cold water with agitation. The reaction mixture settled overnight in a refrigerator and was then concentrated. The residue was washed with methanol or recrystallized from an appropriate solvent to give the product (Table I).

Preparation of (2-Chloroethyl)nitrosoureido Derivative. A urea was dissolved or suspended in 80–99% formic acid. To the mixture, sodium nitrite (2.6–3.1 mol per each ureido group) was added under ice cooling with agitation. After 1 h, the reaction solution was diluted with an equal volume of water and subsequently treated with Amberlite IR-120 (H^+) resin, except in the case of **12**. The solution was concentrated and the residue was washed with an organic solvent to give the product (Table II). In the case of **12**, the reaction mixture was settled overnight in a refrigerator and the precipitated product, **13**, was collected by filtration.

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References and Notes

(1) J. J. Vavra, C. Deboer, A. Dietz, L. J. Hanka, and W. T. Sokolski, *Antibiot. Annu.*, **1959–1960**, 230 (1960).
(2) W. T. Sokolski, J. J. Vavra, and L. J. Hanka, *Antibiot. Annu.*, **1959–1960**, 241 (1960).
(3) C. Lewis and A. R. Barbiers, *Antibiot. Annu.*, **1959–1960**, 247 (1960).
(4) R. R. Herr, T. E. Eble, M. E. Bergy, and H. K. Jahnke, *Antibiot. Annu.*, **1959–1960**, 236 (1960).
(5) R. R. Herr, H. K. Jahnke, and A. D. Arguodelis, *J. Am. Chem. Soc.*, **89**, 4808 (1967).
(6) E. Hardegger, A. Meier, and A. Stoos, *Helv. Chim. Acta*, **52**, 2555 (1969).
(7) T. Suami and T. Machinami, *Bull. Chem. Soc. Jpn.*, **43**, 2953 (1970).
(8) T. Suami and T. Machinami, *Bull. Chem. Soc. Jpn.*, **43**, 3013 (1970).
(9) A. N. Fujiwara, E. M. Acton, and D. W. Henry, *J. Med. Chem.*, **17**, 392 (1974).
(10) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).
(11) T. P. Johnston, G. S. McCaleb, P. S. Opliger, and J. A. Montgomery, *J. Med. Chem.*, **9**, 892 (1966).
(12) T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **18**, 104 (1975).
(13) T. Anderson, M. G. McMenamin, and P. S. Schein, *Cancer Res.*, **35**, 761 (1975).
(14) P. A. Fox, L. C. Panasci, and P. S. Schein, *Cancer Res.*, **37**, 783 (1977).
(15) L. C. Panasci, D. Green, R. Nagourney, P. A. Fox, and P. S. Schein, *Cancer Res.*, **37**, 2615 (1977).
(16) T. Machinami, S. Nishiyama, K. Kikuchi, and T. Suami, *Bull. Chem. Soc. Jpn.*, **48**, 3763 (1975).
(17) T. Hisamatsu and S. Uchida, *Gann*, **68**, 819 (1977).
(18) The nomenclature used in this paper follows the IUPAC-IUB tentative cyclitol nomenclature rules described in *J. Biol. Chem.*, **243**, 5809 (1968). All the compounds except for meso compounds are racemic. All the formulas depict only one enantiomer of the racemic form actually obtained in the experiments.
(19) T. Suami, K. Tadano, S. Nishiyama, and F. W. Lichtenthaler, *J. Org. Chem.*, **38**, 3691 (1973).
(20) T. Suami, Y. Sakota, K. Tadano, and S. Nishiyama, *Bull. Chem. Soc. Jpn.*, **44**, 222 (1971).
(21) T. Suami, S. Nishiyama, K. Tadano, and F. W. Lichtenthaler, *Bull. Chem. Soc. Jpn.*, **46**, 2562 (1973).
(22) T. Suami, S. Ogawa, S. Naito, and H. Sano, *J. Org. Chem.*, **33**, 2831 (1968).
(23) T. Suami, K. Tadano, and S. Horiuchi, *Bull. Chem. Soc. Jpn.*, **48**, 2895 (1975).
(24) F. W. Lichtenthaler and H. O. L. Fischer, *J. Am. Chem. Soc.*, **83**, 2005 (1961).
(25) W. T. Bradner and D. J. Hutchison, *Cancer Chemother. Rep.*, **50**, 79 (1966).
(26) R. I. Geran, N. N. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 9 (1972).

5-Substituted Uracil Arabinonucleosides as Potential Antiviral Agents

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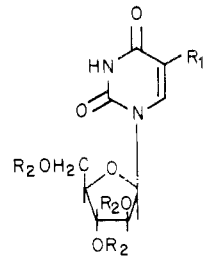
Four 5-substituted analogues of 1-(β-D-arabinofuranosyl)uracil were prepared and evaluated as antiviral agents. 1-(β-D-Arabinofuranosyl)-5-(propynyloxy)uracil (**5**) was prepared by the propargyl bromide alkylation of 1-(β-D-arabinofuranosyl)-5-hydroxyuracil (**6**) which was synthesized by reaction of 1-(β-D-arabinofuranosyl)uracil with bromine–water–pyridine. Compound **6** could also be prepared by bromine–water–pyridine treatment of 1-(2,-3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil, followed by removal of the acetyl groups by NH_3–CH_3OH. 1-(β-D-Arabinofuranosyl)-5-cyanouracil (**4**) was synthesized by basic cleavage of O^2-2′-anhydro-5-cyanouridine which was prepared by reaction of 5-cyanouridine with diphenyl carbonate in hexamethylphosphoramide. 1-(β-D-Arabinofuranosyl)-5-nitrouracil (**1**) was obtained by nitration of 2′,3′,5′-tri-*O*-(3,5-dinitrobenzoyl)uridine (**2**) with fuming HNO_3–H_2SO_4, followed by removal of the protecting groups with NaOEt–EtOH. Compounds **1**, **4**, and **6** were devoid of significant antiviral activity against herpes simplex (type 1) virus, vaccinia virus, and vesicular stomatitis virus in primary rabbit kidney cell cultures and human skin fibroblasts. The propynyloxy analogue, **5**, showed an anti-herpes virus activity comparable to 1-(β-D-arabinofuranosyl)uracil but was substantially less active than 1-(β-D-arabinofuranosyl)thymine.

Recently, the syntheses and antiviral activities of three new 5-substituted thymidine analogues have been reported, namely, 5-nitro-[1], 5-cyano-[2], and 5-(propynyloxy)-2′-deoxyuridines.[3] These substitutents endow 2′-deoxyuridine with unusual properties: 5-cyano-2′-deoxyuridine may be regarded as a specific anti-vaccinia virus agent[2b] that is not incorporated into DNA;[2a] 5-propynyloxy-2′-deoxyuridine possesses potent in vitro anti-herpes activity with remarkably low toxicity toward the replicating host cell;[3,4] 5-nitro-2′-deoxyuridine shows potent inhibitory activity toward both vaccinia virus and herpes simplex virus, and its mode of action seems to be targeted at thymidylate synthetase.[1b,c] *ara*-T [1-(β-D-arabinofuranosyl)thymine] recently has been the subject of a number of papers describing its selective inhibitory effects against herpes simplex and zoster viruses,[5-7] while *ara*-U[8] itself and 5-

thiocyano-[9a] and 5-Et-*ara*-U[9b] show significant in vitro antiviral activity, particularly against herpes simplex virus. We wished to determine what effect the introduction of a nitro, propynyloxy, and cyano group at the 5 position of the pyrimidine ring would have on the antiviral activity of *ara*-U.

Chemistry. We found that the most rewarding approach to **3** followed that developed by Wempen et al.,[10] who used the 3,5-dinitrobenzoyl protecting group for the synthesis of 5-nitrouridine. The approach of employing the 3,5-dinitrobenzoyl protecting group (deactivated toward nitration) succeeds in this instance and with uridine itself but fails in the case of 2′-deoxyuridine.[11] Thus, **1**



1, $R_1 = NO_2$; $R_2 = H$
2, $R_1 = H$; R_2 = 3,5-dinitrobenzoyl
3, $R_1 = NO_2$; R_2 = 3,5-dinitrobenzoyl
4, $R_1 = CN$; $R_2 = H$
5, $R_1 = OCH_2C{\equiv}CH$; $R_2 = H$
6, $R_1 = OH$; $R_2 = H$

could be obtained in an overall yield of 20% by blocking the 2′,3′- and 5′-OH groups of *ara*-U with 3,5-dinitrobenzoyl chloride, nitration of the protected arabinonucleoside **2** with sulfuric acid–fuming HNO_3, followed by deblocking **3** with NaOEt in EtOH. The assigned structure, **1**, was supported by (1) elemental analysis, (2) a yellow color reaction with $SnCl_2$–*p*-dimethylaminobenzaldehyde (diagnostic of aromatic NO_2),[12] (3) the lack of color reaction with periodate–benzidine[13] (no *cis*-glycol), (4) the presence of NO_2 absorption bands in the infrared spectrum, and (5) the ^1H NMR spectrum which showed, most characteristically, the disappearance of the C_5-H doublet of unsubstituted uracils, a singlet corresponding to C_6-H, and the presence of signals that could be assigned to the 2′,3′- and 5′-OH's and the 1′,2′- and 3′-methine protons. Additionally, the UV spectral behavior of the product demonstrated a nitrouracil moiety. 5-Nitrouridine shows a dramatic shift of λ_{max} when the pH changes from 6 to 12. Our nitration product underwent such characteristic changes and had λ_{max} values and ϵ ratios virtually identical to that of 5-nitrouridine.

The cyano analogue **4** was prepared from 5-cyanouridine[2] using the hexamethylphosphoramide–diphenyl carbonate method.[14] The intermediate 2,2′-anhydronucleoside was not isolated but was opened under basic conditions to the arabino nucleoside. The periodate–benzidine negative product, isomeric with 5-cyanouridine by elemental analysis, showed the characteristic 2240-cm^{-1} cyano frequency in the infrared spectrum, and its UV (λ_{max} 276 nm) was typical of 5-cyanouracil derivatives.[2]

The propynyloxy analogue **5** was prepared by the propargyl bromide alkylation[3,15] of 1-(β-D-arabinofuranosyl)-5-hydroxyuracil (**6**), which was prepared by two different synthetic approaches. In the first method, the triacetate of uracil arabinoside was reacted with bromine–water, and the intermediate 5-bromo-6-hydroxydihydrouracil derivative was treated with pyridine to eliminate HBr. Removal of the acetyl groups with CH_3OH–NH_4OH gave the product **5** in 50% yield. 5-OH-*ara*-U (**6**) could also be prepared in one step from *ara*-U by the bromine–water–pyridine procedure to give the desired product in 42% yield. The ferric chloride positive products of both sequences were identical by all criteria, including melting point, TLC chromatographic behavior, and spectral characteristics (see Experimental Section).

Table I. 5-Substituted Pyrimidine Arabinonucleosides Effect on Virus-Induced Cytopathogenicity in Primary Rabbit Kidney Cells[a,c]

compd[d]	X in *ara*-5-X-U	ID_{50},[b] M: vaccinia virus	ID_{50},[b] M: herpes simplex virus
ara-U	-H	8.2×10^{-4}	4.1×10^{-5}
ara-T	$-CH_3$	3.9×10^{-6}	3.9×10^{-7}
1	$-NO_2$	$>6.9 \times 10^{-4}$	$>6.9 \times 10^{-4}$
4	-CN	$>3.7 \times 10^{-4}$	$>3.7 \times 10^{-4}$
5	$-OCH_2F{\equiv}CH$	$>6.7 \times 10^{-4}$	6.7×10^{-5}
6	-OH	$>7.7 \times 10^{-4}$	$>3.9 \times 10^{-4}$

[a] All the above arabinonucleosides were inactive ($ID_{50} > 10^{-4}$ M) against an RNA virus (vesicular stomatitis virus). [b] $ID_{50} \equiv$ dose inhibiting virus-induced cytopathogenicity by 50%. [c] Similar, although not identical, antiviral activities were shown by the above arabinonucleosides when they were assayed for their ability to inhibit the cytopathogenicity of herpes simplex (KOS) or vaccinia viruses in human skin fibroblasts. [d] At the concentrations employed, no discernable microscopic alteration of normal cell morphology occurred in the presence of these arabinonucleoside analogues. In addition, neither compound **1**, **4**, **5**, or **6** exerted an inhibitory effect on DNA synthesis, as monitored by labeled thymidine or 2′-deoxyuridine incorporation.

Antiviral Activity. The antiviral activities of the analogues **1** and **4**–**6** were determined in primary rabbit kidney cell cultures or cultures of human skin fibroblasts infected with either herpes simplex type 1 virus, vaccinia virus, or vesicular stomatitis virus (Table I). In each instance, when the 5-H of *ara*-U was replaced by the CN, NO_2, OH, or propynyloxy substituents, there was a dramatic decrease in antiviral activity. Of all the analogues, only the propynyloxyarabinonucleoside **5** showed moderate antiviral activity in the same concentration range as the parent *ara*-U.

Why should the anti-herpes virus activity of *ara*-U or *ara*-T be abolished by the introduction of a nitro group at pyrimidine C-5? Increase in steric bulk does not seem to be a likely explanation, since the effective radius of the nitro group can be considered as 2.5 Å,[16] a value not much greater than the Van der Waals radii for the iodo (2.15 Å[17]) or methyl (2.0 Å[17]) groups, both of which give active antivirals.[5-7,17] Furthermore, steric bulk at pyrimidine C-5 cannot be invoked to rationalize the loss of activity of the cyano analogue **4**. The cyano group is comparable in size to the chloro and bromo substituents,[16] and both 5-Cl- and 5-Br-*ara*-U have been reported to possess significant antiviral activity.[18]

Electronic factors, manifested as the pK_a of the pyrimidine N_3-H, might explain the inactivity of the nitro analogue **1** and the cyano analogue **4**. The pK_a of **1** is 5.7[19] and the pK_a of **4** is 6.8,[20] compared to pK_a values of 8 ± 0.3 for active antivirals such as 5-I- or 5-Br-*ara*-U.[21] Arguments based on pK_a cannot be made for the 5-hydroxy analogue **6**, however, since its pK_a is 7.8,[22] virtually the same as other active arabinonucleoside antivirals.

The lipophilicity of the 5 substituent also may influence the antiviral activity of the derived arabinonucleoside. If the partitioning hydrophobic substituent constant,[23] π, is used as a measure of group lipophilicity, then it is apparent that those substituents that are hydrophilic in nature, NO_2 ($\pi = -0.85$), CN ($\pi = -0.84$), and OH ($\pi = -1.16$), give

arabinonucleosides with no antiviral activity. Substituents of intermediate lipophilicity, H (π = 0.0) and $OCH_2C{\equiv}CH$ (π = 0.0), give arabinonucleosides with moderate, but significant, antiviral properties. Finally, substituents of increased lipophilicity, CH_3 (π = +0.50), $-CH_2CH_3$ (π = +1.0), Cl (π = +0.39), Br (π = +0.60), and I (π = +1.0), give potent antiviral agents.[5-7,9,18]

In summary, this work, shows that, in contrast to the case of 5-substituted uracil 2'-deoxyribonucleosides where a large variety of functional groups gives rise to specific antiviral agents,[1-3] the antiviral activity of 5-substituted uracil arabinonucleosides is critically dependent on the nature of the 5 substitutent.

Experimental Section

1-(β-D-Arabinofuranosyl)uracil and its thymine analogue were purchased from Terra-Marine Bioresearch (La Jolla, Calif.). 1-(β-D-Arabinofuranosyl)uracil was also prepared by CH_3OH–NH_4OH cleavage of 2,2'-anhydrouridine prepared according to ref 14. 5-Nitrouridine was obtained from Dr. Harry Wood of the National Cancer Institute and was also prepared according to ref 10. 5-Cyanouridine was prepared as previously described.[2b] Propargyl bromide, 3,5-dinitrobenzoyl chloride, and uridine were from Aldrich Chemicals (Milwaukee, Wis.). Melting points (uncorrected) were determined on a Thomas-Hoover apparatus and the following spectra as indicated: UV on a Cary 15, ^1H NMR on a Varian HA-100 or Fourier transform NMR on a FX 100, infrared on a Perkin-Elmer Infracord, and chemical-ionization mass spectra (CIMS) on a Finnigan 1015 D gas chromatograph–mass spectrometer. Me_4Si was the standard for the NMR spectra, and chemical shifts are reported in parts per million (δ). Signals are described as s (singlet), d (doublet), t (triplet), and m (multiplet). Microanalyses were determined by the staff of the microanalytical section of this laboratory. Thin-layer chromatography was performed using silica gel GF plates (Analtech). Methodology used for the assay of antiviral activity has already been described.[9a]

1-[2',3',5'-Tri-*O*-(3,5-dinitrobenzoyl)-β-D-arabinofuranosyl]uracil (2). 1-(β-D-arabinofuranosyl)uracil (1.5 g, 6.14 mmol) was dissolved in anhydrous pryidine (60 mL), and 3,5-dinitrobenzoyl chloride (4.67 g, 20.4 mmol or 3.3 equiv) was added to this solution. The reaction mixture was warmed with exclusion of moisture at 50 °C for 48 h until TLC (cellulose, 2-propanol–1% $(NH_4)_2SO_4$, 2:1, v/v) revealed no starting material. Pyridine was removed by in vacuo evaporation, and then cold H_2O (1000 mL) was added to the viscous residue. The precipitate was stirred and crushed until it was completely granular and then collected by centrifugation and washed twice with H_2O. After in vacuo drying at 60 °C, the crude product was crushed to a powder and suspended in absolute EtOH (200 mL), and the resulting suspension was refluxed with stirring for 3 h. The supernatant was removed by decantation, an additional 200 mL of absolute EtOH was added to the remaining residue, and this mixture was again stirred and refluxed for 3 h. The remaining insoluble residue was removed by filtration and dried in vacuo to give crude **2**. The ^1H NMR spectrum was consistent with tri(*O*-dinitrobenzoyl)ation.

1-[2,3,5-Tri-*O*-(3,5-dinitrobenzoyl)-β-D-arabinofuranosyl]-5-nitrouracil (3). The protected arabinoside (**2**, 6.59 g, 7.97 mmol) was dissolved in concentrated H_2SO_4–fuming HNO_3 (22 mL, 1:1, v/v), and the resulting solution was stirred at room temperature for 30 min. The reaction mixture was then poured into cold H_2O (2000 mL) with vigorous stirring. The precipitate that formed was filtered off, washed with H_2O until the washings were neutral, and then dried in vacuo. The ^1H NMR spectrum of this crude product showed the disappearance of the doublets usually assigned to the 5 and 6 H of uracil and the appearance of a new singlet corresponding to C-6 H.

1-(β-D-Arabinofuranosyl)-5-nitrouracil (1). Sodium ethoxide in absolute ethanol (76 mL of a solution of 1.2 g of Na in 250 mL of EtOH) was added to **3** (7.06 g, 8.1 mmol) suspended in 307 mL of absolute ethanol. This mixture was refluxed for 3 h during which time the color became deep pink. At no time did the solution of the solid become complete. After cooling, the precipitate was filtered off, washed with ethanol and then Et_2O, and dried. The yield of the presumed sodium salt of the arabinoside of 5-nitrouracil was 2.03 g. This solid (2.03 g) was then suspended in absolute EtOH (50 mL) at 70 °C and concentrated H_2SO_4 (ca. 0.3 mL) was added dropwise until the solution was acidic to test paper. The precipitate that formed was removed by filtration, and the filtrate was concentrated to 5 mL in vacuo.

After cooling, the crystals that formed were collected by filtration and recrystallized from methanol to give 480 mg (20.5%) of 1-(β-D-arabinofuranosyl)-5-nitrouracil (**1**): mp 197 °C (dec); UV λ_{max} (pH 1) 240 nm (ϵ 8600) and 306 (ϵ 10 200), λ_{max} (pH 6) 237 (ϵ 7800) and 310 (ϵ 10 900), λ_{max} (pH 12) 280 (sh) and 324 (ϵ 16 900); IR ν_{max} 1340 and 1510 cm^{-1} (NO_2); ^1H NMR (Me_2SO-d_6) δ 12.08 (s, 1, NH), 9.04 (s, 1, H-6), 6.05 (d, 1, J = 4 Hz, H-1'), 5.73 (d, 1, J = 5 Hz, 2'-OH), 5.55 (d, 1, J = 4 Hz, 3'-OH), 5.18 (t, 1, 5'-OH), 4.08 (m, 1, 2'-H), 3.98 (m, 1, 3'-H), 3.85 (m, 1, 4'-H), 3.64 (addition, resolved the multiplet at 4.16–3.88 into two distinct triplets assignable to the C-2' H and C-3' H). The HOD peak obscured the remainder of the multiplet. Anal. C, H, N. The 2', 3' and 4' protons were assigned on the basis of decoupling experiments. The chromatographic behavior of **1** on cellulose TLC was compared with an authentic sample of 5-nitrouridine. In 2-propanol–1% $(NH_4)_2SO_4$ (2:1, v/v) as developing solvent, 5-nitrouridine had R_f 0.63, while the arabinoside IV had R_f 0.86. Furthermore, **1**, like 1-(β-D-arabinofuranosyl)uracil, failed to give a positive *cis*-glycol color reaction with periodate–benzidine spray reagent,[13] whereas both uridine and 5-nitrouridine gave the characteristic white spot on a blue background. Finally, both 5-nitrouridine and the arabinoside **1** gave a light-yellow color with $SnCl_2$–*p*-dimethylaminobenzaldehyde spray reagent[12] under conditions where no reaction occurred with uridine or 1-(β-D-arabinofuranosyl)uracil.

1-(β-D-Arabinofuranosyl)-5-cyanouracil (4). 5-Cyanouridine (269 mg, 1 mmol) and diphenyl carbonate (321 mg, 1.5 mmol) were dissolved in hexamethylphosphoramide (1 mL) and, after addition of $NaHCO_3$ (10 mg), the mixture was heated at 150 °C in an oil bath for 20 min. After cooling, the reaction mixture was poured into H_2O (10 mL) and extracted three times with $CHCl_3$. A 5% NH_4OH solution (5 mL) was added to the aqueous phase, and the clear solution was left at ambient temperature overnight. The residue, obtained by evaporation in vacuo, was dissolved in warm MeOH. Refrigeration gave colorless crystals of **4**: mp 231–232 °C (dec); yield 135 mg (50%); IR ν_{max} 2240 cm^{-1}; CIMS (NH_3) m/e 277 (P + 18, 15%); UV λ_{max} (H_2O) 276 nm (ϵ 13 900); ^1H NMR (Me_2SO-d_6) δ 12.00 (br s, 1, N_3-H), 8.45 (s, 1, H-6), 5.97 (d, J = 4 Hz, 1, H-1'), 5.62 (d, J = 5 Hz, 1, 2'-OH), 5.48 (d, J, = 4 Hz, 1, 3'-OH), 5.17 (br t, $J \sim$ 4 Hz, 1, 5'-OH), ~3.9 (m, 3, H-2'–4', 3.62 (t, J = 4 Hz, 2, H-5'). The hydroxy signals and the H-2'–5' protons could be assigned on the basis of decoupling experiments, and when D_2O was added the spectrum simplified (with loss of hydroxy and NH signals) to δ 4.05 (t, $J_{2'3'}$ = 3 Hz, $J_{1'2'}$ = 4 Hz, 1, H-2'), 3.91 (t, 1, H-3'), 3.82 (m, 1, H-4'). Anal. C, H, N.

1-(β-D-Arabinofuranosyl)-5-hydroxyuracil (6). Method 1. Bromine–water was added dropwise to a solution of 1-(β-D-arabinofuranosyl)uracil (2.44 g, 0.01 mol) in water (150 mL) until a light-yellow color persisted. The solution was then stirred vigorously until it became colorless. Pyridine (112 mL) was added in small aliquots so as to keep the temperature below 25 °C. The solution was stored at room temperature overnight, and then the solvent was removed in vacuo to yield a viscous syrup. Absolute EtOH (200 mL), warmed to 50 °C, was added and the resulting suspension was filtered. The filtrate was evaporated to half its original volume and was refrigerated overnight. This procedure gave 1.12 g (52%) of colorless crystals that had a mp of 252–254 °C and gave a blue color reaction with $FeCl_3$: CIMS (NH_3) m/e 261 (P + 1, 80%) and 278 (P + 18 40%); UV λ_{max} (pH 1) 282 nm (ϵ 8940), λ_{max} (pH 11) 305 nm (ϵ 6750), A_{280}/A_{260} = 1.89 at pH 11 and 0.92 at pH 11; ^1H NMR (Me_2SO-d_6) 11.36 (br s, 1, N_3-H), 8.53 (br s, 1, 5 OH), 7.22 (s, 1, H-6), 6.01 (d, J = 4 Hz. 1, H-1'), 5.52 (d, J = 4 Hz, 1, 2'-OH), 5.43 (d, J = 4 Hz, 1, 3'-OH), 5.04 (t, J = 4 Hz, 1, 5'-OH), 3.93 (m, 2, H-2' and H-3'), 3.62 (m, 3, H-4' and 5'-CH_2). Anal: C, H, N.

Method 2. 1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)uracil (1 g, 3.5 mmol), prepared as described elsewhere, was suspended in 50 mL of H_2O and the mixture warmed. Bromine was added dropwise until the solution retained a yellow tint. The solution was aerated to remove excess bromine, then pyridine (5 mL) was added, and the mixture warmed at 37 °C overnight. Repeated

evaporation from absolute ethanol afforded 1 g of viscous product, 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-hydroxyuracil, which was used directly in the next step.

The acetylated 5-hydroxyuracil derivative (1 g) was dissolved in CH_3OH, and the solution was saturated at 0 °C with NH_3 gas. After overnight reaction, the solution was evaporated to dryness and acetamide was sublimed off at 60 °C (0.1 mmHg). Evaporation of the residue from absolute EtOH produced crystals of **6**. Recrystallization from EtOH–H_2O (1:1) yielded 517 mg (50%) of **6**: mp 252–254 °C.

1-(β-D-Arabinofuranosyl)-5-(propynyloxy)uracil (5). 1-(β-D-Arabinofuranosyl)-5-hydroxyuracil (**6**, 650 mg, 2.5 mmol) was dissolved in methanol–water (1:1) containing KOH (2.5 mmol). Propargyl bromide (439 mg, 3.7 mmol) was added to this stirred solution. After overnight reaction at room temperature, the solvent was evaporated in vacuo. Absolute ethanol was added to the viscous residue, and the resulting solution was stored at 4 °C for several days. The colorless crystals (432 mg, 54%) that formed were collected by filtration: mp 163–166 °C; UV λ_{max} (H_2O) 277 nm (ε 8940); CIMS (NH_3) *m/e* 299 (P + 1, 100%); ^1H NMR (Me_2SO-d_6) 11.46 (br s, 1, N_3-H), 7.52 (s, 1, H-6), 5.97 (d, *J* = 4-Hz, 1, H-1′), 5.54 (d, *J* = 5 Hz, 1, 2′- or 3′-OH), 5.40 (d, *J* = 4 Hz, 1′-, 2′- or 3′-OH), 5.07 (t, *J* = 4 Hz, 1, 5′-OH), 4.56 (d, *J* = 2.5 Hz, 2, propynyl CH_2), 3.98 (m, 2, H-2′ and H-3′), 3.64 (m, 3, H-4′ and H-5′ H's), 3.51 (t, *J* = 2.5 Hz, 1, acetylene H). Anal. C, H, N.

References and Notes

(1) (a) G.-F. Huang and P. F. Torrence, *J. Org. Chem.*, **42**, 3821–3824 (1977); (b) E. De Clercq, J. Descamps, G.-F. Huang, and P. F. Torrence, *Mol. Pharmacol.*, **14**, 422–430 (1978); (c) M. P. Mertes, C. T. C. Chang, E. De Clercq, G.-F. Huang, and P. F. Torrence, *Biochem. Biophys. Res. Commun.*, **84**, 1054–1059 (1978).

(2) (a) R. C. Bleackley, A. S. Jones, and R. T. Walker, *Nucleic Acids Res.*, **2**, 683–690 (1975); (b) P. F Torrence, B. Bhooshan, J. Descamps, and E. De Clercq, *J. Med. Chem.*, **20**, 974–976 (1977).

(3) P. F. Torrence, J. W. Spencer, A. M. Bobst, J. Descamps, and E. De Clercq, *J. Med. Chem.*, **21**, 228–231 (1978).

(4) E. De Clercq, J. Descamps, P. F. Torrence, E. Krajewska, and D. Shugar, *Proc. Int. Congr. Chemother., 10th, 1977*, Zurich, Swizerland, 352–354, 1978.

(5) G. A. Gentry and J. F. Aswell, *Virology*, **65**, 294–296 (1975).

(6) J. F. Aswell, G. P. Allen, A. T. Jamieson, D. E. Campbell, and G. A. Gentry, *Antimicrob. Agents Chemother.*, **12**, 243–254 (1977).

(7) R. L. Miller, J. P. Iltis, and F. Rapp, *J. Virol.*, **23**, 679–684 (1977).

(8) E. De Clercq, E. Krajewska, J. Descamps, and P. F. Torrence, *Mol. Pharmacol.*, **13**, 980–984 (1977).

(9) (a) E. De Clercq, P. F. Torrence, J. A. Waters, and B. Witkop, *Biochem. Pharmacol.*, **24**, 2171–2175 (1975); (b) E. De Clercq, J. Descamps, and D. Shugar, unpublished observations.

(10) I. Wempen, I. L. Doerr, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **82**, 1624–1629 (1960).

(11) G.-F. Huang and P. F. Torrence, unpublished observations.

(12) M. Jurecek, J. Characek, and V. Cervinka, *Mikrochim. Acta*, 102 (1960).

(13) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954). **Caution**: Benzidine is a carcinogen [see *J. Chem. Educ.*, **51**, A322 (1974)].

(14) (a) J. P. H. Verheyden, D. Wagner, and J. Moffatt, *J. Org. Chem.*, **36**, 250–254 (1971); (b) A. Hampton and A. W. Nichol, *Biochemistry*, **5**, 2076–2082 (1966).

(15) (a) B. A Otter, A. Taube, and J. J. Fox., *J. Org. Chem.*, **36**, 1251–1255 (1971); (b) B. A. Otter, S. S. Saluja, and J. J. Fox, *ibid.*, **37**, 2858–2863 (1972).

(16) W. A. Sheppard, in "The Chemistry of the Cyano Group", Z. Rappoport, Ed., Interscience, New York, N.Y., 1970, pp 209–237.

(17) L. Pauling, "The Nature of the Chemical Bond", Cornell University Press, Ithaca, N.Y., 1960, p 260.

(18) F. M. Schabel, Jr., and J. A. Montgomery, *Int. Encycl. Pharmacol. Ther.*, **1**, 231–363 (1972).

(19) The spectrophotometric pK_a of **1** was determined at 25 °C using Teorell–Stenhagen buffers.

(20) The pK_a values of 1-(β-D-arabinofuranosyl)-5-iodouracil and its 5-bromo analogue have not been reported, but it is reasonable to assume that they are approximately the same as the values for the corresponding 2′-deoxyribonucleoside analogues; i.e., 8.2 in the case of iodo and 7.9 in the case of bromo [cf. K. Berens and D. Shugar, *Acta Biochem. Pol.*, **10**, 25–47 (1963)].

(21) Again, the pK_a value for the deoxyribonucleoside (cf. ref 2b) has been used to approximate the pK_a of **4**.

(22) The value for the ribonucleoside was used as an approximation [cf. S. Roy-Burman, P. Roy-Burman, and D. W. Visser, *J. Biol. Chem.*, **241**, 781–786 (1966)].

(23) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525–554. For the sake of argument, aliphatic π values were used here as given in the cited reference or calculated from known values. No allowance has been made for possible effects of intramolecular hydrogen bondings as in the case of the OH substituent.

(24) R. J. Cushley, K. A. Watanabe, and J. J. Fox., *J. Am. Chem. Soc.*, **89**, 394–400 (1967).

Enzyme Affinity of the 5,6-Dihydro Derivatives of the Substrate and Product of Thymidylate Synthetase Catalysis

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The 5,6-dihydro derivatives of 2′-deoxyuridine 5′-phosphate (**2**) and 2′-deoxythymidine 5′-phosphate (**3**) were synthesized and characterized. The affinities of **2** and **3** were compared to those of the substrate (2′-deoxyuridine 5′-phosphate) and product (2′-deoxythymidine 5′-phosphate) of the reaction catalyzed by thymidylate synthetase. In both cases, the enzyme affinity of the 5,6-dihydro derivatives was 50 times less than that of the substrate or product. The conclusions from this study are that a noncovalent complex of enzyme and a dihydro substrate or dihydro product is improbable in thymidylate synthetase catalysis and the covalent enzyme–substrate complex is more reasonable.

Thymidylate synthetase catalyzes the conversion of 2′-deoxyuridine 5′-phosphate (dUMP) to 2′-deoxythymidine 5′-phosphate (dTMP).[1] The cofactor, tetrahydrofolic acid, partakes in the reaction initially as the carbon-transferring agent by the activation of formaldehyde or the equivalent biological source of formaldehyde. Subsequent cofactor involvement is through alkylation of the 5-carbon of the substrate, followed by reduction of the intermediate complex and release of the oxidized cofactor 7,8-dihydrofolate.

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