Redox behaviour of azulenyl nitrones: fully reversible one electron oxidation by cyclic voltammetry at potentials in the range of biological antioxidants

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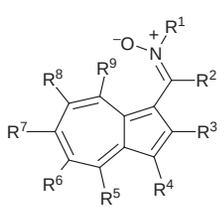
The one electron oxidation chemistry of azulenyl nitrones 4 and 5 employing cyclic voltammetry reveals that, unlike conventional nitrone spin traps such as PBN and S-PBN, such oxidation is electrochemically and chemically reversible at potentials near or within those of important biological chain-breaking antioxidants.

There is presently much interest in exploring the protective effects of antioxidants against free radical damage in biological systems.[1] A wide variety of human pathologies such as atherosclerosis,[2] cancer,[3] stroke,[4] amyotrophic lateral sclerosis[5] and Alzheimer's disease[6] are suspected of having free radicals as significant etiological components. Within the last several years, nitrones have emerged as a class of antioxidants with considerable potential in the treatment of such conditions, particularly as therapeutics for neurodegenerative diseases.[7] Nitrones display a number of distinctive properties in their reactions with oxyl radicals.[8]

Among the hallmarks of potent chain-breaking antioxidants are their possession of low oxidation potentials and their capacity to generate stable non-chain propagating radicals after quenching more harmful paramagnetic species. While conventional nitrones (such as PBN and S-PBN) investigated as antioxidants certainly fulfill the latter role by generating persistent aminoxyls upon capture of deleterious radicals, their ease of oxidation determined by cyclic voltammetry is considerably less than that of natural antioxidants such as β-carotene, glutathione, α-tocopherol and ascorbic acid, whose oxidation potentials (V *vs*. SCE) are +0.76, +0.69, +0.26 and +0.04, respectively.[9] An excellent compilation of the oxidation peak potentials of a variety of nitrone spin traps has been assembled by Janzen and co-workers.[10] Upon inspection of these data, one finds that the lowest such potential among the 16 nitrone spin traps examined is that of S-PBN with a value of +1.34 V *vs*. SCE. The reported value for PBN, the nitrone most frequently employed in biological systems as an antioxidant, is +1.47 V *vs*. SCE, placing it as the third most readily oxidized nitrone of that study. Herein we report that azulenyl nitrones **1** possess



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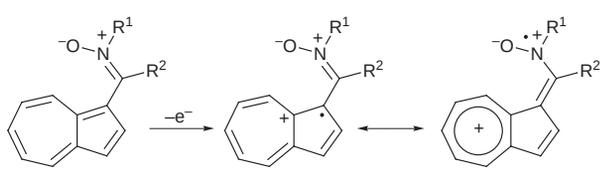
redox behaviour significantly different from that of any nitrones yet measured and on the ramifications of these observations concerning their utility as antioxidants and their efficacy of free radical capture.

Apart from their previously described capacity to function as chromotropic spin trapping agents,[11–14] azulenyl nitrones such as **1** exhibit a number of features that render them appealing candidates as a class of effective antioxidants.[12,15] Azulenes are electron-rich non-benzenoid aromatic compounds that, upon oxidation, readily lose one electron to yield radical cations in which the cationic portion is present as a stabilized aromatic tropylium ion. Indeed, the oxidation potential of azulene (0.96 V *vs*. SCE) is lower than that of most common aromatic carbocycles and heterocycles.[16] In the case of azulene itself, Bargon *et al*. have shown that the fate of the tropylium radical cation produced upon electrochemical oxidation is polymerization.[17] Azulenes bearing alkyl or aryl substituents at both the 1 and 3 positions do not polymerize on electrochemical oxidation, and salts of such tropylium cation radicals have been characterized by EPR spectroscopy.[18]

It was speculated that attachment of a nitrone moiety at C-1 of the azulene nucleus would provide a fortified nitrone antioxidant, **2**, since **2** appears to be exquisitely well-equipped to lose an electron to oxidizing species such as harmful electron-deficient radicals frequently encountered *in vivo*. Thus, inspection of the resonance form **3** of the distonic radical cation



2 **3**

derived from **2** after loss of an electron reveals that, in addition to the presence of a stable aromatic tropylium cation as in other azulene radical cations, *the radical is resonance stabilized as an α,β-unsaturated aminoxyl*.[19] Therefore, radical cation **3** was anticipated to possess unusual stability unlike the radical cations of conventional nitrone spin traps. Recent studies concerning the nature of free radical scavenging by antioxidants support an electron transfer mechanism as the initial event in many instances.[20] Subsequent to the electron transfer, the resulting radical cation can be deprotonated by the newly formed anion as is the case for α-tocopherol, or, in the case of an azulenyl nitrone, the ion pair comprised of the radical cation **3** and the anion would quickly collapse to an aminoxyl spin adduct.[21] Thus, as electron-rich nitrones, the azulenyl nitrones may react more efficiently with radicals than conventional nitrones to produce aminoxyl spin adducts by virtue of such a mechanism.

After achieving the synthesis of **4**,[11] the first representative of the desired new class of nitrones, an investigation was initiated to explore its behaviour upon oxidation by cyclic voltammetry. We report that the oxidation potential of azulenyl nitrone **4** is indeed significantly lower than that of any of the conventional nitrone spin traps previously studied by Janzen and co-workers.[10] The cyclic voltammogram for the oxidation of a 1 mM solution of **4** on a glassy carbon electrode in acetonitrile is presented in Fig. 1. It is evident from Fig. 1 that the oxidation of **4** is electrochemically and chemically reversible, with an $E_{1/2}$

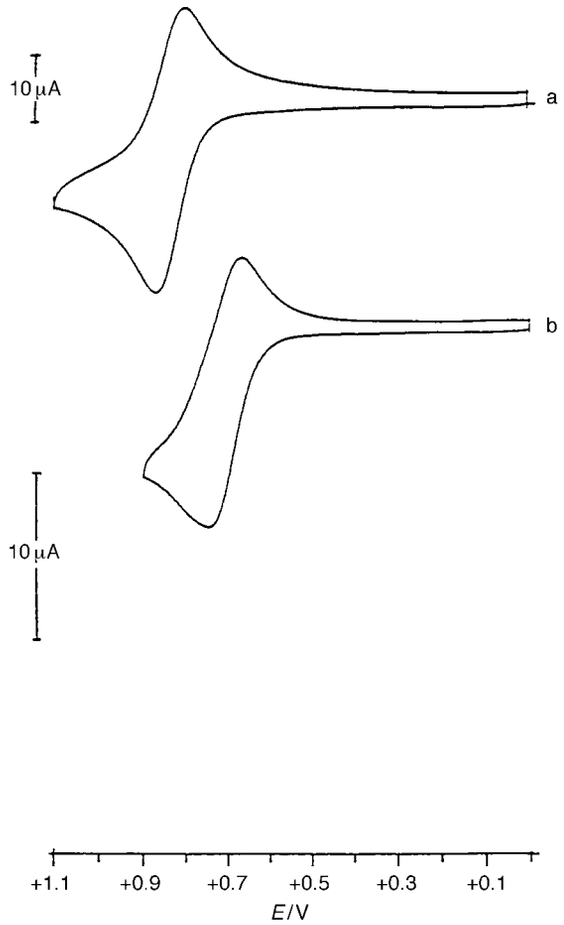
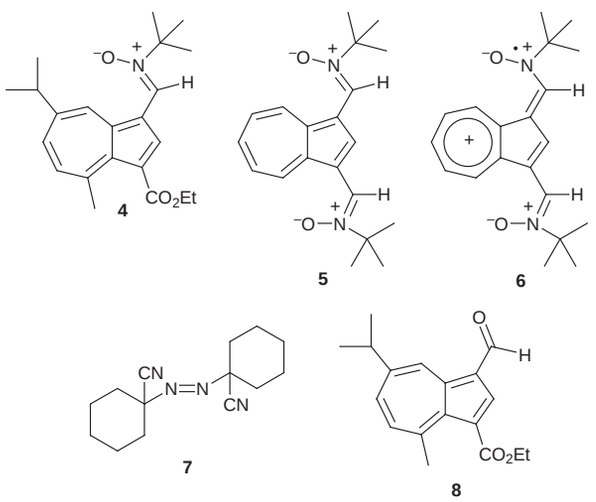


Fig. 1 Cyclic voltammograms in acetonitrile at a scan rate of 100 mV s^{-1}, (a) voltammogram of 1.3 mM of **4**, (b) voltammogram of 1 mM of **5**



of 0.84 V *vs*. SCE, and a cathodic to anodic peak separation of 68 mV. Such a well behaved redox pair is in marked contrast to the behaviour exhibited by other nitrones, such as those reported by Janzen and co-workers.[10] All the nitrones in the Janzen study exhibit chemically irreversible oxidation when analyzed *via* cyclic voltammetry, and thus, only their anodic peak potentials are reported.[10] Although comparing potential values when the redox processes are not chemically reversible is somewhat risky, since true thermodynamic $E_{1/2}$ values cannot be obtained, it is a reasonable approximation to compare peak potentials.[22] The oxidation peak potential for **4** appears at 0.87 V while that corresponding to the irreversible oxidation for PBN appears at 1.47 V, both *vs*. SCE. Thus, compound **4** is oxidized at a potential that is about 0.6 V less positive than that of PBN, which means that the corresponding cation of **4** is

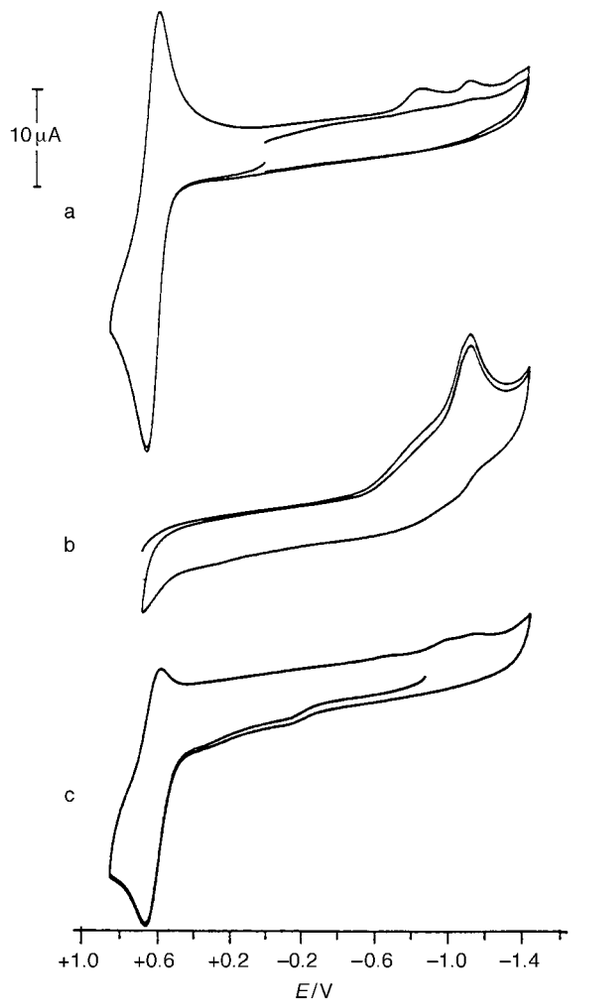


Fig. 2 Cyclic voltammograms recorded at a scan rate of 100 mV s^{-1} after coulometry of **4** in acetonitrile using a silver wire as reference electrode. (a) Voltammograms before coulometry, (b) voltammograms after coulometric oxidation at 0.764 V and (c) voltammograms after coulometric reduction at −1.23 V of the products shown in (b).

about 14 kcal more stable than that of PBN. The anodic peak potential of 0.87 V *vs*. SCE observed for **4** is, to our knowledge, lower than that of any nitrone yet measured, with the exception of azulenyl bis-nitrones such as **5** (*vide infra*). Furthermore, to our knowledge, azulenyl nitrones such as **4** and **5** exhibit the only oxidative processes on nitrones that are known to occur chemically reversibly.

Interestingly, no EPR signal is detected after one electron oxidation of **4**, while **4** can be regenerated from the ensuing diamagnetic product by reduction in what constitutes an electrochemical–chemical–electrochemical (ECE) mechanism (Fig. 2). In contrast, we have now determined by EPR spectroscopy (Fig. 3) that, upon one electron oxidation, azulenyl bis-nitrone **5** ($E_{1/2}$ = +0.72 V *vs*. SCE, Fig. 1) gives a long-lived paramagnetic species believed to be the distonic radical cation **6**. That the oxidation potential of **5** is approximately 240 mV lower than that of azulene suggests that the influence of nitronyl substituents at the 1 and/or 3 positions of an azulene nucleus is such that these moieties do indeed offer stabilization to the corresponding radical cation formed upon one electron oxidation of such species. It is also noteworthy that radical cation **6** is formally a doubly vinylogous member of the structurally interesting class of persistent radicals known as nitronyl aminoxyls, first described by Ullman *et al*. in 1972.[23,24]

The unusual combination of their low oxidation potentials with values near or within the range of crucial cellular antioxidants (such as β-carotene and the glutathione thiolate anion)[9] and the high lipophilicity of azulenyl nitrones such as **4**

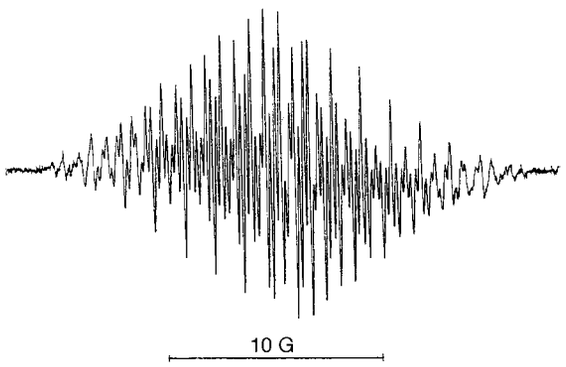


Fig. 3 X-Band EPR spectrum at ambient temperature of $\mathbf{5}^{\bullet+}$ in acetonitrile

makes them attractive candidates as antioxidants to traverse the blood–brain barrier.[12,13] Strategies to reduce the oxidation potential of antioxidants normally involve the incorporation of potent electron-donating functionalities which typically contain heteroatoms and therefore often result in decreased lipophilicity. However, despite their low oxidation potentials, it is important to note that azulenyl nitrones such as **4** and **5** are not readily degraded by atmospheric oxygen.[11]

In light of the relatively low oxidation potentials of the azulenyl nitrones, it was of interest to determine if such nitrones would constitute more efficient free radical trapping agents than conventional nitrone spin traps with higher oxidation potentials such as PBN. Competition experiments were performed in a sealed tube involving the aerobic thermal (90 °C) decomposition of azo compound **7** (1 equiv.) in carbon tetrachloride containing equimolar concentrations of **4** and PBN. After a reaction time of 6 h, the only azulene containing compound detected in a significant amount other than unreacted starting material was aldehyde **8**. The amount of **4** and **8** obtained accounted for the complete mass balance of the starting azulene that was employed. Similarly, in addition to unreacted PBN, the only significant PBN-derived product detected was benzaldehyde. In analogy to previous investigations regarding the nature of the endproducts formed in reactions between peroxyl radical and nitrones, the aldehyde products are expected to arise upon capture of cyanoalkyl peroxyl radicals by the nitrones followed by the known rapid fragmentation of such spin adducts.[25] Inspection of the integral of the NMR signal of the aldehyde proton of **4** *vs*. that of benzaldehyde revealed that the amount of azulenecarbaldehyde **8** produced was nearly 50% greater than the amount of benzaldehyde. Thus, the data suggest that for every two peroxyl radicals scavenged by PBN, azulenyl nitrone **4** scavenges three such radicals. Further studies into the antioxidant properties of **4** and congeners are in progress and will be the subject of future reports.

Experimental

Cyclic voltammetric experiments were carried out under an argon atmosphere using a BAS 100W analyzer (Bioanalytical Systems, Inc., West Lafayette, IN). Acetonitrile (Sigma-Aldrich) was dried over P_2O_5 and distilled under vacuum. Tetrabutylammonium perchlorate (Fluka) was recrystallized twice from ethyl acetate and dried overnight in a vacuum oven at 80 °C. The counter electrode used for the cyclic voltammetric analyses was a platinum wire.

Acknowledgements

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