and washings were concentrated to dryness. A portion of the residue was fractionated by thin layer chromatography (chloroform–methanol, 5:1) to give crystalline material identical (uv, ir, melting point) with authentic **2**. A faster moving component was identified as **8** from its uv spectrum and chromatographic properties.

From 6.—A solution of **6** (10 mg) in 10 ml of 1 *N* hydrochloric acid at 48° was monitored (at pH 12) in the ultraviolet. The initial absorption at 272 mμ shifted over a 50-min period to give a peak at 252 mμ having a final ε value corresponding to a quantitative yield of **2**. Concentration of the reaction mixture and isolation of the product by thin layer chromatography (chloroform–methanol, 5:1) afforded a single component which gave uv and ir spectra identical with those of authentic **2**.

Registry No.—**4**, 24704-26-5; **5**, 24704-27-6; **6**, 24704-28-7; **7**, 24710-89-2; **8**, 24710-90-5; **9**, 24704-29-8; **10**, 24710-91-6; **12**, 24710-92-7; **14**, 24710-93-8; **15**, 24710-94-9.

Acknowledgment.—The authors are grateful to Marvin J. Olsen of this institute for recording the nmr spectra.

Nucleosides. LXVII. The Chemistry of 4-Methyl-2-pyrimidinone Ribonucleosides[1]

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The synthesis of 4-methyl-2-pyrimidinone and 4,5-dimethyl-2-pyrimidinone ribonucleosides **3a** and **3b** is described. The site of glycosylation is determined by two independent routes. Nitrosation of the 4-methyl group converts **3a** and **3b** into their corresponding oxime derivatives (**7a** and **7b**) which, by treatment with acetic anhydride, afford the corresponding nitriles (**8a** and **8b**). The nitrile groups are easily displaced by a variety of nucleophiles. Reduction of oxime **7a** followed by acetylation gives the N-acetylated aminomethyl derivative (**10**) which undergoes facile air oxidation to the 4-carboxymethyl derivative (**11**). In model studies, the structure of **11** is established by an unambiguous synthesis of 1-methyl-2-oxo-4-pyrimidinecarboxylic acid methyl ester (**16**) from 3-methylorotic acid. 1-Methyl-2-oxo-4-pyrimidinecarboxaldehyde oxime (**14**) is also shown to undergo reduction, acetylation, and autoxidation to **16**.

As part of a program directed toward the syntheses of nucleosides of potential biological interest, we have investigated the chemistry of the hitherto unknown ribofuranosyl derivatives of 4-methyl-2-pyrimidinones. Such nucleosides containing a basic aglycon may be viewed as isosteres of cytidine and, since they also contain a potential enamine system, may undergo reactions at the allylic position with electrophilic reagents leading to new types of nucleoside analogs.

Condensation of 4-methyl-2-pyrimidinone (**1a**) or its 5-methyl derivative (**1b**) with tri-*O*-benzoyl-D-ribofuranosyl chloride by the mercuric cyanide–nitromethane procedure[2] gave the blocked nucleosides **2** which were isolated as their hydrochloride salts in good yields (Scheme I). After debenzoylation of **2**, the unblocked nucleosides **3a** and **3b** were obtained as the crystalline hydrochloride salts.

The site of ribosylation (N-1) was established for nucleosides **3** as follows. Condensation of 6-methyluracil (**4**) with the halogenose by the generalized mercuric cyanide–nitromethane procedure[2b] afforded crystalline 3-(tri-*O*-benzoyl-β-D-ribofuranosyl)-6-methyluracil (**5**) in 70% yield, which exhibited an nmr spectrum with values identical with those reported for this product (as a syrup) by Winkley and Robins,[3] and by Prystaš and Šorm.[4] Debenzoylation of **5** followed by acetylation afforded the known[3] crystalline tri-*O*-acetate. Treatment of tri-*O*-benzoate **5** with phosphorus pentasulfide in pyridine, a widely used method for thiation of nucleosides,[5] was accompanied by extensive decomposition. When dioxane instead of pyridine was used as solvent in this reaction, a facile conversion of **5** to 4-thione **6** in above 70% yield occurred.[6] Assignment of the thioxo group to the 4 position of **6** rests on analogy with the thiation of 3-methyluracil (which gave the 4-thione exclusively)[7] and from subsequent reactions of **6**. Reductive desulfurization of **6** with various preparations of Raney nickel under a variety of conditions was complicated by excessive ring reduction. Partial reduction of **6** under mild conditions with activated Raney nickel prepared according to Brown[8] allowed at least the isolation by column chromatography of **2a** from the reaction mixture. Compound **2a**, thus obtained, was identical with that prepared by direct condensation from **1** and afforded the same crystalline picrate. These results establish unambiguously both the site of glycosylation in **2a** at N-1 and the 4-thioxo structure for **6**. Since nucleosides **3** derived from **2** exhibited very similar ultraviolet absorption spectral properties, both **3a** and **3b** are 1-substituted ribosyl derivatives.

Compound **1a** has been shown[9] to undergo nitrosa-

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748).

(2) (a) N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965); (b) K. A. Watanabe and J. J. Fox, *J. Heterocycl. Chem.*, **6**, 109 (1969).

(3) M. W. Winkley and R. J. Robins, *J. Org. Chem.*, **33**, 2822 (1968).

(4) M. Prystaš and F. Šorm, *Collect. Czech. Chem. Commun.*, **34**, 331, 2316 (1969).

(5) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Amer. Chem. Soc.*, **80**, 1669 (1958).

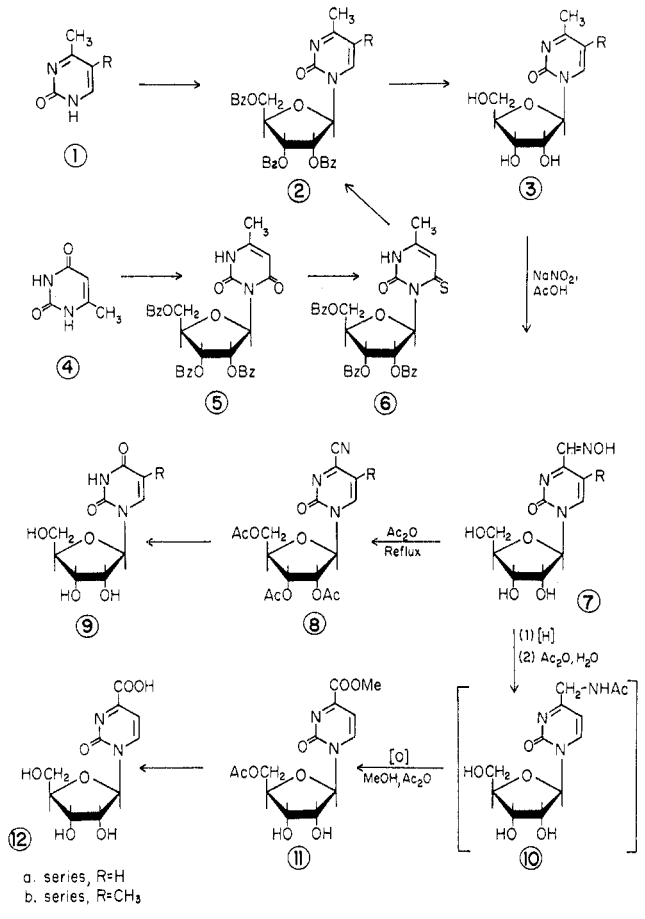
(6) The use of phosphorus pentasulfide in dioxane as a thiating reagent combination has since been applied in our laboratory (E. A. Falco, B. A. Otter, and J. J. Fox, manuscript in preparation) to other nucleosides which are thiated with difficulty in pyridine as solvent. This reagent combination (P_2S_5–dioxane) is described by L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley & Sons, Inc., New York, N. Y., 1967, p 333, and should have wide application in the nucleoside area.

(7) T. Ueda and J. J. Fox, *J. Amer. Chem. Soc.*, **85**, 4024 (1963); K. A. Watanabe, H. A. Friedman, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, **31**, 2942 (1966).

(8) D. J. Brown, *J. Soc. Chem. Ind., London*, **69**, 353 (1950).

(9) G. D. Daves, Jr., D. E. O'Brien, L. R. Lewis, and C. C. Cheng, *J. Heterocycl. Chem.*, **1**, 130 (1964).

SCHEME I



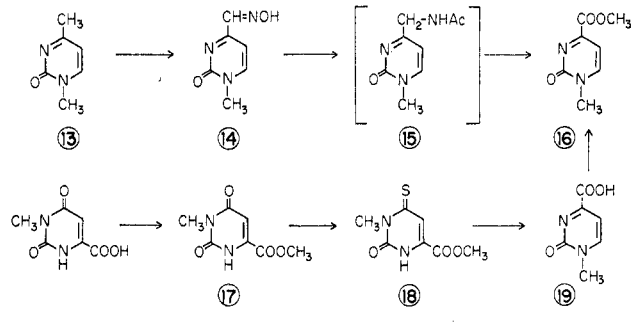
tion on the methyl group. Similar treatment of nucleosides **3** gave crystalline aldoximes **7** in good yields. These were readily converted to the tri-*O*-acetylated carbonitriles (**8**) by refluxing with acetic anhydride. Treatment of **8** with hydrochloric acid in methanol at ambient temperature resulted in displacement of the cyano function to afford crystalline uridine (**9a**) and 5-methyluridine (**9b**), respectively.[10] The exclusive formation of nucleosides **9** from **8** is best explained in this case[11] by initial protonation at N-3 followed by nucleophilic attack by water at C-4 to afford a cyanohydrin-like intermediate which eliminates hydrogen cyanide. The conversion of nucleosides **8** into **9** is also consistent with the 1-β-D-ribofuranosyl structures assigned to nucleosides **2** → **8** and suggests that the cyano derivatives **8** may serve as versatile intermediates for the introduction of other functional groups into the 4 position, thus leading to new nucleoside analogs.[11]

Attempts to tritylate selectively the 5′-hydroxyl function of **3a** resulted in the isolation by column chromatography of the expected 5′-tritylate along with a dityrityl derivative. Nmr spectroscopy (loss of methyl resonance) revealed that the second trityl function had affixed to the methyl group thus reflecting the susceptibility of this group to electrophilic substitution. This susceptibility is supported by the nmr spectrum of **3a** in DMSO-d_6 (methyl resonance at δ 2.28) which shows fairly rapid loss of the methyl resonance upon addition of D_2O. This exchange is catalyzed by acid. A similar situation obtains with the hydrochloride salt in which the protons of the 4-methyl substituent undergo exchange by deuterium. A similar type of exchange has been observed with **1a**.[12]

Attempts to obtain the aminomethyl derivative from **7a** by catalytic hydrogenation were unsuccessful owing to the propensity of the pyrimidine ring to reduction. If the reaction was stopped after the theoretical uptake of hydrogen and the aqueous solution treated with acetic anhydride, the problem of overreduction was partially alleviated. However, the reaction still developed some color and isolation of a product was unsuccessful. It was noted that the uv spectrum of the reaction shifted from that for the oxime ($\lambda_{max}^{pH\ 7}$ 330 mμ) to 303 mμ akin to the $\lambda_{max}^{pH\ 7}$ for **3a** which indicated that compound **10** had formed. The N-acetylation in methanol was monitored spectrophotometrically. Unexpectedly, a further shift on standing of λ_{max} from 303 to 335 mμ (pH 7.0) was observed. This second reaction was accelerated by bubbling oxygen in the mixture thus indicating an autoxidation process.[13] Acetamide was obtained as a by-product of this reaction. The major product, obtained by column chromatography, was shown to have structure **11** by nmr and by comparison of its uv spectrum with that for model compound **16** (see below).

In order to confirm the structure of nucleoside **11**, model studies were carried out with 1,4-dimethyl-2-pyrimidinone (**13**, Scheme II) which was converted into

SCHEME II



the oxime **14** with nitrous acid. When the same reduction and acetylation procedures were applied to this oxime, a crystalline compound identified as the methyl ester of 1-methyl-2-pyrimidinone-4-carboxylic acid (**16**) was obtained. Structure **16** was assigned on the basis of nmr and ir spectra and elemental analysis. In order to confirm its structure, compound **16** was also prepared by an unambiguous route from the methyl ester (**17**) of 3-methylorotic acid.[14] This ester was thiated to **18** then desulfurized with Raney nickel to **19** and esterified to **16** with diazomethane.

The ultraviolet characteristics of **16** were similar to those exhibited by nucleoside **11** obtained by autoxidation of **10** thus establishing the structure of the aglycon moiety of **11**. This product **11** was shown to be acetylated at position 5′ on the basis of its nmr spectrum and

(10) The conversion of 2-chloro-4-cyanopyrimidine to uracil by vigorous conditions had been demonstrated.[9]

(11) For example, reaction of nucleosides **9** with alcoholic ammonia affords cytidine and 5-methylcytidine. This reaction probably proceeds by direct displacement of the cyano group by the stronger nucleophile, ammonia.

(12) T. J. Batterham, D. J. Brown, and M. N. Paddon-Row, *J. Chem. Soc., B*, 171 (1967).

(13) When the reaction was performed under nitrogen, the spectral shift to 335 mμ did not occur.

(14) J. J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, **28**, 295 (1957), and references therein.

by its positive reaction to a periodate–benzidine spray on tlc. Deesterification of **11** afforded the 4-carboxylic acid nucleoside (**12**) which was isolated as the crystalline cyclohexylamine salt.

The exocyclic methylene of **10** may be viewed as the β carbon of a potential enamine–imine system

$$-N{=}C{-}C{-}NHAc \rightleftharpoons NH{-}C{=}C{-}NHAc$$

The autoxidation of the β carbon in such systems has been noted[15] with certain indoles.

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 tetramethylsilane as internal reference. Chemical shifts are reported in parts per million (δ) and signals are described as s (singlet), d (doublet), or m (complex multiplet). Values given for coupling constants are first order. Thin layer chromatography was performed on silica gel GF_{254} (Merck); spots were detected by uv absorbance or by spraying with 20% v/v sulfuric acid–ethanol and heating. Column chromatography was performed[16] on silica gel G under positive pressure. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich. The uv spectra of reactions monitored by changes in their absorbance were recorded on a Unicam SP 800 A spectrophotometer. The reported uv absorption spectral data were determined on a Cary Model 15 spectrophotometer; the apparent pK_a values were determined spectrophotometrically and are accurate to ± 0.05 pH unit unless otherwise indicated. All evaporations were carried out *in vacuo*.

4-Methyl-1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone Hydrochloride (2a).—A suspension of 2.44 g (0.0166 mol) of 4-methyl-2-pyrimidinone hydrochloride and 12.6 g (0.050 mol) of mercuric cyanide in 400 ml of nitromethane was refluxed and 100 ml distilled off. To the clear refluxing solution was added slowly a solution of 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride [prepared from 10.5 g (0.0200 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose]. The red solution was refluxed for 20 min and cooled to room temperature. Any insoluble material was removed by filtration and the filtrate was evaporated. The residual syrup was dissolved in 200 ml of chloroform and the solution was filtered. The filtrate was extracted with 200 ml of 30% aqueous potassium iodide and with 200 ml of water, and the organic layer was dried over sodium sulfate. After filtration the solvent was removed and the residue was redissolved in 75 ml of dry benzene. The solution was cooled in ice and dry hydrogen chloride passed while stirring. Trituration of the precipitated gum gave a white semicrystalline solid which was removed by filtration from the dark red solution and was washed with ether. The procedure afforded 6.6 g (67%) of the crude hydrochloride. The neutralized product in chloroform was chromatographically pure. A small sample gave a picrate, mp 162–164°.

4-Methyl-1-β-D-ribofuranosyl-2-pyrimidinone Hydrochloride (3a).—The crude solid obtained above was partitioned between 100 ml of saturated aqueous sodium bicarbonate and 100 ml of chloroform. The organic layer was again washed with an equal volume of the sodium bicarbonate solution and finally with water. The organic layer was dried (sodium sulfate) and concentrated to a syrup. The residue was dissolved in 125 ml of warm methanol and was treated with 5 ml of alcoholic sodium methoxide (100 mg of sodium) overnight at room temperature. The reaction mixture was neutralized by stirring with 2.5 ml of wet Dowex AG 50 (H^+) resin and was then evaporated to 50 ml, diluted with 15 ml of ether, and dry hydrogen chloride was bubbled through the cold stirred solution. The crystalline precipitate was collected and a second crop was obtained from the mother liquor by further cooling and dilution with ether to give a total of 2.3 g of **3a**. Recrystallization from methanol gave an analytically pure sample: mp 164° dec; uv $\lambda_{max}^{0.1\ N\ HCl}$ 309 mμ (ϵ 9850); λ_{min} 250 mμ (ϵ 850); $\lambda_{max}^{pH\ 7-12}$ 297 mμ (ϵ 6300), sh 215 (8490); λ_{min} 235 mμ (ϵ 550); $pK_a = 2.77$; $[\alpha]^{26}D$ +132° (*c* 1.2, water); nmr (DMSO-d_6) δ 9.15 (1, d, H-6), 6.91 (1, d, H-5), 5.72 (broad s, H-1'), 2.58 (3, s, CH_3), $J_{5,6} = 6.5$, $J_{1',2'} = 1$ Hz.

Anal. Calcd for $C_{10}H_{14}N_2O_5 \cdot HCl$: C, 43.09; H, 5.42; N, 10.05; Cl, 12.72. Found: C, 43.24; H, 5.38; N, 10.08; Cl, 12.82.

4,5-Dimethyl-1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone Hydrochloride (2b).—Condensation of 6.7 g (0.042 mol) of **1b** hydrochloride[17] with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride [prepared from 22.7 g (0.045 mol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose] in the presence of 30 g (0.12 mol) of mercuric cyanide with 1500 ml of nitromethane as solvent was carried out essentially as outlined in the synthesis of **1a** above. The yield of the hydrochloride salt of **2b** was 19.3 g (76%), mp 173–175° eff. A second crop, 0.8 g (3%), was obtained from the mother liquor.

4,5-Dimethyl-1-β-D-ribofuranosyl-2-pyrimidinone Hydrochloride (3b).—The blocked nucleoside hydrochloride **2b** (18.5 g, 0.0306 mol) was debenzoylated to **3b** by a procedure essentially similar to that used in the synthesis of **3a** (*vide supra*). The unblocked nucleoside obtained as a syrup (7.7 g) was converted to the hydrochloride salt. The crude salt was obtained as a purple gummy precipitate which was triturated repeatedly with ether until solidification occurred, affording an amorphous reddish purple product, 4.0 g (45%), mp 152–155° dec. A further 2.45 g of a lower melting product was obtained from the mother liquor. The first crop was recrystallized from a minimum amount of hot ethanol. Crystallization of product was slow, affording 3.2 g of pale violet platelets: mp 157–158° dec; uv $\lambda_{max}^{1N\ HCl}$ 322 mμ (ϵ 9680); λ_{min} 265 mμ (ϵ 2260); $\lambda_{max}^{pH\ 7-12}$ 307 mμ (ϵ 6260); λ_{min} 242 mμ (ϵ 3980); $pK_a = 2.97$; nmr (DMSO-d_6) δ 9.13 (1, s, H-6), 5.76 (broad s, H-1'), 2.59 (3, s, 4-CH_3), 2.13 (3, s, 5-CH_3); $[\alpha]^{26}D$ +96° (*c* 0.3, water).

Anal. Calcd for $C_{11}H_{16}N_2O_5 \cdot HCl$: C, 45.13; H, 5.85; N, 9.57; Cl, 12.11. Found: C, 45.15; H, 5.81; N, 9.61; Cl, 12.05.

It was found unnecessary to use the isolated **3b** hydrochloride for subsequent conversion to the oxime **7b**. For this purpose the unblocked crude syrup was utilized directly.

6-Methyl-4-thio-3-(tri-*O*-benzoyl-β-D-ribofuranosyl)uracil (6).—A suspension of 1.90 g (0.0150 mol) of 6-methyluracil and 5.1 g of mercuric cyanide in 500 ml of nitromethane was heated to reflux and 100 ml distilled off. To the clear mixture was added *dropwise* a solution of 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride (from 0.02 mol of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose) in 50 ml of nitromethane. The reaction mixture was heated for an additional 3 hr. After cooling and filtration from unreacted 6-methyluracil (300 mg), the solution was evaporated to a syrup, redissolved in 250 ml of chloroform, and filtered again from insoluble mercury salts. The chloroform layer was extracted with 250 ml of 30% aqueous potassium iodide and washed with 250 ml of water. The organic layer was dried over sodium sulfate and evaporated. The residue was chromatographed on 500 g of silica gel G (benzene–ethyl acetate 3:1) and 20-ml fractions were collected. Fractions containing the only nucleosidic product were collected and evaporated to give **5** as a brittle foam (6.0 g, 70%). It was crystallized from methanol to give 5.1 g of pure product: mp 164–167°; nmr ($CDCl_3$) δ 2.15 (3, s, CH_3), 4.50–4.90 (3, m, H-5' and H-4'), 5.55 (1, s, H-5), 6.00–6.40 (2, m, H-2' and H-3'), 6.70 (1, s, H-1'), 7.12–7.70, 7.82–8.20 (15, m, benzoate H's), 10.58 (1, broad s, 1-NH), $J_{1',2'} < 1$ Hz. The compound was further characterized by debenzoylation and acetylation[3] to give the crystalline acetate derivative with physical properties identical with those previously reported.[3] To a stirred solution of 3.62 g (0.00635 mol) of **5** in 150 ml of dioxane was added 1.55 g (0.0070 mol) of P_2S_5 and the mixture was refluxed for 30 min. A second charge (1.55 g) of P_2S_5 was then added and heating was resumed for another 30 min. The mixture was cooled to room temperature overnight and filtered to remove some unreacted P_2S_5. The filtrate was evaporated to a gum and heated on a steam bath with 5 ml of water while stirring for 10 min. The resulting mixture was partitioned between chloroform and saturated sodium bicarbonate, and the organic layer was washed with water and dried over sodium sulfate. The chloroform solution was evaporated and the residue was triturated with ethanol to afford a yellow crystalline mass. One recrystallization from 100 ml of ethanol afforded 2.69 g (72%) of **6** as yellow plates,

(15) A. H. Jackson and P. Smith, *J. Chem. Soc., C*, 1667 (1968); Y. Kanaoka, K. Miyashita, and O. Yonemitsu, *Tetrahedron*, **25**, 2757 (1969).

(16) B. J. Hunt and W. Rigby, *Chem. Ind.* (London), 1868 (1967).

(17) S. Sugasawa, S. Yamada, and M. Narahashi, *J. Pharm. Soc. Jap.*, **71**, 1345 (1951); *Chem. Abstr.*, **46**, 8034*d* (1952). A. Albert and F. Reich, *J. Chem. Soc.*, 1370 (1960).

mp 174–178°. A second recrystallization from methanol gave an analytical sample: mp 181–183°; nmr ($CDCl_3$) δ 2.10 (3, s, CH_3), 4.50–5.00 (3, m, H-4′ and H-5′), 5.95–6.37 (2, m, H-2′ and H-3′), 6.47 (1, s, H-5), 7.12–7.65, 7.75–8.14 (16, m, benzoate H's and H-1′), 10.25 (1, broad s, 1-NH).

Anal. Calcd for $C_{31}H_{26}N_2O_8S$: C, 63.47; H, 4.47; H, 4.77; S, 5.46. Found: C, 63.75; H, 4.39; N, 4.80; S, 5.59.

Reduction of 6 to 2a.—A solution of 1.17 g of **6** (0.0020 mol) in 100 ml of ethanol was refluxed and treated with 4 g of activated Raney nickel.[8] After 15 min another 2 g of Raney nickel was added and the reaction was stopped after 5 min. Thin layer chromatography (benzene–ethyl acetate 1:1) shows unreacted material (R_f 0.66) and two major products (R_f 0.16 and 0.30). After filtration through Celite the solution was evaporated to dryness and chromatographed on a column of 100 g of silica gel G (benzene–ethyl acetate 1:1). The slow moving component was collected and the eluent was evaporated to a syrup. It had the same mobility as 4-methyl-1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone in two solvent systems [benzene–ethyl acetate (1:1) and chloroform–methanol (20:1); R_f 0.16 and 0.63, respectively] and formed a crystalline picrate with the same melting point and mixture melting point (162–164°) as **2a**. Ir spectra (KBr) were identical.

2-Oxo-1-β-D-ribofuranosyl-4-pyrimidinecarboxaldehyde Oxime (7a).—To a solution of 1.87 g (0.0077 mol) of **3a** (as the free base) in 8 ml of 50% acetic acid cooled at 0° was added with rapid stirring 0.69 g (0.010 mol) of sodium nitrite. A pale yellow crystalline solid precipitated after *ca.* 10 min. The mixture was stirred for 30 min and the product was filtered, washed with ice water, and dried at room temperature. The crude oxime weighed 1.32 g (63%). Recrystallization of a 1.0 g sample from 110 ml of anhydrous methanol afforded 0.91 g of white prisms: mp 223–224° dec; nmr (DMSO-d_6) δ 3.53–4.18 (5, m, H-2′, H-3′, H-4′, and H-5′), 5.78 (1, d, H-1′), 6.77 (1, d, H-5), 7.80 (1, s, H-4), 8.47 (1, d, H-6), 5.5–7.7 (broad absorption band, sugar OH's), $J_{1',2'}$ = 1.7, $J_{5,6}$ = 6.8 Hz; uv $\lambda_{max}^{1\ N\ HCl}$ sh 223 mμ (ε 4420), 268 (8440), 348 (3090); λ_{min} 303 mμ (ε 3240); $\lambda_{max}^{pH\ 5}$ 225 mμ (ε 12,590), 252 (12,180), 330 (6380); λ_{min} 238 mμ (ε 9820), 293 (2900); $\lambda_{max}^{pH\ 12}$ 219 mμ (ε 10,060), 305 (16,910), 333 (18,050); λ_{min} 250 mμ (ε 1480), 314 (16,370); pK_{a_1} = 1.32, pK_{a_2} = 8.65.

Anal. Calcd for $C_{10}H_{13}N_3O_6$: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.16; H, 4.79; N, 15.42.

2-Oxo-1-(tri-*O*-acetyl-β-D-ribofuranosyl)-4-pyrimidinecarbonitrile (8a).—A solution of 1.00 g (0.0037 mol) of **7a** in 10 ml of acetic anhydride was heated to reflux for 30 min. The mixture was then poured in 100 ml of ice water and stirred for 30 min. The product was extracted with 100 ml of chloroform and the organic layer was washed with aqueous sodium bicarbonate and water. The solution was dried over sodium sulfate and evaporated to dryness. The residue was dissolved in toluene and the solution evaporated again to remove residual acetic acid. The resulting gum was then chromatographed on 120 g of silica gel G (benzene–ethyl acetate, 1:1) and the fractions containing the major product were evaporated to afford 1.26 g (96%) of **8a** as a chromatographically homogeneous syrup: nmr ($CDCl_3$) 2.05–2.10 (9, m, acetate H's), 4.19–4.64 (3, m, H-4′, H-5′), 5.02–5.58 (2, m, H-2′, H-3′), 5.95 (1, d, H-1′), 6.74 (1, d, H-5), 8.33 (1, d, H-6), $J_{1',2'}$ = 2.6, $J_{5,6}$ = 6.8 Hz; $\lambda_{max}^{H_2O}$ sh 248 and 343 mμ. The product fails to give the characteristic nitrile absorption band at 2250 cm^{-1}. This is not without precedent[18] and is probably due to the strong electron-withdrawing effect of the two ring nitrogen atoms.

Hyrolysis of 8a to Uridine.—The product obtained from the acetic anhydride dehydration of 1.00 g (0.0037 mol) of **7a** was dissolved in 4 ml of methanol and treated with 0.4 ml of 12 *N* HCl. Hydrogen cyanide was detected immediately. After standing overnight at room temperature, the solution had an ultraviolet spectrum identical with that of uridine. Examination of a thin layer chromatogram of the mixture (1-butanol saturated with water) indicated the presence of only one product with the same mobility as uridine (R_f 0.35). After evaporation of the solution to dryness, the residue was azeotroped with toluene and after trituration with ethanol afforded a crystalline product. Two recrystallizations from ethanol–water (20:1) gave uridine (0.30 g, 33%) identical in all respects with an authentic sample.

5-Methyl-2-oxo-1-β-D-ribofuranosyl-4-pyrimidinecarboxaldehyde Oxime (7b).—A solution of 7.7 g (~0.03 mol) crude syrupy **3b** in 50 ml of 50% acetic acid was stirred and chilled to 0°. Sodium nitrite (2.4 g, 0.035 mol) was added in one portion. Precipitation started immediately and the reaction mixture became thick. A second 50-ml portion of 50% acetic acid was added and stirring of the suspension was continued for 10 min. The precipitate was filtered and washed with ice water. The damp solid was triturated with a cold methanol–ether solution, and the cream-colored solid was filtered and dried, 5.47 g (59%), mp 149–153° eff. A second crop, 1.0 g (11%), was obtained from the wash liquor. A small sample was recrystallized from methanol (charcoal) and afforded a white crystalline product: mp 150–151° dec; uv $\lambda_{max}^{1\ N\ HCl}$ 222 mμ (ε 6770), 277 (7760), 364 (14,160); λ_{min} 243 mμ (ε 3090), 315 (2680); $\lambda_{max}^{pH\ 4-7}$ 224 mμ (ε 11,270), 258 (9200), 346 (6260); λ_{min} 240 mμ (ε 7240), 300 (2360); $\lambda_{max}^{pH\ 12}$ sh 315 mμ (ε 13,620), 342 (16,450); λ_{min} 218 mμ (ε 12,320), 255 (1280); pK_{a_1} = 1.87, pK_{a_2} = 9.08; nmr (DMSO-d_6) δ 8.40 (1, s, H-6), 7.90 (1, s, 4-CH), 5.77 (1, d, H-1′), 2.20 (3, s, CH_3), $J_{1',2'}$ = 1.8 Hz. The presence of 1 mol of H_2O supports the analytical data.

Anal. Calcd for $C_{11}H_{15}N_3O_6 \cdot H_2O$: C, 43.56; H, 5.65; N, 13.85. Found: C, 43.50; H, 5.67; N, 13.73.

5-Methyl-2-oxo-1-(tri-*O*-acetyl-β-D-ribofuranosyl)-4-pyrimidinecarbonitrile (8b).—A solution of 1.0 g (0.0033 mol) of **7b** in 10 ml of acetic anhydride was refluxed for 30 min. Tlc (ethyl acetate–benzene 2:1) now showed the absence of starting material. The light brown solution was poured into ice water and stirred for 30 min. The product was extracted into methylene chloride which was washed with cold saturated sodium bicarbonate solution, then with water, and dried over sodium sulfate. The solvent was evaporated and the syrupy residue was reconcentrated several times with portions of toluene to remove residual acetic acid and finally with methanol to afford crude **8b** as a yellow syrup which was not further purified: uv $\lambda_{max}^{H_2O}$ 252.5 and 353 mμ; $\lambda_{max}^{pH\ 14}$ 267 mμ.

Hydrolysis of 8b to 5-Methyluridine (9b).—The crude nitrile **8b** was dissolved in methanol containing 1 ml of concentrated hydrochloric acid and was kept at room temperature overnight. The uv spectrum showed loss of the peak at ~355 mμ and the presence of a new peak at 265 mμ. The acid was neutralized with dilute ammonium hydroxide and the reaction mixture evaporated to a syrup. Tlc (butanol–ethanol–water, 40:11:19) of this crude material *vs.* an authentic sample of **9b** showed the same migration. The syrup was dissolved in hot methanol and ethyl acetate was added to incipient turbidity. Crystallization occurred after 4 days in the refrigerator. The precipitate was filtered and washed with cold methanol and ether. The cream-colored solid, 312 mg (37%), gave an undepressed mixture melting point with an authentic sample of **9b** and also an identical ir spectrum.

1-(5′-*O*-Acetyl-β-D-ribofuranosyl)-2-oxo-4-pyrimidinecarboxylic Acid, Methyl Ester (11).—A solution of 0.540 g (0.0020 mol) of **7a** in 100 ml of 0.02 *N* HCl was hydrogenated at atmospheric pressure over 40 mg of 10% Pd–C, and the reaction was stopped after theoretical uptake (0.004 mol). The solution was immediately treated with 10 ml of acetic anhydride for 45 min and then filtered with Celite. The filtrate was then left at 0° overnight. The uv spectrum of the solution exhibited maxima at 303 mμ (pH 7.0) and at 315 mμ (pH 1.0). The solution was treated with 2 ml of 1 *N* NaOH and evaporated to dryness. The residue was redissolved in 50 ml of methanol and 5 ml of acetic anhydride, and the solution was stirred vigorously in an open flask at room temperature overnight. The final uv spectrum of the solution had maxima at 333 mμ (pH 7.0), 315 (pH 12.0), and 328 (pH 1.0) corresponding to the ethyl ester, carboxylate anion, and free carboxylic acid, respectively. The solution was evaporated and the residue was chromatographed on 70 g of silica gel G (methanol–chloroform, 1:5). The major fractions were collected and evaporated to a syrup. The amount of **11** recovered was ~255 mg (calculated spectrophotometrically): nmr (D_2O) δ 1.97 (3, s, CH_3CO–), 3.97 (3, s, $COOCH_3$), 3.62–4.10 and 4.10–4.40 (5, m, H-2′, H-3′, H-4′, and H-5′), 5.84 (1, d, H-1′), 7.15 (1, d, H-5), 8.68 (1, d, H-6), $J_{1',2'}$ ~ 1, $J_{5,6}$ = 6.8 Hz.

2-Oxo-1-β-D-ribofuranosyl-4-pyrimidinecarboxylic Acid (12).—From a methanolic stock solution, 85 mg of **11** was dissolved in 2 ml of 0.5 *N* NaOH and left 3 hr at room temperature. The mixture was then passed through 4.5 ml (wet volume) of Dowex AG 50 (H^+) and eluted with distilled water. The uv absorbing fractions were collected and evaporated to a syrup. A solution of the residue in 1 ml of methanol was treated with 6 drops of freshly distilled cyclohexylamine. The solvent was slowly

(18) P. Sensi and G. G. Gallo, *Gazz. Chim. Ital.*, **85**, 224 (1955); *Chem. Abstr.*, **50**, 3086*a* (1956).

evaporated at room temperature, and the salt crystallized to give, after thorough washing with ethanol and drying (78°, 6 hr, high vacuum), 47 mg of a pure cyclohexylamine salt as a stable adduct, mp 160–163° eff, containing a second mole of cyclohexylamine: nmr (methanol-d_4) 0.80–2.20 (20, m, cyclohexylamine C-H), 3.61–4.26 (5, m, H-2′, H-3′, H-4′, H-5′, and OH), 5.91 (1, d, H-1′), 6.92 (1, d, H-5), 8.73 (1, d, H-6), $J_{1',2'} \sim 1$, $J_{5,6} = 7.0$ Hz. The adduct was hygroscopic and analyzed best for a hydrate: uv $\lambda_{max}^{pH\ 1}$ 213 mμ (ε 14,080), sh 250 (1690), 330 (8600); λ_{min} 272 mμ (ε 1170); $\lambda_{max}^{pH\ 7-12}$ 213 mμ (ε 13,090), 315 (6730); λ_{min} 260 mμ (ε 1380); p$K_a \sim 2.5$.[19]

Anal. Calcd for $C_{10}H_{12}N_2O_7 \cdot 2C_6H_{13}N \cdot {}^1/_2H_2O$: C, 53.10; H, 8.30; N, 11.26. Found: C, 53.02; H, 7.87; N, 11.39.

1-Methyl-2-oxo-4-pyrimidinecarboxaldehyde Oxime (14).—A solution of 1.07 g (0.0086 mol) of 1,4-dimethyl-2-pyrimidinone (**13**)[20] in 30 ml of 50% aqueous acetic acid was treated at 0° with 0.89 g (0.013 mol) of sodium nitrite with rapid stirring. After 30 min the crystalline product was filtered and a second crop was obtained by further evaporation and cooling of the filtrate. The procedure afforded 0.74 g (56%) of crude **14** which was recrystallized from methanol (dec pt 240°) to give an analytical sample: nmr (DMSO-d_6), δ 3.45 (3, s, NCH_3), 6.83 (1, d, H-5), 7.79 (1, s, H-4), 7.98 (1, d, H-6), $J_{5,6} = 6.5$ Hz.

Anal. Calcd for $C_6H_7N_3O_2$: C, 47.06; H, 4.61; N, 27.44. Found: C, 46.98; H, 4.54; N, 27.36.

1-Methyl-2-oxo-4-pyrimidinecarboxylic Acid Methyl Ester (16).

A. From 14.—A solution of 1.22 g of **14** (0.008 mol) in 150 ml of 0.053 *N* HCl was hydrogenated at atmospheric pressure over 40 mg of 10% Pd–C and the reaction was stopped after the theoretical uptake. The solution was filtered through Celite and treated with 25 ml of acetic anhydride. After stirring at room temperature for 40 min another 25 ml of acetic anhydride was added and the mixture was left overnight at 0°. The ultraviolet absorption spectrum showed a maximum at 300 mμ (pH 7.0) with a shift to 310 mμ in acid (pH 1.0). The solution was evaporated to dryness, dissolved in 100 ml of methanol, and neutralized with Amberlite IR-45 (OH^-). The filtrate was then treated with 20 ml of acetic anhydride and stirred at room temperature for 48 hr with ready access to the atmosphere. The ultraviolet maximum had then shifted to 335 mμ (pH 7.0). Thin layer chromatography (chloroform–methanol, 10:1) indicated the presence of one ultraviolet absorbing product (R_f 0.46). The residue after evaporation to dryness was chromatographed on 150 g of silica gel G (chloroform–methanol, 10:1). The major product crystallized on evaporation of the major fractions and was recrystallized from hot ethanol to yield 200 mg of **16**: mp 241–245° eff; nmr (DMSO-d_6) 3.51 (3, s, NCH_3), 3,89 (3, s, $COOCH_3$), 6.77 (1, d, H-5), 8.27 (1, d, H-6), $J_{5,6} = 6.0$ Hz; uv $\lambda_{max}^{pH\ 7}$ 332 mμ (ε 5090), sh 217 (780).

Anal. Calcd for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66. Found: C, 50.42; H, 4.75; N, 16.74.

B. From 3-Methylorotic Acid Methyl Ester (17).—To a solution of 3-methylorotic acid[14] (9.3 g, 0.048 mol) in 35 ml of concentrated H_2SO_4 was added very slowly 65 ml of methanol. The hot solution was left for 1 hr and was diluted with 100 ml of methanol. The mixture was chilled and the white crystalline precipitate was filtered and washed with a cold methanol–ether mixture and then with ether. The crude product (**17**, 6.5 g, mp 203–210°) was used directly for the subsequent step. Recrystallization of a small amount of the crude product from methanol gave the analytical sample: nmr (DMSO-d_6) δ 3.15 (3, s, NCH_3), 3.88 (3, s, $COOCH_3$), 6.17 (1, d, H-5), 11.4 (1, broad s, NH), $J_{NH-5'} \sim 2$ Hz. The signal for H-5 collapses to a singlet with the addition of D_2O.

Anal. Calcd for $C_7H_8N_2O_4$: C, 45.65; H, 4.38; N, 15.21. Found: C, 45.41; H, 4.41; N, 15.02.

To a solution of 3.68 g (0.020 mol) of **17** in 120 ml of dioxane was added 5.55 g (0.025 mol) of P_2S_5; the mixture was refluxed for 1.5 hr. Another charge of 5.55 g of the reagent was added and heating was resumed for 30 min. The mixture was filtered after cooling to room temperature and the filtrate was evaporated to a small volume (*ca.* 20 ml). Methanol (100 ml) was added to the concentrated dioxane solution and the mixture was heated on the steam bath until homogeneous. The solution was chilled and the crude crystalline **methyl ester of 3-methyl-4-thioorotic acid** (4.0 g) was filtered and washed with cold methanol. The product was recrystallized from 600 ml of boiling methanol and afforded 2.7 g of **18** as a yellow crystalline solid: mp 234–235°; nmr (DMSO-d_6) 3.57 (3, s, NCH_3), 3.87 (3, s, $COOCH_3$), 6.83 (1, s, H-5), 11.95 (1, broad s, NH).

Anal. Calcd for $C_7H_8N_2O_3S$: C, 41.99; H, 4.02; N, 13.99; S, 16.01. Found: C, 41.92; H, 4.06; N, 13.82; S, 15.95.

A second crop (0.7 g, mp 226–231°) was obtained from the mother liquor (85% yield).

A solution of 1.0 g (0.010 mol) of **18** in 75 ml of a 10% solution of ammonium hydroxide was heated to reflux with vigorous stirring in the presence of 2.5 g of activated Raney nickel. After 25 hr the reaction was cooled to room temperature and filtered through Celite. The solid was washed several times with small portions of boiling water, and the filtrate and washings were evaporated to a small volume. The solution was put on 60 ml (wet volume) of Dowex AG-50 (H^+) and the product collected by elution with distilled water. The fractions containing ultraviolet absorbing material were evaporated to dryness to afford 0.30 g of crystalline **1-methyl-2-oxo-4-pyrimidinecarboxylic acid (19)**. A small sample was recrystallized from water to give the pure product which decomposes at 209–210° eff: nmr (DMSO-d_6) 3.49 (3, s, NCH_3), 5.12 (COOH), 6.79 (1, d, H-5), 8.39 (1, d, H-6), $J_{5,6} = 6.4$ Hz; uv $\lambda_{max}^{pH\ 1}$ 328 mμ (ε 6950); λ_{min} 272 mμ (ε 530); $\lambda_{max}^{pH\ 7-14}$ 312 mμ (ε 5035); λ_{min} 257 mμ (ε 845); p$K_a \sim 2.80$.[19]

A suspension of 260 mg (0.0016 mol) of crude **19** in 100 ml of methanol was treated with an ethereal solution of diazomethane (from 7.5 g of N-nitrosomethylurea) and stirred at 0° for 20 min. The excess of diazomethane was decomposed with acetic acid and the solution was filtered from unreacted acid. After neutralization of the solution with Amberlite IR-45 (OH^-), it was filtered from the resin and evaporated to dryness to yield 190 mg of **16** in crystalline form. Recrystallization from hot ethanol gave pure product (mp 241–245°) identical in all respects with **16** as obtained by method A.

Registry No.—**2a** hydrochloride, 24744-13-6; **2b** hydrochloride, 24744-14-7; **3a** hydrochloride, 24744-15-8; **3b** hydrochloride, 24744-16-9; **5**, 24744-17-0; **6**, 24744-18-1; **7a**, 24744-19-2; **7b**, 24744-20-5; **8a**, 24744-21-6; **8b**, 24744-22-7; **11**, 24744-23-8; **12**, 24744-24-9; **14**, 24766-53-8; **16**, 24766-54-9; **17**, 24766-55-0; **18**, 24766-56-1; **19**, 24806-53-9.

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(19) This "apparent" pK_a value is an approximation due to the overlap of the dissociation from pH 1–7 with a second "dissociation" in the extremely acidic region for which it was not possible to determine a pK_a value.

(20) D. J. Brown and R. V. Foster, *Aust. J. Chem.*, **19**, 2321 (1966).