JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 8, 587–596 (1995)

RADIOLYTIC STUDIES OF THE REDUCTIVE CYCLIZATION OF 2-NITROARYLAMIDES: CYCLIZATION VIA HYDROXYLAMINE INTERMEDIATES

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The reductive cyclization of several 2-nitroarylamides was studied by radiolytic reduction, examining the effects of substituents on the nitrophenyl ring and on the leaving aniline and variations in the nature of the link between the nitrophenyl ring and the leaving aniline. The stoichiometry of the reduction and the identification of *N*-hydroxylactam and aniline products suggest that the major initial products of such a reduction of the nitroamides are the corresponding hydroxylamines. Under anaerobic conditions, cyclization via the hydroxylamines was considerably faster (up to 160-fold) than via the corresponding amines under comparable conditions, but was similarly influenced by changes in geometry. Unlike cyclization via the amines, rates of cyclization via the hydroxylamines were sensitive to substitution on the leaving aniline, being accelerated by electron-withdrawing groups. The rate-determining step in the cyclization of the hydroxylamines is proposed to be breakdown of the tetrahedral intermediate.

INTRODUCTION

In a recent paper,[1] we evaluated the utility of 2-nitroarylamides as bioreducible prodrugs for the hypoxia-selective release of activated aminoaniline mustard alkylating agents. In this design (Scheme 1), reduction of the nitroamide (**I**) triggers a cyclization reaction, releasing the corresponding lactam or *N*-hydroxylactam (**III**) and an amine-bearing mustard (**IV**). The expelled mustard is a much more active alkylating agent, owing to increased electron release on conversion of the amide substituent into an amine.[2]

Studies of the kinetics of cyclization of model 2-aminoarylamides under aqueous conditions[1,3] has thus far failed to identify an aminoamide which can cyclize sufficiently rapidly under physiological conditions (pH ≈ 7, 37 °C) to be of value as a potential prodrug. The most reactive compounds had $t_{1/2}$ values for the cyclization reaction of *ca* 35 min under these conditions, whereas a value of less than 1 min is probably required. As part of this study,[1] we examined the rate of ring closure of *N*-(3-quinolyl)-3-(2-aminophenyl)propanamide (**2**). The corresponding nitro compound (**1**) has been reported to undergo facile reductive cyclization in refluxing ethanol and cyclohexene, with Pd/C catalyst (90% completely in 30 min),[4] via the aminoamide **2** as intermediate. However, we found that cyclization of the preformed **2** under similar conditions was much slower (only 5% complete in 30 min).

This difference suggested that the reductively triggered ring closure of the nitro amide **1** does not proceed via cyclization of the aminoamide **2**, but through some earlier reduction intermediate, possibly the hydroxylamine. Radiolytic reduction studies were therefore carried out on **1** and related compounds in order to investigate aqueous reactions of the reduction products

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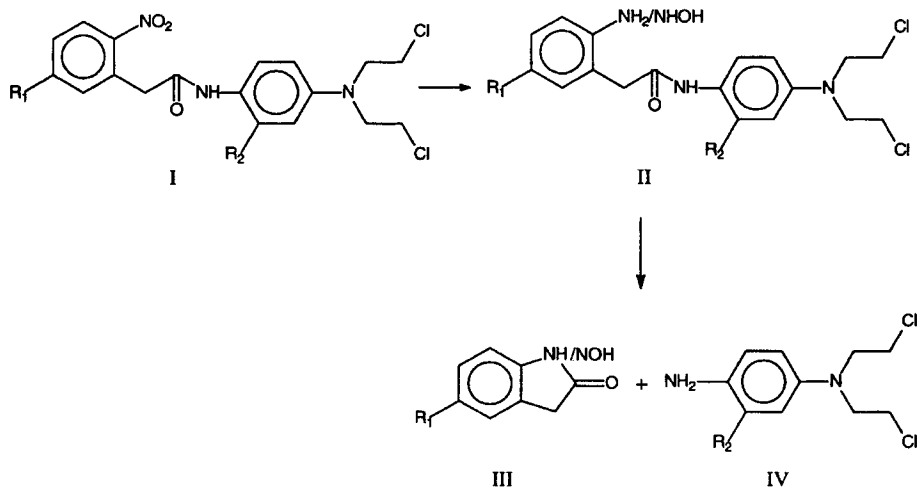
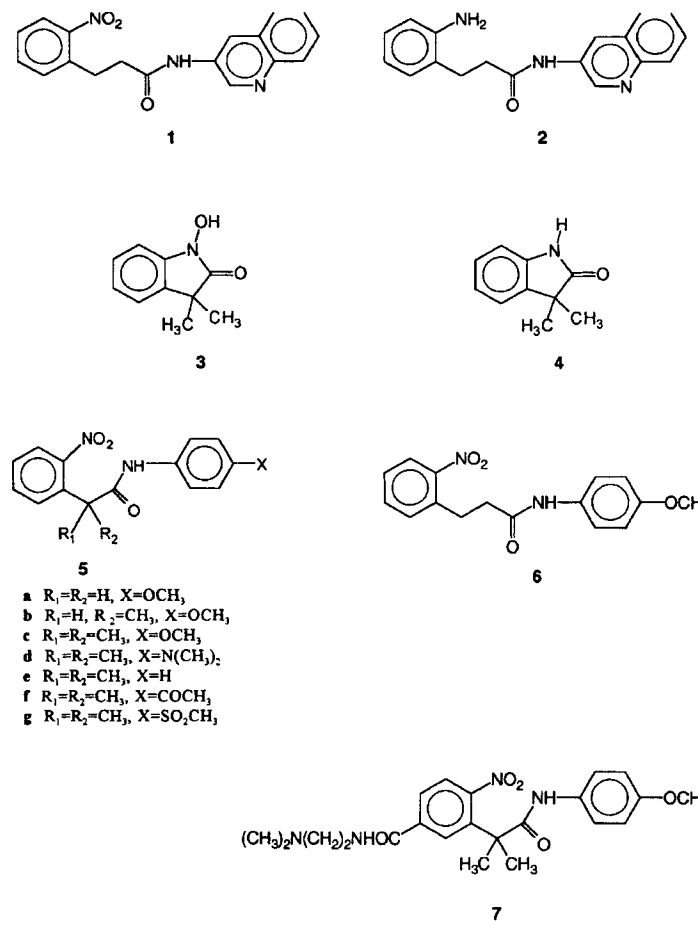
CCC 0894–3230/95/090587–10

Received 8 May 1995
Accepted 30 May 1995



Scheme 1. Reaction pathway for the reductively triggered internal cyclization of 2-(2-aminophenyl)acetamides



Structures 1–7. Structures of the nitroamides studied and of the lactams **3** and **4**

of model nitroamides. This method is particularly suitable for studying the chemical reduction of compounds which form unstable products, since γ-irradiation of aqueous solutions (in the presence of a scavenger of hydroxyl radicals such as propan-2-ol) generates one-electron reducing equivalents in a dose-dependent manner, with a precisely defined stoichiometry.[5]

EXPERIMENTAL

Radiolytic reductions. The radiolysis of water by a low linear energy transfer radiation source (such as ^{60}C γ-rays) produces approximately equal amounts of strongly oxidizing $^{\cdot}OH$ radicals and reducing e^-_{aq}. Totally reducing conditions may be achieved by addition of propan-2-ol, which converts $^{\cdot}OH$ radicals into the reducing radical $(CH_3)_2C^{\cdot}OH$. Oxygen must also be excluded from radiolytic reduction solutions, as it can act as an alternative e^-_{aq} acceptor. This method provides a convenient way of generating reducing species with precise stoichiometry.

Reductions were carried out in Pyrex glass vessels fitted with a B24 cone, to which an adapter containing a tap was fitted. In the case of spectrophotometric monitoring of reduction solutions, the vessels also had a side-arm to which a quartz cuvette was attached. Aliquots (5 ml) of aqueous buffer (0·020 mol l^{-1}) were pipetted into the radiolysis vessel, followed by addition of 193 µl of propan-2-ol (distilled) and the appropriate volume of a concentrated methanolic solution of the nitroamide. The mixture was then de-aerated by shaking continuously for *ca* 30 s while the vessel was evacuated via the opened tap. Temperature control of the radiolysis experiments was impractical, as there was no ready means of thermostating the solutions during irradiation, or while transporting the vessel from the source to the spectrophotometer or the HPLC system. All reactions were therefore carried out at room temperature.

The dose rate of the ^{60}Co source was measured as 0·995 J l^{-1} s^{-1} using an NaCl-modified Fricke dosimeter,[6] and this dose rate was corrected for the decay of the ^{60}Co source in subsequent radiolytic reductions. The time required to produce 1 mol equivalent of reducing radicals to a 50 µmol l^{-1} solution of nitroamide in 5 ml of aqueous buffer was then calculated from

$$t = n/(Gdv) \quad (1)$$

where t is the reduction time, n is the number of moles, G is the radiation chemical yield, taken as 0·62 µmol J^{-1} $[G(e^-_{aq}) + G(^{\cdot}H) + G(^{\cdot}OH)]$, d is the dose rate and v is the volume of the reduction solution.

Spectrophotometric and HPLC methods. Spectrophotometric monitoring was carried out using an HP8452A diode-array spectrophotometer. Radiolysis solutions were transferred to the attached quartz cuvette by appropriate positioning of the apparatus, maintaining anaerobic conditions throughout. Initial experiments involved monitoring the spectral change of the solutions over the wavelength range 190–600 nm with time, to detect isosbestic points and identify a suitable analytical wavelength. Subsequent kinetic measurements were then made using the kinetics software supplied with the instrument, with internal blanking of the absorbance at the analytical wavelength.

HPLC analysis involved injection of a 50 µl sample through a 20 µl sample loop. Assays were carried out on an Econosphere C-18 (particle size 5 µm) column (250 mm × 4·6 m.m. i.d.). Solvents were methanol and phosphate buffer (0·010 mol l^{-1}, pH 7·0) mixtures, and both isocratic and gradient systems were used. The HP1040M Series II detector permitted storage of the spectra of the eluent and retention times, aiding in the identification of chromatographic peaks.

*Preparation of 1,3-dihydro-3,3-dimethyl-1-hydroxy-*H*-indol-2-one* (**3**). 2-Methyl-2-(2-nitrophenyl)propanoic acid[1] (0·115 g) was added to a solution of NH_4Cl (0·05 g) in water, and EtOH was added slowly until all the nitro acid had dissolved. Zinc dust [1 g, activated by washing in HCl (0·1 mol l^{-1}) until gas evolution ceased, then washing well with water and drying] was slowly added to the stirred solution. After 2 h, during which time a white solid formed, the mixture was acidified by dropwise addition of HCl (10%, v/v) until the precipitate dissolved. The mixture was then filtered and the filtrate was extracted with EtOAc to give 1,3-dihydro-3,3-dimethyl-1-hydroxy-*H*-indol-2-one (**3**), m.p. 118–120 °C (lit.[7] m.p. 123–124 °C). IR (KBr disc): 3420 (br, OH), 3109 (m), 1685 (vs, C=O), 1618 (s, (*ortho*-disubstituted aromatic), 1467 (m), 1298 (aromatic C–), 1100 cm^{-1} (br, νN–O). Mass spectrum (CI):m/z (relative intensity, %) 177 (100, M^+), 132 (95), 132 (95), 134 (75), 117 (42).

Hydrogenation of **3** in MeOH (Pd/C, 57 psi, 1 h) gave a quantitative yield of 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (**4**); the spectral characteristics and HPLC retention times were identical with those of an authentic sample.[1]

RESULTS

Aqueous solutions of nitroamides **1**, **5a**, **5c**, **6** and **7** (in 5 ml of 0·020 mol l^{-1} phosphate buffer, pH 7·0) were radiolytically reduced for times corresponding to the addition of 1–8 stoichiometric equivalents of reducing radicals. The nitroamides were present at a concentration of 50 µmol l^{-1}, with the exception of **1**, whose low aqueous solubility necessitated the use of a 5 µmol l^{-1} solution. The compositions of the reduced solutions were investigated by HPLC. Plots of the peak areas of

species in solution with changing stoichiometry of reduction are depicted in Figure 1. A calibration plot of the concentration of the amino amide analogue of **6** (prepared by catalytic hydrogenation) against resultant peak area allowed the quantification of the amount of aminoamide formed in the radiolytic reduction of **6**. The maximum amount (13%) of aminoamide was formed on reduction with sixfold stoichiometry.

The fact that four equivalents of reducing radicals are needed to remove the nitroamide completely implies that the reduction proceeds directly to the hydroxylamine. The relationship between reduction stoichiometry and peak area also implies that the unknown reduction product (■) in each case is the hydroxylaminoamide. The reduction of **5c** was studied in detail, and the major reduction product was found to cyclize, releasing the coupled amine anisidine and forming the *N*-hydroxylactam, 1,3-dihydro-3,3-dimethyl-1-hydroxyindol-2-one (**3**) (see below). This provides further evidence that the major products formed in the radiolysis solutions of the nitro amides **1**, **5a**, **5c**, **6** and **7** reduced with fourfold stoichiometry are the corresponding hydroxylaminoamides, and they are referred to as such from now on.

The influence of oxygen on the reactions of the hydroxylamino amides of **5a–c** were investigated, and the results are presented in Table 1. Solutions of the nitroamides (50 $\mu mol\,l^{-1}$, 0·02 $mol\,l^{-1}$ phosphate, pH 6·8) were radiolytically reduced for the time required to deliver four equivalents of reducing radicals. Solutions were then either opened to the air and shaken, or maintained under vacuum, and monitored spectrophotometrically to determine an analytical wavelength (all spectral changes were found to pass through isosbestic points).

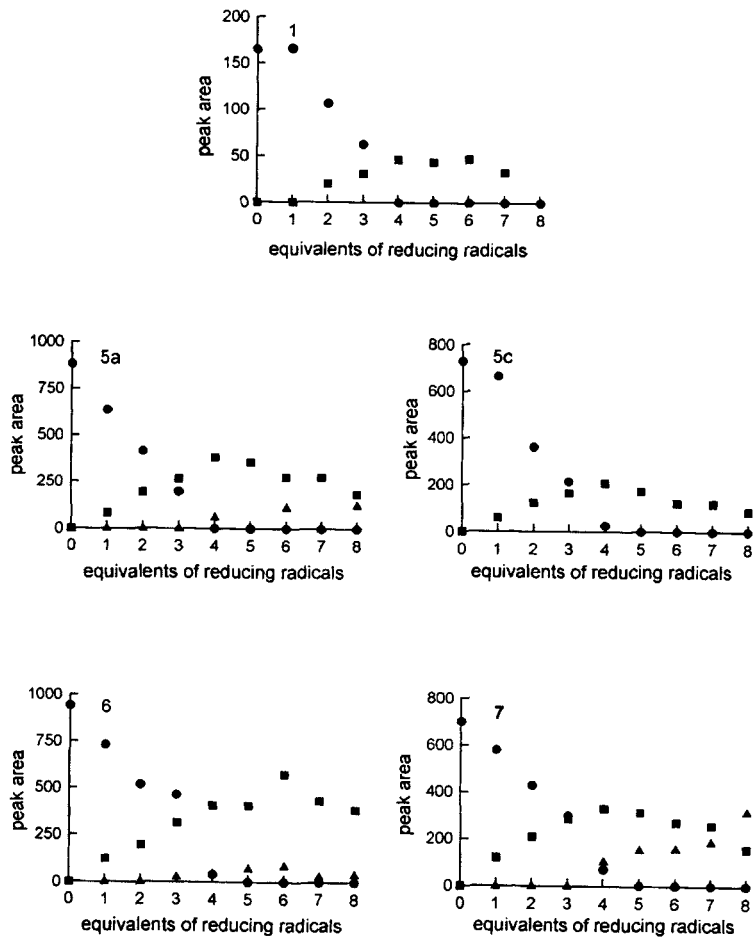


Figure 1. Compositions of radiolytic reduction solutions with changing stoichiometry of added reducing radicals, for compounds **1**, **5a**, **5c**, **6** and **7**. Peaks: ●, nitroamide; ■, unknown product, taken to be the hydroxylaminoamide; ▲, aminoamide

Table 1. Rates and products of the reaction of the hydroxylaminoamides of **5a–c** under aerobic and anaerobic conditions

Compound	Reaction conditions	k_{obs} (min^{-1})	Composition of final reaction solution (HPLC)	Anisidine released (%)
5a	Aerobic	0·021 ± 0·002 0·020 ± 0·001 0·013 ± 0·001	Unreacted **5a**; unknown product of retention time 17·2 min	0
	Anaerobic	Too slow to measure by UV–vis	Unreacted **5a**; aminoamide; hydroxylaminoamide; anisidine; unknown product of retention time 4·1 min	70
5b	Aerobic	0·0172 ± 0·002 0·029 ± 0·002 0·0216 ± 0·0008	Unreacted **5b**; unknown product of retention time 18·6 min	0
	Anaerobic	$(3·80 \pm 0·02) \times 10^{-3}$ $(4·29 \pm 0·08) \times 10^{-3}$ $(4·52 \pm 0·01) \times 10^{-3}$	Anisidine; unknown product of retention time 5·7 min	100
5c	Aerobic	0·079 ± 0·0002 0·111 ± 0·003 0·104 ± 0·005	Unreacted **5c**; anisidine; *N*-hydroxylactam **3**	90
	Anaerobic	0·27 ± 0·01 0·26 ± 0·01 0·215 ± 0·0051	Anisidine; *N*-hydroxylactam **3**	100

Measurements of the observed pseudo-first-order rate coefficient (k_{obs}) were carried out by monitoring the reactions at the analytical wavelength, with the absorbance change internally blanked against the absorbance at 500 nm (where no spectral change was observed). Reactions were carried out in triplicate. The anaerobic reaction of **5a** was too slow to allow spectrophotometric determination of k_{obs}, as the spectrophotometer had a 24 h limit for the continuous monitoring of a single reaction. Once the spectral change was complete, the compositions of the solutions were investigated by HPLC, using authentic samples of the corresponding aminoamides and lactams, anisidine and the *N*-hydroxylactam **3**. The percentage of anisidine released was determined from a calibration plot.

The pH dependences of the rates of cyclization of the hydroxylaminoamides **5c** and **7** were determined over the pH range 6–10, and these data are depicted in Figure 2. These compounds were selected because both possess *gem*-dimethyl substitution, which increases the rate of cyclization compared with the unsubstituted analogue **5a**. Radiolytic reduction of 50 μmol l^{-1} solutions of **5c** and **7** in buffers (0·020 mol l^{-1}), adjusted to various pH values, generated the corresponding hydroxylamines. bis-tris-propane{1,3-bis[tris(hydroxymethyl)methylamino]propane, $pK_1 = 6·8$, $pK_2 = 9·0$} was used for buffering in the pH ranges 5·9–6·4 and 8·5–9·8, and phosphate buffers were used in the intermediate pH range 6·3–8·0. The k_{obs} values of the subsequent anaerobic reactions were determined spectrophotometrically (in triplicate), and the compositions of the final reaction solutions were

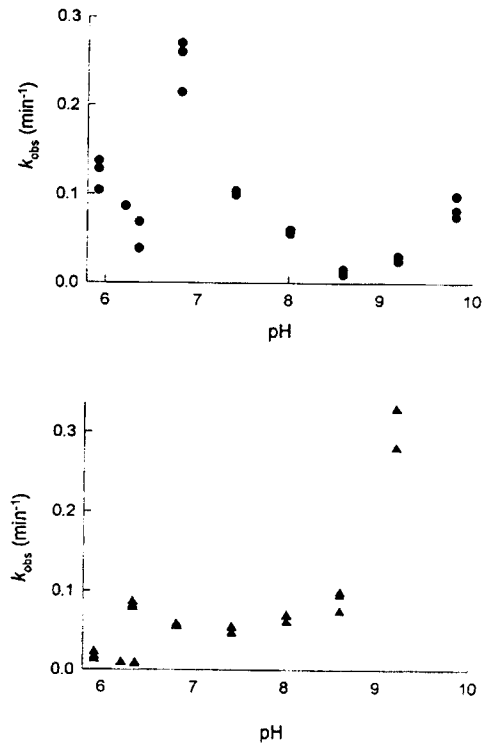


Figure 2. pH dependence of the cyclization of the hydroxylamines of (●) **5c** and (▲) **7**. Triplicate data are represented

investigated by HPLC. Calibration plots for anisidine under each assay condition were constructed and used to determine the amount of anisidine released, which was found to be close to 100% in each case.

The nature of the buffer species appeared to influence the magnitude of the observed pseudo-first-order rate coefficient. The rate coefficient for cyclization of the hydroxylamine of **5c** could not be measured at pH 6·3 in phosphate buffer, as the reaction was complete before the radiolysis solution could be transported to the spectrophotometer. However, at the same pH, a rate coefficient could be determined in bis-tris-propane buffer. For the less reactive analogue **7**, where both values could be determined, the k_{obs} for cyclization at pH 6·3 in phosphate was approximately ten times larger than in bis-tris-propane buffer (Table 2). Investigation of the pH dependences of these reactions was limited by constraints introduced by the radiolytic formation of the hydroxylamine. Reactions having k_{obs} values greater than *ca* 0·4 min^{-1} ($t_{1/2} = 2$ min) could not be effectively monitored, because the spectral changes were essentially complete by the time the radiolysis vessels could be transported to the spectrophotometer.

The influence of changes in the electronic environment of the leaving amine on the k_{obs} of the anaerobic cyclization of the hydroxylamines corresponding to **5c–g** was investigated spectrophotometrically at pH 7·4 (0·020 mol l^{-1} phosphate buffer), and the resulting Hammett plot is depicted in Figure 3. HPLC assays of the final reaction solutions revealed that the *N*-hydroxylactam **3** was formed in all reactions, together with a further product. In the case of **5c** this latter product was identified as anisidine, and it was assumed to be the corresponding 4-substituted anilines in the other cases.

Table 2. Dependence on buffer species of observed pseudo-first-order rate coefficient k_{obs} (min^{-1}) of anaerobic cyclization of the hydroxylamino amide of **7**

Phosphate buffer (pH 6·3)	Bis-tris-propane buffer (pH 6·3)
0·082 ± 0·006	0·0084 ± 0·0002
0·086 ± 0·004	0·0081 ± 0·0001
0·0788 ± 0·0005	0·00741 ± 0·00003

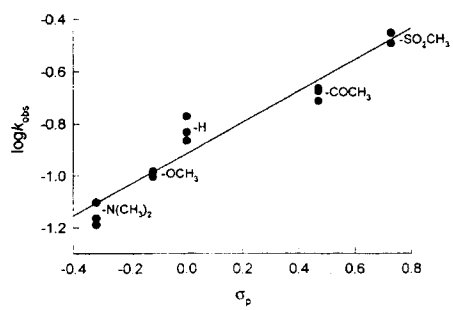


Figure 3. Hammett plot of logarithm of the observed pseudo-first-order rate coefficient of anaerobic cyclization versus the Hammett substituent parameter, σ_p, for the varying X substituents in the hydroxylamines formed from compounds **5c–g**. Triplicate data are presented. A linear regression yields a line of slope (ρ) = 0·60 ± 0·02 (± 1 s.d.) (r = 0·970)

DISCUSSION

Identification of the major reduction product

The stoichiometry of the reactions of the nitroamides induced by radiolytic reduction (controlled addition of stoichiometric equivalents of reducing species) implicate the hydroxylamine as the predominant reduction product. Reduction to the hydroxylamine was observed for nitroamides varying in leaving group (**1**, cf. **6**), alkyl chain length (**5a**, cf. **6**), methyl substitution of the alkyl chain (**5a**, cf. **5c**) and substitution on the nitrophenyl ring (**5c**, cf. **7**). Small amounts of aminoamide formation were detected in some experiments, but no other reduction products were observed. The identity of the major product of radiolytic reduction as the hydroxylamine was verified for **5c**, by identification of the *N*-hydroxylactam formed from its subsequent cyclization. Nucleophile attack by the deprotonated oxygen of the hydroxylamine on an amide has been reported.[8] In contrast, the internal cyclization of the hydroxylamine of **5c** proceeded via the neutral form of the hydroxylamine through nucleophilic attack by nitrogen.

Effect of pH and of buffer species

The effect of pH was investigated over the range 6–10 (Figure 2), but no consistent trends were seen. The nature of the buffer species appeared to influence the magnitude of the observed pseudo-first-order rate coefficient, with cyclizations being much more rapid in phosphate than in bis-tris-propane buffer. However, detailed studies were limited by the requirements of the radiolytic reduction technique.

Effect of the presence and absence of oxygen

The fate of the hydroxylamine appears to be dependent on the presence of oxygen (Table 1). Under anaerobic conditions the hydroxylamines of **5a–c** cyclized, releasing anisidine and a further product (which was identified as the *N*-hydroxylactam **3** in the case of the reaction of **5c**, and is assumed to be the corresponding *N*-hydroxylactam in the case of **5a** and **b**). The anaerobic reaction of **5a** was too slow to determine spectrophotometrically, and the time for completion of the reaction is an estimate. HPLC showed that, even

after 6 days, unreacted hydroxylamine was still present. The release of only 70% of the theoretical amount of anisidine (Table 1) from this solution therefore reflects the fact that hydroxylaminoamide cyclization had not gone to completion, rather than being restricted by a competing reaction.

However, in air no cyclization products were observed in the reduction of **5a** and **b**, with a single species of longer HPLC retention time and more complex UV spectra being formed in each case instead. The rate coefficients of the formation of these species from the hydroxylamines of **5a** and **b** in air are equal within the accuracy of the measurements (average value of $k_{obs} \pm 1$ s.d. of **5a** $= 0{\cdot}018 \pm 0{\cdot}003$ min^{-1}, and for **5b** $= 0{\cdot}023 \pm 0{\cdot}004$ min^{-1}). It is likely that these species are the corresponding azoxybenzenes, formed by oxidation of the hydroxylamines to the nitroso compounds, followed by condensation with further hydroxylamine. The oxygen-dependent formation of azoxybenzene from *N*-phenylhydroxylamine in phosphate buffer is known to occur,[9] and we have recently reported[10] azoxy formation during radiolytic reduction of a dinitrobenzene derivative.

In comparison, the hydroxylamine of **5c** (which possesses a partial trimethyl lock) cyclized to completion under both anaerobic and aerobic conditions. These observations can be understood in terms of competitive cyclization and dimerization reactions. As is the case with cyclization of the corresponding amino amides,[1,3] the rates of cyclization of the hydroxylamino amides are heavily influenced by methyl substitution in the linker chain. For example, the relative rates of cyclization (under anaerobic conditions) for the monomethyl and *gem*-dimethyl-substituted compounds **5b** and **c** are 1:23 at pH 6·8 and room temperature (Table 1). A comparison with the unsubstituted derivative (**5a**) was not possible, because the latter cyclized too slowly to allow spectrophotometric determination of the rate coefficient; however, the relative k_{obs} values for cyclization of the amino amide analogues of **5b** and **5c** have been measured as 189 (pH 6·6, 37 °C).[3]

The rates of aminoamide and hydroxylaminoamide cyclization reactions are therefore similarly influenced by alterations in the stereochemistry of the link between the nucleophilic and electrophilic centres. The *gem*-dimethyl derivative **5c** cyclizes so rapidly that, even in the presence of oxygen, this reaction prevails. However, for the much slower cyclizing derivatives **5a** and **b** under aerobic conditions, the dimerization reaction prevails.

Comparison of rates of cyclization between amino- and hydroxylaminoamides

Table 3 shows a comparison of the k_{obs} values for cyclization of the amino- and hydroxylaminoamides derived from **5c**, together with those derived from the related carboxamide-substituted analogue **7**. These rate coefficients have not been corrected for protonation, because at pH 6·8 both the amine and hydroxylamine groups will be essentially unprotonated. The reactions of amines and hydroxylamines were necessarily carried out at different temperatures; as noted above, the radiolytic reductions have to be conducted at ambient temperature, which was determined as 22 °C for these experiments. Assuming that the rate of reaction will approximately double with a 10 °C rise in temperature, the k_{obs} values for cyclization of the hydroxylamines should be increased approximately 2·5-fold to estimate the rates at 37 °C. If this is done (Table 3), the nitroamides **5c** and **7** are seen to cyclize more rapidly via the hydroxylamines by factors of approximately 160- and 65-fold, respectively.

This provides an explanation as to why the reductive cyclization of the nitroamide **1** reported by Entwistle,[4] which presumably occurred via the hydroxylamine, is faster than the cyclization we observed for the corresponding aminoamide **2**. The increased rate of cyclization of the hydroxylaminoamides over the aminoamides cannot be attributed to a greater basicity of the latter, as the pK_a of phenylhydroxylamine is *ca* 1·5 units lower than that of aniline. However, the

Table 3. Comparison of the rates of cyclization of the hydroxylamino and amino derivatives of compounds **5c** and **7** at pH 6·8

Temperature (°C)	Hydroxylamino		Amino	
	k_{obs} (min^{-1})	$t_{1/2}$ (min)	k_{obs} (min^{-1})	$t_{1/2}$ (min)
22	0·25[a]	2·79[a]		
37		1[a,b]	0·0096[a]	72[a]
22	0·056[c]	12·3[c]		
37		5[b,c]	9×10^{-4} [c]	770[c]

[a] Compound **5c**.
[b] Half-life at 37 °C estimated from value measured at 22 °C (see text).
[c] Compound **7**.

increased reactivity of the hydroxylamino amides may be rationalized in terms of the 'alpha effect,' a general term applied to compounds which possess an electronegative atom with a free electron pair adjacent to the nucleophilic site.[11] While there are many factors which can influence nucleophilicity in such compounds, the most important is considered to be the increase in effective electron density caused by the free electron pair. In the present case, the deficiency in electron density created at the nitrogen of the hydroxylamine on formation of the transition state can be stabilized by electron donation from the lone pairs of the hydroxylamine oxygen.[12] Such stabilization is not available during aminoamide cyclization.

Effect of changes in the electronic environment of the leaving amine

Among the set of compounds **5c–g** possessing different *para*-substituents on the leaving aniline, electron-withdrawing substituents increase the k_{obs} values for hydroxylaminoamide cyclization ($\rho = 0{\cdot}60 \pm 0{\cdot}02$; Figure 3). In contrast, no such effects were observed on the rates of cyclization of the corresponding aminoamides.[1,3] Such an alteration in structure–activity relationships with changing nucleophile suggests a change in the rate-determining step of the cyclization reaction, assuming that the reactions proceed by the same mechanism.

Possible mechanisms for cyclization of hydroxylaminoamides

Kirk and Cohen[13] discussed potential mechanisms for the cyclization of aminoamides, by analogy with the conversion of phthalic acid to phthalic anhydride.[14] Two kinetically equivalent mechanisms were considered, and the hydroxylaminoamide analogues of these pathways are depicted in Figure 4. A four-centre process, bypassing the tetrahedral intermediate (Figure 4, path A) may be rejected, since there is no obvious route for (the observed) buffer catalysis in this mechanism. A mechanism involving protonation of the amide nitrogen (Figure 4, path B) may also be rejected, as the reaction conditions employed are not expected to afford significant protonation of the amide.

More plausible reaction mechanisms involve the formation and breakdown of a tetrahedral intermediate in the reaction path. These can explain the effects of stereochemical changes brought about by methyl substitution in influencing the rate, through the ring-forming equilibria involved in forming the tetrahedral intermediate. No detailed information as to the nature of catalysis is available, and therefore mechanisms requiring differing forms of acid and base catalysis cannot be distinguished. The simplest option simply involves a change in the rate-determining step in the mechanism previously proposed[3,13] for aminoamide cyclization, and this is depicted in Scheme 2.

This mechanism is consistent with several experimen-

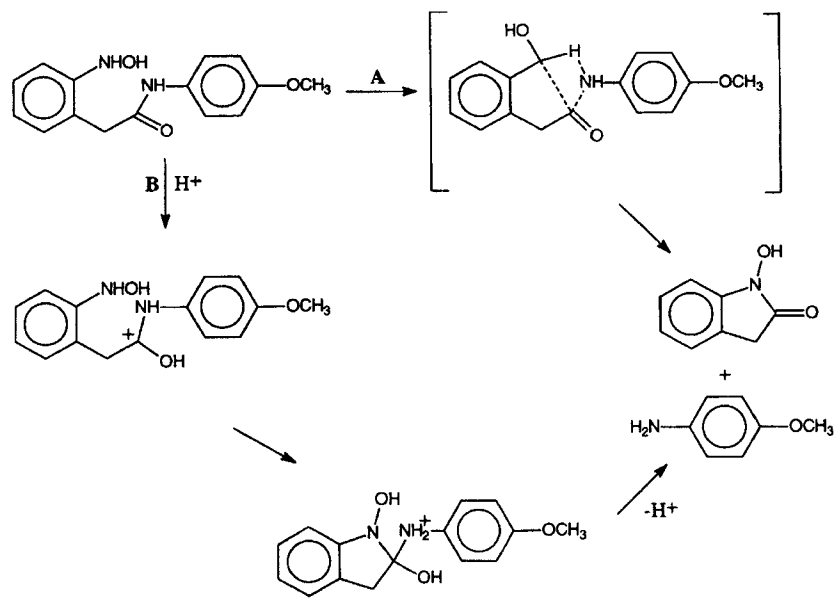
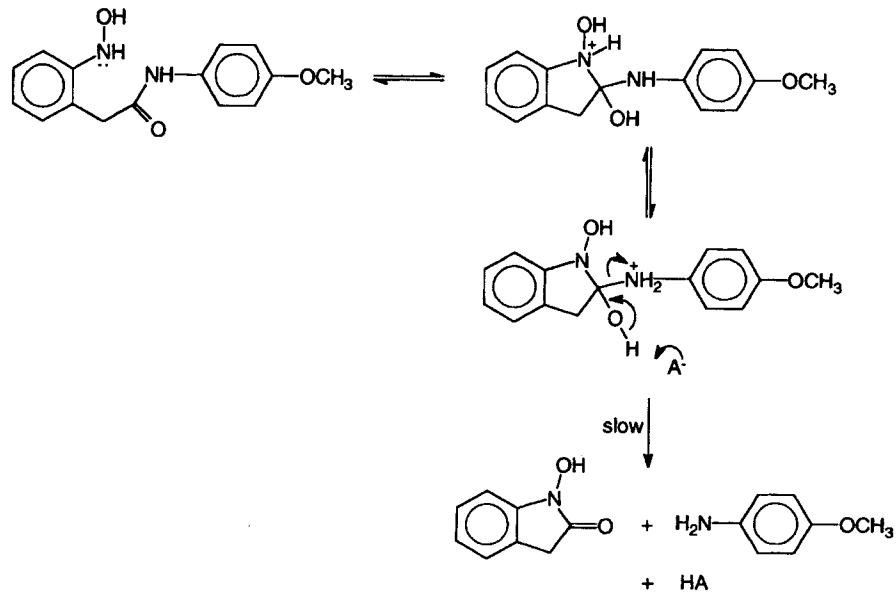


Figure 4. Schematic representation of potential reaction pathways for hydroxylaminoamide cyclization, by analogy with the conversion of phthalic acid into phthalic anhydride.[13] The dotted lines show the incipient bonds



Scheme 2. A potential mechanism for hydroxylamino amide ring closure, involving a change in rate-determining step from that proposed for aminoamide ring closure. This mechanism depicts rate-determining general base-catalysed breakdown of the tetrahedral intermediate

tal observations. In addition to explaining the rate-enhancing effect of methyl substitution, it also accounts for the greater reactivity of hydroxylamino versus amine species. Stabilization of the first transition state can occur through delocalization of the positive charge generated on the hydroxylamine nitrogen by the lone pair of electrons on the oxygen. The concomitant change in the rate-determining step, from formation of the tetrahedral intermediate to its breakdown, accounts for the observed dependence of the hydroxylaminoamide cyclization on the nature of the leaving group. The rate-determining step now involves expulsion of the leaving amine. Substitution of the leaving amine with electron-withdrawing substituents is expected to facilitate this step.

Consequences for prodrug design

It was noted above that previous studies[1,3] of the kinetics of cyclization of 2-aminoarylamides failed to identify a system suitable for use as a bioreductive prodrug, because of the slow rates of cyclization of these compounds under physiological conditions. The demonstration that the hydroxylamino intermediates, which will certainly be formed by cellular nitroreductases, cyclize up to two orders of magnitude more rapidly is of major significance. Thus the half-lives for cyclization of the hydroxylamino derivatives of **5c** and **7** are estimated to be approximately 1 and 5 min, respectively, under physiological conditions (Table 3), with at least that of the former probably being in the acceptable range.

Electron-withdrawing substituents on the nitrophenyl ring (necessary if this concept is to be used as a prodrug for bioreductive release of mustards, to raise the reduction potential of the drug into the range above *ca* −450 mV[15]) do slow the rate of cyclization, but not by a large amount (*ca* fivefold between the hydroxylamino derivatives of **5c** and **7**). Oxygen-dependent reactions (possibly dimerizations) of the hydroxylamines do not significantly compete with the cyclization route for the more reactive compounds (e.g. those containing a *gem*-dimethyl moiety), and are thus unlikely to be a problem.

Modest increases in the rate of cyclization can be effected by substitution of the leaving amine with electron-withdrawing substituents. Within the series of *para*-substituted anilines **5c–g** studies, the rates of cyclization can be estimated to vary about tenfold, from a $t_{1/2}$ of *ca* 0·8 min for the 4-SO_2Me derivative **5g** ($\sigma_p = +0{\cdot}72$) to *ca* 10 min for the 4-NMe_2 derivative **5d** ($\sigma_p = -0{\cdot}82$) under physiological conditions (pH 7, 37 °C). However, increasing the rate of cyclization by this approach may not be desirable, because the use of electron-withdrawing substituents will also lower the cytotoxicity of the released mustard. The best avenue for further increasing the rates of cyclization appears to be by control of the geometry of the prodrug; other

work[16] suggests that application of a full 'trimethyl lock' would result in substantial increases in rate.

ACKNOWLEDGEMENTS

This work was supported by the Auckland Division of the Cancer Society of New Zealand and the Health Research Council of New Zealand. Equipment grants from the Research Committee of the University of Auckland and from the New Zealand Lotteries (Science) Committee are gratefully acknowledged.

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