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

Purine N-Oxides. II. The Structure of Adenine N-Oxide[1]

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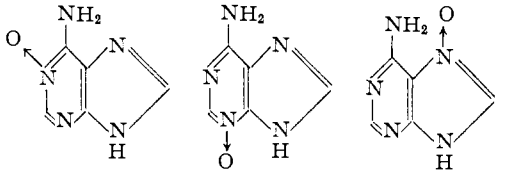
The primary products of the oxidation of adenine and of 9-substituted adenine derivatives have been shown by degradation to be 1-N-oxides.

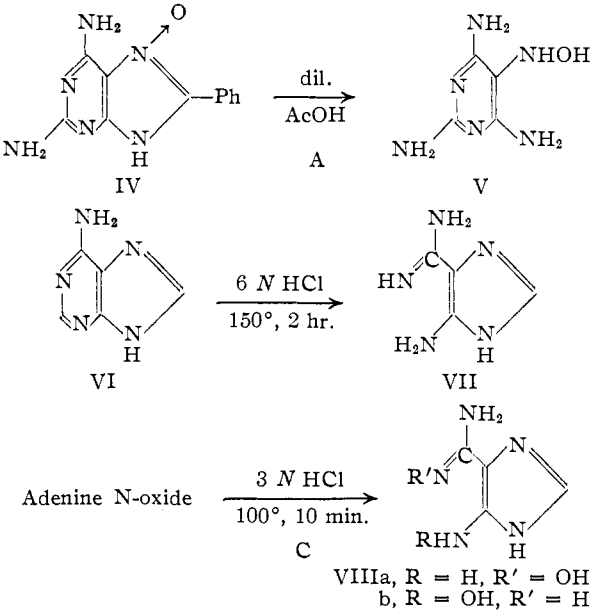
In the previous paper[3] the preparations of certain N-oxides of adenine, adenosine and 2',3'-isopropylideneadenosine were described. Since it was demonstrated that the N-oxides of adenosine and 2',3'-isopropylideneadenosine can be hydrolyzed to adenine N-oxide, these oxides must be, respectively, the 9-β-D-ribofuranosyl and the 9-β-D-(2',3'-isopropylidene)-ribofuranosyl derivatives of adenine N-oxide. Evidence for the structure of adenine N-oxide would thus be applicable to all three compounds.

The fact that 9-substituted derivatives of adenine give N-oxides with great spectral resemblance to adenine N-oxide, and that they can be hydrolyzed to adenine N-oxide, is strong evidence against a 9-N-oxide structure for adenine N-oxide. For this reason adenine N-oxide is most likely to be a 1-, 3- or 7-N-oxide (I, II or III).



I II III

Whether adenine N-oxide possesses an oxidized pyrimidine ring (N-atoms 1 or 3) or an oxidized imidazole ring (N-atoms 7 (or 9)) can be decided by consideration of the products of the hydrolysis of this N-oxide. Timmis[4] found that a substituted purine 7-N-oxide, 2,6-diamino-8-phenylpurine 7-N-oxide (IV), is hydrolyzed readily to give 2,4,6-triamino-5-hydroxylaminopyrimidine (V), by loss of the carbon adjacent to the N-oxide group. On the other hand, upon vigorous hydrolysis, adenine (VI) loses the 2-carbon with the formation of a substituted imidazole,[5,6] 4-aminoimidazole-5-carboxamidine (VII). The oxidation of adenine to adenine N-oxide makes such hydrolytic breakdown of the purine structure much more easy to effect. The product (VIII) of the hydrolysis of adenine N-oxide, upon hydrogenation over Raney nickel, yields 4-aminoimidazole-5-carboxamidine (VII) and not 4,5,6-triaminopyrimidine as would be expected if adenine N-oxide were a 7-N- or 9-N-oxide. The original hydrolysis product must, therefore, arise from adenine N-oxide by loss of carbon 2 as shown in the reaction scheme (reaction C).



IV V VI VII

VIIIa, R = H, R' = OH
b, R = OH, R' = H

Further evidence indicating that adenine N-oxide is cleaved in the pyrimidine ring by hydrolysis in a similar manner to adenine itself is provided by the following: (a) a sample of adenine-N-oxide-8-C^{14}, prepared by oxidation of adenine-8-C^{14}, loses no C^{14} upon hydrolysis; (b) the product of the hydrolysis of adenine N-oxide gives a positive Pauly test[7] which suggests it is an imidazole derivative; (c) the product of the hydrolysis of adenine N-oxide gives color tests with cupric sulfate and with ferric chloride[8] which indicate that it is a hydroxylamino derivative; (d) the hydrolysis product is similar to 4-aminoimidazole-5-carboxamidine in solubility in solvents, in R_f and in ultraviolet spectrum (see Table I).

Consideration has to be given to the possibility that loss of the 2-carbon upon hydrolysis is not proof that adenine N-oxide is a 1-N- or 3-N-oxide. One case where this might not be so would occur if compounds IX or X were formed by hydrolysis of adenine N-oxide. Since Timmis[4] has demonstrated that a 7-N-oxide will lose the 8-carbon upon hydrolysis and since the hydrolysis product of adenine N-oxide appears to be a hydroxylamino compound, that possibility is unlikely.

(1) This investigation was supported in part by funds from the American Cancer Society (Grant #MET-27), 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) Fellow of Sloan-Kettering Institute.

(3) M. A. Stevens, D. I. Magrath, H. W. Smith and G. B. Brown, THIS JOURNAL, **80**, 2755 (1958).

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

(5) J. F. Cavalieri, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3875 (1948).

(6) Although ref. 5 reports yields of purified 4-aminoimidazole-5-carboxamidine of 10% at 150°, lower hydrolysis temperatures lead to somewhat greater yields, which are difficult to separate from undegraded adenine.

(7) H. Pauly, *Z. physiol. Chem.*, **42**, 508 (1904).

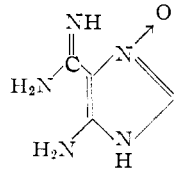
(8) A. Hantzsch and C. H. Besch, *Ann.*, **323**, 23 (1902).

TABLE I

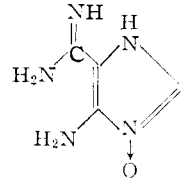
Substance	M.p., °C.	R_f A[a]	R_f B[b]	Pauly test (color)	Ultraviolet absorption Maxima mμ	pH	$A_M \times 10^{-3}$
Adenine	360–365 d.	0.61	0.38	None	262	1.0	13.1
					261	6.47	12.6
Adenine 1-N-oxide	297–307 d.	.48	.48	Transient	258	1.0	11.5
				pink	231, 262.5	7.0	41.5, 8.1
					233, 275	13.0	46.2, 7.4
4-Aminoimidazole 5-carboxamide	Base 168–169	.35[d]	.57	Blue	266, 238	2.0	.....
	HCl 255–256				278	12.0	
4-Aminoimidazole 5-carboxamidine	2HCl 244 d.	.25[d]	.69	Orange	282	2.0	
					285	6.5	11.3
					292	12.0	
4-Aminoimidazole 5-carboxamidoxime	2HCl 175 d.[c]	.31[d]	.57	Orange	277, 222	2.0	7.0, 8.4
1-Methyl-4-aminoimidazole-5-carboxamidoxime	Base 165 d.	.28	.67	Orange	276	1.0	.....
					254	12.0	

[a] 33 ml. of 1% aqueous ammonium sulfate and 66 ml. of isopropyl alcohol (paper previously soaked in 1% ammonium sulfate and dried). [b] 60 ml. of 5% aqueous disodium hydrogen phosphate and 40 ml. of isoamyl alcohol. [c] Darkens 160–175°, starts to melt 175°, foams 179°. [d] The figures are slightly variable because of the weak buffering power of 1% ammonium sulfate.

The problem of whether the primary product of the oxidation of adenine (adenine N-oxide) is a 1-N- or 3-N-oxide can be solved by a study of the



IX



X

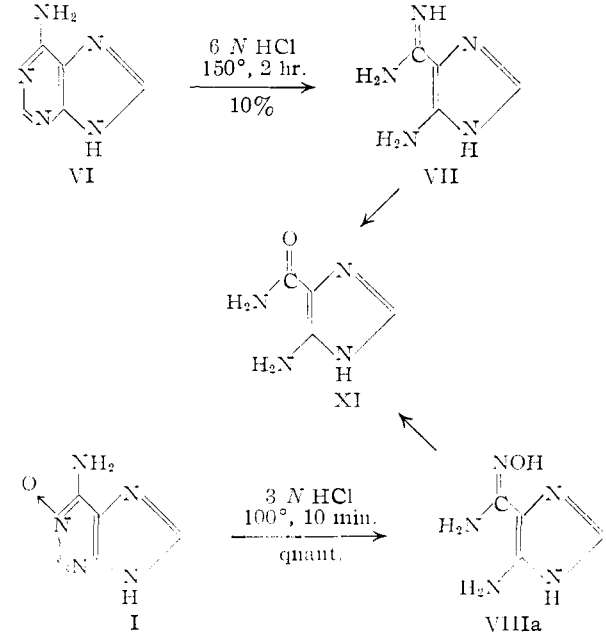
hydrolysis product of adenine N-oxide. The 1-N- and 3-N-oxides would be expected to yield 4-aminoimidazole-5-carboxamidoxime (VIIIa, R = H, R′ = OH) or 4-hydroxylaminoimidazole-5-carboxamidine (VIIIb, R = OH, R′ = H), respectively, on hydrolysis.

Efforts have been made to define the structure of the hydrolysis product of adenine N-oxide by both synthetic and degradative means. Attempts to synthesize 4-aminoimidazole-5-carboxamidoxime from 4-aminoimidazole-5-carboxamide failed because the 5-cyano intermediate required for the synthesis was not obtained. However, the utility of a cyanoimidazole in the synthesis of an imidazole carboxamidoxime has been demonstrated in the case of the known 1-methyl-4-amino-5-cyanoimidazole.[9] This material adds hydroxylamine to give 1 - methyl - 4 - aminoimidazole - 5 - carboxamidoxime, a compound resembling the hydrolysis product of adenine N-oxide in physical and chemical properties (see Table I). That product could, by analogy to adenine oxide, be the hydrolysis product of 7-methyladenine 1-N-oxide. However, treatment of 7-methyladenine under the conditions used for the oxidation of adenine did not yield an oxide.

It has been found from a detailed study of the hydrolysis of adenine N-oxide that hydrolysis with moderately concentrated mineral acid for short duration gives almost completely one hydrolysis product. There is no evidence for the formation of a formylated intermediate during the hydrolysis.

(9) R. N. Prasad and R. K. Robins, THIS JOURNAL, **79**, 6402 (1957). We thank Professor Robins of New Mexico Highlands University for a sample of 1-methyl-4-amino-5-cyanoimidazole.

Continued hydrolysis with dilute mineral acid of the first hydrolysis product of adenine N-oxide slowly gives one further product which differs from the first hydrolysis product in R_f in solvent A (see Experimental), but not in solvent B (see Experimental) and which gives a blue color instead of an orange color with Pauly reagent. The product obtained by prolonged hydrolysis of adenine N-oxide with 0.05 *N* hydrochloric acid can be separated into two fractions, one of which is highly soluble in methanol and is identical with the first hydrolysis product of adenine N-oxide (VIII), and the other which is less soluble. The latter substance was shown to be identical with the known[10] 4 - aminoimidazole - 5 - carboxamide (XI). Chromatographic analysis of the solution during this prolonged hydrolysis shows that 4-aminoimidazole-5-carboxamide is formed from the first hydrolysis product (VIII) and not from adenine N-oxide directly. A 3-N-oxide would lead only to a 4-



VI VII XI I VIIIa

(10) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

hydroxylaminoimidazole (VIIIb, R = OH, R′ = H) derivative and thence by loss of ammonia to 4 - hydroxylaminoimidazole - 5 - carboxamide. Therefore, the identification of the 4-aminoimidazole-5-carboxamide (XI), and thereby of the carboxamidoxime (VIIIa, R = H, R′ = OH), points directly to a 1-N-oxide structure for adenine N-oxide.[11] It is further concluded that adenosine N-oxide and 2′,3′-isopropylideneadenosine N-oxide are also 1-N-oxides.

The spectra of the 1-N-oxides of adenine and adenosine are given in Figs. 1 and 2, respectively. The spectrum of 2,6-diaminopurine N-oxide[3] is presented in Fig. 3 for comparison. In the case of the 2,6-diaminopurine N-oxide the position of the oxide group in the molecule is not known, though the similarity of the spectrum with that of adenine 1-N-oxide suggests that the oxygen is also on the pyrimidine moiety.

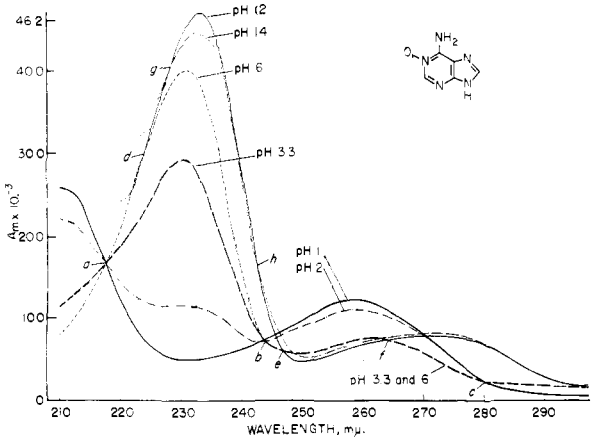


Fig. 1.—Spectrum of adenine 1-N-oxide.

In the spectrum of adenine 1-N-oxide (Fig. 1) at various pH's a change from one species to another is indicated by the major changes in the spectrum between pH < 1 and 6 with all curves passing through isosbestic points a, b and c, for which a pK_a of 2.6 is calculated. The further increases in

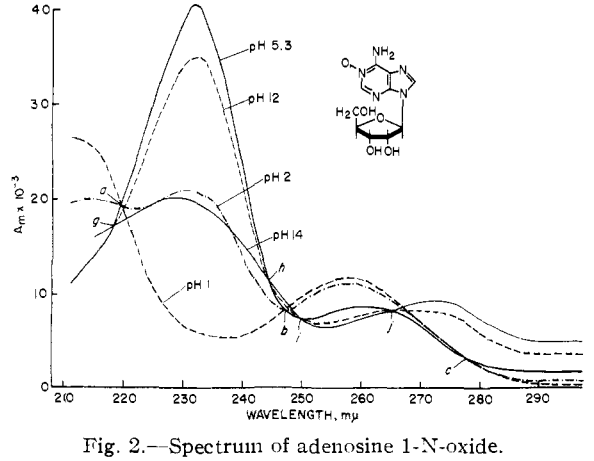


Fig. 2.—Spectrum of adenosine 1-N-oxide.

(11) It is assumed that intramolecular shift of oxygen between the 1- and 3-positions does not take place during the hydrolysis of adenine N-oxide. The fact that adenine N-oxide is completely hydrolyzed to one product during a hydrolysis of short duration suggests that this does not occur.

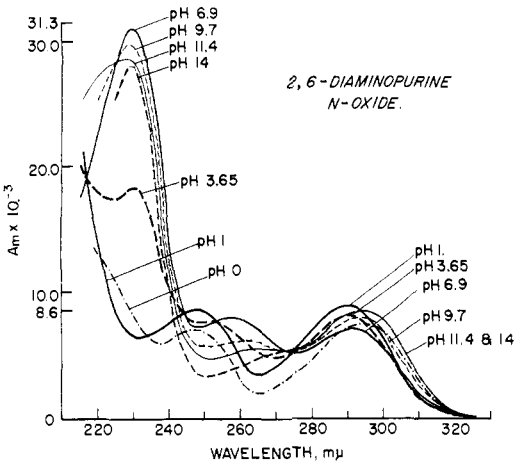


Fig. 3.—Spectrum of 2,6-diaminopurine N-oxide.

absorption and bathochromic shift of each maximum to pH 12, with all curves passing through isosbestic points d, e and f, represent a second pK_a of 9.0. In stronger alkali the shifts, with isosbestic points g and h, are due to a third pK_a of *ca.* 13.

In the spectrum of adenosine 1-N-oxide (Fig. 2) the curves involving isosbestic points a, b and c are those concerned with a pK_a of 2.14, and correspond to those involving points a, b and c for adenine oxide. Changes corresponding to those leading to isosbestic points d, e and f of adenine oxide are missing, but g and h (analogous to those similarly designated on Fig. 1) and i and j are concerned with a pK_a of 12.5. Thus the pK_a 9 for adenine 1-N-oxide must correspond to the removal of a proton from the imidazole ring, and, as would be expected, this pK_a is missing in the oxide of the ribosyl derivative. The pK_a of *ca.* 12–13 found for both adenine oxide and adenosine oxide is not found with adenine nor adenosine, and must be associated with the influence of the N-oxide in labilizing a proton within the molecules.

In the spectrum of 2,6-diaminopurine N-oxide (Fig. 3) the changes with pH indicate similar pK_a's at 3.7, 9.7, *ca.* 12 and an additional pK_a *ca.* 1, attributable to the additional amino group.

Addendum.—Through the courtesy of Dr. A. L. Patterson an X-ray diffraction study has been made on crystals of adenine 1-N-oxide, which were prepared by slow crystallization from hot water. Crystals of adenine 1-N-oxide examined with the Buerger precession camera using Cu Kα radiation were found to be triclinic with two molecules in a cell of dimensions ($\pm 0.2\%$ standard error): a = 6.968 Å., b = 8.232 Å., c = 6.823 Å., and axial angles ($\pm 5'$): $\alpha = 94°28'$, $\beta = 96°20'$, $\gamma = 111°46'$. The cell volume is 358.2 Å.^{3} and the calculated density 1.40 g. cm.$^{-3}$. W. E. Love, Jenny P. Glusker and A. L. Patterson, Institute for Cancer Research, Philadelphia, Pa.

Experimental

All chromatographic analyses were performed ascending on Whatman No. 1 paper at 25° with the developing solvents, A (1% ammonium sulfate–isopropyl alcohol, 1:2 vol./vol.)[12] and B (5% disodium phosphate–isoamyl alcohol,

(12) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).

3:2 vol./vol.)[13]; R_f values are given in Table I. Measurements of ultraviolet absorption were performed on a Beckman DK-2 spectrophotometer, except in the case of accurate determinations of extinction coefficients, etc., which were carried out on a Beckman DU spectrophotometer.

Hydrolysis of Adenine 1-N-Oxide to 4-Aminoimidazole-5-carboxamidoxime.—Adenine 1-N-oxide (500 mg.) was dissolved in 3 *N* hydrochloric acid (10 ml.). The solution was boiled for 10 minutes, then cooled to room temperature and evaporated to dryness *in vacuo*. The residue (0.72 g., quantitative) was almost chromatographically pure. Recrystallization of the product from methanol (5 ml.) gave white crystals of pure 4-aminoimidazole-5-carboxamidoxime dihydrochloride, m.p. 175° dec.

Anal. Calcd. for $C_4H_7N_5O \cdot 2HCl$: C, 22.44; H, 4.24; N, 32.72; Cl, 33.13. Found: C, 22.00; H, 4.24; N, 32.68; Cl, 33.34.

The product gave an orange color with Pauly reagent, an emerald color with 1% copper sulfate solution, and a blue turning to dark brown color with 1% ferric chloride solution.

A minor by-product detected during the hydrolysis of adenosine 1-N-oxide[3] to adenine 1-N-oxide was shown by chromatographic behavior, spectral properties and response to color tests to be 4-aminoimidazole-5-carboxamidoxime.

Further Hydrolysis of Adenine N-Oxide.—Adenine 1-N-oxide (1 g.) was dissolved in 0.05 *N* hydrochloric acid (90 ml.), then brought to reflux as rapidly as possible. Samples were taken from the solution after periods of hydrolysis of 1, 2, 5, 10, 30 and 60 minutes. The first hydrolysis product formed was 4-aminoimidazole-5-carboxamidoxime. There was no evidence for the formation of an intermediate formylated 4-aminoimidazole-5-carboxamidoxime. After a one-hour hydrolysis there began to appear a hydrolysis product with an R_f of 0.42 intermediate between adenine N-oxide and 4-aminoimidazole-5-carboxamidoxime in solvent A. This spot gave a blue color with Pauly reagent compared with an orange-yellow color obtained from 4-aminoimidazole-5-carboxamidine and -5-carboxamidoxime. This further hydrolysis product gave no color with 1% ferric chloride solution which indicates that the hydroxylamino group had been lost.

After having been hydrolyzed for 6 hours the solution was evaported to dryness *in vacuo*. The solid residue was extracted with hot methanol (10 ml.) leaving 100 mg. of material undissolved. After the methanol solution had been cooled 500 mg. of product separated. A further 100 mg. was obtained by evaporating the methanol solution to 5 ml. All of these fractions (total solid 700 mg., 65%) were identical. Recrystallization of the product from methanol gave white crystals which decomposed on heating and which were identical in ultraviolet spectrum, R_f and elemental analysis to 4-aminoimidazole-5-carboxamide, dec. *ca.* 250°.

Anal. Calcd. for $C_4H_6N_4O \cdot HCl$: C, 29.55; H, 4.34. Found: C, 29.62; H, 4.39.

Hydrogenation of 4-Aminoimidazole-5-carboxamidoxime.—4-Aminoimidazole-5-carboxamidoxime dihydrochloride (30 mg.) was dissolved in water (5 ml.). Raney nickel (*ca.* 2 mg.) was added and the mixture was shaken with hydrogen for 6 hours until 3.9 ml. of hydrogen, 1.2-mole proportions, had been absorbed. The solution was filtered and portions of the filtrate were chromatographed in solvents A and B. In solvents A and B the hydrogenation product had R_f's of 0.25 and 0.69 identical to the R_f's of 4-aminoimidazole-5-carboxamidine. The hydrogenation product exhibited ultraviolet absorption maxima at 282 mμ in slightly acid solution and 292 mμ in slightly alkaline solution, which were identical to the maxima of 4-aminoimidazole-5-carboxamidine, and different from the maxima of 4-aminoimidazole-5-carboxamidoxime (see Table I).

Preparation of 1-Methyl-4-aminoimidazole-5-carboxamidoxime.—1-Methyl-4-amino-5-cyanoimidazole (300 mg.) was dissolved in ethanol (10 ml.) and to this solution was added 0.13 ml. of an ethanol solution containing 18.7 mg./ml. of hydroxylamine. The resulting solution was refluxed for 4 hours. The yellow color of the starting solution rapidly disappeared, but toward the end of the reaction period this color reappeared. The solution was evaporated to dryness and the residue recrystallized from ethanol (10 ml.). 1-Methyl-4-aminoimidazole-5-carboxamidoxime (336 mg., 89%) was obtained in buff crystals, dec. point 165°. This carboxamidoxime gave an emerald color with copper sulfate solution and a reddish-purple color with ferric chloride.

Anal. Calcd. for $C_5H_9N_5O$: C, 38.70; H, 5.85; N, 45.14. Found: C, 38.77; H, 5.89; N, 44.66.

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(13) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).