Ready Reduction of Some Nitroxide Free Radicals with Ascorbic Acid

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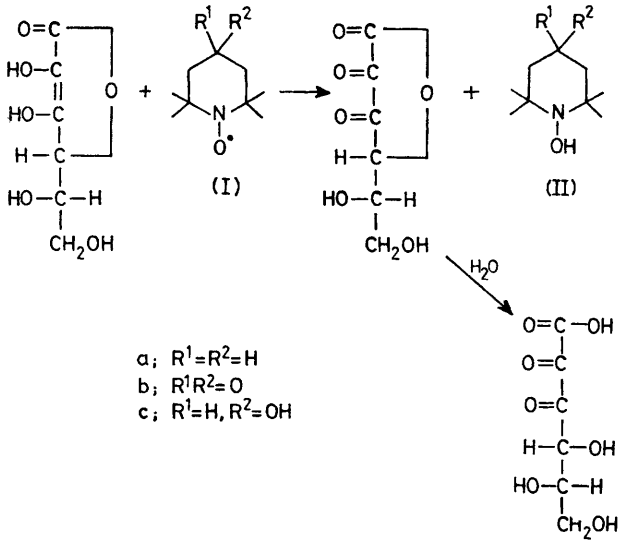
Summary The reduction of some nitroxide free radicals to the corresponding hydroxylamines is reported and, also, a method for their quantitative determination.

FOLLOWING our earlier work on the reduction of 2,2,6,6-tetramethylpiperidine *N*-oxyl (Ia) *via* complex formation with copper(II) perchlorate[1] we report here the reduction of the same radical and its 4-oxo (Ib) and 4-hydroxy derivatives (Ic) to the corresponding hydroxylamines (IIa—c) by ascorbic acid. Such reactions of nitroxides and particularly those with molecules of biological interest are of significance because of the applicability of nitroxides as spin labels[2] in probing biological structures. In general, nitroxides are converted into secondary amines[3] by treatment with strong reducing agents, and into hydroxylamines[3,4] when milder agents are employed.

The hydroxylamines (IIa—c) have been synthesized previously using phenylhydrazine as reducing agent,[5] but the corresponding *N*-phenoxy-piperidines were formed as by-products; (IIb) has also been prepared by a tedious catalytic process,[6] and (IIc) by *in situ* reduction of (Ic) with phenylhydrazine in $CDCl_3$.[7] The reduction of (I; $R^1 = H$, $R^2 = EtO_2C$) by ascorbic acid has been followed by e.s.r. spectroscopy, but the reduction product was not isolated.[8]

The following procedure for the synthesis of (IIb) is typical; the reduction takes place as in the Scheme, dehydroascorbic acid being hydrated to 2,3-diketogulonic acid which cannot be converted back into either ascorbic acid or dehydroascorbic acid under these conditions.[9] Aqueous ascorbic acid (1·25 mmol) was added to a solution of the *N*-oxyl (Ia) (2 mmol) in the minimum amount of water. The solution was decolourized instantaneously; it was then extracted with ether, the ether extract was dried ($MgSO_4$) and evaporated, and the residue was recrystallized from pentane to give the hydroxylamine (IIb),† m.p. 89—90 °C, in 87% yield (yield depends on efficiency of the extraction). Compounds (IIa) and (IIc) were prepared similarly: (IIa), m.p. 37—40 °C, was unstable and reverted to (Ia) in solution or in the solid state; (IIc) had m.p. 155—158 °C (from hexane–$CHCl_3$, 1 : 1).



SCHEME

In the preparation of (IIb), 0·96 mmol of ascorbic acid were consumed, compared with a calculated amount of

† Satisfactory analytical data were obtained for these compounds.

1·00 mmol if the *N*-oxyl (Ib) had been 100% pure, and this provides the basis for a general method for the quantitative estimation of nitroxides. The amount of ascorbic acid which had not been oxidised by the nitroxide can be titrated with standard iodine solution following the procedure in ref. 10, and from this, followed by a blank determination, the amount of nitroxide present can be determined.‡

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‡ Nitroxide content/g = $M(V_0 - V_1)M^I/500$; V_0 = volume (in ml) of iodine solution consumed in the blank, V_1 = volume consumed in the determination itself, M^I the molarity of the iodine solution, and M the molecular weight of the nitroxide.

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