Reactions of 6-Hydrazino-, 6-Hydroxylaminopurines and Related Derivatives

Alfredo Giner-Sorolla

Division of Cell Biochemistry, The Sloan-Kettering Institute for Cancer Research

7*H*-Tetrazolo[5,1-*i*]purine was prepared by nitrosation of 6-hydrazinopurine and by reaction of 6-chloropurine with sodium azide; it was converted to adenine upon catalytic hydrogenation. 6-Hydroxylaminopurine was oxidized to 6-nitrosopurine with manganese dioxide, while alkaline treatment of the former gave 6,6′-azoxypurine. Nitrosation of 6-hydroxylaminopurine afforded 6-(*N*-nitroso)hydroxylaminopurine. Reaction of 6-chloropurine with 6-hydrazinopurine led to 6,6′-bisadenine; the corresponding ribosyl derivatives gave 6,6′-bisadenosine. Upon air oxidation, 6,6′-bisadenine was converted into 6,6′-azopurine. The related 6-thiosemicarbazino- and 6-(*N*-methyl)ureidopurine derivatives are also described. 6-*N*-(Nitroso)hydroxylaminopurine showed an inhibitory activity against several mouse tumors and leukemias.

A variety of 6-substituted purines, such as 6-hydroxylaminopurine (2) and its 9-ribosyl derivative (3), have shown an antitumor activity in mice. Several new purines and nucleosides have now been synthesized from 6-hydroxylamino- and 6-hydrazinopurine derivatives.

Reaction of 6-chloropurine (4) (**1**) with sodium azide led to 7*H*-tetrazolo[5,1-*i*]purine (**4**) in low yield (Scheme I); nitrosation of 6-hydrazinopurine (5) (**5**) afforded the same tetrazolo derivative **4** in 70% yield. Compound **4** was initially formulated as 6-azidopurine (6), but analogous reactions in the pyrimidine series (7) and infrared studies by Montgomery and coworkers (8) indicated that the reaction product had the three-ring formulation **4**. An X-ray diffraction analysis by Glusker and coworkers (9) confirmed the tetrazolo structure.

Oxidation of 6-hydroxylaminopurine (2) (**7**) by activated manganese dioxide (10) gave 6-nitrosopurine (**8**), which was reduced to adenine upon treatment with aniline and transformed into adenine and hypoxanthine with Raney nickel in dilute ammonium hydroxide. The synthesis of a nitrosopurine, 2,6-dihydroxy-9-methyl-8-nitrosopurine, by treatment of 2,6-dihydroxy-9-methyl-8-thiopurine with nitric acid and sodium nitrite was reported by Biltz and Sauer (11). It was later established (12), however, that the preparation of Biltz and Sauer consisted of a mixture of 9-methylxanthine and 9-methyl-8-nitroxanthine.

6-Hydroxylaminopurine (**7**) was smoothly transformed into 6-(*N*-nitroso)hydroxylaminopurine (**9**) by nitrous acid treatment. Compound **9** was readily hydrolyzed in acid solution into hypoxanthine.

Exposure of 6-hydroxylaminopurine (**7**) to aqueous sodium hydroxide led to the rapid formation of the sodium salt of 6,6′-azoxypurine (**10**) the free form of which was obtained by treatment with sodium acetate and 20% acetic acid; direct acidification brought about extensive decomposition. Solutions of **7** in ammonium hydroxide were transformed into 6,6′-azoxypurine (**10**) at 25° after 5 days.

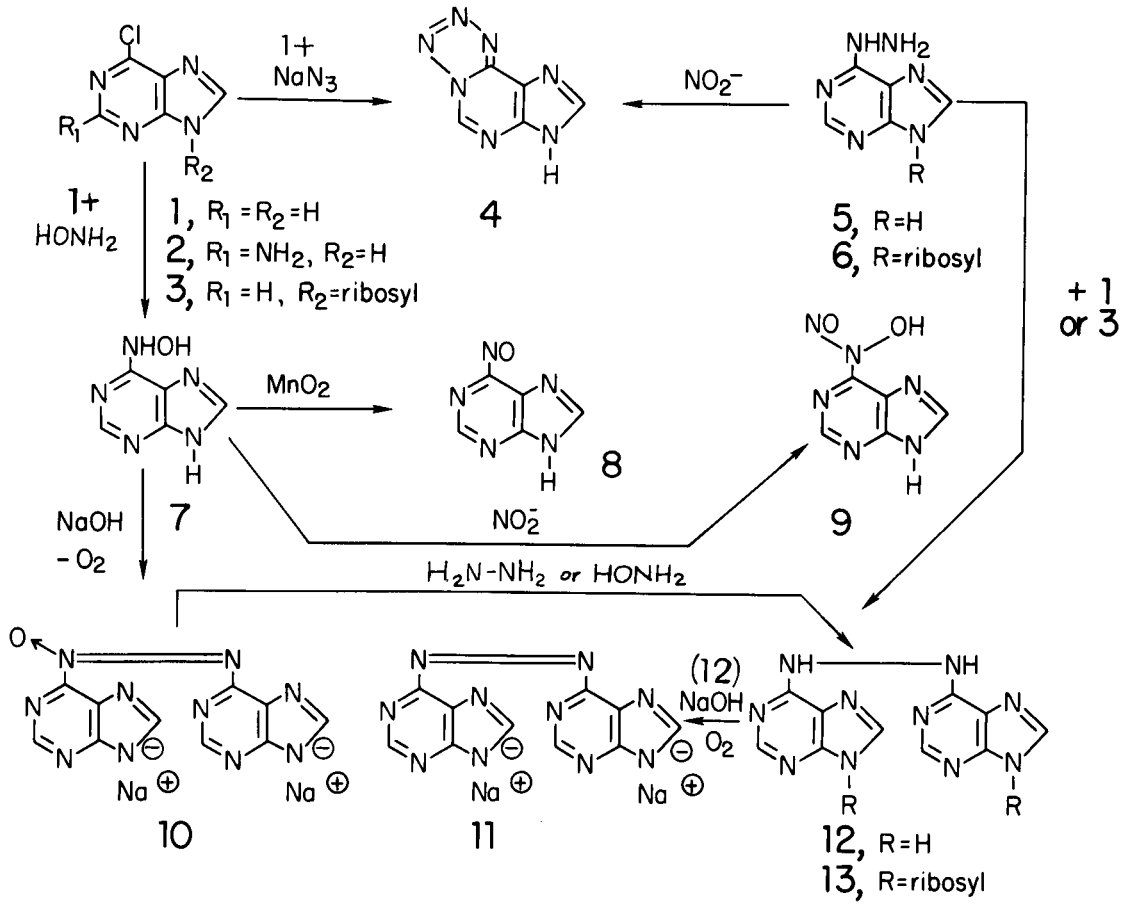
6,6′-Bisadenine (**12**) was obtained by refluxing equimolar amounts of 6-chloropurine (**1**) and 6-hydrazinopurine (**5**) in buffered solution. Bispurines, as well as bispyrimidines and pyrimidinopurines, have been described previously (13). Condensation of equimolar amounts of the 9-ribosyl derivatives of 6-chloro (14) (**3**) and 6-hydrazinopurine (8a) (**6**) resulted in the synthesis of 6,6′-bisadenosine (**13**). Air oxidation of 6,6′-bisadenine (**12**) in alkaline solution led to the sodium salt of 6,6′-azopurine (**11**). This compound was more sensitive to acid treatment than the azoxypurine (**10**), and accordingly could not be isolated as the free base.

Interaction of 6-chloropurine (**1**) or 2-amino-6-chloropurine (**2**) with thiosemicarbazide afforded 6-thiosemicarbazinopurine (**14**) and 2-amino-6-thiosemicarbazinopurine (**15**), respectively. Semicarbazide hydrochloride in a buffered solution did not react with **1**. 6-Ethoxycarbamylpurine (**2**) (**16**) was converted into the 6-monomethylureidopurine (**17**) upon refluxing with methylamine.

EXPERIMENTAL (15)

7*H*-Tetrazolo[5,1-*i*]purine (**4**).

Sodium nitrite (2.4 g., 0.035 mole) dissolved in water (10 ml.) was added dropwise to a solution of 6-hydrazinopurine (5) (**5**), (3.0 g., 0.02 mole) in 0.2 *N* hydrochloric acid (50 ml.) at 5°, with gentle and continuous swirling in a flask free of scratches. A white crystalline precipitate appeared and the mixture was kept at 5° for 30 minutes. The precipitate was collected, washed with cold water



and dried *in vacuo* over sodium hydroxide, colorless needles (1.6 g.) m.p. 185-186° (dec. with explosion). The solution, after standing at 5° for 2 hours afforded a second crop of crystals (0.9 g.), m.p. 183-185° dec., total yield 2.5 g. (70%). After repeated recrystallization from water, long lustrous needles were obtained, m.p. 193-195° dec. An anhydrous sample of **4** was prepared by heating at 105° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_5H_3N_7 \cdot H_2O$ (hydrate): C, 33.52; H, 2.81. Found: C, 33.89; H, 2.70. *Anal.* Calcd. for $C_5H_3N_7$ (anhydrous): C, 37.27; H, 1.88. Found: C, 37.43; H, 1.74 (16).

The refluxing of 6-chloropurine (**1**) (154 mg. 1 mmole) with sodium azide (70 mg., 1.1 mmoles) in ethanol for 1 hour yielded a mixture of starting material **1** and 7*H*-tetrazolo[5,1-*i*]purine (**4**) as determined by paper chromatography and uv spectra. The use of excess of sodium azide or longer heating periods failed to improve the outcome of the reaction; the resulting product always consisted of a mixture of **1** and **4**.

7*H*-Tetrazolo[5,1-*i*]purine (**4**) was converted to adenine by reduction with hydrogen at 25° and atmospheric pressure using 5% palladium/charcoal or Raney nichel as catalysts.

Thermal decomposition at 165-170° of 7*H*-tetrazolo[5,1-*i*]-purine (**4**) resulted in the liberation of 96% of the theoretical amount of nitrogen and an amorphous residue was obtained.

X-Ray diffraction studies on a sample of 7*H*-tetrazolo[5,1-*i*]-purine (**4**) obtained by repeated recrystallization from water, were carried out by Dr. J. P. Glusker and coworkers who found it to have the tetrazolo configuration (9,17).

6-Nitrosopurine (**8**).

A solution of 6-hydroxylaminopurine (2,18) (**7**) (3.0 g. 0.020 mole) in water (2 liter) was prepared by heating at 70° and then cooling to 40°. Sodium acetate (6 g., 0.072 mole) was added, then activated manganese dioxide (10) (7.5 g.), and the suspension was stirred at 30-35° for 1 hour. The precipitate of manganese dioxide was collected and washed with water. Upon adjustment of the *p*H to 6 with 20% acetic acid, the combined filtrates were evaporated *in vacuo*, keeping the temperature below 40°, to a volume of 100 ml. After cooling, the crystalline precipitate was collected, washed with water and dried *in vacuo* over phosphorus pentoxide, yield, 0.63 g. (21%) of short brown yellow prisms, m.p. 195° (with explosion when inserted at 185°); λ max 0.1 *N* hydrochloric acid, 266 and 332 mμ; λ max *p*H 6.8 (0.01 *M* phosphate buffer) 268 (4.62×10^3) and 338 mμ (6.99×10^3); λ max 0.1 *N* potassium hydroxide 249 and 316 mμ. The solubility of **8** in water was 155 mg./l. at 25° (± 1°).

Anal. Calcd. for $C_5H_3N_5O$: C, 40.27; H, 2.03; N, 46.97. Found: C, 40.59; H, 2.49; N, 47.16.

Compound **8** gave a positive Liebermann test (19) (nitroso function) and negative ferric chloride and phosphomolybdate tests (absence of NHOH function).

6-Nitrosopurine (**8**) (30 mg.) was dissolved in 5% ammonium hydroxide (10 ml.) and Raney nichel (100 mg.) was added. The suspension was boiled for 3 hours and filtered. As judged by paper chromatography and uv spectra, the colorless filtrate contained

adenine and hypoxanthine. A sample of **8** (10 mg.) was refluxed with *M* hydroxylamine in 95% aqueous ethanol (25 ml.) for 1 hour. The uv spectra and paper chromatography showed that the reaction product contained a mixture of adenine and hypoxanthine. Similar treatment of a sample of **8** (10 mg.) with 20% ethanolic hydrazine (10 ml.) gave adenine and unidentified products. 6-Nitrosopurine (**8**) (25 mg.) when heated with aniline (0.5 ml.) at 110-120° for 2 hours gave a crude product (17.5 mg.) with uv spectral and chromatographic properties identical to those of adenine. Upon treatment of compound **8** (10 mg.) in *N* hydrochloric acid (5 ml.) at 80° for 1 hour, hypoxanthine was obtained together with unidentified products.

6-(*N*-Nitroso)hydroxylaminopurine (**9**).

Sodium nitrite (3.2 g., 0.046 mole) dissolved in water (15 ml.) was added slowly with stirring to a solution of 6-hydroxylaminopurine (**7**) (3.02 g., 0.02 mole) in 2 *N* hydrochloric acid (40 ml.) at 5°. A yellow product was formed and the resulting suspention, after stirring at 5° for 3 hours, was filtered and washed repeatedly with cold water to yield yellow needles, 3.48 g. (quantitative), m.p. 118-120° (explodes when inserted at 110°); λ max 0.1 *N* hydrochloric acid, 249 (16.64 x 10^3) and 327 mμ (3.24 x 10^3); λ max *p*H 7, 250 mμ (7.51 x 10^3); λ max 0.1 *N* potassium hydroxide, 221 (9.75 x 10^3) and 263 mμ (12.62 x 10^3).

Anal. Calcd. for $C_5H_4N_6O_2 \cdot H_2O$; C, 30.31; H, 3.05; N, 42.42. Found: C, 30.41; H, 3.08; N, 42.40.

Compound **9** when boiled in water or in aqueous acid solutions was converted to hypoxanthine.

6,6′-Azoxypurine (**10**).

Method A.

6-Hydroxylaminopurine (**7**) (12 g., 0.079 mole) was dissolved in 2 *N* sodium hydroxide (120 ml.) with stirring at 25°; after a few seconds the solutions changed in color from pink to violet to red-brown. In a few minutes the reaction mixture congealed to a thick mass with evolution of gas, presumably oxygen (20). After standing at 25° for 24 hours, the precipitate was filtered and dissolved in water (150 ml.). The *p*H of the solution was first adjusted to 7.5 by the addition of solid sodium acetate and then to 6.5 with 20% acetic acid. The yellow-brown precipitate was collected, thoroughly washed with cold water and dried to yield 6.8 g. (61%) of thin brown needles, m.p. > 350°; λ max *p*H 7 (0.01 *M* phosphate buffer) 270 (shoulder) and 385 mμ; λ max *p*H 12, 232 (16.2 x 10^3) and 408 mμ (10.2 x 10^3).

Method B.

A suspension of 6-hydroxylaminopurine (**7**) (0.5 g., 3.2 mmoles) in concentrated ammonium hydroxide (15 ml.) yielded 6,6′-azoxypurine (**10**) (0.26 g., 57%) after standing at 25° for 5 days (21).

Anal. Calcd. for $C_{10}H_6N_{10}O$: C, 42.55; H, 2.14; N, 49.63. Found: C, 42.90; H, 2.54; N, 49.63.

The potassium salt, obtained by treatment of **7** (1.0 g., 7 mmoles) in 2 *N* potassium hydroxide (10 ml.), was washed thoroughly with ethanol to yield 0.44 g. (37%) red needles, m.p. >350°.

Anal. Calcd. for $C_{10}H_4N_{10}K_2O \cdot 2H_2O$: C, 30.45; H, 2.04; N, 35.51. Found: C, 30.37; H, 2.24; N, 35.60.

When the potassium or sodium salts of **10** were treated with acids in the absence of sodium acetate, extensive decomposition occurred and an amorphous product was obtained.

Samples of 6,6′-azoxypurine (**10**) (25 mg. each) were reduced to 6,6′-bisadenine (**12**) by refluxing with 20% ethanolic hydrazine (10 ml.) or *M* ethanolic hydroxylamine (50 ml.) for 3 hours.

6,6′-Bisadenine (**12**).

Solutions of 6-chloropurine (4) (**1**) (4.6 g., 0.030 mole) in 50% aqueous ethanol (120 ml.) at 70° and 6-hydrazinopurine (5) (**5**) (4.5 g., 0.030 mole) in water (200 ml.) at 70° containing anhydrous sodium acetate (2.58 g., 0.030 mole) were combined. The mixture was refluxed with stirring for 6 hours; the suspension was then concentrated by boiling to one fourth of the original volume and cooled. The resulting precipitate was collected, washed with cold water and dried to yield 3.1 g. (77%) of a brown-yellow microcrystalline product, m.p. > 350°; λ max (*p*H 6.8), 274 mμ; λ max 0.1 *N* hydrochloric acid 278 and 338 mμ; λ max 0.1 *N* potassium hydroxide, 406 mμ.

Anal. Calcd. for $C_{10}H_8N_{10} \cdot H_2O$: C, 41.95; H, 3.52; N, 48.93. Found: C, 42.11; H, 3.53; N, 48.88.

Compound **12** is stable in acid solution. However, when dissolved in alkali, the yellow color of the solution turns a deep blue by air oxidation, changing to violet and red (transformation into the azo derivative, **11**).

6,6′-Bisadenosine (**13**).

Solutions of 6-chloro-9-β-D-ribofuranosylpurine (14) (**3**) (0.571 g., 2.0 mmoles) and 6-hydrazino-9-β-D-ribofuranosylpurine (8a) (**6**) (0.562 g., 2.0 mmoles), each in 50% aqueous ethanol (10 ml.) were combined. Sodium acetate (0.2 g., 2.5 mmoles) was added and the mixture refluxed for 3 hours. The resulting precipitate that appeared on cooling was washed with 90% aqueous ethanol to yield long needles, 0.340 g. (30%), m.p. 275° dec.; λ max *p*H 1, 274 (27.5 x 10^3) and 333 mμ (6.3 x 10^3); λ max *p*H 12, 358 mμ (22.3 x 10^3).

Anal. Calcd. for $C_{20}H_{24}N_{10}O_8$: C, 45.11; H, 4.54; N, 26.31. Found: C, 45.02; H, 4.60; N, 26.30.

6,6′-Azopurine (Sodium Salt) (**11**).

6,6′-Bisadenine (**12**) (0.90 g., 3.2 mmoles) was dissolved in 0.5 *N* sodium hydroxide (40 ml.) and air was bubbled through for 5 hours. The resulting brick red crystalline precipitate was collected, washed with a little cold water, and dried to yield 0.73 g. (70%), red needles, m.p. > 350°. An analytical sample was prepared by repeated washing with 90% aqueous ethanol; λ max 0.1 *N* potassium hydroxide, 234 (16.03 x 10^3) and 409 mμ (10.8 x 10^3).

Anal. Calcd. for $C_{10}H_4N_{10}Na_2 \cdot \frac{1}{2}H_2O$: C, 37.62; H, 1.58; N, 43.88. Found: C, 37.90; H, 1.84; N, 43.95.

Aqueous solutions of **11** decomposed when treated with dilute acid. Careful addition of dilute acetic acid to a solution of **11** buffered with sodium acetate afforded yellow needles (presumably the base) which decomposed after a few seconds and amorphous material was obtained. Similar instability was seen following direct acid treatment of 6,6′-azoxypurine (**10**).

6-Thiosemicarbazinopurine (**14**).

Solutions of 6-chloropurine (**1**) (1.54 g., 10 mmoles) in 70% aqueous ethanol (75 ml.), and thiosemicarbazide (1.90 g., 20 mmoles) in hot water (25 ml.) were combined and refluxed for 5 hours. After standing at 25° overnight, the resulting precipitate was collected, washed with a little cold water and dried, yield, 1.85 g. (81%), m.p. 216°. Repeated recrystallization from water gave colorless thin needles, m.p. 227° (22); λ max *p*H 1, 246 (14.2 x 10^3); 270 (17.4 x 10^3); λ max *p*H 6.8 (0.01 *M* phosphate buffer) 264 (18.2 x 10^3); λ max *p*H 12, 314 mμ (16.6 x 10^3).

Anal. Calcd. for $C_6H_7N_7S \cdot H_2O$: C, 31.71; H, 3.99; N, 43.15; S, 14.11. Found: C, 31.88; H, 4.05; N, 42.98; S, 14.21.

2-Amino-6-thiosemicarbazinopurine (**15**).

Solutions of 2-amino-6-chloropurine (**2**) (1.70 g., 10 mmoles) and thiosemicarbazide (1.80 g., 20 mmoles) in 50% aqueous ethanol, (75 ml., each) were combined. Sodium acetate (2.0 g., 26 mmoles) was added and the mixture refluxed for 6 hours. The resulting precipitate was collected to yield 1.85 g. (76%) of a crystalline product, m.p. 350°. An analytical sample was obtained by recrystallization from water (23); λ max *p*H 6.8 (0.01 *M* phosphate buffer), 216 (21.5 x 10^3), 246 (19.5 x 10^3), 287 mμ (13.6 x 10^3); λ max *p*H 1, 217 (20.7 x 10^3), 246 (19.2 x 10^3), 288 mμ (13.4 x 10^3); λ max *p*H 12, 320 mμ (16.6 x 10^3).

Anal. Calcd. for $C_6H_8N_8S$: C, 32.13; H, 3.60; N, 49.97; S, 14.30. Found: C, 32.11; H, 3.99; N, 49.60; S, 14.25.

N-Methyl-6-ureidopurine (**17**.

6-Ethoxycarbamylpurine (**16**) (2a) (0.4 g., 2 mmoles) was dissolved in a saturated methanolic solution of methylamine (40 ml.) and kept at 25° for 3 days. The reaction mixture was concentrated to 10 ml. and the resulting precipitate was collected, washed with a little cold water and dried *in vacuo* over phosphoric pentoxide to yield 0.25 g. (73%) colorless needles, m.p. 320-332° dec. Upon repeated recrystallization from water, the m.p. remained unchanged; λ max *p*H 1, 262 (17.5 x 10^3); λ max *p*H 6.8 (0.01 *M* phosphate buffer), 295 (15.0 x 10^3); λ max *p*H 12, 268 mμ (15.95 x 10^3).

Anal. Calcd. for $C_7H_8N_6O$: C, 43.74; H, 4.20; N, 43.75. Found: C, 43.58; H, 4.02; N, 43.61.

Biological Studies.

The new compounds reported here have been tested in the screening programs for solid tumors and leukemia in the Division of Experimental Chemotherapy and the Division of Applied Therapy. Only 6-(*N*-nitroso)hydroxylaminopurine (**9**) exhibited inhibition of mouse leukemia L1210 (increased life span: 221% at 50 mg./kg.), sarcoma 180 (ascites), Ridgway osteogenic sarcoma, and Murphy-Sturm lymphosarcoma.

Acknowledgment.

The X-ray diffraction analyses of 7*H*-tetrazolo[5,1-*i*]purine were performed by Dr. J. P. Glusker, the late Dr. A. L. Patterson and coworkers of the Institute for Cancer Research, Philadelphia. The tumor screening was carried out by Dr. J. H. Burchenal, Dr. M. R. Dollinger, Dr. G. Tarnowski and Dr. K. Sugiura. The author wishes to thank Dr. C. C. Stock, Dr. G. B. Brown and Dr. A. Bendich for continued stimulating discussions. The author also wishes to thank Miss S. A. O'Bryant, Miss C. Nanos and Mrs. C. Gryte for their valuable technical assistance.

REFERENCES

(1) This investigation was supported in part by funds from the National Cancer Institute (Grant No. CA 08748), The Atomic Energy Commission (Contract No. AT[30-1], 910) and aided by the Grant No. T-128F from the American Cancer Society and the First National City Bank Grant for Research from the American Cancer Society.

(2a) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 3932 (1958); (b) A. Giner-Sorolla, Ph.D. Thesis, Cornell University Medical College, 1958. *Dissertation Abstr.*, **20**, 1148 (1959); (c) A. C. Sartorelli, A. L. Bieber, P. K. Chang, and G. A. Fischer, *Biochem. Pharmacol.*, 13, 507 (1964); (d) P. F. Pecora and M. E. Balis, *ibid.*, 13, 1071 (1964); (e) A. Giner-Sorolla, *Galenica Acta* (Madrid), **19**, 97 (1966).

(3a) A. Giner-Sorolla, L. Medrek, and A. Bendich, *J. Med. Chem.*, **9**, 143 (1966); (b) J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, **16**, 423 (1967).

(4) A. Bendich, P. J. Russell, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(5a) G. B. Elion, E. Burgi, and G. H. Hitchings, *ibid.*, **74**, 411 (1952); (b) J. A. Montgomery and L. B. Holum, *ibid.*, **79**, 2185 (1957).

(6) A. Bendich, A. Giner-Sorolla, and J. J. Fox, in *Ciba Found. Symp. Chem. and Biol. of Purines*, 1957, Churchill Ltd., London, p. 3.

(7) F. R. Benson, L. W. Hartzel, and E. A. Otten, *J. Am. Chem. Soc.*, **76**, 1858 (1954).

(8a) J. A. Johnson, Jr., H. J. Thomas, and H. J. Schaeffer, *ibid.*, **80**, 699 (1959); (b) C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Org. Chem.*, **30**, 3601 (1965); (c) C. Temple, Jr., M. C. Thorpe, W. C. Coburn, Jr., and J. A. Montgomery, *ibid.*, **31**, 935 (1966); (d) T. Itai and G. Ito, *Chem. Pharm. Bull.* (Tokyo), **10**, 1141 (1962); (e) N. B. Smirnova and I. I. Postovskii, *Zh. Vses. Kim. Obshchestva Im. D. L. Mendeleeva*, **9**, 711 (1964).

(9) J. P. Glusker, D. V. d. Helm, W. E. Love, J. A. Minkin, and A. L. Patterson, *Acta Cryst.*, **B24**, 359 (1968).

(10) This oxidation is achieved only when active manganese dioxide prepared according to J. Attenburrow et al. (*J. Chem. Soc.*, 1094 (1952)) is employed. This reagent is available from Winthrop Laboratories, New York, N. Y. Cf. also (a) R. M. Evans *Quart. Rev.* (London), **13**, 60 (1959); (b) E. P. Papadopoulos, A. Jarrar, and C. H. Issidorides, *J. Org. Chem.*, **31**, 615 (1966).

(11) H. Biltz and J. Sauer, *Ber.*, **64**, 752 (1931).

(12) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **82**, 3773 (1960).

(13a) A. Giner-Sorolla, I. Zimmerman, and A. Bendich, *ibid.*, **81**, 2515 (1959); (b) K. W. Merz and H. Stahle, *Arch. Pharm.* (Weinheim), **293**, 801 (1960) and references contained therein; (c) H. G. Mautner, 137th Meeting, Am. Chem. Soc., Cleveland, April 1960, p. 9N; (d) A. Giner-Sorolla, E. Thom, and A. Bendich, 137th Meeting Am. Chem. Soc., Cleveland, April 1960, p. 11N; (e) J. H. Lister, *J. Chem. Soc.*, 3682 (1960); (f) C. Lachman and S. Cohen, *Israel J. Chem.*, **3**, 81 (1965); (g) F. Lingens and H. Schneider-Bernlöhr, *Ann. Chem.*, **686**, 134 (1965); (h) D. H. Hayes and F. Hayes-Baron, *J. Chem. Soc.*, 1528 (1967); (i) A. Giner-Sorolla, *Chem. Ber.*, **101**, 611 (1968).

(14) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953); supplied by H. J. Jensen, Inc., Montclair, N. J.

(15) Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11 and Model 15 (Sadtler Laboratories, Philadelphia, Pa.). Complete uv spectral data for compounds **10**,**11** and **12** were not obtainable because of their instability. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concentrated ammonium hydroxide-water-isopropyl alcohol (10:20:70); 1-butanol-water-acetic acid (50:25:25); and 1 *M* ammonium acetate-ethanol (35-70). The determination of the melting points was carried out with a Thomas Hoover melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(16) N % analysis, Calcd.: 60.85. Found: 59.75. Repeated crystallizations did not improve this figure.

(17) The uv spectral data of compound **4** are contained in ref. 6 where it is referred to as 6-azidopurine exclusively.

(18) Supplied by Nutritional Biochemical Co., Cleveland, Ohio.

(19) F. Feigl, "Spot Tests in Organic Analysis," 6th ed.,

Elsevier, Amsterdam, 1960, p. 165. Compound **8** (15 mg.) was fused with phenol (60 mg.), cooled, and a few drops of concentrated sulfuric acid added. Upon the addition of 0.5 ml. of water, an intense red cherry color appeared which turned blue with excess 5 *N* sodium hydroxide, indicative of the nitroso function (formation of *p*-nitrosophenol).

(20) Cf. alkali treatment of phenylhydroxylamine: (a) E. Bamberger and F. Tschirner, *Ber.*, 32, 342 (1899); (b) E. Bamberger, *ibid.*, 33, 113 (1900).

(21) This behavior contrasts with that observed in a similar reaction of 6-hydroxylaminopurine 3-oxide with concentrated ammonium hydroxide, where in a few seconds a complete reaction into 6,6′-azoxypurine 3,3′-dioxide is obtained (A. Giner-Sorolla, *J. Med. Chem.*, 12, 717 (1969)).

(22) In contrast with 6-chloropurine, its 3-oxide (A. Giner-Sorolla, *J. Org. Chem.*, 34, 2157 (1969)) did not react with thiosemicarbazide. Similar treatment as described of **1** with semicarbazide, guanidine and ethyl carbamate did not yield the corresponding purines.

(23) Samples of Compound **14** and **15** were treated separately with *N* sodium hydroxide at 70-80° for 30 minutes. After cooling, the solutions were acidified to *p*H 1 with 2 *N* hydrochloric acid. The uv spectra in both samples were identical to those of the respective starting materials, thus precluding any possibility of a C_6–S bond (cf: E. F. McInerney and E. J. Kupchik, *J. Med. Chem.*, 10, 741 (1967)).

Received September 6, 1969 New York, N. Y. 10021