adjusted to 6.8 ± 0.2 units for a period of 30 hr. At the end of this time, tlc in solvent systems A (R_f 0.18) and B (R_f 0) indicated the reaction was complete (FUDR R_f 0.68 A, 0.36 B). After removal of the catalyst, the filtrate was deionized by passing it through an Amberlite IR-120 (80 ml, H^+ form) column. The eluent was concentrated and placed on a column of Dowex 1-X2 (70 ml; Cl^- form). The column was eluted with distilled H_2O until free of substances absorbing at 270 mμ, then the column was eluted with 0.01 *N* HCl (total volume, 3300 ml) until the eluent showed no absorption at 268 mμ. Concentration of the eluent on the rotary-evaporator left a crystalline residue which was treated with Darco in hot H_2O, filtered, and subjected to freeze-drying to give 3.2 g of white crystals, mp 218–221° dec, $[\alpha]^{25}D$ +60.7°.

Esters of 5-Fluoro-2′-deoxyuridine-5′-carboxylate. Method A.—A mixture of FUDA (311 mg, 1.20 mmoles) and the alcohol in excess containing a drop of concentrated H_2SO_4 was refluxed for 6 hr. The reaction mixture was concentrated almost to dryness. H_2O was added to give white crystals which were collected and recrystallized from 95% EtOH to give pure product.

Method B.—A mixture of FUDA (0.326 g, 1.25 mmoles), *n*-BuOH (25 ml), and 0.5 g of Amberlite IR-120, H^+ resin were refluxed for 5 hr. The mixture was filtered while hot, and the filtrate was concentrated to dryness. The residue was recrystallized from *n*-BuOH to give white crystals.

N-β-Naphthyl-5-fluoro-2′-deoxyuridine-5′-carboxamide (VII, FUDAN).—To a solution of β-naphthylamine (0.066 g, 0.45 mmole) and dicyclohexylcarbodiimide (0.105 g, 0.51 mmole) in 5 ml of THF was added FUDA (0.120 g, 0.46 mmole) in 5 ml of H_2O. After removal of dicyclohexylurea, which formed almost immediately, the product precipitated after standing overnight. This product was collected and recrystallized (Me_2CO–H_2O) to give slightly pink crystals.

Assay of Compounds on Tissue Culture Cells.—The procedure for the tissue culture assay was based on the protocol of the National Cancer Chemotherapy Service Center[15] using KB cells and a human tumor cell line[16] as test cells and the Lowry protein[17] determination for the quantitative estimation of cell growth. Since the growth of the KB cell control varied slightly from day to day, results were normalized to a standard value for graphic comparison (Figure 1). In each experiment, a control sample of FUDR was included. The amount of each compound tested was calculated in milliequivalents of FUDA. In addition to KB cells, two other cell lines were used for assaying several compounds: a normal human cell line, WI 38,[18] and a mouse tumor cell line, GL-26. KB cells were obtained from Dr. G. L. Miller, Lankenau Hospital, and WI 38 cells from Dr. A. Girardi, Wistar Institute, Philadelphia, Pa. The mouse tumor line, Glioma 26, was started in tissue culture from the solid tumor in C57Bl/6 mice and has retained its ability to produce a tumor when reinjected into mice through several tissue culture passages. The resulting values are tabulated in Table II, based on the average of duplicate protein determinations on duplicate samples.

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Nucleoside Analogs. Synthesis of 6-(6-Amino-9-purinyl)-1,5-anhydro-6-deoxy-L-allitol

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The vast interest in effective cancer chemotherapeutic agents has led to numerous modifications of the purine and pyrimidine nucleosides. In a continuation of this search for anticancer agents, a variety of nucleosides has been prepared with the aim of inhibiting various stages of nucleotide metabolism in the cell.[2] Since ribonucleosides and ribonucleotides are easily cleaved hydrolytically or enzymatically, many nucleosides and nucleotides which may be effective agents in inhibiting the growth of malignant cells become ineffective *in vivo* because they are rapidly destroyed by cleavage into a purine or pyrimidine and a carbohydrate moiety.[3] In order to circumvent this difficulty, some workers have synthesized a novel class of compounds related to nucleosides which are hydrolytically and enzymatically stable.[4] These compounds are purines which contain at the 9 position a cyclopentyl or cyclohexyl ring which is substituted in such a manner that it sterically simulates the sugar moiety.

In order to provide a synthetic route to hydrolytically stable nucleosides which retain the natural purine moiety and a natural sugar, we have prepared 6-(6-amino-9-purinyl)-1,5-anhydro-6-deoxy-L-allitol (VI). Since this nucleoside does not possess the normal glycosidic linkage between the 9-nitrogen of the purine and the 1-carbon of the sugar, it is clear that this molecule will be stable toward hydrolysis and probably enzymatic cleavage.

The synthesis of VI was accomplished by the route outlined in Scheme I. Thus, 6-amino-1,5-anhydro-6-deoxy-L-allitol (II) was prepared from 2,3,4-tri-*O*-benzoyl-β-D-ribopyranosyl cyanide (I)[5] by reduction ($LiAlH_4$) as described by Coxon.[5] When the amine was allowed to react with 5-amino-4,6-dichloropyrimidine (III), a good yield of the corresponding substituted pyrimidine IV was obtained. Cyclization of IV by means of ethyl orthoformate and HCl gave a compound which on the basis of its uv spectrum (265 mμ) was a 6-chloropurine derivative but which exhibited only slight hydroxyl absorption in the ir. This product was assigned the ortho ester structure (V)[6] based on the previous observation that 3-(5-amino-6-chloro-4-pyrimidinylamino)-1,2-propanediol was converted to the ortho ester under similar conditions.[7] Attempts to purify V by crystallization resulted in the formation of a mixture of formate esters. Reaction of V with liquid NH_3 followed by acid hydrolysis gave the desired 6-(6-amino-9-purinyl)-1,5-anhydro-6-deoxy-L-allitol (VI).

Enzyme Inhibition Studies.—Enzymatic evaluation of VI against the enzyme, adenosine deaminase, revealed that the nucleoside does not act as a substrate for this enzyme but does show inhibitory activity. A plot of V_0/V against [I], where V_0 = initial velocity of the uninhibited enzymatic reaction, V = initial velocity of the inhibited reaction, and [I] = the concentration of inhibitor reveals that VI has an $([I]/[S])_{0.5}$ of 4.1.

Antimicrobial Studies.—Antimicrobial testing was

(1) (a) To whom inquiries should be addressed at the University of Minnesota. (b) Undergraduate Research Participant supported by the Undergraduate Research Participation Program of the National Science Foundation at the University of Mississippi, 1967.

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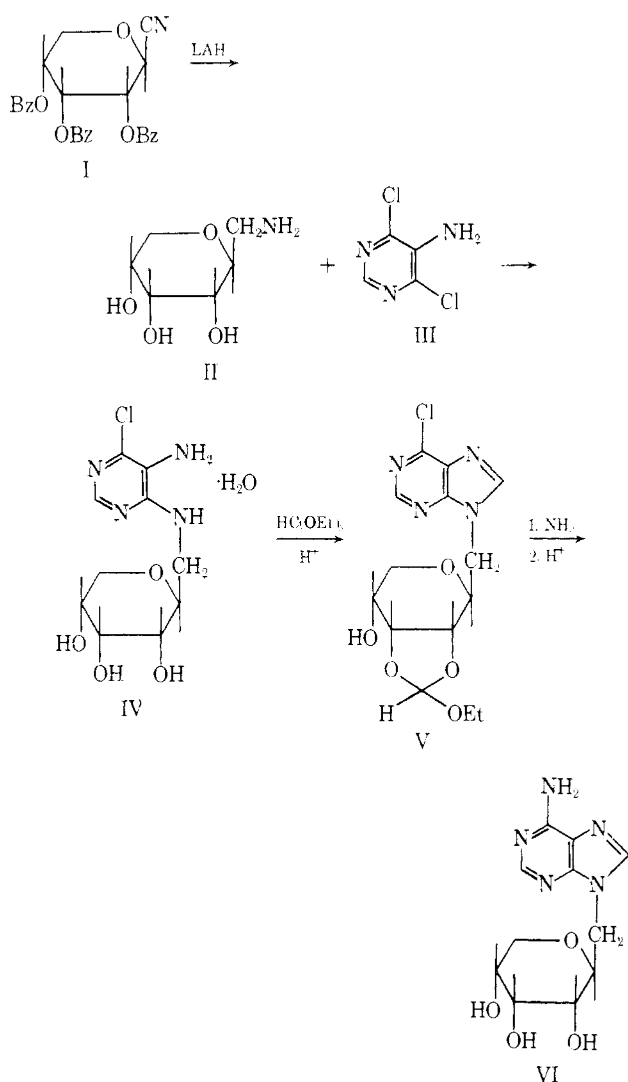
SCHEME I



performed by incorporating the compound into a suitable agar at the concentration of 500 ppm. After solidification, droplets of the test organisms were applied to the surface. The plates were incubated. Observations for kill or inhibition were made after a suitable time.

The only activity observed for VI for the organisms tested was somewhat less than 100% inhibition at 500 ppm against *Mycobacterium phlei*. The other organisms tested were *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, *Trichophyton mentagrophytes*, *Bacillus subtilis*, *Aerobacter aerogenes*, *Candida pelliculosa*, *Pseudomonas sp.* Strain 10, *Salmonella typhosa*, *Pullularia pullulans*, *Pseudomonas aeruginosa*, *Aspergillus terreus*, *Rhizopus nigricans*.

Experimental Section[8]

Reagents and Assay Procedure.—Adenosine and adenosine deaminase were purchased from the Sigma Chemical Co. The general method of assay has been described by Kaplan[9] and involves measuring the rate of disappearance of the absorption band of adenosine at 265 mμ. All enzymatic reactions were performed in 0.05 *M* phosphate buffer at pH 7.6 and 25°. The substrate and the stock solutions of all reagents were prepared in 0.05 *M* phosphate buffer at pH 7.6. For the assay, the cell contained a total volume of 3.1 ml which was 0.066 m*M* with respect to adenosine. To study inhibition, appropriate amounts of buffer were excluded from the cells and were replaced by an equal volume of a solution of the inhibitor in phosphate buffer.

6-(5-Amino-6-chloro-4-pyrimidinylamino)-1,5-anhydro-6-deoxy-L-allitol (IV).—A suspension of 28.0 g (59.4 mmoles) of I[5] in 300 ml of THF was slowly added to a mixture of $LiAlH_4$ (13.3 g) in 150 ml of THF. The mixture was stirred at room temperature for 1 hr and heated under reflux for 1.5 hr. The excess hydride was destroyed by the addition of EtOH, H_2O, and NH_4OH. The mixture was filtered through Filtercel and the filtrate was passed through a column (4.7 × 56 cm) of Dowex 50W-X8 resin. The column was eluted (H_2O, 3000 ml, and then with 2 *N* NH_4OH, 2000 ml). Concentration of the NH_4OH *in vacuo* gave II as a light brown syrup, yield 3.98 g. Compound II was identified by converting a small amount to the known hydrochloride salt.[5]

A mixture of 2.63 g (16.2 mmoles) of unpurified II, 2.42 g (16.2 mmoles) of 5-amino-4,6-dichloropyrimidine (III), and 1.70 g (32.4 mmoles) of NEt_3 in 50 ml of *n*-BuOH was heated under reflux for 20 hr. The reaction mixture was evaporated *in vacuo* and the tan oil was crystallized (H_2O). The white solid was removed by filtration; yield 3.793 g (84%), mp 159–161°. Recrystallization (H_2O) gave 2.90 g of pure product, mp 174–176°, $[\alpha]^{25}D$ −18.4 ± 0.3° (0.1 *N* HCl). *Anal.*[10] ($C_{10}H_{17}N_4ClO_4 \cdot H_2O$) C, H, N.

6-(6-Amino-9-purinyl)-1,5-anhydro-6-deoxy-L-allitol (VI).—To a suspension of 2.30 g (7.48 mmoles) of IV in 25 ml of ethyl orthoformate was added 10.3 mmoles of concentrated HCl. The mixture was stirred overnight at room temperature and evaporated *in vacuo* to a clear oil (V). The unpurified ortho ester was dissolved in 30 ml of liquid NH_3 and heated in a steel bomb at 55–60° for 18 hr. The volatile materials were evaporated and the tan solid was dissolved in 7 ml of 5% HCl and allowed to stand at room temperature for 30 min. The acidic solution was made basic with concentrated NH_4OH and chilled. A white solid was collected by filtration and gave 1.78 g (85%) of VI, mp 248–252°. One recrystallization (H_2O) gave the pure product as a monohydrate, mp 258–259°, $\lambda_{max}^{pH\,7}$ 261 mμ (ε 1.41 × 10^4), $[\alpha]^{25}D$ −13.25 ± 0.3° (H_2O).

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(8) The ir spectra were determined on a Perkin-Elmer Model 137 spectrophotometer; the uv spectra were determined on a Perkin-Elmer Model 4000A spectrophotometer; the enzyme studies were done on a Gilford Model 2000 spectrophotometer. The optical rotations were taken on a Perkin-Elmer Model 141 polarimeter. Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected.

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(10) The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

1-Acylindoles. IX. Syntheses of 1-Cinnamoyl-5-methoxy-2-methyl-3-indolylaliphatic Acids as Potential Antiinflammatory Agents

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Recent interest in the antiinflammatory action of indomethacin prompted us to prepare 1-acyl-3-indolylaliphatic acids for antiinflammatory tests. Various kinds of compounds had been synthesized previously,