Purine N-Oxides. XXVI. The Synthesis and Properties of 6-Halogenopurine 3-N-Oxides[1]

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The 3-N-oxides of 6-chloro- and 6-bromopurine were prepared by halogenation of 6-mercaptopurine 3-oxide and 6-iodopurine 3-oxide from the chloro derivative and HI. Displacement of chloride from 6-chloropurine 3-oxide gave purine-6-sulfonate 3-oxide and 6-methoxypurine 3-oxide. Ethanolic hydroxylamine and 6-methylmercaptopurine 3-oxide led to hypoxanthine 3-oxide. Treatment of 6-chloropurine 3-oxide with NH_3, NH_2NH_2, NH_2OH, and morpholine resulted in substitution and simultaneous loss of oxygen to yield adenine and 6-hydrazino-, 6-hydroxylamino-, and 6-morpholinopurine, respectively. Oxidation of 6-mercaptopurine 3-oxide with butyl nitrite afforded its disulfide. 6-Iodopurine 3-oxide inhibited slightly the growth of carcinoma EO771 in mice; the other compounds were inactive.

N-Oxides of chemotherapeutically effective purines offer[2] a possible improvement of the chemotherapeutic index since they are often less toxic than the parent purine, as in the case of the 6-methylpurine[3] and its 1-oxide[4] and 6-mercaptopurine and its 3-oxide.[5] The halogenopurines such as 6-chloro-[6] and 6-bromopurines[7] have shown encouraging antitumor activity in experimental animals[8] and 6-chloropurine reached clinical trial for the treatment of leukemia.[9] We now report syntheses, some chemical behaviors, and biological testing of several 6-halogenopurine 3-oxides.

Direct oxidation of purines in acetic or trifluoroacetic acids, the usual method for the preparation of purine N-oxides,[10] was not applicable to 6-chloropurine because of its instability in acidic solutions,[6] although a low yield may be obtained with *m*-chloroperoxybenzoic acid under anhydrous conditions.[11] The halogenopurine 3-N-oxides have now been obtained by substitution of the mercapto group of 6-mercaptopurine 3-oxide[5] by the corresponding halogen.

Treatment of 6-mercaptopurine 3-oxide[5] (**1**) with chlorine in methanolic HCl at $-10°$ gave an 89% yield of 6-chloropurine 3-oxide (**2**) (Scheme I). Replacement of the mercapto group by chlorine in the purine series has been previously reported.[12] A synthesis of **2** by another method has recently appeared.[13] Conversion of 6-mercaptopurine 3-oxide (**1**) to 6-bromopurine 3-oxide (**3**) was effected with HBr and Br_2. Treatment of 6-chloropurine 3-oxide (**2**) with concentrated HI afforded 6-iodopurine 3-oxide (**4**). The structure of 6-chloropurine 3-oxide (**2**) was established by its conversion to the starting 6-mercaptopurine 3-oxide (**1**) with thiourea[6] and its reduction to 6-chloropurine[6] (**5**) with Raney nickel. 6-Bromo- and 6-iodopurine 3-oxides (**3** and **4**) were also reduced to the respective 6-bromo- and 6-iodopurines[7a] (**6** and **7**) with Raney nickel.

Equimolar amounts of **2** and sodium sulfite[14] gave purine 6-sulfonate 3-oxide (**8**) which was identical with the product obtained by $KMnO_4$ oxidation of 6-mercaptopurine 3-oxide[15] (**1**). Reaction of 6-chloropurine 3-oxide (**2**) with an excess of sodium methoxide resulted in the formation of 6-methoxypurine 3-oxide (**9**) which was converted to 6-methoxypurine[16] with Raney nickel. On prolonged treatment with aqueous ammonia, 6-chloropurine 3-oxide (**2**) gave adenine **11**. Treatment of **2** with hydrazine resulted in the simultaneous and rapid reduction and hydrazinolysis to 6-hydrazinopurine[17] (**12**). Compound **2**, which reacted more slowly than its parent with ethanolic hydroxylamine, yielded 6-hydroxylaminopurine[18] (**13**). Treatment of 6-chloropurine 3-oxide (**2**) with morpholine afforded 6-morpholinopurine[19] (**14**).

Hypoxanthine 3-oxide (**16**) resulted from the treatment of 6-methylmercaptopurine 3-oxide[5] (**15**) with

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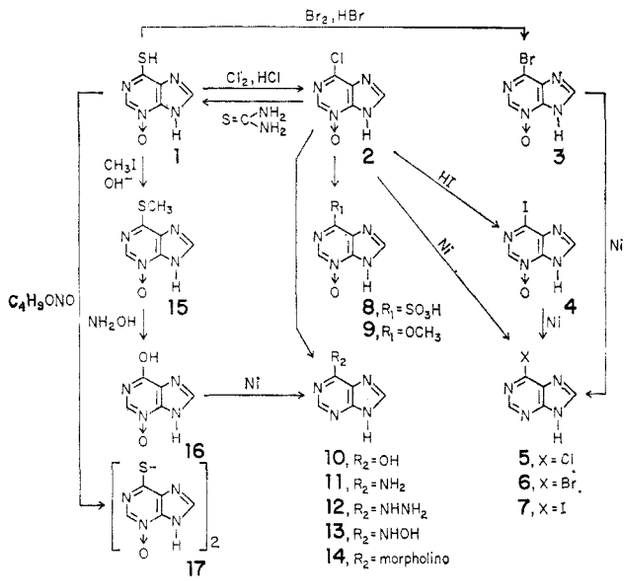
SCHEME I



methanolic hydroxylamine. This compound was identical with the product obtained in this laboratory from purine-6-sulfonate 3-oxide[15] (**8**). 6-Mercaptopurine 3-oxide (**1**) was oxidized to the disulfide **17** with butyl nitrite. Reaction of **2** with molten cyanamide resulted in its conversion to 6-chloro-9-cyanaminopurine 3-oxide.

Biological Activity.—The new purine 3-N-oxides have been tested in the Divisions of Drug Resistance and Experimental Chemotherapy for their effects against several mouse leukemias and Sarcoma 180.

The toxicity of 6-chloropurine 3-oxide toward mice bearing Sarcoma 183 was but slightly less than that of 6-chloropurine, as measured by lack of weight gain. Dosages of 6-chloropurine 3-oxide (**2**) of 125–500 mg/kg/day for 7 days gave the same modest tumorinh ibition as 62.5–250-mg doses of 6-chloropurine (**5**).

Preliminary studies revealed that 6-chloro-, 6-iodo-, and 6-bromopurine 3-oxides were ineffective when tested against mouse leukemia L1210 and Ridgway osteogenic sarcoma. Some slight inhibitory action against carcinoma E0771 in mice was observed with 6-iodopurine 3-oxide (15 days administration, 250 mg/kg/day).

Experimental Section

Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11. Paper chromatograms were run by the ascending method on Whatman No. 1 paper in the following solvent systems: H_2O saturated with *n*-BuOH, *n*-BuOH saturated with H_2O (with or without 10% NH_3); *n*-BuOH–HCOOH–H_2O (77:10:13, v/v). Melting points were taken in a Thomas–Hoover Unimelt apparatus and were corrected. The microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. In all cases, the criteria for the identity of known compounds or the new ones prepared by different methods were based on mixture melting point, uv spectra, and paper chromatography in the solvent systems described above. Th p*K* values were determined electrometrically by methods described.[20] Spectra were determined in 0.01 *M* buffers.[21] Spectral data are shown in Table I.

6-Chloropurine 3-Oxide (**2**).—Chlorine was bubbled through a suspension of 6-mercaptopurine 3-oxide hydrate[5] (**1**, 15 g, 0.081 mmol) in MeOH (15 ml) and concentrated aqueous HCl (45 ml) previously saturated at −10° with HCl. The solid dissolved and later a yellow crystalline product appeared, which redissolved. The addition of chlorine was continued until a sample of the solution permanently bleached a piece of pH indicator paper (~2.5 hr). Crushed ice (100 g) was poured onto the solution and concentrated aqueous NH_3 was added dropwise with stirring to pH 5 while the temperature was maintained below 0°. The thick white crystalline precipitate was kept at 5° overnight, collected, washed with a little cold water, and dried *in vacuo* over P_2O_5; yield 7.8 g of thin needles, mp 160° (explodes when inserted at 150°). Upon concentration of the mother liquors *in vacuo*, a second crop was obtained (5.8 g), mp 160° (explodes when inserted at 150°); total yield 13.6 g (89%). An analytical sample was prepared by repeated washing with 90% aqueous MeOH at 25°.

Anal. Calcd for $C_5H_3N_4OCl$: C, 35.21; H, 1.77; N, 32.85; Cl, 20.80. Found: C, 35.01; H, 1.93; N, 33.76; Cl, 20.71.

Treatment of **2** with mineral acids (HCl, HF) at 80° for 1 hr gave hypoxanthine (**10**).

Preparation of 6-Chloropurine 3-Oxide by Oxidation.[11]—6-Chlorpurine (**5**, 250 mg, 1.6 mmol) was dissolved in ether (400 ml) and *m*-chloroperoxybenzoic acid (85% pure) (0.6 g, 3.2 mmol) was added. The solution was stirred at 25° for 1 week. The resulting crystalline precipitate was collected, washed with ether, and yielded 30 mg (11%). This product was identical with the material prepared above. The residue obtained from the filtrate upon evaporation was washed with C_6H_6 to yield 6-chloropurine containing a small proportion of its 3-N-oxide.

An ether solution of 6-bromopurine (100 mg, 0.5 mmol, in 400 ml ether) and *m*-chloroperoxybenzoic acid (85%) (200 mg, 1 mmol) was stirred at room temperature for 1 week. The starting material was recovered unchanged.

6-Iodopurine was also recovered unchanged from a similar treatment with *m*-chloroperoxybenzoic acid.

Reactions of 6-Chloropurine 3-Oxide (**2**).—Treatment of **2** (100 mg) with aqueous concentrated NH_3 at 70° for 12 hr gave a product which was identified as adenine (**11**). Adenine was also obtained from **2** with saturated EtOH NH_3 at 100° for 18 hr.[15]

Refluxing of **2** (100 mg) with 20% EtOH hydrazine (5 ml) for 30 min gave a 54% yield of 6-hydrazinopurine[17] (**12**).

A solution of 6-chloropurine 3-oxide (**2**) (0.50 g) in 1 *M* EtOH hydroxylamine (250 ml, pH 6.8) was refluxed for 18 hr in the dark. The solution was evaporated to dryness *in vacuo* and the residue was washed with cold MeOH to yield 0.27 g (61%) of a substance which was identified as 6-hydroxylaminopurine[18] (**13**). The rate of transformation of **2** into 6-hydroxylaminopurine (as measured by uv spectral changes and by the time required to show a positive $FeCl_3$ test) was much slower than the rate of reaction of 6-chloropurine with hydroxylamine.[18]

6-Mercaptopurine 3-Oxide (**1**) **from 6-Chloropurine 3-Oxide** (**2**).—6-Chloropurine 3-oxide (**2**) (10 mg, 0.06 mmol) and thiourea (9 mg, 0.12 mmol) in EtOH (3 ml) were refluxed for 5 min. The colorless solution turned yellow after 1 min and exhibited uv spectra (at pH 1, 6.9, and 12) and R_f values (in the three solvent systems), identical with those of 6-mercaptopurine 3-oxide (**1**).[5]

TABLE I

SPECTRAL DATA AND p*K*'s

pH	Charge	λ_{max}, mμ ($\epsilon \times 10^{-3}$)	p*K*
		6-Chloropurine 3-Oxide (**2**)	
2	(0)	229 (22.4), 302 (9.1)	
			5.37 ± 0.07
8	(−)	230 (27.8), 305 (7.8)	
		6-Bromopurine 3-Oxide (**3**)	
2	(0)	233 (22.1), 290[a] (9.2), 303 (11.2)	
			5.28 ± 0.04
8	(−)	233 (29.1), 293[a] (7.3), 306 (8.4)	
		6-Iodopurine 3-Oxide (**4**)	
2	(0)	217 (14.0), 231 (13.9)	
		293[a] (9.5), 311 (14.1)	
			5.35 ± 0.06
8	(−)	238 (20), 295[a] (9.3)	
		311 (10.9), 322[a] (8.6)	

[a] Shoulder.

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6-Bromopurine 3-Oxide (**3**).—6-Mercaptopurine 3-oxide hydrate (**1**) (2.0 g, 12 mmol) was added slowly to a mixture of concentrated HBr (20 ml) and MeOH (15 ml) at −12°. When the addition was complete, bromine (5 ml) was added dropwise with stirring at the same temperature. The temperature was maintained between −10° and −5° and after 1 hr the suspension was adjusted with 50% KOH to pH 7. The yellow precipitate was collected, washed with a little cold H_2O, and dried *in vacuo* over P_2O_5; yield 1.3 g (57%), short yellow needles, mp 178° (explodes when inserted at 170°). An analytical sample was prepared by thorough washing of the product with H_2O and EtOH at 25°.

Anal. Calcd for $C_5H_3N_4BrO$: C, 27.93; H, 1.41; N, 26.06; Br, 37.17. Found: C, 27.79; H, 1.74; N, 25.97; Br, 37.08.

6-Bromopurine 3-oxide (**3**) (100 mg) was recovered unchanged after refluxing with concentrated aqueous NH_3 (50 ml) for 12 hr. Similar treatment with 1 *M* hydroxylamine led to 6-hydroxylaminopurine[18] **13**.

6-Iodopurine 3-Oxide (**4**).—Finely pulverized 6-chloropurine 3-oxide (**2**) (5.0 g, 29.3 mmol) was poured at −15° with stirring into 40 ml of HI (*d* 1.7). Solution occurred and, after a few minutes, an abundant yellow crystalline precipitate appeared. The reaction mixture was kept at −15° for 3 hr. The precipitate was collected and poured into crushed ice (40 g) and the pH carefully was adjusted to 5 with concentrated aqueous NH_3. Colorless needles, mp 175° (explodes when inserted at 165°), 6.2 g (81%), were obtained. When this product was heated slowly, decomposition with evolution of I_2 occurred. An analytical sample was prepared by thorough washing of a sample with H_2O and EtOH.

Anal. Calcd for $C_5H_3N_4IO$: C, 22.92; H, 1.15; H, 21.38; I, 48.44. Found: C, 22.91; H, 1.20; N, 21.37; I, 48.25.

A sample of 6-iodopurine 3-oxide (**4**) gave hypoxanthine (**10**) and iodine when treated with aqueous HF at 80°. When **4** (100 mg) was refluxed with concentrated aqueous NH_3 (50 ml) for 12 hr, no change in the uv spectrum was observed. Upon similar treatment with 1 *M* EtOH, hydroxylamine **4** was converted to 6-hydroxylaminopurine (**13**).

Purine-6-Sulfonate 3-Oxide (**8**).—6-Chloropurine 3-oxide **2** (170 mg, 1 mmol) was suspended in H_2O (5 ml), Na_2SO_3 (126 mg, 1 mmol) was added, and the mixture was heated to 80° for 1 hr.[14] After cooling, EtOH (15 ml) was added, the crystalline precipitate was collected and dissolved in H_2O (2 ml), and the solution was acidified to pH 5 with glacial AcOH. A precipitate, colorless prisms (78 mg, 36%), mp >350°, was obtained. This substance was identical with the product obtained from **1** by $KMnO_4$ oxidation.[15]

6-Methoxypurine 3-Oxide (**9**).—Sodium (1.15 g, 50 mg-atoms) was dissolved in MeOH (100 ml) and finely powdered 6-chloropurine 3-oxide (**2**) (2.5 g, 14.7 mmol) was slowly added with stirring. The mixture was refluxed for 3 hr, cooled, and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in H_2O (20 ml) and neutralized with 20% aqueous AcOH. The precipitate was thoroughly washed with H_2O and EtOH to yield 1.36 g (56%) of a crystalline product, mp 230° (explodes when inserted at 220°). An analytical sample of 6-methoxypurine 3-oxide (**9**) was prepared by solution in a minimum amount of H_2O, charcoal treatment, and precipitation by excess *n*-PrOH; long thin needles, mp 232° (explodes when inserted at 220°).

Anal. Calcd for $C_6H_6N_4O_2 \cdot 0.33H_2O$: C, 41.86; H, 3.90; N, 32.60. Found: C, 41.63; H, 3.80; N, 32.57.

Hypoxanthine 3-Oxide (**16**).—6-Methylmercaptopurine 3-oxide[5] (**15**) (1.0 g, 5.5 mmol) was dissolved in 2 *M* anhydrous hydroxylamine solution in MeOH (200 ml) and refluxed for 18 hr. The precipitate was collected and dissolved in H_2O (10 ml), and the pH was adjusted to 5.5 with 20% aqueous AcOH. The crystalline product was collected and washed thoroughly with H_2O and EtOH; yield 0.55 g (66%) of colorless prisms, mp 330° (explodes when inserted at 320°).

Anal. Calcd for $C_5H_4N_4 \cdot 0.5H_2O$: C, 37.27; H, 3.13; N, 34.78. Found: C, 37.68; H, 3.77; N, 33.68.

This substance was identical with that obtained from purine-6-sulfonate 3-oxide (**8**) by acid hydrolysis.[15]

Disulfide of 6-Mercaptopurine 3-Oxide (**17**).—6-Mercaptopurine 3-oxide hydrate (**1**) (0.30 g, 1.9 mmol) was suspended in EtOH (30 ml) and butyl nitrite (2 ml) was added slowly while stirring. The mixture was refluxed and stirred for 4 hr, and the yellow precipitate was filtered and washed with EtOH giving yellow crystals (0.20 g, 63%) which darkened when inserted at 265° and exploded at 270°. An analytical sample was prepared by repeated washing with 95% EtOH.

Anal. Calcd for $C_{10}H_3N_6O_2S_2$: C, 35.92; H, 1.81; N, 33.52; S, 19.18. Found: C, 35.96; H, 1.81; N, 33.52; S, 19.19.

This product was identical with that obtained by iodine oxidation of **1**.[15]

6-Chloro-9-cyanaminopurine 3-Oxide.—6-Chloropurine 3-oxide (**2**) (0.05 g, 2.9 mmol) was dissolved in molten cyanamide (2.5 g, 0.06 mmol) at 50°. The solution was heated at 80–85° for 30 min and then cooled. The mixture was washed with MeOH to afford 0.46 g (69%) of thin colorless needles, mp >350°. An analytical sample was obtained by recrystallization from EtOH.

Anal. Calcd for $C_6H_3N_6OCl \cdot H_2O$: C, 31.52; H, 2.20; N, 36.76; Cl, 15.51. Found: C, 31.36; H, 3.09; N, 36.52; Cl, 15.37.

In a similar experiment 6-chloropurine (**5**) did not react with cyanamide.

Reduction of 6-Substituted Purine 3-Oxides.—The 3-oxides of 6-chloro- (**2**), 6-bromo-, (**3**), and 6-iodopurine (**4**), purine-6-sulfonate (**8**), 6-methoxypurine (**9**), and hypoxanthine (**16**) were dissolved (10 mg each) in 5% aqueous NH_3 (10 ml) suspension of Raney nickel (100 mg) and boiled for 1 hr (except for **2** which required 72 hr to complete conversion to **5**). After evaporation of the filtrate to 0.5 ml, the reaction products were found to be identical[20] with 6-chloro- (**5**),[6] 6-bromo- (**6**),[7] and 6-iodopurine (**7**)[7a] purine-6-sulfonate,[14] 6-methoxypurine,[16] and hypoxanthine (**10**), respectively.

Registry No.—**2**, 19765-60-7; **3**, 19765-61-8; **4**, 19765-62-9; **8**, 19765-63-0; **9**, 19765-64-1; **16**, 19675-65-2; **17**, 19765-66-3; 6-chloro-9-cyanaminopurine 3-oxide, 19765-67-4.

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