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## Note

Synthesis of 3'-deoxyuridine via transglycosylation of uracil with 3'-deoxyadenosine (cordycepin)\*

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The intermolecular nature of the transglycosylation reaction has been shown by the direct transfer of sugars or pyrimidines, nucleosides, nucleotides, and trimethylsilylated nucleotides to purine bases in the presence of a Lewis acid catalyst<sup>1-9</sup>. The chemical syntheses of 3'-deoxyuridine (1) and other 3'-deoxypurine and pyrimidine nucleosides have been reported<sup>10-12</sup>. Two methods are described here for the synthesis of 1. Method A involves the conversion of D-xylose into methyl 2,5-di-Obenzoyl-3-deoxy- $\beta$ -D-erythro-pentofuranoside<sup>12</sup> (2) and condensation with bis(trimethylsilyl)uracil. Method B involves the direct transfer of the benzoylated 3-deoxy-Derythro-pentosyl moiety of nucleoside 3 to 2,4-bis(trimethylsilyloxy)pyrimidine.



NOTE

Although the 3'-deoxyuridine in earlier studies had limited biological application<sup>12,15,16</sup>, 3'-deoxyuridine is now a very useful biochemical probe in DNA replication. Gumport *et al.*<sup>17</sup> reported that 3'-dATP has limited utility because it competes for ATP as an energy source for DNA synthesis in addition to its ability to block synthesis of primer RNA. 3'-dUTP would not interfere with the ATP-requiring step. Therefore, a rapid, convenient synthesis in high yield of 3'-deoxyuridine is needed in order to pursue the DNA studies with 3'-deoxyuridine 5'-triphosphate.

## EXPERIMENTAL

General methods. — All melting points were taken on a Thomas-Hoover silicone-bath apparatus and are uncorrected. Ultraviolet, n.m.r., and mass-spectral data were recorded on a Gilford model 2400 spectrophotometer, a Varian model XL-100-15, and an AEI MS-9 mass spectrometer, respectively. High-pressure liquid chromatographic separations were performed on a Waters Associates liquid chromatograph model ALC/GPC 202 with a micro-Bondapak C-18 column (4 mm  $\times$  30 cm); flow rate, 2 ml/min; chart speed, 0.5 cm/min; solvent, 0.01M dipotassium hydrogenphosphate (pH 6.0). T.l.c. was performed on silica gel GF plates and Avicel F plates. Solvent volumes are v/v. For the nucleoside derivatives, 4:1 benzene-methanol was used: 1,2-dichloroethane was dried by refluxing it for 2 h over phosphorus pentoxide, distilling it, and storing it over molecular sieve (3A). Silica gel (Ramsey and Patterson) was obtained from Mallinckrodt. Methyl 2,5-di-O-benzoyl-3-deoxy- $\beta$ -Derythro-pentofuranoside was a generous gift from Dr. F. Holly, Merck, Sharp and Dohme Research Laboratories.

Synthesis of 3'-deoxyuridine (1) — Method A. From methyl 2,5-di-O-benzoyl 3-deoxy- $\beta$ -D-ribofuranoside (2). Compound 2 (200 mg, 562  $\mu$ mol) was dissolved in 3 ml of dry 1,2-dichloroethane and 0.5 ml of 2,4-bis(trimethylsilyloxy)pyrimidine  $(1,400 \mu mol)$  was added, followed by stannic chloride (0.2 ml) with stirring in a stoppered flask overnight at room temperature. T.l.c. (4:1 benzene-methanol) indicated the completion of the reaction by the absence of 2 ( $R_F$  0.90). The reaction was stopped by adding 0.5 ml of methanol. After stirring for 10 min at 0°, the suspension was filtered through a coarse, sintered-glass funnel. The solid on the funnel was washed with 30 ml of 1,2-dichloroethane. The filtrate and washings were combined and washed twice with 15-ml portions of cold sodium hydrogencarbonate (water saturated), followed by two 15-ml portions of water. The dichloroethane solution was dried over anhydrous sodium sulfate and evaporated to a syrup in vacuo. The syrup was boiled under reflux for 3 h in methanol (12 ml) containing 1 ml of 2M sodium hydroxide. The solvent was removed in vacuo, and the residue was dissolved in 2 ml of water and put onto a column [AG-1 × 8 resin (formate), 4 ml, bottom layer, and AG-50  $\times$  8 (H<sup>+</sup>) 4 ml, top layer]. The column was developed with water and 10-ml fractions were collected. 3'-Deoxyuridine was eluted in tubes 1-6.

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These fractions were combined and evaporated to dryness *in vacuo*. The residue was taken up in 1:195% ethanol-water (1 ml) and crystallized to give 1; m.p. 178-180° ( $it.^{10-12}$  175.5-179°); yield 116 mg (12% based on D-xylose as starting material). Paper chromatography (Whatman 3MM) and t.l.c. (Avicel F and silica gel GF) showed no contamination by u.v.-absorbing material. Avicel F and paper chromatography used 5:1:4 1-butanol-acetic acid-water as solvent, and silica gel GF used 4:1 benzene-methanol as solvent. The u.v. spectra of 1 in acid, alkali, and at pH 7 were identical with that of uridine, which indicated that the product was the 1-N-ribosyl derivative. N.m.r. spectra of this material in D<sub>2</sub>O showed that 3'-deoxy-uridine has a  $J_{1',2'}$  coupling constant of 1.6 Hz, which is identical with the coupling constant for the  $\beta$ -D anomer of 3'-deoxyuridine as reported by Walton *et al.*<sup>12</sup>. The coupling constant for the  $\alpha$ -D anomer<sup>12</sup> of 3'-deoxyuridine is 3.9 Hz.

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Method B. 3'-Deoxyuridine (1), synthesis via transnucleosidation. Compound 3 was synthesized from 3'-deoxyadenosine and benzoyl chloride in pyridine; yield 546 mg (82%); m.p. 179.5-180.5°.

Anal. Calc. for C<sub>38</sub>H<sub>29</sub>N<sub>5</sub>O<sub>7</sub>: C, 68.38; H, 4.35; N, 10.49; O, 16.80. Found: C, 68.24; H, 4.45; N, 10.01; O, 17.05.

Compound 3 (280 mg, 0.42 mmol) was dissolved in dry 1.2-dichloroethane (3 ml) and the solution was cooled in a Dry Ice-acetone bath. 2,4-Bis(trimethylsilyloxy)pyrimidine (0.6 ml; 1.68 mmol) was added, followed by 0.25 ml of stannic chloride. The mixture was brought to room temperature and stirred for 20 h. The mixture was then cooled to  $-70^{\circ}$  and 0.5 ml of methanol added. After thawing, the mixture was filtered through a coarse, sintered-glass funnel. The funnel was washed with five 10-ml portions of 1,2-dichloroethane. The product was isolated exactly as in method A. 3'-Deoxyuridine was eluted in tubes 2-4. The fractions were combined and evaporated; yield 63 mg. The residue was dissolved in methanol (5 ml) mixed with silicic acid (500 mg) and the methanol was removed by evaporation. Chloroform was added and the slurry was added to a silicic acid column (50 ml. packed with chloroform). The column was washed with 200 ml each of 3:17 acetone-chloroform, 3:7 acetone-chloroform, and 1:1 acetone-chloroform, and finally with 3:7 ethanolchloroform; 20-ml fractions were collected. 3'-Deoxyuridine was eluted with ethanolchloroform. The solvent was removed and the syrup was dissolved in 1 ml of ethanol and crystallized; yield 57 mg (61%). The mass spectrum of 3'-deoxyuridine showed a parent ion (m/e 228) and a molecular ion, m/e 229 (M + H<sup>+</sup>). The peak intensity of the uracil plus H<sup>+</sup> ion (m/e 113) was greater than that of the M + H<sup>+</sup> peak at m/e 229. The properties (m.p., n.m.r., u.v., mass spectrometry, elution by high-pressure liquid chromatography) of 1 synthesized by methods A and B were identical.

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