The Mechanism of Decomposition of *N*-Methyl-*N*-nitrosourea in Aqueous Solution According to ^{13}C and ^{15}N NMR Studies: Quantitative Fragmentation to Cyanate

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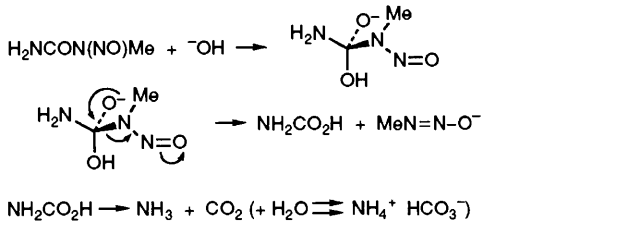
The use of ^{13}C NMR spectroscopy to monitor the decomposition of [^{13}CO]*N*-methyl-*N*-nitrosourea, and of ^{15}N NMR spectroscopy to follow the decomposition of [$^{15}NH_2$]*N*-methyl-*N*-nitrosourea, both in aqueous phosphate buffer at pH 7, shows the initial product to be cyanate rather than carbamate; the cyanate reacts with phosphate to give carbamoyl phosphate.

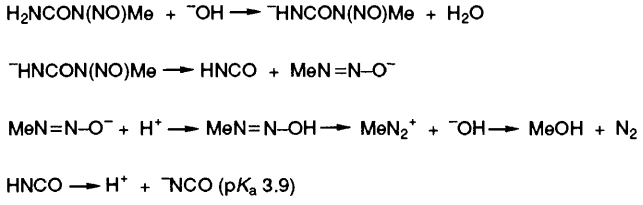
To understand the molecular basis of the carcinogenic and anticancer activities of *N*-alkyl-*N*-nitrosoureas (ANUs) requires definition of the mechanism of their decomposition in water.[1] It has been proposed,[2] primarily on the basis of kinetic studies, that the mechanism of decomposition of *N*-methyl-*N*-nitrosourea **1** (MNU) above pH 5 involves hydroxide attack on the carbonyl group to give a tetrahedral intermediate that decomposes to methanediazoate (MeN=NO$^-$) and carbamate (Scheme 1). The methanediazoate leads to the methanediazonium ion and hence methanol and nitrogen, whilst carbamate gives ammonium hydrogen carbonate. This mechanism has been widely accepted. It forms the basis of an explanation for sequence selectivity in alkylations of DNA effected by ANUs.[3] It was proposed that nucleophilic attack by O^6 of guanine at the imino carbon of the isourea tautomer of ANUs leading to a tetrahedral intermediate is an initial event in the reactions between ANUs and DNA (see also ref. 1).

Using MNU specifically labelled with ^{13}C in its carbonyl group and ^{13}C NMR spectroscopy to monitor reactions, we have found that cyanate is a primary product of the decomposition of MNU in water. This was corroborated by studying the aqueous decomposition of [$^{15}NH_2$]MNU, using ^{15}N NMR spectroscopy to monitor the reaction. These findings require a mechanism (first proposed in ref. 4) in which deprotonation of MNU at its amido function is the initial event. The resulting anion of MNU fragments to cyanic acid and methanediazoate (Scheme 2).

We originally undertook studies of MNU decomposition in water in order to define optimum conditions for studying reactions of MNU and its products of decomposition with



Scheme 1 Mechanism of decomposition of *N*-methyl-*N*-nitrosourea in water, pH > 5 (ref. 2)



Scheme 2 Alternative mechanism of decomposition of *N*-methyl-*N*-nitrosourea (ref. 4)

oligonucleotides (*N.B.* safe procedures for handling MNU are described in ref. 5). We found that 20% (v/v) methanol–aq. phosphate buffer (20 mmol dm^{-3} in phosphate) was a suitable medium for obtaining relatively high concentrations of MNU (*cf.* ref. 6), and using a pH-stat we could maintain pH control within ±0.1 units. Under these conditions with an initial pH of 7.4, we observed initial *consumption* of *ca.* 1 equiv. of hydroxide, and from kinetic studies in phosphate buffer alone (50 mmol dm^{-3}; pH 7.0) we determined k_{obs} (25 °C) = 7.8 × 10^{-5} s^{-1}. The rate constant agrees with previous measurements,[2] but the observed consumption of base seems not to be compatible with the mechanism of Scheme 1, which generates ammonium hydrogen carbonate (*N.B.* a 0.1 mol dm^{-3} solution gives pH 7.8; ref. 7). However, in the mechanism of Scheme 2 protons are liberated by the formation of cyanic acid and the pH of the medium should therefore initially *fall* with this mechanism operating. For the pH of the medium to remain constant the addition of base will be required, as observed under pH-stat conditions.

To probe the aqueous chemistry of MNU further we have used NMR spectroscopy to monitor the appearance and disappearance of intermediates and the formation of products. None of the species of interest bears hydrogen atoms on their carbons and direct observation of these species requires ^{13}C NMR using ^{13}C-enriched MNU [$H_2N^{13}CON(NO)Me$] and ^{15}N NMR using [$^{15}NH_2$]MNU. It was not practical to make observations with non-enriched MNU because of the low sensitivity of detection of the relevant carbon or nitrogen resonances in a system undergoing relatively rapid chemical change. A solution of [^{13}C]cyanate was prepared by oxidising[8] potassium [^{13}C]cyanide (92 atom % ^{13}C) with permanganate. This was treated (*cf.* procedures of refs. 9–11) with methylamine to give [^{13}CO]*N*-methylurea, which was converted[9–11] into [^{13}CO]MNU. This was purified by recrystallisation from dichloromethane–light petroleum [yield 21% based on K^{13}CN, δ_C 26.8 (methyl, natural abundance) and 156.9 (CO)]. Solutions of [^{13}CO]MNU (initial concentration 0.1 mol dm^{-3}) in 20% methanol–0.5 mol dm^{-3} aq. phosphate buffer (pH 7) were monitored by ^{13}C NMR (125 MHz) at 22 °C. We observed (see Fig. 1 for representative spectra) the immediate formation of cyanate (broad triplet, δ 128.9; non-enriched NaNCO in D_2O showed δ 128.8, $^1J_{C\text{-}N}$ 10.5 Hz); its resonance initially progressively increased (up to *ca.* 4 h) and then decreased. Lagging behind the formation of cyanate is the appearance of a doublet ($^2J_{C\text{-}O\text{-}P}$ 5 Hz) at δ 157.0, which is assigned to carbamoyl phosphate. This substance can be prepared from aqueous cyanate and phosphate,[12] and the chemical shift given was reproduced by a solution of authentic non-enriched material. At longer reaction times the resonance from carbamoyl phosphate began to decline and resonances from hydrogen carbonate–carbon dioxide (δ 160.6 and 125, respectively) and methyl carbonate (δ 160.5) began to grow. Similar behaviour to that described was observed at 15 and 35 °C, the processes described occurring either more slowly or rapidly. At 35 °C, all of the cyanate and carbamoyl phosphate had disappeared after 10 h, and the only carbonyl-derived products were hydrogen carbonate–carbon dioxide, methyl carbonate and a minor species showing δ 163 (assigned to

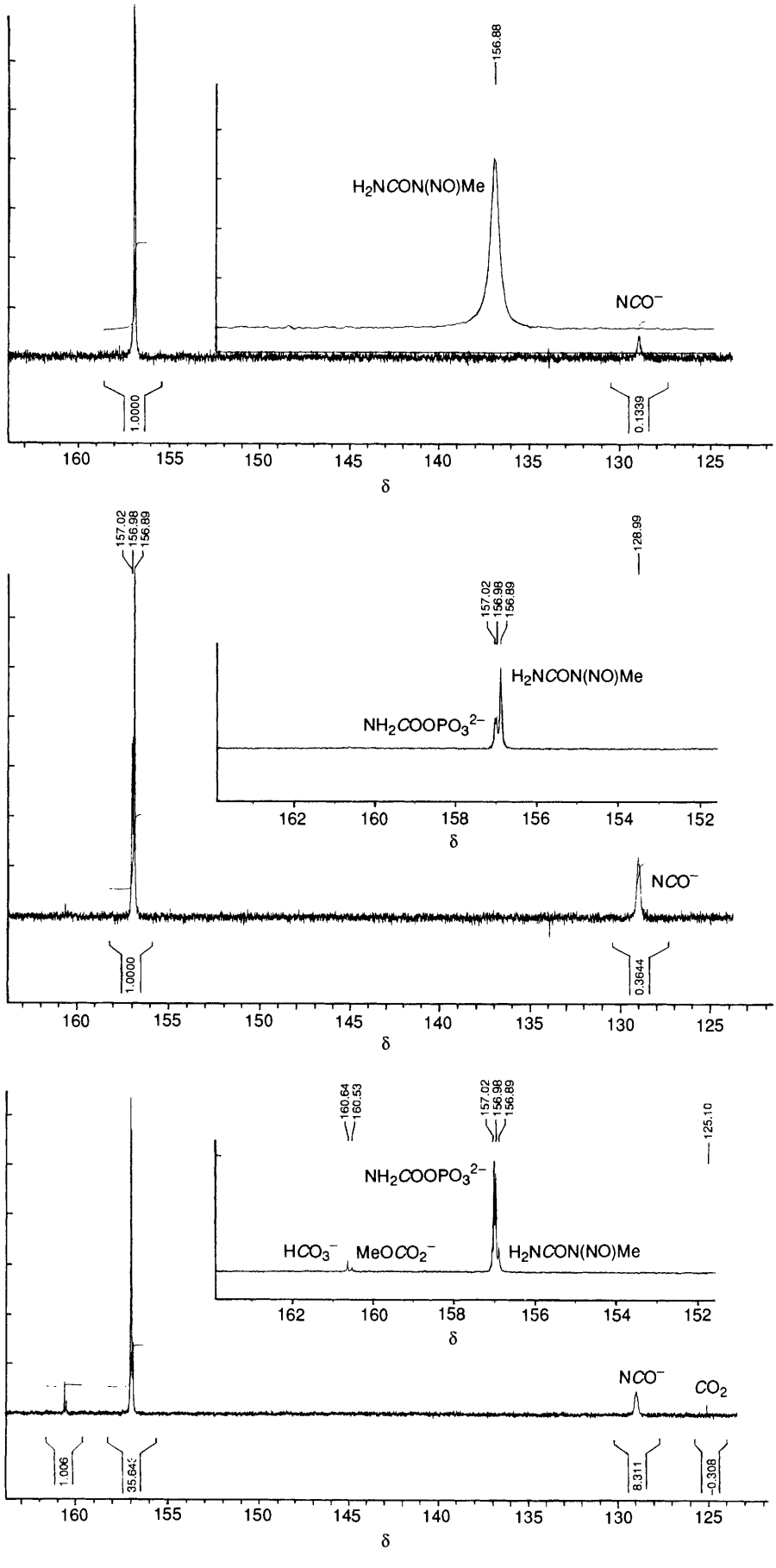


Fig. 1 ^{13}C NMR spectra of the partial decomposition of [^{13}CO]*N*-methyl-*N*-nitrosourea in 20% methanol–0.5 mol dm^{-3} aq. phosphate buffer (pH 7) at 22 °C after 20 min (top), 3 h (middle) and 13 h (bottom spectrum)

urea, reported δ 161.2[13]). Previous studies reported the absence of cyanate (by a spot test) from a reaction of MNU in pH 9.7 borate buffer.[2] Under similar conditions (pH 9.6 in borate buffer) we found that MNU decomposes immediately and from [^{13}CO]MNU we observed [^{13}C]cyanate to be the *exclusive initial product*. We also detected cyanate in our reactions of MNU in the methanol–phosphate buffer by a spot test.[14] Finally, addition of ammonium carbamate to the methanol–phosphate buffer gave hydrogen carbonate–carbon dioxide immediately and no cyanate.

Potassium [^{15}N]cyanate was prepared by fusion of potassium carbonate with [$^{15}N_2$]urea (96 atom % ^{15}N), and converted into MNU as described above. Monitoring by ^{15}N NMR of the decomposition of [$^{15}NH_2$]MNU (at 22 °C under similar conditions to those described for [^{13}CO]*N*-methyl-*N*-nitrosourea) showed the disappearance of a resonance at δ 61.5 {chemical shift relative to external 5 mol dm^{-3} NH_4Cl in D_2O at δ 0; *N.B.* [$^{15}NH_2$]MNU in CD_3OD showed a quintet ($J_{N\text{-}D}$ 14 Hz) at δ 58.9} concomitant with the appearance of a resonance at δ 55.6, which is assigned to cyanate (KCNO in D_2O exhibits δ_N 57.6, *cf.* ref. 15). This resonance initially grew (up to 8 h) but eventually declined as a resonance at δ 59.0, assigned to carbamoyl phosphate, increased in intensity. Finally, this resonance declined and after 2 weeks there was a single resonance at δ −1.0 (NH_4^+).

The results described are in complete accord with the mechanism of Scheme 2. Initial deprotonation of MNU at its amido group is followed by fragmentation to cyanic acid and methanediazoate. The well established[4] mode of decomposition of MNU under anhydrous basic conditions therefore prevails in aqueous solution. Reaction of cyanate with the phosphate buffer gives carbamoyl phosphate, which gradually decomposes to hydrogen carbonate–carbon dioxide and ammonia. Since the medium used contains 20% methanol an equilibrium is also established between carbon dioxide and methyl carbonate.

The results in this paper undermine proposals[3] for mechanisms of DNA methylation that require initial attack by O^6 of G either on the carbonyl carbon of the urea form of MNU or on the imino carbon of the isourea form of MNU. The site or sequence selectivity can be explained by ascribing the enhanced reactivity of a particular site (*e.g.* N^7 of G) towards the methanediazonium ion, to structural factors within the DNA or an oligonucleotide. These factors enhance reactivity towards this alkylating agent in an S_N2 like reaction mechanism.[16]

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