COMMUNICATION

A comparative study of the reduction by ascorbate of 1,1,3,3-tetraethylisoindolin-2-yloxyl and of 1,1,3,3-tetramethylisoindolin-2-yloxyl

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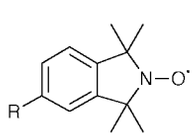
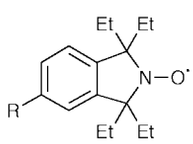
1,1,3,3-Tetraethylisoindolin-2-yloxyl and its water-soluble trimethylammonium and sulfonate derivatives are more resistant to reduction by ascorbate than the corresponding tetramethyl derivatives.

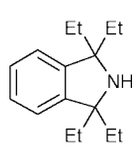
A major problem in the use of the classical aminoxyls (nitroxides) in biological magnetic resonance[1] is their easy reduction by biological media.[2] Radicals of the 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO, **1a**) series have recently been reported to possess excellent thermal and chemical stability,[3] and may not suffer from this drawback. In view of the current interest in **1a** and its water-soluble derivatives **1b**[4] and **1c**,[5] and



1 a R = H
b R = Me_3N^+, I^-
c R = Na^+, SO_3^-



2 a R = H
b R = Me_3N^+, I^-
c R = Na^+, SO_3^-
d R = NH_2



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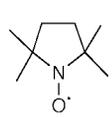

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because the related 1,1,3,3-tetraethylisoindolin-2-yloxyl[6] (TEIO, **2a**) was found to be very resistant to reduction by *p*-phenylenediamine,[7] we have studied the reduction of **1b**, **2a**, **2b** and **2c** by ascorbate (**1a** was not studied because it could not be dissolved in the aqueous buffer solution). The new[8] **2b** and **2c** were obtained respectively from **2d**[9] and **3**,[10] by adaptations of the procedures described for the preparation of **1b**[4] and of **1c**.[5] The reductions were studied under two different conditions (*a* and *b*): after dissolving the sample (*a*: 10^{-3} M, *b*: 10^{-4} M) in a buffered aqueous solution at pH 5.5,[11] L-(+)-ascorbic acid (*a*: 10^{-1} M, *b*: 2.5×10^{-2} M) was added at the initial time $t = 0$ and the EPR spectra were recorded at the temperature of the spectrometer cavity (*ca.* 30 °C) at various time intervals *t*. The results (log *h vs.* *t* where *h* is the height of the high-field line) under conditions (*a*) are presented in Fig. 1 for **1b** and **2b**. As shown by the straight lines obtained, the decay of the aminoxyls follows *pseudo* first-order kinetics. The corresponding *pseudo* first-order constants and the half lives of these aminoxyls in the presence of ascorbic acid are reported in Table 1, where the results previously obtained[12] with 2,2,5,5-tetramethylpyrrolidin-1-oxyl **4** are also given for comparison.

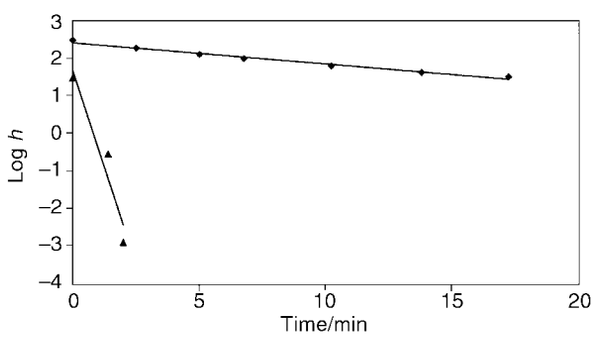

Fig. 1 Decay of the EPR signal (*h* height of the high-field line, arbitrary units) during the reaction of **1b** (▲) and of **2b** (◆), both 10^{-3} M, with L-(+)-ascorbic acid (10^{-1} M) in an aqueous buffered solution at pH 5.5.

Table 1 *Pseudo* first-order rate constants k_{obs} found by EPR for the reduction of aminoxyl radicals dissolved in phosphate buffer (pH 5.5) in the presence of ascorbic acid. (*a*): aminoxyl: 10^{-3} M, ascorbic acid: 10^{-1} M; (*b*): aminoxyl: 10^{-4} M, ascorbic acid: 2.5 10^{-2} M; (*c*): ref. 12.

	(*a*) $10^4\ k_{obs}/s^{-1}$	$t_{1/2}$/min	(*b*) $10^4\ k_{obs}/s^{-1}$	$t_{1/2}$/min
1b	270	0.5		
2a	4.0	29	2.0	60
2b	9.0	13	3.2	35
2c	7.9	15		
4			20 (*c*)	9

Rather than absolute values of reaction rates, these results are to be taken as relative values: the temperature was not controlled and although the spectra were recorded under the same conditions, there was always a systematic error on the time determination. However, it can be concluded that the rates of reduction of the three TEIO derivatives are an order of magnitude smaller than those of the most resistant five-membered ring aminoxyls.[13]

Resistance to reduction of **2a** (*ca.* 4 10^{-2} M) was also observed under various other conditions: no reaction at room temperature in CH_2Cl_2 with 1,2-diphenylhydrazine (10 equiv.) or in methanol–water (2:1) with ascorbic acid (25 equiv.) and only a partial decay of the EPR signal after refluxing 6 hours in methanol with increasing quantities (1 to 7 equiv.) of AIBN added at different time intervals. In total human blood at room temperature, **1a** and **2a** (10^{-3} M) display a half life of 25 and 200 hours respectively.[14]

The great difference between **1b** and **2b** may be attributed to a larger steric hindrance introduced by the ethyl groups. A similar steric protection is probably responsible for the decrease in rate constant induced by complexation with cyclodextrins.[12] The charged aminoxyls **2b** and **2c** were reduced nearly at the

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same rate and slightly more rapidly that neutral **2a**. The most obvious interpretation is that the charge is too distant from the reaction centre to influence the rate-determining step. The difference between these charged radicals and neutral **2a** is more puzzling. It may be related to a possible formation of microscopic aggregates[15] of this less polar molecule, aggregation producing a mutual steric protection for the aminoxyl groups.

These results show that the tetraethyl-substituted isoindolinyloxyl derivatives are more resistant to reduction than the tetramethyl derivatives; at this moment, they are probably the most persistent aminoxyls in the presence of reducing agents.

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Notes and references

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