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Modeling antioxidant properties of polyphenols by the TEMPO-initiated reaction of 3,5-di-*tert*-butylcatechol with dioxygen

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Abstract

The oxidation reaction of 3,5-di-*tert*-butylcatechol ($dbcatH_2$) with dioxygen to 3,5-di-*tert*-butyl-*o*-benzoquinone (dbq) initiated by 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) has been investigated in methanol and CCl_4 at ambient temperature. The oxidation of $dbcatH_2$ was followed by electron spectroscopy and the rate constants were determined according to the rate law $-d[dbcatH_2]/dt = k_{obs}\ [dbcatH_2][TEMPO][O_2]$. The rate constant, activation enthalpy and entropy at 323 K are as follows: $k_{obs}\ (M^{-2}\ s^{-1}) = 1.58 \pm 0.03$, $\Delta H^{\ddagger} = 51 \pm 10\ kJ\ mol^{-1}$ and $\Delta S^{\ddagger} = -84 \pm 32\ J\ mol^{-1}\ K^{-1}$.

Keywords: 3,5-Di-*tert*-butylcatechol; Oxidation; TEMPO catalysis; Kinetics and mechanism

1. Introduction

Antioxidants isolated from natural sources attract considerable attention because several species of active oxygen are thought to be harmful to human health and trigger many diseases, for example, coronary heart disease and cancer (Halliwell, Gutteridge, & Cross, 1992; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog et al., 1995; Perchellet, Perchellet, Gali, & Gao, 1995). Polyphenols, such as flavonoids and catechins, are a large group of antioxidants naturally present in fruits, vegetables and certain beverages, including wine, coffee and tea. Attention has recently focused on this class of compounds as potentially excellent peroxyl radical-trapping, chain-breaking antioxidants compared, for example, to hydroquinones (Foti et al., 2002).

Actually, polyphenols can act as free radical scavengers, quenching hydroxyl radical (·OH) (Hanasaki, Ogawa, & Fukui, 1994), superoxide anion radical ($O_2^{\cdot-}$) (Hatano et al., 1989; Nakagawa & Yokozawa, 2002; Sichel, Corsaro, Scalia, Di Bilio, & Bonomo, 1991), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Hatano et al., 1989; Nanjo et al., 1996). These special features might be explained by the fact that, in catechols and related compounds, which are capable of forming an intramolecular hydrogen-bond, the two hydroxyl groups are certainly not the same. Theoretical calculations have shown that the intramolecular H-bond in the *o*-semiquinone radical is ca. 4 kcal/mol stronger than that in the parent catechol (Korth, de Heer, & Mulder, 2002; Wright, Johnson, & Dilabio, 2001). As a consequence, the ''free'', i.e. non-H-bonded, hydroxyl group in catechol has a significantly lower O–H bond dissociation enthalpy (BDE) than that for hydroquinone (leading to an increase in the intrinsic reactivities for H-atom donation by catechols relative to hydroquinones).

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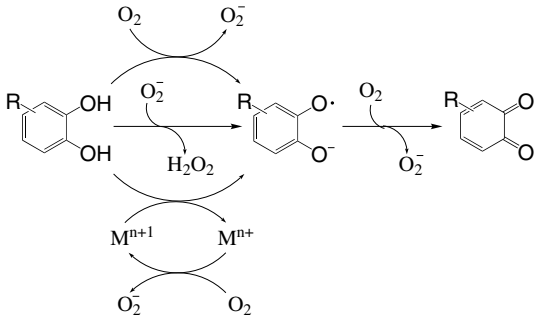


Fig. 1. Proposed mechanism for the autoxidation of polyphenols.

Although these compounds show such biological activities, as antibacterial and antivirial, antimutagenicity, antioxidative activity and enzyme inhibition, it has been recently reported that some polyphenols promote oxidative damage to DNA, lipids, and deoxyribose in the presence of metal ions under certain conditions in vitro (Hayakawa, Kimura, Hoshino, & Ando, 1999; Hayakawa et al., 1997; Sahu & Gray, 1993; Singh, Ahmad, & Rao, 1994; Yamanaka, Oda, & Nagao, 1997). Damage to DNA or chromosomes is important because it influences cell functions, resulting in various diseases and aging. This pro-oxidant action is thought to be due to the attack by reactive oxygen species such as ·OH, O_2^-, and hydrogen peroxide (H_2O_2) generated during the so-called autoxidation process of polyphenols (Cao, Sofic, & Prior, 1997). In general, polyphenols with a catechol moiety are easily oxidized to the corresponding *o*-quinones with dioxygen and a transition metal ion, with concomitant evolution of active oxygen species such as O_2^- and H_2O_2 as shown in Fig. 1 (Kaizer et al., 2002; Mochizuki, Yamazaki, Kano, & Ikeda, 2002).

Since interaction of free radicals with organic substances, especially with metabolic intermediates, can help in understanding the mechanisms of biological oxidations involving molecular oxygen, kinetic studies of TEMPO-catalyzed oxidation of 3,5-di-*tert*-butylcatechol ($dbcatH_2$) have been carried out, in order to obtain a deeper insight into these processes.

2. Materials and methods

2.1. Materials and apparatus

Solvent (methyl alcohol) used for the reactions was purified by literature methods and stored under argon. TEMPO (2,2,6,6-tetramethyl-piperidinyloxyl free radical) and 3,5-di-*tert*-butylcatechol ($dbcatH_2$) were obtained from Aldrich and used without further purification. All reactions were performed by the standard Schlenck technique under dioxygen or argon.

IR spectra were recorded in either Nujol or KBr pellets on a Specord IR-75 (Carl Zeiss) spectrometer. Electron spectra were measured on a Shimadzu UV-160 spectrometer using quartz cells. GC analyses were performed on a HP 5830A gas chromatograph equipped with a flame ionization detector and a CP SIL 8CB column.

2.2. Kinetic measurements

2.2.1. Reactions of $dbcatH_2$

Reactions of $dbcatH_2$ with O_2 in the presence of TEMPO were performed in MeOH solutions. In a typical experiment, $dbcatH_2$ was dissolved, under argon atmosphere, in a thermostated reaction vessel with an inlet for taking samples with a syringe, and connected to a mercury manometer to regulate constant dioxygen pressure. The solution was then heated to the appropriate temperature. The TEMPO catalyst was added after this. A sample was then taken by syringe, and the initial concentration of 3,5-di-*tert*-butyl-*o*-benzoquinone (dbq) was determined by UV–vis spectroscopy, measuring the absorbance of the reaction mixture at 401 nm (log $\varepsilon = 3.2$) [λ_{max} of a typical band of dbq]. Then, the argon was replaced with dioxygen and the formation of dbq was analyzed periodically (ca. every 10–30 min). The rate of the oxidation reaction was independent of the stirring rate, excluding eventual diffusion control effects. Experimental conditions are summarized in Table 1. The temperature was determined with an accuracy of ±0.5 °C; the concentrations of dbq were measured with a relative mean error of ca. ±2%; the pressure of dioxygen was determined with an accuracy of ±1%. The O_2 concentrations at various temperatures were taken from the literature (Kretschmer, Nowakowska, & Wiebe, 1946).

Reactions of $dbcatD_2$ with O_2 in the presence of TEMPO were performed in methyl alcohol-*d* (MeOD) solutions using the same methods as written above.

2.2.2. Preparation of $dbcatD_2$

1.00 g of $dbcatH_2$ was suspended in 20 cm^3 of MeOD and the suspension was stirred at 60 °C for 3 h and then the solvent was removed by vacuum. This operation was repeated three times and the resulting light brown filtrate was dried in a vacuum. Yield: 89%. The purity of $dbcatD_2$ was determined by IR to be 95%.

3. Results and discussion

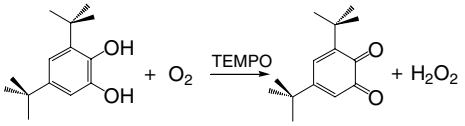
We have found that the oxidation of $dbcatH_2$ to dbq by O_2 is initiated by TEMPO. The reaction takes place at ambient temperature and 1 bar (105 Pa) O_2 pressure in MeOH. According to parallel electron spectroscopic, gas volumetric and iodometric measurements, the stoi-

Table 1
Kinetic data for the TEMPO-initiated oxidation of $dbcatH_2$

Experiment number	Temperature (°C)	10^3 [O_2] (M)[a]	10^3 [$dbcatH_2$] (M)[a]	10^3 [TEMPO] (M)[a]	10^6 k' (s^{-1})	k (M^{-2} s^{-1})
1	50	4.33	12	1.20	8.35 ± 0.21	1.61 ± 0.04
2	50	4.33	18	1.20	9.34 ± 0.36	1.80 ± 0.07
3	50	4.33	24	1.20	7.79 ± 0.26	1.50 ± 0.05
4	50	4.33	30	1.20	7.68 ± 0.26	1.48 ± 0.05
5	50	4.33	36	1.20	8.55 ± 0.16	1.65 ± 0.03
6	50	4.33	36	0.80	6.43 ± 0.51	1.86 ± 0.15
7	50	4.33	36	2.40	14.62 ± 0.87	1.41 ± 0.08
8	50	4.33	36	3.60	22.07 ± 1.54	1.41 ± 0.10
9	50	1.17	24	1.20	2.39 ± 0.15	1.70 ± 0.11
10	50	2.18	24	1.20	3.36 ± 0.26	1.28 ± 0.10
11	50	3.64	24	1.20	6.84 ± 0.13	1.57 ± 0.03
12	50	5.52	24	1.20	10.46 ± 0.66	1.58 ± 0.10
						1.58 ± 0.03[b]
13	31	8.67	24	1.20	4.68 ± 0.62	0.45 ± 0.06
14	38	6.91	24	1.20	5.63 ± 0.25	0.68 ± 0.03
15	44	5.72	24	1.20	6.71 ± 0.34	0.98 ± 0.05
16	50	4.33	24	1.20	5.93 ± 0.27	0.87 ± 0.04[c]
17[a]	50	12.0	24	1.20	4.31 ± 0.21	0.30 ± 0.02[d]

[a] In 30 cm^3 MeOH.
[b] Mean value of the kinetic constant k_{obs} and its standard deviations $\sigma(k_{obs})$ were calculated as $k_{obs} = (\sum_i w_i k_i / \sum_i w_i)$ and $\sigma(k_{obs}) = (\sum_i w_i (k_i - k_{obs})^2 / (n-1) \sum_i w_i)^{1/2}$, where $w_i = 1/\sigma_i^2$.
[c] Oxidation of $dbcatD_2$ in MeOD.
[d] In CCl_4.

chiometry of the oxidation reactions corresponds to Eq. (1). No other oxidation products could be detected.



(1)

The homogeneous redox reactions were performed under pseudo-first-order conditions [Eq. (2)]; ($P(O_2)$ and TEMPO concentrations were kept constant) and the pseudo-first-order rate constants k' were determined spectrophotometrically by monitoring the formation of dbq at 400.5 nm (log $\varepsilon = 3.21$) as a function of time (Fig. 2).

$$-\mathrm{d}[\mathrm{dbcatH_2}]/\mathrm{d}t = k'[\mathrm{dbcatH_2}]^m \quad (k' = k[\mathrm{TEMPO}]^n[\mathrm{O_2}]^q) \tag{2}$$

Measuring the time dependence of the change of concentration of dbq during the oxidation shows that plots of log[$dbcatH_2$] vs. time were linear in experiments 1–5 (Table 1), indicating that the reaction is first-order with respect to substrate concentration. From variations of the reactions rates, plots of $-$d[$dbcatH_2$]/dt vs. the initial $dbcatH_2$ concentration (Fig. 3) were also linear in experiments 1–5 with a correlation coefficient of 99.19%, reinforcing the fact that the reaction is indeed first-order with respect to substrate concentration. This means that $m = 1$.

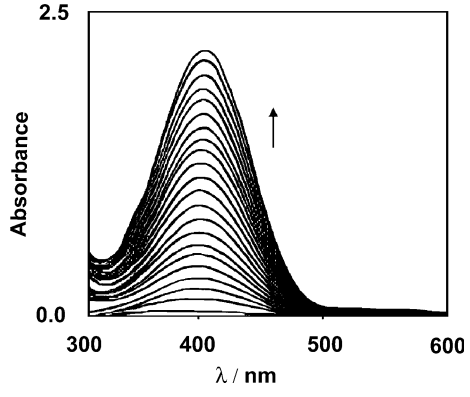


Fig. 2. Time sequence of the increase in the absorption band of dbq at the TEMPO-initiated reaction during experiment 3 in Table 1.

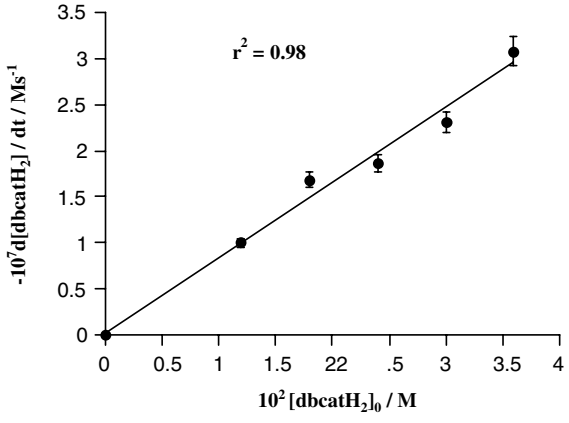


Fig. 3. Plot of oxidation rate of $dbcatH_2$ vs. initial concentration (experiments 1–5, Table 1).

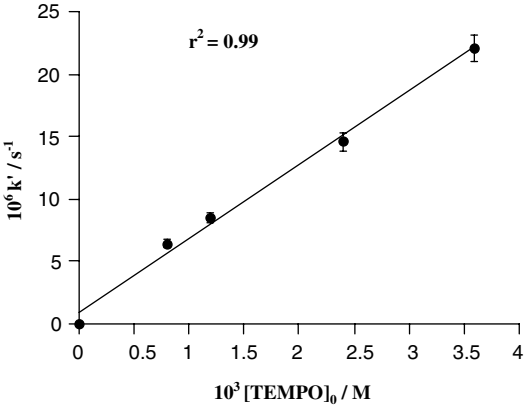


Fig. 4. Plot of pseudo-first-order reaction rate constant (k') vs. the initial TEMPO concentration for the oxidation of $dbcatH_2$ (experiment 5–8, Table 1).

Kinetic measurements of the reaction rate with respect to TEMPO concentration (Table 1; experiments 5–8) indicated a first-order dependence ($n = 1$). Plots of k' vs. $[TEMPO]_0$ (Fig. 4) for the above four experiments gave a straight line with a correlation coefficient of 99.65%.

Experiments conducted at different dioxygen concentrations (calculated from literature data assuming the validity of Dalton's law, the dissolved concentration of O_2 being 4.33×10^{-3} mol dm^{-3} at 50 °C and 760 mm Hg O_2 pressure) show that the dioxygen concentration affects the reaction rate (Table 1; experiments 3, 9–12) and that the reaction is also first-order with respect to dioxygen concentration (Fig. 5). On the basis of the above results, one can conclude that the oxidation of $dbcatH_2$ obeys an overall third-order rate equation with $m = n = q = 1$ (Eq. (2)), from which a mean value for the kinetic constant k_{obs} of 1.58 ± 0.03 M^{-2} s^{-1} at 50 °C was obtained. By the use of $dbcatD_2$ the value of the kinetic constant k_{obs} of 0.87 ± 0.03 M^{-2} s^{-1} and a value for $k_H/k_D = 1.73$ were found at 50 °C (Table 1; experiment

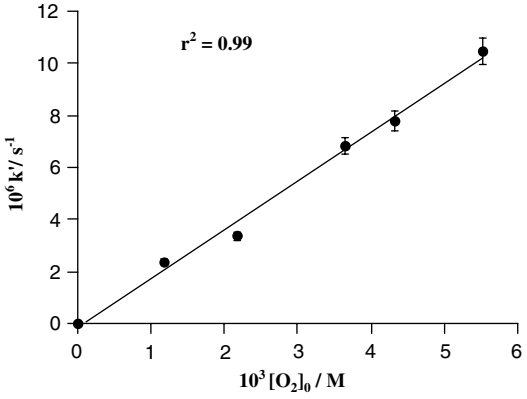


Fig. 5. Plot of pseudo-first-order reaction rate constant (k') vs. the initial dioxygen concentration for the oxidation of $dbcatH_2$ (experiment 3, 9–12, Table 1).

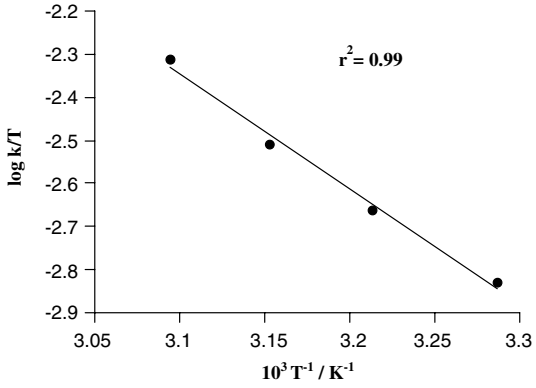


Fig. 6. Eyring plot for the oxidation of $dbcatH_2$ (Table 1).

16). The low value of kinetic isotope effect using $dbcatD_2$ as substrate shows that breaking of the O–H bond precedes the rate-determining step.

The activation parameters for this oxidation reaction were determined from the temperature-dependence of the kinetic constant k_{obs} The Eyring plot $\log(k_{obs}/T)$ vs. $1/T$ obtained at 31, 38, 44, 50 °C (experiments 13–15 in Table 1) gave a straight line (Fig. 6). The slope and the ordinate intercept of this line gave $\Delta H^{\ddagger} = 51 \pm 10$ kJ mol^{-1} and $\Delta S^{\ddagger} = -84 \pm 32$ J mol^{-1} K^{-1}, respectively. Though activation parameters are often not discriminating factors in recognizing the reaction pathway, the large negative entropy of activation, clearly indicates an associative mode of activation in the rate-determining step.

From the experimental data, a mechanism for the oxidation of $dbcatH_2$ catalyzed by TEMPO, as outlined in Fig. 6 can be proposed. This reaction is believed to occur via the intermediation of carbon-centered radicals from the substrate, $dbcatH_2$ produced by the TEMPO radical. Under aerobic conditions, the resulting semiquinone radicals are readily trapped by molecular oxygen to give peroxyl radicals that in turn abstract the hydrogen atom from TEMPOH regenerating TEMPO and giving rise to hydroperoxide. Then the so formed intermediate breaks down into the quinone and hydrogen peroxide (See Fig. 7).

According to this mechanism, we believe, that the key steps of the overall reaction are the abstraction of a hydrogen atom from $dbcatH_2$ by TEMPO and then the reaction of the forming semiquinone radical with dioxygen. These steps take place with rate constants depending on several factors, one of the more important being the intrinsic reactivities of the two reactants and the solvent in which the reaction occurs. The intrinsic reactivities are largely determined by the O–H BDE which can be measured by EPR of the equilibrium constant between couples of phenols and of the corresponding phenoxyl radicals. Since the O–H BDE values in the great majority of phenolic compounds are in the range 80–90 kcal/mol (Lucarini, Mugnaini, & Pedulli, 2002),

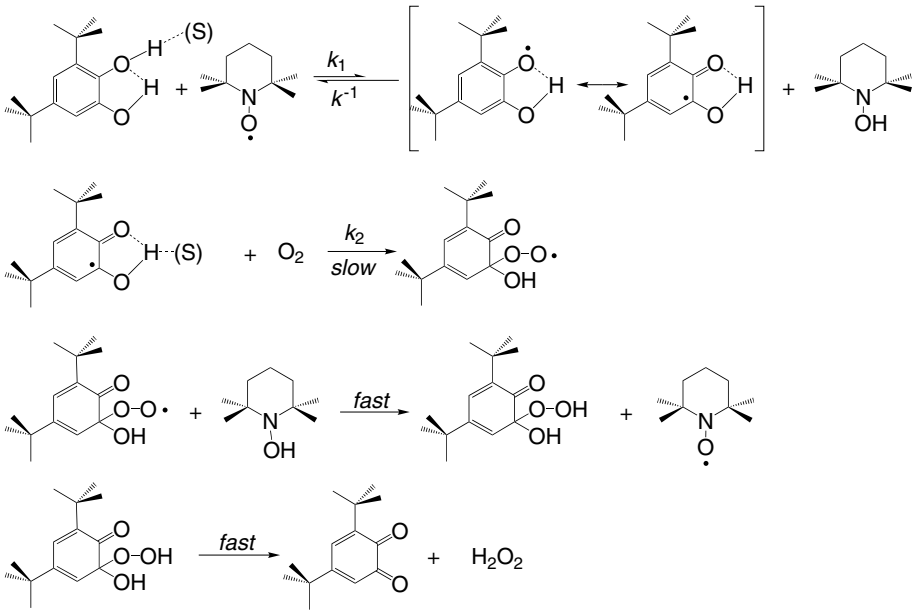


Fig. 7. Proposed mechanism of TEMPO-catalyzed 3,5-di-*tert*-butylcatechol oxidation.

and the experimentally determined O–H BDE value of TEMPO is close to 70 kcal/mol (Minisci, Recupero, Pedulli, & Lucarini, 2003) the abstraction of H-atoms from phenolic compounds by TEMPO is unfavoured. These reactions are strongly endothermic and thus hardly feasible on thermodynamic grounds. Catechols have been found to experience strong kinetic solvent effects on the hydrogen atom abstraction reaction by oxygen-centered radicals. It is now well-established that intermolecularly hydrogen-bonded ArOH molecule are essentially unreactive to all radicals and that only "free", non-hydrogen-bonded ArOH react. 3,5-Di-*tert*-butylcatechol is an outstanding H-atom donor in poor hydrogen-bond acceptor solvent. The rate constant in CCl_4 for the reaction with DPPH · radical is $2.1 \times 10^4\ M^{-1}\ s^{-1}$, while this value in *n*-propanol is $0.007 \times 10^4\ M^{-1}\ s^{-1}$ (Foti, Barclay, & Ingold, 2002). These values are much larger than that was found for the TEMPO-catalyzed $dbcatH_2$ oxidation, suggesting that the rate-determining step in our system is the reaction of the semiquinone radical with dioxygen instead of hydrogen abstraction.

It is also known, that the intramolecular hydrogen bond between the remaining hydroxyl proton and the oxygen radical center is responsible for the relatively large value of radical stabilization energy (4.4 kcal/mol) (Lucarini et al., 2002). Oxidations of $dbcatH_2$ have also been carried out in CCl_4 to see the solvent effect for the rate-determining step and the value of the kinetic constant k_{obs} of $0.30 \pm 0.02\ M^{-2}\ s^{-1}$ was found at 50 °C (experiment 17 in Table 1). This value is five times smaller than that found for methanol, suggesting that, for the rate-determining step, there is an opposite trend; the hydrogen-bonded carbon-centered semiquinone in methanol is much more reactive with dioxygen.

We believe that the low value for the oxidation rate and the antioxidant activity of the catechol-containing polyphenols is largely due to the stabilization of the aryloxyl radicals due to the formation of intramolecular hydrogen bonding.

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