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The C₄ Photocycloadduct Formation of Khellin with Some Olefins

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The photocycloaddition reaction of khellin with several olefins has been investigated. The photocycloadducts are formed regioselectively on furanyl 4',5'-double bond of khellin probably due to the localization of excitation energy on the furanyl 4',5'-double bond in the reactive excited state. The photocycloaddition reaction proceeds through the excited triplet state and the same product was formed when khellin is irradiated with dimethylfumarate or dimethylmaleate indicating the formation of common intermediate. The quantum yields of photocycloadduct formation, fluorescence, and intersystem crossing are very sensitive to proton-donating ability of solvents.

Introduction

The furochromones, khellin and visnagin, two photobiologically active compounds isolated from *Ammi visnaga*¹, closely resemble psoralen in structure and khellin has been used to sensitize λ -phages by 360nm UV light². Recent observations^{3,4} indicate that oral administration of khellin and subsequent exposure to sunlight or long wavelength UV (UVA) light induces repigmentation in vitiligo. Compared with the usual psoralen photochemotherapy recommended for vitiligo, khellin and UVA treatment appears to be equally effective and has the major advantage that khellin produces neither substantial side effects nor phototoxic erythema reactions.

One psoralen molecule intercalated in DNA duplex absorbs photons to form [2+2]-cycloadducts, both monoadducts and biadducts which result in interstrand crosslinks of DNA duplex.^{5,6} The photobiological activities of psoralen have been correlated with this photoreactivity toward pyrimidine bases, especially thymine.^{7,8}

A [2+2]-photocycloadduct on furan ring of khellin has been isolated from the irradiated frozen solution of khellin and thymine and *cis-syn* stereochemistry of cyclobutane ring has been determined.⁹ However, the mechanism of the reaction and action of khellin in vitiligo treatment has not been clearly established.

In this study, photoreaction of khellin with various olefins was investigated to give some insight into the reactive ex-

cited state and site-selectivity in photoaddition reactions of khellin.

Experimental

Materials. Khellin was purchased from Sigma Chemical Company and recrystallized from methanol. 2,3-Dihydrokhellin and 4',5'-dihydrokhellin were prepared by hydrogenation in methanol using Pd-CaCO₃ catalyst and purified by a silica column chromatography. Dimethylfumarate (Aldrich Chemical Co.) was used after recrystallization from methanol. Dimethylmaleate and tetramethylethylene (Aldrich Chemical Co.) were purified by distillation under reduced pressure. Other olefins were used as received from Aldrich Chemical Company. Azulene and benzophenone (Aldrich Chemical Co.) were purified by recrystallization from ethanol.

Kiesel Gel GF254 (Merck) and Kiesel Gel G (Merck) were used for silica gel thin layer and column chromatography, respectively. Chromatographic and spectroscopic grade solvents (Merck) were used for HPLC, absorption, and emission spectra, respectively. Other solvents of extra pure grade were purified according to the literature procedures.¹⁰ Doubly distilled and deionized water was also used for HPLC analysis.

Spectroscopic Measurements. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer and IR spectra were obtained in potassium bromide pellets on a Perkin-Elmer 283B spectrophotometer. Proton NMR and mass spectra were recorded on a Bruker AM-200-SY spectrometer and a Hewlett Packard 5985A GC/MS system, respectively. Fluorescence spectra were recorded on an Aminco-Bowman Spectrophotometer and CD on a JASCO J-500C spectropolarimeter.

Fluorescence Quenching. In the presence of various concentration of olefins, fluorescence spectra of khellin were recorded with high concentration of khellin (about 10⁻³ M) and long wavelength exciting light (λ_{ex} = 370nm) to exclude absorption of light by olefins. The Stern-Volmer constant ($k_q \tau_1$) were obtained from the Stern-Volmer equation; $\phi^0/\phi = 1 + k_q \tau_1 [Q]$, where ϕ^0 and ϕ are fluorescence quantum yields in the absence and presence of quencher, k_q and τ_1 are quenching rate constant and lifetime of excited singlet state of khellin, respectively.

Measurement of [2 + 2]-Photocycloaddition Reaction

Quantum Yields. Samples of 3 ml solution in Pyrex ampoules were degassed by freeze-pump-thaw method with cooling in liquid nitrogen and then sealed. These samples were irradiated in a merry-go-round apparatus with a Hanovia 450 W medium pressure mercury arc lamp (Type 697 A36). To isolate the mercury emission line of 366 nm, Corning glass filters CS 0-52 and 7-37 were used. Light intensity was measured by ferrioxalate actinometry¹¹ and quantitative analysis was carried out by HPLC under following conditions: column, Radial-Pak cartridge C-18(8 mm O.D. × 10 cm); solvent, CH₃OH/H₂O (65/45, v/v); flow rate, 1.0 ml/min; detector, UV (254 nm).

Preparative Photoreaction of Khellin with Olefins.

Khellin and olefins (molar ratio, 1:5) were dissolved in dichloromethane and the solution was deaerated by bubbling with nitrogen gas for about 30 min and irradiated for 48 hr in

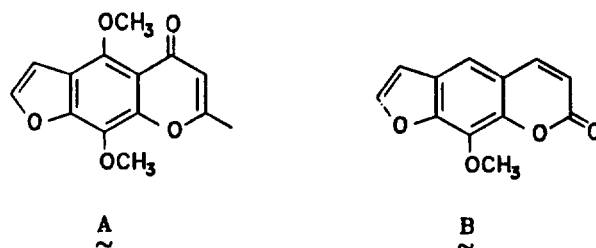


Figure 1. Molecular structures of khellin(A) and 8-methoxypsoralen(B).

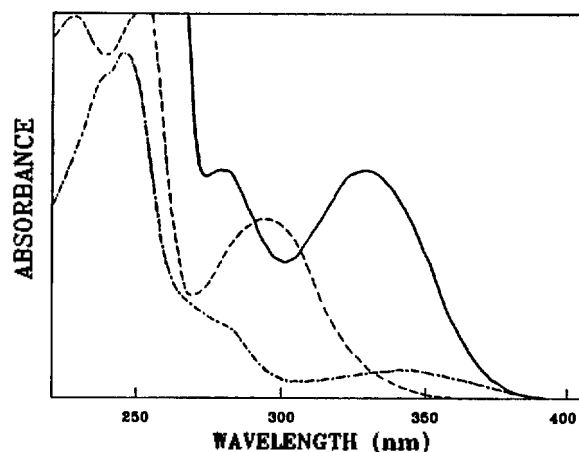


Figure 2. Absorption spectra of 2,3-dihydrokhellin (---), 4',5'-dihydrokhellin (···) and khellin(—) in methanol.

a Rayonet Photochemical Reactor (The Southern New England Ultraviolet Co.) Model RPR-208 equipped with four RUL-350 nm fluorescence lamps. The photoproducts formed were isolated by eluting with dichloromethane-ethyl ether (1:1, v/v) on a silica column.

Photosplitting of photoproducts. Dichloromethane solutions of khellin-dimethylfumarate (KDF) and khellin-dimethylmaleate (KDM) adducts were irradiated with 254 nm light and monitored the reaction by HPLC varying irradiation time. The photosplitting experiments of khellin-dimethylethylidene malonate (KEM) adducts were also carried out the same way in water-methanol solutions.

Results and Discussion

The preparative photoreaction of khellin and olefins was carried out in dichloromethane and the reaction mixture was analyzed by silica gel thin layer chromatography. A pale purple fluorescent spot at slightly lower R_f value than that of khellin on TLC (eluent; dichloromethane/ethyl ether = 4/1 in volume) indicated the photoproduct formation. The photoproducts KDF and KDM were formed quantitatively when dimethylfumarate or dimethylmaleate were irradiated with khellin for 24 hr. Dimethylethylidene malonate gave a small amounts of photoadduct (KEM) and most of the other olefins tested did not give any photoproduct. The photoproducts which are composed of two diastereomers, respectively were isolated by silica gel column chromatography and their diastereomers were separated by reverse-phase HPLC.

Absorption spectra of the photoproducts are very similar to each other and resemble that of 4',5'-dihydrokhellin in

Table 1. Chemical Shifts(ppm) and Coupling Constants(Hz) of [2 + 2]-Cycloadducts of Khellin with Dimethylfumurate and Dimethylethylidene malonate in CDCl₃

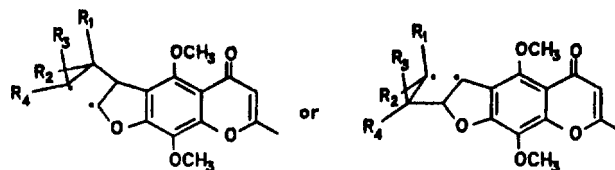
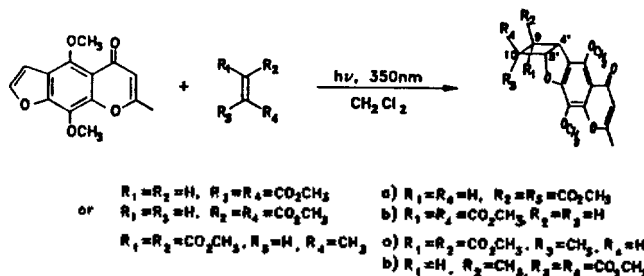
| | Chemical Shift(δ) | | | |
|--|-------------------|--------------|-------------|-------------|
| | KDF-a | KDF-b | KEM-a | KEM-b |
| C-2(CH ₃) | 2.34(s) | 2.34(s) | 2.34(s) | 2.34(s) |
| C-3(H) | 6.02(s) | 6.00(s) | 6.00(s) | 5.98(s) |
| C-5,C-8 (OCH ₃) | 3.79(s) | 3.68(s) | 3.86(s) | 3.83(s) |
| C-4'(H) | 4.39(m) | 4.50(m) | 4.79(dd) | 4.94(m) |
| J _{4',5'} | 7.0Hz | 6.8Hz | 6.7Hz | |
| J _{4',9} | 6.0Hz | 7.2Hz | 1.8Hz | |
| C-5'(H) | 5.57(t) | 5.45(dd) | 5.35(t) | 4.94(m) |
| J _{5',10} | 7.0Hz | 4.0Hz | 6.7Hz | |
| C-9(H) | 3.73(t) | 3.81-3.86(m) | | 3.38-3.5(m) |
| J _{9,10} | 6.0Hz | 4.0Hz | | |
| C-9 (CO ₂ CH ₃) | 3.91(s) | 3.67(s) | 3.98(s) | |
| | | | 3.48(s) | |
| C-10(H) | 3.99(td) | 3.65(t) | 3.35-3.5(m) | |
| C-10 (CO ₂ CH ₃) | 3.93(s) | 3.97(s) | | 3.94(s) |
| C-9(CH ₃) | | | | 3.62(s) |
| J _{CH₃,9} | | | | 1.12(d) |
| C-10(CH ₃) | | | 1.31(d) | 7.4Hz |
| J _{CH₃,10} | | | 7.4Hz | |

indicating that 4',5'-double bond of khellin is saturated in the photoproducts (Figure 2). IR spectra also indicate the saturation of 4',5'-double bond on photoadduct formation. The olefinic C-H stretching bands of furan ring of khellin at 3060 and 3100 cm⁻¹ disappear in the photoproducts and strong C=O stretching bands of esters lying between 1730 and 1760 cm⁻¹ appear in the adducts. Mass spectra of the photoproducts show molecular ion peaks of 1:1 adducts of khellin and olefins at *m/e* = 404 for KDF and KDM, *m/e* = 418 KEM, respectively and their fragmentation patterns are very similar to each other.

When the photoproducts are exposed to short wavelength UV-light, they are easily splitted into khellin and olefins. The reaction is typical for the compounds having a cyclobutane moiety such as dimers of 5,7-dimethoxycoumarin,^{12,13} and C₄-cycloadducts of DMC<>thymine,¹⁴ 8-MOP<>thymidine,^{15,16} and psoralen<>olefin. The results suggest that the photoproducts of khellin and olefins are [2 + 2]-cycloadducts and the fragmentation pattern of mass spectra also support the C₄-adduct formation showing the weak molecular ion peak and base peak at *m/e* = 245 which corresponds to khellin minus CH₃ ion.

Proton NMR spectra of the photoproducts show that C-4' and C-5' olefinic proton peaks of khellin are greatly upfield shifted, while the peak of C-2 allylic methyl and C-3 olefinic protons are slightly upfield shifted, indicating that the adducts are formed on furan side of khellin. Chemical shifts and coupling constants of the photoproducts are summarized in Table 1.

KDF consists of two diastereomers and the major isomer (KDF-a) shows a triplet at 5.57 ppm for C-5' proton and a


Figure 3. Possible intermediates in the [2 + 2]-photocycloaddition reaction of khellin with olefins.

Scheme 1

multiplet at 4.39 ppm for C-4' proton. The minor isomer (KDF-b) shows a AB quartet and near triplet at 5.45 and 4.50 ppm for C-5' and C-4' protons, respectively. Each isomer is photosplitted into khellin and dimethylfumurate indicating that two C-9 and C-10 ester groups have *trans* configuration on the cyclobutane ring. The ¹H-¹H coupling indicates that C-10 proton of KDF-a has *cis*-configuration to C-5' proton and that of KDF-b has *trans*-configuration to C-5' proton. The methoxy protons of C-9 and C-10 ester groups in KDF-a appear at 3.91 and 3.93 ppm, while in KDF-b the same methoxy protons appear at 3.67 and 3.93 ppm. The C-9 ester methoxy proton peaks in KDF-b are greatly upfield shifted suggesting that those protons are located in the shielding zone of the benzene ring of khellin.

In the case of KDM, UV, IR, ¹H-NMR spectra of major (KDM-a) and minor (KDM-b) products are exactly superimposable on those of KDF-a and KDF-b, respectively. Both KDM-a and KDM-b are also photosplitted into khellin and dimethylfumurate, in which dimethylmaleate is not observed. These results indicate that the photocycloaddition reaction of khellin with dimethylfumurate or dimethylmaleate proceeds via a common intermediate, *i.e.* 1,4-biradical (Figure 3) which makes rotation of C=C double bond of olefins possible.

The structure of two photoproducts (KEM-a and KEM-b) of khellin and dimethylethylidene malonate can be assigned as shown in Scheme 1. Difference of chemical shifts of two ester methoxy groups in KEM-a is greater than that in KEM-b. Two methoxy groups in KEM-a appear at 3.48 and 3.98 ppm and in KEM-b at 3.62 and 3.94 ppm. This greater difference between the chemical shifts of these two methoxy groups is due to the configuration that ester group being *cis* to C-10 methyl group lies in shielding zone of benzene ring of khellin. However, the peak of C-10 methyl group in KEM-a (δ = 1.31 ppm) appears slightly downfield relative to that of KEM-b (δ = 1.12 ppm). This is also explained by the effect of anisotropy of the benzene ring. In this case, C-10 methyl group in KEM-a lies in deshielding zone of the benzene in contrast to ester group and thus the peak of C-10 methyl group of KEM-a is downfield shifted than of KEM-b. Protons of C-4' and C-5' in KEM-a show a doublet-of-doublet

Table 2. Solvent Effect on the Fluorescence Quantum Yield (Φ_f), Intersystem Crossing Quantum Yield (Φ_{isc}), Fluorescence Quenching ($k_q \tau_1$) of Khellin by Dimethylfumarate, and the Quantum Yield of [2+2]-Photocycloaddition (Φ_{2+2}) with Dimethylfumarate

| Solvent | Φ_f^a | $k_q \tau_1, M^{-1}$ | Φ_{2+2}^b | Φ_{isc} |
|---------------------------------|------------|----------------------|------------------|--------------|
| CH ₃ OH | 0.0018 | 6.8 | — ^c | |
| EtOH | 0.0036 | 5.6 | 10 ⁻³ | 0.15 |
| CH ₃ CN | 0.013 | 17.4 | 0.022 | |
| CH ₂ Cl ₂ | 0.021 | 13.0 | 0.045 | 0.4 |

^aCited from ref. 17. ^bMeasured below 10% conversion of reactant with 0.016 M and 0.1 M of khellin and olefin. ^cQuantum yield is very low and cannot be measured.

Table 3. Olefin Concentration Effect on [2+2]-Photocycloaddition Reaction Quantum Yield of Khellin with Dimethylfumarate (DF) in CH₂Cl₂ and CH₃CN

| [DF], M | Φ_{2+2}^a | |
|---------|---------------------------------|--------------------|
| | CH ₂ Cl ₂ | CH ₃ CN |
| 0.050 | 0.060 | 0.025 |
| 0.067 | 0.053 | 0.023 |
| 0.10 | 0.045 | 0.022 |
| 0.15 | 0.038 | 0.020 |
| 0.25 | 0.031 | 0.016 |

^aConcentration of khellin is 0.016M and the quantum yields were measured below 10% conversion of reactant by 366 nm irradiation.

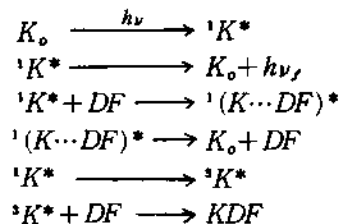
at 4.79 ppm and a triplet at 5.35 ppm, while in KEM-b those are collapsed into a multiplet at 4.94 ppm. Both KEM-a and KEM-b are also splitted into khellin and dimethylethylidene malonate on irradiation with 254 nm UV-light.

Each diastereomer of the photoproducts described must be racemic mixtures and this is confirmed by zero CD throughout the range of all the absorption spectrum.

In the photoreaction of khellin with olefins, one of the important results is that the reaction occurs regio-selectively on the furan ring of khellin rather than the pyrone ring.

The fluorescence quenching of khellin by dimethylfumarate was conducted in various solvents and the results are shown in Table 2. The Stern-Volmer quenching constant ($k_q \tau_1$) increases greatly as the solvent is changed from protic to aprotic and the quantum yields of [2+2] photocycloaddition are also very sensitive to the properties of solvents, especially proton donating ability in hydrogenbonding interactions between solute and solvent. The photocycloaddition quantum yields and the Stern-Volmer constants for fluorescence quenching vary with solvents but they are not correlated with each other. The photocycloaddition quantum yields, furthermore, decreased with increasing the olefin concentration in both acetonitrile and dichloromethane suggesting that the singlet excited state is not the reactive state for the cycloaddition reaction (Table 3). The results are very interesting compared to the general bimolecular photoreaction in which the product formation rate is enhanced with increasing the substrate concentration. It seems that singlet excited state of khellin is quenched by olefins to form exci-

plexes with extensive charge separation and decay to the ground state without giving any cycloadduct and the cycloaddition reaction undergoes through triplet excited state of khellin.



The explanation is supported by the fact that the same photoproducts are formed in the photoreaction of khellin with dimethylfumarate or dimethylmaleate through a common intermediate which is formed from the triplet excited state. The reaction is not concerted reaction which is expected if the reactive state is a singlet excited state. However, the possibility of partial contribution of the excited singlet state cannot be rule out and further investigation is warranted to understand the photoreaction mechanism.

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