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Chromatographic separation and spectrometric identification of the oxidation products from a tetrahydro-isoquinoline alkaloid

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Abstract

The oxidation chemistry of 3′,4′-deoxynorlaudanosoline carboxylic acid, a tetrahydroisoquinoline alkaloid, has been studied by electrochemical approaches. Four reaction products were isolated by semi-preparative high performance liquid chromatography and identified structurally by nuclear magnetic resonance, mass spectrometry, ultraviolet-visible spectrophotometry and electrochemistry studies. An oxidation mechanism was proposed. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Phenylketonuria (PKU) is an inherited disease characterized by the deficiency of the enzyme phenylalanine hydroxylase. As a result, phenylalanine cannot be converted to tyrosine leading to the accumulation of phenylalanine and its metabolites, such as phenylpyruvic acid, in the tissues and blood. As a matter of fact, high level of phenylpyruvic acid was used as a screening test for PKU in the past. PKU could damage developing cells causing mental retardation [1]. In addition, high phenylalanine level also inhibits the production of melanin to cause defects in skin and hair pigmentation, which is characteristic clinical features of PKU. The knowledge about PKU damaging mechanism is still quite limited. It has been suggested that the mental retardation associated with PKU (if not appropriately treated) originates from the disproportionate levels of phenylpyruvic acid which condense with biogenic catecholamines to give tetrahydroisoquinolines (TIQs) [2]. As an example, phenylpyruvic acid condenses with dopamine to generate 3, 4-deoxynorlaudanosoline-1-carboylic acid (DNLCA). Indeed, DNLCA was found to greatly elevate in PKU and accumulate in cerebellum and cortex of hyperphenylalaninic rats [3]. These are precisely

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the brain regions which sustain the most severe neurological damage in PKU [4] implying direct neuropathlogical role for DNLCA. Decreased concentration of all biogenic amine neurotransmitters in PKU provides additional support for this suggestion [5]. It is unlikely unreasonable to expect that the TIQ type DNLCA, as a orthophenol kind compound, is a easily oxidizable species. The aberrant oxidation of DNLCA might lead to the formation of toxins which play roles in the brain cell damage in PKU. Similarly, Collin proposed that oxidation reaction of endogenous TIQs that are elevated in the brain of chronic alcoholics might lead to toxic products which are responsible for neuronal damage [6]. It is clearly important to explore the oxidation chemistry of DNLCA. As a first step toward understanding the chemistry the electrochemical oxidation of DNLCA in aqueous solution was investigated because of the easiness of electrochemistry to provide oxidation potential, oxidation intermediate, oxidation product and mechanism information. It is well known that electrochemistry can often provide very valuable insights into biological oxidation processes. [7,8]. Bobbitt et al. [9] reported that DNLCA can be oxidized electrochemically to its corresponding 3,4-dihydroisoquinoline which was the only product isolated and identified as its methylated and reduced form, and no further mechanistic study was performed. In this report, more detailed investigation on the electrochemical oxidation of DNLCA in physiological pH will be described. The results might be of relevance to the etiology of PKU.

2. Experimental

Dopamine hydrochloride and phenylpyruvic acid were obtained from Sigma (St. Louis, MO). Phosphate salts for the preparation of buffers were of the highest quality available.

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. A conventional three electrode voltammetric cell was used containing a platinum wire counter electrode and a saturated calomel reference electrode (SCE). 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 procedure [10].

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. 160 cm^2. A three-compartment cell was used in which a Nafion member (Type 117, Dupont, Wilmington, DE) separated the working, counter, and reference electrode compartments. The working electrode compartment had a capacity of 70 ml. The counter electrode was pyrolytic graphite suspended into a solution of the supporting electrolyte. A SCE reference electrode was employed. The solution in the working electrode compartment was bubbled vigorously with N_2. All potentials are referred to the SCE at ambient temperature.

^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer. Low and high resolution mass spectrometry (FAB-MS) was carried out on a VG Instruments (Manchester, UK) Model ZAB-E Spectrometer. UV-visible spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer.

High performance liquid chromatograph (HPLC) employed a Gilson (Middleton, WI) gradient system equipped with Model 305 and 306 pumps and a Holochrome UV detector set at 254 nm and a Rheodyne 7125 loop injector. A reversed phase column (Regis, Morton Grove, IN, C18, 10 μm, 25×2.1 cm) and a short guard column (Upchurch, C-1022, 1×1 cm) packed with Bakerbond (Baker, C18, 10 μm) 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 2.6 by addition

of trifluoroacetic acid (TFA). Solvent B was prepared by adding 30 ml concentrated NH_4OH solution to 1 l HPLC grade methanol (MeOH) and 1 l HPLC grade acetonitrile (MeCN) and 2 l deionized water, the pH of the resulting solution was adjusted to 2.6 by adding TFA. For Gradient System I, the following gradient was employed: 0–2 min, 100% solvent A, 2–10 min, linear gradient to 40% solvent B, 10–35 min, linear gradient to 100% B. The flow rate was 7 ml/min. Solvent C was prepared by adding TFA to deionized water till the pH was 2.6. Solvent D was prepared by adding TFA to 50% acetonitrile aqueous solution (v/v) till the pH was 2.6. For Gradient System II, the following gradient was used: 0–2 min, 100% solvent C, 2–35 min, linear gradient to 100% solvent D, 35–45 min, 100% solvent D. The flow rate was 7 ml/min. Gradient System I was used primarily to separate reaction product mixtures. Gradient System II was employed to desalt and purify products.

For product isolation and purification, a typical solution (70 ml) of DNLCA (0.5 mM) in sodium phosphate buffer ($\mu = 0.1$) was electrolyzed at 0.25 V for 120 min. After filtration, the product solution (10–50 ml) was injected onto the HPLC column and eluted with Gradient System I to collect the peaks corresponding to 1, 2, 3 and 4 individually. The collected solutions were injected (5–10 ml) directly onto the column and purified by Gradient System II. The chromatographic peaks corresponding to 1, 2, 3 and 4 were collected and lyophilized to obtain the pure, dry solid compounds.

2.1. Synthesis of 3′,4′-deoxynorlandanosoline carboxylic acid (DNLCA)

A total of 600 mg β-phenylpyruvic acid (sodium salt) was suspended in 12 ml H_2O. The pH of the solution was adjusted to ~ 11 by adding concentrated NH_4OH while purged with N_2 to dissolve the β-phenylpyruvic acid and then the resulting pale yellow clear solution was adjusted to pH 6 by careful addition of concentrated hydrochloric acid. After 567 mg dopamine hydrochloride was dissolved into the above solution, the vessel was sealed and stored in the darkness at room temperature for 4 days to give a white precipitate. The precipitate was filtered with suction and washed thoroughly with ice water and dried under vacuum to give a white powder (yield 42%) with a melting point of 242–244°C (d) ([11], 240°C,). In pH 7.0 phosphate buffer ($\mu = 1.0$), DNLCA showed λ_{max} (log ε_{max}, $M^{-1}cm^{-1}$) at 234 (sh, 3.78) and 286 (3.53). FAB-MS (3-nitrobenzyl alcohol matrix) gave $m/e = 300.1241$ (MH^+, 100%; $C_{17}H_{18}NO_4$, calcd. $m/e = 300.1236$). ^1H NMR (D_2O), δ 7.35 (s, 1H, C(8)-H), 7.35 − 7.34 and 7.24 − 7.20 (m, 5H, the aromatic protons of phenyl group at C(1)), 6.70 (s, 1H, C(5)-H). 3.74 (d, J = 14.6 Hz, 1H, C(α)-H_1), 3.30 (d, J = 14.6 Hz, 1H, C(α)-H_1), 3.45-3.20 (m, 2H, C(3)-H_2), 2.89-2.82 (m, 2H, C(4)-H_2).

2.2. 2,3,4-Trihydro-1-phenyl-7-hydroxy-6-oxy-isoquinoline (1)

Compound 1 was isolated as a yellow powder. In pH 7.0 phosphate buffer ($\mu = 1.0$) λ_{max}, nm (log $\varepsilon_{max,}$ $M^{-1}cm^{-1}$): 252 (3.38), 270 (sh, 3.36), 320 (3.16), 390 (3.66). FAB-MS (3-nitrobenzyl alcohol matrix) gave $m/e = 254.1169$ (MH^+, 100%, $C_{16}H_{16}NO_2$, calcd. $m/e = 254.1181$), 253.1105 (M^+, 20%, $C_{16}H_{15}NO_2$, calcd. $m/e = 253.1103$). ^1H NMR (D_2O) δ 7.46-7.33 (m, 6H, C(8)-H and 5 aromatic protons of the phenyl group at C(1)), 6.87 (s, 1H, C(5)-H), 4.41(s, 2H, C(α)-H_2), 3.76(t, J = 7.9 Hz, 2H, C(3)-H_2), 2.97 (t, J = 7.9 Hz, 2H, C(4)-H_2). ^{13}C NMR (D_2O) δ 178.75 (C = O), 156.31, 146.27, 136.69, 135.21, 132.46, 132.20, 132.07, 131.03, 119.93, 119.36, 118.00, 101.06, 44.03, 41.37, 26.97.

2.3. 1-Phenyl-6, 7-isoquinoline-diol (2)

Compound 2 was isolated as a pale yellow solid. In pH 7.0 phosphate buffer ($\mu = 1.0$) λ_{max}, nm (log $\varepsilon_{max,}$ M^{-1} cm^{-1}): 250 (sh, 3.17), 276 (3.20), 370 (3.09). FAB-MS (3-nitrobenzyl alcohol matrix) gave $m/e = 252.1030$ (MH^+, 100%, $C_{16}H_{14}NO_2$, calcd. $m/e = 252.1025$). ^1H NMR (D_2O) δ 7.89 (d, J = 6.6 Hz, 1H, C(3)-H), 7.73 (d, J = 6.6 Hz, 1H, C(4)-H), 7.63 (s, 1H, C(8)-H), 7.43-7.28 (m, 5H, aromatic protons of the phenyl

group at C(1)), 7.19 (s, 1H, C(5)-H), 4.71 (s, 2H, C(α)-H_2).

2.4. Compound 3

Compound 3 was isolated as yellow fluffy solid. In pH 7.0 phosphate buffer ($\mu = 1.0$) λ_{max}, nm (log $\varepsilon_{max,}$ M^{-1} cm^{-1}): 268 (sh, 4.00), 340 (4.13), 406 (4.26). FAB-MS (3-nitrobenzyl alcohol matrix) gave $m/e = 521.2095$ (MH^+, 100%, $C_{32}H_{29}N_2O_5$, calcd. $m/e = 521.2076$). FAB-MS also gave prominent fragment ions at $m/e = 253$ (60%) and 268 (55%) indicating the presence of an oxygen linkage in the molecule. ^{1}H NMR (acetonitrile-d_3) δ 7.32-7.17 (m, 12 H, 10 aromatic protons of the phenyl groups at C(1) and C(1′), C(8′)-H and C(3)-H). 7.06 (d, J = 6.9 Hz, 1H, C(4)-H), 6.95 (s, 1H, C(8)-H), 6.85 (s, 1H, C(5′)-H). 5.23 and 4.97 (s and s, C(α)-H in 3a and 3b), 4.07 (d, J = 16.2 Hz, 1H, C(α′)-H), 3.59 (d, J = 16.2 Hz, 1H, C(α′)-H), 4.14-4.03, 3.27-3.18 and 2.92-2.86 (m, 4H, C(3′)-H_2 and C(4′)-H_2). Correlated spectroscopy (COSY) experiments revealed that the resonance at 7.06 ppm (C(4)-H) was coupled with a resonance at 7.30 ppm that was masked by a huge multiple peak. The resonance at 7.30 was a doublet corresponding to C(3)-H.

2.5. Compound 4

Compound 4 was a yellow solid. In pH 7.0 phosphate buffer ($\mu = 1.0$), the UV spectrum showed at λ_{max}, nm (log $\varepsilon_{max,}$ $M^{-1}cm^{-1}$), 268 (sh, 4.02), 342 (4.15), 406 (4.28). FAB-MS (3-nitrobenzyl alcohol matrix) gave $m/e = 521.2049$ (MH^+, 100%, $C_{32}H_{29}N_2O_5$, calcd. $m/e = 521.2076$). FAB-MS also gave prominent fragment peaks at $m/e =$ 253 (53%) and 268 (48%) indicating the presence of an oxygen linkage in the molecule. ^{1}H NMR (acetonitrile-d_3) δ 7.52-7.10 (m, 12H, 10 aromatic protons of the phenyl groups at C(1) and C(1′)), C(8′)-H and C(3)-H), 6.90 (s, 1H, C(5′)-H), 6.70 (d, J = 6.6 Hz, 1H, C(4)-H), 6.27 (s, 1H C(5)-H), 5.2 and 4.85 (s and s, C(α)-H of 4a and 4b), 4.44 (d, J = 14.9 Hz, 1H, C(α′)-H), 3.87 (d, J = 14.9 Hz, 1H, C(α′)-H), 4.62-2.46 (m, 4H, C(3′)-H_2 and C(4′)-H_2). COSY experiments revealed that the resonance at 6.70 ppm (C(4)-H) was coupled with a resonance at 7.36 ppm masked by a huge multiple peak. The resonance at 7.36 ppm was a doublet corresponding to C(3)-H.

Because the yields of 3 and 4 were low, isolation of a large quantity of these compounds was difficult. In addition, 3 and 4 were not stable. Both of them decomposed to a complicated mixture that remains to be characterized. All of the spectral data of 3 and 4 were obtained immediately after they were freeze-dried.

3. Results

3.1. Voltammetric studies

Cyclic voltammograms of DNLCA obtained at a pyrolytic graphite working electrode (PGE) in pH 7.0 aqueous buffer indicated that a total of four oxidation peaks (Ia–IVa) were observed on the initial anodic sweep. Fig. 1 presents a series of

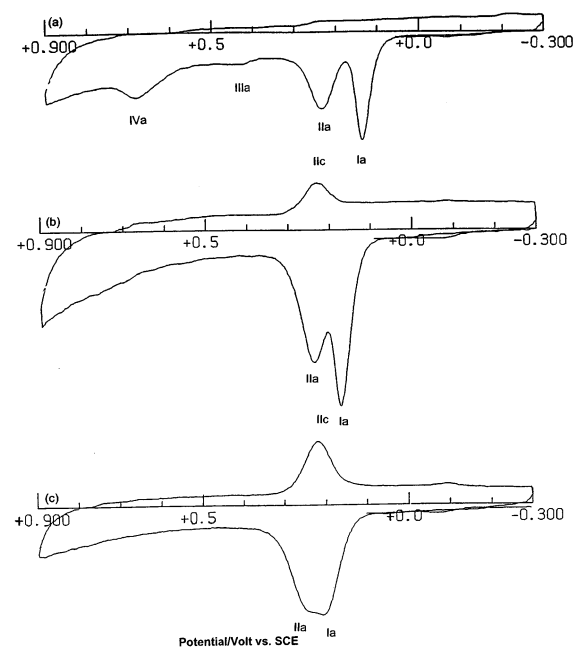


Fig. 1. Cyclic voltammograms at the PGE of 0.03 mM DNLCA in sodium phosphate buffer ($\mu = 1$) at sweep rate of: (a) 100; (b) 1003; and (c) 3531 mV/s, respectively. The initial anodic scan starts at -0.3 V through peaks Ia, IIa, IIIa, IVa and reversed at $+0.9$ V

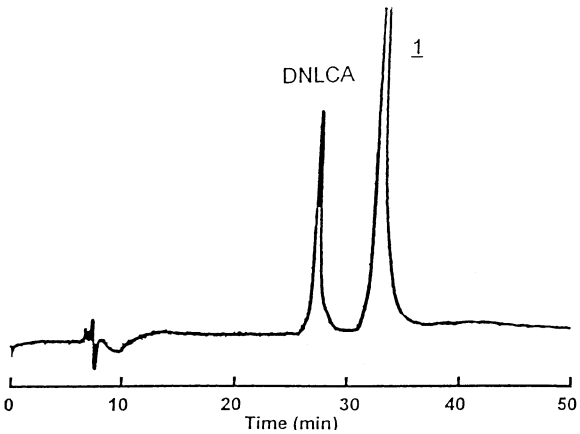


Fig. 2. HPLC chromatogram of the production mixture from oxidation of 0.5 mM DNLCA in pH 7.0 phosphate buffer at 0.15 V for 46 min. Gradient System I; 10 ml injection.

cyclic voltammograms of DNLCA at pH7.0 at increasing values of sweep rate (v). With increasing v, peak IIc appeared on the reversal cathodic sweep, formed a reversible couple with peak IIa; peak IIIa and IVa disappeared. Thus, the proximate electro-oxidation product resulting from the peak IIa reaction must be the precursor of the species responsible for peaks IIIa and IVa. Between sweep rates of 0.1 and 3.5 V/s, Ep for peak IIa remained practically constant. However, Ep for peak Ia systematically shifted to more positive potential and, at $v > 3.5$ V/s, peak Ia merged with peak IIa. Under no circumstances was a reduction peak coupled to oxidation peak Ia observed. Subsequent discussion will focus primarily on the peak Ia and peak IIa oxidations.

3.2. Controlled potential electro-oxidation and product characterization

Controlled potential electro-oxidation of DNLCA (0.5 mM) in pH 7.0 phosphate buffer at 0.15 $\dot{V}$ (Ep for peak Ia) gave a coulometric n-value of 2.3 ± 0.1. HPLC chromatography (Fig. 2) showed that only one product, designated as 1, formed during the oxidation and, after isolation, its structure was identified as 2,3,4-trihydro-1-phenyl-7-hydroxyl-6-oxy-isoquinoline.

Chromatograms of the product mixture formed as a result of controlled potential electro-oxidation of DNLCA at 0.25 V (Ep for peak IIa) in pH 7.0 buffer are presented in Fig. 3. After initiation of the oxidation four products were observed, i.e. 1, 2, 3, and 4, in addition to that of unreacted DNLCA. Compound 2 was identified as 1-phenyl-6,7-isoquinoline. Compounds 3 and 4 were isomers. The coulometric n-value for the oxidation of DNLCA at 0.25 V in pH 7.0 was determined to be 3.5 ± 0.1.

Chromatograms (Fig. 3) indicate that during the controlled potential electro-oxidation of DNLCA at peak IIa potential, the peak corresponding to DNLCA decreased throughout the course of electrolysis while the peaks corresponding to 1, 2, 3, and 4 initially increased and then decreased. This phenomena suggested that at a potential corresponding to peak IIa, 1, 2, 3, and 4 formed form oxidation of DNLCA could be further oxidized. It was found that controlled potential electro-oxidation of 1 at Ep for peak IIa gave 2, 3, and 4 as products. The chromatogram for the mixture from oxidation of 0.5 mM 1 at 0.25V in pH7.0 buffer for 80 min is presented in Fig. 4.

3.3. Cyclic voltammetry of 1, 2, 3, and 4

Cyclic voltammograms (CVs) of DNLCA, 1, 2, 3, and 4 in pH 7.0 are presented in Fig. 5. Based on a comparison of the CVs, it is clear that peak Ia electro-oxidation of DNLCA produces 1 which is responsible for oxidation peak IIa and reduction peak IIc (CVs of 1 showed a reversible couple at potentials of IIa and IIc at $v \geq 1000$ mV/s; data not shown). Oxidation peak IIIa corresponds to that observed with 2. Peak IVa might result from oxidation of 3 and 4. The more negative oxidation peaks observed for 3 and 4 are overlapped with peak Ia and peak IIa.

4. Reaction pathways

It is known that [12] salsolinlol-1-carboxylic acid (A), a close analogue of DNLCA, is electrochemically oxidized to the corresponding orthoquinone intermediate 1,2,3,4-tetrahydro-1-methyl-

1-carboxy-6,7-isoquinoline dione (B) via a 2e-2H$^+$ process in pH 7 aqueous solution. The ortho-quinone B formed is extremely unstable and rapidly decarboxylated to generate the quinone methide tautomer of 3,4-dihydro-1-methyl-6,7-isoquinolinediol (C). The later compound can be further oxidized to give the corresponding quinoid intermediate (D) which is either attached by nucleophilic reagent water or aromatizes to yield 1-methyl-6,7-isoquinonediol (E) (Scheme 1). Cyclic voltammograms of salsolinlol-1-carboxylic acid exhibits two predominant consecutive oxidation peaks which corresponds to the oxidation of salsolinlol-1-carboxylic acid and 3,4-dihydro-1-methyl-6,7-isoquinolinediol along with some ill-defined peaks. The features of cyclic voltammetry of salsolinlol-1-carboxylic acid are quite similar to that of DNLCA. By analogy with the electrochemical oxidation of salsolinlol-1-carboxylic acid, it seems reasonable to expect that the initial step at the peak Ia electrooxidation of DNLAC is also a 2e-2H + abstraction to give ortho-quinone a (Scheme 2). The fact that a reduction peak of a in CVs of DNLCA, which should be reversible to peak Ia, was not observed at v as high as high 3.5 V/s, the highest value of v at which peak Ia can be observed separately from peak IIa, suggested that a is a very unstable species that rapidly decarboxylates to give 1 as conceptualized in Scheme 2. The reaction sequence DNLCA → a → 1 is often

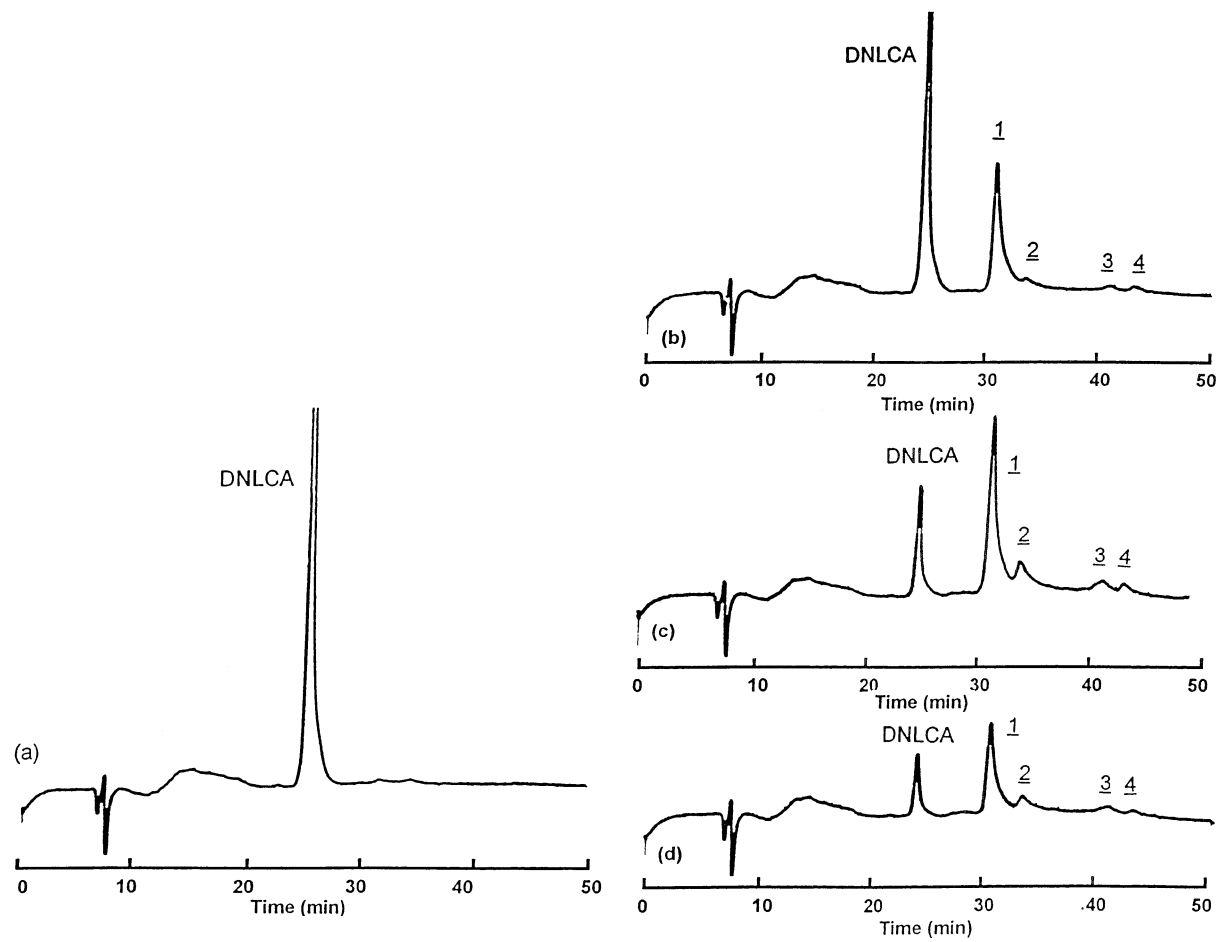


Fig. 3. HPLC chromatograms of the production mixture from oxidation of 0.5 mM DNLCA in pH 7.0 phosphate buffer at 0.25 V for: (a) 0; (b) 20; (c) 240; and (d) 360 min, respectively. Gradient System I; 10 ml injection.

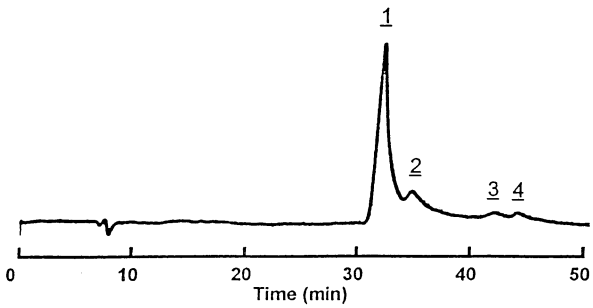


Fig. 4. HPLC chromatogram of the production mixture from oxidation of 0.5 mM 1 in pH 7.0 phosphate buffer at 0.25 V for 80 min. Gradient System I; 10 ml injection.

referred to as a charge transfer followed by an irreversible chemical reaction, i.e. an EC process. The anodic shift of Ep for peak Ia with increasing v is characteristic of such reactions [13], which is consistent with our experimental observation (Fig. 1). In this investigation, it was found that the peak Ia electrooxidation of DNLCA at physiological pH gave 1 as the sole product (Fig. 2) and the voltammetric n-value is 2.3 ± 0.1as expected. ^{13}C NMR spectrum of 1 in D_2O exhibits a strong carbonyl resonance suggesting that this compound exists predominantly under such conditions as a quinone methide tautomer 1a. Further evidence for the pH-dependent tautomerism of 1 over pH 3.0–9.2 was provided by UV spectral study. Thus, a solution of 1 gave a yellow color between pH 3.0 and 9.2. However, the spectra of 1 changed appreciably. With increasing pH, the bands at 246 and 308 nm shifted to longer wavelength and decreased in intensity; while the band at 352 nm shifted to 390 nm and increased in intensity. Increasing pH favors formation of the quinone methide tautomer of 1, i.e. 1a. A similar tautomerism of 3,4-dihydro-1-methyl-6,7-isoquinolinediol that is an analogue of 1 was thoroughly studied in previous reports [12,14].

Voltammetric peak IIa observed for DNLCA is due to the oxidation of 1 formed as the result of the peak Ia oxidation. The oxidation of 1, as a ortho-phenol compound like DNLCA, might also be a 2e-2H + process to produce ortho-quinone b. The intermediate b may then tautomerize to form 2 or be attacked by water to produce c and d. After a series of tautomerizations and oxidations, c and d may be converted to e and f; e and f subsequently coupled with 1 to form 3 and 4. Oxidation of phenols to quinones followed by tautomerization or nucleophilic addition are popular organic reactions. The facts that oxidation of DNLCA at peak IIa potential gave 1, 2, 3, and 4 (Fig. 3) with n-value of 3.5 ± 0.1, while oxidation of DNLCA at peak Ia potential only gave 1 as sole product (Fig. 2) with an n-value of 2.3 ± 0.1, and oxidation of 1 at peak IIa potential produced 2, 3 and 4 (Fig. 4) are all in accordance with the conceptualization in Scheme 2. It is worthwhile to point out that the proposed Scheme II only intends to tentatively interpret the formation of the isolated oxidation products. Additional work is

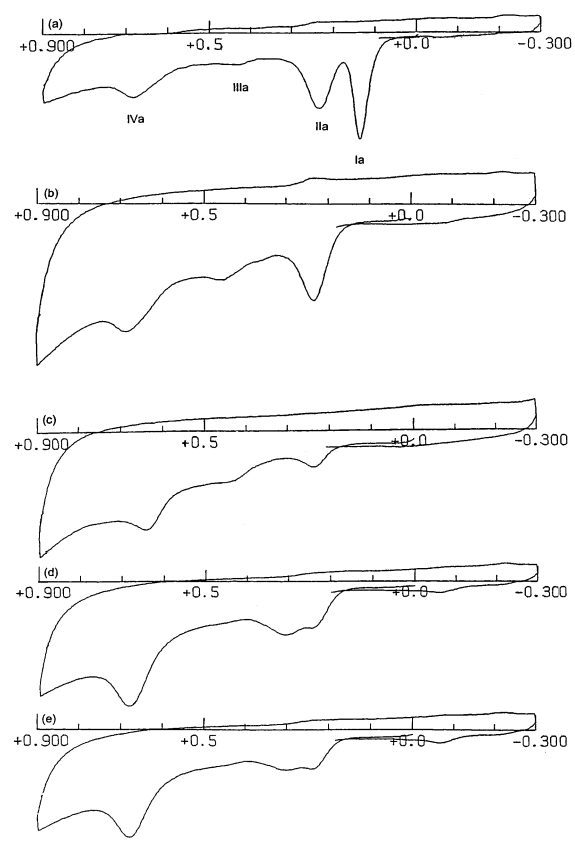
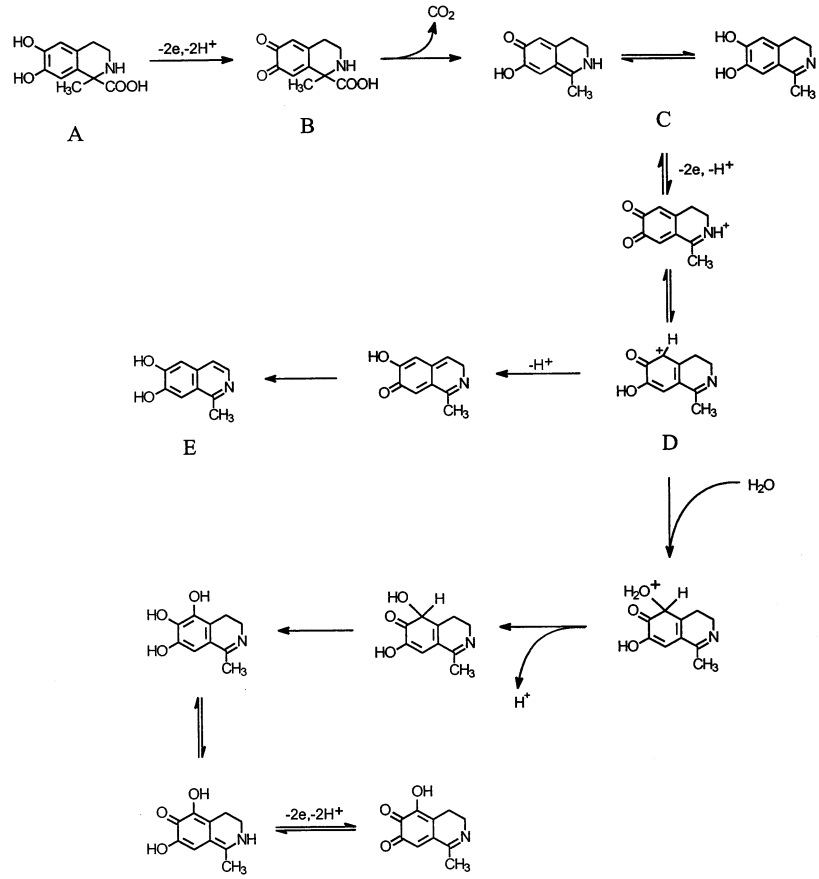


Fig. 5. Cyclic voltammograms at the PGE of: (a) 0.03 mM DNLCA; (b) 0.1 mM 1; (c) 0.1 mM 2; (d) 0.1 mM 3; and (e) 0.1 mM 4 in sodium phosphate buffer ($\mu = 1$) at sweep rate of 100 mV/s. The initial anodic scan starts at 0 V through peaks Ia, IIa, IIIa, IVa and reversed at + 0.9 V.

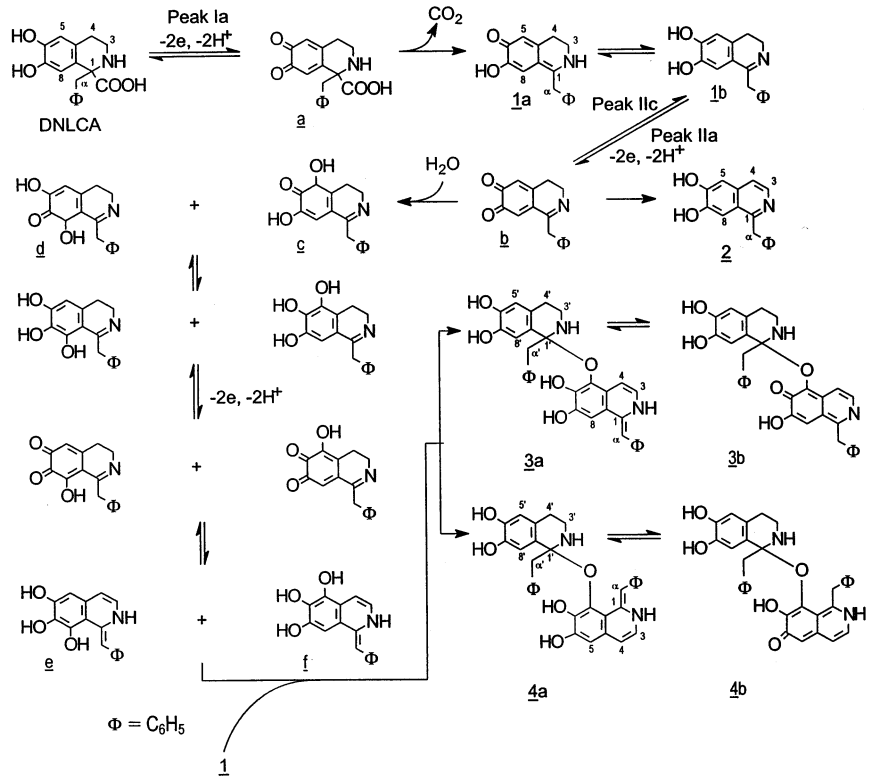


Scheme 1.

necessary for confirmation of this mechanism. Owing to the low stability of 3 and 4, meaningful ^{13}C NMR spectra were not obtained. The UV-visible spectra of 3 and 4 in aqueous solutions of different pH are quite similar to that of 1. In aqueous solution, 3 and 4 gave a yellow color. For both 3 and 4, as the pH of the medium increased, bands at 250 and 320 nm shifted to longer wavelength with decreased intensity while the bands at 360 nm shifted to 406 nm with increased intensity. These behaviors suggest that a process of tautomerization exists for both 3 and 4, i.e. 3a↔3b and 4a↔4b. Increasing pH favors formation of 3b and 4b. Even though the low solubility and poor stability of 3 and 4 in water limited the possibility of getting meaningful ^1H NMR spectra in D_2O, the ^1H NMR spectra of 3 and 4 in acetonitrile-d_3 (see experimental section) indeed revealed the existence of C(α)-H in both quinone methide (3b and 4b) and phenol (3a and 4a) forms.

5. Conclusion

Information bearing on the oxidation of DNLCA at physiological pH has been obtained from both voltammetric data and the chemical structures of isolated products. DNLCA is a quite easily oxidizable species to give the corresponding o-quinone intermediate which decarboxylates immediately at the electrode to yield 1 which is also a relatively easily oxidized compound to produce the fully aromatized 2 or dimer 3 and 4. Both 3



Scheme 2.

and 4 could be further transferred oxidatively to more complicated product mixture owing to their quite low oxidation potentials. The rationale for the work was to explore the oxidative transformation of DNLCA on the assumption that such reactions might occur in the brain and lead to formation of toxic or neuropharmacologically active metabolites that may play role in PKU. It is conceivable that 1–4 might possess toxic properties in the central nervous system if formed.

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