TABLE V

THE SEQUENCE OF AMINO ACIDS IN BOVINE β-MSH ("SERYL-β-MSH")

Method	Substrate	Product	Sequence
FDNB	H[a]		Asp.
LAP	H		Asp.Ser.
PITC	H		Asp.Ser.Gly.Pro.Tyr.Lys.Met
Chymotrypsin	H	Ch-5B[b]	Asp(Ser,Gly,Pro)Tyr
Chymotrypsin	H	Ch-3B[b]	Lys(Met,Glu)His.Phe
LAP	Ch-3B		Lys.Met(Glu,His,Phe)
Chymotrypsin	H	Ch-2B[b]	Arg.Try
Chymotrypsin	H	Ch-4B[b]	Gly(Ser,Pro,Pro,Lys)Asp
LAP	Ch-4B		Gly.
PITC	Ch-4B		Gly.Ser.Pro.Pro(Lys,Asp)
Carboxypeptidase	H		Asp
Complete sequence:			Asp.Ser.Gly.Pro.Tyr.Lys.Met.Glu.His.Phe.Arg.Try.Gly.Ser.Pro.Pro.Lys.Asp
			1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

[a] H = hormone. [b] The N-terminal amino acid was obtained by the FDNB procedure, and the C-terminal amino acid or sequence by the carboxypeptidase method.

that of all the peptides found in the chymotryptic digest, only the dipeptide Arg.Try (Ch-2B) must have its position assigned by elimination, for the contiguity of Ch-5B and Ch-3B is assured by the results of the phenyl isothiocyanate procedure when applied to the intact hormone, and the position of Ch-4B must be C-terminal since it alone possesses a C-terminal aspartic acid. As suspected, the sequence of amino acids in the bovine hormone differs from that found for porcine β-MSH[5,6] only at the second residue. It is therefore suggested that porcine and bovine β-MSH be henceforth called "glutamyl-β-MSH" and "seryl-β-MSH," respectively. The interchange of a neutral amino acid for an acidic one has not been previously encountered among biologically active peptides derived from different species, where experience to date has demonstrated interchanges of the neutral-neutral or basic-basic types.

Finally, in a series of 8 separate comparative assays[14] the porcine hormone has been found to be approximately 2.5 times as active as the bovine hormone. Thus, the interchange of amino acids at the position of the second residue has resulted in a modification of the hormonal activity. Seryl-β-MSH is comparable to the porcine hormone, however, in that upon being heated in 0.1 *N* NaOH for 15 minutes, "protection"[7] occurs with little or no "potentiation,"[7] in confirmation of the findings of Landgrebe and Mitchell[9] with an oxycellulose-eluate of bovine posterior lobe powder.

Acknowledgments.—The authors wish to acknowledge the able technical assistance of Miss Winifred Lilly. This work has been supported in part by grants from the National Institutes of Health of the United States Public Health Service (Grant No. G-2907) and the Albert and Mary Lasker Foundation.

BERKELEY 4, CALIFORNIA

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

Potential Purine Antagonists. VIII. The Preparation of Some 7-Methylpurines[1]

BY RAJ NANDAN PRASAD[2] AND ROLAND K. ROBINS[3]

RECEIVED MAY 13, 1957

A new method of synthesis of 7-methyladenine (III) and 7-methylhypoxanthine (V) has been accomplished from 4-amino-1-methyl-5-imidazolecarbonitrile (II) and 4-amino-1-methyl-5-imidazolecarboxamide (IV), respectively. The chlorination of V yielded 6-chloro-7-methylpurine (VI). Various new 7-methyl-6-substituted purines have been prepared from VI. 2,6-Dichloro-7-methylpurine (XIV) has been prepared from 7-methylxanthine (XIII). Several new 2,6-disubstituted-7-methylpurine derivatives have been prepared from XIV.

In the course of a general program for the synthesis of potential purine antagonists as antitumor agents, it was discovered that 6-chloro-9-methylpurine[4] showed definite activity. It therefore seemed desirable to prepare 6-chloro-7-methylpurine (VI) as a candidate anti-tumor agent. Since preliminary methylation studies of 6-chloropurine gave a difficultly separable mixture of the 7-methyl and 9-methyl isomers, the possible preparation of 6-chloro-7-methylpurine (VI) from 7-methylhypoxanthine (V) was investigated. Earlier methods of preparation of 7-methylhypoxanthine[5] proved unsatisfactory for large scale preparation. This prompted investigation of a new route to the preparation of 7-methylhypoxanthine (V).

(1) (a) 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. (b) Presented in part before the Division of Medicinal Chemistry at the 131st Meeting of the American Chemical Society, April, 1957, at Miami, Florida.

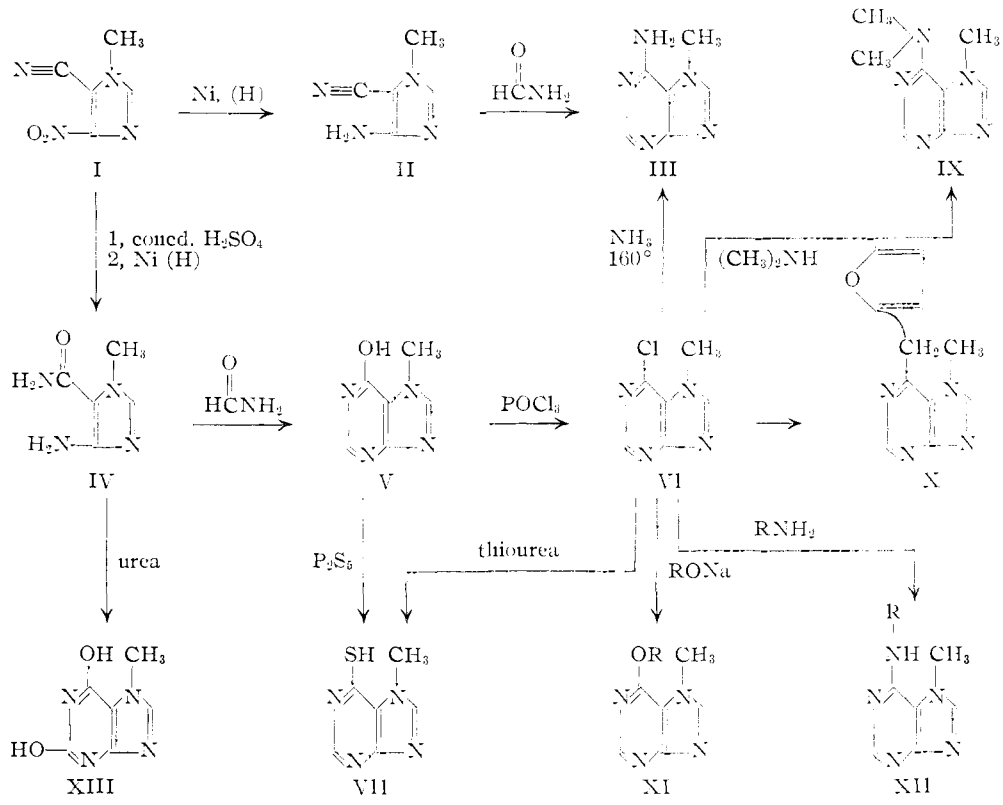
(2) On leave-of-absence from Chemistry Department, B. N. College, Patna University, India.

(3) Department of Chemistry, Arizona State College, Tempe, Arizona.

(4) R. K. Robins and H. H. Lin, THIS JOURNAL, **79**, 490 (1957).

(5) (a) E. Fischer, *Ber.*, **30**, 2400 (1897); (b) **31**, 104 (1898).

REACTION SCHEME 1



Sarasin and Wegmann[6] reported the preparation of 7-methylxanthine from 4-amino-1-methyl-5-imidazolecarboxamide (IV) and diethyl carbonate. The preparation of IV from 1-methyl-4-nitro-5-imidazolecarboxamide was improved considerably in our hands by catalytic reduction with Raney nickel in absolute ethanol, as compared to the tin and hydrochloric acid reduction reported by Sarasin and Wegmann.[6] The corresponding compound, 4-amino-1-ethyl-2-methyl-5-imidazolecarboxamide hydrochloride, was prepared from 1-ethyl-2-methyl-4-nitro-5-imidazolecarboxamide[7] in a similar manner by reduction with Raney nickel catalyst.

4-Amino-1-methyl-5-imidazolecarboxamide (IV) was isolated as the hydrochloride, and this product when heated with formamide gave 7-methylhypoxanthine (V) in 50–60% yield. 1-Methyl-4-nitro-5-imidazolecarbonitrile[5] (I) was reduced catalytically to 4-amino-1-methyl-5-imidazolecarbonitrile (II) in 86% yield. Montequi[8] reported this reduction with tin and hydrochloric acid. When 4-amino-1-methyl-5-imidazolecarbonitrile (II) was heated with boiling formamide, 7-methyladenine (III) resulted. This type of purine ring closure was first accomplished in the preparation of 4-aminopyrazolo[3,4-d]pyrimidine[9] and has recently been employed for the synthesis of 4-aminopyrimido[4,5-b]quinoline.[10]

(6) J. Sarasin and E. Wegmann, *Helv. Chim. Acta*, **7**, 713 (1924).
(7) F. Montequi, *Anales. Soc. españ. fis. quim.*, **24**, 731 (1926).
(8) F. Montequi, *ibid.*, **25**, 182 (1927).
(9) (a) R. K. Robins, THIS JOURNAL, **78**, 784 (1956); (b) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **21**, 1248 (1956).
(10) E. C. Taylor, Jr., and N. W. Kalenda, THIS JOURNAL, **78**, 5108 (1956).

Chlorination of 7-methylhypoxanthine (V) with phosphorus oxychloride gave 6-chloro-7-methylpurine (VI) in above 90% yield. In contrast to the chlorination of hypoxanthine,[11] no tertiary amine was needed for this chlorination. It is interesting to note that when VI was isolated by direct chloroform extraction from the acidic solution which resulted from the phosphorus oxychloride mixture, only 20% yield was obtained. When this acidic solution was first made basic and then acidified and extracted with chloroform, the yield was raised to 90%. This would seem to suggest some type of complex formation which is destroyed by base. A similar situation has been noted in the preparation of 6-chloropurine.[11]

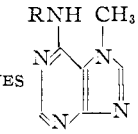
6-Chloro-7-methylpurine (VI) behaves toward nucleophilic attack in much the same manner as 6-chloro-9-methylpurine.[3] 7-Methyl-6-purinethiol[12] (VII) was prepared from VI and thiourea. The synthesis of VII was also accomplished from 7-methylhypoxanthine (V) and phosphorus pentasulfide in pyridine solution.

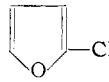
Alkylation of 7-methyl-6-purinethiol (VII) with ethyl iodide gave 6-ethylthio-7-methylpurine. Another new route to the preparation of 7-methyladenine[5b] was by the treatment of 6-chloro-7-methylpurine (VI) with alcoholic ammonia at 160°. Reaction of VI with various primary and secondary amines in alcoholic solution heated on the steam-bath resulted in the preparation of the 7-methyl-6-substituted-aminopurines (XII) listed in Table 1. 6-Dimethylamino-7-methylpurine (IX)

(11) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954).
(12) E. Fischer, *Ber.*, **31**, 431 (1898).

TABLE I

7-METHYL-6-SUBSTITUTED AMINOPURINES

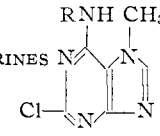


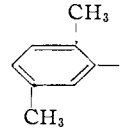
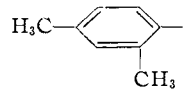
R	Empirical formula	M.p., °C.	Yield, %	Method	Recrystn. solvents	Analyses, % Calcd. C	Calcd. H	Found C	Found H
CH_3–	$C_7H_9N_5$	300	64	B	Methanol	51.5	5.5	51.2	5.6
$CH_3CH_2CH_2$–	$C_9H_{13}N_5$	178	50	B	Toluene + heptane	56.5	6.8	56.3	6.4
	$C_{11}H_{11}N_5O$	214–215	92	C	Toluene + methanol	30.6[b]		30.6[b]	
p-BrC_6H_4	$C_{12}H_{10}N_5Br$	213	60	A	Water	47.4	3.3	47.4	3.4
H_2N–	$C_6H_8N_6$	243	81	A	Water	43.9	4.9	43.9	4.9
p-ClC_6H_4	$C_{12}H_{10}N_5Cl\cdot HCl$	230	65	B	Water	48.6[a]	3.7	48.6	3.9

[a] Calcd. for HCl salt. [b] Nitrogen, %.

TABLE II

2-CHLORO-7-METHYL-6-SUBSTITUTED AMINOPURINES



R	Empirical formula	M.p., °C.	Yield, %	U.v. absorption alcohol λmax	U.v. absorption alcohol *E*	Recrystn. solvents	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
	$C_{14}H_{14}N_5Cl$	230	70	290	15,400	Methanol + water			24.3			24.6
	$C_{14}H_{14}N_5Cl$	258–260	56	287	16,700	Ethanol	58.4	4.9	24.3	58.4	4.9	24.4
m-Cl–C_6H_4–	$C_{12}H_9N_5Cl_2$	219	53	306	20,000	Ethanol	49.0	3.1	23.8	49.2	3.2	23.8

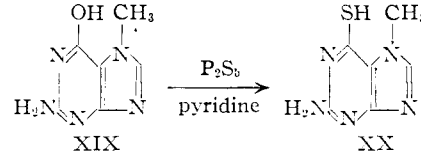
previously has been prepared by Baker, Schaub and Joseph[13] by methylation of 6-dimethylamino-2,8-bis-(methylthio)-purine followed by separation of the isomeric 7- and 9-derivatives and finally desulfurizing the 6-dimethylamino-7-methyl-2,8-bis-(methylthio)-purine with Raney nickel. Comparison of melting point data would indicate that 6-dimethylamino-7-methylpurine (IX) obtained from VI to be identical to that described by Baker, *et al.*[13]

Sodium methoxide and various sodium phenoxides reacted with VI to give the corresponding ethers XI.

Urea fusion of the hydrochloride salt of 4-amino-1-methyl-5-imidazolecarboxamide (IV) gave 7-methylxanthine[6] (XIII) in 64% yield. 7-Methylxanthine was treated with phosphorus oxychloride and phosphorus pentachloride to give 2,6-dichloro-7-methylpurine (XIV). The preparation of XIV previously has been reported by Fischer[5a] by treatment of theobromine with phosphorus oxychloride in a sealed tube at 140°. The treatment of 2,6-dichloro-7-methylpurine (XIV) with various amines under mild conditions has previously been reported to yield the 2-chloro-7-methyl-6-substituted amino derivatives.[5b,14]

Several new 2-chloro-7-methyl-6-substituted aminopurines were prepared by the reaction of XIV and the corresponding amines in ethanolic solution. These compounds are listed in Table II. It is of interest to note that 2,6-dichloro-7-methylpurine (XIV) and ethanolic ammonia warmed gently on the steam-bath yielded 6-amino-2-chloro-7-methylpurine. The sealed tube ammoniation previously employed[5b,14] for the synthesis of this compound is unnecessary. 2-Amino-7-methyl-6-purinethiol (XX) was prepared by thiation of 7-methylguanine (XIX)[5a,15] in pyridine with phosphorus pentasulfide.

7-Methylxanthine (XIII) under similar conditions gave 2-hydroxy-7-methyl-6-purinethiol (XV).



XIX XX

This reaction is similar to that reported for xanthine.[16] The structure of XV was established since this compound was found to be identical to 2-hydroxy-7-methyl-6-purinethiol prepared by Fischer[12] in several steps from XIV. The isomeric 6-hydroxy-7-methyl-2-purinethiol (XVI) was pre-

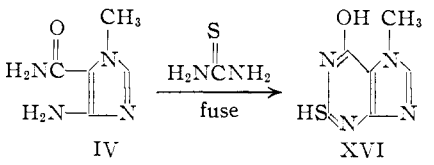
(13) B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).

(14) R. R. Adams and F. C. Whitmore, THIS JOURNAL, **67**, 1271 (1945).

(15) E. Fischer, *Ber.*, **31**, 542 (1898).

(16) A. G. Beaman, THIS JOURNAL, **76**, 5633 (1954).

pared from 2-chloro-6-hydroxy-7-methylpurine[5a] and thiourea in boiling ethanol. Fusion of thiourea and the hydrochloride of 4-amino-1-methyl-5-imidazolecarboxamide (IV) also provided another method for the synthesis of XVI.

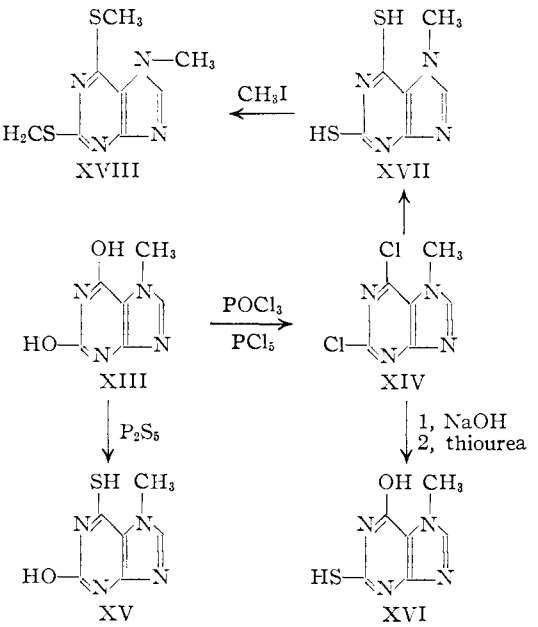


IV XVI

Reaction of XIV and thiourea in boiling ethanol gave 7-methyl-2,6-purinedithiol[12] (XVII). Methylation of XVII with methyl iodide yielded 7-methyl-2,6-bis-(methylthio)-purine (XVIII).

Although ethanolic ammonia at 160° in a bomb converted 6-chloro-7-methylpurine (VI) to 7-methyladenine (III), when the same concentration of ethanolic ammonia was heated with VI on the steam-bath, a completely different product (VIII), $C_{12}H_{11}N_9 \cdot HCl$, was obtained. This was rather unexpected since 6-chloro-9-methylpurine[4] has been recovered unchanged in this Laboratory when heated on the steam-bath with ethanolic ammonia under similar conditions. It would appear that

REACTION SCHEME II



VI under these conditions is partially changed to 7-methyladenine (III) which then reacts further with 6-chloro-7-methylpurine (VI) to give compound VIII, 7-methyl-6-(7-methyl-6-purinyl)-aminopurine. The ultraviolet absorption spectrum of VIII shows rather strong absorption at 354 mμ at *p*H of 1 which suggests a rather strongly absorbing group attached to the 6-position. Further evidence for the structure of VIII is obtained from the infrared spectrum run in pyridine solution. A strong single band was found at 3330 cm.$^{-1}$ which is strongly indicative of the presence of a free –NH– group and the absence of a primary amino-group in the molecule since primary amines generally give rise to two absorption bands in this region. Diphenylamine, a homocyclic model for VIII, shows a free –NH– absorption[17] at 3430 cm.$^{-1}$. The lowering of the –NH– frequency by approximately 100 cm.$^{-1}$ in VIII can be attributed to hydrogen bonding with the pyridine solvent molecule. The ultraviolet absorption spectra of some of the 7-methyl-6-substituted purines are listed in Table III.

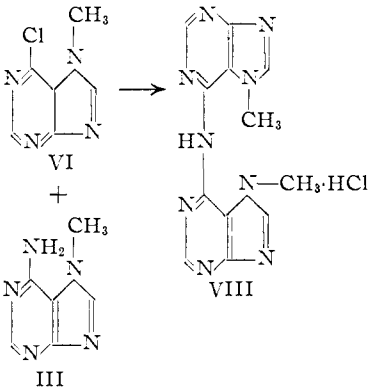
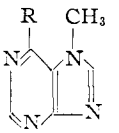


TABLE III

THE ULTRAVIOLET ABSORPTION MAXIMA OF SOME 7-METHYL-6-SUBSTITUTED PURINES



R	Ultraviolet absorption maximum λ_{max}, mμ (*p*H 1)	*E*	λ_{max}, mμ (*p*H 1)	*E*	λ_{max}, mμ (Ethanol)	*E*
Cl	268	8,422	271	8,459		
OH	249	12,000	261	10,620	257	9,000
					259	7,500
SH	333	20,300	319	21,800		
SCH_3	225	9,200	294	16,300		
	301	14,500				
SC_2H_5	225	9,700	297	15,660		
	303	15,600				
OCH_3	256	11,600	258	9,600		
NH_2	272	15,050	270	10,550		
$NH-NH_2$	272	12,300	263	7,700		
$NH-CH_3$	280	16,000	275	14,300	276	18,600
$NHCH_2CH_2CH_3$	281	19,900	277	16,000	277	16,000
	283	18,500	274	16,200	275	16,000
p-Br-C_6H_4-NH	293	20,060	282	27,060		
p-Cl-C_6H_4-NH	290	16,880	290	18,200		
p-Br-C_6H_4-O					259	14,000
p-Cl-C_6H_4-O					264	13,300

Acknowledgment.—The authors wish to thank Dr. Brian M. Lynch for his helpful infrared studies of 7-methyl-6-(7-methyl-6-purinyl)-aminopurine (VIII).

Experimental[18]

4-Amino-1-methyl-5-imidazolecarbonitrile (II).—1-Methyl-4-nitro-5-imidazolecarbonitrile[6] (10 g.) was mixed with approximately 8 g. of wet Raney nickel (the nickel catalyst used in all the experiments described herein was Davison Sponge Nickel Catalyst obtained from The Denver Fire Clay Co., Denver 17, Colo.) in 200 ml. of absolute ethanol.

(17) R. E. Richards and W. R. Burton, *Trans. Faraday Soc.*, **45**, 874 (1949).

(18) All melting points are uncorrected and unless otherwise stated were taken on a Fisher–Johns melting point block.

The mixture was shaken at a pressure of 1.9 atmospheres for 3.5 hr. The solution was then filtered, and dry hydrogen chloride was passed into the ice-cooled solution. The crude yield of light-brown hydrochloride which appeared was 9 g., m.p. 249–256°. The ultraviolet absorption maxima were 233 mμ (*E* 5,104) and 266 mμ (*E* 8,932).

Anal. Calcd. for $C_5H_6N_4 \cdot HCl$: N, 35.3. Found: N, 35.4.

The free base was purified according to Montequi's[8] method. Light yellow-orange crystals were obtained, m.p. 179–180°.

4-Amino-1-methyl-5-imidazolecarboxamide (IV).—The reduction of 1-methyl-4-nitro-5-imidazolecarboxamide was accomplished in a manner similar to that for the preparation of II and was obtained in 90% yield after recrystallization from absolute ethanol to yield crystals of m.p. 197–198°, as compared with that reported by Sarasin and Wegmann[6] of 184–185°.

4-Amino-1-methyl-5-imidazolecarboxamide hydrochloride also was made in the same manner as for II. An analytical sample was obtained by recrystallization from dilute ethanol to give crystals, m.p. 231–232°, reported[6] m.p. 214–215°.

Anal. Calcd. for $C_5H_8N_4O,HCl$: C, 34.0; H, 5.1; N, 31.7. Found: C, 34.5; H, 5.2; N, 31.7.

4-Amino-1-ethyl-2-methyl-5-imidazolecarboxamide Hydrochloride.—1-Ethyl-2-methyl-4-nitro-5-imidazolecarboxamide[7] (2 g.) was shaken with 3 g. of Raney nickel in 200 ml. of absolute ethanol at an initial pressure of 1.36 atmospheres/sq. cm. In 2 hr. absorption had ceased. The reaction mixture was then filtered, and dry hydrogen chloride gas was passed into the filtrate, cooled in ice. The white crystalline hydrochloride which separated out was filtered off and washed with ether to yield 1.3 g. A small amount was recrystallized from boiling 95% ethanol for analysis, m.p. 222–223°. Montequi[7] reports the melting point to be 195°.

Anal. Calcd. for $C_7H_{13}N_4OCl$: C, 41.1; H, 6.3. Found: C, 40.6; H, 6.5.

7-Methyladenine (III). Method 1.—4-Amino-1-methyl-5-imidazolecarbonitrile obtained after reducing 7.5 g. of the corresponding nitro derivative I was heated without further purification with 15 ml. of formamide for about 50 minutes on a hot-plate. The hot reaction product was then poured into an evaporating dish and left for 24 hr. The dark, crystalline residue, after having been washed with absolute ethanol, was recrystallized from boiling 95% ethanol to give 2.5 g. of white crystals, m.p. 344–346° dec. (copper block), reported[5b] m.p. 351°.

Anal. Calcd. for $C_6H_7N_5$: C, 48.3; H, 4.7; N, 47.0. Found: C, 48.3; H, 5.0; N, 47.5.

Method 2.—6-Chloro-7-methylpurine (1 g.) was put in a bomb with 50 ml. of ethanolic ammonia (prepared by saturating absolute ethanol with dry ammonia at 0°) and the solution heated at 160° for 8 hr. On cooling, the reaction mixture was filtered and the insoluble solid washed with ethanol. This was then recrystallized from boiling ethanol to give 0.8 g. of crystalline white solid, m.p. 344–345° dec. (copper block). Mixed m.p. with 7-methyladenine prepared by method 1 showed no depression. The ultraviolet absorption spectra of 7-methyladenine prepared by methods 1 and 2 were found to be identical at *p*H 1 and 11.

7-Methylhypoxanthine (V).—4-Amino-1-methyl-5-imidazolecarboxamide hydrochloride (15 g.) was heated with 25 ml. of C.P. formamide. The reaction mixture was allowed to boil freely for about 45 minutes until the volume was reduced to one-half. The solution was poured into a crystallizing dish and left to cool for 24 hr. The black, crystalline solid which separated out was triturated with cold, absolute ethanol, filtered and dissolved in a large volume of boiling ethanol. The solution was charcoaled, filtered and cooled to give 7 g. of white, fluffy, crystalline needles, m.p. 356–357° dec. (copper block), reported[5a] m.p. 355° dec.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.3. Found: C, 48.1; H, 4.2; N, 37.7.

6-Chloro-7-methylpurine (VI).—Dry, recrystallized 7-methylhypoxanthine (10 g.) was refluxed with 400 ml. of phosphorus oxychloride for 4 hr. By the end of this period a clear, light-brown solution resulted. The excess phosphorus oxychloride (about 370 ml.) was removed under reduced pressure using a steam-bath as a source of heat. The residual sirup was poured slowly, with stirring, onto a mixture of about 400 g. of crushed ice and 50 ml. of 30% sodium hydroxide solution. The final mixture had a *p*H of about 10. While the mixture was carefully cooled with ice, concentrated hydrochloric acid was added until the *p*H was 2. The light-brown, aqueous solution thus obtained was placed in a continuous extractor for extraction with about 600 ml. of chloroform. After 24 hr. the extraction was complete. The chloroform solution was washed several times with cold water and the organic layer dried overnight over anhydrous sodium sulfate. On removal of the solvent by distillation, 10 g. of a light-yellow solid melting at 195–197° was obtained. An analytically pure sample, m.p. 198–199°, was prepared by recrystallization from boiling toluene.

Anal. Calcd. for $C_6H_5N_4Cl$: C, 42.7; H, 3.0; N, 33.2. Found: C, 42.7; H, 3.0; N, 32.7.

7-Methyl-6-purinethiol (VII). Method 1.—7-Methylhypoxanthine (4 g.) was thoroughly mixed with 24 g. of phosphorus pentasulfide and added to 300 ml. of dry pyridine. The mixture was refluxed for 3 hr., during which time a deep-brown solution was obtained. At the end of the 3 hr. period pyridine was removed completely under reduced pressure using steam as the source of heat. The brown residue was mixed very slowly with cold water. When the initial reaction was over, the mixture containing 250 ml. of water was heated on the steam-bath for 3 hr., made alkaline and filtered. The filtrate was acidified with acetic acid and cooled. A light-brown, crystalline solid (3.6 g.) separated. An analytically pure sample, m.p. 306–308°, was obtained by recrystallization from boiling water; reported[12] m.p. 306–307°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.4; H, 3.6; N, 33.8.

Method 2.—A mixture of 1 g. of 6-chloro-7-methylpurine and 2 g. of thiourea was heated in 60 ml. of absolute ethanol under reflux for 4 hr. The solution was evaporated to dryness on the steam-bath and the residue dissolved in dilute potassium hydroxide. The solution was heated with charcoal and the filtrate acidified with acetic acid. Light-yellow crystals (0.6 g.) separated, m.p. 308–310°. The mixed melting point with VII of method 1 was 306–309°. The ultraviolet absorption spectra was identical with that of the product made by method 1.

2,6-Dichloro-7-methylpurine (XIV).—7-Methylxanthine (1.7 g.) was refluxed 5 hr. with a mixture of 75 ml. of phosphorus oxychloride and 5 g. of phosphorus pentachloride. The excess phosphorus oxychloride then was removed under reduced pressure. The residue was thoroughly cooled, and crushed ice was added to it. The aqueous solution was extracted with five 60-ml. portions of chloroform, and the extract was washed with water until free from acid. The dried chloroform solution was distilled and the residue extracted with boiling water. The solution yielded 0.5 g. of cream-colored needles, m.p. 199–201°.[5a] The mixed m.p. with the commercial sample (Cyclo Chemical Corporation, Los Angeles, Calif.) was 200–201°. The compound showed λ_{max} of 280 mμ at *p*H of 1 (*E* 8,100) and 11 (*E* 8,300).

2-Hydroxy-7-methyl-6-purinethiol (XV).—7-Methylxanthine (3 g.) was mixed with 15 g. of phosphorus pentasulfide and the mixture refluxed in 300 ml. of pyridine for about 5 hr. The pyridine was then removed under reduced pressure on the steam-bath, and the nearly dry residue was allowed to decompose slowly by the addition of 550 ml. of water. The mixture was then carefully heated on the steam-bath for 3 hr. and allowed to cool. The insoluble solid was dissolved in dilute, hot potassium hydroxide and the solution treated with charcoal, boiled and filtered. The hot filtrate on acidification with acetic acid gave a white precipitate which was finally purified by recrystallizing from 2-liters of boiling water. On cooling, 1.5 g. of a cream-colored solid was obtained, m.p. 343–344° dec. (copper block), reported[12] m.p. 343°. The compound showed λ_{max} of 244 mμ (*E* 4,900), 336 mμ (*E* 24,900) at *p*H of 1 and 250 mμ (*E* 5,800), 346 mμ (*E* 20,400) at *p*H of 11.

7-Methyl-2,6-purinedithiol[12] (XVII).—Five grams of 2,6-dichloro-7-methylpurine (purchased from Cyclo Chemical Corporation, Los Angeles, Calif.) was heated under reflux in 300 ml. of absolute ethanol with 6 g. of thiourea for nearly 7 hr. On cooling, the mixture was filtered and the yellow residue washed with ethanol. The residue was dissolved in warm, dilute sodium hydroxide and precipitated

by acetic acid. The process was repeated twice to give 4 g. of pure yellow powder, m.p. >300°. The compound showed λ_{max} of 258 mμ (*E* 15,400), 300 mμ (*E* 18,800), 350 mμ (*E* 13,700) at *p*H of 1 and 252 mμ (*E* 21,000), 286 mμ (*E* 19,000), 360 mμ (*E* 12,100) at *p*H of 11.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.0; N, 28.3. Found: C, 36.6; H, 3.4; N, 28.7.

7-Methyl-2,6-bis-(methylthio)-purine (XVIII).—The crude 7-methyl-2,6-purinedithiol (2 g.) was dissolved in 100 ml. of 4% aqueous potassium hydroxide and filtered. The filtrate was diluted with 50 ml. of ice-cold water and mixed with 10 g. of methyl iodide. The mixture was vigorously stirred for about 60 minutes, within which time the reaction was completed. The solid which had separated was filtered off and washed with ice-cold water to give 2 g. of XVIII. On recrystallization from a large volume of water, 1.5 g. of light-yellow needles was obtained, m.p. 176–178°. A sample was recrystallized from water for analysis to give ultraviolet absorption of λ_{max} 220 mμ (*E* 15,400), 263 mμ (*E* 19,000), 318 mμ (*E* 10,400) at *p*H of 1 and 240 mμ (*E* 23,000), 259 mμ (*E* 17,800), 317 mμ (*E* 10,400) at *p*H of 11.

Anal. Calcd. for $C_8H_{10}N_4S_2$: C, 42.5; H, 4.4. Found: C, 42.5; H, 4.7.

6-Hydroxy-7-methyl-2-purinethiol (XVI). Method 1.—2-Chloro-7-methyl-6-hydroxypurine[5a] (5 g.) was refluxed for a period of 15 hr. in 300 ml. of absolute ethanol containing 4 g. of thiourea. After this period the reaction mixture was cooled and the solid separated by filtration. It was purified by dissolving in warm, dilute caustic potash and reprecipitation with acetic acid. The process was repeated to give 4 g. of very light-yellow powder, m.p. > 360° (copper block). The compound showed λ_{max} of 233 mμ (*E* 15,500), 288 mμ (*E* 22,700) at *p*H of 1 and 275 mμ (*E* 19,000) at *p*H of 11.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.3; N, 30.8. Found: C, 39.8; H, 3.5; N, 31.0.

Method 2.—A powdered mixture of 8 g. of 4-amino-1-methyl-5-imidazolecarboxamide hydrochloride and 12 g. of thiourea was fused at 150° for 25 minutes. The cooled residue was dissolved in dilute potassium hydroxide and the solution boiled with a little charcoal and filtered. The filtrate, on acidification with acetic acid, gave 3 g. of a light-brown solid. Reprecipitation gave a pure product which showed ultraviolet spectra identical with XVI made by method 1.

7-Methyl-6-substituted Aminopurines Listed in Table I. Method A.—A solution of about 2 g. of 6-chloro-7-methylpurine (VI) dissolved in 200 ml. of absolute ethanol was mixed with a solution of about 2–3 g. of the amine in 30 ml. of ethanol. The mixed solution was heated gently on the steam-bath for approximately 6 hr. The substituted amino derivative separated from the hot solution. The solid was purified by recrystallization from the indicated solvent and filtered.

Method B.—Method B is the same as method A except that the reaction mixture was evaporated to dryness on the steam-bath, and the substituted amino derivative was isolated from the pasty or dry solid by crystallization from appropriate solvents.

Method C.—Method C is the same as the others but for the isolation of the substituted amino derivative. The pasty reaction mixture after evaporation on the steam-bath, was boiled with a small amount of solid potassium hydroxide pellets, methanol and benzene and the solution filtered and cooled.

2-Chloro-7-methyl-6-substituted Aminopurines Listed in Table II.—The preparation of these compounds is illustrated by the preparation of 2-chloro-7-methyl-6-(2,4-dimethylanilino)-purine.

2,6-Dichloro-7-methylpurine (4 g.) was mixed with 3 g. of 2,4-dimethylaniline in 200 ml. of absolute ethanol. The mixture was heated on a steam-bath until a dry residue was left. This residue was treated several times with ether and the washings decanted. The white residue remaining was dissolved in boiling ethanol. The brownish solution was heated with charcoal and filtered, and the filtrate yielded 1.3 g. of silky needles, m.p. 257.5–258.5°. A further amount of 1.2 g. of the compound was isolated by concentration of the filtrate. Recrystallization of a small amount of sample from absolute ethanol raised the melting point to 258–260°.

Anal. Calcd. for $C_{14}H_{14}N_5Cl$: C, 58.4; H, 4.9; N, 24.4. Found: C, 58.4; H, 4.9; N, 24.4.

The compounds listed in Table II were prepared in a similar manner.

6-Methoxy-7-methylpurine.—6-Chloro-7-methylpurine (2 g) was added to a solution of 2 g. of sodium dissolved in 200 ml. of absolute methanol. The mixture was heated on the steam-bath for 30 minutes and the solution filtered hot. The filtrate, on cooling overnight, deposited long, white needles, m.p. 195°. The solid was recrystallized from benzene–heptane to give 1.0 g. of pure 6-methoxy-7-methylpurine, m.p. 200°.

Anal. Calcd. for $C_7H_8N_4O$: C, 51.2; H, 4.9. Found: C, 51.5; H, 4.6.

6-*p*-Bromophenoxy-7-methylpurine.—One and eight-tenths g. of the crude chloro derivative VI was added to a solution of 2 g. of *p*-bromophenol in 80 ml. of potassium hydroxide solution (containing 2 g. of solid potassium hydroxide in 80 ml. of water). The mixture was stirred for 30 minutes until all the 6-chloro-7-methylpurine dissolved. The solution was heated on the steam-bath for 20 minutes then cooled and filtered. The white product was washed with a little water and recrystallized from boiling aqueous ethanol to give a m.p. of 229–231°. A second recrystallization gave 1.6 g. of long, silky, white threads, m.p. 229–229.5°, with a λ_{max} of 264 mμ in ethanol.

Anal. Calcd. for $C_{12}H_9N_4OBr$: C, 47.2; H, 3.0. Found: C, 47.2; H, 3.3.

6-*p*-Chlorophenoxy-7-methylpurine, m.p. 198°, was prepared in a similar manner.

Anal. Calcd. for $C_{12}H_9N_4OCl$: C, 55.3; H, 3.4. Found: C, 55.3; H, 3.3.

6-Ethylthio-7-methylpurine.—6-Ethylthio-7-methylpurine was prepared from 1.7 g. of 7-methyl-6-purinethiol in a manner similar to that employed by Fischer for the preparation of 7-methyl-6-methylthiopurine.[12] The yield of crude 6-ethylthio-9-methylpurine was 1.2 g., m.p. 157.5°. On recrystallization from hot water, long, white needles were obtained, m.p. 158.5°.

Anal. Calcd. for $C_8H_{10}N_4S$: C, 49.5; H, 5.2; N, 28.9. Found: C, 50.0; H, 5.2; N, 29.0.

2-Amino-7-methyl-6-purinethiol.—A finely powdered mixture of 3 g. of 7-methylguanine[15] and 20 g. of phosphorus pentasulfide was refluxed in 300 ml. of redistilled pyridine for 10 hr. The pyridine was then distilled off at reduced pressure and water added to the residue. When the initial reaction had subsided, the mixture containing about 300 ml. of water was refluxed for 6 hr. and then filtered after cooling. The residue was dissolved in hot potassium hydroxide solution and precipitated by acetic acid. The process of solution and reprecipitation was repeated twice to give 1.5 g. of cream-colored solid, m.p. >300°, λ_{max} at 250 mμ (*E* 11,400), 350 mμ (*E* 21,000) at *p*H of 1 and 277 mμ (*E* 7,300), 327 mμ (*E* 1,300) at *p*H of 11.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.8; H, 3.9. Found: C, 40.2; H, 4.0.

Reaction of 6-Chloro-7-methylpurine with Ethanolic Ammonia to Yield 7-Methyl-6-(7-methyl-6-purinyl)-aminopurine (VIII).—Three grams of VI was heated with 50 ml. of ethanolic ammonia (saturated at 0°) on a steam-bath for 1 hr. At the end of this period, a further amount of ethanolic ammonia (50 ml.) was added and the solution heated for another 2 hr. The mixture was then cooled and filtered and the precipitate washed with ethanol. A yellow, granular substance was left, which was recrystallized from boiling water to give 2.6 g. of very fine, bright-yellow needles. The compound began turning brown at 265° and melted at 280–282°. It gave a positive test for ionizable halogen and showed ultraviolet absorption of λ_{max} 280 mμ (*E* 26,985), 340 mμ (*E* 34,000), 354 mμ (38,400) at *p*H of 1 and 230 mμ (*E* 23,500), 345 mμ (*E* 20,600) at *p*H of 11.

Anal. Calcd. for $C_{12}H_{11}N_9 \cdot HCl$: C, 45.4; H, 3.8. Found: C, 45.5; H, 3.9.

Reaction of 6-Chloro-7-methylpurine (VI) and 7-Methyladenine (III) to give 7-Methyl-6-(7-methyl-6-purinyl)-aminopurine (VIII).—To 200 ml. of absolute ethanol was added 0.5 g. of 7-methyladenine (III) and 0.7 g. of 6-chloro-7-methylpurine (VI). The solution was refluxed for 48 hr. The alcohol was then evaporated to dryness and the residue

recrystallized from 50 ml. water to yield 0.2 g. of yellow needles, m.p. 280–283°. Mixed melting point and ultraviolet absorption data indicated this product to be identical with that obtained from 6-chloro-7-methylpurine and alcoholic ammonia solution when heated on the steam-bath.

TEMPE, ARIZONA

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

Potential Purine Antagonists. IX. Further Studies of Some 4,6-Disubstituted Pyrazolo[3,4-d]pyrimidines[1]

BY ROLAND K. ROBINS[2]

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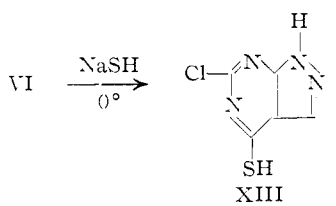
A number of new 4,6-disubstituted pyrazolo[3,4-d]pyrimidines have been prepared. The successful chlorination of 4,6-dihydroxypyrazolo[3,4-d]pyrimidine (V) to give 4,6-dichloropyrazolo[3,4-d]pyrimidine (VI) has provided VI as an intermediate for the preparation of a large number of pyrazolo[3,4-d]pyrimidines previously unreported. Selective replacement of the chlorine atoms of VI under carefully controlled conditions yields the 4-substituted-6-chloropyrazolo[3,4-d]-pyrimidine. A preliminary study has been made with regard to the ease of nucleophilic displacement of various groups in the 4- and 6-positions of the pyrazolo[3,4-d]pyrimidine ring.

The anti-tumor activity[3,4] of several derivatives of the pyrazolo[3,4-d]pyrimidine ring system has prompted a more thorough investigation of these compounds. Although preliminary attempts[5] to convert 4,6-dihydroxypyrazolo[3,4-d]pyrimidine (V) to 4,6-dichloropyrazolo[3,4-d]pyrimidine (VI) were unsuccessful, it has now been discovered that N,N-diethylaniline and phosphorus oxychloride convert 4,6-dihydroxypyrazolo[3,4-d]pyrimidine (V) to VI in 60–70% yield. 4,6-Dichloropyrazolo-[3,4-d]pyrimidine (VI) has proved to be a very useful intermediate in the synthesis of a large number of otherwise inaccessible 4,6-disubstituted derivatives in this series. When VI was treated with the usual nucleophilic reagents under relatively mild conditions, the corresponding 4-substituted-6-chloropyrazolo[3,4-d]pyrimidine was obtained. Thus VI and dilute sodium hydroxide gave 4-hydroxy-6-chloropyrazolo[3,4-d]pyrimidine (VII). The structure of VII was established by refluxing VII with thiourea in ethanol to give 4-hydroxy-6-mercaptopyrazolo(3,4-d)pyrimidine which has previously been prepared[5] by fusion of thiourea and 3-amino-4-pyrazolecarboxamide. 4,6-Dichloropyrazolo(3,4-d)pyrimidine (VI) when treated with sodium hydrosulfide (0.5 *N*) at 0° gave 4-mercapto-6-chloropyrazolo(3,4-d)pyrimidine (XIII). It was evident by inspection of the ultraviolet absorption



XIII

spectrum of XIII that the mercapto group was in position "4" since 4-mercaptopyrazolo(3,4-d)pyrimidine[5] shows an absorption maximum at *p*H of 1, 321 mμ and at *p*H of 11, 314 mμ. The spectrum of XIII shows an absorption maximum at *p*H of 1, 325 mμ and at *p*H of 11, 315 mμ. In all respects the spectra are remarkably similar. The structure of XIII was further established by methylation with methyl iodide to give 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III). The unambiguous synthesis of III was accomplished by chlorination of 6-hydroxy-4-methylmercaptopyrazolo(3,4-d)pyrimidine (II) with dimethylaniline and phosphorus oxychloride. 4-Methylmercapto-6-hydroxypyrazolo(3,4-d)pyrimidine (II) was prepared by careful methylation of 4-mercapto-6-hydroxypyrazolo(3,4-d)pyrimidine (I).[5]

4-Methylmercapto-6-chloropyrazolo(3,4-d)-pyrimidine (III) was also prepared by treatment of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) with potassium methylmercaptide in methanol at 0°. Similarly VI and sodium ethylmercaptide gave 4-ethylmercapto-6-chloropyrazolo(3,4-d)pyrimidine. Another example of the selective replacement of the "4"-chloro atom was the reaction of cold sodium methoxide or cold sodium ethoxide with VI to give 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine (X) or 4-ethoxy-6-chloropyrazolo(3,4-d)pyrimidine, respectively.

The structure assigned X was established by treatment of X with aqueous methylamine which yielded 4-methylamino-6-chloropyrazolo(3,4-d)-pyrimidine (XIV). 4-Methylamino-6-chloropyrazolo(3,4-d)pyrimidine (XIV) was also readily prepared from 4-methylmercapto-6-chloropyrazolo(3,-4-d)pyrimidine (III). Since the structure of III was established by independent synthesis, it follows that XIV must be 4-methylamino-6-chloropyrazolo(3,4-d)pyrimidine.

Treatment of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) with various amines followed the expected course. Treatment of VI with an aqueous or alcoholic solution of a primary or secondary amine, heated briefly on the steam-bath, resulted in the preparation of the corresponding 4-substituted amino-6-chloropyrazolo(3,4-d)pyrimidine. For a number of these derivatives, see Table I. The assignment of the position of the substituted amino group in these compounds was made on the

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(2) Department of Chemistry, Arizona State College, Tempe, Arizona.

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