

calculated using the measured or estimated  $pK_{\text{SRP}}$  values for the imines.

The extent of reaction  $\beta = a_M(\text{sample}) - a_M(\text{pyridoxal})/a_M(\text{imine}) - a_M(\text{pyridoxal})$  was then computed and  $K_{\text{pH}}$  calculated as  $K_{\text{pH}} = 1/r(\beta/1 - \beta)$ .  $K_{\text{pH}}$  was nearly always calculated at 3 or more wave lengths and agreement was usually within 10% or less.

The  $pK_{\text{SRP}}$  for the valine imine was calculated from the change of the imine spectrum with pH in the manner pre-

viously described for pyridoxal.<sup>7</sup> A series of nine solutions 0.5 M in valine of pH 8.3 to 12 and ionic strength 0.5 was employed.  $pK_{\text{SRP}}$  for the glycine imine was estimated in a similar fashion while those for other imines were inferred by a comparison of  $K$  with  $K_{\text{pH}}$  at a pH of about 8 and calculation using eq. 3.

A few of the data used in the calculations are given in Table II.

AMES, IOWA

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, NEW MEXICO HIGHLANDS UNIVERSITY]

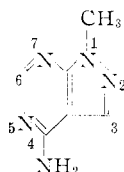
## Potential Purine Antagonists. IV. Synthesis of Some 9-Methyl-6-substituted-purines<sup>1</sup>

BY ROLAND K. ROBINS AND HSI HU LIN

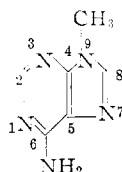
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A new method has been developed for the synthesis of various 9-methyl-6-substituted-purines. 4,6-Dichloro-5-nitropyrimidine (I) has been treated with methylamine to give 6-chloro-4-methylamino-5-nitropyrimidine (V). Reduction of V gave a new route to 5-amino-6-chloro-4-methylaminopyrimidine (VI). Cyclization of VI with ethyl orthoformate and acetic anhydride resulted in the synthesis of 9-methyl-6-chloropurine (VII). Various 9-methyl-6-substituted-purines have been prepared from VII.

In the general program of synthesis of various potential antagonists of the natural purines, it was discovered that 1-methyl-4-aminopyrazolo[3,4-d]-pyrimidine possessed anti-tumor activity<sup>2</sup> against certain animal tumors. It thus seemed desirable to prepare the corresponding purine analog, 9-methyl-6-aminopurine (9-methyladenine) in sufficient quantity for animal testing.



1-Methyl-4-aminopyrazolo-  
[3,4-d]-pyrimidine



9-Methyladenine

9-Methyladenine previously has been synthesized by several different synthetic routes.<sup>3-7</sup> However, various difficulties involved in these procedures led us to investigate a simplified general method for the synthesis of 9-methyl-6-amino- and 9-methyl-6-substituted-aminopurines.

After the present work was complete, a new synthesis of 9-methyladenine was reported by Daly and Christensen<sup>8</sup> from 4,5-diamino-6-methylaminopyrimidine sulfate and boiling formamide.

In the present investigation treatment of 4,6-dichloro-5-nitropyrimidine<sup>9</sup> (I) with an aqueous

solution of methylamine neutralized with acetic acid gave 6-chloro-4-methylamino-5-nitropyrimidine (V) in good yield. A similar modification was employed by Rose<sup>10</sup> for the synthesis of 4-chloro-6-dimethylamino-5-nitropyrimidine. It is interesting to note in this connection that Brown<sup>11</sup> was unsuccessful in an earlier attempt to synthesize 6-chloro-4-methylamino-5-nitropyrimidine (V) by treatment of 4,6-dichloro-5-nitropyrimidine (I) with an alcoholic solution of methylamine. The reduction of V with zinc dust in dilute acetic acid gave 5-amino-6-chloro-4-methylaminopyrimidine (VI). This latter compound has recently been obtained by Brown<sup>11</sup> by another route.

When 5-amino-6-chloro-4-methylaminopyrimidine (VI) was refluxed with formic acid, cyclization took place to give 9-methylhypoxanthine (X) in good yield. The loss of a chlorine atom of various chloro-substituted-4,5-diaminopyrimidines upon formylation and cyclization with formic acid<sup>12</sup> and formamide<sup>13</sup> has been reported previously; thus this behavior is not unexpected.

It was also discovered that when 5-amino-6-chloro-4-methylaminopyrimidine (VI) was heated with ethyl orthoformate and acetic anhydride, cyclization took place without the loss of the chlorine atom to give 9-methyl-6-chloropurine (VII).

Richter and Taylor<sup>14</sup> have reported the use of ethyl orthoformate and acetic anhydride in a new synthesis of hypoxanthine. Montgomery<sup>15</sup> has recently reported the synthesis of 2-chloropurine, 6-chloropurine and 2,6-dichloropurine by cyclization of the appropriate chloro-4,5-diaminopyrimidines under similar conditions.

Treatment of 9-methyl-6-chloropurine (VII) with alcoholic ammonia in a bomb at 150° gave 9-

(1) This investigation was supported in part by research grant C-2845 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(2) H. E. Skipper, R. K. Robins and J. R. Thomson, *Proc. Soc. Exp. Biol. and Med.*, **89**, 594 (1955). For the synthesis of 1-methyl-4-aminopyrazolo(3,4-d)pyrimidine, see "Potential Purine Antagonists, VI," *J. Org. Chem.*, in press.

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(10) F. L. Rose, *ibid.*, 4124 (1954).

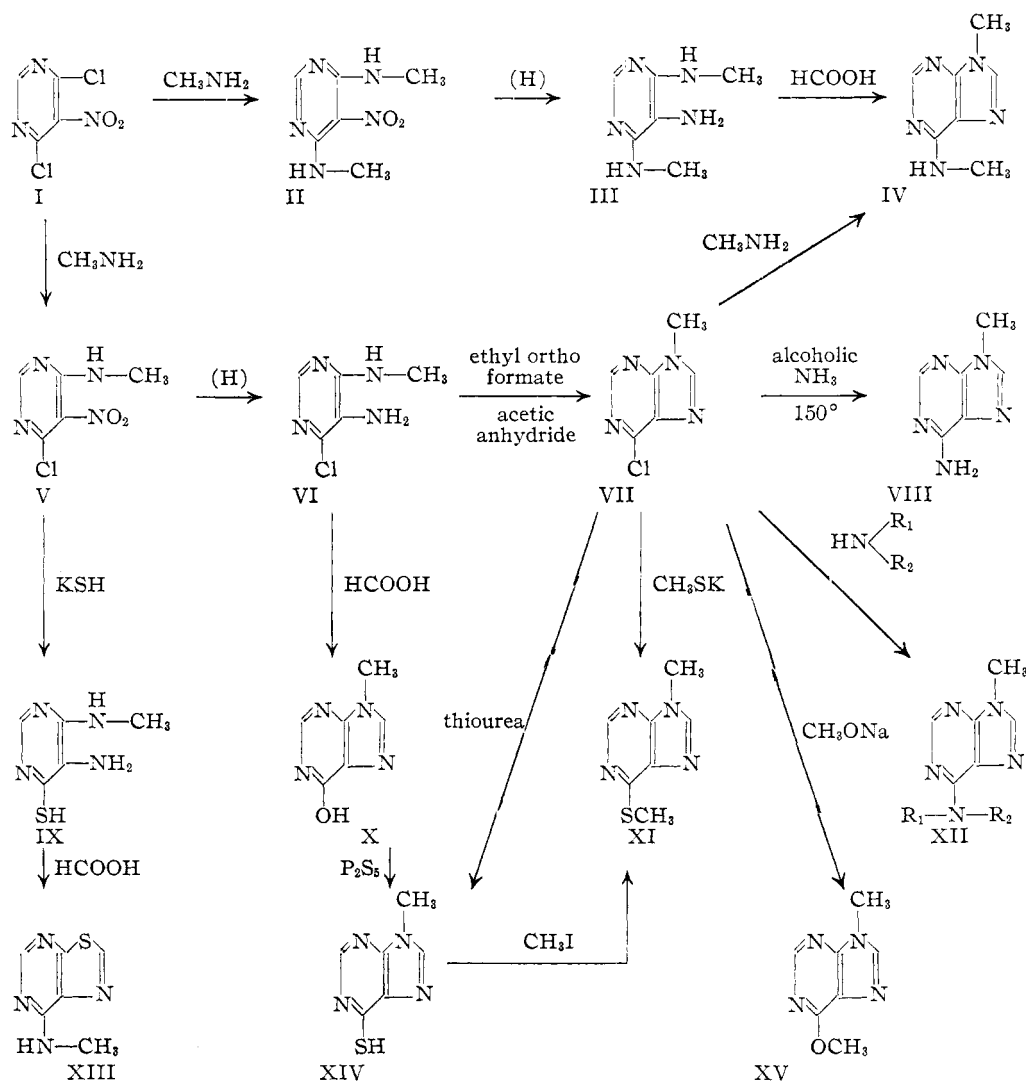
(11) D. J. Brown, *J. Applied Chem.*, **4**, 72 (1954).

(12) R. K. Robins, K. L. Dille and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).

(13) R. K. Robins, K. L. Dille, C. H. Willits and B. E. Christensen, *THIS JOURNAL*, **75**, 263 (1953).

(14) E. Richter and E. C. Taylor, *Angew. Chem.*, **67**, 303 (1955).

(15) J. A. Montgomery, *THIS JOURNAL*, **78**, 1928 (1956).



methyladenine in good yield. Although 9-methyladenine could not be prepared by treatment of VII with aqueous ammonia on the steam-bath, aqueous methylamine under the same conditions yielded 9-methyl-6-methylaminopurine (IV). The synthesis of IV also was accomplished by another route. Treatment of I with excess aqueous methylamine heated on the steam-bath gave 4,6-bis-(methylamino)-5-nitropyrimidine<sup>11</sup> (II). Catalytic reduction of II with Raney nickel and hydrogen gave 4,6-bis-(methylamino)-5-aminopyrimidine (III) which was not isolated but was cyclized directly with formic acid to yield 9-methyl-6-methylaminopurine (IV). This compound was judged to be identical with IV prepared from 9-methyl-6-chloropurine (VII) on the basis of mixed melting points and identical ultraviolet absorption spectra.

Treatment of 9-methyl-6-chloropurine (VII) with various primary and secondary amines in methanol, ethanol or water followed by heating on the steam-bath for several hours resulted in the preparation of the 9-methyl-6-substituted-aminopurines listed in Table I.

9-Methyl-6-chloropurine (VII) and thiourea in

boiling ethanol yielded 9-methyl-6-mercaptopurine (XIV).<sup>16</sup> 9-Methyl-6-mercaptopurine (XIV) was also prepared by treatment of 9-methylhypoxanthine with phosphorus pentasulfide in boiling pyridine. Methylation of XIV with methyl iodide in a basic aqueous solution gave 9-methyl-6-methylmercaptopurine (XI). 9-Methyl-6-methylmercaptopurine (XI) was also prepared by another route in a much better yield by the treatment of 9-methyl-6-chloropurine (VII) with methyl mercaptan dissolved in a methanolic solution of potassium hydroxide.

An unsuccessful attempt was made to prepare 9-methyl-6-mercaptopurine (XIV) by a third route from 4-methylamino-5-amino-6-mercaptopyrimidine (IX). This compound IX was readily obtained in one step by treatment of 4-methylamino-5-nitro-6-chloropyrimidine (V) with potassium hydrosulfide. Refluxing formic acid, however, converted 4-methylamino-5-amino-6-mercaptopyrimidine (IX) to 7-methylaminothiazolo[5,4-d]pyrimidine (XIII) which is isomeric with 9-methyl-6-

(16) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *THIS JOURNAL*, **76**, 6073 (1954).

TABLE I

SOME 9-METHYL-6-SUBSTITUTED-AMINOPURINES,

R <sub>1</sub>	R <sub>2</sub>	M.p., °C.	C	Calcd. H	Analyses, % N	C	Found H	N	Method of prepn.	Recrystln. solvent	Yield, %
CH <sub>3</sub>	CH <sub>3</sub>	119-120	54.2	6.2	39.5	54.0	6.2	39.6	C	<i>n</i> -Heptane	78
H	C <sub>2</sub> H <sub>5</sub>	157-158	54.2	6.2		54.4	6.6		B	Benzene	81
H	CH <sub>3</sub>	190-191	51.9	6.0	42.9	52.1	6.0	43.1	B	Benzene	84
H		175-177	57.6	4.8	30.6	57.8	4.9	30.3	A	Ethanol-water	80
H	<i>iso</i> -C <sub>3</sub> H <sub>7</sub>	136-137	56.6	6.8	36.7	56.7	7.0	37.0	B	Toluene- <i>n</i> -heptane	62
C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	48-50	58.5	7.3	34.1	58.7	7.3	34.3	C	Light pet. ether	53
H	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	130-131	56.6	6.8	36.7	56.5	6.7	36.5	C	Toluene- <i>n</i> -heptane	72
H	NH <sub>2</sub>	210-211	43.9	4.9	51.7	43.2	5.2	50.9	A	Methanol	40
H	NH-CH <sub>3</sub>	100-101	47.2	5.6		47.4	5.4		C	Heptane	30

mercaptapurine (XIV). A similar cyclization of 4,5-diamino-6-mercaptopyrimidine with formic acid under specific conditions has been reported recently by Elion, Lange and Hitchings<sup>17</sup> to give 7-amino-thiazolo[5,4-d]pyrimidine. There are also several other examples recorded in the literature<sup>18,19</sup> of the synthesis of thiazolo[5,4-d]pyrimidines by treatment of mercaptoaminopyrimidines with formic acid.

When 9-methyl-6-chloropurine (VII) was treated with sodium methoxide, 9-methyl-6-methoxypurine (XV) was obtained in good yield. Similarly, when VII was added to a warm aqueous solution of *p*-bromophenol and potassium hydroxide, the compound 9-methyl-6-*p*-bromophenoxypurine rapidly precipitated.

TABLE II

THE ULTRAVIOLET ABSORPTION  
MAXIMA OF SEVERAL 9-METHYL-  
6-SUBSTITUTED-PURINES,

R	$\lambda_{\text{max}}$ $\text{pH} = 1$	$\epsilon$	$\lambda_{\text{max}}$ $\text{pH} = 11$	$\epsilon$
OH	251	6,300	256	7,350
SH	324	19,600	310	16,800
NH <sub>2</sub>	261	14,600	262	11,900
Cl	265	8,100	268	21,100
OCH <sub>3</sub>	254	9,850	254	10,600
SCH <sub>3</sub>	222	12,000	287	20,800
	295	19,500		

The ultraviolet absorption maxima of certain 9-methyl-6-substituted-purines are listed in Table II. Careful inspection of this table and comparison of the spectra published for the 6-substituted-purines reveal that in general the "9-methyl" group results in a hypsochromic shift of 2 to 5  $\mu$ : in other respects the spectra are remarkably similar.

(17) G. B. Elion, W. Lange and G. H. Hitchings, *THIS JOURNAL*, **78**, 2858 (1956).

(18) S. J. Childress and R. L. McKee, *ibid.*, **73**, 3862 (1951).

(19) F. L. Rose, *J. Chem. Soc.*, 3448 (1952).

Preliminary results obtained at the Southern Research Institute have shown that 9-methyl-6-chloropurine (VII) exhibits significant anti-tumor activity against adenocarcinoma 755 in the mouse.

TABLE III

THE ULTRAVIOLET ABSORPTION  
MAXIMA OF SOME 9-METHYL-  
6-SUBSTITUTED-AMINOPURINES,

R <sub>1</sub>	R <sub>2</sub>	$\lambda_{\text{max}}$ $\text{pH} = 1$	$\epsilon$	$\lambda_{\text{max}}$ $\text{pH} = 11$	$\epsilon$
CH <sub>3</sub>	CH <sub>3</sub>	269	14,400	277	16,800
H	C <sub>2</sub> H <sub>5</sub>	265	16,600	268	13,600
H	CH <sub>3</sub>	265	15,300	268	14,000
H		267	20,000	269	18,700
H	<i>iso</i> -C <sub>3</sub> H <sub>7</sub>	266	16,400	269	14,300
C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	271	20,100		
H	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	265	7,100	270	10,900
H	NH <sub>2</sub>	263	16,100		
H	NH-CH <sub>3</sub>	267	16,000	277	13,100

**Acknowledgment.**—The authors wish to acknowledge the helpful suggestions of Dr. B. R. Baker of the Stanford Research Institute.

### Experimental<sup>20</sup>

**6-Chloro-4-methylamino-5-nitropyrimidine (V).**—Thirty ml. of glacial acetic acid was carefully added with cooling to 48 ml. of a 40% aqueous solution of methylamine. The solution was then cooled to 5° and added dropwise to a stirred solution of 22 g. of 4,6-dichloro-5-nitropyrimidine<sup>9</sup> (I) in 45 ml. of 1,4-dioxane previously cooled to 0°. The inside temperature was maintained at 10°, and the addition of the solution of methylamine took 1 hr. After all the methylamine had been added, 300 ml. of water was added dropwise over a 2-hr. period. The inside temperature during this time was kept at 10°. Finally the yellow needles of 6-chloro-4-methylamino-5-nitropyrimidine were filtered and washed with a little ice-water. The yield of product was 18.1 g., m.p. 145-149°. Recrystallization from benzene-petroleum ether raised the m.p. to 150-151°.

(20) All melting points are uncorrected and were taken on a Fisher-Johns melting point block.

*Anal.* Calcd. for  $C_8H_8N_4O_2Cl$ : C, 31.8; H, 2.7; N, 29.7. Found: C, 32.0; H, 2.8; N, 29.7.

**5-Amino-6-chloro-4-methylaminopyrimidine.**—To a constantly stirred 2000 ml. of boiling water containing 200 g. of zinc dust and 100 ml. of glacial acetic acid was carefully added in small portions 60 g. of 6-chloro-4-methylamino-5-nitropyrimidine (V). At the end of the addition which took approximately 15 minutes, the mixture was boiled and stirred 15 minutes more and then filtered while hot. The cooled filtrate yielded 30.4 g. of slightly colored crystalline needles, m.p. 163–166°. A small amount was recrystallized from water to give white needles, m.p. 166–167°. The sample was dried at 130° for analysis.

*Anal.* Calcd. for  $C_8H_7N_4Cl$ : C, 37.9; H, 4.5; N, 35.3. Found: C, 37.8; H, 4.4; N, 35.2.

Brown<sup>11</sup> records 172° as the m.p. of this compound.

**9-Methylhypoxanthine (X).**—A solution of 5 g. of 5-amino-6-chloro-4-methylaminopyrimidine (VI) and 50 ml. of formic acid was refluxed for 8 hr. The solution was then evaporated to near dryness under reduced pressure, and concentrated ammonium hydroxide was added to the residue until pH 10 was reached. The cooled solution yielded 4.1 g. of crude 9-methylhypoxanthine. The crude product was recrystallized from water to give white crystalline needles, m.p. > 300°.

*Anal.* Calcd. for  $C_8H_8N_4O$ : N, 37.3; C, 48.0; H, 4.0. Found: N, 37.2; C, 47.9; H, 4.2.

**9-Methyl-6-chloropurine (VII).**—Thirty-five grams of 5-amino-6-chloro-4-methylaminopyrimidine (VI) recrystallized from water and dried at 110° was added to a mixture of 150 ml. of ethyl orthoformate and 150 ml. of acetic anhydride. The solution was refluxed for 3 hr. and the excess solvent removed under reduced pressure using a water-bath as a source of heat. The solid residue was dissolved in 300 ml. of boiling benzene. To this hot solution was added 100 ml. of heptane and the solution heated with charcoal and filtered. The cooled filtrate yielded 29.0 g. of crystals, m.p. 140–141°. Recrystallization of a small sample from benzene raised the m.p. to 143–144°.

*Anal.* Calcd. for  $C_8H_8N_4Cl$ : C, 42.7; H, 3.0; N, 33.2. Found: C, 42.5; H, 3.0; N, 33.3.

**9-Methyl-6-mercaptopurine (XIV).** **Method (1).**—Three grams of 9-methyl-6-chloropurine (VII) and 3.5 g. of thiourea were dissolved in 90 ml. of absolute ethanol. The resulting solution was refluxed for 3 hr. and then cooled, filtered and the precipitate washed with a small amount of distilled water. The light tan crystals were dissolved in hot dilute potassium hydroxide. The solution was boiled gently for a few minutes with charcoal, and the filtrate was acidified while hot with acetic acid. The resulting precipitate, m.p. > 300°, was washed and dried at 115°.

*Anal.* Calcd. for  $C_8H_8N_4S$ : C, 43.4; H, 3.6; N, 33.7. Found: C, 43.3; H, 3.8; N, 33.2, 34.1.

**Method (2).**—Six grams of phosphorus pentasulfide and 0.8 g. of 9-methylhypoxanthine (X) were added to 100 ml. of dry C.P. pyridine. The solution was refluxed for 2 hr. and the excess pyridine distilled off under vacuum using a steam-bath as a source of heat. To the residue was added 75 ml. of water and the solution heated 2 hr. on the steam-bath. The solution was filtered and the crude product purified by reprecipitation from a hot basic solution with acetic acid. The yield was 0.5 g. This product was found to be identical to XIV prepared by method (1) as judged by identical ultraviolet absorption spectra at pH 1 and pH 11.

**9-Methyl-6-aminopurine (9-Methyladenine) (VIII).**—Ten grams of 9-methyl-6-chloropurine and 120 ml. of alcoholic ammonia (absolute ethanol saturated with dry ammonia at 0°) were placed in a bomb and heated at 150° for 8 hr. The cooled solution was filtered, and the large crystals dissolved in approximately 300 ml. of boiling water. Charcoal was added and the pH of the solution adjusted to 10 with potassium hydroxide. The cooled filtrate yielded 6.9 g. of colorless needles of 9-methyladenine, m.p. 310°.

*Anal.* Calcd. for  $C_8H_7N_5$ : C, 48.3; H, 4.7; N, 47.0. Found: C, 48.4; H, 5.0; N, 46.9.

**9-Methyl-6-methylaminopurine. (IV).** **Method (1).**—Two and a half grams of 4,6-bis-(methylamino)-5-nitropyrimidine<sup>11</sup> was dissolved in 150 ml. of absolute ethanol, and the solution was shaken with Raney nickel catalyst at a hydrogen pressure of approximately 20 lb./sq. in. for 3 hr.

The solution was boiled with charcoal, filtered and the filtrate evaporated to dryness under reduced pressure. The crude orange residue of 4,6-bis-(methylamino)-5-amino-pyrimidine (IV) was not purified but treated directly with 50 ml. of 90% formic acid. The solution was refluxed for 6 hr. and the excess formic acid removed under reduced pressure. The residue was boiled with concentrated ammonium hydroxide and evaporated to dryness on the steam-bath. A dark brown residue remained. The crude material was redissolved in absolute ethanol, boiled with charcoal and the filtrate evaporated to dryness on the steam-bath. The crude yellowish-brown crystals of 9-methyl-6-methylaminopurine thus obtained were recrystallized from a mixture of benzene and ethanol to give 1.5 g. of crystalline needles, m.p. 190–191°.

*Anal.* Calcd. for  $C_9H_{10}N_6$ : N, 42.9; C, 51.9; H, 6.0. Found: N, 43.1; C, 52.1; H, 6.0.

**Method (2).**—Five grams of 9-methyl-6-chloropurine (VII) and 100 ml. of 25% aqueous methylamine were heated to dryness on the steam-bath. The residue was extracted with 3 × 100 ml. of boiling benzene and the benzene evaporated to 100 ml. The cooled solution yielded 3.7 g. of product, m.p. 189–190°. A second recrystallization from benzene raised the m.p. to 190–191°. Mixed m.p. of this product and that prepared by method (1) was 190–191°. Ultraviolet absorption spectra of the two products were identical.

**5-Amino-6-mercapto-4-methylaminopyrimidine (IX).**—To 150 ml. of 1 N potassium hydroxide solution saturated with hydrogen sulfide was added 5 g. of 6-chloro-4-methylamino-5-nitropyrimidine (V) and the solution heated on a steam-bath for 2.5 hr. The solution was then filtered while hot to remove a small quantity of sulfur and the filtrate acidified with acetic acid. The crude product, 3.6 g., was filtered and dried in the oven. For analysis this material was recrystallized from water.

*Anal.* Calcd. for  $C_8H_8N_4S$ : C, 38.4; H, 5.1; N, 35.9. Found: C, 38.5; H, 5.1; N, 36.2.

**7-Methylaminothiazolo[5,4-d]pyrimidine (XIII).**—Two and one-half grams of 5-amino-6-mercapto-4-methylaminopyrimidine (IX) was dissolved in 35 ml. of formic acid and the resulting solution refluxed for 6 hr. The solution was evaporated to near dryness and the pH adjusted to 12 by the addition of concentrated ammonium hydroxide. The yield of crude product was 2 g., m.p. 152–158°. For analysis this product was recrystallized from water to give white needles, m.p. 158–159°,  $\lambda_{max}$ , pH 1, 230 and 270 m $\mu$ . This product analyzed for a monohydrate which lost one mole of water when heated at 130°.

*Anal.* Calcd. for  $C_8H_8N_4S \cdot H_2O$ : C, 39.1; H, 4.4. Found: C, 39.5; H, 4.4.

*Anal.* Calcd. for  $C_8H_8N_4S$ : after drying at 130°, N, 33.7. Found: N, 33.7.

**9-Methyl-6-methoxypurine (XI).**—One gram of 9-methyl-6-chloropurine (VII) was added to 100 ml. of absolute methanol in which had been dissolved one gram of sodium. The solution was heated for 15 minutes on the steam-bath, and a small amount of sodium chloride was filtered off and the volume of the filtrate reduced to 30 ml. on the steam-bath. The cooled solution was then filtered and the white crystals washed with a small amount of methanol. The crude dried product was recrystallized from a benzene-heptane mixture to yield 0.6 g. of small white needles, m.p. 152–153°. The sample was dried 1 hr. at 110° for analysis.

*Anal.* Calcd. for  $C_7H_8N_4O$ : C, 51.2; H, 4.9; N, 34.1. Found: C, 51.5; H, 5.0; N, 34.4.

**Preparation of 9-Methyl-6-methylmercaptopurine (XI).** **Method (1).**—To 100 ml. of methanol containing 5.0 g. of 9-methyl-6-chloropurine was carefully added 15 ml. of a 50% solution of methanethiol dissolved in methanolic potassium hydroxide. The solution was carefully heated on the steam-bath for 1 hr. during which time the volume was reduced to 30 ml. The cooled solution yielded 3.1 g. of light tan needles, m.p. 167–169°. Recrystallization from methanol raised the m.p. to 171–172°.

*Anal.* Calcd. for  $C_9H_{10}N_6S$ : C, 46.6; H, 4.5. Found: C, 46.6; H, 4.8.

**Method (2).**—To a solution of 3 g. of potassium hydroxide in 50 ml. of water was added 4.0 g. of 9-methyl-6-mercaptopurine (XIV). This solution was vigorously shaken at room

temperature with 15 ml. of methyl iodide. The excess methyl iodide was then removed and the aqueous solution shaken twice more with 15-ml. portions of methyl iodide each time. The combined methyl iodide extracts were washed once with water and the excess methyl iodide evaporated on the steam-bath to give 1.5 g. of a light green product, m.p. 165–167°. Recrystallization of this product from a small volume of water raised the m.p. to 171–172°.

*Anal.* Calcd. for  $C_7H_5N_4S$ : C, 46.6; H, 4.5. Found: C, 46.6; H, 4.7.

This product when mixed with XI prepared by method (1) gave a mixed m.p. of 171–172°. Identical ultraviolet absorption spectra were obtained for XI prepared by method (1) and method (2).

**Preparation of 9-Methyl-6-*p*-bromophenoxypurine.**—To 5.0 g. of *p*-bromophenol and 5.0 g. of potassium hydroxide dissolved in 150 ml. of water was added slowly 5.0 g. of 9-methyl-6-chloropurine (VII). The solution was heated on the steam-bath for 0.5 hr., cooled and filtered. The crude product<sup>2</sup> was recrystallized from a mixture of ethanol and water to yield 6.1 g., m.p. 164–165°.

*Anal.* Calcd. for  $C_{12}H_9N_4OBr$ : C, 47.2; H, 3.0; N, 18.3. Found: C, 47.2; H, 3.0; N, 18.7.

The ultraviolet absorption spectra in absolute ethanol showed a maximum at 256 m $\mu$ ,  $\epsilon$  18,300.

**Preparation of the 9-Methyl-6-substituted-aminopurines Listed in Table I.** **Method A.**—Five grams of 9-methyl-6-chloropurine (VII) was dissolved in 150 ml. of methanol. To this solution was added the 10 to 15 ml. of the amine or aqueous solution of the amine. The solution was heated for 1 hr. on the steam-bath, and the solution (volume approximately 40 ml.) was cooled and filtered. The crude product was recrystallized from the solvents indicated.

**Method B.**—To 150 ml. of ethanol was added 20 ml. of the appropriate amine or 20–30 ml. of an aqueous solution of the amine. Five grams of 9-methyl-6-chloropurine (VII) was then carefully added and the solution evaporated to dryness on the steam-bath. The solid residue was extracted with three 100-ml. portions of boiling benzene. The benzene was concentrated and cooled and the desired product allowed to crystallize. The product was purified by recrystallization from the appropriate solvent.

**Method C.**—This method is identical with method B except the final benzene solution which did not yield crystals was evaporated to dryness and the residue recrystallized from a more non-polar solvent.

LAS VEGAS, NEW MEXICO

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, KUNGL. VETERINÄRHÖGSKOLAN]

## Radioactive Tetracycline

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A method for preparation of tritium-labeled tetracycline of high specific activity is described. The  $H^3$ -tetracycline is produced by catalytic hydrogenation of chlorotetracycline with tritium gas in a microhydrogenator with the capacity of 1.5 ml.

The synthesis of radioactively labeled antibiotics has greatly increased the possibilities for investigation of the pharmacology and mode of action of these drugs. Tetracycline has become of foremost importance among the broad spectrum antibiotics and its stability is of considerable advantage in tracer studies.

We have been unable to find in the literature any report of a method for synthesis of radioactive tetracycline.

There are two main alternatives for labeling tetracycline: labeling with  $C^{14}$  and  $H^3$ . It would be possible to label tetracycline with  $C^{14}$  using biosynthetic methods. However, the product would not have a sufficiently high specific activity for many biological investigations unless large amounts of labeled precursor were used and that would make the method prohibitively expensive.

These disadvantages can be avoided by using tritium. The use of tritium offers additional advantages in autoradiography since its particularly short radiation range (energy, 0.017 m.e.v.) provides unique possibilities in obtaining good resolution. This was of particular importance to the authors who desired to extend their previous studies<sup>1</sup> on the distribution of antibiotics in the body using autoradiography.

The authors have modified the method used commercially for large scale production of tetracycline by catalytic hydrogenation of chlorotetracycline.<sup>2,3</sup>

The reaction was performed on a micro-scale using a microhydrogenator. The conditions of the reaction have been altered to permit efficient utilization of tritium in the reaction.

Boothe<sup>2</sup> and Conover<sup>3</sup> used palladium as a catalyst. The present authors preferred platinum oxide which is equally efficient as a catalyst but absorbs less hydrogen. It is essential to use a solvent which does not contain hydrogen (protium) which will freely exchange with the tritium. In dioxane all the hydrogen atoms are bound directly to carbon, and dioxane therefore should fulfill this requirement.

Reaction conditions had to be further modified because of the isotope effect. The heavier isotope tritium may react considerably slower than the lighter protium. The difference in reaction rate between tritium and deuterium is much smaller. Therefore deuterium was used to flush the hydrogenation apparatus before introducing the tritium and at the end of the reaction with tritium, deuterium was introduced in small amounts to complete the hydrogenation.

Since the reaction is irreversible the utilization of tritium is enhanced by prolonging the reaction time and by using a reduction mixture as rich in tritium as possible.

Before the product is used as a tracer the exchangeable tritium must be removed.

**Procedure.**—A hydrogenation apparatus with the capacity of 1.5 ml. was used. One hundred mg. of chlorotetracycline base was dissolved in 1.2 ml. of dioxane in the hydrogenation bottle. Three mg. of platinum oxide was used as a catalyst. An equivalent amount of triethylamine was added to combine with the hydrochloric acid

(1) S. Ullberg, *Acta Radiol.*, Suppl. 118 (1954).

(2) J. H. Boothe, J. Morton, II, J. P. Petisi, R. G. Wilkinson and J. H. Williams, *THIS JOURNAL*, **75**, 4621 (1953).

(3) L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens and F. J. Pilgrim, *ibid.*, **75**, 4622 (1953).