broken by the reducing treatment and that an important fraction of these up to a maximum (in our experiments) equivalent to 42% of the original bonds were replaced by cross-links formed by reaction with the bis-maleimides.

Acknowledgments.—The authors are indebted to I. R. Hunter for advice concerning chromatography, to R. C. Landwehr and Mrs. I. R. Stuart for carrying out some of the physical measurements cited, and to G. E. Secor for the microchemical analyses. They also wish to express gratitude to Dr. H. P. Lundgren for his advice and encouragement during the course of this work.

ALBANY, CAL.

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

Potential Purine Antagonists. II. Synthesis of Some 7- and 5,7-Substituted Pyrazolo[4,3-d]pyrimidines[1]

BY ROLAND K. ROBINS, FREDERICK W. FURCHT, ALAN D. GRAUER AND JESSE W. JONES

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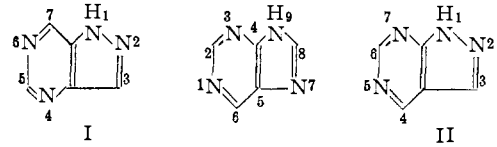
Isomeric structural analogs of various biologically important purines have been synthesized which possess the pyrazolo[4,3-d]pyrimidine ring system. A new route to this ring system has been accomplished beginning with 4-aminopyrazole-3-carboxylic acid. The chemistry of some of the derivatives of pyrazolo[4,3-d]pyrimidine is discussed and a comparison made with the corresponding "isomeric" purine and pyrazolo[3,4-d]pyrimidine ring systems.

In accord with a recent program for synthesis of purine antagonists[2] as potential chemotherapeutic agents against various tumors, it seemed desirable to investigate the synthesis of certain pyrazolo[4,3-d]pyrimidine derivatives. Recent synthesis of the isomeric pyrazolo[3,4-d]pyrimidine[2] ring system has resulted in the preparation of several new compounds with interesting antitumor properties.[3,4]

The structural relationship of pyrazolo[4,3-d]-pyrimidine (I), purine and pyrazolo[3,4-d]pyrimidine (II) is shown below.



I — Pyrazolo[4,3-d]-pyrimidine; Purine; II — Pyrazolo[3,4-d]-pyrimidine

The first reported synthesis of the pyrazolo-[3,4-d]pyrimidine ring was that of Behrend[5] who utilized 5-amino-6-methyluracil for the preparation of 5,7-dihydroxypyrazolo[4,3-d]pyrimidine which he called "isoxanthine."

Rose[6,7] has recently accomplished the synthesis of the pyrazolo[4,3-d]pyrimidine ring system (named 1:2:4:6-tetraazaindene by Rose) by diazotization of a 5-amino-6-methylpyrimidine followed by coupling to form the pyrazolo[4,3-d]-pyrimidine ring. This method although quite satisfactory is definitely restricted since a 5-amino-6-methylpyrimidine substituted at position "4" with a hydroxy, mercapto or amino group upon diazotization, couples to give the corresponding oxadiazole, thiadiazole or triazole ring.[7] Because of this difficulty Rose failed to prepare the analog of hypoxanthine, 6-mercaptopurine and adenine in the pyrazolo[4,3-d]pyrimidine series. In view of the work of Rose it seemed advisable to approach the synthesis of these desired derivatives from another route. Success in the synthesis of the corresponding pyrazolo[3,4-d]pyrimidines[2] from a pyrazole intermediate promptly suggested beginning with the pyrazole ring as a route worthy of investigation. The synthesis of 4-aminopyrazole-3-carboxylic (IV) acid by reduction of 4-nitropyrazole-3-carboxylic acid (III) has been reported by Knorr[8] in a footnote, but no experimental details are given. The study of the reduction of 4-nitropyrazole-3-carboxylic acid (III) required large quantities of 3-methyl-4-nitropyrazole from which III was prepared by oxidation with potassium permanganate.[8] The synthesis of 3-methyl-4-nitropyrazole was best accomplished by large scale decarboxylation of 3-methyl-4-nitropyrazole-5-carboxylic acid[9] rather than by nitration of 3-methylpyrazole.[8]

The reduction of 4-nitropyrazole-3-carboxylic acid (III) with sodium hydrosulfite gave 4-aminopyrazole-3-carboxylic acid (IV), in yield superior to any other method of reduction attempted. 4-Aminopyrazole-3-carboxylic acid heated with boiling formamide gave 7-hydroxypyrazolo[4,3-d]-pyrimidine (V), the analog of hypoxanthine, in approximately 30% yield.

4-Nitropyrazole-3-carboxylic acid (III) was esterified to give 4-nitropyrazole-3-ethylcarboxylate (VI) in above 60% yield. Treatment of VI with concentrated ammonium hydroxide gave almost a quantitative yield of 4-nitropyrazole-3-carboxamide (VII). Reduction of 4-nitropyrazole-3-carboxamide (VII) with hydrogen using a palladium-on-charcoal catalyst yielded 4-aminopyrazole-3-carboxamide (VIII). It might be noted that VIII is an isomeric analog of 5-aminoimidazole-4-carboxamide, a known purine precursor in various

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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

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

(4) T. C. Hsu, R. K. Robins and C. C. Cheng, *Science*, (in press).

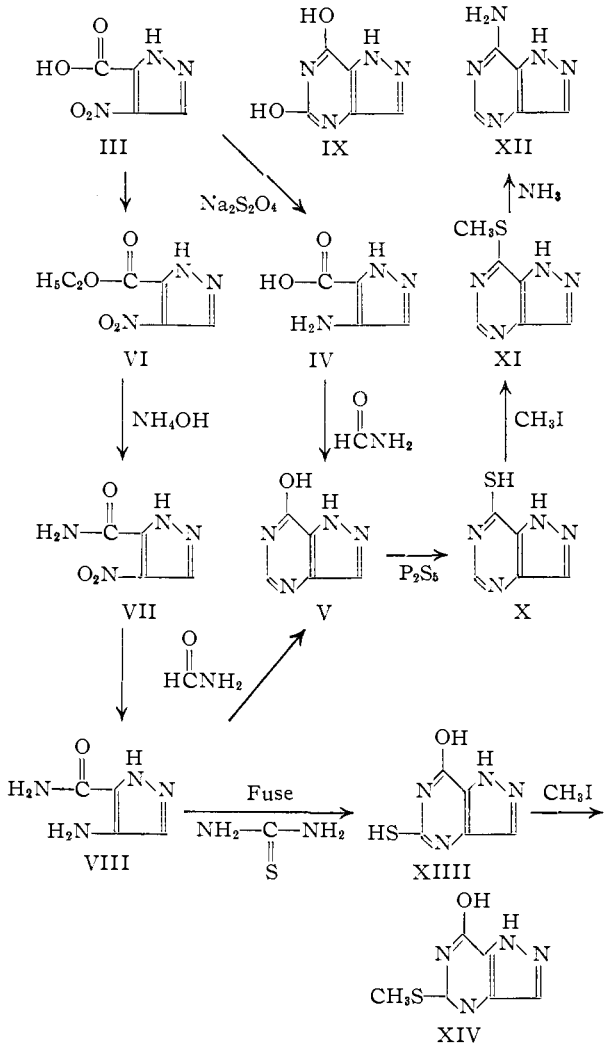
(5) R. Behrend, *Ann.*, **245**, 213 (1888).

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

(7) F. L. Rose, *ibid.*, 4116 (1954).

(8) Knorr, *Ann.*, **279**, 228 (1894).

(9) C. Musante, *Gazz. chim. ital.*, **75**, 121 (1945).



REACTION SCHEME I

biological systems,[10] and is of interest in its own right.

The isolation of VIII was accompanied by considerable discoloration and decomposition of the product. When formamide was added directly to the solution from the reduction of VII and the solution boiled vigorously, approximately 50% yield of 7-hydroxypyrazolo[4,3-d]pyrimidine (VI) was obtained based on the 4-nitropyrazole-3-carboxamide (VII) used.

Fusion of 4-aminopyrazole-3-carboxamide (VIII) with urea gave 5,7-dihydroxypyrazolo[4,3-d]pyrimidine (IX). It was found that this compound was identical to the compound termed "isoxanthine" by Behrend as shown by the identical ultraviolet absorption curves of IX and "isoxanthine" prepared by the method of Behrend.[5]

Treatment of 7-hydroxypyrazolo[4,3-d]pyrimidine (V) with phosphorus pentasulfide in pyridine gave 7-mercaptopyrazolo[4,3-d]pyrimidine (X), the analog of 6-mercaptopurine, in above 80% yield. Methylation of X with methyl iodide yielded 7-methylmercaptopyrazolo[4,3-d]pyrimidine (XI).

(10) M. P. Schulman and J. M. Buchanan, *J. Biol. Chem.*, **196**, 513 (1952).

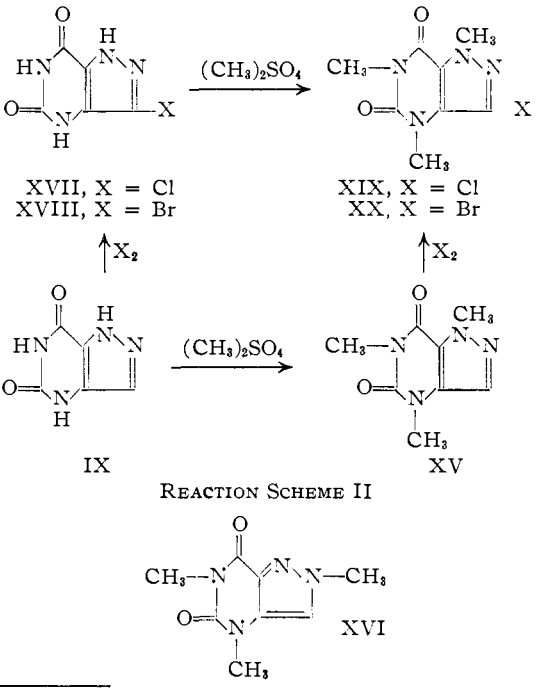
When XI was treated with alcoholic ammonia at 200°, 7-aminopyrazolo[4,3-d]pyrimidine (XII), the analog of adenine, was obtained.

Fusion of crude 4-aminopyrazole-3-carboxamide with thiourea yielded 5-mercapto-7-hydroxypyrazolo[4,3-d]pyrimidine (XIII). Methylation of XIII gave 5-methylmercapto-7-hydroxypyrazalo-[4,3-d]pyrimidine (XIV).

Treatment of XIV with alcoholic ammonia at 200° failed to yield the corresponding analog of guanine.

Methylation of 5,7-dihydroxypyrazolo[4,3-d]-pyrimidine with dimethyl sulfate in the presence of sodium hydroxide gave a compound $C_8H_{10}O_2N_4$ isomeric with caffeine, presumably 1,4,6-trimethylpyrazolo[4,3-d]pyrimidine-5,7-dione (XV), although the possibility of the isomeric structure XVI for this compound has not been entirely eliminated. It is interesting to note that under the conditions of the experiment only one isomer was isolated. Xanthine under similar conditions apparently gives only the 7-methylated isomer, caffeine.[11]

5,7-Dihydroxypyrazolo[4,3-d]pyrimidine (IX) was brominated by Behrend[5] to give 3-bromo-5,7-dihydroxypyrazolo[4,3-d]pyrimidine (XVIII). The preparation of this compound was repeated in this Laboratory. It also was found that 5,7-dihydroxypyrazolo[4,3-d]pyrimidine (IX) could be chlorinated under similar conditions to give 3-chloro - 5,7 - dihydroxypyrazolo[4,3 - d]pyrimidine (XVII). The relative mild conditions necessary for preparation of XVII and XVIII when compared to the sealed tube bromination employed to change xanthine to 8-bromoxanthine[12] led to a further study of these compounds. It was discovered that XVII and XVIII could be methylated to give a trimethyl derivative XIX and XX, respectively. Halogenation of 1,4,6-trimethylpyrazolo[4,3-d]-



REACTION SCHEME II

(11) H. Biltz and A. Beck, *J. Prakt. Chem.*, **226**, 207 (1928).

(12) E. Fischer, *Ber.*, **31**, 2563 (1898).

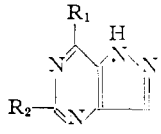
pyrimidine-5,7-dione (XV) gave products which were judged identical to XIX and XX, respectively, on the basis of melting points and mixed melting point behavior. It would thus appear that halogenation of 5,7-dihydroxypyrazolo[4,3-d]-pyrimidine (IX) takes place in position "three" as postulated by Behrend and that methylation of the 3-halogenated derivatives XVII and XVIII follows the same course as for 5,7-dihydroxy-pyrazolo[4,3-d]pyrimidine.

This is not unexpected since by analogy 8-chloroxanthine in the purine series is reported to give 8-chlorocaffeine[13] upon methylation with methyl iodide.

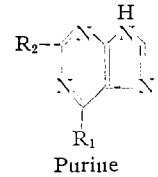
A comparison of the solubility in water of some of the derivatives of pyrazolo[4,3-d]pyrimidine with the corresponding purine derivatives is shown in Table I. In all instances studied the pyrazolo-[4,3-d]pyrimidine was more insoluble in water at 100° than the corresponding purine derivative. This also has been found to be the general case in the pyrazolo[3,4-d]pyrimidine series.[2] This might be explained on a theoretical basis since the tertiary nitrogen in the pyrazole ring should withdraw electrons from the adjacent "imine" nitrogen. This effect should make the hydrogen atom on the "imine" nitrogen more acidic or more readily available for hydrogen bonding in the crystal lattice. Albert, Brown and Cheeseman[14] have postulated that unusually strong crystal lattice forces due to hydrogen bonding are largely responsible for the insolubility of compounds of this type. It would follow that an increase in inter-molecular hydrogen bonding would decrease the opportunity for hydrogen bonding with water molecules.

TABLE I

COMPARISON OF SOLUBILITY OF CERTAIN ANALOGOUS PURINES AND PYRAZOLO[4,3-d]PYRIMIDINES IN AQUEOUS SOLUTION AT 100°



Pyrazolo[4,3-d]pyrimidine

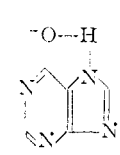


Purine

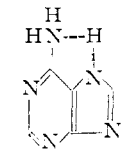
		Solubility in water at 100°, 1 part soluble in X parts water	
R_1	R_2	X Purine[15]	X Pyrazolo[4,3-d]-pyrimidine
OH	H	70	230
OH	OH	400	1000
NH_2	H	40	75
SH	H	180	400
SCH_3	H	20	185

Although the ultraviolet absorption spectra of the pyrazolo[3,4-d]pyrimidine derivatives resemble rather closely those of the corresponding purines, Table II shows that this is not the case with the pyrazolo[4,3-d]pyrimidine series. It can be seen that a rather general bathochromic shift is evident with the pyrazolo[4,3-d]pyrimidines studied. This shift to longer wave length is in general from 10 to 30 mμ when compared to the ultraviolet absorption spectra of the corresponding purines. The bathochromic shift is also accompanied in general by a rather definite hypochromic shift.

Mason[17] has suggested that the similarity of the ultraviolet absorption spectra of the hypoxanthine anion and adenine molecule (which show absorption maxima at 258 and 260 mμ, respectively) can be explained on the basis of similarity of structures XVII and XVIII.

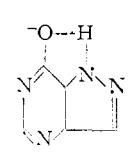


XVII

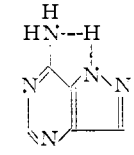


XVIII

If this postulate is true, one would expect that the ultraviolet absorption spectrum of the anion of 7-hydroxypyrazolo[4,3-d]pyrimidine (XIX) would be similar to the spectrum of the molecule, 7-aminopyrazolo[4,3-d]pyrimidine (XX).

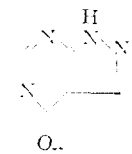


XIX

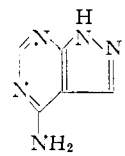


XX

It has now been found that the absorption maxima for XIX and XX are 280 and 294 mμ, respectively. In contrast, the corresponding structures in the pyrazolo[3,4-d]pyrimidine series[2] XXI and XXII in which internal hydrogen bonding of the type shown in XVII and XVIII is impossible, show



XXI



XXII

ultraviolet absorption maxima of 261 mμ for the anion XXI and 260 mμ for the compound XXII. It can be seen that the ultraviolet absorption spectrum of XXI resembles that of the hypoxanthine anion very closely and similarly the spectrum of XXII greatly resembles that of the adenine molecule. From these data it would appear that internal hydrogen bonding of the type suggested by Mason in structures XVII and XVIII does not contribute greatly to the ultraviolet absorption spectra of the hypoxanthine anion and adenine molecule in aqueous solution.

Infrared spectral studies of these compounds are now in progress in a further effort to study the structure of these and related molecules in the solid state.

Biological testing of these derivatives of the pyrazolo[4,3-d]pyrimidine ring system is incomplete and will be reported elsewhere.

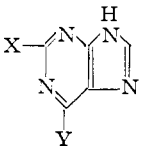
(13) E. Fischer, *Ber.*, **30**, 2336 (1897).

(14) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 4219 (1952).

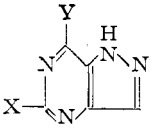
(15) A. Albert and D. J. Brown, *ibid.*, 2060 (1954).

TABLE II

COMPARISON OF ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN PYRAZOLO-[4,3-d]PYRIMIDINES AND THE CORRESPONDING PURINE DERIVATIVES



Purine



Pyrazolo(4,3-d)pyrimidine

X	Y	Pyrazolo[4,3-d]-pyrimidine λmax pH 1	E	Purine λmax	E	pH	Pyrazolo[4,3-d]-pyrimidine λmax pH 11	E	Purine λmax	E	pH
H	OH	274	7,100	248	10,500	0.75[17]	280	6,400	258	11,200	10.35[16]
H	NH_2	294	9,200	264	12,600	1.99[16]	293	7,700	267	12,000	12[16]
H	SH	338	16,100	327	21,300	1[19]	330	11,600	312	19,600	11[18]
OH	OH	283	6,500	227	5,620	1[18]	295	5,200	283	8,700	13[16]
				266	9,340						
H	SCH_3	256	3,300	295	16,200	1[19]	276	4,500	293	16,600	11[18]
		326	16,600								

Experimental[20]

Preparation of 4-Nitro-3-methylpyrazole.[8]—3-Methyl-4-nitropyrazole-5-carboxylic acid[9] was decarboxylated by a modification of the method of Knorr[8] to give 4-nitro-3-methylpyrazole. The exact directions used for large scale operation are given below.

A large evaporating dish containing 204 g. of 3-methyl-4-nitropyrazole-5-carboxylic acid[9] was placed in an oven at 130°. The product slowly melted with the evolution of carbon dioxide. After about 5 hr. decarboxylation was complete. The yield of solid melt was 138 g., m.p. 128–130°. Recrystallization of a small amount from water raised the m.p. to 134°. A mixed melting point taken with this product and an authentic sample prepared by nitration of 3-methylpyrazole[8] showed no depression.

4-Aminopyrazole-3-carboxylic Acid (IV).—4-Nitropyrazole-3-carboxylic acid, 5.0 g., prepared by oxidation of 4-nitro-3-methylpyrazole,[8] was dissolved in 50 ml. of boiling water. To the boiling solution was slowly added 17.5 g. of sodium hydrosulfite. After the addition of sodium hydrosulfite was complete, the solution was boiled for 5 minutes and filtered hot. The cooled filtrate was filtered and the solid washed with water. The yield of crude product was 3.0 g. which slowly decomposed at about 180°. Recrystallization from water gave an analytically pure sample which decomposed slowly above 205°.

Anal. Calcd. for $C_4H_5O_2N_3$: C, 37.8; H, 3.9; N, 33.1. Found: C, 37.2; H, 3.4; N, 33.1.

4-Nitropyrazole-3-ethylcarboxylate (VI).—Fourteen grams of 4-nitropyrazole-3-carboxylic acid[8] was placed in a round-bottom flask to which had been added 23 ml. of absolute ethanol, 37 ml. of dry benzene and 15.5 ml. of concentrated sulfuric acid. This mixture was heated on a steam-bath for 24 hr. The cooled mixture was then poured onto 200 g. of ice and water and the solution extracted several times with ether. The ethereal extracts were washed with water, sodium bicarbonate solution and once again with water and then dried over anhydrous sodium sulfate. Removal of the solvents yielded 10.2 g. of solid which when recrystallized from a benzene–ligroin mixture gave a m.p. 124–126°.

Anal. Calcd. for $C_6H_7O_4N_3$: C, 39.4; H, 3.8; N, 22.7. Found: C, 39.0; H, 3.9; N, 23.0.

4-Nitropyrazole-3-carboxamide (VIII).—To 100 ml. of concentrated ammonium hydroxide was added 5.0 g. of crude 4-nitropyrazole-3-ethylcarboxylate. This solution was heated carefully on the steam-bath while being slowly stirred. After 2 hr. the hot solution was treated with charcoal and filtered and the volume of the filtrate reduced on the steam-bath to 15 ml. The cooled solution yielded 4.0 g. of crude product, m.p. 280–285°. Recrystallization from water raised the m.p. to 291–293°.

Anal. Calcd. for $C_4H_4O_3N_4$: C, 30.9; H, 2.6; N, 35.9. Found: C, 30.8; H, 2.9; N, 36.3.

4-Aminopyrazole-3-carboxamide (VIII).—To 125 ml. of absolute methanol was added 9.5 g. of 4-nitropyrazole-3-carboxamide (VII) and 0.3 g. of 10% palladium-on-charcoal catalyst. The mixture was shaken at 15 lb./sq. in. until the absorption of hydrogen had ceased (approximately 3 hr.). The solution was filtered and the filtrate evaporated to dryness on the steam-bath. The crude residue, 7.0 g., melted at 175–180°. Recrystallization of a small sample from absolute ethanol raised the m.p. to 181–182°.

Anal. Calcd. for $C_4H_6ON_4$: C, 38.1; H, 4.8; N, 44.4. Found: C, 38.2; H, 4.9; N, 44.8.

7-Hydroxypyrazolo[4,3-d]pyrimidine (V). Method (1).—Nine grams of 4-aminopyrazole-3-carboxylic acid (IV) was added to 50 ml. of C.P. formamide. The solution was boiled for 1 hr., at the end of which time the volume was reduced to approximately 25 ml. To the warm solution was carefully added 50 ml. of water and the solution boiled with charcoal and filtered. The cooled filtrate yielded 4.1 g. of a light brown product, a small amount of which was recrystallized from water to give an analytically pure sample, m.p. > 300°.

Anal. Calcd. for $C_5H_4N_4O$: C, 43.1; H, 3.0; N, 41.2. Found: C, 43.1; H, 3.1; N, 41.5.

Method (2).—The methanolic solution containing the 4-aminopyrazole-3-carboxamide (VIII) from the catalytic reduction of 10 g. of 4-nitropyrazole-3-carboxamide (VII) was added to 50 ml. of formamide. The resulting solution was heated gently on a hot-plate until the methanol was boiled off. The formamide was then vigorously boiled for 1 hr. during which time the solution was reduced to approximately 25 ml. and the 7-hydroxypyrazolo[4,3-d]pyrimidine (V) isolated as in method (1) to give a yield of 4.2 g. of light tan product. This product was judged to be identical to that obtained by method (1) on the basis of identical ultraviolet absorption spectra at *p*H 1 and *p*H 11.

7-Mercaptopyrazolo[4,3-d]pyrimidine (X).—A mixture of 11.0 g. of finely powdered 7-hydroxypyrazolo[4,3-d]pyrimidine (X) and 55 g. of powdered phosphorus pentasulfide was added to 275 ml. of dry pyridine. The solution was refluxed for 3 hr. during which time the solid material dissolved and a dark red solution resulted. The excess pyridine was distilled off under reduced pressure using a steam-bath as a source of heat. To the cold solid residue was added 220 ml. of water. The solution was allowed to stand at room temperature for 2 hr. and finally heated for 3 hr. on the steam-bath. The hot solution was acidified with acetic acid and allowed to cool to yield 10.0 g. of crude 7-mercaptopyrazolo[4,3-d]pyrimidine (X). This product was purified by boiling with 200 ml. of water for 5 min. followed by the

(16) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3878 (1948).

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

(18) R. K. Robins, K. J. Dille and B. E. Christensen, THIS JOURNAL, **75**, 263 (1953).

(19) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

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

addition of just enough 2 *N* sodium hydroxide to effect solution. This solution was then heated with charcoal for 5 min. and filtered and acidified while hot with acetic acid to yield 8.5 g. of light tan product.

A small sample was recrystallized from water to give an analytically pure sample, m.p. > 300°.

Anal. Calcd. for $C_6H_4N_4S$: C, 39.5; H, 2.6; N, 36.8. Found: C, 39.7; H, 2.9; N, 36.3.

7-Methylmercaptopyrazolo[4,3-d]pyrimidine (XI).—Twenty grams of 7-mercaptopyrazolo[4,3-d]pyrimidine (X) was dissolved in 280 ml. of 1 *N* potassium hydroxide and 19.2 g. of methyl iodide was added and the solution shaken vigorously until only one phase was present. The solution was then acidified with glacial acetic acid and filtered. The crude product was purified by reprecipitation from a hot basic solution with glacial acetic acid to give 14.7 g., m.p. 200–206°. Recrystallization of this product once from water gave 11.4 g. of white needles, m.p. 209–210°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.3; H, 3.6; N, 33.7. Found: C, 43.3; H, 4.0; N, 34.0.

7-Aminopyrazolo[4,3-d]pyrimidine (XII).—To 3.8 g. of 7-methylmercaptopyrazolo[4,3-d]pyrimidine (XI), m.p. 209–210°, was added 150 ml. of ethanolic ammonia (absolute ethanol saturated with dry ammonia at 0°). This mixture was heated in a bomb at 200° for 20 hr. The cooled solution was then evaporated to dryness on the steam-bath and the residue recrystallized from water to yield 1.8 g., m.p. > 300°.

Anal. Calcd. for $C_5H_5N_5$: C, 44.4; H, 3.7; N, 51.8. Found: C, 44.5; H, 3.9; N, 51.8.

5,7-Dihydroxypyrazolo[4,3-d]pyrimidine (IX).—Two grams of 4-aminopyrazole-3-carboxamide (VIII) and 4 g. of urea were heated at 200–210° for 20 min. until the liquid mass turned solid. After cooling, the solid was dissolved in 50 ml. of 2 *N* sodium hydroxide. The boiling solution was acidified with glacial acetic acid and the warm solution filtered. The crude product, 1.6 g., was reprecipitated twice more from a hot basic solution to give an analytically pure sample, m.p. > 300°.

Anal. Calcd. for $C_5H_4O_2N_4$: C, 39.4; H, 2.6; N, 36.8. Found: C, 39.1; H, 3.0; N, 37.0.

This product was judged identical with Behrend's "isoxanthine" on the basis of identical ultraviolet absorption spectra at *p*H 1 and *p*H 12.

5-Mercapto-7-hydroxypyrazolo[4,3-d]pyrimidine (XIII).—Three grams of 4-aminopyrazole-3-carboxamide (VIII) was heated with 6.0 g. of urea at 200–210° for 20 min. at which time the boiling liquid changed to a semi-solid mass. The cooled solid was dissolved in 2 *N* sodium hydroxide and heated to boiling. The hot solution was acidified with glacial acetic acid and filtered. The crude product was reprecipitated twice more and washed with water to give 2.0 g. of pure product, m.p. > 300°.

Anal. Calcd. for $C_5H_4N_4OS$: C, 35.8; H, 2.4; N, 33.3. Found: C, 36.1; H, 2.9; N, 33.7.

5-Methylmercapto-7-hydroxypyrazolo[4,3-d]pyrimidine (XIV).—To 300 ml. of water was added 10 g. of sodium hydroxide and 3.7 g. of 5-mercapto-7-hydroxypyrazolo[4,3-d]pyrimidine (XIII). The solution was cooled to room temperature and 3.2 g. of methyl iodide added and the solution shaken for 15 min. The solution was then acidified with acetic acid and filtered. The crude precipitate was recrystallized from glacial acetic acid to give 1.4 g. of pure product, m.p. > 300°.

Anal. Calcd. for $C_6H_6N_4OS$: N, 30.8. Found: N, 30.5.

5-Nitro-6-methyluracil.—The following directions for the nitration of 6-methyluracil will be found superior to the brief directions given by Behrend.[5]

Seventy grams of 6-methyluracil[21] was carefully dissolved in 260 ml. of concentrated sulfuric acid with cooling so that the temperature was kept below 40°. The solution was then cooled to 15° and 52.2 ml. of fuming nitric acid (sp. gr. 1.49–1.50) was added dropwise while the temperature was kept below 20°. After the addition was complete the solution was allowed to stand at room temperature for 10 min. and then it was poured onto 1500 g. of crushed ice. The solid was filtered, washed and dried to yield 71 g. A small sample was recrystallized from water.

Anal. Calcd. for $C_5H_5N_3O_4$: N, 24.6. Found: N, 25.1.

5-Amino-6-methyluracil.—The following method of reduction of 5-nitro-6-methyluracil was found superior to that of Behrend.[5]

5-Nitro-6-methyluracil (54.7 g.) was suspended in 500 ml. of water and the solution heated to 80°. To this solution was gradually added 175 g. of sodium hydrosulfite. The temperature of the solution was kept below 90° during the addition of the sodium hydrosulfite after which the solution was boiled for 5 min. with charcoal and filtered. The cooled filtrate yielded 33.6 g. of the desired crystalline product. A small sample was recrystallized from water for analysis.

Anal. Calcd. for $C_5H_7N_3O$: N, 29.8. Found: N, 29.9.

1,4,6-Trimethylpyrazolo[4,3-d]pyrimidine-5,7-dione (XV).—Five grams of 5,7-dihydroxypyrazolo[4,3-d]pyrimidine (isoxanthine) prepared by the method of Behrend[5] from 5-amino-6-methyluracil was dissolved in 40 ml. of 10% sodium hydroxide. The stirred solution was kept at 20° and treated dropwise with 13.3 g. of dimethyl sulfate. The mixture was allowed to stir 1 hr. after the addition of the dimethyl sulfate and then extracted with three 300-ml. portions of chloroform. The chloroform was washed with water and dried over anhydrous sodium sulfate. Distillation of the chloroform extract yielded 4.2 g. of white solid, m.p. 260–265°. An analytical sample was prepared by recrystallization from water to give m.p. 267–269°.

Anal. Calcd. for $C_8H_{10}N_4O_2$: C, 49.5; H, 5.2; N, 28.9. Found: C, 49.2; H, 5.4; N, 28.9.

3-Chloro-5,7-dihydroxypyrazolo[4,3-d]pyrimidine (XVII).—Chlorine gas was slowly passed into 100 ml. of water containing 1.0 g. of suspended 5,7-dihydroxypyrazolo[4,3-d]-pyrimidine (IX). The solution was then heated for 30 min. on the steam-bath and allowed to cool. The crystalline solid, 1.1 g., was filtered and a small amount recrystallized from a water-ethanol mixture for analysis, m.p. > 300°.

Anal. Calcd. for $C_5H_3N_4O_2Cl$: C, 32.3; H, 1.6; N, 30.0. Found: C, 32.1; H, 1.8; N, 30.5.

3-Bromo-1,4,6-trimethylpyrazolo[4,3-d]pyrimidine-5,7-dione (XX). Method (1).—Three grams of 3-bromo-5,7-dihydroxypyrazolo[4,3-d]pyrimidine[5] (XVIII) (bromoisoxanthine) was dissolved in 20 ml. of 10% sodium hydroxide and the stirred solution treated with 7 g. of dimethyl sulfate and the product isolated as for XV. The yield of crude product was 3.2 g., m.p. 175–180°. Recrystallization from a water-ethanol mixture raised the m.p. to 182–183°.

Anal. Calcd. for $C_8H_9N_4O_2Br$: C, 35.2; H, 3.3; N, 20.5. Found: C, 35.1; H, 3.9; N, 20.3.

Method (2).—To 50 ml. of water was added 0.3 g. of 1,4,6-trimethylpyrazolo[4,3-d]pyrimidine (XV), and the solution was heated to 90°, and 20 ml. of bromine water was added. The solution was boiled gently for 5 min. and cooled to yield 0.3 g. of a crystalline solid, m.p. 176–179°. Recrystallization from absolute ethanol raised the m.p. to 180–182°. Mixed melting point with XX prepared by method (1) was 180–182°.

3-Chloro-1,4,6-trimethylpyrazolo[4,3-d]pyrimidine-5,7-dione (XIX). Method (1).—Three grams of 3-chloro-5,7-dihydroxypyrazolo[4,3-d]pyrimidine (XVII) was dissolved in 20 ml. of 10% sodium hydroxide and treated with 6.7 g. of dimethyl sulfate as in the preparation of XV. Chloroform extraction yielded 2.0 g. of crude product, m.p. 170–174°. Recrystallization from a water-ethanol mixture raised the m.p. to 175–177°.

Method (2).—Chlorine gas was passed slowly through a solution of 3 g. of XV in 50 ml. of water at 26°. A crystalline solid appeared, 3.1 g., m.p. 175–177°. Recrystallization of a small sample from a water-ethanol mixture raised the m.p. to 178–179°. Mixed m.p. of this product with that of method (1) was 176–178°.

Anal. Calcd. for $C_8H_9N_4O_2Cl$: C, 42.1; H, 3.9; N, 24.5. Found: C, 41.6; H, 4.2; N, 23.9.

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(21) J. J. Donleavy and M. A. Kise, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 422.