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.

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

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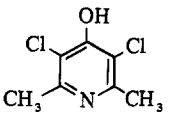
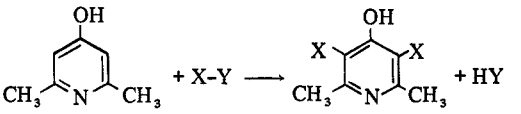
3,5-Dichloro-2,6-dimethyl-4-pyridinol (clopidol, **1**) is the active component in the coccidiostat, COYDEN, sold by The Dow Chemical Co.[1,2] Other 3,5-dihalo-2,6-dimethyl-4-pyridinols including 3,5-dibromo, 3,5-diiodo, and 3-bromo-5-chloro have been synthesized and shown to have good anticoccidial 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

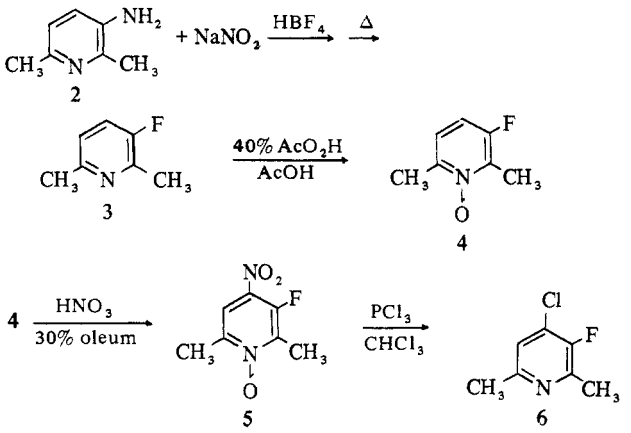
The 3,5-dichloro-, 3,5-dibromo-, and 3,5-diiodo-2,6-dimethyl-4-pyridinols have been synthesized by halogenation of 2,6-dimethyl-4-pyridinol with chlorine,[2] bromine,[3] and iodine chloride,[4] respectively. The synthesis of the fluoro derivatives has required the use of a more indirect approach since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



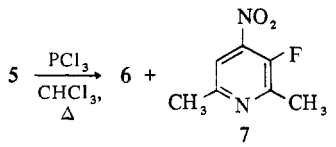
X-Y = Cl_2, Br_2, or I–Cl

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.

Scheme I. Synthesis of 4-Chloro-3-fluoro-2,6-lutidine (6)



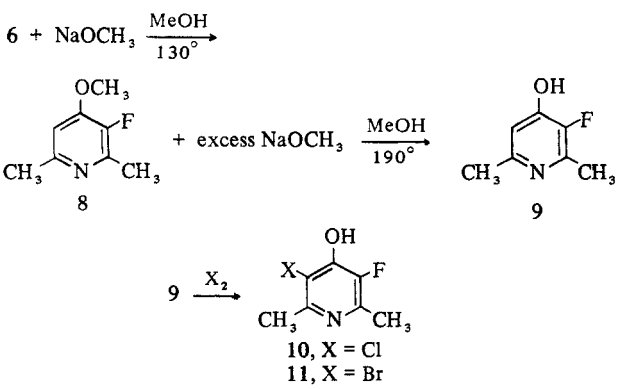
From 4-chloro-3-fluoro-2,6-lutidine (6) we synthesized the desired 3-chloro-5-fluoro-2,6-dimethyl-4-pyridinol (10) and 3-bromo-5-fluoro-2,6-dimethyl-4-pyridinol (11) *via* halo-



genation as outlined in Scheme II. Nucleophilic displacement of the 4-chloro group in 6 with 1 equiv of sodium methoxide took place readily at 130° giving 3-fluoro-4-methoxy-2,6-lutidine (8). Demethylation of 8 was attempted using three different reagents: boron tribromide, hydriodic acid, and sodium methoxide. A quantitative yield of demethylated product, 4-hydroxy-3-fluoro-2,6-lutidine (9), was obtained with excess sodium methoxide at 190°. From 9, the desired products, 10 and 11, were formed by chlorination and bromination, respectively.

Anticoccidial Activity. 3-Chloro-5-fluoro-2,6-dimethyl-4-pyridinol (10) and 3-bromo-5-fluoro-2,6-dimethyl-4-pyridinol (11) were tested against two strains of coccidiosis, *Eimeria necatrix* and *Eimeria acervulina*, using the method given in ref 1. As shown in Table I, both compounds showed activity equal to clopidol (1) against *E. necatrix* but little or no activity against *E. acervulina* at comparable concentrations.

Scheme II. Synthesis of 3,5-Dihalo-2,6-dimethyl-4-pyridinols (10 and 11)



Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ir spectra were recorded on an Infracord spectrophotometer and nmr on a Varian Associates A-60 instrument (Me_4Si) and are in accord with proposed structures. Elemental analyses were obtained on new compounds 9, 10, and 11 and the results were within ±0.3% of the theoretical values of those elements indicated by symbols.

3-Fluoro-2,6-lutidine 1-Oxide (4). To 105 g (0.84 mol) of 3 dissolved in 500 ml of glacial AcOH was added 210 g (1.1 mol of peroxide) of 40% peracetic acid. The reaction mixture was heated at 75–78° for 12 hr and the solvent was then removed *in vacuo*. The liquid residue remaining was dissolved in 400 ml of CH_2Cl_2 and extracted with saturated $NaHCO_3$ and removal of solvent gave a quantitative yield of hydrated 4, mp ~15–20°, which was used without purification. Bellas and Suschitzky[6] reported obtaining the pentahydrate, mp 53°.

4-Chloro-3-fluoro-2,6-lutidine (6) and 4-Nitro-3-fluoro-2,6-lutidine (7). To 114 g (0.615 mol) of 5 dissolved in 250 ml of $CHCl_3$ was added 250 ml (2.86 mol) of PCl_3. After heating the mixture for 3 hr at 65–70° and removal of solvent *in vacuo*, the liquid residue was slowly poured over 600 ml of ice. Neutralization (K_2CO_3) caused precipitation of an immiscible layer which was steam distilled and extracted with CH_2Cl_2. Evaporation of solvent *in vacuo* afforded 84.6 g of liquid which upon distillation gave 48.7 g (50%) of 6 [bp 90–93° (42 mm), $n^{24.5}$D 1.5006 (lit.[5] bp 174–175°)] and 30.3 g (29%) of 7, bp 113° (16 mm).

4-Methoxy-3-fluoro-2,6-lutidine (8). In a stainless-steel bomb was placed 40 g (0.25 mol) of 6 in 50 ml of MeOH and 100 ml of 2.6 *N* $NaOCH_3$–MeOH. After heating for 7 hr at 130°, the contents of the bomb were poured into 200 ml of H_2O. Extraction with CH_2Cl_2 afforded 37.7 g of liquid shown by nmr to be 92% 8 and 8% starting material 6. The product was used without purification since in the next step the starting material 6 is used up.

4-Hydroxy-3-fluoro-2,6-lutidine (9). In a stainless-steel bomb was placed 28 g of 8 and 150 ml of 5 *N* $NaOCH_3$–MeOH and the bomb was heated at 190° for 17 hr. The contents of the bomb were poured into 200 ml of H_2O and 100 ml of MeOH. The solution was neutralized (concentrated H_2SO_4) and the solvent removed *in vacuo* leaving a solid residue which was triturated with 400 ml of boiling MeOH. The filtrate yielded 25 g (98%) of 9, mp 248° dec. Sublimation [125° (0.2 mm)] gave pure product, mp 259° dec. *Anal.* (C_7H_8FNO) C, H, N.

3-Chloro-5-fluoro-2,6-dimethyl-4-pyridinol (10). Into a solution of 10 g of 9, dissolved in 50 ml of H_2O and 25 ml of 5 *N* HCl, was bubbled Cl_2 gas with precipitation of product. The reaction mixture was neutralized (K_2CO_3) and the product 10 was filtered and washed with H_2O and Me_2CO, 4.2 g (34%). Sublimation [150° (0.2 mm)] gave pure product, mp >360°. *Anal.* (C_7H_7ClFNO) C, H, N.

3-Bromo-5-fluoro-2,6-dimethyl-4-pyridinol (11). To 10 g of 9 dissolved in 75 ml of glacial AcOH was added 11.7 g of Br_2 dissolved in 20 ml of glacial AcOH. After stirring 2 hr, the product was filtered and washed with H_2O and Me_2CO: 6.1 g (39%), mp 270° dec. Recrystallization (DMF) gave pure 11, mp 284–286° dec. *Anal.* (C_7H_7BrFNO) C, H, N.

Table I. Activity of Compounds 10 and 11 and Clopidol

	Per cent control of coccidiosis[a]			
	E. necatrix		*E. acervulina*	
No.	125 ppm	250 ppm	125 ppm	250 ppm
10	100	100	0	0
11	100	100	0	70
Clopidol	100	100	95	100

[a] Anticoccidial index = % weight gain – (sum of the mean fecal and lesion scores). % coccidiosis control = (anticoccidial index of experimental group/anticoccidial index of uninoculated-unmedicated control)100.

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