[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, ARIZONA STATE COLLEGE]

Potential Purine Antagonists. XV. Preparation of Some 6,8-Disubstituted Purines[1]

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RECEIVED JUNE 2, 1958

A number of new 6,8-disubstituted purines have been prepared. 6,8-Dihydroxypurine (VI) has been chlorinated with phosphorus oxychloride to yield 6,8-dichloropurine (VII). 6-Hydroxy-8-aminopurine (I) has been prepared by fusion of 4,5-diamino-6-hydroxypyrimidine (II) with guanidine. This represents a new method of introducing the 8-amino group into the purine ring. The preparation of 8-chloro-6-methylthiopurine (XII) and 6-chloro-8-methylthiopurine (VIII) provided useful intermediates for the preparation of a number of interesting purines with substituents in positions 6 and 8.

Prior to the present work relatively few purines have been reported possessing substituents at positions 6 and 8. Fischer and Ach[2] reported the preparation of 6-amino-8-hydroxypurine (XXIV) from the hydrogen iodide reduction of 6-amino-2-chloro-8-hydroxypurine. These authors also reported preparation of 6,8-dihydroxypurine (VI)[2] from the reaction of 6-amino-8-hydroxypurine (XXIV) and nitrous acid. Cavalieri and Bendich[3] improved the preparation of XXIV by treating 4,5,6-triaminopyrimidine (XXV) with phosgene to obtain 6-amino-8-hydroxypurine. Similarly, these investigators obtained 6,8-dihydroxypurine from 4,5-diamino-6-hydroxypyrimidine (II) and phosgene.

More recently Albert and Brown[4] have improved the preparation of 6,8-dihydroxypurine (VI) by urea fusion of 4,5-diamino-6-hydroxypyrimidine (II).

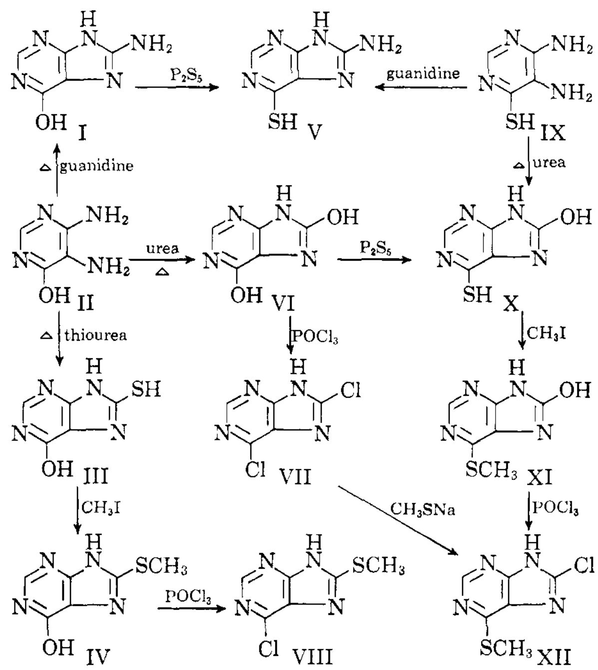
8-Bromo-6-aminopurine has been prepared by the bromination of adenine[5–7] which is reported to give 8-bromo-6-hydroxypurine[5] when treated with nitrous acid. Gabriel and Colman[8] have reported the preparation of 6-methyl-8-hydroxypurine by the urea fusion of 6-methyl-4,5-diaminopyrimidine.

In view of the limited work previously reported it was decided to extend previous efforts and to make a general study of the preparation and properties of 6,8-disubstituted purines.

Chlorination of 6,8-dihydroxypurine (VI) to give 6,8-dichloropurine (VII) was successfully accomplished with phosphorus oxychloride and N,N-diethylaniline. This method recently proved successful in the preparation of the isomeric 4,6-dichloropyrazolo[3,4-d]pyrimidine.[9]

Thiourea fusion of 4,5-diamino-6-hydroxypyrimidine (II)[10] gave 6-hydroxy-8-purinethiol (III) in good yield. The isomeric 8-hydroxy-6-purinethiol (X) was prepared from urea fusion of 4,5-diamino-6-pyrimidinethiol.[11] 8-Hydroxy-6-purinethiol (X) was also prepared when 6,8-dihydroxypurine was treated with phosphorus pentasulfide in boiling pyridine. This reaction is not unexpected since Beaman[12] reported the preparation of 2-hydroxy-6-purinethiol from the isomeric 2,6-dihydroxypurine. Careful methylation of 6-hydroxy-8-purinethiol (III) with methyl iodide gave 6-hydroxy-8-methylthiopurine (IV) in good yield. Treatment of 6-hydroxy-8-methylthiopurine (IV) with phosphorus oxychloride and N,N-diethylaniline gave 6-chloro-8-methylthiopurine (VIII) in excellent yield. The isomeric 8-chloro-6-methylthiopurine (XII) was obtained by treatment of 6,8-dichloropurine (VII) with a basic solution of methanethiol heated on the steam-bath. 8-Chloro-6-methylthiopurine was also prepared in a stepwise fashion by chlorination of 8-hydroxy-6-methylthiopurine with phosphorus oxychloride. 8-Hydroxy-6-methylthiopurine (XI) was in turn prepared from 8-hydroxy-6-purinethiol (X) by methylation with methyl iodide. Thiourea in boiling ethanol converted 8-chloro-6-methylthiopurine (XII) to 6-methylthio-8-purinethiol (XXXV).

REACTION SCHEME I

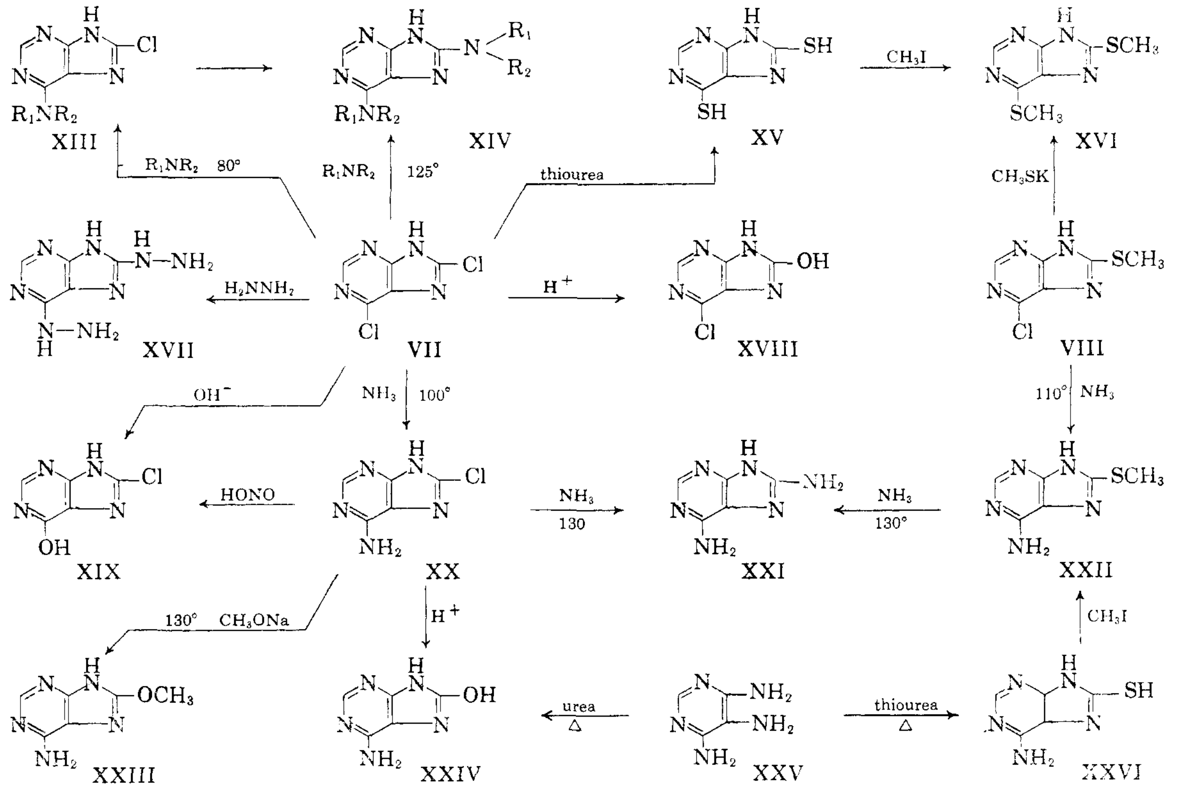


For the preparation of 8-amino-6-hydroxypurine (I) the possibility of introducing an 8-amino group by coupling hypoxanthine with a diazonium salt

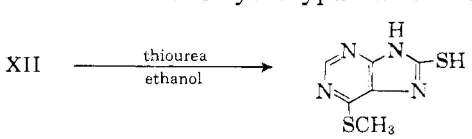
(1) This work was supported in part by Contract SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Institutes of Health, Public Health Service. Presented in part at the 133rd Meeting of the American Chemical Society, Division of Organic Chemistry, April 18, 1958, at San Francisco, Calif.

(2) E. Fischer and L. Ach, *Ber.*, **30**, 2208 (1897).
(3) L. F. Cavalieri and A. Bendich, THIS JOURNAL, **72**, 2593 (1950).
(4) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).
(5) M. Kruger, *Z. physiol. Chem.*, **16**, 5 (1892).
(6) M. Kruger, *ibid.*, **18**, 446 (1894).
(7) Further studies of the reactions of 8-bromo-6-aminopurine have recently been reported by R. M. Burgison at the 133rd Meeting of the American Chemical Society, see Abstracts of the Meeting., page 14-M.
(8) S. Gabriel and J. Colman, *Ber.*, **34**, 1247 (1901).
(9) R. K. Robins, THIS JOURNAL, **79**, 6407 (1957).
(10) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughn, Jr., *ibid.*, **67**, 290 (1945).
(11) G. B. Elion and G. H. Hitchings, *ibid.*, **76**, 4027 (1954).
(12) A. G. Beaman, *ibid.*, **76**, 5633 (1954).

REACTION SCHEME II



was investigated. Burian[13] reports the preparation of "diazobenzenesulfonic acid hypoxanthine" by coupling diazotized sulfanilic acid with hypoxanthine. Attempts to repeat this work in our laboratory were unsuccessful. Cavalieri and Bendich[3] report that 8-amino-6-hydroxypurine and 6,8-di-



XXXV

aminopurine could not be prepared by coupling of 2,4-dichlorobenzenediazonium chloride with adenine or hypoxanthine followed by the usual sodium hydrosulfite reduction to introduce the 8-amino group. This reaction however is quite successful for the introduction of the 8-amino group into xanthine,[3] guanine[3] and isoguanine.[14]

In the search for a new method of preparing 6-substituted-8-aminopurines it was discovered the fusion of guanidine (free base) with 4,5-diamino-6-hydroxypyrimidine (II) at 200° gave a good yield of 8-amino-6-hydroxypurine (I).

The treatment of I with phosphorus pentasulfide in pyridine gave 8-amino-6-purinethiol (V). The latter compound was also prepared from guanidine fusion of 4,5-diamino-6-pyrimidinethiol (IX). The method of introducing an 8-amino group by fusion of a 4,5-diaminopyrimidine is at present under further investigation to determine the generality of this reaction. The preparation of 6,8-diaminopurine (XXI) was accomplished readily by treatment of 6,8-dichloropurine (VII) with aqueous ammonia at 135°. Similarly, 6-amino-8-methylthiopurine (XXII) with aqueous ammonia at 160° gave 6,8-diaminopurine (XXI) in good yield. The preparation of 6-amino-8-methylthiopurine (XXII) was accomplished from 6-chloro-8-methylthiopurine (VIII) and aqueous ammonia at 110°. Also, methylation of 6-amino-8-purinethiol (XXVI) with methyl iodide provided another route to 6-amino-8-methylthiopurine (XXII).

6-Amino-8-purinethiol (XXVI) was readily prepared by the fusion of 4,5,6-triaminopyrimidine (XXV) with thiourea. Similarly, urea fusion of 4,5,6-triaminopyrimidine (XXV) provided a new route to the preparation of 6-amino-8-hydroxypurine (XXIV) which would appear superior to previously reported methods of synthesis.[2,3]

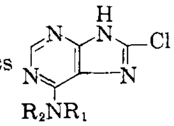
When 6,8-dichloropurine (VII) was heated with concentrated aqueous ammonia at 100°, 6-amino-8-chloropurine (XX) was prepared in excellent yield. The structure of XX was established by hydrolysis of the 8-chlorine atom with concentrated hydrochloric acid to give 6-amino-8-hydroxypurine (XXIV).

When 6-amino-8-chloropurine (XX) was treated with sodium methoxide in methanol at 130°, 6-amino-8-methoxypurine (XXIII) was prepared. Hot nitrous acid converted 6-amino-8-chloropurine (XX) to 8-chloro-6-hydroxypurine (XIX). The latter compound, XIX, was also prepared by treatment of 6,8-dichloropurine in refluxing 4 *N* sodium hydroxide. The isomeric 6-chloro-8-hydroxypurine (XVIII) was prepared from 6,8-dichloropurine by acid hydrolysis with concentrated hydrochloric

(13) R. Burian, *Ber.*, **37**, 705 (1904).

(14) J. R. Spies and T. H. Harris, Jr., THIS JOURNAL, **61**, 351 (1939).

TABLE I

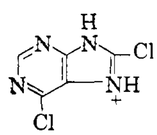
6-ALKYLAMINO-8-CHLOROPURINES 

R_1	R_2	M.p., °C.[a]	Yield, %	Analyses, % C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
CH_3	H	300	88	39.3	39.4	3.3	3.3	38.1	38.0
CH_3	CH_3	264–266	50					35.4	35.0
C_2H_5	H	276–278 d.	50	42.5	42.8	4.1	4.1	35.4	35.4
C_3H_7	H	290–292	22	45.5	45.9	4.7	4.8	33.1	33.2

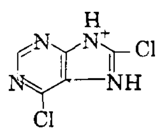
[a] Recrystallization solvent, N,N-dimethylformamide–water.

acid heated on the steam-bath. The structures assigned XVIII and XIX were further confirmed by comparison of the ultraviolet absorption spectra of these compounds with those of the known 8-hydroxy-[15] and 6-hydroxypurine[15] since Mason[15] has shown there is little shift in the wave length maximum due to a chlorine atom in the purine molecule. It is quite interesting that the usual nucleophilic reagents react with 6,8-dichloropurine to give replacement of the 6-chlorine atom preferentially, but treatment with strong acid gives rise to replacement of the 8-chloro group. Similarly, this observation was made by Fischer with 2,6,8-trichloropurine.[16] 2,6,8-Trichloropurine and strong acid gives 2,6-dichloro-8-hydroxypurine[16] while treatment with aqueous potassium hydroxide at 100° yields 2,8-dichloro-6-hydroxypurine.[17]

It would appear that strong acid increases the susceptibility of the 8-chlorine atom toward nucleophilic attack. This phenomenon could be explained if one assumes that in strong acid solution protonation takes place as indicated in formulas XXXVI a and b. The positive charge shown in



XXXVIa



XXXVIb

XXXVIa can become stabilized by being distributed equally over positions 7 and 9 because of resonance with the pyrimidine ring. Such stabilization of a positive charge on position 1 brought about by protonation is impossible. Thus, the electron density at position 8 is lowered, and the susceptibility to an attacking nucleophilic hydroxyl ion becomes greater at position 8 than at position 6.

When 6,8-dichloropurine (VII) was treated with an aqueous solution of an aliphatic primary or secondary amine, heated on the steam-bath, the corresponding 6-alkylamino-8-chloropurine (XIII) was obtained. The structure assigned XIII is based on the fact that the ultraviolet absorption spectra of these derivatives resemble closely those of the corresponding 6-alkylaminopurines.[15,18] For example, the absorption maximum of 8-chloro-6-dimethylaminopurine (XIII, R_1, R_2 = CH_3) is 275 mμ at *p*H 1 and 281 mμ at *p*H 11. 6-Dimethylaminopurine[15] exhibits a maximum of 276 mμ at *p*H 1.7 and 281 mμ at *p*H 13 as compared to 8-dimethylaminopurine[15] which exhibits a maximum at 305 mμ in acid and 306 mμ in basic solution.

Further evidence was obtained when 6-methylamino-8-chloropurine (XXX) was refluxed with concentrated hydrochloric acid to give 8-hydroxy-6-methylaminopurine (XXVII). The ultraviolet absorption spectra of XXVII resembled very closely that of 6-amino-8-hydroxypurine (XXIV) as opposed to that of 8-amino-6-hydroxypurine (I). At *p*H 1, 8-amino-6-hydroxypurine exhibits an absorption maximum at 254 mμ, as compared to approximately 278 mμ for 8-hydroxy-6-methylaminopurine (XXVII) and 279 mμ for 6-amino-8-hydroxypurine (XXIV) at the same *p*H. Similarly, 8-chloro-6-methylaminopurine (XXX) and sodium hydrosulfide heated to 125° gave 6-methylamino-8-purinethiol. The latter compound exhibited an ultraviolet spectrum similar to that of 6-amino-8-purinethiol (XXVI) rather than of 8-amino-6-purinethiol (V), thus providing additional evidence for the structure assigned 8-chloro-6-methylaminopurine (XXX). The 6-alkylamino-8-chloropurines (XIII) thus prepared are listed in Table I. When 6,8-dichloropurine (VII) was heated with aqueous alkylamines at 125° in a bomb, the 6,8-bis-alkylaminopurines (XIV) were formed. These compounds are listed in Table II. It is interesting to note that prolonged treatment with aqueous hydrazine on the steam-bath provided 6,8-bis-(hydrazino)-purine directly. This was the only instance when steam-bath temperature afforded replacement of both chlorine atoms when VII was treated with an aqueous amine.

Treatment of 6,8-dichloropurine (VII) with excess thiourea in boiling ethanol gave 6,8-purinedithiol (XV) in almost quantitative yield. Methylation of XV with methyl iodide gave 6,8-bis-methylthiopurine (XVI). 6,8-Bis-methylthiopurine (XVI) also was prepared readily when 6-chloro-8-methylthiopurine (VIII) was treated with methanethiol in basic solution on the steam-bath.

When VII was treated with sodium methoxide in refluxing methanol, a monomethoxy derivative was prepared. This derivative was judged to be 8-chloro-6-methoxypurine (XXIX) since the ultraviolet absorption spectra of XXIX at *p*H 11 exhibited a maximum at 265 mμ as compared to 6-methoxypurine which exhibits a maximum[15] of 261 mμ at the same *p*H. 8-Methoxypurine[19] at *p*H 10

(15) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(16) E. Fischer, *Ber.*, **30**, 2220 (1897).

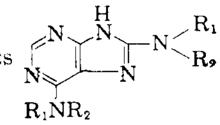
(17) E. Fischer, *ibid.*, **30**, 2227 (1897).

(18) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

(19) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 682 (1957).

TABLE II

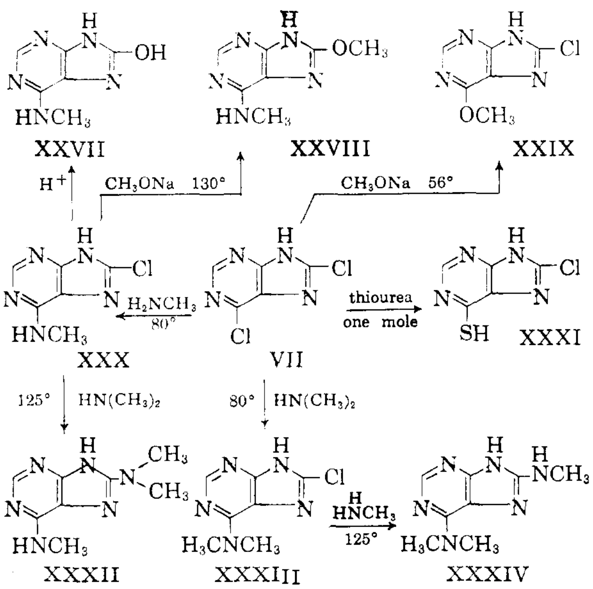
SOME 6,8-BIS-ALKYLAMINOPURINES



R_1	R_2	Formula	M.p., °C.	C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
C_2H_5	H	$C_9H_{14}N_6\cdot H_2O$	215–217	48.3	48.3	7.2	7.2	37.5	37.7
CH_3	CH_3	C_9H_{14}	291–293	52.4	52.7	6.8	6.5	40.8	40.8
CH_3	H	$C_7H_{10}N_6\cdot 2HCl\cdot H_2O$	300–305	31.2	31.7	5.2	5.4	31.2	31.1
NH_2	H	$C_5H_8N_8$	>300	33.4	33.7	4.5	4.7	66.6	65.5

shows a maximum at 279 mμ. Sodium ethoxide in refluxing ethanol reacted similarly to give 8-chloro-6-ethoxypurine.

REACTION SCHEME III



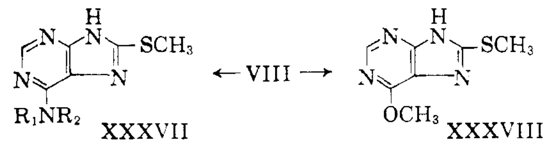
When 6,8-dichloropurine (VII) was treated with one mole of thiourea in refluxing methanol, a mono-chloro-monothiopurine was obtained, presumably 8-chloro-6-purinethiol (XXXI) since the same compound was prepared with refluxing 2 *N* potassium hydrosulfide and 6,8-dichloropurine (VII).

The selective replacement of the chlorine atoms of 6,8-dichloropurine (VII) by aliphatic amines allowed for the synthesis of several interesting derivatives with different alkylamino groups at positions 6 and 8. 8-Chloro-6-methylaminopurine (XXX) when treated with aqueous dimethylamine at 125° gave 8-dimethylamino-6-methylaminopurine (XXXII). The isomeric 6-dimethylamino-8-methylaminopurine was similarly prepared from 8-chloro-6-dimethylaminopurine (XXXIII) and aqueous methylamine at 125°.

8-Chloro-6-methylaminopurine (XXX) and sodium methoxide heated at 130° in methanol resulted in the preparation of 6-methylamino-8-methoxypurine (XXVIII).

8-Chloro-6-ethylthiopurine was prepared from 6,8-dichloropurine and ethanethiol in basic solution heated on the steam-bath. Since 8-chloro-6-methylthiopurine (XII) was similarly prepared and the structure established independently, it would appear to follow that the ethylthio group is in position 6.

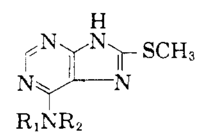
The ease of preparation of 6-hydroxy-8-methyl thiopurine (IV) and the excellent yield of 6-chloro-8-methylthiopurine (VIII) obtained by the treatment of IV with phosphorus oxychloride and N,N-diethylaniline prompted the preparation of several additional 8-methylthio-6-substituted purines. Treatment of 6-chloro-8-methylthiopurine (VIII) with various primary and secondary amines gave



the 6-alkylamino-8-methylthiopurines (XXXVII) listed in Table III. Treatment of VIII with sodium methoxide in boiling methanol gave 6-methoxy-8-methylthiopurine (XXXVIII).

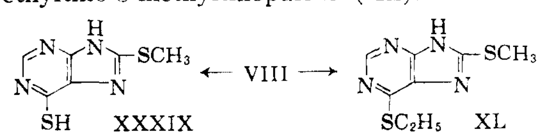
TABLE III

6-ALKYLAMINO-8-METHYLTHIOPURINES



R_1	R_2	Formula	M.p., °C.	Recrystn. solvent	Nitrogen, % Calcd.	Nitrogen, % Found
C_2H_5	H	$C_8H_{11}N_5S$	235–236	Ethanol–water	33.5	33.1
CH_3	CH_3	$C_8H_{11}N_5S$	260	Ethanol	33.5	33.6
p-ClC_6H_4	H	$C_{13}H_{12}N_6SCl$	275–277	Ethoxy-ethanol	22.9	23.3

When 6-chloro-8-methylthiopurine (VIII) was treated with thiourea in boiling ethanol, 8-methylthio-6-purinethiol (XXXIX) was prepared in quantitative yield. Ethanethiol and VIII gave 6-ethylthio-8-methylthiopurine (XL). The ultra-



violet absorption spectra of the 6,8-disubstituted purines prepared are recorded in Table IV.

Acknowledgment.—The author wishes to acknowledge the helpful technical assistance of Cristina Gallegos relative to this work.

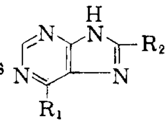
Experimental[20]

Preparation of 6,8-Dihydroxypurine (VI).—The method of Albert and Brown[4] was modified for large-scale operation

(20) Melting points were taken on a Fisher–Johns melting point apparatus, and are uncorrected, unless otherwise indicated.

TABLE IV

ULTRAVIOLET ABSORPTION SPECTRA OF SOME 6,8-DISUBSTITUTED PURINES



R_1	R_2	pH 1 λ_{max}, mμ	pH 1 ε	pH 11 λ_{max}, mμ	pH 11 ε
OH	OH	256	13,800	271	12,900
Cl	Cl	271	13,000	278	12,400
$HNCH_3$	Cl	269	19,000	274	20,000
$N(CH_3)_2$	Cl	277	18,700	282	21,400
NH_2	Cl	262	16,000	270	15,900
HNC_2H_5	Cl	270	16,500	276	16,900
Cl	OH	279	13,300	289	13,600
		243	3,800		
OH	Cl	253	13,800	264	14,300
SCH_3	Cl	228	12,200	228	14,400
		298	16,600	294	20,000
SC_2H_5	Cl	228	10,100	230	12,800
		299	13,900	295	18,600
SH	Cl	346	16,000	332	15,900
OCH_3	Cl	263	12,100	265	11,400
NH_2	NH_2	280	12,000	224	17,500
				280	14,800
OC_2H_5	Cl	264	14,700	266	14,500
OH	SH	234	8,700	234	17,600
		290	27,200	290	18,800
n-NHC_3H_7	Cl	270	17,700	277	18,800
SH	OH	238	13,300	235	24,000
		332	20,600	311	24,200
NH_2	SH	242	12,000	229	18,400
		310	24,700	301	23,400
NH_2	OH	280	11,500	279	16,000
				223	17,700
OH	NH_2	254	14,800	222	15,300
				270	13,400
SH	NH_2	238	17,900	312	26,900
		332	25,400	240	21,000
OH	SCH_3	275	16,600	225	18,000
				280	16,700
Cl	SCH_3	221	12,800	227	18,200
		297	19,800	302	19,200
SH	SCH_3	259	18,000	251	19,600
		342	33,300	328	27,700
$(CH_3)_2N$	SCH_3	302	25,700	233	19,600
				296	24,700
SCH_3	SCH_3	252	12,100	240	18,000
		335	25,400	312	25,700
SCH_3	OH	227	12,200	303	19,300
		299	13,800		
CH_3NH	SCH_3	295	21,600	296	19,700
C_2H_5NH	SCH_3	295	21,300	227	18,200
				290	21,100
C_2H_5S	SCH_3	222	13,300	229	17,600
		297	19,000	304	20,800
		338	7,000		
OCH_3	SCH_3	299	20,000	284	23,100
p-$ClC_6H_4CH_2NH$	SCH_3	302	27,500	226	27,800
				292	24,400
SCH_3	SH	253	18,200	248	15,400
		326	36,200	326	28,700
NH_2	SCH_3	290	20,600	228	21,000
				286	21,000
$NHNH_2$	$NHNH_2$	278	10,400	268	6,700
CH_3NH	CH_3NH	283	14,500	224	18,800
				288	15,300
$(CH_3)_2N$	$(CH_3)_2N$	312	21,000	233	23,200
				302	20,200
C_2H_5NH	C_2H_5NH	285	16,800	225	24,400
				290	21,000
CH_3NH	$(CH_3)_2N$	287	16,200	227	21,000
				292	18,500
$(CH_3)_2N$	CH_3NH	306	16,200	230	21,200
				296	18,500
NH_2	OCH_3	270	13,900	271	14,300
CH_3NH	OCH_3	280	11,100	280	15,800
CH_3NH	OH	277	13,000	283	17,200
CH_3NH	SH	242	11,000	230	21,500
		312	21,700	298	20,100
SH	SH	270	11,900	238	10,500
		358	27,800	259	11,200
				334	23,000

as follows: 400 g. of 4,5-diamino-6-hydroxypyrimidine sulfate, prepared by the method of Roblin, *et al.*[10] (later purchased from Francis Earle Laboratories, Inc., Peekskill, N. Y.), was mixed with 1000 g. of urea. The finely pulverized mixture was heated carefully to 130°. The temperature of the melt was gradually raised to 150° at which temperature the melt became semi-solid after heating for 20 min. Heating was carefully continued to 160°. The cooled solid was dissolved in 2500 ml. of hot dilute potassium hydroxide and the solution boiled with charcoal and filtered. The boiling filtrate was acidified with hydrochloric acid and the hot solution filtered. The crude tan product was reprecipitated from a hot basic solution with dilute hydrochloric acid as before and filtered hot to yield 295 g. of an almost white product. The ultraviolet absorption spectra[4] indicated that the 6,8-dihydroxypurine was above 95% pure. This product was used directly for the experiments here described.

6,8-Dichloropurine (VII).—One hundred grams of very finely pulverized 6,8-dihydroxypurine, dried at 160°, was placed in a 3-liter, 3-necked flask. To this product was added 2000 ml. of redistilled phosphorus oxychloride and 300 ml. of C.P. N,N-diethylaniline. The mixture was refluxed for 3.5 hr., and the excess phosphorus oxychloride was then distilled off under reduced pressure using a steam-bath as a source of heat. When the rate of distillation slowed down to about 1 drop per second, the hot sirupy residue was poured, with vigorous stirring, on crushed ice and the cold aqueous solution made *strongly* basic with concentrated potassium hydroxide solution previously prepared and cooled. The addition of potassium hydroxide was carried out slowly and in the presence of sufficient ice so that the solution remained cold. The solution was allowed to stand for 30 min. and checked to make sure it was still strongly basic. This solution was then placed in a separatory funnel and the diethylaniline allowed to come to the top.

The bottom $^9/_{10}$ of the solution was collected and the top $^1/_{10}$, containing the diethylaniline, was discarded. (This procedure helped to prevent troublesome emulsions.) The basic solution was then extracted once with 2 liters of ether to remove the remaining diethylaniline. The basic aqueous layer then was acidified carefully with concentrated hydrochloric acid. A very fine precipitate appeared at this point which was filtered and discarded. The clear acidic solution was then extracted with ether (5 × 1000 ml.) and the ethereal extract washed once with 500 ml. of cold water. The ether was then dried over anhydrous magnesium sulfate. Distillation of the ether gave 47.0 g. of crude product, m.p. 160° dec. The crude product was used directly for the experiments described in this paper. Further purification was accomplished by recrystallization of the product from a benzene–methanol mixture to yield a product of m.p. 178° dec. Further recrystallization from water gave a white product, a monohydrate, which slowly lost water when carefully heated on the melting point block to give a m.p. 178° dec. When placed on a melting point apparatus preheated to 135°, the hydrate melted instantly and then solidified and remelted at approximately 175° dec. The m.p. of this compound varies somewhat with the rate of heating due to a certain amount of decomposition which precedes the melting point.

Anal. Calcd. for $C_5H_2N_4Cl_2 \cdot H_2O$: C, 29.0; H, 2.0; N, 27.1. Found: C, 29.3; H, 1.96; N, 27.2.

6-Amino-8-chloropurine (XX).—Ten grams of 6,8-dichloropurine (VII) and 100 ml. of concentrated ammonium hydroxide were heated to 100° for 12 hr. in a steel bomb. The contents were cooled and the excess ammonium hydroxide evaporated on the steam-bath. Thirty ml. of water was then added and the product filtered. The crude product was dissolved in dilute sodium hydroxide and boiled with charcoal and the hot filtrate acidified with acetic acid to yield 4.6 g. of white product. This material was dissolved in hot dilute ammonium hydroxide and the excess ammonia evaporated on the steam-bath. Crystals of 6-amino-8-chloropurine appeared from the hot solution and were filtered and washed with water.

Anal. Calcd. for $C_5H_4N_5Cl$: C, 35.4; H, 2.4; N, 41.3. Found: C, 35.7; H, 2.1; N, 41.2.

8-Chloro-6-hydroxypurine (XIX).—Fifteen grams of 6,8-dichloropurine (VII) was added to 100 ml. of 4 *N* potassium hydroxide. The solution was refluxed for 4 hr., diluted with 100 ml. of water and boiled with charcoal. The hot filtrate was acidified with acetic acid and the solution cooled in the refrigerator. The product was filtered and dried to give 10.1 g. Recrystallization from water gave a hydrate which lost water with difficulty.

Anal. Calcd. for $C_5H_3N_4ClO \cdot {}^1/_2H_2O$ (sample dried at 110°): C, 33.5; H, 2.2; N, 31.2. Found: C, 33.5; H, 2.3; N, 31.0. Calcd. for $C_5H_3N_4ClO \cdot {}^1/_4H_2O$ (sample dried at 140°): C, 34.3; H, 2.0. Found: C, 34.3; H, 1.9.

Treatment of 6-amino-8-chloropurine (XX) with nitrous acid at 70° provided another method of preparation of 8-chloro-6-hydroxypurine (XIX) which was identified by ultraviolet absorption spectra.

8-Chloro-6-methoxypurine (XXIX).—Five grams of 6,8-dichloropurine (VII) was added to 150 ml. of absolute methanol containing 4.5 g. of sodium. The solution was refluxed for 3 hr. and the sodium chloride filtered. The filtrate was evaporated to dryness on the steam-bath and the residue dissolved in 80 ml. of water. The solution was neutralized to *p*H 4 with concentrated hydrochloric acid and allowed to cool. The crude product was recrystallized from water to give colorless needles, m.p. 203–204° dec.

Anal. Calcd. for $C_6H_5N_4ClO$: C, 39.2; H, 2.8; N, 30.3. Found: C, 39.4; H, 3.1; N, 30.6.

8-Chloro-6-ethoxypurine.—Similarly, 6,8-dichloropurine (VII) and sodium dissolved in ethanol gave 8-chloro-6-ethoxypurine. The final product was recrystallized from a toluene–ethanol mixture to give a white product, m.p. 197–199° dec.

Anal. Calcd. for $C_7H_7N_4ClO$: C, 42.3; H, 3.5; N, 28.2. Found: C, 42.4; H, 3.8; N, 28.3.

8-Chloro-6-methylthiopurine (XII). Method 1.—To 10.0 g. of 8-hydroxy-6-methylthiopurine (XI) was added 500 ml. of phosphorus oxychloride and 10 ml. of N,N-diethylaniline and the mixture refluxed for 4 hr. The excess phosphorus oxychloride was distilled off under reduced pressure and the residue poured onto crushed ice. The solution was carefully made basic to *p*H 12 with concentrated potassium hydroxide solution and allowed to stand 15 min. The solution was then acidified to *p*H 1 with concentrated hydrochloric acid and then placed in a continuous extractor and extracted 24 hr. with ether. Upon evaporation of the ether, 10.2 g. of product was obtained, m.p. 192–195° dec. Recrystallization from toluene raised the m.p. to 194–196° dec.

Anal. Calcd. for $C_6H_5N_4SCl$: N, 27.9. Found: N, 27.6.

Method 2.—To a solution of 8 g. of potassium hydroxide in 100 ml. of water was added 15 ml. of methanethiol and 4.0 g. of 6,8-dichloropurine (VII). The solution was heated for 1 hr. on the steam-bath and finally neutralized with acetic acid and cooled to yield 2.6 g. of product. Recrystallization from toluene gave a product of m.p. 193–195° dec. A mixed m.p. of this preparation and that obtained by method 1 showed no depression. The ultraviolet absorption spectra of the two preparations were identical.

8-Chloro-6-ethylthiopurine.—Ethanethiol and 6,8-dichloropurine (VII) were allowed to react as for the preparation of XII, method 2, to give 8-chloro-6-ethylthiopurine, which melted at 158–159° after recrystallization from a benzene–heptane mixture.

Anal. Calcd. for $C_7H_7N_4SCl$: C, 39.2; H, 3.3. Found: C, 39.2; H, 3.3.

Preparation of 6-Alkylamino-8-chloropurines (XIII) Listed in Table I.—Five grams of 6,8-dichloropurine (VII) was added to 150 ml. of a 20–40% aqueous solution of the aliphatic amine, and the solution was heated for 8 hr. on the steam-bath. At this time 100 ml. of water was added and the solution heated on the steam-bath until the odor of excess amine was no longer apparent. The aqueous solution was then cooled, filtered and washed with water. The crude product was recrystallized from solvents indicated.

6,8-Purinedithiol (XV).—To 15 g. of thiourea and 300 ml. of absolute ethanol was added 15 g. of 6,8-dichloropurine (XV). The solution was refluxed for 3 hr. and filtered while hot to yield 14.5 g. of 6,8-purinedithiol (XV). This product was further purified by dissolving it in dilute potassium hydroxide solution, boiling the solution with carbon and acidifying the hot filtrate with dilute hydrochloric acid. The product thus obtained was filtered, washed with distilled water and dried at 130°.

Anal. Calcd. for $C_5H_4N_4S_2$: C, 32.6; H, 2.2; N, 30.4. Found: C, 32.5; H, 2.4; N, 30.4.

6,8-Bis-methylthiopurine (XVI). Method 1.—To 10.0 g. of 6,8-purinedithiol (XV), dissolved in 250 ml. of 2 *N* potassium hydroxide, cooled to 10°, was added 15.4 g. of methyl iodide. The solution was vigorously stirred for 15 min. and then gradually heated to 50°. The solution was then acidified with acetic acid and filtered immediately. The precipitate was washed with water and dried to yield 10.3 g., m.p. 254–256°. Recrystallization from ethanol and water raised the m.p. to 257–258°.

Anal. Calcd. for $C_7H_8N_4S_2$: C, 39.6; H, 3.8; N, 26.4. Found: C, 39.5; H, 3.2; N, 26.7.

Method 2.—Ten grams of 6-chloro-8-methylthiopurine (VIII) was dissolved in 100 ml. of 1.5 *N* potassium hydroxide (cooled to 0°) to which had previously been added 20 ml. of methanethiol. The solution was heated 1 hr. on the steam-bath and then acidified while hot with acetic acid. The product was filtered immediately and washed with water and dried to give 10.1 g., m.p. 254–256°. Recrystallization from ethanol–water raised the m.p. to 257–258°. A mixed m.p. of this product and that prepared by method 1 showed no depression. The ultraviolet absorption spectra of the two preparations were identical.

6-Chloro-8-hydroxypurine (XVIII).—Ten grams of 6,8-dichloropurine (VII) was added to 50 ml. of water and 50 ml. of concentrated hydrochloric acid. The solution was evaporated to dryness on the steam-bath. The residue was washed by decantation with 2 × 25 ml. of water, then suspended in 100 ml. of boiling water and enough potassium hydroxide added to effect solution. The solution was boiled with charcoal and the hot filtrate neutralized with acetic acid and allowed to cool. The product thus obtained was recrystallized from water as a final purification to yield 2.3 g. of colorless crystals, which were dried at 110° for analysis.

Anal. Calcd. for $C_5H_3N_4ClO$: C, 35.2; H, 1.8; N, 32.8. Found: C, 34.9; H, 1.9; N, 32.6.

6-Hydroxy-8-purinethiol (III).—Fifty grams of 4,5-diamino-6-hydroxypyrimidine sulfate and 200 g. of thiourea were heated to 200° for 30 min. The solidified product was dissolved in 1000 ml. of 2 *N* sodium hydroxide and treated with charcoal and the boiling filtrate acidified with concentrated hydrochloric acid. The solution was filtered to yield 22.2 g. of product. This product was reprecipitated from a hot basic solution for analysis and dried at 110°. At this temperature the product retained 0.5 mole of water.

Anal. Calcd. for $C_5H_4N_4OS \cdot {}^1/_2H_2O$: C, 33.9; H, 2.8. Found: C, 33.6; H, 2.7.

8-Hydroxy-6-purinethiol (X). Method 1.—Fifty grams of finely powdered 6,8-dihydroxypurine (VI) and 200 g. of pulverized phosphorus pentasulfide were added to 1500 ml. of C.P. pyridine and the solution refluxed for 4 hr. Excess pyridine was distilled off under vacuum using a steam-bath as a source of heat. One liter of water was added and the solution heated on a steam-bath for 2 hr., cooled, and filtered. The crude product was dissolved in 500 ml. of 1 *N* potassium hydroxide. The solution was heated to boiling with charcoal, filtered, then the hot filtrate acidified with hydrochloric acid and allowed to cool overnight. The filtered product was reprecipitated from hot base and the solution cooled as before to give 18.0 g. of product. A small amount was recrystallized from water and acetic acid for analysis.

Anal. Calcd. for $C_5H_4N_4OS \cdot H_2O$: C, 31.9; H, 3.2; N, 30.1. Found: C, 31.7; H, 3.2; N, 29.9.

Method 2.—Five grams of 4,5-diamino-6-pyrimidinethiol (IX)[11] was heated with 15 g. of urea at 190–210° for 15 min. The solid was cooled and dissolved in 300 ml. of dilute potassium hydroxide. The hot solution was acidified with acetic acid and cooled to yield 5.5 g. of 8-hydroxy-6-purinethiol (X). This product was further purified by recrystallization from water. This preparation and the product obtained by method 1 were judged to be identical on the basis of identical ultraviolet absorption spectra.

6-Amino-8-hydroxypurine (XXIV).[3] **Method 1.**—One and five-tenths grams of 6-amino-8-chloropurine was added to 50 ml. of concentrated hydrochloric acid. The solution was refluxed for 4 hr., then adjusted to *p*H 7 with concentrated ammonium hydroxide and allowed to cool. The solution was filtered and the product purified by precipitation from hot dilute sodium hydroxide with acetic acid. The yield of white product was 0.4 g.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.7; H, 3.3; N, 46.4. Found: C, 39.3; H, 3.5; N, 46.0.

Method 2.—Ten grams of 4,5,6-triaminopyrimidine[21] was fused with 30 g. of urea at 200° for 20 min. The solid was dissolved in hot dilute potassium hydroxide and the solution acidified with acetic acid and filtered hot to yield 9.7 g. of 6-amino-8-hydroxypurine (XXIV). Four grams of this product was added to 150 ml. of 5% sulfuric acid and the solution boiled and decolorized with charcoal, filtered and cooled to yield large, white flat needles of sulfate (4.5 g.) of 6-amino-8-hydroxypurine (XXIV).

Anal. Calcd. for $C_5H_5N_5O \cdot {}^1/_2H_2SO_4$: C, 30.0; H, 3.0; N, 35.0. Found: C, 30.3; H, 2.9; N, 35.3.

A small amount of sulfate (2.0 g.) was dissolved in 5% sulfuric acid and the solution heated to boiling. The *p*H of the solution was adjusted to 10 by adding ammonium hydroxide. The white, free base precipitated from the hot solution and was filtered, washed and dried at 120° to yield 1.1 g.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.7; H, 3.3; N, 46.4. Found: C, 39.7; H, 3.5; N, 46.5.

The ultraviolet absorption spectra of XXIV prepared by methods 1 and 2 were identical.

8-Amino-6-hydroxypurine (I).—Thirty-seven grams of 4,5-diamino-6-hydroxypyrimidine[10] (free base) was added to guanidine prepared as follows: Forty-one and six-tenths grams of guanidine hydrochloride was added to a solution of 10 g. of sodium dissolved in 400 ml. of absolute ethanol. The sodium chloride precipitate was filtered, and the clear alcohol filtrate was placed in a 500-ml. round-bottom flask and the excess ethanol removed under reduced pressure using a steam-bath. The remaining sirupy guanidine was used directly.

The reaction mixture was heated in the 500-ml. round-bottom flask by means of a metal-bath at 230° (bath temp.) for 30 min. The temperature of the reaction mixture (inside temp.) was between 200–205°. The cooled residue was dissolved in hot dilute potassium hydroxide; charcoal was added and the solution filtered hot. The boiling filtrate was acidified with acetic acid. The crude yellow product was filtered, washed and reprecipitated from dilute potassium hydroxide as before. The crude product was then dissolved in 500 ml. of 5% sulfuric acid and the solution boiled with charcoal. The filtrate was allowed to cool slowly and deposited 26.1 g. of the crystalline sulfate of 8-amino-6-hydroxypurine (I). For further purification the compound was recrystallized from 5% sulfuric acid to give colorless crystals.

Anal. Calcd. for $C_5H_5ON_5 \cdot {}^1/_2H_2SO_4$: N, 35.0. Found: N, 34.7.

To obtain the free base the salt was dissolved in hot dilute sulfuric acid and the solution neutralized with ammonia. The white product was filtered from the hot solution, washed with distilled water and dried.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.7; H, 3.3; N, 46.4. Found: C, 39.8; H, 3.5; N, 46.4.

(21) R. K. Robins, K. J. Dille, H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 265 (1953).

8-Amino-6-purinethiol (V). Method 1.—Ten grams of 8-amino-6-hydroxypurine (free base) and 45 g. of phosphorus pentasulfide were added to 500 ml. of pyridine and the solution refluxed for 10 hr. The excess pyridine was removed under reduced pressure using a steam-bath as a source of heat. Water (500 ml.) was added to the residue and the solution heated on a steam-bath, cooled and filtered. The crude product was dissolved in dilute sodium hydroxide, boiled with charcoal and filtered. The filtrate was neutralized with dilute acetic acid. Reprecipitation was carried out twice more from dilute potassium hydroxide to yield 2.4 g. of 8-amino-6-purinethiol (V).

Anal. Calcd. for $C_5H_5N_5S$: C, 35.9; H, 3.0; N, 41.9. Found: C, 36.2; H, 3.4; N, 41.5.

Method 2.—Three grams of 4,5-diamino-6-pyrimidinethiol[11] was fused at 200° with 6 g. of guanidine prepared as for the preparation of 8-amino-6-hydroxypurine (I). The cooled residue was dissolved in 250 ml. of dilute potassium hydroxide and the solution boiled with charcoal and filtered. The hot filtrate was neutralized with acetic acid and allowed to cool. The cooled solution gave 0.8 g. of crude product which was identified as 8-amino-6-purinethiol (V) by its absorption spectra.

6-Amino-8-purinethiol (XXVI).—Ten grams of 4,5,6-triaminopyrimidine[21] was thoroughly mixed with 20 g. of thiourea and the mixture heated to 200–220° for 20 min. at which time the mixture became solid. The solid was dissolved in boiling dilute potassium hydroxide and the solution treated with charcoal. The boiling filtrate was acidified with acetic acid and the product filtered hot to yield 10.2 g. of white product. For analysis the sample was purified by recrystallization from 30% acetic acid and finally dried at 110°.

Anal. Calcd. for $C_5H_5N_5S$: C, 35.9; H, 3.0. Found: C, 35.8; H, 3.2.

8-Hydroxy-6-methylthiopurine (XI).—Twenty grams of 8-hydroxy-6-purinethiol monohydrate (X) was added to 400 ml. of *N* potassium hydroxide and the solution cooled to 10° and stirred vigorously. Then 15.4 g. of methyl iodide was added and the solution allowed to stir at 15–20° for 25 min. The solution was warmed to 50°, acidified with acetic acid and allowed to cool. Filtration gave 15 g. of product. Recrystallization of a small sample for analysis was accomplished from 50% acetic acid.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.5; H, 3.3; N, 30.7. Found: C, 39.6; H, 3.5; N, 30.3.

6-Hydroxy-8-methylthiopurine (IV).—To 120 g. of 6-hydroxy-8-purinethiol (III) and 1500 ml. of water was added 100 g. of potassium hydroxide, together with ice to cool the solution to 20°. Then 98 g. of methyl iodide was added and the solution vigorously stirred with a magnetic stirrer for 30 min. until 1 phase appeared. The solution was heated to boiling, acidified with acetic acid and filtered hot. The product was washed and dried at 120° to yield 105 g. of white compound. A small amount was recrystallized from glacial acetic acid for analysis and dried at 130°.

Anal. Calcd. for $C_6H_6N_4SO$: C, 39.5; H, 3.3; N, 30.7. Found: C, 39.9; H, 3.4; N, 30.5.

6-Chloro-8-methylthiopurine (VIII).—To 50 g. of 6-hydroxy-8-methylthiopurine and 1300 ml. of phosphorus oxychloride was added 100 ml. of C.P. N,N-diethylaniline, and the solution was refluxed for 2 hr. The excess phosphorus oxychloride was removed under vacuum and the residue poured onto ice. The solution was made basic with concentrated potassium hydroxide and allowed to stand 10 min., then acidified with concentrated hydrochloric acid to *p*H 1. The total volume was approximately 4 l. The solution was allowed to stand for 1 hr., then filtered and the precipitate washed with distilled water and dried to yield 55 g. of product, m.p. 217–220° dec. Recrystallization from ethanol gave white needles, m.p. 220–222° dec.

Anal. Calcd. for $C_6H_5N_4SCl$: C, 35.9; H, 2.5; N, 27.9. Found: C, 36.2; H, 2.8; N, 27.5.

8-Methylthio-6-purinethiol (XXXIX).—Five grams of 6-chloro-8-methylthiopurine (VIII) and 10 g. of thiourea were added to 200 ml. of ethanol and the solution vigorously refluxed for 1 hr. The solution was filtered while hot and the product washed with ethanol to yield 3.7 g.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.3; H, 3.0; N, 28.3. Found: C, 36.2; H, 2.9; N, 28.2.

6-Methoxy-8-methylthiopurine (XXXVIII).—Seven grams of 6-chloro-8-methylthiopurine (VIII) was added to a 100-ml. solution of 4.5 g. of sodium in absolute methanol and the solution refluxed on the steam-bath for 3 hr. Water, 100 ml., was then added and the solution neutralized with acetic acid and cooled in the refrigerator overnight. The crude product, 1.5 g., was filtered and recrystallized from ethanol and water to give white needles, m.p. 205-206°.

Anal. Calcd. for $C_7H_8N_4OS$: N, 28.6. Found: N, 28.4.

6-Ethylthio-8-methylthiopurine (XL).—To 5.0 g. of 6-chloro-8-methylthiopurine (VIII), dissolved in 100 ml. of 2 *N* potassium hydroxide, was added 20 ml. of ethanethiol and the solution warmed on a steam-bath with occasional shaking for 2 hr. The solution was then acidified with acetic acid and cooled. The crude precipitate was recrystallized from ethanol and water to give 2.6 g., m.p. 175–177°.

Anal. Calcd. for $C_8H_{10}N_4S_2$: C, 42.4; H, 4.4; N, 24.8. Found: C, 42.4; H, 4.4; N, 24.4.

6-Methylthio-8-purinethiol (XXXV).—One gram of 8-chloro-6-methylthiopurine (XII) was added to 50 ml. of ethanol and 1.0 g. of thiourea, and the solution was refluxed for 2 hr. The yellow precipitate was filtered directly from the hot reaction mixture and washed with a small amount of hot ethanol. The yield of 6-methylthio-8-purinethiol was 0.8 g.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.3; H, 3.0; N, 28.3. Found: C, 36.4; H, 3.4; N, 28.5.

8-Chloro-6-purinethiol (XXXI). Method 1.—Twenty-six and five-tenths grams of 6,8-dichloropurine (VII) and 9.1 g. of thiourea were added to 500 ml. of absolute methanol and the solution refluxed for 2 hr. and then cooled overnight. The solution was filtered and the light-orange product washed with methanol. The yield was 16.2 g. Attempts to further purify this product were unsuccessful.

Anal. Calcd. for $C_5H_3N_4SCl$: C, 32.2; H, 1.6; N, 30.0; S, 17.1. Found: C, 32.0; H, 2.5; N, 30.3; S, 16.8.

Method 2.—Three grams of 6,8-dichloropurine (VII) was added to 200 ml. of 2 *N* potassium hydrosulfide and the solution refluxed for 1 hr. The solution was boiled with charcoal and the hot filtrate acidified with hydrochloric acid. The solution was filtered immediately to yield 0.8 g. of 8-chloro-6-purinethiol (XXXI) which was identified by its ultraviolet absorption spectrum which was identical to that of the product prepared by method 1.

6,8-Diaminopurine (XXI). Method 1.—Twenty grams of 6,8-dichloropurine (VII) and 100 ml. of concentrated ammonium hydroxide were heated for 6 hr. at 135° in a high pressure reaction vessel. The ammoniacal solution was cooled and filtered to yield 12.0 g. of 6,8-diaminopurine which was above 95% pure as judged by ultraviolet absorption data. This product was best purified as the monohydrochloride. For this purpose a solution of 6,8-diaminopurine in dilute potassium hydroxide was adjusted carefully to *p*H 5 with hydrochloric acid. The cooled solution was filtered and the salt recrystallized from water.

Anal. Calcd. for $C_5H_6N_6 \cdot HCl \cdot H_2O$: C, 31.5; H, 4.7; N, 36.8. Found: C, 31.0; H, 4.3; N, 36.6.

Method 2.—When 6-chloro-8-methylthiopurine (VIII) was substituted in the above reaction for 6,8-dichloropurine (VII), 6,8-diaminopurine (XXI) was similarly prepared although in lower yield. The identity of XXI prepared by this method was confirmed by the preparation of the hydrochloride and by comparison of the ultraviolet absorption spectra with that obtained by method 1.

6-Amino-8-methoxypurine (XXIII).—To 200 ml. of absolute methanol, containing 4 g. of sodium, was added 3.5 g. of 6-amino-8-chloropurine (XX). The solution was heated in a high pressure bomb at 130° for 5 hr. The solution was then cooled and evaporated on the steam-bath to half its volume; 100 ml. of water was added and the *p*H adjusted to 7 with acetic acid. The cooled solution yielded 2.1 g. of light-tan crystals. A small amount was recrystallized from water for analysis.

Anal. Calcd. for $C_6H_7N_5O$: N, 42.4. Found: N, 42.4.

8-Methoxy-6-methylaminopurine (XXVIII).—The preparation of 8-methoxy-6-methylaminopurine (XXVIII) was accomplished from 8-chloro-6-methylaminopurine (XXX) with sodium methoxide in methanol at 135° and the product isolated in a similar manner as for the preparation of 6-amino-8-methoxypurine (XXIII).

Anal. Calcd. for $C_7H_9N_5O$: N, 39.1. Found: N, 39.3.

Preparation of 6,8-Bis-alkylaminopurines Listed in Table II. 6,8-Bis-dimethylaminopurine (XIV, R_1, R_2 = CH_3).—Eight grams of 6,8-dichloropurine (VII) was dissolved in 200 ml. of 20% aqueous dimethylamine and the solution heated at 125° for 5 hr. in a bomb. The cooled solution was filtered to yield 5.2 g. of colorless needles, m.p. 284–286°. Recrystallization from ethanol raised the m.p. to 286–287°.

Anal. Calcd. for $C_9H_{14}N_6$: C, 52.4; H, 6.8; N, 40.8. Found: C, 52.7; H, 6.5; N, 40.8.

Other 6,8-bis-alkylaminopurines were similarly prepared. In the case of 6,8-bis-methylaminopurine (XIV, R_1 = H, R_2 = CH_3), after reaction was complete the solution was evaporated to dryness and extracted with boiling absolute ethanol. Dry hydrogen chloride passed into the ethanol solution precipitated the dihydrochloride.

Preparation of 6-Alkylamino-8-methylthiopurines Listed in Table III. 6-Dimethylamino-8-methylthiopurine (XXXVII, R_1, R_2 = CH_3).—Five grams of 6-chloro-8-methylthiopurine (VIII) was added to 200 ml. of 30% aqueous dimethylamine. The solution was heated on the steam-bath for 4 hr. and then cooled and filtered. The crude product was suspended in 100 ml. of water and enough concentrated hydrochloric acid to effect solution. Charcoal was added and the solution boiled gently and filtered. The hot filtrate was neutralized with ammonium hydroxide and the product collected. Final recrystallization was accomplished from absolute ethanol to yield 2.6 g. of white needles, m.p. 260°.

Anal. Calcd. for $C_8H_{11}N_5S$: C, 45.9; H, 5.3; N, 33.5. Found: C, 46.0; H, 5.5; N, 33.6.

The other 6-alkylamino-8-methylthiopurines listed in Table III were prepared in a similar manner.

8-Dimethylamino-6-methylaminopurine (XXXII).—Five grams of 8-chloro-6-methylaminopurine (XXX) was dissolved in 150 ml. of 20% aqueous dimethylamine and the solution heated to 125° in a bomb for 5 hr. The solution was evaporated to 40 ml., the *p*H adjusted to 12 with concentrated ammonium hydroxide, and the solution cooled overnight. The crystals were collected and recrystallized from water to give 1.1 g., m.p. 278°.

Anal. Calcd. for $C_8H_{12}N_6 \cdot H_2O$: C, 45.8; H, 6.2; N, 40.0. Found: C, 45.5; H, 7.0; N, 40.4.

6-Dimethylamino-8-methylaminopurine (XXXIV).—Four grams of 8-chloro-6-dimethylaminopurine (XXXIII) was dissolved in 200 ml. of 20% aqueous methylamine and the solution heated at 125° for 5 hr. The solution was evaporated to dryness and the residue extracted with two 100-ml. portions of absolute ethanol. Dry hydrogen chloride was passed into the ethanolic solution and the solution cooled and filtered. The dihydrochloride, 4.2 g., was washed with ethanol. A small sample for analysis was recrystallized from absolute ethanol containing a small amount of dry hydrogen chloride. The product was dried at room temperature and when heated slowly melted at 275–280°.

Anal. Calcd. for $C_8H_{12}N_6 \cdot 2HCl \cdot 1^1/_2H_2O$: C, 30.7; H, 4.8; N, 26.9. Found: C, 30.6; H, 4.9; N, 26.8.

8-Hydroxy-6-methylaminopurine (XXVII).—To 50 ml. of concentrated hydrochloric acid was added 1.2 g. of 8-chloro-6-methylaminopurine (XXX), and the solution was refluxed for 3 hr. The solution was then adjusted to *p*H 8 with ammonium hydroxide. The cooled solution was filtered and the crude product purified by reprecipitation from hot dilute potassium hydroxide with acetic acid. The yield of white crystals of 8-hydroxy-6-methylaminopurine (XXVII) was 0.7 g., m.p. >300°.

Anal. Calcd. for $C_6H_7N_5O \cdot H_2O$: N, 38.3. Found: N, 38.7. Calcd. for $C_6H_7N_5O$ (sample dried at 130°): N, 42.6. Found: N, 42.7.

6-Methylamino-8-purinethiol.—To 200 ml. of 2 *N* sodium hydrosulfide was added 3.0 g. of 8-chloro-6-methylaminopurine (XXX), and the solution was heated for 3 hr. at 125° in a sealed bomb. The cooled solution was neutralized with acetic acid and filtered. The product was purified by reprecipitation from a basic solution with acetic acid to yield

1.6 g. A sample for analysis was recrystallized from 50% aqueous acetic acid and dried at 130°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.8; H, 3.9; N, 38.7. Found: C, 39.5; H, 4.0; N, 38.6.

6-Amino-8-methylthiopurine (XXII). Method 1.—To 150 ml. of concentrated ammonium hydroxide was added 15.0 g. of 6-chloro-8-methylthiopurine (VIII). The solution was heated at 110° for 6 hr. The excess ammonia was then evaporated on the steam-bath, and a solid gradually appeared from the hot solution. The volume was reduced to approximately 70 ml. and the solution filtered to yield 10.5 g. of gray solid. The product was purified by reprecipitation from hot dilute sodium hydroxide with acetic acid. The m.p. was 288–290° dec.

Anal. Calcd. for $C_6H_7N_5S$: N, 38.8. Found: N, 38.7.

Method 2.—To 125 ml. of water was added 10 g. of potassium hydroxide and 3.0 g. of 6-amino-8-purinethiol (XXVI). The solution was cooled to 15° and 3.0 g. of methyl iodide added. The solution was stirred at 15–20° for 30 min. The solution was neutralized with acetic acid and filtered to yield 2.1 g. of product. A small sample was recrystallized from a large volume of 50% aqueous ethanol, m.p. 288–290°.

Anal. Calcd. for $C_6H_7N_5S$: N, 38.8. Found: N, 38.7.

The 6-amino-8-methylthiopurine (XXII) thus prepared was judged to be identical with that prepared by method 1 on the basis of ultraviolet absorption spectra and mixed m.p. data.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE]

Dihydrofolic Acid Reductase[1]

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RECEIVED MAY 13, 1958

An enzyme has been separated from sheep liver which catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid. The reduced form of either di- or triphosphopyridine nucleotide serves as the electron donor in this reduction, the most effective nucleotide being determined by the hydrogen ion concentration of the incubation medium. The Michaelis constants for DPNH, TPNH and dihydrofolic acid were determined. A stoichiometric relationship between dihydrofolic acid reduction and nucleotide oxidation has been demonstrated and a synergistic activity between dihydrofolic acid reductase and choline synthetase has been shown to occur when dihydrofolic acid, rather than tetrahydrofolic acid, is employed as the cofactor for choline methyl group formation from formaldehyde. Dihydrofolic acid reductase preparations have an extremely low activity for folic acid reduction, indicating that the enzyme has been separated from the enzyme system which carries out the complete reduction of folic to tetrahydrofolic acid. Studies with sulfhydryl reagents indicate that free sulfhydryl groups are probably not required for enzyme activity.

Introduction

The reduction of folic acid and FH_2[2] to FH_4 by animal and bacterial enzyme systems has been the subject recently of numerous investigations.[3–7] Wright, *et al.*,[3,4] have studied the reduction of folic acid and teropterin (diglutamylfolic acid) in bacteria and have shown that reduction of these compounds to their dihydro derivatives is coupled to pyruvate oxidation. In avian liver Futterman,[5] Zakrzewski and Nichol[6] and Osborn, *et al.*,[7] have demonstrated a requirement for TPNH as the electron donor for folic acid reduction to the dihydro level. The present investigation is concerned with the reduction of FH_2 to FH_4 by an enzyme separated from sheep liver. Both DPNH and TPNH have been reported[5] to function as cofactors for the reduction of FH_2 to FH_4 whereas only TPNH appears to be implicated in the initial reduction stage from folic acid to FH_2.

FH_2 reduction was measured either by the decrease in optical density at 340 mμ of an incubation mixture containing FH_2-reductase, FH_2 and DPNH (or TPNH) or by the production of a diazotizable amine (see later). The purified FH_2-reductase had only slight DPNH- (or TPNH)-oxidizing activity in the absence of FH_2. In order to determine the optimum conditions for the assay, the rate of DPNH and TPNH oxidation as a function of *p*H was measured, and it was found that the rate of FH_2 reduction by DPNH proceeds most favorably at *p*H values below 5.5 whereas reduction by TPNH is favored at *p*H values above 6 (Fig. 1). The rate of FH_2 reduction was also found to be a linear function of the enzyme concentration.

The unusual *p*H–activity curves (Fig. 1) suggest the possibility that two enzymes are involved in FH_2 reduction, one which utilizes DPNH as cofactor and another which utilizes TPNH. The findings recorded in Table I indicate that both activities probably are associated with one enzyme.

TABLE I

EFFECT OF NUCLEOTIDE SATURATION UPON THE ACTIVITY OF DIHYDROFOLIC ACID REDUCTASE

Incubations were carried out at room temperature in 1 cm. Corex cuvettes for 20 minutes. Nucleotide oxidation was measured by the change in optical density at 340 mμ and was corrected for the optical density change in controls containing no FH_2. Each cuvette contained 0.01 ml. of FH_2-reductase and 0.133 μmole of FH_2 in 3 ml. of 0.1 *M* sodium phosphate buffer, *p*H 5.7.

DPNH added, μmoles	TPNH added, μmoles	Nucleotide oxidized,[a] μmoles
0.32	0	0.044
0.32	0.14	.043
0	0.14	.041

[a] Each value represents the average of duplicate determinations.

(1) Aided by research grants from the National Cancer Institute (CY-3175), United States Public Health Service, and the American Cancer Society, California Division (151).

(2) Abbreviations used are: FH_2, dihydrofolic acid; FH_4, tetrahydrofolic acid; DPN and TPN, di- and triphosphopyridine nucleotides; DPNH and TPNH, reduced nucleotides.

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