precipitation was complete. The slurry was filtered, and the filter cake was washed with cold benzene. The crude product weighed 23.6 Gm.

The product was recrystallized once from an ethanol–ethyl acetate mixture, then twice from tetrahydrofuran. The pure material consisted of shiny, golden flakes melting at 207–209°. The yield was 12.8 Gm.

Anal.—Calcd. for $C_{13}H_{20}ClN_3O_2$: C, 54.63; H, 7.05; N, 14.71. Found: C, 54.93; H, 7.23; N, 14.41.

N - (2 - Dialkylaminoethyl) - 2 - phenylacetanilides.—From 0.020–0.030 mole of substituted ethylenediamine hydrochloride was suspended in 100–150 ml. of benzene. The appropriate acid chloride was added in 10–25% molar excess. The suspension was stirred and heated under reflux 24–72 hr. The mixture was cooled and filtered. The crude product was recrystallized from the appropriate solvent (Table I).

In a few instances the product was soluble in the benzene even after cooling. In these cases, an equal volume of petroleum ether was added to force the salt out of solution.

If the crude hydrochloride salt was liquid and could not be induced to crystallize, it was dissolved in dilute hydrochloric acid and neutralized with 10% sodium hydroxide solution. The free base was extracted into ether. The ethereal solution was dried, and the ether was removed *in vacuo*. The residual base was converted to the perchlorate salt by the method of Caudle *et al.* (5).

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Synthesis of 4-Substituted-7-methylpyrrolo[2,3-*d*]pyrimidines

By RICHARD H. HAMMER

The reactions of 4-chloro-7-methyl-7H-pyrrolo[2,3-*d*]pyrimidine (IV) with ethanolic ammonia, thiourea, and aqueous sodium sulfhydrate solution to give the 4-amino (V) and 4-thione (VI) analogs are described. Ultraviolet data and pKa values for IV, V, and VI are reported.

TUBERCIDIN (I*a*), a naturally occurring nucleoside in streptomyces species (1, 2), has been assigned the structure 4-amino-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-*d*]pyrimidine (7-deazaadenosine) (3–5). It is an inhibitor of several tumor systems, not cross-resistant to 6-mercaptopurine-resistant line tumor systems (6), and incorporated into both DNA and RNA of mouse fibroblasts and several viruses (7, 8). During structural elucidation studies of tubercidin (I*a*), hydrolysis of I*a* to the aglycone (I*b*) and D-ribose was accomplished by refluxing I*a* in 1–3 *N* HCl for 5 hr. (4). From these data the base ribose bond of I*a* appears to be more resistant to acid hydrolysis than a purine base-ribose bond. Subsequently, resistance of I*a* to enzymatic cleavage by *E. coli* nucleoside phosphorylase was demonstrated while 6-mercaptopurine riboside was observed to be rapidly cleaved (9). Significance of the stability of the pyrrolo[2,3-*d*]pyrimidine base-ribose bond in relation to drug distribution and cancer chemotherapy remains to be explained.

Recent studies by Montgomery and Hewson (10, 11) on the cell culture cytotoxicity of 6-mercaptopurine and 6-mercaptopurine-deaza analogs suggests that deaza structures such as 6-mercapto-7-deazapurine (11–13) are not metabolized by the cells to the ribotide form and consequently are 300–500 times less active than 6-mercaptopurine which is readily converted to the ribotide. This raises the question as to whether antitumor activity for pyrrolo[2,3-*d*]-pyrimidine structures may be dependent on a substituent such as a sugar group, cycloalkyl or alkyl group on the 7-nitrogen (corresponding to the 9-position of purine). This is evident by the fact that tubercidin (I*a*) with a β-D-

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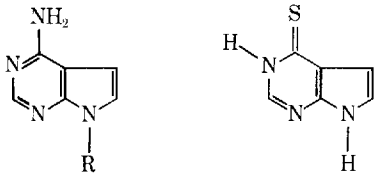
ribose moiety on the 7-position is active while II, which is unsubstituted, is inactive. Pyrrolo [2,3-*d*]pyrimidine structures with a methyl group on the 7-nitrogen and amino or mercapto groups at the 4-position (corresponding to the 6-position of purine) are therefore of biological interest and prompted the described synthesis of 4-amino-7-methyl-7H-pyrrolo[2,3-*d*]pyrimidine (V) and 7 - methyl - 7H - pyrrolo[2,3 - *d*]pyrimidine - 4-(3H)-thione (VI) from 4-chloro-7H-pyrrolo[2,3-*d*] pyrimidine (III).



Ia, R = β-D-Ribose
Ib, R = H

II

EXPERIMENTAL

Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared and ultraviolet analyses were recorded on Beckman IR-5 and model DB spectrophotometers. Titrimetric pKa values (14) were obtained on a Sargent titrator, model D, and spectrophotometric pKa values (15) were obtained using buffered solutions (16) verified with standard buffer solutions on a Beckman expanded scale pH meter, model 76. Microanalyses were conducted by Galbraith Laboratories, Inc., Knoxville, Tenn.

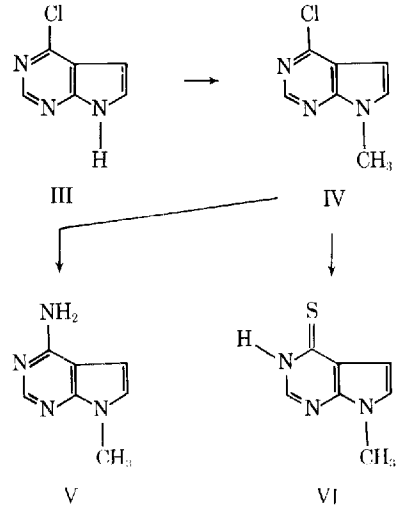
4-Chloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (IV) (17).—To a solution of 1.50 Gm. of crude 4-chloro-7H-pyrrolo[2,3-*d*]pyrimidine (III) (12) in 10.0 ml. of dimethylformamide (dried over petroleum ether extracted sodium hydride) cooled to 5° was added 0.492 Gm. of sodium hydride (represents 0.492 Gm. sodium hydride, 50% dispersed in oil, prior to extraction with petroleum ether). The mixture was kept at room temperature until all the hydrogen gas was liberated (4.5 hr.). The reaction mixture was cooled to 5° and 0.60 Gm. of methyl iodide was added. An immediate tan precipitate was observed with agitation in the ice-bath, and after standing for 12 hr., 10.0 ml. of water was added and cooled for an additional 6 hr. Filtration yielded 1.16 Gm. of crude tan product, m.p. 120–122°. The filtrate gave 0.160 Gm. of additional product, m.p. 118–120°. The combined crops were recrystallized from methanol to give light tan crystals (IV). Yield, 1.009 Gm. (62%), m.p. 126–127°. [Reported m.p. 130° (17).] $\nu_{max.}$, cm.$^{-1}$, 3050, 2880 (CH); 1615, 1580, 1530, 1510 (C=C, C=N).

Anal.—Calcd. for $C_7H_6ClN_3$: C, 50.18; H, 3.58; N, 25.08. Found: C, 49.90; H, 3.70; N, 24.84.

4-Amino-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (V).—To 0.20 Gm. of IV in a Parr stainless steel bomb was added 10.0 ml. of ethanolic ammonia solution (prepared by saturating absolute ethanol at 5° with ammonia). The bomb was sealed and maintained at 125–130° with stirring on a combination hot plate-magnetic stirrer. The reported reaction temperature was obtained by simultaneously heating a beaker of mineral oil adjacent to the Parr bomb on the same hot plate. After 10 hr., the mixture was cooled and filtered 5 times to remove decomposition material. The clear brown filtrate was evaporated to dryness yielding a brown crystalline residue which was recrystallized 2 times from hot water to give tan crystals (V). Yield, 0.044 Gm. (25%), m.p. 207–208° dec. $\nu_{max.}$, cm.$^{-1}$, 3280, 3050, 2890 (NH, CH); 1640, 1580, 1550 (NH, C=C, C=N).

Anal.—Calcd. for $C_7H_8N_4$: C, 56.76; H, 5.40; N, 37.82. Found: C, 56.75; H, 5.60; N, 37.61.

7-Methyl-7H-pyrrolo[2,3-d]pyrimidine-4(3H)-thione (VI).—*Method A.*—To a solution of 0.30



Scheme I

TABLE I.—ULTRAVIOLET ANALYSIS

	$\lambda_{max.}$ mμ (ε)			
	pH 1.0[a]	pH 7.0	pH 13.0	pKa
III		222(20,200)[b]		
		271(4,950)		
IV	229(23,050)	225(25,580)	225(24,310)	2.06[c]
	272(4,550)	270(3,980)	271(3,700)	
V[e]	229(21,710)		224(12,930)	5.25[c]
	274(9,270)	271(9,130)	270(8,810)	5.02[d]
VI	266(6,530)	266(6,530)	221(18,150)	9.35[d] (SH)
	318(19,180)	318(19,450)	306(16,450)	

[a] pH 1.0 (0.1 *N* HCl); pH 7.0 (phosphate buffer) (16); pH 13.0 (0.1 *N* NaOH). [b] Ethanol, absolute (13). [c] Spectrophotometric method (15). [d] Titrimetric method (14). [e] Reported (4): $\lambda_{max.}^{0.01\ N\ HCl}$ 230 (—), 275(10,300); $\lambda_{max.}^{0.01\ N\ NaOH}$ 273(9,400).

Gm. of IV in 8.0 ml. of absolute ethanol was added 0.272 Gm. of thiourea. After 5 min. of refluxing the $\lambda_{max.}$ had shifted from 270 to 325 mμ. Refluxing was continued for an additional 3 hr. at which time the $\lambda_{max.}$ was still 325 mμ. Upon evaporation, the crude residue was recrystallized from methanol (48 hr. at room temperature) to give pale yellow needles (VI). Yield, 0.154 Gm. (52%), m.p. 305–307° dec. $\nu_{max.}$, cm.$^{-1}$, 3125, 3010, 2860 (CH); 1575, 1530 (C=C, C=N).

Anal.—Calcd. for $C_7H_7N_3S$: C, 50.90; H, 4.24; N, 25.44. Found: C, 51.08; H, 4.47; N, 25.20.

Method B.—To a solution of 0.20 Gm. of IV in 10.0 ml. of absolute ethanol was added 30.0 ml. of an aqueous sodium sulfhydrate solution (2.0 *N*). The solution was refluxed for 5 hr. at which time the $\lambda_{max.}$ had shifted from 271 to 319 mμ. The yellow solution was cooled and neutralized to a pH of 7 with 10% acetic acid. After cooling overnight the reaction mixture was filtered to give 0.161 Gm. of a pale yellow residue, m.p. 300–302°. Recrystallization from methanol (48 hr. at room temperature) gave pale yellow needles (VI). Yield, 0.097 Gm. (33%), m.p. 307–308° dec. $\nu_{max.}$, cm.$^{-1}$, 3130, 3000 (CH); 1575, 1545, 1530 (C=C, C=N). (Scheme I.)

Anal.—Calcd. for $C_7H_7N_3S$: C, 50.90; H, 4.24; N, 25.44. Found: C, 50.62; H, 4.27; N, 25.21.

RESULTS AND DISCUSSION

The intermediate compound 4-chloro-7H-pyrrolo-[2,3-*d*]pyrimidine (III) was prepared by a 5-step reaction sequence from starting materials of ethylcyanoacetate and bromoacetal (12). Reaction of III with sodium hydride to form the nucleophile and subsequent reaction with methyl iodide gave the 4-chloro-7-methyl-7H-pyrrolo[2,3-*d*]pyrimidine (IV) compound (17). Nucleophilic displacement of the 4-chloro group of IV by reaction with either thiourea (method *A*) or sodium sulfhydrate (method *B*) gave 7-methyl-7H-pyrrolo[2,3-*d*]pyrimidine-4(3H)-thione (VI). Both reactions proceed smoothly at reflux temperatures. Method *A* gave a yield of 52% of VI compared to 33% by method *B*. Infrared and ultraviolet curves of VI from methods *A* or *B* were superimposable and identical in every respect. Optimum conditions for synthesis of the 4-amino congener (V) required heating IV in ethanolic ammonia for 10 hr. at 125–130° in a stainless steel bomb. The reaction was also run at temperatures of 105–110° and 155–160°. The higher temperature produced more extensive decomposition and the lower temperature gave a lower yield than the 125–130° range. Synthesis of the 4-mercapto analogs of pyrrolo[2,3-*d*] pyrimidines can readily be followed by a bathochromic shift in $\lambda_{max.}$ from 265–275 to 315–325 mμ as previously demonstrated during the synthesis of II (13) and as described here (Table I).

Facile substitution of the 6-chloro group of 6-chloropurine and 6-chloro-9-substituted purines by nucleophilic groups has provided useful synthetic pathways leading to a variety of purine nucleosides and purine analogs. Similarly, 4-chloropyrrolo-[2,3-*d*]pyrimidine moieties, without a 7-nitrogen substituent, are useful intermediates which can be reacted with ammonia (18), alkyl amines and thiourea (12), phosphorus pentasulfide (9), sodium sulfhydrate and sodium methoxide (13), and alkyl mercaptans (19) to give the corresponding 4-substituted derivatives. Even though nucleophilic groups can be substituted in place of the 4-chloro group, on the basis of the conditions required for the amination reactions of III, IV, and 6-chloro-purine compounds, the 4-chloro group appears to be more difficult to displace than the corresponding 6-chloro of purine compounds. Lewis *et al.* (20) reported the synthesis of 6-amino-9-(tetrahydro-2-furyl)purine by treating the 6-chloro precursor with methanolic ammonia at room temperature for 48 hr. However, Hitchings *et al.* (18) reported that to replace the 4-chloro group of III with an amino group it was necessary to heat III in a stainless steel bomb at 155–160° for 20 hr., and as reported here, conversion of IV to V required heating IV in ethanolic ammonia for 10 hr. at 125–130°. For the amination reactions, the reaction conditions indicate that the 4-chloro group of pyrrolo[2,3-*d*]pyrimidines is more difficult to displace than the corresponding 6-chloro group of purines.

The pKa values reported in Table I were obtained by titrimetric (14) or spectrophotometric (15) methods. Due to its insolubility in aqueous perchloric acid, the pKa value for IV was obtained spectrophotometrically. Replacement of the 4-chloro group of IV with an amino group (V) gave an expected shift to a higher pKa which would normally occur when an electron-withdrawing group (Cl) is replaced with an electron-donating group (NH_2). Tubercidin (I*a*) has been reported to have a pKa of 5.3 (4). The pKa of the 4-amino-7-methyl analog (V) was found to be 5.02. Providing V has a favorable partition coefficient, it would be expected to produce high intracellular concentrations. The ratio of unionized to ionized species of V at a pH of 7.4 (plasma) would be 240/1 (pKa 5.02) which should enhance its absorption across cellular membranes. The pKa of the SH group of VI was found to be 9.35. The pKa value was not obtained for the pyrrolo nitrogen of VI.

Screening of these compounds for antitumor properties is being conducted by the Cancer Chemotherapy National Service Center and will be reported at a later date.

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