Analogs of Tubercidin[1]

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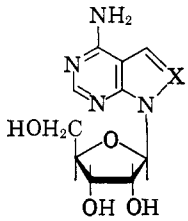
The synthesis of a number of alkyl analogs of tubercidin has been accomplished *via* 4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (**9**). Although somewhat cytotoxic, these analogs showed no activity against leukemia L1210.

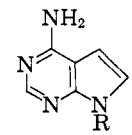
Tubercidin (4-amino-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-*d*]pyrimidine, **1**), an antibiotic analog[2,3] of adenosine in which N-7 has been replaced by a carbon atom,



1, X = CH
2, X = N

is also related to 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) ribonucleoside (**2**),[4,5] in which N-7 and C-8 have been inverted. Since both these structures have shown a high degree of biologic activity,[6-8] and tubercidin has shown significant anticancer activity in experimental animal systems,[6,9] it would appear that N-7 is not a requirement for activity. Furthermore, both 9-alkylpurines[10] and 1-alkylpyrazolo[3,4-*d*]pyrimidines[11] are known to have anticancer activity, and certain 9-alkylpurines inhibit the growth of cells resistant to 6-mercaptopurine.[12] Thus it seemed desirable to prepare and evaluate the biologic activity of a series of 7-alkylpyrrolo[2,3-*d*]pyrimidine analogs (**3**) of tubercidin.

Davoll's procedure[13] for the synthesis of 4-amino-7H-pyrrolo[2,3-*d*]pyrimidine (**3**, R = H), the aglycon of tubercidin, involves ring closure to a 4-aminopyrimidine and cannot be used to make the 4-alkylaminopyrimidines needed to prepare the desired 7-alkyl-

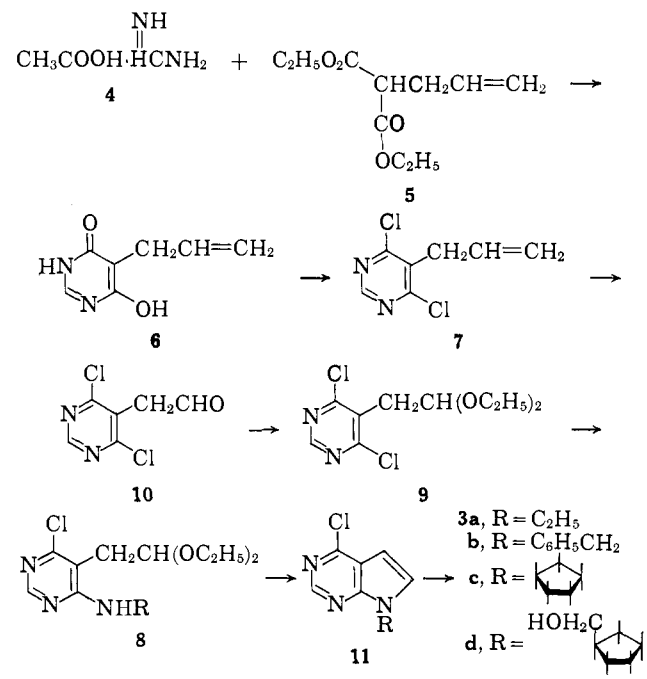


3

pyrrolo[2,3-*d*]pyrimidines (**3**).[14] The acetal function of 5-(2,2-diethoxyethyl)-4,6-dihydroxypyrimidine, which could be prepared by a modification of Davoll's ring-closure procedure, is not compatible with the chlorodehydroxylation procedures that would have to be used to convert it to 4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine, which could be used to prepare the requisite 4-alkylaminopyrimidine intermediates. It was, therefore, necessary to devise a reaction sequence in which the aldehyde function could be introduced after the chlorodehydroxylation step.

5-Allyl-4,6-dihydroxypyrimidine (**6**), prepared by the reaction of formamidine acetate (**4**) with diethyl allylmalonate (**5**), was converted to 5-allyl-4,6-dichloropyrimidine (**7**) by treatment with $POCl_3$ and diethylaniline (Scheme I). The 5-allyl-4,6-dichloropyrimidine (**7**) was ozonized at −75° and the resultant ozonide was reduced with sodium thiosulfate to the desired 4,6-dichloropyrimidine-5-acetaldehyde (**10**). Since reaction of the acetaldehyde (**10**) with benzylamine

SCHEME I



(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

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(14) Recently the methylation of 4-chloro-7H-pyrrolo[2,3-*d*]pyrimidine was described.[15]

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gave a complex reaction mixture from which no pure product could be isolated, **10** was converted to its acetal, 4,6-dichloro-5-(2,2-diethoxyethyl)pyrimidine (**9**), which could be purified by careful vacuum distillation. The acetal **9** reacted smoothly with amines in ethanol solution to give the desired 4-alkylamino-6-chloro-5-(2,2-diethoxyethyl)pyrimidines (**8**), which cyclized to the 4-chloro-7-alkyl-7H-pyrrolo[2,3-*d*]pyrimidines (**11**) on treatment with dilute HCl in dioxane solution. Amination of the chloropyrrolo[2,3-*d*]pyrimidines **11**, which as expected was more difficult than the amination of 9-alkyl-6-chloropurines, gave the tubercidin analogs **3**.

Biological Data.—The cytotoxicity of the tubercidin analogs (**3**) is given in Table I. In the same system tubercidin itself showed an ED_{50} of 0.002 μmole/l. None of these compounds showed any significant activity against leukemia L1210.

TABLE I

Compd **3**	ED_{50}, μM[a]
a	124
b	45
c	25
d	152

[a] The ED_{50} values are the levels of inhibitor required to inhibit the growth of cells to 50% of untreated controls as determined by colony formation counts.

Experimental Section

The ultraviolet spectra were determined with a Cary Model 14 spectrophotometer in aqueous solution. The infrared spectra were determined in KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The pmr spectra were determined in $CDCl_3$ solution with a Varian A-60A spectrometer using Me_4Si as an internal reference. The melting points reported were determined with a Mel-Temp melting point apparatus.

5-Allyl-4,6-dihydroxypyrimidine (6).—Formamidine acetate (10.4 g, 0.1 mole) was added to a cold suspension of $NaOCH_3$ (16.2 g, 0.3 mole) in absolute ethanol (100 ml) and the mixture was stirred at 5° for 10 min before diethyl allylmalonate (18 ml, 0.1 mole) was added. The resulting reaction mixture was stirred at room temperature for 18 hr. The addition of concentrated HCl (25 ml, 0.3 mole) to the cold reaction solution precipitated the crude product, which was collected by filtration and washed with ethanol and water before it was air-dried and recrystallized from water (600–800 ml of boiling water/10 g) to give the pure product in two crops: yield, 7.5 g (54%); mp 258–260°; λ_{max} in mμ (ε × 10^{-3}): pH 1, 259 (13.5); pH 7, 257 (11.0); pH 13, 257 (8.3); σ, in cm^{-1}, 3430 (NH, OH), 2910, 2850, 2770 (CH), 2620 (acidic H), 1650, 1630, 1580 (C=O, C=C, C=N).

Anal. Calcd for $C_7H_8N_2O_2$: C, 55.24; H, 5.30; N, 18.41. Found: C, 55.02; H, 5.32; N, 18.41.

5-Allyl-4,6-dichloropyrimidine (7).—An anhydrous mixture of 4,6-dihydroxy-5-allylpyrimidine (8.8 g, 58 mmoles) and freshly distilled $POCl_3$ (50 ml) was heated to reflux with continuous stirring. After complete solution was effected, a solution of diethylaniline (12.6 ml) in $POCl_3$ (19 ml) was added dropwise to the gently refluxing solution over a period of 1 hr. The reaction solution was refluxed a total of 4 hr before it was concentrated from 50 to 30 ml by distillation *in vacuo* [bp 32° (15–10 mm)]. The concentrate was poured with continuous stirring into ice–water (500 ml) and the resulting mixture was extracted with ether (600 ml in three portions). The combined ether extracts was washed three times with cold water and then successively (cold $NaHCO_3$ solution, H_2O, saturated NaCl) before it was dried ($MgSO_4$). The ether solution was evaporated to dryness *in vacuo* and the residue was distilled *in vacuo* [bp 70–78° (0.8 mm)]. The distillate was redistilled to give the pure product as a colorless oil; yield, 9.8 g (90%); bp 74–76° (0.8 mm); λ_{max} in mμ (ε × 10^{-3}): pH 1, 259 (5.4); pH 7, 13, 258 (5.5); σ, in cm^{-1}, 3080, 3010, 2980, 2925 (CH), 1640, 1590 (C=C).

4-Alkylamino-6-chloro-5-(2,2-diethoxyethyl)pyrimidines (8).—A solution of 4,6-dichloro-5-diethoxyethylpyrimidine (20 mmoles) in absolute ethanol (25 ml) containing 2 molar equiv of the appropriate amine[16] was heated at 80° for 2 to 4 hr. The reaction solution was evaporated to dryness *in vacuo* and the residue was triturated with benzene. The amine hydrochloride that precipitated was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. Trituration of the benzene residue with ligroin dissolved the product which was freed from the amorphous insolubles by filtration through dry Celite. Evaporation of the filtrate to dryness gave the 4-alkylaminopyrimidine as an oil in a 77–98% yield. Since thin layer chromatography indicated only trace contaminants in all cases, the product was used in the next step without further purification. The benzyl and 3-hydroxymethylcyclopentyl compounds were also characterized by their ultraviolet and infrared spectra.

4,6-Dichloro-5-(2,2-diethoxyethyl)pyrimidine (9).—A mixture of 4,6-dichloropyrimidinyl-5-acetaldehyde (19 g, 0.1 mole) and NH_4Cl (540 mg, 0.01 mole) in absolute ethanol (150 ml) was refluxed for 3.5 hr. After treatment with Norit, the reaction solution was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and the solution was extracted with water, dried ($MgSO_4$), and evaporated to dryness. The resulting crude product was distilled *in vacuo*, using a short-path distillation apparatus, to give essentially pure product: yield, 23.7 g (90%); bp 110–120° (0.8 mm). Thin layer chromatography using benzene–ethyl acetate (9:1) as the eluent showed a trace of starting aldehyde as the only contaminant. Although redistillation resulted in the formation of additional aldehyde, a sample of the analytically pure material was obtained; bp 120° (0.8 mm); λ_{max} in mμ (ε × 10^{-3}): pH 1, 7, 259 (5.2); pH 13, 259 (5.1); σ, in cm^{-1}, 2980, 2930, 2900–2880 (CH), 1545, 1515 (C=C, C=N), 1110, 1060 (COC).

Anal. Calcd for $C_{10}H_{14}Cl_2N_2O_2$: C, 45.30; H, 5.33; N, 10.57. Found: C, 45.50; H, 5.48; N, 10.52.

4,6-Dichloropyrimidine-5-acetaldehyde (10).—A solution of freshly distilled 4,6-dichloro-5-allylpyrimidine (53 g, 0.28 mole) in technical methanol (410 ml methanol plus 1 ml of water) was stirred at −78° until crystallization of the pyrimidine was complete (30–40 min). The resulting mixture was ozonized at −75° using a Welsbach ozonizer set at 60 v and 7.5 psi to deliver 4–5% ozone at a rate of 1.5 l./min. After the theoretical amount of ozone had passed through the reaction mixture, the system was flushed with dry nitrogen for 20 min. NaI (168 g) and glacial acetic acid (168 ml) were added simultaneously to the cold reaction mixture and the temperature was allowed to warm up to 20° with continuous stirring over a 20–30-min period. Sodium thiosulfate solution (67 g/100 ml of H_2O) (175–200 ml) was added to the reaction mixture until it became colorless. The resulting emulsion was diluted with water (660 ml) and extracted with ether (400 ml). The cold aqueous layer was extracted with fresh ether (three 250-ml portions) before the pooled ether extracts were washed successively [cold H_2O (three 300-ml portions), excess $NaHCO_3$, H_2O, saturated NaCl]. After the ether solution had been dried ($MgSO_4$), it was evaporated to dryness *in vacuo*. The semisolid residue crystallized after trituration with ligroin and the resulting chromatographically homogeneous product was collected by filtration, washed, and dried *in vacuo* over P_2O_5 and NaOH: yield, 32 g (59%); mp 82–84°. An analytically pure sample was obtained by recrystallization of a sample of this product (1 g) from ligroin (50 ml); mp 84–86°. Thin layer chromatography using benzene–ethyl acetate (9:1) as the eluent showed a single spot: λ_{max} in mμ (ε × 10^{-3}): pH 1, 7, 258 (5.2); pH 13, 330 (10.0); σ, in cm^{-1}, 3070, 2955, 2925, 2850 (CH), 1715 (C=O), 1550, 1520 (C=C, C=N).

Anal. Calcd for $C_6H_4Cl_2N_2O$: C, 37.73; H, 2.11; N, 14.67. Found: C, 37.90; H, 2.27; N, 14.64.

7-Alkyl-4-chloro-7H-pyrrolo[2,3-*d*]pyrimidines (11).—To a solution of the 4-alkylamino-6-chloro-5-diethoxyethylpyrimidine (20 mmoles) in dioxane (120 ml, spectrograde) was added 30 ml of 1 *N* HCl. The reaction solution was allowed to stand at room temperature for 24 hr before it was neutralized with an excess of concentrated NH_4OH (5 ml) and evaporated to dryness *in vacuo*. The residue was dissolved in ethanol and evaporated to dryness *in vacuo* before it was partitioned between benzene ($CHCl_3$ for **11d**) and water. After drying ($MgSO_4$), the benzene

[16] In order to conserve the amine, 1 molar equiv of 3-hydroxymethylcyclopentylamine with 1 molar equiv of triethylamine.

TABLE II

DATA ON COMPOUNDS 3

Compd 3	Yield,[a] %	Recrystn solvent	Mp, °C	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
a	37[b]	Benzene	137–138	59.23	59.35	6.22	6.42	34.57	34.49
b	50	Ethanol	177–179	69.61	69.56	5.40	5.59	24.98	25.25
c	58	Acetone	169	65.31	65.23	6.98	6.79	27.70	27.79
d	31	Acetone	160–162	62.04	61.87	6.95	7.01	24.12	23.92

[a] Analytically pure material. [b] In addition a 16% yield of the hydrochloride salt of **3a** was obtained.

TABLE III

ULTRAVIOLET AND INFRARED SPECTRA OF TUBERCIDIN ANALOGS

Compd 3	λ_{max}, mμ (ε × 10^{-3}) 0.1 N HCl	λ_{max}, mμ (ε × 10^{-3}) pH 7	σ, cm^{-1} NH stretch	σ, cm^{-1} CH stretch	σ, cm^{-1} NH bend	σ, cm^{-1} C=C, C=N stretch	σ, cm^{-1} CH bend
a	230 (24.4)	224 sh	3400, 3320,	2980, 2930,	1645	1590, 1550,	1470, 1450, 940 d,
	275.5 (9.4)	273 (9.1)	3140	2860		1505	900, 790
b	230 (27.4)		3410, 3300,	3020, 2920,	1645	1590, 1550,	1475, 945, 900,
	274 (10.4)	272 (10.6)	3080	2850		1510	795
c	231 (27.0)	228 (17.6)	3340, 3160	2950, 2865	1650	1590, 1550,	1470, 940, 895,
	276 (9.4)	275 (9.3)				1505	790
d	231 (27.0)	225 sh	3380,[a] 3320,[a]	2950, 2870	1650	1590, 1550,	1475, 1010,[b] 950,
	275 (9.4)	273 (9.4)	3140[a]			1505	900, 795

[a] NH and OH stretch. [b] OH bend.

ayer was evaporated to dryness *in vacuo* and the residue was dissolved in ligroin (ethanol for **11d**). The amorphous insolubles were removed by filtration through dry Celite and the filtrate was evaporated to dryness *in vacuo* to give the pyrrolopyrimidine as an oil in 60–100% yield. Thin layer chromatography indicated the presence of only trace impurities. The pmr spectrum confirmed the pyrrolo[2,3-*d*]pyrimidine structure and, therefore, the product was used as an intermediate without further purification. The ultraviolet, infrared, and pmr spectra of **11a**, **c**, and **d** were all very similar, with the expected differences, to the spectra of **11b** (*vide infra*).

On concentration of the ligroin solution, the 4-chloro-7-benzyl-7H-pyrrolo[2,3-*d*]pyrimidine (**11b**) crystallized. The crystals were collected by filtration, washed with ligroin, and dried *in vacuo* to give the pure product in 55% yield; mp 66–67°; λ_{max} in mμ (ε × 10^{-3}): pH 1, 226 (26.6), 273 broad (4.3); ethanol, 224 (28.2), 272 (4.8); pH 7, 13, 226 (27.2), 273 broad (4.4); σ, in cm^{-1}, 3100–2900 (CH), 1575, 1540, and 1500 (C=C, C=N), 915 d, 845, and 710 d (ring CH deformation); δ, in ppm, 8.6 (H_2), 7.2 d (H_5), 6.6 d (H_6), 5.4 (benzyl CH_2), 7.2 m (phenyl H). H_5 and H_6 appear as an AB pair $\nu_0(\delta_5 - \delta_6)$ = 36 cps and $J_{5,6}$ = 3.7 cps.

Anal. Calcd for $C_{13}H_{10}ClN_3$: C, 64.07; H, 4.14; N, 17.24. Found: C, 64.01; H, 4.16; N, 17.21.

7-Alkyl-4-amino-7H-pyrrolo[2,3-*d*]pyrimidines (3).—A solution of the 4-chloro-7-alkyl-7H-pyrrolo[2,3-*d*]pyrimidine in ethanol saturated at 5° with NH_3 (100 ml/20 mmoles) was sealed in a glass-lined Parr bomb and heated at 100–110° for 18 hr. The reaction solution was evaporated to dryness and the oily residue was triturated with ligroin to remove impurities. The insoluble solid was washed with water and recrystallized from the appropriate solvent to give the pure aminopyrrolopyrimidine (Table II). Compounds **3b** and **3c** were washed with water and recrystallized (Table II) to give the pure compounds; **3a** was dissolved in $CHCl_3$, and the $CHCl_3$ solution was extracted with water before evaporation to dryness. The residue was recrystallized to give pure product. Compound **3d** was dissolved in acetone, and the acetone solution was filtered before evaporation to dryness. The residue was recrystallized to give pure product. Ultraviolet and infrared data for **3a–d** are given in Table III.

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