The solid obtained from the second band was recrystallized to give 7, yield 1.3 g.

Method B. Ethyl 5,6-Diamino-4-{[bis(*p*-methoxyphenyl)methyl]amino}-2-pyridinecarbamate (11). A partial solution of **5** (2.2 g) in EtOH (225 ml) containing Raney Ni (~2.5 g, washed with EtOH) was hydrogenated at room temperature and atmospheric pressure. After 16 hr the mixture was heated to 60° under N_2 and filtered. The residue was extracted with an additional amount of hot EtOH (225 ml). The combined ethanol filtrate was evaporated to dryness, and the resulting solid was washed with petroleum ether (bp 60–80°), yield 1.4 g.

In the preparation of **10** and **29** concentrated HCl was added to the ethanol filtrate before evaporation.

5-Amino-7-[(diphenylmethyl)amino]-3*H*-imidazo[4,5-*b*]pyridine (12). A mixture of **13** (9.50 g), KOH (12.6 g), and EtOH (500 ml) protected with $CaCl_2$ and NaOH tubes was refluxed for 18 hr. After filtration the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O (25 ml) and acidified with concentrated HCl to precipitate the HCl of the product, yield 6.50 g.

Ethyl 7-Amino-3*H*-imidazo[4,5-*b*]pyridine-5-carbamate (14). A solution of **13** (2.0 g) in 8% HBr–HOAc (80 ml) was stirred at room temperature for 2 hr. The solid that deposited was collected by filtration, suspended in H_2O (35 ml), and treated with concentrated NH_4OH. After the solid dissolved, the resulting solution deposited the product as white needles, yield 0.79 g.

Method C. Ethyl 7-{[Bis(*p*-chlorophenyl)methyl]amino}-3*H*-imidazo[4,5-*b*]pyridine-5-carbamate (16). A suspension of **10** · 2HCl (3.5 g) in $(EtO)_3CH$ (150 ml) containing concentrated HCl (0.6 ml) was stirred at room temperature for 72 hr. The course of the reaction was followed by tlc of aliquot portions from the reaction mixture. The product was collected by filtration and washed with Et_2O, yield 3.0 g.

Method D. 2,4-Diamino-6-{[bis(*p*-chlorophenyl)methyl]amino}-5-nitropyrimidine (24). A mixture of **22** (2.0 g), bis(*p*-chlorophenyl)-methylamine · HCl (3.1 g), and Et_3N (2.1 g) in MeOH (250 ml) protected by $CaCl_2$ and NaOH tubes was refluxed for 24 hr. After filtration the filtrate was concentrated to about ¼ volume and refrigerated for 18 hr. The solid that deposited was collected by filtration and washed with H_2O, yield 3.0 g.

Method E. 2-Amino-6-(diphenylmethyl)aminopurine (27). A mixture of **26** (2.0 g), diphenylmethylamine (2.2 g), and NaOAc (0.97 g) in BuOH (50 ml) was refluxed for 150 hr and evaporated to dryness *in vacuo*. The resulting residue was washed with H_2O to give crude **27**, yield 2.2 g (59%).

Acknowledgment. The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for the microanalytical results reported and to Dr. W. R. Laster and members of the Cancer Screening Division for the screening data reported.

References

(1) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **31**, 1890 (1966).
(2) C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 866 (1962).
(3) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Amer. Chem. Soc.*, **81**, 3046 (1959).
(4) K. B. deRoos and C. A. Salemink, *Recl. Trav. Chim. Pays-Bas*, **88**, 1263 (1969).
(5) D. E. O'Brien, C. C. Cheng, and W. Pfleider, *J. Med. Chem.*, 9, 573 (1966).
(6) H. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, *Cancer Chemother. Rep.*, **35**, 1 (1964).
(7) H. E. Skipper, F. M. Schabel, Jr., M. W. Trader, and J. R. Thomson, *Cancer Res.*, **21**, 1154 (1961).
(8) A. Goldin, H. B. Wood, Jr., and R. R. Engle, *Cancer Chemother. Rep., Suppl.*, **1** (No. 1), 192 (1968).
(9) A. Haddow and W. A. Sexton, *Nature (London)*, **157**, 500 (1946).
(10) E. Paterson, A. Haddow, I. A. Thomas, and J. M. Watkinson, *Lancet*, **1**, 677 (1946).
(11) H. E. Skipper and C. E. Bryan, *J. Nat. Cancer Inst.*, 9, 391 (1949).
(12) L. W. Blodgett and K. L. Yielding, *Biochem. Biophys. Acta*, **169**, 451 (1968).

Preparation and Biological Activity of Various 3-Deazapyrimidines and Related Nucleosides†

A. Bloch,* G. Dutschman,

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203

Bruce L. Currie, Roland K. Robins,

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

and Morris J. Robins*

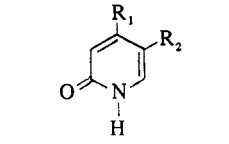
Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada. *Received June 20, 1972*

Among analogs of naturally occurring pyrimidine nucleosides modified in the heterocycle, the 5-aza and 6-aza analogs have demonstrated a broad spectrum of biological activity (see, for example, ref 1). The C^3 ribonucleoside of 6-hydroxy-2-pyridone (1-deazauridine) was rather unstable and showed little biological activity.[2] The 3-deazapyrimidines, substituted analogously to the naturally occurring pyrimidines, constitute another logical class of analogs with potential biological activity. Although a number of *N*-glycosides of pyridines have been prepared (see, for example, ref 3), no 3-deazapyrimidine nucleosides, as defined above, had been synthesized or biologically evaluated prior to our studies (for preliminary accounts, see ref 4).

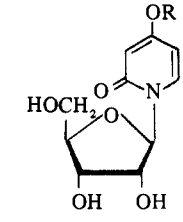
The syntheses of the nucleoside analogs evaluated in this study have been reported previously.[5] A modified decarboxylation of **2** to **1** and a simplified synthesis of 3-deazaC (**3**) from 4-amino-2-chloropyridine *via* the 2-benzyloxy derivative have been accomplished.

Due to the improved antitumor activity reported for arabinosyl cytosine upon 5'-esterification with the adamantoyl group,[6] a monoadamantoyl derivative **5** of 3-deazaUR (**4**) has been prepared.

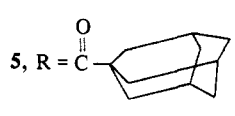
The growth inhibitory activity of these and other 3-deazapyrimidines has been evaluated in microbial and in tumor cell systems, and their effect against leukemia L-1210 *in vivo* has been determined. The results of these studies are reported in this paper. A preliminary account of some of the data has been provided earlier.[4]



1, R_1 = OH; R_2 = H
2, R_1 = OH; R_2 = CO_2H
3, R_1 = NH_2; R_2 = H



4, R = H



5, R = C(=O)–

Results and Discussion

Chemical Results. The reported synthesis[7] of 3-deazaU (**4**-hydroxy-2-pyridone, **1**) employed decarboxylation of 4-hydroxy-2-pyridone-5-carboxylic acid (**2**) in concentrated

†This investigation was supported by Grant CA-12585 from the NCI, U. S. Public Health Service (to A. B.), and by a grant from the National Cancer Institute of Canada (to M. J. R.).

HCl in a sealed tube on a 2-g scale. We have found that heating **2** in distilled H_2O at 200° in a steel bomb gives an 83% yield of **1** on a 20-g scale.

A small sample of 3-deazacytosine (4-amino-2-pyridone,[8] **3**) was prepared by treatment of 4-amino-2-chloropyridine hydrochloride[9] with sodium benzyl oxide to give the 2-benzyloxy intermediate which was hydrogenolyzed directly to yield **3**. The product, **3**, was purified and isolated by preparative tlc in low yield.

Treatment of a pyridine solution of 3-deazaUR (**4**) with adamantane-1-carboxylic acid chloride gave a product whose properties are consistent with the 4-adamantoyloxy structure **5**. The chromatographic mobility, elemental analysis, and nmr spectrum indicated the presence of a monoadamantoyl ester. The uv spectral data indicate substitution on the heterocyclic ring hydroxyl rather than on the sugar. In acidic and neutral solution the maxima exhibit large bathochromic shifts as compared to those of the parent compound but they are identical in basic (saponification) solution. Substitution on O^2 has been observed to give hypsochromic shifts[10] in the uv. The nmr spectrum is also consistent with 4-hydroxyl esterification; the peak corresponding to the 3-hydrogen is shifted downfield, becoming superimposed on the H^5 and anomeric hydrogen peaks, and the hydrogen is not subject to D_2O exchange in DMSO-d_6. An *upfield* shift of the 3-hydrogen peak was seen upon cyclonucleoside formation involving the 2-oxygen,[5a] and no H^3 exchange occurred in systems in which the 3,4-keto-enol tautomerism is blocked.[4,5,11] The pK_a of 3-deazaU (**1**) has been reported[12] to be 6.50 and, thus, in the basic pyridine solution used for the reaction, the phenolic 4-oxygen atom is expected to be the most nucleophilic center for attack at the acyl chloride carbonyl carbon.

Since the adamantoyl ester was to be employed as a possible lipophilic carrier and/or depot form of the nucleoside analog,[6] its position of attachment (4-O *vs.* 5'-O) should be of secondary importance as long as its uptake into the cell and subsequent conversion to the active intermediate can proceed at a rate sufficient to provide the desired inhibitory effect. Chemically, the rate of basic hydrolysis (saponification) is known to be accelerated with increasingly stable (*i.e.*, less basic) leaving alcohol groups[13] such as the phenolic 4-oxygen, which is much less basic than a sugar hydroxyl. In addition, any tendency to go to the keto tautomer would favor hydrolysis of the adamantoyl ester.

That the adamantoyl ester can be cleaved biologically was shown by the finding that, upon incubation of 4-*O*-adamantoyl-^3H-deazaUR with leukemia L-1210 cell extract, free deazaUR was obtained. Under the conditions used (2.5 mg of protein/ml of incubation mixture), approximately 38% of the ester supplied ($1 \times 10^{-4} M$) was cleaved to the nucleoside within 1 hr at 37°, and complete cleavage was obtained following overnight incubation. There was no observable cleavage of the ester in the control buffer solution in the absence of cell extract after 1 hr.

Biological Results. The markedly greater acidity of 3-deazaU (pK_a = 6.50)[12] as compared to that of U (pK_a = 9.38)[12] and the greater basicity of 4-aminopyridine (pK = 9.17)[14] *vs.* that of 4-aminopyrimidine (pK = 5.71)[14] suggest that the deazanucleosides (glycosyl pyridine derivatives) have a greater capacity than the corresponding pyrimidine nucleosides (UR or CR) to form ionic bonds at physiological pH. Furthermore, the altered basicity of the deaza analogs could affect the strength of any hydrogen bonds involving their 2 and 4 substituents. This enhanced binding capacity of the compounds might interfere with the activity of enzymes involved in their metabolic conversion. As shown in Table I, the 3-deaza analogs of UR and CR and the 4-*O*-adamantoyl derivative of deazaUR were markedly inhibitory of the growth of leukemia L-1210 cells *in vitro*. DeazaU, deazaC, or their 2'-deoxyribonucleosides were inactive at $10^{-4} M$, as were the deaza analogs of orotidine, arabinosyl U, and arabinosyl C. This pattern of growth inhibition was essentially duplicated in *Escherichia coli*, except that in this organism 3-deazaCR was much more active than 3-deazaUR, while the adamantoyl derivative was inert. Only in *Streptococcus faecium* did the deoxyribonucleoside analogs demonstrate some, marginal, activity. The reason for this differential activity is likely related to the extent of conversion of these compounds to the biologically active intermediates, suggested to be the nucleotide derivatives.[15]

Because of the inhibitory effect of the deaza analogs of UR and CR *in vitro*, the antileukemic activity of the compounds was also evaluated *in vivo*. Since, in preliminary experiments, deazaUR proved to be the more effective of the two analogs, only this compound was examined. Table II shows that ip doses of 3-deazaUR ranging from 100 to 300 mg/kg/day, 6 days, increased the survival time of male DBA_2/Ha mice bearing leukemia L-1210 by 55–65%. The results shown comprise data obtained with four different batches of the analog, administered to groups of mice from various litters. The effectiveness of the different batches of compound in the different litters varied markedly, the increases in life span ranging from 18 to 168% above control. As a result of this pooling of the data, Table II does not show a definitive dose relationship, although such a relationship does exist when each batch is considered separately. This apparent lack of dose response is also due to the fact that the 30-day survivors were excluded from the calculations. This is not to imply, however, that the compound is completely devoid of toxicity. At 100–200 mg/kg/day, 6 days, a dose-related weight loss was seen ranging, on day 6, from 5 to 16%, respectively. This loss was reduced by approximately one-half on day 10, and no premature deaths occurred.

Of interest is the finding that 3-deazaUR was much more toxic to female DBA_2/Ha mice than it was to the males. The data pertaining to this observation and the hormonal basis for this differential activity will be reported elsewhere.[16]

Table I. Effect of 3-Deazapyrimidines on the Growth of Microbial and Tumor Cells *in Vitro*[a]

Compound	Concn (M) for 50% growth inhibition of: Leukemia L-1210	*S. faecium*	*E. coli*
3-Deazauracil (**1**)	$>10^{-4}$	$>10^{-3}$	$>10^{-3}$
3-Deazauridine (**4**)	6×10^{-6}	2×10^{-4}	4×10^{-4}
3-Deaza-2'-deoxyuridine	$>10^{-4}$	6×10^{-4}	$>10^{-3}$
3-Deazacytosine (**3**)	$>10^{-4}$	$>10^{-3}$	$>10^{-3}$
3-Deazacytidine	5×10^{-5}	2×10^{-4}	3×10^{-7}
3-Deaza-2'-deoxycytidine	$>10^{-4}$	3×10^{-4}	$>10^{-3}$
3-Deazaorotidine	$>10^{-4}$	$>10^{-3}$	$>10^{-3}$
3-Deazaarabinosyluracil	$>10^{-4}$	$>10^{-3}$	$>10^{-3}$
3-Deazaarabinosylcytosine	$>10^{-4}$	$>10^{-3}$	$>10^{-3}$
4-*O*-Adamantoyl-3-deazauridine (**5**)	7×10^{-7}	$>10^{-3}$	$>10^{-3}$

[a] The following compounds were inactive in the above test systems: 2',3',5'-tri-*O*-benzoyl-3-deazauridine, 5-carboxy-3-deazauridine, 5-carbomethoxy-3-deazauridine, 3-deazauridine-5-carboxamide, 3-deazauridine-6-carboxamide, 6-carbethoxy-3-deazauridine, 2',3',5'-tri-*O*-benzoyl-4-*N*-acetyl-3-deazacytidine, 4-*N*-acetyl-3-deazacytidine.

Because of its pronounced inhibition of L-1210 growth *in vitro*, the 4-*O*-adamantoyl derivative **5** of 3-deazaUR was also examined for its antitumor effect in mice. Because of its relative insolubility, the compound was administered sc suspended in saline. At 50 mg/kg/day, 6 days sc, the adamantoyl derivative increased the life span of the tumor-bearing mice to an extent achievable with deazaUR only at the higher ip doses. Subcutaneous doses of deazaUR were less effective than comparable ip doses. The observed cleavage of the adamantoyl group from the nucleoside in the cell extract makes it appear likely that the inhibitory activity of the compound is exerted *via* the nucleoside analog.

Cell-free extracts of *E. coli* or Ehrlich ascites carcinoma which readily catalyze the cleavage of the glycosidic bond of UR do not cleave deazaUR.[15] Similarly, deazaCR is not deaminated by extracts of *E. coli* which effect the rapid deamination of CR. However, as determined with bacterial and tumor cell extracts and with intact cells, both analogs are converted to the nucleoside triphosphate stage but are not incorporated into RNA or DNA. At the nucleoside triphosphate stage the compounds interfere with the activity of RNA polymerase from *E. coli*,[15] and additional sites of interference are under investigation.

From the point of view of structure as it relates to activity, it is worth noting that replacement of nitrogen at position 3 of UR by carbon precluded enzymatic cleavage of the glycosidic bond and that the 3-nitrogen apparently participates in determining substrate specificity for CR deaminase. Replacement of the nitrogen with carbon does not, however, abolish the substrate nature of the deazapyrimidines for the appropriate nucleoside and nucleotide kinases, but their incorporation into RNA and DNA does not occur. On the basis of the marked antitumor activity seen in the L-1210 system, deazaUR has been selected for preclinical evaluation.

Experimental Section

Melting points were determined on a Fisher-Johns block and are uncorrected. Nmr spectra were determined on a Varian A-60 instrument with Me_4Si or DSS as internal standard. Uv spectra were determined on a Beckman DK-2 instrument. Unless specified otherwise, evaporations were carried out with a Büchler rotating evaporator under reduced pressure (aspirator). Analytical results for C, H, and N agreed within ±0.3% of theory.

4-Hydroxy-2-pyridone (3-DeazaU, 1). A mixture of 20 g (0.129 mol) of 5-carboxy-4-hydroxy-2-pyridone[7] (**2**) in distilled H_2O was heated for 6 hr at 200° in a stainless steel bomb. After cooling for 15 hr, the resulting brown solution (usually considerable crystallization of **1** has occurred and this material can be collected directly by filtration) was heated to boiling, treated with Norit, filtered through Celite, and concentrated to 100 ml on the steam bath. The solution was cooled, acidified (pH 4) with HOAc, and refrigerated for several hours. Crystals of **1** (8.87 g, 62%) were collected and a second crop (3.07 g, 21%) raised the yield to 83%. A small sample was recrystallized from MeOH to give colorless prisms: mp 271–273°; uv max (pH 1) 257 nm (ε 4280); uv max (pH 11) 253 nm (ε 6280), 261 sh (6000); uv max (MeOH) 277 nm (ε 4560); nmr (DMSO-d_6) δ 5.61 (d, 1, $J_{3,5}$ = 2 Hz, H_3), 5.89 (d of d, 1, $J_{5,6}$ = 7, $J_{5,3}$ = 3 Hz, H_5), 7.28 (d, 1, $J_{6,5}$ = 7 Hz, H_6). Addition of D_2O caused the peak at 5.61 (H_3) to disappear with corresponding collapse of the peak at 5.89 (H_5) to a doublet with $J_{5,6}$ = 7 Hz. *Anal.* ($C_5H_5NO_2$) C, H, N.

4-Amino-2-pyridone (3-DeazaC, 3). To 5 ml of dry benzyl alcohol was added 0.20 g of clean Na metal in small pieces. The mixture was stirred for 15 hr until solution was complete and 0.60 g (0.0036 mol) of 4-amino-2-chloropyridine hydrochloride and 2 ml of dry bis(2-methoxyethyl) ether [diglyme] were added. The resultant mixture was stirred while protected from moisture and was heated in an oil bath at 170° for 10 hr. The resultant mixture was evaporated to a small volume and dissolved in 6 *N* HCl (30 ml). The acidic solution was extracted with three 30-ml portions of Et_2O, which were discarded. The aqueous layer was then made strongly basic with NaOH pellets and extracted again with three 30-ml portions of Et_2O which were combined, washed once with 30 ml of H_2O, and dried (Na_2SO_4). Evaporation of the ether gave 0.90 g of a crude oil which was dissolved in $CHCl_3$, applied to a silica gel column (1-cm diameter, 30 g, dry packed), and eluted with $CHCl_3$. Fractions (10 ml) 25–40 were combined and evaporated to give 0.23 g of a colorless oil. The oil was dissolved in 20 ml of EtOH, 20 mg of 5% Pd/C was added, and the mixture was hydrogenated at 30 psi on a Parr apparatus for 90 min. The catalyst was removed by filtration using a Celite pad and the filter cake was washed well with EtOH. Evaporation of the solvent gave an oil which was dissolved in MeOH and applied to a preparative tlc plate (2 × 200 × 400 mm Mallinckrodt SilicAR-7GF). The plate was developed in the upper phase of EtOAc–*n*-PrOH–H_2O (4:1:2), and the major band was extracted with hot MeOH. Evaporation of the solvent gave a light yellow crystalline mass which was recrystallized from MeOH–Me_2CO–Et_2O to afford 0.05 g (13%) of **3**: mp 218.5–220° (lit.[8] mp 219–221°); uv max (pH 1) 252, 209 nm (ε 16,000, 18,000); uv max (pH 11) 260 nm (ε 7680), 268 sh (7350); uv max (H_2O) 259, 210 nm (ε 8020, 21,100), 265 sh (7800); nmr (DMSO-d_6) δ 5.27 (d, 1, $J_{3,5}$ = 1.5 Hz, H_3), 5.70 (d of d, 1, $J_{5,6}$ = 7, $J_{5,3}$ = 1.5 Hz, H_5), 7.05 (d, 1, $J_{6,5}$ = 7 Hz, H_6), 6.09 (br s, 2, NH_2), 11.06 (br s, 1, NH). *Anal.* ($C_5H_6N_2O$) C, H, N.

1-β-D-Ribofuranosyl-2-pyridone 4-*O*-(Adamantane-1-carboxylate) (4-*O*-Adamantoyl-3-deazaUR, 5). A solution of 0.5 g (0.0025 mol) of adamantoyl chloride in 2.5 ml of dry $CHCl_3$ was added dropwise over a 15-min period to a magnetically stirred solution of 0.5 g (0.0021 mol) of **4** in 10 ml of dry pyridine. The resulting solution was stirred for an additional 1.5 hr, and 5 ml of saturated aqueous $NaHCO_3$ solution was added. After 5 min, the mixture was diluted with $CHCl_3$ and H_2O, and the phases were separated. The organic phase was washed with 15 ml of $NaHCO_3$, and the combined aqueous phase was washed with 20 ml of $CHCl_3$. The combined organic phase was washed with 5 ml of $NaHCO_3$ solution and 20 ml of H_2O and was then dried over Na_2SO_4. This mixture was filtered, and the filtrate was evaporated *in vacuo*. The resulting white solid was crystallized from absolute EtOH (200 mg/10 ml, avoid long boiling in EtOH) to give 0.6 g (74%) of white crystalline product. This solid was recrystallized from EtOH to give crystalline **7**: mp 197–199°; uv (EtOH) max 300 nm (ε 5300), min 250 nm (ε 800); uv (0.1 *N* HCl) max 296 nm (ε 5600), min 250 nm (ε 1000); uv (0.1 *N* NaOH) max 257 nm (ε 7300), sh 272 (5500), min 237 nm (ε 4000). Acidification of the 0.1 *N* NaOH solution gave a spectrum with max 280 nm and min 250 nm, indicating saponification of the 4-adamantoyloxy ester linkage; nmr (DMSO-d_6–$CDCl_3$) δ 8.08 (d, 1, $J_{6,5}$ = 7.5 Hz, H_6), 6.08 (m, 3, $H_{3,5}$ and $_{1'}$), 5.24 (d, 1, $J_{2'\text{-OH-}2'}$ = 4 Hz, 2'-OH), 4.78 (t, 1, $J_{5'\text{-OH-}5',5''}$ = 5.5 Hz, 5'-OH), 4.41 (d, 1, $J_{3'\text{-OH-}3'}$ = 4 Hz, 3'-OH), 4.3–3.7 (m 5, $H_{2',3',4',5'}$ and $_{5''}$), 2.03 (s, 9, adamantoyl), 1.77 (s, 6, adamantoyl); nmr (DMSO-d_6–D_2O) δ 8.06 (d, 1, $J_{6,5}$ = 7.5 Hz, H_6), 6.15 (m, 3, $H_{3,5}$ and $_{1'}$), 4.09–3.51 (m, 5, $H_{2',3',4',5'}$, and $_{5''}$), 1.96 (s, 9, adamantoyl), 1.70 (s, 6, adamantoyl). *Anal.* ($C_{21}H_{27}NO_7$) C, H, N.

Radioactive 4-*O*-adamantoyl-deazaUR was prepared in the same manner by combining 0.45 g of deazaUR and 0.05 g of ^{3}H-deazaUR (labeled according to the Wilzbach technique by New England

Table II. Effect of 3-Deazauridine and 4-*O*-Adamantoyl-3-deazauridine on Leukemia L1210 in Male DBA_2/Ha Mice

Compound	Dosage, mg/kg/day	Route	Mortality, dead/total Day 5	Mortality, dead/total Day 30	Av life span (days), T/C[a]	% above control	No. of survivors
3-Deazauridine	100 qd 1–6	ip	2/33	31/33	12.7/8.2	55	2
3-Deazauridine	150 qd 1–6	ip	0/26	25/26	14.2/8.6	65	1
3-Deazauridine	200 qd 1–6	ip	0/34	29/34	12.6/7.8	61	5
3-Deazauridine	300 qd 1–6	ip	0/60	54/60	12.5/8.1	54	6
4-*O*-Adamantoyl-3-deazauridine	50 qd 1–6	sc	0/20	17/20	12.7/8.0	59	3

[a] Average life span excludes 30-day survivors. Each mouse received 1 × 10^6 L1210 cells ip 1 day prior to treatment.

Nuclear Corp.) and reacting it with the adamantoyl chloride. The product obtained after work-up had a specific activity of 18 μCi/mg.

Compounds. The 3-deazauridine used for antitumor evaluation in mice was purchased from the ICN Corp., Irvine, Calif.

Microbial Assays. The procedure used for these assays has been published previously.[17]

In Vitro **Antitumor Assays.** The *in vitro* antitumor assays were carried out by our micro technique whereby 0.5-ml aliquots of medium (RPMI 1630 + 10% calf serum) containing the analog are introduced into 16 × 125 mm screw cap culture tubes, followed by 0.5-ml portions of the medium containing 3×10^5 L-1210 cells. The cultures are incubated at 37° for 40 hr, after which time the viable cells are counted by Trypan Blue exclusion. During this time, the cell number in the controls increases *ca.* eight- to ninefold with an average cell viability of 99%.

In Vivo **Antitumor Assay.** On day 0, DBA_2/Ha mice of the same age group (5–7 weeks) were separated into weight groups (17–19 g ± 0.5 g). The ascites fluid from one or more mice (depending on the number of cells needed) inoculated ip with L-1210 cells 6–7 days prior to experiment was collected and the percentage of viable cells determined by Trypan Blue exclusion. The fluid was diluted with saline so that the 0.5-ml aliquot administered ip to each mouse contained 1×10^6 viable L-1210 cells. On day 1, the animals were randomized into the various test groups, and the deazapyrimidines were injected once daily for 6 consecutive days by the route indicated in Table II.

Biochemical Assays. To examine the cleavage of 4-*O*-adamantoyl-3-deazauridine, a total of 2 ml of packed L-1210 cells was collected from the intraperitoneal cavity of DBA_2/Ha mice. The cells were suspended in 10 ml of 0.05 *M* phosphate buffer, pH 7.5, and were disrupted by exposure for 1.5 min to a Bronson III sonifier. The cell debris was removed by centrifugation at 19,500*g* for 30 min, and a 4-ml aliquot of this extract was added to 5 ml of 0.05 *M* phosphate buffer, pH 7.5, containing 1×10^{-4} *M* 4-*O*-adamantoyl-^{3}*H*-deazauridine (specific activity 18.0 μCi/mg) and 1 ml of 0.1 *M* $MgCl_2$. (Such a large volume was used because of the relative insolubility of the adamantoyl derivative.) The mixture was incubated at 37°, and 1-ml aliquots were removed at stated intervals. They were immersed into boiling water for 2 min, the precipitates which formed were removed by centrifugation, the supernatant solutions were reduced in volume to 0.5 ml, and 0.1-ml portions were applied to Whatman No. 3 paper and were chromatographed together with authentic carriers in 1-butanol–H_2O (86:14) and a solvent prepared by dissolving 13.8 g of $Na_2HPO_4 \cdot H_2O$ in 900 ml of H_2O and adjusting the pH to 6.8 with H_3PO_4. The volume was raised to 1 l. and 600 g of $(NH_4)_2SO_4$ and 20 ml of *n*-PrOH were added. In these two solvent systems the R_f of the ester **5** is 0.91 and 0.03 and that of 3-deazauridine (**4**) is 0.44 and 0.50, respectively. Strips 3 cm in width extending from the origin to the solvent front were cut from the chromatogram and were divided into 1-cm segments. Each segment was placed into a scintillation vial to which was added 20 ml of toluene containing 0.4% of 2,5-diphenyloxazole and 0.01% of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene. Radioactivity was measured in a Packard TriCarb Model 3000 liquid scintillation counter. Control experiments were performed by incubating **5** in the same total volume of phosphate buffer, pH 7.5, containing 1 ml of 0.1 *M* $MgCl_2$ but no L-1210 cell extract.

Acknowledgments. The authors are grateful to Miss Rosemary Hromchak and Mr. Gary Bruns for their excellent technical assistance and thank Dr. E. Mihich for helpful discussions.

References

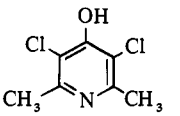
(1) J. Skoda, *Progr. Nucl. Acid Res. Mol. Biol.*, **2**, 197 (1963); C. E. Hoffmann in "Annual Reports in Medicinal Chemistry, 1967," C. K. Cain, Ed., Academic Press, New York, N. Y., 1968, p 120; G. M. Timmis and D. C. Williams, "Chemotherapy of Cancer," Butterworths, London, 1967.
(2) M. P. Mertes, J. Zielinski, and C. Pillar, *J. Med. Chem.*, **10**, 320 (1967).
(3) H. Pischel and G. Wagner, *Arch. Pharm. (Weinheim)*, **300**, 602 (1967), and references cited therein.
(4) M. J. Robins and B. L. Currie, *Chem. Commun.*, 1547 (1968); M. J. Robins, B. L. Currie, R. K. Robins, and A. Bloch, *Proc. Amer. Ass. Cancer Res.*, **10**, 73 (1969).
(5) (a) B. L. Currie, R. K. Robins and M. J. Robins, *J. Heterocycl. Chem.*, 7, 323 (1970); (b) B. L. Currie, M. J. Robins, and R. K. Robins, *ibid.*, **8**, 221 (1971).
(6) G. L. Neil, P. F. Wiley, R. C. Manak, and T. E. Moxley, *Cancer Res.*, **30**, 1047 (1970).
(7) G. Errera, *Ber.*, **31**, 1682 (1898).
(8) J. Baumler, E. Sorkin, and H. Erlenmeyer, *Helv. Chim. Acta*, **34**, 496 (1951).
(9) R. J. Rousseau and R. K. Robins, *J. Heterocycl. Chem.*, **2**, 196 (1965).
(10) H. J. den Hertog and D. J. Buurman, *Recl. Trav. Chim. Pays-Bas*, **75**, 257 (1956).
(11) M. J. Robins, B. L. Currie, R. K. Robins, and A. D. Broom, *Can. J. Chem.*, **49**, 3067 (1971).
(12) A. Albert and J. N. Phillips, *J. Chem. Soc.*, 1294 (1956).
(13) J. Hine, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1956, pp 274–275.
(14) A. Albert, R. Goldacre, and J. N. Phillips, *J. Chem. Soc.*, 2240 (1948).
(15) M. C. Wang and A. Bloch, *Biochem. Pharmacol.*, **21**, 1063 (1972).
(16) A. Bloch, G. Dutschman, and G. Grindey, *Proc. Amer. Ass. Cancer Res.*, **13**, 19 (1972).
(17) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **5**, 3345 (1966).

Synthesis and Anticoccidial Activity of 3-Fluoro-5-chloro- and -bromo-2,6-dimethyl-4-pyridinol

Lowell D. Markley* and Michael K. Eckman

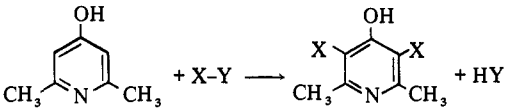
Ag-Organics Department, The Dow Chemical Company, Midland, Michigan 48640. *Received August 18, 1972*

3,5-Dichloro-2,6-dimethyl-4-pyridinol (clopidol, **1**) is the active component in the coccidiostat, COYDEN, sold by The Dow Chemical Co.[1,2] Other 3,5-dihalo-2,6-dimethyl-4-pyridinols including 3,5-dibromo, 3,5-diiodo, and 3-bromo-5-chloro have been synthesized and shown to have good anticoccidial activity.[1] Since many biologically active chemicals contain fluorine, it was of interest to synthesize several 3-fluoro-4-pyridinols and test them for possible anticoccidial activity.



1

The 3,5-dichloro-, 3,5-dibromo-, and 3,5-diiodo-2,6-dimethyl-4-pyridinols have been synthesized by halogenation of 2,6-dimethyl-4-pyridinol with chlorine,[2] bromine,[3] and iodine chloride,[4] respectively. The synthesis of the fluoro derivatives has required the use of a more indirect approach since fluorination of 2,6-dimethyl-4-pyridinol did not seem feasible.



X-Y = Cl_2, Br_2, or I-Cl

In 1965, Talik and coworkers[5] reported synthesizing 4-chloro-3-fluoro-2,6-lutidine (**6**). From this compound, we were able to prepare our desired fluoro-substituted pyridinols. Talik's synthesis of **6** (Scheme I) was utilized with several modifications as given in the Experimental Section. Treatment of 3-fluoro-4-nitro-2,6-lutidine 1-oxide (**5**) with phosphorous trichloride gave 4-nitro-3-fluoro-2,6-lutidine (**7**), previously not reported by Talik, in addition to the desired 4-chloro-3-fluoro-2,6-lutidine (**6**). The two materials were readily separated *via* distillation.