

THE UNIVERSITY OF MANITOBA

"PHOTOCYCLOADDITIONS OF 2',3',5' - TRIACETYLRIDINE"

by

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the University of Manitoba in partial fulfillment of the requirements
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ABSTRACT

Photocycloadditions of different alkenes to 2',3',5' - triacetyluridine are described. The addends used include: cyclohexene, tetramethylethylene, isopropenyl acetate, 1,1-diethoxyethylene, diphenylacetylene and vinylene carbonate. The yields of photocycloadducts are high except in the case of diphenylacetylene which failed to undergo cycloaddition to the substrate.

Product characterization and stereochemical studies on selected adducts are discussed.

The synthesis of ribothymidine by the conversion of 2',3',5'-triacetyluridine — vinylene carbonate adduct was explored in detail. (5-Carboxymethyl)-2',3',5'-triacetyluridine was also synthesized and its decarboxylation to triacetylribothymidine attempted.

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INTRODUCTION

It is well known that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) play an important role in living cells. Since it is also known that ultraviolet irradiation of many microorganisms leads to mutations and death¹, the effect of ultraviolet irradiation on pyrimidines and purines in DNA, RNA and on DNA itself has received attention from photobiologists and photochemists¹⁻⁴. Indeed, the past few years have witnessed an immense effort made to understand the nature and chemical change caused by ultraviolet irradiation on the aforementioned substrates. A review of the literature reveals that most workers chose to begin their investigation by studying the constituent purine and pyrimidine bases rather than DNA and RNA hoping that the photochemical and photobiological results obtained could be appropriately applied to the larger molecules. This assumption seems reasonable and secure since, for example, the absorption spectrum of double-stranded DNA closely resembles the sum of the absorption spectra of the constituent purine and pyrimidine bases in shape but is about 30% less intense⁵.

While reviews emphasizing the biological^{1,6-9} and physical⁴ aspects of the photochemistry of nucleic acids have appeared, it is

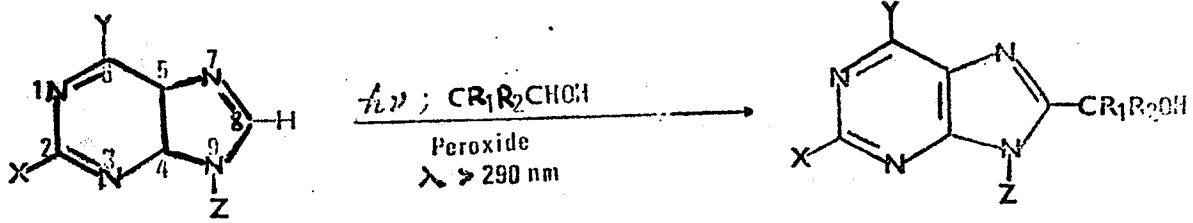
only appropriate here to briefly review the literature from a chemical point of view.

Although some work has been done on both purines and pyrimidines to relate their excited state electron distribution and bond order with some of the photoreactions observed¹⁰⁻¹², most experiments have been dealing with pyrimidines due largely to the relative insensitivity of purines toward ultraviolet light^{2,4}.

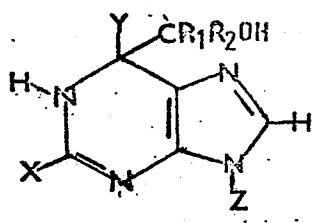
Purines

Purines have been shown to undergo photoalkylation when irradiated at $\lambda > 290$ nm with alcohol in the presence of peroxide. The reaction, therefore, is a radical - addition type. Irradiation results in light - induced fragmentation of the peroxide into free radicals which abstract a hydrogen from the solvent, thus generating alcohol free radicals. These radicals are then scavenged by a purine molecule to yield the photoalkylated products. The addition site on the purine was shown to be mainly at C₈^{13,14}, but adducts at C₆ were also found¹⁵.

Dye-sensitized photooxidation was observed in purine bases.



- X = H, NH₂
- Y = OH, NH₂
- Z = ribose, deoxyribose



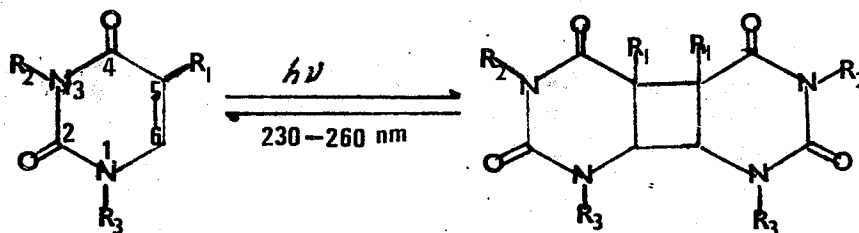
When the solutions of purine containing any one of a variety of dyes were exposed to light of a wavelength absorbed by the dye, the bases were photooxidized. Such a phenomenon is referred as photodynamic action where irradiation is carried out usually, though not always, in the presence of oxygen or air. Sometimes only a particular base was selectively photooxidized^{2,16}, and oxidation products indicated that both rings of the purine bases were involved. It should be noted that dye-sensitized photooxidation was also observed in uracil and a number of its derivatives².

Pyrimidines

Photochemistry of the pyrimidine bases have been more exten-

sively studied than the purines. Most experiments have been centered on photodimerization and photohydration. Others such as photoaddition, photoreduction and photocycloaddition have received relatively less attention.

Irradiation of pyrimidines at 230 nm - 260 nm in either frozen or liquid state leads to the formation of photodimers of cis-fused cyclobutane type. Dimers of different stereoisomeric forms have been isolated and characterized in many instances. Photodimerization of pyrimidines of this type have been extended to polynucleotides, DNA, RNA and a number of other pyrimidine derivatives.



$R_1 = \text{H, CH}_3, \text{Halogen}$

$R_2 = \text{H, CH}_3$

$R_3 = \text{H, CH}_3, \text{ribose, deoxyribose}$

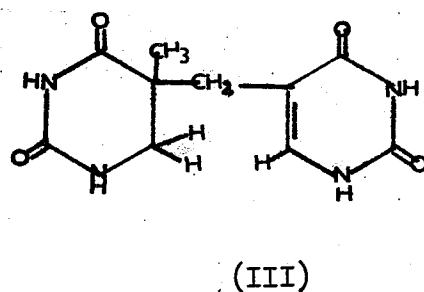
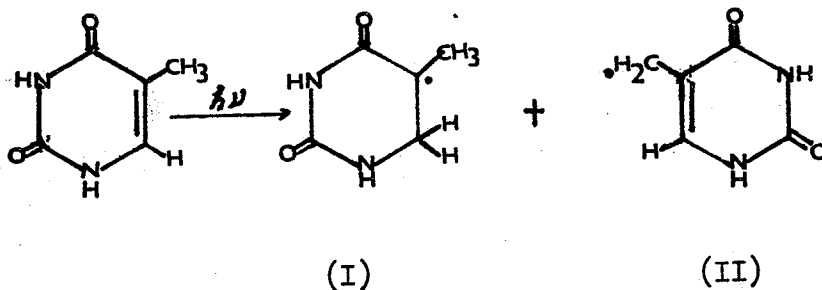
Heterodimers such as the thymine - uracil mixed dimer have also been prepared. Most recently¹⁷, work has been done to prepare photodimers of specifically controlled conformation at the cyclobutane ring

junctions.

When a photodimer was irradiated at the same wavelength as it was prepared the dimer was shown to split into its monomeric components. Such photoreversibility has long been used as one of the various methods for the detection of dimer formation.

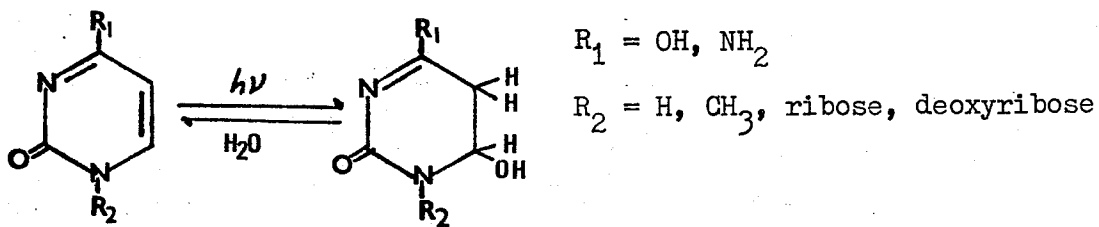
Photodimerization has been shown to take place between one molecule in its lowest excited triplet state and another in its ground state¹⁸⁻²¹. In concentrated solutions or in frozen solutions²⁴ singlet state molecules were also involved.

Another type of photodimer produced via the radical addition pathway was observed in thymine²⁵⁻²⁶. Thus irradiation of thymine produced a thymyl radical (I) by addition of a hydrogen atom at the C₆ position. In situations, such as may exist in dry or frozen DNA or thymidine, the methyl group of an adjacent thymine residue might serve as the hydrogen donor. The resulting thymynyl radical (II) added to the thymyl radical forming the stable adduct (III). Adducts of this type are referred to as "spore products" which are produced upon irradiation of spores and of dried DNA²⁶⁻²⁷. Radical formations were detected in ultraviolet excitation of pyrimidines in frozen solutions²⁸⁻²⁹ and in particular, thymyl radical formation has been



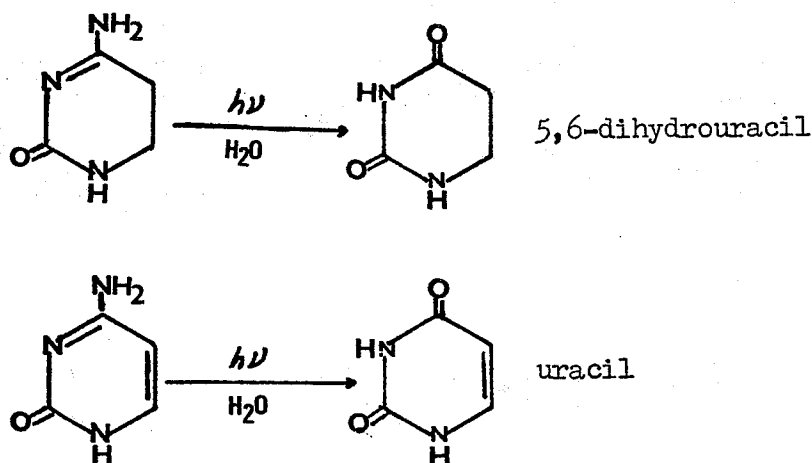
reported by ESR studies on DNA and thymine irradiated by violet light²⁹⁻³⁰.

Along with photodimerization, the photohydration reactions of pyrimidines have a long history and received considerable research efforts. Despite this, the mechanism of photohydration remains obscure². The "photohydrates" are 5,6-dihydro-6-hydroxyl pyrimidines, in which a water molecule is added across the C₅-C₆ double bond with the OH group going to the C₆ - position.



The photohydrates are thermally reversible and dehydrated at high concentrations of hydrogen ions or hydroxyl ions. As the photoreversibility is characteristic of photodimers, the thermal reversibility is characteristic of photohydrates. This is useful for structure elucidation in systems where both photodimer and photohydrate are formed. The photohydration reactions are believed to involve only singlet state intermediates, and they are pH dependent. Excellent discussions on the excited state and possible mechanisms involved are given by Burr² and by Lamola⁴.

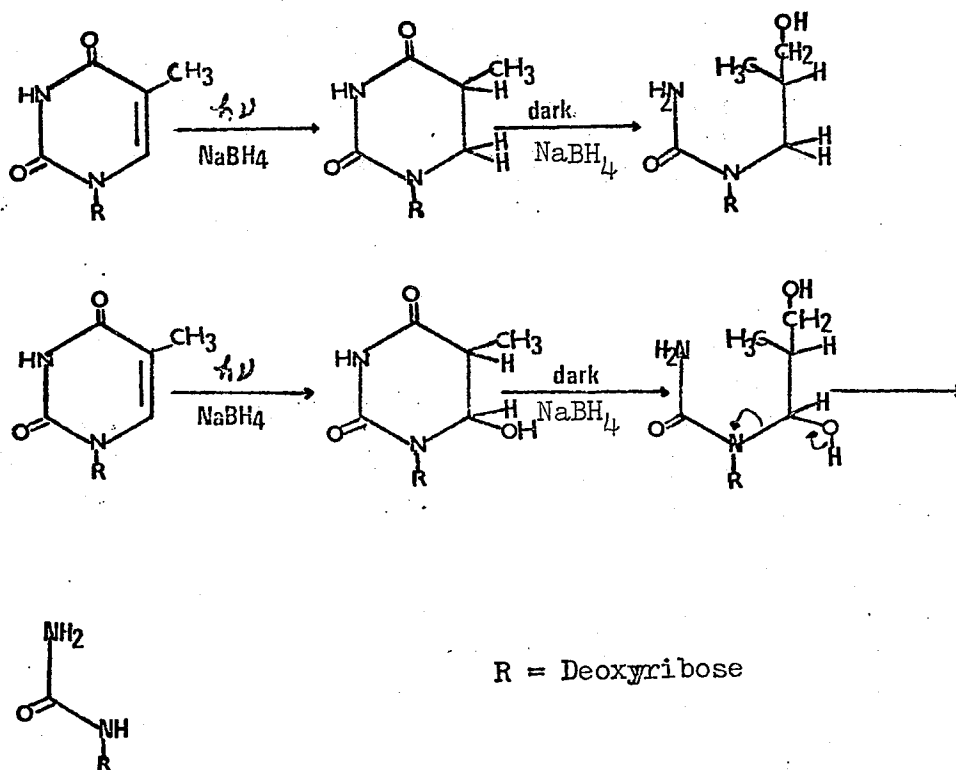
In the cases of cytosine³³, cytidine and their polynucleotides³⁴, the formation of the photohydrate, and thus the saturation of the C₅-C₆ double bond labilizes the amino group in the C₄-position and leads



eventually to the displacement of amino group by a hydroxyl group.

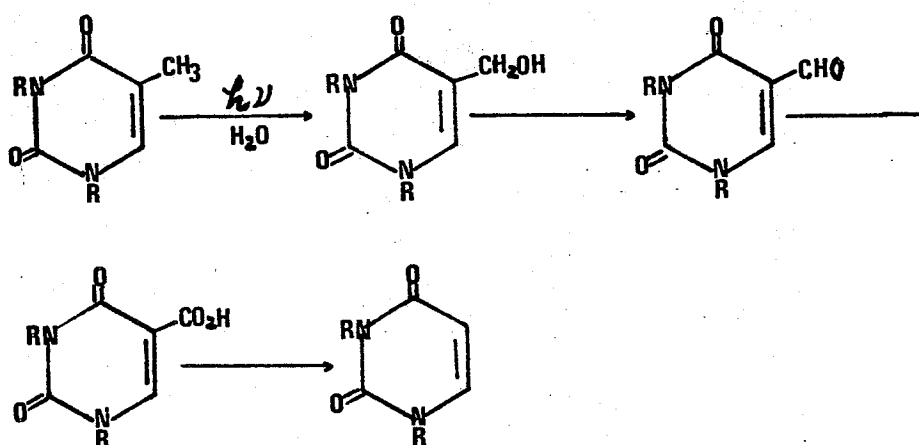
It should be emphasized however, that photodeamination need not necessarily proceed through a photohydrate intermediate. Thus photolysis of 5,6-dihydrocytosine in water gave 5,6-dihydrouracil³² while cytosine gave uracil³³.

Photoreduction of thymidine with sodium borohydride produces γ -hydroxypropyl urea. In the presence of water, the photohydrates was suggested to be the intermediate formed which served as a precursor for the formation of N-deoxyribosyl urea in the subsequent dark reaction³⁵⁻³⁶.

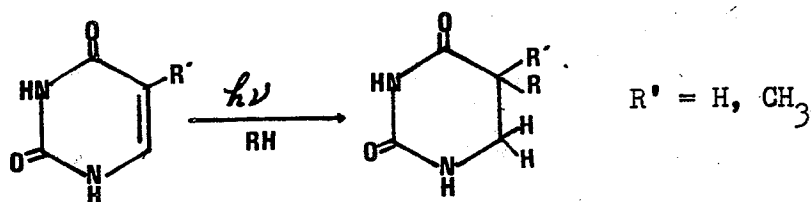


Photoreduction of uridine and dihydrouridine was found to proceed in a similar manner.

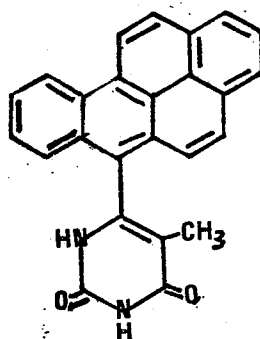
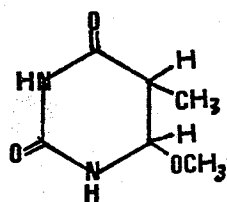
Apart from the dye-sensitized photooxidation mentioned previously, relatively few examples of photooxidation reactions are known for pyrimidines. When thymine or 1,3-dimethylthymine was irradiated in aqueous solution, the corresponding 5-hydroxymethyl uracil, 5-formyl uracil and 5-carboxyuracil were formed³⁷.



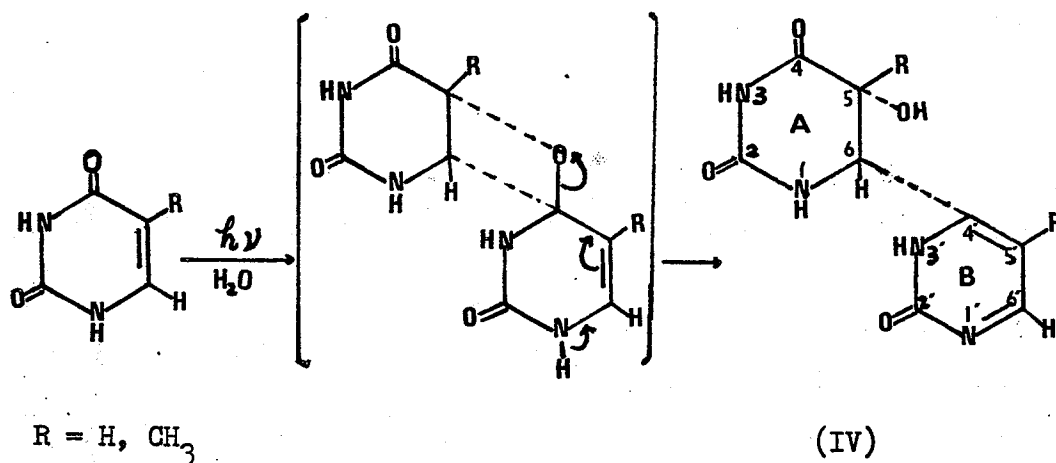
Photoaddition reactions of pyrimidines usually resulted in adducts where the R group (from an added RH) attached to C_5 while the H atom attached to C_6 . Thus irradiation of thymine or uracil in the presence of cysteine gave 5-cysteinyl-6-hydrothymine³⁸ or 5-cysteinyl-5,6-dihydrouracil³⁹, respectively. Similar adducts were obtained in the case of cytosine³³. Recently⁴⁰, glutathione was found to undergo similar addition reactions with uracil and thymine.



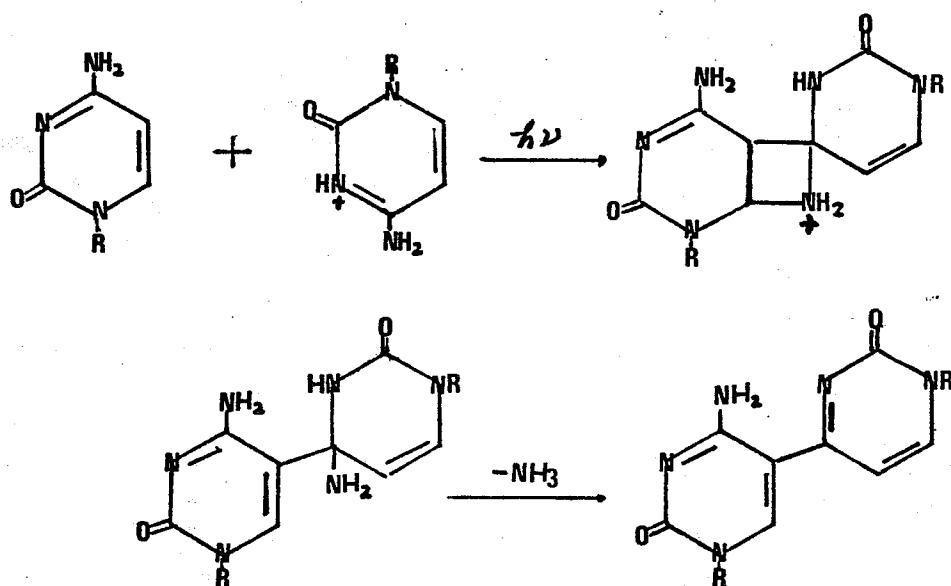
However, exceptions are known. For example, when 5% methanol was present in the irradiation of aqueous solutions of thymine, the OCH_3 added to C_6 and H to C_5 . The photoaddition of 3,4 - benzopyrene to thymine also yielded the 6-substituted derivative⁴¹.



Another type of photoaddition in pyrimidines involves the formation of oxetane. Such oxetane intermediates have generally not been isolated⁷², but are proposed to account for certain photoadducts obtained. Therefore, the final photoadduct isolated had the general structure as shown in (IV)⁴²⁻⁴⁵. The structure of (IV) has been well characterized by spectral data and in the case of the thymine adduct, by X-ray studies as well. The 5-hydroxy group was found to

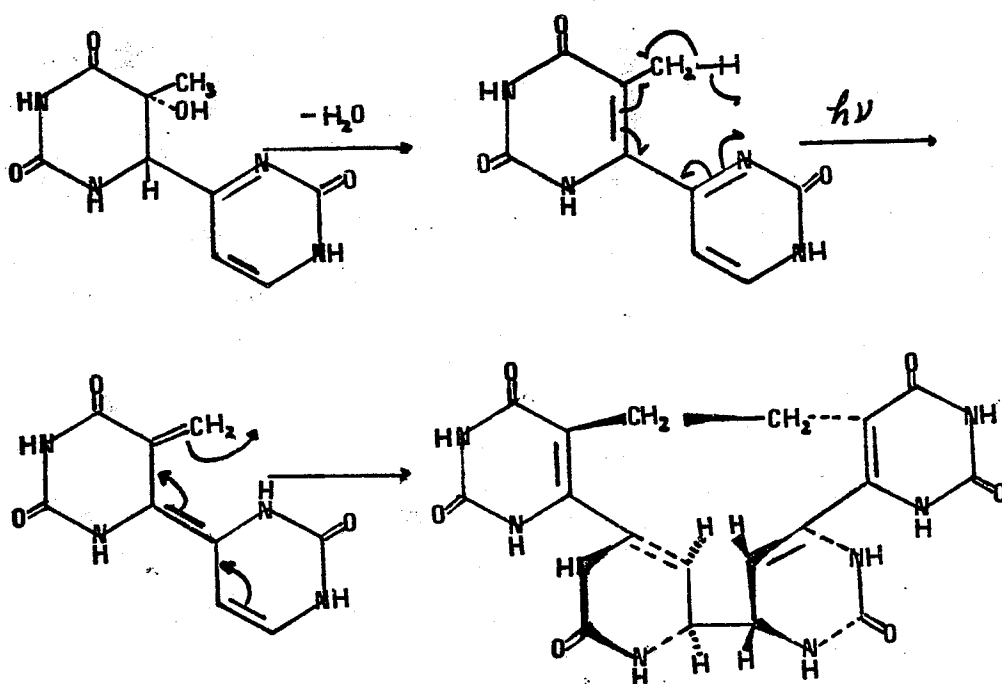


be trans to the C₆-hydrogen as expected; the N-hydrogen in ring B was located at N₃, rather than N₁. . A photoadduct of mixed pyrimidine type such as the thymine - uracil adduct was also observed in which the C₅ - C₆ double bond of the thymine ring was saturated with the uracil ring adding to C₆ and hydroxy group at C₅⁴⁶. Similar adducts were obtained for cytidine. However, an oxetane analogue involving



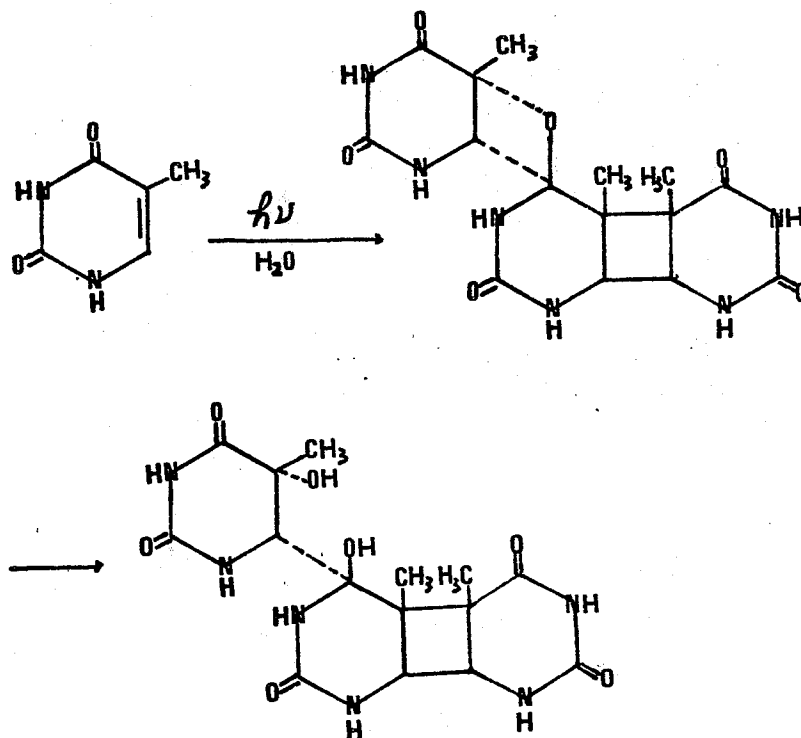
cycloaddition between a double bond of one cytosine ring and an amino nitrogen was virtually without precedent^{47-48a}.

All photoadducts produced via the oxetane intermediates underwent dehydration regenerating the C₅-C₆ double bond in ring A. Further photolysis of such dehydrated products provided interesting chemistry. For example, a thymine - uracil tetramer was isolated from thymine - uracil dimer on irradiation in an aqueous solution at 360 or 313 nm⁴⁹. The authors suggested that the tetramer arose by 1,6 - head - to - head tail - to - tail dimerization; formed by γ -hydrogen transfer analogous to the Norrish Type II process in carbonyl photochemistry.



Equally interesting was the formation of a phototrimer isolated from thymine on irradiation in frozen solution at 254 nm⁵⁰⁻⁵¹. The trimer was generated by hydrolysis of the oxetane - containing trimer. It was suggested^{48b} that the oxetane-containing trimer was formed by addition of thymine to a carbonyl of a thymine-cyclobutane dimer.

However, a mechanism in which a third thymine molecule cyclo-added to the double bond of an oxetane dimer seems more likely.

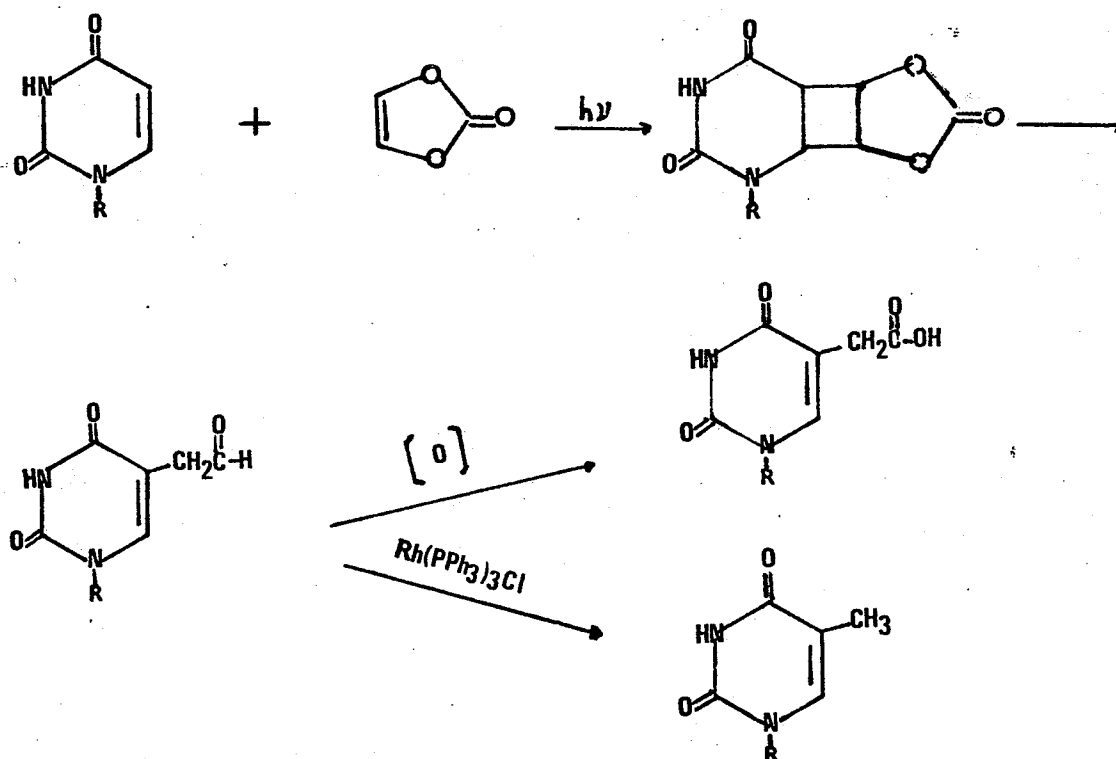


Because of the possible involvement of thietane intermediates in the photochemical cross-linking of 4-thiouridine with cytidine in several *E. coli* tRNAs, light induced interaction between 4-thiouracil derivatives and electron deficient olefins have been studied⁵². Such thietane products have been isolated and characterized.

Photocycloaddition reactions between pyrimidines and olefins are relatively little known. In fact, when this project was begun, only a few such examples were known to us. Some examples were the reactions between: orotic acid and acrylonitrile⁵³; uracil and vinylene carbonate; thymine and skin-photosensitized furocoumarins⁵⁵; and between 1,3-dimethyl uracil and the following olefins: (a) tert-butyl vinyl ether, (b) vinyl acetate and (c) ketene diethyl acetal by Swenton and his co-workers⁵⁶. In 1974, a detailed report by Swenton et al⁵⁷ has appeared. Also another paper has appeared recently on the photocycloaddition of uracil to vinylene carbonate and on the subsequent rearrangement of the photoadduct⁵⁸.

The project was, therefore, designed to demonstrate the feasibility and efficiency of photocycloaddition between uridine and olefins. (So far only one example of photocycloaddition between a nucleoside and an olefin has been reported⁵⁹.) Attempts were made

were made to separate and characterize the different isomeric photocycloadducts formed. However, more emphasis was placed on their potential synthetic utility. The rearrangement of the cyclohexene-vinylene photocycloadduct to the corresponding 5-formylaldehyde derivative⁶⁰ was applied to uracil by Bergstrom et al⁵⁸ and to uridine by us. The availability of 5-formylmethyl triacetyluridine enabled us to synthesize ribothymidine (5-methyl uridine), another naturally occurring but less readily available nucleoside. Also obtained in this work is the 5-carboxymethyl triacetyluridine due to the oxidation of 5-formylmethyl triacetyluridine on silica gel. 5-Carboxymethyl uridine has been isolated from wheat and yeast tRNA.

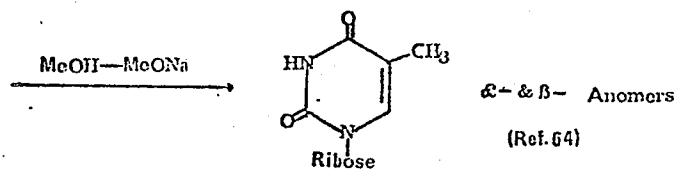
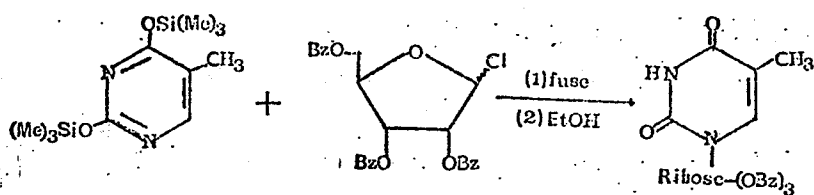
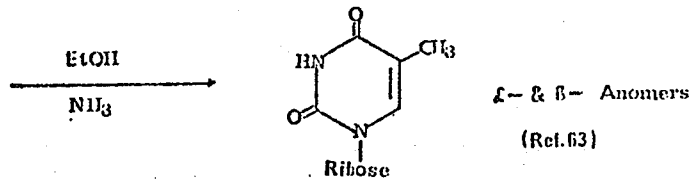
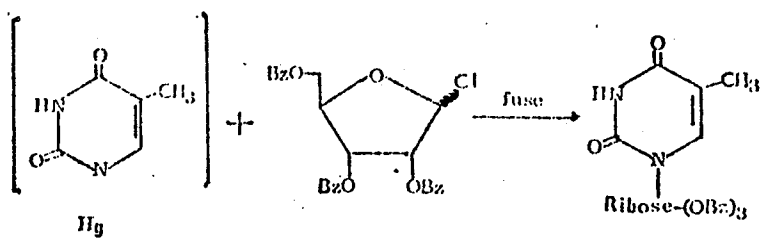


A search of the literature shows that ribothymidine has been synthesized by enzymatic methods⁶¹⁻⁶² or by such tedious conventional chemical methods as condensing the sugar moiety with thymine base. The products were a mixture of α - and β - anomers⁶³⁻⁶⁴. Direct synthesis from uridine is also known but the overall yield is low⁶⁵⁻⁶⁶.

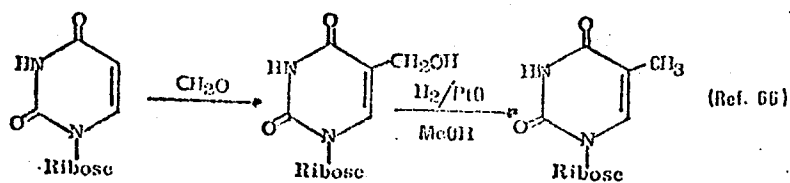
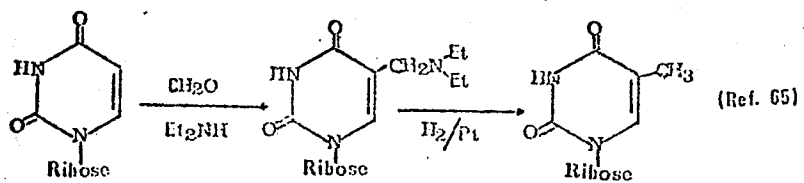
Both 5-carboxymethyl uridine and its corresponding methyl ester have been isolated from hydrolysates of bulk yeast tRNA⁶⁷⁻⁶⁹. The synthesis of 5-carboxymethyl uridine has been reported by Ivanovics et al.⁷⁰, whereas its methylation in vitro to give the corresponding methyl ester has been reported by another group of workers⁷¹. It is perhaps significant to mention that 2-thio-5-carboxymethyl uridine methyl ester has also been isolated from yeast tRNA, and its structure was confirmed by comparison with a synthetic compound.

Our observation of the oxidation of 5-formylmethyl triacetyl uridine to 5-carboxymethyl triacetyluridine on air-exposed silica gel TLC plates indicates the possibility of such conversion by chemical oxidation in the laboratory. Work is continuing in this area of the photocycloadduct.

The potential usefulness of such photocycloadducts leads to exciting speculation of synthesizing natural nucleic acid components



Bz = Benzoyl



via suitable photocycloadducts created from appropriately chosen olefins. The synthesis of natural nucleosides and their analogues via photoadduct pathways is important not only for synthetic work, but the photoadducts themselves might be of biological and chemotherapeutical importance.

DISCUSSIONS

As mentioned in the Introduction, the object of this project was, in part, to demonstrate the feasibility and the efficiency of photocycloaddition between uridine and different olefins. In 1971, Krajewska and Shugar had reported the photocycloaddition of propylene to uridine⁵⁹. In as much as we can find from the existing literature, Shugar's work is the only example of photocycloaddition between a nucleoside and olefin where the photocycloadducts were actually prepared, isolated and characterized.

This discussion will proceed in three parts: firstly, the characterization of photolysis products in general; secondly, the attempts at stereochemical investigation of selected photocycloadducts; and finally, the synthesis of ribothymidine and its implication in the synthetic aspects of natural nucleosides by photocycloadduct rearrangement.

I. Photocycloaddition

Uridine, like its free base, is not soluble in most organic solvents and early work on photoreactions of this compound was conducted in aqueous solution. In order to carry out photocyclo-

additions to the less polar alkenes it was necessary to modify the nucleoside. Our solution to this problem was to acetylate the hydroxyl groups in the ribose moiety. Acetylation is easily accomplished by stirring the pyridine solution of uridine with acetic anhydride overnight, followed by addition of methanol. The acetyl groups can be removed whenever needed by dissolving the acetylated compound in a solution of 15% ammonium hydroxide in methanol. The acetylation reaction proceeds usually in quantitative yield while deacetylation in a typical reaction gives 60 - 80% yield.

The acetylated uridine, 2',3',5'-triacetyluridine is readily soluble in most common organic solvents, however, some of these react photochemically with uridine. It has been found, for example, that irradiation of thymine aqueous solution in the presence of 5% methanol resulted in the formation of the methoxy adduct, 5,6-dihydro-6-methoxy thymine. Other hydroxylic solvents were also rejected on grounds that they too may undergo undesirable photochemical reactions. Preliminary photolysis showed that ethyl acetate was a suitable solvent to use. A very slow photoacetylation occurred to give N₃-acetylated photocycloadduct but such N₃-acetylated product is usually present in a negligible amount.

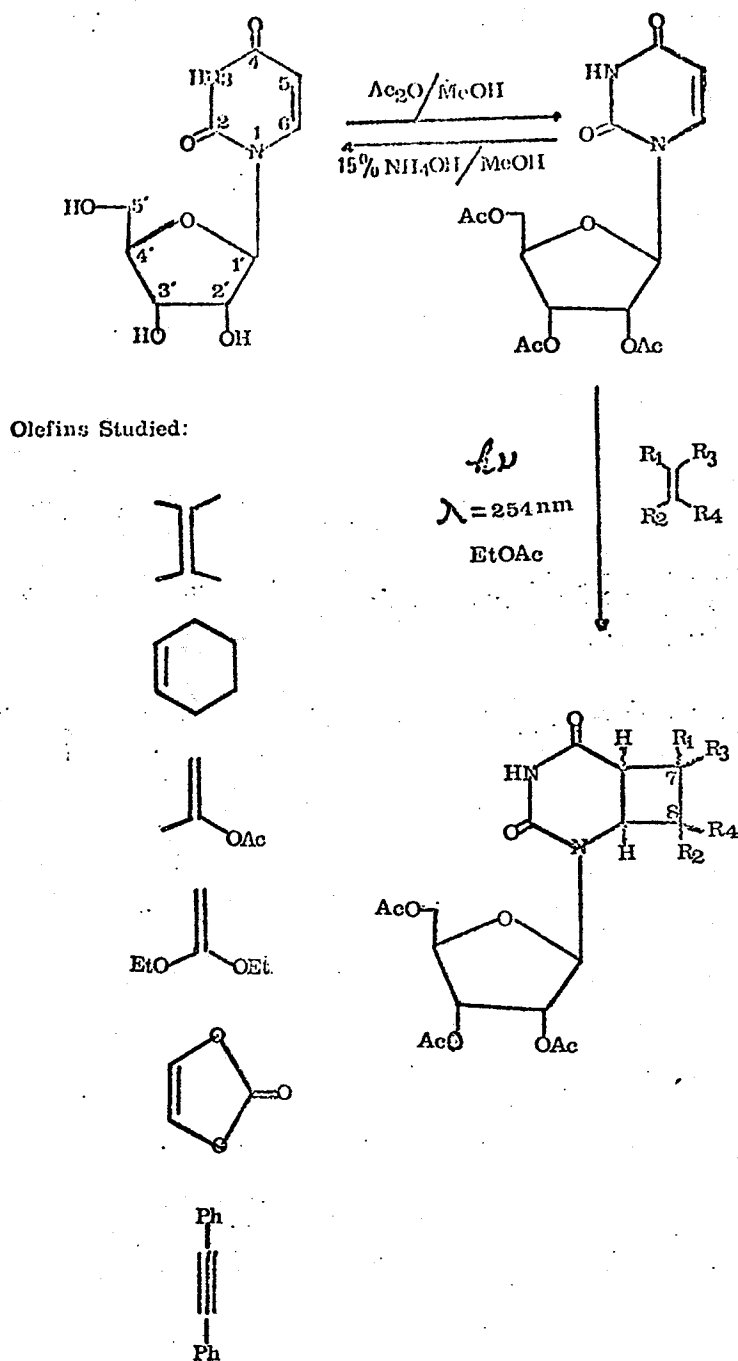


Figure 1. Photocycloadditions of various olefins to 2',3',5'-triacetyluridine

There are numerous citations in literature of the formation of different photoproducts on varying the irradiation wavelength⁷⁴. In the photodimerization studies of dimethylthymine in liquid solution, Morrison et al²² found that the percent composition of formationally different photoproducts was a function of both the excited state precursor (singlet or triplet) as well as the solvent. The percent contribution of each singlet and triplet state was, in itself, a function of solvent²² and solution concentration¹⁸⁻²². For these reasons the experimental conditions for photolysis were kept constant throughout the course of this study. The concentration of the 2',3',5'-triacetyluridine was 2×10^{-2} molar and that of the olefin was 2% by volume. Irradiations were carried out under nitrogen at $\lambda = 254$ nm (Ultraviolet products PCQXI) and at no time was a photosensitizer used.

Photocycloadducts were characterized by elemental analysis in combination with various spectroscopic methods. The cycloadduct formation was indicated by the saturation of the C₅-C₆ double bond, which led to the disappearance of UV absorption at $\lambda_{\text{max}} = 257$ nm (in EtOAc). The excess of olefin was removed by thin layer or column chromatography. Recrystallization of cycloadducts was