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(Journal of the Korean Chemical Society)
Vol. 20, No. 3, 1976
Printed in Republic of Korea

쿠마린과 푸로쿠마린의 광화학반응에 관한 연구

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(1976. 3. 30 접수)

Studies on the Photoreactions of Coumarins and Furocoumarins

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(Received March 30, 1976)

요 약. 광독성 물질인 쿠마린과 푸로쿠마린의 광화학반응을 분광분석, 삼중상태 퀜칭, 형광법을 써서 연구하였다. 크산토톡신과 티민 또는 DNA 수용액의 광화학반응에서 β-카로텐을 퀜처로 썼으나 아무런 퀜칭이 일어나지 않는 것으로 보아 이 반응은 수명이 긴 크산토톡신의 삼중상태보다 수명이 짧은 들뜬 단일상태에서 일어나는 것으로 생각된다.

ABSTRACT. The mechanism of skin-sensitizing photoreactions of coumarins and furocoumarins are studied by spectroscopic, triplet quenching, and fluorescence techniques. The excited singlet mechanism is suggested for xanthotoxin-thymine/or DNA photoreactions from the results of triplet quenching studies utilizing β-carotene as a quencher.

INTRODUCTION

The nature of excited states of skin-photosensitizing coumarins and furocoumarins has received a good deal of attention[1~6]. On the basis of luminescence spectra[4,7] and theoretical calculations[2], it has been proposed that addition of furocoumarins to pyrimidine bases, free or in DNA, results from an attack of the (π, π^*) triplet excited state. This was supported by quenching effect of oxygen and paramagnetic ions on the photodynamic effect of furocoumarins[5]. However, coumarins dimerize to form C_4-cyclo-adduct from both excited singlet (and/or singlet exciplex) and triplet state[8,9]. Furthermore, most of the stereospecific photocycloadditions between olefins are known to be originated from the excited singlet states or singlet exciplexes[10,11]. It is, therefore, suspected that addition of furocoumarins to pyrimidine bases responsible for photosensitization may result from an attack of the (π, π^*) singlet excited state rather than the triplet state. The further study on the mechanism of C_4-photocycloaddition of furocoumarins to pyrimidine bases is carried out to clarify this possibility utilizing spectroscopy, quenching, and fluorescence techniques.

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EXPERIMENTAL

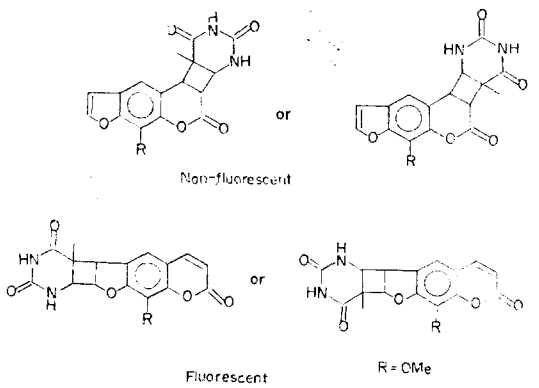
Materials. Coumarin (Aldrich Chemical Company), thymine (Sigma Chemical Co.), xanthotoxin (Sigma Chemical Co.), DNA from herring sperm (Calbiochem), and β-carotene (Eastman Organic Chemicals) were used as received without further purification. All solvents were redistilled prior to use.

Methods. The UV spectra were recorded on a Cary 14 spectrophotometer. The reaction was followed by measuring the change of absorbance at 266 nm and by studying thin layer chromatograms on silica gel G (Merck) plates. A Rayonet photochemical reactor Type RPR-100 (The Southern New England Ultraviolet Company) was used for the light sources. The light intensity was 4×10^{17} and 3×10^{18} quanta/m*l*/min at 300 and 350 nm, respectively. Various ratio of concentraions between furocoumarins ($10^{-3}\sim10^{-5}M$) and pyrimidine bases in water, methanol, ethanol, and aqueous frozen solutions were irradiated at 300 or 350 nm.

Aqueous solutions of nucleic acid (0.01 %) containing 20 mM NaCl were prepared and ethanolic solution of xanthotoxin was added making the concentration of xanthotoxin to be 20 μg/m*l*. The final ethanol content was less than 1.5 % (solution A). After the addition of xanthotoxin, the solution was shaken for an hour at room temperature and filtered. The solution was irradiated at 350 nm in the presence or absence of β-carotene (dioxane solution) for 1.5 hours (at 15 °C). To the irradiated solution, solid NaCl was added to make the final concentration to be 10 %, and ethanol was added to precipitate nucleic acids. The precipitated nucleic acid was separated by centrifugation (5,000 rpm for 25 minutes), washed with 80 % ethyl alcohol and dissolved in distilled water (solution B). The reaction was followed by measuring the absorbance of solution A and B. Fluorescence quenching was monitored by recording the fluorescence spectra of furocoumarin-pyrimidine base solutions in various concentration ratio on an Aminco-Bowman spectrophotofluorometer.

RESULTS and DISCUSSIONS

The ground state complex formation between furocoumarins and pyrimidine bases was studied in aqueous solutions. No complex formation is apparent since there is no change in the UV-VIS spectra when the concentration ratio of coumarin or xanthotoxin and pyrimidine bases, thymine, uracil, and cytosine, is varied. The ground state complex formation, therefore, is not involved in the C_4-photocycloaddition of furocoumarins to thymine. The C_4-photodimerization of coumarin and C_4-photocycloaddition of coumarin to thymine were studied in aqueous solution at room temperature and at the frozen state. The various concentration ratios of coumarin to thymine (1 : 1, 1 : 10, 1 : 100) are used and the results are summarized in *Table* 1 and 2. No change in absorbance was observed when the aqueous solution of coumarin and thymine (concentration ratio of 1 : 10) was irradiated at 350 nm for 1.5~3 hours at the frozen state indicating no reaction between coumarin



C_4-Photoproducts between xanthotoxin and thymine.

and thymine under the condition given. From the results shown in *Table* 1 and 2, it is clear that coumarin undergoes C_4-photodimerization as reported previously but does not react with thymine to give C_4-photocycloaddition product contrary to the theoretical prediction. When xanthotoxin is irradiated with thymine or DNA in aqueous solution, several products are formed as reported by Musajo and Rodighiero[12]. These reactions were thought to undergo via excited triplet state, of xanthotoxin. The quenching of this triplet excited xanthotoxin in the presence of thymine or DNA is attempted with β-carotene as a quencher. The progress of photoreactions were monitored by measuring the change the change of absorbance in the UV-VIS spectra and the results are shown in *Fig.* 1 and 2 for thymine and DNA solution, respectively. From the spectra, it is clear that β-carotene does not quench the photoreaction of xanthotoxin-thymine/or DNA solutions contrary to our expectation for the triplet mechanism. The triplet energy

Table 1. Photochemical reactions of coumarin, coumarin thymine solutions at room temperature

Wavelength	Solution					
	water		ethanol		methanol	
	C&T	C	C&T	C	C&T	C
300 nm (1.6 hours)	−	+	−	+	−	+
350 nm (18 hours)	−	++	−	++	−	+

Coumarin(C): Thymine(T)=1 : 10

Table 2. Photochemical reactions of coumarin and thymine in aqueous solutions irradiated at 350 nm for 72 hours at room temperature.

Photoproduct	Coumarin/Thymine		
	1/1	1/10	1/100
C_4-Coumarin dimer	+++	+	−
C_4-Cycloadduct	−	−	−

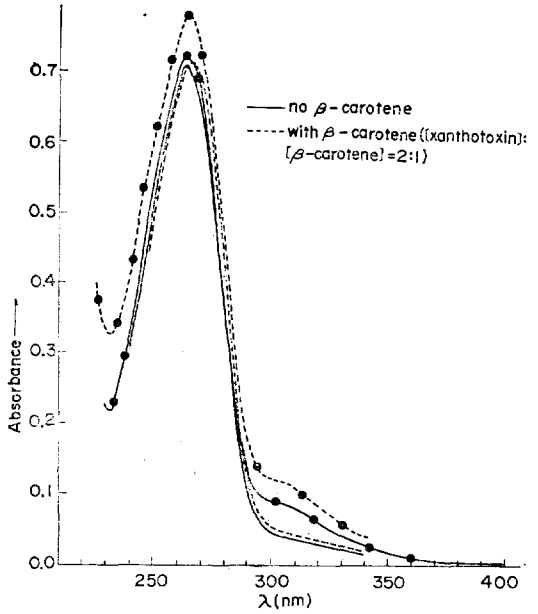


Fig. 1. Spectral changes on irradiation of xanthotoxin-thymine (1 : 10) in water:● ;prior to irradiation, no circles; after irradiation.

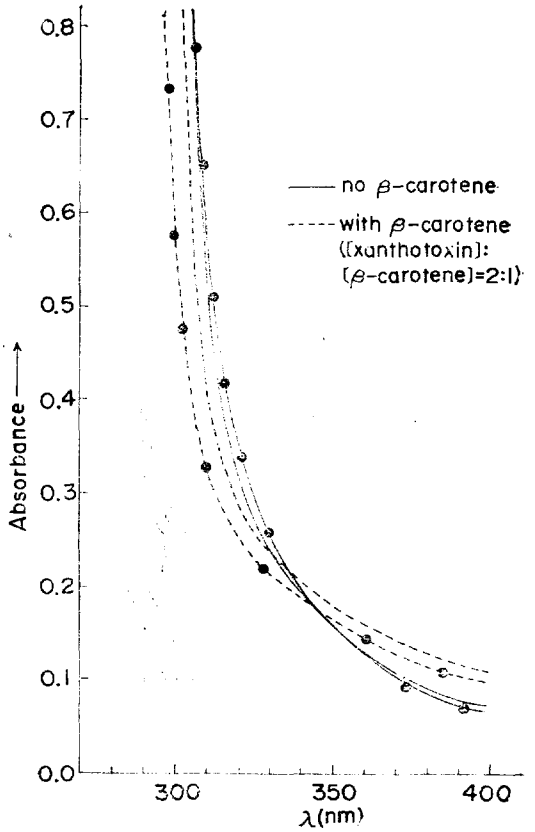


Fig. 2. Spectral changes on irradiation of xanthotoxin-DNA in water:●; prior to irradiation, no circles; after irradiation.

of β-carotene is sufficiently low compared to that of furocumarins (55~62 kcal/mole)[13] since β-carotene even quenches singlet oxygen (37 kcal/mole) through electronic energy transfer[14]. Furthermore, the phosphorescence lifetime of psoralen is relatively long (0.66 sec)[2a] and the triplet state of xanthotoxin is expected to be quenched very efficiently by low energy triplet energy acceptors like β-carotene and molecular oxygen. This quenching study with β-carotene and that of Bevilacqua and Bordin with molecular oxygen and paramagnetic ions suggest that the photoreactions of xanthotoxin with thymine or DNAresult probably from a shorter-lived singlet excited state than the triplet state of the xanthotoxin. This mechanism is also supported by the observation of very low intersystem crossing yields for coumarins ($\Phi_{isc}=6\times10^{-3}$ in EtOAc and 8.8×10^{-3} in acetonitrile for coumarin)[9]. Almost all of the excited coumarin molecules decay from the excited singlet states before crossing to the triplet state.

If the reactive transient is the excited singlet state, the xanthotoxin fluorescence is expected to be quenched by pyrimidine bases in high concentration. The quenching of xanthotoxin fluorescence by thymine and 1,3-dimethyl uracil was tested by monitoring the change of fluorescence intensity versus pyrimidine base concentration in aqueous solution at room temperature. However, no quenching was observed in the range of 0.025~0.4 M pyrimidine base concentrations. This is probably due to the short lifetime of xanthotoxin excited singlet state (1×10^{-11} sec). Thus the fluorescence quenching studies neither prove nor disprove the singlet mechanism and further study is required to elucidate the mechanism of photosensitation reactions of furocoumarins.

ACKNOWLEDGEMENT

The authors thank Professor Pill-Soon Song for the fluorescence spectra and for the helpful-discussions.

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