6,7-Dihydro-5H-pyrrolo[3,4-d]pyrimidines. Syntheses Based on 3-Amino- and 3-Methoxy-1-acyl-4-cyano-3-pyrrolines[1]

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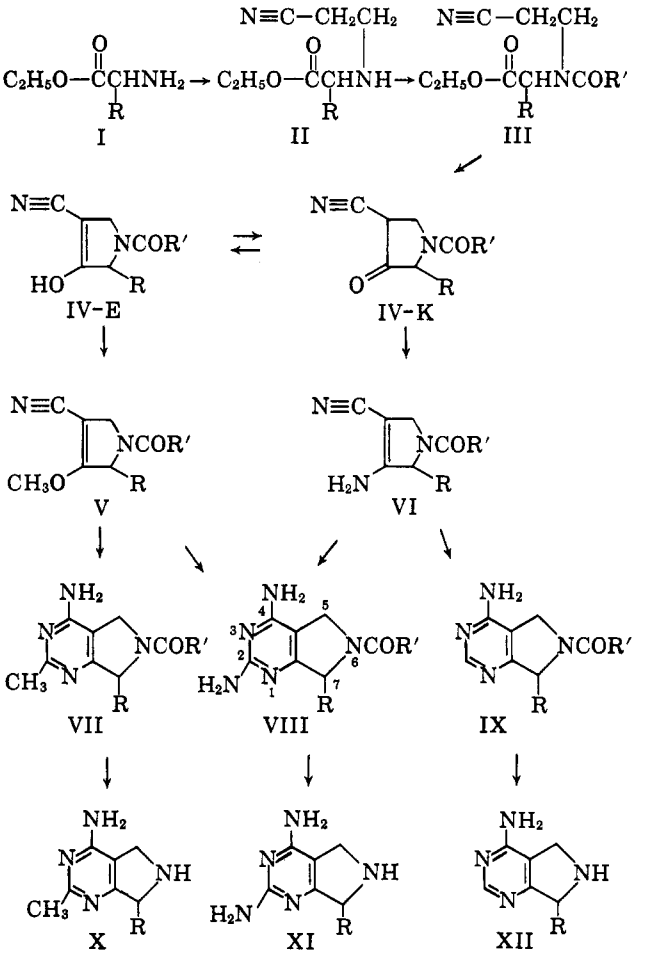
A convenient scheme has been developed for the synthesis of compounds in the 6,7-dihydro-5H-pyrrolo[3,4-*d*]-pyrimidine series from esters of α-amino acids. Successive cyanoethylation and acylation of the amino group, followed by Dieckmann cyclization yielded 2-substituted 1-acyl-4-cyano-3-oxopyrrolidines (IV). Treatment of the latter with ammonium formate afforded 2-substituted 1-acyl-3-amino-4-cyano-3-pyrrolines (VI); treatment with diazomethane gave 2-substituted 1-acyl-4-cyano-3-methoxy-3-pyrrolines (V). Condensation of compounds of types V or VI with guanidine yielded 7-substituted 6-acyl-2,4-diamino-6,7-dihydro-5H-pyrrolo-[3,4-*d*]pyrimidines (VIII). Similar reactions of the amino compounds (VI) with formamidine acetate or ethyl orthoformate and ammonia produced corresponding 4-amino derivatives (IX). The methoxy derivatives (V) yielded 4-amino-2-methyl derivatives (VII) with acetamidine. Except when the 7-substituent was large (benzyl) the 6-acyl groups could be removed from these products readily by alkaline hydrolysis.

Recently the synthesis of a number of compounds in the previously unknown pyrrolo[3,4-*d*]pyrimidine series was described.[2] The starting materials for the synthetic procedures which were employed were 2,3-pyrrolidinediones containing functional groups (carbethoxy or benzylidene groups) in the 4-position. The products incorporated a lactam carbonyl at the 7-position of the pyrrolo[3,4-*d*]pyrimidine structure. In order to broaden the search for compounds with physiological activity in this new heterocyclic series another synthetic scheme has since been devised which makes accessible compounds with other types of substitution in the 7-position and a nitrogen atom with basic character at the 6-position. If the 7-position of this ring system can be regarded as corresponding to the 9-position of the purine system, compounds in the new series having 7-substituents might be particularly interesting, since a number of 9-substituted purines are known to have marked activity against a variety of animal tumors.[3]

Ethyl esters of three amino acids have been used as starting materials in the examples thus far chosen to illustrate the synthesis. The reaction sequences are diagrammed in Chart I. The numbered formulas in the chart denote structural types; individual compounds will be designated in the discussion by appending to the appropriate numeral two letters, the first identifying the group R (h = hydrogen, m = methyl, and b = benzyl), the second identifying the group R′ (m = methyl and p = phenyl). The first step, the cyanoethylation of the amino acid esters, had been described previously,[4] and was found to proceed in yields of 55 to 70%. The second contemplated step, a variation of the Dieckmann cyclization, was regarded as potentially troublesome because of the discouraging nature of the reports of previous efforts to carry out this kind of reaction with cyanoethyl derivatives of ethyl glycinate[5] and ethyl N-methylglycinate.[6] Cook and Reed[6] had cyclized the latter compound to 1-methyl-4-cyano-3-oxopyrrolidine (in unspecified yield) by treatment with sodium ethoxide in toluene, but difficulty was experienced in isolating the product, apparently because of its instability. Cocker, Criss, and McCormick[5a] reported the conversion of ethyl N-cyanoethylglycinate to 4-cyano-3-oxopyrrolidine in 10% yield by use of sodium ethoxide in benzene, but later work by Butskus[5b] indicated that the product obtained was actually N,N′-bis(2-cyanoethyl)-2,5-dioxopiperazine. Our own initial attempts to cyclize the cyanoethyl derivative of *dl*-alanine ethyl ester IIm also failed to yield the desired product. However, the difficulty with the Dieckmann

CHART I



(1) Supported by a research grant (GM-4371) from the National Institutes of Health, U. S. Public Health Service.

(2) P. L. Southwick and G. H. Hofmann, *J. Org. Chem.*, **28**, 3058 (1963).

(3) See R. K. Robbins, *J. Med. Chem.*, **7**, 186 (1964). Robbins discusses the evidence that the active 9-substituted purines exert their inhibitory effect on tumor growth without undergoing removal of the 9-substituent.

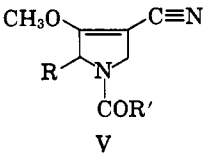
(4) (a) A. P. Terentev and P. F. Butskus, *Zh. Obshch. Khim.*, **23**, 1230 (1953); *Chem. Abstr.*, **47**, 12237*h* (1953). (b) A. P. Terentev and P. F. Butskus, *Zh. Obshch. Khim.*, **27**, 2884 (1957); *Chem. Abstr.*, **52**, 8045*i* (1958).

(5) (a) W. Cocker, B. E. Cress, and J. McCormick, *J. Chem. Soc.*, 1182 (1952); (b) P. F. Butskus, *Zh. Obshch. Khim.*, **40**, 1814 (1960); *Chem. Abstr.* **55**, 7425*i* (1961).

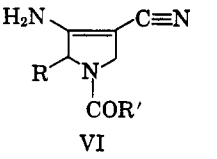
(6) A. H. Cook and K. J. Reed, *J. Chem. Soc.*, 399 (1945).

TABLE I

3-SUBSTITUTED 1-ACYL-4-CYANO-3-PYRROLINES



V



VI

	R	R'	M.p., °C.	Yield, %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Vhp	H	C_6H_5	106–107[a]	90	$C_{13}H_{12}N_2O_2$	68.41	67.82	5.30	5.29	12.27	12.18
Vhm	H	CH_3	71–72[b]	84	$C_8H_{10}N_2O_2$	57.82	57.44	6.06	6.24	16.86	16.85
Vmp	CH_3	C_6H_5	Oil	100[c]	$C_{14}H_{14}N_2O_2$	...	...	..	..	...	...
Vmm	CH_3	CH_3	68–69[b]	88	$C_9H_{12}N_2O_2$	59.98	59.86	6.71	6.61	15.55	15.88
Vbp	$CH_2C_6H_5$	C_6H_5	152[a]	92	$C_{20}H_{18}N_2O_2$	75.45	75.17	5.70	5.74	8.80	8.87
VIhm	H	CH_3	238–239	71	$C_7H_9N_3O$	55.61	55.30	6.00	5.96	27.80	27.85
VImp	CH_3	C_6H_5	203–204	85	$C_{13}H_{13}N_3O$	68.70	68.15	5.77	6.01	18.49	18.11
VImm	CH_3	CH_3	176–177	82	$C_8H_{11}N_3O$	58.16	58.47	6.71	6.67	25.44	25.41
VIbp	$CH_2C_6H_5$	C_6H_5	180–181	85	$C_{19}H_{17}N_3O$	75.22	75.17	5.65	5.56	13.85	13.78
VIbm	$CH_2C_6H_5$	CH_3	192	88	$C_{14}H_{15}N_3O$	69.69	69.79	6.27	6.04	17.42	16.90

[a] Crystallized from 70% ethanol. [b] Crystallized from ether–petroleum ether (b.p. 30–60°). [c] Yield of crude product.

step was overcome by converting the intermediate IIm into the N-benzoyl derivative IIImp, which cyclized to 1-benzoyl-4-cyano-2-methyl-3-oxopyrrolidine (IVmp) in 55% yield when treated with sodium ethoxide in ethanol. The phenylalanine derivative IIIbp afforded a 59% yield of the corresponding 2-benzyl derivative IVbp. Unfortunately, the yield was lower (12%) in the cyclization of the glycine derivative IIIhp, partly because of formation of an unidentified by-product. In later experiments the acetyl derivatives IIIbm, IIIhm, and IIImm were prepared and cyclized by the same procedure used with corresponding benzoyl derivatives. The yields of the cyclization products IVbm and IVmm (*ca.* 53%) were comparable with that of IVbp and the yield of IVhm (55%) was much better than that of IVhp. It seems clear that a variety of acyl derivatives of type III could be used in the synthetic scheme, and that the intermediate of choice does not necessarily contain the same acyl group in every instance.

Unlike all of the 4-substituted-2,3-dioxopyrrolidines which have been examined,[7] the compounds of type IV were not highly enolized under all conditions. Five of the compounds (IVhm, IVmm, IVbm, IVmp, and IVbp) did appear to be almost entirely in the enolic form (IV-E) when in the crystalline state, since the infrared spectra measured on Nujol mulls or potassium bromide pellets showed at the most only very weak absorption corresponding to the ketonic function at 5.6 μ, and the presence of a hydrogen-bonded enolic hydroxyl was indicated by a broad absorption at *ca.* 3.8 μ. These same compounds, however, when examined in chloroform solution, showed no absorption due to enolic hydroxyl and displayed a strong band at 5.6 μ. Thus the infrared data appeared to indicate that the compounds are almost completely enolic (corresponding to formula IV-E) in the crystalline state, but fully ketonic (corresponding to formula IV-K) in chloroform solution. The sixth compound of type IV which was examined, the benzoyl derivative IVhp, constituted an exception to this behavior; it exhibited only the spectrum of the ketonic tautomer either in chloroform solution or in the solid state. The ultraviolet absorption of compound IVmm indicated that the substance was largely in the enol form in 95% ethanol [λ_{max} 233 mμ (ϵ 9830) for IVmm as compared with λ_{max} 232 mμ (ϵ 11,790) for the methyl ether of the enol, Vmm]. In cyclohexane, on the other hand, no strong ultraviolet absorption was evident at wave lengths above 220 mμ; in this medium the compound must be ketonic. An intermediate degree of enolization was indicated by the absorption in ether, λ_{max} 233 mμ (ϵ 5660).

The tendency of these compounds to be fully enolized in the solid state and the manner in which their enolization was affected by solvents is paralleled by the behavior of certain other β-ketonitriles[8a] and of such β-dicarbonyl compounds as dimedone,[8b,c] compounds which give quite acidic enols but are not properly constituted for intramolecular hydrogen bonding. The compounds of type IV, like dimedone, apparently enolize significantly only when the immediate environment of the enolic hydroxyl permits that group to function as a proton donor in hydrogen bonding with an adjacent molecule.[8d] However, all of the compounds of type IV were sufficiently acidic to dissolve in aqueous sodium bicarbonate solutions, and attempts to conduct a reaction of one of them (IVmp) with guanidine failed, presumably because of the resistance of the enolate anion to nucleophilic attack; the starting material was recovered unchanged. In this regard compounds of the type IV resemble the 4-carbethoxy-2,3-dioxopyrrolidines.[2]

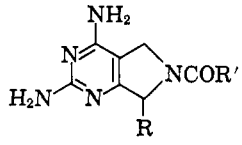
The formation of unreactive enolate anions from compounds of type IV was prevented by converting them into the 1-acyl-3-amino-4-cyano-3-pyrrolines (VI) or into the 1-acyl-4-cyano-3-methoxy-3-pyrrolines (V) (see Table I). The use of the amino derivatives VI in

(7) In the case of 2,3-dioxopyrrolidines a substituent at the 4-position, seemingly regardless of its nature, causes a strong preference for the enol form both in the solid state and in solution. A 5-substituent in that series does not appear to favor enolization. See (a) P. L. Southwick, E. P. Previc, J. Casanova, Jr., and E. H. Carlson, *J. Org. Chem.*, **21**, 1087 (1956); (b) W. L. Meyer and W. R. Vaughan, *ibid.*, **22**, 98, 1554, 1560 (1957); (c) W. R. Vaughan and I. S. Covey, *J. Am. Chem. Soc.*, **80**, 2197 (1958); (d) P. L. Southwick and E. F. Barnas, *J. Org. Chem.*, **27**, 98 (1962); (e) P. L. Southwick and J. A. Vida, *ibid.*, **27**, 3075 (1962).

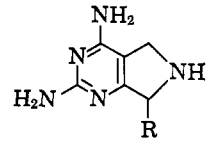
(8) (a) *Cf.* observations and references given by P. B. Russell, *J. Am. Chem. Soc.*, **74**, 2654 (1954), and P. B. Russell and J. Mentha, *ibid.*, **77**, 4245 (1955). (b) C. L. Angell and R. L. Werner, *Australian J. Chem.*, **6**, 294 (1953). (c) B. Eistert and W. Reiss, *Chem. Ber.*, **87**, 92, 108 (1954). (d) Not all α-cyano ketones are highly enolized under similar conditions; 2-cyanocyclohexanone is only 10% enolized in ethanol according to K. von Auwers, *et al.* [*Ann.*, **441**, 68 (1925)], and 2-cyanocyclopentanone is ketonic as the neat liquid according to C. F. Hammer and R. A. Hines [*J. Am. Chem. Soc.*, **77**, 3649 (1955)].

TABLE II

2,4-DIAMINO-6,7-DIHYDRO-5H-PYRROLO[3,4-*d*]PYRIMIDINES



VIII



XI

	R	R′	M.p., °C.	Yield[a]	λmax, mμ (log ε) 95% ethanol	λmax, mμ (log ε) 0.1 *N* HCl	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
VIIIhm	H	CH_3	335–337[b]	62 (A)	282 (3.61)	272 (3.70) 305 (i)[e] (2.76)	$C_8H_{11}N_5O$	49.73	5.74	36.25	49.58	5.62	36.10
VIIImm	CH_3	CH_3	307–308[b]	71 (B)	281 (3.98)	272 (3.91) 302 (i) (3.00)	$C_9H_{13}N_5O$	52.16	6.32	33.80	51.93	6.26	33.57
VIIImp	CH_3	C_6H_5	275–277[b]	65 (A) 71 (B)	282 (3.69)	273 (3.90) 300 (i) (3.43)	$C_{14}H_{15}N_5O$	62.44	5.61	26.01	62.21	5.44	25.87
VIIIbm	$CH_2C_6H_5$	CH_3	246–247[c]	65 (A)	284 (3.87)	273 (3.76)	$C_{15}H_{17}N_5O$	63.58	6.05	24.72	63.46	6.01	24.55
VIIIbp	$CH_2C_6H_5$	C_6H_5	250–251[b]	61 (A) 66 (B)	286 (3.88)	273 (3.77) 306 (i) (2.85)	$C_{20}H_{19}N_5O$	69.54	5.55	20.28	69.34	5.76	20.09
IXh	H		258–259	58	284 (3.90)	271 (3.77) 300 (i) (3.18)	$C_6H_9N_5$	47.67	6.00	46.33	47.38	6.17	46.09
IXm	CH_3		203[d]	69	283 (3.88)	272 (3.75) 300 (i) (3.31)	$C_7H_{11}N_5$	50.89	6.71	42.40	50.66	6.82	42.43

[a] A or B indicates the procedure used; see Experimental. [b] Crystallized from dimethylformamide. [c] Crystallized from ethanol. [d] Obtained at first as a hydrate, m.p. 200°. *Anal.* Calcd. for $C_7H_{11}N_5 \cdot H_2O$: C, 45.89; H, 7.15; N, 38.23. Found: C, 45.97; H, 6.92; N, 38.22. Drying *in vacuo* for 24 hr. at 140° yielded the unsolvated compound. [e] Inflections are indicated by i.

the reaction with guanidine was suggested by the use of analogous compounds in our previous work on pyrrolo[3,4-*d*]pyrimidine synthesis.[2] Enol ethers derived from β-ketonitriles have been employed previously in the synthesis of 2,4-diaminopyrimidines.[9] The amino derivatives VI, which were prepared in yields of 71 to 88% by heating the compounds of type IV with ammonium formate, were all solids easily purified by recrystallization. The enamine formula VI is assigned on the basis of the conjugation revealed by the ultraviolet spectrum of VImm, which showed a maximum at 259 mμ (ε 14,560) in 95% ethanol. The enol ethers V were obtained in yields of 84 to 92% by the action of diazomethane. One of these ethers (Vmp) failed to crystallize and was used as an intermediate without purification.

Condensation of intermediates of types V and VI with guanidine resulted in the formation of 6-acyl-2,4-diamino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (VIII), with yields in the range 61 to 71% (see Table II). In the cases in which a comparison was made, the enol ethers V appeared to react more rapidly than the amino derivatives VI, but to give somewhat lower yields. It was also possible to condense the amino derivatives VI with formamidine acetate in dimethylformamide and the enol ethers V with acetamidine in ethanol to yield the 6-acyl-4-amino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (IX) and the 6-acyl-4-amino-2-methyl-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (VII), respectively. Yields for these condensations are recorded in Tables III and IV. The synthesis developed for the 4-amino derivatives IX is analogous to a synthesis introduced by Taylor and Ehrhart,[10a] who condensed formamidine acetate with an *o*-aminonitrile in the pyrimidine series. However, the reaction of formamidine acetate with 1-acyl-3-amino-4-cyano-3-pyrrolines (VI) requires more severe reaction conditions than the previously described example[10a]; refluxing dimethylformamide (b.p. 152°) rather than 2-ethoxyethanol (b.p. 135°) was necessary as the solvent and a large excess of formamidine acetate was required to obtain reasonable yields of the 4-amino derivatives of type IX. The fact that open-chain β-amino-α,β-unsaturated nitriles (the so-called "dinitriles") often react with basic nitrogen compounds with replacement of the β-amino group[10e] suggests the possibility that it is the 3-amino group of the pyrroline intermediates VI which is eliminated as ammonia in the reaction with formamidine acetate. However, the observation that the enol ether Vbp failed to react with formamidine acetate may indicate that the amino nitrogen of structure VI is essential for the ring closure and therefore not eliminated but rather retained in the 1-position of the pyrrolo[3,4-*d*]pyrimidine ring structure.

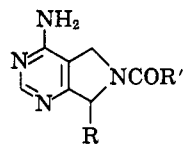
In later experiments it was found that better yields (52 to 90%) of the 4-aminopyrrolopyrimidines (IX) could be obtained by a two-step procedure, also employed previously by Taylor and his associates[10b,c] and by Shaw and Butler[10d] with other *o*-amino nitriles, in which compounds of type VI were treated first with ethyl orthoformate and acetic anhydride, and the products of this reaction were then treated with ammonia in absolute ethanol. It has been shown in other cases[10b-d] that the reaction with ethyl orthoformate and acetic anhydride introduces an ethoxymethylene group on the amino nitrogen; the intermediate products from the compounds of type VI are presumed to be analogous, but they failed to crystallize and were not characterized. Since this second method for the synthesis of the 4-amino derivatives IX is possible only if the amino nitrogen of the amino pyrrolines VI is retained and incorporated in the pyrimidine ring, it is evident that the enamine structure in compounds of type VI has sufficient stability and sufficient reactivity

(9) (a) P. B. Russell and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3763 (1951); (b) B. H. Chase, J. P. Thurston, and G. Walker, *J. Chem. Soc.*, 3439 (1951).

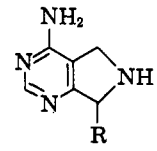
(10) (a) E. C. Taylor and W. A. Ehrhart, *J. Am. Chem. Soc.*, **82**, 3138 (1960); (b) E. C. Taylor and K. S. Hartke, *ibid.*, **81**, 2456 (1959). (c) E. C. Taylor and P. K. Loeffler, *ibid.*, **82**, 3147 (1960). (d) G. Shaw and D. N. Butler, *J. Chem. Soc.*, 4040 (1959). (e) *Cf.* E. v. Meyer, *et al.*, *J. prakt. Chem.*, [2] **52**, 81 (1895); **78**, 497 (1906); **90**, 1 (1914).

TABLE III

4-AMINO-6,7-DIHYDRO-5H-PYRROLO[3,4-*d*]PYRIMIDINES



IX



XII

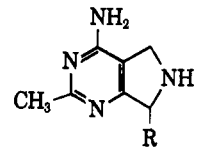
	R	R′	M.p., °C.	Yield[a]	λmax, mμ (log ε) 95% ethanol	λmax, mμ (log ε) 0.1 *N* HCl	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
IXhm	H	CH_3	311–312[b]	17 (A)	237 (4.09)	256 (4.15)	$C_8H_{10}N_4O$	53.92	5.66	31.45	53.65	5.83	31.38
				52 (C)	268 (3.68)								
IXmm	CH_3	CH_3	248	69 (B)	236 (4.12)	249 (3.99)	$C_9H_{12}N_4O$	56.23	6.29	29.15	56.21	6.39	28.96
					269 (3.44)								
IXmp	CH_3	C_6H_5	252–253[c]	6 (A)	236 (4.20)	249 (3.99)	$C_{14}H_{14}N_4O$	66.12	5.55	22.04	65.93	5.52	22.20
				84 (B)	265 (3.76)								
IXbp	$CH_2C_6H_5$	C_6H_5	210–211[c]	34 (A)	237 (4.11)	250 (4.10)	$C_{20}H_{18}N_4O$	72.70	5.49	16.96	72.59	5.41	17.35
				90 (B)	264 (i)[e] (3.68)								
XIIh	H		241	32	234 (3.95)	251 (3.97)	$C_6H_8N_4$	52.92	5.92	41.15	52.76	5.88	41.07
					269 (3.66)								
XIIm	CH_3		125–126[d]	64	235 (3.96)	250 (3.93)	$C_7H_{10}N_4 \cdot 2H_4O$	45.15	7.58	30.09	45.24	7.44	30.65
					269 (3.61)								

[a] A, B, or C indicates the procedure used; see Experimental. [b] Crystallized from dimethylformamide. [c] Crysallized from ethanol. [d] Dihydrate. Drying *in vacuo* at 140° yielded only impure hemihydrate, m.p. 178–179°. [e] Inflections are indicated by i.

TABLE IV

4-AMINO-2-METHYL-6,7-DIHYDRO-5H-PYRROLO[3,4-*d*]PYRIMIDINES

VII



X

	R	R′	M.p., °C.	Yield[a]	λmax, mμ (log ε) 95% ethanol	λmax, mμ (log ε) 0.1 *N* HCl	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
VIIhm	H	CH_3	>360[a]	35	235 (4.00)	248 (4.02)	$C_9H_{12}N_4O$	56.23	6.29	29.15	55.99	6.40	29.40
					268 (3.74)								
VIImp	CH_3	C_6H_5	289–290[a]	32	232 (4.08)	244 (4.12)	$C_{15}H_{16}N_4O$	67.14	6.01	20.88	66.99	5.82	20.69
					266 (3.65)								
VIIbp	$CH_2C_6H_5$	C_6H_5	227–228[b]	32	238 (4.19)	240 (4.11)	$C_{21}H_{20}N_4O$	73.23	5.85	16.27	73.09	5.96	16.18
					265 (3.78)								
Xh	H		205–206	64	234 (3.86)	248 (3.90)	$C_7H_{10}N_4$	55.98	6.71	37.31	55.81	6.80	36.98
					269 (3.63)								
Xm	CH_3		93–94	48	230 (3.86)	255 (3.87)	$C_8H_{12}N_4$	58.51	7.37	34.12	58.40	7.25	33.96
					267 (3.61)								

[a] Crystallized from dimethylformamide. [b] Crystallized from ethanol.

at the nitrogen atom to give some of the normal reactions of an amino function. The readily accessible 1-acyl-3-amino-4-cyano-3-pyrrolines (IV) can therefore be regarded as promising intermediates for the synthesis of a variety of compounds containing the pyrrolidine ring linked to other structures through nitrogen at the 3-position.

Removal of the acyl group from the compounds of types VII, VIII, and IX was accomplished without difficulty by alkaline hydrolysis in ethanol solution when R was hydrogen or a methyl group. The yields of the pyrrolo[3,4-*d*]pyrimidines of the types X, XI, and XII obtained in this deacylation step were in the range 32 to 69% (see Tables II, III, and IV). Thus acetyl or benzoyl proved to be suitable blocking groups in these cases, and there was no need for a more elaborate method of protecting the reactive basic nitrogen of the pyrrolidine ring. However, presumably because of steric hindrance, difficulty arose in the removal of 6-acyl groups from the compounds having a 7-benzyl group; attempted hydrolysis with sodium hydroxide in ethanol led merely to recovery of the starting materials, and more drastic procedures destroyed the pyrrolopyrimidine system. Further investigation will be required to permit the synthesis of compounds Xb, XIb, and XIIb. Preliminary experiments suggested that the use of protecting groups removable by hydrogenolysis might be complicated by competing ring hydrogenation.

In neutral ethanol or in alkaline solutions the ultraviolet spectra of the 2,4-diamino derivatives of types VIII and XI (see Table II) show the maximum at *ca.* 280 mμ characteristic of other compounds in which the 2,4-diaminopyrimidine structure is fused to a saturated ring through the 5- and 6-positions.[11] Similarly, the spectra of the 2-methyl-4-amino derivatives VII and X and the 4-amino derivatives IX and XII (Tables II

(11) (a) L. O. Ross, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3108 (1959); (b) E. J. Modest, S. Chalterjee, and H. Kangur, *J. Org. Chem.* **27**, 2708 (1962); (c) Q. E. Thompson, *J. Am. Chem. Soc.*, **80**, 5483 (1958).

and III) do not differ under neutral or alkaline conditions from the spectra of similarly constituted compounds incorporating the 4-amino-pyrimidine structure[11a]; the maxima are at *ca.* 237 and 265 mμ. However, in 0.1 *N* hydrochloric acid the positions of the absorption maxima for these three types of compounds correspond somewhat less exactly to those of the same related compounds, and it seems probable that the manner and/or extent of protonation of the pyrimidine system is altered by the amino or amido function incorporated into the pyrrolidine portion of the structure. Thus, the acid spectra of the 2,4-diamino derivatives VIII and XI show a shoulder at *ca.* 300 mμ[12] in addition to the expected maximum at *ca.* 272 mμ.[11] The 4-amino derivatives IX and XII and the 2-methyl-4-amino derivatives VII and X show their single maximum in acid solution at 240 to 256 mμ, whereas the maximum is reported to be at 262 mμ in acid solution for the analogous 4-amino-5,6-trimethylenepyrimidine[11a] and 4-amino-2-methyl-5,6-trimethylenepyrimidine.[11c]

With respect to infrared spectra, compounds of types X, XI, and XII show a very close resemblance to such analogous compounds as 2,4-diamino-5,6-trimethylenepyrimidine[11a] and 2,4-diamino-5,6,7,8-tetrahydroquinazoline,[11b] being characterized by bands at 2.9, 3.0, and 3.2 μ in the N–H stretching region and by three distinct bands in the range 6.0 to 6.4 μ. In general the compounds in the 6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidine series have the expected high melting points and rather low solubilities in most organic solvents. Compounds of the types X, XI, and XII displayed appreciable solubility in water and two compounds (IXm and XIIm) crystallized from water in the form of hydrates. No pure anhydrous form of compound XIIm has yet been obtained; it was analyzed in the form of the dihydrate.

Work on the synthesis of additional compounds in this series is in progress; compounds with other substituents, such as a sulfhydryl group in the 4-position, or other combinations of substituents, would be of interest from the standpoint of potential biological activity.[3,13]

Experimental[14]

2-Substituted 1-Acyl-4-cyano-3-oxopyrrolidines (IV).—The N-cyanoethyl derivatives of α-amino acid ethyl esters were acylated with benzoyl chloride or acetic anhydride. The oily acyl derivatives III, which were not fully purified, were treated with 1 mole of sodium ethoxide in absolute ethanol, using approximately 125 ml. of ethanol in the cyclization of 0.1 mole of the acyl derivative. The procedures given for compounds IVmp (a benzoyl derivative) and IVmm (an acetyl derivative) were followed closely with the other derivatives of the same types except for the necessary modifications indicated for certain individual compounds.

The infrared spectra of the enolic forms IV-E were usually observed when the measurements were made on Nujol mulls or potassium bromide pellets (*vide supra*). Characteristic bands in the 2.5- to 6.5-μ range were at 3.8–3.9 (broad), 4.5, 6.0, and 6.2 μ. The spectra of the ketonic forms IV-K were observed when measurements were made on chloroform solutions. In the 2.5- to 6.5-μ range characteristic bands were at 4.5, 5.6–5.7, and 6.2 μ.

1-Benzoyl-4-cyano-2-methyl-3-oxopyrrolidine (IVmp).—Freshly distilled *dl*-N-cyanoethylalanine ethyl ester[4] (42 g., 0.25 mole) was dissolved in dry pyridine (80 ml.). The solution was cooled to 5° and benzoyl chloride (38.5 g., 0.275 mole) was added dropwise to the stirred solution at such a rate that the temperature did not rise above 15° (about 1 hr. required). The mixture was stirred at room temperature overnight. It was then poured into ice–water (500 ml.) 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 (IIImp), was a yellow oil (60 g.) which failed to crystallize.

The oil (52 g., 0.2 mole) was dissolved in absolute ethanol (50 ml.) and a sodium ethoxide solution (0.2 mole, from 4.6 g. of sodium and 200 ml. of ethanol) was added. After a 2-hr. reflux period the ethanol was evaporated under reduced pressure and the solid residue was dissolved in water (150 ml.). The aqueous solution was extracted with ether, cooled, and acidified to congo red with 20% hydrochloric acid. The oil which precipitated was taken up in ether. The oily residue (37 g.), obtained after evaporation of the ether, solidified when allowed to stand overnight, and was crystallized twice from 70% aqueous ethanol to yield 31.5 g. (55% from IIm) of white plates, m.p. 145–147°.

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.13; H, 5.68; N, 12.14.

1-Acetyl-4-cyano-2-methyl-3-oxopyrrolidine (IVmm).—Acetic anhydride (50 ml.) was added with cooling to N-cyanoethylalanine ethyl ester (42 g., 0.25 mole). The mixture was kept at room temperature for 24 hr. Acetic acid and excess acetic anhydride were removed under reduced pressure using a rotary evaporator. (In instances in which the odor of the residue indicated incomplete removal of these materials, ethanol was added, and the evaporation process was repeated.) The oily residue was treated with 0.25 mole of sodium ethoxide as in the procedure given for the cyclization of the benzoyl derivative IVmp. The product was obtained as colorless needles, m.p. 149–150°, following crystallization from water; the yield was 22 g. (52%).

Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.92; H, 6.24; N, 16.81.

1-Benzoyl-4-cyano-3-oxopyrrolidine IVhp.—From 15.5 g. (0.1 mole) of N-cyanoethylglycine ethyl ester the crude product was obtained as a dark red oil. Several crystallizations from benzene yielded 1.86 g. (12%) of colorless cubes, m.p. 132–133°.

Anal. Calcd. for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.04; H, 5.03; N, 12.89.

1-Acetyl-4-cyano-3-oxopyrrolidine (IVhm).—N-Cyanoethylglycine ether ester (39 g., 0.25 mole) was acetylated by the procedure used in the preparation of compound IVmm. After treatment of the product with sodium ethoxide the sodium salt of IVhm began to precipitate. Following a 2-hr. reflux period the solution was cooled and the salt (34 g., 78%) was collected by filtration and washed with ether. Cold hydrochloric acid (1 *N*, 340 ml.) was poured, with cooling and shaking, upon the solid salt. The mixture was cooled for 2 hr. and filtered to collect the product, which was crystallized from water to yield 21 g. (55%) of colorless cubes, m.p. 171–172°.

Anal. Calcd. for $C_7H_8N_2O_2$: C, 55.25; H, 5.30; N, 18.41. Found: C, 55.07; H, 5.21; N, 18.25.

1-Benzoyl-4-cyano-2-benzyl-3-oxopyrrolidine (IVbp.)—Benzoylation and cyclization of 49 g. (0.2 mole) of *dl*-N-cyanoethylphenylalanine ethyl ester yielded, after crystallization from ethanol, 41 g. (59%) of IVbp as colorless needles, m.p. 179–180°. Analysis indicated that the substance was a solvate containing one molecule of ethanol.

(12) The 2-aminopyrimidines display a maximum in acid solution at *ca.* 300 mμ, the position at which this shoulder is found. Thus the spectra of 2,4-diamino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines in 0.1 *N* acid suggest the presence of a chromophore resembling that of the protonated 2-aminopyrimidines as well as that of protonated 2,4-diaminopyrimidines. *Cf.* D. J. Brown and L. N. Short, *J. Chem. Soc.*, 331 (1953); D. J. Brown, E. Hoerger, and S. F. Mason, *ibid.*, 4035 (1955). (In Tables II and III inflections are indicated by i.)

(13) Three of seven compounds tested in the previously described series of 5H-pyrrolo[3,4-*d*]pyrimidin-7(6H)-ones[2] showed some indication of activity in screening arranged by the Cancer Chemotherapy National Service Center. The three compounds were those having the following substitution: (1) 2-amino-4-hydroxy-; (2) 6-benzyl-2,4-dihydroxy-; and (3) 2-amino-6-benzyl-3,4-dihydro-4-phenyl-. All three passed stage one of the CCNSC sequential screen when tested against the solid Friend virus leukemia and the first likewise passed stage one in testing against hepatoma 129. The compounds failed, however, to pass stage two [see "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," *Cancer Chemotherapy Rep.*, **25**, 1 (1962)]. The third compound appeared to be somewhat inhibitory toward sarcoma 180 (tumor weight, test/control was 0.55 at a dose of 250 mg./kg.).

(14) Melting points are corrected. Microanalyses are by Drs. G. Weiler and F. B. Strauss, Oxford, England, and Galbraith Laboratories, Inc., Knoxville, Tenn. Ultraviolet spectra were determined with a Cary recording spectrophotometer; infrared spectra were determined with Perkin-Elmer Model 21 or Infracord spectrophotometers.

Anal. Calcd. for $C_{19}H_{16}N_2O_2 \cdot C_2H_5OH$: C, 71.98; H, 6.33; N, 8.00. Found: C, 72.15; H, 6.30; N, 8.62.

Recrystallization from toluene yielded the unsolvated compound as colorless plates, m.p. 180°.

Anal. Calcd. for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.21. Found: C, 74.50; H, 5.40; N, 9.40.

1-Acetyl-4-cyano-2-benzyl-3-oxopyrrolidine (IVbm).—Acetylation and cyclization of 24.5 g. (0.1 mole) of N-cyanoethylphenylalanine ethyl ester yielded, after crystallization from ethanol, 12.8 g. (53%) of IVbm as colorless cubes, m.p. 180–181°.

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.40; H, 5.83; N, 11.56 Found: C, 69.41; H, 5.81; N, 11.79.

1-Acyl-2-alkyl-4-cyano-3-methoxy-3-pyrrolines (V).—To a vigorously stirred suspension of a compound of type IV (0.05 mole) in ether (150 ml.) was added slowly an ethereal solution of diazomethane (*ca.* 3 g. from 21.5 g. of "Diazald"[15]). The compounds reacted and dissolved with a rapid evolution of nitrogen. The solutions were kept overnight at room temperature and then evaporated under reduced pressure. The residues were crystallized from a suitable solvent as indicated in Table I.

Infrared spectra measured on Nujol mulls showed characteristic bands at 4.5, 6.1, and 6.2 μ in the 2.5- to 6.5-μ range.

1-Acyl-2-alkyl-3-amino-4-cyano-3-pyrrolines (VI).—Solutions of the compounds of type IV (0.02 mole) and ammonium formate (2.52 g., 0.04 mole) in ethanol (20 ml.) were refluxed for 16 hr. The products (VI) precipitated after the solutions were cooled, and were crystallized from ethanol. The compounds prepared are listed in Table I.

Infrared spectra measured on Nujol mulls showed characteristic bands at 2.9, 3.1, 4.5, 6.0, and 6.2 μ in the 2.5- to 6.5-μ range.

6-Acyl-4-amino-2-methyl-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (VII).—Solutions of the compounds of type V (0.01 mole) in dry ethanol (10 ml.) were added to filtered solutions of acetamidine [0.02 mole, from 1.88 g. of acetamidine hydrochloride and 0.46 g. of sodium in dry ethanol (50 ml.)]. The solutions were refluxed for 16 hr., then evaporated under reduced pressure. The residues were washed with water and crystallized from suitable solvents as indicated in Table IV.

6-Acyl-4,4-diamino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (VIII). A. From the Enol Ethers V.—Compounds of type V (0.02 mole) were added to filtered solutions of 0.02 mole of guanidine (from 1.92 g. of guanidine hydrochloride and 0.46 g. of sodium) in 50 ml. of absolute ethanol, and the solutions were refluxed for 4 hr., then evaporated under reduced pressure. The residues were washed with water and crystallized from the solvents indicated in Table II.

B. From the Enamines VI.—Solutions of 0.01 mole of the compounds of type VI and 0.015 mole of guanidine in ethanol (prepared as in procedure A) were refluxed for 16 hr., then evaporated. The residues were washed with water and crystallized.

6-Acyl-4-amino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (IX). A. Formamidine Acetate Procedure.[10a]—Solutions of the compounds of type VI (0.01 mole) and formamidine acetate (10.3 g., 0.1 mole) in dimethylformamide (50 ml.) were refluxed for 1 hr. The dark solutions were concentrated to a volume of 20 ml. under reduced pressure, cooled, and filtered to collect the products in the form of black powders. The compounds were crystallized several times from dimethylformamide or ethanol as indicated in Table III.

B. First Ethyl Orthoformate Procedure.[10b-d]—Solutions of the compounds VI (2 g.) in ethyl orthoformte (8 ml.) and acetic anhydride (8 ml.) were refluxed for 2 hr., then evaporated under reduced pressure. The oily residues were dissolved in dry ethanol (25 ml.) and ethanolic ammonia solutions (excess) were added. After 5 min. the ethanol was evaporated and the residue crystallized.

C. Second Ethyl Orthoformate Procedure.—The following procedure[10a] was adopted for compound IXhm when procedure B proved unsuccessful. A mixture containing compound VIhm (2 g.), ethyl orthoformate (8 ml.), and acetic anhydride (8 ml.) was refluxed for 2 hr. while dry ammonia was bubbled into the solution. Then the mixture was cooled and filtered, and the precipitate was washed well with water to remove formamidine acetate formed. The water-insoluble residue was crystallized from dimethylformamide.

2- and/or 7-Substituted 4-Amino-6,7-dihydro-5H-pyrrolo[3,4-*d*]pyrimidines (X, XI, and XII).—To solutions of the 6-acyl derivatives (VII, VIII, or IX) (0.01 mole) in ethanol (135 ml.) were added solutions of sodium hydroxide (7.5 g.) in water (15 ml.). After a reflux period of 8 hr. the solutions were concentrated under reduced pressure to *ca.* 75 ml., cooled, and diluted with water. The products were collected by filtration and crystallized from water. The compounds of types X, XI, and XII which were prepared are listed in Tables IV, II, and III, respectively.

(15) N-Methyl-N-nitroso-*p*-toluenesulfonamide (Aldrich Chemical Co.) was used as described by T. J. DeBoer and H. J. Backer, *Rec. trav. chim.*, **73**, 229 (1954).

Isomeric Pyrazolo[4,3-*d*]pyrimidinediones

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Diazotization of 1,3,6-trimethyl-5-aminouracil (Ia) followed by cyclization of the diazonium salt with strong alkali gave 4,6-dimethylpyrazolo[4,3-*d*]pyrimidinedione (IV), an isomer of theophylline. Methylation of IV produced 1,4,6-trimethylpyrazolo[4,3-*d*]pyrimidinedione (V), m.p. 211–213°. Since this compound differed greatly from one tentatively assigned the same structure by Robins,[5] the methylation of pyrazolo[4,3-*d*]pyrimidinedione was repeated. Both V and the 2,4,6-trimethylpyrazolo[4,3-*d*]pyrimidinedione (VII), m.p. 261–264°, were obtained. The structure of VII was established by an independent synthesis from 1,3-dimethylpyrazole-5-carboxylic acid (VIII). Corresponding triethylpyrazolo[4,3-*d*]pyrimidinediones were also synthesized. The n.m.r. spectra of the various compounds are consistent with the structures assigned by chemical methods.

Our interest in the possible physiological activity of alkyl derivatives of 1-H-pyrazolo[4,3-*d*]pyrimidine-5,7(4H,6H)-dione (VI), an isomer of xanthine, led to the investigation of methods for the synthesis of the 4,6-dimethyl derivative IV, an isomer of theophylline.[2] We had previously shown that treatment of 5-amino-6-methyluracil (Ib) with excess nitrous acid produced pyrimido[5,4-*d*][1,2,3]triazine-6,8(5H,7H)-dione 3-oxide (IIb).[3] Both Behrend[4] and Robins[5] have reduced IIb with stannous chloride to the pyrazolopyrimidine VI. However, reduction of the corresponding 5,7-dimethylpyrimido[5,4-*d*][1,2,3]triazine-6,8(5H,7H)-dione 3-oxide (IIa) by a similar method failed to give us the desired dimethylpyrazolopyrimidine IV. Instead, a compound analyzing for three nitrogen atoms

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(2) For studies on substituted pyrazolo[3,4-*d*]pyrimidines, see P. Schmidt, K. Eichenberger, and M. Wilhelm, *Helv. Chim. Acta*, **45**, 1620 (1962), and R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6407 (1957), and preceding papers in both series.

(3) V. Papesch and R. M. Dodson, *J. Org. Chem.*, **28**, 1329 (1963).

(4) R. Behrend, *Ann.*, **245**, 213 (1888).

(5) R. K. Robins, F. W. Furcht, A. D. Grauer, and J. W. Jones, *J. Am. Chem. Soc.*, **78**, 2418 (1956).