reflux and HOAc was added until a clear solution was obtained. The mixture was refluxed for 2 hr and allowed to cool. After removing the solvents the residue was recrystallized from EtOAc, 20 g, mp 190–192°; recrystallization from EtOAc, mp 195–196°; mmp 173–185° with acid I.

B.—Acid I (20 g) was refluxed with 50 ml of Ac_2O for 5 min and allowed to cool. After cooling petroleum ether (bp 65–75°) was added and the product was filtered, 11 g. After two recrystallizations from EtOAc–petroleum ether, the melting point was 195–196°.

The filtrates from both A and B on removal of the solvents, followed by hydrolysis (NaOH) of the residue and acidifying gave the original acid I. *Anal.* ($C_{11}H_{14}O_5$) C, H.

4-Carbomethoxycyclohexane-1,1-diacetic Acid Anhydride (III).—Acid II (18 g) was dissolved in 250 ml of THF and allowed to cool to 15°. CH_2N_2 (0.15–0.2 mole) in 400 ml of Et_2O at 10° was added, and as the reaction proceeded most of the product precipitated out of solution. After standing 2 hr the crystals were filtered (14 g), mp 128–129°. Recrystallization from EtOAc–petroleum ether gave 13 g, mp 130–131°. *Anal.* ($C_{12}H_{16}O_5$) C, H.

N-Dimethylaminopropyl-9-carbomethoxy-3-azaspiro[5.5]-undecane-2,4-dione (IV).— The ester anhydride (13 g, 0.054 mole) was mixed with 6 g of 3-dimethylaminopropylamine and when homogeneous was heated at 200° for 1 hr. After cooling the product was distilled, bp 193–200° (0.45 mm), yield 7.6 g. *Anal.* ($C_{17}H_{28}N_2O_4$) C, H, N.

The **methiodide** was prepared in the usual manner, mp 229–230° (from EtOH, MeOH). *Anal.* ($C_{18}H_{31}IN_2O_4$) I.

N-Dimethylaminopropyl-9-hydroxymethyl-3-azaspiro[5.5]-undecane (V).–A solution of 7 g of IV in 200 ml of Et_2O was added to 5 g of $LiAlH_4$ dissolved in 500 ml of Et_2O. After 4 hr the mixture was decomposed (H_2O) in the usual manner, filtered, and dried (Na_2SO_4), and the solvent was stripped off. The residue was distilled, bp 133–138° (0.05 mm), yield 5.5 g. *Anal.* ($C_{16}H_{32}N_2O$) C, H, N.

The **hydrochloride** was prepared with alcoholic HCl, mp 295–296° (EtOH, MeOH). *Anal.* ($C_{16}H_{34}Cl_2N_2O$) Cl.

Synthesis of 5-Fluoro-2′-deoxyuridine-5′-carboxylic Acid and Its Derivatives[1]

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Although 5-fluoro-2′-deoxyuridine (FUDR) is an effective antitumor agent, it is easily hydrolyzed to 5-fluorouracil *in vivo* and *in vitro*, both by phosphorylase and by acid.[2,3] Thus in the clinical use of this drug the toxicity to the gastrointestinal system often masks its chemotherapeutic effectiveness. The present work reports the synthesis of 5-fluoro-2′-deoxyuridine-5′-carboxylic acid which is less toxic and more resistant to hydrolysis than FUDR.

Moss, *et al.*,[4] first reported the synthesis of uronic acid derivatives of nucleosides. They successfully oxidized uridine, thymidine, and adenosine to their 5′-carboxylic acids using Pt as a catalyst.[5] More recently, Imai and Honjo[6] reported the oxidation of deoxyuridine and its 5-bromo and 5-iodo derivatives and pointed out the difficulty in obtaining a product as the halogen substituent became more electronegative.

The synthetic scheme for the oxidation of FUDR to give 5-fluoro-2′-deoxyuridine-5′-carboxylic acid (FUDA) was not as simple as the one-step reaction might indicate. Previous workers[4,6] had used decidedly alkaline conditions (pH 8–9); however, no oxidation product was obtained with analogous treatment of FUDR. Our earlier method[7] for the preparation of phenyl-β-D-glucopyruronoside *via* the stepwise addition of $NaHCO_3$ proved to be fruitful. The pH was maintained between 6 and 7 and the reaction was run for 30 hr at 50–60° to give a 60% yield of FUDA.

The structure of FUDA was established by its ir and nmr spectra and by elemental analysis. Comparison of the nmr spectra of FUDA *vs.* FUDR in D_2O confirms the proposed structure of FUDA, both by the integration of nonexchangeable protons, and by the fact that absorption in the methylene region of FUDR at δ 3.8 is completely absent in FUDA.

While FUDR is sensitive to acid hydrolysis, FUDA has been found stable to acid. Although the mechanism of acid hydrolysis of nucleosides is ambiguous, most workers agree that the sugar ring O atom must be protonated.[8] For example, Garrett and co-workers[3] had suggested that the three steps involved in the acid hydrolysis of nucleosides are (1) protonation of the sugar ring O atom, (2) formation of the Schiff base, and (3) decomposition of the Schiff base. Thus, a plausible explanation for the enhanced stability toward acid hydrolysis of FUDA compared to FUDR can be provided. In the case of FUDA, protonation of the carbonyl O atom can greatly diminish the rate of protonation of the sugar ring O atom, thereby retarding hydrolysis.

FUDA has been shown to have only about one-tenth the toxicity of FUDR *in vivo* in mice. Due to the known lower level of esterase in tumor cells compared to normal cells,[9] the esters shown in Table I were prepared in the hope that, if a more cytotoxic ester could be found, such derivatives would become more permeable to tumor cell membrane, whereas normal cells, by virtue of their esterase content, would hydrolyze it back to FUDA.

The method of synthesis was acid catalysis, both by use of H_2SO_4 and ion-exchange resin, thereby taking advantage of the stability of FUDA toward acid hydrolysis. During these syntheses, no 5-fluorouracil could be detected in the reaction mixture by means of tlc. The use of dicyclohexylcarbodiimide (DCC) as a condensing agent for the preparation of esters was attempted. No product could be found with this method and only the anhydride of FUDA was isolated. Its structure was established by its ir spectrum and complete conversion to FUDA upon hydrolysis. On the other hand, DCC was an effective agent in yielding the amide VII from β-naphthylamine. The facile formation of this amide has led us to our present attempts

(1) This work was supported by U. S. Public Health Service Grant CA 07339; presented at the ACS Mid-Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 1968.

(2) C. Heidelberger, G. D. Birnie, J. Boohar, and D. Wentland, *Biochim. Biophys. Acta*, **76**, 315 (1963).

(3) E. R. Garrett, J. D. Seydel, and A. J. Sharpen, *J. Org. Chem.*, **31**, 2219 (1963).

(4) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963).

(5) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **74**, 5605 (1952).

(6) K. Imai and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **13**, 7 (1965).

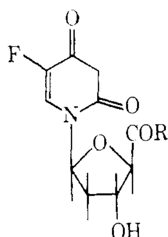
(7) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **75**, 1042 (1953).

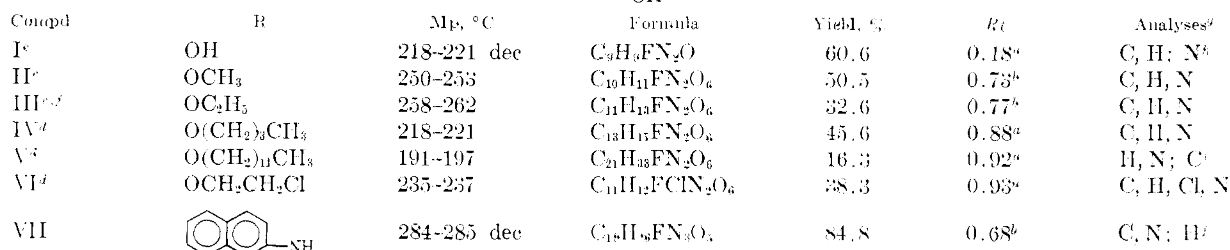
(8) G. W. Kenner, Ciba Foundation Symposium on the Chemistry and Biology of Purines, Little, Brown and Co., Boston, Mass., 1957, p 312.

(9) K. C. Tsou, H. C. Su, C. Segebarth, and U. Mirachi, *J. Org. Chem.*, **26**, 4987 (1961).

TABLE I

DERIVATIVES OF 5-FLUORODEOXYURIDINE-5-CARBOXYLIC ACID





Compd	R	Mp, °C	Formula	Yield, %	R_f	Analyses[g]
I[c]	OH	218–221 dec	$C_9H_9FN_2O$	60.6	0.18[a]	C, H; N[h]
II[c]	OCH_3	250–253	$C_{10}H_{11}FN_2O_6$	50.5	0.73[b]	C, H, N
III[c,f]	OC_2H_5	258–262	$C_{11}H_{13}FN_2O_6$	32.6	0.77[b]	C, H, N
IV[d]	$O(CH_2)_3CH_3$	218–221	$C_{13}H_{15}FN_2O_6$	45.6	0.88[a]	C, H, N
V[d]	$O(CH_2)_{11}CH_3$	191–197	$C_{21}H_{33}FN_2O_6$	16.3	0.92[a]	H, N; C[i]
VI[d]	OCH_2CH_2Cl	235–237	$C_{11}H_{12}FClN_2O_6$	38.3	0.93[a]	C, H, Cl, N
VII	(naphthyl)–NH	284–285 dec	$C_{18}H_{16}FN_3O_5$	84.8	0.68[b]	C, N; H[j]

[a] BuOH saturated with H_2O. [b] $CHCl_3$–*i*-PrOH (3:1). [c] See method A in Experimental Section. [d] See method B in Experimental Section. [e] $[\alpha]^{25}D$ +60.7° (dioxane). [f] $[\alpha]^{25}$ +25.9° (dioxane). [g] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [h] N: calcd, 10.34; found, 10.77. [i] C: calcd, 58.86; found, 59.27. [j] H: calcd, 4.19; found, 4.62.

to prepare other amides that are of possible therapeutic interest.

A comparison of the activity of FUDA and five derivatives, at a level of 50 μg/ml of media, on the growth rate of KB cells is shown in Figure 1. It can be seen that all compounds had some tumor inhibitory activity. The dodecyl ester V showed a growth inhibition rate similar to that of FUDR. This compound was less soluble in the tissue culture media than FUDR, but appeared to go into solution during incubation of the cells at 37°. At 5 μg FUDR was found to be more active than V.

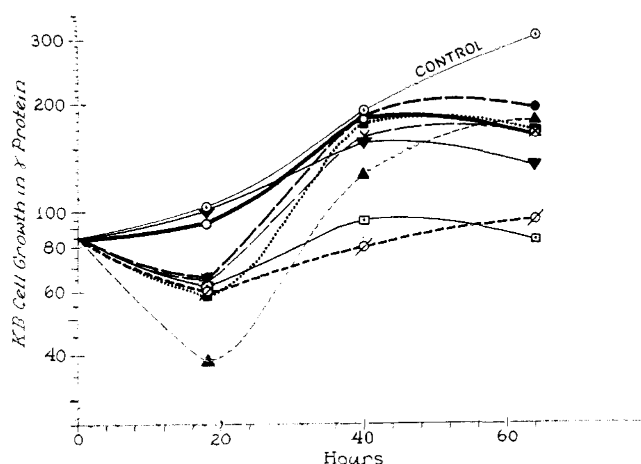


Figure 1.—Inhibitory action of FUDA and derivatives on KB cells in tissue culture: II, III, ——●; VI, –▲; IUDA, ······■; IV, ——×; FUDA, ——○; VII, ——▼; V, --ϕ; FUDR, ⊡.

Comparison of the effect of FUDR, FUDA, and two of its derivatives, in terms of per cent of control cell growth, against two tumor cell lines and a human normal cell line (WI 38) was also carried out (Table II). It is interesting to note that FUDA is moderately toxic to both tumor cell lines, but is relatively nontoxic to the human normal cell line. III shows some activity against the KB cells and normal cells.

The activity of FUDR is considered due either to its conversion to the corresponding 5′-phosphate (FUDRP)[10–12] or its enzymatic cleavage to 5-fluorouracil[13,14] (FU). Since FUDA can not form FUDRP, and is extremely resistant toward hydrolysis by acid or phosphorylase, it is obvious that a different mechanism for its activity must be invoked.

TABLE II

EFFECT OF FUDA AND DERIVATIVES ON DIFFERENT CELL LINES

Compd	% of control cell growth		
	Human tumor cells (KB)	Human normal cells (WI38)	Mouse cells (Glioma 26)
FUDR	27.4	49.1	49.3
FUDA	58.7	83.8	69.0
III	62.7	95.3	108.0
V	30.9	49.1	72.6

Experimental Section

Melting points, uncorrected, were taken on a Rinco apparatus. Ir spectra were determined in KBr with a Perkin-Elmer 137 spectrophotometer. Uv spectra were determined with a Beckman DB-G spectrophotometer. Optical rotation measurements were taken on a Zeiss polarimeter. Tlc was run on Brinkmann silica gel GF_{254} with BuOH saturated with H_2O (system A) and $CHCl_3$–*i*-PrOH (3:1) (system B), and spots were located by visual examination under uv light. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for C, H, and N were within ±0.4% of the theoretical values.

5-Fluoro-2′-deoxyuridine-5-carboxylic Acid (I, FUDA).—Oxygen was bubbled through a solution of 5-fluoro-2′-deoxyuridine (FUDR) (5.0 g, 20.3 moles) in H_2O (750 ml) in a flask equipped with a high-speed stirring motor at 50–60°. The pH of the solution was adjusted to pH 6.7 with $NaHCO_3$ solution (1.6 g/100 ml), at which time Pt black (1.3 g) was added; additional Pt black (0.9 g) was added after 24 hr. The pH was continually

(10) R. Duschinsky, T. Gabriel, W. Tautz, A. Nussbaum, M. Hoffer, E. Grunberg, J. H. Burchenal, and J. J. Fox, *J. Med. Chem.*, **10**, 47 (1967).

(11) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein, *Proc. Natl. Acad. Sci. U. S.*, **44**, 1004 (1958).

(12) K. U. Hartmann and C. Heidelberger, *J. Biol. Chem.*, **236**, 3006 (1961).

(13) K. L. Mukherjee, J. Boohar, D. Wentland, F. J. Ansfield, and C. Heidelberger, *Cancer Res.*, **23**, 49 (1963).

(14) K. L. Mukherjee and C. Heidelberger, *J. Biol. Chem.*, **235**, 433 (1960).

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.

(15) CCNSC, *Cancer Chemotherapy Rept.*, **25**, 22 (1962).
(16) H. Eagle, *Proc. Soc. Exp. Biol. Med.*, **89**, 362 (1955).
(17) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(18) L. Hayflick and P. S. Moorehead, *Exp. Cell Res.*, **25**, 585 (1961).

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.

(2) (a) A. D. Welch, *Cancer Res.*, **21**, 1475 (1961); (b) J. M. Buchanan in "The Nucleic Acids," Vol. III, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1960, p 304; R. E. Handschumacher and A. D. Welch, *ibid.*, p 453.
(3) (a) P. M. Roll, H. Weinfeld, E. Carroll, and G. B. Brown, *J. Biol. Chem.*, **220**, 439 (1956); (b) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967, pp 79, 93.
(4) H. J. Schaeffer, S. Marathe, and V. Alks, *J. Pharm. Sci.*, **53**, 1368 (1964).
(5) B. Coxon, *Tetrahedron*, **22**, 2281 (1966).
(6) The oily product obtained from this reaction is probably a mixture of V and the 2,3 ortho ester.
(7) H. J. Schaeffer, D. Vogel, and R. Vince, *J. Med. Chem.*, **8**, 502 (1965).