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Pyrimidine Nucleosides. II.[1] The Synthesis of Unnatural Pyrimidine Nucleosides saturated at 5,6-Double Bond

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5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (IIIa) and 5,6-dihydroxy-5,6-dihydrouridine (IIIb) were prepared *via* bromination of 2′-deoxyuridine (Ia) and uridine (Ib) respectively, followed by refluxing these unstable intermediates in the neutral conditions to replace the bromo group with the hydroxyl group. In the case of cytidine, however, the 5,6-dihydroxy-5,6-dihydro compound was not obtained by the same procedure as used for preparation of IIIa and IIIb from Ia and Ib.

To our best knowledge, 5,6-dihydrouridine is the only naturally occurring pyrimidine nucleoside saturated at 5,6-double bond. Such 5,6-dihydro compound has offered much interest because of its possible biological role which the compound may take in the metabolism of nucleic acids. By analogy it is anticipated that the 5,6-dihydro-5,6-dihydroxypyrimidine nucleoside might show some biological activities.

Preparation of 5,6-dihydro-5,6-dihydroxy derivatives of pyrimidine bases has been described,[3] but only some of them were isolated and characterized. It was also reported that X-ray irradiation of some pyrimidine bases afforded 5-hydroxy-6-hydroperoxide.[4]

In this study it was found that 5,6-dihydroxy-5,6-dihydro-2′-deoxyuridine (IIIa) could be easily prepared by refluxing an aqueous solution of 5-bromo-6-hydroxy-5,6-dihydro-2′-deoxyuridine[5] (IIa), which in turn was obtained by bromination of 2′-deoxyuridine (Ia) according to a reported procedure.[6] Overall yield of IIIa was 45.2% from Ia. The structural assignment of IIIa rests upon the elementary analysis and the absence of the ultraviolet (UV) absorption spectrum characteristic of 1-substituted pyrimidines. To substantiate the assignment, we measured the nuclear magnetic resonance (NMR) spectrum of IIIa and made a comparison with that of 5-bromo-2′-deoxyuridine. A proton at C_6 of IIIa occurred as a doublet at 5.28 ppm, while a proton at C_6 of 5-bromo-2′-deoxyuridine showed a singlet signal at 8.20 ppm. A signal of a C_5 proton of IIIa appeared as a doublet at higher field (at 4.55 ppm) than a signal due to a C_5 proton of natural pyrimidine nucleosides. Furthermore, IIIa can react with trityl chloride at room temperature to give 5′-O-trityl derivative (IV). This indicates that a hydroxyl group is present in the 5′ position, excluding a possible 6,5′-anhydro structure[7] for IIIa.

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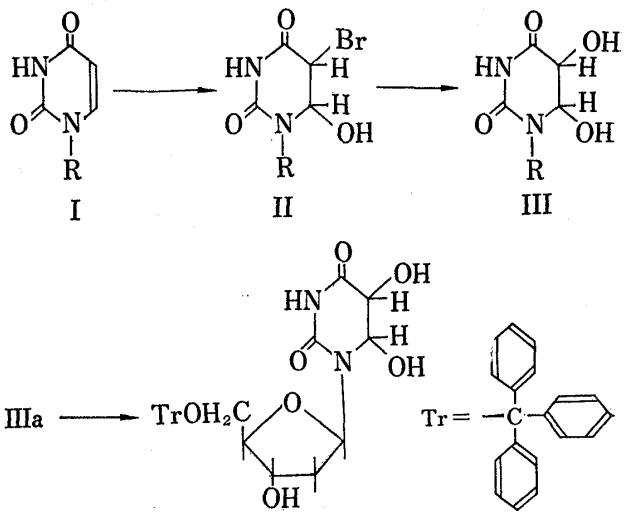
IIIa gave a negative *p*-dimethylaminobenzaldehyde-HCl test[8] for a ureido structure.[9] Therefore it is safe to conclude that two hydroxyl groups introduced are located at vicinal positions C_5 and C_6. It is to be noted that IIIa does not consume periodate on paper chromatogram, suggesting that the glycol system is situated in a trans configuration.

Uridine, having a ribofuranosyl group as a sugar moiety, has afforded by the same treatment a product having no ultraviolet absorption spectrum above 220 mμ, together with a trace of 5-bromouridine and 5-hydroxyuridine.[10] The structure of this compound is supposed to be, by analogy, 5,6-dihydroxy-5,6-dihydrouridine (IIIb).



IV a : R=β– D –2–deoxyribofuranosyl
b : R=β– D –ribofuranosyl

Chart 1

The same treatment of cytidine did not afford 5,6-dihydroxy-5,6-dihydrocytidine. After bromine water was added to cytidine, pH of the reaction mixture (1.3) was adjusted to 5.2 with Dowex 1×2 (HCO_3^-). However, 5-bromocytidine was the only product detectable in this reaction mixture by paper chromatographic technique and ultraviolet light. This indicated that 5-bromo-6-hydroxyhydro compound was rapidly converted to 5-bromocytidine under this condition. It was shown that 5-bromo-6-hydroxyhydrocytidine,[12] the intermediate of this reaction, was more unstable than 5-bromo-6-hydroxyhydrouracil derivatives under this condition.

5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (IIIa) at a concentration of 100 μg/ml showed a mutagenic effect on Myrothecium verrucaria in culture.

Experimental[12]

5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (IIIa)——30.5 ml of bromine water, prepared by 1 ml of Br_2 in 133 ml of H_2O at 5°, was added dropwise with stirring to 1 g of crystalline 2′-deoxyuridine at 5° during 30 minutes to obtain a yellow solution. Air was then bubbled through the solution to remove excess bromine, and the pH of the resulting colorless solution was adjusted to 4.5 with 9 ml of Dowex 1×2 (HCO_3^-) in batch. The resin was filtered and washed with 30 ml of 30% MeOH. Acetone and water were added to this filtrate combined washings as to make 20% aq. acetone solution of 220 ml. This solution was refluxed for 30 minutes on oil bath. After cooling, the pH of the reaction mixture (2.30) was adjusted to 5.5 with 6 ml of Dowex 1×2(HCO_3^-). The resin was filtered and washed with 30% MeOH. Combined filtrate and washings were concentrated *in vacuo* at 20—30°, and to the residue was added EtOH, and evaporated to dryness. This treatment was repeated 3 times. The residue, thus obtained, was dried under reduced pressure over P_2O_5.

Crystallization of the dried residue from 5 ml of EtOH gave prisms, mp 173—174° (decomp.). Recrystallization raised mp to 182° (decomp.). Yield was 515 mg, 45.2%. This crystal had no ultraviolet absorption above 220 mμ, but was recognized to change its structure in 1N NaOH solution because of the gradual appearance of ultraviolet absorption. NMR(D_2O) τ: 3.90 (1H, triplet, $C_{1'}$–H), 4.72 (1H, doublet, C_6–H), 5.45

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12) All melting points were corrected. *Rf*(A) stands for the *Rf* value in the solvent A. Solvents used were: A, *n*-BuOH–H_2O, 86:14; B, *n*-BuOH–pyridine–H_2O, 10:3:3.

(1H, doublet, C_5-H), 5.65 (1H, quintet, $C_{3'}$-H), 6.00—6.40 (3H, multiplet, $C_{4'}$-H and $C_{5'}$-H), 7.75 (2H, triplet, $C_{2'}$-H). *Rf* (A) 0.09 (revealed by cystein hydrosulfate spray test).

Anal. Calcd. for $C_9H_{14}O_7N_2$: C, 41.22; H, 5.38; N, 10.68. Found: C, 41.50; H, 5.39; N, 10.38.

Tritylation of 5,6-Dihydro-5,6-dihydroxy-2'-deoxyuridine (IIIa)——A solution of IIIa (130 mg, 0.5 mmole) and trityl chloride (209 mg, 0.75 mmole) in dry pyridine (5ml) was allowed to stand in a stoppered flask at room temperature for 7 days. Icewater (25 ml) was poured into the reaction mixture with stirring. The granular solid was collected on the filter and dried under reduced pressure. Upon addition of ethyl acetate into the solution of the solid dissolved in a minimum amount of EtOH, crystallization began to occur. Colorless needles was obtained in a yield of 57.54% (145 mg), mp 99° (decomp.).

Anal. Calcd. for $C_{28}H_{28}O_7N_2(H_2O)$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.32; H, 5.87; N, 5.25.

5,6-Dihydro-5,6-dihydroxyuridine (IIIb)——A mixture of 1 g of uridine and 30.5 ml of bromine water, prepared by 1 ml of Br_2 in 133 ml of H_2O at 5°, was treated with the same procedure as used for preparation of IIIa. Crystallization was unsuccessful. The glass, thus obtained, gave three spots having *Rf* (A) 0.37, 0.26 and 0.07 on a paper chromatogram. The former and the middle were minor components assigned as 5-bromouridine and 5-hydroxyuridine respectively by ultraviolet absorption spectrum, the latter was supported to be 5,6-dihydro-5,6-dihydroxyuridine (IIIb) by having no ultraviolet absorption maximum and yet consuming metaperiodate, and by the fact that Beilstein test was negative for the glass obtained by preparative paper chromatography.

A Trial for Conversion of Cytidine to Its Corresponding 5,6-Dihydroxy-5,6-dihydro Compound——30.5 ml of bromine water was added to 1 g of cytidine. Air was bubbled through the solution and the pH of the resulting colorless solution (1.3) was adjusted to 5.2 with Dowex 1×2 (HCO_3^-). The resin was filtered and washed with 30 ml of 30% MeOH. The only one spot (R_{CR} [13](A): 1.6) with slightly heading was obtained by paper chromatography of this solution, which was confirmed to be 5-bromocytidine by ultraviolet absorption spectrum ($\lambda_{max}^{H_2O}$ 290 mμ, $\lambda_{max}^{OH^-}$ 289 mμ, $\lambda_{max}^{H^+}$ 300 mμ).[11,14]

The 20% acetone solution of filtrate and washings prepared by the same way as used for preparation of IIIa, was refluxed for 15 minutes on oil bath, but any changes was not observed in paper chromatographic behavior and ultraviolet absorption.

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