Tetrahedron Letters No. 28, pp 2647 - 2648. 0040-4039/79/0708-2647$02.00/0
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THE REACTIONS OF THIOURACILS AND THIOURIDINES WITH CHLOROACETALDEHYDE

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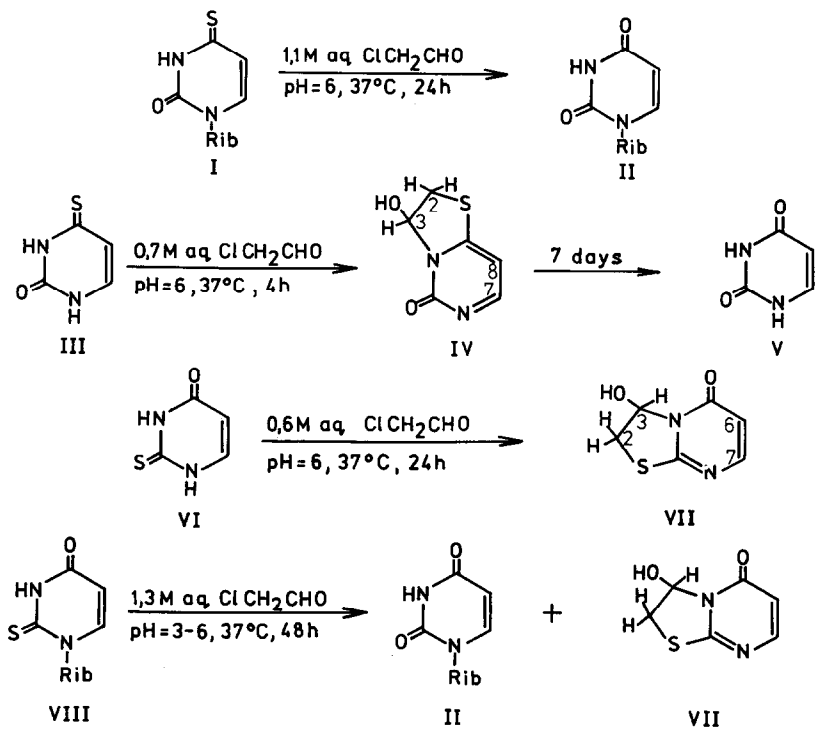
Thiouracils and thiouridines were quantitatively modified under mild conditions with aqueous chloroacetaldehyde. The " hydroxyethano " bridged thiouracils, uracil or uridine were formed .

Since 1971 when chloroacetaldehyde was first applied as a modifying reagent in the field of nucleic acids[1], its reaction[2,3] and fluorescent "etheno" products[4] have found wide applications in biochemistry. Up to now it has been known that only those base residues bearing free or monoalkylated exo-amino group in the vicinity of the endocyclic nitrogen atom /adenine, cytosine, their alkylated analogs and guanine/ could be modified by this reagent under mild conditions to give "ethene" or "hydroxyethano" derivatives, respectively[5-7]. Presently we are able to report that the sulphur-containing components of nucleic acids /2- and 4-thiouracils and their nucleosides/ are also susceptible to chloroacetaldehyde attack yielding "hydroxyethano" bridged thiouracils, uracil or uridine :



The reactions of thionucleosides and their corresponding bases were carried out at 37°C using a 30 - 100 fold molar excess of chloroacetaldehyde. The desired pH was maintained with 1 M aqueous potassium hydroxide or 0.01 M citrate buffer. Tlc analysis on silica gel in two solvent systems : A - nBuOH/H_2O 85:15 and B - nBuOH/H_2O/AcOH 5:3:2, was used to follow the reactions.

4-Thiouridine /I/ showed quantitative, kinetically one-step transformation to /II/ as revealed by tlc in the solvent system A. /II/ was the only reaction product in the whole studied pH range /4.0 - 6.5 / and its identity with uridine was confirmed by the comparison of their UV and PMR spectra. Contrary to /I/, 4-thiouracil /III/ was transformed to /VI via the stable fluorescent intermediate /IV/. Since the first step of the reaction was much faster than the second, it was easy to isolate the intermediate /IV/ in high yield /77%/ by preparative layer chromatography in solvent system A. λ_{max} 268, 329 nm, λ_{min} 287 nm at pH 2; λ_{max} 227, 329 nm, λ_{min} 278 nm at pH 7 - 14; FD-MS M^+ = 170.6; PMR /DMSO - d_6 + TFA/ δ 3.6/sext, 2, 2-H and 2'-H/, 6.1/d, 1, 3-H/, 6.4/d, 1, J=6 Hz, 6-H/, 7.8/d, 1, 7-H/.

2-Thiouracil /VI/ was quantitatively modified by chloroacetaldehyde and /VII/ was the sole reaction product. This reaction was monitored by tlc in solvent system A. The product /VII/ was easily obtained in the crystalline state by crystallization from water[8]. Yield 76%, λ_{max} 233, 294 nm, λ_{min} 260 nm at pH 2 - 7.5; λ_{max} 233 nm at pH 14; FD-MS M^+=170.6; PMR /DMSO - d_6 + TFA/ δ 3.6/sext, 2, 2-H and 2'-H/, 6.6/d, 1, 3-H/, 7.2/d, 1, J=7 Hz, 8-H/, 8.6/d, 1, 7-H/. When 2-thiouridine /VIII/ was allowed to react with chloroacetaldehyde, a mixture of /II/ and /VII/ was formed /tlc in solvent system B/. The ratio of products was found to be pH dependent and /II/ was the main product /70%/ when the reaction was carried out at pH 6, while /VII/ predominated /90%/ at pH 3.

The "hydroxyethane" bridged thiouracils /IV/ and /VII/ should serve as easily obtainable model compounds for the structural studies of thiopyrimidines and as useful intermediates for synthetic work in pyrimidine chemistry. If chloroacetaldehyde is to be used for chemical modification of tRNA molecules containing thiouridines, it is important to know that on the nucleoside level /I/ and /VIII/ are modified at rates similar to those of adenosine and cytidine.

This work was supported by the Polish Academy of Sciences, project MR - I.12.1.7.11. We thank Dr J. Bujarski and Dr J. Łuczak for supplying the analytically pure substrates.

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(Received in UK 9 May 1979)