

Spectrophotometric, EPR and kinetic characterisation of the $>\text{N-O}\bullet$ radical from 1-hydroxybenzotriazole, a key reactive species in mediated enzymatic oxidations

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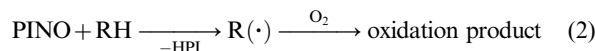
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Characterisation of the aminoxyl ($>\text{N-O}\bullet$) radical BTNO, generated from 1-hydroxybenzotriazole (HBT) by the one-electron oxidant CAN (a Ce^{IV} salt), confirms BTNO as the reactive intermediate in oxidations run with the laccase/HBT system.

Characterisation of reactive intermediates is a fundamental issue in unravelling the mechanism of complex reaction. The aerobic oxidation of organic substrates represents a timely research topic and a fitting example of a reaction proceeding through intermediate species.¹ Recently, the proficiency of *N*-hydroxyphthalimide (HPI, viz. $>\text{N-OH}$) to perform as an oxidation catalyst was documented,² the key intermediate being the phthalimide-*N*-oxyl radical (PINO, viz. $>\text{N-O}\bullet$) formed in the preliminary interaction of HPI with O_2 and a Co(II) salt (eqn. (1)).^{2,3}



The synthetic value of the procedure has prompted the assessment of reactivity features of PINO.⁴ In a kinetic investigation of H-abstraction rates from C–H bonds,^{5,6} PINO was generated from HPI by Pb(OAc)_4 as an inner-sphere oxidant ($E^\circ \geq 1.5$ V/NHE);⁷ the resulting $>\text{N-O}\bullet$ species shows a maximum of absorbance at 380 nm (ϵ 1450 $\text{M}^{-1} \text{cm}^{-1}$) in both MeCN and AcOH solution.^{5,6}

The characterisation of the reactivity of PINO has provided support to the radical route (eqn. (2)) proposed for the oxidation of a number of RH substrates under mild conditions.^{2,3,8,9} Moreover, it also supports an analogous radical route proposed for the ecologically benign oxidation of lignin or non-phenolic lignin-model compounds by the phenoxidase enzyme laccase, where HPI performs as a redox mediator (Med, in Scheme 1).^{10,11} Laccase from the fungus *Poliporus pinsitus*, in view of its redox potential of 0.8 V/NHE,¹² can oxidise HPI (E° 1.09 V/NHE)¹³ to PINO (viz. Med_{ox}) in an electron-abstraction/deprotonation route ($>\text{N-OH}\bullet^+ \rightarrow >\text{N-O}\bullet$), thereby enabling an alternative entrance into the radical oxidation route of eqn. (2).

Among other compounds sharing the $>\text{N-OH}$ moiety of HPI, and performing as laccase mediators through the purported intermediacy of the $>\text{N-O}\bullet$ species, 1-hydroxybenzotriazole (viz. HBT) proved very efficient.^{11,13}

We report here the first UV-Vis characterisation of the aminoxyl radical (dubbed BTNO) from HBT, detected with both a stopped-flow instrument and a diode array spectrophotometer. The BTNO

species was generated in MeCN at 25 °C in a quartz cuvette by adding a solution (0.5 mM) of the outer-sphere one-electron oxidant cerium(IV) ammonium nitrate (viz. CAN; E° 1.3 V/NHE)¹⁴ to a solution (0.5 mM) of HBT (E° 1.08 V/NHE).¹³ A broad absorption band developed almost immediately (15 ms) in the 400–600 nm region (Fig. 1; λ_{max} at 474 nm, ϵ 1840 $\text{M}^{-1} \text{cm}^{-1}$). In analogy to the experimental procedure adopted for the HPI case,⁵ an identical spectrum of BTNO was also obtained in AcOH whenever adding a solution of Pb(OAc)_4 (0.25 mM; a two-electron oxidant) to a 0.5 mM solution of HBT.

The formation of the BTNO radical is followed by its decomposition, which is faster than that reported⁵ for PINO (half-life of 110 and 7900 s, respectively).

Additional support to the characterisation of BTNO rests on the comparable absorption spectrum obtained by laser flash photolysis in MeCN, following the cleavage of dicumyl peroxide by laser pulse at 355 nm, and ensuing H-abstraction from HBT by the cumyloxy radical (Fig. 2).

Thus, a species with the same spectral features and λ_{max} can be generated from HBT by both a radical process (with cumyloxy radical) and an electron-abstraction/deprotonation route with an oxidant of appropriate redox power. Finally, an EPR spectrum of BTNO was obtained in MeCN by adding a solution of CAN (20 mM) to a HBT solution (20 mM) in an EPR tube at 25 °C (Fig. 3). This spectrum was interpreted on the basis of the coupling of the unpaired electron with three different nitrogen nuclei ($a_{\text{N}} = 0.056, 0.152$ and 0.475 mT) and with three unequivocal protons ($a_{\text{H}} = 0.037, 0.203$ and 0.458 mT), consistently with the structure of the BTNO radical.

Following this spectrophotometric/structural characterisation, determination of the second-order rate constants for reaction of BTNO with *para*-substituted benzyl alcohols was achieved by spectrophotometry (Table 1). Under pseudo first-order conditions (10-to-50 times excess of alcohol) the H-abstraction rate was faster than the spontaneous decay of BTNO, and product analysis confirmed the formation of the substituted benzaldehydes from the

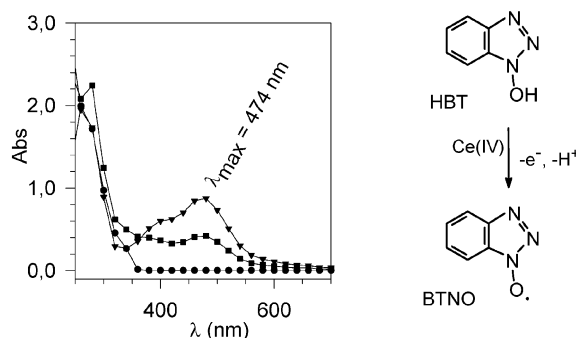
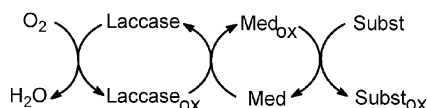


Fig. 1 UV-Vis spectrum of HBT (0.5 mM) in MeCN in the absence of CAN (●); its change after the addition of CAN (0.5 mM): (▼) 15 msec after mixing, (■) 90 sec after mixing.



Scheme 1

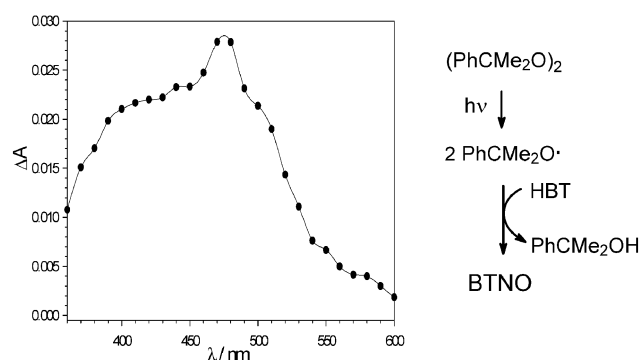


Fig. 2 Absorption spectrum obtained after 355 nm laser flash photolysis of an Ar-saturated MeCN solution containing 1.0 M dicumyl peroxide and 0.03 M HBT, recorded 1.4 ms after the laser flash.

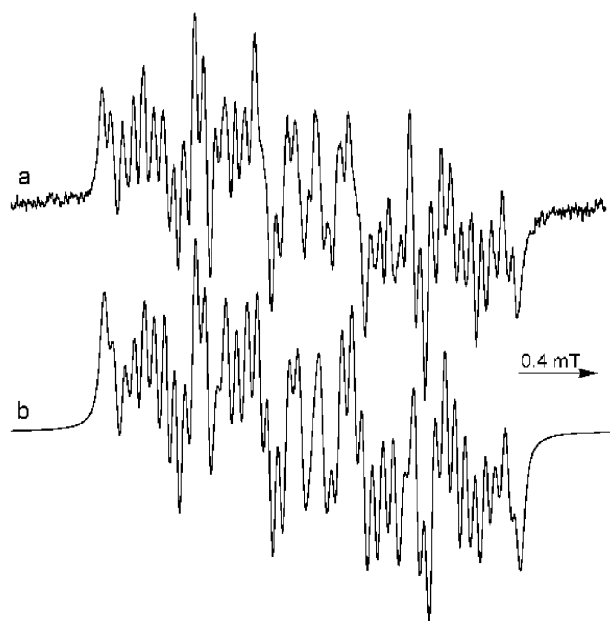
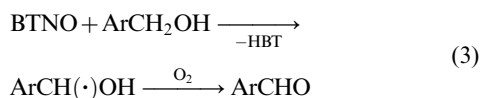


Fig. 3 Experimental EPR spectrum of BTNO obtained at room temperature by reaction of CAN (20 mM) with HBT (20 mM) in MeCN (a), and its computer simulation (b).

parent alcohols (eqn. (3)).



A Hammett analysis of substituents effect on the reaction rate gave a better correlation ($\rho = -0.55$) with the σ^+ than with the σ parameters, in keeping with the known electrophilic character of $>\text{N}-\text{O}\cdot$ radicals.^{3,6,11} The intramolecular kinetic isotope effect was also studied for the oxidation of a suitable α -monodeuterated benzyl alcohol (ArCHDOH), and the $k_{\text{H}}/k_{\text{D}}$ ratio reckoned as 5.6 from mass spectrometric determination of the relative amount of the two aldehydes (ArCDO and ArCHO) produced. The small ρ value obtained confirms the radical nature of this oxidation procedure, and the large $k_{\text{H}}/k_{\text{D}}$ ratio supports a rate determining H-abstraction step.

Table 1 Second-order rate constants for the reaction of BTNO radical with 4-X-C₆H₄CH₂OH in MeCN at 25 °C (followed at 474 nm)

X	$k/\text{M}^{-1} \text{s}^{-1}$
NO ₂	1.24
CF ₃	1.18
Cl	1.93
H	1.87
Ph	4.35
CH ₃	3.80
CH ₃ O	7.80

Both the ρ and the $k_{\text{H}}/k_{\text{D}}$ values obtained here compare very favourably with those (*i.e.*, -0.64 and 6.4 , respectively) obtained in the oxidation of substituted benzyl alcohols with the laccase/HBT system (Scheme 1),¹¹ giving clear-cut support to the formation of the $>\text{N}-\text{O}\cdot$ radical as the Med_{ox} species in those mediated oxidation procedures. An extensive kinetic study of H-abstraction reactivity with BTNO, to compare with available data for PINO,^{5,6} as well as attempts to determine the O–H bond energy of HBT, to compare with that of HPI (BDE_{OH} 88.1 kcal mol^{−1}),⁴ in view of the expected relevance of this parameter upon the relative reactivity of the two aminoxyl radicals, will be reported in a future full paper.

Notes and references

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