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Semiquinone Anion Radicals of Catechol(amine)s, Catechol Estrogens, and Their Metal Ion Complexes

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The characterization and identification of semiquinone radicals from catechol(amine)s and catechol estrogens by electron spin resonance spectroscopy is addressed. The use of diamagnetic metal ions, especially Mg^{2+} and Zn^{2+} ions, to detect transient semiquinone radicals in biological systems and to monitor their reactions, is discussed. A brief account of the identification and reactions of quinones is also presented.

Introduction

Catechols are widely distributed in nature, in both plant and animal systems. Catechol itself is an environmental cocarcinogen present in tobacco smoke (*1–3*). In mammals, catecholamines act as neurotransmitters. They find use in medicine as antiparkinsonism drugs (dopa), antihypertensive agents (α-methyldopa), and as bronchodilators (isoproterenol). Antitumor activity of a number of catechols (γ-glutaminyl-3,4-dihydroxybenzene, 4-methoxycatechol, etc.) and catecholamines (i.e., dopa methyl ester, dopamine, 3,4-dihydroxybenzylamine, and 5-S-cysteinyldopa) in both *in vitro* and *in vivo* systems has been reported (*4–9*). Side effects have been reported for all these drugs, and include hepatic injury (*10*), cardiotoxicity (*11*), hemolysis (*12*) and photosensitivity (*13*). These side effects and the cytotoxicity of these drugs in general are thought to be related to the production of damaging free radicals and *o*-quinones (*14–20*).

Phenols are precursors to catechols (*23–25*); they are widely used as antioxidants (*21,22,26*) and also find use as antitumor agents (*5*). Several phenolic agents also induce skin depigmentation (*27,28*). These effects have been attributed to metabolic activation of phenols to various catechols in pigmented systems (*5,18*). Both estradiol and diethylstilbestrol (DES) are phenolic estrogens and environmental carcinogens (*29,30*). They undergo biotransformation to catechol estrogens in uterus (*31*) and in brain tissues (*32,33*). Free radicals from DES-catechol and 2-hydroxy-estradiol have been implicated in their toxicity (*34–36*).

Several xenobiotics (benzene, 4-bromobenzene, and acetominophen) undergo aromatic hydroxylation by cytochrome P-450 in the liver to form the corresponding catechol(s) (*37–40*). In addition the hematopoietic toxicity of benzene and the nephrotoxicity of bromobenzene has been linked to semiquinone/quinone production from catechol metabolites (*37,38*).

This review addresses the generation, identification, and reactions of *o*-semiquinones and *o*-quinones from catechol(amine)s and catechol estrogens in a biochemical milieu.

Biochemical Toxicology of Semiquinones and Quinones

Catechol(amine)s and catechol estrogens are degraded to semiquinone radicals by both enzymatic and nonenzymatic pathways (Fig, 1). Semiquinone radicals can be formed either directly or indirectly from oxidation of the parent catechol(s) or from reduction of quinones (*41–46*).

Since catechols induce toxicity in several target organs, they are also likely to undergo metabolic activation in several enzyme systems. Enzyme systems capable of oxidizing catechol(amine)s,catechol estrogens, and phenols *in vitro* include: cytochrome P-450/NADPH (*47–49*), horseradish peroxidase (HRP)/H_2O_2 (*50*), PG-synthase/arachidonic acid (*51*), catalase/H_2O_2 (*52*), and tyrosinase/O_2 (*41*). Those that reduce the quinones are: NADPH-P450-reductase, DT-diaphorase, and xanthine/xanthine oxidase (*53–55*). One-electron oxidation of phenols leads to aryloxyl radicals which through sec-

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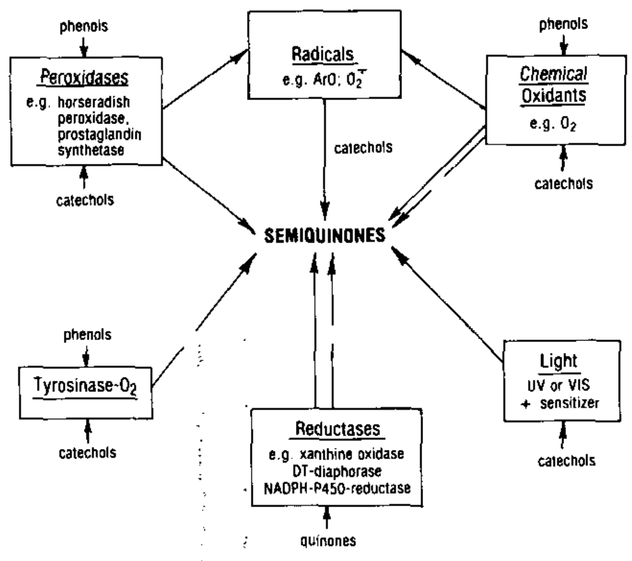

FIGURE 1. Enzymatic and chemical production of semiquinones from their precursors. Reactions that give radicals indirectly are shown by broken arrows.

ondary reactions can in turn form semiquinones (Fig. 1) (*42*). In a biological milieu, semiquinones and quinones can bind covalently to proteins, enzymes or other endogenous constituents producing secondary free radicals and products (*56–60*) (Figs. 2 and 3).

The antitumor effects of several catechol(amine)s have been attributed to inhibition of DNA polymerase (in melanocytes) by *o*-quinones or *o*-semiquinones (*4,5,58*). The neurotoxic effects of 6-hydroxy or amino-substituted catechol(amine)s (i.e., 6-hydroxydopa, 6-aminodopamine, etc.) have also been related to production of free radicals (*61–63*).

Inactivation of enzymes/proteins by *o*-quinones is presumably due to the nucleophilic addition reactions (of quinones) to sulfhydryl or amino groups present in these macromolecules (*16,58*). The production of 5-S-cysteinyl dopa in the urine of melanoma patients (*64*) and the

FIGURE 2. Possible fates of quinones in biological systems.

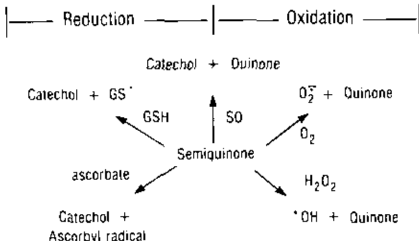

FIGURE 3. Possible oxidation and reduction pathways of semiquinones.

isolation of quinone-alanine adduct(s) in terrestrial humic acid (*65*) provide indirect evidence for occurrence of these addition reactions. Reactions between *o*-quinones and proteins provide a basis for formation of "melano-proteins" (*66*). The toxicity of adrenochrome (a material which leads to myocardial necrosis) has been attributed to formation of the (one-electron) reduced free radical and to the oxy-radicals derived from it (via redox cycling of oxygen) (*67–69*).

Generation of *o*-Semiquinones

Semiquinones are generated from oxidation/reduction of catechols and quinones by a variety of methods, including photooxidation, autoxidation/chemical oxidation and enzymic oxidation (*70–80*).

Photooxidation

Photooxidation provides a simple, clean method for generating semiquinone radicals and studying their reactions. The primary photoreaction in dopa, catechol, and a variety of other catecholamines involves a mixture of photoionization and photohomolysis (*81*). This was established by a quantitative spin trapping procedure that we developed using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), which scavenges both hydrated electrons and hydrogen atoms formed during the photooxidation.

$$\text{catechol (HO, HO, R)} \xrightarrow{h\nu} e^-_{aq},\ \text{H}\cdot + \text{semiquinone (}\cdot\text{O}, {}^-\text{O, R)} \quad (1)$$

The quantum yield for semiquinone formation is ca. 0.04 at pH 7, increasing to about 0.08 at higher pH where the catechol moiety is ionized.

Sensitized photolysis of catechols and catecholamines has been demonstrated using a variety of dyes. Visible irradiation in the presence of hematoporphyrin, Rose Bengal, methylene blue, and other sensitizers gives intense spectra of corresponding semiquinones (*70*).

In general, photooxidation (using a slow flow) can be used for (1) generating specific primary radicals for characterization purposes without interference from secondary radicals and (2) obtaining kinetic data for

their reactions, e.g., termination rate constants for *o*-semiquinones.

Autoxidation/Chemical Oxidation

Free-radical chain reactions have been proposed during autoxidation of catechol(amines)s (*82*). From the pH dependence of oxygen consumption, the initiating step of an autoxidation reaction appears to involve electron transfer from the mono-anion of the catechol(amine) to molecular oxygen, e.g.,

$$\text{HO, HO, R} + O_2 \xrightarrow{-2H^+} \cdot O, {}^-O, R \; (1\text{–}6) + O_2^{\overset{\cdot}{-}} \quad (2)$$

A more recent study, however, suggests that this electron transfer to oxygen is metal-catalyzed (*83*). The superoxide radical formed in the initiating reaction also is scavenged by catechol(amine) (*84*).

$$\text{HO, HO, R} + O_2^{\overset{\cdot}{-}} \rightarrow \cdot O, {}^-O, R + H_2O_2 \quad (3)$$

Radicals can be generated by either static or flow measurements (*85*). Static oxidation involves addition of the catechol(amine) or catechol estrogen to an oxygen-saturated solution of sodium hydroxide (*76,78,79*). Semiquinones are observed fairly easily at high pH because of the slow rate of dismutation of radical anions. Autoxidation also allows the monitoring of secondary radical formation, i.e., semiquinones from 6-hydroxy-substituted catechol(amine). Alkaline hydrogen peroxide (a source of superoxide anion) is another effective way of oxidizing catecholamines and, possibly, catechol estrogens (*84*). Both sodium periodate and silver oxide are often used to oxidize catechols. Periodate is a two-electron oxidizing agent, but silver oxide appears to act as a one-electron oxidant (*75,76*).

Enzymatic Oxidation

o-Semiquinones formed during enzymatic oxidation have previously been detected by ESR using continuous flow methods (*85*). This procedure entails the use of large volumes of substrate and enzyme. Recently, we have developed a spin-stabilization procedure by which o-semiquinones (formed enzymatically) can be stabilized by chelation through the use of diamagnetic di- or trivalent ions (*73*).

$$\cdot O, {}^-O, R \xrightarrow{M^{n+}} M^{n+} \text{-chelate}, R \quad (4)$$

Although several metal ions (e.g., Al^{3+}, Y^{3+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, and Zn^{2+}) have been employed to stabilize *o*-semiquinones in aqueous (*86*) and nonaqueous media (*87*), we feel that either Mg^{2+} or Zn^{2+} is more likely to be useful in biological systems, and consequently only Zn^{2+}- and Mg^{2+}-complexed *o*-semiquinones in aqueous media are discussed in this review.

Except at high pH, *o*-semiquinone radicals are transient, decaying rapidly via disproportionation to give the catechol and *o*-quinone.

$$2 \; \cdot O, {}^-O, R \underset{}{\overset{2H^+}{\rightleftarrows}} O, O, R + HO, HO, R \quad (5)$$

For dopa, the measured radical half-life was 2 msec for a steady-state concentration of 2.5 μM, corresponding to a second-order termination rate constant of $2 \times 10^8 \; M^{-1} \, sec^{-1}$ (Fig. 4). However, Zn^{2+}-complexed dopa semiquinone radicals are much less transient than the uncomplexed ones (*88*). Decay remains second-order, but the radical lifetime is now several seconds for a steady-state concentration of ca. 10^{-5} M. The calculated second-order rate constant is $1.1 \times 10^4 \; M^{-1} sec^{-1}$.

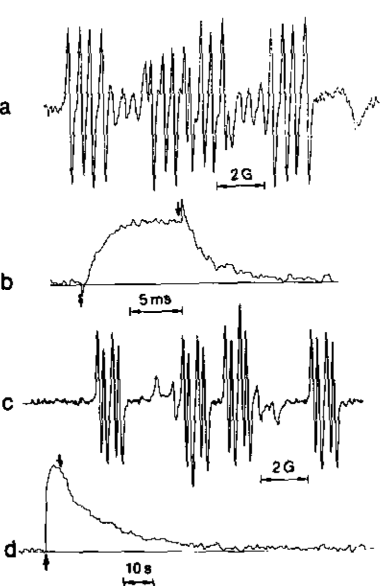

FIGURE 4. ESR spectra (*a,c*) and kinetic profiles (*b,d*) for uncomplexed and spin-stabilized *o*-semiquinones produced by the UV photolysis of dopa. Conditions: (*a,b*), 25 mM dopa, pH 7.0; (*c,d*), 25 mM dopa plus 0.08 M Zn^{2+} in acetic acid–acetate buffer, pH 5.0.

Chelation (or complexation) is therefore extremely effective in decreasing the rate of radical termination. The uncomplexed *o*-semiquinone at neutral pH has a rate constant over 10,000-fold greater. Thus, the complexed radical can be detected at rates of radical formation 10,000 times lower than are necessary to detect

the uncomplexed *o*-semiquinone. This allows the use of static rather than flow systems (*73*).

Whereas HRP/H_2O_2-dependent oxidation of catechol(amine) and catechol estrogens involves *o*-semiquinones as obligate intermediates (*88*), the tyrosinase-catalyzed oxidation proceeds via a two-electron oxidation (*41*) with the formation of *o*-semiquinones in a secondary reaction.

HO, HO (catechol, R) $\xrightarrow[-1e]{HRP/H_2O_2}$ ·O, −O (semiquinone, R) + 2H⁺ (6)

HO, HO (catechol, R) $\xrightarrow[-2e]{Tyrosinase,\ O_2}$ O, O (o-quinone, R) + 2H⁺ (7)

HO, HO (catechol, R) + O, O (o-quinone, R) ⇄ 2 ·O, −O (semiquinone, R) + 2H⁺ (8)

The enzyme activity in each system is not affected to any marked extent by the presence of Zn^{2+} or Mg^{2+} ions (*73*).

Characterization and Identification of *o*-Semiquinones

Production of primary and secondary free radicals from the oxidation of catechols and the dopa and epinephrine classes of catecholamines is shown in Figure 5.

Whereas the identification of semiquinones from catechols has been fairly straightforward (*71,74*), there existed several inconsistencies with regard to interpretation of spectra of *o*-semiquinones from dopa (*89*) and epinephrine (*91*). The major reasons for the observed inconsistencies were: the presence of more than one spectral species; the presence of magnetically inequivalent methylene protons in the amino acid side chain; and acid-base equilibria in the radicals.

For example, oxidation of dopa and its analogs can, depending on the conditions employed, give three major types of radical (D1-D3). The primary radical (D1) does not show the expected multiplicities or linewidths because of a combination of the magnetic inequivalence referred to and restricted rotation (*46,90*). This phenomenon is illustrated in Figure 6. At low temperature only one-half of the spectral lines are clearly visible, whereas when the temperature is increased the "missing lines" that were previously broadened are apparent and complete spectral analysis becomes possible. An additional complexity in the system is provided by the ionization of the amino group ($pK_a \approx 9$) which causes a shift in the spectral parameters at high pH (Fig. 7).

Data for uncomplexed and complexed primary *o*-semiquinones of catechol(amine)s are given in Tables 1 and 2. The magnetic parameters of the complexes (Table 2) are modified from those of the uncomplexed species, with the differences between them being fairly constant (*74*). For example, for the Zn^{2+}-complexed species a_3^H and a_6^H typically decrease by about 0.28 G in going to the complex while a_5^H increases by about the same amount. a_β^H also increases for Zn^{2+}. Observation of satellite peaks from magnetic isotopes present in natural abundance [e.g., from ^{67}Zn (4%, I = 5/2)] verified that complex formation is occurring. Also indicative of com-

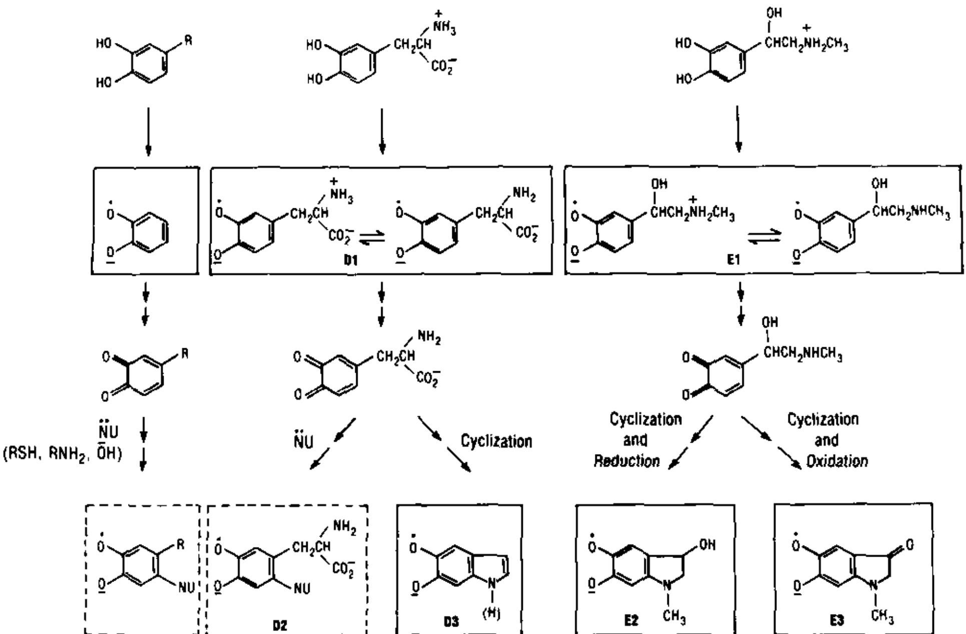

FIGURE 5. Free radicals from oxidation of catechol, dopa, and epinephrine.

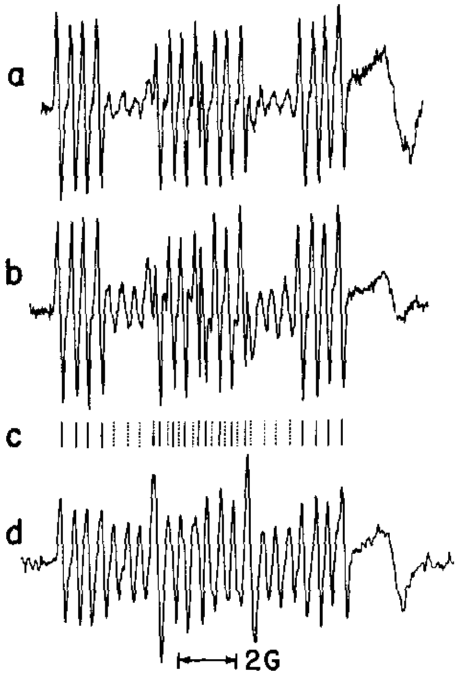

FIGURE 6. ESR spectra of the primary semiquinone anion from dopa as a function of temperature. The spectrum has a total of 32 lines because of magnetic inequivalence of the methylene protons in the side chain. 16 of the lines are selectively broadened as a result of restricted rotation of the methylene hydrogens, and sharpen as the temperature is increased (*a*→*c*).

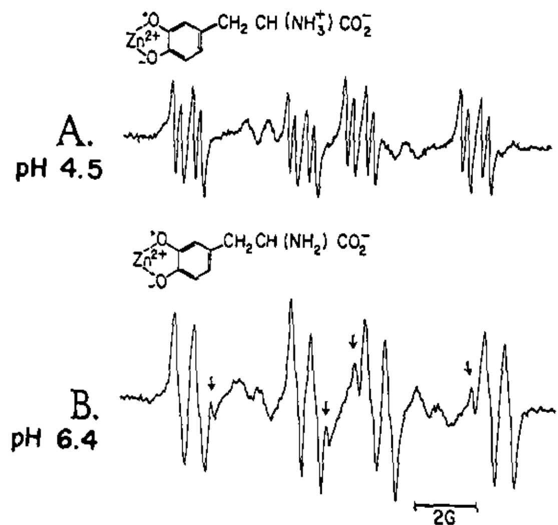

FIGURE 7. ESR spectra obtained (*A*) upon the addition of 40 nM HRP to an incubation mixture containing 175 μM H_2O_2, 8 mM dopa and 225 mM Zn^{2+} in acetate-acetic acid buffer (pH 4.5) under nitrogen; (*B*) upon the addition of 5 nM HRP to an incubation mixture containing 100 μM H_2O_2, 8 mM dopa and 380 mM Zn^{2+} in acetate solution (pH 6.4) under nitrogen.

plex formation are the observed changes in *g* value. *g* Values are decreased in the complexes, consistent with spin density in a vacant metal orbital.

Although hyperfine couplings to aromatic protons, in particular a_5^H, do not differ markedly for the majority of radicals studied, couplings to the methylene protons in a substituent at position 4 vary considerably, suggesting that the rotations of these protons can be quite restricted (Tables 1 and 2). For example, whereas the protons in a freely rotating alkyl substituent (such as the methyl group in 4-methyl o-benzosemiquinone) have a hyperfine splitting of about 4.8 G, the protons in the α-methyl dopa semiquinone radical [where R = $CH_2C(CH_3)(NH_3^+)(CO_2^-)$] have couplings as low as 2.2 G (the mean of splittings from two inequivalent protons). In general, the methylene proton couplings decrease as the bulk of the substituent on the carbon atom bearing the methylene protons increases.

Secondary radicals C2 from catechols and D2 and D3 from dopa analogs have been detected during autoxidation (*44,76*) and enzyme oxidation (with tyrosinase); they are derived from hydroxycatechols, 6-hydroxydopa, and 5,6-dihydroxyindole respectively (Table 3). The unusual spectrum of radical D2 again is a consequence of magnetic inequivalence and restricted rotation in a single radical (Table 3). The spectrum of the cyclized radical, D3, is quite distinctive, showing the hyperfine coupling to nitrogen (Table 4).

Oxidation of epinephrine and its analogs in aqueous solution gives transient, broad, poorly resolved spectra under most conditions (*91*). Use of ESR-spin stabilization, however, enabled us to obtain three types of radical (*75*). Their spectra and, for the most part, their structures, are distinct from the dopa series.

The primary radical E1 lacks a second methylene hydrogen (Fig. 5) in the side chain, so that its spectrum is much narrower than that of D1; spectra of the secondary radicals (E2 and E3) are, however, much greater because of a large hyperfine splitting from nitrogen following cyclization (note that the nitrogen coupling in D3 is much lower than in either E2 or E3 possibly due to additional spin-delocalization in the indole ring). Also notable is the failure to detect hydroxy-substituted semiquinones from the epinephrine class unlike the dopa or catechol series (Fig. 5). This is possibly due to a more rapid rate of cyclization of the precursor quinones. Magnetic parameters that characterize these secondary radicals are give in Table 5.

Adrenochrome is the stable 4-electron oxidation product of epinephrine. There is evidence for increased toxicity (e.g., myocardial necrosis) from adrenochrome in the presence of the reducing agents, ascorbic acid and cysteine (*67*). Ascorbic acid previously has been shown to reduce adrenochrome to leucoadrenochrome via a free radical mechanism. Adrenochrome forms both one-electron reduced and one-electron oxidized radicals.

·O, H, OH, $^-$O, N, CH_3

Reduction radical

·O, O, $^-$O, N, CH_3

Oxidation radical

Table 1. Electron spin resonance data for primary *o*-semiquinones (uncomplexed) from catechol, dopa, and epinephrine and their analogs.

I

Parent catechol(amine)	R in radical I	Hyperfine couplings, G						
		a^H_3	a^H_5	a^H_6	a^H_β	a^H_γ	a_{other}	g
Catechol	H	0.76	3.66	0.76	3.66(a^H_4			2.0046
3,4-Dihydroxybenzoic acid	CO_2^-	0.72	3.16	1.22				2.0047
3,4-Dihydroxyhydrocinnamic acid	$CH_2CH_2CO_2^-$	0.22	3.73	0.88	3.56			2.0045
3,4-Dihydroxybenzyl amine	$CH_2NH_3^+$	0.82	3.48	0.93	2.75		1.38(a^N)	2.0045
	CH_2NH_2	0.34	3.72	0.79	4.13		0.79(a^N)	2.0045
3,4-Dihydroxyphenylacetic acid	$CH_2CO_2^-$	0.29	3.71	0.89	3.12			
4-Methoxycatechol	OCH_3	0.61	4.19	1.30			1.15($a)^{OCH_3}$)	2.0045
Dopa	$CH_2CH(NH_3^+)CO_2^-$	0.51	3.58	0.94	1,94,3.34			2.0045
	$CH_2CH(NH_2)CO_2^-$	0.35	3.71	0.88	2.32, 3.15			
α-Methyldopa	$CH_2C(CH_3)(NH_3^+)CO_2^-$	0.59	3.55	0.94	1.70,2.70			2.0045
	$CH_2C(CH_3)(NH_2)CO_2^-$	0.42	3.69	0.89	2.00,2.82			2.0045
Dopa methyl ester	$CH_2CH(NH_2)CO_2CH_3$	0.42	3.65	0.93	2.54,2.66			2.0045
Dopamine	$CH_2CH_2NH_3^+$	0.46	3.58	0.94	2.98			2.0045
	$CH_2CH_2NH_2$	0.27	3.73	0.89	3.13			2.0045
3,4-Dihydroxymandelic acid	$CH(OH)CO_2^-$	0.55	3.53	0.86	1.89			2.0045
3,4-Dihydroxyphenyl serine	$CH(OH)CH(NH_2)CO_2^-$	0.48	3.71	0.85	3.12			2.0045
6-Hydroxydopamine	$CH_2CH_2NH_2$,5-O^-	0.58	—	0.87	3.24			2.0043
6-Aminodopamine	$CH_2CH_2NH_2$,5-NH_2	0.77	—	1.60	2.75		2.75(a^N), 1.34(a^{NH})	
Epinephrine	$CH(OH)CH_2NHCH_3$	0.60	3.62	0.83	2.31			2.0044
	$CH(O^-)CH_2NHCH_3$	0.56	3.58	0.86	2.26	0.18,0.26		
Norepinephrine	$CH(OH)CH_2NH_2$	0.60	3.62	0.82	2.30			2.0044
	$CH(O^-)CH_2NH_2$	0.46	3.58	0.84	2.32	0.12,0.26	0.09(a^N)	
Isoproterenol	$CH(OH)CH_2NHCH(CH_3)_2$	0.60	3.60	0.80	2.28	0.20,0.20		2.0044
	$CH(O^-)CH_2NHCH(CH_3)_2$	0.48	3.53	0.82	2.20	0.18,0.26		
3,4-Dihydroxynorephedrine	$CH(OH)CH(CH_3)NH_2$	0.56	3.61	0.85	1.96			2.0045

The one-electron oxidized species has recently been identified during peroxidatic oxidation of adrenochrome (*75*). However, there is evidence for one-electron reduction of adrenochrome in microsomes containing NADPH (*68*). This proposed species apparently is oxidized by molecular oxygen forming superoxide and the parent compound (*68*). We have again chosen the spin stabilization approach in a chemical system (*92*) (Fig. 8). Magnetic parameters for the Zn^{2+}- and Mg^{2+}-complexed species are in Table 6.

Semiquinone radicals have been detected for the first time from auto- and enzymatic oxidation of catechol estrogens (*93*); the spin stabilization approach was again crucial to detect semiquinones produced enzymatically. Using either Zn^{2+} or Mg^{2+} as complexing agents, we showed the production of semiquinones (VII (R = H), VIIa (R = H), VII (R = OH) and VIIa (R = OH)) during peroxidase/tyrosinase oxidation of catechol estrogens and estrogens (Figs. 9 and 10). The species VII (R = H) is characterized by three large hyperfine couplings to β-alicyclic protons (at C-6 and C-9), whereas VII (R = OH) exhibits only two large couplings. The species VIIa (R = H) is characterized by only one large coupling to an alicyclic β-proton (C-9) and a significant coupling to an aromatic proton; species VIIa (R = OH) exhibits a similar pattern. From these spectra, one can unequivocally assign the coupling to specific alicyclic protons. The *g*-values are close to those reported for metal complexes of *o*-semiquinones from simple catechols (Fig. 10) (*71,74*). Magnetic parameters that characterize each species are given in Table 7.

Reactions of *o*-Semiquinones

The spin stabilization approach has enabled us to study radical reactions of *o*-semiquinones in two enzymatic systems (HRP/H_2O_2 and tyrosinase/O_2). *o*-Semiquinone radicals were detected in high steady-state con-

Table 2. Electron spin resonance data for Zn^{2+}-complexed primary *o*-semiquinones from catechol, dopa, and epinephrine and their analogs.

II

Parent catechol(amine)	R in radical II	Hyperfine couplings, G						g
		a_3^H	a_5^H	a_6^H	a_β^H	a^H	a_{other}	
Catechol	H	0.5	3.9	0.5				2.0040
4-Methoxycatechol	OCH_3	0.75	4.51	0.97			1.35(a^{OCH_3})	
3,4-Dihydroxybenzoic acid	CO_2^-	0.47	3.45	0.85				2.0040
3,4-Dihydroxyhydrocinnamic acid	$CH_2CH_2CO_2^-$	—	4.02	0.62	4.02			2.0038
3,4-Dihydroxybenzylamine	$CH_2\overset{+}{N}H_3$	0.52	3.65	0.71	3.05		1.28(a^N)	2.0040
4-Methylcatechol	CH_3							
Dopa	$CH_2CH(NH_3^+)CO_2^-$	0.25	3.83	0.67	2.65, 3.11			2.0039
	$CH_2CH(NH_2)CO_2^-$	<0.2	3.9	0.6	2.2, 4.2			
α-Methyldopa	$CH_2C(CH_3)(NH_3^+)CO_2^-$	0.31	3.82	0.66	1.84, 2.96			2.0039
Dopa methyl ester	$CH_2CH(NH_3^+)COOCH_3$	0.31	3.76	0.71	2.63, 2.95			2.0039
Dopamine	$CH_2CH_2\overset{+}{N}H_3$	0.16	3.88	0.67	3.36			2.0039
6-Hydroxydopamine	$CH_2CH_2\overset{+}{N}H_3$,5-O$^-$	0.22	—	0.66	3.36			2.0040
Epinephrine	$CH(OH)CH_2\overset{+}{N}H_2CH_3$	0.38	3.62	0.65	3.15	0.23		2.0039
Norepinephrine	$CH(OH)CH_2\overset{+}{N}H_3$	0.40	3.65	0.70	3.20	≈0.15 (2H)		2.0039
Isoproterenol	$CH(OH)CH_2\overset{+}{N}H_2CH(CH_3)_2$	0.38	3.70	0.65	3.10	0.26		2.0039
Dihydroxynorephedrine	$CH(OH)CH(CH_3)\overset{+}{N}H_3$	0.36	3.70	0.60	3.70	0.26		2.0039
Adrenalone	$C(=O)CH_2\overset{+}{N}H_2CH_3$	0.55	2.90	1.65	—	0.23,0.20		2.0041

centration during peroxidatic oxidation of catechol(amine)s and catechol estrogens (*88*, *93*). The square-root relationship between the enzyme and the steady-state radical concentration suggested that *o*-semiquinones decay predominantly via dismutation.

$$2\ \text{(semiquinone, R)} \xrightarrow{2H^+} \text{(o-quinone, R)} + \text{(catechol, R)} \quad (9)$$

From photolytic systems, the rate constant k_d was calculated as ca. 10^4 M^{-1} sec^{-1} (for complexed semiquinones) and as 10^8 M^{-1} sec^{-1} for uncomplexed semiquinone radicals.

During peroxidatic oxidation, with H_2O_2 limiting and constant substrate concentrations, the duration of the steady-state (t_{ss}) is linearly dependent on the initial concentration of hydrogen peroxide, $[H_2O_2]$. This allowed an estimate (Fig. 11) of the rate of the rate of removal of hydrogen peroxide in this system, since

$$-\frac{d[H_2O_2]}{dt} = v = \frac{[H_2O_2]_o}{t_{SS}}$$

Correlation of this rate with the measured rate of radical formation (by obtaining the termination rate constant and steady-state radical concentrations) verified that the semiquinone is an obligate intermediate in the reaction.

In systems containing catecholamine, HRP/H_2O_2, and phenol in moderate concentrations, the rate of semiquinone production showed a marked increase as a function of phenol concentration. This is consistent with the production of phenoxyl $PhO^\bullet$ radicals which subsequently oxidizes the catecholamine:

$$PhOH \xrightarrow{-e^-} PhO^\bullet \quad (10)$$

$$PhO^\bullet + QH_2 \rightarrow PhOH + Q^\bullet + 2H^+ \quad (11)$$

In contrast, ascorbate (Fig. 12) and glutathione resulted in a lag time for semiquinone detection that was proportional to the concentration of reductant added. During the lag time, only radicals from the reducing agent are detected. Reaction between *o*-semiquinone and ascorbate was reported to be too slow to measure by pulse methods, although there is ESR evidence for the reduction of ascorbate by a semiquinone radical from 6-hydroxydopa (*94*).

FIGURE 8. Free radical from reduction of adrenochrome.

Table 3. Electron spin resonance data for secondary *o*-semiquinones from the autoxidation of catechol(amine)s.

III

Parent catechol(amine)	R in radical III	Hyperfine couplings, G					g
		a_3^H	a_6^H	a_β^H	a^H	a_{other}	
3,4-Dihydroxyhydrocinnamic acid	$CH_2CH_2COO^-$	0.55	0.81	4.22	0.11		2.0044
3,4-Dihydroxycinnamic acid	$CH{=}CHCOO^-$	0.40	1.37	2.92	1.80		2.0044
4-Methylcatechol	CH_3	0.58	0.65	4.95			2.0043
4-Methoxycatechol	OCH_3	0.55	1.0			1.15(a^{OCH_3})	
3,4-Dihydroxybenzylamine	CH_2NH_2	0.62	0.93	4.21		0.62(a^N)	2.0043
3,4-Dihydroxyphenylacetic acid	$CH_2CO_2^-$	0.47	0.89	3.47			2.0043
Dopamine	$CH_2CH_2NH_2$	0.56	0.86	3.31			2.0043
Dopa	$CH_2CH(CO_2^-)NH_2$	0.55	0.93	2.39,3.87	0.20		2.0043
α-Methyldopa	$CH_2C(CH_3)(CO_2^-)NH_2$	0.51	0.96	2.64,3.25			2.0043

Table 4. Electron spin resonance data for Zn^{2+}-complexed secondary *o*-semiquinones (indolesemiquinones) from epinephrine and its analogs.

IV

Parent catecholamine	R_1, R_2 in derived radical IV			Hyperfine couplings, G				g
	Solvent	R_1	R_2	a^N	a^{NH} or a^{ND}	a_1^H	a_2^H or a_2^D	
Epinephrine	H_2O	H	CH_3	4.44		5.10(3H)	0.91(2H)	2.0040
Epinephrine-α,α,β-d3[a]	D_2O	D	CH_3	4.44		5.10(3H)	≤0.15(2D)	
Norepinephrine	H_2O	H	H	3.40	3.40(1H)		1.2(2H)	2.0040
	D_2O	H	D	3.40	0.52(1D)		1.2(2H)	
Isoproterenol	H_2O	H	$CH(CH_3)_2$	4.58		1.90(1H)	0.90(2H)	2.0040
Dihydroxynorephedrine	H_2O	CH_3	H	3.40	3.30(1H)		0.90(1H)	2.0040
	D_2O	CH_3	D	3.40	0.5(1D)		0.90(1H)	

[a] *In this structure, the other ring hydrogen also is replaced by deuterium.*

Table 5. Electron spin resonance data for indolesemiquinones from photooxidation or autoxidation.

V

Parent compound	R in radical V	Hyperfine couplings, G		g
		a^N	a^H	
5,6-Dihydroxyindole	H	1.20	4.62, 0.52, 0.64(2H)	2.0041
α-Methyldopa	CH_3	1.27	5.45(3H), 0.55, 0.70	2.0041

Table 6. Magnetic parameters of one-electron reduced adrenochrome-metal ion complexes.

VI

Radical	Hyperfine couplings, G							g
	a^N	$a^N_{CH_3}$	$a^N_{CH_3}$	$a_2^{H'}$	a_3^H	a_4^H	a_7^H	
VI-Mg^{2+}	5.36	5.70	4.78	5.75	3.45	0.62	0.38	2.0038
VI-Zn^{2+}	5.62	5.88	5.35	6.00	3.84	0.51	0.23	2.0035

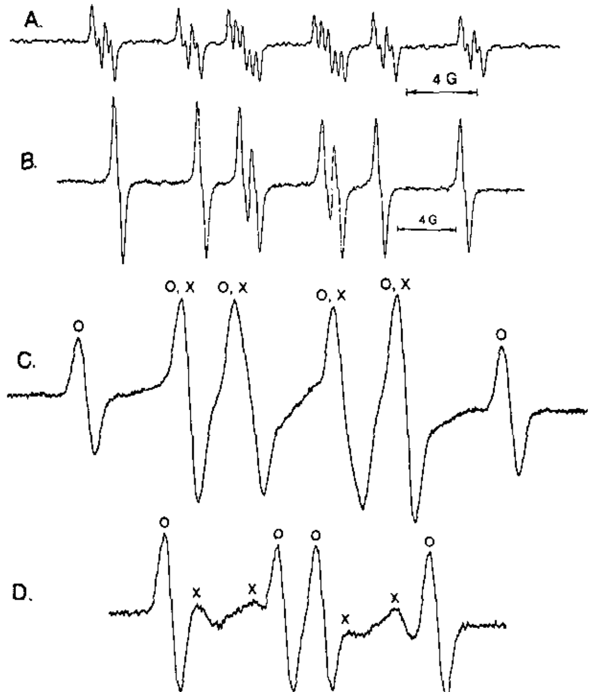

FIGURE 9. ESR spectra of (*A*), the uncomplexed semiquinone from 2-hydroxyestradiol obtained by autoxidation; (*B*)Zn^{2+}-complexed semiquinones from HRP/H_2O_2 oxidation of 2-hydroxyestradiol; (*C*) Mg^{2+}-complexed semiquinones from the hydroxylation/oxidation of β-estradiol by tyrosinase/O_2; (*D*) Mg^{2+}-complexed semiquinones from the hydroxylation/oxidation of 6α-hydroxyestradiol by tyrosinase/O_2. In spectrum *C*, radicals from 2- and 4-hydroxyestradiol are denoted by (×) and (○), respectively.

We verified in photolysis experiments that reactions of phenoxyl radicals with catechols, and of semiquinones with ascorbate, indeed occur. Formation of semiquinone is promoted by the presence of phenols, showing the ability of phenoxyl radical to oxidize catechols (Fig. 13). In contrast, semiquinone concentrations are strongly quenched by ascorbate and thiols. Radical decay is pseudo-first-order, indicating a direct reaction between the semiquinone and the reducing agent.

Although reactions between these primary *o*-semiquinones and oxygen have been postulated, we did not observe an increased oxygen consumption above background levels from autoxidation during peroxidatic oxidation of catechols. Since the *o*-semiquinone is an obligate intermediate in the peroxidase system, significant electron transfer to oxygen can therefore be ruled out.

FIGURE 10. Enzymatic oxidation of β-estradiol.

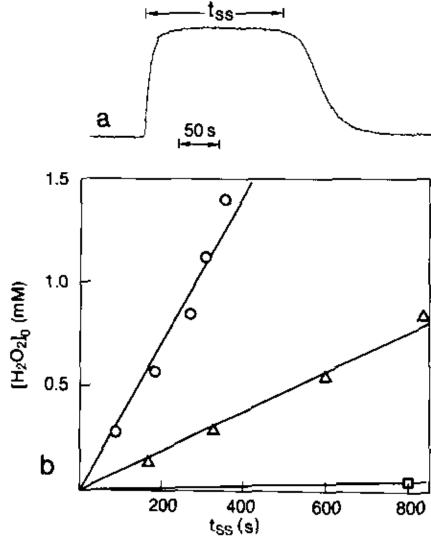

FIGURE 11. Effect of hydrogen peroxide on the duration of the steady state in radical concentration. (*a*) Steady state for o-semiquinone radicals from horseradish peroxidase-catalyzed oxidation of norepinephrine. Abscissa: reaction time; ordinate: ESR signal amplitude (proportional to free radical concentration). Conditions: 6mM norepinephrine, 140 mM H_2O_2, 28 nM horseradish peroxidase, 227 mM Zn^{2+} in acetic acid-acetate buffer, pH 5.0. The duration of the steady state, t_{ss}, is proportional to the initial hydrogen peroxide concentration. Individual measurements of t_{ss} were reproducible to ± 10%. (*b*) Dependence of the duration of the steady state in semiquinone radical concentration on the initial hydrogen peroxide concentration $[H_2O_2]$ for the three peroxidase substrates: (○) dopamine, (Δ) norepinephrine, and (□) dopa. Conditions: 6 mM substrate, 28 nM horseradish peroxidase, 227 mM Zn^{2+} in acetic acid-acetate buffer, pH 5.0.

Table 7. Electron spin resonance data for o-semiquinones from catechol estrogens.

VII VIIa

Starting material	Generating system[a]	Radical detected	M^{n+}	Hyperfine splittings, G				g
				$a^H_{C\text{-}6}$	$a^H_{C\text{-}6}$	$a^H_{C\text{-}9}$	$a^H_{aromatic}$	
2-Hydroxyestradiol	A	VII (R = H)	Zn^{2+}	8.60	5.70	9.40[a]		2.0039
		VII (R = H)	Mg^{2+}	8.50	5.75	9.40	0.3	2.0042
	B	VII (R = H)		7.70	4.88	8.08	0.3,0.73	2.0044
β-Estradiol	C	VII(R = H)	Mg^{2+}	8.50	5.65	9.45		2.0042
		VIIa (R = H)	Mg^{2+}			8.60	3.00	2.0042
6α-Hydroxyestradiol	C	VII (R = H)	Mg^{2+}	7.00		9.40		2.0042
		VIIa (R = H)	Mg^{2+}			9.00	3.60	2.0042
		VII (R = H)	Zn^{2+}	7.00		9.40		2.0039
		VIIa (R = H)	Zn^{2+}			8.60	4.00	2.0039

[a] A = HRP-H_2O_2, pH 5.0; B = autoxidation (1 M NaOH); C = tyrosinase-O_2, pH 7.4.

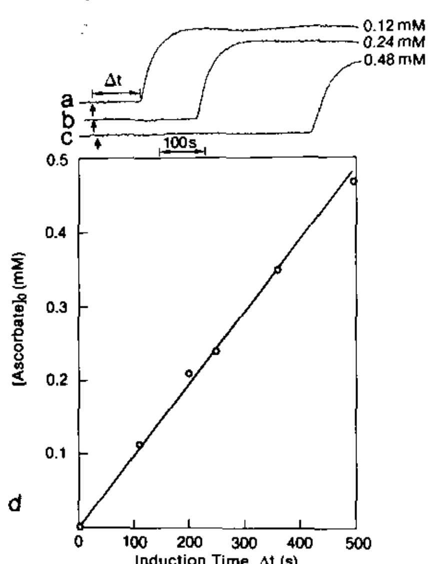

FIGURE 12. Induction time for detection of semiquinones: (*a*,*b*,*c*) effect of ascorbate on the level of semiquinone radical detected during horseradish peroxidase-H_2O_2 oxidation of norepinephrine. *Abscissa: reaction time; ordinate: ESR signal amplitude (proportional to free radical concentration).* The time at which enzyme was added is indicated by the arrows. Individual measurements of Δt were reproducible to ± 10%. Conditions: 6 mM norepijnephrine, 28 nM horseradish peroxidase, 0.28 mM H_2O_2, 227 mM Zn^{2+} in acetic acid-acetate buffer, pH 5.0. The lag in semiquinone detection that is observed is proportional to the initial concentration of ascorbate (*d*).

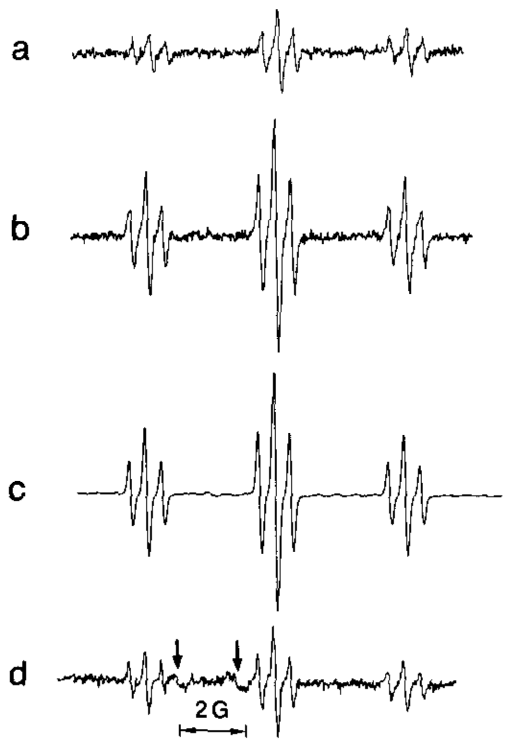

FIGURE 13. Effects of phenol and ascorbate on concentrations of spin-stabilized *o*-semiquinones from catechol: (*a*) 0.1 mM catechol; (*b*) 0.1 mM catechol + 10 mM phenol; (*c*) 20 mM catechol; (*d*) 20 mM catechol + 0.2 mM ascorbate. Radical generation was by UV photolysis of solutions in acetic acid acetate buffer, pH 5.5, containing 0.2 M Zn^{2+}. In (*a*),(*c*), and (*d*) the majority of the absorbed light is absorbed by catechol; in (*b*) the majority of the absorbed light is absorbed by phenol.

Since semiquinones also can catalyze the direct reduction of H_2O_2, the feasibility of reduction of H_2O_2 by *o*-benzosemiquinone and *p*-semiquinone (e.g., daunorubicin semiquinone) was compared (*95*). We were able to demonstrate this reaction for daunorubicin semiquinone, but not for *o*-benzosemiquinone. While *o*-semiquinones derived from simple catechols do not, therefore, appear to reduce H_2O_2, *o*-semiquinones from hydroxy or amino substituted catechols may behave differently (by acquiring *p*-semiquinone character through resonance).

Generation, Identification and Reactions of *o*-Quinones

Melanogenesis (i.e., the production of melanin) involves at least three types of *o*-quinones (dopaquinone, dopachrome and 5,6-indole quinone) (*96–98*). Each of these *o*-quinones possesses a characteristic visible absorption spectrum. Based on visible absorption characteristics, *o*-quinones from catecholamines in general can be classified as catecholamine *o*-quinone, aminochrome, and aminoquinones or topaquinones (Tables 8–10).

o-Quinones are formed directly from two-electron oxidation of catechol(amine) by tyrosinase or from one-electron oxidation (of catechol(amine)) by HRP/H_2O_2. Whereas aminochromes are formed via intra-molecular cyclization of catecholamine o-quinone (dopachrome from *o*-dopaquinone, adrenochrome from epinephrine *o*-quinone), the aminoquinones and topaquinones are formed by the addition of nitrogenous compounds and water, respectively, to *o*-quinones (*66, 107–109*).

Quinones are electrophilic in nature (Fig. 2). They undergo addition reactions with the sulfhydryl groups present in DNA polymerase and also cause the inactivation of the enzyme (*18,58*). Indeed the selective toxicity of melanin precursors against melanoma cells has been shown to be due to formation of *o*-quinones (*18*). Production of pheomelanin involves the addition of cysteine to *o*-dopaquinone followed by subsequent polymerization (*64*). Catecholamine o-quinones have been shown to be more toxic to melanocytes than are aminochromes (probably due to lack of electrophilic reactive sites in the latter) (*18*). Reactions between *o*-quinones and amino acids (leading to "melanoproteins") form the

Table 8. Optical data for primary *o*-quinones of catechol(amine)s.

VIII

Parent catechol(amine)	R in quinone VIII	λ_{max}, nm	Extinction coefficient, M^{-1} cm^{-1}	Reference
Catechol	H	300,390	$\epsilon_{390} = 1834$	(*97–99*)
			$\epsilon_{420} = 2040$	(*99*)
4-Methylcatechol	CH_3	300,400		(*66*)
4-*Tert*-butylcatechol	$C(CH_3)_3$	420		(*100*)
Dopa	$CH_2CH(NH_2)CO_2^-$	278,395	$\epsilon_{390} = 1250$	(*101*)
Dopamine	$CH_2CH_2NH_2$	303,394		(*18, 102, 103*)
Norepinephrine	$CH(OH)CH_2NH_2$	296,384		(*103*)
Epinephrine	$CH(OH)CH_2NHCH_3$	302,387		(*103*)
N-Acetyldopamine	$CH_2CH_2NHCOCH_3$	392	$\epsilon_{392} = 1300$	(*102*)

Table 9. Optical data for hydroxyquinones from oxidation of catechol(amine).

o *p*

Parent catechol(amine)	R in quinone	Form	λ_{max}, nm	Extinction coefficient, M^{-1} cm^{-1}	Reference
1,24-Trihydroxybenzene	H	*p*	485	$\epsilon_{485} = 2042$	(*104*)
4-*Tert*-butylcatechol	$C(CH_3)_3$	*p*	480		(*100*)
6-Hydroxydopa (TOPA)	$CH_2CH(NH_2)COO^-$	*p*	495	$\epsilon_{495} = 2000$	(*102, 103, 105*)
6-Hydroxydopa (TOPA)	$CH_2CH(NH_2)COO^-$	*o*	465		(*102*)
6-Hydroxydopamine	$CH_2CH_2NH_2$	*p*	495	$\epsilon_{495} = 2200$	(*105, 106*)

Table 10. Optical data for aminochromes.

Parent catechol(amine)	R_1, R_2, R_3 in aminochrome: R_1	R_2	R_3	λ_{max}, nm	Extinction coefficient, M^{-1} cm^{-1}	Reference
Adrenochrome	OH	H	CH_3	480	$\epsilon_{480} = 4020$	(*92*)
Noradrenochrome	OH	H	H	297,477		(*103*)
Dopachrome	H	CO_2^-	H	302,475	$\epsilon_{475} = 3600$	(*97, 102*)
Dopaminochrome	H	H	H	303,479		(*103*)

basis of "browning reactions" that occur in fruits and vegetables (*109*).

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