excited singlet states occurs as the polarity of solvents changes. As shown in Table 2, the fluorescence maximum of the monoadduct is red–shifted by 19 nm when the solvent is changed from water to methanol. Its quantum yield decreases substantially from 0.044 to 0.007 but, as the solvent is changed from methanol to ethyl ether, the fluorescence quantum yield decreases slowly. The rations of phosphorescence to fluorescence quantum yield also decrease greatly as solvents are changed from ethanol to ethanol: ethyl ether (2:5, v/v).

This observation suggests that the lowest excited singlet state is (π, π^*) and (n, π^*) states in water and methanol, respectively and the triplet state to which the singlet state intersystem–crosses is the (n, π^*) state. Although the lowest singlet state in methanol is the (n, π^*) state, fluorescence emission is observed. This is because the lowest singlet $^1(n, \pi^*)$ and upper $^1(\pi, \pi^*)$ states are very similar in energy and these two states are intermixed very much, and the upper excited singlet (π, π^*) state fluoresces. The overlap of the two states decreases gradually when the solvent is changed from methanol to ethyl ether. Thus, the lowest singlet state

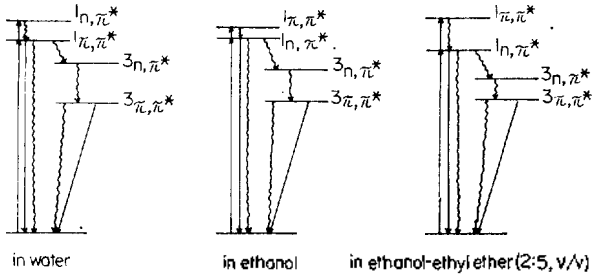


Figure 5.

becomes more (n, π^*) statelike and the fluorescent quantum yield decreases. As shown in Table 2, the fluorescence quantumyields are affected significantly by temperature because of the proximity of $^1(n, \pi^*)$ and $^1(\pi, \pi^*)$ states. Figure 5 illustrates three possible Jablonski diagrams which can explain the solvent and temperature effect on the fluorescence and phosphorescence.

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A Short Synthesis of (Z)-13-Eicosen-10-one the Principal Component of the Peach Fruit Moth Pheromone

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(Z)-13-Eicosen-10-one (**1**), one of the active components of the female pheromone of the peach friut moth, *Carposina niponensis* Walsingham, was synthesized from 2-nonanone, succinic anhydride, and n-nonyllithium. The key step involves the preparation of 13-eicosyn-10-one from an epoxyketone via Eschenmoser cleavage.

Introduction

Peach fruit moth, *Carposina niponensis* Walsingham, is a major economic pest of apple, peach, and other fruits in Japan and in Korea,[1,2] Population monitoring of this species has depended entirely on field observations of injured fruits, since the moths are not captured light traps. Therefore the establishment of a convenient monitoring system for this species by using traps baited with a synthetic sex pheromone is desired.

In 1976, Tamaki and coworkers identified (Z)-13-eicosen-10-one (**1**) and (Z)-12-nonadecen-9-one as the major components of the female sex pheromone of peach fruit moth.[1] A number of different synthetic schemes for the preparation of **1** have since been published,[3] the most notable being the addition of (Z)-1-octenyl cuprate reagent to 1-dodecen-3-one.[3d]

We wish to report an alternative scheme for the synthesis of **1** which employs Eschenmoser cleavage[4] of an epoxyketone in the preparation of 13-eicosyn-10-one.