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]

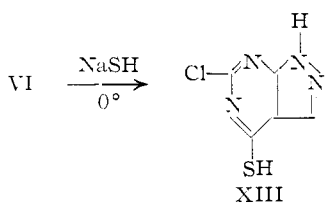
Potential Purine Antagonists. IX. Further Studies of Some 4,6-Disubstituted Pyrazolo[3,4-d]pyrimidines[1]

BY ROLAND K. ROBINS[2]

RECEIVED MAY 15, 1957

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

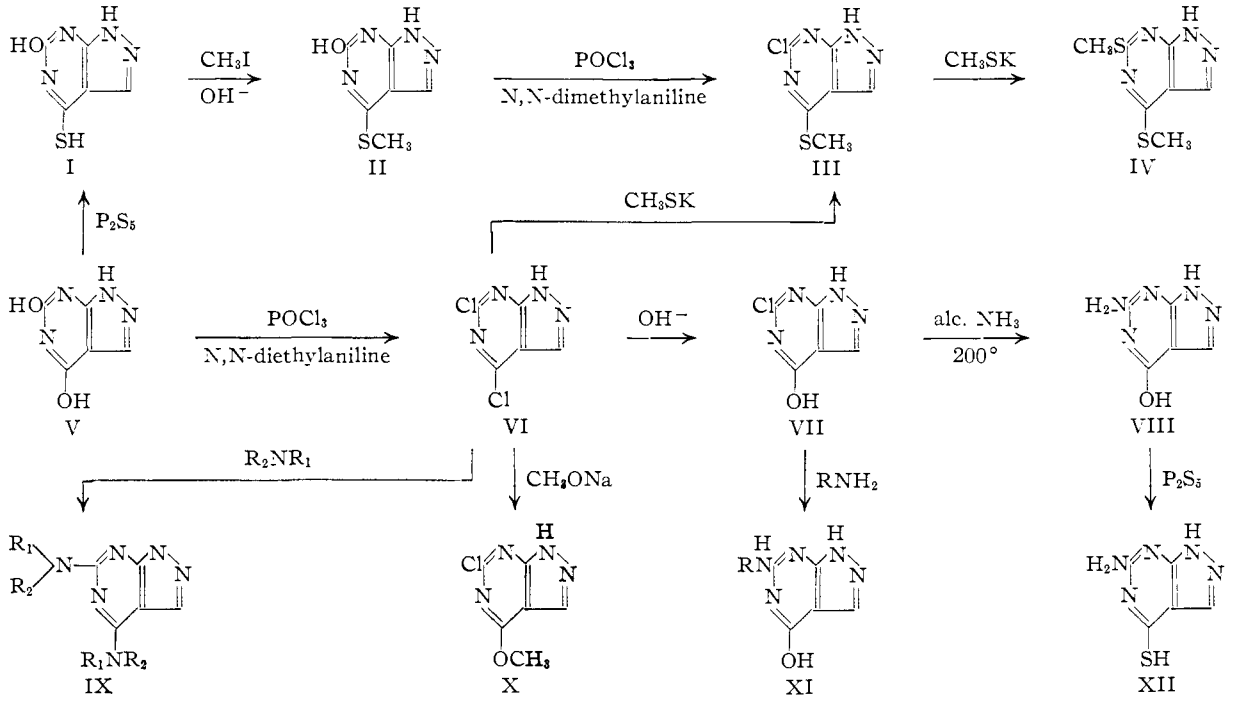
(1) This investigation was supported in part by research grant C-2105(C2) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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

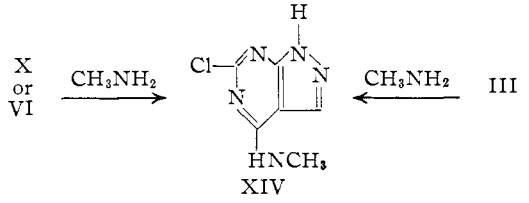
(3) H. E. Skipper, R. K. Robins and J. R. Thomson, *Proc. Soc. Exp. Biol. and Med.*, **89**, 594 (1955).

(4) H. E. Skipper, R. K. Robins, J. R. Thomson, C. C. Cheng, R. W. Brockman and F. M. Schabel, Jr., *Cancer Research*, **17**, 579 (1957).

(5) R. K. Robins, THIS JOURNAL, **78**, 784 (1956).

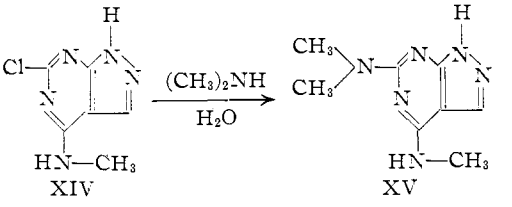


REACTION SCHEME I

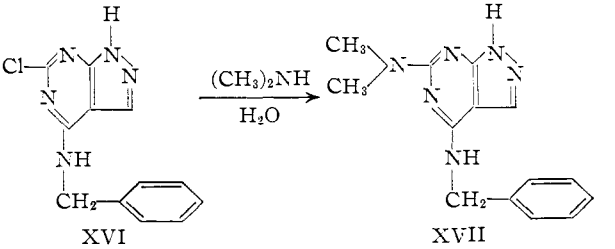


basis of comparison of the ultraviolet absorption spectra at *p*H 11 with the corresponding 4-alkylaminopyrazolo(3,4-d)pyrimidines.[5] In all cases the spectra were remarkably similar. This would be expected if the alkylamino group was in position "4" since it has been noted throughout this work that the "6"-chloro atom effects little change in the ultraviolet spectrum. The preparation of XIV from VI and aqueous methylamine confirms the assigned structure of the compounds in Table I since the structure of XIV has been independently determined by its preparation from III. The replacement of the 4-chloro atom took place usually from 10 to 20 minutes after heating the solution on the steam-bath. The completion of this reaction was often evidenced by the appearance of the product as a precipitate in the hot solution. Prolonged heating from 24 to 36 hr. with an excess of the amine resulted in the corresponding 4,6-bis-(substituted-amino)-pyrazolo(3,4-d)pyrimidine (IX). The 4,6-bis-(substituted-amino)-pyrazolo(3,4-d)-pyrimidines prepared are listed in Table II. The only exception encountered in the reaction of VI with amines was with aqueous dimethylamine. In this case 4,6-bis-(dimethylamino)-pyrazolo(3,4-d)pyrimidine (IX, R_1, R_2 = CH_3) was the only compound that could be isolated. All attempts to isolate 4-dimethylamino-6-chloropyrazolo(3,4-d)-pyrimidine failed. This bis compound resulted in a reaction time of less than 5 minutes with very dilute solutions of dimethylamine. This type of reaction with dimethylamine previously has been noted in other condensed pyrimidine systems which contain active chlorine atoms.[6]

The selective replacement of the chlorine atoms in VI in a stepwise fashion by various amines gave rise to a number of interesting substituted-amino derivatives. For example, treatment of XIV with aqueous dimethylamine on the steam-bath gave 4-methylamino-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XV).



In a similar manner 4-benzylamino-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XVII) was prepared from 4-benzylamino-6-chloropyrazolo(3,4-d)pyrimidine (XVI) and aqueous dimethylamine.

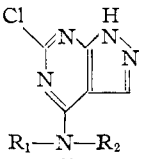


The treatment of 4,6-dichloropyrazolo(3,4-d)-pyrimidine (VI) at higher temperatures and for a longer period of reaction time with an excess of the nucleophilic reagent as in the case of the amines

(6) R. K. Robins and G. H. Hitchings, THIS JOURNAL, **78**, 973 (1956).

TABLE I

4-SUBSTITUTED AMINO-6-CHLOROPYRAZOLO(3,4-d)PYRIMIDINES



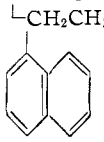
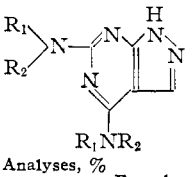
R_1	R_2	Method of prepn.	Yield, %	Recrystn. solvents	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	U.v. absorption λ_{max}, mμ pH 1	pH 11
H	CH_3	A	84	Dimethylformamide–water	39.2	3.3	38.1	39.4	3.3	37.7	278	278
H	i-C_3H_7	A	92	Ethanol–water	45.5	4.8	33.1	45.0	5.1	32.8	280	279
H	C_2H_5	A	80	Ethanol–water	42.5	4.1	35.4	42.6	4.2	35.5	278	279
H	n-C_3H_7	A	95	Ethanol–toluene	45.5	4.8	33.1	45.6	5.1	33.3	279	279
H	i-C_4H_9	A	83	Ethanol–toluene	47.9	5.3	31.1	47.8	5.2	31.3	280	280
H	$CH_2C_6H_5$	B	78	Dimethylformamide–ethanol	55.6	3.9		55.6	3.9			
H	CH_2CH_2OH	A	60	Dimethylformamide–water	39.4	3.8		39.8	4.1		278	270
H	$CHCH_2CH_2CH_2$ (ring)	B	73	Ethanol	52.5	5.6		52.3	5.8		285	275
H		B	70	Ethanol	61.0	3.4	23.7	61.0	3.7	23.1	285	290

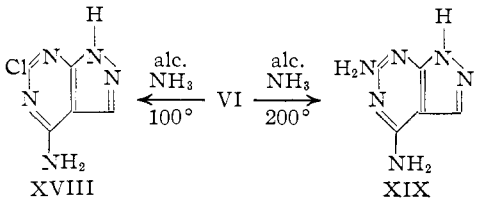
TABLE II

4,6-BIS-(SUBSTITUTED AMINO)-PYRAZOLO(3,4-d)PYRIMIDINES



R_1	R_2	Yield, %	M.p., °C.	Recrystn. solvents	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	U.v. absorption maximum, mμ pH 1	pH 11
CH_3	CH_3	78	249–250	Ethanol	52.5	6.8	40.8	52.0	6.7	40.6	270	242 284
H	C_2H_5	85	238–240	Ethanol–water	52.5	6.8	40.8	52.4	6.9	40.3	261	280
H	CH_3	82	240–242	Water	47.2	5.6	47.2	46.8	5.6	47.2	260	252 278
H	n-C_4H_9	73	182–183	Ethanol–water	59.8	8.6	32.2	60.2	8.4	32.2	..	..
H	n-C_3H_7	62	194–195	Ethanol–water	56.2	8.1		56.3	7.9		262	280 240
H	$HNCH_2CH_2OH$	56	214–215	Water	40.3	6.0		40.4	6.1		270	285

usually gave rise to disubstitution. Alcoholic ammonia and VI at 100° gave 4-amino-6-chloropyrazolo(3,4-d)pyrimidine (XVIII). The structure of XVIII was assigned because of the similarity of the ultraviolet absorption spectra of XVIII and 4-aminopyrazolo(3,4-d)pyrimidine.[4] Alcoholic ammonia and VI at 200° gave 4,6-diaminopyrazolo-(3,4-d)pyrimidine (XIX) as the only product.



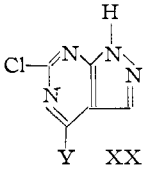
XVIII XIX

The preparation of XIX also was accomplished by a similar treatment of III or 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine[5] with alcoholic ammonia at 200°.

Treatment of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) with an excess of sodium methoxide in boiling methanol 36 hr. gave 4,6-dimethoxypyrazolo(3,4-d)pyrimidine. Sodium methylmercaptide under similar conditions gave 4,6-bis-(methylmercapto)-pyrazolo(3,4-d)pyrimidine (IV). This compound was also prepared from 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III) in a similar manner by the use of sodium methylmercaptide. The preparation of IV has recently been reported by Falco and Hitchings[7] by methylation of 4,6-dimercaptopyrazolo(3,4-d)pyrimidine. Although good agreement was obtained in comparison with the ultraviolet absorption spectra recorded for IV and that obtained in this Laboratory, the melting point of an analytically pure sample was 196–197° as compared to 189–190° as recorded by Falco and Hitchings.[7]

Thiourea and VI heated in ethanol on the steam-bath gave 4,6-dimercaptopyrazolo(3,4-d)pyrimidine.[5,7]

Compounds of formula XX present an interesting study with regard to nucleophilic displacement.



Y XX

Y = Cl, OCH_3, SCH_3, SH, OH, NH_2, $HNCH_3$

As in other condensed pyrimidine systems[8] it can be stated generally that the '4''-position is more

(7) E. A. Falco and G. H. Hitchings, THIS JOURNAL, **78**, 3145 (1956).

(8) (a) R. K. Robins and G. H. Hitchings, *ibid.*, **77**, 2256 (1955); (b) R. K. Robins and G. H. Hitchings, *ibid.*, **78**, 973 (1956), and other work listed in these references.

susceptible to nucleophilic attack than is position "6." Since the course of any given reaction is also dependent on the particular group being replaced, compounds of the formula XX could react with a nucleophilic reagent to give displacement of the group "Y" in position "4" or the "Cl" atom of position "6." All nucleophilic reagents which were tried with XX, Y = Cl, gave, as expected, replacement of the chlorine atom in position "4." Most of these reactions already have been discussed. The case Y = OCH_3 is an interesting one since Bunnett and Zahler[9] list the methoxyl group as much less readily replaced by nucleophilic attack than the chlorine atom. However, when 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine (XX, Y = OCH_3) was treated with alcoholic ammonia at 100°, 4-amino-6-chloropyrazolo(3,4-d)-pyrimidine (XVIII) was the only compound isolated. Similarly, aqueous methylamine heated on the steam-bath with XX, Y = OCH_3, gave 4-methylamino-6-chloropyrazolo(3,4-d)pyrimidine (XIV). As the case with VI when 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine was heated with aqueous dimethylamine, the only product isolated was 4,6-bis-(dimethylamino)-pyrazolo(3,4-d)pyrimidine (IX, R_1, R_2 = CH_3).

When 4-methylmercapto-6-chloropyrazolo(3,-4-d)pyrimidine (XX, Y = SCH_3) was treated with alcoholic ammonia or aqueous alkylamines, the methylmercapto group was similarly removed preferentially. It is interesting to note that the "4"-methylmercapto group can be replaced in this instance by simply heating an aqueous amine on the steam-bath. In the purine series replacement of the methylmercapto group[10] by amines has been achieved in sealed tubes at elevated temperatures. It would therefore seem that the chlorine atom in position "6" of III exerts a definite activating influence on the methylmercapto group in position "4."

Reaction of III and boiling normal sodium hydroxide gave 4-hydroxy-6-chloropyrazolo(3,4-d)-pyrimidine (VII). As with XX, Y = OCH_3, 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III) and aqueous dimethylamine gave 4,6-bis-(dimethylamino)-pyrazolo(3,4-d)pyrimidine (IX, R_1, R_2 = CH_3) as the only product isolated.

Compound XX, Y = SH, presents an interesting case since nucleophilic replacement of the mercapto group in position "4" of the pyrazolo(3,4-d)-pyrimidine ring already has been reported.[7] Treatment of 4-mercapto-6-chloropyrazolo(3,4-d)pyrimidine (XX, Y = SH) with aqueous methylamine on the steam-bath resulted in replacement of the "6"-chloro atom to give 4-mercapto-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XXI) instead of the bis compound IX, R_1, R_2 = CH_3.

Confirmation of the structure assigned to XXI was obtained by treating 4-hydroxy-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XXII) with phosphorus pentasulfide in pyridine to yield a compound identical to XXI.

4-Hydroxy-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XXII) was prepared by treatment of 4-hydroxy-6-chloropyrazolo(3,4-d)pyrimidine (VII) with aqueous dimethylamine for 24 hr. on the steam-bath. Aqueous methylamine and other amines reacted with VII under similar conditions.

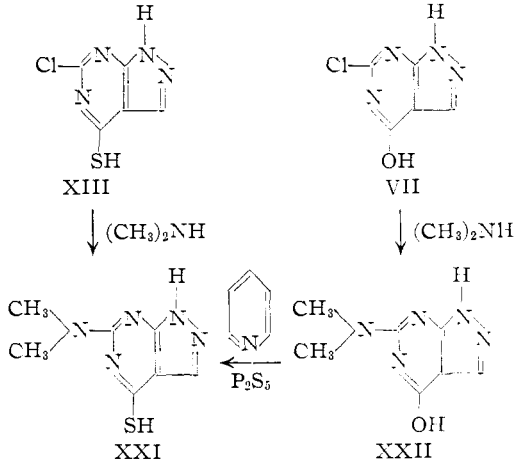


Table III lists the 4-hydroxy-6-substituted-aminopyrazolo(3,4-d)pyrimidines prepared in this manner. Reaction of VII and alcoholic ammonia heated to 200° gave a good yield of 4-hydroxy-6-aminopyrazolo(3,4-d)pyrimidine (VIII), the analog of guanine. Thiation of VIII in a manner similar to that employed by Elion and Hitchings[11] for the preparation of "6-thioguanine" gave 4-mercapto-6-aminopyrazolo(3,4-d)pyrimidine (XII). A preliminary report[12] describing the properties of a compound assigned the structure VIII has already appeared. Later studies carried out in this Laboratory have revealed that this compound is in reality 4-amino-6-hydroxypyrazolo(3,4-d)pyrimidine, the analog of isoguanine.

A careful comparison of the ultraviolet absorption spectra of 4-amino-6-hydroxypyrazolo(3,4-d)-pyrimidine previously synthesized[5] by fusion of 4-amino-3-cyanopyrazole and urea and that reported for 4-hydroxy-6-aminopyrazolo(3,4-d)pyrimidine[12] revealed the compounds to be identical. Paper chromatographic analysis carried out in two different solvent systems showed identical R_f values as further confirmation. The erroneous preliminary report[12] was based on the reaction of aqueous ammonia at 100° with 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III). It was at first expected in earlier experiments that III and aqueous ammonia at 100° would give 4-methylmercapto-6-aminopyrazolo(3,4-d)pyrimidine (XXIV). Since a compound was obtained which analyzed for $C_6H_7N_5S$, it was assumed to be XXIV. The product thus obtained has now been shown to be 4-amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIII) rather than the expected isomer, 6-amino-4-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIV). The structure XXIII was established by comparison of ultraviolet and infrared spectra of this preparation and the compound, 4-amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine, previously reported[5] prepared from 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine and

(9) J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).

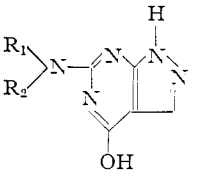
(10) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

(11) G. B. Elion and G. H. Hitchings, *ibid.*, **77**, 1676 (1955).

(12) R. K. Robins, *J. Org. Chem.*, **21**, 489 (1956).

TABLE III

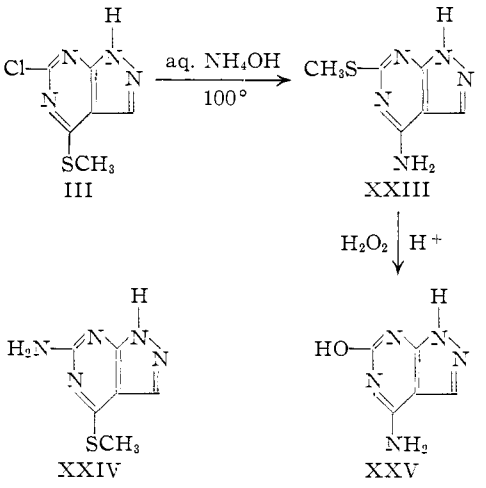
4-HYDROXY-6-SUBSTITUTED AMINOPYRAZOLO(3,4-d)PYRIMIDINES



R_1	R_2	Formula	Yield, %	Recrystn. solvents	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	U.v. absorption λ_{max}, mμ pH 1	U.v. absorption λ_{max}, mμ pH 11
H	CH_3	$C_6H_7N_5O$	62	Dimethylformamide–water	43.7	4.2	42.4	43.4	4.5	42.8	251	250 266
H	n-C_3H_7	$C_8H_{11}N_5O$	76	Ethanol–water	49.8	5.7		50.0	6.1		251	250
H	NH_2	$C_5H_6N_6O\cdot{}^1/_2H_2O$	70	Ethanol–water	34.3	4.0		34.4	4.0		252	255
CH_3	CH_3	$C_7H_9N_5O$	67	Dimethylformamide–water	46.9	5.0	39.1	47.1	5.2	39.4	252	252 275
H	$(CH_2)_3N(CH_3)_2$	$C_{10}H_{16}N_5O\cdot2HCl$	86	Ethanol	38.8	5.9	27.2	38.8	6.1	27.8	254	255 267

aqueous ammonia. The reaction product of III and aqueous ammonia at 100° when treated with nitrous acid at 80° gave 4-hydroxy-6-methylmercaptopyrimidine[5] as final evidence of structure XXIII.

This reaction of 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III) and aqueous ammonia at 100° would appear to involve either a rearrangement of some type in the pyrimidine ring or conceivably nucleophilic displacement of the methylmercapto group in position "4" followed by displacement of the chlorine atom in position "6" by the CH_3S^- ion which is not permitted to escape from the sealed container. A number of experiments are presently being conducted to investigate this reaction further.

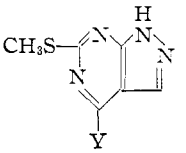


III XXIII XXIV XXV

The isomeric compound 4-methylmercapto-6-aminopyrazolo(3,4-d)pyrimidine(XXIV) has now been prepared successfully by methylation of 4-mercapto-6-aminopyrazolo(3,4-d)pyrimidine (XII) with methyl sulfate. Since XXIII was obtained from III with aqueous ammonia at 100° instead of XXIV, it is readily explained why hydrogen peroxide oxidation of the reaction product gave the isoguanine analog XXV instead of the guanine analog VIII.

A further consideration of nucleophilic attack on XX, Y = OH, NH_2 and $HNCH_3$ has shown that replacement of the "6"-chloro atom occurred before replacement of group "Y." This is not unexpected since the groups OH, NH_2 and $HNCH_3$ are not replaced readily by nucleophilic reagents.

In view of the ease of replacement of "Y" in compounds of the type XX when "Y" was the methoxyl or methylmercapto group, several additional experiments were carried out with compounds of the general formula XXVI.



XXVI, Y = OCH_3, SCH_3

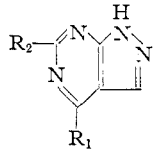
The synthesis of XXVI, Y = OCH_3, has previously been reported.[5] Aqueous methylamine and aqueous dimethylamine and XXVI, Y = OCH_3, heated on the steam-bath resulted in the preparation of 4-methylamino-6-methylmercaptopyrazolo-(3,4-d)pyrimidine and 4-dimethylamino-6-methylmercaptopyrazolo(3,4-d)pyrimidine,[5] respectively. Similarly, with XXVI, Y = SCH_3, under identical reaction conditions, the 4-methylmercapto group was replaced to yield the same 4-substituted-amino - 6 - methylmercaptopyrazolo(3,4 - d)pyrimidines as obtained with XXVI, Y = OCH_3. In the case of the reactions of XXVI with aqueous dimethylamine only the group in position "4" was replaced in contrast to the reaction of VI, III and X with dimethylamine under the same experimental conditions. 4-Methylamino-6-methylmercaptopyrazolo(3,4-d)pyrimidine also was synthesized by the reaction of 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine and aqueous methylamine in a manner previously described[5] for the preparation of 4-substituted-amino-6-methylmercaptopyrazolo(3,4-d)pyrimidines.

The ultraviolet absorption spectra of a number of the 4,6-disubstituted pyrazolo(3,4-d)pyrimidines are described in Table IV. When compared with the spectra of the corresponding purine analogs where data are published, there is marked similarity in most instances.

With regard to biological activity of the compounds described here, to date only the guanine an-

TABLE IV

ULTRAVIOLET ABSORPTION SPECTRA OF SOME 4,6-DISUBSTITUTED PYRAZOLO(3,4-d)PYRIMIDINES



R_1	R_2	λ_{max} pH 1	E	λ_{max} pH 11	E
Cl	Cl	271	7,600	278	6,200
SCH_3	Cl	228	13,000	254	6,400
		297	13,000	292	12,000
SCH_3	OH	259	7,850	260	7,300
		316	7,300	310	9,100
OH	Cl	253	8,500	263	9,900
OH	NH_2	252	7,200	251	9,100
$HNCH_3$	Cl	278	11,900	278	12,500
H_3CNCH_3	H_3CNCH_3	270	27,900	242	21,800
				284	8,900
SC_2H_5	Cl	297	15,800	255	14,100
			6,800	293	
NCH_3	H_3CNCH_3	264	13,000	235	29,000
				284	11,400
$HNCH_3$	SCH_3	235	14,600	244	25,400
		275	21,500	275	14,200
OCH_3	Cl	276	12,000	260	8,400
SH	Cl	325	18,400	315	15,200
OCH_3	OCH_3	261	14,900	262	9,500
		233	12,800	250	8,700
SH	NH_2	258	7,500	277	10,500
		327	18,500	328	16,400
NH_2	Cl	265	10,000	265	10,000
				289	10,000
$NHCC_6H_5$	H_3CNCH_3	265	22,000	240	25,700
H_3CNCH_3	SCH_3	240	11,500	246	25,000
		275	20,600	280	14,200
NH_2	NH_2	255	21,100	255	8,100
				274	9,400
		235	16,200	233	25,100
SH	$HNCH_3$	265	9,300	284	16,500
		328	18,000	314	14,000
		240	18,700	236	26,000
SH	H_3CNCH_3	269	11,900	255	14,200
		330	17,200	292	17,500
				321	12,500
SCH_3	NH_2	285	19,800	300	8,700

alog 4-hydroxy-6-aminopyrazolo(3,4-d)pyrimidine (VIII) has shown significant anti-tumor activity.[4] However, further screening is in progress, the detailed results of which will be reported elsewhere.

Acknowledgment.—The author wishes to thank Jack D. Davidson and Philip Feigelson of the Department of Biochemistry and Medicine, Columbia University, for their helpful assistance and suggestions relative to this work.

Experimental[13]

Preparation of 4,6-Dichloropyrazolo(3,4-d)pyrimidine (VI).—To a 3-liter 3-necked round-bottom flask was added 200 g. of purified and finely powdered 4,6-dihydroxypyrazolo(3,4-d)pyrimidine (V),[5] 1300 ml. of redistilled phosphorus oxychloride and 500 ml. of C.P. N,N-diethylaniline (mono free). The flask was equipped with three condensers and the reaction mixture cautiously heated. When the vigorous initial reaction had subsided, the reaction mixture was gently refluxed for 2 hr. By this time all the solid material had gone into solution. Approximately 900 ml. of excess phosphorus oxychloride was removed under reduced pressure using a steam-bath as a source of heat. The dark, sirupy residue was carefully poured, with stirring, onto crushed ice and water. The cold aqueous solution was extracted with ether (3 × 1000 ml.). The ethereal solution was washed well with water and dried over anhydrous sodium sulfate. Removal of the ether by distillation left 128 g. of crude, tan product, m.p. 143° dec. This product was further purified by extraction in a Soxhlet extractor for 5 hr. with a solvent mixture consisting of 250 ml. of dry benzene and 250 ml. of cyclohexane. This solution was then evaporated to 200 ml. and poured slowly, with stirring, into one liter of low boiling petroleum ether. The yield of light-yellow solid was 105 g., m.p. 145° dec. This product was used directly for most of the reactions described in this paper. Recrystallization from a benzene–heptane mixture gave colorless crystals melting with decomposition at 145°.

Anal. Calcd. for $C_5H_2N_4Cl_2$: C, 31.8; H, 1.1; N, 29.6. Found: C, 31.7; H, 1.3; N, 29.6.

Preparation of 4-Hydroxy-6-chloropyrazolo(3,4-d)pyrimidine (VII). Method 1.—To 400 ml. of a boiling and vigorously stirred 2 *N* solution of potassium hydroxide was carefully added, a little at a time, 40 g. of purified 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI). The addition took 15 minutes, after which time charcoal was added, and the solution was gently boiled and stirred for 15 minutes more. The solution was then filtered and the hot filtrate acidified with acetic acid and allowed to cool to 30°. The light-green solution was then filtered from approximately 1 g. of solid impurities and the filtrate further cooled for 48 hr. in the refrigerator. The yield of almost colorless product was 24.1 g. The ultraviolet absorption spectrum showed this material to be approximately 95% pure, judged on the basis of extinction coefficients. This material was recrystallized from water to give colorless crystals, m.p. >300°. This compound was dried at 130° for analysis.

Anal. Calcd. for $C_5H_3N_4OCl$: C, 35.2; H, 1.8; N, 32.9. Found: C, 35.4; H, 2.1; N, 33.0.

VII also was obtained in 60% yield when III was refluxed 3 hr. with normal sodium hydroxide solution.

Preparation of 4-Methylmercapto-6-hydroxypyrazolo(3,-4-d)pyrimidine (II).—To 3 liters of water was added 135 g. of 4-mercapto-6-hydroxypyrazolo(3,4-d)pyrimidine.[5] To this suspension was added 100 g. of solid potassium hydroxide, and the solution was stirred for 1 hr. By this time all the 4-mercapto-6-hydroxypyrazolo(3,4-d)pyrimidine had dissolved, and the temperature of the solution was 32°. Then 115 g. of C.P. methyl iodide was added and the solution vigorously stirred for 45 minutes. During this time the temperature rose to 37°. The solution was then carefully warmed to 50° and then neutralized with acetic acid and the product filtered immediately. The crude precipitate was washed with water and dried at 100° to give a yield of 120 g. A small sample was recrystallized from water for analysis.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.5; H, 3.3. Found: C, 39.1; H, 3.6.

Preparation of 4-Methylmercapto-6-chloropyrazolo(3,-4-d)pyrimidine (III). Method 1.—To 1200 ml. of redistilled phosphorus oxychloride were added 108 g. of 4-methylmercapto-6-hydroxypyrazolo(3,4-d)pyrimidine (II) and 100 ml. of N,N-dimethylaniline. The solution was refluxed gently for 1 hr. and then treated as for the isolation of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) to yield 63 g. of a tan product, m.p. 185–190° dec. This product was recrystallized from toluene to give 49.5 g. of light-tan needles, m.p. 197–199° dec. A second recrystallization from toluene gave an analytically pure sample, white needles, m.p. 200–201° dec.

Anal. Calcd. for $C_6H_5N_4ClS$: C, 35.9; H, 2.5; N, 27.9. Found: C, 36.1; H, 2.7; N, 28.1.

Method 2.—A solution of 5.0 g. of potassium hydroxide and 100 ml. of methanol and 20 ml. of methanethiol was cooled to 0°. To this solution was added 4.0 g. of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI). The solution was kept at 0° for 15 minutes. Finally 50 ml. of ice-water was

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

added and the solution acidified immediately with acetic acid. A yellow precipitate appeared which was filtered, dried and recrystallized from toluene. The yield of colorless needles was 3.5 g., m.p. 198–199° dec. A second recrystallization from toluene raised the m.p. to 200–201° dec. A mixed m.p. of this compound and the 4,6-bismethylmercaptopyrazolo(3,4-d)pyrimidine (IV) was 165–170°. Mixed m.p. of this compound and that prepared by method 1 was 200–201° dec.

Anal. Calcd. for $C_6H_5N_4ClS$: C, 35.9; H, 2.5. Found: C, 35.9; H, 2.4.

Method 3.—To 100 ml. of water containing 4.4 g. of sodium hydroxide cooled to 10° were added 5.0 g. of 4-mercapto-6-chloropyrazolo(3,4-d)pyrimidine (XIII) and 6 g. of methyl iodide. This solution was vigorously shaken for 10 minutes, stirred with charcoal and filtered. The filtrate was acidified with acetic acid and the product filtered to give 3.6 g. of crude III. Two recrystallizations from toluene gave 1.1 g. of pure material, m.p. 200–201° dec. Mixed m.p. data and ultraviolet absorption spectra showed this compound to be identical to that prepared by methods 1 and 2.

Preparation of 4-Mercapto-6-chloropyrazolo(3,4-d)pyrimidine (XIII).—To 200 ml. of 0.5 *N* sodium hydroxide previously saturated with hydrogen sulfide and cooled to 0° was carefully added 5.0 g. of powdered 4,6-dichloropyrazolo-(3,4-d)pyrimidine (VI). The solution was stirred for 15 minutes at 0° and then gradually allowed to warm to 10°. A small amount of charcoal was added and the solution filtered. The cold filtrate was immediately acidified with acetic acid and the yellow product filtered and washed with water. The yield was 4.9 g. A small quantity was precipitated with acetic acid from a hot very dilute sodium hydroxide solution to give an analytically pure sample after drying at 130°.

Anal. Calcd. for $C_5H_3N_4ClS$: C, 32.2; H, 1.6. Found: C, 32.5; H, 1.3.

Preparation of 4-Methylamino-6-chloropyrazolo(3,4-d)-pyrimidine (XIV). Method 1.—To 75 ml. of 25% aqueous methylamine was added 5.0 g. of 4,6-dichloropyrazolo(3,-4-d)pyrimidine (VI). The solution was heated for 20 minutes on the steam-bath, and the initially clear reaction mixture gradually deposited yellow crystals. The solution was filtered hot and the precipitate washed with water. The crude yield was 3.9 g., m.p. >300°. The product was dissolved in dimethylformamide and a small amount of water added. The solution was boiled with charcoal, filtered and cooled to yield white crystals, 3.1 g.

Anal. Calcd. for $C_6H_6N_5Cl$: C, 39.2; H, 3.3; N, 38.1. Found: C, 39.4; H, 3.3; N, 37.7.

Method 2.—4-Methylmercapto-6-chloropyrazolo(3,4-d)-pyrimidine (III) and aqueous methylamine were allowed to react as in method 1 to give 85% yield of XIV.

Anal. Calcd. for $C_6H_5N_5Cl$: N, 38.1. Found: N, 38.3.

Method 3.—4-Methoxy-6-chloropyrazolo(3,4-d)pyrimidine (X) and aqueous methylamine were allowed to react as in method 1 to give an 80% yield of XIV. The products obtained by methods 1, 2 and 3 showed identical ultraviolet absorption spectra at *p*H 1 and 11.

Preparation of 4-Methoxy-6-chloropyrazolo(3,4-d)pyrimidine (X).—To a solution of sodium methoxide made by dissolving 5.0 g. of sodium in 150 ml. of absolute methanol were added, in small portions, 6.0 g. of 4,6-dichloropyrazolo(3,-4-d)pyrimidine (VI). The solution was warmed until all the VI had dissolved. The insoluble sodium chloride was filtered and the filtrate immediately evaporated to dryness under reduced pressure. To the residue was added 100 ml. of water, and the clear, yellow solution was then acidified with acetic acid. After cooling overnight, the solution was filtered to give 4.8 g. of crude product, m.p. 175–180°. Recrystallization from benzene raised the m.p. to 181–182°.

Anal. Calcd. for $C_6H_5N_4ClO$: C, 39.0; H, 2.7. Found: C, 39.4; H, 2.8.

Preparation of 4-Ethoxy-6-chloropyrazolo(3,4-d)pyrimidine.—Five grams of VI was treated with sodium ethoxide in ethanol in a manner similar to that employed in the preparation of X. After recrystallization from benzene, 1.8 g. of product was obtained, m.p. 212–214°.

Anal. Calcd. for $C_7H_7N_4ClO$: C, 42.4; H, 3.5. Found: C, 42.1; H, 3.6.

Preparation of 4,6-Bis-methylmercaptopyrazolo(3,4-d)pyrimidine (IV). Method 1.—To a solution of 5 g. of potassium hydroxide, 10 ml. of methanethiol and 100 ml. of water was added 2.0 g. of 4-methylmercapto-6-chloropyrazolo-(3,4-d)pyrimidine (III). The solution was heated on the steam-bath for 30 minutes and then acidified with acetic acid. The product was filtered, dried and recrystallized from toluene to give 1.1 g., m.p. 197–198°.

Falco and Hitchings[7] record 188–189° for this compound. The mixed m.p. of this product and III was 165–170°.

Anal. Calcd. for $C_7H_8N_4S_2$: C, 39.6; H, 3.8; N, 26.4. Found: C, 39.5; H, 3.8; N, 26.5.

Method 2.—To an aqueous solution of 10 g. of potassium hydroxide, 100 ml. of water and 15 ml. of methylmercaptan was added 5.0 g. of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI). The solution was heated on the steam-bath for 4 hr. The solution was acidified with acetic acid and the product filtered, dried and recrystallized from toluene as in method 1 to yield a product, m.p. 197°; mixed m.p. of this compound and that prepared by method 1 showed no depression.

Anal. Calcd. for $C_7H_8N_4S_2$: N, 26.4. Found: N, 26.4.

Preparation of 4,6-Dimethoxypyrazolo(3,4-d)pyrimidine.—The preparation of this compound was carried out in a manner identical to that for the preparation of 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine (X), except that the reaction mixture was refluxed 36 hr. on the steam-bath, then evaporated to 50 ml., and 100 ml. of water was added. The solution was then acidified with acetic acid and allowed to cool. The yield from 5.0 g. of VI was 3.2 g., m.p. 210–214°. Recrystallization from a benzene–ethanol mixture raised the m.p. to 222–223°.

Anal. Calcd. for $C_7H_8N_4O_2$: C, 46.7; H, 4.4; N, 31.1. Found: C, 46.5; H, 4.5; N, 31.5.

Preparation of 4-Hydroxy-6-aminopyrazolo(3,4-d)pyrimidine (VIII).—Ten grams of 4-hydroxy-6-chloropyrazolo-(3,4-d)pyrimidine (VII) was added to 100 ml. of alcoholic ammonia (absolute ethanol saturated with dry ammonia at 0°). The solution was placed in a high pressure bomb and heated at 200° (inside temperature) for 12 hr. The solution was cooled and filtered. The white product was further purified by suspending the material in 300 ml. of boiling water followed by the addition of just enough concentrated hydrochloric acid to effect solution. To the clear solution was carefully added ammonium hydroxide until a *p*H of 9 was reached. The product was immediately filtered from the hot solution and washed with distilled water. The yield of purified product was 7.6 g.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.8; H, 3.3; N, 46.4. Found: C, 39.6; H, 3.4; N, 46.2.

Preparation of 4-Mercapto-6-aminopyrazolo(3,4-d)pyrimidine (XII).—Six grams of 4-hydroxy-6-aminopyrazolo-(3,4-d)pyrimidine (VIII) was finely powdered and mixed with 35 g. of powdered phosphorus pentasulfide. This mixture was added to 300 ml. of C.P. pyridine and the solution refluxed for 3 hr. The solids went into solution upon heating, and gradually a yellow solid precipitated from the hot solution. The reaction mixture was allowed to cool and was then filtered. The yellow solid thus obtained was added to 500 ml. of water and heated on the steam-bath overnight. Then 50 ml. of concentrated ammonium hydroxide was added and the solution heated on the steam-bath until the odor of ammonia no longer could be detected. The cooled solution was filtered and the solid added to 700 ml. of boiling water, and concentrated ammonium hydroxide was carefully added until solution was effected. The solution was boiled with charcoal and the hot filtrate acidified with acetic acid to yield 4.1 g. of light-green needles, m.p. > 300°.

Anal. Calcd. for $C_5H_5N_5S$: C, 35.9; H, 3.0; N, 41.9. Found: C, 36.0; H, 3.2; N, 42.3.

Preparation of 4-Hydroxy-6-mercaptopyrazolo(3,4-d)pyrimidine from 4-Hydroxy-6-chloropyrazolo(3,4-d)pyrimidine (VII).—Two grams of 4-hydroxy-6-chloropyrazolo(3,4-d)-pyrimidine (VII) and 4.0 g. of thiourea were added to 100 ml. of absolute ethanol. The solution was heated for 4 hr. then cooled and filtered. The ultraviolet absorption spectra at *p*H 1, as previously reported,[5] showed a maximum at 248 mμ, and 287 mμ at *p*H 11. 4-Hydroxy-6-mercapto-

pyrazolo(3,4-d)pyrimidine prepared by fusion of thiourea and 3-amino-4-pyrazolecarboxamide[5] and the product obtained from VII exhibited identical ultraviolet absorption spectra.

Preparation of 4-Substituted-amino-6-chloropyrazolo(3,4-d)pyrimidines Listed in Table I. Method A.—Five grams of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) were carefully powdered and added carefully to 150 ml. of 20–40% aqueous solution of the appropriate primary amine. The clear solution was then heated on the steam-bath for 10 to 30 minutes. During this time a precipitate of the product usually appeared in the hot solution. The solution was allowed to cool and then filtered. The yield recorded is the crude yield obtained at this point. For purification the compound was recrystallized from the indicated solvents. None of these products possessed a definite melting point. Decomposition gradually occurred above 200°.

Method B.—The procedure employed was to dissolve the appropriate amine, 10–15 g., in absolute ethanol and then add 5.0 g. of VI and heat on the steam-bath for 15 to 30 minutes. The cooled solution yielded the product.

Preparation of 4,6-Bis-(substituted-amino)-pyrazolo(3,4-d)pyrimidines Listed in Table II.—The synthesis of these compounds is probably best illustrated by the method used in the preparation of 4,6-bis-(methylamino)-pyrazolo(3,4-d)pyrimidine (IX, R_1 = H, R_2 = CH_3). One gram of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) and 150 ml. of 30% aqueous methylamine were heated on the steam-bath for 12 hr. The initial precipitate of XIV slowly dissolved. More methylamine was added and the solution heated another 12 hr. on the steam-bath. The resulting, clear solution was cooled overnight to yield 0.7 g. of white crystals, m.p. 245–247°. Recrystallization from water raised the m.p. to 248–250°.

Anal. Calcd. for $C_7H_{10}N_6$: C, 47.2; H, 5.6; N, 47.2. Found: C, 46.8; H, 5.6; N, 47.2.

Preparation of 4-Hydroxy-6-substituted-aminopyrazolo(3,4-d)pyrimidines Listed in Table III.—The general method of synthesis can be illustrated by the preparation of 4-hydroxy-6-methylaminopyrazolo(3,4-d)pyrimidine (XI, R_1 = H, R_2 = CH_3). Two grams of 4-hydroxy-6-chloropyrazolo(3,4-d)pyrimidine (VII) was added to a solution of 150 ml. of 40% aqueous methylamine. The solution was heated for 8 hr. on the steam-bath; 100 ml. more of 40% aqueous methylamine was added and the solution heated 8 hr. more. This process was repeated again for a total of 24 hr. reaction time. The solution was then cooled and filtered and the crude product washed with water and recrystallized from a mixture of dimethylformamide and water and finally dried at 130°.

Anal. Calcd. for $C_6H_7N_5O$: C, 43.7; H, 4.2; N, 42.4. Found: C, 43.3; H, 4.5; N, 42.8.

Preparation of 4-Methylamino-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XV).—Three grams of 4-methylamino-6-chloropyrazolo(3,4-d)pyrimidine (XIV) was heated with 150 ml. of 20% aqueous solution of dimethylamine on the steam-bath for 8 hr. Then 100 ml. more of aqueous dimethylamine was added and the reaction heated an additional 8 hr. The solution was then filtered from a very small amount of insoluble material and the filtrate allowed to cool. The solid was filtered and recrystallized twice from an ethanol–water mixture to yield 1.9 g., m.p. 272–273°.

Anal. Calcd. for $C_8H_{11}N_6$: C, 50.0; H, 6.3; N, 43.7. Found: C, 50.4; H, 6.4; N, 43.4.

Preparation of 4-Benzylamino-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XVII).—To 100 ml. of 30% aqueous dimethylamine was added 2.0 g. of 4-benzylamino-6-chloropyrazolo(3,4-d)pyrimidine (XVI). This solution was heated on the steam-bath for 8 hr. An additional 100 ml. of aqueous dimethylamine were added and the solution heated an additional 16 hr. on the steam-bath. The solution was then cooled and filtered and the crude product recrystallized from ethanol and water to give 1.5 g., m.p. 255–256°.

Anal. Calcd. for $C_{14}H_{16}N_6$: C, 62.7; H, 6.0. Found: C, 62.5; H, 6.3.

Preparation of 4-Amino-6-chloropyrazolo(3,4-d)pyrimidine (XVIII). Method 1.—To 100 ml. of alcoholic ammonia (absolute ethanol saturated with dry ammonia at 0°) was added 12.0 g. of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI). The solution was heated in a bomb at 100° (inside temperature) for 12 hr. The cooled solution was filtered and the product washed with water. The crude product was suspended in 800 ml. of boiling water. Just enough potassium hydroxide was added to effect solution, and the solution was boiled gently with charcoal for 10 minutes. The hot filtrate was acidified with acetic acid and the product filtered immediately to yield 6.4 g. of white crystals. This product was above 90% pure as judged by the ultraviolet absorption spectra. Further purification was effected by reprecipitation from hot dilute basic solution. Finally, for analysis a sample was recrystallized from dimethylformamide and water. The compound gradually decomposed above 250°.

Anal. Calcd. for $C_5H_4N_5Cl$: C, 35.4; H, 2.4; N, 41.3. Found: C, 35.8; H, 2.8; N, 41.6.

Method 2.—Two grams of 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine (X) was heated with alcoholic ammonia at 100° in a bomb for 8 hr. and the product isolated as in method (1) to yield 1.1 g. of purified product.

Anal. Calcd. for $C_5H_4N_5Cl$: N, 41.3. Found: N, 41.8.

4-Methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III) under similar conditions yielded the same product XVIII as judged by the ultraviolet absorption spectra of the product and that obtained by methods 1 and 2.

Preparation of 4,6-Dimercaptopyrazolo(3,4-d)pyrimidine from 4,6-Dichloropyrazolo(3,4-d)pyrimidine (VI).—A solution of 10 g. of thiourea, 150 ml. of ethanol and 5.0 g. of VI was refluxed 3 hr. on the steam-bath. The cooled solution was filtered to yield 4.1 g. of yellow 4,6-dimercaptopyrazolo(3,4-d)pyrimidine. The product was identified by its ultraviolet spectra.[5,7]

Preparation of 4-Mercapto-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XXI). Method 1.—Two grams of 4-mercapto-6-chloropyrazolo(3,4-d)pyrimidine (XIV) and 100 ml. of 20% aqueous dimethylamine were heated on the steam-bath for 6 hr. The solution was then cooled and filtered to give 1.8 g. of crude product. Recrystallization from water and dimethylformamide gave an analytically pure sample.

Anal. Calcd. for $C_7H_9N_5S$: C, 43.1; H, 4.6; N, 35.9. Found: C, 43.0; H, 4.2; N, 35.8.

Method 2.—Three grams of 4-hydroxy-6-dimethylaminopyrazolo(3,4-d)pyrimidine (XXII) and 15 g. of phosphorus pentasulfide were added to 500 ml. of pyridine. The solution was refluxed for 6 hr. and the excess pyridine removed under reduced pressure using a steam-bath as a source of heat. To the residue was added 300 ml. of water and the solution warmed gently on the steam-bath. The solution was cooled and filtered and the product purified by reprecipitation from hot dilute base and finally recrystallized from water and dimethylformamide to yield 0.8 g. of product. This material and that obtained by method 1 were shown to have identical ultraviolet absorption spectra at *p*H 1 and 11.

Preparation of 4-Amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIII). Method 1. From 4-Methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III).—Three grams of 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III), m.p. 199–200° dec., and 100 ml. of concentrated ammonium hydroxide were placed in a bomb and heated at 100° (inside temperature) for 8 hr. The cooled solution was filtered and the white product washed with water. The crude yield was 2.5 g. of product, m.p. 280–285°. Two recrystallizations from ethanol raised the m.p. to 297–298°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.7; H, 3.9; N, 38.6. Found: C, 39.4; H, 3.7; N, 39.0.

Method 2. From 4-Chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine.—The preparation of 4-amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIII) from 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine with aqueous ammonia heated on the steam-bath already has been reported.[5] Because of the relatively low yield obtained, this reaction was repeated with concentrated ammonium hydroxide at 100°.

Three grams of 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine,[5] m.p. 178–179° dec., was added to 100 ml. of concentrated ammonium hydroxide and the solution heated at 100° for 8 hr. in a bomb. The cooled solution

was filtered to give 2.6 g. of white product which melted at 297–298° when recrystallized from ethanol.

The mixed melting point of this product and that prepared by method 1 showed no depression. The ultraviolet absorption spectra of these two preparations at *p*H 1 and 11 were identical as were the infrared spectra.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.7; H, 3.9. Found: C, 39.6; H, 4.0.

Preparation of 4-Hydroxy-6-methylmercaptopyrazolo(3,4-d)pyrimidine from 4-Amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIII).—One gram of XXIII was added to 250 ml. of boiling water and enough hydrochloric acid added to effect solution (3 ml.). To the hot solution were carefully added 3 g. of sodium nitrite and the solution heated on the steam-bath for 10 minutes and then allowed to cool.

Filtration yielded 0.4 g. of 4-hydroxy-6-methylmercaptopyrazolo(3,4-d)pyrimidine which was identified by ultraviolet absorption spectra[5] at *p*H 1 and 11.

Preparation of 4-Amino-6-hydroxypyrazolo(3,4-d)pyrimidine (XXV) from 4-Amino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXIII).—To 100 ml. of water was added 1.5 g. of XXIII prepared by method 1, 10 ml. of concentrated hydrochloric acid and 10 ml. of 30% hydrogen peroxide. The solution was boiled for 20 minutes and then carefully neutralized while hot with concentrated ammonium hydroxide. The solution was filtered immediately and the precipitate washed with water to yield 0.8 g. of white product.

Anal. Calcd. for C_5H_5NO: C, 39.8; H, 3.3; N, 46.4. Found: C, 39.8; H, 3.5; N, 46.2.

A paper chromatogram of this compound was made using an 18-hr. descending method with propanol and ammonium hydroxide and Whatman #1 paper as described by Hanes and Isherwood.[14] An R_f value of 0.036 was obtained for this compound and 4-amino-6-hydroxypyrazolo(3,4-d)-pyrimidine[5] prepared by fusion of urea and 4-amino-3-cyanopyrazole. The R_f value for these two compounds in a concentrated hydrochloric acid–isopropyl alcohol–water system described by Wyatt[15] was found to be 0.50. The ultraviolet absorption spectra at *p*H 1 and 11 of XXVIII and that previously reported[5] for 4-amino-6-hydroxypyrazolo(3,4-d)pyrimidine were found to be identical.

Preparation of 4-Methylamino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXVI, Y = $HNCH_3$). Method 1.—Two grams of 4-methoxy-6-chloropyrazolo(3,4-d)pyrimidine[5] and 100 ml. of 30% aqueous methylamine were heated on the steam-bath for 4 hr. The solution was then cooled and filtered and the crude product recrystallized from ethanol–water to yield 1.2 g., m.p. 253–254°.

Anal. Calcd. for $C_7H_9N_5S$: C, 43.1; H, 4.6; N, 35.9. Found: C, 42.9; H, 4.8; N, 35.7.

Method 2.—To 100 ml. of 30% aqueous methylamine was added 1.5 g. of 4,6-bis-methylmercaptopyrazolo(3,4-d)pyrimidine (IV). The solution was heated on the steam-bath for 4 hr., then cooled and filtered. The crude product was recrystallized from 90% ethanol–water to give 0.9 g., m.p. 254–255°. Mixed m.p. of this compound and that prepared by method 1 showed no depression.

Method 3.—Three grams of 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine[5] and 150 ml. of 30% aqueous methylamine were heated for 4 hr. on the steam-bath. The solution was cooled, and the crude product filtered and recrystallized from dimethylformamide and water to give 1.7 g., m.p. 254–255°. The mixed m.p. of this product and that prepared by methods 1 and 2 showed no depression. The ultraviolet absorption spectra of all three preparations were identical at *p*H 1 and also at *p*H 11.

Preparation of 4-Dimethylamino-6-methylmercaptopyrazolo(3,4-d)pyrimidine (XXVI, Y = $N(CH_3)_2$). Method 1.—Two grams of 4-methoxy-6-methylmercaptopyrazolo(3,4-d)pyrimidine was heated with 130 ml. of 30% aqueous dimethylamine on the steam-bath for 1 hr. A precipitate appeared from the hot solution. The solution was cooled and filtered and the crude product recrystallized from ethanol–water to yield 1.1 g., m.p. 262–265°.

Anal. Calcd. for $C_8H_{11}N_5S$: C, 45.8; H, 5.3; N, 33.5. Found: C, 46.2; H, 5.6; N, 34.0.

Method (2).—To 100 ml. of 30% aqueous dimethylamine was added 1.5 g. of 4,6-bis-methylmercaptopyrazolo(3,4-d)-pyrimidine (IV). The solution was heated on the steam-bath for 3 hr. and the product isolated and recrystallized as in method 1 to give 0.8 g., m.p. 263–265°.

Anal. Calcd. for $C_8H_{11}N_5S$: C, 45.8; H, 5.3; N, 33.5. Found: C, 45.4; H, 5.8; N, 33.7.

The melting point for 4-dimethylamino-6-methylmercaptopyrazolo(3,4-d)pyrimidine previously has been reported[5] as 263–265°. The ultraviolet absorption spectra of the product prepared by methods 1 and 2 corresponded with that for the earlier reported[5] product.

Preparation of 4-Ethylmercapto-6-chloropyrazolo(3,4-d)-pyrimidine.—Three grams of VI was added to a solution of 3 g. of sodium hydroxide in 100 ml. of ethanol containing 15 ml. of ethanethiol. The solution was allowed to stand 30 minutes at room temperature and acidified with acetic acid. The crude product was filtered and dried and recrystallized from benzene–heptane mixture to give 1.6 g. of white needles, m.p. 149–150°.

Anal. Calcd. for $C_7H_7N_4ClS$: C, 39.1; H, 3.3; N, 26.1. Found: C, 39.5; H, 3.6; N, 26.3.

Preparation of 4-Methylmercapto-6-aminopyrazolo(3,4-d)pyrimidine (XXIV).—One gram of 4-mercapto-6-aminopyrazolo(3,4-d)pyrimidine (XII) was dissolved in 30 ml. of water containing 2.0 g. of sodium hydroxide. To this solution was added 0.5 ml. of dimethyl sulfate, and the solution was stirred for 20 minutes. The solution was then carefully acidified with acetic acid and allowed to stand. The crude product, 0.8 g., m.p. 235–237°, was filtered and recrystallized from water to raise the m.p. to 240–241°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.7; H, 3.9. Found: C, 39.3; H, 3.9.

Preparation of 4,6-Diaminopyrazolo(3,4-d)pyrimidine (XIX).—Six grams of 4,6-dichloropyrazolo(3,4-d)pyrimidine (VI) was added to 50 ml. of alcoholic ammonia (absolute ethanol saturated with dry ammonia at 0°) and the solution placed in a bomb and heated to 200° (inside temperature) for 24 hr. The solution was then evaporated to dryness on the steam-bath and the residue dissolved in 100 ml. of water containing 5 g. of sodium hydroxide. This solution was boiled with charcoal and the hot filtrate neutralized with acetic acid. The filtrate, after standing, deposited white needle-like crystals. This product was recrystallized from water and then dried in the oven at 130° for analysis.

Anal. Calcd. for $C_5H_6N_6$: C, 40.0; H, 4.0; N, 55.9. Found: C, 39.8; H, 3.9; N, 55.3.

The preparation of XIX also was accomplished in a similar manner when 4-methylmercapto-6-chloropyrazolo(3,4-d)pyrimidine (III) or 4-chloro-6-methylmercaptopyrazolo(3,4-d)pyrimidine[5] was substituted in this reaction for VI.

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(15) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).