[CONTRIBUTION FROM THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Purine *N*-Oxides. X. The Effect of Some Substituents on Stability and Reactivity[1]

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From studies of additional purine *N*-oxides, it is apparent that the stabilities vary with the strength of the electron-donating character of the substituents on the ring. 6-Methylpurine 1-*N*-oxide, obtained in high yield from 6-methylpurine, resembles adenine 1-*N*-oxide in that it hydrolyzes to an imidazole, 4-acetyl-5-aminoimidazole, and with xanthine oxidase it leads to 8-hydroxy-6-methylpurine 1-*N*-oxide. *N*-Oxides of purine, 9-ribosylpurine, and 8-hydroxypurine have been obtained, the last in good yield. The relation between the structures of the oxides of these three purines has been established by enzymatic interconversions by means of a purine nucleoside hydrolase and xanthine oxidase. In analogy to the behaviors of pyridine or picoline *N*-oxides with acetic anhydride, 6-methylpurine 1-*N*-oxide gives 6-acetoxymethylpurine, and 8-hydroxypurine *N*-oxide rearranges to 6,8- and 2,8-dihydroxypurine.

Although amino substituted purines yield stable *N*-oxides with peroxyacetic acid,[2] treatment of unsubstituted purine,[3] or 9-β-D-ribosylpurine[4] under similar oxidizing conditions,[5] leads to products not showing specific absorption of ultraviolet light. However, the presence of a methyl group which, like the amino group, is an electron donor, does stabilize the purine *N*-oxide formed.

The oxidation of 6-methylpurine[6] (Fig. 1, I) with acetic acid–hydrogen peroxide leads, as with 6-aminopurine (adenine),[2] to a good yield of a mono-

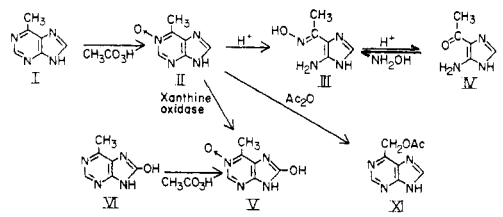

Figure 1

N-oxide, even under the vigorous (*ca.* 80°) conditions used for the oxidation of methylpyrimidines.[7] This is a 1-*N*-oxide (II) as is demonstrated by analysis, by its hydrogenation to 6-methylpurine with one molar proportion of hydrogen, and by its hydrolysis to 4-acetyl-5-aminoimidazole (IV), which involves the loss of the oxygen and the 1-nitrogen. IV gives a Pauly[8] test for an imidazole, no test[9] for a hydroxylamino group and, *via* its oxime (III) and treatment with butyl nitrite, leads to 6-methyl-2-azapurine 1-*N*-oxide.[10] The oxime of 4-acetyl-5-aminoimidazole (III), which is the expected intermediate in the hydrolysis, is occasionally observed on chromatograms of hydrolysates of II and is chromatographically identical with that obtained[10] from the ketone (IV) and hydroxylamine hydrochloride.

In further analogy to adenine 1-*N*-oxide,[11] the 6-methylpurine 1-*N*-oxide is converted by xanthine oxidase to a monohydroxy derivative. This was shown to be 8-hydroxy-6-methylpurine 1-*N*-oxide (V) by its identity with the product of peroxyacetic acid oxidation of 8-hydroxy-6-methylpurine (VI).[6] VI must therefore have also been oxidized at the 1-nitrogen.

Although unsubstituted purine and its ribosyl derivative are rapidly converted to nonultraviolet-absorbing products by acetic acid–hydrogen peroxide, it is possible to obtain from each, in small yield, single ultraviolet-absorbing products which are not the starting materials. These products (VII and VIII) can be separated by paper chromatography and eluted from the papers. They show fluorescence in ultraviolet light and strong absorption in the 230-mμ region which is characteristic of all the *N*-oxides of purines thus far studied, and also absorption at 260 mμ as with purine itself. In addition, there is considerable absorption shown by these two oxides in the 310-mμ region.

In an oxidation of purine with anhydrous perbenzoic acid, there was less decomposition to nonultraviolet-absorbing products, and again there was formation of an oxide with a high 230-mμ absorp-

(1) This investigation was supported in part by funds from the American Cancer Society (Grant E-8), National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190), and from the Atomic Energy Commission [Contract AT(30-1)-910].

(2) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958); M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2759 (1958).

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

(4) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(5) Oxidized in 30 hours *vs.* 10 days for 6-aminopurine under the same conditions.

(6) S. Gabriel and J. Colman, *Ber.*, **34**, 1234 (1901).

(7) E. Ochiai and H. Yamanaka, *Pharm. Bull. (Japan)*, **3**, 175 (1955).

(8) H. Pauly, *Z. Physiol. Chem.*, **42**, 508 (1904).

(9) A. Hantzsch and C. H. Besch, *Ann.*, **323**, 23 (1902).

(10) M. A. Stevens, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **82**, 3189 (1960).

(11) G. B. Brown, M. A. Stevens, and H. W. Smith, *J. Biol. Chem.*, **233**, 1513 (1958).

tion. This oxidation product of purine was identical with the product (VIIa) of the oxidation of purine with mixtures of acetic acid–hydrogen peroxide in R_f (0.43 in solvent A) and ultraviolet spectrum. Lyophilization of the product of the oxidation of purine with acetic acid–hydrogen peroxide gave a powder which still contained only the oxide component R_f 0.43 in solvent A, with maxima at 230 and 260 mμ. If this powder was allowed to stand in contact with the atmosphere for a day, the fluorescent oxide component (VIIa) was replaced by a substance with an R_f of 0.67 in solvent A and which was ultraviolet absorbent and not fluorescent. Heating the lyophilized powder while it still contained peroxide components caused a slow change to another fluorescent oxidation product (VIIb) with an R_f identical to VIIa in solvent B and in aqueous butanol (85%)–concentrated ammonium hydroxide (99:1) (R_f 0.12) and a similar R_f to VIIa in solvent A (0.41 *vs.* 0.43) and in solvent C (0.32 *vs.* 0.36). When the product of the oxidation of purine with perbenzoic acid was worked up by evaporation, partition of the residue between ether and water, evaporation of water, and recrystallization from methanol, it was found that the whole of the original oxide (VIIa) with the 230- (and 260-mμ absorption had been converted to the second substance (VIIb). This product (VIIb) was isolated in the crystalline state and found to have an analysis corresponding to a hydrate[12] of purine oxide ($C_5H_4N_4O \cdot H_2O$) after having been dried at 80°, and a nitrogen analysis corresponding to that expected for purine *N*-oxide after having been dried at 110°. This product (VIIb) had a spectrum showing absorption at 222 and 291 mμ at *p*H 4.0 which was quite different from that shown by the oxide (VIIa) at the same *p*H.

Evidently the first oxidation product of purine goes over to other compounds[13] extremely easily. The nature of transformations occurring is not known, but it has been determined that the first oxidation product of purine (oxidation product VIIa) is stable in cold 0.05*N* hydrochloric acid, in cold 0.05*N* sodium hydroxide, and in hot acetic acid and water. The tendency to convert to further products seems to be related to contact with peroxides during work-up stages.

The stabilizing effect[11] of the 8-hydroxy group on purine *N*-oxides is again demonstrated by the fact that an oxide (IX) of 8-hydroxypurine (X) is obtained in good yield from an oxidation with a mixture of acetic acid and hydrogen peroxide. From analysis, spectrum, its reduction to 8-hydroxypurine, and its nonidentity with known dihydroxypurines, it is demonstrated to be a mono-*N*-oxide. It is stable in hot concentrated hydrochloric acid and information as to the position of the *N*-oxide could not be ascertained through hydrolysis.

It has been possible to interrelate the *N*-oxides obtained by oxidation of the three purines, 8-hydroxypurine, 9-ribosylpurine, and purine itself (Fig. 2). With the nucleoside hydrolase from *Lactobacillus delbruckii*,[14] the ribosylpurine *N*-oxide (VIII) could be cleaved to a product identical in its spectrum and behavior on paper chromatography, in two solvents, with the *N*-oxide (VIIa) from purine. With xanthine oxidase, the purine *N*-oxide (VIIa) was oxidized in part to a product apparently identical, by the same criteria, with 8-hydroxypurine *N*-oxide (IX), although there was accompanying loss of ultraviolet absorption and formation of other ultraviolet absorbing products. Thus the *N*-oxides from purine, ribosylpurine, and 8-hydroxypurine all appear to bear the oxygen in the same position. It was observed, quite inexplicably, that the altered oxidation product (VIIb) obtained from purine also gives some 8-hydroxypurine *N*-oxide upon hydroxylation with xanthine oxidase.

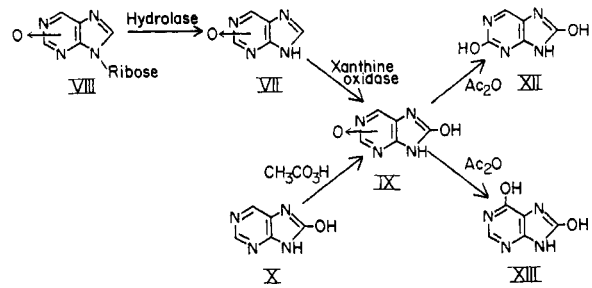

Figure 2

It is becoming possible to make some predictions as to the position on a purine ring at which oxidation by peroxyacetic acid may take place. If the first step is addition of the peroxy acid,[15] this would be expected to occur on the most nucleophilic nitrogen which is also the first to be protonated. Formation of a 1-*N*-oxide of adenine[2] parallels the lines of evidence[16–19] that nitrogen is the one

(12) E. C. Taylor, C. C. Cheng, and O. Vogl, *J. Org. Chem.*, **24**, 2019 (1959), found great difficulty in obtaining anhydrous hypoxanthine 1-*N*-oxide.

(13) A complexity of the instability of purine and certain of its derivatives to acid and alkali has been reported [M. P. Gordon, V. S. Weliky, and G. B. Brown, *J. Am. Chem. Soc.*, **79**, 3245 (1957)].

(14) Y. Takagi and B. L. Horecker, *J. Biol. Chem.*, **225**, 77 (1957). This enzyme will cleave ribose from many purine nucleosides, including 9-β-D-ribofuranosylpurine. The present data indicate that its action, like that of xanthine oxidase, is not prevented by the presence of an *N*-oxide function.

(15) Although the mode of reaction of ketones and peracids has been studied, little is known about the mechanism of *N*-oxide formation [W. Von E. Doering and E. Borfman, *J. Am. Chem. Soc.*, **75**, 5595 (1953)].

(16) W. Cochran, *Acta Cryst.*, **4**, 81 (1951); J. M. Broomhead, *Acta Cryst.*, **4**, 92 (1951).

(17) D. O. Jordan, in Chargaff and Davidson, *The Nucleic Acids*, Vol. 1, Academic Press, New York, 1955, p. 451.

(18) A. Bendich, A. Giner-Sorolla, and J. J. Fox, in *The Chemistry and Biology of Purines*, G. E. W. Wolstenholme, Ed., Little, Brown and Co., 1957, pp. 3 and 18.

which is first protonated. In the other studies where an *N*-oxide function has been proved to be introduced in the 1- position, the theoretical calculations of Pullman[20] have also indicated that it is the 1-nitrogen which is the most basic, including purine itself. In addition, rearrangement products described below are in accord with a 1-*N*-oxide structure; a 9-*N*-oxide is unlikely in view of the interrelation with the oxide of 9-ribosylpurine; a 7- in view of the acid lability of the imidazole ring in one such *N*-oxide.[21] Other 8-hydroxypurines have yielded 1-*N*-oxides upon oxidation. It is thus most probable the three are 1-*N*-oxides.

The present compounds permit some comparisons of behaviors of purine *N*-oxides with earlier known[22] reactions in the pyridine series. In studies of adenine[23] and other 6-aminopurines,[24] there were distinct contrasts with the pyridine series. In contrast to adenine, the amino group of 2-aminopyridine has to be protected by acetylation[25] before hetero-*N* can be oxidized, and it could be argued that the substituent amino group is most basic and is attacked in preference to the nuclear nitrogen,[26] although Albert's discussions[27] do not favor this interpretation for the 2- or 4-aminopyridines. With adenine 1-*N*-oxide and hot acetic anhydride a rearrangement to the very stable 5-methyl-3-[5′-(4′-acetamido)imidazolyl]-1,2,4-oxadiazole resulted,[23] but with the aminopyridine *N*-oxides no analogous rearrangement product has been reported, possibly because there is no opportunity to form a product stabilized by a second ring.

However, both the rearrangement of pyridine *N*-oxide to a pyridone[28] and of 2-methylpyridine to 2-acetoxymethylpyridine,[29] have been found to have counterparts in the purine *N*-oxide series. 6-Methylpurine *N*-oxide (II) is found to yield 6-acetoxymethylpurine (XI, Fig. 1) upon reaction with hot acetic anhydride. When that product is oxidized with hydrogen peroxide–acetic acid, the ester group is hydrolyzed and 6-hydroxymethylpurine *N*-oxide results instead of an acetoxymethyl *N*-oxide as is obtained[28] in the pyridine series.

In analogy to the rearrangement of pyridine *N*-oxide to pyridone, 8-hydroxypurine *N*-oxide can be rearranged by long refluxing with acetic anhydride. The reaction can be speeded and the by-products diminished by adding sodium acetate and acetic acid. Two products were formed in a total yield of about 30% and in roughly equal amounts.[30] These were compared with authentic samples by chromatography in several solvents and by spectra, and identified as 2,8-dihydroxypurine (XII, Fig. 2) and 6,8-dihydroxypurine (XIII). From analogy to the rearrangement of pyridine *N*-oxide[28] (where both carbons adjacent to the nitrogen are identical), this suggests that rearrangement of the oxygen was occurring from the 1-nitrogen to the carbons on either side of it.

Several comparisons have been made of the biological activities of purine *N*-oxides with their parent purines.[31] With 6-methylpurine, Dr. D. A. Clarke and associates have observed an extremely high toxicity (LD_{50} = *ca.* 2 mg. per kg. per day on a repeated dose basis) in Swiss mice[32] and in C57 black mice, but with 6-methylpurine *N*-oxide a much larger dose was tolerated (LD_{50} = 63 mg. per day) by either strain of mice. At the LD_{50} level 6-methylpurine led to modest inhibition of Crocker mouse Sarcoma 180,[33] and negligible inhibition of Carcinoma 755. With 6-methylpurine *N*-oxide, there was a modest inhibition of Carcinoma 755 without evidence of intoxication at 32 mg. per kg. per day, although with Sarcoma 180 significant inhibition occurred only at dosages greater than the LD_{50}. In view of the observed reduction of adenine *N*-oxide *in vivo*[31,34] it was suggested[35] that dosage schedules might be altered by providing reservoir doses in the form of *N*-oxides, and the result with Carcinoma 755 might be so interpreted.

EXPERIMENTAL

Chromatographic analyses were performed, ascending, on Whatman No. 1 paper at 25° with the developing solvents,

(19) The 1-nitrogen of a purine derivative is not always the most nucleophilic. J. Lawley [*Proc. Chem. Soc.*, 290 (1957); *Chem. & Ind.*, 633 (1957)] has evidence that the 7-nitrogen of guanosine is the nitrogen first quaternized.

(20) T. Nakajima and B. Pullman, *Bull. soc. chim.*, 1502 (1958); B. Pullman, *J. Chem. Soc.*, 1621 (1959).

(21) G. M. Timmis, I. Cooke, and R. G. W. Spickett in *Ciba Foundation Symposium on the Chemistry and Biology of Purines*, Little, Brown and Co., Boston, Mass., 1957, p. 139.

(22) A. R. Katritzky, *Quart. Rev.*, **10**, 395 (1956).

(23) M. A. Stevens, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **82**, 1148 (1960).

(24) M. A. Stevens, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **82**, 3189 (1960).

(25) A. R. Katritzky, *J. Chem. Soc.*, 191 (1957).

(26) 2-Aminopyridine and 2,6-diaminopyridine are found to break down completely upon oxidation with peroxyacetic acid. Attack on the 2-amino group as well as on the 1-nitrogen of 2,6-diaminopurine would explain the low yield[2] (15%) of 2,6-diaminopurine 1-*N*-oxide.

(27) A. Albert, *Heterocyclic Chemistry*, Athlone Press, 1959, pp. 47, 54, 86.

(28) M. Katada, *J. Pharm. Soc. (Japan)*, **67**, 51 (1947).

(29) V. Boekelheide and W. J. Lynn, *J. Am. Chem. Soc.*, **76**, 1288 (1954).

(30) In early experiments of shorter duration, only 2,8-dihydroxypurine had been detected (M. A. Stevens, H. W. Smith, A. Giner-Sorolla, and G. B. Brown, Abstracts, 134th Meeting of the American Chemical Society, Chicago, 1958, p. 15P).

(31) G. B. Brown, D. A. Clarke, J. J. Biesele, L. Kaplan, and M. A. Stevens, *J. Biol. Chem.*, **232**, 1509 (1958).

(32) F. S. Philips, S. S. Sternberg, L. Hamilton, and D. A. Clarke, *Ann. N. Y. Acad. Sci.*, **60**, 283 (1954).

(33) D. A. Clarke, F. S. Philips, S. S. Sternberg, and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954).

(34) D. Dunn, M. H. Maguire, and G. B. Brown, *J. Biol. Chem.*, **234**, 620 (1959).

(35) G. B. Brown, 4th International Congress Biochemistry, Vol. XIII, Vienna, 1958, p. 111.

TABLE I

Substance	M.P.	R_f A	R_f B	Spectral Data: Maxima	pH	$a_M \times 10^{-3}$	Ref
Purine	216	0.71	0.54	260	0.23	6.26	a,b
				262.5	5.94	8.16	
				270	11.9	7.98	
Purine *N*-oxide	—	0.43	0.54	Shoulder at 230, 262, 318	1.2	—	c
				230, 260, and 316	6.0		
9-Ribosylpurine	181–182	0.60	0.71	263	1.0	—	d
				263	6.0		
				263	13.0		
9-Ribosylpurine *N*-oxide	—	0.48	0.77	238, shoulder at 260, 322	6.0	—	c
8-Hydroxypurine	305–307 dec.	0.71	0.53	280	0	10.5	e
				235 and 277	5.4	3.2 and 11.2	
				285	10.1	12.9	
				284	13.0	12.6	
8-Hydroxypurine *N*-oxide	Starts dec. 293	0.53	0.62	225, 270, and 317	2.0	25.3, 6.8, and 7.9	f
				226, 275, and 320	7.0	22.8, 3.35, and 9.3	
				236 and 322	12.3	15.2 and 5.35	
6-Methylpurine	228–233	0.77	0.55	265	0	7.6	e
				261	5.85	8.3	
				271	11.53	8.5	
6-Methylpurine 1-*N*-oxide	265 dec.	0.60	0.57	272	0	9.7	g
				226, 260, and 311	2.0	33.7, 7.54, and 8.42	
				227, 260, and 311	5.8	36.5, 8.13, and 9.41	
				231 and 310	8.1	44.4 and 10.7	
				231 and 310	13.0	39.7 and 9.99	
4-Acetyl-5-aminoimidazole oxime	236–238	0.58	0.58	284	12.0		
				281	7.0		
				274	3.8		
				272	2.0		
4-Acetyl-5-aminoimidazole.HCl	235–237 dec.	0.74	0.64	247 and 312	12.0		
				shoulder at 232, 302	7.0		
				235 and 303	4.6		
				243 and 292	2.0		
6-Acetoxymethylpurine	239 dec.	0.50	0.48	245 and 265	5.5		
				275	12.0		
6-Methyl-8-hydroxypurine	228 dec. sublimes 242–244	0.70	0.47	232 and 282	2.0		
				274	7.0		
				283	12.0		
6-Methyl-8-hydroxypurine 1-*N*-oxide	288–298	0.55	0.62	228, 269, and 309	2.0		
				230, 273, and 314	7.0		
				236 and 317	12.0		
2,8-Dihydroxypurine	Dec.	0.38[h]	0.62	220 and 316	13.0	36.2 and 9.91	e
				262 and 306	10.0	9.55 and 7.4	
				230 and 310	5.1	9.91 and 8.45	
6,8-Dihydroxypurine	Dec.	0.41	0.47	271	12.00	13.8	
				265	8.67	10.96	e
				257 and 280	5.08	12.0 and 5.75	

[a] A. Bendich, D. J. Russell Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954). [b] pK's are 2.52 and 8.92. [c] Spectral data determined from material eluted from a chromatographic spot. [d] See ref. 4. [e] S. F. Mason, *J. Chem. Soc.*, 2071 (1954). [f] a_M figures and decomposition point are for hemihydrate. [g] pK's are 1.1, 7.0, and 12.5. [h] R_f in solvent C, 0.47; in solvent D, 0.43.

all prepared by volume: A, 1% ammonium sulfate–isopropyl alcohol, 1:2, paper previously soaked in 1% ammonium sulfate and dried[36]; B, 5% disodium hydrogen phosphate–isoamyl alcohol, 3:2[37]; C, *n*-butyl alcohol–water–acetic acid, 4:1:1; and D, hydrochloric acid (sp. gr. 1.19) –isopropyl alcohol–water, 4:17:4.[38] R_f values are in Table I. Measurements of ultraviolet absorption were made on a Beckman DK-2 spectrophotometer, except for accurate determinations of extinction coefficients, which were done with a Beckman DU spectrophotometer.

Preparation of 6-methylpurine 1-N-oxide. 6-Methylpurine (0.5 g.) in acetic acid (2.5 ml.) and 30% hydrogen peroxide (0.4 ml.) was kept at 75° to 80° for 3 hr.; 0.25 ml. of 30% hydrogen peroxide was added and heating continued for 9 hr. The solution was cooled and evaporated to dryness *in vacuo;* traces of hydrogen peroxide were removed from the residue by addition and evaporation of water. The residue was recrystallized from 95% ethanol to yield 6-methylpurine 1-*N*-oxide as white needles (0.3 g., 70%), m.p. 246° to 248° dec. Three recrystallizations raised the m.p. to 265° dec.

Anal. Calcd. for $C_6H_6N_4O$: C, 47.99; H, 4.03; N, 37.31. Found: C, 47.86; H, 3.98; N, 37.77.

Hydrogenation of 6-methylpurine 1-N-oxide. A solution of 6-methylpurine 1-*N*-oxide (75 mg.) in water (3 ml.), containing Raney nickel (50 mg., wet with ethanol), was shaken with hydrogen at atmospheric pressure for 6 hr. Approximately one mole proportion of hydrogen (11.2 ml.) was absorbed. Nickel was removed by filtration and washed with water. Filtrate was evaporated *in vacuo* at room tempera-

(36) N. Anand, V. M. Clark, R. H. Hall, and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).
(37) C. E. Carter, *J. Am. Chem. Soc.*, **72**, 1466 (1950).
(38) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

ture to a white solid (56.0 mg., 83%), m.p. 220°. The R_f's of this material in solvents A and B, its spectra at various *p*H's, its m.p. and mixed m.p. were identical with those of authentic 6-methylpurine.

Hydrolysis of 6-methylpurine 1-N-oxide. A solution of 6-methylpurine 1-*N*-oxide (0.52 g.) in 2*N* HCl (6 ml.) was brought to reflux rapidly (4 min.), held there for 6 min., then rapidly cooled. The solution was evaporated to dryness *in vacuo* at room temperature. The yellow powder was dissolved in hot ethanol (55 ml.). When the solution was cooled, 4-acetyl-5-aminoimidazole monohydrochloride crystallized in shining plates, m.p. 235–237° dec. The first two crops from the crystallization (228 mg. and 20 mg.) represented a yield of 44.5%.

Anal. Calcd. for $C_5H_8N_3OCl$: C, 37.16; H, 4.99; N, 26.01. Found: C, 37.04; H, 5.21; N, 25.92.

4-Acetyl-5-aminoimidazole gives a blue color with Pauly's reagent on a chromatogram developed with solvent A, and brick red on one developed with B.

6-Acetoxymethylpurine. 6-Methylpurine 1-*N*-oxide (3.0 g.) was suspended in a solution of acetic anhydride (15 ml.) and anhydrous dioxane (40 ml.). The suspension was heated with stirring for 90 min. at 65°. The material in suspension dissolved almost completely, and the solution turned red. The crystalline precipitate (0.18 g.), m.p.2 00° dec., was purine 6-carboxylic acid.[39] This was collected by filtration, and the filtrate concentrated to a sirup *in vacuo*. Ethanol was twice added and removed under reduced pressure at 40–50° to remove excess acetic anhydride. The sirup was dissolved in cold ethanol, filtered, and cooled. The dark yellow crystalline precipitate (1.2 g.), m.p. 180–185° dec., was washed with hot methanol to yield 6-acetoxymethylpurine as yellow needles, m.p. 239° dec.

Anal. Calcd. for $C_8H_8N_4O_2$: C, 49.99; H, 4.19; N, 29.15. Found: C, 50.21; H, 3.96; N, 29.08.

Preparation of 6-Methyl-8-hydroxypurine 1-N-Oxide. A. *From 6-methyl-8-hydroxypurine.* A solution of 6-methyl-8-hydroxypurine (1.5 g.) in acetic acid (75 ml.) containing 30% hydrogen peroxide (6 ml.) was stirred for 5 days. Paper chromatography showed most of the starting material had been converted into the oxide. Five per cent palladium-charcoal (200 mg.) was added and stirring continued for a day. The solution was filtered, evaporated, and the residue crystallized from water (15 ml.). The crystals obtained (1.0 g., 62%) were recrystallized twice more from water to give chromatographically homogeneous 6-methyl-8-hydroxypurine 1-*N*-oxide, m.p. 288–298° dec.

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.37; H, 3.64; N, 33.72. Found: C, 44.00; H, 3.91; N, 32.57.

B. *From 6-methylpurine 1-N-oxide.* 6-Methylpurine 1-*N*-oxide (2.7 mg.) was dissolved in 0.015*M* phosphate buffer, *p*H 7.5 (25 ml.). Xanthine oxidase (*ca.* 2500 units in 0.5 ml. of *p*H 7.5 phosphate buffer) was added to the solution, which was kept at 37° for 3.5 hr.[40] Samples (0.1 ml.) withdrawn during the course of the reaction and diluted 20-fold with water showed a rise of absorption in the 310-mμ region from 0.20 to 0.23, a shift in the position of this maximum toward 314 mμ, and decreases in the 260- and 228-mμ maxima from 0.105 to 0.06 and 0.78 to 0.75, respectively. The changes were largely complete after 3.5 hr. The solution was boiled for 15 min., filtered, evaporated to 5 ml., then filtered again to remove protein. The filtrate was evaporated to dryness and the residue dissolved in water (1 ml.). The spectrum of this solution was identical with that of 6-methyl-8-hydroxypurine 1-*N*-oxide. This product had the same R_f in solvents A, B, C, and D as the synthetic 6-methyl-8-hydroxypurine 1-*N*-oxide.

Preparation of 8-hydroxypurine N-oxide. 8-Hydroxypurine[41] (5.6 g.) was dissolved in hot acetic acid (100 ml.). The solution was cooled to about 40°, 30% hydrogen peroxide (11 ml.) was added, and the solution allowed to stand at room temperature. After 2 days, chromatographic analysis of the solution showed that about half the starting material had been converted to oxide. After 6 days, with a trace of starting material still present, the solution was treated with 10% palladium-charcoal (1 g.) overnight. The solution was filtered and evaporated to dryness at room temperature under vacuum. The white solid residue was broken up, dissolved in hot 90% propanol–water (300 ml.), and an insoluble residue (0.15 g.) discarded. The propanol–water solution was cooled and seeded, and after a day, light buff crystals of 8-hydroxypurine *N*-oxide (1.89 g.) slowly formed. These were chromatographically pure. When heated, these crystals decomposed at 293° and sublimed at 300°. Upon evaporation of the mother liquors to 90 ml., another 0.54 g. of white crystalline oxide separated, and after long standing, a further 0.34 g., for a total yield of 8-hydroxypurine *N*-oxide hemihydrate of 2.23 g. (33.6%).

Anal. Calcd. for $C_5H_4N_4O_2 \cdot 1/2H_2O$: C, 37.26; H, 3.13; N, 34.77. Found: C, 37.19; H, 3.31; N, 34.76.

Reaction of 8-hydroxypurine N-oxide with acetic anhydride. 8-Hydroxypurine *N*-oxide (24.5 mg.) and sodium acetate (4 mg.) in a 1:1 mixture of acetic anhydride and acetic acid (10 ml.) were refluxed for 3.5 hr. and evaporated to dryness. The brown residue was broken up under ethanol, the ethanol removed, and the residue treated with hot 25% ammonium hydroxide to hydrolyze any acetylated hydroxyl groups. Ammonium hydroxide was evaporated and the solid dried in a desiccator. The amounts of 2,8-dihydroxypurine, 6,8-dihydroxypurine, and 8-hydroxypurine *N*-oxide in this residue were determined by dissolving the residue in water (15 ml.) and chromatographing 100 μg. fractions on paper in solvents A, B, and C. The paper section corresponding in R_f (Table I) to 6,8-dihydroxypurine on the chromatogram developed with solvent B was eluted with water (3 ml.), and the resulting solution was adjusted to a *p*H of 8.6. Its optical density (0.30) at 265 mμ indicated that 6,8-dihydroxypurine was formed in 15.2% yield. The paper section corresponding in R_f (Table I) to 2,8-dihydroxypurine on the chromatogram developed with solvent A was eluted with water (3 ml.), and the resulting solution was adjusted to a *p*H of 9.9. The optical density (0.25) of the solution at 262 mμ indicated that 2,8-dihydroxypurine was formed in 13.6% yield. With solvent C, a compact and well separated spot of the starting material resulted, and this indicated that 22% of 8-hydroxypurine *N*-oxide remained unchanged. It was not possible to get a component to crystallize from the complex mixture. 6,8-Dihydroxypurine and 2,8-dihydroxypurine, though formed in only modest yield, were the major ultraviolet absorbing products. They were identical in R_f in solvents A, B, and C and in spectra at varying *p*H with authentic samples.

Oxidation of purine. A. 30% Hydrogen peroxide (0.25 ml.) was added to a solution of purine (10 mg.) in acetic acid (1.0 ml.). The mixture was kept at 37° for 30 hr., then frozen and lyophilized to a white powder. This substance gave one ultraviolet fluorescent spot on chromatograms in solvents A or B. Upon elution from paper the spot possessed the typical oxide spectrum with high 230 mμ absorption and peaks at 260 and 318 mμ. After exposure to air for a day, the lyophilized powder suffered a slow change. A component, ultraviolet absorbing but not fluorescent, appeared to form in the mixture, perhaps by action of atmospheric moisture. The new component possessed an R_f 0.67 in solvent A.

B. *With perbenzoic acid.* A saturated solution of purine in dry chloroform was prepared by shaking 1.0 g. with 1700 ml. and decanting from the 100 mg. remaining undissolved. This was treated with 66 ml. of chloroform containing 3.5 g. of perbenzoic acid,[42] freshly dried over anhydrous sodium sulfate. After 7 days at 4°, a further 33 ml. of perbenzoic acid solution was added. After approximately 2 weeks at 4°,

(39) L. B. Mackay and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 3511 (1956).

(40) 6-Methylpurine is also oxidized by xanthine oxidase very much less rapidly than is purine.

(41) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(42) G. Braun, *Org. Syntheses*, Col. Vol. I, 431 (1941).

chromatography indicated that the purine had been almost entirely oxidized to an ultraviolet fluorescent oxide absorbing at 230, 260, and 318 mμ at *p*H 6.0, identical with the product obtained in the oxidation of purine with peracetic acid. The chloroform solution was evaporated at room temperature to a volume of 50 ml. This turbid yellow solution was shaken with a mixture of 300 ml. of water and 370 ml. of ether. The water layer was separated and washed twice with 50-ml. portions of ether.[43] The water fraction was evaporated at a temperature under 40° to a light yellow solid (640 mg.). This solid had an R_f of 0.41 in A, 0.56 in B, and 0.33 in C, was fluorescent in ultraviolet, and adsorbed at 222 and 291 mμ at *p*H 4.0. The material was difficult to crystallize. The light yellow solid was crystallized from hot methanol (10 ml.) to yield a light yellow crystalline powder which sublimed and partially decomposed at *ca.* 200° and blackened at *ca.* 260°. Recrystallization was repeated from 10 ml. of hot methanol to give 15 mg. of a substance of sublimation and partial decomposition point 258°, softening and strong decomposition point 272–278°. Additional material separated when ether was added.

Anal. Calcd. for $C_5H_4N_4O \cdot H_2O$: C, 39.47; H, 3.95; N, 36.83. Found, after drying at 80°: C, 39.92; H, 3.84; N, 37.23.

If this substance was dried at 110° (boiling toluene), the N value rose to 41.03%. Anhydrous purine oxide requires N, 41.18%.

Action of xanthine oxidase on purine N-oxide. A sample of crude, purine-free, purine *N*-oxide (lyophilized powder) prepared from oxidation of purine (10 mg.) with peroxyacetic acid was dissolved in 0.05*M* disodium hydrogen phosphate buffer (*p*H 7.5). A 1-ml. portion of the solution was diluted to 10 ml. with phosphate buffer, one drop of a dilute aqueous catalyse solution (O.D. increase at 260 mμ caused by such an amount of catalase was 0.003) was added, and the solution was incubated for 10 min. to cause destruction of peroxide. A solution of xanthine oxidase (0.03 ml. *ca.* 5000 units) was next added to the solution which was incubated at 37° for 16 hr. A portion, diluted fivefold, was used to follow the small O.D. and spectral changes during incubation. The concentrated solution was applied to a chromatogram after 30 min. and again after 2.5 hr. After development of these chromatograms in solvent B, a fluorescent spot was observed approximately at the R_f of the fluorescent spot of 8-hydroxypurine *N*-oxide, and considerably higher than the R_f of the fluorescent spot of purine *N*-oxide, which was closer to the level of the ultraviolet absorbing spot of purine. A chromatogram in solvent A gives a fluorescent spot at R_f 0.56, or approximately the same level as authentic 8-hydroxypurine *N*-oxide (R_f 0.53). This evidence strongly suggests that purine *N*-oxide is hydroxylated to 8-hydroxypurine *N*-oxide by xanthine oxidase. It is not rigid, since the presence of the enzyme, either before or after heat denaturation, has a considerable effect on R_f's. Spots from chromatograms were eluted with water and their spectra determined. The material eluted from the spot with the same R_f as 8-hydroxypurine *N*-oxide on a chromatogram developed with solvent A has a spectrum with maxima at 274 and 324 mμ, closely resembling that of 8-hydroxypurine *N*-oxide at *p*H 6.0. Material eluted from a chromatogram developed with solvent B showed maxima at 281 and 320 mμ corresponding to maxima of 8-hydroxypurine *N*-oxide at *p*H 9.0. Both differ from the spectrum of the starting purine *N*-oxide.

The purine oxide VIIb was also treated with xanthine oxidase under the conditions used with the oxide VIIa. During the reaction with this enzyme, appearance of a shoulder in the spectrum at 320 mμ was indicative of 8-hydroxypurine *N*-oxide formation. Chromatography of the hydroxylation mixture gave a spot corresponding to 8-hydroxypurine *N*-oxide in spectra and R_f, and the substance in this spot was stable to strong acid hydrolysis, as expected of 8-hydroxypurine *N*-oxide. At least three other products were formed in this reaction.

Oxidation of 9-ribosylpurine. 9-Ribosylpurine (10 mg.) in a solution of acetic acid (1.0 ml.) and 30% hydrogen peroxide (0.25 ml.), was kept at 37° for 30 hr. Lyophilization of the resulting solution yielded a white solid which gave only one ultraviolet absorbing spot upon chromatography. This spot (R_f in solvent A, 0.48 and R_f in solvent B, 0.77) exhibited a blue fluorescence in ultraviolet light. The solution, obtained by eluting the spot, gave a spectrum at *p*H 6.0, with a characteristic high absorption at 238 mμ and also absorption at 322 mμ and a shoulder at 260 mμ. The mixture, obtained by lyophilization of the oxidation mixture, contained a large amount of nonultraviolet absorbing material which prevented crystallization of 9-ribosylpurine *N*-oxide.

Action of nucleoside hydrolase on 9-ribosylpurine N-oxide. The impure 9-ribosylpurine *N*-oxide, obtained by oxidation of 9-ribosylpurine (10 mg.), was dissolved in 0.05 *M* citrate buffer, *p*H 6.0 (5 ml.). The solution was treated with a trace of catalase and incubated at 37° for 30 min. to destroy residual hydrogen peroxide. Purine nucleoside hydrolase solution (0.02 ml.) was added and the solution incubated for a further 2 hr. During this time, there was observed a decrease in absorption at 262 mμ accompanied by an increase at 312 mμ. After 64 hr. at room temperature, the solution was lyophilized and chromatographed. The R_f's of the product obtained by this technique in solvents A and B were identical to R_f's of the product obtained by a 30-hr. oxidation of purine with peroxyacetic acid.

The activity of the hydrolase solution used was verified[14] by its activity in conversion of adenosine to adenine. Hydrolase, 0.02 ml., added to 5 ml. of a solution of adenosine (0.01 mg./ml.) in 0.05*M*, *p*H 6.0, citrate buffer caused a drop in O. D. at 260 mμ of 0.59 to 0.55 during an incubation at 37° for 1 hr.

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(43) The peroxide-containing ether was discarded immediately.