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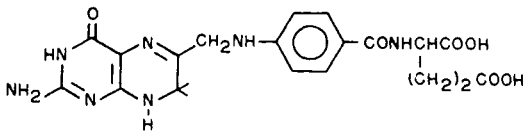
Irreversible Enzyme Inhibitors. LXXXI. Further Observations on the Mode of Binding of the Anilino Moiety of 2-Amino-5-(anilinopropyl)-6-methyl-4-pyrimidinol to Dihydrofolic Reductase and Thymidylate Synthetase (1,2)

B. R. Baker and Jaroslav Novotny

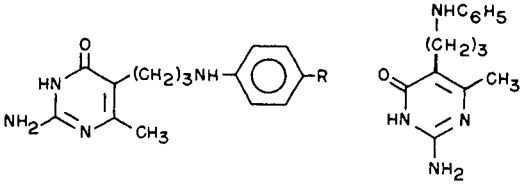
Replacement of phenyl group of 2-amino-5-(anilinopropyl)-6-methyl-4-pyrimidinol (III) by benzyl (XI) led to a large loss in binding to both dihydrofolic reductase and thymidylate synthetase; the binding by XI returned when the protonated benzylamino group was *N*-acetylated to XII, which removes the charge at *p*H 7.4 and changes the ground-state conformation of the benzene ring. Replacement of the benzyl group of the acetamide, XII, with the polar 2-, 3-, or 4-, picolyl groups also led to a loss in binding. Substitution of *p*-fluoro or *m*-trifluoromethyl on the anilino group of III, or replacement of the anilino of III by 3-pyridylamino, gave little -- if any -- enhancement in binding to the enzymes.

Early in our program on synthesis and evaluation of inhibitors of (dihydro)folic reductase (3), it was observed that the pyrimidyl analog (II) of tetrahydrofolic acid was a good inhibitor of the enzyme; with folic acid as a substrate, II was complexed 5-fold better than the substrate (4), while with dihydrofolate (II) as substrate, II was complexed 16-fold less effectively than II (5). Similarly, II was an inhibitor of thymidylate synthetase, being complexed 23-fold less effectively than the substrate, *l*-tetrahydrofolate (6). Removal of the carboxy-L-glutamate moiety from II to give III (7) resulted in only an 8-fold loss in binding to dihydrofolic reductase (5) and a 2-fold loss in binding to thymidylate synthetase (6); these results opened up the possibility of studying the mode of binding of the anilino moiety of III to the two enzymes. One such study on these two enzymes indicated that the NH of the anilino moiety of III was not necessary for binding and that the binding by the phenyl ring could be increased by a *p*-chloro substituent (8); with these results in hand, the current study on the mode of phenyl binding of III to the two enzymes was initiated and the results are the subject of this paper.

Near the completion of the work reported here, the strong hydrophobic bonding region on dihydrofolic reductase was discovered (9) with its effect on the possible rotomeric conformations of inhibitor



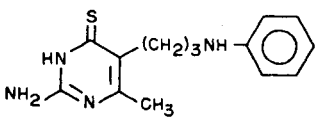
I



II, R = -CONHCHCOOH
(CH2)2COOH

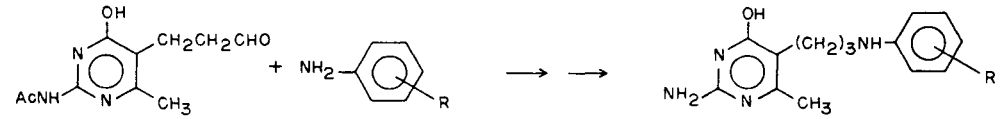
III B

III A, R = H



IV A

binding to the enzyme (3,10-16). If the pteridine ring of dihydrofolate (I) is arbitrarily assigned the conformation I, then the anilinopropyl moiety of III is believed to be hydrophobically bonded in conformation III*B*, rather than the normal conformation III*A* (15,16); in contrast, the 4-thiopyrimidine, IV, is more probably complexed normally in the IV*A* conformation (17). Therefore the report of the current study was delayed due to the ambiguity of binding of the 5-anilinopropyl side-chain of these

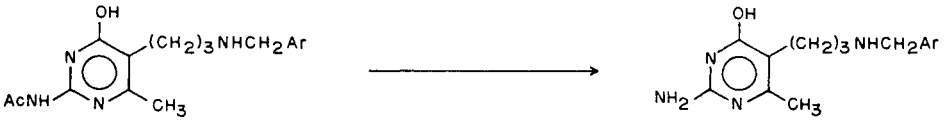


XVIII XIX

VII, R = p-F

VIII, R = m-CF_3

XVII, R = 3,4-CH=CHCH=CH-



XX, Ar = C_6H_5

XXI, Ar = 2-pyridyl

XXII, Ar = 3-pyridyl

XXIII, Ar = 4-pyridyl

XXIV, Ar = 2-furyl

XI, Ar = C_6H_5

XXV, Ar = 2-pyridyl

XXVI, Ar = 3-pyridyl

XXVII, Ar = 4-pyridyl

XXVIII, Ar = 2-furyl

XXIX, Ar = C_6H_5

XXX, Ar = 2-pyridyl

XXXI, Ar = 3-pyridyl

XXXII, Ar = 4-pyridyl

XXXIII, Ar = 2-furyl

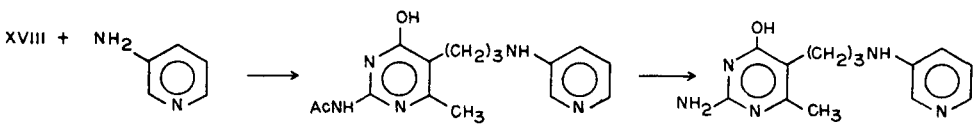
XII, Ar = C_6H_5

XIII, Ar = 2-pyridyl

XIV, Ar = 3-pyridyl

XV, Ar = 4-pyridyl

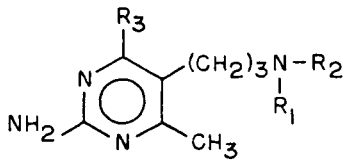
XVI, Ar = 2-furyl

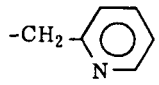
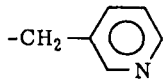
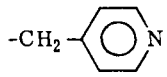
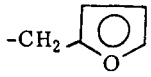
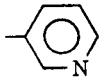


XXXIV XXXV

TABLE I

Inhibition of Dihydrofolic Reductase and Thymidylate Synthetase By

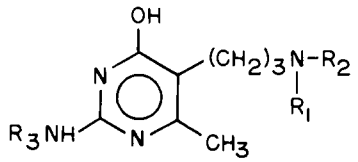


No.	R_1	R_2	R_3	Dihydrofolic Reductase (a) mM Conc.	% Inhib.	$[I/S]_{0.5}$ (c)	Thymidylate Synthetase (b) mM Conc.	% Inhib.	$[I/S]_{0.5}$ (d)
III (e)	H	C_6H_5-	OH	0.60	43	130	1.2	50	50
IV (f)	H	C_6H_5	SH	0.022 (g)	50	7.3	0.080 (h)	38	11
V (f)	H	p-ClC_6H_4-	SH	0.014	50	2.3	0.018 (h)	50	1.4
VI (f)	H	p-$Me_2NC_6H_4$	SH	0.030 (g)	50	10	0.080 (h)	40	8.6
VII	H	p-FC_6H_4-	OH	0.64	50	110	0.25	34	19
VIII	H	m-$CF_3C_6H_4$-	OH	0.36	50	60	0.050	0	> 8 (i)
IX (j)	H	p-$NO_2C_6H_4$	OH	0.15	33	52			
X (f,k)	$H_2^{\oplus}$	n-C_4H_9-	OH	6.0	0	> 4000	4.5	50	350
XI (k,l)	$H_2^{\oplus}$	$C_6H_5CH_2$-	OH	4.0	0	> 2700	6.0	35	400
XII	CH_3CO-	$C_6H_5CH_2$-	OH	0.60	50	100	1.0	50	39
XIII	CH_3CO		OH	1.8	50	300	12.7	50	490
XIV	CH_3CO		OH	3.6	50	600	4.8	50	190
XV	CH_3CO		OH	3.6	50	600	5.3	50	200
XVI	CH_3CO		OH	0.21	50	35	4.5	50	170
XVII	H	β-naphthyl	OH	0.28	50	47	0.13 (m)	13	~30
XXXV	H		OH	1.0	50	110	1.0 (m)	37	47

(a) Dihydrofolic reductase was a 45-90% ammonium sulfate fraction from pigeon liver prepared and assayed with 6 μM dihydrofolate and 12 μM TPNH in 0.05 M Tris buffer (pH 7.4) containing 10 mM mercaptoethanol and 10% N,N-dimethylformamide as previously described (6). (b) Thymidylate synthetase was a 45-90% ammonium sulfate fraction from *E. coli* B prepared and assayed with 51.4 μ *dl*-tetrahydrofolate and 80 μM 2'-deoxyuridylate in 0.05 M Tris buffer (pH 7.4) containing 10 mM mercaptoethanol and 5% 2-methoxyethanol as previously described (6). (c) Estimated ratio of concentration of inhibitor to 6 μM dihydrofolate giving 50% inhibition. (d) Estimated ratio of concentration of inhibitor to 25.7 μM *l*-isomer of tetrahydrofolate giving 50% inhibition. (e) Data previously reported (6). (f) Data previously reported (8). (g) Assayed with 3 μM dihydrofolate. (h) Assayed with 25.7 μM *dl*-tetrahydrofolate. (i) Since 20% inhibition is readily detectable, the concentration of inhibitor for 50% inhibition is at least 4-times greater than that measured. (j) Synthesis previously described (20). (k) No organic solvent used in assay. (l) Similar results were obtained when the phenyl ring was replaced by 2-, 3-, or 4-pyridyl groups as in XXV-XXVII. (m) Maximum solubility.

The technical assistance of Barbara Baine, Maureen Baker, Ann Jaqua and Karen Smith with these assays is acknowledged.

TABLE II

Physical Constants of 

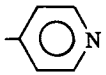
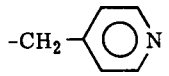
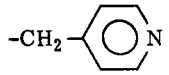
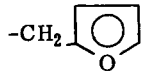
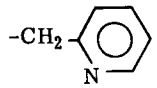
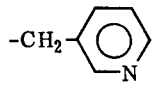
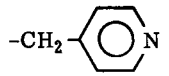
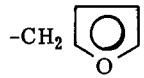
Compound No. (a)	R_1 (b)	R_2	R_3 (b)	Method	% Yield	M.p. °C	Anal. Calcd. C	H	N	Found C	H	N
VII	H	*p*-FC_6H_4-	H	A	75	200-201 (c)	60.9	6.20	20.3	60.6	6.05	20.1
VIII	H	*m*-$CF_3C_6H_4$-	H	A	85	195-196 (c)	55.2	5.25	17.2	54.9	5.30	17.0
XI (d)	H	$C_6H_5CH_2$-	H	B	65	242-243 (d, e)	44.4 (d)	3.59	19.2	44.1	3.71	19.0
XI (f)	H	$C_6H_5CH_2$	H	C	63	271-272 (f, g)	52.2	6.42	16.2 (h)	52.2	6.31	16.1 (h)
XII	Ac	$C_6H_5CH_2$	H	H	45	93-95 (i)	65.0	7.05	17.8	65.0	7.10	17.6
XIII	Ac	-CH_2- 	H	H	77	amorphous (j)	43.5 (j)	3.52	19.9	43.5 (j)	3.53	19.9
XIV	Ac	-CH_2- 	H	H	50	amorphous (k)	43.5 (k)	3.52	19.9	43.3 (k)	3.55	19.8
XV	Ac	-CH_2- 	H	H	62	106-108 (i, l)	60.9	6.71	22.2	60.7	6.77	22.9
XVI	Ac	-CH_2- 	H	H	40	81-82 (i)	59.2	6.62	18.4	59.2	6.57	18.4
XVII	H	β-naphthyl	H	G	36	232-233 (m)	70.1	6.53	18.2	69.9	6.70	18.0
XXV	H	-CH_2- 	H	G	65	182-183 (i, n)	59.0 (n)	7.19	24.6	59.1 (n)	7.30	24.6
XXVI	H	-CH_2- 	H	G	70	200-201 (i, o)	59.6 (o)	7.14	24.8	59.7 (o)	7.23	24.7

TABLE II (Continued)

Compound No. (a)	R_1 (b)	R_2	R_3 (b)	Method	% Yield	M.p. °C	Anal. Calcd. C	H	N	Found C	H	N
XXVII (f)	H		H	C	53	281-282 (p)	48.6 (f)	6.11	20.2	48.4 (f)	6.24	20.0 (q)
XXVII	H		H		76 (r)	69-70 (i)	57.6	7.16	24.1	57.7	7.26	24.0
XXVIII (f)	H		H	H	54	287-288 (s)	46.6 (f)	6.01	21.1 (v)	46.3	5.94	21.0 (v)
XXIX	Ac	$C_6H_5CH_2$-	Ac	E	49	128-129 (t)	64.0	6.79	15.6	63.9	7.00	15.6
XXX	Ac		Ac	F	69	187-188 (p)	60.5	6.49	19.6	60.3	6.70	19.4
XXXI	Ac		Ac	F	72	178-179 (p)	60.5	6.49	19.6	60.2	6.41	19.5
XXXII	Ac		Ac	F	64	153-154 (p)	60.5	6.49	19.6	60.2	6.29	19.4
XXXIII	Ac		Ac	F	53	83-85 (u)	58.9	6.40	16.2	58.7	6.37	16.0
XXXIV	H		Ac	D	62	210-211 (e)	59.8	6.36	23.2	60.0	6.57	23.3
XXXV	H		H	H	65	236-237 (i)	60.2	6.61	27.0	60.0	6.68	26.8

(a) All compounds had infrared and ultraviolet spectra compatible with their assigned structures and moved as single spots on TLC. (b) Ac = acetyl. (c) Recrystallized from methanol-water. (d) Dipicrate. (e) Recrystallized from 50% aqueous ethanol. (f) Dihydrochloride. (g) Recrystallized from methanol-acetone. (h) Calcd. Chlorine: 20.5; found, 20.4. (i) Recrystallized from water. (j) Characterized as dipicrate formed in 86% yield from ethanol, m.p. 213-214°. (k) Characterized as dipicrate formed in 85% yield from ethanol, m.p. 189-190°. (l) Gave an 82% yield of crystalline dipicrate, m.p. 220-221°. (m) Recrystallized from aqueous 2-methoxyethanol. (n) Sinters at 52-54° and was a 2/3 hydrate after drying. (o) Sinters at 66-68° and was a 1/2 hydrate after drying. (p) Recrystallized from ethanol-acetone-ether. (q) Calcd. Chlorine: 20.5; found, 20.5. (r) By neutralization of dihydrochloride in water. (s) Recrystallized from aqueous acetone. (t) Recrystallized from ethanol-ether-petroleum ether. (u) Recrystallized from ethyl acetate-ether. (v) Chlorine.

pyrimidines; rationalizations of the data of dihydrofolic reductase in Table I are still difficult to make since it is not certain when the 5-side-chain is complexed in the hydrophobic region or when it is complexed in the *p*-aminobenzoyl binding locus for I (14).

The benzylaminopropyl side-chain (XI) -- being fully protonated at the *p*H 7.4 of the assays -- was poorly complexed to either enzyme as previously noted with the butylaminopropyl side-chain (X) (8). However, when the NH group of this side-chain of XI was acetylated, the resultant XII was as good an inhibitor of both enzymes as the prototype anilinopropyl pyrimidine, III. It is of interest that this longer side-chain of XII complexes satisfactorily; the conformation of the phenyl group of XII is different than in XI since the amide group of XII places the methylene group of the benzyl in the same plane as the nitrogen of the amide. Thus the phenyl group of III and XII are in about the same plane, but that of the phenyl ring of the amine, XI, is not.

Although the *p*-chloro group of V gave an increment in binding to both enzymes compared to IV, such an increment was not observed with the *m*-trifluoromethyl (VIII) or *p*-fluoro (VII) groups. Similarly, replacement of the phenyl group of III by the more polar and more electronegative 3-pyridyl group (XXXV) had little effect on binding.

Replacement of the phenyl group of XII by the highly polar 2-, 3-, or 4-pyridyl rings (XIII-XV) led to considerable loss in binding to both enzymes; however, the same replacement by a furan ring (XVI) led to a better inhibitor of dihydrofolic reductase but a poorer inhibitor of thymidylate synthetase. The β-naphthyl group (XVII) gave better binding than phenyl (III) to dihydrofolic reductase. These results on dihydrofolic reductase are difficult to rationalize with only a single mode of binding since the furan is smaller and more polar than phenyl, the naphthyl is larger and less polar than phenyl, and pyridyl is about the same size as phenyl, but more polar.

The two substituted anilinopropyl pyrimidines, VII and VIII, were synthesized by the previously developed general route (4,7) for the synthesis of III; reductive condensation of the pyrimidine-5-propionaldehyde, XVIII, and the requisite amine (XIX) with sodium borohydride gave VII and VIII in excellent yield. Reductive condensation of XVIII with benzylamine in the presence of sodium borohydride gave, after hydrolysis of the N_2-acetyl group, crude XI which could not be crystallized; however, a crystalline dipicrate could be isolated in 65% yield which was converted to the crystalline dihydrochloride of XI in good yield. This procedure was unsatisfactory for the picolylamines such as XXV since the picrates could not be purified due to being a mixture of di- and tri-picrates and being contaminated with the dipicrate of the picolylamine; therefore catalytic reductive condensation methods were investigated.

Reductive condensation of XVIII with a 4:1 ratio of benzylamine using a Raney nickel catalyst gave a mixture of XI and XX, due to partial deacetylation by the excess benzylamine; hydrolysis of the mixture with aqueous hydrochloric acid gave XI in 63% overall yield (from XVIII) isolated as the dihydrochloride. The crystalline free base of XI was then readily prepared from the dihydrochloride with aqueous sodium hydroxide.

A lengthy study on selective side-chain acetylation of XI to XII proved unsuccessful; when the reactions were followed by thin layer chromatography, it was noted that when the reaction was run long enough to remove all the starting material, XI, an almost equal amount of diacetyl derivative, XXIX, was formed. Therefore, the full acetylation of XI to XXIX followed by selective removal of the N_2-acetyl group was investigated.

With excess acetic anhydride in boiling acetone, XI was converted to its crystalline diacetyl derivative, XXIX, in good yield; the N_2-acetyl group could then be removed selectively with *n*-butylamine in boiling methanol (18) to give pure crystalline XII in 45% yield. The overall procedure was then further simplified by direct acetylation of the reduction mixture of XVIII and benzylamine to give XXIX in 49% yield for the two steps. Further studies show that the catalyst of choice for the reductive condensation was platinum oxide; Raney nickel was also effective, but the product was removed from the catalyst with difficulty. Palladium-charcoal required one-two days for complete reduction, whereas platinum oxide was complete in a few minutes.

The 3-pyridylaminopropyl (XXXV) and the β-naphthylaminopropyl (XVII) pyrimidines were also prepared by the catalytic method followed by removal of N_2-acetyl group with *n*-butylamine in methanol. Attempts to prepare XXXV by reductive condensation of XVIII with 3-aminopyridine mediated by sodium borohydride gave mixtures from which pure XXXV could not be isolated.

EXPERIMENTAL

Melting points were determined in capillary tubes on a Mel-temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer. Thin layer chromatograms (TLC) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light.

2-Amino-6-methyl-5-(*m*-trifluoromethylanilinopropyl)-4-pyrimidinol (VIII). Method A (4,7).

A solution of 112 mg. (0.5 mmole) of XVIII (4,19) and 402 mg. (2.5 mmoles) of *m*-trifluoromethylaniline in 2 ml. of *N,N*-dimethylformamide was allowed to stand 30 minutes, then diluted with 10 ml. of ethanol. With magnetic stirring, 0.80 g. sodium borohydride was added in portions over 30 minutes. After being stirred for about 18 hours, the mixture was treated with 5 ml. of 0.1 *N* aqueous sodium hydroxide, then spin-evaporated *in vacuo*. The residue was dissolved

in a minimal amount of 3 *N* aqueous hydrochloric acid; the filtered solution was adjusted to *p*H 8-9 with 1.5 *N* aqueous sodium hydroxide. The product was collected on a filter, washed with water, then recrystallized from aqueous methanol; yield, 138 mg. (85%) of white needles, m.p. 195-196°; ν max 3480, 3300, 3100 (NH); 1675, 1650, 1615 (NH, C=O, C=N, C=C); 1340, 1155, 1105 (CF_3); 785, 693 cm^{-1} (*m*-C_6H_4); λ max (*p*H 1): 227 (ϵ, 11,100), 265 mμ (ϵ, 9,100); (*p*H 7): 246 (ϵ, 15,200), 290 mμ (ϵ, 6,400); (*p*H 13): 244 (ϵ, 16,300), 281 mμ (ϵ, 8,300). See Table II for analytical data.

2-Amino-5-benzylaminopropyl-6-methyl-4-pyrimidinol (XI) Dipicrate. Method B.

A mixture of 112 mg. (0.5 mmole) of XVIII and 268 mg. (2.5 mmoles) of benzylamine was reductively condensed with 0.20 g. of sodium borohydride as described in Method A. After the hydrochloric acid solution was adjusted to *p*H 9, no product separated. The solution was spin-evaporated *in vacuo*; the residue was extracted with boiling chloroform (5 x 10 ml.). The combined extracts, dried with magnesium sulfate, were spin-evaporated *in vacuo* leaving a glass that could not be crystallized.

The glass was dissolved in a minimal amount of hot ethanol, then the solution was treated with 10 ml. of saturated alcoholic picric acid. The mixture was heated to boiling, then allowed to stand at ambient temperature. The dipicrate (260 mg.) was collected on a filter, washed with ice-cold ethanol, then recrystallized from 50% aqueous ethanol; yield, 237 mg. (65%) of yellow needles, m.p. 242-243°. See Table II for analytical data.

A mixture of 237 mg. (0.32 mmole) of XI dipicrate and 36 ml. of 1 *N* aqueous hydrochloric acid was heated on a steam-bath until solution was complete. The cooled solution was washed with chloroform (3 x 10 ml.) until the washings were colorless. Spin-evaporation of the aqueous solution *in vacuo* left 86 mg. (97%) of crystalline XI dihydrochloride. Recrystallization from methanol-acetone gave 50 mg. (57%) of white prisms, m p. 271-272°; ν max 3350, 3150 (broad NH); 2950, 2800, 2600, 2440, 2380 (NH^+); 1680 (C=NH^+); 1650, 1540 (NH, C=N, C=O, C=C); 755, 698 cm^{-1} (C_6H_5); λ max (*p*H 1): 227 (ϵ, 12,000), 265 mμ (ϵ, 9,300); (*p*H 7): 225 (sh., ϵ, 12,200), 272 (ϵ, 5,500), 290 mμ (sh., ϵ, 4,800); (*p*H 13): 229 (sh., ϵ, 10,800), 281 mμ (ϵ, 7,600). See Table II for analytical data.

To a solution of 172 mg. (0.5 mmole) of XI dihydrochloride in 1 ml. of water was added 1 ml. of 1 *N* aqueous sodium hydroxide. The crystalline free base (128 mg.) was collected on a filter and washed with ice water. Recrystallization from water gave 82 mg. (60%) of white rosettes, m.p. 208-211°. A second recrystallization gave the analytical sample, m.p. 210-211°; ν max 3380, 3100 (NH); 1650, 1620, 1570, 1540 (NH, C=C, C=O); 740, 700 cm^{-1} (C_6H_5).

Anal. Calcd. for $C_{15}H_{20}N_4O$: C, 65.9; H, 7.40; N, 20.6. Found: C, 65.9; H, 7.28; N, 20.4.

2-Amino-6-methyl-5-(4-picolylaminopropyl)-4-pyrimidinol (XXVII) Dihydrochloride. Method C.

A solution of 1.11 g. (5 mmoles) of XVIII and 1.30 g. (5 mmoles) of 4-aminomethylpyridine in 100 ml. of absolute ethanol was shaken with hydrogen at 2-3 atmospheres in the presence of about 10 g. of Raney nickel for about 18 hours during which time reduction was complete. The catalyst was removed by filtration through a pad of Celite; the catalyst cake was extracted with hot ethanol (3 x 100 ml.) to remove absorbed product from the catalyst. The combined filtrate and extracts were spin-evaporated *in vacuo*. The oily residue was triturated with ethyl acetate; yield, 0.911 g. (67%) of crude XXIII.

A solution of 200 mg. of crude XXIII in 10 ml. of 3 *N* hydrochloric acid was heated on a steam-bath for 15 minutes, then spin-evaporated *in vacuo*. Recrystallization of the residue from ethanol-acetone gave 202 mg. (82%) of the hydrochloride as white prisms, m.p. 281-282°; ν max 3350, 3230 (broad, NH); 2950, 2800, 2760, 2700, 2560, 2450, 2400 (NH^+); 1680 (C=NH^+); 1650, 1600, 1570, 1550, 1520 cm^{-1} (C=O, NH, C=C, C=N); λ max (*p*H 1): 229 (ϵ, 8,800), 263 mμ (ϵ, 11,200); (*p*H 7): 225 (sh., ϵ, 10,000), 265 (ϵ, 6,200), 290 mμ (sh., ϵ, 3,600); (*p*H 13): 235 (ϵ, 7,600), 280 mμ (ϵ, 6,000). See Table II for analytical data.

2-Acetamido-6-methyl-5-(3-pyridylaminopropyl)-4-pyrimidinol (XXXIV). Method D.

A solution of 223 mg. (1 mmole) of XVIII and 94 mg. (1 mmole) of 3-aminopyridine in 100 ml. of ethanol was shaken with hydrogen at 2-3 atmospheres in the presence of 150 mg. of 5% palladium-charcoal. Reduction was complete after 80 hours. The filtered solution was spin-evaporated *in vacuo*, yield, 312 mg. (98%) of crystalline residue, m.p. 203-204°. Recrystallization from ethanol gave 196 mg. (62%) of white needles, m.p. 210-211°; ν max 3380, 3200 (NH); 1690 (amide C=O); 1650, 1620, 1595, 1583 (NH, C=O, C=N, C=C); 820, 800, 780, 730, 705 cm^{-1} (*m*-$C_5H_{10}N$); λ max (*p*H 1): 248 (ϵ, 15,800), 264 (ϵ, 20,400), 344 mμ (ϵ, 3,400); (*p*H 7): 250 (ϵ, 17,800), 290 mμ (ϵ, 7,200); (*p*H 13): 248 (ϵ, 17,800), 275 mμ (sh., ϵ, 8,800). See Table II for analytical data.

2-Acetamido-5-(*N*-acetyl-3-benzylaminopropyl)-6-methyl-4-pyrimidinol (XXIX). Method E.

An ethanolic solution of 1.116 g. (5 mmoles) of XVIII and 2.14 g. (20 mmoles) of benzylamine was reductively condensed as described in method C. The crude XX was triturated with acetone; yield, 945 mg. (70%), m.p. 190-195°.

A mixture of 928 mg. of crude XX, 20 ml. of acetone and 5 ml. of acetic anhydride was refluxed for 4 hours when TLC showed the reaction was complete. The solution was spin-evaporated *in vacuo*. The residue was crystallized from ethanol by addition of one volume of ether and four volumes of petroleum ether; yield, 0.854 g. (70%), m.p. 124-127°. Recrystallization from the same solvent system gave white crystals, m.p. 128-129°; ν max 3230, 3100 (NH); 1690 (amide C=O); 1660, 1640, 1620, 1570, 1540 (NH, C=O, C=C, C=N); 730, 720, 698 cm^{-1} (C_6H_5); λ max (*p*H 1): 245 (ϵ, 11,400), 265 mμ (ϵ, 9,000); (*p*H 7): 246 (ϵ, 8,800), 290 mμ (ϵ, 5,200); (*p*H 13): 247 (ϵ, 8,600), 278 mμ (ϵ, 7,400). See Table II for analytical data.

2-Acetamido-5-(*N*-acetyl-2-picolylaminopropyl)-6-methyl-4-pyrimidinol (XXX). Method F.

A solution of 1.116 g. (5 mmoles) of XVIII and 1.298 g. (11 mmoles) of 2-aminomethylpyridine in 100 ml. of methanol was shaken with hydrogen at 2-3 atmospheres in the presence of 75 mg. of platinum oxide; reduction was complete in 90 minutes. The filtered solution was spin-evaporated *in vacuo*. The residue was acetylated in acetone as in method E. Trituration of the residue with acetone gave 0.863 g. (69%) of product, m.p. 182-184°, suitable for the next step. Recrystallization of a sample from ethanol-acetone gave white prisms, m.p. 187-188°; ν max 3220, 3100 (NH); 1690 (amide C=O); 1660, 1640, 1620, 1570, 1540, 1520 (NH, C=O, C=C, C=N); 772 (*p*-$C_5H_{10}N$); λ max (*p*H 1): 245 (sh., ϵ, 11,100), 257 (sh., ϵ, 12,500), 261 (infl., ϵ, 12,700), 266 (sh., ϵ, 13,300), 290 mμ (sh., ϵ, 3,800); (*p*H 7): 245 (sh., ϵ, 8,900), 256 (ϵ, 9,500), 262 (ϵ, 8,300), 268 (ϵ, 7,300), 290 mμ (sh., ϵ, 4,600); (*p*H 13): 245 (sh., ϵ, 6,800), 258 (sh., ϵ, 7,500), 263 (ϵ, 7,500), 270 (ϵ, 6,900), 277 mμ (sh., ϵ, 5,400). See Table II for analytical data.

2-Amino-6-methyl-5-(2-furfurylaminopropyl)-4-pyrimidinol (XXVIII) Dihydrochloride. Method G.

Reductive condensation of XVIII with furfurylamine by method F gave an 87% yield of crude XXIV. A solution of 645 mg. of crude XXIV in 20 ml. of methanol containing 0.15 ml. of *n*-butylamine was refluxed for 1 hour then spin-evaporated *in vacuo*. The residue was heated on a steam-bath with 15 ml. of 1 *N* aqueous hydrochloric acid for 30 minutes, then spin-evaporated *in vacuo*. Recrystallization from ethanol-acetone gave 217 mg. (42%) of the hydrochloride as white needles, m.p. 282-284°. The analytical sample was prepared by one more recrystallization and had m.p. 287-288°; ν max 3380, 3200 (NH); 2950, 2800, 2760, 2700, 2580, 2450, 2400 (NH^+); 1690 (C=NH^+); 1650, 1600, 1570, 1550, 1530, 1500 (NH, C=C, C=O, C=N); 884, 820, 750 cm^{-1} (furan); λ max (*p*H 1): 230 (infl., ϵ, 12,000), 268 mμ (ϵ, 7,900); (*p*H 7): 230 (infl., ϵ, 10,200), 272 (ϵ, 4,100), 290 mμ (sh., ϵ, 3,300); (*p*H 13): 235 (sh., ϵ, 9,800), 280 mμ (ϵ, 7,100). See Table II for analytical data.

In some cases, such as XIV, the free base was recrystallized from water rather than being converted to the dihydrochloride.

2-Amino-5-(*N*-acetyl-3-picolylaminopropyl)-6-methyl-4-pyrimidinol (XIV). Method H.

A solution of 715 mg. (2 mmoles) of XXXI in 20 ml. of methanol containing 0.12 ml. of *n*-butylamine was refluxed for 1 hour, then evaporated *in vacuo*. Trituration with ether gave 582 mg. (93%) of crude product, m.p. 80-85°. Recrystallization from ethanol-ethyl acetate-ether gave 385 mg. (62%) of white crystals, m.p. 106-108°; λ max 3400, 3180 (NH); 1650, 1620, 1570, 1550-1530 cm^{-1} (NH, C=C, C=O, C=N). See Table II for analytical data.

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