neutral fraction (495 mg.) separated from it. The benzene-soluble part of the neutral fraction (340 mg.) dissolved in a small volume of benzene was poured onto a column of 20 g. of Fisher alumina and the chromatogram developed with absolute petroleum ether. The first 10 fractions (each 50 ml.) yielded 137 mg. of colorless oil which was converted into the trinitrobenzene complex. This compound was repeatedly crystallized from ethanol to a constant melting point of 157°; the yield of the analytically pure sample was 30 mg. This melting point is 20° higher than the melting point of 1-methyl-7-ethylphenanthrene trinitrobenzolate and no depression of melting point was observed on admixture of an authentic sample of pimanthrene trinitrobenzolate which melted also at 157°. The sample was decomposed by chromatography on a small column of alumina and the crystalline hydrocarbon sublimed for infrared and ultraviolet spectrum. These were identical with the spectra of an authentic specimen of pimanthrene.

FREDERICTON, NEW BRUNSWICK, CANADA

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis and Properties of 6-Chloropurine and Purine[1]

BY AARON BENDICH, PERCY J. RUSSELL, JR., AND JACK J. FOX[2]

RECEIVED JULY 2, 1954

6-Chloropurine has been prepared in good yield by the treatment of hypoxanthine with phosphorus oxychloride in the presence of dimethylaniline. The chloro atom was removed by catalytic hydrogenation in a new synthesis of purine. Purine also has been prepared by Raney nickel desulfurization of 6-mercaptopurine, the latter of which was synthesized *via* a new route directly from 6-chloropurine by reaction with thiourea. A synthesis of 4,5-diaminopyrimidine—which has been converted to purine—is described. A study of the ionization and ultraviolet absorption spectral behavior of these purines as well as of 7- and 9-methylpurine has been made. Some physical and chemical properties of these purines are given. A preliminary account of the effect of these compounds on tumor tissue is included.

Simple structural alteration of naturally occurring purines has made available a variety of purine analogs which are potent antagonists of many biological systems.[3] Outstanding examples of such purine derivatives which, *in addition*, exhibit an anti-tumor activity have resulted from the introduction of an amino group or a chlorine atom at position-2 of adenine,[4] the replacement of carbon-8 of guanine by nitrogen[5] or of carbon-2 of adenine or hypoxanthine by nitrogen[6] and by the replacement of the amino group of adenine by the mercapto group.[7]

(1) This investigation was supported by grants from the National Cancer Institute, National Institutes of Health, Public Health Service, Grant No. C-471, and from the Atomic Energy Commission, Contract No. AT(30-1)-910.

(2) Damon Runyon Memorial Fund Fellow, 1952-1954.

(3) (a) See, for example, R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, THIS JOURNAL, **67**, 290 (1945); (b) G. W. Kidder and V. C. Dewey, *J. Biol. Chem.*, **179**, 181 (1949); (c) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *ibid.*, **183**, 1 (1950); (d) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell and H. VanderWerff, *Ann. N. Y. Acad. Sci.*, **52**, 1318 (1950); (e) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951); (f) M. Williamson, W. Jacobson and C. C. Stock, *ibid.*, **197**, 763 (1952); (g) V. L. Ryzhkov and H. K. Marchencko, *Doklady Akad. Nauk. S.S.S.R., Leningrad*, **86**, 637 (1952); (h) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **194**, 641 (1952); (i) C. Miller, *Proc. Soc. Exptl. Biol. Med.*, **83**, 561 (1953); (j) R. E. F. Matthews, *Nature*, **171**, 1065 (1953).

(4) (a) J. H. Burchenal, A. Bendich, G. B. Brown, G. B. Elion, G. H. Hitchings, C. P. Rhoads and C. C. Stock, *Cancer*, **2**, 119 (1949); (b) J. J. Biesele, R. E. Berger, M. Clarke and L. Weiss, *Exptl. Cell Research*, **3**, Supp. 2, 279 (1952).

(5) (a) G. W. Kidder, V. C. Dewey, R. E. Parks, Jr., and G. L. Woodside, *Science*, **109**, 511 (1949); (b) A. Gellhorn, N. Engelman, D. Shapiro, S. Graff and H. Gillespie, *Cancer Research*, **10**, 170 (1950); (c) K. Sugiura, G. H. Hitchings, L. F. Cavalieri and C. C. Stock, *ibid.*, **10**, 178 (1950).

(6) (a) D. W. Woolley and E. Shaw, *J. Biol. Chem.*, **189**, 401 (1951); (b) J. J. Biesele, *Cancer*, **5**, 787 (1952).

(7) (a) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952); (b) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock, G. B. Elion and G. H. Hitchings, *Cancer Research*, **13**, 593 (1953); (c) J. H. Burchenal, M. L. Murphy, R. R. Ellison, M. P. Sykes, T. C. Tan, L. A. Leone, D. A. Karnofsky, L. F. Craver, H. W. Dargeon and C. P. Rhoads, *Blood*, **8**, 965 (1953); (d) C. P. Rhoads and G. H. Hitchings, "Conference on 6-Mercaptopurine," *Ann. N. Y. Acad. Sci.*, in press.

An examination of the structures of a few hundred purine derivatives tested for possible effectiveness in cancer chemotherapy[8] reveals that (a) the active analogs of naturally occurring purines are those in which the new group or atom introduced is not greatly different in size from the one replaced, (b) the more active compounds seem to result from an alteration at a single site of the structure of adenine, hypoxanthine or guanine, and (c) active analogs have resulted from replacement in adenine, hypoxanthine or guanine of carbons-2 or 8 by nitrogen or by substitution at carbons-2 or 6, but not at any other position thus far tested. (Although the introduction of the ribofuranosyl group at N_9 may yield active derivatives, compounds of the nucleoside type[8b] are outside the present discussion.) The replacement of the amino group of adenine (or the hydroxyl of hypoxanthine (I)) by a chlorine or hydrogen atom would give rise, respectively, to the compounds 6-chloropurine (II) and purine (III) which embody some of the structural features listed above. This communication deals with the preparation and properties of these and related purines.

Synthetic Studies.—Neither a Sandmeyer-type reaction with adenine nor the treatment of hypoxanthine (I) with phosphorus oxychloride alone afforded the desired 6-chloropurine (II). Since the use of dimethyl- or diethylaniline greatly improves the chlorination of many hydroxypyrimidines[9-12] and uric acid[13] with phosphorus oxychloride, this reaction was applied to hypoxanthine, and 6-chloropurine (II) was obtained in good yields.[14]

(8) See references 3 to 7, and (a) C. C. Stock, *Cancer Research*, Supp. No. 2 (1953); Supp. No. 1 (1955), in press; also (b) G. B. Brown, in C. P. Rhoads and A. Bass, "Antimetabolites in Cancer. AAAS Monograph," in press.

(9) J. Baddiley and A. Topham, *J. Chem. Soc.*, 678 (1944).

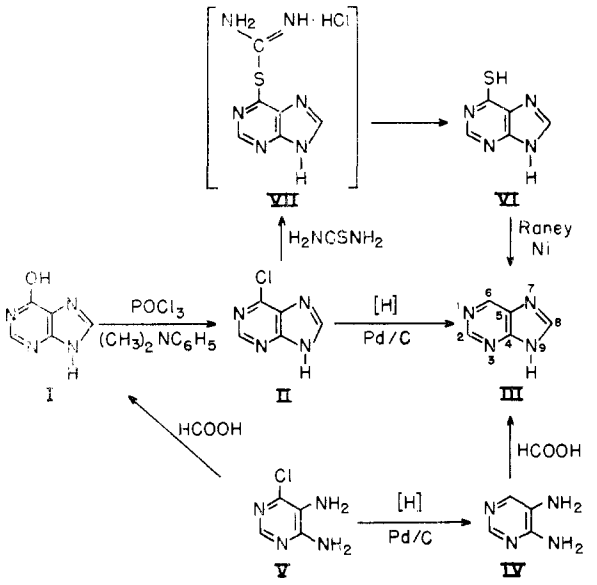
(10) P. Bitterli and H. Erlenmeyer, *Helv. Chim. Acta*, **34**, 835 (1951).

(11) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

(12) N. Whittaker, *ibid.*, 1565 (1951); 1646 (1953).

(13) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 2936 (1951).

(14) 6-Diethylaminopurine was formed when hypoxanthine and phosphorus oxychloride were refluxed in the presence of triethylamine; R. K. Robins and B. E. Christensen, THIS JOURNAL, **74**, 3624 (1952).



An earlier attempt[7a] to prepare this compound by this method was unsuccessful probably because of the formation of a variously colored complex between the product and dimethylaniline from which 6-chloropurine is not extractable with ether. However, the complex is easily broken up by mixing with crushed ice and subsequent treatment with strong alkali *in the cold*. The dimethylaniline is then removed by ether extraction from the chilled *alkaline* solution and the 6-chloropurine is recovered, in turn, by ether extraction of the *acidified* residue.

Another reaction which has a counterpart in the pyrimidine series[15] is the dechlorination of 6-chloropurine by catalytic hydrogenation (atmospheric pressure and room temperature) from which purine (III) is obtained thereby in 83% yield. Purine was first prepared by a circuitous route (in about 2% yield) from uric acid by Fischer[16] in 1898. Later, Isay[17] transformed uracil into 4,5-diaminopyrimidine (IV) which was converted, in turn, into purine (unspecified yield) upon reaction with formic acid in a Traube-type synthesis. Refinements in the preparation of 4,5-diaminopyrimidine from uracil have been described recently.[18] A synthesis of this intermediate (IV) *via* 6-chloro-4,5-diaminopyrimidine (V)[19] is reported here.[20]

In a reinvestigation of the Isay synthesis of purine, this compound III was prepared (in 69% yield) by heating 4,5-diaminopyrimidine (IV) with 98–100% formic acid. The greater yield (85%) in this reaction observed by Albert and Brown[21] appears to depend upon the decomposition of an intermediate formic acid salt of purine by boiling the salt with a suspension of calcium carbonate in ethanol.

In another type of reaction encountered in the pyrimidine series,[22] 6-mercaptopurine (VI)[7a] was obtained directly from 6-chloropurine, without the isolation of the intermediate thiuronium salt (VII), by boiling with an ethanol solution of thiourea. Purine resulted from 6-mercaptopurine upon dethiolation with Raney nickel. Similar reductive dethiolations have been carried out in the pyrimidine series. For a discussion of the various reactions described above, see ref. 15.

Spectral Studies.—The ultraviolet absorption spectra of purine and its 6-chloro and 7- and 9-methyl derivatives as a function of *p*H are shown in Fig. 1: apparent dissociation constants are listed in Table I. Previous studies of this kind in the pyrimidine and purine series[23–25] have frequently permitted an assignment to be made as to the specific molecular or ionic species which are responsible for the absorption and have furnished a basis for the determination of apparent dissociation constants. It has not, however, been possible to learn, from the present data, what the structures of the cationic forms might be.

Purine itself exhibits two equilibria spectrophotometrically as a function of *p*H, the first (involving curves passing through isosbestic points a and b) refers to that between the purine cation and the neutral molecule, while the second (through isosbestic points c and d) deals with the dissociation of the –NH– group to $-N^{\ominus}-$ of the imidazole ring. The curve for *p*H 5.94 represents the neutral species and is common to all isosbestic points. 6-Chloropurine presents a spectral pattern similar to that of the parent compound, purine, with the qualification that in the acid ranges the chloro derivative is slowly hydrolyzed to hypoxanthine thus making a spectral determination of its basic pK_a impossible.

7- and 9-methylpurine, as would be expected, exhibit but one pK_a each involving the equilibrium between their neutral and cationic forms. A very close resemblance is seen between the spectral patterns of 9-methylpurine and the equilibrium curves involved in the lower pK_a of purine (curves between *p*H values 0.23 and 5.94). Both patterns are characterized by isosbestic point pairs at ~230 and ~275 mμ and both exhibit very slight shifts to shorter wave lengths as the *p*H of the medium is decreased. 7-Methylpurine, on the other hand, shows isosbestic points at 256 and 281 mμ and gives a pattern of appreciable hypsochromic shifts with decrease in *p*H. The fact that the absorption maxima of the neutral forms of 9-methylpurine and

(15) For references, see A. Bendich, "Chemistry of Purines and Pyrimidines," in Chargaff and Davidson, "Nucleic Acids, Chemistry and Biology," Academic Press, Inc., New York, N. Y., 1954, in press.

(16) E. Fischer, *Ber.*, **31**, 2550 (1898).

(17) O. Isay, *ibid.*, **39**, 250 (1906).

(18) D. J. Brown, *J. Appl. Chem.*, **2**, 239 (1952).

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

(20) An attempt (unpublished results) to convert 6-chloro-4,5-diaminopyrimidine (V) into 6-chloropurine (II) by refluxing with concentrated formic acid led to the formation of hypoxanthine and, apparently, 4-amino-5-formylamino-6-hydroxypyrimidine. In a similar fashion, hypoxanthine has been obtained by refluxing 6-chloro-4,5-diaminopyrimidine in anhydrous formamide (R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 263 (1953); see *ibid.*, **75**, 6359 (1953) for errata).

(21) A. Albert and D. J. Brown, *J. Chem. Soc.* 2060 (1954). We are indebted to these investigators for many helpful discussions and for their kindness in making their observations available to us prior to publication.

(22) J. F. W. McOmie and M. P. V. Boarland, *Chem. and Ind.*, 602 (1950); *J. Chem. Soc.*, 1218 (1951); 3722 (1952).

(23) J. J. Fox and D. Shugar, *Bull. soc. chim. Belg.*, **61**, 44 (1952).

(24) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

(25) L. F. Cavalieri, J. J. Fox, A. Stone and N. Chang, THIS JOURNAL, **76**, 1119 (1954).

TABLE I

SPECTROPHOTOMETRIC DATA

Purines	Mol. species	pH	Maxima λ, mμ	Maxima ε × 10⁻³	Isosbestic points pH range	Isosbestic points λ, mμ	Isosbestic points ε × 10⁻³	pK_a[a]
Purine	Cation	0.23	260	6.26	0.23–5.94	225	1.65	2.52
	Neutral	5.94	262.5	8.16		275	3.66	
	Anion	11.90	270	7.98	5.94–11.90	228	1.78	8.92
						267	7.57	
7-Methyl	Cation	0.23	257.5	6.71	0.23–9.15	256.3	6.65	2.29
	Neutral	9.15	266.5	8.14		281	3.67	
9-Methyl	Cation	0.62[b]	262.5	5.87	0.62–8.50	232	1.92	2.48
	Neutral	8.50	264	7.90		277	2.66	
6-Chloro[c]	Neutral	5.23	265	9.12	5.23–13	230	2.29	7.68
	Anion	13	274	8.79		269.5	8.37	

[a] Apparent pK_a values ±0.05. [b] The curve for pH 0.62 is identical with that for 0.1 *N* HCl. In 1 *N* HCl, the spectrum changes slowly with time. [c] This compound is hydrolyzed in acidic solutions to hypoxanthine. Data for the cationic species are therefore omitted from this table.

purine occur at shorter wave lengths than does the corresponding maximum of 7-methylpurine seems to be characteristic of other purine derivatives involved in the nucleic acids. (The extent to which this positional spectral relationship between 7- and 9-substituted purines may be general is under investigation.[25a])

The similarity of the equilibrium patterns for the cationic dissociation of purine and 9-methylpurine would suggest that proton addition in both these compounds involves the same position of the purine ring. The spectral pattern of 7-methylpurine, which differs from the above-mentioned purines, *may* indicate that with the 7-alkylated purine proton addition to the heterocyclic ring takes place at a position different from that which occurs with purine or its 9-alkylated analog.

Biological Activity.—Testing of these compounds has been carried out in the Division of Experimental Chemotherapy. In tissue culture experiments, 6-chloropurine shows a selective inhibition of mouse sarcoma 180 cell division; purine is more toxic to such cells than to cells of embryonic mouse skin, and 7- and 9-methylpurine are essentially inactive.[26] Purine and 6-chloropurine retard the growth of sarcoma 180 in mice[27] and in preliminary tests retard the growth of a number of tumors upon which they have been tested.[28]

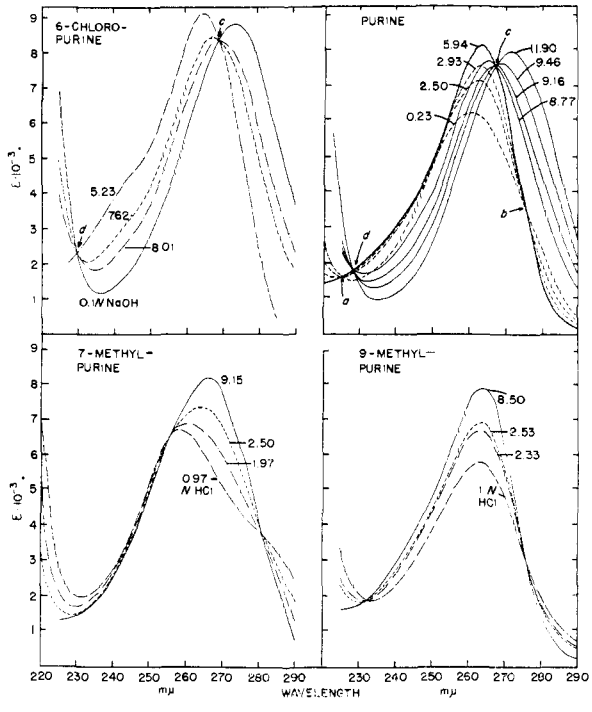


Fig. 1.—Ultraviolet absorption spectra of some purines in aqueous solutions at pH values indicated on the curves.

Experimental

All melting and decomposition points recorded here are corrected, and were determined on the hot stage, with one exception.

6-Chloropurine.—A number of commercial samples[29] of hypoxanthine containing adenine (about 20%) were tried, but these consistently gave poor yields of 6-chloropurine. To 10 g. of hypoxanthine,[30] dried over P_2O_5 *in vacuo*, was added 25 ml. of freshly distilled N,N-dimethylaniline and 300 ml. of freshly distilled phosphorus oxychloride. The mixture was refluxed under anhydrous conditions for 4.5 hours and, after standing overnight at room temperature, was concentrated *in vacuo* to a viscous sirup, which was slowly poured, with continuous mixing, onto 500 g. of crushed ice. The mixture was thoroughly agitated and carefully triturated *in the cold* with 10 *N* NaOH until strongly alkaline. Depending on the amount of residual phosphorus oxychloride remaining in the viscous sirup, a thick paste of crystals may form at this point and may be dispersed by the addition of cold water. The crystals need not be dissolved. The alkaline solution was extracted in the cold with small portions of ether until the ether extract was nearly colorless. The chilled aqueous solution was then acidified with concd. HCl and continuously extracted with ether. Acidic conditions (not lower than pH 1) must be maintained throughout the ether extraction, which proceeds to completion in about 2 days. Crystals of 6-chloropurine deposited when 500 ml. of ether was used. The ether was removed by distillation and the residue was dissolved by boiling in water and the hot solution, after treatment with charcoal, was taken to dryness *in vacuo*. The yield of colorless or faintly yellow residue varied from 7 to 8 g. (61–70%). Nearly

(25a) NOTE ADDED IN PROOF.—The data of B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954), show a similar spectral relationship between 7- and 9-substituted 6-dimethylaminopurines.

(26) J. J. Biesele, M. C. Slautterback and M. Margolis, *Cancer*, in press, ref. 7d.

(27) D. A. Clarke, F. S. Philips, S. Sternberg and C. C. Stock, in press, ref. 7d.

(28) K. Sugiura and C. C. Stock, unpublished results.

(29) Many commercially available samples of "hypoxanthine" are prepared by nitrous acid deamination of adenine (from yeast nucleic acid hydrolysates). A commercial sample was reported to contain over 50% of adenine: see R. E. F. Matthews, *J. Gen. Microbiol.*, **8**, 277 (1953).

(30) An excellent synthetic hypoxanthine was obtained from Dougherty Chemicals, Richmond Hill 18, New York.

quantitative yields were obtained when the scale was reduced to $^1/_{10}$.

Further purification was effected by recrystallization (charcoal) from a minimum volume of hot water. The resulting small blunt needles were dried at room temperature over P_2O_5 *in vacuo*. The melting point behavior was anomalous and was difficult to reproduce. When placed on the hot stage at about 170°, melting with decomposition could be observed at about 175–177°. The melting point of its eutectic with dicyandiamide[31] was 180–181° dec.

Anal. Calcd. for $C_5H_3N_4Cl$: N, 36.2; Cl, 22.9. Found: N, 36.1; Cl, 22.9. Solubility: 1.00 g. in 182 g. H_2O at 24°.

6-Chloropurine Picrate.—This slowly formed from aqueous solution and was recrystallized from water in the form of long needles. The melting point behavior was also anomalous; placed on the hot stage at 155°, it melted at 164–165° dec.

Anal. Calcd. for $C_5H_3N_4Cl \cdot C_6H_3O_7N_3$: N, 25.5; Cl, 9.25. Found: N, 25.7; Cl, 9.14.

Stability of 6-Chloropurine. a. Toward Boiling Water.—6-Chloropurine remained unchanged on boiling in dilute aqueous solution for 4 hours.

b. **Toward Acid and Alkali.**—Quantitative conversion of 6-chloropurine to hypoxanthine occurred upon boiling either in 0.1 *N* NaOH for 4 hours or 0.1 *N* HCl for one hour.

c. **Toward Ammonia.**—No change was noted when 6-chloropurine in concentrated aqueous ammonia solution was heated in sealed tubes at 100° for 4 hours. Conversion to adenine was observed upon heating for 10 hours in 10% ammoniacal butanol solution at 150°. A second product, isolated as a crystalline sulfate and showing ultraviolet absorption maxima at 240 and 318 mμ in neutral aqueous solution, was not further investigated.

Conversion to 6-Mercaptopurine.[7a]—A suspension of 0.98 g. of 6-chloropurine (6.3 mmoles) and an equimolar quantity of thiourea in 14 ml. of absolute ethanol was refluxed. The solids dissolved and soon a yellow crystalline product deposited. After refluxing for one hour, the mixture was chilled and 0.64 g. of 6-mercaptopurine (67% yield) was collected. Opaque yellow elongated prisms of the *hydrate* were obtained by neutralizing a warm alkaline solution of the product with acetic acid. The hydrate did not lose its water of crystallization *in vacuo* at room temperature over P_2O_5.

Anal. Calcd. for $C_5H_4N_4S \cdot H_2O$: N, 32.9. Found: N, 33.3.

It was found to be identical with an authentic specimen[7a] when examined by paper chromatography in a variety of solvent systems. It melted at 313–315° dec.; mixed m.p. 312–315° dec. with an authentic specimen [7a] that melted at 312–315° dec. It showed an absorption maximum at 325 mμ, ε 19,200 at *p*H 1 and a maximum at 312.5 mμ, ε 18,500, at *p*H 10.8.

Anal. Calcd. for $C_5H_4N_4S$: (*dried over P_2O_5 in vacuo at 100°*): N, 36.8; S, 21.0. Found: N, 36.6; S, 20.5.

Hydrogenation of 6-Chloropurine: Partial Synthesis of Purine.—6-Chloropurine (9.0 g.) was shaken at room temperature with 3.0 g. of 5% palladium–charcoal in 250 ml. of water under one atmosphere of hydrogen. A theoretical uptake of one mole of hydrogen occurred during 90 minutes of treatment (*after which time there was a further, but much slower absorption of hydrogen*). The mixtures from two such hydrogenations were combined and the whole was adjusted to neutrality with aqueous ammonia, filtered and the filtrate taken to dryness *in vacuo*. The residue was continuously extracted with about 500 ml. of boiling toluene in a Soxhlet apparatus. Nearly colorless elongated prisms of purine (11.3 g.) deposited in the hot toluene after extracting for 2 days. An additional 0.3 g. was recovered from the solution; total yield 83% of theory. The crude purine was homogeneous as judged by paper chromatography and melted one degree lower than a highly purified sample. For analysis, a sample was sublimed at 100–150° (0.1 mm.), m.p. 216°. The sample could be remelted repeatedly without change.

Anal. Calcd. for $C_5H_4N_4$: C, 50.0; H, 3.36; N, 46.7. Found: C, 49.9; H, 3.47; N, 47.2. Solubility: greatly in excess of 1.0 g. in 5.0 g. H_2O at 23°.

(31) K. Dimroth and H. G. Meyer-Brunot, *Biochem. Z.*, **323**, 343 (1952).

Purine Picrate.[16]—The picrate formed readily in thin yellow plates from concentrated solutions of purine and more slowly in sturdy needles from dilute solution, m.p. 207–209° dec.

Anal. Calcd. for $C_5H_4N_4 \cdot C_6H_3O_7N_3$: C, 37.8; H, 2.02; N, 28.1. Found: C, 37.7; H, 2.22; N, 28.2.

Purine from 6-Mercaptopurine.—The use of a great excess of Raney nickel is to be avoided since the resulting purine adheres very tenaciously to the nickel. 6-Mercaptopurine (1.0 g.) was taken up in 50 ml. of boiling water and small amounts of Raney nickel were added to the boiling mixture until the ultraviolet absorption above 310 mμ just disappeared. The mixture was filtered and the filtrate was taken to dryness *in vacuo* to yield 0.32 g. (41% of theory) of a pale yellow crystalline residue that appeared homogeneous and indistinguishable from purine when examined by paper chromatography. A sublimed sample melted alone or on admixture with purine at 216°.

Purine from 4,5-Diaminopyrimidine.[21]—A solution of 4.00 g. of 4,5-diaminopyrimidine (for preparation, see below) in 23 ml. of 98–100% of formic acid was heated in an oil-bath at 100° for 30 minutes, a stream of CO_2 bubbling through the solution. The temperature was raised to 210° during 45 minutes and maintained at this temperature for 30 minutes. The temperature was lowered to 110° and was kept at 110° until the formic acid was driven off (2 hours) in the stream of CO_2. A quantity of crystalline purine that had sublimed during this time was collected. The residue was sublimed at 100–150° (0.1 mm.) and both sublimates were combined and resublimed to give 3.03 g. (69%); m.p., alone or on admixture with a specimen prepared as above from 6-chloropurine, 216°.

Hydrogenation of Purine. (a).—One gram of purine was dissolved in 25 ml. of water to which 0.3 g. of 5% palladium–charcoal was added. The mixture was shaken under one atmosphere of hydrogen at 23°, but no absorption was observed until an equivalent quantity of acid (2.1 ml. of 4 *N* HCl) was added; a total of 175 ml. of hydrogen (94% of theory) was taken up during 90 hours.

The mixture was filtered and a spectroscopic examination of the filtrate revealed no selective absorption in the ultraviolet. A specimen of the filtrate gave a positive Bratton–Marshall test[32] indicating the presence, in the product, of an "aromatic" amino group, probably a 4-aminoimidazole derivative. A crystalline picrate, m.p. 174–175° dec., was prepared from the product. Further characterization of the reduction product and those described below will be reported elsewhere.

(b).—A specimen of purine was heated (100°) for 30 minutes with zinc dust and *N* H_2SO_4. The filtrate, which showed no specific ultraviolet absorption, gave a positive Bratton–Marshall test.

(c).—0.36 g. of 2-hydroxypurine[33] was hydrogenated (one atmosphere) at room temperature in 25 ml. of water containing 0.12 g. 5% palladium–charcoal. The absorption of a molar equivalent of hydrogen (29 hours) was accompanied by a loss of the ultraviolet absorption maximum at about 315 mμ at neutral *p*H. The resulting dihydropurine derivative did not give a positive Bratton–Marshall reaction until after prior brief treatment with dilute hydrochloric acid.

(d).—Neutral or acidic solutions of adenine or hypoxanthine did not absorb hydrogen (1 atmosphere) at room temperature in the presence of palladium–charcoal.

Stability of Purine toward Acid and Alkali.—No change was observed when purine was heated (100°) for one hour either with 0.1 *N* HCl or NaOH.

9-Methylpurine.—5-Amino-4-methylaminopyrimidine[34] was converted to 9-methylpurine by reaction with formic acid[21]; m.p. 163–164° (Fischer[16] reported 162–163° (cor.)). We have confirmed the observations of Albert and Brown that 9-methylpurine darkens slowly on storage.

Anal. Calcd. for $C_6H_6N_4$: N, 41.8; C, 53.7; H, 4.51. Found: N, 41.9; C, 53.7; H, 4.62.

7-Methylpurine.—This compound was prepared by catalytic hydrogenation of 2,6-dichloro-7-methylpurine[35]; m.p. 183–184° (Fischer[16] found m.p. 184° (cor.)).

(32) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

(33) We are indebted to the Wellcome Research Laboratories for a gift of this compound.

(34) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).

(35) E. Fischer, *Ber.*, **30**, 2400 (1897).

TABLE II

R_f VALUES OF PURINES (DESCENDING CHROMATOGRAPHY)[a]

Solvent system	Reference	Adenine	Hypoxanthine	Purine	6-Chloropurine	6-Mercaptopurine	7-Methylpurine	9-Methylpurine
n-Butanol satd. with H_2O (NH_3)	*b*	0.28	0.08	0.32	0.35	0.06	0.17	0.57
H_2O satd. with *n*-butanol		.51	.63	.76	.73	..	.74	.81
n-Butanol–diethylene glycol (NH_3)	*b*	.55	.35	.68	.78	.41	.46	.83
t-Butanol–20% acetic acid		.42	.39	.56	.67	.42	.42	.64
2 *N* HCl–isopropyl alc.	*c*	.18	.18	.24	.56	.21	.27	.32
6 *N* HCl isopropyl alc.–propylene glycol	*d*	.40	.34	.46	.68	.37	.49	.60
70% Ethanol		.63	.59	.74	.78	.59	.67	.79
3% Aqueous ammonium chloride	*e*	.75	.66	.76	.69	..	.73	.84

[a] These chromatograms were developed in duplicate at room temperature. Sheets of (17.5 × 41 cm.) Schleicher and Schuell filter paper #597 were used. In most instances, the solvent front moved about 30 cm. from the "starting line." The "spots" were examined under ultraviolet light. The purines, with the exception of 6-chloro- and 6-mercaptopurine, appeared as dark "spots" against a light fluorescent background. In certain solvent systems, the latter two purines appeared much lighter. [b] E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 703 (1948). [c] G. R. Wyatt, *Biochem. J.*, **48**, 481, 484 (1951). [d] D. L. Woodhouse, *ibid.*, **56**, 349 (1954). [e] A. Albert and D. J. Brown, ref. 21, and R. Tschesche and F. Korte, *Ber.*, **84**, 641, 801 (1951).

A suitable ultraviolet lamp (General Electric mercury lamp No. G8T5) equipped with Corning glass filter No. 9863 is supplied (Model #MR4) by G. W. Gates and Co., Inc., Franklin Square, Long Island, N. Y.

4,5-Diaminopyrimidine.—5-Amino-4,6-dichloropyrimidine[34] m.p. 146–147° (5.0 g., 0.03 mole), was heated in a sealed tube at 125–130° for 6 hours with 38 ml. of 10% ammonia in ethanol (w./v.). After chilling, 5.1 g. of long yellow needles was collected by filtration. An additional crop (0.3 g.) was obtained following concentration of the filtrate. The crude 6-chloro-4,5-diaminopyrimidine, poorly soluble in either water or ethanol, reduced alkaline phosphomolybdate solution but not alkaline dichlorophenolindophenol, indicating the absence of significant amounts of 4,5,6-triaminopyrimidine.[36]

For purification, the product was dissolved in 50 ml. of warm 0.5 *N* HCl; after treatment with charcoal, the warm solution was neutralized with concentrated ammonia and 3.33 g. of long colorless needles was obtained (76% yield). The compound (6-chloro-4,5-diaminopyrimidine) sublimed on the hot stage at 248–251° and melted in a capillary tube at 249–250° dec. Albert, *et al.*,[19] who reported a decomposition point of *ca.* 248°, first prepared the compound by the hydrogen–Raney nickel reduction of 4-amino-6-chloro-5-nitropyrimidine in methanol. It was later prepared (m.p. 251° dec.) by water–zinc dust reduction.[37]

Anal. Calcd. for $C_4H_5N_4Cl$: N, 38.8. Found: N, 39.1.

When catalytic dehalogenation of 6-chloro-4,5-diaminopyrimidine was attempted in the presence of magnesium oxide,[12] the uptake of hydrogen did not discontinue after one molar proportion of it had been absorbed. The following procedure was therefore adopted.

6-Chloro-4,5-diaminopyrimidine (0.82 g.) and 0.32 g. palladium–charcoal(5%) in 25 ml. of water were shaken under one atmosphere of hydrogen (24°). The uptake ceased (60 minutes) after one molar proportion of hydrogen was absorbed. The filtrate was neutralized with concentrated ammonia and evaporated to dryness *in vacuo*. The residue was continuously extracted with ethyl acetate (75 ml.) in a Soxhlet apparatus and a 74% yield of stout, pale yellow needles of 4,5-diaminopyrimidine was obtained. A sample was recrystallized twice from ethyl acetate[38] to give square and rectangular plates, m.p. 205–206°. A convenient synthesis from uracil on a larger scale has been described.[18]

Anal. Calcd. for $C_4H_6N_4$: C, 43.63; H, 5.50; N, 50.89. Found: C, 43.81; H, 5.41; N, 50.78.

Spectrophotometric Studies.—Measurements were made with a Beckman Model DU spectrophotometer using techniques and buffers previously described.[39] In addition, glycine buffers were employed between *p*H values 8.80 and 10.41, bicarbonate buffer for *p*H 11 and 0.01 *N* sodium hydroxide for *p*H 12, all solutions of which were of ionic strength 0.05. The apparent pK_a values of these compounds were determined spectrally by procedures previously employed[23,24] and are given along with other pertinent spectrophotometric data in Table I.

Chromatographic Properties.—The chromatographic properties of several purines in a number of media are listed in Table II.

Acknowledgment.—The authors wish to express their gratitude to Dr. George Bosworth Brown for valuable support and advice and to Mrs. N. C. Yung for assistance. The microanalyses were performed by the late Mr. R. C. Funk, Jr., and Dr. J. Alicino.

NEW YORK 21, N. Y.

(36) A. Bendich and G. C. Clements, *Biochim. Biophys. Acta*, **12**, 462 (1953).

(37) See reference cited in footnote 20.

(38) Solution of 4,5-diaminopyrimidine in hot ethyl acetate required a refluxing period of 10 to 20 minutes.

(39) J. J. Fox, L. F. Cavalieri and N. Chang, THIS JOURNAL, **75**, 4315 (1953).