(b) Antibacterial. Nutrient broth was used to grow the cultures and nutrient agar for the plates. Incubation was at 37° overnight.

(c) Antifungal. Sabouraud agar was used for the cultures and the plates. Sabouraud broth was used to wash off the culture for inoculation. Incubation was at 27° for 3–4 days.

(d) Plant Disease. Foliage Tests. Test plants were sprayed with aqueous emulsions of the compounds followed by inoculation with cultures of plant pathogens. The test plants were grown in a greenhouse under standard conditions of temperature and humidity, and after a suitable period the severity of the plant diseases was measured with reference to controls.

(e) Plant Disease. Soil Results. Compounds were incorporated into soil at the levels indicated. Test plants treated with plant pathogenic cultures were grown under standard conditions in a greenhouse, and after a suitable period disease conditions were measured with reference to controls.

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5-Hydroxymethyltubercidin. Synthesis, Biological Activity, and Role in Pyrrolopyrimidine Biosynthesis†

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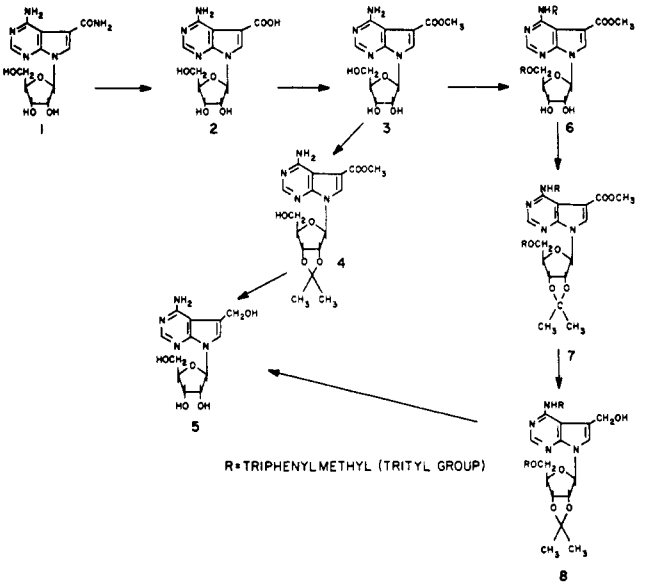
Three naturally occurring 4-amino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine nucleosides have been isolated from the *Streptomyces* (for review see ref 2). They are tubercidin, toyocamycin, and sangivamycin. These three nucleoside antibiotics are structural analogs of adenosine in which N-7 of the imidazole ring has been replaced by a carbon atom. These antibiotics have significant antibacterial, antiviral, and anticancer activity in experimental animal systems.[2-11] The chemical reactivity of a number of pyrrolopyrimidine ribonucleoside analogs has also been studied.[2,12-15] Studies on the biosynthesis of these pyrrolopyrimidine nucleosides show that the carbon-8 of GTP is lost as formic acid; carbons 1′, 2′, and 3′ of the ribosyl moiety serve as the carbons of the pyrrole ring and the cyano group of toyocamycin.[2,16] These biological and biosynthetic properties of the pyrrolopyrimidine nucleosides prompted the present study to determine the effect of a hydroxymethyl group on C-5 of tubercidin and to determine if 5-hydroxymethyltubercidin is involved in the biosynthesis of toyocamycin and if there is a difference in the toxicity of toyocamycin and 5-hydroxymethyltubercidin against bacterial and mammalian cells. The results describe (1) the synthesis of 5-hydroxymethyltubercidin by two methods, (2) the biological properties of 5-hydroxymethyltubercidin in leukemia L-1210 cells and bacterial cells, and (3) studies on the biosynthesis of toyocamycin (Scheme I).

Experimental Section‡

Method 1. Tubercidin-5-carboxylic acid (**2**) and methyl tubercidin-5-carboxylate (**3**) were prepared from sangivamycin§ by the method of Rao.[18]

Scheme I. Synthesis of 5-Hydroxymethyltubercidin (**5**) by Methods 1 and 2



Methyl 2′,3′-*O*-Isopropylidenetubercidin-5-carboxylate (4). Compound **3** (371 mg, 1.14 mmol) was added to a solution of *p*-toluenesulfonic acid monohydrate (400 mg, 2.29 mmol) and 2,2-dimethoxypropane (1.5 ml) in 14 ml of acetone. The reaction mixture was passed through an AG 1-X8 acetate column (10 ml, 1 cm diameter). Elution of **4** was done with 150 ml of methyl alcohol. The effluent was concentrated to syrup *in vacuo* (45°). The syrup was dissolved in chloroform (10 ml) and added to a silicic acid column (50 ml, 8 × 2.5 cm, in chloroform). The column was washed with 300 ml of chloroform. Compound **4** was eluted with 135 ml of ethyl acetate–chloroform (25:75, v/v). The eluent was concentrated to a syrup (under vacuum, 40°). Compound **4** was crystallized from ethyl ether and recrystallized from ethanol–water (30:70, v/v): yield, 381 mg (1.046 mmol); 91.5%; mp 194.5–195.5°; uv λ_{max} (chloroform) 282 nm (ε 17,600). **4** was analyzed correctly for $C_{16}H_{20}N_4O_6$. Unreacted compound **3** was eluted from the column with 200 ml of methanol–chloroform (50:50, v/v): yield, 14 mg (0.05 mmol).

5-Hydroxymethyltubercidin (5). Compound **4** (58 mg, 0.16 mmol) was suspended in 5 ml of ethyl ether (distilled over lithium aluminum hydride). The suspension was stirred and three 20-mg portions of lithium aluminum hydride were added at 2, 4, and 6 hr. The mixture was stirred an additional 12 hr. The excess lithium aluminum hydride was filtered through silica gel on a millipore filter. The insoluble residue was washed with 100 ml of acetone–water (50:50, v/v). The combined filtrates and washings were concentrated to dryness. The residue was taken up in 10 ml of acetic acid–water (80:20, v/v) and refluxed for 2.5 hr. The mixture was evaporated to dryness and dissolved in 5 ml of water. The solution was added to an AG hydroxide column (5 ml, 1 cm diameter). The column was washed with 100 ml of methanol–water (30:70, v/v). Compound **5** was eluted with methanol–water (70:30, v/v) in tubes 17–28 (10-ml fractions). The tubes were combined and evaporated to dryness under vacuum (40°). **5** was crystallized from water: yield, 23 mg; 48%; mp 229–230°; uv λ_{max} (water) 272 nm. There was no melting point depression of a mixture of **5** synthesized by method 2. The mass spectra of **5** showed a parent ion at 296.

Method 2. Methyl N^4,5′-*O*-Dityltubercidin-5-carboxylate (6). Compound **3** (440 mg, 1.36 mmol) was added to 7 ml of dry pyridine. Triphenylmethyl chloride (630 mg, 2.10 mmol) was added with stirring at room temperature; an additional 510 mg (17 mmol) of triphenylmethyl chloride was added at 50 hr and the reaction continued for an additional 39 hr. Methyl alcohol (5 ml) was added; the mixture was allowed to stand for 3 hr and evaporated *in vacuo* to a colorless syrup. Chloroform (50 ml) was added and mixed with 2 g of silicic acid and dried *in vacuo* (under 40°). This mixture was added to a column of silicic acid (100 ml, 2.5 × 10 cm). The column was washed with 200 ml of lig-

†Paper 15. For the previous paper in this series, see ref 1.

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‡ Infrared, ultraviolet, and mass spectra were recorded with a Perkin-Elmer 437 gradient spectrophotometer and a Beckman Model DB spectrophotometer. Samples were introduced by direct inlet probe at 274°. Melting points were taken with a Thomas-Hoover silicone bath apparatus and are uncorrected. Elemental analyses were performed by Huffman Laboratory, Wheatridge, Colo.

§Sangivamycin was isolated from *Streptomyces rimosus*; see ref 17.

Table I. Differences in Sensitivity of Mammalian Cells and Bacterial Cells to Toyocamycin and 5-Hydroxymethyltubercidin

Nucleoside	% inhibition[a] Leukemia L-1210	*E. coli* B, 1×10^{-4} *M*	*M. phlei* 1×10^{-5} *M*	*M. phlei* 1×10^{-4} *M*
Toyocamycin	50	100	100	100
5-Hydroxymethyltubercidin	50	0	0	0

[a] The *in vitro* antitumor assays were carried out as described by Bobek, *et al.*[9] The concentrations of toyocamycin and 5-hydroxymethyltubercidin were 4×10^{-8} and 4×10^{-7} *M*, respectively. *E. coli* B was grown on minimal synthetic medium (concentration in g/l. of distilled water–disodium hydrogen phosphate 2.2 g, potassium dihydrogen phosphate 1.0 g, magnesium sulfate 0.1 g, ammonium sulfate 10 g, glucose 2 g, pH 7.2). *M. phlei* was grown on Difco broth base (1.3 g/180 ml of tap water).

roine and 200 ml of ethyl acetate–chloroform (5:95, v/v). Compound **6** was eluted with 400 ml of ethyl acetate–chloroform (20:80, v/v). The acetate–chloroform was removed under vacuum (40°) and **6** was crystallized from carbon tetrachloride–heptane (50:50, v/v): yield, 616 mg (0.785 mmol); 60%; mp 126–136°; uv λ_{max} (chloroform) 260 nm (ε 10,100), 293 (19,800), 302 sh (15,700); analyzed correctly for $C_{51}H_{44}N_4O_6$.

Methyl N^4,5′-*O*-Dibityl-*O*-2′,3′-isopropylidenetubercidin-5-carboxylate (7). Compound **6** (405 mg, 0.4 mmol) was dissolved in 6 ml of acetone and 0.6 ml of 2,2-dimethoxypropane and stirred at room temperature for 4 hr. The reaction mixture was poured onto an AG 2-X8 acetate column (2.5 ml, 1 cm diameter). The column was washed with 200 ml of acetone. Compound **7** passed through the column and was taken to dryness *in vacuo* below 45°. The residue was dissolved in 50 ml of *n*-heptane and 300 ml of ethyl acetate–*n*-heptane (10:90, v/v) was used to elute compound **7**. Fractions (13 ml) were collected. Compound **7** was distributed in tubes 10–30. The tubes were combined and evaporated to dryness *in vacuo* below 45°. The colorless residue was crystallized from ligroine (bp 65–75°) to give 367 mg (0.439 mmol) of **7**: 85%; mp 125°; uv λ_{max} (chloroform) 259.5 nm (ε 10,460), 292.5 (18,080); infrared max at 1700 (for the C=O) and 1075 cm^{-1} (for the C–O–C); correctly analyzed for $C_{54}H_{48}N_4O_6$.

N^4,5′-*O*-Dibityl-2′,3′-*O*-isopropylidene-5-hydroxymethyltubercidin (8). Compound **7** (42.4 mg, 0.05 mmol) was added to 20 ml of ether with a condenser and drying tube (calcium sulfate). Lithium aluminum hydride (3 mg) was added with stirring. A white precipitate formed. Two additional portions of lithium aluminum hydride (2.5 mg each time) were added at 75 and 120 min. The reaction mixture was added to a silicic acid column (2.5 g, 1 cm diameter) to which ligroine (bp 65–75°) had been added. Compound **8** was eluted with 200 ml of chloroform (10-ml fraction). The eluate was concentrated *in vacuo* (below 40°) to a syrup and crystallized twice from ligroine and once from ligroine (bp 90–100°)–ethyl acetate (60:40, v/v) to give 32 mg of **8**: 39 mmol; 78%; mp 162–165°; correctly analyzed for $C_{53}H_{48}N_4O_5 \cdot C_2H_5OCOCH_3$. There was no infrared absorption peak at 1700 cm^{-1} (C=O moiety); uv λ_{max} (chloroform) 286, 295 (sh) nm (ε 17,150); infrared 1750 cm^{-1} (ethyl acetate).

5-Hydroxymethyltubercidin (5) from N^4,5′-*O*-Dibityl-2′,3′-*O*-isopropylidene-5-hydroxymethyltubercidin (8). Compound **8** (47 mmol) was refluxed in 5 ml of acetic acid–water (80:20, v/v) for 2 hr. The mixture was extracted with 5 ml of ether (two times). The ether extract was discarded. The solution was evaporated to dryness *in vacuo* (under 50°). The residue was taken up in 10 ml of water and added to a AG 1-X8 hydroxide column (8 ml, 1 cm diameter). The column was washed with 200 ml of methanol–water (39:61, v/v); compound **5** was eluted with 120 ml of methanol–water (70:30, v/v). The solvent was removed *in vacuo* (below 40°); **5** was crystallized from water: yield 8 mg, 27 μmol; 57%; mp 229–230°; analyzed correctly for $C_{12}H_{16}N_4O_5$; uv and infrared spectra and melting points were identical with those of **5** prepared by method 1.

[U-^{3}H]Sangivamycin was prepared by the tritium gas exchange method by New England Nuclear. It was purified by paper chromatography (water) and column chromatography [Dowex 1-OH^-, elution with methanol–water (70:30, v/v)] and crystallized from water–methanol (10:90 v/v) to constant specific activity. [U-^{3}H]-5-Hydroxymethyltubercidin was prepared by method 2.

Biological. The effect of 5-hydroxymethyltubercidin on the growth of leukemia L-1210 cells is shown in Table I. 5-Hydroxymethyltubercidin was about ten times less effective an inhibitor to L-1210 cells than was toyocamycin. There is a marked difference in the selectivity of inhibition of 5-hydroxymethyltubercidin with L-1210 cells and bacterial cells that is not exhibited when the hydroxymethyl group of tubercidin is replaced with the cyano group (toyocamycin). For example, toyocamycin inhibits L-1210 cells and bacterial cells, while 5-hydroxymethyltubercidin is selective in that it is only inhibitory to L-1210 cells. When *Escherichia coli* is grown on Kirchner's medium, there is no inhibition by either toyocamycin or tubercidin at concentrations greater than 100 μg/ml of medium.[19,20] In an attempt to determine the metabolic fate of 5-hydroxymethyltubercidin in L-1210 cells, [U-^{3}H]-**5** (2.77 μCi/μmol, 0.1 μg/ml) was added. After 24 hr the cells were collected as described by Bobek, *et al.*[9] The radioactivity in the acid-soluble pool, the RNA, and DNA were determined by the procedure described earlier in this laboratory.[21] Although a considerable amount of [U-^{3}H]-**5** was found in the acid-soluble pool of L-1210 cells, there was no 5′-mono-, di-, or triphosphate.

Tritium-labeled **5** from the RNA hydrolysate was detected by paper chromatography (R_f 0.7) [Whatman 3 MM; solvent, 1-butanol–concentrated ammonium hydroxide–water (86:1:13, v/v/v)]. Authentic **5** had the same R_f. A radioactive compound (R_f 0.75) was detected from the DNA hydrolysate by paper chromatography (Whatman 3 MM; solvent, water–ammonia, pH 10). The R_f of authentic **5** was 0.85. The radioactive compound, isolated from the hydrolyzed DNA, is presumed to be 5-hydroxymethyl-2′-deoxytubercidin. L-1210 cells can phosphorylate 5-hydroxymethyltubercidin and incorporate it into RNA. The nucleotide analog can then be reduced to the 2′-deoxy derivative and incorporated into DNA. To elucidate the biosynthetic mechanism for the formation of the cyano group of toyocamycin, the hydroxymethyl derivative of tubercidin (compound **5**) was considered as a precursor. When [U-^{3}H]-**5** was added to cultures of *Streptomyces rimosus* at the time of toyocamycin production, there was uptake of **5** but there was no conversion to toyocamycin.

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Amino Acid Analogs. 2. 3-Fluoroamino Acids. 1. Chain Length Three to Seven Carbon Atoms[1]

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The synthesis of 3-fluoroamino acids has presented an intriguing challenge for many years. 3-Fluoroalanine as the hydrochloride was first reported in 1959,[2] but the validity of the synthesis was questioned.[3] It was disclosed in 1972 that the compound was prepared by the same as well as other methods, but none of the products or intermediates were characterized;[4a] however, the same author prepared and characterized 3-fluoro-D-alanine from D-alanine by photofluorination with trifluoromethyl hypofluorite in liquid HF.[4b] In 1967 a synthesis of 3-fluoroalanine was reported which was carried out by ammonolysis of 2-bromo-3-fluoropropionic acid obtained from the bromination of 3-fluoropropionic acid.[5] 3-Fluoro-2-methylalanine was also synthesized in 1963 by two independent methods.[6,7] None of these methods were readily adaptable for the preparation of other 3-fluoroamino acids.

A general approach to the preparation of 2-bromo-3-fluorocarboxylic acids was devised, which upon ammonolysis yielded a series of 3-fluoroamino acids. By extending the method of bromo fluorination of methyl acrylate[8] to substituted acrylic acids with subsequent ammonolysis, the following amino acids were prepared: 3-fluoroalanine, 2-amino-3-fluorobutyric acid, 3-fluorovaline, 3-fluoronorvaline, 3-fluoronorleucine, and 2-amino-3-fluoroheptanoic acid.

The bromo fluorination of acrylic, crotonic, and 3-methylcrotonic acids was carried out by dissolving the substrate in liquid HF, followed by addition of *N*-bromoacetamide (NBA). The reaction proceeded smoothly without formation of excessive complicating side products, and the three bromofluoro compounds were purified easily by distillation. Upon bromo fluorination of 2-pentenoic, 2-hexenoic, and 2-heptenoic acids, mixtures of products were obtained which could not be separated conveniently by distillation. Purification of the desired products was achieved by formation of cyclohexylamine salts followed by repeated recrystallizations until the required degree of purity was obtained, as indicated by gas chromatographic analysis of the trimethyl silyl esters of the acids.[9] The salts were subsequently decomposed by mineral acid, and the bromofluoro acids were distilled. No attempt was made at this time to determine structures of by-products produced in these reactions.

3-Fluoroamino acids were obtained by ammonolysis of the bromofluoro acids in liquid ammonia by modifications of the method of Lettré and Wölcke.[5] 3-Fluoroalanine and 2-amino-3-fluorobutyric acid were obtained by ammonolysis at room temperature. The remaining four 2-amino acids were prepared by reaction with liquid ammonia at 65°. Although it is recognized that four of the described fluoroamino acids contain two centers of asymmetry, no attempt was made at separating the erythro and threo diastereoisomers.

The data characterizing the bromofluoro acids and the fluoroamino acids are contained in Table I, and the 60-MHz nmr spectral features of the compounds are listed in Table II. Infrared spectra for the 3-fluoroamino acids have been obtained.†

All of the 3-fluoroamino acids were tested against *Aspergillus niger, Trichoderma viride,* and *Myrothecium verrucaria* in Sabouraud dextrose agar (Difco) at pH 4.0 and 5.6 and against *Trichophyton mentagrophytes* in the same medium at pH 5.6 and 7.0, according to published methods.[10] Very little fungitoxic activity was observed under those conditions. 3-Fluoroalanine inhibited *A. niger* and *M. verrucaria* at concentrations between 10^3 and 10^4 ppm at pH 4.0 and *T. mentagrophytes* at a concentration of between 10^2 and 10^3 ppm at pH values of 5.6 and 7.0. 3-Fluorovaline was less active and inhibited *M. verrucaria* at a concentration between 10^3 and 10^4 ppm at pH values of 4.0 and 5.6 and *T. mentagrophytes* at the same concentration at pH values of 5.6 and 7.0. Of the remaining compounds, 3-fluoronorvaline inhibited *M. verrucaria* at pH 4.0 at a concentration between 10^3 and 10^4 ppm, and 3-fluoronorleucine inhibited *T. mentagrophytes* at concentrations between 10^2 and 10^3 ppm at pH 5.6 and 10^3 and 10^4 ppm at pH 7.0.

Experimental Section‡

2-Bromo-3-fluoropropionic Acid. Acrylic acid (18 g, 0.25 mol) was dissolved in 25 ml of liquid HF kept at −30 to −10° in a polyethylene bottle. To the mixture was added NBA (37.3 g, 0.27 mol) in small portions with stirring during the course of 0.5 hr. Stirring was continued overnight, allowing the mixture to come to room temperature. The excess HF was removed under a stream of air, and the residue was poured into a slurry of ice and H_2O. The product was extracted with Et_2O, dried (Na_2SO_4), and distilled under vacuum.

2-Bromo-3-fluorobutyric acid was prepared from crotonic acid in the same manner as 2-bromo-3-fluoropropionic acid.

2-Bromo-3-fluoro-4-methylbutyric acid was prepared from 3,3-dimethylacrylic acid in the same manner as 2-bromo-3-fluoropropionic acid.

2-Bromo-3-fluoropentanoic Acid. 2-Pentenoic acid (89 g, 0.89 mol) was bromo fluorinated in 200 ml of liquid HF with NBA (124 g, 0.9 mol) in the same manner as acrylic acid. The aqueous solution of product was extracted with ether which in turn was extracted three times with 200-ml portions of 10% NaOH. The basic extract was acidified with HCl and extracted with $CHCl_3$. Upon evaporation of the solvent, 120 g of residue remained which was then dissolved in 250 ml of ether. A solution of cyclohexylamine (60 g, 0.6 mol) in 200 ml of ether was added slowly to the bromofluoro compound with stirring. After stirring for 2 hr, the mixture was refrigerated overnight. The cyclohexylamine salt was obtained by filtration (yield 113 g, 42%). It was recrystallized sever-

†See paragraph at end of paper regarding supplementary material.

‡Melting points were taken in a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 221 spectrophotometer, and nmr spectra were taken with a Jeolco JNM-C-6HL spectrometer. Gas chromatography was performed on a Varian Aerograph Model 1200 gas chromatograph with a flame ionization detector to which was attached a Varian Aerograph Model 20 recorder. The purity of the bromofluoro acids was established by gas chromatographing the trimethyl silyl esters[8] on a column containing 1% Apiezon L on acid-washed Chromosorb W (80–100 mesh), previously treated with dimethyldichlorosilane.