The double wave tended to disappear with increasing water concentration, and at intermediate peroxide concentrations it was either absent or the first wave was very poorly developed as the water concentration approached 30% of the total volume. The reason for the double wave was unclear, but it appeared likely that slow diffusion of the reduction products from the region of the dropping electrode may have been responsible since even vigorous mixing for several seconds was often insufficient to prevent layering when additions were made to the glycol solutions. In fact, a fairly persistent "streamer" due to the passage of the mercury droplet through the solutions could be visualized by adding a small amount of a dye to the more concentrated glycol solutions. A rotating microelectrode might have been superior to the dropping electrode in the media used.

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

Synthesis of Purine-6-carboxaldehyde and Related Derivatives[1]

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RECEIVED NOVEMBER 10, 1958

Treatment of 6-methylpurine with iodine and pyridine afforded purine-6-methylenepyridinium iodide which upon reaction with *p*-nitrosodimethylaniline gave *p*-phenylenediamine-N,N-dimethyl-N′-(6-purinylmethylene)-N′-oxide. Acid hydrolysis of the latter gave a solution of purine-6-carboxaldehyde from which the aldehyde could be isolated only in low yield. Direct treatment of the acid hydrolysate of *p*-phenylenediamine-N-N dimethyl-N′-(6-purinylmethylene)-N′-oxide with the appropriate carbonyl reagents led to the hydrazone, oxime, thiosemicarbazone, semicarbazone and phenylhydrazone of purine-6-carboxaldehyde. Good yields of purine-6-carboxaldehyde were obtained by treatment of its hydrazone with ethyl nitrite and hydrochloric acid; when ethyl nitrite and acetic acid were used, purine-6-carboxaldehyde azine was obtained. The catalytic hydrogenation of 6-cyanopurine in the presence of semicarbazide afforded purine-6-carboxaldehyde semicarbazone. Similar treatment with hydroxylamine, hydrazine and phenylhydrazine gave N-amino-6-purinylamidine, 6-hydroxyamidinopurine and N-phenyl-6-purinylamidine, respectively. Treatment of N-amino-6-purinylamidine with nitrous acid led to purine-6-carboiminoazide which was converted into 4,5-diamino-6-carboiminopyrimidine with aqueous ammonia. The reaction of purine-6-carbohydrazide with benzenesulfonyl chloride gave purine-6-phenylsulfocarbohydrazide.

Several purines related to purine-6-carboxylic acid and 6-cyanopurine have been synthesized.[2,3] It was of interest to prepare purine-6-carboxaldehyde. As this compound can be considered an oxidation product of the carcinolytic and extremely toxic 6-methylpurine,[4,5] it was of further interest to determine whether the conversion of the methyl group to the carboxaldehyde function would affect the toxicity and anti-tumor properties. Purine carboxaldehydes have not been reported previously, although several aldehydes or their derivatives in the pyrimidine[6-13] and pteridine (see review by Albert[14]) series are known. Little is known of the effect of such aldehydes on biological systems, although 2-amino-4-hydroxypteridine-6-carboxaldehyde is well known as a potent inhibitor of xanthine and pteridine oxidase.[15-17]

The desired compound was an elusive target which could not be prepared by any of the conventional means tried. The synthesis of purine-6-carboxaldehyde was achieved by the application of methods which have recently been developed.

Synthetic Studies.—A total synthesis of purine-6-carboxaldehyde from the corresponding pyrimidine by classical methods[18] was not attempted since it was expected that neither the carboxaldehyde group nor its acetal would withstand the drastic conditions used in the synthesis. Instead, syntheses of purine-6-carboxaldehyde by transformation of substituents at the 6-position of purine were investigated.

An adaptation of the method of preparation used for substituted β-keto-alkylpyridinium iodides by the reaction of methyl ketones with iodine and pyridine[19] was found suitable in the first step of the synthesis, and the preparation of purine-6-methylenepyridinium iodide (II) (Scheme 1) from 6-methylpurine[20] (I) was achieved in 63% yield. This type of reaction was adapted[21] to the preparation of carboxylic acids and esters and extended

(1) This investigation was supported by grants from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190); the Atomic Energy Commission (Contract No. AT (30-1)910); the Ann Dickler League and the Damon Runyon Fund.

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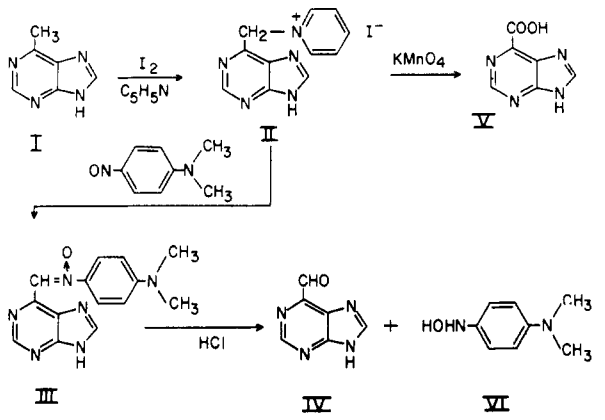


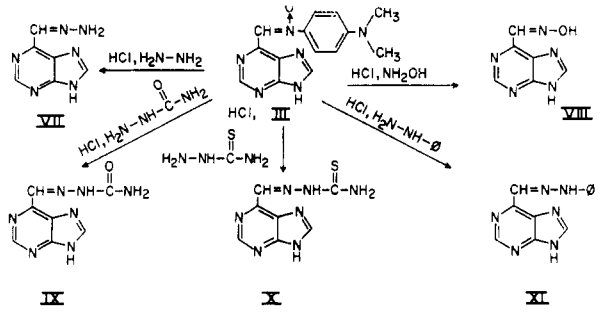
SCHEME 1.

by Ried and Bender[22] to the synthesis of aldehydes of benzthiazol, benzoxazol and other heterocycles. Their method was used for the preparation of *p*-phenylenediamine-N,N-dimethyl-N'-(6-pyrinylmethylene)-N'-oxide (III), which represents the second step of the synthesis from II and *p*-nitrosodimethylaniline in 94% yield.

The identity of the compounds which result from the reaction between aromatic nitroso derivatives and compounds which contain reactive methylene groups has been discussed by de Waal and Brink[23] and Kröhnke and his colleagues.[24]

The hydrolysis of III with HCl gave the sparingly soluble *p*-dimethylaminophenylhydroxylamine (VI), and a 4% yield of purine-6-carboxaldehyde (IV) was obtained. An improved synthesis of the aldehyde was carried out *via* the hydrazone VII (see below). The oxidation of II with $KMnO_4$ led to a new synthesis of the known,[2,25] purine-6-carboxylic acid (V) in a 77% yield.

After acid hydrolysis of III, several derivatives were made with the usual carbonyl group reagents (Scheme 2). For example, when III was acidified



SCHEME 2.

with hydrochloric acid, the insoluble *p*-dimethylaminophenylhydroxylamine (VI) removed by filtration and the filtrate treated with thiosemicarbazide, purine-6-carboxaldehyde thiosemicarbazone (X) was formed in 85% yield. Similar treatment with semicarbazide hydrochloride gave purine-6-carboxaldehyde semicarbazone (IX) in 34% yield.

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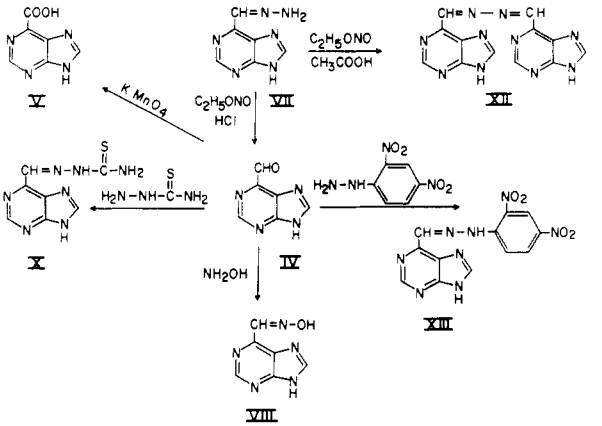
When hydrazine was added to the acid hydrolysate of III, the corresponding purine-6-carboxaldehyde hydrazone (VII) was formed in 87% yield. The thiosemicarbazone X was transformed into the hydrazone VII in 72% yield when refluxed with an aqueous solution of hydrazine. The filtered acid hydrolysate of III gave purine-6-carboxaldehyde oxime (VIII) (15% yield) upon treatment with ethanolic hydroxylamine, and the phenylhydrazone XI in 60% yield upon treatment with phenylhydrazine.

The treatment of purine-6-carboxaldehyde hydrazone (VII) with nitrous acid was found to be a suitable method for the preparation of purine-6-carboxaldehyde (IV) (Scheme 3). Nitrogen was



SCHEME 3.

evolved and the aldehyde was readily obtained in analytically pure form in 67% yield. This method is an adaptation of that of Auwers and Ottens[26] for the preparation of aldehydes by treatment of aromatic semicarbazones with a hot solution of sodium nitrite and acetic acid. Succinic dialdehyde has been obtained in this manner from its semicarbazone by Goldschmidt.[27] However, treatment of purine-6-carboxaldehyde semicarbazone (IX) with nitrous acid resulted in a poor yield of the aldehyde IV, as judged by spectroscopic analysis.

Purine-6-carboxaldehyde (IV) was easily oxidized with $KMnO_4$ to purine-6-carboxylic acid (V) in 82% yield. The preparation of the latter compound was previously reported[2,25] (see Scheme 1 also for its preparation from II by oxidation with $KMnO_4$). By treatment of the aldehyde IV with thiosemicarbazide, purine-6-carboxaldehyde thiosemicarbazone (X) was obtained in 87% yield. Analogously, the use of 2,4-dinitrophenylhydrazine gave the corresponding hydrazone XIII in 92% yield, and the reaction with hydroxylamine afforded the oxime VIII in 73% yield.

Purine-6-carboxaldehyde hydrazone (VII) upon treatment with hot dilute acetic acid and ethyl nitrite afforded purine-6-carboxaldehyde azine (XII) in 70% yield. This transformation can be formulated as a reaction between the aldehyde IV which was formed and the hydrazone VII still present.

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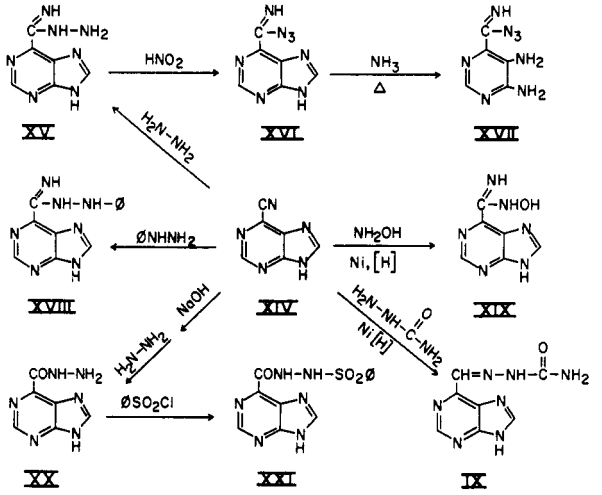
(27) St. Goldschmidt and W. L. C. Veer, *Rec. trav. chim. Pays Bas.* **65**, 796 (1946).

The derivatives of purine-6-carboxylic acid which were prepared in this work represent other approaches to the synthesis of purine-6-carboxaldehyde. According to Plieninger and Werst,[28] a variety of aromatic nitriles can be transformed into the semicarbazones of the corresponding aldehydes by hydrogenation with Raney nickel in the presence of semicarbazide hydrochloride and sodium acetate. When this reaction was applied to 6-cyanopurine (XIV),[2] a low yield (24%) of the semicarbazone (IX) was obtained (Scheme 4).



SCHEME 4.

The catalytic hydrogenation of 6-cyanopurine (XIV) in the presence of semicarbazide presumably proceeds through the formation of an intermediate such as purine-6-carboxaldehyde imine which is converted into purine-6-carboxaldehyde semicarbazone (IX) by reaction with semicarbazide. Reactions of this type have been studied by several authors.[29-31]

Reaction between 6-cyanopurine and hydroxylamine in the presence of hydrogen and Raney nickel gave the known[3] 6-hydroxyamidinopurine (XIX) instead of the desired oxime.

The reduction of aromatic nitriles to aldehydes by means of hydrazine and Raney nickel has been carried out in good yields by Pietra and Trinchera.[32,33] The rate of reduction at atmospheric pressure of a buffered mixture of 6-cyanopurine and hydrazine was very low, and a mixture of N-amino-6-purinylamidine (XV) and purine-6-carboxaldehyde hydrazone (VII) was obtained. Similar reduction experiments with phenylhydrazine hydrochloride, 6-cyanopurine and sodium acetate gave only N-phenyl-6-purinylamidine (XVIII).

The treatment of the known N-amino-6-purinylamidine (XV)[3] with nitrous acid led to purine-6-carboiminoazide (XVI) in 65% yield (Scheme 4). The ammonolysis of XVI at 180° gave 4,5-diamino-6-carboiminoazidopyrimidine (XVII).

The reaction of purine-6-carbohydrazide (XX)[3] with benzenesulfonyl chloride afforded purine-6-phenylsulfonecarbohydrazide (XXI) in 83% yield. This compound (XXI) was synthesized with the aim of preparing purine-6-carboxaldehyde (IV) from it by treatment with sodium carbonate and ethylene glycol, but only a small amount of purine-6-carboxylic acid was obtained and starting material was recovered unchanged from the reaction mixture. This method was first described by McFadyen and Stevens[34] for the preparation of aromatic aldehydes and was further developed by Buchman and Richardson[35] and Erne and coworkers.[36]

Among other approaches which were studied, but which did not yield either the desired aldehyde IV or its derivatives, were the oxidation of purine-6-carbohydrazide (XX) with sodium metaperiodate, following the method of synthesis of certain heterocyclic aldehydes by Wingfield and coworkers.[37] Purine-6-carboxylic acid and starting material were isolated from the reaction product. The oxidation of purine-6-carbohydrazide (XX) with potassium ferricyanide gave purine-6-carboxamide as already described[3] instead of the aldehyde which might have been expected.[38] Reduction of 6-cyanopurine (XIV), in the presence of N,N'-diphenylethylenediamine, acetic acid, hydrogen and Raney nickel, according to the method of Plieninger and Werst[28] (see also ref. 39) failed to give the desired 1,3-diphenyltetrahydroimidazole intermediate. 6-Aminomethylpurine[3] was formed and N,N'-diphenylethylenediamine was recovered unchanged from the reaction mixture.

Application of the Stephen method[40] (reaction with stannous chloride) was equally unsuccessful for the preparation of the aldehyde from 6-cyanopurine (XIV). Attempts to obtain the aldehyde by the oxidation of 6-methylpurine with selenium oxide[41-43] were also unsuccessful.

The exchange reactions of aldehydes with semicarbazones and thiosemicarbazones have been useful in the synthesis of some otherwise difficultly attainable aldehydes. Among the several descriptions of this type of reactions found in the literature, the recent work by Felder and Pitré[44] deals with the transaldehydation of pyridine semicarbazones in acid solution with *m*-nitrobenzaldehyde. The use of either this reagent or benzaldehyde for the exchange reaction with purine-6-carboxaldehyde thiosemicarbazone (X) or semicarbazone (IX) gave only partial conversion into the aldehyde IV as determined by ultraviolet spectrophotometry. The aldehyde IV could not be iso-

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lated. The aldehyde exchange reaction was applied to purine-6-phenylsulfonecarbohydrazide (XXI) by use of an excess of benzaldehyde in hot acid solution. The starting material XXI was recovered from the reaction mixture.

Thiosemicarbazones of aromatic aldehydes have been found active against acid-fast bacteria,[45] and heterocyclic thiosemicarbazones have shown an activity against vaccinia virus.[46] Pyridine-2-carboxaldehydes and related compounds have been found to be active against mouse leukemia.[47]

The inhibitory effect of pteridine aldehyde thiosemicarbazone and semicarbazone on the growth of *Streptococcus faecalis* and *Lactobacillus arabinosus* has been reported recently.[48]

The biological effects of the compounds described in this communication are under study in the Division of Experimental Chemotherapy, and the results will be presented elsewhere.

Experimental[49]

Purine-6-methylene-pyridinium Iodide (II).—6-Methylpurine (I)[50] (13.4 g., 0.1 mole) was dissolved in dry pyridine (200 ml.). A solution of iodine (19 g., 0.15 mole) in dry pyridine (100 ml.) was added and a dark precipitate appeared in a few seconds. The mixture was heated at 110° with continuous stirring for 6 hr. After cooling, benzene (400 ml.) was added, and the mixture was stirred for 5 minutes. The suspension was filtered, and the precipitate washed twice with benzene (100 ml., each time) and dried. A brown crystalline precipitate, 38.5 g. m.p. 210° dec., was obtained. The crude material was recrystallized from water with charcoal to give 16.2 g. of pale yellow plates, m.p. 246° dec. From the mother liquors, 5.4 g. of additional crystals was obtained, m.p. 242° dec., total yield 63%. Recrystallization from water gave colorless plates, m.p. 246° dec.

Anal. Calcd. for $C_{11}H_9N_5I$: C, 38.95; H, 2.97; N, 20.65; I, 37.42. Found: C, 38.89; H, 3.25; N, 20.40; I, 37.67.

***p*-Phenylenediamine-N,N-dimethyl-N'-(6-purinylmethylene)-N'-oxide (III).**—Finely powdered *p*-nitrosodimethylaniline (4.0 g., 0.027 mole) was added to a suspension of purine-6-methylenepyridinium iodide (II) (9.0 g., 0.027 mole) in pyridine (30 ml.). To this suspension, 2 *N* NaOH (25 ml.) was added with stirring, at room temperature. The dark reaction product turned red, the solids dissolved, and the temperature rose to about 45°. After a few minutes, the reaction mixture turned orange-brown and thickened. The stirring was continued for 5 minutes and the resulting paste was allowed to stand overnight at room temperature. The orange-colored precipitate was collected and dried in air to yield 9.7 g., m.p. 170–172° dec. The product, which gave a strongly alkaline reaction in aqueous solution, was suspended in water (10 ml.) and the suspension acidified to *p*H 5 with 5% acetic acid. The orange-red product which resulted was collected, dried *in vacuo* over NaOH and yielded 7.1 g. (94%) of crystals, m.p. 176–178° dec. A sample of this material was recrystallized from ethanol to give orange-red rhomboidal crystals, m.p. 180–182° dec.

Anal. Calcd. for $C_{14}H_{14}N_6O$: C, 59.56; H, 4.99; O, 5.66. Found: C, 59.55; H, 5.06; O, 6.12.

Purine-6-carboxaldehyde (IV). Method A.—Ethyl nitrite (2 ml.) was added at 0° to a stirred solution of purine-6-carboxaldehyde hydrazone (see below) (VII) (0.60 g., 3.6 mmoles) in 1 *N* HCl (3 ml.) and 50% aqueous ethanol (4 ml.) kept at 0°. An effervescence occurred, and after 5 minutes of stirring, at the same temperature, the solution was filtered and evaporated *in vacuo*. To the residue 10 ml. of 50% aqueous ethanol was added, evaporated to dryness *in vacuo* and washed with cold alcohol to yield 0.50 g. (67%) of tiny colorless plates which charred at 235–240° and melted above 350° dec.

Anal. Calcd. for $C_6H_4N_4O \cdot HCl \cdot H_2O$ (IV): C, 35.56; H, 3.48; N, 27.65; Cl, 17.50. Found: C, 35.23; H, 3.78; N, 27.94; Cl, 17.33.

This compound (IV) was very soluble in cold water and easily oxidized by boiling in aqueous ethanolic solution containing charcoal; the resulting product gave an ultraviolet spectrum corresponding to that of purine-6-carboxylic acid.[2] The m.p. of a recrystallized sample (from H_2O) was 195–200° dec. and it was chromatographically indistinguishable from an authentic sample of purine-6-carboxylic acid.[2]

A 5% aqueous solution of semicarbazide hydrochloride was added to a sample of IV dissolved in 1 *N* HCl and the solution heated for 5 minutes at 80°. Upon cooling, a white crystalline precipitate was obtained, m.p. 270–275° dec., which gave the same ultraviolet spectra as purine-6-carboxaldehyde semicarbazone (IX).

The aldehyde IV could be obtained with the same yield by carrying out the reaction at a higher temperature (up to 60°), for 10 to 15 minutes, although the reaction product was darker.

Ultraviolet Spectral Properties.—The ultraviolet spectra of purine-6-carboxaldehyde (IV) at various values of *p*H are shown in Fig. 1. Purine-6-carboxaldehyde (IV) exhibited a basic dissociation, pK_{a_1} 2.4 ± 0.1, and an acidic dissociation, pK_{a_2} 8.8 ± 0.1. The anion (*p*H 11.9), neutral (*p*H 3.9 to 7.2) and mainly cationic species (*p*H 1.61) showed, respectively, single maxima at 277 (A_M 6,380), at 267 (A_M 8,610) and 264 mμ (A_M 7,590). Glycine buffers in the *p*H region 8 to 10 gave erroneous spectral data presumably due to an interaction with the aldehyde.

Method B.—A solution of 1 *N* HCl (10 ml.) was added to a suspension of *p*-phenylenediamine-N,N-dimethyl-N'-(6-purinylmethylene)-N'-oxide (III) (1 g., 3.5 mmoles) in water (5 ml.). The suspension changed from dark red to violet and green. The precipitate which formed was collected and dried to yield 0.3 g. of green crystals of *p*-dimethylaminophenylhydroxylamine (VI),[22] m.p. 120–122° dec. This product gave a deep violet color when its aqueous solution was treated with ferric chloride. The filtrate from the above suspension was heated at 70–75° for 30 minutes, cooled, evaporated to a sirup *in vacuo* and ethanol (10 ml.) was added three times to eliminate the excess of HCl. The sirupy residue was filtered to remove a small amount of insolubles and the thick filtrate was allowed to stand for 48 hours at room temperature. The small amount of solid (25 mg., 4%) which separated as colorless plates, was collected; it charred at 235–240° and melted with decomposition at >350°. This material was readily soluble in water. Treatment of an acidic solution of this material with semicarbazide hydrochloride gave a product which melted at 270–275° dec. and possessed the same ultraviolet spectra as purine-6-carboxaldehyde semicarbazone (IX). The mixed m.p. of this material with a sample of IX showed no depression. The R_f values in different solvent systems and the ultraviolet absorption data indicated that the product obtained by method B was identical with that obtained by method A.

Purine-6-carboxylic Acid (V). Method A.—A solution of $KMnO_4$ (0.6 g., 3.8 mmoles) in water (10 ml.) was added dropwise and with continuous stirring at 0° to a cooled solution of purine-6-methylenepyridinium iodide (II) (0.338 g., 1 mmole) in water (5 ml.). After complete addition of the $KMnO_4$ solution the mixture was stirred at 2° for 30 minutes and at room temperature for 1.5 hours. Celite (0.3 g.) was added and the suspension was adjusted to *p*H 10 by the addition of 2 *N* NaOH. The resulting precipitate was collected, extracted twice with hot water (10 ml. each) and the extract neutralized with 2 *N* HCl. The solution was evaporated *in vacuo* to dryness, and the residue dissolved in water (2 ml.), treated with charcoal and acidified with 2 *N* HCl to *p*H 2. A precipitate of colorless needles appeared, which was collected and dried to yield 0.12 g. (77%) of product, m.p. 199° dec. A mixed m.p. of this product with an authentic sample of purine-6-carboxylic acid[2] gave no de-

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(49) All melting points are uncorrected. Microanalyses were carried out by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Spang Microanalytical Laboratory, Ann Arbor, Mich.

(50) Supplied by Cyclo Chemical Corp., Los Angeles, Calif.

pression. This product had the identical R_f in different solvent systems as an authentic sample of V.

Method B.—A solution containing purine-6-carboxaldehyde (IV) (20.0 mg., 0.1 mmole) in 3 ml. of 1 *N* NaOH was cooled at 0° and a 5% aqueous solution of $KMnO_4$ (0.2 ml.) was added dropwise with stirring. The temperature was kept at 0° for 1 hour after the addition of the $KMnO_4$, and at room temperature for 2 hours. A little diatomaceous earth was added, the suspension filtered and the precipitate washed with a little hot water. The combined filtrates were acidified with concentrated HCl to *p*H 2 and cooled to 0°. A crystalline precipitate appeared after a few minutes. The precipitate was collected, washed and dried at 105°, to yield 13.5 mg. (82%) of colorless needles, m.p. 200° dec. This product was found to be identical with an authentic specimen of V[2] when examined by paper chromatography in several solvent systems and by ultraviolet absorption spectrophotometry.

Purine-6-carboxaldehyde Semicarbazone (IX). Method A.—Hydrochloric acid (40 ml., 5 *N*) was added to a suspension of *p*-phenylenediamine-N,N-dimethyl-N′-(6-purinylmethylene)-N′-oxide (III) (5.64 g., 0.02 mole) in 50 ml. of water. After standing at room temperature for 30 minutes the green precipitate of *p*-dimethylaminophenylhydroxylamine (VI) which formed was removed by filtration. The filtrate was boiled 1 minute, charcoal was added, and filtered off, and a solution of semicarbazide hydrochloride (3.5 g.,0.034 mole) in water (10 ml.) was added to the filtrate. The mixture was boiled for 5 minutes and then cooled while the walls of the flask were scratched with a glass rod. The cream-white needles (1.4 g., 34%) which separated were collected, washed with water and dried and had m.p. 270–275° dec. A sample was recrystallized three times from 50% aqueous ethanol and dried at 105° over P_2O_5 *in vacuo* to constant weight. White needles were obtained, m.p. 270–275° dec.

Anal. Calcd. for $C_7H_7N_7O$: C, 40.97; H, 3.44. Found: C, 41.26; H, 3.94.

Method B.—Semicarbazide hydrochloride (0.56 g., 5 mmoles) was added to a solution of 6-cyanopurine (XIV)[2] (0.725 g., 5 mmoles) and sodium acetate (0.41 g., 5 mmoles) in 50% aqueous methanol (10 ml.). The mixture was hydrogenated at room temperature using Raney nickel (0.5 g.) as catalyst. After absorption of the theoretical amount of hydrogen, the mixture was filtered, the catalyst extracted 5 times with boiling methanol (10 ml. each time) and the combined filtrates concentrated *in vacuo*. On cooling, a light yellow precipitate appeared which was collected to yield 0.25 g. (24%) of crystals, m.p. 270° dec. By repeated recrystallizations from methanol, needles were obtained, m.p. 270–275° dec. Mixed m.p. determinations, R_f values in different solvent systems and ultraviolet absorption data indicated that this product was identical with that obtained by method A.

Ultraviolet Spectral Properties.—Purine-6-carboxaldehyde semicarbazone (IX) showed three apparent dissociation constants, pK_{a_1} 2.1 ± 0.1, pK_{a_2} 9.1 ± 0.1 and $pK_{a_3} > 13$. At *p*H 1.05, there was a single maximum at 334 mμ with A_M 17,900; at *p*H 7.47 the maximum was at 318.5 mμ, A_M 17,600.

Purine-6-carboxaldehyde Phenylhydrazone (XI).—Hydrochloric acid (5 ml., 2 *N*) was added to a suspension of *p*-phenylenediamine-N,N-dimethyl-N′-(6-purinylmethylene)-N′-oxide (III) (0.40 g., 1.4 mmoles) in water (5 ml.). The dark green precipitate of VI which formed was separated by filtration. A solution of phenylhydrazine (0.3 g., 0.028 mole) in ethanol (2 ml.) was added to the filtrate and the mixture refluxed. A precipitate of orange needles appeared after 15 minutes and the refluxing was continued for 1 hour. The suspension was cooled and the precipitate filtered off. The precipitate was suspended in a little water, the *p*H of the suspension adjusted to 7 with solid sodium bicarbonate, the orange colored needles (0.2 g., 60%) filtered off, washed with a little cold water and dried; m.p. 155–157° dec.

Anal. Calcd. for $C_{12}H_{10}N_6.H_2O$: C, 56.24; H, 4.72; N, 32.80. Found: C, 56.24; H, 4.82; N, 32.43.

Ultraviolet Spectral Properties.—The ultraviolet spectrum of purine-6-carboxaldehyde phenylhydrazone (XI) was the same from *p*H 10.7 to *ca.* 14, and showed three maxima: 242 (A_M 13,400), 294 (A_M 6,440) and at 381 mμ (A_M 22,700). The three maxima at *p*H 7.26 were at 244 (A_M 13,500), 273 to 290 (shoulder, A_M 5,800) and at 388 mμ (A_M 22,000).

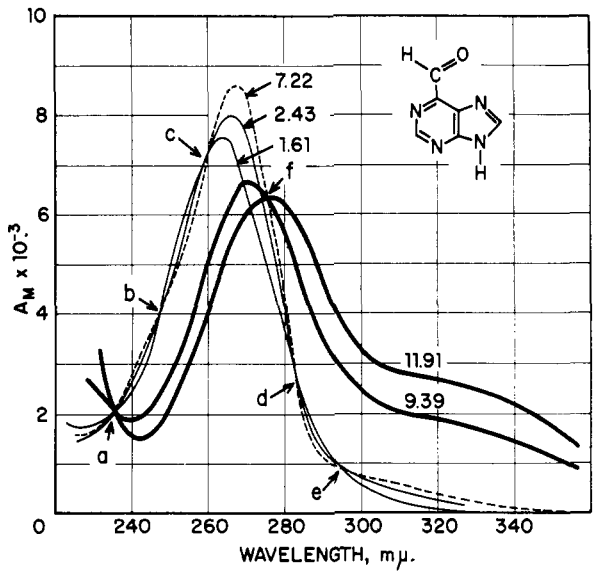

Fig. 1.—Ultraviolet absorption spectra of purine-6-carboxaldehyde at *p*H values indicated. Isosbestic points *a*, *b*, *c*, *d* and *e* are for pK_{a_1} 2.4; and *a* and *f* are for pK_{a_2} 8.8.

Purine-6-carboxaldehyde Thiosemicarbazone (X). Method A.—To a suspension of *p*-phenylenediamine-N,N-dimethyl-N′-(6-purinylmethylene)-N′-oxide (III) (0.45 g., 1.6 mmoles) in water (5 ml.), 2 *N* HCl (10 ml.) was added and the dark green precipitate of VI which appeared was removed by filtration. The filtrate was heated at 80° for 5 minutes and a solution of thiosemicarbazide (0.165 g., 1.8 mmoles) in hot water (5 ml.) was added. A yellow crystalline precipitate appeared immediately. The precipitate was collected after cooling, and dried to yield 0.32 g. (85%) of yellow needles, m.p. 250° dec. Upon recrystallization from ethanol, light cream needles, m.p. 250° dec., were obtained. A sample of X gave a positive test for sulfur (lead acetate) after sodium fusion.

Anal. Calcd. for $C_7H_7N_7S$: C, 38.00; H, 3.19; N, 44.32. Found: C, 37.97, 38.04; H, 4.39; N, 44.69.

Ultraviolet Spectral Properties.—Purine-6-carboxaldehyde thiosemicarbazone (X) showed the following maxima, respectively, at *p*H 1.02, 6.10 and 0.1 *N* NaOH: 283 and 355 mμ (A_M 3,500 and 9,500), 337 mμ (A_M 12,200) and 373 mμ (A_M 11,900).

Method B.—A solution of thiosemicarbazide (91.0 mg., 1.0 mmole) in water (5 ml.) was added to a solution of purine-6-carboxaldehyde hydrochloride hydrate (IV) (43.0 mg., 2 mmoles) in 0.5 *N* HCl (0.7 ml.). The mixture was heated at 70° for 5 minutes and allowed to stand at room temperature for 1 hour. The yellow product which appeared was collected, washed with cold water (0.5 ml.) and dried to yield 41.3 mg. (87%) of yellow needles, m.p. 245–250° dec. The ultraviolet absorption data and R_f in different solvent systems indicated that this product was identical to that obtained by method A.

Purine-6-carboxaldehyde Hydrazone (VII). Method A.—Hydrochloric acid (5 ml., 2 *N*) was added to a suspension of *p*-phenylenediamine-N,N-dimethyl-N′-(6-purinylmethylene)-N′-oxide (III) (0.4 g., 1.4 mmoles) in water (5 ml.). The precipitate of VI which formed was removed by filtration. The filtrate was heated at 80° for 5 minutes, and sufficient 20% aqueous hydrazine solution was added to adjust the *p*H to 7 and then 0.75 ml. more. A crystalline yellow precipitate appeared immediately. After cooling, the precipitate was collected, washed with a little cold water and dried. A yield of 0.2 g. (87%) of yellow needles, m.p. 240–242°, was obtained. By repeated recrystallization from ethanol, light cream colored needles, m.p. 255° dec., were obtained.

Anal. Calcd. for $C_6H_6N_6$: C, 44.46; H, 3.73; N, 51.86. Found: C, 44.41; H, 3.71; N, 51.60.

Method B.—Purine-6-carboxaldehyde thiosemicarbazone (X) (1.7 g., 7.7 mmoles) was suspended in a solution of hydrazine hydrate (3.8 g., 0.077 mole) in water (25 ml.), and the mixture refluxed for 20 minutes. After cooling, the crystalline precipitate which appeared was collected, washed with a little cold water and dried *in vacuo* over P_2O_5 to yield 0.91 g. (72%) of colorless needles, m.p. 253–254° dec. A mixed m.p. with a specimen prepared by method A showed no depression. The ultraviolet spectra data and R_f values of this product in different solvent systems were indistinguishable from that obtained by method A.[51]

Ultraviolet Spectral Properties.—Purine-6-carboxaldehyde hydrazone (VII) exhibited two dissociations, with apparent pK_{a_1} 2.8 ± 0.1 and pK_{a_2} 9.2 ± 0.1. In 1 *N* HCl, there was a single maximum at 367 mμ, A_M 11,700; at *p*H 7.50, there was a single maximum at 320 mμ, A_M 17.94 and at *p*H 11.0 it was at 327 mμ, A_M 4,660.

Purine-6-carboxaldehyde Oxime (VIII). Method A.—Hydrochloric acid (10 ml., 2 *N*) was added to a suspension of *p*-phenylenediamine-N,N-dimethyl-N′-(purinylmethylene)-N′-oxide (III) (0.8 g., 2.8 mmoles) in water (10 ml). The precipitate of VI which formed was filtered off, and 70 ml. of ethanolic solution of hydroxylamine[3] was added to the filtrate. The mixture was allowed to stand overnight. The greenish gelatinous precipitate was collected, washed with a little cold water and recrystallized from 50% aqueous ethanol to give 0.07 g. (15%) of colorless needles, m.p. 251–252° dec.

Anal. Calcd. for $C_6H_5N_5O$: C, 43.90; H, 3.68; N, 42.67. Found: C, 44.36; H, 3.32; N, 42.99.

Method B.—To a solution of purine-6-carboxaldehyde hydrochloride hydrate (11.0 mg., 0.05 mmole) in water (0.2 ml.), 5 ml. of an ethanolic solution of hydroxylamine[3] was added. The mixture was refluxed for 1 hour and concentrated *in vacuo*. On cooling, colorless needles appeared, yield 6.0 mg. (73%), m.p. 245° dec. After recrystallization from water the m.p. was 249° dec. Mixed m.p. determination, R_f values in different solvents systems and ultraviolet absorption data indicated that this product was identical with that synthesized by method A.[52]

Ultraviolet Spectral Properties.—Purine-6-carboxaldehyde oxime (VIII) exhibited a maximum at 298 mμ (A_M 10,500) at *p*H 7.28 and at 319 mμ (A_M 16,450) in 0.1 *N* NaOH.

Purine-6-carboxaldehyde 2,4-Dinitrophenylhydrazone (XIII).—A solution of 2,4-dinitrophenylhydrazine (133.0 mg., 7 mmoles) in ethanol and concentrated HCl (1:1, v./v.) (35 ml.) was added to a solution of purine-6-carboxaldehyde hydrochloride hydrate (IV) (86.0 mg., 5 mmoles) in water (2 ml.). The mixture was heated at 70° for 15 minutes. Upon cooling, a yellow orange precipitate appeared, which was collected, washed with water and ethanol, and then dried to yield 128 mg. (92%) of yellow needles, m.p. 308–310° dec.

Anal. Calcd. for $C_{12}H_8N_8O_4$: C, 43.90; H, 2.45; N, 34.14. Found: C, 43.75; H, 2.46; N, 33.93.

Purine-6-carboxaldehyde Azine (XII).—Ethyl nitrite (2 ml.) dissolved in an equal volume of ethanol was added slowly to a chilled suspension of purine-6-carboxaldehyde hydrazone (VII) (0.2 g., 1.2 mmoles) in 30% aqueous acetic acid (3 ml.). When the addition was completed, the solution was heated at 80° for 5 minutes. The resulting cream suspension was cooled and the precipitate was collected, washed with a little water and dried to yield 0.14 g. (70%) of brown crystals, m.p. >350° dec. Upon recrystallization from water, yellow needles were obtained, m.p. >350° dec.

Anal. Calcd. for $C_{12}H_8N_{10}$: C, 49.31; H, 2.76; N, 47.93. Found: C, 49.47; H, 3.13; N, 47.49.

N-Phenylamino-6-purinylamidine (XVIII).—A solution of phenylhydrazine (0.43 g., 2 mmoles) in ethanol (6 ml.) was added to 6-cyanopurine (0.29 g., 2 mmoles), dissolved in ethanol (4 ml.). The mixture was refluxed for 2 hours. Yellow crystals appeared on cooling, 0.42 g. (82%), m.p. 156°. After repeated recrystallization from 50% aqueous ethanol, yellow needles were obtained, m.p. 156°.

Anal. Calcd. for $C_{12}H_{11}N_7$: C, 55.90; H, 4.38; N, 38.83. Found: C, 56.18; H, 4.69; N, 38.83.

The same product (XVIII, instead of the desired purine-6-carboxaldehyde phenylhydrazone (XI)), was obtained when a mixture of 6-cyanopurine (0.29 g., 2 mmoles), phenylhydrazine hydrochloride (0.29 g., 2 mmoles) and sodium acetate (0.35 g., 2.5 mmoles) were dissolved in 20 ml. of a 50% aqueous solution of methanol and hydrogenated at atmospheric pressure and room temperature in the presence of 0.2 g. of Raney nickel. A yellow crystalline precipitate was formed (0.2 g.), m.p. 156°. Mixed m.p. determinations, R_f values in different solvents and ultraviolet absorption data indicated that this compound was identical with the product XVIII made by the procedure described above.

Purine-6-phenylsulfonecarbohydrazide (XXI).—Benzenesulfonyl chloride (2.2 g., 0.013 mole) was added dropwise with stirring at 20° to a suspension of purine-6-carbohydrazide (XX)[3] (1.78 g., 0.01 mole) in pyridine (25 ml.). After the complete addition, the reaction product was stirred for 3 hours at 20°, filtered, and concentrated *in vacuo*. The resulting sirup was treated with cold water (20 ml.), and the precipitate which formed was collected and dried *in vacuo* over NaOH to yield 2.70 g. (83%) of colorless needles, m.p. 226° dec. By repeated recrystallizations from ethanol, the m.p. of the product rose to 235° dec.

Anal. Calcd. for $C_{12}H_{10}N_6O_3S.{}^1/_2H_2O$: C, 44.03; H, 3.38; N, 25.67; S, 9.80. Found: C, 44.24; H, 3.47; N, 26.01; S, 10.06.

Purine-6-carboiminoazide (XVI).—N-Amino-6-purinylamidine (XV)[3] (1 g., 5.6 mmoles) was added to a solution of sodium nitrite (1.94 g., 0.028 mole) in water (14.7 ml.) was added slowly with stirring. The reaction mixture was allowed to stand at room temperature for 1 hour. The precipitate was filtered and washed twice with 5 ml. of water and dried *in vacuo* over P_2O_5 to yield 0.68 g. (65%) of colorless needles, m.p. 285° dec. This compound gave a negative test with phosphomolybdate.[53]

Anal. Calcd. for $C_6H_4N_8$: C, 38.30; H, 2.14; N, 59.56. Found: C, 37.89; H, 2.17; N, 59.11 (Kjeldahl), 58.97 (Dumas).

4,5-Diamino-6-carboiminoazidopyrimidine (XVII).—A uspension of purine-6-carboiminoazide (XVI) (0.5 g., 2.8 mmoles) in a saturated aqueous solution of ammonia was heated in a sealed tube at 180° for 10 hours. The resulting olution was evaporated *in vacuo* to yield 0.38 g. (70%) of colorless crystalline product, m.p. 325° dec. The crude product was recrystallized from 0.1 *M* NH_4OH to yield thin needles, m.p. 325° dec. A sample of these crystals gave a positive test with phosphomolybdic acid reagent.[53]

Anal. Calcd. for $C_6H_6N_8$: C, 33.71; H, 3.38; N, 62.90. Found: C, 33.87; H, 3.73; N, 63.18.

Spectrophotometric Studies.—The measurements were made with a Cary model 11 recording spectrophotometer using techniques previously described.[54,55] Discussions con-

(51) The catalytic reduction of 6-cyanopurine in the presence of buffered hydrazine sulfate was carried out by dissolving 6-cyanopurine (XIV) (0.45 g., 3 mmoles) hydrazine sulfate (0.39 g., 3 mmoles) and sodium acetate (0.45 g., 3.3 mmoles) in 30 ml. of a 50% aqueous solution of methanol. To this solution 0.2 g. of Raney nickel was added and the mixture was hydrogenated at room temperature and atmospheric pressure. A yellow precipitate was obtained (0.5 g., m. p. 220° dec.) which was identified by ultraviolet spectrophotometry as a mixture of about 70% of N-amino-6-purinylamidine (XV) and 30% of purine-6-carboxaldehyde hydrazone (VII).

(52) An attempt was made to obtain VIII from 6-cyanopurine by catalytic hydrogenation in presence of hydroxylamine. 6-Cyanopurine (0.45 g., 3 mmoles), hydroxylamine hydrochloride (0.23 g., 3 mmoles) and sodium acetate (0.45 g., 3.3 moles) were dissolved in 20 ml. of 50% aqueous methanol and subjected to hydrogenation at room temperature and atmospheric pressure in the presence of Raney nickel. After a few minutes a crystalline precipitate appeared which was identified as the known[3] 6-hydroxyamidinopurine (XIX), m.p. 274–276° dec. The mixed m.p. of this product with XIX showed no depression. It exhibited the same ultraviolet spectra and R_f values in different solvent systems as XIX. The yield of the product was 0.6 g. (98%).

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cerning the significance of isosbestic points have appeared.[18,56]

Acknowledgments.—The authors wish to express their gratitude to Dr. George B. Brown for his advice and encouragement, and to Dr. Jack J. Fox for valuable discussions.

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NEW YORK 21, N. Y.

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Synthesis of 5-Substituted Pyrimidines *via* Formaldehyde Addition[1]

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RECEIVED NOVEMBER 7, 1958

A reinvestigation of the synthesis and properties of 5-hydroxymethyluracil has revealed that this compound is not unstable and can be prepared in 80% yield by the simple addition of formaldehyde to uracil. The 5-hydroxymethyl compounds, including those prepared from orotic acid, uridine and deoxyuridine, were oxidized to 5-formyl- and 5-carboxypyrimidines, hydrogenated to 5-methyl-, 5-hydroxymethyldihydro- and 5-methyldihydropyrimidines and condensed with alcohols and acids (including amino acids). Radioactive 5-hydroxymethyl derivatives of uracil and uridine were used in biological studies.

Formaldehyde and its addition products with pyrimidines are presently of considerable interest in connection with problems of nucleic acid metabolism. Wyatt and Cohen identified 5-hydroxymethylcytosine as a unique constituent of the DNA of T-even bacteriophage,[2] and Flaks and Cohen noted that phage-infected *E. coli* contain enzymes which can utilize formaldehyde to convert deoxycytidylic acid to the 5-hydroxymethyl derivative, or deoxyuridylic acid to thymidylic acid.[3] The latter transformation has also been studied by Friedkin using extracts of normal *E. coli*,[4] and a similar formation of thymidine from formaldehyde and deoxyuridine has been found to occur with extracts of thymus gland.[5,6] The addition product of formaldehyde with tetrahydrofolic acid appears to be an important cofactor in these biosyntheses. The conversion of 5-hydroxymethylcytosine to thymine has been observed in various bacteria.[7] Thymine biosynthesis has been reviewed recently.[8]

In a previous communication from this Laboratory[9] it was reported that incubation of C^{14}-labeled thymine with rat liver slices led to the formation of 5-hydroxymethyluracil (5-HMU) and 5-methyluridine as radioactive products. Because of puzzling literature reports concerning the preparation and properties of such compounds their proper identification required initiation of the studies reported in this paper. For example, it was surprising to find that 5-HMU prepared by deamination of 5-hydroxymethylcytosine was stable to prolonged boiling in aqueous solution, for the instability of 5-HMU and some other 5-hydroxymethylpyrimidines, in contrast to 6-hydroxymethylpyrimidines, appeared well documented.[10] Johnson and Litzinger, believing the lability of 5-HMU prevented its preparation from uracil and formaldehyde, devised a round-about synthesis involving deamination of thyminylamine,[11] although Kircher had been able to make 5-hydroxymethyl-6-methyluracil in good yield from formaldehyde and 6-methyluracil.[12]

The addition of formaldehyde to uracil (I) was reinvestigated with the aid of chromatographic and other techniques which were unavailable to the earlier workers and which greatly facilitate the detection and isolation of products present in complex reaction mixtures. After a mixture of paraformaldehyde and I were allowed to stand in warm 0.4 *N* KOH, there was obtained a 70–80% yield of product showing the elementary analysis expected for 5-HMU (II) and having R_f values which were identical with those of the 5-hydroxymethylcytosine deamination product in all chromatographic solvents tested. A lower yield and more by-products were obtained when the reaction was carried out in acidic media. Stability studies described in the Experimental section showed that although extreme conditions were generally required to reverse the simple addition of formaldehyde to pyrimidines, such as the conversion of II to I, much milder conditions sufficed to bring about self-condensation reactions of hydroxymethylpyrimidines with the evolution of a little formaldehyde.

The infrared absorption spectrum of II was measured by Ulbricht and interpreted as being consistent with the presence of the hydroxymethyl group.[13] Comparison of the ultraviolet absorption

(1) For a preliminary report on portions of this work see R. M. Fink, R. E. Cline and K. Fink, *Federation Proc.*, **15**, 251 (1956). A later report was presented before the Division of Organic Chemistry at the 133rd Meeting of the American Chemical Society, San Francisco, California, April, 1958. Financial support was provided in part by U. S. Public Health Service Grant C-1669 and by Cancer Research Funds of the University of California.

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(13) We are indebted to Dr. T. L. V. Ulbricht of Yale University for infrared data on 5-HMU (II). In common with two other 5-hydroxymethylpyrimidines, II shows a hydrogen-bond hydroxyl stretching vibration at 2.9 μ and a C–O vibration at 9.9 μ characteristic of pri-