Nitric Oxide Reactions with Antioxidants in Model Systems: Sterically Hindered Phenols and α-Tocopherol in Sodium Dodecyl Sulfate (SDS) Micelles

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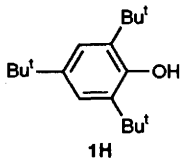
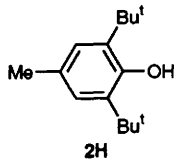
Reaction of nitric oxide with antioxidant phenols in SDS micelles produces phenoxyl radicals which subsequently form covalent adducts with excess of nitric oxide.

The free radical chemistry of nitrogen monoxide, commonly known as nitric oxide (·NO)[1] is essentially unknown. Text books and monographs on free radical chemistry ignore the subject of ·NO.[2] Little can be found about the EPR characteristics of ·NO in the condensed phase,[3] although the EPR spectrum of ·NO in the gas phase has been reported.[4] Increasing interest in ·NO as a stable free radical in biological systems[5,6] prompted us to initiate a program of research designed to study the fundamental reaction chemistry of ·NO.

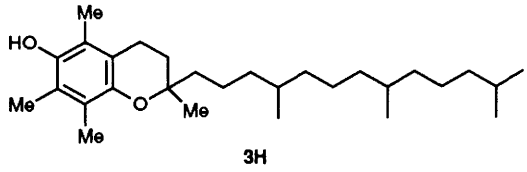
Three phenols were selected for this study: a sterically hindered phenol which produces persistent phenoxyl free radicals upon hydrogen atom abstraction, 2,4,6-tri-*tert*-butylphenol (**1H**); a commercially used antioxidant, 2,6-di-*tert*-butyl-4-methylphenol or 'butylated toluene', BHT (**2H**); a



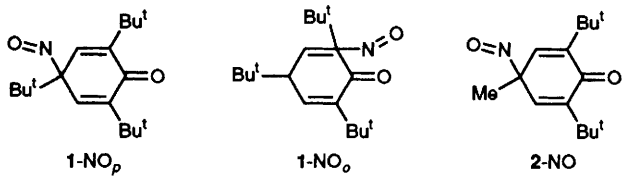
1H



2H



3H



naturally occurring biologically significant phenol, vitamin-E or α-tocopherol (**3H**).

Reactions of these phenols with ·NO were examined in SDS micelles to similate the environment of phospholipid membranes. Micelle solutions were prepared in N_2-saturated deionized water above the critical micelle concentration of SDS. EPR spectroscopy was used to characterize the reactivity of ·NO with phenols. Commercial ·NO was bubbled through aqueous sodium hydroxide to remove any $\cdot NO_2$ impurity immediately prior to its use in reactions.

The EPR spectrum obtained when ·NO was bubbled through an SDS micelle solution of **1H** slowly decayed in the presence of ·NO, but sharpened to a 1 : 2 : 1 triplet with 2.0 G (1 G = 10^{-4} T) hyperfine splitting characteristic of tri-*tert*-butylphenoxyl (**1**·)[7] when the solution was saturated with N_2. The spectrum more than doubled in intensity after 30 min following saturation with N_2. This was followed by a slow broadening and decrease in intensity. When **1**· was prepared by sonicating **1H** in N_2-saturated SDS micelles the spectrum did not decrease over the time course of the experiments.

These results can be accounted for by reactions (1) and (2). The phenoxyl radical **1**·, is slowly produced from reaction of **1H** with ·NO. The EPR spectrum is broadened by excess of ·NO indicating that coupling between the phenoxyl radical and ·NO is slow. When N_2 is introduced to remove excess of ·NO, the concentration of **1**· increases, indicating reaction (2) is reversible.

$$\mathbf{1H} + \cdot NO \rightarrow \mathbf{1}\cdot + HNO \quad (1)$$

$$\mathbf{1H} + \cdot NO \rightarrow \mathbf{1}\text{–}NO \quad (2)$$

Structures assigned to **1**–NO adducts are cyclohexadienone isomers resulting from ·NO attack at the *ortho*- and *para*-positions of **1**·. Support for structures of the cyclohexadienone adducts (**1**–NO) includes proton NMR studies of the reaction of **1H** and ·NO.† Coupling of **1**· and ·NO was confirmed by reaction with **1**· produced from ferricyanide oxidation of **1H**.[8] Decomposition of **1**· does not significantly contribute to the reactivity of **1**· over the time course of the experiment.

No products were detected by EPR when ·NO was bubbled for 30 min through SDS micelles containing **2H**. After 60 min a weak EPR spectrum of a 1 : 3 : 3 : 1 quartet (11.2 G) of 1 : 2 : 1 triplets (1.8 G) was detected when the solution was saturated with N_2. The spectrum is assigned to 2,6-di-*tert*-butyl-4-methylphenoxyl **2**·.[7] The spectrum of **2**· slowly decayed over 60 min. Formation of a **2**–NO adduct was confirmed by ^1H NMR analysis of the reaction in benzene.‡

We concluded nitric oxide oxidizes **2H** to **2**·, which quickly couples with excess of ·NO to produce **2**–NO. The formation of **2**–NO appears to be more favourable than formation of the analogous **1**–NO adducts because of smaller steric hindrance at the 4-position of **2**·. In fact the only product in ·NO-saturated solutions of **2H** observed by NMR is **2**–NO. When the concentration of ·NO is reduced, **2**–NO dissociates to **2**· and ·NO. Decomposition of **2**· to a quinone methide and a dimer have been reported.[9] Thus, formation of **2**–NO may be reversible under certain conditions.

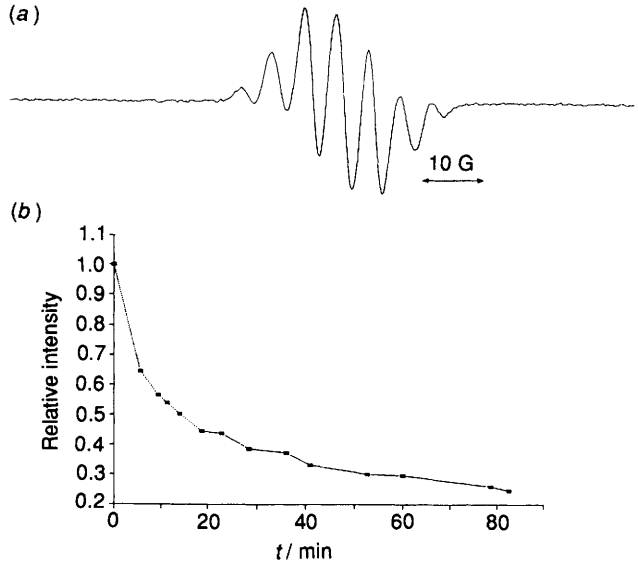


Fig. 1 (*a*) EPR spectrum from **3H** (10 mmol l^{-1}) in SDS (300 mmol l^{-1}) bubbled with ·NO for 60 min after saturation with N_2. (*b*) Intensity of spectrum in (*a*) as a function of time. Bruker 300E EPR spectrometer: receiver gain: 5.00 × 10^5; modulation frequency: 100 kHz; modulation amplitude: 1.053 G; sweep time: 84 s.

The intensity of the EPR spectrum of tocopheroxyl (**3**·) increased over 60 min as an SDS solution of **3H** was bubbled with ·NO. A typical spectrum of **3**· from the reaction in SDS micelles is shown in Fig. 1(*a*). When ·NO bubbling was stopped, the spectrum of **3**· decayed during 90 min as shown in Fig. 1(*b*). The spectrum decayed to baseline in 5 min when the ·NO saturated solution was bubbled with N_2. The decay of **3**· is slower in the presence of ·NO than after the solution is saturated with N_2, indicating competition between formation and decomposition of **3**· in ·NO-saturated micelles. The rate of formation of **3**–NO is expected to be slow by analogy to reaction of **3**· and O_2, which has been shown not to compete with decomposition of **3**·, regardless of O_2 concentration.[10] However, radical addition to **3**· occurs in reactions with peroxyl radicals.[11]

There are two possible mechanisms for production of phenoxyls from reaction of ·NO with phenols. H-atom abstraction produces HNO, but little is known about the chemistry of HNO. In aqueous media, HNO dimerizes and is dehydrated to N_2O.[12] It is also possible that phenols reduce ·NO by single electron transfer to produce the phenol radical cation and nitroxyl anion as has been suggested for reaction of **3H** and ozone[13] as well as for oxidation of phenols by iron complexes.[14] Phenoxyl would form by loss of a proton from the phenol radical cation. Nitroxyl anion is converted to N_2O by dimerization,[15] but can oxidize protein sulfhydryl groups.[16] Nitroxyl anion may also coordinate to ferric heme complexes. Reaction of ·NO with phenols may therefore be biologically significant not only by destroying ·NO but also by converting ·NO to NO^-, resulting in oxidative destruction of cellular components or altering enzymatic activity by coordinating to protein-bound iron. In blood plasma, *S*-nitrosothiols constitute the major source of ·NO and these compounds may serve to regulate ·NO production.[17] Our results in micellar model systems show that ·NO reacts with phenols to produce phenoxyls and reversible coupling between ·NO and phenoxyls may serve to attenuate the concentration of ·NO *in vivo*.

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† Analysis of NMR spectra in benzene showed a 4 : 1 ratio of 1–NO_p to 1–NO_o.

‡ Analysis by NMR showed formation of one product, the *para*-nitrosocyclohexadienone adduct **2**–NO.

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