when necessary to keep the solution homogeneous. The solution was washed with ethyl acetate (100 ml.) and extracted at pH 3 with two 100-ml. portions of the same solvent. The combined extract was washed with 50 ml. of water. Fresh water (50 ml.) was added to the ethyl acetate solution, and the mixture was adjusted to pH 6.8 with saturated aqueous sodium carbonate. The last extraction was repeated with 35 ml. of water; the combined pH 6.8 aqueous solution was washed with ether (30 ml.) and evaporated briefly to remove solvent. The aqueous solution was cooled to 5° and treated with a cold solution of procaine hydrochloride (7.2 g.) in 8 ml. of water. After refrigerating the hazy solution for 1 hr., crystalline procaine salt was recovered by filtration, washed with cold water and ether, and dried: yield, 13.6 g. (49%); m.p. 139–143° dec.; $[\alpha]^{24}D$ +143° (*c* 1, 70% acetone); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{25}H_{41}N_4O_7PS_2$: C, 49.65; H, 6.84; N, 9.27. Found: C, 49.58; H, 6.78; N, 9.23.

6-Diphenoxyphosphinylaminopenicillanic Acid N-Ethylpiperidine Salt (4).—Diphenyl chlorophosphate (13.5 g., 0.05 mole) and 6-APA (10.8 g., 0.05 mole) were made to react as described for **10**. The first ethyl acetate extract (pH 5.5) yielded 3.0 g. (11%) of crystalline **4**; m.p. 133–136° dec. For analysis a sample was recrystallized from acetone; m.p. 133–136° dec.; $[\alpha]^{24}D$ +173° (*c* 1, water); λ_{max} 5.64 μ (vs) in KBr.

Anal. Calcd. for $C_{27}H_{36}N_3O_6PS$: C, 57.74; H, 6.46; N, 7.48. Found: C, 57.91; H, 6.48; N, 7.42.

6-Bis-*p*-methylphenoxyphosphinylaminopenicillanic Acid N-Ethylpiperidine Salt (6).—Bis-*p*-tolyl chlorophosphate (14.8 g., 0.05 mole) and 6-APA (10.8 g., 0.05 mole) reacted as described for **4** yielded 3.4 g. (12%) of crystalline **6**; m.p. 128–130° dec.; $[\alpha]^{24}D$ +149° (*c* 1, ethanol); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{29}H_{40}N_3O_6PS$: C, 59.07; H, 6.84; N, 7.13. Found: C, 58.99; H, 6.91; N, 6.91.

6-Phenoxyphenylphosphinothioylaminopenicillanic Acid N-Ethylpiperidine Salt.—Phenoxyphenylphosphinothioyl chloride, $C_6H_5PS(C_6H_5O)Cl$ [13.5 g., 0.05 mole; b.p. 131–135° (0.01 mm.); $n^{26}D$ 1.6190] and 6-APA (10.8 g., 0.05 mole) were allowed to react as described for **4** to give 10.4 g. (37%) of crystalline product. For analysis a sample was recrystallized from acetone; m.p. 106–111° dec.; $[\alpha]^{23}D$ +214° (*c* 1, water); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{27}H_{36}N_3O_4PS_2$: C, 57.73; H, 6.46; N, 7.48. Found: C, 57.92; H, 6.70; N, 7.54.

6-Ethylthiophenylphosphinothioylaminopenicillanic Acid Potassium Salt (44).—A solution of 6-APA (4.32 g., 0.02 mole) in 30 ml. of water with the addition of solid potassium bicarbonate (4 g.) was treated with a solution of ethylthiophenylphosphinothioyl chloride, $C_6H_5PS(C_2H_5S)Cl$ [4.72 g., 0.02 mole; b.p. 114–118° (0.02 mm.); $n^{25}D$ 1.6310] in 30 ml. of acetone. The solution was adjusted to pH 6 with 10% potassium bicarbonate (14 ml.) and stirred for 5 hr. (final pH 6.6). After clarification the solution was extracted with 2 volumes of ethyl acetate at pH 5.5. The extract was washed with 0.5 volume of water, dried, and adjusted to pH 8 with *N* methanolic potassium hydroxide. The solution was evaporated to dryness, and the residue was triturated with absolute ether to an amorphous solid, which was dried over phosphorus pentoxide under vacuum. The yield of **44** was 1.55 g. (12%); calculated chemical assay for $C_{16}H_{20}N_2O_3PS_2K$, 1310 sodium penicillin G units per mg.; found (hydroxylamine colorimetric method[5]), 935 units per mg.; purity, 71%.

6-Diphenoxyphosphinylaminopenicillanamide (II).—A solution of diphenyl chlorophosphate (6.7 g., 0.025 mole) in 50 ml. of acetone was added rapidly with stirring to a cooled solution of 6-aminopenicillanamide *p*-toluenesulfonate[6] (9.8 g., 0.025 mole) in a mixture of 50 ml. of 0.067 *M* disodium hydrogen phosphate (adjusted to pH 7.5) and 20 ml. of acetone. After stirring for 3 hr. at room temperature (pH kept at 7.5), the reaction solution was extracted with 1 volume of ethyl acetate. The extract was washed with one-half volume of water, three times with 0.5 volumes of 5% sodium bicarbonate, and again with water. Solvent was removed from the dried extract by evaporation, and the residue on trituration with benzene yielded 1.1 g. (10%) of crystalline II. For analysis a sample was recrystallized from acetone–hexane: m.p. 105–108°; $[\alpha]^{23}D$ +197° (*c* 1, chloroform); λ_{max} 5.60 (vs) and 5.99 μ (vs) in KBr.

Anal. Calcd. for $C_{28}H_{22}N_3O_5SP$: C, 53.68; H, 4.96; N, 9.39. Found: C, 53.37; H, 5.20; N, 9.14.

Acknowledgments.—We thank R. J. Sawicki, Jr., E. P. Grant, and G. L. Peterson for expert technical assistance. We gratefully acknowledge acute toxicity studies by Dr. S. Y. P'an and Dr. C. S. Delahunt, antiviral tests by Dr. C. D. Brandt, physical measurements and microanalyses by Dr. R. L. Wagner, Jr., paper chromatography by Dr. T. M. Lees and Miss B. M. Cummings, and penicillin chemical assays by Miss M. J. Loughlin. We are indebted to Dr. J. F. Snell and Mrs. L. Cheng for the penicillinase studies cited.

Analogs of Tetrahydrofolic Acid. VIII.[1,2] Synthesis of N-[1-(2-Amino-4-mercapto-6-methyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid, a Mercapto Analog

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Received May 3, 1963

The title compound (II) has been synthesized in nine steps starting with ethyl acetoacetate *via* the key intermediates 2-acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII) and 2-amino-5,6-dihydro-7-hydroxy-4-methyl-7H-thiopyrano[2,3-d]pyrimidine (XVIII). Conversion of 2-amino-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinol (X) to the corresponding 4-pyrimidinethiol XV or 4-chloropyrimidine XI could not be accomplished due to the instability of the 5-side chain; in contrast, the N^2-acetyl of X, namely XII, underwent fast conversion to 2-acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII) under mild conditions due to the greater reactivity and better solubility of XII compared to X. The key intermediate 2-amino-4-mercapto-6-methyl-5-pyrimidylpropionaldehyde (XX) existed almost completely in the hemiacetal form XVIII in neutral solution, but existed in the open chain aldehyde form XXII when converted to an anion at pH 13.

Since fifteen enzymes that use folic acid, tetrahydrofolic acid, or derivatives of tetrahydrofolic acid as substrates are known,[3-5] a program was initiated on analogs of tetrahydrofolic acid. The rationale[6] for, as

(1) This work was generously supported by Grants CY-5845 and CY-06624 of the National Cancer Institute, U. S. Public Health Service. Part of this work was presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962.

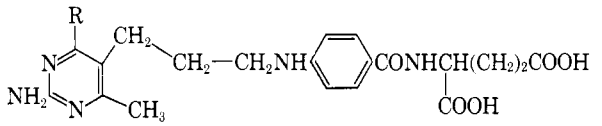
(2) For the previous paper of this series see B. R. Baker and C. E. Morreal, *J. Pharm. Sci.*, **52**, 840 (1963).

(3) T. H. Jukes and H. P. Broquist, "Sulfonamides and Folic Acid Antagonists" in "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel, Ed., Academic Press, Inc., New York, N. Y., 1963, pp. 481–534.

(4) F. M. Huennekens, M. J. Osborn, and H. R. Whitely, *Science*, **128**, 120 (1958).

(5) J. F. Holland, *Clin. Pharm. Therap.*, **2**, 374 (1961).

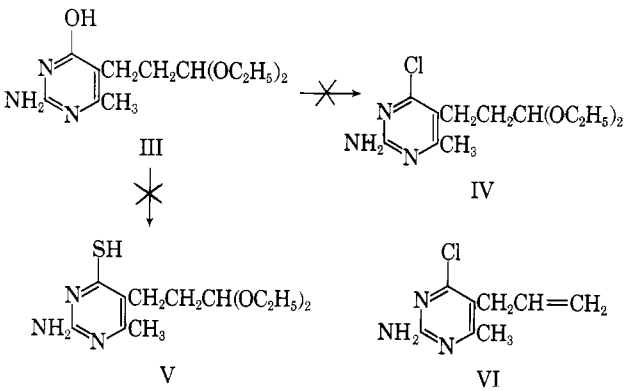
well as the synthesis[2,7] of, a pyrimidine analog of tetrahydrofolic acid (I) has been previously presented. Since I could inhibit some of these enzymes, we embarked on the synthesis of the 4-mercaptopyrimidine analog II of tetrahydrofolic acid; to our knowledge no



I, R = OH; II, R = SH

mercapto analog closely related to the folic acid structure has been synthesized.

A key intermediate in the synthesis of I was the pyrimidine-5-propionaldehyde acetal III which also appeared to be a likely intermediate for the synthesis of the required 4-mercaptopyrimidine (V). Unfortunately, III was quite unstable to phosphorus oxychloride, giving carbonaceous material even at room temperature; under a variety of conditions including the use of dimethylaniline, IV could not be obtained. Similarly, III was unstable to phosphorus pentasulfide, gave only tarry materials in hot pyridine or xylene, and no V could be isolated. That the difficulty of the phosphorus oxychloride reaction could not be attributed to the 5,6-dialkyl structure of the pyrimidine ring was shown by the conversion of 5-allyl-2-amino-6-methyl-4-pyrimidinol[8] to the chloropyrimidine VI, albeit only in 13% yield. Thus, the main instability of III to phosphorus oxychloride was attributed to the acid-sensitive acetal group.



Since the dioxolane blocking group is considerably more acid-stable than the ethyl acetal blocking group, the synthesis of X, the pyrimidine-5-propionaldehyde blocked with a dioxolane group, was undertaken in order to study its conversion to a mercaptopyrimidine (XV). Reaction of ethyl 2-acetylglutaraldehydate (VIII)[2,9] with ethylene glycol in benzene containing *p*-toluenesulfonic acid, *but without azeotropic removal of water*, gave the desired dioxolane (VIII) in 55% yield as a distillable oil. As one could anticipate,[10] the same conditions with removal of water led to the bisdioxolane (IX).

(6) B. R. Baker, Preprints of the Scientific Session of the American Pharmaceutical Association, Las Vegas, Nevada, 1952; paper V of this series.

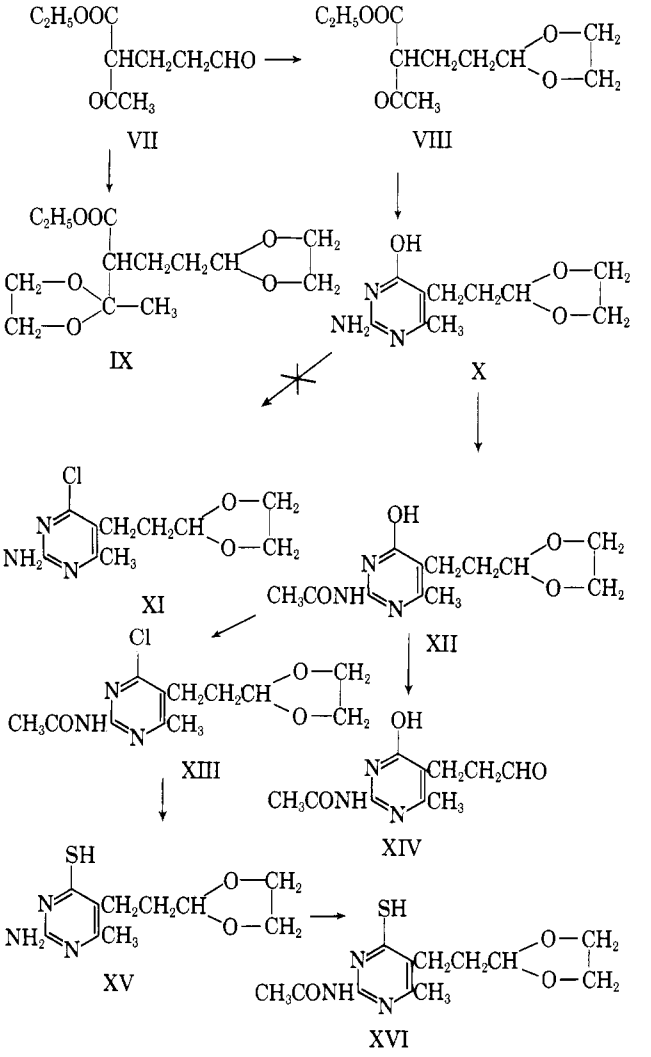
(7) B. R. Baker and C. E. Morreal, *J. Pharm. Sci.*, **51**, 596 (1962).

(8) V. Hacht, *Chem. Listy*, **45**, 459 (1951).

(9) O. A. Moe and D. T. Warner, U. S. Patent 2,610,204; *Chem. Abstr.*, **47**, 5961 (1953).

(10) L. Willman and H. Schinz, *Helv. Chim. Acta*, **32**, 2151 (1949).

Condensation of VIII with guanidine in boiling ethanol gave the crystalline pyrimidyl dioxolane (X) in 69% yield; the over-all yield from VII was increased by 18% when the intermediate blocked keto ester (VIII) was not isolated. Acetylation of X with acetic anhydride in pyridine at 80–90° afforded the N-acetyl



derivative XII in 57% yield; with boiling acetic anhydride the yield was lowered to 45%. In contrast to 2-acetamido-4-hydroxy-6-methylpyrimidine-5-propionaldehyde acetal, which was readily hydrolyzed to 2-acetamido-4-hydroxy-6-methylpyrimidyl-5-propionaldehyde (XIV) with boiling water,[2] XII was recovered unchanged under these conditions, indicating the desired increase in the stability of the dioxolane blocking group. That the dioxolane blocking group of XII could be hydrolyzed to XIV was shown by treatment of XII with 90% formic acid at room temperature.

In spite of the increased stability of the dioxolane blocking group, treatment of XII with phosphorus oxychloride with or without the presence of such bases as pyridine or dimethylaniline to form XI caused extensive decomposition, and no organic materials with the expected solubility could be recovered; similar results were obtained with phosphorus pentasulfide in pyridine in attempts to synthesize XV. Since the N-acetyl derivative XII was considerably more soluble in nonpolar solvents than the 2-aminopyrimidine X, reaction of XII with phosphorus oxychloride was

studied. Traces of the desired 4-chloropyrimidine (XIII) could be obtained by heating in phosphorus oxychloride for a short period of time. The yield was improved to the order of 5% if the excess phosphorus oxychloride was not removed by distillation *in vacuo*, but the reaction mixture added directly to ice. Use of benzene as a diluent and decomposition of the excess reagent in cold alkali gave 10–30% yields of XIII. Finally, by proper control of the reaction time and temperature, work-up with cold aqueous sodium acetate gave the crystalline 4-chloropyrimidine (XIII) in 96% yield. The increase in rate of reaction of phosphorus oxychloride with the 2-acetamido-4-pyrimidinol (XII) compared to other 2-amino-4-pyrimidinols such as X cannot be contributed solely to increased solubility; apparently, the hydroxyl group of XII is considerably more reactive.

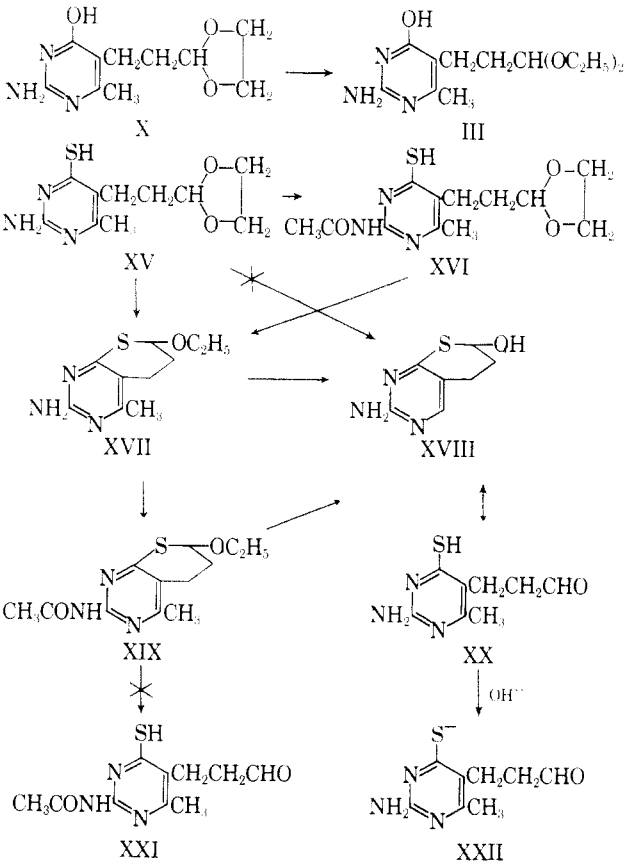
When the 4-chloropyrimidine (XIII) was treated with potassium hydrosulfide in boiling methanol, the chloro group was replaced by mercapto and the N-acetyl group was cleaved to give the desired 2-amino-4-mercaptopyrimidine (XV) in 75% yield; thus either XI or XVI, or both were intermediates in the conversion of XIII to XV. Other studies in this Laboratory[11] have shown that the chloro group of the 2-acetamidopyrimidine (XIII) can be displaced readily by nucleophiles under conditions where the 2-amino-4-chloropyrimidine (XI) is recovered unchanged, indicating a tremendous difference in reactivity of the halogen in the two compounds. That the reaction proceeded predominantly through XVI was indicated by further treatment of the methanol-freed reaction mixture of potassium hydrosulfide and XIII with acetic anhydride and pyridine; in this way pure XVI was obtained in 88% yield, presumably by reacetylation of the XI to reform XIII followed by further reaction with hydrosulfide or thioacetate ion.

If inadvertently not all the potassium hydroxide was converted to potassium hydrosulfide, the more alkaline solution caused more rapid deacetylation and the 2-amino-4-chloropyrimidine (XI) was the main product. By use of the neutral nucleophile, thiourea, in *t*-butyl alcohol, which does not give rise to alcoholysis, rapid replacement of the chloro group took place without loss of the N-acetyl group and the intermediate, weakly-acidic thiouronium salt was isolated in 82% yield. Hydrolysis of the N-acetyl and thiouronium groups was complete in 2 hr. at room temperature in *N* NaOH, as shown by the change in the ultraviolet spectrum; acidification gave an 84% yield of XV. Moreover, the 2-amino-4-chloropyrimidine (XI) was recovered essentially unchanged under similar conditions. The route through the thiouronium salt was the method of choice.

The higher reactivity of groups in the 4-position of a 2-acetamidopyrimidine compared to a 2-aminopyrimidine is not only a noteworthy difference in the character of the 2-aminopyrimidine system, but a highly useful difference that should find utility in other aminopyrimidine transformations.

Since the dioxolane blocking group of XII was hydrolyzed to the aldehyde XIV with difficulty only with acid, and since hydrolysis of 4-mercaptopyrimidyl dioxolane (XVI) with acid to XXI gave difficultly separable mixtures, the reconversion of the dioxolane blocking group to the acetal blocking group was studied. Treatment of the 4-hydroxypyrimidyl dioxolane (X) with refluxing 0.5% alcoholic sulfuric acid gave the acetal III in 30% yield; this reaction was not studied in further detail, but attention was turned to the mercapto series.

Acid-catalyzed alcoholysis of XV or XVI did not give a diethyl acetal, but formed the cyclic acetal XVII in near-quantitative yield. That this compound, giving combustion values for the cyclic structure XVII, had this structure was confirmed readily by its ultraviolet absorption spectrum; XV has maxima in neutral solution at 260 and 347 mμ as expected for a 2-amino-4-mercapto-5,6-dialkylpyrimidine,[12] whereas XVII has maxima at 230 and 307 mμ, typical of a 2-amino-4-alkylthio-5,6-dialkylpyrimidine[12] (see Table I). Acetylation of XVII with acetic anhydride in pyridine gave XIX. Attempts to hydrolyze XIX with water or 90% formic acid to the pyrimidyl aldehyde (XXI) were again unpromising.

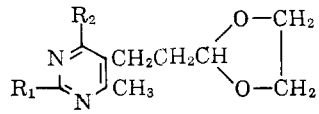
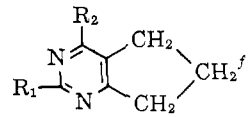
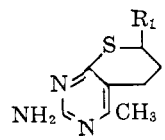


Short aqueous sulfuric acid hydrolysis of XVII or XIX gave a crystalline compound in 65 or 74% yield, respectively, that gave combustion values in agreement with the 2-aminopyrimidyl aldehyde XX structure. However, the ultraviolet spectrum showed that in neutral solution the supposed aldehyde XX was mostly in the hemiacetal form since its maximum at 307 mμ is characteristic of a 2-amino-4-alkylthio-5,6-dialkylpyrimidine[12]; a small peak also occurred at 350 mμ due to the presence of some open chain aldehyde XX (see Table I). The ratio of the 307 to 350 mμ peaks indicated that the ratio of the hemiacetal form XVIII to the open aldehyde form XX at pH 7 was about

(11) B. R. Baker and B. Ho, unpublished results.

(12) L. O. Ross, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3108 (1959).

TABLE I
ULTRAVIOLET SPECTRA OF THE PYRIMIDINES

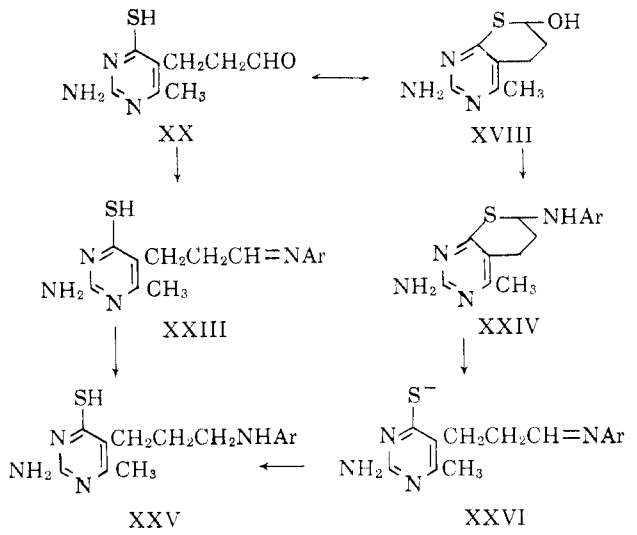
No.	Compounds R_1	R_2	$\lambda_{max}^{pH\ 1}$ ($\epsilon \times 10^3$)	λ_{max} ($\epsilon \times 10^3$) (solvent)[a]	$\lambda_{max}^{pH\ 13}$ ($\epsilon \times 10^3$)
					
X	NH_2	OH	228 (9.65) 266 (8.52)	227 (shoulder, 13.0) (B) 291 (9.15) (B)	(279 (7.52)
XII	AcNH	OH	245 (11.08) 262 (8.6) (inflection)	220 (18.96) (A) 245 (9.92) (A)[b]	245 (8.82) 276 (7.80)
XIII	AcNH	Cl	242 (18.74)[c]	242 (18.27) (A)[d]	
XVI	AcNH	SH		223 (15.30) (B) 295 (10.15) (B) 354 (10.5) (B)	
XV	NH_2	SH	266 (6.22) 332 (12.38)	260 (4.15) (A) 347 (12.99) (A) 346 (10.37) (C)[e] 317 (12.42) (D)[e]	260 (4.4) (inflect.) 317 (10.61)
					
	NH_2	OH	230 (9.30) 267 (8.20)	229 (9.10) (A) 287 (8.30) (A)	232 (8.20) 277 (7.20)
	NH_2	SH	252 (4.64) 337 (14.04)	242 (5.83) (A) 344 (14.36) (A)	237 (8.53) 312 (11.23)
	NH_2	CH_3S	236 (10.05) 271 (11.60) 315 (12.89)	234 (14.91) (A) 308 (10.04) (A)	234 (14.91) 308 (10.04)
					
XVII	C_2H_5O		274 (9.71) 316 (10.31)	230 (12.72) 307 (6.89)	307 (6.89)
XVIII	OH		274 (9.54) 317 (10.73)	307 (7.09) (A) (C) 350 (1.20) (A) (C) 312 (11.12) (D)	317 (12.86)[g]
XXIVb	C_6H_5NH		219 (18.70) 276 (9.73) 317 (10.34)[h]	231 (20.67) (A) 307 (8.06) (A)	260 (8.6) (inflect.) 317 (13.68)

[a] A, pH 7; B, 95% ethanol; C, pH 8.4; D, pH 11. [b] Inflections centering at 261 and 290 mμ. [c] Plateau at 266–281 mμ (ε 4700). [d] Inflection centering at 270 mμ (ε 5200); at pH 13 the compound rapidly underwent spectral changes presumably by hydrolysis to X. [e] Shoulder at 260 mμ (ε 6000). [f] See ref. 12. [g] Inflection centering at 258 mμ (ε 5200). [h] Inflection centering at 232 mμ (ε 12,000).

10:1. At pH 13 the spectra of both XVIII and XV were identical with a peak at 317 mμ, showing that the anionic form was completely in the open chain form (XXII and/or its ionically tautomeric forms). Although pH 11 was sufficiently basic to convert all of XV to the anionic form with a peak at 317 mμ, pH 13 was required for complete ionization of XVIII to XXII. The shift in alkali to 317 mμ for a 2-amino-4-mercapto-5,6-dialkylpyrimidine has been recorded previously.[12]

Condensation of XVIII ↔ XX with *p*-aminobenzoyl-L-glutamic acid in dimethylformamide followed by reduction with methanolic sodium borohydride, the procedure used for synthesis of the 4-hydroxy analog I,[2,7] gave the final product (II = XXVa) in only 3% yield. If the condensation was carried out with XVIII in methanol containing three equivalents of sodium methoxide so that the open aldehyde anion (XXII) was the reactive intermediate, no XXVa could be isolated. Since the 4-mercapto series behaved so differently from the hydroxy series,[2,7] a study of the chemistry of the condensation of XVIII with aniline and reduction of the intermediate was undertaken.

When aniline was condensed with XVIII in boiling ethanol, a pure recrystallized condensation product was obtained in 73% yield. That this condensation product had the cyclic structure XXIVb, and not the anil structure XXIIIb, was readily shown by its ultraviolet spectrum; the compound showed a maximum at 307 mμ at pH 7 which is compatible with structure XXIVb and not XXIIIb; the latter could be expected to have a maximum at about 350 mμ. At pH 13 XXIVb readily formed the anion of the open chain anil (XXVIb) since its maximum was at 317 mμ (see Table I). When XXIVb was converted to the anionic anil



a series, Ar = p-$C_6H_4CONHCH(COOH)CH_2CH_2COOH$; b series, Ar = C_6H_5

XXVIb with methanolic sodium methoxide and then the anil group reduced with sodium borohydride, the desired anilinopropylpyrimidine XXVb was obtained in 84% yield; at pH 7 it had an ultraviolet maximum at 348 mμ as expected for a 2-amino-4-mercapto-5,6-dialkylpyrimidine such as XXVb.

With conditions now established for conversion of XVIII to XXVb, the same conditions were used for synthesis of the 4-mercaptofolic analog (II = XXVa); the overall yield of analytically pure IIa was 54%.

The mercapto analog II was a good inhibitor of the folic reductase from rat liver[13] at pH 6.1 with an inhibitor–enzyme dissociation constant (K_i) of 1.1×10^{-6} M; II binds 9 times better than the substrate, folic acid, which had $K_m = 1.0 \times 10^{-5}$. However, the binding was only twofold increased compared to the 4-hydroxy analog I ($K_i = 2.0 \times 10^{-6}$). In contrast, when I and II were tested as inhibitors of the 5,10-methylenetetrahydrofolic dehydrogenase from pigeon liver,[4] II was bound five times better than I. The mercapto analog II had $K_i = 1.8 \times 10^{-4}$, I had $K_i = 10 \times 10^{-4}$, and the substrate had $K_m = 0.30 \times 10^{-4}$ M.

Also of interest was the relatively minor contribution of the carboxy-L-glutamate moiety of I and II to the binding to folic reductase; XXVb had $K_i = 4 \times 10^{-6}$, still twice as good as folic acid. In contrast, XXVb showed no measurable inhibition of 5,10-methylenetetrahydrofolate dehydrogenase at 0.23 mM (7 times substrate concentration), the highest concentration still allowing full light transmission.

Experimental[14]

5-Allyl-2-amino-4-chloro-6-methylpyrimidine (VI).—A solution of 1.0 g. of 5-allyl-2-amino-6-methyl-4-pyrimidinol[8] in 30 ml. of phosphorus oxychloride protected from moisture was heated in a bath at 90–100° for 30 min., then poured into 50 ml. of cold water. After the excess phosphorus oxychloride had hydrolyzed, the solution was filtered from a small amount of insoluble material. Adjustment of the filtrate to pH 10 with 10% sodium hydroxide gave a white precipitate which was collected on a filter and dried. Two recrystallizations from benzene gave 0.149 g. (13%) of white crystals, m.p. 145–146°; λ_{max}^{KBr} 3.00 (NH), 6.14 μ (pyrimidine double bond, NH).

Anal. Calcd. for $C_8H_{10}ClN_3$: C, 52.3; H, 5.49; N, 22.9; Cl, 19.3. Found: C, 52.6; H, 5.25; N, 22.6; Cl, 19.1.

Ethyl α-[2-(1,3-Dioxolan-2-yl)ethyl]acetoacetate (VIII).—A mixture of 41 g. (0.22 mole) of VII,[2,9] 25 g. of ethylene glycol, 0.5 g. of p-toluenesulfonic acid, and 200 ml. of benzene was refluxed for 1 hr., during which time a lower layer of water had formed. Washed with 50 ml. of water and dried with anhydrous $MgSO_4$, the solution was evaporated to a sirup *in vacuo;* this crude material was suitable for conversion to X. Distillation gave 28 g. (55%) of a colorless oil, b.p. 104–108° (0.10 mm.); λ_{max}^{film} 3.38 (enolic OH), 5.79, 5.86 (C=O), 6.12 (enolic C=C), 8.10 (ester C–O–C), 8.80, 9.75 μ (ether C–O–C).

Anal. Calcd. for $C_{11}H_{18}O_5$: C, 56.9; H, 7.87. Found: C, 57.3; H, 7.93.

If water was continuously removed with a Dean–Stark trap, the bisdioxolane IX was formed, as can be anticipated by the conversion of ethyl acetoacetate to a dioxolane.[10]

2-Amino-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinol (X).—A mixture of 23 g. (0.1 mole) of VIII, 100 ml. of absolute ethanol, 9.5 g. (0.1 mole) of guanidine hydrochloride, and 5.4 g. (0.1 mole) of sodium methoxide was refluxed with magnetic stirring for 2 hr. The solvent was removed by spin-evaporation *in vacuo* and the residue triturated with water to give 11.4 g. (51%) of insoluble product, m.p. 246–247°. After standing for several days at 3°, the filtrate deposited an additional 4.1 g. (total 69%) of product, m.p. 246–248°. Recrystallization from water gave white crystals of unchanged m.p.; λ_{max}^{KBr} 2.82, 3.03, 3.26 (OH, NH), 6.11, 6.55 (NH, pyrimidine), 8.90, 9.90 μ (ether C–O–C).

Anal. Calcd. for $C_{10}H_{15}N_3O_3 \cdot 0.5H_2O$: C, 51.3; H, 6.90; N, 18.0. Found: C, 51.6, 51.6; H, 6.85, 6.69; N, 18.0, 18.0.

Preparatively, crude, undistilled VIII gave 55% over-all yield based on VII; the over-all yield from VII with purification of VIII was 38%.

2-(Acetamido)-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinol (XII).—A mixture of 11.4 g. (0.053 mole) of X, 22 ml. of acetic anhydride, and 43 ml. of reagent pyridine was heated in a bath at 90° for 1 hr. The solution was spin-evaporated *in vacuo,* and the evaporation repeated after the addition of 50 ml. of toluene. The solid residue, m.p. 189–191°, was recrystallized from ethyl acetate to give 7.9 g. (57%) of white crystals, m.p. 188.5–189°; λ_{max}^{KBr} 3.18, 3.38 (OH, NH), 6.15 (C=O, pyrimidine), 8.84, 9.72 μ (ether C–O–C).

Anal. Calcd. for $C_{12}H_{17}N_3O_4$: C, 53.9; H, 6.42; N, 15.7. Found: C, 54.0; H, 6.22; N, 15.8.

Without pyridine, the reaction mixture was more difficult to crystallize and the yield dropped to 45%. This compound XII could be recovered unchanged when boiled 1 hr. in water, in contrast to the corresponding diethyl acetal (III), which gave XIV under these conditions.[2,7] With 90% formic acid for 2 hr. at room temperature, a 25% yield of XIV was obtained.[2]

2-Acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII).—A stirred mixture of 10 g. (0.0347 mole) of XII, 100 ml. of reagent benzene, and 10 g. (0.0652 mole) of reagent phosphorus oxychloride was surrounded by an oil bath preheated and maintained at 85°. All of the solid dissolved in 5 min.; as soon as turbidity appeared (an additional 5 min.), the mixture was immediately poured onto a stirred solution of 64 g. of anhydrous sodium acetate in 200 ml. of water plus 50 g. of ice. The mixture was stirred *exactly 5 min.,* then the organic layer was separated. The aqueous layer was extracted with three 30-ml. portions of dichloromethane. The combined organic solutions, dried with anhydrous $MgSO_4$, were spin-evaporated to dryness *in vacuo;* yield, 9.5 g. (96%) of yellowish white solid, m.p. 115–117°, suitable for further transformations. For analysis a sample was continuously extracted with boiling hexane. Evaporation of the hexane gave a 16% recovery of white solid, m.p. 126–127°. Recrystallization from benzene–hexane afforded white crystals of unchanged m.p.; λ_{max}^{KBr} 3.08 (NH), 5.95 (amide C=O), 6.47, 6.65 (NH, pyrimidine), 9.62 μ (ether C–O–C).

Anal. Calcd. for $C_{12}H_{16}ClN_3O_3$: C, 50.4; H, 5.64; N, 14.7. Found: C, 50.5; H, 5.64; N, 14.8.

The crude product, m.p. 115–117° was used for further transformations since purification at this stage led to high losses. If the reaction time was increased to 40 min., the yield of crystal-

(13) We are grateful to Dr. W. C. Werkheiser, Roswell Park Memorial Institute, for the folic reductase assays.

(14) Melting points were taken on a Fischer–Johns apparatus; melting points below 230° are corrected, those above are not. Boiling points are uncorrected. Infrared spectra were taken with a Perkin–Elmer Model 137B spectrophotometer. Ultraviolet spectra were taken with either a Cary Model 11 or a Perkin–Elmer Model 202 spectrophotometer.

line material obtained by trituration with alcohol was about 40% and considerable brown oil was formed. The yield was also lowered if the benzene solution was stirred with the aqueous sodium acetate for a longer period of time.

2-Acetamido-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidylisothiuronium Chloride.—A mixture of 10 g. (0.035 mole) of crude 2-acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII), 2.8 g. (0.0368 mole) of thiourea, and 100 ml. of *t*-butyl alcohol was refluxed with stirring for 1 hr. When the solvent started refluxing a considerable amount of solid was dissolved, but a clear solution was not obtained. Crystallization began after 5 min. and the amount of solid was increasing during the last 55 min. of heating. The resulting mixture was allowed to cool to room temperature. Acetone (20 ml.) was added and the mixture was chilled. The product was collected on a filter and washed with acetone; yield, 10.3 g. (81%), m.p. 163–164°.

From a previous run the crude product, after one recrystallization from absolute ethyl alcohol had a constant m.p. at 162–163: λ_{max}^{KBr} 3.11 3.19 (NH), 5.88 (C=NH$^+$), 6.07, 6.37 (pyrimidine, amide), 9.72, 9.92 μ (ether C–O–C), $\lambda_{max}^{pH\ 1}$ 242 (ϵ 19,800), 293 mμ (ϵ 8,200); $\lambda_{max}^{pH\ 7}$ 242 (ϵ 19,400), 292 mμ (ϵ 8,200); $\lambda_{max}^{pH\ 13}$ 309 (ϵ 12,400), inflection centering at 242 mμ (ϵ 17,513); the pH 13 curve may be inaccurate due to conversion to XVI.

Anal. Calcd. for $C_{13}H_{20}ClN_3O_3S$: C, 43.1; H, 5.57; N, 19.4; S, 8.86. Found: C, 43.2; H, 5.83; N, 19.4; S, 9.03.

2-Amino-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinethiol (XV). A.—To a solution of 0.20 g. (3.56 mmoles) of potassium hydroxide in 3 ml. of methanol saturated with hydrogen sulfide was added 400 mg. (1.4 mmoles) of pure XIII. After being refluxed for 9 hr., the mixture was spin-evaporated *in vacuo*. The residue was dissolved in 10 ml. of water and filtered from traces of dipyrimidinyl sulfide formed in some runs; then the filtrate was acidified to pH 4 with acetic acid; yield, 257 mg. (76%), m.p. 218–219°. Recrystallization from water gave yellow crystals, m.p. 218–219°; λ_{max}^{KBr} 3.38 (NH), 6.04, 6.42 (NH, pyrimidine), 8.85, 9.42, 9.70 μ (ether C–O–C).

Anal. Calcd. for $C_{10}H_{15}N_3O_2S$: C, 49.7; H, 6.26; N, 17.4; S, 13.3. Found: C, 49.5; H, 6.47; N, 17.3; S, 13.1.

Preparatively, it was more expedient to use crude XIII, m.p. 115–117°, which gave 60% of XV, m.p. 217–218°.

B.—A solution of 50 mg. (0.138 mmole) of 2-acetamido-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidylisothiuronium chloride in 2.5 ml. of *N* sodium hydroxide was allowed to stand at room temperature for 2 hr. when spectral changes were complete. Acidification to pH 7 with acetic acid precipitated 28 mg. (84%) of product identical with preparation A.

2-Acetamido-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinethiol (XVI). A.—A mixture of 5.0 g. (21.3 mmole) of XV, 5 ml. of acetic anhydride, and 10 ml. of reagent pyridine was heated in a bath at 85° for 2 hr., then spin-evaporated *in vacuo*. Recrystallization of the residue from ethanol gave 5.3 g. (89%) of product in two crops, m.p. 216–217°; λ_{max}^{KBr} 3.10 (NH), 6.00 (amide C=O), 6.22, 6.48 (NH, pyrimidine), 8.83, 9.75 μ (ether C–O–C).

Anal. Calcd. for $C_{12}H_{17}N_3O_3S$: C, 50.8; H, 6.16; N, 14.9; S, 11.3. Found: C, 51.0; H, 6.20; N, 15.0; S, 11.2.

B.—To a solution of 9.6 g. of potassium hydroxide in 150 ml. of reagent methanol saturated with hydrogen sulfide at 0° was added 19.1 g. of crude XIII. After being refluxed for 6 hr. the mixture was spin-evaporated to residue *in vacuo*. To the semisolid was added 50 ml. of acetic anhydride and 100 ml. of pyridine. After the exothermic reaction had subsided, the dark orange solution was heated in a bath at 80–90° for 2 hr. The solution was spin-evaporated *in vacuo* and the residue was extracted with boiling 95% ethanol. The hot solution was filtered, then chilled; yield, 16.6 g. (88%), m.p. 213–214°.

C.—A solution of 10 g. (0.0276 mole) of 2-acetamido-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidyl isothiuronium chloride in 110 ml. (0.11 mole) of *N* NaOH was allowed to stand at room temperature for 2 hr. and then adjusted to pH 7 with glacial acetic acid. After chilling, the yellow precipitate was collected, washed with ice water, and dried; yield, 6.9 g. (88%), m.p. 201–203°, that was suitable for further transformations.

Conversion of X to 2-Amino-5-(3,3-diethoxypropyl)-6-methyl-4-pyrimidinol (III).—A solution of 2.0 g. (9.6 mmoles) of X in 100 ml. of absolute ethanol containing 0.5 g. of 96% sulfuric acid was refluxed for 6 hr., then neutralized with a solution of 0.64 g. of potassium hydroxide in absolute ethanol. The mixture was spin evaporated *in vacuo*, then the residue was extracted with boiling dichloromethane. The filtered solution was evaporated and the residue was crystallized from ethyl acetate to give 0.60 g. (30%) of III, m.p. 179–180°, identical with an authentic sample.[2] No attempt was made to establish optimum conditions.

2-Amino-5,6-dihydro-7-ethoxy-4-methyl-7H-thiopyrano[2,3-d]-pyrimidine (XVII). A.—A solution of 5.5 g. (19.5 moles) of XVI in 200 ml. of absolute ethanol containing 1.94 g. of 95% sulfuric acid was refluxed for 8 hr., then neutralized with ethanolic potassium hydroxide. The mixture was spin-evaporated *in vacuo* and the residue was extracted with 200 ml. of hot chloroform. Spin-evaporation of the filtered chloroform solution *in vacuo* and recrystallization from absolute ethanol gave 4.1 g. (94%) of product, m.p. 181–182°; λ_{max}^{KBr} 2.98, 3.12 (NH, pyrimidine), 9.12, 9.47 μ (ether C–O–C).

Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.3; H, 6.72; N, 18.7; S, 14.2. Found: C, 53.2; H, 6.81; N, 98.5; S, 14.3.

B.—By the same procedure, 440 mg. (1.82 mmoles) of XV gave 400 mg. (98%) of chloroform residue, m.p. 180–181°. Recrystallization from absolute ethanol gave 263 mg. (64%) of product, m.p. 181–182°, that was identical with preparation A. No attempt was made to obtain a second crop.

2-Acetamido-5,6-dihydro-7-ethoxy-4-methyl-7H-thiopyrano-[2,3-d]pyrimidine (XIX).—Acetylation of 0.5 g. of XVII as described for XV, then 3 recrystallizations from ethanol gave 38% of analytically pure product, m.p. 129–130°; λ_{max}^{EtOH} 235 (ϵ 26,900); 292 mμ (ϵ 8,200); μ_{max}^{KBr} 3.15, 3.45 (NH), 6.02 (amide C=O), 6.50 (NH, pyrimidine), 9.20, 9.40 μ (ether C–O–C).

Anal. Calcd. for $C_{12}H_{17}N_3O_2S$: C, 53.9; H, 6.44; N, 15.7; S, 12.0. Found: C, 54.0; H, 6.43; N, 15.5; S, 11.8.

2-Amino-5,6-dihydro-7-hydroxy-4-methyl-7H-thiopyrano-[2,3-d]pyrimidine (XVIII).—A solution of 5 g. (22.2 mmoles) of XVII in 50 ml. of 5% sulfuric acid was refluxed for 30 min., cooled to 25°, and neutralized with solid sodium bicarbonate. The separated product was collected on a filter and washed with water. Recrystallization from absolute ethanol gave 2.4 g. (65% of pure product, which gradually decomposed above 280° without melting; on a Kofler hot-bench, it showed partial melting near 235°; λ_{max}^{KBr} 2.98, 3.02, 3.18 (NH, OH), 6.15, 6.54 μ (NH, pyrimidine).

Anal. Calcd. for $C_8H_{11}N_3OS$: C, 48.2; H, 5.63; N, 21.3; S, 16.3. Found: C, 48.2; H, 5.67; N, 21.2; S, 16.2.

Similarly, hydrolysis of XIX gave 74% of recrystallized product. Hydrolysis of XV under the same conditions gave a product with the same ultraviolet spectrum as XVIII, but the material was insoluble in hot alcohol, had slightly shifted peaks in the 6 μ region of its infrared spectrum, and traveled differently on a thin layer silica gel chromatogram using methanol–water–acetic acid (8:1:1); the same properties were observed with this material prepared from XV after solution in dilute alkali and reprecipitation with acetic acid at about pH 8.

The best route to XVIII from XIII is *via* isothiouronium chloride → XVI → XVII.

2-Amino-7-anilino-5,6-dihydro-4-methyl-7H-thiopyrano-[2,3-d]pyrimidine (XXIVb).—To a solution of 98.6 mg. (0.5 mmole) of XVIII in 30 ml. of boiling absolute alcohol was added 480 mg. (5.1 mmoles) of aniline. After being refluxed for 1 hr., the solution was spin-evaporated *in vacuo*. Recrystallization of the residue from toluene gave 100 mg. (73%) of analytically pure material, m.p. 177–178°; λ_{max}^{KBr} 2.89, 3.07, 3.22 (NH), 6.17, 6.32, 6.53 (NH and aromatic double bonds), 13.8, 14.5 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{13}H_{16}N_4S$: C, 61.7; H, 5.92; N, 20.6. Found: C, 61.6; H, 5.86; N, 20.4.

2-Amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinethiol (XXVb).—To a solution of 100 mg. (0.37 mmole) of XXIVb in 15 ml. of reagent methanol protected from moisture was added 0.37 ml. (0.37 mmole) of *N* methanolic sodium methoxide. After 30 min. at room temperature, the solution was treated with 500 mg. (13.2 mmoles) of sodium borohydride, then refluxed for 90 min. The solvent was removed by spin-evaporated *in vacuo;* the residual sodium salt was dissolved in 10 ml. of water and the solution was acidified to pH 7 with hydrochloric acid; yield, 85 mg. (85%) of product, m.p. 204–206°. Recrystallization from aqueous alcohol gave 68 mg. (68%) of pure product, m.p. 206–207°; $\lambda_{max}^{pH\ 1}$ 259 (5,880), 338 mμ (ϵ 15,600); $\lambda_{max}^{pH\ 7}$ 242 (15,900), 348 mμ (ϵ 15,600); $\lambda_{max}^{pH\ 13}$ 317 (14,100), shoulder at 240 mλ (ϵ, 29,400); λ_{max}^{KBr} 3.02, 3.27 (NH), 6.06, 6.34, 6.45 (NH and aromatic double bonds), 13.2, 14.5 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{14}H_{18}N_4S$: C, 61.3; H, 6.61; N, 20.4. Found: C, 61.1; H, 6.69; N, 20.3.

The recrystallized product gave a negative Bratton–Marshall test for arylamine; the crude product contained 3% Bratton–Marshall positive material calculated as starting material (XXIVb).

N-[1-(2-Amino-4-mercapto-6-methyl-5-pyrimidiyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid (II).—To a hot solution of 98.6 mg. (0.5 mmole) of XVIII in 30 ml. of absolute ethanol was added 133 mg. (0.5 mmole) of *p*-aminobenzoyl-L-glutamic acid. After being refluxed with stirring for 1 hr., the solution was filtered from a trace of insoluble material, then spin-evaporated *in vacuo* leaving 231 mg. (100%) of crude XXIVa; $\lambda_{max}^{pH\ 1}$ 222, 282, 317 mμ, $\lambda_{max}^{pH\ 8.4}$ 307 mμ; $\lambda_{max}^{pH\ 13}$ 312, shoulder at 270 mμ.

A solution of 230 mg. (0.5 mmole) of the crude XXIVa in 20 ml. of reagent methanol and 1.7 ml. of *N* methanolic sodium methoxide was allowed to stand at room temperature protected from moisture for 1 hr. After the addition of 0.7 g. (18.7 mmoles) of sodium borohydride, the mixture was refluxed with stirring for 90 min., then spin-evaporated *in vacuo*. The residue was dissolved in 25 ml. of water and the solution adjusted to pH 3 with 6 *N* hydrochloric acid. The precipitate was collected by centrifugation, then washed successively with two 5-ml. portions of water and two 5-ml. portions of hot water; yield, 146 mg. (69%), which contained 4% of Bratton–Marshall positive material calculated as the intermediate thiopyran (XXIVa). For purification, 142 mg. was dissolved in 20 ml. of 1% aqueous sodium bicarbonate; the filtered solution was acidified to pH 3 and the product collected and washed as before; yield, 119 mg. (55%) of amorphous solid that contained 2.6% Bratton–Marshall positive material and melted at 180–189° dec.; $\lambda_{max}^{pH\ 1}$ 337 mμ (ε 16,200); $\lambda_{max}^{pH\ 8.4}$ 310 (20,200), inflection centering at 350 mμ (ε 7200); $\lambda_{max}^{pH\ 13}$ 312 mμ (ε 24,900).

Anal. Calcd. for $C_{20}H_{25}N_5O_6S$: C, 53.7; H, 5.63; N, 15.6; S, 7.16. Found: C, 53.5; H, 5.87; N, 15.6; S, 7.48.

The pH for reprecipitation of XXVa is critical; precipitation at pH 4.5 caused loss of nearly half the product. In contrast to I, XXVa (II) is somewhat soluble in alcohol and acetone; therefore washing with these solvents was avoided.

Acknowledgment.—We wish to thank Starks Associates, Inc. and the Cancer Chemotherapy National Service Center, National Cancer Institute for large-scale preparation of certain intermediates, mediated by Contract No. SA-43-ph-4346.

Analogs of Tetrahydrofolic Acid. IX.[1,2] Synthesis of N-[1-(2-Amino-4-hydroxy-6-phenyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid, a "Nonclassical" Inhibitor of Some Folic Cofactor Area Enzymes

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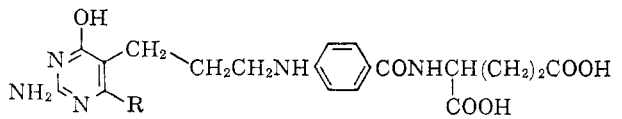
Received May 25, 1963

The title compound II has been synthesized in six steps from ethyl benzoylacetate. Due to the restricted rotation of the phenyl ring in 2-amino-5-(3,3-diethoxypropyl)-6-phenyl-4-pyrimidinol (VI), the synthesis of VI from ethyl α-(3,3-diethoxypropyl)benzoylacetate (VII) and guanidine presented difficulty; the rate of reaction of formation of the pyrimidine (VI) was sufficiently slow that alcoholysis of the keto ester VII was the predominant reaction. By use of dimethyl sulfoxide as a solvent, the yield in the condensation reaction was increased from 5 to 52%. II was an excellent inhibitor of folic reductase and was bound 100 times more tightly to the enzyme than the substrate folic acid. In addition, II was a good inhibitor of 5,10-methylenetetrahydrofolate dehydrogenase.

The important B-family vitamin, folic acid, is intracellularly reduced to its cofactor form, tetrahydrofolic acid, by the enzyme, folic reductase. The resultant tetrahydrofolic acid then participates as a cofactor in a series of enzymatic reactions for acceptance and transfer of "one-carbon" fragments involving at least 14 known



I, R = CH_3; II, R = C_6H_5

enzymes.[3–5] In enzymic transfer reactions between substrate and cofactor (or cosubstrate), the atoms to which the transfer group is attached are unlikely to be binding points of the molecule to the enzyme.[6] Therefore, it was postulated that the N-5 of the tetrahydrofolic acid molecule, involved in some of the transfer reactions in the folic cofactor area, should be replaced by a methylene in order to obtain an inhibitor.[7] The first such compounds synthesized were 5,6,7,8-tetrahydroquinazoline analogs of tetrahydrofolic acid[7–9] which showed good inhibition of folic reductase.[5,9] A simpler molecule was needed which could lend itself to the type of molecular manipulation needed for design of "nonclassical antimetabolites" and irreversible inhibitors of the "*exo*-alkylating" type.[6,10] Such a proposed[5] compound was the pyrimidyl analog I of tetrahydrofolic acid; I was synthesized and found to bind to folic reductase about as well as the substrate folic acid.[11]

(1) This work was generously supported by the U. S. Public Health Service by Grant No. CA-06624 and Training Grant No. 2G-555.

(2) For the previous paper of this series see B. R. Baker, C. E. Morreal, and B. Ho, *J. Med. Chem.*, **6**, 658 (1963).

(3) T. H. Jukes and H. P. Broquist, "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel Ed., Academic Press, Inc., New York, N. Y., 1963, pp. 481–534.

(4) F. M. Huennekens, M. J. Osborn, and H. R. Whitely, *Science*, **128**, 120 (1958).

(5) B. R. Baker, paper V of this series, Preprints of the Scientific Session of the American Pharmaceutical Association Meeting, Las Vegas, Nevada, 1962.

(6) B. R. Baker, *Cancer Chemotherapy Rept.*, **4**, 1 (1959).

(7) R. Koehler, L. Goodman, J. DeGraw, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5779 (1958); paper I of this series.

(8) J. I. DeGraw, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1156 (1961); paper III of this series.

(9) J. DeGraw, L. Goodman, B. Weinstein, and B. R. Baker, *ibid.*, **27**, 576 (1962); paper IV of this series.

(10) (a) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962); (b) B. R. Baker, *Biochem. Pharmacol.*, **11**, 1155 (1962); (c) B. R. Baker and R. P. Patel, *Biochem. Biophys. Res. Commun.*, **9**, 199 (1962); B. R. Baker, *Biochem. Pharmacol.*, **12**, 293 (1963).

(11) (a) B. R. Baker, and C. E. Morreal, *J. Pharm. Sci.*, **51**, 596 (1962); paper VI of this series; (b) B. R. Baker and C. E. Morreal, *ibid.*, **52**, 804 (1963); paper VII of this series.