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A FACILE CONVERSION OF AMINO TO THIONO GROUP IN CERTAIN NUCLEOBASES

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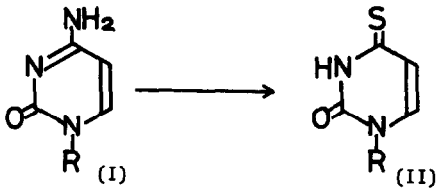
4-Thiouridine, initally synthesized by Fox and co-workers[1], has been identified as one of the minor nucleoside components of transfer ribonucleic acid[2]. Later, several 2-thiouridines and 2-thiocytidine have been reported as minor components[3,4]. The replacement of a carbonyl function by a thiocarbonyl in nucleobases may provide analogs with interesting chemical and biological activities. In the course of our synthetic studies on 2-thiopyrimidine nucleosides[5-8] we have observed a facile replacement reaction of an amino group with thiono group in cytidine derivatives by treatment with hydrogen sulfide.



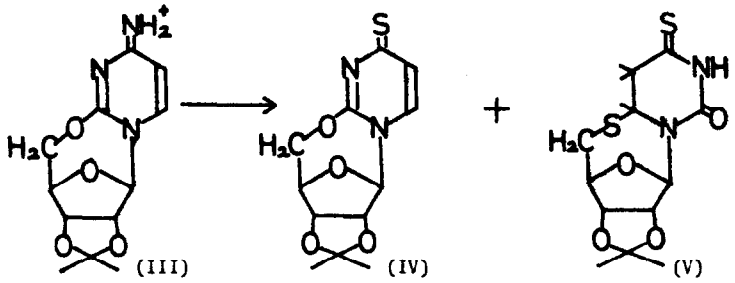
(I) (II)

R= 5-Phosphoribosyl, Ribofuranosyl or H.

To cytidine 5'-phosphate disodium salt(I, 400 mg in 5 ml of water) was added liquid hydrogen sulfide solution (H_2S gas was conducted into 5 ml of pyridine at -60%~-70° to a volume of 15 ml) in a steel container and heated at 60° for 41 hours. After evaporation of solvents the residue was triturated in water, filtered, and the filtrate was concentrated to a small volume from which yellow needles precipitated. Ethanol was slowly added to complete the crystallization. 4-Thiouridine 5'-phosphate[9] disodium salt(II, 300 mg, 70%), thus obtained, showed the expected chromatographic behavior and UV(λmax [mμ],ε: 333, 23500; 246, 4300 in 0.05 N HCl; 318, 19500 in 0.05 N NaOH) and elemental analyses(Anal. Calcd for $C_9H_{11}N_2O_8Na_2PS$-1.5 H_2O: C, 26.27; H, 3.41; N, 6.81. Found: C, 26.30; H, 3.90; N, 6.82). In a similar manner, cytidine(1.5g) in 50 ml of DMF with 50ml of pyridine-H_2S(1:1) at 50° for 3 days was converted into 4-thiouridine[1,9a](mp 135-138°, 800 mg, 50 %) after silicic acid column chromatography. 2',3'-O-Isopropylidenecytidine also gave the 4-thio derivative, mp 181-184°, in 82 % yield. Cytosine

itself afforded 4-thiouracil as expected. The present method of preparation of 4-thiouracil derivative is unique when compared with the prevalent thiation procedures because of its simplicity ; prior protection of the sugar moiety is unnecessary[1,9].

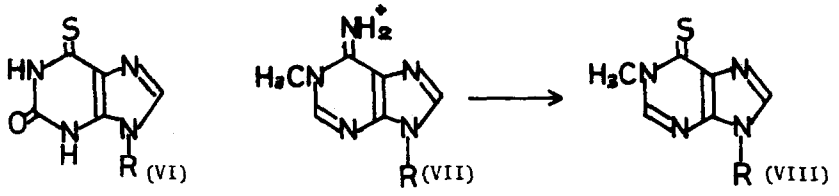
The exchange reaction of an amino by a thiono group was also achieved with other cytidine derivatives. 2-Thiocytidine[5,6] in H_2S-pyridine-water at 60° for 60 hours was completely converted into 2,4-dithiouridine, mp 170.5-171.5°[5]. 1-β-D-Ribofuranosyl-2,4-diaminopyrimidine[6] also gave 2,4-dithiouridine along with an unidentified product. 3-Methylcytidine methosulfate[10] was converted to 3-methyl-4-thiouridine[11], mp 147-148°, under very mild conditions. 2',3'-O-Isopropylidene-O^2,5'-cyclocytidine p-toluenesulfonate[12](III) gave two products depending on reaction conditions. When



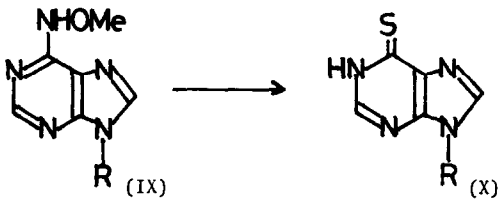
(III) (IV) (V)

the reaction was carried out in DMF at 50° for 19 hours, O^2,5'-cyclo-4-thiouridine derivative(IV) was formed as the main product. This reaction, carried out in pyridine at room temperature for 3 days, gave 5'-deoxy-5',6-epithio-5,6-dihydro-2',3'-O-isopropylidene-4-thiouridine(V, mp 177-179.5°, Mass spectrum, m/e; 316(M^+); Anal. Calcd for $C_{12}H_{16}N_2O_4S_2$: C, 45.55; H, 5.11; N, 8.86; S, 20.25. Found: C, 45.74; H, 4.96; N, 8.89; S, 20.25). The UV spectra of V, λ_{max}^{EtOH} at 284mμ, shifted to 320mμ on addition of sodium hydroxide solution and acidification of the solution changed the maximum to 330mμ. This absorption gradually decreased in concomitant with an increase at 285mμ. A similar phenomenon has been exemplified with 5'-deoxy-5'-mercaptouridine[13], in which the 5'-sulfhydryl group adds at 5,6-double bond of the uracil moiety in acidic medium. Isoguanosine[14], in which the pyrimidine portion is identical with the cytosine structure, was treated with H_2S-pyridine-water at 60° for 45 hours to yield 6-thioxanthosine(VI)[15].

From the above results it seemed that the displacement of the amino group in cytosine derivatives would be facilitated by the quarternization(such as in 1-methyl



cytidine and III). Therefore it follows that the relatively inactive amino group in adenosine(and guanosine) could be replaced if the ring nitrogen of purine nucleus is alkylated. Treatment of 1-methyladenosine hydroiodide(VII)[16] with H_2S-pyridine-water system at 60° for 46 hours afforded 1-methyl-6-thioinosine(VIII) as expected: mp 217-218°, Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.30; H, 4.70; N, 18.79; S, 10.67. Found: C, 44.39; H, 4.88; N, 18.74; S, 10.67. UV $\lambda_{max}^{H_2O}$ 320, 230mμ; $\lambda_{max}^{N\ HCl}$ 322, 230mμ. Adenosine itself afforded 6-thioinosine in very poor yield even under forced condition(100°. one week). However, when the leaving ability of the amino function is enhanced as in 6-methoxyadenosine(IX)[17], treatment with H_2S at 50° for 57 hours afforded 6-thioinosine(X)[18] in 77% yield.



This new method of preparation of pyrimidine and purine thiones should be applicable to other nitrogen-containing heterocyclic amines and studies along these lines are being undertaken. Furthermore, the treatment of ribonucleic acid with H_2S-pyridine-water at 37° for 60 hours converted most of the cytosine moieties to their 4-thiouracil counterparts without appreciable decomposition of phosphodiester linkages thus exhibiting a potential use of this method for the specific modification of nucleic acids.

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