Studies on the Mechanism of the Photosensitized Dimerization of Pyrimidines
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Thymine-2-¹⁴C was irradiated with UV light (>300 nm) in water solutions in the presence of different sensitizers. Pyrimidines upon irradiation with wavelengths of 300—320 nm in the presence of some ketones as sensitizers, yield cyclobutyl pyrimidine dimers. The reaction occurs through a triplet-triplet energy transfer from the sensitizer to the substrate. The energy transfer, however, does not occur through a simple physical mechanism. The ability of a donor (e.g. ketone molecule) in its excited state to form a complex with the acceptor (pyrimidine molecule) appears to be a prerequisite in this type of photodimerization reaction. A correlation of the photochemical behavior of different ketones in this reaction with the nature of their excited states has been proposed. Carbonyl compounds possessing n, π* lowest triples are active in inducing pyrimidine dimerizations. By introducing some different electron donating groups to the ketone molecules that lower its π, π* state below its n, π* level, the sensitizing ability of the molecule appears to decrease or disappears completely. Ethylacetoacetate was found to be a more effective sensitizer than acetone or acetonaphone. Likewise, dihydroxyacetone was found to be a potent photosensitizer in dimerization of thymine. Urocanic acid (UCA), a major UV absorbing compound in mammalian skin, did not show any sensitizing ability to induce cyclobutyl pyrimidine dimers on irradiation in the presence of thymine, with wavelengths greater than 300 nm.

Proposals concerning the reaction mechanisms of the sensitized photodimerization of pyrimidines have been suggested 1, 2; these include population of the triplet state of thymine or uracil from the triplet state of a suitable sensitizer. In our earlier work it was proposed that photosensitized dimerization of pyrimidines involving acetone, or acetonaphone takes place through a mechanism of the triplet energy transfer from the sensitizer to the substrate. The essential hypothesis in this reaction mechanism, besides the triplet energy transfer, is an intermediate formation of a complex between the donor (e.g. ketone molecule) and the acceptor (pyrimidine molecule), giving an unstable adduct of Type I (Fig. 1), which can react with another pyrimidine molecule in its ground state. If this proposal is correct, then only a few selected sensitizers could be active in promoting pyrimidine dimerization. In order to explore the validity of this hypothesis, it was decided to screen some different classes of compounds having similar triplet energies, and check their ability to induce photosensitized dimerization of pyrimidines. Urocanic acid (UCA) II (Fig. 1), a deaminated product of histidine, was included in this investigation, as it is an important ultraviolet-light absorbing constituent of mammalian skin 3. Dihydroxy acetone, on the other hand was investigated because it is a physiologic product of the body formed and utilized in the Embden-Meyerhof pathway of glycolysis, and also it is a major constituent of cosmetic products extensively used all over the western world for imparting artificial color to the skin. Recently, it has been established that in vivo irradiation of mammalian skin with wavelengths of 290—320 nm, which are involved in eliciting sunburn reaction and carcinogenesis, produces cyclobutyl pyrimidine dimers 4. Relevant to this is the question of how pyrimidine dimers are produced in vivo by 290—320 nm irradiation, i.e. whether the dimerization is the result of direct absorption of ultraviolet light by DNA or whether it...
proceeds through a photosensitized reaction by triplet-triplet energy transfer from some other molecules (e.g. urocanic acid or dihydroxy acetone). The triplet energy of UCA has not yet been calculated.

**Materials and Methods**

Ultraviolet irradiation (UV) was carried out with a 500 Watt high pressure mercury lamp (Christie Electric Co., Los Angeles). U.V. spectra of the compounds listed in Table 1 were recorded on a Perkin-Elmer 350 spectrophotometer. The samples were irradiated in an open quartz cuvette (1 cm light path) at room temperature in presence of air. The cuvette was placed directly behind a glass filter (window glass 7.5 x 2.5 x 0.2 cm) that transmitted wavelengths greater than 300 nm. Thymine-2-14C (Tracer Lab., specific activity 2.65 mCi/mmol in water 2 x 10^-3 M) was used through this study. To the thymine-2-14C solution the different sensitizers listed in Table 1, dissolved either in water, or in water/ethanol or water/dioxane mixtures were added; the final concentrations of the sensitizers ranged from 10^-1 to 10^-4 M. The details are shown in Table 1.

**Results and Discussion**

The triplet energies\(^6\) of the compounds used as sensitizers in this study are summarized in Table 2\(^7\). The yields of thymine dimers using these compounds as sensitizers are shown in Table 1. As can be seen from \(\Delta^1\) yields in Table 1, only with very few of the potential sensitizers, has a measurable thymine dimerization occurred. About 1 — 2% of thymine dimers were detected even without any sensitizer, following the applications of UV doses at the level indicated in Table 1. Acetone, ethylacetocetate and dihydroxy acetone were found to be more potent sensitizers than acetophenone and benzophenone. The relative efficiency of these potent photosensitizers cannot be established with the data presented in this study and can be ascertained only when a concentration versus the effect curve is obtained under identical conditions. The maximum yields of thymine dimers in our study were obtained with UV. The dried paper chromatograms were then sliced into 1 cm strips, eluted with 1 ml water in Packard vials and the radioactivity was measured after addition of 10 ml Butler-dioxane based scintillation fluid (8 g Omnifuor, NEN, 90 g naphthalene in 1 liter dioxane) in a Packard Tri-Carb (Model 500 B) liquid scintillation spectrophotometer. RF values of thymine (T) and of thymine dimers (TT) under these experimental conditions were 0.40 and 0.60, respectively.

<table>
<thead>
<tr>
<th>Sensitizer</th>
<th>% TT yield formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1 — 2</td>
</tr>
<tr>
<td>Acetone(^b)</td>
<td>30 — 40</td>
</tr>
<tr>
<td>Dihydroxy acetone(^b)</td>
<td>25 — 30</td>
</tr>
<tr>
<td>Acetophenone(^c)</td>
<td>5 — 10</td>
</tr>
<tr>
<td>Benzophenone(^d)</td>
<td>5 — 8</td>
</tr>
<tr>
<td>4-Methoxyacetophenone(^d)</td>
<td>2 — 3</td>
</tr>
<tr>
<td>Ethylacetocetate(^e)</td>
<td>35 — 45</td>
</tr>
<tr>
<td>Phenyl cyanide(^f)</td>
<td>1 — 3</td>
</tr>
<tr>
<td>Carbazole(^g)</td>
<td>2 — 3</td>
</tr>
<tr>
<td>Fluorene(^g)</td>
<td>1 — 3</td>
</tr>
<tr>
<td>Benzene, naphthalene(^g)</td>
<td>1 — 3</td>
</tr>
<tr>
<td>Xanthene-9-one(^g)</td>
<td>1 — 3</td>
</tr>
<tr>
<td>Urocanic acid(^h)</td>
<td>1 — 3</td>
</tr>
</tbody>
</table>

Table 1. Irradiation of thymine-2-14C, \(2 \times 10^{-3} \text{ M}\) with different sensitizers. Total dose of UV delivered \(1.8 \times 10^6 \text{ erg/cm}^2 \cdot \lambda > 350 \text{ nm}\). All irradiations were carried out in water (sensitizer No. 1), as well as in water/ethanol (3:1) (sensitizer No. 2). Compounds No. 4, 5, 7, 8 and 9 were also irradiated in water/dioxane (3:1) (sensitizer No. 5). \(^a\) Measured with a black ray UV intensity meter (UV products, San Gabriel, Calif.) \(^b\) \(10^{-1} \text{ M}\) and \(10^{-2} \text{ M}\) in Solvent No. 1 and 2. \(^c\) \(10^{-2} \text{ M}\) in Solvent No. 1; \(5 \times 10^{-2} \text{ M}\) in Solvent No. 2 and 3. \(^d\) \(10^{-4} \text{ M}\) in Solvent No. 1; \(10^{-2} \text{ M}\) in Solvent 2 and 3. \(^e\) \(10^{-1} \text{ M}\) and \(5 \times 10^{-2} \text{ M}\) in Solvent No. 2 and 3. \(^f\) Saturated Solution in Solvent No. 1; \(5 \times 10^{-2} \text{ M}\) in Solvent No. 2 and 3. \(^g\) Saturated Solution in Solvent No. 1 and 2. \(^h\) \(5 \times 10^{-3} \text{ M}\) in Solvent No. 1 and 2.

Table 2. The lowest triplet energies of some different classes of compounds used as sensitizers. \(^a\) Taken from: A. A. LAMOLA, in: ref. 11, p. 92. \(^b\) Estimated value. \(^c\) A. A. LAMOLA, M. GUERON, T. YAMANE, J. EISINGER, and R. G. J. SHULMAN, Chem. Phys. 47, 2210 [1967].
aliphatic ketones. The aromatic ketones, on the other hand gave only poor yields of \( \bar{\text{T}}T \). Ethyl acetooacetate and dihydroxy acetone, we believe, are examined and reported for the first time as sensitzers in studies involving thymine dimerization.

The low sensitizing ability of the aromatic ketones can be rationalized in two ways:

1. **The concentration effect.** The aromatic ketones are practically insoluble in water. With acetophenone, concentrations up to \( 10^{-2} \text{ M} \) can be obtained, whereas with the other aromatic ketones used in this investigation, maximum concentration between \( 10^{-2} - 10^{-4} \text{ M} \) could be obtained. The sensitizing efficiency of a donor, however, is concentration dependent. In order to increase the solubility of some of these sensitizers, water/ethanol and water/dioxane mixtures were used as solvents for dissolving thymine and the sensitizers. In spite of the increased solubility of the sensitizers, no measurable improvement of the \( \bar{\text{T}}T \) yields could be observed after irradiation. One of the possible explanations of this failure lies in the known photo-reduction of aromatic ketones in some organic solvents, where hydrogen abstraction can become an important reaction, yielding pinacols as major products. With aliphatic ketones as sensitizers, however, appreciable yields of \( \bar{\text{T}}T \) were obtained even in water/ethanol or water/dioxane solutions (see Table 1).

2. **The nature of the exited states of the aromatic ketones.** The nature of the excited state of a molecule (n, \( \pi^* \) or \( \pi, \pi^* \)) can influence the sensitizing ability of a ketone to a much greater degree than its solubility property. The n, \( \pi^* \) state, even of the alkyl ketones, possesses some \( \pi, \pi^* \) character. The triplet state of such a molecule can be described as

\[
T_1 = \alpha(n, \pi^*) + \beta(\pi, \pi^*)
\]

where \( \alpha \) and \( \beta \) are factors expressing the extent to which each configuration n, \( \pi^* \) or \( \pi, \pi^* \) contributes to the actual \( T_1 \) state of the molecule. For acetone or ethylacetooacetate \( \alpha \) is significantly greater than \( \beta \). Only in the case of n,\( \pi^* \) states, however, is the triplet excitation energy largely localized in the carbonyl group. Furthermore, an n, \( \pi^* \) excitation results in a decrease of negative charge at the oxygen atom of the carbonyl group whereas \( \pi, \pi^* \) excitation causes an electronic shift toward the oxygen of the carbonyl and results in an increased negative charge on the oxygen. Conjugation of a carbonyl group with an aromatic nucleus, or an electron releasing group (e.g., methoxy) reduces the positive character of the excited carbonyl oxygen and lowers the energy of the \( \pi, \pi^* \) transition to a larger extent than that of the corresponding n, \( \pi^* \) transition. In the latter case the photochemistry typical for the n, \( \pi^* \) state may be altered or become negligible.

This reasoning implies that in the case of aromatic ketones: a) the excited carbonyl oxygen is less electrophylic than in the corresponding aliphatic ketones; b) the excitation energy is delocalized into the \( \pi \) system and may therefore not be available to overcome the activation energies for reaction at the carbonyl moiety.

Based on these considerations and the results obtained in this work, we can suggest that one of the requirements of ketones to induce sensitized pyrimidine dimerization is that their triplet state energy should be highly localized in the carbonyl group. The electron-deficient carbonyl oxygen is the primary reaction site in this type of photosensitization, and it reacts with the pyrimidine molecule in its ground state yielding a complex of the type I (Fig. 1). This concept is also supported by the fact that the cross sections for triplet-triplet energy transfer in solutions are of the order of molecular diameters, so that electronically excited molecular complexes appear as quite likely intermediates. Recently some radical complexes in the photoreduction of benzophenone, involving hydrogen bondings, have also been reported. In irradiated solutions, the occurrence of species characteristic of the acetone photochemistry in the gas phase (i.e. the production of methyl radicals) has been eliminated.

Having all this in mind, we can postulate that the energy transfer in the case of the photosensitized dimerization of pyrimidines does not occur through a simple physical mechanism, as it was proposed generally for some photosensitized reactions in solution. Simple triplet energy transfer might not be involved in this dimerization since some sensitizers (e.g., 4-methoxy-acetophenone, xanthene-9-one and others listed in Table 1) having triplet energies higher than thymine did not show any significant ability for thymine dimerization. Our results, however, support the “relay” mechanism for sensitized reactions given by Schenck; they are also in general accordance with the findings of some other investigators in this field.
The failure of 4-methoxy-acetophenone to promote thymine dimerization can be explained by the observation that its lowest triplet state has predominantly a $\pi, \pi^*$ configuration. The molar absorbance (300 nm) of 4-methoxy-acetophenone is about three times greater than the corresponding value for acetophenone. It should be also realized, that in some cases the solvent can play a major role in establishing the nature of the excited state. Xanthene-9-one in hydrocarbon solvents gives $n, n^*$ whereas in polar solvents the $\pi, \pi^*$ population prevails. Carbazole showed a low sensitizing activity and it is likely that the excited carbazole molecule, through the lone electron pair on the nitrogen, can react with a pyrimidine molecule in its ground state.

The observation that ethylacetoacetate and dihydroxy acetone are efficient in inducing photosensitized thymine dimerization is of considerable interest in as much as these types of compounds are always present in biologically active tissues as intermediates of carbohydrate and fatty acid metabolism. Urocanic acid (UCA) on the other hand, did not show any sensitizing property to promote pyrimidine dimerization under the experimental conditions applied in this work. It would thus appear that UCA does not play any role of a sensitizer in inducing TT formation under in vivo conditions; the absorbed ultraviolet energy by UCA is probably used to induce cis-trans isomerizations. The results obtained in this preliminary investigation should be helpful in predicting which compounds and chromophores, normally present in biological material, can be active in promoting photosensitized pyrimidine dimerizations in DNA of skin with wavelengths particularly between 290 – 320 nm.

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6 The values listed in this Table have been obtained from phosphorescence spectra recorded at 77 °K. It should be understood that these values are not strictly applicable to aqueous solutions at room temperature.
7 As reported by: W. G. Herdstreeter, A. A. Lamola, and G. S. Hammond, J. Amer. chem. Soc. 86, 4537 [1964].