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.

Acknowledgment.—Our special thanks go to Dr. Saburo Suzuki of the Institute for Physical and Chemical Research, Tokyo, for supplying us with tubercidin and for his stimulating interest and encouragement throughout the work. This work was supported, in part, by a grant-in-aid for fundamental scientific research from the Ministry of Education of the Japanese Government to which our thanks are also due.

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.

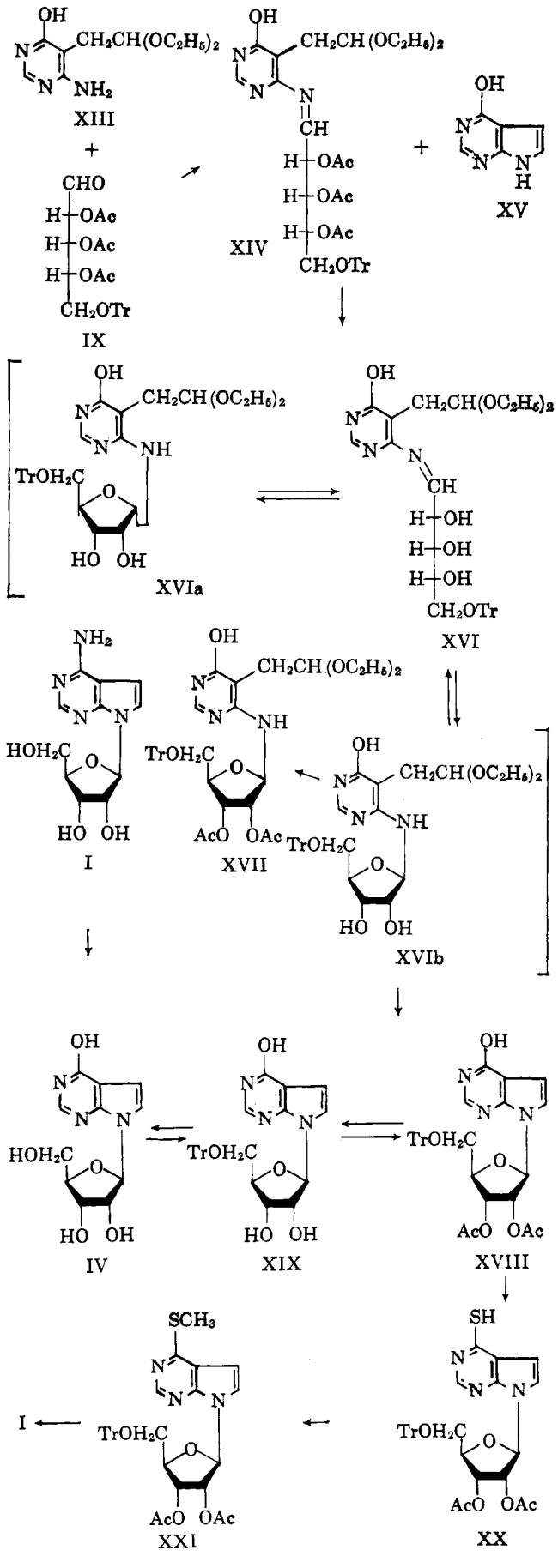
(7) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914); J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

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

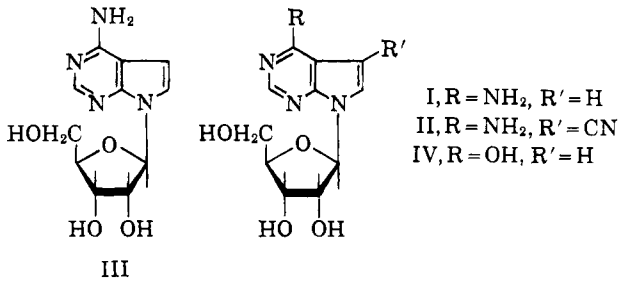
(9) G. Shaw and D. V. Wilson, *ibid.*, 2937 (1962).

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

FLOW SHEET I

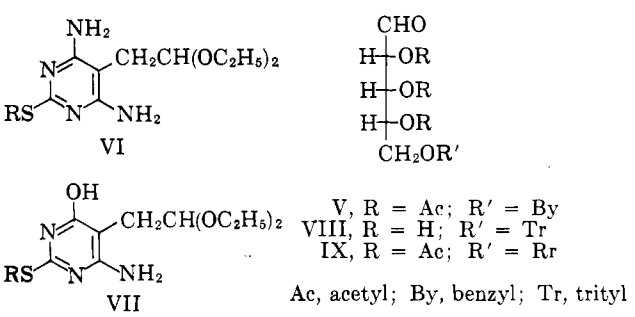


approaches to tubercidin (I). An application of the general method[7] to the synthesis of tubercidin, that is,



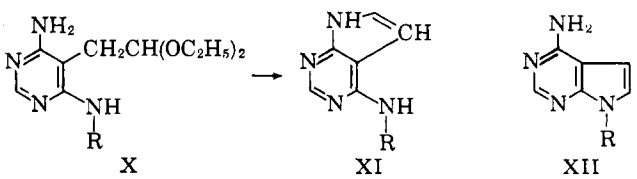
the reaction of heavy metal salts of 4-chloro- or 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine[11] gave an intractable resinous mixture. In view of the successful synthesis of IV using an adaptation of method E[6,8] (see Experimental), no experiment employing 4-acylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine was made, because of the inaccessibility of derivatives of the corresponding 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine by a standard procedure.[12] An adaptation of method F[6,9,10] for the synthesis of I did not appear attractive because the prerequisite key intermediates (for instance, 1-D-ribosyl derivative of 2-amino-3-pyrrolocarboxamidine or of 2-amino-3-pyrrolocarboxamide) are not easily accessible.

An adaptation of the method of Kenner, Taylor, and Todd[8] (method E[6]) for the synthesis of I or IV appeared the most promising, because both kinds of prerequisite starting materials (VI[11] or VII[11] for the base moiety; V,[8] VIII,[13] or IX[14] for the sugar moiety) have already



Ac, acetyl; By, benzyl; Tr, trityl

been described. In addition, because of the ambiguity which might be associated with pyrrole ring formation of an intermediate of type X, namely, ring closure of X to either XI or XII or to a mixture of both, 4-amino-



5-(2,2-diethoxyethyl)-6(1*H*)-pyrimidinone (XIII) was selected as being a more favorable starting compound. Thus, the design for the total synthesis of I was worked out as shown in Flow Sheet I.

XIII, prepared by a Traube type of condensation[15] of ethyl (2,2-diethoxyethyl)cyanoacetate with formami-

(11) J. Davoll, *J. Chem. Soc.*, 131 (1960).

(12) Acylation of 4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine with acetic anhydride or benzoyl chloride in pyridine gave the N^4,7-diacyl derivative. Specific removal of the 7-acyl group was found to be impossible.

(13) F. W. Holl, C. H. Shunk, E. W. Peel, J. J. Cahill, J. B. Lavigne, and K. Folkers, *J. Am. Chem. Soc.*, **74**, 4521 (1952).

(14) H. Zinner, *Ber.*, **86**, 817 (1953).

(15) W. Traube, *ibid.*, **26**, 2551 (1893); **37**, 4544 (1904).

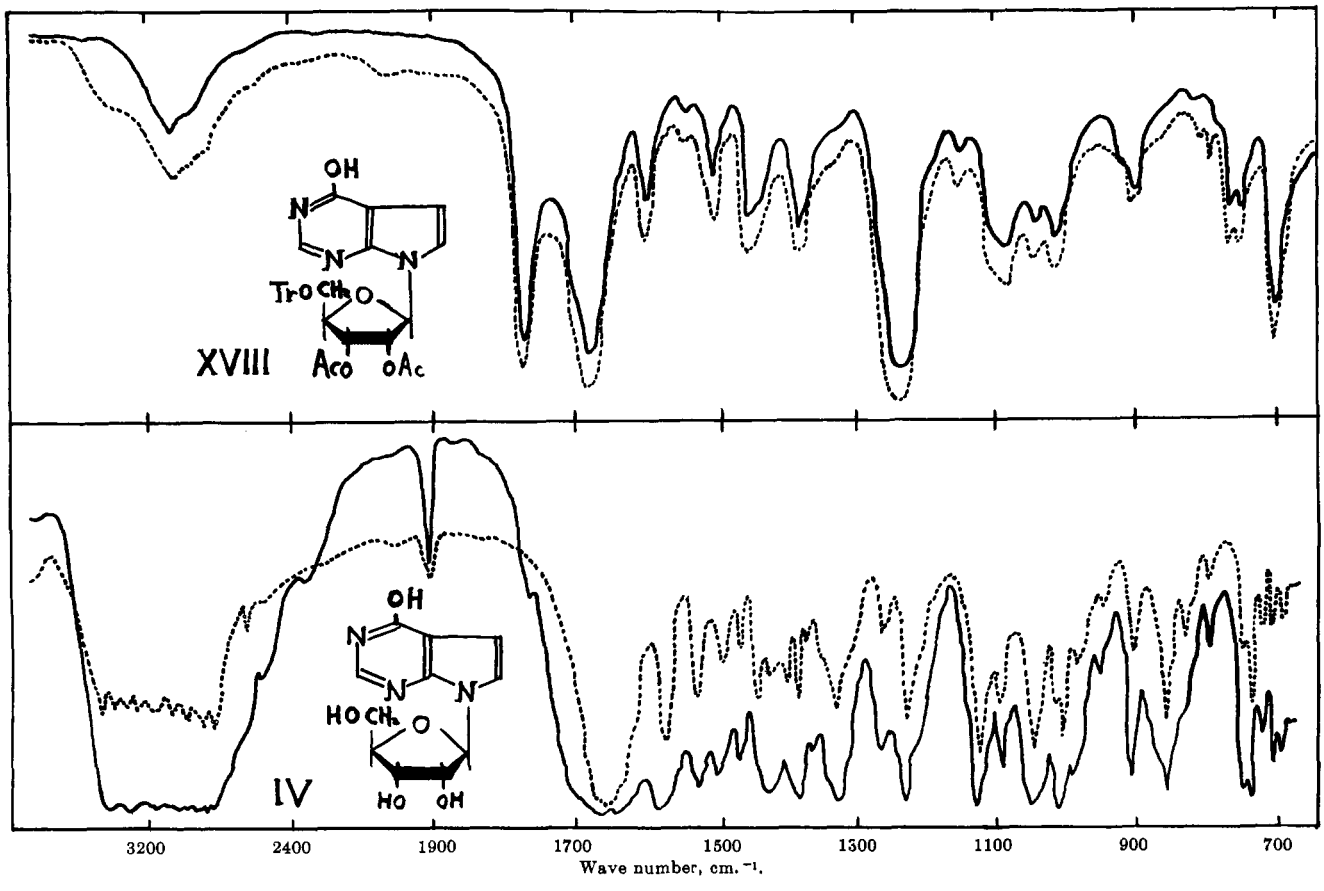


Fig. 1.—Infrared absorption spectra of IV and XVIII: ———, from tubercidin; ———, *de novo* synthetic.

dine acetate,[16] was treated with IX in refluxing ethanol, in the presence of a trace of ammonium chloride, for 6 hr. to give XIV (R_f 0.48)[17] together with 4-hydroxy-1*H*-pyrrolo[2,3-*d*]pyrimidine (XV). The mode of the formation of the latter (XV) was not clear, but, presumably, it may come from 4-amino-5-(formylmethyl)-pyrimidinone (resulting from acid-catalyzed acetal exchange between XIII and IX). The condensation was followed chromatographically and was found to be completed in less than 6 hr. (see Table II). The product XIV was separated from XV by dissolving XIV in chloroform. The chloroform solution of XIV gave, on evaporation, a residue which, without further purification, was treated with methanol saturated with ammonia. Removal of the solvent gave a yellow glass XVI which may exist in solution as tautomeric structures XVI, XVIa, and XVIb (see Flow Sheet I). The yellow glass XVI was acetylated with acetic anhydride and pyridine at 0°. Excess acetic anhydride was removed by distillation with methanol *in vacuo*, to afford a dark colored residue XVII which was applied to an alumina column. The elution pattern is given in Fig. 4. The first two fractions, eluted by benzene, contained ethyl trityl ether. The third and fourth fractions, eluted by a mixture of benzene and chloroform, contained nitrogen free sugar derivative(s) not further investigated. Fractions eluted by chloroform gave, after removal of the solvent, purified XVII as a glass in a yield of 21% on the basis of IX or XIII. Purified XVII melted at 231–232° and the infrared absorption spectrum exhibited bands characteristic of imino (ν 3330), acetyl (ν 1770), heteroaromatic ring (ν 1500), and trityl (ν 770, 750, and 700 cm.$^{-1}$); and the ultraviolet absorption maximum appeared at 260 mμ. XVII was treated with a mixture (pH 2.8) of dioxane and 80% aqueous acetic acid for 2 hr. at room temperature (to cause a pyrrole ring closure) to give XVIII which was purified by repeated reprecipitation from ethanol with petroleum ether (b.p. 60–80°) to give a glass XVIII.[18] The yield from the ring closure was 48.5%. The infrared absorption spectrum of XVIII is given in Fig. 1, together with that of a sample XVIII[19] derived from tubercidin (I) *via* IV and XIX (tritylation of IV afforded XIX which was, in turn, acetylated with acetic anhydride and pyridine in the presence of sodium acetate to give XVIII of natural origin). The *de novo* synthetic XVIII was identical with the sample of natural origin on the basis of elementary analyses and infrared (see Fig. 1) and ultraviolet absorption spectra. In addition, the specific rotations of the two were also identical, which indicates that the *de novo* synthetic XVIII possess β-D-glycosyl configuration. Therefore, the pyrrole ring-closure reaction may be stereospecific. However, since the yield from the reaction was not more than 50% and the rest of the product has not been fully characterized, a definite decision as to whether or not the reaction is really stereospecific is reserved until further studies have been completed.[20]

(16) E. C. Taylor and W. A. Ehrhart, *J. Am. Chem. Soc.*, **82**, 3188 (1960).
(17) The solvent system, 1-butanol–water, 84:16.
(18) The sample is referred to as *de novo* synthetic XVIII.
(19) The sample is referred to as XVIII of natural origin.
(20) A series of parallel reactions starting from XIII and 2,3,4-tri-*O*-acetyl-5-*O*-trityl-D-arabinose leading to a 7-D-arabinofuranosyl derivative of this ring system also is being carried out in our laboratories. Our attention is focused on the stereochemistry of the pyrrole ring closure reaction in the D-arabinose series.

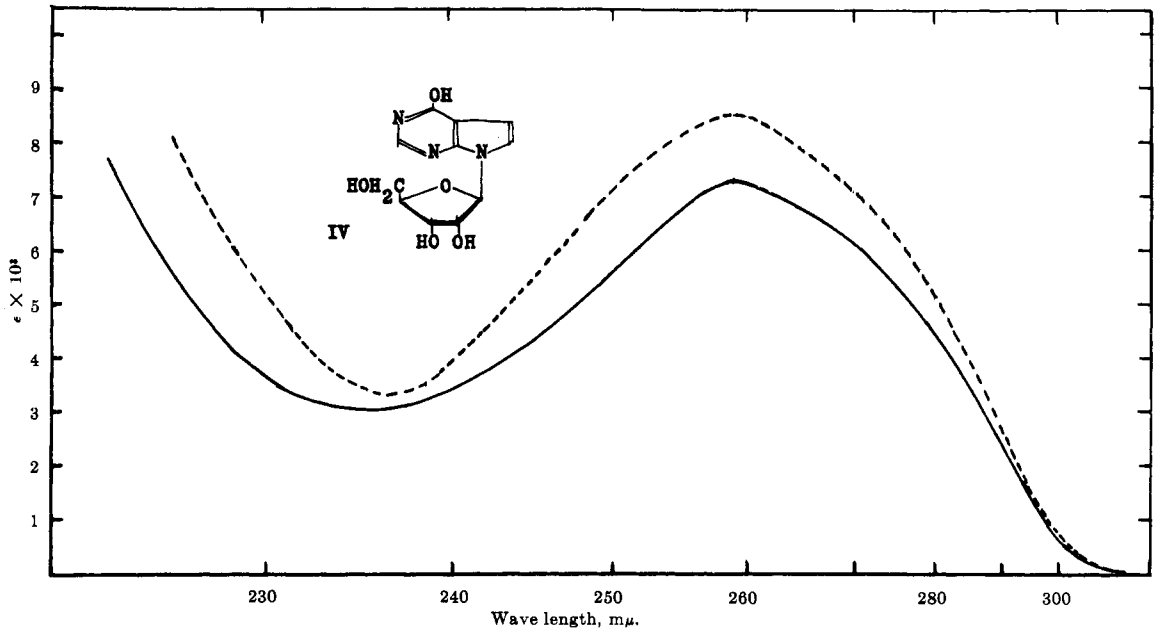


Fig. 2.—Ultraviolet absorption spectra of IV: ———, IV prepared from tubercidin; ———, *de novo* synthetic IV.

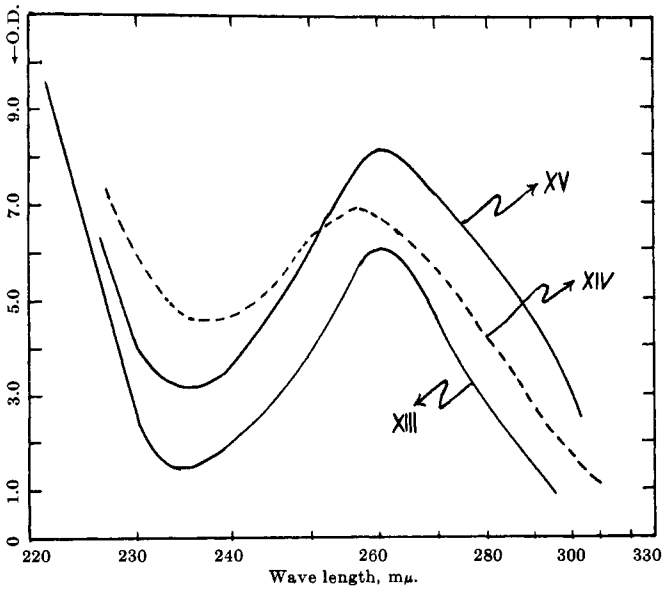


Figure 3.

The *de novo* synthetic XVIII was treated with phosphorus pentasulfide in refluxing pyridine, essentially according to Mizuno, Ikehara, and Watanabe's procedure,[21] to give XX as a yellow powder whose absorption maximum appeared at 328 mμ and was almost identical with that of 7*H*-pyrrolo[2,3-*d*]pyrimidine-4-(3*H*)-thione (see Experimental). XX was methylated with an equivalent amount of methyl iodide in the presence of methanolic sodium methoxide (one equivalent) to afford XXI. The 4-methylthio group was found to be inert toward the nucleophile; for example, it was inert to methanolic ammonia, even under forcing conditions (165° for 10 hr. in a sealed tube).[22] An attempt to replace the hydroxyl group of IV with an amino group by this route was abandoned. Another approach to the synthesis of I was to convert XXI to the corresponding 4-chloro derivative by a procedure originally developed by Robins[23] and improved by Ikehara, *et al.*,[24] followed by amination of the chloro derivative; however, chlorination of XXI by this procedure failed to give the desired product.[25]

The *de novo* synthetic XVIII was treated with methanolic ammonia at 0° for 2 days to afford XIX (as a glass) in good yield. Detritylation of XIX by a standard procedure gave 4-deaminohydroxytubercidin (7-deazainosine), m.p. 242–243°. The *de novo* synthetic sample was identical with deaminohydroxytubercidin of natural origin on the basis of ultraviolet and infrared absorption spectral data, R_f values, specific rotation, and the mixture melting point (see Fig. 1 and 2 and Table I). The over-all yield of IV was 6% on the basis of XIII.

TABLE I

	R_f (H_2O, pH 10)	$[\alpha]_D$	M.p., °C.	λ_{max} mμ
IV (*de novo* synthetic)	0.62	−6.72	242–243	259
IV (of natural origin)	0.62	−5.20	241–242	259

Experimental[26]

Reaction between 4-Amino-5-(2,2-diethoxyethyl)-6(1*H*)-pyrimidinone (XIII) and 2,3,4-*O*-Acetyl-5-*O*-trityl-D-ribose (IX). Synthesis of XIV and XV.—A solution of XIII[11] (3.90 g., 17.2 mmoles) and IX[14] (8.9 g., 17.2 mmoles) in absolute ethanol (250 ml.) was heated in the presence of freshly sublimed ammonium chloride (150 mg.) at the refluxing temperature. The reaction was followed by paper chromatography (Toyo filter paper 51A, solvent system 1-butanol–water, 84:16 v./v., ascending technique). At

(21) Y. Mizuno, M. Ikehara, and K. A. Watanabe, *Chem. Pharm. Bull.*, **10**, 647 (1962).

(22) The process was followed by ultraviolet spectral examination. The absorption maximum of XXI (λ_{max} 328 mμ) did not vary throughout the reaction.

(23) R. K. Robins, *J. Am. Chem. Soc.*, **82**, 2654 (1960).

(24) M. Ikehara, A. Yamasaki, and T. Fujieda, *Chem. Pharm. Bull.*, **10**, 1075 (1962).

(25) A shift of the absorption maximum was observed. However the maximum of the product was entirely different from that expected for 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine.[11]

(26) All melting points are corrected. Ultraviolet spectra were recorded with a Beckman Model DK-2 recording spectrophotometer. Except where noted, removal of the solvent was performed *in vacuo* (15–18 mm.). Paper chromatography was performed using the ascending technique. Infrared spectra were determined in potassium bromide disks using a Koken Model DS-301 infrared recording spectrophotometer.

TABLE II

PAPER CHROMATOGRAPHIC ANALYSIS OF THE REACTION BETWEEN IX AND XIII

	Reaction time, hr.						
	0	1	2	3	4	5	6
R_f values for the upper spot	0.69[a]	0.67	0.64	0.68	0.68	0.67	0.67
		(317)[c]	(1.156)	(779)	(492)	(300)	(32)
R_f values for the lower spot		0.51[b]	0.46	0.42	0.45	0.46	0.45
		(102)	(712)	(1353)	(1669)	(1397)	(233)
Ratio of total optical density of the lower spot to that of the upper spot		0.32	0.616	1.61	3.39	4.66	7.28
Yield, in % of a product (XIV)[d]		24.2	38.6	61.6	77.2	82.3	87.9

[a] R_f of XIII. [b] R_f of a product (XIV). [c] Numbers in parentheses indicate total optical density. [d] Yields (in %) were calculated by the ratio multiplied by a factor (ratio of two molecular extinction coefficients).

1-hr. intervals, the reaction mixture (1 ml.) was pipeted out and an aliquot was diluted to twice its volume and a certain definite volume was put on the paper. Compounds on the developed chromatograms were detected as two dark spots when viewed under an ultraviolet lamp. These spots were cut out, extracted for 1 min. with hot ethanol (5 ml.), the extract cooled to 20°, and the optical density at 260 mμ determined using an ethanol extract of a blank paper as the control (the same area as the dark spot, with the same volume of hot ethanol). The results obtained are shown in Table II, and the ultraviolet absorption spectrum of the ethanol extract of a spot (R_f 0.56) is presented in Fig. 3.

After the reaction was almost complete, which required about 6 hr., the reaction mixture was allowed to stand for 19 hr. at room temperature, during which period, brown needles separated. These needles were filtered off, washed with ethanol, and dried (1.45 g.), after recrystallization from water, m.p. 320–325° dec. The compound was identical with 4-hydroxy-7*H*-pyrrolo[2,3-*d*]pyrimidine prepared by a reported method[11] from the criteria of ultraviolet and infrared absorption spectra and elementary analysis.

Anal. Calcd. for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 53.40; H, 3.96; N, 31.15.

The combined filtrate and washings were concentrated *in vacuo* at 30° to dryness, to afford a residue which was dissolved in chloroform and the suspension filtered. The chloroform was removed *in vacuo*. The residue was azeotropically dried by distillation *in vacuo* with benzene (40 ml.). The dried residue weighed 9.30 g.; λ_{max}^{MeOH} 255–256 mμ, λ_{min}^{MeOH} 244 mμ; R_f 0.46.

Deacetylation of XIV (R_f 0.46). Conversion of XIV to XVI.—The dried residue (9.30 g.) was dissolved in methanol saturated with ammonia at 0° (200 ml.). The color of the solution gradually turned to brown. The solution was kept at room temperature for 2 days, and the solvent was removed *in vacuo* at 40° to afford a residue which was dried azeotropically three times by distillation with three 10-ml. portions of benzene. The dried residue was dissolved in pyridine (20 ml.) and treated with acetic anhydride (10 ml.) at 0°. The solution was kept at room temperature for 2 days, absolute ethanol (10 ml.) was added at 0°, and the solution was concentrated *in vacuo* at 40° to half its volume. The process was repeated three times. Finally the solution was concentrated to dryness *in vacuo*. The residue was dissolved in chloroform (30 ml.). The solution was washed with water (30 ml.), and the chloroform layer was separated, dried over sodium sulfate (10 g.), filtered from salts, and concentrated *in vacuo* at 40° to give a residue which weighed 8.64 g.

Purification of the Residue (Crude XVII) by Alumina Column Chromatography.—The residue (8.64 g.) was dissolved in chloroform (60 ml.) and the solution was applied to an alumina column (alumina, 110 g., 21 cm. × 2.7 cm. diameter). The column was washed successively with benzene (600 ml.), chloroform (1.1 l.), chloroform–ethanol (95:5 v./v.), and chloroform–ethanol (8:2 v./v.). One hundred milliliters of eluate was collected as each fraction. The elution pattern is shown in Fig. 4. The first two fractions gave after evaporation of the solvent a nitrogen-free residue (1.25 g.). after recrystallization from ethanol, m.p. 81–82°. Its infrared absorption spectrum showed the presence of a trityl group and the absence of carbonyl. Mixture melting point with an authentic sample (of ethyl trityl ether) showed no depression. The yield was 25% on the basis of IX.

Anal. Calcd. for $C_{21}H_{20}O$: C, 87.50; H, 6.94. Found: C, 87.38; H, 6.97.

The third, as well as, the fourth fraction afforded after evaporation of the solvent another nitrogen-free compound (1.10 g.);

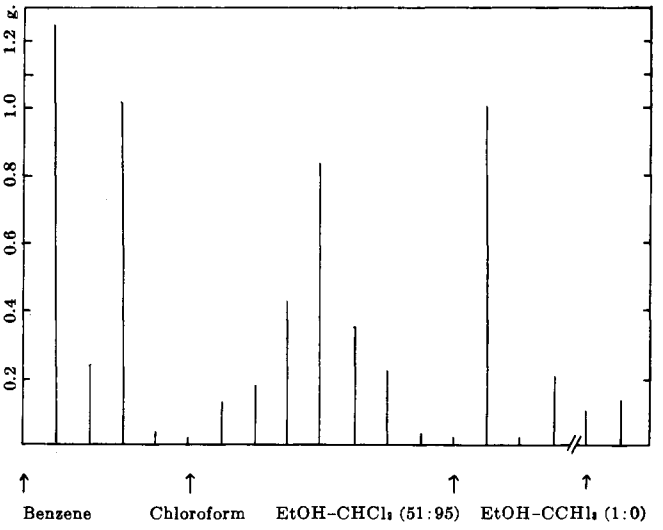


Fig. 4.—Elution pattern of alumina chloromatography of acetylated XVI (one fraction, 100 ml.).

its infrared absorption spectrum had bands characteristic of a sugar. The sample was not examined further. Fractions 5 to 12 gave after evaporation of the solvent *in vacuo* a glass, 2.37 g. of purified XVII (21% yield on the basis of XIII or IX). XVII (256 mg.) was trituated for 0.5 min. with dioxane (11.8 ml.) and decanted. The residue (52 mg.) was recrystallized from ethanol and petroleum ether (1:1 v./v.) to give needles, m.p. 232–233°; $[\alpha]^{17.5}D$ 18.8° (*c* 1.08, MeOH–$CHCl_3$, 2:1 v./v.)

Anal. Calcd. for $C_{38}H_{43}N_3O_9$: C, 66.56; H, 6.27; N, 6.17, Found: C, 66.80; H, 6.42; N, 6.57.

Preparation of XVIII.—Purified XVII (256 mg.) was dissolved in dioxane (11.8 ml.), and the solution was filtered. The filtrate was adjusted to pH 2.8 by addition of 75% aqueous acetic acid and the solution was kept at room temperature for 2 hr. Benzene (36 ml.) was added and the solvent was quickly removed *in vacuo* to give a residue (162 mg.) which was dissolved in benzene (2 ml.), the solution was filtered and the filtrate concentrated to dryness. The residue was dissolved in methanol (1 ml.) and the solvent was removed *in vacuo* to afford a colorless glass which was dissolved in methanol and reprecipitated with water. The process (reprecipitation) was repeated three times. Finally the precipitate was dissolved in ethanol and precipitated with petroleum ether to give an amber glass (50.5 mg.); $[\alpha]^{19.5}D$ +20.0° ± 2.2 (*c* 0.45, MeOH); ultraviolet absorption, λ_{max}^{MeOH} 259 mμ (ϵ 8.4 × 10^3). The infrared spectrum is shown in Fig. 1.

Anal. Calcd. for $C_{34}H_{31}N_3O_7$: C, 68.80; H, 5.23; N, 7.08. Found: C, 68.73; H, 5.20; N, 7.28.

4-Hydroxy-7-(5-*O*-trityl-β-D-ribofuranosyl)-7*H* pyrrolo[2,3-*d*]-pyrimidine (XIX).—XVIII (143 mg., 0.24 mmole) was dissolved in absolute methanol (30 ml.) and the solution was saturated with ammonia at 0°. The solution was kept for 3 days at room temperature. Removal of the solvent *in vacuo* gave a residue (122.9 mg.) quantitatively; $[\alpha]^{15.5}D$ +16.7° (*c* 1.32 MeOH); λ_{max}^{MeOH} 260 mμ.

4-Hydroxy-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (IV).—A solution of XIX (112 mg., 0.22 mmole) in 80% aqueous acetic acid (v./v.) was refluxed for 30 min. Removal of the solvent *in vacuo* gave a residue which was treated with three 3-ml.

portions of ether. The extract gave after evaporation of the solvent tritanol (m. p. 159°, 48.5 mg.). The ether-insoluble material (46.9 mg.) was dissolved in water (15 ml.); the solution was divided in two portions and each portion was applied to a 40 cm. wide Toyo filter paper 51A and developed (adjusted to pH 10 with ammonia). A band corresponding to IV (R_f 0.61) was cut out, and eluted with two 25 ml. portions of hot water. The combined extracts were concentrated to dryness to afford 34.6 mg. of IV which was recrystallized from 1.5 ml. of water to give 29.9 mg. of needles (m.p. 242–243°, 51.1% yield). Further extraction of the band with water (150 ml.) in a Soxhlet apparatus gave after removal of water a further crop (8 mg., m.p. 241°). Total yield 37.9 mg. (64.6%). Over-all yield of IV from XIII was 6%. Mixture melting point with an authentic sample[2] showed no depression, $[\alpha]^{18.5}D$ −6.72 (*c* 0.5, H_2O). The ultraviolet absorption is shown in Fig. 2, $\lambda_{max}^{H_2O}$ 259 mμ (ε 8.5 × 10³). The infrared spectrum is shown in Fig. 1.

Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C, 49.43; H, 4.90; N, 15.73. Found: C, 49.20; H, 5.02; N, 15.48.

4 Hydroxy-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine from Tubercidin (I).—A suspension of tubercidin (I, 250 mg., 0.94 mmole) in glacial acetic acid (15 ml.) and water (0.375 ml.) was heated until the nucleoside was dissolved and then cooled; barium nitrite (525 mg., 2.12 mmoles) was added and the solution was kept for 2 days in the dark at room temperature. The solution was heated at 70–75° for 1 hr., and then an additional 0.375 ml. of glacial acetic acid was added. The process was followed by paper chromatography (water adjusted to pH 10). The solution was heated for a further 8 hr. and cooled. Sulfuric acid (1.01 *N*, 4.25 ml.) was added and the precipitated barium sulfate was filtered off and washed with ten 15-ml. portions of water. The combined filtrate and washings were concentrated *in vacuo* to afforded a residue which was recrystallized from water, and dried (200 mg., 80% yield); m.p. 242–243° (lit.[2] m.p. 240–241°); $[\alpha]^{18.5}D$ −5.20 (*c* 0.895, H_2O).

Preparation of XVIII of Natural Origin.—4-Deaminohydroxytubercidin (124 mg., 0.47 mmole) and trityl chloride (148 mg., 0.53 mmole) were dissolved in pyridine (1.5 ml.). The solution was kept at 31° for 20 hr. (after this period, paper chromatography showed the absence of starting material), and then freshly fused sodium acetate (61 mg.) was added; the solution was kept at 31° for 5 hr. Acetic anhydride (0.8 ml.) was added, the solution was kept for a further 16 hr., and poured on ice–water (10 g.), to deposit a solid; this was filtered off, washed with five 1.5-ml. portions of water, air-dried, and dried over phosphorous pentoxide at 30° (255 mg., 91.1%). For purification, the sample was dissolved in methanol and precipitated with light petroleum ether; the process was repeated twice. The residue thus obtained was dissolved in methanol and the solvent was removed *in vacuo* to give a glass (214 mg., 77%); $[\alpha]^{19.6}D$ +21.8° (*c* 0.55, MeOH); ultraviolet, λ_{max}^{MeOH} 259 mμ (ε 8.5 × 10³). The infrared spectrum is shown in Fig. 1.

Anal. Calcd. for $C_{34}H_{31}N_3O_7$: C, 68.80; H, 5.23; N, 7.08. Found: C, 69.12; H, 5.57; N, 6.98.

Thiation of XVIII. 7-(2,3-Di-*O*-acetyl-5-*O*-trityl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-thione (XX).—XVIII (130 mg., 0.219 mmole), was treated with phosphrus pentasulfide (29 mg.) in refluxing pyridine (3.3 ml.); the reaction was followed by ultraviolet absorption spectral analysis. During the first 50 min., no significant shift of the spectrum was observed. After the mixture had been heated for 2.5 hr., pyridine (2 ml.) containing water (0.02 ml.) and phosphorus pentasulfide (120 mg.) were added, and the heating was continued until a complete disappearance of the starting material (λ_{max} 259 mμ) was observed and a new band corresponding to a product (λ_{max} 328 mμ) appeared. A total of 9.5 hr. of heating was required. The solution was kept at room temperature overnight; a small amount of an insoluble material which separated was freed from the liquid by decantation and washed with three 3-ml. portions of pyridine. The combined supernatant liquor and washings were concentrated *in vacuo* to afford a tar which was dissolved in chloroform (5 ml.); the solution was washed with water, dried with sodium sulfate (700 mg.), and filtered. The sodium sulfate was washed with five 2-ml. portions of chloroform. The filtrate and washings were combined and concentrated *in vacuo* to afford a residue which was dried azeotropically by distillation with benzene (5 ml.). This process was repeated twice. The residue (117 mg., 87.6% yield) was 83.2% pure (determined on the basis of the molecular extinction coefficient). Further purification was effected by dissolving in ethanol and subsequent precipitating with petroleum ether to give a yellow powder; ultraviolet, λ_{max}^{MeOH} 328 mμ (ε 16.2 × 10³); λ_{min}^{MeOH}, 293 mμ (ε 5.3 × 10³).

Anal. Calcd. for $C_{34}H_{31}N_3O_6S$: C, 67.00; H, 5.09; N, 7.26. Found: C, 67.23; H, 5.43; N, 7.10.

7-(2,3-Di-*O*-acetyl-5-*O*-trityl-β-D-ribofuranosyl-4-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine (XXI).—XX (115 mg., 0.19 mmole) was treated with an absolute methanol solution (4.6 ml.) of methyl iodide (28 mg.) in the presence of sodium methoxide (0.94 equivalents) in methanol (1 ml.) at room temperature for 2 hr., during which time the ultraviolet absorption maximum was shifted from 328 to 291 mμ. The solvent was removed *in vacuo* at room temperature to afford a residue (130 mg.) which was dissolved in benzene (5 ml.). The solution was filtered and the filtrate was concentrated to dryness, giving a yellow powder (74 mg., 63%); ultraviolet, λ_{max}^{MeOH} 291 mμ (ε 9.66 × 10³).

Anal. Calcd. for $C_{35}H_{33}N_3O_6S$: C, 67.41; H, 5.29; N, 6.74. Found: C, 67.28; H, 5.23; N, 6.98.

7*H*-Pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-thione.—4-Hydroxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (XV, 1.35 g.) was treated with phosphorus pentasulfide (1.11 g.) in refluxing pyridine (40 ml.) for 3 hr. The solution was cooled, the supernatant layer was separated and the residue was washed with three 5-ml. portions of pyridine. The combined supernatant liquor and washings were concentrated *in vacuo* to dryness (1.8 g.). The residue was suspended in water (40 ml.) and ammonium hydroxide was added until the sample was almost dissolved. The suspension was filtered and glacial acetic acid was added to the filtrate precipitating material which on recrystallization from a large proportion of water gave a pure product as needles, m.p. 280° (1.21 g., 80%); ultraviolet, λ_{max}^{MeOH} 324 (ε 19.7 × 10³) and 270 mμ (4.9 × 10³), λ_{min}^{MeOH} 234 (ε 4.2 × 10³) and 243 mμ (0.9 × 10³).

Anal. Calcd. for $C_6H_5N_3S$: C, 47.68; H, 3.31; N, 27.82. Found: C, 47.65; H, 3.31; N, 27.78.

4-(Methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine.—7*H*-Pyrrolo-[2,3-*d*]pyrimidine-4(3*H*)-thione (151 mg.) was treated at room temperature with methyl iodide (154 mg.) in absolute methanol (5 ml.) in the presence of sodium methoxide (0.94 *N* solution in methanol, 1.06 ml.). After the solution had been kept for 2 hr. at room temperature, the ultraviolet absorption maximum had shifted from 324 mμ to 298 mμ. The solution was neutralized with acetic acid, to precipitate a product which was filtered off and recrystallized from aqueous methanol to give a powder (122 mg., 73.9%); ultraviolet; $\lambda_{max}^{aq.\ MeOH}$ 294 (ε 11.5 × 10³) and 250 mμ (4.5 × 10³); $\lambda_{min}^{aq.\ MeOH}$ 261 (ε 5.3 × 10³) and 240 mμ (2.6 × 10³).

Anal. Calcd. for $C_7H_7N_3S$: C, 50.90; H, 4.24; N, 25.45. Found: C, 51.13; H, 4.32; N, 25.45.

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