Synthesis of 4-Substituted Pyrrolo[2,3-*d*] Pyrimidines

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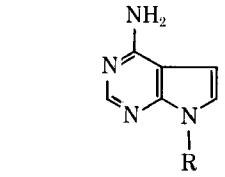
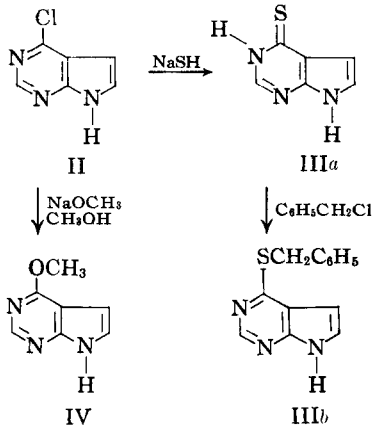
Synthesis and ultraviolet analysis of 7H-pyrrolo[2,3 - *d*]pyrimidine - 4(3H) - thione (III*a*), 4-benzylthio-(III*b*), and 4-methoxy-7H-pyrrolo[2,3-*d*]pyrimidines (IV) are described.

ISOLATION OF tubercidin (I*a*), a new antibiotic nucleoside, by Anzai *et al.* from a species of streptomyces has recently been reported (1). The nucleoside has also been found to be co-produced with the new antibiotic sparsomycin in a fermentation broth of *Streptomyces sparsogenes* (2). Tubercidin inhibits the growth of Sarcoma 180, Ehrlich ascites tumor, and Jensen Sarcoma *in vivo*, and is not cross-resistant with 6-mercaptopurine-resistant line tumor systems (3).

Degradation and configuration studies have led to the assignment of 4-amino-7-β-D-ribofuranosyl-7H-pyrrolo[2, 3-*d*]pyrimidine (I*a*) for the nucleoside (4–6). Synthesis of tubercidin has not been accomplished,[1] although the aglycone 4-amino-7H-pyrrolo[2, 3-*d*]pyrimidine (I*b*) (7-deazaadenine) has been obtained independently by Davoll (7) and Taylor and Hendess (8) by different methods. Owing to the structural similarity of tubercidin and its aglycone to adenosine and adenine, and its demonstrated antitumor activity and lack of cross-resistance to 6-mercaptopurine, structures possessing a pyrrolo[2, 3-*d*]pyrimidine ring system are of interest. Purine compounds substituted in the 6-position, corresponding to the 4-position of pyrrolo[2, 3-*d*]pyrimidine, have shown antitumor activity when the substituent has been either a methoxy or benzylthio group (9, 10). This paper therefore describes the synthesis of 4-(benzylthio)-7H-pyrrolo[2, 3-*d*]pyrimidine (III*b*) and 4-methoxy-7H-pyrrolo[2, 3-*d*]pyrimidine (IV) from the intermediate, 4-chloro-7H-pyrrolo[2, 3-*d*]-pyrimidine (II).



I*a*, R = β-D-Ribose
I*b*, R = H



Scheme I

EXPERIMENTAL[2]

7H - Pyrrolo[2,3 - d]pyrimidine - 4(3H) - thione (IIIa).—The following procedure is similar to the method employed to prepare 6-mercaptopurine analogs (11). To 0.31 Gm. of crude 4-chloro-7H-pyrrolo[2, 3-*d*]pyrimidine (II) (7) was added 50 ml. of an aqueous 2 *N* sodium hydrosulfide solution and 10.0 ml. of ethanol. The solution was refluxed for 1.5 hr., at which time the $\lambda_{max.}$ of the reaction mixture had shifted from 273 to 323 mμ. The reaction mixture was cooled in an ice bath and neutralized to pH 7 with dilute acetic acid. The precipitate was filtered and recrystallized from water to give tan crystals (III*a*). Yield, 0.18 Gm. (60%) m.p. 308° dec.

Anal.—Calcd. for $C_6H_5N_3S$: C, 47.63; H, 3.33; N, 27.78. Found: C, 47.58; H, 3.41; N, 27.67.

4 - (Benzylthiol) - 7H - pyrrolo[2, 3 - d]pyrimidine (IIIb).—To a solution containing 0.40 Gm. of 7H-pyrrolo[2, 3-*d*]pyrimidine-4(3H)-thione (III*a*), 5 ml. of ethanol, and 30 ml. of aqueous 2 *N* sodium hydroxide solution was added dropwise 0.34 ml. (0.38 Gm.) of benzyl chloride over a 5-min. period. A precipitate began to settle out after 30 min.; and after 3 hr. of stirring the mixture was neutralized with dilute acetic acid. The crude residue was recrystallized from absolute ethanol to give gray crystals (III*b*). Yield, 0.198 Gm. (31%), m.p. 168–170°.

Anal.—Calcd. for $C_{13}H_{11}N_3S$: C, 64.70; H, 4.59; N, 17.45. Found: C, 64.58; H, 4.71; N, 17.42.

4 - Methoxy - 7H - pyrrolo[2, 3 - d]pyrimidine (IV).—To a solution of 0.5 Gm. of crude 4-chloro-7H-pyrrolo[2, 3-*d*]pyrimidine (II) (7) in 20 ml. of anhydrous methanol was added 5.0 ml. (2.2 *N*) of methanolic sodium methoxide solution. The solution was refluxed for 2 hr. and an additional 5.0 ml. of methanolic sodium methoxide solution was added, and refluxing was continued for a total of 8 hr., at which time the $\lambda_{max.}$ had shifted from 274 to 268 mμ. The solution was neutralized to pH 7 with dilute acetic acid and evaporated to a small volume yielding a tan colored residue upon filtration. Crude yield 0.354 Gm. (72%), m.p. 195–197° dec. Recrystallized from methanol; yield, 0.145 Gm. (30%) white crystals, m.p. 208–210° dec.

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[1] Synthesis and kinetic studies of tubercidin are under investigation in this laboratory.

[2] 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. Microanalyses were conducted by Schwarzkopf Microanalytical Laboratory, Wodside, N. Y. (See Table I for ultraviolet analyses.)

TABLE I.—ULTRAVIOLET ANALYSIS

	λmax. mμ(ε)		
	Ethanol	0.1 *M* HCl	0.1 *M* NaOH
II[a]	222(20,200)	[b]	[b]
	271(4,950)		
III*a*[c]	266(3,000)	265(5,300)	227(13,400)
	324(16,400)	319(12,600)	307(13,500)
III*b*	246(6,200)	[b]	[b]
	291(14,000)		
IV	263(8,500)	266(5,800)	264(7,600)

[a] Reported (7): $\lambda_{max.}^{pH\ 6.8}$ 223(26,000), 275(4,500). [b] Insoluble. [c] Reported (7): $\lambda_{max}^{pH\ 6.8}$ 267(5,500), 322(20,200); $\lambda_{max.}^{HCl}$ 267(5,600); 323(19,600); $\lambda_{max.}^{NaOH}$ 228(17,400), 310 (17,800).

Anal.—Calcd. for $C_7H_7N_3O$: C, 56.38; H, 4.73; N, 28.17. Found: C, 56.46; H, 4.64; N, 28.18.

RESULTS AND DISCUSSION

The intermediate, 4-chloro-7H-pyrrolo[2, 3-*d*]-pyrimidine (II), was prepared by a five-step reaction sequence starting with bromoacetal and ethylcyanoacetate (7). Similar to nucleophilic substitution on 6-chloropurine, the 4-chloro group of II can be displaced by nucleophilic groups such as *n*-propyl amine as demonstrated earlier by Davoll (7) and by the methoxide and sulfhydryl ions as described here. Unsuccessful attempts were made to replace directly the 4-chloro with an amino group (I*b*) by heating II in methanolic ammonia in a sealed tube at 110 and 120°.

Synthesis of III*a* has been accomplished by Davoll (7) by refluxing II with thiourea and by Mizuno *et al.* (12) by treating 4-hydroxy-7H-pyrrolo[2, 3-*d*]-pyrimidine with phosphorus pentasulfide. Synthesis of III*a* can also be accomplished through nucleophilic displacement of the 4-chloro group of II by the sulfhydryl group (Scheme I) and can be followed by measuring the $\lambda_{max.}$ of the reaction mixture at different time intervals. Reaction of III*a* with benzyl chloride in aqueous base gave III*b* in low yields. The $\lambda_{max.}$ of III*b* (290 mμ) is indicative of substitution on the 4-mercapto group (III*a*) as shown by the synthesis of the 4-(methylthio)-7H-pyrrolo[2, 3-*d*]pyrimidine (12) which has a $\lambda_{max.}$ at 294 mμ *versus* a $\lambda_{max.}$ of 324 mμ for III*a*.

Substitution of a methoxy group for the 4-chloro group of II proceeds at reflux temperatures and can be followed by a shift in $\lambda_{max.}$ of II from 274 to 268 mμ indicating substitution had taken place. This shift to a lower wavelength in the basic reaction media was anticipated as a comparison of the ultraviolet analysis of 6-methoxypurine and 6-chloropurine in base indicates a similar difference in $\lambda_{max.}$ (13).

Antitumor results of these compounds and further studies on tubercidin currently under investigation in this laboratory will be reported at a later date.

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