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TRIAZENE DRUG METABOLITES: BASE CATALYSED FORMATION OF *N*-ALKYLTRIAZENES FROM *N*-HYDROXYMETHYL-*N*-ALKYLTRIAZENES

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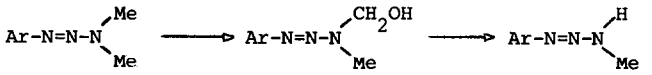
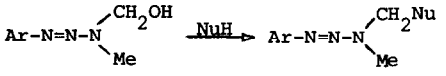
Abstract: Decomposition of the title compounds is base catalysed and requires the presence of a heteroatom - hydrogen bond in the catalyst and depends on the pKa of the base; aminomethylation of the base is not observed.

The *N*-hydroxymethyl-*N*-methyltriazene (2) is the central intermediate between the cancer chemotherapeutic *N,N*-dimethyltriazene prodrug (1) and its ultimate cellular methylating agent, the corresponding monomethyltriazene (3)[1]. The metabolism of (1) to (2) occurs in



(1) (2) (3)

the liver and the active metabolite (2) is then thought to be transported *via* the bloodstream to its site of action where it decomposes to (3)[2]. An alternative hypothesis[3] is that (2) reacts with nucleophiles, such as DNA, RNA or protein, to form aminomethylated products (4).



(2) (4)

Studies in aqueous buffer solutions[4] have shown that the *N*-hydroxymethyltriazenes (2) decompose to the corresponding aniline rather than (3), though the kinetics of such reactions have indicated that the monomethyltriazene (3) is formed instantaneously. Recently, we attempted, on the basis of product analysis, to assess the importance of amine basicity on the catalytic ability of various amines to bring about the conversion of (2) to (3)[5]. Our study was frustrated, however, by the lack of a spectroscopic method for differentiating between (2) and the corresponding (3)[6] and by the use of suspensions of (2) rather than solutions.

Now we report that we have developed an hplc method that can separate and quantify (2; Ar = 3-pyridyl) and (3; Ar = 3-pyridyl) and that we have used this to determine catalytic rate constants, k_2, in very dry ethanol for various bases for the conversion of (2; Ar = 3-pyridyl) to the corresponding (3). We have used ethanol as solvent because compound (2) is too unstable in water. Our goal has been to elucidate those structural features essential for catalytic activity *in vivo*. Chromatographic separation was achieved using a 25 cm x 5 mm i.d. column containing Spherisorb S5-ODS2 packing with a pH6.5 0.5M ammonium acetate acetonitrile: methanol: water (2:3:5) eluting solvent. The method could also separate 3-aminopyridine but in no reaction mixture was this detected. Reactions were first order in both [(2)] and [base] and the catalytic rate constants, k_2, obtained using this procedure are given in the Table.

Table. Catalytic rate constants for the conversion of (2; Ar = 3-pyridyl) to (3; Ar = 3-pyridyl) in dry ethanol at 37 °C.

Entry Number	Base	$10^5 k_2/1mol^{-1}s^{-1}$
1	Water	10.2
2	Ethanol	1.9
3	Pyridine	0
4	Piperidine	718
5	Morpholine	531
6	Piperazine	28.3
7	Imidazole	4.3
8	N-Methylimidazole	0
9	N-Methylpiperidine	0
10	2,2,6,6-Tetramethylpiperidine	2460
11	Triethylamine	0
12	Tri(n-butyl)amine	0

The most striking observation from these results is the lack of any catalysis by the tertiary bases, including pyridine. That this is not due to steric hindrance is dramatically shown by 2,2,6,6-tetramethylpiperidine which has the largest k_2. Such a lack of catalytic effect rules out both the catalyst acting as a general base and the solvent as a general acid.

The dependence of the k_2 values on base pKa (Figure) indicates that the rate constants increase rapidly above pKa 10. This is in good accord for the calculated pKa of a *ca* 9-10 for the alcohol proton in aminomethanols[7].

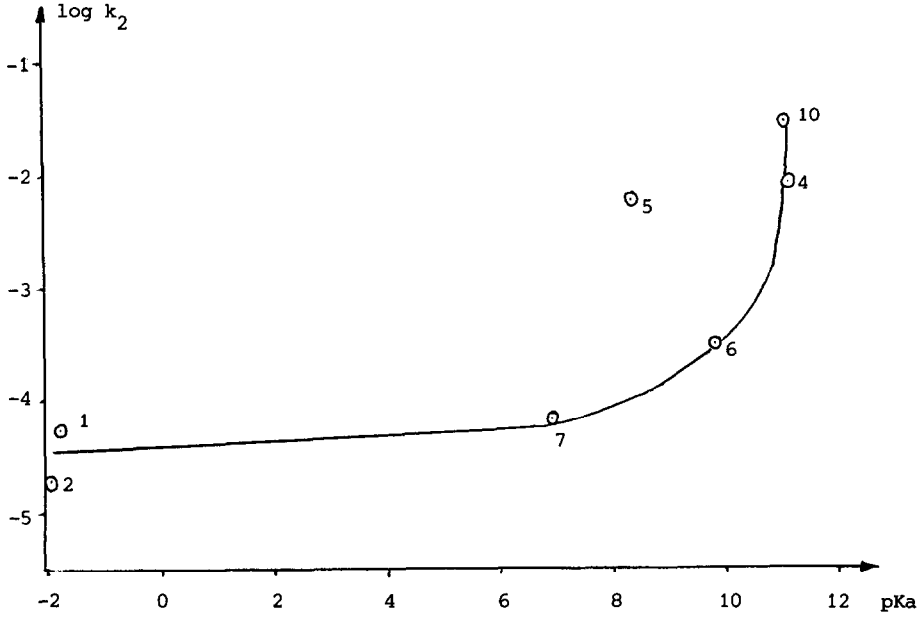
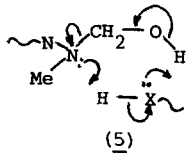


Figure. A plot of log k_2 *vs.* aqueous pKa for the conversion of (2; Ar = 3-pyridyl) to (3; Ar = 3-pyridyl).

It appears that the base requires both a lone pair of electrons *and* an X-H bond (where X = *O* or *N*) and the data point to a mechanism involving a cyclic system such as (5). Furthermore, for the alkyl homologues of (2) (where Me has been replaced by Et or Pr), the values of k_2 at 25 °C decrease in the order $k_2^{Pr} \overset{1}{>} \overset{3}{} k_2^{Et} \overset{4}{>} k_2^{Me}$ for both the ethanol and piperidine catalysed reactions. This emphasises that the electron density at the terminal triazene nitrogen plays a part in the reaction.



(5)

In sum, this work discounts the recently proposed possibility that *N*-hydroxymethyltriazenes (2) aminomethylate nucleophiles. We[8] and others[9], have shown that a better leaving group than OH^- is required for such a reaction. Moreover, it suggests that an X-H bond is an essential requirement for catalytic activity and that a pKa > 10 is required for efficient catalysis.

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