tion. The mixture was stirred for an additional 2 hr. and then was allowed to stand for 2 days in the refrigerator. The solid was collected by suction filtration, washed with water, and dried *in vacuo*, 6.1 g., m.p. 287–289°. Recrystallization proved difficult, solubility in petroleum ether, benzene, tetrahydrofuran, chloroform, and water being poor, but it was finally effected from absolute methanol containing a few drops of absolute ethanol, in which this triamide (XVIc) is more soluble, m.p. 260.0–261.2° dec. (red color).

Anal. Calcd. for $C_{11}H_{15}N_3O_3$: C, 55.58; H, 6.37; N, 17.71. Found: C, 55.42; H, 6.46; N, 17.62.

Finally a 0.24-g. sample (1 mmole) of XVIc was very finely ground in a mortar and placed in a vacuum sublimation apparatus along with 1.14 g. of phosphorus pentoxide (8 mmoles) and a number of glass beads. The components were intimately mixed by agitation for several minutes, and then the apparatus was heated slowly to 130° and finally to 160° at 0.005 mm. Heating was continued for 3 hr., during which time the mixture became dark brown and some of the phosphorus pentoxide sublimed along with the product (50.0 mg., crude). The crude sublimate was recrystallized from absolute methanol to which was added a trace of water, m.p. 129.0–130.5°.

Anal. Calcd. for $C_{11}H_9N_3$: C, 72.27; H, 4.92. Found: C, 71.94; H, 5.07.

Synthetic Studies of Potential Antimetabolites. IX. The Anomeric Configuration of Tubercidin

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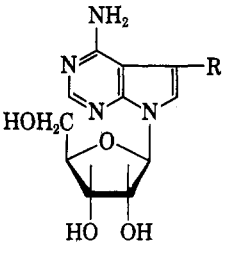
The glycosyl center of the antibiotic nucleoside, tubercidin (I), has been established as β-D on the basis of a series of reactions. The nucleoside I was converted to 2,3-*O*-isopropylidenetubercidin (III) which was in turn converted to the 2,3-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl derivative IV. Treatment of IV with boiling acetone gave rise to a water-soluble, intramolecularly quaternized nucleoside V. 3-(2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (IX) was prepared and converted to X by intramolecular alkylation. The chromatographic properties and spectral characteristics of V were compared with those of X and VII (the latter is the similar intramolecularly quaternized derivative in the adenosine series).

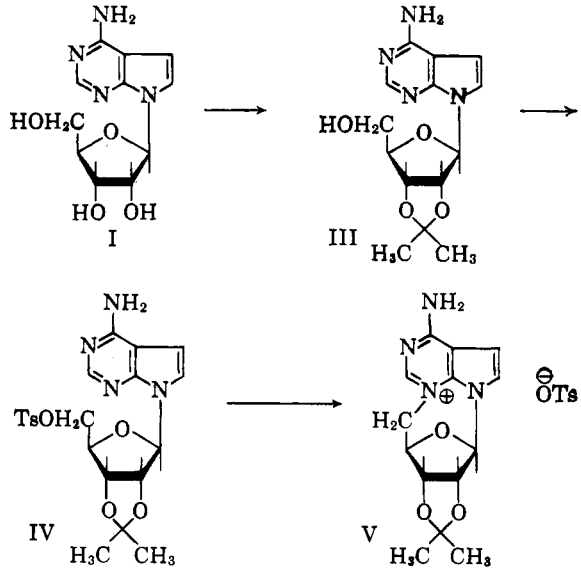
The antibiotic nucleoside, tubercidin, was isolated from *Streptomyces tubercidicus* by Anzai and Marumo[2] and has been assigned the 4-amino-7-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine structure (I).[3] This is the first example of the natural occurrence of a pyrrolo[2,3-*d*]pyrimidine ring system. Because of its unique structure, tubercidin as well as toyocamycin (5-cyanotubercidin, II)[4] has attracted interest. The establish-



I, R=H
II, R=CN

ment of the structure of I[3] was based on degradative work, elementary analyses, and spectral examination, and is quite reasonable. However, assignment of the β-D configuration[3] to the anomeric carbon atom in I was not unequivocal. Since we are interested in the total synthesis of I and in the preparation of potential antimetabolites containing this ring system, we have felt it mandatory to establish unequivocally the configuration at the glycosyl center of tubercidin. The present paper reports an investigation on the glycosyl linkage.

Tubercidin (I) was converted to the 2,3-*O*-isopropylidene acetal III in almost quantitative yield by a reported procedure.[5] Paper chromatography of the product gave in three solvent systems (1-butanol–water, ethanol–ammonia–water, and water) a single spot whose R_f values were 0.16, 0.38, and 0.43, respectively. These ultraviolet-absorbing spots on paper chromatograms failed to give a positive test with the periodate spray reagent.[6] The ultraviolet absorption spectrum of the spot was very similar to that of tubercidin (I), see Fig. 1, indicating that the chromophore of I was not appreciably affected on conversion of I to III. On the basis of these facts, the product was assigned the 2,3-*O*-isopropylidene structure III, although elementary analyses were not done because of the small amount of the material available. The compound III was then converted to the 5-*O*-*p*-tolylsulfonate IV with *p*-toluenesulfonyl chloride in pyridine. The ultraviolet absorption maximum of IV was



(1) Part VIII of this series: Y. Mizuno, M. Ikehara, T. Itoh, and K. Saito, *Chem. Pharm. Bull.* (Tokyo), **11**, 1091 (1963).

(2) K. Anzai and S. Marumo, *J. Antibiotics* (Tokyo), *Ser. A*, **10**, 20 (1957).

(3) S. Suzuki and S. Marumo, *ibid.*, **14**, 34 (1961).

(4) K. Ohkuma, *ibid.*, **14**, 343 (1961).

(5) A. Hampton and D. I. Magrath, *J. Am. Chem. Soc.*, **79**, 3250 (1957).

(6) A combination spray of 2% aqueous metaperiodate, followed by the benzidine spray (benzidine-glacial acetic acid–absolute ethanol, 500 mg./200 ml./80 ml.).

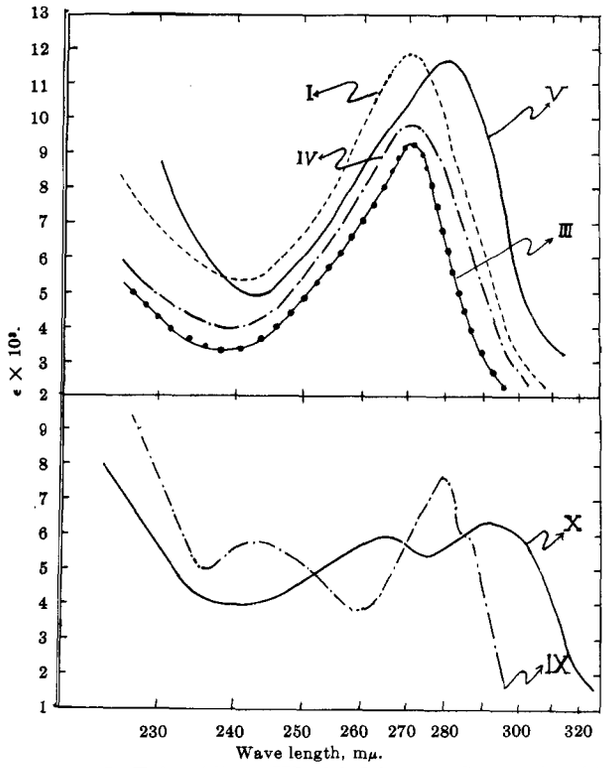


Fig. 1.—I, Tubercidin; III, 2,3-*O*-isopropylidenetubercidin; IV, 2,3-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyltubercidin; V, quaternized IV; IX, 3-(2,3-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine; X, quaternized IX.

also similar to III, strongly indicating again that this conversion did not affect the chromophoric moiety of III (see Fig. 1). Paper chromatography of IV gave a single spot (R_f: 0.76 in ethanol–ammonia–water). Treatment of IV with boiling acetone gave rise to V. The solubility properties of V differed markedly from IV. Thus, IV was insoluble in water and soluble in acetone, but V was insoluble in acetone and soluble in water. The ultraviolet absorption maximum of V was shifted bathochromically from that of I or III by ~9 mμ, characteristic of an intramolecularly quaternized nucleoside[7,8] (see Fig. 1 and Table I). Chromatographic properties of V were also very similar to those of the same type of intramolecularly quaternized derivatives of 2,3-*O*-isopropylideneadenosine[7,8] or 3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]-pyridine (VIII),[9] see Table I. These facts clearly indicate that intramolecular quaternization took place on position 1 of IV to afford V. This type of intramolecular quaternization of the 5-*O*-sulfonyloxy derivative of I is feasible only if tubercidin possessed the β-D configuration. These reactions, therefore, established that the anomeric configuration of tubercidin (I) is β-D.

TABLE I

	R_f (EtOH–NH_3–H_2O, 80:4:16 v./v./v.)	λ_{max}^{MeOH} (mμ)
2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyladenosine (VI)	0.78	260
Quaternized VI (VII)	0.53	271
2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyltubercidin (IV)	0.76	272
Quaternized IV (V)	0.51	281
	R_f (BuOH–H_2O, 84:16 v./v.)	$\lambda_{max}^{H_2O}$ (mμ)
3-(2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine[a] (IX)	0.88	282, 287
Quaternized IX (X)[a]	0.65	266, 293

[a] Preparation of these compounds (IX and X) have been reported by us in a preliminary form.[9]

Experimental[10]

7-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (III).—The procedure employed was essentially that reported by Hampton and Magrath.[6] To a solution of tubercidin (I, 29.4 mg., 0.11 mmole) in acetone (1.1 ml.) was added 2,2-dimethoxypropane (0.11 ml.) and *p*-toluenesulfonic acid (189 mg., 1.1 mmoles). The mixture was kept for 12 hr. at room temperature, after which it was poured, with stirring, into an ice-cold sodium hydrogen carbonate solution (saturated). The flask was washed with five 2-ml. portions of acetone, and the washings were added to the neutralized solution; this was stirred at room temperature for 30 min. and concentrated *in vacuo* at 49° (bath temp.) to dryness. The residue was dried azeotropically by codistillation with five 5-ml. portions of benzene, and further dried *in vacuo* (1 mm.) at 60° for 1 hr. The dried sample was extracted with ten 5-ml. portions of chloroform. Removal of the solvent *in vacuo* gave a 33.6-mg. yield (quantitative) of a glass; ultraviolet absorption, λ_{max}^{MeOH} 271 mμ (ε 9.1 × 10³); paper chromatography, R_f 0.16 (BuOH–H_2O, 84.16), 0.33 (EtOH–NH_3–H_2O, 80:4:16), 0.43 (H_2O, pH 10). III on paper chromatography was detected as a dark spot under an ultraviolet lamp and failed to give a positive test with the periodate spray reagent.[7]

7-(2,3-*O*-Isopropylidene-5-*O*-tolylsulfonyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (IV).—To a solution of III (23 mg., 0.075 mmole) in dry pyridine (0.5 ml.) was added *p*-toluenesulfonyl chloride (12.1 mg., 0.075 mmole) at 0°. The mixture was stirred for 4 hr. at 0° and kept for 12 hr. at room temperature. To the reaction mixture was added, with stirring at 0°, a saturated solution of sodium hydrogen carbonate (3 ml.) and then chloroform (3 ml.). The chloroform layer was separated and the aqueous layer was extracted with three 3-ml. portions of chloroform. The combined extracts were washed with two 5-ml. portions of saturated sodium hydrogen carbonate solution and with three 5-ml. portions of water, dried with sodium sulfate (0.8 g.), and filtered. Removal of the solvent *in vacuo* at room temperature gave a product (10.4 mg.); paper chromatography, R_f 0.76 (EtOH–NH_3–H_2O. 80:4:16); ultraviolet absorption, λ_{max}^{MeOH} 272 mμ (ε 9.6 × 10³), λ_{min}^{MeOH} 253 mμ. The product was quite soluble in acetone or chloroform and insoluble in water.

Anal. Calcd. for $C_{21}H_{24}N_4O_6S$: C, 54.78; H, 5.21; N, 12.17. Found: C, 54.66; H, 5.20; N, 12.22.

Internal Alkylation of IV. Preparation of V.—IV (4.2 mg.) in dry acetone (0.84 ml.) was refluxed for 3 hr. during which time a white precipitate deposited on the surface of the flask. After filtration, the precipitate was washed with a small amount of cold acetone. The product weighed 3.9 mg. and was insoluble in acetone or chloroform, but soluble in water or ethanol; ultraviolet absorption, λ_{max}^{MeOH} 281 mμ (ε 11.7 × 10³); λ_{min}^{MeOH} 265 mμ; R_f 0.51 (EtOH–NH_3–H_2O).

Anal. Calcd. for $C_{21}H_{24}N_4O_6S$: C, 54.78; H, 5.21; N, 12.17. Found: C, 54.71; H, 5.22; N, 12.22.

2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyladenosine (VI).—VI was prepared according to a reported method from 2,3-*O*-isopropylideneadenosine (m.p. 212–215°, 85.9 mg., 0.28 mmole)[7,8] as a white glass (55.5 mg., 43.3%); R_f 0.78 (EtOH–NH_3–H_2O, 80:4:16), lit.[7] 0.86, in the same solvent system; ultraviolet absorption, λ_{max}^{MeOH} 260 mμ (ε 12.3 × 10³).

(7) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).
(8) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **121**, 131 (1937).
(9) Y. Mizuno, M. Ikehara, T. Itoh, and K. Saito, *Chem. Pharm. Bull.*, **11**, 265 (1963).

(10) All melting points were corrected. Ultraviolet absorption spectra were run with a Beckman Model DK-2 recording spectrophotometer. Molecular extinction coefficients were determined with a Shimadzu manual spectrophotometer. Except where noted, removal of the solvent was done *in vacuo* (water aspirator). Paper chromatography was performed using the ascending technique.

Quaternization Reaction of VI. Formation of Quaternized Salt (VII).—According to the reported method,[7] VI (27.7 mg.) in acetone (2 ml.) was converted into VII (21.1 mg.); R_f 0.53 (EtOH–NH_3–H_2O, 80:4:16), lit.[7] 0.86, in the same solvent system; ultraviolet absorption, λ_{max}^{MeOH} 271 mμ (ε 16.3 × 10^3).

3-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (VIII).—Finely powdered and well dried 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (VII), 543 mg., was added to freshly distilled acetone (54 ml.) containing *p*-toluenesulfonic acid (5.4 g.) and the mixture was vigorously stirred for 30 min. at room temperature. Sodium hydrogen carbonate (5.4 g.) was then added, with stirring, to the reaction mixture during 1 hr. The insoluble material was filtered off and washed with three 20-ml. portions of hot acetone, and the combined filtrate and washings were concentrated to dryness *in vacuo* to give a residue which was treated with dry acetone (30 ml.). A small amount of insoluble material was filtered off, and the filtrate was evaporated *in vacuo* to afford a clear resinous residue (580 mg., 90.2%); this was dissolved in a hot mixture of acetone and hexane (1:3 v./v.). The resulting solution was kept for several days at room temperature, depositing an amorphous solid (53 mg.) which was collected by centifugation; m.p. 184–189°; ultraviolet absorption, λ_{max}^{MeOH} 282 mμ. Concentration of the supernatant liquor *in vacuo* gave a further crop (420 mg.) as a resin.

3-(2,3-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (IX).—To a dry pyridine solution (10 ml.) of VIII (420 mg.) was added, with stirring at 0°, *p*-toluenesulfonyl chloride (300 mg., 1.1 equivalent). The mixture was kept overnight at room temperature. Water (3 ml.) and, subsequently, a saturated solution of sodium hydrogen carbonate (20 ml.) was added. The neutralized solution was extracted with three 30-ml. portions of chloroform. The combined chloroform extracts were washed successively with two 30-ml. portions of ice cold sodium hydrogen sulfate solution (5%) and with two 30-ml. portions of water. The chloroform layer was separated, dried with magnesium sulfate (20 g.), and concentrated to dryness *in vacuo* to give a residue (320 mg.) which was recrystallized from 4 ml. of a 1:3 mixture of acetone and hexane. After standing at room temperature overnight, prism crystals (32 mg.) separated and were filtered. From the filtrate, after two days' standing at room temperature, a further crop (colorless needles, 30 mg.) was obtained; R_f 0.88 (BuOH–H_2O, 84:16); m.p. 154–154.5°; ultraviolet absorption, $\lambda_{min}^{H_2O}$ 241 (ε 5660), 282 (7950), 287 mμ (6280, sh); $\lambda_{min}^{H_2O}$ 235 (ε 1860), 258 mμ (3860).

Anal. Calcd. for $C_{11}H_{23}N_3O_6S$: C, 56.61; H, 5.21; N, 9.41. Found: C, 56.72; H, 5.24; N, 9.19.

Intramolecular Quaternization of 3-(2,3-*O*-isopropylidene-5-*O*-tolylsulfonyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (IX). Preparation of X.—IX (30 mg.) was dissolved in a mixture (1 ml.) of acetone and hexane (1:3 v./v.). The solution was refluxed for 2 hr. to give X as prisms which were soluble in water, but insoluble in acetone or chloroform; m.p., 228–231°; ultraviolet absorption, $\lambda_{max}^{H_2O}$ 293 (ε 6460), 266 mμ (5930); $\lambda_{min}^{H_2O}$ 273 (ε 5650); 236 mμ (3490); R_f 0.65 (BuOH–H_2O, 84:16), 0.81 (*i*-C_3H_7OH–NH_3–H_2O).

Anal. Calcd. for $C_{11}H_{23}N_3O_6S$: C, 56.61; H, 5.21; N, 9.41. Found: C, 56.60; H, 5.30; N, 9.31.

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Synthetic Studies of Potential Antimetabolites. X.[1] Synthesis of 4-Hydroxy-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine, a Tubercidin Analog

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The synthesis of 4-hydroxy-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (IV, "7-deazainosine"), an analog of the antibiotic tubercidin ("7-deazaadenosine") has been achieved starting with the condensation of 4-amino-5-(2,2-diethoxyethyl)-6(1*H*)-pyrimidinone (XIII) with 2,3,4-tri-*O*-acetyl-5-*O*-trityl-D-ribose. The condensation product XIV was ring closed in dioxane–acetic acid to the 5-*O*-trityl-2,3-di-*O*-acetyl derivative XVIII of IV which, after removal of the blocking groups, gave IV. Thiation followed by methylation of XVIII yielded the 4-methylthio analog XXI of IV. Attempts to convert either IV or XXI to tubercidin were unsuccessful.

The antibiotic nucleoside, tubercidin has been assigned the 4-amino-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine structure (I).[1,2] This is the first example of the natural occurrence of a substance possessing the pyrrolo[2,3-*d*]pyrimidine ring system, and the antibiotic, as well as a related antibiotic, toyocamycin (II, 5-cyanotubercidin),[3] recently has attracted interest because of their unique structure and their close structural relationship to adenosine (III).

We are now interested in the synthesis of tubercidin, toyocamycin, and related nucleosides possessing this heterocyclic ring system. In the present paper, we report the synthesis of 4-hydroxy-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (IV) along with some attempts to convert the nucleoside IV to tubercidin (I).

For the synthesis of purine nucleosides, methods which might be extended to the synthesis of a nucleoside of the pyrrolo[2,3-*d*]pyrimidine series can be divided into three general classes,[4-10] depending on what type of starting materials (bases and sugar derivatives) are employed in the synthesis. Corresponding to the three types of synthetic methods for purine nucleosides,[4-10] there are, theoretically, three possible

(1) Part IX of this series: Y. Mizuno, M. Ikehara, K. A. Watanabe, S. Suzaki, and T. Itoh, *J. Org. Chem.*, 3329 (1963).

(2) S. Suzuki and S. Marumo, *J. Antibiotics* (Tokyo), *Ser. A*, **14**, 34 (1961).

(3) K. Ohkuma, *ibid.*, **14**, 343 (1961).

(4) Synthetic methods of purines have been elegantly covered in recent articles by Michelson[5] and Montgomery and Thomas.[6]

(5) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, Inc., New York, N. Y., 1963.

(6) J. A. Montgomery and H. J. Thomas, "Advances in Carbohydrate Chemistry," Vol. 17, W. L. Wolfrom, Ed., Academic Press, Inc., New York, N. Y., 1962, pp. 301–341, where they have classified the method of preparation of purine nucleosides into several classes. Among them, method A (condensation of a heavy metal salt of a purine with an acylglycosylhalide, the Fischer–Helferich procedure[7]), method E (ring closure of a 5-amino-4-glycosylaminopyrimidine[8]), and method F (ring closure of an imidazole nucleosides[9,10]) are pertinent to our discussion.

(7) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914); J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

(8) For a leading reference, see G. W. Kenner, C. W. Taylor, and A. R. Todd *J. Chem. Soc.*, 1620 (1949).

(9) G. Shaw and D. V. Wilson, *ibid.*, 2937 (1962).

(10) G. A. Howard, A. C. MacLean, G. T. Newbold, F. S. Spring, and A. R. Todd, *ibid.*, 232 (1949).