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RATES OF FORMATION OF ANHYDRONUCLEOSIDES AND COMPARISON WITH THEIR CORRESPONDING OXY ANALOGUES

G.R. NIAZ AND FASIHULLAH KHAN
Department of Applied Chemistry, University of Karachi, Karachi-75270

ABSTRACT:

Anhydronucleosides are reactive intermediates in the synthesis of modified nucleosides. Their rates of cyclization of 2,2'; 2,3' and 2,5' - anhydro 2-thiouridine were studied and compared with their oxy analogues. The order of displacement of the leaving group is as follows: 2'>5'> 3'. It was also observed that the rates of cyclization onto the 5' or 3'-position was about 70 times faster in 2-thiouridine series as compared with the uridine series. Similarly the rate of formation of 2, 2' - anhydronucleoside is nearly 2 orders of the magnitude faster in the case of thionucleosides.

INTRODUCTION

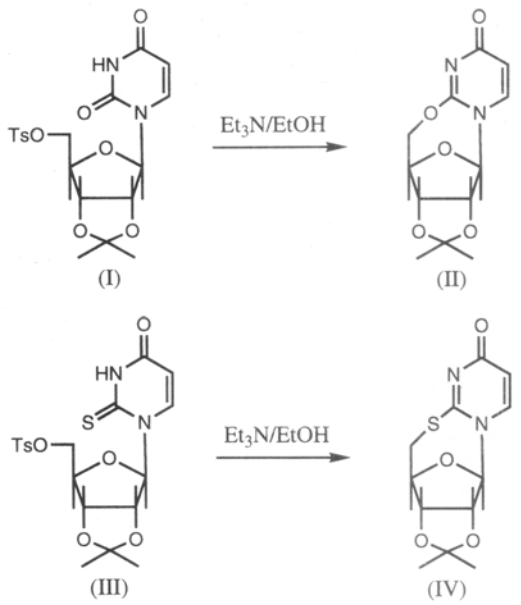
Anhydro or cyclonucleosides are useful intermediates in nucleoside chemistry. The first compound of this type was reported in 1951 by Todd and co-workers who observed that when 2', 3'-0- isopropylidene 5'-O-tosyladenosine was heated in acetone it yielded 2',3-O-isopropylidene 3,5'-cycloadenosine tosylate. These compounds have been used to establish the B-configuration of the nucleosides (Todd *et al.,* 1957). In addition one can achieve the desired alteration of the configuration of the sugar moiety and thereby obtain arabino-lyxo- or xylo pentofurauoside (Bristow and Lyurgu, 1949). Nucleosides in which 2' or 3' position are halogen substituted or compounds in which unsaturation exists at this position have also been prepared via anhydronucleoside transformations, modification of the base moiety to prepare unusual, nucleoside has also been achieved by this route (Todd *et al.,* 1951).

An active agent, against HIV virus 3'-azido 3'-deoxythymidine AZT was synthesized by treating 2, 3'-anhydrothymidine with trimethylsilyl azide and BF3 in ether. This compound is used as a potent anti HIV virus (Mandai and Acbari, 1993, Sha *et al.,* 1993).

The significance of anhydronucleosides in ribonucleoside chemistry has therefore led us to investigate some synthetic approaches to the formation of anhydronucleosides of uridine and 2-thiouridine and to compare their rates of formation.

MATERIAL AND METHODS

When 2', 3'-O-isopropylidene 5'-O-tosyluridine (I) was treated with triethylamine in ethanol solution at room temperature TLC revealed the formation of a product with lowere Rr value. This material was found to be 2', 3'-O-isopropylidene O^2, 5'- anhydrouridine (11), on comparison with authentic material prepared following the method of Brown and Todd, 1957. The half-time of the cyclization reaction was found to be approximately 12 hours and the reaction went to completion within 108 hours. For preparation purposes the above reaction was carried out at 100°C and it was observed that it went to completion in 6 hours. This approach is advantageous since it leads to a convenient synthesis of 2', 3'-O-isopropylidine O^2, 5'-anhydrouridine (II) by a shorter route, which avoids the iodination step.



The cyclization of 2', 3'-O-isopropylidene-5'-Otosyl-2- thiouridine (III) was studied under similar experimental conditions. The reaction was followed by TLC and a lower Rr value product was observed, the ultraviolet spectrum of which had $\lambda_{max.}$ at 239 nm. The reaction mixture after work-up was purified by chromatography, and the product was identified as 2',3'-O-isopropylidene-S^2,5'anhydro-uridine (IV). The half-time of the reaction was found to be ca. 10 minutes and the reaction went to completion within 3 hours at room temperature. the product crystallized from ethanol-benzene mixture and its properties were identical to those described for this compound by Ueda, 1970, who had reported the formation of this compound by heating with triethylamine in dioxan solution at 100°C for I hour. We have also studied this reaction at 100°C with triethylamine in ethanol solution, and have found that the reaction is complete within 10 minutes. It is clear from the above cyclization reactions that 2',3'-O-isopropylidene-5'-O-tosyl-2-thiouridine (Ii) cyclizes ca. 70 times as fast as its oxyanalogue.

We then undertook a study of the comparative rates of cyclizations of uridine and 2-thiouridine derivatives where the leaving group (tosylate) was (i) at the 2'-position and (ii) at the 3'-position of the ribose moiety, the remaining two hydroxyl groups being protected by acetylation. 3',5'-Di-O-acetyl 2'-O-tosyluridine (XII) was prepared following the method of Brown and Todd, 1957. Treatment of the latter compound under similar experimental conditions with triethylamine in ethanol solution at 20°C yielded a product which, on the basis of its spectral and analytical data, indicated it to be 3',5'-di-O-acetyl-O^2,2'-anhydrouridine (XIII). The half-time of the reaction was found to be ca. 3 hours, and the reaction went to completion within 36 hours.

In order to compare its rate of cyclization with that of its corresponding thio-analogue, 3',5'-di-O-acetyl-2'-O-tosyl-2-thiouridine (X) was prepared. 2-Thiouridine (V) was treated with trimethyl orthoacetate in dioxan solution at room temperature in the presence of toluene-p-sulphonic acid. TLC revealed the formation of 2',3'-O-methoxyethylidene-2-thiouridine (VI) as the main product. This was acetylated with acetic anhydride in pyridine solution. Complete acetylation at 5'-hydroxyl group was found to occur within 30 minutes. After work-up the product (VII) was treated with 90% acetic acid for 20 minutes at room temperature. In this way a mixture of 3',5'-di-O-acetyl-2-thiouridine (IX) and 2',5'-di-O-acetyl-2-thiouridine (XIII) was obtained. This mixture was purified by chromatography. The resulting glass containing (XIII) and (IX) was treated with toluene-p-sulphonyl chloride in pyridine solution. The products (XI) and (X) from the tosylation reaction were purified by chromatography, but it was not found possible to separate the two

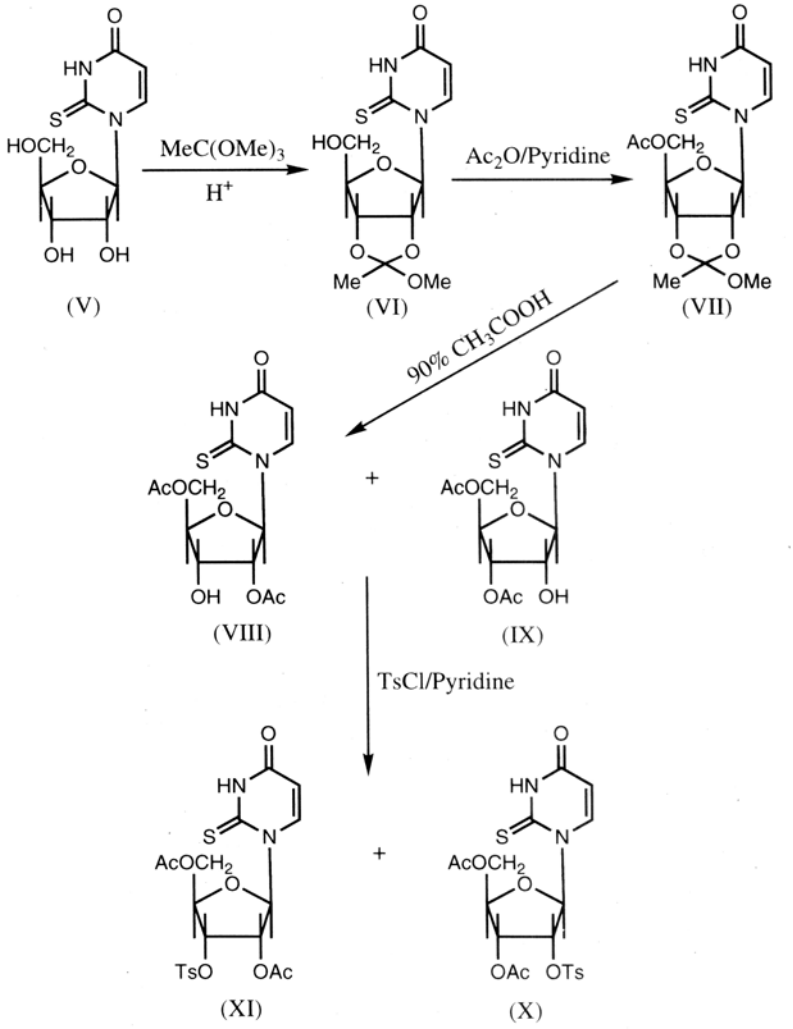
isomers.

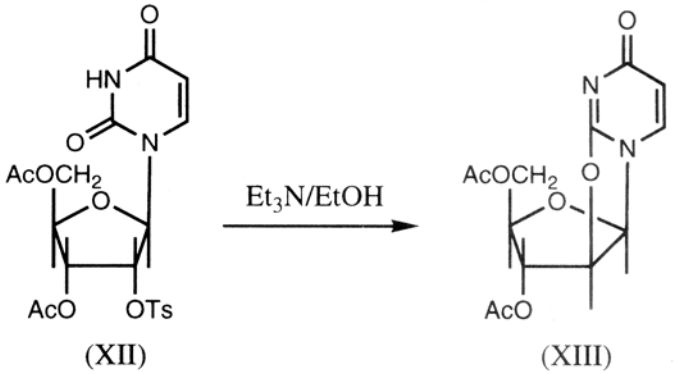
It was anticipated that 3',5'-di-O-acetyl-2'-O-tosyl-2-thiouridine (X) would cyclize more rapidly than 2',5'-di-O-acetyl-3'-O-tosyl-2-thiouridine (XI). When such a cyclization reaction was attempted on the mixture of the two tosylates with triethylamine in ethanol solution at room temperature, TLC revealed that cyclization of 3',5'-di-O-acetyl- 2'-O-tosyl-2-thiouridine (X) was complete in ca. 20 minutes (Ira of this reaction was found to he ca. 2 minutes). However, it was not found possible to get an accurate value for the half-time of the cyclization reaction. The products were chromatographed and evaporation of the appropriate fractions afforded 2',5'-di-O-acetyl-3'-O-tosyl-2-thiouridine as a crystalline compound. The later fractions contained 3',5'-di-O-acetyl-S-,2'-anhydrouridine (XI), which was crystallized and characterized. It is evident from the above cyclization reactions that the rate of cyclization of the thio-compound (X) is ca. 90 times faster than its corresponding oxy-analogue.

We next directed our efforts towards the comparison of the ease of cyclization of 2',5'-O-acetyl-3'-O-tosyl-2-thiouridine (XI) and its corresponding oxy-analogue (XV). In order to prepare the latter compound (XV), 3',5'-di-O-acetyluridine was stirred in 50% aqueous pyridine solution at room temperature for 5 minutes. An equilibrium mixture of 2',5'-di-O-acetyluridine and 3',S'-di-O-acetyluridine was obtained. The material was dried and treated with toluene-p-sulphonyl chloride in pyridine solution at room temperature to yield a mixture of 3',5'-di-O-acetyl-2'-O-tosyluridine (XII) and 2', 5'-di-O-acetyl-3'-O-tosyluridine (XV). These products had slightly different RI values on TLC and were separable by careful chromatography. The product (XV) crystallized from ethanol and gave satisfactory analytical and spectral data. When the latter was treated with triethylamine in ethanol solution ai room temperature and the reaction followed by TLC the formation of a lowere Rf value product was evident. No starting material was detectable after II days. The reaction mixture after work-up was purified by chromatography and the product was identified on the basis of its spectral and analytical data, as 2',5'-di-O-acetyl-0^2,3'-anhydrouridine (XVI). The half-time of the cyclization reaction was found to be ca. 36 hours.

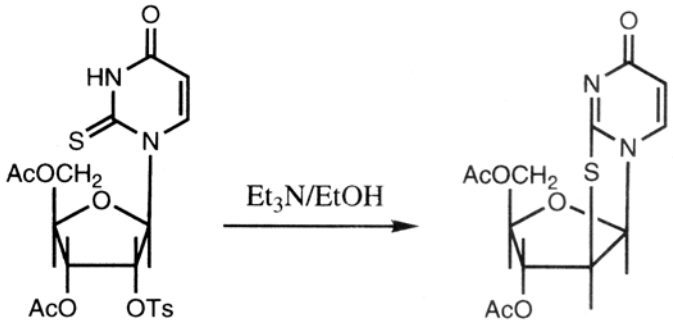
2',5'-di-O-acetyl-3'-O-tosyl-2-thiouridine (XI) was cyclized under similar experimental conditions. The main product of the reaction had a lower Rf value on TLC and was anticipated to be 2',5'-di-O-acetyl-S",3'-anhydrouridine (XVII). No starting material was detectable after 6 hours. The product (XVII) was purified by chromatography and its structure confirmed on the basis of spectral and analytical

data. The half-time of the reaction was found to be ca. 30 minutes at room temperature; thus the rate of cyclization was ca. 70 times as fast as that of its oxy-analogue.





(XII) (XIII)



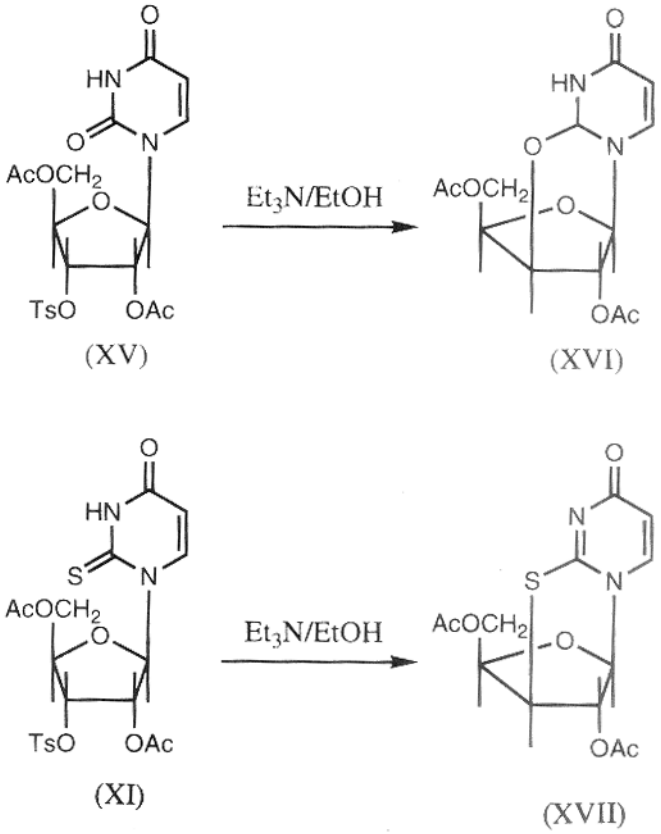
(X) (XIV)

RESULTS AND CONCLUSIONS

One can conclude from the above cyclization reactions that both in the uridine and 2-thiouridine series, the order of the displacement of the 2',3'- and 5'-leaving groups is:-

2'>5'>3'

These results are summarized in Table 1.



The above results are in accordance with expectation since sulphur compounds are more reactive than their oxy-analogues. This is due to the greater polarisability of the electrons on the sulphur atom. It should be noted that the rates of cyclization onto the 5'- or 3'- positions is ca. 70 times faster in the 2- thiouridine series as compared with the corresponding uridine derivatives. Similarly the rate of formation of the 2, 2'- anhydronucleosides is nearly 2 orders of magnitude faster in the case of the thionucleoside. The relative rates of 2, 2'-, 2, 3'-, and 2,5'-anhydronucleoside formation are in accord with previous findings.

Rates of Forma*t*ion of anhyd*ro*-u*ri*dine and -2-thiouridine Derivatives in Et3N/EtOR 0:9 *vlv)* at 20°C.

Compound	t ½	Time for Complete Reaction
2',3'-O-Isopropylidene-5'-O-tosyluridine (I)	12 hours	108 hours
2',3'-O-Isopropylidene-5'-O-tosyl-2-thiourrdine (III)	10 min	3 hours
2',5'-Di-O-acetyl-3'-O-tosyluridine (XV)	36 hours	11 days
2',5'-Di-O-acetyl-3'-O-tosyl-2-thiouridine (XI)	30 min	6 hours
3',5'-Di-O-acetyl-2'-O-tosyluridine (XII)	3 hours	6 hours
3',5'-Di-O-acetyl-2'-O-tosyl-2-thiouridine (X)	2 min	20 min

The comparative ease of formation of the S. 5'-anhydronucleoside system suggests that it might be possible to cleave a t-RNA molecule or other polyribonucleotide containing a 2-thiouracil (or substituted 2-thiouracil) moiety specifically as desired. Although phosphate is a poorer leaving group than tosylate, this process might occur at a reasonable rate in neutral or slightly alkaline solution. This would clearly he valuable in the elucidation of the structure and the mode of action of such t-RNA molecules.

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