

Israel Scheinfeld, James C. Parham\* (2) and George Bosworth Brown

Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Received March 1, 1982

*N*-Oxidation of 2-amino-6-chloropurine to the 3-oxide provided a convenient intermediate for the synthesis of 2-amino-6-substituted purine 3-oxides, including the previously unavailable 2,6-diaminopurine 3-oxide. Thiation of the 6-halogen was accompanied by reduction of the *N*-oxide. The properties of the 1- and 3-oxides of 2,6-diaminopurine are compared.

*J. Heterocyclic Chem.*, **19**, 1231 (1982).

*N*-Oxidized purine derivatives have exhibited a variety of biological properties, including inhibition of several relatively substrate-specific enzymes involving purine nucleotides (3-5). Some have been investigated as potential cancer chemotherapeutic agents (6-9), while several *N*-oxidized purines proved to be potent oncogens when administered to rodents (11-18). The greatest oncogenic activity was found in purines oxidized at the 3 or 7 positions, e.g., 3- and 7-hydroxyxanthine and 3-hydroxyguanine. As part of studies on the structural requirements for and the mechanism of tumor induction by these compounds, a route for the synthesis of 2,6-disubstituted purine 3-oxides was desired.

The substituent at the 6-position of purines can direct the position of *N*-oxidation in the pyrimidine ring. Electron-donating substituents at C-6 direct *N*-oxidation to the adjacent 1-nitrogen, as in the oxidation of adenine and 2,6-diaminopurine (19). Strongly *s*-withdrawing substituents at C-6 render the molecule less susceptible to *N*-oxidation and direct reaction to the 3-nitrogen, as shown by the *N*-oxidation of 6-chloro- (20,21) and 6-methoxypurines (20,22) solely to the 3-oxides. To obtain a possible intermediate for the synthesis of 2-amino-6-substituted purine 3-oxides, the peroxidation of 2-amino-6-chloropurine, (**1**) (Scheme 1) was investigated. If the position of *N*-oxidation of **1** is regulated by the 6-halogen even in the presence of a 2-amino group, then the corresponding 3-oxide, **2**, would be anticipated. The availability of this compound would permit the synthesis of a variety of otherwise inaccessible 2-amino-6-substituted purine 3-oxides.

Initial attempts to *N*-oxidize 2-amino-6-chloropurine were frustrated by the facile displacement of the 6-halogen in hydroxylic solvents, the low solubility of **1** in organic solvents and its poor susceptibility to *N*-oxidation. We now report that the use of *m*-chloroperoxybenzoic acid at low temperature in 10% aqueous ethyleneglycol dimethyl ether (glyme) permits *N*-oxidation of 2-amino-6-chloropurine to the 3-oxide **2**.

The 6-chloro substituent of **2** is readily displaced in aqueous base to yield guanine 3-oxide, **3** (23) (3-hydroxyguanine) (24) and by sodium methoxide in methanol to afford 2-amino-6-methoxypurine 3-oxide, (**4**) (25). The identity of these products to the known 3-oxides obtained by

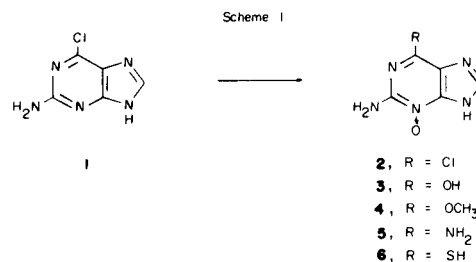


Table 1

Ultraviolet Absorption and  $pK_a$  Values  
2-Amino-6-chloropurine 3-Oxide (**2**)

pH	Charge (a,b)	$\lambda$ max, nm ( $\epsilon$ ) (c)	Apparent $pK_a$ Values (d,e,f)
-0.4	(+)	217 (20,600), 258 (3,500), 318 (7,100)	1.71 ( $\pm 0.07$ ) (d)
4.0	(0)	233 (22,800), [273] (c) (4,300), 280 (4,600), 331 (4,900)	6.51 ( $\pm 0.05$ ) (d)
9.0	(-)	231 (22,600), 286 (6,000), 321 (5,300)	
2,6-Diaminopurine 3-Oxide ( <b>5</b> )			
2.0	(+)	220 (22,300), 243 (9,600), 281 (11,200)	4.44 ( $\pm 0.02$ ) (d)
6.47	[0]	227 (33,300), 259 (5,500), 298.5 (7,800)	8.38 ( $\pm 0.03$ ) (e)
12	(-)	229 (33,000), 263 (5,400), 296 (9,500)	
2,6-Diaminopurine 1-Oxide			
1	+	248 (8,600), 290 (8,700)	< 1 (f)
6.9	0	230 (31,300), 260 (8,400), 290 (7,000)	3.7 (f)
11	-	229 (28,000), [265] (c) (6,000), 295 (8,500)	9.7 (f)

(a) Parentheses indicate the spectrum is of a pure species. (b) Brackets indicate that a pure ionic species is not available. (c) Brackets indicate a shoulder. (d) Determined at  $24 \pm 1^\circ$  spectrophotometrically with 0.01 *M* buffers (31) and a Beckman DU spectrophotometer. (e) Determined at  $24 \pm 1^\circ$  electrometrically with 0.001 *N* solutions and the use of a Beckman Research Model pH meter by procedures described (32). (f) Estimated from isosbestic spectra.

direct peracid oxidation of the parent purines (23,25) provided the assignment of structure for **2**. Additional confirmation was provided by hydrolysis of **2** in 2*N* hydrochloric acid to 3-hydroxyxanthine (23,25). The 3-oxide, **2**, was also reactive with aqueous ammonia at elevated temperature to afford the previously unavailable 2,6-diaminopurine 3-oxide, **5**, in low yield. Attempts to convert **2** to 6-thioguanine 3-oxide, **6**, with thiourea in hot ethanol, caused loss of the 3-oxide function to give only 6-thioguanine.

The ultraviolet and  $pK_a$  values of the *N*-oxides, **2** and **5**, are given in Table 1. Unlike most purine *N*-oxides, 2-amino-6-chloropurine 3-oxide and 2,6-diaminopurine 3-oxide do not completely lose the high extinction uv absorption band near 230 nm in acid. With adenine 1-oxide or 2,6-diaminopurine 1-oxide, as well as most purine 3-oxides, that band is completely lost in acid (19,21,22,25). This spectral change accompanying acidification has been interpreted to mean that protonation occurs on the available electron pairs of the *N*-oxide function. Thus, **2** and **5** are evidently only slightly protonated on the *N*-oxide function in dilute acid. This behavior parallels that observed with 6-chloropurine 3-oxide (unpublished data) and thus might be anticipated for **2**, but was not expected for **5**. With this exception, the uv spectral values of the cation, neutral form and anion of the 2,6-diaminopurine 1- and 3-oxides are remarkably close (Table 1). This is unusual since most of the purine 1- and 3-oxide pairs possess distinctly different uv spectral patterns (19,21-23, 24-29). Apparently the 2- and 6-amino groups exert such a dominating effect on the purine chromophore (28) that the introduction of an *N*-oxide function at either pyrimidine nitrogen produces little additional effect.

#### EXPERIMENTAL (30)

##### 2-Amino-6-chloropurine 3-Oxide (**2**).

A solution of 75 g of *m*-chloroperoxybenzoic acid (0.43 mole) and 20.5 g of 2-amino-6-chloropurine (0.15 mole) in 500 ml of ethyleneglycol dimethyl ether (glyme)-water (9:1) was allowed to react at 0° for 4 days. Progress of the reaction could be monitored by tlc (silica gel, 3% sodium bicarbonate; Rf of **1** = 0.5; Rf of **2** = 0.8). The resulting fine suspension was centrifuged. The precipitate was collected, washed 3 times with 200 ml portions of ether and then dissolved in 1800 ml of ammonium hydroxide at pH > 10. A small amount of insoluble material was removed by filtration through celite. The filtrate was then evaporated under reduced pressure until a precipitate began to deposit. The solution was chilled overnight. The precipitate of starting material was collected and the procedure was repeated (with addition of 55% ammonium hydroxide to maintain pH > 10) until no further precipitation of **1** occurred. Further reduction of volume under reduced pressure of the 2-amino-6-chloropurine-free filtrate without addition of ammonium hydroxide caused precipitation of the product **2** at pH ~ 8. The precipitate was collected and purified by dissolving the crude sample in ammonium hydroxide, treating the solution with charcoal, filtering and removing the ammonia under reduced pressure until pure **2** began to precipitate. The solution was then chilled, the precipitate was collected, washed with ether and air dried to afford **2**, yield 6.1 g (39%), mp > 157°; grad dec; nmr:  $\delta$  8.08 (s, 1, CH), 7.45 (s, 2 exchangeable, NH<sub>2</sub>); **2** gave a dark blue-green ferric

chloride test. A sample for elemental analysis was purified as described above twice to remove residual traces of **1**.

*Anal.* Calcd. for C<sub>5</sub>H<sub>4</sub>ClN<sub>5</sub>O: C, 32.36; H, 2.17; N, 37.73. Found: C, 32.07; H, 2.21; N, 37.56.

##### Proof of Position of *N*-Oxidation.

A sample of the *N*-oxidation product of 2-amino-6-chloropurine **2** was dissolved in 1*N* sodium hydroxide and heated at 80° for 3 hours. Acidification of the solution afforded a precipitate which was shown to be identical with guanine 3-oxide, **5**, (23,25) rather than 1-hydroxyguanine (26), by the identity of the uv spectral values in acid, neutral and base. Similarly, refluxing a sample of **2** in sodium methoxide/methanol afforded 2-amino-6-methoxypurine 3-oxide **6**, which was identical with the *N*-oxidation product of 2-amino-6-methoxypurine (**25**) by the same criteria. The *N*-oxide, **2**, could also be hydrolyzed by heating in 2*N* hydrochloric acid to 3-hydroxyxanthine (23,25) rather than to the isomeric 1-hydroxyxanthine (27), as shown by a comparison of uv spectral values at several pH's.

##### 2,6-Diaminopurine 3-Oxide (**5**).

2-Amino-6-chloropurine 3-Oxide (**2**, 1.0 g, 5.4 mmoles) was suspended in 35 ml of 5 *M* ammonium hydroxide in a glass-lined high pressure reaction vessel. The reaction mixture was heated at 90-100° for a day. The solution was then allowed to cool to 25° and the solvent was evaporated under reduced pressure until the product began to precipitate. The pH of the solution was adjusted to pH 8.0 and the precipitate was collected. This procedure was repeated until no further precipitation occurred at pH 8. The crude product was purified by dissolving it in ammonium hydroxide, treating with charcoal and evaporating the solvent under reduced pressure until precipitation occurred. Compound **5** was obtained in a yield of 160 mg (16%), mp > 197° grad dec; nmr:  $\delta$  7.66 (s, 1, CH), 7.33 (s, 2, exchangeable, NH<sub>2</sub>), 7.18 (s, 2, exchangeable, NH<sub>2</sub>); Rf = 0.0 on silica gel tlc developed in 3% sodium bicarbonate solution.

*Anal.* Calcd. for C<sub>5</sub>H<sub>6</sub>N<sub>6</sub>O·H<sub>2</sub>O: C, 32.61; H, 4.38; N, 45.64. Found: C, 32.57; H, 4.24; N, 45.65.

##### Acknowledgements.

We thank Mr. Marvin J. Olsen for determining the nmr values and Mr. Gerald M. Reiser for assistance in determining the spectral and pK data in Table 1.

#### REFERENCES AND NOTES

- (1) This investigation was supported in part by funds from the National Cancer Institute (No. CA 08748) and from the Atomic Energy Commission (Contract No. AT (30-1)-910).
- (2) To whom correspondence should be addressed, at the Walker Laboratory, 145 Boston Post Road, Rye, NY 10580.
- (3) D. B. McCormick, *Biochemistry*, **5**, 746 (1966).
- (4) H. H. Mantsch, I. Goia, M. Kezdi, O. Bärzu, M. Dansoreau, G. Jebeleanu and N-G. Ty, *ibid.*, **14**, 5593 (1975).
- (5) H. P. Schnebli, D. L. Hill and L. L. Bennett, Jr., *J. Biol. Chem.*, **242**, 1997 (1967).
- (6) E. J. Reist, D. F. Calkins and L. Goodman, *J. Med. Chem.*, **10**, 130 (1967).
- (7) J. A. Montgomery and H. J. Thomas, *ibid.*, **15**, 182 (1972).
- (8) C. Case, T. Tan and M. Selner, *Cancer Res.*, **39**, 1563 (1979).
- (9) G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly, G. S. Tarnowski, F. A. Schmid, M. N. Teller and C. C. Stock, *J. Med. Chem.*, **8**, 190 (1965).
- (10) G. B. Brown, in "Antagonists and Nucleic Acids", M. E. Balis, ed, North Holland, Amsterdam, 1968, p 241.
- (11) G. B. Brown, K. Sugiura and R. M. Cresswell, *Cancer Res.*, **25**, 986 (1965).
- (12) K. Sugiura and G. B. Brown, *ibid.*, **27**, 925 (1967).
- (13) M. N. Teller, G. Stöhrer and H. Dienst, *ibid.*, **30**, 179 (1970).
- (14) K. Sugiura, M. N. Teller, J. C. Parham and G. B. Brown, *ibid.*, **30**,

184 (1970).

(15) G. B. Brown, M. N. Teller, T. Smullyan, N. J. M. Birdsall, T. C. Lee, J. C. Parham and G. Stöhrer, *ibid.*, **33**, 1113 (1973).

(16) M. N. Teller, J. M. Budinger, G. Zvilichovsky, A. A. Watson, J. J. McDonald, G. Stöhrer and G. B. Brown, *ibid.*, **38**, 2038 (1978).

(17) L. M. Anderson, J. J. McDonald, J. N. Budinger, I. M. Mountain and G. B. Brown, *J. Natl. Cancer Inst.*, **61**, 1405 (1978).

(18) L. M. Anderson, M. N. Teller, J. N. Budinger and G. B. Brown, *ibid.*, **61**, 1411 (1978).

(19) M. A. Stevens, D. I. Magrath, H. W. Smith and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).

(20) H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Japan*, **42**, 750 (1969).

(21) A. Giner-Sorolla, C. Gryte, A. Bendich and G. B. Brown, *J. Org. Chem.*, **34**, 2157 (1969).

(22) I. Scheinfeld, J. C. Parham, S. Murphy and G. B. Brown, *ibid.*, **34**, 2153 (1969).

(23) U. Wolcke and G. B. Brown, *ibid.*, **34**, 978 (1969).

(24) This compound exists in the neutral species as an almost equal

mixture of *N*-hydroxy- and *N*-oxide tautomers (25).

(25) J. C. Parham, T. G. Winn and G. B. Brown, *J. Org. Chem.*, **36**, 2639 (1971).

(26) A. A. Watson, S. C. Nesnow and G. B. Brown, *ibid.*, **38**, 3046 (1973).

(27) J. C. Parham, J. Fissekis and G. B. Brown, *ibid.*, **32**, 1151 (1967).

(28) J. C. Parham, J. Fissekis and G. B. Brown, *ibid.*, **31**, 966 (1966).

(29) The  $\lambda$  max ( $\epsilon$ ) of 2,6-diaminopurine at pH 1 is 280 nm (12,000) and at pH 11 are 224 (17,500), 280 (14,800) (R. K. Robins, *J. Am. Chem. Soc.*, **80**, 6671 (1958)).

(30) The nmr spectra were determined in DMSO- $d_6$  with a JEOL PFT-100 NMR Spectrometer using TMS as an internal standard. The uv spectra were determined with a Cary 15 recording spectrophotometer. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Micro analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Michigan.

(31) D. D. Perrin, *Aust. J. Chem.*, **16**, 572 (1963).

(32) A. Albert and E. D. Sergeant "Ionization Constants of Acids and Bases", Wiley, New York, NY, 1962.