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Decomposition of the Antitumour Drug Temozolomide in Deuteriated Phosphate Buffer: Methyl Group Transfer is accompanied by Deuterium Exchange

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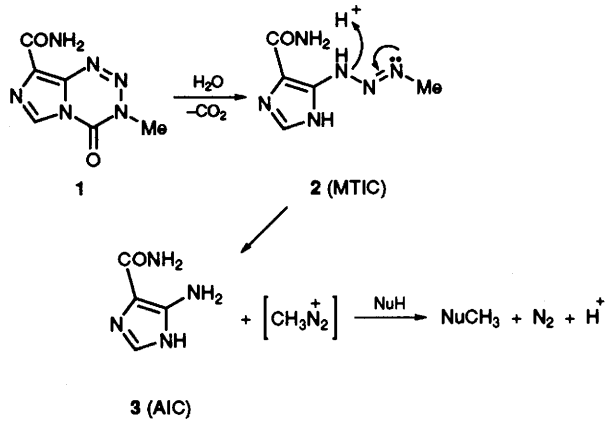
The antitumour prodrug temozolomide **1** undergoes ring-opening in deuteriated phosphate buffer; deuterium incorporation into the methyl group transferred from the reactive species 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) **2** has been monitored by observing D–H and P–H couplings in the NMR spectra of the products.

The antitumour agent temozolomide **1** has shown encouraging activity against brain tumours in clinical trials conducted under the auspices of the Cancer Research Campaign, UK.[1] Activation of the prodrug temozolomide involves hydrolytic ring-opening of the tetrazinone ring to generate 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC; **2**), the bioactive species:[2] we have proposed that this event is facilitated by the basic microenvironment of guanine-rich sequences in the major groove of DNA.[3] MTIC subsequently transfers a methyl group to a bionucleophile (Nu), *e.g.* to the O-6 or N-7 positions of guanine residues,[4] in a reaction where the leaving groups are a molecule of dinitrogen and 5-aminoimidazole-4-carboxamide (AIC; **3**), Scheme 1.

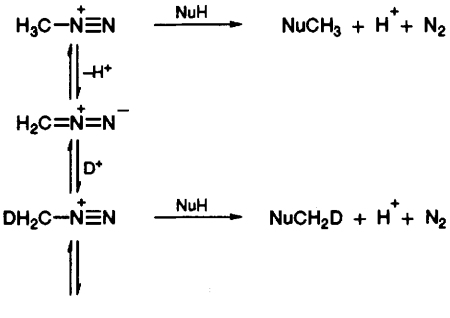
We have conducted a model study of this reaction involving the decomposition of **1** in deuteriated phosphate buffer with monitoring of the outcome by ^1H and ^{31}P NMR.† As previously reported,[5] the aromatic region of the spectrum shows an exponential decay of the imidazole proton of **1** (δ 8.60) and concomitant emergence of a signal for the corresponding proton of **3** (δ 7.24) without any significant signals for intermediate species (*e.g.* **2**). We now report that the methyl region of the spectrum is complex (Fig. 1); exponential decay of the methyl singlet of **1** at δ 3.94 was as expected but, surprisingly, a plethora of signals corresponding to methylation of solvent and buffer, with or without deuterium incorporation, was observed. A closer analysis of the spectrum (Fig. 2) revealed the presence of six alkylated products: CH_3–OD, CH_2D–OD and CHD_2–OD from the solvent; and CH_3–OPO_3, CH_2D–OPO_3 and (undeuteriated) dimethylphosphate from the buffer. Identification of the phosphorous containing products was confirmed by spiking the sample with authentic methyl- and dimethyl-phosphate, re-running both ^1H and ^{31}P NMR spectra and observing the signal enhancements.

† NMR spectra were recorded on a Bruker AC-250 spectrometer. A typical procedure was as follows: 0.4 ml of 0.2 mol dm^{-3} phosphate buffer in D_2O (pD = 7.8 equivalent to pH 7.4) was incubated in the probe at 37 °C. A sample of **1** (0.1 ml of a 40 mg per ml solution) in $(CD_3)_2SO$ at 37 °C was added and the magnet shimmed. Blocks of 32 transients with a sweep width from 9–1 ppm were recorded at 0.5 h intervals for 14 h, by which time all **1** had reacted. The data were transformed and referenced assuming δ 2.5 for $(CD_3)_2SO$. The ^{31}P NMR spectrum was recorded after the addition of 2 drops of 10 mol dm^{-3} DCl in D_2O.

The extent of deuterium incorporation was the same in methanol and methylphosphate: hence the isotope exchange occurred before attachment of the methyl group to the substrate. No deuterium exchange occurred in the methyl group of intact temozolomide **1** and when an authentic sample of MTIC **2** was incubated under conditions to which it is stable for several hours (10% Na_2CO_3 in D_2O–Me_2SO, 4 : 1 at 18 °C) the same pattern of deuteriation was seen in the ^1H NMR spectrum of the transferred methyl group but the signal due to the methyl group of intact **2** remained a sharp singlet (data not shown).



Scheme 1



Scheme 2

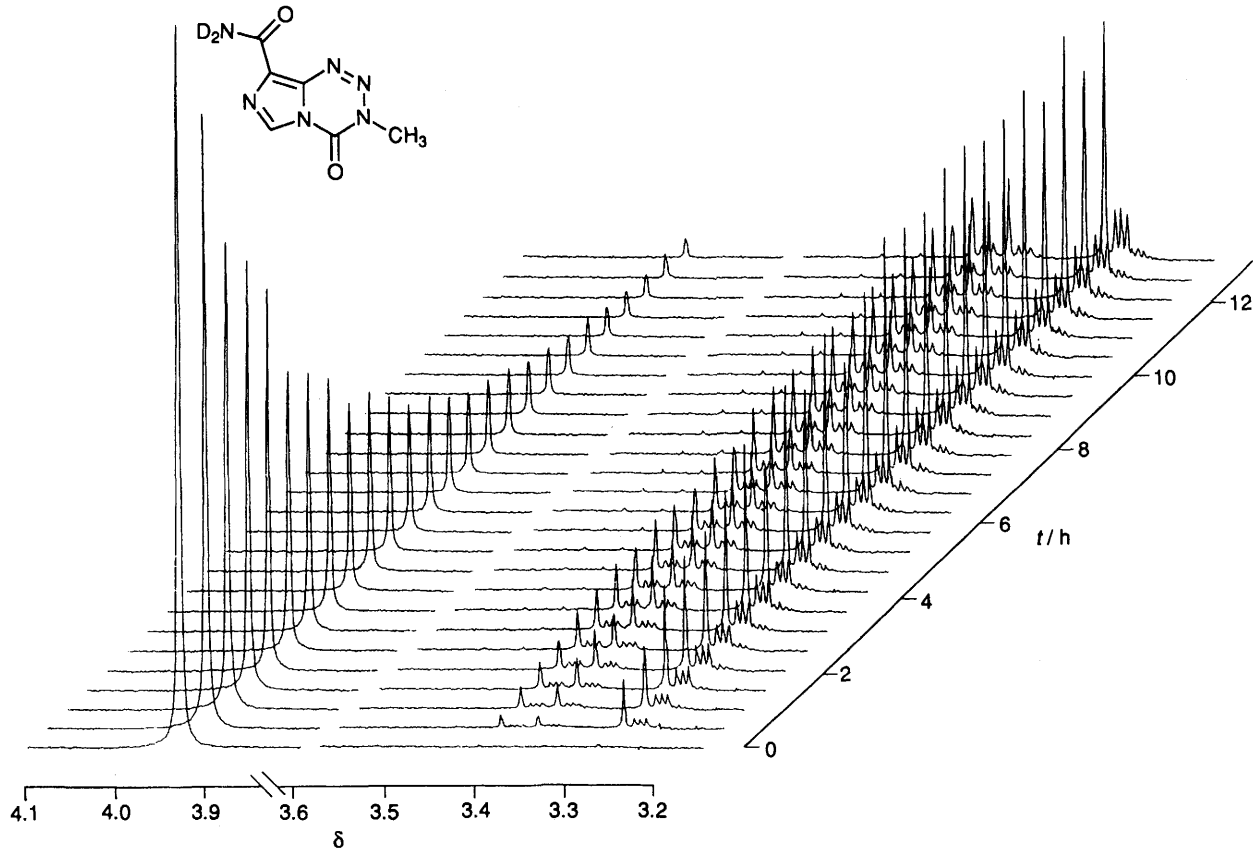


Fig. 1 Partial ^{1}H NMR spectra of the time-dependent decomposition of **1** in deuteriated phosphate buffer

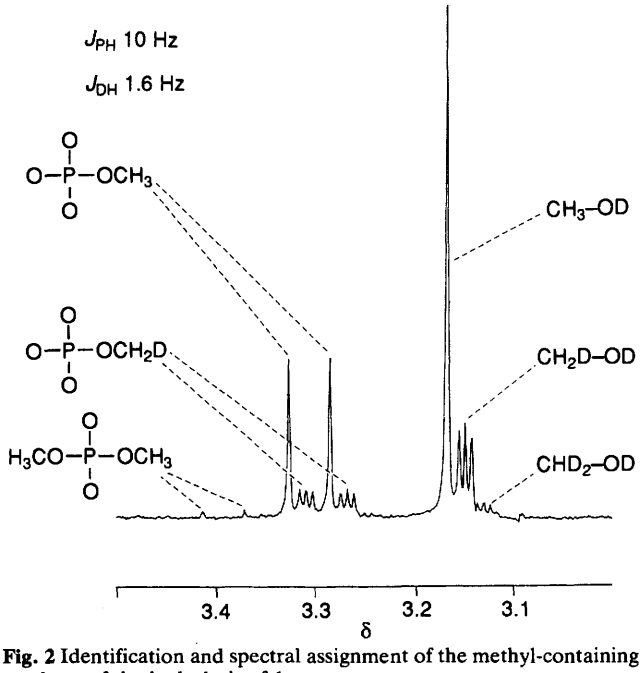


Fig. 2 Identification and spectral assignment of the methyl-containing products of the hydrolysis of **1**

Although the present work does not clarify the precise timing of the bond-forming and bond-breaking events during methyl group transfer and leaving group(s) departure, particularly within the vicinity of guanine residues in the major groove, we propose that the reaction between MTIC and DNA may be dominated by a unimolecular fragmentation of MTIC to form a highly reactive methyl-diazonium species rather than by direct nucleophilic attack of the bionucleophile on MTIC. This proposal is consistant with the known thermal breakdown of other triazenes[6] and the aqueous decomposition of the related anticancer prodrug *N*-methyl-*N*-nitrosourea.[7]

Deuterium incorporation probably occurs because of the equilibration of the methyldiazonium species with diazomethane (Scheme 2), the extent of deuteriation depending on the proportion of exchangeable H^+ and D^+ in the solvent.

Although the molar ratio of the D_2O and phosphate nucleophiles in the buffer was 276:1 at the start of the decomposition, the alkylated products were formed in the ratio Me–OD (1.6):Me–OPO_3 (1):dimethylphosphate (trace). These values were quantified by integration of the ^{1}H NMR spectrum and correcting for signal loss due to deuterium exchange. This result reflects the greater nucleophilicity of phosphate anion over D_2O. The identification of dimethylphosphate supports the prediction that temozolomide will react with phosphodiester linkages as well as with guanine bases in DNA.

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