vent B), leaving only the spot R_f 0.04, which was the cyclic tosylate. Evaporation to dryness gave a colorless foam. This had, in the uv spectrum, $\lambda_{max}^{H_2O}$ 274 mμ (ε 12,300). In the ir spectrum, new bands at 685, 1010, and 1210 cm^{-1} indicated the presence of the tosylate anion.

Anal. Calcd for $C_{17}H_{18}N_5O_5FS$: C, 48.22; H, 4.28; N, 16.54; F, 4.49; S, 7.57. Found: C, 48.20; H, 4.51; N, 16.38; F, 4.35; S, 7.55.

Registry No.—**3b**, 20187-72-8; **4a**, 20187-73-9; **4b**, 20187-74-0; **6**, 20187-75-1; **7**, 20187-76-2; **8**, 20187-77-3; **9**, 20187-78-4; **10**, 20187-79-5; **11**, 20187-80-8; **12**, 20227-40-1; **13**, 20187-81-9; **14**, 20227-41-2; **15**, 20187-82-0; **16**, 20187-83-1; **17**, 20187-84-2; **18**, 20187-85-3.

Acknowledgment.—The authors are indebted to Mr. Marvin J. Olsen for recording the pmr spectra.

Nucleosides. LXI. Transformations of Pyrimidine Nucleosides in Alkaline Media. IV. The Conversion of 5-Hydroxyuridines into Imidazoline Nucleosides[1,2]

BRIAN A. OTTER, ELVIRA A. FALCO, AND JACK J. FOX

Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York, New York 10021

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Isopropylidene-5-hydroxyuridine (**2**) and 1-methyl-5-hydroxyuracil (**8**) undergo a benzilic acid type of rearrangement and dehydration in 0.1 *N* NaOH at 100° to give the corresponding 1-substituted 2-oxo-4-imidazoline-4-carboxylic acids **5** and **9**. 1,3-Dimethyl-5-hydroxyuracil (**10a**) and 1-methyl-3-benzyl-5-hydroxyuracil (**10b**) are converted under these conditions into the corresponding 1,3-disubstituted 4-hydroxy-2-oxoimidazolidine-4-carboxylic acids **11a** and **b**. Compound **11b** was converted into the crystalline methyl ester **14** by treatment with diazomethane. The 4-hydroxyimidazolidines **11a** and **b** undergo acid-catalyzed dehydration to give the 1,3-disubstituted 2-oxo-4-imidazoline-4-carboxylic acids **12a** and **b**. Evidence for the existence of the tautomeric 5-keto forms of the 5-hydroxyuracil derivatives necessary for benzilic acid rearrangement is presented. The 5-hydroxyuracil derivatives are prepared by treatment of the corresponding 5-bromouracils with CO_2-buffered sodium bicarbonate solution at 100°. In unbuffered sodium bicarbonate solution, isopropylidene-5-bromouridine (**1**) and 2′-deoxy-5-bromouridine are converted *via* their 5-hydroxy derivatives into the 2-oxo-4-imidazoline-4-carboxylic acid nucleosides. The potential application of this rearrangement to DNAs containing 2′-deoxy-5-bromouridine instead of thymidine is discussed. An *in situ* method for the conversion of uridine into the imidazoline nucleoside **6** is described. Ultraviolet spectral and pK_a data for the 5-hydroxyuracil derivatives are given.

We have previously reported[3] that 5-halogeno derivatives of isopropylideneuridine (**1**, X = F, Br, I) undergo rearrangement in 1 *N* sodium hydroxide to give the 2-oxo-4-imidazoline-4-carboxylic acid nucleoside **5** in varying yield. This rearrangement involves participation of the 5′-hydroxyl group, and it was suggested that the reaction proceeds *via* the 5′,6-anhydro acyclic ureide **4** (X = F, Br, I). We now wish to report that certain derivatives of 5-hydroxyuracil (isobarbituric acid) also undergo base-catalyzed rearrangement to 2-oxo-4-imidazoline-4-carboxylic acids. This new rearrangement does not involve participation of a sugar hydroxyl group and proceeds by a mechanism different from that of the rearrangement **1** → **4** → **5** in 1 *N* sodium hydroxide (Scheme I).

Our interest in the alkaline stability of 5-hydroxyuracil derivatives resulted from experiments which indicate that isopropylidene-5-hydroxyuridine (**2**) is stable in 1 *N* sodium hydroxide but unstable in 0.1 *N* sodium hydroxide. First, compound **2** was formed along with the imidazoline nucleoside **5** (20% yield) when a 0.1 *M* solution of the 5-bromo nucleoside **1** in 1 *N* sodium hydroxide was heated at 55° for 20 hr.[3] Moreover, compound **2** appeared to be stable under these reaction conditions, as shown by a gradual increase in the intensity of the uv absorption maximum of **2** at ~305 mμ. Secondly, isopropylidene-5-hydroxyuridine (**2**) was also formed when a 0.02 *M* solution of **1** (X = Br) in 0.1 *N* sodium hydroxide was heated at 100°. In this case, however, the concentration of **2** as monitored spectrally first increased and then gradually decreased with the concomitant formation of the imidazoline nucleoside **5**. After acidic hydrolysis of the isopropylidene group, the known[3] 1-(β-D-ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid (**6**) was obtained in 45% yield. This finding suggests the possibility that isopropylidene-5-hydroxyuridine (**2**) is an intermediate in the formation of **5** from **1** (X = Br) in 0.1 *N* sodium hydroxide. Evidence supporting the intermediacy of **2** was obtained when an attempt was made to synthesize this compound by using the procedure of Wang.[4] Accordingly, when **1** (X = Br) was heated under nitrogen in dilute sodium bicarbonate solution, the formation of **2** was indicated by the appearance of an absorption peak at 305 mμ. During the 22-hr reaction period, however, the pH of the reaction mixture increased from ~8.3 to ~10 and the slow disappearance of **2** and concomitant formation of **5** was again noted. The unblocked nucleoside **6** was isolated in 54% yield. Formation of **5** was considerably reduced when the reaction mixture of **1** (X = Br) with sodium bicarbonate was buffered (~ pH 8.3) with carbon dioxide gas. After a reaction period of 5 hr, crystalline **2** was isolated in 46% yield and characterized by conversion into the known 5-hydroxy-

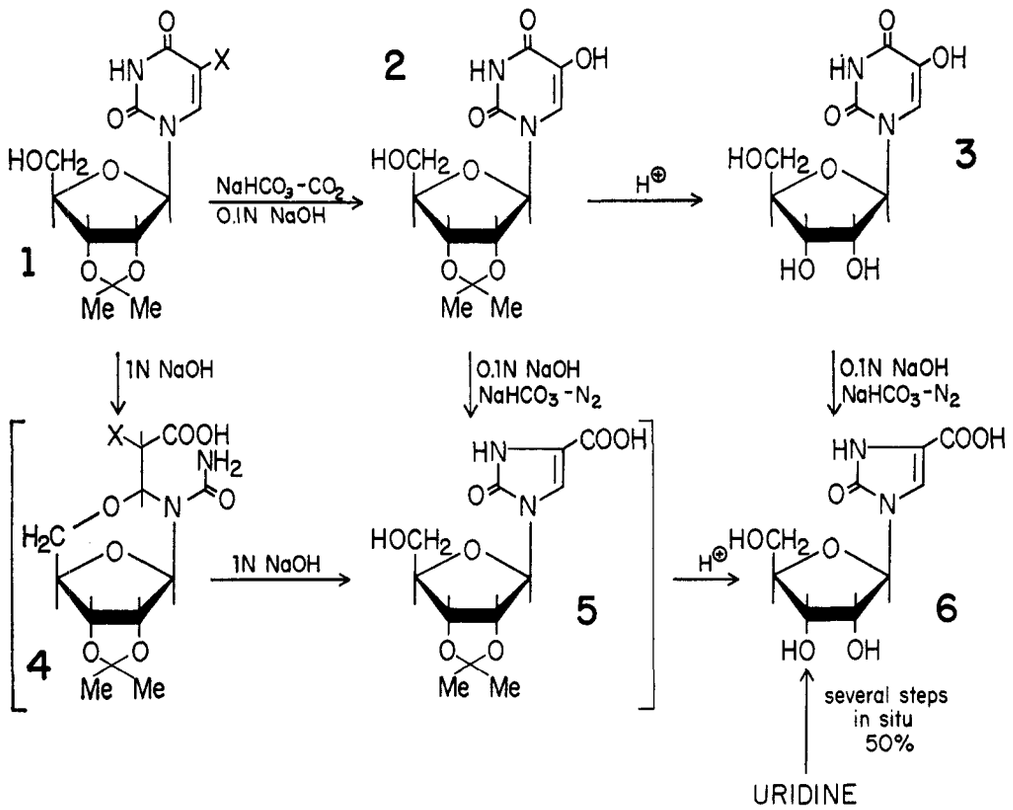
(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Publich Health Service Grant CA 08748.

(2) A preliminary account of part of this work has been published: B. A. Otter, E. A. Falco, and J. J. Fox, *Tetrahedron Lett.*, 2967 (1968).

(3) B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **34**, 1390 (1969).

(4) S. Y. Wang, *J. Amer. Chem. Soc.*, **74**, 668 (1952).

SCHEME I



uridine (**3**).[5] Treatment of **2** with either 0.1 *N* sodium hydroxide or unbuffered sodium bicarbonate solution at 100° resulted in smooth conversion into the imidazoline **5**, which was isolated as the unblocked nucleoside **6** in good yield. Similar treatment of 5-hydroxyuridine (**3**) afforded **6** directly. Moreover, the rearrangement **3** → **6** proceeds at the same rate as that of the rearrangement **2** → **5**, indicating that neighboring-group participation of the 5′-hydroxyl group is not involved in these reactions.[6] However, isopropylidene-5-hydroxyuridine (**2**, 0.02 and 0.1 *M*) proved to be stable in 1 *N* sodium hydroxide at 55° as shown by the constancy of the uv spectrum over a 24-hr period. These data show that the imidazoline nucleoside **5** can be formed from **1** (X = Br) *via* two routes. One pathway, operating in 1 *N* sodium hydroxide, does not involve **2** as an intermediate but probably proceeds *via* the acyclic ureide **4** as suggested previously.[3] The other pathway, operating in 0.1 *N* sodium hydroxide[7] or unbuffered sodium bicarbonate solution, proceeds *via* **2** which is itself converted into **5**. Based on these considerations, we devised a simple *in situ* synthesis of the imidazoline nucleoside **6** directly from uridine in 50% yield. Treatment of uridine with bromine water afforded 5-bromo-6-hydroxy-5,6-dihydrouridine, which on treatment with sodium bicarbonate[4] was converted into 5-hydroxyuridine (**3**) and thence to imidazoline **6**.

The ring contraction of 5-hydroxyuracil derivatives is not restricted to the nucleoside series, but can be used for the preparation of a variety of 1-substituted and 1,3-disubstituted-2-oxo-4-imidazoline-4-carboxylic acids. Thus, treatment of 1-methyl-5-hydroxyuracil (**8**, Scheme II) with refluxing 0.1 *N* sodium hydroxide afforded the known[8] 1-methylimidazoline **9** in 80% yield. However, the reactions of 1,3-disubstituted 5-hydroxyuracils in sodium hydroxide differ from those of the 1-substituted compounds (**2**, **3**, and **8**) in that only small amounts of the corresponding imidazolines are formed directly. Instead, the 1,3-disubstituted compounds are slowly converted into non-uv-absorbing intermediates (**11**) which afford the imidazolines after treatment with acid. Thus, uv spectral examination (pH ~10) of the reaction of 1,3-dimethyl-5-hydroxyuracil (**10a**) with refluxing 0.1 *N* sodium hydroxide revealed an 80% decrease in the intensity of the peaks at 242 and 310 mμ over a 19-hr period. Only a small increase in absorption at 255 mμ, attributable to the formation of **12a**, was noted. Acidification of the solution, however, resulted in the rapid formation of a large peak at 269 mμ, a value corresponding to the known[8] absorption of **12a** in acid solution. On a preparative scale, compound **12a** was isolated in 71% yield.

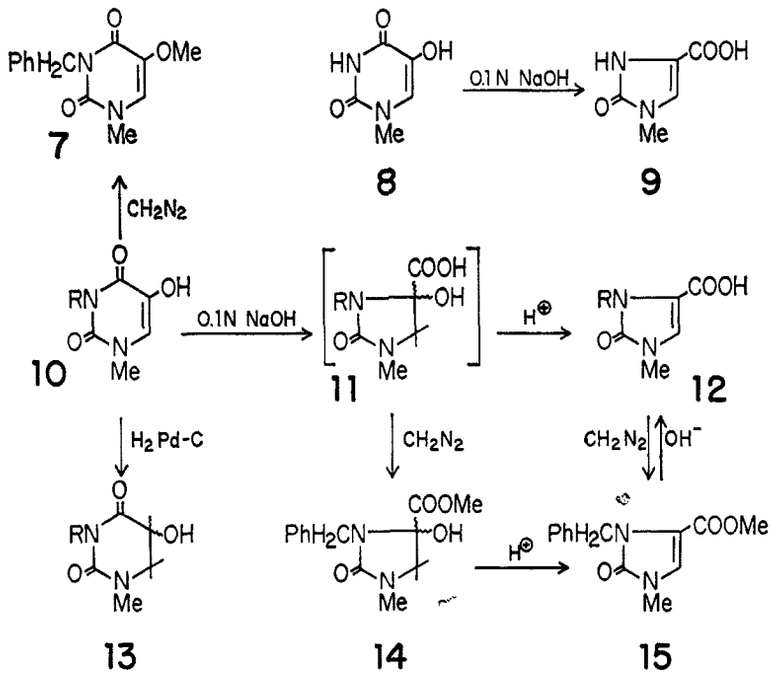
(5) Further study of the formation of 5-hydroxyuridine (**3**) from 5-bromouridine in $NaHCO_3$–CO_2 has shown, contrary to our previous report,[2] that this reaction and the analogous conversion of isopropylidene-5-bromouridine (**1**) into isopropylidene-5-hydroxyuridine (**2**) proceed at similar rates. These reactions do not, therefore, involve participation of the 5′-hydroxy group[6] (formation of a 5-bromo-5′,6-anhydro-5,6-dihydro intermediate) but probably proceed *via* 5-bromo-6-hydroxy-5,6-dihydrouridines. The latter intermediates are analogous to those suggested by Wang[4] for the conversion of 5-bromouracil and 1,3-dimethyl-5-bromouracil into their corresponding 5-hydroxy compounds.

(6) It has been demonstrated previously that reactions involving interaction between the 5′-hydroxyl group and the aglycon of uridine derivatives are greatly facilitated by the presence of a 2′,3′-*O*-isopropylidene group. For a discussion of this point, see ref 3 and other citations therein.

(7) It is possible that the formation of **5** from **1** (X = Br) in 0.1 *N* NaOH involves both routes (**1** → **4** → **5**) and (**1** → **2** → **5**). It should be noted that in aqueous alkali (0.1 → 1*N* hydroxide) a competing reaction involving direct attack by hydroxide on C-6 leading to barbituric acid nucleosides is also operative. This affects the over-all yields of imidazoline nucleoside from **1**. As mentioned previously,[3] such barbituric acid nucleosides are unstable under these reaction conditions, leading to nonchromophoric degradation products.

(8) B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **33**, 3573 (1968).

SCHEME II



Similar results were obtained with 3-benzyl-1-methyl-5-hydroxyuracil (**10b**) which gave the corresponding imidazoline (**12b**) in 74% yield. The intermediate (**11a**) in the formation of **12a** from **10a** proved to be unstable and attempts to isolate it resulted in formation of a considerable amount of **12a**. The analogous intermediate (**11b**) was more stable and was isolated as a crude, amorphous sodium salt which was contaminated with small amounts of starting material **10b** and imidazoline **12b**. Careful neutralization of this material with acetic acid and methylation with a large excess of diazomethane afforded a mixture containing the methyl ether **7**, the methyl ester **15**, and a crystalline, non-uv-absorbing compound which was shown to be 3-benzyl-4-hydroxy-1-methyl-2-oxoimidazolidine-4-carboxylic acid methyl ester (**14**). The intermediates **11a** and **11b** are therefore the corresponding 1,3-dimethyl- and 3-benzyl-1-methyl-carboxylic acids, respectively. The structure of **14** was established from the combustion analysis ($C_{13}H_{16}N_2O_4$); from the infrared spectrum, which shows peaks at 3400 (hydroxyl), 1750 (ester carbonyl), and 1680 cm^{-1} (ureide carbonyl); and from the nmr spectrum in DMSO-d_6. In addition to the expected 1-methyl and 3-benzyl resonances, the nmr spectrum of **14** shows an additional methyl signal at δ 3.30 (CO_2Me) and an exchangeable proton (OH) at δ 7.0 which is not coupled to either of two protons (H-5, H-5) appearing as an AB system at δ 3.31 and 3.79. The lack of coupling of the hydroxyl proton indicates the tertiary alcohol structure; for secondary alcohols, such as **13a** and **13b**, coupling (~5 Hz) is observed. The chemical shifts of the geminal protons of **13a** and **13b** are similar to those of the C-5 protons of **14**. Treatment of an aqueous solution of **14** with dilute hydrochloric acid resulted in the rapid formation of the imidazoline ester **15** which could be converted into the imidazoline carboxylic acid **12b** by alkaline hydrolysis.

Neither 5-hydroxyuracil nor 3-methyl-5-hydroxyuracil is converted into the corresponding imidazoline when treated with 0.1 *N* sodium hydroxide at 100°. In both cases, ultraviolet absorption was lost over a 24-hr period but no indication of imidazoline formation was obtained, either before or after acidification of the solutions. The instability of 5-hydroxyuracil in alkali has been reported previously.[4,9]

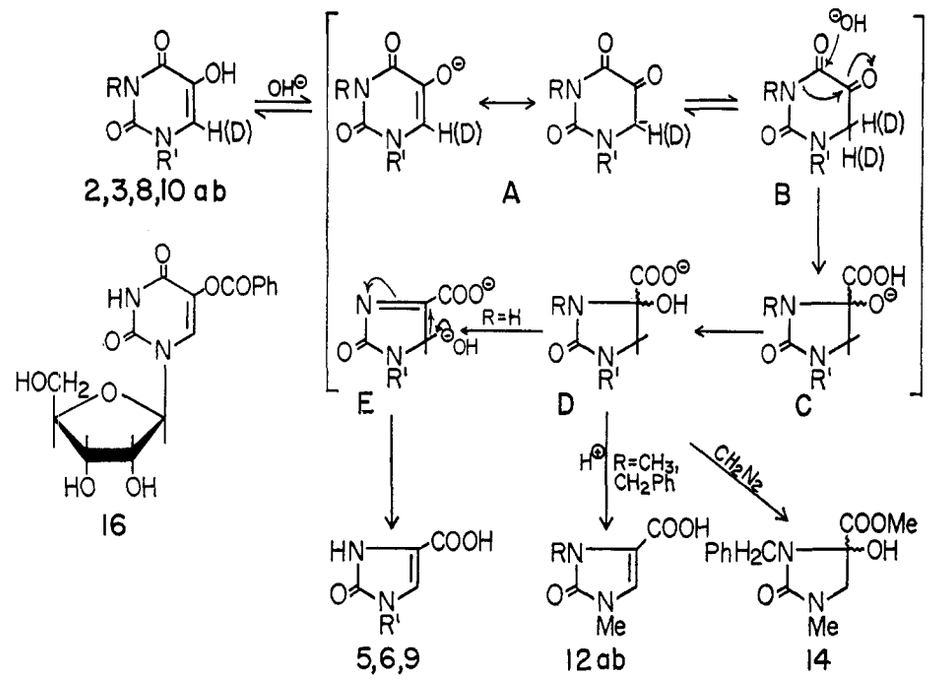
Identification of the intermediates **11a** and **11b** as α-hydroxy acids allows the formulation (Scheme III) of the ring contraction of 5-hydroxyuracil derivatives as a benzilic acid type of rearrangement. A requirement of this rearrangement is that the 5-hydroxyuracils exist partly in the tautomeric 5-keto form (**B**). Attack of hydroxide ion on C-4 of **B** and subsequent migration of the C–N bond[10] would give intermediate **C**, which would undergo a proton shift to give the imidazolidine **D**. In the 1,3-disubstituted series, intermediate **D** (corresponding to **11a** and **b**) is stable in base but undergoes rapid acid-catalyzed dehydration to the imidazolines **12a** and **12b**. Base-catalyzed dehydration of 1,3-disubstituted **D** takes place only to a small extent. In the 1-substituted series (**D**, R = H), however, base-catalyzed dehydration involving abstraction of the labile N-3 protons would give the 2 oxoisoimidazolines

(9) E. R. Garrett, H. J. Nestler, and A. Somodi, *J. Org. Chem.*, **33**, 3460 (1968).

(10) This process could take place in either the concerted manner shown or stepwise with the formation of a ureido α-keto acid which would then undergo ring closure to **C**. Attack of hydroxide ion on C-5 of **B** followed by migration of the 5,6 bond would also give **C**, but this is considered to be unlikely because the benzilic acid rearrangement of isotopically labeled alloxan to alloxanic acid has been shown[11] to involve exclusive C–N bond cleavage.

(11) H. Kwart, R. W. Spayd and C. J. Collins, *J. Amer. Chem. Soc.*, **83**, 2579 (1961). See also P. A. S. Smith and R. O. Kan. *ibid.*, **83**, 2580 (1961).

SCHEME III



E. Rearrangement of **E** would then give the 1-substituted imidazolines **5**, **6** and **9**.

Although 5-hydroxyuracil derivatives exist in solution predominately in the 5-enol form (see later discussion of uv spectra), the presence of the 5-keto tautomers (**B**) is indicated by the following data. Treatment of isopropylidene-5-hydroxyuridine (**2**) with refluxing deuterium oxide results in exchange of H-6 for deuterium, as shown by a gradual decrease in the intensity of the H-6 resonance relative to the nmr signals of protons which did not undergo exchange. This exchange reaction involves ionization of the 5-hydroxyl group (pK_a = 7.7) of **2** to give the mesomeric anion **A**. Deuteration of **A** at the C-6 position gives **B**, which then reverts to isopropylidene-5-hydroxyuridine-6-*d*. Evidence supporting this mechanism is that deuterium exchange does not take place when the enolate ion–keto equilibrium **A** → **B** is precluded by substitution of the 5-hydroxyl group. Thus, 5-benzoyloxyuridine (**16**) does not incorporate deuterium at C-6 when treated with refluxing deuterium oxide. Rapid deuterium exchange of isopropylidene-5-hydroxyuridine (**2**), and hence formation of the 5-keto tautomer **B**, takes place under the alkaline conditions which lead to the formation of the imidazoline nucleoside **5**. Thus, when the reaction of **2** (0.02 *M*) in 0.1 *N* sodium deuterioxide at 100° was stopped after 30 min, the remaining starting material had undergone complete exchange of H-6 for deuterium. Under identical conditions, the 1,3-dimethyl-5-hydroxyuracil (**10a**) remaining after 2 hr had undergone ~30% exchange. The slower rate of exchange observed for **10a**, compared with **2**, is consistent with the slower rate at which **10a** undergoes ring contraction.

As mentioned proviously, isopropylidene-5-hydroxyuridine (**2**, 0.1 *M*) is stable in 1 *N* sodium hydroxide at 55°. The rate of deuterium incorporation into **2** is low under these conditions; over a 24-hr period in 1 *N* sodium deuterioxide, only 80% exchange of H-6 was observed. The lack of formation of the imidazoline nucleoside **5** from **2** in 1 *N* sodium hydroxide is therefore a reflection of the very low concentration of the 5-keto tautomer **B**. Similarly, 1,3-dimethyl-5-hydroxyuracil (**10a**, 0.1 *M*) is stable in 1 *N* sodium hydroxide as 55°, and the extent of deuterium incorporation (~10% in 24 hr) is again smaller than that observed for isopropylidene-5-hydroxyuridine (**2**) under these conditions. The slower rates of exchange of **10a** may be due to a decrease in the carbanion character of the mesomeric ion **A** caused by the inductive effect of the methyl substituents. Substituent effects on the enol–keto equilibria of 5-hydroxyuracils, together with studies of electrophilic substitution of the C-6 position, are currently under investigation in this laboratory.

The ultraviolet absorption and apparent pK_a data of the 5-hydroxyuracils used in this study are listed in Table I. The similarity of the spectrum of 1,3-dimethyl-5-hydroxyuracil to those of 5-hydroxyuracil and its mono-N-methyl derivatives in the pH range of 1–10 (neutral to monoanion) show conclusively, as would be expected, that the first dissociation of all these compounds is due to ionization of the 5-hydroxyl group. The similarity of the spectrum of 1-methyl-3-benzyl-5-methoxyuracil (pH 1–14) to those of the other 5-hydroxyuracils (at pH 1) in Table I shows that the neutral species of all these compounds exist predominantly in the 2,4-dicarbonyl-5-hydroxy form (due allowance given to the effects of alkylation at N-1 and N-3). The bathochromic shifts produced by N-1 substitution (*vs.* N-3 substitution) are similar to effects noted previously[12] with uracil and its N-methyl derivatives. As expected, the substitution of a sugar moiety on N-1 in place of a methyl group exerts an acid-strengthening effect.

General Considerations.—5-Hydroxyuridine is a normal component of the ribonucleic acids of yeast (*Torula utilis*).[13] In this regard, the chemistry of 5-

(12) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).
(13) A. W. Lis and W. E. Passarge, *Arch. Biochem. Biophys.*, **114**, 593 (1966).

TABLE I

ULTRAVIOLET AND APPARENT pK_a DATA[a] FOR 5-HYDROXYURACIL AND DERIVATIVES

	Neutral species				Monoanion				Dianion				
	pH 1				pH 10				pH 14				
Compd	λ_{max}, mμ	ε × 10^{-3}	λ_{min}, mμ	ε × 10^{-3}	λ_{max}, mμ	ε × 10^{-3}	λ_{min}, mμ	ε × 10^{-3}	λ_{max}, mμ	ε × 10^{-3}	λ_{min}, mμ	ε × 10^{-3}	pK_{a_1}
5-Hydroxyuracil	277	7.10	245	2.24	305	6.12	272	3.04	302	~5.25[b]	270	~2.52[b]	8.09[c]
					240	7.62	220	4.86	sh 240	~7.29[b]			
1-Methyl-5-hydroxyuracil	284	7.90	247	1.73	310	6.75	272	2.55	304	6.03	269	2.54	7.94[d]
					240	7.38	220	4.74	sh 240	6.95			
Isopropylidene-5-hydroxyuridine	280	8.67	244	2.40	306	7.50	270	3.50	303	7.10	268	2.87	7.72[e,f]
					247	6.40	224	5.10					
3-Methyl-5-hydroxyuracil	277	6.70	245	2.23	304	6.13	272	3.15	322	~7.90[b]	275	~1.65[b]	8.22
					242	7.19	222	4.71	242	~7.77[b]	222	~4.24[b]	
1,3-Dimethyl-5-hydroxyuracil	283	7.35	247	1.83	310	6.73	273	2.55	...	...	...	...	8.18
					242	6.90	225	5.17					
1-Methyl-3-benzyl-5-hydroxyuracil	286	7.22	249	1.93	312	6.66	275	2.54	...	...	...	...	8.06
					243	7.01	228	6.18					
1-Methyl-3-benzyl-5-methoxyuracil	283[g]	7.13	249[g]	2.07	...	...	...	...	...	...	...	...	...

[a] Determined spectrophotometrically by methods previously described [J. J. Fox and D. Shugar, *Bull. Soc. Chim. Belges*, 61, 44 (1952)]. pK_{a_1} values refer to ionization of the 5-hydroxyl group and are accurate to ±0.05 pH unit. Accurate pK_a values (ionization of N–H groups) were not determined. [b] Compound unstable in 1 *N* NaOH making pK_{a_2} determination impossible. [c] pK_{a_1} 8.11 and pK_{a_2} 11.48 (potentiometric titration) previously reported [A. Albert and J. N. Phillips, *J. Chem. Soc.* 1294 (1956)]. [d] pK_{a_2} ~ 12. [e] pK_{a_2} ~ 11.5. [f] pK_{a_1} 7.8 (potentiometric titration) previously reported for 5-hydroxy-2′-deoxyuridine [T. Y. Shen, J. F. McPherson, and B. O. Linn, *J. Med. Chem.*, 9, 366 (1966)]. [g] pH 1–14.

hydroxyuridine described herein should be taken into account in studies on the alkaline degradation of ribonucleic acids containing 5-hydroxyuridine or of 5-hydroxyuridylic acids.[13,14]

We also find that 5-bromo-2′-deoxyuridine is converted in dilute alkali (*via* its 5-hydroxy derivative) into the 2′-deoxy analog of **6**. This finding suggests that the over-all conversion may be applicable under mild conditions to known[15] deoxyribonucleic acids in which 2′-deoxy-5-bromouridine replaces some of the thymidine residues. Such a chemical rearrangement may alter the base pairing and, conceivably, the biological properties of the deoxyribonucleic acid.

Experimental Section

General Procedures.—Melting points were determined on a Thomas–Hoover apparatus (capillary method) and are uncorrected. The nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal reference. Chemical shifts are reported in parts per million (δ) and signals are expressed as s (singlet), d (doublet), t (triplet), or m (complex multiplet). Values given for coupling constants (hertz) and chemical shifts are first order unless the spin system is designated AB or ABX. Thin layer chromatography was performed on silica gel GF_{254} (Merck); separated materials were detected with ultraviolet light and by spraying with 10% v/v sulfuric acid in ethanol followed by heating at 110°. Evaporations were carried out *in vacuo* with bath temperatures kept below 45°. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, and by Spang Microanalytical Laboratory, Ann Arbor, Michigan. For reactions that were monitored by changes in the uv spectra, 0.1-ml aliquots were diluted with water to give a concentration of 1×10^{-4} *M* in starting material; uv spectra were then recorded at pH ~ 10 on a Cary Model 15 spectrometer.

1-(β-D-Ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid (6) from Isopropylidene-5-bromouridine[16] (1). Method A.—A solution of 363 mg (1 mmol) of 1 (X = Br) in 50 ml of 0.1 *N* NaOH was refluxed for 6 hr. A rapid decrease in the uv absorption of 1 at 275 mμ was followed by the appearance of peaks at ~305 (**2**) and 253 mμ (**5**). The 305-mμ peak reached a maximum value of OD 0.11 at 90 min and then decreased, while the 252-mμ peak increased to a maximum OD of 0.53. Hydrolysis of the isopropylidene group of **5** to give **6** took place during the isolation procedure. The dark yellow solution was cooled and passed through a column containing an excess of Dowex AG-50W-X8 (H^+). The colorless, acidic effluent and washings were concentrated to ~2 ml. Acetone was added and the solution cooled to give crystals of the dihydrate of **6** (135 mg, 45%), mp and mmp 107–110° (resolidifies and melts at 195–200°, eff, dec). The uv and ir spectra of this material were identical with those of **6** prepared previously.[3]

Method B.—Isopropylidene-5-bromouridine (**1**) (18.15 g, 0.05 mol) was added to a solution of sodium bicarbonate (0.15 mmol) in 1 l. of water. The solution was refluxed under nitrogen for 22 hr. Uv spectral examination revealed a gradual loss of **1** (λ_{max} 275 mμ) and formation of **2** (λ_{max} 305 mμ) which reached a maximum concentration (OD 0.21) at ~3 hr. During this time the pH of cooled samples of the reaction mixture increased from 8.5 to 10. After 3 hr, the 305-mμ peak (**2**) gradually decreased with the concomitant formation of a peak at 252 mμ (**5**) which reached a maximum value of OD 0.72 at 22 hr. The brown reaction mixture was cooled and deionized by passage through a column containing ~200 ml of Dowex AG 50W-X8 (H^+). The effluent and washings were concentrated to ~100 ml; acetone (50 ml) was added and the solution was cooled, whereupon crystalline material separated. The product (8.0 g, 54%) had mp and mmp 107–110° (resolidifies and melts at 195–200°, eff, dec) and gave ir and uv spectra identical with those of **6** prepared *via* method A.

2′,3′-*O*-Isopropylidene-5-hydroxyuridine (2).—Carbon dioxide gas was bubbled into a suspension of 5.45 g (0.015 mol) of **1** (X = Br) in 300 ml of water containing 3.78 g (0.045 mmol) of sodium bicarbonate. The mixture was heated to reflux temperature, whereupon **1** dissolved. Heating was continued for 6 hr, at which time the absorption of **2** at 305 mμ reached a maximum value of OD 0.45. The pH of the cooled reaction mixture was ~8.5. The solution was passed through a column (3 × 30 cm) containing Dowex AG1, X-8 (OH^-, 100–200 mesh) and the column was washed with water until the effluent was neutral. The column was then eluted with 0.05 *M* NH_4HCO_3, and 25-ml fractions were collected. Fractions 50–100, which gave a positive ferric chloride test, were combined and concentrated to 30 ml. Crystallization of **2** (1.80 g, 40%) commenced on cooling. A second crop of 280 mg, 6% (total yield 46%), was obtained by concentration of the filtrate. Both crops gave only a single spot on tlc (MeOH–$CHCl_3$, 1:4 v/v). A sample recrystallized from water for analysis had mp 215–217°; nmr δ 11.43 broad s (1, NH), 8.64 s (1, 5-OH), 7.32 s (1, H-6), 5.89 d (1, H-1′, $J_{1',2'}$ = 2.2), 5.06 broad t (1, 5′-OH), 4.84 m (2, H-2′, H-3′),

(14) D. A. Smith and D. W. Visser, *J. Biol. Chem.*, **240**, 446 (1965).

(15) For a discussion of DNAs containing 5-bromouracil see review by R. E. Handschumacher and A. D. Welch, "The Nucleic Acids," E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1960, Vol. 3, p 453.

(16) Purchased from Zellstoff-fabrik Waldhof, Mannheim, W. Germany.

4.07 m, (1, H-4'), 3.61 m (2, H-5', H-5'), 1.50 and 1.31 ppm s (2, 6-H, isopropylidene methyls).

Anal. Calcd for $C_{12}H_{16}N_2O_7$: C, 48.00; H, 5.37; N, 9.33. Found: C, 47.90; H, 5.40; N, 9.19.

Compound **2** was converted into 5-hydroxyuridine (mp 238–240°, lit.[17] mp 238–240°) by treatment with 80% acetic acid at 100° for 1 hr.

1-(β-D-Ribofuranosyl)-2-oxo-4-imidazoline-4 carboxylic Acid (6) from Isopropylidene-5-hydroxyuridine (2). Method A.—A solution of 300 mg (1 mmol) of **2** in 50 ml of 0.1 *N* NaOH was refluxed for 6 hr, at which time the absorption peak at 252 mμ (**5**) reached a maximum value of OD 0.90. The cooled solution was passed through a column containing an excess of Dowex AG-50W-X8 (H^+), and the neutral eluate was acidified (to pH ~1) with HCl to ensure complete hydrolysis of the isopropylidene group. Concentration of the solution to ~1 ml and addition of acetone afforded crystals (213 mg, 72%) of **6**, mp and mmp 107–110° (resolidifies and remelts at 195–200° eff, dec).

Treatment of 5-hydroxyuridine (**3**, 1 mmol) with 0.1 *N* NaOH (50 ml) at 100° results in formation of **6**. Uv spectral examination showed that the rearrangements **3** → **6** and **2** → **5** proceed at the same rate and give similar yields of imidazoline.

Method B.—A sample of **2** (300 mg, 1 mmol) was added to a solution of sodium bicarbonate (252 mg, 3 mmol) in 20 ml of water. The solution was refluxed for 22 hr, cooled, and processed as described in method A above. The yield of pure **6** was 207 mg (70%).

1-(2-Deoxy-β-D-ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic Acid.—2'-Deoxy-5-bromouridine (3 g, 0.01 mol) was dissolved in a solution of sodium bicarbonate (2.52 g, 0.03 mol) in 200 ml of water. The solution was buffered with CO_2 gas (pH ~8.5) and refluxed for 9 hr. During this time, the conversion of starting material into 2'-deoxy-5-hydroxyuridine was monitored by the increase in absorption at ~302 mμ. The flow of CO_2 was stopped after 9 hr, and refluxing continued for a further 12 hr. Spectral examination of the black reaction mixture (pH ~10) showed the presence of the 2'-deoxy-imidazoline nucleoside together with some 2'-deoxy-5-hydroxyuridine. The cooled solution was passed through an excess of Dowex AG-50W-X8 (H^+) and the eluate and washings were concentrated almost to dryness. Most of the water was removed by codistillation with ethanol, and the product was precipitated by addition of ether. The dried precipitate was crystallized with difficulty from 95% methanol–ether to give 610 mg (40%) of the hemihydrate, mp 187–190°.

Anal. Calcd for $C_9H_{12}N_2O_6 \cdot {}^1/_2H_2O$: C, 42.68; H, 5.13; N, 11.06. Found: C, 42.97; H, 4.91; N, 11.14.

1-Methyl-2-oxo-4-imidazoline-4-carboxylic Acid (9).—1-Methyl-5-hydroxyuracil[18] (**8**) (1.42 mg, 1 mmol) was dissolved in 50 ml of 0.1 *N* NaOH and the solution was refluxed. Uv spectral examination showed that the product (**9**) reached a maximum concentration (OD = 0.85) at ~23 hr. The solution was acidified with HCl, concentrated to 25 ml, and cooled. The resulting precipitate was recrystsllized from water to give 114 mg (80%) of pure **9**, mp and mmp 274–276° eff, dec (but dependent on rate of heating). The uv, nmr, and ir spectra of this material were identical with those of authentic[8] **9**.

1,3-Dimethyl-2-oxo-4-imidazoline-4-carboxylic Acid (12a).—A solution of 780 mg (5 mmol) of **10a**[4] in 250 ml of 0.1 *N* NaOH was refluxed for 19 hr. During this time, the uv absorption max of **10a** (~310 mμ) decreased by 84%. Acidification (to pH 3) of the 1×10^{-4} solution used for following uv changes resulted in the appearance of a peak at 269 mμ which increased to OD 0.95 in 10 min. Acidification of the cooled reaction mixture with HCl (to pH ~1) and concentration to ~50 ml afforded **12a** (550 mg, 71%) in two crops, mp and mmp 230–232°. The uv and ir spectra were identical with those of authentic[8,19] **12a**.

1-Methyl-3-benzyluracil.—1-Methyluracil (7.2 g, 5.7 mmol) was added to a solution of KOH (6.4 g, 11.4 mmol) in 150 ml of ethanol. Benzyl bromide (9.7 g, 5.7 mmol) was added and the mixture was refluxed for 7 hr. The cooled solution was concentrated almost to dryness, diluted with 100 ml of water, and neutralized with acetic acid. The solution was extracted with ether (thrice, 100 ml); the ether extracts were dried and concentrated to dryness. Recrystallization of the residue from 50% ethanol afforded pure material (9 g, 73%), mp 105–106°.

Anal. Calcd for $C_{12}H_{12}N_2O_2$: C, 66.66; H, 5.55; N, 12.96. Found: C, 66.71; H, 5.56; N, 12.93.

1-Methyl-3-benzyl-5-bromouracil.—Bromine (6.6 g, 4.1 mmol) was added to a solution of 1-methyl-3-benzyluracil (9.0 g, 4.2 mmol) in 175 ml of glacial acetic acid. The pale yellow solution was heated on a steam bath for 30 min, concentrated to ~50 ml, and then poured into 500 ml of water. The resulting precipitate was recrystallized from 50% ethanol to give pure material (12 g, 97%), mp 124–125°.

Anal. Calcd for $C_{12}H_{11}BrN_2O_2$: C, 48.81; H, 3.73; N, 9.49. Found: C, 48.82; H, 3.68; N, 9.39.

1-Methyl-3-benzyl-5-hydroxyuracil (10b).—Carbon dioxide gas was bubbled through a solution of 9 g (0.033 mol) of 1-methyl-3-benzyl-5-bromouracil in 600 ml of 50% ethanol containing 7.5 g (0.09 mol) of sodium bicarbonate. The solution was refluxed for 22 hr, at which time the absorption of **10b** at 312 mμ reached a maximum value. The solution was cooled, neutralized with 1 *N* HCl, and concentrated to remove the ethanol. The product crystallized when the aqueous solution was acidified with 1 *N* HCl. Recrystallization from 50% ethanol afforded pure **10b** (5.7 g, 80%): mp 163–164°; nmr δ 8.66 s (1, OH), 7.33 and 7.31 two barely resolved singlets (6, phenyl and H-6), 5.07 s (2, CH_2), and 3.29 ppm s (3, CH_3).

Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.07; H, 5.17; N, 12.07. Found: C, 62.21; H, 5.09; N, 11.96.

1-Methyl-3-benzyl-2-oxo-4-imidazoline-4-carboxylic acid (12b) was prepared from 1.26 g (5 mmol) of **10b** as described for the preparation of **12a**. The yield of pure **12b** (recrystallized from water), mp 224–226°, was 74%; nmr δ ~12.5 broad s (1, COOH), 7.30 s (5, phenyl), 7.55 s (1, H-5), 5.16 s (2, CH_2), and 3.28 ppm s (3, NCH_3).

Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.07; H, 5.17; N, 12.07. Found: C, 62.03; H, 5.14; N, 11.99.

3-Benzyl-4-hydroxy-1-methyl-2-oxoimidazolidine-4-carboxylic Acid Methyl Ester (14).—A solution of 2.32 g (10 mmol) of **10b** in 300 ml of 0.1 *N* NaOH was refluxed for 17 hr. The uv absorption maximum of **10b** (312 mμ) decreased by 85% during this period. The cooled solution was passed through a column (2.5 cm diameter) containing 16 g of Amberlite IRC-50. The eluate was made weakly alkaline by the addition of 1.5 ml of 1 *N* NaOH. The eluate and washings (100 ml) were concentrated to dryness (with the bath temperature kept below 20°) and the residue was dried *in vacuo* over KOH pellets. The amorphous material (2.3 g) was suspended in 10 ml of methnol and 400 mg of unidentified gelatinous material removed by filtration. To the methanol solution (5°) was added three charges of diazomethane (~30 mmol each in 50 ml of ether) at 4 hr intervals after just neutralizing the reaction mixture each time with glacial acetic acid. The solution was stored at 5° for 4 hr after the final addition of diazomethane, and then concentrated to 10 ml, whereupon **14** (250 mg) crystallized. Concentration of the methanol filtrate to dryness and suspension of the residue (800 mg) in cold methanol afforded a second crop (400 mg) of insoluble **14**. Recrystallization of the combined crops from boiling methanol gave 460 mg of pure **14**: mp 169–170°; ir ν_{max} (KBr) 3400 (OH), 1750 (ester carbonyl), 1680 cm^{-1} (ureide carbonyl). The nmr spectrum of **14** showed an AB subspectrum for the C-5 protons (δ 3.79, 3.31; $J_{5,5}$ = 10.5 Hz) and another AB system for the benzyl methylene protons (δ 4.44, 4.24; J_{gem} = 16.0 Hz). Other nmr signals were at δ 7.28 s (5, phenyl), 7.0 s (1, OH, exchanges on addition of D_2O), 3.30 s (3, ester methyl), and 2.78 ppm s (3, NCH_3).

Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.09; H, 6.06; N, 10.65. Found: C, 59.16; H, 6.01; N, 10.55.

Tlc (MeOH–$CHCl_3$, 1:30 v/v) showed that the combined mother liquors from above contained mostly **14** and small amounts of **7** and **15**. These compounds were not isolated, but their identities were established by comparison of chromatographic mobility and uv spectra of eluted materials with those of **7** and **15** described below. A 1×10^{-4} *M* solution of **14** in water showed only end absorption (below 220 mμ) in the uv. Acidification of this solution resulted in formation of the imidazoline ester **15** ($\lambda_{max}^{pH\ 1}$ 271 mμ) which underwent hydrolysis to **12b** (shift of λ_{max} to 258 mμ) on treatment with alkali.

1-Methyl-3-benzyl-5-hydroxy-5,6-dihydrouracil (13b).—A sample of **10b** (1 g, 4.3 mmol) was dissolved in a suspension of 10% Pd–C (~50 mg) in 200 ml of ethanol, and the mixture was hydrogenated on a Parr apparatus for 6 hr. The catalyst was removed and the filtrate concentrated to a colorless syrup which

(17) D. W. Visser, "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorbach and R. S. Tipson, Ed., Interscience Publishers, New York, N. Y., 1968, Vol. 1, p 428.

(18) Prepared from 1-methyluracil as described by Z. Budesinsky, J. Prikryl, and E. Svatek, *Coll. Czech. Chem. Commun.*, **29**, 2980 (1964).

(19) G. E. Hilbert, *J. Amer. Chem. Soc.*, **54**, 3413 (1932).

crystallized spontaneously to give pure **13b**, mp 75–78°. The nmr spectrum of **13b** contained peaks at 7.25 (5, s, phenyl), 6.09 (1 broad peak, OH) and 4.82 (2, s, benzyl CH_2). The geminal H-6 and H-5 protons appeared as an ABX subspectrum (after removal of the H-5, OH coupling by addition of D_2O) with H-5, δ_X 4.32; H-6, δ_A 3.29; H-6, δ_B 3.51 (J_{AB} = 12.5; J_{AX} = 9.7; J_{BX} = 5.1 Hz). The 5-OH group in the spectrum of 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil[20] (**13a**) appeared as a doublet (δ 5.97, $J_{5,OH}$ = 5.5 Hz).

Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 5.98; N, 11.97. Found: C, 61.29; H, 6.03; N, 11.96.

1-Methyl-3-benzyl-5-methoxyuracil (**7**).—A solution of **10b** (232 mg, 1 mmol) in methanol (20 ml) was treated with an excess of diazomethane in ether. The solution was concentrated to dryness after 12 hr, and the residue was crystallized from ethyl acetate to give 210 mg (85%) of **7**: mp 146–147°; nmr δ 7.45 s (1, H-6), 7.28 s (5, phenyl), 5.01 s (2, CH_2), 3.65 s (3, OCH_3), and 3.30 ppm s (3, NCH_3).

Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.37. Found: C, 63.42; H, 5.72; N, 11.21.

1-Methyl-3-benzyl-2-oxo-4 imidazoline-4-carboxylic acid methyl ester (**15**) was prepared by methylation of 232 mg (1 mmol) of **12b** with an excess of diazomethane. The yield of pure material (from ethyl acetate–30–60° petroleum ether) was 220 mg (90%), mp 147–149°; nmr δ 7.61 s (1, H-5), 7.25 s (5, phenyl), 5.10 s (2, CH_2), 3.70 s (3, $COOCH_3$), and 3.27 ppm s (3, NCH_3).

Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.37. Found: C, 63.44; H, 5.68; N, 11.26.

5-Benzoyloxyuridine (**16**).—Benzoic anhydride (226 mg, 1 mmol) was added to a refluxing solution of **3** (260 mg, 1 mmol) in 25 ml of methanol. Heating was continued for 1 hr and then a further charge of benzoic anhydride (1 mmol) was added. This procedure was repeated; after a reaction period of 3 hr, tlc ($CHCl_3$–MeOH, 5:1 v/v) showed only a trace of starting material. Concentration of the solution afforded a colorless syrup which crystallized after addition of ether. The solid was washed liberally with ether and the dried residue was dissolved in 10 ml of 50% ethanol. Crystallization commenced during concentration of the solution to 5 ml, and was completed by cooling. The yield of pure material, mp 218–220°, was 197 mg (54%) [crystallization of **16** remaining in the mother liquor was inhibited by the presence of a small amount of starting material (**3**)]: uv $\lambda_{max}^{H_2O}$ 237, 270 mμ; $\lambda_{min}^{H_2O}$ 214, 252 mμ; nmr δ 11.8 s (1, NH), 8.30 s (1, H-6), 8.2–7.4 m (5, phenyl), 5.83 d (1, H-1′, $J_{1',2'}$ = 4.5 Hz), ~5.0 broad peak (3, hydroxyls), 4.0 m (3, H-2′, H-3′, H-4′), 3.6 broad s (2, H-5′, H-5′).

Anal. Calcd for $C_{16}H_{16}N_2O_8$: C, 52.75; H, 4.43; N, 7.69. Found: C, 52.96; H, 4.46; N, 7.66.

Stability of Isopropylidene-5-hydroxyuridine (2) and 1,3-Dimethyl-5-hydroxyuracil (10a) in 1 *N* NaOH.—Solutions of **2** (0.1 and 0.02 *M*) and **10a** (0.1 *M*) in 1 *N* NaOH were heated at 55°. Aliquots (0.1 ml) taken immediatedly and at 24 hr were diluted with water to 1×10^{-4} *M*. In each case, the uv absorption maxima decreased by less than 5% in 24 hr.

(20) S. Y. Wang, *J. Amer. Chem. Soc.*, **80**, 6196 (1958).

Deuterium Exchange of Isopropylidene-5-hydroxyuridine (2) and 1,3-Dimethyl-5-hydroxyuridine (10a). A. In D_2O.—A solution of 60 mg of **2** in 2 m lof D_2O was heated under reflux at 100°. Integration of the H-6 and H-1′ signals in the nmr spectrum of **2** revealed that 75% and 100% exchange of H-6 for deuterium had taken place at 2 and 4 hr respectively. 5-Benzoyloxyuridine (**16**, 30 mg) showed no decrease of the H-6 signal, relative to the H-1′ resonance, when refluxed in D_2O (1 ml) for 2 hr.

B. In 0.1 *N* NaOD.—A solution of **2** (30 mg, 0.1 mmol) in 5 ml of 0.1 *N* NaOD in D_2O was refluxed for 30 min. The solution was cooled and concentrated to 0.5 ml. Integration of the nmr spectrum showed that complete exchange of H-6 had taken place. When 15.6 mg (0.1 mmol) or **10a** was refluxed in 5 ml of 0.1 *N* NaOD for 2 hr, the nmr spectrum of the concentrated solution showed that ~30% exchange of H-6 had taken place. In this case, the intensity of the H-6 signal was compared to those of the N-1 and N-3 methyl groups.

C. In 1 *N* NaOD.—A solution of **2** (15 mg, 0.05 mmol) in 0.5 ml of 1 *N* NaOD in D_2O was heated at 55° for 23 hr. Integration of the nmr spectrum at 5 and 23 hr showed that 36% and 80% exchange, respectively, of H-6 had taken place. 1,3-Dimethyl-5-hydroxyuracil (**10a**), 15.6 mg (0.1 mmol), was heated at 55° in 1 ml of 1 *N* NaOD for 23 hr. The solution was concentrated to 0.3 ml; integration of the nmr spectrum indicated ~10% exchange of H-6.

Conversion of Uridine into 1-(β-D-Ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic Acid (**6**).—A small excess of bromine was dissolved in a solution of uridine (4.84 g, 20 mmol) in 300 ml of water. The excess bromine was removed by aeration, and sodium bicarbonate 6.7 g (80 mmol) was added in portions. The solution was diluted to 400 ml and refluxed for 20 hr. The brown solution was deionized by passage through a column containing ~100 ml of Dowex AG 50W-X8 (H^+). The effluent and washings were concentrated to 30 ml; acetone was added and the solution was cooled. The resulting crystals (2.3 g, 39%), mp 107–110° (resolidifies and melts 195–200°, eff, dec), gave ir and uv spectra identical with those of dihydrated **6** prepared as above. A second crop was obtained as follows. The filtrate was neutralized with acetic acid and the solution was passed through a column containing 50 ml of Dowex AG1-X8 (OAc^-). The column was eluted with 0.1 *N* HOAc until the effluent was free of uv absorbing material; nucleoside **6** was then obtained by elution with 0.1 *N* HCl. Concentration of the appropriate fractions afforded crystalline **6** dihydrate (600 mg, total yield 49%).

Registry No.—**2**, 20406-82-0; 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid, 20406-83-1; 1-methyl-3-benzyluracil, 20406-84-2; 1-methyl-3-benzyl-5-bromouracil, 20406-85-3; **7**, 20462-27-5; **8**, 15585-47-4; **10a**, 20406-86-4; **10b**, 20406-87-5; **12b**, 20406-88-6; **13b**, 20406-89-7; **14**, 20406-90-0; **15**, 20407-04-9; **16**, 20407-06-1; 5-hydroxyuracil, 4628-37-9; 3-methyl-5-hydroxyuracil, 1671-14-3.