Cyclohepta-amylose-catalysed Hydrolysis of 2-Oxo-4,4,5,5-Tetramethylimidazolidin-1-oxyl and of Related Carbamoyl and Ester Nitroxides. Nitroxide as Spin Label and Activating Group

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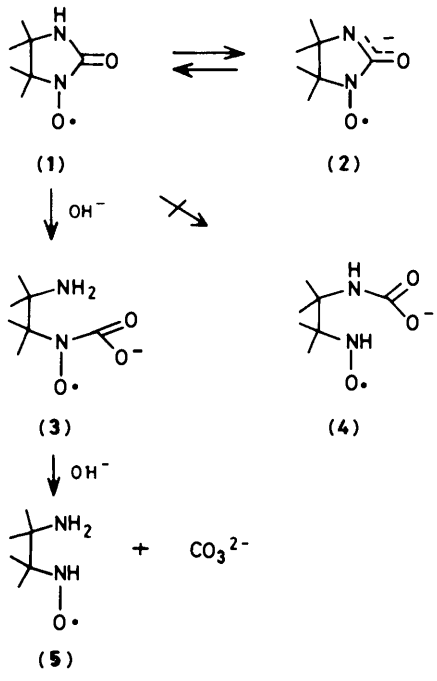
Base hydrolysis and cyclohepta-amylose-catalysed hydrolysis of 2-oxo-4,4,5,5-tetramethylimidazolidin-1-oxyl (**1**), its thiocarbonyl analogue (**6**), the carbamoyl nitroxides (**7a**—**c**), and the ester nitroxide (**8**) were studied by e.s.r. spectroscopy. On base hydrolysis in the absence of cyclohepta-amylose, the intermediate radical anions $RN(-O^{\bullet})CO_2^-$ (R = Bu^t and $NH_2CMe_2CMe_2-$) were detected before decarboxylation. Hydrolysis of the amide group of the carbamoyl nitroxides is faster than that of 4,4,5,5-tetramethylimidazolidin-2-one (**9**) and therefore there is activation by the nitroxide group. For rate-determining nucleophilic addition to the carbonyl group, values of $k_{e.s.r.}$ correlate inversely with a_{N-1} values, and this is explained in terms of the predominant activating inductive effect of the nitroxide group. When the ester (**8**) reacted, the carbonyl nitroxide group was hydrolysed before the ester group. Complexing of the compounds (**1**), (**6**), (**7a**—**c**), and (**8**) with cyclohepta-amylose caused anisotropic effects in the e.s.r. spectra, reduced a_{N-1} values, and increased rates of hydrolysis. These effects were greatest for carbamoyl nitroxide (**1**). In the cyclohepta-amylose-catalysed reaction, cycloamylose alkoxide ion acts as nucleophile but no intermediates are detected since both bonds of the carbamoyl and ester nitroxide systems are rapidly hydrolysed.

Most of the ionic reactions of nitroxide radicals that have been studied involve sites remote from the nitroxide group. There are few exceptions, such as reactions in which the nitroxide behaves as a Lewis base towards protons [1] and metal ions,[2] and reactions that occur at the position of conjugated nitrone [3] and imino [4] nitroxides. The number of such reactions is limited because of the necessity of having complete α-substitution in unconjugated nitroxides, to prevent decomposition through H-transfer.[5] Therefore the use of nitroxides as spin labels [6] derives mainly from measurement of the extent of a reaction or molecular interaction in terms of anisotropic–motional effects on the e.s.r. spectra. Rarely has e.s.r. spectroscopy been used to measure electronic effects in ionic reactions of nitroxides.

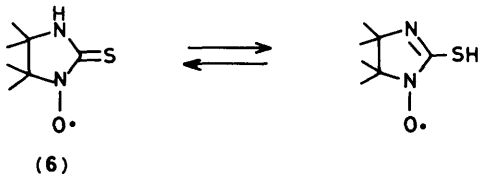
Reactions of the carbamoyl nitroxide (**1**) have interested us, since e.s.r. spectroscopy reveals the electronic effects of the nitroxide group on its hydrolysis, and on tautomerism [7] of the thiocarbonyl analogue (**6**). We have published preliminary evidence [8] that hydrolysis of the amide group in (**1**) is accelerated by binding with cyclohepta-amylose; and that the spin label reports not only on the anisotropic effects due to binding, but also on electronic effects and structures of intermediates. In this paper we compare similar hydrolyses of acyclic carbamoyl nitroxides and of an ester nitroxide in order to evaluate the influence of the radical centre as an activating group in these ionic reactions.



Scheme 1.

Results and Discussion

Intermediates in the Uncatalysed Hydrolysis of Carbamoyl Nitroxides.—At pH 13 (70 °C) the e.s.r. spectrum of the radical anion (**2**) (a triplet of triplets) [4] disappeared at a first-order rate, and a transitory spectrum appeared, which consisted of three equal lines with coupling constant 13.8 G. The neutral radical (**1**), rather than its anion (**2**), is expected to react [9] (Scheme 1), and the formation of two radicals, (**3**) and (**4**), must be considered. Radical (**3**) would show hyperfine coupling with one nitrogen atom to give a triplet spectrum as observed, and it should also be long lived by analogy with the known radical anion $MeN(-O^{\bullet})CO_2^-$.[10] Radical (**4**), however, would show coupling for one nitrogen and one hydrogen atom and would have a short lifetime. Radical anions of the form $RN(-O^{\bullet})CO_2^-$ (though not that with R = Bu^t) have been generated by carbonation of hydroxylamines in potassium carbonate buffer followed by oxidation with ferricyanide. We attempted to prepare the *N*-t-butyl radical anion (**10**) (Scheme 2) by this method, for comparison with the radical anion produced during hydrolysis of nitroxide (**1**), but found that the carbonation did



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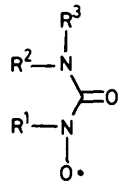
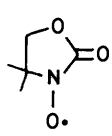


(7)

a; $R^1 = R^2 = Bu^t$, $R^3 = H$
b; $R^1 = Bu^t$, NR^2R^3 = pyrrolidinyl
c; $R^1 = R^2 = Ph$, $R^3 = H$



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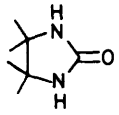

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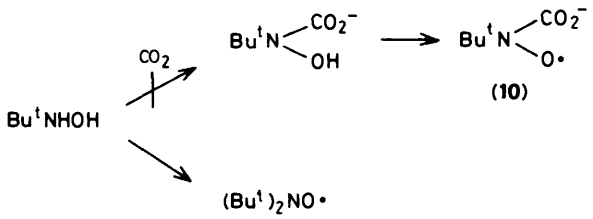


(10)

Scheme 2.

not take place and only di-t-butyl nitroxide was obtained on oxidation. However, both *N,N'*-di-t-butylcarbamoyl nitroxide (**7a**) and *N*-t-butyl-*N*-chorocarbonyl nitroxide, on hydrolysis at pH 13, gave a spectrum that we assign to the radical anion (**10**). On hydrolysis of the ester nitroxide (**8**) a spectrum tentatively assigned to the radical anion (**16**) was obtained as described below.

The collected e.s.r. data for the *N*-carboxylate radical anions are shown in Table 1. The a_N values are lower than that of di-t-butyl nitroxide. Compared with the carbonyl nitroxide, *N*-acetyl-*N*-t-butyl nitroxide (**12**), the a_N values are higher and *g* values lower (the latter in spite of the possibility of delocalisation on to an additional oxygen atom).

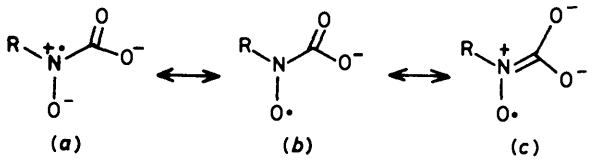
The resonance form $RN^+(-O^\cdot)=C(-O^-)R$ has been shown to be responsible for the low spin on the nitrogen atom in carbonyl nitroxides.[14] It is therefore probable that the analogous form (*c*) for the carboxylate nitroxides (Scheme 3) is less important than form (*a*). Form (*a*) would cause the observed higher a_N and lower *g* values as compared with carbonyl nitroxides, by shift of spin from oxygen to nitrogen.

It can also be seen from Table 1 that the inductive effect of the *N*-substituent R increases a_N by favouring form (*a*). The opposite effect has been reported for electron-withdrawing

Table 1. E.s.r. spectroscopic data of carboxylate, and related, nitroxides in aqueous solution

Radical		a_N/G	*g*
$Me_3CN(-O^\cdot)CMe_3$	(**11**)	17.2[a]	2.0056[b]
$Me_3CN(-O^\cdot)COMe$	(**12**)	8.0	2.0068[c,d]
$Me_3CN(-O^\cdot)CO_2^-$	(**10**)	14.7	2.0055
$Me_2CHN(-O^\cdot)CO_2^-$	(**13**)	11.2	2.0060[e]
$MeN(-O^\cdot)CO_2^-$	(**14**)	10.8[f]	
$HN(-O^\cdot)CO_2^-$	(**15**)	9.2[f]	
	(**3**)	13.8	2.007[g]
	(**16**)	15.8	2.0058

[a] Reference 11*a*. [b] Reference 11*b*. [c] In benzene. [d] Reference 12. [e] Reference 13. [f] Reference 10. [g] ±0.001 Owing to overlap of spectra.



(*a*) (*b*) (*c*)

Scheme 3.

substituents to a nitroxide group[15] and for protonation of α-substituents.[16] The large increase in a_N for the radicals (**3**) and (**16**), as compared with that for the isopropyl nitroxide (**13**), is probably due to hydrogen-bond donation from the amino and hydroxy groups to the oxygen atom of the nitroxide group, again favouring form (*a*).

Hydrolysis of the Thiocarbamoyl Nitroxide (**6**).—The thiocarbamoyl nitroxide (**6**) hydrolysed at approximately one-tenth the rate of its oxygen analogue (**1**) (Table 2). The sulphur compound exists mainly in the ene-thiol form,[7] which would undergo slower nucleophilic addition. During hydrolysis no three-line spectrum was observed for a thiocarbonyl analogue of the radical anion (**3**). Since it would not be expected to decarboxylate much slower than (**3**), it is assumed that its steady-state concentration is not high enough for detection.

Hydrolysis of the Ester Nitroxide (**8**).—This radical decomposed at a significant rate at neutral pH. The first-order rate increased with pH (Table 2) indicating that the reaction involved was hydrolysis even though no triplet spectrum for intermediate anion (**16**) was observed during this hydrolysis. This suggested that the carbonyl–nitrogen bond is hydrolysed before the ester group. Occasionally a triplet spectrum of equal lines (a_N = 15.8 G) appeared after *ca.* 12 h. This spectrum may tentatively be assigned to the radical anion (**16**), as discussed above for Table 1, and the observations accounted for by the reaction Scheme 4.

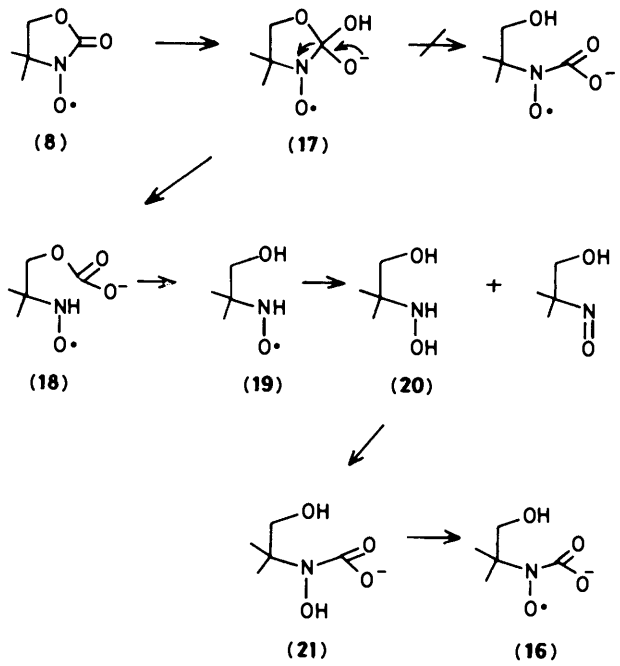
If the carbonyl–nitrogen bond is hydrolysed first, then this reaction, by analogy with amides, proceeds *via* slow addition of the nucleophile. The intermediate (**17**) is therefore not observed since it opens rapidly to the reactive radical (**18**). There follows hydrolysis of the ester group. Slow appearance of the radical anion (**16**) is possible, by disproportionation of the radical (**19**) produced by this hydrolysis, to give the hydroxylamine (**20**) and nitroso compound. Slow carbonation of the hydroxylamine[10] gives the carboxylate anion (**21**) which would be oxidised by air to the radical anion (**16**) finally observed.

Factors Affecting Rates of Hydrolysis.—The urea (**9**) did not hydrolyse under the conditions used for the carbamoyl nitroxide (**1**): it was recovered quantitatively. Therefore the

Table 2. Effects of cyclohepta-amylose on e.s.r. spectra and hydrolysis rates of nitroxides

Compound	$10^{11}\ \tau_c$[a]/s	$a_N(H_2O)$/G	$a_N(H_2O$–cycloa)/G[a]	$10^3\ k_{OH}$[b]/s^{-1}	$10^3\ k_c$[b]/s^{-1}	Hydrolysis conditions: Temperature/°C	Hydrolysis conditions: pH
(1)	65	10.3	9.7	14.5	80.2	70	13
					60.1[c]	70	13
					25.1[d]	70	13
(6)	57	9.7[e]	9.4[e]	0.7	3.1	70	12
(7a)		10.8[f]		16[g]	*h*	20	13
(7b)	39	13.0	12.7	1.9	3.6	70	13
(7c)		9.6		*h*	*h*	20	13
(8)	16	8.2	8.1	1.8	1.9	20	7
				7.4		20	8
				67		20	9
				h		20	10

[a] Measured for 10^{-3}M radical and 5×10^{-3}M cycloamylose. [b] ±15%. This does not take into account uncertainty in the measurement of pK_a values used for correction: pK_a (1) = 11.3, pK_a (6) = 12.0 ± 0.2. [c] Radical, 10^{-3}; cycloamylose, 0.7×10^{-3}; (9), 10^{-3}M. [d] Radical, 10^{-3}; cycloamylose, 0.7×10^{-3}; (9), 2.5×10^{-3}M. [e] Ene-thiol form. [f] In ethanol. [g] Not corrected for ionisation. [h] Too fast to measure.



Scheme 4.

amide group of the radical is activated by the nitroxide group. The hydrolysis and the e.s.r. spectrum of this radical were compared with those for the open-chain carbamoyl nitroxides (7a—c), to see if the spectra would make it possible to account for the activating effect.

A strong electron-withdrawing inductive effect on the 2-position is known for similar nitrone nitroxides,[17] and the electron-donating resonance effect of the nitroxide group operates in acyl nitroxides, where an analysis of spin distribution has shown the importance of the canonical form $R\overset{+}{N}(-O^{\cdot})=C(-O^{-})R$.[14] In the cyclic nitroxide (1), the resonance effect due to coplanarity of the nitroxide and amide groups is evident from the large a_{N-3} value (2.0 G) produced by spin on C-2 and N-3. In contrast, the open-chain nitroxides (7a—c) have higher a_{N-1} and lower a_{N-3} values (Table 2). The spectra of similar open-chain radicals have been shown to be consistent with a conformation in which the amide group is *not* coplanar

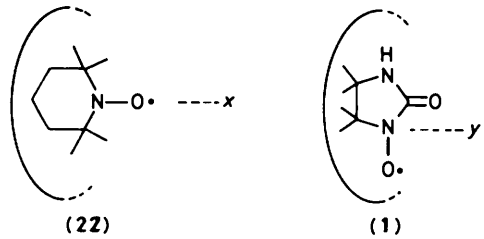


Figure. Complexes of radicals (1) and (22)

with the nitroxide group.[18] This conformation reduces the resonance contribution of the form $R\overset{+}{N}(-O^{\cdot})=C(-O^{-})R$ and increases a_{N-1}.

The resonance effect of the nitroxide group is therefore reduced in the open-chain compounds, which have generally higher rates of hydrolysis compared with (1) (Table 2).* Also, the increase in rate in going from (9) to (1) shows that in contrast to the NH group, the inductive effect of the nitroxide group rather than the resonance effect determines the nucleophilicity of C-2.

The relation between electronic effects and a_{N-1} values may be analysed as follows. The coupling constant for N-1 is given by the equation (1).[20] A similar equation may be written for

$$a_{N-1} = Q^{N}_{N-1}\rho_{N-1} + Q^{N}_{ON}(1 - \rho_{N-1} - \rho_{N-3}) \quad (1)$$

N-3, and since Q_{N-1} is 10—20 times as great[21] as Q^{N}_{ON}, the following approximations in equations (2) and (3) hold.

$$a_{N-1} \simeq Q^{N}_{N-1}\rho_{N-1} \quad (2)$$

$$a_{N-3} \simeq Q^{N}_{N-3}\rho_{N-3} \quad (3)$$

Spin on the oxygen atoms in these carbamoyl nitroxides must be low, considering the much higher total spin on nitrogen atoms as compared with cyclic (for 2-oxo-5,5-dimethyl-

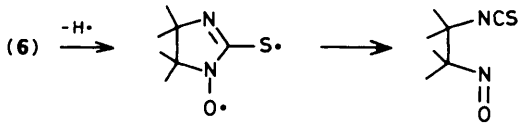
* The higher rates of hydrolysis of the open-chain compounds discount the importance of strain in the five-membered ring of compound (1) in increasing rate by lowering amide resonance or favouring formation of the intermediate with tetrahedral carbon. Of these two effects, only the latter is considered significant for the relatively high rates of hydrolysis of the more strained four-membered lactam ring of penicillins.[19]

pyrrolidin-1-oxyl, $a_N = 6.5$[22]) or open-chain carbonyl nitroxides. This means that the C=N (nitrone) bond order is low, and this includes the cyclic nitroxide (**1**) in spite of its planarity. In this molecule the relatively high a_{N-3} value of 2.0 G is due to spin on C-2 and N-3 and the a_{N-1} value may be normalised to a_{N-1} for the open-chain compounds by the factor $(a_{N-1} + a_{N-3})/a_{N-1}$, to give the value 12.3 G. Then, the lower the value of a_{N-1} for these carbamoyl nitroxides, the higher the rate of nucleophilic addition to the carbonyl group (Table 2): k_{OH} (**7c**) > (**7a**) > (**1**) > (**7b**).

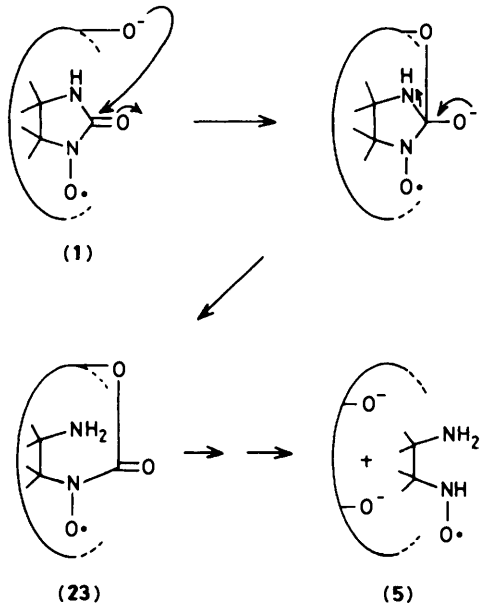
This supports the conclusion above, that the inductive effect of the nitroxide group is the important rate factor. In the open-chain carbamoyl nitroxides, there is obviously little conjugation between the nitroxide and amide groups. Variations (9.6—13 G) in the large nitroxide coupling constants must be due to redistribution of the unpaired spin density within the nitroxide group, brought about by inductive effects of substituents (Ph and Bu^t) and polar effects depending on the overall molecular conformation. The latter would also depend on substituents on the amide nitrogen.* The greater the neutralisation of charge on nitrogen in the resonance form $R_2\overset{+\cdot}{N}-O^-$, the greater is the spin density on the nitrogen atom. Correspondingly, the strong electron-withdrawing inductive effect of the nitroxide group is reduced and the rate of hydrolysis lowered.

The low a_N value for the ester nitroxide (**8**) also corresponds to a high rate of nucleophilic addition (Table 2).

Complexing by Cyclohepta-amylose.—The dissociation constant (5.0×10^{-4}) for the complex between the cyclic carbamoyl nitroxide (**1**) and cyclohepta-amylose is comparable to that for 2,2,6,6-tetramethylpiperidino-oxyl (**22**) (6.0×10^{-4}). This is surprising in view of the polar groups in radical (**1**): a 4-carbonyl group, for example, increases[24] the dissociation constant of the piperidino-oxyl to 6×10^{-3}. The tetramethyl grouping in (**1**) may give a better fit than the wider hydrophobic group (ring and methyls) of the piperidino-oxyl. There is, however, evidence that the NH and nitroxide groups have a positive effect on binding. It was observed that the value of the rotational correlation time τ_c, a measure of immobilisation, was greater for radical (**1**) ($\tau_c = 65 \times 10^{-11}$ s) than for the piperidino-oxyl ($\tau_c = 32 \times 10^{-11}$ s) under conditions of saturation by the cycloamylose. This effect was not inherent in the measurements from the more complex spectrum of the carbamoyl nitroxide, since in glycerol, which produced the same degree of anisotropy as cycloamylose in the spectrum of the piperidino-oxyl, the same τ_c value was obtained for both radicals. The more pronounced anisotropic effect for the carbamoyl nitroxide complexed with cycloamylose is therefore probably due to anisotropic motion. The piperidino-oxyl radical (**22**) complexes as shown (Figure) and can rotate about the *x*-axis, defined as that of the N–O bond by the usual notation for these compounds.[25] The *y*- and *z*-components of the *a* and *g* values are therefore averaged out. When nitroxide (**1**) complexes, rotation takes place about the C=O bond, parallel to the *y*-axis, to that the *x*- and *z*-components are averaged out.† If, however, this motion were restricted, then there would be an increase in τ_c, since it is strongly affected by non-averaging of the *z*-component. This may occur by hydrogen-bonding between the NH and nitroxide groups and the secondary hydroxy groups of the cycloamylose.



Scheme 5.



Scheme 6.

The thio-radical (**6**) is indefinitely stable in neutral aqueous solution when complexed with cycloamylose, although it is normally short-lived when prepared in water. The cycloamylose protects it probably from reaction with another molecule of the radical. It is longer-lived in deuterium oxide than in water, which points to transfer of the amide hydrogen atom to the nitroxide group of another radical as in Scheme 5.‡ Such transfer would take place more readily from the thio group of the ene-thiol form than from the NH group of the (more stable) amide (**1**). The more rapid decomposition in water, as compared with hydrocarbon solvents where it also exists as the ene-thiol,[7] may be due to greater self-association.

Hydrolysis of Carbamoyl Nitroxide (**1**) *in the Presence of Cyclohepta-amylose and Base.*—In the presence of excess of cyclohepta-amylose at pH 13, the e.s.r. spectrum of the radical anion (**2**) disappeared at a first-order rate, which is six times greater than in the presence of base only. Since the cycloamylose is present in large excess, it may be assumed that only the complexed nitroxide reacts. The anion (**2**) does not react, and the observed rate constant, measured from disappearance of the anion, was adjusted to the correct rate constant for reaction of the nitroxide using the pK_a value of the nitroxide. There was no rate increase when cyclohexa-amylose was used instead of cyclohepta-amylose, since the smaller cycloamylose does not complex with the radical.[8] On addition of a urea (**9**), which would be expected to complex with cyclohepta-amylose, the rate increase was less (Table 2).

* The small coupling constants for aromatic hydrogen nuclei in *N,N'*-diaryl carbamoyl nitroxides[23] show that there is also little spin delocalisation to the phenyl ring of the PhNO· group.

† This explanation assumes that most of the unpaired spin density in radical (**1**) is located on the nitroxide group in consideration of the large nitrogen coupling constant and the expected small spin density on the carbonyl group.[14]

‡ These results are in agreement with the suggestion that the instability of carbamoyl nitroxides such as (**1**) is due to the presence of the NH group.[26]

The three-line spectrum observed during simple base hydrolysis, and ascribed above to the intermediate carboxylate nitroxide (**3**), was not observed in the cycloamylose-catalysed hydrolysis. This result implies a different mechanism (Scheme 6). Hydrolysis of the first C–N bond by a deprotonated secondary hydroxy group of cycloamylose would give the intermediate (**23**). This would hydrolyse further to (**5**) by a similar mechanism, and at a high rate [of the same order as for the ester (**8**), Table 2], and would not therefore be detected. The product (**5**), expected from both catalysed and non-catalysed hydrolysis, would not be observed in either case under the conditions used, because of the reactive NH–O˙ group.[10]

The rate increases produced by cycloamylose are compared in Table 2 with rotational correlation times τ_c, which are a measure of immobilisation on complexing, and with decreases in a_N values due to complexing with the hydrophobic cavity. For the ester (**8**), where the hydrolysis rate is not affected, both τ_c and Δa_N are small by comparison with the cyclic nitroxides (**1**) and (**6**). The open-chain compounds (**7a**) and (**7b**) can conceivably complex by using either end of the chain, and the possible orientations of the carbonyl groups towards the cycloamylose differ from that obtaining with the cyclic nitroxides. Although its τ_c value is high, (**7b**) shows little rate increase with cycloamylose. This may be due to (unfavourable) complexing through the pyrrolidine ring, since the open-chain compound (**7a**), with a t-butyl group at either end, shows an appreciable rate increase.

Conclusions

The hydrolysis of the carbamoyl nitroxide (**1**) shown in Scheme 6 is the first example of a cycloamylose-catalysed hydrolysis in which the substrate is a spin-labelled amide.[27] It is similar to other examples of cycloamylose- or chymotrypsin-catalysed hydrolyses in that nucleophilic addition to the amide carbonyl is rate determining.[28] The resulting tetrahedral intermediate transforms to a cycloamylose ester (**23**) which hydrolyses rapidly to yield the original cycloamylose.

System (**1**) may also be regarded as a urea in which both C–N bonds are cleaved. The C–N bond which is slower to react in the presence of base only (that with N as nitroxide nitrogen) is also rapidly hydrolysed in the presence of cycloamylose. Phosphonate diesters hydrolyse in a similar fashion,[29] the second P–O bond cleaving slowly in the presence of base only, but more rapidly in the presence of cycloamylose.

Other studies have shown that cycloamylose accelerates the hydrolysis of an amide only when the amide is activated by a neighbouring trifluoromethyl[28] or imidazolyl[30] group or is part of a strained four-membered ring.[31] Thus, the nitroxide group in the carbamoyl nitroxides studied in the present work appears to exert a comparable activating effect.

Experimental

E.s.r. spectra were recorded on a Decca X-1 spectrometer with 100-kHz modulation, amplitude 1.3 G. Mn^{II} in magnesium oxide was used as standard (separation of lines 3 and 4 = 86.8 G). Samples for e.s.r. spectra were deoxygenated with a stream of nitrogen.

Samples of radicals for spectra and kinetic measurements were freshly prepared by treating a solution (1 ml) of the appropriate precursor (10^{-4}—10^{-3}M) with lead dioxide (20 mg) for 30 s and removing the reagent by centrifugation; for the ester nitroxide (**8**), nickel peroxide (20 mg) was used as oxidant. For attempted preparation of the radical (**10**), aqueous potassium ferricyanide (10^{-2}M) was mixed in a 1:1 ratio with a solution of t-butylhydroxylamine (2×10^{-2}M) in aqueous potassium carbonate (10^{-1}M).

The pK_a values of radicals were measured from spectra run over a range of pH values in sodium hydroxide solution and showing the presence of both ionised and un-ionised forms.

Aqueous solutions of cyclohepta-amylose were prepared by dissolving the compound in acetonitrile (5% of total solvent) and diluting. Acetonitrile was omitted for solutions in strong base.

Dissociation constants measured by e.s.r. spectroscopy were calculated[32] from plots of the ratio of overall heights of the second and third triplets (or quartets) *versus* cycloamylose concentration, and were not corrected for complexing by the acetonitrile.[33] Values of τ_c were calculated according to literature methods.[34]

For measurement of hydrolysis rates, the centre line of the spectrum was taken as the measure of concentration. First-order kinetics were observed over at least three half-lives.

N-t-Butylhydroxylamine[35] and the *N*-hydroxy precursors to radicals (**6**),[7] (**7b**),[26] (**7c**),[23] and (**8**)[26] were prepared by methods described in the literature.

1-*Hydroxy*-4,4,5,5-*tetramethylimidazolidin*-2-*one*.—To 1-hydroxy-4,4,5,5-tetramethylimidazolidin-2-thione[7] (1 g) in methanol (20 ml) was added dropwise, with cooling, aqueous hydrogen peroxide (30% v/v; 5 ml). During the addition, the solution turned violet, then colourless again. T.l.c. (SiO_2; eluant 95:5 ethyl acetate–hexane) revealed that all of the starting material had reacted and that a major product (R_F *ca.* 0.6) was present. This (0.25 g) was isolated by preparative t.l.c., with silica and the same solvent system, and was recrystallised from ethyl acetate–methanol to yield the ketone (0.15 g), m.p. 226—229 °C (lit.,[4] 225—230 °C); $\nu_{max.}$(KBr) 3 210 (broad, NH, OH) and 1 700 (C=O) cm^{-1}.

N,N′-*Di*-*t*-*butyl*-N-*hydroxyurea*.—A cooled solution of t-butylhydroxylamine (1 g) in dry benzene (40 ml) under nitrogen was stirred during gradual addition (10 min) of t-butyl isocyanate in dry benzene (10 ml). The mixture was left at room temperature (2 h), then the solvent was evaporated to yield the colourless crystalline *product* (2 g), m.p. 46—48 °C (Found: C, 57.1; H, 10.5; N, 15.1. $C_9H_{20}N_2O_2$ requires C, 57.5; H, 10.6; N, 14.9%); $\nu_{max.}$(KBr) 3 330 (OH), 3 260 (NH), 1 730 (NC=O), and 1 698 (O–C=O) cm^{-1}; $\delta_H(CDCl_3)$ 1.17 (9 H, s, 3 CH_3), 1.38 (9 H, s, 3 CH_3), 6.03 (1 H, br s, exchangeable), and 6.25 (1 H, br s, exchangeable).

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References

1 (*a*) A. R. Forrester, J. M. Hay, and R. H. Thomson, 'Organic Chemistry of Stable Free Radicals,' Academic Press, London, 1968, p. 225; (*b*) J. N. Helbert, P. W. Kopf, E. H. Poindexter, and B. H. Wagner, *J. Chem. Soc., Dalton Trans.*, 1975, 998.
2 (*a*) N. M. Karayannis, C. M. Paleos, C. M. Mikulski, L. L. Pyttewski, H. Blum, and M. M. Labes, *Inorg. Chim. Acta*, 1973, **7**, 74; (*b*) A. H. Cohen and B. M. Hoffman, *Inorg. Chem.*, 1974, **13**, 1484.
3 E. F. Ullman, J. H. Osiecki, D. G. B. Boocock, and R. Darcy, *J. Am. Chem. Soc.*, 1972, **94**, 7049.
4 E. F. Ullman, L. Call, and J. H. Osiecki, *J. Org. Chem.*, 1970, **35**, 3623.
5 A. R. Forrester, J. M. Hay, and R. H. Thomson, 'Organic Chemistry of Stable Free Radicals,' Academic Press, London, 1968, p. 193.
6 'Spin Labelling, Theory and Applications,' ed. L. J. Berliner, Academic Press, New York, 1975.
7 R. Darcy, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1089.
8 R. Lyons and R. Darcy, *Nouv. J. Chim.*, 1982, **6**, 71.

9 (*a*) R. M. Pollack and M. L. Bender, *J. Am. Chem. Soc.*, 1970, **92**, 7190; (*b*) M. Bergon and J.-P. Calmon, *J. Chem. Soc., Perkin Trans. 2*, 1978, 493.
10 C. J. W. Gutch and W. A. Waters, *J. Chem. Soc.*, 1965, 751. Decay of the transitory spectrum was not much slower than decay of the spectrum for the radical anion (**2**), and it could not be confirmed that intermediate (**3**) was formed to the exclusion of (**4**).
11 (*a*) B. R. Knauer and J. J. Napier, *J. Am. Chem. Soc.*, 1976, **98**, 4395; (*b*) T. Kawamura, S. Matsunami, and T. Yonezawa, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 1111.
12 A. Mackor, Th. A. J. W. Wajer, and Th. J. De Boer, *Tetrahedron*, 1968, **24**, 1623.
13 D. J. Edge and R. O. C. Norman, *J. Chem. Soc. B*, 1970, 1083.
14 T. C. Jenkins, M. J. Perkins, and B. Terem, *Tetrahedron Lett.*, 1978, 2929.
15 A. H. M. Kayen, Th. A. B. H. Bolsman, and Th. J. De Boer, *Recl. Trav. Chim. Pays-Bas*, 1976, **95**, 14.
16 J. F. W. Keana, M. J. Accaregui, and S. L. M. Boyle, *J. Am. Chem. Soc.*, 1982, **104**, 827.
17 D. G. B. Boocock, R. Darcy, and E. F. Ullman, *J. Am. Chem. Soc.*, 1968, **90**, 5945.
18 E. G. Janzen, E. R. Davis, and D. E. Nutter, *Tetrahedron Lett.*, 1978, 3309.
19 N. P. Gensmantel, D. McLellan, J. J. Morris, M. I. Pafe, P. Proctor, and G. S. Randahawa, in 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' ed. G. I. Gregory, Royal Society of Chemistry, London, 1981, p. 227.
20 (*a*) E. W. Stone and A. H. Maki, *J. Chem. Phys.*, 1963, **39**, 1635; (*b*) P. H. Rieger and G. K. Fraenkel, *ibid*, p. 609.
21 (*a*) A. Carrington and J. dos Santos-Veiga, *Mol. Phys.*, 1962, **5**, 21; (*b*) P. B. Ayscough and F. B. Sargent, *J. Chem. Soc. B*, 1966, 907.
22 H. G. Aurich and J. Trosken, *Chem. Ber.*, 1973, **106**, 3483.
23 V. S. Griffiths and G. R. Parlett, *J. Chem. Soc. B*, 1969, 997.
24 J. Michon and A. Rassat, *J. Am. Chem. Soc.*, 1979, **101**, 995.
25 P. C. Jost and O. H. Griffith, in 'Methods in Enzymology,' eds. C. H. Hirs and S. N. Timasheff, Academic Press, New York, 1978, vol. 49, ch. 17.
26 P. F. Alewood, S. A. Hussain, T. C. Jenkins, M. J. Perkins, A. H. Sharma, N. P. Y. Siew, and P. Ward, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1066.
27 For reviews of previous systems studied, see: (*a*) D. W. Griffith and M. L. Bender, in 'Advances in Catalysis,' eds. D. D. Eley, H. Pines, and P. B. Weisz, Academic Press, New York, 1973, vol. 23, p. 209; (*b*) M. L. Bender and M. Komiyama, in 'Concepts in Organic Chemistry,' eds. K. Hafner, J.-M. Lehn, C. W. Rees, P. v. R. Schleyer, B. M. Trost, and R. Zahradnik, Springer Verlag, Berlin, 1978, vol. 6; (*c*) W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344; (*d*) R. Breslow, *Chem. Br.*, 1983, **19**, 126.
28 M. Komiyama and M. L. Bender, *J. Am. Chem. Soc.*, 1977, **99**, 8021.
29 H. J. Brass and M. L. Bender, *J. Am. Chem. Soc.*, 1973, **95**, 5391.
30 M. Komiyama and M. L. Bender, *Biorg. Chem.*, 1977, **6**, 323.
31 D. E. Tutt and M. A. Schwartz, *J. Am. Chem. Soc.*, 1971, **93**, 767.
32 A. G. Marshall, 'Biophysical Chemistry: Principles, Techniques and Applications,' Wiley, New York, 1978.
33 R. I. Gelb, M. S. Lowell, M. Radeos, R. B. Edmonds, and D. A. Laufer, *J. Am. Chem. Soc.*, 1982, **104**, 6283.
34 A. S. Waggoner, O. H. Griffith, and C. R. Christensen, *Proc. Natl. Acad. Sci. USA*, 1967, **57**, 1198; O. H. Griffith, D. W. Cornell, and H. M. McConnell, *J. Chem. Phys.*, 1965, **43**, 2909.
35 A. Calder, A. R. Forrester, and S. P. Hepburn, 'Organic Syntheses,' Wiley, New York, 1972, vol. 52, p. 77.

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