Isotopic labelling studies of the deaminative fragmentation of *N*-nitrosohydroxylamines and related compounds by kinetics, millimetre-wave spectroscopy and ^{17}O NMR spectroscopy

Johanna Haider, M. N. Stuart Hill, Iain D. Menneer, Howard Maskill* and John G. Smith

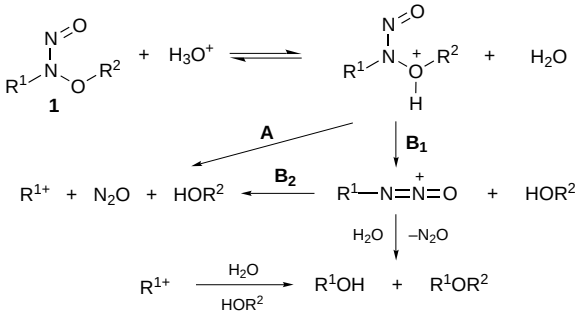
Department of Chemistry, Bedson Building, University of Newcastle upon Tyne, Newcastle upon Tyne, UK NE1 7RU

Nitrous oxide enriched with ^{18}O has been identified by millimetre-wave spectroscopy from the acid-induced hydrolysis of N^1-(2-adamantyl)-N^2-[^{18}O]hydroxydiazen-1-ium N^1-oxide 2 which, along with ^{17}O NMR studies and an α-deuterium kinetic isotope effect of k_H/k_D = 0.98 (±0.02) for *N*-nitroso-*N*-(2-[2-^{2}H]adamantyl)-*O*-benzylhydroxylamine 4, confirms (i) that nitrous oxide is liberated in these reactions, and (ii) that the fragmentations in these reactions are step-wise and not concerted.

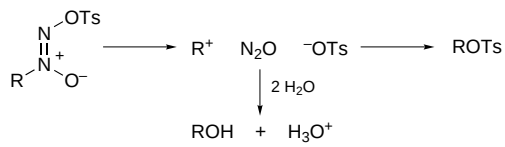
A decade ago, the biological importance of nitric oxide (NO) was first recognised,[1] and this led to the search for compounds capable of releasing NO under mild physiological conditions. Promising first results were reported for some rather complex *N*-nitrosohydroxylamine derivatives,[2] whereas simpler analogues were shown to undergo a new deamination type of reaction.[3] Since related *N*-nitrosamines are known to be carcinogenic,[4] and deamination reactions involve highly reactive electrophilic intermediates with mutagenic potential, the borderline between the possibly benevolent homolytic reaction and the almost certainly hazardous heterolytic alternative from *N*-nitrosohydroxylamines required exploration, and both mechanisms need better characterization.

The mechanism we proposed for the acid-induced deamination type of solvolysis of *N*-nitroso-*N*,*O*-dialkylhydroxylamines **1** is shown in Scheme 1.[3] Our earlier results did not allow us to distinguish between the concerted fragmentation (path A) and the step-wise alternative (path B). We had previously established, however, that the related fragmentation of alkylazoxy arenesulfonates, shown in Scheme 2 for azoxy tosylates, is concerted.[5] N^1-(2-Adamantyl)-N^2-hydroxydiazene-1-ium N^1-oxide **2**, the product of nitrosation of *N*-(2-adamantyl)hydroxylamine, also undergoes acid-induced hydrolysis with kinetic features very similar indeed to those of several *N*-nitroso-*N*,*O*-dialkylhydroxylamines.[3] Consequently, a mechanism involving isomerization of **2** into its *N*-nitroso isomer **3** followed by protonation and fragmentation was proposed, and is shown in Scheme 3. In this mechanism also, the fragmentation could be concerted (path A) or step-wise (path B). Additionally, however, the direct mechanism from **2** shown in Scheme 4 in which the other oxygen departs as part of the nucleofuge appeared a viable alternative, especially in view of the above-mentioned fragmentation of alkylazoxy tosylates (Scheme 2). We now report that, by a combination of isotopic studies and spectroscopic methods, we have been able to distinguish between the concerted and step-wise alternatives for compounds **1**, and between the mechanisms of Schemes 3 and 4 for N^1-(2-adamantyl)-N^2-hydroxydiazene-1-ium N^1-oxide **2**.

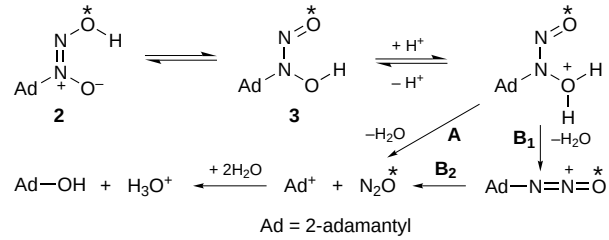
Compound **2** was prepared by reaction of *N*-(2-adamantyl)-hydroxylamine with aqueous nitrous acid enriched with ^{17}O following a procedure established by Dahn,[6] and the labelled atom, marked with an asterisk in Scheme 3, was confirmed by ^{17}O NMR spectroscopy [δ_O 198 (relative to $^{17}OH_2$), the other oxygen giving a signal at δ_O 334].[3]† The ^{17}O NMR spectrum of the adamantan-2-ol (δ_O = 30), isolated from acid-induced hydrolysis of this labelled substrate, was then recorded and shown to be identical to a sample of natural abundance adamantan-2-ol,‡ *i.e.* none of the ^{17}O in the substrate ended up in the adamantanol. Since appreciable quantities of the ether R–O–R′ are formed from the hydrolysis of the *N*-nitroso-*N*,*O*-dialkylhydroxylamines **1**, we are confident that the water molecule which departs as nucleofuge from **2** (or from **3**–whichever is involved) would be trapped to some extent by the 2-adamantyl cation. We concluded on the basis of this admittedly negative evidence that the isotopically labelled oxygen departs entirely as nitrous oxide according to the mechanism in Scheme 3. To obtain positive evidence, we also



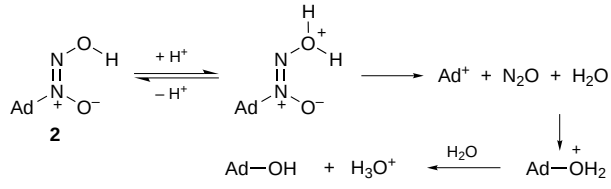
Scheme 1



Scheme 2



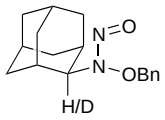
Scheme 3



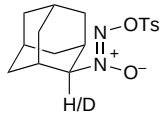
Scheme 4

prepared compound **2** specifically enriched with ^{18}O at the oxygen marked with an asterisk in Scheme 3. The acid-induced hydrolysis was then carried out for more than eight half-lives at 65 °C, and the evolved gas was flushed through by a stream of nitrogen, dried by a potassium hydroxide tower, and trapped at liquid nitrogen temperature. It was then transferred *via* normal vacuum line procedures to a millimetre-wave spectrometer and part of its rotational spectrum was recorded.[7] In addition to the band at 125 614 MHz due to the J 5 ← 4 rotational transition in the N_2O containing ^{16}O, we also observed the band at 71 155 MHz corresponding to the J 3 ← 2 transition of the N_2O molecule containing ^{18}O; both frequencies are in excellent agreement with values calculated from literature data.[8] This result corroborates the ^{17}O NMR result described above, and together they are compatible with the mechanism of Scheme 3 and rule out that in Scheme 4.

We also prepared *N*-nitroso-*N*-(2-adamantyl)-*O*-benzylhydroxylamine **4** specifically labelled with deuterium at C-2 of the

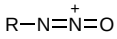


4

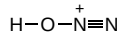


5

6a R = 2-adamantyl
b R = H



7a R = H
b R = 2-adamantyl



8

adamantyl system, and measured the α-deuterium kinetic isotope effect (KIE). Results of k_H/k_D = 1.09–1.13 for the α-deuterium KIE for solvolysis of 2-adamantylazoxy tosylate **5** were part of the evidence that cleavage of the C–N bond is involved in the rate-limiting transition state for the solvolysis of **5** (Scheme 2).[5] Similar results would be expected for **4** if the mechanism follows path A in Scheme 1 (or if the reaction is step-wise with step B_2 rate limiting). The step-wise mechanism with the initial step B_1 rate limiting would be expected to have an α-deuterium KIE of approximately unity since only minor perturbations of the α-C–H vibrations are expected in the formation of the transition structure from the reactant molecule. Reactions were measured at two temperatures and two acid concentrations as shown in Table 1, and results of k_H/k_D = 0.98 (±0.02) were obtained; these are compatible only with the stepwise mechanism and rate-limiting departure of the nucleofuge.

The protonated form of **2** in Scheme 4 is clearly analogous to **5** in that both have good nucleofuges, H_2O and TsO^- respectively, bonded to the terminal nitrogen of the azoxy group. Stepwise fragmentation of **5** and of the protonated form of **2** would involve **6a** as an intermediate. In a theoretical investigation, however, we have already shown that **6b** exists only as a transition structure in the gas-phase isomerization of **7a** into **8**,[9] and that no bonded structure corresponding to **6a** is expected to exist. So, whereas **5** avoids formation of **6a** by a concerted fragmentation, we have now shown that formation of **6a** from **2** in acidic solution is avoided by rearrangement and a step-wise route involving **7b** (Scheme 3, path B).

Table 1 Rate constants and α-deuterium kinetic isotope effects for the acid-induced hydrolysis of *N*-nitroso-*N*-(2-adamantyl)-*O*-benzylhydroxylamine[a]

Compound	T/°C	$[HClO_4]$/mol dm^{-3}	$k/10^{-3}$ s^{-1}	k_H/k_D
4-H	34.68	0.10	1.14	
4-D	34.68	0.10	1.16	0.98
4-H	44.67	0.10	2.90	
4-D	44.67	0.10	2.92	0.99
4-H	44.68	0.50	4.86	
4-D	44.68	0.50	5.04	0.96

[a] Reaction rates were measured by our normal UV spectrophotometric method which has already been described (refs. 3, 5). Simultaneous reactions were monitored for about three half-lives with two cells containing the protium compound and two cells containing the deuterium analogue in the same thermostatted four-cell block. Identical solvolytic media were used for H and D compounds in one four-cell experiment, and the ionic strength in all reactions was 1.0 mol dm^{-3} ($NaClO_4$). The rate constant for a given compound was reproducible to within 1%, and standard deviations on individual rate constants were always less than 1%. The maximum error in k_H/k_D is estimated to be 0.02.

Footnotes and References

* E-mail: h.maskill@ncl.ac.uk

† ^{17}O NMR spectra were recorded using a Bruker WM 300 WB spectrometer operating at 300 MHz for ^1H and 40.670 MHz for ^{17}O; the solvent was deuteriochloroform.

‡ For both the natural abundance (non-solvolytic) sample of adamantan-2-ol and that isolated from the hydrolysis of 2-Ad-N(O)^{17}OH, practically the same signal-to-noise ratio was achieved for the signal at δ_O 30 after 880 000 scans using identical spectrometer conditions and similar substrate solution concentrations.

1 A. R. Butler, *Chem. Br.*, 1990, 419; A. R. Butler and D. L. H. Williams, *Chem. Soc. Rev.*, 1993, 233; P. L. Feldman, O. W. Griffith and D. J. Stuehr, *Chem. Eng. News*, December 20, 1993, p. 26.
2 See, for example, C. M. Maragos, D. Morley, D. A. Wink, T. M. Dunams, J. E. Saavedra, A. Hoffman, A. A. Bove, L. Isaac, J. A. Hrabie and I. K. Keefer, *J. Med. Chem.*, 1991, **34**, 3242 and citations in ref. 1.
3 I. D. Menneer, H. Maskill and D. I. Smith, *J. Chem. Soc., Chem. Commun.*, 1995, 1855; I. D. Menneer, PhD Thesis, Newcastle, 1996.
4 D. L. H. Williams, *Nitrosation*, Cambridge University Press, Cambridge, 1988.
5 H. Maskill, J. T. Thompson and A. A. Wilson, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1693; I. M. Gordon and H. Maskill, *J. Chem. Soc., Chem. Commun.*, 1989, 1358.
6 H. Dahn and M.-N. Ung-Truong, *Helv. Chim. Acta*, 1988, **71**, 241.
7 J. H. Carpenter, J. D. Cooper, J. B. Simpson, J. G. Smith and D. H. Whiffen, *J. Phys. E.*, 1974, **7**, 678.
8 B. A. Andreev, A. V. Burenin, E. N. Karyakin, A. F. Krupnov and S. M. Shapin, *J. Mol. Spectrosc.*, 1976, **62**, 125.
9 P. M. W. Gill, H. Maskill, D. Poppinger and L. Radom, *J. Chem. Res. (S)*, 1987, 54.

Received in Cambridge, UK, 2nd June 1997; 7/03801A