Synthesis of Antifols Related to 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo-[3,4-*d*]pyrimidine. Enhancement of Antiparasitic Selectivity by Nitrogen-Linked Mono- and Dichlorobenzoyl Groups or the 3,4-Dichlorophenylthiocarbamoyl Group (1)

Philip L. Southwick, Venkataraman Amarnath (2) and R. Madhav (3)

Department of Chemistry, Carnegie-Mellon University,
Pittsburgh, Pennsylvania 15213

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Improved procedures have been developed for the synthesis of 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2a**), its 7-methyl derivative (**2b**), and 6-(chloro-substituted phenyl) derivatives of 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**4**). Direct acylation of compounds **2a** or **2b** with acid chlorides or mixed anhydrides derived from chloro-substituted benzoic or cinnamic acids gave 6-(chloro-substituted benzoyl or cinnamoyl) derivatives. Lithium aluminum hydride reduction of 6-(chloro-substituted benzoyl) derivatives under controlled conditions permitted preparation of 6-(chloro-substituted benzyl) derivatives (3). Compound **2a** also reacted with aryl isothiocyanates to yield 6-arylthiocarbamoyl derivatives. Antimalarial assays for *in vivo* activity against murine malaria (*P. berghei*) and avian malaria (*P. gallinaceum*) revealed that a somewhat enhanced *in vivo* antiparasitic effect above that of parent compound **2a** without any evident increase in host toxicity was conferred by introduction of certain of the 6-chloro-substituted benzoyl groups or the 6-(3,4-dichlorophenylthiocarbamoyl) group. Corresponding 6-(chloro-substituted benzyl) derivatives more frequently displayed host toxicity.

Among synthetic fused-ring systems incorporating the pyrimidine structure the pyrrolo[3,4-*d*]pyrimidine system is of relatively recent advent (4). 2,4-Diamino derivatives in this system, like other compounds containing the 2,4-diaminopyrimidine structure, are of interest as potential folic acid antagonists (5). Inhibition of folic acid-dependent metabolic processes is believed to be the basis of the antimalarial activity of the 2,4-diaminopyrimidine drugs pyrimethamine and trimethoprim (5).

Although a limited number of 2,4-diamino derivatives of 6,7-dihydro-5*H*-pyrrolo[3,4-*d*]primidine had been prepared in this laboratory (6) and elsewhere (7) before the present work was initiated, no antimalarial screening results had been reported. Very recently, however, Elslager, Curry and Werbel (8) have described four additional derivatives of 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine and have reported that one of these compounds, the 6-(2,4-dichlorobenzyl)-7-methyl derivative (**3**, R = methyl, R′ = 2,4-dichlorophenyl) is active against *Plasmodium berghei* infections in mice, as demonstrated by the screening procedure of Osdene, Russell and Rane (9). We describe here the results of our independent search for antimalarial activity in this series. Among the compounds chosen for synthesis and screening were a series of 6-(chloro-substituted phenyl) derivatives and a series of 6-(chloro-substituted benzoyl and cinnamoyl) derivatives. The latter compounds were selected on the basis of their own potential for biological activity and also for the possible utility of some of them as intermediates for the synthesis of a parallel series of 6-(chloro-substituted benzyl) derivatives.

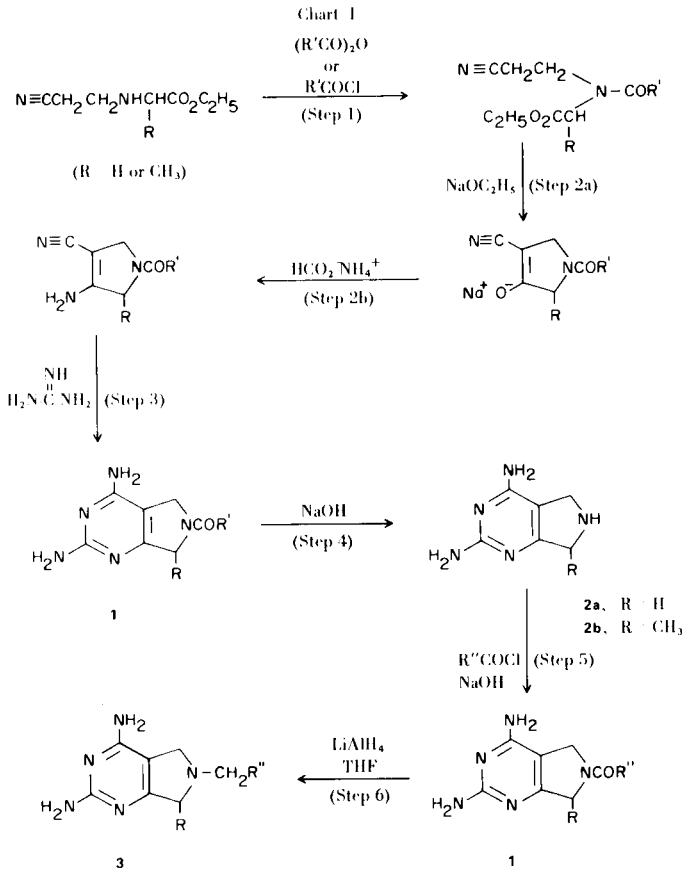
Chemistry.

The preparation of the 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidines **2a** and **2b** had been described by Sheradsky and Southwick (6a). The reaction sequence given in Chart I represents an improved version of the original synthesis, with the Dieckmann cyclization and enamine formation (steps 2a and 2b) carried out sequentially in the same reaction vessel to avoid losses attendant upon isolation of a cyano ketone intermediate. The last of the intermediates in this sequence were the 6-acyl derivatives **1**. It therefore appeared feasible to obtain derivatives with desired chlorobenzoyl and chlorocinnamoyl groups at the 6-position using the appropriate acid chlorides for acylation of the amino acid ester at step 1 of the sequence. However, preliminary experiments

aimed at the synthesis of a 6-*p*-chlorobenzoyl derivative indicated that chloro-substituted benzoyl groups might undergo removal under the basic conditions used in condensation with guanidine in step 3. For this reason, direct acylation of compounds **2a** and **2b** was examined in the hope that reaction would take place selectively at the 6-nitrogen. It was easily demonstrated that direct acylation of **2a** and **2b** (step 5) with benzoyl chloride led to the same products obtained by carrying through the sequence of three steps in Chart I beginning with benzoylation in step 1. Therefore the chloro- and dichlorobenzoyl and cinnamoyl derivatives were prepared by acylation carried out on **2a** or **2b**, using acid chlorides in pyridine or in the Schotten-Baumann procedure, or mixed anhydrides obtained from appropriate acids with triethylamine and ethyl chloroformate in chloroform. Infrared spectra of all of these acylation products resembled closely the spectra of the 6-benzoyl derivatives of established structure.

It was anticipated that 6-(chloro-substituted benzyl) derivatives of 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]-pyrimidine could be obtained by lithium aluminum hydride reduction of 6-(chloro-substituted benzoyl) derivatives. Such reductions (indicated by step 6 in Chart I) were carried out successfully for the preparation of the desired chlorobenzyl derivatives (**3**). Yields in the reduction step

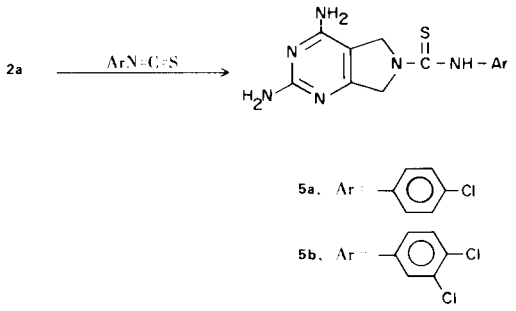
Chart I



were generally only fair, but except in some experiments in which an extended reaction period permitted significant cleavage of chlorine, the initial products were nearly free of by-products and were very easily purified.

The 6-(chloro-substituted phenyl) derivatives (**4**) were made by the scheme employed by Southwick, Madhav and Fitzgerald (6b) but with considerable changes in procedure. One of the compounds (**4a**) had been described in the earlier publication (6b). For the synthesis of **4b** and **4c** Dieckmann cyclization and enamine formation were carried out in sequence in a single operation, as in the improved synthesis represented in Chart I for compounds of type 1. The complete synthetic sequence was conducted as represented in Chart II.

The 6-arylthiocarbamoyl derivatives of type **5** were obtained by the reaction of intermediate **2a** with aryl isothiocyanates in pyridine solution, as indicated in the equation given below. The products were high-melting,



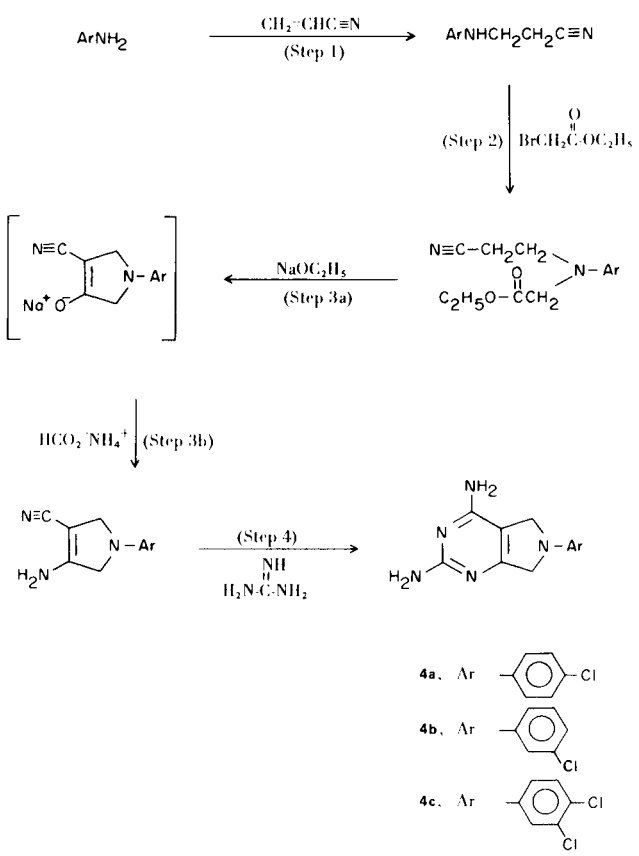
relatively-insoluble compounds closely resembling the 6-acyl derivatives of type **1** with respect to infrared spectra as well as other properties. The 7-methyl derivative **2b** failed to react in this manner with aryl isothiocyanates under similar conditions, presumably because of steric hindrance.

Biological Activity.

Somewhat surprisingly, the parent compound of the series, 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2a**), produced a statistically significant increase in the survival time of mice subjected to blood-induced infections with *Plasmodium berghei* (9). At the highest dose level examined (640 mg./kg.) the mean increase was 3.9 days. No toxicity was evident at 640 mg./kg. The compound failed to show either a reproducible suppression of the infection or a toxic effect in avian malaria assays (9,10). However, dose levels above 120 mg./kg. were not investigated in the avian assays. The 6-methyl derivative **2b** was inactive in all assays.

The utility of such drugs as pyrimethamine and trimethoprim demonstrates the possibility of selecting substituent groups so as to create dihydrofolate reductase inhibitors having effective toxicity for certain pathogenic organisms at concentrations several orders of magnitude less

Chart II



than those producing observable toxicity to the host animal. The results of attempts to enhance the selective toxicity of the parent structure **2a** by introduction of appropriate substituent groups are summarized in Table I, which records both avian and murine malaria assays. It is evident that increases in parasite toxicity were produced, although the relatively high doses required for the antiparasitic effect have deterred secondary evaluation of the compounds. These compounds have been separated in Table I into a group (Class A) in which toxicity toward the murine or avian parasite was increased without any evident increase in host toxicity (at least to mice), and a second group (Class B) in which toxicity to mice was increased, so that any increased antiparasitic activity may have been masked in the *in vivo* assay. A third group, described in Table II but not included in Table I, showed neither increased toxicity to the animals nor any improvement over compound **2a** in apparent antiparasitic effect *in vivo*.

The most interesting testing results were obtained against a sporozoite-induced *Plasmodium gallinaceum* infection of chicks in an assay procedure which has recently been introduced to assist in the selection of experimental drugs that might have prophylactic activity and act against tissue forms of the malarial parasite (10). The most active compound in this assay was the 7-methyl-6-*m*-chlorobenzoyl derivative **1a**, which yielded some cures at a dose level as low as 15 mg./kg., and showed no animal toxicity in this or any other assay. At somewhat higher doses the 7-methyl-6-(2,4-dichlorobenzoyl) (**1c**) derivative also produced cures in the same assay for prophylactic activity, and also showed suppressive activity at high dose levels against *Plasmodium berghei* in an established murine malaria assay (9). The *N*-(3,4-dichlorophenylthiocarbamoyl) derivative **5b** showed activity in suppressing murine malaria, but has not been assayed against avian malaria.

The compounds in Class B all showed toxicity to mice at one or more of the examined dose levels. Except in the case of the 3,4-dichlorocinnamoyl derivative (**1d**), any toxicity the derivatives may have had to the murine parasite failed to become clearly evident on the basis of increased survival time, quite possibly because of the toxicity to the host. These derivatives were also usually toxic to the chicks used in the avian malaria assays. However, one of the toxic compounds, the *p*-chlorobenzyl derivative **3b**, was active against a blood-induced *Plasmodium gallinaceum* infection in chicks, producing a 9.8 day increase in survival time at 320 mg./kg. In the assay employing the sporozoite-induced infection in chicks evidence of antiparasitic activity seemed to be obscured by host toxicity at the higher dose levels. This compound was also observed to suppress markedly the *Plasmodium cynomolgi* infection in the Rhesus monkey at a dose as low as 3.16 mg./kg., but marked toxicity to the host was also evident at this dose level (personal communication from Walter Reed Army Institute of Research). The observed toxic effects (vomiting, anorexia, depression) seem not unlike those produced by methotrexate and other effective antifols.

Comparison of the structures of the remainder of the compounds in Table II with closely related members of the A and B groups shows how sensitive both antiparasitic activity and host toxicity were to changes in the number and positions of the chlorine atoms and the presence or absence of the 7-methyl group. However, these results suggest that chloro-substituted benzoyl or chloro-substituted arylthiocarbamoyl groups linked to nitrogen should be added to the frequently exploited nitrogen or carbon-linked chloro-substituted phenyl or benzyl groups in the construction of derivatives for examination as potential antiparasitic agents.

EXPERIMENTAL (11)

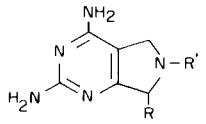
Improved Synthesis of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]-pyrimidine (**2a**) and its 7-Methyl Derivative (**2b**).

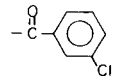
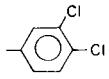
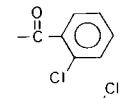
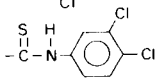
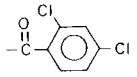
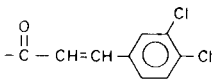
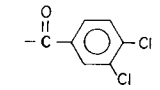
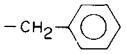
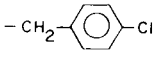
1-Acetyl-3-amino-4-cyano-3-pyrroline.

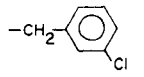
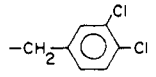
Acetic anhydride (100 ml.) was added with cooling to 78 g.

Table I

In Vivo Antimalarial Assay Results (a)



Compound No.	R	R′	P. berghei Assays (b)			P. gallinaceum Assays (c) (Blood induced (BI) or Sporozoite induced (SI) infections)			
			Dose (mg./kg.)	ΔMST (days)	Cures (C) or Toxic Deaths (T)	Assay	Dose (mg./kg.)	ΔMST (days)	Cures (C) or Toxic Deaths (T)
Parent Compound									
2a	H	H	320	3.3		BI	320	0.0	
			640	3.9		SI	100	0.9	
						SI	120	0.4	4/5 C
Class A Derivatives -- Increased Toxicity to Murine and/or Avian Parasites:									
1a	CH_3		320	1.1		BI	160	2.8	
						BI	320	3.4	
						SI	15	3.4	3/5 C
						SI	30	2.4	3/5 C
						SI	60	2.4	5/5 C
						SI	120		5/5 C
						SI	320		5/5 C
4c	H		320	1.5		BI	320	0.0	
						SI	480	1.9	1/5 T
1b	CH_3		320	1.3					
			640	5.8, 5.9					
5b	H		160	1.9, 2.1					
			320	5.9					
			640	9.1, 9.3					
1c	CH_3		320	4.3		BI	120	0.0	
			640	14.1		SI	60	4.2, 3.8	
						SI	120	-0.2	9/10 C
						SI	240	-0.2	9/10 C
						SI	480		10/10 C
Class B Derivatives -- Increased Toxicity to Murine Host:									
1d	H		640	3.9	1/5 T	SI	480	0.2	
1e	CH_3		320	0.9	1/5 T	BI	320	0.0	
			640	1.4	3/5 T	SI	240	0.2	
3a	H		320	0.7		BI	160	0.0	
			640	0.0	4/5 T				
3b	H		80	0.4		BI	160	1.8	
			160	0.9	4/5 T	BI	320	9.8	
			320	0.0	4/5 T	SI	20	3.5, 1.8	3/10 T
						SI	40	3.2, 2.0	2/10 T
						SI	80	0.5, 1.2	5/10 T

3c	H		160	0.3						
			320		5/5 T					
			640		5/5 T					
3d	H		40	0.4	1/5 T	BI	160			5/5 T
			80	0.9	3/5 T	SI	120	1.5		1/5 T
			160	---	5/5 T					

(a) Antimalarial assays were conducted by Dr. L. Rane, University of Miami. The result is given in Table I only for highest does examined, unless some possible activity or toxicity for the compound was suggested by a change >1.0 day in mean survival time. In the latter event enough results are quoted to indicate the lowest dose level at which a discernible biological effect may be evident. (b) Assays conducted on groups of five mice; mean survival time of controls was 6.1 days; ΔMST is increased survival beyond controls. Deaths before the sixth day were attributed to drug toxicity. (c) Assays conducted on groups of five chicks; mean survival time of controls with blood induced infection was 4 days; mean survival time of controls with sporozoite-induced infection varied from 7 to 9.1 days. Chicks surviving beyond 30 days were recorded as cures, chicks dying earlier than controls were recorded as toxic deaths.

(0.5 mole) of *N*-cyanoethylglycine ethyl ester (12). The mixture was kept at room temperature for 24 hours. Acetic acid and excess acetic anhydride were removed by distillation under reduced pressure. (If the odor of the residue indicated remaining acetic acid or anhydride, ethanol was added and the evaporation repeated.) The residual oil was then dissolved in 250 ml. of absolute ethanol and added to a sodium ethoxide solution prepared from 11.5 g. (0.5 mole) of sodium and 500 ml. of absolute ethanol. The mixture was held at the reflux temperature for 2 hours, then ammonium formate (79 g., 1.25 moles) was added together with an additional 500 ml. of absolute ethanol, and refluxing was continued for 48 hours on a steam bath, with care to insure adequate mixing from vigorous refluxing. The mixture was cooled in an ice bath and the crystalline product was collected by filtration. The yield was 68 g. (90%), m.p. *ca.* 236°. Recrystallization from 70% aqueous ethanol raised the m.p. to 237-239°. (Reported m.p. 238-239° (6a).)

6-Acetyl-2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine.

1-Acetyl-3-amino-4-cyano-3-pyrroline (75.5 g., 0.5 mole) dissolved in 500 ml. of absolute ethanol was added to an ethanolic solution of guanidine prepared from 95.5 g. (1.0 mole) of guanidine hydrochloride, 23 g. (1.0 mole) of sodium and 600 ml. of absolute ethanol. Prior to use the guanidine solution was filtered with the aid of Celite to remove precipitated sodium chloride, and approximately 200 ml. of additional absolute ethanol was added in the process of washing the filter cake. The mixture was refluxed for 16 hours on a steam bath, then cooled in an ice bath and filtered to collect the crystalline product. The yield was 67.5 g. (70%), m.p. *ca.* 335°. (An analytical sample, m.p. 335-337°, had been obtained by recrystallization from dimethylformamide (6a).)

2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2a**).

To 19.3 g. (0.1 mole) of the above 6-acetyl derivative (**1**, R = H, R′ = CH_3) dissolved in 1350 ml. of ethanol was added 75 g. of sodium hydroxide dissolved in 150 ml. of water. The mixture was refluxed for 12-14 hours, then concentrated under reduced pressure to *ca.* 500 ml., cooled in an ice bath for several hours, and filtered to collect the crystalline product. The yield was 8.8 g. (58%) of crude **2a**, m.p. 235-240°. Recrystallization from water raised the melting point to 258-259° (reported m.p. 258-259° (6a)).

3-Amino-1-benzoyl-4-cyano-2-methyl-3-pyrroline.

Freshly distilled *dl*-*N*-cyanoethylalanine ethyl ester (12) (85 g., 0.5 mole) was dissolved in dry pyridine (160 ml.). The solution was cooled to 5° and benzoyl chloride (77 g., 0.55 mole) was added dropwise to the stirred solution at such a rate that the temperature did not rise above 15°. The mixture was stirred at room temperautre overnight. It was then poured into ice-water (1 ℓ.) and the oily layer was taken up in ether. The ether solution was washed twice with 5% hydrochloric acid, then with 5% sodium bicarbonate, and finally with water, dried over sodium sulfate, and evaporated. The residue, *N*-benzoyl-*N*-cyanoethylalanine ethyl ester, was a yellow oil which failed to crystallize. The oil was dissolved in 250 ml. of absolute ethanol and added to a sodium ethoxide solution (0.5 mole, from 11.5 g. of sodium and 500 ml. of absolute ethanol). After refluxing for 2 hours, excess ammonium formate (79 g., 1.25 moles) in 300 ml. of absolute ethanol was added and the refluxing was continued on a steam bath, initially shaking the solution occasionally to prevent the formation of large lumps of solid material. The flow of steam was fast enough to promote rapid boiling and agitate the whole solution. After a reflux period of 48 hours the solution was cooled to room temperature and the undissolved solid (sodium formate) was filtered out, extracted with small amounts of ethanol and discarded. The filtrate and extracts were cooled in ice and the precipitated solid was collected by filtration. The filtrate was concentrated to about one-third its volume, and cooled overnight to recover additional product. The yield was 85 g. (75%). The melting points of the crude products were usually within 1 or 2° of that of purified material. Crystallization from ethanol yielded white plates, m.p. 203-204° (6a)).

6-Benzoyl-2,4-diamino-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]-pyrimidine.

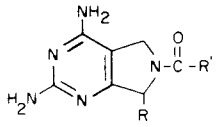
3-Amino-1-benzoyl-4-cyano-2-methyl-3-pyrroline (68 g., 0.3 mole) in 300 ml. of absolute ethanol was added to a solution of guanidine in absolute ehtanol. The guanidine solution was prepared from 57.3 g. (0.6 mole) of guanidine hydrochloride, and 13.8 g. (0.6 mole) of sodium dissolved in 400 ml. of absolute ethanol. Precipitated sodium chloride was removed before use of the guanidine solution by filtration with the aid of Celite, and approximately 100 ml. of additional absolute ethanol was used to extract guanidine from the filter cake into the filtrate. The mixture was refluxed for 16 hours on a steam bath, then was cooled in an ice bath and filtered to collect the crystalline product. A second crop was obtained after concentration of the filtrate to about one-third of the original volume, followed by cooling. The total yield was 56.5 g. (70%) of material melting at 275-277° (reported m.p. 275-277° (6a)).

2,4-Diamino-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2b**).

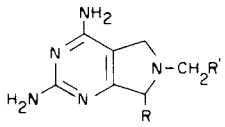
Basic hydrolysis of 26.9 g. (0.1 mole) of the above 6-benzoyl-7-methyl derivative (**1**, R = CH_3, R′ = C_6H_5) carried out as in the procedure described above for the 6-acetyl derivative of **2a** yielded 11.5 g. (69%) of the crude 7-methyl derivative **2b**, m.p. 203° (d). Crystallization from water did not change this melting point (reported 203°) (d) (6a)).

Table II

6-Acyl and 6-Benzyl Derivatives of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine



1



3

	R	R'	M.P.	%, Yield (a)	Solvent (b)	Formula	%, Carbon Calcd.	%, Carbon Found	%, Hydrogen Calcd.	%, Hydrogen Found	%, Nitrogen Calcd.	%, Nitrogen Found
1a	CH_3	m-ClPh-	284-285°	55 (S)	ethanol	$C_{14}H_{14}ClN_5O$	55.36	55.13	4.65	4.52	23.06	22.82
1b	CH_3	*o*-ClPh-	279-281°	59 (P)	ethanol	$C_{14}H_{14}ClN_5O$	55.36	55.32	4.65	4.51	23.06	22.84
1c	CH_3	2,4-Cl_2Ph-	282-284°	52 (S)	ethanol	$C_{14}H_{13}Cl_2N_5O$	49.72	49.65	3.87	3.79	20.71	20.75
1d	H	3,4-Cl_2PhCH=CH-	315-317°	71 (A)	DMF	$C_{15}H_{13}Cl_2N_5O$	51.44	51.41	3.74	3.63	20.00	19.89
1e	CH_3	3,4-Cl_2Ph-	271-272°	62 (S)	ethanol	$C_{14}H_{13}Cl_2N_5O$	49.72	49.71	3.87	3.84	20.71	20.55
1f	H	*p*-ClPh-	290-291°	52 (S)	ethanol	$C_{13}H_{12}ClN_5O$	53.89	53.70	4.18	4.03	24.17	24.37
1g	H	m-ClPh-	>350	52 (S)	DMF	$C_{13}H_{12}ClN_5O$	53.89	53.69	4.18	3.89	24.17	24.38
1h	H	o-ClPh-	290-295°	43 (P)	ethanol	$C_{13}H_{12}ClN_5O$	53.89	53.64	4.18	4.00	24.17	24.06
1i	H	2,4-Cl_2Ph-	295-296°	60 (S)	ethanol	$C_{13}H_{11}Cl_2N_5O$	48.16	47.93	3.42	3.21	21.60	21.65
1j	H	3,4-Cl_2Ph-	>350	63 (S)	DMF	$C_{13}H_{11}Cl_2N_5O$	48.16	49.94	3.42	3.44	21.60	21.76
1k	CH_3	3,4-Cl_2PhCH=CH-	332-334°	50 (S)	DMF	$C_{16}H_{15}Cl_2N_5O$	52.76	52.57	4.15	4.34	19.23	19.14
1l	CH_3	p-ClPh	290-291°	60 (S)	ethanol	$C_{14}H_{14}ClN_5O$ (c)	55.36	55.25	4.65	4.71	23.06	23.08
1m	CH_3	3,5-Cl_2Ph-	>300°d	30 (S)	ethanol	$C_{14}H_{13}Cl_2N_5O$	49.72	49.78	3.87	3.81	20.71	20.70
1n	CH_3	2,5-Cl_2Ph-	>300°d	18 (S)	ethanol	$C_{14}H_{13}Cl_2N_5O$	49.72	50.07	3.87	3.76	20.71	20.48
1o	CH_3	2,6-Cl_2Ph-	178-180°	25 (S)	ethanol	$C_{14}H_{13}Cl_2N_5O$	49.72	50.01	3.87	3.68	20.71	20.91
1p	H	2,4-Cl_2PhCH=CH-	316-319°	71 (A)	DMF	$C_{15}H_{13}Cl_2N_5O$	51.44	51.59	3.74	3.91	20.00	19.87
1q	CH_3	m-ClPhCH=CH-	312-314°	52 (S)	ethanol	$C_{16}H_{16}ClN_5O$	58.27	58.33	4.89	4.73	21.24	21.09
1r	CH_3	2,4-Cl_2PhCH=CH-	262-264°	83 (S)	ethanol	$C_{16}H_{15}Cl_2N_5O$	52.76	52.82	4.15	4.34	19.23	19.27
1s	H	Ph-	294-295°d	50 (S); 43 (G)	ethanol	$C_{13}H_{13}N_5O$	61.16	61.17	5.13	5.16	27.44	27.62
3a	H	Ph-	225-227°	26.5	ethanol	$C_{13}H_{15}N_5$	64.71	64.89	6.27	6.19	29.03	29.00
3b	H	p-ClPh-	216-217°	33	ethanol	$C_{13}H_{14}ClN_5$ (d)	56.62	56.73	5.12	4.97	25.40	25.37
3c	H	m-ClPh-	209-210°	63	ethanol	$C_{13}H_{14}Cl_2N_5$	56.62	56.89	5.12	5.40	25.40	25.48
3d	H	3,4-Cl_2Ph-	243-244°	27	ethanol	$C_{13}H_{13}Cl_2N_5$	50.34	50.13	4.22	4.32	22.58	22.32
3e	CH_3	*p*-ClPh-	202-203°	33	ethanol	$C_{14}H_{16}ClN_5$	58.01	57.87	5.57	5.36	24.16	24.35
3f	H	2,4-Cl_2Ph-	196-197°	52	ethanol	$C_{13}H_{13}Cl_2N_5$	50.34	50.53	4.22	4.23	22.58	22.64

(a) S means Schotten-Baumann method; P, pyridine method; A, mixed anhydride method. Two yields quoted for compound **1s** are for direct Schotten-Baumann benzoylation (S) of **2a** and for condensation of guanidine with 1-benzoyl-3-amino-4-cyano-3-pyrroline (G). The latter compound, m.p. 230-231°, was obtained by a procedure parrallel to that described in the Experimental section for the corresponding 2-methyl derivative. *Anal.* Calcd. for $C_{12}H_{11}N_3O$: C, 67.58; H, 5.20; N, 19.20. Found: C, 67.62; H, 5.22; N, 19.79. (b) Solvents used for recrystallization; DMF is dimethylformamide. (c) Ir (Nujol) μ 2.92, 3.04, 3.18, 6.00, 6.08-6.14, 6.24-6.32, 7.66, 7.80, 8.54, 9.18, 9.60, 9.82, 10.60, 11.18, 11.90, 12.58, 12.94, 13.10. Uv (ethanol) λ max: (log ϵ): 222 nm (4.41); 286 nm (3.84). Nmr: (δ) 1.60 (d, 3, J = 6 Hz, CH_3), 4.48 (s, 2, ring CH_2), 4.80-5.12 (m, 1, $CH(CH_3)$) and 7.30 (s, 4, aromatic). (d) Ir (Nujol): μ 2.90-3.02, 3.22, 5.98, 6.12, 6.24-6.28, 7.00, 7.42, 8.72, 9.10-9.30, 9.60, 9.80, 11.26, 11.54, 12.08, 12.50, 12.74. Uv (ethanol) λ max: (log ϵ): 216 nm (4.39), 237 nm sh (4.15), 288 nm (3.88). Nmr: (δ) 4.66, 4.76 and 4.86 (overlapping s, 6, three CH_2) and 7.46 (s, 4, aromatic).

6-Acyl Derivatives of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]-pyrimidine (**1**). Schotten-Baumann Procedure.

2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2a**) or its 7-methyl derivative (**2b**) and excess sodium hydroxide were dissolved in water and the acid chloride was added dropwise to the stirred solution. For each 0.01 mole of pyrrolo[3,4-*d*]pyrimidine derivative 1.5 g. of sodium hydroxide, 35-50 ml. of water and *ca.* 0.012 mole of the acid chloride were employed. Stirring was continued for at least 30 minutes, then the precipitated product was collected by filtration and washed with small amounts of cold ethanol. The products were crystallized from ethanol or dimethylformamide. Preparation by the Schotten-Baumann procedure is indicated by (S) following the figure for the yield in Table II.

Pyridine Procedure.

To 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2a**) or its 7-methyl derivative (**2b**) (0.01 mole) in 40 ml. of dry pyridine cooled in an ice bath, an acid chloride (0.012 mole) was added very slowly with stirring. Stirring was continued for 16 hours at room temperature. After removal of the pyridine by distillation under reduced pressure, the residue was dissolved in water and the solution was basicified with sodium hydroxide and cooled. The product was collected by filtration and purified by crystallization from ethanol. The results with individual compounds are recorded in Table II, in which preparations using the pyridine procedure are indicated by (P) following the yield figure.

Ethyl Chloroformate-Mixed Anhydride Procedure.

After cooling a solution of the carboxylic acid (0.01 mole) and triethylamine (1.01 g., 0.01 mole) in 30-40 ml. of chloroform to -5° ethyl chloroformate (1.09 g., 0.01 mole) was added dropwise and the resulting mixture was stirred at 15° for 45 minutes. The pyrrolo[3,4-*d*]pyrimidine **2a** (1.51 g., 0.01 mole) was added and the resulting suspension was stirred vigorously for 30-40 minutes at -5° and then for 16 hours at room temperature. After addition of cold ethanol (and, if necessary for neutralization, a small amount of sodium hydroxide) the solid was collected by filtration, washed with cold ethanol, and recrystallized. In Table II preparations using this mixed anhydride procedure are indicated by (A) following the yield figure.

6-(Mono- and Dichloro-benzyl) Derivatives of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**3**).

The 6-(mono- or dichloro-benzoyl) derivatives (**1**) were reduced by treatment with lithium aluminum hydride (LAH) in purified tetrahydrofuran. An excess (2:1 molar proportion) of LAH was used and the volume of solvent was 50 ml./g. of starting material. The mixtures were heated and stirred at the reflux temperature for 1 hour. They were then cooled in an ice bath and saturated aqueous sodium sulfate solution was added dropwise at a slow enough rate to avoid a large temperature rise. When the original dark suspension had become entirely white the mixtures were filtered and the filtrates were concentrated to small volumes, cooled, and filtered to collect the reduction products. Extraction with hot ethanol of the filter cake obtained from the sodium sulfate treatment yielded additional amounts of product in some cases. Results with individual compounds are given in Table II.

6-(Mono- and Dichloro-phenyl) Derivatives of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**4**).

Preparation of 3-Amino-1-aryl-4-cyano-3-pyrrolines.

Equimolar amounts of the appropriate chloro-substituted aniline and acrylonitrile were refluxed gently at 100-110° in the presence of copper acetate (8.0 g. per mole of acrylonitrile) for 2.5 hours (13). The copper salt was removed by filtration through a bed of Celite and washed with ethanol. The dark-colored ethanol solutions were decolorized with Norit, and concentrated by distillation. From *m*-chloroaniline a residual oil was obtained which was distilled at 200-230°/20 mm to afford a 51% yield of almost colorless (3-*m*-chloroanilino)propionitrile, having the expected nmr spectrum. From 3,4-dichloroaniline 3-(3,4-dichloroanilno)propionitrile was obtained as pale-yellow needles, m.p. 78-80°. The expected nmr spectrum was observed in deuteriochloroform; the yield was 65%. The 3-anilinopropionitriles were refluxed for 48 hours with a 2:1 molar proportion of ethyl bromoacetate dissolved in 95% ethanol (50 ml. of ethanol/mole of ethyl bromoacetate). The reaction mixtures were poured onto crushed ice and basicified with aqueous sodium hydroxide (40%) below 0° with stirring until the acrid odor of ethyl bromoacetate disappeared. The products were then extracted into ether, dried (sodium sulfate) and concentrated to yield residual oils, which were refluxed for 3 hours with sodium ethoxide solutions (25 g. of sodium in 1250 ml. of absolute ethanol per mole of original anilinopropionitrile). Excess ammonium formate (200 g. per mole or original anilinopropionitrile) was then added in two portions over a 48 hours period of refluxing on a steam bath. The solvent was removed by distillation under reduced pressure and water was added. The water-insoluble products were collected by filtration and crystallized from ethanol.

3-Amino-1-(*m*-chlorophenyl)-4-cyano-3-pyrroline.

This compound, m.p. 226-227°, was obtained in 40% yield from 3-*m*-chloroanilinopropionitrile in an experiment run on a 0.1-mole scale.

Anal. Calcd. for $C_{11}H_{10}ClN_2$: C, 60.14; H, 4.59; N, 19.13. Found: C, 60.35; H, 4.45; N, 19.05.

3-Amino-5-cyano-1-(3,4-dichlorophenyl)-3-pyrroline.

This compound, m.p. 236-237°, was obtained in 47.5% yield from 3-(3,4-dichloroanilino)propionitrile in an experiment run on a 0.2-mole scale.

Anal. Calcd. for $C_{11}H_9Cl_2N_3$: C, 51.98; H, 3.56; N, 16.53. Found: C, 51.76; H, 3.67; N, 16.76.

Conversion of 3-Amino-1-aryl-4-cyano-3-pyrrolines to 2,4-Diamino-6-aryl Derivatives of 6,7-Dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**4**).

The 3-amino-1-aryl-4-cyano-3-pyrrolines were heated with equimolar amounts of guanidine carbonate in 2-ethoxyethanol (250-300 ml. per mole of pyrroline) at the reflux temperature for 6-7 hours. The mixtures were poured into cold (*ca.* 0°) water and the insoluble products were collected by filtration. They were recrystallized from dimethylformamide (DMF)-ethanol or DMF-water mixtures.

2,4-Diamino-6-(*m*-chlorophenyl)-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]-pyrimidine (**4b**).

This compound, m.p. 275-276° (from DMF-ethanol), was obtained in 95% yield (crude) in an experiment run on a 0.01-mole scale.

Anal. Calcd. for $C_{12}H_{12}ClN_5$: C, 55.07; H, 4.62; N, 26.76. Found: C, 54.97; H, 4.52; N, 26.88.

2,4-Diamino-6-(3,4-dichlorophenyl)-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]-pyrimidine (**4c**).

This compound, m.p. 304-305° (from DMF-water), was obtained in 77% yield in an experiment run on a 0.02-mole scale.

Anal. Calcd. for $C_{12}H_{11}N_5Cl_2$: C, 48.66; H, 3.74; N, 23.65. Found: C, 48.46; H, 3.98; N, 23.57.

6-Arylthiocarbamoyl Derivatives of 2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**5**).

Purified 2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (1.51 g., 0.01 mole) was partially dissolved in 80-100 ml. of dry pyridine. An equimolar amount of aryl isothiocyanate was added, and the mixture was stirred at room temperature for 1 hour. The 6-arylthiocarbamoyl derivatives were isolated by distillation of the pyridine at reduced pressure, followed by washing of the residue with acetone and collection of the product by filtration (14).

6-*p*-Chlorophenylthiocarbamoyl-2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**5a**).

From *p*-chlorophenyl isothiocyanate (prepared from *p*-chloroaniline by the method of Dains, Brewster and Olander (15)), the yield was 2.8 g. (87%), m.p. 280-281° (from ethanol); ir (Nujol): μ 2.88, 3.00, 3.10, 5.96, 6.10, 6.18, 6.28, 6.50, 6.68, 7.06, 7.44, 7.88, 8.00, 9.14, 9.80, 10.18, 12.02, 12.74, 13.88; uv (ethanol): λ max (log ϵ) 246 nm (4.61), 281 nm (4.16); nmr: (δ) 5.12 and 5.28 (4, ring CH_2), 7.26 and 7.60 (q, AB type, 4, J = 9 Hz, aromatic).

Anal. Calcd. for $C_{13}H_{13}ClN_6S$: C, 48.67; H, 4.09; N, 26.20. Found: C, 48.48; H, 4.03; N, 25.94.

6-(3,4-Dichlorophenylthiocarbamoyl)-2,4-diamino-6,7-dihydro-5*H*-pyrrolo[3,4-*d*]pyrimidine (**5b**).

From 3,4-dichlorophenyl isothiocyanate (prepared from 3,4-dichloroaniline by the method of Dains, Brewster and Olander (15)), the yield was 3.2 g. (90%), m.p. 297-299° (from ethanol).

Anal. Calcd. for $C_{13}H_{12}Cl_2N_6S$: C, 43.95; H, 3.42; N, 23.66. Found: C, 44.15; H, 3.30; N, 23.44.

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(3) Postdoctoral Research Associate.

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