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NUCLEOSIDES LI. NOVEL TRANSFORMATIONS OF THE PYRIMIDINE MOIETY OF RIBONUCLEOSIDES

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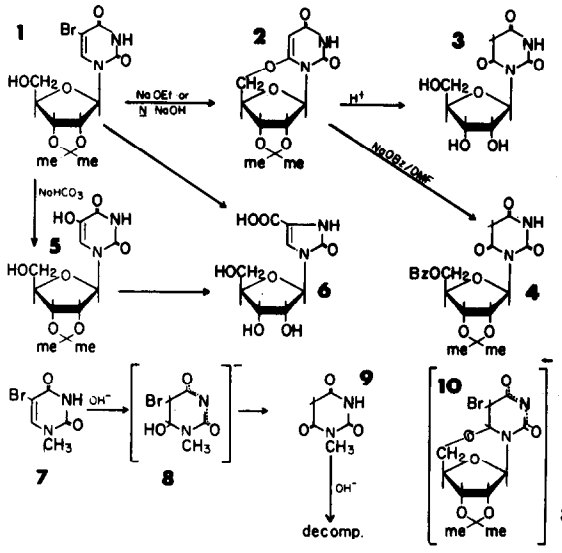
1-β-D-Ribofuranosylbarbituric acid (3) ("6-hydroxyuridine") and its derivatives offer many possibilities for chemical and biochemical investigation. The fact that such investigation has been sparse is a reflection of the difficulty in preparing nucleosides of this type. The recently reported methods [2,3] for the preparation of N-ribosyl barbiturates are adaptations of the classical barbiturate synthesis. These syntheses are lengthy and require complicated isolation procedures. The reported overall yields are relatively low and, in the synthesis of 3, the product is of questionable purity.

We report herein a simple, high-yielding synthesis of ribosyl-barbituric acid (3), from the readily-available isopropylidene-5-bromouridine, as part of a study of the base-catalyzed transformations of 5-halogenouracil nucleosides. [4,5]

Treatment of the known [6] isopropylidene-5-bromouridine (1) with an excess of sodium ethoxide in hot ethanol for 17 hours afforded a 90% yield of the 6,5'-anhydro nucleoside [7] (2), mp 251-253°. [8] The structure of 2 was convincingly demonstrated by its NMR spectrum in DMSO-d_6 which shows completely resolved signals including a single vinylic proton for H-5 (δ = 5.41) and a widely-spaced quartet (centered at δ = 4.4, $J_{5',5'}$ = 13 cps). This quartet has been found to be characteristic of the $H_{5'}$ signals in anhydronucleosides containing an oxygen bridge from the aglycon to C5'. [9]

Treatment of 2 with warm, dilute HCl cleaved the anhydro bridge as well as the isopropylidene group to give an 85% yield of 1-β-D-ribofuranosylbarbituric acid (3) as the monoethanolate, mp 116-118° (eff), basic pK_a = 3.75 ± 0.05 (spectrophotometrically-determined). The ultraviolet spectrum of (3) [$\lambda_{max}^{pH\ 7}$ 260 mμ (ϵ, 22,050); $\lambda_{max}^{pH\ 14}$ 265 mμ (ϵ, 15,220); $\lambda_{max}^{pH\ 0}$ 214, 260 mμ (ϵ, 7,400, < 300)] is similar to that given [10] by 1-methylbarbituric acid (9) (pK_a = 4.2). Compound 3 consumed 2 moles of metaperiodate within 5 min., whereas 9 consumed only one mole during this period. Both 3 and 9 then exhibited a much slower uptake of an additional mole of oxidant within 48 hours. The rapid uptake of the second mole by 3 is consistent with a ribofuranosyl (α-cis glycol) structure. The total synthesis of the Na salt of 3



(probably impure)[11] in 37% yield has been reported.[2]

Treatment of anhydronucleoside (2) with sodium benzoate in DMF yielded 4, the 5'-benzoate of 1-(2,3-O-isopropylidene-β-D-ribosyl)barbituric acid, mp 163-166°. Compound 4 is conveniently substituted with both acid and base labile protecting groups, and is suitable for further studies on transformations of the sugar moiety.

The reactions of 1 with aqueous base gave mixtures, the composition of which depended upon the alkaline conditions employed. Spectral examination (pH 10) of the reaction of 1 in 0.1N NaOH at 100° shows a rapid partial loss of ultraviolet absorption at 275 mμ followed by the appearance of a small peak at 305 mμ. The intensity of this peak increases and then decreases with the concomitant formation of a larger peak at 252 mμ, a process which is essentially complete in 6 hours. As will be shown later, the 305 mμ peak is due to formation of isopropylidene-5-hydroxyuridine (5). After removal of the isopropylidene group with dilute acid, a 45% yield of 1-(α-D-ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid (6) (λ_{max} 252 mμ) was obtained as the monohydrate, mp 107-110° (eff). The structure of 6 was established by the similarity of its u.v. spectrum to the corresponding arabinofuranosyl analog[5] and by total synthesis. Thus, condensation of tri-O-benzoyl-D-ribofuranosyl chloride with 2-oxo-4-imidazoline-4-carboxylic acid methyl ester (by the nitromethane-mercuric cyanide method)[12] followed by de-esterification of the blocked intermediate afforded crystalline material identical with 6.

When 1 was refluxed in weaker base (0.15 N $NaHCO_3$ under CO_2 gas, pH ∽ 8.3) the major product after 5 hours was the 5-hydroxyuridine derivative (5). This isobarbituric acid nucleoside was isolated as an amorphous solid which, after acid hydrolysis, gave the known[13] 5-hydroxyuridine. However, when the reaction of 1 with hot $NaHCO_3$ solution was performed under nitrogen[14] instead of carbon dioxide, the pH of the reaction mixture increased to ca. 10, and the formation and slow disappearance of 5 (λ_{max} 305 mμ) was noted along with formation of the isopropylidene derivative of 6 (λ_{max} 252 mμ). These data suggest the surprising transformation of an isobarbituric acid nucleoside to an imidazoline. In order

to confirm this unexpected transformation, 5 (or 5-hydroxyuridine itself) was refluxed in dilute bicarbonate for 24 hours. After removal of the isopropylidene group a 60% yield of imidazoline (6) was obtained. Similar results were obtained when 5 was refluxed in 0.1N NaOH. However, 5 was stable in N NaOH at 55° over a 24 hour period as evidenced by the constancy of its u.v. spectrum. Under these conditions, 5 must exist predominately as a dianion which apparently does not undergo ring contraction to the imidazoline. This fact is of interest because treatment of isopropylidene-5-bromouridine (1) with N NaOH at 55° for 24 hours afforded a mixture containing predominately isopropylidenated 6, the anhydro compound 2, and a slight amount of 5. In this case, the formation of 6 does not proceed via 5.

A unifying mechanism for the formation in alkali of 2, 5 and 6 from 1 is best accounted for by intermediate 10 which is formed by nucleophilic addition on C6 by the 5'-OH. Intermediate 10 can lose HBr to form 2. Alternatively, replacement of the bromine atom by hydroxyl followed by elimination of HOR (R = sugar alcohol) would give 5. The formation of imidazoline 6 from 10 would occur by ring opening at the 3,4 position followed by amide attack on C5 as previously proposed [5] for the arabino analog. The mechanism of the conversion of 5 → 6 is currently under study. Evidence for the participation of the 5'-OH group in the conversion of 1 → 5 and 1 → 6 is that treatment of the 5'-deoxy analog of 1 with 0.1N NaOH at 100° afforded only trace amounts of the corresponding imidazoline. Moreover, participation of the 5'-OH group is greatly facilitated by the presence of the isopropylidene group. Thus 5-bromouridine itself affords only small amounts of imidazoline 6 when treated with 0.1N NaOH. Similarly, the reaction of 5-bromouridine with $NaHCO_3/CO_2$ proceeds much less readily than the analogous reaction of 1 → 5.

It is conceivable that the isopropylidene derivative of the barbituric acid nucleoside (3) could have also been formed directly in the reactions of 1 in NaOH. In model studies, treatment of 1-methyl-5-bromouracil (7) with N NaOH for 6 hours at 55° afforded a 50% yield of 1-methylbarbituric acid (9). However, 9 is unstable under these alkaline conditions and after a 24 hour period it decomposes to products devoid of selective u.v. absorption. The formation of 9 from 7 probably proceeds via intermediate ion 8. Thus, any barbituric acid nucleoside (3) formed by a similar process in the reaction of 1 with warm N NaOH would have decomposed during the 24 hour reaction period employed. A study of the stability of 3 over a 24 hour period shows that it, too, is completely decomposed in N alkali at 55°.

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