The purified base was dissolved in hot EtOH and ethereal HCl was added to the turbidity point. After the mixture was cooled, the salt was collected and recrystallized from 2-propanol to give 12.3 g (65% overall yield), mp 235–237 °C dec.

1-(4-Chloro-3-sulfamylbenzamido)-6-chloro-5-indolinesulfonamide (12). To a stirred solution of 2.84 g (0.010 mol) of 11 and 3.03 g (0.030 mol) of triethylamine in 100 mL of THF was added 3.75 g (0.015 mol) of 4-chloro-3-sulfamylbenzoyl chloride[8] in portions over a 30-min period. After the mixture was stirred overnight, the precipitate salt was removed by filtration and the filtrate was evaporated to dryness. The residual solid was triturated with Na_2CO_3 and with H_2O and then dried. Recrystallization from EtOAc furnished 2.5 g (45%) of pure **12**, mp 177–179 °C.

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Phosphonate Analogue of 2′-Deoxy-5-fluorouridylic Acid

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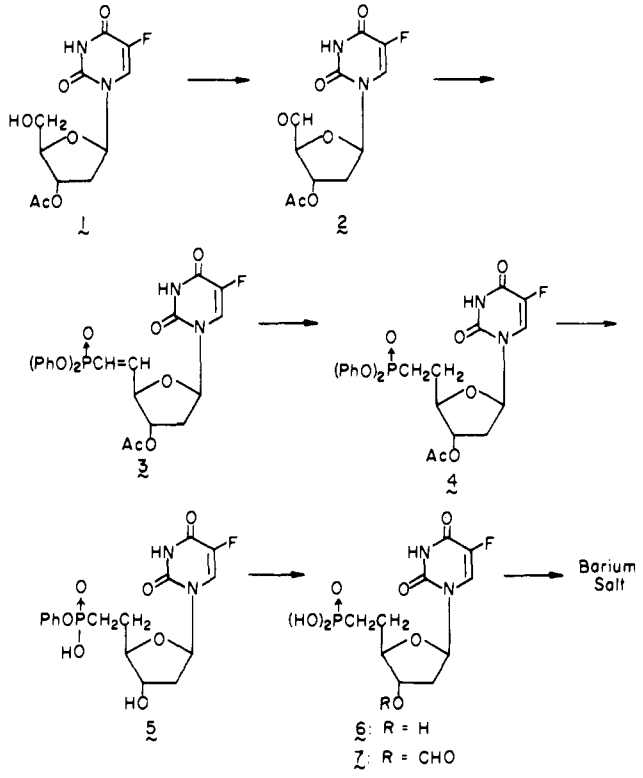
The phosphonate analogue (**6**) of 2′-deoxy-5-fluorouridylic acid has been prepared via a Pfitzner–Moffatt oxidation and Wittig reaction. This compound was found to inhibit thymidylate synthetase from three sources and to be cytotoxic to H.Ep.-2 cells in culture.

It is widely accepted that 5-fluorouracil (FU) exerts its cytotoxic action and anticancer effects by its inhibition, after conversion to 2′-deoxy-5-fluorouridylic acid (FdURP), of thymidylate synthetase.[1] Further, resistance to FU is thought to be due to loss of the anabolizing enzymes that carry out its conversion to FdURP.[1,2] Efforts to enhance the activity of FU and to circumvent the resistance problem by the use of 2′-deoxy-5-fluorouridine have been essentially negative, probably because it is rapidly degraded to FU, but attempts to overcome this phosphorolysis have so far met with little success.[3]

Another approach to overcoming this problem involves the preparation of a metabolically stable derivative of FU that will penetrate cells and inhibit thymidylate synthesis. A logical candidate would appear to be the phosphonate analogue of FdURP (**6**). This compound should be sterically very similar to FdURP; yet, it should not be dephosphorylated because of the stability of the C–P bond. However, since nucleotides are not phosphorolyzed, the glycosyl bond should also be metabolically stable. In fact, Moffatt and co-workers have shown that phosphonates of this type are substrates for the phosphodiesterase of snake venom[4] and that uridine cyclic 2′,3′-phosphonate binds more tightly to ribonuclease A than the cyclic phosphate.[5]

The recently established antitumor activity of PALA[6] clearly indicates that a phosphonate is capable of penetrating mammalian cells sufficiently to cause cell death by the inhibition of a specific enzyme[7] and provided impetus for the present work.

The synthesis of this phosphonate derivative was based on the work of Jones and Moffatt.[4] 3′-*O*-Acetyl-2′-deoxy-5-fluorouridine (**1**)[8] was oxidized to the corresponding aldehyde **2** by treatment with Me_2SO, DCC, pyridine, and trifluoroacetic acid. The aldehyde was



allowed to react with diphenyl (triphenylphosphoranylidene)methylphosphonate[9,10] and the resultant olefin **3**, predominantly the trans isomer (ca. 7:3, NMR), reduced

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catalytically with 5% palladium on barium sulfate catalyst.[4] Unfortunately, reduction was incomplete even at 51 psi, a high catalyst-to-substrate ratio, and a long reduction period. Complete reduction of the double bond was accomplished with Adam's catalyst but only with concomitant loss of fluorine from about one-half of the substrate. Reduction of the trans isomer of **3** could be accomplished with diimide,[9] and the cis isomer that remained could then be reduced with palladium on barium sulfate, indicating that in the original attempt to reduce **3** catalytically only the cis isomer was reduced. Treatment of the saturated compound **4** with aqueous 1 N sodium hydroxide resulted in removal of one phenyl group[6] to give **5**. More vigorous conditions (3 N NaOH at 105 °C for 3 h) resulted in the formation of a new compound, in addition to the desired product **6**, tentatively identified by its UV spectrum as an imidazoline formed by contraction of the pyrimidine ring.[11] The remaining phenyl group could be facilely removed by treatment of **5** with snake venom[4] at pH 8.5 (the reaction was considerably slower at pH 7). Purification of the target compound **6** was accomplished on an ion-exchange resin. The product was eluted with 2.5 N formic acid, but this treatment resulted in the formylation of the 3′-OH to give an unstable compound **7**, which was deformylated and converted to the barium salt for storage. Elemental analyses indicate that salt formation occurred at both the phosphonate moiety and the acidic lactam of the pyrimidine ring, which is not surprising in view of the known acidity of 5-fluorouracil.

Biologic Activity. The phosphonate analogue **6** is a potent inhibitor of thymidylate synthetase from *Lactobacillus casei*, *Escherichia coli*, and Coliphage T_2 (Table I). With all three synthetases it was necessary to preincubate the enzyme with the analogue to obtain inhibition. Preincubation times required to inhibit the *L. casei* enzyme are shown in Table II. Fluorodeoxyuridylate inhibits the *L. casei* enzyme 50% at 1.2×10^{-7} M without preincubation.

Analogue **6** was moderately cytotoxic to H.Ep.-2 cells in culture (ED_{50} = 45 μM),[15] although whether this results from its inhibition of thymidylate synthetase is not established.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. The preparative separations were carried out on Brinkmann 2-mm silica gel F-254 plates. The analytical sample was essentially TLC homogeneous. UV absorption spectra were determined in 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH with a Cary 17 spectrophotometer; the maxima are reported in nanometers ($\epsilon \times 10^{-3}$). The NMR spectra were determined with a Varian XL-100-15 spectrometer in the solvent indicated with tetramethylsilane as an internal reference; chemical shifts (δ in parts per million) quoted in the case of multiplets are measured from the approximate center. Mass spectral data were taken with a Varian MAT 311A instrument equipped with a combination EI/FI/FD ion source. Analytical results obtained were within ±0.4% of the theoretical values.

Diphenyl [1-(2,5,6-Trideoxy-β-D-*ribo*-hex-5-enofuranosyl)-5-fluorouracil]-6′-phosphonate (3). A solution of 3′-*O*-acetyl-2′-deoxy-5-fluorouridine (**1**, 2.18 g, 7.56 mmol) and dicyclohexylcarbodiimide (4.69 g, 22.7 mmol) in Me_2SO (34 mL) was treated with pyridine (0.64 mL, 7.56 mmol) followed by trifluoroacetic acid (0.29 mL, 1.68 mmol), and the reaction mixture was stirred for 24 h at room temperature. After removal of the precipitated dicyclohexylurea, diphenyl (triphenylphosphoranylidene)methylphosphonate[9,10] (7.7 g, 15.1 mmol) was added with stirring to the Me_2SO solution of the aldehyde and the resulting solution stirred for 2 days at ambient temperature before it was evaporated to dryness in vacuo. The resulting residue was purified by chromatography on thick silica gel plates using 9:1 $CHCl_3$–MeOH as eluate. Extraction of the silica gel with methanol gave the product as a light orange solid: yield 3.29 g (84%); MS 516 $(M)^+$, 477 $(M + 1 - CH_3CO)^+$, 456 $(M - CH_3CO_2H)^+$, 423 $(M - OC_6H_5)^+$, 387 $(sugar)^+$; NMR ($CDCl_3$) δ 2.1 (s, CH_3CO), 1.95–2.4 (m, $2H_{2'}$), 4.6 (m, $H_{4'}$), 5.07 (m, $H_{3'}$), 6.3 (m, $H_{1'}$ and $H_{6'}$), 6.9 and 7.3 (2 m, phenyl, $H_{5'}$ and H_6), 8.35 (br, NH). The approximate location of $H_{5'}$ and assignments of $H_{1'}$, $H_{3'}$, $H_{4'}$, and $H_{6'}$ were confirmed by spin decoupling.

Table I. Inhibition of Thymidylate Synthetase by the Phosphonate Analogue of Fluorodeoxyuridylate[a]

thymidylate synthetase from	concn, M, for 50% inhibn	enzyme concn, M
L. casei	7×10^{-6}	8×10^{-9}
E. coli	6×10^{-7}	8×10^{-9}[b]
Coliphage T_2	6×10^{-8}	4×10^{-9}

[a] The analogue was preincubated with the enzyme in the assay mix,[12] 0.11 M 2-mercaptoethanol, 3×10^{-4} M tetrahydrofolate, 0.012 M formaldehyde, and 0.021 M $MgCl_2$, at 30 °C for 20 min, and the reaction was initiated with dUMP, 4×10^{-5} M. [b] Calculated assuming that the *E. coli* enzyme has the same turnover number as the *L. casei* enzyme.[13] We are grateful to Drs. Frank and Gladys Maley and Dr. J. Galivan of the Public Health Research Institute of New York for providing the *E. coli* and Coliphage T_2 enzymes.[14]

Table II. Effect of Preincubation Time on Inhibition of *L. casei* Thymidylate Synthetase by the Phosphonate Analogue of Fluorodeoxyuridylate[a]

preincubation time, min	% inhibn
0	0
5	45
10	64
20	75
40	85

[a] Conditions as in Table I. Phosphonate present at 2×10^{-5} M.

Diphenyl [1-(2,5,6-Trideoxy-β-D-*ribo*-hexofuranosyl)-5-fluorouracil]-6′-phosphonate (4). To a mixture of the olefin **3** (3.27 g, 6.33 mmol) and potassium azodicarboxylate (6.67 g, 34 mmol) in pyridine (60 mL) was added dropwise with stirring under a nitrogen atmosphere a solution of 2.92 mL (50.8 mmol) of acetic acid in 14 mL of pyridine. The mixture was stirred for 24 h before it was filtered into 1 L of water, which was extracted with chloroform. Evaporation of the dried chloroform extract gave 2.14 g of partially reduced material. A solution of this material in ethanol (30 mL) containing 428 mg of 5% palladium on barium sulfate catalyst was reduced overnight at atmospheric pressure. The catalyst was removed by filtration and the filtrate evaporated to dryness in vacuo to give a yellow glass, yield 2.0 g (61%), that was used in the next step without further purification: MS 519 $(M + 1)^+$, 474 $[518 - (H_2NCO)]^+$, 458 $(518 - MeCO_2H)^+$, 425 $(518 - OC_6H_5)^+$, 389 $(sugar)^+$, 365 $(518 - MeCO_2H - OC_6H_5)^+$, 329 $(389 - MeCO_2H)^+$. The MS showed the absence of any of the olefin **3**.

[1-(2,5,6-Trideoxy-β-D-*ribo*-hexofuranosyl)-5-fluorouracil]-6′-phosphonic Acid (6) Barium Salt 1.5 Hydrate. A solution of the diphenyl ester **4** (2.0 g, 3.9 mmol) in 20 mL of 1 N sodium hydroxide was stirred and heated at 105 °C for 1 h (to give **5**), then taken to pH 8.5 with 0.5 M sulfuric acid, and treated with 100 mg of phosphodiesterase (snake venom from *Crotalus atrox*). After stirring overnight at room temperature, the mixture was filtered and introduced on a column of Dowex 1-X2 (formate) resin (200–400 mesh, 57 × 2.5 cm). After elution with 800 mL of 0.1 N, 200 mL of 0.5 N, and 600 mL of 1 N formic acid, the product was obtained by elution with 2.5 N formic acid. Evaporation of the formic acid solution by freeze-drying gave a mixture of **6** and the 3′-formyl compound **7** as an unstable purple solid: yield 255 mg; MS 325 (M + 1) and 353 (M + 1 of **7**). A solution of the solid in 10 mL of water was taken to pH 8.5 with

barium hydroxide solution, which was then filtered, and the filtrate diluted with 20 mL of ethanol to give the product as a white precipitate. It was collected by filtration, washed with absolute ethanol, and dried 4 h at 100 °C (0.07 mm): yield 308 mg (15%); UV (0.1 N HCl) 268 nm (log ϵ 8.63), (pH 7) 268 (8.78), (0.1 N NaOH) 268 (7.20); NMR (D_2O) δ 1.3-2.1 (m, $2H_{5'}$ and $2H_{6'}$), 2.3 (m, $2H_{2'}$), 3.96 (m, $H_{4'}$), 6.26 (t, $H_{1'}$), 7.5 (d, J_{HF} = 6 Hz, H_6); LC, C_{18} column (Waters), 0.1 M $NH_4H_2PO_4$ (pH 3.5), retention time 7.8 min. Anal. ($C_{10}H_{11}FN_2O_7P \cdot 1.5Ba \cdot 1.5H_2O$) C, H, N.

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Correlation of Carbonic Anhydrase Inhibitory Activities of Benzenesulfonamides with the Data Obtained by Use of Nitrogen-14 Nuclear Quadrupole Resonance

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Nitrogen-14 nuclear quadrupole resonance (NQR) spectra of several benzenesulfonamides in their solid state are reported and analyzed in the framework of the Townes and Dailey theory. Satisfactory correlations between the ($\sigma_{NH} - \sigma_{NS}$) electron densities at the sulfamyl nitrogen and the in vitro carbonic anhydrase inhibitory activities of the sulfonamides have been found. The correlations are in accord with the results of other studies that show the carbonic anhydrase inhibitory activities to be largely influenced by the electronic property of the sulfamyl group.

It has been well established[1,2] that sulfonamides are active as inhibitors of carbonic anhydrase, the enzyme responsible for the conversion of carbon dioxide and water to hydrogen ion and bicarbonate ion. Since the original observation by Mann and Keilin,[3] other reports[4] have confirmed the fact that high carbonic anhydrase inhibition was obtained in these compounds where the unsubstituted sulfamyl group ($-SO_2NH_2$) was attached directly to an aromatic group (phenyl, naphthyl, or heterocyclic) and that *N*-sulfamyl substitution abolished activity for practical purposes. Kakeya et al.[5] have shown that carbonic anhydrase inhibitory activity of the sulfonamides depends largely on the electronic property of the sulfamyl group. These authors correlate the in vitro inhibitory activity of the sulfonamides with various parameters such as the Hammett σ value, NMR chemical shift of the sulfamyl protons, and pK_a.

In the present work, several benzenesulfonamides have been investigated by nitrogen-14 nuclear quadrupole resonance (NQR) spectroscopy. NQR techniques are well suited to probe the electronic environment of a nucleus and, thus, can be employed to determine the electron distribution at the site of the atom containing the nucleus.[6,7] Correlations of NQR data with the in vitro carbonic anhydrase inhibitory activities of the benzenesulfonamides are considered.

Experimental Section

The NQR signals were detected by using the spin-echo technique.[8,9] Two radio-frequency (rf) pulses of height about 4 kV peak to peak were applied to a coil containing the sample, one pulse (90°) at $t = 0$ and the second pulse (180°) at $t = \tau$, and an echo was observed at $t = 2\tau$. The 90 and 180° pulses were of widths about 20 and 40 μs, respectively, and the interval between the pulses was on the order of 2-3 ms.

The experiments were performed on polycrystalline samples obtained from commercial sources and used as such. All the measurements were made at liquid nitrogen temperature (77 K). Very weak resonances were detected with the aid of a signal averager.[10]

Experimental Results

For nitrogen-14 ($I = 1$), the NQR spectrum consists, in general, of three lines[6,7]

$$\nu_{\pm} = \tfrac{3}{4}e^2qQ(1 \pm \eta/3) \quad (1)$$

and

$$\nu_d = \nu_+ - \nu_- \quad (2)$$

where eQ is the quadrupole moment of the nucleus; e^2qQ and η are, respectively, the quadrupole coupling constant and asymmetry parameter. The quantities q_{xx}, q_{yy}, and $q = q_{zz}$ are the diagonalized components of the electric-field gradient (EFG) tensor at the site of the nitrogen in the principal axis system chosen such that

$$|q_{zz}| \geq |q_{yy}| \geq |q_{xx}|$$

In this notation

$$\eta = (q_{xx} - q_{yy})/q_{zz} \quad (3)$$

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