Pyrrolopyrimidine Nucleosides. 1. The Synthesis of 4-Substituted 7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines from Tubercidin[1]

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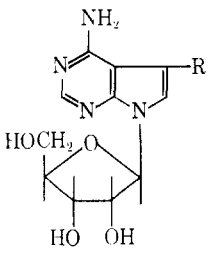
Received January 16, 1967

The synthesis of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa) has been accomplished by treatment of 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (V) with $POCl_3$. Nucleophilic substitution of the 4-chloro group resulted in the synthesis of new analogs of tubercidin. Direct methylation of tubercidin (I) afforded 4-amino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (X) which rearranged to 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (XI) when treated with NaOH. The synthesis of XI was also accomplished directly from VIa with methylamine.

Isolation[2] of the nucleoside antibiotic tubercidin from *Streptomyces tubercidicus* provided the first naturally occurring derivative of the pyrrolo[2,3-*d*]pyrimidine ring system. The structure of tubercidin was later established[3] unequivocally as 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (7-deazadenosine, I). Tubercidin has subsequently demonstrated[4]



I, R = H
II, R = CN
III, R = $CONH_2$

growth inhibition of certain experimental animal tumors. The *in vivo* inhibition[5] of mouse fibroblast multiplication and definite inhibition[6] of several human tumors *in vitro* has also been noted. Tubercidin inhibits *de novo* purine biosynthesis by preventing the synthesis of 5-phosphoribosylpyrophosphate (PRPP).[7] It has been shown[8] that the binding of certain aminoacyl-RNA's to ribosomes was significantly stimulated when tubercidin was incorporated into the aminoacyl-s-RNA. The isolation and characterization of other derivatives of the pyrrolo[2,3-*d*]pyrimidine ring system, toyocamycin[9] (II) and sangivamycin[10] (III), from naturally occurring sources has created considerable interest in the chemical synthesis of these compounds and their derivatives. It has been reported[11] that 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (IV, 7-deazainosine), a derivative of tubercidin, also possesses significant antitumor activity. It therefore seemed of interest to prepare a number of 4-substituted 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines to study structure–activity relationships.

Acetylation of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (IV, 7-deazainosine)[12] with a mixture of acetic anhydride and pyridine furnished a good yield of 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (V). Chlorination with $POCl_3$ and redistilled N,N-diethylaniline has been previously reported[13] to afford 6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine from 9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinone. However, these reaction conditions with V produced a highly colored solution from which none of the desired VIb could be isolated. It was subsequently discovered that by simply excluding N,N-diethylaniline from the reaction mixture a 70% yield of the desired product, 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIb), was readily obtained (Chart I). It is of interest that an attempted conversion of 4-methylthio-7-(2′,3′-di-*O*-acetyl-5′-*O*-trityl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine to the corresponding 4-chloro derivative using a previously reported procedure[14] was also unsuccessful, presumably due to oxidation of the pyrrole ring by excess chlorine. Removal of the blocking groups on the carbohydrate moiety with methanolic ammonia furnished the versatile compound, 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa). The recent finding[15] that 6-chloro-9-(β-D-ribofuranosyl)purine is dechlorinated to inosine by heart, red cell, and intestinal mucosa adenosine deaminases would indicate that the chemotherapeutic activity of 6-chloro-9-(β-D-ribofuranosyl)purine

(1) This work was supported by Research Contract PH 43-65-1041 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service. A preliminary report of this work was presented before the Division of Medicinal Chemistry at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966.

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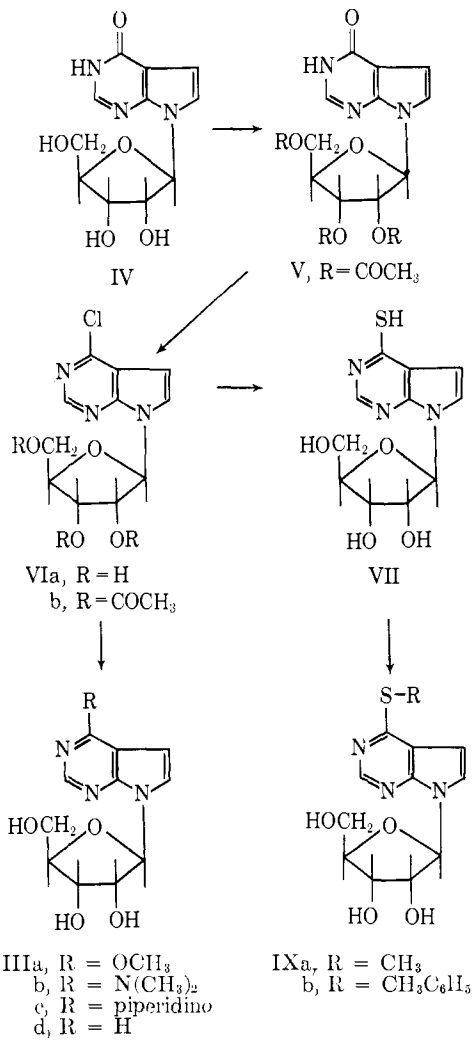
CHART I



might be increased if this reaction could be inhibited or eliminated. It is of interest that tubercidin demonstrated a complete resistance to these three deaminases under the same conditions and, therefore, by analogy VIa might well resist enzymatic dechlorination by adenosine deaminase.

Treatment of VIa with aqueous thiourea produced 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thiol (VII) which was identical in all respects with the same compound previously prepared[12c] from the thiation of V with P_2S_5 followed by removal of the blocking groups on the carbohydrate moiety. 4-Benzylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IXb) was produced when VII was treated with benzyl chloride in dilute aqueous ammonia. It is of considerable interest that 6-methylthiopurine ribonucleoside has been postulated[16] to act as a substrate for adenosine kinase which converts it to the nucleotide form in mouse tissues, Ehrlich ascites carcinoma cells *in vivo*, and tumor cells *in vitro*. 6-Methylthiopurine ribonucleoside has also demonstrated[17] significant *in vivo* activity against leukemia L1210 which had become resistant to 6-mercaptopurine therapy. This activity has prompted an investigation to determine if a correlation between the ring nitrogens and cytotoxicity might exist. While it has been determined that 3-deaza-6-methylthiopurine ribonucleoside[18] possesses far greater activity than does 1-deaza-6-methylthiopurine ribonucleoside,[19] neither compound compares favorably with the parent compound, 6-methylthiopurine ribonucleoside. The only remaining deaza nucleoside derivative is the compound, 4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IXa) which was prepared in our laboratory by alkylation of VII with methyl iodide.

Dehalogenation of VIa with hydrogen and Pd–C catalyst has resulted in the preparation of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIId, 7-deazanebularine). This compound is of interest since the active constituent from *Agaricus nebularis* Batch has been isolated[20] and later characterized[21] as the nucleoside antibiotic 9-(β-D-ribofuranosyl)purine (nebularine). Nebularine has also been isolated[22] from *Streptomyces yokosukaensis* and has demonstrated[23] some activity in tissue cultures. In fact it has been recently suggested[24] that 7-deazanebularine (VIId) should be synthesized and its biochemical behavior compared with that of nebularine.

Nucleophilic displacement of the 4-chloro group of VIa in refluxing sodium methoxide afforded 4-methoxy-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIIa). Treatment of VIa with dimethylamine and piperidine under anhydrous conditions produced 4-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIIb) and 4-(1-piperidyl)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (VIIIc), respectively. It is of interest that the corresponding purine ribonucleoside, 6-(1-piperidyl)-9-(β-D-ribofuranosyl)purine possesses[25] significant antitumor activity against Walker 755 and 756 carcinoma and inhibits the growth of *Vicia faba*. Treatment of VIa with methanolic ammonia at 150° resulted in a displacement of the chloro group to produce 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (I, tubercidin).

Actually this provides the first total chemical synthesis of tubercidin since 7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]-4-pyrimidone has been previously prepared[12b] by chemical synthesis.

Methylation of tubercidin (I) with methyl iodide in dimethylacetamide (Chart II) furnished a monomethyl derivative as the iodide salt which was assumed to be 4-amino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (X). The pmr spectrum indicated that alkylation of a ring nitrogen had occurred since the absorption peak observed for the methyl group was a singlet and it has been previously shown[26] that under similar reaction conditions adenosine undergoes alkylation on the ring nitrogen adjacent to the exocyclic

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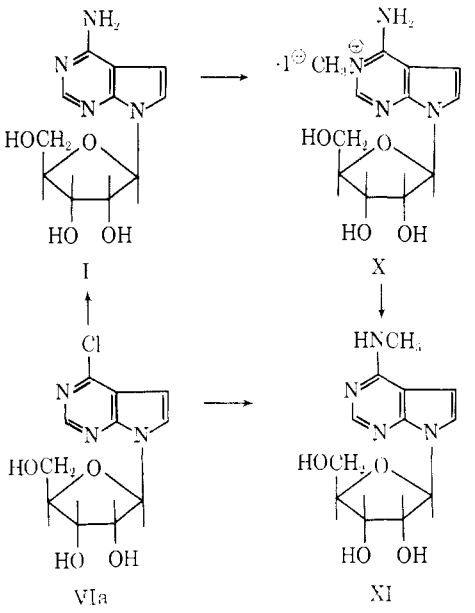
CHART II



amino group to afford 1-methyladenosine. Treatment of X with strong base produced another compound with a different melting point, pmr (methyl group occurs as a doublet), and ultraviolet spectra. The structure of this compound was unequivocally established as 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (XI) when the product obtained from the treatment of VIa with anhydrous methylamine was identical in all respects. This firmly established the structure of the unrearranged compound as 4-amino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (X).

All of the compounds prepared have purine nucleoside counterparts which possess a wide variety of biological and chemotherapeutic activity. One of the most serious disadvantages in the utilization of purine nucleosides in chemotherapy is the ease with which the glycosidic linkage is cleaved by the enzyme nucleoside phosphorylase. Tubercidin has been found[12c] to be completely resistant toward glycosidic cleavage by this enzyme and it is tempting to postulate that N-7 of the purine ring is therefore an essential binding site for this enzymatic action. Thus all the 7-deazapurine nucleosides prepared in this investigation should possess the advantage of being stable *in vitro* or *in vivo* to nucleoside phosphorylase.

Antitumor Evaluation.[27]—The results obtained from the antitumor testing of compounds prepared in this investigation are shown in Table I and evaluation of the activity is in accordance with the criteria[28] of the Cancer Chemotherapy National Service Center. From the testing data available (Table I) at the present time it is evident that this series possesses very little potential as inhibitory agents of lymphoid leukemia L1210. Although VIIIb possessed a T/C of 120%, this fails to pass stage 1 of the sequential screen.

Preliminary testing results against Walker carcinosarcoma 256 (intramuscular), however, have been rather encouraging. These tests were performed on randomly bred albino rats and the compounds were administered intraperitoneally using saline as the vehicle. All compounds tested thus far have exhibited some inhibition; in fact, IV has demonstrated sufficient activity to pass stage 1, 2, and 3 of the sequential screen of step I and the three successive tests at sequential dose of step II confirmation, and 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][4-pyrimidone (IV) can now be considered a confirmed active compound in this test system. Additional testing results of this series of compounds are at the present time incomplete. However, it does appear that derivatives of the pyrrolo[2,3-*d*]pyrimidine nucleosides may indeed possess important biological activity.

(27) Testing was performed under the auspices of the Cancer Chemotherapy National Service Center.

(28) The requirements for passing different stages of the sequential screen where the value of T/C in a single test at a nontoxic dose must be as follows: lymphoid leukemia L1210, stage 1 T/C ≧ 125% and stage 2 T/C ≧ 156%; Walker carcinosarcoma 256 (intramuscular), stage 1 T/C ≦ 0.53, stage 2 T/C ≦ 0.19, and stage 3 T/C ≦ 0.07.

Experimental Section

Melting points were taken on a Thomas–Hoover melting point apparatus and are uncorrected. Chromatograms were developed using Whatman No. 1 chromatography paper in the following solvent systems: A, 5% aqueous NH_4Cl; B, ethanol–water (7:3 v/v); C, 5% aqueous $NaHCO_3$; D, 2-propanol–concentrated NH_4OH–water (4:3:3, v/v); all systems descending. The ultraviolet spectra are given for some of the compounds in Table II.

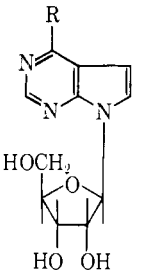
7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (V).—Ten grams of IV was added to a solution of 100 ml of pyridine and 50 ml of acetic anhydride. This mixture was allowed to stand at 5° for 24 hr with occasional shaking until all the solid was in solution. The solution was then evaporated to a thick syrup *in vacuo*, and 400 ml of anhydrous ethanol was added. This solution was refluxed for 1 hr, the ethanol was removed *in vacuo*, and the syrup was dissolved in 400 ml of methylene chloride. The CH_2Cl_2 solution was washed once with 1 *N* HCl (300 ml), twice with 300-ml portions of saturated $NaHCO_3$, and once with 300 ml of H_2O. Another 300 ml of H_2O was added and the mixture was allowed to stir vigorously overnight. The layers were separated and the CH_2Cl_2 portion was dried ($MgSO_4$). The CH_2Cl_2 was removed *in vacuo* and the residue was dried to a foam *in vacuo*; yield 12 g.

Anal. Calcd for $C_{17}H_{18}N_2O_8$: C, 51.79; H, 4.87; N, 10.68. Found: C, 51.87; H, 4.79; N, 10.48.

4-Chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIb).—7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (V, 3.0 g) was dissolved in $POCl_3$ (15 ml) and the solution was heated on a hot water bath at 70–75° for 30 min. The amber solution was then poured over crushed ice (150 ml) with vigorous stirring. The mixture was stirred until the excess $POCl_3$ was decomposed and additional ice was added if necessary to keep the temperature between 0–5°. The cold mixture was then extracted with two 75-ml portions of CH_2Cl_2 and the combined extracts were allowed to stir overnight with 150 ml of water. The CH_2Cl_2 layer was then separated, dried ($MgSO_4$), and evaporated *in vacuo* to yield 2.0–2.2 g (70%) of product as an oil which was used directly in the next step.

4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa).—4-Chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (VIb, 3.6 g, 0.88 mole) was dissolved in 100 ml of methanolic NH_3 (saturated at −10°) and the solution was allowed to stand at 5° for 24 hr. The solution was then evaporated *in vacuo* at 50–60° to yield a thick syrup. The syrup was dissolved in 35–50 ml of boiling water and the solution was allowed to cool to room temperature. A colorless solid, which crystallized from the solution, was filtered from the mixture, washed with water, and dried. A yield of 1.8 g of colorless product, mp 161–163°, was obtained. An additional 350 mg of product, mp 159–162°, was obtained by partial evaporation of the filtrate and a total yield of 2.15 g (86%) was obtained. This product was essentially pure but can be recrystallized from water or a ligroin (bp 90–120°)–acetone mixture to give an analytically pure product, mp 161–163°, $[\alpha]^{25}D$ −63.2° (*c* 1, 1:1 ethanol–H_2O).

TABLE I
TESTING DATA OF SOME 4-SUBSTITUTED 7-(β-D-RIBOFURANOSYL)PYRROLO[2,3-*d*]PYRIMIDINES



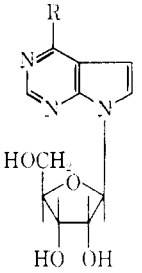
Compd	R	Dose, mg/kg	Survivors	Animal wt diff (T − C)	Survival days T/C	%	Stage index	Test status
				Lymphoid Leukemia L1210				
VII	SH	500	6/6	−2.6	10.7/9.2	116	116	2
VIIIa	OCH_3	400	0/6					1
		200	0/4					22
		100	3/4	−5.3	8.5/8.4	101		22
		50	4/4	−3.7	8.5/8.4	101		22
		25	3/4	−6.0	7.3/8.4	86		22
VIa	Cl	400	5/6	−4.2	6.2/8.2			1
		200	4/4	−3.8	9.3/8.4	110		22
		100	4/4	−2.8	9.3/8.4	110		22
		50	4/4	−0.8	8.4/8.4	104		22
		25	4/4	0.2	9.3/8.4	110		22
IXb	$SCH_2C_6H_5$	400	1/6	−5.2	6.0/8.2			1
		200	4/4	−2.2	9.8/8.4	116		22
		100	4/4	−0.4	8.3/8.4	98		22
		50	4/4	−1.6	9.0/8.4	107		22
		25	4/4	−2.2	9.3/8.4	110		22
IV	OH	250	4/6	−4.8	6.0/8.9			3
		100	0/6					3
		500	0/6					1
		12.5	6/6	−2.0	9.5/9.3	102		
VIIIc	NC_5H_{10}	400	6/6	−0.2	8.5/8.9	95		2
		400	4/4	0.4	8.3/8.9	93		22
		200	4/4	−0.1	8.8/8.9	98		22
		100	4/4	−0.2	8.5/8.9	95		22
		50	4/4	0.5	8.3/8.9	93		22
XI	$NHCH_3$	400	1/4	−6.0	9.0/8.9			22
		200	0/4					22
		100	1/4	−5.9	11.0/8.9			22
		50	4/4	−1.8	9.8/8.9			22
		45	0/6					1
VIIIb	$N(CH_3)_2$	500	0/6					1
		125	0/6					3
		30	1/6	−6.9	7.0/8.9			3
		7.5	6/6	−1.7	11.2/9.3	120		4
				Walker Carcinosarcoma 256 (intramuscular)				
					Tumor wt (g) T/C			
VIIIb	$N(CH_3)_2$	7.5	5/5	−19	5.2/9.9	52	52	11
		7.5	6/6	−12	8.0/9.4	85	44	6
VII	SH	500	2/6	−24.0	1.0/7.2			1
		250	5/6	−12.0	1.8/5.0	36	36	13
IV	OH	65	7/7	−2.0	4.4/9.0	48	48	11
		12.5	5/7	−26.0	0.9/9.0	10	10	11
		12.5	6/6	−22.0	1.8/5.1	35	3	15
		12.5	4/6	−25.0				
		12.5	3/6	−23.0	2.1/5.5	38	38	20
		10.0	4/6	−21.0	2.9/9.3	31		20A
		10.0	6/6	−19.0	1.6/5.0	32		20C

Anal. Calcd for $C_{11}H_{12}ClN_3O_4$: C, 46.3; H, 4.23; N, 14.7; Cl, 12.4. Found: C, 46.1; H, 4.07; N, 14.8; Cl, 12.4.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (Tubercidin, I).—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (500 mg, 1.75 mmoles) was dissolved in 100 ml of methanolic NH_3 (saturated at −10°) and the solution was heated in a steel bomb at 150° for 3 hr. The solution was evaporated to dryness and the residue was recrystallized twice from water to yield 250 mg (54%) of analytically pure tubercidin as colorless crystals.

Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.7; H, 5.30; N, 21.0. Found: C, 49.8; H, 5.46; N, 20.9.

TABLE II
ULTRAVIOLET ABSORPTION OF SOME 4-SUBSTITUTED 7-(β-D-RIBOFURANOSYL)PYRROLO[2,3-*d*]PYRIMIDINES



Compd	R	λ_{max}^{pH1}, mμ	ε	λ_{max}^{pH7}, mμ	ε
VII	SH	322	(22,300)	309	(20,700)
		267	(6,000)	230	(17,800)
VIa	Cl	273	(1,450)	273	(4,600)
		223	(27,400)	224	(22,600)
VIIIb	$N(CH_3)_2$	278	(15,400)	281	(17,700)
		232	(7,800)		
IXb	$SCH_2C_6H_5$	308	(10,300)	296	(13,800)
		264	(8,900)	247	
		222	(22,800)		
X	$NHCH_3$	272	(13,600)	273	(13,600)
		228	(18,500)		
IXa	SCH_3	304	(11,000)	293	(13,700)
		260	(12,000)	240	(5,930)
VIIId	H	264	(17,800)	263	(18,100)
		227	(32,100)		
VIIIa	OCH_3	270	(6,200)	263	(7,600)
		223	(21,300)		
VIIIc	NC_5H_{10}	281	(17,000)	287	(18,900)
		237	(11,100)		

This compound was found to possess the same ultraviolet, infrared, and pmr spectra and identical R_f values in two chromatographic solvent systems (B, 0.51, and C, 0.53) as naturally occurring tubercidin.[29] There was also no depression observed by mixture melting point.

4-Amino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (X).—Tubercidin[29] (I, 5.0 g, 0.0188 mole) was added to 50 ml of dimethylacetamide and 5 ml of methyl iodide was added to the mixture. The mixture was stirred at room temperature for 24 hr (after less than 1 hr all the solid had dissolved). The solution was poured into 800 ml of ethyl ether with vigorous stirring and an oil formed which solidified upon standing. The solid was crushed, filtered from the mixture, and dried to yield 7.6 g (100%) of the hydriodide salt. This product was dissolved in ethanol (1.0 g/50 ml) and the volume was reduced to two-fifths of the original volume on a hot plate. The product slowly crystallized to yield 5.6 g (74%) of pure compound: mp 203–205°; $[\alpha]^{25}D$ −41.5° (*c* 1, H_2O); ultraviolet, λ_{max}^{pH1} 272 mμ (ε 9900) and 220 (36,300); λ_{max}^{pH7} 227 mμ (ε 22,900), 270 (12,300), and 295 (sh) (5300).

Anal. Calcd for $C_{12}H_{16}N_4O_5 \cdot HI$: C, 35.4; H, 4.20; N, 13.70. Found: C, 35.2; H, 4.27; N, 13.6.

4-Methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (XI). Method A.—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (VIa, 500 mg, 1.76 mmoles) was dissolved in ethanol (25 ml) containing anhydrous methylamine (2 ml) and the solution refluxed for 1 hr. The solution was evaporated to dryness and the residue was recrystallized from water to yield 380 mg (77%) of analytically pure product, mp 173–175.5°, $[\alpha]^{25}D$ −69.9° (*c* 1, 1% HCl). The product was dried at 100° (0.1 mm) over P_2O_5 for analysis.

Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.4; H, 5.76; N, 20.0. Found: C, 51.2; H, 5.9; N, 19.8.

Method B.—4-Amino-3-methyl-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine hydriodide (X, 5.0 g) was dissolved in 30 ml of 1 *N* NaOH and the solution was heated on a steam bath for 1 hr. The solution was then filtered and the filtrate was neutralized with 6 *N* HCl. The product crystallized from solution after standing at 5° overnight and was removed by filtration. The colorless solid was washed with a small amount of cold water and dried. Recrystallization from water furnished 3.08 g of product, mp 175–177°.

Anal. Calcd for $C_{12}H_{16}N_4O_5 \cdot H_2O$: C, 48.3; H, 6.18; N, 18.8. Found: C, 48.20; H, 6.18; N, 19.2.

(29) Purchased from the Upjohn Co., Kalamazoo, Mich.

The products obtained from methods A and B possessed identical ultraviolet, infrared, and pmr spectra and the same R_f values in three chromatographic solvent systems (A, 0.66; B, 0.77; D, 0.80). A mixture melting point showed no depression.

4-Methoxy-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIIa).—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa, 4.0 g, 0.014 mole) was dissolved in 70 ml of 0.5 *M* methanolic NaOCH$_3$ and the solution refluxed for 1 hr. The solution was then cooled and neutralized with 6 *N* HCl. The neutral mixture was evaporated to a thick slurry *in vacuo* over a hot-water bath and the slurry was dried by repeated evaporation *in vacuo* with 2-propanol. The dry residue was extracted with five 25-ml portions of acetone. The acetone extracts were concentrated and the product crystallized. A yield of 3.0 g (76%) of analytically pure product was obtained. A small sample was recrystallized from acetone, mp 158–160°, $[\alpha]^{25}D$ −69.8° (*c* 1, H_2O).

Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 51.4; H, 5.38; N, 15.0. Found: C, 51.5; H, 5.43; N, 15.2.

4-Dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIIb).—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa, 2.9 g, 0.007 mole) was dissolved in methanol (80 ml) containing anhydrous dimethylamine (5 ml) and the resulting solution was allowed to stand at room temperature for 1 hr. The solution was then evaporated *in vacuo* to yield an oil which was dissolved in 10 ml of ethanol and again evaporated *in vacuo*. This procedure was repeated until the oil solidified and all trace of dimethylamine was removed. The crude product was dissolved in 50 ml of boiling anhydrous ethanol and the solution was concentrated to 30 ml. The solution was allowed to cool and the product crystallized. The solid was removed by filtration, to yield 1.7 g (81%) of analytically pure product, mp 192–195°, $[\alpha]^{25}D$ −67.6° (*c* 1, H_2O).

Anal. Calcd for $C_{13}H_{19}N_3O_5$: C, 53.1; H, 6.17; N, 19.1. Found: C, 53.3; H, 6.60; N, 19.3.

4-(1-Piperidyl)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIIc).—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa, 5.0 g, 0.0175 mole) was dissolved in 100 ml of ethanol containing 3.5 ml (3.02 g, 0.035 mole) of piperidine. The solution was refluxed for 1 hr and then evaporated *in vacuo* to yield an oil. The oil was thoroughly triturated with ethyl ether until the product solidified. The solid was completely pulverized in ethyl ether and after being removed by filtration was washed thoroughly with ethyl ether. The dried, colorless product was recrystallized twice from water to yield 3.3 g (55%) of colorless, analytically pure needles, mp 168–170°, $[\alpha]^{25}D$ −73.5° (*c* 1, 0.1 *N* HCl).

Anal. Calcd for $C_{16}H_{23}N_3O_5$: C, 57.5; H, 6.62; N, 16.7. Found: C, 57.5; H, 6.75; N, 16.50.

7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIIId, 7-Deazanebularine).—4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2.5 g, 8.76 mmoles) was dissolved in a solution of 100 ml of ethanol and 5 ml of concentrated NH_4OH. To this solution was added 1.0 g of 5% Pd/C. The mixture was hydrogenated at 2.8 kg/cm^2 (40 psi) at room temperature for 4 hr and filtered through a Celite pad and the carbon was washed with five 5-ml portions of hot ethanol. The filtrate was taken to dryness *in vacuo*. The residue was dissolved in acetone and carefully precipitated with petroleum ether (bp 90–110°) to yield 1.5 g of a white solid (68%), mp 117°. A sample was dried at 100° over P_2O_5 (0.1 mm) for analysis.

Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 52.59; H, 5.22; N, 16.72. Found: C, 52.58; H, 5.48; N, 16.57.

7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thiol (VII). 4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VIa, 2.5 g) was dissolved in 30 ml of warm water containing 2.0 g of thiourea. One drop of 25% aqueous formic acid was added to the solution and the solution was heated for 15 min on a steam bath. After the first 5 min of heating the pH of the solution was adjusted to approximately 3 with concentrated NH_4OH. The solution was then allowed to cool and the product crystallized. A yield of 1.7 g of crystals, mp 206–208°, was obtained. An additional 400 mg of product was obtained by partial evaporation of the filtrate. The combined products were recrystallized from water to yield 1.7 g (68%) of analytically pure needles, mp 206–208°, $[\alpha]^{25}D$ −117.5° (*c* 0.65, 0.1 *N* NaOH), lit.[16] mp 204–207°.

Anal. Calcd for $C_{11}H_{12}N_2O_4S$: C, 46.7; H, 4.63; N, 14.9. Found: C, 46.5; H, 4.44; N, 14.7.

4-Benzylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IXb).—7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thiol

(VII, 500 mg, 1.77 mmoles) was dissolved in 4 ml of 14% aqueous NH_3. Benzyl chloride (230 mg, 1.8 mmoles) in 2.0 ml of dioxane was added to the basic solution and the mixture was stirred at room temperature for 3 hr. The mixture was diluted with 6 ml of water and then extracted five times with 7.5-ml portions of ethyl acetate. The ethyl acetate solution, after drying ($MgSO_4$), was evaporated *in vacuo* to yield an oil which solidified when triturated with ligroin (bp 90–120°). The crude solid was recrystallized from an acetone–ligroin mixture to yield 500 mg (76%) of analytically pure product, mp 143–144°, $[\alpha]^{22}D$ −66.3° (*c* 1, ethanol).

Anal. Calcd for $C_{18}H_{19}N_3O_4S$: C, 57.9; H, 5.13; N, 11.3. Found: C, 58.1; H, 5.03; N, 11.1.

4-Methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IXa).—To a solution of 0.55 g of $NaOCH_3$ in 30 ml of methanol was added 2.8 g of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thiol (VII). After VII had dissolved, 0.6 ml of methyl iodide in 5 ml of methanol was added and the solution was stirred at room temperature for 2 hr. The pH was then adjusted to 6 and the solution (some solid present) evaporated to dryness *in vacuo* at room temperature. The resulting yellow oil was triturated with acetone. The white solid was collected by filtration, dried, and recrystallized from methanol to yield 2 g (65%) of analytically pure compound, mp 193–194°.

Anal. Calcd for $C_{12}H_{15}N_3O_4S$: C, 48.4; H, 5.05; N, 14.14. Found: C, 48.53; H, 5.20; N, 14.27.

Nucleosides. V. 2-Thiopyrimidine β-D-Arabinofuranosides

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Received December 7, 1966

The reaction of 2,2′-anhydro-1-(5-*o*-trityl-β-D-arabinofuranosyl)uracil (IIa) with H_2S in alkaline medium followed by detritylation produced 1-(β-D-arabinofuranosyl)-2-thiouracil (IX). Thiation of the triacetate of IV yielded the corresponding 2,4-dithiouracil nucleoside VI. Reaction of VI with NH_3 yielded the 2-thiocytosine arabinoside VII, cytosine arabinoside VIII, or the 2,2′-anhydrocytosine nucleoside IX depending on the conditions used. Reaction of IV or VII with bromine water resulted in the formation of 2,2′-anhydronucleosides IIb and IX, respectively. Iodination of IV gave a similar result. 1-(β-D-Arabinofuranosyl)-2-thiocytosine (VII) showed antiviral activity against vaccinia in tissue culture. The other thiopyrimidine nucleosides were inactive as antiviral agents.

Interest in thiopyrimidine nucleosides was heightened recently by the isolation of 4-thiouridylic acid[1] and a 2-thiopyrimidine nucleotide[2,3] from *E. coli* t-RNA as "odd" nucleotides and by the demonstration of an enzymatic thiolation of t-RNA.[4] It has been suggested that[2] the facile formation and cleavage of a disulfide bond in t-RNA may provide a chemical mechanism to the "adapter modification hypothesis,"[5] although no difference in the maximum tyrosine-accepting ability was detectable between the native and disulfide forms of *E. coli* tyrosine t-RNA.[6] More recently, evidence was presented for reversible conformational changes and ribosome binding efficiency upon iodine oxidation of lysyl t-RNA from *B. subtilis*.[7] A model involving sulfhydryl–disulfide interconversion of thiopyrimidines was again postulated. In our study of nucleoside antimetabolites, it was noted that the free base, 2-thiouracil, has been reported to suppress the production of infective turnip yellow mosaic viral nucleoprotein,[8] possibly *via* a preferential inhibition of viral-RNA-dependent RNA synthesis.[9] It was also incorporated into RNA of tobacco leaves and tobacco mosaic virus. The physicochemical difference between 2-thiouracil and uracil was indicated by recent nmr studies which concluded that 2-thiouracil exists essentially in the thiol form.[10] The ultraviolet absorption spectrum of 2-thiouridine suggests that it may act as an analog of cytidine as well as of uridine in RNA synthesis.[9] Following our previous investigations of pyrimidine nucleosides as potential antiviral agents the synthesis of 2-thiopyrimidine β-D-arabinosides was considered of some interest.

In contrast to the 4-thiopyrimidine nucleosides which are readily available from the corresponding 4-oxopyrimidine nucleosides by thiation with P_2S_5,[11,12] the 2-thiopyrimidine nucleosides have been prepared by more circuitous routes.[13] Methods involving the use of glycosyl amines[14,15] and glycosylthioureas[16] as starting points for building the 2-thiouracil ring system have been reported.

The formation of 2-thiouridine by the reaction of H_2S with 2,5′-anhydrouridine under mildly alkaline conditions has been described by Todd[17] and by others.[18,19] The bacterial synthesis of thiouridylic acid has been demonstrated.[20]

Taking advantage of our recent experience with 2,2′-

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