Analogs of Tetrahydrofolic Acid XXIV

Further Observations on the Mode of Pyrimidyl Binding to Dihydrofolic Reductase and Thymidylate Synthetase by the 2-Amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol Type of Inhibitor

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2-Amino-6-methyl-5-(3-phenylpropylamino)-4-pyrimidinol (VI) was found to be as effective as 2-amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol and 2-amino-6-methyl-5-(4-phenylbutyl)-4-pyrimidinol (IV) as an inhibitor of thymidylate synthetase. The effectiveness of IV was reduced when the 6-methyl group was replaced by 6-amino (VIII). In contrast, VIII was about a fourfold better inhibitor of dihydrofolic reductase than IV. Similarly, 5-(4-phenylbutyl)-2,4,6-triaminopyrimidine (IX) was about a ninefold better inhibitor of dihydrofolic reductase than IV; however, IX was only about one-eightieth as effective as 2,4-diamino-6-methyl-5-(4-phenylbutyl)pyrimidine (XVII). With dihydrofolic reductase, 5-(3-phenylpropyl)-2,4,6-triaminopyrimidine (XV) was nearly as effective as IX. Compounds VIII, IX, and XV bind to dihydrofolic reductase about as well as the substrate, dihydrofolate, and are easily synthesized in two steps from commercially available materials.

In a previous study the inhibition of thymidylate synthetase by the pyrimidyl analogs (I, II) of tetrahydrofolate was reported (1); furthermore, removal of the carboxyl-L-glutamate as in II gave only a twofold loss in binding compared to I. However, the ratio of I to the *l*-isomer of tetrahydrofolate necessary for 50% inhibition of thymidylate synthetase was a relatively high 23, whereas I was bound to folic reductase tighter than the substrate, folic acid (2). Since the 5 and 8 nitrogens of tetrahydrofolate (III) are absent in the analog (I), it was considered possible that one or both of these nitrogens might contribute to the binding to thymidylate synthetase, thus possibly accounting for the poorer binding of I. The synthesis of inhibitors containing either the 5- (VI) or 8-nitrogen (VIII) in an open-chain system related to II and their evaluation as inhibitors of thymidylate synthetase and dihydrofolic reductase is the subject of this paper.

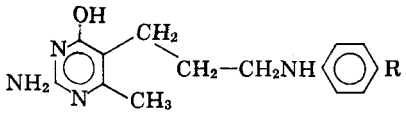
DISCUSSION

2 - Amino - 6 - methyl - 5 - (3 - phenylpropylamino)-4-pyrimidinol (VI) was about as effective an inhibitor of thymidylate synthetase as the phenylbutyl analog (IV) and the anilinopropyl analog (II) (Table I). Replacement of the 6-methyl group of the phenylbutyl analog (IV) by 6-amino (VIII) led to a loss in inhibition, VIII being less than one-quarter as effective an inhibitor as IV. Thus, neither the 5-nitrogen of VI, nor the 6-amino group of VIII (corresponding to N-8 of tetrahydrofolate, III) alone, accounted for the 23-fold difference in binding between I and III.

It is interesting to note that the 5-cinnamylaminopyrimidine (VII), an intermediate in the preparation of VI, is almost as effective as VI. Since VII has restricted rotation about the *trans*-double bond, it is clear that when the phenylpropylamino group of VI is bound to thymidylate synthetase, it must have a conformation about the same as any of the more restricted conformations that the cinnamylamino group of VII can assume.

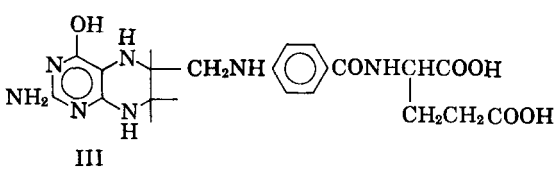
Since compounds VI–VIII were available from this thymidylate synthetase study, they were also investigated as inhibitors of dihydrofolic reductase with some useful results.

2 - Amino - 6 - methyl - 5 - (3 - phenylpropylamino)-4-pyrimidinol (VI) was as good an inhibitor



I, R = —CONHCHCOOH
CH$_2$CH$_2$COOH

II, R = H



III

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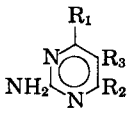
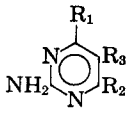
TABLE I.—INHIBITION OF THYMIDYLATE SYNTHETASE BY



Compd.	R_1	R_2	R_3	μM Concn. *dl*-FAH_4	mM Concn. Inhibitor	% Inhibition[a]	Inhibitor: Substrate[b]
II[c]	OH	CH_3	$C_6H_5NH(CH_2)_3$—	25.7	0.62	50	50
				51.4	1.2	50	50
IV[d]	OH	CH_3	$C_6H_5(CH_2)_4$—	25.7	0.20	30	35
V	OH	CH_3	$C_6H_5(CH_2)_3$—	51.4	0.50	27	110
VI	OH	CH_3	$C_6H_5(CH_2)_3NH$—	51.4	1.5	50	58
VII	OH	CH_3	C_6H_5CH=$CHCH_2NH$—	51.4	1.5	42	80
VIII	OH	NH_2	$C_6H_5(CH_2)_4$—	51.4	0.90	0	>130
IX	NH_2	NH_2	$C_6H_5(CH_2)_4$—	51.4	1.0	0	>150
X[c]	NH_2	CH_3	$C_6H_5NH(CH_2)_3$—	25.7	0.80	50	63

[a] The thymidylate synthetase was a 45–90% ammonium sulfate fraction prepared from *Escherichia coli* B as previously described (1), except cells were broken with a French press. The assay was performed with 25.7 or 51.4 μM *dl*-tetrahydrofolate as indicated, 80 μM deoxyuridylate, and formaldehyde in Tris buffer (pH 7.4) with 5% 2-methoxyethanol present, as previously described (1). [b] Ratio of concentrations of inhibitor to the *l*-isomer of tetrahydrofolate giving 50% inhibition. [c] Data from *Reference 1*. [d] Data from *Reference 3*.

TABLE II.—INHIBITION OF DIHYDROFOLIC REDUCTASE BY



Group	Compd.	R_1	R_2	R_3	μM Concn. for 50% Inhibition	Inhibitor: Substrate[a]
A	II[b]	HO	CH_3	$C_6H_5NH(CH_2)_3$—	800	130
	IV[c]	HO	CH_3	$C_6H_5(CH_2)_4$—	30	5.0
	V	HO	CH_3	$C_6H_5(CH_2)_3$—	160	27
	VI	HO	CH_3	$C_6H_5(CH_2)_3NH$—	690	120
	VII	HO	CH_3	C_6H_5CH=$CHCH_2NH$—	>3600	>600
	XII	HO	CH_3	H	19,000	3200
B	VIII	NH_2	OH	$C_6H_5(CH_2)_4$—	8.4	1.4
	XI	NH_2	OH	$C_6H_5(CH_2)_3$—	60	10
	XIII	NH_2	OH	H	13,000	2200
C	X[b]	NH_2	CH_3	$C_6H_5NH(CH_2)_3$—	2.2[d]	0.32
	XIV	NH_2	CH_3	H	1100	180
	XVII[e]	NH_2	CH_3	$C_6H_5(CH_2)_4$—	0.027[d]	0.0045
D	IX	NH_2	NH_2	$C_6H_5(CH_2)_4$—	3.5	0.59
	XV	NH_2	NH_2	$C_6H_5(CH_2)_3$—	7.1[d,f]	1.2
	XVI	NH_2	NH_2	H	1200	200

Dihydrofolic reductase was a 45–90% ammonium sulfate fraction of pigeon liver which was prepared and assayed in Tris buffer (pH 7.4) with 6 μM dihydrofolate and 12 μM TPNH as previously described (1); all assays were performed in 10% *N,N*-dimethylformamide unless otherwise indicated. [a] Ratio of concentration of compounds to dihydrofolate giving 50% inhibition, the I_{50}. [b] Data from *Reference 1*. [c] Data from *Reference 2*. [d] No *N,N*-dimethylformamide used in assay. [e] Baker, B. R., and Ho, B.-T., unpublished data. [f] Same result within experimental error obtained with the presence of 10% *N,N*-dimethylformamide.

of dihydrofolic reductase as the prototype 5-(3-anilinopropyl)-4-pyrimidinol (II) (Table II). However, VI and II must also be considered in relationship to the 5-(4-phenylbutyl)-4-pyrimidinol (IV) (3); both VI and II are about 27-fold less effective than IV, thus showing that introduction of either nitrogen corresponding to N_5 or N_{10} in the folic acid substrate molecule leads to a decrease in binding to the enzyme. In contrast to the case with thymidylate synthetase, introduction of the *trans*-double bond into the molecule (VII) leads to a greater than 4.5 loss in binding compared to VI, indicating that the conformation of VI when complexed to dihydrofolic reductase is different from any of the more restricted number of conformations that VII can assume.

2,6 - Diamino - 5 - (4 - phenylbutyl) - 4 - pyrimidinol (VIII) was about a fourfold better inhibitor than the corresponding 6-methyl-4-pyrimidinol (IV). Since VIII can presumably bind to the enzyme as the equivalent 2,4-diamino-6-pyrimidinol (VIII*a*), this fourfold tightening of binding is rather poor when one compares the 350-fold increase in binding when 2 - amino - 5 - (3 - anilinopropyl) - 6 - methyl - 4-pyrimidinol (II) is converted to the corresponding 2,4-diaminopyrimidine (X) (1); however, the relative binding of VIII and X is the same magnitude. Zakrzewski (4) has previously reported that 2,4-diamino-6-methylpyrimidine (XIV) is an inhibitor of folic reductase (folic acid as substrate) at pH 6.0 with $K_i = 2.6 \times 10^{-5}$, but that 2-amino-6-methyl-4-pyrimidinol (XII) shows no inhibition of folic reductase at 4.4×10^{-4} M; it can be estimated from his data that the K_i of XIII is greater than 90×10^{-4} M, a greater than 35-fold difference in binding between XIII and XIV. 2,4-Diamino-6-methylpyrimidine (XIV) has now been measured as an inhibitor of the dihydrofolic reductase from pigeon liver under the conditions specified in Table II and

showed 50% inhibition of the enzyme at 1.1 mM; the corresponding 4-pyrimidinol (XIII) showed 50% inhibition at 19 mM, thus showing that XIV was 19 times as effective as XIII under these conditions. Parenthetically, it should be noted that if the substrate concentration is greater than four times the K_m, then the ratio of the K_i's of any pair of compounds is the same as the ratio of the 50% inhibition concentrations (2, 4, 5), since $(V_0/V_I) = 1 + (K_m/S) \times (K_m/K_I) \times (I/S)$, where V_0 = velocity without inhibitor, V_I = velocity in the presence of inhibitor, S = substrate concentration, I = inhibitor concentration, K_i = the inhibitor-enzyme dissociation constant, and K_m = the apparent enzyme-substrate dissociation constant. If S is greater than four times K_m and I = concentration for 50% inhibition ($V_0 = 2V_I$), the term K_m/S becomes relatively negligible compared to experimental error and then $K_i/K_m = I/S$. The ratio of I/S for 50% inhibition is termed the I_{50}.

At first glance, 5-(4-phenylbutyl)-2,4,6-triaminopyrimidine (IX) could be considered to be a relatively good inhibitor of dihydrofolic reductase, showing 50% inhibition at 3.5 μM, about the same as X and the 6-pyrimidinol (VIII). However, if one considers that 2,4-diamino-6-methylpyrimidine (XIV) and 2,4,6-triaminopyrimidine (XVI) are almost equally effective inhibitors, one might anticipate that insertion of the 5-phenylbutyl group to give XVII and IX, respectively, ought to give a pair of compounds that show the same magnitude of inhibition. That this is not the case is seen in Table II; the 6-methylpyrimidine (XVII) is a 130-fold better inhibitor of dihydrofolic reductase than is the corresponding 6-aminopyrimidine (IX). When compared to XVII, even 2,4-diamino-5-(4-phenylbutyl)-4-pyrimidinol (VIII) might have been anticipated to be a thirtyfold better inhibitor than actually found, since 2,4-diamino-6-pyrimidinol (XIII) is only 11 times less effective an inhibitor than the corresponding 6-methylpyrimidine (XIV). It is obvious that with the 2,4-diamino-5-(4-phenylbutyl)pyrimidines either the 6-hydroxy or 6-amino group has a great detrimental effect on enzyme inhibition compared to a 6-methyl. Recent unpublished observations in this laboratory have shown that hydrophobic bonding to dihydrofolic reductase can occur with the alkyl group of 5-alkylpyrimidines related to XII, XIII, XIV, and XVI; if this hydrophobic bonding region on the enzyme extends to the 6-position of the pyrimidine, then a hydroxyl or amino group on the 6-position of the pyrimidine moiety would be repulsed from this hydrophobic region of the enzyme.

2 - Amino - 6 - methyl - 5 - (4 - phenylbutyl) - 4-pyrimidinol (IV) is a sixfold better inhibitor of dihydrofolic reductase than the corresponding 5-(3-phenylpropyl)-4-pyrimidinol (V). Similarly, in the 2,6-diamino-4-hydroxy series, the phenylbutyl pyrimidine (VIII) is a sevenfold better inhibitor than the phenylpropyl pyrimidine (XI). In contrast, in the 2,4,6-triamino series, the phenylpropyl pyrimidine (XV) is nearly as effective as the phenylbutyl pyrimidine (IX), and 4,6-diamino-2,2-dihydro-2,2-dimethyl-1-(3-phenylpropyl)-*s*-triazine is as effective as the phenylbutyl analog (6). Whether these differences can be due to hydrophobic effects is under investigation.

Regardless of mechanism of binding, several useful results emerge from this study: (*a*) the 5-phenylpropylamino side chain on a 2-amino-4-pyrimidinol (VI) gives as good an inhibitor of both enzymes as the 5-(3-anilinopropyl) side chain (II), (*b*) 5-(phenylalkyl)-2,4,6-triaminopyrimidines (IX, XV) and the 5-(4-phenylbutyl)-2,4-diamino-6-pyrimidinols (VIII, XI) are good inhibitors of dihydrofolic reductase with I_{50}'s near 1 (6 μM) that are easily synthesized in two steps from commercially available materials.

CHEMISTRY

Methods

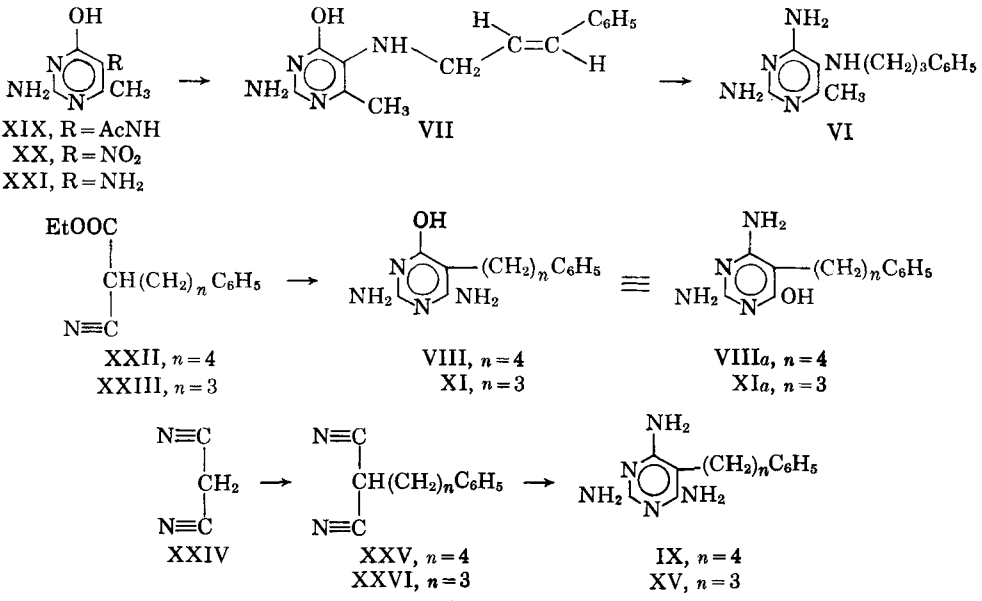
Two general routes are available for the preparation of 2,5-diamino-6-methyl-4-pyrimidinol (XXI). Condensation of ethyl α-phenylazoacetoacetate with guanidine, followed by catalytic reduction of the resultant pyrimidine, afforded XXI (7). A modification of this route, reported from this laboratory (8), involved condensation of ethyl α-acetamidoacetoacetate (9) with guanidine to give the 5-acetamido-4-pyrimidinol (XIX) which was then hydrolyzed with acid to the desired XXI. The second route reported involved conversion of the nitrate salt of 2-amino-6-methyl-4-pyrimidinol to the 5-nitro derivative (XX) with concentrated sulfuric acid, followed by electrolytic reduction to XXI (10). Although this two-step procedure *via* XX would appear to be shorter than the route *via* XIX, the reported conditions are rather awkward for general laboratory use.

It has now been found that the commercially available 2-amino-6-methyl-4-pyrimidinol can be smoothly nitrated in 96% sulfuric acid with one equivalent of aqueous nitric acid (d. = 1.5). After purification through the insoluble sodium salt, the nitropyrimidine (XX) (66% yield) was sufficiently pure for catalytic reduction; the reduction proceeded smoothly with a palladium-charcoal catalyst in 1.25 N hydrochloric acid. Pure, recrystallized 5-aminopyrimidine (XXI) was obtained in 67% yield.

Condensation of the 5-amino group of XXI with cinnamaldehyde in 2-methoxyethanol, followed by sodium borohydride reduction of the resultant yellow anil, gave the 5-cinnamylpyrimidine, one of whose possible conformations is indicated by VII. Catalytic hydrogenation of the side-chain double bond with Raney nickel catalyst proceeded smoothly to VI without concomittant reduction of the 5,6-double bond as shown by the ultraviolet spectrum of the product. The product (VI) was further differentiated from starting material (VII) by mixed melting points and by their different ultraviolet spectra. (Scheme I.)

Until recently, direct alkylation of malononitrile (XXIV) proceeded in poor yields due to competitive reactions of XXIV with alkoxide ion (11). Thus, it was necessary to alkylate malonic ester, then convert the ester to a diamide, and finally dehydrate to a nitrile (12). Recently, Bloomfield (13) has reported that malononitrile can be directly dialkylated in dimethyl sulfoxide; the latter solvent not only accelerates the alkylation reaction, but also no alkoxide ion is present to give side reactions.

It has now been found that when malononitrile in dimethyl sulfoxide was converted to the anion with sodium hydride, mono-alkylation with 3-phenylpropyl bromide or 4-phenylbutyl bromide (14) to



Scheme I

XXVI and XXV, respectively, proceeds to completion in about 3 min. at ambient temperature. Condensation of the crude substituted malononitriles with guanidine in ethanol gave the desired 5-aralkyl-2,4,6-triaminopyrimidines (IX and XV) in over-all yields of 35–41% of analytically pure material, based on malononitrile. Similarly, alkylation of ethyl cyanoacetate to XXII or XXIII, followed by guanidine condensation, gave VIII and XI in 48–53% over-all yield of analytically pure material.

Synthesis

Melting points were determined on a Mel-Temp block in capillary tubes, and those below 230° are corrected. Ultraviolet spectra were determined in water at the pH indicated with a Perkin-Elmer 202 spectrophotometer. Infrared spectra were run on a Perkin-Elmer 137B spectrophotometer in KBr pellet unless otherwise indicated. Thin-layer chromatograms (TLC) were run with Brinkmann Silica Gel G with ethanol, and spots were detected with iodine vapor. Paper chromatograms were run on Whatman No. 1 paper and spots were detected under ultraviolet light.

2-Amino-6-methyl-5-nitro-4-pyrimidinol (XX).—To a vigorously stirred solution of 12 ml. of nitric acid (d. = 1.5) in 75 ml. of 96% sulfuric acid cooled in an ice bath was added 25 Gm. (0.2 mole) of 2-amino-6-methyl-4-pyrimidinol (Aldrich Chemical Co.) in small portions over a period of 3 hr. Stirring was continued at 0° for 3 hr., then at ambient temperature ror 1.5 hr. The light yellow solution was poured on about 2 L. of crushed ice with hand stirring. To the mixture was added concentrated ammonium hydroxide until the solution was slightly basic as indicated by the bright yellow color of the mixture. Acetic acid was added until slightly acidic as indicated by the discharge of the bright yellow color. The crude, buff product (32 Gm.) was collected on a filter and washed well with water. At this stage of purity catalytic reduction would not go to completion due to the presence of catalyst poisons.

The crude product was dissolved in 500 ml. of hot 1.75 *N* aqueous sodium hydroxide and the solution was clarified with 3 Gm. of decolorizing carbon. After being chilled overnight, the mixture was filtered and the yellow sodium salt was washed with 25 ml. of ice cold 1.75 *N* aqueous sodium hydroxide. The moist salt was dissolved in about 200 ml. of hot water and the solution added to excess 25% aqueous acetic acid. The product was collected on a filter and washed with water; yield, 22.4 Gm. (66%) of pure product that showed a single spot on paper chromatography with *tert*-butanol–methyl ethyl ketone–concentrated ammonium hydroxide–water (40:30:20:10) (15); $\nu_{max.}$ 3600, 3250–3100 (OH, NH); 1710, 1660, 1620, 1590 (NH, C=O, pyrimidine); 1550, 1325 cm.$^{-1}$ (NO_2); $\lambda_{max.}$ (pH 13) 254, 358 (broad) (O.D. ratio 358/254 = 0.80); $\lambda_{max.}$ (pH 1) 268, 340 (broad inflection) (O.D. ratio 340/268 = 0.46).

The use of fuming nitric acid or sodium nitrate in 96% sulfuric acid for nitration led to oxidation to water-soluble products.

2,5-Diamino-6-methyl-4-pyrimidinol (XXI).—A mixture of 17 Gm. (0.1 mole) of purified XX, 1.7 Gm. of 5% palladium-charcoal, and 200 ml. of 1.25 *N* aqueous hydrochloric acid (0.25 mole) was shaken with hydrogen at 2–3 Atm. until reduction was complete (about 4 hr.). The filtered solution was spin-evaporated *in vacuo;* the residual hydrochloride salt was triturated with 50 ml. of methanol, filtered, then dissolved in 100 ml. of water. The aqueous solution was neutralized with solid sodium bicarbonate with vigorous magnetic stirring. The solid was collected on a filter and recrystallized from water; yield, 11.1 Gm. (79%), m.p. 270–275° dec. A second recrystallization from water gave 9.4 Gm. (67%) of white needles, m.p. 276–280° dec.; $\nu_{max.}$ (Nujol) 3400, 3200–3100 cm.$^{-1}$ (OH, NH); 1700, 1675, 1640

cm.$^{-1}$ (NH, C=O, pyrimidine). The compound moved as a single spot on paper chromatography in methyl ethyl ketone–*tert*-butanol–formic acid–water (30:40:15:15) and in *tert*-butanol–methyl ethyl ketone–concentrated NH_4OH–water (40:30:20:10) (16). [Lit. m.p. 280° dec. (7).]

Less volume can be used in the catalytic reduction if the ratio of HCl to XX is kept constant.

2 - Amino - 5 - cinnamylamino - 6 - methyl - 4-pyrimidinol (VII).—To a suspension of 2.80 Gm. (20 mmoles) of XXI in 100 ml. of 2-methoxyethanol containing 0.3 ml. of acetic acid was added 3.58 (27 mmoles) of cinnamaldehyde; the mixture was diluted with 7 ml. of water while being heated in a steam bath. When solution was complete, it was cooled to 0° when the yellow anil separated; then 6.5 Gm. of sodium borohydride was added portionwise over a period of 30 min. After being stirred an additional 18 hr. at ambient temperature, the mixture was spin-evaporated *in vacuo* to a gelatinous mass. The mixture was cooled in an ice bath and sufficient ice cold 3 *N* hydrochloric acid was added cautiously with mixing to destroy the excess sodium borohydride and bring the pH near 7. Water was added to a total volume of about 300 ml. The product was collected on a filter, washed with 50 ml. of 0.1 *N* hydrochloric acid and 200 ml. of water. The solid was dissolved in 30 ml. of 1 *N* aqueous potassium hydroxide by warming to 80°. The hot solution was clarified with carbon, then poured into 30 ml. of cold 2 *N* aqueous acetic acid. The product was collected on a filter, washed with water, then recrystallized from 2-methoxyethanol by addition of water; yield, 2.87 Gm. (56%), m.p. 210–213° dec. A second recrystallization from ethanol gave pure material, m.p. 213–215° dec. Recrystallization of a pilot run from methanol gave 237 mg. (31%) of analytically pure, white crystals, m.p. 210° dec.; $\lambda_{max.}$ (pH 1) 255 (ϵ 20,600), 283 (inflection), 293 mμ (ϵ 5000); $\lambda_{max.}$ (pH 13) 249 (ϵ 22,000), 285 (ϵ 8600); 293 mμ (ϵ 8100); $\lambda_{max.}$ (EtOH) 250 (ϵ 21,000), 294 mμ (ϵ 7500); $\nu_{max.}$ 3400, 3150 (NH, OH); 1650, 1645, 1550 (C=O, C=N, C=C, NH_2); 740, 690 cm.$^{-1}$ (C_6H_5). TLC showed one spot.

Anal.—Calcd. for $C_{14}H_{16}N_4O$: C, 65.7; H, 6.31; N, 21.9. Found: C, 66.0; H, 6.07; N, 22.0.

2 - Amino - 6 - methyl - 5 - (3 - phenylpropylamino)-4-pyrimidinol (VI).—A solution of 256 mg. (1 mmole) of VII in 25 ml. of 2-methoxyethanol was shaken with hydrogen at 2–3 Atm. in the presence of 1 Gm. of No. 28 Raney nickel. After 21 hr., 1 mmole of hydrogen had been consumed. The filtered solution was spin-evaporated *in vacuo*. Two recrystallizations from ethanol gave 181 mg. (72%) of analytically pure, buff-colored crystals, m.p. 201–203°; $\lambda_{max.}$ (pH 1) 258 mμ (ϵ 14,700); $\lambda_{max.}$ (pH 13) 248 (ϵ 14,700), 290 mμ (broad, ϵ 7100); $\lambda_{max.}$ (EtOH) 249 (ϵ 13,600), 295–305 mμ (plateau, ϵ 6600); $\nu_{max.}$ 3350, 3250, 3050 (OH, NH); 1650, 1620, 1590 (NH, C=O, C=N, C=C); 745, 725, 695 cm.$^{-1}$ (C_6H_5). TLC showed one spot with about the same mobility as VII.

Anal.—Calcd. for $C_{14}H_{18}N_4O$: C, 65.1; H, 7.02; N, 21.7. Found: C, 64.9; H, 6.80; N, 21.4.

Attempts to prepare VI by reductive condensation of hydrocinnamaldehyde with XXI, as described for the preparation of VII, gave a mixture of four compounds, detectable by TLC; VI could not be readily isolated.

2 - Amino - 6 - methyl - 5 - (3 - phenylpropyl) - 4-pyrimidinol (V).—By condensation of ethyl acetoacetate with 3-phenylpropyl bromide, then reaction with guanidine, as described for the preparation of the corresponding 5-(4-phenylbutyl)-4-pyrimidinol (IV) (3), was obtained 1.04 Gm. (43% over-all from the bromide) of analytically pure material, m.p. 260–261° dec.; $\lambda_{max.}$ (pH 13) 279; $\lambda_{max.}$ (pH 1) 230, 266; $\nu_{max.}$ (Nujol) 3400, 3050–3200 (broad) (NH, OH); 1690, 1650, 1610 (NH, C=C, C=N, C=O); 720, 695 cm.$^{-1}$ (C_6H_5). TLC showed one spot.

Anal.—Calcd. for $C_{15}H_{17}N_3O$: C, 69.1; H, 7.04; N, 17.3. Found: C, 69.0; H, 6.93; N, 17.2.

5 - (4 - Phenylbutyl) - 2,4,6 - triaminopyrimidine (IX).—To a stirred suspension of 0.240 Gm. (10 mmoles) of sodium hydride (56.5% suspension in mineral oil) in 5 ml. of dimethyl sulfoxide immersed in a cold water bath was added 0.727 Gm. (11 mmoles) of malononitrile (XXIV). After about 15 min., hydrogen evolution was complete and solution had taken place. Then 3.20 Gm. (15 mmoles) of 4-phenylbutyl bromide (14) was added. Within 3 min., the mixture had jelled and had become neutral. The mixture was warmed on a steam bath for a few minutes to dissolve the gel, then poured into about 50 ml. of benzene. The benzene solution was washed thoroughly with water, dried with magnesium sulfate, and spin-evaporated *in vacuo;* the last traces of dimethyl sulfoxide were removed in a hot water bath at less than 1 mm.

To the residual oily XXV was added a filtered solution of 0.595 Gm. (11 mmoles) of sodium methoxide and 1.05 Gm. (11 mmoles) of guanidine hydrochloride in 20 ml. of absolute ethanol. After being refluxed with stirring for 3 hr. protected from moisture, the hot solution was filtered. On cooling, the solution deposited white crystals. Recrystallization from ethanol gave 1.06 Gm. (41% from XXIV) of white leaflets, m.p. 148–149°; $\lambda_{max.}$ (pH 1) 244 (shoulder, ϵ 6200), 284 mμ (ϵ 18,600); $\lambda_{max.}$ (pH 13) 241 (shoulder, ϵ 6200), 276 mμ (ϵ 10,400); $\nu_{max.}$ 3400, 3190 (NH); 1640, 1600, 1560 (NH, C=C, C=N); 747, 695 cm.$^{-1}$ (C_6H_5). TLC showed one spot.

Anal.—Calcd. for $C_{14}H_{19}N_5$: C, 65.3; H, 7.44; N, 27.2. Found: C, 65.1; H, 7.60; N, 27.1.

Although 4-phenylbutyl bromide was used, it is probable that the commercially available 4-phenylbutyl chloride and sodium iodide would be equally effective with a longer reaction time.

5 - (3 - Phenylpropyl) - 2,4,6 - triaminopyrimidine (XV).—This compound was prepared from 3-phenylpropyl bromide as described for the preparation of IX; yield, 0.85 Gm. (35%) of analytically pure, white leaflets, m.p. 146–147°; the infrared spectrum and the ultraviolet spectrum in basic solution were similar to those of IX; $\lambda_{max.}$ (pH 1) 276 mμ (ϵ 15,900).

Anal.—Calcd. for $C_{13}H_{17}N_5$: C, 64.2; H, 7.04; N, 28.8. Found: C, 64.3; H, 7.05; N, 28.6.

2,6 - Diamino - 5 - (4 - phenylbutyl) - 4 - pyrimidinol (VIII).—Condensation of 4-phenylbutyl bromide (14) with ethyl cyanoacetate and reaction of the intermediate XXII with guanidine was performed as described for the preparation of IX; at the end of the reaction with guanidine the solution was neutralized with glacial acetic acid. Recrystallization from ethanol gave 1.38 Gm. (54% over-all) of white leaflets, m.p. 225–226°; $\nu_{max.}$ (Nujol) 3460, 3350, 3200 (OH, NH); 1660, 1630, 1600 (C=O,

C=N, C=C, NH); 757, 705 cm.$^{-1}$ (C_6H_5); $\lambda_{max.}$ (pH 13) 272; $\lambda_{max.}$ (pH 1) 276. TLC showed one spot.

Anal.—Calcd. for $C_{14}H_{18}N_4O$: C, 65.1; H, 7.02; N, 21.7. Found: C, 64.9; H, 6.83; N, 21.5.

2,6-Diamino-5-(3-phenylpropyl)-4-pyrimidinol (XI).—This compound was prepared from 3-phenylpropyl bromide as described for VIII; yield, 1.24 Gm. (48%) of analytically pure, white leaflets, m.p. 220–221°; $\nu_{max.}$ (Nujol) 3400, 3200–3050 (NH, OH); 1690, 1650, 1610, 1550 (NH, C=O, C=C, C=N); 740, 720, 695 cm.$^{-1}$ (C_6H_5). The ultraviolet spectrum was the same as that of VIII, and TLC showed one spot.

Anal.—Calcd. for $C_{13}H_{16}N_4O$: C, 63.9; H, 6.60; N, 22.9. Found: C, 63.9; H, 6.50; N, 22.9.

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Base-Catalyzed Addition and Solvolysis Reactions of *N*-Phenylmaleimide in Methanol

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The base-catalyzed reaction of methanol with *N*-phenylmaleimide provides mainly methyl α-methoxysuccinanilate (III), which is formed from the intermediate methyl maleanilate (II). The course of the reaction was followed gas chromatographically, and the role of various possible intermediates is discussed. Observations concerning the relative thermal stabilities of III and its β-methoxy isomer (IV) are also presented. Nuclear magnetic resonance spectra were recorded for III, *N*-phenyl-α-methoxysuccinimide (V), and dimethyl α-methoxysuccinate (VI), all of which exhibit *ABX*-type patterns. Calculations carried out on the spectrum of V indicated J_{ax}, J_{bx}, and J_{ab} to be 7.85, 4.5, and 18.4 c.p.s., respectively.

THE CONJUGATE addition of thiols (1–3) and amines (2) to the double bond of *N*-alkyl- and *N*-arylmaleimides is well established. Indeed, the reaction is used for both the qualitative and quantitative estimation of thiols (3–5). These addenda also react with *N*-carbamylmaleimides (6) but give rise to a complex mixture of products apparently derived by ring opening as well as addition to the activated double bond. The reaction of alcohols, however, with this latter type of acceptor leads exclusively to ring opened products (6). The authors had occasion to observe, during the course of another investigation, the reaction of methanol with *N*-phenylmaleimide (I) in the presence of a catalytic amount of aqueous sodium hydroxide, which led apparently to a single product in high yield. Microanalysis and molecular weight determinations indicated that this product was the result of the addition of 2 moles of methanol to 1 mole of I, and the infrared spectrum showed both ester and amide carbonyl bands as well as absorption due to a phenyl ring and an N—H group. These data are consistent either with the structure III, methyl α-methoxysuccinanilate or the β-methoxy isomer (IV), and the NMR spectrum of the product also accords with this conclusion. As will be shown, both isomers are actually formed in this reaction, although one is present in only a very minor amount. The authors envisioned three possible pathways by which III and IV could be formed from I. Path *A* involves first ring opening to give methyl maleanilate (II), followed by addition to the double bond. Path *B* consists of the reverse order of these events, that is: first, addition to

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