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THE PHOTOCHEMICAL MECHANISM OF PYRIMIDINE CYCLOBUTYL DIMERIZATION†

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Abstract—Irradiation of 6MU with 254 nm light, 6MU=6MU are formed with *cis-syn* and *cis-anti* configurations in frozen aqueous solutions and with predominantly *cis* configurations in frozen t-butanol solutions; no dimerization occurs in methanol "puddles". Irradiated with 254 nm light, 6MU=6MU with predominantly *trans* configurations are obtained in H_2O and D_2O solutions; dimer yields in D_2O are three times that in H_2O. Irradiated with 313 nm light, acetone photosensitized dimerization gives only *trans-syn* and *trans-anti* dimers. The total dimer yields decrease as the solvent is changed from 90% CH_3COOH, CH_3OH, CH_3CN to non-polar solvent p-dioxane. Our results contradict the general belief that if dipole moment increases in the transition state leading to *head-to-head* or *syn* dimers, the dimer yield increases in a solvent with high dipole moment. The study of pH effects indicated that keto-enol equilibrium of 6MU probably controls the dimer yields and distributions of these photosensitized dimerization processes. With 1,3,6- trimethyluracil, the total dimer yields decrease with increasing pH; however, the product compositions remained unchanged over the entire pH range. This finding suggests that the ketonic or protonated ketonic form of an α,β-unsaturated ketone molecule is the favored structure for photodimerization and keto-enol equilibrium influences the compositions of dimers.

THIS PAPER describes the study of photoinduced dimerization of 6-methyluracil (6MU) and relates the results to those of similar dimerization processes of α,β-unsaturated ketones,[1] particularly in the pyrimidine series.[2] Previously, mechanistic understanding of the photodimerization reaction was impeded by difficulty in elucidating the detailed stereochemistry of the dimeric products.[1] In this study, we have separated four stereoisomeric products; two of these structures have been established by X-ray analyses[3,4] and the other determinations are in progress.‡ Furthermore, it has been found that each isomer has its own characteristic NMR spectrum. This facilitates ascertainment of the isomer distribution,[5] which, in turn, supplies additional information, unobtainable in a study based only on *head-to-head* and *head-to-tail* assignments.

RESULTS AND DISCUSSION

Dimeric products are formed when pyrimidine bases are irradiated with UV light under various conditions. So far, four distinct types of dimeric products have been identified. In addition to the widely studied cyclobutyl dimers of thymine, discovered by Wang[7,10] and by Beukers and Berends,[11] and of uracil, discovered by Wang,[7] the formation of azetidine adducts from cytosine and other pyrimidines has recently been recognized.[12–14] Compounds formed *via* oxetanes can be isolated from derivatives of

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thymine,[15,16] uracil[17] and a mixture of thymine and uracil.[18] A fourth type of adduct is formed by the addition of photo-induced thyminyl radicals[19] to other pyrimidines.[20,21] While all four kinds of products may be important for an understanding of the photobiology of nucleic acids and all can occur simultaneously in certain photoreactions, this paper will deal only with cyclobutyl dimerization.

The use of 6MU to study the mechanism of cyclobutyl dimerization was based on preliminary results with 6MU, which indicated few side reactions. It is indeed a fortunate choice because the experimental results are far more concise than those obtained with uracil,[22] thymine[23] and their derivatives.[24] For this reason, more definitive conclusions could be drawn regarding the mechanism.

Characterization of dimers

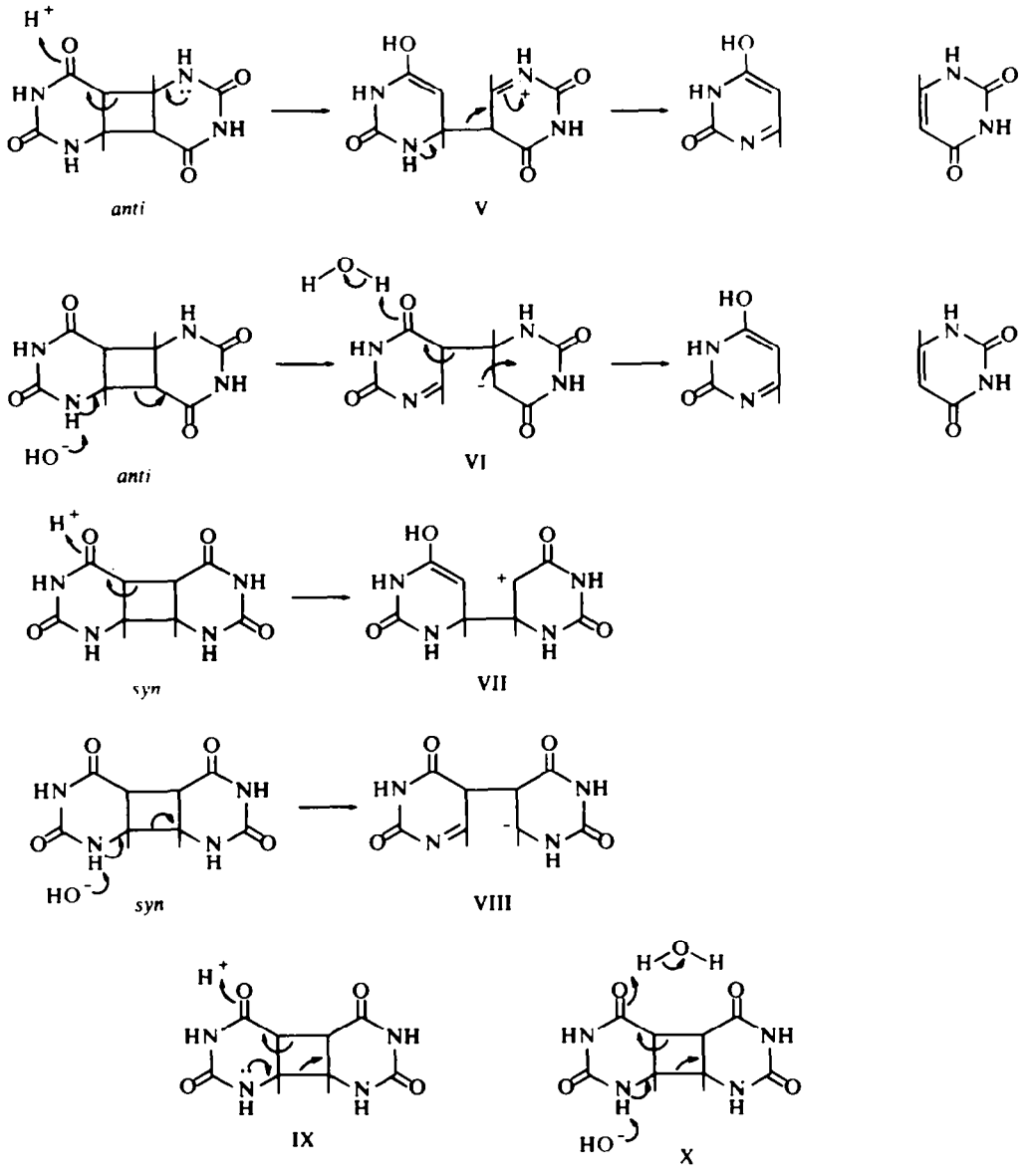
Theoretically, twelve stereoisomers of cyclobutyl dimers[25] may result; however, only the stable and energetically favored isomers with *cis* ring junctions (between the cyclobutyl and a pyrimidine ring) are isolated. Since no *trans* ring junction isomers are detected, there exist only four isomers, namely: head-to-head (*syn*) and head-to-tail (*anti*) isomers with both pyrimidine rings on the same side (*cis*) and opposite side (*trans*) of the cyclobutyl ring.

Separation of several stereoisomers from a mixture usually presents difficulties similar to those encountered in the separation of cyclobutyl dimers of thymine and uracil.[22-24,26] Four thymine dimers have been separated.[27] Two of these were obtained from acid hydrolysates of thymidine dimers and two from dinucleotides, and are not from a single starting material. On the other hand, the separation of 6MU cyclobutyl dimers (6MU=6MU) presents few problems. Under certain conditions, a single isomer can be isolated; under others, a pair of *cis* or *trans* isomers are obtained (see Tables 3–6). These results help to elucidate the basic mechanism of photodimerization.

All four 6MU=6MU have similar UV spectra with only end absorption. In dilute aqueous solution irradiated with 254 nm light, each isomer gives a spectrum characteristic of 6MU, indicating the reversion of these dimers to 6MU—a phenomenon noted with thymine[7,11] and uracil[7] dimers. This reversibility is also observed with the two *anti* dimers in acid and in base, although the two *syn* dimers are stable. Again, this behavior parallels that of thymine dimers.[27]

$$6MU \xrightarrow[254\ nm,HOH]{245\ nm,\ ice} {}^{c,t}MU{=}6MU$$

A generalized mechanism can be formulated to explain this differential reactivity. As shown in the following scheme, the *anti* dimers may form intermediate V by acid catalysis or VI by base catalysis. Both species, quaternary nitrogen ions and enolate ions, are relatively stable intermediates, which may give rise to the monomers. On the other hand, the carbonium ions VII and carbonions VIII formed from the *syn* dimers are much less stable, and further reaction *via* VII or VIII is less likely. Or, it may be argued that the monomerization of *syn* dimers may proceed *via* a concerted mechanism IX and X, as shown. However, this is unlikely since both of these processes necessitate *cis*-elimination at the cyclobutyl ring junctions and are, therefore, energetically unfavorable. Indeed, if the cyclobutyl ring junctions were *trans*, this



monomerization process might occur readily. In short, two interesting generalizations may be drawn: First, stability in acid or in base may be used to diagnose the two types of dimers, *i.e.*

anti dimers $\xrightarrow{H^+ \text{ or } {}^-OH}$ monomer

syn dimers $\xrightarrow[\text{mild conditions}]{H^+ \text{ or } {}^-OH}$ no change

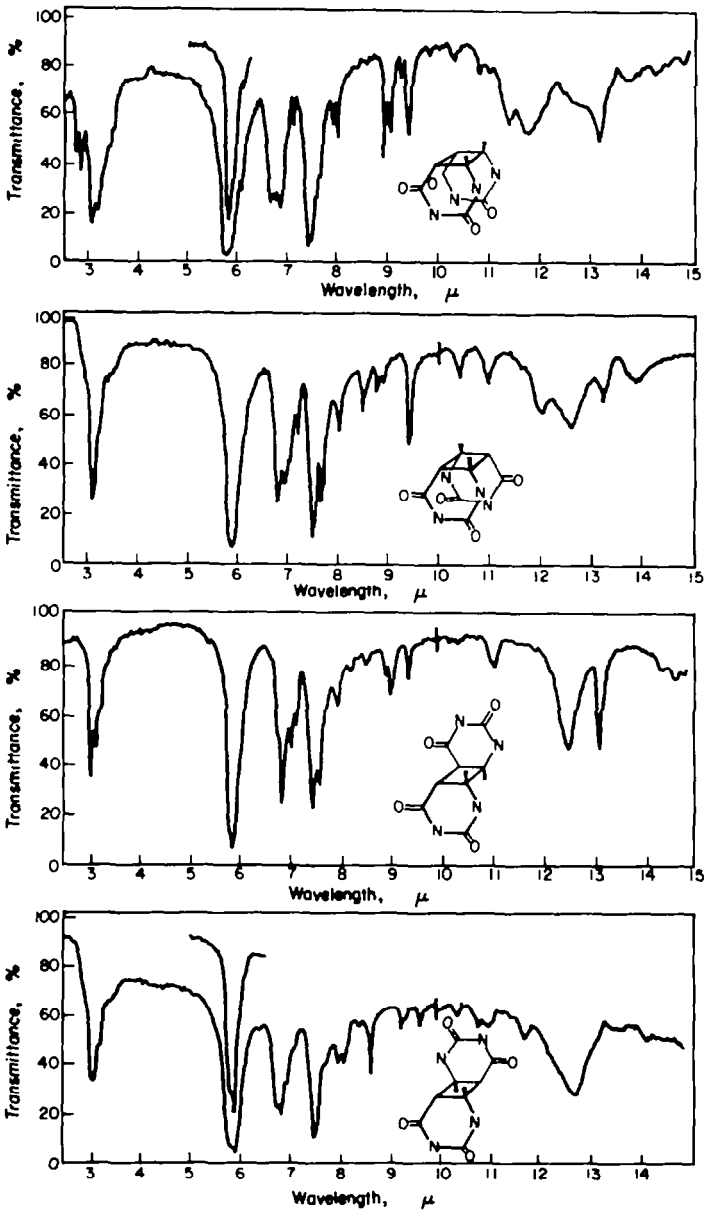


FIG 1. IR spectra of *cis-syn*, *cis-anti*, *trans-syn*, and *trans-anti* 6MU=6MU in KBr pellets.

Second, even if dimers with *trans* ring junctions also form as photoproducts, the process of reversion may be favorable, making their isolation less probable. Thus, only dimers with *cis* ring junctions have been obtained so far.

While each isomer displays a characteristic IR spectrum (Fig. 1), few diagnostic features for either the cyclobutyl structure or the *anti* or *syn* configuration can be

identified unambiguously. It must be pointed out, however, that the IR spectrum of *cis-trans* 6MU=6MU in KBr pellets has certain anomalous features, which are unusual and unexplainable for cyclobutyl dimers. Two strong bands at 2·80 μ and 2·90 μ suggest that this compound may have a diol structure. The band at 2·80 μ disappears completely upon heating at 100° in solid state or upon crystallization from TFA. Also, crystallization from D_2O results in the appearance of bands at 4·13 μ and 4·20 μ and in the disappearance of the bands below 3 μ. However, the NMR spectrum evidences the compound as *cis-syn* 6MU=6MU and reveals that the OH bands are probably due to water of crystallization. In order to understand the nature of this unusual water of crystallization, an X-ray diffraction study was carried out.[3] It showed that channels containing H_2O molecules are formed in the crystal lattice. Each H_2O molecule participates in hydrogen bonding to three dimer molecules. This represents the first reported structure of a cyclobutyl dimer obtained from irradiation of thymine, uracil or their derivatives, which contains water in the crystal lattice.

The NMR spectra of the four dimers were measured in two solvent systems; their chemical shifts are indicated in Table 1.

TABLE 1. NMR SPECTRA OF 6MU AND 6MU=6MU

Compound	in TFA* (δ)		in $(CD_3)_2SO$† (δ)			
	CH singlet	CH_3 singlet	CH singlet	CH_3 singlet	N_1 singlet	N_3 singlet
6MU	3·34	1·15	5·50	2·01	—	10·95
cis-syn 6MU=6MU	2·28	0·98	3·27	1·25	7·53	10·20
cis-anti 6MU=6MU	2·13	1·10	3·05	1·25	7·53	10·20
trans-syn 6MU=6MU	2·20	1·00	2·82	1·23	7·86	10·23
trans-anti 6MU=6MU	2·17	1·10	3·05	1·47	7·70	10·12

* $CHCl_3$ is used as internal reference with CH resonance at δ 7·27.
† TMS is used as internal reference (δ 0·00)

Although it is possible to assign the stereoconfigurations of these dimers on the basis of the chemical and spectral evidence discussed above, their identities were further established by X-ray analyses.[3,4] Dimers I, II, III and IV are identified as *cis-syn*, *cis-anti*, *trans-syn* and *trans-anti*, respectively (see Fig. 1).

Effects of irradiation conditions

Prior to this study, few compounds have been examined under widely varied conditions for the purpose of understanding photodimerization reactions. Distinct further established by X-ray analyses.[3,4] Dimers I, II, III and IV are identified as reactions.

Irradiation of 6MU *in frozen state.* The "Molecular Aggregates—Puddle Formation" hypothesis was proposed by Wang[7, 28] to explain the formation of photodimers from thymines and uracils irradiated in ice. Several laboratories have provided additional evidence to support this hypothesis.[29] One aspect of the hypothesis

TABLE 2. TLC R_f VALUES OF 6MU AND DIMERS

Compound	Eluent A	B	C	D
6MU	0·70	0·54	0·64	0·68
cis-syn 6MU=6MU	0·38 (0·23)*	0·25	0·41	0·40
cis-anti 6MU=6MU	0·48 (0·32)*	0·29	0·43	0·50
trans-syn 6MU=6MU	0·42	0·33	0·45	0·57
trans-anti 6MU=6MU	0·50	0·33	0·48	0·59
Adduct	0·42	0·33	0·47	0·45

* R_f values in parentheses for paper chromatography

emphasizes that while a solution is freezing, crystallization of ice forces the solute to form microcrystals interspersed among the ice crystals. Thus, irradiation of compounds in frozen state should be analogous to irradiation in solid state. In the case of thymine, only *cis-syn* dimer was obtained. Apparently, water of crystallization influences the stability of thymine crystalline structure, which, in turn, determines the ease (or quantum yield) and the stereochemistry of thymine dimerization.[30] Irradiation with 254 nm light of 6MU in ice (Table 4) results in the formation of both *cis-syn* and *cis-anti* dimers; whereas, that in frozen t-BuOH results in three different isomers. From these results is may be deduced that at least two crystalline structures of 6MU in water and three in t-BuOH favor dimerization. Another aspect of the above

TABLE 3. EFFECTS OF SOLVENTS AND pH ON THE 6MU=6MU DIMER COMPOSITION

Solvent	Total Yield	Composition *cis-syn*	*cis-anti*	*trans-syn*	*trans-anti*
		(1) Effect of Solvent			
t-BuOH	70%	0%	0%	85%	15%
MeOH	57	0	0	67	33
MeCN	60	0	0	67	33
p-dioxane	45	0	0	55	45
		(2) Effect of pH			
90% AcOH	100%	0%	0%	100%	0%
pH 3 AcOH	80	0	0	80	20
pH 5 AcOH	50	0	0	75	25
HOH	40	0	0	65	35
pH 8 NaOH	40	0	0	100	0
ph 10 NaOH	40	0	0	100	0
pH 12 NaOH	0	0	0	0	0

Photosensitizer: Acetone 50%
Light Wavelength: 313 nm for 20 hr
Concentration of Solutions: 10 mM
Temperature: Ambiant

hypothesis states that in an aqueous solution containing a small percentage of a solvent with a low melting point, such as MeOH, the solute molecules together with some organic solvent will be excluded from the water crystals and form "puddles" as the solution freezes.[7, 28] Thus, irradiation of compounds in the "puddles" of frozen solutions would be different from irradiation in solid state and similar to that in concentrated solutions. Indeed, when 6MU was irradiated in 5% MeOH "puddles", no dimerization was observed; instead, the adducts of MeOH—6MU [as indicated by acid reversal] and 6MU—6MU [as indicated by 320 nm absorbancy increase] were obtained. Thus, irradiation with 254 nm light of 6MU in solid or frozen state results in the formation of dimers with a predominantly *cis* configuration. This configuration is stereochemically more crowded than the *trans* and is unlikely to be formed by free molecular collision. Rather, isolation of *cis* dimers strengthens the belief that crystalline structure directs the stereochemical course of photodimerization reactions.[28, 30]

Irradiation of 6MU *in concentrated solutions.* Irradiation of 6MU in H_2O and D_2O solutions with 254 nm light resulted in the formation of three isomeric dimers (Table 4). These were predominantly in the *trans* configuration. This indicates that most of the excited molecules of 6MU must have a halflife long enough to permit the bimolecular reaction to occur, with the stereochemically favored isomers as the major products.

TABLE 4. EFFECTS OF IRRADIATION CONDITIONS ON 6MU DIMER COMPOSITIONS
CONCENTRATION OF SOLUTIONS: 2 mM TIME OF IRRADIATION: 2 hr

Condition	Temperature	Dimer Yield	Light Source	6-Methyluracil Dimer Composition			
				cis-syn	*cis-anti*	*trans-syn*	*trans-anti*
Ice	−70°	20%	254 nm	85%	15%	0	0
t-BuOH	−70°	10%	254 nm	40%	40%	20%	0
5% MeOH* puddle	−70°	0	254 nm	0	0	0	0
Acetophenone	−70°	0	313 nm	0	0	0	0
Water	30°	10%	254 nm	0	20%	30%	50%
D_2O	30°	30%	254 nm	0	33%	17%	50%

* MeOH adduct and 6MU—6MU adduct formation observed.

The above data provide evidence supporting the belief that in frozen solutions or solid states the chromophores cohere in certain geometric relationships,[28, 30] which favor dimerization *via* an excited singlet precursor[31] (although a triplet precursor cannot be precluded). However, the lifetime of excited singlet molecules is not long enough to favor bimolecular reactions in solution. Thus, if dimers are to form with reasonable yields in solutions, they most likely have triplet precursors. Furthermore, the dimer yield in D_2O is three times that in H_2O, which conforms with our earlier observation[32] in the study of product compositions in photohydration and photodimerization of uridine and 1,3-dimethyluracil.

Photosensitized reactions. With acetone as a photosensitizer, 313 nm light was used to induce dimerization of uracil and thymine derivatives.[8] It is interesting to point out that in the present study only dimers with *trans* configurations were obtained; (*cis* dimers are stable under these conditions). Since the precursors of photosensitized dimerizations are known to be of excited triplet state,[8] the absence of *cis* dimers in these reaction products may again suggest that *cis* dimers are formed *via* excited singlet precursors. However, the yields and compositions of products varied considerably with changing conditions (see below).

Effects of solvents[33, 1] (Table 3). The total yields of dimers decrease as the solvent changes from 90% AcOH to non-polar solvent, dioxane. The same trend was observed in the *trans-syn* isomer yields. On the other hand, the *trans-anti* isomer yield increases. However, the relative yields (Table 5) of *trans-anti* isomers reach a maximum at about

TABLE 5. RELATIVE YIELDS AND PRODUCT RATIOS OF *trans-syn* AND *trans-anti* 6MU=6MU

Solvent	Total Yield	Relative Yield *trans-syn*	*trans-anti*	Ratio
		(1) Effect of Solvent		
90% AcOH	100%	100%	0%	∞
t-BuOH	70	60	11	5
MeOH	57	38	19	2
MeCN	60	40	19	2
p-dioxane	45	25	20	1
		(2) Effect of Acidic pH		
90% AcOH	100%	100%	0%	∞
pH 3	80	64	16	4
pH 5	50	37	13	3
HOH	40	26	14	2
		(3) Effect of Basic pH		
HOH	40%	26%	14%	2
pH 8	40	40	0	∞
pH 10	40	40	0	∞
pH 12	0	0	0	—

20% and no further increase is noted with a less polar solvent. Thus, the ratios of the two isomers vary from ~1 in *p*-dioxane to higher values in other solvents. (It must be noted that under certain conditions, only a single isomer was isolated.) Our findings are in accord with other reports of photodimerization reactions.[1] However, one important difference in this study is the recognition that only one type of *syn* dimer, rather than both, from a triplet precursor is involved in this reaction. Subsequent experiments seem to indicate that the polar effect alone cannot adequately explain the variation of products. Some investigators have suggested that solvent-dependent

aggregation of the molecules influences the course of photodimerization.[34] Our present data does not permit us to evaluate this possibility.

Effects of acetone concentration or dipole moments of solvents[5,1] (Tables 3 and 6). In considering the dipole moments of t-BuOH (1·67 D), MeOH (1·66 D), MeCN (3·18 D) and *p*-dioxane (0·45 D) (all measured in benzene)[35] in relation to 6MU dimerization, neither the total nor the individual isomer yields appear to follow a definite trend. Therefore, it would be interesting to examine this reaction in water with various concentrations of acetone, *i.e.* in different solvent dipole moments.[5] Since only two *trans* dimers are formed under these conditions, the relative magnitude of the dipole moments of these two dimers and of 6MU can be easily assigned, i.e. *trans-syn* dimer > 6MU > *trans-anti* dimer. The latter should have a dipole moment close to zero. It has been thought[1] that if there is an increase in dipole moment in the

TABLE 6. EFFECTS OF CONCENTRATION OF THE PHOTOSENSITIZER, ACETONE

Solvent		Total Yield	Composition of 6MU = 6MU			
Acetone	HOH		*cis-syn*	*cis-anti*	*trans-syn*	*trans-anti*
80%	20%	60%	0	0	80%	20%
50%	50%	40%	0	0	65%	35%
10%	90%	10%	0	0	67%	33%

Light Wavelength: 313 nm for 20 hr
Concentration of Solutions: 10 mM
Temperature: Ambiant

transition state leading to *head-to-head* or *syn* dimers, such a dimer yield should be increased in a solvent with a higher dipole moment (μ = 75 D, 10% acetone–90% HOH). However, our results contradict this belief. Conversely, it is believed that if there is a decrease in dipole moment in the transition state leading to *head-to-tail* or *anti* dimers, such a dimer yield should be decreased in a solvent with a lower dipole moment (μ = 32 D in 80% acetone–20% HOH). Again our findings are in opposition to this hypothesis (*cf.* 5). Therefore, the exact role played by solvent dipole moments in the determination of photodimer structures requires further close examination. Also observed in this study was a decrease in total yields of dimers with decreasing concentrations of the photosensitizer, acetone. This is not unexpected.

The effect of solution pH

The effect of solution pH was studied in order to understand the possible importance of lactam-lactim or keto-enol equilibrium of the pyrimidine and of solute-solute and solute-solvent hydrogen bonding in controlling dimer distributions in photodimerization processes. As shown in Table 3, total dimer yields decrease with increasing solution pH. The yield is 100% in acetone–90% acetic acid (1:1) and is reduced to 40% at neutral or alkaline pH. At pH 12, no dimer formation was observed. These results suggest that the molecules in their ketonic or lactam form favor dimerization; whereas, the anionic or lactim moiety gives no cyclobutyl dimers. The compositions of the two *trans* isomers also varied with the pH of the solutions. In 90%

AcOH, pH 8 and pH 10 solutions, *trans-syn* dimer was obtained. Otherwise, the *trans-syn* dimer yields decrease with increasing pH. The *trans-anti* dimer yields, however, are practically constant, 13–16%, over the entire pH range. Thus, the relative yields are between 2–4. The above observation suggests that solution pH, *i.e.* the lactam-lactim equilibrium influences the total yields and product compositions of a photodimerization reaction.

TABLE 7. EFFECT OF pH ON PHOTOSENSITIZED DIMERIZATION OF 1,3,6-TRIMETHYLURACIL

Solvent	Total Yield	Composition Dimer A	Dimer B	Dimer C
90% AcOH	45%	55%	30%	15%
pH 3	36%	55%	30%	15%
pH 5	36%	55%	30%	15%
HOH	30%	55%	30%	15%
pH 8	25%	55%	30%	15%
pH 10	25%	55%	30%	15%
pH 12	20%	55%	30%	15%

Photosensitizer: Acetone
Light Wavelength: 313 nm for 20 hr
Concentration of Solution: 10 mM
Temperature: Ambiant

To further pursue this point, 1,3,6-trimethyluracil was prepared. The N-Me substitutions at 1 and 3 would prevent lactam-lactim equilibrium at various solution pH. Photosensitized dimerization of 1,3,6-trimethyluracil at various pH (Table 7) again showed a decrease in total yields. However, the product compositions remained unchanged over the entire pH range. Thus, keto-enol equilibrium is a dominant factor in determining the dimer compositions, and the solution pH determines the total dimer yields. The latter suggests that the ketonic or protonated ketonic form of an α,β-unsaturated ketone molecule is the favored structure for photosensitized photodimerization *via* triplet precursors.

EXPERIMENTAL

IR (KBr pellets) and UV absorption spectra were recorded on a Perkin-Elmer Infrared Spectrophotometer Model 21, and a Beckman recording spectrophotometer Model DK-1, respectively. NMR spectra in trifluoroacetic acid (TFA) and in dimethyl sulfoxide (DMSO-d_6) were obtained on a Varian HR-60 or a Varian HA-100 spectrophotometer operating in the field-sweep mode and using chloroform ($CHCl_3$ = 7·27 ppm) and tetramethylsilane (TMS = 0·00 ppm) as internal standards.

Irradiation apparatus. These have been described previously for solution irradiation[6] and for frozen state irradiation.[7] General Electric germicidal tubes (G15T8), which emit mainly 254 nm light, were used. General Electric BL (black-light) tubes, which transmit light longer than 300 nm, were used for the photosensitization study.

Chromatography. Whatman No. 1 or No. 3 (for isolation) paper was used with descending technique. Eastman chromatogram sheets, No. 6471, cellulose-coated, were used. A 2 × 50 cm column of Dowex

50W-X12, H^+ form, 100–200 mesh, was used. The following systems, given by volume, were used for paper and TLC.

(A) sec-BuOH and H_2O (50:20)
(B) n-BuOH, AcOH and H_2O (80:12:30)
(C) t-BuOH, methyl ethyl ketone (MEK), HCOOH and H_2O (40:30:15:15)
(D) t-BuOH, MEK, conc. NH_4OH and H_2O (40:30:15:30).

After elution, UV absorbing materials or spots were determined by UV spectra or located with an UV viewer. The presence of cyclobutyl dimers was detected by the appearance of 6MU spectra after irradiation of the solutions with 254 nm light and by the appearance of dark spots after irradiation of the dried plates.

6-Methyluracil. Chromatographically pure samples of 6MU were obtained by crystallization of the commercial product twice from water. λ_{max}^{HOH} 258 nm, ε 9·6 × 10^2; λ_{min}^{HOH} 228 nm, ε 2·2 × 10^3.

t-Butanol, methanol, dioxane, acetonitrile, and acetone. Baker's analytical reagents were used for purifications but spectro grades were used for irradiation experiments.

Irradiation of frozen aqueous solutions. (A similar procedure is applicable with frozen t-BuOH and with frozen aqueous solution containing 5% MeOH.) An aqueous solution of 6MU (2 mM) was irradiated in the frozen state for 2 hr with the germicidal lamps. Under these conditions, 20% dimerization was observed. After thawing, the irradiated solution was evaporated until dry at < 40°. The residue was taken up in a small volume of hot water and, after cooling, was applied on a column. The column was eluted with distilled water. The first 200 ml eluates displaying end absorption were combined as the "dimer fraction"; the next 400 ml eluates exhibiting 6MU spectrum were collected as the unreacted material. Finally, the column was eluted with 2N HCl, and the eluates with absorbancy maxima at ~320 nm were collected as the "adduct fraction".

Dimer fraction. The solution was concentrated under reduced pressure to 25 ml. After it had been allowed to stand at 5° overnight, large, colorless, cubic crystals (Dimer I) were obtained. [The properties of this material and of other isomers are given in Tables 1 and 2.] Dimer I (40 mg collected by filtration) migrated as a single spot on TLC in each of the four solvent systems. It was stable in 6N HCl and in 2N NaOH but reverted quantitatively to 6MU in solution irradiated with 254 nm light.

The filtrate obtained after the removal of Dimer I was concentrated and applied to Whatman No. 3 paper. The chromatograms were developed with eluent A. The material was separated into two bands: The R_f 0·23 material was found to be identical to Dimer I; the R_f 0·32 material was eluted from the paper and crystallized from water. TLC showed that this material, Dimer II, was a single compound. Dimer II was unstable in 6N HCl and in 2N NaOH, as well as in solution reirradiated with 254 nm light. In all cases, the product was isolated in yields > 95% and was shown to be 6MU by UV and IR spectra.

Photosensitized dimerization of 6MU. First, the compound, 6MU, was dissolved in the solvent (see Table 3). Then, an equal volume of acetone was added as sensitizer[8] to give a final 10 mM concentration of 6MU. Oxygen was removed from the solutions either by using a stream of N_2 during irradiation or by flushing with argon for 30 min prior to irradiation; (the loss of acetone and the solvent was kept at a minimum). The solutions were irradiated with G.E. BL lamps in 2 × 30 cm pyrex containers for 20 hr. After irradiation, both the solvent and the sensitizer were removed by flash evaporation at ~40°. The residue was redissolved in distilled water. The absorbancy readings at 260 nm of this solution before and after irradiation with 254 nm light, which compare with those of the unirradiated solution, were used to determine the total dimer yields. These values were further confirmed by NMR study.

Isolation of dimers III *and* IV. In a t-BuOH acetone system, the gradual appearance of a crystalline material was observed during irradiation. After 20 hr, these crystals were collected by filtration and washed with abs. EtOH. TLC and NMR study evidenced a single compound, Dimer III. Purification of this dimer was accomplished by recrystallization twice from water. Dimer III was stable in 6N HCl and in 2N NaOH. However, upon irradiation in solution with 254 nm light for 4 min, Dimer III reverted quantiatively to 6MU, which was identified by the IR spectrum.

The filtrate, after removal of Dimer III, was evaporated until dry. The NMR spectrum of the residue indicated that it was a mixture of three compounds, i.e. 6MU, Dimer III and another dimeric product. In order to isolate this additional product, the residue was dissolved in a small volume of boiling water and, after cooling, was applied on a column. It was eluted with distilled water and fractions of 10 ml were collected. A single compound, Dimer IV, was isolated from fractions 5 to 8. Dimer III was isolated from fractions 12 to 20. Fractions 9 to 12 contained a mixture of Dimers III and IV. The unreacted 6MU was recovered from fractions 21 to 40. Dimer IV, like Dimer II, was unstable in 6N HCl and in 2N NaOH, as

well as in solution reirradiated with 254 nm light. In each instance, 6MU was isolated in > 90% yield, as shown by UV and IR spectra.

Synthesis of 1,3,6-*trimethyluracil.* It was prepared from 6MU by methylation with dimethyl sulfate,[9] m.p. 110–111°. λ_{max}^{HOH} 267 nm, ε 10·1 × 10^3; λ_{min}^{HOH} 237 nm, ε 2·0 × 10^3.

REFERENCES

[1] O. L. Chapman, P. J. Nelson, R. W. King, D. J. Trecker and A. A. Griswold, *Rec. Chem. Progr.* **28**, 167 (1967)

[2] A. A. Lamola and J. P. Mittal, *Science* **154**, 1560 (1966); C. L. Greenstock, I. H. Brown, J. W. Hunt and H. E. Johns, *Biochem. Biophys. Res. Comm.* **27**, 431 (1967); P. J. Wagner and D. J. Bucheck, *J. Am. Chem. Soc.* **92**, 181 (1970)

[3] J. Konnert, J. W. Gibson, I. L. Karle, M. N. Khattak and S. Y. Wang, *Nature* **227**, 953 (1970); J. W. Gibson and I. L. Karle, *J. Cryst. Mol. Struct.* **1**, 115 (1971)

[4] L. M. Amzel, H. P. Avey, L. N. Becka, R. J. Poljak, M. N. Khattak and S. Y. Wang, (submitted for publication)

[5] B. H. Jennings, S. C. Pastra and J. L. Wellington, Photochem. *Photobiol.* **11**, 215 (1970)

[6] S. Y. Wang, *J. Am. Chem. Soc.* **80**, 6196 (1958)

[7] S. Y. Wang, *Nature* **190**, 690 (1961)

[8] A. A. Lamola, M. Gueron, T. Yamane, J. Eisinger and R. G. Shulman, *J. Chem. Phys.* **47**, 2210 (1967)

[9] D. Davidson and O. Baudisch, *J. Am. Chem. Soc.* **48**, 2379 (1926)

[10] S. Y. Wang, *Nature* **188**, 844 (1960)

[11] R. Beukers and W. Berends, *Biochim. Biophys. Acta* **41**, 550 (1960); **49**: 181 (1961)

[12] S. Y. Wang and A. J. Varghese, *Biochem. Biophys. Res. Comm.* **29**, 543 (1967); A. J. Varghese and S. Y. Wang, *Science* **156**, 955 (1967)

[13] A. J. Varghese and M. H. Patrick, *Nature* **223**, 299 (1969)

[14] D. F. Rhoades and S. Y. Wang, *J. Am. Chem. Soc.* **93**, 3779 (1971); *Biochemistry* (in press)

[15] A. J. Varghese and S. Y. Wang, *Science* **160**, 186 (1968)

[16] I. L. Karle, *Acta Cryst.* **B25**, 2119 (1969); I. L. Karle, S. Y. Wang and A. J. Varghese, *Science* **164**, 183 (1969)

[17] M. N. Khattak and S. Y. Wang, *Science* **163**, 1341 (1969)

[18] D. F. Rhoades and S. Y. Wang, *Biochemistry* **9**, 4416 (1970)

[19] R. Alcantara and S. Y. Wang, *Photochem. Photobiol.* **4**, 465; 473 (1965); S. Y. Wang and R. Alcantara, *Ibid.* **4**, 477 (1965)

[20] A. J. Varghese, *Biochem. Biophys. Res. Commun.* **38**, 484 (1970); *Biochemistry* **9**, 4781 (1970)

[21] D. F. Rhoades and S. Y. Wang, *J. Am. Chem. Soc.* **93**, 2554 (1971)

[22] K. H. Donges and E. Fahr, *Z. Naturforsch* **21b**, 87 (1966); G. M. Blackburn and R. J. H. Davies, *Tetrahedron Letters* **37**, 4471 (1966); H. Ishihara, *Photochem. Photobiol.* **2**, 455 (1963); I. H. Brown and H. E. Johns, *Ibid.* **8**, 273 (1968)

[23] H. L. Gunther and W. H. Prusoff, *Methods in Enzymology* Vol. XII [Eds. L. Grossman and K. Moldave], Acad. Press, 1967, p. 19–30; A. Wacker, H. Dellweg and D. Weinblum, *J. Mol. Biol.* **3**, 787 (1961); R. Setlow, *Biochim Biophys. Acta* **49**, 237 (1961); K. C. Smith, *Photochem. Photobiol.* **2**, 503 (1963); J. Eisinger and A. A. Lamola, *Mol. Photochem.* **1**, 209 (1969)

[24] G. Furst, E. Fahr and H. Weiser, *Z. Naturforsch.* **22b**, 354 (1967); E. Fahr, G. Furst, G. Daerhoefer and H. Popp, *Angew. Chem.* **6**, 250 (1967); E. Ben-Hur, D. Elad and R. Ben-Ishai, *Biochem. Biophys. Res. Comm.* **32**, 355 (1968); R. Ben-Ishai, E. Ben-Hur and Y. Hornfeld, *Israel J. Chem.* **6**, 769 (1968); I. Rosenthal and D. Elad, *Biochem. Biophys. Res. Comm.* **32**, 599 (1968); I. Rosenthal and D. Elad, *Photochem. Photobiol.* **8**, 145 (1968); M. Charlier, C. Helene, *Ibid.* **6**, 501 (1967); H. Morrison, A. Feeley and R. Kleopfer, *Chem. Comm.* 7, 358 (1968); H. Morrison and R. Kleopfer, *J. Am. Chem. Soc.* **90**, 5037 (1968); I. von Wilucki, H. Matthaus and C. H. Krauch, *Photochem. Photobiol.* **6**, 497 (1967); C. H. Krauch, D. M. Kraemer, P. Chandra, P. Mildner, H. Feller and A. Wacker, *Angew. Chem.* **79**, 944 (1967); A. Kornauser, J. N. Herat and N. Trinajstic, *Chem. Comm.* **18**, 1108 (1968)

[25] R. O. Kan, *Organic Photochemistry*, p. 157, McGraw-Hill (1966)

[26] M. N. Khattak and S. Y. Wang, (unpublished results)

[27] D. Weinblum and H. E. Johns, *Biochim. Biophys. Acta* **114**, 450 (1966) (*cf.* Kunieda and Witkop, *J. Am. Chem. Soc.* **93**, 3493 (1971))

[28] S. Y. Wang, *Fed. Proc.* **24**, S-71 (1965)
[29] S. Apelgot and M. Frilley, *J. Chim. Phys.* 838 (1965); A. R. Butler and T. C. Bruice, *J. Am. Chem. Soc.* **86**, 313 (1964); C. Helene, R. Santus and P. Douzou, *Photochem. Photobiol.* **5**, 127 (1966); R. E. Pincock and T. E. Kiovsky, *J. Chem. Educ.* **43**, 358 (1966); S. Davis and I. Tinoco, *Nature* **210**, 1286 (1966); W. Fuchtbauer and P. Mazur, *Photochem. Photobiol.* **5**, 323 (1966)
[30] S. Y. Wang, *Nature* **200**, 879 (1963)
[31] A. A. Lamola and J. Eisinger, *Proc. Nat. Acad. Sci. U.S.* **59**, 46 (1968)
[32] J. C. Nnadi and S. Y. Wang, *Tetrahedron Letters* **27**, 2211 (1969)
[33] H. Morrison and R. Kleopfer, *J. Am. Chem. Soc.* **90**, 5037 (1968)
[34] C. H. Krauch, S. Farid and G. O. Schenck, *Chem. Ber.* **99**, 625 (1966)
[35] A. L. McClellan, *Tables of Experimental Dipole Moments*, San Francisco: W. H. Freeman (1963)