

Oxidation Chemistry of the Endogenous Central Nervous System Alkaloid Salsolinol-1-carboxylic Acid

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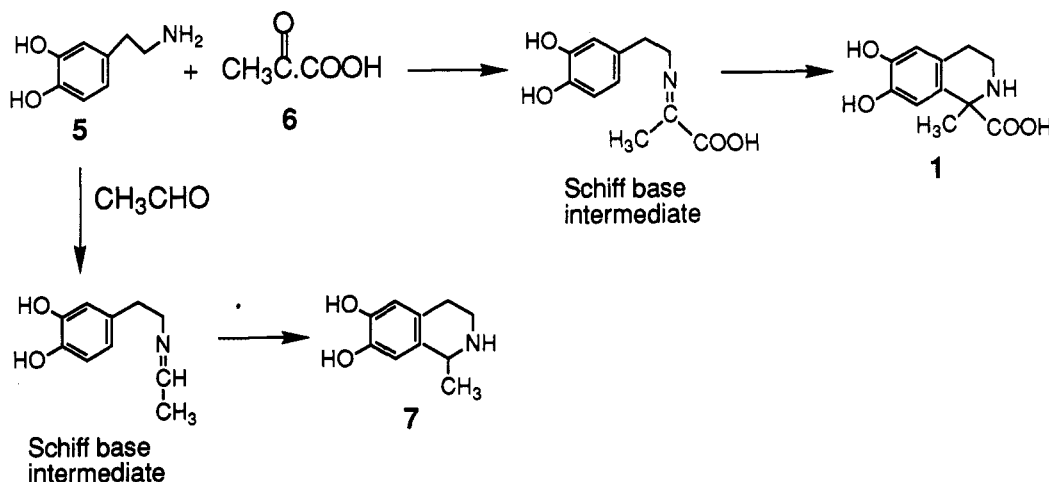
The oxidation chemistry of salsolinol-1-carboxylic acid (1), an alkaloid endogenous to the central nervous system which is elevated as a result of ethanol consumption, has been studied by electrochemical approaches at pH 7.0 in aqueous solution. The first voltammetric oxidation peak of 1 at pH 7.0 occurs at $E_p = +0.116$ V, indicating that this alkaloid is a very easily oxidized compound. The peak I_a reaction is a $2e-2H^+$ oxidation of 1 to 1,2,3,4-tetrahydro-1-methyl-1-carboxy-6,7-isoquinolinedione (8), which rapidly decarboxylates ($k > 10^3 s^{-1}$) to give predominantly the quinone methide tautomer of 3,4-dihydro-1-methyl-6,7-isoquinolinediol (2). The latter compound is responsible for the second observed oxidation peak II_a observed with 1. This peak is a $2e$ oxidation of 2 to a quinoid intermediate (9) which can either be attacked by water to yield 3,4-dihydro-1-methyl-5,7-dihydroxyisoquinolin-6-one (13b) (which is readily further oxidized to 3,4-dihydro-1-methyl-5-hydroxyisoquinoline-6,7-dione (3)) or aromatizes to yield 1-methyl-6,7-isoquinolinediol (4). Preliminary in vivo experiments have revealed that 2 and 13b are behavioral toxins when injected into the brains of laboratory mice. The in vitro oxidation reactions of 1 and 2 reported here might be of relevance to the neurodegenerative, behavioral, and addictive consequences of chronic alcoholism.

Salsolinol-1-carboxylic acid (1; 1,2,3,4-tetrahydro-1-methyl-1-carboxy-6,7-isoquinolinediol) has recently been detected as a trace constituent of the rat striatum, human urine, and the caudate nucleus (a part of the striatum) of post-mortem human brain.¹ In the latter brain region the levels of 1 have been found to be significantly higher in the brains of alcoholics who at autopsy had ethanol present in the blood.¹ Levels of 1 are also elevated in the urine of human volunteers given acute doses of ethanol.²⁻⁴ The biogenesis of 1 in the brain in the absence of ethanol intake is not known definitively although it is likely that it is formed by the Pictet-Spengler condensation reaction between the catecholamine neurotransmitter dopamine (5) and pyruvic acid (6)^{1,5,6} as shown in Scheme I. Pyruvate is a key substance involved in carbohydrate metabolism. The exact role of ethanol or its metabolites in stimulating the elevation of brain levels of 1 remains to be determined. A related alkaloid, salsolinol (7; 1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol), is also a trace endogenous constituent of the mammalian central nervous system.⁷⁻⁹ Levels of 7 also increase in certain brain regions of the rat which chronically consume ethanol.¹⁰⁻¹² In humans, urinary levels of 7 are elevated during ethanol intoxication and decline during detoxification.^{9,13} Such observations have led to the suggestion that acetaldehyde, the proximate metabolite of ethanol in the liver, might enter the brain via the circulatory system and react with 5 and perhaps other biogenic catecholamines to form 7 and other tetrahydroisoquinoline (TIQ) alkaloids (Scheme I). Furthermore, it has been suggested that 7 and related TIQs elevated in the brain as a result of chronic ethanol consumption might contribute to the behavioral changes, physical dependence, and addictive properties of ethanol.¹⁴ Provocative evidence in support of this suggestion has been provided from the observation that chronic intracerebroventricular infusion of 7 to the rat evoke a dramatic increase in the preference of the animal for ethanol

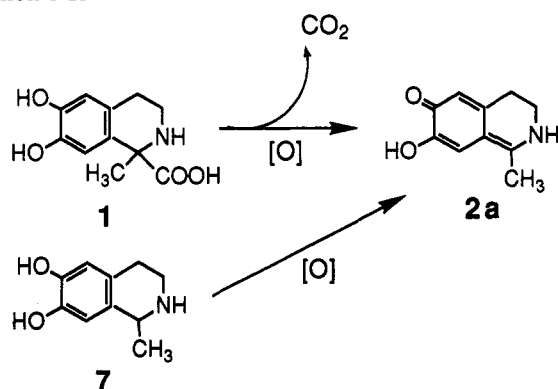
consumption,¹⁵ an effect which continues long after the discontinuance of drug administration.¹⁶⁻¹⁷ The presence of 1 and 7 in brain, even in the absence of ethanol intake, has resulted in the speculation that the former TIQ is decarboxylated to give the latter.^{1,2,6,18} However, incubations of 1 with the supernatant of a rat brain homogenate did not give 7 but rather 3,4-dihydro-1-methyl-6,7-isoquinolinediol (2).¹⁹ In such homogenates the oxidative decarboxylation of 1 was not enzyme catalyzed; the same reaction also occurred at the same rate in the buffer system employed. The stimulation of formation of 2 from 1 in aqueous buffer by transition metal ions, and inhibition by EDTA and superoxide dismutase suggests a role for a process mediated in some fashion by superoxide radical anion, $O_2^{\cdot-}$.^{19,20} The fact that supernatants of brain homogenates do not catalyze the oxidative decarboxylation of 1 does not necessarily imply that brain enzyme systems do not exist which can mediate such reaction. Indeed, Coultas et al.²¹ have demonstrated that several related tetrahydroisoquinoline-1-carboxylic acids (TIQ-1-CAs) with C(6)- or C(7)-hydroxy substituents are oxidatively decarboxylated in the presence of peroxidase/ H_2O_2 and fungal laccase enzymes to the corresponding 3,4-dihydroisoquinolines. Electrochemical studies on a number of TIQ-1-CAs in basic nonaqueous or partially aqueous solutions also indicate that a free hydroxyl group at C(6) or C(7) is necessary for oxidative decarboxylation to the corresponding 3,4-dihydroisoquinolines to occur although 1 was not studied.²² Bobbitt and Cheng²² have noted, at an unspecified working electrode, that voltammograms of the various TIQ-1-CAs which were investigated exhibited two oxidative peaks. The first, more negative peak was responsible for the oxidative decarboxylation of the TIQ-1-CAs. The second, more positive peak was speculated to be associated with the oxidation of the 3,4-dihydroisoquinoline moieties formed in the first peak reaction. The products and mechanisms associated with the second peak, however, were not investigated.

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Scheme I



Scheme II



Chronic ethanol consumption can result in impaired learning and intellectual abilities, effects which have been attributed to organic brain damage.²³ Neuronal damage and loss, particularly in the hippocampus, has been noted in rats following prolonged ethanol consumption.²⁴ The effects of chronic alcoholism have been likened to a premature aging of the brain.²⁵ Collins²⁶ has proposed that oxidative transformations of 1, 7, and other TIQs that are elevated in the brains of alcoholic individuals might result in the in situ formation of toxic products which are responsible for this neuronal damage. For example, it was suggested that oxidation of 1 or 7 might lead to 3,4-dihydro-1-methyl-6,7-isoquinolinediol, which in the form of its quinone methide tautomer (2a, Scheme II) has a structural resemblance to the electrophilic *p*-quinone believed to be the toxic product of intraneuronal oxidation of the catecholaminergic neurotoxin 6-hydroxydopamine.^{27,28} The latter *p*-quinone has been proposed to be toxic to catecholamine neurons as a result of binding to the sulfhydryl residues of cellular proteins. However, quinone methide 2a is not conjugated by the tripeptide glutathione.²⁹ We have recently demonstrated that at physiological pH 2 is a rather easily oxidized compound,³⁰ although the details of this reaction were not studied. In this paper the electrochemically-driven oxidations of 1 and 2 at physiological pH are described. Preliminary results on the effects of 2 and one of its major oxidation products when centrally administered to laboratory mice are also presented.

Results

Voltammetric Studies. Cyclic voltammograms of 1 obtained at a pyrolytic graphite working electrode (PGE)

in aqueous buffers between pH 3.0 and 9.2 are presented in Figure 1. At the sweep rate (ν) employed (100 mV s⁻¹) a total of four oxidation peaks (I_a–IV_a) can be observed on the initial anodic sweep. The peak potentials (E_p) for oxidation peaks I_a–IV_a are dependent upon pH according to the following relationships (pH 3.0–9.2):

$$\text{peak I}_a: E_p = [+0.62 - 0.072 \text{ pH}] \text{ V}$$

$$\text{peak II}_a: E_p = [+0.79 - 0.077 \text{ pH}] \text{ V}$$

$$\text{peak III}_a: E_p = [+1.02 - 0.088 \text{ pH}] \text{ V}$$

$$\text{peak IV}_a: E_p = [+1.03 - 0.057 \text{ pH}] \text{ V}$$

These relationships are based upon E_p values measured from linear sweep voltammograms obtained at the PGE using 0.22 mM 1 and $\nu = 5 \text{ mV s}^{-1}$. The cyclic voltammograms presented in Figure 1 reveal that after scanning through the various oxidation peaks a small reduction peak V_c appears on the reverse sweep. On the second anodic sweep, a new oxidation peak V_a appears and forms a quasi-reversible couple with peak V_c. The peak V_c/peak V_a couple is most pronounced at pH ≥ 7 (Figure 1C,D). Peak clipping experiments reveal that the peak V_c/peak V_a couple cannot be observed when the initial anodic sweep is reversed after scanning only through oxidation peak I_a. However, if the initial anodic sweep is reversed after scanning through oxidation peaks I_a and II_a, then the peak V_c/peak V_a couple can be observed (Figure 2A). Thus, the species responsible for the latter couple is formed at the electrode surface as a result of the peak II_a electrooxidation reaction. Subsequent discussion will focus primarily on the peak I_a and peak II_a oxidation reactions of 1 at physiological pH (7.0). Figure 2 presents a series of cyclic voltammograms of 1 at pH 7.0 at increasing values of ν . Thus, at $\nu \geq 1 \text{ V s}^{-1}$ the peak currents (i_p) for peaks II_a and II_c become equal and the peak V_c/peak V_a couple and peaks III_a and IV_a (data not shown) disappear. Clearly, therefore, the proximate electrooxidation product resulting from the peak II_a reaction must be the precursor of the species responsible from the peaks V_c, III_a, and IV_a. Between sweep rates of 0.1 and 10 V s⁻¹ E_p for peak II_a remains constant at $+0.263 \pm 0.007 \text{ V}$. However, over the same range of ν , E_p for peak I_a systematically shifts to more positive potentials ($\Delta E_p = +33 \text{ mV}$ per decade increase in ν). One consequence of this anodic shift is that, at $\nu > 10 \text{ V s}^{-1}$, peak I_a merges with peak II_a (Figure 2F). Under no circumstances was a reduction peak coupled

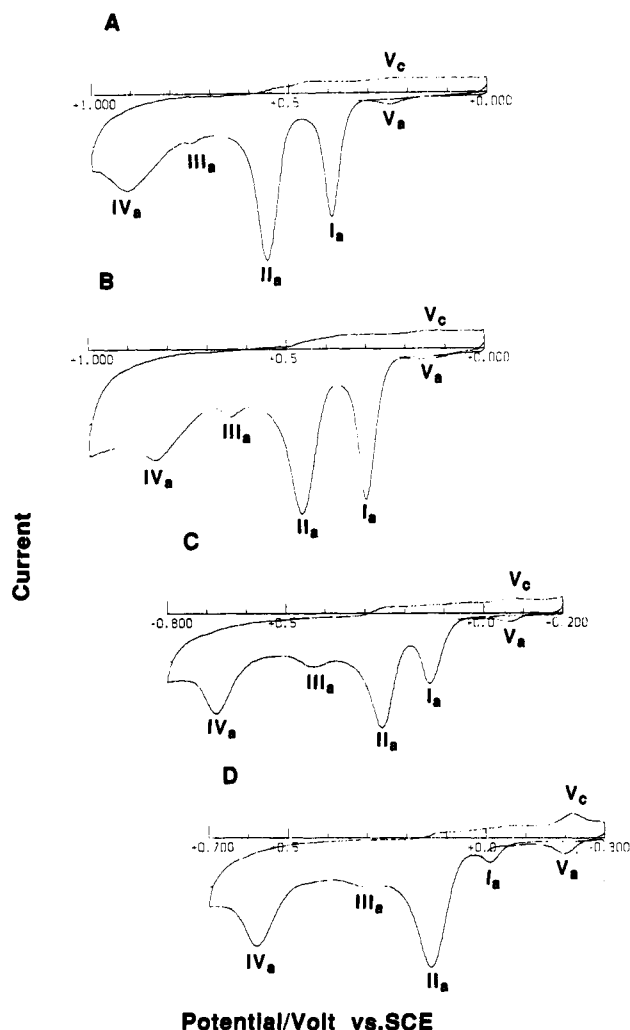


Figure 1. Cyclic Voltammograms at the PGE of 0.22 mM salsolinol-1-carboxylic acid (1) in phosphate buffers ($\mu = 1.0$) at pH (A) 3.0, (B) 5.0, (C) 7.0, and (D) 9.2. Sweep rate: 100 mV s^{-1} .

to oxidation peak I_a observed even when the scan was reversed after sweeping through only the latter peak.

Controlled Potential Electrooxidation and Product Characterization. Controlled potential electrooxidation of 1 (0.2–0.9 mM) in pH 7.0 phosphate buffer at +0.14 V (24 mV more positive than E_p for peak I_a ; 111 mV more negative than E_p for peak II_a) gave a coulometric n value of 2.1 ± 0.2 . Chromatograms recorded periodically throughout the electrolysis demonstrated the progressive disappearance of 1 and the appearance and growth of a single major product (Figure 3). The product responsible for the chromatographic peak having a retention time (t_R) of 27.5 min was isolated, and on the basis of 1H and ^{13}C NMR spectroscopy, mass spectrometry, and UV spectrophotometry and by comparison with the independently synthesized compound, it was identified as 2.

Representative chromatograms of the product solutions formed as a result of the controlled potential electrooxidation of 1 at +0.25 V (E_p for peak II_a) in pH 7.0 phosphate buffer are presented in Figure 4. Thus, shortly after initiation of the reaction three major product peaks can be observed in addition to that of unreacted 1. Using preparative-scale HPLC, compounds 2 and 4 were purified, desalted, and isolated as pure solids. Compound 4 was spectroscopically (1H NMR, ^{13}C NMR, FAB-MS, UV) characterized as 1-methyl-6,7-isoquinolinediol.³⁰ Compound 3 was not sufficiently stable for it to be isolated.

However, the HPLC mobile phase containing chromatographically pure 3 could be quantitatively reduced with an excess of ascorbic acid to 3,4-dihydro-1-methyl-5,6,7-isoquinolinetriol (13), which was isolated and spectroscopically characterized as its quinone methide tautomer 13b (see Experimental Section). Cyclic voltammograms of 3 and 13 at pH 7.0 were identical (see later discussion). Furthermore, controlled potential electroreduction of 3 at -0.30 V at pH 7.0 resulted in the quantitative formation of 13. Conversely, controlled potential electrooxidation of 13 at +0.10 V under otherwise identical conditions resulted in the formation of 3. Coulometric measurements indicated that the latter reactions were both 2e processes. Accordingly, 3 and 13 represent the oxidized and reduced forms, respectively, of a reversible redox system.

The chromatograms shown in Figure 4 indicate that during the controlled potential electrooxidation of 1 at peak II_a potentials the chromatographic peak corresponding to 2 increases during the first 15 min of the electrolysis (Figure 4B–D) and then decreases and ultimately disappears (Figure 4G). The chromatographic peaks corresponding to 3 and 4, however, particularly the latter, increase in height throughout the course of the electrolysis. This behavior suggested that at potentials corresponding to peak II_a 1 is initially electrooxidized to 2, which is then further oxidized to 3 and 4. Controlled potential electrooxidation to 2 at +0.25 V in pH 7.0 phosphate buffer did in fact result in the formation of 3 and 4 (Figure 5).

Cyclic Voltammetry of 2, 3, and 4. Cyclic voltammograms of the various identified products of electrochemical oxidation of 1 at pH 7.0 are presented in Figure 6B–F. A comparison of these voltammograms with that of 1 (Figure 6A) supports the conclusion that the peak I_a electrooxidation of the latter compound yields 2 which is responsible for peak II_a (Figure 6B) and, at sufficiently fast sweep rates, reduction peak II_c (Figure 6C). Oxidation peak III_a corresponds to that observed with 4 (Figure 6D). Peak IV_a corresponds to a peak observed for oxidation of 3 (Figure 6E) and 13 (Figure 6F). The peak V_a /peak V_c couple observed in cyclic voltammograms of 1 (Figure 6A) is clearly identical to that observed for 3 and 13 (Figure 6E,F).

Reaction Pathways. The peak I_a electrochemical oxidation of 1 at physiological pH is, overall, a 2e–2H⁺ reaction to give the 3,4-dihydroisoquinoline 2 as the sole product. Because of the shift of E_p for peak I_a to more positive potentials by about 33 mV per decade increase in ν , the latter peak merged with peak II_a at $\nu > 10$ V s^{-1} (Figure 2). As a result, it was not possible to search for a transient electroactive intermediate generated in the peak I_a reaction using very fast sweep cyclic voltammetry. Nevertheless, the peak I_a reaction is clearly an electrochemically-driven oxidative decarboxylation of 1. Such reactions which result from the direct oxidation of the carboxylate function are widely known as the Kolbe reaction and occur at very much more positive potentials than is required to oxidatively decarboxylate 1.³¹ This no doubt derives from the very poor electron-donating properties of the carboxyl function.³² Previous workers have noted that a number of TIQ-1-CAs containing hydroxy substituents at the C(6) or C(7) positions are oxidatively decarboxylated at anomalously low potentials²² at a graphite anode to give 3,4-dihydroisoquinolines. Thus, it seems probable that the oxidative decarboxylation of 1 to 2 derives from the ease of oxidation of the 6,7-dihydroxy residue. Catecholamines, such as dopamine (5), from

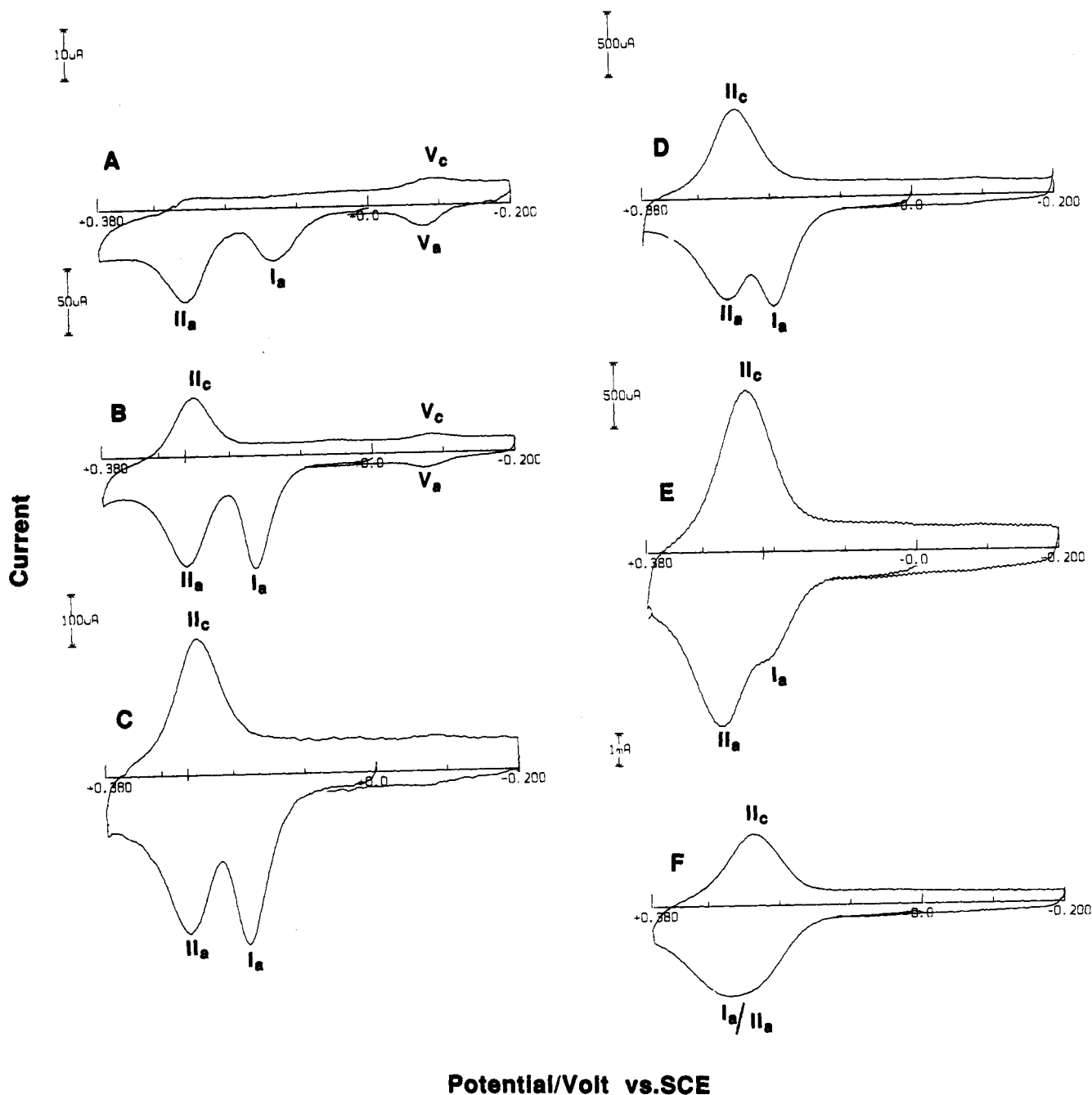


Figure 2. Cyclic voltammograms at the PGE of 0.22 mM **1** in pH 7.0 phosphate buffer ($\mu = 1.0$) at sweep rates of (A) 100 mV s^{-1} , (B) 0.5 V s^{-1} , (C) 2.0 V s^{-1} , (D) 5.0 V s^{-1} , (E) 10.2 V s^{-1} , and (F) 20.5 V s^{-1} .

which **1** is derived (Scheme I), are known to be electrochemically oxidized to the corresponding *o*-quinone.³⁵ Accordingly, it seems reasonable that the initial step in the peak I_a electrooxidation of **1** is a $2e-2H^+$ abstraction to give *o*-quinone **8** (Scheme III). By analogy with structurally similar *o*-quinones, such as that formed as the proximate electrooxidation product of salsolinol (**7**),³⁰ *o*-quinone **8** should be readily detected in cyclic voltammograms of **1** as a reduction peak reversibly coupled to peak I_a provided it had sufficient stability. That such a reduction peak cannot be observed at sweep rates as high as 10 V s^{-1} (the largest value of ν at which peak I_a can be observed separate from peak II_a) suggests that **8** is a very unstable species which rapidly decarboxylates to give **2a** as conceptualized in Scheme III. The reaction sequence $1 \rightarrow 8 \rightarrow 2a$ shown in Scheme III is often referred to as an EC mechanism, i.e., charge transfer followed by an irreversible chemical reaction. The anodic shift of E_p for

peak I_a with increasing ν is in qualitative accord with that theoretically predicted for such a reaction.³⁶ Based upon the fact that a peak corresponding to the reduction of **8** cannot be observed in cyclic voltammograms of **1**, it can be calculated that the first-order rate constant for the decarboxylation of **8** to **2a** is $>10^3 \text{ s}^{-1}$.³⁷ Collins and Cheng¹⁹ have previously noted that the transition metal ion catalyzed oxidative decarboxylation of **1** might proceed by either a concerted or coupled (i.e., via intermediate **8**) reaction. The lack of competitive inhibition of the catalyzed conversion of **1** to **2** by excess phenolic TIQ-1-CA tended to support the coupled mechanism. The very poor electron donating properties of the carboxyl group would also tend to argue against a concerted reaction.

The ^{13}C NMR spectrum of **2** in D_2O exhibited a strong carbonyl resonance, suggesting that this compound exists predominantly under such conditions as the quinone methide tautomer **2a** (Scheme III). Further evidence for

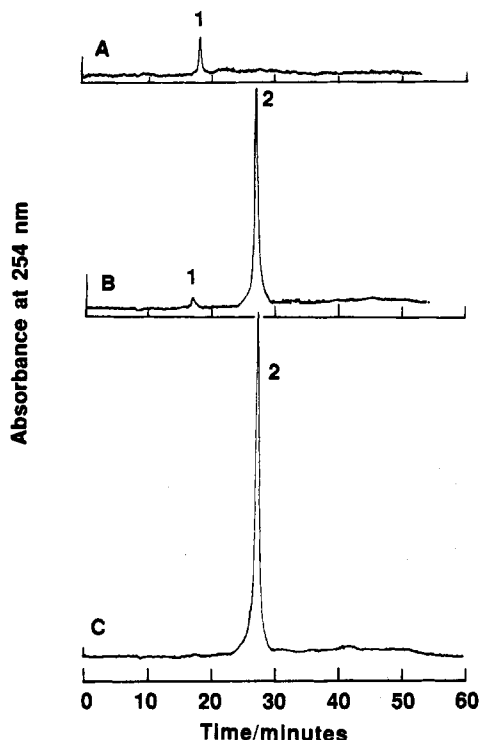
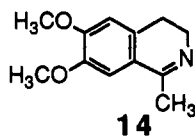


Figure 3. HPLC chromatograms recorded during the controlled potential electrooxidation of 0.3 mM 1 in pH 7.0 phosphate buffer ($\mu = 0.15$) at +0.14 V. The chromatograms were recorded after (A) 0, (B) 30, and (C) 90 min electrolysis and employed HPLC method I. Injection volume: 2 mL.

the pH-dependent tautomerism of 2 over the pH range of interest in this study was provided by UV spectral data. Figure 7A–D shows spectra of 2 in aqueous solution between pH 3 and 9.2. At pH ≤ 5 the spectrum of 2 exhibits bands at $\lambda_{\max} = 348, 300$, and 244 nm (Figure 7A,B). These spectra are virtually identical to those of 3,4-dihydro-1-methyl-6,7-dimethoxyisoquinoline (14) between pH 3 and



9 (Figure 7E,F). Thus, it can be concluded that at pH ≤ 5 the predominant tautomer of 2 in solution is 2b. At pH > 5 , however, the spectrum of 2 changes appreciably. Thus, the band initially at 348 nm (pH ≤ 5) shifts to longer wavelengths and increases in intensity (Figure 7C,D). The band at 300 nm (pH ≤ 5) similarly shifts to longer wavelengths but decreases in intensity with increasing pH, and the band at 248 nm shifts to 264 nm. Thus at pH 9.2 the spectrum of 2 ($\lambda_{\max} 382, 318, 264$ nm) is quite different from that at pH ≤ 5 and, therefore, corresponds to that of tautomer 2a. Based upon studies of structurally similar dihydroxyphenols,^{37,38} it is unlikely that the spectral changes observed for 2 between pH 3 and 9.2 are associated with extensive dissociation of a hydroxyl group. At pH 7.0 the spectrum of 2 exhibits bands characteristic of 2b (244 nm) and 2a (264 and 380 nm). Assuming that the spectra of 2 at pH 3.0 and 9.2 correspond almost exclusively to tautomers 2b and 2a, respectively, it has been calculated that at pH 7.0 compound 2 exists about 70% as quinone methide tautomer 2a. On the basis of fluorescence methods, Jonsson³⁹ and Corrodi and Jonsson⁴⁰ concluded that the 3,4-dihydro-6,7-isoquinolinediol formed by exposure of 5 in neuronal tissue to formaldehyde and

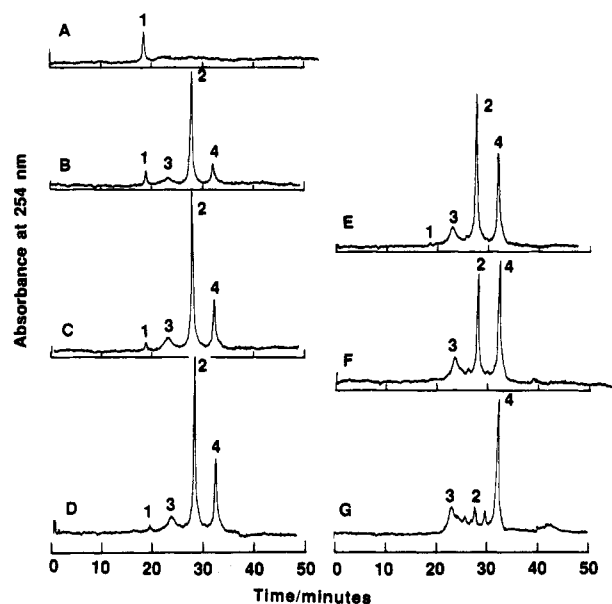


Figure 4. HPLC chromatograms recorded during the controlled potential electrooxidation of 0.32 mM 1 in pH 7.0 phosphate buffer ($\mu = 0.15$) at +0.25 V. Chromatograms were recorded after (A) 0, (B) 5, (C) 10, (D) 15, (E) 20, (F) 30, and (G) 60 min and employed HPLC method I. Injection volume: 2.0 mL.

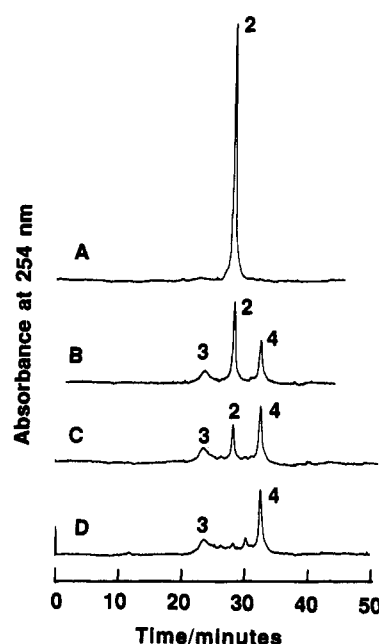


Figure 5. HPLC chromatograms recorded during the controlled potential electrooxidation of 0.3 mM 2 in pH 7.0 phosphate buffer ($\mu = 0.15$) at +0.25 V. Chromatograms were recorded after (A) 0, (B) 15, (C) 30, and (D) 60 min and employed HPLC method I. Injection volume: 2.0 mL.

oxidizing conditions exists largely as a quinone methide tautomer structurally analogous to 2a. Cheng et al.⁴¹ have used similar techniques and concluded that 2a is the predominant tautomer at physiological pH.

Voltammetric peak II_a observed for 1 is clearly due to the oxidation of 2 formed as a result of the peak I_a reaction. A major product deriving from the electrooxidation of 2 is 3 (Scheme III). Accordingly, it can be concluded that the proximate 2e–1H⁺ oxidation product of 2 is 9a/9b. Nucleophilic attack by water on the C(5)-centered carbocation resonance form 9b of this putative intermediate would be expected to yield the 5,6,7-trihydroxy-3,4-dihydroisoquinoline 13a as outlined in Scheme III. However, the ¹³C NMR spectrum of 13 in D₂O exhibits an

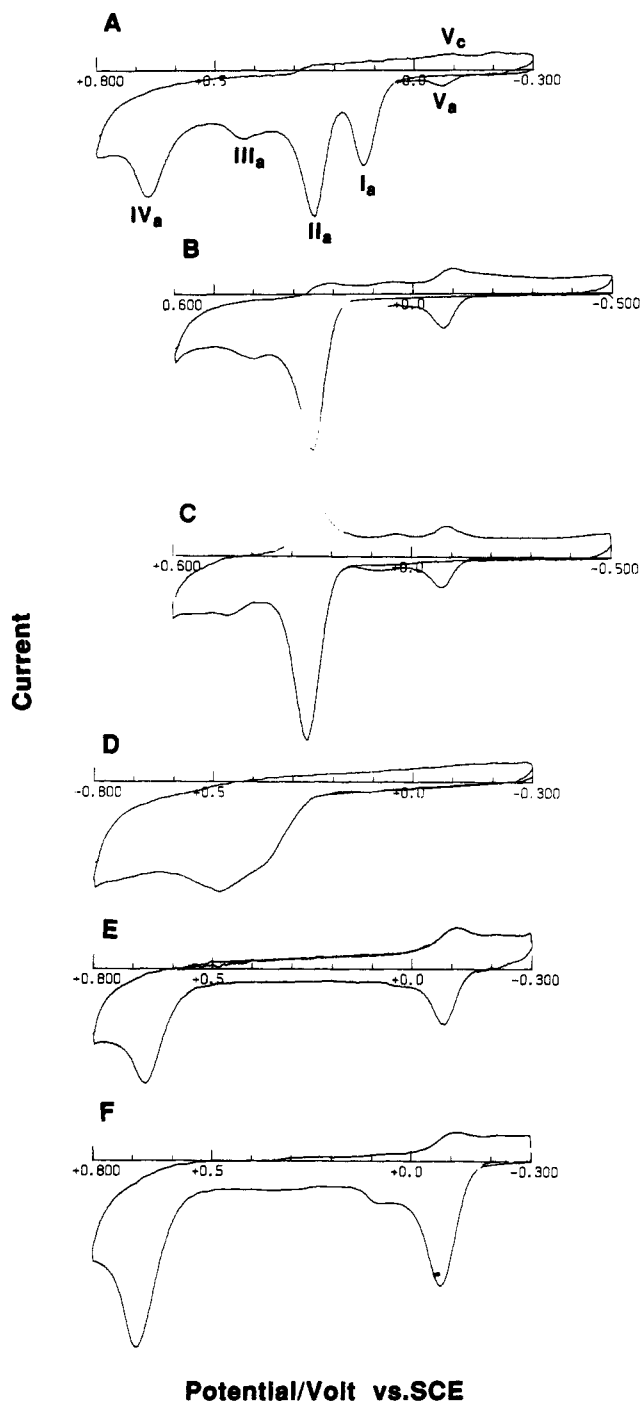


Figure 6. Cyclic voltammograms at the PGE of (A) 0.15 mM 1, (B) and (C) 0.3 mM 2, (D) 0.3 mM 4, (E) 3, and (F) 0.3 mM 13 in pH 7.0 phosphate buffer ($\mu = 1.0$). For A, B, D–F the sweep rate was 100 mV s⁻¹; for C the sweep rate was 1.0 V s⁻¹. The solution for 3 was prepared by dilution of the HPLC eluent containing 3 with pH 7.0 (1:1 v/v) phosphate buffer ($\mu = 1.0$); the pH of the resulting solution was 7 and the concentration of 3 was ca. 0.1 mM.

intense carbonyl resonance indicating that at pH values in the vicinity of 7 this compound exists predominantly as the quinone methide tautomer 13b. Compound 13 is a very easily oxidized species (Figure 6F), and hence, as soon as it is formed in the peak II_a electrooxidation of 1, it is immediately oxidized to 3. Thus, at relatively small values of v , cyclic voltammograms of 1 exhibit reduction peak V_c following sweep reversal after scanning peak II_a. Peak V_c corresponds to the 2e–2H⁺ reduction of 3 to 13. On the second anodic sweep oxidation peak V_a appears, which corresponds to the reverse reaction.

The major product of the electrochemical oxidation of 2 is 4. This compound exists in the same oxidation state as putative *o*-quinone intermediate 9a and hence is formed as a result of the rearrangement (aromatization) of this species as conceptualized in Scheme III.

Preliminary Biological Studies. The sole peak I_a electrochemical oxidation product of 1 is 2. At peak II_a potentials 2 is further electrooxidized to 3 and 4. Preliminary experiments were carried out to assess the toxicity and behavioral effects evoked when 2, 4, and the reduced form of 3, i.e., 13, were centrally administered to laboratory mice. Compounds 2, 4, and 13 were administered intracranially, with animals maintained under a light ether anesthesia, in the vicinity of the right lateral ventricle in 5 μ L of a vehicle consisting of isotonic saline (0.9% NaCl in deionized water).

LD₅₀ Determination. The LD₅₀ was defined as that dose of drug dissolved in 5 μ L of isotonic saline and injected intracranially which resulted in the death of 50% of the treated animals within 1 h. The statistical methods of Dixon^{42,43} were employed to measure the LD₅₀ of 2 and 13; compound 4 was not toxic up to the maximum dose employed (100 μ g), and hence an LD₅₀ was not determined. The experimental LD₅₀ values for 2 and 13 were 31.1 \pm 1.1 (mean \pm standard deviation) μ g and 38.9 \pm 1.1 μ g, respectively.

Behavioral Effects. Central administration of 2 (15–100 μ g dissolved in 5 μ L of isotonic saline) evoked marked behavioral effects in the mouse. Thus, after recovery from the anesthetic (\leq 5 min), the animals generally were unable to stand and lay on their sides. All limbs often moved erratically and wildly, the animal's necks curved backwards, and the tail was stiff. During this period, which persisted for times up to 2 h (>15 μ g dose), animals' bodies trembled continuously. Periodically, animals rolled over along the head–tail axis, each episode typically involving 3–5 revolutions within a few seconds. The larger the dose of 2, the more violent were the above behavioral responses. At doses \geq 60 μ g death occurred in <10 min. At doses \leq 31 μ g animals which survived for longer than 1 h had great difficulty in standing for the next several hours and appeared to have lost coordination of the movement of their limbs. After 12 h, all surviving animals appeared to fully recover normal behavioral patterns.

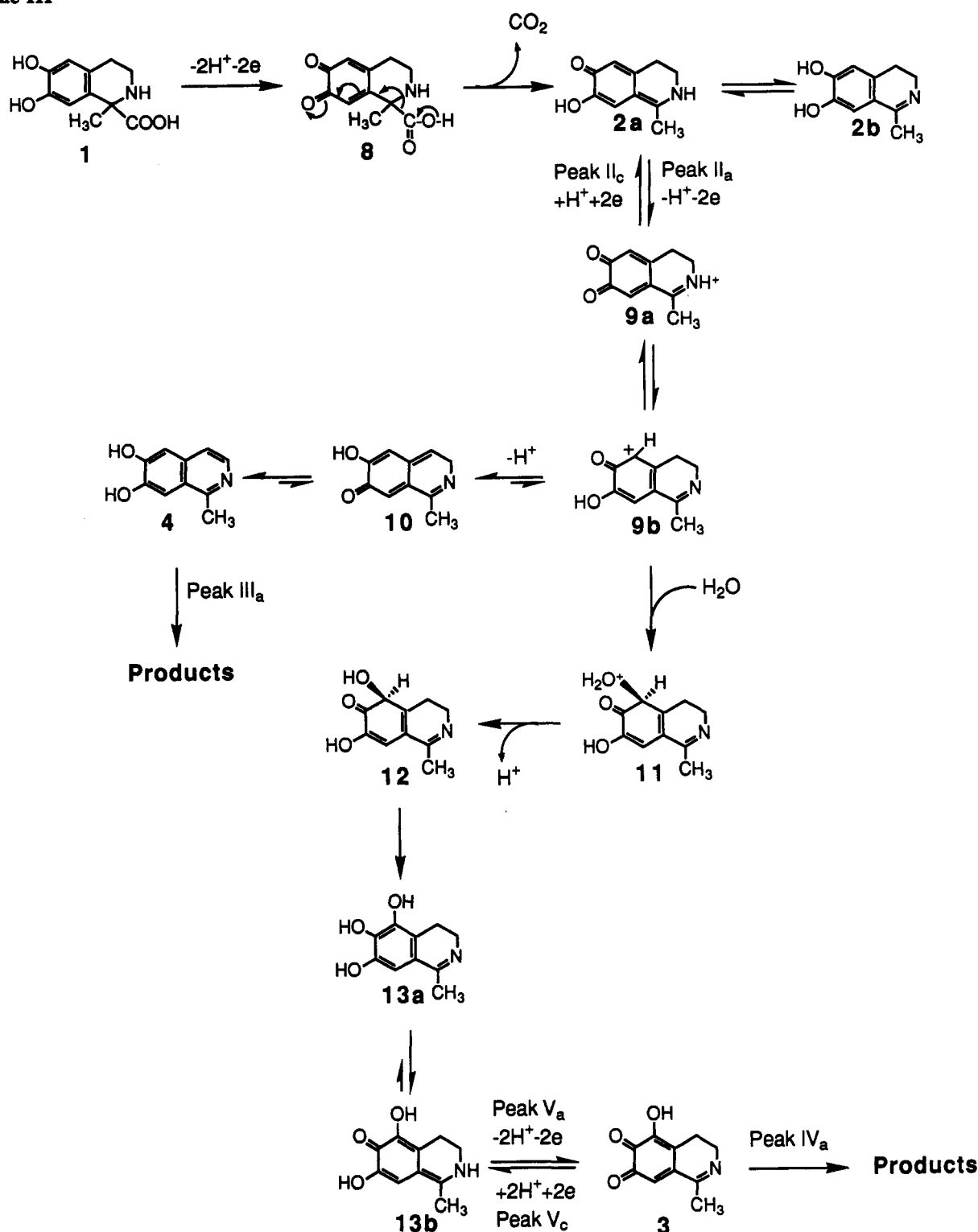
When animals recovered from the anesthetic following central administration of 13 (30–63 μ g), they were unable to stand and lay on their sides. Their limbs moved wildly and occasionally episodes of violent jumping or rolling along the head–tail axis occurred. These episodes lasted for 1–3 s. During this initial phase the neck was held in a rigid, backward posture and the tail was stiff. For animals which survived for \geq 1 h, the above violent behavior slowly subsided, but standing was difficult and both the body and limbs exhibited severe shaking and trembling. After 1.5–2 h animals became lethargic and continued to walk with difficulty. After 12 h all surviving animals appeared to fully recover. At the maximum dose employed (63 μ g) all treated animals died within 15–20 min.

Intracranial injection of vehicle alone (5 μ L) resulted in none of the behavioral responses described following administration of 2 or 13. Animals treated with vehicle alone exhibited apparently identical behavior to totally untreated animals once they recovered from the anesthetic.

Discussion

The rationale for the work reported above was to explore the oxidative transformations of 1 on the assumption that

Scheme III



such reactions might occur in the brain and result in formation of toxic or neuropharmacologically active compounds which play roles in the neurodegenerative, behavioral, and addictive consequences of chronic ethanol consumption. The results reported in this paper indicate that the peak I_a oxidation product from 1 is 2, which is indeed toxic and evokes unusual behavioral responses when centrally administered to mouse. Furthermore, 3 (the anticipated autooxidation product of 13 in brain tissue), one of the two oxidation products derived from the peak I_a oxidation of 2, is similarly toxic and results in profound behavioral responses in the mouse. The low peak oxidation potential of 1 at pH 7.0 (+0.116 V; $\nu = 5 \text{ mV s}^{-1}$) is in

accord with the reported ease of autoxidation of 1 to 2.^{19,20} However, it is important to note that the peak oxidation potential for 2 at pH 7.0 (+0.25 V; $v = 5 \text{ mV s}^{-1}$) is also quite low, i.e., 2 is also a relatively easily oxidized compound. Incubations of 1 with the supernatant of a rat brain homogenate results in the slow formation of 2 although there is apparently no enzyme catalytic activity present in such preparations, i.e., oxidative decarboxylation occurs by an autoxidation pathway.¹⁹ However, the lack of oxidative enzyme activity in such brain homogenate supernatants does not unequivocally discount the possibility of enzyme systems in the brain that might catalyze the oxidation of 1 and 2. Furthermore, it is of relevance

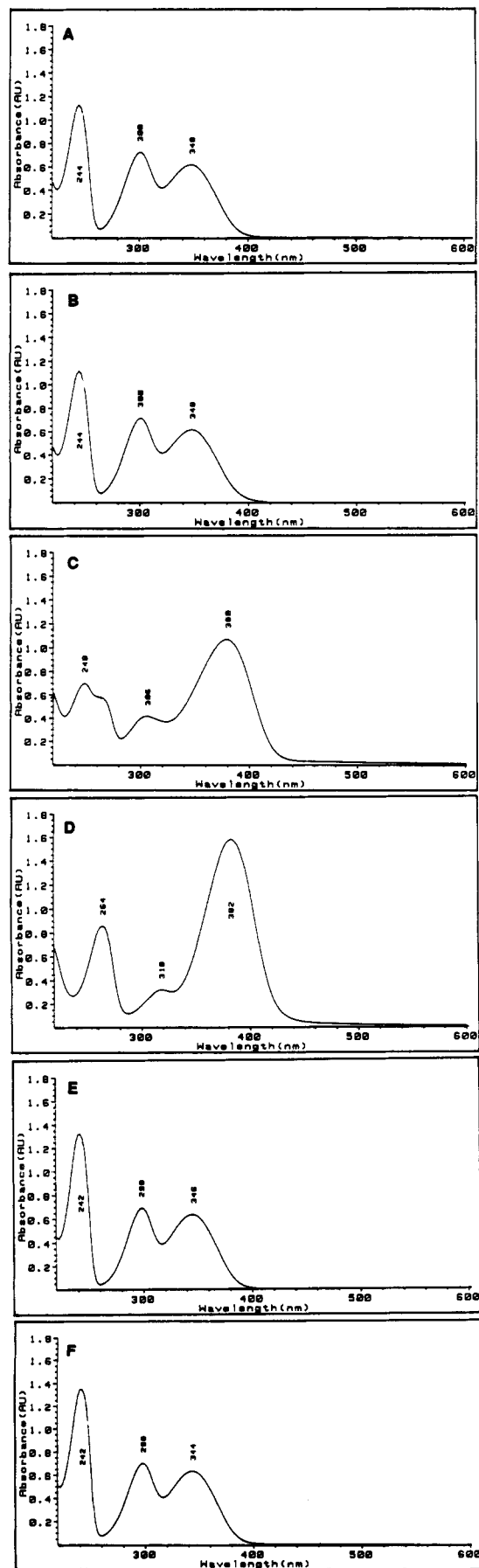


Figure 7. UV spectra of 0.157 mM **2** in phosphate buffers ($\mu = 1.0$) at pH (A) 3.0, (B) 5.0, (C) 7.0, (D) 9.2 and 0.157 mM **14** at pH (E) 3.0 and (F) 7.0. Spectral measurements employed 0.5 cm pathlength cells.

to note that salsolinol (**7**), which exhibits a voltammetric oxidation peak at the PGE at pH 7.0 with $E_p = 0.222$ V ($\nu = 10$ mV s $^{-1}$)³⁰ is readily oxidized in the presence of a number of oxidative enzyme systems.²⁹ Indeed, Coultas et al.²¹ have demonstrated that several TIQ-1-CAs with C(6)- or C(7)-hydroxy substituents are oxidized in the presence of peroxidase/H₂O₂ and fungal laccase enzymes. Ung-Chhun et al.³ have obtained evidence for traces of **2** in extracts of human brain although the precise source of this compound and its ultimate fate in this organ are currently not known.

The underlying chemical and/or biochemical mechanisms related to the toxicity and behavioral responses evoked by **2** and **3** are currently unknown. Cheng et al.⁴¹ have reported that **2** is a potent inhibitor of catechol-*O*-methyltransferase (COMT) in vivo. Based on the observation that **2a** is not attacked by the tripeptide glutathione, it seems unlikely that the toxicity of this quinone methide is related to alkylation of cellular nucleophiles as suggested by Collins.²⁶ However, it is provocative to note that the electrochemical oxidation of **2a** does yield an electrophilic intermediate (**9a/9b**, Scheme III) which is readily attacked by water, a relatively weak nucleophile, to yield, ultimately, **3**.

In summary, both **1** and its initial oxidation product **2a** are rather easily oxidized species. In vivo experiments have revealed that quinone methide **2a** and one of its major oxidation products, **13**, are behavioral toxins in mouse brain. Whether oxidations of **1** or the related endogenous alkaloid salsolinol (**7**), both of which are elevated in brain as a result of chronic, heavy ethanol consumption, actually occur in the brain is not yet known with any certainty. However, if such oxidation reactions do indeed occur and they follow pathways similar to those elucidated in this study then toxic, neuropharmacologically active metabolites would be formed. These, in turn, might play some roles in the neurodegenerative and other behavioral and biochemical consequences of chronic alcoholism.

Experimental Section

Dopamine hydrochloride, pyruvic acid, 3,4-dimethoxyphenethylamine, and trifluoroacetic acid were obtained from J. T. Baker (Phillipsburg, NJ). Phosphorus oxychloride, hydrobromic acid, and L-ascorbic acid were obtained from Aldrich (Milwaukee, WI).

Voltammograms were obtained at a pyrolytic graphite electrode (PGE; Pfizer Minerals, Pigments and Metals Division, Easton, PA) having an approximate surface area of 12.5 mm². A conventional three-electrode voltammetric cell was used containing a platinum wire counter electrode and a saturated calomel reference electrode (SCE). Linear sweep and cyclic voltammograms were obtained using a BAS-100A (Bioanalytical Systems, West Lafayette, IN) instrument. All voltammograms were corrected for iR drop. The PGE was always resurfaced prior to recording each voltammogram using published procedures.⁴⁴

Controlled potential electrolyses employed a Princeton Applied Research Corp. (Princeton, NJ) Model 173 potentiostat and Model 379 digital coulometer. The working electrode used for controlled potential electrolyses consisted of several plates of pyrolytic graphite having a total surface area of ca. 240 cm². A three-compartment cell was used in which the working, counter, and reference electrode compartments were separated by a Nafion membrane (Type 117, Du Pont Co., Wilmington, DE). The working electrode compartment had a capacity of 70 mL. The counter electrode consisted of several plates of pyrolytic graphite and was suspended into a solution of the supporting electrolyte. An SCE reference electrode was employed. The solution in the working electrode compartment was stirred with a Teflon-coated magnetic stirring bar and N₂ was bubbled vigorously. All potentials are referred to the SCE at ambient temperature (22 \pm 2 $^{\circ}$ C).

^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer. Low- and high-resolution fast atom bombardment mass spectrometry (FAB-MS) were carried out on a VG Instruments (Manchester, UK) Model ZAB-E spectrometer. Mass spectrometry on samples dissolved in a chromatographic mobile phase was carried out with a Kratos MS 25/RFA mass spectrometer equipped with a thermospray source. The solvent was 0.1 M ammonium acetate in deionized water adjusted to pH 4.1 with acetic acid; a flow rate of 1 mL/min was employed. Samples (1–2 mL) were introduced into this solvent using a Rheodyne Model 7125 loop injector. The thermospray capillary was maintained at 150 °C and the source at 250 °C. UV-visible spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer.

High-performance liquid chromatography (HPLC) employed a Gilson (Middleton, WI) gradient system equipped with Model 305 and 306 pumps, a Holochrome UV detector set at 254 nm, and a Rheodyne 7125 loop injector. A reversed-phase column (Regis, Morton Grove, IN; C_{18} , 10- μm particle size, 25×2.1 cm) and a short guard column (Upchurch, C-1022, 1×1 cm) packed with Bakerbond (J.T. Baker, C_{18} , 10- μm particle size) were used. In order to separate the products formed for either analytical or preparative purposes, two binary gradient mobile phase solvents were employed. Solvent A was prepared by adding 30 mL of concentrated ammonium hydroxide solution (NH_4OH) to 4 L of deionized water; the pH of the resulting solution was adjusted to 3.0 by addition of concentrated trifluoroacetic acid (TFA). Solvent B was prepared by adding 15 mL of NH_4OH to 2 L of HPLC-grade methanol (MeOH) and 2 L of deionized water; the pH of the resulting solution was adjusted to 3.0 by addition of TFA. For HPLC method I, the following gradient was employed: 0–2 min, 100% solvent A; 2–35 min, linear gradient to 100% solvent B; 35–45 min, 100% solvent B. The flow rate was 6 mL/min. Solvent C was prepared by adding TFA to deionized water until the pH was 3.0. Solvent D was prepared by adding 1 L of MeOH and 1 L of HPLC-grade acetonitrile (MeCN) to 2 L of deionized water; the pH was adjusted to 3.0 with TFA. For HPLC method II, the following gradient was employed: 0–2 min, 100% solvent C; 2–20 min, linear gradient to 60% solvent C and 40% solvent D; 20–30 min, linear gradient to 100% solvent D; 30–40 min, 100% solvent D. The flow rate was 6 mL/min. HPLC method I was used primarily to separate reaction product mixtures. HPLC method II was employed to desalt and purify products.

Outbred adult male mice of the Hsd:ICR albino strain (Harlan Sprague-Dawley, Madison, WI) weighing 28–36 g were used in animal experiments. Animals were housed 10 per cage, were allowed access to Purina Rat Chow and water ad libitum, and were maintained on a 12 h light/dark cycle with lights on at 7:00 a.m. Animals were used only after at least 7 days following receipt from the supplier. Experimental animals were treated with 2 or 13 dissolved in 5 μL of isotonic saline (0.9% NaCl in deionized water). Animals were first anesthetized with ether for 60 s. Injections were performed free hand, the puncture being as close as possible to a point 3.5 mm anterior to the interaural line and 1 mm right lateral of the midline according to Lehman et al.⁴⁵ The injection at this point was perpendicular to the scalp to a depth of 3 mm. The depth of the injection was controlled by a collar on the needle of a 10- μL microsyringe. The dose was injected over the course of ca. 10 s; the needle was held in place for ca. 10 s before removal. Control animals were treated with 5 μL of vehicle alone. The above experimental protocols were approved by the University of Oklahoma Institutional Animal Care and Use Committee.

Synthesis of 1,2,3,4-Tetrahydro-1-methyl-1-carboxy-6,7-isoquinolinediol (1). Dopamine hydrochloride (5 g) and pyruvic acid (2 g) were dissolved in deionized water (25 mL). The pH of this solution was adjusted to 4.0 by dropwise addition of concentrated NH_4OH solution. The resulting solution was purged with N_2 , and then the vessel was sealed and stored in the dark at room temperature for 4 days. Following evaporation on a rotary evaporator (50 mmHg; 80 °C), the product obtained was recrystallized from water to give a white solid (yield 68%): mp 235 °C dec (lit.⁴⁶ mp 230–235 °C); FAB-MS (3-nitrobenzyl alcohol matrix) m/e 246 (MNa^+ , 29), 224 (MH^+ , 100), 178 ($\text{MH}^+ - \text{HCOOH}$, 44); accurate mass measurements on MH^+ 224.0917 ($\text{C}_{11}\text{H}_{14}\text{NO}_4$; calcd m/e 224.0923); ^1H NMR (D_2O) δ 7.04 (s, 1 H,

C(8)–H), 6.70 (s, 1 H, C(5)–H), 3.50–3.35 (m, 2 H, C(3)–H₂), 3.00–2.83 (m, 2 H, C(4)–H₂), 1.77 (s, 3 H, CH₃); ^{13}C NMR (D_2O) δ 177.70 (C=O), 146.78, 145.73, 128.27, 126.45, 118.14, 117.04, 101.03, 66.74, 41.98, 26.98.

Synthesis of 3,4-Dihydro-1-methyl-6,7-dimethoxyisoquinoline (14) and 3,4-Dihydro-1-methyl-6,7-isoquinolinediol (2). Compound 2 was synthesized by demethylation of 3,4-dihydro-1-methyl-6,7-dimethoxyisoquinoline which was prepared via the Bischler-Napieralski reaction from *N*-acetylhomoveratrylamine [*N*-[2-(3,4-dimethoxyphenyl)ethyl]acetamide]. *N*-Acetylhomoveratrylamine was prepared by adding 50 mL of acetic anhydride to a stirred solution of 70 mL of 3,4-dimethoxyphenethylamine in 37 mL of pyridine at a rate such that the temperature never exceeded 90 °C (ca. 1 h was required). The reaction solution changed from yellow to orange during the course of this reaction. After standing overnight at room temperature, the solution was evaporated using a rotary evaporator (50 mmHg/70–80 °C). The residue was recrystallized from ethyl acetate to give *N*-acetylhomoveratrylamine as a white powder (55 g, 60% yield), mp 104–105 °C (lit.⁴⁷ mp 100 °C). The latter compound (15 g) was dissolved in 77 mL of toluene, and then 15 mL of POCl_3 was slowly added to the resulting solution over 30 min. The resulting solution was stirred and refluxed for 2 h and then stored in ice-water overnight to give a white precipitate. The precipitate was vacuum filtered and then washed with methanol and ethyl acetate. Recrystallization from ethyl acetate gave 3,4-dihydro-1-methyl-6,7-dimethoxyisoquinoline (14) (12 g, yield 74%), mp 202–204 °C (lit.⁴⁷ mp 202–203 °C). A solution of the latter compound (2.7 g) in hydrobromic acid (48%) was refluxed for 3 h. The resulting solution was evaporated with a rotary evaporator. The residue obtained was crystallized from methanol/ether to give pale yellow 2.HBr: electron-impact MS m/e 177.0790 (M^+ , 100; $\text{C}_{10}\text{H}_{11}\text{NO}_2$; calcd m/e 177.0790); ^1H NMR (D_2O) δ 7.34 (s, 1 H, C(8)–H), 6.85 (s, 1 H, C(5)–H), 3.75 (t, $J_{3,4} = 7.8$ Hz, 2 H, C(3)–H₂), 2.97 (t, $J_{3,4} = 7.8$ Hz, 2 H, C(4)–H₂), 2.63 (s, 3 H, CH₃); ^{13}C NMR (D_2O) δ 177.84 (C=O), 155.99, 146.21, 135.81, 120.16, 114.77, 117.80, 43.86, 26.84, 22.84.

3,4-Dihydro-1-methyl-5-hydroxy-6,7-isoquinolinedione (3). In the mobile phase employed with HPLC method I (apparent pH 3.0) 3 gave a yellow solution with $\lambda_{\text{max}} = 410$, 328, and 222 nm. Thermospray mass spectrometry on this solution showed $m/e = 192$ (MH^+ , 100). After desalting a solution of 3 (HPLC method II) followed by freeze-drying, HPLC analysis (method I) of the resulting product revealed the presence of a complex mixture of decomposition products of 3, which have not been identified. Accordingly, the solution containing 3 in the HPLC method I mobile phase was reduced by the addition of an excess of L-ascorbic acid which caused the yellow solution to become colorless. HPLC analysis (method I) of the reduced product showed a single peak at $t_R = 31$ min (t_R for 3 was 22.5 min). The compound eluted under this peak was collected and the solution freeze-dried. The resulting solid was dissolved in the minimum volume of HPLC mobile phase solvent C and desalted using HPLC method II. The single product (13) eluting at $t_R = 22$ min was collected and freeze-dried to give a very pale yellow solid. In pH 7.0 buffer a solution of 13 showed λ_{max} (log ϵ_{max} , $\text{M}^{-1} \text{cm}^{-1}$) at 380 sh (3.20), 3.28 (3.59), and 244 nm (3.40); FAB-MS (3-nitrobenzyl alcohol matrix) $m/e = 194.0844$ (MH^+ , 100; $\text{C}_{10}\text{H}_{12}\text{NO}_3$; calcd $m/e = 194.0817$); ^1H NMR (D_2O) δ 6.44 (s, 1 H, C(8)–H), 3.61 (t, $J_{3,4} = 7.2$ Hz, 2 H, C(3)–H₂), 2.87 (t, $J_{3,4} = 7.2$ Hz, 2 H, C(4)–H₂), 2.74 (s, 3 H, CH₃). Correlated spectroscopy (COSY) 2D- ^1H NMR experiments revealed that the resonances at δ 6.44 and 2.74 (CH₃) were strongly coupled, indicating, therefore, that the former resonance must correspond to C(8)–H. ^{13}C NMR (D_2O) δ 177.78 (C=O), 156.57, 154.76, 135.43, 133.35, 110.44, 109.86, 43.34, 28.27, 26.22. These spectral data for 13 are in accord with the structure of 3,4-dihydro-1-methyl-5,7-dihydroxyisoquinolin-6-one as the reduced form of 3. Cyclic voltammograms of 3 and 13 were identical (Figure 6E,F). Furthermore, 13 could be quantitatively converted to 3 by controlled potential electrooxidation at 0.1 V in pH 7.0 phosphate buffer. Conversely, 3 was quantitatively converted to 13 by controlled potential electroreduction at –0.3 V.

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