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SYNTHESIS OF 2'-DEOXY-2'-MERCAPTOURIDINE

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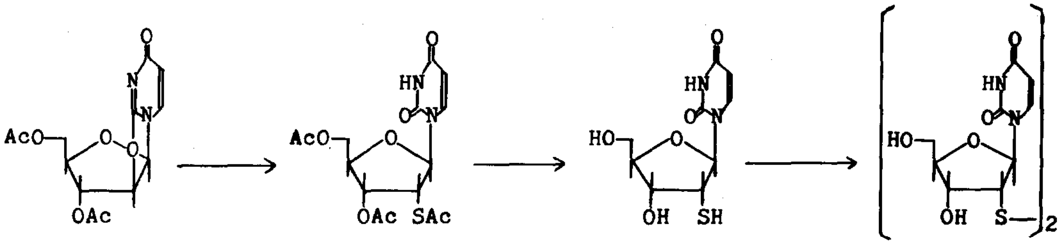
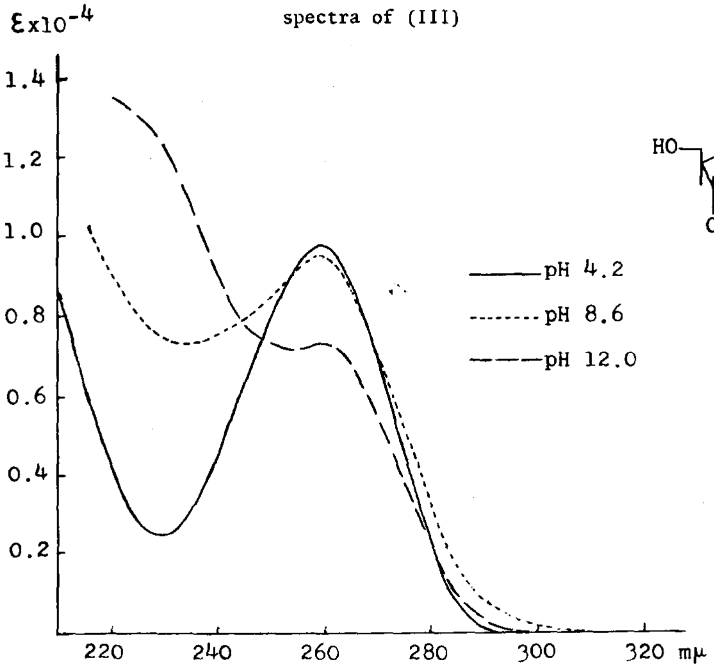
The introduction of thiol function in the sugar moiety of nucleoside is of great interest for the study of chemical and biochemical nature of nucleosides. While a number of C-5'- and C-3'-mercapto derivatives have been prepared (1)-(6), no 2'-deoxy-2'-mercaptonucleosides are known except a 3'-deoxy-3'-amino derivative (7). This paper deals with the first synthesis of 2'-deoxy-2'-mercaptouridine (III).

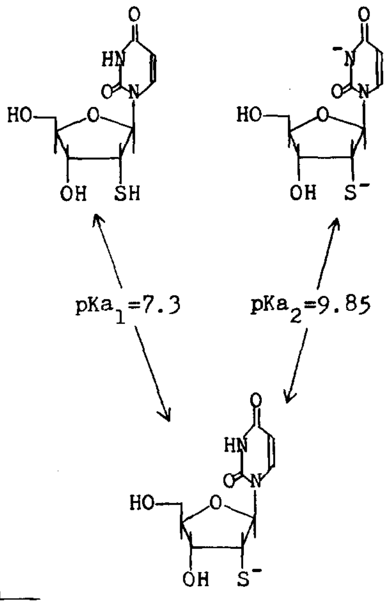


I II III IV

A mixture of 4.4 g of 2,2'-anhydro-3',5'-di-O-acetyluridine (I) (8), 25 g of thioacetic acid and 25 ml of N,N-dimethylformamide (DMF) was heated at 110° for 6 hr. in a steel container. Paper chromatography (solvent system, nBuOH-H_2O-AcOH, 90:18:2) of the reaction mixture revealed four spots. The slowest moving UV-absorving spot (Rf, 0.49) corresponded to that of the starting material. One of the major products, compound (II) having a Rf value of 0.79 showed characteristic ultraviolet absorption spectra, suggesting one thiolacetyl residue ($\varepsilon_{231m\mu}=4.5\times10^3$ (9)) was introduced to the ribose moiety of the compound (I) with concomitant cleavage of 2,2'-anhydro linkage. This product was estimated as triacetate of 2'-deoxy-2'-mercaptouridine. After purification through a silicic acid (250 g) column, product (II) was obtained in 4 %-ethanol-chloroform effluent (yield, 30 %; assuming $\varepsilon_{260m\mu}$ of the product is 10000). This solution was evaporated and compound (II) was deacetylated in 1N KOH-EtOH (1:1) at 10° for 30 minutes. After neutralization by Dowex 50 (H^+) and evaporation of the solvent, the residue was purified by cellulose column chromatography (solvent system, same as that of the paper chromatography). From the fraction of the main product 1.01 g of 2'-deoxy-2'-mercaptouridine (III) was obtained as white plates from water (yield, 27 % based on (I)); mp 171-174°; ir 2560 cm^{-1} (SH); mass spectrum m/e 260 (M^+), 149, 112. Anal. Cald for $C_9H_{12}N_2O_5S$: C, 41.54; H, 4.65; N, 10.67; S, 12.32. Found: C, 41.49; H, 4.76; N, 10.74; S, 12.22. The ultraviolet absorption spectra are shown in Figure 1. Similarly to 1-(2,3-dideoxy-3-mercapto-β-D-threo-pentofuranosyl)-thymine (6), this nucleoside (III) exhibited two equilibria with two sets of isosbestic points in the pH 4-12 region, with apparent pKa values of 7.3 and 9.85. The lower pKa value must represent ionization of the 2'-mercapto group, as thiolate ion has a strong absorbancy (ε=5000) about 235 mμ where absorption of the

Figure 1. Ultraviolet absorption spectra of (III)





undissociated thiol group is negligeble (10). The higher pKa is of N_3 dissociation.

Treatment of aqueous solution of (III) with iodine gave the disulfide (IV), which was purified by cellulose column chromatography and crystallized from 95 % ethanol (yield, 51 %); mp 161-164°; λ_{max}^{water} 260.5$_{m\mu}$ (ε; 17700); pKa, 9.2. Anal. Cald for $C_{18}H_{22}N_4O_{10}S_2$: C, 41.69; H, 4.28; N, 10.80; S, 12.37. Found: C, 41.51; H, 4.36; N, 10.66; S, 12.16. 2-Mercaptoethanol treatment caused complete conversion of (IV) to the starting material (III). Desulfurization of (IV) with activated Raney nickel yielded uracil and 2'-deoxyuridine (the ratio, 6:4). The structure of the latter was confirmed by reductive hydrolysis of glycosyl bond by Burke's method (11) and subsequent identification of the deoxy sugar with 2-deoxyribose by electrophoresis in a borate buffer (12). This fact clearly attest that compound (III) has a 2'-deoxy-2'-mercapto structure. In fact, compound (III) was not identical with 1-(3-deoxy-3-mercapto-β-D-threo-pentofuranosyl)-uracil, which was recently synthesized in our laboratory (13).

The reaction of 2,2'-anhydrouridine derivatives with thiobenzoate had been investigated (14) and gave no sulfur containing product with a partial liberation of uracil. We observed the similar cleavage in the reaction of (I) with potassium thioacetate and thioacetic acid in DMF at 110°. In fact, compound (II) yielded uracil very easily by treatment with thioacetate or acetate in DMF at 110°. Compound (III) also gave uracil by heating at a melting point temperature. It has been reported that a methyl-2-deoxy-2-benzoylthio-3-O-tosyl-β-D-xylofuranoside was converted to the 2,3-epithio derivative on treatment with sodium benzoate at 110° (15). The lability of glycosyl bond in (II) and (III) should well be explained analogously by assuming uracil as the leaving group. The bases such as thioacetate, acetate, thiobenzoate and benzoate anion may accerelate the cleavage of glycosyl bond of the product (II), while (II) was stable in thioacetic acid-DMF at 110°.

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