Tetrahedron Letters No.13, pp. 1543-1546, 1968. Pergamon Press. Printed in Great Britain.

SYNTHESIS OF 6-AZAPSEUDOURIDINE FROM THE PRODUCT OF OZONOLYSIS OF 2',3',5'-TRI-O-ACETYLPSEUDOURIDINE

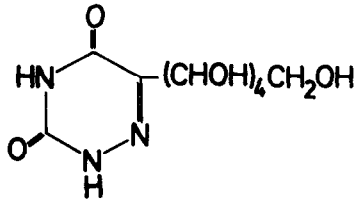
M.Bobek, J.Farkaš and F.Šorm

Institute of Organic Chemistry and Biochemistry

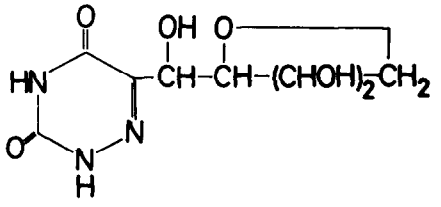
Czechoslovac Academy of Sciences, Prague

(Received in UK 4 December 1967)

It was found that 5-(D-altro-pentahydroxypentyl)-6-azauracil (I) treated with diluted hydrochloric acid yields an anhydroderivative[1] which, by analogy with a similar cyclisation of the sugar chain in the synthesis of pseudouridine[2], was tentatively identified as 5-(D-ribofuranosyl)-6-azauracil. On the basis of NMR spectra it has been found that the previously suggested structure of the anhydroderivative is erroneous. The isopropylidene derivative of the product obtained by cyclisation of I (m.p. 240-241.5°; for $C_{11}H_{15}N_3O_6$ calcd: 46.31 %C, 5.30 %H, 14.73 %N; found: 46.40 %C, 5.36 %H, 14.92 %N) dissolved in dimethylsulfoxide-d_6 (ref.3) exhibits in the NMR spectrum a signal of the hydroxyl proton split into a doublet (τ 4.84, $J_{CH,OH}$ 5.5 Hz at 100 mHz, Varian HA-100). The presence of a secondary hydroxyl group thus indicates that the cyclisation of I in an acid medium yields, contrary to expectation, 5-(2,5-anhydro-D-altro-pentahydroxypentyl)-6-azauracil (II). Cyclisation without inversion at C_2' is assumed on the basis of the well-known steric course of cyclisation of alditols[4].



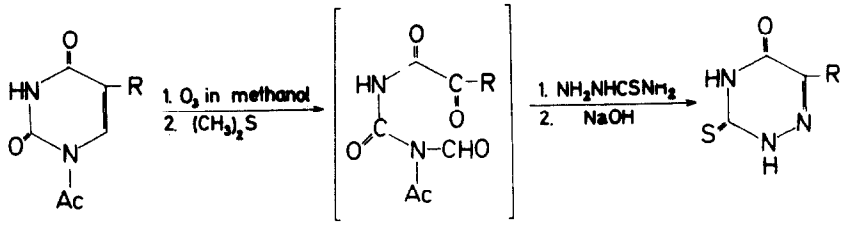
I D-altro-



II D-altro-

For this reason, the synthesis of 6-azapseudouridine was attempted by the method starting from 3,6-anhydro-D-allo-heptulosonic acid. Ozonolytic cleavage of pseudouridine was considered as a suitable method of preparation of this acid.

In model experiments, 2-thio-6-azathymine (V) and 2-thio-5-methoxymethyl-6-azauracil (VI) were prepared from α-keto acids obtained by ozonolytic cleavage of 1-acetylthymine[5] (III) and 1-acetyl-5-methoxymethyluracil[+] (IV).



III R = CH_3
IV R = CH_2OCH_3

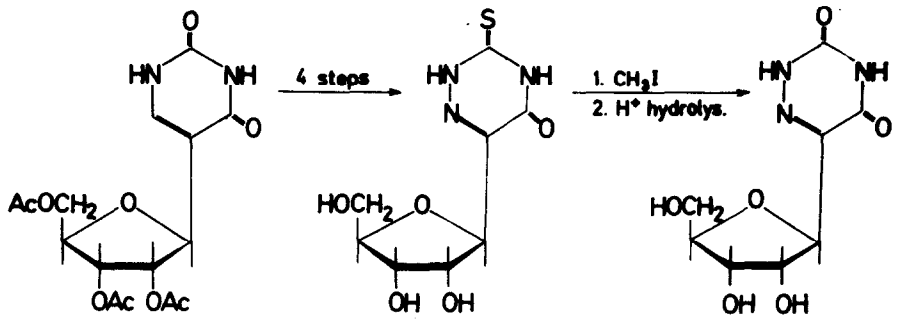
V R = CH_3
VI R = CH_2OCH_3

A slow stream of ozonized oxygen was introduced into a solution of III in methanol at -40° until absorption at 260 nm disappeared (75 min.). The ozonide formed was reduced by dimethylsufide[6] in methanol for 12 hours at 0°. The crude reaction mixture was heated with an aqueous solution of thiosemicarbazide to 80° for 20 min. and the thiosemicarbazone formed wax cyclised by treatment with 1 M sodium hydroxide for 20 min. at 100°. 2-Thio-6-azathymine[7] (V) melting 218-219° (water) was obtained in a total yield of 37 %. The same procedure was used for preparing 2-thio-5-methoxymethyl-6-azauracil (VI) from IV, the total yield being 42 %.M.p. 230-233° (water). UV spectrum: λ_{max}214 nm(logϵ 4.18), λ_{max}268 nm(logϵ 4.34) in 0.1 M HCl; λ_{max}282 nm(logϵ 4.33),

---

[+] Prepared in the same way as 1-acetylthymine[5].M.p. 164° (etnanol); UV spectrum: λ_{max}211 nm(logϵ 3.89), λ_{max}260 nm(logϵ 3.97) in ethanol.

λ_{max}256 nm(logε 4.27), λ_{max}306 nm(logε 3.69) in 0.1 M NaOH. For $C_5H_7N_3O_2S$ calcd: 34.67 %C, 4.07 %H, 24.27 %N, 18.48 %S, 17.92 % CH_3O; found: 34.64 %C, 4.14 %H, 24.19 %N, 18.32 %S, 18.18 % CH_3O.



VII VIII IX

By an analogous procedure, 2.59 g of 2',3',5'-tri-O-acetyl-pseudouridine[+] (VII) were ozonized at -70° for 20 min. until absorption at 260 nm disappeared. The ozonide formed was treated as described for preparation of V and VI. Chromatography of the reaction mixture on Dowex 1 (acetate) and subsequent chromatographic separation on cellulose in ethyl acetate-acetic acid-water (3:1:1) and on silica gel in ethyl acetate-ethanol (9:1) yielded 510 mg of 6-(β-D-ribofuranosyl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine-5-one (VIII). M.p. 197-198° (ethanol). Optical rotation: α_D^{25} -3.7° (c 0.5 water). ORD spectrum: M_{284} -4100°, M_{250} 0°, M_{222} +10 300° in water. UV spectrum: λ_{max}214 nm(logε 4.05), λ_{max}268 nm(logε 4.31) in 0.1 M HCl; λ_{max}258 nm(logε 4.17), λ_{max}313 nm(logε 3.75) in 1 M NaOH. For $C_8H_{11}N_3O_5S$ calcd: 36.78 %C, 4.24 %H, 16.08 %N, 12.27 %S; found: 37.05 %C, 4.46 %H, 15.78 %N, 11.98 %S. 2',3'-O-Isopropylidene derivative of VIII m.p. 188-190° (ethanol). For $C_{11}H_{15}N_3O_5S$ calcd: 43.84 %C, 5.02 %H, 13.95 %N, 10.64 %S; found: 44.17 %C, 5.25 %H, 13.76 %N, 10.57 %S.

[+] Prepared by acetylation of pseudouridine[8] as chromatographically homogeneous sirup. Optical rotation α_D^{25}+42.0°/c 0.5 chloroform/. UV spectrum: λ_{max}262 nm(logε 3.90) in ethanol.

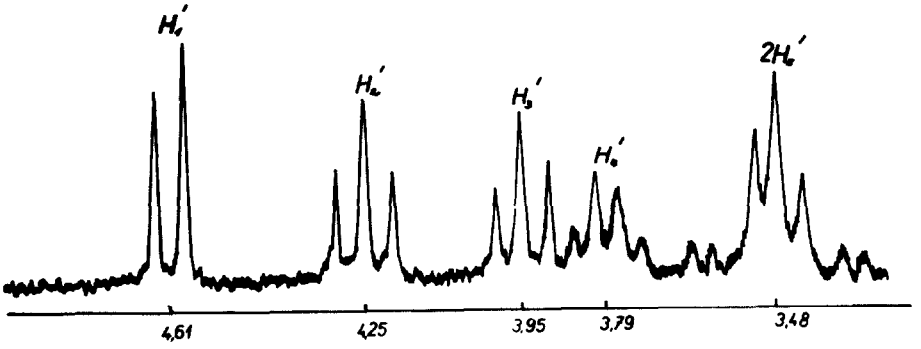


Fig. 1

NMR spectrum of IX in dimethylsulfoxide-d_6 at 25° (100 MHz) in p.p.m., TMS used as standard. Values of coupling constants: $J_{1',2'}$ 5.5 Hz, $J_{2',3'}$ 5.0 Hz, $J_{3',4'}$ 5.0 Hz, $J_{4',5'}$ 3.7 Hz, $J_{4',5''}$ 4.5 Hz.

Treatment of VIII with methyl iodide in an aqueous medium and subsequent acid hydrolysis yielded 76 % of 6-azapseudouridine (IX).M.p. 138-139° (ethanol). Optical rotation: a_D^{25} -24.9° (c 0.5 water). UV spectrum: λ_{max} 263 nm(logε 3.84) in water. Consumption of periodic acid at pH 4.7 at 25° after 24 hr. was 1.05 moles. For $C_8H_{11}N_3O_6$ calcd: 39.19 %C, 4.52 %H, 17.14 %N; found: 38.95 %C, 4.65 %H, 16.94 %N. NMR spectrum (Fig.1) is in agreement with the suggested structure.

Ozonolytic cleavage of nucleic acids and of their components is the subject of further research.

REFERENCES

1. M.Bobek, J.Farkaš and F.Šorm: Tetrahedron Letters, 3115 (1966).
2. D.M.Brown, M.G.Burdon and R.P.Slatcher: Chem.Commun. 5, 77 (1965).
3. O.L.Chapman, R.W.King: J.Am.Chem.Soc. 86, 1256 (1964).
4. R.C.Hockett, M.Conley, M.Yusem and R.I.Mason: J.Am.Chem.Soc. 68, 902 (1946); L.F.Wiggins: Advances in Carbohydrate Chemistry 5, 191 (1950).
5. M.Hoffer: Chem.Ber. 93, 2777 (1960).
6. J.J.Pappas, W.P.Keaveney, E.Gancher and M.Berger: Tetrahedron Letters 4273 (1966).
7. J.Gut: Coll.Czechoslov.Chem.Commun. 23, 1588 (1958).
8. W.E.Cohn, V.Kurkov and R.W.Chambers: Biochem. Prep. 10, 135 (1963).