

# Methylated Purines and Pyrimidines. I. Syntheses of 8-Hydroxy-7-methylguanine and Methylation of a 5-(Benzylideneamino)pyrimidine\*

I. J. Borowitz,<sup>†</sup> S. M. Bloom,<sup>‡</sup> J. Rothschild, and D. B. Sprinson<sup>§</sup>

**ABSTRACT:** Several methylated purines and pyrimidines were prepared, and some useful procedures were developed in attempts to synthesize 2,6-diamino-(5-methylamino)-4-pyrimidinol (compound II), which was desired as an intermediate for the synthesis of 5-methylfolic acid. Ethyl 2-(*N*-methylacetamido)cyanoacetate (compound III), which would be expected to form *N*-(2,4-diamino-6-hydroxy-5-pyrimidinyl)-*N*-methylacetamide by condensation with guanidine, could not be prepared by a variety of procedures. Among these was the attempted dehydration of ethyl 2-(*N*-methylacetamido)malonamate (compound IVa), and of ethyl 2-(benzylmethylamino)malonamate (compound IVb).

The known conversion of uric acid by acetic anhydride to acyluracils suggested the possibility of similar ring opening of 2-amino-7-methylpurine-6,8-dio-(8-hydroxy-7-methylguanine) (compound V), which was synthesized by two independent routes and subjected to similar procedures. However, compound V failed to yield II. Attempts to methylate the azomethine nitrogen of 2,4-diamino-5-(benzylideneamino)-6-methoxypyrimidine (compound XI), leading eventually to compound II by hydrolysis of the Schiff base, resulted in alkylation of N-1. Good yields were obtained of 2,5,6-triamino-1-methyl-4(1*H*)-pyrimidinone (compound XIV), which was identified by conversion to 3-methylguanine (compound XV).

The important role of the  $\beta$ -carbon of serine in the biosynthesis of methyl groups, and the observation that the  $\beta$ -hydrogens are transferred with the carbon atom to the newly formed methyl group, suggested that 5,10-methylene-5,6,7,8-tetrahydrofolate is an intermediate in this process (Elwyn *et al.*, 1955). The present studies were stimulated by the hypothesis that while the general methyl group donor is *S*-adenosylmethionine the formation of a methyl group *de novo* occurs by intramolecular reduction of the 5,10-methylene group. The resulting betaine, 7,8-dihydro-*N*<sup>5</sup>-methylfolate, or its oxidation product, *N*<sup>5</sup>-methylfolate, was considered a likely intermediate in the biosynthesis of methionine from homocysteine. Efforts, therefore, have been directed in this laboratory since 1954 toward the synthesis of *N*<sup>5</sup>-methylfolic acid. Although the latter compound was not obtained, several methylated purines and pyrimidines were synthesized, and some useful procedures were developed. These are the subject of this and the following paper (Sprecher and Sprinson, 1965). It has, of course, been shown recently by Bu-

chanan and his collaborators (Larrabee *et al.*, 1963; Cathou and Buchanan, 1963) that 5-methyl-5,6,7,8-tetrahydrofolate is an intermediate for methionine biosynthesis, the methyl group being transferred to homocysteine by a cobalamin enzyme. Certain strains of *Escherichia coli* have been shown by Woods and his collaborators to utilize only 5-methyltetrahydropteroyl-triglutamate for methionine synthesis, in the absence of cobalamin (Guest *et al.*, 1964; Foster *et al.*, 1961), and this pathway probably occurs in plants and molds. Whether the oxidized, betaine derivatives of these compounds can transfer their methyl group to homocysteine under some conditions is unknown. A portion of the present paper was published in preliminary form (Borowitz *et al.*, 1959).

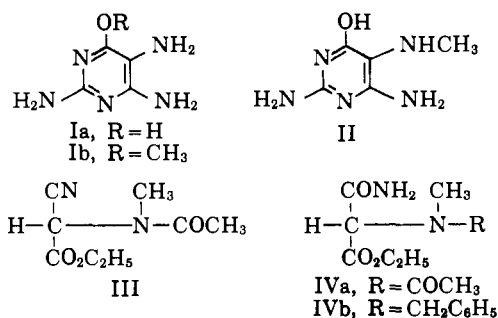
The ease with which 2,5,6-triamino-4-pyrimidinol (compound Ia) reacts in aqueous solution with *p*-aminobenzoylglutamate and a three-carbon compound, such as  $\alpha,\beta$ -dibromopropionaldehyde, to form folic acid (Waller *et al.*, 1948) suggested a similar course for the 5-methylamino analog (compound II). In the first attempts to synthesize compound II it was hoped to condense ethyl 2-(*N*-methylacetamido)cyanoacetate (compound III) with guanidine to form *N*-(2,4-diamino-6-hydroxy-5-pyrimidinyl)-*N*-methylacetamide. However, all efforts to prepare compound III or a suitable precursor failed. The following were some of the methods tried: (1) displacement of bromine on ethyl bromocyanoacetate with methylamine, benzylmethylamine, or dibenzylmethylamine; (2) condensation of ethyl glyoxylate or glyoxylic acid with methylamine and

\* From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City. Received June 29, 1964; revised December 7, 1964. This work was supported by grants from the American Cancer Society, the American Heart Association, and the National Institutes of Health of the U.S. Public Health Service.

<sup>†</sup> Present address: Department of Chemistry, Lehigh University, Bethlehem, Pa.

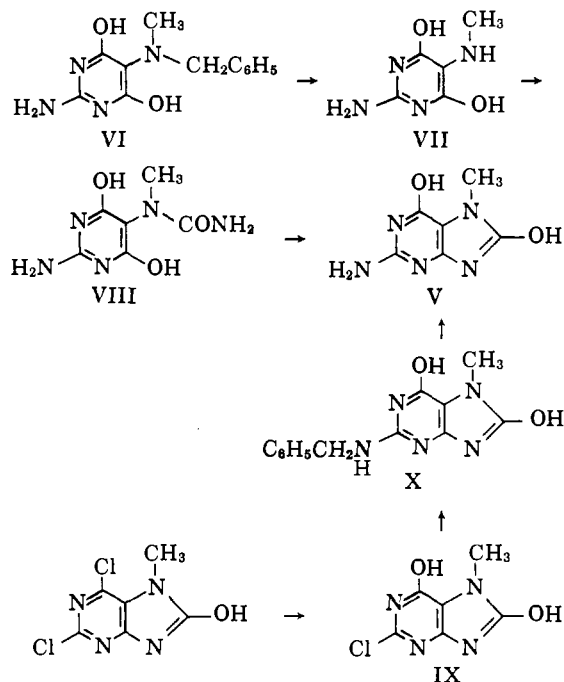
<sup>‡</sup> Present address: Polaroid Corporation, Cambridge, Mass.

<sup>§</sup> Career Investigator of the American Heart Association.



HCN under anhydrous conditions or in aqueous solution; (3) dehydration of ethyl 2-(*N*-methylacetamido)malonamate (compound IVa),<sup>1</sup> or ethyl 2-(benzylmethylamino)malonamate (compound IVb);<sup>1</sup> (4) carbethoxylation of (*N*-methylacetamido)acetonitrile or (*N*-methylbenzamido)acetonitrile with diethyl carbonate or ethyl chlorocarbonate under a variety of conditions.

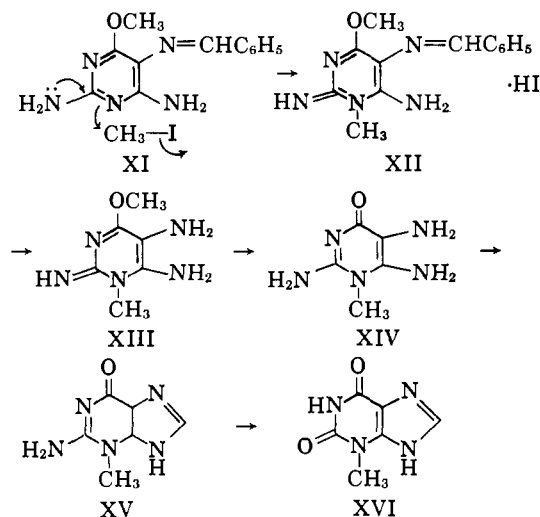
The conversion of uric acid to 5,6-diacetamidouracil with acetic anhydride and pyridine (Bredereck *et al.*, 1953) suggested the possibility of a similar ring opening of 8-hydroxy-7-methylguanine (compound V) which was prepared by two independent methods. Diethyl 2-(benzylmethylamino)malonate and guanidine were condensed in the usual manner to 2-amino-5-(benzylmethylamino)-4,6-pyrimidinediol (compound VI). Catalytic hydrogenolysis to compound VII, followed by treatment with cyanate, gave the 5-(1-methylureido)pyrimidine (compound VIII), which was cyclized to compound V by refluxing with 6 *N* HCl. In a second procedure, 2-chloro-7-methylpurine-6,8-diol (compound



<sup>1</sup> Unpublished procedures. Compound IVb (mp 84–86°) was prepared from ethyl 2-bromomalonamate (Wohl, 1919; Pinner, 1895) and benzylmethylamine in the usual manner. Compound IVa (mp 90–91°) was prepared from compound IVb by hydrogenolysis over Pd in acetic anhydride.

IX) was prepared, apparently for the first time, from 2,6-dichloro-7-methylpurin-8-ol by hydrolysis with alkali, and was refluxed with benzylamine to yield 2-(benzylamino)-7-methylpurine-6,8-diol (compound X). Catalytic hydrogenolysis of compound X afforded V. However, attempts to convert compound V to the desired pyrimidine, compound II, failed.

The relatively stronger basicity of the 5-amino group of 2,5,6-triamino-4-pyrimidinol (compound Ia) is known to result in the formation of 5-(*N*-arylidene-amino)pyrimidines (Dick *et al.*, 1956). Since azomethines can be alkylated and then hydrolyzed to the alkylated amines (Woodruff *et al.*, 1940; Morrison and Rinderknecht, 1950), an attempt was made to apply this route to the synthesis of compound II. However, although the benzylidene derivative (compound XI) of 2,4,5-triamino-6-methoxypyrimidine (compound Ib) reacted with methyl iodide, alkylation occurred on N-1 to give compound XII. The formulation of compound XII as a hydriodide was supported by its acidity in aqueous solution, in which the Schiff base was slowly hydrolyzed. The methoxy 1-methylpyrimidine (compound XIII) was recovered from compound XII by treatment with acid at room temperature, and further treatment with HBr in glacial acetic acid gave the 2,5,6-triamino-1-methyl-4(1*H*)-pyrimidinone (compound XIV).



The structure of compound XIV was demonstrated by conversion to 3-methylguanine (compound XV), the only unknown *N*-methylguanine at the time of this investigation (Borowitz *et al.*, 1959). This purine was characterized by its absorption spectrum, which differed from the other *N*-methylguanines, and the hydrolysis of its 2-amino group in concentrated ammonia to yield 3-methylxanthine (compound XVI) (cf. Elion, 1957, 1962). The conversions XIV → XV → XVI were also reported recently by Townsend and Robins (1962).

Although the 5-*N*-benzylidene derivative (compound XI) of the 4-methoxypyrimidine is methylated under relatively mild conditions, and undergoes some reaction even at room temperature, 2,4,5-triamino-

6-methoxypyrimidine (compound Ib) and the 5-*N*-benzylidene derivative of the 4-hydroxypyrimidine (compound Ia) do not react with methyl iodide under similar conditions.

While this work was in progress Weissmann and Gutman (1956) isolated a purine from human urine to which the structure V was assigned. This structure was confirmed by comparison with 8-hydroxy-7-methylguanine prepared in the present investigation (Weissmann and Gutman, 1957). Another urinary purine, 7-methylguanine, occurs in transfer RNA (Dunn, 1963). The availability of 8-hydroxy-7-methylguanine (compound V) may aid in investigating the suggested relationship between these two purines, or the independent existence of 8-hydroxy-7-methylguanine in nucleic acids.

The successful synthesis of compound II is described in the following paper (Sprecher and Sprinson, 1965).<sup>2</sup>

### Experimental<sup>3</sup>

**Diethyl 2-(Benzylmethylamino)malonate.** A solution of 23.9 g (0.10 mole) of diethyl bromomalonate in 40 ml of absolute alcohol was treated with 24.2 g (0.20 mole) of *N*-benzylmethylamine as described for the synthesis of the dimethyl ester by Uhle and Harris (1956). Fractional distillation of the crude product gave 16.7 g (60% yield), bp 135–137°/1.0–2 mm. For analysis the compound was redistilled at 0.7 mm and a middle fraction, bp 128–129°, was used.

*Anal.* Calcd for  $C_{15}H_{21}NO_4$  (279.3): C, 64.5; H, 7.58; N, 5.01. Found: C, 64.6; H, 7.63; N, 4.98.

**2-Amino-5-(benzylmethylamino)-4,6-pyrimidinediol (Compound VI).** A solution of 9 g (0.39 mole) of sodium in absolute ethanol was treated with 36 g (0.37 mole) of guanidine hydrochloride and filtered by suction. To the filtrate was added a solution of 9 g of sodium in ethanol and 100 g (0.36 mole) of diethyl 2-(benzylmethylamino)malonate. The mixture was refluxed under nitrogen for 2 hours and evaporated to dryness *in vacuo*. The residual violet gum was triturated with 10% aqueous acetic acid, and the resulting pink solid was removed by filtration, washed with cold water, and dried. Yield, 80 g of material which could be used in the next step.

When dissolved in the minimum amount of 4 *N*  $H_2SO_4$  and allowed to crystallize first at room temperature and then in the cold, this solid gave a neutral

sulfate in 80% yield. For analysis it was recrystallized twice by dissolving in the minimum amount of hot water, adding ethanol to the cloud point, and seeding. Ultraviolet spectrum:  $\lambda_{max}^{H^+}$  253 m $\mu$  ( $\epsilon$  13,500);  $\lambda_{max}^{OH^-}$  267 m $\mu$  ( $\epsilon$  8500).

*Anal.* Calcd for  $C_{12}H_{14}N_4O_2 \cdot \frac{1}{2}H_2SO_4$  (295.3): N, 19.0. Found: N, 19.1.

The picrate of compound VI was prepared by dissolving the free base in 0.1 *N* HCl and adding an aqueous solution of picric acid. It was recrystallized twice from methanol and finally from hot water. Yellow platelets, mp 175–178° decomp. Ultraviolet spectrum,  $\lambda_{max}^{H^+}$  250 and 354 m $\mu$ .

*Anal.* Calcd for  $C_{12}H_{14}N_4O_2 \cdot C_6H_3N_3O_7$  (475.4): C, 45.6; H, 3.61; N, 20.6. Found: C, 45.7; H, 3.74; N, 20.3.

**2-Amino-5-(methylamino)-4,6-pyrimidinediol (Compound VII).** A solution of 3.0 g (12 mmoles) of compound VI in 100 ml of glacial acetic acid was stirred magnetically under 1 atmosphere of hydrogen in the presence of 0.2 g of 10% Pd on charcoal. After 6 hours, hydrogen uptake (310 ml) ceased (calcd 274 ml). The catalyst was removed by filtration, a small excess of 2 *N* HCl (6.5 ml) was added, and the solution was taken to dryness *in vacuo*. (Twice as much compound VI could be hydrogenated in 100 ml of glacial acetic acid. However, the product tended to precipitate, and it was necessary to add the HCl prior to removal of the catalyst.) The residual white solid (2.2 g) could be used in the next step. For analysis it was recrystallized from hot methanol and methanol-benzene, yielding a microcrystalline, hygroscopic hydrochloride which was dried at 75°/0.05 mm. Ultraviolet spectrum,  $\lambda_{max}^{H^+}$  253 m $\mu$  ( $\epsilon$  14,000);  $\lambda_{max}^{OH^-}$  272 m $\mu$ , unstable.

*Anal.* Calcd for  $C_8H_8N_4O_2 \cdot HCl$  (192.6): C, 31.2; H, 4.71; N, 29.1. Found: C, 30.9; H, 4.86; N, 28.8.

**1-(2-Amino-4,6-dihydroxy-5-pyrimidinyl)-1-methylurea (Compound VIII).** After hydrogenating 1.3 g (5.2 mmoles) of compound VI as described, the glacial acetic acid was removed *in vacuo* (without adding HCl). The residual solid, which turned pink on exposure to air, was treated with 0.48 g (6 mmoles) of KCNO in 15 ml of water and heated on a steam bath under nitrogen for 45 minutes. The cooled solution was cleared by filtration and evaporated to dryness. The residue was taken up in 6 *N* HCl and allowed to crystallize. First crop, 0.57 g; on standing, a second crop of 0.40 g was obtained from the filtrate. Ultraviolet spectrum, assuming pure hydrochloride of compound VIII (mw 235.5),  $\lambda_{max}^{H^+}$  258 m $\mu$  ( $\epsilon$  13,700);  $\lambda_{max}^{OH^-}$  258 m $\mu$  ( $\epsilon$  8350). This was used in the next step without further purification.

**2-Amino-7-methylpurine-6,8-diol (8-Hydroxy-7-methylguanine) (Compound V).** Method A: A solution of 3.5 g of compound VIII in 40 ml of 3.5 *N* HCl was refluxed for 1 hour and evaporated to dryness. The residue was recrystallized twice by dissolving in the minimum amount of hot 6 *N* HCl and adding five times the volume of cold water, and finally from the minimum volume of hot 6 *N* HCl with slow cooling. Yield, 1.9 g of white platelets. Ultraviolet spectrum,  $\lambda_{max}^{H^+}$

<sup>2</sup> During preparation of this manuscript we became aware that 2,5,6-triamino-1-methyl-4(1*H*)-pyrimidinone (compound XIV) had been prepared previously by Roth *et al.* (1951a) who, however, assigned to it the structure of the 3-methylpyrimidinone. A reinvestigation of this compound by Boon and Bratt (1957) led to the correct structural assignment (compound XIV). Although their paper is abstracted in *Chem. Abstr.* 51, 13875 (1957), 2,5,6-triamino-1-methyl-4(1*H*)-pyrimidinone is not mentioned in the abstract, and does not occur in the subject and formula index for 1957 or in the formula index for 1957–1961. This paper had therefore escaped our attention until recently.

<sup>3</sup> Ultraviolet spectra in aqueous solution were taken in 0.1 *N* HCl and 0.1 *N* NaOH.

248 m $\mu$  ( $\epsilon$  10,900), 293 m $\mu$  ( $\epsilon$  9900);  $\lambda_{\text{max}}^{\text{OH}^-}$  255 m $\mu$  (plateau,  $\epsilon$  5400), 286 m $\mu$  ( $\epsilon$  11,700).

*Anal.* Calcd for  $\text{C}_6\text{H}_7\text{N}_5\text{O}_2 \cdot \text{HCl}$  (217.7): C, 33.1; H, 3.70; Cl, 16.3. Found: C, 33.4; H, 4.03; Cl, 16.2.

**2-Chloro-7-methylpurine-6,8-diol (Compound IX).** A large-scale preparation of 2,6,8-trichloro-7-methylpurine was adapted from the original method of Fischer (1895). A mixture of 100 g of theobromine (0.55 mole), 500 g of  $\text{PCl}_5$ , and 750 g of  $\text{POCl}_3$  was heated with shaking at  $150^\circ$  for 3 hours in a stainless steel 1-liter vessel of an Aminco hydrogenation apparatus. After cooling overnight the vessel was removed from the heating jacket, chilled in ice, and opened. About 500 ml of liquid was withdrawn by suction through a fritted-glass filter stick, and the residue was washed by stirring successively with 500 ml of ether and 500 ml of 1.5 N aqueous ammonia, fluid being removed through the filter stick. The product was removed from the vessel and crystallized from 1 liter of hot ethanol. Yield 54 g (41%) of light-yellow crystals, mp  $155\text{--}157^\circ$ .

The trichloromethylpurine (24 g, 0.1 mole) was refluxed for 10 minutes in 960 ml of 6 N HCl. A clear solution resolute briefly, followed by precipitation. After chilling for 1 hour the 2,6-dichloro-7-methylpurin-8-ol was removed by filtration, washed with water, and dried. Yield 20 g (90%), mp  $265\text{--}270^\circ$  (cf. Fischer, 1895). This material was suspended in 35 ml of water, treated with 465 ml of 2 N NaOH, and refluxed for 8 hours. Titration of an aliquot by the Volhard procedure showed the release of 1 equivalent of  $\text{Cl}^-$ . On cooling the mixture, a crystalline precipitate formed which was dissolved by adjusting the pH to 7.0 with concentrated HCl. A gelatinous precipitate was removed by centrifugation and discarded, and the supernatant solution was brought to pH 1–2 with concentrated HCl. The resulting suspension was heated on a steam bath for 15 minutes and chilled, and the product was removed by filtration, washed with water, and dried. Yield 16.9 g (92%), mp above  $300^\circ$ . To prepare a sample for analysis a suspension of the purine in water was treated dropwise with 17 M  $\text{NH}_4\text{OH}$ . Solution took place and was followed quickly by precipitation of a white amorphous product, which was removed by centrifugation, dissolved in water, and reprecipitated by the addition of 2 N HCl. Ultraviolet spectrum,  $\lambda_{\text{max}}^{\text{H}^+}$  263 m $\mu$  ( $\epsilon$  9800), 283 m $\mu$  (shoulder,  $\epsilon$  7400);  $\lambda_{\text{max}}^{\text{OH}^-}$  278 m $\mu$  ( $\epsilon$  14,300).

*Anal.* Calcd for  $\text{C}_6\text{H}_5\text{N}_4\text{O}_2\text{Cl}$  (200.6): C, 35.9; H, 2.51; N, 27.9. Found: C, 35.5; H, 2.31; N, 27.4.

**2-(Benzylamino)-7-methylpurine-6,8-diol (Compound X).** The 2-chloro-7-methylpurine-6,8-diol (compound IX) (15 g, 74 mmoles) was refluxed for 18 hours under nitrogen in 120 ml of dry freshly distilled benzylamine. On cooling, the white crystals which separated were removed by filtration and washed with ethanol and  $\text{CCl}_4$ . Yield 15 g (74%). For analysis a portion of the product was dissolved in 0.1 N NaOH and the solution was clarified by filtration. The compound was precipitated by the addition of acetic acid, removed by filtration, and dried. It was recrystallized from the minimum amount of 6 N HCl and dried at  $100^\circ$ . Ultraviolet spectrum,  $\lambda_{\text{max}}^{\text{H}^+}$  250 m $\mu$  ( $\epsilon$  14,500),

298 m $\mu$  ( $\epsilon$  11,600);  $\lambda_{\text{max}}^{\text{OH}^-}$  255–265 m $\mu$  (plateau,  $\epsilon$  8900), 288 m $\mu$  ( $\epsilon$  12,900).

*Anal.* Calcd for  $\text{C}_{13}\text{H}_{13}\text{N}_5\text{O}_2 \cdot \text{HCl}$  (307.5): C, 50.8; H, 4.59; N, 22.8. Found: C, 50.9; H, 4.77; N, 22.5.

**2-Amino-7-methylpurine-6,8-diol (Compound V).** Method B: A solution of 15 g (55 mmoles) of 2-(benzylamino)-7-methylpurine-6,8-diol (compound X) in 1 liter of formic acid was stirred magnetically with 1.5 g of 10% Pd on charcoal under 1 atm of hydrogen. Uptake of  $\text{H}_2$  was 1450 ml (calcd 1350 ml). The catalyst was removed by filtration, and the formic acid by distillation *in vacuo*. The residue was washed with ethyl acetate, dried (9 g, 91%), and recrystallized by dissolving in boiling 6 N HCl, filtering the solution, and cooling the filtrate. For analysis a portion was recrystallized again in the same manner. The ultraviolet spectrum was identical with the compound synthesized by Method A, described previously.

*Anal.* Calcd for  $\text{C}_6\text{H}_7\text{N}_5\text{O}_2 \cdot \text{HCl}$  (217.7): C, 33.1; H, 3.70; N, 32.2; Cl, 16.3. Found: C, 33.4; H, 4.00; N, 32.4; Cl, 16.1.

Efforts were made to open the imidazole ring of 7-methylguanine<sup>4</sup> or of 8-hydroxy-7-methylguanine (compound V) by treatment with acetic anhydride (Bredereck *et al.*, 1953). Only starting material could be recovered. When compound V was treated with benzoyl chloride and aqueous NaOH it was decomposed to products which did not absorb in the ultraviolet.

**2,4-Diamino-5-(benzylideneamino)-6-methoxypyrimidine (Compound XI).** A solution of 46.5 g (0.3 mole) of 2,4,5-triamino-6-methoxypyrimidine<sup>5</sup> in 600 ml of 1 N HCl was prepared from the free base. (When the pyrimidine sulfate monohydrate was used, 1 g of the salt in 1 ml of water was treated with the calculated amount of  $\text{BaCl}_2$  and the  $\text{BaSO}_4$  was removed by centrifugation.) To the aqueous solution were added 123 g (0.9 mole) of sodium acetate trihydrate, 600 ml of ethanol, and 34 ml (0.3 mole) of redistilled benzaldehyde in 600 ml of 50% aqueous ethanol. The mixture was chilled in an ice bath, and the yellow crystalline precipitate was removed by filtration, washed with cold 50% ethanol and cold absolute ethanol, and dried rapidly *in vacuo* over NaOH. Yield 71 g (97%), mp  $146\text{--}148^\circ$ . This product was recrystallized by dissolving in 300 ml of hot ethyl acetate, adding hot cyclohexane or ligroin to incipient crystallization, and cooling slowly, yield 45 g, mp  $147\text{--}149^\circ$ . For analysis a portion was recrystallized again in the same manner; mp  $153\text{--}154^\circ$ . Ultraviolet spectrum (read immediately after solution in absolute ethanol),  $\lambda_{\text{max}}$  245 m $\mu$  ( $\epsilon$  15,400),  $\lambda_{\text{max}}$  284 ( $\epsilon$  11,500),  $\lambda_{\text{max}}$  352 ( $\epsilon$  23,300),  $\lambda_{\text{max}}$  365 (shoulder,  $\epsilon$  21,200).

<sup>4</sup> Prepared according to Fischer (1897, 1898).

<sup>5</sup> Prepared as the sulfate monohydrate according to Roth *et al.* (1951b). Ultraviolet spectrum,  $\lambda_{\text{max}}^{\text{H}^+}$  228 m $\mu$  ( $\epsilon$  8400), 279 m $\mu$  ( $\epsilon$  14,600). This compound was also a generous gift of Dr. J. M. Smith, Jr., American Cyanamid Co. In addition it was furnished by Cyclo Chemical Corp. both as the free base and as the sulfate. The 2,5,6-triamino-4-pyrimidinol sulfate monohydrate was a gift of Dr. J. M. Smith, Jr. Ultraviolet spectrum  $\lambda_{\text{max}}^{\text{H}^+}$  262 m $\mu$  ( $\epsilon$  15,900).

*Anal.* Calcd for  $C_{12}H_{13}N_5O$  (243): C, 59.3; H, 5.39; N, 28.8. Found: C, 59.4; H, 5.34; N, 28.9.

*6-Amino-5-(benzylideneamino)-1,2-dihydro-2-imino-4-methoxy-1-methylpyrimidine Hydriodide (Compound XII).* A solution of 7.3 g (0.03 mole) of the 2,4-diamino-5-(benzylideneamino)-6-methoxypyrimidine (compound XI) in 100 ml of redistilled ethyl acetate was treated with 18.5 ml (0.3 mole) of reagent grade methyl iodide and refluxed for 6 hours with magnetic stirring in an oil bath kept at 75°. The reaction mixture was chilled, and the yellow crystalline precipitate was removed by filtration, washed with cold ethyl acetate and absolute ethanol, and dried quickly over NaOH. Yield 4.6 g (40%), mp 205–207°. This material was analytically pure. It could be recrystallized from hot absolute ethanol by the addition of petroleum ether (mp 207–209°). Ultraviolet spectrum (absolute ethanol),  $\lambda_{\max}$  300–310 m $\mu$  (plateau,  $\epsilon$  14,300),  $\lambda_{\max}$  330 (shoulder,  $\epsilon$  18,900),  $\lambda_{\max}$  345 ( $\epsilon$  23,500),  $\lambda_{\max}$  363 (shoulder,  $\epsilon$  18,100).

*Anal.* Calcd for  $C_{13}H_{15}N_5O \cdot HI$  (385.2): C, 40.5; H, 4.18; N, 18.2; I, 32.9. Found: C, 40.7; H, 4.21; N, 17.9; I, 32.8 (sample dried at 80°).

*5,6-Diamino-1,2-dihydro-2-imino-4-methoxy-1-methylpyrimidine (Compound XIII).* A solution of 3.85 g (10 mmoles) of the hydriodide (compound XII) in 30 ml of 6 N HCl was kept at room temperature for 10 minutes and extracted four times with petroleum ether. The aqueous solution was evaporated to dryness *in vacuo*, and the crystalline residue was dissolved in boiling absolute ethanol. The solution was clarified by filtration, ethyl acetate was added to the cloud point, and crystallization was completed by chilling. Yield 2 g (83%). A portion was recrystallized twice for analysis. Ultraviolet spectrum,  $\lambda_{\max}^{H^+}$  231 m $\mu$  ( $\epsilon$  7400), 277 m $\mu$  ( $\epsilon$  13,900).

*Anal.* Calcd. for  $C_6H_{11}N_5O \cdot 2HCl$  (241.1): C, 29.9; H, 5.44; Cl, 29.4. Found: C, 30.2; H, 5.35; Cl, 29.2.

*2,5,6-Triamino-1-methyl-4(1H)-pyrimidinone (Compound XIV).* A solution of 3.5 g (9 mmoles) of the methylated Schiff base hydriodide (compound XII) in 30 ml of 6 N HCl was treated as described above. After removal of the HCl *in vacuo*, absolute ethanol was added to the residue and taken to dryness three times. It was then treated with 60 ml of a cold 36% (w/w) solution of HBr in glacial acetic acid, and kept at room temperature for 1 hour with frequent shaking.<sup>6</sup> The precipitate was removed by centrifugation, washed once with cold glacial acetic acid and twice with chloroform, and dried overnight *in vacuo* over NaOH and  $P_2O_5$ . The essentially pure dihydrobromide (2.4 g) was moistened with 2 ml of water and treated with 10 ml of 4 N  $H_2SO_4$ . On warming to approximately 50° a clear solution was obtained which was chilled in ice. The crystals were removed by filtration, washed twice with cold 4 N  $H_2SO_4$  and three times with cold absolute

ethanol, and dried *in vacuo* overnight. Yield 1.7 g (65%) of analytically pure material, which could be recrystallized from 2 N  $H_2SO_4$ . The same product was obtained even if solution did not take place in the 4 N  $H_2SO_4$  prior to crystallization. Ultraviolet spectrum,  $\lambda_{\max}^{H^+}$  264 m $\mu$  ( $\epsilon$  16,200), 240 m $\mu$  (slight shoulder,  $\epsilon$  5400).

*Anal.* Calcd for  $C_5H_9N_5O \cdot H_2SO_4 \cdot H_2O$  (271.2): C, 22.2; H, 4.83; N, 25.8; S, 11.8; N-CH<sub>3</sub>, 5.2. Found: C, 22.1; H, 4.89; N, 25.6; S, 12.0; N-CH<sub>3</sub>, 4.9.

*3-Methylguanine (Compound XV).* 2,5,6-Triamino-1-methyl-4(1H)-pyrimidinone (compound XIV) sulfate was refluxed in 98% formic acid, and the product was refluxed in dimethylformamide. The resulting 3-methylguanine (compound XV) gave the expected elemental analysis and ultraviolet spectrum (Elion, 1962). It was converted to 3-methylxanthine (compound XVI) by heating with concentrated  $NH_4OH$  in a sealed tube at 145° (cf. Townsend and Robins, 1962).

#### Acknowledgments

We are pleased to acknowledge the assistance of Mrs. N. H. Bernhardt, Mrs. E. W. Gardner, and Mr. P. Lemanski. We are grateful to Dr. J. M. Smith, Jr., for gifts of 2,5,6-triamino-4-pyrimidinol and 2,4,5-triamino-6-methoxypyrimidine, and to Dr. G. B. Elion for 3-methylxanthine.

#### References

- Ben-Ishai, D., and Berger, A. (1952), *J. Org. Chem.* 17, 1564.
- Boon, W. R., and Bratt, G. (1957), *J. Chem. Soc.*, 2159.
- Borowitz, I. J., Bloom, S. M., Rothschild, J., and Sprinson, D. B. (1959), *Federation Proc.* 18, 195.
- Bredereck, H., Heunig, I., and Pfeleiderer, W. (1953), *Ber.* 86, 321.
- Cathou, R. E., and Buchanan, J. M. (1963), *J. Biol. Chem.* 238, 1746.
- Dick, G. P. G., Wood, H. C. S., and Logan, W. R. (1956), *J. Chem. Soc.*, 2131.
- Dunn, D. B. (1963), *Biochem. J.* 86, 15P.
- Elion, G. B. (1957), *Ciba Found. Symp. Chem. Biol. Purines*, 39.
- Elion, G. B. (1962), *J. Org. Chem.* 27, 2478.
- Elwyn, D., Weissbach, A., Henry, S. S., and Sprinson, D. B. (1955), *J. Biol. Chem.* 213, 281.
- Fischer, E. (1895), *Ber.* 28, 2480.
- Fischer, E. (1897), *Ber.* 30, 2402.
- Fischer, E. (1898), *Ber.* 31, 117, 544.
- Foster, M. A., Tejerina, G., and Woods, D. D. (1961), *Biochem. J.* 81, 1P.
- Guest, J. R., Friedman, S., Dilworth, M. J., and Woods, D. D. (1964), *Ann. N.Y. Acad. Sci.* 112, 774.
- Larrabee, A. R., Rosenthal, S., Cathou, R. E., and Buchanan, J. M. (1963), *J. Biol. Chem.* 238, 1025.
- Morrison, A. L., and Rinderknecht, H. (1950), *J. Chem. Soc.*, 1478.
- Pinner, A. (1895), *Ber.* 28, 473.

<sup>6</sup> Cf. Ben-Ishai and Berger (1952), who used this procedure for removing carbobenzoxy groups. Removal of the *O*-methyl group by refluxing with aqueous 2 N HCl was very slow, whereas refluxing with 6 N HCl led to destruction of the pyrimidine.

- Roth, B., Smith, J. M., Jr., and Hultquist, M. E. (1951a), *J. Am. Chem. Soc.* 73, 2864.
- Roth, B., Smith, J. M., Jr., and Hultquist, M. E. (1951b), *J. Am. Chem. Soc.* 73, 2869.
- Sprecher, M., and Sprinson, D. B. (1965), *Biochemistry* 4, 655 (this issue; following paper).
- Townsend, L. B., and Robins, R. K. (1962), *J. Am. Chem. Soc.* 84, 3008.
- Uhle, F. C., and Harris, L. S. (1956), *J. Am. Chem. Soc.* 78, 381.
- Waller, C. W., Hutchings, B. L., Mowat, J. H., Stockstad, E. L. R., Boothe, J. H., Angier, R. B., Semb, J., Subbarow, Y., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. (1948), *J. Am. Chem. Soc.* 70, 19.
- Weissmann, B., and Gutman, A. B. (1956), *Federation Proc.* 10, 170.
- Weissmann, B., and Gutman, A. B. (1957), *J. Biol. Chem.* 229, 239.
- Wilson, W. (1948), *J. Chem. Soc.*, 1157.
- Wohl, A. (1919), *Ber.* 52, 51.
- Woodruff, E. H., Lambooy, J. P., and Burt, W. E. (1940), *J. Am. Chem. Soc.* 62, 922.

## Methylated Purines and Pyrimidines. II. Synthesis and Properties of 2,6-Diamino-5-(methylamino)-4-pyrimidinol\*

M. Sprecher† and D. B. Sprinson‡

**ABSTRACT:** 2,6-Diamino-5-(methylamino)-4-pyrimidinol (compound VIII) was synthesized by an application of the Hinsberg reaction. Sulfonylation of 2,4,5-triamino-6-methoxypyrimidine (compound Ib) gave the 5-sulfonamidopyrimidines II and VI, which were methylated to the corresponding 5-(*N*-methylsulfonamido)pyrimidines (compounds III and VII). Removal of the sulfonyl group by reduction with sodium in liquid ammonia, and of the *O*-methyl group with HBr in acetic acid or with concentrated aqueous acid, gave compound VIII in good yields. The 5-(methylamino)pyrimidine was considerably more stable than the unmethylated compound (Ia) in hot acids, or in alkali in the presence of oxygen. Rather vigorous conditions appeared neces-

sary to cyclize the 5-(*N*-methylformamido)- and 5-(1-methylureido)pyrimidines derived from compound VIII to 7-methylguanine and 8-hydroxy-7-methylguanine, respectively. Heating of compound VIII with ethyl oxalate gave 5-methylleucopterin (compound IX). Preliminary attempts failed to convert compound VIII to 5-methylfolic acid. Unlike 2,5,6-triamino-4-pyrimidinol (compound Ia), compound VIII did not appear to react with 2,3-dibromopropanal plus *p*-aminobenzoyleglutamate. When compound VIII was treated with 2-bromo-3,3'-diethoxypropanal the reaction was very slow compared to that observed with compound Ia. There was no evidence for the formation of a dihydropteridin or a pteridin.

As discussed in the preceding paper (Borowitz *et al.*, 1965), interest in the possible role of 5-methylfolic acid in methionine formation stimulated several unsuccessful attempts to synthesize 2,6-diamino-5-(methylamino)-4-pyrimidinol (compound VIII), which it was hoped would afford 5-methylfolic acid by conventional means (Waller *et al.*, 1948). Further efforts, described in the present communication, were directed toward methylation of 5-sulfonamidopyrimidines and hydrolysis or reduction of the 5-(*N*-methylsulfonamido)pyrimi-

dines to compound VIII. It has been demonstrated conclusively by Wilson (1948) that acylation of 2,5,6-triamino-4-pyrimidinol (compound Ia) afforded the 5-acylaminopyrimidine. Sulfonylation of 2,4,5-triamino-6-methoxypyrimidine (compound Ib), therefore, appeared to be a convenient method to prepare intermediates for methylation. The 6-methoxypyrimidine (compound Ib) was chosen as starting material in order to avoid nuclear substitution, owing to anion formation on N-3, during subsequent alkylation reactions in basic medium.

No difficulties were encountered in preparing the *p*-toluenesulfonamide II or the  $\alpha$ -toluenesulfonamide VI by usual methods. These were methylated smoothly to the corresponding *N*-methylsulfonamides III and VII with one equivalent of methyl iodide and sodium methoxide in methanol solution, and the products were freed of unmethylated starting material by extraction

\* From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City. Received June 29, 1964; revised December 7, 1964. This work was supported by grants from the American Cancer Society, the American Heart Association, and the National Institutes of Health of the U.S. Public Health Service.

† Present address, Bar Ilan University, Ramat Gan, Israel.

‡ Career Investigator of the American Heart Association.