THE UNIVERSITY OF MANITOBA

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

by

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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 death[1], the effect of ultraviolet irradiation on pyrimidines and purines in DNA, RNA and on DNA itself has received attention from photobiologists and photochemists[1-4]. Indeed, the past few years have witnessed an immense effort made to understand the nature and chemical change caused by ultraviolet irradiation on the afroementioned 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 intense[5].

While reviews emphasizing the biological[1,6-9] and physical[4] aspects of the photochemistry of nucleic acids have appeared, it is

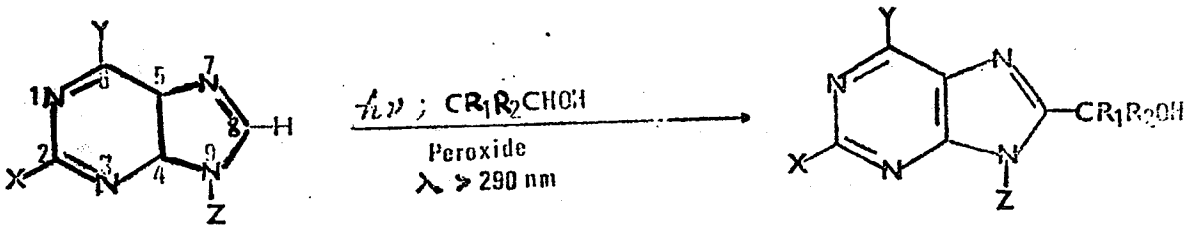
only appropriate here to briefly review the literature from a chemical point of view.

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 observed[10-12], 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 C_8[13,14], but adducts at C_6 were also found[15].

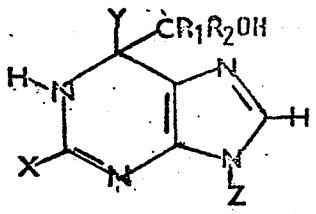
Dye-sensitized photooxidation was observed in purine bases.



X = H, NH_2

Y = OH, NH_2

Z = ribose, deoxyribose



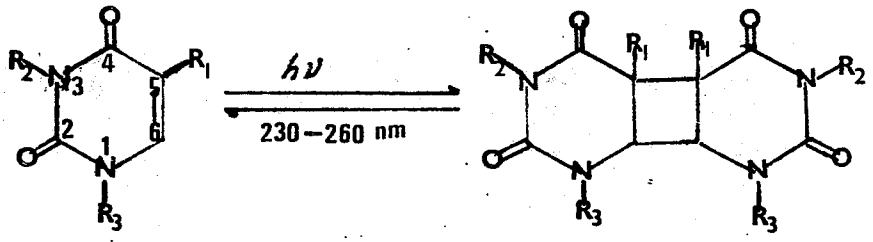
When the solutions of purine containing any one of a variety of dyes were exposed to light of a wavelength absorbed by the dye, the bases were photooxidized. Such a phenomenon is referred as photodynamic action where irradiation is carried out usually, though not always, in the presence of oxygen or 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 derivatives[2].

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 = H, CH_3, Halogen

R_2 = H, CH_3

R_3 = H, CH_3, ribose, deoxyribose

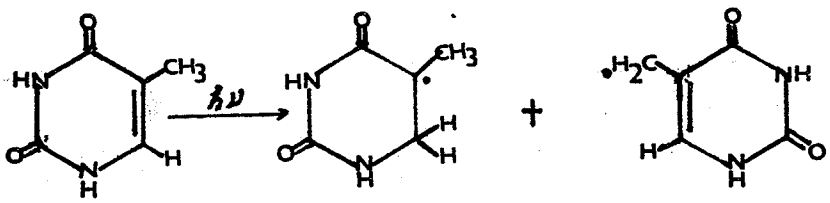
Heterodimers such as the thymine - uracil mixed dimer have also been prepared. Most recently[17], work has been done to prepare photodimers of specifically controlled conformation at the cyclobutane ring

junctions.

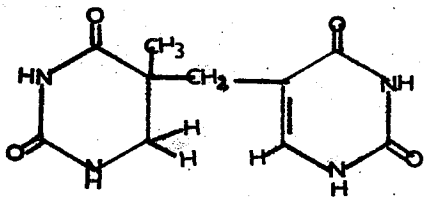
When a photodimer was irradiated at the same wavelength as it was prepared the dimer was shown to split into its monomeric components. Such photoreversibility has long been used as one of the various methods for the detection of dimer formation.

Photodimerization has been shown to take place between one molecule in its lowest excited triplet state and another in its ground state[18-21]. In concentrated solutions or in frozen solutions[24] singlet state molecules were also involved.

Another type of photodimer produced via the radical addition pathway was observed in thymine[25-26]. Thus irradiation of thymine produced a thymyl radical (I) by addition of a hydrogen atom at the C_6 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 thyminyl 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 DNA[26-27]. Radical formations were detected in ultraviolet excitation of pyrimidines in frozen solutions[28-29] and in particular, thymyl radical formation has been



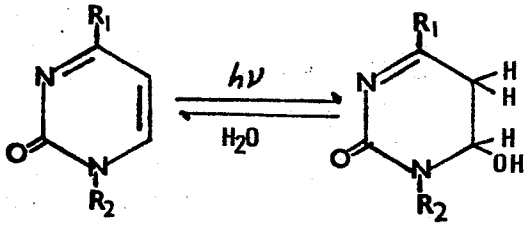
(I) (II)



(III)

reported by ESR studies on DNA and thymine irradiated by violet light[29-30].

Along with photodimerization, the photohydration reactions of pyrimidines have a long history and received considerable research efforts. Despite this, the mechanism of photohydration remains obscure [2]. The "photohydrates" are 5,6-dihydro-6-hydroxyl pyrimidines, in which a water molecule is added across the C_5- C_6 double bond with the OH group going to the C_6 - position.

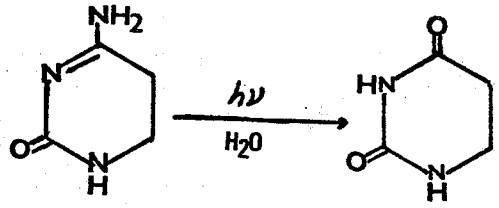


R_1 = OH, NH_2

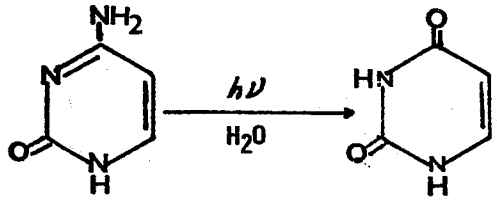
R_2 = H, CH_3, ribose, deoxyribose

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 Burr[2] and by Lamola[4].

In the cases of cytosine[33], cytidine and their polynucleotides[34], the formation of the photohydrate, and thus the saturation of the C_5-C_6 double bond labilizes the amino group in the C_4 -position and leads



5,6-dihydrouracil

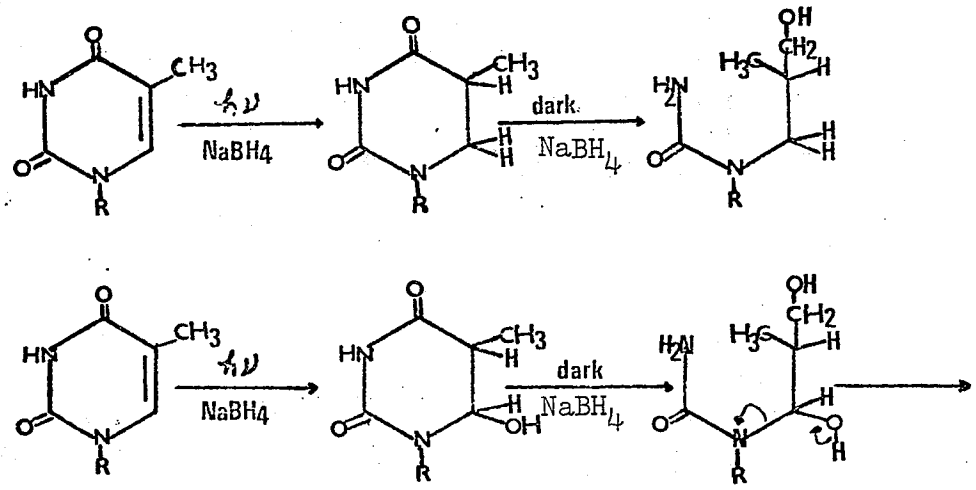


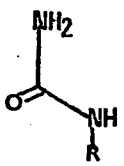
uracil

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-dihydrouracil[32] while cytosine gave uracil[33].

Photoreduction of thymidine with sodium borohydride produces Y- 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 reaction[35-36].

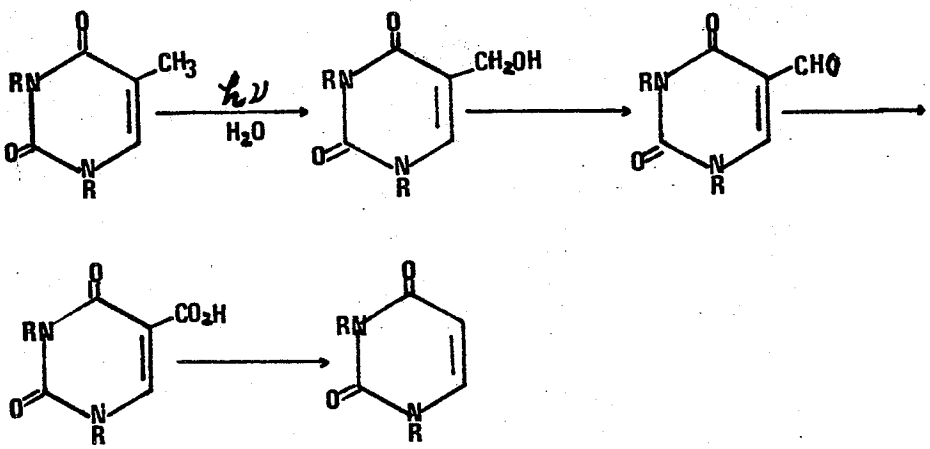




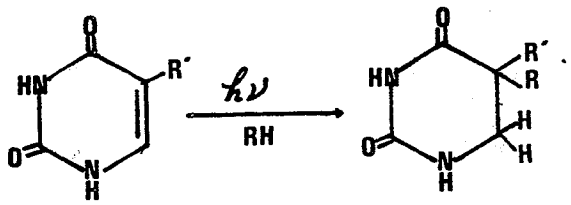
R = Deoxyribose

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 formed[37].

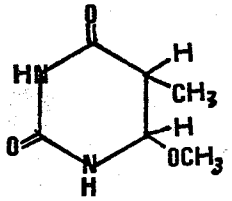


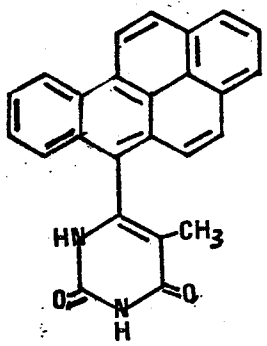
Photoaddition reactions of pyrimidines usually resulted in adducts where the R group (from an added RH) attached to C_5 while the H atom attached to C_6. Thus irradiation of thymine or uracil in the presence of cysteine gave 5 - cysteinyl - 6 - hydrothymine[38] or 5-cysteinyl-5,6-dihydrouracil[39], respectively. Similar adducts were obtained in the case of cytosine[33]. Recently[40], glutathione was found to undergo similar addition reactions with uracil and thymine.



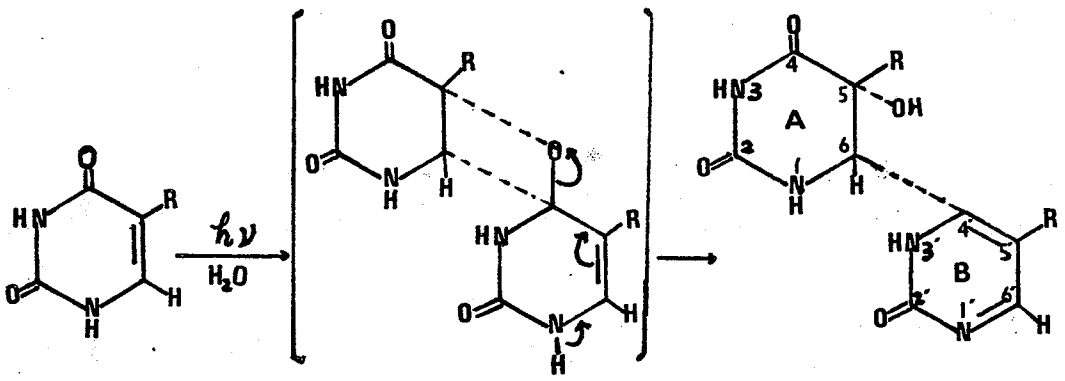
R' = H, CH_3

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 derivative[41].



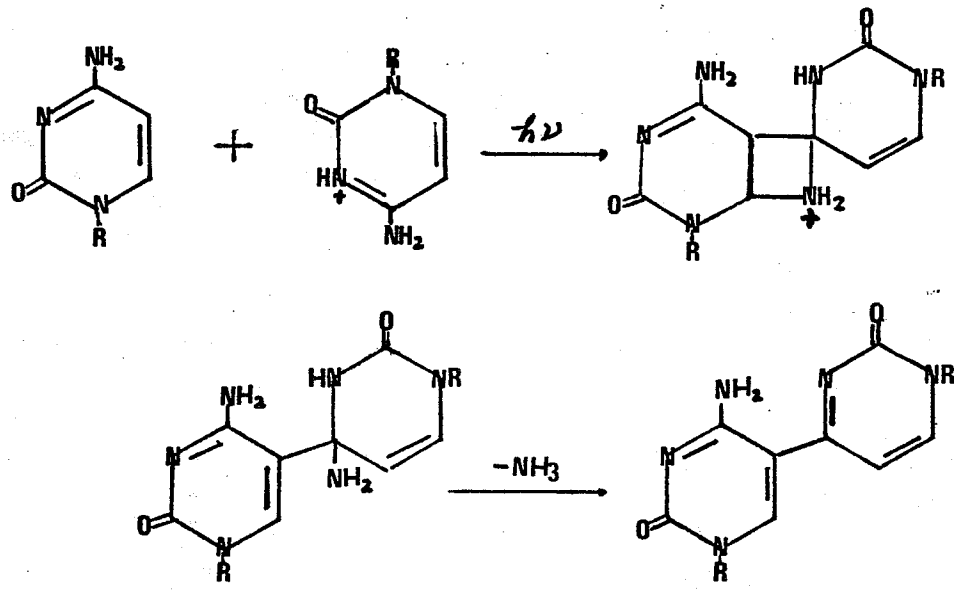


Another type of photoaddition in pyrimidines involves the formation of oxetane. Such oxetane intermediates have generally not been isolated[72], but are proposed to account for certain photoadducts obtained. Therefore, the final photoadduct isolated had the general structure as shown in (IV)[42-45]. 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



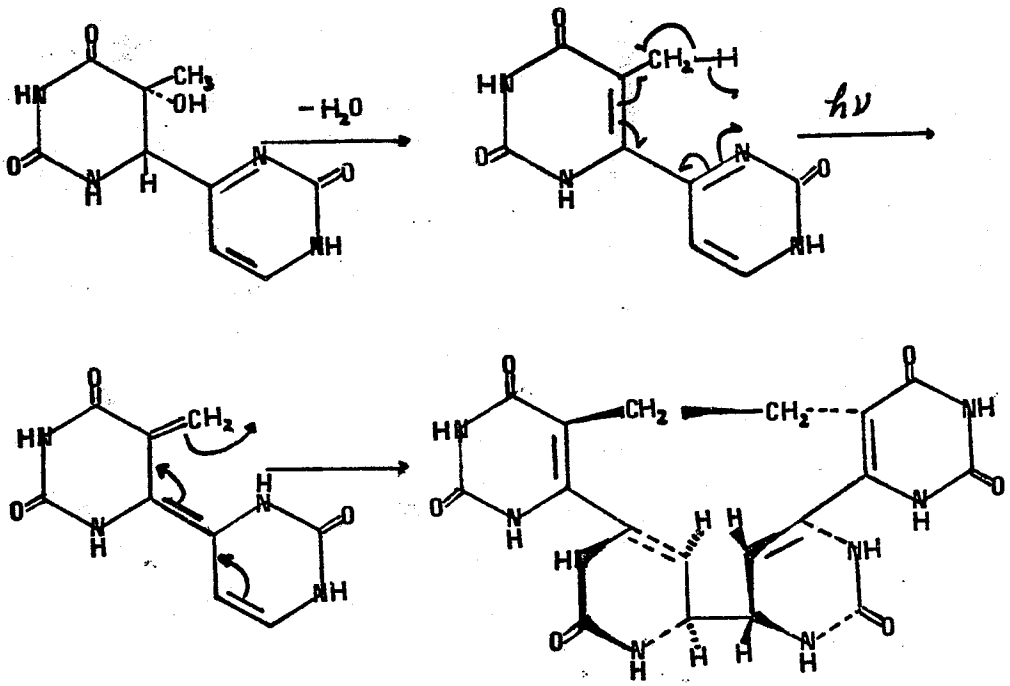
R = H, CH_3 (IV)

be trans to the C_6-hydrogen as expected; the N-hydrogen in ring B was located at N_3, rather than N_1. . A photoadduct of mixed pyrimidine type such as the thymine - uracil adduct was also observed in which the C_5 - C_6 double bond of the thymine ring was saturated with the uracil ring adding to C_6 and hydroxy group at C_5[46]. Similar adducts were obtained for cytidine. However, an oxetane analogue involving



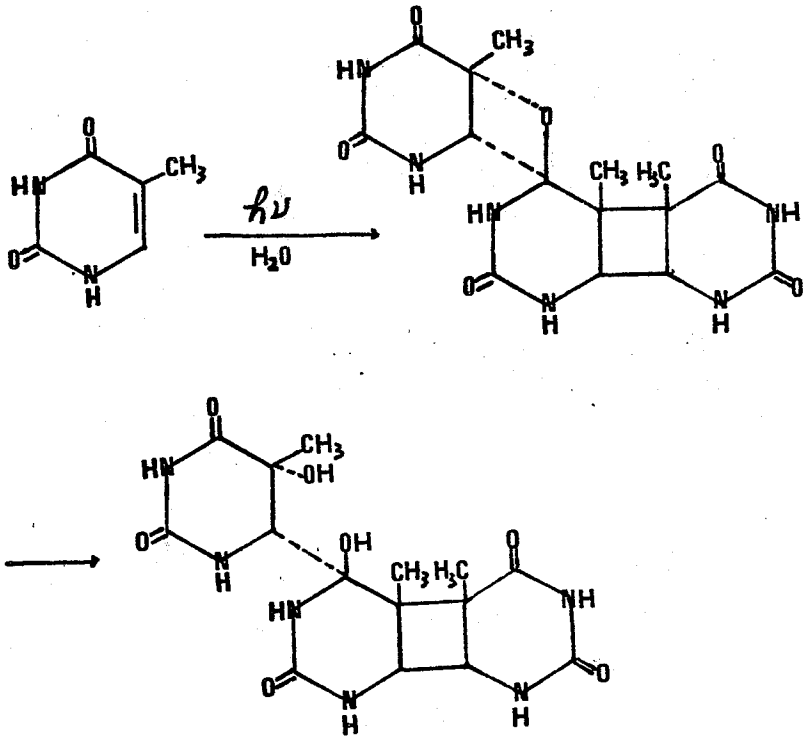
cycloaddition between a double bond of one cytosine ring and an amino nitrogen was virtually without precedent[47-48a].

All photoadducts produced via the oxetane intermediates underwent dehydration regenerating the C_5-C_6 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 nm[49]. 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 nm[50-51]. 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 cycloadded to the double bond of an oxetane dimer seems more likely.

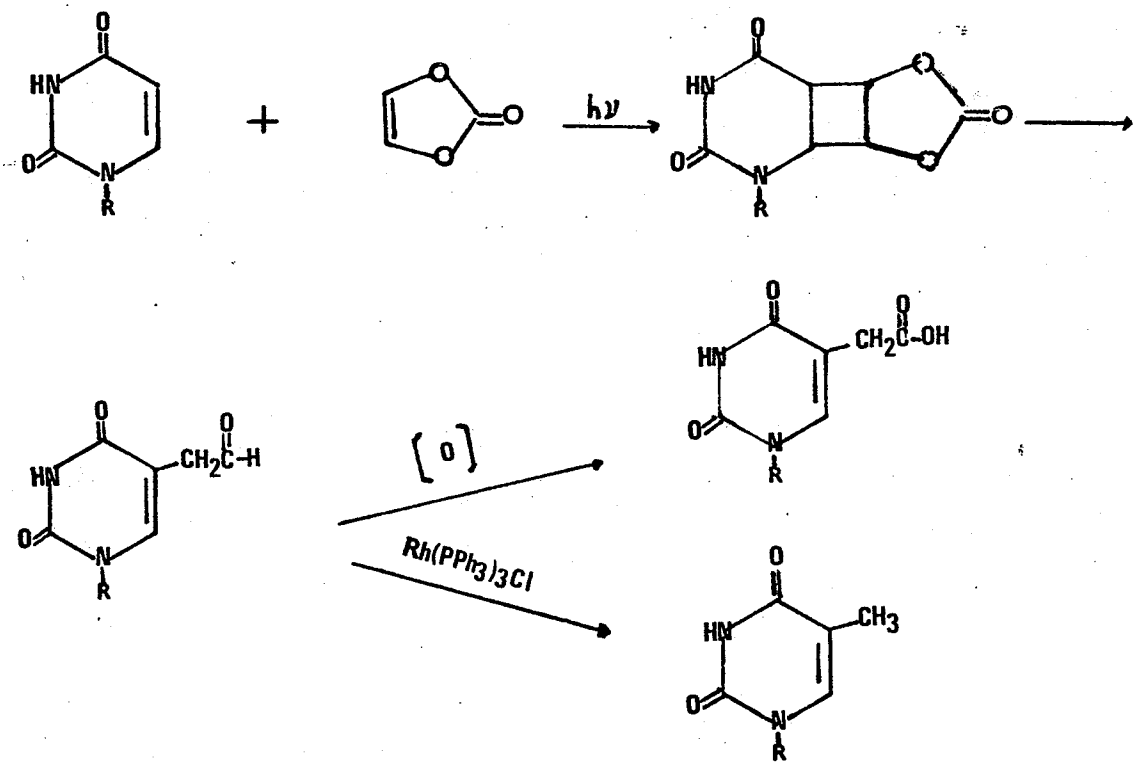


Because of the possible involvement of thietane intermediates in the photochemical cross-linking of 4-thiouridine with cytidine in several E.coli tRNAs, light induced interaction between 4-thiouracil derivatives and electron deficient olefins have been studied[52]. Such thietane products have been isolated 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 acrylonitrile[53]; uracil and vinylene carbonate; thymine and skin-photosensitized furocoumarins[55]; 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-workers[56]. In 1974, a detailed report by Swenton et al[57] has appeared. Also another paper has appeared recently on the photocycloaddition of uracil to vinylene carbonate and on the subsequent rearrangement of the photoadduct[58].

The project was, therefore, designed to demonstrate the feasibility and efficiency of photocyloaddition between uridine and olefins. (So far only one example of photocycloaddition between a nucleoside and an olefin has been reported[59].) 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 cyclohexane -vinylene photocycloadduct to the corresponding 5-formylaldehyde derivative[60] was applied to uracil by Bergstrom et al[58] and to uridine by us. The availability of 5-formylmethyl triacetyluridine enabled us to synthesize ribothymidine (5-methyl uridine), another naturally occuring 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.

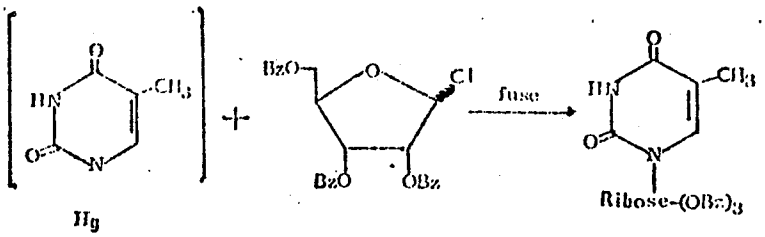


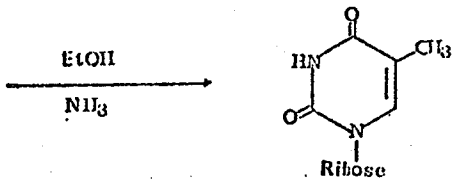
A search of the literature shows that ribothymidine has been synthesized by enzymatic methods[61-62] or by such tedious conventional chemical methods as condensing the sugar moeity with thymine base. The products were a mixture of α- and β- anomers[63-64]. Direct synthesis from uridine is also known but the overall yield is low[65-66].

Both 5-carboxymethyl uridine and its corresponding methyl ester have been isolated from hydrolysates of bulk yeast tRNA[67-69]. 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 workers[71]. 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.

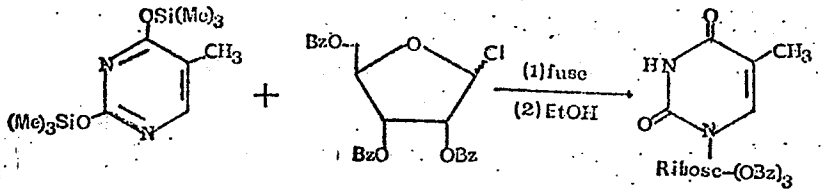
The potential usefulness of such photocycloadducts leads to exciting speculation of synthesizing natural nucleic acid components

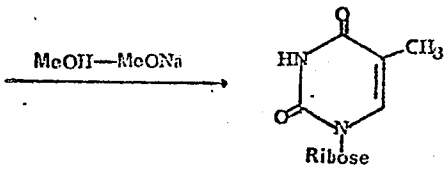




α– & β– Anomers

(Ref.63)

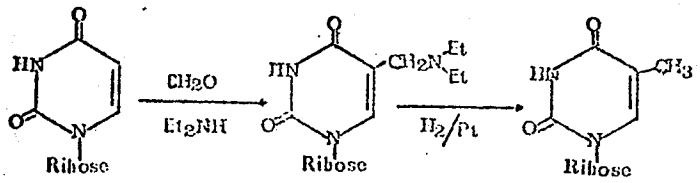




α– & β– Anomers

(Ref.64)

Bz = Benzoyl



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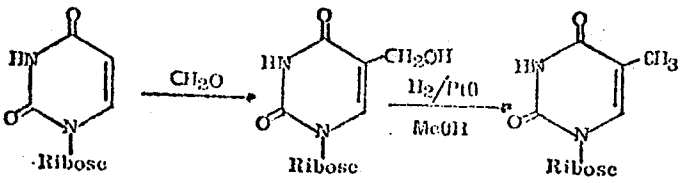


(Ref. 66)

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 uridine[59]. 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 photocloadduct 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 N_3-acetylated photocycloadduct but such N_3-acetylated product is usually present in a negligible amount.

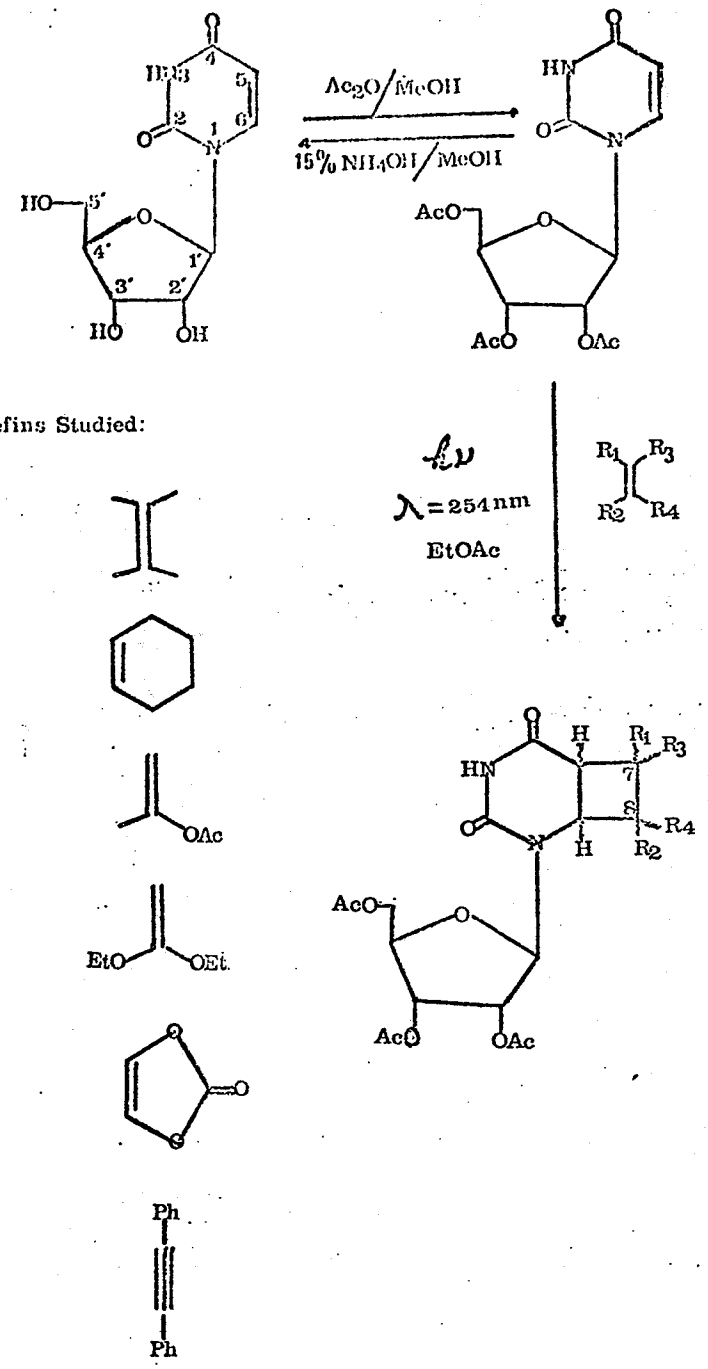


Figure 1. Photocycloadditions of various olefins to 2',3',5'-triacetyluridine

There are numerous citations in literature of the formation of different photoproducts on varying the irradiation wavelength[74]. In the photodimerization studies of dimethylthymine in liquid solution, Morrison et al[22] found that the percent composition of conformationally different photoproducts was a function of both the excited state precursor (singlet or triplet) as well as the solvent. The perecent contribution of each singlet and triplet state was, in itself, a function of solvent[22] and solution concentration[18-22]. 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 λ= 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 C_5-C_6 double bond, which led to the disappearance of UV absorption at λ_{max} = 257 nm (in EtOAc). The excess of olefin was removed by thin layer or column chromatography. Recrystallization of cycloadducts was

achieved by evaporation of its chloroform solution or by precipitation from an ether/hexane mixed solvent.

Mass spectrometry has been an indispensable tool for structural elucidation. Mass spectral evidence proved that the photoproducts obtained were 1:1 adduct of 2',3',5'-triacetyluridine and the olefins. Coupled with n.m.r. data which will be discused later, the adducts were shown to be cyclobutanyl derivatives fused at the nucleoside C_5-C_6 double bond and the olefin double bond. Mass spectral peaks of diagnostic value were those characterisitc to nucleosides as first studied by Biemann and McCloskey[75]. In addition to those characteristic nucleoside fragments, electron impact caused the parent photocycloadduct (P^+ or SBA^+) to "split" into the original triaceyluridine $[SB]^+$ and the olefin A (Figure 2). The relative intensity of the parent ion is usually low and sometimes even unobserved. This could be attributed to the low volatility of the adduct. Sometimes, instead of P^+. $[P + 1]^+$ was observed, probably due to chemical ionization taking place when the pressure in the ion chamber was high[76]. The same argument could be applied to the mass peak m/e 371, corresponding to the ion $[SB + H]^+$. Although hydrogen transfer from the olefin is possible,such a process is not preferred over the elimina-

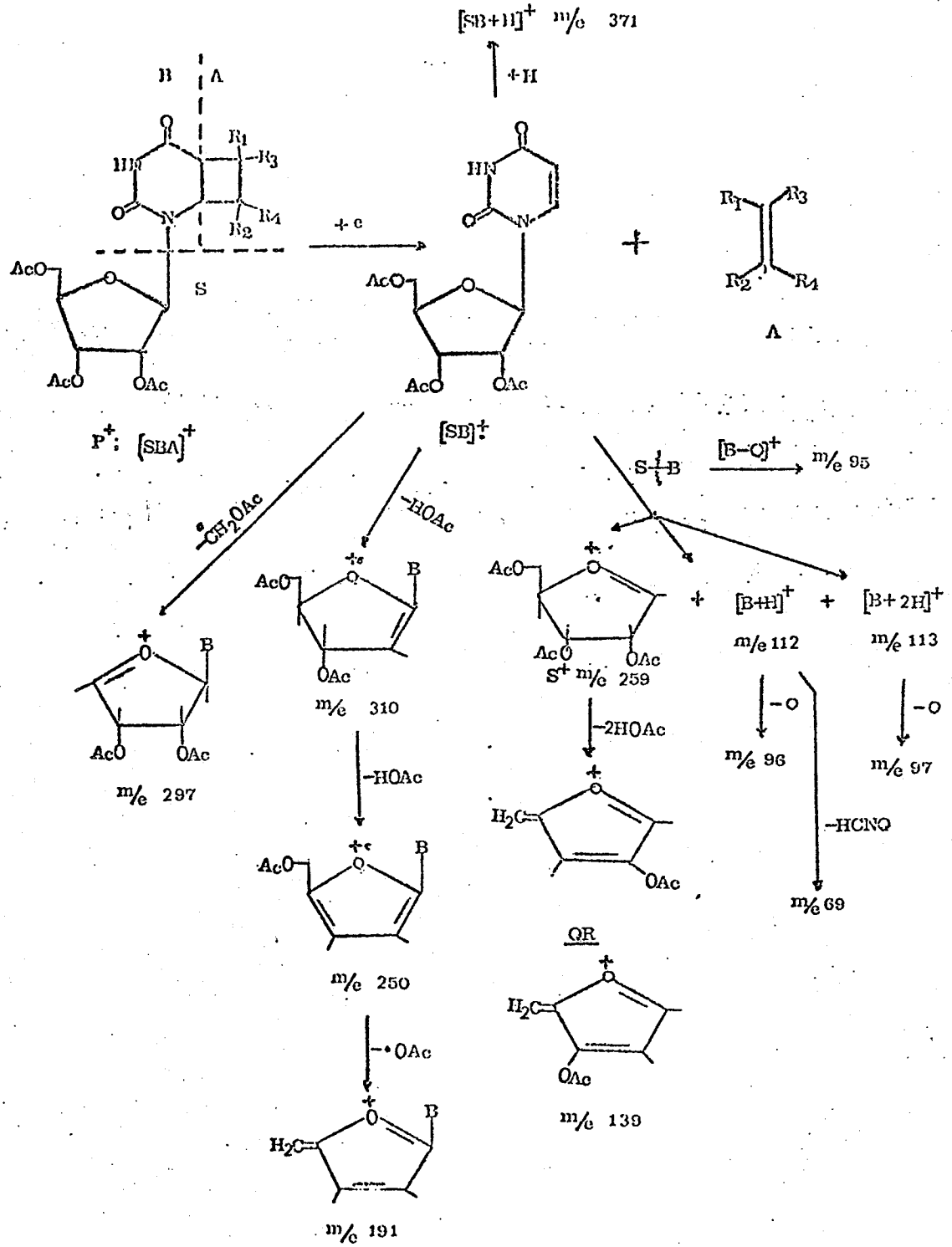


Figure 2. Major mass spectrometeric fragmentations of 2',3',5'-triacetyluridine photocycloadduct

tion of the stable neutral molecule (the olefin) formed. Furthermore, the spectrum of triacetyluridine itself usually gave the peak m/e 371.

The mass peaks at m/e 310, 250 and 191 were due to the successive loss of two HOAc molecules and a .OAc radical, respectively from the ion $[SB]^{+\cdot}$. 1',2'-Eliminations of neutral species such as CH_3OH were also observed in O-methylated nucleosides[77].

The most prominent peaks observed for all of the adducts prepared were ions at m/e 259, 139 and 97. Formation of the ion S^+ (m/e 259) and $B+2H^+$ (m/e 113) indicated the ready cleavage of the N-glycosidic bond. The lower intensity of the ion $[B+H]^+$ (m/e 112) than the ion $[B+2H]^+$ (m/e 113) could be attributed to the higher stability of $[B+2H]^+$ ion[78]. On the other hand, $[B+H]^+$ readily underwent elimination of HNCO to give the ion m/e 69. Both ions $[B+H]^+$ and $[B+2H]^+$ are characteristic of nucleoside mass spectra where transfer of 1 and 2 hydrogen(s) from the ribose skeleton are required[78].

A series of mass peaks occured at m/e 95, 96 and 97 consecutively. These have been assigned to the ions $[B-O]^+$, $[B+H-O]^+$ and $[B+2H-O]^+$ respectively[79]. Among them, m/e 97 was of the highest intensity.

The ion of mass 139 was assigned to the structure as shown (Figure 2). It has a similar structure to the ion at m/e 191. Similar ions were observed in the anhydronucleoside derivatives[79].

Elimination of a $\cdot CH_2OAc$ radical from the $[SB]^{+\cdot}$ gave rise to the ion of mass 297 with an intensity of similar magnitude to that of m/e 310. Elimination of other radicals such as $\cdot CH_3$ from the acetyl group was negligible though sometimes observed.

This left two other prominent peaks in the region higher than 100. These were m/e 115 and 195. The former has been assigned to a dioxane derivative in anhydronucleosides[79] and cyclonucleosides[80] studies. Another possible route to its formation from the S^+ ion is proposed here, in which an acetyl group is retained. The structure may be one involving a three-membered ring (Figure 3). If (V), the dioxane, was the ion produced, then it would not have had required the presence of acetyl groups. In fact, while the mechanism may be true for anhydronucleosides and cyclonucleosides, the mass peak at 115 is absent in uridine itself. This tends to support the route leading to the formation of (VI) proposed here.

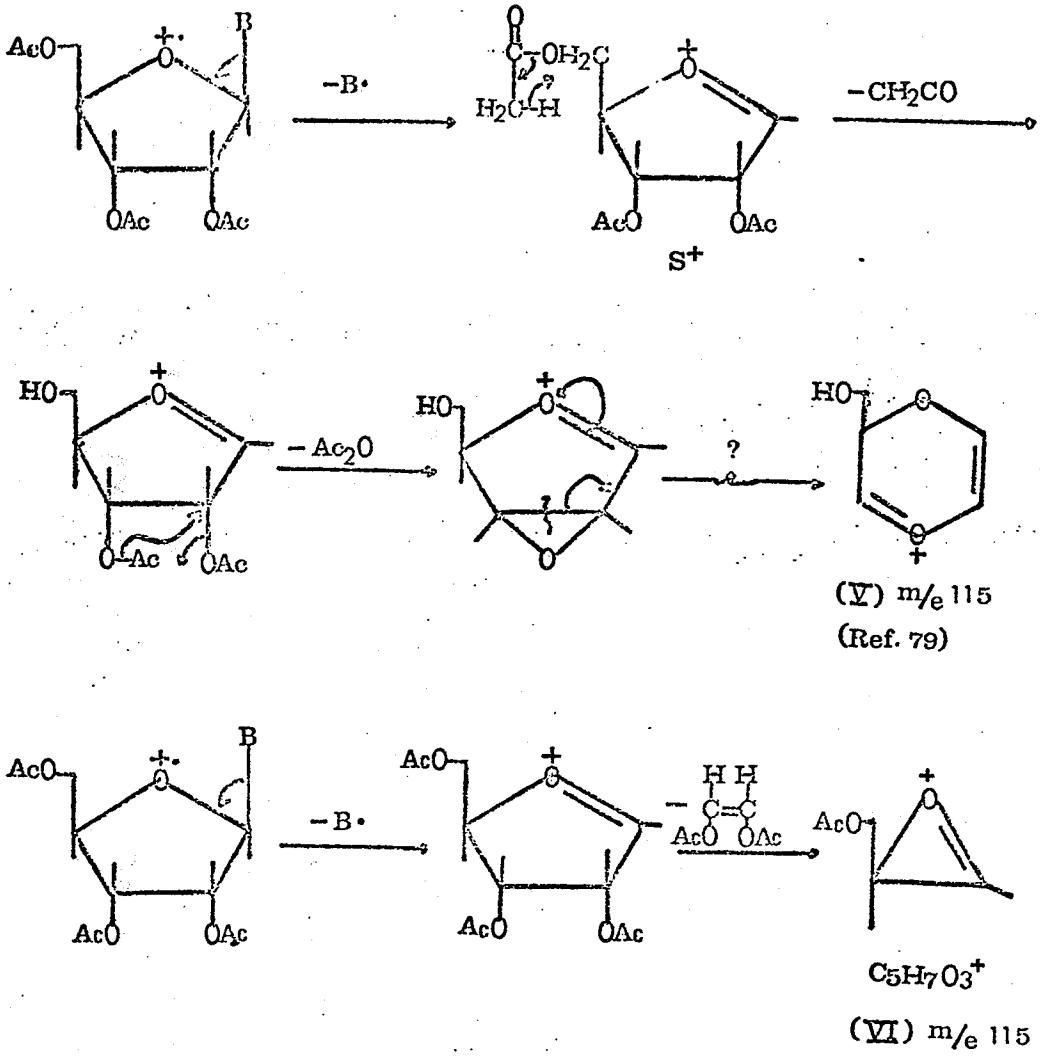


Figure 3. Possible routes leading to the ion m/e 115

The ion of mass 195 may be assigned to a structure (VII) analogous to that assigned in anhydronucleosides[79] and cyclonucleosides[80]. In both anhydronucleosides and cyclonucleosides the ion of mass 195 was always accompanied by a peak at m/e 177 due to the ready loss of a water molecule. No peak at m/e 177 was observed in our compounds since elimination of a water molecule from the ion of 195 (VII) is impossible. However, evidence could be found to suggest an alternate pathway leading to m/e 195. This involved the intermediate (VIII) (m/e 222) which was sometimes observed in a very low intensity presumably due to the ready loss of a water molecule to give the ion (IX) at m/e 195 (see Figure 4). Also suggesting such a pathway were the corresponding ions observed in 2'- and/or 3'-methylated nucleosides[77].

Fragments in the region lower than 100 are not diagnostically important. They are formed from the base and the sugar moiety.

The foregoing fragmentations discussed may be applicable to the cycloadduct $[SBA]^+$ too. However the ions so formed were usually of so low abundance that they were often unobserved. This further indicated that the photocycloadducts were not stable upon electron impact. Fragmentations tended to take place after dissociation of

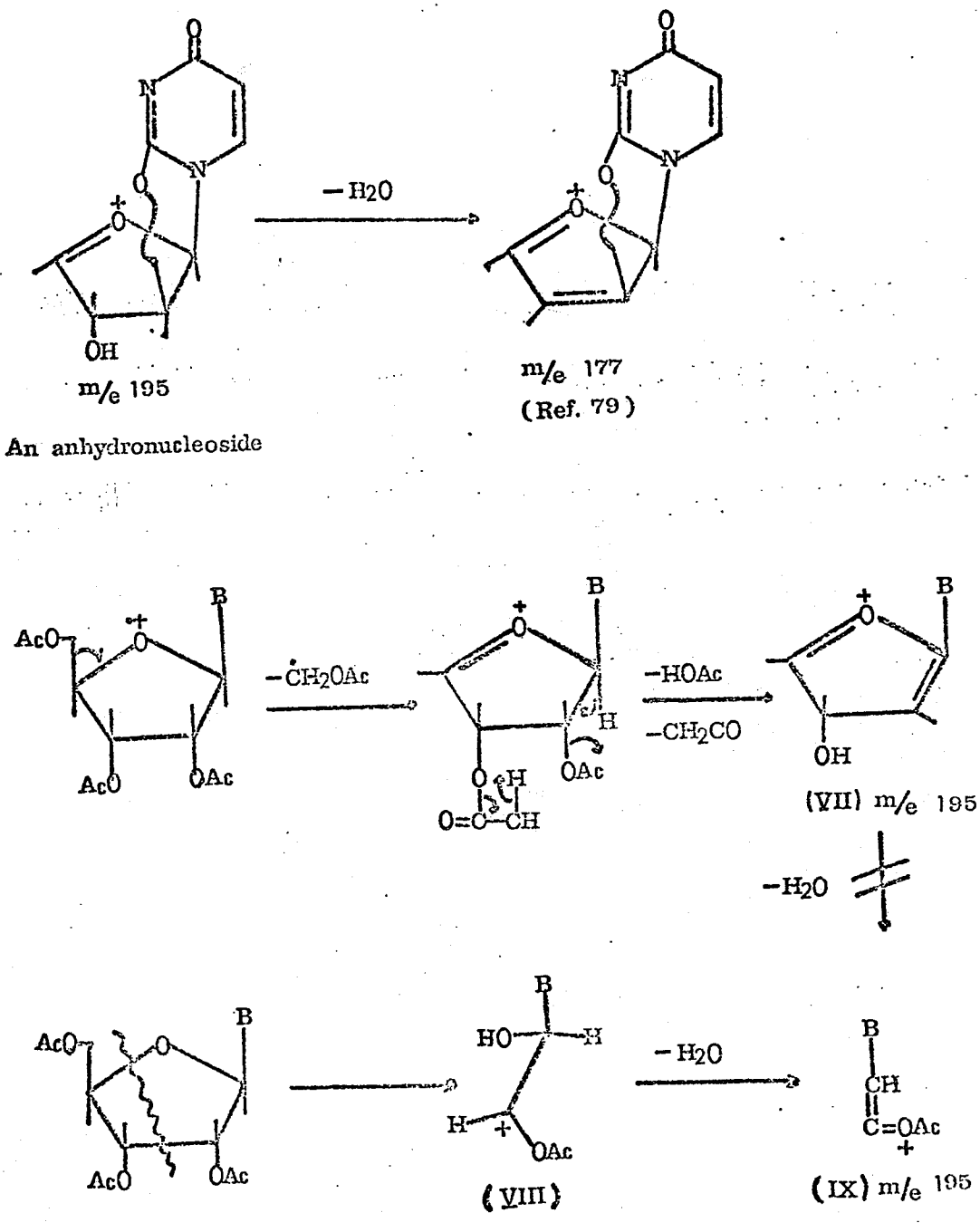


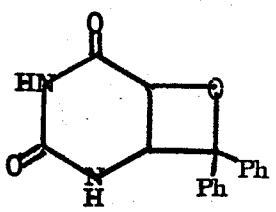
Figure 4. Possible routes leading to the ion m/e 195

the cyclobutane ring.

The n.m.r. spectrum of 2',3',5-triacetyluridine was useful as a reference for comparison purposes. Its N_3-H appeared at 10.2 ppm downfield from the TMS internal standard while the H_5 and H_6 appeared as a characteristic olefinic doublet (J= 8.0 Hz) at 6.05 and 7.70 ppm respectively. The formation of photocycloadduct led to the saturation of C_5-C_6 double bond thus shifting the H_5 and H_6 protons upfield to the region 2.0 - 4.5 ppm. The N_3-H was also shielded by the saturation of the double bond. The H_5 , H_6 protons were often masked by the complicated sugar protons which ranged from 4.2 to 6.1 ppm and sometimes by the acetyl groups as well (~ 2.1 ppm). The coupling pattern became very complicated especially if there were protons at C_7 and/or C_8 as there are in most cycloadducts.

A first priority for a good n.m.r. interpretation of the structure is a highly purified cycloadduct. While it may be argued that oxetane formation would have given the same parent mass and perhaps even a similar fragmentation pattern as it does in cyclobutane adduct, such doubts can be cleared up by comparison between the product and the triacetyluridine n.m.r. spectra. An oxetane n.m.r. spectrum would have shown the presence of olefinic H_5 and

H_6 protons with equal coupling and that their chemical shifts should not differ much from the corresponding values in triacetyluridine itself. The oxetane formed between thymine and benzophenone was isolated by von Wilucki et al[72] and it has the structure shown in (X). The only olefin in our study having a crabonyl function is iso-



(X)

propenyl acetate. However, the carbonyl in isopropenyl acetate is an ester type and the n $-\pi^*$ transition is shifted from 270 mu (for a carbonyl not attached to an electronegative atom) to 215 mu[81]. Since irradiation is carried out at 254 mu, the isopropenyl acetate carbonyl is not likely to be excited. Even if excitation did take place, an energy transfer process would dominate since the carbonyl possesses a higher triplet state than the unsaturated substrate[82].

The experiments have demonstrated that photocycloadditions of uridine to olefins result in cyclobutane photoadducts in high

yield. The olefins used in this study are tetramethylethylene, cyclohexene, isopropenyl acetate, 1,1-diethoxyethylene, vinylene carbonate and diphenylacetylene. An exception was found in the case of diphenylacetylene which failed to give any photoproduct. It is believed that diphenylacetylene acted as a quencher of the uridine triplet state. However, no autoadduct or rearranged product of diphenylacetylene was detected. Recovered from the irradiated solution were 2',3',5'-triacetyluridine and diphenylacetylene. When irradiated in n-hexane under N_2 , diphenylacetylene is shown to give products believed to be derived from fearrangement of the tetraphenylcyclobutadiene intermediate[82-83].

II. Stereochemical Studies of Selected Photocycloadducts

(A) 2',3',5'-triacetyluridine ⇌ cyclohexene adducts

While the stereochemistry of the biologically active nucleosides and their analogues may be crucial in enzymatic systems, the specific conformation of 2',3',5'-triacetyluridine photocycloadducts proved to be challenging and extremely difficult. The problems involved become immediately obvious as shown in Figure 5. Although the anti-uridine (A), where the base carbonyl is oriented away from the ribose ring, exists in conformational equilibrium with its syn isomer(S), the anti isomer predominates. The two conformations can be interrelated by a 180° rotation about the glycosidic bond. When cyclohexene approaches the excited uridine molecule each of these conformers could theoretically give rise to four different stereoisomers. If the cyclohexene molecule were to approach the syn conformer from the back-side (from behind the paper), the cycloadduct formed would be S_b^t (base ring trans to cyclohexene) and/or S_b^c (base ring cis to cyclohexene). On the other hand, front-side attack (from above the paper) would give S_f^t (trans rings) and/or S_f^c (cis rings). Similarly the anti conformer could theoretically give rise to A_b^t /A_b^c and A_f^t /A_f^c respectively.

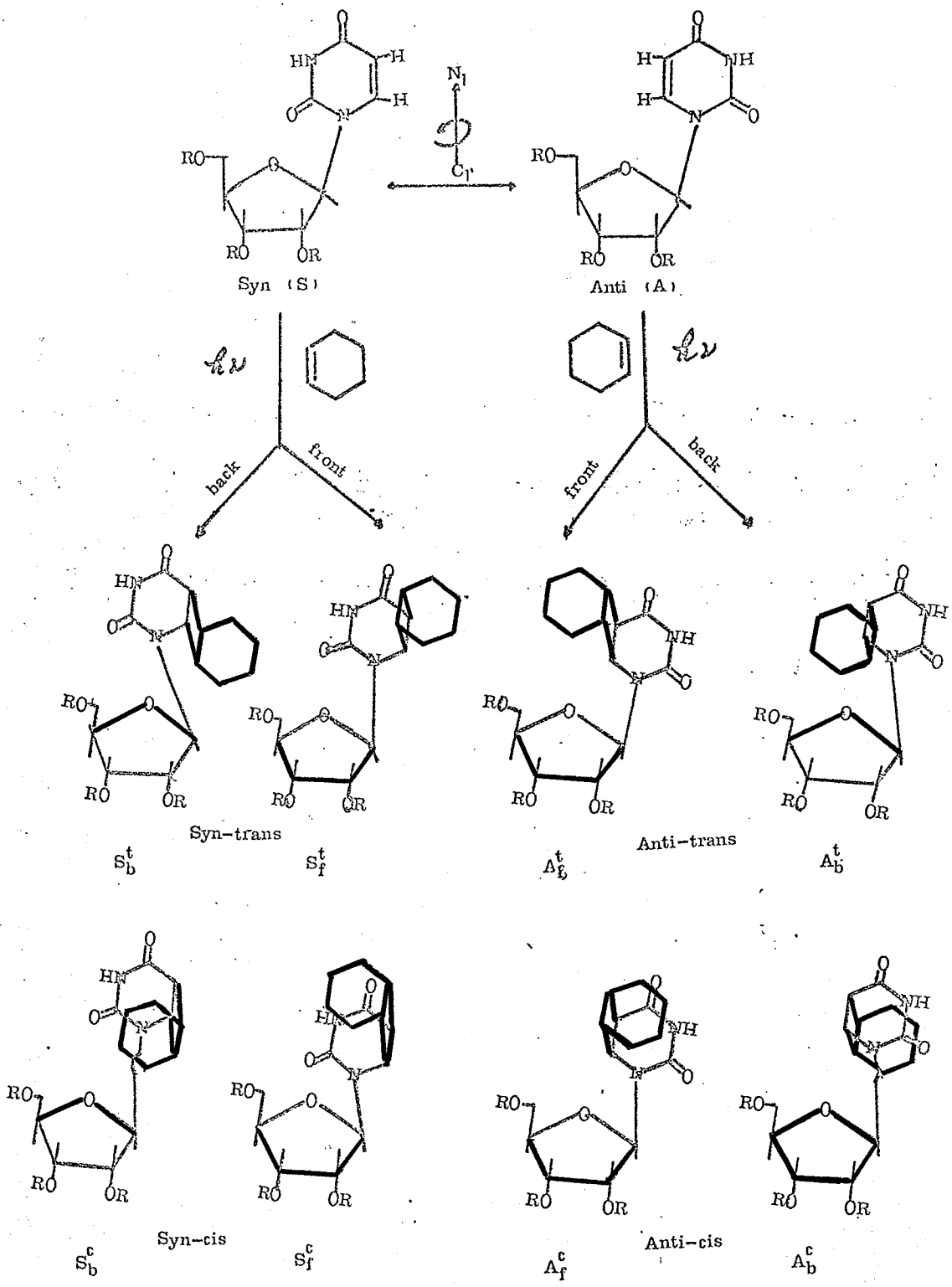


Figure 5. Different possible stereoisomers of 2',3',5'-triacetyluridine — cyclohexene photocycloadduct

It is seen that S_b^t is related to A_f^t ($S_b^t \longleftrightarrow A_f^t$) the same way as the parent syn-uridine is related to the anti-uridine. By analogy, from Figure 5 $S_f^t \longleftrightarrow A_b^t$, $S_b^c \longleftrightarrow A_f^c$ and $S_f^c \longleftrightarrow A_f^c$ Bonds marked with red ink indicate they are on the same side of the $N_1 - C_{1'}$ bond. Thus back-side attack results in isomers where the addend is oriented above the $C_{1'} - O$ bond of the sugar ring (S_b^t, S_b^c; A_b^t, A_b^c); wherease front-side attack gives isomers with the addend oriented above the $C_{1'} - C_{2'}$ bond of the sugar ring (S_f^t, S_f^c; A_f^t, A_f^c).

Despite repeated attempts, TLC gave only partial separation of the isomers. The combined fractions yielded correct elemental compositions for structures of the type shown in Figure 5. The n.m.r. spectrum was complicated. It indicated that there were at least three isomers formed as shown by three-downfield N-H peaks. The complexity of $H_{2'}$ and $H_{3'}$ couplings yielded no information except the likelihood of mixed isomers. Addition of a bulky group is likely to force the molecule to adopt a predominantly syn conformation so that the steric interaction between cyclohexane and the sugar ring is less than it is in the anti form. Based also on steric grounds, cis isomers may be more stable than their trans

counterparts.

It was thought that by a series of chemical degradations, the number of isomers could be reduced by reducing the number of asymmetric carbon centers in the photoadducts. One method attempted was to cleave the glycosidic bond by acid hydrolysis. This will render all trans isomers identical to each other; and all cis isomers identical to each other. The hydrolyzed isomers can then be compared with adducts prepared by photocycloaddition of uracil to cyclohexene (Figure 6). However, this raised another problem in that adducts without the triacetylated sugar moiety are insoluble in most organic solvents. Modification of adducts such as methylation at N_1 and N_3 might have solved this problem, but this reaction was found to give a mixture of mono- and di-methylated compounds. It was clear that while glycosidic cleavage may have been useful for determining the stereochemistry at the cyclobutane ring junctions, it would not enable one to distinguish the stereochemistry with respect to the $C_{1'}$ chiral center in the original cycloadducts. For example, it would not help to distinguish S_b^t from S_f^t, or S_b^c from S_f^c. Similar arguments could be used against the usefulness of, for instance, oxidizing $C_5 - C_6$ to make the base ring coplanar to the cyclobutane,

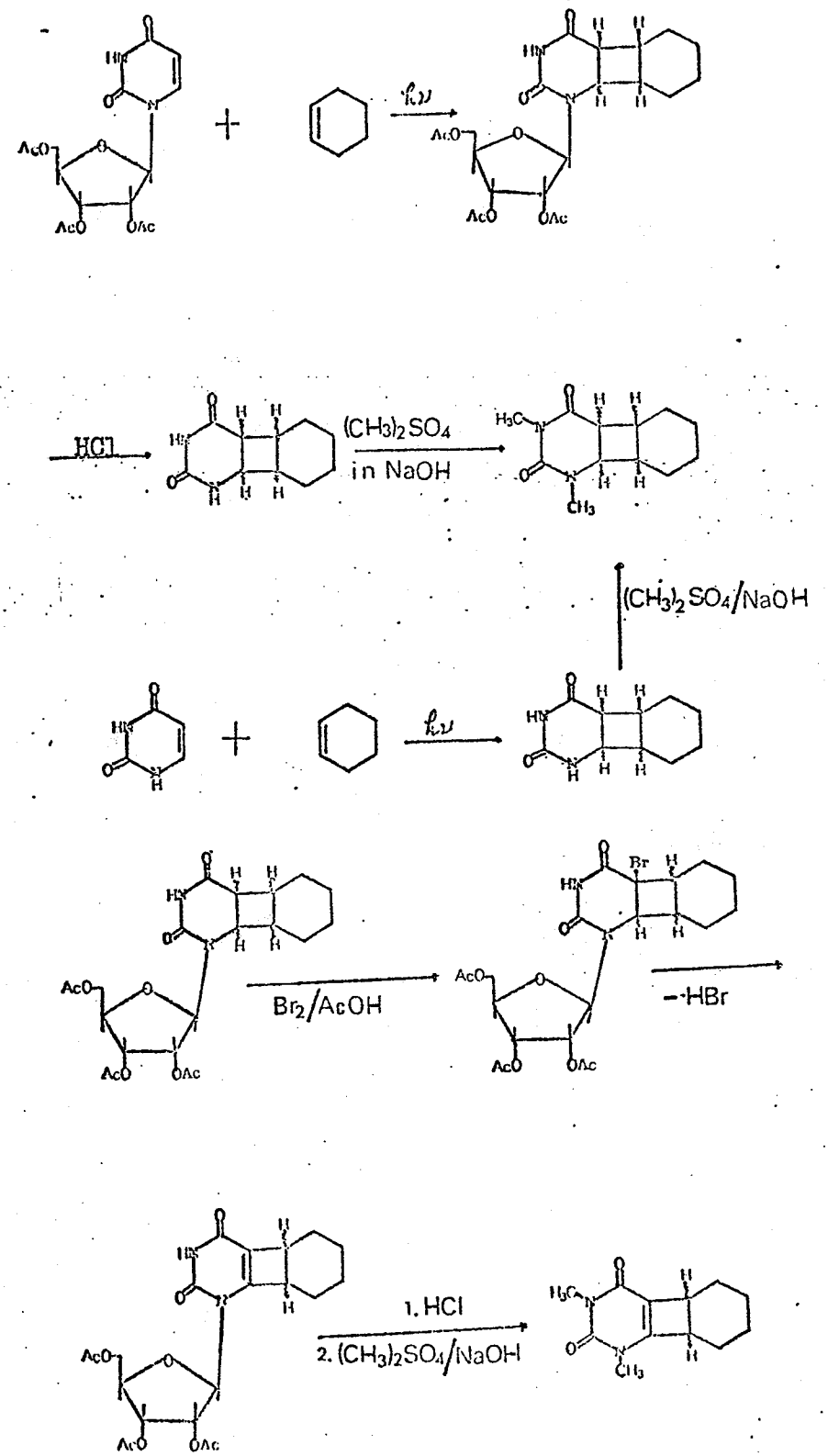


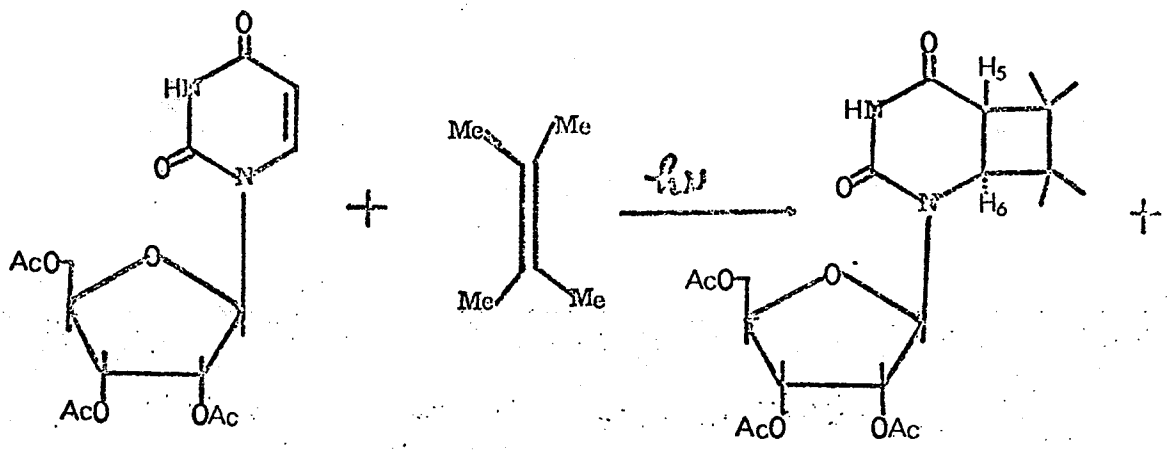
Figure 6. Reducing the number of possible stereoisomers by chemical degradations

although such an oxidized cycloadduct would be of more biological significance since it would more closely resemble uridine.

(B) 2',3',5'-triacetyluridine — tetramethylethylene adducts

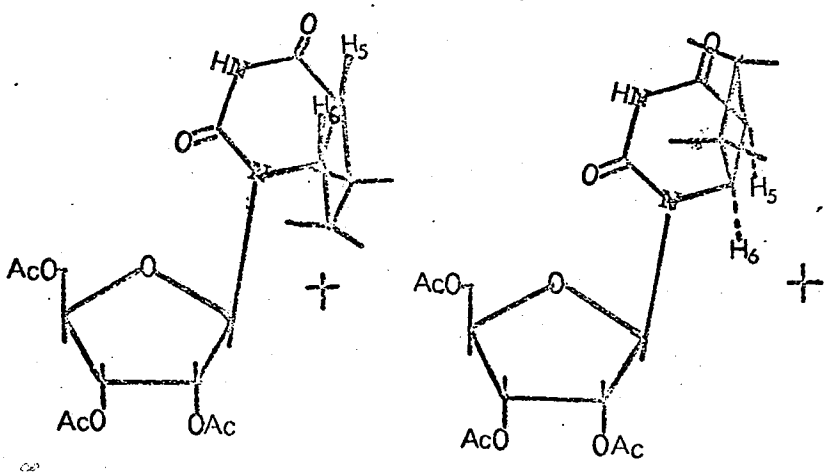
Good separation of isomers is necessary but not sufficient for successful stereochemical elucidation. This is best demonstrated in the tetramethylethylene cycloadducts where isomers were adequately well separated. Four products were obtained with the first compound (the order follows its relative elution from the column) unidentified. This compound exhibited only methyl and methylene n.m.r. absorptions. In contrast to the other three products, it could not be recrystallized and existed as a yellow oil. No further investigation was made although the compound could be a polymer of tetramethylethylene produced by uridine radicals. Acrylonitrile is known to polymerize when irradiated in the presence of orotic acid (6-carboxyuracil). It has also been shown that such polymerization was induced by excited orotic acid radicals[53]. Pyrimidine radicals produced by UV excitation have been detected[28-29].

The second product had an elemental analysis consistent with a cyclobutane adduct. It exhibited some unusual n.m.r. peaks.



XI-C

$J_{5,6}$ = 13.3 Hz



XI-A or XI-B

$J_{5,6}$ = 9.8 Hz

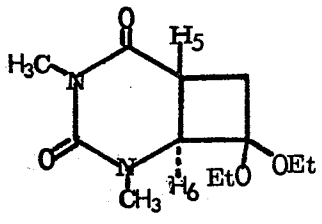
XI-B or XI-A

$J_{5,6}$ = 9.8 Hz

Unidentified product; highest m/e = 446

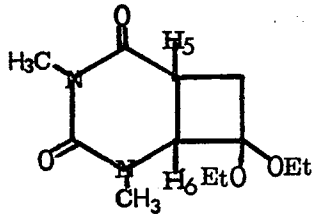
Figure 7. Different stereoisomers of 2',3',5'-triacetyluridine — tetramethylethylene photoproducts obtained.

The H_1 (δ = 4.83 ppm) and NH (δ = 7.48 ppm) were very much shielded compared to those of the third and fourth compounds and triacetyl-uridine. Its H_5 and H_6 appeared at δ = 2.56 ppm and δ = 3.75 ppm respectively. Also striking was the fact that $J_{1',2'}$ (3.03 Hz) was unusually small. The reason for such a large shielding effect on NH and the sugar protons is not known. The large coupling between H_5 and H_6 ($J_{5,6}$ = 13.3 Hz) may be indicative of a trans-fused ring. Based on this coupling, the compound was assigned a structure as shown in (XI-C) (see Figure 7). It is relevant to quote some J_{trans} values from the literature as supporting evidence. The corresponding values for (XII-A) and (XII-B) were found to be 13.0 Hz and 9.6 Hz respectively[57]. An even larger value ($J_{A,B}$ = 15.0 Hz) was found in the corresponding 4,4-dimethylcyclohexenone adduct (XIII)[85]. While the coupling value seems to support a trans-fused



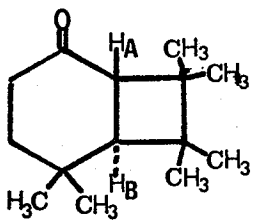
(XII-A)

$J_{5,6}$ = 13.0 Hz



(XII-B)

$J_{5,6}$ = 9.6 Hz



(XIII)

$J_{A,B}$ = 15.0 Hz

adduct, the assignment is only tentative until more rigorous proof is available. It will be of interest to see if the trans isomer will undergo base catalyzed isomerization to give the cis isomer, and if it will, will it be (XI-A) or (XI-B), or a mixture of both. Both (XII-A) and (XIII) are known to isomerize to their cis counterpart.

The third and fourth products had very similar n.m.r. spectra. While the latter was isolated pure, the third compound was not further separated from the fourth in a mixed fraction. The compound in the mixed fraction had an n.m.r. spectrum nearly superimposable with that of the pure fourth compound. Both compounds had $J_{5,6}$ = 9.8 Hz , characteristic of a cis hydrogen coupling. Therefore they were assigned as (XI-A) and (XI-B). However, as far as the stereochemical nature with respect to the $N_1 - C_1$, bond is concerned, (XI-A) is indistinguishable from (XI-B). That is, no evidence is available to tell whether (XI-A) or (XI-B) is the fourth compound, which has the slowest chromatographic mobility and a mp = 48 - 53 ^{o}C. The n.m.r. data of the tetramethylethylene cycloadducts are summarized in Table I.

Fraction	Compound	NH	$H_{1'}$	$H_{2'}$	$H_{3'}$	H_6	H_5	$J_{5,6}$	$J_{1'2'}$	$J_{2'3'}$
1	?	(Hydrocarbon, highest m/e = 446)								
2	XI-C	748	482.6			375.3	256.4	13.3	3.03	
3(major)	XI-A	878	579	530.1	512	400	295	9.8	5.6	
	and									
(minor)	XI-B	878	596	535.2	512	393	295	9.8	5.6	5.8
4	XI-B	861	595	535	514	392	296.4	9.8	5.6	5.8

TABLE I. - N.m.r. data of 2',3',5'-triacetyluridine-tetramethylethylene cycloadducts. It is seen that the minor compound in fraction 3 is the same compound in fraction 4.

- values approximated to $\pm$ 0.15 Hz .

- values of methyl and acetyl groups not included.

(C) Conclusion On the Stereochemistry of Photocycloadducts

It has now become clear that stereochemical investigation of this kind of photoproducts is tedious and laborious work. Complete and thorough structural elucidation requires not only a good separation of the isomers involved, but certainly also requires sophisticated spectroscopic techniques such as n.m.r. Overhauser effect, chemical labelling or exhaustive chemical degradation. In fact, it is doubtful, for example, that photocycloadducts such as S_b^t will ever be distinguishable from A_f^t (see Figure 5) since the problem involved is a rapid thermodynamic equilibrium between the two. Stereochemistry may be of crucial importance for enzymatic recognition of nucleosides and their analogues[86-88], and perhaps a more exhaustive study of the present compounds would be warranted if they were found to be biologically active.

III. Synthesis of Ribothymidine and Isolation of 5-Carboxymethyl Uridine

Thymidine exists in the deoxyribose series of nucleic acids, while uridine exists in the ribose series; and they have been used accordingly in the studies of nucleic acids chemistry. The ready availability of ribothymidine would certainly increase its role in nucleic acids studies. Ribothymidine, like most other nucleosides, has been conventionally prepared by condensing the sugar moiety and the thymine base[63,64,70]. Although synthesis from uridine is also known, the yield is usually low[65,66]. Furthermore, both methods are laborious and tedious. A direct synthesis of ribothymidine from uridine is therefore highly desirable.

In 1971, Wiesner et al[60] demonstrated the synthesis of 1,4-dicarbonyl compounds by fragmentation of the photocycloadducts between vinylene carbonate and α,β-unsaturated cyclic ketones (see Figure 8). The reactins proceeded smoothly with good yields. This led to the prediction that similar rearrangement should be applicable in a uridine-vinylene carbonate adduct. This was indeed recently found to be the case in the uracil-vinylene carbonate adduct[58] and in our present study. In the case of alkenone adduct,

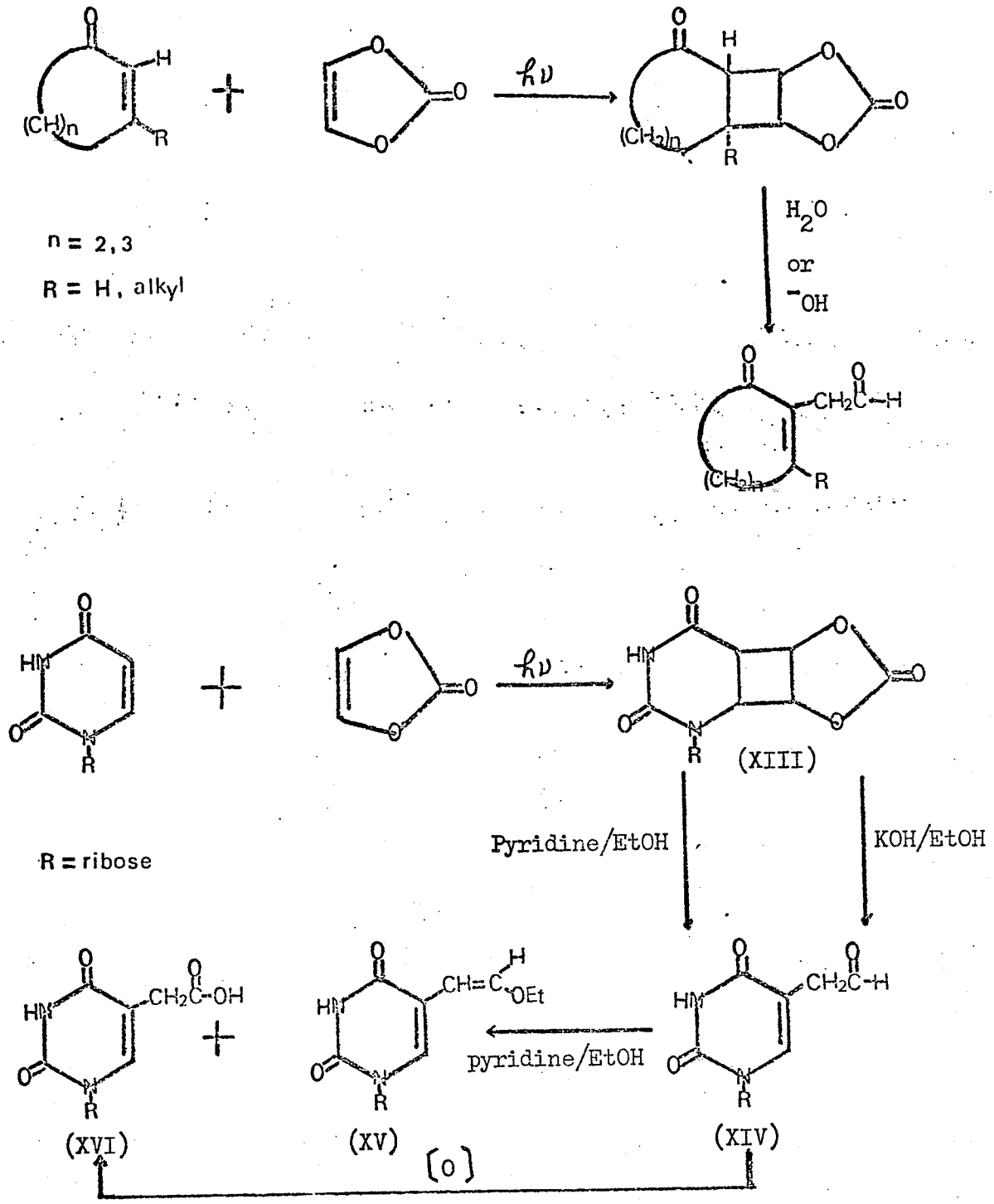


Figure 8. Rearrangement of 2',3',5'-triacetyl-uridine — vinylene carbonate adduct to 5-formylmethyl and its subsequent oxidation to 5-carboxymethyl.

the trans isomer was found to convert into the aldehyde more readily and in a higher yield than the cis isomer. The nearly quantitative conversion of triacetyluridine - vinylene carbonate cycloadduct into the 5-formylmethyl derivative (XIII⟶XIV) may be indicative of the existence of the predominant isomer(s). The conversion (XIII⟶ XIV) was optimal with treatment of 10^{-4} molar potassium hydroxide in 95% ethanol for 30 minutes. The reaction could be followed by TLC since formation of the aldehyde gave rise to UV absorption at 264 nm. The adduct (XIII) was so susceptible that even a few hours in 95% ethanol at room temperature gave rise to the aldehyde. The transformation (XIII ⟶ XIV) was also attempted with pyridine/ethanol solution; this, however, gave two UV absorbing compounds at different R_f values. The compound with higher R_f had a λ_{max}(EtOH) = 265 nm and showed a mass peak at m/e 446. This was believed to be the 5-vinylene ethoxy derivative (XV) of triacetyluridine, although no additional firm evidence was available. The slower running compound having a λ_{max}(EtOH) = 260 nm and a mass peak at m/e 429 seemed to suggest the 5-carboxymethyl triacetyluridine structure (XVI). However, no further investigation was made of its identity.

Despite several attempts to characterize the aldehyde (XIV), no satisfactory n.m.r. spectrum could be obtained. The compound underwent oxidation readily to give the 5-carboxymethyl derivative. 5-Carboxymethyl triacetyluridine gave a satisfactory elemental analysis and spectroscopic data. The n.m.r. spectrum showed a vinylic proton due to H_6 at $\delta = 7.46$ ppm and a singlet due to the methylene protons at $\delta = 3.45$ ppm. Its melting point was 84 - 89 °C. This compound has been isolated from wheat and yeast tRNAs[67-69]. The previous synthesis of this compound involved fusion between 5-carboxymethyluracil and the ribose moiety. The procedure also involved protecting and subsequent deprotecting of the sugar hydroxyl groups. The overall yield was 45% but the method was time consuming and laborious.

5-Carboxymethyl uridine has also been shown to undergo decarboxylation upon treatment with copper powder and quinoline. Our attempt to carry out the same reaction on the triacetyl derivative was unsuccessful.

Because of its susceptibility to oxidation, 5-(formylmethyl)-2',3',5'-triacetyluridine was indirectly characterized by its conversion to triacetylribothymidine, a useful but less readily

available natural nucleoside. Decarbonylation of the aldehyde was accomplished by refluxing with tris(triphenylphosphine) rhodium (I) chloride[90] in ethanol/benzene (2/10) solution for 3 hours. The yield of decarbonylation ranged from 28% to 42% and it was optimal when the rhodium complex to the aldehyde ratio was 1:1. Methylen chloride and acetonitrile was also used to replace benzene; but the yield was lower in the case of methylene chloride and it failed to decarbonylate in the case of acetonitrile.

The decarbonylated product, 2',3',5'-triacetylribothymidine had a λ_{max}(EtOH) = 266 nm (Lit. 267 nm). Its mass spectrum was identical to that of the authentic compound[89]. The authentic compound was prepared by triacetylation of ribothymidine, which in turn was made fusing together the thymine and the sugar. The process involved protecting the ribose hydroxyl groups with COC_6H_5 groups. The only difference between the decarbonylated and the authentic compounds was a peak which appeared at m/e 105 in the spectrum of the latter. This was believed to be $COC_6H_5^+$ due probably to incomplete debenzoylation. The triacetylribothymidine had an n.m.r. spectrum showing a methyl peak at δ= 1.90 ppm and a vinylic proton due to H_6 at δ = 7.2 ppm. Deacetylation produced a compound which gave an identical

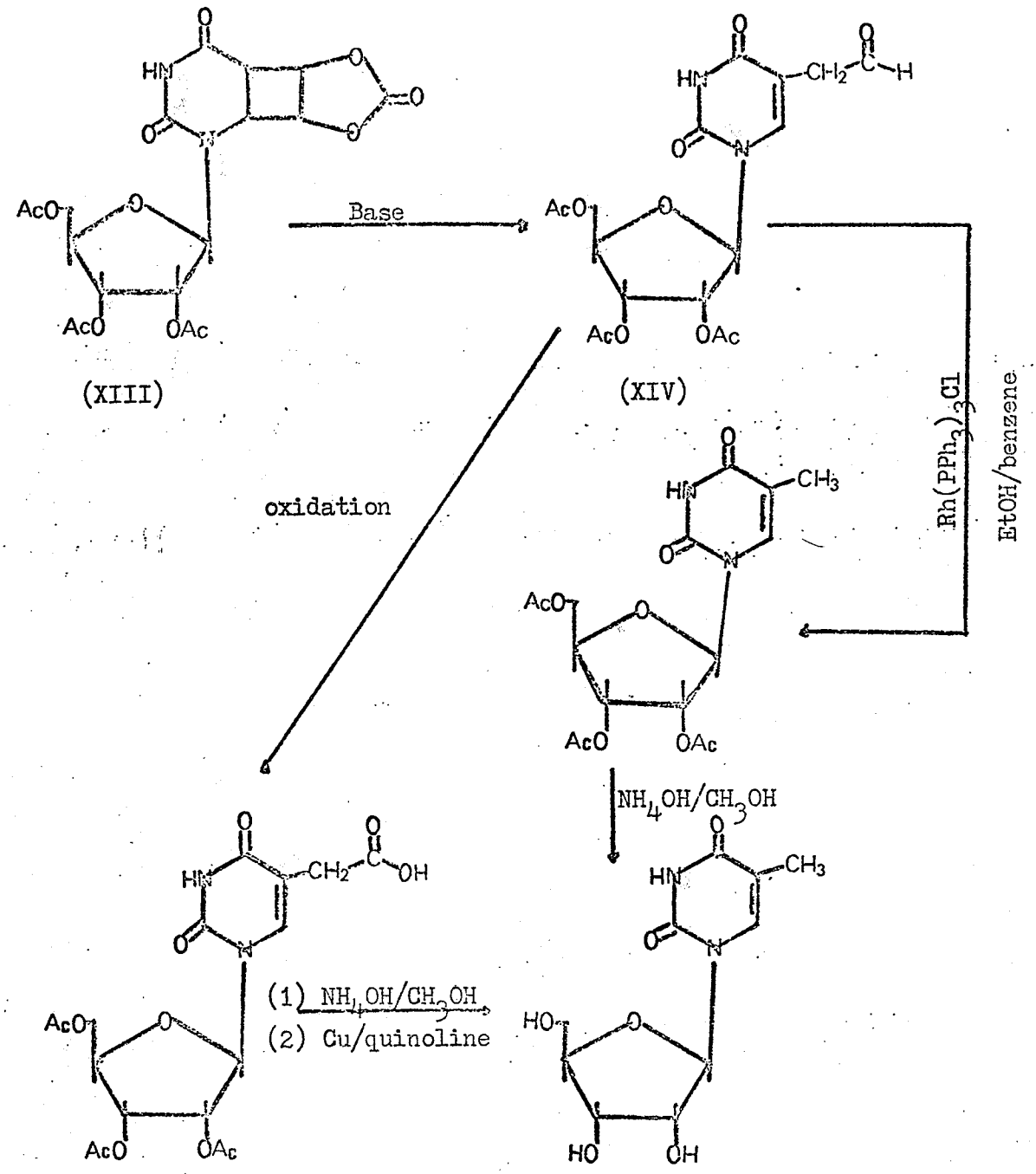


Figure 9. Synthesis of ribothymidine

mass spectrum compared to that of authentic ribothymidine.

The transformations discussed so far are summarized in Figure 9. The procedure involved certainly is less tedious than the conventional methods and the yields are reasonably good. It is hoped that continuing work in this area will improve the synthesis. A good oxidation method is highly desirable to convert the 5-formylmethyl to the 5-carboxymethyl derivative of uridine. Decarboxylation of 5-carboxymethyl uridine to ribothymidine will be attempted in the near future. It is believed that alternate routes to 5-formylmethyl uridine are also feasible as demonstrated by Hunter et al[91].

The role of suitably prepared photocycloadducts in the synthesis of naturally occuring nucleosides and their analogues is thus enlightening and promising. Not only is the availability of rare nucleosides desirable but their analogues may prove to be useful in biological and medincinal activities.

EXPERIMENTAL

General

Photocycloadditions were achieved by photolyzing the ethyl acetate solution of 2',3',5'-triacetyluridine in the presence of the addends. Irradiations were carried out in a Hanovia quartz probe with a low pressure mercury arc, 254 nm, by Ultraviolet Products Inc., PCQXI.

Column chromatography was done using a slurry of silica gel containing 5% calcium sulfate, DSF-5, manufactured by Camag, Switzerland. The same support was also employed for thin layer chromatography plates.

Elementary analyses were done by Chemalytics Inc., Tempe, Arizona.

Ultraviolet spectra were recorded on a Cary-14 Recording Spectrophotometer. Mass spectra were obtained on a Finnigan 1015 Mass Spectrometer at an ionization energy of 70 eV. The mass ratio were calculated from relative peak heights and were precise only to ±2%. Nuclear magnetic resonance spectra were obtained on a Varian A56-60 or varian HA-100 spectrometer. The chemical shifts were recorded in ppm with respect to tetramethylsilane as an internal standard. Deutero-chloroform was the solvent used unless otherwise specified.

Melting points were taken on a Reichert (Austria) Melting Point apparatus and reported uncorrected.

2',3',5'-triacetyluridine

Uridine (1.006 gm, Sigma Chemicals) was dissolved in dry pyridine (10.0 ml). Distilled acetic anhydride (40.0 ml) was then added and the solution was stirred overnight with a magnetic stirrer. An instantaneous and vigorous reaction took place when methanol (20.0 ml) was added to the solution. The solution was then heated on a steam bath for about $\frac{1}{2}$ hour. Removal of excess pyridine and acetic acid was achieved by repeatedly adding 95% ethanol followed by its removal on a rotary evaporator. The product crystallized from an ether/hexane solvent has mp = 126 - 128 °C[92]. The yield was quantitative.

Mass spectrum and m/e (relative intensity):
371(2), 310(19), 259(>100), 250(34), 195(37), 191(74), 139(>100), 115(>100), 113(>100), 97(>100), 86(100).

N.m.r. δ: 2.15(9H,S), 4.30(3H,m), 5.35(2H,m), 5.75(1H, d, J=8.0Hz), 5.96(1H, low lump), 7.48(1H,d,J=8.0Hz), 9.60(1H, low lump).

2',3',5'-triacetyluridine - cyclohexene adduct

A 2.0×10^{-2} molar solution of 2',3',5'-triacetyluridine in ethyl acetate containing 2% (by volume) cyclohexene (Eastman Kodak) was irradiated as described above. Irradiation was terminated at the end of $3\frac{1}{2}$ hours when the UV absorbance at 257 nm had decreased by 90%. Evaporation of the solvent followed by recrystallization gave a yellow product which showed five bands on TLC (Benzene/ethylacetate = 50/50).

The first fraction (lowest band) gave the same R_f on TLC as spotted against authentic 2',3',5'-triacetyluridine. Its identity was also confirmed by mass spectrum and UV spectroscopy. The fourth and fifth bands gave only trace amounts of material and their mass spectra did not show the characteristic ribose peaks (e.g. m/e 259). No further investigation was made of their identity.

The second band had the following mass spectral peaks:

m/e (relative intensity): 452(< 1), 371(< 1), 259(59), 139(100), 115(13), 113(16), 97(55).

The mass peaks in the spectrum of the third band were:

m/e (relative intensity): 452(< 1), 370(1.8), 259(53), 139(100), 115(9), 113(16), 97(42).

The second and third bands were recombined and treated with charcoal in ethylacetate. Mp = 60 - 65 °C. Analysis calculated for $C_{21}H_{28}N_2O_9$:

C: 55.76, H: 6.22, N: 6.19, O: 31.89

Found:

C: 55.90, H: 6.32, N: 5.99, O: 31.79

N.m.r. δ: 1.1 - 1.8(m), 2.10(s), 4.15 - 4.0(m), 5.10 - 5.40(m), 5.75(m), 6.10(t), 8.42(s), 8.86(s), 8.95(s).

Hydrolysis of the Glycosidic Bond of Triacetyluridine — cyclohexene Adduct[31]

Triacetyluridine - cyclohexene adduct (51 mg) was dissolved in methanol (5 ml) followed by addition of 0.1N HCl (10 ml). The solution was heated on a steam bath for 1½ hours. A saturated $NaHCO_3$ solution was used to neutralize the solution. Further heating was applied to remove excess methanol, however this cause some precipitation to occur. Water was added and this was extracted with ether. Both the ether and aqueous layers were evaporated to dryness. A mass spectrum indicated the ethanol solution might contain some triacetylribosyl residue but this was not positively confirmed. A slightly yellow residue was obtained from the aqueous layer; but no mass spectral evidence of a

uracil base derivative was obtained.

Deacetylation of 2',3',5' - triacetyluridine — cyclohexene adduct

Triacetyluridine — cyclohexene adduct (9 mg) was dissolved in 15% NH_4OH in methanol (2 ml) and stirred overnight. The solution was evaporated to dryness with several portions of 95% ethanol. A white crystalline residue was recovered (5 mg).

M/e (relative intensity): 226(10), 133(50), 113(100), 97(24).

Hydrolysis of glycosidic bond of uridine — cyclohexene adduct

Uridine — cyclohexene adduct (5 mg) obtained from deacetylation of the triacetyl derivative was heated in 0.1 N HCl (5 ml) on a steam bath for about 90 minutes. Ether was added, swirled and then filtered through $MgSO_4$.

M/e (relative intensity): 227(20), 150(16), 113(100).

Uracil — cyclohexene adduct

Absolute ethanol (50 ml) was used to dissolve uracil (100 mg,

Sigma Chemicals) by gentle heating. The solution was cooled down to room temperature and irradiated in the presence of cyclohexene (1 ml. distilled; Fisher Sci. Co.). The reaction was terminated when the UV absorbance at 262 nm (in EtOH) had decreased by 85%. Evaporation of the solvent gave a pale yellow solid in 80% yield. The product was not detectable on TLC. It was barely soluble in ethanol and pyridine and insoluble in CCl_4 , $CDCl_3$ or other n.m.r. solvents.

m/e (relatively intensity): 195(60), 113(100), 96(100).

N-methylation of Uracil - cyclohexene Adduct

(1) With Dimethyl Sulfate and Potassium Carbonate[93]

The adduct (34 mg.) was dissolved in a minimal amount of anhydrous acetone. Potassium carbonate (0.026 gm.) was added followed by intermittent addition of distilled dimethyl sulfate (0.02 mg). The solution was refluxed on a steam bath for 4 hours and then rotary evaporated. Acetone was used to sweep out excess dimethyl sulfate. N.m.r. showed no N-methyl peaks in the expected region.

(2) With dimethyl sulfate and sodium hydroxide[94]

The adduct (198 mg) was dissolved in a solution of 2.08N NaOH (5 ml). The flask was kept in an ice bath while dimethyl sulfate (1.0 ml) was added dropwise with constant swirling. Magnetic stirring was continued first at 0°C for 4½ hours then at room temperature overnight. Evaporation of the chloroform extract gave a brown oil.

m/e (relative intensity):

220(9), 141(100), 140(71), 127(>100), 98 (100).

The n.m.r. spectrum showed more than two N-methyl peaks in the region δ = 3.0 - 3.8 ppm.

Bromination and subsequent dehydrobromination of triacetyluridine - cyclohexene adduct[95]

(1) Bromination

Triacetyluridine - cyclohexene adduct (492 mg) was dissolved in acetic acid (7 ml) and treated with bromine (0.16 gm). The solution was heated on a steam bath for 1 hour and then evaporated several times with ethanol. The dark brown residue was redissolved in ethanol, treated with charcoal, and evaporated to dryness. The compound gave the following mass spectral peaks:

m/e: 411/413/413 (1:2:1), 391/393/395 (1:2:1), 373,330/332/334(1:2:1), 311, 259.

(2) Dehydrobromination

The brominated product from (1) was treated with pyridine (10 ml) and heated on a steam bath for 1½ hours. The solution was filtered hot and quickly transferred into ice-cold water (20 ml), and then extracted with benzene (20 ml). The benzene extract was treated with charcoal and evaporated to give a brownish yellow residue. The following mass spectral peaks were observed:

m/e: 393, 330, 279, 259, 112.

UV λ_{max}(EtOH) = 255 nm.

Bromination and Subsequent Dehydrobromination of Uracil - cyclohexene Adduct

(1) Bromination

Uracil - cyclohexene adduct (0.17 gm), acetic acid (5 ml) and bromine (0.16 gm) were treated as described above for the nucleoside adduct to give a brown compound which showed the following major

spectral peaks:

m/e : 199/201/203 (1:2:1), 158/160/162(1:2:1), 113, 82.

(2) Dehydrobromination

The brominated product from (1) (0.3 gm.) was treated with pyridine (4 ml) as described previously. But both ethanol and aqueous extracts gave similar compounds which showed virtually identical mass spectra.

m/e : 178, 149, 113(base peak), 79.

Only multiplet absorptions (centered at $\delta = 1.5$) were observed in the n.m.r. spectrum (in DMSO) of the residue obtained from ethanol extraction.

Photolysis with diphenylacetylene

Diphenylacetylene (0.37 gm; Baker Chemicals) was dissolved in the ethylacetate solution of 2',3',5'-triacetyluridine (50 ml; 2×10^{-2} M) and photolyzed for $4\frac{1}{2}$ hours. Evaporation of solvent yielded a yellow

oil, crude weight 0.735 gm. This was chromatographed against authentic diphenylacetylene on TLC. The bottom fraction (Rf = 0.11) gave the following spectroscopic data:

UV λ_{max}(EtOAc) = 255 nm.

Mass spectrum m/e:

371, 310, 259, 250, 195, 191, 139, 115, 113, 97.

N.m.r. δ: 2.10(9H,S), 4.37(3H;m), 5.40(2H,m), 5.83(1H, d, J=8.0 Hz), 6.08(1H,m), 7.50(1H,d, J=8.0 Hz), 10.0(1H,broad).

The top fraction gave the following UV and mass spectral data:

UV λ(EtOAc;nm) = 298, 287, 278, 264.

Mass spectrum m/e: 178, 101, 77.

Photolysis with tetramethylethylene

Triacetyluridine (2.0×10^{-2}M) was irradiated in the presence of tetramethylethylene (2% by volume; Aldrich Chemicals). The reaction was complete in 2 hours. Evaporation of solvent gave a glassy solid with crude weight 1.193 gm. The crude material was chromato-

graphed on 70 gm. silica gel and eluted with benzene/ethyl acetate (B/E):

B/E (100/0; 150 ml) 12.8 mg of (1);

B/E (95/5; 300 ml) 44 mg. of (1);

B/E (90/10; 270 ml) 23 mg. of (1);

B/E (80/20; 400 ml) 26 mg of (1);

B/E (60/40; 190 ml) 78.5 mg. of (2);

B/E (60/40; 100 ml) 398 mg. of (3) and (4) in a ratio of 3:1;

B/E (60.40; 375 ml) 346.5 mg. of (4);

B/E (40/60; 1.1 litre) 176 mg. of (4);

B/E (0/100;260 ml) 6.5 mg. of (4).

Compound (1) could not be recrystallized and it existed as a yellow oil.

Mass spectrum m/e:

446(<1), 307(10), 279(23), 261(5), 167(100), 141(26).

N.m.r. Multiplets at 0.85 and 1.10 - 1.70. ppm.

Compound (2) could be crystallized from ether/ hexane to give white crystals; mp. 59 - 61 °C. Analysis for $C_{21}H_{30}N_2O_9$:

Calculated	C: 55.50,	H: 6.65,	N:6.16,	O: 31.69
Found	C: 55.23,	H: 6.69,	N:5.93,	O: 32.15

Mass spectrum m/e:

455(2), 396(15), 371(5), 259(>100), 139(100), 84(77).

N.m.r. δ: 1.05(3H,S), 1.08(3H,S), 1.21(6H,S), 2.08(9H,S), 2.56(1H,d,J=13.3 Hz), 3.75(1H,d,J=13.3 Hz), 4.05 - 4.41(3H,m), 4.82(1H,d,J=3.03 Hz), 7.48(1H,S).

Compound (4) was obtained as white cyrstals by recrystallization from ether/hexane. mp. 48 0 53 °C. Analysis for $C_{21}H_{30}N_2O_9$:

Calculated	C: 55.50,	H:6.65,	O: 31.69	N: 6.16
Found	C: 55.29,	H:6.71,	O: 32.09,	N: 5.91

Mass spectrum m/e:

455(<1), 371(1.5), 259(21), 139(100), 113(52), 97(>100), 84(>100).

N.m.r. δ: 0.96(3H,S), 1.00(6H,S), 1.22(3H,S), 2.06(9H,S), 2.96(1H,d,J=9.8Hz), 3.92(1H,d,J=9.8 Hz), 4.15 - 4.36(3H,m), 5.14 (1H,t, $J_{3'2'}$ = 5.8 Hz), 5.35(1H, $J_{2'3'}$ = 5.8 Hz), 5.95(1H, S, J = 5.6 Hz), 8.61(1H,S).

Compound (3) was not further separated from compound (4) in a mixed fraction obtained from column chromatography. The mixture could be crystallized in the same manner as described for compounds (2) and (4). The mass spectrum was virtually identical to that of (4).

The n.m.r. data of compound (3) as obtained from the spectrum of the mixture are as below.

δ; 1.02(6H,S), 1.10(3H,S), 1.25(3H,S), 2.10(9H,S), 2.95(1H,d,J = 9.8 Hz), 4.00(1H,d,J = 9.8 Hz), 4.10 - 4.38(3H,m) 5.12(1H,t, $J_{2'3'}$ = 5.8 Hz), 5.30 (1H,t, $J_{2'3'}$ = 5.8 Hz), 5.79(1H, d,J = 5.6 Hz), 8.78(1H,S).

Photolysis with isopropenyl acetate

An ethyl acetate solution of 2',3',5'-triacetyluridine (2 x 10^{-2} M) was photolyzed with 2% (by volumes) of isopropenyl acetate (Eastman Kodak). The reaction was followed by UV and was complete in 3 hours. Evaporation of solvent followed by purification on TLC (Ethyl acetate/benzene = 50/50) gave a band with Rf = 0.23. The band was eluted with ethyl acetate, treated with charcoal and evaporated to dryness to give yellow solids. Yield 90%. Analysis for $C_{20}H_{26}N_2O_{11}$:

Calculated	C: 51.11,	H: 5.57,	N: 5.95,	O:37.71
Found	C: 51.38	H: 5.67,	N: 5.78,	O:31.17.

Mass spectrum m/e:

471(< 1), 371(36), 259(>100), 191(21), 139(>100), 115(66), 113(100), 97(>100).

N.m.r. δ: 1.33(2H), 1.70(3H), 2.10(12H), 2.40 - 1.72 (2H), 4.30(3H), 5.35(2H), 5.85(1H), low field (1H, broad).

Photolysis with 1,1-diethoxyethylene

(A) Preparation of 1,1-diethoxyethylene

(i) Bromoacetaldehyde diethylacetal (45 gm; Aldrich Chemicals) was refluxed in a 1:2 mixture of ethylene glycol/KOH solution (62 gm/ 112 gm) at 110 - 115 °C for 15 minutes. When KBr ceased to precipitate out, the mixture was distilled. A two-phase distillate was collected at 80 - 120 °C. The mixture was shaken in a separatory funnel and the aqueous layer was drained off, while the orgainc layer was filtered through $MgSO_4$. This separatory procedure was repeated by salting out more water with salt. The organic material was redistilled. The first fraction was collected at 74 - 80 °C and was identified as ethylacetate. The second fraction, collected at 180 °C was identified as the starting material.

m/e : 153, 117 and 103.

N.m.r. δ: 1.20(6H,t), 3.70(4H,q), 3.40(2H, d) 4.75(1H,t).

(ii) Cleaned sodium metal (10.0 gm.) and previously dried and distilled (over anhydrous sodium sulfate) cyclohexanol (300 gm.) were gently heated in a three-neck flask until the sodium was completely reacted. The solution was brought to reflux temperature. Bromoacetaldehyde diethylacetal (27 gm.) was then added from a dropping funnel and the solution refluxed for $\frac{1}{2}$ hour. The first fraction collected at 160 - 165 °C gave spectral data consistent with that of cyclohexanol (highest m/e = 100; multiplets at δ = 1.10 - 1.80 ; singlet at δ = 4.20), while the other collected at ~180 °C consisted approximately equal amounts of cyclohexanol and the unreacted starting material (by b.p., mass spectrum and n.m.r.).

(iii) By the method of McElvain and Kundiger[96], dry t-Butyl alcohol (refluxed with and distilled from Sodium ; 350 ml) was refluxed with potassium metal (20gm.) under nitrogen until all potassium was reacted (8 hours). To the warm solution, bromoacetaldehyde

diethylacetal (20 gm) was added. A partial take-off distillation head was attached to the flask through a short column and the pot then heated in an oil bath. Temperature was maintained at 124 - 127 °C while the distillate was collected at a reflux ratio (at still heat) of 1:40. After 24 hours, the bath temperature was gradually raised to 160 °C and maintained there until the flask was about to dry up. Up to this point, all material was collected at 86 °C, the b.p. of t-butyl alcohol (highest m/e = 74; δ = 1.20, 9H, S., δ= 4.15 ppm, 1H, S). The flask was cooled before vacuum pressure was applied for further distillation. More material was collected at 40 - 69 °C under 85 - 95 mm pressure. This material was redistilled under atmospheric pressure. The fraction collected at 130 - 160 °C gave a compound (of 85% purity, the other component being t- butyl alcohol) with the following spectral data:

Mass spectrum m/e:

117(18), 116(16), 89(37), 61(60), 60(58), 43(100).

N.m.r. δ: 1.25(6H,t), 3.10(2H,S), 3.84(4H,q).

yield: 60%

(B) Photolysis

Irradiation of a 2 x 10^{-2} molar solution of 2',3',5'-triacetyl

uridine containing 2% (by volume) 1,1-diethoxyethylene led to the disappearance of λ_{max} at 257 nm in 3 hours as evidenced by UV spectroscopy. Evporation of the solvent gave a colorless solid. The TLC plates (ethylacetate/benzene = 75/25) show 3 bands. The first band had the same Rf (0.40) and identical mass spectrum as triacetyluridine. The second band (Rf = 0.62) was eluted with ethyl acetate, treated with charcoal and recrystallized from ether/hexane to give 240 mg. of white solids. Analysis for $C_{21}H_{30}N_2O_{11}$:

Calculated C: 51.85, H: 6.22, N:5.76, O: 36.17

Found C: 51.63, H: 6.20, N:5.70, O: 36.47.

Mass spectrum m/e:

441(7), 259(70), 157(100), 139(>100), 116(>100), 112(93).

N.m.r. δ: 1.11 - 1.40 (8H,m), 2.15(9H,3S), 2.40(2H,m), 3.50(4H,q), 4.24(4H,m), 5.50(1H,m), 5.80(1H,m), 6.18(1H, broad), 8.58(1H, broad).

The third band (Rf = 0.8) after recovery as described above yielded 10 mg. of solids.

Mass spectrum m/e: 259(12), 139(100), 97(87).

Photolysis With Vinylene Carbonate

A 2×10^{-2} molar solution of 2',3',5'-triacetyluridine containing 2% vinylene carbonate (Aldrich Chemicals) was irradiated under N_2 for 3 hours. Evaporation of the solvent gave a glassy solid. Recrystallization from benzene was attempted and a small amount of white crystals was obtained which showed two prominent spectral peaks at m/e 173 and m/e 86 respectively.

The rest of the benzene solution of the crude product was chromatographed through a silica gel column and eluted with benzene/ethylacetate at different ratios progressively from 100% benzene to 100% ethylacetate. The product was treated with charcoal and recovered with 90% yield. Analysis for $C_{18}H_{20}N_2O_{12}$:

Calculated	C:	47.37,	H:	4.42,	N:	6.14,	O: 42.07
Found	C:	47.70,	H:	4.80,	N:	5.75,	O: 41.12

Mass spectrum m/e (relative intensity): 457(3), 384(6), 371(6), 310(36), 259(>100), 250(75), 191(100), 139(>100), 97(>100).

N.m.r. δ: 2.18(9H,s), 3.94 - 3.14(2H,m), 4.32(3H,m), 5.15 - 5.50(4H,m), 6.15(1H,broad), 9.10(1H,broad).

Formation of (5-formylmethyl)- and (5-carboxymethyl)-2'3'5'-triacetyl uridine on TLC plates

During attempts to purify the vinylene carbonate adducts of 2',3',5'-triacetyluridine by TLC (ethylacetate/benzene = 75/25), it was found that rearrangement occured on silica gel plates. The bands were each eluted with ethylacetate, treated with charcoal and recovered by evaporation of solvent to dryness.

The top band (Rf = 0.45) had a λ_{max}(EtOAc) = 264 nm; and shows the following mass spectral peaks:

m/e: 383, 310, 259, 139, 115, 113 and 97.

This compound (later identified as 5-formylmethyl aldehyde) was not stable enough to give a satisfactory elementary analysis nor could the aldehydic proton and the adjacent methylene protons be detected by n.m.r. spectrum.

The bottom band (Rf = 0.25) was recovered as described above and recrystallized from ether/hexane to give a yellow product. mp = 84 - 89 °C. It had a UV λ_{max}(EtOAc) = 260 nm. Analysis for $C_{17}H_{20}N_2O_{11}$:

Calculated	C: 47.67,	H: 4.71,	N: 6.54,	O: 41.09
Found	C: 47.89,	H: 4.86,	N: 6.13,	O: 41.12

Mass spectrum m/e:

413, 384, 310, 259, 157, 115; 113 and 97.

N.m.r. δ: 2.10(9H,S), 3.43(2H,S), 4.34(3H,m), 5.30(2H,m), 6.04(1H,d), 7.46(1H,S), 9.69(1H,S), 9.77(1H,S).

Separation of (5-formylmethyl)-and (5-carboxymethyl)-2',3',5'-triacetyluridine

In cases when TLC separation of (5-formyl methyl)- and (5-carboxymethyl)-2',3',5'-triacetyluridine did not give sharp and isolated bands, the two compounds could be separated from each other by dissolving the mixture in $CHCl_3$ and extracting with saturated $NaHCO_3$. The $CHCl_3$ layer now contained the aldehyde while the acid went into the aqueous layer. This was confirmed by taking a mass spectrum of the recovered materials from each layer.

Rearrangement of the Vinylene Carbonate Adduct to 5-Formylmethyl Aldehyde

(i) With KOH/95% EtOH

2',3',5' - triacetyluridine — vinylene carbonate adduct

(208 mg) was dissolved in 95% EtOH (20 ml) and warmed slightly above room temperature. KOH (0.1N, 0.2 ml) was added to the solution with constant swirling. The reaction was followed by UV and TLC and was complete in 30 minutes. A small amount of acetic acid was added to neutralize the solution followed by rotatory evaporation with 95% ethanol. The yield was nearly quantitative. The UV and mass spectrum were identical with those obtained from rearrangement on TLC plates.

An analytical method for the detection of aldehyde was developed by using TLC technique. The reaction solution of the adduct was from time to time spotted on TLC and developed in ethyl acetate / benzene (75/25). The formation of aldehyde would give a distinct spot at Rf ~ 0.45. When the plate was warmed slightly another spot at a slightly higher Rf value appeared under UV light. This spot was presumably due to the rearrangement of the adduct (to aldehyde) which was invisible before warming of the plate.

(ii) With pyridine/EtOH

The adduct (10 mg.) was dissolved in 95% EtOH (3 ml). Distilled pyridine (~ 100 µl) was added and the solution brought to reflux temperature for 2 hours. The UV and mass spectrum were identical with

those obtained in KOH/EtOH catalyzed conversion of the compound.

Prolonged refluxing of the solution (overnight) gave two compounds with different Rf values on TLC (ethyl acetate/benzene - 50/50) and different λ_{max} in UV. The one with higher Rf has a λ_{max}(EtOH) = 265 nm; m/e 441, 371, 259, 139 and 103. The compound with low Rf has a λ_{max}(EtOH) = 260 nm; m/e 429, 371, 259, 139, 103, and 97.

(iii) With pyridine/ethyl acetate

Distilled pyridine (2 ml) and ethylacetate (1 ml) were added to a flask containing the cycloadduct (10 mg.). The solution was refluxed for 2 hours and then evaporated to dryness. The compound had a λ_{max} (EtOH) = 264 nm.

However, when the reaction was carried out on a larger scale (adduct = 117 mg.) no conversion was observed.

(iv) With hydrated silica gel and ethyl acetate/ benzene

The adduct (10 mg.) was dissolved in ethyl acetate (2 ml) and poured into a flask containing hydrated silica gel (DSF-5, 1 gm.). The mixture was dried slowly with vacuum line suction. Then a 1:1 mixed

solvent of ethyl acetate/ benzene (5 ml) was added and the mixture stirred at room temperature for about 1 hour. The solvent was filtered off and evaporated to dryness. UV λ_{max}(EtOH) = 263 nm. Yield 55% assuming $\epsilon = 10^4$.

m/e: 384, 310, 259, 139, and 97.

(v) By column chromatography with hydrated silica gel

The crude photocycloadduct from photolysis of 2',3',5' - triacetyluridine with vinylene carbonate was chromatographed through a column packed with a slurry of hydrated silica gel (prepared by washing DSF-5 silica gel with distilled water followed by suction filtration and air drying) in benzene. This was eluted first with benzene, then benzene/ethyl acetate (50/50), benzene/ethyl acetate (20/80) and finally ethyl acetate. Obtained were:

Compound	Amount	Eluant
Vinylene carbonate	101 mg.	benzene
Photoadducts	22 mg.	benzene/ethyl acetate (50/50)
Photoadducts	431 mg.	benzene/ethyl acetate (20/80)
Photoadducts	5 mg.	ethylacetate

(vi) With dimethylformamide/triethylamine

The adduct (165 mg.) was dissolved in a 1:1 mixture of dimethylformamide/ triethylamine solution (10 ml) and stirred at room temperature. A TLC test taken immediately after dissolution of the adduct showed one single dark spot. No additional spot appeared upon heating of the plates. The solution was stirred for $1\frac{1}{2}$ hours and then evaporated with ethanol. Purification by TLC (ethyl acetate/benzene = 75/25) gave a long strip of dark band running from the base line to Rf ~ 0.40. The band was eluted with ethyl acetate and rotatory evaporated to dryness. A yellow solid was obtained and recrystallized from chloroform/hexane. Recovered was 102 mg. white crystals. Mp. 84 - 88 °C. UV λ_{max}(EtOH) = 260 nm. The n.m.r. spectrum was identical to that of 5-carboxymethyl 2',3',5'-triacetyluridine obtained previously.

(vii) With 95% ethanol at room temperature overnight

The adduct (10 mg.) was dissolved in 95% ethanol (100 ml) in a volumetric flask and left overnight at room temperature. By UV absorbance, about 50% conversion (assuming $\epsilon = 10^4$ for aldehyde) of the adduct to aldehyde took place after 18 hours. The absorbance did not increase significantly by longer standing (48 hours) of the

solution. UV λ_{max}(EtOH) = 263 nm.

Effect of acids on 2',3',5'-triacetyluridine —— vinylene carbonate Adduct

(i) With HCl

A solution was prepared by dissolving the adduct (10 mg.) in 95% ethanol (100 ml) and trace of 0.1 N HCl was then added. No change in UV absorption was observed.

(ii) With acetic acid/95% ethanol

95% ethanol (3 ml) and previously distilled acetic acid (0.1 ml) were used to dissolve the adduct (5 mg). The solution was refluxed for $2\frac{1}{2}$ hours and then evaporated repeatedly with ethanol until all acid was gone. The solid material recovered gave virtually no UV absorption.

The reaction was repeated at room temperature for 2 hours. Again no UV absorption was obtained.

(iii) With p-Toluenesulfonic Acid/95% Ethanol

The adduct (194.5 mg.) was refluxed with p-toluenesulfonic acid

(20 mg) in 95% ethanol (8 ml) for $2\frac{1}{2}$ hours. The solution was diluted with some saturated $NaHCO_3$ solution. Extraction with $CHCl_3$ gave 78 mg. solid material after evaporation of the solvent. The n.m.r. spectrum showed a spectrum virtually identical to that of the adduct before acid treatment, except that peaks due to some p-toluenesulfonic acid were also present.

Acetylation of Ribothymidine (5-Methyluridine)

Synthetic ribothymidine[89] (5 mg.) was stirred overnight (18 hours) in a 4:1 mixture of acetic anhydride/pyridine solution (each freshly distilled; 1.25 ml) at room temperature. Methanol (4 ml) was added carefully and the solution was heated briefly (~ 10 minutes) on a steam bath followed by evaporation with ethanol. The identity of the product was confirmed by its mass spectrum.

Mass spectrum m/e (relative intensity): 384(10), 324(6), 311(4), 259(>100), 208(20), 204(27), 157(100), 139(>100), 127(75), 115(67), 113(37), 105(37), 97(>100).

Preparation of tris(triphenylphosphine) rhodium (I) chloride

The method of Wilkinson et al.[90] was followed. Rhodium trichloride hydrate (2 gm., Alfa Chemicals)and a 6 molar excess of triphenylphosphine (12.6 gm., MCB) were dissolved in 95% ethanol (70 ml). The solution was brought to reflux. Orange crystalline material appeared almost instantaneously upon refluxing. After 40 minutes, the crystals turned dark red and the reaction was stopped 10 minutes later. The crystalline product was collected by suction filtration to give quantitative yield (7.5 gm.).

Decarbonylation of (5-formylmethyl) 2',3',5'-triacetyluridine

(i) In benzene/95% ethanol

The freshly made aldehyde (200 mg.) was separated on TLC plates (ethyl acetate/benzene = 75/25) and eluted with ethylacetate, rotatory dried and promptly dissolved in benzene/95% ethanol (10 ml/2 ml). About 1 equivalent of tris(triphenylphosphine) rhodium chloride (460 mg.) was added. The mixture was refluxed on a steam bath for 3 hours. As the reaction proceeded, the solution color turned lighter from its original dark red. The reaction was checked by TLC (ethyl acetate /

benzene = 75/25) against the authentic triacetylribothymidine (Rf = 0.5) until further refluxing did not show darker appearance of the spot under UV lamp. The rhodium complex was removed by suction filtration and the solution was evaporated to give a yellow crude product. Ethyl-acetate was used to precipitate out triphenylphosphine oxide (m/e 277). This was repeated several times. The remaining triphenylphosphine oxide could be removed from triacetylribothymidine by chromatographing on TLC plates with ethyl acetate/benzene in a 9:1 ratio.

The TLC band gave recovered product, after treatment with charcoal in 42% yield (76 mg.). UV λ_{max}(EtOH) = 266 nm.

Mass spectrum m/e (relative intensity):

384(<1), 324(3), 311(2), 259(>100), 208(17), 204(23), 157(100), 139(>100), 127(76), 115(61), 113(23), 97(>100).

The n.m.r. spectrum was similar to that of 2',3',5'-triacetyl uridine except that the H_5 proton at δ= 5.75 was absent and instead a singlet appeared at δ= 1.9 ppm (3H) and H_6 now appeared at = 7.2 ppm (1H).

(ii) In acetonitrile

The aldehyde (82.5 mg.) freshly prepared by KOH catalysis

was dissolved in acetonitrile (16.6 ml). The solution was refluxed with tris(triphenylphosphine) rhodium chloride (297.5 mg.) under nitrogen for 6 hours. The rhodium complex was removed by suction filtration and the triphenylphosphine oxide was crystallized out with ethyl acetate as described above. No triacetylribothymidine was detected on TLC or by mass spectrometry.

(iii) In methylene chloride

The freshly prepared aldehyde (71 mg.) was refluxed under nitrogen with tris(triphenylphosphine) rhodium chloride (350 mg.) in methylene chloride (15 ml) for 5 hours. The rhodium complex was removed by suction filtration. The filtrate was evaporated to give a yellow residue from which triphenylphosphine oxide was repeatedly crystallized out with ethyl acetate. The residue was dissolved in a small amount of ethyl acetate and chromatographed on TLC with ethyl acetate/benzeze (9:1).

The band with the same Rf value as the authentic triacetylribothymidine was eluted with ethylacetate, treated with charcoal and evaporated to give 28% yield. The mass spectrum showed a similar pattern to the authentic triacetylribothymidine; however, an additional

peak was observed at m/e = 446.

Deacetylation of synthetic triacetylribothymidine

2',3',5'-triacetylribothymidine (36 mg.) synthesized by decarbonylation of (5-formyl methyl)-2',3',5'-triacetyluridine was dissolved in 15% NH_4OH in methanol (3 ml). The solution was stirred at room temperature overnight. Evaporation of the solvent gave a gummy solid. The crude product was redissolved in hot ethanol, chromatographed on TLC in tetrahydrofuran. The band was eluted with tetrahydrofuran, treated with charcoal and recrystallized by addition of n-hexane. The melting point was rather wide, 150 - 180 °C. (Lit. mp. 183 - 185 °C)[73]. Further recrystallization did not improve the melting point. UV λ_{max}(EtOH) = 266 nm. (Lit. 267 nm)[73].

The mass spectrum was identical to that of ribothymidine[73]; m/e: 258, 155, 133, 125, and 97.

Attempted decarboxylation of (5-carboxymethyl)-2',3',5'-triacetyluridine

(i) Direct pyrolysis

(5-carboxymethyl) 2',3',5'-triacetyluridine (10.2 mg.) separated

from (5-formylmethyl)-2',3',5'-triacetyluridine was placed in a test tube. The test tube was heated to 250 °C for 10 minutes in a metal crucible filled with lead. The test tube was cooled and a mass spectrum of the crude sample was taken. Although there was a peak at m/e 384, other fragments seemed to be different from that in authentic triacetylribothymidine.

(ii) With pyridine

The acid (10 mg.) was warmed with dried pyridine (1 ml) on a steam bath for 30 minutes. The solution was diluted with $CHCl_3$ and extracted 3 times with 0.1 N HCl. The $CHCl_3$ layer was filtered through $MgSO_4$ and treated with activated charcoal. The compound showed a peak 4 mass units less (m/e 380) than triacetylribothymidine.

(iii) With benzene/iodine

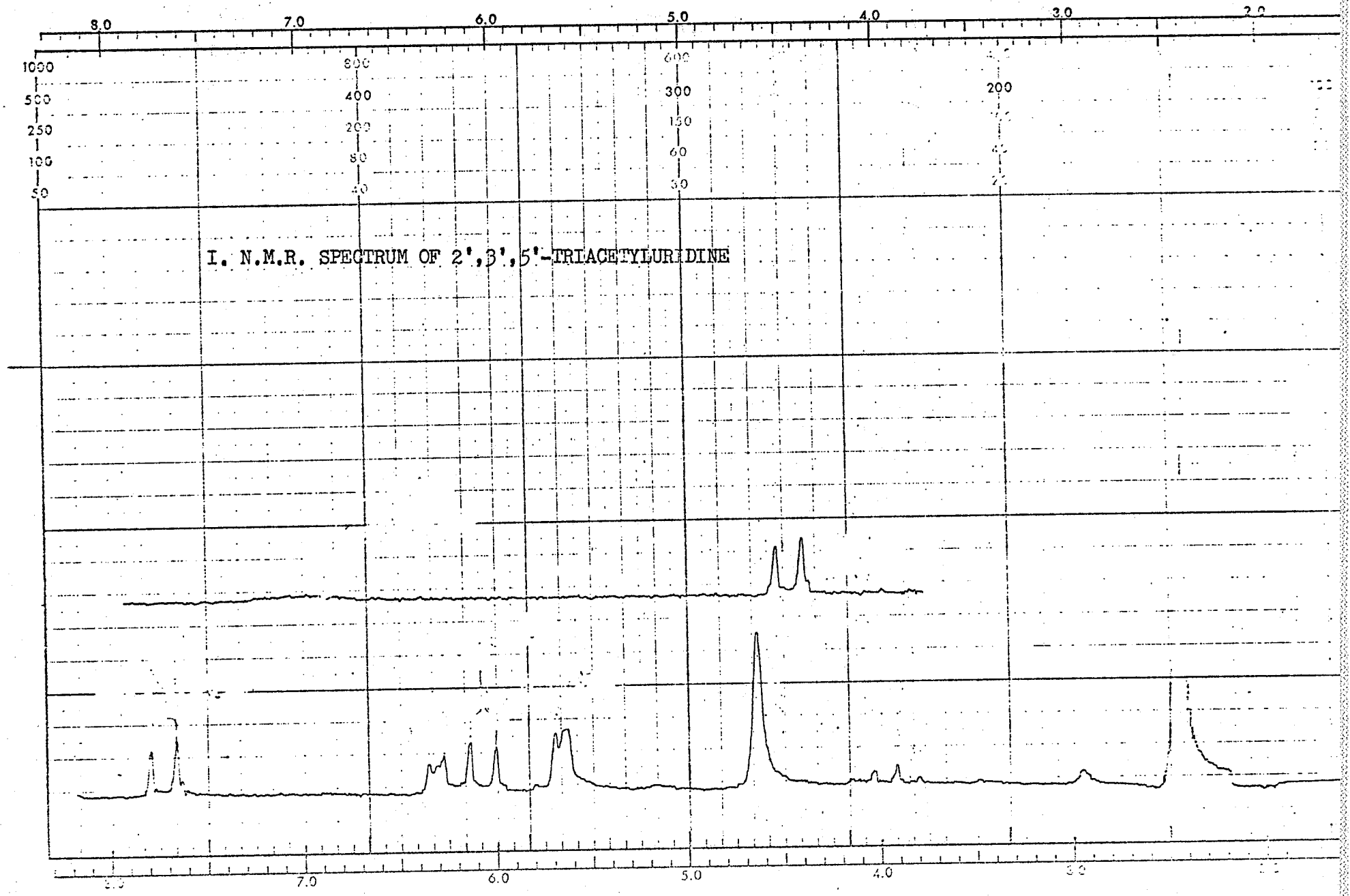
The acid (5 mg.) was not very soluble in benzene (5 ml). A minimal amount of $CHCl_3$ was added to dissolve the compound. The solution was refluxed gently overnight with a single crystal of iodine. $CHCl_3$ was added to wash the residue and filtered through $MgSO_4$/charcoal.

Evaporation of the solvent gave brownish solid with a highest mass peak at m/e 279.

(iv) With copper/quinoline

The acid (10.0 mg.) was refluxed with copper powder (30 mg.) and quinoline (2 ml) at 230 ^{o}C for 20 minutes. $CHCl_3$ was added and this was then washed with 0.1 N HCl. The $CHCl_3$ layer was filtered through $MgSO_4$ and treated with activated charcoal. Although TLC seemed to show the presence of decarboxylated product, only the sugar peak (m/e 259) was observed in the mass spectrum..

N.M.R. SPECTRA


8.0
7.0
6.0
5.0
4.0
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1000
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200
80
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600
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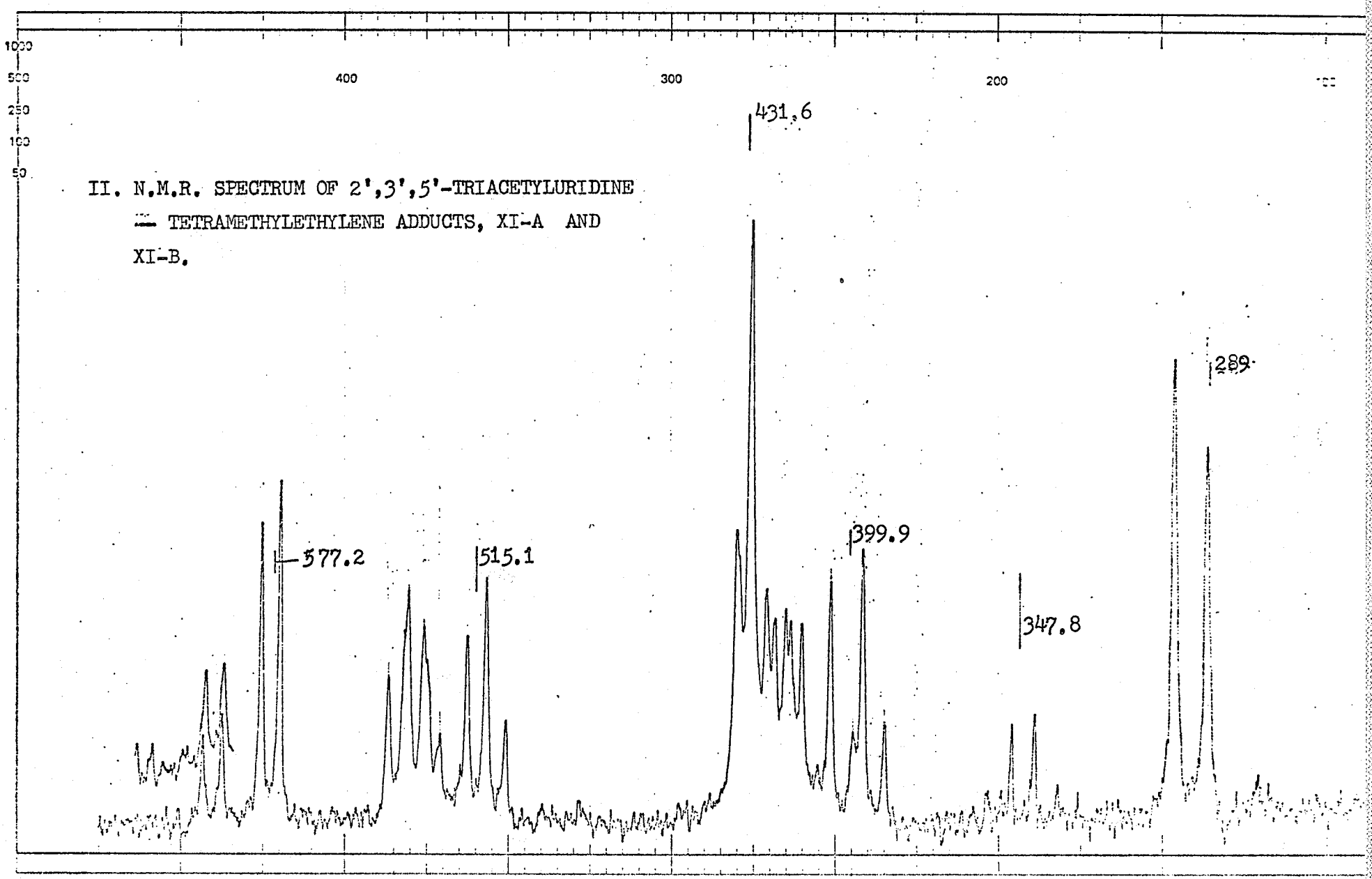
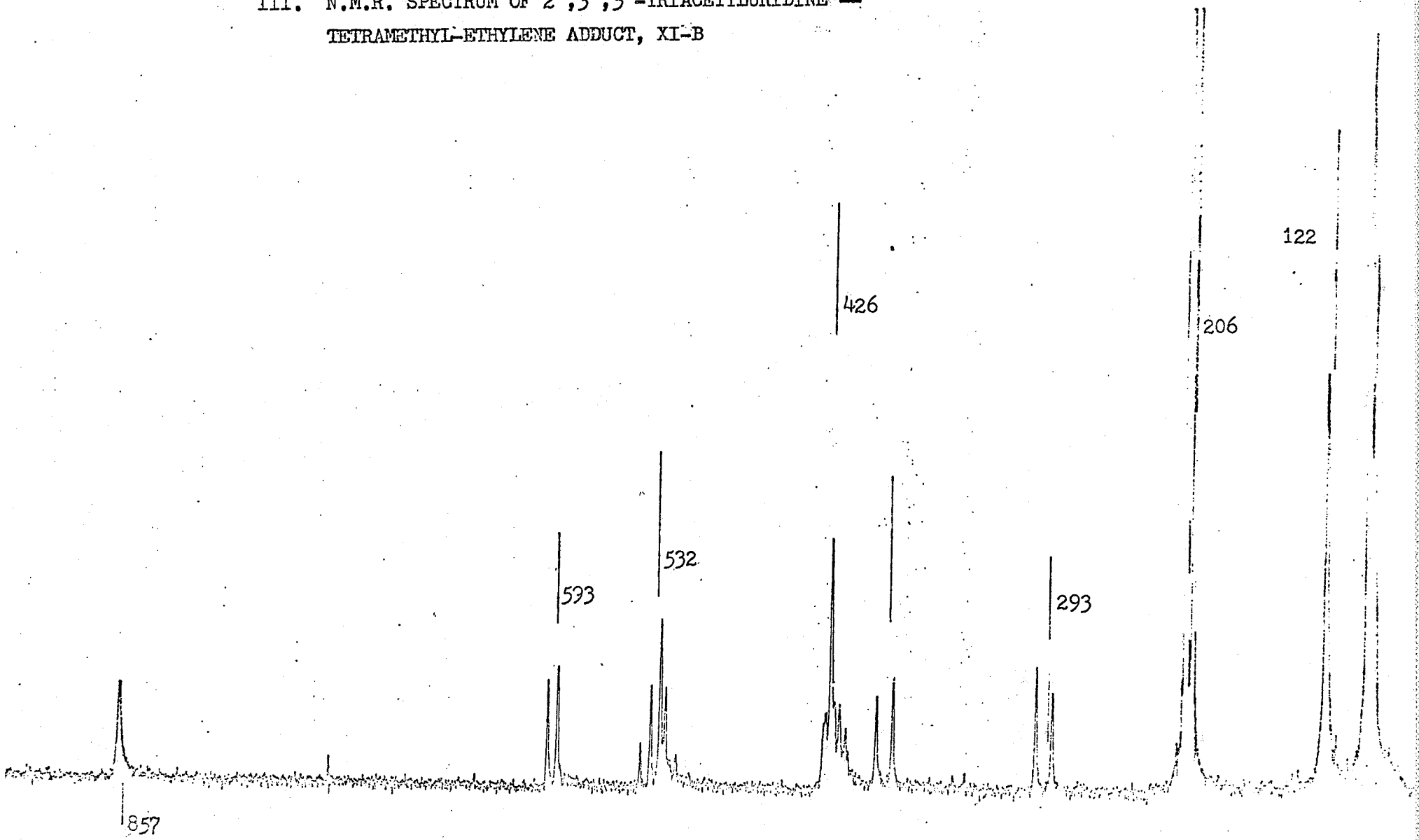
150
60
30
200
I. N.M.R. SPECTRUM OF 2',3',5'-TRIACETYLURIDINE

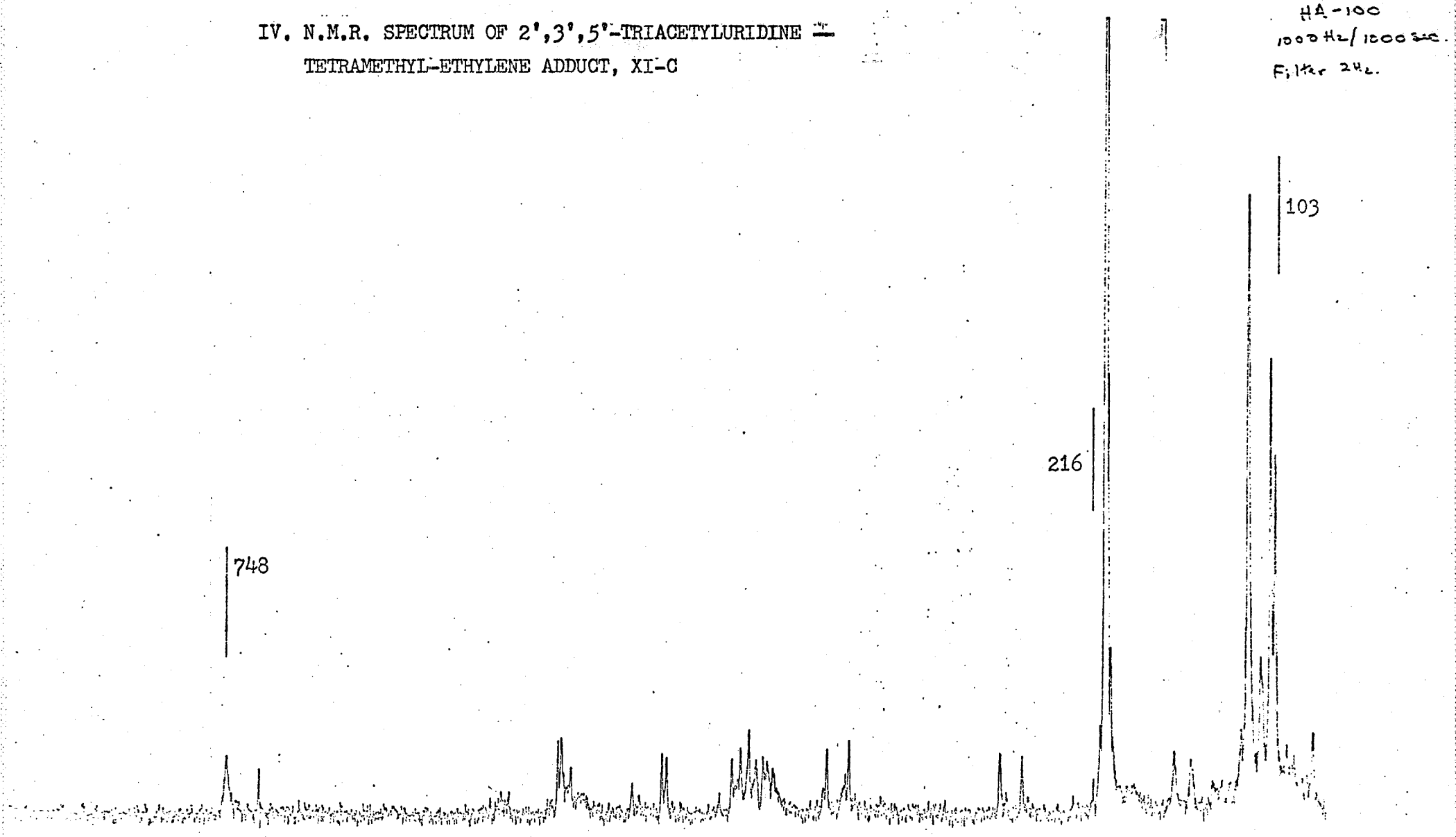
II. N.M.R. SPECTRUM OF 2',3',5'-TRIACETYLURIDINE — TETRAMETHYLETHYLENE ADDUCTS, XI-A AND XI-B.

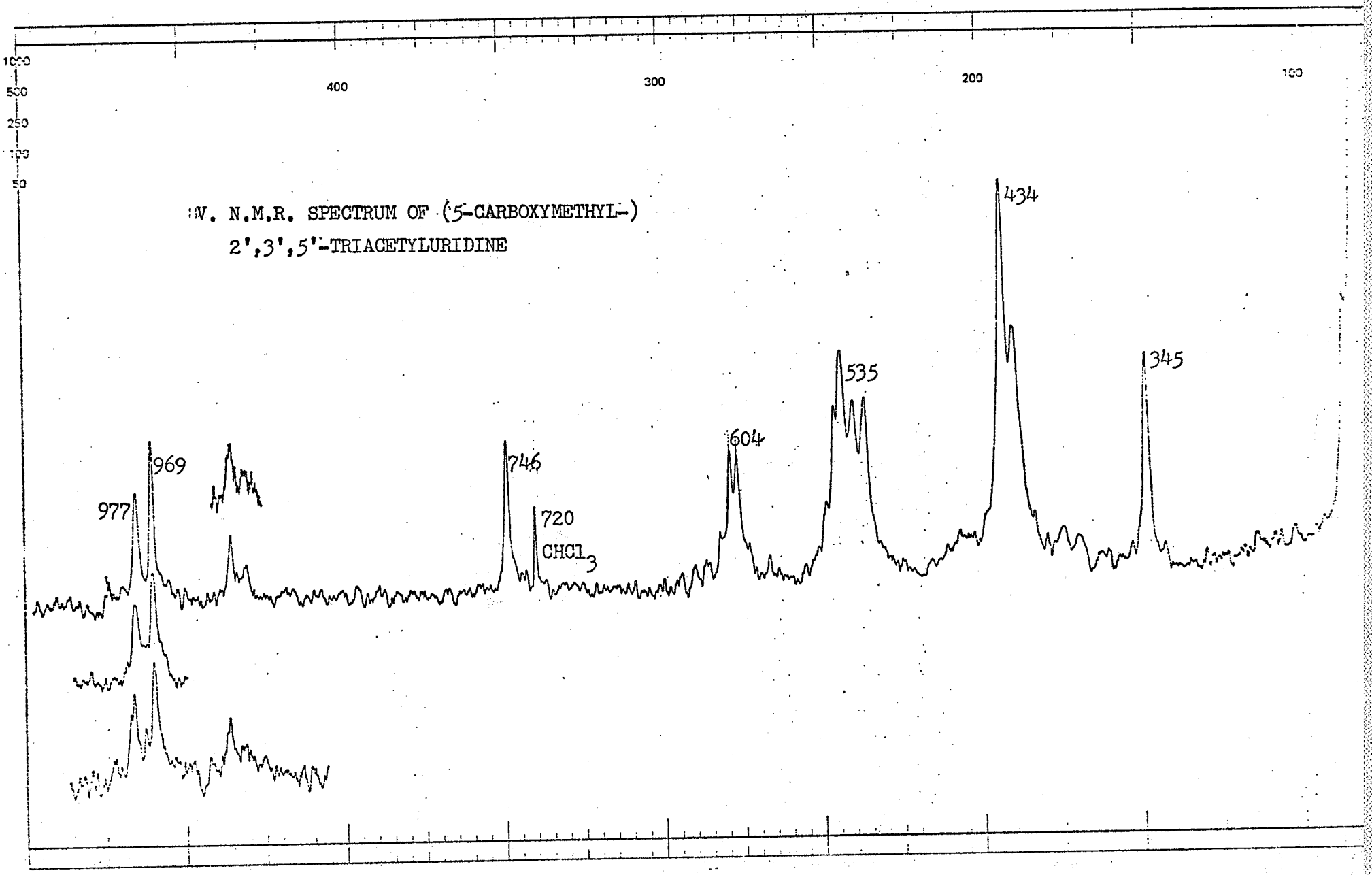


III. N.M.R. SPECTRUM OF 2',3',5'-TRIACETYLURIDINE — TETRAMETHYL-ETHYLENE ADDUCT, XI-B



IV. N.M.R. SPECTRUM OF 2',3',5'-TRIACETYLURIDINE — TETRAMETHYL-ETHYLENE ADDUCT, XI-C





IV. N.M.R. SPECTRUM OF (5-CARBOXYMETHYL-)
2',3',5'-TRIACETYLURIDINE

REFERENCES

1. A.D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic acids", Pergamon, New York, 1964. p.328

2. J.G. Burr, "Advances in photochemistry", Ed. Pitts, Hammond and Noyes, Wiley Interscience, New York, 1968. Vol. 6, p.193.

3. E. Fahr, Angew. Chem. Internatl. Edt., 8, 578 (1969).

4. A.A. Lamola, Pure and Applied Chem., 34, 281 (1973).

5. J. Eisinger and R.G. Shulman, Sci., 161, 1311(1968)

6a. K.C. Smith, "Photophysiology", Ed. A.C. Giese, Academic Press, New York, Vol. 2, p.329.

6b. K.C. Smith, Photochem. and Photobiol., 3, 787(1961)

7. A. Nacker, "Progress in Nucleic acids Research", Ed. J.N. Davidson and W.E. Cohn, Academic Press, New York, 1963, Vol. 1, p. 369.

8. R.B. Setlow, Sci., 153, 179(1966).

9. J.K. Setlow, "Current Topics in Radiation Research", Ed. M. Ebert and A. Howard, North Holland Publ. Co., Amsterdam, 1966. Vol. 2, p.596.

10. V.I. Danilov, Y.A., Kruglyak, V.A. Kuprievich and V.V. Ogloblin, Theoret. Chem. Acta. 14, 242(1969).

11. A. Imamura, H. Fujita and C. Nagata, Bull. Chem. Soc. (Japan), 40, 21(1967).

12. Z. Neiman, Israel J. Chem., 9, 119(1971).

13. H. Steinmans, I. Rosenthal and D. Elad, J. Org. Chem., 36, 3594(1971)

14. J. Salomon and D. Elad, J. Org. Chem., 38, 3420(1973).

15. H. Linschitz and J.S. Connolly, J. Am. Chem. Soc., 90, 2979(1968).

16. J.D. Spikes and B.W. Glad, Photochem. and Photobiol., 3, 471(1964).

17. N.J. Leonard and C.R. Frihart, J. Am. Chem. Soc., 96, 5895 (1974).

18. E. Sztumpt - Kulikovska, D. Shugar and J.W. Bong, Photochem. and Photobiol., 6, 41(1967)

19. I.H. Brown and H.E. Johns, Photochem. and Photobiol., 8, 273(1960).

20. M. Charlier, C. Helene and M. Dourlent, J. Chem. Phys., 66, 700(1969)

21. M. Charlier and C. Helene, Photochem. and Photobiol., 6, 601(1967).

22. H. Morrison and R. Kleopfer, J. Am. Chem. Soc., 90, 5037 (1968)

23. R. Kleopfer and H. Morrison, J. Am. Chem. Soc., 94, 255 (1972)

24. A.A. Lamola and J. Eisinger, Proc. Natl. Acad. Sci., U.S., 59, 46(1968).

25. A.J. Varghese, Biochem., 9, 4781(1970)

26. A.J. Varghese, Biochem. Biophys. Res. Comm., 38, 484(1970)

27. J.E. Donnellan Jr. and R.B. Setlow, Sci., 149, 30*(1965)

28. P.S. Pershan, R.G. Shulman, B.J. Wyluda and J. Eisinger, Physics. 1, 163(1964).

29. M. Lacroix and A. Van de Vorst, Photochem. and Photobiol., 2, 477(1968)

30. B. Pruden, W. Suipes and W. Gordy, Proc. Natl. Acad. Sci., U.S. 53, 917(1965)

31. W.E. Cohn and D.C. Doherty, J. Am. Chem. Soc., 78, 2863(1956).

32. M. Green and S.S. Cohen, J. Biol. Chem., 228, 601(1968).

33. N.C. Yang, R. Okazaki and F.T. Liu, Chem. Comm., 462(1974).

34. D.F. Rhoades and S.Y. Wang, Biochem., 10, 4603(1971)

35. B. Witkop, Photochem. and Photobiol., 7, 813(1968)

36. Y. Kondo and B. Witkop, J. Am. Chem. Soc., 90, 764(1968)

37. R. Alcantara and S.Y. Wang, Photochem. and Photobiol., 4, 465(1965)

38. K.C. Smith, Biochem. Biophys. Res. Comm., 39, 1011(1970)

39. K.C. Smith and R.T. Aplin, Biochem., 5, 2115(1966)

40. A.J. Varghese, Photochem. and Photobiol., 20, 339(1074).

41. G.M. Blackburn, R.G. Fenwick and M.H. Thompson, Tetra. Lett., 589(1972)

42. M.N. Khattak and S.Y. Wang, Sci., 163, 1341(1969)

43. A.J. Varghese and S.Y. Wang, Sci., 160, 186(1968).

44. S.Y. Wang and A.J. Varghese, Sci., 164, 183(1969)

45. I.L. Karle, Acta. Cryst. B25, 2119(1969)

46. R.F. Rhoades and S.Y. Wang, Biochem. 9, 4416(1970)

47. ibid., 10, 4603(1971)

48a. "Photochemistry", J. Chem. Soc., (London) Vol. 4, P798(1973).

48b. ibid, P550(1973)

49. S.Y. Wang and R.F. Rhoades, J. Am. Chem. Soc., 93, 2554(1971)

50. S.Y. Wang, J. Am. Chem. Soc., 93, 2768(1971)

51. J.L. Flippen and I.L. Karle, J. Am. Chem. S c., 93, 2762(1971)

52. J.L. Fourrey, P. Jouin and J. Moron, Tetra. Lett. 3003(1974) and references therein.

53. C. Helene and F. Brun, Photochem. and Photobiol., 11, 77 (1970)

54. R. Bengelmans, J.L. Fourray, S.D. Gero, M.T. Legoff, D. Meriur and V. Ratoveimanana, Compt. rend. 274 C, 882(1972)

55. L. Musajo and G. Rodighiero, Photochem. and Photobiol., 11, 27(1970)

56. J.A. Hyatt and J.S. Swenton, J. Am. Chem. Soc., 94, 7605 (1972)

57. J.S. Swenton, J.A. Hyatt, J.M. Lisy and J. Clardy, J. Am. Chem. Soc., 96, 4885(1974).

58. D.E. Bergstrom and W.C. Agosta, Tetra. Lett. 1087(1974).

59. E. Krajewska and D. Shugar, Sci., 173, 453(1971)

60. P.T. Ho, S.F. Lee, D. Chang and K. Wiesner, Experientia, 27, 1377(1971)

61. J. W. Littlefield and D.B. Dunn, Biochem. J., 70, 642(1958)

62. P. Reichard, Acta . Chem. Scad., 9, 1275(1955)

63. J.J. Fox, N. Yung, J. Davoll and G.B. Brown, J. Am. Chem. Soc., 78, 2117(1956)

64. T. Nishimura, B. Shimizu and I. Iwai, Chem. Pharm. Bull.(Tokyo) 12, 1471(1964)

65. E.I. Budowsky, V.N. Shibaev and G.I. Elisceva, "Synthetic Procedures in Nucleic acids chemistry", Ed. W. Werner, Wiley Interscience, 1968. Vol. 1, p.436.

66. R.C. Cline, R.M. Fink and K. Fink, J. Am. Chem. Soc., 81, 2521(1959)

67. M.W. Gray and B.G. Lane, Biochim. Biophys. Acta., 134, 243(1967)

68. M.W. Gray and B.G. Lane, Biochem. 7, 3441(1968)

69. T.D. Tumaitis and B.G. Lane, Biochim. Biophys. Acta, 224, 391(1970)

70. G.A. Ivanovics, R.J. Rousseau and R.K. Robins, Physiol. Chim. Phys., 3, 489(1971)

71. P. Brouskill, T.O. Kennedy and B.G. Lane, Biochim. Biophys. Acta., 262, 275(1972)

72. To the best of our knowledge, only one oxetane has actually been isolated namely, that formed between benzophenone and thymine. I. Von Wilucki, H. Matthaus and C.H. Krauch, Photochem. and Photobiol., 6, 497(1967).

73. R. Hall, "The Modified Nucleosides of Nucleic Acids", Columbia University Press, 1971. p.162.

74. F.E. Ullman, Accounts. Chem. Res. 1, 335-359(1968)

75. K. Biemann and J.A. McCloskey, J. Am. Chem. Soc., 84, 2005 (1962)

76. F.W. McLafferty, "Interpretation of Mass Spectra", 2nd ed., W. A. Benjamin Inc., 1973, p.31.

77. M.J. Robins, S.R. Naik and A.S.K. Lee, J. Org. Chem., 39, 1891(1974).

78. J.M. Rice and G.O. Dudek, Biochem. Biophys. Res. Comm., 35, 383(1969)

79. D.C.K. Lin, Ph.D. Thesis, University of Manitoba, 1972.

80. S. Tsuboyama and J. H. McCloskey, J. Org. Chem., 37, 166(1972)

81. H.H. Jaffe and M. Orchin, "Theory and Application of Ultraviolet Spectroscopy", Wiley and Sons Inc., 1962, p.175.

82. N.J. Turro, "Molecular Photochemistry", Benjamin, 1965, p.208.

83. G. Buchi, C.W. Perry and E.W. Robb, J. Org. Chem., 27, 4106 (1962)

84. D. Bryce - Smith and J.E. Lodge, J. Chem. Soc., 695(1963).

85. P.J. Nelson, D. Ostrem, J.D. Lassila and O.L. Chapman, J. Org. Chem., 34, 811(1969)

86. A.M. Kaupler C. Monny and A.M. Michelson, Biochim. Biophys. Acta. 217, 18(1970).

87. A.M. Kaupler and E. Reich, Biochem., 9, 4050(1971)

88. M. Ikehara, I. Tazawa and T. Fukui, Biochem., 8, 736(1969)

89. We thank Dr. K.K. Ogilvie for a gift of this compound.

90. M.C. Baird, C.J. Nyman and G. Wilkinson, J. Chem. Soc., A, 348(1968).

91. N.R. Hunter, G.A. MacAlpine, H.J. Liu and Z. Valenta, Can. J. Chem., 48, 1436,(1970)

92. D.M. Brown, A.R. Todd and S. Varadarajan, J. Chem. Soc., 2388(1956)

93. L.E. Fieser and M. Fieser, "Reagents for organic synthesis", Wiley and Sons Inc., 1966, p.295.

94. D. L. Wulff and G. Fraenkel, Biochim. Biophys. Acta. 51, 332 (1961)

95. J. Schmidt and F. Leipprand, Chem. Ber., 38, 3751 (1905)

96. S. M. McElvain and D. Kundiger, "Organic Synthesis", Wiley and Sons Inc., New York, 1964, p.506.