Synthesis of 1-β-D-Arabinofuranosylorotate and an Investigation of the Rearrangement of 2,2′-Anhydroorotidine Derivatives

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2,2′-Anhydroorotidine ethyl ester (**2**) was prepared by cyclization of 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (**1**) with diethyl acetylenedicarboxylate. Treatment of compound **2** with 2 *N* HCl afforded 1-β-D-arabinofuranosylorotic acid 2′,6-lactone (**4**) which gave, after titration with 0.5 *M* KOH, potassium 1-β-D-arabinofuranosylorotate (**7**). Compound **2** was readily converted to 2,2′-anhydro-1-β-D-arabinofuranosyluracil-6-carboxamide (**3a**) by treatment with methanol saturated with ammonia at 0°. Prolonged treatment of compound **2** with methanolic ammonia gave 2-amino-2′,6-anhydro-1-β-D-arabinofuranosyl-5,6-dihydrouracil-6-carboxamide (**6**). A similar rearrangement was observed when compound **7** was treated with base or allowed to stand at room temperature in solution for a few days. Treatment of compound **3a** with trifluorcacetic acid, which had been saturated with dry hydrogen bromide at 0°, afforded, not the expected 3′-bromo-3′-deoxyorotidine derivative, but a 3-β-D-anhydronucleoside isomeric with **3a**, which was subsequently hydrolyzed to 3-β-D-arabinofuranosyluracil-6-carboxamide (**11**). The combined nature of the arabinosyl 2′-OH group and the pyrimidine 6-carboxyl group in close proximity has led to a number of unusual interactions and rearrangements, for which mechanisms have been proposed.

The importance of orotic acid in the biological synthesis of nucleic acid, and pyrimidine nucleotides in particular, has been well established.[1] Orotidine was first isolated from a mutant of *Neurospora crassa* by Michelson, Drell, and Mitchell,[2] who showed that it was composed of orotic acid and ribose. Subsequently, Lieberman, *et al.*,[1g] by enzymatic studies, and Fox, *et al.*,[3] by ultraviolet absorption spectroscopy proved that the ribose moiety was affixed to the N-1 position of the pyrimidine. To date, however, the only synthetic method of preparing orotidine (~10%) is that reported by Angier and Curran.[4] This consideration prompted an investigation of new synthetic routes to various derivatives of orotic acid nucleosides.

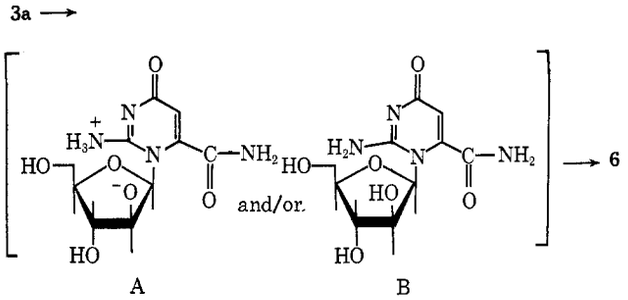
In 1968, Ferris, Sanchez, and Orgel[5] reported a synthesis of cytosine and uracil from cyanoacetylene and later, by a similar procedure, Sanchez and Orgel[6] obtained α-ribosyl- and β-arabinosylpyrimidines. Following a similar approach in our laboratory, 2,2′-anhydroorotidine ethyl ester (**2**) was synthesized by treatment of 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline (**1**) with diethyl acetylenedicarboxylate in 53% yield. This compound, like other anhydronucleosides in the literature,[7] is an important intermediate in stereochemically controlled reactions involving alteration of the sugar configuration, as well as that of the aglycon.

The potassium salt of 2,2′-anhydroorotidine (**5b**) was readily prepared by treatment of **2** with 1 equiv of potassium hydroxide at room temperature. Neutralization with Dowex 50 (H^+) furnished the corresponding free acid (**5a**).

Compound **2** was readily converted to 2,2′-anhydro-1-β-D-arabinofuranosyluracil-6-carboxamide (**3a**) by treatment of **2** at 0° with methanol saturated with ammonia (0°); the morpholino derivative **3b** was prepared in a similar manner with the objective of obtaining a more stable protecting group for future reactions.

It was found that prolonged treatment of compound **2** at 0° with methanolic ammonia afforded 2-amino-2′,6-anhydro-1-β-D-arabinofuranosyl-5,6-dihydrouracil-6-carboxamide (**6**) as a side product. The structural assignment of **6** was based on the following data: uv absorption above 240 nm was lost; the nmr spectrum showed a broad singlet at δ 8.47 assigned to NH_2 and a widely separated broad doublet (22 Hz) which was attributed to the carboxamido group. This assignment was substantiated by the disappearance of the H-5 signal at δ 6.28 (singlet) and the appearance of a singlet at δ 2.8 which integrated for two protons and exchanged upon the addition of D_2O and NaOD. That the 5′ position is not involved in the anhydro linkage of **6** follows from the nmr spectrum, which showed the H-5′ protons as a broad singlet at δ 3.60. In a typical 5′-anhydro derivative the H-5′ signals appear at lower field, for example at δ 4.08,[8] and as a quartet with $J_{5',5''}$ ~ 13 Hz. Compound **6** was obtained in 82% yield from **2** when the same reaction was performed at room temperature in a sealed vessel for 3 days.

The formation of compound **6** can be explained by the displacement of the 2,2′-anhydro linkage of **3a** through nucleophilic attack of ammonia on C-2 to form A. Attack of the initially formed 2′-O anion of A or the 2′-



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(2) A. M. Michelson, W. Drell, and H. K. Mitchell, *Proc. Nat. Acad. Sci. U. S.*, **37**, 396 (1951).

(3) J. J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, **23**, 295 (1957).

(4) W. V. Curran and R. B. Angier, *J. Org. Chem.*, **31**, 201 (1966).

(5) J. P. Ferris, R. A. Sanchez, and L. E. Orgel, *J. Mol. Biol.*, **33**, 693 (1968).

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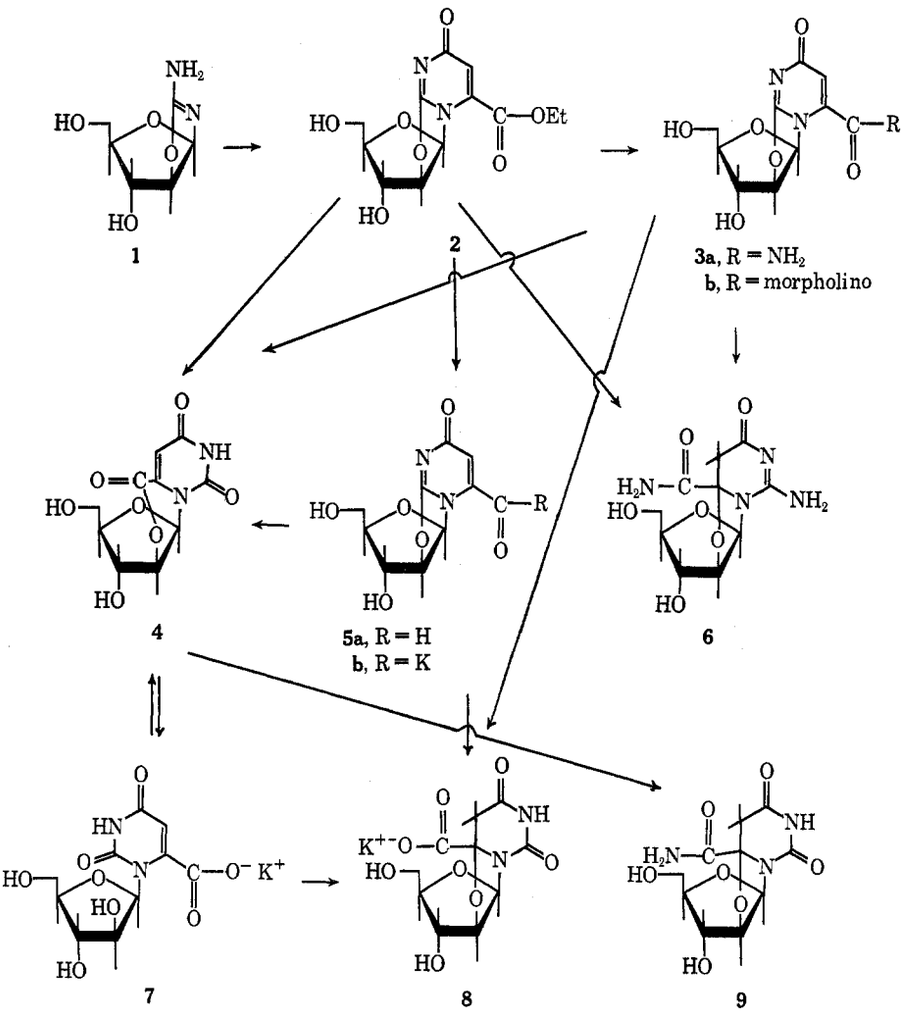
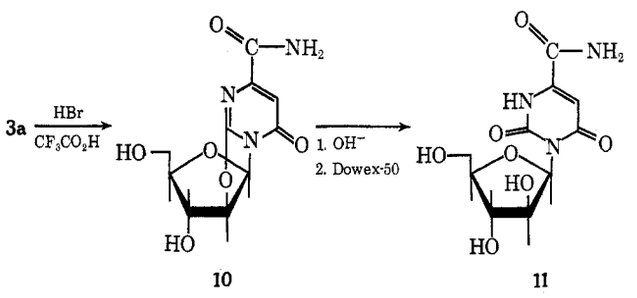


OH group of B on C-6 would lead to the formation of the dihydro derivative **6**.

The possibility of the utilization of 2,2′-anhydroorotidine as a starting material for the synthesis of 2′-bromo-2′-deoxy nucleosides was investigated following the method described by Fox and coworkers.[9] This involves treatment of the anhydronucleoside with trifluoroacetic acid saturated with dry hydrogen bromide at 0°. This procedure, however, led to the rearrangement of compound **3a** to the corresponding 3-β-D isomer (**10**). The structure assigned to **10** is supported



by the following data: the uv absorption spectrum showed a significant bathochromic shift of 14 nm relative to compound **3a**; and the ir spectrum of compound **10** was markedly different from that of **3a**, indicating a difference in the electronic structure of the pyrimidine moiety. In addition, the similarity of the nmr spectra of **3a** and **10** (except H-5 and H-1′) implies that both compounds have the same conformation and therefore, contain anhydro rings of the same size. Elemental analysis supports the fact that these two compounds are isomers. This structural assignment was further substantiated by the alkaline hydrolysis of compound **10** to 3-β-D-arabinofuranosyluracil-6-carboxamide (**11**). Compound **11** showed a bathochromic shift of 39 nm in alkaline solution accompanied by an increase in ϵ_{max}, characteristic of an N-3 substituted orotic acid.[3,10]

A mechanism that accounts for the above rearrangement involves protonation of **3a** to form **12**. Nucleophilic attack by bromide ion on C-1 leads to **13**, which subsequently undergoes an intramolecular displacement of the bromide ion to give compound **10**.

Further evidence for the preferential rearrangement to N-3 can be seen from orotate methylation studies.[3,4] Orotic acid may be methylated by dimethyl sulfate in alkaline medium to 3-methylorotic acid, but treatment of 3-methylorotic acid with dimethyl sulfate afforded only meager yields of 1,3-dimethylorotic acid. 1-Methylorotic acid, however, may be methylated by the same process in good yields. From an examination of a molecular model it is quite evident that the 6-carboxy function of orotic acid creates a considerable amount of steric hindrance at the N-1 position of the pyrimidine ring, and this fact would explain the difficulty in

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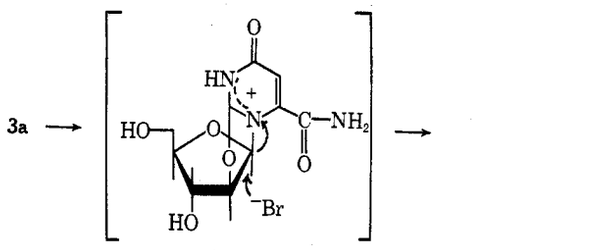
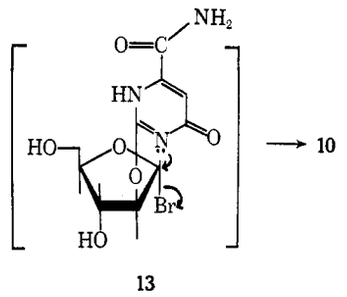
(10) M. W. Winkley and R. K. Robins, *ibid.*, **33**, 2822 (1968).





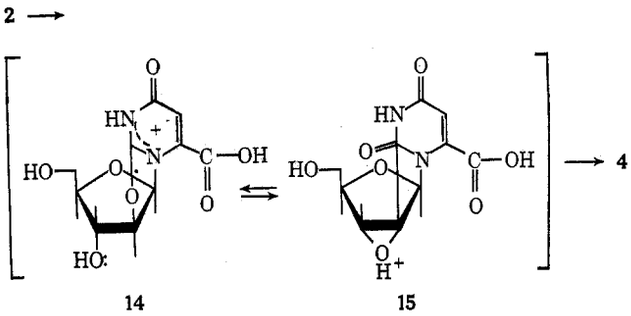
methylating orotic acid at N-1. Hence, by the same token, once glycosylic cleavage has been effected, intramolecular glycosylation will favor the formation of the more stable N-3 isomer (**10**).

Studies have shown[11] that arabinosyl nucleosides are of considerable biological interest. These conditions prompted the synthesis of 1-β-D-arabinofuranosylorotic acid from the easily accessible 2,2′-anhydroorotidine derivative. Aqueous acidic (2 *N* HCl) hydrolysis of **2** at room temperature afforded the lactone **4**.

The nmr spectrum of **4** showed an NH (δ 12.08) and H-5 as a broad singlet ($J_{5,NH}$ = 1.2 Hz) which becomes a sharp singlet after *deuteration*. It has been observed[12] that an NH, H-5 coupling in DMSO-d_6 is characteristic of many pyrimidine nucleosides, of which the orotidines are an example.

Titration of **4** with dilute potassium hydroxide readily furnished the potassium salt **7**. Potassium 1-β-D-arabinofuranosylorotate (**7**) is unstable for extended periods of time in solution at room temperature and is spontaneously converted to a corresponding dihydro derivative **8**; it is stable, however, at 0° in the dry state as the potassium salt. Attempts made to convert salt **7** to the corresponding carboxylic acid led only to reconversion to the original lactone (**4**).

It is suggested that the reaction of aqueous hydrochloric acid with the 2,2′-anhydronucleoside **2** involves as a first step hydrolysis of the ester **2** to the acid **5a** and



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(12) (a) R. S. Klein, J. Wempen, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, **35**, 2330 (1970). (b) Nmr spectra of orotidine and orotidine ethyl ester, kindly provided by Dr. B. A. Otter, showed $J_{HN,H-5}$ = 2 Hz.

protonation of the pyrimidine ring to give intermediate **14**.

Intramolecular attack by the 3′-OH on C-2′ leads to the formation of a protonated "down" 2′,3′-epoxide (**15**) which can revert to **2**. Subsequent attack by the 6-carboxylic acid oxygen on C-2′ of the epoxide **15** gives lactone **4**, which upon base hydrolysis gives arabinosylorotate (**7**).

The stability of compounds **3** and **10** to acidic conditions can be explained by the fact that although the "down" epoxides can be formed, they can only be converted back to the 2,2′-anhydro starting materials, since the amide linkage is stable to acidic conditions. This acid stability of **3** and **10** rules out the possible formation of **4** by acidic hydrolysis of the anhydro linkage followed by transesterification with the C-2′ hydroxyl moiety.

In 1961 Yung and Fox[13] suggested the formation of a similar "down" protonated epoxide to account for the formation of arabinosyluracil when 2,3′-anhydroxylosyluracil was heated under reflux temperature in dilute hydrochloric acid.

A similar attempt to open lactone **4** using methanolic ammonia afforded the dihydro carboxamide (**9**). The structural assignment was based, again, on the disappearance of the H-5 vinylic proton at δ 6.42, which was accompanied by the appearance of a quartet centered at δ 2.85 (2 H, $J_{5a,5b}$ = 18.5 Hz), which exchanged upon the addition of D_2O and NaOD, and which were assigned to the 5-methylene moiety ($-CH_2$). Furthermore, loss of uv absorption at wavelengths higher than 250 nm is consistent with the dihydro structure proposed.

The combined nature of the arabinosyl 2′-OH and the pyrimidine 6-carboxyl group in close proximity have led to unusual interactions between these groups. It is interesting to point out that ring opening of the 2,2′-anhydro linkage under *acidic* conditions was accomplished only when substituents at position 6 were the ethyl ester or the free carboxylic acid group. When similar conditions were used for the hydrolysis of compound **3** and **10** (carboxamido derivatives), only the starting materials were isolated after 5 days. These observations can probably be explained by the fact that ethanol and water are better leaving groups than ammonia.

Experimental Section

General Procedure.—Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter with a 1-dm path length. Ultraviolet spectra were recorded on a Cary Model 15 spectrometer, ir spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets), and nuclear magnetic resonance spectra with a Hitachi R20a spectrometer using DMSO-d_6 as a solvent and sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Values given for coupling constants (Hz) and chemical shifts (δ) are first order. Microanalyses were performed by M-H-W-Laboratories, Garden City, Mich., and by Galbraith Laboratories, Inc., Knoxville, Tenn. Evaporations were carried out under reduced pressure with bath temperatures below 40°.

2,2′-Anhydroorotidine Ethyl Ester (**2**).—Diethyl acetylenedicarboxylate (12.1 g, 0.71 mol) was added to a well-stirred suspension of 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline[6] (**1**, 12.0 g, 0.69 mol) in DMAc (30 ml) and the resulting mixture was stirred overnight at room temperature. The greenish suspension

(13) N. C. Yung and J. J. Fox, *J. Amer. Chem. Soc.*, **83**, 3060 (1961).

was diluted to about 40 ml with chloroform and allowed to stand for 1 hr at 4°. Filtration of the mixture afforded white crystals, mp 234–236° (11 g, 53%). A portion was recrystallized from aqueous methanol (with a trace of ethyl acetate) to furnish colorless needles: mp 235–236°; $[\alpha]^{25}D$ −194.8° (*c* 1.0, DMF); λ_{max}^{MeOH} 276 nm (ε 6000); $\lambda_{max}^{pH\ 1}$ 275 nm (ε 7000); $\lambda_{max}^{pH\ 11}$ 264 nm (ε 7200); nmr δ 6.85 (1, d, H-1′, $J_{1',2'}$ = 6.0 Hz), 6.48 (1, s, H-5), 5.92 (1, d, 3′-OH, $J_{3'\text{-OH}}$ = 4.2 Hz), 5.25 (1, d, H-2′), 4.97 (1, t, 5′-OH, $J_{5'\text{-OH}}$ = 4.5 Hz), 4.6–4.06 (4, m, H-3′, H-4, CH_2), 3.15–3.45 (2, m, H-5′, H-5″), 1.28 (3, t, CH_3).

Anal. Calcd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39. Found: C, 48.25; H, 4.90; N, 9.25.

2,2′-Anhydro-1-β-D-arabinofuranosyluracil-6-carboxamide (3a).—Methanol saturated with ammonia at 0° (200 ml) was added to compound **2** (5 g, 0.17 mol) and the suspension was stirred at 0° until complete solution was achieved. The solvent and excess ammonia were evaporated *in vacuo* and 50 ml of methanol was added to the resulting residue to afford white, crystalline **3** (4 g, 89%) mp 236° dec (softens at >221°). Recrystallization of a small portion from methanol afforded colorless crystals: mp 249° dec (softens at >225°); $[\alpha]^{22}D$ −280.0° (*c* 1.0, DMF); λ_{max}^{MeOH} 263 nm (ε 5900), $\lambda_{max}^{pH\ 11,1}$ 263 nm (ε 6300); nmr δ 8.39 and 8.01 (2, s, NH_2), 6.85 (1, d, H-1′, $J_{1',2'}$ = 6 Hz), 6.28 (1, s, H-5), 5.88 (1, d, 3′-OH, $J_{3'\text{-OH}}$ = 5 Hz), 5.22 (1, d, H-2′), 4.87 (1, t, 5′-OH, $J_{5'\text{-OH}}$ = 5 Hz), 4.42 (1, broad d, H-3′), 4.05 (1, broad s, H-4′), 3.25 (2, broad s, H-5′).

Anal. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.61; H, 4.12; N, 15.61. Found: C, 44.64; H, 4.09; N, 15.55.

2,2′-Anhydro-1-β-D-arabinofuranosyl-6-morpholinocarbonyluracil (3b).—Compound **2** (0.5 g, 1.67 mmol) was suspended in absolute ethanol (15 ml), and morpholine (5 ml) was added. The resulting mixture was heated at reflux for 3–4 hr and then allowed to stand, at room temperature, for several days; progress of the reaction was followed by tlc on silica gel GF_{254} using 1-butanol–ethanol–water (2:1:1) as solvent system. Evaporation of the solvent and excess morpholine gave a syrup which crystallized from methanol containing a few drops of 2-propanol. Recrystallization from methanol afforded white crystals of **13** (0.5 g, 88%): mp 218–220°; $[\alpha]^{22}D$ −167.6° (*c* 1.0, DMF); $\lambda_{max}^{H_2O}$ 256 nm (ε 8700), $\lambda_{max}^{pH\ 1}$ 256 nm (ε 9200), $\lambda_{max}^{pH\ 11}$ 256 nm (ε 8900); nmr δ 6.36 (1, d, H-1′, $J_{1',2'}$ = 6 Hz), 6.02 (1, s, H-5), 5.99 (1, d, 3′-OH), 5.27 (1, d, H-2′), 5.0 (1, t, 5′-OH), 4.43 (1, broad d, H-3′), 4.16 (1, broad peak, H-4′), 3.8–3.2 (10, m, H-5′, morpholino protons).

Anal. Calcd for $C_{14}H_{17}N_3O_7$: C, 49.55; H, 5.05; N, 12.39. Found: C, 49.40; H, 4.92; N, 12.43.

1-β-D-Arabinofuranosylorotic Acid 2′,6-Lactone (4). Method A.—Compound **2** (1.00 g, 0.003 mol) was suspended in freshly prepared 2 *N* hydrochloric acid and the mixture was stirred at room temperature for 4 days. Evaporation of the solvent and excess acid to dryness afforded a white foam. Two successive additions and evaporations of ethanol gave a syrup which was dissolved in methanol. Crystallization occurred upon standing at 4°; filtration and subsequent washing with cold methanol afforded white crystals (0.43 g, 53%). An analytical sample was obtained by recrystallization (twice) from methanol: mp 249–250.5°; $[\alpha]^{22}D$ −25.0° (*c* 1.0, DMF); λ_{max}^{MeOH} 290 nm (ε 7600), $\lambda_{max}^{pH\ 1}$ 290 nm (ε 7800), $\lambda_{max}^{pH\ 11}$ 270 nm (ε 5400); nmr δ 12.08 (1, broad s, NH), 6.42 [1, broad s (becomes a sharp s after deuteration), H-5], ~5.97 [2, H-1′ (d) overlapped by 3′-OH at 5.95, $J_{1',2'}$ = 2.2 Hz], ~4.95 (2, H-2′ d at 4.97 overlapped by 5′-OH at 4.95), 4.1–4.3 (1, m, H-3′), 3.7–4.0 (1, m, H-4′), 3.47 (2, broad t, H-5′, H-5″).

Anal. Calcd for $C_{10}H_{10}O_7N_2$: C, 44.45; H, 3.73; N, 10.37. Found: C, 44.48; H, 3.92; N, 10.48.

Method B.—Compound **3a** (0.6 g, 2 mmol) was suspended in 15 ml of 0.5 *M* potassium hydroxide, and the resulting solution was stirred for 1 hr at room temperature. Treatment of the reaction mixture with Dowex 50 (H^+) (~1 g), filtration, and subsequent evaporation of the resulting filtrate afforded a foam. Water was removed by azeotropic distillation with absolute ethanol. Crystallization was effected by dissolving the crude material in ethanol and storing the solution at ~4° overnight. A small portion of this material was recrystallized from methanol to afford white crystals whose physical properties were identical with those of an authentic sample of compound **4**.

2,2′-Anhydroorotidine (5a).—Compound **2** (2.0 g, 0.006 mol) was dissolved in 25 ml of 0.5 *M* potassium hydroxide. After stirring for 5 min, the solution was treated with Dowex 50 (H^+). The solvent was evaporated and the gel was broken by azeotropic distillation with absolute ethanol. Treatment of the white residue with methanol effected crystallization (1.5 g, 81%). A pure sample was prepared by recrystallization from methanol and subsequent washing with cold methanol (with a few drops of water): mp 235–239°; $[\alpha]^{22}D$ −178.6° (*c* 1.0, DMF); $\lambda_{max}^{H_2O}$ 267 nm (ε 6500), $\lambda_{max}^{pH\ 1}$ 273 nm (ε 6800), $\lambda_{max}^{pH\ 11}$ 265 nm (ε 6800); nmr δ 6.89 (4, d, H-1′ at 6.89 overlapped by 3′-OH, 5′-OH, and CO_2H at ~6.8, $J_{1,2'}$ = 6 Hz), 6.40 (1, s, H-5), 5.22 (1, d, H-2′), 4.40 (1, broad s, H-3′), 4.08 (1, broad t, H-4′), 3.28 (2, d, H-5′, H-5′).

Anal. Calcd for $C_{10}H_{10}N_2O_7$: C, 44.45; H, 3.73; N, 10.37. Found: C, 44.20; H, 3.43; N, 10.18.

2,2′-Anhydroorotidine Potassium Salt (5b).—Compound **2** (1.0 g, 0.003 mol) was suspended in 0.1 *M* potassium hydroxide (30 ml, 0.003 mol) and the mixture was stirred at room temperature until the reaction was complete by tlc (about 30 min). Methanol (25 ml) was added to the warm solution and crystallization began immediately, providing 0.93 g of product (90%): mp 264° dec; $[\alpha]^{25}D$ −154.2° (*c* 1.0, H_2O); $\lambda_{max}^{pH\ 1}$ 273 nm (ε 6200), λ_{max}^{MeOH} 268 nm (ε 5900), $\lambda_{max}^{pH\ 11}$ 270 nm (ε 6200); nmr (D_2O) δ 7.08 (1, d, H-1′, $J_{1',2'}$ = 6.2 Hz), 6.47 (1, s, H-5), 5.45 (1, d, H-2′), 4.8–4.3 (2, m, H-3′, H-4′), 3.57 (2, d, H-5′, H-5″).

Anal. Calcd for $C_{10}H_9O_7N_2K$: C, 38.96; H, 2.94; N, 9.09. Found: C, 38.70; H, 3.00; N, 8.87.

2-Amino-2′,6-anhydro-1-β-D-arabinofuranosyl-5,6-dihydrouracil-6-carboxamide (6).—Compound **2** (1.0 g, 0.003 mol) was suspended in 100 ml of methanol saturated with ammonia (0°) in a pressurized bottle and the solution was allowed to stand for 3 days (RT). Evaporation of the solvent and excess ammonia gave a syrup. This residue was crystallized from methanol, with a few drops of 2-propanol, to furnish colorless crystals (0.75 g, 82%). An analytical sample was obtained by recrystallization of a small portion of the material from water: mp 192–194°; $[\alpha]^{22}D$ − 68.0° (*c* 1.0, DMF); λ_{max}^{MeOH} 234 nm (ε 12,600), $\lambda_{max}^{pH\ 11}$ 232 nm (ε 12,900), $\lambda_{max}^{pH\ 1}$ end absorption; nmr δ 8.47 (2, broad s, NH_2), 7.17 and 6.8 (2, broad s, $CONH_2$), 5.77 (1, d, H-1′, $J_{1',2'}$ = 4.5 Hz), 5.6 (1, d, 3′-OH, $J_{3'\text{-OH}}$ = 5 Hz), 4.9–5.2 (1, m, 5′-OH), 4.30–4.6 (1, m, H-2′), 3.8–4.2 (1, m, H-3′), 3.60 (3, broad s, H-4′, H-5′, H-5″), 3.39 (2, s, H_2O), 2.80 (2, s, H-5).

Anal. Calcd for $C_{10}H_{14}O_8N_4 \cdot H_2O$: C, 39.47; H, 5.30; N, 18.42. Found: C, 39.44; H, 5.50; N, 18.52.

Potassium 1-β-D-Arabinofuranosylorotate (7).—Compound **4** (0.15 g, 0.5 mmol), suspended in 17 ml of water, was titrated with potassium hydroxide (2 ml of 0.5 *M* solution). The solution was evaporated to dryness and the white residue was crystallized by adding a small amount (~15 ml) of methanol. Filtration and washing (twice with cold methanol with a few drops of water) afforded, after freeze-drying, white crystals of **7** (0.12 g, 74%): $[\alpha]^{22}D$ +26.9° (*c* 1.0, H_2O); $\lambda_{max}^{\sim pH\ 4.5}$ 268 nm (ε 8100), $\lambda_{max}^{pH\ 1}$ 285 nm (ε 6400), $\lambda_{max}^{pH\ 11}$ 269 nm (ε 4700); nmr δ 11.25 (1, broad s, N_3H), 5.98 (1, d, H-1′, $J_{1',2'}$ = 6 Hz), 5.35 (2, H-5, broad singlet at 5.35 overlapped by an OH), ~4 (2, H-2′, H-3′), 3.62 (3, H-4′, overlapped by the H-5′).

Anal. Calcd for $C_{10}H_{11}O_8N_2K \cdot 1/2H_2O$: C, 35.81; H, 3.58; N, 8.35. Found: C, 35.86; H, 3.50; N, 8.39.

Titration of **4** (0.15 g) with potassium hydroxide (2 ml of 0.5 *M* solution) followed by treatment with Dowex 50 (H^+) gave 0.082 g of white crystals. Recrystallization of this material from methanol afforded a pure sample, the physical properties of which were identical with those of the starting material **4**.

Potassium 2′,6-Anhydro-1-β-D-arabinofuranosyl-5,6-dihydroorotate (8). Method A.—A small portion of **7** was recrystallized from methanol containing a small amount of water to give colorless **8** which did not show any uv absorption above 240 nm: mp 218° (sinters), 222° dec; $[\alpha]^{22}D$ +29.6° (*c* 1.0, H_2O); nmr (with two drops of trifluoroacetic acid to effect solution) δ 10.72 (1, broad s, N^3H), 8.75 (all of the OH's overlapped by trifluoroacetic acid peak), 5.97 (1, d, H-1′, $J_{1',2'}$ = 4.7 Hz), 4.73 (1, d of d, H-2′), 4.15 (1, m, H-3′), 3.5 (3, H-5′ singlet at 3.55 overlapped by H-4′ at 3.68), 2.88 (2, q, H-5, $J_{5a,5b}$ = 17 Hz).

Anal. Calcd for $C_{10}H_{11}O_8N_2K$: C, 36.81; H, 3.30; N, 8.50. Found: C, 37.10; H, 3.50; N, 8.69.

Method B.—Crystalline **7**, when allowed to stand at room temperature, gradually lost uv absorption above 240 nm (complete loss of absorption was observed after 5 days). Nmr, ir, and uv spectra of this material are superimposable upon those of the product by method A.

Method C.—Compound **3a** (1.3 g, 0.004 mol) was suspended in 25 ml of 0.5 *M* potassium hydroxide and the resulting solution was stirred for 6 hr. The solution was neutralized by stirring

with Dowex 50 (H^+) (~1.5 g). Filtration of the resin and subsequent evaporation of the filtrate to dryness gave a white solid which crystallized from methanol to give **8** (0.9 g, 78%), mp 219°. An analytical sample was obtained by recrystallization from methanol to give white crystals, mp 222° dec. Physical properties of this material were identical with those of an authentic sample prepared previously.

Concentration of the mother liquor to about 10 ml afforded white crystals (0.20 g). Nmr, ir, and uv spectra, as well as the melting point of this material, are identical with those of lactone **4**.

Method D.—Compound **2** (0.3 g, 1 mmol) was dissolved in 10 ml of 0.5 *M* potassium hydroxide and the resulting solution was stirred at room temperature until the absorption above 240 nm was completely gone. Dilution with water (~50 ml), treatment with Dowex 50 (H^+), and subsequent evaporation of the solvent afforded a gel. This residue upon azeotropic distillation with absolute ethanol gave a syrup which crystallized from methanol after standing at ~4°. This material was shown (by ir, uv, nmr, and melting point) to be identical with compound **8**.

2′,6-Anhydro-1-β-D-arabinofuranosyl-5,6-dihydrouracil-6-carboxamide (**9**).—Compound **4** (0.2 g, 0.69 mmol) was stirred with 50 ml of methanol saturated with ammonia (0°). As soon as solution was effected, the solvent and excess ammonia were immediately evaporated. Addition of a small amount of methanol (~10 ml) furnished colorless crystals (0.19 g, 95%). Recrystallization from methanol (with a few drops of water) afforded pure **9**: mp 172–174° dec; $[\alpha]^{22}D$ +47.9° (*c* 1.0, DMF); nmr δ 10.35 (1, broad s, N^3H), 7.52 (2, broad s, CNH_2), 5.88 (1, d, H-1′, $J_{1',2'}$ = 4.4 Hz), 5.60 (1, broad peak, 3′-OH), 5.06 (1, broad s, 5′-OH), 4.65 (1, d, H-2′), 4.20 (1, broad peak, H-3′), ~3.64 (3, H-4′ at ~3.65 overlapped by the 2 H-5′ at ~3.64), 2.85 (2, q, H-5, $J_{5a,5b}$ = 18.5 Hz).

Anal. Calcd for $C_{10}H_{13}O_7N_3$: C, 41.81; H, 4.56; N, 14.63. Found: C, 41.67; H, 4.54; N, 14.54.

2,2′-Anhydro-3-β-D-arabinofuranosyluracil-6-carboxamide (**10**).—Compound **3a** (0.6 g, 0.002 mol) was suspended in 35 ml of trifluoroacetic acid saturated with hydrobromic acid (0°) and the resulting mixture was allowed to react at room temperature in a pressurized bottle overnight. Evaporation of the solvent and excess hydrobromic acid gave a foam which gradually crystallized from ethanol upon standing at ~4°. Filtration of the dark suspension afforded light gray crystals (400 mg, 67%); a second crop (~50 mg) was obtained from the mother liquor. Recrystallization from methanol with decolorization afforded white crystals (300 mg, 50%): mp 170° (sinters), 210° dec; $[\alpha]^{22}D$ −168.0° (*c* 0.4, DMF); $\lambda_{max}^{MeOH,\ pH\ 1\ and\ 11}$ 296 nm (ε 5000); nmr δ 7.85 (2, broad d, $CONH_2$), 6.58 (1, s, H-5), 6.45 (1, d, H-1′, $J_{1',2'}$ = 6 Hz), 5.91 (1, d, 3′-OH, $J_{3'\text{-OH}}$ = 5 Hz), 5.26 (1, d, H-2′), 4.95 (1, t, 5′-OH, $J_{5'\text{-OH}}$ = 5 Hz), ~4.45 (1, broad s, H-3′), ~4.14 (1, broad s, H-4′), 3.35 (2, broad t, H-5′, H-5″).

Anal. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.61; H, 4.12; N, 15.61. Found: C, 44.45; H, 4.21; N, 15.35.

3-β-D-Arabinofuranosyluracil-6-carboxamide (**11**).—Compound **10** (0.5 g, 0.002 mol) was dissolved in 10 ml of 0.5 *M* potassium hydroxide and progress of the reaction at ambient temperature was followed by tlc on silica gel GF-254 using the solvent system ethyl acetate–1-propanol–water (4:1:2, upper phase). After about 4 hr the solution was treated with Dowex 50 (H^+) (~1 g). Evaporation of the solvent to dryness afforded a foam which crystallized upon addition of methanol (0.4 g, 80%). A small portion was recrystallized from ethanol to give colorless crystals: mp 172–174°; $[\alpha]^{22}D$ −59.5° (*c* 1.0, DMF); λ_{max}^{MeOH} 282 nm (ε 5500), $\lambda_{max}^{pH\ 1}$ 282 nm (ε 5900), $\lambda_{max}^{pH\ 11}$ 321 nm (ε 6400); nmr δ 10.77 (1, broad s, N^3H), 8.31 and 8.03 (2, broad s, $CONH_2$), 6.48 (1, d, H-1′, $J_{1',2'}$ = 7 Hz), 5.45–5.05 (2, m, 5′-OH), 4.00–4.50 (3, m, H-2′, H-3′, H-4′), 3.65 (2, broad s, H-5′, H-5″).

Anal. Calcd for $C_{10}H_{13}O_7N_3$: C, 41.81; H, 4.56; N, 14.64. Found: C, 42.02; H, 4.62; N, 14.42.

Registry No.—**2**, 33780-80-2; **3a**, 33780-81-3; **3b**, 33780-82-4; **4**, 33886-19-0; **5a**, 33886-20-3; **5b**, 33872-65-0; **6**, 33886-21-4; **7**, 33780-83-5; **8**, 33886-22-5; **9**, 33886-23-6; **10**, 33780-84-6; **11**, 33886-24-7.

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Mesoionic Compounds. XVI. 1,4-Dipolar Type Cycloaddition Reactions Utilizing Pyrimidinium Betaines[1]

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N,N′-Disubstituted amidines and carbon suboxide gave in excellent yield *anhydro*-4-hydroxy-6-oxo-1,2,3-trisubstituted pyrimidinium hydroxides which underwent 1,4-dipolar type cycloadditions with dimethyl acetylenedicarboxylate. In the case of *anhydro*-1,3-diphenyl-4-hydroxy-2-methyl-6-oxopyrimidinium hydroxide, the primary adduct, dimethyl 2,6-diaza-3,5-dioxo-2,6-diphenyl-1-methylbicyclo[2.2.2]oct-7-ene-7,8-dicarboxylate, was isolated; on heating, it lost phenyl isocyanate, forming dimethyl 6-methyl-2-oxo-1-phenylpyridine-4,5-dicarboxylate.

1,3-Dipolar cycloaddition reactions utilizing mesoionic ring systems as the source of the 1,3-dipole are well documented in the literature.[2] Both five-membered[2] and six-membered[3] ring systems have been utilized in these reactions. In most instances during the reaction the primary cycloadduct readily lost species such as carbon dioxide,[4] carbonyl sulfide,[5] isocyanates,[6,7] or sulfur[7] leading to substituted heterocycles often difficult to obtain by alternative routes. In other cases the primary cycloadduct was quite stable but, by standard procedures, could be converted into interesting ring systems.[3b,8]

In a recent communication[9] we showed how *anhydro*-

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(2) Summaries of this general area may be found in M. Ohta and H. Kato, "Non Benzenoid Aromatics," J. P. Snyder, Ed., Academic Press, New York, N. Y., 1969, Chapter 4; R. Huisgen, "Aromaticity," Chemical Society Special Publication No. 21, p 51.

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