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Investigation on the fluorescence quenching of 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) by certain estrogens and catechols

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Abstract

The fluorescence quenching of singlet excited 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), a fluorescent probe for antioxidants, by various estrogens and certain catechols has been investigated. The time resolved and steady state fluorescence quenching experiments were conducted in acetonitrile and dichloromethane. The bimolecular quenching rate constant (k_q) values are in the range of 10^7–10^9 M^{-1} s^{-1} for estrogens and 10^9–10^{10} M^{-1} s^{-1} for catechols. In the case of estrogens, a direct hydrogen atom abstraction is proposed, while exciplex induced quenching becomes competitive for catechols with electron donating (ED) substituents. The quenching mechanism was analysed on the basis of exciplex formation, deuterium isotopic effects and cyclicvoltammetric studies. Further, the invitro-antioxidant activity of estrogens and catechols were evaluated with rat liver catalase by gel electrophoresis. The data suggest the involvement of hydrogen atom transfer in the fluorescence quenching of DBO by estrogens and catechols.

Keywords: 2,3-Diazabicyclo[2.2.2]oct-2-ene; Fluorescence quenching; Estrogens; Catechols

1. Introduction

The photochemical reactions of ketones with n,π^* states [1] resembling simple alkoxyl radicals [2–4] in their reactivity are well studied. Such reactive radicals are responsible for cellular damage in biological systems [5]. Antioxidants play a vital role in medicine, biology, polymer chemistry, cosmetics and in food industry. By intercepting oxidizing species, predominantly reactive radicals, they prevent cellular damage and polymer or food degradation. The important aim in such research areas is the quantification of their reactivity [6,7]. The reactivity of *t*-butoxyl (*t*-BuO$^\bullet$) radical towards antioxidants [8,9] has been studied by transient absorption spectroscopy. Alternatively, the reactivity of n,π^* triplet-excited ketones, mostly benzophenone (Ph_2CO), has been analysed by laser flash photolysis to obtain direct information on antioxidant reactivity [8,10]. The hydrogen abstraction by n,π^*-excited chromophores, in particular ketones, is the most extensively studied photoreaction [11]. The mechanism of charge transfer processes [12,13] is vital to the understanding of many photochemical and photobiological reactions. The reactions of singlet-excited states, which occur often on faster time-scales, and the reactions of other n,π^* chromophores except the carbonyl group (C=O), which may be of minor practical relevance, have received comparatively less attention. Some aspects of hydrogen abstraction reactions of n,π^*-excited states have appeared recently [14–16]. Of late there is great interest on fluorescent probe for biomolecules [17–19]. However, the fluorescence lifetime of common fluorophores are typically in the range of several nanoseconds or even less. This is too short to monitor nanosecond-to-microsecond processes, which is important to understand the functions and dynamics of many biomolecules.

The azoalkane, 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) has an extremely long fluorescence lifetime (up to 1 μs) [20]. Its n,π^*-excited states behave in a radical-like way, thus the reactivity resembles simple alkoxyl radicals [2–4,21] and has been extensively employed as a fluorescent probe for antioxidants [22–24]. DBO shows a pronounced tendency to undergo direct

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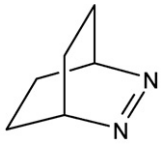
Table 1
Fluorescence quenching rate constants and thermodynamic data of DBO by estrogens

S. No.	Quencher (Estrogens)	$k_q/(10^9\ M^{-1}\ s^{-1})$[a]			D	$E^{OX}_{1/2}$ vs. SCE (V)[b]	ΔG_{et} (eV)[c]
		A	*B*	*C*			
1	DES	1.38 [1.46]	1.03 [1.18][d]	1.05	1.46	1.83	1.27
2	EST	0.70 [0.75]	0.47 [0.82][e]	0.55	1.91	1.42	0.86
3	EED	0.65 [0.69]	0.44 [0.18][f]	0.39	1.08	1.57	1.01
4	BEST	0.77 [0.83]	0.43	0.58	1.72	1.44	0.88
5	EEDME	0.09 [0.09]	0.09	0.06	1.12	1.60	1.04

[a] Bimolecular fluorescence quenching rate constants (*A*) measured by steady state method in CH_3CN ($\tau = 620$ ns); values in brackets are measured by time resolved method in CH_3CN, (*B*) measured by steady state method in CH_2Cl_2 ($\tau = 185$ ns), (*C*) measured by steady state method in wet acetonitrile (10% H_2O) (*D*) deuterium isotopic effect measured by steady state fluorescence quenching in CH_3CN containing 10% H_2O or D_2O, i.e. [k_q (O—H)/k_q (O—D)].
[b] Irreversible oxidation potential of estrogens in V vs. SCE in CH_3CN.
[c] Calculated by Rhem–Weller equation $\Delta G_{et} = E^{OX}(D) - E^{red}(A) - E^* + C$, the reduction potential of DBO is −2.8 V vs. SCE, $E^* = 3.3$ eV and $C = -0.06$ (for acetonitrile). Error ±10%.
[d] Data for model compounds from [20].
[e] Data for model compounds from [20].
[f] Data for model compound from [48].

hydrogen abstraction reactions [25–28] with alcohols, ethers, alkanes, phenols and alkylbenzenes. Taking advantage of this property, DBO has been used in this work to probe the reactivity of the mentioned estrogens and catechols.



2,3-Diazabicyclo[2.2.2]oct-2-ene (DBO)

Steroidal estrogens have been reported to function as antioxidants and free radical scavengers under a variety of experimental conditions. For example, phenolic and catecholic estrogens prevent lipid peroxidation induced by diverse pro-oxidants in microsomes [29–31] and liposomes [32,33]. They exert their protective effects to different extents with different efficiencies depending on the phenolic and catecholic structure of the molecule [5]. In the present work, we have studied the fluorescence quenching of DBO by various estrogens such as estrone (EST), β-estradiol (BEST), diethylstilbesterol (DES), 17-α-ethynylestradiol (EED), ethynylestradiol-3-methyl ether (EEDME) as well as certain catechols such as catechol (CAT), tetrachlorocatechol (TCC), 3,5-di-*tert*-butylcatechol (DTBC), 4-nitrocatechol (4-NC), 4-*tert*-butylcatechol (4-TBC), 3-methylcatechol (3-MC). The quenching rate constants (k_q) of DBO were determined by both steady state and time resolved fluorescence technique in acetonitrile and dichloromethane (Tables 1 and 2). Rehm–Weller equation was applied to measure free energetics for electron transfer process.

2. Experimental

2.1. Materials

DBO was obtained as a gift sample from Prof. W.M. Nau, International University of Bremen, Germany. The estrogens, catechols and D_2O were obtained from Sigma–Aldrich, USA. Potassium ferricyanide, ferric chloride, H_2O_2, KH_2PO_4, K_2HPO_4, glycerol and Tris-buffer were obtained from Merck, India. The spectroscopic grade solvents, CH_3CN and CH_2Cl_2 were used for preparing the solutions. Fresh rat liver tissue homogenate was used as enzyme source for gel electrophoresis. All measurements were performed at ambient temperature.

Table 2
Fluorescence quenching rate constants and thermodynamic data of DBO by catechols

S. No.	Quencher (Catechols)	$k_q/(10^9\ M^{-1}\ s^{-1})$[a]			D	$E^{OX}_{1/2}$ vs. SCE (V)[b]	ΔG_{et} (eV)[c]
		A	*B*	*C*			
1	CAT	1.10 [1.19]	0.92	0.95	1.85	0.95	0.39
2	TCC	2.64 [2.77]	1.77	2.13	2.10	1.36	0.80
3	DTBC	2.42 [2.36]	4.10	2.08	1.45	1.40	0.84
4	4-NC	43.6 [40.1]	42.9	42.2	1.62	2.09	1.53
5	4-TBC	1.73 [1.57]	2.83	1.39	1.32	1.28	0.72
6	3-MC	1.24 [1.40]	1.95	1.02	1.19	1.17	0.61

[a] Bimolecular fluorescence quenching rate constants (*A*) measured by steady state method in CH_3CN ($\tau = 620$ ns); values in brackets are measured by time resolved method in CH_3CN, (*B*) measured by steady state method in CH_2Cl_2 ($\tau = 185$ ns), (*C*) measured by steady state method in wet acetonitrile (10% H_2O) (*D*) deuterium isotopic effect measured by steady state fluorescence quenching in CH_3CN containing 10% H_2O or D_2O, i.e. [k_q (O—H)/k_q (O—D)].
[b] Irreversible oxidation potential of catechols in V vs. SCE in CH_3CN.
[c] Calculated by Rhem–Weller equation $\Delta G_{et} = E^{OX}(D) - E^{red}(A) - E^* + C$, the reduction potential of DBO is −2.8 V vs. SCE, $E^* = 3.3$ eV and $C = -0.06$ (for acetonitrile). Error ±10%.

2.2. Methods

2.2.1. Fluorescence quenching experiments

Samples were prepared by dissolving DBO (ca. 0.1 mM) in proper (acetonitrile and dichloromethane) solvents and administering the appropriate amounts of estrogens and catechols with a GC syringe. The samples were carefully degassed using pure nitrogen gas for 30 min. Quartz cells (4 cm × 1 cm × 1 cm) with high vacuum Teflon stopcocks were used for degassing. For the (Stern–Volmer) quenching plots, various concentrations of quenchers were chosen. Lifetimes and fluorescence intensities were obtained for different quencher concentration and plotted according to the equation: $\tau_0/\tau = I_0/I = 1 + k_q\ \tau_0$ [Q]. The slopes afforded the bimolecular quenching rate constant (k_q). The lifetime (τ_0) of DBO in degassed acetonitrile and dichloromethane without any added quencher was 620 ns and 185 ns, respectively. Deuterium isotope effects for estrogens and catechols were measured in wet acetonitrile (either with 10% H_2O or D_2O) assuming a complete and fast OH/OD exchange of hydrogen.

2.2.1.1. Steady-state measurements. The steady-state fluorescence quenching measurements were carried out in a Perkin-Elmer LS55 Luminescence spectrometer. The excitation wavelength was 365 nm and the emission was monitored at 420 nm. The excitation and emission slit widths (15 nm) and scan rate (500 nm) were maintained constant for all the experiments.

2.2.1.2. Time-resolved experiments. Fluorescence lifetime measurements were carried out in a picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source is the tunable Ti-sapphire laser (TSUNAMI, Spectra Physics, USA). The diode laser pumped millennia V (Spectra Physics) CW Nd-YVO_4 laser was used to pump the sapphire rod in the Tsunami mode locked picosecond laser (Spectra Physics). The diode laser output was used to pump the Nd-YVO_4 rod in the Millennia. The DBO was excited by the laser pulse at 365 nm. The time resolved fluorescence emission was monitored at 420 nm. The emitted photons were detected by a MCP-PMT (Hamamtsu R3809U) after passing through the monochromator (f/3). The laser source is operated at 4 MHz and the signal from the photodiode is used as a stop signal. The signal from the MCP-PMT is used as start signal in order to avoid the dead time of the TAC. The difference between the start and stop signal is due to the time taken by the pulses traveling through the cables and electronic relaxation of the excited state. The data analysis was carried out by the software provided by IBH (DAS-6). The kinetic trace was analyzed by non-linear least square fitting of monoexponential function.

2.2.2. Cyclic voltammetric measurements

The reduction potential for DBO was reported as −2.8 V versus SCE [34] in acetonitrile. In the present study, the oxidation potential of estrogens and catechols were measured in acetonitrile with tetrabutylammonium perchlorate (0.1 M) as electrolyte. The experimental setup consisted of a platinum working electrode, a graphite counter electrode and a silver reference electrode. Irreversible peak potentials were measured at different scan rates (50–100 mV/s). All values are reported relative to ferrocene as internal standard. All samples were deaerated by bubbling with pure nitrogen gas for ca. 5 min at room temperature.

2.2.3. Invitro-antioxidant activity

Liver sample (100 mg/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.0); and then centrifuged at 10,000 rpm for 15 min; the supernatant thus obtained was used for this experiment as a source of catalase enzyme. This experiment was designed as follows:

Sample 1 contains liver homogenate (30 μl), sample 2 contains liver homogenate (30 μl) and 10 μl of H_2O_2 (30%). Samples 3–8 contain 30 μl of liver homogenate, 10 μl of H_2O_2 (30%) and 10 μl of antioxidant compounds as DES, EST, EED, BEST and EEDME, respectively. All samples were shaken well and incubated at 37 °C for 30 min. After the incubation period, equal volume (50 μl) of sample buffer (7.25 ml distilled water, 1.25 ml of 0.5 M Tris buffer (pH 6.8) and 1 ml of glycerol) were added to all the samples and were subjected to non-denaturing polyacrylamide gel electrophoresis (Native – PAGE).

Non-denaturing polyacrylamide gel electrophoresis was performed essentially as described by Laemmli [35], except that SDS was omitted from all the buffers and the samples were not boiled before electrophoresis. Electrophoretic separation was performed on 8% gel at 4 °C with a constant power supply of 50 V for stacking gel and 100 V for separating gel.

Catalase activity was detected by the method of Woodbury et al. [36]. The gel was soaked in 5 mM H_2O_2 solution for 10 min and was washed with water and stained with a reaction mixture containing 1% potassium ferricyanide (w/v) and 1% ferric chloride. The enzyme appeared as a yellow band superimposed on a dark green background. The reaction was terminated by adding water and the gel was photographed at once. Quantification of the enzyme bands was performed by a densitometer (GS-300 transmittance/reflectance scanning densitometer, Hoefer Scientific Instruments, USA).

3. Results and discussion

The absorption and emission spectrum of DBO are shown in Fig. 1. The structures of the quenchers used in the present study are given below.

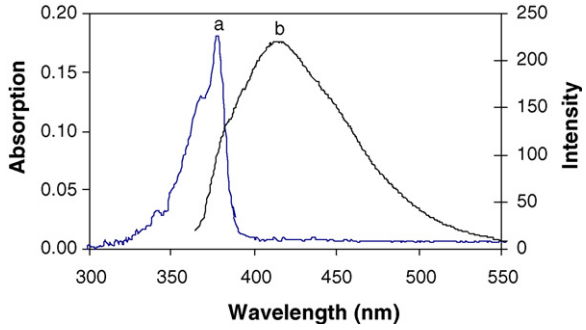
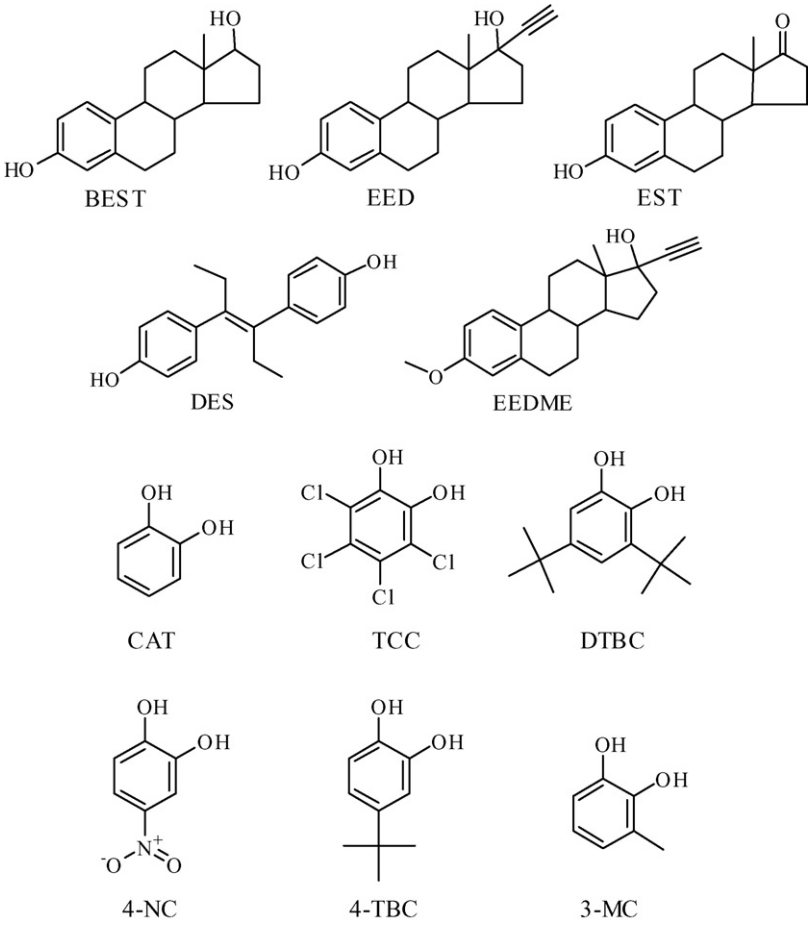


Fig. 1. Absorption (a) and emission (b) spectrum of DBO.



3.1. Fluorescence quenching of DBO by estrogens

The time resolved and steady state quenching of DBO by estrogens provide the same quenching rate constants (within experimental error) as shown in Figs. 2 and 3. The Stern–Volmer quenching plots obtained by the plot of τ_0/τ or I_0/I versus $[Q]$ were linear, indicating that the quenching process is dynamic in nature. As can be seen from literature [20], estrogens possess less k_q values compared with that of glutathione, ascorbic acid, uric acid and α-tocopherol. From the k_q values given in the Table 1, the following trend is observed in acetonitrile among the estrogens:

$$\text{DES} > \text{BEST} \approx \text{EST} \approx \text{EED} > \text{EEDME}$$

In the estrogen structure, the hydroxyl group attached to the aromatic ring is an important feature for the contribution of the antioxidant activity. DES possesses highest k_q among the estrogens because DES contains two monohydroxylated benzene rings (phenolic units) separated by an ethane bridge. Almost similar reactivities among BEST, EST and EED are in accord with the fact that phenolic estrogens possess similar antioxidant activities [37]. These three estrogens possess only one phenolic

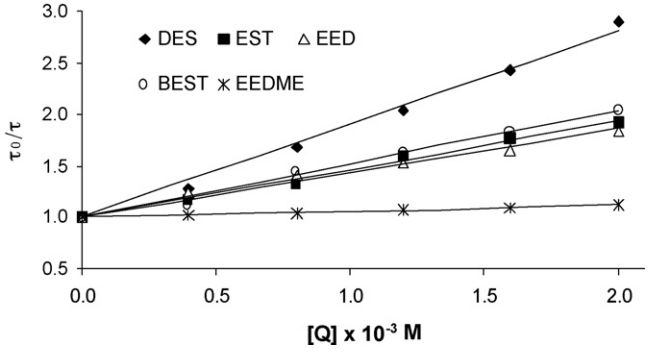


Fig. 2. Stern–Volmer plots for the time resolved fluorescence quenching of DBO (1×10^{-4} M) by estrogens in acetonitrile.

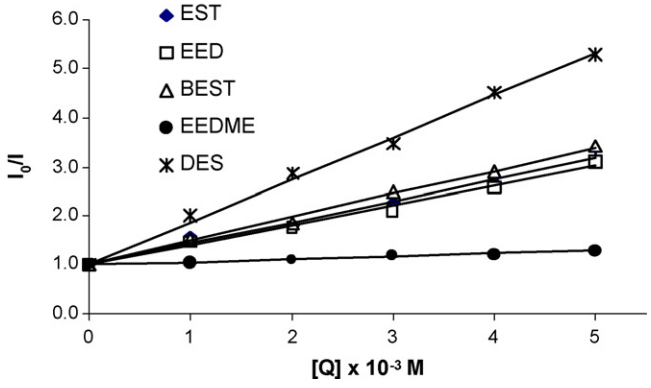


Fig. 3. Stern–Volmer plots for the steady state fluorescence quenching of DBO (1×10^{-4} M) by estrogens in acetonitrile.

group unlike DES. Even though BEST and EED possess one additional hydroxyl group in the "*D*" ring, the k_q values remain almost the same. Hence, the hydroxyl group in the "*D*" ring does not have any significant effect on the antioxidant activity. While analyzing the k_q values of BEST, EST and EED, only a slight variation among these three compounds could be identified. EEDME possesses one order lower magnitude k_q, because the reactive 3-hydroxyl group of the aromatic ring is replaced by $-OCH_3$ group, which proves the importance of aromatic O–H hydrogen in the fluorescence quenching of DBO by estrogens. It may be noted that the antioxidant activity of the estrogens was already established based on the reduction of the blue–green 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonicacid) radical cation ($ABTS^{\bullet+}$) as indicated by the suppression of its characteristic absorption at 734 nm [38]. Such dependence of antioxidant activity on the availability of –OH groups has been reported in the case of flavonoids [39] which also possess more than one aromatic ring just like estrogens. The similar trend with low k_q values has been observed in the case of low polar dichloromethane solvent. The k_q decreases with decreasing solvent polarity [40,49,50], which clearly indicates that the quenching mechanism of singlet excited DBO involves hydrogen atom abstraction. In the present study, the observed trends suggest that the phenolic ring of estrogens could be a crucial component for their antioxidant activity.

3.2. Fluorescence quenching of DBO by catechols

The fluorescence quenching experiments were conducted in dichloromethane and acetonitrile which have different polarities. The Stern–Volmer plots (Figs. 4 and 5) clearly suggest the absence of any static component in quenching. Table 2 provides the fluorescence quenching rate constant for catechols and shows the following trend for quenching rate constants in acetonitrile:

$$4-NC > TCC > DTBC > 4-TBC > 3-MC > CAT$$

Among catechols, 4-NC shows an unusually high quenching rate constant, which is not expected from the electron withdrawing nature of nitro group. To get more insight into the high quenching rate constant, 4-nitrobenzene was used as a quencher to infer the importance of hydroxyl groups. It was found that there is no significant reactivity

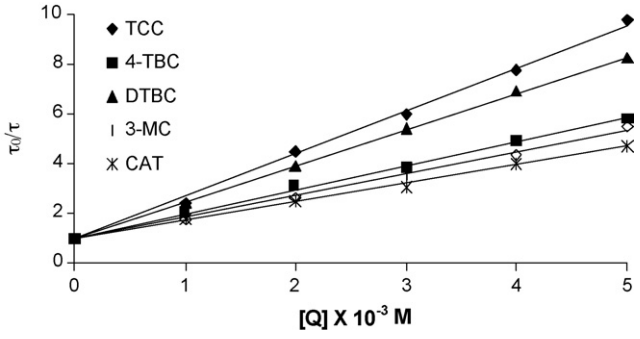


Fig. 4. Stern–Volmer plots for the time resolved fluorescence quenching of DBO (1×10^{-4} M) by catechols in acetonitrile.

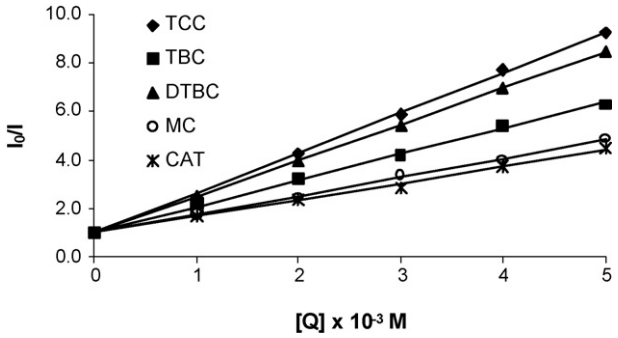


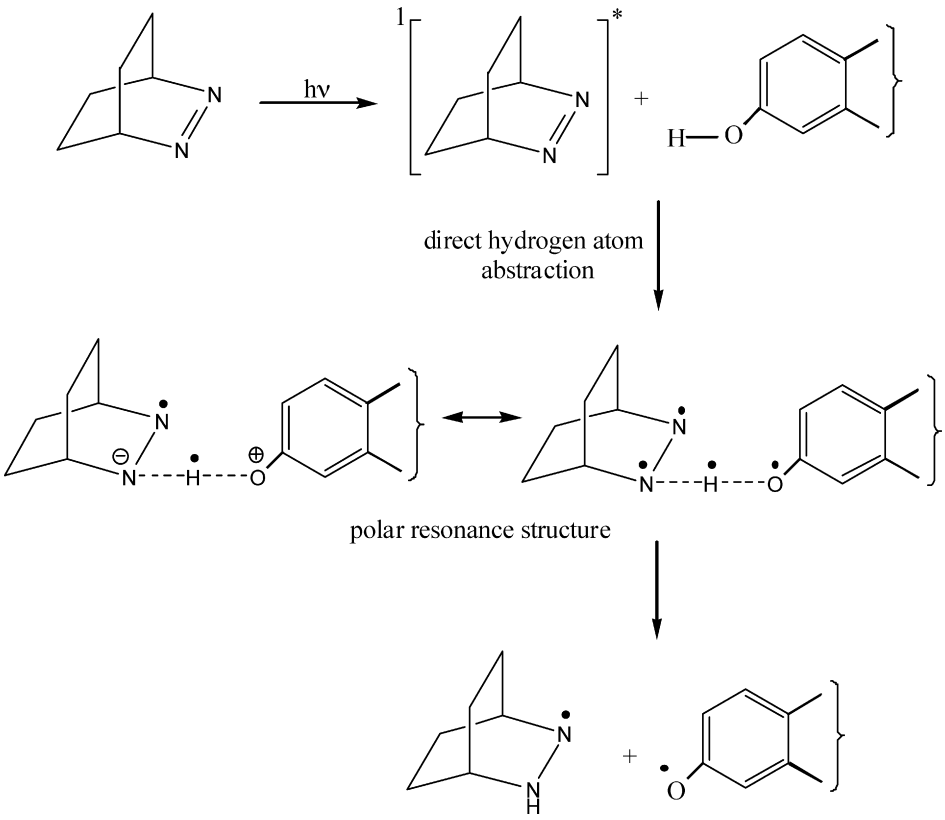
Fig. 5. Stern–Volmer plots for the steady state fluorescence quenching of DBO (1×10^{-4} M) by catechols in acetonitrile.

difference between 4-nitrocatechol ($43.6 \times 10^9\ M^{-1}\ s^{-1}$) and 4-nitrobenzene ($37.8 \times 10^9\ M^{-1}\ s^{-1}$) which speaks against the involvement of aromatic hydroxyl group in hydrogen abstraction. Energy transfer from the excited DBO to 4-NC presents an efficient competitive quenching mechanism in this case [28]. All the substituted catechols show higher k_q than the parent catechol. The k_q values of DTBC, 4-TBC and 3-MC were reduced in high polar solvent (acetonitrile) and increased in low polar solvent (dichloromethane), that is, inverted solvent effect is observed [40]. It is presumed that the initial reaction step involves the formation of an exciplex with partial charge transfer character [11,41–43]. The exciplex is stabilized to a lesser degree by polar solvents than the reactants. These catechols may undergo through the exciplex formation mechanism as electron donating groups tend to stabilize the partial development of positive charge in the aromatic ring. The electron donating groups generally tend to increase the electron density of the aromatic ring by +I effect which might facilitate exciplex formation [Scheme 2] mediated hydrogen atom transfer. The parent catechols and TCC have shown increase in the k_q values with increasing solvent polarity from dichloromethane to acetonitrile. Hence, these catechols may undergo direct hydrogen abstraction mechanism.

The DTBC ($2.42 \times 10^9\ M^{-1}\ s^{-1}$) shows higher k_q than 4-TBC, 3-MC and catechol, which reveals that the fluorescence quenching of DBO increases quite systematically with increase in the number of alkyl groups due to electron donating ability. All catechols show slightly higher k_q value than those of estrogens. The above results infer that catechols are potential antioxidants than estrogens. From these observations, it is proposed that catechols may undergo two different mechanisms (Schemes 1 and 2) based on electronic effects of the substituents on the aromatic ring.

3.3. Deuterium isotope effects

Fluorescence quenching of DBO by estrogens and catechols shows substantial deuterium isotope effects in (10% H_2O or D_2O) CH_3CN/H_2O and CH_3CN/D_2O mixtures (Tables 1 and 2). A small but significant deuterium isotope effect was observed for estrogens between 1.08 and 1.91, which are characteristic for hydrogen abstraction close to diffusion controlled limit [14]. The parent catechol has isotope effect of 1.89 and quenching rate con-



Scheme 1. Fluorescence quenching mechanism of DBO through a direct hydrogen atom transfer.

stant of $1.10 \times 10^9\ M^{-1}\ s^{-1}$. A high isotope effect is observed for TCC (2.10) with relatively high quenching rate constant ($2.64 \times 10^9\ M^{-1}\ s^{-1}$). This possibly suggests a direct hydrogen abstraction leading to dominant quenching. The low values of isotope effect for DTBC, 4-TBC and 3-MC (catechols with electron donating substitutions) with relatively high quenching rate constants compared to that of the parent catechol justifies competitive quenching through exciplex formation. The deuterium isotope effects provide evidence that hydrogen abstraction is involved in the quenching process of singlet excited DBO by estrogens and catechols.

3.4. Invitro-antioxidant activity

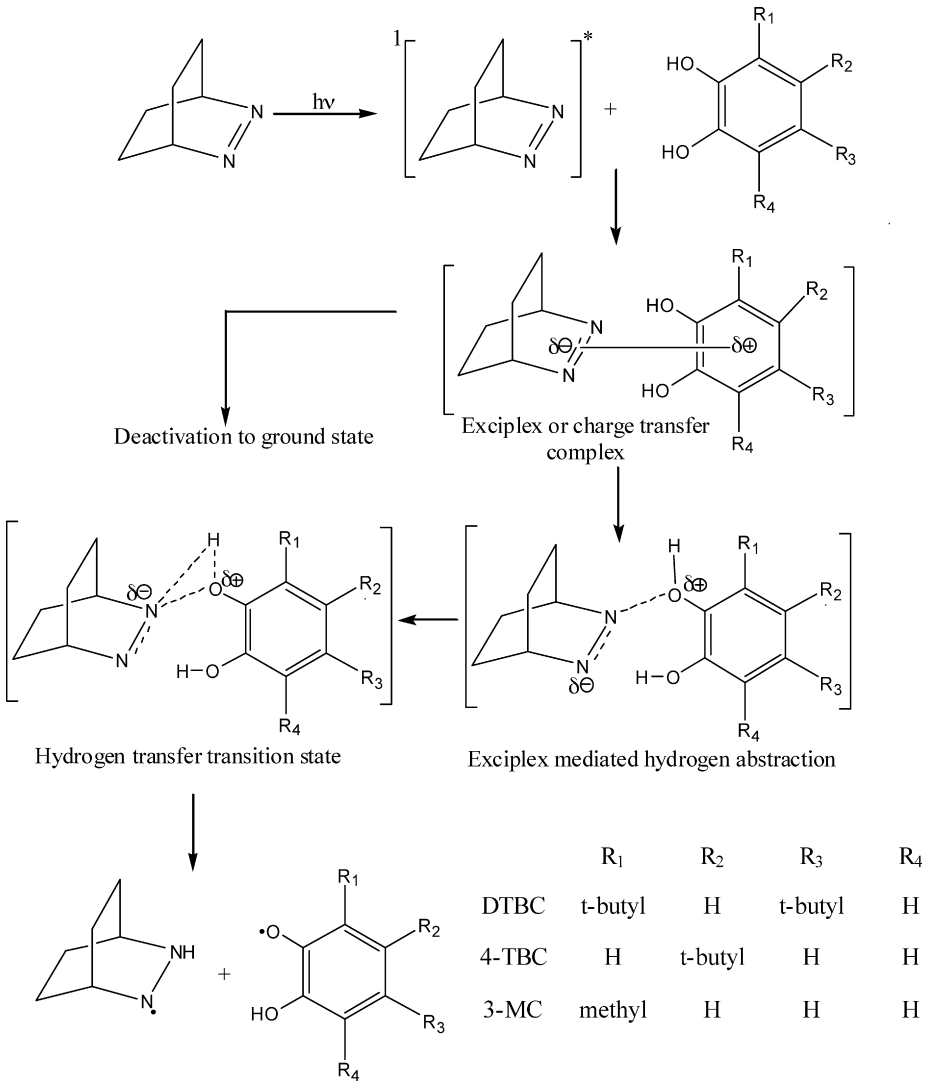
Antioxidant activity of various estrogens and catechols were determined by the electrophoretic method. An analysis of the electrophoretic pattern of catalase enzyme in the liver homogenate revealed one band with variations in the staining intensity of the band among the seven samples of estrogens (Fig. 6) and eight samples of catechols (Fig. 7), the lane 1 (only liver homogenate) exhibited a single band of high intensity (band area 230), while that of lane 3– 7 exhibited a band of almost similar intensity in estrogen compounds (Fig. 6a) (band area 226.00, 217.68, 216.82, 218.16 & 212.98 respectively) and in catecholic compounds (Fig. 7a) (band area 213.90, 220.12, 218.97, 228.14, 217.85 and 216.72, respectively). However, lane 2 (liver homogenate damaged by H_2O_2) revealed a single band with less intensity (band area 211.29).

Among the estrogen compounds tested DES possess high antioxidant activity whereas compounds EST, EED and BEST show similar activity. However, EEDME has low activity than the other compounds. In the case of catechol compounds, 4-NC possesses high antioxidant activity whereas the compounds TCC, DTBC, 4-TBC and 3-MC possess antioxidant activity in the following order: TCC > DTBC > 4-TBC > 3-MC. However, CAT has low activity than the other catechol compounds. The ability to scavenge free radicals by the estrogen and catechol compounds is correlated with the number of hydroxyl group bound with the aromatic ring and their structure, position of aromatic –OH ring.

Catalase is an antioxidant enzyme, which is present in most cells and catalyses the decomposition of H_2O_2 to water and O_2. In the present study the enzyme catalase was inactivated by H_2O_2 (lane 2) and then activated by the estrogen and catechol compounds (Figs. 6 and 7). This might be due to the potential antioxidant activity of the estrogens and catechols. The H_2O_2 could be scavenged by estrogen and catechol compounds. This might be the reason for increasing intensity of the bands in lanes 3– 8. This result strongly indicates the potential antioxidant activity of studied estrogens and catechol compounds.

3.5. Thermodynamic Properties of Estrogens and Catechols

The oxidation potential of estrogens and catechols are given in the Tables 1 and 2, respectively. The relevant mechanistic pathways are illustrated in Schemes 1 and 2. The question



	R_1	R_2	R_3	R_4
DTBC	t-butyl	H	t-butyl	H
4-TBC	H	t-butyl	H	H
3-MC	methyl	H	H	H

Scheme 2. Exciplex-induced quenching of DBO by electron donating (ED) substituents of catechols.

whether the photoreduction operates through sequential electron and proton transfers or by direct H-atom abstraction from the estrogens and catechols by the excited azoalkane can only be answered speculatively on the basis of the free energetics for electron transfer estimated by the Rehm–Weller equation [44]

$$\text{i.e.,} \quad \Delta G_{et} = E^{OX}(D) - E^{red}(A) - E^{*} + C$$

where E^{OX} (D) is the oxidation potential of donor, E^{red} (A) is the reduction potential of acceptor, E^{*} is the excitation energy of the acceptor and C is the coulomb term which describes the electrostatic attraction within the contact ion pair and has a value of ca.— 0.06 eV in acetonitrile. The calculated ΔG_{et} values are listed in Tables 1 and 2.

The electrochemical data imply positive ΔG_{et} values for excited ^{1}DBO with estrogens and catechols (ΔG_{et} values in Tables 1 and 2). The variation in the oxidation potential of estrogens and catechols arises due to their structure. Among the estrogens studied, DES possesses higher oxidation potential because of its readily oxidisable nature and resonance stabilization. EED and EEDME possess almost similar oxidation potential due to its similarity in structure (presence of –C≡CH– group). EST and BEST shows lower oxidation potential.

Among catechols, 4-nitrocatechol shows highest E_{ox} (2.09 V versus SCE) due to its electron withdrawing nature of the nitro group (inductive effect). The alkyl substituted catechols show higher E_{ox} values than the parent catechol. Among the alkyl substituted catechols the following trend is noticeable: DTBC > 4-TBC > 3-MC. The E_{ox} increases with increasing number of alkyl group. The driving force for electron transfer is significantly endergonic for estrogens and catechols. An endergonic thermodynamics reveals that quenching through hydrogen abstraction may be a possible mechanism.

The overall mechanistic pathways of the photoreduction of DBO by estrogens and catechols are illustrated in Schemes 1 and 2. The photoinduced electron transfer can be excluded on the basis of endergonic energetics. The quenching may occur along two pathways: (i) the formation of an excited state charge transfer complex (exciplex) or (ii) direct hydrogen abstraction. In the transition state for direct hydrogen abstraction, polar resonance structure becomes important,

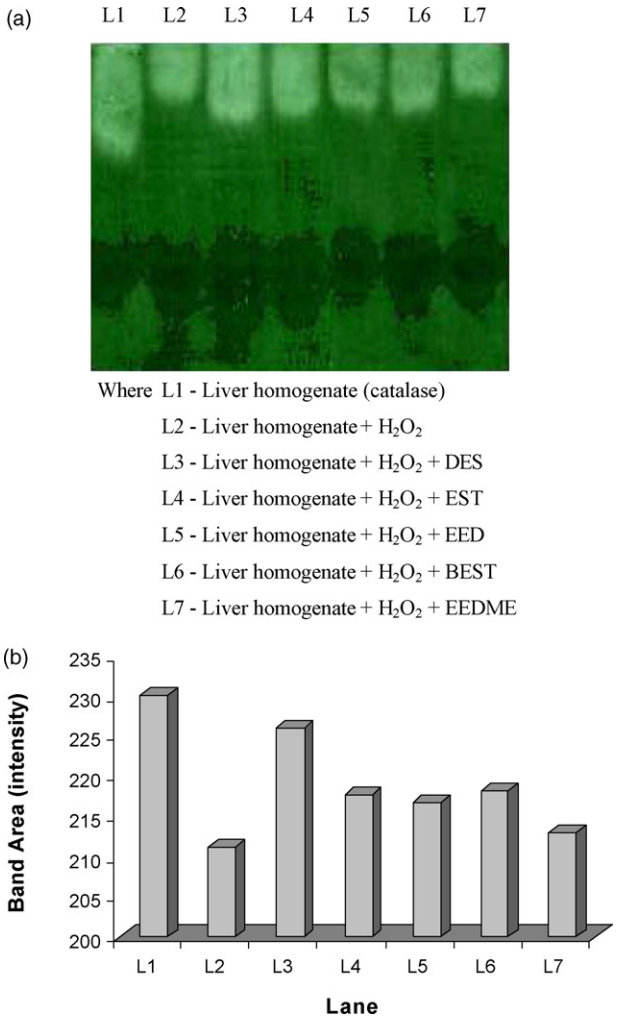


Fig. 6. Invitro-antioxidant activity of estrogens by gel electrophoresis (a) densitometric pattern of catalase enzyme from liver homogenate treated with estrogens.

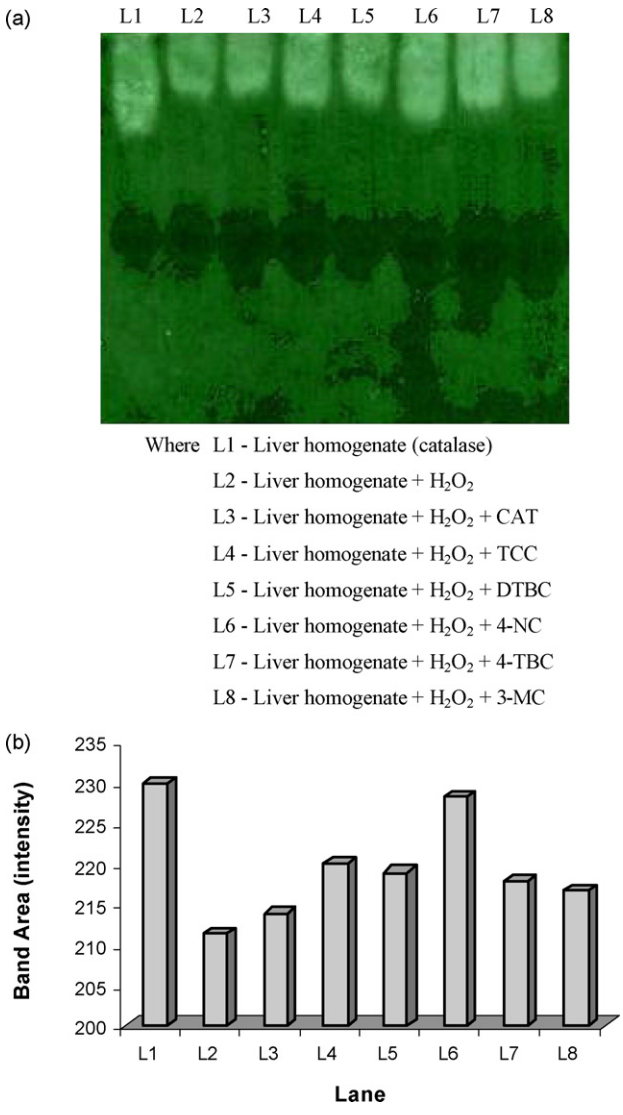


Fig. 7. Invitro-antioxidant activity of catechols by gel electrophoresis (a) densitometric pattern of catalase enzyme from liver homogenate treated with catechols.

which accounts for the possible observation of polar substituent effects in the hydrogen abstraction. In the case of estrogens, parent catechols, and TCC, quenching involves direct hydrogen abstraction. Excited state hydrogen abstraction not only may be achieved by direct abstraction, but also may occur from exciplex (mediated abstraction) involving lone pair coordination. Catechols with electron donating substituents involve hydrogen abstraction through formation of exciplex. Alternatively, the exciplex may induce deactivation to the ground state. Hydrogen atom abstraction from the phenolic hydroxyl group is the proposed quenching mechanism. The estrogen phenoxyl radical formed after the donation of hydrogen atom to DBO would be relatively long-lived and stabilized by internal delocalization of the electron deficiency around its aromatic structure.

The same quenching mechanism of singlet excited DBO has been characterized in theoretical and experimental detail with respect to the involvement of conical intersections along the quenching pathways [11,25,34,45]. Quenching of DBO proceeds via hydrogen atom transfer [26,44,46] as does the scavenging of reactive radicals by vitamin E in vitro [47]. From the quenching rate constant and endergonic thermodynamics, the fluorescence quenching of DBO by estrogens and catechols occurs through a reaction mechanism that "mainly" involves hydrogen atom from the hydroxyl group. The experimental data obtained from various experiments i.e., quenching studies (steady state and time resolved), cyclic voltammetric studies and gel electrophoresis studies are fully consistent with this interpretation.

3.6. Tentative mechanism

Scheme 1.

4. Conclusions

The compound DBO is quenched by estrogens and catechols in acetonitrile and dichloromethane and the corresponding k_q (fluorescence quenching rate constant) depends on the availability of –OH group in the aromatic '*A*' ring of the estrogens. The involvement of exciplexes of DBO and catechols with electron

donating substituents, i.e. partial charge transfer intermediates, is suggested by the observation of an "inverted solvent effect". The trend in quenching efficiency and antioxidant capacity was similar in all the three methods studied. It clearly suggests that the quenching of singlet excited DBO by estrogens and catechols involves hydrogen atom abstraction based on the experimental data obtained from time resolved and steady state fluorescence quenching technique, cyclic voltammetric and invitro-antioxidant activity studies.

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