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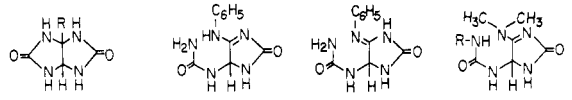
The Product of the Air Oxidation of Uric Acid. An Intermediate Formed in the Presence of Dimethylamine*

P. Heinrich Stahl

ABSTRACT: Uric and 1-methyluric acids were oxidized by air in 20% aqueous dimethylamine. The reaction is catalyzed by cupric ions or by manganese dioxide. Through nuclear magnetic resonance spectra the oxidation products have now been established as 2,5-dihydro-4-dimethylamino-2-oxo-5-ureido-1*H*-imidazole (IV) and 2,5-dihydro-4-dimethylamino-2-oxo-5-(3-methylureido)-1*H*-imidazole (V). The "isoallantoin-anilide" of Frèrejacque and Fosse (Frèrejacque, M., and Fosse, R. (1931), *Compt. Rend. 193*, 860), prepared by oxidation of uric acid in aqueous potassium hydroxide in the presence of aniline, has proved to have a similar structure, rather than the symmetrical imidazolo[4,5-*d*]imidazole structure I previously proposed.

It is apparent that the imidazole portions of uric acid and of 1-methyluric acid, respectively, remain unaffected during the formation of IV and V, in contrast to the oxidations to allantoin, *in vivo* or *in vitro*, which involve a symmetrical intermediate.

The oxidation of uric acid in aqueous alkali metal hydroxides leads to different reaction products depending upon the oxidizing agent (Schuler and Reindel, 1933). Oxidation by molecular oxygen is catalyzed by metal ions (Schuler and Reindel, 1933; Griffiths, 1952), or with charcoal present (Frèrejacque and Delepine, 1930), leading to compounds which display only end absorption or short-wavelength absorption maxima (*e.g.*, allantoin, λ_{max} 225 mμ at pH 10) in the ultraviolet region. However, in the presence of primary and secondary amines, solutions of uric acid exposed to air show an increased absorbance at about 250 mμ, and a decrease at 293 mμ at about the same rate. This oxidation, involving subsequent reaction with the amine, is catalyzed by cupric ions (Griffiths, 1952) or manganese dioxide. The oxidation product of uric acid in the presence of dimethylamine can be obtained by passing a stream of air or oxygen through a solution of uric acid in 20% aqueous dimethylamine containing suspended manganese dioxide, or with very low concentrations of cupric sulfate. The conditions are similar to those used by Frèrejacque and Fosse (1931) who oxidized uric acid in aqueous potassium hydroxide in the presence of aniline. They obtained a product to which they assigned structure I and which they called "isoallantoinanilide."



I, R = -NHC$_6$H$_5$
II, R = -N(CH$_3$)$_2$

III a

III b

IV, R=H
V, R=CH$_3$

By analogy, the oxidation product of uric acid in the presence of dimethylamine would have the structure II.

The nuclear magnetic resonance spectra of the present oxidation product of uric acid and that of "isoallantoin-anilide" are compared in Table I. The results are not consistent with the symmetrical structures I and II, but favor imidazolone structures III and IV, respectively. Instead of showing two singlets, each integrating for two NH protons as to be expected for II, the spectrum of the uric acid oxidation product exhibits two singlets and a doublet integrating for a total of four exchangeable protons. One singlet integrates for two protons. Comparison with the nuclear magnetic resonance spectra of hydantoin and of the 1- and 3-methylallantoins permits assignment of all signals, so that the structure of the oxidation product of uric acid in dimethylamine solution was established as 2,5-dihydro-4-dimethylamino-2-oxo-5-ureido-1*H*-imidazole (IV, R=

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TABLE I: Chemical Shifts, τ (parts per million), and Coupling Constants (J) (cycles per second).

Compound	Ring Protons			Ureido Substituent Protons		Methyl Protons
	N_1-H	N_3-H	C_5-H	N_1-N	N_3-H	
Hydantoin	2.30 s[a]	−0.63 s	6.08 d, $J \simeq 1$			
Allantoin	1.95 s	−0.56 s	4.69 q, $J = 8$, $J \simeq 1.5$	3.05 d, $J = 8$	4.18 s	
1-Methyl-allantoin		−0.63 s	4.85 d, $J = 8$	3.05 d, $J = 8$	4.14 s	7.35 s
3-Methyl-allantoin	1.74 s		4.74 q, $J = 8$, $J \simeq 1.5$	3.16 d, $J = 8$	4.24 s	7.24 s
IV	2.42 s		4.05 q, $J = 9$, $J \simeq 1$	3.08 d, $J = 9$	4.24 s	6.92 s, 6.97 s
V	2.44 s		4.06 d,[b] $J = 9$	3.13 d, $J = 9$	~4.0 s[c]	6.98 s, 7.02 s, 7.39 d, $J = 5$
III	2.08 s	−0.18[d]	4.21 q, $J = 9$, $J \simeq 1$	3.03 d, $J = 9$	4.24 s	Aromatic protons, ~2.6 m

[a] s, singlet; d, doublet; q, quartet; m, multiplet. [b] No further splitting observable because of superimposed signals. [c] Signal superimposed on C_5-H doublet. [d] Definite assignment not possible. See text.

H). The signal of the N-1′ proton of the ureido substituent appears as a doublet (J = 9 cps) coupled with the C-5 proton. The C-5 proton also interacts with the proton attached to N-1 of the imidazole ring (J = 1 cps) to give rise to a quartet for the signal of the C-5 proton. The quartet collapses to a sharp singlet upon addition of D_2O. This coupling between the N-1 and C-5 proton is also present in the spectrum of hydantoin and of 3-methylallantoin but not in that of 1-methylallantoin. The small coupling between the C-5 and N-1 protons can be expected from the dihedral angle, as estimated from models, between the two protons (φ ~60°). The splitting cannot be observed in the signal of the N-1 proton due to the width of the doublet. The methyl groups of the dimethylamino substituent (τ 6.92 and 6.97) are magnetically nonequivalent. The coalescence of the two singlets to a single absorption signal upon heating indicates that the nonequivalence is due to hindered rotation about the C_4–N bond.

The nuclear magnetic resonance spectrum of "isoallantoinanilide" is similar to that of IV, as in III. However, it was not possible to decide between the tautomers IIIa and IIIb. The proton signal at τ −0.18 has a chemical shift similar to that of the acidic proton of the hydantoin derivatives which suggests that IIIb may be more probable.

Oxidation of 1-methyluric acid under the same conditions gave compound V, analogous to IV. In the nuclear magnetic resonance spectrum of V the signal corresponding to that of the ureido NH_2 group in the methylallantoins and in IV integrates for only one proton. A third methyl group has its signal at τ 7.39 (doublet; J = 5 cps). The good agreement between this τ value and that of the methyl group in *N*-methylurea (τ 7.45; doublet, J = 5 cps) indicates that the site of this methyl group is the terminal nitrogen of the ureido substituent and that the imidazole ring of V represents the unchanged imidazole moiety of 1-methyluric acid. This result is in contrast to the formation of only 3-methylallantoin, in high yield, when 1-methyluric acid is oxidized by lead dioxide (Fischer and Ach, 1899).

It becomes obvious from the structure of V that the catalyzed air oxidation of uric acid in the presence of primary and secondary amines does not proceed *via* an intermediate produced by the cleavages of both the imidazole and pyrimidine portions of the purine ring (Brandenberger, 1956). Since the origins of V and IV must be similar, there is no reason to consider a secondary ring closure of a diureide intermediate, such as the symmetrical (even with ^{15}N) intermediate predicated in the alkaline oxidation of uric acid to allantoin *in vitro* (Schuler and Reindel, 1933; Cavalieri and Brown, 1948) and from metabolism *in vivo* (Brown *et al.*, 1947).

Experimental Section

Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratories, Ann Arbor, Mich. Melting points were determined in a Fisher-Johns melting point apparatus. Ultraviolet spectra (Table II) were determined with a Unicam SP800A spectrometer, and nuclear magnetic resonance spectra with a Varian A-60 with dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. Chromatograms were developed, ascending, on Whatman No. 1 paper and viewed under ultraviolet light of 254 mμ, then sprayed with freshly diluted (1:10 with Me_2CO) Ehrlich's reagent (10% solution of *p*-dimethylaminobenzaldehyde in 37% HCl). Compound IV gives a weak, III a strong yellow spot. The solvent systems were (A) acetonitrile–H_2O (3:1, v/v), (B) isopropyl alcohol–H_2O–50% NH_4OH (7:2:1, v/v), and (C) organic layer of 5% Na_2HPO_4–isoamyl alcohol–EtOH (3:2:1, v/v) (Table II).

Oxidation of Dilute Solutions of 1-Methyluric Acid. The initial rate of oxidation of 1-methyluric acid (Fischer and Clemm, 1897) in the 0.01 M buffers of Perrin (1963) gradually increases with pH to 10.4 in $NaHCO_3$ buffer and from 11.3 to 13 in KOH, and the slopes of the rates of oxidation interpolate to meet each other. In the butylamine buffer, from pH to 10.5 to 11.6 the initial rates of oxidation are somewhat higher, but the

TABLE II

Compound	R_F Value in Solvent			Spectral Data		
	A	B	C	$\lambda_{max}{}^b$ ($\epsilon \times 10^{-3}$)	$\lambda_{min}{}^b$ ($\epsilon \times 10^{-3}$)	pH
IV	0.51	0.45	0.09	249 (11.6)	226 (6.20)	7–10
V	0.65	0.53	0.21	249 (10.9)	223 (6.10)	7–10
III	0.81	0.70	0.33	271 (11.4)	236 (4.91)	7–10
				246 (7.3)[a]	226 (6.4)[a]	1.0

[a] Values approximate due to rapid decomposition. [b] mμ

slope is parallel. These results suggest that the same basic change is taking place from pH 10 to above 13, but that the amine is trapping an intermediate. The spectral changes of uric acid at *ca.* 10^{-4} M were also indicative for the formation of a derivative in the amine buffer. Isolation of an amine derivative proved more satisfactory with dimethylamine than with the butylamine of the buffer system.

2,5-Dihydro-4-dimethylamino-2-oxo-5-ureido-1H-imidazole (IV). A. COPPER II CATALYST. A stream of air was passed by means of a gas dispersion tube through a solution of uric acid, 4.2 g (2.5×10^{-2} mol), in 20% aqueous dimethylamine solution (420 ml) containing 2.5×10^{-4} mol of $CuSO_4$. The progress of the reaction was observed by ultraviolet spectra and paper chromatography. In 5 hr about 5% of the uric acid was left, and the solution was evaporated to dryness. The residue was dissolved in 350 ml of H_2O and acidified with HCl to precipitate 150 mg of unreacted uric acid. The filtrate was treated dropwise with H_2O saturated with H_2S and the CuS was removed by filtration. After concentration to a volume of 10 ml, 50 ml of EtOH was slowly added to yield a white precipitate. After this was cooled overnight 2.9 g (65%) was isolated. A pure sample was obtained by chromatography of 500 mg of the sample on a Dowex 50W-X8 (H^+) column (7.5 ml) by elution of impurities with H_2O and the compound with 0.5 N HCl. To remove the acid from the 200 ml of acidic eluate containing IV, it was passed through an Amberlite IR-45 (OH^-) column (85 ml) which was, in turn, washed with 400 ml of H_2O. Evaporation of the eluate gave 290 mg of chromatographically pure IV (dec above 230°). Further elution of the column with H_2O gave hydrolysis products of IV.

B. MANGANESE DIOXIDE CATALYST. A solution of $KMnO_4$ (0.6 g) in 1 N KOH (50 ml) was treated with an excess of H_2O_2. The MnO_2 was washed free from alkali with H_2O and then suspended in 10 ml of H_2O. This suspension (1 ml) was added to a solution of uric acid (2.5 g) in 20% aqueous dimethylamine solution (250 ml) and oxidized as described above. The solution was filtered after 4 hr and concentrated to dryness. The residue was partially dissolved in 50 ml of H_2O and 80 mg of unreacted uric acid was removed by filtration. Concentration of the filtrate to a volume of 15 ml and addition of 50 ml of EtOH caused crystallization of IV (1.9 g, 71%) as small crystals which were recrystallized from 50% EtOH to obtain an analytical sample.

Anal. Calcd for $C_6H_{11}N_{15}O_2$: C, 38.91; H, 5.99; N, 37.82. Found: C, 38.93; H, 5.88; N, 38.06.

2,5-Dihydro-4-dimethylamino-2-oxo-5-(3-methylureido)-1H-imidazole (V). A. COPPER(II) CATALYST. A solution of 1-methyluric acid (0.96 g, 5×10^{-3} mol) in 20% aqueous dimethylamine solution (100 ml) containing 5×10^{-5} mol of $CuSO_4$ was treated with air as described above. After 4 hr, the reaction solution was evaporated to dryness and the residue was treated with 25 ml of H_2O. Unreacted methyluric acid (10 mg) was removed by filtration. Copper ions were removed from the filtrate by dropwise addition of H_2S in H_2O and subsequent filtration. This filtrate was evaporated to dryness and the residue was dissolved in 100 ml of hot EtOH. Filtration and concentration to a volume of 5 ml followed by addition of the same volume of EtOAc, and cooling, gave V (0.46 g, 48.5%). Two recrystallizations from EtOH, once with charcoal added, gave white microcrystalline needles, mp 202–205° dec. Purification by passage through a cation-exchange column was unsuccessful because the product was hydrolyzed under these conditions.

B. MANGANESE DIOXIDE CATALYST. The oxidation catalyzed by manganese dioxide yielded unreacted methyluric acid (20 mg) and V (590 mg, 60%) which was recrystallized from EtOH, mp 207–210° dec.

Anal. Calcd for $C_7H_{13}N_5O_2 \cdot 0.5H_2O$: C, 40.37; H, 6.77; N, 33.63. Found: C, 40.30; H, 6.65; N, 33.48 (dried at 100°). Calcd for $C_7H_{13}N_5O_2$: C, 42.20; H, 6.58; N, 35.16. Found: C, 41.81; H, 6.57; N, 34.58 (dried at 110°).

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Polynucleotides. VII. Synthesis of Ribopolynucleotides Containing 8-Substituted Purine Nucleotides by Polynucleotide Phosphorylase*

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ABSTRACT: Polymerization of 8-substituted purine ribonucleoside diphosphates, such as 8-bromoadenosine diphosphate, 8-oxyadenosine diphosphate, 8-bromoguanosine diphosphate, 8-oxyguanosine diphosphate, and 8-dimethylaminoguanosine diphosphate, catalyzed by polynucleotide phosphorylase was studied. Although homopolymerization of these diphosphate analogs failed, copolynucleotides were obtained from these analogs and adenosine diphosphate or guanosine diphosphate. The rate and extent of the polymerization decreased according to the amount of analog diphosphates used in the polymerization reaction. Polynucleotides containing 8-substituted purine in either polyadenylic acid or polyguanylic acid chains had lower T_m's relative to the parent polyadenylic acid or polyguanylic acid, respectively. Melting temperature of the complex of the copolymer, (poly A,BrG)–(poly U)$_2$, was also lower than that of (poly A)–(poly U)$_2$ complex due presumably to the looping out of nucleotide analog residues.

Synthesis of ribopolynucleotides from naturally occurring ribonucleotide diphosphates as well as from a variety of analog diphosphates catalyzed by polynucleotide phosphorylase (Grunberg-Manago and Ochoa, 1955) has been extensively investigated (Steiner and Beers, 1961; Grunberg-Manago, 1963; Michelson *et al.*, 1967). Although it was generally accepted that polynucleotide phosphorylase had relatively loose substrate specificity, several ribonucleoside diphosphates, such as 6-azauridine diphosphate (Skoda *et al.*, 1959), 6-mercaptopurine 9-riboside diphosphates (Carbon, 1962), and arabinosyluracil diphosphate (Michelson *et al.*, 1962), were known to inhibit polynucleotide phosphorylase action.

Recently, methods for the introduction of various substituents into the 8 position of purine nucleosides and nucleotides have been developed (Holmes and Robins, 1964; Ikehara and Muneyama, 1965; Ikehara *et al.*, 1967; Long *et al.*, 1967). Since some of these nucleosides having 8 substituents were found to be inhibitory toward growth of the cancer cells (Kuretani and Fukuoka, 1966; Bloch *et al.*, 1966), the mode of their incorporation into ribopolynucleotides would be of interest. This paper deals with the polymerization of 8-bromo-ADP, 8-oxy-ADP, 8-bromo-GDP, 8-oxy-GDP, and 8-dimethylamino-GDP catalyzed by polynucleotide phosphorylase and with the nature of the resulting polynucleotides containing analogs. It was found that: (i) 8-Substituted ADP and 8-substituted GDP were very poor substrates for this enzyme and essentially no homopolynucleotides were formed. (ii) In the copolymerization of these analog diphosphates with ADP or GDP, the rate of the polymerization and the amount of polynucleotide synthesized decreased according to the content of analog diphosphates in the incubation mixture. (iii) The copolynucleotides containing analogs showed lower T_m's in their acid form and in the complex with poly U.

Materials

Synthesis of 8-Substituted Diphosphates (Ikehara *et al.*, 1968). 8-BrAMP and 8-BrGMP were obtained by bromination of AMP and GMP with bromine–water (1.2 equiv) in sodium acetate buffer of pH 3–4 (Ikehara *et al.*, 1967; Ikehara and Uesugi, 1968). Resulting 8-bromo compounds were isolated by ion-exchange column chromatography in the yield of 64–78%.

8-Oxy-AMP and 8-oxy-GMP were synthesized from the 8-bromo compounds obtained as above. Reflux with sodium acetate in acetic acid for 2 hr (Ikehara *et al.*, 1965) and chromatography on Dowex 1-X8 column

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