EVIDENCE FOR DIFFERENT ACTIVATION MECHANISMS IN THE OXIDATION OF 6-OXO- AND 6-THIOPURINES BY MAMMALIAN XANTHINE OXIDASE *

Felix Bergmann, Hannah Burger-Rachamimov, and Meir Tamari

Department of Pharmacology, The Hebrew University-Hadassah Medical School, Jerusalem (Israel)

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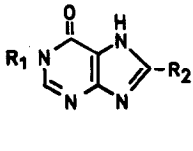
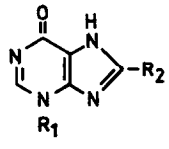
Hypoxanthine (I, $R_1 = R_2 = H$), in contrast to all other 6-substituted purines tested so far as substrates of mammalian xanthine oxidase (XO) (Wyngaarden and Dunn, 1957; Bergmann and Ungar, 1960; Bergmann, Ungar-Waron, Goldberg and Kalmus, 1961), is attacked exclusively at position 2. Successful oxidation of hypoxanthine is dependent on tautomerization to II ($R_1 = R_2 = H$). This is demonstrated by the fact that 1-methylhypoxanthine (I, $R_1 = CH_3$; $R_2 = H$) is refractory, while the 3-methyl derivative (II, $R_1 = CH_3$; $R_2 = H$) is converted to 3-methylxanthine (Berg-



I



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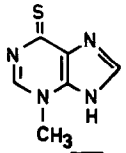

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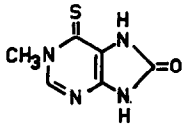

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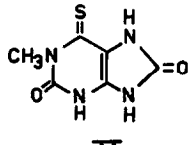

V

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mann, Kwietny, Levin and Brown, 1960). The analogous mode of activation was found in the 6,8-dihydroxypurine series, where again the 1-methyl derivative (I, R_1 = CH_3; R_2 = OH) resisted enzymic attack, while the 3-methyl derivative (II, R_1 = CH_3; R_2 = OH) was oxidized to 3-methyluric acid (Bergmann, Levin, Kalmus and Kwietny-Govrin, 1961).

We now present evidence that this process of activation is specific only for 6-oxopurines, while 6-thiopurines form a different type of enzyme-substrate (ES) complex. Thus 3-methyl-6-purinethione (III) is not attacked by XO, while 1-methyl-8-hydroxy-6-purine thione (IV) is oxidized slowly to 1-methyl-6-thiouric acid (V). The latter is a new compound and is identified by its absorption spectrum, which closely resembles that of 6-thiouric acid, and by the fact that its R_F value is considerably lower than that of IV, as is to be expected by comparison with the corresponding pair of non-methylated purines (Table 1). It is

TABLE 1: PROPERTIES OF PURINES

Compound	$\lambda max^{(m\mu)}$ at pH 8.0	R_F	Enzyme dilution used	Relative rate (%) of oxidation
8-Hydroxy-6-purine-thione	311	0.42	1:600	23
6-Thiouric acid	348	0.13		
1-Methyl-8-hydroxy-6-purinethione (IV)	333	0.58	1:50	~0.01
1-Methyl-6-thiouric acid (V)	346	0.30		
6,8-Dihydroxypurine	263	0.28	1:600	100
3-Methyl-8-hydroxy-6-purinone	284	0.20	1:50	~0.05

R_F values were determined by descending paper chromatography, using the following solvent: 95% ethanol:water:glacial acetic acid = 85:10:5, by vol. Spots were located under a Mineralight

ultraviolet lamp emitting light of about 255 mμ. Enzymic oxidations were carried out at 29° in 0.01M phosphate buffer of pH 8.0. Purified milk xanthine oxidase, at a dilution of 1:600, produced 1 μg/ml of uric acid, when 6.5×10^{-5}M xanthine served as substrate. In order to prevent inactivation of XO by H_2O_2, catalase (Worthington) 1:500 was added. Rates were measured by following the initial change of absorption at the appropriate wavelengths. All rates are expressed as percentage of the rate of xanthine oxidation, extrapolated for the same enzyme dilution.

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of interest that the ratio of the enzymic rate of IV to that of 8-hydroxy-6-mercaptopurine is similar to the ratio reported previously for the pair 3-methyl-8-hydroxy-6-purinone (II, $R_1 = CH_3$; $R_2 = OH$) and 6,8-dihydroxypurine (see Table). In either case a N-methyl group, adjacent to the C=N-bond undergoing oxidation, slows the reaction down to about the same degree, presumably by steric interference.

It is concluded that 6-thiopurines require fixation of a double bond at $\overset{2}{C} = \overset{3}{N}$ in order to undergo oxidation at C-2. The ES-complex of 6-thiopurines thus differs essentially from that of 6-oxopurines, in which enzymic attack at position 2 proceeds only with the tautomeric form of the pyrimidine ring, bearing a double bond at $\overset{2}{C} = \overset{1}{N}$.

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