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Irreversible Enzyme Inhibitors. XCIV. Inhibition of Dihydrofolic Reductase with Derivatives of 2,6-Diaminopurines (1,2)

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In order to gain additional information on the hydrophobic bonding region of dihydrofolic reductase, some derivatives of 2,6-diaminopurine with aryl or aralkyl groups at the N^6, C^8 and N^9-positions were investigated as inhibitors. Since none of the six compounds gave an increment in binding over the parent 2,6-diaminopurine, hydrophobic bonding to dihydrofolic reductase could not be detected with this ring system. Furthermore, 2,6-diaminopurine bridged from its 8-position with methylene groups to the amino group of *p*-aminobenzoic acid also failed to show an increment in binding.

The 8-substituted 2,6-diaminopurines were synthesized by base-catalyzed cyclodehydration of the appropriate 5-acylamido-2,4,6-triaminopyrimidines; the latter compounds were readily prepared by selective acylation of tetraaminopyrimidine.

Although the discovery of a hydrophobic bonding region on dihydrofolic reductase (3) explained many of the anomalous results on binding to this enzyme (4), the presence of this region considerably complicated the design of active-site-directed irreversible inhibitors (4,5) for this enzyme (4). Some seventeen different approaches were started simultaneously in order to understand and use bonding to the hydrophobic region in order to design an active-site-directed irreversible inhibitor. Some questions answered were (a) where was the hydrophobic bonding region with respect to the binding loci for the substrate, dihydrofolate (4,6,7), and (b) how could effective active-site-directed irreversible inhibitors be designed (4,8-11)? 2,6-Diaminopurine was one of a number of known 2,4-diaminoheterocycles that could inhibit dihydrofolic reductase (12-14); therefore, 2,6-diaminopurine was selected as one of the seventeen problem areas for further study on hydrophobic bonding to dihydrofolic reductase. The results are the subject of this paper.

Phenyl and phenylalkyl groups were introduced on the N^6, C^8, and N^9-positions of 2,6-diaminopurine. Of these six compounds (II-VII) (Table I), none showed an appreciable increase in binding to dihydrofolic reductase over that shown by the parent 2,6-diaminopurine (I); if hydrophobic bonding were present a 10 to 40,000-fold increment in binding should have been observed (3,4,7).

Since hydrophobic bonding could not be detected with 8-aralkyl substituents (VI, VII) on the 2,6-diaminopurine, it was considered possible that the 2,6-diamino-8-substituted purines, when complexed to the enzyme, assumed a conformation similar to dihydrofolate; if such were the case then enhanced binding should be shown with *p*-aminobenzoic acid bridged to the 8-position of the 2,6-diaminopurine with an alkyl group (IX, X). Again no increment in binding was observed; therefore the binding of 2,6-diaminopurine to dihydrofolic reductase remains anomalous at this time. Since some new methods for synthesis of substituted 2,6-diaminopurines have evolved from this study, these methods make up the main body of this paper.

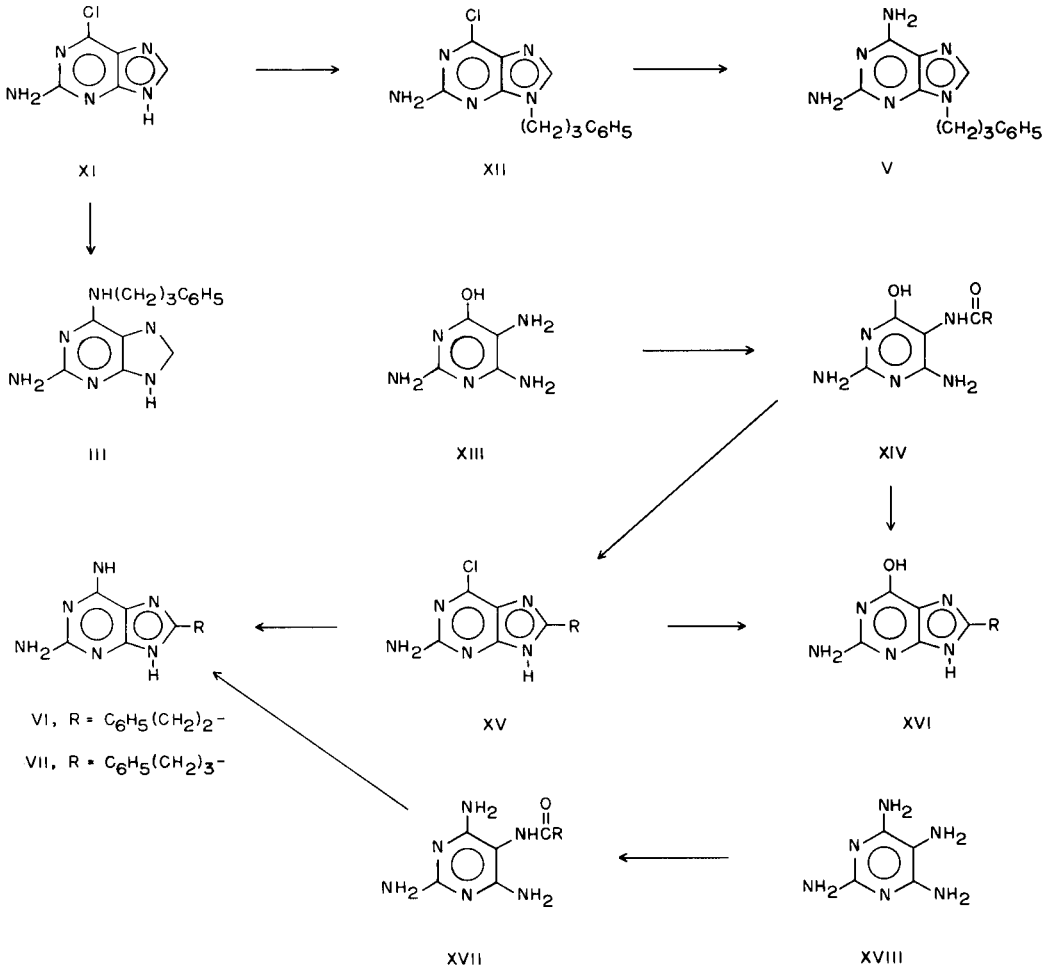
Fusion of 2-amino-6-chloropurine (XI) with phenylpropylamine at 150° gave a 50% yield of pure III that showed the proper ultraviolet spectra for a 2-amino-6-alkylaminopurine (16). Alkylation of 2-amino-6-chloropurine (XI) with benzyl chloride has been reported to give a mixture of the 7- and 9-benzyl derivatives (17). When XI was converted to its sodium salt with sodium hydride in *N*,*N*-dimethylformamide, then reacted with 3-phenylpropylbromine, two major products were observed by TLC. The slower moving product (XII) was readily crystallized from benzene in 30% yield and had ultraviolet spectra in agreement with 2-amino-9-benzyl-6-chloropurine (17); furthermore, acid hydrolysis gave a product with the ultraviolet spectra of a 9-alkylguanine (18). Reaction of XII with methanolic ammonia at 140° gave a 62% yield of V.

The most facile method of converting a preformed pyrimidine to a purine consists of treatment of a 4,5-diaminopyrimidine with an orthoester (19); unfortunately, the necessary orthoester precursors for more complex 8-substituted purines such as VI and VII are not easily obtained. The synthetic approach having the most latitude insofar as variation of an 8-alkyl or aralkyl substituent of 2,6-diamino (such as VI) and 2-amino-6-hydroxypurines (XVI) appears to be via dehydrocyclization of the 4-amino-5-acylamidopyrimidines of type XIV and XVII. The most

general procedure of effecting this ring closure consists of heating the 4-amino-5-acylamidopyrimidine at or above its melting point until dehydration occurs (13); unfortunately, purines of type XVI and VI are obtained in low yield by this method due to decomposition. A number of 2,6-diamino-8-arylpurines have been prepared by ring closure of 5-arylamido-2,4,6-triaminopyrimidines (XIVa) with phosphorous oxychloride (13); similarly, 2,6-diamino-5-arylamido-4-pyrimidinols (XIVa) have been converted to the 2-amino-6-chloropurines (XVa) which can be aminated to give the 2,6-diaminopurines (VI, R = aryl) or hydrolyzed to 8-arylguanine derivatives (XVIa) (13). Recently, 8-arylpurines have been obtained in good yield by the polyphosphoric acid catalyzed dehydrocyclization of 4-amino-5-arylamidopyrimidines (20).

The 5-arylamidopyrimidinol (XIVa) precursors can readily be synthesized by treatment of the bisulfite salt of 2,5,6-triamino-4-pyrimidinol (XIII) with an acid chloride under Schotten-Baumann conditions (13, 21). In a similar manner, the 5-aralkylamidopyrimidinols (XIVb and XIVc) were prepared by treatment of the sulfate salt of XIII with hydrocinnamoyl chloride or 4-phenylbutyroyl chloride in 2 *N* sodium hydroxide. Since the 4,5-diaminopyrimidine system is reported to be susceptible to oxidation in aqueous base (22) the precaution of including sodium bisulfite in the reaction mixture was taken in the initial experiments performed; it was later found that the omission of this anti-oxidant had no significant effect.

The elegant dehydrocyclization of 4-amino-5-arylamidopyrimidines with polyphosphoric acid (20) gives the corresponding 8-arylpurines in higher yields and in one less step than the earlier phosphorous oxychloride ring closure (13). By utilization of the former procedure, XVIa could readily be obtained in 60% yield (23). When the aralkylamidopyrimidinols (XIVb and XIVc) were treated with polyphosphoric acid in an identical manner as used for the preparation of XVIa, intractable gums were isolated which did not exhibit absorption in the ultraviolet



region characteristic of 8-substituted guanine derivatives or the starting acylamidopyrimidinols (XIV). Although no further attempt was made to identify the reaction products, it was apparent that cyclization of XIVb and XIVc with polyphosphoric acid was not a feasible route to the desired 8-aralkylpurines (XVIb and XVIc) (23).

Treatment of the phenylpropionamidopyrimidinol (XIVb) with phosphorus oxychloride gave the crude chloropurine (XVb) in 81% yield (23). As reported in the preparation of XVa (13,24), rigorous exclusion of moisture from the reaction mixture was essential to prevent formation of the isomeric oxazolopyrimidine. Acid hydrolysis of crude XVb gave pure 8-phenylethylguanine (XVIb) in 50% yield (23). The diaminopurine (VI) could be obtained in 57% yield after treatment of the 6-chloropurine (XVb) with concentrated ammonium hydroxide. In a similar manner, XIVc was converted to 8-phenylpropylguanine (XVIc) (23) and 2,6-diamino-8-phenylpropylpurine (VII) via the chloropurine, XVc.

A number of 4-amino-5-acylamidopyrimidines have been cyclized to purines by mild treatment with aqueous base (25-27); this method has not been utilized for the synthesis of 2,6-diaminopurines or 2-amino-6-hydroxypurines. The simplicity of this method, along with the consideration that 2,6-diaminopurines are quite stable in basic media (28), prompted a study of the direct synthesis of the 2,6-diaminopurines (VI and VII) via the base catalyzed dehydrocyclization of XVII. Furthermore, since the conversion of XVII to VI is accompanied by a bathochromic shift of about 20 mμ in the ultraviolet spectrum at high *p*H, the progress of the reaction could be easily monitored.

The 5-acylamido-2,4,6-triaminopyrimidines (XVIIb, XVIIc) were readily prepared by acylation of tetraaminopyrimidine (XVIII) in 2 *N* sodium hydroxide; XVIIb and XVIIc separated from solution as almost pure amorphous powders in 35 to 45% yields.

After heating a solution of XVIIb in 1 *N* sodium hydroxide at 80° for one hour, the diaminopurine (VI) could not be detected in the ultraviolet spectrum and XVIIb was recovered unchanged upon neutralization of the reaction mixture. Since it was believed at this time that more drastic reaction conditions would result in hydrolysis of the amide bond of XVIIb, attention was turned to the base catalyzed dehydrocyclization in nonaqueous media.

When a solution of XVIIb in *t*-butyl alcohol containing 1.1 equivalents of potassium *t*-butoxide was heated at reflux for twelve hours, the ultraviolet

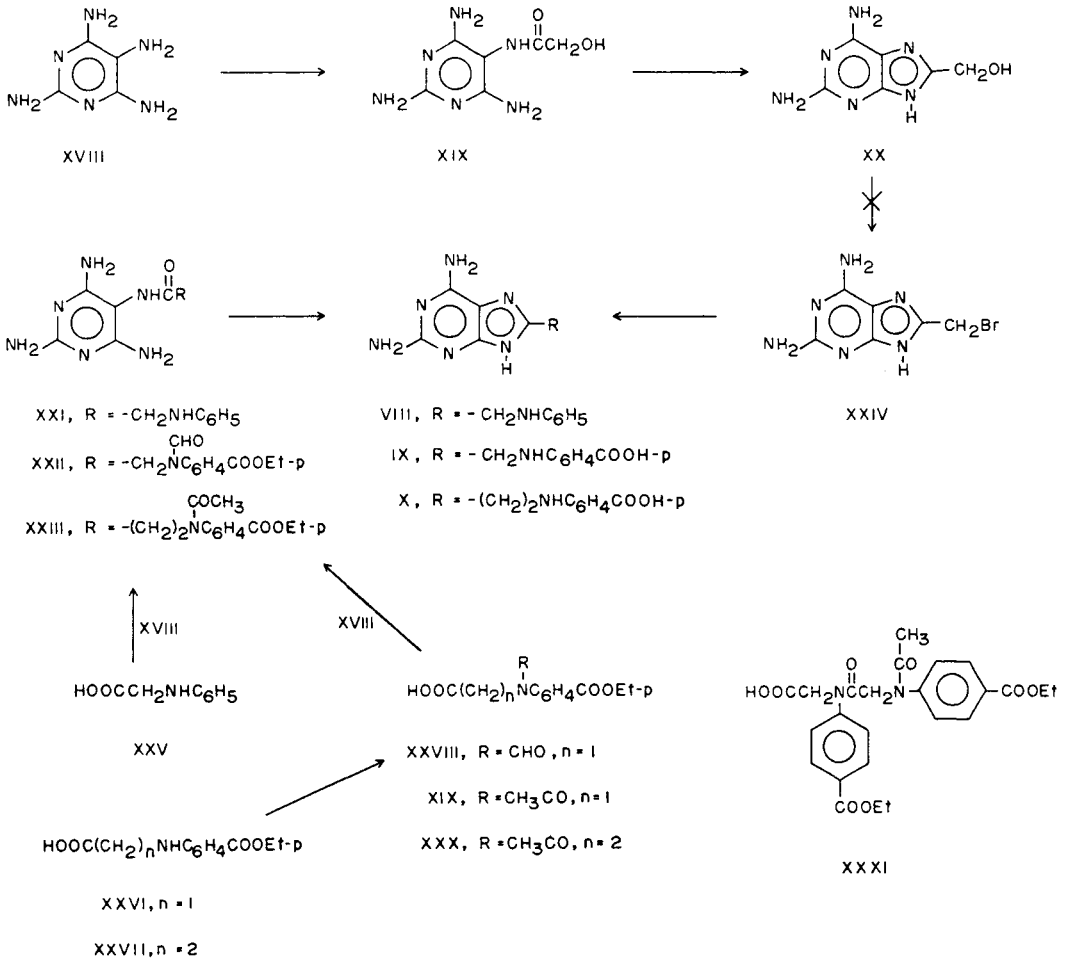
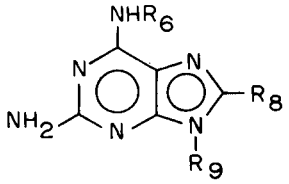


TABLE I

Inhibition of Dihydrofolic Reductase by



Compound	R_6	R_8	R_9	μM Conc. for 50% Inhibition (a)
I	H	H	H	0.34
II (b)	C_6H_5	H	H	1.6
III	$C_6H_5(CH_2)_3$-	H	H	0.20
IV (b)	H	H	C_6H_5	0.85
V	H	H	$C_6H_5(CH_2)_3$-	0.30
VI	H	$C_6H_5(CH_2)_2$-	H	0.43
VII	H	$C_6H_5(CH_2)_3$-	H	0.25
VIII	H	$C_6H_5NHCH_2$-	H	0.53
IX	H	p-$HOOCC_6H_4NHCH_2$-	H	0.33 (c)
X	H	p-$HOOCC_6H_4NH(CH_2)_2$-	H	0.50 (c)

The technical assistance of Maureen Baker, Shirley Humphrey, Ann Jaqua and Karen Smith is acknowledged. (a) Dihydrofolic reductase was a 45-90% saturated ammonium sulfate fraction from pigeon liver; it was prepared and assayed with 6 μM dihydrofolate and 12 μM TPNH in Tris buffer (pH 7.4) containing 10% N,N-dimethylformamide as previously described (15). (b) We wish to thank Professor Roland K. Robins for a gift of this compound. (c) Sample dissolved in Tris buffer by readjustment of pH to 7.4.

spectrum of an aliquot of the reaction mixture indicated complete conversion to the phenylethylpurine (VI). After removal of the reaction solvent and dissolution of the product in 1 N sodium hydroxide, aqueous sulfuric acid was added to precipitate the hydrated hemisulfate salt of VI in 59% yield. Recrystallization from 2-methoxyethanol-aqueous sulfuric acid gave the hydrated sulfate salt of VIb which was shown to be identical with the product previously obtained by amination of the chloropurine XVb. Dehydrocyclization of XVIIc in an identical manner gave the hydrated hemisulfate salt of VII in 46% yield.

In view of the successful ring closure of XVIIb and XVIIc to the 2,6-diaminopurines (VI, VII) in t-butyl alcohol, it was considered worthwhile to reinvestigate the cyclization of XVIIb in aqueous media under more vigorous reaction conditions than previously used. After a solution of the phenylpropionamidopyrimidine (XVIIb) in 4 N sodium hydroxide was heated at reflux for seven hours, the shift in ultraviolet spectrum had become constant and the diaminopurine (VI) was readily isolated in 79% yield; this result was rather surprising since the vigorous conditions used to effect ring closure could be anticipated to hydrolyze the amide bond.

The facile synthesis of 8-phenylethylpurine (VI) by the base catalyzed ring closure of XVIIb, prompted an attempt to convert the 4-pyrimidinol (XIV) to the corresponding 8-substituted guanine derivative (XVI) by the same method. When the phenylpropionamido-4-pyrimidinol (XIVb) was treated with 4 N sodium hydroxide or potassium t-butoxide in an identical manner as used for the synthesis of VI, only end absorption could be detected in the ultraviolet spectrum; the similar results were obtained with the benzamido-4-pyrimidinol (XIVa). It was therefore concluded that in basic media the 5-acylamido-4-pyrimidinols underwent cleavage of the pyrimidine ring at a greater rate than dehydrocyclization.

The two synthetic routes considered for the synthesis of 8-anilinoalkyl derivatives of 2,6-diaminopurines were: (a) alkylation of an aromatic amine with 8-bromomethyl-2,6-diaminopurine (XXIV); and (b), acylation of tetraaminopyrimidine (XVIII) with a preformed precursor of the 8-anilinoalkyl substituent followed by base catalyzed ring closure. The former route appeared to offer the most latitude insofar as variation of the anilino moiety was concerned and was therefore chosen for initial investigation.

Reactive alkylating functions and strongly basic heterocycles are most compatible under conditions where the heterocycle is protonated and therefore not susceptible to alkylation. The synthetic route selected to the 8-bromomethylpurine (XXIV) was via

the 8-hydroxymethylpurine (XX) by reaction with hydrogen bromide in glacial acetic acid; a similar route has been successfully used for the synthesis of a 6-bromomethyl-2,4-diaminopyrimidine (29).

Initially, it was believed that the synthesis of the glycolamidopyrimidine (XIX) precursor of XX would require an activated derivative of glycolic acid in which the hydroxyl group had been masked; however, a number of 5-acylamidopyrimidines have been reported to be obtained by direct acylation of 5-aminopyrimidines with carboxylic acids bearing an electron withdrawing group at the α-carbon atom (26,27). For example, treatment of 5,6-diamino-4-pyrimidinol with glycolic acid, malonic acid, or oxalic acid gave the corresponding 5-acylamidopyrimidinols which could be subsequently cyclized to the corresponding 8-substituted hypoxanthine derivatives by treatment with aqueous base (26). In a similar manner, 8-hydroxymethylpurine could easily be obtained starting from glycolic acid and 4,5-diaminopyrimidine (28).

When tetraaminopyrimidine (XVIII) was treated with a concentrated aqueous solution of glycolic acid at 80° for fifteen minutes, the ultraviolet spectrum of an aliquot of the reaction mixture indicated that complete conversion to XIX had occurred; however, the water soluble nature of the glycolamidopyrimidine (XIX) hindered its isolation from the reaction mixture. When a mixture of XVIII and purified glycolic acid was heated as a melt at 100° for one hour and poured into a mixture of ethanol-ethyl acetate, crude XIX readily precipitated as an amorphous white solid. Since all attempts to further purify XIX resulted in the formation of intractable gums, the crude glycolamidopyrimidine (XIX) was directly converted to the 8-hydroxymethylpurine (XX) by treatment with aqueous sodium hydroxide. Upon neutralization of the reaction mixture, crude XX precipitated from solution in 59% overall yield from XVIII which could be further purified as its acetate salt.

When the hydroxymethylpurine (XX) was treated with 10% anhydrous hydrogen bromide in glacial acetic acid at reflux for 24 hours, a highly insoluble product was obtained that showed the characteristic ultraviolet spectra of 2,6-diaminopurines, but could not be moved on thin layer or paper chromatography with a number of solvent systems. The chromatograms of this material did not show the positive *p*-nitrobenzylpyridine test characteristic of active halogen compounds (30,31), nor did they correspond to the starting 8-hydroxymethylpurine (XX). Since these results indicated that polymerization had occurred upon treatment of XX with hydrogen bromide, an alternate synthesis of the desired 8-anilinoalkyl-2,6-diaminopurines was investigated. The synthetic route to the 8-anilinoalkylpurines (VIII-X) most likely to be successful was considered to be via acylation of the tetraaminopyrimidine (XVIII) with a preformed blocked precursor of the 8-anilinoalkyl substituent, and subsequent base catalyzed dehydrocyclization.

Although 5-acylamido-2,4,6-triaminopyrimidines can readily be prepared by treatment of XVIII with an acid chloride under Schotten-Baumann conditions (13,21), the activation of the carboxyl group of *N*-phenylglycine (XXV) requires prior blocking of the aromatic amine. Since the glycolamidopyrimidine (XIX) was readily formed upon treatment of XVIII with glycolic acid, it was considered expedient to attempt the direct acylation of XVIII with *N*-phenylglycine (XXV).

When a mixture of tetraaminopyrimidine (XVIII) and *N*-phenylglycine (XXV) was heated at 140° a homogeneous melt was obtained; within fifteen minutes the mixture had solidified and the predominant presence of acylamidopyrimidine (XXI) could be shown by its ultraviolet spectrum. After dissolving the crude reaction mixture in sodium hydroxide, sulfuric acid was added to cause the precipitation of an insoluble sulfate salt of XXI. Without further purification, this crude product was treated with 2 *N* sodium hydroxide to give pure VIII in 17% overall yield from XVIII.

Since *N*-(*p*-carboxyphenyl)glycine and *N*-(*p*-carboxyphenyl)-β-alanine decompose at their melting points, and since each possesses two potential sites of reaction, the direct acylation of tetraaminopyrimidine with these compounds was not attempted. For the synthesis of the acylamidopyrimidine precursors of XXII and XXIII, it was considered necessary to use carboxyl activated derivatives in which the aromatic carboxylic acid and amino groups were suitable protected.

Acetylation of *N*-(*p*-carbethoxyphenyl)-β-alanine (XXVII) with acetic anhydride in glacial acetic acid gave the protected β-alanine derivative (XXX) in nearly quantitative yield. The mixed carbonic-carboxylic anhydride was readily formed by treatment of a solution of the triethylammonium salt of XXX in tetrahydrofuran with ethyl chloroformate at -5°; the mixed anhydride was obtained as a clear viscous oil which was characterized by its infrared spectrum and used directly in the subsequent reaction.

When a solution of tetraaminopyrimidine (XVIII) sulfate in 2 *N* sodium hydroxide was treated with the mixed anhydride from XXX in an identical manner as used for the preparation of XVIIb, the acylamidopyrimidine (XXIII) did not separate from solution. Since the ultraviolet spectrum of an aliquot of the reaction mixture showed the predominant presence of a 5-acylamido-2,4,6-triaminopyrimidine, it was apparent that the product had been solubilized by hydrolysis of the ester function of XXIII in the strongly basic reaction medium. If the *p*H of the reaction mixture was maintained at about 10 by the careful addition of sodium hydroxide, ester hydrolysis was avoided and the blocked acylamidopyrimidine (XXIII) readily separated from solution. Treatment of XXIII with 4 *N* sodium hydroxide resulted in dehydrocyclization and concomitant removal of the blocking groups to give the 8-(*p*-carboxyanilino)ethyl-

purine (X) in 65% yield.

Treatment of *N*-(*p*-carbethoxyphenyl)glycine (XXVI) with acetic anhydride in the identical manner used for the preparation of XXX resulted in the formation of a high melting solid that gave combustion values in agreement with structure XXXI. Attempted acetylation of other *N*-arylglycines have resulted in the formation of similar products (32), which have been suggested to arise from intermediates akin to a mixed anhydride of XXVI and acetic acid. Based on this conjecture, it was rationalized that a mixed anhydride derived from a carboxylic acid having a highly polarized carboxyl group, such as formic acid or trifluoroacetic acid, would yield an *N*-acylated derivative of XXVI regardless of whether an unsymmetrical anhydride were formed.

After treatment of *N*-(*p*-carbethoxyphenyl)glycine (XXVI) with the highly reactive formic-acetic anhydride, pure XXVIII could be readily obtained in 84% yield. Preparation of the mixed anhydride with ethyl chloroformate and subsequent acylation of XVIII under the controlled conditions used for the preparation of XXIII gave the blocked 5-acylamidopyrimidine (XXII). Since minor impurities, detectable by TLC, were not removed after numerous recrystallizations of XXII, the crude product was directly treated with 4 *N* sodium hydroxide to give the 8-(*p*-carboxyanilinomethyl)purine (IX) in 25% yield.

EXPERIMENTAL

Methods.

Melting points were determined in capillary tubes in a Mel-temp block and those below 230° are corrected. Ultraviolet spectra were determined in 10% ethanol with a Perkin-Elmer 202 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer 137B or 337 spectrophotometer. Thin layer chromatograms were run on Brinkmann silica gel GF (unless otherwise indicated) and spots were detected by visual examination under ultraviolet light.

2-Amino-6-chloro-9-phenylpropylpurine (XII).

To a stirred suspension of 0.73 g. (4.3 mmoles) of XI in 4.3 ml. of *N*,*N*-dimethylformamide was added 0.103 g. (4.3 mmoles) of sodium hydride as a 50% dispersion in mineral oil. After hydrogen evolution was complete, 1.195 g. (6 mmoles) of 3-phenylpropylbromide and 0.150 g. (1 mmole) of sodium iodide were added. The mixture was stirred at ambient temperature for 24 hours, then evaporated *in vacuo*. The residue was dissolved in 50 ml. of chloroform, washed successively with two 50 ml. portions of 0.5 *N* sodium hydroxide and two 50 ml. portions of water, then dried with magnesium sulfate. The solution was evaporated *in vacuo* to a white solid which was extracted with two 20 ml. portions of boiling benzene. Upon standing at ambient temperature for 48 hours, the solution deposited 0.370 g. (30%) of white crystals, m.p. 136-140°. A portion was recrystallized from benzene to give the analytical sample, m.p. 138-140°; λ max (*p*H 1): 244, 325 mμ; (*p*H 7): 247, 314 mμ; (*p*H 13): 247, 314 mμ.

Anal. Calcd. for $C_{14}H_{14}ClN_5$: C, 58.4; H, 4.90; N, 24.3. Found: C, 58.3; H, 5.08; N, 24.5.

2-Amino-6-phenylpropylaminopurine (III).

A mixture of 0.255 g. (1.5 mmoles) of 2-amino-6-chloropurine (XI) and 2.02 g. (15 mmoles) of 3-phenylpropyl amine was heated at 150-155° for 20 hours. After being allowed to cool to room temperature, the dark solution was poured into 50 ml. of petroleum ether (b.p. 60-110°. The viscous residue was separated by decantation and partitioned between 25 ml. of 1 *N* aqueous sodium hydroxide and 25 ml. of chloroform. The aqueous solution was washed with three 25 ml. portions of chloroform, then neutralized with glacial acetic acid to give 0.240 g. (60%) of white crystals, m.p. 145-148°. Recrystallization from ethyl acetate afforded 0.220 g. (55%) of product that contained 0.2 mole of acetic acid, m.p. 170-171°.

Anal. Calcd. for $C_{24}H_{16}N_6 \cdot {}^1/_5CH_3CO_2H$: C, 61.7; H, 6.04; N, 30.0. Found: C, 61.5; H, 5.98; N, 30.0.

A portion of this product was further dried *in vacuo* at 100° for 48 hours to give solvate-free product, m.p. 170-171°; λ max (*p*H 1): 254, 286 mμ; (*p*H 13): 254, 291 mμ.

Anal. Calcd. for $C_{14}H_{16}N_6$: C, 62.7; H, 6.02; N, 31.3. Found: C, 62.6; H, 6.20; N, 31.5.

2,6-Diamino-9-phenylpropylpurine (V).

A mixture of 0.180 g. (0.63 mmoles) of XII and 15 ml. of 20% methanolic ammonia was heated in a steel bomb at 140-145° for 24 hours. The reaction mixture was concentrated to about 3 ml., treated with decolorizing carbon, and chilled to give 0.104 g. (62%) of analytically pure white crystals, m.p. 135-137°; λ max (*p*H 1): 257, 296 mμ; (*p*H 13): 258, 284 mμ.

Anal. Calcd. for $C_{14}H_{16}N_6$: C, 62.7; H, 6.02; N, 31.3. Found: C, 63.0; H, 6.05; N, 31.5.

5-Phenylpropionamido-2,4,6-triaminopyrimidine (XVIIb).

To a vigorously stirred solution of 4.8 g. (20 mmoles) of 2,4,5,6-tetraaminopyrimidine (XVIII) sulfate and 2.1 g. (20 mmoles) of sodium bisulfite in 120 ml. of 2 *N* aqueous sodium hydroxide cooled in an ice bath was added 1.6 (20 mmoles) of hydrocinnamoyl chloride; 15 minutes later a second 1.6 g. (20 mmoles) of acid chloride was added. After being stirred for an additional 1 hour in the ice bath the mixture was filtered on a sintered glass filter and the product washed with water; yield, 2.6 g. (48%) of crude product. One recrystallization from water with the aid of decolorizing carbon gave 2.0 g. (37%) of pure product as white crystals, m.p. 264-265° dec.; λ max (*p*H 1): 274 mμ; (*p*H 13): 270 mμ. The compound moved as a single spot in chloroform-ethanol (5:3) on TLC. In a pilot run the analytical sample was obtained as an acetate salt by recrystallization from glacial acetic acid, m.p. 260-261° dec.

Anal. Calcd. for $C_{13}H_{16}N_6O \cdot CH_3CO_2H$: C, 54.2; H, 6.12; N, 25.3. Found: C, 54.3; H, 6.33; N, 25.4.

5-Phenylbutyramido-2,4,6-triaminopyrimidine (XVIIc).

This was prepared in the same manner as the above homolog (XVIIb); yield, 34% of analytically pure material, m.p. 227-229° dec.; λ max (*p*H 1): 274 mμ; (*p*H 13): 270 mμ. The compound moved as a single spot in chloroform-ethanol (5:3) on TLC.

Anal. Calcd. for $C_{14}H_{18}N_6O$: C, 58.7; H, 6.32; N, 29.4. Found: C, 58.7; H, 6.42; N, 29.5.

2,6-Diamino-8-phenylethylpurine (VI).

Method A.

A mixture of 1.3 g. (4.8 mmoles) of XVb (23) and 15 ml. of 28% ammonium hydroxide was heated at 150° in a Parr bomb for 7 hours. After the mixture was chilled in an ice bath, the crude product was collected on a filter; a yield, 1.1 g. (90%). To a solution of 0.125 g. of this product in 10 ml. of water was added 2 ml. of 2 *N* sulfuric acid; after chilling overnight, 0.128 g. of analytically pure product was obtained as a hydrated sulfate salt, m.p. 224-225° dec.; λ max (*p*H 1): 246, 289 mμ, O. D. ratio 260/280 = 0.79; λ max (*p*H 13): 288 mμ; O. D. ratio 260/280 = 0.36.

Anal. Calcd. for $C_{13}H_{14}N_6 \cdot H_2SO_4 \cdot H_2O$: C, 42.2; H, 4.90; N, 22.6. Found: C, 42.3; H, 4.78; N, 22.7.

Method B.

To a solution of 0.123 g. (1.1 mmole) of potassium *t*-butoxide in 5 ml. of *t*-butyl alcohol was added 0.272 g. (1.0 mmole) of XVIIb. After being refluxed for 12 hours the mixture was evaporated *in vacuo*. The residue was dissolved in 5 ml. of 1 *N* potassium hydroxide and clarified by filtration. The solution was slowly acidified with 6 *N* sulfuric acid to give 0.206 g. (66%) of white crystals, m.p. 200-201°. One recrystallization from aqueous 2-methoxyethanol gave 0.185 g. (59%) of analytically pure product as a hemi-hydrate, hemi-sulfate salt, m.p. 205-206° dec.; ultraviolet spectra were identical to those reported in Method A. This compound moved as a single spot in chloroform-ethanol (5:3) of TLC.

Anal. Calcd. for $C_{13}H_{14}N_6 \cdot {}^1/_2H_2SO_4 \cdot {}^1/_2H_2O$: C, 50.0; H, 5.16; N, 26.9. Found: C, 49.9; H, 4.62; N, 27.1.

A portion of this product was recrystallized from 2-methoxyethanol 1 *N* sulfuric acid (4:1) to give the hydrated sulfate salt, identical to that obtained in Method A, m.p. 224-225° dec.

Method C.

A solution of 0.272 g. (1.0 mmole) of XVIIb in 5 ml. of 4 *N* sodium hydroxide was refluxed for 7 hours then acidified with 6 *N* sulfuric acid. The product was recrystallized from aqueous 2-methoxyethanol with the aid of decolorizing carbon to give 0.300 g. (79%) of pure product that was identical to that obtained in Method B, m.p. 204-205° dec.; the product moved as a single spot with chloroform-ethanol (5:3) on TLC.

2,6-Diamino-8-phenylpropylpurine (VII).

This was prepared in the same manner as described in Method B for preparation of VI; yield, 56% of analytically pure material, m.p. 193-195° dec.; λ max (*p*H 1): 246, 289 mμ; (*p*H 13): 288 mμ. This compound moved as a single spot with chloroform-ethanol (5:3) on TLC.

Anal. Calcd. for $C_{14}H_{16}N_6 \cdot {}^1/_2H_2SO_4 \cdot {}^1/_2H_2O$: C, 51.5; H, 5.57; N, 25.8. Found: C, 51.5; H, 5.81; N, 26.1.

2,6-Diamino-8-hydroxymethylpurine (XX).

An intimate mixture of 4.8 g. (20 mmoles) of 2,4,5,6-tetraaminopyrimidine (XVIII) sulfate and 7.6 g. (0.10 mole) of purified glycolic acid was heated at 100-105° for one hour. The yellow reaction mixture was slowly poured into 150 ml. of ethanol-ethyl acetate (1:1) and chilled. The product was collected on a filter and washed with ethyl acetate. The crude glycolamidopyrimidine (XIX) was refluxed with 100 ml. of 2 *N* sodium hydroxide for 5 hours. After clarification by filtration, the solution was brought to neutrality with glacial acetic acid. The product that separated from solution was collected on a filter and washed with water. The product was extracted with 150 ml. of boiling glacial acetic acid to give, after cooling, 2.4 g. (50%) of an amorphous powder. Recrystallization from glacial acetic acid, with the aid of decolorizing carbon gave 1.7 g. (35%) of product which showed trace impurities on TLC with chloroform-ethanol (3:5). A portion was twice more recrystallized from glacial acetic acid to give the analytically pure acetate salt, m.p. >300°; λ max (*p*H 1): 245, 288 mμ, O. D. ratio 260/280 = 0.51; (*p*H 13): 291 mμ, O. D. ratio 260/280 = 0.64.

Anal. Calcd. for $C_6H_8N_6O \cdot CH_3CO_2H$: C, 40.0; H, 5.05; N, 35.0. Found: C, 39.9; H, 5.10; N, 35.0.

8-Anilinomethyl-2,6-diaminopurine (VIII).

An intimate mixture of 4.8 g. (20 mmoles) of 2,4,5,6-tetraaminopyrimidine (XVIII) sulfate and 9.1 g. (60 mmoles) of *N*-phenylglycine (XXV) was heated at 140-145° in an oil bath for 30 minutes with intermittent hand stirring. The solidified mass was allowed to cool to room temperature and then dissolved in 150 ml. of 0.5 *N* sodium hydroxide. After clarification by filtration, the solution was acidified with 6 *N* sulfuric acid to give, after chilling, 3.69 g. of a crude sulfate salt of XXI; λ max (*p*H 1): 274 mμ, O. D. ratio 260/280 = 0.59; (*p*H 13): 270 mμ, O. D. ratio 260/280 = 1.56.

Without further purification, this crude XXI was refluxed with 70 ml. of 2 *N* sodium hydroxide for 1.5 hours. After clarification by filtration, the solution was brought to neutrality with glacial acetic acid to give 1.5 g. (30%) of crude product. Two recrystallizations from aqueous ethanol, with the aid of decolorizing carbon gave 1.0 g. (19%) of analytically pure crystals, m.p. 262-263°; λ max (*p*H 1): 246, 289 mμ, O. D. ratio 260/280 = 0.87; (*p*H 13): 290 mμ, O. D. ratio 260/280 = 0.74. The compound moved as a single spot in chloroform-ethanol (1:1) on TLC.

Anal. Calcd. for $C_{12}H_{13}N_7$: C, 56.5; H, 5.14; N, 38.4. Found: C, 56.6; H, 5.20; N, 38.4.

N-Acetyl-*N*-(4-carbethoxyphenyl)-β-alanine (XXX).

A solution of 11.2 g. (47 mmoles) of *N*-(4-carbethoxyphenyl)-β-alanine (XXVII) (33) in 25 ml. of glacial acetic acid and 25 ml. of acetic anhydride was heated at 85° for one hour. After allowing the solution to cool, 100 ml. of water was added and the mixture was evaporated *in vacuo*. The residual oil was crystallized from ethyl acetate-petroleum ether (b.p. 60-110° to give 12.9 g. (99%) of pure white crystals, m.p. 98-99°; lit. (34) m.p. 99-100°.

5-[*N*-Acetyl-*N*-(4-carbethoxyphenyl)-β-alanylamido]-2,4,6-triaminopyrimidine (XXIII).

To a vigorously stirred solution of 12.3 g. (44 mmoles) of XXX and 4.45 g. (44 mmoles) of triethyl amine in 100 ml. of dry tetrahydrofuran cooled in an ice-salt bath (-5°) was added slowly 4.4 ml. (44 mmoles) of ethyl chloroformate. After an additional 30 minutes at -5°, the precipitated triethylamine hydrochloride was removed by filtration and washed with 25 ml. of dry tetrahydrofuran. The combined filtrate and washings were evaporated *in vacuo* at ambient temperature to give a clear viscous oily mixed anhydride which showed strong absorption at 1840 cm^{-1} in the infrared.

To a vigorously stirred solution of 9.6 g. (40 mmoles) of 2,4,5,6-tetraaminopyrimidine (XVIII) sulfate and 2.1 g. (20 mmoles) of sodium bisulfite in 60 ml. of 2 *N* sodium hydroxide cooled in an ice-rocksalt bath (-5°) was added the crude mixed anhydride in one portion; the vessel that contained the mixed anhydride was washed with 5 ml. of acetone which was also added to the reaction mixture. The reaction was allowed to proceed until the *p*H fell to about 9 (about 30 seconds), after which just enough 2 *N* sodium hydroxide was added to adjust the *p*H to about 10. This process was repeated until the *p*H of the reaction mixture remained constant over a period of 5 minutes, at which time a total of 23 ml. of 2 *N* sodium hydroxide had been added to the reaction mixture over a period of 6 minutes. After an additional 30 minutes at -5°, the mixture was filtered on a sintered glass filter and the product washed with 25 ml. of water; yield, 9.8 g. (62%) of a crude amorphous powder. Two recrystallizations from water, with the aid of decolorizing carbon, gave 4.9 g. (33%) of analytically pure white crystals; λ max (*p*H 1): 273 mμ, O. D. ratio 260/280 = 1.00; λ max (*p*H 13): 240, 265 mμ, O. D. ratio 260/280 = 2.78. This compound showed one spot with chloroform-ethanol (5:3) on TLC.

Anal. Calcd. for $C_{18}H_{23}N_7O_4$: C, 53.9; H, 5.76; N, 24.4. Found: C, 53.8; H, 5.95; N, 24.5.

N-Formyl-*N*-(4-carbethoxyphenyl)glycine (XXVIII).

To a stirred suspension of 1.0 g. (4.5 mmoles) of *N*-(4-carbethoxyphenyl)glycine (XXVI) (35) in 6.0 ml. of 97% formic acid was added dropwise 2.0 ml. of acetic anhydride. The solution was heated at 60° for one hour, then slowly added to 50 ml. of vigorously stirred ice-water over a period of 5 minutes to give 1.1 g. (100%) of white crystals, m.p. 160-162°. Recrystallization from ethyl acetate gave 0.92 g. (84%) of analytically pure product, m.p. 160-162°; ν max (Nujol) 1740 (ester C=O); 1710 (acid C=O); 1650 (amide C=O); 1600 cm^{-1} (phenyl C=C).

Anal. Calcd. for $C_{12}H_{13}NO_5$: C, 57.4; H, 5.22; N, 5.58. Found: C, 57.7; H, 5.37; N, 5.54.

5-[*N*-Formyl-*N*-(4-carbethoxyphenyl)glycylamido]-2,4,6-triaminopyrimidine (XXII).

This was prepared from 11.1 g. (44 mmoles) of XXVIII and 9.6 g. (40 mmoles) of XVIII in the same manner described for XXIII. The crude product obtained after filtration of the reaction mixture (6.7 g.) was recrystallized from aqueous ethanol, with the aid of decolorizing carbon, to give 4.8 g. (32%) of yellow crystals that were sufficiently pure for subsequent conversion to IX; λ max (*p*H 1): 273 mμ; (*p*H 13): 265 mμ. TLC on cellulose powder showed one major spot along with slower moving contaminants with 0.2 *N* acetate buffer (*p*H 3.6) as eluent.

8-(4-Carboxyanilinoethyl)-2,6-diaminopurine (X).

A suspension of 2.00 g. (5 mmoles) of XXIII in 25 ml. of 4 *N* sodium hydroxide was refluxed for 5 hours. After clarification with the aid of decolorizing carbon, the hot solution was neutralized with glacial acetic acid to give 1.20 g. (77%) of a crude yellow powder. Recrystallization from *N*,*N*-dimethylformamide-water gave 0.80 g. (51%) of product that showed minor contaminants on TLC. Reprecipitation of this product from hot 1 *N* ammonium hydroxide with glacial acetic acid with aid of decolorizing carbon gave 0.61 g. (41%) of an analytically pure amorphous solid, m.p. >300°; λ max (*p*H 1): 244, 290 mμ; (*p*H 13): 288 mμ. This product showed one spot on TLC on cellulose powder with 1 *N* ammonium hydroxide-ethanol (1:1).

Anal. Calcd. for $C_{14}H_{15}N_7O_2 \cdot {}^2/_3H_2O$: C, 51.8; H, 5.05; N, 30.2. Found: C, 52.0; H, 5.07; N, 30.4.

8-(4-Carboxyanilinomethyl)-2,6-diaminopurine (IX).

This was prepared from XXII in the same manner described for X; yield, 25% of an analytically pure product as an off-white powder, m.p. >300°; λ max (*p*H 1): 244, 290 mμ, O. D. ratio 260/280 = 0.51; (*p*H 13): 290 mμ, O. D. ratio 260/280 = 0.45. This product moved as a single spot on TLC on cellulose powder in 1 *N* ammonium hydroxide-ethanol (1:1). Although this compound is mentioned in the literature (36), its synthesis and physical properties were not described.

Anal. Calcd. for $C_{13}H_{13}N_7O_2 \cdot {}^2/_3H_2O$: C, 50.2; H, 4.63; N, 31.4. Found: C, 50.3; H, 4.60; N, 31.5.

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