Reactivity of adrenaline toward alkoxyl radicals and carbonyl triplet states

Gonzalo Cosa*[a] and J. C. Scaiano*[b]

Received 26th June 2008, Accepted 1st September 2008
First published as an Advance Article on the web 31st October 2008
DOI: 10.1039/b810765c

The reactivities of adrenaline toward hydrogen abstraction by the *tert*-butoxyl radical and the excited triplet state of benzophenone have been examined in solution using laser flash photolysis techniques. The rate constants obtained in acetonitrile-rich solvents are 13 and 1.5×10^8 M^{-1} s^{-1} for benzophenone triplet and the *tert*-butoxyl radical, respectively. Adrenaline is a better hydrogen donor than phenol, but not as efficient as α-tocopherol.

Introduction

Adrenaline, together with noradrenaline and dopamine, belongs to the general class of compounds known as catecholamines. These molecules act both as neurotransmitters in the brain, and as hormones in the blood system. They are produced in the organism by the enzyme tyrosine hydroxylase, which transforms tyrosine into dopa, a first intermediate in their synthesis (see Fig. 1).[1]

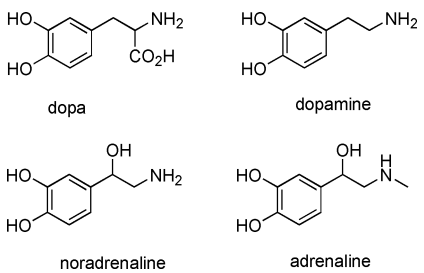
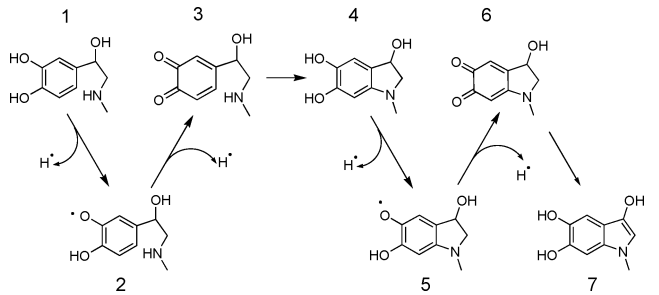


Fig. 1 Structure of various catecholamines.

Catecholamines may readily undergo oxidation ultimately leading to melanin pigments which are known for their protection against solar radiation.[2–6] The protective effect is due not only to a screening effect of the solar radiation, but also the antioxidant effect of melanin plays an important role as a scavenger of radicals.[3,7–9] In the case of dopamine it is also worth noting that the immediate products following its oxidation are precursors of the potent neurotoxin 6-hydroxydopamine, a compund involved in oxidative stress neuronal degeneration.[10]

Catecholamines share a common oxidation pathway; Scheme 1 illustrates it with adrenaline.[11–16] It is initiated by the oxidation of the dihydroxybenzylamine (**1**) to the amine semiquinone radical (**2**); the latter disproportionates to form aminoquinone (**3**) and **1**. Upon cyclization aminoquinone forms leucoaminochrome (**4**). Leucoaminochrome further oxidizes to form leucoaminochrome semiquinone radical (**5**), which upon disproportionation generates aminochrome (**6**) and **4**. Aminochrome then rearranges to aminoleutine (**7**).



Scheme 1 Oxidation of adrenaline. 1 adrenaline, 2 adrenaline semiquinone radical, 3 adrenoquinone, 4 leucoadrenochrome, 5 leucoadrenochrome semiquinone radical, 6 adrenochrome, 7 adrenoleutine.

The oxidation of catecholamines may be initiated by free radical attack on the dihydroxybenzene moiety resulting in H-abstraction and formation of an aryloxyl or semiquinone radical.[17,18] The kinetics of formation of the different transients involved in the oxidation of dopa semiquinone radical (dopa equivalent of **2**) to dopachrome (dopa equivalent of **6**), as well as their spectroscopy, have been described following pulse radiolysis studies in water.[12,15] The reactivity of catecholamines has also been analyzed in the presence of peroxyl radicals.[18]

Here we report absolute rate constants for the reaction of the catecholamine adrenaline with *t*-butoxyl radicals and carbonyl triplets, specifically, triplet benzophenone. Our goal is to learn on the reactivity of adrenaline towards these highly reacticve intermediates in order to explore the feasibility of radical mediated degradation of catecholamines. The choice of *t*-butoxyl radicals and triplet benzophenone as the oxidating agents of adrenaline is not casual. Previous results existing in the literature, where the former are quenched by well known radical scavengers such as phenol,[19,20] α-tocopherol,[21,22] aminoleutins[7] or melatonin,[23] will allow us to compare the free radical scavenging activity of these phenols to that of adrenaline.

Experimental section

Materials

Adrenaline ((±) 4-[1-hydroxy-2-methylamino) ethyl]-1,2-benzenediol), benzhydrol, di-*tert*-butyl peroxide (treated in alumina column before use) and 1,4-cyclohexadiene (distilled before use) were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada).

[a]*Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, QC, H3A 2K6, Canada. E-mail: gonzalo.cosa@mcgill.ca; Fax: (514) 398-3797; Tel: (514) 398-6932*
[b]*Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, ON, K1N 6N5, Canada. E-mail: tito@photo.chem.uottawa.ca; Fax: (613)562-5633; Tel: (613)562-5896*

Halostachine ((±)1-hydroxy-1-phenyl-2-methylaminoethane) was purchased from Lancaster (Windham, NH, USA). Benzophenone from BDH Chemicals Ltd. (Poole, England) was recrystallized in ethanol before use. Acetonitrile was HPLC-grade and purchased from OmniSolv (Gibsstown, NJ, USA), it was used without further purification. Acetic acid, glacial, was purchased from Anachemia (Montreal, QC, Canada).

Nanosecond Laser Flash Photolysis

The laser flash photolysis (LFP) system has been previously described.[24,25] Samples were excited with a Molectron UV-24 nitrogen laser generating pulses at 337 nm of 6 ns and < 6 mJ output at the source, or with the third harmonic of a Surelite Nd/YAG laser generating pulses at 355 nm of 8 ns duration and 25 mJ output. The signals from the monochromator/photomultiplier system were initially captured by a Tektronix 2440 digitizer and transferred to a PowerMacintosh computer that controlled the experiment with a software developed in the LabVIEW 4.1 environment from National Instruments (Austin, TX, USA).

All transient spectra, kinetics, and power dependence studies were recorded employing a flow system with a 7 × 7 mm, 2 ml capacity, fused silica cuvettes from Luzchem Research (Ottawa, Canada). Samples were purged in a storage tank for 30 minutes with N_2. A minimum of 8 shots were acquired at each wavelength to record transient absorption spectra. For the power dependence studies 40 to 60 shots were accumulated for each measurement, to improve the signal to noise ratio due to the low intensity of the signals. The quenching rate constant for hydrogen abstraction by *t*-butoxyl radical was determined from the signal growth monitored at 370 nm. Up to 30 shots were accumulated. In the case of studies performed with benzophenone, the 355 nm laser was employed. In this case no more than 4 shots were accumulated to obtain each kinetic trace. Details of the techniques employed and related data analysis are available in recent reviews.[26,27]

Fresh solutions were prepared every day before experiments. The solvent employed in all cases was prepared by mixing 60 parts in volume of acetonitrile with 2.25 parts in volume of acetic acid glacial, and bringing to 100 parts with di-*t*-butyl peroxide (approximately 38 parts in volume). These values ensured an absorbance of 0.3–0.5 for di-*t*-butyl peroxide in the laser cell at the excitation wavelength. Sample stability was checked under nitrogen atmosphere. There was no observed change after 3 hours, as determined by steady state absorption spectroscopy.

Results

Reaction with *t*-butoxyl radicals

The irradiation of di-*tert*-butyl peroxide (di-*t*-butyl peroxide), with a short wavelength nanosecond laser pulse produces *t*-butoxyl radicals within the duration of the pulse. Upon their formation these radicals undergo slow β-cleavage with elimination of acetone and production of a methyl radical (Step 2 in Scheme 2). Alternatively, in the presence of a hydrogen donor (H-donor) *t*-butoxyl radicals will be reduced to *t*-butanol concomitant with the oxidation of the H-donor (Step 3, 4 or 5 in Scheme 2).

Following irradiation of di-*t*-butyl peroxide with 337 nm laser pulses in the presence of adrenaline we can readily monitor *via* LFP the appearance of a transient with an absorption band shoulder at 380 nm (see Fig. 2). This transient also presents a band in the visible region which extends in the near IR, *i.e.*, from 500 nm and over 700 nm. We assign the observed spectrum to the semiquinone radical resulting from H-abstraction from adrenaline (Step 3 in Scheme 2). The spectrum obtained here matches spectra obtained following pulse radiolysis studies of dopa in aqueous solution containing NaN_3 at pH 6,[15] and of 3,5-di-*tert*-butyl-1,2-benzoquinone under N_2O atmosphere at pH 3.[28]

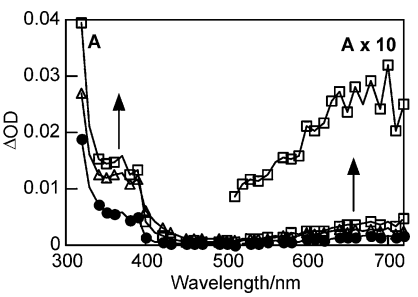


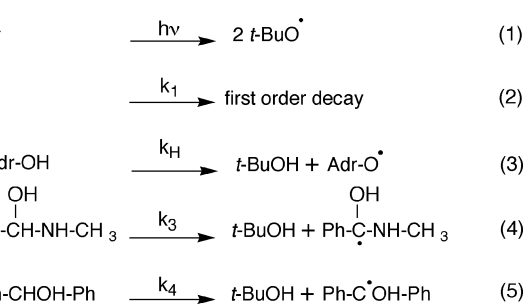
Fig. 2 Transient absorption spectra of 2.9×10^{-3} M adrenaline under N_2 atmosphere, in 38:60:2 di-*tert*-butyl peroxide:acetonitrile:acetic acid. Laser wavelength 337 nm. Time windows: (●) 0.56 μs; (△) 4.72 μs; and (□) 17.00 μs after the laser pulse.

The rate constant for the reaction of *t*-butoxyl radicals with adrenaline (k_H) was estimated using Equation 1, where k_{exp} is the observed growth rate constant of the semiquinone radical, and k_1 is the rate constant for *t*-butoxyl radical β-cleavage.

$$k_{exp} = k_1 + k_H \times [AdrO - H] \quad (1)$$

By plotting k_{exp} values for increasing adrenaline (Adr-OH) concentrations (Fig. 3), we obtained a k_H value of 1.5×10^8 M^{-1} s^{-1} from the slope of the plot and a k_1 value of 2.6×10^5 s^{-1} from its intercept. Given that *t*-butoxyl radicals are rapidly quenched by adrenaline the formation of methyl radicals *via* β -cleavage and their subsequent reaction with adrenaline is negligible under our experimental conditions. Thus our measured reactivity is characteristic for the reaction of *t*-butoxyl radicals with adrenaline (Step 3 in Scheme 2).

We have previously shown that in alkylamines the C-H bond in position α to the amino group is reactive towards *t*-butoxyl radicals.[29] It is therefore important to establish which role, if any, the amino group plays in the oxidation of the catecholamines. In fact, the methodology we employ in our experiments provides kinetic, not mechanistic information.[27,30] Although we only observe the semiquinone radical spectra, we cannot rule out on this



Scheme 2 Steps involved in *t*-butoxyl radical formation and consumption.

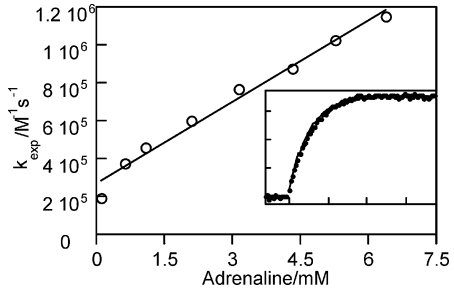


Fig. 3 Observed growth rate constant for the formation of the adrenaline semiquinone radical measured at 370 nm, in the presence of different concentrations of adrenaline in 38:60:2 di-*tert*-butyl peroxide:acetonitrile:acetic acid as solvent. Laser wavelength 337 nm, N_2 bubbled. The inset shows the time dependence of the absorption at 370 nm for an adrenaline concentration of 1.09×10^{-3} M.

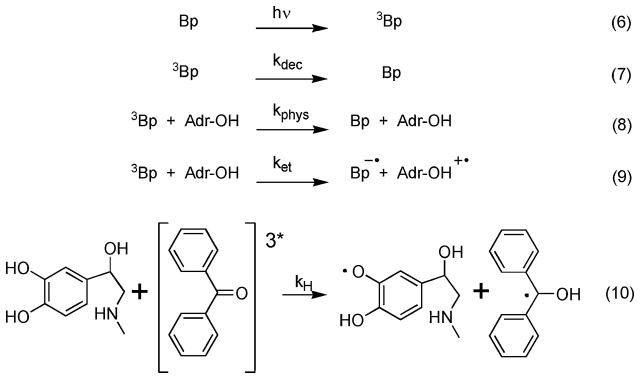
sole basis the possibility of hydrogen abstraction at the α-amino position, which would in turn result in a transparent radical in our system, and an overestimation of the reactivity at the phenol moiety.

In order to establish if H-abstraction in position α to the amino group is viable in our system, we performed kinetic measurements with 1-hydroxy-1-phenyl-2-methylaminoethane (halostachine, Step 4, Scheme 2) in the presence of a constant concentration of benzhydrol, employed as a signal carrier (Step 5, Scheme 2).[29,31] Even at concentrations as high as 42 mM on halostachine, the observed growth rate (monitored at 330 nm) was the same as that obtained with no halostachine, *ca.* 3.3×10^5 $M^{-1}s^{-1}$. At the same concentration of adrenaline, the observed rate would be *ca.* 6.3×10^6. Thus we estimate an upper limit of 5% for hydrogen abstraction at centers other than the phenylhydroxy moiety (Step 4 in Scheme 2).

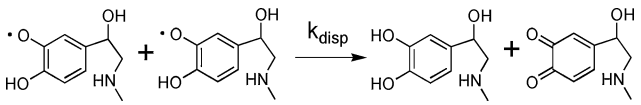
Reaction with triplet benzophenone

Following excitation, benzophenone (Bp) undergoes efficient intersystem crossing to the n,π* lowest excited triplet state.[32] The triplet state is very reactive towards hydrogen abstraction, rate constants for benzophenone triplet quenching by phenols ranging between 8×10^7 to 5×10^9 $M^{-1}s^{-1}$ have been measured in acetonitrile and benzene.[19] However, not only hydrogen abstraction, but also electron transfer,[33] energy transfer and other type of physical deactivation may be operational in the case of triplet benzophenone, as shown in Scheme 3.[19,22]

The quenching rate constant of triplet benzophenone is 1.3×10^9 $M^{-1}s^{-1}$. In order to establish to which extent the observed quenching is due to hydrogen abstraction (Step 10), and how important are the other possible mechanisms proposed in Scheme 3 (Steps 7 to 9), we evaluated the ratio of absorbances from the triplet state and from the ketyl radical at 500 nm in order to establish how much of the triplet state is converted to ketyl radical following H-abstraction. The triplet state absorption was obtained immediately after the laser pulse, and the ketyl radical absorption was measured after the decay of the triplet state is complete.[22,34] Given that the absorption of adrenaline semiquinone radical has a band extending from *ca.* 500 nm and to the red, (Fig. 2), we used the ratio of absorbances measured at 500 nm. We then compared the value obtained following 95% quenching by adrenaline, to that obtained following 95% quenching by 1,4-cyclohexadiene, under



Scheme 3 Deactivation mechanism for triplet benzophenone in the presence of adrenaline.



Scheme 4 Disproportionation of adrenaline semiquinone radical.

the same conditions. The latter is a good hydrogen donor, and the allylic radical obtained following its oxidation is transparent over the whole visible range.[35] The decay traces obtained for both quenchers are shown in Fig. 4.

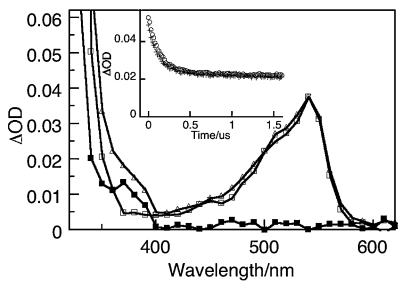


Fig. 4 Transient absorption spectra obtained 0.9 μs after the laser pulse, in the presence of (△) adrenaline 5.9×10^{-4} M, and (□) 1,4-cyclohexadiene 2.05×10^{-2} M. Also shown is the difference between both traces, which were normalized at 500 nm (■). Inset: Time dependence for the absorption at 500 nm following excitation of benzophenone in the presence of (+) adrenaline 5.9×10^{-4} M, and (○) 1,4-cyclohexadiene 2.05×10^{-2} M. Experiments were performed under N_2 atmosphere, in 98:2 acetonitrile:acetic acid V:V. The Laser wavelength employed was 355 nm.

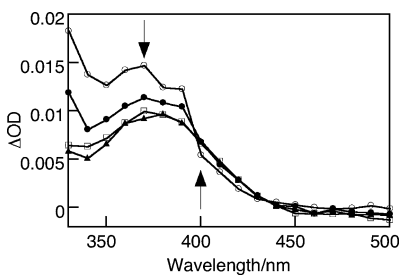


Fig. 5 Transient absorption spectra obtained for 3.0×10^{-3} M adrenaline under N_2 atmosphere in 38:60:2 di-*t*-butyl peroxide:acetonitrile:acetic acid v:v:v. Spectra correspond to time windows recorded (○) 23 μs, (●) 113 μs, (□) 449 μs and (▼) 812 μs after a 337 nm laser pulse.

Based on our results we conclude that over 95% of the reaction with adrenaline is due to hydrogen abstraction by benzophenone triplet state. Thus, we obtained values of 2.21 and of 2.33 for the triplet absorption/ketyl radical absorption when employing adrenaline and 1,4-cyclohexadiene as hydrogen donors, respectively. These values are within a 5% error difference, that for adrenaline being smaller than that for the alkene, where hydrogen abstraction is the only operational mode of quenching.

A comparison of the ketyl radical spectra obtained with 1,4-cyclohexadiene to that obtained with adrenaline evidences the presence of the semiquinone radical of adrenaline, as can be observed following the subtraction of both spectra in Fig. 4. From the data in Fig. 4 we can determine the molar absorptivity for the semiquinone radical at 370 nm (ε_{SQ} 370) employing the known value for the ketyl radical at 550 nm *i.e.*, $3{,}300 \pm 700$ $M^{-1}cm^{-1}$.[36] A value of 1400 ± 300 $M^{-1}cm^{-1}$ is obtained for ε_{SQ} 370. This value is within experimental error, the same as that previously determined for catechol in benzene at 383 nm, *ca.* 1500 ± 300 M^{-1} cm^{-1}.[20]

Decay of the semiquinone radical

As part of our experiments we also monitored the fate of the semiquinone radical over time. Fig. 5 shows the transient absorption spectra at long times following excitation. Note the drop and increase in absorption at 370 nm and 400 nm, respectively. In this case the experimental traces are best fitted by a second order function. The transient spectra obtained at 0.812 ms after the laser pulse matches that reported for aminoquinones formed following the oxidation of their respective catecholamines (not shown).[12,15] These aminoquinones are formed following disproportionation of two semiquinone radicals,[12,13,15,16,37] as exemplified in Scheme 1 for adrenaline.

A disproportionation is supported in our case both by the observed temporal evolution of the semiquinone radical absorbance, fitted with a second order rate expression, and by the quadratic power dependence for the absorbance measured at 400 nm, 1.7 ms after the laser pulse (data not shown).[27]

The disproportionation rate constant k_{disp}, may be obtained from Equation 2:

$$k_{obs} = \frac{k_{disp}}{\varepsilon_{SQ} \times l} \quad (2)$$

where ε_{SQ} is the molar absorptivity for the semiquinone radical, (l) is the optical path length of the cell, k_{obs} is the observed disproportionation rate constant, determined by changes in transient absorbance, and k_{disp} is the rate constant of disproportionation, which accounts for the actual changes in the concentration of the semiquinone radical (rather than changes in absorbance).

From the ε_{SQ} determined at 370 nm, and from the observed decay second order rate constant k_{obs} monitored at 370 nm, we calculate a k_{disp} value of $35 \pm 0.8 \times 10^8$ $M^{-1}s^{-1}$. The value reported in the literature for dopa in buffer at pH 7.7 is 0.98×10^8 $M^{-1}s^{-1}$.[12]

Discussion

The antioxidant effect of phenols has received considerable attention, where a major emphasis has involved the free radical scavenging reactivity of phenols.[38–46] We have employed *t*-butoxyl radicals and benzophenone in its excited triplet state to establish the activity of adrenaline as a radical scavenger.

Table 1 Hydrogen abstraction rate constants from different radical scavengers by benzophenone and *t*-butoxyl radical in units of 10^{-8} M^{-1} s^{-1}

	Benzophenone triplet		*t*-butoxyl radical	
Substrate	Benzene	CH_3CN	Benzene	Polar sv.
phenol	13[19]	0.8[19]	3.3[20]	[a]0.22[20]
p-methoxyphenol	45[19]	39[19]	16[20]	[a]1.1[20]
adrenaline	—	13	—	[b]1.5
aminoleutin	69[7]	84[7]	—	[b]8.1[7]
melatonin	—	76[23]	—	[b]0.34[23]
α-tocopherol	51[22]	60[21]	38[22]	[b]6.6[22]

[a] In methanol and di-*t*-butyl peroxide; [b] in acetonitrile and di-*t*-butyl peroxide. To perform our experiments it was necessary the addition of acetic acid (2.2% V:V; *ca.* 0.4 M) in acetonitrile to increase adrenaline solubility. The latter is insoluble in benzene, and therefore we could not determine the kinetics of hydrogen abstraction in this solvent.

The rate constants for H-abstraction obtained here can further be compared to those obtained under similar experimental conditions for well known free radical scavengers such as α-tocopherol,.[21,22] aminoleutins[7] or melatonin,[23] These values are listed in Table 1 together with the rate constants for H-abstraction from phenol and p-methoxyphenol.

Reaction with *t*-butoxyl radicals

Under our experimental conditions reactions involved solely the phenol moiety and we did not observe hydrogen abstraction on the CH_2 position α to the amino group. This can be rationalized on the basis that the lone pair of electrons in the nitrogen atom is protonated. The resonance stabilization of the α-amino radical by this lone pair of electrons will not be operational under this condition,[29] neither is the stabilization of the transition state leading to this radical.

Quantitative predictions have been formulated for kinetic solvent effects (KSE) that permit the extrapolation of the data for hydrogen atom abstraction by a radical (for example *t*-butoxyl) from a phenol in a given solvent, based on the known value in a different media.[40,42] These predictions are based on three assumptions; each substrate hydrogen bonds to only one solvent molecule; the equilibrium rate constant for this bonding is independent of the surrounding media; and finally, due to steric reasons, the hydrogen atom of the donor cannot be directly abstracted by the acceptor, rather the acceptor has first to replace the solvent molecule in the complex formed by the hydrogen donor-solvent molecule.[40,42]

The three assumptions account for the difference observed in the reactivity of phenol and p-methoxyphenol with *t*-butoxyl radicals in methanol vs. benzene. Qualitatively, we would expect a similar increase in the radical scavenging activity of adrenaline in going from acetonitrile (where hydrogen bonding is strong) to benzene. The low solubility of adrenaline in benzene however did not permit us to test this hypothesis.

Reaction with triplet benzophenone

The k_H value in acetonitrile for benzophenone is roughly an order of magnitude larger than that for *t*-butoxyl radical. This difference has been previously described with other phenols.[20,22] Thus we observe in Table 1 that an order of magnitude difference

in reactivity in acetonitrile also applies to α-tocopherol, phenol and p-methoxyphenol, as well as melatonin, an indole derivative of similar structure to aminoleutines, although lacking the dihydroxy functionality of the latter. This is explained by the stabilization, due to polar effects, of the transition state leading to the photoreduction of benzophenone, which in this case may involve charge transfer and offsets the strong hydrogen bonding of the radical scavengers with acetonitrile.[20,22] These polar effects are less important in the case of hydrogen abstraction by *t*-butoxyl radical and other radicals.[40]

Our results show that the consequence of quenching of the benzophenone triplet state is only hydrogen abstraction. Under the conditions employed the nitrogen atom in the catecholamine is protonated (*vide supra*), and therefore electron transfer from this species to benzophenone does not occur. As in the case of the reaction with *t*-butoxyl radicals, no reaction to form α-aminoradicals takes place in our media. This is due to protonation of the nitrogen atom at the amine functional group.

We have been able to determine the molar absorptivity of adrenaline semiquinone radical at 370 nm following its reaction with benzophenone triplets, and from this value we have calculated the second order rate constant for disproportionation. Our value for this disproportionation is *ca.* 35 fold larger than that in water. At the present time we cannot determine if this difference is due to solvent effects, or whereas the accummulated errors with which the values have been obtained also play a role.

Free radical scavenging activity, a comparison

We consider it worth analyzing at this time subsequent intermediates formed in the oxidation of adrenaline and other catecholamines. In this sense, both leucoadrenochrome and aminoleutin pose very interesting structures in terms of radical scavenging potential.

The rate constants for hydrogen abstraction from phenols depend on the strength of the O–H bond, *i.e.*, on the O–H bond dissociation energy (BDE).[46] Electron donating groups substituted ortho or para to the reactive hydroxyl group stabilize the aryloxyl radical formed, and also the transition state leading to its formation.[45] This stabilization accounts for the higher reactivity of adrenaline and *p*-methoxyphenol in comparison to that of phenol which lacks an electron donating group. In the case of α-tocopherol, a 6 member heterocyclic ring reduces the dihedral angle existing between the lone pair of the *p*-oxygen atom in the heterocyclic ring and the hydroxyl group, this results in its enhanced antioxidant activity.[44] A similar rigid structure with an electron donating N atom located in position para to a hydroxyl group can be observed both in the case of adrenoleutine (**7**) and leucoadrenochrome (**4**) (see Scheme 1), we may therefore anticipate that these species will be more reactive radical scavengers than their catecholamine precursors.[39]

The reactivity of aminoleutine as a hydrogen donor has been reported with quenching experiments done with benzophenone and fluorazophore-P.[7] From the results obtained with benzophenone we observe that aminoleutine is about as reactive as α-tocopherol in acetonitrile, and *ca.* 6.5 times more reactive than adrenaline (Table 1). However care should be taken in analyzing the reactivity of these molecules with benzophenone, given that they are affected by charge transfer effects.[23]

An analysis based on the reactivity of these molecules with *t*-butoxyl radicals presents a much clearer picture; thus from the values measured for dopaleutine we establish that its reactivity is as high as that of α-tocopherol, and *ca.* 5 times higher than that of the catecholamine (Table 1). The low reactivity of melatonin, an indole analog of dopaleutine that lacks the hydroxyl groups, further illustrates that the radical scavenging reactivity of dopaluetine relies on its hydroxyl moiety.

Conclusions

We report the reactivity of the catecholamine adrenaline towards *t*-butoxyl radicals and benzophenone triplets. Adrenaline is a reasonably good hydrogen donor, however we predict that the reactivity of the products of its oxidation will exhibit an enhanced radical scavenging activity. This in view of the good overlap existing between the lone pair of electrons in the nitrogen atom of the newly formed heterocyclic ring, and the hydroxy group in the benzene moiety. This overlap (and the heterocycle) is nonexistent in adrenaline and the other catecholamines, previous to their oxidation. In the presence of large free radical concentrations we believe that the oxidation of adrenaline into melanin will proceed towards complete polymerization following the steps shown in Fig. 1, where each new hydroxybenzene species will have enhanced antioxidant power compared to its immediate precursor. This enhanced reactivity would ensure the complete elimination of undesired intermediates in adrenaline oxidation, following radical mediated lesions.

Acknowledgements

This work was generously supported by the Natural Sciences and Engineering Research Council of Canada and by the Canadian Foundation for Innovation. J.C.S. is grateful to the Government of Ontario for support and to McGill University for its hospitality during the sabbatical leave when this work was completed. G.C. is additionally thankful to Le Fonds Québécois de la Recherche sur la Nature et les Technologies and McGill University for funding.

Notes and references

1 R. Rhoades and R. Pflanzer, *Human Physiology*, Saunders College Publishing, Orlando, Second Edition edn., 1992.
2 N. Kollias, R. M. Sayre, L. Zeise and M. R. Chedekel, *J. Photochem. Photobiol. B: Biology*, 1991, **9**, 135–160.
3 S. Schmitz, P. D. Thomas, T. M. Allen, M. J. Poznansky and K. Jimbow, *Photochem. Photobiol.*, 1995, **61**, 650–655.
4 J. Cadet, S. Courdavault, J.-L. Ravanat and T. Douki, *Pure Appl. Chem.*, 2005, **77**, 947–961.
5 M. Brenner and V. J. Hearing, *Photochem. Photobiol.*, 2008, **84**, 539–549.
6 Y. Yamaguchi, M. Brenner and V. J. Hearing, *J. Biol. Chem.*, 2007, **282**, 27557–27561.
7 X. Zhang, C. Erb, J. Flammer and W. M. Nau, *Photochem. Photobiol.*, 2000, **71**, 524–533.
8 K. J. Reszka, Z. Matuszak and C. F. Chignell, *Free Rad. Biol. Med.*, 1998, **25**, 208–216.
9 K. Stepién, M. Porebska-Budny, A. M. Hollek and T. Wilczok, *J. Photochem. Photobiol. B: Biology*, 1992, **15**, 223–231.
10 A. Palumbo, A. Napolitano, P. Barone and M. D'Ischia, *Chem. Res. Toxicol.*, 1999, **12**, 1213–1222.
11 S. Adak, U. Bandyopadhyay, D. Bandyopadhyay and B. R., *Biochemistry*, 1998, **37**, 16922–16933.

12 M. R. Chedekel, E. J. Land, A. Thompson and T. G. Truscott, *J. Chem. Soc. Chem. Commun.*, 1984, 1170–1172.
13 R. P. Ferrari, E. Laurenti, E. M. Ghibaudi and L. Casella, *J. Inorg. Biochem.*, 1997, **68**, 61–69.
14 B. Kalyanaraman, C. C. Felix and R. C. Sealy, *J. Biol. Chem.*, 1984, **259**, 354–358.
15 C. Lambert, T. G. Truscott, E. J. Land and P. A. Riley, *J. Chem. Soc. Faraday Trans.*, 1991, **87**, 2939–2942.
16 F. Marques, R. O. Duarte, J. J. G. Moura and M. P. Bicho, *Biol. Signals*, 1996, **5**, 275–282.
17 D. G. Graham, S. M. Tiffany, J. W. R. Bell and W. F. Gutknecht, *Mol. Pharmacol.*, 1978, **14**, 644–653.
18 K. Ohkubo, Y. Moro-oka and S. Fukuzumi, *Org. Biomol. Chem.*, 2006, **4**, 999–1001.
19 P. K. Das, M. V. Encinas and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 4154–4162.
20 P. K. Das, M. V. Encinas, S. Steenken and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 4162–4166.
21 M. V. Encinas, E. A. Lissi and A. F. Olea, *Photochem. Photobiol.*, 1985, **42**, 347–352.
22 C. Evans, J. C. Scaiano and K. U. Ingold, *J. Am. Chem. Soc.*, 1992, **114**, 4589–4593.
23 J. C. Scaiano, *J. Pineal Res.*, 1995, **19**, 189–195.
24 J. C. Scaiano, *J. Am. Chem. Soc.*, 1980, **102**, 7747–7753.
25 J. C. Scaiano, M. Tanner and D. Weir, *J. Am. Chem. Soc.*, 1985, **107**, 4396–4403.
26 G. Cosa, *Pure Appl. Chem.*, 2004, **76**, 263–275.
27 G. Cosa and J. C. Scaiano, *Photochem. Photobiol.*, 2004, **80**, 159–174.
28 S. V. Jovanovic, K. Kónya and J. C. Scaiano, *Can. J. Chem.*, 1995, **73**, 1803–1810.
29 D. Griller, J. A. Howard, P. R. Marriott and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 619–623.
30 J. C. Scaiano, in *Degradation and Stabilization of Polymers*, ed. H. H. G. Jellinek and H. Kachi, Elsevier, Amsterdam, 1989, vol. 2, pp. 1–39.
31 J. C. Scaiano, in *Reactive Intermediate Chemistry*, ed. R. A. Moss, M. S. Platz and M. J. Jones, Jr., Wiley, Hoboken, New Jersey, 2004, pp. 847–872.
32 N. J. Turro, *Modern Molecular Photochemistry*, University Science Books, Sausalito, 1991.
33 K. Bhattacharyya and P. K. Das, *J. Phys. Chem.*, 1986, **90**, 3987–3993.
34 M. J. Muldoon, A. J. McLean, C. M. Gordon and I. R. Dunkin, *Chem. Commun.*, 2001, 2364–2365.
35 M. V. Encinas and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 6393–6397.
36 L. J. Johnston, D. J. Lougnot, V. Wintgens and J. C. Scaiano, *J. Am. Chem. Soc.*, 1988, **110**, 518–524.
37 C. Giulivi and E. Cadenas, *Free Rad. Biol. Med.*, 1998, **25**, 175–183.
38 L. R. C. Barclay, C. E. Edwards and M. R. Vinqvist, *J. Am. Chem. Soc.*, 1999, **121**, 6226–6231.
39 D. A. Pratt, G. A. DiLabio, G. Brigati, G. F. Pedulli and L. Valgimigli, *J. Am. Chem. Soc.*, 2001, **123**, 4625–4626.
40 D. W. Snelgrove, J. Lusztyk, J. T. Banks, P. Mulder and K. U. Ingold, *J. Am. Chem. Soc.*, 2001, **123**, 469–477.
41 L. R. C. Barclay and M. R. Vinqvist, in *Chemistry of Phenols*, 2003, vol. 2, pp. 839–908.
42 G. Litwinienko and K. U. Ingold, *Acc. Chem. Res.*, 2007, **40**, 222–230.
43 L. Valgimigli, R. Amorati, M. G. Fumo, G. A. DiLabio, G. F. Pedulli, K. U. Ingold and D. A. Pratt, *J. Org. Chem.*, 2008, **73**, 1830–1841.
44 G. W. Burton and K. U. Ingold, *Acc. Chem. Res.*, 1986, **19**, 194–201.
45 G. Brigati, M. Lucarini, V. Mugnaini and G. F. Pedulli, *J. Org. Chem.*, 2002, **67**, 4828–4832.
46 D. D. M. Wayner, E. Lusztyk, K. U. Ingold and P. Mulder, *J. Am. Chem. Soc.*, 1996, **61**, 6430–6433.

