The Synthesis of Orotidine and Its Isomer, 3-β-D-Ribofuranosylorotic Acid, and the Methylation of Orotic Acid[1]

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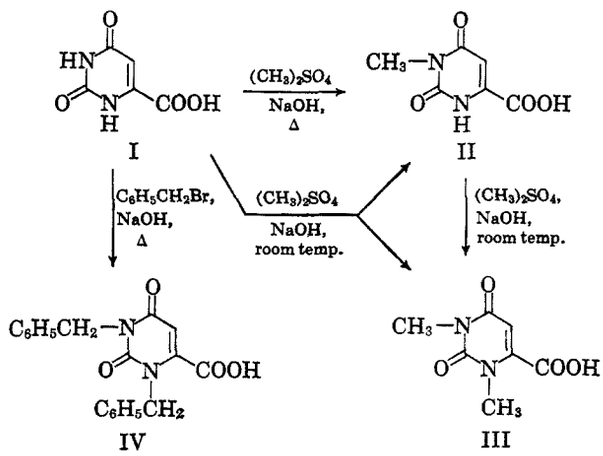
Orotidine (X) and its isomer, 3-β-D-ribofuranosylorotic acid (XI), have been synthesized by the reaction between 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride and the mercury derivative of butyl orotate. Some studies on the methylation of orotic acid which led to the above synthesis are also described.

The methylation of orotic acid (I) in hot, dilute base with excess dimethyl sulfate has been reported[2] to give the 3-methyl derivative II. In addition, methylation of 3-methylorotic acid using the same conditions afforded only meager amounts of the 1,3-dimethyl derivative III. These results and an examination of molecular models led these investigators[2] to conclude that "the 6-carboxyl function of orotic acid caused a considerable amount of steric hindrance at the N_1 position" and, "that N_1-ribosylation of a mercuri derivative of orotic acid (or its ester) would be difficult if not unlikely."

During a preparation of 3-methylorotic acid (II) by the above-mentioned method, a profuse amine evolution was detected. A small yield (20%) of the 3-methyl derivative II was isolated, but paper chromatographic examination of the mother liquor revealed the presence of two additional products. In order to avoid the decomposition that took place in the hot solution, orotic acid was treated with the same reagents at room temperature. Rather unexpectedly, this experiment gave 1,3-dimethylorotic acid (III) in 58% yield while no 3-methyl compound (II) was obtained. Under similar conditions the 3-methyl derivative II was also converted to the dimethyl compound III.[3] In an effort to improve the yield of 3-methylorotic acid (II), orotic acid was treated with 1.5 molar equiv. of dimethyl sulfate at room temperature. In this instance a 29% yield of II was obtained along with a very small mount of the isomeric 1-methylorotic acid. Paper chromatography of the reaction mixture revealed that starting material (I) and 1,3-dimethylorotic acid (III) were also present. These results explain the appearance of other ultraviolet-absorbing spots on paper chromatograms of the reaction carried out by the procedure of Fox, *et al.*[2] The identity of the amine previously detected during this reaction was clarified when it was found that upon heating 1,3-dimethylorotic acid (III) in alkali, methylamine was given off. It is quite evident, from the conversion of both orotic acid and the 3-methyl derivative II to 1,3-dimethylorotic acid (III) at room temperature, that the N-1 position of orotic acid (I) is not nearly as hindered by the 6-carboxyl group as was previously supposed.

These results prompted us to investigate some synthetic routes to orotidine, the naturally occurring riboside of orotic acid. Initially, we felt that the best approach would be to block the 3-position of the



pyrimidine ring in order to force ribosylation of the 1-position.[4] The benzyl group appeared attractive, since it could ultimately be removed by a reductive process. Attempts to treat benzyl bromide with orotic acid in the cold gave only starting material while the same reagents on the steam bath afforded only 1,3-dibenzylorotic acid (IV) in 20% yield. The low yield is undoubtedly due to the fact that this compound, like the 1,3-dimethyl analog III, is unstable in hot alkaline solution.[5] However, the successful introduction of a benzyl group into position 1 reinforced our opinion concerning the steric situation at this position.

3-Benzylorotic acid (VI) was finally prepared by substantially improving an early literature procedure[6] involving the condensation of N-benzylurea with diethyl oxalacetate to afford 1-benzyl-4-carbethoxymethylidenehydantoin (V) followed by rearrangement under alkaline conditions to VI.[8] This product was converted to its ethyl ester VII and then to the bispyrimidylmercury compound. However, attempts to prepare a ribosyl derivative were not successful.

(1) A portion of this work has been reported previously: W. V. Curran and R. B. Angier, *Tetrahedron Letters*, **No. 8**, 533 (1963).

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

(3) The water solubility of 1,3-dimethylorotic acid contributes to the low yields obtained.

(4) When this work was initiated, no blocking groups for the pyrimidine moiety had been reported in the literature. However, after the completion of this portion of the work, the benzhydryl group was utilized in a synthesis of 6-azauridine by M. Prystas, J. Gut, and F. Sorm, *Chem. Ind.* (London), 947 (1961).

(5) Some insight into the mechanism of this reaction was obtained when it was found that alkaline solutions of 1,3-dibenzylorotic acid (IV) slowly deposit N,N′-dibenzylurea.

(6) M. Bachstez [*Chem. Ber.*, **64**, 2683 (1931)] has reported that 1.2 g. of N-benzylurea gave 0.2 g. of recrystallized, "benzyl-uracil-carbonsäure-äthylester" (m.p. 137°). Subsequent workers[7] have shown that the product of this type reaction is a hydantoin. Therefore, this product corresponds to our 1-benzyl-4-carbethoxymethylidenehydantoin (V), m.p. 136–138°. Bachstez also reports the free acid (V) as microcrystals, m.p. 247°, in unstated yield.

(7) H. K. Mitchell and J. F. Nyc, *J. Am. Chem. Soc.*, **69**, 674 (1947).

(8) The location of the alkyl group at the N-3 position has been conclusively proved.[2]

Although these initial failures were discouraging, further examination of the literature revealed that the use of the mercuri procedure with 2,4-dihydroxypyrimidines always gave N-1 rather than N-3-glycosides[9] with the exception that 6-alkyl derivatives afforded O-glycosides.[10] However, 6-alkyl compounds are methylated only in the 3-position[11] using the same conditions that produced 1,3-dimethylorotic acid (III). With these facts in mind, we felt that an attempt to ribosylate a simple orotic acid ester was warranted even though the literature records several unsuccessful attempts in this direction.[12,13] We used *n*-butyl orotate with the hope that the *n*-butyl function might have a beneficial effect on the solubility of the mercury derivative in refluxing xylene.

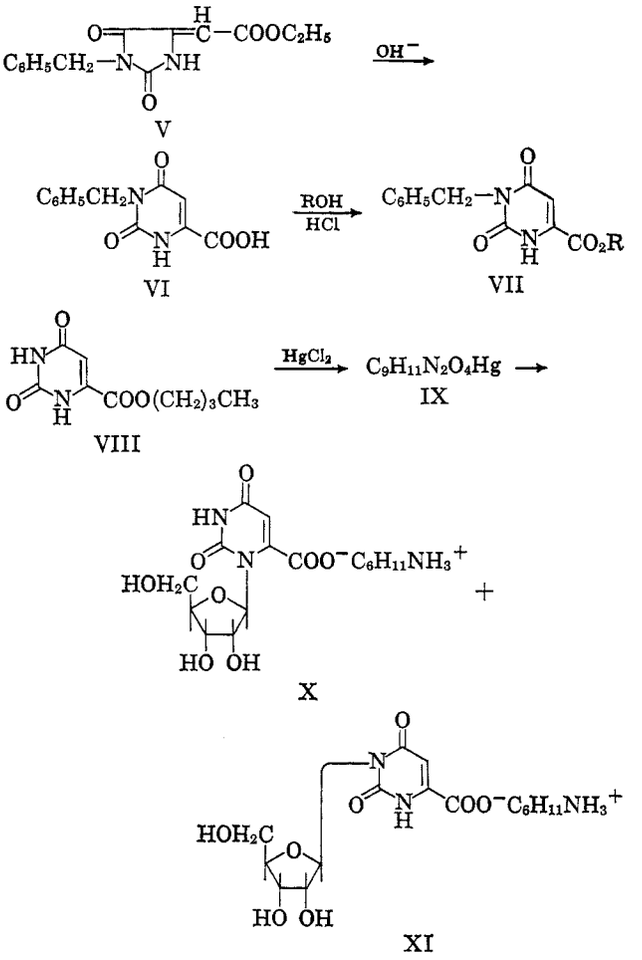
n-Butyl orotate (VIII)[15] was converted to a monomercury derivative IX in good yield. A mixture of the mercury compound IX and tribenzoylribofuranosyl chloride in xylene was refluxed for 5 hr., and the crude product was isolated by pouring the cooled reaction mixture into a large volume of low-boiling petroleum ether. Deblocking was accomplished by treating with methanolic sodium methoxide followed by aqueous base, and the product was separated into two isomeric nucleosides by ion-exchange chromatography on Dowex-1 using a linear water–0.1 *M* ammonium bicarbonate eluent. Both products were converted to their corresponding cyclohexylammonium salts and the one which came off the column first was crystallized from a mixture of ethanol–ethyl acetate. This proved to be identical with an authentic specimen of orotidine cyclohexylammonium salt (X)[16] as judged by infrared and ultraviolet absorption spectra, mixture melting point, and paper chromatography. The corrected yield of recrystallized product was 8.7%. The other compound, 3-β-D-ribofuranosylorotic acid (XI), obtained in 10% yield, resisted all attempts at crystallization, perhaps because trace amounts of X were present as shown by paper chromatography. The structure of XI is based upon elemental analyses, optical rotation, and the similarity of the ultraviolet spectra with those of the corresponding 3-methyl derivative. (See Table I.)

TABLE I

Compound	λ_{max}, mμ (ε × 10⁻³) 0.1 *N* HCl	0.1 *N* NaOH
3-β-D-Ribofuranosylorotic acid (XI)	285 (5.41)	306 (5.66)
3-Methylorotic acid (II)	282 (6.38)	298 (6.64)
Orotidine (X)	265 (9.70)	265 (7.75)
1-Methylorotic acid	272 (6.93)	270 (5.72)

This appears to be the first time a 3-substituted pyrimidine has been obtained using the mercuri procedure.

One other synthesis using the same procedure, except that the condensation reaction was carried out by refluxing for 3 instead of 5 hr., gave orotidine (X) in a 14.5% yield (corrected). Isolation of the isomer XI was not attempted in this experiment.



Orotidine was first isolated from a mutant of *Neurospora crassa* by Michelson, Drell, and Mitchell,[14] who showed it was composed of orotic acid and ribose. Subsequently Lieberman, *et al.*,[17] by enzymatic studies, and Fox, *et al.*,[2] by ultraviolet absorption spectroscopy, proved that the ribose moiety was affixed to the 1-position of the pyrimidine. The present work provides a synthetic confirmation of the structure of orotidine; the mode of synthesis also supports the assignment of the β configuration at the anomeric center.[18] 3-β-D-Ribofuranosylorotic acid (XI) has also been assigned the β configuration on similar grounds.

Experimental Section

Paper chromatographic experiments were carried out on Whatman No. 1 paper using the descending technique. A zinc silicate plate coated with DuPont phosphor No. 609235 was used to aid in detecting the ultraviolet-absorbing spots.

3-Methylorotic Acid (II).—Orotic acid (3.1 g., 0.02 mole) was dissolved in 20 ml. of water containing 2.4 g. (0.06 mole) of sodium hydroxide, and 2.8 ml. (0.03 mole) of dimethyl sulfate was added with stirring during a 30-min. period. The reaction

(9) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 336 (1959).

(10) J. J. Fox and N. C. Yung, unpublished data in ref. 9, p. 335.

(11) J. P. Scannell, A. M. Crestfield, and F. W. Allen, *Biochim. Biophys. Acta*, **32**, 412 (1959).

(12) R. K. Ralph, G. Shaw, and R. N. Naylor [*J. Chem. Soc.*, 1169 (1959)] reported that in preliminary experiments they could find no evidence of condensation between metal derivatives of ethyl orotate and 2,3,5-tribenzoylribosyl chloride.

(13) Michelson, *et al.*,[14] stated that several unsuccessful attempts have been made to synthesize glycosides and especially a ribofuranoside of orotic acid.

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

(15) Prepared by the method of L. O. Ross, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **25**, 1950 (1960).

(16) Purchased from California Corporation for Biochemical Research.

(17) I. Lieberman, A. Kornberg, and E. S. Simms, *J. Biol. Chem.*, **215**, 403 (1955).

(18) B. R. Baker, The Chemistry and Biology of Purines, A Ciba Foundation Symposium, J. and A. Churchill Ltd., London, 1957, p. 120.

mixture was stirred for an additional 30 min., then acidified with 5.0 ml. of concentrated hydrochloric acid and chilled overnight. The crystals were collected and dried: yield 1.85 g., m.p. 286–297° with gas evolution. Paper chromatography in isopropyl alcohol–1 *N* ammonium hydroxide (7:3) revealed this to be a mixture of orotic acid, 3-methylorotic acid, and a small amount of 1-methylorotic acid.[19] The crude product was extracted with 100 ml. of boiling ethanol and the extract was chilled overnight. The product was collected and dried to give 1.0 g. (29.4%) of chromatographically pure 3-methylorotic acid, m.p. 316–323° gas evolution.[20] The ultraviolet absorption spectra were the same as given by Fox, *et al.*[2]

1,3-Dimethylorotic Acid (III). Method A.—Orotic acid (3.15 g., 0.02 mole) was dissolved in 100 ml. of 1 *N* sodium hydroxide using a magnetic stirrer and 9.3 ml. (0.10 mole) of dimethyl sulfate was added dropwise over a 1-hr. period. The stirring was continued for an additional 1 hr. at which time the pH had dropped to 1.5. After 20 ml. of concentrated hydrochloric acid had been added to the solution, it was chilled for several days. No crystallization occurred. The solution was evaporated to one-half the original volume *in vacuo* and extracted with five 250-ml. portions of chloroform. The extracts were dried over magnesium sulfate, evaporated to 100 ml., and diluted with petroleum ether (b.p. 30–60°) to give crystals of 1,3-dimethylorotic acid, yield 0.40 g., m.p. 148–150°.

The aqueous reaction mixture was again extracted with eight 50-ml. portions of ethyl acetate. These extracts were combined, dried over magnesium sulfate, and concentrated *in vacuo* to afford 0.85 g. of product, m.p. 147–150°. Addition of petroleum ether to the filtrate gave an additional crop of 0.90 g., m.p. 146–150° (total yield, 58%). All three crops were chromatographically homogeneous in isopropyl alcohol–1 *N* ammonium hydroxide (7:3) and butanol–5 *N* acetic acid (7:3). The ultraviolet absorption spectra of the first crop were the same as previously reported.[2]

Method B.—3-Methylorotic acid (2.0 g., 11.8 mmoles) was dissolved in 36 ml. of 1.0 *N* sodium hydroxide and 3.3 ml. (35.4 mmoles) of dimethyl sulfate was added to the solution with stirring during a 20-min. period. The stirring was continued for an additional 10 min. at which time the pH was 1.0. Paper chromatography of the reaction mixture in isopropyl alcohol–1 *N* ammonium hydroxide (7:3) revealed that the major product was the 1,3-dimethyl derivative III along with a small amount of the starting 3-methyl compound (II). The reaction mixture was extracted with five 100-ml. portions of ethyl acetate which were combined and dried over magnesium sulfate. The ethyl acetate was concentrated to about 20 ml. and chilled overnight. The crystals of 1,3-dimethylorotic acid were collected and dried: yield 0.70 g. (21%), m.p. 147–150° with some previous wetting. For analyses the product was recrystallized from ethyl acetate, m.p. 149–151°.[21]

Anal. Calcd. for $C_7H_8N_2O_4$ (184): C, 45.7; H, 4.38; N, 15.2. Found: C, 45.9; H, 4.42; N, 15.1.

1,3-Dibenzylorotic Acid (IV).—Orotic acid (11.5 g., 0.067 mole) was added to 100 ml. of water, stirred, and placed in a water bath at 65°. Benzyl bromide (40 ml., 0.337 mole) and 80 ml. of 4.2 *N* sodium hydroxide (0.336 mole) were added in eight equal portions at 15-min. intervals. The mixture was stirred for an additional 30 min. at 65° and then acidified to pH 1 with 10 ml. of concentrated hydrochloric acid. An oil separated which was extracted into 150 ml. of ether. The aqueous layer was extracted with two more 150-ml. portions of ether and the combined extracts were dried over magnesium sulfate. Evaporation of the ether left an oil which was taken up in 200 ml. of water containing 15.0 g. of sodium bicarbonate. The solution was extracted with 100 ml. of chloroform to remove excess benzyl bromide and then acidified with 20 ml. of concentrated hydrochloric acid to give an oil which crystallized. The product was collected and dried: yield 12.5 g., m.p. 160–180°. This impure product was dissolved in 200 ml. of water containing 10 g. of sodium bicarbonate and filtered to remove a small amount of insoluble material. The filtrate was acidified to pH 6.5–7.0 with 5 ml. of acetic acid, warmed on a steam bath, treated with Norit, and filtered. This solution was brought to boiling and further acidified with 15 ml. of concentrated hydrochloric acid to give an oil which crystallized immediately: yield 8.0 g. (21%); m.p. 188–190° with some previous wetting; R_f 0.87 in isopropyl alcohol–1 *N* ammonium hydroxide (7:3). For analyses a small portion of this product was recrystallized from 50% ethanol; the melting point was raised to 194–196°: λ_{max}^{pH7} 272 mμ (ε 9400), $\lambda_{max}^{1\ N\ HCl}$ 273 mμ (ε 8200), the absorption in 0.1 *N* NaOH was the same as in pH 7 buffer.

Anal. Calcd. for $C_{19}H_{16}N_2O_4$ (336): C, 67.9; H, 4.8; N, 8.3. Found: C, 67.8; H, 4.9; N, 8.2.

1-Benzyl-4-carbethoxymethylidenehydantoin (V).—Diethyl oxalacetate sodium salt (56 g., 0.266 mole) was added to 400 ml. of water, covered with 200 ml. of ether, and stirred. To this mixture was added a solution of 7.0 ml. of concentrated sulfuric acid (0.252 mole) in 25 ml. of water. The ether layer was separated and the aqueous layer was extracted twice with 200 ml. of ether. The combined ether extracts were washed with 200 ml. of water and dried over magnesium sulfate. Removal of the ether gave 41 g. of an oily liquid (0.218 mole, calculated as diethyl oxalacetate).

The diethyl oxalacetate was dissolved in 50 ml. of glacial acetic acid containing 32.7 g. (0.218 mole) of N-benzylurea and heated on a steam bath for 1.5 hr. while a rapid stream of anhydrous hydrogen chloride was passed into the solution. After the reaction mixture had been chilled over the weekend, the crystals were collected and dried: yield 23.0 g., m.p. 121–130°. Recrystallization from ethanol gave 13.8 g. (23%) of product, m.p. 133–135°.[6] For analytical purposes a small portion of this product was again recrystallized from ethanol (melting point was raised to 136–138°).

Anal. Calcd. for $C_{14}H_{14}N_2O_4$ (274): C, 61.3; H, 5.15; N, 10.2. Found: C, 61.2; H, 4.89; N, 10.3.

3-Benzylorotic Acid (VI).—1-Benzyl-4-carbethoxymethylidenehydantoin (13.6 g., 0.05 mole) was dissolved in 150 ml. of ethanol containing 30 ml. of 20% aqueous potassium hydroxide solution and refluxed for 0.50 hr. The solution was concentrated *in vacuo* to a volume of about 25 ml. and diluted with 250 ml. of water. This solution was warmed on a steam bath, treated with Norit, and filtered. The filtrate was reheated to 85° and acidified with 10 ml. of concentrated hydrochloric acid to give a white crystalline product. After having been chilled, the crystals were collected and dried: yield 11.5 g., m.p. 210–217°.[6] The yield, after recrystallizing from 75 ml. of ethanol, was 10.6 g. (86%): m.p. 226–227.5° with some previous softening; $\lambda_{max}^{0.1\ N\ NaOH}$ 300 mμ (ε 7400); $\lambda_{max}^{0.1\ N\ HCl}$ 283 mμ (ε 7100); $\lambda_{max}^{pH\ 7}$ 279 mμ (ε 7400).

Anal. Calcd. for $C_{12}H_{10}N_2O_4$ (246): C, 58.5; H, 4.09; N, 11.4. Found: C, 58.6; H, 4.43; N, 11.5.

Ethyl 3-Benzylorotate (VII).—3-Benzylorotic acid (3.0 g., 12.2 mmoles) was added to 150 ml. of absolute ethanol, cooled in an ice–salt bath, and saturated with anhydrous hydrogen chloride. The mixture was refluxed for 1 hr., re-treated with hydrogen chloride in an ice–salt bath, and stored at −5° overnight followed by 2 hr. of refluxing. This was evaporated to a crystalline solid, dissolved in 80 ml. of absolute ethanol, and again evaporated to crystals. After having repeated this procedure, the resulting crystals were recrystallized from 25 ml. of absolute ethanol using Norit: yield 2.4 g. (72%), m.p. 139–141°. For analysis a small portion was recrystallized from 70% ethanol. The melting point did not change.

Anal. Calcd. for $C_{14}H_{14}N_2O_4$ (274): C, 61.3; H, 5.2; N, 10.2. Found: C, 61.3; H, 5.0; N, 10.1.

Bis(ethyl 3-benzylorotate)mercury.—Ethyl 3-benzylorotate (1.5 g., 5.5 mmoles) was stirred for 10 min. in a solution of 80 ml. of water containing 6 ml. of 1 *N* sodium hydroxide and filtered to remove a small amount of undissolved compound. To the filtrate was added a solution of 0.75 g. (2.75 mmoles) of mercuric chloride in 20 ml. of ethanol, dropwise over a 10-min. period, and stirred for an additional 15 min. The product was collected and recrystallized from 135 ml. of ethanol using Norit: yield 1.0 g. (24%).

Anal. Calcd. for $C_{28}H_{26}HgN_4O_8$ (748): C, 45.0; H, 3.5; N, 7.5. Found: C, 45.3; H, 3.7; N, 7.7.

(*n*-Butyl orotate)mercury (IX). Method A.—*n*-Butyl orotate (10 g., 0.047 mole)[15] was added to 3.0 l. of water and stirred vigorously. Sodium hydroxide (50 ml., 1 *N*) was added to the mixture which was stirred for about 3 min. and filtered to remove undissolved *n*-butyl orotate (0.35 g.). To the filtrate was added, with stirring, 13.0 g. (0.047 mole) of mercuric chloride dissolved in 100 ml. of ethanol. A white, flocculent precipitate came out almost immediately. The suspension was stirred for 10 min., warmed to 50°, and allowed to stand at room tempera-

(19) In one experiment a small amount of 1-methylorotic acid (m.p. 277–278° dec., lit.[2] 273–275° dec.) was isolated. The ultraviolet absorption spectra (see Table I) were in agreement with those reported.[2]

(20) Lit.[2] m.p. 306–311°.

(21) Lit.[2] m.p. 149–151°.

ture overnight. The product was collected by filtration, washed with water until free from chloride ion, and dried over phosphorus pentoxide *in vacuo*: yield 7.75 g. (40%).

Anal. Calcd. for $C_9H_{11}HgN_2O_4$ (412): C, 26.3; H, 2.69; N, 6.8. Found: C, 26.6; H, 2.85; N, 6.6.

Method B.—Mercuric acetate (3.2 g., 10 mmoles) was dissolved in 60 ml. of boiling methanol. To this solution was added a hot solution of 2.1 g. (10 mmoles) of *n*-butyl orotate in 100 ml. of water. A white flocculent precipitate formed immediately: yield 3.85 g. (94%).

Anal. Calcd. for $C_9H_{11}HgN_2O_4$ (412): C, 26.3; H, 2.7; N, 6.8. Found: C, 25.9; H, 2.9; N, 6.6.

Orotidine Cyclohexylammonium Salt (X). A. Condensation Reaction.—(*n*-Butyl orotate)mercury (IX) (6.2 g., 15 mmoles) was ground to a fine powder and added to 250 ml. of xylene. The mixture was azeotropically dried by distilling 80 ml. of solvent using a Dean–Stark trap. To the hot, stirred mixture was added 2,3,5-tribenzoylribofuranosyl chloride [prepared as previously described[22] from 15 g. (29.8 mmoles) of 1-acetyl-2,3,5-tribenzoylribofuranose] dissolved in 120 ml. of xylene. The solution was refluxed for 3 hr. with 120 ml. of xylene being removed by distillation at the beginning of this period. A white solid precipitated on cooling: yield 2.26 g. (*Anal.* Found: C, 21.8; H, 2.50; N, 5.27.) The analyses and the infrared spectrum indicated that this material was an impure mercury derivative of *n*-butyl orotate. On the basis of the nitrogen analysis it contained 40% (4.25 mmoles) of the *n*-butyl orotate.

The filtrate was poured into 3 l. of petroleum ether (b.p. 30–60°) to give a thick gum. After having been chilled for several hours, the solution deposited a crystalline precipitate. The flask was swirled several times and allowed to stand in the cold for 2 days. The crystals were decanted from the gum and dried: yield 3.0 g., m.p. 117–123°. This material did not contain nitrogen and was not further investigated. The residual gum was dissolved in 250 ml. of ethyl acetate and extracted with three 100-ml. portions of 30% aqueous potassium iodide solution followed by two 100-ml. portions of water. After having been dried over magnesium sulfate, the ethyl acetate was removed *in vacuo* on a water bath (70–75°) to give a syrup: yield 10.59 g.

B. Deblocking.—The above syrup was added to 1500 ml. of anhydrous methanol containing 4.0 g. of sodium methoxide. The mixture was protected with a tube of Drierite, stirred for several hours, and allowed to stand at room temperature overnight. A small amount of insoluble material was collected and discarded. The filtrate was neutralized to pH 5 with Dowex 50 W-X4 which had previously been washed with water and methanol, the resin was removed by filtration, and the filtrate was evaporated to an oil *in vacuo*. The oil was slurried in 100 ml. of water and extracted with three 100-ml. portions of ether to remove methyl benzoate. Paper chromatography of the aqueous layer in *n*-propyl alcohol–water (3:1) showed four ultraviolet-absorbing spots, two of which traveled close to orotidine and orotic acid (R_f *ca.* 0.25 and 0.30) and the other two at R_f *ca.* 0.70 and 0.75. On treatment with base, both of the higher R_f spots disappeared, indicating they were being converted to the lower R_f compounds.

The aqueous layer was evaporated to 22 ml. *in vacuo* and cooled to room temperature, and 25 ml. of 1 *N* sodium hydroxide was added to give a turbid solution. The turbidity disappeared after 10–15 min. of stirring. After having been stirred for 1 hr. (pH 13.4), the solution was treated with a small amount of Norit, stirred for an additional 5 min., and filtered through a pad of Celite. The filtrate was acidified to pH 5 with 1.0 ml. of acetic acid and allowed to stand at room temperature overnight. Some crystals came out which were collected and dried after several hours in the cold: yield 146 mg., 250 mμ/260 mμ = 0.61, 280 mμ/260 mμ = 1.8 in 0.1 *N* HCl. These values are in good agreement with those published for orotic acid.[23] The fact that the material came out at pH 5 can only mean it is a sodium salt. This amounts to 0.82 mmoles calculated as a monosodium salt.

The filtrate from this product was evaporated *in vacuo* on a water bath to 12–15 ml. and chilled overnight. A small crop (6 mg.) of crystals was filtered off and discarded. The filtrate was adjusted to 100 ml. with water and treated as described below.

C. Gradient Elution Chromatography.—The above solution (85 ml.) was added to 500 ml. of water and brought to pH 10.5 with concentrated ammonium hydroxide. This solution was applied to a Dowex 1-x8 (Cl) column (4 × 11.5 cm., 200–400 mesh) and washed with 2 l. of water. The column was then eluted with a linear 0.1 *M* ammonium bicarbonate gradient. The progress of the column was followed by reading the optical densities of the fractions at 260 and 280 mμ.

The main orotidine-containing fractions (135 to 168), as determined by ultraviolet, were combined and evaporated to dryness *in vacuo* at 70–75°. The residue was dissolved in a 100-ml. portion of water and evaporated until a constant weight was obtained: yield 1.07 g. This material traveled as a single spot alongside authentic orotidine in isopropyl alcohol–1 *N* ammonium hydroxide (7:3), *n*-butyl alcohol–formic acid–water (77:10:13), and isopropyl alcohol–concentrated hydrochloric acid–water (77:4:4). This product gave a positive test for chloride ion; therefore, it was treated as described below.

D. Charcoal Adsorption.—The 1.07 g. of crude product was dissolved in 250 ml. of water and adjusted to pH 2.5–3.0 with 1 *N* hydrochloric acid. The volume was brought to 300 ml., and 100 ml. of a 4% suspension of acid-washed Norit A was added with stirring. Additional amounts (25 and 50 ml. of the 4% suspension and 1.0 g. of acid-washed Norit A) were added after 1, 2, and 3 hr. After a total of 4 hr., approximately 90% of the orotidine had been adsorbed as judged by reading the optical density at 260 mμ. The Norit was filtered off and stirred in 500 ml. of 0.3% ammonia in ethanol–water (1:1) for 30 min. and then filtered through a pad of Celite. The Norit–Celite mixture was extracted three more times with 500-ml. portions of the alcoholic ammonia solution, the last one being heated on a steam bath. The combined extracts were evaporated to dryness *in vacuo* at 70–75°, and the residue was dissolved in 500 ml. of water. This solution was passed through a column (25 ml. wet volume, 2 × 6 cm.) of Dowex 50W-x4 (cyclohexylammonium form) and washed with 200 ml. of water. The eluate was evaporated to dryness *in vacuo* at 75°, extracted with water, filtered from a small amount of insoluble material, and again evaporated to dryness *in vacuo:* yield 591 mg. This product was dissolved in 15 ml. of absolute ethanol, treated with a very small amount of Norit, and filtered through a pad of Celite. The Celite was washed with three 5-ml. portions of ethanol. The combined filtrate and washings were evaporated to 10 ml. and filtered to remove a small amount of amorphous material. Ethanol (5 ml.), followed by 10 ml. of ethyl acetate, was added to the filtrate. This mixture stood at room temperature for 2 days and deposited a small amount of a white solid which was filtered off and discarded. Ethyl acetate was added to the filtrate to incipient turbidity and seeded with orotidine cyclohexylamine salt. Very little crystallization occurred after 24 hr. About 60–70 ml. of ethyl acetate was added to precipitate a thick syrup and the mixture was brought to boiling. The syrup crystallized on cooling: yield 345 mg., m.p. 173–174.5° dec. Recrystallization from ethanol–ethyl acetate afforded 284 mg., m.p. 178.5–180° dec. This material was identical with authentic orotidine cyclohexylammonium salt as judged by mixture melting point, paper chromatography, and infrared and ultraviolet absorption spectra: $[\alpha]_D$ +15 ± 5° (*c* 1.00, water).

Anal. Calcd. for $C_{16}H_{25}N_3O_8$ (387): C, 49.6; H, 6.5; N, 10.9. Found: C, 49.5; H, 6.5; N, 10.7.

Additional crops of 94 mg. (m.p. 182–183.5° dec.), 28 mg. (m.p. 178–179° dec.), and 68 mg. (m.p. 180–183.5° dec.) were obtained from various mother liquors.[24] The identity of these materials with the main crop was confirmed by infrared spectroscopy. Thus, the total yield of isolated orotidine cyclohexylammonium salt was 474 mg. However, since only 85 of 100 ml. of the solution of deblocked nucleoside was subjected to gradient elution, the actual yield should be 557 mg. Correcting for the amount of *n*-butyl orotate (4.25 mmoles) recovered as an impure mercury salt and the sodium orotate (0.82 mmoles) obtained after deblocking, this amounts to a 14.5% yield.

3-β-D-Ribofuranosylorotic Acid Cyclohexylammonium Salt (XI).—(*n*-Butyl orotate)mercury (9.5 g., 23 mmoles) was added to 300 ml. of xylene and distilled until 100 ml. of distillate had

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been collected. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl chloride [prepared from 25.0 g. (49.5 mmoles) of the corresponding acetate] dissolved in 125 ml. of xylene was added followed by removal of 130 ml. of solvent by distilling. The reaction mixture was refluxed for 5.0 hr. and then worked up and deblocked as described in the previous experiment.

The crude deblocked mixture was adjusted to 500 ml. with water, brought to pH 10.5 with concentrated ammonium hydroxide, and applied to a Dowex 1-X8 (Cl^-) column (6 × 13 cm., 200–400 mesh). The column was eluted with a water–0.1 *M* ammonium bicarbonate gradient and 400 fractions (from 70 to 85 ml./fraction) were collected. The orotidine-containing fractions as shown by the 280 mμ/260 mμ ratio (fractions 113–227) were treated as described previously. A total of 686 mg. (8.7%) of recrystallized orotidine cyclohexylammonium salt was obtained. The other main component (fractions 290–383), whose ultraviolet absorption indicated a 3-substituted orotic acid, was adsorbed onto charcoal and converted to the cyclohexylammonium salt: yield 1.34 g., $\lambda_{max}^{0.1\ N HCl}$ 284 mμ, $\lambda_{max}^{0.1\ N NaOH}$ 306 mμ. Paper chromatography using isopropyl alcohol–1 *N* ammonium hydroxide (7:3) indicated that this product was contaminated with a small amount of orotidine. This product was dissolved in 15 ml. of hot absolute ethanol, treated with Norit, and filtered. Attempted crystallization by the addition of ethyl acetate was unsuccessful. The solvents were evaporated at reduced pressure; the residue was taken up in water and chromatographed again on Dowex-1 as described above. No separation was accomplished, as shown by ultraviolet monitoring. The product was again isolated by adsorption onto acid-washed Norit: yield 0.87 g. Paper chromatography in several solvent systems indicated that this compound was still contaminated with a small amount of orotidine. The compound was dissolved in 25 ml. of hot absolute ethanol treated with a small amount of Norit and filtered. About 100 ml. of ethyl acetate was added to precipitate a syrup which was isolated by decanting the supernatant liquid after chilling: yield 571 mg. An additional 260 mg. was obtained by evaporating the supernatant liquid. The first crop was dissolved in 250 ml. of water and passed through a 2 × 6 cm. column of Dowex 50W-X8 (cyclohexylammonium form) and then evaporated to dryness *in vacuo* to afford 555 mg. of product: $[\alpha]^{25}D$ −19.7 ± 4.9° (*c* 1.015, water); R_f 0.40 with trace of slightly lower R_f material in *n*-butyl alcohol–formic acid–water (77:10:13) (solvent B).

The second crop was treated similarly: yield 240 mg.; $[\alpha]^{25}D$ −16.3 ± 4.8° (*c* 1.045, water); R_f 0.40 in solvent B. The infrared spectra of the two products were the same. These two crops correspond to a 10% yield (corrected for the recovered sodium orotate). The 240-mg. crop was dissolved in 25 ml. of water, treated with a small amount of Norit, filtered, and evaporated to an oil: yield 175 mg.

Anal. Calcd. for $C_{16}H_{25}N_3O_8$ (387): C, 49.6; H, 6.5; N, 10.9. Found: C, 49.3; H, 7.3; N, 10.8.

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Nucleosides. IX. The Formation of 2′,3′-Unsaturated Pyrimidine Nucleosides *via* a Novel β-Elimination Reaction[1,2]

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The study reports the direct introduction of 2′,3′-unsaturation in the carbohydrate moiety of pyrimidine nucleosides *via* base-catalyzed elimination reactions. 2,3′-Anhydronucleosides (II) derived from both 2′-deoxyuridine and thymidine are converted to the corresponding 2′,3′-unsaturated nucleoside (IV) in high yield on treatment with potassium *t*-butoxide in dimethyl sulfoxide. The same base–solvent system applied to 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentosyl)pyrimidines (XIV, pyrimidines = thymine, uracil, and 4-thiouracil) also provides 2′,3′-unsaturated nucleosides (V) in excellent yields. The oxetane derivatives (XIV) and anhydro nucleosides (II) apparently both undergo β-proton abstraction (C′-2) followed by decyclization of the resulting carbanion. The close analogy of these transformations to the alkaline cleavage of related cyclic ethers is discussed. A novel double elimination reaction leading to 1-[2-(5-methylfuryl)]thymine (XVIII) is described.

Reichard, *et al.*,[3] have demonstrated the direct deoxygenation of both purine and pyrimidine ribonucleotides to corresponding 2′-deoxyribonucleotides by cell-free preparations from *E. coli* and from chick embryo. These observations have prompted the suggestion[3c,4] of 1′,2′- and 2′,3′-unsaturated nucleotides as possible intermediates in the biosynthetic pathway leading to 2′-deoxyribonucleotides. This possibility stimulated our interest in corresponding unsaturated nucleosides which to our knowledge were unknown prior to the inception of the present investigation.

It has been demonstrated that sulfonate esters of cyclic and secondary acyclic alcohols afford high yields of alkenes on treatment with potassium *t*-butoxide (*t*-BuOK) in dimethyl sulfoxide (DMSO)[5] at ambient temperatures. In fact, olefin formation is readily effected from a relatively wide spectrum of aliphatic functional derivatives in *t*-BuOK–DMSO.[6] The present communication describes the successful application of this base–solvent system to the synthesis of 2′,3′-unsaturated pyrimidine nucleosides from 3′-mesylates of 1-(2-deoxy-β-D-*threo*-pentosyl)pyrimidines. Moreover, the scope of base-promoted reactions in DMSO

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