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Oxidation Chemistry of the Endogenous Central Nervous System Alkaloid Salsolinol

I. Electrochemical Studies

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The electrochemical oxidation of salsolinol (SAL; 1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol) has been studied in aqueous solution at physiological pH. The initial step in the oxidation is a reversible $2e$–$2H^+$ oxidation of SAL to the corresponding *ortho*-quinone (**E**). This species has a short lifetime at pH 7 (ca. 1 s). Reaction pathways leading from **E** to four major initial products, 2,3,4-trihydro-1-methyl-7-hydroxy-6-oxyisoquinoline (**C**), *cis*- and *trans*-1,2,3,4-tetrahydro-1-methyl-4,6,7-isoquinolinetriol (**A** and **B**), and 1-methyl-6,7-isoquinolinediol (**D**), are described. The potential relationships of the oxidation reactions of SAL to nervous system damage caused in chronic alcoholism are discussed.

The Pictet–Spengler condensation reaction between the catecholamine neurotransmitter dopamine (DA) and acetaldehyde (ACH) results in the formation of salsolinol (SAL; 1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol) (Scheme 1) (*1*, *2*). SAL and its 7-*O*-methyl derivative are normal, albeit trace, constituents of the mammalian central nervous system (*3–5*). Levels of SAL increase in certain brain regions of rats which chronically consume ethanol (*6–8*). And, in humans, urinary levels of SAL become elevated during ethanol intoxication and decline during detoxification (*5*, *9*). Such observations have led to the suggestion that in chronic alcoholism ACH, the proximate metabolite of ethanol in the liver, might escape into the circulatory system, enter the brain, and react with DA and other biogenic catecholamines to form tetrahydroisoquinoline (TIQ) alkaloids. Furthermore, it has been suggested that these TIQs might contribute to the behavioral changes, physical dependence, and addictive properties of ethanol (*10*). Indeed, chronic intracerebroventricular infusions of SAL to the rat evoke a dramatic increase in the preference of the animal for ethanol consumption (*11*), an effect which continues long after termination of drug administration (*11–13*). The half-life of SAL in rat brain is 12.5 min (*14*). Accordingly, it is conceivable that the alkaloid might be rapidly metabolized to an active compound(s) which is responsible for the long-lasting effect of the drug on ethanol preference.

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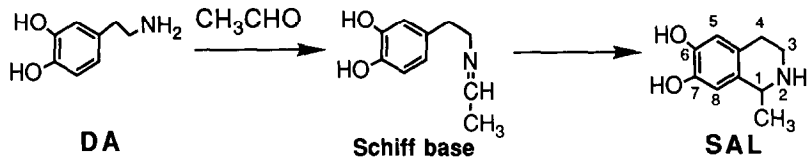
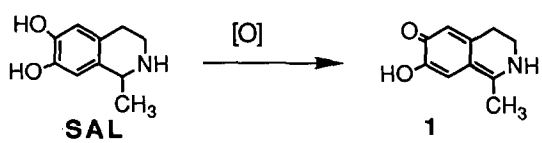


SCHEME 1

Ethanol consumption not only evokes behavioral changes and is addictive but, at least in the case of long-term chronic alcoholism, results in impaired learning ability and a general decline of intellectual abilities. The latter effects have been attributed to organic brain damage (*15*). Neuronal damage and loss, particularly in hippocampal regions of the brain, have been noted (*16*). Indeed, the effects of chronic alcoholism have been likened to a premature aging of the brain (*17*). Recently, Collins (*18*) proposed that oxidation reactions of SAL and other endogenous TIQs that are elevated in the brains of chronic alcoholics might lead to toxic products which are responsible for neuronal damage. For example, it was suggested that a potential toxic oxidation product of SAL might be the quinone **1** (Scheme 2), which has a striking structural similarity to the electrophilic *para*-quinone thought to be a toxic product of intraneuronal autoxidation of 6-hydroxydopamine, a widely used catecholaminergic neurotoxin (*19, 20*). However, there is currently very little information available regarding the oxidation reactions of SAL, or indeed other endogenous TIQs. If, in fact, oxidation reactions of SAL and other TIQs which are elevated in the brains of chronic alcoholics do play important roles in the etiology of the disease by forming CNS toxins and/or other neuropharmacologically active compounds, it is clearly important to understand such reactions. As a first step toward understanding such chemistry the electrochemical oxidation chemistry of SAL in aqueous solution has been investigated. Electrochemical techniques were selected for these initial studies because of their ability to rapidly provide information regarding the oxidation potentials of SAL and the nature and lifetime of key intermediates. Furthermore, it is relatively easy to isolate the initial major products of electrochemically driven reactions without the potential involvement of reactions of electrophilic intermediates with complex nucleophiles (e.g., peptides and proteins) which could well occur in biological systems. There is now a considerable body of evidence to support the view that electrochemistry can often provide very valuable insights into biolgoical oxidation (and reduction) processes (*21–23*).



SCHEME 2

EXPERIMENTAL

L-1,2,3,4-Tetrahydro-1-methyl-6,7-isoquinolinediol hydrochloride (L-SAL · HCl) was obtained from Sigma (St. Louis, MO) and was used without additional purification. Phosphate buffers of known ionic strength (μ) were prepared according to Christian and Purdy (*24*).

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 mm^2 and were recorded on a BAS-100A (Bioanalytical Systems, West Lafayette, IN) instrument. All voltammograms were corrected for *iR* drop. The PGE was resurfaced before each voltammogram was recorded using standard procedures (*25*). Conventional three-electrode electrochemical cells were used both for voltammetric studies and for controlled potential electrolyses (CPE) and contained platinum counterelectrodes and saturated calomel reference electrodes (SCE). All potentials are referred to the SCE at ambient temperature (22 ± 3°C). Two cells were employed for CPE; one had a working electrode compartment capacity of 35 ml, and the other a capacity of ca. 200 ml. Several plates of pyrolytic graphite served as the working electrode. All CPEs were performed on solutions which were stirred by a Teflon-coated magnetic stirring bar and with N_2 bubbling vigorously through the solution.

High performance liquid chromatography (HPLC) was performed with a Bio-Rad (Richmond, CA) gradient system equipped with a Gilson (Gilson Medical Electronics, Middleton, WI) Holochrome uv detector set at 280 nm. Both a semipreparative reversed phase column (Brownlee Laboratories RP-18, 5 μm particle size, 25 × 0.7 cm) and a preparative reversed phase column (J. T. Baker, Phillipsburg, NJ, Bakerbond C_{18}, 10-μm particle size, 25 × 2.12 cm) were employed. Both columns were always protected with guard columns during use.

The products formed upon electrooxidation of SAL were initially separated using a binary gradient mobile phase system (Gradient System I) and the preparative HPLC column. The two mobile phases used with Gradient System I were prepared as follows: Solvent A was prepared by adding 15 ml of concentrated ammonium hydroxide (NH_4OH) and 1200 ml of HPLC grade methanol (MeOH) to 2785 ml of deionized water. The resulting solution was adjusted to pH 3.0 with trifluoroacetic acid. Solvent B was prepared by adding 30 ml NH_4OH to 3970 ml of water and then the pH of this solution was adjusted to 3.0 with trifluoroacetic acid. Gradient System I employed the following conditions: 0–20 min, linear gradient from 100% solvent B to 60% solvent B and 40% solvent A; 20–30 min, linear gradient to 100% solvent A; 30–40 min, 100% solvent A; 40–50 min, linear gradient to 100% solvent B; 50–55 min, 100% solvent B. The flow rate was constant at 8 ml min^{-1}. Gradient Systems II and III were employed for desalting and purification of the products using the semipreparative HPLC column. Two mobile phase solvents were employed. Solvent C was prepared by adding 8 ml of concentrated formic acid (HCOOH) and 800 ml of HPLC grade acetonitrile (MeCN) to water to make a total volume of 4000 ml. Solvent D was prepared by adding 4.0 ml HCOOH to 4 liters of water. For Gradient System II the following conditions were employed: 0–5 min, 100% solvent D; 5–15 min, linear gradient to 100%

solvent C; 15–25 min, 100% solvent C; 25–30 min, linear gradient to 100% solvent D; 30–40 min, 100% solvent D. The flow rate was constant at 3 ml min^{-1}. For Gradient System III the following conditions were employed: 0–15 min, linear gradient from 100% solvent D to 100% solvent C; 15–25 min, 100% solvent C; 25–30 min, linear gradient to 100% solvent D; 30–35 min, 100% solvent D. The flow rate was constant at 3 ml min^{-1}.

Fast atom bombardment mass spectrometry (FAB-MS) was carried out with a VG Instruments (Manchester, UK) Model ZAB-E instrument. The matrix employed for all FAB-MS measurements was 3-nitrobenzylalcohol (3-NBA). Liquid chromatography–mass spectrometry (LC-MS) was performed on a Kratos Model MS-25RF spectrometer equipped with a thermospray interface. Electron impact mass spectrometry (EI-MS) was carried out on a Hewlett–Packard 5985B spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer. Assignments of resonances in ^1H NMR spectra were based on homonuclear decoupling experiments and homonuclear 2D-correlated spectroscopy (COSY) experiments. Ultraviolet–visible spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. Infrared spectra were recorded on a Sirius 100 spectrophotometer (Mattson Instruments, Inc., Madison, WI) using a KBr pellet matrix.

Product Isolation and Purification

In a typical CPE, SAL · HCl (6 mg) was dissolved in 300 ml of pH 7.0 phosphate buffer ($\mu = 0.1$) (i.e., ca. 1 mM SAL) and electrolyzed at +0.29 V. A chromatogram of an incompletely oxidized solution is shown in Fig. 3. Chromatographic peaks **A, B, C,** and **D** correspond to the major initial products of the oxidation. Preparative HPLC (2- to 50-ml injections) of product solutions, using Gradient System I, was employed to separate **A, B, C,** and **D.** Chromatographic peaks corresponding to these products were collected individually and the resulting solutions were then lyophilized. The resulting solid was dissolved in a minimum volume of water and desalted and purified using a semipreparative reversed phase HPLC column. Compounds **A** and **B** were desalted and purified using Gradient System II; compounds **C** and **D** were desalted and purified using Gradient System III. The resulting desalted and purified solutions of each product were then lyophilized to obtain the pure, dry solid compounds.

cis-1,2,3,4-Tetrahydro-1-methyl-4,6,7-isoquinolinetriol (*A*). Compound **A** was isolated as a light brown solid. In pH 7.0 phosphate buffer **A** gave a pale yellow solution, λ_{max}, nm (log ε_{max}): 352 (2.51), 282 (3.21), 230 (sh 3.49). FAB-MS (3-NBA matrix) gave *m/e* = 218 (MNa^+, 34%), 196 (MH^+, 100%), 195 (M^+, 29%). Accurate mass measurements on MH^+ gave *m/e* = 196.0930 ($C_{10}H_{14}NO_3$; calcd. *m/e* = 196.0947). Thus, **A** has a molar mass of 195 g and a molecular formula $C_{10}H_{13}NO_3$. ^1H NMR (pyridine-d_5) δ 7.51 (s, 1H, C(5)–H), 7.13 (s, 1H, C (8)–H), 4.86 (m, 1H, C(4)–H), 4.25 (q, J = 6.6 Hz, 1H, C(1)–H), 3.59 (dd, $J_{3,3'}$ = 13.2 Hz, $J_{3,4}$ = 3.6 Hz, 1H, C(3)–H), 3.30 (dd, $J_{3,3'}$ = 13.2 Hz, $J_{3',4}$ = 3.3 Hz, 1H, C(3')–H), 1.52 (d, J = 6.6 Hz, 3H, CH_3). COSY experiments revealed that the resonances at δ

4.86 (C(4)–H) and δ 4.25 (C(1)–H) were coupled, indicating that **A** has the *cis*-configuration with respect to the C(1)-methyl and C(4)-hydroxyl residues.

trans-1,2,3,4-Tetrahydro-1-methyl-4,6,7-isoquinolinetriol (**B**). Compound **B** was isolated as a light brown solid. In pH 7.0 phosphate buffer **B** gave a pale yellow solution, λ_{max}, nm (log ε_{max}): 356 (2.60), 282 (3.27), 230 (sh 3.51). FAB-MS (3-NBA matrix) gave *m/e* = 218 (MNa^+, 28%), 196 (MH^+, 100%), 195 (M^+, 22%). Accurate mass measurements on MH^+ gave *m/e* = 196.0947 ($C_{10}H_{14}NO_3$; calcd *m/e* = 196.0947). Thus, **B** has a molar mass of 195 g and a molecular formula $C_{10}H_{13}NO_3$. ^1H NMR (pyridine-d_5) δ 7.68 (s, 1H, C(5)–H), 7.09 (s, 1H, C(8)–H), 5.07 (t, $J_{3/3',4'}$ = 6.0 Hz, 1H, C(4)–H), 4.30 (q, J = 6.6 Hz, 1H, C(1)–H), 3.64 (m, 1H, C(3)–H), 3.37 (dd, $J_{3,3'}$ = 12.3 Hz, $J_{3',4}$ = 6.3 Hz, 1H, C(3')–H), 1.53 (d, J = 6.6 Hz, 3H, CH_3). COSY experiments showed no coupling between the resonances at δ 5.07 (C(4)–H) and 4.30 (C(1)–H), indicating that **B** has the *trans*-configuration with respect to the C(1)-methyl and C(4)-hydroxyl residues.

2,3,4-Trihydro-1-methyl-7-hydroxy-6-oxyisoquinoline (**C**). Compound **C** was isolated as a pale yellow solid. In pH 7.0 phosphate buffer λ_{max}, nm (log ε_{max}): 380 (3.93), 316 (3.34), 262 (3.69). In EtOH, λ_{max}, nm (log ε_{max}): 4.02 (3.95), 312 (3.47), 272 (3.62), 256 (3.64), 240 (3.61), 204 (3.89): in 1 ml EtOH plus 0.01 ml HCl: 360 (3.69), 310 (3.73), 252 (4.01), 212 (3.78). EI–MS (12 eV, 120°C) gave *m/e* = 178 (11%), 177 (M^+, 100%). Accurate mass measurements on M^+ gave *m/e* = 177.0778 ($C_{10}H_{11}NO_2$; calcd *m/e* = 177.0789). ^1H NMR (pyridine-d_5) δ 7.43 (s, 1H, C(5)–H), 7.18 (s, 1H, C(8)–H), 3.59 (t, $J_{3,4}$ = 7.5 Hz, 2H, C(3)–H_2), 2.57 (t, $J_{3,4}$ = 7.5 Hz, 2H, C(4)–H_2), 2.53 (s, 3H, CH_3). ^{13}C NMR (D_2O) δ 176.96 (C(6)=0), 159.55, 146.90, 136.54, 119.06, 118.55, 118.25, 43.70, 27.20, 22.08. Infrared spectrum (KBr pellet, cm^{-1}) 2912, 2848, 1664, 1570, 1472, 1424, 1392, 1344, 1312. These spectral data, especially the ^{13}C NMR and ir results, clearly indicate the presence of one carbonyl residue in **C** and are, therefore, in accord with the proposed structure.

1-Methyl-6,7-isoquinolinediol(**D**). Compound **D** was isolated as a pale pink solid. At pH 7.0 λ_{max}, nm (log ε_{max}): 344 (4.19), 254 (4.62). EI–MS (70 eV, 110°C) gave *m/e* = 176 (12%), 175 (M^+, 100%). Accurate mass measurements on M^+ gave *m/e* = 175.0634 ($C_{10}H_9O_2N$; calcd *m/e* = 175.0633). Thus, **D** has a molar mass of 175 g and a molecular formula $C_{10}H_9O_2N$. ^1H NMR (pyridine-d_5) δ 8.37 (d, $J_{3,4}$ = 6.0 Hz, 1H, C(3)–H), 7.54 (s, 1H, C(5)–H), 7.43 (d, $J_{3,4}$ = 5.7 Hz, 1H, C(4)–H), 7.17 (s, 1H, C(8)–H), 2.85 (s, 3H, CH_3). ^{13}C NMR (Me_2SO-d_6) δ 153.8, 152.0, 148.1, 136.9, 132.3, 122.1, 117.7, 108.4, 107.3, 21.2.

RESULTS

Cyclic Voltammetry

Representative cyclic voltammograms of SAL at the PGE are presented in Fig. 1. Oxidation peak I_a appears on the initial anodic sweep. At low pH, after scan reversal even at quite slow sweep rates (ν), reduction peak I_c appears and is reversibly coupled to oxidation peak I_a (Fig. 1A). With increasing pH, however,

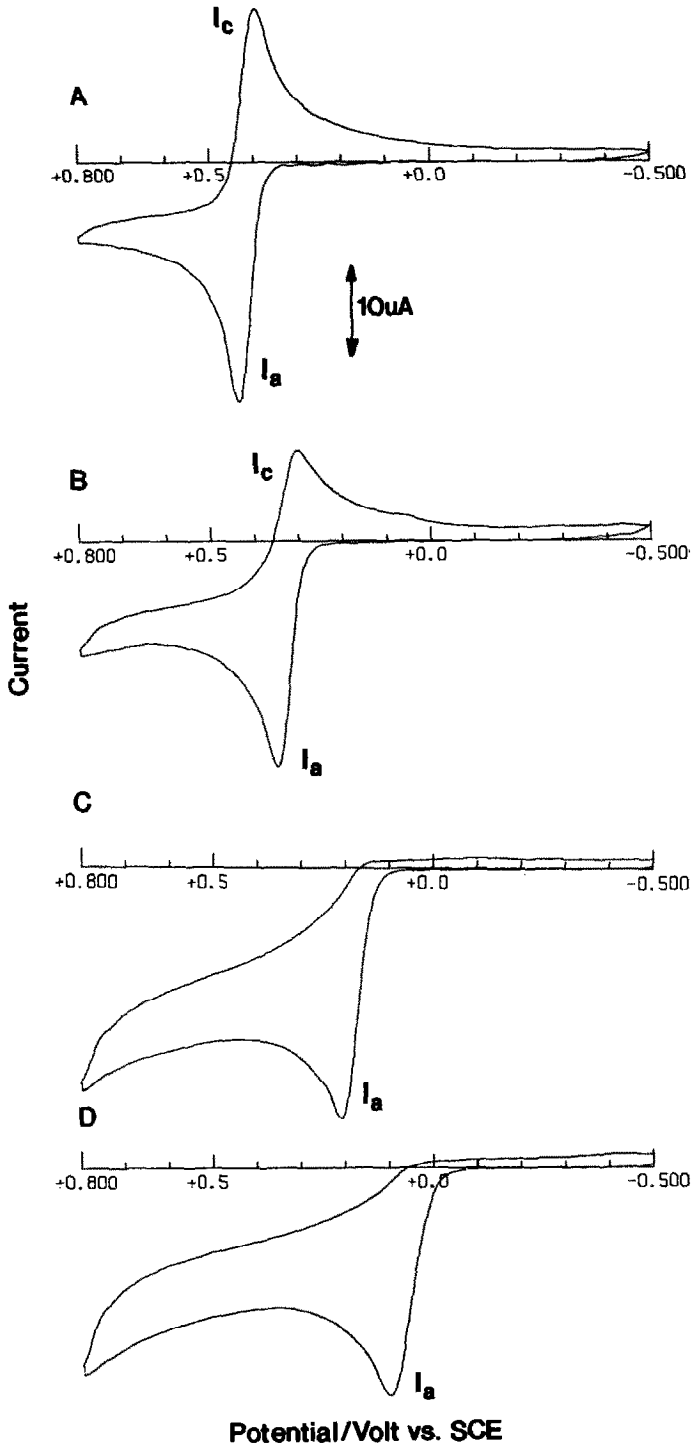


FIG. 1. Cyclic voltammograms at the PGE of 1.39 mM salsolinol (SAL) in phosphate buffers (μ = 1.0) at pH (A) 3.0, (B) 5.0, (C) 7.0, and (D) 9.2. Sweep rate: 10 mV s^{-1}.

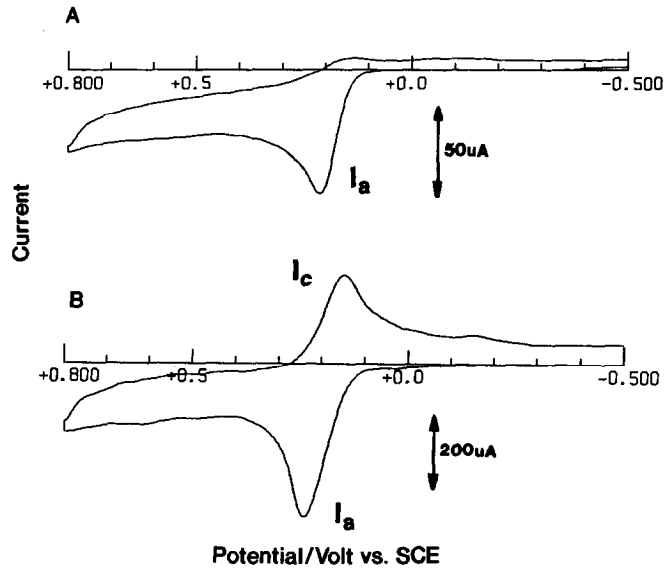


FIG. 2. Cyclic voltammograms at the PGE of 1.39 mM salsolinol (SAL) in pH 7.0 phosphate buffer (μ = 1.0). Sweep rate: (A) 50 mV s^{-1}; (B) 1000 mV s^{-1}.

peak I_c decreases relative to peak I_a (Fig. 1B) and at pH $\geq$7 it disappears (Figs. 1C, 1D). At sufficiently fast sweep rates at pH $\geq$7, however, I_c can be observed (Figs. 2A, 2B). These data indicate that the proximate oxidation product of SAL responsible for reduction peak I_c is significantly more stable in acidic solution than in neutral or basic solutions.

The peak potential (E_p) for peak I_a is dependent upon pH according to

$$E_{p(pH\ 1.5-10.8)} = [0.635 - 0.059\,pH]V, \qquad [1]$$

where V is measured at ν = 10 mV s^{-1}. Thus, at physiological pH (7.4) SAL is a rather easily oxidized compound, E_p = +0.19 V. Over the pH range studied (1.5–10.8) the experimental peak current function, $i_p/AC\nu^{1/2}$ (all terms have their usual electrochemical meaning (*21*)), increased with ν, whereas the $(i_p)_{I_a}/(i_p)_{I_c}$ ratio and ΔEp decreased. Such behaviors indicate that SAL is adsorbed at the PGE (*26, 27*).

Controlled Potential Electrolysis

A chromatogram of the product solution obtained following an incomplete controlled potential electrooxidation of SAL at +0.29 V (i.e., 76 mV more positive than E_p for peak I_a) at pH 7.0 is shown in Fig. 3. Approximately 85% of the initial SAL present had been oxidized when this chromatogram was recorded. Four major products, **A, B, C,** and **D**, are clearly formed. A similar product profile was observed when the product solution was analyzed at earlier stages of the electrolysis. However, exhaustive electrooxidations of SAL resulted in the disap-

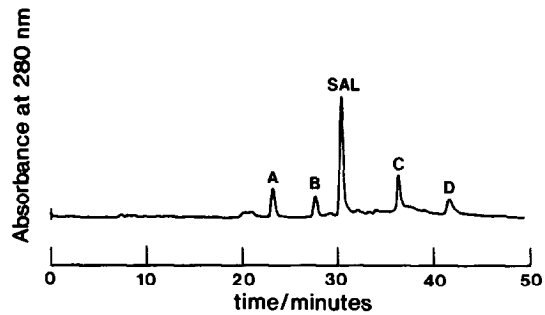


FIG. 3. HPLC chromatogram of the product solution formed upon controlled potential electrooxidation of 0.94 mM salsolinol (SAL) in pH 7.0 phosphate buffer (μ = 0.1) for 6 h at 0.29 V. Chromatography employed Gradient System I (see Experimental). Injection volume: 2.0 ml.

pearance of **A, B,** and **C** and formation of a very large number (>20) of secondary products and a black insoluble, presumably polymeric, material.

Cyclic voltammetry indicated that the proximate oxidation product of SAL, responsible for reduction peak I_c, was most stable at low pH. Accordingly, in order to obtain information about this product, CPE of SAL (ca. 1 mM) in 0.1 M HCl at +0.62V (ca. 60 mV more positive than E_p for peak I_a) was carried out. After ≤30 min electrolysis HPLC analysis of the resulting solution showed a single major product **E** (Fig. 4A). LC-MS of the solution eluted under chromatographic peak **E** showed ions at *m/e* = 180 (MH_2H^+, 58%), 178 (MH^+, 100%), and 177 (M^+,

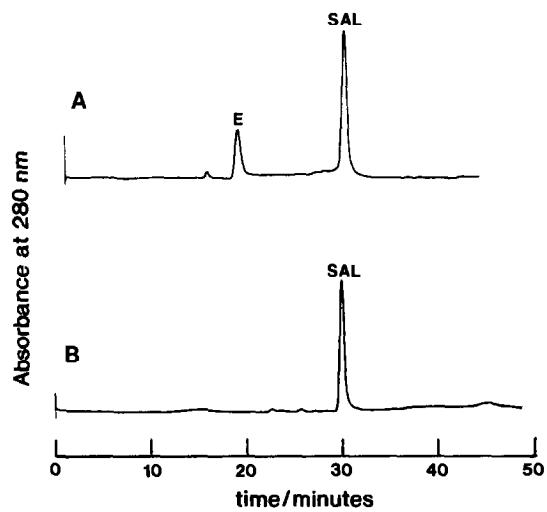


FIG. 4. HPLC chromatogram of (A) the product solution formed upon controlled potential electrooxidation of 0.93 mM salsolinol (SAL) in 0.1 M HCl at +0.62 V for 20 min; (B) the solution formed following controlled potential electroreduction of the product eluted under peak E in (A) at 0.30 V for 30 min. Chromatography employed Gradient System I (see Experimental). Injection volume: 2 ml.

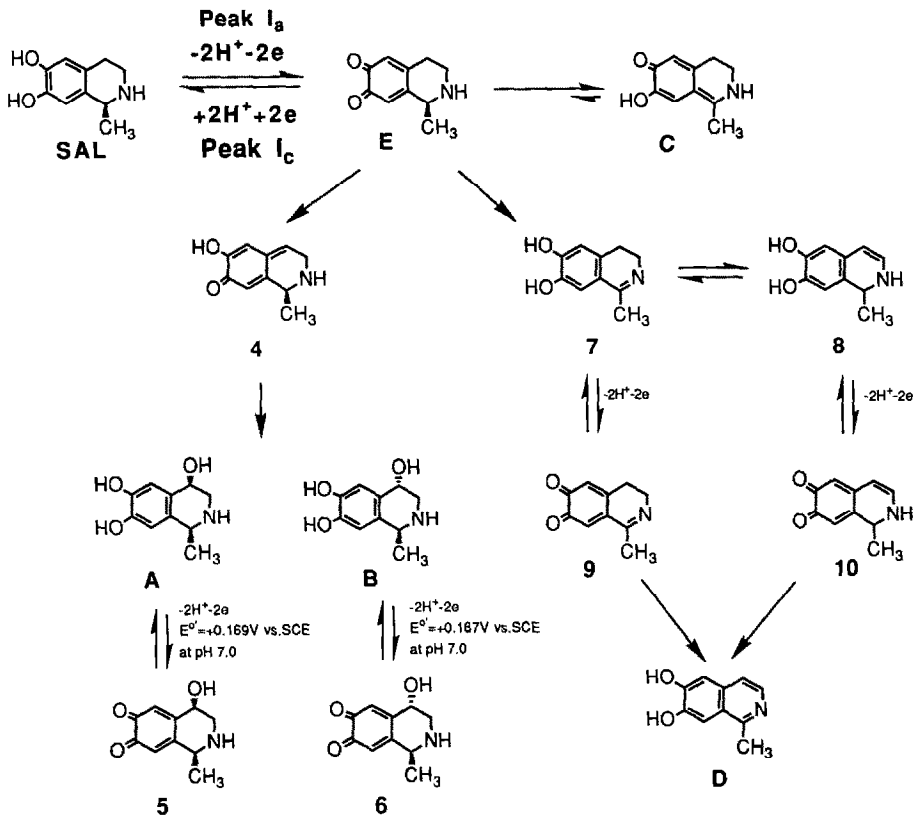
65%). A solution of **E** in the chromatographic mobile phase (pH 3.0) was pale yellow, λ_{max} = 400, 280, 220 nm. Efforts to either prepare concentrated solutions of **E** or to isolate **E** were unsuccessful owing to its rather facile decomposition to, in acid solution, unknown products. However, cyclic voltammograms of freshly separated **E** dissolved in the chromatographic mobile phase were identical to those of SAL under the same conditions. Controlled potential electroreduction of **E** (0.30 V in the chromatographic mobile phase, pH 3.0) gave SAL as the major product (e.g., Fig. 4B). Accordingly, **E** (molar mass = 177 g), the proximate oxidation product of SAL (molar mass = 179 g), must be formed in a reversible $2e$–$2H^+$ electrochemical reaction.

REACTION PATHWAYS

Cyclic voltammetry indicates that SAL is electrochemically oxidized in a reversible electrode reaction although adsorption processes complicate the voltammetric response of the alkaloid. The proximate oxidation product responsible for reduction peak I_c in cyclic voltammograms of SAL is **E** and is formed as a result of a $2e$–$2H^+$ electrode reaction. The reversible nature of the electrode process and the observation that **E** is more stable in acidic solution than in neutral and basic solution are consistent with the conclusion that **E** is an *ortho*-quinone (*28*).

At pH 7.0 the lifetime of **E** is quite short (estimated from cyclic voltammetry to be about 1 s) and, based upon the identities of the ultimate products formed, it must rapidly enolize. A major isolated product is the quinone methide **C** (Scheme 3). At pH 7 **C** is quite stable in solution and exhibits no tendency to rearrange to more reactive tautomers. It seems likely that both resonance effects and hydrogen bonding (between the adjacent carbonyl and hydroxyl groups) act to stabilize **C.** Resonance and steric effects, furthermore, protect the C(8a)–C(1) double bond of **C** from nucleophilic attack by water. Spectral studies (uv and fluorescence) on 1-methyl-3,4-dihydro-6,7-isoquinolinediol also suggest the predominance of tautomer **C** at neutral pH (*29*). Quinone methide **4** is another tautomer of **E** (Scheme 3). Neither resonance nor steric effects protect the C(4)–C(4a) double bond of this tautomer from nucleophilic attack. Thus, attack by water yields the *cis*- and *trans*-4,6,7-trihydroxy-TIQs **A** and **B** (Scheme 3). Another major initial product from SAL is the fully aromatized isoquinoline **D.** Controlled potential electrooxidations of **A, B,** or **C** did not give **D** as a product. Accordingly, it can be concluded that tautomers **7** and/or **8** are the intermediates which are further oxidized to **D,** presumably via the putative *ortho*-quinone intermediates **9** and/or **10,** respectively.

At sufficiently fast sweep rates cyclic voltammograms of **A, B,** and **C** at pH 7 show reduction peaks which form quasireversible couples with the primary oxidation peak (Fig. 5). While the oxidation chemistry of **A** and **B** has not been investigated in detail, it seems probable that the primary voltammetric oxidation peaks of these isomers correspond to a $2e$–$2H^+$ reaction giving *ortho*-quinones **5** and **6,** respectively (Scheme 3). Based upon the cyclic voltammetric behaviors of the *L*-SAL/**E, A/5, B/6,** and **C** and its, as yet unknown, proximate oxidation product, the $E^{0\prime}$ values at pH 7.0 are +0.194, +0.169, +0.167, and +0.266 V, respectively.



SCHEME 3

Such $E^{0'}$ values suggest that *o*-quinone **E**, the proximate oxidation product of SAL, should be capable of chemically oxidizing **A** and **B**. Indeed, when a solution of **E**, prepared by controlled potential electrooxidation of SAL in 0.1 M HCl followed by HPLC purification, was adjusted to pH 7.0 and analyzed by HPLC, the resulting solution contained SAL as the major product along with some **C** and a large number of unidentified minor products. This solution contained no traces of **A** and **B**. Accordingly, it can be concluded that at pH 7.0 **E** reacts to give initially **A**, **B**, and **C** (and possibly other tautomers) by the routes shown in Scheme 3. Compounds **A** and **B** are then rapidly oxidized by the remaining **E** to generate putative *o*-quinones **5** and **6** which subsequently decompose to the ultimate reaction products as conceptualized in Scheme 4.

The $E^{0'}$ values noted above indicate that not only SAL but also **A**, **B**, and **C** are quite easily oxidized compounds. It is, no doubt, the ease of oxidation of **A**, **B**, and **C** that accounts for their appearance as products only during the initial stages of the electrolysis of SAL. As the electrolysis progresses these initial products are further oxidized, resulting in formation of many secondary products and, ultimately, an insoluble polymeric material.

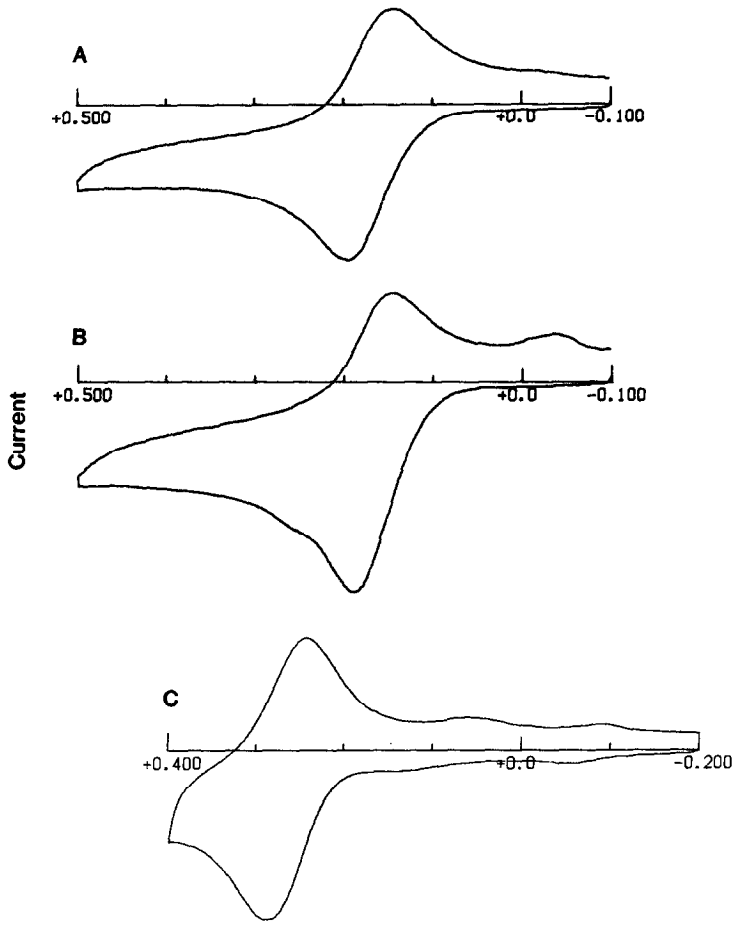
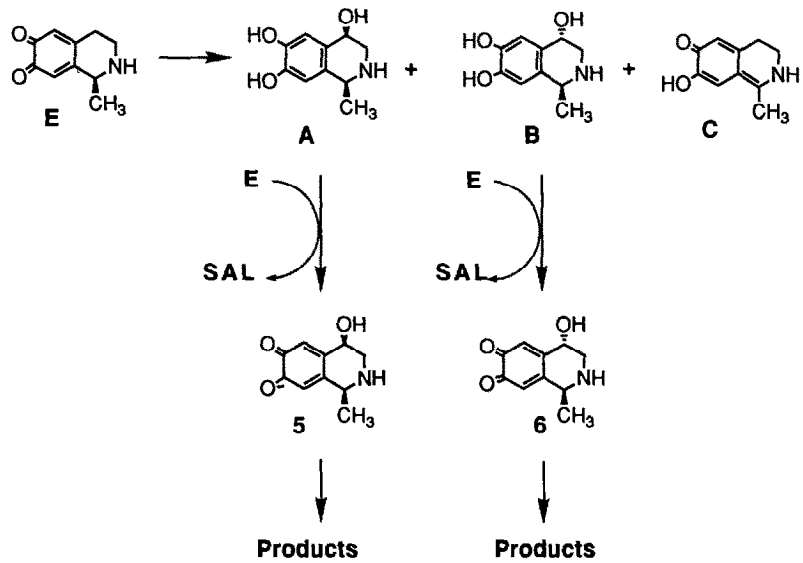


FIG. 5. Cyclic voltammograms at the PGE of (A) 1.28 mM *cis*-1,2,3,4-tetrahydro-1-methyl-4,6,7-isoquinolinetriol, **A**; (B) 1.79 mM *trans*-1,2,3,4-tetrahydro-1-methyl-4,6,7-isoquinolinetriol, **B**; and (C) 3,4-dihydro-1-methyl-6,7-isoquinolinediol, **C**, in pH 7.0 phosphate buffer (μ =1.0). Sweep rates: (A) and (B): 100 mV s^{-1}; (C) 5.12 V s^{-1}. Initial sweeps were toward positive potentials.

CONCLUSIONS

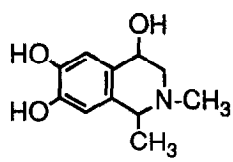
The results presented in this report provide information bearing on the initial oxidation reactions of the CNS alkaloid L-SAL. In aqueous solution at physiological pH the primary electrode reaction is a $2e$–$2H^+$ oxidation of L-SAL to the key *ortho*-quinone intermediate **E**. This is a rather short-lived species (ca. 1 s) at pH 7 because of its rapid tautomerization. Quinone methide **C** is a quite stable tautomer of **E** and has been isolated and structurally characterized. However, another tautomer, quinone methide **4**, is rapidly attacked by water to give *cis*- and *trans*-4,6,7-trihydroxy TIQs **A** and **B**. The exact reaction pathway leading to the fully



SCHEME 4

aromatized isoquinoline **D** is not at this stage known. However, because **A, B,** and **C** are not oxidized to **D** it is tentatively assumed that other tautomers of **E,** i.e., **7** and/or **8,** are further oxidized to **D.**

It is of interest to note that a crude condensate of epinephrine and ACH, which presumably contained 1,2,3,4-tetrahydro-1,2-dimethyl-4,6,7-isoquinolinetriol (**11**), when administered into the CNS of laboratory animals caused profound behavioral effects, depletion of hypothalamic norepinephrine (*30*), and selective degeneration of adrenergic neurons (*31*). These effects are similar to those evoked by the catecholaminergic neurotoxin 6-hydroxydopamine (*32*). Compounds **A** and **B,** initial electrooxidation products of L-SAL at physiological pH, are structurally very similar to **11,** suggesting, therefore, that they might also be CNS toxins. It is planned to describe the behavioral effects evoked by central administration of **A, B,** and **C** to mice and the relationships between the electrochemically driven and enzyme-mediated oxidations of L-SAL in a future report (*33*).



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