Synthesis and Biological Activities of Some Uronic Acids, Uronates, Uronamides, and Urononitriles of Pyrimidine Nucleosides[1]

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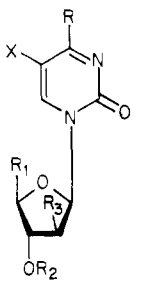
The 5′-hydroxymethylene function of several uracil and cytosine nucleosides has been modified to produce a variety of uronic acids, uronates, uronamides, and urononitriles of 2′-deoxy-β-D-*erythro*-pentofuranosyl- and β-D-*arabino*-pentofuranosylpyrimidines. In addition, the 5 position in many of these nucleosides has been substituted by a halogen atom. Twenty-one of the 35 compounds synthesized and examined for biological activity have not been previously reported. The purity of the products was measured by a high-pressure liquid chromatographic method. They were then evaluated as potential growth inhibitors of murine Sarcoma 180 cells in culture, of herpes simplex virus type 1 in vitro, and of *Streptococcus faecium*, a folic acid or deoxythymidine dependent bacterial strain. The ability of these nucleoside analogues to inhibit the phosphorylation of deoxythymidine by herpes simplex virus type 1 encoded pyrimidine deoxyribonucleoside kinase was also investigated and a structure–activity relationship examined.

We have been interested in the synthesis of nucleoside and nucleotide analogues that are capable of being transported into a cell with a subsequent inhibition of enzymes involved in the synthesis of DNA. Recent interest in the modification of the hydroxymethylene moiety in the 5′ position of nucleosides[2] prompted us to reexamine the biological activity of a variety of compounds in which this group was replaced with a carboxyl, ester, amide, or nitrile in several pyrimidine nucleosides, some of which were substituted at C-5 with a halogen (Chart I). The biological activity of nucleosides modified in this position has been reported.[3,4] For instance, 9-(β-D-*erythro*-pentofuranuronic acid)adenine and several of its ester and amide derivatives are potent, nontoxic, coronary[5,6] and renal vasodilators.[7] Similarly, the uronic acid and uronate analogues of 5-fluoro-2′-deoxyuridine (FdUrd) are toxic to some neoplastic cell lines.[8] Studies of deoxythymidine variants as deoxythymidylate kinase inhibitors[9] have shown that the urononitrile analogue of deoxythymidine had comparable activity to 5′-fluoro-2′,5′-dideoxythymidine.[10] However, the uronic acid and methyl uronate analogues of deoxythymidine were found to be poor inhibitors of deoxythymidine kinase isolated from Walker 256 carcinoma.[11] Although some of these derivatives have been tested against a wide variety of bacterial, viral, and tumor strains[2,8] and their enzymic inhibitory properties determined, no systematic biological and structural examination has been made. The present report describes the synthesis of some new pyrimidine nucleosides in which the 5′-hydroxymethylene moiety has been modified and the effect of these, as well as other previously synthesized 5′-modified analogues, on the reproduction of herpes simplex virus type 1, Sarcoma 180 cells, and *Streptococcus faecium*. Furthermore, the effect of these compounds on the phosphorylation of deoxythymidine by the herpes simplex virus type 1 encoded pyrimidine deoxyribonucleoside is also described, and the structure–activity relationships of some of these compounds are discussed.

Chemistry. Oxidation of hydroxymethylene groups to carbonyls is one of the most common transformations of carbohydrates.[12] Several methods are available for the selective and mild oxidation of the C-5′ hydroxyl group of nucleosides.[13-15] The products obtained are useful precursors for synthesizing 3′,4′-unsaturated nucleosides[16,17] and nucleoside-specific synthetic antigens[18,19] and for effecting the stepwise degradation of oligonucleotides.[20] In this present study, the 5′-hydroxymethylene groups of the pyrimidine nucleosides were oxidized using platinum[21] as catalyst utilizing a modification of the methods described by Moss et al.[13] and Imai and Honjo.[22] Thus, the uronic acids of both the uracil and cytosine series, **1–8**, were formed from the corresponding 5′-hydroxymethylene-

Chart I. Structural Modifications of the Pyrimidine Nucleosides



R_1 = COOH (uronic acid)
R_1 = COOMe (methyl uronate)
R_1 = $CONH_2$ (uronamide)
R_1 = CN (urononitrile)
R = OH, NH_2
R_2 = H, COMe
R_3 = H, OH
X = H, F, Br, I, Me

pyrimidine nucleoside at 70 °C in bicarbonate-buffered solutions at pH 8 or 9 (the lower pH value was necessary for the more base-sensitive nucleosides). Oxidation was more efficient in dilute solutions of nucleosides. The reaction was monitored by TLC and paper electrophoresis and proceeded faster for the uracil than for the cytosine series. The ease of oxidation of both series of nucleosides varied with the nature of the substituent in the 5 position of the pyrimidine moiety and was in the order H > CH_3 > F > Br > I. Before proceeding with the next step in the synthesis it was necessary to ascertain that the 5-halogen-substituted pyrimidineuronic acids, which were isolated by repetitive anion-exchange chromatography, did not contain traces of either starting material or hydrolysis product. This is very important since these impurities cannot be detected by conventional methods and some are biologically active even at minute concentrations. Using a high-pressure liquid chromatographic method, the acids were clearly separated from the parent pyrimidine bases and nucleosides on a reverse-phase column using a methanol–water gradient system. The retention times increased linearly with the size of the substituent on the pyrimidine base (Table I). The concentrations of the respective impurities in the uronic acids were less than 0.005%. Because of the very potent inhibitory activity of FdUrd, a more sensitive assay utilizing radioactive nucleoside was devised (see the Experimental Section).

The chromatographically pure uronic acids were converted sequentially into their respective uronates **10–17**, uronamides **19–27**, and urononitriles **33–35** by the method of Baker et al.[2] The uronic acids were esterified to the methyl esters with absolute methanol containing a catalytic

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Table I. LC of Parent Nucleosides, Bases, and Uronic Acids[a]

compd[c]	retention time, min	compd	retention time, min
FUra	5.29	3	2.97
FdUrd	7.93	BrdCyd	16.53
5	2.43	8	3.05
BrUra	7.69	IdCyd	19.87
BrdUrd	12.16	7	3.35
4	2.56	*ara*-C	2.07[b]
IUra	8.85	9	2.56[b]
IdUrd	14.41		

[a] Du Pont 830 liquid chromatograph. Conditions: Partisil-10 ODS (Whatman) column, 25 cm × 4.6 mm i.d., ambient temperature; mobile phase A = H_2O and B = 20% MeOH in H_2O, linear gradient 0–100% at 5% B/min; inlet pressure 500 psi; flow rate 0.75 mL/min; detection at 254 nm; reproducibility within ± 2%. [b] Conditions: Partisil-10 SCX (Whatman) column; mobile phase 0.2 M sodium acetate, pH 5; isocratic; inlet pressure 800 psi; flow rate 1.4 mL/min. [c] See ref 28.

amount of concentrated sulfuric acid, and treatment of the uronates with aqueous ammonia gave the amides. Acetylation of the uronamides with acetic anhydride gave the 3′-*O*-acetyl derivatives which were dehydrated with phosphoryl chloride in pyridine at 0 °C, and the resulting 3′-O-acetylated urononitriles were isolated by chromatography on silica. Deblocking with dilute ammonia gave the appropriate urononitrile in good overall yield. By the same route, 1-(β-D-*arabino*-pentofuranosyl)cytosine (*ara*-C) was oxidized to uronic acid **9** and converted, via methyl ester **18**, to amide **27**. The structures of the products were fully supported by conventional analytical data (Table II).

The profound deshielding observed in the ^1H NMR spectra for H-6, compared to little or none for H-5, is best explained by intramolecular hydrogen-bond stabilization caused by the 5′-carboxylic acid, ester, and amide functions on the carbohydrate moiety of the uracil and cytosine series, localized at H-6 (i.e., 6-C—H···O=C—C-4′), and suggests a preferred anti-base conformation for these analogues in solution.[23,24] This deshielding effect increased in the order amide > acid > ester for all the pyrimidine 2′-deoxyribonucleosides. The presence of an electron-withdrawing group adjacent to H-6 caused additional polarization. The *ara*-C variants, however, had an increased deshielding effect in the order acid > ester > amide, and this may be due to the 2′-hydroxyl function participating in hydrogen bonding with the 5′ substituent.[25] Interestingly, the amide protons in all the uronamides were observed as two peaks separated by about 0.4 ppm which deuterated very slowly (almost 1 month) at ambient temperature and may be ascribed to restricted rotation of the 5′-amide C–N bond.[26] The nitrile function caused the strongest downfield shift of H-4′, followed by that of the ester, acid, and amide functions, respectively, in all the nonhalogen-containing nucleosides. There was no clear correlation for the halogen-containing variants. These results indicate that it is not only the nature of the group on the C-5′ position[2,9] but also the substituents on C-5 and C-2′ that determine the extent of the interaction between the glycosyl and the aglycon and the preferred conformation in Me_2SO-d_6 for this series of compounds.

Biological Results and Discussion. A. Enzyme Studies. Table III shows the kinetic constant (K_i) for various nucleoside analogues with herpes simplex virus type 1 encoded pyrimidine deoxyribonucleoside kinase. All the compounds tested showed a competitive inhibition pattern against deoxythymidine as the substrate. The specific substituent (H, Me, F, Br, or I) at the 5 position of the pyrimidine moiety of the nucleoside analogues affects their affinity for the enzyme. Although the inductive effect of the methyl function is opposite to that of the halogens, the changes in the affinity of the compounds for the enzyme are probably mainly due to the van der Waals radius of the group at the 5 position rather than to the electron distribution in the pyrimidine ring. The state of ionization at N-3 probably has no significant effect on the binding of the compounds to the enzyme since the pK_a values of the parent compounds[27] are, respectively, 9.3 (dUrd), 9.8 (dThd), 7.7 (FdUrd), and 8.2 (IdUrd).[28] As anticipated, all the 5-unsubstituted uracil nucleoside derivatives, **1**, **10**, and **19**, have poor binding affinity.

Modification of the 5′ position from a hydroxymethylene to a carboxylic acid group greatly decreases the affinity of the analogues for the enzyme relative to the parent compound. Under the assay conditions (pH 7.8) used, the carboxylic acid function is fully ionized. The uronic acid analogue of deoxythymidine, compound **2**, is a weak inhibitor of deoxythymidine kinase derived from Walker 256 carcinoma[11] and Sarcoma 180 ascites cells[29] and of deoxythymidylate kinase derived from Sarcoma 180 ascites cells.[30] Esterification of uronic acids **1**–**5** to methyl uronates **10**–**14** does not change significantly the affinity of the compounds, except for FdUrd analogue **14** which has a fourfold decreased affinity. Although the steric volume of the methyl group is much larger than the carboxylic hydrogen atom which it replaced, the affinity for the enzyme is not affected by this parameter per se. Furthermore, the fact that the uronic acid analogues are ionized does not account for the decreased affinity, since the un-ionized methyl uronates have a similar K_i. The relatively low affinity for ester **14** is reversed in amide analogue **23**, as shown by the 20-fold increase in the binding affinity. Amination of the other carboxylic acid analogues produced, at best, a small increase in the affinity of these compounds for the enzyme. The steric effect of the amide function would be similar to that of the carboxylic acid group; hence, it would appear that the neutral charge on the amide or nitrile function, compared to the negative charge on the carboxylic acid, slightly decreases the K_i value. The nitrile analogues have about the same affinity for the enzyme as the amide variants.

A comparison has been made with substituents in the 5′ position that are of less steric volume than the modification described in the present report. 2′,5′-Dideoxythymidine relative to deoxythymidine has a 30-fold decreased binding affinity to *Escherichia coli* deoxythymidine kinase.[31] This has been interpreted as being due to the loss in binding by the group normally present in the 5′ position. Steric hindrance is not a factor since the bulkiness of the hydrogen atom at C-5′ is considerably less than that of the hydroxyl group. Furthermore, we have found the K_i for 5′-fluoro-2′,5′-dideoxy-5-iodouridine (5′-FIdUrd) to be 1.5 μM.[32] The covalent radii of fluorine and hydroxyl are 0.72 and 1.10 Å, respectively. The binding affinity of 5′-FIdUrd is similar to the K_i of IdUrd analogues with bulky substituents at the 5′ position, such as the carboxylic acid, methyl ester, carboxamide, or nitrile, and, hence, the size of the moiety in the 5′ position does not appear to be a major factor in the binding of these analogues to the kinase.

The K_i of 3′-*O*-acetyl-2′-deoxythymidine is 58 μM, a decrease of about 100-fold in affinity for the enzyme when compared to deoxythymidine.[32] This decrease in affinity is further illustrated by the 3′-O-acetylated derivatives of the uronamides and urononitriles (Table III). The importance of a 3′-hydroxyl group, which is not only un-

Table II. Characterization of the Pyrimidine Nucleoside Analogues

compd	R	R_1	R_2	R_3	X	recrystn solvent	yield,[a] %	mp, °C (lit. mp; ref)[b]	formula[c]	^{1}H NMR (J, Hz)[d] 6-H	4'-H	other
1	OH	COOH	H	H	H	EtOH	67	226–227 dec (222–223; 22)	$C_9H_{10}N_2O_6$	8.23 (d, 7.7)	4.35	
2	OH	COOH	H	H	Me	EtOH	60	250–251 dec (263–265; 13)	$C_{10}H_{12}N_2O_6$	8.06	4.30	1.76 (5-Me)
3	OH	COOH	H	H	I	EtOH	51	187–188 dec (187–189; 22)	$C_9H_9IN_2O_6$	8.79	4.38	
4	OH	COOH	H	H	Br	EtOH	38	186–187 dec (185; 22)	$C_9H_9BrN_2O_6$	8.77	4.39	
5	OH	COOH	H	H	F	H_2O	51	218–219 dec (218–221; 8)	$C_9H_9FN_2O_6 \cdot 0.75H_2O$	8.54 (d, 6.9)	4.36	
6	NH_2	COOH	H	H	H	H_2O	26	186–187 dec (188; 14)	$C_9H_{11}N_3O_5 \cdot H_2O$	8.19 (d, 7.4)	4.31	
7	NH_2	COOH	H	H	I	H_2O	34	203–204 dec (192–194; 22)	$C_9H_{10}IN_3O_5$	8.70	4.36	
8	NH_2	COOH	H	H	Br	H_2O	51	199–200 dec	$C_9H_{10}BrN_3O_5$	8.70	4.40	
9	NH_2	COOH	H	OH	H	H_2O	21	241–242 dec (235–238; 22)	$C_9H_{11}N_3O_6 \cdot H_2O$	8.21 (d, 7.4)	4.30	
10	OH	COOMe	H	H	H	EtOH	96	227–228	$C_{10}H_{12}N_2O_6$	8.09 (d, 7.7)	4.42	3.72 (COOMe)
11	OH	COOMe	H	H	Me	MeOH	63	237–238 dec (247; 11)	$C_{11}H_{14}N_2O_6$	7.95	4.41	1.80 (5-Me) 3.75 (COOMe)
12	OH	COOMe	H	H	I	EtOH	89	251–252 dec	$C_{10}H_{11}IN_2O_6$	8.64	4.45	3.75 (COOMe)
13	OH	COOMe	H	H	Br	EtOH	96	258–259 dec	$C_{10}H_{11}BrN_2O_6$	8.62	4.47	3.75 (COOMe)
14	OH	COOMe	H	H	F	MeOH	88	248–249 (250–253; 8)	$C_{10}H_{11}FN_2O_6$	8.43 (d, 7.1)	4.47	3.76 (COOMe)
15	NH_2	COOMe	H	H	H	MeOH	95	207–208	$C_{10}H_{13}N_3O_5$	8.08 (d, 7.3)	4.40	3.72 (COOMe)
16	NH_2	COOMe	H	H	I	MeOH	77	220–221 dec	$C_{10}H_{12}IN_3O_5$	8.58	4.45	3.74 (COOMe)
17	NH_2	COOMe	H	H	Br	MeOH	78	213–214 dec	$C_{10}H_{12}BrN_3O_5$	8.54	4.46	3.74 (COOMe)
18	NH_2	COOMe	H	OH	H	MeOH	74	219–220 dec	$C_{10}H_{13}N_3O_6 \cdot H_2O$	8.10 (d, 7.5)	4.30	3.68 (COOMe)
19	OH	$CONH_2$	H	H	H	MeOH	85	257–258 dec	$C_9H_{11}N_3O_5$	8.32 (d, 8.1)	4.21	7.71, 7.34 ($CONH_2$)
20	OH	$CONH_2$	H	H	Me	EtOH	89	251–252 dec (240; 9)	$C_{10}H_{13}N_3O_5$	8.18	4.19	1.77 (5-Me) 7.71, 7.37 ($CONH_2$)
21	OH	$CONH_2$	H	H	I	EtOH	98	249–250 dec	$C_9H_{10}IN_3O_5$	8.97	4.25	7.80, 7.39 ($CONH_2$)
22	OH	$CONH_2$	H	H	Br	MeOH	96	240–241 dec	$C_9H_{10}BrN_3O_5$	8.99	4.26	7.82, 7.39 ($CONH_2$)
23	OH	$CONH_2$	H	H	F	H_2O	98	253–254 (265–266; 2)	$C_9H_{10}FN_3O_5$	8.81 (d, 7.3)	4.24	7.81, 7.43 ($CONH_2$)
24	NH_2	$CONH_2$	H	H	H	EtOH	98	225–226	$C_9H_{12}N_4O_4$	8.32 (d, 8.1)	4.22	7.72, 7.35 ($CONH_2$)
25	NH_2	$CONH_2$	H	H	I	MeOH	93	198–199 dec	$C_9H_{11}IN_4O_4 \cdot H_2O$	8.79	4.21	7.84, 7.35 ($CONH_2$)
26	NH_2	$CONH_2$	H	H	Br	EtOH	57	189–190 dec	$C_9H_{11}BrN_4O_4 \cdot 1.5H_2O$	8.82	4.25	7.89, 7.34 ($CONH_2$)
27	NH_2	$CONH_2$	H	OH	H	EtOH	98	202–203 dec	$C_9H_{12}N_4O_5 \cdot 0.25H_2O$	7.93 (d, 7.4)	4.10	7.41, 7.36 ($CONH_2$)
28	OH	$CONH_2$	COMe	H	I	EtOH	44	227–228 dec	$C_{11}H_{12}IN_3O_6 \cdot 0.5EtOH$	8.79	4.42	7.78, 7.54 ($CONH_2$) 2.09 (COMe)
29	OH	$CONH_2$	COMe	H	Br	EtOH	97	210–211	$C_{11}H_{12}BrN_3O_6 \cdot 0.5H_2O$	8.82	4.44	7.81, 7.55 ($CONH_2$) 2.10 (COMe)
30	OH	$CONH_2$	COMe	H	F	EtOH	98	133–135 (137–138; 2)	$C_{11}H_{12}FN_3O_6$	8.64 (d, 7.5)	4.42	7.77, 7.54 ($CONH_2$) 2.10 (COMe)
31	OH	CN	COMe	H	I	EtOH	57	83–84	$C_{11}H_{10}IN_3O_5 \cdot 1.5EtOH$	8.17	5.13 (d, 2.7)	2.07 (COMe)
32	OH	CN	COMe	H	Br	EtOH	61	84–86	$C_{11}H_{10}BrN_3O_5 \cdot 0.5H_2O$	8.21	5.14	2.08 (COMe)
33	OH	CN	H	H	I	EtOH	40	239–240 dec	$C_9H_8IN_3O_4$	8.00	4.77	
34	OH	CN	H	H	Br	EtOH	43	221–222 dec	$C_9H_8BrN_3O_4$	8.03	4.78	
35	OH	CN	H	H	F	EtOH	47	177–178 (190; 2)[e]	$C_9H_8FN_3O_4$	7.92 (d, 7.1)	4.75	

[a] The yields quoted here have generally been derived from multiple experiments. [b] Uncorrected. [c] Analytical results (C, H, and N) are within ±0.4%. [d] NMR spectra were recorded on a Bruker 270 HX spectrometer in Me_2SO-d_6; chemical shifts δ (ppm); d = doublet; where there is no symbol a singlet was observed. [e] Mass spectrum m/e 241 (M^+), 214 (M^+ – HCN), 130 (base + H).

Table III. Kinetic Constants for Herpes Simplex Virus Type 1 Encoded Pyrimidine Deoxyribonucleoside

		analogue K_i, μM					
parent compd[a]	K_i, μM	uronic acid	methyl uronate	uronamide	3'-O-acetyl-uronamide	3'-O-acetyl-urononitrile	urononitrile
dThd	0.6-1.0[b]	5.4	4.0	1.6			
dUrd	9.5	500	500	310			
FdUrd	1.5	98	410	18	960		75
BrdUrd	0.2	2.7	2.9	0.8	192	28	1.8
IdUrd	0.2	4.2	1.5	1.0	20	18	1.3

[a] See ref 28. [b] K_m.

substituted but also in the erythro configuration, is supported by the following. 2',3'-Dideoxythymidine exhibits an 80-fold loss in binding[31] to *E. coli* deoxythymidine kinase relative to deoxythymidine, which has been suggested to be due to the loss of binding by the 3'-hydroxy function. Furthermore, the K_i of 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-iodouracil against deoxythymidine as substrate for the HSV-1 encoded pyrimidine deoxyribonucleoside kinase is 66 μM,[32] which may indicate that the 3'-hydrogen of IdUrd is not involved, at least to a major extent, in the binding to the enzyme.

Several uronic acid derivatives of FdUrd, compounds **5**, **14**, **23**, and **35**, as well as the uronamide of 2'-deoxyuridine, compound **19**, were tested by the methods of Roberts[33] as potential inhibitors of deoxythymidylate synthetase derived from two sources, L1210 ascites cells and *Lactobacillus casei*. None of these compounds showed significant inhibition at 250 μM.[34] These results indicate that the carboxyl, methyl ester, amide, and nitrile functions are unable to mimic the 5'-phosphate group, since it is known that 2'-deoxyuridine 5'-monophosphate is an excellent substrate and the corresponding 5-fluoro analogue is a potent inhibitor of these enzymes.

B. Cell Culture, Viral, and Bacterial Studies. None of the compounds in the cytosine series, **6–9**, **15–18**, and **24–27** (Table II), was active at 400 μM (final concentration used in all the biological testing) when evaluated as inhibitors of the reproduction of Sarcoma 180 (S-180) cells in vitro, herpes simplex virus type 1 (HSV-1) in vitro, and *S. faecium* with the exception of the arabino compound **9** (Table IV). This carboxylic acid analogue of *ara*-C inhibited growth of S-180 cells by 76%, and this activity, like that of *ara*-C, was prevented by 2'-deoxycytidine or cytidine. No further studies were conducted with this series.

In the uracil series **1–5**, **10–14**, **19–23**, and **28–35**, there was no significant inhibition for the derivatives tested with the exception of the uronamides **21** and **22** which inhibited the yield of HSV-1 by 96 and 92%, respectively. A 10-fold decrease in concentration reduced the activity of these analogues to 78 and 42%, respectively (Table IV). A moderate 74% inhibition of HSV-1 was produced by urononitrile **33**. Although the uronic acid analogue of FdUrd, compound **5**, was previously reported[8] to be moderately toxic to human tumor cells (KB), mouse cells (Glioma 26), and human normal cells (WI 38), it had no detectable activity against the reproduction of S-180. However, methyl uronate **14** produced a slight inhibition (32%) of S-180.

The same uracil series was tested for inhibitory activity against *S. faecium*. The cells were grown in the presence of either folic acid (4.53 nM) or deoxythymidine (7 μM), and the growth was measured as a function of turbidity of the cells in the stationary phase. None of the compounds exhibited significant activity with the exception of the uronamide analogue of dUrd, compound **19**, which inhibited growth by 71% when the medium contained deoxythymidine. An inhibition analysis indicated that maximum inhibition was attained with compound **19** at 600 μM and the ED_{50} was 200 μM. The inhibition of growth by the uronamide of dUrd (500 μM) can be prevented by deoxythymidine (ED_{50} = 11 μM), 2'-deoxyuridine (ED_{50} = 37 μM), and 2'-deoxycytidine (ED_{50} = 50 μM), as well as by purine 2'-deoxyribonucleosides. Surprisingly, the uronamide analogue of deoxythymidine, compound **20**, had no inhibitory activity against the above microorganism.

Table IV. Biological Activity of Several 5'-Substituted Pyrimidine Nucleosides

		inhibition, %			
				S. faecium	
compd	concn, μM	S-180	HSV-1	+dThd	+folic acid
9	400	76	0	0	0
14	400	32	0	0	0
19	400	0	0	71	22
21	400	0	96	0	28
	40		78		
22	400	0	92	0	0
	40		42		
33	400	0	74	0	14
ara-C	200		91		
	5	98			
FdUrd	400			96	95
	0.1		88		
	0.001	95			
BrdUrd	400			76	91
	10		99.6		
IdUrd	400			12	71
	50		99.7		

Whether the uronamide analogue of IdUrd or BrdUrd has any unique property which merits further investigation as an antiviral agent remains to be elucidated. It is encouraging that both of these analogues are rather potent inhibitors of the HSV-1 encoded pyrimidine deoxyribonucleoside kinase with K_i values of 1.0 and 0.8 μM, respectively, whereas the inhibition constants of two very potent antiviral agents, IdUrd and BrdUrd, for this enzyme are 0.2 μM. The range of antiviral activity, toxicity, and mechanism of action of these two amides remains to be established.

Experimental Section

Chemical Methods and Materials. Melting points were taken on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker 270 HX spectrometer in Me_2SO-d_6 solution at a concentration of ≤30 mM. Chemical shifts are reported in δ units, parts per million downfield from internal tetramethylsilane. Chemical shifts and coupling constants (*J* values in hertz) are first order. Double-resonance studies support the assignment of protons for the compounds. The OH and NH protons were confirmed by deuterium exchange and integrations were consistent with peak assignments. Mass

spectra were obtained on an AEI MS9 high-resolution instrument at an ionization potential of 70 eV. TLC was performed on Eastman 6060 precoated silica gel sheets with fluorescent indicator. In the LC analysis, distilled, deionized water and methanol distilled in glass (Burdick & Jackson Labs, Inc., Mich.) were both degassed. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, Conn. 5-Iodo- and 5-bromo-2′-deoxycytidine were synthesized by the method of Cheng and Welch.[35] 5-Fluoro-2′-deoxyuridine was a gift from Dr. W. E. Scott of Hoffmann-La Roche. DEAE-cellulose (DE52) was obtained from Whatman, Kent, U.K. All other chemicals were obtained from commercial sources.

General Method for Preparation of Uronic Acids 1–9. Platinum oxide (1 g) was reduced at room temperature in glacial acetic acid (20 mL) with hydrogen at 20 psi (107.9 kN/m^2) for 5 min. The suspension was carefully filtered, washed first with water until neutral and then with bicarbonate buffer (pH 9, prepared from 2.2 g of sodium bicarbonate and 0.355 g of sodium carbonate in 860 mL of water), and stored moist under nitrogen. To a three-necked flask fitted with a condenser, overhead stirrer, and oxygen inlet (fritted head) were added the powdered nucleoside, platinum, and bicarbonate buffer. For optimum yield, the ratio of nucleoside to platinum and buffer should be of the order 1 mmol:0.25 g:45 mL. Oxygen was bubbled into the rapidly stirred suspension maintained at 70–75 °C for 10–18 h. The reaction was monitored by TLC ($CHCl_3$–EtOH, 2:1) and paper electrophoresis (0.05 M phosphate or citrate buffer, pH 6.8 and 3.8, respectively). The warm suspension was filtered and the platinum was recovered. The filtrate containing colloidal platinum was reduced to about half its volume in vacuo and then adjusted with glacial acetic acid to pH 3. It was then filtered through Celite and the clear solution reduced to almost dryness. The acid slowly crystallized at room temperature or was recovered and purified as described below.

Purification of Uronic Acids 1–9. The solution of the crude uronic acid was adjusted to pH 8 with 1 M NaOH and applied to a column of DEAE-cellulose in the bicarbonate form (CO_2 free). The column was eluted with distilled water until the eluant showed no absorption at 268 nm. The column was then eluted with a freshly prepared solution of triethylammonium bicarbonate (0.2 M, pH 7.5–8). The UV-absorbing fractions were evaporated under reduced pressure and the moist solid was coevaporated several times with methanol. The residue was redissolved in water and the pH of the solution adjusted to 3 with AG50W-X2 (H^+) resin. The mixture was filtered and the filtrate evaporated to dryness in vacuo to give an oil which slowly crystallized. The crystals were washed with cold ethanol, filtered, and then dried. Recrystallization in the appropriate solvent gave the pure uronic acid. The purity was confirmed using a LC analytical system before submitting samples for bioassay.

Methyl Uronates 10–18. Application of the method of Baker et al.[2] gave the methyl uronates in 63–96% yield. However, compounds containing the cytosine residue were neutralized, after esterification, with a 1 M K_2CO_3 solution in order for the product to separate. The methyl uronate **15** had to be desalted by chromatography on a short silica column eluting with $CHCl_3$–EtOH (2:1), since this compound is very soluble in both water and methanol.

Uronamides 19–27. The uronates were converted to the uronamides by the method of Baker and co-workers[2] in almost quantitative yields. The course of the reaction was followed on TLC ($CHCl_3$–EtOH, 4:1).

Ununonitriles 33–35. The ununonitriles were obtained via the 3′-*O*-acetyluronamides **28–30** by the method of Baker and coworkers[2] in 10–47% yield (based on the unprotected uronamide). Care should be taken in hydrolyzing the excess phosphoryl chloride since both deacetylation and dehydration can occur.

1-(2-Deoxy-β-D-*erythro*-pentofuranuronic acid)-5-fluorouracil (5). This compound was prepared according to the method of Tsou et al.[8] However, when the oxidation was completed, the suspension was filtered and to the filtrate was added [2-^{14}C]-5-fluoro-2′-deoxyuridine as a marker. This was then loaded onto an AG1-X8 (Cl^- form, 200–400 mesh) resin column. The column was first eluted with water until the eluant showed neither absorption at 268 nm nor radioactive counts when concentrated (99.7% of the radioactivity was recovered). The column was then eluted with 0.01 M HCl and the fractions were collected. Early fractions containing low counts were discarded. The remaining UV-absorbing fractions were pooled and evaporated to dryness under reduced pressure. The solid obtained was recrystallized twice from water to give white prisms, mp 218–219 °C dec (lit.[8] mp 218–221 °C). A sample of this material (15 mg) was found to have no radioactivity above background.

Biological Evaluation. Compounds reported in this paper were screened for activity against murine Sarcoma 180 and HSV-1 (yield reduction assay). The methodology used as has been described previously.[36]

Antimicrobial and Inhibition Analysis. *S. faecium* (ATCC 8043) was obtained from the American Type Culture Collection and grown overnight at 37 °C in tomato juice, yeast extract (Difco), and skim milk (Carnation) in the ratio 100 mmol:100 mL:5 g in 1 L of distilled water at pH 7. The stock bacteria were maintained by transfers from medium to medium, medium to agar slant, or slant to slant. Every week a new culture was started from agar. For the experiment, an inoculum from agar was transferred to 10 mL of inoculum broth [glucose (5 g), yeast extract (20 g), and anhydrous sodium acetate (5 g) in water (1 L)] and incubated at 37 °C overnight. One-tenth milliliter of cell suspension was transferred to 10 mL of inoculum broth and incubated for 5–6 h. The cells were harvested by centrifugation at 800g for 10 min. These were then washed with saline (0.9% w/v, 2 × 5 mL) and the pellet was resuspended in enough saline to yield a turbidity of 100 units (1 Klett unit corresponded to 2 × 10^6 cells/mL) as measured on a Klett–Summerson photoelectric colorimeter using a red filter (640–700 nm). A 10-fold dilution in saline was used as the inoculum. All the reagents were sterilized either by autoclaving at 121 °C for 15 min at 15 psi (103.4 kN/m^2) or by sterile filtration for heat-sensitive compounds.

A solution of the compound to be tested, basal medium[37] (2.5 mL), and deoxythymidine (7 μM final concentration) or folic acid (4.53 nM final concentration) was added into sterile Klett tubes covered with metal caps (final volume 4.9 mL). To this mixture 0.1 mL of the previously prepared *S. faecium* suspension was added, the tubes were thoroughly mixed, and the transmission was measured. The tubes were then incubated at 37 °C for 15–17 h. The extent of the growth was determined by measuring the difference in turbidity before and after incubation. Prevention of inhibition of uronamide **19** (500 μM) by deoxyribonucleosides was carried out by adding dThd, dUrd, or dCyd in increasing concentrations to the incubation medium which contained deoxythymidine (7 μM). ED_{50} values of the deoxyribonucleosides were estimated from dose–response curves compiled from two separate experiments and represent the concentration needed to prevent cell growth by 50%. Appropriate controls were performed for all the experiments including positive controls using the known *S. faecium* antimetabolites (e.g., FUra, FdUrd, or BrdUrd).

Enzyme Assay. Herpes simplex virus type 1 encoded pyrimidine deoxyribonucleoside kinase was purified from LMTK$^-$ cells by the procedure of Cheng and Ostrander.[38]

Assays of the kinase activity were performed at 37 °C by measuring the amount of [2-^{14}C]deoxythymidine phosphorylated. Initial velocity experiments were carried out as follows: the final volume of 100 μL contained 3 mM ATP·Mg^{2+}, 50 mM Tris (pH 7.8), 0.3 mg/mL of BSA, 0.3 mM 2-mercaptoethanol, 2% v/v glycerol, varied amounts of [2-^{14}C]deoxythymidine, and the nucleoside analogue. The reaction was initiated by the addition of enzyme followed by incubation at 37 °C. At several time intervals, a 15-μL aliquot was removed from the incubated mixture, and the amount of phosphorylated [2-^{14}C]deoxythymidine formed was determined as described previously.[39] All kinetic constants (K) were calculated from double-reciprocal plots.

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References and Notes

(1) This work was presented in part at the 174th National Meeting of the American Chemical Society, Chicago, Ill., Aug 29–Sept 1, 1977, CARB 20.
(2) J. J. Baker, A. M. Mian, and J. R. Tittensor, *Tetrahedron*, **30**, 2939 (1974), and references cited therein.
(3) W. H. Prusoff and D. C. Ward, *Biochem. Pharmacol.*, **25**, 1233 (1976).
(4) A. Bloch in "Drug Design", Vol. IV, E. J. Ariens, Ed., Academic Press, New York and London, 1973, pp 285–378.
(5) R. N. Prasad, A. Fung, K. Tietje, H. H. Stein, and H. D. Brondyk, *J. Med. Chem.*, **19**, 1180 (1976), and references cited therein.
(6) J. F. Henderson and M. L. Battell, *Biochem. Pharmacol.*, **25**, 1915 (1976).
(7) O. Kraupp, German Patent 2610985 (1977); *Chem. Abstr.*, **88**, 7312d (1978).
(8) K. C. Tsou, N. J. Santora, and E. E. Miller, *J. Med. Chem.*, **12**, 173 (1969).
(9) J. J. Baker, P. Mellish, C. Riddle, A. R. Somerville, and J. R. Tittenson, *J. Med. Chem.*, **17**, 764 (1974).
(10) P. Langen and G. Kowollik, *Eur. J. Biochem.*, **6**, 344 (1968).
(11) J. P. Neenan and W. Rohde, *J. Med. Chem.*, **16**, 580 (1973).
(12) R. W. Binkley, *J. Org. Chem.*, **42**, 1216 (1977), and references cited therein.
(13) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and Lord A. Todd, *J. Chem. Soc.*, 1149 (1963).
(14) A. S. Jones, A. R. Williamson, and M. Winkley, *Carbohydr. Res.*, **1**, 187 (1965), and references cited therein.
(15) P. Howgate, A. S. Jones, and J. R. Tittensor, *J. Chem. Soc. C*, 276 (1968).
(16) J. Zemlicka, R. Gasser, J. V. Freisler, and J. P. Horwitz, *J. Am. Chem. Soc.*, **94**, 3213 (1972); *J. Org. Chem.*, **38**, 990 (1973).
(17) A. A. Akhrem, A. G. Lapko, I. A. Mikhailopulo, and V. A. Timoshehuk, *Carbohydr. Res.*, **54**, C1–C2 (1977).
(18) M. Sela and H. Ungar-Waron in "Methods in Enzymology", L. Grossman and K. Moldave, Ed., Academic Press, New York, N.Y., 1968, pp 900–902.
(19) H. Pischel, A. Holy, and G. Wagner, *Collect. Czech. Chem. Commun.*, **39**, 3773 (1974).
(20) C. B. Reese, K. Schofield, K. Shapiro, and A. R. Todd, *Proc. Chem. Soc., London*, 290 (1960).
(21) K. Heyns and H. Paulsen in "Newer Methods in Preparative Organic Chemistry", Vol. II, W. Foerst, Ed., Academic Press, New York, N.Y., 1963, pp 303–335.
(22) K. Imai and M. Honjo, *Chem. Pharm. Bull.*, **13**, 7 (1965).
(23) J. Zemlicka and J. P. Horwitz, *J. Am. Chem. Soc.*, **97**, 4089 (1975).
(24) H. Follmann and H. Witzel, *Eur. J. Biochem.*, **77**, 451 (1977).
(25) E. Darzynkiewicz, M. Remin, A. Dworak, and D. Shugar, *Cancer Biochem. Biophys.*, **1**, 85 (1975).
(26) B. Sunners, L. H. Piette, and W. G. Schneider, *Can. J. Chem.*, **38**, 681 (1960).
(27) B. R. Baker in "Design of Active-Site-Directed Irreversible Enzyme Inhibitors", B. R. Baker, Ed.. Wiley, New York, N.Y., 1967, p 75.
(28) Abbreviations: FUra, 5-fluorouracil; BrUra, 5-bromouracil; IUra, 5-iodouracil; dUrd, 2′-deoxyuridine; FdUrd, 5-fluoro-2′-deoxyuridine; BrdUrd, 5-bromo-2′-deoxyuridine; IdUrd, 5-iodo-2′-deoxyuridine; IdCyd, 5-iodo-2′-deoxycytidine; BrdCyd, 5-bromo-2′-deoxycytidine.
(29) Y. C. Cheng and W. H. Prusoff, *Biochemistry*, **13**, 1179 (1974).
(30) Y. C. Cheng and W. H. Prusoff, *Biochemistry*, **12**, 2162 (1973).
(31) B. R. Baker, T. J. Schwan, and D. V. Santi, *J. Med. Chem.*, **9**, 66 (1966).
(32) W. H. Prusoff, M. S. Chen, T. S. Lin, G. T. Shiau, and R. F. Schinazi, unpublished results.
(33) D. Roberts, *Biochemistry*, **5**, 3546 (1966).
(34) We thank Dr. K. Scanlon of this department and Dr. T. Kalman of SUNY at Buffalo for these data.
(35) P. K. Chang and A. D. Welch, *Biochem. Parmacol.*, **8**, 327; **6**, 50 (1961).
(36) T. S. Lin and W. H. Prusoff, *J. Med. Chem.*, **21**, 106 (1978).
(37) M. Hashmi in "Assays of Vitamins in Pharmaceutical Preparations", Wiley, London and New York, 1973, p 225.
(38) Y. C. Cheng and M. Ostrander, *J. Biol. Chem.*, **251**, 2605 (1976).
(39) M. S. Chen and W. H. Prusoff, *Biochemistry*, **16**, 3310 (1977).

Phosphorus–Nitrogen Compounds. 22. Synthesis and Antitumor Activity of Arylsulfonylhydrazone Analogues

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A series of pyridine-2-carboxaldehyde *N*-oxide and pyridine-2-carboxaldehyde (thio)phosphoric hydrazones and two cupric chelates was synthesized. The hydrazones, chelates, and combinations of hydrazones and cupric chloride were tested against mice bearing P388 lymphocytic leukemia, Sarcoma 180, or Ehrlich carcinoma ascites cells. The effects of various structural modifications of the hydrazones on antineoplastic activity for this latter system were determined. In general, the pyridine-2-carboxaldehyde thiophosphoric monohydrazones containing *P*-phenyl or *P*-phenoxy substituents possessed the highest activity when concurrently administered with cupric ion, whereas the ligands themselves were inactive. Two of the compounds were prepared with *P*-hydroxyl groups to permit increased hydrophilicity. The ability of the hydrazones to chelate cupric, ferrous, and cobaltous salts was investigated, and discrepancies between determined and calculated log *P* values for three compounds are discussed.

A series of arylsulfonylhydrazones of aromatic aldehydes has been investigated for murine antineoplastic properties, with the establishment of relationships between such activity and structure.[1,2] In general, the greatest effects resulted when the aldehyde portion was 2-formylpyridine *N*-oxide and when bulky substituents were on the sulfur atom. In the case of the related 5-hydroxy-2-formylpyridine thiosemicarbazones, relatively large groupings on the N^4 are also beneficial with regard to the effect against the Sarcoma 180 animal model system but not as concerns inhibition of ribonucleoside diphosphate reductase.[3] Thiophosphoric hydrazones related to the highly investigated oncolytic 2-formylpyridine thiosemicarbazones have been prepared and tested against Ehrlich carcinoma, with significant activity noted with certain cupric chelates and with concurrently administered ligands and cupric chloride.[4]

The present paper describes the synthesis and biological evaluation against three murine tumor systems of phosphoric and thiophosphoric hydrazones of 2-formylpyridine

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