Hydrogen–Deuterium Exchange in Hypoxanthines

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Summary The rate of hydrogen–deuterium exchange of the "aromatic" protons of hypoxanthine and some of its derivatives has been measured at various pH levels and a mechanism is suggested for these reactions.

IT has been reported[1] that hypoxanthine, when heated with D_2O at 100°, exchanges the hydrogen at position 8. However, this conclusion was based on the analogy with purine rather than on experimental evidence to distinguish between 2- and 8-H in hypoxanthine. A systematic study of *N*-methylhypoxanthines has shown that the *N*-alkyl substituent has a pronounced influence on the direction of deuteriation. Our results are summarised in Table 1.

Assignment of the two signals for ring hydrogens to either 2- or 8-H in 1- (**3**) and 3-methyl-hypoxanthine (**5**) is based on comparison with their 8-methyl homologues (**4**) and (**6**) respectively.[2] In compound (**5**), position 2 undergoes rapid deuteriation. In the 1-methyl derivative (**3**), reliable results have not yet been obtained, because here decomposition of the molecule takes place simultaneously

TABLE 1

Deuterium exchange of hypoxanthines (70°)

No.	Substituents	p*K* for anion formation	Exchange at position	Reaction at pH	$t_{\frac{1}{2}}$
(**1**)	—	8·6	8	10·5	120 min.
		ca. 12·5	8	14	*ca.* 50 hr.
(**2**)	8-Methyl	*ca.* 10		14	——[a]
		> 13			
(**3**)	1-Methyl	8·8		13	——[b]
(**4**)	1,8-Dimethyl		2	13	45 min.
(**5**)	3-Methyl	8·4	2	13	19 min.
(**6**)	3,8-Dimethyl		2	13	5 min.
(**7**)	7-Methyl	9·4	8	13	20 min.
(**8**)	2-Chloro-7-methy. ..		8	13	26 min.
(**9**)	9-Methyl	10·3	8	13	26 min.

[a] No exchange during 72 hr. observation time.
[b] The compound underwent slow decomposition when heated at pH 13 to temperature 70°.

with the exchange. However, in compound (**4**), deuteriation occurs at position 2. It should be recalled that in 3-methyl-6-pyrimidone, exchange takes place at C-2.[3]

For 7-methylhypoxanthine (**7**), assignment of the n.m.r. signals to individual ring hydrogens is based on comparison with the 2-chloro-derivative (**8**) (Table 2). Thus, it was

TABLE 2

N.m.r. spectra of 7-methylhypoxanthines[a]

	7-Methylhypoxanthine			2-Chloro-7-methylhypoxanthine	
pH	7-CH_3	2-H	8-H	7-CH_3	8-H
0	259	505	545	258	548
13	240	485	475	240	475

[a] On a Jeol 60 MHz instrument. All values are expressed in Hz., relative to DSS as internal standard.

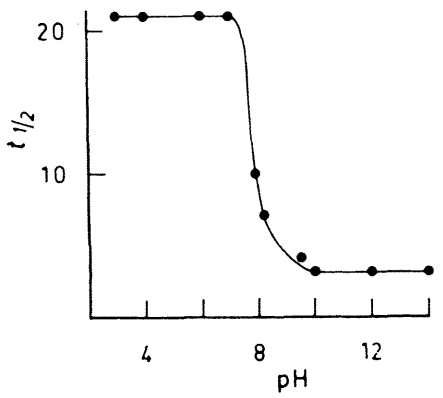

FIGURE 1. *pH-Dependence of the half-time of deuteriation of* 3-*methylhypoxanthine*, 85°

established that here deuteriation occurs at position 8. For the 9-methyl derivative (**9**), no direct experimental evidence is available so far. However, if one assumes that an *N*-methyl group directs the exchange towards the neighbouring CH group, it may be that in compound (**9**) also, 8-H reacts with D_2O. This problem is now under study.

Deuteriation is most rapid at alkaline pH; exchange is slow at neutral pH and is lacking altogether at pH 0. The favourable influence of alkaline environment may be due to one or both of the following factors: (i) the anion of the hypoxanthine may be the reactive species; or (ii) the reaction may be catalysed in some different way by OH^-. Figure 1 shows the half-time of deuteriation of 3-methyl hypoxanthine as a function of pH. It appears that increasing C_{OH^-} is mainly to increase the anion concentration of the purine, and that the anion is more reactive than the neutral form. The favourable effect of anionisation may be rationalised by assuming that in the anion the electron density at the reacting carbon atom is increased. On the other hand, Table 1 shows that the dianion of hypoxanthine exchanges at a much lower rate than the monoanion. Thus, it appears that the mechanism of deuteriation of hypoxanthines is complex and requires further studies.

Figure 2 demonstrates that the activation energy for the exchange in compound (**5**) is independent of the pH.

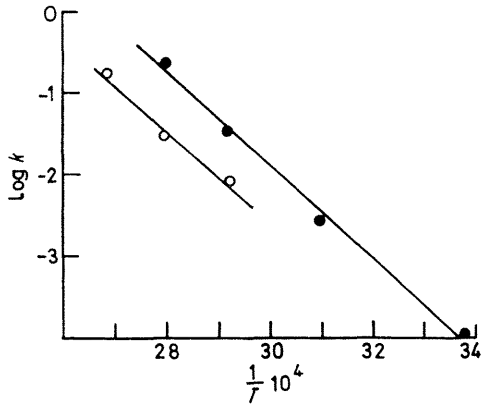

FIGURE 2. *Arrhenius function for the deuteriation of* 3-*methylhypoxanthine in neutral and anionic forms. Full circles*: *pH* 13. *Activation energy* 28 *kcal./mole*; *open circles*: *pH* 7. *Activation energy* 25 *kcal./mole.*

Thus, it may be assumed that, at least in this case, the same mechanism prevails for deuteriation of the neutral and the negatively charged forms.

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